THE CONTRIBUTIONS OF HAMAO UMEZAWA TO SCIENCE AND MEDICINE

Dr. Hamao Umezawa's scientific life has concentrated on the secondary metabolites produced by microorganisms. His prime concern has been to detect such metabolites through their biological activities, isolate them, determine their structure, study their mechanism of action and encourage clinical trials of those having medical significance. This work was started in 1944 and continues today. In the early stages of the work methodology was relatively crude. Dr. Umezawa and associates have played an extremely important role in refining the methodology so that today very sophisticated techniques permit studies thought impossible just a few years ago. In the course of his work, Dr. Umezawa has had associated with him a number of young researchers who received the benefit of his thinking, philosophy and training. These men in turn have made numerous contributions both in academic and industrial research. Thus, Dr. Umezawa has had a profound effect in the development of microbial chemistry in Japan and has played a very important role in setting the high standard of such studies in his native land.

Japan, of course, has had a long tradition in using fermentation processes for various aspects of its food and drink consumption. In a sense Dr. Umezawa has followed this tradition but his emphasis was to make fermentation of use in medicine. He started his work with the study of antimicrobial agents produced by microorganisms. In the course of this work he has discovered almost one hundred such agents. One of these agents, kanamycin, was found to be useful in treating human infections caused by a variety of microorganisms and is in worldwide use today.

As an indication of Dr. Umezawa's degree of penetration into scientific problems, he studied the very interesting question as to why certain pathogens became resistant to this and other aminoglycoside antibiotics. He and his group studied in detail the mechanism of such resistance and found that there were specific inactivating enzymes produced by various organisms which reacted at certain sites of the molecule. With this information available Dr. Umezawa studied how such antibiotics could be modified so that they would be resistant to the inactivating enzyme but still retain activity. This type of work could be carried out only by a person who had great capability in several fields, since success in this area involved intricate studies in microbiology, enzymology, structure determination and organic synthesis. It is indeed rare to find a person with great competence in such a variety of scientific fields. This integration of several disciplines permitted Dr. Umezawa to rationally plan the synthesis of an antibiotic which would be active against resistant micro-organisms. This led to the discovery of dideoxykanamycin B which is now being used in Japan and is under clinical study in many countries of the world. This new drug probably is the first example of a useful drug developed rationally as a result of understanding fundamental biochemical mechanisms.

Dr. Umezawa's interest broadened into the antitumor area and again he brought to bear his penetrating approach to the problem. Recognizing specificity with respect to the pharmacological distribution of a potentially toxic compound, Dr. Umezawa concentrated much work on an agent, bleomycin, which showed a differential distribution in certain tissues as well as tumors. He showed that this distribution resulted in part, at least, from the presence of inactivating enzymes in certain tissues. The fact that there was accumulation in various tissues led to the hope that such an agent would not show the typical blood dyscrasias associated with such agents. Clinical studies of this agent did indeed indicate utility against certain types of human

cancers and the agent is used worldwide today in helping control this disease.

Dr. Umezawa's studies also led to the discovery of an agent very useful in controlling a disease of rice caused by the organism, Pyricularia oryzae. This drug is used widely in Asian countries in controlling the disease and has led to increased productivity of rice. In today's food short world this contribution takes on increasing importance.

Much of Dr. Umezawa's recent work has concentrated in finding unusual metabolites which had the properties of inhibiting specific enzyme systems. Again seeking a rational way to develop new drugs, Dr. Umezawa delved into the biochemical mechanisms of various disorders and focused his attention on enzyme systems that may be key steps. He then set up refined methods to detect such activities and isolate and determine the structure of metabolites having the activity. As a result of these studies a variety of specific enzyme inhibitors have been found. A number of these are under current study clinically and such substances are also extremely useful probes into studying mechanisms of enzyme action. They also provide useful tools in determining whether postulated mechanisms of certain disease states are indeed the basic responsible events.

Dr. Umezawa's work thus represents an attempt to find through a rational approach agents of clinical importance. This has been the hope of many scientists working in this area and Dr. Umezawa's findings are a stimulus for workers to do the same. His success in discovering new useful drugs sets an example for such an approach which will have a profound and long lasting effect on future research in the medical area.

A more detailed description of some of Dr. Umezawa's more important findings are presented below along with a curriculam vitae, a biography of Dr. Umezawa's most important publications, a list of new antibiotics discribed by Dr. Umezawa and a list of structures of enzyme inhibitors discovered by Dr. Umezawa.

Discovery of Bleomycin and Its Action

In 1956, H. Umezawa discovered phleomycin*1 which exhibits inhibition against Ehrlich carcinoma with a high therapeutic index*2. However, this antibiotic produced irreversible renal toxicity in dogs*3. Therefore, H. Umezawa continued the study of antibiotics of similar types and discovered bleomycin(1) which was separated into individual components by chromatography(2). The bleomycins caused hepatotoxicity in dogs but not renal toxicity(3) and were differentiated from phleomycin by paper chromatography and by stability in acid and alkaline solutions. Umezawa(4,5) studied the distribution of ^{3}H-bleomycin in organs of mice, measuring the radioactivity and the antibacterial activity in organ extracts, and proved that bleomycin is inactivated in organs and tissues. This inactivation is significantly slower in skin and lung than in other organs, especially in old mice(4). This inactivation proved to be due to an enzyme which hydrolyzed the carboxamide bond in the bleomycin molecule(4,5). Bleomycin is effective against squamous cell carcinoma induced in mouse skin by 20-methylcholanthrene, but not against sarcoma of mouse skin induced by the same agent(4). Umezawa(4) confirmed that the content of the bleomycin-inactivating enzyme is significantly lower in the former than in the latter. He purified this enzyme, which hydrolyzes not only the carboxamide of β-aminoalanine moiety of bleomycin molecule but also lysinamide,

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*1 Maeda, K.; H. Kosaka, K. Yagishita & H. Umezawa: A new antibiotic, phleomycin. J. Antibiotics, 9A, 82 (1956). *2 Umezawa, H.; M. Hori, M. Ishizuka & T. Takeuchi: Studies on antitumor effect of phleomycin. J. Antibiotics, 15A, 274 (1962). *3 Ishizuka, M.; H. Takayama, T. Takeuchi & H. Umezawa: Studies on antitumor activity, antimicrobial activity and toxicity of phleomycin. J. Antibiotics, 19A, 260 (1966).

lysyl-β-naphthylamide and argininyl-β-naphthylamide and which was found to be a new aminopeptidase[6]. Moreover, ^{3}H-bleomycin is distributed in squamous cell carcinoma in a significantly higher concentration than in sarcoma. Thus, the selective effect on squamous cell carcinoma was shown to be due to the low content of the bleomycin-inactivating enzyme and a high concentration of the antibiotic in this tumor. The data also indicates that initial toxicity should appear in the skin and lung because of weaker inactivation of bleomycin by these organs, especially in old animals[4].

As described in a review on bleomycin[7], Umezawa clarified the structural feature of bleomycin molecule*1 and found that bleomycin reacts with DNA and causes single strand scission[7]. This reaction occurs even at 0°C*2.

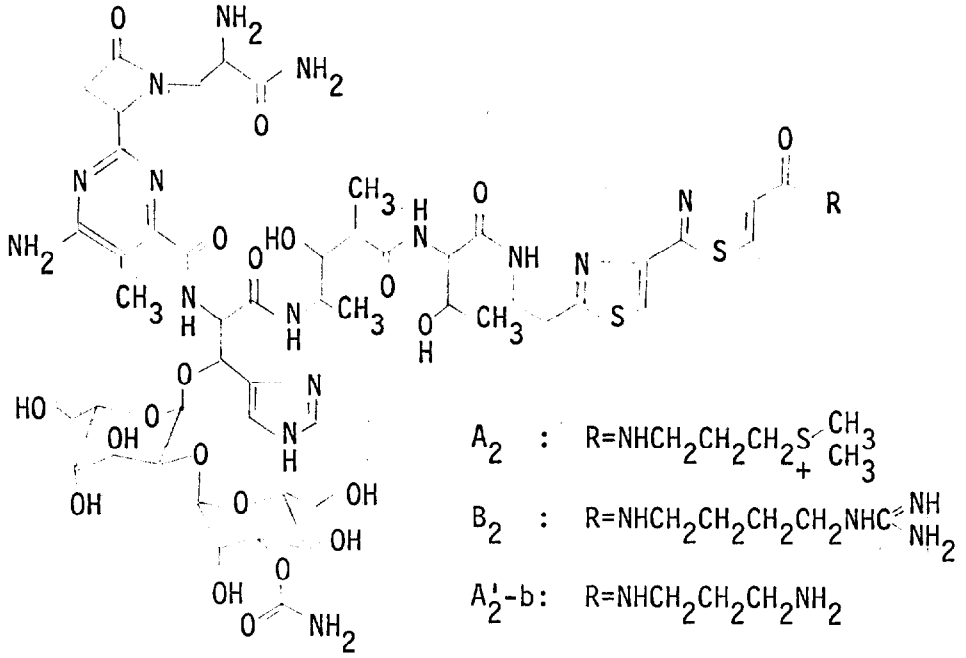

Fig. 1. Structural Features of Bleomycins A_2, B_2, A_2'-b and Bleomycinic Acid

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*1 Takita, T.; Y. Muraoka, T. Yoshioka, A. Fujii, K. Maeda & H. Umezawa: The chemistry of bleomycin. IX. The structure of bleomycin and phleomycin. J.

Antibiotics, 25, 755 (1972). *2 Umezawa, H.; H. Asakura, K. Oda & M. Hori: Characteristics of bleomycin action which produces a single scission in a superhelical form of SV40 DNA. J. Antibiotics, 26, 521 (1973).

Various bleomycins are different from one another in the terminal amine moiety (R in the structure)[5]. The addition of an amine to the fermentation medium suppresses the production of natural bleomycins and causes the production of bleomycin containing the added amine[5,7]. Bleomycinic acid can be obtained by an enzymatic hydrolysis (a new enzyme, agmatine amidohydrolase*1) of bleomycin B_2 and is used for chemical synthesis of new bleomycins[7].

Bleomycin is now widely used for the treatment of squamous cell carcinoma. It is especially effective on the well-differentiated type. There are patients who were treated with bleomycin who survived for more than 5 years*2. Bleomycin has also been found to be an effective agent in the treatment of malignant lymphoma*2.

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*1 Umezawa, H.; Y. Takahashi, A. Fujii, T. Saino, T. Shirai & T. Takita: Preparation of bleomycinic acid: Hydrolysis of bleomycin B_2 by agmatine aminohydrolase of a Fusarium sp. J. Antibiotics, 26, 117 (1973). *2 New Drug Seminar on Bleomycin. National Cancer Institute, Division of Cancer Treatment, Bethesda, Maryland, (1974).

Enzymatic Mechanism of Resistance and Synthesis of Useful Agents Against Resistant Infections

The enzymatic mechanism of the selective effect of an antibiotic agent on different microbes can be applied to the development of active agents against resistant infections. Umezawa clarified the enzymatic mechanism of resistance, predicted the active structure and succeeded in the synthesis of such a compound with practical usefulness. This is very important, because the probability of finding new useful antibacterial antibiotics has become very small.

In the study of water-soluble basic antibiotics, Umezawa discovered kanamycin in 1957, which was evaluated for its effect against infection of staphylococci resistant to drugs at that time and against tuberculosis resistant to streptomycin, as shown in papers reported at the New York Academy Symposium in 1958*1. Later, when resistant Gram negative organisms appeared and synthetic penicillins were introduced, the usefulness of kanamycin in treating infections caused by resistant Gram negative bacteria became especially important, as described in papers at the New York Academy Symposium in 1965*2. Kanamycin resistant strains appeared in 1965. In 1967, Umezawa(8) extracted enzymes from disrupted cells of E. coli K12 ML1629 which he obtained from S. Mitsuhashi (Medical School, Gumma University) and E. coli K12 R5 which he obtained from H. Okamoto (National Institute of Health, Tokyo). He(8) isolated and purified the reaction products of these enzymes and elucidated their structures, clarifying the nature of the enzyme reactions. Thus, the enzyme which was obtained from most common resistant strains was shown to transfer phosphate from ATP to the

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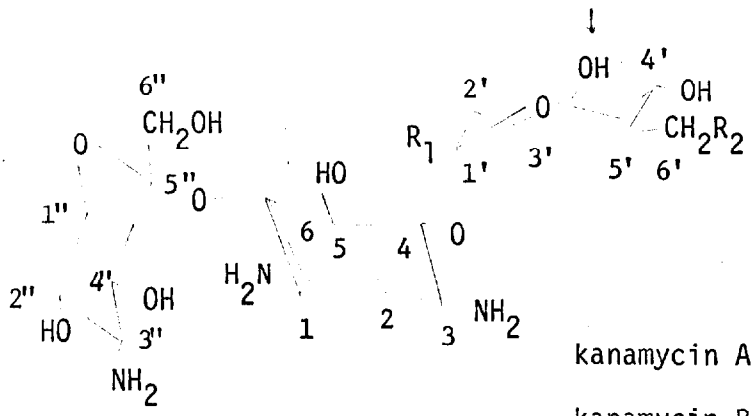
*1 The basic and clinical research of the new antibiotic kanamycin. Ann. N. Y. Acad. Sci., 76, Art. 2, 19-408 (1958). *2 Kanamycin: Appraisal after 8 years. Ann. N. Y. Acad. Sci., 132, Art. 2, 773-1090 (1966).

3'-hydroxyl group of kanamycins. Based on these findings, 3',4'-dideoxy-kanamycin B, which does not contain the 3'-hydroxyl and does not undergo the reaction of the phosphotransferase, was synthesized[9]. It was shown that this compound inhibits most resistant staphylococci, resistant Gram negative organisms and Pseudomonas aeruginosa. The usefulness of 3',4'-dideoxykanamycin B in the treatment of resistant infections was confirmed by large-scale clinical studies in Japan in 1970-1971.

In another aspect, the confirmation of the ability of 3',4'-dideoxykanamycin B to unhibit resistant organisms was proof of the involvement of the enzyme in the mechanism of resistance.



kanamycin A: R_1=OH, R_2=NH_2

kanamycin B: R_1=NH_2, R_2=NH_2

kanamycin C: R_1=NH , R_2=OH

↓

OH is phosphorylated by kanamycin-neomycin phosphotransferase.

Besides 3',4'-dideoxykanamycin B, other interesing derivatives have been synthesized. 1-N-(4-amino-2-hydroxybutyryl)-6'-N-methyl-3',4'-dideoxykanamycin B is active against resistant organisms producing kanamycin-neomycin phospho-transferases I and II, kanamycin-gentamicin nucleotidyltransferase, gentamicin acetylase, and kanamycin acetylase.

Umezawa and his coworkers also elucidated the mechanism of resistance

to the other aminoglycosidic antibiotics (streptomycin, lividomycin, etc.) and determined the structures of most of the products of enzymes which are involved in the mechanism of resistance to aminoglycosidic antibiotics[10].

The Study on Enzyme Inhibitors of Microbial Origin[11]

H. Umezawa extended his antibiotic studies to include enzyme inhibitors produced by microorganisms*1. Compared with inhibitors obtained from animal and plant tissues which generally are of macromolecular nature, the inhibitors that he found in microbial culture filtrates are small molecules of unique structural types which no one had detected before. He found leupeptin inhibiting plasmin, trypsin, papain and cathepsin A, antipain inhibiting trypsin, papain and cathepsins A and B, chymostatin*2 inhibiting chymotrypsin, and elastatinal*3 inhibiting elastase. These inhibitors contain a C-terminal aldehyde group and he showed that argininal in leupeptin, phenylalaninal in chymotrypsin, alaninal in elastatinal werenecessary for inhibition of the enzymatic cleavage at the carboxyl side of arginine, phenylalanine, and alanine (or glycine). He found pepstatin inhibiting pepsin, cathepsin D and renin. Pepstatin is the first small molecule found to inhibit these enzymes.

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*1 Umezawa, H.: Enzyme inhibitors of microbial origin. University of Tokyo Press, Bunkyo-ku, Tokyo, 1973. Umezawa, H.: Chemistry of enzyme inhibitors of microbial origin. Pure and Applied Chemistry, 33, 129 (1973). *2 Tatsuta, K.; N. Mikami, K. Fujimoto, S. Umezawa, H. Umezawa & T. Aoyagi: The structure of chymostatin, a chymotrypsin inhibitor. J. Antibiotics, 26, 625 (1973). *3 Umezawa, H.; T. Aoyagi, A. Okura, H. Morishima, T. Takeuchi & Y. Okami: Elastatinal, a new elastase inhibitor produced by actinomycetes. J. Antibiotics, 26, 787 (1973).

Phosphoramidon[*1] inhibiting thermolysin was found to be a new inhibitor against a metal protease which cleaves the amino side of a hydrophobic amino acid.

He and his coworkers[(11)] also isolated inhibitors of enzymes which are involved in norepinephrine biosynthesis and elucidated their structures: oudenone and aquayamycin inhibiting tyrosine hydroxylase; fusaric acid and dopastin inhibiting dopamine β-hydroxylase, methylspinazarin and dihydro-methylspinazarin inhibiting cathecol-O-methyl transferase. Inhibitors of dopamine β-hydroxylase and tyrosine hydroxylase showed hypotensive effects. Structures of enzyme inhibitors are shown in an attached paper. Thus, H. Umezawa has demonstrated that microorganisms are rich sources of enzyme inhibitors of small molecular nature which exhibits pharmacological activities.

Pepstatin has been shown to be effective in treatment of gastric ulcer. Leupeptin ointment applied immediately after burn suppresses pain and blister-formation. The hypotensive effect of fusaric acid has been confirmed clinically. The study of enzyme inhibitors, thus, has been shown to be a rational approach to the discovery of useful drugs.

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*1 Suda, H.; T. Aoyagi, T. Takeuchi & H. Umezawa: A thermolysin inhibitor produced by actinomycetes: phosphoramidon. J. Antibiotics, 26, 621 (1973).

A Short Curriculum Vitae of H. Umezawa

Date of Birth: October 1, 1914

Address: 23, Toyotama-kita 4-chome, Nerima-ku, Tokyo, Japan

Present positions

Member, The Japan Academy
Director, The Institute of Microbial Chemistry (Kamiosaki, Shinagawa-ku, Tokyo)
Director, The Department of Antibiotics, The National Institute of Health, Japan (Kamiosaki, Shinagawa-ku, Tokyo)

Curriculum vitae

March, 1937	:	Graduated from Medical School, University of Tokyo
June, 1944-July, 1947	:	Associate Professor, University of Tokyo and Member, The Institute for Infectious Diseases, University of Tokyo
April, 1945	:	Doctorate of Medical Science, University of Tokyo
July, 1947 -	:	Director, Department of Antibiotics, The National Institute of Health, Japan
August, 1954 - March, 1975	:	Professor, University of Tokyo, Institute of Applied Microbiology
May, 1962 -	:	Director, Institute of Microbial Chemistry
April, 1963 -	:	Honorary Member, Japanese Association of Medical Science
November, 1969 -	:	Member, The Japan Academy
August, 1973 -	:	Member, Deutsche Akademie der Naturforscher Leopoldina
April, 1974 -	:	Honorary Member, Pharmaceutical Society of Japan

Prizes

Culture Medal (Bunka-Kunsho) conferred by the Emperor, November, 1962

Japan Academy Prize, May, 1962

Fujiwara Prize, June, 1971

Asahi Prize, January, 1959

Commandeur de l'Ordre de la Santé Publique (France), December, 1960

List of New Antibiotics and Their Derivatives Discovered

by H. Umezawa

Antitumor Antibiotics:

No. 289 Substance (1953), Sarkomycin (1953), Actinoleukin (1954), Ractinomycins A and B (1955), Pluramycins A and B (1956), Phleomycin (1957), Raromycin (1957), No. 418 Substance (1959), Peptimycin (1961), Enomycin (1963), Labilomycin (1963), Hilamycin (1963), Formycin A (1964), Bleomycins (1965), Plurallin (1966), Phenomycin (1967), Macromomycin (1968), Acrylamidine (1968), Coriolins (1969), Neopluramycin (1970), Diketocoriolin B (1971).

Antimicrobial Antibiotics:

Two Streptothricin B of S. fradiae (neomycins) (1948), Aureothricin (1949), Griseolutein A and B (1950), Nitrosporin (1951), Actinomycin J (1951), Abikoviromycin (1952), Exfoliatin (1952), Moldin (1952), Phaeofacin (1952), Sarcidin (1953), Achromoviromycin (1953), Thiazolidone antibiotic (1953), Pyridomycin (1953), Azomycin (1953), Phthiomycin (1953), Seligocidin (1954), Aureothin (1954), Mediocidin (1954), Tertiomycin A and B (1955), Antitoxoplasmic Substance No. 534 (1955), Mesenterin (1955), Kanamycin A, B and C (1957), Althiomycin (1957), Alboverticillin (1958), Mikamycin (1959), Blastmycin (1957), Niromycin A and B (1960), Unamycin A and B (1960), Amidinomycin (1960), Emimycin (1960), Ilamycin A_1, A_2, B_1, B_2, C_1 and C_2 (1961), Cytomycin (1961), Griseococcin (1962), Monazomycin (1963), Bottromycin A_1, A_2 and B (1965), Kasugamycin (1965), Spinamycin (1966), Josamycin (1967), Leucinamycin (1967), Pepthiomycin (1968), Ablastmycin (1968), Laspartomycin (1968), Oryzoxymycin (1968), Gougeroxymycin (1969), Deoxynybomycin (1970), Macarbomycin (1970), Negamycin (1970), Leucylnegamycin (1971), Requinomycin (1972), Minosaminomycin (1974), Amiclenomycin (1974).

Derivatives of Aminoglycosidic Antibiotics:

3',4'-Dideoxykanamycin B (1971), 5"-Deoxylividomycin A (1972), 5"-Amino-5"-deoxylividomycin A (1972), 5"-Deoxylividomycin B (1972), 3',4'-Dideoxy-6-N-methylkanamycin B (1972), 3'-Deoxykanamycin (1972), 3'-O-Methylkanamycin (1972), 1-N-(4-Amino-2-hydroxybutyryl)lividomycin A (1973), 3',4'-Dideoxybutirosin B (1973), 1-N-(4-Amino-2-hydroxybutyryl)-kanamycin B (1973), 3'-Deoxybutirosin B (1973), 1-N-(4-Amino-2-hydroxy-butyryl)-6-N-methyl-3',4'-dideoxykanamycin B (1973), 6'-Amino-6'-deoxy-lividomycin B (1973), 6'-Deoxy-6'-methylaminolividomycin B (1973), 6'-Deoxy-6'-hydroxyethylaminolividomycin B (1973), 1-N-Isoserylkanamycins (1974), 4'-Deoxykanamycin (1974).

Enzyme Inhibitors:

Leupeptin (1969) inhibiting trypsin, plasmin, papain, thrombokinase and cathepsin B, Antipain (1972) inhibiting trypsin, papain, thrombokinase, cathepsin A and cathepsin B, Chymostatin (1970) inhibiting chymotrypsins, papain and cathepsin B, Elastatinal (1973) inhibiting elastase, Pepstatin (1970) inhibiting pepsin, gastricsin, cathepsin D and renin, Hydroxy pepstatin (1973) inhibiting pepsin, gastricsin, cathepsin D and renin, Pepstanone (1972) inhibiting pepsin, gastricsin, cathepsin D and renin, Phosphoramidon (1973) inhibiting thermolysin, Panosialin (1971) inhibiting trypsin, plasmin, pepsin, sialidase, acid phosphatase and polygalacturonase, Aquayamycin (1968) inhibiting tyrosine hydroxylase, Oudenone (1970) inhibiting tyrosine hydroxylase, Fusaric acid (1969) inhibiting dopamine β-hydroxylase, Dopastin (1972) inhibiting dopamine β-hydroxylase, Oosponol (1972) inhibiting dopamine β-hydroxylase, Pimprinine (1973) inhibiting monoamine oxidase, Cinnamic acid amide (1973) inhibiting monoamine oxidase, Methylspinazarin (1973) inhibiting catechol-O-methyltransferase, 7-O-methylspinochrome B (1973) inhibiting catechol-O-methyltransferase, Dihydromethylspinazarin (1973) inhibiting catechol-O-

methyltransferase, 6-(3-hydroxy-n-butyl)-7-O-methylspinochrome B (1973) inhibiting catechol-O-methyltransferase, 1-[2-(3,4,5,6-tetrahydropyridyl)]-1,3-pentadiene (1974) inhibiting N-methyltransferase, Lecanoric acid (1974) inhibiting histidine decarboxylase, 5-Formyl uracil (1972) inhibiting xanthine oxidase, Coformycin (1967) inhibiting adenosine deaminase, β-Lactamase inhibitor (1974).

A Bibliography of H. Umezawa's Most Important Publications

1) Umezawa, H.; K. Maeda, T. Takeuchi and Y. Okami: New antibiotics bleomycins A and B. J. Antibiotics, 19A, 200 (1966).

In the study of antibiotics resembling phleomycin, new antibiotics which were differentiated from phleomycin by their stability in acid and their behaviour in paper chromatography were isolated by cation exchange resin chromatography, and named bleomycins A and B.

2) Umezawa, H.; Y. Suhara, T. Takita and K. Maeda: Purification of bleomycins. J. Antibiotics, 19A, 210 (1966)

Bleomycins A and B were further separated into A_1 - A_6 and B_1 - B_6, and each of them completely purified by chromatography using a gradient of ammonium formate.

3) Ishizuka, M.; H. Takayama, T. Takeuchi and H. Umezawa: Activity and toxicity of bleomycin. J. Antibiotics, 20A, 15 (1967)

Bleomycin A and B exhibited inhibition of Ehrlich carcinoma with a high therapeutic index. They also inhibited microorganisms. Bleomycin showed no irreversible renal toxicity in dogs and exhibited a therapeutic effect against round cell carcinoma near the vagina in dogs.

4) Umezawa, H.; T. Takeuchi, S. Hori, T. Sawa, M. Ishizuka, T. Ichikawa and T. Komai: Studies on the mechanism of antitumor effect of bleomycin on squamous cell carcinoma. J. Antibiotics, 25, 409 (1972)

^{3}H-bleomycin was prepared and after subcutaneous injection in mice, all organs were homogenized and concentrations of bleomycin in each organ homogenate were determined by radioactivity and antibacterial measurements. The results indicated that all tissues contain a bleomycin-inactivating enzyme. The inactivation was significantly slower in skin and lung, especially in old mice. Squamous cell carcinoma and sarcoma in the skin

of mouse was induced by methylcholanthrene. These tumors were homogenized, and the activity in inactivating bleomycin was shown to be significantly higher in the carcinoma than in the sarcoma. The bleomycin-inactivating enzyme was extracted from mouse liver homogenate by successive application of protamine treatment, precipitation with 60% saturated ammonium sulfate and Sephadex 100 chromatography. The enzyme was shown to hydrolyze the carboxamide group of bleomycin. The inactivated bleomycin was isolated and was shown to have a carboxylic acid group. During inactivation, one mole of ammonia was released.

5) Umezawa, H.: Natural and artificial bleomycins: Chemistry and antitumor activity. Pure and Applied Chemistry, 28, 665 (1971)

Sequential hydrolysis of bleomycin resulted in each amino acid component and indicated that various bleomycins are different from one another in the terminal amine moiety. Addition of an amine to the culture suppressed the production of natural bleomycins and caused the production of bleomycin containing the added amine. This technique was useful in providing a bleomycin mixture containing various bleomycins in a constant ratio. This paper also describes the isolation of the inactivated bleomycin.

6) Umezawa, H.; S. Hori, T. Sawa, T. Yoshioka and T. Takeuchi: A bleomycin-inactivating enzyme in mouse liver. J. Antibiotics, 27, 419 (1974)

The enzyme which hydrolyzes the carboxamide group of bleomycin was purified form mouse liver by affinity chromatography using Sepharose 4B-lysinamide. The enzyme thus purified also hydrolyzed L-lysinamide, L-lysyl-β-naphthylamide and L-arginyl-β-naphthylamide, but not leucyl-β-naphthylamide. Thus, this enzyme was shown to be aminopeptidase B. However, in mice this bleomycin-inactivating enzyme was very unstable. Using rat liver it was differentiated from known aminopeptidase B by chromatographic separation.

7) Umezawa, H.: Studies on bleomycin: Chemistry and biological action. Biomedicine, 28, 459 (1973)

H. Umezawa's study on the discovery, isolation, chemistry, mechanism of action, mechanism of the effect on squamous cell carcinoma, and production of artificial bleomycins are reviewed in this paper. H. Umezawa first found that bleomycin binds with DNA and causes single strand scission.

8) Umezawa, H.; M. Okanishi, S. Kondo, K. Hamana, R. Utahara, K. Maeda and S. Mitsuhashi: Phosphorylative inactivation of aminoglycosidic antibiotics by E. coli carrying R factor. Science, 157 (3796), 1559 (1967)

Resistant E. coli carrying R factor were shown to contain intracellular enzymes inactivating kanamycins, streptomycin, and neomycins. These enzymes were present in the supernatant of disrupted cells, and the products of the enzyme reactions were isolated. Thus, kanamycin resistance was shown to be due to a phosphotransferase which phosphorylates 3'-OH of kanamycins.

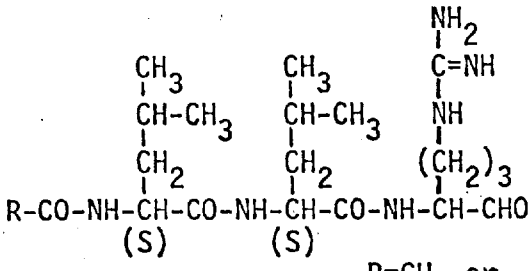
9) Umezawa, H.; S. Umezawa, T. Tsuchiya and Y. Okazaki: 3',4'-Dideoxykanamycin B active against kanamycin-resistant E. coli and Ps. aeruginosa. J. Antibiotics, 24, 485 (1971)

3'-OH kanamycin B underwent phosphorylation by the enzyme in resistant organisms. Therefore, 3'-OH and 4'-OH were removed chemically from kanamycin B. 3',4'-Dideoxykanamycin B thus prepared inhibited the resistant organisms. This proves the involvement of enzymatic inactivation in resistance. The compound represents the first successful application of knowledge of the biochemical basis of resistance to the development of an active agents useful in the treatment of resistant infections.

10) Umezawa, H.: Biochemical mechanism of resistance to aminoglycosidic antibiotics and development of active derivatives. Advances in Carbohydrate Chemistry and Biochemistry. Academic Press, New York and London, 1974.

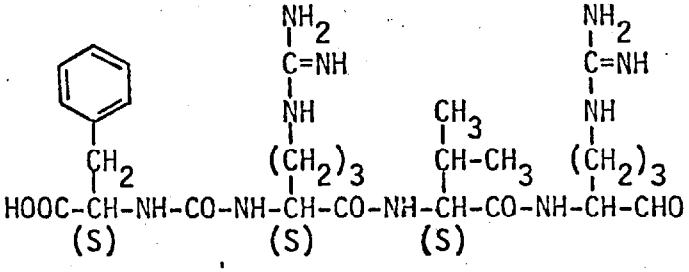
11) Umezawa, H.: Enzyme Inhibitors of Microbial Origin. University of Tokyo Press, Bunkyo-ku, Tokyo, 1973. Umezawa, H.: Chemistry of enzyme inhibitors of microbial origin. Pure and Applied Chemistry, 33, 129 (1973).

List of Structures of Enzyme Inhibitors Discovered by H. Umezawa



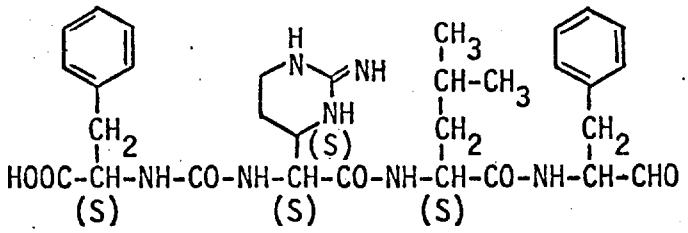
Leupeptin $R=CH_3$ or C_2H_5

inhibiting trypsin, plasmin, papain, thrombokinase, cathepsin B



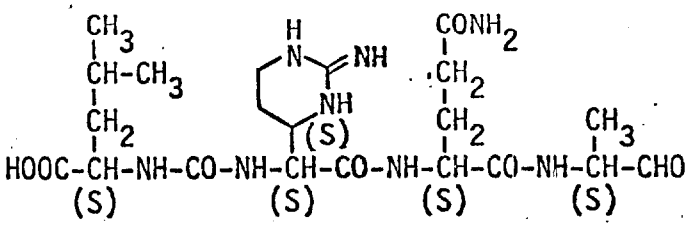
Antipain

inhibiting trypsin, papain, thrombokinase, cathepsin A and B



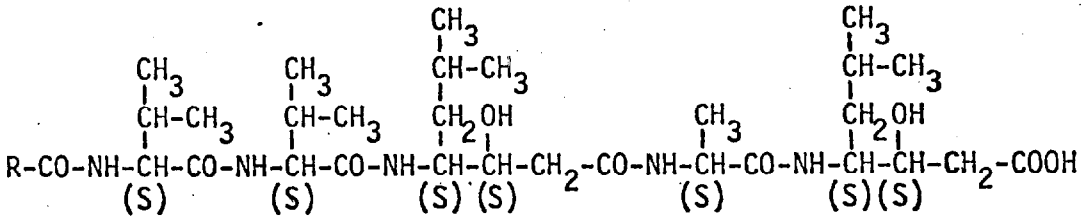
Chymostatin

inhibiting chymotrypsins, papain, cathepsin B



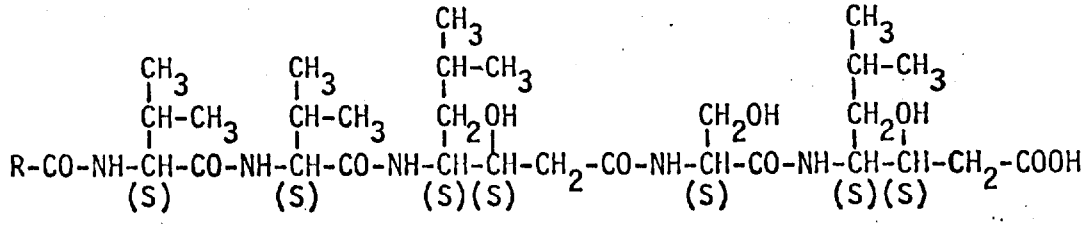
Elastatinal

inhibiting elastase



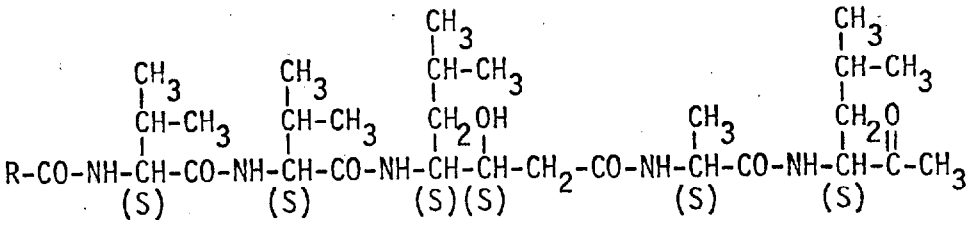
Pepstatin $R=CH_3$, C_2H_5, C_3H_7, C_4H_9, C_5H_{11} ..... etc.

inhibiting pepsin, gastricsin, cathepsin D, renin



Hydroxy pepstatin $R=CH_3$, C_2H_5, C_3H_7, C_4H_9, C_5H_{11} ..... etc.

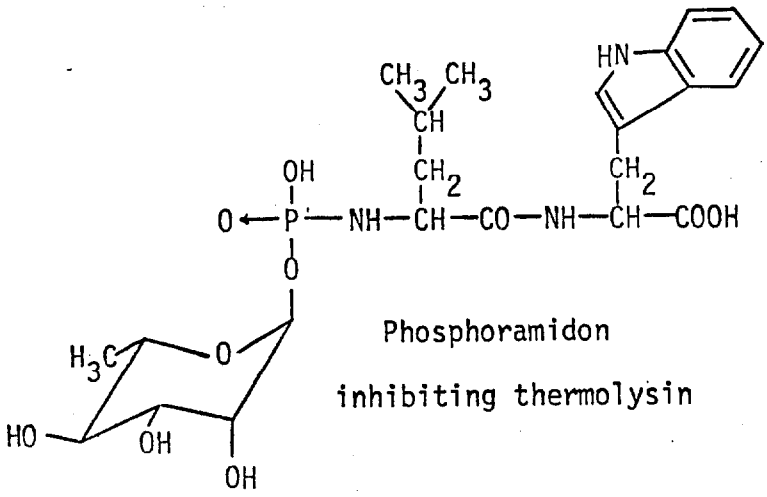
inhibiting pepsin, gastricsin, cathepsin D, renin



Pepstanone

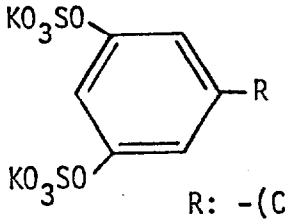
R=CH_3, C_2H_5, C_3H_7, C_5H_{11} ... etc

inhibiting pepsin, gastricsin, cathepsin D, renin



Phosphoramidon

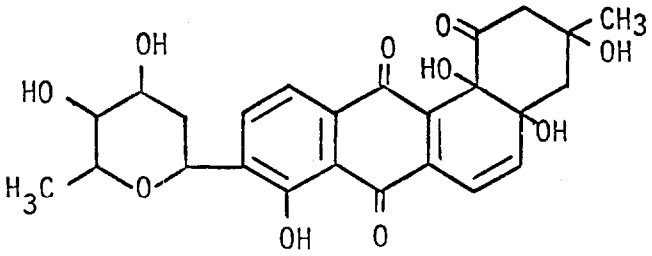
inhibiting thermolysin



R: $-(CH_2)_{12}-CH(CH_3)_2$,
$-(CH_2)_{14}-CH_3$,
$-(CH_2)_{13}-CH(CH_3)_2$

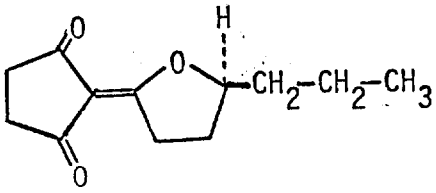
Panosialin

inhibiting trypsin, plasmin, pepsin, sialidase, acid phosphatase, polygalacturonase



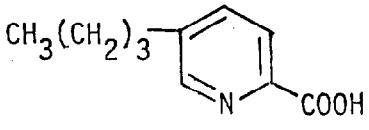
Aquayamycin

inhibiting tyrosine hydroxylase



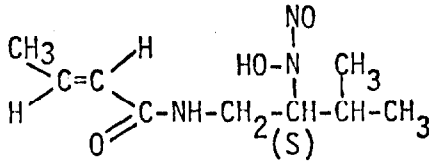
Oudenone

inhibiting tyrosine hydroxylase



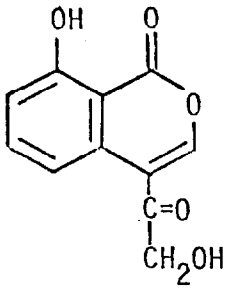
Fusaric acid

inhibiting dopamine β-hydroxylase



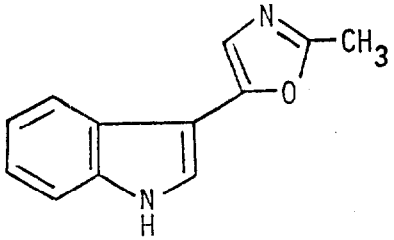
Dopastin

inhibiting dopamine β-hydroxylase

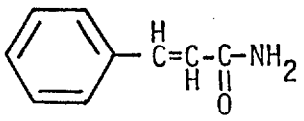


Oosponol

inhibiting dopamine β-hydroxylase

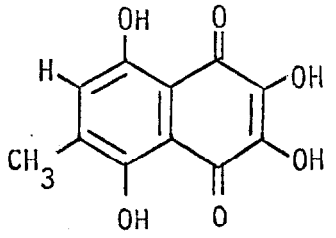


Pimprinine



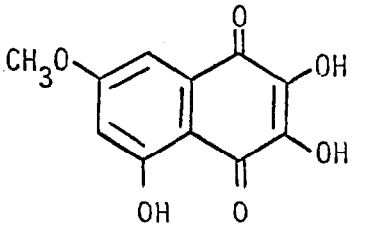
Cinnamic acid amide

inhibiting monoamine oxidase



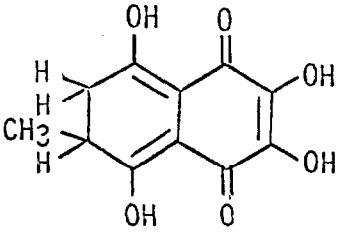
Methylspinazarin

inhibiting catechol-O-methyl-transferase



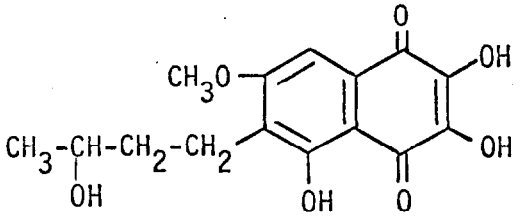
7-O-methylspinochrome B

inhibiting catechol-O-methyltransferase



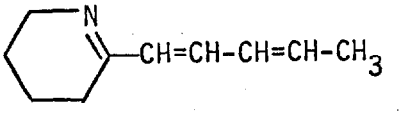
Dihydromethylspinazarin

inhibiting catechol-O-methyltransferase



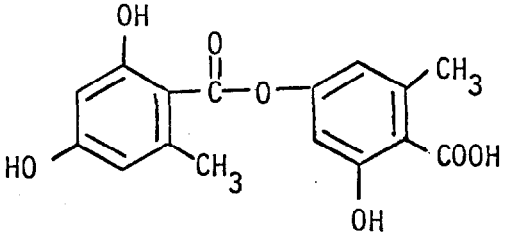
6-(3-hydroxy-n-butyl)-7-O-methylspinochrome B

inhibiting catechol-O-methyltransferase



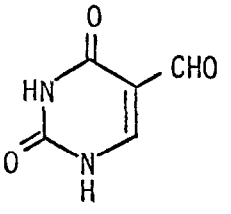
1-[2-(3,4,5,6-tetrahydropyridyl)]-1,3-pentadiene

inhibiting N-methyltransferase



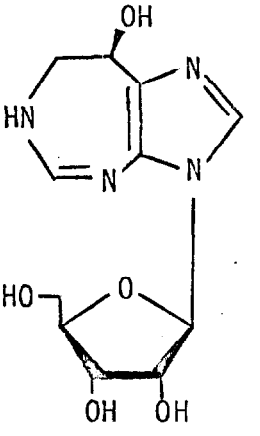
Lecanoric acid

inhibiting histidine decarboxylase



5-Formyl uracil

inhibiting xanthine oxidase



Coformycin

inhibiting adenosine deaminase