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# Research 22

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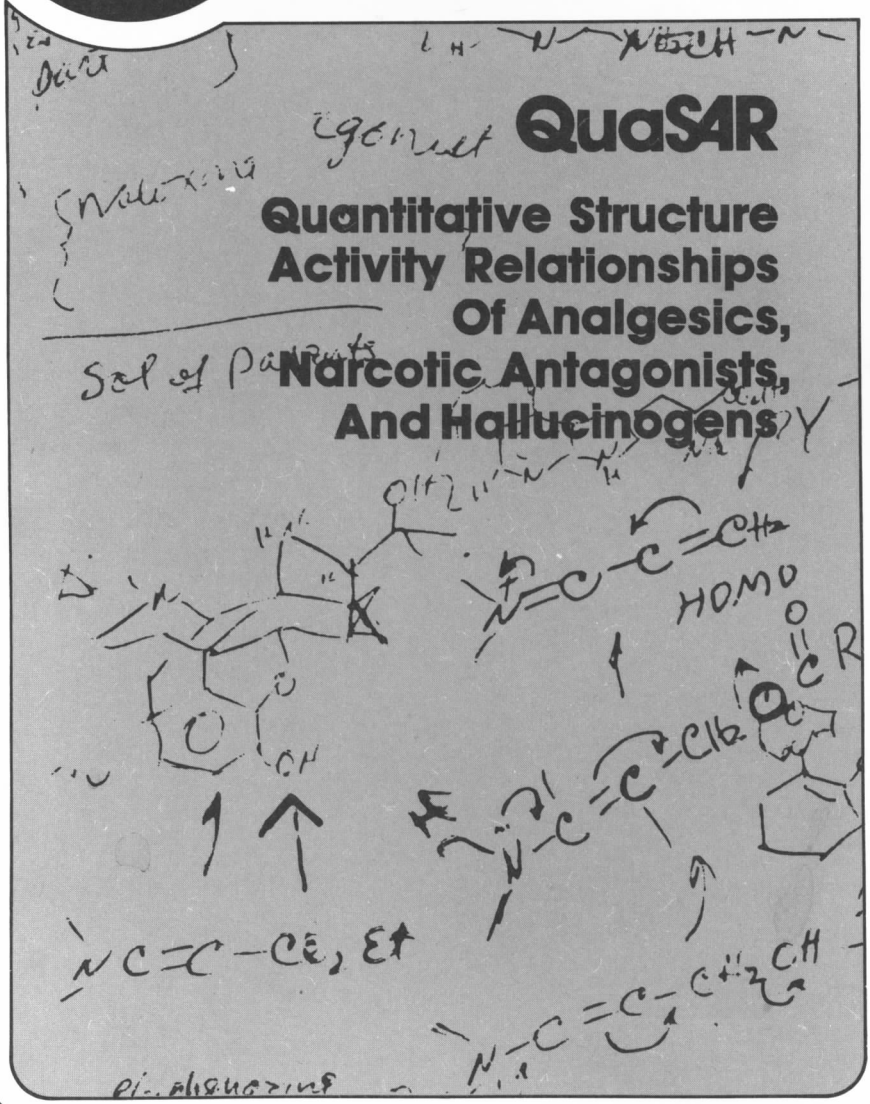

# QuaSAR

## Quantitative Structure Activity Relationships Of Analgesics, Narcotic Antagonists, And Hallucinogens

U S DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE • Public Health Service • Alcohol, Drug Abuse, and Mental Health Administration

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Editors:

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Milan Trsic, Ph.D.
Robert E. Willette, Ph.D.

**NIDA Research Monograph 22**

**DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE**
**Public Health Service**
**Alcohol, Drug Abuse, and Mental Health Administration**

**National Institute on Drug Abuse**
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# Quantitative Structure Activity Relationships of Analgesics, Narcotic Antagonists, and Hallucinogens

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# Foreword

The narcotic analgesics and the hallucinogens, two of the many classes of drugs used by mankind for many centuries, are still objects of substantial research, and the source of numerous questions as to the nature of their biological activity. Features that these two groups of drugs have in common include a strong predisposition to abuse, considerable overlap in physicochemical and pharmacochemical properties, and some possibly similar mechanisms of action. It is therefore no coincidence that we held a meeting to discuss these compounds together.

Man, in his quest for greater knowledge and understanding, demonstrates extreme resourcefulness as he deliberately turns to every means and technique available in order to help in the pursuit of revealing the secrets of nature. This is dramatically illustrated in this volume, where use is demonstrated of a wide variety of physicochemical, pharmacochemical, and theoretical methods. The monograph is one result of an intensive three-day meeting sponsored by the National Institute on Drug Abuse to bring together research scientists involved in the investigation of biological properties of analgesics, narcotic antagonists, and hallucinogens.

It was recognized by the Institute, as well as by others, that the use of such techniques as quantum mechanics, molecular spectroscopy, tissue and receptor binding studies, chemical modification of molecular structures, and correlation analysis can be of significant aid in understanding the basic mechanisms of drug action at the molecular level. For this reason NIDA has judiciously supported a considerable amount of research using these approaches on drugs of major concern and interest. Much of the work reported in this volume has been a product of that support.

The recent convergence of the discovery of the endorphins and the development of significant new and refined research techniques led us to conclude that it was essential, at this time, to bring together this interdisciplinary group of both esteemed and newly budding scientists in order that they could discuss their findings.

It will be apparent to the reader that final answers and definitive conclusions, both about the drugs of interest and the methods of study, are still not abundant. However, it is clear that much has been achieved and our continuing interest and support are necessary to assure that further advances are possible. It is our eventual goal that, in learning how these useful but abused drug molecules interact with the human body, we will one day be able to tailor their structures to provide a more favorable balance of properties, in order to direct us toward better means of treating drug addiction or abuse, and perhaps eventually to learn how one can prevent drug abuse altogether.

William Pollin, M.D.
*Director*
*Division of Research*
*National Institute on Drug Abuse*

# Contents

# Introduction

This monograph contains the proceedings of a technical review meeting held by the National Institute on Drug Abuse during April 20-22, 1978, entitled Quantitative Structure Activity Relationships (QuaSAR) of Narcotic Analgesics, Narcotic Antagonists and Hallucinogens. The meeting agenda is given in Appendix A and a list of participants is given in Appendix B. The meeting consisted of 6 half-day sessions with the first three sessions devoted to the opiates while the last half of the meeting was given to considerations of the hallucinogens. The chairmen were asked to provide an overview of the discussions for their respective sessions which were then used in preparing the general commentary at the beginning of each of the 4 sections of the monograph. The sections are defined according to primary quantitative method used as follows: I. Pharmacochemical Methods: II. Hansch Analvsis and other Empirical Methods; III. Molecular Mechanics; IV. Spectroscopic Methods. This classification is admittedly arbitrary due to both the interdisciplinary nature of the scientists gathered for this meeting and the complexity of the problems under consideration.

The initial quantitative approach to studying a problem as complex as those being discussed in these proceedings is to begin with the simplest model which when well defined, in terms of both functionality and parameterization, gives a reasonable zeroth order description of the system. Thus the kinetics of drug absorption and tissue penetration are initially described by Fick's law. Likewise, the time dependence of drug disposition from site of administration to sites of action may be considered as a classical kinetic process. The structure of the drug molecule is initially described in

simplest quantum mechanical terms with an approximate Hartree-Fock procedure. The relation between molecular structure and biological action is roughly correlated on a one-to-one level. Molecule interaction with the biosystem is initially described by a simple key and lock model for the concept of drug receptor interaction. From these elementary or crude approximate models one hopes to gain sufficient intuitive insight to provide a rational basis for a more refined quantitative investigation of the problem.

When one begins to refine the approach, the quantitative investigator will likely turn to a superposition principle. On the basis of preliminary findings a simple functional description $\phi i$, the i th of a set of valid ones, is weighted in terms of its assumed or actual importance by a factor Xi and the true solution is expressed as

$$F_{True} = \sum_{i=1}^{\infty} \lambda_i \Psi_i$$

At this point the infinite sum must be truncated to practical limits by some means, be it experimentation, trial and error, or intuition.

At this higher level of quantitative sophistication the simplicity of the Fickian description for molecular transport must eventually be replaced by procedures utilizing the Phenomenological Equations of irreversible thermodynamics, which have been developed and applied successfully to biosystem transport. This more complex method of description has identified concomitant and interacting driving forces and flows existent in the transport process where one considers a combination (not necessarily linear) of flows and of driving forces that necessarily obey the Onsager reciprocal relations. The process of drug disposition within the biosystem likewise becomes more complex. The current pharmacokinetic description of drug absorption, distribution, transformation and elimination is described by a superposition of first order kinetic processes where one often needs to consider nonlinear components in the process as well when the quantitative level is increased. Likewise the correlation between molecular properties and biological response is a more complex procedure involving superposition of parameters. In molecular structure calculations the quantitative advances have used the superposition of atomic orbitals to calculate more accurate molecular orbitals and the Hartree-Fock form of the quantum state function may be

replaced by a configuration interaction function when a more sophisticated quantitative description is warranted.

Finally, the concept of a drug receptor is becoming necessarily more complex and several different types of receptors are considered. Although the concept of multiple modes of interaction of opioids with different recognition loci on a single type of receptor or a group of closely related receptors was proposed over ten years ago to account for this complexity (P.S. Portoghese, J Med Chem, 8:609 (1965); W.R. Martin, Pharmacol Rev, 19:463 (1967)), until recently this concept had remained an idea whose time had not arrived. Multiple hallucinogen receptors have now been proposed as well (J.P. Green et al., this monograph; A.T. Shulgin et al., this monograph). The complexity of a biomembrane in terms of molecular architecture and transport properties, its structure and function, makes the superposition of receptors a reasonable concept. A drug receptor R(r,t) has an observed response that is both spatial and time dependent. It is appealing to describe this general receptor in terms of contributions from localized receptors, $r_i(r,t)$, we have not yet begun (or are only now beginning) to resolve sufficiently. Then the observed drug response is expressed in terms of a general (global) receptor that is a superposition of localized receptors,

$$R(r,t) = \sum_i \lambda_i \, r_i(r,t)$$

where $\lambda_i$ is likely to be time dependent, $\lambda_i(t)$.

Conclusions that can be drawn from the three days of presentations and discussions offer not only guidance for new directions for further research but also caution in the use of some of the many procedures and methods that are a necessary part of interdisciplinary research.

(1) The experimental pharmacologists have urged extreme caution in utilizing data obtained from some of the currently applied tests for biological activity, especially from whole animal or other complex systems. It was felt that further research and development is urgently needea both for new methods and to make the old methods more quantitative.

(2) The Hansch analysis method came under considerable criticism but was also ably defended. It was concluded that the method does have a domain of validity in the type of research discussed here. The reason that this type of method is so controversial, while little

criticism is aimed at the quantum chemical methods, is both apparent and one of the virtues of the Hansch analysis. This method is straightforward and quite easy to comprehend although it was urged that greater care be exercised in the choice of methods and parameters and that greater emphasis be placed on orienting such studies so as to provide mechanistic insight. The quantum chemical method, on the other hand, is couched in the language of the Schroedinger Equation, partial differential equations, molecular integrals, matrix elements and jargon such as HOMO, LUMO, SCF-HF-LCAO-HO, CNDO, etc. Therefore, this difficulty tends to lead the nontheoretician to accept calculated results with minimal objection.

(3) The theoretical physicists have urged caution in the application and interpretation of quantum chemical approaches to research. Because of the complexity of the methods and the ready availability of packaged computer programs it has been concluded that a critical technical review of the role of quantum mechanics in biomedical research may be beneficial in the near future.

(4) There is a far greater role for spectroscopic methods in QuaSAR research than indicated by the small number of presentations included in these proceedings. The methods presented here study properties of isolated molecules and solvent-solute interactions. The conclusion is warranted that such studies be extended to the molecule-receptor complex by utilization of synthetic model membranes as well as in vitro, in vivo and extracted receptors. This advance is needed to provide some measurement of the chemicophysical interactions involved and thus allow more quantitative modeling of the molecule-receptor complex.

(5) Studies should be directed toward better opioid receptor characterization. This would include receptor isolation, studying their chemical and biochemical properties, and ultimately determining their constitution. Ventures of this magnitude are formidable tasks and require a long term commitment to basic research.

(6) In conjunction with the above, a concerted effort should be made to develop new opioid receptor probes that would facilitate receptor characterization and be of aid in other types of pharmacologic studies. This would include design and synthesis of highly selective affinity labeling agents to tag opioid receptors. One needs only to scan the literature or a pharmacology text to recognize the broad impact that agents such as phenoxybenzamine have made in elucidating mechanisms of drug action. The development of selective

receptor probes may have a similar effect in the opioid field.

(7) The area of endorphins is of fundamental importance and should continue to be pursued with vigor. This would include projects directed toward (a) the isolation and characterization of new endorphins and antiendorphins, (b) biosynthesis and catabolism of endorphins, and (c) synthesis of novel endorphins.

(8) There is a lack of basic knowledge concerning the pharmacokinetics of the transfer of ligands into and out of the brain. While many reports have dealt with the relationship between uptake and lipophilicity, no systematic study has addressed the role of pharmacokinetic parameters in this process. For example, there are no systematic studies that provide information on the relationship (if any) between the individual transfer constants (entry and exit constants) and lipophilicity. Clearly, a knowledge of such rate processes would be useful in the design of opioids or opioid antagonists having a predictable time-course of action. This would be particularly relevant to the develoment of long-acting antagonists.

(9) With respect to the hallucinogen areas discussed, it is apparent that the evolution of new molecules continues to provide further specificity or separation of pharmacological properties. The work on identifying receptors and further delineating the properties of this little understood group of compounds should help to provide greater insight into the intimate processes of the brain and mind.

The proceedings reported in this monograph concentrate on questions of molecular structure, correlation of molecular properties with biological activity, and molecular interactions with the receptor(s). While these proceedings demonstrate progress in achieving an understanding of the basic problems, each participant would readily agree that there is still a long, tortuous, high resistance pathway that must be traversed before full knowledge is gained.

The Editors

**ACKNOWLEDGMENT**

We are pleased to acknowledge the special assistance provided by Dr. Philip Portoghese on scientific matters.

# Section I

## Pharmacochemical Methods

The relationship between structure and biological activity of ligands that act at opioid receptors is extremely complex. The fact that opioids possess diverse chemical constitution, divergent absolute stereoselectivities, and a spectrum of pharmacologic profiles attests to the magnitude of this complexity. The advent of new testing procedures has opened a new chapter in this active research area, leading not only to superior agonists and antagonists, but also to the discovery of the enaorphins ("The Endorphins," E. Costa and M. Trabuchi, ed., Raven Press, 1978). Mounting evidence supports a complex picture consisting of multiple recognition sites with regard to location on the nerve cells and location in the CNS. This being the case, one wonders whether the comparison of congeners from distantly related series is valid and whether a unified analysis of opioids is tenable.

Though a new chapter has been opened, our knowledge of opioid receptors is still rather rudimentary. After these recognition sites have been characterized further and additional basic information concerning the endorphins is obtained, quantum mechanics may be able to make substantial contributions toward the understanding of events at the level of the receptor. However, until a critical mass of basic knowledge is acquired, it would be unreasonable to expect definitive answers from this approach, although it should provide guidance along the way.

To a lesser degree, basic work on the identification and isolation of hallucinogen receptors and the measurement of drug-receptor bindings is beginning to provide a more quantitative understanding of the structural requirements for such activity. Indeed, in his presentation, Dr. Hartin cautioned that structure-activity studies

must carefully take into account the existence of multiple receptors or families of receptors. The work presentea by Dr. Nichols and by Dr. Barfknecht on rigid or restricted molecules as probes appears helpful. The work by Green and associates illustrates a good combination of the interrelationship of in vivo and in vitro data and calculated values. Dr. Shulgin and collaborators have used more traditional approaches in their attempts to shed light on the subtle distinction in effects brought about by minor structural changes. Correlations of these many factors often provide clues if not answers.

At our present level of knowledge, it appears that multiple regression analysis on series of compounds might be useful in assisting in the search for non-addictive analgesics or therapeutically useful psychotomimetics if the pitfalls and limitations of this technique are recognized by its users. One of the problems with multiple regression analysis as it is presently applied to analgesics is that no attempts have been made to sort out analgesic action from side effects such as addiction liability. Studies of this type might be done retrospectively if reliable biological data are available but it would seem that a combined program of synthesis, testing and regression analysis might be more rewarding. Extreme caution should be exercised in such studies because of the possibility that different ligands might bind to multiple receptors. In this connection, regression analysis should be confined to a series of closely related compounds in an effort to avoia such pitfalls. Projects of this type might best be categorized as development work because the limitations of the approach and the complexity of the biological system would make very difficult the factoring of receptor-related physical parameters for a basic understanding of the drug-receptor interaction.

'QuaSAR' Research Monograph 22
G. Barnett, M. Trsic. and R. Willette, eds.
National Institute on Drug Abuse, 1978

# Absolute Configuration and Psychotomimetic Activity

George M. Anderson, III, Gisela Braun, Ulrich Braun, David E. Nichols, and Alexander T. Shulgin

Most of the known psychotomimetic agents have at least one chiral center within their structures but have been stuaied only as the racemic mixtures. All of those which have been studied in optically active form are consistent in that the more potent isomer is the isomer with the absolute "R" configuration at the chiral center carrying the nitrogen that corresponds to the amino group of the phenethylamine moiety. With LSD, the 5-"R",8-"R" isomer is effective in man within the dosage range of 0.05-0.1 mg, whereas the diastereo-isomeric 1-iso-LSD (5-"S", 8-"R") is inactive in man at twenty times tnis dosage (Hofmann 1959). Four of the ring-substituted phenylisopropylamine psychotomimetics have been studied in their optically active forms. With 4-bromo-2,5-dimethoxyphenylisopropylamine (DOB) the "R" isomer is effective at 0.5 mg, approximately twice the potency of the racemate (Shulgin et al. 1971), whereas the "S" isomer is only about a fifth as potent. The "R" isomer of 4-methyl-2,5-dimethoxyphenylisopropylamine (DOM, STP) is reported as being at least four times more potent as a psychotomimetic than the "S" isomer (Shulgin 1973). These observations with both DOB and DOM are parallel to those observed in biochemical studies (Dyer et al. 1973) and in animal models (Benington et al. 1973), although these latter studies were conducted at nearly lethal dosages. The ethyl homolog of DOM, 4-ethyl-2,5-dimethoxyphenylisopropylamine (DOET), has been studiea as its optical isomers (Snyder et al. 1974) and here again the "R" isomer is approximately four times the activity of the "S" counterpart. Finally, the "R" isomer of 3,4-methylenedioxyphenylisopropylamine (MDA) is reported to be three-fold more potent than its optical enantiomer (Marquardt 1978).

Amphetamine itself, on the other hand, is at nominal dosages a stimulant rather than a psychotomimetic drug. In direct comparisons of its optical isomers in man, it

is the "S" or dextrorotatory form that is the more active. In most titration measurements in clinical stuaies it is accepted as being about twice as potent as the "R" isomer (Smith and Davis 1977), although the levo, or "R", isomer has been reported to be as effective as tne "S" isomer in the development of a psychotic syndrome (Angrist et al. 1971). These comparisons are presented in table I.

**TABLE I**

RELATIVE POTENCIES OF THE OPTICAL ISOMERS OF SEVERAL CNS AGENTS

| Drug | CNS Action | Chiral carbon | Order of potency | Factor |
|---|---|---|---|---|
| LSD | Psychotomimetic | 5 | R > S | >x20 |
| DOB | Psychotomimetic | α | R > S | x10 |
| DOM | Psychotomimetic | α | R > S | x4 |
| DOET | Psychotomimetic | α | R > S | x4 |
| MDA | Psychotomimetic | α | R > S | x3 |
| Amphetamine | Stimulant | α | S > R | x2 |

Several other clinically used CNS stimulants contain the same chiral center as does amphetamine but they have been studied in man only as the "S" form (as with phenaimetrazine) or in the racemic form (as with fenfluramine, mephentermine, methylphenidate and fenethylline). There are no reports with these drugs of the "R" isomer having been evaluated in man.

In addition to optical properties, there is another structural feature that can be employed to separate the various phenethylamine-type psychotomimetics into two groups, i.e., the effect on the potency of the drug with methylation of the primary amino group. Six pairs of homologs have been studied and can be compared as to tneir psychotogenic potency. Four of these compound pairs (all trisubstituted on the aromatic ring) show a arop of from a half to a whole order of magnitude upon N-methylation. Two of these, DOB and DOM, upon N-methylation are less effective as psychotomimetic agents by a factor of ten. The other two, 3,4,5-trimethoxy-pnenylisopropylamine and the 2,4,5-counterpart (TMA and TMA-2, respectively), are decreased in potency by a factor of three. The remaining psychotomimetic, MDA, is unique in that upon N-methylation, it maintains substantially an unchanged potency (Shulgin and Nichols 1978) although undergoing subtle changes in the qualitative nature of the induced intoxication. The drug amphetamine, although a stimulant at normal clinical dosages, can exhibit dramatic psychotomimetic properties when used chronically. The N-methyl homolog methamphetamine is similarly psychotoxic and thus these represent a pair of compounds resembling in this respect MDA but being

quite dissimilar from the other phenethylamine psychotomimetics. These relationships are summarized in table II.

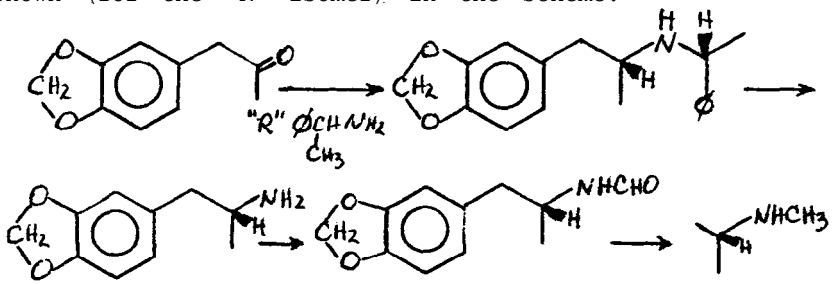
**TABLE II**

RELATIVE POTENCIES OF SEVERAL PRIMARY AMINES AND THEIR N-METHYL HOMOLOGS AS PSYCHOTOMIMETIC AGENTS

| Parent $NH_2$ drug | Relative potencies $NH_2$ vs. NHCH3 |
|---|---|
| dl-DOB | ×10 |
| dl-DOM | ×10 |
| dl-TMA | >×3 |
| dl-TMA-2 | >×3 |
| dl-MDA | = |
| d-Amphetamine | = |

It is apparent that the N-methyl homolog of MDA, N-methyl 3,4-methylenedioxyphenylisopropylamine or MDMA, might be a drug with unusual properties. Its parent, MDA, is a drug with the expected absolute configuration for psychotomimetic activity, but it is unlike the other psychotomimetics in that it does not suffer a diminution of potency upon N-methylation.

This report discusses the comparative pharmacology and psychopharmacology of MDMA which has been prepared in the form of its optical isomers. The two bases "R" (-) MDMA and "S" (+) MDMA were prepared synthetically as snown (for the "R" isomer) in the scheme:




"R"-MDA "R"-MDMA

3,4ace-Methylenedioxybenzyl methyl ketone (piperonylacetone) was coupled with (-) "R" benzylmethylamine, and the resulting Schiff base reduced with Raney nickel. Catalytic reduction of this "R,R" secondary base proviaea "R"-MDA, (a)D= -24.7, agreeing in physical and chromatographic properties with a sample obtained from NIDA, Rockville, Maryland. This base was formylated in methyl formate in a sealed tube and tne resulting forma-

mide had a reversed rotation $(\alpha)D$= +12.4 (in ethanol) and m.p.= 99-101. Reduction of this amide in THF with LAH provided the desired "R"-MDMA, $(\alpha)D$=-18.2, m.p.= 181-183 as the HCl salt. The "S" isomer was prepared in an exactly parallel manner, with the primary amine showing $(\alpha)D$= +25.3, the amide $(\alpha)D$= -12.6, m.p.= 101-102, and the final "S"-MDMA with $(\alpha)D$= +17.2 and a m.p.= 184-185. Racemic MDMA m.p.= 150-151.

An evaluation of the two optically active isomers, in comparison with the racemic form of MDMA, was made in rabbits, using the evoked rectal hyperthermia as a measure of central activity. The literature procedure (Aldous et al. 1974) was followed, and the two methods suggested for the evaluation of potency were employed: Methoa A utilized the mean maximum temperature rise (determined by a plot of $\Delta T$ vs. log dose), Method B employed an integration of the temperature-time curve as a measure of effectiveness. All compounds were assayed in at least three doses, in duplicate animals, and all values were normalized against racemic DOM, which was given the arbitrary value of 100. The results for MDMA in its optically active form and as the racemate are given in table III. The values obtained for MDA are presented for purposes of comparison.

**TABLE III**

RABBIT HYPERTHERMIA, RELATIVE POTENCY OF TEST COMPOUNDS, WITH dl-DOM = 100

| Compound | Method A (Mean Maximum) | Method B (Integral) |
|---|---|---|
| dl-MDMA | 1.96 | 1.15 |
| "R"-MDMA | 0.71 | ~0.5* |
| "S"-MDMA | 4.81 | 1.30 |
| dl-MDA | 2.46 | 1.51 |
| "R"-MDA | 3.48 | 2.29 |
| "S"-MDA | 2.93 | 0.91 |

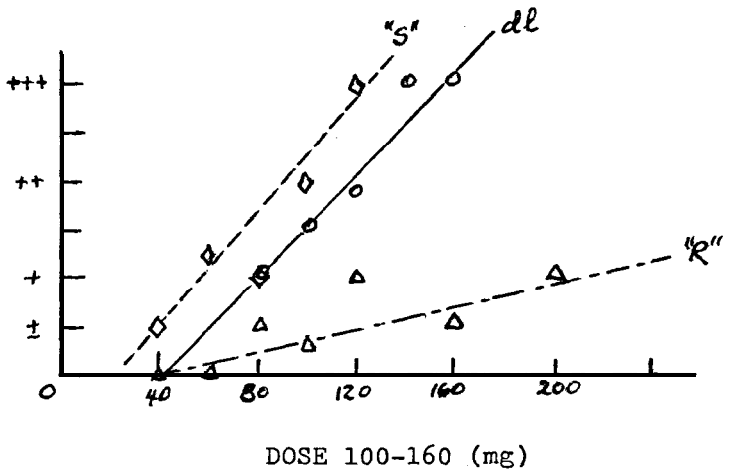
*Assays were conduced near the $LD_{50}$, and several animals were lost during the experiment.

Two features are immediately apparent from table III, both of which are in exact accord with the human intoxication aspects which will be discussed below. First, it is apparent, employing either method of evaluation, that MDMA is about two orders of magnitude less potent than the reference compound DOM and that it is slightly less potent than the N-desmethyl homolog MDA. Second, although it is clearly apparent that the "R" isomer of MDA is more effective than the "S" isomer with either method of calculation, with MDMA this poten-

cy assignment is reversed. "S"-MDMA is more effective as a CNS agent than is "R"-MDMA. Less apparent from the table, but a point to be brought out during the discussion below concerning human clinical evaluation, is the disconcerting absense of additivity between the component isomers in comparison with the activity of the racemate.

The three chemical species racemic MDMA, "R"-MDMA and "S"-MDMA, were evaluated in normal human subjects as their hydrochloride salts, orally, by established procedures (Shulgin et al. 1969). A plot of the quantitative response factor (judged as any of five points, -,±,+,++, and +++, depending upon the degree and the disruptiveness of the induced intoxication) against aosage, for each of the three chemical species, is shown in figure I. All responses at a given dose for a given compound are averaged and plotted as a single data point.

**FIGURE I**
HUMAN RESPONSE TO "R", "S" AND dl-MDMA




The data in figure I represent 35 clinical trials. The effective dosage for racemic MDMA has been presented as being in the range 75-150 mg (Shulgin and Nichols 1978) and 100-160 mg (Shulgin et a1.1978). Both values range in complete accord with the response of ++ being representative of a nominal intoxication as seen in the figure. The "S" isomer is more active than its optical enantiomorph, being effective within the dosage range of 80-120 mg. This range, however, is a larger dosage than is calculated (50-80 mg) by halving the racemate. As the contribution of the "R" isomer is little if any at

these levels, it must be concluded that the racemate is more effective as a CNS agent than would be expected or calculated from the separate activities of the component optical isomers. No acceptable value for the effective dose of the "R" isomer is clear from these data, but it would-appear that an effective dose might lie in the vicinity of 300 mg. Qualitatively, most of the sensory and interpretative properties reported for the racemate are seen in the "S" isomer, including the frequent physical toxicity manifestations of mydriasis and jaw-clenching. The "R" isomer is free of both side effects, even at the highest doses assayed. However, two subjects who experienced color enhancement on the racemate observed this peculiarity only with the (otherwise ineffective) "R" isomer. It must be concluded, within the data presently in hand, that with this one psychotomimetic compound, MDMA, the active optical isomer is the absolute "S" isomer with the configuration of dextro-amphetamine, in contrast with the previous generality that the activity of racemic psychotomimetic compounds could be largely accounted for by the "R" isomer.

The inability of the component isomers to adequately account for the action of the racemate is unusual but not without precedent with similar compounds in the literature. There are numerous reports where there is a clear interaction between the component isomers of racemic compounds in in vitro studies; this is well established in the metabolism of both amphetamine (Gal et al. 1976) and DOM (McGraw et al. 1977). In vivo studies in animals with 3,4-dimethoxyphenylisopropylamine (3,4-DMA) (Barfknecht and Nichols 1972) showed that the "R" isomer was only a third as active as the "S" isomer, and that neither could duplicate the characteristic effects of the racemic mixture.

The purpose of this report is not to explain or theorize on the persistence of activity following the N-methylation of MDA or on the unexpected reversal of activity assignment to the optical isomers. It is, rather, to present these findings and to let them provoke the necessary changes in receptor-site theory that must be made. Since the effects of MDA to some extent, and of MDMA to a very large extent, are far removed from that constellation of symptoms usually associated with psychotomimetic drugs such as TMA, DOM, and LSD, it is appealing to explain the difference of assymetric requirements in accord with these differences in qualitative responses, and to conclude that there is some different receptor site being acted upon in this case. There is no insistence that all psychotomimetic agents act by a single mechanism. It has been proposed (Cheng et al. 1974) that hallucinogenic drugs might be usefully classified either as direct acting or as indirect acting releasing agents. Most psychotomimetics appear to act

as direct serotonin agonists, but materials such as para-methoxyphenylisopropylamine (PMA) and MDA do not fit this model quantitatively (Nichols et al. 1977). Although no human studies have been conducted with the isomers of PMA, both PMA and MDA have been demonstrated to be indirect acting sympathomimetics (Nichols et al. 1974; Paton et al. 1975).

MDMA probably does not act by demethylation, as the reversal of optical requirements speaks against such a mechanism. This is further supported by the absense of cross-tolerance between these two drugs in man. Perhaps there is a stimulant action from one of the isomers which enhances or potentiates the (otherwise) dormant potential of the other. Such questions can be answered only by further experiments.

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# Congeners of DOM: Effect of Distribution on the Evaluation of Pharmacologic Data

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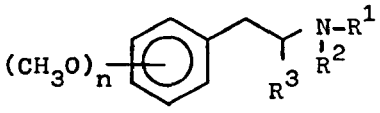
## INTRODUCTION

The study of structure-activity relationships (SAR) has intrigued medicinal chemists for decades. In the class of compounds termed psychotomimetics, the group of mescaline-type agents (1) has been studied extensively. The SAR of the group may be summarized as (Shulgin 1976; Barfknecht, Nichols and Dunn 1975):



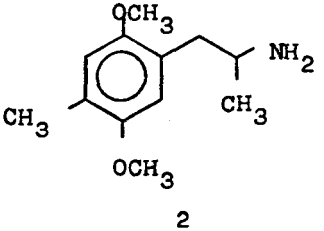

1

1. The presence of an $\alpha$-methyl ($R^3$) enhances the potency, while $R^3$ = H or $R^3$ = ethyl or larger alkyl group yield compounds which may be psychoactive, but not psychotomimetic.
2. Three aromatic ring substitutents enhance the potency over mono-or disubstituted ring systems. The location of the three substitutents in the 2,4,5 position is optimal.
3. In the compounds with an a-methyl, the para position is the primary site of metabolism (O-demethylation for p-methoxy and p-hydroxylation for p-hydrogen).

p-Methyl or p-halo substitutents retard the metabolism at this site. In addition, compounds with these para-substitutents are significantly more potent than the p-methoxy or p-hydrogen analogs.
4. Substitution on the nitrogen abolishes psychotomimetic activity. The N-methyl analog of DOM is an antagonist.
5. When the α-methyl group is present, two enantiomers exist. The R(-) is more potent than the S(+) enantiomer. The absolute stereochemistry of the more potent enantiomer correlates with the stereochemistry of the corresponding position in LSD.
6. The ability of a compound to reach its site of action is dependent upon the drug distribution between lipid and aqueous phases. Changes in the ring substitution have a major influence on distribution.

In the group, the prototype synthetic agent is called DOM (2, STP), 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane. Although many studies have been reported on the pharmacologic and biochemical effects of methoxylated phenylisopropylamines, the mechanisms by which these molecules exert the psychotomimetic effect remain unclear.

A breakthrough in the SAR was the discovery that 1-(2,5-dimethoxy-4-methylphenyl)-2-aminobutane (3) does not exhibit psychotomimetic effects (Standridge et al. 1976). Preliminary evaluation indicated that its actions could be exploited for therapeutic purposes.



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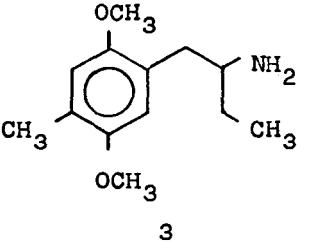
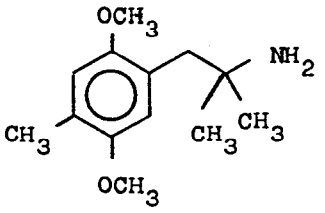


3

A research effort was launched to explore the SAR of 3 and to develop animal models for differentiating between the effects of 2 and 3. This paper will present the synthesis of two congeners, the evaluation of the compounds in the whole animal model and by in vitro assays, and finally the interpretation and correlation of these results in light of the distribution studies.

3 differs from 2 only by a single additional methylene in the α-side chain. This rather minor structural modification has a pronounced effect on pharmacologic

effects. 2-Amino-1-(2,5-dimethoxy-4-methylphenyl)-2-methyl propane (4, BL-4041A) and 1-amino-1-(2,5-dimethoxy-4-methylbenzyl)-cyclopropane (5, BL-4358A) were designed to incorporate aspects of both 2 and 3. From an empirical formula standpoint, 4 has one additional hydrogen and 5 has one less hydrogen than 3. From steric and electronic viewpoints, these molecules-should differ from both 2 and 3 and from each other.

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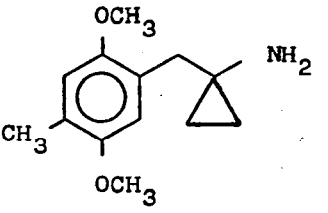
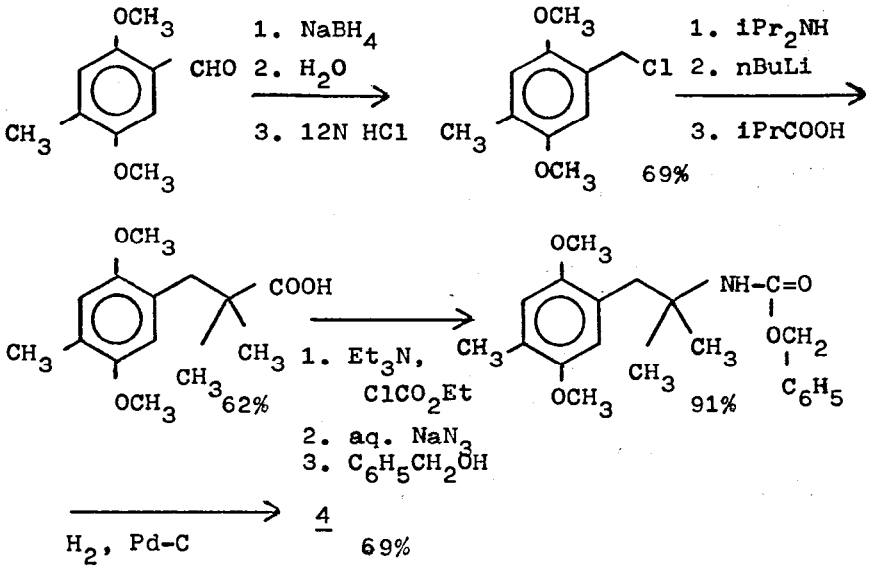


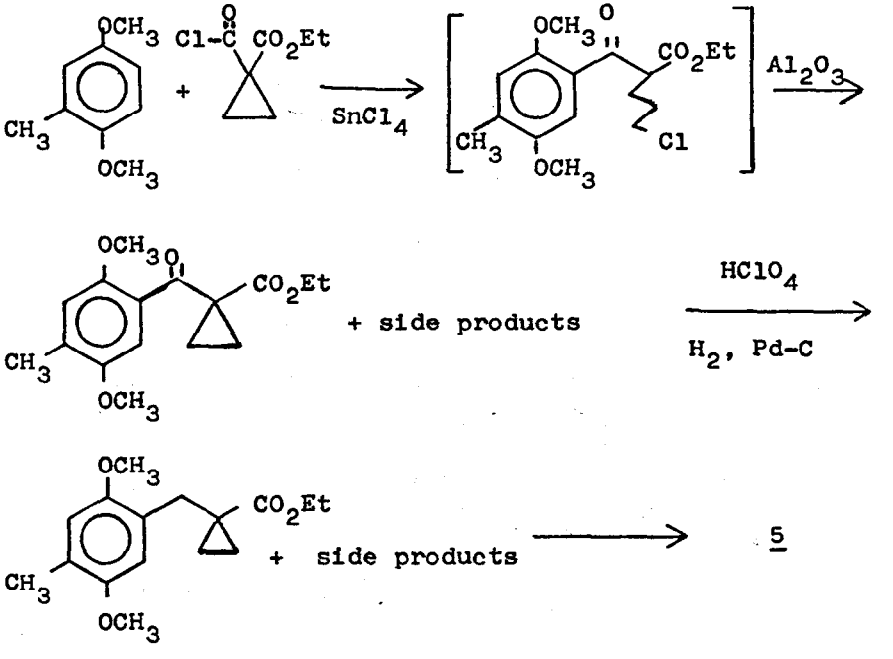

5

**CHEMISTRY**

Preparation of 4 was accomplished by a route previously used for a closely related compound (Standridge et al. 1976). 2,5-Dimethoxy-4-methylbenzyl chloride was condensed smoothly with isobutyric acid to yield 2-(2,5-dimethoxy-4-methylbenzyl)-2-methylpropanoic acid. This acid was then converted to the amine (4) through the carbobenzyloxy derivative.

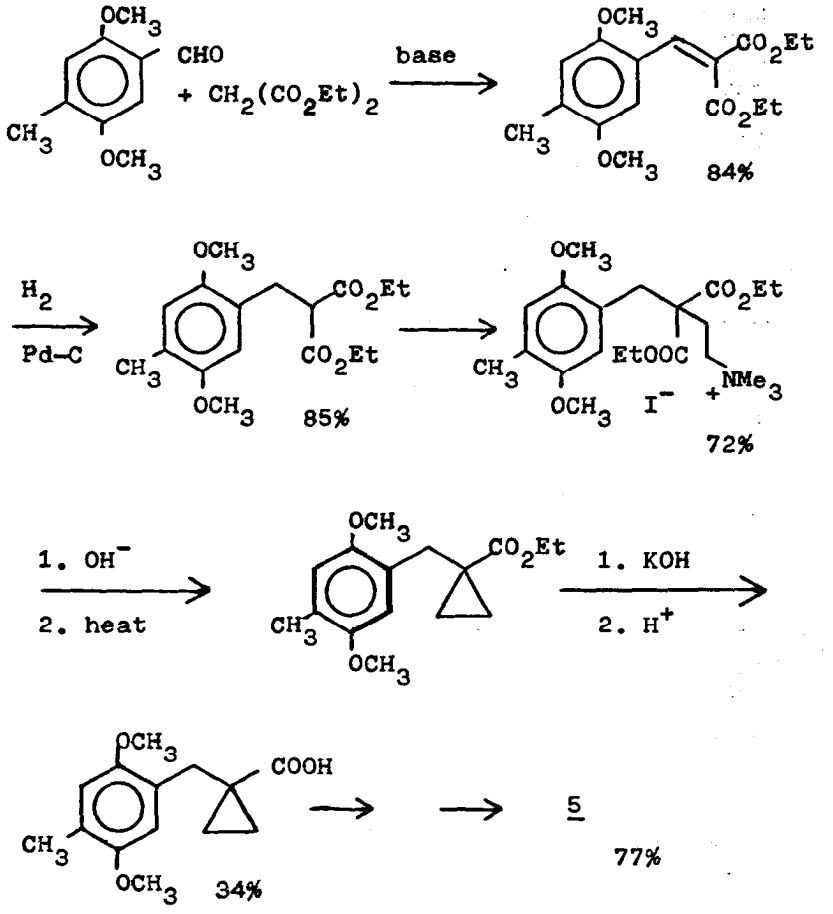


Application of the previous synthetic scheme to the preparation of 5 failed. Another route involved condensation of 2,5-dimethoxytoluene with 1-carbethoxycyclopropanecarbonyl chloride to give the substituted cyclopropyl phenyl ketone. The ketone could be removed and the ester converted to the amine (5) via the acid. The actual execution of this proposal proved to be considerably more complicated than anticipated.




While the scheme provided the desired intermediate, the side reactions and purification difficulties which were encountered necessitated the search for a more satisfactory route.

The successful route involved modification of a synthetic procedure developed by Kaiser and Weinstock (Kaiser and Weinstock 1972). The substituted benzaldehyde was condensed with diethyl malonate. After catalytic hydrogenation, the substituted malonate was alkylated with dimethylaminoethyl chloride and quaternized. The quaternary hydroxide underwent a Hoffman elimination - condensation, yielding the desired cyclopropanecarboxylic ester. After saponification and purification, the acid was converted to 5 in a manner similar to that used for 4.




**PHARMACOLOGY**

The goal of the biological evaluation in this group of compounds is to differentiate between those compounds which may have useful psychoactivities and those which are psychotomimetic and thus are eliminated from further consideration. The pharmacology of substituted phenylalkylamines is highly complex and will not be explored here.

Earlier, the spasmogenic effect of 2 and 3 on smooth muscle of the rat fundus was reported (Standridge et al. 1976). 2 was substantially more potent than 3 and was approximately one quarter the potency of serotonin. It

has been suggested that stimulation of serotonin receptors may be related to psychotomimetic activity (Barfknecht et al. 1973; Dyer et al. 1973). The relative potencies of 2, 3, 4 and 5 on the rat fundus system are summarized in Table I. Compounds 2 and 5 exhibited greater potencies than 3 and 4.

Table I. Comparative Pharmacological Effects on the Cat Behavior and Rat Gastric Fundus Tests

| | | Cat Behavior | | Rat Gastric Fundus | |
|---|---|---|---|---|---|
| Compound | N | Dose Mg/kg sc | Mean ± SE | ED50 Molar | Relative Potency |
| 2 | 4 | 1 | 13.3 ± 1.8 | $1.28 \times 10^{-7}$ (0.81 - 2.02) | 26.8 |
| 3 | 4 | 10 | 1.3 ± 0.50 | $7.14 \times 10^{-5}$ (5.06 - 10.1) | 0.048[1] |
| 4 | 4 | 10 | 0.75 ± 0.48 | $4.69 \times 10^{-6}$ (2.81 - 8.20) | 0.73 |
| 5 | 2 | 5 | 15 (14,16)[2] | $5.21 \times 10^{-7}$ (1.52 - 10.2) | 6.58 |

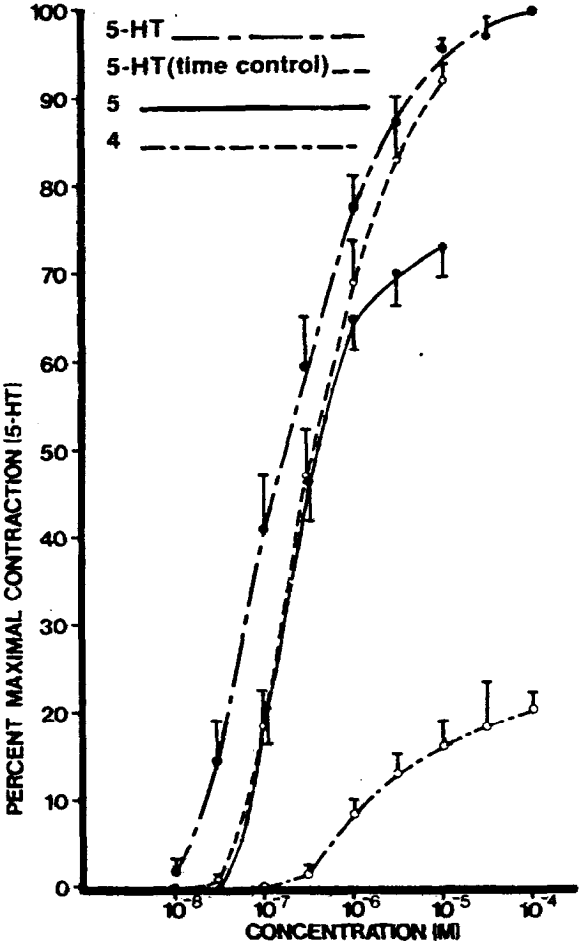
[1] Relative spasmogenic potency in terms of serotonin (100); $ED_{50} = 3.43 \times 10^{-8}$m, (2.49 - 4.91), 95 percent confidence limits in parenthesis.

[2] Mean and individual values in parenthesis.

The cat behavior test, a modification of a published procedure (Wallach, Friedman, and Gershon 1972), has been used to determine psychotomimetic activity. The animal reactions to drugs are observed and categorized in several classes: body posture (arching of body and tail), limb extension (legs and claws), body muscle rigidity, abnormal limb position (cataleptic-like reactions), motor coordination, mouth opening or tongue protrusion, hostility reactions (clawing, biting, hissing) , loss of contact to environment, autonomic reactions (piloerection, myosis, salivation, emesis). The drug effects are quantitated by assigning a point value for the occurrence. The effects of the compounds in this test are summarized in Table I. Both 3 and 4 were weak and exhibited a similar potency in contrast to 2 and 5 which induced markedly stronger effects. The conclusion drawn from these studies was that 5 had undesirable psychotomimetic properties, while its chemical relative (4) did not. Since the structural similarities of 4 and 5 are so great, further demonstration of the different pharmacologic effects was needed.

The sheep umbilical artery strip preparation has been shown to be serotonergic and to be stimulated by 2 and other psychotomimetic compounds. By way of contrast, 3 was found to be ineffective as an agonist (Dyer 1976). The dose-response relationships of serotonin, 4, 5 on the sheep umbilical arteries are presented in Figure I. Consistent with the data in Table 1, 5 was shown to be an agonist, while 4 was much less effective.

**Figure I.** Comparative Pharamacological Effects of 4 and 5 on the Sheep Umbilical Artery Preparation



**DISCUSSION**

With the results of three pharmacologic studies in agreement that 5 was psychotomimetic and 4 was not, it was tempting to ascribe these differences to interactions at the receptor site. However, from inspection of molecular models there were no obvious dissimilarities in steric requirements or preferred conformations. If we were to propose conformational differences as the source of the pharmacologic effects, detailed studies of the crystal structures, solution conformations, and molecular orbital calculations of preferred conformations would have to be undertaken. Even then, it would be necessary to show that these data were applicable to the pharmacologic assay situations.

For the past decade, the importance of the physical distribution of molecules on their apparent pharmacological actions and their relative potencies has received greater attention. Lead by the work of Hansch and his colleagues (Hansch 1969), we have become aware of partition coefficients and their influence on the interpretation of pharmacologic data. Recently, the use of the distribution coefficient has been shown to offer advantages over the partition coefficient in assessing the role of distribution (Scherrer and Howard 1977). The distribution coefficient, D, is defined as the ratio of the concentration of drug in the lipid phase to the concentration of total ionized and unionized drug in the aqueous phase at a given pH. It is assumed that only the unionized drug partitions into the lipid phase.

The relationship between log D and log P is shown in the following equation:

$$\text{Log } D_{\text{bases}} = \log P + \log \left[ \frac{1}{1 + 10^{pKa - pH}} \right]$$

The significance of this relationship is embodied in the second term on the right-hand side of the equation. This term is the correction factor for ionization of the involved species. When the pKa of the molecule is equal to the pH of the aqueous phase, this factor approaches zero. When the pKa becomes greater than the pH, the correction term becomes a larger negative value. Thus, the log P of an amine will always be equal to or greater than the log D when the pH is 7.4 (physiological pH). The distribution coefficients were determined in 1-octanol/water according to published procedure at ambient temperature (Fujita, Iwasa, Hansch 1964). The aqueous phase was buffered to pH 7.4 with phosphate buffer. The distribution data are shown in Table II.

Table II. Distribution Data on 4 and 5

| Compound | D | LogD | S[1] |
|---|---|---|---|
| 4[2] | 0.542 | -0.266 | 0.038 |
| 5[3] | 14.55 | 1.162 | 1.028 |

[1] S is the standard deviation

[2] D was calculated as the mean of six determinations

[3] D was calculated as the mean of four determinations

If one were to explain the pharmacologic data without considering the distribution data, one might say that the evidence clearly suggests that 5 is hallucinogenic and 4 is not. Then one would be tempted to invoke some receptor interaction difference to explain the situation. However, with the distribution data available, one has such a clear difference in distribution that any thought of ascribing the pharmacologic effects solely to receptor site interactions must await further studies of binding or molecular efficacy. Thus both molecules (4 and 5) may well exhibit the hallucinogenic profile, If they both were able to attain the necessary concentration at the receptor site.

Pharmacological screening is designed to find useful effects without regard to mechanism of action or the factors that may be contributing to the potency. It is only necessary to investigate distribution, metabolism, and molecular factors when the events on the receptor level are to be analyzed. These two strategies (pharmacological screening and molecular investigation) are designed to answer different questions and provide complementary information.

In applying the information gained in this study to other situations, one must not expect the cyclopropyl analog of every compound to have a significantly different distribution from that of its gem-dimethyl analog. Rather, the data presented here shows that one must consider the effect of the pKa on the distribution and the effect of structure on the pKa. The cyclopropyl group of 5 affects the pKa of the amino function by means of its pseudo-$\pi$ character. The result is that less of the compound is protonated at pH 7.4 and less is present in the aqueous phase. The gem-dimethyl grouping does not affect the pKa of the amino appreciably. Thus more of the compound is protonated at pH 7.4 and more is present in the aqueous phase, resulting in a distribution of 4 similar to that anticipated for 2 or 3.

This study has provided an insight into the complex process of evaluating the results of various pharmacologic tests and interpreting the data with and without considering the physical distribution of molecules in biological fluids. It is evident that distribution may play a significant role in all pharmacologic test systems, including the isolated in vitro preparations where traditionally it has not been considered. Further work to study the influence of physical factors in isolated systems is under investigation.

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# Mescaline Analogs: Substitutions at the 4-Position

Ulrich Braun, Gisela Braun, Peyton Jacob III,
David E. Nichols, and Alexander T. Shulgin

Mescaline, 3,4,5-trimethoxyphenethylamine, is one of the longest known and best studied of the psychotomimetic drugs. It has served as the structural paradigm for the synthesis and study of a large number of analogs. Many of the molecular changes have resulted in increased potency, and have established structural parameters felt to be necessary for maximum neurotoxic activity. One of these is the extension of the carbon chain from two carbons to three, by the addition of an alpha-methyl group adjacent to the basic nitrogen. This simple homologation appears to protect the nitrogen atom from metabolic removal, and to effectively increase the potency of the drug. In the examples in the earlier literature where direct comparisons between the two-carbon and the three-carbon counterparts were made, there was certainly an increase in potency. However, the metabolic argument is clouded by the fact that in those examples where the chain was extended to four carbons (providing as complete a structural hindrance to metabolic attack as a three-carbon chain) there was also a consistent decrease in biological activity.

A second parameter is the positioning of the groups of the aromatic ring. The relocation of substituents from the 3,4,5-orientation of mescaline to the 2,4,5-pattern has, again in the earliest reports (Shulgin 1964), resulted in a substantial increase in psychotomimetic potency. As a result of these generalizations, both the two-carbon phenethylamines and the 3,4,5-"mescaline-like" substitution pattern have been largely ignored in the synthesis and evaluation of psychotomimetic drugs and have played only a small role in structure activity relationship studies. In recent years, several discoveries have renewed interest in compounds more closely allied to mescaline in structure. First, a number of potent ring-substituted phenethylamines have been reported, chemicals that are the two-carbon analogs of

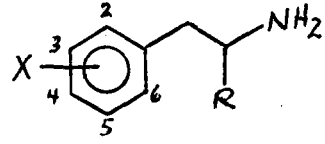
known psychotomimetic phenylisopropylamines. Second, there has been an increasing awareness of the importance of the 4-position in the substitution pattern of the 2,4,5-orientation. Third, there have been recent correlations between psychotomimetic potency and physical properties such as lipophilicity, which have suggested that modest chain-lengthening within a set system of aromatic substituents might affect biological potency (Barfknecht et al. 1975). The purpose of this paper is to review these parameters, to present experimental data concerning several new compounds, and to discuss the possible reasons for their activity.

Initially, mescaline was the only two-carbon psychotomimetic known. Although many variations on its structure had been made leading to a variety of compounds with greatly increased potency, it was only recently that the logical step was taken of investigating the two-carbon counterparts of the more potent phenylisopropylamines. The phenethylamines which have been studied, and their reported potencies in human subjects, are presented in table I in direct comparison to the corresponding substituted amphetamine homologs. It is immediately apparent that, in all cases, the three-carbon homolog is more potent than the corresponding phenethylamine, sometimes by as much as an order of magnitude. In some entries, the absence of defined action in man makes the comparison between the two groups uncertain.

Structural changes at the 4-position of the psychotomimetic phenylisopropylamines can modify both the quantitative and the qualitative effects that are produced. The potency increases as the nature of the group in the 4-position varies from H< OR < SR< R < X wherein R is an alkyl group and X is a halogen. Within each of these families, small groups of close homologs have been studied and the comparative quantitative relationships of these are shown in table II. In general, those series that represent progressive homologous sets of compounds have their maximum potency with the methyl or the ethyl substituent. However, of particular interest to this study is the unusual enhancement of activity seen in the ethoxy compound 3,5-dimethoxy-4-ethoxyphenylisopropylamine. The relatively minor change of potency seen upon replacing a methoxy group with an ethoxy group, or a methyl group with an ethyl group, at the 4-position (as seen in the comparison of TMA-2 to MEM, of para-DOT to aleph-2, and of DOM to DOET) is exaggerated when this alkoxy group is flanked with methoxy substituents (see table II). The unusual five fold increase in activity of 3,5-dimethoxy-4-ethoxyphenylisopropylamine over the 4-methoxy counterpart TMA may emphasize the importance of steric considerations in the action of these drugs. In the case of the 3,4,5-

**TABLE I**

HUMAN ACTIVITY OF 2-CARBON AND ANALOGOUS 3-CARBON-CHAIN PSYCHOTOMIMETICS (a)




| X = | 2-Carbon R=H | | 3-Carbon R=$CH_3$ | | Rel. potency $CH_3$ v. H |
|---|---|---|---|---|---|
| 4-$OCH_3$ | MPEA | >400mg (b) | PMA | 60mg (c) | >5x |
| 3,4-$OCH_3$ | DMPEA | 1500mg (d) | 3,4-DMA | ~400mg (e) | 4x |
| 3,$OCH_2O$-4 | | >200mg (f) | MDA | 100mg (e) | >2x |
| 3,4,5-$OCH_3$ | mescaline | 350mg (g) | TMA | 200mg (h,i) | 2x |
| 2,3,4-$OCH_3$ | 2,3,4-TMPEA | ~400mg (j) | TMA-3 | >100mg (e) | ? |
| 2,4,5-$OCH_3$ | TMPEA | >300mg (k,l) | TMA-2 | 20mg (m) | >15x |
| 2,5-$OCH_3$-4-Br | | 10mg (n) | DOB | 1mg (o) | 10x |
| 2,5-$OCH_3$-4-I | | >8mg (e) | DOI | 1mg (e) | >8x |
| 2,5-$OCH_3$-4-$CH_3$ | | 20mg (n) | DOM(STP) | 5mg (e) | 4x |
| 2,5-$OCH_3$-4-Et | | 20mg (p) | DOET | 4mg (e) | 5x |
| 2-$OCH_3$-3-$OCH_2O$-4 | | >60mg (c) | MMDA-3a | 30mg (e) | >2x |
| 3-$OCH_3$-4-$OCH_2O$-5 | | >250mg (q) | MMDA | 150mg (r) | >2x |
| 3,5-$OCH_3$-4-OEt | escaline | 60mg (e,s) | | 40mg (p) | 1½x |
| 3,5-$OCH_3$-4-OPr | proscaline | 60mg (e,s) | | ? | ? |
| 3,5-$OCH_3$-4-$SCH_3$ | thiomescaline | 30mg (s) | | ? | ? |

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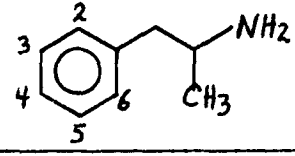
s. Present paper.

## TABLE II

RELATIVE POSTENCIES IN MAN OF DIMETHOXYPHENYLISOPROPYLAMINE PSYCHOTOMIMETICS WITH VARIOUS SUBSTITUENTS ON THE 4-POSITION




| Substitution Pattern on the Phenylisopropyl Amine | R = | Name | Potency (total dose mg/man |
|---|---|---|---|
| 2,5-$OCH_3$-4-OR | $CH_3$ | TMA-2 | 20 mg (a) |
| | $C_2H_5$ | MEM | 30 mg (b) |
| | $C_3H_7$ (n) | MPM | 50 mg (c) |
| 2,5-$OCH_3$-4-SR | $CH_3$ | para-DOT | 10 mg (d) |
| | $C_2H_5$ | aleph-2 | 5 mg (b) |
| | $C_3H_7$ (i) | aleph-4 | 8 mg (c) |
| 2,5-$OCH_3$-4-R | $CH_3$ | DOM(STP) | 5 mg (b) |
| | $C_2H_5$ | DOET | 4 mg (b) |
| | $C_3H_7$ (n) | DOPR | 5 mg (e) |
| | $C_4H_9$ (n) | DOBU | 10 mg (e) |
| | $C_4H_9$ (t) | DOTB | >25 mg (d,f) |
| | $C_5H_{11}$ (n) | DOAM | 40 mg (e) |
| 2,5-$OCH_3$-4-X | Br | DOB | 1 mg (g) |
| | I | DOI | 1 mg (b) |
| 3,5-$OCH_3$-4-R | $OCH_3$ | TMA | 200 mg (h,i) |
| | $OCH_2C_6H_5$ | | 150 mg (b) |
| | $OC_2H_5$ | | 40 mg (c) |
| | Br | | 6 mg (j) |

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f. A > symbol indicates the absence of any activity at the stated dosage.
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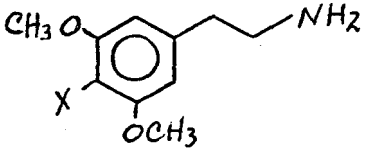
j. Nichols, D.E., Shulgin, A.T., and Dyer, D.C. Life Sci, 21:569, 1977.

substitution pattern, an ethoxy group in the 4-position is of necessity directed away from the plane of the aromatic ring.

These possible steric effects have been evaluated by an approach involving partition coefficients. In this way, an estimate of comparative lipophilicity can be made since this property is felt to influence the ease of membrane transport and thus eventual availability to the site of action. A number of psychotomimetic phenylisopropylamines have been studied in an octanol-water partition system, and the correlation of the resulting values with central activity has provided a relationship that suggests an optimum lipophilicity for maximum biological activity (Barfknecht et al. 1975). These partition values have been correlated to serotonin receptor stimulation capability (Nichols and Dyer 1977) and have recently been extended to a number of phenethylamine compounds (Nichols et al. 1977).

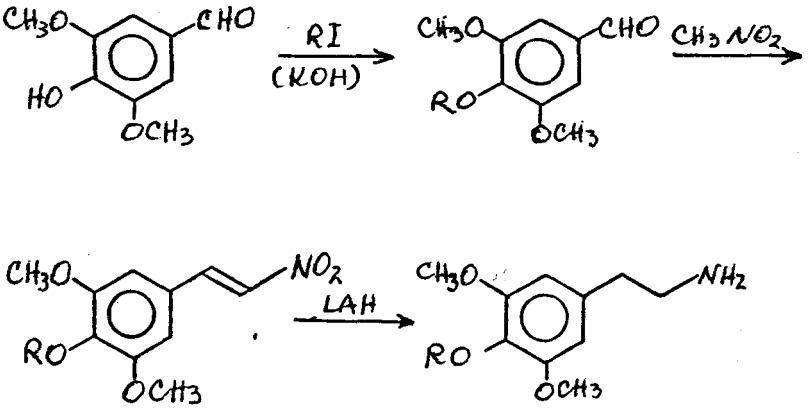
We have undertaken a project directed towards an investigation of a number of compounds that represent a return to the 3,4,5-orientation and the two-carbon chain features of mescaline, but that are modified in some way by the substituent that is found at the 4-position. These are compounds of the general structure:




wherein X = R, OR, SK and halogen. In this report we will discuss the chemistry and the psychopharmacology of the first three compounds studied in this direction.

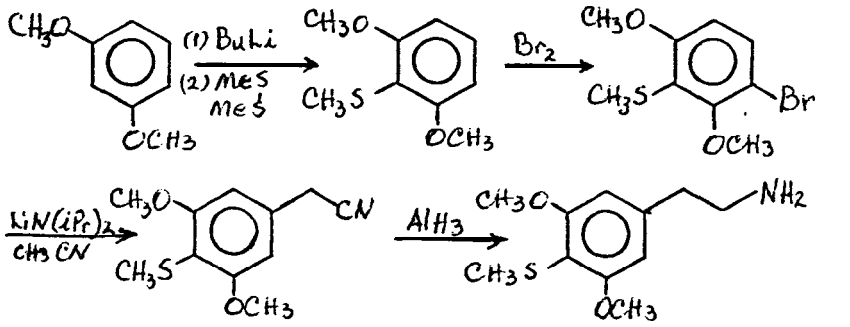
Two of the compounds described here are 4-alkoxy homologs of mescaline. These have been prepared by the appropriate alkylation of syringaldehyde with either ethyl or propyl iodide followed by the formation of a nitrostyrene with nitromethane. These intermediates were then reduced with LAH to form the product amines 4-ethoxy-3,5-dimethoxyphenethylamine (1) and 4-propoxy-3,5-dimethoxyphenethylamine (2), respectively. These products have been called escaline and proscaline in keeping with the well-established trivial name mescaline for the 4-methoxy counterpart. These reactions are shown in scheme I.

**SCHEME I**




The third compound studied is the 4-thio analog of mescaline, 4-methylthio-3,5-dimethoxyphenethylamine. This was prepared (see scheme II) by the reaction of lithiated m-dimethoxybenzene with dimethyldisulfide to form 2,6-dimethoxythioanisole. This underwent bromination uniquely adjacent to the methoxy group, and the resulting bromodimethoxythioanisole underwent a smooth benzyne reaction with acetonitrile to form the benzyl cyanide shown, which was reduced to the desired end product (3) with aluminum hydride in THF.

**SCHEME II**




These three mescaline analogs (table I) have been assayed in normal subjects by procedures previously outlined (Shulgin et al. 1969). Both of the alkoxy homologs are effective as psychotomimetics at dosages of 60 mg orally, with clear threshold effects being noted

in some subjects at levels as low as 10 mg. These bases differ from mescaline in that the onset of action occurs sooner (within the first hour) and there is no nausea noted, but otherwise the time course and much of the qualitative content of the intoxication are similar to those of mescaline. The sulfur compound thio-mescaline (3) is also of unexpectedly high potency and is an effective psychotomimetic in man at oral doses of 30 mg. The initial indicators of intoxication are apparent during the second half of the first hour following administration, and a plateau of intoxication is maintained for approximately three hours. The qualitative content of the experience resembles LSD more closely than it does mescaline in that there are few reports of color enhancement but rather considerable involvement with intellectualization. The overall content of the intoxication is closely related to the "aleph" effects which are characterteristic of the several 4-thioalkyl-2,5-dimethoxyphenylisopropylamine compounds listed in table II.

There are several appealing explanations for the unexpectedly high potency of these three compounds. The most direct explanation is the one suggested above, that the presence of two methoxy groups adjacent to the 4-position (the 3,4,5-orientation) forces the group in that position completely out of the plane of the aromatic ring. With the 4-methoxy group as found in mescaline, there is only a minor protuberance from the plane of the ring and there is a relatively low order of potency. However, with the ethoxy and the propoxy groups the alkyl "tail" of the alkoxy group is conspicuously inserted into the surrounding area. This steric necessity may inordinately affect both the pharmacokinetics and the pharmacodynamics of the molecule. The aliphatic nature of this "tail" could modify the local lipophilicity of the molecule, which will influence its bioavailability. These effects might be related to partition properties, as mentioned earlier. There could also be a major dissymetry introduced into the molecule by this out-of-plane forcing which would potentially change the closeness of fit of the drug at some receptor site. Further, the change of the orbital hybridization of the heteroatom at the 4-position, especially in the case of the 4-methylthio example, could allow a change of availability of the molecule to metabolism and thus to eventual distribution in the body. An immediate challenge to these possibilities may be found in the 4-thioethyl compound, which has not yet been evaluated pharmacologically. If the enhancement seen from the methoxy to the higher alkoxy (6-fold increase over mescaline) and the enhancement seen from the metnoxy to the methylthio (12-fold increase over mescaline) may be expected to be general in applicability,

the ethylthio compound may be expected to be an exceptionally potent psychotomimetic.

Yet another group at the 4-position deserves more attention than it has received. The 4-alkyl analog of mescaline, 4-methyl-3,5-dimethoxyphenethylamine, has been prepared and studied in cats (Benington, Morin, and Clark 1960). Although the geometric change induced by the replacement of the methoxy group with the methyl group is minor, there was nonetheless a dramatic change in animal response observed. A rage reaction was elicited, a property not observed with mescaline itself. With longer-chain homologs, the "tail" of the molecule would again be forced out of the plane of the aromatic ring, but there would be less metabolic susceptability.

Studies of alkyl and thioalkyl analogs of mescaline are currently underway.

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# Defining the Histamine $H_2$-Receptor in Brain: The Interaction With LSD

Jack Peter Green, Harel Weinstein, and Saul Maayani

Numerous studies have shown that LSD (D-lysergic acid diethylamide) affects serotonergic, dopaminergic, adrenergic, and tryptaminergic systems in the central nervous system (see Mandell and Geyer, 1976; Martin and Sloan 1977; Freedman and Halaris 1978). To this list is now added a histaminergic system. For LSD is a competitive antagonist of the histamine $H_2$-receptor in brain (Green et al. 1977).

In describing this action of LSD, we giva an account of the established criteria that we followed to define the receptor with which LSD reacts. This definition fostered an analysis of the relationship between the molecular structure of known $H_2$-antagonists and LSD. This results in the correct prediction of a new $H_2$-antagonist.

The action of LSD on a histamine receptor in brain may be relevant to the neural effects of LSD. For histamine may be a transmitter in brain. Evidence for this function of histamine remains circumstantial but perhaps no more so than that offered for most other biogenic amines that are classified as putative transmitters. In fact, as recently reviewed (Green, Johnson and Weinstein 1978), studies of mammalian brain show that histamine meets most criteria for having transmitter function. It has non-uniform distribution with highest concentration in the hypothalamus. Subcellular fractions of brain containing nerve-endings are rich in histamine. Brain contains the specific enzyme that decarboxylates histidine to form histamine as well as the specific enzyme that metabolizes histamine. After destroying fibers in the median forebrain bundle or the afferent fibers to the hippocampus the activity of the specific histidine decarboxylase falls in brain regions distal to the lesion. Histamine is released from brain slices by potassium ions in a process that is dependent on ionic calcium. Histamine turns over rapidly: unlike other aromatic biogenic amines, it is not taken up by presynaptic terminals but is instead metabolized to 3-methylhistamine which is then oxidatively deaminated by monoamine oxidase B. Neurons respond to histamine, e.g., it decreases the firing rate of cerebral cortical and brainstem neurons and increases the firing rate of hypothalamic neurons. Especially provocative is the observation that electrical stimulation of

afferent fibers to the cerebral cortex or hippocampus reduces their firing rate, and part of this effect of electrical stimulation can be blocked by histamine $H_2$-antagonists. As described below, histamine increases adenylate cyclase activity in brain, and this effect is blocked by specific antagonists.

Evidence that LSD and D-2-bromo-LSD block the histamine $H_2$-receptor is presented here, and the implications are discussed.

**THE CLASSIFICATION OF A RECEPTOR IN A TISSUE**

Studies aimed at revealing the mechanisms of action of a drug by observing effects in intact animals are especially difficult when applied to the brain because of the awesomely complex multineuronal interactions. For example, treating rats with either LSD or mescaline decreased the firing rate of the raphe neurons but only LSD did this when applied directly to the neurons (Haigler and Aghajanian 1973), mescaline affecting the firing rate by acting indirectly (Gallagher and Aghajanian 1976). The receptors that a drug acts upon can be revealed less ambiguously by studies on isolated preparations in vitro. Towards this end, two systems have been widely used in recent years for studies of receptors in brain and other tissues. One is the study of high affinity binding of ligands to membranes. The other is the measurement of adenylate or guanylate cyclase activity which can reflect interactions of ligands with the receptor if the receptor is coupled to the enzyme. Direct actions of drugs on the cyclase can be ruled out. High affinity binding requires a ligand of high specific activity and of high affinity for the receptor. Both these criteria mere met with tritlated pyrilamine, an $H_1$-antagonist, which was used to label the Hi-receptor in guinea pig (Hill, Young, and Marrian 1977). The low affinity of the known $H_2$-antagonists for the $H_2$-receptor requires a very highly labeled ligand to study the $H_2$-receptor, and this is not available. Adenylate cyclase activity was therefore used.

Whatever system is used, it is imperative that, whenever possible, the receptor regulating a physiological or biochemical function be defined, and that the kinetics of the interaction with the agonist and antagonist be described. Agonists with high selectivity for a specific receptor, e.g., isoproterenol for the ß-adrenergic receptor or dimaprit for the histamine $H_2$-receptor, are useful but uncommon tools. The receptor may be defined by measuring the relative potencies of a series of agonists. For example, histamine stimulates rat gastric acid secretion, relaxes the rat uterus, and increases guinea pig atria1 rate. The agonist activities relative to histamine of a series of agonists on these effects were indistinguishable, which suggested that the receptors were the same or at least very similar (Ash and Schild 1966; Black et al. 1972): they were classified as histamine $H_2$-receptors. Earlier, the relative potencies of agonists in contracting the guinea pig ileum and the rat stomach were in close agreement, implying that the receptors were very similar or the same: they were classified as histamine $H_1$-receptors. Such classifications demand that the slope of the dose-response curves for each agonist be the same; that all agonists

produce the same level of maximum response, differing only in affinity; and that the time of onset and offset of each agonist be similar. These criteria were met in a study on the relative potencies of a series of tryptamines in contracting the isolated stomach strip of the rat, an effect blocked by LSD and its 2-bromo analogue; the relative potencies paralleled their potencies in blocking LSD binding to brain membranes (Green et al. 1978).

While agonists have proved extremely useful in helping to classify receptors, they may yield discordant results. For example, the potency of dimaprit, an $H_2$-agonist, varies as much as fiftyfold relative to histamine on different $H_2$-receptors (Durant, Ganellin, and Parson 1975; Parsons et al. 1977). The use of competitive antagouists has provided less ambiguity in the classification of receptors. The effect of a substance directly on the tissue respouse, e.g., a direct action on muscle contraction is not annulled by a competitive antagonist, while the effect mediated by activation of the receptor is. Furthermore, the displacement of the dose-response curves by the antagonist is not dependent on the affinity of the agonist. Parallel displacement of the log dose-response curve by the antagonist suggests that the agonist and antagonist are acting on the same receptor. From the $ED_{50}$ values in the presence and absence of antagonist, one can estimate the apparent dissociation constant, $K_B$, by estimating the concentration of antagonist required to give a dose ratio of two, i.e., the concentration of antagonist that requires doubling the concentration of agonist to obtain the same effect as obtained in the absence of antagonist. A more rigorous way to classify a receptor is to measure the displacement of the dose-response curves of an agonist by different concentrations of the antagonists. Antagonism is expressed by the dose-ratios (DR) of agonist needed to produce equal responses, e.g., the $ED_{50}$, in the presence and absence of different concentrations of antagonists (B). Simple competitive antagonism results in a straight line of 1 when log (DR-1) is plotted against log (B); the intercept with the abscissa is -log $K_B$, which is referred to as $pA_2$ (Schild 1947; Arunlakshana and Schild 1959). Yet more reassuring is to estimate the $pA_2$ value of an antagonist with different agonists. The $H_1$-receptor (Ash and Schild 1966) and the $H_2$-receptor (Black et al. 1972) were defined in this way. Agonists with very different relative potencies yielded indistinguishable $pA_2$ values. The use of more than one agonist - and of more than one antagonist as well- can help to avoid false inferences due to adventitious effects, e.g., effects on release, uptake, metabolizing enzymes - any of which can vitiate the estimate of $pA_2$.

A judicious and mindful use of these methods, this "taxonomy of receptors" (Black 1976), has led to order and understanding and fecundity. It yields new drugs such as the ß-adrenergic blockers and, more recently, cimetidine, the histamine $H_2$-antagonist which is proving so effective in the treatmeut of duodenal ulcer. This and other $H_2$-antagonists were then used as tools to reveal the $H_2$-receptor and its associated functions in many tissues (Chand and Eyre 1975) and the action of known drugs on this receptor - one consequence of which was the discovery of new $H_2$-antagonists as described below. Knowing that a receptor for a mediator is

***Table 1.*** *Apparent dissociation constants ($K_B$) & $pA_2$ values of histamine $H_2$-antagonists on adenylate cyclase in the guinea pig hippocampus homogenates compared with $pA_2$ values on pharmacological preparations*

| Antagonists | Adenylate cyclase activity: Hippocampus (guinea pig) $K_B$ | Adenylate cyclase activity: Hippocampus (guinea pig) $pA_2$ | $H_2$-receptor in pharmacological preparations* $pA_2$ |
|---|---|---|---|
| Imidazolylpropyl-methylthiourea | $4.68 \times 10^{-4}$ | 3.33 | 3.50 |
| $N^{\alpha}$-guanylhistamine | $7.94 \times 10^{-5}$ | 4.14 | 3.90 |
| Imidazolylpropyl-guanidine | $3.16 \times 10^{-6}$ | 5.50 | 4.65 |
| Thiaburimamide | $2.39 \times 10^{-6}$ | 5.62 | 5.50 |
| Metiamide | $8.71 \times 10^{-7}$ | 6.06 | 6.03 |
| Cimetidine | $6.03 \times 10^{-7}$ | 6.22 | 6.10 |

*Guinea pig atrium and rat uterus (Ganellin 1978)

homogeneous in different tissues provides prediction of side-effects and contraindications, e.g., that ß-adrenergic blockers would be contraindicated in patients with asthma. Showing that the receptor for a mediator in different tissues is homogeneous provides convenience: the search for antagonists of histamine at the $H_2$-receptor was facilitated after it was established that the receptor associated with gastric secretion was indistinguishable from that associated with guinea pig atrial rate, for effects on the latter are more accurately and rapidly measured. Of more pervasive importance, the demonstration of a homogeneity of a receptor in different tissues offers the opportunity to study the receptor in a more tractable milieu. This is salient to the subject of this discussion: the histamine $H_2$-receptor linked to adenylate cyclase in brain is not distinguishable from that in the guinea pig heart, in the rat uterus or in the parietal cells of the stomach. The cyclase system and the peripheral systems can now be studied as a target of psychotropic drugs. And the relationship between chemical structure and biological activity becomes amenable to study on a well defined system.

Although these procedures, which rest on receptor theory, have been used for over fifty years, there appears to be a surprising innocence of them. For example, it has been repeatedly asserted that the histamine stimulated adenylate cyclase in brain slices is linked to the $H_1$-receptor. The evidence offered to support this is that the effect of histamine on cyclase is blocked by $H_1$-antagonists. In all these studies, the $H_1$-antagonists were used at concentrations of $10^{-6}$ M to $10^{-4}$M. The $pA_2$ values for the commonly used Al-antagonists, pyrilamine (also called mepyramine) and tripellenamine, are 9.4 and 8.5 respectively or, in $K_B$ values, $4 \times 10^{-10}$ M and $3.2 \times 10^{-9}$ M. Testing an antagonist at a concentration greater than 100 times the $K_B$ is likely to block more than the specific receptor. Non-specificity of many antagonists at high doses is well known; the $H_1$-antagonists in suitable concentrations are anticholinergic (see Nauta and Rekker 1978; van den Brink and Lien 1978). More to the point, at concentrations of $10^{-6}$ M and greater, $H_1$-antagonists block the $H_2$-receptor

*Table 2. Effect of histamine on adenylate cyclase activity in fresh membranes from different regions of the guinea pig brain**

| Brain Area | Basal | Histamine Concentration, M | | | |
|---|---|---|---|---|---|
| | | $10^{-6}$ | $10^{-5}$ | $10^{-4}$ | $10^{-3}$ |
| | | Per cent increase in activity (±s.e.) | | | |
| Cortex | 48.3±0.9 | 8.5±1.0† | 1.1±0.4§ | 106.2±1.4§ | 117.6±2.4§ |
| Hippocampus | 73.8±0.6 | 5.7±0.85† | 29.1±0.76¶ | 86.7±1.1§ | 103.9±0.3§ |
| Thalamus | 18.3±0.52 | 7.2±1.7† | 32.8±3.7¶ | 49.4±1.1¶ | 46.6±2.1¶ |
| Striatum | 89.4±1.04 | 3.6±1.0† | 10.5±0.8¶ | 20.7±5.7‖ | 28.0±7.4‖ |
| Hypothalamus | 42.9±0.15 | 3.9±0.3¶ | 9.6±1.4¶ | 15.3±2.0¶ | 15.0±0.5¶ |
| Central Gray | 56.8±0.7 | 2.2±0.7† | 4.9±1.8† | 10.0±1.8‖ | 8.4±1.6‖ |

*This represents one series of experiments on membranes. Each region was studied at least twice with similar results.
†pmoles of c-AMP formed/min/mg protein (±s.e.)
‡not significantly different from basal activity
§significantly different ($p<0.001$) from basal activity
¶significantly different ($p<0.005$) from basal activity
‖significantly different ($p<0.025$) from basal activity

linked to adenylate cyclase in homogenates of both brain and heart ventricle of the guinea pig (see below).

The $H_2$-antagonists have lower affinity for their receptor than have the Hl-antagonists for the $H_1$-receptor. Cimetidine has a $pA_2$ value of 6.10 ($K_B$ = 8 x $10^{-5}$ M). Clearly, a higher concentration of $H_2$-antagonist, e.g., cimetidine, is needed to block the $H_2$-receptor, than the concentration of $H_1$-antagonist needed to block the $H_1$-receptor. Yet in some work purporting to define a receptor, equal concentrations of each type of antagonist is used, commonly $10^{-6}$ M, which is near the $K_B$ of the $H_2$-antagonist but a thousand times the $K_B$ of the $H_1$-antagonist. Not surprisingly, at these concentrations the $H_2$-antagonist has no or slight effect while the $H_1$-antagonist blocks histamine, prompting the (false) inference that the activity is linked to the $H_1$-receptor. Confusion can also result from the use of only one concentration of agonist, e.g., histamine. As the antagonism is competitive and surmountable by increasing doses of agonist, one dose of agonist is unrevealing, for if the concentration is too high, it will surmount the effect of the antagonist, as shown by a glance at the dose-response curves below.

**THE HISTAMINE RECEPTOR IN BRAIN**

Parallel displacement of the dose-response curves to histamine by metiamide, an $H_2$-antagonist, indicated that histamine-stimulated adenylate cyclase in guinea pig brain homogenates is linked to an $H_2$-receptor. The $pA_2$ of metiamide on this system was the same as that on known $H_2$-systems. The relative potencies of two other agonists, 4-methylhistamine and 2-methylhistamiae, were also consonant with stimulation of the $H_2$-receptor (Hegstrand, Kanof, and Greengard 1976). This conclusion was supported by observations on the same preparation with additional agonists and six $H_2$-antagonists. The $pA_2$ values of the six antagonists (Green et al. 1977) were very similar to those on the $H_2$-receptors in pharmacological

preparations (see Ganellin 1978) and on the $H_2$-receptor linked to adenylate cyclaae in guinea pig cardiac ventricle (C.L. Johnson, unpublished work). Table 1 shows the results on the guinea pig hippocampus. Simple competitive antagonism was further shown by a Schild plot with cimetidine as antagonist which resulted in a straight line of slope 1. When dimaprit was used as agonist instead of histamine, the points fell on the same line (Green et al. 1977). Dimaprit is virtually an exclusive $H_2$-agonist, having less than 0.0001 per cent of the activity of histamine on $H_1$-receptors (Parsons et al. 1977). Homogenates of the cerebral cortex and hippocampus were not distinguishable in their response to agonists and antagonists. It is thus clear that histamine stimulated adenylate cyclase in homogenates of guinea pig hippocampus and cortex is linked to an $H_2$-receptor. (No extrapolations can be made to brain slices.)

Table 2 shows that the histamine stimulated adenylate cyclase activity is more sensitive in homogenates of the cerebral cortex and hippocampus than in other parts of the brain. Neither of these regions is especially rich in histamine. This distribution confirms previous findings (Hegstrand, Kanof, and Greengard 1976), except that we showed activity in the hypothalamus and central gray. In the cortex of guinea pigs, activity was highest in subcellular fractions containing nerve endings (Kanof, Hegstrand, and Greengard 1977). The activity was present in homogenates of the rat hippocampus (Green et al. 1977) and the rat cortex (Hegstrand, Kanof, and Greengard 1976), but the corresponding portions of guinea pig brain were more sensitive.

It may be worth noting that adenylate cyclase in the hippocampus homogenates is more sensitive to histamine than to norepinephrine and dopamine. At a concentration of $10^{-4}$ M, the respective percentage increases were 107.5, 37.0, and 24.7. Cimetidine, $10^{-4}$ M, reduced histamine activation by 65.5 per cent without reducing activation by the catecholamines (Green et al. 1977).

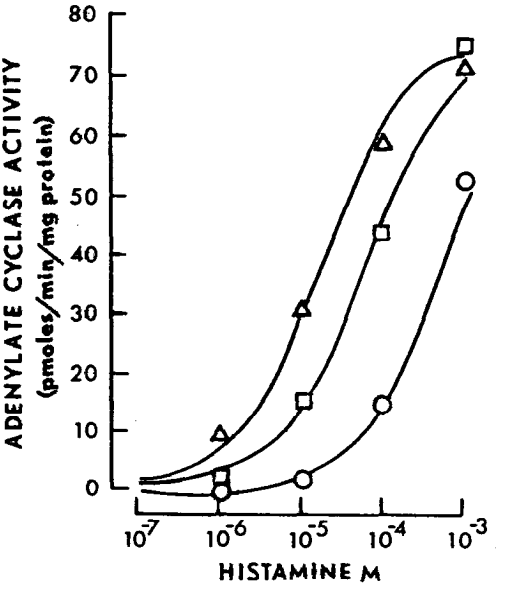
The $H_1$-antagonists also antagonized histamine stimulated adenylate cyclase, as shown for cyproheptadine (Fig. 1). But to do so required concentrations of pyrilamine and tripellennamine about a thousand times those required to block the $H_1$-receptor. For cyproheptadine, the corresponding ratio was ten times (Table 3). These differences in $pA_2$ values show that the $H_1$-antagonists were not blocking the $H_1$-receptor linked to cyclase. Further, the $H_1$-antagonists blocked stimulation of the cyclase by dimaprit which is selective for the $H_2$-receptor and is virtually devoid of activity on the $H_1$-receptor. Hence, the $H_1$-antagonists at high concentration block the $H_2$-receptor.

Cyproheptadine has greater affinity for the $H_2$-receptor (KB = $3.7 \times 10^{-8}$ M) than any substance yet described, including the $H_2$-antagonists that have been used clinically, metiamide and cimetidine.

**CYPROHEPTADINE AS ANTAGONIST OF MANY RECEPTORS**

Although often called a "serotonin antagonist", cyproheptadine has no special penchant for serotonin receptors. Table 4 shows the $K_B$

***Fig. 1.*** *Adenylate cyclase activity of the guinea pig hippocampus in response to varying concentrations of histamine in the absence* (Δ) *and presence of* $10^{-7}$ *M* (•) *or* $10^{-6}$ *M* (o) *cyproheptadine. Each point represents the increase in adenylate cyclase activity above basal level (no histamine) and is the mean of triplicate determinations on a single enzyme preparation.*




(and $pA_2$) values of cyproheptadine on different receptors, listed in order of affinity. It has highest affinity for the $H_1$- and $H_2$-receptors and least for the serotonin receptor. The values for the serotonin and LSD receptors were estimated from published $IC_{50}$ values as noted in the table. Cyproheptadine also antagonizes acetylcholine (Rocha e Silva and Leme 1972) and epinephrine (Stone et al. 1961).

**COMPETITIVE BLOCKADE OF THE $H_2$-RECEPTOR BY LSD AND D-2-BROMOLYSERGIC ACID DIETHYLAMIDE (BrLSD)**

Because cyproheptadine has high affinity for the $H_2$-receptor and it blocks 5-HT, it seemed appropriate to test LSD which also has affinity for 5-HT receptors. LSD alone did not increase adenylate cyclase activity in homogenates of the guinea pig hippocampus and cortex [as it does in rat striatum (Von Hungen, Roberts, and Hill 1975)]. But it blocked histamine or dimaprit stimulated cyclase in a competitive number as shown by a Schild plot. The $pA_2$ value of LSD on hippocampal homogenates was 5.95, on cortical homogenates 6.07, not significantly different. This value is very nearly that of cimetidine, 6.22, and metiamide, 6.06, the two potent and selective $H_2$-antagonists. Inactive in this system were L-LSD,

***Table 3.*** *The $pA_2$ values* of histamine *$H_1$ antagonists on the histamine $H_2$ receptor linked to adenylate cyclase in guinea pig hippocampus homogenates compared with the $pA_2$ values on the $H_1$ receptor in guinea pig ileum*

| Antagonist | $pA_2$ on $H_2$ linked adenylate cyclase activity: hippocampus, guinea pig | $pA_2$ on $H_1$ receptor: ileum, guinea pig |
|---|---|---|
| Pyrilamine | 5.18 | 9.4* |
| Tripellenamine | 5.4 | 8.5† |
| Cyproheptadine | 7.43 | 8.3‡ |

*Arunlakshana and Schild 1959
†Marshall 1955
‡Rocha e Silva and Garcia Leme 1972

psilocin and mescaline, all at $10^{-4}$ M. But Br-LSD was a competitive antagonist, as shown by the Schild plot. Its $pA_2$ value was 7.16, thus showing an affinity about tenfold that of either LSD, cimetidine, or metiamide.

**PREDICTION OF A NEW $H_2$-ANTAGONIST**

There is no ostensible similarity in chemical structure between LSD (or BrLSD) and the selective $H_2$-antagonists when they are drawn in the conventional way (Fig. 2). But one of the likely conformers of metiamide and cimetidine has a topological congruency with LSD, as shown for cimetidine in Fig. 3. The molecules neatly superimpose, the oxygen of the amide group of LSD being congruent with the nitrogen of the cyano group of cimetidine or the sulfur atom of the throurea group in metiamide. After making this observation, we wrote:

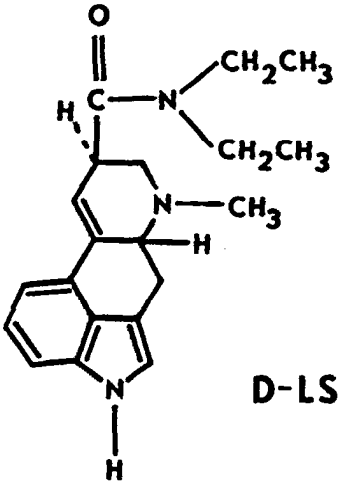
> The residue in D-LSD that is sterically congruent with metiamide is hexanoic acid-4-aza-4-methyl-6-(3-pyrrolyl)-N,N-diethylamide. This and the indolyl and imidazol analogs and their derivatives may have $H_2$-antagonists activity (Green et al. 1977).

We tested hexanoic acid-4-aza-4,5-dimethyl-6-(3-indolyl)-N,N-diethylamide (SK&F 10856), obtained from Dr. Carl Kaiser. The structure is shown (Fig. 4). A Schild plot proved it to be a competitive antagonist of histamine and dimaprit at the $H_2$-receptor linked to adenylate cyclase, with $pA_2 = 6.10$ ($K_B = 7.9 \times 10^{-7}$ M), not different from that of LSD. We plan to test the pyrrolyl and imidazoyl analogs.

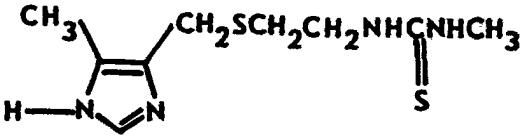
**THE $H_2$-RECEPTOR AND THE PHARMACOLOGY OF LSD AND BrLSD**

Both LSD and BrLSD are competitive antagonists of histamine at the $H_2$-receptor linked to adenylate cyclaee in guinea pig hippocampus and cortex. The $pA_2$ value of D-LSD was 6.0, very nearly that of cimetidine, 6.22, and of metiamide, 6.06, the two potent $H_2$-antagonists. The L-isomer, which has no measureable central effects, did

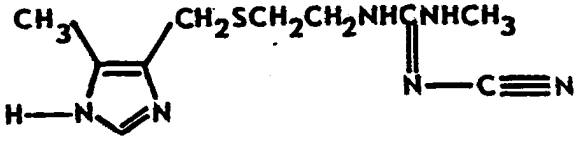
***Fig. 2.*** *The structural formulas of LSD, metiamide, and cimetidine*




D-LSD




METIAMIDE




CIMETIDINE

***Fig. 3.*** *Comparison ofthe molecular structures of LSD and cimetidine*

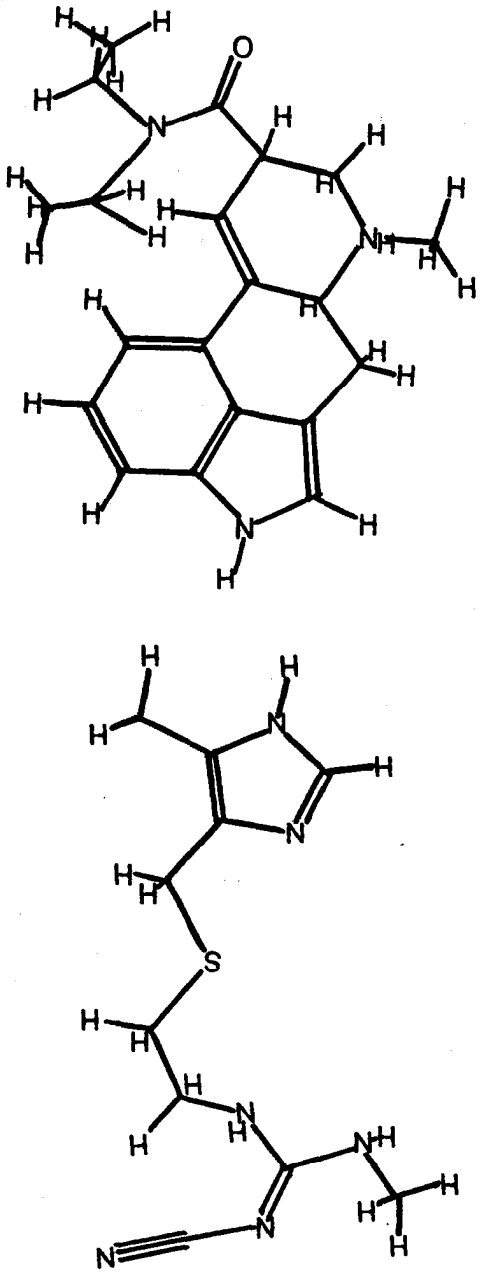


***Table 4.*** *Estimated $K_B$ and $pA_2$ values of cyproheptadine for different receptors*

| Receptor | $K_B$ (M) | $pA_2$ |
|---|---|---|
| Histamine $H_1$* | $5 \times 10^{-9}$ | 8.3 |
| Histamine $H_2$† | $3.7 \times 10^{-8}$ | 7.4 |
| LSD ‡ | $5.0 \times 10^{-8}$ | 7.3 |
| Bradykinin* | $6.3 \times 10^{-8}$ | 7.2 |
| Haloperidol§ | $6.5 \times 10^{-8}$ | 7.2 |
| Angiotensin* | $2.5 \times 10^{-7}$ | 6.6 |
| Dopamine§ | $1.0 \times 10^{-6}$ | 6.0 |
| Serotonin‡ | $1.6 \times 10^{-6}$ | 5.8 |

*Rocha e Silva and Leme 1972
†Green et al. 1977
‡Estimated from IC50 values of high affinity binding by rat cortex, from Bennett and Snyder 1976; according to: $K_B = IC_{50}/1 + [A]/K_A$ where [A] is the agonist concentration and $K_A$ is the dissociation conetant of the agonist
§Creese, Burt and Snyder 1976

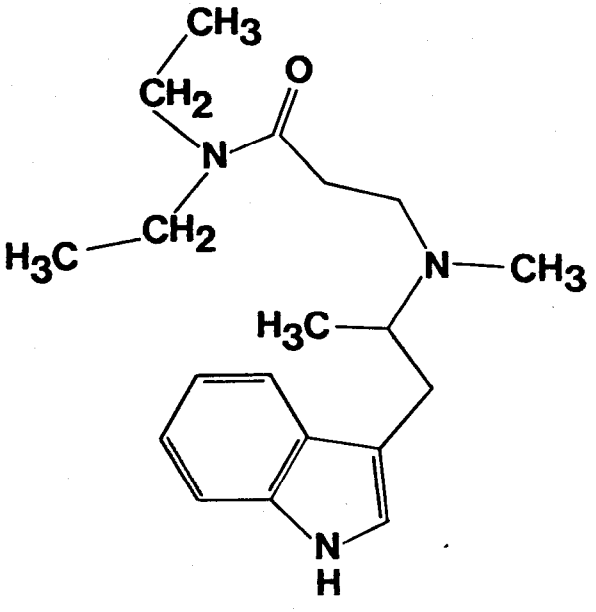
not antagonize the histamine-activated adenylate cyclase. BrLSD ($pA_2$=7.2) was about ten times more potent than D-LSD (or cimetidine) in inhibiting histamine-stimulated adenylate cyclase activity (Green et al. 1977).

The affinity of LSD for the $H_2$-receptor linked to adenylate cyclase cannot be directly compared with its affinities for other receptors linked to adenylate cyclase in brain because the $pA_2$ values are not available. From Table 5 it would appear that LSD and BrLSD may be more effective in blocking histamine stimulated cyclase than in blocking norepinephrine or dopamine stimulated cyclase.

However, the $pA_2$ values (Table 6) show that LSD has a greater affinity for the dopamine receptor then for the $H_2$-receptor. LSD has an even greater affinity for the serotonin receptor. BrLSD has the came $pA_2$ values for the serotonin, dopamine and $H_2$-receptors. And it has a greater affinity than LSD for both the haloperidol binding sites and for the $H_2$-receptor. Other work has shown that LSD and BrLSD have the same affinity for LSD binding sites in cortex (Bennett and Snyder, 1976).

Although adenylate cyclase measurements suggest that BrLSD has a greater affinity for the dopamine receptor (Table 5), high affinity binding studies of dopamine show that BrLSD has slightly less affinity than LSD for the dopamine binding sites (Creese, Burt, and Snyder, 1976; Table 6). This paradox suggests the presence of dopamine receptors that are not linked to cyclase, a receptor that has a greater affinity for LSD than for BrLSD. LSD, is contrast with BrLSD, is an agonist as well as an antagonist at striatal receptors linked to cyclase (Von Hungen, Roberts, and Hill 1975; Bockaert et al. 1976). Perhaps BrLSD has leas affinity for another (presynaptic?) dopamine receptor. BrLSD has about five times more affinity for haloperidol binding sites than has LSD (Creese, Burt, and Snyder 1976). As haloperidol binds to the postsynaptic dopaminergic receptors, it was inferred that BrLSD is a better dopamine

***Fig. 4.*** *The structure of hexanoic acid-4-aza-4,5-dimethyl-6-(3-indolyl-N,N-diethylamide. It is shown in a conformation congruent with LSD and cimetidine in fig. 3.*




**SK&F 10856**

antagonist than is LSD. Supporting this inference are experiments in rat striatum showing that BrLSD is more potent than LSD in increasing the lwels of DOPA (Persson 1977a) and of a dopamine metabolite, 3,4-dihydroxyphenylacetic acid (Persson 1977b). All these biochemical studies are consistent with observations on unit cell activity of the subatantia nigra, where LSD was more effective than BrLSD in decreasing the firing rate of dopamine-containing neurons (Trulson, Stark, and Jacobs 1977; Christoph, Kuhn, and Jacobs, 1977). Perhaps correlating with this difference in affinity for postsynaptic dopamine receptors is that head twitches induced in mice by LSD (Corne and Pickering 1967) and LSD stimulated striatal cyclase (Von Hungen, Roberts, and Hill 1975) were blocked by BrLSD.

Agonist activity of LSD is also greater than that of BrLSD on the flexor reflex of the dog. LSD, like tryptamine, enhanced the flexor reflex (Martin and Sloan 1974), but a dose of BrLSD 100

***Table 5.*** *Comparative effects of LSD, L-LSD, and BrLSD in blocking the adenylate cyclase activity in broken cell preparations, stimulated by histamine, norepinephrine and dopamine, all at $10^{-4}$ M. Values are percentage inhibition.*

| Drug | Histamine* | Norepinephrine† | Dopamine |
|---|---|---|---|
| LSD, $10^{-4}$ M | - | 75 | 81† |
| LSD, $10^{-5}$ M | 56 | - | - |
| L-LSD, $10^{-4}$ M | 0 | - | 0‡ |
| BrLSD, $10^{-4}$ M | - | 90 | 98† |
| BrLSD, $10^{-5}$ M | 97 | - | - |

*Guinea pig cortex and hippocampus (Green et al. 1977)
†Rat cortex (Von Hungen, Roberts, and Hill 1975)
‡Rat striatum (Von Hungen, Roberts, and Hill 1975)

times higher did not (W.R. Martin, personal communication).

The differences between the $pA_2$ values for LSD and BrLSD on the serotonin receptors could also reflect a difference between affinities for presynaptic (agonist) and postsynaptic (antagonist) serotonin sites. LSD has greater affinity for the serotonin receptor than does BrLSD. LSD, like serotonin, completely inhibits the firing of neurons in the midbrain raphe at low doses (10 µg/kg) whereas BrLSD only partially inhibits these neurons even at a dose 200 times greater (Aghajanian, Foote, and Sheard 1970). Microiontophoretically applied BrLSD also failed to totally inhibit the firing (Aghajanian 1976). Both BrLSD and LSD inhibited postsynaptic sites on the ventral lateral geniculate and amygdala (Aghajanian 1976). Some of this work recalls studies of peripheral systems. LSD and BrLSD are about equally potent antagonists at the postsynaptic serotonin receptors in the rat uterus (Cerletti and Doepfner 1958) and stomach (Vane 1959). On one peripheral tissue, the effects of LSD and BrLSD are very neatly defined. LSD is a serotonin agonist in human and sheep umbilical vasculature, and BrLSD is an antagonist of both LSD and serotonin (Dyer and Gant 1973).

It thus seems clear from studies on neural tissue using biochemical and eleetrophysiological techniques that LSD and BrLSD have affinities for the same receptors and that LSD has greater activity at (and perhaps a greater affinity for) functionally "presynaptic" sites, and BrLSD has greater affinity for some "postsynaptic" sites.

The sites that LSD and BrLSD share are very likely to be implicated in the hallucinogenic action of LSD. For BrLSD blocks the hallucinogenic effect of LSD in man (Ginzel and Mayer-Gross 1956; Bertino, Klee, and Weintraub 1959) at a dosage interval before cross-tolerance is seen (Isbell, Miner, and Logan 1959). The effect of a dose of LSD of 1 µg/kg was blocked by a dose of BrLSD of 32 to 64 µg/kg (Bertino, Klee, and Weintraub 1959).

Perhaps of greater interest is that BrLSD causes behavioral changes in laboratory animals and in man. Larger doses of BrLSD than LSD are needed to show changes in animal behavioral paradigms but they occur (e.g., Uyeno 1968; Uyeno and Mitoma 1969). In man there is one report of hallucinations occurring after ingestion of 0.5 mg of

***Table 6.*** *$pA_2$ values of LSD and BrLSD for different receptors*

| Receptor | LSD | BrLSD |
|---|---|---|
| Serotonin* | 8.3 | 7.2 |
| Haloperidol† | 7.7 | 8.4 |
| Dopamine† | 7.5 | 7.2 |
| Histamine-$H_2$‡ | 6.0 | 7.2 |

*Estimated from $IC_{50}$ value of high affinity binding by rat cortex, from Bennett and Snyder 1976; according to: $K_B = IC_{50}/1 + [A]/K_A$ where [A] is the agonist concentration and $K_A$ is the dissociation constant of the agonist
†Creese, Burt, and Snyder 1977
‡Green et al. 1977

BrLSD (Richards et al. 1958). Others have observed severe psychic effects without hallucinations. Bertino, Klee. and Weintraub (1959) wrote:

> Six of the 10 subjects receiving 128 or 256 µg./Kg. exhibited mental effects. These consisted of a "drunk feeling," "things seem bright," "devil-may-care attitude," "a feeling of tiredness, euphoria, anxiety, and impaired concentration."
>
> Psychophysiological effects of 2-brom-LSD were reported by subjects at dosages as low as 32 µg./Kg. and increased in severity with increasing dosage. The psychic effects, though milder, were qualitatively the same as those with lysergic acid diethylamide (LSD-25).

Schneckloth et al. (1957) wrote:

> Thus, when constant intravenous infusions of bromo-LSD were given to 2 normal subjects (cases 5 and 6) both experienced psychic changes, which became more severe as the infusion continued and persisted for 3 to 4 hours after the infusion was stopped. No hallucinations were noted but there were feelings initially of drowsiness, depression, anxiety and apprehension, followed by feelings of irritation, restlessness, and tenseness, and later, intensely disagreeable sensations of unreality and depersonalization, inexplicable feelings of strangeness and mild confusion.

What contribution blockade of the histamine $H_2$-receptor makes to the behavioral effects of BrLSD and LSD is not known. The availability of an $H_2$-antagonist that penetrates the brain could lead to understanding. Cimetidine, an $H_2$-receptor antagonist, which is now being used in humans, does not normally enter brain (Ganellin 1978). It has, however, produced rare and idiosyncratic responses that may be revealing of central function. There are reports of mental confusion after cimetidine - patients described as agitated, delirious, disoriented - all rapidly reversed when the drug was withdrawn (Delaney and Ravey 1977; Robinson and Mulligan 1977; Menzies-Gow 1977; Nelson 1977; McMillen et al. 1978; Quap 1978). Reports of

fever after cimetidine (Ramboer 1978; McLoughlin, Callender, and Love 1978) also recall that LSD raises temperature in humans (Gorodetzky and Isbell 1964; Klock, Boerner, and Becker 1975) and rabbit (Carino and Horita 1977); in some animals, $H_2$-receptor stimulation causes hypothermia (Cox and Lomax 1977). Further attesting to central effects is that increased plasma prolactin levels have been observed especially when the drug is given by intravenous injection (Della Pave et al. 1977; Carlson and Ippoliti 1977; Burland et al. 1978; Daubresse, Meunier and Ligny 1978); animal studies have suggested that the histamine $H_2$-receptor mediates events that inhibit prolactin release (Arakelian and Libertun 1977).

The $H_1$-antagonists at suitable concentrations interact with both $H_2$- (Table 3) and cholinergic receptors (e.g., Nauta and Rekker 1978; van den Brink and Lien 1978). It is therefore risky to attribute their behavioral effects to blockade of any one reeeptor. It may be interesting to note, however, that $H_1$-antagonists produce arousal in dogs (Shaw, Gershon, and Bentley 1957; Gershon and Shaw 1958). In man, high dose of the $H_1$-antagonists have produced disorientation, euphoria (Gott 1968), visual hallucinations (Soleymanikashi and Weiss 1970), and behavior characterized as schizophrenic-like (Nigro 1968). Diphenhydramine, an $H_1$-antagonist, on intravenous administration (30 or 45 mg to each male subject) produced disturbance of body image and thought. When administered at the height of the LSD reaction (25 µg of LSD by mouth), diphenhydramine intensified most of the LSD effects - especially hallucinations, depersonalization, and disturbances of body image and thinking - without prolonging the effects (Yamada, Tsunoda, and Takashina 1957).

It is becoming increasingly clear that the complex behavioral and neural effects of LSD or BrLSD cannot be explained on the basis of any single effect on any single neurophysiological system or site. The effect elicited at the lowest dose in the rat is a decreased firing of the raphe nuclei (Aghajanian 1976). This effect cannot explain the behavioral effect of LSD (Freedman and Halaris 1978; Trulson, Ross, and Jacobs 1977; Pieri et al. 1978). In humans, LSD - and, as quoted above, BrLSD - produce an array of neural effects, which range from activation of the galvanic skin response at the lowest dose (Greiner, Burch, and Edelberg, 1958). a sign of neurophysiological arousal; to marked changes in perception, affect, thought, body image; perseveration; heightened awareness; depersonalization; illusions and hallucinations (Freedman, 1968; Mandell and Geyer, 1976). One effect, hallucinations, is complex enough (Editorial, 1977). It is very unlikely that all the effects can be attributed to action at one receptor at one site. How the visual cortex processes a spot of light is still not known, but it is clear that the

> response properties of most neurones depend on subtle interactions within an intricate system of more and less specific excitatory and inhibitory influences, whose cooperative details determine most aspects of neuronal behaviour (Movshon 1978).

This complexity must apply to the action of drugs, which have access to all neurons and all receptors. Almost certainly the behavioral effects produced by any drug, e.g., LSD, BrLSD, are the final consequences of a conflation of events occurring at different neural sites and by different mechanisms. The basis for the neural and behavioral effects will be learnt after the receptor sites at which these drugs act are explicitly and rigorously delineated. Then will follow the concatenation of the neural and behavioral events.

## SUMMARY

Two aspects of the complexities of the mode of action of drugs are described. One is the criteria and pitfalls of defining the interaction with specific receptors. The other is the need to consider each of the pharmacological effects of a drug as a concatenation of receptor events, because it has become clear that each drug may have substantial affinity for many specific receptors.

Illustrating these ideas is a characterization of the histamine receptor linked to adenylate cyclase in brain. The activities of a series of $H_2$-antagonists and $H_2$-agonists were shown to be the same on the histamine receptor linked to adenylate cyclase as on known $H_2$-receptors. The $K_B$ values of antagonists and $ED_{50}$ values of agonists were not distinguishable among these receptors.

Notably, at high concentrations, the $H_1$-antagonists are also competitive antagonists of the $H_2$-receptor.

Cyproheptadine has especially high affinity for the $H_2$-receptor. It is the most potent $H_2$-antagonist yet reported. Other published results are reviewed to show the variety of receptors that cyproheptadine has affinity for. Its affinity for serotonin receptors led us to examine other serotonin antagonists.

On this $H_2$-receptor linked to adenylate cyclase in homogenates of guinea pig hippocampus and cortex, D-LSD and D-2-bromo-LSD (BrLSD) were shown to be competitive antagonists of histamine. L-LSD, mescaline and psilocin were inactive.

Noting congruency in the molecular structure of D-LSD and known $H_2$-antagonists, we predicted a new $H_2$-antagonist. This prediction is shown to be correct: the compound has similar affinity to the $H_2$-receptor as has LSD.

The affinities of D-LSD and BrLSD for the $H_2$-receptor are compared with their affinities for other receptors. The pharmacology of D-LSD and BrLSD is reviewed. Evidence is assembled that BrLSD has considerable central effects.

## ACKNOWLEDGMENTS

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# The Nature of Opioid and LSD Receptors: Structural Activity Relationship Implications

William R. Martin

The structure activity relationships of the drugs under consideration are inexorably entwined with receptors and receptor theory since they have been clearly demonstrated to have agonistic and antagonistic activity. It also appears that their pharmacologic effects are in some way related to neurotransmitters and neuromodulators. The discovery of the endorphins and enkephalins and the similarities of some of their effects to those of the narcotic analgesics has given rise to a most exciting chapter of pharmacology. Over a period of nearly three decades, a pharmacology of the LSD-like hallucinogens has been emerging which suggests that they too may interact with several receptors. In this presentation, I wish to discuss some concepts that are emerging concerning receptor topology and drug selectivity which bear directly on receptor classification and which have relevance to SAR of these two groups of drugs.

**NARCOTIC ANALGESICS**

A number of investigators have studied the SAR of narcotic analgesics. For the most part, the data on which these studies have been based have been generated in several species using different measures of pharmacologic effects such as analgesia. Table 1 presents data on seven critical drugs that have great theoretical significance in discussing their structural activity relationships. Morphine is used as a standard drug against which the others are compared. However, it should be recognized that morphine may have several modes of action and interact with more than one receptor (Martin et al. 1976; Gilbert and Martin 1976).

Normorphine is a weak analgesic in the mouse, is 1/6 to 1/3 as potent as morphine in man and in binding assays, equipotent to morphine on the guinea pig ileum but devoid of morphine like activity in the dog.

Metazocine is an agonist approximately equipotent to morphine in the muse, dog and man. It is clearly a strong agonist in the dog, but has been characterized as an agonist-antagonist by Pert and Snyder (1974) on the basis of data from binding studies having a relatively low NaCl/no NaCl ratio (6).

Phenazocine is some 10 times more potent than morphine on measures

presented in Table 1, yet was only 1/5 as potent as morphine in suppressing abstinence in the monkey.

N-allyl normetazocine (SKF 10047) is virtually devoid of analgesic activity but produces delirium and hallucinations in man and delirium in the dog. These effects can be antagonized by naloxone.

Meperidine is an effective analgesic in man and the mouse; is much less potent relative to morphine on the guinea pig ileum and in binding studies. In man it will partially suppress abstinence in morphine dependent subjects (Himmelsbach 1942). Further, it has a limited ability to produce physical dependence in man (Himmelsbach 1942, 1943). Of particular interest is the observation that nalorphine was nearly ineffective in precipitating abstinence in meperidine dependent patients (Isbell 1955). In the dog, meperidine is devoid of morphine-like activity (Gilbert and Martin 1976) and will not produce physical dependence (Carter and Wikler 1955). Although it is relatively impotent in inhibiting the electrically stimulated guinea pig ileum and in preventing binding of naloxone to rat brain homogenate, it is qualitatively similar to morphine in these preparations.

These data have been analyzed by calculating correlation coefficients between the various measures and this analysis is presented in Table 2. As can be seen, the only significant regression was between the mouse and dog. Many of the correlation coefficients were highly significant which stresses the weakness of this technique for analysis of this type of data.

**LSD-LIKE HALLUCINOGENS**

There are both theoretical and practical problems of defining the group of drugs considered LSD-like hallucinogens. The discussion of these drugs will be restricted largely to data generated in the spinal dog, man and rat. An LSD-like hallucinogen should, of course, have pharmacologic activity like LSD. The effects of LSD can be divided into several categories including: (1) Somatomotor and autonomic; facilitation of the patellar (man) and flexor reflex (spinal dog), evocation of the stepping reflex (spinal dog), tachycardia (dog and man); increased blood pressure (man); mydriasis (man and dog), tachypnea (man and dog) and increased body temperature (man and dog). (2) Subjective and behavioral changes; euphoria, delusion and hallucinations (man); arousal, restlessness, tracking and starting (dog) and the alteration of operant behavior (rat). (3) The development of tolerance and the conferring of cross tolerance. (4) The blocking of the effects with more or less specific antagonists. Thus chlorpromazine and cyproheptadine will antagonize this effect of LSD-like hallucinogens but not phenoxybenzamine.

As can be imagined the permutations of these attributes are large and from a practical point of view resources allow only the study of certain ones. Because of differences in biases of investigators, certain aspects of the action of certain putative LSD-like hallucinogens are focused on. For this reason, very little quantitative comparable data has been generated which allows hard

**TABLE 1**

POTENCY OF SEVERAL OPIOID AGONISTS AND ANTAGONISTS
ON DIFFERENT TEST SYSTEMS

| | MOUSE HOT PLATE[a] | DOG[b,c] MAN[d] | | GUINEA PIG[e] ILEUM | RAT BRAIN[f,g] BINDING SITES NO NaCl | 100 Mo NaCl |
|---|---|---|---|---|---|---|
| MORPHINE | 1 | 1 | 1 | 1 | 1(3) | 1(110) |
| NORMORPHINE | .05 | 0 | .35 | 1 | .2(15) | .16(700) |
| METAZOCINE | .9 | 1.3 | .8 | .4 | .3(10) | 1.8(60) |
| PHENAZOCINE | 13 | 8.3 | 3 | 10 | 5(.6) | 14(8) |
| PENTAZOCINE | 0.1 | .3 | .17 | .45 | .2(15) | 2.2(50) |
| N-ALLYL NOR-METAZOCINE | 0 | 0 | 0 | 1 | 1.5(2) | 37(3) |
| MEPERIDINE | .21 | 0 | .13 | .06 | .001(3000) | .002(50,000) |

POTENCIES ARE EXPRESSED AND THE NUMBER MGS OF MORPHINE EQUIVALENT TO 1 MG OF THE DRUG. THE FIGURES IN PARENTHESIS ARE THE CONCENTRATIONS IN nM THAT PRODUCE 50% INHIBITION OF BINDING. THE VALUES 0 INDICATE THAT THE DRUG HAS NO ACTIVITY ON THE GIVEN MEASURE.

a. Pert et al. 1976; b. Martin et al. 1976; c. Gilbert and Martin 1976;
d. data from the work of Houde; e. Kosterlitz and Waterfield 1975;
f. Pert and Snyder 1973; g. Pert and Snyder 1974.

TABLE 2

PRODUCT MOMENT CORRELATION COFFICIENTS (r), SLOPES (b) WITH STANDARD ERROR OF ESTIMATE ($s_{y,x}$) FOR DATA PRESENTED IN TABLE 1

| | | Mouse Analgesia Hot Plate | Dog Depression of the Flexor Reflex | Man Analgesia | Guinea Pig Ileum | Rat Brain Binding No NaCl |
|---|---|---|---|---|---|---|
| DOG | r | $.99^{.01}$ | | | | |
| | b | $.63^{.05}$ | | | | |
| | $s_{y.x}$ | .27 | | | | |
| MAN | r | $.96^{.01}$ | $.98^{.01}$ | | | |
| | b | .21 | 1.17 | | | |
| | $s_{y.x}$ | .26 | .59 | | | |
| GUINEA PIG | r | $.99^{.01}$ | $.98^{.01}$ | $.94^{.01}$ | | |
| | b | .75 | 1.15 | 3.14 | | |
| | $s_{y.x}$ | .44 | .67 | .73 | | |
| BINDING (RAT) NO NaCl | r | $.95^{.01}$ | $.94^{.01}$ | $.90^{.01}$ | $.97^{.01}$ | |
| | b | .35 | .55 | 1.52 | .48 | |
| | $s_{y.x}$ | .50 | .56 | .73 | .39 | |
| BINDING (RAT) 100 NM NaCl | r | .16 | .13 | .01 | .23 | .42 |
| | b | .45 | .60 | .17 | .90 | 3.28 |
| | $s_{y.x}$ | 12.51 | 12.55 | 12.67 | 12.31 | 11.47 |

SUPERSCRIPT INDICATES LEVEL OF SIGNIFICANCE

comparisons which are useful for SAR considerations. At the present time, it seems that LSD-like hallucinogens may act through at least two mechanisms of action; a tryptaminergic and serotonergic mechanism (Martin and Sloan 1977). The relative importance of these two mechanisms in mediating different signs and systems has not been ascertained at this time.

Table 3 and table 4 present comparisons of the effects of several important drugs that are related in one way or another to LSD. LSD is the prototypic drug against which the others are compared.

Psilocin and its phosphorylated congener, psilcybin, appear to be very similar to LSD in their pharmacology. However, there is a marked difference between the potency of psilocin in man and its ability to prevent the saturable binding of 5HT and LSD in rat brain homogenate on the one hand and in the dog on the other. Further, LSD tolerant rats were not cross tolerant to psilocin. The time course of brain concentrationhas not been studied (or even plasma level) in these species so differences in distribution, metabolism or excretion cannot be excluded as reasons for differences in potency. Since both LSD and psilocin have a rapid onset and a prolonged duration of action, differences in distribution, metabolism or excretion probably do not explain the differences in potency in different test systems. Differences in affinity between psilocin and LSD for the receptor are another possibility which, if true, could indicate a subtle species difference in receptor topography.

DMT is less potent in man than it is in the dog. Further, cross tolerance to DMT in both LSD tolerant man and chronic spinal dogs is not complete and is seen for certain signs and symptoms but not for others.

A critical drug is BOL. It is questionable whether it can produce LSD-like effects in man. Chronically administered BOL conferred some cross tolerance to LSD in man (Isbell et al. 1959). In doses up to 1 mg/kg intravenously BOL is devoid of LSD-like activity. In the rat, BOL inhibits operant behavior and tolerance develops to this effect with chronic administration (Appel and Freedman 1968). Whether the BOL tolerant rat is cross tolerant to LSD cannot be answered. Some tolerance was seen but it was not statistically significant. The LSD tolerant rat may exhibit some tolerance to BOL but it too was not statistically significant. BOL on the other hand is nearly as potent as LSD in inhibiting the binding of both LSD and 5HT to the brain. BOL is neither an agonist or antagonist in man (Isbell et al. 1959).

**DISCUSSION**

The first point that these data suggest is that there are small but significant differences in receptors between species. To illustrate this point for both the opioid-like analgesics and LSD-like hallucinogens the following contrasts are reviewed:

(1) Normorphine appears to resemble morphine in the mouse and guinea pig ileum but is devoid of morphine activity in the dog.

**TABLE 3**

POTENCY OF SEVERAL LSD-LIKE HALLUCINOGENS IN MAN, DOG AND RAT AS WELL AS THEIR ABILITY TO INDUCE TOLERANCE AND CROSS TOLERANCE

| | MAN | DOG[a] | RAT[b] | RAT BRAIN BINDING[c] 5HT | RAT BRAIN BINDING[c] LSD |
|---|---|---|---|---|---|
| LSD | 1 (T) | 1 (T) | (T) | 1 | 1 |
| PSILOCIN | .014 (T;CT) | 1 (CT) | | .01 | .008 |
| PSILOCYBIN | | | T (CT) | | |
| MESCALINE | .0003 (T;CT) | .004 (CT) | T (CT) | | |
| DMT | .003 (Partial T) | .1 (Partial T) | | .05 | .004 |
| 2-BROMO-LSD (BOL) | 0 (CT) | 0 | T (no Sig. CT) | .1 | .8 |

T in parenthesis indicates that chronic administration of the drug induced tolerance. CT indicates that the LSD tolerant animal was cross tolerant to the drug. The papers of Isbell which were summarized by Martin and Sloan (1977) and Martin et al. (1978) are the primary source of the data in man. (a) Martin et al. (1978); (b) Appel and Freedman (1968); (c) Bennett and Snyder (1976). 0 indicates that the drug was without effect.

**TABLE 4**

PRODUCT MOMENT CORRELATION COEFFICIENTS (r), SLOPES (b) WITH STANDARD ERROR OF ESTIMATE ($s_{y,x}$) FOR DATA PRESENTED IN TABLE 3

| | | MAN | DOG | RAT |
|---|---|---|---|---|
| DOG | r | .62 | | |
| | b | .74 | | |
| | $s_{y.x}$ | .37 | | |
| RAT 5HT | r | $1.00^{.01}$ | .52 | |
| | b | .95 | .44 | |
| | $s_{y.x}$ | .04 | .35 | |
| RAT LSD | r | .69 | .07 | 0.74 |
| | b | .72 | .06 | 0.82 |
| | $s_{y.x}$ | .33 | .45 | 0.30 |

SUPERSCRIPT INDICATES LEVEL OF SIGNIFICANCE

(2) Metazocine is a typical morphine-like drug in man, dog, mouse and guinea pig ileum but appears to resemble other agonists-antagonists in rat brain binding studies.

(3) Phenazocine is a very potent morphine-like agonist in man and dog, but a much weaker and less effective agonist in the monkey.

(4) N-allylnormetazocine (SKP 10047) is devoid of morphine-like activity in the mouse, dog and man, but is equipotent to morphine on the guinea pig ileum.

(5) BOL produces effects similar to LSD in the rat but is nearly if not totally devoid of LSD-like activity in the man and the dog.

The most probable explanation of these differences is that the receptors differ in their intimate details from one preparation to another. The critical dimension could be species or tissue (e.g. brain vs. gut) or even different brain regions or different functional systems. It may thus be important in SAR studies to treat species or even varieties as an independent variable.

Perhaps the next level of receptor classification should be subspecies. Recently there has been increasing evidence that there are subspecies of opioid receptors (Martin 1967; Martin et al. 1976; Lord et al. 1977). I suspect that it will be demonstrated that there are subspecies of the serotonin and tryptamine receptors also and that these subspecies may also be in different functional systems. Thus with regard to the opioid receptors, μ agonists produce one type of analgesia as measured by the tail flick or hot plate while κ agonists produce another which can be measured using the writhing test or the flexor reflex. It is important for SAR consideration that the data be homogeneous and indicative of one pharmacologic action. This is important because many agonists and antagonists are mixed, having several modes of actions.

Another important attribute of agonists is their intrinsic activity. Partial agonists of the μ type (profadol, propiram and buprenorphine) and κ type (nalorphine) have been identified. An important attribute of partial agonists as they relate to SAR considerations is that potency determinations will underestimate affinity.

As we attempt to understand the relationship of the chemical structure of drugs, the nature of receptors and pharmacologic actions, our theories have become more sophisticated which in turn necessitates more precise and well designed experiments.

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# The Use of Rigid Analogues to Probe Hallucinogen Receptors

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Over the past several years effort in our laboratory has been directed toward the design and synthesis of hallucinogen analogues which would be useful as probes of the hallucinogen receptor(s). Although this has involved some investigation of aromatic substituent effects, major efforts have been directed toward the elucidation of the stereochemistry of side chain binding for the phenethylamine hallucinogens. We have tried to answer the question, "how can two relatively dissimilar structures such as LSD and mescaline act at the same receptor, if indeed they do?" The ability to identify functional similarities between the indolealkylamines and the phenethylamines may ultimately lead to an understanding of possible similarities between catecholamine and indoleamine receptors and to underlying principles of receptor activation.

As an attack on this problem, we have used the rigid analogue approach. In this context rigid analogue is construed to mean any compound which has fewer degrees of conformational freedom than its open chain counterpart. The design of such molecules however, can present considerable difficulties. For example, N-alkylation of the phenylisopropylamine (amphetamine) hallucinogens generally leads to loss of activity (Shulgin 1973; Ho et al. 1970). Extension of the a methyl of the phenylisopropylamines to longer alkyl homologues likewise abolishes hallucinogenic activity (Shulgin 1963; Standridge et al. 1976). How then is one to design appropriate rigid analogues which avoid these deleterious changes? One must have some confidence that this is possible since, after all, LSD is a very rigid molecule. The real question seems to be how to make appropriate changes in the rigid molecule which compensate for loss of activity due to amine or side chain alkylation. At the extreme, one is talking about the synthesis of new classes of hallucinogens where structure-activity relationships (SARs) must be sufficiently developed to allow predictive arguments to be made.

Structure activity studies involve two separate inquiries. One seeks to determine how changes in molecular structure effect (1) mechanism of action and (2) intensity of action, or potency. With respect to the first point it seems likely that hallucinogens may interact with various monoamine pathways in the CNS. Unfortunately,

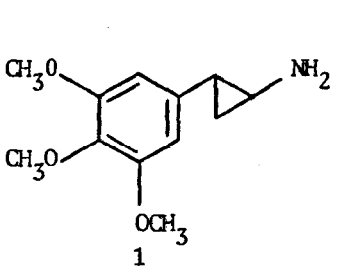
it is impossible to correlate chemical properties with particular biological responses when varying mechanisms of action are involved. Entirely different structure activity relationships may define the different mechanisms.

On the other hand, one often may make simplifying assumptions. Hansch (1969) began by assuming that for a congeneric series, one particular biological reaction might be crucial and could act as a "rate determining" step. The ability to identify such a critical step can be a very useful asset. If this is an enzyme for example, activity can be directly measured. At the level of the whole organism, multiple steps may distort quantitative structure activity relationships.

We have attempted to identify functional similarities between the various type of hallucinogens which could be related to some critical biological interaction. Based on numerous pharmacological studies, this would appear to be an interaction with central serotonin receptors (Brawley and Duffield 1972). However, as suggested by many workers, the overall intoxication may result from additional interactions with central pathways involving neurotransmitters other than serotonin (Nichols 1976).

The use of carefully designed rigid analogues can be of great utility in developing structural comparisons. For example, studies of 2-amino-1,2,3,4-tetrahydronaphthalenes (2-aminotetralins; 2-ATs) have led to important findings about the conformational requirements of dopamine receptors (Goldberg et al. 1977). Similar studies have not been as effective in elucidating requirements of hallucinogen receptors. The major difficulty in developing useful analogues has been the catch words "carefully designed" and is a result of lack of knowledge about the receptor. One can see that this is a cyclic paradox where design initially plays a relatively unimportant role and active compounds are the result of trial and error sequences.

Nevertheless, the rigid analogue approach has been used with a certain degree of success. As an important example of this approach, early workers attempted to identify similarities between LSD and mescaline by proposing that the side chain of mescaline was able to assume a cisoid conformation which would resemble the indole nucleus of LSD (Snyder and Richelson 1968). Although unlikely on theoretical grounds, the proof against such a conformation came from studies by Cooper and Walters (1972). These workers demonstrated mescaline-like activity in animals for trans-2-(3,4,5-trimethoxyphenyl)-cyclopropylamine 1. In contrast, the cis isomer would have been expected to be more active on the basis of the indole mimicry hypothesis. More recently, Aldous et al. (1974) examined a series of 2-phenylcyclopropylamines as potential hallucinogens. Notable in this study were the results obtained for trans-2-(2,5-dimethoxy-4-methylphenyl)-cyclopropylamine 2, an analogue of DOM. This compound was reported to have about one-third the potency of DOM. These studies led to the clear conclusion that the side chain of the phenethylamine hallucinogens must assume a transoid conformation.

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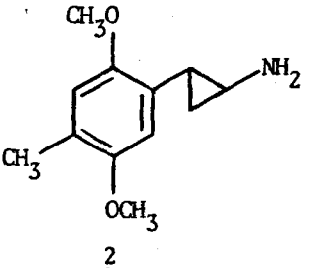
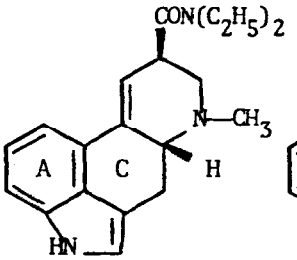
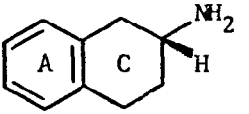



2

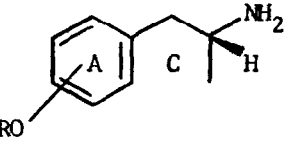
At about the same time our group, and others, developed hypotheses which attempted to relate the stereochemistry of the phenylisopropylamines to that of LSD. The basis for these proposals apparently had its origin in earlier work by Baltzly and co-workers (1949), by Bovet et al. (1951) and by Marini-Bettolo et al. (1952). In the late 1940's and early 1950's these workers had proposed that the oxytocic activity of the ergot alkaloids was related to the aminotetralin fragment, or A-C ring portions of lysergic acid, as illustrated below. Kang and Green (1970) suggested extension of this approach to consideration of LSD. Similarly, work was pub-




(+)-5R,8R-LSD 3




(+)-R-2-amino tetralin 4



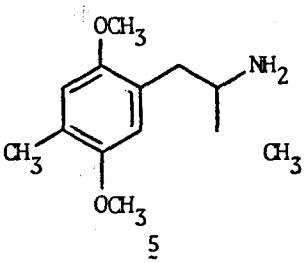

(-)-R-phenylisopropylamines

lished from our laboratory which included recognition of the possible relationship between LSD and the R enantiomer of phenylisopropylamines (Barfknecht and Nichols 1972). These predictions were confirmed by clinical studies carried out by Shulgin (1973) using the optical isomers of DOM. The R-(-) enantiomer showed clear stereoselective activity at a dose level one-half that required for the racemate. The development (Nichols et al. 1973) of an asymmetric synthesis for the enantiomers of various substituted phenylisopropylamines allowed several subsequent studies with the isomers. These clearly demonstrated that in animals and in man the R-(-) enantiomer was the more active (Benington et al. 1973; Snyder et al. 1974). Certain in vitro serotonin receptor preparations showed parallel stereo selectivity for the R enantiomer (Dyer et al. 1973).

Concurrently, a number of aminotetralin derivatives were re-examined for potential psychotomimetic activity (Violland et al. 1971; Barfknecht et al. 1973; Green, Dressler and Khazan 1973). The sympathomimetic action of 2-aminotetralin had been known

since 1889 (Bamberger and Filehne 1889) and later studies focused on the potential oxytocic and pressor effects of 2-AT derivatives. These studies invariably drew parallels to the structures of the ergot alkaloids. It was not surprising therefore, that the possibility was explored that the aminotetralins potentially represented the hallucinogenic pharmacophore in LSD. To date however, none of these attempts has led to compounds with clear-cut psychotomimetic activity. In animal models 2-aminotetralin and its 7-hydroxy derivative do show certain effects characteristic of hallucinogens (Barfknecht et al. 1973; Green, Dressler and Khazan 1973). There is no evidence however, that 2-AT or any of its derivatives is hallucinogenic in man.

On the other hand, some interesting results have been obtained from studies using aminotetralin derivatives. Cheng et al. (1974), using a smooth muscle preparation, showed that aminotetralins are, in general, about 20-30 times more potent in eliciting contractions than are the correspondingly substituted phenylisopropylamines. A direct comparison between DOM 5 and the rigid analogues 6 (DOM-AT) and 7 (DOM-AI) showed 6 to be a more potent agonist at serotonin



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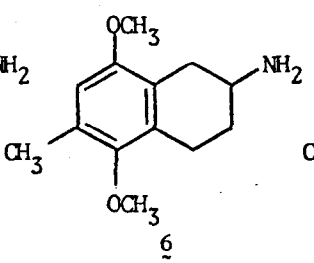

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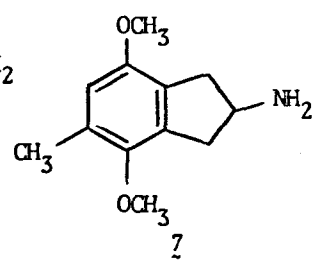
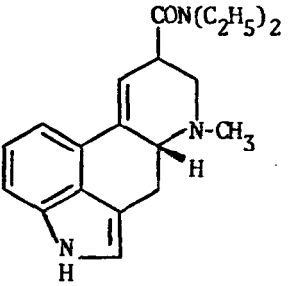


7

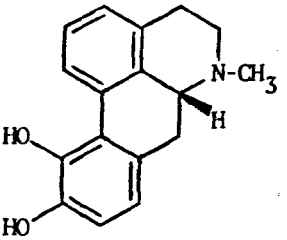
receptors than either DOM or the indan 7. The contractions induced by DOM or DOM-AT were completely blocked by cinanserin, a 5-HT antagonist. In the rat fundus, DOM-AT was much more potent than 7 and the contraction was blocked by BOL (Nichols et al. 1974). Behavioral tests in rats did not reveal a typical psychotomimetic-like profile for either 6 or 7. In cats, DOM-AT does elicit a sham rage reaction and in rabbits produces excitement and hyperthermia (unpublished results).

Although nothing definite can be extrapolated from these studies to hallucinogenic effects in humans, it may be reasonable to conclude that there is a requirement for an extended planar side chain conformation in order to obtain effective binding at peripheral serotonin receptors and to elicit certain behavioral changes in animals.

In view of recent data which indicate that LSD is able to act as an agonist at central dopamine receptors, a potential structural similarity between LSD 3 and the dopamine agonist apomorphine 8 has been proposed (Nichols 1976). This hypothesis and the implied relationship between the structures of serotonin and dopamine led us to reevaluate the theories relating the structure of LSD to mescaline and other phenethylamine hallucinogens. Examination of space filling (CPK) molecular models clearly seemed to indicate




LSD 3




Apomorphine 8

that previously proposed aminotetralin-like conformations for phenylisopropylamines were sterically unfavorable. Conformations which seemed energetically favorable were not superimposable on the structure of LSD using the A-C ring analogy. Since this was the criterion used to develop the aminotetralin analogy, means were sought to resolve this issue.

This proves to be a case where rigid analogues can be usefully employed. Our first approach was to resolve and test the enantiomers of 2-aminotetralin (Nichols et al. 1977). This was partially prompted by the practical consideration of synthetic availability. However, 2-AT does show a hallucinogen-like profile in behavioral tests in rats (Barfknecht et al. 1973). We first examined the effect of R-(+) and S-(-) 2-AT on spontaneous activity in mice. While current models predicted activity for the (+) isomer, the data in figure 1 clearly show that the (-) isomer is responsible for the previously reported depressant action of racemic 2-AT. As an additional indicator of hallucinogenic activity, we also carried out studies using rabbit EEG. The quantitative parameters total power, P, and median frequency in the power spectrum, $f_{50}$, were examined. Mescaline (20 mg/kg) in our experiments produced a slight initial depression in $f_{50}$ and an initial increase in P. The S-(-) isomer of 2-AT produced nearly identical changes at 4 mg/kg. On the other hand, the (+) isomer of 2-AT produced a dramatic and long lasting decrease in $f_{50}$. While these studies cannot be judged to be conclusive, when combined with the stereochemical arguments they justified more extensive efforts to test the validity of the A-C ring hypothesis. In particular, in view of the proposed relationship between LSD and the dopamine agonist apomorphine 8 we speculated that similar logic might apply to the phenethylamine hallucinogens.

It was decided to examine a rigid analogue which more nearly resembled the structure of a known hallucinogen in man. For this we undertook the synthesis and resolution of the two isomers of trans-2-(2,5-dimethoxy-4-methylphenyl)-cyclopropylamine (IMCPA). As indicated earlier, this is the DOM analogue which had been reported as the racemic material by Aldous et al. (1974). It was reasoned that the A-C ring analogy would predict activity for the 1S, 2R isomer. In contrast, using reasoning based on our results with isomers of aminotetralin, we predicted activity for the 1R, 2S

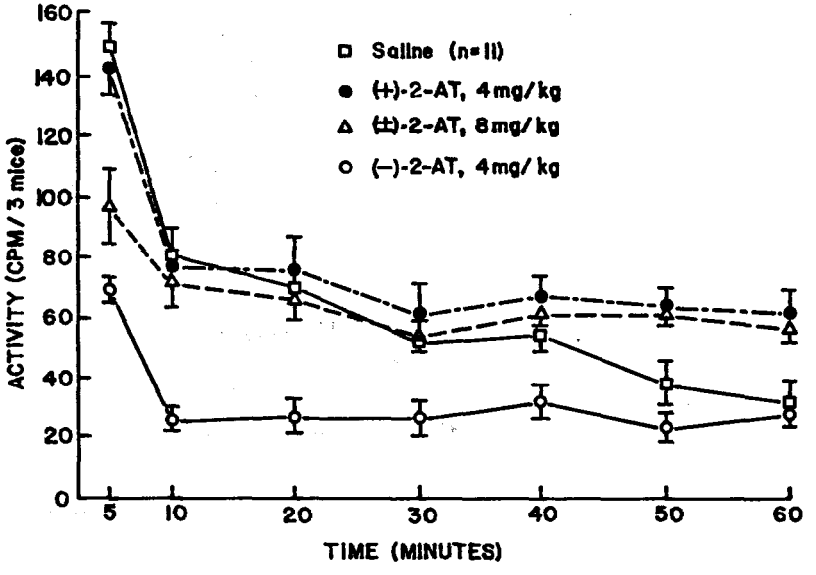



***Figure 1.*** *Effects of R-(+) and S-(-) 2-aminotetralin as compared to saline controls. Aminotetralin isomers were administered intraperitoneally as the HCl salts (Nichols et al. 1977).*

enantiomer. Effects of the isomers of DMCPA were examined in mice (5 mg/kg) and in cats (0.125 mg/kg). The results are shown in figure 2. Figure 2A represents the cumulative spontaneous activity for groups of three mice (n=4). Parallel studies were carried out with the isomers of DOM. The reduction in activity in mice was apparently not due to general depression but was a result of more time spent in various stereotypic behaviors.

Figures 2B and 2C show the effects of the isomers of DMCPA and DOM on the cat limb flick and head/body shake response reported by Jacobs et al. (1976). These results are typical for several other behavioral symptoms which were elicited. In all cases, the (-) isomer selectively elicited effects which were characteristic of hallucinogens (Jacobs et al. 1976).

We argue that the (-) isomer of DMCPA has the 1R, 2S absolute configuration (depicted in figure 3c) for the following reasons. Levoamphetamine is well-known to have the R configuration (Smith, Cook and Warren 1964). The cyclopropyl analogue of amphetamine, tranylcypromine, is also known to possess the (-)-1R,2S configuration (Riley and Brier 1972). Hence, the configuration at the alpha carbon is R in the levorotatory isomer of both compounds. Substituent introduction into the aromatic ring of amphetamine does not affect sign of rotation (Nichols et al. 1973). For example, DOM is basically a "substituted amphetamine" and has the R-(-) configuration (Matin et al. 1974). We reasoned that con-

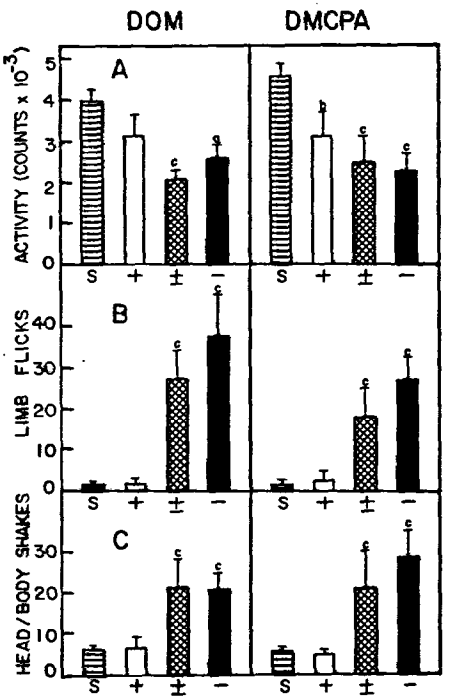



***Figure 2.*** *The effect of (+), (-), and racemic DOM and DMCPA on (A) cumulative motor activity in mice, (B) the limb flick response in the cat, and (C) head/body shakes in the cat. Motor activity was measured for 60 min. Limb flicks and head/body shakes in cats were measured for 60 min. Values are mean ± s.e.*

version of DOM into the corresponding cyclopropyl compound would parallel the amphetamine-tranylcypromine case.

Based on the results with (-) 2-AT and (-) DMCPA we present the hypothesis that the phenethylamine hallucinogens correspond to the structure of LSD as illustrated in figure 3. We have assumed that binding occurs from the underside face of the molecule (Kang and Green 1970) so that the relative side chain orientation allows the a methyl of DOM or the methylene of DMCPA to project away from the binding surface. This could explain the stereoselective activity observed for hallucinogenic amphetamine derivatives. On the basis of the earlier findings with 2-AT isomers, we propose correlation of the ortho methoxy of the phenethylamines with the 2 position of the tryptamines or LSD, rather than with the region at position 12 of LSD as suggested by earlier studies.

These arguments deal only with the ability of phenethylamines to bind at serotonin receptors. This analogy obviously does not apply to cases where the drug acts indirectly by the release of endogenous transmitter (Menon, Tseng and Loh 1976; Cheng et al. 1974).

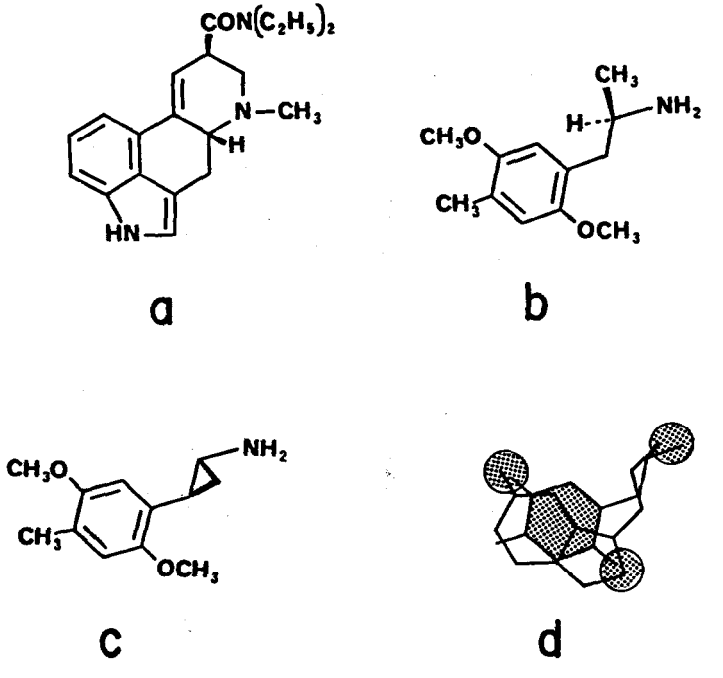



***Figure 3.*** *A comparison of the structures of* (a) *5R, 8R-(+)-LSD; (b) R-(-)-1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM); (c) 1R,2S-2-(2,5-dimethoxy-4-methylphenyl)-cyclopropylamine and (d) a framework superposition of a phenethylamine and tryptamine moiety using the hypothesis discussed in the text.*



Although we initially suggested (Nichols et al. 1977) that the aromatic ring of the phenethylamines corresponded to the pyrrole region in the indole nucleus of LSD, a more general view is now proposed in light of the following observations:

(1). The interatomic distance between the centers of the 02 and 05 atoms of DOM and the C2 and 05 atoms of serotonin is virtually identical, being approximately 5.5-6Å.

(2). Molecular orbital calculations have shown a high electron density at C2 in LSD (Karreman, Isenberg and Szent-Györgyi 1959). This has been cited as evidence for charge-transfer complex formation. The 02 atom in the phenethylamines, with its unshared electrons, could also interact with an electrophilic site at this region of the receptor.

(3). The 05 atom in the phenethylamines probably binds at the same site which accomodates the 5-hydroxy of serotonin. In the phenethylamines extension of the 5-methoxy to an ethoxy abolishes hallucinogenic activity (Shulgin 1968). Similarly, homologation of 5-methoxy tryptamine to 5-ethoxy or longer alkyl homologues decreases 5-HT agonist activity (Arutyunyan et al. 1964; Buznikov

et al. 1965). It also seems possible that the 9,10 double bond of LSD could interact with this site.

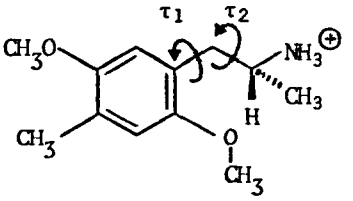
(4). The description of a lipophilic region on the receptor which binds the para substituent of phenethylamine hallucinogens (Nichols, Shulgin and Dyer 1977) is consistent with an area of the proposed receptor which normally accomodates the C6-C7 edge of serotonin.

(5). The receptor is probably flexible. The cyclopropyl analogues are relatively rigid and complete superposition of LSD is impossible. For a planar interaction, the amino group of IMCPA would lie approximately 1 Å from N(6) of LSD.

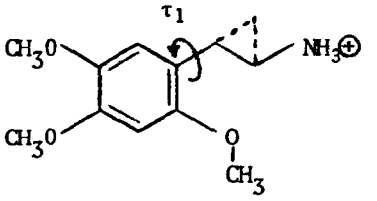
The para substituent seems to play a unique role in defining activity. As indicated above, this substituent may bind at an area of the serotonin receptor which is "incompletely filled." However, since metabolic deactivation appears to occur by oxidative attack at this position (Ho et al. 1971) the ability of the substituent to resist oxidation probably influences activity. The contrasting duration of action for DOB (4-bromine) and para-DOT (4-methylthio) may reflect this.

**THEORETICAL CALCULATIONS**

We have also performed theoretical conformational calculations for R-DOM and 1R,2S-DMCPA. The following structures indicate starting orientation and direction of rotation for the major side chain torsional angles. (Note that this does not correspond to the standard convention for "torsional angles"). For this work we



R-DOM



1R,2S-DMCPA

employed the CAMSEQ software system developed by Weintraub (1975), but using a revised solvation model for charged species in solution (Weintraub and Nichols 1978). The conformational energy maps for the two compounds are shown in figure 4. The active conformation which we propose for DOM would lie in the region $\tau_1 \sim 180°$; $\tau_2 \sim 0°$. Although we cannot assign a strict value to $\tau_1$ we believe it lies within ± 30° of this value. It will be noted from the conformational energy maps that this lies within a region approximately 1-4 kcal above the global minimum.

In DMCPA $\tau_2$ is fixed at about 328° (our convention). If one assumes that in the active conformation this is an appropriate value of $\tau_2$ for flexible compounds, and that the amino group lies approximately in the plane of the aromatic ring, as it does in LSD, a value for

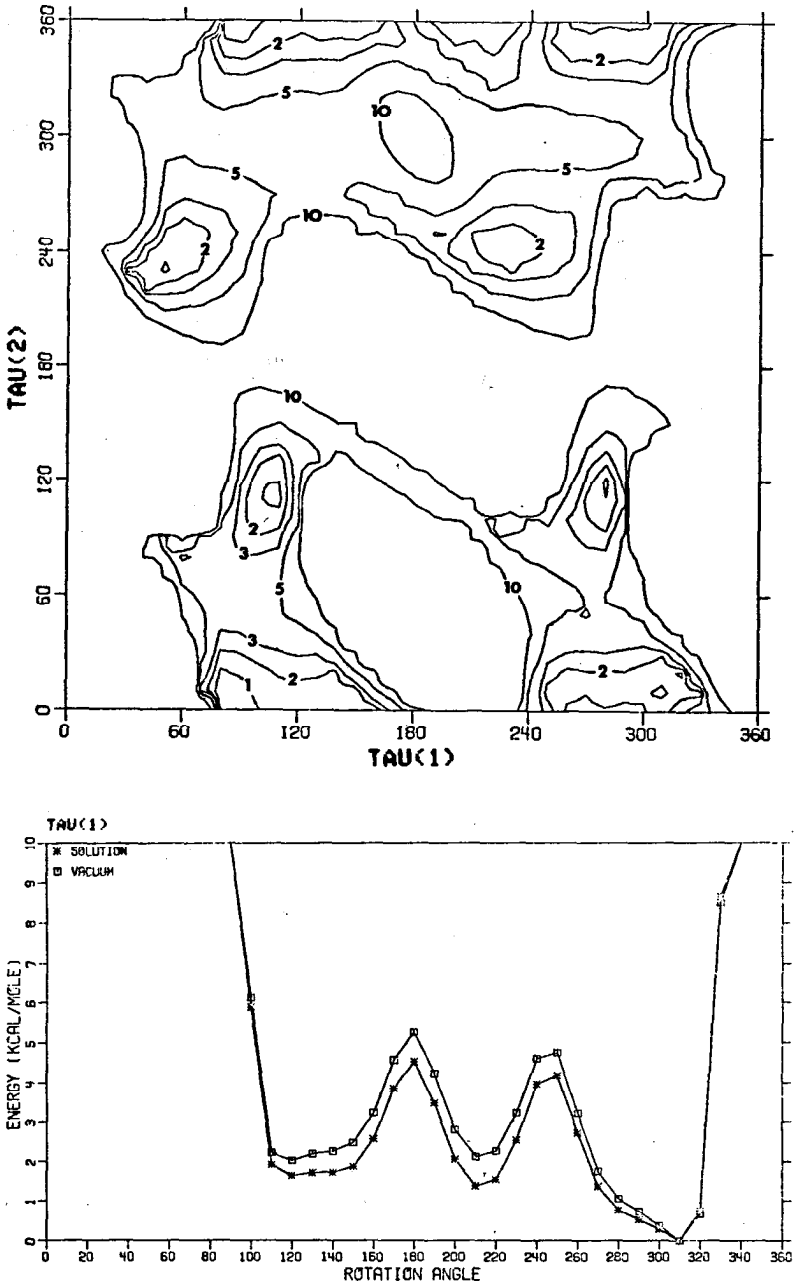



***Figure 4.*** *A. Conformational isoenergy contour map for DOM in solution and B. the conformational energy profile for rotation of* $\tau_1$ *in DMCPA.*

$\tau_1$ of approximately 155° is required. This places $\tau_1$ in a relatively low energy region of the conformational energy map for both DOM and DMCPA.

Similar calculations for the non-hallucinogenic a ethyl homologue of DOM (Standridge et al. 1976) resulted in a conformational profile very similar to DOM. This finding seems to indicate that the lack of hallucinogenic activity for the a ethyl homologue is probably not due to an inability to assume the active conformation. This work is reported in detail elsewhere (Weintraub and Nichols 1978).

**FOOTNOTE**

1. Reprinted with permission, from Nichols, D.E., Pfister, W.R., and Yim, G.K.W. LSD and phenethylamine hallucinogens: New structural analogy and implications for receptor geometry. Life Sciences, Vol. 22, No. 24. © 1978, Pergamon Press, Inc.

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# Section II

## Hansch Analysis and Other Empirical Methods

This portion of the meeting appeared to accomplish two objectives. The papers highlighted several important techniques of applying physicochemical parameters to the problem of understanding the nature of the effects of substituent variation upon the drug activity observed. The areas where these parameters are weakest--most notably in stereochemical differences and in some steric effects--were also pointed out. But perhaps the second objective might, in the long run, prove more valuable to the attendees. That objective was to clearly define just what should be expected of this approach to QuaSAR; i.e., an acceptable statement of philosophy.

In a paper given at an earlier session, Dr. Sydney Archer put forth this challenge: Of the three levels of drug design--(1) finding a totally new pharmacophore; (2) circumventing a basic shortcoming of an established one (e.g., eliminating dependence on drugs that have analgesic action); and (3) improving potency--QuaSAR methods addressed only the latter. Then in the discussion Dr. Jack Green added the further challenge that the Hansch type of correlation analysis may not be appropriate to investigate basic questions of mechanism of action but was rather intended to obtain "straight lines" that have predictive value.

As was evident from the discussions clinicians do not consider potency the "bottom line" in choosing which drug to use, but rather look at the entire profile of efficacy weighed against various side effects. And, of course, "constructing straight lines with predictive value" was not seen by those who gave papers utilizing correlation analysis as the end and final goal of QuaSAR. Each speaker came to present and defend some rather narrow areas of their own expertise, and so the challenge to defend the entire concept could not be met

with well-organized material prepared in advance. But it was evident that the purpose of Dr. Archer and Dr. Green was to force the Hansch analysis people to bring out for open scrutiny some of the basic methods they had established long ago and perhaps had taken for granted, but which, for some of the conferees, would be crucial in deciding how much credence should be given to the results reported. If this was meant to be an application of the "gadfly" technique, it worked. The discussions were much more lively and enlightening than they otherwise would have been.

In the general discussion periods there also appeared to be a difference of opinion amongst the conferees as to what tools or language one must be using before he "really gets down to molecular mechanism." An example was cited where the significance of an electronic effect was appreciated through correlation analysis using a sigma parameter. This prompted the investigators to do a study with molecular orbital calculations which enabled them to see where the sigma correlation came from. Only then were they getting down to molecular mechanisms. Naturally the question was raised: "Why, if M.O. methods at their present level of feasibility are more basic in describing molecular mechanisms, have they not been used to better effect than sigma constants in uncovering these electronic correlations?"

The session ended with a discussion of the various interpretations which could be given to a parabolic relationship between hydrophobicity and activity. Since this is seen in some in vitro experiments, such as in brain homogenates and even in semi-purified enzymes, there is the strong possibility that part or all of the parabolic dependency may not arise from transport through cellular compartments, but, alternatively, the drug may be involved in several competing reactions which have different dependence upon hydrophobicity. Occasionally these competing reactions can be studied via Hansch analysis, and the knowledge put to use in solving problems connected with area (2) in Dr. Archer's scheme of drug design.

'QuaSAR' Research Monograph 22
G. Barnett, M. Trsic, and R. Willette, eds.
National Institute on Drug Abuse, 1978

# QSAR: A Critical Appraisal

Sydney Archer

The pioneering work of Hansch on QSAR has led to an avalanche of papers, as witnessed by the fact that the first three papers in the January 1978 issue of the Journal of Medicinal Chemistry deal with this subject. QSAR has become so popular that two chapters in the first three volumes of the series "Drug Design" edited by E.J. Ariens are devoted to this subject (Hansch 1972; Verloop 1972). In his Smissman Award Address, Alfred Burger (1978) devoted the last several paragraphs of this talk to QSAR. Because of the attention that this aspect of medicinal chemistry is receiving, a critical appraisal of the significance of the role played by QSAR in drug aesign is the subject of this communication.

Before proceeding, it would be worthwhile to define what is meant by drug design. Ariens (1972) defines the term as follows: "Drug design is an effort to develop drugs on as rational a basis as possible. This implies a reduction of the trial and error factor in the procedure to the absolute minimum. However, the fact that trial and error is not completely excluded in the efforts to develop a drug does not mean that drug design is not involved." This writer finds this definition unsatisfactory for the following reasons: first, design is not an effort; second, the implied goals in the definition are far too modest; third, it does not conform to the dictionary definition of the word design; fourth, it involves the word rational, whose meaning is also clouded with ambiguity; and fifth, it employs the word, drug, which is to be defined in the definition.

What is a drug? According to Webster's 2nd International Edition a drug is "any substance used as a medicine or in the making of medicines for internal or external use." According to the Pure Food and Drug Act, the term "drug" includes all medicines and preparations recognized in the U.S.P. and N.F. for internal and

external use and "any substance or mixture of substances intended to be used for the cure, mitigation or prevention of disease in either man or animals." The same authority defines design as "a plan formed in the mind, of something to be done or produced; a mental project or scheme in which the means to an end are laid down..." Finally, rational is defined as, 1) having reason or understanding, reasoning; 2) of or pertaining to the reason or reasoning process; of the nature of, based upon, derived from, concerned with or characterized by reason; as rational insight.

Since a drug is intended to cure or mitigate disease in man or animals and design is a plan or scheme to produce something, drug design may be defined as a mental process wherein plans are formulated to produce substances which will cure or mitigate disease.

The problems facing medicinal chemists who are interestea in drug design may be classified into three categories. Category I encompasses those diseases for which there are no known or satisfactory treatments. Here the medicinal chemist has no known models at his aisposal so that drugs must be designed de novo if the purely empirical approach is to be avoided. Black's work on H2 receptor antagonists and the Nachmanson-Wilson development of nerve-gas antagonists are examples of solutions to problems which fit into this category. Category II involves those diseases for which therapeutic agents are known but suffer from obvious clinical disadvantages. The development of penicillinase-resistant penicillins and strong analgesics relatively free of a morphine-like dependence syndrome may serve as examples here. Category III is concerned with increasing potency of clinically satisfactory drugs. The super-potent anti-inflammatory steroids, the highly active thiazide diuretics, the ultrapotent morphine-like analgesics fall into this class.

QSAR studies are for the most part concerned with the last category. It is difficult to impress upon the minds of many medicinal chemists and pharmacologists that it is only in rare instances that potency per se is of paramount concern. One example is in the development of long-acting depot preparations. If a drug can be adminstered conveniently in a suitable dosge form. milligram potency is of little concern to the clinician.

Supporters of QSAR studies like to quote Spinks who is reported to have estimated that one new drug arises out of each 200,000 new compounds and that one anticancer drug will emerge from 400,000,000 randomly tested compounds. The implication is that QSAR studies can reduce these odds.

First, one must ask, what does Spinks mean by a new drug? Does he mean one that belongs in Category I or II or a simple molecular modification which would place the compound in Category III for which QSAR may be of help? If Spinks means by new drugs those which fit only in Categories I and II, by far the more difficult to develop, then the reality of the situation belies these estimates. Using antimalarial agents as examples of Category I and II drugs, the therapeutic arsenal contains examples of the following: quinolinemethanols (quinine), amino-quinolines (primaquine, chloroquine), acridines (mepacrine), dihydrotriazines (cycloguanil), aihydro-pyrimidines (pyrimethamine), sulfonamides (sulphormethoxine), sulfones (DDS). If the 1:200,000 ratio is valid, then about $1.4 \times 10^6$ compounds would have to be screened to yield this crop of drugs, a number which is more than 1/3 of all known organic compounds.

The fact of the matter is that truly random screening rarely, if ever, takes place. In the antimalarial field screening is limited, with rare exceptions, to N containing substances which also contain oxygen. Furthermore, the vast majority of compounds are either aromatic (carbocyclic or heterocyclic) or combinations thereof. Functionality such as COOH, $SO_3H$ and quaternary ammonium are usually avoided to minimize absorption and distribution problems. Thus on an almost intuitive basis true, random screening is usually avoided so that the Spinks' estimate is at best a statistical exercise.

In one of the more important QSAR papers Hansch et al. (1968) analyzed a large group of hypnotics, with special emphasis on barbiturates. The biological data, obtained from various literature sources, was amassed over the period 1923-1949, hardly the golden age of modern pharmacology. Not only were different methods of assessing hypnosis used but different species were employed as test animals. Several equations were developed and the most interesting conclusion that emerged from this collection of inhomogeneous data was that log Po for the oxybarbiturates equaled 2. The authors concluded that the hypnotic activity of groups of barbiturates depend almost entirely on the relative lipophilic character as defined by their octanol-water partition coefficients. In the case of the thiobarbiturates the mean log Po was 3.1. The authors state, "The thiobarbiturates quite definitely do not fit into the same pattern shown by other barbiturates or the other hypnotics. Their maximum activity is attained wnen their partition coefficients are about 10 times that of the barbiturates. This strongly implies a different overall mechanism of action. That the thiobarbiturates have quite different biological action from

the oxybarbiturates has been pointed out. by Aldridge and Parker."

In 1960, eight years before the publication of the Hansch paper, B.B. Brodie and his colleagues published a paper (Brodie et al. 1960) entitled, "The importance of dissociation constant and lipid solubility in influencing the passage of drugs into the cerebrospinal fluid" wherein the penetration of drugs into the CNS was carefully examined. This paper was not even mentioned by Hansch (Hansch et al. 1968).

Broaie was able to determine penetration constants (P) into the CSF using Fick's law for diffusion:

$$d(Cp-Cc)/dt = -P(Cp-Cc) \qquad (1)$$

where cp = concentration of drug in plasma
cc = concentration of drug in the CSF.

If Cp is kept constant as it can, by maintaining a constant rate of infusion, then integration of (1) gives equation (2):

$$\ln((Cp-Cc)/Cp) = -Pt \qquad (2)$$

Plotting log ((Cp-Cc)/Cp) vs. time gives a straight line, the negative slope of which equals P. Using a variety of drugs these authors developed a family of curves from which the penetration constants were calculated. Goldstein (Goldstein et al. 1974) constructed table I using data generated earlier by others. It is clear from inspection of this table that lipophilicity as measured by the effective partition coefficient (column e) correlates well with P and the penetration half-time. Thus the effects of lipophilicity on penetration into the CNS was well appreciated some time before the QSAR treatment and it would have been surprising if Hansch's conclusions differed from those of Brodie.

More aisturbing is Hansch's interpretation of the results obtained with the thiobarbiturates. On the basis of the different log Po values for oxybarbiturates and thiobarbiturates it is claimed that this result "strongly implies a different mode of action" and cites Aldridge and Parker (1960) as support. What these authors do say is: 1) "The oxybarbiturates...inhibit but do not uncouple oxidative phosphorylation of liver mitochondria with pyruvate as a substrate." 2) "The thiobarbiturates inhibit and to a <u>certain</u> <u>extent</u> uncouple oxidative phosphorylation. The uncoupling appears to be correlated with their ability to activate mitochondrial ATPase." 3) "It is possible that thio-

TABLE 1

| (a) Drug | (b) PKa | (c) Fraction non-ionized at pH 7.4 | (d) Partition coefficient n-heptane/ water of non-ionized form | (e) Effective Partition Coefficient (c) x (d) (x 100) | (f) Penetration rate constant P ($min^{-1}$) | (g) Penetration half-time (min) |
|---|---|---|---|---|---|---|
| Thiopental | 7.6 | 0.613 | 3.3 | 2000 | 0.50 | 1.4 |
| Aniline | 4.6 | 0.998 | 1.1 | 1100 | 0.40 | 1.7 |
| Aminopyrine | 5.0 | 0.996 | 0.21 | 210 | 0.25 | 2.8 |
| Pentobarbital | 8.1 | 0.834 | 0.05 | 42 | 0.17 | 4.0 |
| Antipyrine | 1.4 | >0.999 | 0.005 | 5.0 | 0.12 | 5.8 |
| Barbital | 7.5 | 0.557 | 0.002 | 1.1 | 0.026 | 27 |
| Mecamylamine | 11.2 | 0.016 | >400 | >4.8 | 0.021 | 32 |
| N-Acetyl-4-aminoantipyrine | 0.5 | >0.999 | 0.001 | 1.0 | 0.012 | 56 |
| Salicylic Acid | 3.0 | 0.004 | 0.12 | 0.48 | 0.006 | 115 |
| Sulfaguanidine | >10.0 | >0.998 | <0.001 | <1.0 | 0.003 | 231 |

barbiturates have two unrelated mechanisms of action, one inhibiting respiration by a similar mechanism to that of the oxybarbiturates and the other activating ATPase." (Emphasis added).

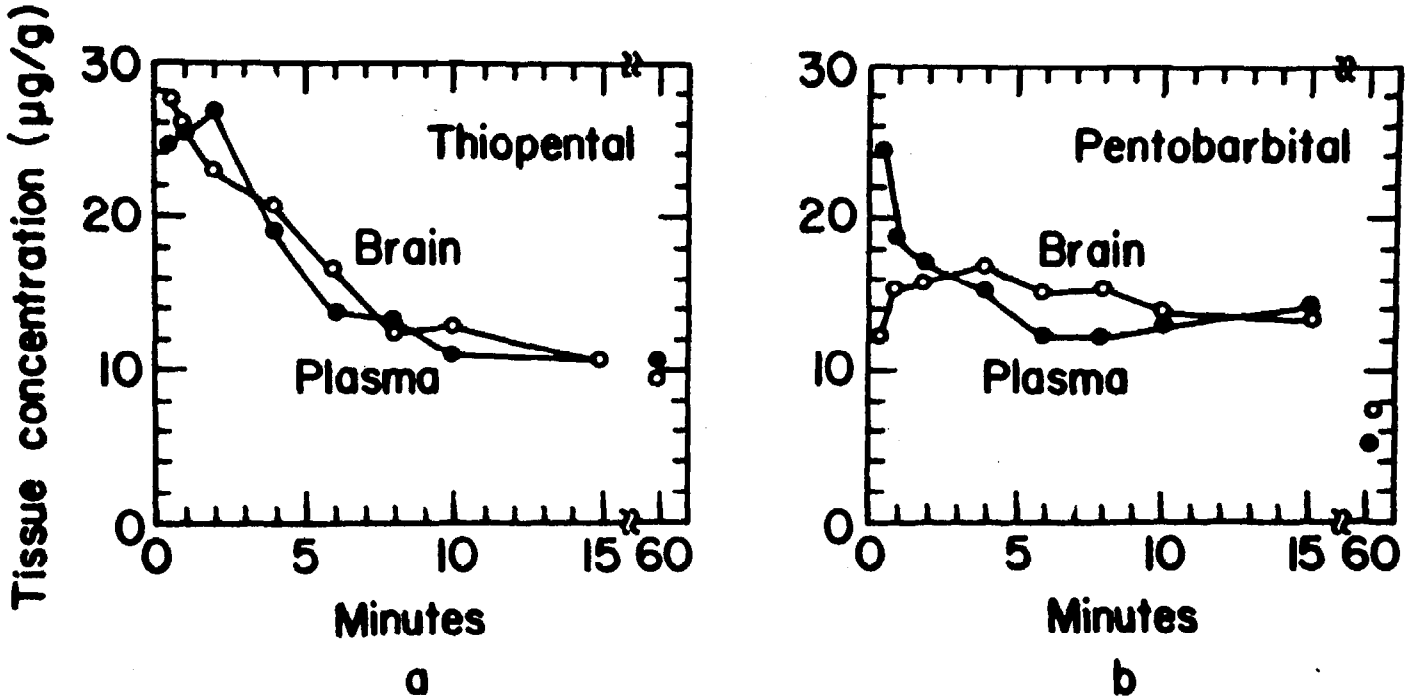
The last two statements are a far cry from Hansch's interpretation of the Aldridge-Parker paper, "that the thiobarbiturates have quite different biological action from the oxybarbiturates."

Another questionable interpretation of the literature is the statement, "The possibility that more lipophilic centers in the brain are involved in the case of the thiobarbiturates can be inferred from the work of Goldstein and Aronow (1960) and Roth and Barlow (1961). Their efforts have shown that the concentration of the tniobarbiturates rises in the brain considerably above that in blood plasma in a rather short time." Actually Goldstein and Aronow showed that the brain and plasma concentrations of thiopental are equal within a minute or so after drug administration as a result of rapid equilibration. Roth and Barlow agreed that whole brain levels and those in plasma were about equal but they believed the concentration of thiopental in the cerebral cortex was twice that in the plasma at very early times.

The Goldstein-Aronow experiments were carried out to account for the ultrashort duration of thiopental in comparison with pentobarbital. It was known that thiopental and pentobarbital were equipotent, that is to say, that equal brain concentrations of the two drugs produced the same degree of anesthesia. It was known that thiopental concentrates in fat depots, but the kinetics of the fat depot plasma interchange could not account for the ultrashort action of the hypnotic.

Owing to the favorable lipid solubility pentothal rapidly penetrates into the brain and brain levels and plasma levels are essentially identical in a very short time (figure 1). The brain level of the drug is above the threshold level required to produce anesthesia so that the pharmacological response is very rapid. As plasma levels decay, brain levels follow so that at the end of about 5 minutes brain levels of drug are insufficient to maintain the hypnotic state. In contrast pentobarbital equilibrates relatively slowly and in the Goldstein-Aronow experiment this oxybarbiturate did not reach a sufficiently high concentration in brain to produce anesthesia. The experiment showed why a higher dose of pentobarbital is needed to produce anesthesia, why it is slow in onset and why it is protracted in duration. The conclusions drawn from this study are at variance with those indicated by QSAR studies, in the

FIGURE 1




*Plasma and brain concentration of thiopental (a) and pentobarbital (b) after intravenous injection in rats (Goldstein and Aronow 1960). Reprinted with permission of William and Wilkins Publising Co.,* © *1960. From* Journal of Pharmacology and Experimental Therapy, *128:1, by A. Goldstein and L. Aronow.*

sense that no difference in mechanism of action between the thiobarbiturates and oxybarbiturates is implied.

## II. QSAR AND STRONG ANALGESICS

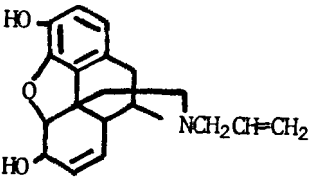
The reason for the intense interest in research in this field was well stated by Eddy and May (1966): "The ever present stimulus to the recurring search for new synthetic analgesics has been the hope that new active compounds might be found free of the undesirable side effects of morphine itself." By far the most important side effect of this classical opiate is its ability to produce physical dependence.

Burger (1978) has cautioned that "Quantitative structure activity relationships are only as good as the biological test methods against which physicochemical parameters are plotted." There is no better illustration of this point than in the field of strong analgesics.

In the mid-1950's the laboratory models for testing strong analgesics were the D'Amour-Smith test or some slight modification thereof in rats and the Eddy-Leimbach "hot plate" test in mice. Both procedures were capable of detecting morphine-like analgesic activity and were extremely accurate predictors of milligram potency in man. At that time, thanks largely to the efforts of Beecher and his collaborators, assays of analgesic activity in man could be carried out accurately with a relatively small patient population. Investigators at the Addiction Research Center in Lexington were devising ways to determine addiction liability in man in a quantitative manner. With all the proper bioassays in place, the stage was set to find a potent agonist with little or no addiction liability. Unfortunately the goal remained elusive despite the fact that a number of structurally distinct series of compounds, some members of which showed strong'analgesic action in animals and man, were synthesized. These included compounds in the methadone, meperidine, levorphanol, benzomorphan and etonitazene series. Despite the aiversity of chemical types the similarity in biological effects was extremely frustrating and disappointing. Compounds several hundred times as potent as morphine were synthesized but the pharmacological profiles were essentially identical with morphine.

The partial solution to this problem stemmed from two related serendipitous observations. The first was that replacement of the N-methyl group of morphine resulted in a compound (I) which antagonized the pharmacological effects of morphine. Nalorphine was essentially inactive in the D'Amour-Smith and Eddy-Leimbach tests and thus was considered not to be an analgesic. The second

important observation was made by Beecher and Lasagna in 1954, who found that in man nalorphine was a strong analgesic equal in milligram potency to morphine. Yet when given after morphine the agonist effects of the latter were abolished. Unfortunately, the drug produced such bizarre effects in man that it was not considered suitable for clinical use. Preliminary studies on its ability to induce physical dependence were negative.




I

Clinical studies on other narcotic antagonists were carried out by Arthur Keats. A few were found to be good analgesics but those that were also produced unacceptable CNS side effects. The significance of the Keats work lay in the fact that it clearly demonstrated that the clinical behavior of nalorphine was not unique and that a careful investigation of other narcotic antagonists might lead to a useful drug.

In our laboratory, analysis of the available animal and clinical data led to the conclusion that the laboratory models for evaluating strong analgesics were more reliable predictors of addiction liability than of clinical analgesic potency.

The design of potential analgesics involved the synthesis of putative narcotic antagonists in the benzomorphan series, testing for analgesic antagonism in rats and also for activity in the D'Amour-Smith test. A positive result in the latter test resulted in rejection of a compound from further study. At that time the only reliable assessment of analgesia was trials in humans. Fortunately the severe restrictions on early clinical investigations which followed in the aftermath of the passage of the Kefauver-Harris bill were not yet in force so that clinical research could be carried out with far less toxicological, biochemical and metabolic data than are required now. It should be added that our collaborating clinical pharmacologists employed all the necessary clinical safeguards and there were no greater dangers to the patient than we have now. Several narcotic antagonists were tested in a relatively short period of time. One of these, pentazocine, a weak antagonist was found to be a clinically acceptable drug and is presently marketed. It was also found that

TABLE 2

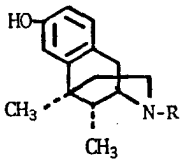
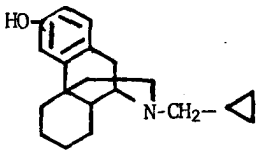
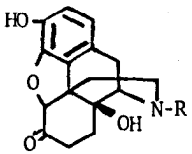
| STRUCTURE | POTENCY AS AN ANTAGONIST IN ANIMALS AND MAN | POTENCY AS AN AGONIST IN MAN RELATIVE TO MORPHINE |
|---|---|---|
|  | | |
| R = $CH_2CH = C(CH_3)_1$ Pentazocine | weak | 1/4 - 1/3 x morphine |
| R = $CH_2$– | strong | 20-40 x morphine |
|  Cyclorphan | strong | 40 x morphine |
|  R = $-CH_2-CH = CH_2$ Naloxone | strong | none |
| R = $-CH_2$– Naltrexone | strong | none |

**TABLE 2** (cont.)

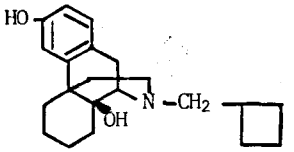
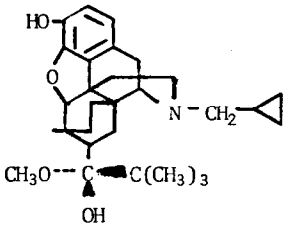
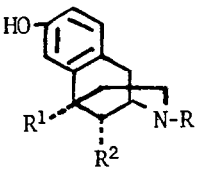
| | | |
|---|---|---|
| R =  nalbuphine | moderate | 1 x morphine |
| 3 OH instead of 0 | | |
|  Butorphanol | moderate | 2-5 x morphine |
|  Buprenorphine | strong | several times morphi |

**TABLE 3**

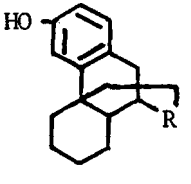
| Compound | | | $ED_{50}$ | Clinical Potency mg equivalent to 10 mg of morphine |
|---|---|---|---|---|
|  | $R^1$ | $R^2$ | | |
| R = $-CH_2-HC=CH_2$ | Me | Me | Inactive | ca 15 |
| R = $-CH_2HC=C(CH_3)_2$ | Me | Me | Inactive | ca 30-40 |
| R = $-CH_2$  | Me | Me | 23.1 | ca 0.25 |
| R = $-CH_2CH=C(CH_3)_2$ | Me | Et | 15.9 | ca 40-60 |

powerful antagonists could also be potent clinically active analgesics. Cyclorphan and cyclazocine are two such examples. These drugs did not produce a morphine-like physical dependence in monkeys and even more important, in man. These developments stimulated other workers to look at other narcotic antagonists as possible analgesics and table II shows some results of their efforts.

In light of this history it was surprising to see the recent paper by Katz et al. (1977) on a QSAR study of narcotic analgesics in which the assay method was the Eady-Leimbach test procedure. One could safely predict that none of the compounds in table II would show significant activity in that test. Table III reports some results from the Eddy-Leimbach test and clinical potency of some benzomorphans which are known to be mixed agonist-antagonists.

It should be clear from the few examples shown in table III that the Eddy-Leimbach test is virtually useless as a predictor of useful analgesic activity.

Since the early studies on narcotic antagonists, pharmacological tests have been devised which can detect the agonist potency of this group of analgesics. However, one must be careful in the choice of assays because of difficulties with internal correlations. For example, it is well known that quaternary ammonium salts of strong analgesics generally are inactive in whole animal tests. Yet in isolated systems such as the guinea pig ileum and isolated receptors Opheim and Cox (1976) have shown that the methiodide of levorphanol (III) was about 1/3 as active as levorphanol (II) using the myenteric plexus of the longitudinal muscle of the guinea pig ileum, a preparation popularized by Kosterlitz (Kosterlitz and Waterfield 1975). The activity in this preparation was reversed by naloxone.




II R = $NCH_3$
III R = $^{+}N(CH_3)_2I^{-}$

Using guinea pig brain homogenates the methiodide was able to depress the stereospecific binding of ($^{3}$H)-etorphine. The dextro isomer was far less active.

Recently Kosterlitz (Lord et al. 1977) found a new opiate receptor in the mouse vas deferens. As can be seen from table IV there is a difference in potency of Met-enkephalin analogs in these two preparations.

TABLE IV

| Compound | Guinea Pig Ileum | Mouse Vas Deferens | Ratio Guinea Pig Ileum/ Mouse Vas Deferens |
|---|---|---|---|
| Met-enkephalin | 1 | 1 | 1 |
| Met-enkephalin amide | 1.47 ± 0.18 | 0.71 ± 0.08 | 2.1 |
| NCH -Met-enkephalin | 1.45 ± 0.19 | 0.23 ± 0.01 | 6.3 |
| NCH -Met-enkephalin amide | 3.72 ± 0.96 | 0.33 ± 0.04 | 11 |
| DAla -Met-enkephalin | 6.01 ± 0.31 | 0.56 ± 0.62 | 11.1 |

Another striking difference between these two preparations is the amount of naloxone necessary to antagonize the action of some opioid peptides as shown in table V.

TABLE V

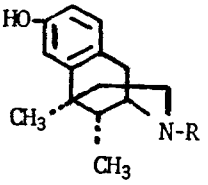
| Agonist | Guinea Pig Ileum Ke (nM) | Mouse Vas Deferens Ke (nM) |
|---|---|---|
| Nor-morphine | 1.89 ± 0.20 | 1.84 ± 0.20 |
| Tyr-Gly-Gly-Phe | 1.73 ± 1.77 | 29.2 ± 2.7 |
| Met-enkephalin | 2.45 ± 0.26 | 22.6 ± 0.5 |
| -Endorphin (61-76) | 1.56 ± 0.15 | 22.5 ± 2.9 |
| -Endorphin (61-91) | 2.86 ± 0.42 | 21.7 ± 1.2 |
| N-Me-Met-enkephalin | 2.07 ± 0.08 | 15.6 ± 3.8 |
| N-Me-Met-enkephalin amide | - | 16.6 ± 2.6 |
| D-Ala -Met-enkephalin | 2.55 ± 0.20 | 22.4 ± 2.7 |

The guinea pig ileum assay results for agonist potency correlate well with clinical results, but some discrepancies could surface if compounds such as quaternary salts are ever tested in man, a highly unlikely situation. It was pointed out earlier that potency is not of primary concern to clinicians--nor should it be to medicinal chemists. There are many unanswered questions in the field of strong analgesics, the answers to which should be of greater concern than problems involving maximizing potency. Some of these are: 1) Why is it that, in this field, the structural requirements for agonist activity are relatively few while those for antagonists are highly restrictive? In other areas of pharmacology such as adrenergic, serotoninergic and cholinergic systems, the reverse is usually true, that is, that the agonists are represented by few structural types whereas greater diversity can be found in the antagonists. 2) Why is it that lengthening the chain on nitrogen in the morphinan and benzomorphan series converts a compound which is a pure agonist to one that is either a pure antagonist or a mixed agonist-antagonist? Explanations based on conformational differences are not convincing since it is difficult to see why the methyl and amyl analogs should be conformationally similar and both differ from the n-propyl compound (table VI). 3) How can one account for the unusual effects of 14-OH substitution in the morphinans and the corresponding 11-OH substitution in the benzazocines? For example nalorphine is a mixed agonist-antagonist while the structurally similar agent, naloxone, is devoid of agonist action but is essentially a pure antagonist. Similarly naltrexone, a 14-OH-N-cyclopropylmethyl derivative, is a pure antagonist whereas other non-14-hydroxylated derivatives such as cyclorphan and cyclazocine are highly potent agonists and antagonists. Replacement of the N-cyclopropylmethyl radical with the cyclo-butylmethyl radical in the 14-hyaroxy-morphinans reduces antagonist action but agonist activity reappears as in the case of nalbuphine and butorphanol.

Even if one accepts that the modest goal of QSAR studies is to reduce the trial and error approach to the maximization of potency then such investigations must have some heuristic value; otherwise QSAR will become a sterile exercise in data manipulation. However, Verloop (1972) states "Examples of predictions of bioactivity from regression equations are still rare. As far as the present author is aware, there are no examples of cases in which the prediction made by one author was later substantiated in another paper, with the exception of the 'thyroxine case' quoted by Hansch."

**TABLE VI**

**Agonist Potencies of Some Benzomorphans in the Eddy-Leimbach Test**

| Compound | $ED_{50}$ (mg/kg, s.c.) |
|---|---|
|  | |
| R= | |
| $-CH_3$ | 3.0 |
| $-CH_2CH_3$ | Inactive |
| $-CH_2CH_2CH_3$ | Inactive |
| $-CH_2CH_2CH_2CH_3$ | Inactive |
| $-CH_2CH_2CH_2CH_2CH_3$ | 2.2 |

A report (Humber et al. 1975) prepared by an ad hoc Committee of the Section on Medicinal Chemistry of the IUPAC entitled, "'Predicted' Compounds with 'alleged' Biological Activities from Analyses of Structure-Activity Relationships" lists 15 such cases of compounds whose activity was predicted on the basis of QSAR studies, but which were never synthesized and tested for the pharmacological activity indicated. It would seem to this writer that it would be useful to put these predictions to the test. Positive results of such an exercise could give greater credence to the implicit and explicit claims that QSAR have an important place in drug design.

**ACKNOWLEDGMENT**

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# QSAR of Agents Involved in Serotonin and LSD Binding Sites

Y.L. Chan, E. J. Lien, and J. C. Shih

## INTRODUCTION

Using cerebral cortex membrane preparations of rat brain, Bennett and Snyder (1976) studied the displacement of specifically bound [$^3$H]-serotonin (5-HT) and d-[$^3$H]-lysergic acid diethylamide (LSD) by various drugs. It was concluded that both binding sites have similar structural specificity, with a more crucial role played by the 5-hydroxy substituent in the binding of various tryptamines for the [$^3$H]-5-HT binding sites than for the d-[$^3$H]-LSD sites.

The compounds studied by Bennett and Snyder include LSD analogues; tryptamines and anti-serotonin drugs. Since lipophilicity (Lien 1975; 1974), type of amines (Hansch and Lien 1968) and molecular size (Lien 1974; Lien 1976) have been shown to be important factors in drug uptake, distribution and drug action, it was felt worthwhile to apply multiple regression analysis to the data of Bennett and Snyder (1976) using 1-octanol/water partition coefficient as a measure of lipophilicity and molecular weight as a measure of the molecular size, and $n_H$ to represent different types of amines (Hansch and Lien 1968).

## METHODS

The biological data were converted to log $1/IC_{50}$ (moles/1) (Table 1). Whenever available the experimentally determined log $P_{(octanol,w)}$ values of the undissociated forms were used. When the log P values were not available, they were calculated from the log P of structurally related congeners and the $\pi$ values of the substituents

TABLE 1

Displacement of [$^3$H]-Serotonin and d-[$^3$H]LSD from Rat Cerebral Cortex Membranes by Various Drugs and Their Physicochemical Constants

| Compound | log $1/IC_{50}$ (moles/l) | | | | | | |
|---|---|---|---|---|---|---|---|
| | [$^3$H]5-HT | | d-[$^3$H]LSD | | | | |
| | obsd.[1] | calcd.[2] | obsd.[1] | calcd.[3] | log $P_{oct}$ | log M.W. | $n_H$ |
| **LSD Analogues** | | | | | | | |
| d-LSD | 8.00 | 7.41 | 8.10 | 7.44 | 2.95[4] | 2.51 | 1 |
| 2-Br-LSD | 7.00 | 6.69 | 8.00 | 7.59 | 3.81[5] | 2.60 | 1 |
| d-Isolysergic acid amide | 7.00 | 7.21 | 6.70 | 6.55 | 0.95[5] | 2.43 | 1 |
| Methysergide | 6.52 | 7.61 | 7.00 | 7.54 | 2.34[5] | 2.55 | 1 |
| **Tryptamines** | | | | | | | |
| 5-Hydroxytryptamine | 8.00 | 6.46 | 6.00 | 5.38 | 0.21[4] | 2.25 | 3 |
| 5-Methoxytryptamine | 7.30 | 7.09 | 6.00 | 5.96 | 0.81[5] | 2.28 | 3 |
| Tryptamine | 6.00 | 7.15 | 5.70 | 5.75 | 0.88[5] | 2.20 | 3 |
| 5-Hydroxy-N,N-dimethyltryptamine | 7.70 | 7.31 | 6.52 | 6.22 | 1.10[5] | 2.31 | 1 |
| 5,6-Dihydroxytryptamine | 6.22 | 5.62 | 5.15 | 4.89 | -0.37[5] | 2.28 | 3 |
| 5,7-Dihydroxytryptamine | 5.00 | 5.49 | 4.52 | 4.80 | -0.45[5] | 2.28 | 3 |
| N,N-Dimethyltryptamine | 5.70[6] | (7.61) | 5.70 | 6.45 | 1.78[5] | 2.27 | 1 |
| **Neurotransmitters and Analogues** | | | | | | | |
| Dopamine | 4.40 | 4.50 | 4.22 | 3.83 | -0.98[4,7] | 2.18 | 3 |
| l-Norepinephrine | 3.52 | 3.95 | 3.00 | 3.64 | -1.24[4,7] | 2.23 | 3 |

Table 1. (Continued)

| Compound | log $1/IC_{50}$ (moles) | | | | | | |
|---|---|---|---|---|---|---|---|
| | [$^3$H]5-HT | | d-[$^3$H]LSD | | | | |
| | obsd.[1] | calcd.[2] | obsd.[1] | calcd.[3] | log $P_{oct}$ | log M.W. | $n_H$ |
| Other Drugs | | | | | | | |
| Fluphenazine | 5.70 | 5.98 | 7.00 | 7.49 | 4.36[4] | 2.64 | 1 |
| Chlorpromazine | 4.30 | 4.18 | 7.00 | 6.36 | 5.35[4] | 2.50 | 1 |
| Promethazine | — | — | 6.00 | 6.65 | 4.73[4] | 2.45 | 1 |
| Haloperidol | — | — | 5.70[6] | 7.32 | 4.30[8] (4.20)[5] | 2.58 | 1 |

[1]From Reference 1.
[2]Calculated from Eq. 3 of Table 2.
[3]Calculated from Eq. 8 of Table 2.
[4]From A. Leo and C. Hansch, Computer Tabulation of Partition.Coefficients, Pomona College, 1975.
[5]Calculated value, see the section under Methods.
[6]Not included in the regression, outliers.
[7]Private communication from J. Schaeffer, Department of Chemistry, University of Missouri, Kansas City. These values are for the unprotonated forms.
[8]From P. Laduron, J Pharm Pharmac, 28: 250, 1976.

(Leo, Hansch and Elkin 1971; Hansch et al. 1973; Hansch 1971). For example: log P (2-Br-LSD) = log P (LSD) + $\pi_{Br}$ = 2.95 + 0.86 = 3.81. Log P (d-Isolysergic acid amide) = log P (LSD) - $2\pi_{C_2H_5}$ 2.95-2.00 = 0.95. log P (methysergide) = log P (d-lysergic acid amide + log P (butanol) + $\pi_{branching}$ + $\pi_{CH_3}$ = 0.95 + 0.88 - 0.20 + 0.71 = 2.34. log P (5-methoxytryptamine) = log P (5-HT - $\pi_{OH}$ + $\pi_{CH_3O}$ = 0.21 - (-0.67) + (-0.02) = 0.86.
log P (tryptamine) =- log P (5-HT) - $\pi_{OH}$ = 0.21 - (-0.67) = 0.88
log P (5-OH-N, N-dimethyltryptamine) = log P (5-HT) - $\pi_{CH_2NH_2}$ + $\pi_{CH_2N(CH_3)_2}$ = 0.21 + 0.15 - (-1.04) = 1.10.
log (5,6-dihydroxytryptamine) = log P (5-HT) + log P (catechol) - log P (phenol) = 0.21 + 0.80 - 1.46 = -0.45
log P (haloperidol) = 2 log P (benzene) + $\pi_F$ + $\pi_{COCH_3}$ + $\pi_{-CH_2CH_2-}$ + log P (HN⟨⟩) + log P (cyclohexanol) - log P (cyclohexane) + $\pi_{Cl}$ = 2 × 2.13 + 0.14 + 1.00 + (-0.55) + 0.85 + 1.23 - 3.44 + 0.71 = 4.20.

The logarithmic scale of the molecular weight was used in order to be in line with the biological data. The method of least squares was used in deriving the equations using an IBM 370/155 computer. The number of hydrogens of the protonated amino group is represented by $n_H$, i.e., $n_H$ = 3 for a primary amine and $n_H$ = 1 for a tertiary amine. Cyproheptadine, methiothepin, mianserine, psilocybin, and psilocin were not included in the regression analysis since no partition data were available for these molecules or their analogs. Also excluded from the analysis was [-]LSD, since a fair number of both enantiomorphs would be needed to allow a meaningful analysis of optical isomers (Lien 1976).

**RESULTS AND DISCUSSION**

The equations correlating the displacement data with the physicochemical paramters are summarized in Table 2. For the displacement of [$^3$H]-5=HT, a parabolic equation of log P gives a statistically highly significant correlating (Eq. 3, r = 0.873, s = 0.766, $F_{1, 11}$ = 34.05; $F_{1, 11.9995}$ = 23.6). About 76 percent ($r^2$ = 0.76) of the variance in the data can be "accounted for" by this equation.

TABLE 2

Equations Correlating the Displacement Data with the Physicochemical Constants

| | Displacement of [$^3$H]-5-HT | n | r | s |
|---|---|---|---|---|
| 1. | $\log 1/IC_{50} = 0.111 \log P + 6.033$ | 14 | 0.158 | 1.483 |
| 2. | $\log 1/IC_{50} = 1.831 \log M.W. + 1.843$ | 14 | 0.201 | 1.472 |
| 3. | $\log 1/IC_{50} = -0.330 (\log P)^2 + 1.391 \log P + 6.181$<br>$\log P_0 = 2.11$ (1.76-2.52) | 14 | 0.873 | 0.766 |
| 4. | $\log 1/IC_{50} = -0.335 (\log P)^2 + 1.483 \log P - 1.090 \log M.W. + 8.662$ | 14 | 0.875 | 0.797 |
| | **Displacement of d[$^3$H]LSD** | | | |
| 5. | $\log 1/IC_{50} = 0.487 \log P + 5.238$ | 16 | 0.752 | 0.922 |
| 6. | $\log 1/IC_{50} = 6.964 \log M.W. - 10.479$ | 16 | 0.779 | 0.877 |
| 7. | $\log 1/IC_{50} = -0.182 (\log P)^2 + 1.222 \log P + 5.265$<br>$\log P_0 = 3.36$ (2.77-4.83) | 16 | 0.906 | 0.613 |
| 8. | $\log 1/IC_{50} = -0.171 (\log P)^2 + 0.980 \log P + 3.283 \log M.W. - 2.206$<br>$\log P_0 = 2.87$ (2.08-4.08) | 16 | 0.929 | 0.559 |

Addition of the log M.W. term does not improve the correlation significantly (Eq. 4). The ideal lipophilic character log $P_o$ for maximum binding derived from Eq. 3 is 2.11 with a 95 percent confidence interval of 1.76 to 2.52 (see Figure 1).

For the displacement of d-[$^3$H]-LSD, log M.W. gives a slightly better correlation than log P (Eq. 6 vs. Eq. 5). A parabolic equation of Log P again gives a highly significant correlation (Eq. 7). About 82 percent ($r^2$ = 0.82) of the variance in the data is "explained" by this non-linear equation. An F-test indicates that the $(\log P)^2$ term is significant at 99.9 percentile level ($F_{1,13}$ = 20.05; $F_{1,12}$ = 18.7). Addition of the log M.W. to Eq. 7 yields Eq. 8, which is significant at a level between 90 and 95 percentile. ($F_{1,12}$ = 3.65; $F_{1,12.90}$ = 3.18). The log $P_0$ from Eq. 8 is 2.87 (2.08-4.08), while that from Eq. 7 is 3.36 (2.77-4.83), significantly highers than that of Eq. 3 for the displacement of d[$^3$H]LSD. Furthermore, (see Figures 2 and 1), indicating greater degree of hydrophobic interactions involved in the displacement of d[$^3$H]LSD. Furthermore, the dependence on log M.W. is positive in Eq. 8 while that in Eq. 4 is negative. This reflects that while large molecules like chlorpromazine and promethazine will be effective in displacing d[$^3$H]LSD from the binding site but not so effective in displacing [$^3$H]5-HT. This is also partly due to the difference in log $P_0$ values as well as the qualitatively different dependence on log M.W. Addition of nH term does not result in any significant improvement in correlation for both sets of data.

It is interesting to note that log $P_0$ values obtained are very much in line with the log $P_0$ values observed for the CNS activities of various drugs observed in vivo (Lien 1976; Hansch and Clayton 1973). Since the displacement of binding data were obtained from membrane preparations, the difference in log $P_0$ values reflects most likely the different hydrophobic characters of the binding sites of serotonin and LSD.

It is important to note that for unlabelled 5-hydroxytryptamine displacing [$^3$H]5-HT (see Table 1) the observed log $1/IC_{50}$ value is 1.54 units higher than the predicted value, while for the displacement of d[$^3$H]LSD the difference between the observed and the calculated value is only 0.62 unit. The 5-methoxytryptamine, on the other hand is well predicted for both binding sites. This is in agreement with the original qualitative conclusion of Bennett and Snyder concering the crucial role of the 5-OH group in the binding site. Similar activity enhancement by the OH group has also been observed in series of anticholinergic compounds (Lien, Ariëns and Bled 1976).

The important of the side chain amino group in binding with both sites is clearly indicated by the extremely low binding activities of

FIGURE 1

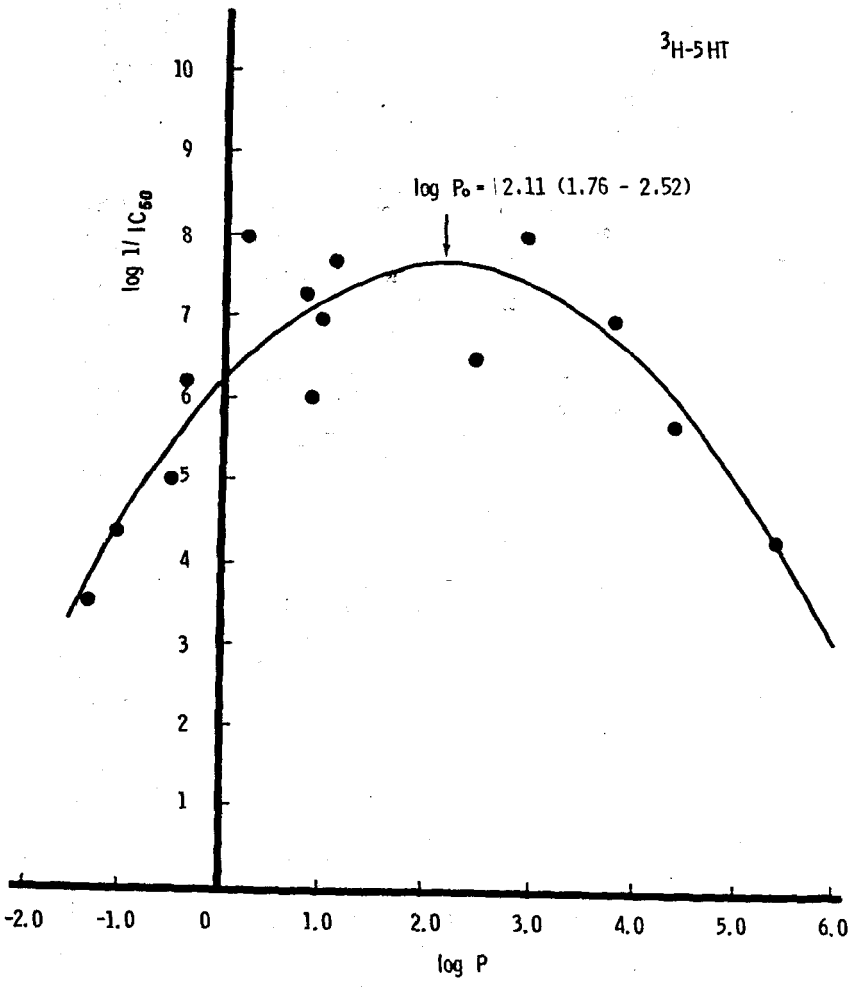
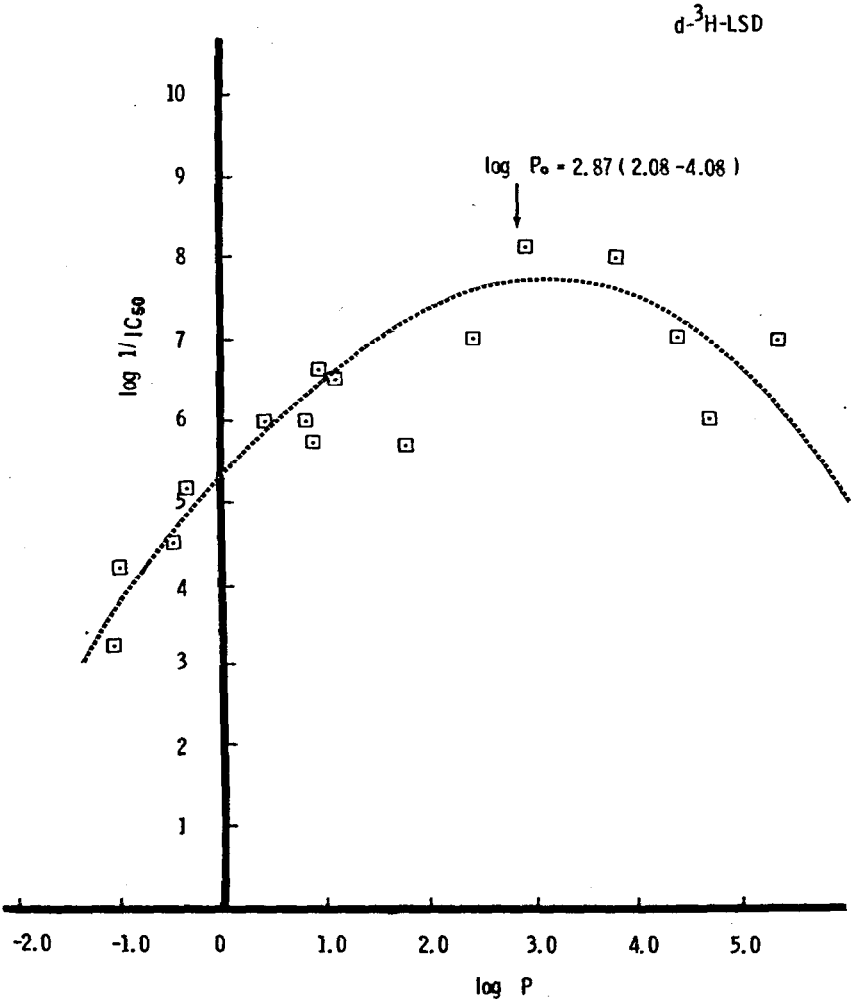

$^3$H-5HT
log $P_o$ = 2.11 (1.76 - 2.52)
log 1/ $IC_{50}$
10
9
8
7
6
5
4
3
2
1
-2.0
-1.0
0
1.0
2.0
3.0
4.0
5.0
6.0
log P

FIGURE 2


d-$^{3}$H-LSD
log $P_o$ = 2.87 ( 2.08 -4.08 )
log $1/IC_{50}$
10
9
8
7
6
5
4
3
2
1
-2.0
-1.0
0
1.0
2.0
3.0
4.0
5.0
log P

5-hydroxytryptophol and 5-hydroxyindole acetic acid ($IC_{50} \geq 10^5$ nm). It is conceivable that an anionic site is involved in the binding of the protonated form of the side chain amino group under physiological pH.

The positive dependence on the log M.W. and the higher log $P_0$ for the displacement d H LSD suggest a greater area of accessory binding site available for less specific type of intermolecular interactions (e.g., van der Waals forces)(Ariëns and Simonis 1967). It is quite conceivable that the two different conformations with the critical binding site (an anionic site) being surrounded by two different hydrophobic environments. The one for the agonist (e.g., 5-HT) then appears to be less hydrophobic than the one for the antagonist (e.g., methysergide, cyproheptadine, etc.). It is well known that the log $P_0$ for anticholinergics and antihistaminergics are also higher than that of the respective agonists (Lien, Ariëns and Beld 1976; van den Brink and Lien 1977). LSD having an intermediate lipophilicity can occupy both receptors and behave as agonist-antagonist.

Shih and Rho (1977) have shown that the serotonin-binding protein obtained from affinity chromatograph (Shih et al. 1974) caused a bathochromic shift in the fluorescence and the excitation wavelength of LDS, while bovine serum albumin did not cause such a change. This was intepreted an extensive delocalization of the LSD molecular orbital electrons when LSD was bound to the serotonin-binding protein.

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# Structure Activity Studies by Means of the SIMCA Pattern Recognition Methodology

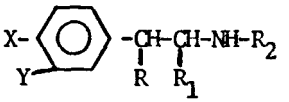
J. Dunn and Svante Wold

## INTRODUCTION

Since the publication of the linear models by Free and Wilson (Free and Wilson 1964) and Hansch (Hansch 1969) there have been many published examples of QuasAR using these methods. Such examples illustrate that biological-structure activity data can behave in a continuous manner and can give consistent results when analyzed by appropriate statistical methods. The method of Hansch can be very helpful in attempts to determine how subtle changes in structure can effect changes in the level of biological response of structurally similar substances.

This method has limitations, however, and cannot be used, for example, to classify compounds according to their type of biological response. For solutions to such problems one must look to methods of classification such as linear discriminant analysis and the various methods of pattern recognition (PaRC). In the past few years the use of such methods in the solution and development of QuaSAR has been reported (Martin et al. 1974; Ting et al. 1973; Kowalski and Bender 1974; Chu et al. 1975; Soltzberg and Wilkens 1977). Such methods also have limitations and their use in developing QuaSAR has been criticized (Perrin 1972; Mathews 1975).

In a recent report (Dunn, Wold and Martin 1977) we have described the use of the SIMCA method of PaRC in the classification of phenethylamine beta adrenergic agonists and antagonists of general structure (I).




(I)

In this report we describe the SIMCA method, briefly compare it to other methods of PaRC and illustrate its utility in classifying the above mentioned agonists and antagonists. The data analyzed are those reported by Lefkowitz (Lefkowitz et al. 1976).

**PHILOSOPHY OF THE USE OF PaRC IN THE DEVELOPMENT OF QuaSAR**

The basic assumption underlying the application of PaRC methods to the development of QuaSAR is that structurally similar substances can be described mathematically to be similar. If it is assumed that the type of pharmacological response that a compound elicits is a highly specific function of its structure and that if seemingly similar substances give different (opposite) responses, then mathematical rules based on the chemical structure of the two types (pharmacological) of compounds should be derivable which could be used to distinguish or classify these compounds. The use of PaRC, therefore, requires two considerations: 1) a description of the compounds by appropriate structural parameters and 2) a method which will derive rules based on these parameters that will distinguish one pharmacological type from the other.

The compounds of known pharmacological type, in this case, agonists and antagonists are placed in sets called training sets. From each training set the methodology detects patterns of similarity which exist between the compounds and derives the mathematical ruleswhichdefine eachclass. In a second step, compounds of unknown class assignment, the test set, are then compared with the rules of each class: They are assigned to the class which they are calculated to be similar to. From the calculation the probability of the prediction should also be obtained.

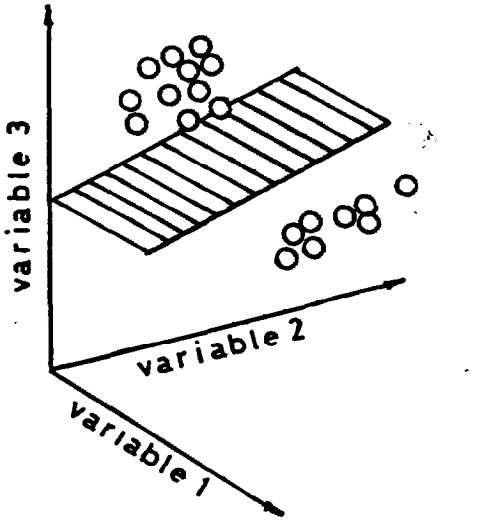
The description of the compounds by structural parameters is usually the most critical aspect of a PaRC study. If the compounds to be classified are not described as completely as possible the method chosen to generate the mathematical rules might not be able to distinguish the different classes. For use with the linear learning machine and linear discriminant methods it has been suggested that molecular descriptors derived from the structure, such as the presence or absence ofcertainfragments or functional groups be used (Brugger, Stuper and Jurs 1976). The generation of such descriptors from connection tables and their use in PaRC have been discussed (Brugger, Stuper and Jurs 1976).

For the description of each compound in this report, physicochemically based substituent parameters such as the Hansch p constant, the Hanmett s constant and the Verloop steric constants were used. These were chosen because it is assumed that agonist and antagonist activity result from specific drug receptor interactions and we believe that parameters derived from systems which model these interactions should be used as the basis of differentiation. Quantum mechanically based descriptors might also be useful since they, along with the other descriptors mentioned above, are theoretically derivable and their use precludes synthesizing compounds and making measurements of them. Measured physicochemical parameters such as pKa, log P, pKi, etc., can also be included as descriptors if available. This overall approach to compound description has the additional advantage of using variables which are derived from continuous functions.

The separation of classes of pharmacological substances on the basis of such descriptors is illustrated graphically in Figure 1. Here in

3-dimensional variable space two classes of compounds are well separated. Each compound is represented by a point (vector) in the 3-space.

**FIGURE 1**

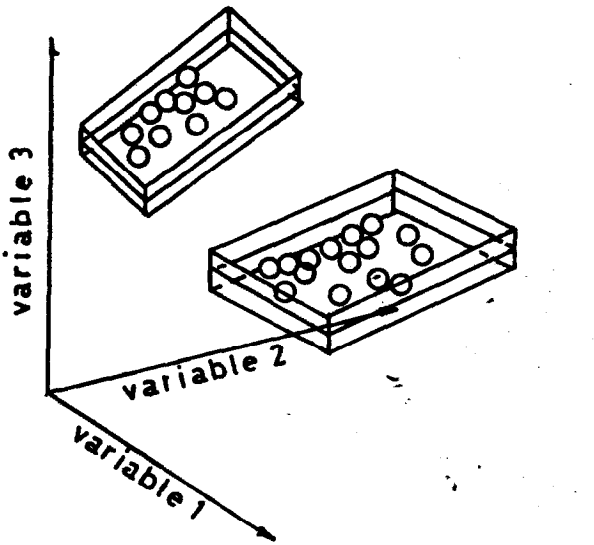



***Graphical representation of classification by a hyperplane***

This brings us to the second consideration in the use of PaRC methods and that is the selection of a method which can be used to separate the two or more classes in a study. The commonly used linear learning machine and linear discriminant analysis methods classify on the basis of a hyperplane which is inserted between the classes as shown in Figure 1. These methods are sensitive to the number of variables (N) relative to the number of compounds (M) since if N > M a separation plane will always exist. In addition if N approaches M the probability of finding such a plane increases which can lead to the observation of artifacts. This limitation, which also applies to multivariable regression methods, requires that M > N/3 where N is the initial number of variables in the analysis.

The SIMCA method, differently from the linear learning machine or linear discriminant analysis, classifies by deriving a classification function that contains each class in a closed mathematical structure, or 'hyperbox'. This is shown in Figure 2. A more detailed discussion of the method will be presented later. At this time a comparisonofthe two approaches will be presented in the context of different levels of classification which are possible in QuaSAR.

FIGURE 2




*Classification by "hyperboxes"*

**FORMULATION OF A PROBLEM AS ONE OF CLASSIFICATION**

Once it has been recognized that a problem can be treated as one of classification, there are different levels of classification which can be achieved (Dunn, Wold and Martin 1977). The levels are very much method dependent and the ambition of a classification study can determine the method of classification required. Four levels of classification can be recognized (I-N).

**Level I**

At this level the objective is to classify an unknown as a member of one class or another. All methods of data analysis can classify at this level.

**Level II**

At this level the objective is to classify an unknown as a member of one class or the other with the possibility of the unknown being a member of none of the defined classes. If the unknown is classified as being a member of neither class it is considered to be an outlier and may be a member of a third, as yet undescribed class. Since SIMCA confines the classes in closed mathematical structures it always works on at least level II.

**Level III**

At level III classification includes that of level II accompanied by the observation of a relationship between the position of the compounds in the class structure and the level of activity of the compounds. Such a relationship, if observed, would constitute a QuaSAR. Therefore, at level III one would not only be able to tell what type of activity an unknown will have but also one can predict the level of response expected from the unknown.

**Level IV**

On this, or highest, level sweral effect variables such as measures of side effects, may be available for objects in the classification study. One might find, for example, that toxicity of certain compounds could be related to their position in the class structure derived from classification of the agents as agonists of the beta receptor. The more toxic substances may be positioned differently from the more active substances and one could conclude that the processes have different structural requirements and specificities.

Since SIMCA encloses the classes in the aforementioned mathematical structures or 'hyperboxes' it can operate at the higher levels of classification. For the classification of agonists and antagonists of the beta adrenergic receptor we formulate the problem at level III in which a closed structure is defined for each class. After classification the position of each compound in each class is related to its activity as agonist or antagonist.

**DESCRIPTION OF SIMCA**

The basis for the separation of classes by SIMCA is that the descriptor variables for the compounds which compose each class can be described by a principal components (PC) model. This is based on the assumption that the function generating the variables can be Taylor expanded (Wold 1976; Wold and Sjöstrom 1977). The PC model is shown in equation 1. Referring to Figure 2 this corresponds

$$y_{ik}^{(q)} = m_i^{(q)} + \sum_{a=1}^{A} b_{ia}^{(q)} u_{ak}^{(q)} + e_{ik}^{(q)} \qquad 1)$$

to an A-dimensional plane of closest fit of the data points in the space chosen to describe the compounds. The indices i and k refer to variables and compounds, respectively, while y refers to a specific variable. a refers to the specific component in the PC model for the g-th class. $m_i$ is the mean value for variable i, b is a term which is model specific and u is a compound specific term. $e_{ik}$ are the residuals for the variables y. The compound specific term, u, is a measure of the pisition of the compound in the variable-space. For each class a standard dwiation can be calculated relative to the plane of closest fit. A similar standard deviation can be calculated for each object in each class. Fit to the class is determined by this parameter which is approximately F-distributed. If a compound has a standard deviation for its residuals which is

less than that for the class, it is a member of that class and it falls within the "hyperbox" for that class.

In classification studies a complete description as possible of the compounds in each class is desirable. Therefore, a large number of variables, some of which may be irrelevant, can result. A rationale must be devised by which the irrelevant variables can be eliminated from the class description as they can lead to "noise" in the analysis. SIMCA uses information from the residuals, $e_{ik}$, in assessing variable significance. Variables which contribute to the determination of class structure are said to have modelling power while those which contribute to class separation are said to have discrimination power. In SIMCA analyses variables with low modelling and low discrimination power are deleted. This has the advantage of selecting variables which optimize class description rather than those which optimize class separation.

As in most methods of PaRC, SIMCA can use variables which are regularized. In this study data are regularized by placing them on a scale such that each variable has zero mean and variance of unity. This prevents the masking of variables with little variation by those with large variation. Other variable transformations such as conversion to logs, etc., can be used.

**DATA SET OF BETA ADRENERGIC AGONISTS AND ANTAGONISTS**

The biological activities and descriptor variables for the compounds in this study are given in Tables I and II. The compounds are classified in the training sets according to that of Lefkowitz (Lefkowitz et al. 1976). The biological measurements were made on racemic mixtures with the affinities (binding constants) for the beta receptor determined by displacement of -($^3$H)-alprenalol from the receptor binding sites of partially purified frog erythrocytes. The agonist activity was determined as the ability to stimulate beta adrenergic coupled adenylate cyclase while antagonist activity was determined as the ability to inhibit agonist stimulation. The agonist used was isoproterenol.

The positions of variation in structure (I) were described by Rekker hydrophobic fragment constants (Nys and Rekker 1974), Es constants (Taft 1956) and Verloop steric constants (Verloop, Hoogenstraaten and Tipker 1976). Electronic effect of the amino group was characterized by σ- star (Taft 1956) and the estimated pKa (Clark and Perrin 1964). In addition the binding constant to the beta receptor was included as a variable for each compound.

**RESULTS**

Using the regularized data as previously discussed, SIMCA was applied to these data. Analysis with all variables included led to a PC model with 2 (A=2) components describing the agonists and one with 1 component describing the antagonists. On the basis of low modelling and low discrimination power the variables $\sigma_m$, $f_\phi$, $L_m$ and $B_m$ were deleted for the compounds in both classes. SIMCA application then led to a PC model with 3 components (A=3) describing each class.

TABLE I

| Compound | X | Y | R | $R_1$ | $R_2$ | Class | Activity[1] | pKb[1] | pKa[2] | $f_\phi$[3] | RSD[4] | $u_1$ | $u_2$ | $u_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 18 | 18 | OH | 2 | 1 | 2 | 4.39 | 4.55 | 8.93 | 1.14 | 0.18 | 3.54 | 1.22 | 0.45 |
| 2 | 18 | 18 | OH | 3 | 1 | 2 | 4.42 | 4.74 | 8.93 | 1.14 | 0.28 | 4.04 | 0.90 | -.79 |
| 3 | 18 | 18 | OH | 1 | 2 | 2 | 5.00 | 5.07 | 9.29 | 1.14 | 0.42 | 0.84 | 0.79 | 0.71 |
| 4 | 18 | 18 | OH | 1 | 4 | 2 | 5.85 | 5.77 | 9.90 | 1.14 | 0.40 | -.58 | 0.07 | 0.40 |
| 5 | 18 | 18 | OH | 3 | 4 | 2 | 4.35 | 4.62 | 9.90 | 1.14 | 0.24 | 1.03 | -1.49 | -1.53 |
| 6 | 18 | 18 | OH | 3 | 5 | 2 | 4.51 | 4.41 | 9.93 | 1.14 | 0.09 | 0.78 | -1.89 | -1.48 |
| 7 | 18 | 18 | OH | 1 | 6 | 2 | 6.33 | 6.17 | 9.19 | 1.14 | 0.31 | -1.29 | 0.63 | -.10 |
| 8 | 18 | 18 | H | 1 | 7 | 2 | 6.37 | 6.17 | 9.19 | 1.14 | 0.25 | -1.18 | 0.71 | -.10 |
| 9 | 18 | 18 | H | 1 | 8 | 2 | 4.68 | 4.33 | 10.03 | 1.14 | 0.20 | 0.23 | -.91 | 1.24 |
| 10 | 18 | 18 | OH | 1 | 9 | 2 | 5.04 | 4.62 | 10.29 | 1.14 | 0.19 | -1.05 | -1.60 | 0.89 |
| 11 | 18 | 18 | H | 1 | 10 | 2 | 7.10 | 7.22 | 9.29 | 1.14 | 0.20 | -2.32 | 1.01 | -.76 |
| 12 | 18 | 18 | OH | 1 | 11 | 2 | 5.04 | 4.64 | 10.22 | 1.14 | 0.33 | -.15 | -1.08 | 1.22 |
| 13 | 18 | 19 | OH | 1 | 4 | 2 | 6.00 | 5.62 | 9.94 | -.07 | 0.38 | -.51 | -.02 | 0.45 |
| 14 | 18 | 19 | OH | 1 | 4 | 2 | 5.48 | 6.19 | 9.77 | -.07 | 0.51 | -.25 | 0.67 | 0.37 |
| 15 | 18 | 19 | OH | 1 | 12 | 2 | 7.10 | 7.85 | 9.29 | -.07 | 0.39 | -3.14 | 0.98 | -.98 |
| 16 | 17 | 20 | OH | 1 | 1 | 1 | 3.51 | 4.08 | 8.93 | 2.66 | 0.21 | 3.65 | 0.66 | -1.31 |
| 17 | 17 | 19 | OH | 1 | 2 | 1 | 3.66 | 4.19 | 9.29 | 0.55 | 0.25 | 1.89 | 0.48 | 0.98 |
| 18 | 17 | 18 | OH | 1 | 2 | 1 | 3.87 | 4.28 | 9.29 | 1.36 | 0.23 | 1.87 | 0.45 | 0.98 |
| 19 | 17 | 18 | OH | 1 | 3 | 1 | 4.29 | 4.66 | 9.61 | 1.36 | 0.15 | 1.33 | 0.28 | 1.51 |
| 20 | 20 | 18 | OH | 1 | 4 | 1 | 5.89 | 5.38 | 9.90 | 2.04 | 0.50 | -1.31 | 1.43 | 0.82 |
| 21 | 18 | 17 | OH | 1 | 4 | 1 | 4.96 | 4.82 | 9.90 | 1.36 | 0.80 | -.59 | -.30 | 1.04 |
| 22 | 17 | 18 | OH | 2 | 1 | 1 | 4.52 | 4.46 | 8.93 | 1.36 | 0.58 | 3.11 | 0.70 | -1.19 |
| 23 | 20 | 20 | OH | 1 | 4 | 1 | 6.40 | 6.24 | 9.90 | 3.34 | 0.74 | -1.52 | 1.14 | 0.79 |
| 24 | 19 | 17 | OH | 1 | 4 | 1 | 5.80 | 5.89 | 9.90 | 0.55 | 0.89 | -3.59 | 1.46 | -.88 |
| 25 | 17 | 17 | OH | 16 | 1 | 1 | 3.85 | 4.29 | 8.46 | 1.90 | 0.39 | 3.66 | 0.73 | -1.12 |
| 26 | 17 | 17 | OH | 2 | 2 | 1 | 4.07 | 5.04 | 9.29 | 1.90 | 0.62 | 1.45 | 0.00 | 0.56 |
| 27 | 21 | 17 | OH | 3 | 4 | 1 | 5.35 | 4.85 | 9.90 | -.94 | 0.70 | -4.28 | 2.46 | -1.85 |
| 28 | 18 | 17 | OH | 2 | 8 | 1 | 5.74 | 5.06 | 9.03 | 1.36 | 0.50 | -.28 | -1.35 | -.64 |
| 29 | 18 | 17 | OH | 2 | 13 | 1 | 6.62 | 5.85 | 8.18 | 1.36 | 0.41 | -.73 | -2.59 | -.86 |
| 30 | 18 | 17 | OH | 2 | 14 | 1 | 6.89 | 6.74 | 9.29 | 1.36 | 0.50 | -1.73 | -2.57 | 0.24 |
| 31 | 18 | 22 | OH | 2 | 13 | 1 | 7.22 | 7.12 | 8.16 | 1.04 | 0.50 | -1.05 | -3.02 | -.95 |

(Table I continued)

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 32 | 17 | 23 | OH | 2 | 15 | 1 | 5.64 | 5.11 | 10.26 | 1.96 | 0.58 | -1.87 | 0.02 | 1.84 |
| 33 | 18 | 18 | OH | 1 | 1 | 0 | 4.04 | <3.70 | 8.93 | 1.14 | 0.33 | 3.35 | 1.12 | 1.86 |
| 34 | 18 | 17 | OH | 1 | 1 | 0 | <3.00 | <3.70 | 8.93 | 1.36 | 0.35 | 3.36 | 1.15 | 1.85 |
| 35 | 18 | 17 | H | 1 | 1 | 0 | <3.00 | <3.70 | 8.93 | 1.36 | 0.35 | 3.36 | 1.15 | 1.85 |
| 36 | 18 | 18 | H | 1 | 1 | 0 | <3.00 | <3.70 | 8.93 | 1.14 | 0.35 | 3.36 | 1.15 | 1.85 |
| 37 | 17 | 17 | H | 1 | 1 | 0 | ---- | <3.70 | 9.80 | 1.90 | 0.61 | 3.15 | 1.75 | 0.04 |

[1](Lefkowitz et al. 1976); [2](Clark and Perrin 1964); [3]( Nys and Rekker 1974); [4]RSD for object when fit to model for its class; RSD class 1 = 0.53, RSD class 2 = 0.31

TABLE II

Substituents and descriptor variables

| Substituent number | Formula | f | σ-star | Es-$R_2$ | $\sigma_p$ | $\sigma_m$ | L | B-4 |
|---|---|---|---|---|---|---|---|---|
| 1 | H | 0.19 | 0.49 | 1.24 | | | | |
| 2 | $CH_3$ | 0.70 | 0.00 | 0.00 | | | | |
| 3 | $C_2H_5$ | 1.23 | -.10 | -.07 | | | | |
| 4 | $CH(CH_3)_2$ | 1.64 | -.19 | -.47 | | | | |
| 5 | $CH(CH_2CH_2)_3$ | 2.35 | -.20 | -.51 | | | | |
| 6 | $CH(CH_3)CH_2C_6H_4$-4-OH | 2.83 | -.13 | -.93 | | | | |
| 7 | $CH(CH_3)CH_2C_6H_4$-3,4-$OCH_2O$- | 2.56 | -.13 | -.93 | | | | |
| 8 | $CH_2CH_2C_6H_4$-4-OH | 2.42 | -.08 | -.38 | | | | |
| 9 | $CH(CH_3)(CH_2)_2C_6H_4$-4-OH | 3.36 | -.13 | -.93 | | | | |
| 10 | $C(CH_3)_2CH_2C_6H_4$-4-OH | 2.43 | -.30 | -1.60 | | | | |
| 11 | $(CH_2)_3C_6H_4$-4-OH | 2.95 | -.08 | -.38 | | | | |
| 12 | $C(CH_3)_2CH_2C_6H_5$ | 3.80 | -.30 | -1.60 | | | | |
| 13 | $CH(CH_3)CH_2OC_6H_5$ | 2.77 | -.13 | -.93 | | | | |
| 14 | $CH(CH_3)(CH_2)_2C_6H_5$ | 3.90 | -.13 | -.93 | | | | |
| 15 | $C(CH_3)_3$ | 2.24 | -.30 | -1.60 | | | | |
| 16 | $CH_2CH_2OH$ | 0.02 | --- | --- | | | | |
| 17 | H | | | | 0.00 | 0.00 | 2.06 | 1.00 |
| 18 | OH | | | | -.37 | 0.12 | 2.74 | 1.93 |

(Table II continued)

| | | | | | |
|---|---|---|---|---|---|
| 19 | $NHSO_2CH_3$ | 0.03 | 0.20 | 4.06 | 3.08 |
| 20 | Cl | 0.23 | 0.37 | 3.52 | 1.80 |
| 21 | $CH_3SO_2NH_2$ | 0.28 | ---- | 5.05 | 3.58 |
| 22 | $CH_3SO_2N(CH_3)_2$ | ---- | 0.23 | 5.05 | 3.58 |
| 23 | $OCH_3$ | ---- | 0.12 | 3.98 | 2.87 |

TABLE III

$b_{ia}$ Values

| | pKb | $f_{R1}$ | $f_{R2}$ | σ-star | pKa | $E_{sR2}$ | $\sigma_p$ | L | B-4 |
|---|---|---|---|---|---|---|---|---|---|
| Class 1 | | | | | | | | | |
| $m_i$ | -.14 | 0.02 | -.20 | 0.07 | -.26 | 0.13 | 0.64 | -.14 | 0.00 |
| $b_{i1}$ | -.24 | -.16 | -.28 | 0.36 | -.25 | 0.33 | -.02 | -.51 | -.52 |
| $b_{i2}$ | -.33 | -.14 | -.42 | 0.08 | 0.40 | 0.25 | 0.58 | 0.14 | 0.30 |
| $b_{i3}$ | -.03 | -.28 | 0.06 | -.44 | 0.56 | -.35 | 0.05 | -.40 | -.34 |
| Class 2 | | | | | | | | | |
| $m_i$ | 0.15 | -.03 | 0.23 | -.08 | 0.29 | -.14 | -.73 | 0.16 | 0.00 |
| $b_{i1}$ | -.42 | 0.41 | -.44 | 0.45 | -.10 | 0.51 | 0.00 | 0.00 | 0.00 |
| $b_{i2}$ | 0.61 | -.31 | -.33 | 0.21 | -.60 | 0.16 | 0.00 | 0.00 | 0.00 |
| $b_{i3}$ | -.33 | -.84 | -.01 | 0.28 | 0.27 | 0.20 | 0.00 | 0.00 | 0.00 |

On the basis of these models, the compounds in the training sets were classified with 15/15 (100%) of the agonists being correct and 15/17 (88%) of the antagonists being correct. These results were validated by omitting every fourth object from the training set for each class and from the 3 component model calculated for each class, the deleted compounds were classified. This process was repeated until all compounds had been deleted one and only one time. The results of the validation was correct classification of 30/32 (94%) of the compounds in the classes. The results of the classification are given in Table II and the statistical parameters in Table III.

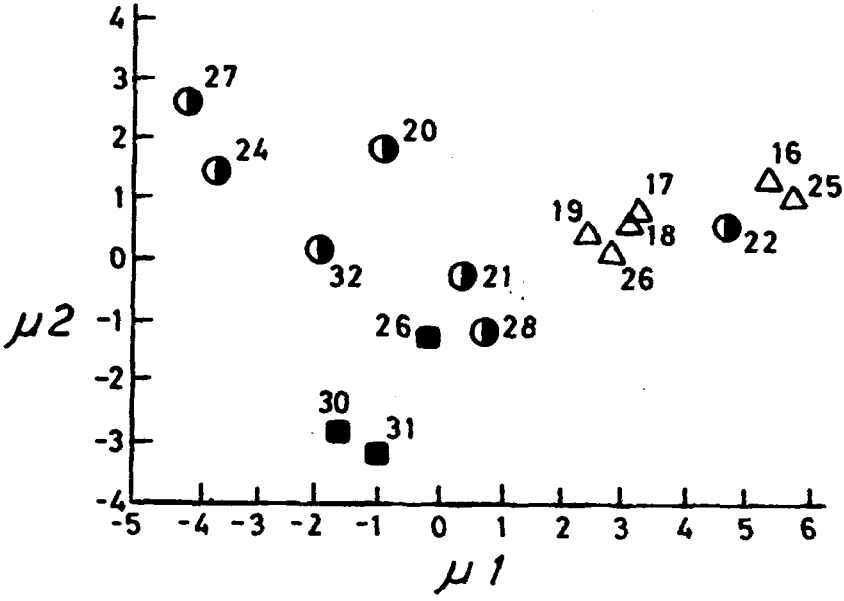
On the basis of the PC models objects 33-37 of the test set were classified. It can be seen in Table II that compound 33 (norepinephrine) is correctly classified as an agonist while compounds 34-36, tyramine, octopamine and dopamine were also classified as agonists. Dopamine is classified by Lefkowitz as an agonist even though its intrinsic activity could not be determined. Its affinity was determined from the assay for antagonists. Tyramine and octopamine were both classified as antagonists by Lefkowitz. Compound 37, phenethylamine, had no detectable activity in either assay; SIMCA classifies it as an antagonist. Classification, in itself, does not provide sufficient information for identification of these compounds.

**GRAPHICAL ANALYSIS OF THE $u_i$ PARAMETERS**

The $u_i$ values for the PC models which describe each class are measures of the position of the objects in the variable space for that class. It is reasonable to assume that those objects with similar levels of activity will cluster in the variable space as do the objects with similar types of activity. This is shown in Figures 3 and 4 in which $u_1$ vs $u_2$ is plotted for the classes 1 and 2, respectively. For class 1 the less active substances are in the region with positive $u_1$ and $u_2$ coordinates while the more active compounds have negative $u_1$ and positive $u_2$ coordinates. For the agonists (class 2) the less active analogs cluster with negative $u_1$ and $u_2$ coordinates A plot of $(u_1 + u_2)$ vs activity for antagonists is given in Figure 5 while Figure 6 shows a plot of $(u_1 - u_2)$ for the agonists. Inclusion of the test compounds shows that they are predicted to be weekly active.

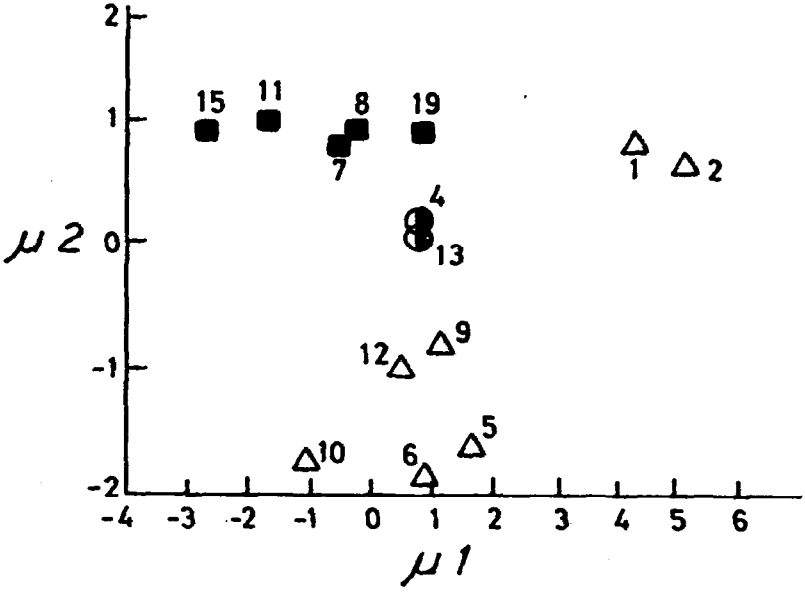
PaRC, as a predictive tool, should be trained using variables which are theoretical in the sense that they can be derived for a compound without the necessity for synthesizing and making measurements on it. The experimentally determined receptor binding constant was included in this study as a descriptor in the training sets. The relevant case would be that in which a hypothetical molecule is created in order that its type and level of activity could be predicted. In this circumstance, to test our PC models derived with the binding constant included the models were applied to the training sets with pKb set to zero. The classification that resulted was significant with 88% (two compounds from each class incorrect) classification. The result of a similar graphical analysis as discussed above are given in Figures 7 and 8 and it can be seen that the graphical analysis holds. The antagonists are well predicted while the agonists are not quite as well fit.

FIGURE 3



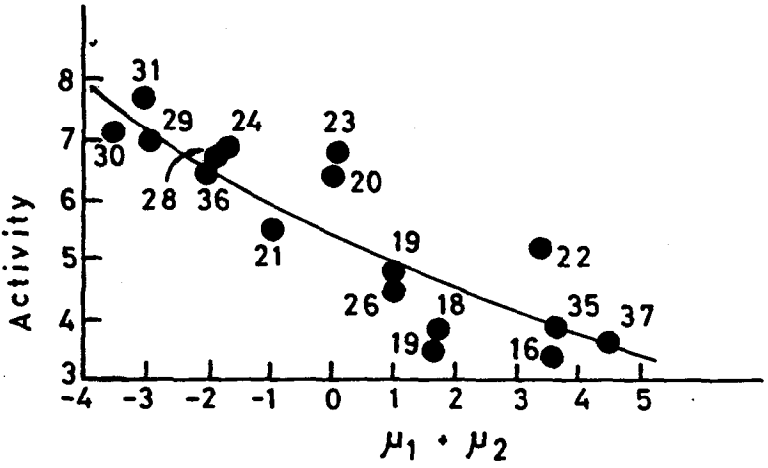

$u_i$ plot of class 1

FIGURE 4



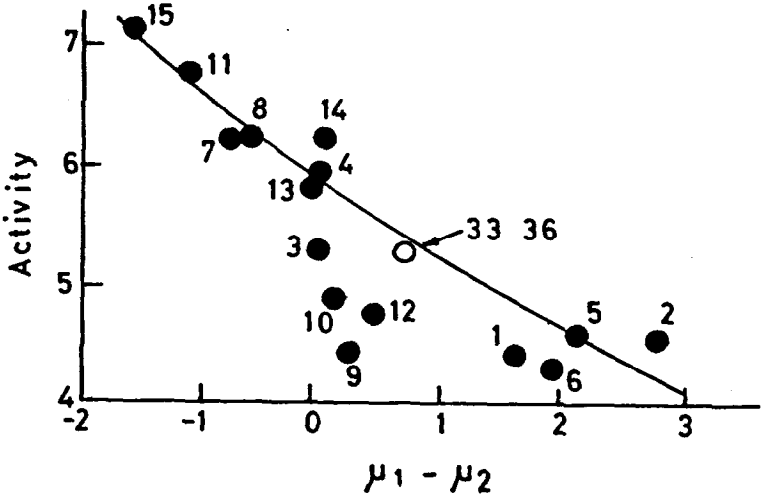

$u_i$ plot of class 2

FIGURE 5



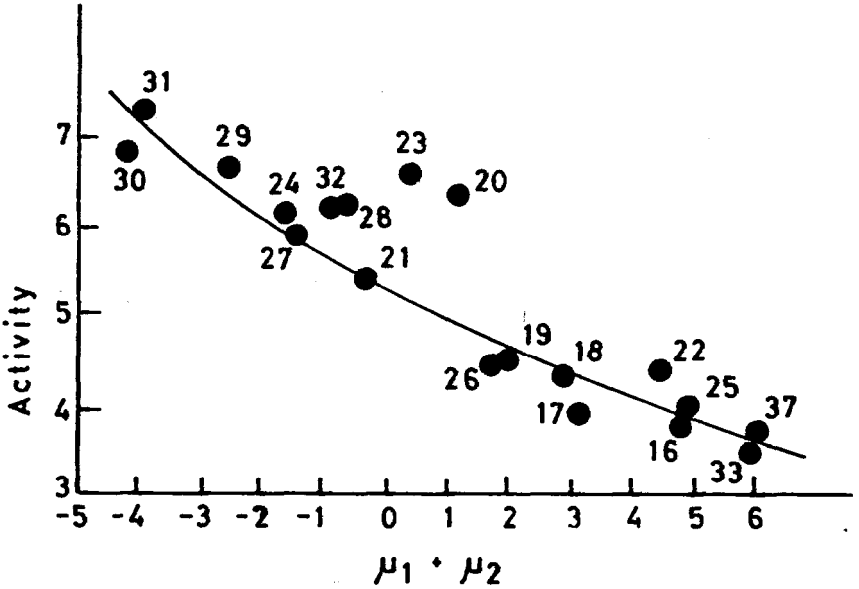

*Activity as a function of $u_i$ for class 1*

FIGURE 6



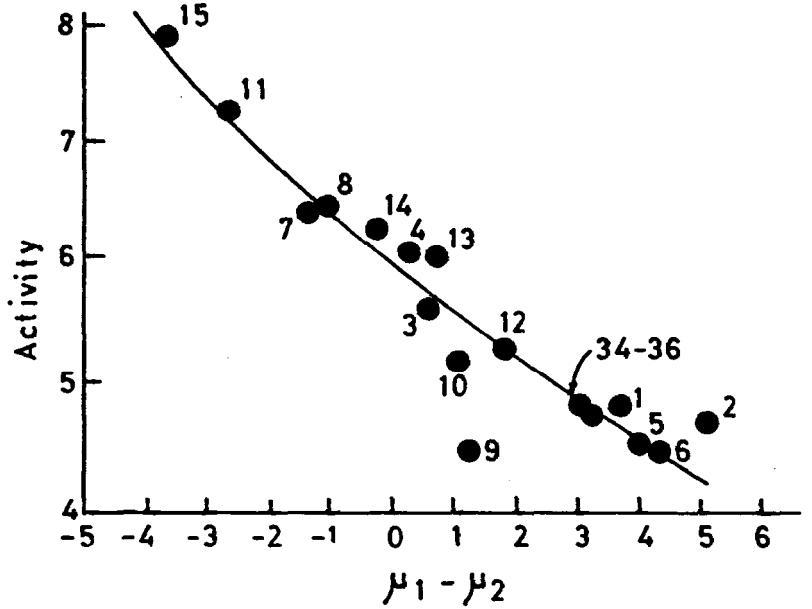

*Activity as a function of $u_i$ for class 2*

FIGURE 7




*Prediction of class 1 activities on the basis of theoretical variables*

FIGURE 8




*Prediction of class 2 activities on the basis of theoretical variables*

The identification of the test objects can be better understood in terms of the graphical analysis. Their classification was based on pKb values set as upper limits of 3.70 since they could not be experimentally determined to be above this value. Their positions in their predicted class are shown in the Figures 5 and 6 and it can be seen that they are estimated to have very low activities.

SIMCA as shown by this graphical analysis can lead to significant information further than that of classification. The objective of some PaRC studies has been classification at level I in our proposed scheme. Such studies may sometimes be trivial to the trained pharmacologist. Level II and III studies are usually less trivial. In this first application of SIMCA to study the QuaSAR of biologically significant compounds, we are pleased with its performance and its predictive ability, not only of pharmacological type but of the level of activity for each type.

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# Quantitative Relationship Between Antinociceptive Activity and Opiate Receptor Affinity: The Importance of Lipophilicity

Arthur E. Jacobson

Medicinal chemists have synthesized a multitude of opioids over the past century and a half. The term "opioids" has been defined as substances which have morphine-like pharmacological effects. I will use "opioids" and "narcotic agonists" as interchangeable terms. There are, then, a considerable number of these compounds of diverse structural type, all of which have at least one pharmacological property in common. The number of compounds, and the defined biological effect which can be easily measured in small animals, should lead theoretically inclined medicinal chemists to the opioids in their search for the relationships between molecular structures and a biological property. Unfortunately, apparently the very diversity of these molecules, and their rather peculiar pharmacologic effects, have militated against successful QuaSAR.

## OPIATE RECEPTORS

The discovery of the opiate receptor in verterbrate brain has given us a tool with which we can measure receptor mediated events. These receptors may be there to interact with endogenous ligands, the enkephalins (Hughes et al. 1975) and endorphins (Ross et al. 1977). The endogenous ligands are small, or large, peptides, and presumably the opioids mimic at least one of their pharmacological effects, that of analgesic action. The opioids, on interaction with the opiate receptor, can produce several effects in vivo. Almost all opioids have been shown (Kosterlitz, Waterfield, and Berthoud 1973) to have some antagonist effect, as well as narcotic agonist or opioid effect. They also have "side effects" in vivo, such as tolerance, dependence of the opiate type, some nausea, cough suppressant effect, respiratory depressant effect, etc. The "side effects"

and the narcotic antagonist activities, may be mediated by the same receptor.

**Multiplicity of Receptors**

There has been discussion in the literature on the possibility of a number of receptors with which the opioids and their antagonists can interact. Several scientists (Martin et al. 1976; Lord et al. 1977) believe that there are mu receptors for morphine types of opioids, sigma receptors for agonist antagonist types like N-allylnormetazocine, kappa receptors for ketocyclazocine-like compounds and, perhaps, other types of receptors as well. It should be noted that Martin's work is-based on symptomatology from in vivo dog work, and Kosterlitz (Lord et al. 1977) has compared the rank order of binding affinity of a series of compounds in the mouse vas deferens and in the guinea pig ileum. Our recent work on a few "nonclassical" narcotic antagonists (Jacobson and Klee 1978) may have some bearing on the concept of multiple receptors. I will return to that subject at the end of the paper. It is important, howerer, to maintain the idea that we have the possibility of multiple receptors, and that opioids tend to have more than one effect on interaction with the receptor or receptors, if we are going to speak of the relationship of molecular structure to biological action. My topic is necessarily limited to the relationship of receptor binding to antinociceptive action, since I have not succeeded in relating structure to biological effect quantitatively.

**Receptor Interaction And Biological Activity**

The main point which I should like to make is that the assumption of parallelism between receptor interaction and biological activity can be misleading. I do not believe that the measurement of receptor binding affinities always relates directly to the biological effect of the opioids in vivo. This is a very simple point, but one which I believe has been unfortunately ignored by many pharmacologists, and perhaps by some medicinal chemists. In order to relate receptor binding to biological activity, a series of compounds should be chosen which have mostly the same pharmacological activities. The type of pharmacological effect that will occur when a drug interacts with the opiate receptor cannot be presumed a priori, but must be ascertained from in vivo experiments.

The compounds which I will discuss have, overuhelmingly, antinociceptive effects in vivo, in the Eddy hot plate test (Eddy and Leimbach 1953; Jacobson and Nay 1965). Most of these compounds have been examined in the rhesus

monkey, under the aegis of the Committee on Problems of Drug Dependence (CPDD), Inc., and have been found to have little or no narcotic antagonist activity. That is, they do not appear to exacerbate the opiate dependence syndrome in single dose suppression studies in the Rhesus monkey, or precipitate abstinence in nonwithdrawn monkey studies. They do have "side effects", which I shall ignore, and they all presumably interact with the classical mu receptor. Thus, we will discuss compounds which have a measurable antinociceptive effect (in one laboratory, by the same technician), and which, hopefully, interact with mainly one type of receptor.

## QUALITATIVE RELATIONSHIP OF BINDING AND ACTIVITY

The initial attempts to relate receptor binding to antinociceptive activity were done by pharmacologists who were merely interested in showing that the opiate receptor interacted stereospecifically with opioids and their antagonists. These were qualitative relationships established to indicate that there actually were receptors for the opioids in vertebrates.

## LINEAR DEPENDENCE IN HOMOLOGOUS MOLECULES

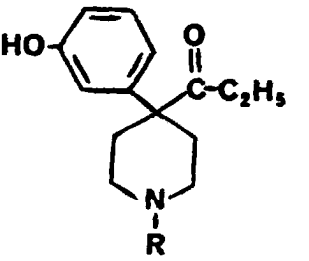
The first quantitative relationship which was published, that I am aware of, was from my laboratory. This was the work of Yilson et al. (1975). They determined the antinociceptive activity of a series of homologous ketobemidones and related that activity to the receptor binding affinity. I have replotted their data, in figure 1, so that the log of the activity, in nM/kg, can be seen to be directly related to the binding constant. The binding constant is the concentration of drug, in nM/L, which is required to inhibit stereospecific binding of (3H) dihydromorphine, or (3H) naloxone, or (3H) naltrexone, etc., in 1.0 nM concentration, by 50 percent, to the opiate receptors in rat brain homogenates. They obtained a correlation coefficient of 0.95, and 90 percent of the variance was "explained" by this direct relationship, in the absence of sodium.

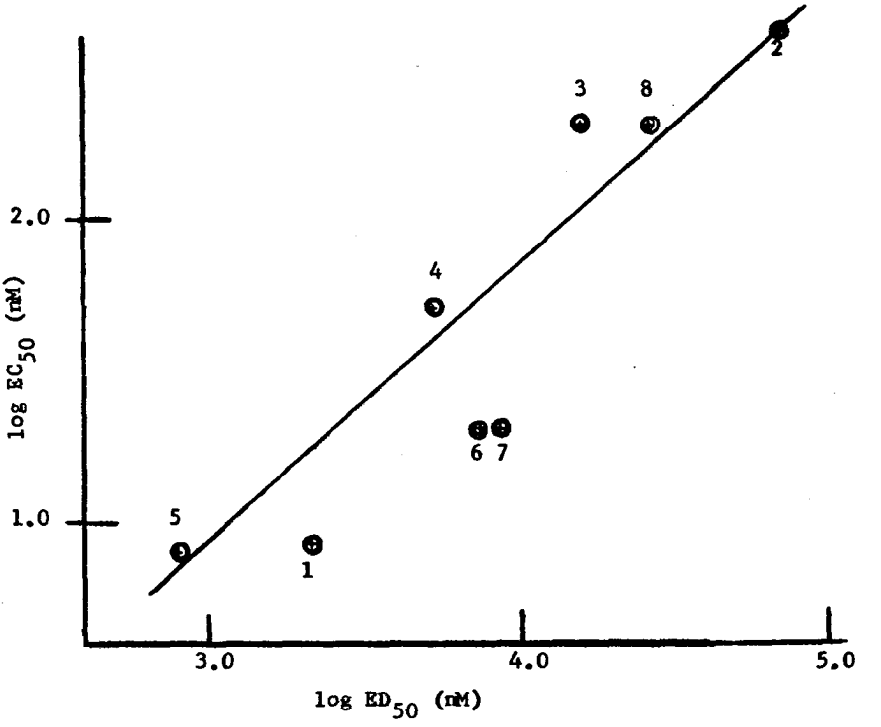
### Effect of Sodium on Binding

A reasonable correlation in this, and in other work (Stahl et al. 1977), could not be obtained in the presence of added sodium. Sodium salts have been noted to effect the binding affinity of antagonists preferentially. They had an inhibiting effect on narcotic agonists (Pert, Pasternak, and Snyder 1973: Simon 1975). The suggestion was made that this is due to a conformational change in the receptor. If the NaCl to no NaCl (+Na/-Na) ratio is indicative of antagonist

FIGURE 1

| R | $ED_{50}$ [a,b] | $EC_{50}$ [b,c] |
|---|---|---|
| Methyl (1) | 3.32 | 0.93 |
| Ethyl (2) | 4.83 | 2.60 |
| Propyl (3) | 4.20 | 2.30 |
| Butyl (4) | 3.66 | 1.70 |
| Amyl (5) | 2.90 | 0.90 |
| Hexyl (6) | 3.88 | 1.30 |
| Heptyl (7) | 3.95 | 1.30 |
| Octyl (8) | 4.42 | 2.30 |




[a] From Wilson et al. 1975; [b] log units, in nM; [c] under "no sodium" binding conditions.

Relationship Between Antinociceptive Activity and Opiate Recptor Affinity for N-Alkylnorketobemidones

activity, as it has been said to be, then in the presence of NaCl some of the ketobemidones should have a small +Na/-Na ratio. That is, a few of the several ketobemidones tested in rhesus Monkeys by the CPDD showed antagonist activity, or, at least, caused behavioral signs in the monkey which might be interpreted as a physical effect of a narcotic antagonist.

The ratio did change for some of these compounds, since some affinities decreased less than others, and none showed increased binding affinity in the presence of NaCl. The +Na/-Na ratios predicted that the hexyl and higher homologs would be antagonists. Of these, only the hexyl and heptyl homologs mere shown to have antagonist activity in the monkey, and the N-amyl compound was noted to have "atypical" antagonist activity (Swain, Villarreal, and Seevers 1973). However, the N-amyl compound could not be shown to have antagonist activity by rat infusion techniques (Dewey and Harris 1977). The N-hexyl and heptyl compounds are presently being examined for antagonist activity by rat infusion. These new binding affinities, in the presence of NaCl, apparently aid not reflect antinociceptive activity in vivo. The correlation was excellent only in the absence of NaCl.

**Hot Plate Test for Antinociception**

It was noted some time ago that the hot plate test for antinociceptive activity fails with agonist antagonist molecules. That is, the antinociceptive activity of agonist antagonists will generally be underestimated by that test, However, the hot plate assay, evidently, did not underestimate the antinociceptive activity of these ketobemidones, since the correlation with binding in the absence of sodium was successful. This suggests that the ketobemidones do not behave as agonist antagonists in the hot plate test, or had very little antagonist activity compared with their antinociceptive activity, in partial disagreement with the results from monkeys and with the +Na/-Na binding ratio.

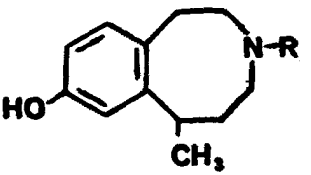
**Further Quantitative Relationships**

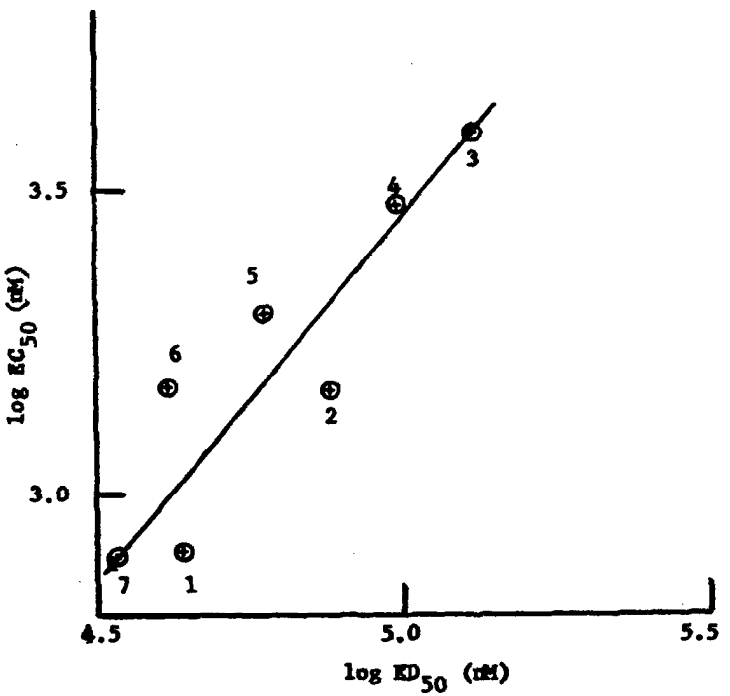
In that same year in our laboratory, Rogers, Ong, and Nay (1975) found a quantitative correlation between receptor binding affinity and antinociceptive activity in a set of homologous N-alkyl-3-benzazocines. A replot of their data is shown in figure 2. The correlation coefficient obtained was 0.95, the proportion of variance "explained" by the regression was 89 percent.

The third example of quantitation is from a series of

FIGURE 2

| R | $ED_{50}$ [a,b] | $EC_{50}$ [b] |
|---|---|---|
| Methyl (1) | 4.64 | 2.90 |
| Ethyl (2) | 4.88 | 3.18 |
| Propyl (3) | 5.11 | 3.60 |
| Butyl (4) | 4.99 | 3.48 |
| Amyl (5) | 4.77 | 3.30 |
| Hexyl (6) | 4.62 | 3.18 |
| Heptyl (7) | 4.53 | 2.90 |




[a] From Rogers et al. 1975; [b] log units, in nM.

Relationship Between Antinociceptive Activity and Opiate Recptor Affinity for N-Alkylnorbenzazocines

diastereoisomeric prodine analogs by Iorio and Klee (1977): Iorio prepared the series of prodine analogs shown in figure 3. Their correlation coefficient was 0.99. About 98 percent of the variance was rationalized by the direct correlation of antinociceptive activity obtained from hot plate assay, and the receptor binding from rat brain homogenate data. The data was transformed into log units for consistency with the other sets.

**CORRELATION IN STRUCTURALLY DIVERSE ANALGESICS**

These three examples of quantitative correlation between activity and binding affinity, in three different subsets of compounds, certainly appear to indicate that the antinociceptive activity of opioids is directly related to their binding to the opiate receptor, presumably of the mu type. We thought, however, that before the concept became too firmly established with medicinal chemists, as it had become with pharmacologists before any quantitative work had been completed, we should examine the question of whether the relationship would be valid over a much wider range of structurally different opioids (Jacobson, Klee, and Dunn 1977).

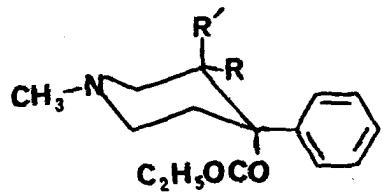
lie chose compounds from'the endoethenooripavine, propionamide, methadone, pethidine, morphinan, and morphine families of opioids, which had previously had their partition coefficients determined by Kutter et al. (1970). The molecular structures of the ten compounds are shown in figure 4. The data for the regression analyses, and the experimental and calculated antinociceptive activities are shown in table 1. A direct relationship between antinociceptive activity determined by hot plate assay, and receptor binding affinity did not exist among these heterogenous analgesics.

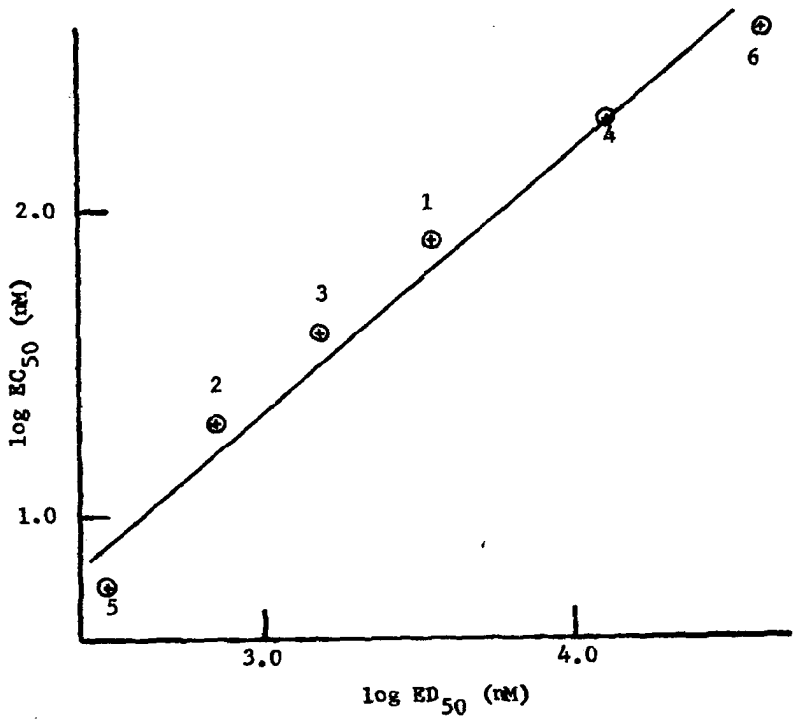
**Importance of Lipophilicity**

The equations determined by the regression are shown in table 1. Only 41 percent of the variance could be accounted for by a direct correlation tetween binding and activity. A direct relationship also did not exist between antinociceptive activity and lipophilicity (equation 2, table 1), nor does a correlation exist between receptor binding and lipophilicity. In the latter case, the correlation coefficient uas 0.26. In the correlation between antinociceptive activity and binding affinity, the explained variance was increased to 82 percent, with a correlation coefficient of 0.91, when the partition coefficient (log P) was included in

FIGURE 3

| | R | R′ | $ED_{50}$ [a,b] | $EC_{50}$ [b] |
|---|---|---|---|---|
| (1) | Methyl | H | 3.54 | 1.90 |
| (2) | H | Methyl | 2.84 | 1.30 |
| (3) | Ethyl | H | 3.18 | 1.60 |
| (4) | H | Ethyl | 4.11 | 2.30 |
| (5) | Allyl | H | 2.49 | 0.78 |
| (6) | H | Allyl | 4.61 | 2.60 |
| (7) | H [c] | Hexyl [c] | 2.21 | 2.0 |

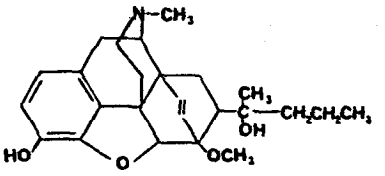


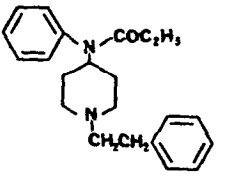

[a] From Iorio and Klee 1977; [b] log units, in nM; [c] eliminated from correlation due to poor dose response relationship (95% SE limits = 1.72-2.84).

Relationship Between Antinociceptive Activity and Opiate Recptor Affinity for Prodine Related Compounds

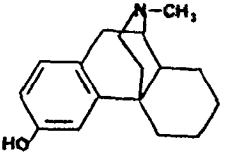
FIGURE 4



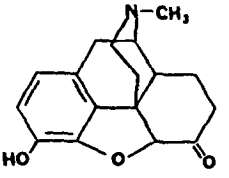

ETORPHINE



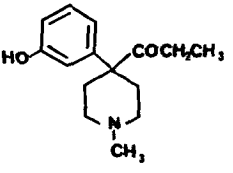

FENTANYL



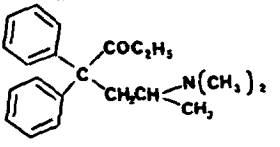

LEVORPHANOL



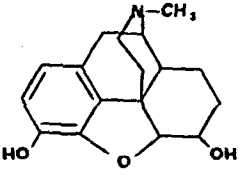

HYDROMORPHONE



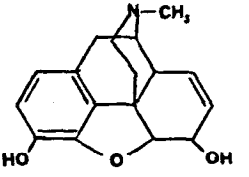

KETOBEMIDONE



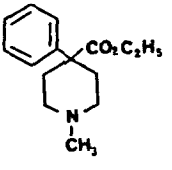

METHADONE



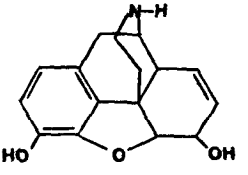

DIHYDROMORPHINE




MORPHINE




PETHIDINE




NORMORPHINE

**Molecular Structures For Regression Analyses**

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## TABLE 1

Data and Equations From Regression Analyses

| Compound | $EC_{50}$ [a] | Log P [b] | $ED_{50}$ [c] | Log 1/B [d] | Log 1/C [e] Obs. | Log 1/C [e] Calcd. [f] |
|---|---|---|---|---|---|---|
| Etorphine | 0.3 | 0.15 | 0.00096 | 0.52 | 5.67 | 4.89 |
| Fentanyl | 1.0 | 1.29 | 0.015 | 0.0 | 4.55 | 4.66 |
| Levorphanol | 0.7 | -2.04 | 0.19 | 0.15 | 3.33 | 3.77 |
| Hydromorphone | 1.0 | -4.00 | 0.17 | 0.0 | 3.28 | 2.99 |
| Ketobemidone | 2.0 | -3.06 | 0.60 | -0.30 | 2.67 | 2.95 |
| Methadone | 20.0 | 1.65 | 0.80 | -1.30 | 2.64 | 3.34 |
| Dihydromorphine | 3.0 | -5.00 | 0.89 | -0.48 | 2.56 | 2.14 |
| Morphine | 3.0 | -5.00 | 1.17 | -0.48 | 2.44 | 2.14 |
| Pethidine | 700.0 | 0.53 | 4.69 | -2.85 | 1.78 | 1.27 |
| Normorphine | 4.0 | -5.00 | 19.2 | -0.60 | 1.21 | 2.00 |

| # | Equation | $R$ [g] | $R^2$ [h] | $s$ [i] | $N$ [j] |
|---|---|---|---|---|---|
| 1 | $\log 1/C = 3.478\ (\pm 0.897) + 0.870\ (\pm 0.855) \log 1/B$ | 0.64 | 0.41 | 1.06 | 10 |
| 2 | $\log 1/C = 3.457\ (\pm 1.138) + 0.209\ (\pm 0.344) \log P$ | 0.46 | 0.21 | 1.22 | 10 |
| 3 | $\log 1/C = 4.254\ (\pm 0.699) + 1.107\ (\pm 0.528) \log 1/B + 0.317\ (\pm 0.184) \log P$ | 0.91 | 0.82 | 0.62 | 10 |
| 4 | $\log 1/C = 4.313\ (\pm 0.842) + 1.079\ (\pm 0.605) \log 1/B + 0.231\ (\pm 0.542) \log P - 0.023\ (\pm 0.134\ (\log P)^2$ | 0.91 | 0.83 | 0.66 | 10 |

Table 1 continued

[a] Receptor binding affinity in nM; [b] partition coefficient determined by Kutter et al. 1970; [c] effective dose level in mg/kg at which antinociception is obtained in 50% of the mice, as obtained by probit analysis; [d] B = $EC_{50}$ in nM; [e] C = $ED_{50}$ in nM; [f] calculated from equation 3; [g] correlation coefficient, for a perfect correlation R = 1; from Jacobson, Klee, and Dunn 1977; [h] proportion of variance "explained" by the regression; [i] standard deviation; [j] number of compounds used in the regression.



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the regression (equation 3, table 1). The use of a Squared log P term did not improve the correlation (equation 4, table 1). We concluded from these data that when a number of different types of opioids are examined it is necessary to account for their differential ability to reach the receptors in the brain. This transport phenomenon can, apparently, be reasonably simulated by partition coefficients.

**Possible Contradiction**

In contrast to this work, if not in apparent contradiction to it, is a paper by Stahl et al. (1977). They measured antinociceptive activity through intravenous application in a tail withdrawal reflex assay of a number of disparate analgesics (including etotphine, levorphanol, fentanyl, morphine and a number of neuroleptics and antidiarrheal agents). They obtained a good correlation, directly, with the binding affinities for the compounds in rat brain homogenates, without sodium. The correlation coefficient which they reported was 0.907, with 82% of the variance explained by the correlation. In the presence of sodium, the correlation was not as good, which would be in agreement with the data from the ketobemidones.

If the data from Stahl et al. (1977) are closely examined for four of the compounds which have had their partition coefficients determined by Kutter et al. (1970), morphine, etorphine, fentanyl and levorphanol, it can be seen that incorporation of partition coefficients might be advantageous. In the paper of Stahl, these four compounds were not predicted to be in their correct order for antinociceptive potency when compared with their binding affinities. For a quick estimation of the effect of the incorporation of partition coefficients into their data, the coefficients shown in equation 3, table 1 were utilized, since it was not possible to determine new coefficients based on only four data points. The effect on the rank order of antinociceptive potencies is shown in table 2. When partition coefficients are included, the correlation is improved. The calculated and observed activities agree nicely, if fortuitously, by the incorporation of a constant. With these four compounds, in any case, the antinociceptive activity did not parallel receptor binding, unless partition coefficients were included, in agreement with my data. Overall, with their large number of compounds, the statistics proved to be acceptable. It is possible, however, that if the partition coefficients of the compounds were determined and incorporated in the regression, a greater proportion of the variance would be accounted for.

Thus, although a direct correlation between binding affinity and in vivo opioid activity exists within limited homologous series of opioids, the inclusion of partition coefficients is advantageous, and sometimes necessary, to achieve reasonable correlations among opioids with radically different structures. It is likely that if we knew more about the metabolism of the various types of opioids, and could incorporate these data in the regression, we would obtain a further increase in "explained" variance.

Table 2. Relationship Betweeen Antinociceptive Activity And Receptor Binding Affinity After Incorporation of Partition Coefficients

| Compound | Log 1/IC50[a] | Log 1/ED50[a] | |
|---|---|---|---|
| | | Obs. | Calcd.[b,c] |
| Etorphine | 0.40 | 2.42 | 2.49 |
| Pentanyl | -1.40 | 1.48 | 0.86 |
| Levorphanol | -0.60 | 0.07 | 0.69 |
| Morphine | -1.43 | -0.92 | -1.17 |

[a] Original data from Stahl et al. 1977, transcribed into log units; [b] from equation 3 in table 1; [c] includes a constant (+2.0), added to obtain correspondence with observed data.

**BIOLOGICAL TESTS FOR NARCOTIC ANTAGONISTS**

The work which has been examined merely relates biological activity to receptor binding. Although we have shown that partition coefficients should not be neglected when a disparate group of opioids are examined, the molecular structures of these opioids have not been quantitatively related to either binding affinity or to antinociceptive activity. The relationship of the molecular structure of agonist antagonists to in vivo antagonist biological activity may also prove to be difficult. Although there are

several assay systems which can quantitatively relate in vivo antinociceptive activity of opioids, and these are generally relatable to each other, there are relatively few assay systems available for determining the antagonist activity of agonist antagonists.

**Assay Systems**

Ue obtain qualitative antagonist activities from studies in monkeys, through the Committee on Problems of Drug Dependence, Inc. Also, Aceto et al. (1977) have reported their quantitative results from a tail flick antagonist assay vs. morphine to the Committee on Problems of Drug Dependence, Inc. In a rapid review of the last three years, I have found at least twenty very different antagonists for which they have determined antagonist activity. It should be noted that a number of these have standard error limits an order of magnitude greater than we normally obtain in the hot plate assay for antinociceptive activity, and that some compounds which show antagonist activity in the monkey do not show antagonist activity in the tail flick assay. I do not know which is more dependable, when compared with the effect of the compound in man. Unfortunately, few partition coefficients, or other physicochemical data, and little binding affinity data are knoun for most of these compounds,

Another set of compounds, some of which overlap those obtained by Aceto et al. (1977), have been examined by Kosterlitz, Waterfield, and Berthould (1973) in the guinea pig ileum assay and in the mouse vas deferens. These two sets of data are the most comprehensive available in the literature. It would be of interest, as more compounds are added to both sets, to see whether these quite different assays correlate well with each other.

**Atypical Narcotic Antagonists**

We have noted that odd results can be obtained from the guinea pig ileum and the mouse vas deferens assays with a new type of antagonist. We have prepared opioid antagonists based on substitution in the 9-alpha position of the benzomorphans, rather than on the nitrogen atom (Rice et al. 1977). Classical, potent, opioid antagonists had been prepared by replacing the N-methyl group of potent, structurally rigid, opioids by certain, moieties.

our non-classical type of opioid antagonist appears to interact with the receptors in the ileum, and even more so in the mouse vas deferens, to give above normal increases in the amplitude of the twitch after naloxone

treatment (Jacobson and Klee 1978), unlike the classical antagonists which do not produce this supranormal increase. The experiments were run by first obtaining a decrease in twitch tension by normorphine (10-7 M), followed by reversal (but not quite back to baseline levels) with the non-classical antagonist (10-6 to 10-7 M). Introduction of naloxone (10-7 M) at that point resulted in a supranormal increase in twitch tension.

These experiments can be interpreted as an interaction of these "non-classical" antagonists with receptors other than the mu receptor or, more simply, by assuming that these antagonists have stimulant activity as well as opioid antagonist activity. We may be seeing a complex mixture of biological actions which would not be easy to dissociate, rendering QuaSAR with these compounds much more difficult. Thus, QuaSAR with structurally disparate antagonists should be critically examined when biological activities are obtained from ileum or vas deferens measurements. Some of the binding which is measured may not be due to the antagonist activity of the molecule.

**QuaSAR IN THE FUTURE**

If QuaSAR with disparate opioids or agonist antagonists are attempted, they should include the newer, and apparently quite potent, peptides which have recently been reported in, for example, the Basel Zeitung. Some of these peptides, from the Sandoz Pharmaceutical Co., are said to have ten thousand tines the activity of morphine in vivo. It would be of great interest to obtain physicochemical constants of different kinds for these and other antinociceptive agents with such remarkable structural diversity, so that we might, one day in the future, try to relate structure to activity with a greater hope for success.

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# Quantitative Stereo-Structure-Activity Relationships I. Opiate Receptor Binding

Howard Johnson

One of the central problems in the design of safer and more effective drugs is that of better defining physicochemical properties and structural attributes which contribute to selective high affinity for specific receptors. While modern methods of analysis of quantitative structure-activity relationships (QSAR) effectively address the relationship of structure and bulk physical properties of molecules (such as partition coefficierits) to macromolecular binding and transport phenomena these methods are generally deficient in not adequately addressing steric relationships of molecular geometry in relation to specific receptor binding and biological activity. Yet it is to be expected that the geometrical distribution of physicochemical properties is of paramount importance with regard to binding to specific receptors. Indeed this is well substantiated by many examples of stereospecificity in drug action and in receptor binding as illustrated by the narcotic analgesics (Goldstein 1974).

The present study represents a reasonably successful attempt to correlate opiate receptor affinities of a rather diverse group of narcotic drugs with their three dimensional structures in terms of the geometrical distribution of additive components of various physical properties. The structures considered are too diverse for a simple substituent constant approach, and it is shown that an adequate correlation is not obtained by considering bulk properties as represented by molecular parameters such as log P, molecular wieght, and molar regraction (Hansch 1968). However, the advantages of multiple linear regression methods are retained in the method described which is conceptually similar to pattern recognition approaches. Significant correlations were obtained for forty two compounds whose affinities for the opiate receptor span a range of more than then logs (-11.5 to 0.69).

## METHODS

Experimental Data. For purposes of multiple regression analysis, in vitro affinity for the opiate receptor was defined as log $1/IC_{50}$ Values for opiate concentrations which cause a 50% displacement of

tritiated naloxone in stereospecific binding assays ($IC_{50}$ values) in the presence of 100 mM NaCl are those of Pert and snyder (1974), Pert, Snyder and portoghese (1976), Pert, Snyder, and May (1976), and Wilson et al. (1975). Although several studies were involved in the generation of such data, duplication of results indicated sufficient consistency to justify treatment of the combined results as a single database. Results from binding studies conducted in the presence of 100 mM NaCl were selected for analysis because receptor conformation is believed to best mimic the normal physiological state under such conditions (Pert and Snyder 1974; Creese and Snyder 1975). Furthermore, receptor affinities with and without 100 mM NaCl were found to be highly correlated: log $(1/IC_{50})$ = 1.103, log $(1/IC_{50})$ = 1.628 ($IC_{50}$ = $IC_{50}$ in the absence of 100 mM (NaCl);

R= 0.9196; F= 219.02.

Type structures for the compounds included in the present study are shown in figures 1 and 2.

**FIGURE 1**

*PRIMARY STRUCTURAL TYPES*

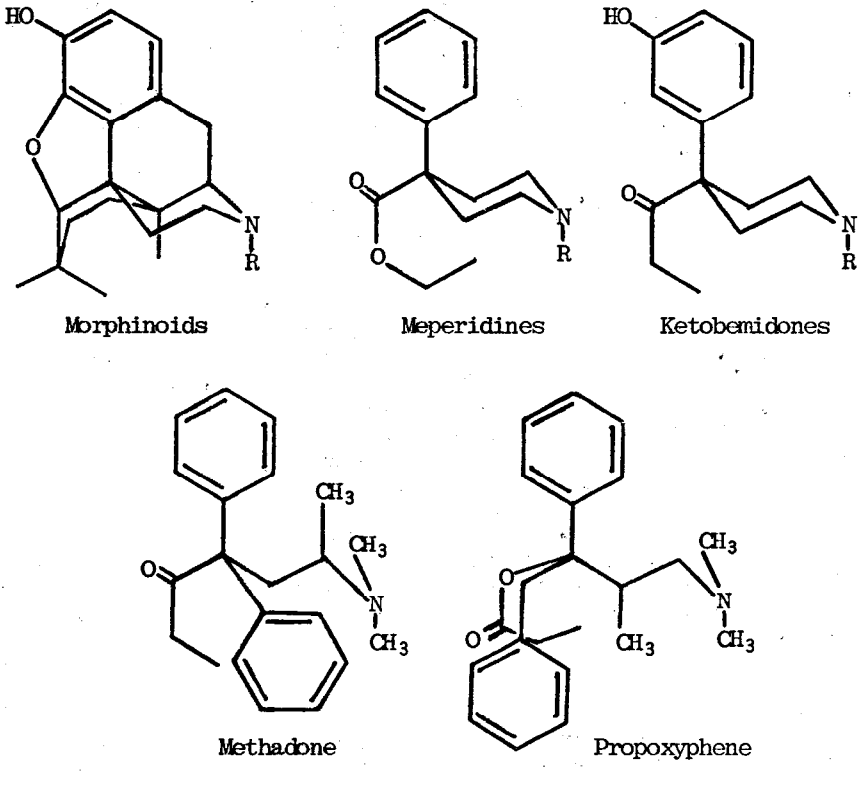
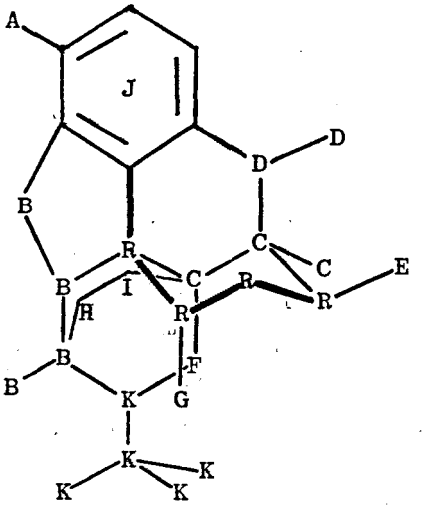


**FIGURE 2**

*ORIPAVINE GENERAL STRUCTURE AND MOLECULAR REGION DEFINITIONS*




Analytical Procedures. The oripavine general structure (figure 2) was used as a basis for selecting regions in molecular space for assignment of structural fragments. The fragments which are common to all of the compounds examined are the phenyl ring(J) and the propylamine moiety (R) shown in heavy lines.

Because most of the structures examined contain the phenylpiperidine moiety, their superimposition on figure 2 is relatively nonarbitrary (coincidence of R and J detemines the locations of other portions of the molecule). In the case of methadone and propoxyphene, however, the propylamine chain could be aligned with either R or C in figure 2. Alignment with R was arbitrarily chosen on the basis of receptor interaction concepts discussed by Feinberg et al. (1976). This resulted in the assignment of one of the N-methyl groups to the C region with the second phenyl group of methadone and the benzyl group of propoxyphene then distributed between the C and F regions. In methadone the phenyl moiety was assigned to the C region; in proxphene the entire phenyl ring was assigned to the F region.

Subsequent to such alignment and regional assignment of structural fragment to the various regions in molecular space as shown in figure 2, various parameter values were then calculated for each of these regional fragments.

The parameters used were hydrophobicity (log P), molar refraction and molecular weight. Regional hydrophobicity values (f) were calculated using the fragmental constants of Leo et al. (1975).

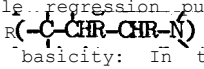
Similarly, regional fragment molar refraction values (m) were obtained or calculated from the compilation by Hansch et al. (1973). Fragment molecular weight values are designated as w.

The summation of regional fragment parameter values (A+BtC...+K) yields the calculated molecular parameter values log P, MR (molar refraction) and MW (molecular weight) (table 1):

$$\Sigma f = \log P; \Sigma m = MR; \Sigma w = MW.$$

These molecular parameters were used both alone and in combination with the corresponding fragmental values in stepwise multiple regression amputations using log ($1/IC_{50}$) as the dependent variable. Computations were performed both within and across parameter classes.

Regressions were performed stepwise interactively using a combination of arbitrary and program-guided insertion and deletion of terms.[1]

There are numerous possibilities with regard to the manner in which the oripavine structure could be segmented for designation of geometrical regions. It should be noted that a number of conditions implicit in the regional designations chosen for this study (figure 2) are important in interpretation of results. The value of J, for example, is inversely proportional to the extent of substitution of the phenyl ring because such substitution eliminates ring protons. Thus, J is smallest in the morphinoid compounds due to their fused rings and is greatest in the meperidine analogs. Values of J, however were used only for summation purposes to obtain molecular parameter values. For multiple regression purposes J was considered a constant. Selection of R  as a constant neglects any differences in nitrogen basicity: In the structurally less complicated compounds, values for a number of regions are zero: H>O only when region H is part of the ring defined by B, H, I, C and R; K>O only when it includes substituents attached to the BHICR ring; thus K>O only in the oripavines of which only two are included in the present study. Fragment G was considered identical in all compounds included in this study and R differs only in the case of propoxyphene and methadone. Regions G, J and R were omitted from regression equations.

**RESULTS**

Parameter coefficients and statistical data for correlations involving bulk molecular parameters (log P, molecular weight, and molar refraction) are shown in table 2. Rather poor correlations were obtained with these parameters and even the inclusion of all three accounted for only 25% of the affinity variance ($R^2$ = .25). Even with the inclusion of second order terms the best correlations obtainable (in terms of R) accounted for no more than 36% of the variance. Furthermore, parameter coefficient values indicated variable qualitative (+ or -) contributions by each parameter depending upon which of these were included in the equation.

In contrast to the use of molecular parameters, regional parameters yielded correlations accounting for 65-80% of the variance in receptor affinity ($R^2$ = 0.65 to 0.80). Furthermore, such

**TABLE 1**

*OPIATE RECEPTOR AFFINITIES AND CALCULATED MOLECULAR PARMETER VALUES*

| Compound | Log $1/IC_{50}$ 100mMNACL | Log P | Mol. Wt. $x10^{-2}$ | Mol. Ref. $x10^{-1}$ |
|---|---|---|---|---|
| Etorphine | -1.79 | 1.92 | 4.12 | 11.49 |
| Diprenphine | .69 | 2.09 | 4.24 | 11.74 |
| Naltrexone | .69 | -.01 | 3.41 | 9.39 |
| Naloxone | -.41 | -1.07 | 3.27 | 9.08 |
| Normorphine | -6.55 | -.75 | 2.71 | 7.51 |
| Oxymorphone | -3.40 | -1.26 | 3.01 | 8.14 |
| Dihydromorphine | -4.94 | 1.12 | 2.87 | 7.92 |
| Morphine | -4.70 | .11 | 2.85 | 7.98 |
| Nalorphine | -1.39 | .30 | 3.11 | 8.86 |
| Levallorphan | -.69 | 4.25 | 2.83 | 8.72 |
| Levorphanol | -2.70 | 4.06 | 2.57 | 7.84 |
| Metazocine | -5.30 | 3.65 | 2.31 | 7.1 |
| Etazocine | -4.94 | 4.73 | 2.59 | 7.09 |
| Propylnormetazocine | 4.38 | 4.73 | 2.59 | 8.03 |
| Allylnometazocine | -1.71 | 3.84 | 2.57 | 7.96 |
| Phenylnormetazocine | -7.82 | 4.66 | 2.93 | 9.54 |
| Ketocyclazacine | -4.09 | 3.24 | 2.85 | 8.47 |
| Et-Ketocyclazacine | -4.08 | 3.78 | 2.99 | 8.93 |
| Pentazocine | -3.91 | 4.79 | 2.65 | 8.5 |
| Cyclazocine | -.41 | 4.90 | 2.71 | 7.88 |
| Phenazocine | -2.08 | 5.32 | 3.07 | 8.6 |
| Meperidine | -10.82 | 3.04 | 2.47 | 7.45 |
| Ethylnormeperidine | -10.82 | 3.58 | 2.62 | 7.91 |
| Propylnormeperidine | -11.51 | 4.12 | 2.76 | 8.38 |
| Butylnormeperidine | -10.30 | 4.66 | 2.9 | 8.85 |
| Phenethynormeperidine | -9.62 | 5.20 | 3.04 | 9.32 |
| Hexylnormeperidine | -7.61 | 5.74 | 3.18 | 9.79 |
| Heptylnormeperidine | -6.48 | 6.28 | 3.33 | 10.26 |
| Octylnormeperidine | -6.69 | 6.82 | 3.47 | 10.73 |
| Nonylnormeperidine | -8.99 | 7.36 | 3.61 | 11.2 |
| Ketobemidone | -4.25 | 1.98 | 2.47 | 7.39 |
| Ethylnorketobemidone | -7.60 | 2.50 | 2.61 | 7.85 |
| Propylnorketobemidone | -6.91 | 3.04 | 2.75 | 8.32 |
| Butylnorketobemidone | -6.55 | 3.58 | 2.89 | 8.79 |
| Pentylnorketobemidone | -3.40 | 4.12 | 3.03 | 9.26 |
| Hexylnorketobemidone | -3.69 | 4.66 | 3.17 | 9.73 |
| Heptylnorketobemidone | -3.91 | 5.20 | 3.32 | 10.2 |
| Octylnorketobemidone | -5.29 | 5.74 | 3.46 | 10.67 |
| Nonylnorketobemidone | -6.55 | 6.28 | 3.6 | 11.14 |
| Decylnorketobemidone | -6.55 | 6.82 | 3.74 | 11.61 |
| Propoxyphene | -9.39 | 5.14 | 3.39 | 10.46 |
| Methadone | -5.30 | 4.54 | 3.09 | 9.95 |

correlations could be obtained with inclusion of a minimum of three or a maximum of six regional terms in the parameter class equations (table 3) and the qualitative contribution of a given region remained constant among the various correlation equations for a given parameter type.

The similarities among equations suggest that f, m, and w are highly colinear. That this is true was evident from examination of the correlation matrix. Fragment weights and refractions were highly colinear except for regions A and I. Fragment hydrophobicities were highly colinear with weight except for region A and were highly colinear with refractions except for regions A and I. Furthermore, regions D and I were highly colinear with respect to weight and refraction. Also evident in the correlation matrix was the generally high colinearity between compound molecular weights and molar refractions.

The inclusion of region R values (constant except for propoxyphene and methadone) in regression equations was discontinued early in the study because it had little influence on correlation statistics.

The inclusion of molecular parameters ($\Sigma f$, $\Sigma w$, $\Sigma m$) in the parameter class equations (table 4) did not result in better correlations. It did, however, result in substantial changes in regional coefficients. If it is true that regional properties are more important in stereospecific binding than total molecular properties, inclusion of the latter would be expected to dampen the sensitivity of correlations to regional influences.

The inclusion of second order terms produced some improvement in correlation (R and F values) but such improvements required a greater number of terms.[2] Similarly, regressions which were not restricted to individual parameter classes produced somewhat better correlations in terns of values of R and F, but generally with greater numbers of terms (table 5).

## DISCUSSION

The use of molecular hydrophobicity and steric bulk parameters without regard to the steric distribution of these properties (log P, molecular weight, molar refraction) did not result in satisfactory correlations (table 2). In general, one might expect such parameters to suffice for a simple homologous series because parameter changes would then reflect fragmental changes which are restricted sterically to a small number of regions in molecular space. In such a case the use of log P, for example, is quite analogous to the use of $\pi$ for the substituents. Similarly, log P should suffice when the dependent variable is primarily a reflection of some phenomenon which in turn is dependent primarily upon bulk hydrophobicity; presumably this would be the case where drug transport by passive diffusion is of major importance. For binding to specific receptors, however, bulk hydrophobicity could hardly be expected to adequately define affinities which are based upon steric distribution of molecular binding regions. The validity of this reasoning appears to be substantiated by the fact that considerably better correlations

TABLE 2

*CORRELATION EQUATIONS BASED ON MOLECULAR PARAMETERS*

| Equation | Parameter Coefficients | | | | | | Constant | Statistics | |
|---|---|---|---|---|---|---|---|---|---|
| | Log P | Mol. Wt. | Mol. Ref. | $(\text{Log P})^2$ | $(\text{Mol. Wt.})^2$ | $(\text{Mol. Ref.})^2$ | | R | F |
| 1 | -0.395 | — | — | — | — | — | -3.15 | 0.286 | 3.570 |
| 2 | -0.540 | 2.594 | — | — | — | — | -10.3 | 0.428 | 4.364 |
| 3 | — | 11.90 | -3.478 | — | — | — | -9.71 | 0.517 | 7.126 |
| 4 | — | 1.64 | — | — | — | — | -10.1 | 0.211 | 1.871 |
| 5 | — | — | 0.100 | — | — | — | -6.05 | 0.041 | 0.066 |
| 6 | -0.57 | — | 0.62 | — | — | — | -7.86 | 0.359 | 2.88 |
| 7 | 10.65 | -2.965 | -0.153 | — | — | — | -9.81 | 0.523 | 4.777 |
| 8 | -0.625 | — | — | -0.026 | — | — | -2.79 | 0.291 | 1.807 |
| 9 | — | -10.13 | — | — | 1.859 | — | 8.16 | 0.245 | 1.248 |
| 10 | — | — | — | — | 1.998 | -0.198 | -7.30 | 0.547 | 8.318 |
| 11 | — | — | — | -0.018 | 1.819 | -0.170 | -7.38 | 0.553 | 5.581 |
| 12 | 0.034 | -47.04 | 18.64 | 0.034 | 9.456 | -1.195 | -19.47 | 0.597 | 3.222 |
| 13 | — | — | -0.497 | — | — | 0.032 | -3.31 | 0.044 | 0.038 |

TABLE 3

*CORRELATION EQUATIONS BASED ON REGIONAL FRAGMENT PARAMENTERS*

| | Equations and Coefficients | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | All Fragments | | | Minimum Fragments | | | | |
| Region | f | w | m | f | w | w | m | m |
| A | -6.35 | 18.03 | 17.77 | -5.11 | 15.81 | 16.70 | 17.18 | 18.79 |
| B | -0.003 | -5.35 | -1.78 | -2.02 | -7.19 | -6.47 | -2.04 | -2.53 |
| C | 9.80 | 23.23 | 6.29 | — | — | 29.50 | — | 6.98 |
| D | 0.39 | -1.41 | 4.12 | — | — | — | — | — |
| E | 0.32 | 2.67 | 0.79 | — | 2.42 | 2.28 | — | 0.65 |
| F | -1.14 | 2.78 | 0.45 | — | 4.37 | — | — | — |
| H | 3.26 | 22.70 | 4.32 | 4.08 | 24.88 | 25.56 | 4.49 | 4.70 |
| I | 2.25 | 6.29 | -1.06 | — | — | — | — | — |
| K | 2.68 | 3.49 | 1.11 | — | 3.84 | 4.09 | — | 1.35 |
| Constant | -15.36 | -13.37 | -14.22 | -4.189 | -5.908 | -13.57 | -7.037 | -12.67 |
| $R_{Correl.}$ | 0.845 | 0.889 | 0.878 | 0.805 | 0.880 | 0.881 | 0.805 | 0.870 |
| $F_{Correl.}$ | 8.875 | 13.37 | 11.97 | 23.32 | 19.96 | 20.30 | 23.31 | 18.23 |

TABLE 4

*CORRELATION EQUATIONS BASED ON REGIONAL FRAGMENT PARAMENTERS*

| | Equations and Coefficients | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | All Fragments | | | Minimum Fragments | | | | |
| Region | f | w | m | f | w | w | m | m |
| A | 2.40 | 57.04 | 15.21 | — | 13.06 | 22.44 | 16.79 | 18.37 |
| B | 6.55 | 36.48 | -7.31 | 4.88 | -8.40 | — | -3.31 | -4.14 |
| C | 22.56 | 109.2 | -3.56 | 19.41 | — | 40.07 | — | — |
| D | 6.43 | 39.34 | 0.04 | 4.88 | — | — | — | — |
| E | 6.62 | 44.94 | -4.77 | 5.01 | — | 2.79 | — | -0.82 |
| F | 4.79 | 44.47 | -4.93 | 3.27 | — | — | — | — |
| H | 8.31 | 62.88 | -0.23 | 7.01 | 21.10 | 17.95 | 4.04 | — |
| I | 7.01 | 46.72 | -6.75 | 5.73 | — | 16.08 | — | — |
| K | 8.04 | 45.80 | -4.43 | 6.65 | — | — | — | — |
| Σ* | -6.08 | -42.27 | 5.56 | -4.52 | 2.89 | — | 0.87 | 1.55 |
| Constant | -11.78 | 31.46 | -34.68 | -12.79 | -11.85 | -21.51 | -13.04 | -16.40 |
| $R_{Correl.}$ | 0.864 | 0.889 | 0.879 | 0.862 | 0.879 | 0.864 | 0.863 | 0.856 |
| $F_{Correl.}$ | 9.105 | 11.71 | 10.50 | 10.32 | 31.45 | 21.24 | 26.86 | 25.26 |

*Σf = log P, Σw = mol. wt., Σm = molar refraction.

TABLE 5

*CORRELATION EQUATIONS BASED ON REGIONAL FRAGMENT PARAMENTERS*

| Region | Equations and Coefficients | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| A | 15.8w | 15.8w | 19.2w | 22.5w | 24.9w | 13.1w | — | — | -0.8m | 2.1m | 4.2f |
| B | -7.2w | -7.2w | -4.1w | — | — | -8.4w | -13.2w | 14.0w | -6.4w | -11.1w | -3.4w |
| C | — | — | 6.2f | 7.9f | 32.2w | — | — | — | — | — | 14.5f |
| D | — | — | — | — | — | — | 1.5f | 1.5f | — | 1.3f | 3.7f |
| E | — | — | -1.9f | -2.4f | -3.3f | — | — | — | 2.8m | — | — |
| E | 2.4w | 2.4w | 10.9w | 13.0w | 16.4w | — | — | — | -2.4f | — | 3.6f |
| F | 4.4w | 4.4w | — | — | — | — | — | — | — | — | 2.1f |
| H | 24.9w | 25.0w | 21.1w | 19.7w | 4.1f | 21.1w | — | 2.7f | 3.0f | — | 7.5f |
| I | — | — | — | 2.1f | — | — | — | — | — | — | — |
| K | 3.8w | 1.3m | 1.3m | 3.2w | 3.9w | — | — | — | — | — | — |
| Σ | — | — | — | — | — | — | -0.7f | -0.8f | — | -0.6f | — |
| Σ | — | — | — | — | — | 2.9w | 5.5w | 1.9m | 3.9w | 4.9w | -3.2f |
| Constant | -5.91 | -5.93 | -11.3 | -15.95 | -17.6 | -11.9 | -11.8 | -11.5 | -12.8 | -12.4 | -5.00 |
| $R_{Correl.}$ | 0.880 | 0.879 | 0.897 | 0.901 | 0.891 | 0.879 | 0.877 | 0.892 | 0.896 | 0.885 | 0.860 |
| $F_{Correl.}$ | 19.96 | 19.87 | 19.88 | 20.93 | 22.54 | 31.45 | 30.81 | 27.95 | 23.82 | 26.14 | 10.10 |

were obtained using fragmental parameter values for sterically defined molecular regions.

The regional fragmentation pattern chosen for this study was intended to specify major fragments which are absent or altered in various opiate classes while simultaneously minimizing the number of separate fragments to be considered in regression analysis. Aside from such considerations, the pattern chosen was quite arbitrary. It is possible that better correlations could be obtained by using a different pattern. The fact that inclusion of all fragments (except J, G and R) in regression equations did not result in any vanishingly small fragment coefficients suggests that the regional pattern chosen was reasonable.

It should be noted that the present molecular model method for steric specification of regional parameters neither implies nor requires that the compounds with high degrees of conformational freedom must bind to the receptor in a conformation closely approximating that of the model. Such conformtional modeling is merely a methodological tool for steric description. However, the extent to which such binding conformations are involved among the compounds included may indeed determine how good a correlation is obtained. Thus, the inclusion of a "conformational probability" term might result in improved correlations.

When all variable regions are included in equations the resulting regional coefficients provide both a qualitative and quantitative indication of the relative importance of regional properties to opiate receptor binding. The results obtained in this manner are generally in agreement with common concepts of opiate structure-activity relationships. Thus hydrophobicity appears to be of little value or even disadvantageous in regions A and B which frequently contain either no substituents or polar oxygen functions. The presence of polar steric bulk appears to be important in region A. Similarly, hydrophobicity appears to be desirable, though of modest importance, at E (the N-substituent), and undesirable at F which is the 14-hydroxyl group in some morphinoids. The importance of hydrophobicity in the K region coincides with the high activities and receptor affinities of the oripavines. Similarly, the importance of region H hydrophobicity is quantitated.

The relatively minor importance of E region hydrophobicity in spite of the great variation in N-substituents among the compounds included in this analysis is perheps unexpected. However, this result is consistent with the possibility that the N-substituent is primarily of importance in determining agonist-intrinsic activity versus antagonist properties rather than receptor affinity. On the other hand, the K region appears to contribute substantially to both receptor affinity and agonist/antagonist properties.

The apparent importance of both steric bulk and hydrophobicity in region H suggests an area for synthetic exploration among nonmorphinoid compounds such as the benzomorphans, meperidine analogs, etc.

These conclusions are further emphasized in the minimum fragment equations (table 3) which indicate that the regions most determinant of receptor affinity are A, B, E, H, and K.

In addition to addressing the problem of steric relationships to biological activity, the present method combines the quantitative and statistical advantages of multiple linear regression with the broader applicability and power of pattern recognition approaches. As such it is not unrelated to the Free-Wilson method and, in some respects, is a further extension of the substituent constant and dummy parameter variations of the basic Hansch analysis. The steric modeling aspect is only broadly defined but provides ample opportunity for the preception and creativity of the individual investigator. Work currently in progress involves completely computerized superimposition and comparison of structures in three dimensional molecular space with computer assignment of regional fragment parameter values; such assignments are made on the basis of parent model defined regional substructure searches wherein the regional search limits are defined by the investigator as a function of atomic radii.

## FOOTNOTES

1. "The organization and analysis of the data base associated with this investigation were carried out using the PROPHET System, a unique national computer resource sponsored by the NIH. Information about PROPHET, including how to apply for access, can be obtained from the Director, Chemical/Biological Information-Handling Program, Division of Research Resources, National Institutes of Health, Bethesda, Maryland 20014."

2. For example: $\log 1/IC_{50} = 5.97\ f_A + 10.70\ f_E + 2.65\ f_E - 0.31\ f_E^2 + 42.30\ f_H - 55.86\ f_H^2 + 3.44\ f_I - 20.0$

$R = 0.893 \quad F = 19.12$

## ACKNOWLEDGMENT

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# Progress with Several Models for the Study of the SAR of Hallucinogenic Agents

Lemont B. Kier and Richard A. Glennon

At the present time, the studies in our laboratories are directed. toward several facets of the SAR of hallucinogenic agents. It is desirable to take this opportunity to disclose the broad objective of our efforts and, at the same time to record the progress in each aspect of our research.

We can study the action of hallucinogenic agents by the use of models, each one providing a framework for analysis and research into the structure activity relationships (SAR) governing observable phenomena.

Using as a model just the conservative molecule, one considers properties and events arising directly as a consequence of molecular structure. At this molecular level, we ignore interactions with other molecules, in a milieu or with receptors. The model here arises from a reduction of variables until the molecule in the isolated state remains as the governing structure dictating the magnitude of observable phenomena. The extraction of SAR from this model leads to information which is necessarily limited by certain exclusions of reality, but which is frequently the most attainable kind of a relationship. The expectation is that modest success in this category of SAR study may lead to prediction of alternate structures with predictable experimental properties.

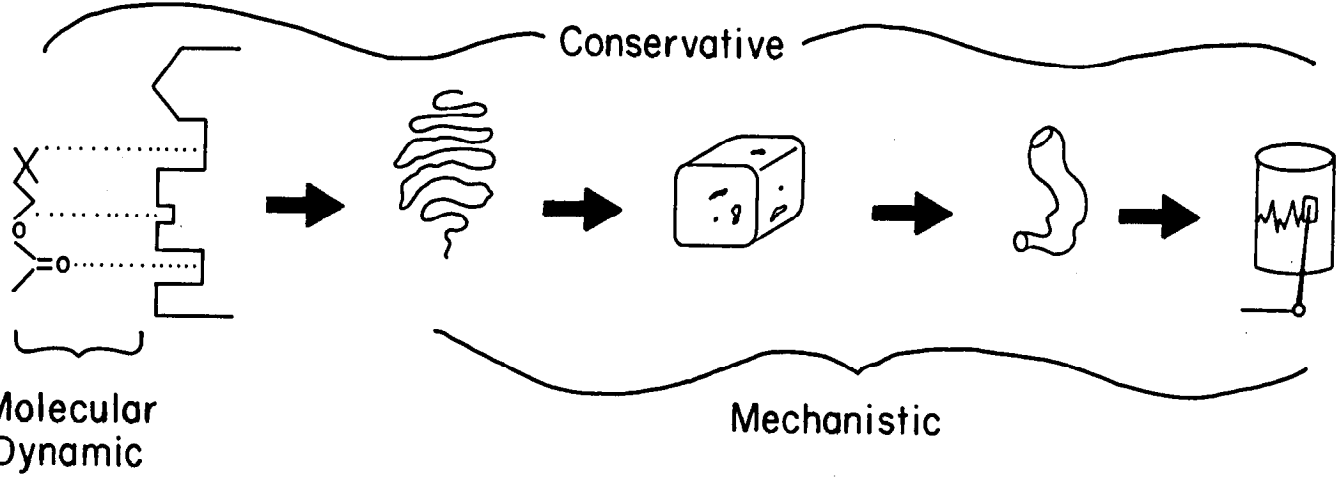
SAR studies in this molecular framework involve the definition of structure using molecular orbital theory or topological descriptions such as molecular connectivity. In its simplest form, structural influence on activity may be defined by a count of carbon atoms in a normal alkane series (Kier and Hall 1976).

From successful studies with this model, we may presume to have some insight into molecular structure influencing activity. Denied to us is the ability to reflect on intermediate mechanisms involving aggregates of molecules.

A second model for SAR studies involves some attempt to simulate the dynamic intermolecular events taking place as a biological agent encounters a tissue-based receptor molecule. In the absence of any real understanding of the structure of a receptor, surrogate molecules may be used to simulate the *in vivo* part Of an interacting molecular pair.

A presumption inherent in this stochastic model is that the in-

# SAR MODELS



teraction energy emerging from the molecular pair bears some relationship to the magnitude of the observed biological phenomena. Theoretical calculations using molecular orbital theory or empirical calculations quantitating categorized forces lead to energy values as a function of intermolecular distance and model structure.

This general approach to SAR may lead to information about molecular structure and possible molecular level mechanisms of action. The information is much harder to obtain than from conservative molecule SAR considerations since all of the spatial aspects of interacting pairs must be probed and a suitable receptor model established.

A third model of SAR analysis involves the development of quantitative relationships between suspected parallel properties or actions of molecules. This general approach relies heavily upon the quantitation of pharmacological, biochemical, and physicochemical properties. The interplay of these properties in the search for relationships presumes the existence of models depicting mechanisms of action at the level of bulk solutions, tissues and even organs.

Structural information arising from successful correlations using this general model is limited. The real benefit evolves from a greater possibility of mechanistic interpretation.

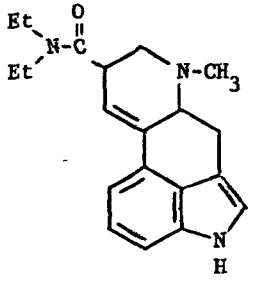
Because of facilities and capabilities it is possible in our laboratories to utilize all three of these general SAR models. Thus we have utilized molecular structural description, stochastic modeling and pharmacological and physicochemical measurement, to probe on a broad front the phenomenon of hallucinogenic activity. We describe here some of the results in each category.

**CONSERVATIVE MOLECULE SAR**

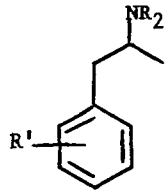
The pioneering work of Karreman, Isenberg and Szent-Gyorgyi (1959), and of Snyder and Merril (1965) ushered in theoretical studies of the influence of molecular structure on the hallucinogenic potency of LSD (1), phenylisopropylamines (2) and tryptamines (3). Using primarily the human data of Shulgin, Sargent and Naranjo (1969) a relationship with molecular orbital energy parameters was reported. Later studies by Green and collaborators (Green 1967; Kang and Green 1970a; Green and Kang 1970; Kang and Green 1970b) sought to improve electronic correlates of hallucinogenic potency by using more sophisticated molecular orbital methods, and by examining additional parameters.

An electronic parameter to which hallucinogenic activity was initially related was the energy of the compounds' highest occupied molecular orbital($E_{Homo}$), an index thought to reflect the compounds' ability to donate electrons in a charge-transfer type of interaction. More recently this relationship between $E_{Homo}$ and activity has been questioned with respect to LSD analogs (Kumbar and Siva Sankar 1973; Green, Johnson and Kang 1974) and in respect to N, N-dialkyl tryptamines (Glennon 1974). Green

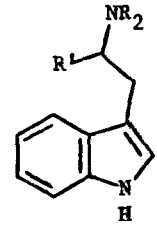
has commented that such a molecularly gross characteristic as $E_{HOMO}$ probably cannot explain so specific a biological activity. To date, no direct electronic correlate relating the hallucinogenic activity of phenylisopropylamines, tryptamines and LSD analogs has been reported.



1



2



3

Topological structure of hallucinogens has been studied using molecular connectivity (Kier and Hall 1977). A direct correlation was found between connectivity indices and hallucinogenic potency, which was of a quality at the upper limit of the value of the experimental data. Thus for 23 ring substituted phenylisopropylamines (amphetamines) (table 1), an equation was found:

$$\log MU = \frac{45.16}{{}^{3}\chi_P} + 1.29\,{}^{6}\chi_P - \frac{4.30}{{}^{4}\chi^{V}_{PC}} - 5.59 \qquad \text{Eqn I}$$

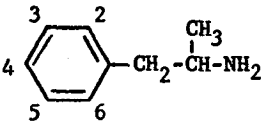
$$r = 0.92 \quad S = .251 \quad N = 23 \quad F = 35$$

Two important consequences of this work emereged. The first was that statements, comprehensive to the medicinal chemist, could be made about molecular structure and its influences on hallucinogenic potency. The equation delineates structural fragments which are of significance in governing the experimental potency.

No understanding of intervening mechanisms or biopharmaceutical events is possible from the equation. The Information from molecular connectivity calculations is purely structural. Nevertheless, the Information makes it possible to make predictions of potency, the second important consequence of this work. Table 2 lists six satisfactory potency predictions reported from the equation, compounds 27 to 32 (Kier and Hall 1977), plus six additional predictions, compounds 33 to 38 (Glennon and Kier,unpublished data) since that publication. The potency of LSD (1) can also be

TABLE 1

Substituted Phenylisopropylamines and Predicted hallucinogenic Activity [a]




| No. | Ring position and group 2 | 3 | 4 | 5 | 6 | Exptl. log MU | Calcd. log MU |
|---|---|---|---|---|---|---|---|
| 4 | | | $OCH_3$ | | | 0.59 | 0.55 |
| 5 | $OCH_3$ | | $OCH_3$ | | | 0.67 | 0.87 |
| 6 | $OCH_3$ | | | $OCH_3$ | | 0.87 | 1.06 |
| 7 | | $OCH_3$ | $OCH_3$ | $OCH_3$ | | 0.37 | 0.55 |
| 8 | $OCH_3$ | $OCH_3$ | | $OCH_3$ | | 0.63 | 1.01 |
| 9 | $OCH_3$ | | $OCH_3$ | | $OCH_3$ | 1.03 | 1.12 |
| 10 | $OCH_3$ | $OCH_3$ | | | $OCH_3$ | 1.14 | 1.06 |
| 11 | $OCH_3$ | | $OCH_3$ | $OCH_3$ | | 1.26 | 1.00 |
| 12 | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | | 0.86 | 0.92 |
| 13 | | $-OCH_2O-$ | | | | 0.41 | 0.21 |
| 14 | | $OCH_3$ | $-OCH_2O-$ | | | 0.43 | 0.62 |
| 15 | $-OCH_2O-$ | | $OCH_3$ | | | 0.48 | 0.80 |
| 16 | $OCH_3$ | $-OCH_2O-$ | | | | 1.00 | 0.71 |
| 17 | $OCH_3$ | | $-OCH_2O-$ | | | 1.08 | 0.71 |
| 18 | $OCH_3$ | $OCH_3$ | $-OCH_2O-$ | | | 0.75 | 1.09 |
| 19 | $OCH_3$ | $-OCH_2O-$ | | $OCH_3$ | | 1.13 | 1.29 |
| 20 | $OCH_3$ | | $OCH_2H_5$ | | | 1.22 | 0.93 |
| 21 | $OCH_3$ | | Br | $OCH_3$ | | 2.71 | 2.92 |
| 22 | $OCH_3$ | | $CH_3$ | $OCH_3$ | | 1.89 | 1.85 |
| 23 | $OCH_3$ | | $C_2H_5$ | $OCH_3$ | | 2.01 | 1.70 |
| 24 | $OCH_3$ | | n-$C_3H_7$ | $OCH_3$ | | 1.94 | 1.60 |
| 25 | $OCH_3$ | | n-$C_4H_9$ | $OCH_3$ | | 1.63 | 1.34 |
| 26 | $OCH_3$ | | n-$C_5H_{11}$ | $OCH_3$ | | 1.09 | 1.09 |

a Kier and Hall 1977

predicted quite well (table 2) using Equation I. It might be pointed out that the molecular connectivity method does not consider stereochemistry and that it cannot account for variation in hallucinogenic potency resulting from alteration in the route of administration.

In a more recent study, a series of phenethylamine (mescaline) analogs was analyzed using molecular connectivity (Glennon, Kier and Shulgin, unpublished data). The hallucinogenic activity of phenethylamines was found to relate with two connectivity indices according to the following expression:

$$MU = 129\,{}^{3}\chi_{c}^{v} - 4.45\,{}^{4}\chi_{p} - 14.54$$

$$r = 0.97 \quad S = 3.02 \quad N = 10 \qquad \text{Eqn II}$$

Again, with this equation, a higher percent of the variation in potency is found, than is probably warranted by the experimental data.

A number of structural conclusions are possible from these findings (table 3). From the relating equation, several generalizations can be derived about the impact of structural variation on activity. The ${}^{3}\chi_{c}^{v}$ (term suggests that increasing the number of nuclear substituents will generally result in an increase in potency; compare the activities of 39 and 40 with, for example, those of 41 and 42. This is also accord, biologically, with the findings of Clarke, Bennington and Morin (1965) that those phenethylamines with high degrees of methoxylation serve less well as substrates for enzymic degradation. In addition, the presence of the ${}^{3}\chi_{c}^{v}$ term implies that the lower the $\delta^{v}$ in the substituent, the higher the potency, i.e. Br > $CH_3$ > $OCH_3$; compare 42, 45 and 46.

The ${}^{4}\chi_{p}$ term is related to the substitution pattern of the phenethylamine ring. Because the coefficient for this term in the relating equation is negative, the greater the value of the term, the lower the potency of the compound. In the monosubstituted phenethylamines, an ortho substituent contributes two additional terms and a meta substituent one additional term as compared to a para substituent. Therefore the position of the stistituent would be expected to enhance activity in the order: ortho < meta < para.

Considering phenethylamines with more than one ring substituent, the ${}^{4}\chi_{p}$ term suggests that activity is enhanced when two substituents are para to one another (para > meta > ortho). Such substitution can only be realized, in disubstituted molecules for example, when the phenethylamine is 2,5-disubstituted.

The above statements, which can be quantified using the chi terms, appear to echo those structure activity relationships, which have evolved, in a qualitative manner, by inspection of a series of

TABLE 2

Predictions of Hallucinogenic Potency for Compounds Not Use in Generating Equation I

| | Position and Substituent | | | | Exptl. MU | Calcd. MU |
|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | | |
| | | | Phenylisopropylamines[a] | | | |
| 27 | $OCH_3$ | $OCH_3$ | $OCH_3$ | | 2 | 2 - 7 |
| 28 | | $OCH_3$ | $OCH_3$ | | 2 | 1 - 4 |
| 29 | $OCH_3$ | - O $CH_2$O - | | | 1 | 2 - 7 |
| 30 | $OC_2H_5$ | | | $-OCH_2O-$ | 7 | 2.5 - 8 |
| 31 | $OCH_3$ | | $OCH_3$ | | 7 | 4.8 - 15.1 |

| | Tryptamines | MU | Exptl. log MU | Calcd. log MU |
|---|---|---|---|---|
| 32[a] | 4-OH DMT (Psilocin) | 32 | 1.51 | 1.69 |
| 33 | DMT (N, N-Dimethyltryptamine) | 5[b] | .70 | 1.47 |
| 34 | DET (N,N-Diethyltryptamine) | 5[b] | .70 | .98 |
| 35 | DPT (N,N-Dipropyltryptamine) | 3.5-4.7[c] | .61 | .53 |
| 36 | 5-OMe DMT | 40[b] | 1.60 | 1.60 |
| 37 | α-Methyltryptamine | 7-18[c] | 1.10 | 1.19 |
| 38 | 5-OMe α-Methyltryptamine[d] | 100[b] | 2.00 | 1.33 |
| 1 | LSD | | 3.57 | 3.60 |

[a] Kier and Hall 1977

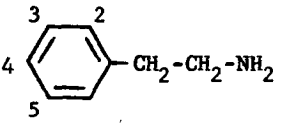
[b] MU data obtained from A. T. Shulgin, personal communication

[c] Dose range obtained from A. T. Shulgin and then converted to MU range

[d] Active orally

TABLE 3

Chi Terms and Activities of Various Phenethylamine Analogs




| | Position | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | $^3\chi^v_c$ | $^4\chi_p$ | Mescaline Units |
| 39 | | | $OCH_3$ | | .186 | 2.34 | < 1 |
| 40 | | $OCH_3$ | | | .236 | 3.13 | < 1 |
| 41 | $OCH_3$ | | $OCH_3$ | $OCH_3$ | .279 | 3.77 | 1 |
| 42 | | $OCH_3$ | $OCH_3$ | $OCH_3$ | .287 | 3.94 | 1 |
| 43 | | $OCH_3$ | $OCH_2CH_3$ | $OCH_3$ | .287 | 4.07 | 7 |
| 44 | | $OCH_3$ | $OCH_2CH_2CH_3$ | $OCH_3$ | .287 | 4.18 | 6 |
| 45 | $OCH_3$ | | $CH_3$ | $OCH_3$ | .364 | 3.47 | 20 |
| 46 | $OCH_3$ | | Br | $OCH_3$ | .509 | 3.47 | 35 |
| 47 | $OCH_3$ | $-O-CH_2-O-$ | | | .263 | 4.34 | > 5 |
| 48 | | $OCH_3$ | $-O-CH_2-O-$ | | .287 | 4.22 | 2 |
| 49[ac] | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | | | b |
| 50[a] | $OCH_3$ | | I | $OCH_3$ | .593 | 3.47 | b |

[a] Not used to derive the relating equation (Equation II)

[b] Effective human intoxication levels have not yet been fully evaluated

[c] Compd. 49 also possesses a 6-methoxy group

phenethylamines. Shulgin (1978) has reported that if the para-methoxy group is replaced with another substituent (higher alkoxy, methyl, halo) there is an unquestioned Increase in potency. Shulgin (1978) has further commented that, in general, it appears that 2, 4, 5-orientation is more effective than 3, 4, 5-orientation although there are two few examples to establish this generality.

Investigations of SAR can serve to highlight those structural features which Influence activity. However, because hallucinogenic activity can not be measured accurately, one can not expect to accurately predict hallucinogenic potency. This is especially true with those compounds that are only weakly active as hallucinogens and is reflected by the standard deviation of equation II. Application of the relating equation (equation II) should, nevertheless, afford a relative measure of predictable activity, particularly when activity is moderately high. Smythies and co-workers (Smythies et al. 1967) employing a modified Bovet-Gatti profile, have found that 2, 3, 4, 5, 6-pentamethoxyphenethylamine (49) possesses an approximate activity of eight mescaline units in animal models. Using the relating equation (equation II), 49 is predicted to have a potency of 4.5 MU. As another example, an iodinated derivative, 4-iodo-2,5-dimethoxyphenethylamine ( 50) , has recently been titrated In humans although effective intoxication levels have not yet been explored (Shulgin 1978). The iodo group, which is para to the side chain, possesses a $\delta^{v}$ which is even lower than that of a bromo group. In addition, this molecule possesses two methoxy groups which are para to one another (i.e. 2,5 dimethoxy substitution). On this basis, 50 is predicted by the relating equation to be approximately 45 times more active than mescaline. Shulgin (1978) has found that threshold effects of 50 are clearly noted at a total dose of 8 mg.; this corresponds to an activity of about 44 MU.

**INTERACTION MODEL SAR**

The goal in this endeavor is to deduce a model of interacting species, to estimate the energy of interaction at reasonable distances of separation and then to attempt to correlate the energies for a series of such pairs with an observed biological property. The hypothesis that the energetics so computed govern the measured biological response underlies this approach.

In practice, the drug molecule structure is known while the receptor must be found. By elimination and speculation, this frequently is taken to be a portion of a polypeptide chain, usually an amino acid side chain.

We have reported such a study on 17 hallucinogenic phenylisopropylamines (DiPaola, Kier and Hall 1978). Calculations of interaction energy using the Claverie and Rein (1969) procedure have deduced a receptor model, an interaction distance and orientation and a relating equation for activity in log mescaline tits:

$$\text{Log MU} = 0.28\ E_T + - 0.26\ I_{3,4} - 0.63 \qquad \text{Eqn III}$$

where $E_T$ is the total calculated interaction energy computed for the model and orientation mode optimizing the relationship and $I_{3,4}$ an indicator variable depicting the presence of substituents in the 3 and 4 ring positions.

The optimized model, shown in figure 1, is based on the amphetamine ring separated from an indole receptor surrogate by 4.5A.

A further sophistication of this approach involves the estimation of the electrostatic potential field around each atom. The interaction calculations thus consider a changing charge mosaic as the molecules approach. Current work considers these charge fields.

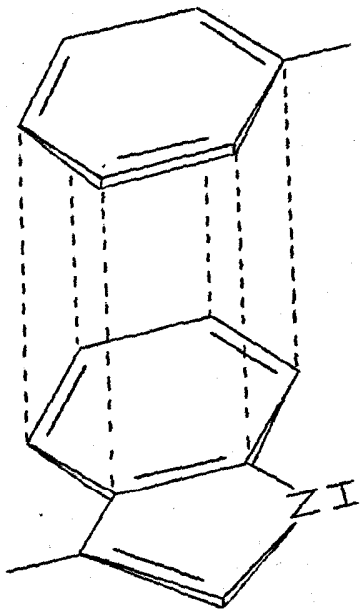
**MECHANISTIC MODEL SAR**

The probing of potential pharmacological mechanisms is the objective of structure activity studies using this general model. Determination of the mechanism of action of agents whose biological effect is essentially subjective in nature, i.e. a psychopharmacological response, is inherently fraught with the problems of measuring this effect. Much of the comparative human data on the hallucinogenic activity of various substances has been accumulated by Shulgin and co-workers who have estimated a variation in activity data of about 25% (Shulgin, Sergeant and Naranjo 1969). While the use of so-called "Mescaline Unit" data is necessitated by the lack of any other type of comparative human data, Shulgin is quick to point out that such data do not reflect, for example, route of administration, time of onset, duration of action, and, are extrapolated from dose ranges. What is needed is a model or surrogate measure of the hallucinogenic response, or rather, a model which might reflect or yield results which reasonably parallel hallucinogenic activity. Though such an idea is not new,a study of drug/model-receptor interactions might still be useful inasmuch as similar interactions might be responsible for the hallucinogenic response. Regarding the validity of using model receptor systems, Steinsland and Hieble (1978), for example, have recently reported on a rabbit ear artery dopamine receptor model. The affinities determined for various neuroleptic agents, using their model system, closely parallel the dopamine receptor binding affinities which were determined using calf brain homogenates.

In these studies, then, pharmacological and physicochemical correlates are sought; these, in turn,can be analyzed using previously described SAR methods, i.e. molecular orbital theory or molecular connectivity. Various mechanistic models might be constructed which relate hallucinogenic activity to familiar drug receptor systems or to measurable physicochemical phenomena known to occur <u>in vivo.</u>

What model systems might be useful? Tryptamine, phenalkylamine

**FIGURE 1**




Optimum mode of interaction predicted for amphetamine rings and the methylindole receptor model.

and lysergamide hallucinogens are known to interact with various neurotransmitter systems, for example, serotonergic and dopaminergic systems. Because of the close structural similarity between these hallucinogens and serotonin (5-hydroxytryptamine, 5-HT), we have chosen the 5-HT receptors for our initial. investigations. This does not preclude the possibility that hallucinogens might produce some facets of their activity by interaction with other types of receptors.

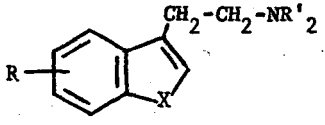
With this as a working hypothesis we have examined various model 5-HT receptor interactions of hallucinogenic agents. Woolley initialy suggested that 5-HT/hallucinogen interactions might be implicated in their mechanism of action (Wolley and Shaw 1954; Woolley 1962). Later, others (Gaddum et al. 1955; Vane 1959; Earlow and Khan 1959; Offermeier and Ariens 1966; Winter, Gessner and Godse 1967) examined the ability of tryptamines and related compounds to interact as agonists or antagonists with the 5-HT receptors of various isolated tissue preparations. These investigations, however, were not solely directed toward finding a correlate of hallucinogenic activity but were, rather, studies of drug-receptor interactions. One of the first studies,using model receptors, designed expressly for investigating hallucinogenic activity was that of Green and Kang (1970). Employing nitrogen-unsubstituted tryptmines and using an isolated rat stomach fundus preparation, several potential quantum chemical correlates of agonistic activity were implicated.

Using a somewhat different approach, Glennon and Gessner (1975;1977) investigated the binding affinities (p A2's) of N, N-dialkyltryptamines and related analogs for the 5-HT receptors of this same model system (table 4). Although further work is indicated from the results, several interesting findings have emerged: (a) increasing the steric bulk on the terminal amine decreases affinity, (b) methoxyl-substitution is most favorable at the 5-position, moving this methoxy group to the 4- or 6-position results in about a 10-fold decrease, (c) replacement of the indole nitrogen by a sulfur atom has no apparent effect on affinity while replacement by an $sp^3$-hybridized carbon atom results in a two-fold decrease in affinity.

The electronic properties of a number of these compounds were examined by various molecular orbital methods (Glennon and Gessner 1977). No significant correlation was observed between affinity and $E_{HOMO}$ or between affinity and any other electronic parameter, for that matter. However, if the 7-methoxy analog is deleted, a correlation is observed between affinity and the electrophilic frontier orbital electron density at the 4-position ($f_r^+$) ($r = .96$, $n = 8$). Only eight compounds were examined; because the variation in $f_r^+$ small, the significance of this correlation is not clear and may even be fortuitous. A larger series of compounds should be examined. Such correlations between electronic parameters and 5-HT receptor binding affinities remain to be fully developed, Interestingly, using different compounds and a different procedure to measure activity, Green and co-workers have found relationships be-

TABLE 4

Binding Affinities of N,N-Dialkyltryptamines and Related Compounds[a, b]




| | | X | R | R' | $pA_2$ [c] |
|---|---|---|---|---|---|
| 51 | 5-OH DMT | NH | 5-OH | $CH_3$ | 7.41 (±.05) |
| 36 | 5-OMe DMT | NH | 5-$OCH_3$ | $CH_3$ | 7.10 (±.04) |
| 52 | 5-OMe DET | NH | 5-$OCH_3$ | $C_2H_5$ | 6.94 (±.06) |
| 53 | 5-OMe DPT | NH | 5-$OCH_3$ | n$C_3H_7$ | 6.53 (±.05) |
| 54 | 4-OMe DMT | NH | 4-$OCH_3$ | $CH_3$ | 6.17 (±.01) |
| 55 | S-DMT | S | H | $CH_3$ | 6.03 (±.03) |
| 33 | DMT | NH | H | $CH_3$ | 6.00 (±.08) |
| 56 | 5-Ac DMT | NH | 5-$COCH_3$ | $CH_3$ | 5.86 (±.06) |
| 34 | DET | NH | H | $C_2H_5$ | 5.79 (±.03) |
| 57 | 6-OMe DMT | NH | 6-$OCH_3$ | $CH_3$ | 5.77 (±.01) |
| 58 | C-DMT | $CH_2$ | H | $CH_3$ | 5.68 (±.06) |
| 59 | 7-OMe DMT | NH | 7-$OCH_3$ | $CH_3$ | 5.33 (±.02) |

a Glennon and Gessner 1975.
b Glennon and Gessner, unpublished data.
c Determined in duplicate. $pD_2$ for 5-HT = 7.45 (±.16), n=33.

tween activity and various electronic parameters, including $f_4^E$, both in their 1970 study (Green and Kang 1970) and in later investigations (Johnson and Green 1974; Weinstein et al. 1976).

Two questions emerge from these studies. First, is there a receptor related parameter which might be used to investigate the hallucinogenic phenalkylamines as well as the N,N-dialkyltryptamines, and secondly, are agents within these two,classes of hallucinogens capable of producing similar behavioral effects? In other words, is it justifiable to examine these compounds as one large group as opposed to examing each class separately. Though each of these classes may elicit unique behavioral responses, an investigation of a common component of their activity might afford the most useful data. Evidence that such an approach might be warranted comes from molecular connectivity studies. Using the equation relating hallucinogenic activity to the structure of a series of phenylisopropylamines, satisfactory predictions were made (table 2) concerning the hallucinogenic potency of several N,N-dialkryltryptamines (which were not used in generating the equation).

In addition, in collaboration with Dr. J. Rosecrans of MCV, we are examining the behavioral effects of various hallucinogens employing a discriminative stimulus model. Rats are trained to discriminate between a drug and non-drug (saline) state until a high level of performance is attained. These animals are then challenged with a new drug to detemine if the challenge-drug is perceived by the animals as producing; an effect similar to the test-drug. If generalization occurs,that is, if the animals cannot discriminate between the effects of the challenge-drug and the test-drug, this is an indication that both agents are producing similar behavioral effects and, furthermore, that they might be operating via a similar mechanism (Kuhn, White and Appel 1977).

The discriminative stimulus properties of several hallucinogens have been recently reviewed (Kuhn, White and Appel 1977). LSD, for example, will serve as a discriminative stimulus in animals. Though generalization will occur with psilocybin, it does not occur with morphine,amphetamine, PCP, or phenobarbital. Rosecrans and co-workers (Hirschhorn, Hayes and Rosencrans 1975) have also shown that LSD will generalize to electrical stimulation of midbrain raphe.

We have found that 5-OMe DMT possesses discriminative stimulus properties and we have trained rats to discriminate between this compound and saline. When challenged with DOM,the animals are unable to discriminate between DOM and the 5-OMe DMT stimulus. Thus, generalization is occurring (table 5) and both compounds are presumed to be producing similar behavioral effects. Additional studies involving other hallucinogens are currently underway, to determine if there are certain common components of their behavioral effects.

Phenylisopropylamines are know to affect serotonergic systems both <u>in vivo</u> and <u>in vitro.</u> There is an obvious structural similarity

**TABLE 5**

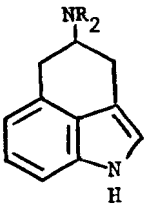
Generalization of DOM to 5-OMe DMT stimulus [a]

| Drug | | n | % 5-OMe DMT Correct Responses [b] |
|---|---|---|---|
| 5-OMe DMT [c] | 1mg/kg | 48 | 85% |
| Saline | 0.9 1ml/kg | 42 | 20% |
| DOM [c] | 0.25 mg/kg | 6 | 39% |
| | 0.50 mg/kg | 12 | 53% |
| | 0.75 mg/kg | 5 | 60% |
| | 1.00 mg/kg | 6 | 85% |
| | 2.00 mg/kg | 6 | d |

a Glennon, Rosecrans and Gaines; unpublished data
b Average responses of n animals
c In 0.9% Saline
d Behavior disrupted, animals did not respond

between these structures and the structures of 5-HT and the N,N-dialkyltryptamines. Superimposition of these structures, as shown in figure 2 may be conceived as one way (or conformation) in which these compounds might interact with 5-HT receptors. Others have

**FIGURE 2**




Phenylisopropylamine structure superimposed over a tryptamine structure

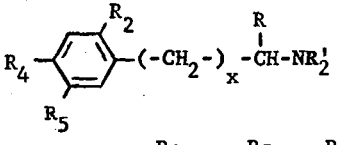
also noted these structural similarities. Therefore it might be expected that the phenylisopropylamines might possess binding affinity chacteristics similar to those of the N,N-dialkyltryptamines. We have examined this possibility (Glennon, Liebotitz and Mack 1978) and some of the results are presented in table 6. Though this study is far from complete, several generalizations might be made.

2,5-Dimethoxyphenylisopropylamine (6) possesses a pA2 of 6.83. Para-methoxylation does not appear to alter affinity (compound 11 ). This in line with the suggestion that p-methoxylation increases potency by blocking metabolism at that position. Para-methylation, on the other hand, results in a two-fold increase affinity (compound 22 ). Nichols, et al. (1977) have recently demonstrated a hydrophobic site in the region of this para-position, in the isolated sheep umbilical artery 5-HT receptor model. A similar site in the rat fundus model would explain the observed enhancement in affinity upon methylation of the 2,5-dimethoxy compound. If such is the case with our model and if the interaction is similar to that shown in figure 2, then the hydrophobic region would be in the vicinity of the tryptamine 7-position. Green et al. noticed, as we did, the detrimental effect that charge-related terms, involving the 7-position of the tryptamines, had on attempted activity correlations employing the rat fundus preparation (Green, Johnson and Kang 1974). One explanation which they offered was that this position might be coming into contact with a hydrophobic Site. On this basis and because p-methylation doubled the affinity of 2,5-dimethoxyphenylisopropylamine, the 7-methyl analog of DMT was synthesized (Glennon et al. 1978) and evaluated. As expected, 7-methylation of DMT resulted in a two-fold increase in affinity (compound 65)

Another finding is that there is no direct relationship between the affinity of the phenalkylamines for 5-HT receptors of the model and their hallucinogenic potency. This is demonstrated by the similar

TABLE 6

Binding Affinities of Phenylalkylamines [a]



| | $R_2$ | $R_4$ | $R_5$ | R | R' | X | $pA_2$ [b] | n [c] |
|---|---|---|---|---|---|---|---|---|
| 6 | $OCH_3$ | H | $OCH_3$ | $CH_3$ | H | 1 | 6.83 (±.09) | 4 |
| 11 | $OCH_3$ | $OCH_3$ | $OCH_3$ | $CH_3$ | H | 1 | 6.81 (±.08) | 2 |
| 22 | $OCH_3$ | $CH_3$ | $OCH_3$ | $CH_3$ | H | 1 | 7.12 (±.07) | 2 |
| 13 | H | $-OCH_2O-$ | | $CH_3$ | H | 1 | 6.45 (±.04) | 2 |
| 60 | $OCH_3$ | H | $OCH_3$ | H | H | 1 | 6.85 (±.19) | 3 |
| 61 | $OCH_3$ | H | $OCH_3$ | $CH_3$ | $CH_3$ | 1 | 6.50 (±.08) | 2 |
| 62 | $OCH_3$ | H | $OCH_3$ | H | $CH_3$ | 1 | 6.52 (±.19) | 3 |
| 63 | $OCH_3$ | H | $OCH_3$ | H | $CH_3$ | 2 | 5.45 (±.02) | 2 |
| 64 | H | H | H | H | H | 1 | 5.26 (±.02) | 2 |
| 33 | DMT | | | | | | 6.00 (±.08) | 2 |
| 65 | 7-Methyl DMT | | | | | | 6.29 (±.08) | 2 |
| 66 | 2-Methyl DMT | | | | | | 6.02 (±.03) | 2 |

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a Glennon, Liebowitz and Mack 1978
b Values are ± standard deviation
c Number of determinations

affinities of 6 and 60 , while it is known that removal of the α-methyl group of 6 decreases its potency. So, while affinities may be similar, other explanations, such as relative rates of oxidative deamination in vivo might be necessary to account for differences in activity. It might be noted that in those compounds where stereoisomers exist, the racemic mixtures were used.

What other support is there for similarities in phenylisopropylamine and N,N-dimethyltryptamine binding? Demethylation of 5-OMe DMT, to yield 5-OH DMT, results in a two-fold increase in binding affinity. Evoking our structure in figure 2, this would correspond to the 2-position of the phenylisoproylamines. As shown in table 7, demethylation of the appropriate methoxy groups results in an increase in binding affinity in each case(Glennon and Liebowitz 1978). Not enough data have yet been collected to examine the results by molecular orbital or molecular connectivity methods, yet the affinity data appear interesting and informative in their own right.

However, as these data were being collected, Nichols, Shulgin and Dyer (1977) reported their results on the agonistic activity of seventeen phenalkylamines on the 5-HT receptors of the isolated umbilical artery preparation (table 8). They suggested that such activity, coupled with lipophilicty, might be predictive of hallucinogenic potential in man. We explored the structural aspects of these seventeen phenalkylamines by use of the molecular connectivity method (Kier and Glennon 1978). Agonistic activity was found to relate to three molecular connectivity indices according to the following expression:

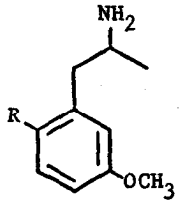
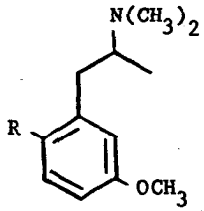
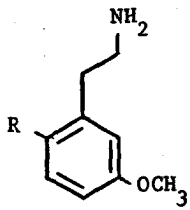
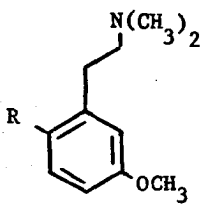
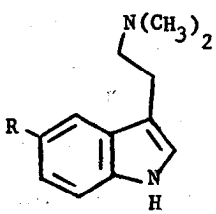
$$\log RBR = 11.07\ {}^{3}\chi^{v}_{p} - 2.78\ ({}^{3}\chi^{v}_{p})^{2} + {}^{4}\chi^{v}_{p} - 21.19 \qquad \text{Eqn IV}$$

$$r = 0.95 \qquad S = .196 \qquad N = 17$$

The predicted activities, using equation IV, are shown in table 8. Of the seventeen compounds for which activity was reported, variation in structure divides these compounds into several structural types. The compounds (a) either possess (phenylisopropyl analogs) or are devoid (phenethylamine analogs) of an α-methyl group and (b) possess either a 2-methoxy or a 3-methoxy substituent. The major structural alteration is at the 4-position, which includes bromo,alkoxy,methlthio and alkyl derivatives and one example of a nitro derivative. It has been demonstrated that the 3, 4, 5-trisubstituted compounds are about as active as 2, 4, 5-trisubstituted compounds of comparable log P in this model system (Nichols, Shulgin and Dyer 1977). For this reason, and because the major structural variation is at the 4-position it might be expected that molecular connectivity indices will focus on these 4-position substituents. Both ${}^{3}\chi^{v}_{p}$ and ${}^{4}\chi^{v}_{p}$ can be related to the α-methyl and suggest an enhanced actitity when this group is present. The ${}^{3}\chi^{v}_{p}$ index also increases by one subgraph as the length of the substituent in the 4-position increases. Because, in the second term, this index is negative and squared, increasing the length of the chain will result in a rapidly diminishing effect on ac-

TABLE 7

Binding Affinities of Methoxy Derivatives and Their Corresponding Hydroxy Analogs [a]

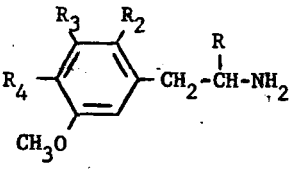
| | $pA_2$ [b] | |
|---|---|---|
| | R = $OCH_3$ | R = OH |
|  | 6.83(±.09) 4 | 7.00(±.08) 6 |
|  | 6.50(±.08) 2 | 6.78(±.09) 3 |
|  | 6.85(±.19) 3 | 7.10(±.09) 3 |
|  | 6.52(±.19) 3 | 6.84(±.13) 2 |
|  | 7.09(±.10) 5 | 7.41(±.05) 2 |

[a] Glennon and Liebowitz 1978

[b] The $pA_2$ values are followed by the standard deviation (in parentheses) and the number of determinations

TABLE 8

Serotonin Receptor Agonist Activity



| | $R_2$ | $R_3$ | $R_4$ | R | Log RBR Obs.[a] | Calc. | Δ |
|---|---|---|---|---|---|---|---|
| 42 | H | $OCH_3$ | $OCH_3$ | H | 0.00 | -0.06 | .06 |
| 43 | H | $OCH_3$ | $OC_2H_5$ | H | 0.31 | 0.40 | .09 |
| 44 | H | $OCH_3$ | $OC_3H_7$ | H | 0.56 | 0.48 | .07 |
| 46 | H | $OCH_3$ | Br | H | 0.88 | 0.78 | .10 |
| 67 | H | $OCH_3$ | O-i-$C_3H_7$ | H | 0.45 | 0.74 | .29 |
| 68 | $OCH_3$ | H | $SCH_3$ | H | 0.83 | 0.97 | .14 |
| 69 | $OCH_3$ | H | $SCH_3$ | $CH_3$ | 1.31 | 1.15 | .16 |
| 70 | $OCH_3$ | H | $NO_2$ | $CH_3$ | 0.67 | 0.64 | .03 |
| 21 | $OCH_3$ | H | Br | $CH_3$ | 1.57 | 1.64 | .07 |
| 22 | $OCH_3$ | H | $CH_3$ | $CH_3$ | 1.00 | 0.78 | .22 |
| 23 | $OCH_3$ | H | $C_2H_5$ | $CH_3$ | 1.59 | 1.31 | .28 |
| 24 | $OCH_3$ | H | n$C_3H_7$ | $CH_3$ | 1.84 | 2.06 | .22 |
| 25 | $OCH_3$ | H | n$C_4H_9$ | $CH_3$ | 1.62 | 1.30 | .32 |
| 26 | $OCH_3$ | H | n$C_5H_{11}$ | $CH_3$ | .88 | 1.02 | .14 |
| 71 | $OCH_3$ | H | t$C_4H_9$ | $CH_3$ | 1.39 | 1.44 | .05 |
| 72 | H | $OCH_3$ | O-n-$C_4H_9$ | H | 0.10 | 0.30 | .20 |
| 73 | H | $OCH_3$ | $OCH_2C_6H_5$ | H | 0.48 | 0.51 | .03 |

[a] Data from Nichols, Shulgin and Dyer 1977, employing an isolated sheep umbilical artery preparation

tivity. Thus, this second term $(^3x_p^v)^2$ governs a maximum potency value with an intermediate length side chain. This appears with 44 in the phenethylamine series with 24 in the phenylisopropylamine series. The great size of the benzyloxy side chain of compound 73 contributes adversely to activity.

The emergence of valence chi terms in the equation indicates that the nature of atoms is of more than passing importance. That means that heteroatoms are playing a role more than just space filling comparable to carbon isosteres. The valence chi terms indicate that ring substituents with heteroatoms are going to differ in potency from alkyl substituents of the same length. The magnitude of the difference depends on the overall size of the substituent and whether its length is less than or exceeds the maximum value governed by the two $^3x_p^v$ indices.

Nichols, Shulgin and Dyer (1977) have discussed the importance of lipophilicity and have found that this parameter correlates with activity if an indicator parameter, necessary to limit the steric length of the 4-position substituent, is additionally introduced into the equation. It would thus appear that this model might be useful in predicting hallucinogenic potency, and that the hydrophobic region of the receptor will need to be considered. Molecular connectivity can be used to describe the structure of molecules in an SAR study of these compounds and so might also prove to be a useful tool in predicting agonistic activity.

In another study by Glennon and Kier (1978) a modelwas invoked in which hallucinogenic potency of LSD analogs was presumed to arise from interaction with 5-HT receptors. Cerletti and Doepfher (1958) have examined the 5-HT antagonistic activity of a series of lysergamides (table 9) which differ only in respect to their amide substituents. Molecular connectivity studies on these molecules produced an equation correlating potency (log relative biological response) with three connectivity indices:

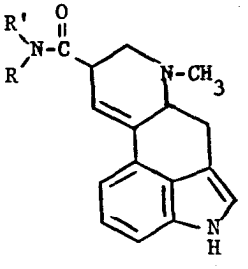
$$\log RBR = 24.94 - 0.835\,{}^2\chi - .917\,{}^6x_p - \frac{1}{0.007\,{}^0\chi^v} \qquad \text{Eqn V}$$

$$r = 0.94 \qquad S = .196 \qquad N = 16$$

A number of conclusions are possible concerning the important structural features contributing to activity. The 5-HT antagonistic activity of the lysergamides is influenced by their amide substituents. Molecular connectivity indices quantify the effect of these substituents which might otherwise have been revealed,only in a qualitative manner, by inspection of the structures. The $^2x$ term suggests that branching of the amide substituents will have an unfavorable effect on activity. An even greater unfavorable effect results from the incorporation of the amide substituent into a cyclic structure as revealed not only by the $^2x$ term but also by the $^6x_p$ term. In addition, the $^6x_p$ indicates that the diethyl amide structure of LSD is optimal for 5-HT antagonism, and that lengthen-

TABLE 9

Observed and Calculated Activities of Lysergamides as 5-HT Antagonists



| | R | R' | Activity (Log RBR) Observed | Calculated |
|---|---|---|---|---|
| 74 | H | H | 0.63 | 0.47 |
| 75 | $CH_3$ | H | 0.81 | 1.10 |
| 76 | $C_2H_5$ | H | 1.08 | 1.35 |
| 77 | $C_3H_7$ | H | 1.60 | 1.49 |
| 78 | $iC_3H_7$ | H | 1.35 | 1.27 |
| 79 | $C_4H_9$ | H | 1.81 | 1.90 |
| 80 | $C_5H_{11}$ | H | 1.87 | 1.65 |
| 81 | $CH_3$ | $CH_3$ | 1.36 | 1.20 |
| 1 | $C_2H_5$ | $C_2H_5$ | 2.00 | 1.84 |
| 82 | $C_3H_7$ | $C_3H_7$ | 1.62 | 1.72 |
| 83 | $iC_3H_7$ | $iC_3H_7$ | 1.37 | 1.53 |
| 84 | $C_4H_9$ | $C_4H_9$ | 1.49 | 1.44 |
| 85 | $-(CH_2)_4-$ | | 0.67 | 0.70 |
| 86 | $-(CH_2)_5-$ | | 0.93 | 0.77 |
| 87 | $-CH_2-CH = CH-CH_2-$ | | 0.61 | 0.51 |
| 88 | $-CH_2\ CH_2-O-CH_2\ CH_2-$ | | 0.31 | 0.57 |

ing both amide substituents will have an increasingly detrimental effect on activity. Shortening both diethyl substituents by one carbon atom, to yield the dimethyl homolog, is even more deleterious than lengthening the diethyl substituents to the dipropyl or dibutyl homlogs. The valence term, $^{o}x^{v}$, indicates that the incorporation of nonaliphatic cyclic substituents, such as those containing a heteroatom or uhsaturation, further reduce activity. In this manner,the influence of each structural change can be quantitated.

Most of the studies now underway in our laboratories are still in their infancy. The object of these investigations is to find biological and/or physicochemical correlates of hallucinogenic activity and then to examine these correlates by means of molecular orbital or molecular connectivity methods. Though hallucinogenic potency can be studied by these methods directly, the use of models might enhance precision and might also yield information on the nature of the binding of these compounds to various receptors.

The direction of our future research, then, will be to accumulate such data by synthesizing and examining various compounds, particularly, modifications of tryptamines and phenylisopropylamines. For example, if both classes of compounds interact with 5-HT receptors in the manner shown in figure 2, then the optimal stereochemistry about the carbon atom bearing the terminal amine might be expected to be opposite in comparing a phenylisopropylamine with an α-methyptamine.

In addition, the behavioral studies will continue, to determine if compounds with high 5-HT receptor affinities will generalize to 5-OMe DMT and DOM stimuli.

It should be stressed that we have not implicated serotonergic mechanisms as playing a role in the hallucinogenic response. Rather, we are using a 5-HT receptor model to investigate the SAR of hallucinogens agents. With such data in hand, molecular orbital, and in particular molecular connectivity investigations should not only provide us with SAR, but will allow us to quantitate these data.

## CONCLUSION

These studies can be discussed at two levels. At the level of results, we can review, as we have just done, a mosaic of studies ranging across several molecular types and several biological responses. The mosaic, as yet, is incomplete. We cannot conclude that the 5-HT receptor is the hallucinogenic receptor, for example, and yet these studies point to trends of information which will surely form a basis for definitive conclusions about mechanism and structure activity relationships.

At another level, we can comment on the broad spectrum of SAR methodology utilized in these studies. It should be clearly noted that we have not confined ourselves to only one model in our research.

We have, for example, not relied solely on isolated molecule studies using sophisticated M. O. theory. Neither have we looked exclusively at affinity data.

The entire gamut of SAR models must be utilized, conservative, dynamic, and mechanistic, in order hopefully to approach the goal of understanding of the complex processes at work in hallucinogenesis.

## ACKNOWLEDGMENTS

We wish to acknowledge the U.S. Public Health Service (grants DA-02046 and DA-01642) for their support of this work. In addition we would like to express our appreciation to NIDA and the Psychopharmacology Research Branch of NIMH for supplying us with certain compounds, to Dr. Max von Strandtmann of WIRI for compound 56 and to Drs. P.K. Gessner, J.A. Rosecrans and J.F. Stubbins for their co-operation and/or helpful discussions. Use of the Data Acquisition facilities at MCV/VCU are greatly appreciated.

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# QSAR of Narcotic Analgetic Agents

E. J. Lien, G. L. Tong, D. B. Srulevitch, and C. Dias

## INTRODUCTION

Analgetics are drugs which alleviate pain without significantly imparing consciousness. Lim et al. (1964) have proposed that analgetics groups into (a) peripherally acting, nonnarotic (e.g., salicylates); (b) centrally acting, nonnarcotic (e.g., d-propoxyphene); and (c) centrally acting, narcotic (e.g., morphine, meperidine, etc.).

The major sites of action of centrally acting narcotic analgetics are the cerebrum and medulla (Korolkovas and Burckhalter 1976); therefore, their ability to pass the blood brain barrier is extremely important. It is now well recognized that this ability is intimately related to the lipophilicity of the drug as measured by lipoid/water partition coefficient (Lien 1975).

Although the precise mechanism of drug-receptor interaction for analgetics has not been elucidated several common structural features have been recognized:(a) a quaternary carbon atom;(b)a phenyl or isostere ring attached to this carbon atom; (c) a tertiray amino group two carbon atoms removed, and (d) a phenolic hydroxyl group in meta position relative to the point of attachment to the quaternary carbon atom if the tertiary nitrogen is part of a six-membered ring (Korolkovas and Burckhalter 1976).

It becomes apparent that both electronic and steric parameters are important besides the lipophilicity of the drug molecule for selective drug-receptor interaction. The main purpose of this report is to examine how the physicochemical properties of the compounds, namely, partition coefficient, molecular weight, molar refraction, and steric constants may affect the analgetic potency or their binding with the receptor.

## METHODS

The first set of data was from the work of Bukckett (1964) on esters of 14-hydroxycodeinone. The relative activity (morphine = 1) was converted to the logarithmic scale in order to be in line with the linear-free energy related parameters like log P.

The partition coefficients of the uuprotonated forms were calculated from the experimental value of morphine and codeine, and the $\pi$ constants of the substituents (Leo, Hansch and Elkins 1971). The log MW and log MR of the side chain (Hansch et al. 1973) were also included in the regression analysis (see Table 1).

The second set of data was from the excellent work of Pert, Snyder and Portoghese (1976) on meperidine homologues. The apparent partition coefficients in octanol-phosphate buffer pH 7.4 (Table 2) (Larson and Portoghese 1976) were used in the correlation. Since the pKa's of the homologues do not vary substantially because of the close $\sigma^*$ values of the R group, the correlations obtained will differ from those using true partition coefficient only by a constant term (Hansch and Lien 1968).

The method of least squares was used in deriving the equations using an IBM 370/155 computer executed through a Tektronix 4012 terminal.

**RESULTS AND DISCUSSION**

The equations correlating the analgetic activities of esters of 14-hydroxycodeinone are summarized in Table 3. The equations correlating the analgetic activities and the receptor binding of N-alkylnormeperidine homlogues are given in Table 4.

From Equations 1 and 4 of Table 3 one can see that there is a parabolic dependence of the analgetic activity on the lipophilicity as represented by 1-octanol/water partition coefficient (log P). The $(\log P)^2$ term in Equation 4 is statistically highly significant as indicated by an F-test ($F_{1,10}$=76; $F_{1,10\ .9995}$=25.5).

Combination of log P with log MW or log P with log MR also gives highly significant correlations (Equations 2 and 3). Addition of log MW to Equation 4 yields Equation 5 which is significant at 90 percentile level ($F_{1,9}$ =4.3; $F_{1,9\ .90}$ =3.4).

The optimum lipophilicity ($\log P_o$) derived from Equation 4 is 3.23 with a 95 per cent confidence interval of 2.92 to 3.46. The $\log P_o$ for Equation 5 is 1.45 with a wider 95 per cent confidence interval. The positive dependence on log MW and log MR probably reflects fairly non-specific Van der Waals interactions with the receptor in addition to hydrophobic interactions. Several possible explanations of the parabolic dependence of activity on log P have been proposed by Hansch and Clayton (1973) and by Lien (1975). It is gratifying to see that the relative analgetic activities of all 13 compounds with side chain R ranging from one to 12 carbon atoms and even aromatic side chains were well correlated with Equation 5 (see Table 1).

For the analgesic activities of N-alkylnormeperidine homologues Equations 6-8 were obtained (Table 4). The correlation coefficient of the parabolic equation (Eq. 7) is only 0.824. This is primarily due to the apparent "biphasic" curve of the log 1/C vs. log P plot as indicated by Figure 1. Meperidine, the lowest member in the series, appears to have activity higher than expected from the

regression. However, when the method of least squares was applied, meperidine did not appear as an outlier. The deviation was only slightly higher than the standard deviation of the regression (0.264 vs. 0.239). Pert, Snyder, and Portoghese (1976) have attributed the relatively high analgetic activity of meperidine to its ability to penetrate the brain (600-fold higher brain level relative to morphine).

From Figure 1 one can also see that meperidine also has relatively high affinity in the inhibition of naloxone binding, especially in the absence of NaCl. Addition of the Hancocks corrected steric constant $E_S^C$ (1961) to the parabolic equation improved the correlation significanrly as indicated by an F-test ($F_{1,5}$ = 124.96; $F_{1,5\ .9995}$ = 63.6). Positive dependence on $E_S^C$ suggests that the bulk tolerance oh the N-substituent is very small.

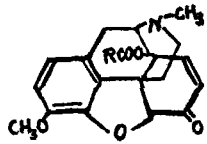
Figure 2 clearly shows that the $E_S^C$ constant changes quickly from $C_1$ to $C_3$ but remains fairly constant from $C_3$ and above. This suggests that perhaps in addition to greater penetrability to the brain, meperidine, having the least hindered N-$CH_3$, will bind more tightly to the receptor site (presumably a negative site). This interpretation is in agreement with the finding that the receptor affinity of meperidine in the absence of sodium is more drastically enhanced than in the presence of sodium ion. The $E_S^C$ terms in Equations 12 and 14 are statistically significant at 99 and 27.5 percentile levels, respectively, ($F_{1,5}$ = 17.76 for Equation 12 and 12.96 for Equation 14). Similar "biphasic" dependence of the analgetic activity of N-alkylnorketobemidones on the length of the N-R side chain has been observed by several investigators (Oh-ishi and May, 1973; Wilson, Rogers, Pert and Snyder, 1975). This again can be attributed to the difference in $E_S^C$ constants .

The log $P_o$values for maximum analgesia obtained from Equations 7 and 8 are around 3. This also agrees with the Peak for inhibition of naloxone binding in the presence of NaCl as seen from Figure 1, as well as from Equations 12 and 14.

From the above-mentioned analysis, it may be worthwhile studying the modification of meperidine and ketobemidone on the benzene ring or the ester or keto side chain to reach a log P of 3 (octanol/buffer pH 7.4) but keep the N-CH group unchanged in order to minimize the steric effect.

**TABLE 1**

Physicochemical Properties and Analgetic Activities of
Esters of 14-hydroxycodeinone in Mice.

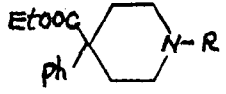



| RCO | log (Relative Activity) obsd.[a] | log (Relative Activity) calcd.[b] | log P[c] | log MW(RCO.) | log MR(RCO) |
|---|---|---|---|---|---|
| $CH_3CO^-$ | 0.60 | 0.46 | 1.40 | 1.18 | 0.75 |
| $C_2H_5CO^-$ | 1.27 | 1.24 | 1.90 | 1.46 | 1.01 |
| $n\text{-}C_3H_7CO^-$ | 1.46 | 1.60 | 2.40 | 1.63 | 1.17 |
| $n\text{-}C_4H_9CO^-$ | 1.59 | 1.74 | 2.90 | 1.76 | 1.29 |
| $n\text{-}C_5H_{11}CO^-$ | 1.67 | 1.67 | 3.40 | 1.85 | 1.38 |
| $n\text{-}C_6H_{13}CO^-$ | 1.78 | 1.45 | 3.90 | 1.93 | 1.46 |
| $n\text{-}C_7H_{15}CO^-$ | 0.71 | 1.13 | 4.40 | 2.00 | 1.53 |
| $n\text{-}C_9H_{19}CO^-$ | 0.05 | 0.03 | 5.40 | 2.10 | 1.63 |
| $n\text{-}C_{11}H_{23}CO^-$ | -1.47 | -1.51 | 6.40 | 2.19 | 1.72 |
| $ph\text{-}CH_2CO^-$ | 1.72 | 2.01 | 3.36 | 1.96 | 1.48 |
| $ph\text{-}CH_2CH_2CO^-$ | 2.06 | 1.76 | 3.86 | 2.02 | 1.54 |
| $phCH{=}CHCO^-$ | 2.25 | 1.91 | 3.66 | 2.01 | 1.53 |
| $CH_3CH{=}CHCO^-$ | 1.49 | 1.61 | 2.20 | 1.61 | 1.16 |

[a] Morphine = 0. Tail clip method. From Buckett, 1964.
[b] Calculated from Eq. 5.
[c] Octanol/water system, calculated from log $P_{morphine}$ = 0.76, log $P_{codeine}$ = 0.76+0.65 = 1.41, where 0.65 = log $P_{C_6H_5OMe}$ - log $P_{C_6H_5OH}$, $\pi CH_3COO$ = -0.01, $\pi CH_2$ = 0.50.

TABLE 2

Physicochemical Properties and Analgetic Activities of N-Alkylnormeperidine Homologues




| R | Analgesia (log 1/Cmoles/kg) | | Opiate Receptor Inhib. (log 1/C) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | (-NaCl) | | (+NaCl) | | | |
| | obsd.[a] | calcd.[b] | obsd.[a] | calcd.[c] | obsd.[a] | calcd.[d] | 30 log $P_{(app)}$ | $E_S^c$ (R) |
| $CH_3^-$(meperidine) | 4.48 | 4.49 | 6.30 | 6.18 | 4.40 | 4.51 | 1.28[e] | 0.00 |
| $C_2H_5^-$ | 4.39 | 4.37 | 5.30 | 5.59 | 4.30 | 4.08 | 1.56 | -0.38 |
| n-$C_3H_7^-$ | 4.34 | 4.34 | 5.40 | 5.26 | 4.00 | 3.88 | 1.85[e] | -0.67 |
| n-$C_4H_9^-$ | 4.70 | 4.73 | 6.05 | 5.89 | 4.52 | 4.62 | 2.13[e] | -0.70 |
| n-$C_5H_{11}^-$ | 5.00 | 5.03 | 6.40 | 6.44 | 4.82 | 5.23 | 2.41 | -0.71 |
| n-$C_6H_{13}^-$ | 5.30 | 5.26 | 6.70 | 6.98 | 5.70 | 5.76 | 2.78[e] | (-0.71)[f] |
| n-$C_7H_{15}^-$ | 5.30 | 5.27 | 7.26 | 7.18 | 6.19 | 5.91 | 3.06 | (-0.71)[f] |
| n-$C_8H_{17}^-$ | 5.00 | 5.08 | 7.52 | 7.20 | 6.10 | 5.77 | 3.43 | (-0.71)[f] |
| n-$C_9H_{19}$ | 4.82 | 4.79 | 6.82 | 7.01 | 5.10 | 5.41 | 3.71 | (-0.71)[f] |

[a] From Pert, Snyder and Portoghese, 1976.
[b] Calculated from Eq. 8.
[c] Calculated from Eq. 12.
[d] Calculated from Eq. 14.
[e] Experimentally determined in octanol-phosphate buffer, pH 7.4, from Larson and Portoghese, 1976. The others were calculated values using $\pi CH_2 = 0.28$, $\pi CH_2CH_2 = 0.65$.
[f] Estimated value, using the $E_S^c$ of n-$C_5H_{11}$.

**TABLE 3**

Physicochemical Properties and Analgetic Activities of Constants of Esters of 14-Hydroxycodeinone in Mice

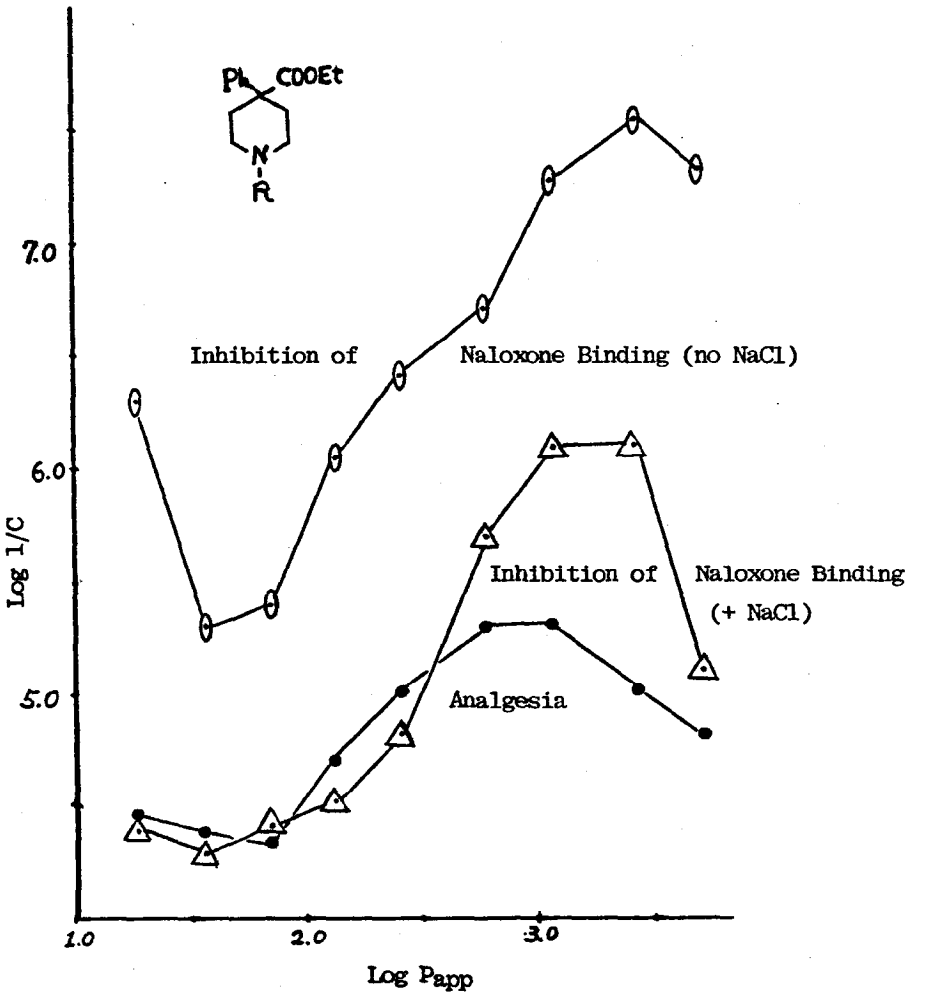
| Eq. No. | | n | r | s |
|---|---|---|---|---|
| 1 | $\log RA = -0.408 \log P + 2.585$ | 13 | 0.569 | 0.860 |
| 2 | $\log RA = 6.267 \log MW_{(R)} - 1.568 \log P - 4.811$ | 13 | 0.952 | 0.337 |
| 3 | $\log RA = 6.853 \log MR_{(R)} - 1.630 \log P - 2.471$ | 13 | 0.950 | 0.342 |
| 4 | $\log RA = -0.339 (\log P)^2 + 2.193 \log P - 1.745$<br>$\log P_0 = 3.23$ (2.92 to 3.46) | 13 | 0.960 | 0.307 |
| 5 | $\log RA = -0.200 (\log P)^2 + 0.581 \log P + 2.924 \log MW_R - 3.412$<br>$\log P_0 = 1.45$ (-22.13 to 3.35) | 13 | 0.973 | 0.267 |

**TABLE 4**

Physicochemical Properties and Analgetic Activities of Constants of N-Alkylnormeperidine Homologues

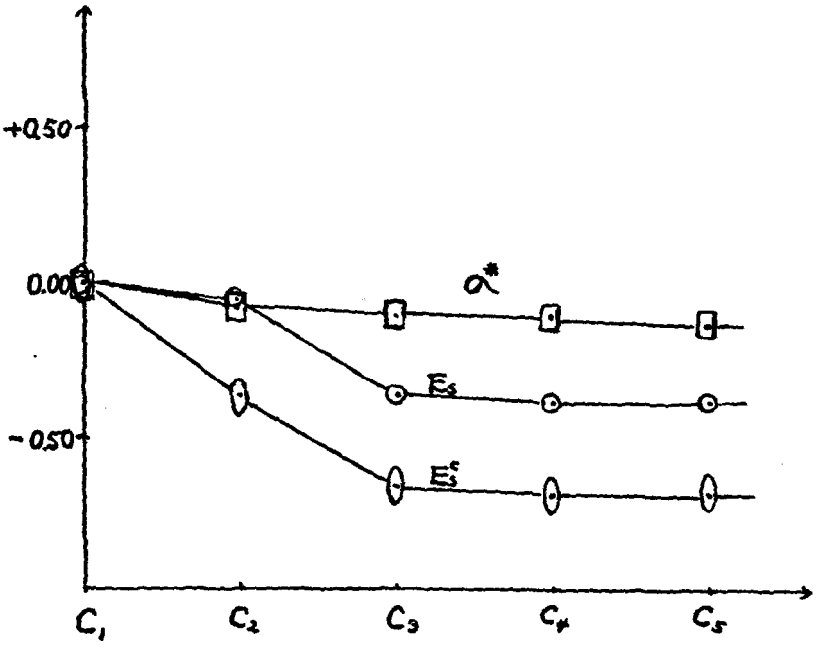
| Eq. No. | | n | r | s |
|---|---|---|---|---|
| | Analgesia (in mice, hot-plate technique) | | | |
| 6 | $\log 1/C = 0.306 \log P_{app} + 4.060$ | 9 | 0.703 | 0.278 |
| 7 | $\log 1/C = -0.275 (\log P_{app})^2 + 1.681 \log P_{app} - 2.515$ | 9 | 0.824 | 0.239 |
| | $\log P_0 = 3.05 (\pm \infty)$ | | | |
| 8 | $\log 1/C = -0.854 (\log P_{app})^2 + 5.041 \log P_{app} + 2.246 E_S^c - 0.563$ | 9 | 0.994 | 0.051 |
| | $\log P_0 = 2.95 (2.90 \text{ to } 3.01)$ | | | |
| | Inhibition of [$^3$H] naloxone binding (without NaCl) | | | |
| 9 | $\log 1/C = 0.718 \log P_{app} + 4.646$ | 9 | 0.796 | 0.491 |
| 10 | $\log 1/C = 1.015 \log P_{app} + 1.436 E_S^c + 4.757$ | 9 | 0.860 | 0.446 |
| 11 | $\log 1/C = 0.077 (\log P_{app})^2 + 0.333 \log P_{app} + 5.078$ | 9 | 0.798 | 0.528 |
| 12 | $\log 1/C = -1.075 (\log P_{app})^2 + 7.015 \log P_{app} + 4.471 E_S^c - 1.043$ | 9 | 0.959 | 0.271 |
| | $\log P_0 = 3.26 (3.01 \text{ to } 4.85)$ | | | |
| | (with NaCl) | | | |
| 11 | $\log 1/C = 0.751 \log P_{app} + 3.161$ | 9 | 0.780 | 0.541 |
| 12 | $\log 1/C = -0.207 (\log P_{app})^2 + 1.787 \log P_{app} + 1.997$ | 9 | 0.794 | 0.568 |
| 13 | $\log 1/C = 0.891 \log P_{app} + 0.675 E_S^c + 3.213$ | 9 | 0.794 | 0.509 |
| 14 | $\log 1/C = -1.398 (\log P_{app})^2 + 8.698 \log P_{app} + 4.622 E_S^c - 4.333$ | 9 | 0.947 | 0.328 |
| | $\log P_0 = 3.11 (2.91 \text{ to } 3.97)$ | | | |

FIGURE 1




Dependence of the Analgesic Activity and Receptor Binding Affinity on the Apparent Partition Coefficient

**FIGURE 2**




Dependence of the Electronic and Steric Constants on the Chain Length, where $E_S^C = E_S + 0.306\ (n-3)$

**ACKNOWLEDGMENT**

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# Section III

# Molecular Mechanics

The presentations using quantum mechanical calculations are primarily intended to predict drug activity. The rather large number of papers using this method of approachillustrates the extent to which this is a current tool in QuaSAR studies. These calculations can be classified into two categories: The isolated molecule model, which provides information on the early stages of the drug-receptor interaction; and molecule-receptor-complex models, which are designed to obtain insight on relevant features of the drug molecule at the receptor site. In both cases the quantum mechanical models explore "intrinsic" activity at the receptor site rather than overall in vivo activity.

One special application of the isolated model approach is tne calculation of molecular conformations. Similarities in conformation, charge distribution and size of atoms or groups of atoms may suggest similar binding to the receptor. However, inferences from calculated conformations should be made cautiously: the molecule may possess several close lying conformations and varying aegrees of flexibility; the active conformation(s) is (are) not known; and the calculated geometries are "method dependent." This last point is actually true for any quantum mechanical index but the situation is nevertheless not hopeless: ab initio Hartree-Fock calculations are generally reliable and f'ull configuration interaction calculations, when possible to carry out from a practical standpoint, give the exact non-relativistic energies; on the other hand, the simple semi-empirical procedures (e.g., IEHT), carefully calibrated and judiciously applied can be well correlated to experimental data. It was pointed out by Dr. Kaufman that perhaps the greatest caution is necessary in using the intermediate calculational schemes (e.g., CNDO). The paper of Katz et al. (this monograph) is an illustration of this point.

An alternative method of calculating molecular geometries via classical potential functions has also been used on isolated and solvated molecules. While this method has the advantage of being relatively simple to use, it does have the limitation of being an empirical approach.

Some concern was expressed about conclusions that are based on properties of individual molecular orbitals (e.g. HOMO and LUMO). Within the Hartree-Fock functional form for a molecular wave function, the orbital energies and orbital symmetries are both invariant quantities and thus quantum mechanical observables. The shape of a molecular orbital is not an invariant property and in fact is strongly dependent on the orbital basis set used in the calculations. However, the careful use of individual molecular orbitals in predicting chemical behavior is clearly justified by its practical success over the years.

'QuaSAR' Research Monograph 22
G. Barnett, M. Trsic, and R. Willette, eds.
National Institute on Drug Abuse, 1978

# Quantitative Structure-Activity Relationships in the 2,4,5-Ring. Substituted Phenylisopropylamines

George M. Anderson III, Neal Castagnoli, Jr., and Peter A. Kollman

**ABSTRACT**

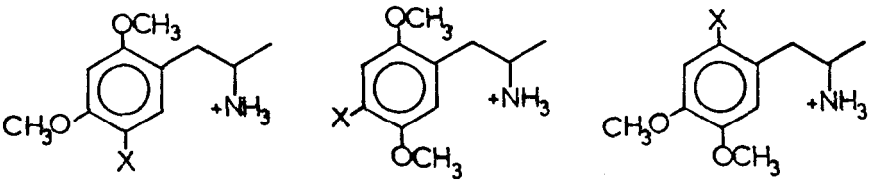
The potency of a series of 2,4,5-ring substituted phenylisopropylamines was examined using the rabbit hyperthemia assay. An excellent correlation (r=0.99) was found between the rabbit hyperthermic and human psychotomimetic potencies. In the hyperthermic model, the 4-X-substituted-2,5-dimethoxyphenylisopropylamines were found to be one to two orders of magnitude more potent than the 2-X- or 5-X-substituted positional isomers (X=-H,-$CH_3$,-$SCH_3$,-Br). Conformational perturbations induced by substituents ortho to the ethylamine side chain were studied with the PCILO and ab initio molecular orbital methods. The variations in the biological activities could not be rationalized in terms of the ability of the ortho substituents to stabilize conformations which mimic LSD. The electronic structures of the positional isaners were examined in the corresponding toluene analogues using the CNDO/2 method. A reasonable correlation (r=0.98) was found between the Highest Occupied Molecular Orbital (HOMO) energy and the ionization potentials reported from photoelectron spectroscopy studies. In the case of the positional isomers, the HOMO energies were ordered as follows: 4-X>5-X>2-X. However, the regression analysis of the relationship between these orbital energies and Log Biological Activity (B.A.) was not impressive. Examination of the partitioii coefficients (octanol/water) of the positional isomers indicated that the 4-X- and 5-X-substituted compounds have almost equivalent Log P's, but that the 2-X-substitute-4,5-dimethoxyphenylisopropylamines are unusually hydrophilic. The regression of Log H.P. to the HOMO energies resulted in a marginally significant relationship; addition of the Log P's resulted in no significant improvement. Qualitative models based on both regiospecific lipophilicity or electron densities and also metabolic conversion to reactive intermediates are presented.

**INTRODUCTION**

Structural manipulation of the aromatic moiety of amphetamine has provided numerous methoxy-substituted analogues and a variety of 4-substituted-2,5-dimethoxyphenylisopropylamines. Attempts to relate

the psychotomimetic potency of these compounds to physical properties have elaborated three "themes." The first approach is based on the basis of physical properties suggested by Karreman et al. (1959) and further elucidated by Snyder and Merrill (1965) and Kang and Green (1970) proposing that potency is related to the electron donor properties of the aromtic system. In the second scenario, addressed by Snyder (1970), Kang and Green (1970), Baker et al. (1973), and Nichols et al. (1978), the potency of various classes of agents having the arylethylamine structure is related to the ability of the side chain to assume conformations which mimic the potent psychotomimetic LSD. Finally, Barfknecht et al. (1975) and Nichols et al. (1978) have pointed to the correlation of activity with lipid solubility and implicated the importance of distribution effects in modulating the psychotomimetic potency of the ring substituted phenethylamines and phenylisopropylamines.

In the intact animal, psychotomimetic activity appears to be a complex function involving all of these variables. Since this is the case, it is difficult to draw conclusions about molecular events from the structure activity relations. We have attempted to simplify the structure activity relations by designing a novel series of "rearranged"' phenylisopropylamines bearing substituents at the 2,4, and 5 positions:




The logic behind our approach is that positional isomers might be expected to be isolipophilic. We had therefore hoped to factor the electronic and conformational aspects of the activity equation from distribution considerations. With these expectations in mind, we began our theoretical investigation into the physical parameters of conformation and electronic structure using molecular orbital methods. Specifically, we have studied the side chain conformation of various ortho-substituted phenethylamines and attempted to relate this information to the biological activities of 2-X-substituted-4,5dimethoxyphenylisopropylamines. In parallel with this, we present rabbit hyperthermic potencies of these rearranged isomers and demonstrate a good correlation between these numbers and human psychotomimetic potencies. Next, we have analyzed the effects of substituents on the orbital energies and electron densities of the aromatic ring. We have evaluated the validity of the assumption that positional isomers are isolipophilic and have attempted to explain the deviations in the partition coefficients in terms of electronic effects. The data have also been interpreted in terms of how structural effects may influence the metabolic fate of these compounds. Finally, our studies have led us to propose potentially interesting analogues for further analysis.

## METHODS AND PROCEDURES

Synthesis of the "rearranged" positional isomers was afforded by condensation of the appropriately substituted benzaldehydes with nitroethane followed by reduction of the phenylnitropene with lithium aluminum hydride. Details of the synthetic scheme will be described elsewhere.

The biological activities of compounds (1)-(14) were measured using the rabbit hyperthermia assay and the potencies are reported in Standard Rabbit Units (SRU) in which racemic DOM (5) is taken as the reference. Details of the assay have been reported by Jacob III et al. (1977).

The PCILO semiempirical molecular orbital method (Diner et al. 1967) was used to study the conformational structure of the ethylamine side chain in a variety of ring substituted phenethylamines. Standard bond lengths and angles were used and parameters for the bromine atom were taken from the previous assignments of Kollman et al. (1973). Extensive applications of this method to problems of biological interest have been made by Pullman et al. (1973), who have demonstrated the reliability of the calculated conformational minima. Rotations were made about the $T_1$ and $T_2$ axes of the phenethylamine side chain in increments of 30°, and the energies of the localized Kekúle structures were averaged. The energy of several of the PCILO minima were also calculated with the ab initio program Gaussian 70 (Hehre et al. 1974) using the STO-3G basis set

The electronic structure of the variously substituted analogues was examined in the corresponding substituted toluenes using CNDO/2. The Highest Occupied Molecular Orbital energies were taken at the conformational minima with respect to 30° rotation of the methoxy substituents. Calculated HOMO energies have been shown by Domelsmith, et al. (1977) to correlate with ionization potentials measured by photoelectron spectroscopy. The technique is also appropriate for describing rotations about a bond with partial bond character as occurs with methoxy substituted benzenes.

The distribution characteristics of compounds (1)-(14) were modeled by partition coefficients (octanol/water). Substituent contributions to the overall molecular lipophilicity were derived from the measured Log P of the substituted phenylisopropylamine or from a study of model compounds. When partition coefficients were determined in solvents other than octanol, both the substituted and reference values were taken from the same solvent, converted into the Log P in octanol according to the equations of Leo et al. (1971), and subtracted to give the substituent $\pi$ values.

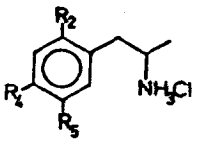
## RESULTS AND DISCUSSION

### Rabbit Hyperthermia

The rabbit hyperthermia assay has been employed in the pharmacological evaluation of a series of LSD analogs (Cerletti 1959), substituted tryptamines (Brimblecombe 1967), and phenylisopropylamines (Aldous

et al. 1974). The applicability of this animal model to psychoactive agents can be measured in terms of the correlation between the animal and human potencies. The hyperthermic potencies of compounds (1)-(14) are summarized in table 1; an excellent correlation exists between the hyperthermic and human potencies (n=8, r=0.99). As can be seen, the most potent compounds were the 4-X-substituted-2,5-dimethoxyphenyl-isopropylamines. In this series, substitution of the para-H with a $-OCH_3$, $-SCH_3$ $-CH_3$ and -Br group results in large increases in hyperthermic potency. Similar increases in activity are not achieved in the 5-X-substituted-2,4-dimethoxyphenylisopropylamines which all have

**Table 1. Comparison of Human Psychotomimetic Potency, Rabbit Hyperthermic Potency, Ionization Potentials, CNDO/2 Calculated Highest Occupied Molecular Orbital Energies, and Octanol-Water Partition Coefficients for the "Rearranged" Phenylisopropylamines**




| Compound | $R_2$ | $R_4$ | $R_5$ | Human Potency (MU) | Hyperthermic Potency (SRU) | I.P. (ev) | MNDO Energy (a.u.) | Log P |
|---|---|---|---|---|---|---|---|---|
| (1) | $OCH_3$ | $OCH_3$ | H | 5[a] | 3 | 7.91[g] | -0.410 | 1.75[h] |
| (2) | $OCH_3$ | H | $OCH_3$ | 8[a] | 3 | 7.70[g] | -0.409 | 1.72[i], 1.88[h] |
| (3) | H | $OCH_3$ | $OCH_3$ | <1[b] | 0.3 | 8.03[g] | -0.419 | 1.20[i], 1.00[h] |
| (4) | $OCH_3$ | $OCH_3$ | $CH_3$ | --- | 1 | --- | -0.403 | 2.24[j] |
| (5) | $OCH_3$ | $CH_3$ | $OCH_3$ | 80[a] | 100 | 7.62[g] | -0.400 | 2.24[i], 2.08[h] |
| (6) | $CH_3$ | $OCH_3$ | $OCH_3$ | --- | 0.5 | --- | -0.407 | 1.76[k] |
| (7) | $OCH_3$ | $OCH_3$ | $SCH_3$ | --- | 3 | --- | -0.409 | 2.17[l] |
| (8) | $OCH_3$ | $SCH_3$ | $OCH_3$ | 50[c] | 54 | 7.64[g] | -0.405 | 2.17[i] |
| (9) | $SCH_3$ | $OCH_3$ | $OCH_3$ | --- | 2 | --- | -0.412 | 1.81[m] |
| (10) | $OCH_3$ | $OCH_3$ | Br | 4[d] | 2 | --- | -0.375 | 2.54[n] |
| (11) | $OCH_3$ | Br | $OCH_3$ | 400[e] | 405[f] | 7.94[g] | -0.375 | 2.54[i], 2.58[h] |
| (12) | Br | $OCH_3$ | $OCH_3$ | --- | 3 | --- | -0.377 | 2.06[o] |
| (13) | $OCH_3$ | $OCH_3$ | $OCH_3$ | 16[a] | 12 | 7.66[g] | -0.397 | 1.10[i], 1.74[h] |
| (14) | H | H | H | --- | 1 | 8.99[g] | -0.473 | 1.63[p] |

[a]Shulgin et al. (1969); [b]Shulgin, (1978); [c]Shulgin et al (1976); [d]Sepulveda et al. (1972); [e]Shulgin et al (1971); [f]Aldous et al (1974); [g]Domelsmith and Houk (1978); [h]Barfknecht et al. (1975); [i]Nichols, (1977); [j]Assumed to be isolipophilic with compound (5). [k]Log P = Log $P_{3,4-(OCH_3)_2\text{-phenylisopropylamine}} + \pi_{CH_3}$ = 1.20 + 0.56. [l]Assumed to be isolipophilic with compound (8). [m]Log P = Log $P_{3,4-(OCH_3)_2\text{-phenylisopropylamine}} + \pi_{SCH_3}$ = 1.20 + 0.61. [n]Assumed to be isolipophilic with compound (11). [o]Log P = Log $P_{3,4-(OCH_3)_2\text{-phenylisopropylamine}} + \pi_{Br}$ = 1.20 + 0.86. [p]Vree, et al (1969).

comparable potencies, except in the case of the methoxy analogue which clearly is more potent. In the 2-X-substituted-4,5-dimethoxyphenylisopropylamines, replacement of the ortho-H with any group increases activity, but only the $-OCH_3$ analogue has any substantial potency.

**Analysis of Conformational Properties**

The hyperthermia results indicated that potent activity was generally associated with analogues bearing an ortho methoxy group. Since ortho substituents could be expected to influence the arylethylamine conformation, we have attempted to explain the inactivity of the ortho -H, $-SCH_3$, $-CH_3$ and -Br analogues in conformational terms. Historically, thel psychotomimetic potency of the ring-substituted phenethylamines, phenylisopropylamines and tryptamines has been rationalized in terms of the ability of the ethylamine side chain to assume conformations which mimic certain aspects of the LSD structure. In the model proposed by Snyder et al. (1975), the crucial phenethylamine conformations are those which mimic the B and C ring of LSD. The "B Ring Mimic" was envisioned to arise from a gauche conformation ($T_1$=180°, $T_2$=0°) in which the side chain was planar with respect to the aromatic ring and the amine was folded back toward an ortho-H substituent. The C Ring of LSD was thought to be mimicked by a folded gauche conformation ($T_1$=0°, $T_2$=40°) in which the arylethylamine protons were involved in an intramolecular interaction with the electron density centered on the oxygen atom of an ortho methoxy substituent. Kang and Green (1970) have suggested that the LSD structure could be mimicked by an extended trans conformation ($T_1$=150°, $T_2$=170°) in which the phenethylamine portions both LSD and the ring substituted phenethylamines and phenylisopropylamines were directly superimposed. Baker et al. (1973) have analyzed the X-ray structure of LSD and 2,4,5-trimethoxyphenylisopropylamine (HCl salt) and have proposed a mimic conformation ($T_1$=40° , $T_2$=240°) in which the amine portions of the phenethylamines were superimposed with the N(6) of LSD and an ortho methoxy group was paired with the 9,10 double bond of LSD. Recently Nichols et al. (1978a) have proposed a mimic conformation which was similar to a trans conformation proposed by Kang and Green (1970), but the phenyl ring oriented differently with respect to the indole ring of LSD ($T_1$=150°, $T_2$=-120°). Nichols superimposed the N(6) and C(2) portions of the LSD structure on the amine and ortho methoxy groups of the ring substituted phenethyl and phenylisopropylamines,

Since these conformational theories were based on consideration of analogues bearing either hydrogen or methoxy substituents at the ortho position, it was of interest to reexamine the applicability of these conformational predictions for the novel series of 2-X-substituted-4,5-dimethoxyphenylisopropylamines studied by us. Thus, a PCILO conformational analysis of these analogues has been carried out and is summarized in table II.

There have been many theoretical studies of phenethylamine conformation using a variety of methods (Martin et al. 1975; Weintraub and Hopfinger 1973; Hall et al. 1975 and Pullman and Pullman 1975). These theoretical calculations have generally indicated that the side chain of these variously substituted phenethylamines was conformationally quite

unrestricted and that the trans conformation was slightly less favorable than the gauche. The location of the global and local minima for these compounds was similar to those obtained by us using PCILO.

Our PCILO calculations predict that in the gas phase or in an inert solvent, the folded gauche conformation ($T_1$=90°, $T_2$=60°) was about 1 kcal/mole more stable than the extended trans conformation ($T_1$=90°, $T_2$=180°). In the neutral phenethylamine,this can be rationalized by N-H...$\pi$ attractions which preferentially favor the folded forms. In the protonated phenethylammonium, these attractions result in stabilizationofthe gauche foms relative to the trans conformations. The introduction of electron donating groups such as a methoxy groups in the para position further increases the electron donating capacity of the aromatic ring and lowers the energry of the gauche confomers. Comparison of the unsubstituted phenethylammonium with compounds substituted in the ortho position by a -$OCH_3$, -OH, -$SCH_3$ groups revealed that in these compounds, one of the gauche conformations ($T_1$=120°, $T_2$=90°) was stabilized via N-H...$OCH_3$, N-H...OH, N-H...$SCH_3$ intramolecular attractions. In the orthomethoxyphenethylammonium molecule, PCILO found the global minimum to be the gauche conformation in which the ammonium hydrogen points to the oxygen electrons and predicted this conformation to be 6.5 kcal/mole more stable than the trans orientation. The intramolecular stabilization was considerably weaker in the orthothiomethyl case where the gauche conformation is energetically only 1.7 kcal/mole more favorable the trans. In the ortho -$CH_3$ and -Br substituted phenethylammoniums, gauche conformation ($T_1$=90°, $T_2$=-60°) in which the amine is folded toward the aromatic ring on the side opposite to the ortho substituent was found to be the global minimum and slightly more stable than the local trans minimum.

Ab initio calculation of the trans and gauche PCILO minima for phenethylammonium showed the gauche to be preferred over the trans form by 0.24 kcal/mole, in excellent agreement with the PCILO energies (0.30 kcal/mole). A similar comparison of the energies calculated by the ab initio and PCILO methods at the trans and gauche minima of orthomethoxyphenethylammonium found the gauche conformation to be more stable than the trans by 16.5 kcal/mole and 6.5 kcal/mole respectively.

The relevance of gas phase conformations in biological systems is indirect at best and calculations at all levels which have considered solvent effects have shown the trans conformations to be preferentially stabilized relative to the gauche (Weintraub and Hopfinger 1973; Pullman 1974). Determination of the $T_2$ rotamer populations of phenethylamine (Ison et al. 1973), dopamine (Bustard and Egan 1971) and numerous poly-substituted phenylisopropylamines (Bailey et al. 1971) via NMR have shown almost equal populations of trans and gauche conformations in solution. X-ray structures have been reported for the salts of phenethylamine (Tsoucaris 1961), dopamine (Bergin and Carlstrom 1968), mescaline (Ernst and Cagle 1973), amphetamine (Bergin and Carlstrom 1971) and 2,4,5-trimethoxyphenylisopropylamine (Baker et al. 1973). In each crystal structure, an extended trans conformation ($T_1$=70-89°, $T_2$=171-175°) was found except for the 2,4,5-trimethoxy compound ($T_1$=67°, $T_2$=50°).

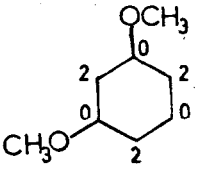
A number of workers have proposed phenethylamine conformations which allow it to mimic LSD at a hypothetical hallucinogenic receptor. We have used our PGILO calculations to evaluate the energy of these conformations and have attempted to rationalize the conformational effects of the ortho substituents in terms of these mimic structures. If the phenethylamine binds to the receptor 103 times weaker than LSD and has the same total intrinsic attraction at its LSD mimic conformation, one would expect that a reasonable upper bound for the conformational energy would be about 4 kcal/mole relative to the global minimum ($\Delta G=-2.3RT \log(10)^3$). If LSD has a greater intrinsic affinity for the receptor than the LSD mimic conformation of the analog, then the upper bound of the conformational energy must be correspondingly less to compensate for this.

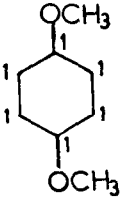
Since our calculations employed fixed internal bond lengths and angles, the energy of conformations in which there are significant steric effects ($T_1$ between -40° and 40° or between 140° and 220°) is probably an overestimate of the true energy for these conformations. Table II lists the energies for the two LSD mimic conformations proposed by Snyder and the mimics proposed by Kang and Green (1970) , Baker et al. (1973) and Nichols (1978).

It is clear that the Snyder conformations are impossibly high in energy but that the other three are energetically quite reasonable. However, comparison of the energies of the latter conformational mimics of the ortho-H and -$OCH_3$ analogues predicts that the ortho-$OCH_3$ phenethylammonium should bind correspondingly weaker than phenethylammonium, a prediction not supported by the biological activities. Further, no explanation for the low potency of the ortho substituted -$SCH_3$, -$CH_3$, and -Br analogues can be clearly defined in terms of LSD mimic conformations.

**Electron Donating Properties**

The apparent inability of conformational arguments to rationalize the variations in hyperthermic potency led us to a detailed investigation of the electronic structure of compounds (1) - (14). A very simple minded glance at the rearranged isomers might suggest that the electron donating properties of the aromatic rings would be very similar. However, CNDO/2 calculations as well as recent photoelectron spectroscopy measurements (Domelsmith and Houk 1978) have indicated to the contrary (see table I). On a qualitative level, these electronic differences are apparent from the resonance structures. For example, the dimethoxy benzenes, shown below, are accompanied by the number of resonance structures which place excess charge on the carbon atoms of the aromatic ring.



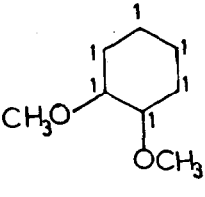
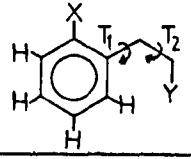



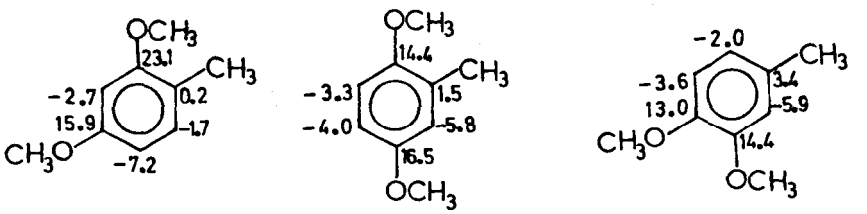
**Table II.** PCILO Conformational Analysis of a Series of Substituted Phenethylamines




| X= | Y= | Global Minimum[a] | Local Minimum[a] | Energy (kcal/mole)[b] S I[c] | S II[d] | KG[e] | B[f] | N[g] |
|---|---|---|---|---|---|---|---|---|
| -H | $NH_3(+)$ | G I = G II | T (0.9) | 253 | 480 | 1.3 | 5.5 | 1.4 |
| $-OCH_3$ | $NH_3(+)$ | G I | G II (6.9)<br>T (6.5) | 260 | 278 | 8.5 | 15.3 | 8.7 |
| -OH[h] | $NH_3(+)$ | G I | G II (7.6)<br>T (7.1) | 1705 | 474 | 9.6 | 12.3 | 13.4 |
| -OH[i] | $NH_2$ | G I | G II (1.4)<br>T (0.8) | 10088 | 21.7 | 90.4 | 4.5 | 36.6 |
| $-SCH_3$ | $NH_3(+)$ | G I | G II (0.9)<br>T (1.7) | 256 | 981 | 3.5 | 5.7 | 4.0 |
| $-CH_3$ | $NH_3(+)$ | G II | G I (1.1)<br>T (0.7) | 251 | 2117 | 1.6 | 5.5 | 4.9 |
| -Br | $NH_3(+)$ | G II | G I (0.3)<br>T (0.5) | 253 | 1061 | 2.2 | 5.4 | 4.3 |

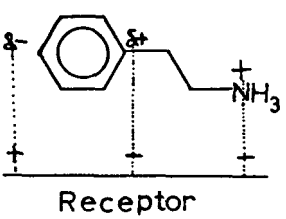
[a] G I = gauche conformation with $NH_3(+)$ group toward X ($T_2>0$) G II = gauche conformation with $NH_3(+)$ group away from X ($T_2>0$); T = trans conformation. [b] Energy above the conformational minimum. [c] "B ring mimic" of Snyder (1970). [d] "C ring mimic" of Snyder (1970). [e] LSD mimic of Kang and Green (1973). [f] LSD mimic of Baker et al. (1973). [g] LSD mimic of Nichols (1978). [h] -OH pointed away from side chain. [i] -OH pointed toward side chain.

The trends seen in the $\pi$ densities are also reflected by the CNDO/2 calculated Mulliken population densities which account for both the $\sigma$ and $\pi$ electrons.




Calculation of the HOMO energies for compounds (1)- (11) (excluding the Br isomers which are suspect) has been made and a satisfactory correlation (n=7, r=098) can be shown between these orbital energies and the ionization potentials determined by photoelectron spectroscopy in agreement with Koopmans Theorem. While the orbital energy differences between the various rearranged isomers were small, the CNDO/2 results indicated that the ionization potentials were ordered as follows: 4-X<5-X<2-X. However, the regression of these HOMO energies with the hyperthermic potencies (log H.P.) was quite impoverished (r=0.65) and barely significant at the 95% confidence level. The good correlations reported by others for the methoxy substituted phenylisopropylamines were not to be found among the rearranged isomers studied by us.

While quantitative electronic models seemed outside our grasp, a qualitative picture of drug-receptor inter-reaction did emerge:




Two pharmacophoric sites are postulated in the drug binding, the first involving the charged ammonium group and the second resulting from an electronic association between the receptor and the aryl-moiety. It is clear that good binding results from electron rich aromatic systems which have both large negative electrostatic potentials at the van der Waals radius (=1.7Å) and also favorable electrostatic potential gradients parallel to the aromatic plane as have been described by Weinstein at al. (1976) for the 4,5,6, and 7-OH tryptamines. In general, addition of electron donating substituents such as a methoxy group was found to augment the $\pi$ electron density of the aromatic ring and presumably results in favorable electronic associations. The inplane electrostatic gradients were, however, quite sensitive to the position of the methoxy substituents. For example, in the

positional dimethoxy benzenes discussed previously, the resonance arguments predicted low $\pi$ density at $C_4$ and high density at $C_1$ for the 2,4-dimethoxy substituted benzene, but little gradient was found in the 2,5- or 3,4-dimethoxy substituted benzenes. (The Mulliken populations are also consistent with this reasoning.) These gradients predict that analogues which are electron rich at the 3,4, and 5 positions and relatively electron poor at the 1,2, and 6 positions might be most potent at our diagrammatic receptor. While the unfavorable electrostatic gradient of the 2,4-dimethoxy orientation might be invoked to account for the low potency of the 5-X-substituted-2,4-dimethoxyphenisopropylamines, apparently other arguments must be advanced to separate the very potent 4-X-substituted-2,5-dimethoxy-phenylisopropylamines from the weakly potent 2-X-substituted-4,5-dimethoxyphenylisopropylamines.

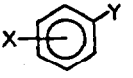
**Distribution Properties**

If the molecular partition coefficient is assumed to be the linear sun of the component $\pi$ substituent values, then the Log P's of the mono-, di-, tri-, and tetramethoxy substituted phenylisopropylamines would be almost equivalent (±0.10). Were this actually the case, one would expect a rather poor correlation between the small differences in the distribution characteristics and the rather large variations in the biological activity of this class of phenylisopropylamines. Further, the various positional isomers would be isolipophilic and hence would be incorrectly predicted to be equiactive. These expectations have not been supported by experiment.

Barfknecht et al. (1975) have measured the partition coefficients of various methoxy substituted phenylisopropylamines and have shown conclusively that nonadditativity of $\pi$ values was more the rule than the exception for these compounds. Some Precedent for these findings could be taken from the work of Leo et al. (1971) in which the $\pi$ value of the central methoxy group in 1,2,3-trimethoxybenzene was derived to be -0.56 and much closer to the $\pi$ value of an aliphatic methoxy group (-0.47) than an aromatic methoxy group (-0.02). It was postulated that unfavorable steric repulsions forced the central methoxy group out of the plane making the hybridization of the oxygen more "sp3" and hence resembling an aliphatic methoxy group.

Because of the nonadditive nature of these substituents, we have analyzed the $\pi$ values of the various positional mono- , di-, and trimethoxy configurations in a number of model compounds and have summarized these results in table III. As can be seen, the positional monomethoxy isomers had almost equivalent $\pi$ values ($\pi$=0.03 - 0.09) and were reasonably close to the traditional value of -0.02. Similar results were obtained for the 2,3-, 2,4-, and 2,5-dimethoxy orientations. However, in the case of the 3,4-dimethoxy grouping, a very large neighboring group interaction substantially lowered the $\pi$ substituent value. In the trimethoxy arrangements, the 2,4,6- pattern appears to have a normal $\pi$ value, whereas the 2,4,5-trimethoxy $\pi$ value derived from Nichols et al. (1978) measurement resembles the 3,4-dimethoxy $\pi$ value in that it is considerably more hydrophilic than expected. The 2,3,4- and 3,4,5-trimethoxy $\pi$ values are very similar ($\pi$=0.20) and somewhat less lipophilic than expected.

**Table III.** Model System Study of the Positional Monomethoxy $\pi$ Substituent Values, and the Positional Di- and Trimethoxy $\pi$ Multisubstituent Values




| X = Monomethoxy | $\pi_{4\text{-}OCH_3}$ | $\pi_{3\text{-}OCH_3}$ | $\pi_{2\text{-}OCH_3}$ |
|---|---|---|---|
| Y = $-COOH$[a] | 0.09 | 0.15 | --- |
| $-CHCHNO_2$[b] | -0.06 | 0.09 | 0.33 |
| $-CHC(CH_3)NO_2$[b] | -0.34 | -0.06 | -0.07 |
| $-CH_2COOH$[a] | 0.01 | 0.09 | --- |
| $-OCH_2COOH$[a] | 0.12 | -0.03 | -0.33 |
| $-OH$[a] | -0.12 | 0.12 | --- |
| $-NH_2$[a] | 0.05 | 0.05 | 0.03 |
| $-CONH_2$[a] | 0.22 | 0.30 | 0.23 |
| $-CH_2CH(CH_3)NH_2$[c] | 0.14 | --- | --- |
| $-CH_2CH(CH_3)NH_2$[d] | 0.15 | --- | --- |
| Average= | 0.03 | 0.09 | 0.04 |
| (Stand. Dev.) | $\pm$0.19 | $\pm$0.10 | $\pm$0.25 |

| X = Dimethoxy | $\pi_{2,3\text{-}(OCH_3)_2}$ | $\pi_{2,4\text{-}(OCH_3)_2}$ | $\pi_{2,3\text{-}(OCH_3)_2}$ | $\pi_{3,4\text{-}(OCH_3)_2}$ |
|---|---|---|---|---|
| Y = $-CHCHNO_2$[b] | 0.13 | 0.23 | -0.01 | -0.86 |
| $-CHC(CH_3)NH_2$[c] | -0.11 | -0.14 | -0.19 | -0.92 |
| $-CH_2CH(CH_3)NH_2$[c] | --- | 0.12 | 0.25 | -0.43 |
| $-CH_2CH(CH_3)NH_2$[d] | --- | --- | 0.07 | -0.75 |
| Average = | 0.01 | 0.07 | 0.03 | -0.74 |
| (Stand. Dev.) | $\pm$0.12 | $\pm$0.15 | $\pm$0.16 | $\pm$0.19 |

| X = Trimethoxy | $\pi_{2,3,4\text{-}(OCH_3)_3}$ | $\pi_{2,4,5\text{-}(OCH_3)_3}$ | $\pi_{2,4,6\text{-}(OCH_3)_3}$ | $\pi_{3,4,5\text{-}(OCH_3)_3}$ |
|---|---|---|---|---|
| Y = $-CH_2CH(CH_3)NH_2$[c] | -0.27 | (0.11) | -0.06 | -0.34 |
| $-CH_2CH(CH_3NH_2$[d] | -0.12 | -0.53 | --- | -0.12 |
| Average = | -0.19 | ? | (-0.06) | -0.23 |
| (Stand. Dev.) | $\pm$0.07 | --- | --- | $\pm$0.11 |

[a]Values derived from experimental Log P collected by Leo *et al* (1971). [b]Values derived from experimental log P measured by Curie *et al* (1966). [c]Values derived from experimental log P measured by Barfknecht *et al* (1975). [d]Values derived from experimental log P measured by Nichols *et al* (1978).

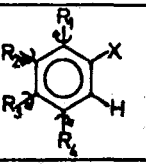
These unusual results have prompted us to explore neighboring group effects in numerous other ortho oxygen substituted model compounds. The substituent $\pi$ values along with the CNDO/2 calculated conformational minima of these groupings have been summarized in table IV. As can be seen from the table, the minimum energy conformation of both 2,3- and 3,4-dimethoxytoluene and also 3-methoxy-4-thiomethyltoluene was predicted by CNDO/2 to be nonplanar; however, in the latter two substitution patterns, a low-lying planar conformation was also found. In 3-methoxy-4-hydroxytoluene, a nonplanar orientation similar to the conformational minima of 3,4-dimethoxytoluene was computed to be slightly more stable than the planar form which differs from the dimethoxy case in that the hydrogen of the phenol is directed toward a methoxy group whose methyl substituent is pointed away. A planar global minima was found in 3,4-dihyroxytoluene whereas nonplanar conformations were favored in both 2,3,4- and 3,4,5-trimethoxytoluene. The variations in the lipid solubility of these groupings appear to be related to the solvation of the lone pair electrons of the methoxy groups by water. The hydrophilicity of the 3,4-dimethoxy grouping can be explained in one of two ways: 1) lone pair-lone pair repulsions in adjacent methoxy groups favor nonplanar conformations which are more strongly solvated than the planar structures, or 2) the planar orientation which exists as a low-lying local minima results in the buildup of electron density between the methoxy substituents, and this region provides a good site of solvation. It is difficult to rule out one of these explanations. Since both the 2,3- and 3,4-dimethoxytoluenes exist in nonplanar forms and yet only the latter has an abnormal $\pi$ value, one might conclude that a specific solvation site is implicated. However, the differences between the $\pi$ values in these two cases could also be explained by steric inhibition of solvation in the 2,3-dimethoxy compounds by the ortho substituent (such as an ethylamine side chain). Conversely, the methylenedioxy grouping, which is more lipipophilic than the component substituents, is both planar and the specific solvation site is blocked by the methylene moiety. Similarly, 3-methoxy-4-hydroxy pattern is more lipophilic than expected due to intermolecular hydrogen bonding in the low-lying planar conformational minima.

In any case, it is clear that our initial assumption that the "rearranged" positional isomers were isolipophilic was in error. The adjacent methoxy group interaction makes the 4,5-dimethoxy-2-X-substituted analogues much more hydrophilic than the nearly isolipophilic 2,4-dimethoxy-5-Xand 2,5-dimethoxy-4-X-substituted compounds. Regression analysis showed a rather poor correlation between Log H.P. and HOMO energies; addition of the Log P resulted in no significant improvement. Scrutiny of the log-log plot showed a fairly straight line for the 2,5-dimethoxy-4-X-substituted compounds, but the H.P. of the other positional isomers was not correlated with Log P.

**Metabolism**

A potentially important consequence of the electronic and distribution properties of the "rearranged" isomers concerns their susceptibility to biotransformation in vivo. Investigation of the metabolic fate of DOM (5) (Zweig and Castagnoli 1977) had established the conversion of

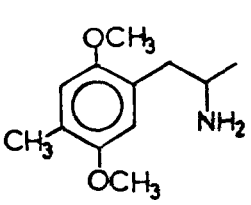
**Table IV.** Model System Study of the π Substituent Values and the CNDO/2 Calculated Conformational Structure of Various ortho-Oxygen Substituted Groups



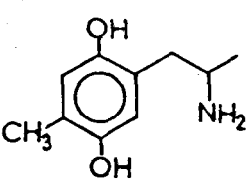

| $R_1$ | $R_2$ | $R_3$ | $R_4$ | $\pi_{add.}$[a] | $\pi_{obs.}$[b] | $\pi_{add.}-\pi_{obs.}$ | CNDO/2 Minima[c] Global | CNDO/2 Minima[c] Local |
|---|---|---|---|---|---|---|---|---|
| $OCH_3$ | $OCH_3$ | H | H | -0.04 | 0.01 (±0.12) | -0.05 | NP[d] | P[e](ΔE=78) |
| H | H | $OCH_3$ | $OCH_3$ | -0.04 | -0.74 (±0.19) | 0.70 | NP[d] | P[e](ΔE=0.65) |
| H | H | $SCH_3$ | $OCH_3$ | 0.59 | 0.37 (±0.12) | 0.22 | NP[d] | P[e](ΔE=1.06) |
| H | H | $OCH_2CH_3$ | $OCH_3$ | 0.36 | -0.65 (±0.17) | 1.01 | | |
| H | H | OH | $OCH_3$ | -0.69 | -0.31 (±0.07) | -0.38 | NP[f] | P[g](ΔE=0.23) |
| H | H | O--$CH_2$--O | | -0.50 | -0.02 (±0.03) | -0.48 | -------P------- | |
| H | H | OH | OH | -1.34 | -1.42 (±0.10) | 0.08 | P[h] | NP[d](ΔE=2.50) |
| $OCH_3$ | $OCH_3$ | $OCH_3$ | H | -0.06 | -0.19 (±0.07) | 0.04 | NP[i] | P[j](ΔE=15.94) |
| H | $OCH_3$ | $OCH_3$ | $OCH_3$ | -0.06 | -0.23 (±0.11) | 0.17 | NP[k] | P[m](ΔE=0.02) |

[a]Substituent π values taken from Hansch et al. (1973). [b]Determined from measured Log P of model compounds. [c]Energy in kcal/mole and X=$CH_3$; NP=nonplanar, P=planar. [d]$T_a$=120°, $T_b$=180°(a<b). [e]$T_a$=0°, $T_b$=180°(a<b). [f]$T_3$=180°, $T_4$=90°. [g]$T_a$=180°, $T_b$=180°(a<b). [h]$T_3$=0°, $T_4$=0°. [i]$T_1$=225°, $T_2$=135°, $T_3$=180°. [j]$T_1$=90°, $T_2$=0°, $T_3$=180°. [k]$T_2$=135°, $T_3$=45°, $T_4$=180°. [m]$T_2$=0°, $T_3$=0°, $T_4$=180°.

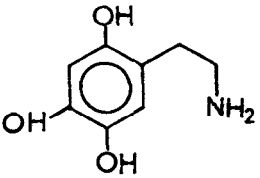
this compound into the bis-O-demethylated metabolite (15), a close analogue of the selective noradrenergic toxin 6-hydroxydopamine (16). The chemical behavior of the hydroquinone (15) and 6-hydroxydopamine (16) is analogous in that both readily undergo spontaneous oxidation leading to electrophilic intermediates (Zweig and Castagnoli 1974) which in the case of 6-hydroxydopamine are likely to be responsible for the observed destruction of noradrenergic terminals (Malnifors and Thoenen 1971).




(5)




(15)




(16)

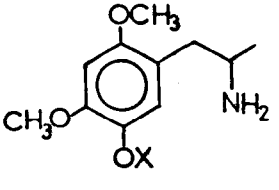
In this regard, the exceptional hyperthermic potency of phenylisopropylamines (5), (8), (11), and (13) (100, 54, 405, and 12 SRU respectively), all of which are 2,5-dimethoxy substituted, compared to the low hyperthermic potency of the positional isomers (4), (6), (7) and (12) (1, 0,5, and 3 respectively), none of which are 2,5-dimethoxy substituted and are incapable of metabolic conversion to a para-hydroquinone, focused attention on the special structural features associated with the para-oxygen substitution pattern.

At the present time, no attempts have been made to determine if these substituent effects relate, in general, to the metabolic profiles of this class of compounds. Nonetheless, the potential association of psychotomimetic activity with the metabolic formation of 6-hydroxydopamine like intermediates from the potent para-oxygen substituted compounds studied here is intriguing. In vivo metabolic oxidation of psychotomimetic amines to electrophilic species capable of covalent interactions with brain enymes responsible for the control of central amines could lead to biochemical lesions and account for the toxic CNS effects of these analogues.

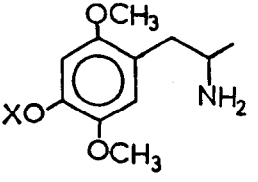
**Summary and Future Directions**

The excellent correlation between rabbit hyperthermia and human psychotomimetic potencies for a variety of psychotomimetic agents strongly asserts the utility of this animal model in the pharmacological evaluation of this class of compounds. Consideration of the conformational, electronic, metabolic, and distribution properties of the "rearranged" isomers has led us to the conclusion that the psychotomimetic potencies cannot be related satisfactorily to overall molecular properties, such as Log P or the HOMO energies, but instead appears to be dependent upon the regiospecific properties such as in plane electrostatic gradients, local group lipophilicity, or metabolic conversion to reactive intermediates. While trends can be found in both the HOMO energies and Log P which do reflect the variations in psychotomimetic potency, the numerical formulation of the SAR using these variables results in

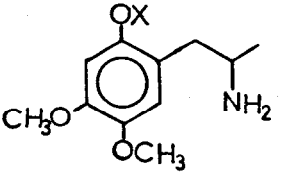
unimpressive regressions which have only marginal predictive capabilities and contribute little to our understanding of these novel compounds. Indeed, further analysis into the regiospecific properties of the "rearranged" isomers and integration of these factors into a QuaSAR equation constitute the direction of our researches. Towards these ends, we have extended the analogue set by including both the $OCH_2CH_3$ (17), (18), (19) and OH (20), (21), (22) substituted phenylisopropylamines, In the $OCH_2CH_3$ analogues, the overall electronic and distribution properties of these positional isomers are very nearly equivalent and may provide insights into the potential relationships between the inplane electrostatic gradient and psychotomimetic potency.




X = $-CH_2CH_3$(17) -H(20)




$-CH_2CH_3$(18) -H(21)




$-CH_2CH_3$(19) -H(22)

The -OH substituted phenylisopropylamines provide an interesting example of positional isomers which are probably much more lipophilic than previously anticipated due to the intramolecular hydrogen bonding of the adjacent OH...$OCH_3$ groups in compounds (20) and (21), and similarly between the OH...$NH_2$ moieties in the 2-OH analogue (22). In these examples, the involvement of regiolipophilicity and metabolic effects will be investigated.

Clearly, the "rearranged" phenylisopropylamines represent a unique. set of analogues whose SAR are quite different from the methoxy substituted phenlisopropylamines or 2,5-dimethoxy-4-alkylphenylisopropylamines studied previously. Further research into these analogues may well reveal the critical factors related to the molecular expression of the complex phenomenon of psychotomimesis.

"In the infancy of a science generalizations are rarely true beyond narrow and too often undefined limits. Always the question, How? punctures the bubble of theory, and the answer is to be sought in analysis and ever more analysis."

*K. S. Lashley, 1933*

**ACKNOWLEDMENTS**

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# Assessment of Quantum Mechanical Techniques for Use in Structure Activity Relationship Development, and Application to Analgesics and Other Drugs

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## I. INTRODUCTION

In the past fifteen years, the combination of advances in theoretical methodology and computing capabilities has created a major new, avenue of reseacrh. This research, Which can be described as the application of theoretical chemical techniques to problerms of biological interest, is now moving rapidly toward quantifying concents and providing significant insight in areas where only very qualitative discussions have been possible in the past. For example, in the period between and 1975, no less than 154 studies using quantum mechanical techniques in the area of drug design appeased in the literature (Christoffersen 1976 a ). These studies encompass at least 27 different areas, including 25 studies on drugs having analgesic and/or hallucinogenic action.[1] During the last two years, both the interest and activity in these areas have increased substantially, as this and the other papers in this symposium will indicate.

Since most of the papers that follow this one will deal with the use of one (or several) specific technique(s) to develop quantitative structure-activity relationships on narcotic analgesics, antagonists, and hallucinogens, this paper will describe several of the techniques that are currently available or being developed, and the general "state of the art." Included will be a discussion of the general capabilities and deficiencies of the techniques, as well as an indication of the nature and extent of studies that are necessary if they are to be used profitably within a program of drug design. In addition, application of ab initio techniques to a variety of narcotic agonists and antagonists will be described.

## II. METHODOLOGY ASSESSMENT

In order to place into proper context the vast majority of studies already in the literature and most of those which are expected in the near future, it is useful first to consider some of the major ingredients that are needed if a reasonable description of drugs and drug-receptor interactions is to be obtained using theoretical methods. In addition, it is important to describe at least some of the available theoretical techniques, so that their advantages and

limitations when applied to problems in drug design can be taken into account.

First, it should be recognized that, for nearly all theoretical studies, only one or at most several of the important factors dealing with drug activity are considered. In particular, most studies deal either with characterization of the isolated (or solvated) drug, or with of model of the drug-receptor interaction. While these aspects are indeed very important components of overall drug activity, it most also be remembered that a proper understanding of overall activity will include many factors, e.g., absorption, excretion, catabolism, binding to plasma protein, penetration of the blood-brain barrier, affinity for the receptor, intrinsic activity, and perhaps others. Hence, if only isolated drugs or model drug-receptor interections are considered, an adequate understanding of overall drug activity can be expected only if other factors play a relatively constant role. In addition, if only isolated (or solvated) drug molecules are, considered, then only a description of the initial stages of the drug-receptor interaction can be expected. These initial stages will be important considerations only if the transition state(s) resemble the reactants. However, in spite of the complexity of the problem and the many effects that need consideration in principle, remarkable progress is now occurring in providing insight into drug action mechanisms, or at least, to provide very valuable information for use in the direction of synthetic efforts.

In characterizing the available theoretical methods for correlating overall drug action with molecular characteristics of drugs, (called "quantitative structure-activity relationships"-QSAR studies), it is of interest to note that there are essentially only two or three approaches in use currently. The first of these, exemplified by the studies of Hansch (Hansch 1969) and several of the papers at this symposium, employs the use of calculated or measured molecular properties of drugs, and attempts to find statistically valid linear (or non-linear) relationships between activity and molecular properties related to the thermodynamic free energy. The second approach, which is similar in intent but differs in the methodology used, is generally referred to as pattern recognition (Kowalski, Bender 1974). In this approach much larger data bases are used in general, frequently including simply basic molecular information such as the nature of the atoms, bond lengths, bond angles, etc. Mathematical searching and sorting techniques quite different from linear (or non-linear) free energy correlation techniques are then used to construct correlations with biological data.

However, in both of these approaches there is an important similarity. Since the resulting correlation(s) and/or equation(s) are derived on a statistical basis, it cannot be expected that they will necessarily give insight into the actual mechanism of action. Instead, they should be viewed as relationships that may be quite useful in directing synthetic efforts toward development of increased activity in a given series ("lead optimization"), but will not ordinarily be capable of creating that is chemically unrelated to the series used to develop the initial structure-activity relationship. To do the

latter ("lead generation") typically requires that information concerning the receptor as well as the drug be included in the study. Since few receptors have to date been isolated, purified and characterized, studies such as these have been quite limited in number to date (Scheiner, Kleier, Lipscomb 1975). However, it is expected that theoretical studies of drug-receptor interactions will be of considerable importance and expanded significantly in the future.

In generating molecular properties for use in QSAR studies, one of the techniques most commonly employed is molecular quantum mechanics. Unfortunately, not only one technique is implied by this terminology, but a whole variety of approaches that differ both in level of accuracy attainable in principle and in the particular manner in which the method has been implemenented.

In terms of the accuracy attainable in principle, the available techniques can be classified as:

1. Hartree-Fock (SCF-MO) techniques
2. Correlation energy techniques
3. Empirical techniques

In the first case, the accuracy is limited to that of the molecular orbital model, where the detailed correlation between electrons cannot be described (Schaeffer 1972). This deficiency is corrected in the second case where, at least in principle, all non-relativistic interactions can be described accurately. The third approach eschews the first two entirely, and uses available experimental data to develop a set of empirical potentials that accurately describe molecular properties of test systems. Then, as long as the drugs (or drug-receptor models) of interest resemble the test system in terms of the kinds of interactions expected, an adequate description of the energetics of these systems can be obtained.

In the large majority of studies on drugs to date, the Hartree-Fock model has been used, at various levels of approximation. This includes several semi-empirical approaches, including Extended Huckel Theory (EHT) (Lohr,Lipscomb 1963; Hoffman 1963; Hoffman 1964), Interacted Extended Huckel Theory (IEHT) (Rein, Win, Clarke 1966; Carroll, Armstrong, McGlynn 1966; Zerner, Gouterman 1966), various methods based on neglect of diatomic differential overlap (CNDO/2), INDO, PRDDO, and MINDO) (Pople 1970; Halgren, Lipscomb 1973; Dewar, Haselbach 1970), as well as ab initio approaches using a variety of basis sets(Christoffersen 1972). Each of these methods has its own set of idiosyncracies (Pullman 1976), and a potential user of any of these must become familiar with the characteristics of the method before application to drug system, if serious pitfalls are to be avoided.

In the second class of techniques, only two methods are in common usage, the PCILO technique (Diner, Malriu, Jordan, et al 1969) and ab initio formulations (Hackmeyer, Whitten 1971). The first of these is a semi-empirical procedure that is designed primarily for conformational studies of isolated molecules. It is based on a configuration interaction approach which allows, at least in principle, incorporation of correlation effects into the parameters that are chosen. In the case of ab initio approaches, only a few applications of configuration interaction techniques to systems of biological interest have occurred

to date(Hackmeyer, Whitten 1971), but represent an important trend for the future that will allow, at least in principle, the proper description of reaction surfaces and spectra as well as the ground state. In the third class of techniques, a variety of potentials has been created (Scheraga 1968; Balasubramanian, Chidambaram, Ramachandran 1970; Engler, Andose 1973) each designed to describe a particular class of molecules. This class of techniques provides a potentially powerful methodology for examination of relatively large numbers of conformational degrees of freedom, and hence is of substantial interest to problems involving large molecular systems. However, there remains a great need for development of a consistent set of potentials that includes both intra- and intermolecular interactions over a broad range of molecules.

In addition to the three basic classes of methods mentioned above, other developments related to these should be noted, that add substantially to the techniques available for theoretical studies. Perhaps the most useful and promising of these involves the use of isopotential maps and interaction potentials(Weinstein 1975a,b) that. are derived from wavefunctions obtained using Hartree-Fock and/or correlation energy techniques to describe drug-receptor interactions.

Even if one or more methods can be identified as being suitable for application to a given problem, significant difficulties remain in the usual manner in which studies are carried out. As an example, consider the methadone molecule, shown in Figure 1. Considering conformational questions only, there are at least 11 dihedral angles that should be varied simultaneously to find minima. If bond angle and bond distance variations are also allowed, the dimension of the non-linear parameter space that must be searched is beyond the size that can be accommodated with contemporary computer systems. Even if the energy evaluation at each point in this space is very rapid (e.g., using empirical potentials), the techniques available for optimizing non-linear parameters begin to fail for systems containing >20 independent variables. Hence, serious simplifying assumptions are typically required in order to devise a workable problem. Fortunately, chemical experience and experimental data are frequently available to aid in introducing realistic simplifying assumptions.

Other examples where currently used methodology is frequently inadequate include the proper use of statistical mechanical concepts when interpreting conformational maps and the inclusion of solvent effects. The first of these is relatively easily remedied(Washel, Levitt 1976; Warshal 1977), but the second adds yet another serious dimension to the problem. Work in the solvent effect area is currently quite active(McCreery, Christoffersen, Hall 1976; Beveveridge, Kelly, Radna 1974; Beveridge, Schnuelle 1975; Pullman,Pullman 1975; Kollman, Kuntz 1976), and several approaches are becoming both computationally feasible and chemically realistic. However, proper consideration of, e.g., solvent displacement at a model receptor site, is a very difficult problem to treat properly using current techniques and technology.

Hence, special care should be exercised in theoretical studies to choose a suitable method, to include as many aspects as available technology and resources allow, and systemtically study a relatively large data base to avoid artifacts.

As difficult as such a procedure may appear, one current technological development of great potential for alleviating several of the major areas of difficulty is now available, and needs to be exploited. In particular, the introduction during the last several years of minicomputer systems that have both high speed and high internal precision at relatively low cost is an event of particular importance. Even using "off-the-shelf" hardware and software, minicomputer systems that have comparable precision and are only 10-15 times slower than near-state-of-the-art large scale computer systems (e.g., CDC 7600s) can be purchased. Considering the access that is reasonably available to a dedicated system such as this, it is easy to show that such a minicomputer system is far more cost effective for many problems than large scale systems that are available, and that the total amount of work that can be accomplished (e.g., in number of molecules studied, number of degrees of freedom considered etc.) is greatly enlarged. If the possibility of specialized hardware design and ease of implementation on minicomputers is also considered, the cost-effectiveness of a minicomputer system is further enhanced. Hence, considering the likely advances and decrease in cost of minicomputer technology in the near future, it is clear that substantial advances in the extent of studies possible and general availability of theoretical studies to the chemical and biological community are now possible.

## III. APPLICATION TO ANALGESICS AND RELATED DRUGS

### A. Geometric Studies.

As noted as early as 1949(Shaumann 1949), there are several structural features shared by nearly all strong analgesics(the "morphine rule"), including: 1) a quaternary carbon atom, 2) an aromatic ring linked to the quaternary carbon atom, 3) a tertiary amino group separated from the quaternary carbon by two saturated carbon atoms, and 4) a phenolic hydroxyl group situated meta to the quaternary carbon atom if the tertiary nitrogen atom is part of a fused piperidine system. In addition, replacement of the methyl substituent attached to the quaternary nitrogen with an allyl or other substituents frequently introduces a degree of antagonist activity to the molecule. Hence, it is of interest first to see if these characteristics can be rationalized on a molecular basis using electronic and geometric features resulting from quantum mechanical and other theoretical studies.

In order to begin such investigations, six classes of compounds that exhibit various degrees of analgesic agonism and/or antagonism have been examined. The 16 specific molecules iincluded in the study are depicted in Figure 1, and include 1) the morphines, 2) oxymorphones, 3) morphinans, 4) benzomorphans, 5) 1,1-diphenylaminoalkanes, and 6) 4-phenylpiperidines.

In order to establish reasonable starting points for study of these molecules, geometric structure data was sought from available x-ray crystallographic studies. For morphine (Gylbert 1973; Mackay, Hodgkin 1971), naloxone(Karle 1974; Sime, Forehand, Sime 1975), cyclazocine (Karle, Gilardi, Fratini et al 1969), methadone(Hanson,Ahmed 1958; Burgi, Dunitz, Shefter 1973; Bye 1974), propoxyphene(Bye 1973), and meperidine (Van Koningsveld 1970), x-ray studies have been reported earlier. In the current studies, four new, high precision, single crystal x-ray Studies have been carried out. The molecules studied include morphine, nalorphine, fentanyl, and meperidine (Duchamp, Chidester, Olsen 1977). Since one of the questions of interest pertains to the degree to which these various molecules resemble each other, it was decided that, before attempting electronic structural studies, it would be important first to ascertain whether these molecules could be placed in similar conformations without excessive energetic cost. To do this, the techniques of molecular mechanics(Scheraga 1968; Balasubramanian, Chidambaram, Ramachandran 1970;Engler, Andose,Schlever 1973) were employed but using a set of potential functions developed specifically to be applied to large molecular systems such as the analgesics. The form of the potential that was used is given by

$$E = E_{ang} + E_b + E_{nb} + E_{hb} + E_{op} + E_{tor} + E_{dd} \quad , \tag{1}$$

where $E_{ang}$ is an angle deformation energy, defined as

$$E_{ang} = 0.021926\ f_\theta(\Delta\theta^2 - 0.00002924\Delta\theta^3) \quad , \tag{2}$$

$E_b$ is a bond deformation energy, defined as

$$E_b = 71.985\ f_d\ (r-r_0)^2 \quad , \tag{3}$$

$E_{nb}$ is a non-bonded interaction energy, defined as

$$E_{nb} = fe^{-gr} - (e/r^6) \tag{4}$$

$E_{hb}$ is a hydrogen bond energy term, defined by Schroeder and Lippincott(Schroeder,Lippincotte 1957),

$E_{op}$ is an out-of-plane deformation energy(Duchamp unpublished) (for bending at $sp^2$) planar hybridized atoms), defined as

$$E_{op} = f \cdot D^2, \tag{5}$$

$E_{tor}$ is a torsional strain energy term, defined as

$$E_{tor} = \sum_n v_n\ (1 \pm \cos n\phi) \tag{6}$$

and $E_{dd}$ is an electrostatic interaction energy term, defined as

$$E_{dd} = Kq_iq_j/Dr_{ij}. \tag{7}$$

The parameters for these potentials were chosen by studying experimental data for small molecules, and tested by predicting geometric

parameters for large molecules in the crystal. The level of accuracy expected in predicted geometric parameters is ~0.01Å for bond distances, ~1° for bond angles, ~3° for torsion angles, and ~0.05Å for nonbonded distances. Details of the parameter development will be given elsewhere(Oie,Duchamp,Christoffersen, to be published).

One of the first questions investigated was: How flexible is morphine itself? While the molecule is frequently thought to be quite rigid because of the presence of five rings, there is still some flexibility. To illustrate this flexibility, rotation about the C12-C13 bond was investigated, minimizing all other geometric parameters for each value of the $C_4$-$C_{12}$-$C_{13}$-$C_{14}$ torsion angle. The results of this Study are summarized in Figure 2. The first point of interest that is evident in the figure is that there is a considerable amount of flexibility in morphine about the $C_{12}$-$C_{13}$ bond, in that less than 3 kcal/mole is required for a variation of ±15° from the minimum. This is an important flexibility, since it greatly changes the distance of the basic nitrogen from the plane of the aromatic ring, a feature which is inferred to be important in the drug-receptor interactions(Hende,Nelson 1967). This flexibility is also exhibited in other molecules, whose observed $C_{12}$-$C_{13}$ angles are also indicated in Figure 2. In particular, it is seen that, in addition to the minimum at ~141° found here for the free, protonated morphine molecule, a value of 135.7° is found in the morphine hydrochloride crystal (A)(Gylbert 1973;Mackay,Hodgkin 1971), 134.1° is found in free base morphine (B)(Duchamp,Chidester,OlSen 1977), 132.5° is found in the nalorphine hydrochloride crystal (C-current study), and ~125° is found for the O-$CH_3$ derivative of etorphine (D)(Hende,Nelson 1967). Thus, one of the first conclusions of interest is that morphine itself has some flexibility, and when compared to other molecules, its geometry must also be varied and should not be assumed to be rigid.

Next let us consider the fentanyl molecules, which has considerable conformational flexibility, starting from the crystallographic data (Duchamp,Chidester,Olsen 1977). In order to investigate whether fentanyl and morphine can achieve similar conformations at reasonable energetic cost, four points were matched, including the quaternary nitrogen in both molecules, plus $C_3$, $C_1$, and $C_{12}$ in morphine with the para and two meta positions of the phenyl of the phenthyl moiety in fentanyl, respectively. The technique used in this matching has been described elsewhere (Duchamp 1977). By allowing the coordinates of all other atoms to vary, a geometry for each molecule which maximized the overlap of the four atom just mentioned was obtained. The result for this case is that, for an energy cost of 2.6 kcal/mole in morphine and 5.2 kcal/mole in fentanyl(compared to the minimum energy for each of the molecules taken as isolated, free molecules), an overlay of the two molecules can be obtained in which the three atoms chosen from the phenyl groups are positioned within 0.1, 0.2, and 0.2Å of each other, and the nitrogen nuclei are within 0.5Å of each other. It should be noted that the fentanyl geometry found in the match to morphine is Substantially different than the geometry found in the crystal. Of course, if higher energies were allowed, even closer fits could be obtained. The conclusion that is reached is that, for energies well within the range that might be

expected to be present in a drug-receptor interaction, both fentanyl and morphine can be matched quite well.

For the case of meperidine,which was also studied crystallographically in the current investigations, it was found that meperidine hydrochloride has 2 molecules in the asymmetric unit, which differ primarily in the orientation of the ethyl group. The other point of interest is that, in both the current studies and the earlier study of meperidine hydrobromide by Van Konigsveld(Van Koningsveld 1970). the phenyl group is in an equatorial position relative to the cyclohexyl ring system.

Earlier discussions of meperidine activity have involved suggestions that an axial phenyl geometry might be the active form(Beckett,Casey 1965). As a free molecule, the equatorial phenyl form is found to be favored by 0.8 kcal/mole over the axial phenyl form using the potential functions described earlier. Both the axial and equatorial forms of meperdine were matched to morphine, using the 4-point model described earlier. The result is that both the axial and equatorial geometries can be made to fit morphine, although the fit for the axial form is somewhat better. In particular, for essentially a perfect match of the phenyl moieties, a 0.38Å difference in nitrogen position results at an energetic cost of -3.6 kcal/mole for the axial form of meperidine, and requires 2.1 kcal/mole for morphine. For the equatorial form of meperidine, 6.9 kcal/mole is required, and 2.7 kcal/mole required for morphine, in order to achieve a nitrogen match within 0.4Å It should be noted that, in considering the axial phenyl case, (which involves a piperidine ring flip), the $N-CH_3$ moiety is kept in an equatorial form by inverting the configuration of the proton and methyl group at the nitrogen. Thus, either axial or equatorial phenyl meperidine can be matched to morphine without excessive energetic cost. This result should be combined with the observation that, in the equatorial case, the protron attached to the quaternary nitrogen is no longer oriented in the same position as the corresponding proton in morphine after matching (while the corresponding proton orientation in the axial case is the same). Thus, it appears that the axial form of meperidine is favored, at least when comparison to morphine is made, even though the crystal geometry and lowest energy form is the equatorial phenyl form.

Now let us consider geometric features of antagonists, using nalorphine as an example. One of the geometric features of particular interest relates to the position of the allyl group, or of similar moieties (e.g., the cyclopropyl group in cyclazocine). In the crystal, it is found that the allyl group conformation in the current study of nalorphine is different than that found in naloxone(Karle 1974; Sime,Forehand,Sime 1975), but similar to the conformation found for the cyclopmpyl moiety in cyclazocine(Karle, Gilardi, Fratini et al 1969).

To examine the extent to which the allyl (or cyclopropyl) group orientation is variable, a scan of the two relevant torsion angles ($\tau 1 = C_{16}-N-C_{17}-C_{18}$ and $\tau_2 = N-C_{17}-C_{18}-C_{19}$) was carried out. The results for nalorphine are presented in Figure 3, and the results for cyclazocine are presented in Figure 4. These particular calculations

were carried out using rigid rotations, varying only the two dihedral angles The minima that are found are described in Table 1.

It is seen that the conformational energy surfaces for the two molecules look quite similar, with four minima less than 1.0 kcal/mole above the global minimum found for each molecule for approximately the same ($\tau_1$ $\tau_2$) values. In addition, interconversion among the four low-lying minima in each molecule should be relatively easily accomplished, since a barrier of only ~3 kcal/mole is encountered between adjacent minima. The only difference between these two molecules from the point of view of ($\tau_1$,$\tau_2$) flexibility is the presence of 2 additional local minima at 3-5kcal/mole above the global minimum, while cyclazocine has only 1 such additional minimum. Hence, and nalorphine and cyclazocine look quite similar, at least with respect to the possible orientations of the allyl or cyclopropyl groups.

In addition, nalorphine and naloxone were matched using the technique described earlier. Using the global minimum found for ($\tau_1$ $\tau_2$) in nalorphine as a starting point, a good match of the two molecules can be obtained. In particular, the aromatic rings and quaternary nitrogen can be placed within 0.2Å and the allyl groups within 0.6Å, at an energetic cost of only 1.8 kcal/mole for nalorphine and 1.2 kcal/mole for naloxone. Similar results can be expected for the 3 other energetically favorable conformations. Hence, it is seen that nalorphine and naloxone can assume quite similar geometries (e.g., for a drug-receptor interaction), even though their crystal structures are different.

B. Electronic Structural Studies

We have now seen that, for several of the important classes of analgesic agonists and antagonists, quite similar geometric environments for atoms believed to be important to the drug-receptor interaction can be attained at only modest energetic cost. It is thus of interest to see whether electronic structural features can be found which will distinguish e.g., agonists from antagonists, even though geometric features may be similar.

To examine this possibility, ab initio SCF calculations using the molecular fragment basis(Christoffersen 1972) have been carried out on each of the 16 molecules illustrated in Figure 1. The geometries chosen reflect several considerations. For morphine, meperidine, and fentanyl, the geometries used were those found in the matching process described in the previous section. For nalorphine, naloxone, and cyclazocine, methadone,and propoxyphene, the x-ray structure was used, while in oxymorphone and naltrexone the basic geometric data from the naloxone crystal structure was used with appropriate substitutions at the quaternary nitrogen. Similarly, for metazocine, N-allyl normetazocine and pentazocine, the cyclazocine crystal strtucture was used with appropriate modifications at the quaternary nitrogen. Levorphanol and levallorphan were built from the cyclazocine structure, using molecular mechanics to orient the added substituents.

The results of these calculations are reported in part in Table 2, where the molecular orbital (MO) energies for the five highest energy occupied MOs and five lowest energy unoccupied MOs are given. In addition, to indicate the shape and location of MOs of interest, electron density maps for several MOs that are representative are given in Figures 5-10. In each calculation, the protonated form of the molecule was used.

## IV. DISCUSSION

With the reasonably extensive geometric and electronic structural data described above, obtained in a consistent fashion using the same techniques, it is of interest to examine it further from at least two different perspectives. First, since these techniques are, relatively speaking, in their infancy, it is of interest from a methodology point of view to compare the results obtained here with other theoretical studies. Next, it is of interest to examine whether the electronic and geometric structural features that are identified as being of importance in these studies can be related to the observed pharmacolcgical differences in the molecules, and add to the understanding of these pharmacological differences based upon the molecular properties of the drugs.

Turning first to geometric features, the minima found here for the $N\text{-}C_{18}$ and $C_{18}\text{-}C_{19}$ dihedral angle variations in nalorphine are consistent with those found by Loew and Berkowitz(Loew,Jester 1975) and by Kaufman and Kerman(Kaufman,Kerman 1977) using the PCILO technique, including both the location of the minima and the approximate energetic barrier separating them. This similarity of results using different methodologies is of interest, both from the point of view of the adequacy of the techniques to describe these geometric features in analgesics, and in the likelihood that the results represent the actural physical situation (for the isolated molecule).

In the case of meperidine, the finding that the lowest energy form is the equatorial phenyl form is consistent with PCILO studies(Loew, Jester 1975;Beckett,Casey 1965). However, in contrast to earlier results in which only two dihedral angles were varied(Loew,Jester 1975; Loew,Jester,Berkowitz et al. 1975), the ability to relax all bond distances, bond angles and dihedral angles using the molecular mechanics technique indicates that a good fit to the morphine structure can be achieved at reasonable energetic cost, for both the axial and equatorial forms of meperidine. This is consistent with the observation that significant potencies are observed for both phenyl-equatorial and phenyl-axial compounds(Portoghe,Mikhail 1968) and indicates that additional investigation is needed before identification of only one form (whether axial or equatorial) as being solely responsible for meperidine activity is appropriate.

For the case of fentanyl, the results obtained here are consistent with the features proposed by Feinberg(Feinberg,Creese,Snyder 1976) to be important factors in determining its potency. In particular, one of the reasons why the "F" ring plays such an important role in fentanyl and similar molecules is that it can be closely matched with the "A" ring of morphine without excessive energetic requirements. However, it would then appear that the "A" ring region (and not the

"F" ring) of fentanyl and similar molecules is the "special feature" that confers unusually high potency in the model proposed by Feinberg et. al(Feinberg,Cresse,Snyder 1976).

While additional geometric features may play important roles in determining the potency and/or efficacy of these molecules(Loew, Berkowritz,Newth 1976;Loew,Berkowitz 1978), it is clear from the few examples presented here in abbreviated form that, even though the molecules included here represent a rather broad range of chemical structures of analygesics, they can be matched to morphine with only modest energetic requirements. Hence, further examination of electronic structural features may be appropriate in order to discern reasons for potency differences.

First, comparison of the HOMO in morphine and naloxone or nalorphine (Figs. 5 and 6) indicates that the general shape of the MO is nearly identical in the two molecules, even though one is agonist and the others are partial or pure antagonists. In addition, the MO energies are nearly the same, as has also been noted earlier(Kaufman ,Kerman,Koski 1974;Popkie,Koskie,Kaufman 1976). This suggests that, if the analgesic receptor does indeed exist in two allosterically related conformations(Feinberg,Creese,Snyder 1976), the binding site for the phenol moiety of either agonists and antagonists is unaffected by the receptor being in either "agonist" or "antagonist" form, if the receptor acts as an electron acceptor.

On the other hand, comparison of the LUMO shape in morphine and naloxone reveals important differences. In particular, the LUMO in morphine is located in the phenyl region of ring "A", but in naloxone the LUMO is associated with the allyl group attached to the quaternary nitrogen. This is an important distinction, for it allows a direct rationalization of the introduction of antagonist behavior when $N\text{-}CH_3$ in morphine is replaced by N-allyl to form nalorphine. Specifically, if the receptor serves as au electron donor in the drug-receptor complex, then introduction of the allyl group produces a low-lying unfilled MO in the region of the quaternary nitrogen that is well suited to the proposed drug-receptor interaction. In other words, one of the features that appears to be important for introduction of antagonist behavior is the presence of a low-lying unfilled MO in the region of the quaternary nitrogen.

Not only the allyl moiety can introduce "hole" density in the region of the quaternary nitrogen to introduce antagonist characteristics. For example, nlrexone and cyclazocine have a methyl cyclopropyl moiety in place of the allyl moiety. In this case, the "hole" density is still placed in the proper region of space, as indicated in Figure 11. In this figure it is seen that the general shape of the MO is quite similar to that of the corresponding allyl unoccupied MO, in spite of the chemical differences, and the introduction of antagonist characteristics to naltrexone and cyclazocine is not surprising.

These observations immediately allow a qualitative rationalization of the behavior of many of the molecules in Figure 1, including morphine vs nalorphine, oxymorphone vs naloxone (or

naltrexone), levorphanol vs levallorphan, and metazocine vs N-allylnormetazocine (or pentazocine or cyclazocine).

It is of interest to note that the above observations are in contrast to conclusions reached in earlier studies involving morphine and nalorphine(Loew,Berkowitz 1975), in which it was concluded that "the electronic properties of the fused ring skeleton including specifically the cationic region around the nitrogen are relatively unaffected by varying N-substitueuts." The primary reason that the effect was not seen in earlier studies is associated with the lack of ability of INDO to describe the natrue of unoccupied MOs properly. This is not surprising, since the parameterization of INDO was done to provide a balanced description of the filled MOs only.

Also, the current suggestion that nalorphine and similar antagonists act as electron acceptors (not donors) is in contrast to the suggestion that the receptor "interacts with the $\pi$-electrons of the N-allyl" group(Feinberg,Creese,Snyder 1976). Instead, the receptor electrons are proposed to interact with the N-allyl "hole density", in the form of the LUMO in nalorphine.

Finally, it should be remembered that the observation that the location and energy of the LUMO may be an important factor in determining antagonist behavior does not imply that it is the sole factor of importance. Other electronic and geometric features may also need to be taken into account, and the overall activity of a molecule at the analgesic receptor will be determined by the detailed balance of the various effects.

Clearly, much additional analysis and quantitative study is needed. However, it is already clear that both geometric and electronic structural features of the drugs play an important role in determining analgesic potency, and that currently available theoretical techniques axe capable of probing these features in detail.

**ACKNOWLEDGMENTS**

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**Table 1.** Energetic Minima Found in Allyl (or Cyclopropyl) Rotations

| | $(\tau_1,\tau_2)$ | $\Delta E$(kcal/mole)[a] |
|---|---|---|
| Nalorphine | A=(180,120) | 0.0 |
| | B=(180,278) | 0.26 |
| | C=(70,255) | 0.43 |
| | D=(70,98) | 0.88 |
| | E=(315,245) | 3.69 |
| | F=(340,105) | 4.48 |
| Cyclazocine | A=(175,165) | 0.0 |
| | B=(65,265) | 0.65 |
| | C=(175,290) | 0.73 |
| | D=(75,155) | 0.74 |
| | E=335,165) | 4.35 |

a. Energies measured relative to the global minima for the molecule.

**Table 2.** Compilation of Selected Molecular Orbital Energies of Analgesics[a]

| Molecular Orbital | Morphine | Nalorphine | Oxymorphone | Naloxone | Naltrexone | Levorphanol | Levallorphan |
|---|---|---|---|---|---|---|---|
| LUMO[b]+4 | +0.4366 | +0.4225 | +0.4160 | +0.4158 | +0.4220 | +0.4777 | +0.4492 |
| LUMO +3 | +0.4164 | +0.2572 | +0.4043 | +0.3289 | +0.3482 | +0.4546 | +0.4339 |
| LUMO +2 | +0.2558 | +0.2298 | +0.3278 | +0.2398 | +0.3307 | +0.4210 | +0.2378 |
| LUMO +1 | +0.2282 | +0.2132 | +0.2383 | +0.2109 | +0.2411 | +0.2358 | +0.1977 |
| LUMO | +0.2118(#77) | +0.1706(#84) | +0.2095(#81) | +0.1686(#88) | +0.2124(#92) | +0.1959(#71) | +0.1716(#78) |
| HOMO[c] | -0.2146(#76) | -0.2132(#83) | -0.2238(#80) | -0.2225(#87) | -0.2211(#91) | -0.2467(#70) | -0.2448(#77) |
| HOMO -1 | -0.2636 | -0.2622 | -0.2692 | -0.2679 | -0.2662 | -0.3015 | -0.2996 |
| HOMO -2 | -0.2867 | -0.2856 | -0.2840 | -0.2827 | -0.2812 | -0.4021 | -0.4002 |
| HOMO -3 | -0.3349 | -0.3337 | -0.3485 | -0.3459 | -0.3426 | -0.4096 | -0.4076 |
| HOMO -4 | -0.3579 | -0.3567 | -0.3843 | -0.3828 | -0.3807 | -0.4148 | -0.4123 |

a. Orbital energies are given in Hartree atomic units. MO numbers are given in parentheses.
b. LUMO=Lowest unoccupied molecular orbital.
c. HOMO=Highest occupied molecular orbital.

| Molecular Orbital | Metazocine | N-allyl Normetazocine | Pentazocine | Cyclazocine | Methadone | Propoxyphene | Fentanyl | Meperidine (axial phenyl) | N-allyl Normeperidine |
|---|---|---|---|---|---|---|---|---|---|
| LUMO+4 | +0.4686 | +0.4586 | +0.4664 | +0.4693 | +0.3377 | +0.3641 | +0.3782 | +0.4304 | +0.4312 |
| LUMO+3 | +0.4409 | +0.4366 | +0.4421 | +0.4442 | +0.2155 | +0.2375 | +0.1979 | +0.4190 | +0.3482 |
| LUMO+2 | +0.4109 | +0.2318 | +0.2342 | +0.3650 | +0.2077 | +0.2320 | +0.1970 | +0.3457 | +0.1872 |
| LUMO+1 | +0.2296 | +0.1961 | +0.2099 | +0.2337 | +0.1968 | +0.2008 | +0.1939 | +0.1865 | +0.1848 |
| LUMO | +0.1941 (#64) | +0.1724 (#71) | +0.1984 (#79) | +0.1979 (#75) | +0.1860 (#85) | +0.1953 (#93) | +0.1934 (#92) | +0.1841 (#68) | +0.1633 (#75) |
| HOMO | -0.2533 (#63) | -0.2513 (#70) | -0.2490 (#78) | -0.2495 (#74) | -0.2528 (#84) | -0.2611 (#92) | -0.2495 (#91) | -0.2678 (#67) | -0.2657 (#74) |
| HOMO-1 | -0.3138 | -0.3118 | -0.3095 | -0.3099 | -0.2804 | -0.2733 | -0.2630 | -0.3010 | -0.2991 |
| HOMO-2 | -0.4111 | -0.4094 | -0.3822 | -0.3861 | -0.2920 | -0.2790 | -0.3080 | -0.3162 | -0.3153 |
| HOMO-3 | -0.4252 | -0.4230 | -0.4074 | -0.3950 | -0.2990 | -0.2993 | -0.3095 | -0.3238 | -0.3232 |
| HOMO-4 | -0.4454 | -0.4265 | -0.4206 | -0.4080 | -0.3129 | -0.3107 | -0.3165 | -0.3494 | -0.3476 |

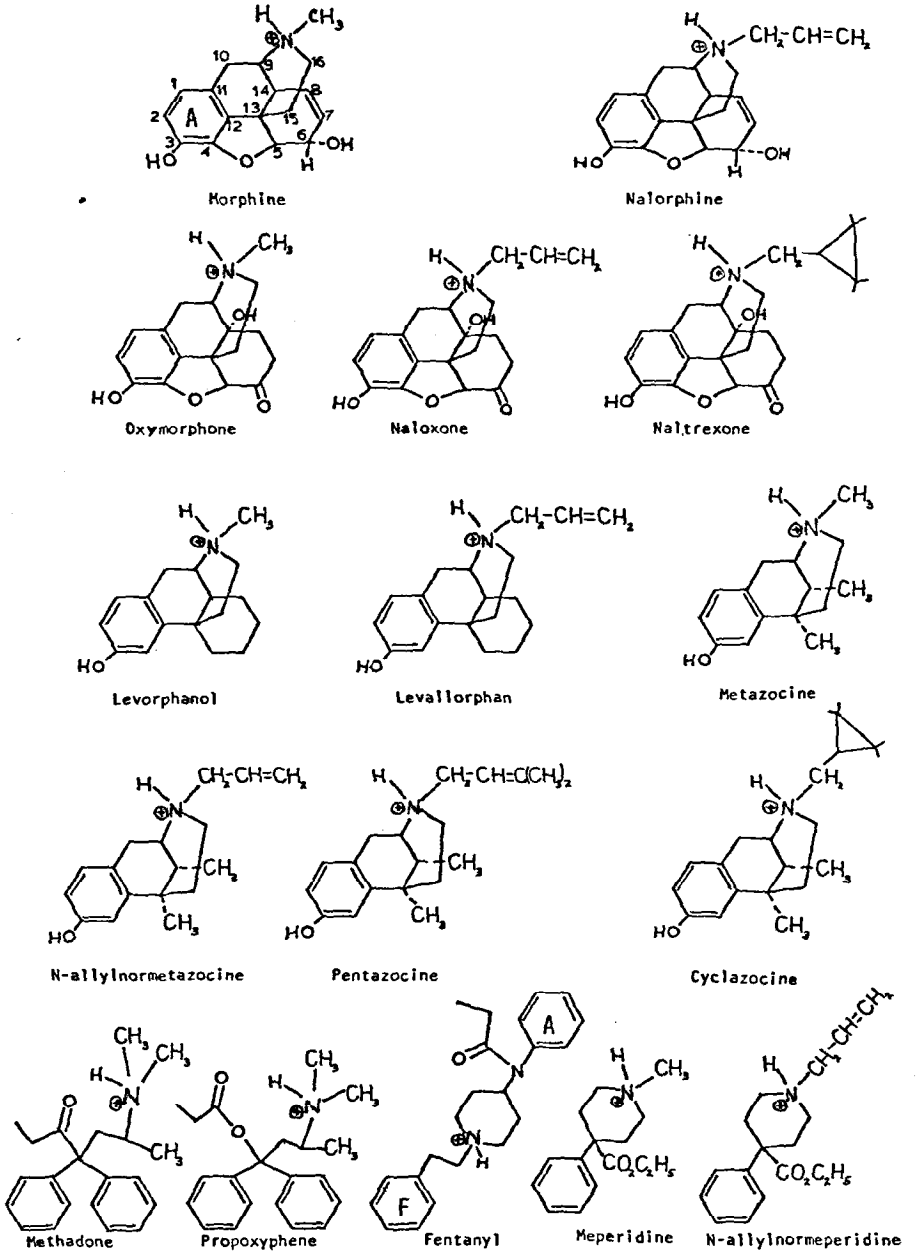


***Figure 1.*** *Molecules Studied.*

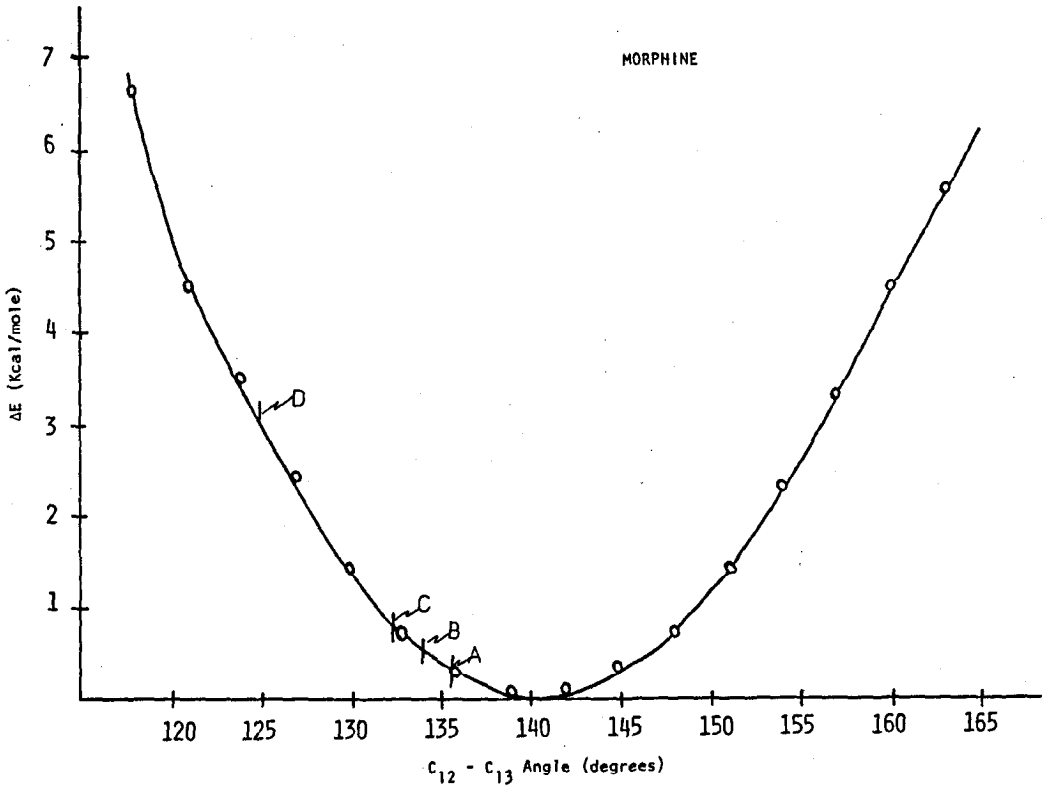


***Figure 2.*** *Study of Morphine Flexibility About the $C_{12}$-$C_{13}$ Bond. A=morphine hydrochloride; B=free base morphine; C=nalorphine hydrochloride; D=o-methyl etorphine.*

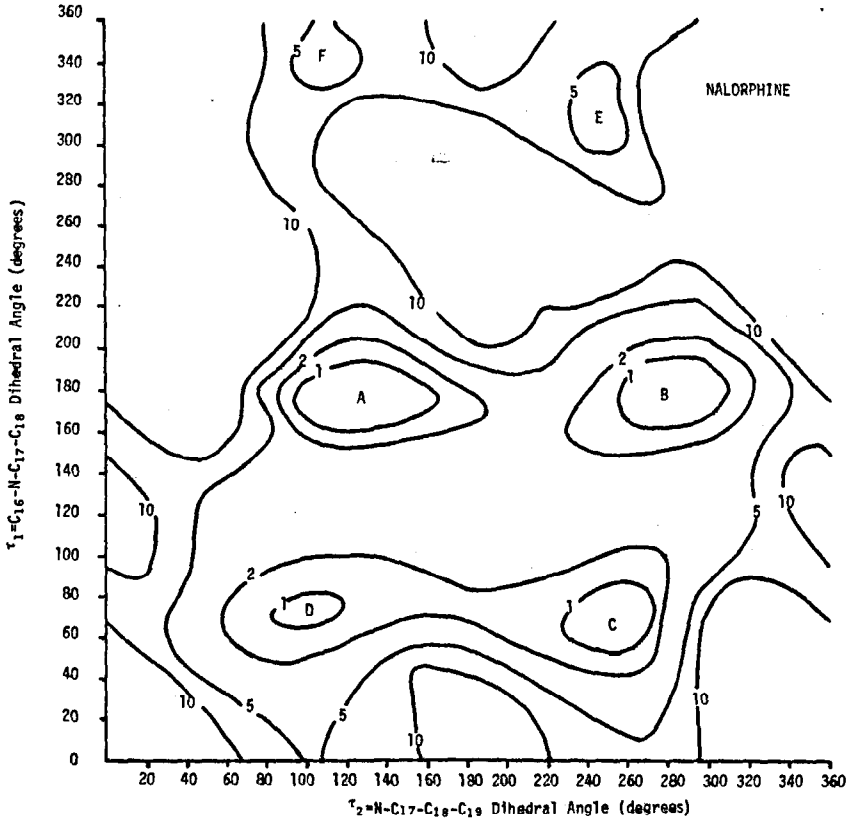


***Figure 3.*** *Isoenergy contours for nalorphine. Contours are are drawn for 1.0, 2.0, 5.0 and 10.0 kcal/mole above the global minimum at (180, 120) - labelled "A" in the figure.*

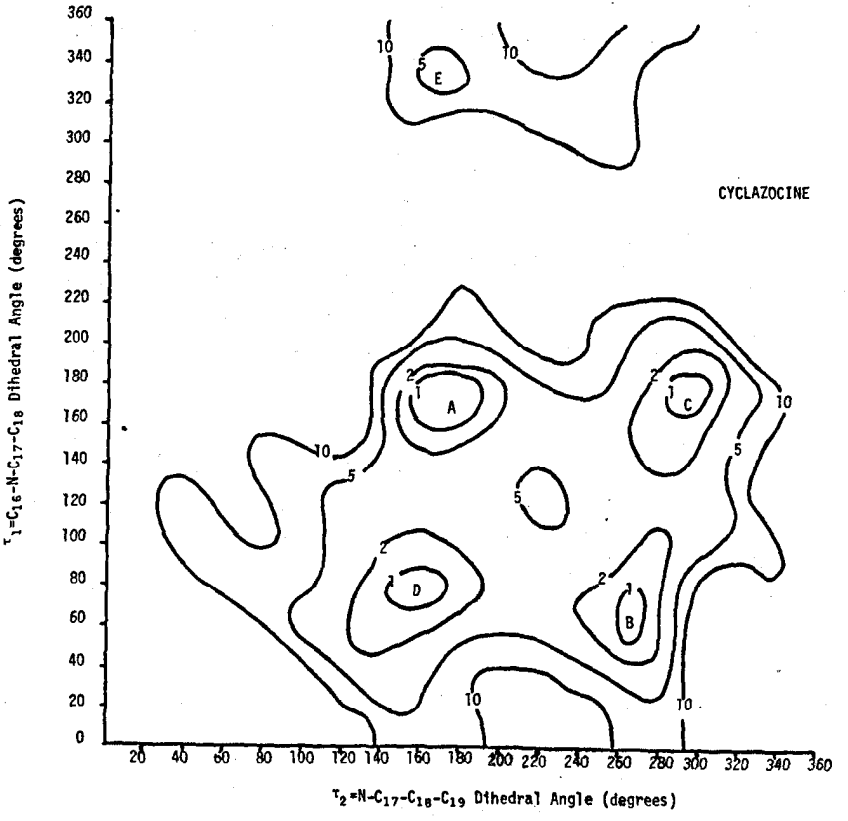


***Figure 4.*** *Isoenergy contours for cyclazocine. Contours are drawn for 1.0, 2.0, 5.0, and 10.0 kcal/mole above the global minimum at (175, 165) - labelled "A" in the figure.Text*

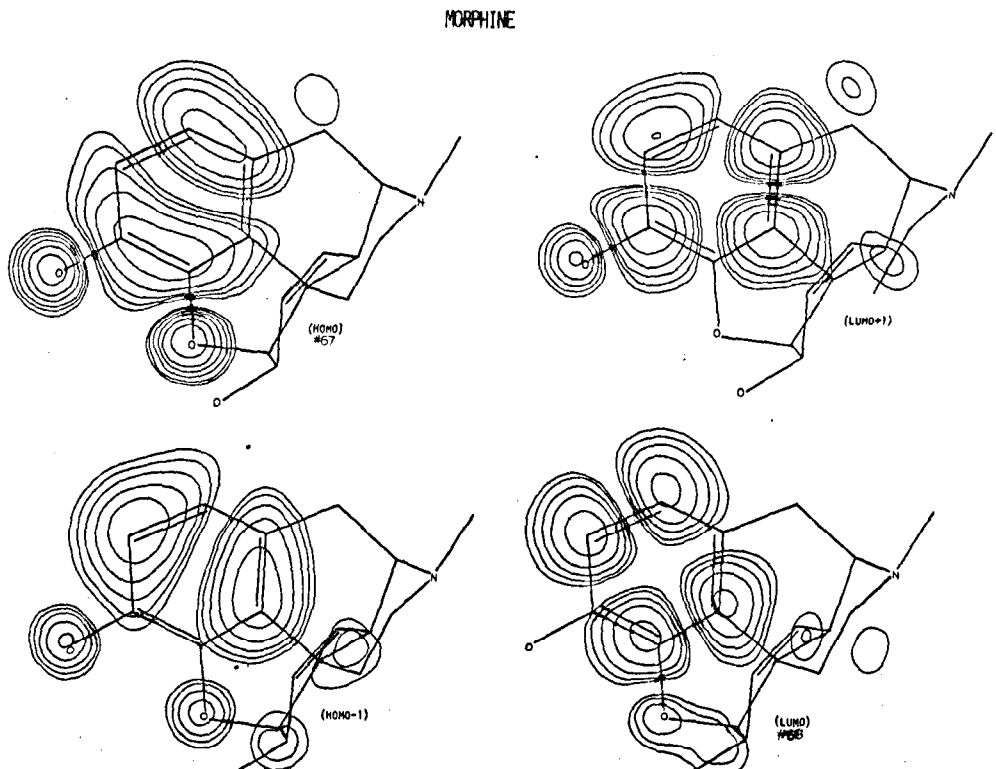


***Figure 5.*** *Electron Density Contour Maps for the HOMO-1, HOMO, LUMO, and LUMO+1 Molecular Orbitals in Morphine.*

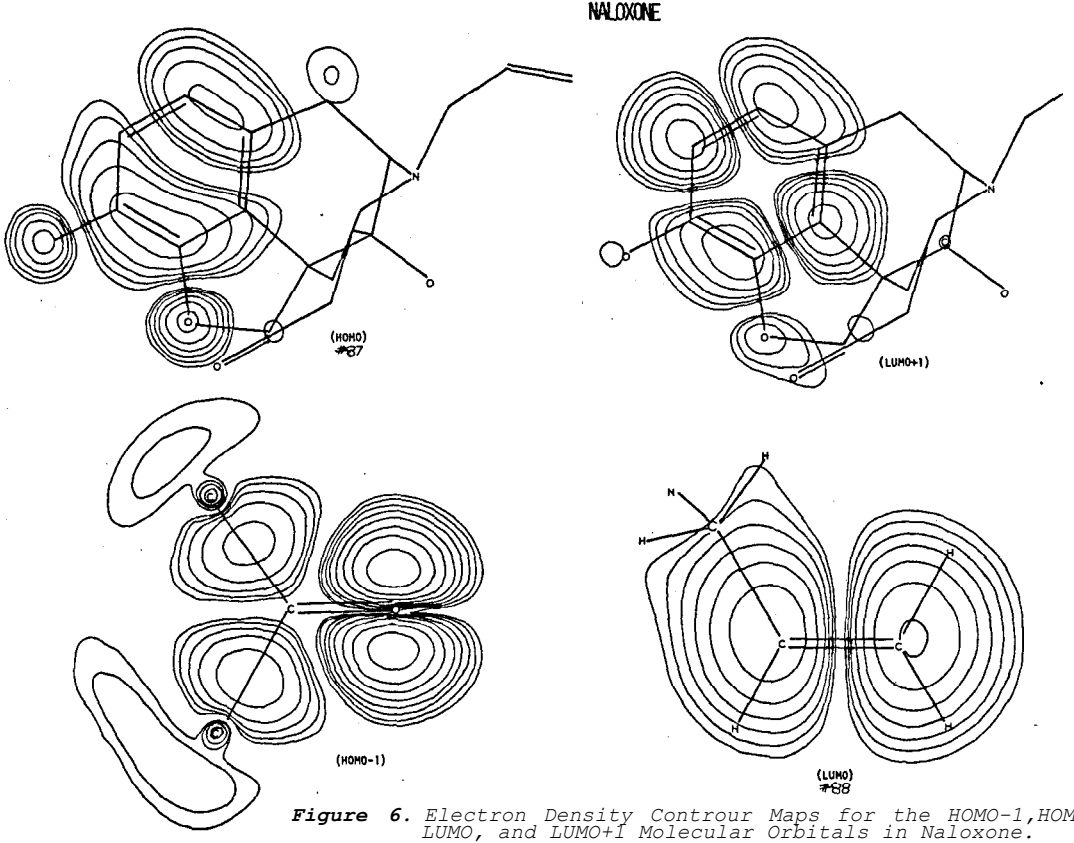


***Figure 6.*** *Electron Density Controur Maps for the HOMO-1,HOMO, LUMO, and LUMO+1 Molecular Orbitals in Naloxone.*

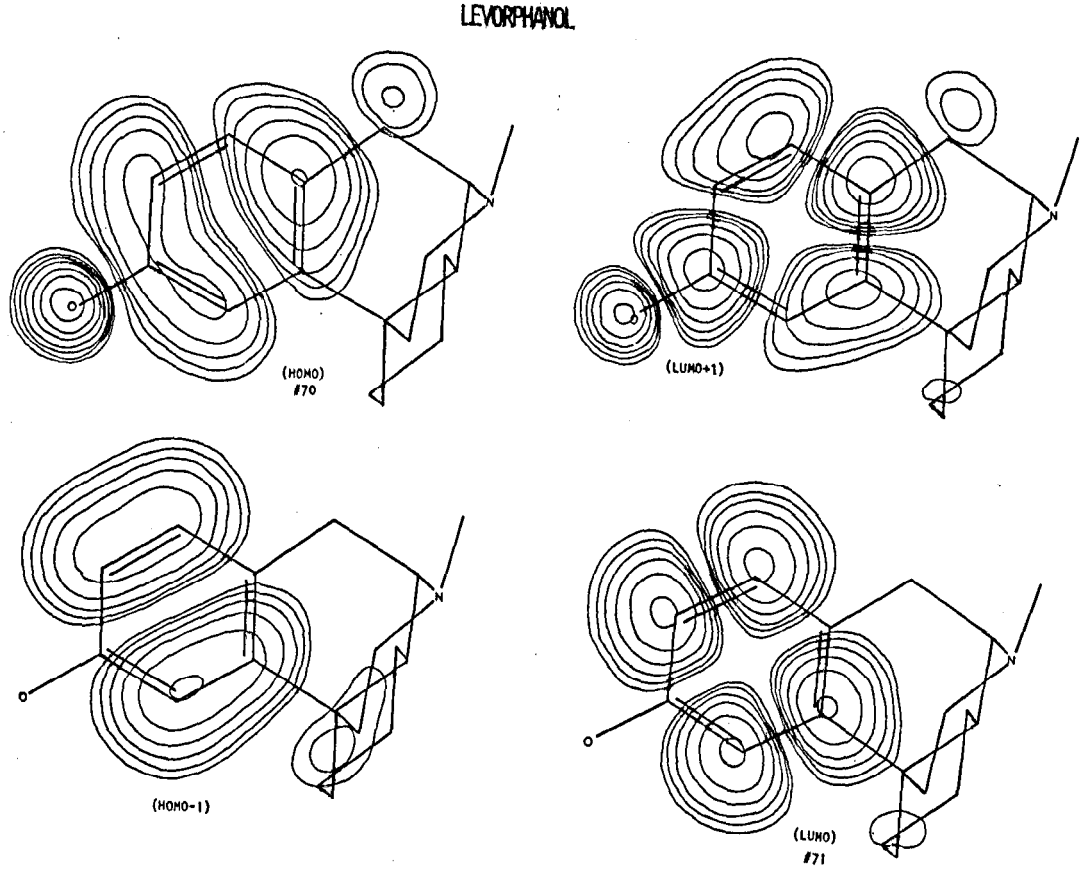


***Figure 7.*** *Electron Density Contour Maps for the HOMO-1, HOMO, LUMO, and LUMO+1 Molecular Orbitals in Levorphanol.*

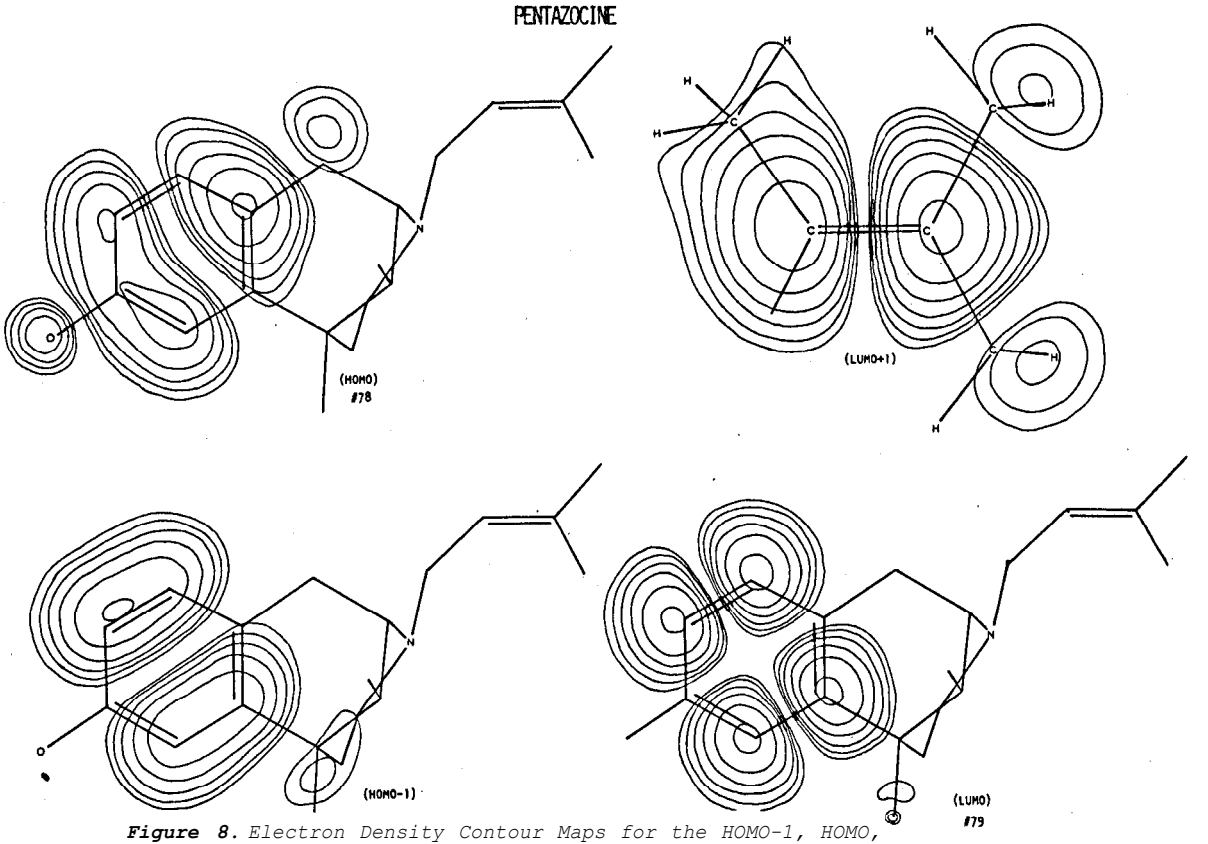


***Figure 8.*** *Electron Density Contour Maps for the HOMO-1, HOMO, LUMO, and LUMO+1 Molecular Orbitals in Pentazocine.*

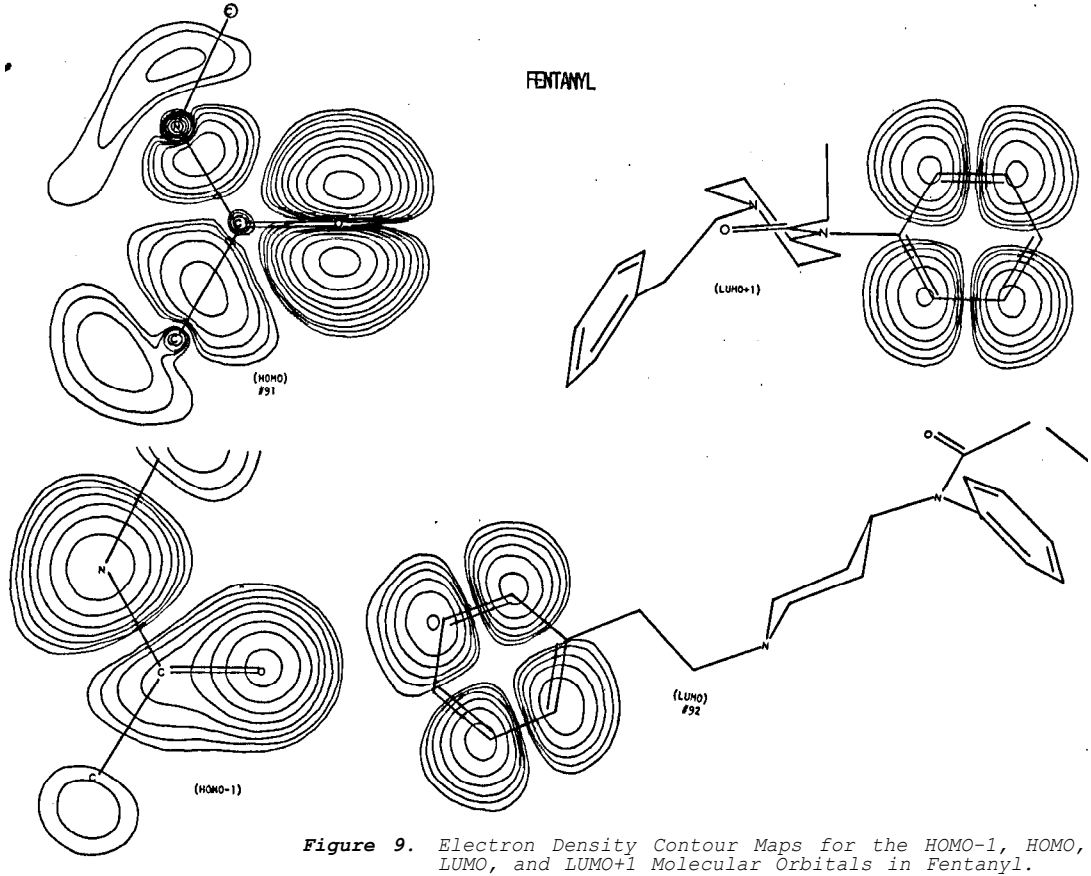


***Figure 9.*** *Electron Density Contour Maps for the HOMO-1, HOMO, LUMO, and LUMO+1 Molecular Orbitals in Fentanyl.*

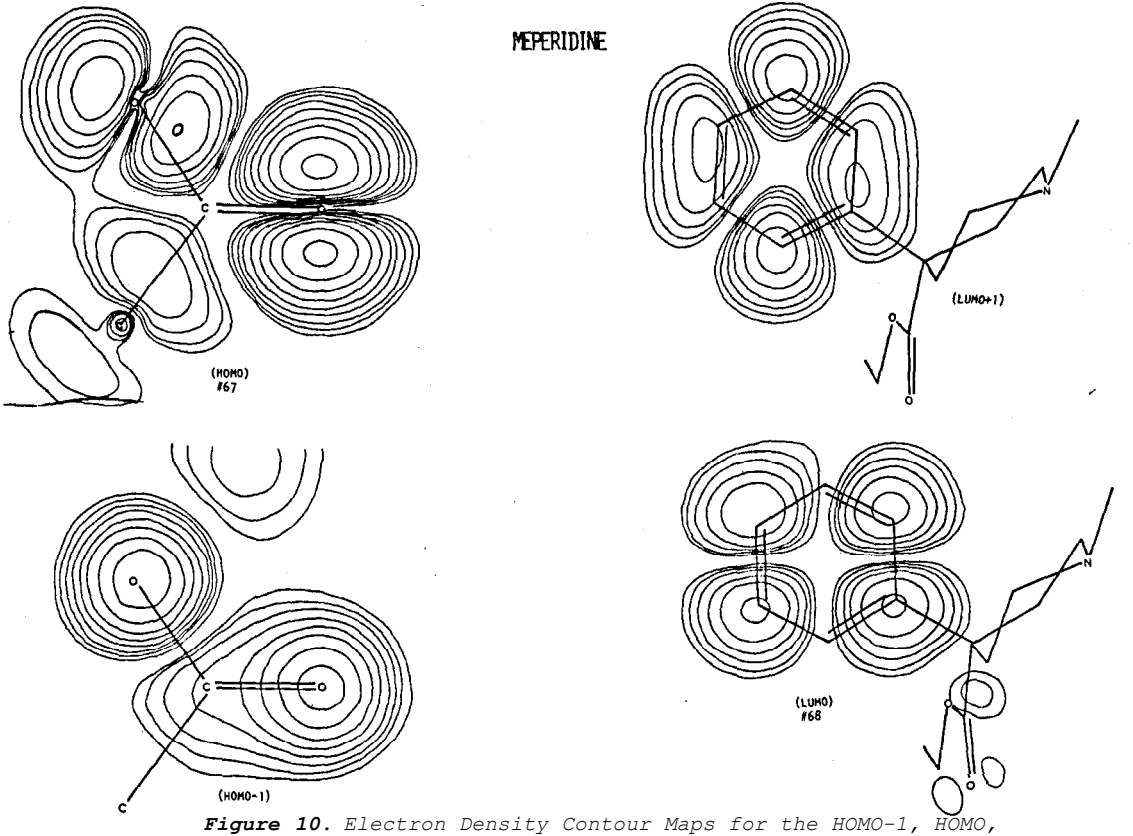


***Figure 10.*** *Electron Density Contour Maps for the HOMO-1, HOMO, LUMO, and LUMO+1 Molecular Orbitals in Meperidine.*

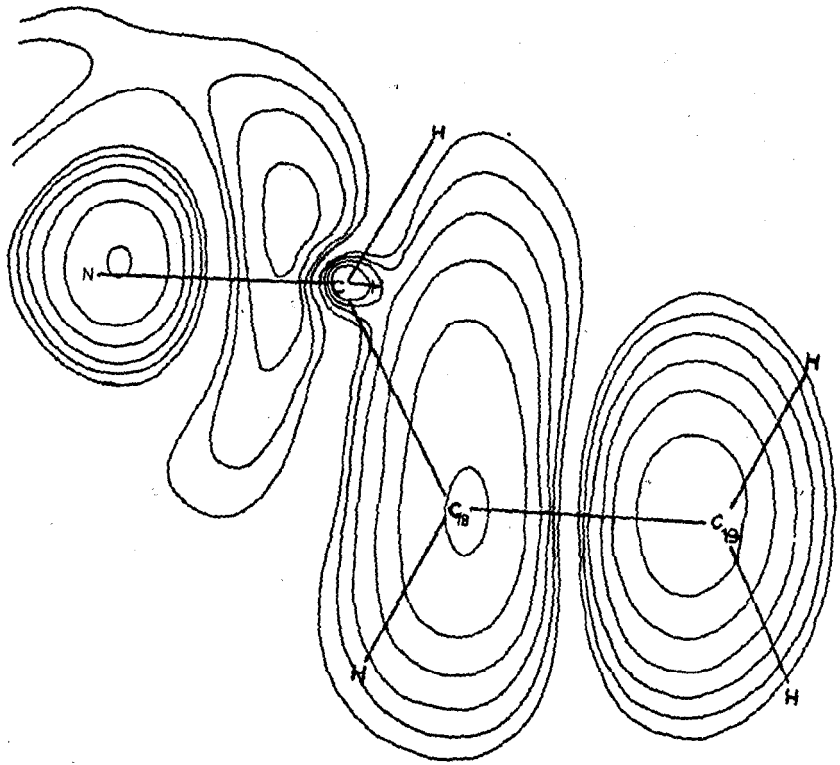


***Figure 11.*** *Density Contour Plot for MO#77 in Cyclazocine. The third carbon atom of the cyclopropyl moiety is connected to $C_{18}$ and $C_{19}$, and in a plane perpendicular to the plane of the paper.*

**FOOTNOTE**

1. See the following articles:


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# Recent Physicochemical and Quantum Chemical Studies on Drugs of Abuse and Relevant Biomolecules

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## I. INTRODUCTION

The objective of quantitative structure activity relation (QSAR) studies is to establish a reliable quantitative relationship between the structure of drug molecules and their pharmacological activity. It is necessary in these considerations to distinguish between the extremes of intrinsic activity (the activity of a drug at its locus of action) (Ariens and Simonis 1964) as opposed to whole animal or man in vivo potency which is influenced by transport, metabolism and intrinsic activity. One of the most striking examples of such a distinction and one which is illustrative of general phenomena in the field of central nervous system(CNS) drugs is exemplified in the pioneering work of Herz and coworkers (Kutter et al. 1970). In these studies they compared the analgesic potency in a series of narcotics by intravenous and by direct intraventricular injection. The intrinsic activity of the narcotics as exemplified by direct intraventricular injection appeared to be primarily governed by the structure and polarity of the narcotic molecule. The whole animal potency of narcotics observed from intravenous injection was greatly influenced by the lipophilicity as well as being dependent on structure and polarity. [The in vitro inhibition by a narcotic of the induced release of acetylcholine from the isolated guinea pig ileum (Kosterlitz, Lord and Watt 1973) more closely reflects intrinsic activity while binding studies on the endogenous opiate receptor reflect affinity (Pert and Snyder 1973).]

Thus quantitative structure activity (potency) relations are governed by a combination of several factors:

i) Physicochemical properties of the drug, primarily its lipophilicity

ii) Topological, topographical and substructure analysis (and when appropriate. those of the relevant biomolecules)

iii) Electronic structure of the drug (and when appropriate those of the relevant biomolecules)

iv) Systems analyses and control theory of how drugs interveneinto the pathways of normal biomolecules and the various influences these drugs exert on the normal biomolecules, on their synthesizing and metabolizing enzymes and processes, on their release from and reuptake back into the presynapse and their effect on the postsynaptic receptors.

In our group we have been calculating or measuring the various factors that contribute to QSAR. Our studies encompass the gamut (Kaufman and Koski 1975a 1975b; Kaufman 1977a) from ab initio quantum chemical calculations on large drug molecules (Popkie and Kaufman 1976b, Popkie, Kaufman and Koski 1976) and improved techniques for such calculations for large drug and biomolecules (Popkie and Kaufman 1975, 1976a, 1977a, 1977b, 1977c; Kaufman 1977b) to theoretical approaches such as systems analyses, control theory and catastrophe theory (Kaufman and Koski 1975a, 1975b; Kaufman, Koski and Peat 1975), physicochemical studies (Kaufman, Semo and Koski 1975) to experimental pharmacology, teratology studies and interactions with physicians in the clinic (Kaufman et al. 1975; Benson, Kaufman and Koski 1976; Kaufman et al. 1975/76; Kaufman, Koski and Benson 1977).

In this presentation we consider QSAR aspects of drug action by moans of experimentally measured $pK_a$'s, oil-water partition and drug distribution coefficients as a function of pH for some hallucinogenic drugs of abuse, some additional narcotics as well as some relevant endogenous biomolecules. Examples will be given of ab initio quality wave functions for large drug and biomolecules calculated by incorporating new desirable techniques. In addition quantum chemical calculations have been carried out in an endeavor to establish the utility of electrostatic molecular potential contour maps in the study of drug action.

## II. PHYSICOCHEMICAL PROPERTIES

The physicochemical properties such as $pK_a$'s, partition coefficients, distribution coefficients, and pH dependences were determined using the same procedures previously outlined in detail (Kaufman,Semo and Koski 1975) so these will not be presented here. The results for some hallucinogenic drugs of abuse, sane barbiturates as well as for some relevant endogenous biomolecules are included in Table I and the results for etorphine are contained in Table II. Aknowledge of these parameters is of interest in QSAR studies since, in general, such investigations follow the approach of Hansch and coworkers (Hansch et al. 1973) which involves the use of linear free energy (LFER) relations

$$\log \left(\frac{1}{c}\right) = -k_1 (\log P) + \text{constants}$$

where c is the concentration in the biophase and P is the partition coefficient,

However, a number of years ago we realized that, while the partition coefficient P (which we call $D_{o/w}$) is an appropriate index for rationalizing the pharmacology of a neutral molecule, the partition coeffiecent as defined by convention is not in general the appropriate index for rationalizing the pharmacology of drugs which can be protonated or deprotonated. The appropriate lipophilicity index for drugs which can be protonated or deprotonated is the apparent partition coefficient $P_{app}$, (which we call $P_{pH}$ in our tables to indicate its pH dependence) (which reflects the drug distribution coefficient).

For a base:

$$\text{Apparent partition coefficient} = P_{app} = \frac{[\text{free base + acid salt}]_{lipid}}{[\text{free base + acid salt}]_{aqueous}}$$

$$\simeq \frac{[\text{free base}]_{lipid}}{[\text{free base + acid salt}]_{aqueous}}$$

For an acid:

$$\text{Apparent partition coefficient} = P_{app} = \frac{[\text{undissociated acid + anion}]_{lipid}}{[\text{undissociated acid + anion}]_{aqueous}}$$

$$\simeq \frac{[\text{undissociated acid}]_{lipid}}{[\text{undissociated acid + anion}]_{aqueous}}$$

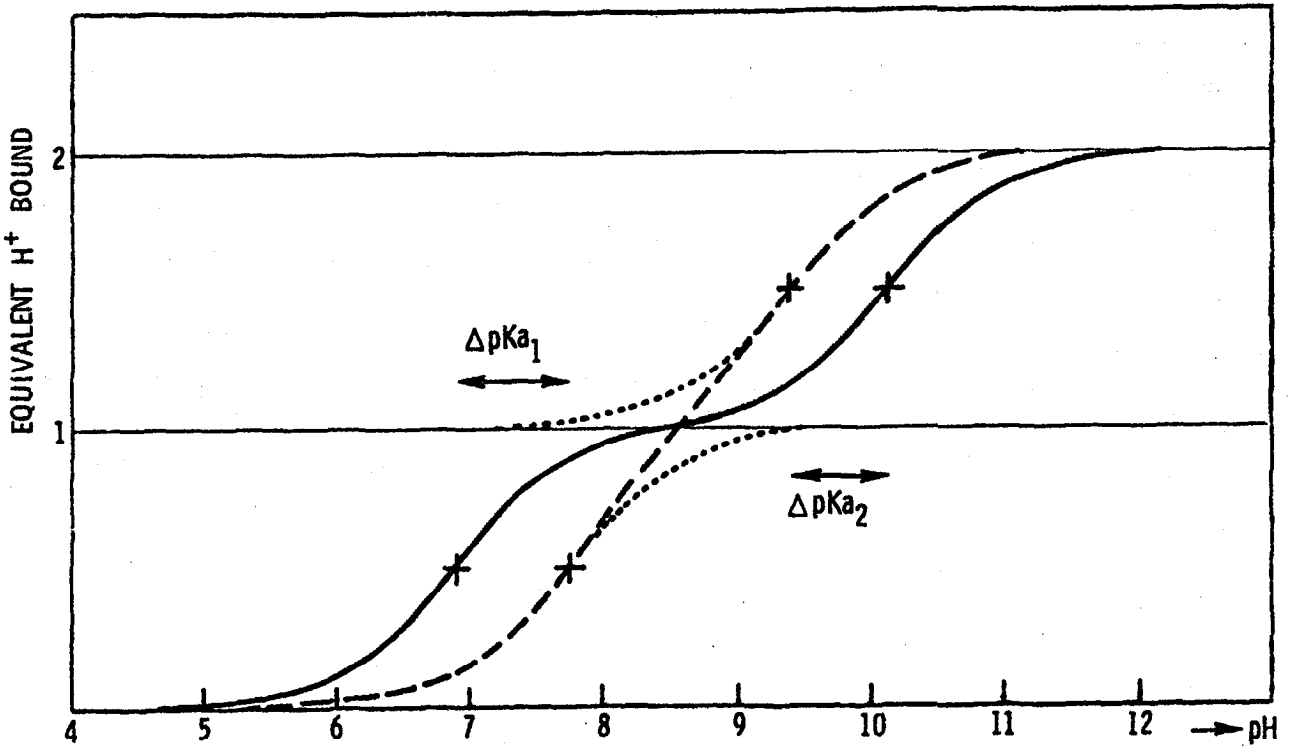
We thus set up a microelectrometric titration method and a related mathematical. deconvolution procecure (Kaufman, Semo, and Koski 1975) which permit

i) Deconvolution of overlapping $pK_a$'s not possible to resolve experimentally alone;

ii) Determination of both the partition coefficient and the apparent partition coefficient from the shift in the measured $pK_a$'s obtained by running the titration in a mixed lipid-aqueous solution.

Often when there are two $pK_a$'s in a molecule, one deprotonating an amine acid salt from a charged to a neutral species and the other deprotonating a phenolic OH from a neutral to a charged anion, it has been difficult to decide which measured $pK_a$ corresponds to which process. By the method we use this ambiguity never arises. The shift in the measured $pk_a$ (figure 1) in mixed oil-water is always toward lower pH in going from a protonated amine (a charged species) to the more lipophilic free base (a neutral species) and toward higher pH going from the more lipophilic neutral species (such as phenol or acid) to an anion (a charged species).

FIGURE 1




Comparison of the aqueous titration and 10% oil-water titration of nalorphine, HCl. *Dashed line is the aqueous titration, solid line is the octanol-water titration. The crosses mark the $pK_a$ values and the $pK_a$'s represent the shifts.*

Our very first experimental results on the narcotics and narcotic antagotists showed several general phenomena crucial to their QSAR and potencies.

i) Strong pH dependencies within the range of attainable human physiologic pH's;

ii) Marked temperature dependencies which were widely differing percentages (from 21 percent to 300 percent) even for related compounds;

iii) Lipophilicities (apprrent partition coefficients) which could differ by several hundred percent for such closely related congeners that the molecules differed by only one C and one H atom in a total of about 50 atoms.

We have established these results on narcotics and narcotic antagonists to clinical practice and clinical observations confirmed our findings.

We have extended such determinations of the pH dependence of lipophilicities to a variety of drugs and biomolecules and to compounds potentially teratogenic (Kaufman, Koski and Benson 1977).

We report here results on some CNS agents and biomolecules which have relevance to the area of drug abuse:

A. Dissociative anesthetics: phenyclidine, ketamine and some ketamine metabolites
B. Barbiturates
C. Normal neurotransmitters
D. Narcotics

Some comments on the results and their significance are in order at this point

The dissociative anesthetics cause hallucinations in adults and thus are not customarily employed in adult anesthesia although they can be used clinically for children. These dissociative anesthetics are among the latest and perhaps most violent drugs of abuse. From our results (Table I) it will be noted that the lipophilicities of the dissociative anesthetics have a similar pH dependence as do the narcotics and narcotic antagonists previously reported. Since the $pK_a$ listed corresponds to deprotonation of an acid salt of an amine, a rise in pH leads to more neutral free base and thus to a higher drug distribution coefficient which in turn influences those pharmacologic actions that are dependent on the drug concentration in the lipid. On the other hand, the metabolites listed in Table I exhibit a mild dependence on pH compared to ketamine. They are considerably less lipophilic than ketamine and thus will be excreted more rapidly.

In the case of barbiturates the $pK_a$ which is relevant to the pharmacological effects of the drugs is that of a deprotonation of the

TABLE 1

$pK'_a$, Partition Coefficient and Drug Distribution Coefficient as a Function of pH (Temp = 37°C)

| Compound | $pK'_a$ | Drug Distribution Coefficient $D_{o/w}$ | $P_{7.10}$ | $P_{7.35}$ | $P_{7.40}$ | $P_{7.45}$ | $P_{7.50}$ | $P_{7.60}$ | $P_{7.70}$ |
|---|---|---|---|---|---|---|---|---|---|
| A. Dissociative Anesthetics | | | | | | | | | |
| Ketamine | 7.205 | 145.60 | 64.03 | 84.84 | 88.87 | 92.80 | 96.62 | 103.80 | 110.31 |
| Ketamine Metabolite I | 6.483 | 57.81 | 46.56 | 50.90 | 51.57 | 52.18 | 52.74 | 53.71 | 54.50 |
| Ketamine Metabolite II | 5.616 | 28.64 | 27.73 | 28.12 | 28.18 | 28.23 | 28.27 | 28.34 | 28.40 |
| Phencyclidine | 9.43* | 1646.66 | 76.38 | 135.36 | 151.72 | 170.04 | 190.55 | 239.18 | 299.95 |
| B. Barbiturates | | | | | | | | | |
| Pentobarbital | 7.870 | 125.90 | 107.62 | 96.69 | 94.03 | 91.22 | 88.25 | 81.91 | 75.11 |
| Hexobarbital | 8.108 | 40.82 | 37.12 | 34.75 | 34.13 | 33.47 | 32.75 | 31.15 | 29.35 |
| Thiopental sodium | 7.495 * | 705.70 | 503.44 | 411.17 | 391.36 | 371.09 | 350.83 | 310.39 | 271.08 |

* $pK_{a_{H_2O}}$ = pK (50% EtOH - $H_2O$) + 0.5 pH

*(Kaufman, Semo and Koski 1975)

**Table I** (continued0

pK'a, Partition Coefficient and Drug Distribution Coefficient as a Function of pH (Temp = 37°C)

| Compound | pK'a | $D_{o/w}$ | Drug Distribution Coefficient $P_{7.10}$ | $P_{7.35}$ | $P_{7.40}$ | $P_{7.45}$ | $P_{7.50}$ | $P_{7.60}$ | $P_{7.70}$ |
|---|---|---|---|---|---|---|---|---|---|
| D. Normal Neuro-transmitters | | | | | | | | | |
| Dopamine | 8.706 (10.25) | 0.92 | $8.98x10^{-1}$ | $8.81x10^{-1}$ | $8.77x10^{-1}$ | $8.72x10^{-1}$ | $8.66x10^{-1}$ | $8.53x10^{-1}$ | $8.37x10^{-1}$ |
| Norepinephrine | 8.423 (9.363) | 0.23 | $2.22x10^{-1}$ | $2.12x10^{-1}$ | $2.10x10^{-1}$ | $2.08x10^{-1}$ | $2.06x10^{-1}$ | $2.00x10^{-1}$ | $1.93x10^{-1}$ |



*$pK_{a_{H_2O}}$ = pK (50% EtOH - $H_2O$) + 0.5 pH

*(Kaufman, Semo and Koski 1975)

neutral phenolic compound to form the anion. Consequently. as the pH goes up into the range of physiologically attainable pH the drug distribution coefficient is expected to decrease. This is supported by the data in Table I for pentobarbital, hexabarbital and thiopental.

Table I also includes data for the neurotransmitters dopamine and norepinephrine. It will be noted that there are 2 $pK_a$'s for each molecule (dopamine: 8.706 and 10.25; norepinephrine: 8.423 and 9.363). The data indicates that the physiologically significant $pK_a$ refers to deprotonation of the phenolic group since the lipophilicity rises with decreasing pH.

In our earlier research (Kaufman, Semo and Koski 1975) we measured the pH and temperature dependence of the lipophilicities of a number of narcotics and narcotic antagonists. The lipophilicities of narcotics and narcotic antagonists were found to be extremely sensitive to pH within the range of attainable human physiologic pH. For an acidotic patient, only about 60 percent as much of a narcotic or narcotic antagonist would penetrate the blood brain barrier as it would at a physiologically normal pH. On the other hand. in a patient whose pH is alkaline about 60 percent more of a narcotic or narcotic antagonist would penetrate the blood brain barrier than at normal pH. This has significant implications in the clinical usage of such drugs as well asinthe drug abuse area (Benson, Kaufman and Koski 1976; Kaufman, et al. 1975/76; Kaufman, Koski and Benson 1977).

Etorphine is about 500 times more potent than morphine when given intravenously but only about 34 times more "potent" when given intraventricularly (Kutter et al. 1970). The high intravenous potency is due in large part to the lipophilicity of etorphine (Table II) which is of the order of 1000 times greater than that of morphine under physiological conditions. Etorphine would be expected to have a rapid onset of action due to its high lipophilicity and preliminary clinical trials confirm this (Jasinski, Griffith and Carr; Lewis[1]). The high intravenous potency of etorphine makes a knowledge of the pH dependence of the lipophilicity an important property, especially in avoiding accidental overdose. The lipophilicity of etorphine is 46% greater at pH 7.7 than at the physiologic norm of 7.4. Consequently, care must be taken in the clinical use of etorphine especially during surgery when this narcotic might be used concomitantly with a general anesthetic under which the patient might hyperventilate and have a rise in blood pH to 7.7.

Since there was interest from the veterinarians in the lipophilicity of etorphine at the higher body temperatures of the larger animals we also carried out the determinations as a function of temperature (20°, 37°, 38°, 40°C) as well as of pH.

**TABLE II**

pK'a, Partition Coefficient and Drug Distribution Coefficient as a Etorphine as a Function pH and Temperaturea

| Narcotic | Temp. °C | $pK_a$ | $D_{o/w}$ | $P_{7.10}$ | $P_{7.35}$ | $P_{7.40}$ | $P_{7.45}$ | $P_{7.50}$ | $P_{7.60}$ | $P_{7.70}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Etorphine | 20° | 7.92 | 2496 | 328.12 | 529.37 | 578.98 | 631.74 | 687.60 | 808.03 | 938.35 |
| | 37° | 7.63 | 3587 | 817.46 | 1234.77 | 1329.50 | 1426.81 | 1527.03 | 1731.17 | 1937.77 |
| | 38° | 7.62 | 3680 | 853.61 | 1285.79 | 1383.42 | 1484.44 | 1587.55 | 1797.72 | 2008.95 |
| | 40° | 7.61 | 3731 | 876.22 | 1317.42 | 1416.99 | 1519.75 | 1624.29 | 1837.92 | 2051.67 |

## III. ELECTRONIC STRUCTURE

### A. LCAO-MO-SCF,MC-SCF and CI

We have long had an interest in quantum chemical calculations of the electronic structure of large drugs and biomolecules (Giordano et al. 1967; Giordano et al. 1968; Kaufman and Kerman 1972; Kaufman 1973a, 1973b; Preston and Kaufman 1973; Preston et al. 1973; Kaufman and Kerman 1974a, 1974b, 1974c; Kaufman, Koski and Kerman 1974; Kaufman and Koski 1975a, 1975b; Saethre et al. 1975; Kaufman and Kerman 1976; Kaufman and Kerman 1977; Popkie and Kaufman 1976b; Popkie, Koski and Kaufman 1976;. Petrongolo, Preston and Kaufman, in press 1977; Petrongolo et al. submitted 1978). In our first paper dealing with pharmacological research in 1964 we performed extended Huckel calculations on the pyridine aldoxime antagonists to the organophosphorus intoxicants to determine both their conformational energy profiles and their charge distributions (Giordano et al. 1967; Giordano et al. 1968). In our earlier studies on the neuroleptics and the narcotics (Kaufman and Kerman 1972; Kaufman 1973a, 1973b; Kaufman and Kerman 1974a, 1974b, 1974c; Saethre et al. 1975; Kaufman and Kerman 1976) we used the CNDO and INDO methods (Pople and Beveridge 1970). Probably the most reliable use of such CNDO or INDO calculations seems to be in comparison of calculated charges on the same atom in closely related molecular environments (Saethre et al. 1975). We have used the semirigorous PCILO method (Diner et al. 1969) (perturbative configuration interaction based on localized orbitals) for conformational analysis (Kaufman and Kerman 1977). We also carried out completely ab initio calculations for narcotics (Popkie, Koski and Kaufman 1976) (morphine and nalorphine) and for neuroleptics (Popkie and Kaufman 1976b) (chlorpromazine and promazine).

However, more recently our major attention has been devoted to the development and implementation of procedures for calculating nonempirical ab initio quality wave functions for the valence electrons of a molecule (Popkie and Kaufman 1975, 1976a, 1977a, 1977b, 1977c, Kaufman 1977b, 1978a, 1978b) by incorporating into our own new fast ab initio computer programs (Popkie, 1974; 1975; 1976) two desirable options: ab initio effective core model potentials (MODPOT) (Bonifacic and Huzinaga 1974) for the inner shell electrons which allow one to treat only the valence electrons explicitly and a nonempirical charge conserving integral prescreening approximation [which we have named VRDO - variable retention of diatomic differential overlap (Popkie and Kaufman 1975)] especially effective for spatially extended molecules.

The charge conserving integral prescreening approximation cuts down significantly on the number of two electron integrals which have to be calculated, stored or processed in the calculation of a large molecule. If too many of the millions of two electron integrals are thrown away by size alone, then the SCF calculation will not converge. This is because there is a delicate balance between one and two electron integrals and while there are relatively few one electron integrals, many of them are large. Thus an effective procedure to discard integrals must still

retain the delicate balance between one- and two-electron intergrals. Our nonempirical VRDDO approximation (Popkie and Kaufman 1975) (variable retention of diatomic differential overlap) was inspired in part by a suggestion from solid state physics by Wilhite and Euwema (Wilhite and Euwema 1974; Euwema and Green 1975). They had proposed several approximations. These speed up considerably minimum basis set SCF calculations even to the point where they might become competitive with less rigorous techniques. These approximations reduce the number of integrals that have to be determined in an ab initio LCAO-MO-SCF calculation on a large molecule. Their first approximation consists of neglecting all one- and two-electron integrals that involve basis function products $\phi_i(1)\phi_j(1)$ whose pseudo-overlap

$$S^*_{ij} = \int \phi_i^*(1)\phi_j^*(1)dv_1$$

is less than some tolerance $\tau_1$ The pseudio-function $\phi_i^*(1)$ is represented by a single normalized spherically averaged Gaussian function whose exponent is equal to that of the most diffuse primitive in the contracted Gaussian function $\phi_i(1)$. A second threshhold $\tau_2$ controls the accuracy of the two-electron repulsion integrals (integrals are accurate to n decimal places); those integrals whose absolute magnitude is less than $\tau_2$ are neglected. Alternatively, if the pseudo overlap of any pair of orbitals in the two electron integrals is less than $\tau_3$ the integrals are neglected. The results (Popkie and Kaufman 1975,1976a, 1977a, 1977b;1977c; Kaufman 1977b, 1978a, 1978b)show both these approximations to be numerically accurate. The computer CPU time required to generate the two-electron integral list for a large molecule is substantially reduced as $\tau_2$ $\tau_3$ are increased. The introduction of the VRDDO approximation with $\tau_1 = 10^{-2}$ and $\tau_2 = 10^{-4}$ a.u. (1 a.u. = 27.21 ev) hardly affects at all the accuracy[2] of the computed properties. The valence orbital energies and gross atomic populations are essentially accurate to 3 decimal places (in a.u.). The error in the total energy increases slightly on going from the smaller to the larger molecules. The maximum error found in the total overlap populations is 0.008 and in the dipole moment is 0.009 a.u.. In the alternative procedure the same accuracy is found if $\tau_1 = 10^{-2}$ and $\tau_3 = 10^{-2}$.

Recently Bonifacic and Huzinaga have given a new formulation of the model potential (MODP0T) method of the core projection type for carrying out LCAO-MO-SCF calculation considering only the valence electrons (Bonifacic and Huzinaga 1974, 1975a, 1975b, 1976, 1977; McWilliams and Huzinaga, 1975). The effect of the core electrons is taken into account through the use of a modified model potential (MODPOT) Hamiltotian:

$$\mathcal{H} = \sum_{i}^{n_v} h(i) + \sum_{i>j}^{n_v} 1/r_{ij}; \qquad (1)$$

$$h(i) = -\frac{1}{2}\Delta_i - \sum_{k}^{n_h} 1/r_{ik} \qquad (2)$$

$$- \sum_{k}^{n_c} (Z_k - N_c^k)(1 + A_{1k} e^{-\alpha_{1k} r_{ik}^2} + A_{2k} e^{-\alpha_{2k} r_{ik}^2})/r_{ik}$$

$$+ \sum_{k}^{n_c} \{B_{1s}^k |1s_k><1s_k| + B_{2s}^k |2s_k><2s_k|$$

$$+ B_{2p}^k (|2p_{xk}><2p_{xk}| + |2p_{yk}><2p_{yk}| + |2p_{zk}><2p_{zk}|)\} .$$

In equations (1) and (2), $n_v$ is the number of valence electrons, $n_h$ is the number of hydrogen atoms and $n_c$ is the number of atoms with core electrons. $N_c^k$ is the number of core electrons in the k-th core. $|1s_k>$, $|2s_k>$ and $|2p_k>$ are inner shell atomic orbitals for the k-th core. The parameters $A_{1k}$, $A_{2k}$, $\alpha_{1k}$, $\alpha_{2k}$ and $B^k$ have been tabulated by Bonifacic and Huzinaga (Bonafacic and Huzinaga 1975a) for the atoms Li to Ne (for the particular atomic basis sets they chose). The $A_{1k}$, $A_{2k}$, $\alpha_{1k}$ and $\alpha_{2k}$ parameters are virtually independent of basis set. The $B_k$ parameters must be optimized from atomic calculations for each different choice of Atomic basis set.

We have implemented the coding for the ab initio effective core model potential for inner s, p (Popkie 1974; 1975) and d (Popkie 1975) orbitals with s, p and d orbitals. We extended the derivation to inner f orbitals in our f orbital package (Popkie 1976).

We verified the accuracy of the MODPOT method for a variety of molecules by carrying out the completely ab initio reference calculations with two-electron integrals accurate to 6 decimal places. Then the MODPOT calculations were carried out with valence shell two-electron integrals accurate to 6 decimal places and the MODPOT modified one-electron integrals. The same reference valence atomic basis set was used for both calculations. Comparison of the MODPOT results to the completely ab initio ones (including inner shell electrons) showed the valence orbital energies and gross atomic populations are accurate to better than 2 decimal places (the average error is 0.005 a.u.). The maximum error in the orbital energies for the largest molecule studied to date is only 0.011 a.u. and in the gross atomic populations is only 0.010 a.u.. It should be emphasized that the accuracy obtained with the MODPOT approximation is more than acceptable if one wants to reproduce ab initio results for large molecules. Both these options (MODPOT and VRDO) cut down significantly on the time necessary for quantum chemical calculations on large molecules yet retain the chemical accuracy.

Since both the MODPOT and VRDDO techniques are independent, they can be used together. The combined MODP0T/VRDDO method is just as accurate compared to the completely ab initio calculations as is the MODPOT method itself. The relative accuracy of the MODPOT/ VRDDO method along a potential energy surface, such as for molecular conformational changes or interactions between two species, (0.0001 - 0.0002 a.u.) is even greater than its absolute accuracy.

The results of our testing of the MODPOT, VRDDO and MODPOT/VRDDO methods on a variety of molecules including heteroaromatics and substituted aromatics indicated that these methods give excellent agreement with the completely ab initio results using the same valence atomic basis set. Part of the excellent agreement arises from our careful prior optimization of the atomic model potential parameters in both the isolated atom and in the small prototype molecules containing these atoms.

Moreover we have previously devoted considerable attention to optimization of small completely ab initio atomic basis sets to match as closely as possible the results of larger completely ab initio atomic basis sets and to examining the influence of basis set size and composition on calculated orbital energies and charge distributions.

We have been carrying out nonempirical MODPOT/VRDDO calculations on a variety of drugs, endogenous biomolecules, and other compounds suspected of being teratogens and carcinogens. As an example, the timings for some prototype endogenous biomolecules (Kaufman 1977b; 1978a, 1978b) using a carefully optimized MODPOT atomic basis set corresponding to a 6-3G full basis set, indicate the speed of this method.

| MOLECULE | NO. ATOMS | | NO. BASIS FNS. | TIME (Minutes) | |
|---|---|---|---|---|---|
| | C,N,O, | H | | Integrals* | S C F† |
| Neurotransmitters | | | | | |
| Norepinephrine | 12 | 11 | 59 | 16.9 | 8.4 |
| 6-Hydroxydopamine | 12 | 11 | 59 | 17.2 | 8.3 |
| Histamine | 8 | 9 | 41 | 6.8 | 4.1 |
| Serotonin (5-HT) | 13 | 13 | 65 | 22.9 | 10.9 |

* CDC 6600 computer, FIN, OPT = 0. (The integrals run even 50 percent faster under OPT = 2 on a CDC 6600 computer which supports a full OPT = 2 compiler).

† CDC 6000 computer, FIN, OPT = 2.

More recently we have been carrying out MODPOT/VRDDO calculations on even larger molecules (teratogens and carcinogens and their metabolites) and the increase in computer time with the number of basis functions (n) appears to rise by much less than by the theoretical $n^4$ for the completely ab initio calculations retaining all integrals. The timings by the MODPOT/VRDDO method (as well as by completely ab initio calculations) are influenced also by the size and shape of the molecules. In particular, there is very little increase in integral or SCF time for the almost planar polyclic aromatic hydrocarbon methyl cholathrene (100 basis functions) over that for the closely related benzopyrene (92 basis functions).

While many of the phenomena of interest for large drug and biomolecules involve properties related to the ground states of these molecules, there are certain biological and chemicophysical phenomena which involve excited states. Thus several potential energy surfaces of different spin or symmetry may be involved. To calculate appropriately molecular interactions one needs configuration interaction (CI) (Shavitt 1976) or multiconfiguration SCF (MC-SCF) (Hinze 1973) potential energy surfaces. To interpret measured UV and visible spectra it is necessary to calculate excitation energies, which can only be carried out properly by CI or MC-SCF calculations.

Thus we meshed the MODPOT, VRDDO and MODPOT/VRDDO methods into the MC-SCF and CI programs (Shavitt and Pipano). We tested the combined MODPOT/CI program and the results are accurate to 0.005 ev compared to a completely ab initio calculation including the inner shells and the MODPOT/CI results are accurate to 0.01 ev compared to the experimental values. The most time and space consuming part of a CI calculation is the transformation of integrals from integrals over atomic basis orbitals to integrals over molecular

orbitals. A procedure has been implemented which allows folding into a new effective CI Hamiltonian the influence of the molecular orbitals from which no excitations are allowed (Raffenetti 1973-74; Raffenetti 1974-76). This permits us to transform only over molecular orbitals from and to which excitations are allowed.

Thus, with the combination of MODPOT, VRDDO and MODPOT/VRDDO SCF, MC-SCF and CI procedures, rapid and reliable calculations on large drug and biomolecules are more than feasible at present.

**B. Electrostatic Molecular Potential Contour Maps**

In this phase of the study we present the calculations of electrostatic molecular potential contour maps and illustrate their application to drug action.

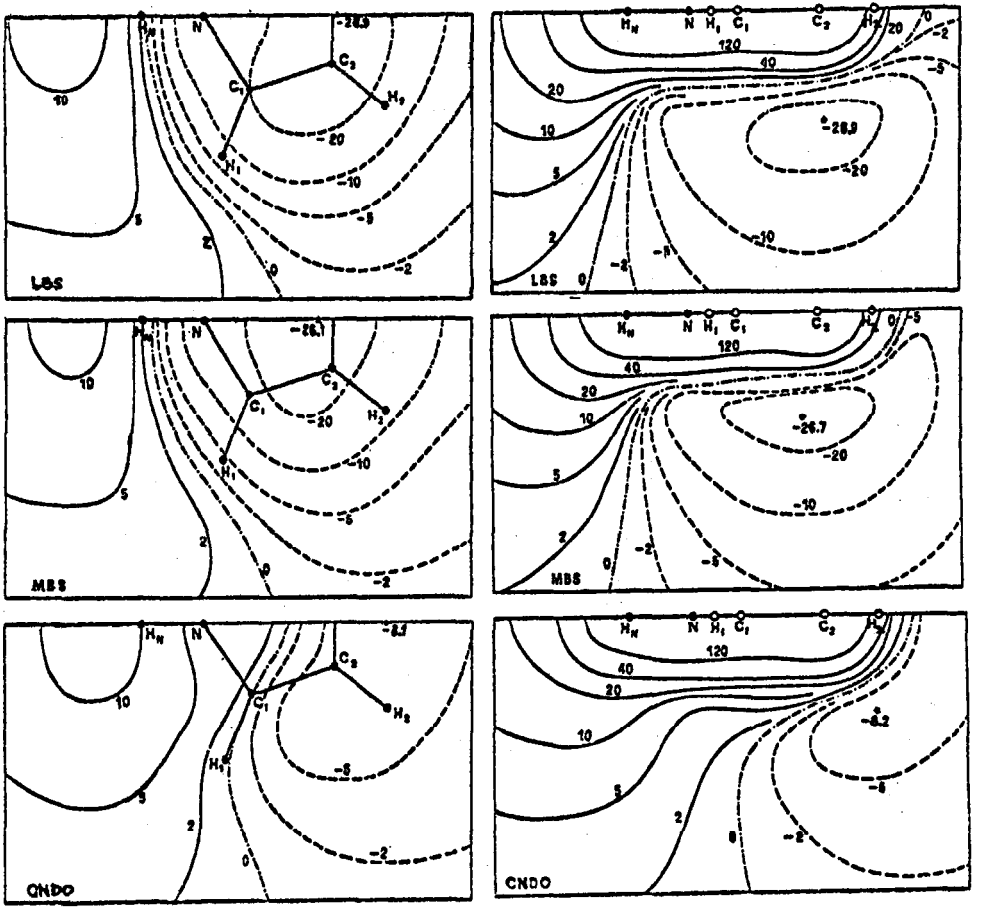
The electrostatic potential V(r), arising from molecule A is completely defined at every point of the space if one knows the charge distribution (electronic and nuclear) of the molecule (Scrocco and Tomasi. 1973).

In general it is preferable to use ab initio [or now ab initio effective core model Potential (Kaufman 1978a, 1978b; Pullman 1977, Weinstein and Osman 1977; Osman and Weinstein 1977)] wave functions employing a well balanced basis set from which to calculate electrostatic molecular potential contour maps. Frequently it is desirable if the molecules are quite large to use small atomic basis sets and the question arises whether maps generated from such basis sets using completely ab initio wave functions match the results of those using larger atomic basis sets. We have calculated the electrostatic potential map for pyrrole (Petrongolo, Preston and Kaufman, in press 1977) using both the results of our previous large basis set ab initio calculations (Preston and Kaufman 1973) and those using a minimal basis set which had been carefully optimized to reproduce closely the properties calculated from the wave functions with a large basis set. The results are illustrated in figure 2 and they show that the large basis set (LBS) and the matching minimal basis set (MBS) wave functions generate maps virtually identical in shape and in numerical values. It will be noted, on the other hand, the electrostatic molecular potential contour maps generated from deorthgonalized CNDO wave functions as suggested by Pullman (Giessner-Prettre and Pullman 1968) do not appear similar in shape let alone in numerical values (figure 2) confirming the results of others (Giessner-Prettre and Pullman 1972; Petrongolo and Tomasi 1975).

It would appear reasonable that one of the theoretical requisites for a drug that acts as a direct agonist to a normal neurotransmitter is that the electrostatic molecular Potential contour map which is generated around the pharmacophorically significant parts of the drug molecule must resemble very closely in shape and numerical magnitude the contour that is generated around the corresponding pharmacophorically significant parts of the normal

**FIGURE 2**

Pyrrole - molecular plane
Electrostatic molecular potential contour map




LBS - large basis set

MBS - minimal basis set

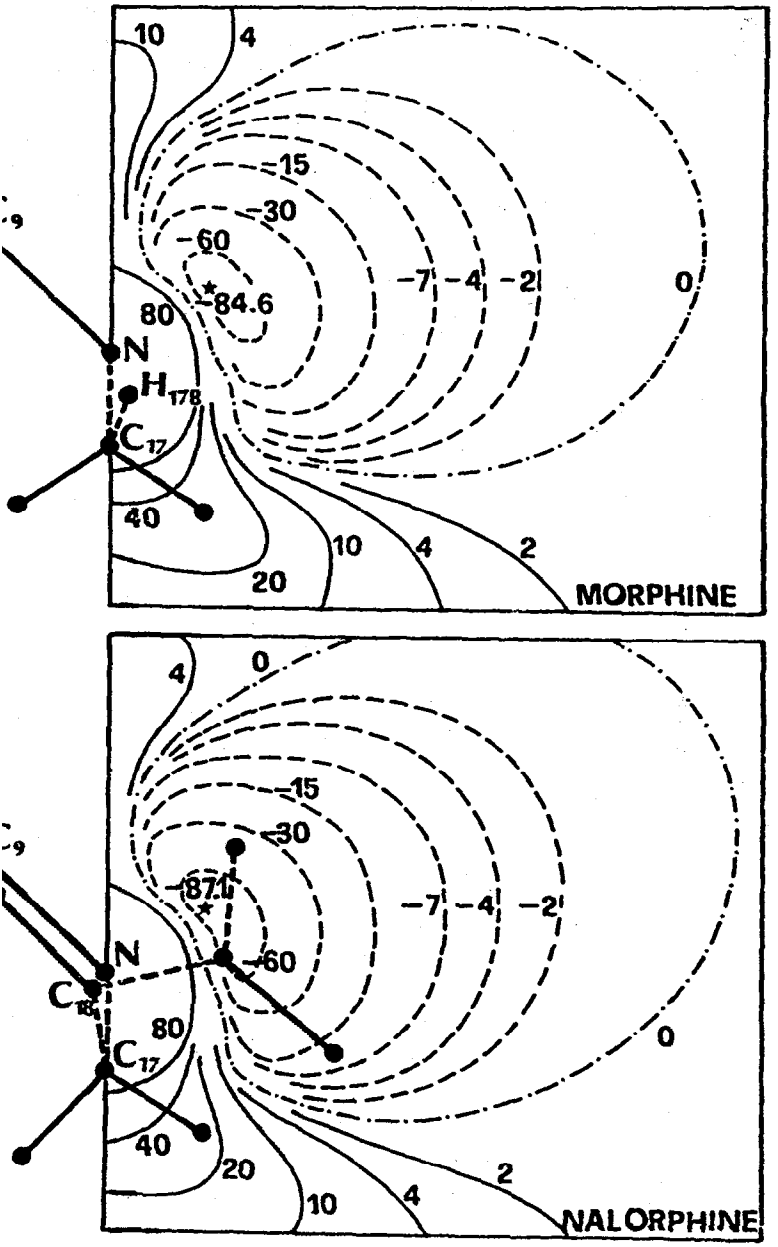
CNDO - CNDO deorthogonalized

neurotransmitter itself. In these applications one must be careful to distinguish between direct and indirect agonists. In the former case the direct agonist binds to the postsynaptic receptor site and so perfectly mimics the true endogenous normal neurotransmitter that the receptor cannot distinguish the agonist from the neurotransmitter. In the case of the indirect agonist the concentration of the normal neurotransmitter is raised by an indirect means at the postsynaptic receptor either by decreasing the metabolism, increasing its synthesis, increasing its release from the presynapse or interferring with its reuptake back into the presynapse. It is clear that in the latter case potential contour maps would not be applicable.

An interesting example of this concept is shown in figure 3 where we have generated (Petrongolo, et al. Submitted 1978) the electrostatic molecular potential contour map around the pharmacophorically significant N atoms of morphine (an N-methyl narcotic agonist) and nalorphine (an N-allyl narcotic agonist-antagonist) from our ab initio calculations on morphine and nalorphine (Popkie, Koski and Kaufman 1976). Using the criterion defined above, namely that two related drugs which extert a common pharmacological effect must generate similar or identical maps around their relevant pharmacologically significant pharmacophores, it is clear in figure 3a that the molecular potential contour maps generated around the nitrogen in the lone pair direction are nearly identical in shape as well as in numerical value. This suggests that it is in this direction from the N atom that both morphine and nalorphine exert their common narcotic agonist effect. On the other hand, maps generated around the substituent groups on the N atoms in morphine and nalorphine from a different perspective (figure 3b) are completely different. In particular there is a region around the isolated double bond in the allyl group of nalorphine which is attractive to an electrophilic reagent in contrast to the completely repulsive region around the methyl group in morphine. This corresponds also to a low lying unoccupied C = C antibonding orbital in all of the narcotic antagonists. This low lying unoccupied orbital contributes to the paramagnetic polarizability type term which causes the $^{15}N$ NMR shift of nalorphine to be different than that of morphine (Kaufman 1977a).

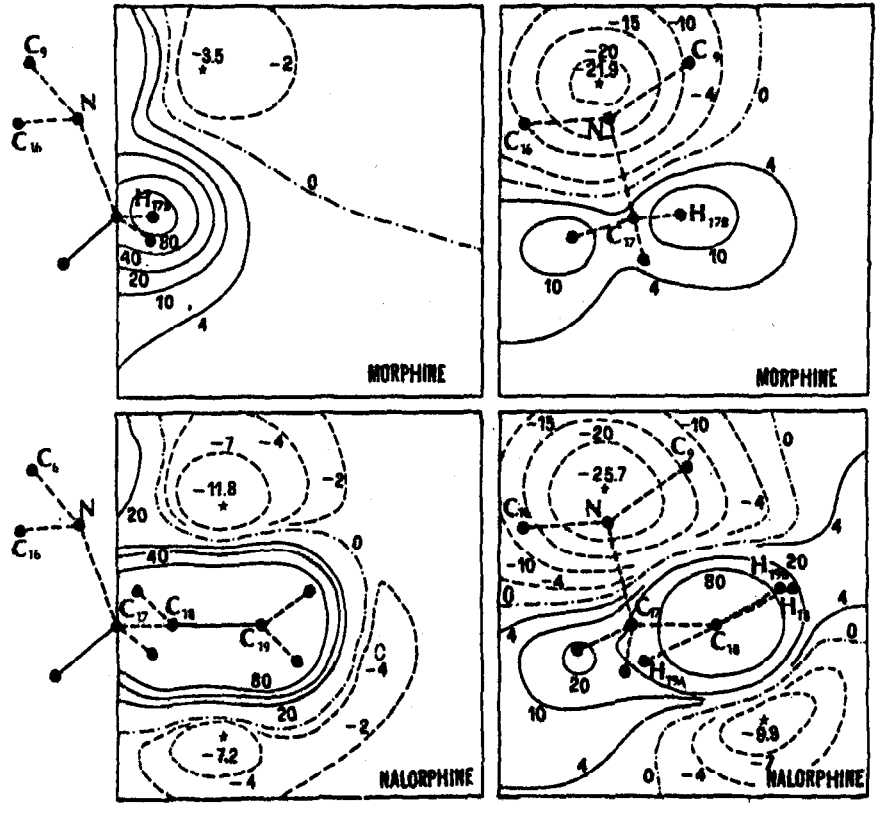
Moreover, to investigate in a thorough and comprehensive manner the agreement or disagreement of electrostatic molecular potential maps generated from CNDO or INDO wave functions (either orthogonalized or deorthogondlized), to determine the influence of the various approximations when CNDO or INDO wave functions are used and to assess the influence of the GTO expansion to STO'S, in collaboration with Petrongolo (Petrongolo et al. Submitted for publication) we generated the maps using both approximations III and IV(Giessner-Prettre and Pullman 1972) and STO-3G and STO-6G expansions. Striking changes are obtained deorthogonalizing both the CNDO and INDO eigenvectors. Different expansions, STO-3G or STO-6G, give the same quantitative results. The significant conclusion is that if the minimum around the N atom is compared

**FIGURE 3a**




Morphine and Nalorphine - N lone pair direction
Electrostatic molecular potential contour map

FIGURE 3b




Morphine and Nalorphine - N substituent direction Electrostatic molecular potential contour map

with the minimum around the O atoms, the semehipirical relative ordering is greatly changed with respect to the ab initio one. Near the oxygen atoms of the OH groups the semiempirical potential holes are as deep as near the N atom or even deeper. This is in contrast with the trend found in present and previous (Scrocco and Tomasi 1973; Petrongolo and Tomasi 1975) ab initio calculations. Also, ab initio and semiempirical calculations, give even different qualitative results in the $\pi$ region of the benzene ring in morphine and nalorphine since the ab initio attractive region due to the $\pi$-electron distribution of the benzene ring becomes repulsive. In the $\pi$-region of the allyl group, only the semempirical results using the approximation IV and the STO-6G expansion are in agreemerit with the ab initio ones. From these and other results (Scrocco and Tomasi 1973; Petrongolo and Tomasi 1975; Petrongolo, Preston and Kaufman - in press 1977; Petrongolo et al, - submitted for publication) the conclusion remains that the CNDO and INDO methods can only be used with caution to get V (r) and that it seems difficult to derive any general rules.

## IV. SYSTEMS ANALYSIS, CONTROL THEORY AND CATASTROPHE THEORY

We formulated (Kaufman, Kerman and Koski 1974; Kaufman and Koski 1975a, 1975b; Kaufman, Koski and Peat 1975; Kaufman 1976; Kaufman, Koski and Peat 1977) a system analysis and control theory and catastrophe theory (Thom 1972; Thom 1975) framework in order better to clarify the dynamic balance of the influence of endogenous diseases or exogenous CNS drugs and for combined interactions of several CNS drugs with diverse modes of action (Kaufman 1976). Using this we have been able to delineate the conditions for normal, abnormal, and "catastrophic" dynamic balance of neurotransmitters. We have also been able to program this for an interactive computer system that ties into a patient clinical test and drug record keeping system and allows computer generated diagnostics and prescriptions.

Moreover, drugs which cause physiological addiction appear to be categorized by causing a particular type of neurotransmitter imbalance while drugs which may be addictive psychologically but not physiologically present a different neurotransmitter profile.

## FOOTNOTES

[1]J. Lewis. Private communication, 1975.

[2]Where accurate implies accuracy with respect to the completely ab initio calculations with the same atomic basis set retaining all integrals $10^{-6}$ a.u. or greater.

[3]Figure 1 is reprinted with permission, from Kaufman, J. J., Semo, N. M., and Koski, W. S. Microelectrometric titration measmement of the $pK_a$'s, partition and drug distribution coefficients of narcotics and narcotic antagonists and their pH and temperature dependence. J Med Chem 18(7), 1975, p. 649. © American Chemical Society, 1975:

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# Structure-Activity Studies of Narcotic Agonists and Antagonists from Quantum Chemical Calculations

Gitda H. Loew, Donald S. Berkowitz, and
Stanley K. Burt

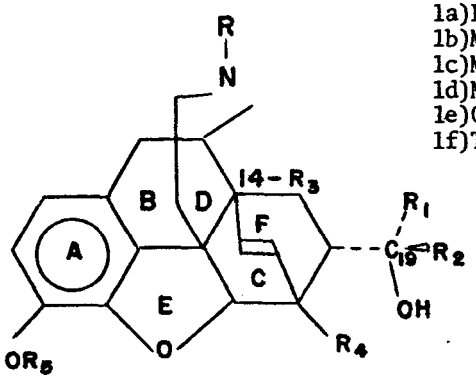
In this paper, the application of quantum chemical methods in elucidating electronic and structural requirements for opiate narcotic analgesic agonism and antagonism will be discussed. In particular the relevance of this method to two specific problems of opiate activity will be described.

One major effort centers on characterizing molecular features which determine the extent of agonism and antagonism in a given opiate. This problem is of clinical importance since there is growing evidence that a clinically useful analgesic with little or no addiction liability will be a mixed agonist/antagonist with just the "right" mixture of both activities. It is also particularly suited to quantum chemical studies, since the ratio of agonism to antagonism in a given compound should be a receptor-related event. To help understand how chemical modifications in a series of opiate analogues alter their agonist/antagonist potency ratios, calculations have been done on N-substituent variations in a series of rigid opiates with and without an axial $C_{14}$-OH group and for a series of oripavine derivatives with varying $C_7$ substituents. In addition, interaction of these different agonist/antagonist with a model for an anionic receptor site have been studied.

Another focus of attention has been on the diversity of chemical structures which have narcotic analgesic activity. Conformational studies have been made for a number of different types of flexible opiates including methadone, meperidine and enkephalins. Since all of these classes are thought to act at the same receptor as rigid morphine like opiates, low energy conformers for each type of opiate were characterized and compared with each other and with rigid opiates. Studies of this type should help understand how these more flexible opiates interact at the receptor site. They should also indicate to what extent results of structure activity studies for one class of opiates should carry over to another class.

## I. STRUCTURAL FEATURE OF RIGID OPIATES THAT MODULATE AGONIST/ANTAGONIST ACTIVITY

It is now generally acknowledged that compounds possessing potent analgesic activity yet having some degree of antagonist properties are good candidates for clinically useful anagesics with low addition potential. N-substituents of fused ring opiates (1a-f)




| Class | Rings |
|---|---|
| 1a)Benzomorphans | A,B,D |
| 1b)Morphinans | A,B,C,D |
| 1c)Morphine | A,B,C,D,E |
| 1d)Morphone | A,B,C,D,E |
| 1e)Oripavines | A,B,C,D,E,F |
| 1f)Thebaines | A,B,C,D,E,F |

play a central role in determining the relative analgesic agonist/antagonist potencies of these molecules. However, other structural modifications such as an axial $C_{14}$-OH group in the morphone (6 keto 7,8 dihydro morphine) series and ong chain ($R_1$,$R_2$) tertiary carbinol substituents on $C_7$ in the thebaine ($R_5$=$CH_3$) and in the oripavine series ($R_5$=H) mitigate this ratio for a given N-substituent.

To help understand how these chemical modifications in a series of opiate analogues alter their agonist/antagonist potency, semiempirical quantum chemical calculations have been done for N-substituent variations in a series of rigid opiates with (oxymorphone) and without (morphine) an axial $C_{14}$-OH group and for a series of oripavine derivatives with varying substituents on $C_7$. In addition, preliminary studies of the interaction of different agonist/antagonist with models for anionic receptor site have been made. The goal of these studies was to identify structural properties which might explain the observed variation in relative agonist/antagonist potencies.

In early work (Loew, Berkowtiz, *et al* 1975) the effect of polar groups on the relative agonist potency of morphine derivatives was explored by mapping of electrostatic potentials of low energy conformers of morphine, codeine, heroin, 6 monoacetyl and 2 amino morphine, in the presence of a model anionic receptor site (fig. 1 morphine). This work established the relevance of a dynamic interaction pharmacophore (Weinstein, *et al* 1973) for opiates. Only by initial interaction of the quaterinized amine group with an anionic receptor site do the remainder of the polar groups

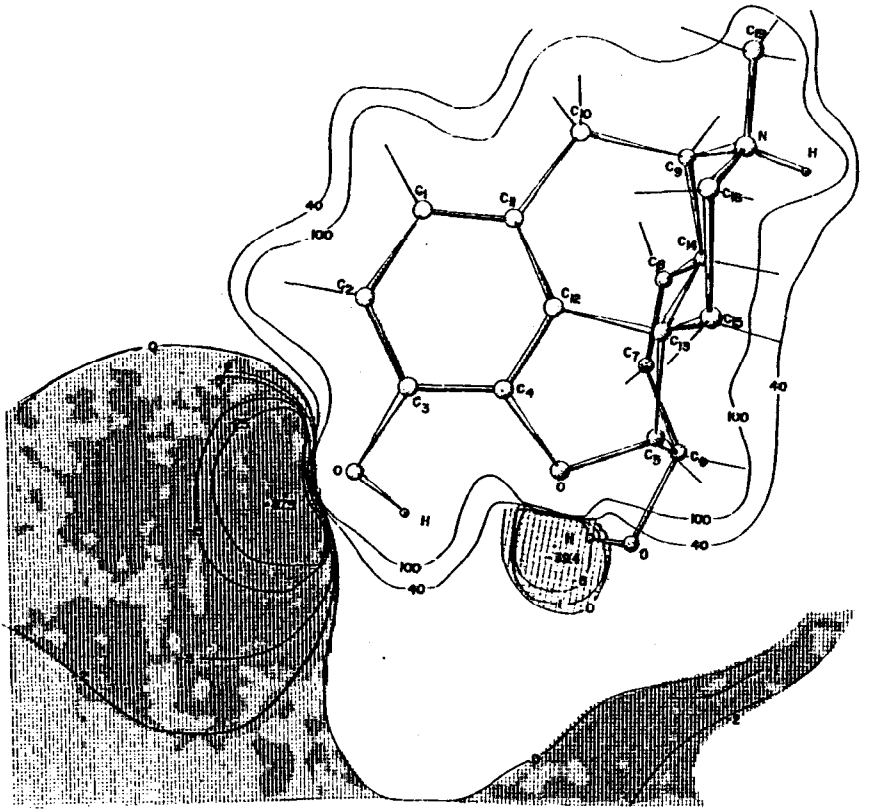



***Figure 1.*** *Calculated electrostatic potential pattern of cis morphine in the plane of the benzene ring*
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(e.g., 3-OH, furan oxygen) develop significant negative potential to effectively interact with cationic receptor sites. These electron rich regions are greatly diminished in neutral species and in the isolated opiate cation.

In another study (Loew, Weinstein, Berkowitz 1976) to help understand the origin of the enhanced agonist activity of a $C_{14}$-OH group in pure agonists we have investigated the ionization and partition behavior of the related opiates hydromorphone and oxymorphone including the effect of solvent on them. The $C_{14}$-OH group was shown to enhance the proton affinity of the nitrogen in the presence or absence of solvent, and to increase the pKa in agreement with experiment (Jolliffe, Ahmad 1971; Kaufman, Semo, Koski 1975) and contrary to the usual inductive effect of an OH group. By studies of hydration energies, we were also able to conclude that both the apparent and intrinsic partition coefficients of the $C_{14}$-OH oxy compounds should be lower than the $C_{14}$-H analogue. If our predictions are correct, the intraven ricular potency of oxymorphone

relative to hydromorphone should be greater than the apparent two fold differences obtained from standard $ED_{50}$ measurements (Mellet, Woods 1963).

In contrast to agonist potency, relative agonist/antagonist potency in a given compound is a receptor related event. In a series of related compounds, variations in this ratio would most likely be due to differences in binding and interaction at the receptor site. Thus correlations of structural features of the isolated molecule with this clinically important behavior should be particularly successful since the influence of such factors as transport and metabolism are kept to a minimum. To establish such correlations all of the conformational calculations reported here. for N-substituents with and without $C_{14}$-OH and for $C_7$ substituents were done on the isolated protonated form of the Opiate though; to be the active form (Casy 1973). Solvent effect on conformation are not expected to be substantial at the lipophilic receptor site.

The possibility of induced conformational change at the receptor site is included in all our studies by considering many low energy conformers rather than a single global minimum as receptor site candidates. All calculations were performed using the semiempirical all valence electron program called PCILO (Perturbative Configuration Interactive. using Localized Orbitals) developed in the Laboratory of Professor Bernard Pullman (Diner; *et al* 1969) specifically for energy conformational studies of large molecules. It has been used extensively to study a variety of pharmacological and biological molecules ( Christoffersen 1976) and has proven its reliability by consistent agreement with x-ray structures and results of more sophisticated calculations.

## A. EFFECT OF N-SUBSTITUENT AND AXIAL $C_{14}$-OH GROUP ON AGONIST/ANTAGONIST POTENCY

### 1. Origin of Mixed Agonism/Antagonism in Nalorphine.

In our first study of the effect of N-Substituent on agonist/antagonist potency (Loew, Berkowitz 1975), nalorphine and N-phenethyl morphine were chosen as prototype compounds. From the results of those studies, we proposed that the dual agonist antagonist behavior of nalorphine was due to the existence of two low energy “types” of equatorial N-allyl conformations at the receptor (fig. 2). Differences in activity between agonist and antagonist could not be attributed to electronic effects (Loew, Berkowitz 1975; Kaufman, Kerman 1974; Popkie, Koski, Kaufman 1976). Such differences in drug conformation, as well as in receptor conformation, could account for the differences between agonist and antagonist binding associated with the observed Na+ effect (Pert, Snyder 1974).

### 2. Origin of Mixed Agonism/Antagonism in Oxymorphone Series.

In addition to our hypothesis for nalorphine, several other investigators have speculated on the roles of N-substituents and the axial

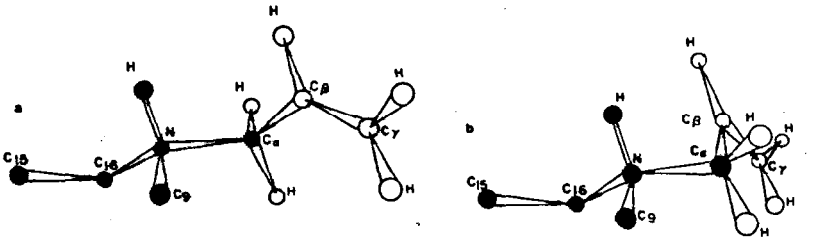

***Figure 2.*** *Two types of low energy allyl group conformers in nalorphine: (a) low energy, noninterfering (agonist) conformer; (b) minimum energy, protruding (antagonist) conformer*
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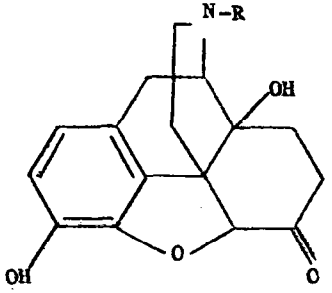
$C_{14}$-OH group in conferring agonist or antagonist activity on the him-like fused ring structure. It has been suggested (Smythies 1976) that axial $C_{14}$-OH groups might act by direct steric interference with certain equatorial N-substituent conformations. In another widely publicized hypothesis (Feinberg, Cresse, Snyder 1976), it has been suggested that agonist and antagonist activities are associated with axial and equatorial N-substituents, respectively, and the efffect Of the $C_{14}$-OH group enhancing antagonism is explained by assuming its presence is responsible for a shift from an axial to equatorial N-substituent conformation. Although there is evidence for rapid interconversion between axial and equatorial N-substituent orientation of unhindered piperidines in solution (Jones, Katritsky, *et al* 1970), nitrogen inversion is unverified in the highly rigid fused ring opiates. Further, in all crystal structures of neutral and protonated 3,4,5 and 6 fused-ring opiates reported to date N-substitents have been found to be equatorial.

To further explore these hypothesis and the role of the $C_{14}$-OH group and various N-substituents in mediating agonism and antagonism, a systematic quantum chemical study has been made of six oxymorphone derivatives (2a-f)




2a, R = $CH_3$ (oxymorphone)
b, R = $CH_2CH = CH_2$ (naloxone)
c, R = $CH_2CH = C(CH_3)_2$ (nalmexone)
d, R = $CH_2$-Δ (naltrexone)
e, R = $CH_2$-◇ (nalbuphone)
f, R = $CH_2CH_2\phi$

This series is a good example of the complex role the N-substituent plays in controlling pharmacological behavior ranging from potent agonism (2a and 2f) to mixed agonism-antagonism (2c, 2e) to pure

potent antagonism (2b, 2d) (Kosterlitz, Waterfield 1975; Blumberg, Dayton 1974; Pert, Snyder 1974; Kosterlitz, Lord, Watt 1972; Deneau, Villareal, Seevers 1966). While there are three structural differences between the morphine and oxymorphone parent compounds, it is the $C_{14}$-OH group that appears to most drastically alter the morphine-like pharmacological profile for varying N-substituents (Cordon, Priciro, *et al* 1974; Kutter, Herz., *et al* 1970; Knoll 1975). In mixed agonist-antagonist, the $C_{14}$-OH drastically diminishes agonism while increasing antagonism.

The geometry of the oxymorphone fused ring structure was taken from a recent crystal structure of naloxone (Karle 1974) and is comparable to other recent oxymorphone derivative structures (Sime, Dobler, Sime 1976). With this basic ring structure, energy conformation calculations including geometry relaxation were made for both axial and equatorial N-substituents. Energy differences between optimized axial and equatorial and barriers to inversion conformers were obtained for each compound (2a-f) in their protonated and neutral form as well as for similar forms of morphine and nalorphine. Possible interaction of the $C_{14}$-OH group with these substituents was also considered.

The results summarized in table I do not support the hypothesis that axial N-substituents are required for agonist activity while equatorial substituents confer antagonist activity. No correlation exists between agonist/antagonist potency variation and calculated axial-equatorial energy differences in either the base or protonated form of the opiates studied. For all compounds studied, the equatorial conformer is the lowest energy form. This result is consistent with crystal structure data as are the optimized values of equilibrium bond angles about the nitrogen. Morphine and nalorphine have comparable axial-equatorial N-substituent energy differences, as do oxymorphone and naloxone. Some effect of the $C_{14}$-OH can be seen by larger energy differences in the oxymorphone series over those in morphine and nalorphine. However, axial conformations are equally accessible in naloxone and oxymorphone indicating an equally hindering effect of the $C_{14}$-OH group on agonists and antagonists. In general there is no systematic increase in axial conformational energies as agonist potency decreases.

The inversion barriers calculated for the neutral molecules are insensitive to N-substitution and to the presence or absence of $C_{14}$-OH and hence show no correlation to agonist/antagonist potencies. The inversion barriers correspond to the calculated differences in energy between the lowest energy (equatorial) and planar structures. Calculated barrier heights for ammonia and aniline were 12 and 7 kcal/mole respectively, compared to known values of 6 and 2. Thus while the PCILO method overestimates these quantities, their relative values are reasonably reliable.

Comparison of the calculated inversion energies (table I) with those calculated for ammonia and aniline indicates that the inversion process can occur in these fused ring opiates at about the same rate as in ammonia and that the axial conformer is accessible, particularly

**TABLE I:** ANIAL-EQUATORIAL ENERGY DIFFERENCES IN THE PROTONATED AND NEUTRAL SPECIES AND NEUTRAL MOLECULE INVERSION ENERGIES

| compd / Energy | Morphine | Nalorphine | Oxymorphone | Naloxone | Nalmexone | Naltrexone | Nalbuphone | N-phenyl Noroxymorphone |
|---|---|---|---|---|---|---|---|---|
| $\Delta E^a$ (protonated) | 0.4 | 1.2 | 1.8 | 1.9 | 1.0 | 2.0 | 1.9 | 2.2 |
| $\Delta E^b$ (neutral) | 2.9 | 2.1 | 5.1 | 5.4 | 4.5 | 4.0 | 3.5 | 3.9 |
| $\Delta E^{*c}$ (inversion) | 14.9 | 12.1 | 15.0 | 13.2 | 13.4 | 11.8 | 12.3 | 15.2 |

[a] Energy differences in kcal/mol between axial and equatorial minimum energy protonated conformations using optimized N bond angles.

[b] Energy differences in kcal/mol between axial and equatorial minimum energy neutral molecule conformations using optimized bond angles.

[c] Energy differences in kcal/mol between planar and equatorial minimum energy neutral molecule conformations using optimized geometries.

in the protonated form of the isolated molecule. There is no evidence, however, that this inversion or the presence of axial conformers is related to drug action at the receptor. No direct interaction of the $C_{14}$-OH group with low energy axial or equatorial conformations of the N-substituents occurs and hence cannot explain the effect of $C_{14}$ substitution on pharmacological activity.

The rotational behavior of the equatorial N-allyl group in naloxone, with the $C_{14}$-OH in its minimum energy conformation, is identical within computational accuracy to that calculated for the allyl group in nalorphine. The allyl group in naloxone retains the two types of low energy conformers found in nalorphine which we previously associated with its dual agonist and antagonist behavior (Loew, Berkowitz 1975). In low energy regions of axial nalorphine and naloxone the same statements can be made. Thus, the total abolition of agonist activity in naloxone cannot be explained by differing accessible allyl conformations from those in nalorphine nor by direct interaction of the axial $C_{14}$-OH with low energy N-allyl conformers.

As mentioned above we have suggested that agonist/antagonist behavior could be regulated by different low energy equatorial N-substituent conformers (Loew, Berkowitz 1975). We have also recently reported calculations (Berkowitz, Loew 1976) which suggest that the $C_{14}$ OH group and various N-substituents cannot interact directly but might interact indirectly through a common anionic receptor site such as R-$SO_4$ or $R_1R_2PO_4$ Both of these hypotheses together can be used to help understand the variation of agonist/antagonist potency in the series of oxymorphone derivates studied.

Drug receptor complex formation could occur by simultaneous interaction of the $C_{14}$-OH, cation nitrogen, and certain N-substituents with a model antonic receptor site (sulfate or phosphate). The ternary complex formed is stable only for a small range of N-substituent conformations. These could correspond to effective antagonist-receptor interaction and account for the relatively strict structural requirements for pure antagonism. The role of the $C_{14}$-OH group in diminishing agonism then would be to cause preferential selection of only one type of low energy conformer for receptor complex formation from among the several hypothesized “agonist” and “antagonist” N-substituent conformations.

With this suggested hypothesis, allyl and methylcyclopropyl N-substituents would be prototypes for optimum formation of such ternary complexes since they have a comparatively high degree of felxibility and $\pi$ character. Nalmexone and nalbuphone are less flexible and have less $\pi$ character and thus would form weaker ternary complexes reducing the effect of the $C_{14}$-OH group (i.e., creating a less pure antagonist).

If this hypothesis is correct, any substitution that detracts from the strict geometric requirements found for ($C_{14}$-OH)- (anion)- (N-allyl) antagonist complexing should enhance the agonist/antagonist potency ratio. Two such alterations, the $C_{14}$-$OCH_3$ derivative of naloxone and

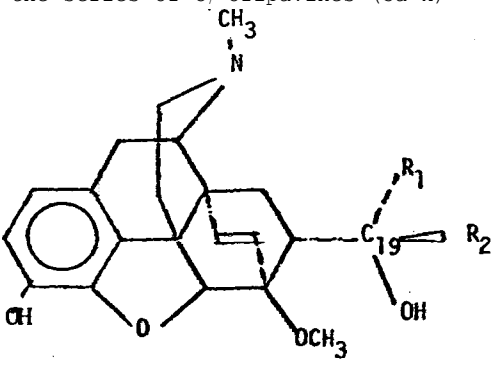
the $C_{15}$-OH analogue, should restore agonism. Removal of the $C_{14}$-OH group together to make N-allyl hydromorphone would also produce a agonist/antagonist. The ability of the axial $C_{14}$-OH group alone to reduce intrinsic agonism has been recently demonstrated by the decreased agonism of naltrexone relative to its hydromorphone analogues in the guinea pig ileum. Also, one would predict that substitution on cyclopropyl ring carbons would alter the locations of conformational local minimas and reduce the pure agonism of naltrexone by reducing overlap with the local minima of the allyl group.

The lack of antagonist activity of methyl and phenethyl N-substituents could then be explained by their lack of interaction with the receptor anionic site. The N-phenethyl substituent could, however, interact with a more distant binding site enhancing its binding and agonist patency. Further characterization of the interaction of different N-substituents with a model anionic site is now in progress.

**B. EFFECT OF $C_7$ SUBSTITUENTS ON AGONIST/ANTAGONIST IN ORIPAVINES**

The oripavine and thebaine series of opiates synthesized by Bentley, Lewis and co-workers (Lewis, Bentley, Cowan 1971) in the search for clinically useful narcotic agonists and antagonists are structurally similar to morphine and dihydromorphine but contain a $C_6$-$C_{14}$ etheno bridge and $C_7$ substituents. Among other reasons, the series became a focus of attention because some of the compounds had unexpectedly high agonist [>1000 x morphine (M)] and antagonist [>100 x nalorphine (N)] potencies, and because the relative agonist/antagonist potencies of N-substituted compounds were dramatically sensitive to substitution at $C_7$ and because some of the compounds showed unique pharmacological profiles.

To provide additional insight into causes of sane of these variations in activity, we have made a conformational study of the series of $C_7$ oripavines (3a-k)




| | $R_1$ | $R_2$ |
|---|---|---|
| 3a | H | H |
| b | $CH_3$ | H |
| c | H | $CH_3$ |
| d | $CH_3$ | $CH_3$ |
| e | $CH_2CH_3$ | $CH_3$ |
| f | $CH_3$ | $CH_2CH_3$ |
| g | $CH_2CH_3$ | $CH_2CH_3$ |
| h | $(CH_2)_2CH_3$ | $CH_3$ |
| i | $CH_3$ | $(CH_2)_2CH_3$ |
| j | $C(CH_3)_3$ | $CH_3$ |
| k | $CH_3$ | $C(CH_3)_3$ |

Calculations were performed cm compounds 3a-k to determine the most energetically feasible conformations of the $C_7$ substituents. In

these studies attention was given to possible hydrogen bonding interaction between the $C_6$-$OCH_3$ and the $C_7$-OH group. Although the calculations reported here were done on compounds in the oripavine series ($C_3OH$) the results are equally applicable to compounds in the bridged thebaine series ($C_3$-$OCH_3$) since the $C_3$ and $C_7$ positions are separated enough to preclude conformational interaction.

The pharmacology of this and related series has been reviewed (Lewis, Bentley, Cowan 1971) and only illustrative points will be mentioned here. The compound most similar to M, differing only by a $C_{14}$-$C_{16}$ ethyl bridge and a methoxy group on $C_6$ is 30 x M in the rat tail pressure test (Lewis Redhead, Smith 1973) and only six times as potent as $C_6$-$OCH_3$ morphine (Soine, Willette 1966). Thus the greatly increased agonist activity of etorphine (31) [1,100 x M RTP] (Lewis, Redhead 1970) must be strongly linked to the additional substituents present at the $C_7$ position. The relative agonist/antagonist potencies of the well-studied N-methyl cyclopropyl and N-allyl derivatives of (3) are also highly sensitive to $C_7$ substitution. For example, in the N-methyl cyclopropyl derivatives of 3d and 3i lengthening $R_2$ from a $CH_3$ group to $CH_2CH_2CH_3$ (i) changes the activity from strong antagonism (50 x N RTP1) to potent agonism (1,000 x MRTP1). In the N-allyl derivatives, the same variation in $R_2$ causes a similar change in activity from moderate antagonism (2 x N RTP1) to strong agonism (60 x M RTR1).

The sensitivity of agonist/antagonist potency ratio to $C_7$ substitution is most likely related to binding and interaction of the $C_7$ substituent at the receptor site. To explain the effect of $C_7$ substituents on the observed activity of oripavines, several hypotheses have been presented. Based on the Beckett and Casy receptor model (Beckett, Casy 1954) it has been proposed (Lewis, Bentley, Cowan 1971) that the $C_7$ substituents interact with a lipophilic receptor site since polar $C_7$ substituents reduce agonist activity (Bentley, Lewis, Smith 1972) while large lipophilic substituents substantially enhance it (Lewis, Bentley, Cowan 1971). This idea has been extended to suggest that in phenethyl etorphine, ($R_1$=$CH_3$, $R_2$=$CH_2CH_2\phi$) the phenethyl moiety, by complementarity, describes a receptor site which stabilizes a conformation of the receptor associated with agonism (Feinberg, Creese, Snyder 1976). Much has been made of the "F" ring binding but without results of extensive binding studies the importance of aromaticity is unclear since at least five other compounds with $R_2$ saturated have been synthesized (Bentley, Hardy 1976) with higher *in vivo* potencies than the phenethyl derivatives. It is hoped that conformational studies of the $C_7$ substituents 3a-k would provide some insight into how they modulate agonist/antagonist activity.

Torsion angles for substituents at all positions which would not interact with the $C_7$ substituent or with each other were determined by independent variation. For the $C_6$-$OCH_3$ group whose conformation could be coupled to the $C_7$ substituent conformation, rotations were performed in etorphine with the $C_7$ substituent in hydrogen bonding and nonbonding conformations. In all cases only one conformer was

the only local minima and was held fixed in all subsequent calculations. The most extensive calculations were performed for the lowest energy intramolecularly hydrogen bonded structures ($\tau_3$ = 300°, $\tau_1$ = 60°, $\tau_{10}$ = 60°) where energies were determined for $C_{19}$ alkyl group(s) using all combinations of rotation angles for each optical isomer. Local minima were determined in this way for hydrogen bonded forms. These, as well as those determined using models and less complete rotational variations, were then optimized to determine local minima accurate to a few degrees and energies accurate (within the computational method) to a few tenths of a kcal/mole.

The results of conformational studies (table II) indicate that intrarmolecular hydrogen bonding to the $C_6$-$OCH_3$ in oripavines does not appear to play a dominant role in determining the conformation of $C_{19}$ carbinol substituents on $C_7$ if $R_1$ are $R_2$ hydrogen or methyl groups (3a-d). This result is in keeping with the observation of several conformers of these $C_7$ substituents in the NMR spectra (Fulmor, *et al* 1967). With these small groups, the $C_7$ substituents have substantial conformational freedom in binding to the receptor. Thus it is not surprising that their apparent potencies are similar, increasing somewhat with lipophilicity of the alcohol group (17, 15, 37 and 63 x M RTP for 3a-d, respectively), and that the relative

Table II

Energy Differences Between H Bonded and Non H Bonded* Conformers of $C_7$ Substituents of Oripavine

| Compound | ΔE (HBond-NoHBond) |
|---|---|
| 3a | -0.4 kcal/mol |
| 3b | +0.3 |
| 3c | +0.3 |
| 3d | +0.1 |
| 3e | +0.9 |
| 3f | +1.9 |
| 3g | +3.4 |
| 3h | +1.4 |
| 3i | +2.3 |

*H bond between $C_6OCH_3$ + $C_7$ OH groups.

agonist/antagonist activities conferred by different N-substituents are consistent with structure activity relationships found in other fused-ring opiates.

In compounds with tertiary carbinol substituents of the type $C_{19}CH(CH_2)\eta CH_3OH$ with $\eta \geq 1$ (table II) hydrogen bonding is favored in both diastereoisomers. This constraint fixes the $C_7$ substituent in one of two distinct spatial regions which are different for the (R)- and (S)-diastereoisomers (fig. 3). It is suggested that this difference in orientation determines the extent to which the long

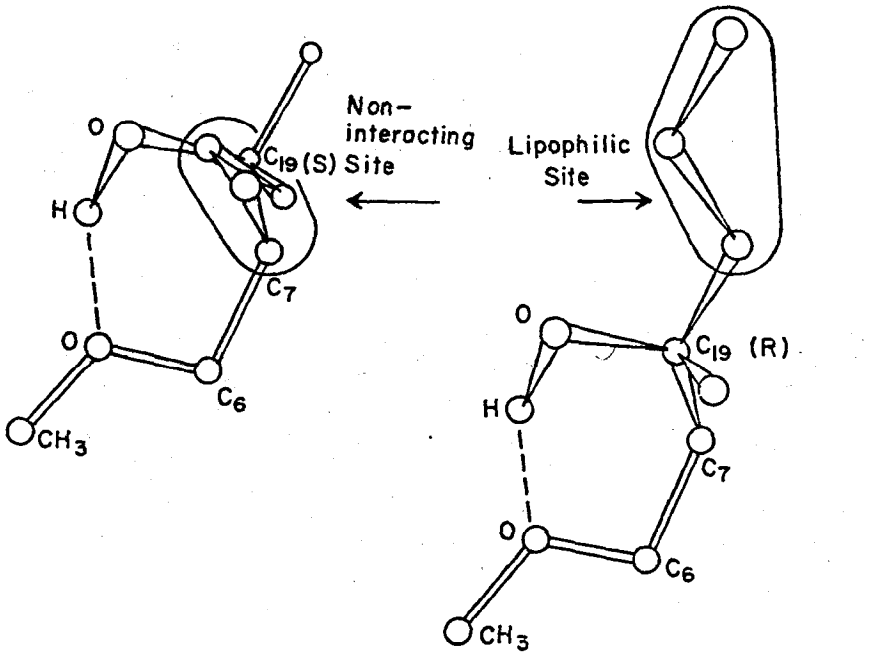
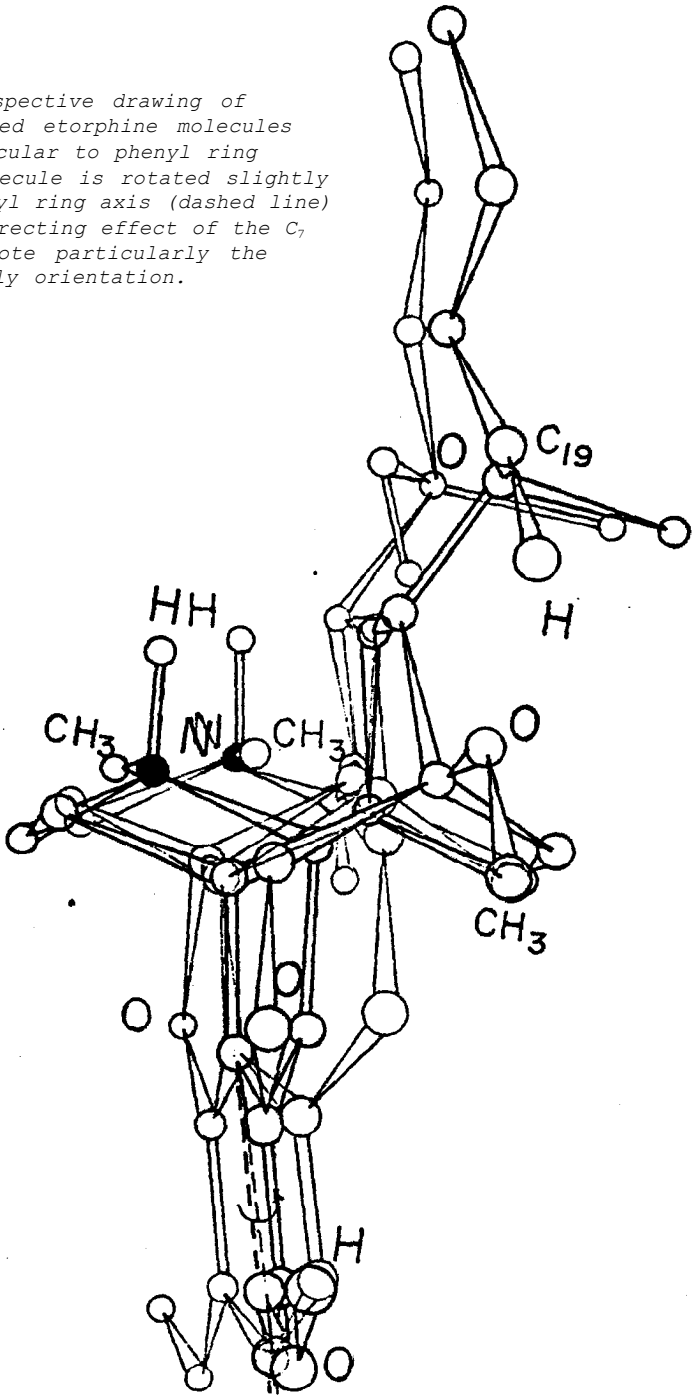



***Figure 3.*** *Lowest energy conformers of $C_7$ R- and S-substituent diastereoisomers for etorphine showing by complementarity the lipophilic site for accommodation of the most potent isomer*

alkyl group of the $C_{19}$-carbinol can be accommodated at the postulated lipophilic receptor could account for the difference in observed agonist potencies in the (R) and (S) isomers of N-$CH_3$ compounds. For example, in compounds 3e, f, h and i the R-diatereoisomers are much more potent than either the (S) diastereoisomers or compounds (3a-d) with smaller alkyl groups. The calculated low energy conformers of the more potent (R) isomers place the longer alkyl chain in one spatial region. This region should, by complementarity, define optimum binding to a lipophilic receptor site. We further suggest that it is the interaction of these conformationally restricted groups with this site that directs the overall orientation of these compounds at the receptor and imposes a change in N-substituent binding relative to morphine like compounds. Specifically, in keeping with our hypothesis, the change in orientation imposed on the N-substituent by binding of the $C_{19}$ n-$CH_2$ $CH_3$ group in a rigid conformation could interfere with the antagonist "type" of N-substituent binding mode while enhancing the agonist "type" (fig. 4). The anomalous structure-activity profiles of the N-allyl and N-methyl cyclopropyl derivatives of etorphine (3i) could then be due to this altered overall drug receptor interaction. If this hypothesis is correct, replacement of the $C_6OCH_3$ or the $C_{19}$-OH group by a H or $CH_3$ substituent should enhance or restore antagonist potency to N-allyl and N-cyclopropyl-methyl derivatives of oripavines and thebaines with long chain, $C_7$ carbinol substituents.

***Figure 4.*** *Perspective drawing of two superimposed etorphine molecules shown perpendicular to phenyl ring plane. One molecule is rotated slightly about the phenyl ring axis (dashed line) to show the directing effect of the $C_7$ substituent. Note particularly the altered N-methly orientation.*

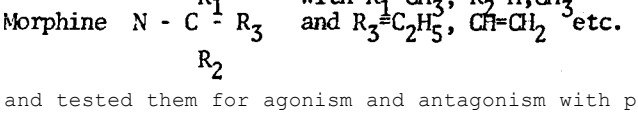


In compounds 3j and 3k, related to $C_{19}$ (R) and (S) diatereoisomers of buprenorphine, the bulky t-butyl substituents must be accommodated at nearly identical positions in the two diastereoisomers thus allowing an intramolecular $C_6$-O---H-O-$C_{19}$ hydrogen bond in the (S) isomer (3k) and not in the other. The pharmacology of the (S) isomer of buprenorphine has been extensively studied (Cowan, Lewis, MacFarlane 1977; Cowan, Doxey, Harry 1977) but there appears to be no published data on the (R) diastereoisomer or any such pair of $C_{19}$ t-butyl isomers. In contrast to the $C_{19}$ straight chain deriivated compounds (such as 3h and 3i ) we predict that intrinsic activity in the t-butyl compounds 3j and 3k should have a rather low sensitivity to $C_{19}$ optical isomerism as the t-butyl group occupies similar positions in th isomers.

**SUMMARY AND PRACTICAL IMPLICATIONS**

These studies have given us some insight into important opiate structural features which modulate agonist/antagonist potencies. Specifically, in the morphine series we have suggested that restriction of the conformational freedom of the N-substituent to. two types of low energy equatorial conformers causes the molecule to adopt binding modes favoring either agonism or antagonism.

Our results refute any connection between agonist/antagonist potency ratios and the extent of axial/equatorial N-substituent conformers. The role of $C_{14}$-OH group in modulating agonism and antagonism of N-substituent in oxymorphone is not by direct interaction but possibly thorugh their simultaneous binding to the anionic receptor site to form a stable tertiary complex. In oripavines, the agonist/antagonist profile of N-substituents is altered relative to morphine by the directing effect of the $C_7$-substituents that hydrogen bond to the $C_6$-$OCH_3$ group. These $C_7$-substituents are relatively rigid in both diatereoisomers with long alkyl chains on $C_{19}$. This rigidity could also account for the differences in potency between the (R)- and (S)- isomers. On the basis of these hypotheses a number of predictions of altered agonist/antagonist with specific chemical modifications have been made. For example, in the morphine series we have suggested that further restrictions of the conformational freedom of the N-substituent could cause the molecule to adopt binding modes favoring either agonism or antagonism. Placing of substituents at appropriate locations on the alkyl side chains should create such conformational restrictions. Colleagues at SRI International have thus synthesized a series of morphine compounds:




and tested them for agonism and antagonism with promising results (DeGraw, Lawson, Crase 1978). Other compounds in this series are also being investigated.

In the oripavine series we have suggested that the replacement of the $C_6$-$OCH_3$ by a nonhydrogen substituent would restore antagonism to N-allyl and N-methylcyclopropyl derivatives. In the oxymorphone series we predict that replacement of $C_{14}$-OH by $C_{14}$-$CH_3$ or $C_{14}$-$OCH_3$ would restore agonism to naloxone and naltrexone as would substitutions on the cyclopropyl ring of naltrexone. These prediction, if true, would further verify our hypothesis and could lead to synthesis of other clinically useful analgesics.

## II. CONFORMATIONAL STUDIES OF FLEXIBLE OPIATES AND THEIR RESEMBLANCE TO RIGID OPIATES

### A. Methadone and 4-Phenyl Piperidines

Rigid opiates (la-d) have in common the three fused rings (A, B, D) of the 6,7-benzomorphan nucleus. The salient common features of such opiates which appear to be necessary for analgesic activity are: a tertiary amine group which is part of a piperidine ring; an aromatic ring which is constrained to be axially connected to the piperidine ring at the central carbon atom ($C_{13}$) *para* to the nitrogen, and polar oxygen groups, particularly an OH group at the $C_3$ position of the phenyl ring.

In the 4-phenyl piperidine class of opiates, the phenyl ring can be axial or equatorial to the piperidine ring with free rotation about the bond between them. Meperidine (fig. 5)

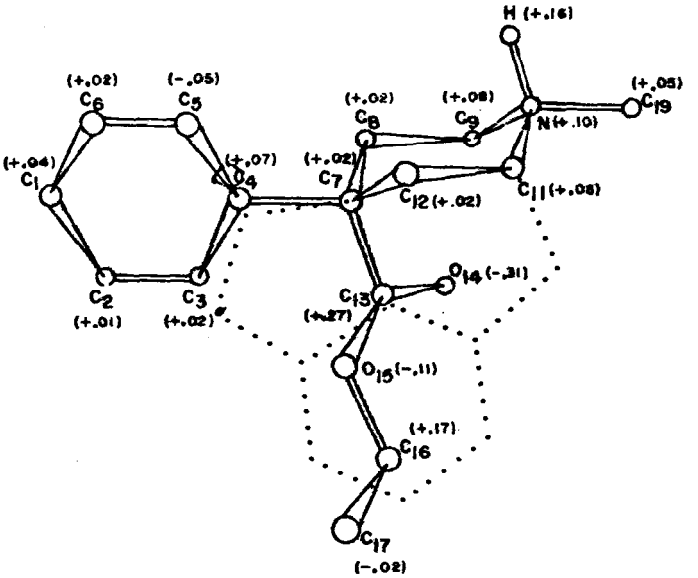



***Figure 5.*** *Minimum energy conformer of meperidine with piperidine ring superimposed on that of morphine and calculated net atomic charges.*

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and its reverse ester desmethyl prodine (fig. 6) are common examples

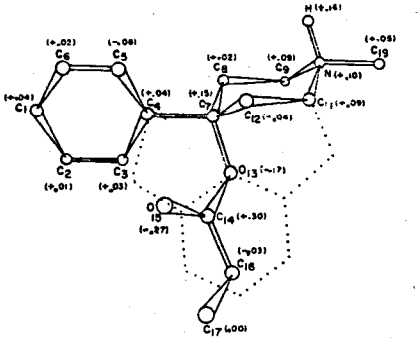

***Figure 6.*** *Desmethyl (or (+) α) prodine with piperidine ring superimposed on that of morphine and calculated net atomic chaarges*

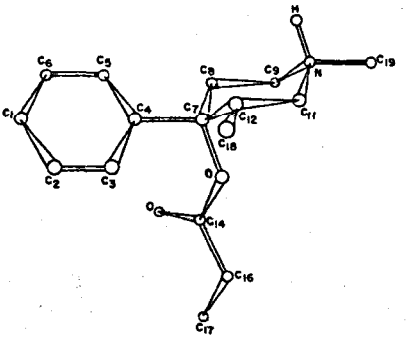

***Figure 9.*** *Minimum energy conformer for (-) α, (-) ß prodine with piperidine ring superimposed on that of morphine*

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of such opiates. In addition to the relative orientation of the two rings, another defining conformational feature in these compounds is the relative orientation of the ester chain.

Meperidine is 1/10 as potent as morphine when administered subcutaneously in mice and tested by a modified hot plate method (Portoghese, Larson 1973). However, intraventricular data determined by a tooth pulp test in rabbits indicate that it is only 1/60 as potent as morphine when transport and distribution factors are eliminated (Kutter, Herz, Teschemacher 1970). Evidence for meperidine-type opiates acting at the morphine receptor is given by the fact that cross tolerance is observed and both are antagonized by nalorphine and naloxone (Aceto, McKean, Pearl 1969).

The reversal of the ester chain from meperidine to desmethyl prodine enhances *in vivo* potency by a factor of 10, making desmethyl apparently equal to morphine in potency (Portoghese, Larson 1973). As seen in table III they span a potency range of 100 with (+) isomers more potent than (-). While no intraventricular potency data is available for these compounds, metabolic and brain concentration studies (Portoghese, Abdel-Monen, Larson 1972) indicate that differences in potency among them is due solely to receptor events.

Despite the fact that crystal structures indicate that protonated meperidine (Van Konigsvel 1970), (+)α (Ahmed, Barnes, Kartha 1960) and (-) ß (Ahmed, Barnes 1963) prodines are all in a phenyl-equatorial conformer, there was a tendency to believe that because of its greater similarity to morphine an axial conformer was implicated at the receptor (Beckett, Casy 1954) and that the lower its energy the more potent the drug (Porteghese 1966). The recent synthesis of active 4-phenyl piperidine analogues constrained to

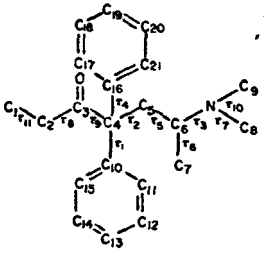
Table III. Salient Energy-Conformation Characteristics of Six Compounds Studied

| Compound | X-Ray structure | | Low-energy conformers | | | | | | ΔE (eq-ax), kcal/mol | Potency $ED_{50}$ mg/kg |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | φ equatorial | | | ϕ axial | | | | |
| | $\tau_1$[a] | $\tau_2$[a] | $\tau_1$[a] | $\tau_2$[a] | $\Delta E$[b] | $\tau_1$[a] | $\tau_2$[a] | $\Delta E$[b] | | |
| Meperidine | 0 | 90 | 60 (0–90)[c] | 0 | 0 | 30 | ±45 | 0 | 5.3 | 13.1 |
| | | | 120 (60–150) | ±90 | 0 | 30 | All values | 2 | | |
| | | | 60 | 0 ± 90 | 4 | | | | | |
| | | | 150 | ±45 | 4 | | | | | |
| Desmethylprodine | | | 60 | 0 | 0 | 30 | ±45, 180 | 0 | 6.6 | 1.3 |
| | | | 150 | ±45 | 0 | | | | | |
| | | | 30 (0–30) | ±45 | 3 | 30 | All values | 3 | | |
| | | | 120 (60–120) | ±90 | 4 | | | | | |
| α-(+)-Prodine | 58 | 294 | 105 (75–135) | 300 | 0 | 45 (30–45) | 0 (300–60) | 0 | 8.6 | 0.91 |
| α-(−)-Prodine | (182)[d] | (66) | 135 (105–165) | 60 | 0 | 15 (15–30) | 0 (300–60) | 0 | 8.6 | 22.4 |
| β-(+)-Prodine | (62)[d] | (248) | 90 (45–105) | 300 | 0 | 0 | 180[e] | 0 | 21 | 0.25 |
| | | | 45 | 0 (300–60) | 2.5 | 120 (105–120) | 180 (180–300) | 1 | | |
| β-(−)-Prodine | 158 | 62 | 150 (135–195) | 60 | 0 | 0 | 180[e] | 0 | 21 | 3.3 |
| | | | 195 | 0 (300–60) | 2.5 | 120 (120–135) | 180 (60–180) | 1 | | |

[a] $\tau_1$, $\tau_2$ in degrees as defined in text. [b] $\Delta E$ relative to minimum energy conformer in kcal/mol. [c] Values of angles in parentheses indicate range of broad minima. [d] Inferred values from X-ray structure of isomer. [e] In these conformers the 3-$CH_3$ group was eclipsed rather than staggered.

be in a phenyl equatorial position has altered such hypotheses (Portoghese, Mihail, Kupferber 1968). No conformational studies of 4-phenyl piperidine compounds exist to further explore the general question of similarities between morphine, meperidine, and prodines in their low energy conformers.

Methadone as shown below (4) has a central carbon atom ($C_7$) to

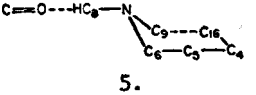


4.

which are attached two phenyl rings, a $COC_2H_5$ carbonyl group and a $CH_2CH(CH_3)$ $N(CH_3)$ amine group This compound retains the feature of a phenpropyl amine group in common with both morphine and 4-phenyl piperidines . The tertiary amine is acyclic. Moreover methadone has 11 degrees of rotational freedom as shown above with defining torsion atoms for each angle listed in table IV.

There is evidence that methadone also acts at the same receptor site as morphine (Goodman, Gilman 1970; Smits, Takemori 1970). Tests for analgesic activity in mice have shown an $ED_{50}$ for methadone approximately equal to morphine and five times stronger than meperidine (Smits, Takemori 1970; Eddy, Halback, Braenden 1956). However, in intraventricular administration (Kutter, Herz, Teschemacher 1970) the potency of methadone is 1/30 that of morphine and only twice that of meperidine. These results are consistent with its enhanced lipophilicity (Kutter, Herz, *et al* 1370) and a less efficacious mode of action at the receptor site than morphine.

Speculation over the past 20 years on morphine-like conformers for methadone has centered on intramolecular interaction, particularly of the amine and carbonyl groups. In 1954, Gero proposed a protonated confomer of methadone with a hydrogen bond between one of the N-methyl groups and the oxygen presumably confining the molecule in a rigid position and causing formation of pseudorpiperidine ring (5).



5.

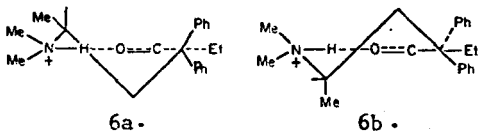
Another structure for the protonated form has been proposed based on intramolecular bonding between the nitrogen proton and the

**Table IV.** Minimum Energy Conformers of Cadidate Methadone Structures

| Defining atoms[h,j] | $\tau_i$[i] | Protonated conformers | | | | | | | | Nonprotonated conformers | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1[a] | 2[b] | 3[c] | 4[d] | 5[e] | 6[f] | 7[f] | 8[g] | I[a] | II[b] | III[h] | IV[f] |
| $C_{Ph_1}$[j]-$C_{10}$-$C_4$-$C_3$ | 1 | 95.0 | 86.0 | 176.0 | 168.0 | 101.0 | 65.0 | 70.0 | 20.0 | 143.0 | 86.0 | 37.0 | 166.0 |
| $C_{10}$-$C_4$-$C_5$-$C_6$ | 2 | 189.0 | 50.0 | 312.0 | 309.0 | 190.0 | 79.0 | 317.0 | 310.0 | 296.0 | 51.0 | 296.0 | 354.0 |
| $C_5$-$C_6$-N-$H^l$ | 3 | 321.0 | 72.0 | 315.0 | 46.0 | 315.0 | 303.0 | 46.0 | 30.0 | 158.0 | 45.0 | 27.0 | 37.0 |
| $C_{10}$-$C_4$-$C_{16}$-$C_{Ph_2}$[j] | 4 | 32.0 | 160.0 | 36.0 | 16.0 | 98.0 | 6.0 | 50.0 | 55.0 | 95.0 | 161.0 | 69.0 | 19.0 |
| $C_4$-$C_5$-$C_6$-N | 5 | 230.0 | 197.0 | 221.0 | 259.0 | 73.0 | 93.0 | 263.0 | 285.0 | 292.0 | 192.0 | 301.0 | 287.0 |
| $C_5$-$C_6$-$C_7$-H | 6 | 58.0 | 54.0 | 71.0 | 49.0 | 76.0 | 82.0 | 50.0 | 60.0 | 52.0 | 33.0 | 55.0 | 51.0 |
| $C_6$-N-$C_9$-H | 7 | 76.0 | 57.0 | 69.0 | 50.0 | 71.0 | 63.0 | 50.0 | 60.0 | 58.0 | 44.0 | 60.0 | 51.0 |
| $C_4$-$C_3$-$C_2$-$C_1$ | 8 | 159.0 | 260.0 | 251.0 | 154.0 | 259.0 | 200.0 | 203.0 | 225.0 | 192.0 | 260.0 | 100.0 | 109.0 |
| $C_{10}$-$C_4$-$C_3$-$C_2$ | 9 | 69.0 | 299.0 | 272.0 | 291.0 | 28.0 | 182.0 | 211.0 | 170.0 | 180.0 | 299.0 | 188.0 | 79.0 |
| $C_6$-N-$C_9$-H | 10 | 67.0 | 80.0 | 55.0 | 60.0 | 60.0 | 38.0 | 61.0 | 60.0 | 49.0 | 59.0 | 55.0 | 68.0 |
| $C_3$-$C_2$-$C_1$-H | 11 | 60.0 | 60.0 | 64.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 63.0 | 60.0 | 65.0 | 61.0 |
| $\Delta E$,[k] kcal/mol | | 9.8 | 11.6 | 4.3 | 0.0 | 12.8 | 29.5 | 12.1 | 120.1 | 11.4 | 4.2 | 0.0 | 3.6 |

[a]X-ray structure of conjugate acid. [b]Extended chain. [c]Structure 5. [d]Structure 6a. [e]Structure 6b. [f]Morphine like structure. [g]Morphine like structure not energy optimized. [h]Angles tabulated are defined ($\tau$ABCD) by clockwise rotation of atom A into D along B→C axis. Values given in degrees. [i]Axis number as shown in structure 4. [j]$C_{Ph}$ is either Ph carbon atom once removed from pivoting atom ($C_{10}$ or $C_{16}$) on $Ph_i$. [k]Energy relative to minimum energy protonated structure.

oxygen leading to a seven membered "piperidine" ring with the methyl group on the asymmetric carbon equatorial (6a) or axial (6b) to the pseudopiperidine ring.




6a. 6b.

A methadone conformer with maximum atom for atom overlap with morphine has also been proposed (Goldstein, Aronow, Kollman 1968).

However, no conclusive evidence supporting or refuting the existence of any of the postulate conformers has been obtained (Henkel, Bell, Portoghese 1974), and an x-ray crystallographic study of (+) methadone hydrobromide showed no intramolecular hydrogen bonding between the nitrogen proton and carbonyl oxygen (Hanson, Ahmed 1958) or siginificant morphine overlap.

Thus, in spite of longstanding consideration of the problem, the question of the most likely conformation of methadone at the receptor site and its similarity to morphine remains open.

In this study, using the PCILO method, we have investigated the energy conformational behavior of meperidine, desmethyl, $\alpha$ and ß prodines, and methadone. The objectives of the study are: to identify low energy conformers of each compound as candidates for interaction at the receptor site; to correlate these conformers, together with calculated net atomic charges, with observed analgesic potencies; to compare these conformers with morphine; to understand how and with what degree of overlap they can act at the morphine receptor site.

For meperidine, desmethyl and $\pm\alpha$ and $\pm$ß prodine, a regularized geometry based on the x-ray structure of the prodines (Kartha, Ahmed, Barnes 1960: Ahmed, Barnes 1963) was used and nested rotations about $\tau_1$ ($C_3C_4$–$C_7C_8$ in figs. 5 and 6) and $\tau_2$ ($C_4C_7$–$C_{13}C_{15}$ in fig. 5 and $C_4C_7$–$O_{13}C_{14}$ in fig. 6) were perfomed for phenyl axial and phenyl equatorial positions. The ester chain was kept in the extended, staggered conformation found in the crystal structure. Rotations of the methyl group on $C_8$ ($C_{12}$) in the prodines were performed as needed to identify minima. Table III summarizes the salient energy-conformation behavior for the six compounds studied. Figures 5 and 6 show meperidine and desmethyl prodine in their minimum energy conformers with calcualted net atomic charges. For comparison, similar results for morphine obtained from a previous calculation (Loew, Berkowitz, Weinstein 1974) are given in figure 7.

For methadone, with 11 degrees of rotational freedom, low energy conformers could not feasibly be identified by systematic nested rotations about each axis. Instead, a plausibility approach was taken to find a candidate low energy conformer.

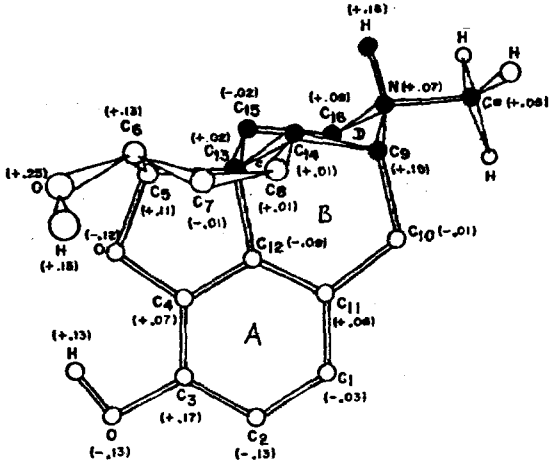



***Figure 7.*** *Minimum energy conformer of morphine from x-ray structure with calculated net atomic charges.*

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Eight protonated structures Mere considered: the x-ray structure; an extended chain structure to calibrate the effect of the proposed intramolecular hydrogen bonding structure; internal hydrogen bonding ($NCH_2H-O \rightleftharpoons C$) between a carbonyl group and a hydrogen of the $N-CH_3$ group as proposed by Gero (Gero 1954); structures 6a and 6b with internal hydrogen bonding ($N - H \cdots O \rightleftharpoons C$) between the two carbonyl groups and the proton of the nitrogen as previously suggested; and three structures with an increased similarity of methadone to morphine. Table IV summarizes the results obtained from energy optimization of all of these structures except the last (conformer 8), which was constructed to maximize similarity to morphine instead. Energies are all expressed relative to the minimum energy conformer.

For each compound shown in Table III the calculated low energy conformers are consistent with the x-ray data, but our results show additional low energy local minima in $\tau_1$ and $\tau_2$. In each case the energy of the best phenyl equatorial conformer was significantly lower than the best phenyl-axial one. These results, combined with the fact that significant potencies have been measured in forced phenyl-equatorial 4-phenyl piperidine compounds (Portoghese, Mikhail, Kupferberg 1968), dispel the idea that a phenyl-axial conformation per se is more efficacious at the receptor.

Since complete superposition of a phenyl-equatorial form of these compounds on morphine is impossible, it is evident that they occupy only partially overlapping receptor sites, which also agrees with observed differences in some of the physiological effects of meperidine and morphine (Portoghese 1966). The question remains as to the actual orientation of the phenylpiperidine compounds at the

receptor. We have considered two likely orientations of these compounds at the opiate receptor.

In one orientation (I) (figures 5 and 6) the piperidine ring of these compounds is directly superimposed on the piperidine ring of morphine, used as the prototype rigid opiate, implying a fixed cationic receptor for both types of compounds. The phenyl ring is then displaced to a position identical with that proposed for the phenyl substituent of 5-phenylbenzomorphan (GPA 1657) (Clarke, Hill *et al.* 1974). This displacement is reasonable without a m-OH group in the phenyl ring corresponding to the crucial 3-OH group in morphine. However, the presence of a m-OH group could direct the phenyl group of 4ϕ-piperidines to the same receptor site as in morphine. In this orientation (II) (figure 8) the

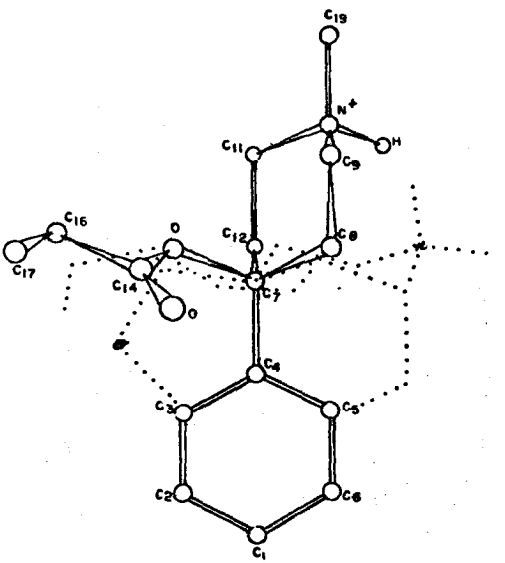


***Figure 8.*** *Pharmacophore II. Minimum energy conformer of prodine with phenyl ring superimposed on that of morphine.*
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phenyl rings of the two types of compounds are assuned to make the same receptor contact, displacing the piperidine rings. An optimum correlation between observed potencies and calculated properties is obtained using the first orientation which is the more appropriate one without a m-OH group.

While meperidine and desmethyl prodine have similar low energy conformers, the reversal of the ester chain places the polar O═C—O groups in different relative positions, which could account for their 10-fold difference in apparent potency. In desmethyl prodine (fig. 6) the O═C—O group more closely mimics the charge distribution in morphine. The highly negative carbonyl oxygen ($O_{15}$) is very close to the negative furan oxygen of

morphine (fig. 7); the positive carbonyl carbon ($C_{14}$) is near the positive $C_4$ of morphine; and the negative ester oxygen ($O_{13}$) is nearly superimposed on the negative $C_{12}$ of morphine. By contrast, the reversal of the ester group to meperidine (fig. 5) seems less advantageous. The polar O═══ C—O group is superimposed on the relatively neutral part of the phenyl ring in morphine far from either the negative furan oxygen, the positive $C_3$, or the phenolic oxygen, therefore possibly. accounting for its lower potency.

The reasons for (+)$\alpha$, (+)ß, desmethyl prodine potency differences do not appear to be conformational, as all three compounds have accessible energy minima at similar values of $\tau_1$ and $\tau_2$ (fig. 6) and have the same electron distribution even on $C_8$. An explanation has been offered (Portoghese, Gomma, Larson 1973), however, that a hydrophobic pocket is present in the receptor which preferentially fits the methyl group in the axial position. Recent data have been obtained (Portoghese, Mihail, Kupferberg 1968; Casy 1968; Casy, McErlane, Jones 1973) on the potencies of the 3-allyl and 3-propyl prodine derivatives confirming this idea.

The differences in potency between the (+) and (-) isomers of prodine may, however, be due to conformational differences since both $\tau_1$ and $\tau_2$ have different values for low energy conformer of the two diastereosiomers (table III, figs. 6 and 9). This torsion angle difference in $\tau_1$ for the (+)/(-) isomers confirms previous speculation based on x-ray structures (Portoghese, Larson 1973). However, since the proposed phenyl binding site is different from morphine, it is difficult to assess how the relative phenyl position ($\tau_1$) affects potency. Nevertheless, the nitrogen is 7Å in front of the phenly ring plane in the (-) isomers, and is .7Å behind the plane in the (+) isomers, as in morphine.

Most convincing evidence for the enhanced potency of the (+) isomers comes from the results of the variation of $\tau_2$. The minimum energy. conformers of the least potent isomers (-) have $\tau_2 = 60°$, causing the ester chain to protrude into the original phenyl receptor site on the same side as the piperidine ridge atoms (fig. 9, page 293), possibly hindering receptor interaction with the phenyl, and piperidine rings. A value of $\tau_2 = 300°$ in the most potent (+) isomers (fig. 6) places the ester chain in a nonhindering position away from the receptor (on the opposite side as the piperidine ridge atoms) but still close enough to the phenyl receptor site to favorably interact with the receptor. It is interesting to note that the least potent isomer (-) is "locked" into the unfavorable value of $\tau_2 = 60°$ whereas (-), though favored at $\tau_2 = 60°$, has an accessible energy minima at 300° as well. Thus on the basis of $\tau_2 = 60°$, has an accessible predicted order of potency would be (+)$\alpha$ = (+)ß > (-)ß$^2$> (-)$\alpha$, in good agreement with potencies.

From the results summarized in table IV, it is clear that morphine like conformers of methadone (conformers 6, 7, 8) are high energy forms and that methadone cannot occupy the identical receptor sites as morphine.

The minimum energy conformer was one based on the hydrogen-bonded (N—H---O—C) structures proposed by Portoghese. It was 11.6 kcal/mole more stable than the extended chain. Figure 10 shows this minimum energy conformer of protonated methadone in an orientation with its nitrogen superimposed on that of morphine, implying a fixed cationic receptor site. Also shown in this figure are the net atomic charges calculated for methadone. In this orientation the central carbon atom ($C_7$) superimposes on its counterpart in morphine and meperidine. In fact, the minimum energy conformer of methadone greatly resembles the minimum energy conformer for meperidine shown in fig. 10 in the same orientation. The ester chain in meperidine and the carbonyl chain of methadone could occupy similar positions at the

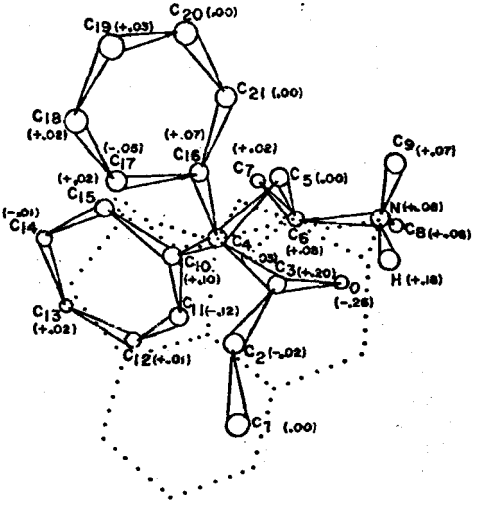


***Figure 10.*** *Minimum energy conformer of protonated methadone with net atomic charges showen with a nitrogen atom superimposed on that of morphine.*
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receptor site reinforcing the possibility that the receptor can, to some extent, accommodate a polar group in this vicinity. The displaced phenyl groups are also in similar positions in the two compounds. The two fold increase in methadone potency over meperidine in intraventricular studies could be attributable to the interaction of the second phenyl group with a new receptor site. This $\phi$ group is in the vicinity of the tertiary alcohol substituents on $C_7$ in the potent etorphine type rigid opiates. Comparing net atomic charges for meperidine and methadone, the cationic head atoms (nitrogen and the atoms binding to it) have similar charges, as do the C═O groups, consistent with the idea that these two types of analgesics could act in a similar fashion at the opiate receptor.

The net charges on the cationic head atoms are in fact, similar in all three compounds: meperidine, methadone and morphine. Thus these molecules could have the same delocalized interaction with

a diffuse anionic receptor site as has been previously proposed for morphine. However, surprisingly, in methadone the proton on the nitrogen points down towards the phenyl ring position in morphine, a consequence of hydrogen bonding to the C=O group, while for meperidine and prodine with a true piperidine ring, the proton is in a more morphine like position. Thus it appears that a morphine like position of the nitrogen proton is not required for activity, at least in flexible opiates where the phenyl binding site to the receptor is apparently displaced.

**B. PEPTIDE OPIATES**

Two endogenous pentapeptides with morphine-like activity have been isolated from mammalian brain (Hughes, Smith, Morgan 1975; Simantov, Snyder 1976). These pentapeptides have been characterized as methionine-enkephalin and leucine-enkephalin, and have the amino acid sequence Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu, respectively. Both pentapeptides mimic the actions of morphine in the guinea pig ileum (Hughes, Smith, Morgan 1975; Simantov, Snyder 1976) and the mouse vas deferens assays (Morgan, Smith, Waterfield 1976), exhibit cross tolerance to morphine (Waterfield, Hughes Kosterlitz 1976), and inhibit the stereospecific receptor binding of $^{3}$H-naloxone in rat brain homogenates (Simatov; Snyder 1976). In addition, intracerebroventricular injections of enkephalin can produce transient analgesia (Jacquet, Marks 1976; Feldberg, Smyth 1977) which can be blocked by the potent opiate antagonist naloxone (Beluzzi, et al 1976). These findings, plus the fact that enkephalin appears to be localized in synaptosomal fractions that are rich in nerve terminals (Simantov, Snowman, Snyder 1976) provide evidence that the enkephalins may be involved in analgesia or pain suppression.

Since the enkephalins and rigid opiates appear to compete for the same receptors, it would seem that there must be chemical and conformational similarity between these two different classes of opiates. The most striking feature common to both the enkephalins and the rigid opiates is the presence of a p-OH phenethylamine (tyramine) moiety formed by the terminal amino group and the tyrosine residue. It has been established by structure-activity studies that for potent opiate activity the tyrosine residue is critical and, in common with rigid opiates, the amine and p-OH groups must remain intact (Chang et al. 1976).

A number of conformational comparisons with rigid opiates have been made by model building and comparing overlaps of different assumed critical regions of Met-enkephalin and rigid opiates such as 7-[1-phenyl-3-hydroxybutyl-3-]endothenotetrahydrothebaine (PET) (Bradbury, Smith,Snell 1976; Horn, Rodgers 1976; Smith, Griffin 1978). In addition, extensive conformational analyses of Met-enkephalin have been made by means of empirical energy calculations (Isogai, Nemethy, Scheraga 1977; Momany 1977). Of the fifty-two conformations reported (Isogai, Nemethy, Scheraga 1977), the lowest energy structures, i.e., those with $\Delta E < 2.5$ Kcals/mole were found to be G-PßII' type bends with glycine[3] and phenylalanine[4] taken as

the central residues. Many other conformers were in the relative energy range of $5<\Delta E<11$ Kcal/mole. The lowest energy structure was found to be stabilized by a hydrogen bond between the tyrosine OH group and the glycine backbone C=O group. This low energy structure is consistent with chemical shifts and coupling constants in reported NMR studies of Met-enkephalin (Garbray-Janeguiberry, Roques, Oberlin 1976; Jones, Gibbons, Garsky 1976).

While valuable in describing numerous low and medium energy conformers of Met-enkephlain, none of the optimized conformers previously reported (Isogai, Nemethy, Scheraga 1977) have significant spatial overlap with several functional groups in morphine like opiates. On the other hand the conformers proposed by overlap with rigid opiates are based on model building with no estimate of their relative energies. Deviations from minimum energy conformations of the isolated molecule could occur by an induced fit at the receptor site allowing enhanced resemblance to rigid opiates, but with sane energy required.

In order to determine the most likely conformers for interaction at the receptor site, we have made systematic energy conformational studies by both empirical and quantum mechanical methods. The aim was to determine the energy of met-enkephalin conformers with varying degrees of similarity to rigid opiates and select as most likely those conformers with modest energy expenditure and significant overlap with rigid opiates. An addititonal requirement in our selection strategy was the accommodation of D-ala$^2$ but not L-ala$^2$ in place of the Gly$^2$ residue. This second criterion is consistent with the observation of the essential retention of activity when Gly$^2$ is replaced by D-ala$^2$ (Walker, Bernston, Sandman 1977) but a large decrease of receptor affinity upon replacement by L-ala$^2$ (Pert, Pert, Chang 1976).

Starting with the fifty-two low and medium energy conformers previously obtained(Isogaii, Nemethy, Scheraga 1977), successive imposition of these criteria allowed the selection of a small number of low energy conformers as most likely candidates for receptor site interaction. While the empirical and quantum mechanical energy calculations did not give identical results, a number of likely candidates common to both methods were obtained. Both methods also showed a trend towards higher energies for conformers with increasing resemblance to rigid opiates.

The conformational energy calculations reported here were performed a CDC 7600 using the Empirical Conformational Energy Program for Peptides (ECEPP) (Momany *et al* 1975) and the Perturbative Configuration Interaction using Localized Orbitals (PCILO) (Diner, Malrieu *et al.* 1969) method of quantum chemistry. Energy minimizations for ECEPP were carried out by a Quasi-Netwon method (Gill, Murray, Pitfield 1972) which employs a gradient search. The convergence criterion was 0.01 kcal/mole. Local minima were also found by parabolic fits to sequentially varied sets of torsion angles. In the PCILO method, local minima were found only by parabolic fits.

**Table V.**[6] Energy of enkephalin conformers with maximum overlap with the rigid opiate PET[a]

| Conformer | $\Delta E$[h] | $\Delta E$[i] |
|---|---|---|
| Maximum PET overlap ($w_1$=0)[b] | -10[7] | 613 |
| Relaxed PET overlap ($w_1$=0)[b] | 135 | 25 |
| Modeled GG II ($w_1$=180°)[b] | 628 | ---g |
| Modified GG II (no optimization)[B] | 119 | ---g |
| Opitmized GG II ($\psi_1$,$x_1x_2$ fixed)[c] | 25.5 | 14.4 |
| Optimized GG II ($x_1x_2$ fixed)[d] | 17.3 | 9.7 |
| Totally optimized GG I'($x_1x_2$=-110,-130)[e] | 13.3 | 10.7 |
| Totally optimized GG II'($x_1x_2$=-168-100)[f] | 6.5 | 5.4 |

[a]PET = 7-[1-phenyl-3-hydroxybutyl-3-]endoethenotetrahydrothebaine

[b]These conformers were chosen by similarity to PET and were not optimized.

[c]Optimized GG II keeping $\psi_1$, $x_1$ and $x_2$ at values which mimiced PET.

[d]Optimized GG II letting $\psi_1$ relax.

[e]Totally optimized GG bend which gave closest tyramine overlap.

[f]Lowest energy GG bend. It has no similarity to PET.

[g]Energy not calculated.

[h] $\Delta E$ in Kcal/mole calculated by ECEPP method.

[i]$\Delta E$ in Kcal/mole calculated by PCILO method.

The faster ECEPP method was used to obtain totally optimized conformations and energies for each conformer considered. The PCILO method was also used to calculate the energies of the totally optimized conformations obtained from ECEPP calculations with local optimization for important structural features such as the tyramine p-OH phenethylamine overlap with morphine.

As shown in table V, a conformer constructed to have maximum overlap with PET, and not energy optimized, gave extremely high energies by both the ECEPP and PCILO methods. These high energies are due to the crowding of the terminal nitrogen and hydrogen atoms with the $Gly^2$ carbonyl carbon and oxygen atoms. Relaxation of the $Tyr^1$ and $Gly^2$ backbone angles to a local minima, while maintaining $w_1 = 0°$, decreased the energy significantly. Step-wise relaxation of another starting structure with w angles $\sim$ 180° yielded a series of relatively high energy structures by both methods.

In another approach it was assumed that a minimum viable overlap of Met-enkephalin with rigid opiates should involve the tyramine ($NH_2-C_\alpha-C_\beta-\phi-OH$) moiety of both opiates. Imposition of this constrain on the fifty-two previously optimized Met-enkephalin conformers, resulted in twenty-two with relative energy 9-20 kcal/mole above the lowest energy conformer without the imposed overlap. These relative energies calculated by the PCILO method ranged from 1.0 to 8.5 kcal/mole, implying many more accessible cmformers. The there condition that $D\text{-}ala^2$ be accommodated more readily than $L\text{-}ala^2$ in place of $Gly^2$ reduced the number of viable conformers from twenty-two to twelve. These twelve conformers were reoptimized with the constraint of accommodating both the $Gly^2$ and $D\text{-}ala^2$ residues and the tyramine overlap with morphine.

The results of this procedure were that the minimum energy conformers calculated by both the ECEPP and PCILO methods did not have any overlap with rigid opiates beyond the imposed tyramine overlap. On the other hand, the lowest energy conformer with signigicant additional rigid opiate overlap was the same by both methods: a G-G ßII' bend with a hydrogen bond between the carbonyl group of tyrosine and the amine group of phenylalanine. This is a particularly viable candidate for interaction at the receptor site since it is only 3 kcal/mole above the minimum energy form as calculated by the PCILO method.

Figure 11 shows the overlap of this structure with the potent agonist PET. Not only do the important phenethylamine moieties overlap, but the phenylalanine side chain overlaps with the phenethyl $C_{19}$ substituent of PET and the methionine backbone C=O group and side chain are in the region of the $C_6$ methoxy group of PET. This conformer is very similar to the one previously proposed by model building (Bradbury, Smyth, shell 1976) and is confirmed by these studies as a very likely candidate at the receptor site.

Further dramatic verification that this type of enkephalin conformer can interact with the opiate receptor is provided by a just published x-ray crystal structure determination of

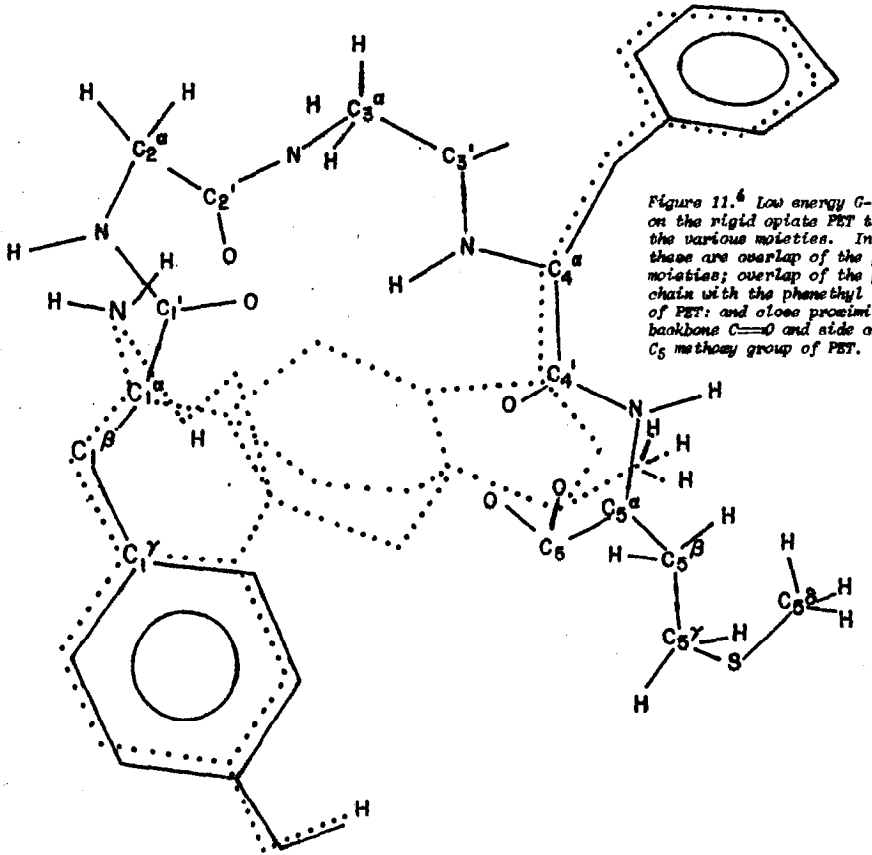

*Figure 11.[6] Low energy G-G & II' superimposed on the rigid opiate PET to show overlap of the various moieties. In particular, these are overlap of the phenethylamine moieties; overlap of the phenylalanine side chain with the phenethyl $C_{19}$ substituent of PET: and close proximity of the methionine backbone C=O and side chain with the $C_5$ methoxy group of PET.*

Leu-enkephalin (Smith, Griffin 1978). The structure reported is a G-G ß bend stabilized by hydrogen bonding between the tyrosine and phenylalanine residues; This structure is different from the minimum energy conformer obtained by previous calculations and from structures deduced from NMR studies in solution. It. is consistent with the lowest energy confomer we have chosen by a combination of energy calculations and a selection rationale based on resemblance to rigid opiates and accomodation of D-ala. This approach to conformational studies thus appears to be a powerful tool for determining important opiate peptide receptor interactions. An implication from this study is that enkephalin binds to the receptor by an induced fit initiated at the crucial tyramine region, followed by binding of the C-terminal residues. This mode is similar to a proposed "zipper" mechanism (Burgen, Roberts, Feeney 1975) or the concept of a dynamic pharmacophore (Weinstein 1975) which our previous work (Loew, Berkowitz, Weinstein 1975) indicated was appropriate even for rigid opiates.

**C. SUMMARY**

Three classes of flexible opiates have been studied: 4-phenyl piperdines, methadone and enkephalins. Our results show that low energy conformers of the 4-phenyl piperidines have equatorial phenyl rings and cannot completely overlap with rigid opiates at the receptor. A combination of calculated conformational and electronic properties could account for observed potency differences in meperidine, desmethyl, $\alpha$+, $\alpha$-, ß+ and ß- prodines.

Our results also indicate that both meperidine and its reverse ester bind to the receptor in a similar mode with the $\phi$ ring in approximmately the same position as the phenyl substituent in 5-phenyl benzomorphans.

Conformers of methadone which maximally resemble morphine have very high relative energies. The lowest energy conformer has a partial H-bond between the NH and O=C groups. In this conformation methadone resembles meperidine more than morphine. The electronic structure of all three types of opiates indicate a similar cationic charge distribution around the amine nitrogen and imply that their binding to an anionic receptor site could be similar.

The determination of peptide opiate conformations present a challenge of a different order of magnitude than the most flexible exogenous opiates. Because of the extremely large number of possible conformations, search strategies based on energy optimized conformations alone are not adequate to select plausible receptor site candidates. Other criteria such as consistency with known structure activity data and similarities to rigid opiates must be used. With this rationale, we have predicted and characterized a low energy conformer of Met-enkephalin and D-ala$^2$ Met-enkephalin as a likely candidate at the receptor site. With a modest energy input ($\Delta E \sim 3$ kcal/mole) significant overlap of this conformer with the potent opiate PET was obtained. The tyrosine and phenyalanine side chains

and the terminal amine and carboxyl groups play crucial role in this overlap. It is hoped that this calculation will help establish a template for peptide opiate receptor interactions.

## III. POTENTIALLY USEFUL AREAS OF QUANTUM CHEMICAL RESEARCH IN OPIATE NARCOTICS

The results reported here have established the reliability of semiempirical quantum chemical methods for conformational studies of both flexible and rigid opiates. Crystal structure conformers are always obtained as low energy local or global minima. More subtle conformations effects such as intramolecular hydrogen bonding and conformational mixtures determined by NMR spectra have also been verified.

Quantum chemical calculations supply additional information not readily accessible to experimental techniques relevant to pharmacological behavior. These include: characterization of compounds not yet synthesized; characterization of separate diastereoisomers; and characterization of a large number of stable conformers within a range of energies, many of which are beyond detection in the isolated molecule but which could be viable candidates for induced fit at a receptor site.

These studies can be made relatively efficiently and can serve as a guide in many ways to more costly and time consuming synthesis and testing. Thus one area of productive use of theory is in predictive structural-activity efforts, ideally in collaboration with medicinal chemists and pharmacologists. Such calculations can also be used to compare different classes of opiates for region of similarities and differences. Thus they can be of use in delineating crucial regions of the opiate receptor common to many classes of opiates and those unique to specific ones.

Future effort is thus envisioned in two related areas: predictive medicinal chemistry and opiate-receptor theory. Specifically, we plan to use the working hypothesis we have developed for rigid opiates which relate their structure to their agonist/antagonist potency ratios to continue to predickt potentially useful rigid opiate analogues. We also plan to extend this effort to unusual classes of flexible exogenous opiates such as ketobemidone type 4-phenyl piperidines and to peptide opiates. Our central goal is to understand requirements for agonism/antagonism in all these series of compounds and to compare these requirements among members of different classes of opiates. If successful this effort should lead to prediction of other potentially useful analgesics and also help to understand the nature and degree of flexibility of the opiate receptor.

## FOOTNOTES

1. From Loew, G.H., Berkowitz, D.S., Weinstein, H., and Srebrenik, S. Quantum chemical studies of morphine-like opiate narcotics: Effect of polar group variations. In: Molecular and Quantum Pharmacology, Bergmann, E., and Pullman, B., eds. Copyright 1975. Reprinted by permission of D. Reidel Publishing Company, Dordrecht-Holland.

2. Reprinted with permission, from Loew, G.H., and Berkowitz, D.S. Quantum chemical studies of morphine-like opiate narcotic analgesics: I. effect of N-substituent variations. J Med Chem, 18(7):656-662, 1975. Copyright by the American Chemical Society.

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5. Reprinted with permission, from Loew, G.H., Berkowitz, D.S., and Neuth, C. Quantum chemical studies of methadone. J Med Chem, 19(7): 863-869, 1976. Copyright by the American Chemical Society.

6. Reprinted from Loew, G.H., and Burt, S.K. An energy conformation study of Metenkephalin and its D-ala$^2$ analogue and their resemblance to rigid opiates. Proc Natl Acad Sci USA, 75(11):7-11, 1978.

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# An Extended Isolated Molecule Method and Its Applications to the Design of New Drugs: General Aspects

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## INTRODUCTION

The aim of the isolated molecule method is the study of chemical reactions in which the attacking species is always the same (Greenwood and McWeeny 1966). As the isolated molecule method has been successfully used in the study of chemical reactivities for various types of chemical reactions, these reactivities are then considered to depend only on the differences in the substrates. Also the isolated molecule method is convenient for the study of biological activities (BA) of drugs, because here only the substrates are known. The receptor or any other interactive species which might be involved are subject to speculation.

In a previous work (Brown and Simas 1978), many of the $\pi$ indices used in the isolated molecule method were justified for the more advanced semiempirical methods where all of the valence electrons, antisymmetry, and interelectronic repulsions were included. This allows a more credible study of nonplanar and heteropolar systems. The objective of this paper is to extend such an advanced isolated molecule method to the study of Quantitative Structure Activity Relationships (QSAR) of drugs.

Approaches to QSAR, such as the isolated molecule method, have rarely been employed. Many of the studies which have been reported in the literature have used only $\pi$ electron approaches focussing on the calculation of electronic charge distributions (Brownlee and Taft 1968; Kang and Beveridge 1971; Kang and Cho 1973). Two works, however, are of special importance. The first is a paper by Wohl where the extended Hückel method was used to calculate the indices for the $\pi$ system of a family of benzothiadiazines (Wohl 1971). Using a similar approach, which also yielded good results, Peradejordi studied the tetracycline family of drugs (Peradejordi 1971).

Many criticisms have appeared against this kind of approach including the meaningfulness of the indices employed, the exaggerated importance given to the statistical analysis, the nonexistence of a solid theoretical foundation to the BA phenomena, the necessity of utilizing good experimental data for correlating the MO indices, etc. However, the theoretical calculations possess two great advantages over the purely experimental correlations. First, these calculations provide information about submolecular properties such as charge distributions and polarizabilities in the atoms. These properties are of obvious importance in understanding the differing chemical aspects in a series of substrates. Second, and probably more important, these calculations provide a reasonably objective way to predict the properties of a drug before it has been synthesized.

Here a conceptual set of hypotheses which can extend the applicability of the all valence isolated molecule method to the study of BA is presented and discussed. In an extension of our previous work (Brown and Simas 1978), a new rationalization of the superdelocalizabilities is also presented. In a subsequent paper, this methodology, including these newly derived indices, will be applied to the chloramphenicol family of drugs (Simas et al. 1978).

**BASIC APPROXIMATIONS**

The objective of the isolated molecule approach is to determine the differences in the free energies of activation of the reactions associated with the various compounds of a homologous series from the initial steps of the reactions. Suppose that a set of compounds belonging to a homologous series are subjected to one type of reaction. The rate constant for the ith compound, $k_i$, is given by

$$k_i = K \frac{kT}{h} e^{-\Delta A^{\neq}/kT}$$

where K, k, h, T and R are the transmission coefficient, the Boltzmann constant, Planck's constant, the absolute temperature and the ideal gas constant (Glasstone, Laidler and Eyring 1941). The molar free energy of activation, $\Delta A^{\neq}$, can be expressed in terms of the entropy and the energy of activation in the usual manner. The free energy has the important property of being proportional to the logarithm of the rate constant, and is the unique quantity which varies largely from one reaction to another.

With a homologous series it is always possible to make the initial free energies of the substrates equal, such as in figure 1. The free energy profile can be, at least in principle, approximated by a straight line. Its slope is then proportional to the free energy of activation:

$$\Delta A^{\neq} = \Delta\lambda \tan\theta \qquad (2)$$

where the proportionality constant $\Delta\lambda$ represents the change in the reaction coordinate from $\lambda_0$, the states of the reagents, to $\lambda^{\neq}$ the transition state. Further, let us consider that the free energy profiles do not cross from the state of the reagents to the transition state, and that AA is constant through the series, which implies that the mechanisms of the reactions are always the same for the homologous series. In this way it is always possible to find a $\lambda'$ between $\lambda_0$ and $\lambda^{\neq}$ where the derivatives of the free energy with respect to the reaction coordinate can be described by a linear transformation

$$\Delta A_i^{\neq} \simeq a \left.\frac{\partial A_j}{\partial \lambda}\right|_{\lambda=\lambda'} + b \quad . \tag{3}$$

Equation 3 yields good estimates for the free energies of activation of the reactions. In this equation, a and b are constant through the series.

The above relation allows the study of processes which are kinetically controlled. In order to study equilibrium controlled processes, one can assume that there exists a relationship of the type expressed in equation 3 involving $\Delta A^0$, the standard free energy of reaction, instead of $\Delta A^{\neq}$ Reactions where the free energy curves are approximately parallel past the transition state could be common for a homologous series, and we assume this here. This is particularly true where the product resembles somewhat the transition state. For both of these processes, a compensating effect appears. The derivative of the free energy at the beginning of the reaction, at $\lambda_0$, where one finds a minimum in the reaction profile, must be zero. For this point, considering the relations between A, E and S, the equation

$$\left.\frac{\partial E}{\partial \lambda}\right|_{\lambda_0} = T \left.\frac{\partial S}{\partial \lambda}\right|_{\lambda_0} \tag{4}$$

is valid for both the equilibrium and kinetically controlled reactions. For this reason an index related to a first order perturbation does not always correlate well with the reactivities of the compounds, whereas a second order One does (e.g., induced charge and self atom polarizability). The change in energy associated with the first order index was completely absorbed by the change in entropy in the initial steps of the reaction. This implies another approximation; the second order changes in entropy will be very similar for all reactions since the attacking species, solvent, etc., are always the same.

Finally, with these hypotheses, equation 3 can be rewritten by substituting the derivative of E for the derivative of A to obtain

$$\Delta A_i^{\neq} \text{ or } \Delta A_i^0 \simeq a' \left.\frac{\partial E_i}{\partial \lambda}\right|_{\lambda=\lambda'} + b' \quad . \tag{5}$$

As attempts will be made to find indices which are proportional to the changes in energy at the beginning of the reactions, one must also consider that each index will be related to the same displacement in the reaction coordinate. Generalizing equation 5, one has

$$\Delta A_i^{\neq} \text{ or } \Delta A_i^0 \simeq a'' X_i + b'' \tag{6}$$

where $X_i$ is the value of index X for compound i.

Although some large assumptions have been made up to this point, it is important to note that this isolated molecule method is consistent with the Hammett treatment, where the constant of reaction, is constant for a homologous series and the substituent constant $\sigma_i$ accounts for the differences in the reactivity.

**INDICES FOR THE ALL-VALENCE ISOLATED MOLECULE METHOD**

As a first step in the perturbation procedure, consider only the electrostatic interaction of the attacking species with the substrate. The electrostatic field and its potential have been derived using the point charge approximation as discussed elsewhere (Brown and Simas 1978). The coulombic interaction energy between the electrostatic potential $V_s$ of the substrate and the charge distribution $Y_A$ of the attacking species is

$$\Delta E_{coul} = \sum_i V_S(\vec{r}_i) \cdot Y_A(\vec{r}_i) \tag{7}$$

$$V_S(\vec{r}) = \sum_a Q_a/|\vec{r}-r_a| \tag{8}$$

where $r_a$ and $Q_a$ are the position and net charge of atom a of substrate. For points distant from the substrate, the point charge approximation is adequate.

An advantage of studying the electric field around the substrate is that reasonably far from the molecule, this field can be considered to be relatively constant in a small region of space. If the attacking species has a permanent dipole moment, $\vec{\mu}_A$, its interaction with the electric field may be written as

$$\Delta E_{coul} \simeq \vec{E}_S(\vec{r}) \cdot \vec{\mu}_A \tag{9}$$

$$\vec{E}_S(\vec{r}) = \sum_a Q_a(\vec{r}-\vec{r}_a)/|\vec{r}-\vec{r}_a|^3 \tag{10}$$

In this way the electric field can be decomposed into its components and the differing correlations of the components of $\vec{E}$ with the reactivities provide information about the direction of attack.

Equations 7 through 10 illustrate how the potential, field components and net atomic charges are proportional to the energy of interaction.

The gross environmental effects on the drug molecule can be considered, in a first approximation, as the interaction of an electric field with the substrate. The interaction energy can then be written as:

$$\delta E_{el} = -\vec{E}\cdot\vec{\mu}_S - \vec{E}\cdot\alpha_S\cdot\vec{E}^t \tag{11}$$

where $\vec{\mu}_S$ is the dipole moment of the substrate and $\alpha_S$ is its polarizability tensor. The derivations and a discussion concerning the applicabilities of these indices have been reported (Brown, Simas and Bruns 1977; Brown and Simas 1978).

Now consider the attacking molcule as a charged or polar species which can produce some perturbation to the substrate molecule. We treat this as an empirical perturbation to only one atom where the energies of the atomic orbitals on atom v are modified by the amount $\delta\alpha_\mu$. For advanced semiempirical methods such as the CNDO method, it was previously discussed and derived that the modification to the electronic density at atom v and the energy of interaction are given respectively by (Brown and Simas 1978),

$$\delta q_\nu = \Pi_{\nu\mu}\,\delta\alpha_\mu \tag{12}$$

$$\delta E = q_\mu\delta\alpha_\mu + \frac{1}{2}\Pi_{\mu\mu}(\delta\alpha_\mu)^2 \tag{13}$$

The quantity $\Pi_{\nu\mu}$ is the atom-atom polarizability, $\Pi_{\mu\mu}$ is the self-atom polarizability and $q_\mu$ is the electronic density at atom $\mu$. Obviously both $q_\mu$ and $\Pi\mu\mu$ are proportional to the energy of interaction. As MO indices, they have proven useful in parameterizing reactivities.

If the attacking species approaches the substrate more closely, it must not be described simply as a distant point charge or polar species, but as an atom or molecule possessing electronic energy levels. Bond formation through exchange or charge transfer must be considered. Suppose that we have two species, the attacking reagent T and the substrate S, and that each satisfies the eigenvalue equations,

$$H_S^{(0)} \cdot \psi^{(0)S} = E_S^{(0)} \cdot \psi^{(0)S} \tag{14}$$

$$H_T^{(0)} \cdot \psi^{(0)T} = E_T^{(0)} \cdot \psi^{(0)T} \tag{15}$$

these zeroth order wave functions $\psi^{(0)A}$ are the usual normalized determinantal functions.

We have similar expressions for the excited state functions $\Psi_i^{sS}$ and $\Psi_j^{tT}$ where an electron is promoted from the occupied MO i of S or j of T to the unoccupied MO s and t respectively. These excited state functions are defined as

$$\Psi_i^{aA} = \frac{1}{\sqrt{(2\cdot 2n!)}} \det\{\psi_1, \bar{\psi}_1, \ldots, \bar{\psi}_{i-1}\} \cdot \{\psi_i \bar{\psi}_a - \bar{\psi}_i \psi_a\}\psi_{i+1} \cdots \bar{\psi}_n\} \tag{16}$$

The MO with bars denote those with ß spin, and the two singly occupied MO are singlet coupled. The MO are expanded in terms of the atomic orbitals $\chi_p^\mu$

$$\psi_j = \sum_{\mu=1}^{NA} \sum_{p}^{N\mu} C_{pj}^{\mu} \chi_p^{\mu} \tag{17}$$

where NA is the number of atoms in the molecule, and Nμ is the number of valence AO's for the μth atom. Such excited state functions as described in equation 16 are approximate representations of the excited states of S and T with energies approximately $\varepsilon_s-\varepsilon_i$ and $\varepsilon_t-\varepsilon_j$ respectively above that of their ground state functions. Suppose that upon approach of the attacking species, there is an interaction between atom σ of S and atom τ of T and that every atomic orbital of atom σ can interact with every atomic orbital of atom t with the same energy of interaction, $\delta ß_{\sigma\tau}$. Then the empirical perturbation can be written as,

$$\langle \chi_a^{\lambda} | H^{(1)} | \chi_b^{\phi} \rangle = \delta\beta_{\sigma\tau}\{\delta_{\sigma\lambda}\cdot\delta_{\tau\phi} + \delta_{\sigma\phi}\cdot\delta_{\tau\lambda}\} \tag{18}$$

where σ, λ, τ and ϕ are atoms, δ is the Kronecker delta and $\delta ß_{\sigma\tau}$ is the change in the one electron resonance integral due to the interaction between σ and τ. Note that the resonance integral was zero prior to the reaction. The $\chi_a^{\sigma}$ and $\chi_b^{\tau}$ are one of several atomic orbitals on the respective atoms.

Now the appropriate zeroth order functions for the total complex, S-T. must be constructed which represent the eigenfunctions of the total zeroth order Hamiltonian, $H_{S-T}^{(0)} = H_S^{(0)} + H_T^{(0)}$. These are the antisymmetrized and normalized products of the wavefunctions for S and T which we represent as: $\{\Psi^{(0)}S\Psi^{(0)}T\}$, $\{\Psi_i^{sS}\Psi^{(0)}T\}$ and $\{\Psi^{(0)}S\Psi_j^{tT}\}$. Multiple promotions do not contribute in first and second order since the perturbation is a one electron operator. We will also include the charge transfer functions where an electron in MO i on S or MO j on T is transferred to MO t on T or s on S respectively. The resulting singly occupied orbitals are then singlet coupled in the same manner as in equation 16. We

represent these charge transfer functions as $\{\Psi_i^{S+}\Psi^{tT-}\}$ and $\{\Psi^{sS-}\Psi_j^{T+}\}$ We include their effect in the same perturbational scheme as the other zeroth order functions described above. In all cases above, we use the difference of the initial and final orbital energies to approximate the difference in the energies of the zerothorder functions.

Since such an empirical perturbationis taken as nonzero only between atoms on different molecules, the first order correction is zero. That is, all integrals involve MO localized on either S or T only.

The second order correction to the energy is given as:

$$E^{(2)S+T} = \langle\Psi^{(0)}|H^{(1)}|\Psi^{(1)}\rangle \tag{19}$$

$$\Psi^{(1)} = \sum_{i,t} \frac{\langle\Psi^{(0)}|H^{(1)}|\{\Psi_i^{S+}\Psi^{tT-}\}\rangle}{\varepsilon_i - \varepsilon_t}\{\Psi_i^{S+}\Psi^{tT-}\} + \tag{20}$$

$$\sum_{s,j} \frac{\langle\Psi^{(0)}|H^{(1)}|\{\Psi^{sS-}\Psi_j^{T+}\}\rangle}{\varepsilon_j - \varepsilon_s}\{\Psi^{sS-}\Psi_j^{T+}\}$$

The other zeroth order functions involving promotions localized on only S or T do not contribute for the same reason; the perturbation is nonzero only between atoms σ and τ on the two molecules. Upon substituting in the expression for the zeroth order functions and the MO and then evaluating the integrals, we obtain the simple result,

$$E^{(2)S+T} = \sum_s \sum_t \sum_p \sum_q \sum_r \sum_m \frac{(ns-nt)C_{rt}^{\tau}C_{ps}^{\sigma}C_{qs}^{\sigma}C_{mt}^{\tau}}{\varepsilon_s - \varepsilon_t}(\delta\beta_{\sigma\tau})^2 \tag{21}$$

Here the energy levels, both the occupied and unoccupied, of S and T are represented by s and t respectively, with ns and nt being the number of electrons in the s and t levels.

Now it is of interest to derive an index which depends only on the characteristics of the substrate, such as the one used in the Hammett equation. Suppose that the attacking species possesses just one level which interacts strongly with the substrate. If the attack is electrophilic, the level t is empty and will interact only with occupied levels of S. An electrophilic interaction energy can be defined as

$$EE^{(2)S+T} = \{\sum_i^{occ} \sum_p \sum_q \frac{C_{pi}^{\sigma}C_{qi}^{\sigma}}{\varepsilon_i - \varepsilon_t}(\delta\beta_{\sigma\tau})^2\}\{2\sum_{rm} C_{rt}^{\tau}C_{mt}^{\tau}\} \tag{22}$$

The coefficient of the atom $\tau$ factors out because it has only one normalized level. If the attack is nucleophilic, the analogous interaction energy is

$$EN^{(2)S+T} = \{ \sum_{a}^{unocc} \sum_{p} \sum_{q} \frac{C^{\sigma}_{pa} C^{\sigma}_{qa}}{\varepsilon_a - \varepsilon_t} (\delta\beta_{\sigma\tau})^2 \} \tag{23}$$

It is now possible to define the superdelocalizabilities which are assumed to be proportional to the interaction energy between S and T but independent of T as described in the equations as follows

$$EE^{(2)S+T} = S_{E\sigma} \cdot \rho_{E\tau} \tag{24}$$

and

$$EN^{(2)S+T} = S_{N\sigma} \cdot \rho_{N\tau} \tag{25}$$

Here $S_{E\sigma}$ and $S_{N\sigma}$ are the electrophilic and nucleophilic superdelocalizabilities of atom $\sigma$ in S. The quantities $\rho_{N\tau}$ and $\rho_{E\tau}$ are considered to be constants of reaction in the same spirit as found in the Hammett equation. The denominators of equations 22 and 23 prevents such a separation. However, assuming the Hammett equation valid, the superdelocalizabilities can be defined as

$$S_{E\sigma} = \sum_{i}^{Occ} \sum_{p} \sum_{q} \frac{C^{\sigma}_{pi} C^{\sigma}_{qi}}{\varepsilon_i} \tag{26}$$

and

$$S_{N\sigma} = \sum_{a}^{unocc} \sum_{p} \sum_{q} \frac{C^{\sigma}_{pa} C^{\sigma}_{qa}}{\varepsilon_a} \tag{27}$$

It is obvious that such a separation is arbitrary. It is based on the validity of experimental relations, such as the Hammett equations. Attempting to expand the denominators in equation 22 or 23 in terms of the ratios $\varepsilon_t/\varepsilon_i$ or $\varepsilon_t/\varepsilon_a$ respectively, gives a convincing argument only when the ratio is less than unity in magnited. (It also indicates that perhaps higher order terms in $1/\varepsilon_i$ and $1/\varepsilon_a$ should be included in any correlations.) For reactions where we are proposing weak bond formation, i.e., only a partial transfer of charge, this is not uncommon since the energy level $\varepsilon_t$ may lie close to the zero level in either case. However, for the general case, such an argument is pure speculation. These new formulae are only slightly different from those used previously (Brown and Simas 1978) to successfully parameterize the chemical reactivities of several homologous series of compounds.

If the molecule being studied has a plane of symmetry which contains atoms forming unsaturated bonds, the total wave function of the molecule satisfies the $\sigma$-$\Pi$ separability conditions of Parr (Parr 1963). It can then be written as an antisymmetrized product of the antisymmetrized functions $\{\Sigma\}$ and $\{\Pi\}$ which contain MO of only $\sigma$ and $\Pi$ symmetry respectively.

$$\Psi = \{\{\Sigma\}\{\Pi\}\} \tag{28}$$

The total electronic energy can then be divided into a contribution from the $\{\Pi\}$ function and another from the $\{\Sigma\}$ function. If the plane of symmetry is conserved during thecourse of the reaction, the transition state electronic energy can also be divided into $\sigma$ and $\pi$ contributions. Indices calculated using only the $\{\Pi\}$. functions might more accurately reflect the trends in reactivity. But these arguments are only valid for substitution reactions in which the attacking species is chemically equivalent to the leaving species and the plane of symmetry is conserved during the reaction. However, if the principle established in the Hammett equation is considered to be valid, the indices which can describe this reaction must also describe others in which the attacking species is not chemically equivalent to the leaving species and vice-versa. All indices calculated using the MO of only one symmetry are indicated with the appropriate superscript, e.g., $Q_a^{\pi}$ and $S_{N\sigma}^{\pi}$.

One can also use frontier indices to estimate trends in reactivity. The frontier electron treatment (Greenwood and McWeeny 1966) has been very successful in describing satisfactorily not only the aromatic hydrocarbon reactivities but also the stereoselectivity of reactions and the mechanisms of transition metal catalysis. To use a frontier treatment one can compute the indices previously described using only the HOMO (highest occupied molecular orbital) and the LUMO (lowest unoccupied molecular orbital). However, there is a fundamental conceptual difficulty associated with frontier treatments. Frontier indices, like molecular orbitals, are not observables andthereareno fundamental reasons to assume that relationships exist between them and observable quantities, such as biological activities. As such, the interpretation of frontier indices must involve a high degree of caution.

**SOME SIMPLE APPLICATIONS**

Two of these MO indices, the polarizabilities and superdelocalizabilities, have been extended from the simple Hückel theory to the more advanced CNDO method and should be tested on simple systems to verify their effectiveness in parameterizing chemical reactivities. Since the CNDO method includes all the valence electrons and is parameterized for heteropolar molecules, all of these indices should be applicable to the study of reactivities of both cyclic and exocyclic sites in either polar or nonpolar systems.

Four simple homologous series were studied to verify the effectiveness of these indices. These were the aromatic hydrocarbons, the substituted benzene compounds, the substituted benzoic acids, and the substituted phenyl amines. Since the details of these calculations can be found elsewhere (Brown and Simas 1978) we present here only a short description of these results. The MO indices described above gave acceptable correlations with the chemical reactivities as measured by the appropriate Hamett-like parameter. For example, correlations against the Hamett $\sigma_+$ parameter for the 14 unique reactive sites in the first group which includes benzene, biphenyl, napthalene, anthracene and phenanthrene gave results very similar to those of the Hückel indices. Since the CNDO method was parameterized for the more general case, this is an acceptable result. For the other three series, the results were as good or better. This is particularly significant in that these other series are not only polar, but in the case of the benzoic acids and phenyl amines, have the reactive site off of the aromatic ring.

For all of these cases, the best indices were the superdelocalizabilities which showed good correlations for both perturbationally and electrostatically controlled reactions. It is also significant that the frontier indices gave poor results, which only confirms the suspicion that one should exercise caution in the use of frontier indices. Only with the aromtatic hydrocarbons did the $\pi$-components of the indices show good correlations with the reactivities, however the total indices gave comparable results. The self-atom polarizabilities were good parameters in addition to the electrophilic polarizability. For the other three series, all of which are polar, some potential field and group charge indices produced correlations nearly as good as those of the electrophilic superdelocalizabilities.

In general, correlation coefficients between 0.86 and 0.90 were obtained with the best indices used in these studies. The number of data points used in these regressions were 14, 25, 19 and 19 respectively for the aromatic hydrocarbons, the substituted benzenes, the benzoic acids and the phenyl amines. These results engender confidence in the application of these CNDO indices to the study of more complicated problems such as QSAR of drugs.

## AN EXTENSION OF THE ALL-VALENCE ISOLATED METHOD TO THE STUDY OF BIOLOGICAL ACTIVITIES OF DRUGS

A model which can satisfactorily describe the biological activities of drugs, based on quantum theory, must have the following important characteristics:

(1) As BA are observable quantities, the model must be based also on observables, such as the dipole moment, electric fields around the molecules, etc., and

(2) the parameters or the indices used must provide a consistent set of physico-chemical quantities, useful in the interpretation of the results.

As pointed out by Lin (Lin 1974), the drug molecule may follow a very complicated path, passing through a great number of stages including dissolution, absorption, distribution, penetration, binding, metabolism and excretion in order to give a particular response. It is clear that the success of the drug as a whole is related to its behaviour in all these stages (if they can really be distinguished from each other).

Now, consider the molecule in one general stage of the pharmacological process. The system may be divided into two subsystems; the drug molecule and the biological environment which contains all the biological complexes which surround the drug. The central idea is that the success of the drug in each step will depend on its recognition by the environment. This recognition will be associated with a measuring process where the measuring apparatus is the biological environment and what is being measured is the drug molecule. In this way the drug molecule can be seen as a collection of observables. During the steps, the various environments will recognize the drug with the degree of success depending on the expectation values of the observables. The metabolism is assumed to occur only after all the previous steps, including the interaction with receptor, have been completed.

So, the set of the observable expectation values will determine unequivocally the drug's biological activity. There are two problems here: the lack of knowledge of the biological environmentand the large quantity of observables pertaining to the drug molecule, many of which mimic each other. Therefore in the application of this method, it will be necessary to know the BA of some compounds of the homologous series of drugs, and to have a guarantee that the global mechanism of action is identical for all cogeners. Only observables related in some way to the interaction energies between the molecule and the environment will be considered. These are the indices previously described.

There is one additional problem. For which geometrical conformation will the indices be calculated? To solve this we will use the Kier hypothesis of "Remote Recognition of the Preferred Conformation" (RRPC) (Kier 1973, 1975). This hypothesis states that there is always a significant amount of binding between the two molecules when they approach each other from infinity, yet both initially retain their preferred isolated conformations. Also, this binding is almost always greater than kT (where T is about 310°k). Moreover, their change in conformation only has significance when they are very near each other.

First consider the drug molecule inside a cavity of infinite radius whose surface reflects the biological environment. Letting the system relax it will progress from $\lambda_0$ (the initial state) to $\lambda_R$ (the state which really occurs) with the corresponding changes in the biological environment and the geometry of the substrate. Supposing that for the interval including $\lambda_0$ and $\lambda'$, Kier's hypothesis is valid and that the reaction profiles for every compound of the homologous series do not cross, then all the concepts of the isolated molecule method can be applied.

To be consistent with the indices considered here, the BA must be expressed as a quantity which can be related to the free energy such as the logarithm of rate constants or equilibrium constants.

A step of the biological process, for the purposes of this method, can be defined operationally as a stage which can be explained by only one index or an expansion of indices which represent just one perturbation. Suppose that the ith step can be explained by a set Of indices $X_{i\mu}$ calculated in the manner pointed out previously for each compound μ of the homologous series. Following the isolated molecule method, a linear transformation of the index will give a good estimate of the free energy of interaction of the μth compound, $\Delta A_{i\mu}$

$$\Delta A_{i\mu} = a_{i\mu} \cdot X_{i\mu} + b_i \tag{29}$$

We now define $r_\mu$ as the BA of the μth compound, which is proportional to the free energy, $\Delta A_\mu$ , being considered from the administration of the drug until the interaction with the receptor and subsequent metabolism. ΔAμ can be written as a summation of the free energies involved in each step i, $\Delta A_{i\mu}$ , plus the interaction free energy between the various steps, $\Delta A_{i,j\mu}$

$$r_\mu \propto \Delta A_\mu = \sum_i \Delta A_{i\mu} + \sum_{i<j} \sum \Delta A_{i,j\mu} \tag{30}$$

Many steps will possess the same free energy for all the homologous compounds with the exception of a small number of them represented by $\Delta A_{k\mu}$ which will differentiate the compounds. Neglecting the free energies of interaction between the steps, one can write:

$$r_\mu \propto \Delta A_\mu = \Delta A_{const} + \sum_k \Delta A_{k\mu} \tag{31}$$

Applying equation 29 in equation 31 one has,

$$r_\mu = b + \sum_k a_k \cdot X_{k\mu} \tag{32}$$

This is the basic equation of the extended isolated molecule method.

**CHANGES OF MECHANISM THROUGH THE HOMOLOGOUS SERIES**

Eventually, some subset of compounds of the homologous series can have their BAs well explained by a small number of indices. If the BA of the remaining compounds are not well explained by those indices, this implies that the mechanism of BA has changed, or that the steps which are rate determining for one subset are different for the other subset. This implies the crossing of the reaction

profiles for these compounds. In many cases, this is caused by steric effects of the substituents. In the domain of the extended isolated molecule method, recognition of this change of mechanism is possible. Also attempts to establish rules allowing the differentiation of those compounds belonging to this or that subset can be made.

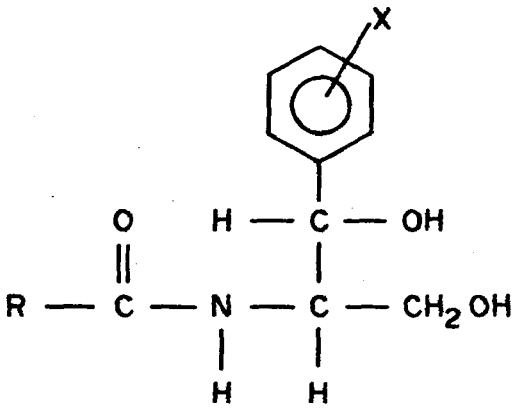
## CONCLUSION

From the initial set of hypotheses established here, it is not possible to deduce which of the more highly correlated indices is related with one or more particular stages of drug action. As a result all of these stages are taken into account. The more highly correlated indices reflect the importance of strategic positions in the molecule, or which of its global properties, such as the dipole moment, are significant. Of course, some indices will be more closely related to one stage than to others, but in the domain of the extended isolated molecule method, it is not possible to make this discrimination. The model can only reflect the complete biological process. On the other hand, this is the model's great advantage, as the particular stages such as solvent effects cannot be considered explicitly.

It is important to note that all the experimental parameters used in free energy relationships (such as the Hansch analysis), are compatible with equation 32 and can be employed together with the theoretical indices. This outlook is very promising for the method since a complete set of indices (a set which can span every perturbation in a molecule) is difficult to obtain. However, looking at the mixed equations, one must be careful because the physical models involved in the two sets of parameters are different, although they can complement one another.

One additional point must be made. The free energy relationships such as in the Hansch analysis, the Free-Wilson analysis, etc., explain in principle only those parts of the BA that are equilibrium controlled. The extended isolated molecule method can treat not only these, but also the kinetically controlled processes. In this sense it is, at least in principle, more powerful.

The extended isolated molecule method has been applied to the chloramphenicol family of drugs to clarify the mechanism of the drug potency and to predict new active drugs in this family. We comment briefly on these results. The chloramphenicol drug as illustrated here is an effective antibiotic, particularly for the treatment of typhoid fever. The parent chloramphenicol has X= p-$NO_2$ and R = $CHCl_2$. None of the 40 odd cogenors which have been synthesized have proven to be more active. The propanediol moiety, the phenyl group and the acylamino side chain are critical to the drug potency. However, substitutions can be made for the p-nitro group and the dichloromethyl group without destroying the biological activity.




Statistically significant correlations have been obtained for the X-series for X = p-$NO_2$, CN, $OCH_3$, Cl, H and $NH_2$. The single variable regressions are given below where the biological activities are fitted to equation 32.

| INDEX | A | B | R |
|---|---|---|---|
| $E_\perp$ | 0.343 | 0.593 | .98 |
| $Q_X$ | -6.930 | 0.594 | .96 |
| $V_\perp$ | -0.952 | 0.272 | .95 |
| $Q_{HOCH}$ | 99.70 | 2.290 | .95 |
| $S_{NC}$ | 1.260 | -14.2 | .95 |

R denotes the correlation coefficient and $S_{NC}$ the superdelocalizability of the benzyl carbon. $E_\perp$ and $V_\perp$ are the orthogonal component of the electric field and the coulombic potential at a point 4.5A below the center of the benzene ring. These parameter do not strengthen the argument that the mechanism involves the formation of a radical by the loss of the $\alpha$-hydrogen atom. An electrostatic mechanism agrees with these results. The potential field below the benzyl carbon and $\alpha$-hydrogen increases with the drug potency for a positive charge which indicates that an attack by a nucleophile them is indeed probable. The high correlation of $S_{NC}$ agrees with this. Electron withdrawal by the X-group is also important since $Q_x$ has a high correlation coefficient.

However, α-deutero-chloramphenicol experiments show a 20% drop in activity. In all, these results indicate that the most probable mechanism involves a loss of the α-hydrogen as a proton rather than a radical. The importance of indicates that an accompanying interaction between the benzene ring and the receptor is also likely. The details of this work as well as further applications to the prediction of new drugs can be found elsewhere (Brown, Simas and Bruns 1977; Simas et al.1978).

These results give only a glimpse of the way in which this method can be used. It can be seen that the isolated molecule method, as translated to an all valence formalism and extended to the study of the BA of drugs can be used in clarifying the mechanism of drug action as well as in predicting the activities of new drugs. This method represents a potentially useful combination of experimental and theoretical techniques.

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# Recognition and Activation Mechanisms on the LSD/Serotonin Receptor: The Molecular Basis of Structure Activity Relationships

Harel Weinstein, Jack Peter Green, Roman Osman, and W. Daniel Edwards

Most studies on the relation between the molecular structure of drugs and their biological effects are aimed at deducing what structural characteristics of the drug are recognized by the receptor and what characteristics evoke the tissue response - all based on the assumption that the interaction between drugs and receptors can be described in the same terms as those used for chemical interactions and reactions. Early work - and to a great extent, work beiug done today - focussed almost exclusively on functional groups such as a hydroxyl group, a phenyl ring, a tertiary mine, and on the geometrical and stereometrical relationships among them. Implicit in such an approach is the tacit assumption that the functional group necessarily reacts directly with the receptor, and that its role is independent of its effect on the physical and chemical properties of the entire molecule. A pertinent example is the conclusion that because 5-hydroxytryptamine (5-HT) is more potent than other tryptamine derivatives, the hydroxyl group at the C5 position interacts directly with the receptor. In fact the hydroxyl substitution markedly alters the electronic properties and thereby the reactivity pattern of the molecule so that, for example, the magnetic circular dichroism spectrum of 5-HT in solution is markedly different from that of tryptamine (Sprinkel et al. 1975) and the self association of 5-HT in solution is very different from that of 6-hydroxytryptamine (6-HT) (Sapper and Lohman 1976). It became evident, even from very early theoretical structure-activity studies that the differences in potency in the tryptamine series are attributable to changes in the electronic structure of the entire indole ring (Green and Kang 1970; Kang and Green 1970; Johnson and Green 1974). This level of understanding of the molecular determinants for drug activity is closely related to the development of new physicochemical and theoretical methods of analysis that provide the proper tools for the study of molecular mechanisms.

The classical theoretical method from which a quantitative measure is obtained in relating molecular properties to biological activity is the "quantitative structure activity relationship" (QSAR) procedure pioneered by Hansch (for reviews see Hansch 1971; Van Valkenberg 1972). The basic assumption underlying this approach is that the pharmacological activity is a numerically quantifiable function of several mutually independent parameters which are themselves

dependent on the molecular structure. For example, experimentally measurable partition coefficients between model media (e.g., oil:water) are considered to be dependent on substituents on the molecule, and they determine the amount of drug that reaches the receptor site (the $\pi$ coefficient). Other parameters considered to affect the biological response are electronic affects of substituents (the $\sigma$ coefficient from Hammett's models), steric affects (the $E_s$ parameter introduced by Taft) and recently also quantum mechanical indices such as frontier electron densities ($f_i$) and energies of the highest occupied molecular orbital ($E_{HOMO}$) and the lowest unoccupied molecular orbitals ($E_{LUMO}$). Assuming that the effects of the parameters are independent of each other, one formulates the dependence of the biological response (BR) on the parameters ($P_i$) as Log (BR) $= \sum_i C_i P_i$ where $C_i$ is a coefficient.

With the parameters measured or calculated, and the use of multiple regression analysis, it is possible to obtain the coefficients (Ci) by fitting the biological data for a series of compounds to the (Pi) values for each molecule. The resulting equation is then assumed to predict the biological potency of any other drug for which the $P_i$ values are known. This quantitative correlation between molecular properties and potency has proved useful in drug design and can, in principle, indicate the molecular structural factors that are most likely to affect biological activity (see review quoted above; also, Hansch 1971; Redl et al. 1974).

The main shortcomings in this approach are related to the nature of the parameters used, the assumption that they are mutually independent, and the inherent impossibility to yield Inferences on the molecular mechanisms of interaction between the drug and a receptor. Thus, au important question is the meaning of parameters such as the Hammet sigma constant. These parameters reflect the effects of structural changes at a particular reaction center in a model compound. But such parameters cannot indicate which sites in the molecule are involved in the reaction. This problem is overcame to a certain extent by the use of parameters obtained from quantum mechanical calculations. For example, the quantum chemical concepts of Fukui's frontier orbital theory (for a review see Fukui 1964) provide a means of defining site-localized reactivity parameters by the use of frontier densities ($f_i$). These numbers represent the electronic charge localized on a certain atom and originating from a Mulliken population analysis involving only the Highest Occupied or Lowest Unoccupied orbitals (HOMO or LUMO). Such parameters have been used in QSAR studies on tryptamine and LSD (Johnson and Green 1974; Green et al. 1978), and with success in predicting biological aetivities: the mescaline-like activity of 2-amino-7-hydroxyteralin (Green, Dressler, and Khazan 1973) was predicted in a study (Kang and Green 1970) that also led to the correct prediction that the R-enantiomers of the hallucinogenic amphetamines were the active forms (Kang, Johnson, and Green 1973).

However, reactivity criteria from point charge distributions generated by a Mulliken population analysis, such as frontier densities,

cannot provide a physically rigorous description of the fundamental mechanisms that control the reactions of drugs with macromolecular receptors. Such an understanding requires a description of the structural features of the molecules , their electronic distributions and the energies required to modify and perturb them during interactions. From these basic elements one can obtain a more complete characterization of the molecular reactivity of the drugs and of the modes of interaction, the electronic consequences and the energetic characteristics of drug-receptor complexes. In principle and in fact, most of the information required for such a dynamic representation of biological interactions can be acquired by a judicious use of quantum mechanical and physicochemical methods.

The success of quantum mechanics in describing the physical and chemical properties of organic compounds and their reactions is evident in every facet of chemical research and is very well documented. The main advantage of theoretical studies with quantum mechanical methods are: the ability to predict processes and mechanisms of interaction with the use of model systems that cannot readily be obtained experimentally but are amenable to experimentation once the most probable mechanism is established (for a very recent assessment of this successful role in a specific example see Maugh 1976); and the ability to analyze, interpret and rationalize all experimental observations on molecular processes in a unified language based on the primary principles of physics. During the past decade, quantum chemical methods have been increasingly applied to drugs and related compounds (Kier 1971; Bergmann and Pullman 1974), and these studies have recently been reviewed by us and others (Green, Johnson and Kang 1974; Christoffersen 1976).

Until very recently, most theoretical studies on drug molecules have concentrated on the calculation of conformation in an attempt to predict the most probable geometry of the drug at the receptor and the relation between conformation and activity. With very few exceptions, notably the calculation of aeetylcholine by a classical-quantum chemical method (Gelin and Karpilus 1975), the total energy of the molecules is calculated for a small number of variable parameters and most bond lengths and bond angles fired at their initial values. Unfortunately. freezing most of the molecule in its initial geometry may influence the outcome of the calculation: an ab-initio calculation of acetylcholine (ACh) using the crystal geometry of AChCl showed a gauche form to-be 3.2 kcal/mole more stable than the trans form, whereas with a standard geometry the trans form was 3.4 kcal/mole more stable than the gauche (Port and Pullman 1973). Analogously, PCILO calculations based on crystal geometries of AChCl and AChBr produced different preferred conformers (Pullman and Courriere 1972). However, the energy differences are generally small and do not invalidate conclusions as to which conformations are energetically permissible and which improbable: the single conformation with the lowest calculated energy is not necessarily the conformer at the receptor, as the many possible interactions of the drug with its environment may stabilize other allowed conformations that are only a few kcal less stable than the optimal one. Moreover, a certain degree of flexibility may be a requirement for agonist

activity (Weinstein et al. 1975; Richards et al. 1975). In our studies we have regarded the conformational data obtained from quantum mechanical calculations as a starting point rather than an end. By indicating which conformations should be considered for further work, they simplify the problem of defining the reactive regions of the molecule and the sites of likely intermolecular interaction. Whatever ambiguities evolve from theoretical studies on conformation can often be eliminated or at least reduced by studying a series of similar compounds and by the judicious use of experimental date from x-ray and NMR studies as well as pharmacological data from studies of rigid analogs.

Good theoretical calculations on molecular structure provide more than conformational date: the electronic structure in each conformation of the molecule can be obtained from the corresponding wave function and the chemical behavior of the drugs can be studied from the changes in this electronic structure upon interaction with model reagents such as models for receptor sites. On this basis, the wealth of methods and formulations developed in quantum chemistry, which were shown to provide reliable predictions and understanding of chemical reactivity, can be directly used to study drug receptor interactions (Weinstein 1975). Parameters related to the reactivity of biologically active molecules may be sought from the molecular wave functions and from the calculated properties, providing insight into the likely molecular mechanisms of drug receptor interactions and to the biological implications of such interactions. As with experimental studies, theoretical studies carry the obvious risk that model results may be extrapolated too far and the test of resulting hypotheses may be hampered by the inherent ambiguity of crude Biological correlations. Nevertheless, considerable evidence is at hand proving the relative success of theoretical approaches in elucidating specific mechanisms of drug action, in providing a basis for rational drug design, and in leading to conclusions about the nature of drug-receptor complexes and about the consequences of drug-receptor interactions (see Green, Johnson and Kang 1974; Christoffersen 1976). Yet more recently, new theoretical methods have been developed and applied to biologically active substances. This continuous develovpment, combined with more rigorous quantum chemicals methods (e.g.; ab-initio) for calculating the wave function, has provided new insight into the basic molecular events determining drug activity, and, at the same time, furnished a useful tool in drug design.

**LINEAR CORRELATIONS BETWEEN BIOLOGICAL ACTIVITY AND ELETRONIC CHARGES: TRYPTAMINE DERIVATIVES**

QSAR studies including electronic parameters from total valence (INDO) calculations on nine ring-substituted tryptamine derivatives that contract the rat fundus strip, demonstrated several correlations of potency with charge densities and frontier electron densities at the N-1, C-4, and C-5 positions of the indole ring ($f_1$, $f_4$ and $f_5$, respectively). It was suggested that the indoles react with the fundus receptor (see Green et al. 1978 for a review of the biological data used in the correlations) through charge-transfer

forces involving these atoms, and that the greater potency of 5-HT was due to the effect of the hydroxyl substitution at the C-5 position on the electronic structure of the ring (Green and Kang 1970). These correlations predicted accurately the potencies of the 4-$NH_2$, 5-F and 5-OH-7-Cl derivatives measured subsequently for their ability to contract the fundus. In the correlation with $f_4$, the carbon, sulfur and oxygen isosteres of tryptamine and 5-HT are predicted to be less active than 5-HT on the fundus, in agreement with measurements. To test further the validity of the prediction we expanded the study to eight new tryptamine derivatives which we tested on the same biological system. The activity of the new compounds was found to correlate with the old indices $f_1$, $f_5$ and with another parameter, $f_3$. In addition, evidence was obtained for a specialized interaction with the receptor of substituents at the 7-position, probably involving hydrophobic forces (Johnson and Green 1974). Figure 1 shows the correlation between the measured activity on the fundus strip and the activity calculated from the regression equation vith $f_3 + f_5$. The main deviant, 5,6-dihydroxytryptamine (circled) was not used on the regression equation, for it was shown by spectrofluorometric analysis to be destroyed in the assay medium probably by oxidation (even in the presence of ascorbic acid). Similar deviation of the 7-$OCH_3$ derivative from the correlation with the experimentally measured ionization potential was reported at this symposium by Domelsmith and Houk (1978).

The rank order of potencies of these tryptamines in contracting the fundus was nearly identical to their potencies in blocking high affinity binding of LSD to brain membranes, implying that the two receptors are very similar (Grew et al. 1978). Not surprisingly, the potencies in this latter system correlated with the same electronic indices, fl, $f_3$ and $f_5$.

These correlations prwided additional support for the hypothesis of an "electron-donor" role for tryptamines in the drug-receptor complex. The QSAR approach, Based on indices derived from molecular orbital calculations, therefore successfully accounts for the potencies of tryptamines acting on an LSD receptor, and thereby offers both a guide for the synthesis of new compounds and a hypothesis for the molecular basis of their activity. However pleasing and practical these results are, they are subject to the fundamental question that pharmacological activity (and chemical reactivity) may not be determined by such derived quantities as point charge dietributione, frontier densities, etc. This consideration prompted us to introduce to our studies reactivity criteria that are based on the combined contribution from electrons and nuclei for the molecule as a whole. These led to a representation of the interaction characteristics of the molecule such as the mutual orientation in space of its active sites, orientation of the molecular active sites towards reacting agents representing receptor sites, and the response of the molecule, on an electronic level, to perturbations caused by the environment or by the receptor itself. Clearly, this is a more realistic attempt towards understanding molecular properties that determine biological activity but its success requires overcoming complex theoretical and numerical problems. At the present stage it can be dealt with within the frame of the approximations that

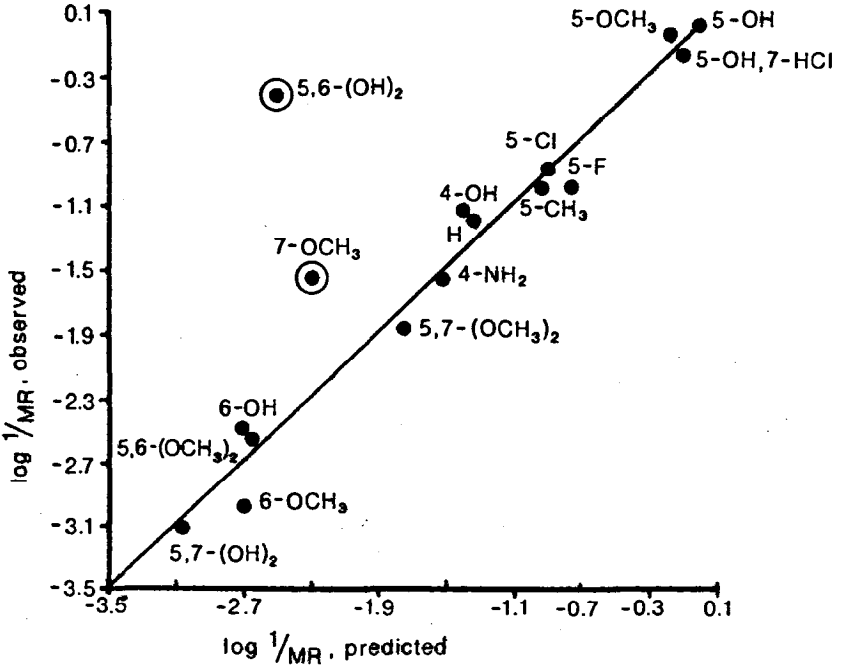


***Figure 1.*** *Correlations between the observed and predicted values for the activity of tryptamine derivatives on the rat fundus. The correlation equation is pMR = 1.542 (±0.129)$\pi_7$ + 20.63 (±1.23)$f_3$+ 33.14 (±2.46)f5 - 6.28 for n = 14. The correlation coefficient is r = 0.990. The 5,6-$(OH)_2$ and the 7-$OCH_3$ derivatives (circled in the figure) were not used in the regression. See Green et al. 1978 for additional details.*

are inherent even in the most elaborate theoretical approaches. In view, however, of what could be considered early signs of progress obtained with the use of such theoretical approaches and because they represent some of the most recent and powerful advances in the analysis of molecular processes, we apply them to the elucidation of structure activity relationships.

Since molecular systems represent a spatial distribution of electronic and nuclear charges (an averaged time independent distribution within the workable approximations of quantum mechanics), the natural approach is to seek a description of the molecular reactive sites and interaction ability based on the long-range intermolecular electrostatic forces. By describing the electrostatic potential field generated by the distribution of charge in the molecule, a "picture" could be expected representing the forces that the molecule can apply on another charge or on the continuous electron density distribution of another molecule. Regions of space in which the molecule generates negative potentials (i.e., where the contributions from all the electrons in the molecules are being felt stronger than that from all its nuclei) should attract positively charged species or fragments. A positive region (where the nuclear contribution is stronger) would repulse positive, and attract negatively charged fragments. A molecular specie approaching the investigated molecule should therefore adopt an orientation that will bring its charged fragments into matching attractive interaction with the positive and negative potential pattern. On this basis, the structural requirement for the interaction with the receptor can be inferred from the potential maps of active drugs, and used as criteria for the ability of other compounds to mimic this reactive pattern when approaching the receptor. These molecules should thereby be able to achieve the same mutual orientation as the active drug and interact with the same sites of the receptor. The pattern of the potential can therefore be regarded as an "interaction pharmacophore" and should prove to be a basic analytic tool in the study of molecular determinants of pharmacological activity. Since the criterion established by the interaction pharmacophore is independent of the nature of the groups generating the potential, it can be applied to molecules which do not have atom-to-atom correspondence thereby broadening the scope of the analysis to compounds derived from unrelated basic structures. These properties have been used by us in the characterization of the physicochemical basis and the molecular mechanism of action of several drugs of abuse such as phencyclidine (Weinstein et al. 1973) and psychotomimetic anticholinergics (Maayani et al. 1973; Weinstein et al. 1975; Weinstein et al. 1977); morphine-like narcotic analgesics (Loew et al. 1974; Loew, Weinstein, and Berkowitz 1976) and tryptamine congeners acting on an LSD/serotonin receptor (Weinstein et al. 1976; Green et al. 1978). Other laboratories have also begun to approach specific pharmacological problems with methods based on electrostatic potentials (Petrongolo et al. 1974; Politzer, Daiker, and Donnelly 1976; Hayes and Kollman 1976; see also Anderson and Kollman, and Kaufman - in this volume.

**The Relation to Rigorous Quantum Chemical Calculations**

On the basis of newly developed quantum chemical methods (Weinstein 1979) it becomes possible to formulate a more rigorous link between the electronic structure of the active compounds and the mechanisms of action proposed on the basis of QSAR studies as described above. Thus, the excellent correlation observed between frontier densities and the ability of the tryptamines to contract the rat fundus strip and to inhibit high affinity LSD binding to brain membranes indicated that the tryptamines might interact with the receptor through a complex involving mainly electron polarization from the drugs toward the receptor. The importance of polarizability and electrostatic forces in complexation of indoles has been emphasized before (Green and Malrieu 1965). The QSAR results (Johnson and Green 1974) indicate that the main sites of interaction [besides the side-chain amino group, which is essential] would be the indole nitrogen; a region including the C-4 and C-5 bond; and the C-3 atom (see numbering scheme). Strong indications for the importance of these sites for complexation are also obtained from experimental studies in a variety of media. Thus, C-3 end C-5 are sites of close contact in crystalline complexes of 5-HT with both picrate and creatinine sulfate (Thewalt end Bugg 1972). NMR studies on complexes of methylated indoles and trinitrobenzene in solution show that the 3 position of the indole (especially in 3-methylindole) makes the greatest contribution to complexation (Foster and Fyfe 1966). Proton NMR studies (Daly and Witkop 1967; Kang, Witherup, and Gross 1977) indicate that the proton at the C-4 position is exchanged fastest in 5-HT and 5-methoxytryptamine.

We have shown that the positions at which a high frontier electron density was correlated with high biological potency, correspond to the sites at which the density of the highest occupied molecular orbital is localized in 5-HT (Weinstein et al. 1976; Green et al. 1978). We explained these correlations in terms of a multiple perturbation expansion (Bartlett and Weinstein 1975; Chang, Weinstein, and Chou 1976) of the interaction energies of 5-HT with positively charged reagents: the highest contribution to the polarization term in the interaction energy comes from the HOMO (Weinstein et al 1976; Green et al. 1978). Since the electronic density is localized on specific centers in the HOMO of the various 5-HT congeners, this finding explained the relation between high frontier density at the same sites at which HOMO is localized in 5-HT, and a more potent 5-HT-like activity. Similarly, it became clear why earlier studies found a negative correlation between potency and the frontier density on certain atoms (Johnson and Green 1974): the localization of HOMO and the orbital Next to HOMO (NHOMO) differs, and a requirement for high frontier density on the atoms on which the HOMO of 5-HT is localized is tantamount to a requirement for low frontier density on the atoms on which the NHOMO of 5-HT is localized. We have shown (Green et al. 1978) that the localization pattern in the 5-HT NHOMO is precisely on those sites on which Johnson and Green (1974) found a negative correlation between frontier density and biological activity.

The relation between the different localization patterns of HOMO in the various congeners and the biological activity also indicate some of the reasons for the apparent failure to find a direct correlation between the energy of HOMO (or the molecular ionization potential) and 5-HT-like biological activity (Green et al. 1978).

The predictive value of the HOMO localization pattern was further demonstrated in a study of the relation between experimentally obtained C-13 chemical shifts of tryptamine derivatives and the biological activity of the compounds: When the total electronic charges on the indole moieties in a series of 5-HT congeners were compared, the two most similar compounds were found to be 5-HT and 5-fluorotryptamine (5-FT). This similarity in total charges (calculated with the ab-initio method) was echoed by a comparison of the C-13 chemical shifts for the atoms in the indole fragments of the same molecules. Table 1 shows that the highest correlation is obtained between the shifts on 5-HT and 5-FT. These similarities do not correlate with the biological potencies of the compounds: figure 1 shows that 5-FT is closer in its activity to tryptamine than to 5-HT. This similarity in potency is predicted by the correlation with frontier charges: the distribution of HOMO (and NHOMO) charges on the atoms of the indole fragments of 5-FT is much more similar to that of tryptamine than to the distribution of these charges in 5-HT. This is indicated here by the high correlation coefficient between the distribution of HOMO (and NHOMO) charges in 5-FT and tryptamine, as shown in table 2.

The important insight prwided by these results from quantum chemical studies into the basic relation between QSAR parameters, molecular structure, and biological activity prompted a more thorough analysis of the molecular mechanisms of action of 5-HT congeners on the LSD/serotonin receptor.

**MOLECULAR REACTIVITY CRITERIA FOR THE ACTION OF TRYPTAMINES ON THE LSD/SEROTONIN RECEPTOR**

Our quantum chemical studies aim to establish end compare the molecular determinants for the rank order of the affinity of tryptamine congeners for the LSD/serotonin receptor. Some molecular structural factors that may be responsible for the differences between measured affinities of molecules in the tryptamine series for the LSD/serotonin receptor were identified from the reactivity characteristics of these molecules (Weinstein et al. 1976). These characteristics were obtained from ab-initio LCAO-SCF-MO calculations with (5s,3p/2s,p) minimal gausian basis sets.

The studies shoved that as a result of the interaction of the protonated side chain amine with a negative site or an electron donating group, the indole portion of the tryptamine congener cations would assume the same reactivity characteristics that this moiety would have in the neutral species (free base form). The importance of this primary interaction with an anionic site has been stressed by the experimental data: pharmacological SAR studies demonstrated the necessity of a cationic side chain for activity on the rat fundus (Vane 1959; Johnson and Green 1974), while proton NMR experiments

Table 1. CORRELATION COEFFICIENT[a]

| | Between total charges on atoms[b] | | Between C-13 chemical shifts[c] | |
|---|---|---|---|---|
| | TRYP | 5-HT | 5-FT | 6-HT |
| TRYP | 1.0 | 0.824 | 0.830 | 0.908 |
| 5-HT | 0.989 | 1.0 | 0.9970 | 0.630 |
| 5-FT | 0.990 | 0.9998 | 1.0 | 0.652 |
| 6-HT | 0.993 | 0.966 | 0.966 | 1.0 |

[a] Atoms included in the correlations are: C2, C3, C4, C7, C8, and C9 on the indole portion of the molecules.
[b] The correlation coefficients result from a linear fit of the total charges on the same atoms in the different molecules.
[c] The correlation coefficients result from a linear fit of the measured C-13 chemical shifts of the same atoms in different molecules.

Table 2. CORRELATION COEFFICIENT[a]

| | Between charges on atoms from HOMO[b] | | Between charges on atoms from NHOMO[b] | |
|---|---|---|---|---|
| | TRYP | 5-HT | 5-FT | 6-HT |
| TRYP | 1.0 | 0.649 | 0.9428 | 0.744 |
| 5-HT | 0.578 | 1.0 | 0.841 | 0.149 |
| 5-FT | 0.9549 | 0.783 | 1.0 | 0.506 |
| 6-HT | 0.856 | 0.162 | 0.698 | 1.0 |

[a] Atoms included in the correlation are: C2, C3, C4, C7, C8, and C9 on the indole portion of the molecules.
[b] The correlation coefficients result from a linear fit of charges on the same atoms in the different molecules.

and fluorescence spectroscopy studies indicated that the ring-stacking interaction of these molecules with the bases in poly-A, DNA or ATP is dependent upon the ionic interaction of the side chain (Helene, Dimicoli, and Brun 1971; Nogrady, Hrdina, and Ling 1972). The theoretical finding on the effect of such an interaction is not peculiar to the tryptamine series and has been shown to be important in the determination of the physicochemical and biological properties of other substances such as histamine (Weinstein et al. 1976). The relevant reactivity characteristics of the tryptamine derivatives were therefore determined from the electronic structures of the isolated neutral drugs, or from the structure of the complex between the cationic form of the drug and an anionic group.

A comparison of the interaction pharmacophores (obtained from the molecular electrostatic potentials) of the tryptamine derivatives indicated that there will be differences in the preferred orientation that each of the congeners will assume towards a polar receptor site following the primary interaction with the anionic site. It was shown, for example, that after the interaction with the anionic site, the vector describing an optimal electrostatic alignment between 5-hydroxytryptamine (5-HT) and other parts of a putative receptor would be almost perpendicular to the vector describing the optimal alignment of 6-HT with the same receptor site. Figure 2 shows the electrostatic potentials in planes at 1.6Å above the indole portions of 5-HT and 6-HT, respectively. Figure 2 also shows the directions of the orientation vectors obtained from the gradients of the electrostatic-potential maps (i.e., the lines connecting the sites of strongest negative potentials through the areas of steepest change in the potential). These discrepancies in optimal electrostatic orientations for the various tryptamine derivatives (see Weinstein et al. 1976 for further examples) are fully consistent with NMR data on the complexes of these molecules: different geometries are observed for the different congeners (Sapper and Lohman 1976). Consequently, when the cationic head of the tryptamines is anchored at the same anionic site, the difference in optimal alignment will necessitate a change in the conformation of the side chain if optimal interaction is to be achieved by both 5-HT and 6-HT with the same reactant (such as another part of a receptor). The energy expenditure required for such a conformational change affects the probability for optimal binding and should be measurable as "lover affinity" or "lower potency" of one drug relative to another. If 6-HT is to assume an orientation that is electrostatically equivalent to that of 5-HT at the receptor, its apparent affinity should be lower. This is in fact the experimental observation. A hypothesis for the interaction of tryptamine congeners with the LSD/serotonin receptor resulted from these findings: "the difference between the affinity of 5-hydroxytryptamine (5-HT) andthatof any other congener is related to the discrepancy between the preferred electrostatic orientation of 5-HT and that of the congener in the field of the receptor" (Weinstein et al. 1978).

The definition of molecular reactivity parameters that have such a proved predictor function is not only of immediate practical value in the design of specific drugs, but is a most important element in

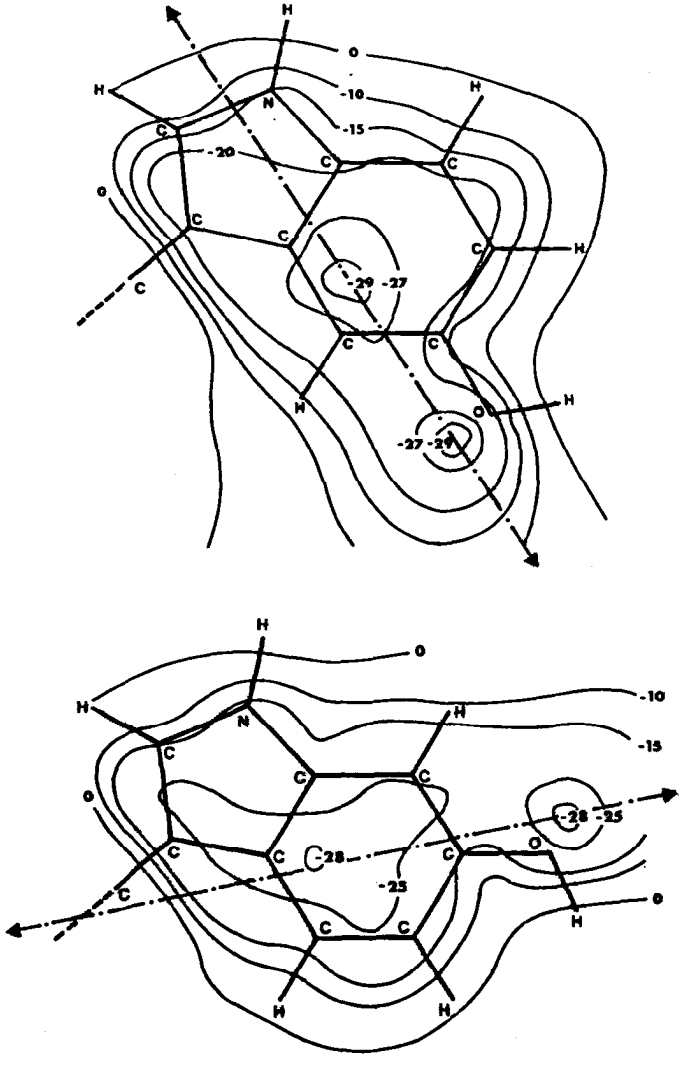


***Fig. 2.*** *Molecular electrostgtic potential maps for 5-HT and 6-HT. The potential in a plane 1.6Å above the indole fragment of the neutral (free base) forms of the molecules was calculated from ab-initio wave functions with the (5s, 3p/2s, p) basis set. Values ar kcal/mole. the arrows indicate the orientation of optimal electrostatic alignment ("orientation vectors"). See Weinstein et al. 1976 for details on the choice of planes, the geometry of the molecules and methods of calculation.*

the elucidation of the molecular structural factors that are responsible for the biological activity of the molecules. It was therefore reassuring to find that the reactivity criteria proposed on the basis of calculations on the isolated molecules were validated by calculations on complexes of 5-HT with imidazolium cation (Weinstein and Osman 1977). Calculations on supermolecular models of complexes with imidazolium were therefore carried out to test the working hypothesis for the interaction with the LSD/serotonin receptor and to identify a possible electronic basis for the activation mechanism.

## MODELS FOR MOLECULAR MECHANISMS

### Methods and Background for the Simulation

The studies of Shinitzky, Katchalski and coworkers on the complexes between indole and imidazole derivatives (for an elegant review see Shinitzky and Katchalski 1968) have described inter- as well as intramolecular complexes that are of particular relevance to the reactivity of indoles in biological systems. These careful studies have demonstrated the formation of complexes between imidazole, histidine, or histamine, and indole, tryptophan, aminotryptophan, or methoxytryptophan. All the complexes are formed when the imidazole ring protonated and "the binding forces between the indole and imidazole moieties are weak and strongly dependent on pH and orientation" (Shinitzky and Katchalski 1968). Although "no final proof as to the nature of the complex" was provided by the authors, they interpreted their finding as suggestive of "a complex of the charge-transfer type". The complexes of tryptamine derivatives with imidazolium cation were therefore chosen as models for the test of the reactivity criteria and the related hypothesis. For the theoretical simulation of interactions in systems that have also been studied experimentally can provide additional insight into the properties of the molecules and their mechanisms of interaction. Because the reactivity of a substituted tryptamine cation interacting with an anionic site has been shown to be identical to that of the neutral tryptamine (free base) (Weinstein et al. 1976), we studied the interaction of the neutral tryptamine derivatives with the imidazolium cation. Such a model simulates the simultaneous interaction of the cationic tryptamine derivatives (all these molecules would be almost fully protonated at pH = 7.4) with an anionic site and with other molecules (or "receptor sites") that can form complexes with the indole portion.

The first calculations on these models (Weinstein and Osman 1977) were performed with a near ab-initio pseudopotential (model potential) method devised by Van Wazer coworkers (Ewig and Van Wazer 1975; Ewig, Osman, and Van Wazer 1977). The applicability of this method to molecules such as tryptamine derivatives was tested by comparisons with full ab-initio calculations using the same gaussian basis set (5as,3p2,s) contracted to minimal basis (Osman and Weinstein 1977).

These calculations indicated that the 5-HT complex with imidazolium cation (IMID) is basically electrostatic in nature: the amount of

charge transferred from 5-HT to imidazolium was very small (0.0046e) and the molecular orbitals of the complex could be assigned back to either molecule based on their very pronounced localization. It appeared, therefore, that there would be a valid theoretical basis for the we of reactivity criteria obtained from the electrostatic potential maps of the molecules (in the form of the electrostatic orientation vectors). This possibility was tested by calculations on the [5-HT + IMID] and [6-HT + IMID] complexes in mutual configurations that align the electrostatic vectors of IMID (see figures 4 in Weinstein et al. 1976, and 1 in Weinstein and Osman 1977) with that of the corresponding tryptamine derivative. In order to test the relative importance of vector alignment for the stabilization of complexes with IMID, we studied the following supermolecular systems:

a) [5-HT + IMID] in a mutual configuration that aligns the vector of IMID with that of 5-HT (to be referred to as A-5);

b) [6-HT + IMID] in a configuration that aligns the vector of IMID with that of 6-HT (referred to as A-6);

The geometrical arrangements of the imidazolium (IMID) relative to the indole portion of the 5-HT congeners is shown in figure 3 for configurations A-5 and A-6. We have shown that an optimal interaction between a relatively positive hydrogen and the indole nitrogen may be important for the enhancement of the polarization interaction (Chou and Weinstein 1978), due to the special properties of nitrogen as a "charge transducer." Consequently, the configurations are chosen according to the additional criterion that one of the hydrogens in IMID is positioned above N1 in both the A-5 and the A-6 configuration. Note, however, that due to geometrical constraints the hydrogen placed above N1 differs in the two configurations: in configuration A-5 it is H2; in configuration A-6 it is H4. It should be noted that H2 carries a more positive charge than H4 and is therefore expected to have a stronger effect on the polarization at the indole nitrogen.

**Stabilization Energies and the Nature of the Complexes**

Table 3 presents the stabilization energies of the complexes of 5-HT and 6-HT with IMID in the geometries described in figure 3. It is clear that in the complex with 5-HT, the preferred geometry is that in which the imidazolium cation aligns its most positively charged regions with the electrostatic orientation vector of 5-HT (i.e., configuration A-5), whereas the A-6 configuration is preferred for the [6-HT + IMID] complex. These results are in full agreement with the predictions that evolved from the reactivity critertia regarding the differences in the preferred configurations that 5-HT and 6-HT would adopt towards the same polar site. It appears now that the electrostatic orientation vectors that were generated as force vectors from the molecular electrostatic potentials are useful indicators of the reactivity characteristics of the 5-HT congeners in interactions with molecules of the type of imidazolium cation. The underlying reason for the validity of these reactivity criteria was suggested by our findings (Weinstein and Osman 1977) that the complexes [5-HT + IMID] and [5-HIND + IMID] are electrostatic in

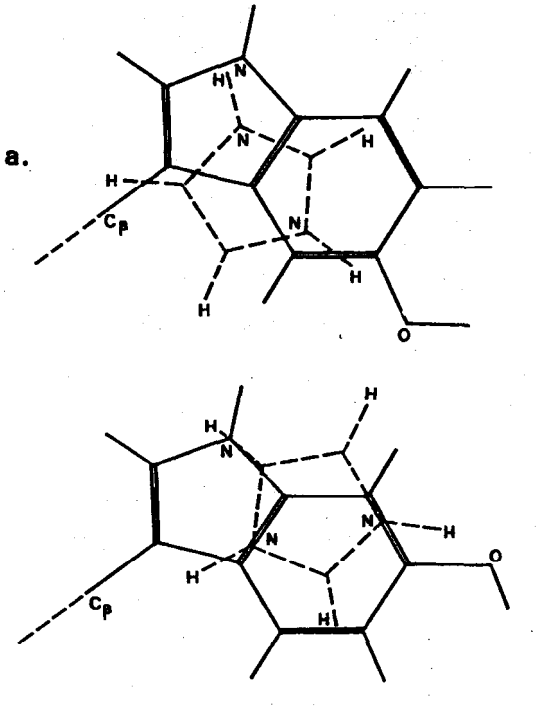


***Figure 3.*** *The orientation of imidazolium (IMID) in complexes with 5-HT and 6-HT: a) [5-HT + IMID] in the A-5 configurations; b) [6-HT + IMID] in the A-6 configuration. See text for the definition of A-5 and A-6.* ©1978, John Wiley & Sons, Inc.[1]

nature. A more complete elucidation is given by the results of the decomposition of the interaction energies in the complex that was performed according to the scheme proposed by Morokuma and coworkers (Morokuma 1971; Kitaura and Morokuma 1976). Table 3 shows that for all the complexes, in all the configurations, the electrostatic interaction (ES) is the largest term. Moreover, it is evident from table 3 that the total energetic preferences for a given configuration (i.e., A-5 for the 5-HT complex, and A-6 for the 6-HT complex) is determined by the electrostatic contribution (ES): for a given configuration, either A-5 or A-6, the only energy term that is different in the 5-HT complex from the 6-HT complex is ES, while all the others (PL, EX, CT + mix) are virtually identical. Also, for the same complex (e.g., [5-HT + IMID]), only the ES values differ significantly between the A-5 and A-6 configurations. The very small contribution of the (CT + mix) term indicates that the main changes in the electron distributions of the molecules upon complex formation are in the form of charge polarization (see below).

The predictive value of the reactivity characteristics is thus confirmed and explained by the calculation of the supermolecular model complexes: The anchoring of the side chain in the tryptamine molecule

**Table 3.** Energy Decomposition in Complexes with Imidazolium[a)]

| Type of contribution | 5-HT + IMID at A-5 | 5-HT + IMID at A-6 | 6-HT +IMID at A-6 | 6-HT +IMID at A-5 |
|---|---|---|---|---|
| E S | -4.52 | -3.70 | -4.51 | -3.93 |
| P L | -1.00 | -0.98 | -0.96 | -1.00 |
| E X | 2.39 | 2.27 | 2.28 | 2.38 |
| CT(+ mix) | -0.59 | -0.38 | -0.38 | -0.59 |
| Total stabilization | -3.72 | -2.79 | -3.57 | -3.14 |

[a]*Energy values are from calculations with the STO-3G set. Units are Kcal/mole.* © 1978, John Wiley & Sons, Inc.[1]

determines a fixed direction with respect to which the substituted indole portion can orient itself as determined by the electrostatic orientation vector, in order to achieve optimal electrostatic interaction. The preferred orientations are different in different congeners, and can be predicted by the orientation vectors.

Since the orientation vectors are defined only on the indole portion of the neutral (or neutralized) tryptamine congeners, it was interesting to find that the same configurational preferences hold for the complexes between indole derivatives (i.e. 5-hydroxy and 6-hydroxyindole: 5-HIND and 6-HIND) and imidazolium. The energetic differences between the tryptamine and the indole complexes with imidazlium are insignificant both in the total stabilization energies and in most of the components. The only noticeable difference, albeit small, is in the polarization term (PL) which is greater in the tryptamine complexes than in the corresponding indole models. This difference is directly attributable to the inductive effect of the saturated, neutrel side chain. It appears, therefore, that in addition to its role in defining the directionality constraints of the interaction of the indole portions in these molecules, the side chain reinforces the electrostatic nature of the interaction. The preferred orientation is, however, determine by the electrostatic reactivity charecteristics in the idole portion. Table 4 shows how the complex stabilization energy, and its decomposition, very with the interplanar distance for the [5-HIND + IMID] complex. The optinal interplanar distance in the A-5 geometry is 3.5Å i.e., slightly greater than the "close contact" distance from crystallographic data, but very close to the average interring distance in the complexes of 5-HT (Thewalt and Bugg 1972). It is evident from the energy decomposition presented in table 4 that the electrostatic nature of the complex is preserved at all relevant distances, as the ES component is the determinant factor in the total stabilization energy at distances greater than 3.1Å Identical conclusions hold for the [6-HIND + IMID] complex in the A-6 configuration.

**The Electron Charge Redistribution in the Complexes as an Element of the Activation Mechanism.**

The electrostatic nature of the complexes and the relative contribution of polarization, exchange repulsion and "charge transfer" terms to the stabilization energies indicated that the chargere disttibution

*Table 4. Energy Decomposition in complexes 5-HIND + IMID at A-5*[a)]

| Type of contribution | Interplanar distance (Å) | | | |
|---|---|---|---|---|
| | 3.796 | 3.51 | 3.301 | 2.971 |
| ES | -3.60 | -4.11 | -4.62 | -6.13 |
| PL | -0.51 | -0.63 | -0.74 | -0.99 |
| EX | 0.24 | 0.83 | 2.24 | 9.24 |
| CT + mix | -0.08 | -0.24 | -0.57 | -1.99 |
| Total stabilization | -3.95 | -4.15 | -3.69 | +0.13 |

[a] *Energy values are from calculations with the STO-3G basis set. Units are Kcal/mole.*

accompanying complex formation would be mainly "intramolecular" in character: the polarization within each molecule will enhance the electrostatic interaction but there will be little "overlap" or actual transfer of charge. With the STO-3G basis set in an ab-initio calculation, the Mulliken population analysis (which at an interplanar distance of 3.3Å can be expected to reproduce well the charges within each component of the complex) indicates a minimal amount of charge transferred from 5-HT to IMID (0.0029 at A-5, and 0.0018 at A-6). The same results are obtained for the [6-HT + IMID] complex: 0.0029 and 0.0017 for the A-5 and A-6 configurations; respectively.

In order to study the detailed characteristics of the charge redistribution we used partial integrations of the total electron density distribution function ($\rho$). The "planar density" ($\rho P$), obtained by integrating ($\rho$) in a given plane, has been calculated with Module PA60 of the POLYATOM program. An expression of the charge redistribution upon complex formation is obtained from the difference between the $\rho P$ values for the complex and the sum of the $\rho P$ values of the separated molecules. This difference function ($\Delta\rho P$) is plotted in figure 4, and indicates that the charge in 5-HT has been strongly polarized towards IMID, while the charge in imidazolium cation is polarized away from 5-HT. This result is in full agreement with the basic idea that the polarizability of the 5-HT molecules is a major determinant of its ability to form molecular complexes, especially with positively charged species. The polarization of the electronic eharge in IMID will cause an increase in the positive electrostatic field generated by the molecule in the direction of 5-HT; this further increases the interaction with the excess electronic charge introduced in the interplanar region by the polarization of 5-HT.

Figure 4 also shows that the polarization pattern of the [5-HT + IMID] complex in the A-6 configuration will be very similar to that in the A-5 arrangement (although very slightly less accentuated). This is in full agreement with the contribution of polarization (PL) to the stabilization of the complexes in the two configurations (see tables 3 and 4). Since the contributions of the PL terms to the total stabilization of the [6-HT + IMID] and [6-HIND + IMID] complexes were nearly identical to those for the corresponding

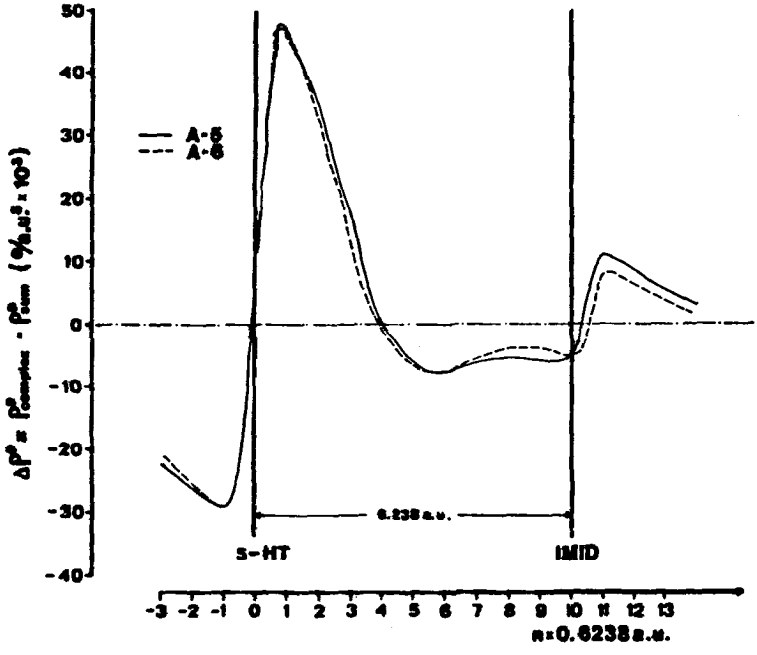


***Fig. 4.*** *Differences in planar densities showing the electron density redistribution in [5-HT + IMID] complexes in the A-5 and A-6 configurations. At each distance (r = n x 0.6238 a.u.),* $\rho P(r)$ *is the total (integrated) density in a plane parallel to the indole portion of 5-HT.* $\Delta\rho P(r)$ *is the difference (at each point r) between the total density in the plane calculated from the wave function of the complex, and the sum of densities in that plane calculated from the wave functions of 5-HT and IMID, separately.*

complexes of 5-HT and 5-HIND, it is not surprising that the same polarization pattern is observed in the complexes of 6-HT. This near identity holds not only for the integrated values of the charge distribution function, but also for the charge redistribution pattern in localized parts of the complex. This chatacteristic is exemplified in figures 5 and 6 in which the changes in the total density upon complex formation ($\Delta\ \rho$) are calculated in equivalent planes for the [5-HT + IMID] complex (figure 5), and the [6-HT + IMID] complex (figure 6).

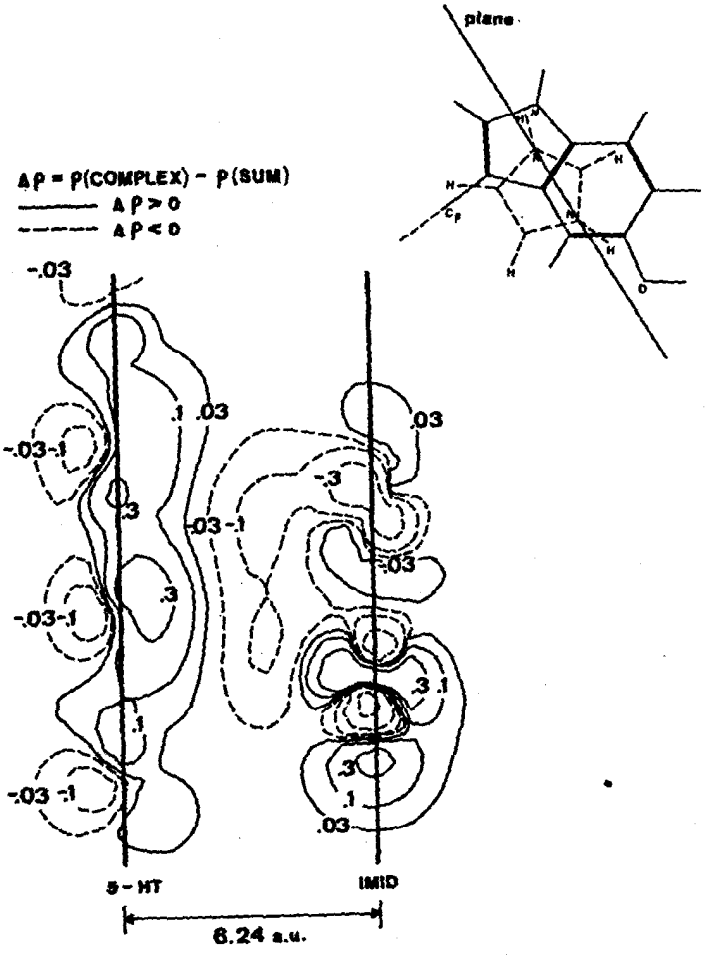


***Figure 5.*** *Density difference map in a plane perpendicular to the rings in the [5-HT + IMID] complex, in the A-5 configuration, The insert in the upper right corner shows the position of the plane intersecting the complex.* ©1978, John Wiley & Sons, Inc.[1]

This finding is most important in view of the biological activity exhibited by 5-HT and 6-HT: the mechanism of receptor activation must be the same for these compounds since they have the same maximal activation ability ("intrinsic activity").

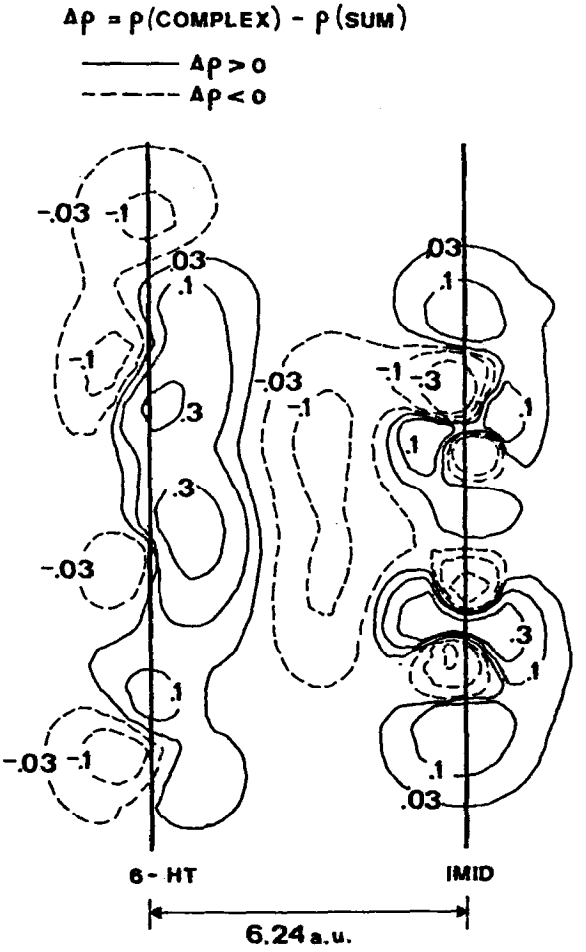


***Figure 6.*** *Density difference map in a plane perpendicular to the rings in the [6-HT + IMID] complex, in the A-6 configuration. The plane intersects the complex through the two nitrogens in the imidazolium ring, and is perpendicular to the rings. See figure 5.*

It appears therefore that polarization complexes of the type analyzed here provide a good model for the interaction mechanism of these molecules with the LSD/serotonin receptor: the considerable difference in affinity is a direct result of the molecular reactivity pattern of the molecule and is expressed in the difference of preferred interaction geometries with the same reactant. However, once a congener (e.g., 6-HT) adopts au interaction geometry that aligns its vector with that of the common reactant (albeit with lover probability), the consequences of the interaction are identical. Finally, it is not difficult to realize that the other members of the congeneric series of singly substituted tryptamine derivatives will all exhibit the same type of reactivity characteristics: i) formation of polarization complexes with a variable degree of probability (dependent on their molecular structure and predictable by the reactivity parameters); ii) generation of charge redistribution patterns that are very similar to those of 5-HT once an equivalent interaction geometry is adopted with the receptor site. This hypothesis can be tested at the present level of biological experimentation by the prediction, based on the reactivity parameters identified above, of structures that can have similar electrostatic orientation preference to 5-HT and generate the same charge redistribution in a model reactant (such as IMID) without necessarily sharing the molecular structure of tryptamine derivatives.

## FOOTNOTE

1. Figures 3-6 aNd tabie 3, © 1978 by John Wiley & Sons, Inc., are reprinted by permission of the publisher, from Weinstein, H., Osman R., Edwards, W.D., and Green, J.P. Theoretical models for molecular mechanisms in biological systems: tryptamine congeners acting on an LSD/serotonin receptor. Int J Quant Chem, QBS5, 1978. In press.

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# Conformational Energies and Geometries of Narcotics, Using a Potential Function Method

Mark Froimowitz

## INTRODUCTION

As anyone who reads the scientific literature must be aware, conformational enerygy calculations are an increasingly popular approach to the analysis of biologically interesting molecules. The apparent objective of much of this work is to characterize possible conformations for molecules which have significant rotations about single bonds. Sometimes, it is also explicitly or implicitly assumed that the conformation with the lowest computed energy should be the one that is responsible for biological activity. However, this is incorrect for a number of reasons. First and most importantly, there is no evidence that the results of these calculations are quantitative. This problem is worsened by the recent demonstration that bond angle bending and stretching, which certainly occurs in a molecule, can significantly affect the quantitative results of an energy calculation (Gelin and Karplus 1975). In contrast to this, almost all calculations make the assumption of rigid bond angles and lengths. Moreover, it can be documented that the semiempirical quantum mechanical PCILO, CNDO and INDO methods may produce unrealistic results under certain conditions (see below). A second difficulty with these calculations is that they are performed with unrealistic molecular environments, since they generally consider only a single isolated molecule. The computed energy surface in such an environment is unlikely to be quantitatively relevant for a molecule in its biological system where there are unknown and unspecified intermolecular interactions. Finally, these calculations generally compute conformational energies rather than the thermodynamically relevant free energies. For all of these reasons, it is clear that calculated conformational energy differences of several kilocalories/mole should not be considered to be significant. However, the results of energy calculations can provide useful qualitative information about the likelihood of various molecular conformations. In particular, it should be possible to exclude those regions of conformation space that are unlikely to be important for a molecule due to the energetically unfavorable overlap of nonbonded atoms.

Conformational energy calculations can be performed with methods that are based on either quantum mechanics or on a more classical. approach using potential functions. Some of the most commonly used methods are the semiempirical quantum mechanical PCILO, CNDO, and INDO computer programs whose results can be unrealistic under certain conditions. In comparing the results of an INDO calculation on acetylcholine (Beveridge and Radha 1971) with that obtained by a potential function method (Froimowitz and Gans 1972), it was noted that the former produced low energy minima for conformations in which there should have been serious energetically unfavorable overlaps of nonbonded atoms. Indeed the lowest energy minimum found by the INDO calculation, at $\tau$(C6-01-C5-C4) = 270° and $\tau$(01-C5-C4-N) = 50° ([270°, 50°]), appears to be a totally fictitious result. Two PCILO conformational energy maps for acetylcholine using different molecular geometries (Pullman, Courrière, and Coubeils 1971; Pullman and Courrière 1972) show the corresponding extra pair of minima at [270°, 60°] and [90°, 300°]. In the second of these two calculations, these are computed to be the global minima. That these minima are fictitious is reinforced by the results of *ab initio* calculations on acetylcholine which do not show these conformations as minima (Genson and Christoffersen 1973; Pullman and Port 1973). A more recent study on model systems has also indicated that the CNDO and INDO methods produce insufficient repulsion between nonbonded atoms (Gregory and Paddon-Row 1976). It was also found that extended Hückel, which is a full overlap method although it is the most simple of the various semiempirical quantum mechanical methods, does have the corect repulsive behavior between nonbonded atoms, though it is well known to have other deficiencies.

A more recent illustration of these difficulties is provided by the PCILO investigation of the methadone molecule (Loew, Berkowitz, and Newth 1976). Using a mathematical search algorithm to locate minima in the conformational energy surface, a total of twelve minima were reported, along with some of their associated interatomic distances. Of the twelve, eight have interatomic distances that are within the van der Waals' radii of those atoms (table 1). There are likely to be additional overlaps which were not reported. While nonbonded atoms can approach to within their van der Waals' radii under some conditions, such as when hydrogen bonding occurs or if other molecular interactions are so favorable that they can counterbalance the expected repulsion, the severity and ubiquity of the atomic overlaps argues against this explanation. Thus, it appears that these semiempirical quantum mechanical programs consistently report low energies for conformations which should be energetically unfavorable. This is not to say that these methods do not provide a great deal of useful information. However, it would be helpf'ul if users of these program excluded those conformtions which are clearly unrealistic.

These computional difficulties may also provide an explanation for the results of a recent investigation of the enkephalins, in which conformational energies were computed by both the PCILO method

**TABLE 1**

| Conformation | Atom Pair | Reported interatomic distances (A) | Van der Waals' radii for atom pair* (A) |
|---|---|---|---|
| 4 | N-O | 2.46 | 3.05 |
| 4 | H-O | 1.58 | 2.70 |
| 5 | H-O | 1.76 | 2.70 |
| 6 | C-C | 2.40 | 3.47 |
| 7 | C-N | 3.27 | 3.32 |
| 8 | C-N | 2.67 | 3.32 |
| I | N-O | 2.97 | 3.05 |
| I | N-C | 2.58 | 3.25 |
| III | N-O | 2.65 | 3.05 |
| III | N-C | 2.46 | 3.25 |
| IV | C-N | 2.84 | 3.32 |
| IV | N-C | 2.91 | 3.32 |

*The short interatomic distances of various atoms of some "low energy conformations that were reported from a PCILO investigation of methadone (Loew, Berkowitz, and Newth 1976). These conformations are unlikely to be of low energy since many of the atoms are within their van der Waals' radii, which is the interatomic distance at which atoms begin to repel each other.*

**It should be noted that there are different van der Waals' radii for phenyl and alkyl carbon and hydrogen atoms (Bondi 1964).*

and a potential function method (Loew and Burt 1978). It was reported that local minima found by the potential function method were also minima for the PCILO method but that the latter produced additional minima. It would appear that the likely cause of these extra "minima" is the qualitatively incorrect behavior that is associated with the PCILO method when nonbonded atom overlap.

In contrast to these difficulties, potential diction methods do appear to give qualitatively correct conformational energies. For

example, in regions of conformation space in which there are no atomic overlaps, a potential function method gave similar results to an INDO calculation in that minima and a maximum appeared in similar locations (Froimowitz and Gans 1972). similarly, the results detained for the dopamine molecule are consistent with intuitive expectations that there are minima associated with a *trans* and two *gauche* conformations (see below). Using this method, it was also possible to account for the crystal conformations of a large number of phenothiazine derivatives relative to a restricted dihedral angle range (Froimowitz and Matthysse, to be published). That is, all of the observed values of the dihedral angle were close to the three computed minima for this dihedral angle.

Another very important advantage to using a potential function method is the relative speed of the calculation. It has been noted that this method is 10,000 times as fast as an INDO calcula tion on the same molecule and computer (Froimowitz and Gans 1972). This enormous increase in speed allows one to compute conformational energies on a finer grid for larger molecules with increased numbers of variable dihedral angles.

### Conformational Geometries

While the relative energies of various conformations are undoubtedly important for the analgesic activities of the narcotics, an addi- tional factor which should also be important are conformational geometries, since the narcotics have been shown to interact with receptors (Gyang and Kosterlitz 1966; Pert and Snyder 1973). In order for them to do so, they must be satisfying specific molecular and geometrical requirements that allow them to mimic the charac- teristics of an endogenous substance and successfully compete with it for its receptors. One such requirement is that the narcotics must be the correct size and shape to fit into the receptor site. A second requirement is that they and their endogenous equivalents must contain specific atom or atomic groups which attract comple- mentary atoms or atomic groups in the receptor. This molecular and geometrical specificity insures that biologically active substrates only bind to certain receptors in the complex regulation of biological processes. Therefore, in addition to computing the conformational energy as a function of variable dihedral angles, various intramolecular geometrical paramters will also be monitored. This should allow the characterization of the various conformational geometries.

## METHODS

### Potential Function Method

A number of different possible interactions should be considered in computing intramolecular conformational energies using potential functions (Wilson 1972). For the molecules to be considered here, these include nonbonded, electrostatic, torsional, and bond bending and stretching forces.

The nonbonded interaction between a pair of atoms i and j that are at a distance of $r_{ij}$ can be simulated by a Lennard-Jones 6-12 potential function

$$U_{ij} = a_{ij}/r_{ij}^{12} - b_{ij}/r_{ij}^{6} \quad (1)$$

where $a_{ij}$ and $b_{ij}$ are constants which depend on the atomic types (Scott $a_{ij}$ Schersga 1966a; Brant, Miller, and Flory 1967; Froimowitz and Gans 1972). The parameter $b_{ij}$ which controls the attractive van der Waals' forces can be calculated from

$$b_{ij} = 3e\hbar\, \alpha_i\alpha_j/2m^{1/2}\, [(\alpha_i/N_i)^{1/2} + (\alpha_j/N_j)^{1/2}] \quad (2)$$

where e and m are the electron charge and mass, $\hbar$ is Planck's constant, and $\alpha_i$ and $N_j$ are the atomic polarizability and effective number of valence electrons of atom i. Using this formulation for the van der Waals' forces, it was possible to account for the heats of formation and energies of isomerization of small hydrocarbon chains (Pitzer 1959). Since the "effective" number of valence electrons is unclear for most atoms, the actual number will be used in this work. The parameter $a_{ij}$ which controls the repulsive part of equation (1) that is attributed to the overlap of nonbonded atoms can be obtained by minimizing the nonbonded energy at the van der Waals' radii of the pair of interacting atoms. Thus,

$$a_{ij} = b_{ij}\, r_{vdw}^{6}/2 \quad (3)$$

where $r_{vdw}$ is the sum of the van der Waals' radii of atoms i and j (Scott and Scheraga 1966b; Froimowitz and Gans 1972). The qualitatively correct behavior of equation (1) is to have a sharply repulsive energy within $r_{vdw}$, an energy minimum at $r_{vdw}$, and a slight attractive energy outside of $r_{vdw}$. Atomic polarizabilities and van der Waals' radii to be used in equations (2) and (3) can be obtained as before (Ketelaar 1958; Bondi 1964). We have previously shown that equation (1) tends to define the permissible regions of conformation space (Froimowitz and Gans 1972). In particular, it assigns high energies to those regions in which there is significant overlap of nonbonded atoms.

A second force which should also be important for the conformational energies of many molecules is the electrostatic interactions that can occur between partially charged atoms. The usual method of representing this is by

$$E_{ij} = q_i q_j/Dr_{ij} \quad (4)$$

where $q_i$ and $q_j$ are partial charges that are centered on atoms i and j at a distance of $r_{ij}$ and where D is the dielectric constant (Scott and Scheraga 1966b; Brant, Miller, and Flory 1967; Froimowitz and Gans 1972). There are uncertainties associated with the values and locations of the partial charges and the appropriate dielectric

constant (Froimowitz and Gans 1972; Momanyy *et al* 1975). For these reasons, a quantitative number will not be assigned for the electrostatic interaction. However, it will be possible to recognize molecular conformations that could be electrostatically favored.

A third set of terms that is typically added to these computations is used to represent torsional forces. While the theoretical basis for these forces is uncertain, they are added to give the correct barrier height for the rotation about a single bond, since the use of equation (1) alone generally underestimates the experimentally observed barrier (Scott and Scheraga 1966a). However, terms for the torsional forces will not be included in this work since they add at most only one or two kilocalories/mole to the rotation about a single bond. As with the electrostatic interactions, certain molecular conformations such as *gauche trans* along a hydrocarbon chain, can be identified as being preferred.

The effect of bond bending and stretching on the computation of conformational energies using potential functions has recently been examined (Gelin and Karplus 1975). Their results indicate that structural flexibility can affect the quantitative results of such calculations, especially in regions in which there is steric hindrance. This is due to the very steep repulsive nature of the first term of equation (1) since it is possible for a molecule to decrease this energy by bond bending and strecthing. However, it was also found that the location of local minima were unchanged by these forces. For this work, bond bending and stretching will be neglected. However, to ensure that possibly important conformations are not missed, regions of conformation space that are within 20 kilocalories/mole of the global minimum will be considered in our analysis.

To summarize the above discussion, the conformational energy will be computed by summing the nonbonded interactions that are represented by equation (1) over all atoms whosee distances can change relative to each other. This equation defines the permissible regions of conformation space, since it assigns high energies to conformations in which there is significant atomic It should be noted that other interactions that are discussed above are not being explicitly included, since there is uncertainty in the correct quantitative assignment of their values. Instead, these interactions will be incorporated qualitatively by the direct examination of the conformations. It should be realized that, for this work, we are only interested in computing qualitatively accurate energies since our primary concern is elimination of those conformations which cannot be significant due to the overlap of nonbonded atoms.

**Intramolecular Geometrical Parameters**

The minimal features that are common to all narcotics appear to be a basic nitrogen separated from an aromatic ring, which is almost

always benzene, by several bonds (Beckett and Casy 1954). Very often, the presence of a hydroxy group in a particular position of the benzene ring leads to enhanced activity (Jacobson, May, and Sargent 1970). Thus, it appears that the critical intramolecular geometrical parameters would include the distance of the basic nitrogen atom (1) from the center of the aromatic ring, (2) to the plane of the aromatic ring, and (3) to the hydroxy oxygen on the ring. Another structural feature which is particularly important for the potencies of the meperidines and the methadones is a carbonyl group (Jacobson, May, and Sargent 1970). Therefore, distances between the electronegative carbonyl oxygen and the positively charged nitrogen may be important and will also be monitored.

The tyrosine is the most likely portion of the enkephalins to be relevant to the structures of narcotics, since it contains the phenyl hydroxy group and basic nitrogen appear to be important for analgesic activity. The only other phenyl group in the enkephalins is that of phenylalanine while there are no other basic nitrogens or hydroxy groups. While it will not be possible to analyze the enkephalins in detail because of the large number of single bonds about which rotation can occur, analysis of the tyrosine fragment will be feasible.

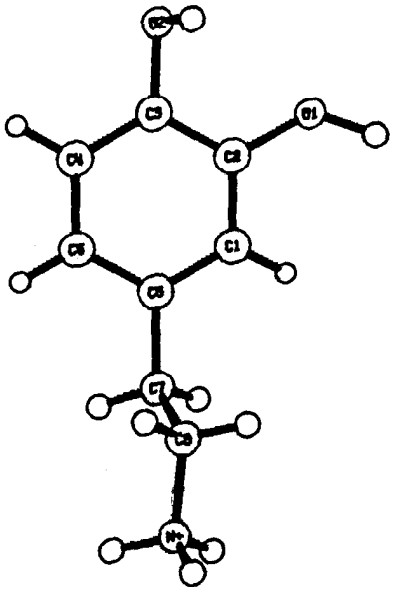
**RESULTS**

While we are only beginning our calculations on conformationally flexible narcotics, our general method is illustrated by our investigation of the phenothiazine and thioxanthene classes of neuroleptic (antipsychotic) drugs, some of whose results are briefly presented here. More detailed results will be published elsewhere (Froimowitz and Matthysse, to be published). As with the narcotics, neuroleptic drugs are believed to compete with an endogenous substrate in the central nervous system. There is considerable evidence that the relevant endogenous substrate for the neuroleptics may be dopamine (Matthysse 1977).

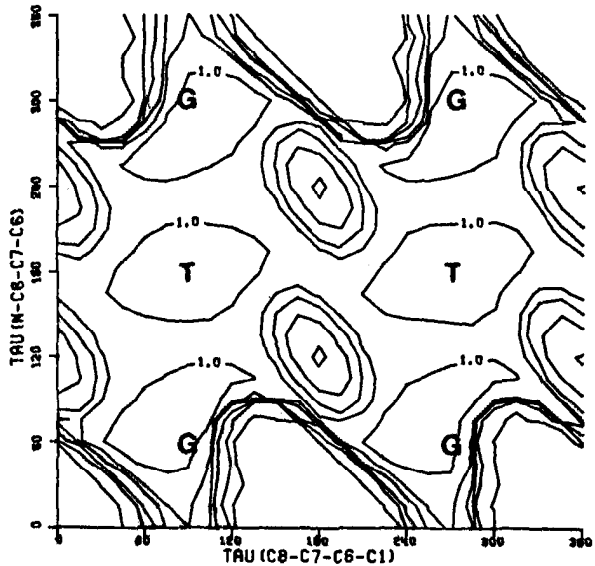
The conformational energy surface for dopamine (figures la and lb) contains two equivalent sets of minima due to molecular symmetry. Each set consists of one *trans* and two *gauche* conformations, which is the expected result for the rotation about the C7-C8 bond. Various intramolecular geometrical parameters, which happen to be identical to those defined above for the narcotics, were measured for these preferred conformations of dopamine, and the conformational volumes of the neuroleptics were then searched for possible geometrical analogues to them.

Chlorpromazine (figure 2a), which is the prototypical phenothiazine, contains three single bonds about which rotation can take place and which can affect the relative orientation of the sidechain nitrogen to the phenothiazine structure. Of the three, rotation about the C13-Nl bond was found to be the most hindered with two relatively broad minima at $\tau$(C14-C13-Nl-C12) = 210° and 300° and a very narrow

FIGURE 1




*1a. Crystal conformation of dopamine (Bergin and Carlström 1968. Hydrogen atom (unlabeled), which were not determined, have been added.*



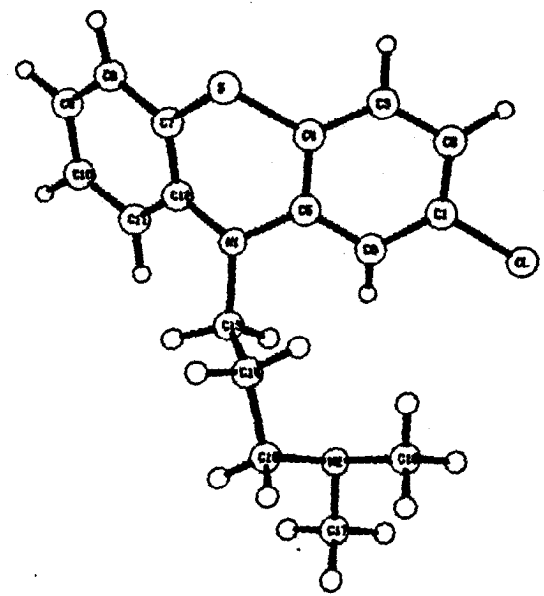

*1b. Variation of the conformational energy for dopamine. G and T indicate gauche and trans conformations. Contour levels are 1, 3, 5, 10, 20, and 100 Kilocalories/mole. Unlabeled extrema are maxima.*

one at 75°. A number of possible geometrical analogues to the dopamine conformations were found, some of which are noted in figure 2b, which is the energy surface for chlorpromazine with τ(C14-C13-N1-C12 = 210°. The two analogues in the lower right hand side of the figure occur when the N2 atom approaches the Cl substituent, with the S atom in an equivalent position to one of the dopamine oxygens. These conformations would be expected to be electrostatically favored since the N2 atom, which would be protonated at physiological pH, approaches the electronegative Cl atom. The *trans* analogue is also very close to the conformation that chlorpromazine assumes in the crystal state (McDowell 1969; Horn and Snyder 1971), though different conformations occur in the crystal structures of other phenothiazines which are also potent neuroleptics (Horn, Post, and Kennard 1975). The two dopamine analogues which appear in the upper left side of figure 2b occur when the N2 atom swings toward the other benzene ring and the Cl atom assumes an equivalent position to one of the dopamine oxygens. We have considered the latter to be possible dopamine analogue even though the N2-benzene plane distance for them is about 2 Å further than the equivalent parameter in dopamine. However, it appears that this parameter is sensitive to slight tilts of the benzene ring. In addition to these, equivalent analogues also appear at the minima at τ(C14-C13-N1-C12) = 75° and 300°. However, as shall be seen from our results for the thioxanthenes, it is unlikely that the latter are crucial for antipsychotic activity.

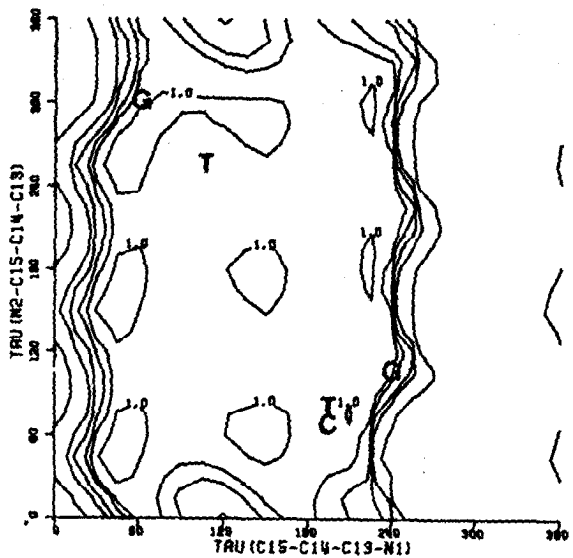
The thioxanthenes, of which α-flupenthixol(figure 3a) is an example, are neuroleptics that are structurally related to the phenothiazines, with the only difference between them being that the first sidechain N-C single bond of the latter (the N1-C13 bond in chlorpromazine) is replaced by a C-C double bond. The restricted rotation about a double bond results in a pair of geometrical isomers with *cis* isomers (relative to a substituent on the tricyclic structure) consistently having greater potency as neuroleptics than the equivalent *trans* isomer (Kaiser, Warren, and Zirkle 1974). Since *cis* isomers, such as the relatively potent neuroleptic α-flupenthixol, have a value of τ(C17-C16-C9-C14) near 180°, this suggests that the crucial dopamine analogues occur at the equivalent dihedral angle of 210° in the phenothiazines. The similarity in these dihedral angles also shows up in the geometrical and conformational energy results (figure 3b) for a molecule based on the crystal structure of α-flupenthixol (Post *et al.* 1975). However, the *gauche* dopamine analogue in which the protonated N1 atom approaches the tricyclic substituent is absent for steric reasons. Thus, there are still three possible dopamine analogues for neuroleptics of this class.

Diethazine (figure 4a) is typical of low potency neuroleptics in which there are only two carbon atoms between the sidechain nitrogen and the phenothiazine structure. The possible dopamine analogues figure 4b) for such a molecule (with a Cl atom in the approptiate position) are ones in which the Cl atom is in an equivalent position to one of the dopamine oxygens. Other analogues found previous, in

**FIGURE 2**

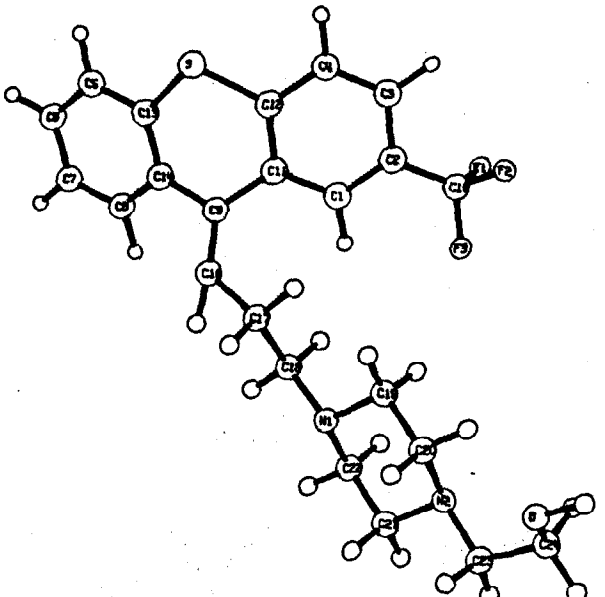


*2a. Crystal conformation of chlorpromazine (MoDowell 1969). Hydrogen atoms (unlabeled), which were not determined, have been added.*

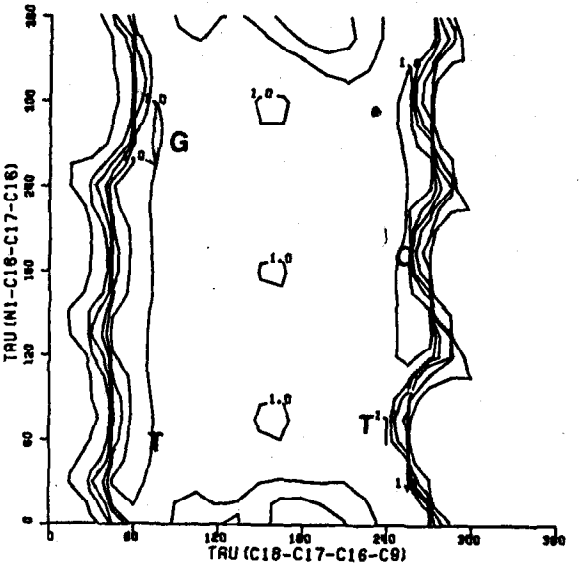



*2b. Variation of the conformational energy for molecule based on crystal structure of chlorpromasine with τ (C14-C13-N1-C12) = 210° contour levels are 1, 3, 4, 10, 20 and 100 kilocalories/mole. Unlabeled extrema are maxima. G and T indicate the location of analogues to* gauche *and* trans *dopamine conformations while C indicates the approximate crystal state conformation.*

FIGURE 3

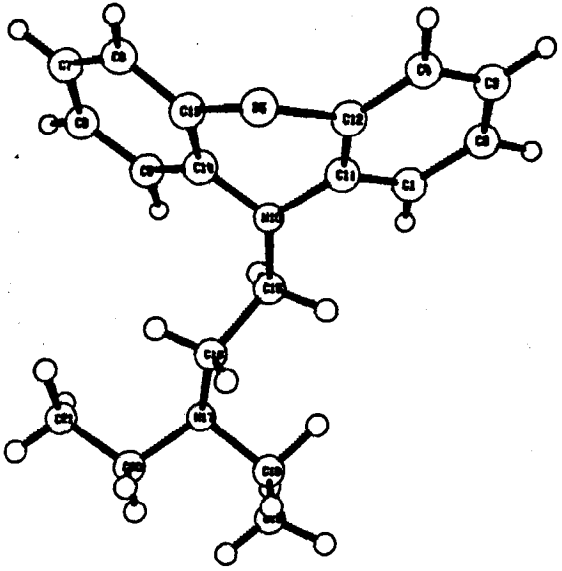


*3a. Crystal conformation of α-flupenthixol (Post et at. 1975).*

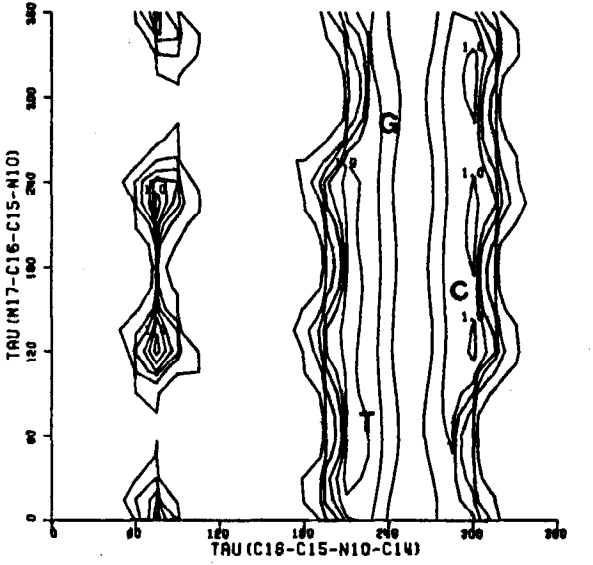



*3b. Variation of the conformational energy for molecule based on crystal structure of α-flupenthixol. Contour levels are 1, 3, 5, 10, 20, and 100 kilocalories/mole. Unlabeled extema are maxima. G and T indicated the location of analogues to gauche and trans dopamine conformation while C indicates the crystal state conformation.*

FIGURE 4



4a. Crystal conformatio of disthazine (Marsau 1971).




4b. Variation of the conformational energy for molecule based on crystal structure of diethazine. Contour levels are 1, 3, 5, 10, 20, and 100 kilocalories/moles. G and T indicate location of analogues to gauche and trans confromations of dopamine, while C indicates the crystal state conformation.

which the protonated sidechain nitrogen approaches the phenothiazine substituent, do not appear because the N17 atom cannot swing over far enough to assume that position. The two analogues that do appear should be preferred since they are close to $\tau$(N17-C16-C15-N10) = 60° and 300°, which are preferred conformations for a hydrocarbon chain, though the *gauche* analogue is about 4 kilocalories/mole over the global minimum. Since these possible analogues appear with reasonably low energies for phenothiazines like diethazine, which have very weak neuroleptic activity, it suggests that the remaining *trans* analogue of chlorpromazine, in which the sidechain nitrogen approaches the phenothiazine substituent and in which the sulfur atom takes an equivalent position to one of the dopamine oxygens, is the relevant one for antipsychotic activity. This is the same chlorpromazine conformation that was suggested previously on the basis of geometrical similarities between the crystal structures of chlorpromazine and dopamine (Horn and Snyder 1971).

In summary, a number of possible geometrical analogues to dopamine can be found in the low energy regions of chlorpromazine. Because of the uncertainties associated with conformational energy calculations, it is not possible to clearly differentiate which of these analogues is responsible for the antipsychotic activity of the neuroleptics. However, by relating low energy geometries of structurally related molecules with varying pharmacological potencies, it was possible to make a prediction as to the biologically active conformation.

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# Conformational Study of Lysergic Acid Derivatives in Relation to their Hallucinogenic and Antiserotonin Activities

Mahadevappa Kumbar

Conf'ormational analysis of five lysergates in six different solvents has been carried out by an empirical method. The energy maps are developed for all molecules in all solvents. Further, the receptor site conformations are identified for both hallucinogenic and antiserotonin activities. It appears that all the molecules do not act on the same site. Effect of solvent is examined by comparing the solvated conformation with that of vacuum and it can be concluded that this effect definitely exists, but depends on both solute and solvent. Various properties such as free energy, entropy, percent population, dipole moment, translational and rotational diffusion coefficients, and relaxation time are obtained for receptor site conformations and correlated with the observed activities. It is concluded that the mode of action in eliciting the hallucinogenic response is different from that of antiserotonin response.

## INTRODUCTION

The ergot alkaloids have always been a fascinating class of drug molecules due to their diversified effects and unusual structure activity relationships. There have been numerous investigations regarding the pharmacological actions of LSD (d-lysergic acid diethylamide) and other similar compounds. Among all the phamracological actions, the hallucinogenic (H) action has dram most of the attention (Sankar 1975). The main interest lies in understanding the mechanism of hallucinogenic and/or antiserotonin (S) actions in terms of molecular structure under existing physiological conditions. Earlier molecular orbital studies (Karreman et al. 1959; Snyder and Merrill 1965; Kang and Green 1970) have indicated a charge transfer reaction for the hallucinogenic activity of LSD (being a donor) due to high degree of correlation between the actitity and the highest occupied molecular orbital energy which was later found to be not true (Johnson et al. 1975; Kumbar and Sankar 1973; Sankar and Kumbar 1974). In recent years, however, attention has been turned to utilize the concept of "structural resemblance" in explaining the activities of various hellucinogens other than LSD. This concept has been examined by

using molecular models (Snyder 1970), x-ray crystallography (Baker et al. 1973), and molecular orbital methods (Kier 1971; Green et al. 1974; Pullman et al. 1974). All these studies, however, have been confined to congeners of LSD, but not to the derivatives of d-lysergic acid.

The quantitative values of hallucinogenic and antiserotonin effects are known for about 20 lysergates (Cerletti and Doepfner 1958) These include mono and disubstituted amides, cycle and 1, 2, and 8 substituted derivatives of LSD. In the present study, only five lysergates ( figure 1) have been selected due to their structural differences in side chains which might help to assess the role of the side chain in producing the pharmacological response. Any change in the drug molecule and in the medium in which the molecule exists can dramatically effect the active (receptor site) conformations. Therefore, these molecules are studied in different solvents varying in physiochemical properties to see how the conformational behavior changes from solvent to solvent. In the course of this action, the empirical method in conjunction with reaction field theory (Sinanoglu and Abdulnur 1965) has been used. Within the framework of reaction field theory, an approach to solvation effect involves the computation of the interaction energy of solute with its surrounding, making use of a continuum model for solvent. However, considerable doubt exists (Linder 1967) about the applicebility of this continuum model, because of its treatment of the solvent as a uniform medium inside and outside the cavity of the molecule. In reality, the dielectric constant inside and outside of the cavity may be different. Previously (Kumbar 1976), it was pointed out that the effective dielectric constant (inside the cavity) is about one half of the bulk dielectric constant (outside the cavity). With this in mind, a conformational analysis is carried out and properties such as free energy, entropy, percent population, dipole moment, translational and rotational diffusion coefficients, and relaxation times are deduced for active conformations, with the hope of obtaining a quantitative relation between structure and activity.

The translational diffusion coefficient describes how the molecule travels in a given medium, while the rotational diffusion coefficient gives an indication of how the molecule orients itself at the receptor site. When an interaction between the drug molecule and the receptor site begins, the molecule that is present in a random state in a solution near the receptor site mist come to an order state (specific orientation) at the receptor site for a proper interaction. Therefore the relaxation time may be defined as the time required for the molecule to relax itself from an ordered state into a random state. In other words, the relaxation time may be considered as the time spent by the drug molecule at the receptor site.

**METHOD OF CALCULATION**

(a) Conformational energy: The total energy of a molecule for a particular geometry under the influence of a solvent is calculated as

$$E_{total} = E_{solute} + E_{solvation} \quad (1)$$

where $E_{solute}$ is the total energy of a solute molecule in the free space approximation and $E_{solvation}$ is the energy of solute solvent interaction. $E_{solute}$ is computed as the sum of various contributions as given in the following equation:

$$E_{solute}(\phi, \psi, \tau_i) = E_{nonbonded} + E_{torsional} + E_{electrostatic} \quad (2)$$

where $\phi, \psi$, and $\tau_i$ are the dihedral angles defined in figure 1. The nonbonded interaction energy between the pair of interacting atoms is calculated by means of the Lennard-Jones 6-12 potential function, which is,

$$E_{nonbonded} = \sum_{i,j} d_{ij} r_{ij}^{-12} - e_{ij} r_{ij}^{-6} \quad (3)$$

The first term in this equation represents the repulsive forces and the second term describes the attractive forces. The term $r_{ij}$ is the distance between the ith and the jth interacting atoms, and $e_{ij}$ and $d_{ij}$ are coefficients which have been determined according to the method described previously (Scherega 1968; Kumbar 1975) The torsional potential energy about C-C single bonds and the C-N single bond is added via the following equation

$$E_{torsional} = (E^o_\phi/2)(1+\cos 3\phi) + E^o_\psi/2)(1+\cos 2\psi) + \sum_i (E^o_{\tau i}/2)(1+\cos 3\tau_i) \quad (4)$$

where $E^o_\phi$, $E^o_\psi$, and $E^o_{\tau i}$ are the energy barriers which are respectively set equal to 1.0 kcal $mole^{-1}$, 1.9 kcal $mole^{-1}$, and 0.58 kcal $mole^{-1}$ (Volkenstein 1963; Flory 1969; Scheraga 1968). In recent years various crystallographic studies (Cody et al. 1973) and theoretical investigations (Renugopalkrishnan and Rein 1976) have indicated that the peptide bond deviates from its planar structure. Besides, the assumption of the planar structure in d-lysergic acid diethylamide introduces considerable steric interaction between the side chain and the ring portion. In order to understand how the side chain behaves if this constraint is relaxed, and how this effects the orientation of -C=O bond; the peptide bond is treated as a single bond. In addition, the potential barrier is lowered to about one tenth of its true value. Therefore, the results obtained here should be used cautiously, due to hypothetical nature of the present model. The electrostatic energy is evaluated using the formula

$$E_{electrostatic} = 332\, e_i e_j / \varepsilon' r_{ij} \quad (5)$$

where $\varepsilon'$ is the effective dielectric constant that is applicable inside the cavity and $e_i$ is the charge on the ith atom, which is the sum of $\pi$ and $\sigma$ charges. The $\pi$ and $\sigma$ charges are computed respectively by the Huckel method (Pullman and Pullman 1963) and the procedure suggested by Del Re (1958).

$E_{solvation}$ energy is the sum of three contributions, namely,

$$E_{solvation} = E_{es} + E_{dis} + E_{cav} \tag{6}$$

where $E_{es}$ is the electrostatic solute solvent interaction, $E_{dis}$ is the solute solventt interaction energy due to dispersion forces, and $E_{cav}$ is the energy required to create a solvent cavity. The $E_{es}$ is computed by treating the solute as a sphere of radius a with a point dipolar ion of charge q and the total dipole moment $\mu$ positioned at the center. The solute sphere is imbeded in a cavity of identical size, making the dielectric constant of the medium inside and outside the cavity different. According to Onsager's reaction field theory (Onsager 1936), the electrostatic energy is given by

$$E_{es} = -(\mu^2 L\, \bar{a}^3)(1- \bar{\alpha}\, \bar{a}^3/L)^{-1} \tag{7}$$

$$\text{with } L = 2(\varepsilon-1)/(2\varepsilon+1) \tag{8}$$

where $\varepsilon$ is the bulk dielectric constant of the solvent outside the cavity and $\alpha$ is the average polarizability of the medium, which is $\alpha = 1/3\ (\alpha_{11} + 2\alpha_{\perp})$. The solute-solvent interaction energy due to dispersion forces is

$$E_{dis} = \frac{4\pi\rho}{2} \int_0^{\infty} V_{AB}^{eff}(r)\, g^2(r)\, r^2 dr \tag{9}$$

where $\rho$ is the number density of the solvent, $g^{(2)}(r)$ is the radial distribution function for solvent molecule about a central solute, whlch is taken as zero for r<a and unity for r>a. The effective pair potential between a solute A and a solvent B at a separation r is

$$V_{AB}^{eff} = V_{AB}(r)\, B'_{AB}(r) \tag{10}$$

where $V_{AB}(r)$ and $B'_{AB}(r)$ are pairwise interaction potentials for a molecule in a gas phase and a liquid phase correction factor (Sinanoglu 1967) respectively. The $V_{AB}(r)$ is taken in the form of a Kihara potential (Kihara 1953), assuming each molecule as a spherical core of diameter 1. This potential function is

$$V_{AB} = C_{AB}\,(\sigma_{AB}^{6}\ P_{AB}^{-12} - P_{AB}^{-6}) \tag{11}$$

In the above equation, $C_{AB}$ is the London dispersion coefficient, $P_{AB}$ is the intercore distance, and $\sigma_{AB}$ is the collision diameter.

The intercore distance is given by

$$P_{AB} = r_{AB} - l_{AB} \tag{12}$$

with $l_{AB} = \frac{1}{2}(l_A + l_B)$

and $\sigma_{AB} = \frac{1}{2}(\sigma_A + \sigma_B)$

These core size parameters are evaluated from the semiempirical relations

$$l_A = a\,\beta^{-1}(3.24 + 7\omega_A)^{-1} \tag{15}$$

$$\sigma_A = 2^{-1/6} a\,\beta^{-1}\,(2.24 + 7\omega_A)(3.24 + \tag{16}$$

where $w_A$ is Pitzer's "accentric factor" (Dannon and Pizer 1962), $\beta \approx 1.15$, and a is an effective solute cavity radius which is taken as (Amos and Burrows 1973)

$$a^3 = r'^3_A\,(1+r')^4\,(\pi r')^{-1};\ r' = r'_A/r'_B \tag{17}$$

The $r_A$ is generated from the radius of gyration for a particular conformation and $r_B$ is determined from the critical densities of solvents. The liquid phase correction factor for the gas phase potential in equation (10) (Sinanoglu 1967) is

$$B'_{AB} = 1 - \frac{1}{2}\,\frac{\Delta'_{AB}\,D'_B\,L'_{AB}}{1 - \{\sigma_{AB}/(r - l_{AB})\}^6} \tag{18}$$

where $\Delta_{AB}$ is a function of ionization energies $I_A$ and $I_B$, as shown in the following equations:

$$\Delta'_{AB} = (I_A + 2I_B)/\{2(I_A + I_B)\} \tag{19}$$

and $D'_B = D_B/(1 + D_B)$ (20)

with $D_B = (n_B^2 - 1)/(n_B^2 + 2)$ (21)

where $n_B$ is the refractive index of the solvent. The London dispersion coefficient $C_{AB}$ is set equal to

$$C_{AB} = (3/2)(1.35)\,\bar{\alpha}_A\bar{\alpha}_B\,I_A I_B/(I_A + I_B) \tag{22}$$

Finally, the energy required to create a solvent cavity to accomodate a spherical solute of volume v is expressed as

$$E_{cav} = M\,v_A^{2/3}\,(1 + N\,v_A^{-2/3}) \tag{23}$$

$$\text{with } M = 6.96 \times 10^{-3} \gamma_B \left(1 - \frac{\partial \ln \gamma_B}{\partial \ln T} - \frac{2T\tau}{3}\right) \quad (24)$$

$$\text{and } N = v_B^{2/3}(\kappa_B(1)-1) \quad (25)$$

where $k_B$ is the constant dependent of the volume fraction, $v_B/v_A$, T is the thermal expansion of the solvent, $\gamma_B$ is the surface tension of the solvent, and T is the absolute temperature of the system which is set equal to 310°, a physiological temperature.

(b) Conformational properties: The Helmnoltz free energy is calculated by

$$A = E - TS \quad (26)$$

where E is the conformational energy in a particular solvent, and S is the entropy, which is computed by the following set of equations:

$$S = R \ln z + \langle E \rangle / T \quad (27)$$

$$\langle E \rangle = z^{-1} \Sigma_i E_i \exp(-E_i/RT) \quad (28)$$

$$\text{and } z = \Sigma \exp(-E_i/RT) \quad (29)$$

The dipole moment, on the other hand, is predicted by using the equation, $\mu q = \Sigma q_i r_i$, where $q_i$ is the charge on the ith atom and $r_i$ is the distance of the ith atom from the origin of the chosen reference coordinate system. The translation and rotational diffusion coefficients are obtained from the well known equations applicable to spherical molecules in an unbounded fluid of viscosity $\eta$. These equations are

$$D_t = kT/6\pi\eta r \quad \text{and} \quad D_r = kT/8\pi\eta r \quad (30)$$

Here, k is the Boltzmann constant. Further, the rotational diffusion coefficient is related to the relaxation time ($\tau$) through the equation, $\tau = 1/2\ D_r$. The percent population of the nth minimum is estimated according to the equation:

$$Pn = \Sigma_i \exp(-E_i^n/RT) / \Sigma\Sigma_{ni} \exp(-E_i^n/RT) \quad (31)$$

where $E_i^n$ is the energy of the ith conformation in the vicinity of the nth minimum. The most probable properties associated with a given energy valley are determined by

$$\langle p \rangle = z^{-1} \Sigma P_i \exp(-E_i/RT) \quad (32)$$

In addition, the most probable properties averaged over various solvents are computed through the following equation:

$$\langle\langle p \rangle\rangle = z'^{-1} \Sigma \Sigma P_i \exp(-E_i/RT) \quad (33)$$

with $z' = \Sigma \Sigma \exp(-E_i/RT)$. (34)

**RESULTS AND DISCUSSION**

Energy maps were obtained for all five molecules, (figure 1) in six diffferent solvents: heptane, carbon tetrachloride, aniline, ethanol, methanol, and water which have a wide range of dielectric constants. The LSD molecule has a bulky side chain with six degrees of freedom. It is difficult (see figure 1) to rotate all six dihedral angles simultaneously, due to computer time. Hence, a different approach was taken. Firstly, the angles $\tau_3$ and $\tau_4$ in all the molecules were fixed at 60° in a staggered position with the preceeding group (minimum interactions). Secondly, the angles $\tau_1$ and $\tau_2$ were rotated in 10° interval to determine the most stable values by considering only the interactions in the side chain. The energy maps produced as a result of rotations of $\tau_1$ and $\tau_2$ contained two minima centered around $\tau_1 = \tau_2 = 90°$, and $\tau_1 = 70°$ and $\tau_2 = 250°$. The minimum energy conformations may not represent the energy valley in which they exist. Therefore, most probable conformations were determined as indicated in equation (32). It happened that the most probable values did not deviate considerably from their respective minimum values. So these values were used in further computations in LSD and dimethylamide The same strategy was also adopted for ethylamide and found that $\tau_2$ had three different most probable values, namely 60°, 90°, and 180°. Similarly, the most probable value of $\tau_2$ in methylamide was determined to be 180°. Using these values, the rotations around $\phi$ and $\psi$ were carried out in 10° increment in various solvents mentioned above. The most probable conformations for each molecule in all six solvents are shown in figures 2(a) to 2(e).

**Effect of solvation**

In order to understand the influence of the solvent medium or to gain some information about the conformational behavior in presence of external forces (solvent medium), the vacuum (gaseous phase) conformations were also generated by setting $\varepsilon' = 1.0$ in $E_{solute}$ equation (2). The most probable conformations are also indicated in figures 2(a) to 2(e). These vacuum conformations may be considered as the most ideal situation conformations (ideal conformations). Some of the properties of ideal and solvated conformations are compared in figure 3. It is appparent that the solvated conformation is quite different from an ideal conformation. The degree of influence of the solvent medium depends on the nature of the solute as well as the nature of the solvent. In all cases, the conformational energies of ideal conformations are lower than the corresponding solvated conformations. The conformational entropy decreases when molecule is solvated, due to a loss of conformational freedom. A further decrease in entropy is observed when the dielectric constant of the medium is increased. The dipole moment is higher when the molecule is solvated except for dimethylamide, where it is lower. This property

slightly varies as the dielectric constant of the medium changes. The volumes of all the molecules except dimethylamide are smaller in a given solvent and also to some extent depend on the dielectric constant of the medium. Therefore, it is clear that the solvent definitely influences the conformational behavior of the solute, depending upon the nature of the solute as well as that of the solvent.

**Solvated conformations**

For a given molecule, number of minima appearing in energy maps depends on the nature of the solvent. As the dielectric constant of the medium increases, some of the minima disappear due to an increased interaction between the solute and the solvent that favors certain conformational regions. As a result, the conformational entropy decreases. Even though the most probable values of the dihedral angles $\phi$ and $\psi$ have altered to some extent from solvent to solvent, they have grouped together into a small region as shown by the circles in figures 2(a) to 2(e). This behavior is observed in spite of the fact that $\tau_1$ and $\tau_2$ have been set at different values. This might be an indication that these subsidiary angles have less influence on the main two dihedral angles, $\phi$ and $\psi$. On the whole, the LSD has eight conformational regions, dimethylamide has four, ethylamide has four, methylamide has two, and an amide has four. In reality, the LSD should have only 4 conformational regions, the dimethylamide should have only 2, and the amide should have only 2 due to the symmetry nature of the side chain over the dihedral angle $\psi$. The asymmetry over the angle $\psi$ is due to fixing of $\tau_1$ and $\tau_2$ at certain values when $\phi$ and $\psi$ were rotated.

Free energy, entropy, dipole moment, percent population, translational and rotational diffusion coefficients, and relaxation times are important aspects of drug receptor interactions. All these properties truly depend on the nature of the solvent medium. Some of these properties of each molecule as a whole, without differentiating various minima, are considered in figure 3. It is obvious that these properties are functions of the nature of the medium and the molecule. For a given property, all molecules except dimethylamide have behavior similar to the dielectric constant of the medium increases. These changes inproperties point out that the solvent medium that exists around the receptor site(s) may play an important role. The effect of the solvent depends on the nature of the energy valley. If the valley is deeper, for example, IV(VIII) in LSD, the effect is smaller. On the other hand, if the valley is shallow like I or III or IV in ethylamide, the effect is greater. The shallowness, however, is dictated by the conformational flexibility. It means that a molecule with a considerable flexibility might experience a greater effect of the solvent.

**Receptor site conformations**

Among the five molecules studied here, the LSD is highly

potent in terms of hallucinogenic and antiserotonin activities. The magnitudes of these activities are also shown in figure 1. The differences in the activities of these molecules may be attributed to differences in behaviors of side chains. The structural differences, together with differences in various physiochemical factors, might account for the variability of their activities. Therefore, it is essential to identify those conformations which are believed to be present at the receptor site(s). This is, of course, a diffcult task. To get around this problem, the following approach is taken. Since the LSD possesses the maximum activities (H=100 and S=100), it is assumed that all four conformations (figure 2(a)) are involved in producing these activities. However, it is difficult to assess their degree of contribution towards the total activity. When a drug molecule binds to the receptor site, the molecular conformation might be complementary to the receptor site(s). On this basis, it is assumed that the LSD binds to four different sites corresponding to four sterically different conformations labelled as I to VIII in figure 2(a). Region III(VII) appears only in high dielectric constant medium as a result of dissappearce of region II(VI). It means that there are only three sites in low dielectric medium and three in high dielectric medium. If there is a continous fluctuation in dielectric constant, or if the receptor site region exists in an inhomogeneous medium, then all four sites might be activated simultaneously as shown below:

| site | I(V) | II(VI) | III(VII) | IV(VIII) |
|---|---|---|---|---|
| low ε | O | O | | O |
| high ε | O | | O | O |
| fluctuation in ε | O | O | O | O |

In order to obtain the receptor site conformations for other molecules, the conformational regions of each molecule are compared with those of the LSD in figures 4(a) to 4(d). It is clear that some of the regions lie close to those of the LSD. Thus it appears that there exists a conformational resemblance between LSD and other molecules. Therefore, it is conceivable to assume that these molecules ellcit their pharmcological response by resembling the conformations of LSD. However, in methylamide the resemblance is found only for regions II and IV, and in amide only for I and IV. In addition, all molecules do not act on the same site (table I). To have some kind of overall representation of each region shown in figure 2(a) to 2(e), a further averaging over all solvents is carried out end resulting diherdal angles, together with various properties, are tabulated in table II. These overall conformations are presented in figures 5(a) to 5(e).

A close look at the LSD molecule reveals that it contains various centers for interactions as schematically shown in figure 6. Various intracenter distances for all the overall conformations as are listed in table III. These distances might be complementary

to the interacting centers existing in receptor site(s). Since the ring portion of the LSD is common to all molecules, the interactions in this portion are retained in all molecules. Therefore, the variation in antiserotonin and hallucinogenic activities might be explained in terms of the variability that exists in side chain interactions such as the hydrogen bonding, hydrophobicity, free energy, etc. The hydrophobicities for these molecules have been calculated by Dunn and Bederka (1974) which are 2.16, 1.16, 1.16, 0.66 and 0.16 respectively for LSD, dimethylamide, ethylamide, methylamide, and amide. This property decreases as the activities decrease. In addition to hydrophobicity, other factors might also influence the drug receptor interaction. We have looked into the possibility of hydrogen bonding by the carbonyl oxygen of the side chain and the receptor site. Since the LSD molecule has the maximum activities among the molecules considered here, it is assumed that the oxygen atom in all the receptor site conformations (I . . . IV, table II) of LSD is situated in an optimum position. The deviations of this atom from the optimum positions (measured in terms of the angle, $\theta$, figure 6) for only receptor site conformations (shown in figures 4(a) to 4(e)) are also tabulated in table III. This angle increases as activities decrease, suggesting that the strength of hydrogen bonding is also an important factor. This strength decreases as activities decrease. Further, it is possible to distinguish the conformations responsible for hallucinogenic and antiserotonin activities using the angle $\theta$. The antiserotonin activity is always higher than the hallucinogenic activity for a given molecule, which might be due to a close congruity between the molecule and the receptor site(s). It means that a stronger hydrogen bonding is necessary for antiserotonin activity compared to hallucinogenic activity. Thus, the conformations with smaller $\theta$ values might be responsible for antiserotonin activity while larger $\theta$ for the hallucinogenic activity. This distinction is noted in table III by the letters S and H. This is further supported by a good correlation obtained between the $\theta$ and the differences in activities (table IV).

In order to investigate the role of other properties in drug receptor interaction, a correlation is also carried out between these properties and the activities. From the correlation coefficient, few conclusions may be drawn. Entropy and percent population do not seem to play any role. On the contrary, the free energy, rotational diffusion (relaxation times), hydrophobicity, and the strength of the hydrogen bonding appear to be an important determinants. The dipole moment and translational diffusion coefficients to sane degree are also important factors only in hallucinogenic activity. The amide has no hallucinogenic activity. Therefore, we assume that the conformations falling in region II(IV) are responsible (figure 2(e)) for this activity. Hence, the properties of this region are used in correlating the hallucinogenic activity. From the correlation coefficients, one can see that even among the correlated properties, the degree of correlation is not the same for both activities. Besides, the dipole moment is better correlated in hallucinogenic activity. Therefore, it is concluded that the mode of action in eliciting the hallucinogenic action is quite different from that of antiserotonin activity.

**TABLE 1**

Receptor site conformations.
The Roman numerals indicate the conformational regions shown in figures 2(a) to 2(e) and 4(a) to 4(d)

| Molecule | LSD Sites | | | |
|---|---|---|---|---|
| | I(V) | II(VI) | III(VII) | IV(VIII) |
| d-lysergic acid dimethylamide | | | | II(IV) |
| d-lysergic acid ethylamide | I | III | II | IV |
| d-lysergic acid methylamide | | | I | II |
| d-lysergic acid amide | | I(III) | | II(IV) |

TABLE II

Over all conformations (averaged over all solvents) with their various properties

| Site | $\langle\langle\phi\rangle\rangle$ deg | $\langle\langle\psi\rangle\rangle$ deg | $\langle\langle A\rangle\rangle$ kcal mole$^{-1}$ | $\langle S\rangle$ cal deg$^{-1}$ mole | $\langle\langle v\rangle\rangle$ A$^{o3}$ | $\langle\langle\mu\rangle\rangle$ D | %P | $\langle\langle D_t\rangle\rangle \times 10^{5}$ cm$^2$ sec$^{-1}$ | $\langle\langle D_r\rangle\rangle \times 10^{-9}$ sec$^{-1}$ | $\langle\langle\tau\rangle\rangle \times 10^{11}$ sec |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | LSD | | | | | |
| I | 140 | 82 | -11.651 | 4.922 | 292 | 9.450 | 66 | 1.421 | 6.287 | 8.009 |
| II | 201 | 94 | -10.627 | 4.635 | 263 | 9.062 | 13 | 1.459 | 6.927 | 7.348 |
| III | 257 | 60 | -6.446 | 2.808 | 233 | 10.460 | 1 | 1.004 | 5.165 | 10.200 |
| IV | 333 | 86 | -8.270 | 2.393 | 252 | 9.316 | 1 | 1.448 | 7.076 | 7.379 |
| V | 127 | 260 | -9.078 | 6.193 | 315 | 5.669 | 1 | 1.389 | 5.847 | 8.584 |
| VI | 201 | 274 | -10.627 | 4.635 | 263 | 9.062 | 13 | 1.459 | 6.927 | 7.348 |
| VII | 268 | 257 | -9.852 | 4.832 | 258 | 11.938 | 4 | 1.043 | 5.013 | 10.009 |
| VIII | 333 | 266 | -8.270 | 2.393 | 252 | 9.316 | 1 | 1.448 | 7.076 | 7.379 |
| | | | | | dimethylamide | | | | | |
| I | 62 | 85 | -17.739 | 8.235 | 263 | 5.554 | 35 | 1.479 | 7.021 | 7.125 |
| II | 326 | 99 | -17.300 | 6.198 | 220 | 12.606 | 17 | 1.570 | 8.395 | 5.957 |
| III | 60 | 265 | -17.735 | 8.051 | 262 | 5.678 | 35 | 1.481 | 7.046 | 7.100 |
| IV | 330 | 281 | -17.151 | 5.876 | 222 | 12.335 | 13 | 1.565 | 8.320 | 6.012 |
| | | | | | ethylamide | | | | | |
| I | 149 | 81 | -39.379 | 6.340 | 256 | 5.850 | 2 | 1.494 | 7.237 | 6.923 |
| II | 259 | 77 | -41.295 | 6.327 | 213 | 7.822 | 31 | 1.587 | 8.669 | 5.769 |
| III | 182 | 279 | -41.314 | 5.998 | 243 | 8.854 | 37 | 1.519 | 7.602 | 6.585 |
| IV | 317 | 273 | -41.082 | 5.576 | 208 | 7.398 | 30 | 1.600 | 8.896 | 5.629 |

**Table II.** Continued

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | methylamide | | | | |
| I | 275 | 87 | -47.262 | 7.019 | 196 | 13.714 | 32 | 1.632 | 9.424 | 5.306 |
| II | 307 | 275 | -47.733 | 6.917 | 193 | 14.027 | 68 | 1.640 | 9.570 | 5.225 |
| | | | | | | amide | | | | |
| I | 170 | 94 | -45.651 | 9.507 | 190 | 7.102 | 39 | 1.649 | 9.722 | 5.143 |
| II | 289 | 91 | -44.839 | 8.930 | 181 | 8.220 | 11 | 1.675 | 10.205 | 4.900 |
| III | 170 | 274 | -45.051 | 9.507 | 190 | 7.102 | 39 | 1.049 | 9.722 | 5.143 |
| IV | 289 | 271 | -44.839 | 8.930 | 181 | 8.220 | 11 | 1.675 | 10.205 | 4.900 |

TABLE III

Various intracenter distances and the angle θ (see Fig. 6) for various sites.

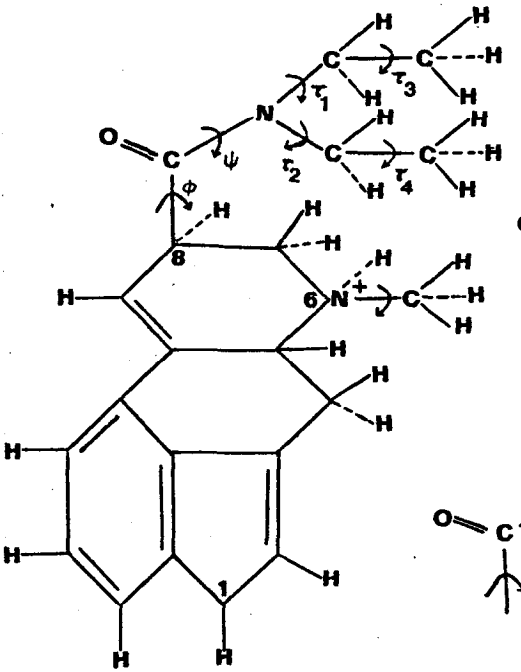
| Site | b $A^o$ | c $A^o$ | d $A^o$ | θ deg | Site | b $A^o$ | c $A^o$ | d $A^o$ | θ deg |
|---|---|---|---|---|---|---|---|---|---|
| | | | LSD | | | | ethylamide | | |
| I | 4.215 | 3.145 | 5.681 | | I | 4.193 | 2.813 | 5.487 | 8(S) |
| II | 4.236 | 3.173 | 5.580 | | II | 4.531 | 2.789 | 4.785 | 5(S) |
| III | 4.519 | 2.985 | 4.618 | | III | 4.187 | 2.812 | 5.505 | 16(H) |
| IV | 4.830 | 3.174 | 4.663 | | IV | 4.798 | 2.846 | 4.563 | 14(H) |
| V | 4.259 | 3.130 | 5.587 | | | | | | |
| VI | 4.236 | 3.174 | 5.580 | | | | methylamide | | |
| VII | 4.583 | 3.107 | 4.700 | | | | | | |
| VIII | 4.830 | 3.174 | 4.663 | | I | 4.621 | 2.846 | 4.670 | 16(S) |
| | | | | | II | 4.766 | 2.834 | 4.599 | 22(H) |
| | | dimethyl | | | | | amide | | |
| I | 4.605 | 3.158 | 4.925 | | I | 4.176 | 2.840 | 5.502 | 27(S) |
| II | 4.818 | 3.136 | 4.834 | 6(H) | II | 4.692 | 2.858 | 4.614 | 38(H) |
| III | 4.616 | 3.165 | 4.912 | | III | 4.176 | 2.840 | 5.502 | 27(S) |
| IV | 4.826 | 3.121 | 4.751 | 3(S) | IV | 4.692 | 2.858 | 4.614 | 38(H) |

TABLE IV

Summary of the properties of the receptor site conformations for both activities along with correlation coefficients

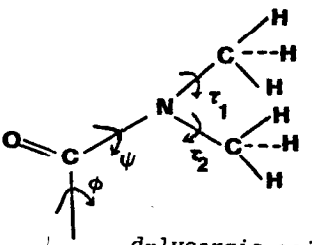
| Molecule | ΔA kcal mole$^{-1}$ | ΔS kcal mole$^{-1}$ deg | $\|\Delta\mu\|$ D | $\|\Delta D_t\| \times 10^5$ cm$^2$ sec$^{-1}$ | $\|\Delta D_r\| \times 10^{-9}$ sec$^{-1}$ | Δ Hydrophobicity | $\|\Delta\%P\|$ | θ deg | Δ Activity |
|---|---|---|---|---|---|---|---|---|---|
| | | | | antiserotonin activity | | | | | |
| dimethylamide | 8.881 | -3.483 | 3.019 | 0.119 | 1.244 | 1.0 | 12.67 | 3 | 76.80 |
| ethylamide | 27.728 | -1.418 | 3.600 | 0.073 | 0.950 | 1.0 | 66.74 | 8 | 88.10 |
| | 34.849 | -3.519 | 2.638 | 0.583 | 3.504 | 1.0 | 30.98 | 5 | 88.10 |
| methylamide | 40.816 | -4.20 | 3.254 | 0.628 | 4.259 | 1.50 | 31.98 | 16 | 93.70 |
| amide | 35.024 | -4.872 | 1.960 | 0.190 | 2.795 | 2.0 | 26.10 | 27 | 95.70 |
| corr. coeff. | 0.93 | -0.38 | -0.35 | 0.38 | 0.62 | 0.75 | 0.29 | 0.82 | |
| | | | | hallucinogenic activity | | | | | |
| dimethylamide | 9.03 | -3.805 | 3.29 | 0.122 | 1.319 | 1.0 | 16.68 | 6 | 90 |
| ethylamide | 30.687 | -1.363 | 0.208 | 0.06 | 0.675 | 1.0 | 24.10 | 16 | 96 |
| | 32.862 | -3.189 | 1.918 | 0.152 | 1.820 | 1.0 | 29.67 | 14 | 96 |
| amide | 36.569 | -6.537 | 1.098 | 0.227 | 3.129 | 2.0 | 10.0 | 38 | 100 |
| corr. coeff. | 0.96 | -0.41 | -0.75 | 0.53 | 0.63 | 0.73 | -0.19 | 0.90 | |

FIGURE 1



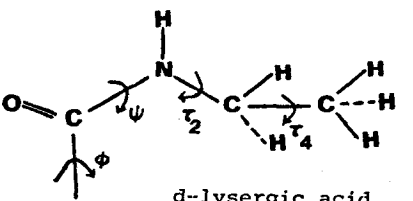
d-lysergic acid diethylamide

H=100 S=100



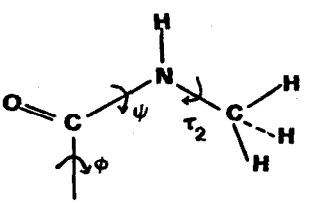
d-lysergic acid dimethylamide

H=10 S=23.2



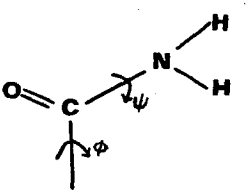
d-lysergic acid ethylamide

H=3.4,5 S=12



d-lysergic acid methylamide

H= - S=6.3



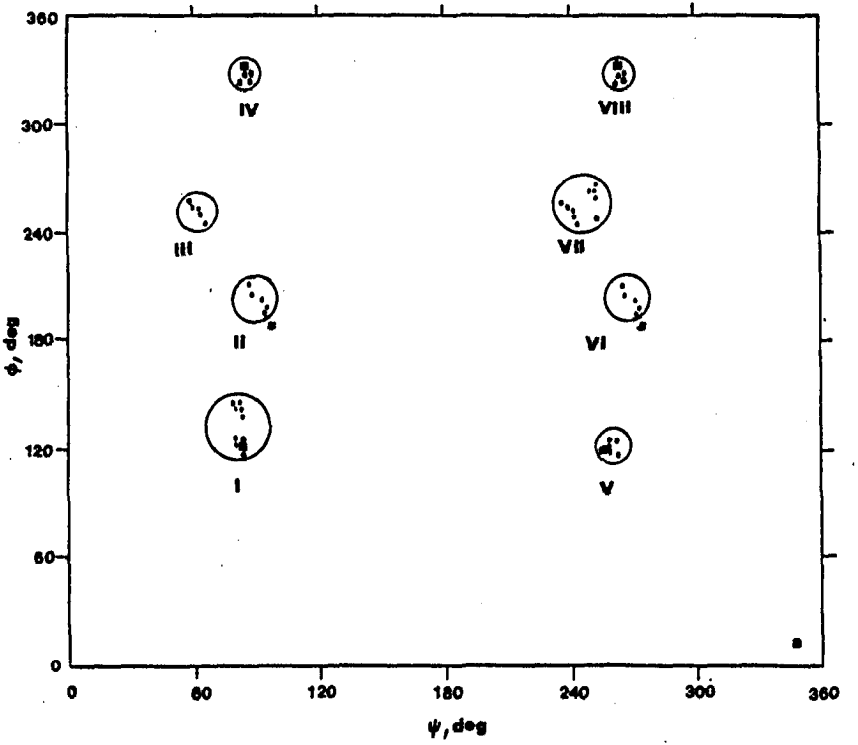
d-lysergic acid amide

H= 0 S=4.3

*Definition of dihedral angles.* $\phi$=0 *when* $C_7$-$C_8$ *bond is in planar cis position with* C-N *bond,* $\psi$=0 *when* $C_8$-C *bond is in planar cis position with N-C bond.* $\tau_1$ ($\tau_2$) =0 *when* C-N *bond is in planar cis position with* C-C *bond,* $\tau_3$ ($\tau_4$)=0 *when* N-C *bond is in planar cis position with* C-H *bond. the calculations were performed for* $\tau_3 = \tau_4 = \omega = 60^\circ$. *The magnitude of the hallucinogenic* (H) *and antisertonin* (S) *activities is also indicated.*

**FIGURE 2**

*(a) d-lysergic acid disthylamide (*)*




*The most probable conformations in all six solvents. Each minimum is identified by Roman numeral. The vacuum conformation is shown by: (a) d-lysergic acid diethylamide, (b) d-lysergic acid dimethylamide (c) d-lysergic acid ethylamide (d) d-lysergic acid methylamide, and (e) d-lysergic acid amide.*

**FIGURE 2**

*(b) d-lysergic acid dimethylamide (•)*

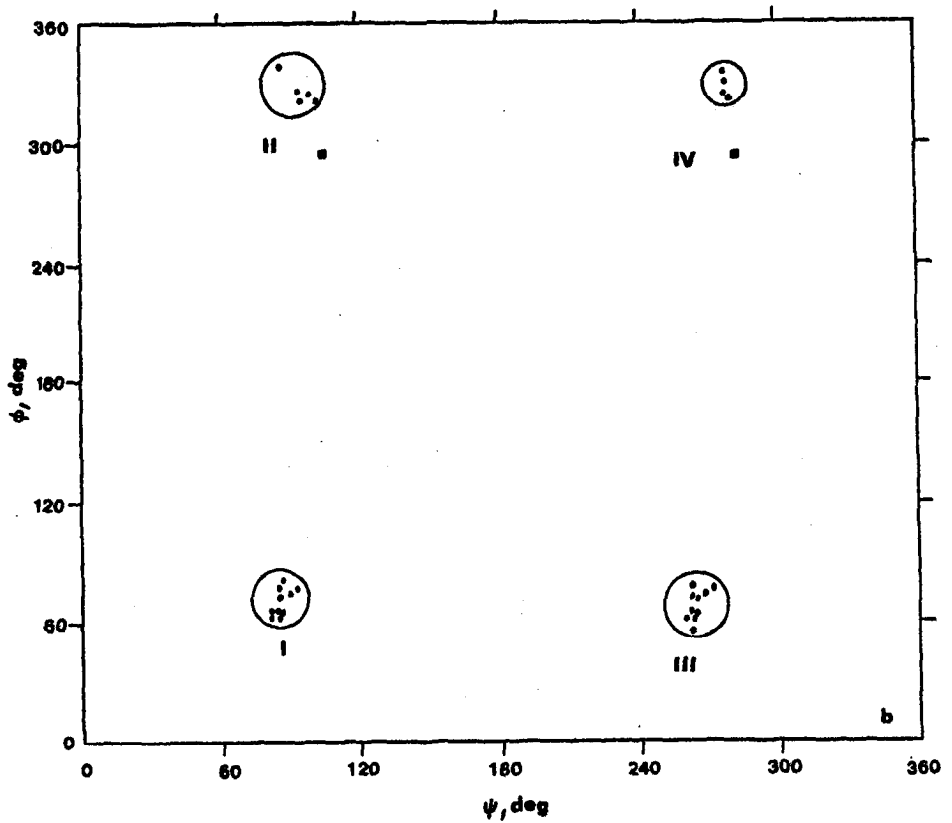


**FIGURE 2**

*(c) d-lysergic acid ethylamide* (•)

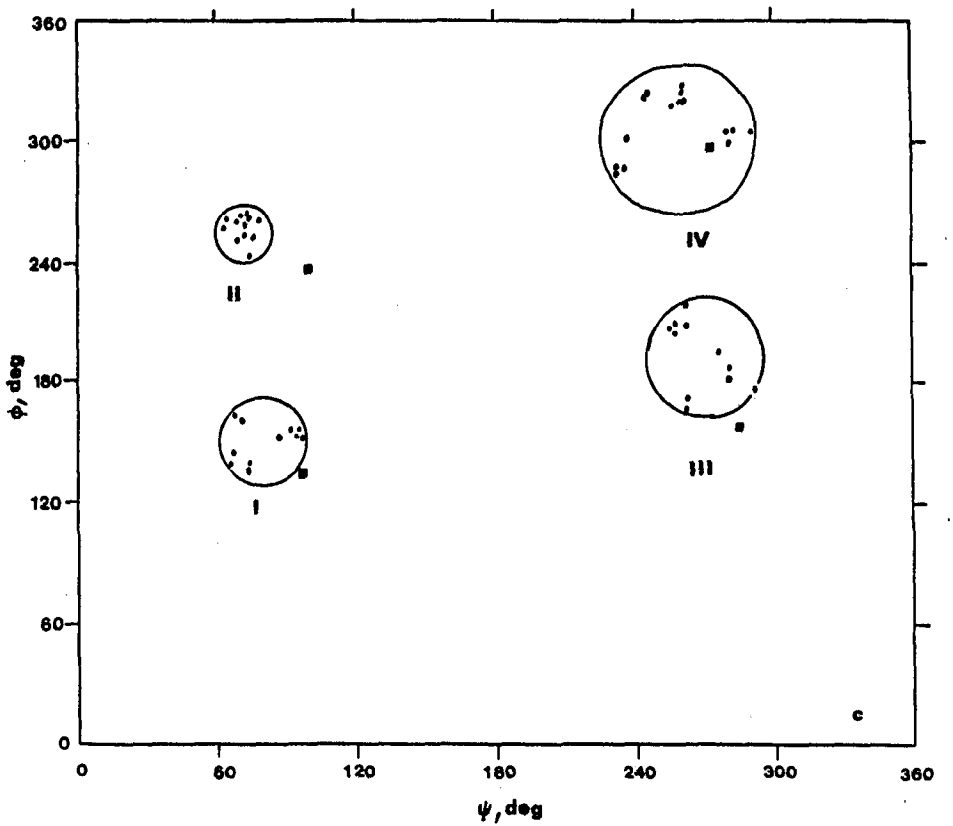


FIGURE 2

*(d) d-lysergic acid methylamide* (•)

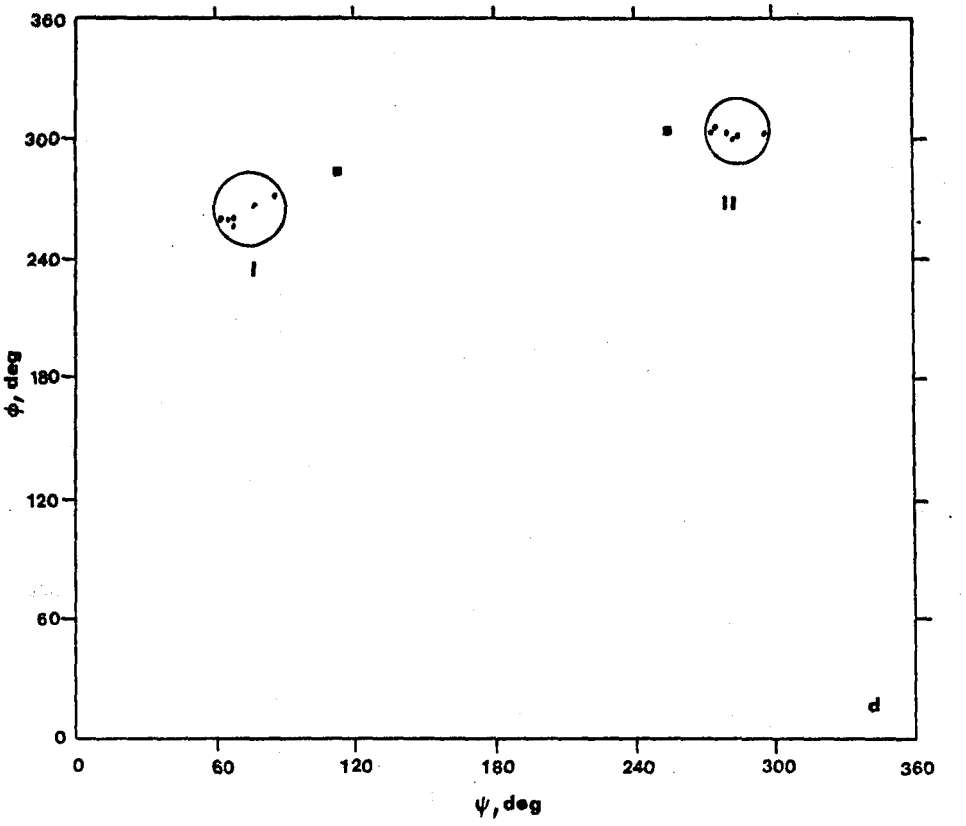


**FIGURE 2**

*(e) d-lysergic acid amide* (•)

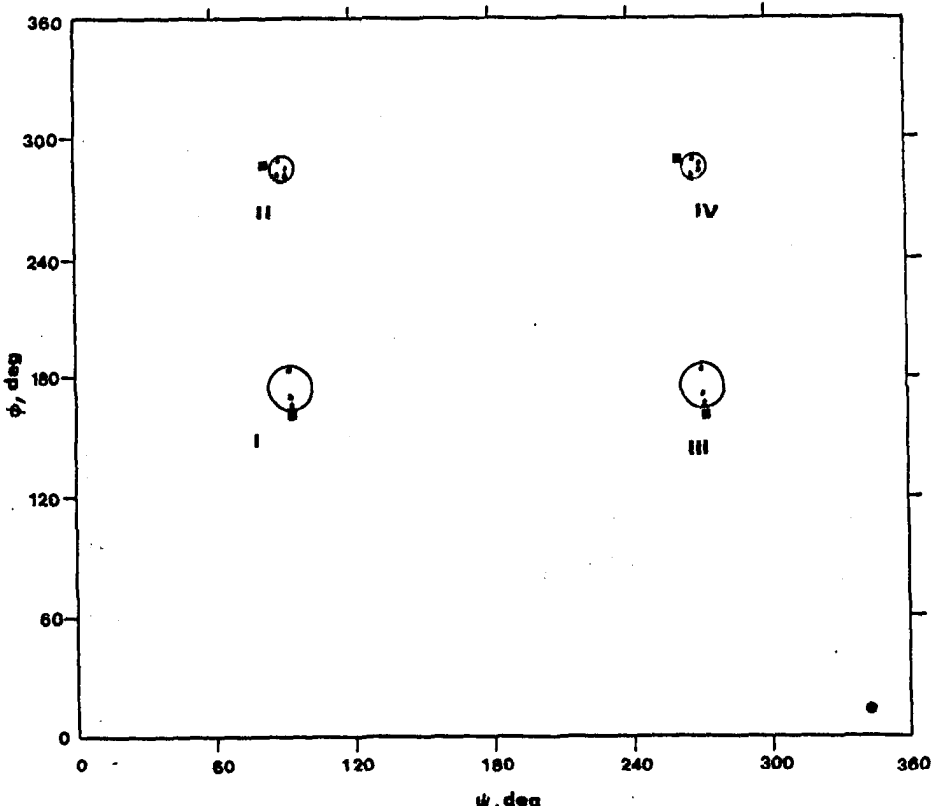
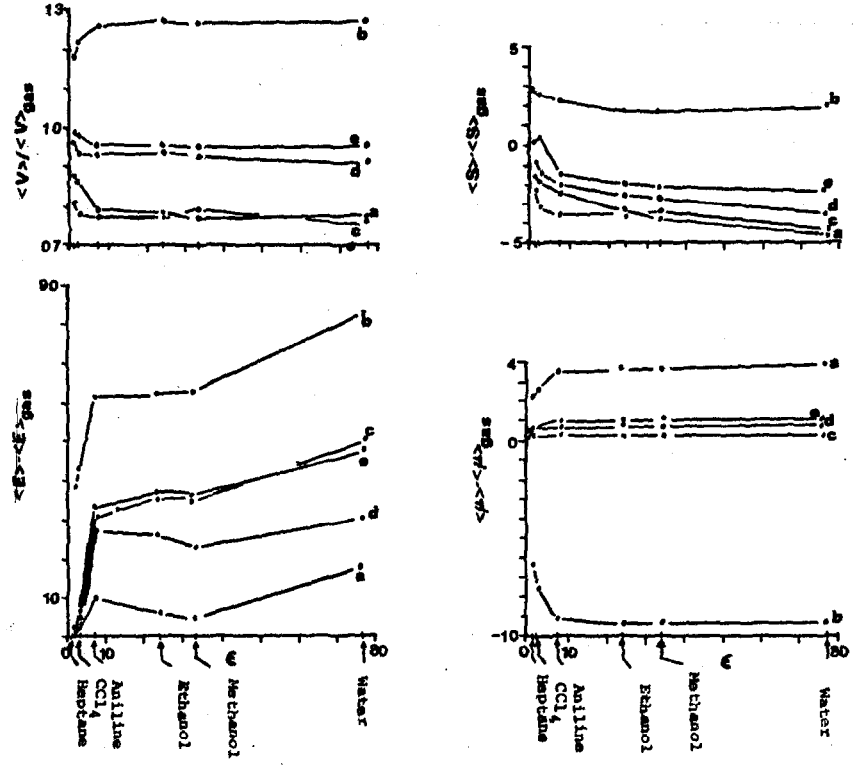


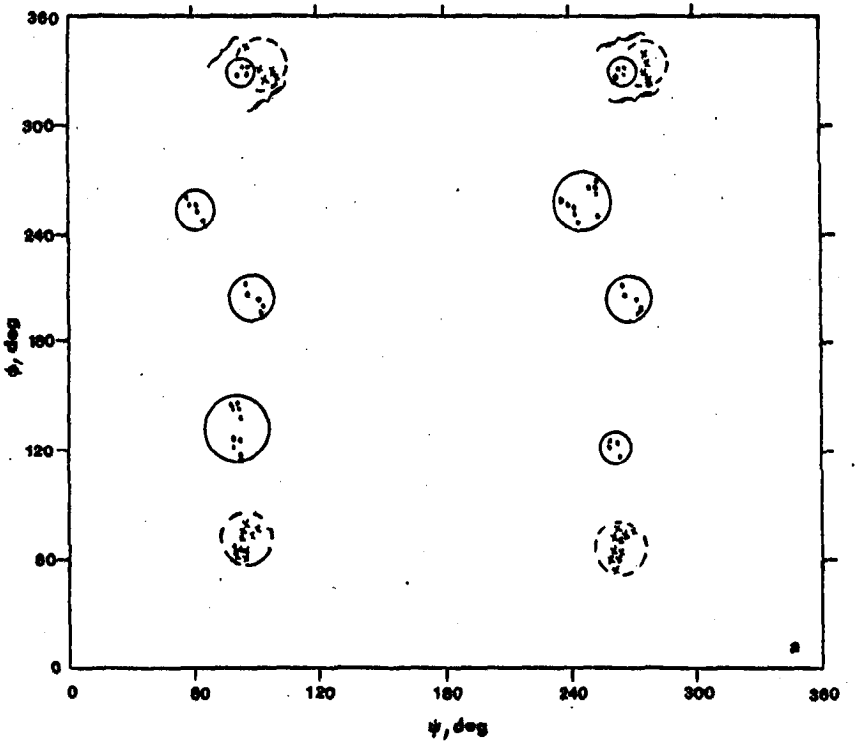
FIGURE 3




*Comparison of various properties in various solvents relative to the vacuum conformations: (a) LSD, (b) dimethylamide, (c) ethylamide, (d) methylamide, and (e) amide.*

**FIGURE 4**

*(a) LSD (*) and dimethylamide (x)*




*Comparison of various most probable conformational regions of a given molecule with those of LSD. .- LSD x - a given molecule: (a) LSD and dimethylamide, (b) LSD and ethylamide, (c) LSD and methylamide, and (d) LSD and amide.*

**FIGURE 4**

*(b) LSD (•) and ethylamide (x)*

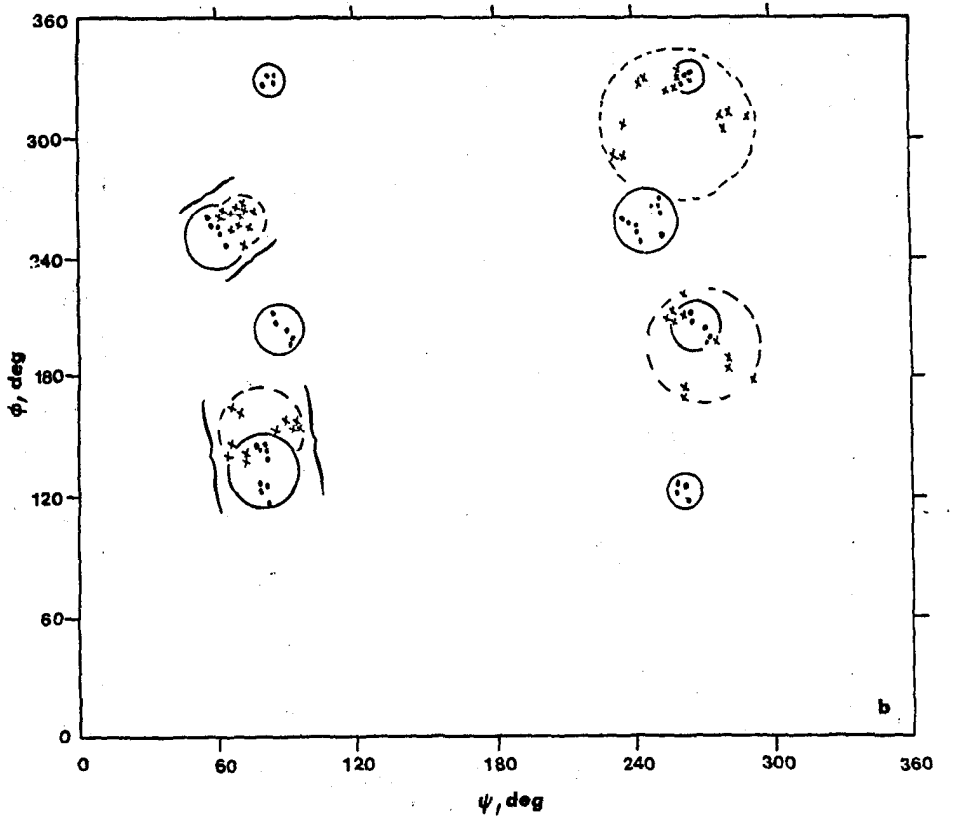


**FIGURE 4**

*(c) LSD (•) and methylamide (x)*

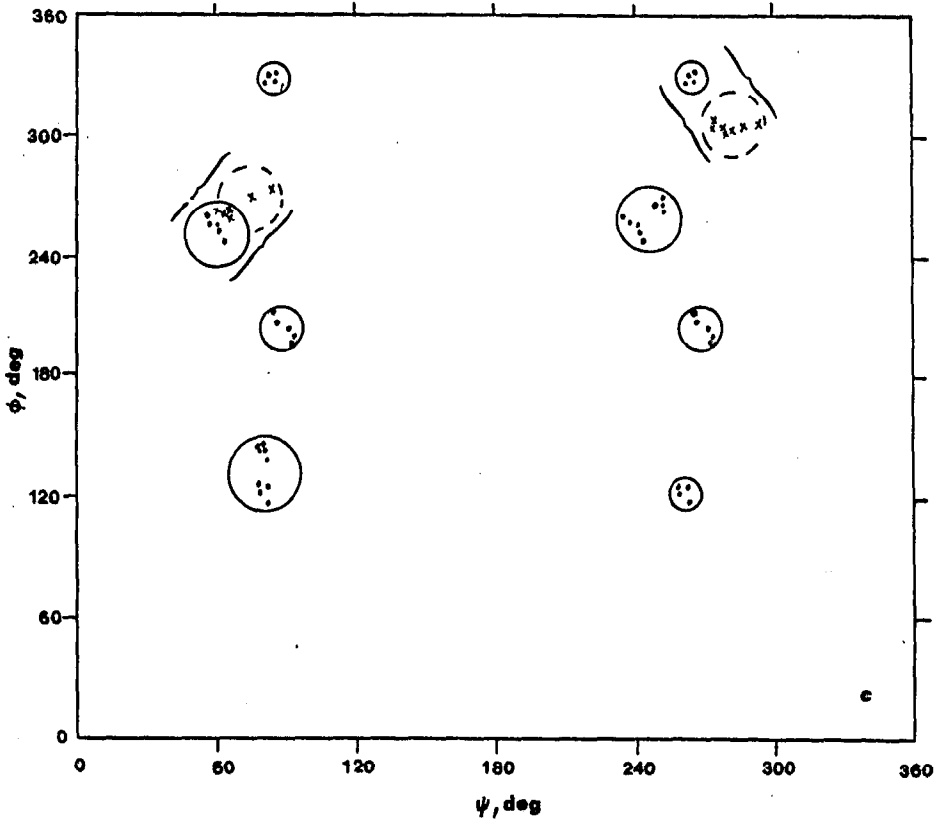


**FIGURE 4**

*(d) LSD (•) and amide (x)*

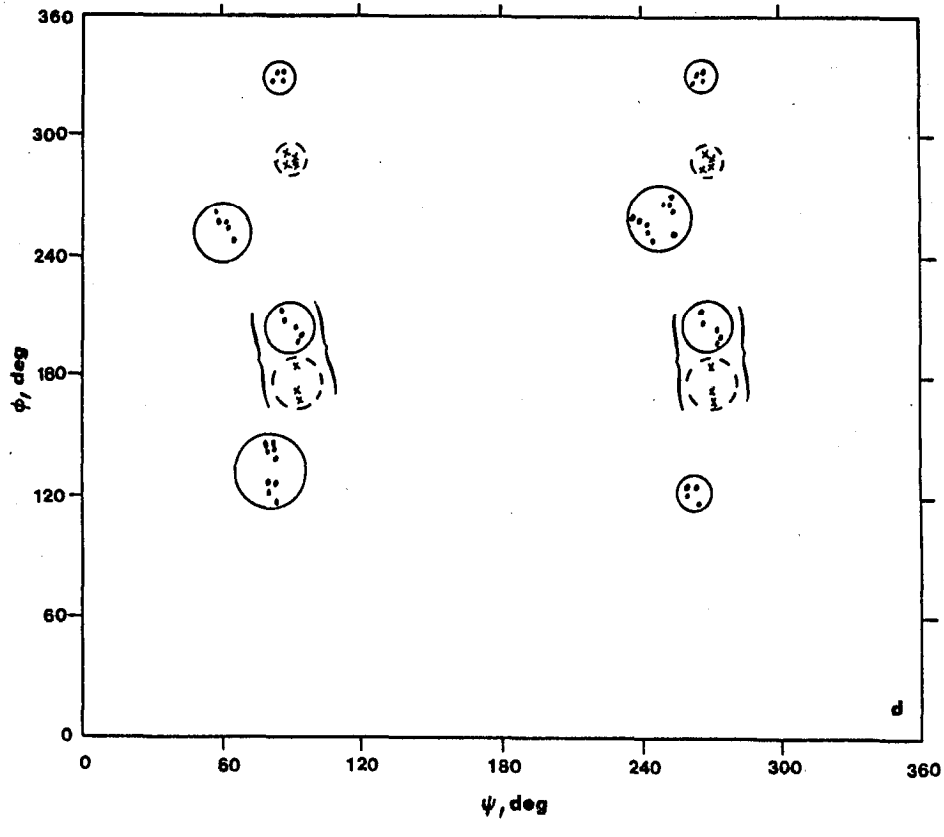
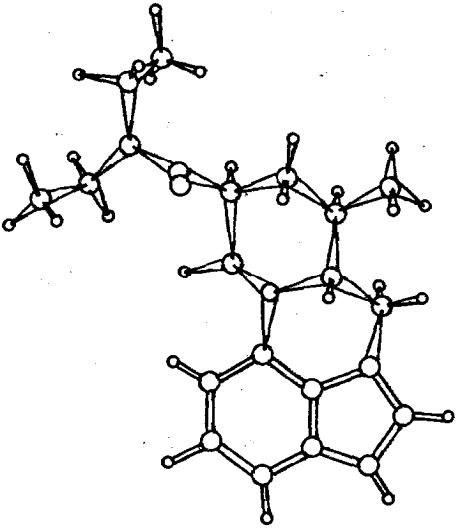


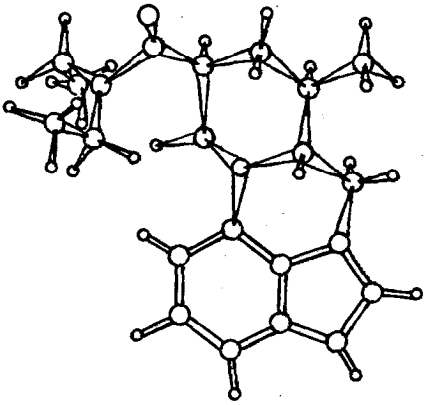
**FIGURE 5**

*Over all conformations (see text and also table III) of molecules studied here. The conformation(s) responsible for each activity (both activities) is (are) indicated by S and/or H: (a) LSD, (b) dimethylamide, (c) ethylamide, (d) methylamide, and (e) amide.*

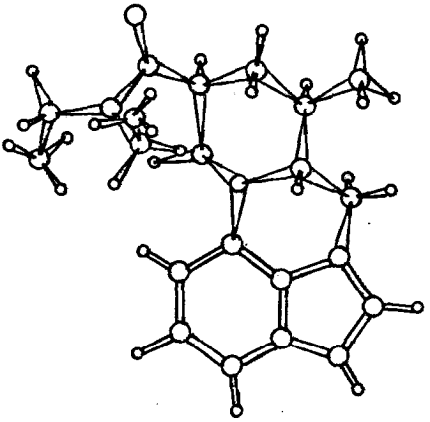
*(a) LSD*



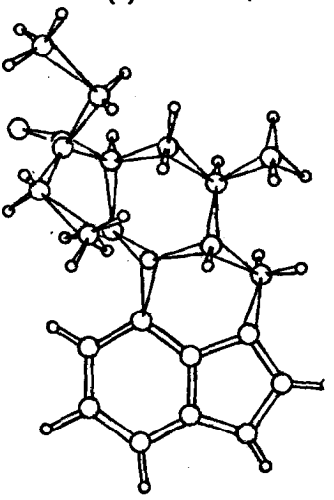
(a) I(V) S



(a) II(VI) H,S



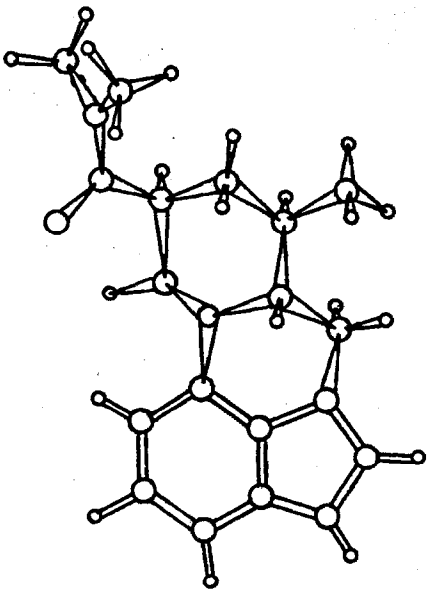
(a) III(VII) S



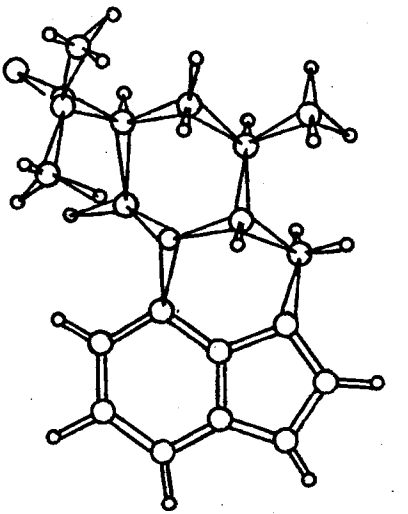
(a) IV(VIII) H,S

**FIGURE 5**

*(b) dimethylamide*



(b) K(III)



(b) K(IV) H,S

**FIGURE 5**

*(c) ethylamide*

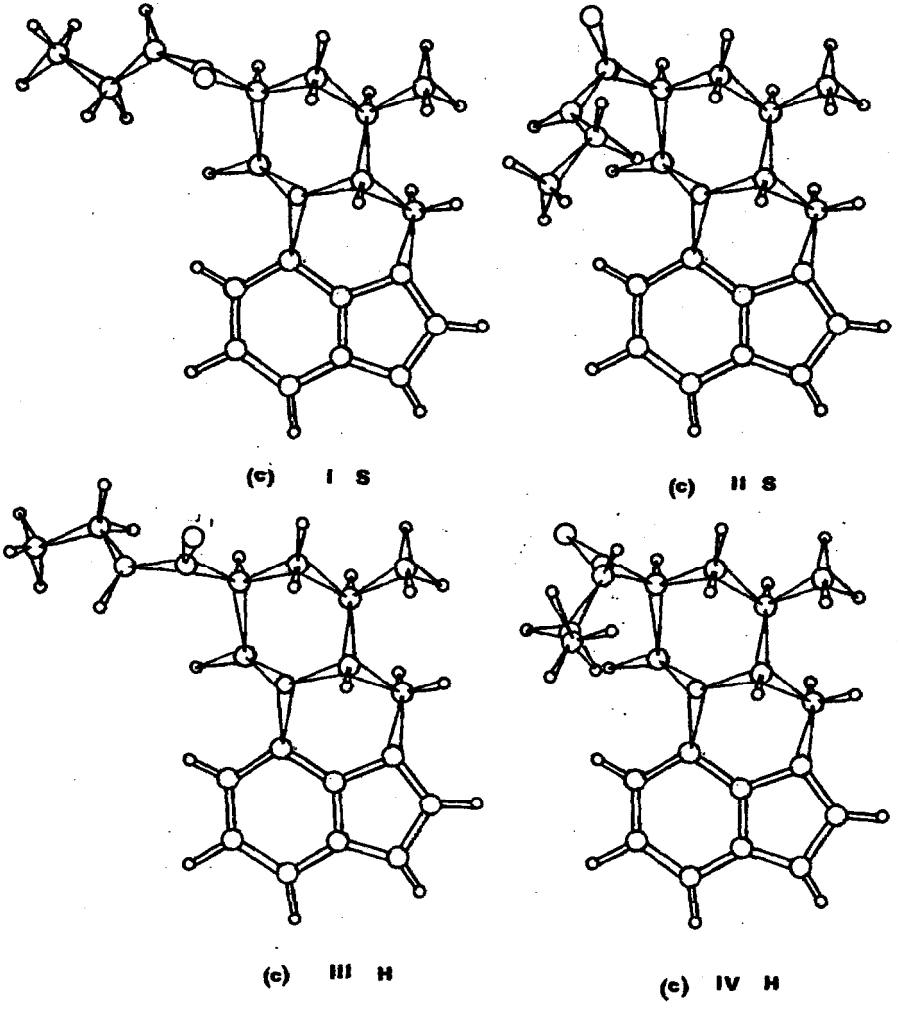
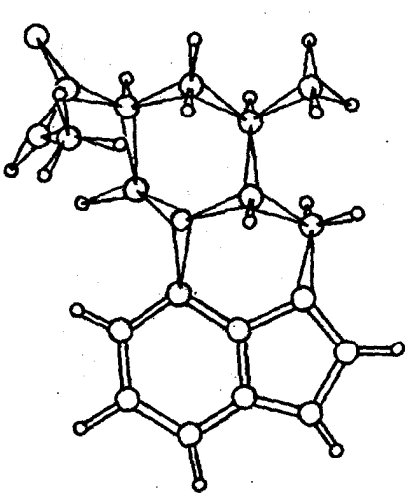
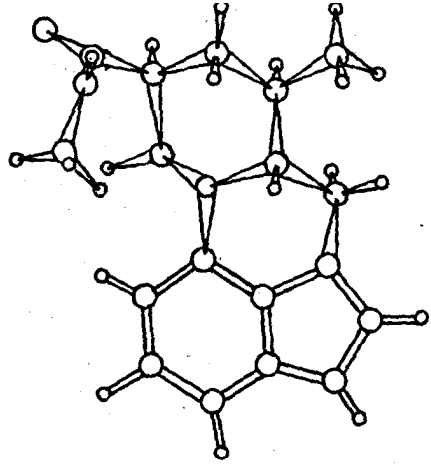


**FIGURE 5**

*(d) methylamide*

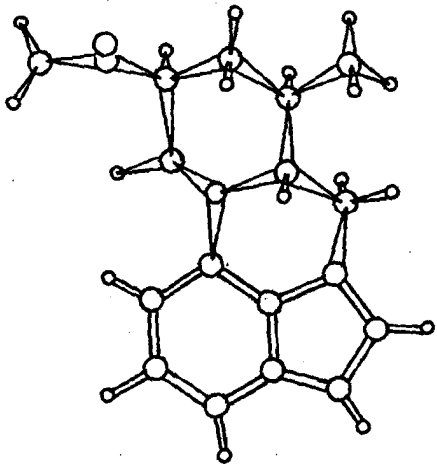


(d) I S

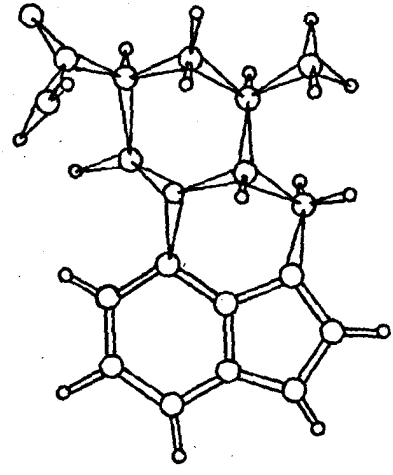


(d) II H

*(e) amide*

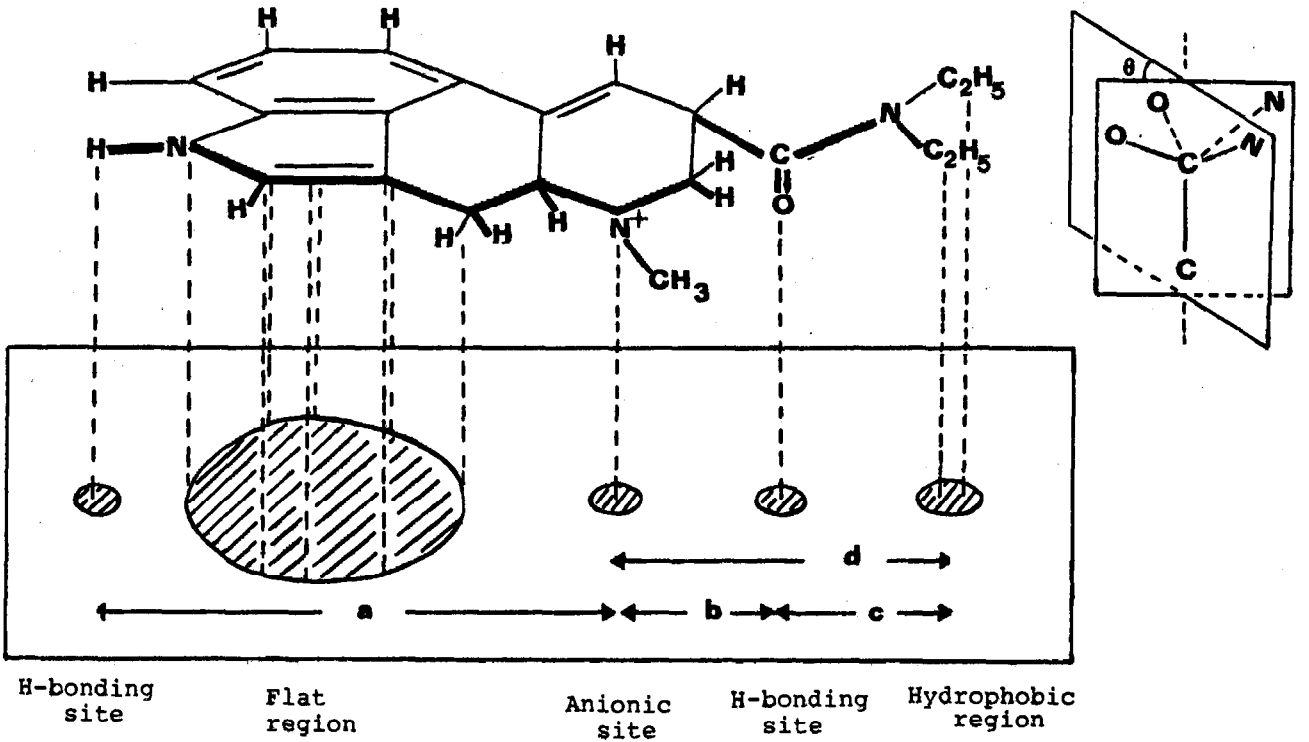


(e) I S



(e) II H

FIGURE 6




*Schematic representation of various centers for interaction in LSD. The intracenter distances are listed in Table III. The angle θ measures the deviation of -C=0 bond in receptor conformations (see figures 4(a) to 4(d) It is zero for LSD conformations. The values may be found in table III.*

**ACKNOWLEDGMENT**

I am thankful to Dr. Gene Barnett of the National Institute On Drug Abuse for valuable discussions, This work is supported by the National Institute on Drug Abuse grant number 1R01 DA01542-01A1.

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# Section IV

# Spectroscopic Methods

The power of the many and varied techniques of spectroscopy needs to be fully applied in the QuaSAR area. The papers presented here have been primarily concerned with properties of the isolated drug molecule and questions of molecular conformations and solvent interactions. It is hoped that studies will be applied to more complex systems to model the drug at the site of the receptor. These techniques have been extensively applied to the problem of biomembrane structure and the next step in complexity will be to obtain some physicochemical parameters for the drug receptor complex at the physiological site of action.

'QuaSAR' Research Monograph 22
G. Barnett, M. Trsic, and R. Willette, eds.
National Institute of Drug Abuse 1978

# Carbon-13 Nuclear Magnetic Resonance Study of the a- and ß-Isomers of Methadol and Acetylmethadol Hydrochlorides

F. Ivy Carroll, Charles G. Moreland, George A. Brine, and Karl G. Boldt

## INTRODUCTION

In 1954 Beckett and Casy (Beckett and Casy 1954) proposed that the opiate receptor topography consisted of an essentially inflexible site possessing a flat surface, a cavity, and an anionic site to accommdate an aromatic ring, a hydrocarbon moiety, and a protonated nitrogen, respectively. Over the years this proposal has been modified in attempts to account for the high activity of certain synthetic narcotics including some acyclic compounds which did not fit the receptor area proposed by Beckett and Casy. More recently, the proposal has been further modified to account for the morphine like activity of the pentapeptides (Met-enkephalin, H-Tyr-Gly-Gly-Phe-Met-OH and Leu-enkephalin, H-Tyr-Gly-Gly-Phe-Leu-OH) isolated from pig brain (see Horn and Rodgers 1977, Smith and Griffin 1978). Thus, any structure activity relationship proposal will have to account for the analgesic activity of flexible acyclic narcotics and enkephalins, as well as the rigid opiates.

The flexibility of the acyclic narcotics makes it very difficult to derive definitive structure activity relationships. However, it is apparent that the conformation(s) of the acyclic narcotics will be required to arrive at such a relationship.

Both $^{1}$H- and $^{13}$C-NMR spectral studies have been used to determine the conformation of morphine and similar compounds in solution (F. I. Carroll et al. 1976). Since these compounds possess rigid structures, it is likely that the conformation indicated by the spectral studies are the actual conformers of the drug at the "opiate receptor."

There have also been a number of pKa, $^{1}$H-NMR, and other physical chemical studies conducted on the acyclic narcotics, methadone, α-methadol, and ß-methadol, in an attempt to define the conformer(s) that are present in solution (Henkel, Bell, and Portoghese 1974; Portoghese and Williams 1970; Portoghese and Williams 1969). From the $^{1}$H-NMR data reported it appears that methadone and α- and ß-methadol in solution exist as mixtures of interconverting

conformers. Due to limitation of the low field $^{1}$H-NMR technique, it was not possible to determine accurately the conformational population or the structure of the individual conformers.

The solid state structure [conformation(s)] of methadone hydrobromide (Hanson and Ahmed 1958), isomethadone hydrochloride, α-methadol hydrochloride and α-acetylmethadol hydrochloride (Shefter 1974) have been determined by X-ray single crystal analysis. The most interesting feature of these studies was the lack of conformational similarity among these acyclic narcotics. Of the compounds studied only methadone hydrobromide and isomethadone hydrochloride showed similar conformations.

The present study was undertaken to ascertain whether $^{13}$C-NMR could provide additional information about the conformation of narcotic agonists in different solvents and, furthermore, to determine whether or not specific differences between the conformation of α- and ß-methadol hydrochlorides and α- and ß-acetylmethadol hydrochloride could be defined. Since chemical shift differences are generally larger for carbon resonances than those of protons, it was also anticipated that low temperature $^{13}$C-NMR would show separate resonances for the individual conformers.

## CARBON-13 NMR STUDIES

### a-Methadol Hydrochloride

Table 1 gives the $^{13}$C-NMR chemical shifts of α-methadol hydrochloride and ß-methadol hydrochloride in DMSO-$d_6$ and in $CD_2Cl_2$. A comparison of the chemical shifts for α-methadol hydrochloride in DMSO-$d_6$ with those in $CD_2Cl_2$ shows large differences for both C-3 (∿7 ppm) and C-5 (∿7 ppm). The change that is observed for C-3 could be due to the fact that DMSO-$d_6$ would, by means of hydrogen bond formation with the C-3 hydroxyl group, interact more strongly than $CD_2Cl_2$ However, the fact that the shift at C-3 is accompanied by an analogous shift at C-5 leads one to believe that DMSO-$d_6$, through such hydrogen bonding interactions, has effectively reduced the ratio of internally hydrogen bonded cyclic conformer(s) to acyclic conformer(s). Thus, it is proposed for α-methadol hydrochloride that acyclic conformer(s) are dominant in DMSO-$d_6$ and that cyclic conformer(s) are dominant in $CD_2Cl_2$.

These propositions are supported by the observations of two N-methyl carbon resonances for α-methadol hydrochloride in $CD_2Cl_2$ and one N-methyl carbon resonance in DMSO-$d_6$ at 30°C. Further evidence to support these arguments can be seen in figures 1A, B and C. As shown in figure 1C, two conformers of α-methadol hydrochloride in $CD_2Cl_2$ have been "frozen out" at -95°C and, furthemore, the conformer present in the larger amount has two N-methyl carbon signals while the one present in the smaller amount has only one. In the low temperature $^{13}$C spectrum the lesser conformer has resonances for C-3 at 73.8 ppm, for C-S at ∿39.5 ppm, and for the N-methyl carbons at ∿39.5 ppm. The close similarity of these chemical shifts to those for the same carbons of α-methadol hydrochloride in

**TABLE 1**

$^{13}$C-NMR Chemical Shifts[a] for α-Methadol Hydrochloride and ß-Methadol Hydrochloride[b] in Dimethylsulfoxide-$d_6$ and in Methylene Chloride-$d_2$

$CH_3-CH_2-CHOH-C(C_6H_5)_2-CH_2-CH(CH_3)-N(CH_3)_2 \cdot HCl$

1 2 3 4 5 6 7 8 9

| | α-Methadol•HCl | | ß-Methadol•HCl | |
|---|---|---|---|---|
| Carbon | $CD_2Cl_2$ | DMSO-$d_6$ | $CD_2Cl_2$ | DMSO-$d_6$ |
| 1 | 11.41 | 11.56 | 11.43 | 11.24 |
| 2 | 25.75 | 26.15 | 26.16 | 25.80 |
| 3 | 81.70 | 74.80 | 73.21 | 73.62 |
| 4 | 55.67 | 54.96 | 55.17 | 54.45 |
| 5 | 46.50 | 39.53 | 41.61 | 39.31 |
| 6 | 60.51 | 58.93 | 58.88 | 58.54 |
| 7 | 14.66 | 15.87 | 12.94 | 14.16 |
| 8 | 36.71[c] | 38.21 | 35.52[c] | 39.31 |
| 9 | 41.83[c] | 38.21 | 42.30[c] | 39.31 |

[a]All values are in ppm downfield from TMS at 30°C.

[b]The α- and ß-methadol hydrochloride samples were dried at 90°C under vacuum.

[c]The N-methyl chemical shifts cannot be assigned to a particular methyl group.

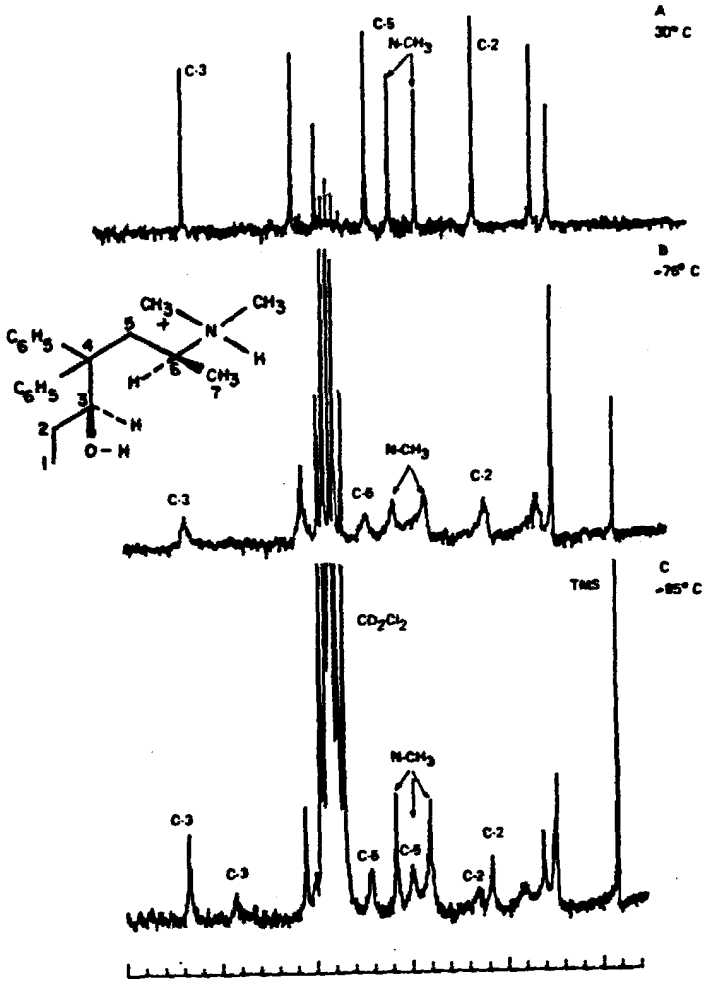
DMSO-$d_6$ (table 1) along with the presence of one N-methyl carbon resonance indicates that the lesser conformer is acyclic. If temperature effects on the chemical shifts can be ignored, then the $^{13}$CNMR chemical shifts of each signal at 30°C (figure 1A) should be the weighted average of the chemical shifts of the corresponding signals observed for the conformers at -95°C (figure 1C). Using chemical shifts for C-3, C-5, and the N-methyl carbons to calculate a mole ratio of cyclic to acyclic conformers gives an average value of approximately 5 to 1.

**ß-Methadol Hydrochloride**

In the case of ß-methadol hydrochloride an examination of the chemical shifts (table 1) shows a very small change with solvent for C-3 (0.4 ppm) and a somewhat larger change for C-5 (2.3 ppm). In addition, two N-methyl carbon resonances are observed for ß-methadol hydrochloride in $CD_2Cl_2$ as compared to one in DMSO-$d_6$. In order to be consistent with the conclusions reached for the α-isomer, the latter result should be taken to mean that the ß-isomer is acyclic in DMSO-$d_6$ and mainly cyclic in $CD_2Cl_2$. Additional evidence in support of acyclic conformers for the α- and ß-isomers in DMSO-$d_6$ is that there are small differences in their chemical shifts in DMSO-$d_6$ (table 1). One is then left to explain why the changes in chemical shifts with solvent at C-3 and C-5 for the ß-isomer are much less than the changes observed for α-isomer. The only rationale that seems plausible is that the chemical shifts for the acyclic conformer(s) are indeed very similar to the chemical shifts of the cyclic conformer(s) for the ß-isomer but not for the α-isomer. This could possibly indicate that the acyclic conformers of the α- and ß-isomers and the cyclic conformer(s) of the ß-isomer are similar.

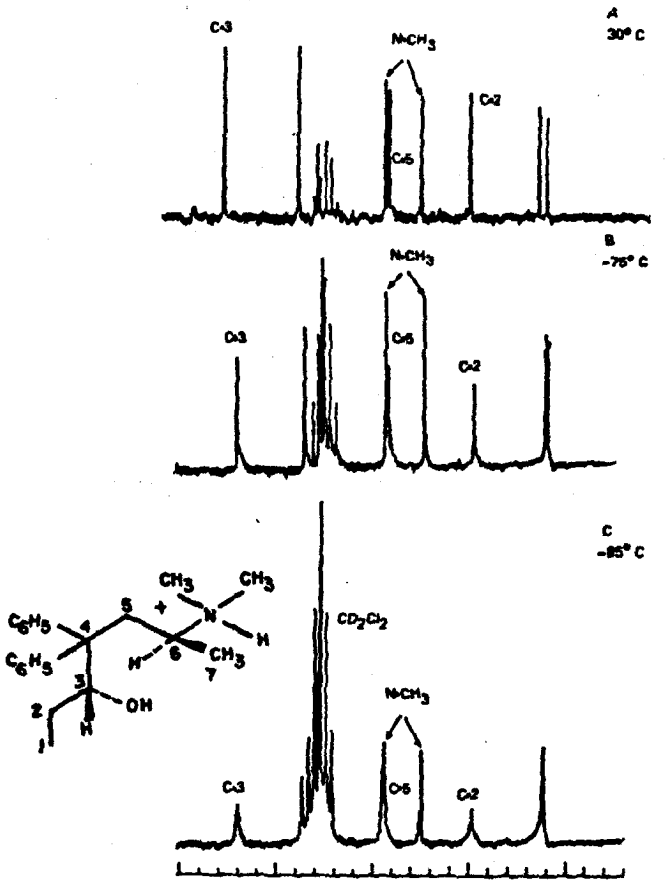
The low temperature spectra of ß-methadol hydrochloride in $CD_2Cl_2$ are presented in figures 2B and 2C. As can be seen, C-2, C-3 and C-5 (and other carbon resonances) have broadened and shifted in comparison to the same signals in the spectrum at 30°C (figure 2A). The N-methyl resonances, on the other hand, have shifted and broadened only sightly. Unfortunately, due to low temperature limitations on both the $^{13}$C probe and the solvent it was not possible to reach a low enough temperature where separate signals from the contributing conformers could be observed. Even with this limitation it is possible to suggest the following explanations. There is little or no acyclic conformer(s) for the ß-isomer in $CD_2Cl_2$ since <u>all</u> carbon resonances including the N-methyl carbons should have broadened and shifted. There is a strong possibility that the ß-isomer is a mixture of cyclic conformers that have N-methyl groups with very similar $^{13}$C-NMR chemical shifts and dissimilar chemical shifts for the other carbon resonances such as C-3, C-5, C-2, etc. As expected, another more definite conclusion is that the cyclic conformer(s) of the ß-isomer is different from the cyclic conformer of the α-isomer. This is based primarily on the large difference in chemical shifts between the α- and ß-isomers in $CD_2Cl_2$.

FIGURE 1




*$^{13}$C-NMR Spectra of α-Methadol Hydrochloride in $CD_2Cl_2$ at Various Temperatures*

**FIGURE 2**




*$^{13}$C-NMR Spectra of ß-Methadol Hydrochloride in $CD_2Cl_2$ at Various Temperatures*

**a-Acetylmethadol Hydrochloride**

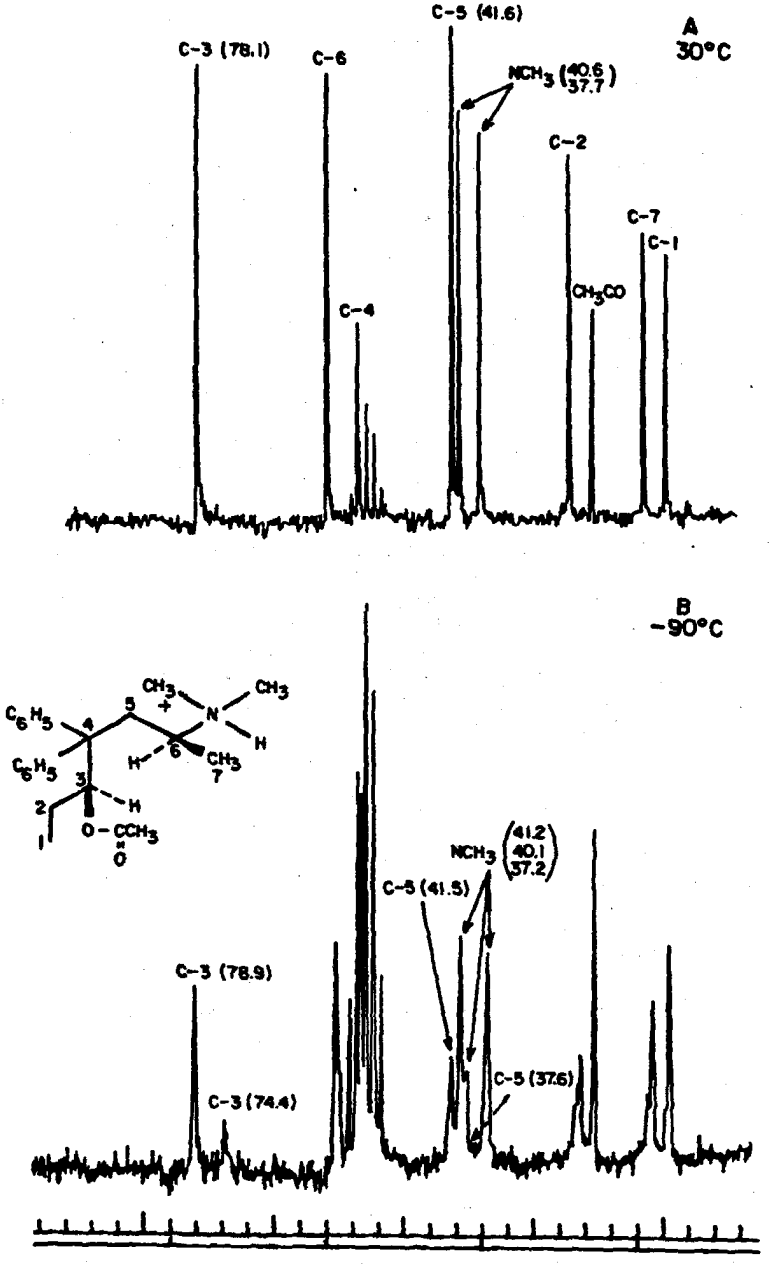
The $^{13}C$-NMR spectra of α-acetylmethadol hydrochloride and ß-acetylmethadol hydrochloride have been run in $CD_2Cl_2$ at 30° and -90°C. figures 3A and 3B show the spectra of α-acetylmethadol hydrochloride at 30° and -90°C respectively. Examination of figure 3 shows that C-3 appears as one signal at 30° and two signals at -90°C. If the chemical shifts of the two peaks obtained at -90°C are weighted 80/20, the calculated chemical shift for C-3 is identical to that in the 30°C spectrum. If the two C-S peaks in the -90°C spectrum are also weighted 80/20, the observed chemical shift for C-5 in the 30°C spectrum is obtained. The N-methyl groups appear as two signals separated by 2.9 ppm in the 30°C spectrum. The -90°C spectrum shows two major N-methyl peaks separated by 4.0 ppm and in addition a third smaller peak which is located between the two major peaks. If the $^{13}C$ chemical shift of the smaller N-methyl signal is weighted by 20% and the larger N-$CH_3$ resonances are weighted by 80%, the-result gives two signals separated by ~3.0 ppm which is the separation in the 30°C spectrum. Combining these observations with the conclusions reached for α-methadol hydrochloride, it is proposed that the α-acetylmethadol hydrochloride in $CD_2Cl_2$ is a 4 to 1 mixture of cyclic to acyclic conformers.

The $^{13}C$-NMR spectra of α-acetylmethadol hydrochloride in deuterium oxide ($D_2O$) were also obtained at 30° and +5°C and are shown in figure 4. The most interesting feature of these spectra is the appearance of two distinct N-methyl resonances when the temperature is reduced to +5°C. This is different from what was observed for α-methadol hydrochloride in $D_2O$ or in DMSO-$d_6$ where only one N-methyl resonance was observed (see table 1). By comparison to the results discussed previously for α-methadol hydrochloride and the observation of a single N-methyl resonance for the acyclic conformer in $CD_2Cl_2$ at -90°C for the α-acetylmethadol hydrochloride, one is led to the conclusion that the α-acetylmethadol hydrochloride is not totally acyclic in $D_2O$. Furthermore, analysis of the separation of the N-methyl signals for α-acetylmethadol hydrochloride in $D_2O$ compared to that in $CD_2Cl_2$ predicts that it is about a 1 to 1 mixture of cyclic to acyclic conformers.

ß-Acetylmethadol Hydrochloride

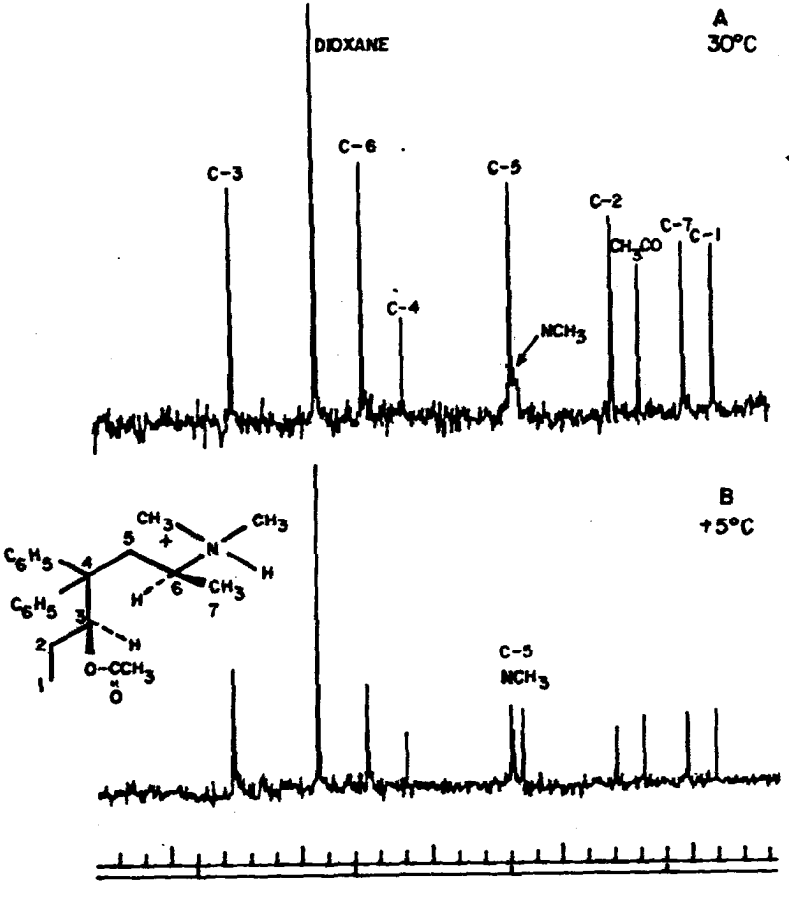
Figures 5A and 5B show the $^{13}C$-NMR spectra of ß-acetylmethadol hydrochloride in $CD_2Cl_2$ at 30° and -90°C respectively. Inspection of the low temperature spectrum shows two signals for the C-1, C-2 and the acetyl methyl group indicating the presence of two conformers. However, since C-3 is not well resolved, and the N-methyl and C-5 carbons give a complicated set of signals, it is not possible to analyze the date in relation to cyclic and acyclic structures. The picture is further complicated by the $^{13}C$-NMR spectrum of ß-acetylmethadol hydrochloride in $D_2O$ (figure 6) which shows two N-methyl resonances and very sharp signals for all carbons. Additional experiments at higher fields and lower temperatures will be required in order to explain these results.

FIGURE 3



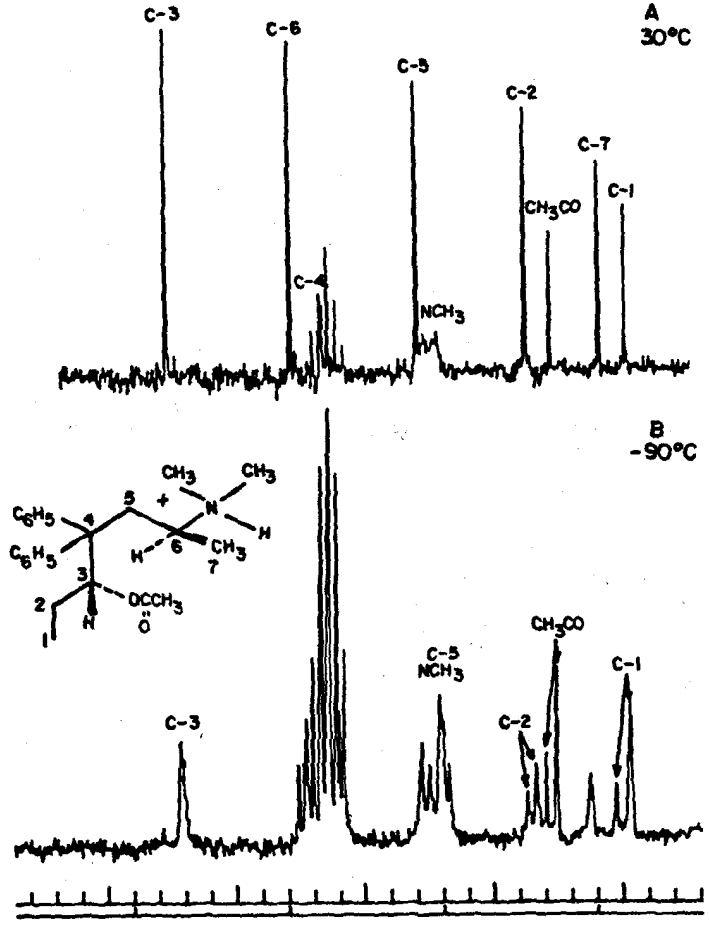

*$^{13}$C-NMR Spectra of α-Acetylmethadol Hydrochloride in $CD_2Cl_2$ at 30° and -90°C*

**FIGURE 4**



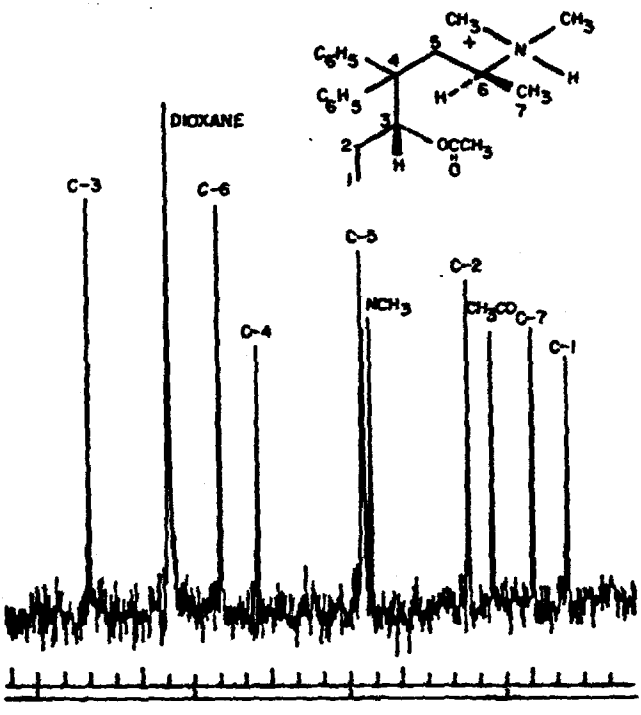

*$^{13}C$-NMR Spectra of α-Acetylmethadol Hydrochloride in $D_2O$ at 30° and + 5°C*

**FIGURE 5**




*$^{13}C$-NMR Spectra of ß-Acetylmethadol Hydrochloride in $CD_2Cl_2$ at 30° and -90°C*

**FIGURE 6**




*$^{13}$C-NMR Spectra of ß-Acetylmethadol Hydrochloride in $D_2O$ at 30°*

**SUMMARY**

The $^{13}$C-NMR spectra reported in these studies give direct evidence for the presence of two contributing conformers for α-methdol hydrochloride, α-acetylmethadol hydrochloride and ß-acetylmethadol hydrochloride. Indirect evidence is also available for the presence of more than one conformer for ß-methadol hydrochloride. However, in order to be more descriptive about the structures of the conformers, it is necessary to obtain $^{13}$C-NMR spectra that have a higher degree of resolution than is available from our present NMR system. Such systems are available, and plans are currently in progress to obtain $^{13}$C-NMR spectra on these compounds at high enough magnetic fields and low enough temperatures to give us the necessary data.

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# Photoelectron Spectroscopic Studies of Hallucinogens: The Use of Ionization Potentials in QSAR

L. N. Domelsmith and K. N. Houk

Studies of the electronic structures of hallucinogenic drugs (Snyder and Merril 1965; Snyder 1970; Green and Kang 1970; Weinstein et al. 1976) have provided various lines of evidence for the role of electronic characteristics, such as molecular orbital energies and electrostatic potentials, in the causation of hallucinogenic activity. Because of the power of photoelectron spectroscopy as a tool for elucidation of electronic structure (Rabalais 1977), we have studied a variety of hallucinogenic substances and inactive analogs by this technique. Here, we wish to: (1) demonstrate the utility of photoelectron spectroscopy, in conjunction with various types of quantum mechanical calculations, for the determination of orbital energies and shapes, as well as conformations of side chains and substituents, even for relatively larger molecules ranging in size from phenethylamine to LSD; (2) describe the results of our studies of phenethylamines, amphetamines, tryptamines and ß-carbolines (hamines); (3) show that ionization potentials are important empirical parameters useful in the quantitative correlation between physical properties and hallucinogenic activities, as well as other types of potencies in biological systems; and, (4) suggest that these correlations are not fortuitous, but, instead, confirm the role of complexation by the aromatic moiety at biological amine receptor sites.

## ELECTRONIC INFORMATION FROM PHOTOELECTRON SPECTROSCOPY

Photoelectron spectroscopy is a technique for measurement of ionization potentials, or orbital energies, of molecules. Our instrument is equipped with a He(I) discharge source, which produces photons of 21.21eV energy. Upon collision with vapors of a sample, the photons cause excitation to a free electron and various states of the radical cation of the molecule. The energy required for ionization, the ionization potential (IP), is measured as the difference between the incident photon energy and the energy of the ejected electron. With a He(I) source, IPs only up to 21eV can be measured, but this allows determination of the energies of the low-lying valence IPs of organic molecules,

The connection between the IPs and the orbital energies of a molecule was made by Koopmans (1934). That is, the energy of an electron in a

molecule relative to the energy of a free electron according to SCF molecular orbital calculation is the kinetic energy of the electron plus the attraction of the electron for the molecular nuclei (the so-called "one electron orbital energy") minus the repulsion of that electron for all other electrons in the molecule. Upon removal of an electron from a molecule, all of these terms go to zero. By convention, IPs are positive, whereas orbital energies are negative. Koopmans' theorem is based on two assumptions: (1) after ionization, the orbitals are unchanged from those of the neutral molecule, and (2) correlation energy (the inescapable error in SCF calculations) is the same in ground and radical cation states. Neither of these assumptions is correct, but they tend to cancel, and in any case become less troublesome as the size of the molecule increases. Throughout this paper, Koopmans' theorem language will be used: the negatives of orbital energies will be considered synonomous with the IPs of a molecule.

Photoelectron spectroscopy can reveal more than the IPs, or orbital energies, of molecules. For example, vibrational structure in the spectra can reveal considerable information about the type of orbital involved in that ionization process, and the magnitudes of changes in IPs caused by substitution of a molecule can provide evidence about the shapes, or positions of electron densities, in orbitals. An example relevant to the subject of this paper is given in Figure 1.

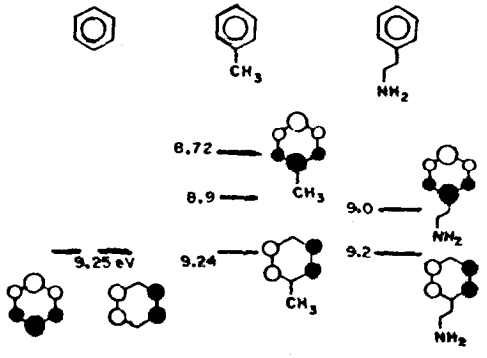



***Figure 1.*** *Ionization potentials of benzene, tolune, and phenethylamine*

Benzene has two degenerate highest occupied molecular orbitals which give rise to identical IPs of 9.25eV. Upon methylation of benzene, the degeneracy of these orbitals is lifted dramatically. One is not changed at all, since there is a node at the site of methylation, while the HOMO is raised by about 0.35eV (8 kcal/mol) by the weakly electron-donating methyl substituent. Two values, 8.72 and 8.9eV, are given for the first IP of toluene, and these result from population of different vibrational states of the radical cation ground state. The vertical IP, 8.9eV, represents ionization to form the radical cation in the same geometry as the ground stat of toluene. Since the vertical IPs will represent the best indicator of reactivity, they will be used here. Phenethylamine has IPs very similar to toluene, and for the same reason: changes in IPs or orbital energies are

largest when substituents are attached at the site of highest orbital electron density. According to perturbation theory, the change in orbital energy is proportional to the square of the orbital coefficient at the site of substitution.

Having given this all too brief introduction to photoelectron spectroscopy, let us pause for a moment to consider the relevance of ionization potent to chemical phenomena, namely the reactivity and complexing abilities of molecules. It can be shown that IPs are useful correlators and predictors of chemical reactivity, and this leads us to believe that quantitative structure-reactivity relationships (QSRR) for these simple system will be paralleled by quantitative structure-activity relationships (QSAR) in biological systems.

Figure 2 shows a plot of the logs of the second-order rate constants for acid-catalyzed hydrations of alkenes (Oyama and Tidwell 1976) and styrenes (Schubert and Keefee 1972) versus the IPs of the alkenes (McAlduff and Houk, unpublished). Over a range of $10^{18}$ in reactivity,

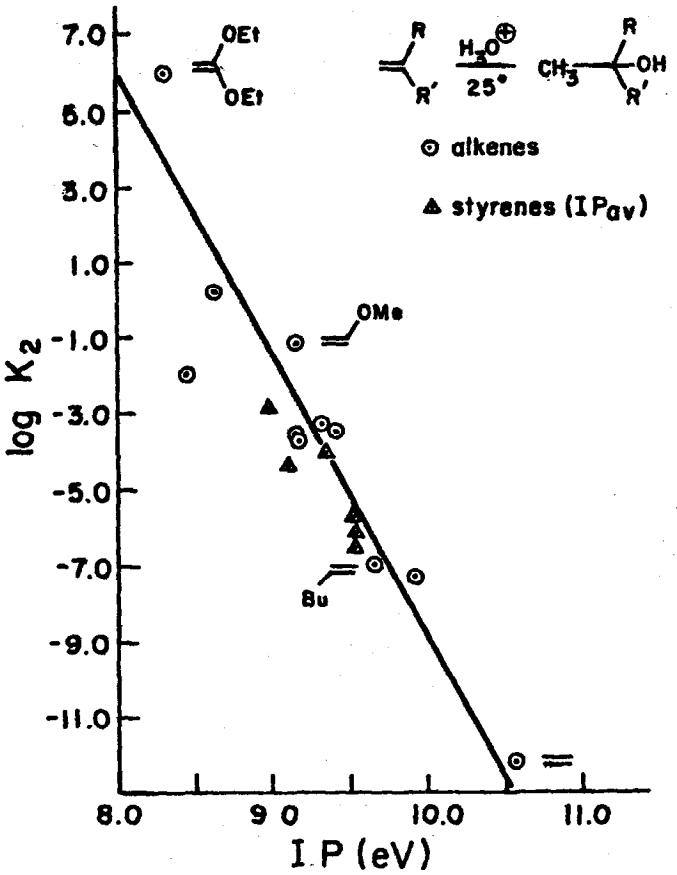
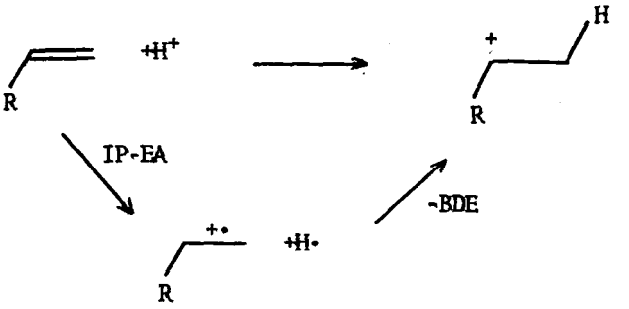



***Figure 2.*** *Plot of log k of acid-catalyzed hydrations of alkenes versus the IPs of the alkenes*

there is a reasonably linear plot--those molecules with the lowest IPs react fastest and vice versa. Why should such a plot be linear? There are two ways to think about such a plot. From the point of view of perturbation theory, we may say that the transition state for proton transfer from hydronium to an alkene is stabilized by interaction of the alkene HOMO and the hydronium LUMO. According to perturbation theory, the stabilization resulting from this interaction is inversely proportional to the HOMO-LUMO gap, or, using Koopmans' theorem,the difference between the alkene IP and the electrophile EA. Alternatively, the correlation between rates and IPs may be considered to arise in the following way:




Assuming the transition state is near the carbonium ion intermediate in structure, the energy of the transition state may be estimted in step, First, an electron is transfered to the electrophile. The energy of this step is equal to IP(alkene) - EA($H^+$). Second, the CH bond is formed between the two radicals. If this second step has a constant energy (-BDE), then the difference between the energies of two transition states will be directly proportional to the difference between the alkene IPs, for the same electrophile. Linear correlations of the type observed in Figure 2 suggest that the constancy of this BDE is a reasonable assumption.

An even better correlation is found for the rates of bromination reactions of alkenes (Olah and Hockswender 1974). shown in Figure 3. These reactions involve the formation of the bromonium, and the transition state resembles a relatively loose molecular complex.

A third example, somewhat closer to the subject at hand, comes from our work on cycloaddition reactions. The transition states for Diels-Alder reactions are believed to be akin to $\pi$ complexes, stabilized by interaction of the diene HOMO with the alkene LUMO, and we and others have found very good correlations between the IPs of dienes and their rates of reactions with alkenes (Houk 1975).

Finally, and probably of greatest relevance to the use of IPs to correlate activities of hallucinogens, a correlation exists between IPs of aromatics and stabilities of molecular complexes formed with good acceptors. We have shown earlier that (Domelsmith, Munchausen, and

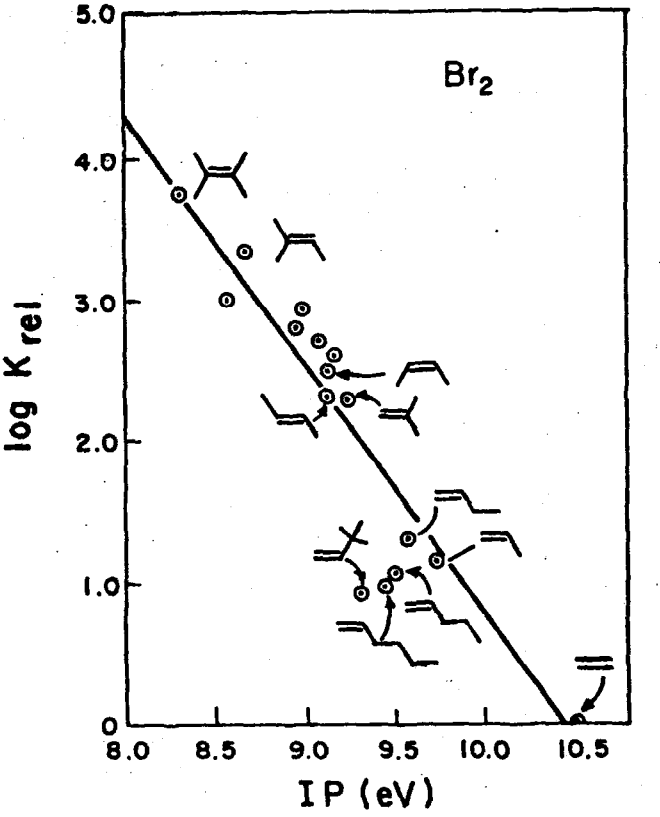



***Figure 3.*** *Plot of log k of bromination of alkenes versus the IP of the alkene*

Houk 1977a) there is an excellent linear correlation between the electron-donor ability of a substituted aromatic, as measured by the average of the first and second IPs, and the stability of the complex with TCNE. In this case, a 6 kcal/mol (or 0.25eV, in more conventional IP units) decrease in IP is accompanied by a 1 kcal/mol increase in the stability of the complex.

Because of the success of these quantitative structure-reactivity relationships (QSRR), we had some hope at the outset of this work that IPs would be useful quantities for QSAR. As will be shown later, this is, indeed, the case.

## PHOTOELECTRON SPECTRA OF HALLUCINOGENS

### Tryptamines and LSD

Indole has five occupied $\pi$ orbitals, two of which are considerably higher in energy than the others, and are expected to dominate the

chemical characteristics of indole and its derivatives. In the photoelectron spectrum of tryptamines, three π IPs are resolved, along with that due to the amine lone pair on the side-chain nitrogen (Domelsmith, Munchausen, and Houk 1977a). The two highest π orbitals of indole, which has a similar π system, are shown in Figure 4. These pictures,

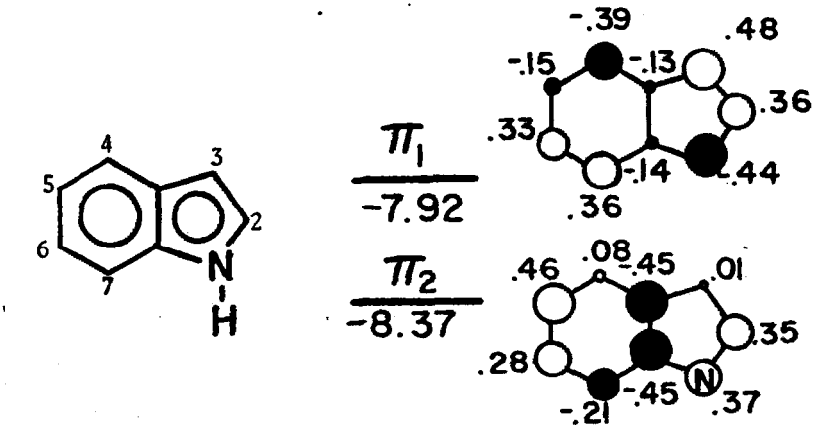



***Figure 4.*** *HOMO and SHOMO of indole (CNDO/2)*

which indicate the p orbital coefficients by the size of the circles, are obtained by CNDO/2 calculations, but qualitatively similar orbital shapes were obtained by Weinstein et al. (1976) using higher quality ab initio calculations. As mentioned earlier, the coefficient magnitudes at various positions in a given orbital are indicators of the influence that substituents will have on the IP. The HOMO has large coefficients at carbons 4, 6, and 7, but a very small coefficient at carbon 5. By contrast, the second highest occupied MO (SHOMO) has the largest coefficient at carbon 5. The first IP should not be influenced much by substitution at carbon 5, while the second should be influenced substantially. The opposite effect should be observed for substitution at carbon 4. To date, we have only measured the effects of substituents at the 5 position. Table I lists the first and second π IPs of tryptamines we have studied. The first IP remains essentially constant, with changes in apparent values due, in part, to overlapping of bands. The second IP is influenced significantly by methyl and even more so by methoxy substitution. The MOs of the substituted complex will not, of course, be unchanged from those of the parent compound. For example, Weinstein et al. (1976) have shown the significant reorientation which occurs upon substitution of tryptamine by hydroxyl groups at various positions. It is also interesting that the ability of tryptamines to inhibit binding of D-LSD to rat brain membranes (IC-50, see Table I) increases as the IP of the tryptamine decreases. The first IP changes little, hut the second decreases

**Table I.** IPs (eV) of Tryptamines[1]

| Substituent | $IP_1$ | $IP_2$ | IC-50[2] |
|---|---|---|---|
| None | 7.69 ± 0.08 | 8.25 ± 0.08 | $8.0 \pm 10^{-6}$ |
| N-Methyl | 7.60 ± 0.08 | 8.25 ± 0.08 | |
| N,N-Dimethyl | 7.57 ± 0.05 | 8.22 ± 0.06 | |
| 5-Methyl | 7.64 ± 0.05 | 8.03 ± 0.08 | $7.6 \pm 10^{-6}$ |
| 5-Methoxy | 7.68 ± 0.12 | 7.79 ± 0.12 | $2.3 \pm 10^{-6}$ |
| 5-Methoxy-N,N-Dimethyl | 7.61 ± 0.14 | 7.8 ± 0.2 | |
| LSD | 7.24 ± 0.05 | 8.04 ± 0.08 | $1.1 \pm 10^{-8}$ |

[1]Domelsmith, Munchausen, and Houk 1977a,b.
[2]Inhibition of the binding of D-LSD by rat brain membranes (Green et al. 1976).

substantially upon going from parent to methyl to methoxy. So, also, does the ability to bind to the LSD site, as shown by the IC-50.

LSD has a much lower IP than simpler tryptamines (Domelsmith, Munchausen and Houk 1977b). The first two IPs are due to aromatic π orbitals. The first IP here changes most from the value for tryptamine, since conjugation of the vinyl group, which lowers IPs, is much greater in the HOMO than in the SHOMO, the oppositetothe effect at carbon 5. LSD, the best electron-donor in the series of tryptamines we. have studied, is the most potent binder to rat brain membranes (Table I).

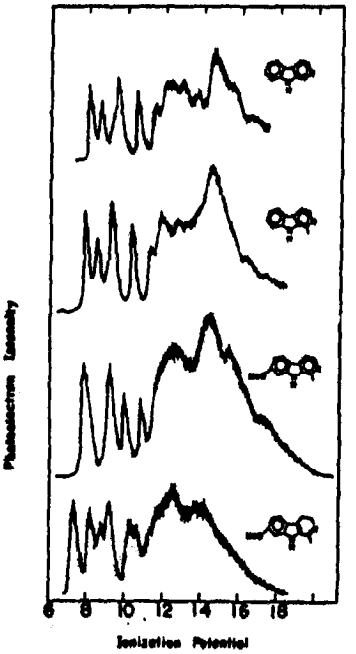
**Harmala Alkaloids**

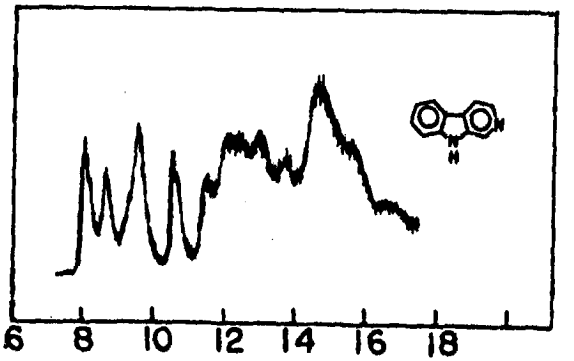
Harmine and four of its derivatives have been studied. The spectra of these compounds, shown in Figure 5, exhibit four well resolved bands with IPs in the range of 7-10.5eV. In addition to these bands, which are due to π orbitals, the pyridine-like nitrogen lone pair appears as a shoulder at ~9.3eV. These IPs are given in Table II.

**Table II.** Ionization Potentials (±0.06eV) of Harmala Alkaloids

| Compound | $\pi_1$ | $\pi_2$ | $\pi_3$ | $\pi_4$ | $n_N$ |
|---|---|---|---|---|---|
| Norharmane | 7.99 | 8.57 | 9.42 | 10.39 | 9.3 ± 0.2 |
| Harmane | 7.83 | 3.46 | 9.20 | 10.21 | ~9.3 |
| Harmol | 7.92 | 8.17 | 9.24 | 10.06 | ~9.3 |
| Harmine | 7.78 | ~8.0 | 9.12 | 9.84 | ~9.3 |
| Harmaline | 7.38 | 8.20 | 8.81 | 10.18 | ~9.2 |

The substituent effects can be interpreted in a fashion similar to that used earlier for tryptmines. That is, the two highest occupied MOs of norharmane are such that the site of hydroxy or methoxy substitution has a near-node in the HOMO and a large coefficient in the SHOMO, as shown in Figure 6.




*Norharmane: enlarged spectrum*

***Figure 5.*** *Photoelectron spectra of (top to bottom) norharmane, harmane, harmine, and harmaline*

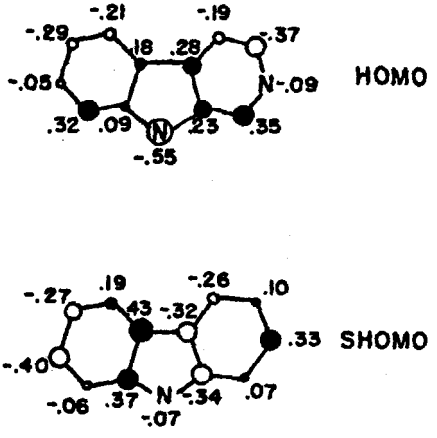



***Figure 6.*** *HOMO and SHOMO of norharmane (MINDO/3)*

In harmol, substitution of a hydroxy group has caused the first IP to increase slightly and the second to decrease substantially as compared to the IPs of harmane. This behavior is experimental evidence for a node in the HOMO at the site of substitution, and a relatively large orbital electron density at this site in the SHOMO. In phenol, the HOMO is raised substantially from the position in benzene, and the SHOMO is slightly lowered. The methoxy group of harmine lowers both $IP_1$, and $IP_2$, but the second to the greatest extent. It is interesting to note that this behavior is identical to thatobservedfor substitution at the carbon 5 of tryptamine.

Although it has been observed that harmines and related ß-carbolines are hallucinogenic, quantitative data are mainly available on the ability of these compounds to inhibit MAO.

**Phenethylamines**

Each of these molecules has two high-lying aromatic orbitals, resembling those of benzene, and the side-chain amino lone-pair orbital. These give rise to IPs between 8 and 10eV, and are listed in Table III (Domelsmith, Munchausen, and Houk 1977).

**Table III.** Ionization Potentials of Phyenthylamines

| Substituents | π $IP_1$ | π $IP_2$ | n IP |
|---|---|---|---|
| None | 8.99 ± 0.20 | 9.35 ± 0.16 | ~9.3 |
| N-Methyl | 9.08 ± 0.16 | 9.32 ± 0.20 | ~8.7 |
| N,N-Dimethyl | 8.95 ± 0.16 | 9.27 ± 0.20 | 8.35 ± 0.14 |
| 4-Hydroxy | 8.41 ± 0.12 | 9.35 ± 0.12 | ~9.3 |
| 4-Methoxy | 8.16 ± 0.08 | 9.19 ± 0.10 | ~9.3 |
| 3,4-Dimethoxy | 8.03 ± 0.16 | 8.86 ± 0.16 | ~9.3 |
| 3,4,5-Trimethoxy | 8.18 ± 0.24 | 8.18 ± 0.24 | ~9.3 |

In the parent and p-substituted compounds, the HOMO and SHOMO are related to those of toluene, shown earlier in Figure 1. Both 3,4-dimethoxyphenethylamine and mescaline have lower IPs due to the presence of additional donor substituents. Interestingly, mescaline has a higher IP than 3,4-dimethoxyphenethylamine, which indicates to us that the central methoxyl group has become non-planar, and thus a poorer electron donor. This variation in methoxyl conformations will be discussed in more detail later.

**Amphetamines**

The most extensive series of hallucinogens we have stuedied are amphetamines. The aromatic rings of phenethylamine and amphetamine are electronically indentical, as reflected by th photoelectron spectra show in Figure 7. Indeed, the same can be said for toluene, a

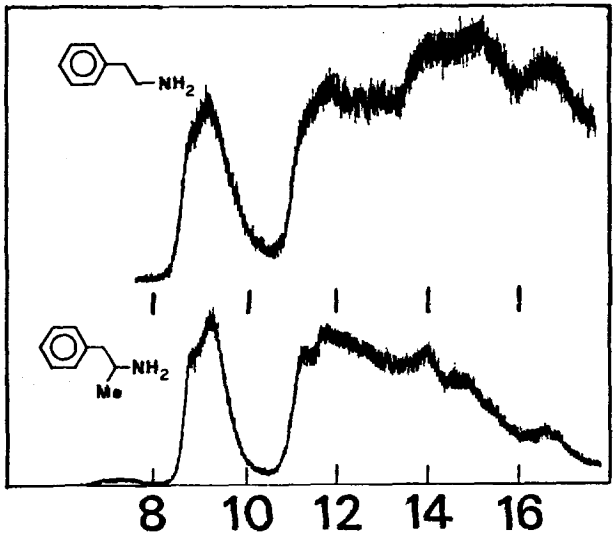



***Figure 7.*** *Photoelectron spectra of phenethylamine and amphetamine*

finding which not only has implications about the activities of these compounds, but shows that ionization potentials of one series may be estimated very accurately from ionization potentials of of another series. The lowest two ionization potentials of the amphetamines we have studied are given in Table IV, along with quantities which are to be disscussed later. These data confirmed many expectations, but also produced some surprises. In order to investigate these compounds in more detail, ab initio calculations using the STO-3G basis set were carried out on polymethoxybenzenes. Figure 8 shows the results of these calculations for dimethoxybenzenes, along with the IPs measured for these molecules.

**Table IV.** IPs (±0.06eV) Partition Coefficients, and Hallucinogenic Activities of Amphetamines

| | Substituent | π $IP_1$ | π $IP_2$ | log $P^1$ | Activity, MMU[5] |
|---|---|---|---|---|---|
| 1 | None | 8.99 | 9.35 | --- | 0 |
| 2 | 3,4-diOH[3] | 8.18 | 8.90 | --- | |
| 3 | 2,6-diMeO | 8.18 | 8.18 | --- | |
| 4 | 4-MeO | 8.16 | 9.19 | 1.77 | 3.91 |
| 5 | 3,4,5-triMeO | 8.16 | 8.16 | 1.48[2] | 2.13 |
| 6 | 2,3,4-triMeO | 8.09 | 8.36 | 1.36 | 2.13 |
| 7 | 3,4-diMeO[3] | 8.03 | 8.86 | 1.00 | 0.92 |
| 8 | 3,4-Methylenedioxy | 8.01 | 8.97 | 1.68[2] | 2.54 |
| 9 | 4-Br-2,5-diMeO | 7.94 | 8.92 | 2.58 | 519[6] |
| 10 | 2,4-diMeO | 7.91 | 8.75 | 1.75 | 4.62 |
| 11 | 2,4,6-triMeO | 7.76 | 8.19 | 1.57 | 10.66 |
| 12 | 2,5-diMeO | 7.70 | 8.86 | 1.88 | 7.39 |
| 13 | 2,4,5-triMeO | 7.66 | 8.69 | 1.74 | 18.12[7] |
| 14 | 4-MeS-2,5-diMeO | 7.64 | 8.37 | 2.49[4] | 49.1[1] |
| 15 | 4-Me-2,5-diMeO | 7.62 | 8.68 | 2.08 | 79.2[7] |

[1]Experimental octanol-water partition coefficients (Barfknecht, Nichols, and Dunn 1975).
[2]Calculated, (Barfknecht, Nichols, and Dunn 1975).
[3]Corresponding phenethylamine IPs used to estimate those of amphetamine.
[4]Calculated (Castagnoli, Anderson, and Shulgin, unpublished).
[5]Human (Shulgin, Sargent, and Naranjo 1969; Jacob et al. 1977).
[6]Also human data by Sepúlveda, Valenzuela, and Cassels (1972).
[7]Also rabbit hyperthermia, Castagnoli, Anderson, and Shulgin, unpublished.

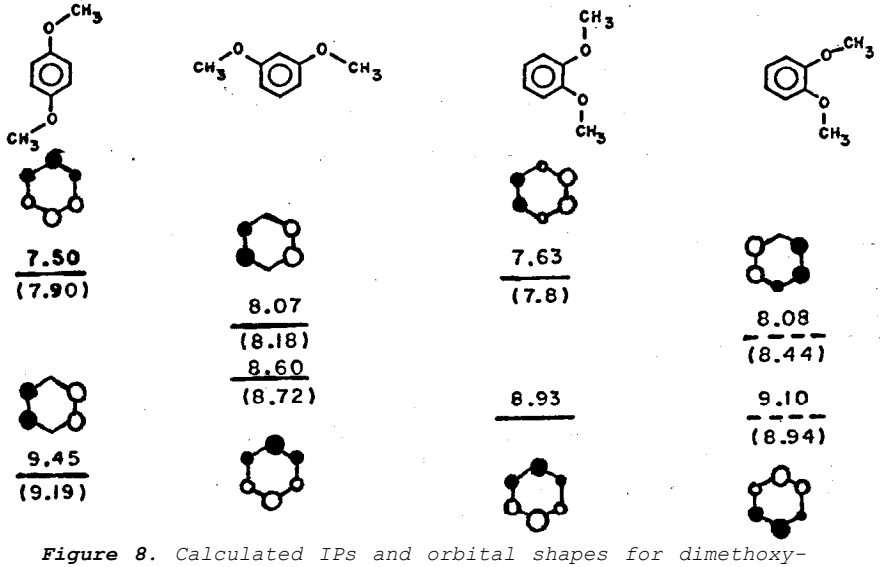



*Figure 8. Calculated IPs and orbital shapes for dimethoxybenzenes. Experimental values are given in parentheses.*

The para-dimethoxy compound has a very large split in degeneracy due to the fact that one orbital is raised both substituents, while the second is essentially unaffected. The first IP is 1.35eV lower than that of benzene, while the second is essentially identical to that of benzene. Meta-dimethoxybenzene has a much higher IP and much closer first and second IPs due to the smaller and more nearly equal influence of the two methoxys on both orbitals. The meta-disubstitution pattern produces a much less potent electron-donating aromatic ring than the para.

According to the simple analysis we have used so far, ortho- and meta-dimethoxybenzene should have identical IPs. Higher order effects change this simple picture. While we have discussed this in more detail elsewhere (Domelsmith and Houk 1978), this effect can be summarized simply. In the meta case, the HOMO is lowered somewhat by mixing with the LUMO, and the SHOMO is raised by mixing with the lowest benzene $\pi$ orbital. The HOMO and SHOMO of the ortho compound are affected in opposite fashion. In the photoelectron spectrum of ortho-dimethoxybenzene, a small shoulder is observed at 7.8eV, but the first strong band appears at 8.44eV, considerably above the calculated IP of 7.63eV. Much better agreement between calculation and experiment is obtained when the calculation is carried out on a geometry in which one of the methoxyls is rotated by 90°. This is shown at the far right of Figure 8. In such a conformation, the methoxy is a much poorer donor, since the $\pi$ orbital perpendicular to the COC plane of the ether linkage is rotated away from ideal conjugation with the aromatic $\pi$ orbitals. We do not as yet know why ortho-dimethoxybenzene prefers a conformation in which at least one of the methoxyls is rotated out of the benzene ring plane, but the photoelectron spectrum seems to demand to this for the major conformer. It is worth noting that the calculated IPs, although obtained from ab initio calculations, scaled to fit experimental IPs by a least squares treatment and done for several conformations, still give relatively inaccurate predictions of IPs even when calculations on many different geometries are performed. We feel that this is an important reason for obtaining experimental IPs rather than only calculating these.

The various dimethoxyamphetamines show a similar pattern (Table IV). The 2,5-dimethoxyamphetamine, which has the para-dimethoxy pattern, has much lower IPs than those amphetamines with the ortho- and meta-dimethoxy patterns. This has some significance in understanding hallucinogenic activities of amphetaines. The 2,5-dimethoxyamphetamines have been long recognized to be more potent hallucinogens than isomeric analogs (Shulgin, Sargent and Naranjo 1969). The lower IPs and higher electron-donating abilities of the 2,5-dimethoxy compounds are possibly the origin of higher activities of these compounds.

Turning to the more highly substituted amphetamines, this special potency of the p-dimethoxy pattern persists. Inspection of Table IV shows that the best electron-donors are those with the 2,5-dimethoxy substitution pattern.

**THE USE OF IONIZATION POTENTIALS IN QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS**

Photoelectron spectral studies have revealed a great deal about the

electronic structures of hallucinogenic drugs. The ionization potentials obtained in this way have also been found to be very useful indicators of activity. One of our early correlations is shown in Figure 9.

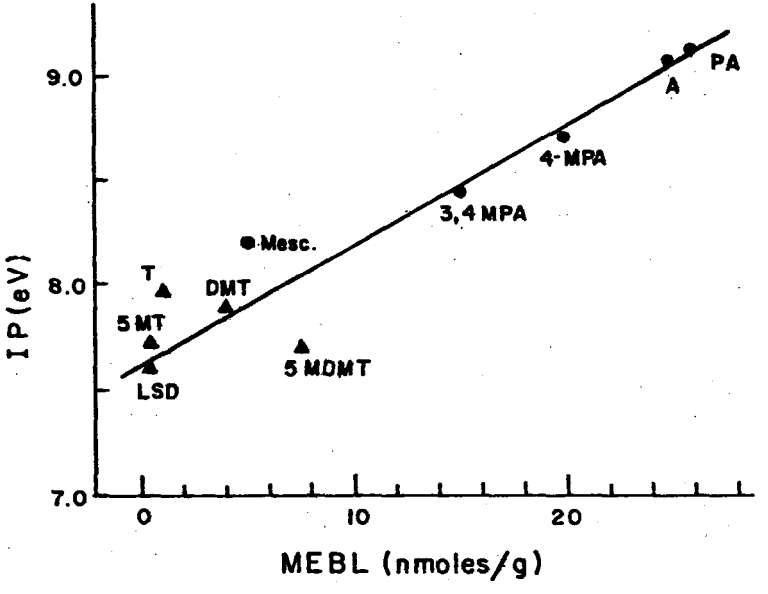



***Figure 9.*** *Correlation between minimum effective brain levels (MEBL) to interfere with conditioned avoidance respnse in rats (Vogel and Evans 1977) and average of the first and second IPs*

This plot includes both phenethylamines and tryptamines and is surprising for that reason, since most workers in this area believe that these compounds influence different receptors. More extensive structure-activity relationships have been tested for the hallucinogenic amphetamines, on which a variety of physical measurements have been made, and many good correlations between physical properties and activities have been found. Of course, the availability of human activity data measured by Shulgin and coworkers (1969) has provided, for this series of molecules, an unprecedented fund of information with which to test the importance of various physical properties upon hallucinogenic activity. Correlations between these hallucinogenic activities reported by Shulgin and coworkers (in molar mescaline units, MMU) and the IPs of amphetamines were tested.

For the first IP alone, a moderate correlation is found:

$$\log \text{MMU} = -2.37\ IP_1 + 19.53,$$
$$(n = 11,\ r = 0.86)$$

which indicates a substantial increase in activity with a decrease in IP, in accord with the expected increase in the complexing ability of the aromatic ring as the IP decreases. If $IP_2$, is also used, no sig-

nificant improvement is obtained, in light of the two independent parameters now included in the correlation equation:

$$\log MMU = -2.35\ IP_1 - 0.13\ IP_2 + 20.42$$
$$(n = 11,\ r = 0.86).$$

The most significant deviation from either of these equations is observed for 4-bromo-2,5-dimethoxyamphetamine, and this molecule was not included in the correlation. Even for those compounds fitting this equation better, there are some notable deviations which give a clue to how the correlation can be improved. For example, although the first two IPs for 3,4-dimethoxyamphetamine and 3,4-methylenedioxyamphetamine are very close, 3,4-methylenedioxyamphetamines is at least 2.8 times more active. Similarly, 2,4,5-trimethoxyamphetamine and 4-methyl-2,5-dimethoxyamphetamine have essentially identical IPs, but 4-methyl-2,5-dimethoxyamphetamine is approximately 4.4 times more active than 2,4,5-trimethoxyamphetamine. In each of these cases, the more active drug is the one which has a significiantly higher 1-octanol: water partition coefficient (see Table I); log P for 3,4-methylenedioxyamphetamine is 1.38 while that for 3,4-dimethoxyamphetamine is only 1.00 and log P for 4-methyl-2,5-dimethoxyamphetamine is 2.08 compared with 1.74 for 2,4,5-trimethoxyamphetamine.

Correlations between partition coefficients and hallucinogenic activities have been shown by Barfknecht, Nichols, and Dunn (1975), and more recently Nichols, Shulgin, and (1977) have described correlations of partition coefficients of both phenethylamines and amphetamines with 5HT stimulation in sheep umbilical artery preparations.

The Barfknecht et al. correlation, uses a set of amphetamines Similar

$$\log MU = 3.15 \log P - 0.50 \log P^2 - 3.17$$
$$(n = 26,\ r = 0.79)$$

to, but more exstensive than the set used in correlation. The Nichols et al. correlation, could be modified to eliminate the unusual

$$\log RBR = -0.89 \log P + 0.95(\log P)^2 - 0.16(\log P)^3 - 0.23$$
$$(n = 15,\ r = 0.98),$$

third term by using an indicator parameter to account for steric bulk of large alkyl substituents. Since both log P and IP are of value in Predicting activities, correlations combining both of these physical properties were tested. The relationships between hallucinogenic activity and $IP_1$, $IP_2$, and log P for the same eleven compounds are given by the equations:

$$\log MMU = -1.48\ IP_1 + 0.78 \log P + 11.15$$
$$(n = 11,\ r = 0.94)$$

$$\log MMU = -1.42\ IP_1, - 0.19\ IP_2 + 0.80 \log P + 12.30$$
$$(n = 11,\ r = 0.94)$$

Once again, inclusion of $IP_2$ gave no significant improvement. A plot the observed versus calculated activity according to the equation

using only $IP_1$ and log P is given in Figure 10. The 4-Br-2,5-dimethoxyamphetamine is included in this figure, but was excluded from the regression analysis.

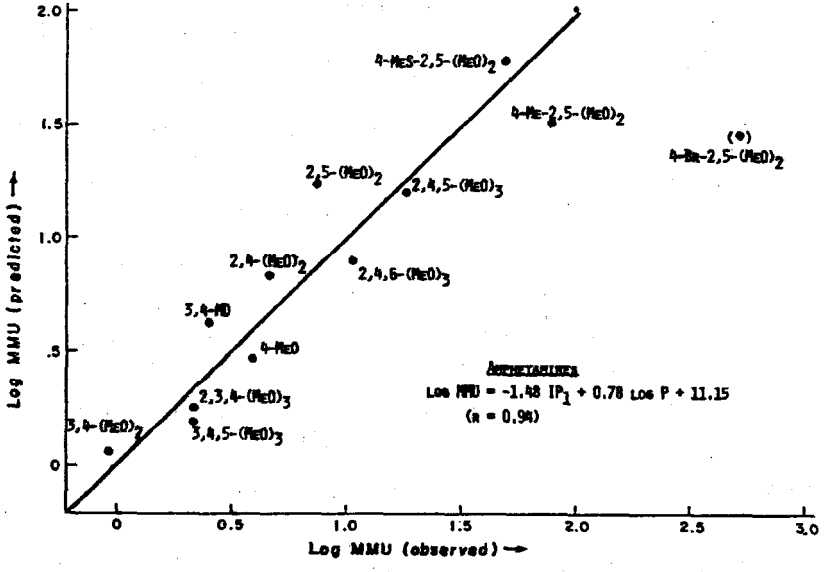



***Figure 10.*** *Plot of predicted versus observed hallucinogenic activities based on $IP_1$ and log P*

Ionization potentials have also been found to provide valuable indicators of potencies of various amphetamines and tryptamines. Green and coworkers have determined the inhibition of high affinity d-LSD binding by various amphetamines, tryptamines, mescaline and LSD in rat brain homogenates (Green et al. 1976). The relationship between the inhibition constant, $IC_{50}$, and $IP_1$ and $IP_2$ for the drugs mescaline, 3,4,5-, 2,4,6- and 2,4,5-trimethoxytryptamines, 2,4- and 2,5-dimethoxyamphetamine, 5-methyl- and 5-methoxytryptamine, tryptamine, and d-LSD, is given by

$$-\log IC_{50} = -3.81\ IP_1 - 1.64\ IP_2 + 47.78$$
$$(n = 10,\ r = 0.85)$$

Similarly, Bennett and Aghajanian (1974) measured drug concentrations required to displace d-LSD in rate brain homogenates for a wide variety of psychotropic drugs, including several that we have investigated by photoelectron spectroscopy: the phenothiazine tranquilizers, promethazine and chloropromazine (Domelsmith, Munchausen, and Houk 1977c). LSD, dimethyltryptamine, 4-methyl-2,5-dimethoxyamphetamine, mescaline,

and dopamine. The relationships between the effective dose necessary to displace 50 percent o f d-LSD, $ED_{50}$, and IPs are given by:

$$-\log ED_{50} = -3.49\ IP_1 + 32.50 \quad (n = 7,\ r = 0.92)$$

$$-\log ED_{50} = -2.79\ IP_1 - 1.93\ IP_2 + 43.26 \quad (n = 7,\ r = 0.97)$$

These relationships indicate that ionization potentials are quite promising predictors of the ability of hallucinogens to bind to the LSD binding site. We hope to test the generality of this conclusion with a more extensive series of compounds.

## CONCLUSION

These correlations suggest that the complexing abilities of the aromatic rings of amphetamines are very imporant in the production of hallucinogenic activity. Furthermore, even for the less closely related compounds, ionization potentials can predict the ability of molecules to bind to biological receptors.

Although only scratching the surface of compounds available for investigation, our studies show that photoelectron spectroscopy is not only capable of providing valuable experimental information about the electronic structures of hallucinogenic molecules, but the ionization potentials of the aromatic portions of these molecules can be used profitably in developing quantitative structure-activity relationships. From a more general point of view, the correlations we have observed help to restore some of the lost faith in the importance of electronic characteristics of molecules in producing biological activities.

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# An Assessment of Parameters in QuaSAR Studies of Narcotic Analgesics and Antagonists

Robert Katz, Steven Osborne, Florin Ionescu, Peter Andrulis, Jr., Robert Bates, William Beavers, Paul C. C. Chou, Gilda Loew, and Donald Berkowitz

Antinociceptive activity of narcotic agonists "in vivo," was recently quantitatively related to lipophilicity (log P). In both studies (Kutter et al. 1970; Jacobson, Klee and Dunn 1977), the types of analgesics included in analyses belonged to different classes with diverse chemical structures. They were, however, carefully selected to behave mainly as pure agonists "in vivo." The first part of our presentation concentrates on narcotic drugs capable of showing both agonist and antagonist effects, and the possible dependence of these opposite effects, inherent in the same structure, on log P. On the other hand, numerous previous studies (e.g., Kaufman and Kerman 1974; Kaufman, Popkie, and Koski 1976; Loew and Berkowitz 1975; Loew, Berkowitz, and Newth 1976) elaborated on the relationship between activity and minimum energy conformations of molecules or activity and charge densities calculated by various molecular orbital methods. We shall discuss the correspondence between charge densities (calculated by the INDO, PCILO, and IEHT molecular orbital methods) and the 13C NMR chemical shifts ($\delta$) of the protonated molecule measured in $D_2O$. We also extend the 13C NMR data of various mixed agonist-antagonists to structure and activity studies. Chemical structures for all the compounds included in the various studies (1a-6b) are presented in figure 1.

## LIPOPHILICITY AND BIOLOGIC ACTIVITY

Drug distribution coefficients (DDC) for compounds in table 1 were measured by dissolving the narcotic drug in n-octanol saturated with buffer (~pH 7.40 at 37°C). An approximately 10 fold excess of buffer was added and the mixture vigorously agitated (2 minutes), centrifuged (2000 rpm, 75 min.), and placed in a water bath (37°C, 90 min.). Five ml. aliquots were examined at 284 nm. against standard (20 mg. of sample in pretreated octanol). $pK_a$ values for (-) BC 2888 and for norketobemidone. HCl were determined in double distilled water under $N_2$ at 37°C. Values were calculated from the titration curve by using a computer program entitled PHPAIR. All other $pK_a$ values from table 1 were determined by others (see legend to table 1). Partition coefficients were calculated from DDC and $pK_a$ values by use of the following formula:

$$\log P = \log \left( \frac{DDC}{1 - [1 + \log^{-1} (pH - pK_a)]^{-1}} \right).$$

As for the biological activity data, receptor bindtng affinity in rat brain homogenates ($K_{RB}$) was measured by replacing specifically bound [$^3$H] dihydromorphine following the method of Klee and Streaty (1974). $K_{RB}$ (nmoles/i) is the concentration of dru required to inhibit 50 percent of the stereospecific binding of [$^3$H] dihydromorphine in the rat brain homogenates. Antagonist potency in the guinea pig ileum (Ke) and agonist binding ($ID_{50}$)(equivalent to agonist potency in the same "in vitro" system) were used as previously determined by Kosterlttz and his group (Kosterlitz and Watt 1968; Kosterlitz, Lord, and Watt 1973

Plots of log P vs. log Ke and log P vs. log $ID_{50}$ are shown in figures 2 and 3. A parabolic dependence of both agonist and antagonist activities on log P is obvious from these plots. Naloxone (2e). naltrexone (2a), nalorphine (la), oxymorphone (2b), and norketobemidone (5a) are members of a group with log P < 2.0, while the rest of the compounds (see table 1) are members of a group with log P > 2.5. The plot of log P vs. log agonist or antagonist potency shows good linear dependence in this second group. Optimum log P values between 2 and 2.5 can be approximated for both activittes.

Linear Hansch type correlations between log 1/Ke vs. log P and log $1/ID_{50}$ vs. log P provided extremely low correlation coefficients (0.46 and 0.066 respectively). The quadratic correlation with log P is presented in table 2. Equations 1 and 5 from this table are statistically significant, but still explain only 67 or 60 percent respectively of the variance present. A similarly "mediocre" correlation results when log 1/Ke is correlated with log $1/K_{RB}$ (eq. 3). However, combining log P and log $1/K_{RB}$ results in a significant improvement (eg. 2) R = 0.91, $R^2$ = 0.82.

It can-be said that a correlation coefficient of 0.88 for log $ID_{50}$ vs. log $1/K_{RB}$ tends to support the fact that there are fewer inherent possibilities for errors in measurement of $ID_{50}$ than in case of Ke. Thus, It is not surprising that eq. 4, combining both log P and log $1/K_{RB}$, provides the best correlation in table 2 (although statistically significant only at the 92 percent level). Eq. 2 and 4 yielded an ideal log P = 2.25 ± 0.44 for log 1/Ke and of 2.42 ± ∞ for log $1/ID_{50}$ in accordance with plots fmm figures 2 and 3.

The correlation reported by Kutter et al. (1970) is quadratic in log P. The biological activity correlated with log P is a ratio of two different "in vivo" agonist effects (in rabbits), and is considered to be a measure of different capability of analgesics to cross the blood brain barrier. The correlation is given below:

$$\log (C_{iventr}/C_{iv}) = 0.673 + 0.036 \log P - 0.090 (\log P)^2$$

n = 11, s = 0.297, $R^2$ = 0.941, R = 0.970 (eq. 8)

In this equation, as pointed out by Jacobson, Klee, and Dunn (1977), the log P term is statistically not warranted. However, a modified correlation, statistically valid, still contains the $(\log P)^2$ term.

Unfortunately, log P values in this study were measured in heptane-water system and therefore the calculated ideal log P = O is not directly comparable with ours. As mentioned above, Jacobson et al. obtained a correlation between antinociceptive activity (as measured by the standard hotplate assay in mice) and log P. Addition of a binding term quantitattng the binding affinity of the narcotic agonist to rat brain homogenate(identical with our $K_{RB}$), to log P, provided a good, statistically valid linear equation:

$$\log 1/C = 4.254 + 1.107 \log 1/K_{RB} + 0.317 \log P \quad \text{(eq. 9)}$$

$$n = 10,\ S = 0.62,\ R^2 = 0.82,\ R = 0.91$$

As the authors state, this correlation suggests that besides lipophilicity, antinociceptive activity depends on other parameters adequately expressed by "in vivo" receptor binding affinity. Returning to table 2, we can see that equations 2 and 4, the best for "in vitro" agonist as well as antagonist activities, are quadratic in log P and also contain the term $K_{RB}$ which quantitates 'in vitro" receptor binding affinity.

There is no contradiction between our findings and those discussed in eq. 8 and 9. While log P "in vivo" is synonymous with transport, "in vitro" it could be interpreted to represent a hydrophobic "pocket" on the receptor. Equations 2 and 4 could thus be considered extensions of eq. 8 and 9 to the receptor level. As reflected by eq. 2 and 4, the parallelism between agonist and antagonist behaviour at the receptor level is remarkable. No major differences am discernible, although by comparing equations 5, 1, 2, and 4, it appears that $K_{RB}$ is more relevant to agonist activity than to antagonist activity.

In summary, we can state that similarities between agonist and antagonist behaviour of a drug. as expressed by eq. 2 and 4, tend to support the presently accepted view of a receptor possessing agonist and antagonist conformations in equilibrium.

## ELECTRONIC FACTORS AND BIOLOGICAL ACTIVITY

We tntended to compare atomic charges (Q) calculated by means of various molecular orbital methods for compounds in figure 1 to their 13C NMR chemical shifts ($\delta$) ($D_2O$). This comparison could validate the use of $\delta$ values in structure and activity studies, as well as in Hansch type correlations, instead of Q values. Naloxone•HCl (2e), naltrexone•HCl (2a), nalbuphone•HCl (2c), and nalmexone•HCl (2g), all oxymorphone type antagonists or mixed agonist-antagonists, were Selected for study.

A recent crystal structure of naloxone (Karle 1974) pmvided the oxymorphone fused ring structure. Hydrogens were placed by using equalized standard bond length (Pople and Beveridge 197O) and bond angles (CCH, HCH, etc.). For methyl groups, tetrahedral geometry was used. The phenyl hydroxyl group was set in plane, trans to the furan oxygen at C4, a minimum energy conformation calculated by PCILO (Diner et al. 1969). The C14 hydroxyl group is ß and trans to C9. Heavy atoms were positioned as in recent naloxone crystal structures with the cyclopropyl methyl group of naltrexone . HCl following the cyclazocine•HCl geometry. The N-side chain geometries were

based on the following two torsion angles: $\tau_1$ defined as C9-N-C17-C18 and $\tau_2$ as N-C17-C18-19, ($\tau_1$ ABCD = clockwise rotation angle to superimpose A on D while looking from atom B to atom C). Cyclazocine•HCl (4a) was the only benzomorphan derivative included in the study.

PCILO charges were calculated for the six lowest local minimum energy conformations. The same conformations were chosen for both naltrexone and cyclazocine. INDO calculations were performed on the two lowest local minimum energy conformations of both molecules, while IEHT calculations were performed only on the lowest energy conformation of each molecule. Calculations for nalbuphone•HCl and nalmexone•HCl followed a similar pathway. The internal error far net charges is not clearly defined for INDO and PCILO results, although it appears smaller than that in IEHT, where charge inconsistency is the controlling force in the iteration procedure. For IEHT, the $\Delta Q$ due to internal error was set to be no greater than 0.005 electrons/atom.

13C NMR measurements for all of the above compounds, as well as others included in table 6 were taken on the protonated base in $D_2O$, as opposed to previous reported work (Terui et al. 1975; Carroll et al. 1976; Brine et al. 1976) where free bases and organic solvents were used, Broadband decoupled and offresonance NMR spectra were run on each sample at 22.63 MHz, on a Bruckner WH-90 spectrometer at 30-33°. The chemical shifts obtained were referenced to internal dioxane (at $\delta = 67.9$). The assigning of carbons to peaks was greatly aided by comparison with similar substances and by well established substituent effects (Levy and Nelson 1972; Wehrit 1973).

Comparison of the shifts measured in this study on protonated amines in $D_2O$ with shifts an related free amines in $DCCl_3$ and $_{d6}$-DMSO shows small differences, and no cases which suggest revision of assignments. In the case of naltrexone•HCl and naloxone•HCl (Carroll et al. 1976) the largest differences come in the $sp^2$ carbons of the N-allyl group; C19 is shifted downfield by protonation of the nitrogen, while C18 is shifted upfield. The shifts for the free allyl amine are similar to those in allyl ethyl ether, whereas those in the corresponding ammonium salt are much closer to those of ethyl acrylate (Levy and Nelson 1972).

13C NMR chemical shifts in $D_2O$ for naltrexone•HCl are compared in table 3 with atomic charges calculated by the INDO, PCILO, and IEHT methods in one minimum energy conformation (Martin, Martin, and Idiot 1975). When 13C-$\delta$ values of nonaromatic carbons with $sp^3$ hybridization (C5, C7-C16) are plotted against atomic charges (Krandall and Sojka 1972), it becomes apparent that 13C-$\delta$ vs. Q correlates better in the order INDO, PCILO, and IEHT. This improvement is evident from figures 4. 5, and 6. Only the atomic charges of the two quaternary carbons C13 and C14 are underestimated by IEHT. PCILO overestimates atomic charge on C5, C13, and C7 while underesti-. mates C14 and C16. INDO does well only with C8, C9, C1O, and C15.

Table 4 summarizes 13C-δ and $Q_{IEHT}$ values for naloxone•HCl, nalbuphone•HCl, and nalmecone•HCl. If plotted, these data show consistently good correlations comparable to the one for naltrexone•HCl from figure 5.

Results obtained for cyclazocine•HCl are shown in table 5. In this case net charge densities calculated by IEHT are shown to correlate best with the 13C-δ values of this benzomorphan derivative.

The above study suggests that 13C-δ values are valid measures of atomic charges possibly for a broad variety of narcotic structures. It is of interpst to point out that such 13C-δ vs. atomic charge plots have been used recently to validate consistency of CNDO/2 charges for carbonyl carbons in a series of neuroleptic drugs (Riga et al. 1977).

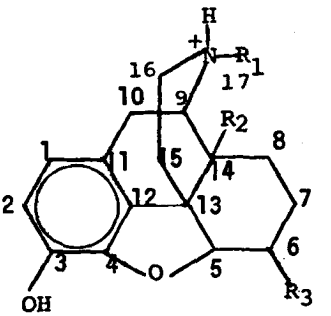
Let us then look at 13C-NMR data of various mixed agonist-antagonists as shown in table 6. C9, C16 and C17 are the three carbons neighbouring the nitrogen atom required for the activity of these compounds. C18-C21 are various side chain carbon atoms starting ß from the nitrogen. We could find no meaningful relationship between any of these chemical shifts, taken individually, and antagontst potency Ke, whether compounds are compared according to similar side chains or similar ring structures. The same lack of correlation was found when the sum C9, 16, 17-δ was compared with Ke.

However, across several classes with similar side chains, a certain pattern emerges. Thus, benzomorphans 4c, 4d, 4e, and 4f, with an isobutenyl moiety contained in the side chain, show an increase in ΣC9, 16, 17-δ, which paralleled an increase in the respective Ke values (see table 7). This could mean that an increased shielding of the carbon atoms α to nitrogen (as evidenced by the lower sum of chemical shifts) results in an increased antagonist potency of the drug. This can be rephrased to say that the less the inductive release of electrons by C9, C16 and C17 (presumably toward the nitrogen), the better antagonist the drug is,

Two other subgroups of compounds exist, each with a different side chain, but bath possessing the 146 hydroxyl group. It appears that both in subgroup one (2a and 3b, in table 8) and in subgroup two (3c, 2c, and 2d, in table 8), with a 148 hydroxyl group present, it is a decrease shielding that corresponds to increased antagonist potency. Thus, the dependence of potency on 13-δ is reversed for compounds with 148 hydroxyl group. The special role of this hydroxyl group in drastically reducing agonist effect of mixed agonist-antagonists, while increasing their antagonist effect, has been recently established (Gordon et al. 1974). These effects could result from the electron withdrawing properties of this group as they appear in table 8. Assumtng that the 148 hydroxyl group is the only reason for the above reversed dependence of potency on ΣC9,16,17-δ, a direct involvement of the 14ß hydroxyl in receptor binding could be

then tentatively hypothesized. Such an involvement has been recently considered a possibtlity by another group, based on considerations of conformational nature (Loew and Berkowitz 1977). We would like to emphasize that these conclusions should be considered extremely tentative until a more vigorous study with larger sets of compounds will be undertaken.

Figure 1. *Structures for morphine (1a-2g) and morphinan (3a-3e) derivatves*

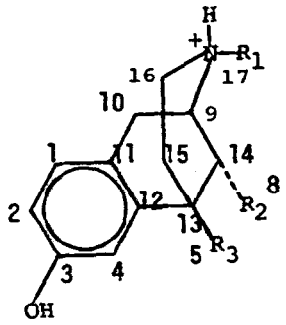



| # | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 1a | $-CH_2-CH=CH_2$ | -H | -OH ($\Delta$7-8) |
| 2a | $-CH_2$-cy-propyl | -OH | =O |
| 2b | $-CH_3$ | -OH | =O |
| 2c | $-CH_2$-cy-butyl | -OH | =O |
| 2d | $-CH_2$-cy-butyl | -OH | -OH |
| 2e | $-CH_2-CH=CH_2$ | -OH | =O |
| 2f | $-CH_2-CH=CH_2$ | -OH | $=CH_2$ |
| 2g | $-CH_2-CH=C(CH_3)_2$ | -OH | =O |
| 3a* | $-CH_2-CH=CH_2$ | -H | -H |
| 3b | $-CH_2$-cy-propyl | -OH | -H |
| 3c | $-CH_2$-cy-butyl | -OH | -H |
| 3d** | $-CH_2$-cy-propyl | -OH | -H |
| 3e | $-CH_3$ | -OH | -H |

*Compounds 3a-3e are morphinans and thus do not possess the $C_4$-$C_5$ oxygen bridge.

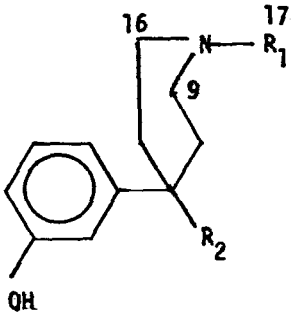
**This is the 3-nicotinyl derivative of compound 3b.

Figure 1. (continued) *Structures for benzomorphans (4a-4g), norketobemidone (5a) and phenylpyrrolidines (6a-6b)*

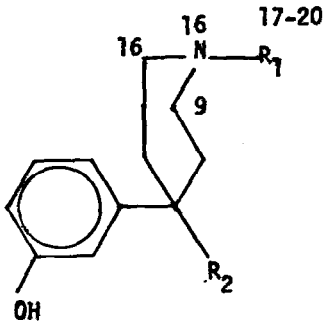



| # | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 4a | $-CH_2$-cy-propyl | $-CH_3$ | $-CH_3$ |
| 4b | $-CH_2$-cy-propyl | -H | $-C_6H_5$ |
| 4c* | $-CH_2$-3-furyl | $-CH_3$ | $-CH_3$ |
| 4d ** | $-CH_2$-3-furyl | $-CH_3$ | $-CH_3$ |
| 4e | $-CH_2$-2-furyl | $-CH_3$ | $-CH_3$ |
| 4f | $-CH_2-CH=C(CH_3)_2$ | $-CH_3$ | $-CH_3$ |
| 4g*** | $-CH_2$-3-furyl | $-CH_3$ | $-CH_3$ |

* = (-)-isomer; ** = (±)-mixture;
*** = 3-acetyl derivative




| # | $R_1$ | $R_2$ |
|---|---|---|
| 5a | H | $-OCOCH_2CH_3$ |




| # | $R_1$ | $R_2$ |
|---|---|---|
| 6a | $-CH_2$-cy-propyl | $-CH_2CH_2CH_3$ |
| 6b | $-CH_2-CH=CH_2$ | $-CH(CH_3)_2$ |

Figure 2. *Dependence of log antagonist potency the guinea pig ileum (log Ke) on octanol-water partition coefficients (log P)*

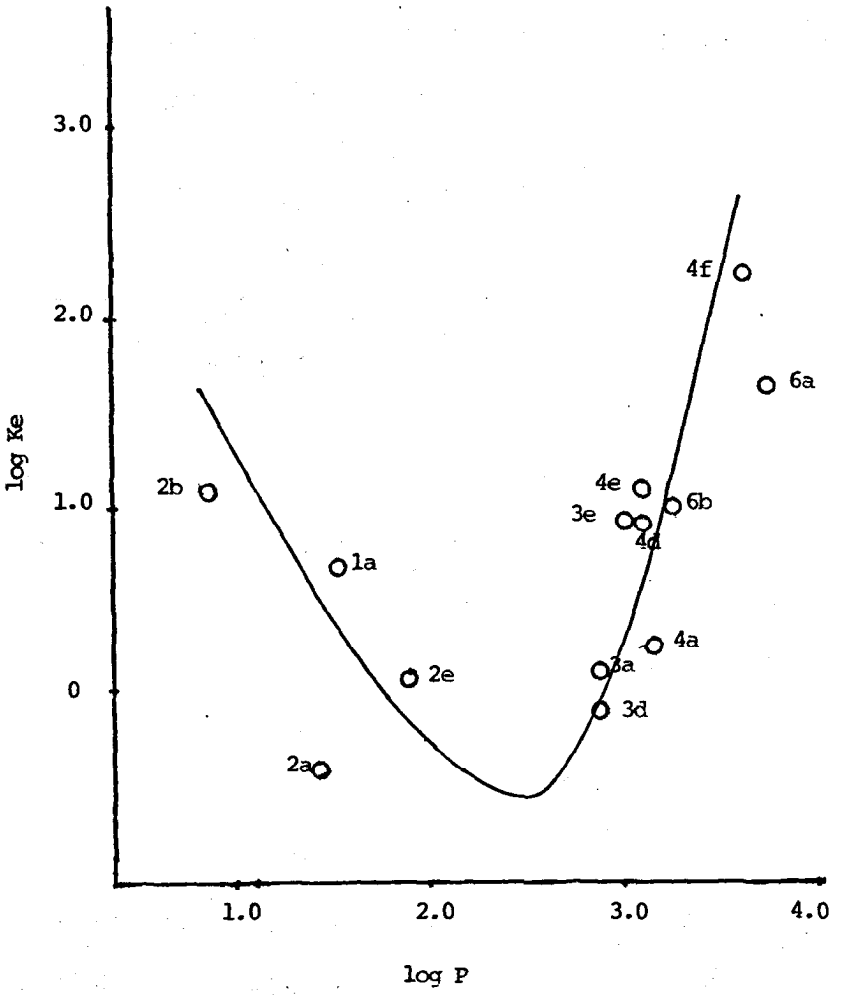


Figure 3. *Dependence of log agonist potency in the guinea pig ileum ($ID_{50}$) on octanol-water partition coefficients (log P)*

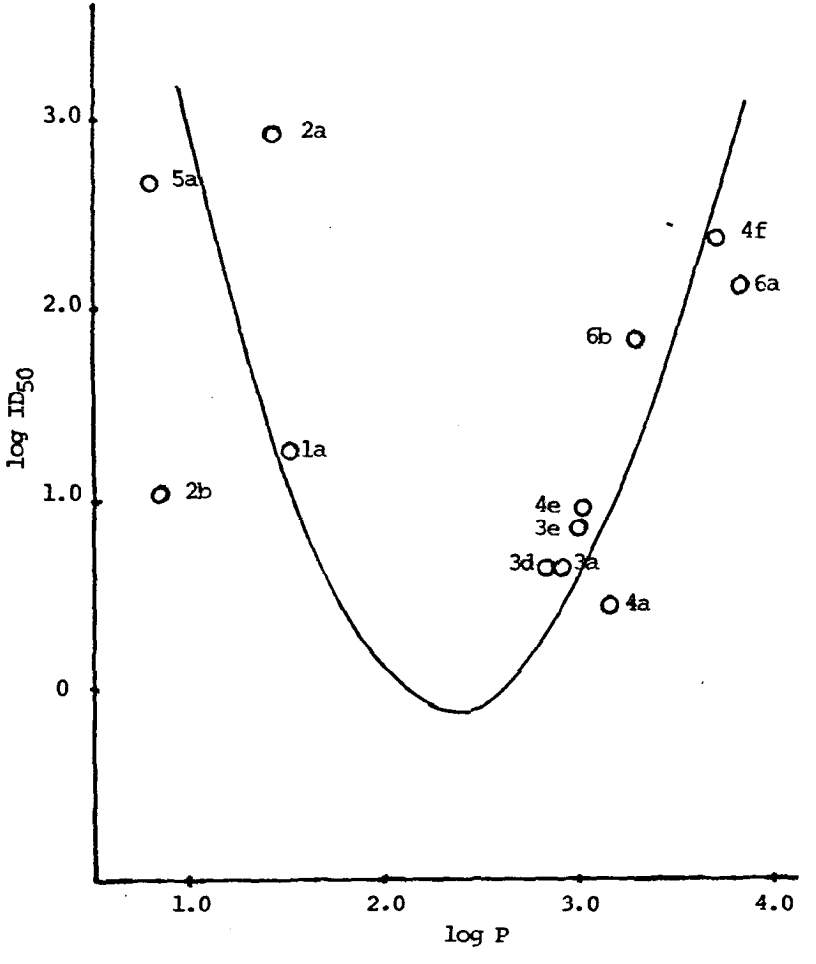


Figure 4. *Correlation between atomic charges (by INDO) and chemical shifts of nonaromatic carbon atoms in Naltrexone•HCl*

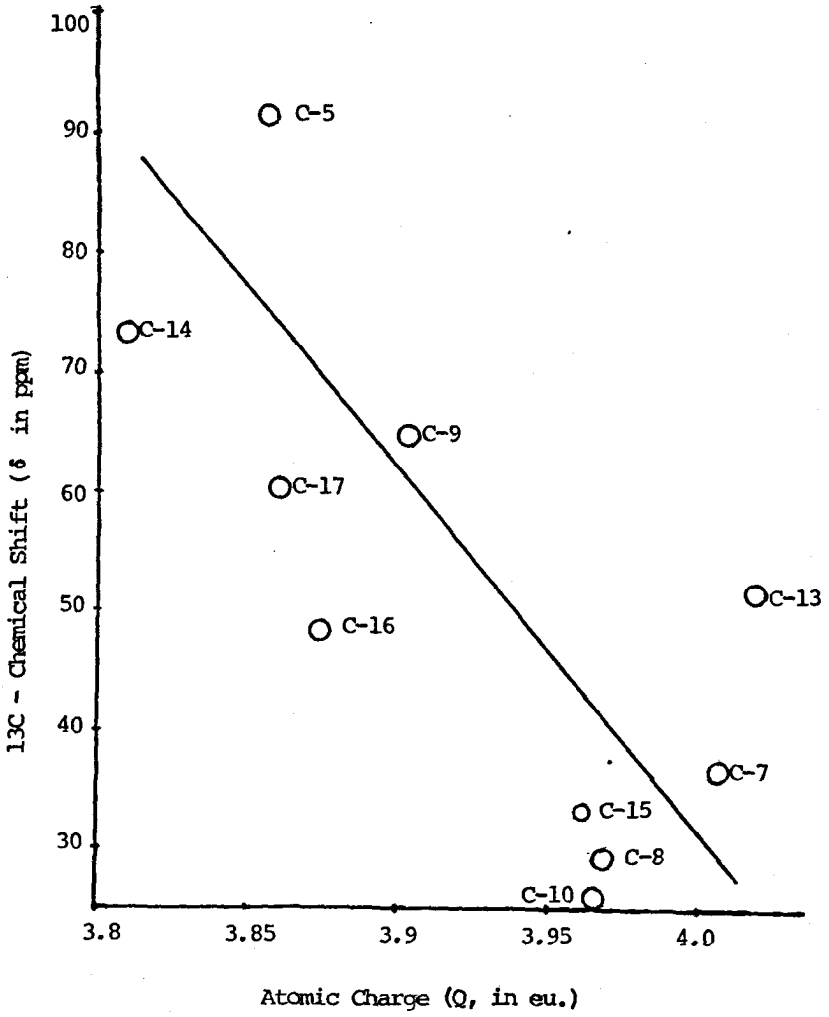


Figure 5. *Correlation between atomic charges (by PCILO) and chemical shifts (in $D_2O$) of nonaromatic carbon atoms in Naltrexone•HCl*

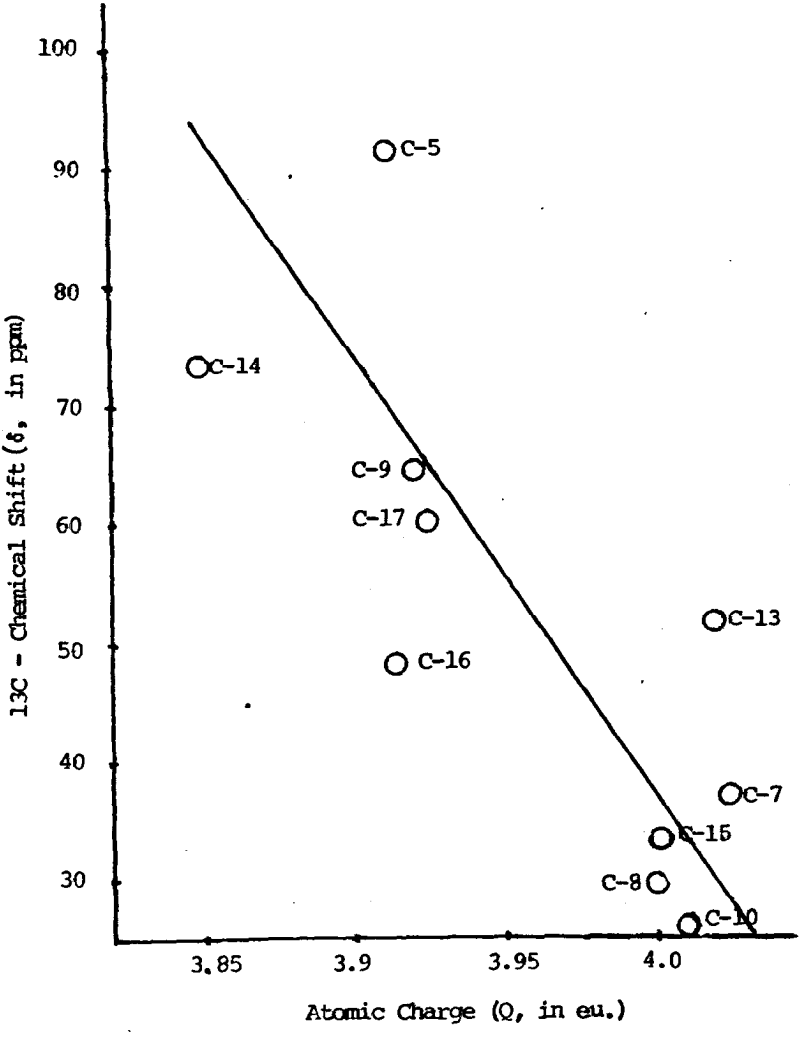


Figure 6. *Correlation between atomic charges (by IEHT) and chemical shifts (in $D_2O$) of nonaromatic carbon atoms in Naltrexone•HCl*

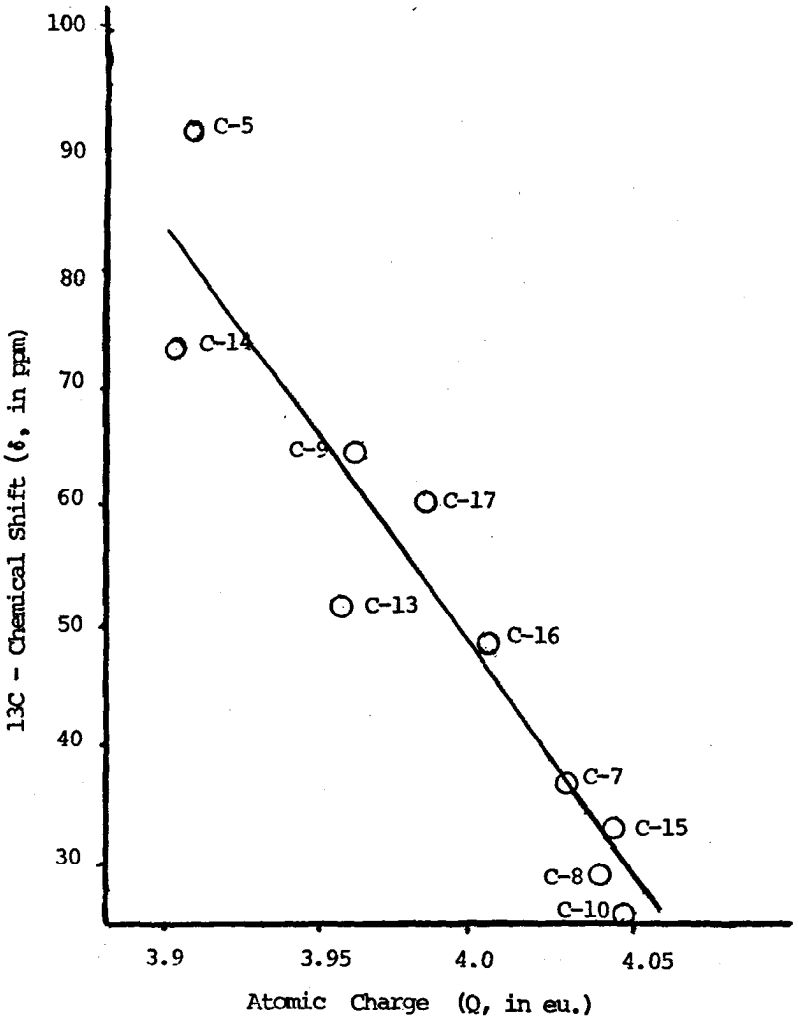


Table 1. *Drug distribution coefficients (DDC), $pk_\alpha$, octanol-water partition coefficients (log P), antagonist and agonist potency in the guinea pig ileum (Ke and $ID_{50}$) and binding to the rat brain homogenate ($K_{RB}$) for 14 narcotic agonists, mixed agonist-antagonists and antagonists*

| # | Compound | DDC[a] | $pK_a$ | log P | Ke[j] | $K_{RB}$ | $ID_{50}$[j] |
|---|---|---|---|---|---|---|---|
| 1a | Nalorphine . HCl | 14.99[b] | 7.59[h] | 1.58 | 4.47 | 1.5[k] | 24.30 |
| 2a | Naltrexone . HCl | 4.47[c] | 8.13[h] | 1.47 | 0.38 | 0.2[l] | 850.00 |
| 2e | Naloxone . HCl | 20.72[d] | 7.82[h] | 1.91 | 1.22 | 1.0[k] | -- |
| 3a | Levallorphan . tartrate | 53.80[d] | 8.43[h] | 2.84 | 1.12 | 0.6[k] | 4.28 |
| 4a | Cyclazocine . HCl | 14.50[d] | 9.38[h] | 3.19 | 1.48 | 0.8[k] | 3.60 |
| 4f | Pentazocine . HCl | 95.53[d] | 9.16[h] | 3.74 | 151.00 | 10.0[k] | 250.00 |
| 4d | (±) MR 1256 MS | 111.80[e] | 8.42[h] | 3.08 | 6.83 | 3.0[k] | -- |
| 4e | (±) MR 1029 MS | 323.93[e] | 7.86[h] | 3.08 | 10.40 | 2.5[k] | 10.70 |
| 3e | Levorphanol . tartrate | 10.80[d] | 9.37[h] | 3.05 | 7.00 | 0.5[l] | 9.18 |
| 3d | (-) BC 2888 | 15.35[f] | 9.02 | 2.82 | 0.71 | 0.2[k] | 4.25 |
| 2b | Oxymorphone . HCl | 1.35[g] | 8.17[h] | 0.89 | 11.20 | 0.5[l] | 12.10 |
| 5a | Norketobemidone . HCl | 0.21[g] | 9.01 | 0.87 | -- | 10.0[l] | 491.00 |
| 6a | PD-1 . monosuccinate | 51.76[f] | 9.50[i] | 3.82 | 29.80 | 4.0[l] | 141.00 |
| 6b | PD-3 . HCl | 48.53[f] | 9.00[i] | 3.30 | 8.80 | 10.0[l] | 73.20 |

a) DDC determined @ 37° ±0.1°C; b) Determined at pH 7.41; c) pH 7.38; d) pH 7.36; e) pH 7.43; f) pH 7.40; g) pH 7.49; h) Kaufman et al. 1975, in water or in 50% EtOH and adjusted according to the relationship $pK_a$ ($H_2O$) = $pK_a$ (50% EtOH) + 0.5 pH; i) Parke & Davis 1954, in 50% MeOH adjusted as in h); j) from Kosterlitz and Watt 1968 and Kosterlitz, Lord & Watt 1973; k) from Ionescu, Klee & Katz 1975; l) measured in our laboratory.

Table 2. *Hansch type correlations between biological activities (Ke, $ID_{50}$, $K_{RB}$) and octanol-water partition coefficients of narcotic durgs from table 1.*

| | Equation* | N | S | $R^2$ | R |
|---|---|---|---|---|---|
| 1. | $\log 1/Ke = -3.0016 + 2.8979 \log P - 0.6839 (\log P)^2$ | 13 | 0.45 | 0.67 | 0.82 |
| 2. | $\log 1/Ke = -2.6848 + 0.6582 \log 1/K_{RB} + 2.26 \log P - 0.5 (\log P)^2$ | 13 | 0.35 | 0.82 | 0.91 |
| 3. | $\log 1/Ke = -0.5694 + 0.9929 \log 1/K_{RB}$ | 13 | 0.46 | 0.62 | 0.78 |
| 4. | $\log 1/ID_{50} = -2.8223 + 0.8612 \log 1/K_{RB} + 1.6452 \log P - 0.3406 (\log P)^2$ | 11 | 0.29 | 0.89 | 0.95 |
| 5. | $\log I/ID_{50} = 4.4133 + 3.3867 \log P - 0.7419 (\log P)^2$ | 11 | 0.53 | 0.60 | 0.78 |
| 6. | $\log 1/ID_{50} = -1.1409 + 1.1067 \log 1/K_{RB}$ | 11 | 0.37 | 0.78 | 0.88 |
| 7. | $\log 1/K_{RB} = -1.5954 + 1.825 \log P - 0.434 (\log P)^2$ | 14 | 0.51 | 0.38 | 0.62 |

*All equations except #4 are statistically significant at the 95% confidence level or above. Equation 2 is significant at the 92% confidence level.

Table 3

*13C NMR chemical shifts in D2O and atomic charges for Naltrexone ·HCl (Q) as calculated by the INDO, PCILO, and IEHT molecular orbital methods*

| # Carbon Atoms | 13C-δ (in ppm) | Q (eu.) | | |
|---|---|---|---|---|
| | | INDO | PCILO | IEHT |
| 1 | 123.6 | 3.9748 | 4.0433 | 4.0538 |
| 2 | 121.5 | 4.0300 | 4.1406 | 4.0422 |
| 3 | 146.7* | 3.7978 | 3.8171 | 3.9290 |
| 4 | 142.1* | 3.8486 | 3.9272 | 3.9199 |
| 11 | 124.4 | 4.0031 | 3.9364 | 4.0092 |
| 12 | 130.1 | 4.0367 | 4.0811 | 3.9857 |
| 5 | 91.9 | 3.8588 | 3.9124 | 3.9080 |
| 6 | 214.2 | 3.7242 | 3.8312 | 3.8720 |
| 7 | 37.0 | 4.0085 | 4.0251 | 4.0344 |
| 8 | 29.8 | 3.9685 | 4.0061 | 4.0449 |
| 9 | 64.9 | 3.9040 | 3.9208 | 3.9625 |
| 10 | 25.8 | 3.9662 | 4.0108 | 4.0555 |
| 13 | 51.8 | 4.0114 | 4.0200 | 3.9592 |
| 14 | 73.2 | 3.8107 | 3.8512 | 3.9032 |
| 15 | 33.2 | 3.9613 | 4.0017 | 4.0446 |
| 16 | 48.7 | 3.8746 | 3.9155 | 4.0047 |
| 17 | 60.7 | 3.8599 | 3.9223 | 3.9873 |
| 18 | 7.0 | 3.9893 | 3.9950 | 4.0348 |
| 19 | 5.2* | 3.9523 | 3.9829 | 4.0785 |
| 20 | 7.4* | 3.9715 | 4.0021 | 4.0800 |

*δ values for pairs 3, 4 and 19, 20 are interchangeable. Minimum energy conf. for side chain:

INDO (orthogonal) $\tau_1 = 300$ $\tau_2 = 210$ ($\tau_1 = 210$;

PCILO $\tau_1 = 300$ $\tau_2 = 210$ $\tau_2 = 90°$).

IEHT $\tau_1 = 300$ $\tau_2 = 210$

Table 4. *13C NMR chemical shifts and atomic charges for other mixed mixed agonist-antagonist members of the oxymorphone family*

| # of Carbon Atoms | Naloxone* HCl (2e) 13C-δ (ppm) | Naloxone* HCl (2e) $Q_{IEHT}$ (e.u.) | Nalbuphone** HCl (2c) 13C-δ (ppm) | Nalbuphone** HCl (2c) $Q_{IEHT}$ (e.u.) | Nalmexone*** HCl (2g) 13C-δ (ppm) | Nalmexone*** HCl (2g) $Q_{IEHT}$ (e.u.) |
|---|---|---|---|---|---|---|
| 5 | 92.4 | 3.9074 | 91.8 | 3.9081 | 91.5 | 3.9074 |
| 6 | 214.0 | 3.8709 | 214.3 | 3.8716 | 214.0 | 3.8709 |
| 7 | 37.5 | 4.0367 | 37.1 | 4.0391 | 30.8 | 4.0367 |
| 8 | 30.2 | 4.0417 | 29.6 | 4.0440 | 29.5 | 4.0418 |
| 9 | 65.3 | 3.9525 | 64.9 | 3.9533 | 64.4 | 3.9534 |
| 10 | 25.8 | 4.0574 | 25.7 | 4.0582 | 25.5 | 4.0574 |
| 13 | 52.0 | 3.9585 | 51.6 | 3.9589 | 51.5 | 3.9586 |
| 14 | 73.5 | 3.9033 | 73.1 | 3.9036 | 72.9 | 3.9036 |
| 15 | 33.5 | 4.0449 | 33.3 | 4.0475 | 32.9 | 4.0452 |
| 16 | 49.0 | 3.9894 | 49.1 | 3.9907 | 48.0 | 3.9911 |
| 17 | 58.7 | 3.9869 | 60.5 | 3.9917 | 53.9 | 3.9938 |

*Minimum energy conformation (MEC) of side chain: $\tau_1 = 180°$, $\tau_2 = 240°$

**MEC of side chain: $\tau_1 = 300°$, $\tau_2 = 300°$ ***MEC of side chain: $\tau_1 = 180°$, $\tau_2 = 210°$

Table 5. *13C NMR chemical shifts (in $D_2O$) and net charge densities for the benzomorphan derivative Cyclazocine•HCl in the respective minimum energy conformation*

| #C | 13C-δ (in ppm) | $Qx10^2$ INDO | PCILO | IEHT |
|---|---|---|---|---|
| 5 | 25.6 | 4.84 | -1.16 | -11.37 |
| 6 | - | - | - | - |
| 7 | - | - | - | - |
| 8 | 14.8 | 4.83 | -0.62 | -11.64 |
| 9 | 60.3 | 11.40 | 9.19 | 3.12 |
| 10 | 24.2 | 3.84 | -0.52 | -6.77 |
| 13 | 36.6 | 3.60 | 4.81 | -1.60 |
| 14 | 40.6 | 4.15 | 3.43 | -3.68 |
| 15 | 40.6 | 2.92 | -0.01 | -5.90 |
| 16 | 48.1 | 13.00 | 8.66 | 0.90 |
| 17 | 60.3 | 14.19 | 7.90 | 0.89 |

**Regression on all above 13C-δ vs Q x $10^2$ provided the following equations:**

10. 13C-δ = 20.72 + 2.6 INDO
    n = 9, S = 11.12, $R^2$ = 0.56, R = 0.75
11. 13C-δ = 27.66 + 3.22 PCILO
    n = 9, S = 8.06, $R^2$ = 0.77, R = 0.88
12. 13C-δ = 49.8 + 2.70 IEHT
    n = 9, S = 6.76, $R^2$ = 0.84, R = 0.92

Table 6. *13C NMR data for mixed narcotic agonist-antagonists (in $D_2O$)*

| | | 13C-NMR chemical shifts and sums of chemical shifts (δ in ppm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| # | Compound | C-9 | C-16 | C-17 | C-18 | C-19 | C-20 | C-21 | ΣC-9, 16,17 | Antagonist Potency, Ke |
| 4a | Cyclazocine HCl | 60.3 | 48.1 | 60.3 | 7.8 | 6.1 | 6.1 | -- | 168.7 | 1.22 |
| 4b | GPA 1833 HCl | 57.4 | 49.1 | 61.6 | 8.6 | 6.7 | 6.7 | -- | 168.1 | 4.11 |
| 4c | MR 1452 (-)$MeSO_3H$ | 59.4 | 47.7 | 49.1 | 114.7 | 145.8 | 145.8 | 112.1 | 156.2 | 6.05 |
| 4d | MR 1256 $MeSO_3H$ | 60.3 | 48.4 | 50.1 | 115.7 | 146.5 | 146.5 | 113.0 | 158.8 | 6.83 |
| 4e | MR 1029 $MeSO_3H$ | 61.3 | 48.5 | 51.6 | 145.9 | 147.9 | 113.7 | 116.9 | 161.4 | 10.40 |
| 4f | Pentazocine HCl | 61.7 | 48.7 | 54.9 | 114.5 | 148.0 | 28.0 | 20.5 | 165.3 | 151.00 |
| 4g | MR 1405 HCl | 59.0 | 48.3 | 48.9 | 114.8 | 145.7 | 145.7 | 112.2 | 154.7 | 7.07 |
| 3a | Levallorphan Tartrate | 60.4 | 48.5 | 58.5 | 128.5 | 128.8 | -- | -- | 167.4 | 1.12 |
| 2a | Naltrexone HCl | 64.9 | 48.7 | 60.7 | 7.0 | 5.2 | 7.4 | -- | 174.3 | 0.38 |
| 3b | Oxilorphan Tartrate | 63.1 | 48.6 | 59.6 | 7.3 | 9.4 | 6.6 | -- | 171.3 | 0.86 |
| 3c | Butorphanol Tartrate | 64.9 | 48.1 | 60.5 | 33.1 | 27.1 | 20.5 | 31.1 | 174.7 | 2.14 |
| 2c | EN 1655A HCl | 64.0 | 50.0 | 60.9 | 32.8 | 27.8 | 20.6 | 29.1 | 174.5 | 3.00 |
| 2d | EN 2234A HCl | 64.3 | 48.1 | 60.1 | 32.5 | 27.4 | 20.2 | 29.1 | 172.5 | 9.00 |
| 2e | Naloxone HCl | 65.3 | 49.0 | 58.7 | 128.6 | 129.2 | -- | -- | 173.0 | 1.22 |
| 2f | EFH-I-27-2 HCl | 65.4 | 49.1 | 58.3 | 128.5 | 128.9 | -- | -- | 173.1 | 3.00 |
| 2g | EN 1620A HCl | 64.4 | 48.1 | 53.9 | 113.9 | 147.5 | 27.4 | 20.0 | 166.3 | 43.00 |

**Table 7**

*Dependance of antagonist potency (Ke), on the sum of C-9,16,17 chemical shifts in compounds without 14ß-OH substituent*

| Number | ΣC-9,16,17 (δ in ppm) | Ke |
|---|---|---|
| 4c | 156.2 | 6.05 |
| 4d | 158.8 | 6.83 |
| 4e | 161.4 | 10.40 |
| 4f | 165.3 | 151.00 |

**Table 8**

*Dependence of antagonist potency (Ke), on the sum of C-9,16,17 chemical shifts in compounds with 14ß-OH substitent*

| Number | ΣC-9, 16, 17 (δ in ppm) | Ke |
|---|---|---|
| 2a* | 174.3 | 0.38 |
| 3b* | 171.3 | 0.86 |
| 3c** | 174.9 | 2.14 |
| 2c** | 174.5 | 3.00 |
| 2d** | 172.5 | 9.00 |

*Compounds which have cyclopropyl methyl substituents on the nitrogen atom

**Compounds which have cyclobutyl methyl substituents on the nitrogen atom

## ACKNOWLEDMENT

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# Conformational Studies on Phenethylamine Hallucinogens: The Role of Alpha Alkyl Substitution

Alexandros Makriyannis and James Knittel

## INTRODUCTION

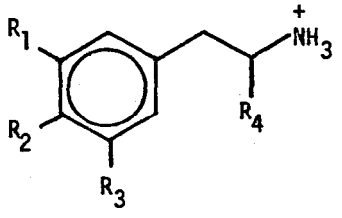
A large number of methoxyphenethylamine analogs have been synthesized (Shulgin 1970; Barfknecht and Nichols 1971) and evaluated in humans (Shwlgin, Sargent, and Naranjo 1969; Shulgin 1973) and animals (Aldous, Barrass, and Brewster 1974; Smythies, Bradley, and Johnston 1967) for psychotomimetic activity. The overall picture which emerges from these evaluations shows that small structural variations can produce drastic changes in the psychotropic potency of these compounds, making them particularly suitable for studies in structure activity relationships.

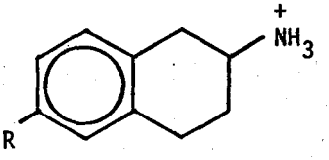
The dramatic effect that alpha alkyl substitution has on the pharmacological activity of these phenethylamines is of particular interest. It is thus well known (Shulgin 1970) that the presence of an alpha methyl group increases considerably the psychotomimetic potency of the corresponding phenethylamine. On the other hand, alpha ethyl substituted analogs are devoid of psychotomimetic activity and act as functional antagonists for their alpha methyl counterparts (Shulgin 1968; Standridge et al. 1976). Activity is recovered if the alpha ethyl group is tied back on the phenyl ring to give tetralin analogs which are weaker agonists (Violland, Violland-Duperet and Pacheco 1971; Nichols, Barfknecht et al. 1974).

In the present study we have used model methoxyphenethylamine analogs (1-5; figure 1) to study the effect of alpha alkyl substitution on the conformational properties of the phenethylamine component in these molecules. The information thus obtained was used to explain their observed divergent pharmacological properties.

The conformation of a drug is relevant to any consideration of its interaction with the receptor. Like many small molecules, phenethylamines and their alpha alkyl analogs are potentially flexible, and thus, may exist in solution in a number of conformations in equilibrium with one another. Of these, the most stable are the perfectly staggered conformations shown in figure 2, Since it is generally recognized that a flexible drug has only one *pharmacophoric conformation* in its complex with the receptor, the formation of this com-

FIGURE 1




1 $R_1, R_3, R_4$=H; $R_2$=$OCH_3$

2 $R_1, R_3$=H; $R_2$=$OCH_3$; $R_4$=$CH_3$

3 $R_1, R_2, R_3$=$OCH_3$; $R_4$=$CH_3$

4 $R_1$=H; $R_2, R_3$=$OCH_3$; $R_4$=$C_2H_5$

5 R=$OCH_3$

plex must involve a *conformational selection* which will influence the kinetics and energetics of complex formation. If the drug is present in more than one conformation, the extent to which the drug exists in the *pharmacophoric conformation* should influence its reactivity.

To study the conformational distribution of the model methoxyphenethylamine salts in solution, we used nuclear magnetic resonance (NMR) spectroscopy, which is clearly the experimental method of choice. We have also sought to obtain information on the role of the solvent in the conformational behavior of phenethylamines. by conducting our studies in two different solvents, $D_2O$ and $CDCl_3$, whenever possible. Because of its lower polarity, $CDCl_3$ can be expected to represent more accurately the hydrophobic environment of the nerve membrane.

It has been argued (Makriyannis 1974; Mautner 1974) that, in addition to conformation, molecular flexibility may play an important role during the drug-receptor interaction. To better evaluate the role that the conformational distribution of phenethylamine salts in solution plays on their biological activity, it is important to have information on the rate of conformational interconversion. If the compound exists predominantly in its *pharmacophoric conformation,* restricted flexibility will enhance its interaction with the receptor. On the other hand, if the predominant drug conformation is not the *pharmacophoric* one, the restricted rotation will hinder the conformational change necessary for the drug to achieve its proper conformation and will thus affect adversely the drug-receptor interaction. Such an observation (Gannelin 1973) served to explain the different pharmacological activities of histamine and 4-methylhistamine.

In this study, we have obtained a reasonable semiquantitative index for the molecular flexibility of phenethylamine salts in $D_2O$ solu-

tion by measuring the $^{13}C$ spin-lattice relaxation times ($T_1$) of individual carbon atoms. The information on molecular flexibility was then integrated into the results obtained from conformational analysis. The outcome was a more accurate dynamic picture for the conformational behavior of each molecule in solution. Such a picture gives us more insight into the possible mode of drug-receptor interaction (figure 3).

## METHODS

### A. Conformational Analysis

No single measurement can provide enough information for the unambiguous conformational analysis of potentially flexible molecules in solution. We have thus sought more than one criterion in our conformational assignments. As a result, we were able to clear. some remaining ambiguities existing in the literature related to the conformational analysis of alpha methylphenethylamines in solution (Neville et al. 1971).

#### 1. $^1H$ Coupling Constants

The measureument of vicinal $^1H$-$^1H$ coupling constants is the most direct and still the most important method used in the conformational analysis of small molecules in solution. The principle of this method is described in the Karplus equation (Karplus 1959) which states that the spin-spin coupling constant between proton attached to two adjacent carbon atoms is proportional to $\cos^2\phi$, the dihedral angle about the C-C band. In our system, the dihedral angle of interest is the one about the central Ar-C-C-N bond of the arylphenethylamine.

The two benzylic protons in the alpha alkylphenethylamine analogs (2, 3, 4) and those on the $C_1$ carbon of compound 5 constitute the AB portion of an ABC spin system. The four protons on the $ArCH_2CH_2CH_2N$ fragment of the phenethylamine analog are of the AA'BB' type. Initial estimates of the spectral parameters were obtained by standard methods (Pople, Schneider, and Bernstein 1969; Makriyannis et al. 1972) assuming that the corresponding spectra were approximately ABX and AA'XX'. The parameters were then refined by spectral simulation and iteration using Nicolet ITRCAL program.

The coupling constants thus obtained were considered to be averaged values arising from a mixture of three possible perfectly staggered rotamers. The relative population of each rotamer was then extracted from simple analogies which include $J_g$ and $J_t$ terms for the coupling constants of perfectly staggered *gauche* and *trans* vicinal protons.

While calculating the conformer distribution for the compounds under study, we made a special effort to consider the uncertainties inherent in the above method. The first of these uncertainties is the spectral assignment for each of the two benzylic protons in alpha alkylphenethylamines. Previous assignments (Neville et al. 1971; Bailey et al. 1971) in similar spin systems depended on pre-

dicting trends in the relative distribution of conformers among a series of analogs, based on stereochemical considerations. We have, in this study, provided additional spectral evidence which confirmed the previous assignments.

A second uncertainty is introduced with the choice of $J_g$ and $J_t$ values. to be used in our calculations. These values vary and to a large extent are dependent on the substituents of the C-C bond. They can thus either be obtained from model compounds in which the HCCH dihedral angle is fixed, or can be calculated from a number of semiempirical equations. In this study we have chosen those $J_g$ and $J_t$ values which gave results with the best internal consistency. We have used a combination of values (Table I, page ), some obtained from model compounds and others calculated from an empirical relationship between vicinal coupling constants and substituent electronegativities. This relationship (Abraham and Gatti 1969) has given us satisfactory results in the past (Makriyannis et al. 1972).

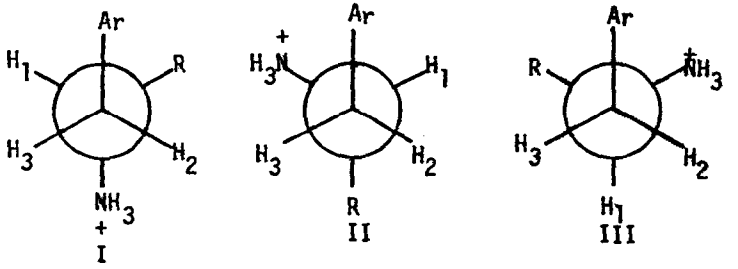
The final uncertainty considered here is related to the assumption that measured vicinal coupling constants with intermediate values, in between those of $J_g$ and $J_t$, are the result of the population-weighted average of the $J_g$ and $J_t$ values in three perfectly staggered rotamers. Although perfectly staggered rotamers are generally accepted as the most stable, the possibility must be considered that an off-staggered stable rotamer could exist in solution. The presence of such a conformer, with dihedral angles different from the usually assigned values of 60° and 180°, could lead to serious errors in our calculations. It could also mean that a single distorted conformer may account for observed coupling constants with intermediate values. Such a possibility is more likely in the case of sterically crowded compounds with large free energy differences among individual conformers.

In order to settle this uncertainty we undertook low temperature studies on compound 5 and other alpha ethylphenethylamine analogs. Since the coupling constants of a rotamer are usually minimally affected by temperature, a change in the observed vicinal coupling constants is an indication of a change in rotamer distribution. No change in the coupling constants with temperature indicates that, most probably, the compound exists solely in one conformation. Our low temperature experiments on alpha ethylphenethylamines in $CD_3OD$ and in $CDCl_3$ showed a considerable variation in the vicinal coupling constants with temperature, and indicated that these compounds exist in solution as an equilibrium mixture of two or more conformers. We shall report on these experiments in greater detail elsewhere. The results can be extrapolated to the other open chain phenethylamine analogs considered in this study and used as evidence against the presence of any of these compounds in solution as a single distorted conformer. Further evidence is, however, necessary to rule out the possibility that any of the compounds could exist as an equilibrium mixture of two or more off-staggered conformers.

## 2. Chemical Shifts

A careful examination of the $-CH_2CH-$ component in the $^1H$ NMR spec-

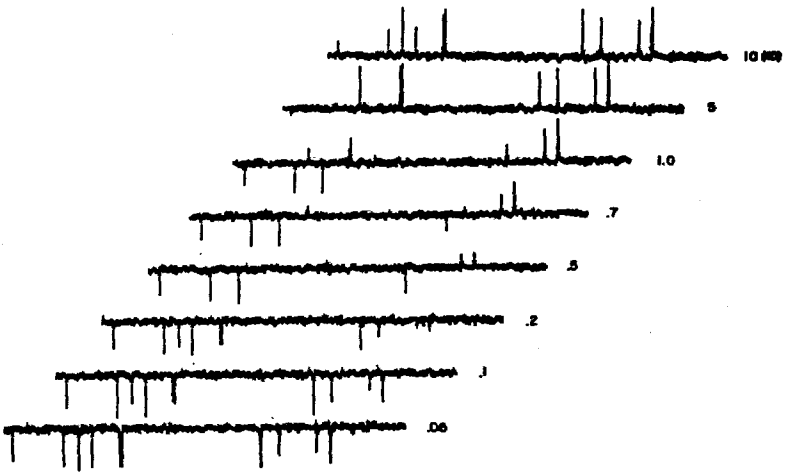
FIGURE 2




R = H, $CH_3$ or $C_2H_5$

Ar = 4-$OCH_3C_6H_5$; 3,4-di$OCH_3C_6H_5$; 3,4,5-tri$OCH_3C_6H_5$

FIGURE 3




Selected spectra from a $^{13}C$ spin-lattice relaxation tine ($T_1$) experiment on 2-amino-6-methoxytetralin hydrochloride. Determinations were made with complete $^1H$ decoupling using a $(-180°-\tau-90°-T-)_n$ inversion recovery pulse sequencewhere t is experimentally varied ($\tau$=0.05, .1, .2, .5, .7, 1.0, 5 and 10 sec) and T is equal to at least five times the longest $T_1$ to be measured.

trum of the alpha alkyl arylethylamines under consideration revealed that valuable information could be learned from the chemical shift differences between the two $H_2$, $H_3$ benzylic protons in this ABC system. These AB protons, which frequently give rise to two individual "quartets", have different chemical environments as a result of their different positions relative to their two vicinal substituents: the $NH_3$ and the alkyl groups. These substituents exert respectively deshielding and shielding influences with the more pronounced effects being experienced by the proton situated *gauche* to the substituent. The population-weighted average of these effects in the three possible rotamers determines the relative chemical shifts for each of the benzylic protons.

Additional useful information was gained by observing the spectra of the phenethylamine analogs in $D_2O$ and in $CDCl_3$ solutions. Solvent variation was usually accompanied by considerable changes in the chemical shifts and the coupling constants of the side chain protons. Chemical shift changes can be attributed to a reduction in the deshielding influence of the $NH_3$ group upon the neighboring *gauche* proton when $CDCl_3$ is replaced with $D_2O$. Such a modification of the deshielding influence of a charged nitrogen by solvent has been already reported (Reynolds and Priller 1968). The highly polar $D_2O$ presumably (Reynolds and Priller 1968) decreases the effect of the positive charge through direct solvation of the positive ion. The result is that, in the less polar $CDCl_3$, we observe a downfield shift relative to the chemical shift in $D_2O$. We have recently demonstrated (Makriyannis and Hite 1978), using a tropane derivative, that the magnitude of the chemical shift differences in the two solvents can be related to the relative distances between the protonated nitrogen and the neighboring protons. We were able to successfully apply this principle here, in the phenethylamine systems under investigation.

The information obtained from chemical shift measurements was used: a. to distinguish between the two diastereotopic $H_2$,$H_3$ benzylic protons in the spectra of the alpha alkylphenethyl analogs; b. to provide supporting evidence for the results on rotamer populations which were calculated from the vicinal coupling constants.

B. Molecular Dynamics

The method we have used to study the microdynamic behavior of the phenethylamine analogs in solution is based on the determination of effective correlation times ($\tau_{eff}$) for individu 1 carbon atoms which can be calculated from the corresponding $^{13}C$ spin-lattice relaxation times ($T_1$). The correlation times measure the period of molecular reorientation of a C-H vector through a given angular displacement. They can also serve as a measure for the motions of individual $^{13}C$ atoms and thus provide. a description of the molecule's dynamic behavior in solution. Such measurements allow us to make semiquantitative comparisons on the flexibility of closely related molecules in solution and also give us information about specific intramolecular interactions which affect their flexibility.

In intermediate sized molecules, spin-lattice relaxation, for carbons directly attached to protons, is usually dominated by the

$^{13}C$-H dipole-dipole mechanism. In such instances, the $T_1$ of a $^{13}C$ nucleus can be related to its effective correlation tine using the following equation (Allerhand et al. 1971) which assumes isotropic motion:

$$T^{-1} = mm^2\gamma_H\gamma_C r_{CH}^{-6} = n2.2065x10^{10}x\tau_{eff}$$

where n = number of protons directly attached on the $^{13}C$ nucleus under consideration. The only possible exceptim in our systems are fast rotating methyl groups where spin-rotation can be a contributing mechanism in the relaxation process. In such instances, the calculated $\tau_{eff}$ values should be considered only as upper limits.

## RESULTS AND DISCUSSION

The conformational and dynamic properties in solution for each of the compounds examined, are discussed, with special emphasis on the effects of alpha alkyl substitution. Chemical shifts, coupling constants and relaxation parameters are listed in tables I, II and III.

### 4-Methoxyphenethylamine hydrochloride (1)

This compound exists in solution as a mixture of two conformers. The conformational preference is slightly in favor of the *trans* conformer, which makes up slightly over me third of the total population. One third is the expected ratio for the *trans* conformer,if there is no conformational preference.

In p-nethoxyphenethylamine hydrochloride, the side chain carbons have similar correlation times indicating that both carbons are moving in unison. The movement of the side chain appears to be faster than the tumbling of the phenyl ring, as indicated by the longer correlation tine values for the phenyl carbons. This is an indicatim that rotation along the C-Ph bond is faster than along the C-C bond of the side chain. The methyl carbon in the para methoxy group has a $T_1$ value considerably longer than that of the rest of the protonated carbons and indicates very fast motion. Its calculated correlation time is probably only an upper limit, because of a possible contribution of spin-rotation in the relaxation of this carbon.

### 4-Methoxyamphetamine (2) and 3,4,5-trimethoxyamphetamine (3) hydrochlorides.

Amphetamines can occur as mixtures of two major (fig. 2, I. II) and one minor (fig. 2, III) conformers. In $D_2O$ compound 2 and compound 3 (which was included in this study because of its solubility in chloroform) occur as a mixture of equally populated conformers I and II with mly a modest cmtribution from conformer III. This last conformer has all three vicinal substituents *gauche* to one another and is, therefore, understandably not favored. When the solvent was changed to $CDCl_3$ in the trimethoxy analog 3) the conformational distribution was shifted strongly in favor of rotamer I which has the ammonium group *trans* and the methyl group *gauche* to the aromatic ring. This is an indication that hydrophobic media favor a *trans* phenethylamine configuration.

**TABLE I**

Proton Chemical Shifts[a] for the

Phenethylamine Hydrochloride Analogs

| Compound | Solvent | Chemical Shift (ppm) $H_1$ | $H_2$ | $H_3$ | $R_4[CH_2]$ | $[CH_3]$ | Difference[c] $V_3$-$V_2$ ($H_Z$) |
|---|---|---|---|---|---|---|---|
| 1 | $D_2O$[b] | 3.199 | 2.910 | | | | |
| 2 | $D_2O$[b] | 3.442 | 2.725 | | | 1.154 | 0 |
| 3 | $D_2O$[b] | 3.425 | 2.733 | | | 1.183 | 0 |
| | $CDCl_3$ | 3.573 | 2.816 | 3.166 | | 1.406 | 94.7 |
| 4 | $D_2O$ | 3.442 | 2.789 | 2.998 | (1.659,1.710) | 1.006 | 56.4 |
| | $CDCl_3$ | 3.375 | 2.880 | 3.183 | (1.708,1.727) | 1.092 | 81.7 |
| 5 | $D_2O$ | 3.449 | 2.639 | 2.962 | (1.667,2.020) | | 87.3 |
| | $CDCl_3$ | 3.613 | 3.044 | 3.266 | (2.041,2.379) | | 60 |

[a]Chemical shifts were measured from TMS which was used as an internal standard in the $CDCl_3$ solutions and as an external standard in a concentric tube in the $D_2O$ solutions. Unless stated otherwise spectra were obtained at 270 $MH_z$ using 0.10M solutions at an ambient temperature of 20°; [b]Spectra taken at 60 $MH_z$ using 0.30M solutions at 20°; [c]Frequency differences for spectra measured at 270 $MH_z$.

The accidental equivalence of the $H_2$ and $H_3$ benzylic protons in both amphetamine analogs when examined in $D_2O$ reflects the weighted deshielding influence of the ammonium group and the shielding influence of the methyl group on each of the two protons, in all three conformers. The net sum of these effects, in the above two examples, is apparently equal for both protons. The change to a hydrophobic solvent, in compound 3, is accompanied with a downfield shift for both $H_2$ and $H_3$ protons. This behavior reflects the increased deshielding influence of the positively charged nitrogen when going from aqueous to hydrophobic media. Furthermore, examination of the spectrum in $CDCl_3$ revealed that the $H_3$ proton undergoes a stronger downfield shift an the $H_2$ proton. he presence of the $H_2$-frequencies at higher field relative to the $H_3$ signal is compatible with the observed increase in the population of rotamer I. In this rotamer, the $H_2$ proton experiences the preferential shielding influence of the *gauche* methyl group. The above observed solvent effect is therefore evidence that the spectral assignments for the $H_2$ and the $H_3$ protons are correct.

The addition of an alpha methyl group on the side chain of the phenethylamine salt analog appears to slow down the overall movement of the molecule, as indicated by the general increase in the correlation times. In this amphetamine analog, the alpha methine carbon is the slowest moving carbon in the side chain and has a correlation time very similar to those of the ring carbons. The beta methylene carbon moves only slightly faster, indicating some flexibility around the C-Ph bond. Finally, the alpha methyl carbon ro-

tates cmsiderably faster than any other carbon on the side chain. Nevertheless, this methyl carbon has a correlation time at least three times as long as that of the freely rotating O-methyl group carbon. This indicates serious restriction in its rotation.

3,4-Dimethoxy-a-ethylphenethylamine Hydrochloride 4

This compound occurs in two principal conformations (I,II). Of these, the *trans* phenethylammonium conformation which has the ethyl group *gauche* to the aromatic ring is favored. As with the alpha methyl analog, the change to hydrophobic solvents enhances this conformation. The change of solvent also causes the signals for the $H_2$ and $H_3$ protons to shift to lower fields; the strongest effect is experienced by the $H_3$ proton. All of the above effects can be explained using the same arguments used for 4-methoxyamphetamine.

In this molecule (4), all but the three methyl carbons have very short relaxation times which correspond to long correlation times and indicate sluggish overall reorientation. Again the alpha methine carbon is the slowest, while the benzylic carbon has a slightly shorter correlation time. Such $\tau_{eff}$ values are compatible with slightly freer mobility around the C-Ph bond compared to the central C-C phenethylamine bond. The two carbons of the alpha ethyl substituent differ greatly in their dynamic behavior. While the methylene carbon is cmsiderably restricted, the methyl carbon has a very fast rotation, as shown by its very short correlation time. The free rotation of the above mentioned methyl group gives us some

TABLE II

Spin-Spin Coupling Constants and Conformational Distributions for the Phenethylamine Hydrochloride Analogs

| Compound | Solvent | $J_{1,2}$(Hz) | $J_{1,3}$(Hz) | $J_{2,3}$(Hz) | pI | pII | pIII |
|---|---|---|---|---|---|---|---|
| 2 | $D_2O$ | 7 | 7 | - | .47 | .47 | .06 |
| 3 | $D_2O$ | 7 | 7 | - | .47 | .47 | .06 |
| | $CDCl_3$[a] | 9.2 | 5.2 | 13.3 | .76 | .23 | .01 |
| 4 | $D_2O$[a] | 8.5 | 5.9 | 14.3 | .67 | .32 | .01 |
| | $CDCl_3$[a] | 9.3 | 5.1 | 13.4 | .77 | .21 | .01 |
| 5 | $D_2O$ | 9.5 | 4.6 | 15.5 | 1 | - | - |
| | $CDCl_3$ | 10.9 | 4.5 | 14.7 | 1 | - | - |
| 1 | $D_2O$[b] | 6.8($J_{AB}$) | 7.6($J_{AB'}$) | 13.5($J_{AA'}$) | .42 | .58 | |

[a] Conformer ratios were calculated assuming $J_t$=11.0Hz and $J_g$=3.5Hz; values were obtained from the model compound γ-1,2,5-trimethyl-4-phenyl-4-piperidinol (Casy 1971); [b] Conformer ratios were calculated assuming $J_t$=13.11 Hz and $J_g$=3.63 Hz (Abraham, and Gatti 1969).

TABLE III

Carbon-13 Spin-Lattice Relaxation Times[a] (sec) and Effective Correlation Times ($x10^{-12}$ sec) for Phenethylamine Hydrochloride Analogs

| Compound | $C_2$ | $C_3$ | $C_4C_5$ | $C_6$ | $C_\beta$ | $C_\alpha$ | $CH_2$ | $CH_3$ | $OCH_3$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.76 | 1.76 | 1.76 | 1.76 | 1.02 | 1.02 | - | - | 2.31 |
| | (26) | (26) | (26) | (26) | (22) | (22) | | | (<6.5) |
| 2 | 1.07 | 1.04 | 1.04 | 1.07 | .58 | 1.04 | | .74 | 2.06 |
| | (42) | (44) | (44) | (42) | (39) | (44) | | (20) | (<7.7) |
| 4 | .34 | - | .39 | .33 | .27 | .44 | .40 | 1.33 | 0.93 .93 |
| | (133) | | (116) | (137) | (84) | (103) | (57) | (<11) | (<16)(<16) |
| 5. | - | .68($C_5$) | .80($C_7$).74($C_8$).42($C_1$).68($C_2$).41($C_3$).41($C_4$) | | | | | | 1.76 |
| | | (67) | (57) | (61) | (54) | (67) | (55) | (55) | (<8.6) |

[a] Measurements were made using .75M solutions of the compounds in $D_2O$. The results given are the average of three determinations.

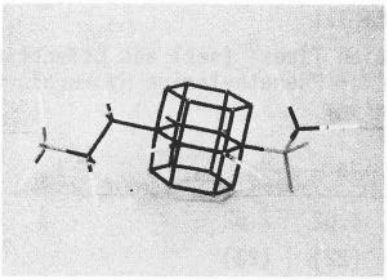
insight into the conformation around the HC-$CH_2CH_3$ bond. Of the three possible conformers, only one will allow the methyl group to experience free rotation. This conformer is one in which the methyl group is *gauche* to the ammonium group and *trans* to the benzylic methylene. According to the evidence, this conformation is the most favored. The chosen conformation is compatible with the downfield shift experienced by the methyl group when the solvent is changed from $D_2O$ to $CDCl_3$. The slightly increased value in the correlation tines of the two ortho O-methyl groups reflects some mutual interference in the rotation of the two groups.

2-Amino-6-methoxytetralin Hydrochloride (5)

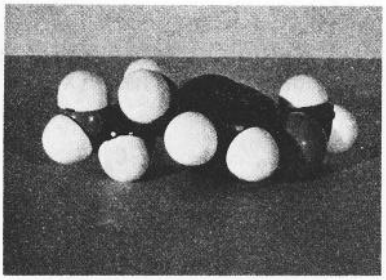
In addition to the coupling constants shown in table II, we have obtained approximate estimates for the vicinal coupling constants of the protons on carbon $C_3$ when coupling with the methine proton on carbon $C_2$ of this compound. These coupling constants were found to be equal to: $J_{vic}$=1OHz; 4Hz. The four vicinal coupling constants at hand are consistent with the two $H_{ax}$,-$H_{ax}$ and two $H_{ax}$-$H_{eq}$ couplings. Such coupling indicates that the saturated ring exists in a chair in which the methine proton on $C_2$ occupies an axial position and the ammonium group is equatorial.

The accurately measured vicinal coupling constants between the two benzylic protons on carbon Cl and the methine proton on $C_2$ indicate that this chair conformation is slightly deformed. If we assume that compound 5 exists in one dominant conformation in solution, we can then evaluate the ring distortion. This can be done by comparing the measured vicinal coupling constants with the coupling constants of

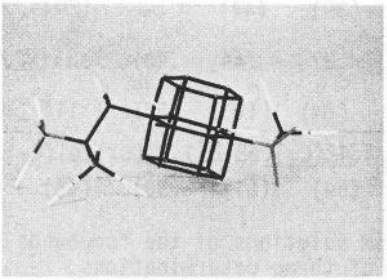
FIGURE 4



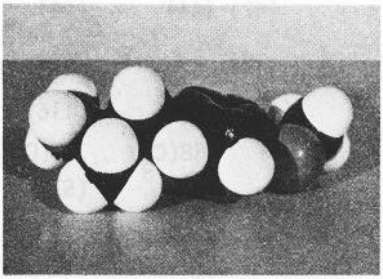
1a



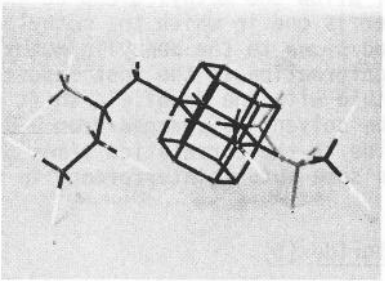
1b



2a



2b



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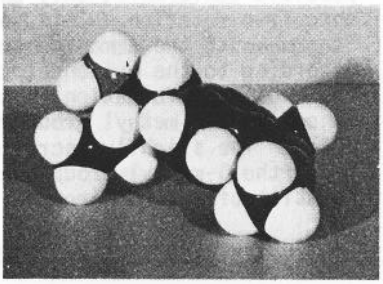

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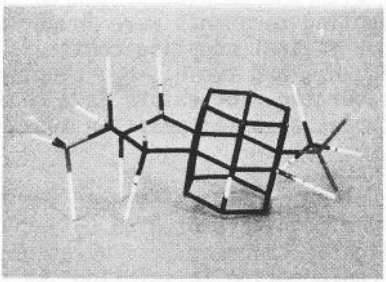

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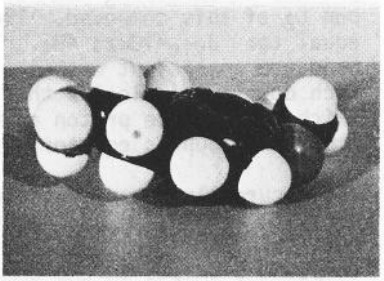

5b

cyclohexane ($J_{ax,ax}$=13.31, $J_{ax\ eq}$=3.59; Abraham and Gatti 1969) which is considered to be a perfectly staggered chair. The following are the calculated dihedral angles for the $C_1H_2C_2H$ fragment of 2-amino-6-methoxytetralin hydrochloride in $D_2O$ and in $CDCl_3$ respectively: $\phi(H_{ax}C_1C_2H_{ax})$155°,158°; $\phi(H_{eq}C_1C_2H_{ax})$=55°, 56°.

The differing relaxation time values of the phenyl ring carbons in this tetralin analog are an indication of a certain degree of anisotropic motion. In this molecule, rotation appears to be favored around an axis which runs very near the $C_6$-O and the $C_2$-N bonds. The consistently shorter correlation times of the aliphatic carbons are an indication of some flexibility in the ring.

## CONCLUSION

Stick as well as space-filled models for compounds 1, 2, 4,and 5, in their preferred conformations, are shown in figure 4. The stick models provide a more accessible representation of the molecular conformation, while the space-filled models give a realistic portrayal of the structural features that may affect the drug-receptor interaction. The conformations around the C-O and the C-Ph bonds are only predicted conformations. Work is currently under progress for their experimental verification.

The evaluation of the results obtained in this study is given below:

1. This study has helped to put the conformational assignments for alpha alkylphenethylamine salts on more solid ground, with the use of variable temperature and solvent experiments. It was found that, in all of the compounds studied here, the *trans* phenethylammonium conformer makes up a sizeable portion of the total conformer population in solution.

2. The *trans* phenethylammonium conformer appears to be enhanced a) by the presence of a small alkyl group in the alpha position; b) by hydrophobic solvents.

3. The addition of alpha alkyl substituents introduces a considerable degree of rotational restriction. This restriction is more pronounced around the central C-C bond, while the C-Ph bond appears to maintain more rotational freedom in all the open chain compounds studied here. The deceased flexibility in the molecule allows a more important role to be played by the conformer distribution in the determination of the drug's biological activity.

4. It is tempting to suggest, on the basis of the evidence provided here, that the *trans* phenethylammonium conformation is essential for psychotomimetic activity. Such a suggestion is compatible with the finding that in the 2-(3.4,5-trimethoxyphenyl) cyclopropylamines, psychotomimetic activity resides with the *trans* isomer (Walters and Cooper 1968; Cooper and Walters 1972).

   No conclusions can be drawn from our experiments about the *pharmacophoric conformation* around the C-Ph bond.

This question will be investigated using ortho substituted phenethylamine analogs. A *trans pharmacophoric conformation* does not rule out the possibility that these drugs may produce their effects by interacting with more than me receptor.

5. The presence of an alpha methyl group *gauche* to the phenyl ring does not seem to obstruct the interaction of the phenethylammonium component with the receptor. On the contrary it may enhance its affinity through some hydrophobic interaction of the methyl group with the receptor site. The possibility of such a specific interaction is strengthened by the existing evidence about the stereospecific requirements of methoxyamphetamines for activity (Shulgin 1973; Standridge et al. 1976). When the alpha methyl is substituted with an ethyl group, the steric requirements of the receptor for productive interaction are not satisfied any longer. As can be seen from the models, the preferred conformation of the ethyl group is such,that the methyl group is protruding beyond the level of the ammonium group and probably obstructs its interaction with the receptor site. However,the molecule can still bind to the receptor although not productively because of its other structural features and therefore acts as an antagonist (probably competitive). The conformation of the alpha $CH_2CH_2$ chain is quite different in the tetralin analog. Such a conformation allows the interaction between the protonated nitrogen with the receptor site to occur and accounts for the psychotomimetic properties of this molecule.

We have currently extended our studies to include other phenethylamine analogs. We are seeking to understand how other structural modifications can affect the conformational properties of phenethylamines and how these conformational changes correlate with their psychotomimetic activity.

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# Appendix A.

## QuaSAR Program

TECHNICAL REVIEW ON
THE QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS (QuaSAR)
OF NARCOTIC ANALGESICS, NARCOTIC ANTAGONISTS,
AND HALLUCINOGENS

AGENDA

THURSDAY, APRIL 20, 1978
COUNSEL ROOM, SHERATON SILVER SPRING

| | | |
|---|---|---|
| 9:00 - 9:15 | Welcome and Introductory Remarks | Bob Willette<br>*Gene Barnett* |
| | Chairman - Milan Trsic | |
| 9:15 - 9:45 | New Aspects and Applications of the Extended Isolated Molecule Method to the Study of Drugs | Richard Brown--State University of New York |
| 9:45 - 10:00 | Questions and Answers | |
| 10:00 - 10:30 | Assessment of Quantum Mechanical Techniques for Use in Structure-Activity Relationship Development and Application to Analgesics and Other Drugs | Ralph Christoffersen--University of Kansas |
| 10:30 - 10:45 | *Questions and Answers* | |
| 10:45 - 11:00 | Coffee Break | |
| 11:00 - 11:30 | Structure Activity Studies by Means of the SIMCA Pattern Recognition Methodology | William Dunn--Umeå University |
| 11:30 - 11:45 | Questions and Answers | |
| 11:45 - 12:15 | QSAR: A Critical Appraisal | Sydney Archer--Rensselaer Polytechnic Institute |
| 12:15 - 12:30 | Questions and Answers | |
| 12:30 - 2:00 | Lunch | |
| | Chairman - Phillip Portoghese | |
| 2:00 - 2:30 | The Nature of Opioid and LSD Receptors: Structural Activity Relationship Implications | William Martin--University of Kentucky |
| 2:30 - 2:45 | Questions and Answers | |
| 2:45 - 3:15 | Carbon-13 Nuclear Magnetic Resonance Study of the $\alpha$- and $\beta$-Isomers of Methadol and Acetylmethadol Hydrochlorides | F. Ivy Carroll--Research Triangle Institute |
| 3:15 - 3:30 | *Questions and Answers* | |
| 3:30 - 3:45 | *Coffee Break* | |
| 3:45 - 4:15 | Conformational Energies and Geometries of Narcotics, Using a Potential Function Method | Mark Froimowitz--McLean Hospital |
| 4:15 - 4:30 | Questions and Answers | |
| 4:30 - 5:00 | Structure-Activity Studies of Narcotic Agonists and Antagonists from Quantum Chemical Calculations | Gilda Loew--Stanford University |
| 5:00 - 5:15 | Questions and Answers | |
| 5:15 - 8:00 | Cash Bar in the "El Fontenal" Room--2nd floor | |

## AGENDA

FRIDAY, APRIL 21, 1978
COUNSEL ROOM, SHERATON SILVER SPRING

**Chairman - Albert Leo**

| | | |
|---|---|---|
| 8:30 - 9:00 | Hammett-Type Substituent Constants: What They Are, What What They Aren't, What They Tell Us, What They Don't | Robert Davidson--Amherst College |
| 9:00 - 9:15 | Questions and Answers | |
| 9:15 - 9:45 | Quantitative Relationship Between Antinociceptive Activity and Opiate Receptor Affinity: The Importance of Lipophilicity | Arthur Jacobson--National Institutes of Health |
| 9:45 - 10:00 | Questions and Answers | |
| 10:00 - 10:30 | Quantitative Stereo-Structure-Activity Relationships L Opiate Receptor Binding | Howard Johnson--Stanford Research Institute |
| 10:30 - 10:45 | Questions and Answers | |
| 10:45 - 11:00 | Coffee Break | |
| 11:00 - 11:30 | An Assessment of Parameters in QuaSAR Studies of Narcotic Analgesics and Antagonists | Robert Katz--Bethesda, Maryland |
| 11:30 - 11:45 | Questions and Answers | |
| 11:45 - 12:15 | QSAR of Narcotic Analgetic Agents | Eric Lien--University of Southern California |
| 12:15 - 12:30 | Questions and Answers | |
| 12:30 - 2:00 | Lunch | |

**Chairman - Earl Usdin**

| | | |
|---|---|---|
| 2:00 - 2:30 | Structural Definition Among Hallucinogenic Phenylalkylamines | Lemont Kier, Richard Glennon--Medical College of Virginia |
| 2:30 - 2:45 | Questions and Answers | |
| 2:45 - 3:15 | Recognition and Activation Mechanisms on the LSD/Serotonin Receptor: The Molecular Basis of Structure Activity Relationships | Harel Weinstein--Mount Sinai School of Medicine |
| 3:15 - 3:30 | Questions and Answers | |
| 3:30 - 4:00 | D-LSD and the Histamine $H_2$-Receptor in Brain | Jack Peter Green--Mount Sinai School of Medicine |
| 4:00 - 4:15 | Questions and Answers | |
| 4:15 - 4:30 | Coffee Break | |
| 4:30 - 5:00 | Mescaline Analogs: Substitutions at the 4-Position | Alexander Shulgin--Lafayette, California |
| 5:00 - 5:15 | Questions and Answers | |
| 5:15 - 5:45 | Congeners of DOM: Effect of Distribution on the Evaluation of Pharmacologic Data | Charles Barfknecht--University of Iowa |
| 5:45 - 6:00 | Questions and Answers | |

**AGENDA**

SATURDAY, APRIL 22, 1978
COUNSEL ROOM, SHERATON SILVER SPRING

Chairman - Richard Brown

| | | |
|---|---|---|
| 9:00 - 9:30 | Quantitative Structural Activity Relations in 2, 4, 5-Substituted 1-phenyl-2-amino Propanes | Peter Kollman--University of California, S. F. |
| 9:30 - 9:45 | Questions and Answers | |
| 9:45 - 10:15 | QSAR of Agents Involved in Serotonin and LSD Binding Sites | Eric Lien--University of Southern California |
| 10:15 - 10:30 | Questions and Answers | |
| 10:30 - 10:45 | Coffee Break | |
| 10:45 - 11:15 | Conformational Study of Lysergic Acid Derivatives in Relation to Their Hallucinogenic and Anti-5HT Activities | Mahadevappa Kumbar--Adelphi University |
| 11:15 - 11:30 | Questions and Answers | |
| 11:30 - 12:00 | Recent Physicochemical and Quantum Chemical Studies on Drugs of Abuse and Biomolecules | Joyce Kaufman--Johns Hopkins University |
| 12:00 - 12:15 | Questions and Answers | |
| 12:15 - 1:45 | Lunch | |

Chairman - Milan Trsic

| | | |
|---|---|---|
| 1:45 - 2:15 | Conformational Studies on Phenethylamine Hallucinogens | Alexandros Makriyannis--University of Connecticut |
| 2:15 - 2:30 | Questions and Answers | |
| 2:30 - 3:00 | The Use of Rigid Analogues to Probe Hallucinogen Receptors | David Nichols--Purdue University |
| 3:00 - 3:15 | Questions and Answers | |
| 3:15 - 3:45 | Absolute Configuration and Psychotomimetic Activity | Alexander Shulgin--Lafayette, California |
| 3:45 - 4:00 | Questions and Answers | |
| 4:00 - 4:30 | Photoelectron Spectroscopic Studies of Hallucinogens: The Use of Ionization Potentials in QSAR | Kendall Houk--Louisiana State University |
| 4:30 - 4:45 | Questions and Answers | |
| 4:45 - 5:15 | General Discussion | |
| 5:15 - 5:45 | Concluding Remarks | Bob Willette<br>Gene Barnett |

# Appendix B.

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