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SPECIAL REPORT

RESULTS FROM THE CHEMICAL ANALYSIS OF OILY RESIDUE SAMPLES TAKEN FROM STRANDED JUVENILE SEA TURTLES COLLECTED FROM PADRE AND MUSTANG ISLANDS, TEXAS

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RESULTS FROM THE CHEMICAL ANALYSES OF OILY RESIDUE SAMPLES TAKEN FROM STRANDED JUVENILE SEA TURTLES COLLECTED FROM PADRE AND MUSTANG ISLANDS, TEXAS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

A joint United States-Mexican project has been initiated with the goal of argumenting the natural breeding stock of the endangered Kemp's Ridley sea turtles (Lepidochelys kempi). Eggs were hatched and the hatchlings raised to juveniles by the United States National Marine Fisheries Service in Galveston, Texas. The juvenile Kemp's Ridleys were released June 7, 1983, in sargassium patches situated along the 10 fathom depth contour offshore of Padre and Mustang Islands. Between June 8 and 13, 1983, many of the juveniles were found dead or distressed and stranded on Padre and Mustang Islands. Upon inspection, the turtles were found to have small oily residues, with the appearance of tar in the mouth, esophagus, and stomach. Approximately thirty samples of the oily residues from these stranded turtles were collected and sent to the Center for Bio-Organic Studies at the University of New Orleans for chemical analyses.

Oily residue samples were collected with metal spatulas and stored frozen until they were analyzed. Quantities of the oily material in the various samples ranged from approximately one hundred milligrams to approximately three thousand milligrams. Of the thirty samples, the U.S. Department of Interior's Mineral Management Service contracted to have six samples subjected to various types of chemical analyses. It was decided not to composite sample material collected from different turtles since this technique could result in the mixing of oils from two or more different sources. Because of this decision not to composite the oily residues, the selection process simplified to the task of finding six samples that contained enough oily residue material for the analyses. The analytical scheme, which was requested by the Mineral Management Services, required approximately 1000 milligrams of the oily residue material per sample and included:

a) High resolution gas chromatographic (HRGC) analyses of the saturate fractions.

b) High resolution gas chromatographic-mass spectrometric (HRGC-MS) analyses of the aromatic fractions.

c) Stable carbon, hydrogen, and sulfur isotope analyses.

d) Nickel and vanadium analyses.

e) American Petroleum Institute (API) gravities.

EXPERIMENTAL

HRGC, HRGCMS, carbon 13, hydrogen 2 (deuterium), nickel and vanadium analyses require approximately 1000 milligrams of oily material per sample for processing. However, the other analytical tests (stable sulfur 34 and API gravity) require between 1 and 100 ml of sample per analysis. Consequently, no sulfur 34 stable isotope analyses were performed and specific gravities were obtained using an alternate procedure to the API method. Brief descriptions of the analytical techniques that were used for analyses of the oily residue samples are found below.

Twenty to 30 milligram aliquots of the six samples were dissolved in hexane and fractionated using liquid-solid column chromatographic techniques (activity 1 silica gel) into saturate (f1) and aromatic (f2) fractions. The f1 fractions were eluted with three bed volumes of n-hexane while the f2 fractions were eluted with three bed volumes of 30% methylene chloride in n-hexane. Both fractions were analyzed by high resolution gas chromatography using 30 meter by 0.32 mm I.D. SE-52 fused silica capillary columns and Hewlett-Packard (HP) 5711 gas chromatographs. The aromatic fractions were further analyzed under identical chromatographic conditions by fused silica capillary column gas chromatography-mass spectrometry on a repetitively scanned HP 5985 GCMS instrument.

Procedures for stable isotope analyses were as follows. Unfractionated samples, sent to Globial Geochemistry Corporation, Canaga, California, were washed to remove salts, freeze-dried, and then analyzed for carbon and hydrogen stable isotope ratios. One milligram aliquots of each sample were combusted at 900°C in the presence of cupric oxide and silver metal. The produced CO_2 vapors were purified and collected for isotope analyses. The waters produced by the combustions were converted to hydrogen gas by reaction with uranium turnings at 800°C and collected on activated charcoal at liquid nitrogen temperatures in sample tubes. Stable isotopic ratio determinations for C and H were carried out using a Varian MAT 250 isotope ratio mass spectrometer. All isotopic data were expressed in the standard notation:

$$X \text{ sample } (\text{o/oo}) = \frac{R \text{ sample} - R \text{ standard}}{R \text{ standard}} \times 1000$$

were X represents the element of interest and R the ratio of the rare to the most abundant isotope of that element (e.g., $^{13}C/^{12}C$). The isotopic standards used for carbon and hydrogen are Chicago PeeDee Beleminite (PDB) and standard mean ocean water (SMOW), respectively. Analytical precision of the reported values is $0.10^{o}/oo$ for carbon and $1.0^{o}/oo$ for hydrogen. Sulfur 34 stable isotope analyses require between 1 and 4 milliliters of sample material per analysis and, consequently, were not attempted.

Additional aliquots of several hundred milligrams from each sample were dissolved in xylene and then analyzed by graphite furnace atomic absorption spectrometry, using a Perkin-Elmer 5000 instrument. Their nickel and vanadium concentrations were determined using the modified procedures as outlined in the Perkin-Elmer applications manual.

Specific gravities were determined by weighing approximately five hundred milligrams of each sample in small graduated cylinders and then measuring the oily residue's volume by the water displacement method. The API gravity method requires approximately 100 ml of sample material per analysis and, consequently, was not feasible for use with these samples.

RESULTS

Table I contains data which summarizes information that is pertinent to when and where the oily residue samples were collected. Four of the samples were taken from deceased organisms while the other two samples were taken from turtles that were eventually re-released into the Gulf.

The goals of this project were to identify the nature of the oily residues, determine if they contained toxic components and, if possible, establish sources for the oily materials. Chemical data from the specific gravity, carbon 13, hydrogen 2, nickel and vanadium analyses are contained in Table II. Specific gravity results support the conclusion that the oily residues were buoyant and available to the turtles as foodstuffs. Stable isotope data are within the range of those values reported by Van Vleet *et al*. (1983) for floating oil residues in the eastern Gulf of Mexico. The isotopic data, however, support the conclusion that the oily residues did not originate from a single diagenetic source such as IXTOC or south Louisiana crude. Nickel and vanadium concentrations, and their ratios, were similar to those reported by Jeffery *et al*. (1975) in floating tars collected from the Gulf. In general, nickel and vanadium concentrations were higher than is found in crude oil sources situated in either the Gulf of Mexico and the Arabian Gulf (Pancirov, 1974).

Table III contains information on the quantities of hydrocarbons found in the saturate (f1) and aromatic (f2) fractions and, by inference, the quantities of asphaltenic and polar materials in these samples. It is interesting to note, for example, that sample NNM101 contained almost all of it's hydrocarbon components in the f1 and f2 fractions while sample NNM843 contained more asphaltenes and polar materials than f1 and f2 hydrocarbons. In general, the oily residue samples contain greater quantities of f1 components than f2 components by factors of 4 to 7. These quantitative data are in agreement with the fact that the oily residue samples contained relatively large quantities of high molecular weight waxy hydrocarbon components in their f1 fractions.

Figures 1 and 2 show portions of the computer reconstructed gas chromatograms of the saturate fractions from samples NNM035 and NNM101. These data provide clear evidence to support the conclusion that the oily residue samples contained high levels of the "waxy" hydrocarbons (paraffins) in the n-C20 to n-C32 range. Also, the samples' chromatograms did not exhibit pronounced humps in their chromatographic baselines. A chromatographic hump, commonly referred to as a complex unresolved mixture (CUM) (Farrington and Tripp, 1977), is an important characteristic which indicates the environmental weathering of crude oils that have been spilled into the marine environment. Figure 3 shows the high resolution chromatographic data from analysis of a sample of weathered IXTOC crude. This chromatogram is typical of analytical data which is obtained when samples of spilled crude oil are weathered in the marine environment. Note that the chromatogram contains a pronounced hump and has a distribution of saturate hydrocarbons from *n*-C16 to *n*-C30 with the abundant normal hydrocarbon being *n*-C20. Figure 4 shows high resolution gas chromatographic data from the analysis of a "waxy" crude oil from an Indonesian source. This crude contained only small quantities of saturate hydrocarbons below *n*-C22 with the most abundant normal hydrocarbon being *n*-C28. Note the absence of a hump in the chromatogram of this waxy crude that had not been environmentally weathered.

Table IV contains the tabular data on the concentrations of selective saturate hydrocarbons in all six oily residue samples. Examination of the chromatograms in Figures 1 to 4, and the chromatographic data contained in Table IV leads to the following observations. First, the oily residue samples were not simply weathered crude oils. Second, the residues contained high quantities of "waxy" hydrocarbons that are commonly found as residues from tanker cleaning operations. Third, based on differences in the pristane/phytane ratios of the various samples, there were probably more than one diagenetic source for these samples.

Tables V and VI contain ratios of the parent and C1 to C3 alkyl homologs of phenanthrene-dibenzothiophene and chrysene-naphthylbenzothiophene aromatic hydrocarbons. These data were derived from the GCMS analyses of the aromatic fractions of the oily residue samples. This type of data has been used to differentiate crude oils from several diagenetic sources and is based on the fact that some crude oils have elevated levels of sulfur containing aromatic hydrocarbon compounds when compared to other oils from different diagenetic sources (Overton _et al_., 1981). Examination of the data revealed that sample NNM101 contained significantly lower amounts of sulfur containing aromatic compounds than the other oily residue samples. Also, even though the pristane-phytane data contained evidence that there may be three different crude oil sources among the six oily residue samples, this GCMS data could be used to segregate the samples into only two distinct groups.

Based on our examination of all the analytical data, the following observations can be made.

1) Stable carbon and hydrogen isotope analyses do not support a single source for the six oily residue samples.

2) Data on the quantities of nickel and vanadium are similar to those found in heavy crude oils or residues and within the range of reported concentrations found in tarballs collected throughout the Gulf of Mexico.

3) The absence of pronounced chromatographic humps is supportative of the fact that these oily residue samples were not simply heavily weathered crude oils.

4) High resolution gas chromatographic data support the fact that the oily residues contained elevated levels of "waxy" paraffins in the _n_-C20 to _n_-C32 range.

5) Pristane-phytane data ratios and high resolution gas chromatographic profile data support the following grouping of samples as having similar sources:

a) NNM035 and NNL268
b) NNM833, NNM843 and NNM922
c) NNM101

6) Other parameters, such as the ratio of saturate to aromatic hydrocarbons, do not provide conclusive evidence to support this matching of sample sources.

7) GCMS derived alkyl homolog data support the conclusion that sample NNM101 has a lower sulfur content than the other samples.

8) GCMS analyses revealed only low quantities of the more acutely toxic alkyl benzenes and naphthalenes in the samples.

9) Specific gravities of all samples were low enough to allow the samples to be buoyant in seawater.

CONCLUSIONS

Based on our examination of the data, and an appreciation of the effects of weathering on spilled crude oils, we have reached the following conclusions.

First, the samples were most probably waxy residues that had been discarded after tanker cleaning operations.

Second, there were insufficient data to accurately identify the diagenetic sources of the waxy residues.

Third, the samples did not contain appreciable quantities of the more acute toxic (to marine organisms) petroleum hydrocarbons such as the alkyl benzenes and naphthalenes.

Fourth, the samples were buoyant and available to the turtles as foodstuffs.

ACKNOWLEDGEMENTS

The authors would like to express their appreciation to: the National Park Service and National Marine Fisheries Service for their careful collection of the oily residue samples and their information concerning the circumstances of this incident; Mr. Dale Hall of the U.S. Fish and Wildlife Service for transmittal of the samples to New Orleans; Mineral Management Service for funding of the analyses; Dr. Robert Avent and Ms. Gail Rainey of the Mineral Management Service for their many helpful discussions and technical assistance in the preparation of this report; and Ms. Diane Trembley for her assistance in preparation of this document.

REFERENCES

1. Farrington, J.W. and B.W. Tripp (1977). Hydrocarbons in Western North Atlantic Surface Sediments. *Geochim et Coschochim Acta 41*:1627-1641.
2. Jeffrey, L.M., Powel, N., and Vos, A. (1975). Progress Report on the Chemistry of Environmental Tars of the Gulf of Mexico and Atlantic, *Study of Naturally Occurring Hydrocarbons in the Gulf of Mexico*, Report of the U.S. Department of Interior, Bureau of Land Management.

3. Overton, E.B., McFall, J.A., Mascarella, S.W., Steele, C.J., Antoine, S.R., Politzer, I.R., and Laseter, J.L. (1981). Identification of Petroleum Residue Sources After a Fire and Oil Spill, *Proceedings of the 1981 Oil Spill Conference*, Atlanta, GA.
4. Pancirov, R.J. (1974). Compositional Data on API Reference Oils Used in Biological Studies: A # Fuel Oil, A Bunker C, Kuwait Crude Oil and South Louisiana Crude Oil, Report No. AID.IBA.74, Esso Research and Engineering Company, Linden, N.J.
5. Van Vleet, E.S., Sackett, W.M., Reinhardt, S.B. and Mangini, M.E. (1983). Distribution, Sources and Fates of Floating Oil Residues in the Eastern Gulf of Mexico, accepted for publication, *Marine Pollution Bulletin*.

TABLE I

DATA USED TO IDENTIFY SPECIFIC OILY RESIDUE SAMPLES CHOSEN FOR CHEMICAL ANALYSES

Tag Number	Date Recaptured (Live/Dead)	Tar Specimen Taken From
NNM035	6/18/83 (D)	Mouth Esophagus
NNM101	6/9/83 (D)	Mouth
NNM833	6/11/83 (D)	Mouth
NNM922	Prior to 6/14/83	Mouth, Throat Esophagus
NNL268	6/21/83 (L)	Mouth
NNM843	6/9/83 (L)	Mouth

TABLE II

RESULTS FROM THE SPECIFIC GRAVITY, CARBON AND HYDROGEN STABLE ISOTOPE AND NICKEL AND VANADIUM ANALYSES OF OILY RESIDUES TAKEN FROM JUVENILE SEA TURTLES

Parameters Measured	SAMPLE TAG NUMBERS					
	NNM 035	NNM 101	NNM 833	NNM 922	NNL 268	NNM 843
Specific Gravity	0.97	0.72	0.96	0.89	0.73	0.81
^{13}C(o/oo)	-28.56 ppt	-26.48 ppt	-27.11 ppt	-25.87 ppt	-27.03 ppt	-27.03 ppt
^{2}H (o/oo)	-113 ppt	-123 ppt	-109 ppt	-100 ppt	-97 ppt	-100 ppt
δNi	20 ppm	12 ppm	22 ppm	32 ppm	19 ppm	24 ppm
δV	37 ppm	20 ppm	92 ppm	130 ppm	82 ppm	61 ppm
Ni/V	0.54	0.60	0.25	0.23	0.24	0.39
V/Ni	1.9	1.7	4.0	4.3	4.2	2.6

TABLE III

QUANTITATIVE DATA AND RESULTS FROM THE LIQUID-SOLID CHROMATOGRAPHIC FRACTIONATIONS OF OILY RESIDUE SAMPLES TAKEN FROM JUVENILE SEA TURTLES

Parameters Measured	SAMPLE TAG NUMBERS					
	NNM 035	NNM 101	NNM 833	NNM 922	NNL 268	NNM 843
Amount Fractionated*	24 mg	19 mg	30 mg	24 mg	23 mg	18 mg
Amount in F1 Fraction**	10 mg	14 mg	12 mg	14 mg	9.6 mg	5.3 mg
Amount in F2 Fraction**	2.7 mg	3 mg	2 mg	2 mg	1.3 mg	0.8 mg
Total F1 & F2	13 mg	17 mg	14 mg	16 mg	11 mg	6.1 mg
Total F1 & F2 / Amt Fract.	0.54	0.89	0.47	0.67	0.48	0.34
Amount F1 / Amount F2	3.7	4.7	6.0	7.0	7.4	6.6

*Determined by gravimetric methods.

**Determined from gas chromatographic data.

TABLE IV

CONCENTRATIONS IN PPM* OF SELECTED HYDROCARBONS IN OILY RESIDUE SAMPLES TAKEN FROM JUVENILE SEA TURTLES

SAMPLE/ COMPOUND	NNM035	NNM101	NNM833	NNM922	NNL268	NNM843
n-C16	3600.	2800.	1000.	2300.	1300.	590.
n-C17	3800.	3600.	1400.	3000.	1600.	380.
pristane	1800.	3300.	1400.	1500.	1300.	770.
n-C18	3500.	4100.	1600.	3200.	1700.	480.
phytane	2000.	930.	880.	940.	1200.	560.
n-C19	3200.	4900.	1700.	3500.	1600.	480.
n-C20	3100.	5400.	1900.	3900.	1600.	430.
n-C21	2900.	6200.	2000.	4500.	1400.	410.
n-C22	2620.	6900.	2100.	4600.	1400.	380.
n-C23	2300.	7700.	2200.	4700.	1300.	430.
n-C24	2300.	8200.	2400.	4800.	1300.	530.
n-C25	1900.	8900.	2600.	4900.	1400.	660.
n-C26	2100.	8600.	2600.	4700.	1400.	730.
n-C27	2300.	9100.	2700.	4700.	1400.	760.
n-C28	3300.	10000.	2600.	4300.	1400.	940.
n-C29	4300.	7700.	2600.	4000.	1400.	940.
n-C30	5800.	6100.	2100.	3300.	1200.	700.
n-C31	7200.	5600.	2100.	2900.	1300.	1000.
n-C32	5300.	3800.	1900.	2100.	850.	620.

PPM = micrograms per gram

TABLE V

RATIOS OF THE PARENT AND C1 TO C3 ALKYL HOMOLOGS FOR PHENANTHRENE AND DIBENZOTHIOPHENE DERIVED FROM GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC DATA

Parameters Measured	SAMPLE TAG NUMBERS					
	NNM 035	NNM 101	NNM 833	NNM 922	NNL 268	NNM 843
$\frac{\text{Phen}}{\text{DBT}}$	1.20	6.97	2.80	1.15	1.47	2.14
$\frac{C_1\text{Phen}}{C_1\text{DBT}}$	1.09	5.18	1.99	1.13	1.24	1.94
$\frac{C_2\text{Phen}}{C_2\text{DBT}}$	0.78	5.36	1.42	0.86	0.94	1.32
$\frac{C_3\text{Phen}}{C_3\text{DBT}}$	0.75	4.51	1.24	0.74	0.81	1.01

Phen = Phenanthrene (MW = 178).

DBT = Dibenzothiophene (MW = 184).

C1, C2, and C3 denotes respectively the C1, C2 or C3 alkyl homologs of the parent aromatic compounds.

TABLE VI

RATIOS OF THE PARENT AND C1 TO C3 ALKYL HOMOLOGS FOR CHRYSENE AND NAPTHYLBENZOTHIOPHENE DERIVED FROM GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC DATA

Parameters Measured	SAMPLE TAG NUMBERS					
	NNM 035	NNM 101	NNM 833	NNM 922	NNL 268	NNM 843
$\frac{\text{Chry}}{\text{NBT}}$	0.56	1.32	0.98	0.82	0.76	0.94
$\frac{C_1\text{Chry}}{C_1\text{NBT}}$	0.56	1.94	0.94	0.55	0.66	0.78
$\frac{C_2\text{Chry}}{C_2\text{NBT}}$	0.46	2.21	0.94	0.54	0.62	0.64
$\frac{C_3\text{Chry}}{C_3\text{NBT}}$	0.45	2.17	0.86	0.54	0.52	0.61

Chry = Chrysene (MW = 228).

NBT = Naphthylbenzothiophene (MW = 234).

C_1, C_2, and C_3 denotes respectively the C_1, C_2, and C_3 alkyl homologs of the parent aromatic compounds.

Figure 1. Computer Reconstructed High Resolution Gas Chromatogram of the Saturate Fraction from Oily Residue Sample NNM035 (from 20 to 70 minutes).

SAMPLE NUMBER NNM 035
24 mg FRACTIONATED
SILICA GEL FRACTION 1

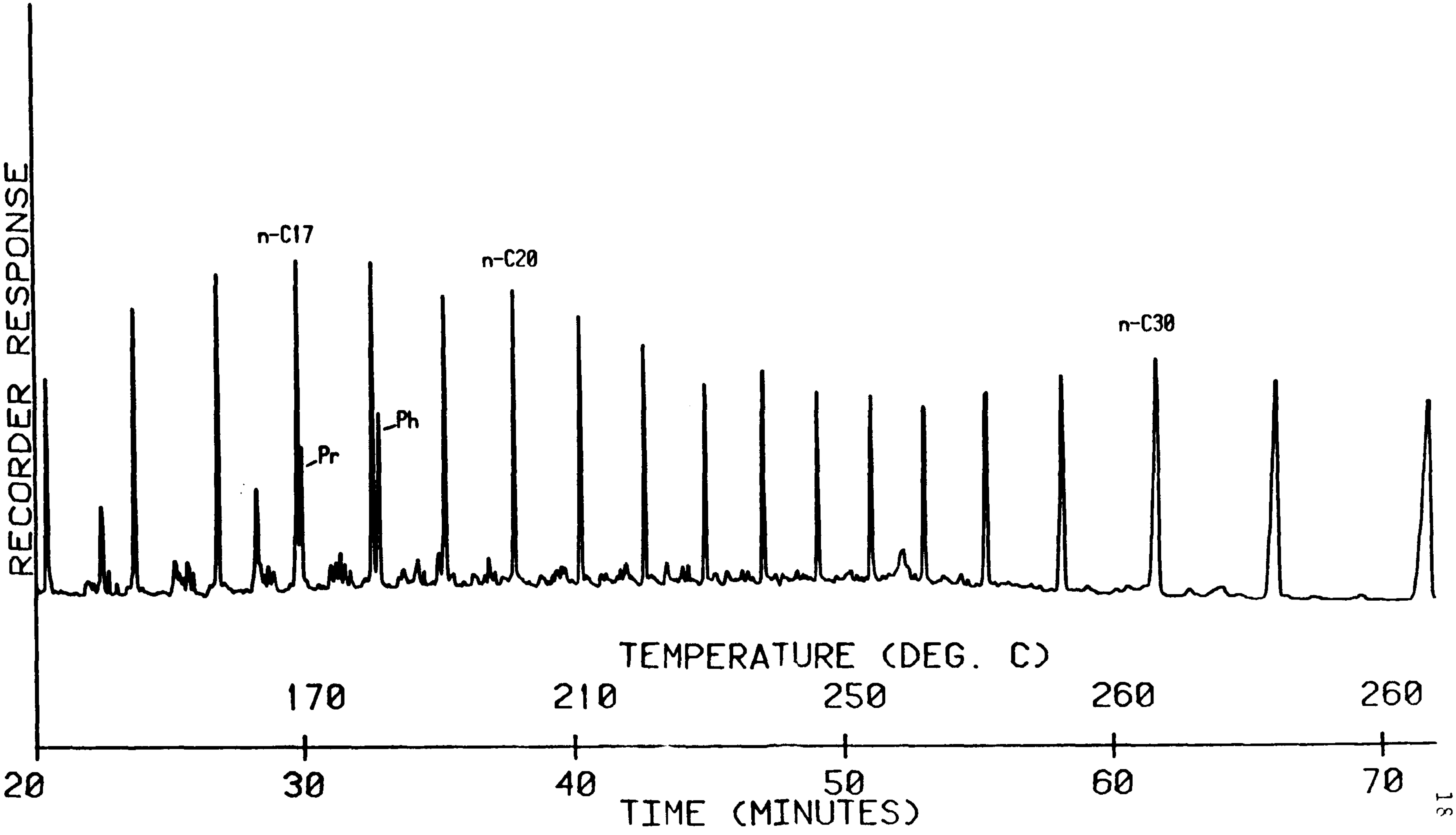


Figure 2. Computer Reconstructed High Resolution Gas Chromatogram of the Saturate Fraction from Oily Residue Sample NNM101 (from 20 to 70 minutes).

SAMPLE NUMBER NNM 101
19 mg FRACTIONATED
SILICA GEL FRACTION 1

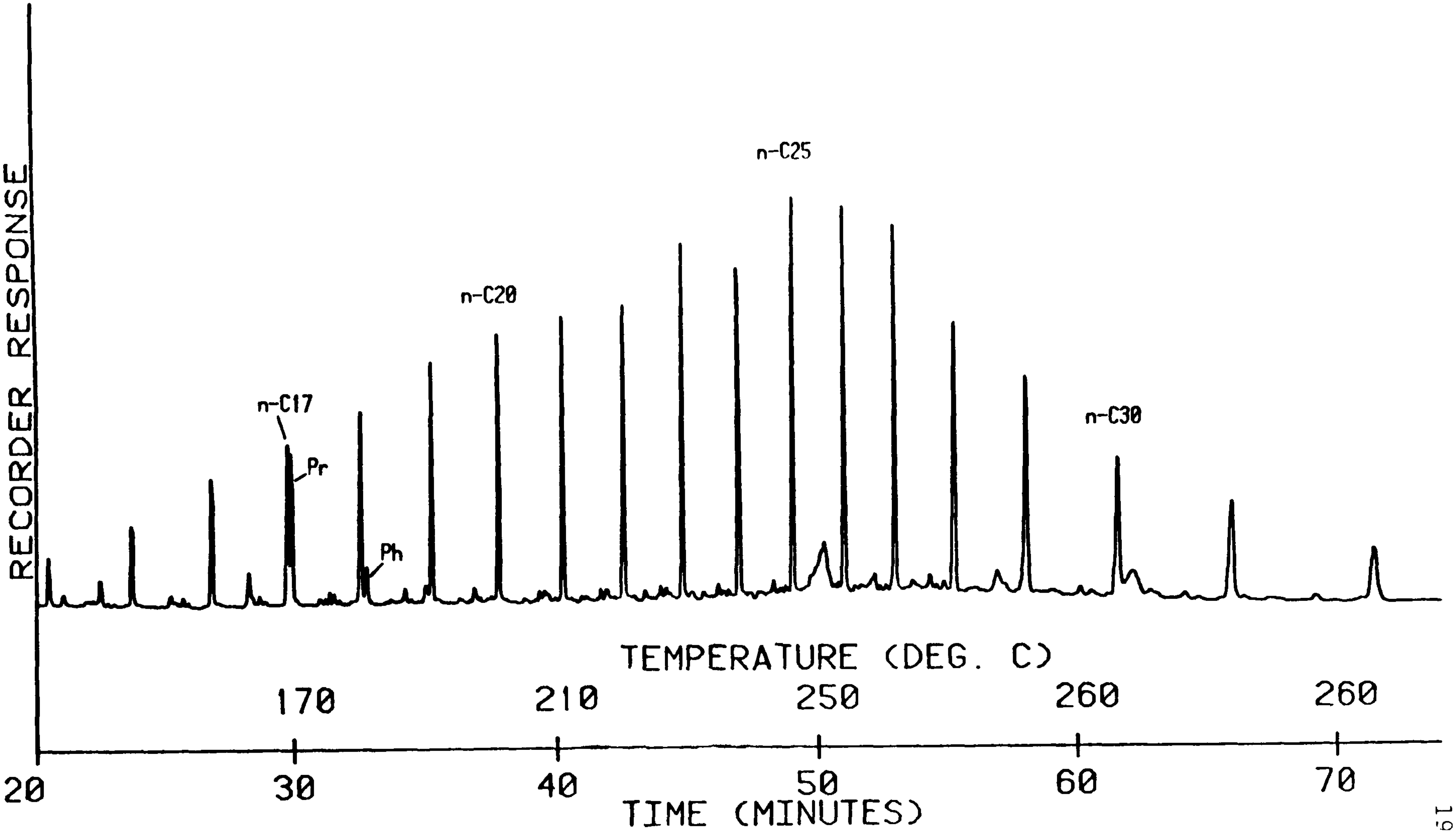


Figure 3. Computer Reconstructed High Resolution Gas Chromatogram of the Saturate Fraction from a Sample of Weathered IXTOC Oil.

WEATHERED IXTOC OIL (MOUSSE)

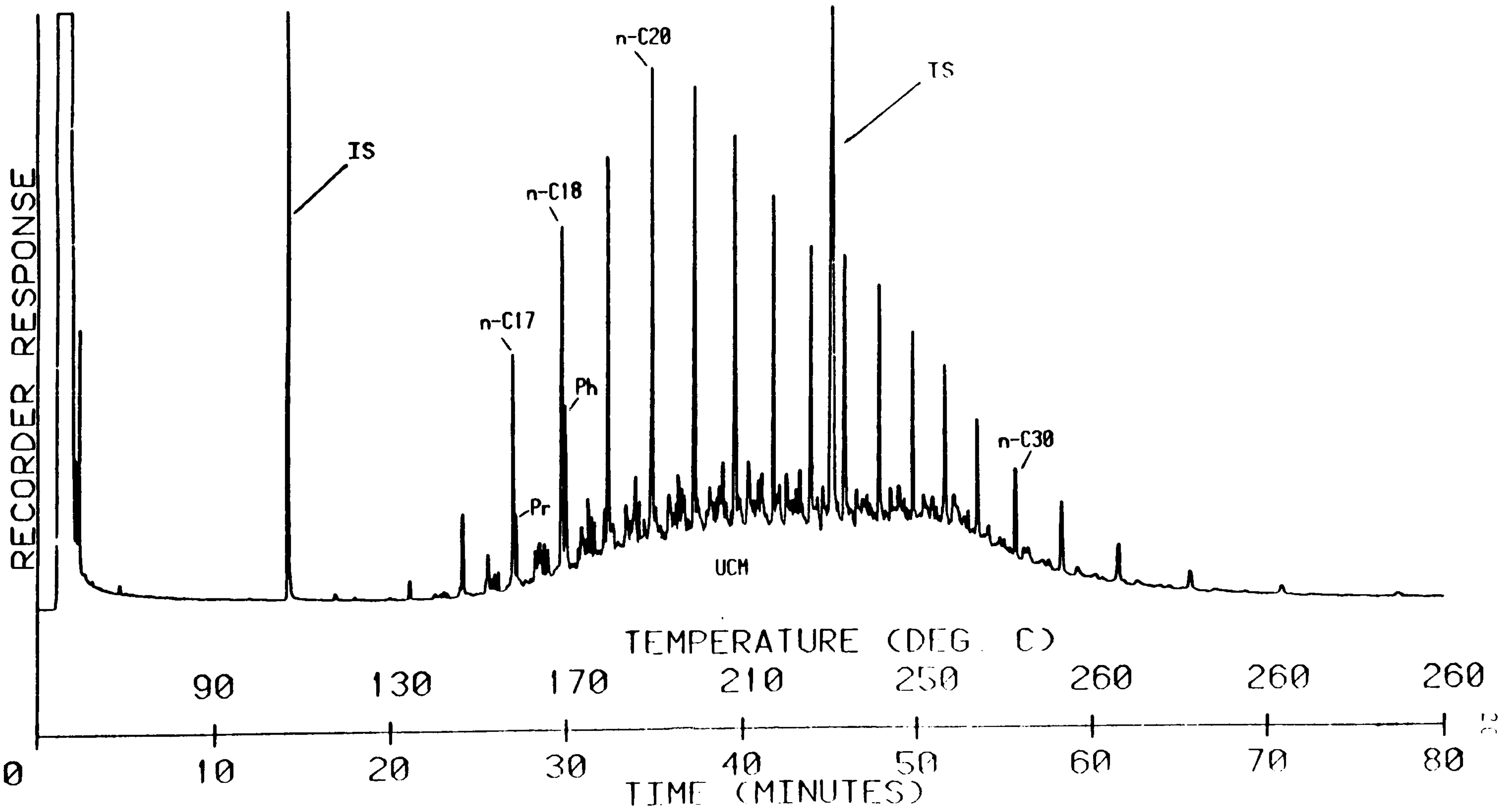
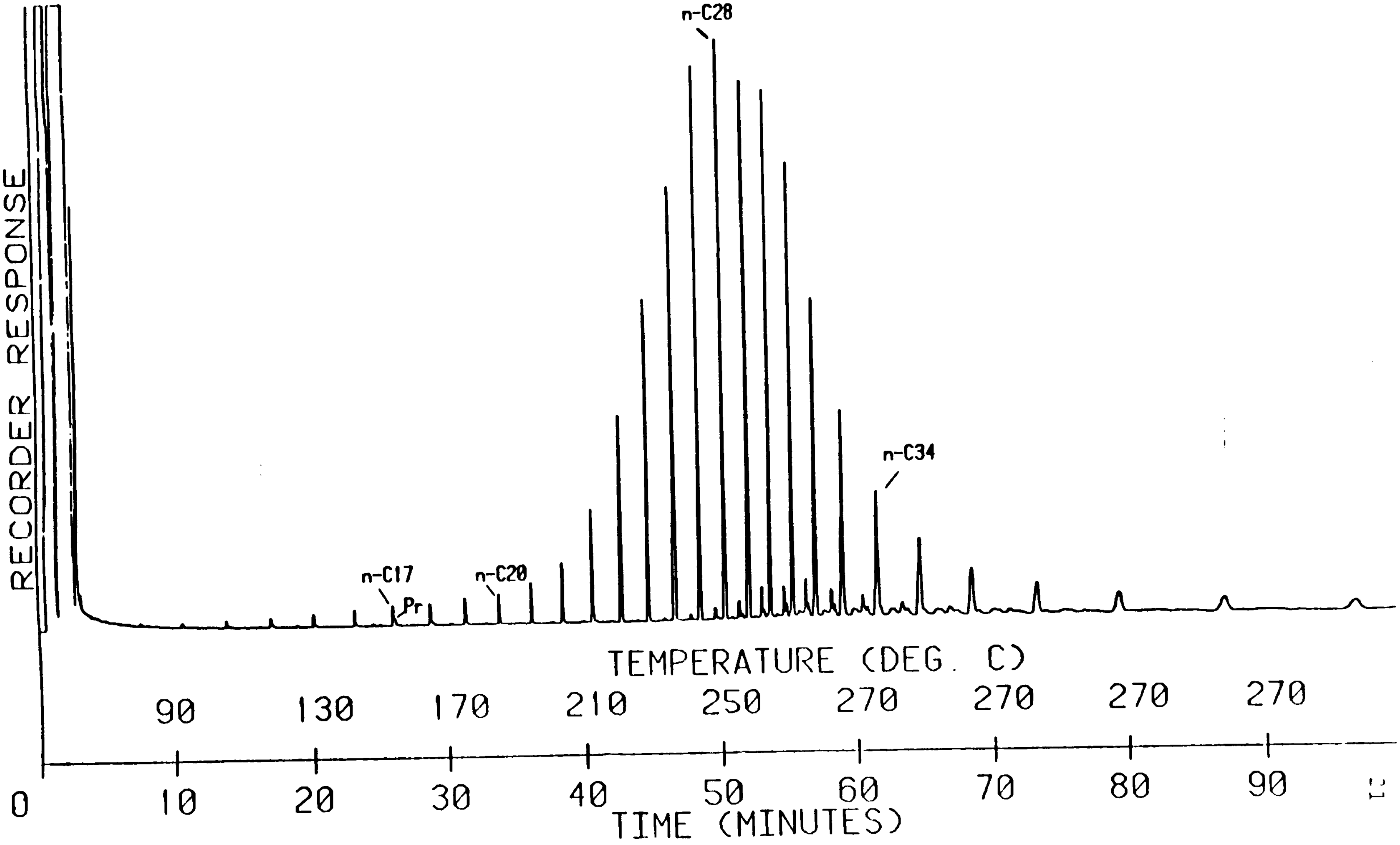


Figure 4. Computer Reconstructed High Resolution Gas Chromatogram of the Saturate Fraction from a Sample of a Waxy Crude Oil of Indonesian Origin.

INDONESIAN WAXY CRUDE OIL



MARCH 3, 1849

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.