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Applications of Mass Spectrometry to Organic Geochemistry

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Applications of Mass Spectrometry to Organic Geochemistry

By

Patricia Ann Haug

B.S. (Columbia University) 1963

DISSERTATION

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Committee in Charge

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Degree conferred.....

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Dedicated to
Professor George Jura

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Introduction

How did life originate? Modern evolutionary theories postulate a continuum extending from abiotically formed macromolecules to those capable of reproducing themselves and mutating in a manner which can be considered characteristic of the processess of metabolism, growth, reproduction, sensitivity, and evolution. In this sense, life consists of a small segment of possible chemical reactions. The basic element of these reactions, carbon, is unique because of its ability to form four equivalent sp^3 bonds. The strength of the s character is essential for the formation of stable polymers, while the ability to form four bonds permits the greatest degree of structural complexity and thus maximum possible specificity.¹

One experimental approach to the fundamental question of how life originated has been to attempt to create life from the components of a primitive environment.²⁻¹¹ A second scientific approach is to search for the remains of primitive forms of life buried in ancient sediments, i.e. to investigate the complex organic molecules preserved in inorganic rock matrices of varying ages in an attempt to extrapolate backward to how life originated. Two assumptions made in such an approach are that the organic matter extracted from these sediments is indigenous to the sediment (and has not migrated in from another source at a time different from the deposition of the sediment) and that hydrocarbon skeletons of biological molecules can be distinguished from abiologically formed

molecules. The problem is complicated by the lack of knowledge in the intertwined areas of abiological synthesis, biosynthesis, and diagenesis.

Abiological Synthesis. Distinction between biogenetically derived compounds and abiogenetically created compounds is rendered complex by the continuum postulated to exist between naturally occurring (abiogenetic) chemical reactions and the biosynthetic reactions of living organisms. For example, amino acids which serve as the building blocks for proteins (essential for all metabolic and growth processes) have been synthesized in quantity via electric discharges, a possible source of energy, in a primitive atmosphere of water, methane, and ammonia.¹²⁻²² Further experiments confirm the ability of these components under certain conditions to polymerize, form protective membranes, assimilate organic compounds, and divide.²²⁻²⁷

Porphyrins were until very recently accepted as biological evidence of the photosynthetic processes;²⁸⁻³² however, current studies have indicated that certain porphyrin skeletal types can be formed via simple chemical reactions.³³⁻³⁵ Also understood are the roles played by such catalysts and conditions as clay³⁶ and water-oil interfaces.³⁷

Recently it was postulated that the exposed edges of the graphite lattice might serve as active sites for catalytic hydrogenation producing hydrocarbons such as the isoprenoids, and normal alkanes.³⁸ Indeed one might even postulate mechan-

isms for the formation of certain triterpanes. Experimental data substantiate the claim that the naively calculated thermodynamic equilibrium of saturated hydrocarbons is not necessarily the result obtained. In particular, sufficient quantities of isobutane are lacking from graphite pyrolysis.³⁹

Biosynthesis. As has been pointed out the distinction of biogenetic from abiogenetic compounds by relying on compound types is difficult. The optical activity commonly possessed by biologically synthesized molecules has been taken as an indication (although the effects of diagenesis would be difficult to assess) of life processes (see further discussion in Part II).

The isotopic ratios, particularly C_{12}/C_{13} , may be a way of distinguishing abiological molecules from biological molecules since such ratios are extremely sensitive to reaction paths and membrane fractionation.⁴⁰⁻⁵¹ However, they can also be related to factors such as temperature.⁵²⁻⁵³ Unfortunately, there have been no studies of this type on individual hydrocarbons.

In every area of biochemical investigation there remain many unsolved problems. It is difficult, therefore, to extrapolate backwards and draw conclusions about the chemistry of extinct organisms; some of the difficulty may simply reflect our fragmented knowledge of existing systems.

Diagenesis. The fact that many reactions occur after the death of any organism further complicates the already fragmented picture. Sediment conditions can vary from oxidizing to

reducing, may possess high thermal or pressure gradients, as well as varied bacterial conditions. An understanding of diagenetic reactions in sediments is an important prerequisite for drawing conclusions on the origin of a class of compounds isolated from it. Many studies have been undertaken in an attempt to piece together possible diagenetic pathways.⁵⁴⁻⁶⁵ Geochemical studies are, by their nature, closely related to the field of diagenesis and, therefore, geochemical results can be expected to give rise to theories of diagenetic processes. Throughout this thesis, therefore, structural similarities between the components of living organisms and those identified in ancient sediments are noted. In the case of the Green River Shale, where past life is well documented by fossils and there is no question of life having existed, a primary purpose of geochemical investigation is to gain information about the diagenetic processes which have taken place since sediment deposition.

This research is primarily concerned with the identification of those compounds which are present in sediments and covers several areas of geochemical interest: Part I, the analysis of hydrocarbons from ancient sediments; Part II, the determination of steranes and triterpanes from the Colorado Green River Shale; Part III, the characterization of the acids and bases of the Colorado Green River Shale; and Part IV the exploration of high resolution mass spectrometry as a tool for the preliminary analysis of geochemical mixtures.

In all of these studies mass spectrometry was used as the principle physical technique. The research had as its goal two main objectives: the exploration of mass spectrometry in regard to its application to organic geochemistry and the exploitation of mass spectrometric data for the characterization of organic compounds, and consequent contribution of meaningful geochemical results.

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Part I

Hydrocarbons from Geological Sediments

Mass Spectrometric Analysis of Hydrocarbons

Mass spectrometry was the principle tool used for identification of isolated compounds. Whereas mass spectrometry has been explored as an analytical instrument for standard analyses by petroleum chemists (particularly for characterizations of crude mixtures)¹, its great potential for detailed structure analysis on individual compounds has been exploited only in recent years. In geochemical research the mass spectrometer is an absolutely essential analytical method since it is the only technique which can provide detailed and unambiguous structure information on extremely small samples (microgram-nanogram). Indeed one might say that modern geochemistry would be almost impossible without utilization of this instrument. Mass spectra are particularly useful for recognition of structural types and for identifying rapidly the members of a homologous series of compounds whose structural complexity is not great.

This research has concentrated on the isolation and identification of hydrocarbons from carbonaceous shales and sediments for three main reasons. First, hydrocarbons appear to be the most abundant end products of diagenesis (the biological and inorganic processes by which living material is degraded). Secondly, many of the hydrocarbons found possess a high degree of structural specificity, i.e. isoprenoids and steranes, such that they can be related to biological precursors. Thirdly, hydrocarbons are relatively easily analyzed. Often a single mass spectrum is sufficient to identify a

compound even when no standards are available. For such compounds no other physical method can provide clear cut structural information as conveniently. The widespread application of mass spectrometry to organic geochemical problems has in turn led to intensive investigations into the fragmentations produced upon electron impact of organic compounds. Results from these studies have considerably broadened the knowledge of the mass spectrometry of organic molecules, permitting fairly conclusive structural deductions from the mass spectrometric fragmentation pattern of an unknown compound. Furthermore, in those cases where the mass spectrum does not identify a compound completely, it often provides definite information on the type of structure. In general, then, it can be said that mass spectrometry provides basic information for any organic geochemistry problem.

Sediments Analyzed

Of the sediments examined in this laboratory,²⁻⁸ the youngest (3×10^6 years) was the Abbott Source Rock from Lake County in Northern California. Attention was first drawn to the Abbott Mercury Mine Seep Oil by a paper suggesting that the mode of deposition in silica required an "abiogenic" origin.⁹ Analysis of this oil, however, yielded gas chromatograms which have some noticeable maxima, thus vaguely resembling those from known biological sediments rather than the smooth curve of the abiogenic oil examined by Eglinton et al. (Fig. I-II). The isolation and identification of the C_{19} isoprenoid, pristane, (2,6,10,14-tetramethyl penta-decane)⁴ further strengthened the suggestion that it was a normally formed biological oil. A sample of the siliceous source rock was provided by Dr. E. Bailey of the U.S. Geological Survey, Menlo Park, California, and compounds were isolated by Dr. R. B. Johns.^{3,5}

A sample of a typical San Joaquin Valley Oil was provided by courtesy of Dr. L. Lindeman of California Research Corporation, Richmond. Samples were isolated from the oil, considered to be about 30×10^6 years old,¹⁰ by E. D. McCarthy.⁵

In an attempt to extend the investigation of organic components of ancient sediments to another continent, the Moonie Crude Oil from Queensland, Australia (provided by Mr. A. S. Keller, Resident Geologist of the Union Oil Development Corporation, Toowoomba, Queensland, Australia) was examined. It is believed that the oil had its source in Permian rocks,

probably sedimentary, and later migrated along an unconformity at the bottom of the reservoir basin into Jurassic and Triassic sandstone, dated on the basis of spores.¹¹⁻¹⁵ Samples were isolated by Bill Van Hoeven.⁵

A sample of Antrim Shale from Midland County, Michigan, was provided by Mr. R. D. Matthews of Dow Chemical Company in the form of a core taken from a depth of 2,608 feet. This shale has been dated from spores as Late Devonian in age, about 265×10^6 years¹⁴ and is the northern part of a large deposit which extends to the South where it is referred to as the Chattanooga Shale.¹⁵ The carbonaceous Antrim Shale is rich both in total carbon content and in extractable organic material (Table I). Samples were isolated by Eugene D. McCarthy.⁵

The Nonesuch Seep Oil was previously analyzed in detail for isoprenoids, the C_{15} , C_{16} , C_{19} and C_{20} were found.⁴ Samples of some of the minor hydrocarbon components were provided by Dr. R. B. Johns for identification.⁵ The source rock is estimated to be two billion years of age.

A sample of the Soudan Shale was provided by Professor P. E. Cloud, Jr., of the University of California at Los Angeles, and compounds were isolated by Ted Belsky.^{3,5,2,7} It is the oldest carbonaceous Precambrian sediment known on the North American continent and is located in northeastern Minnesota in the Lake Superior region.¹⁶ This shale has an age of deposition approaching 3 billion years, determined by the age of granitic intrusion which is known by isotopic

methods to be $2.7 \pm .2 \times 10^9$ years. The geological relationships of the Soudan Iron Formation are given by Goldich.¹⁷ The Soudan Shale is an example of possible migration, despite the fact that the strange C_{13}/C_{12} ratios have been extremely well rationalized.⁵ The sample which has been analyzed was cut from a surface exposure and is stratigraphically related to the 21st level of the Soudan Iron Mine, Soudan, Minnesota, at a depth of 1,800 feet below ground. The surface sample has approximately four hundred times as much hexane soluble extractable organic matter as the mine sample (Table I). There is about two hundred and fifty times as much sulfur in the mine sample as in the surface sample. Water could conceivably have washed away the sulfur of the surface sample. Organic matter may have seeped in during this process. Figures III and IV compare the gas chromatograms of the two samples; the surface sample shows a more selective pattern, the spikes of pristane and phytane standing out compared to the chromatogram from the mine which shows some resemblance to the chromatogram of a methane discharge (Figure II). The shale is known to be metamorphosed and perhaps subjected to a temperature as high as 350° or 400°C which does not argue well for the preservation of original organic matter. Figure V and VI compare the chromatogram of the total hydrocarbon fraction after HF digestion of the surface sample with the chromatogram of the organic material obtained by solvent extraction from the surface sample. It does not argue against migration of organic

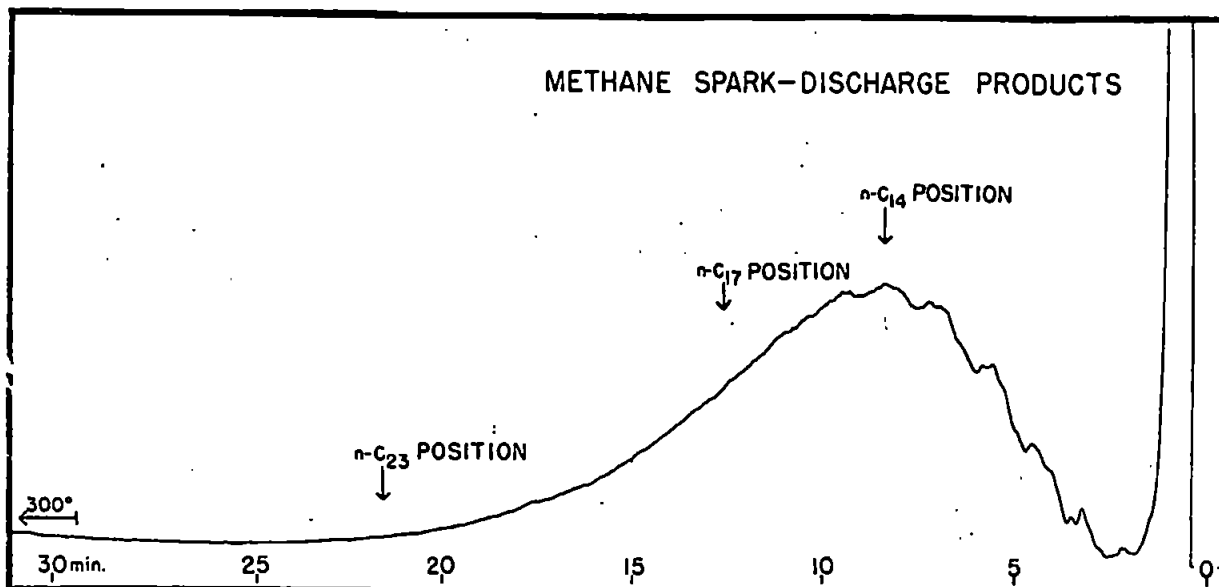
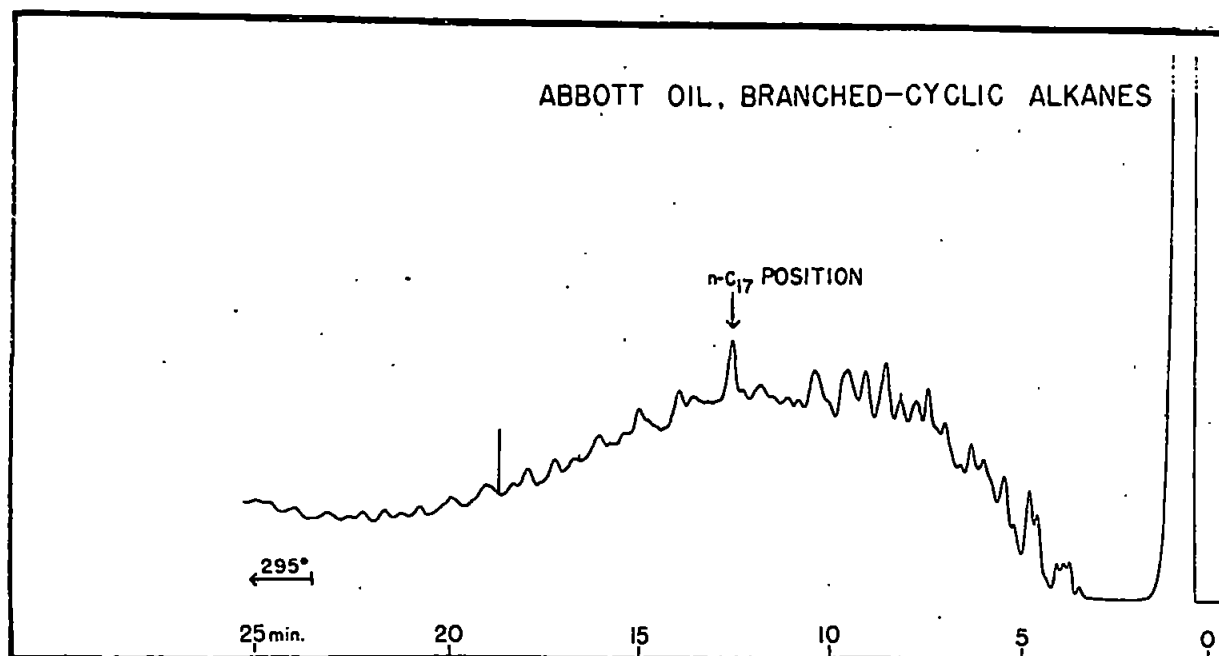
components into the shale. This question does not arise in other sediments examined which are encompassed by comparatively impermeable rock matrices.

Also analyzed was a sample of the abiological Fischer-Tropsch product (the oil resulting from passing hydrogen and carbon monoxide through a heated ring), obtained from Dr. A. G. Sharkey, Jr. of the Pittsburgh Coal Research Center, which was separated into fractions and collected by Ted Belsky.

These sediments, then, are the points of reference upon which a reconstruction of chemical evolution may be based. Since these geological sources are of considerably varied age and location they may be thought of as reference points for future investigations. While the evolution of living organisms from the Precambrian period to the present can be traced on the basis of plentiful and convincing fossil records, such morphological remains from the Precambrian (represented in this study by the Soudan Formation) are scarce or lacking and for the early Precambrian ($3.5-1.5 \times 10^9$ years) in particular, only two very recent reports of well preserved micro fossils provide evidence for the existence of life.^{18,19} It is felt, therefore, that a chemical approach, i.e. the isolation of organic compounds which could be related confidently to biological precursors might provide essential (and independent) information on the existence and possibly the evolution of living organisms in that period. Furthermore, the analysis of sediments or oils of different geological history and age may eventually permit some deduction concerning the diagenesis of organic compounds.

Figure I. Gas Chromatogram of Abbott Oil

Figure II. Gas Chromatogram of Methane Spark-Discharge
Products



MUB-3949

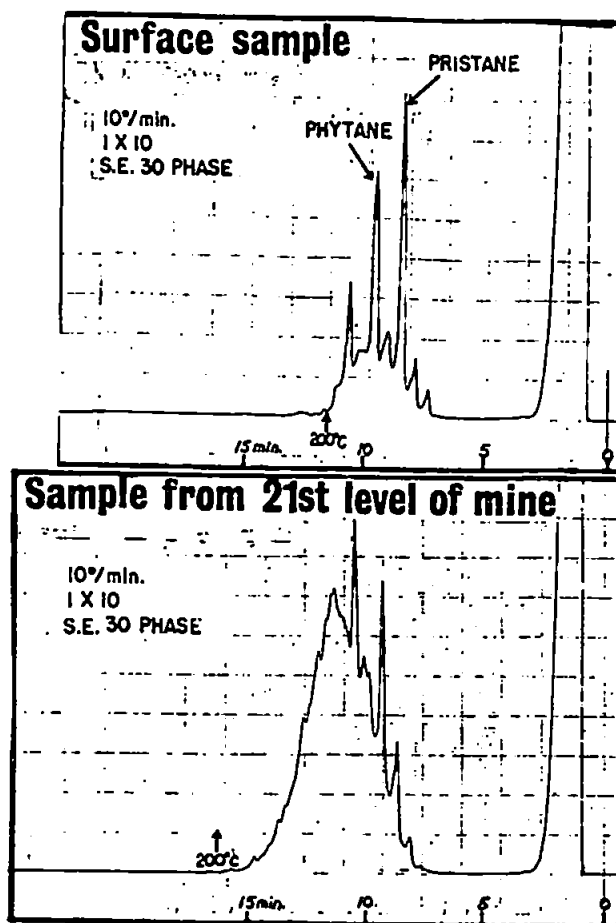


Figure III. Gas Chromatogram of Soudan Surface Sample

Figure IV. Gas Chromatogram of Soudan Mine Sample

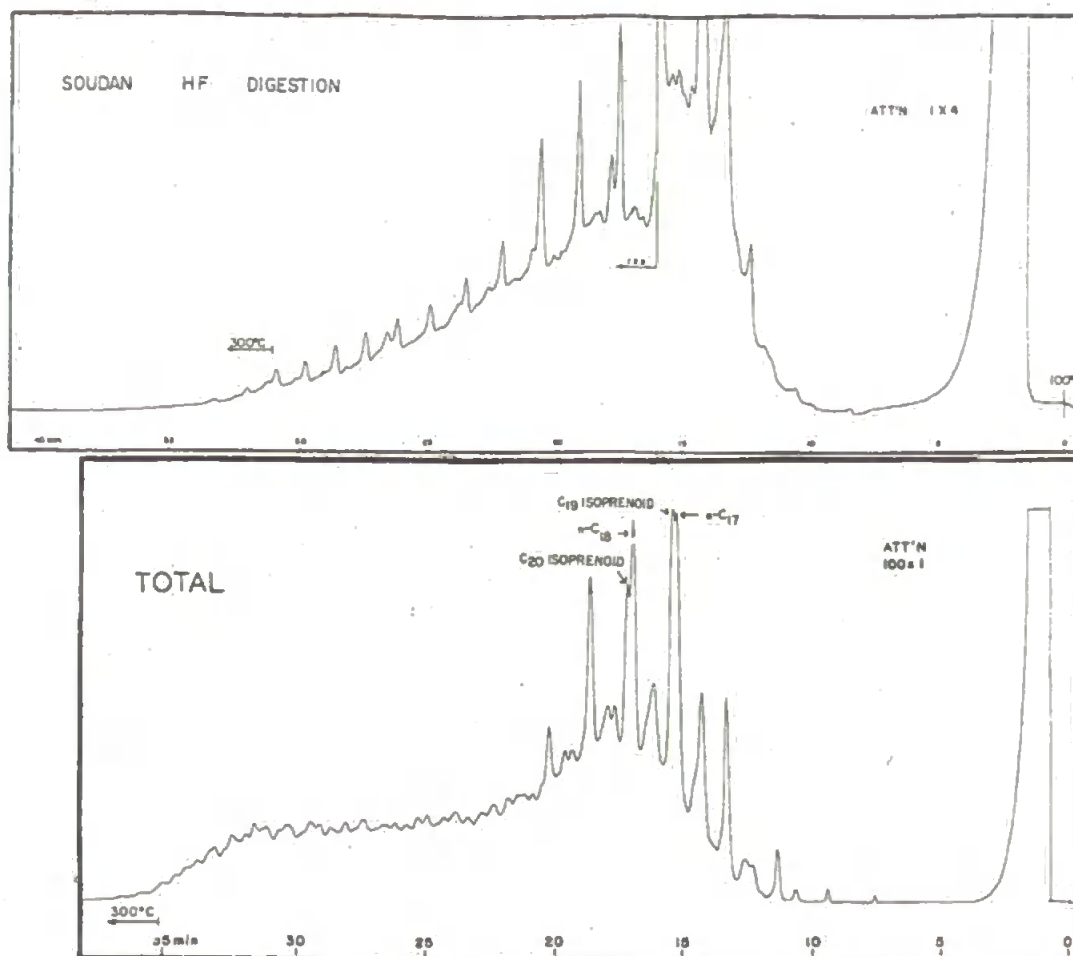


Figure V. Gas Chromatogram of Soudan Surface Sample HF Digestion

Figure VI. Gas Chromatogram of Soudan Surface Sample Solvent Extraction

Extraction and Isolation

The detailed method of extraction and isolation of hydrocarbons and the method of separation into three major fractions: "Total," "Normal" and "Branched-Cyclic" has been extensively reported,^{4,5,7} and will be discussed in more detail in Part II. Relevant data are summarized in Table I. Individual compounds of the "Branched-Cyclic fraction" were, in general, separated on a 3% SE-30 gas-liquid chromatography column (10 ft. x 1/4 in.) programed at 4°/min. and subsequently isothermally purified successively on 7-ring metapolyphenyl-ether and on tetracyanoethylated pentaerythritol (25 ft. x 1/4 in.) columns.

Mass Spectrometric Instrumentation

A modified Consolidated Electrodynamics Corporation Model 21-103C mass spectrometer²⁰ was used to elucidate the structures of isolated compounds.* In Figures VII-IX the original mass spectra of the first three compounds isolated from the Moonie Oil are presented. These are original mass spectra illustrating the component purity attained by the isolation procedures (described above) and proper mass spectrometric handling techniques. In Figures XXXIX (page 31), XLVI (page 34), and LII (page 36), the normalized bar graph spectra drawn from these originals are presented. (Relative intensities for all peaks which are off scale due to the choice of the reference peak used for normalization are given in Table III.)

* Mass spectra were run by Miss Sheri Firth and spectra were measured by Edward DeFranceschi and Craig Weinstein.

Three requirements for the determination of geochemical samples which were not incorporated in the unmodified 21-103C are fast scanning, a direct inlet system, and high sensitivity. Usually geochemical samples of components isolated using gas chromatographic techniques are in the microgram range. The unmodified C.E.C. with its Faraday cup and DC amplifier (capable of detecting a minimum ion current of only approximately 3×10^{-4} ampere), slow electrical scanning and 800 cc reservoir and inlet line volume gave a mass spectrum on a minimum sample of about 25 ug - clearly quite an unsatisfactory arrangement. First, a direct inlet system (See Figure X) was added which permitted samples to be placed in a direct line with the electron beam (within six inches) avoiding the pressure differential of the gold leak. This not only decreased the amount of sample needed, but also permitted determination of the mass spectra of compounds which easily decomposed or pyrolyzed. Since many samples (particularly the C_{15} , C_{16} , and C_{17} alkanes) are quite volatile at pressures of 10^{-6} mm Hg, a liquid nitrogen cooled probe was constructed (Figure XI) to permit their analysis.

The AC ion source filament has been replaced by a DC filament effecting reduction of cycle modulation in the ion beam. The 60 liter/second mercury diffusion pump on the analyzer has been replaced by two S.S. 115 liter/second 5-ring polyphenylether pumps positioned at the source and at the ion multiplier, respectively. This improved the vacuum system and decreased the pumpdown time between samples. The

ion source exit slit has been reduced from 5 to 1 mil and the two collector slits of 7 and 14 mils changed to one fixed slit of 1 1/2 mil effecting an increase of the focused resolution from 1/120 to 1/400 and the non-focused from 1/350 to 1/1200.

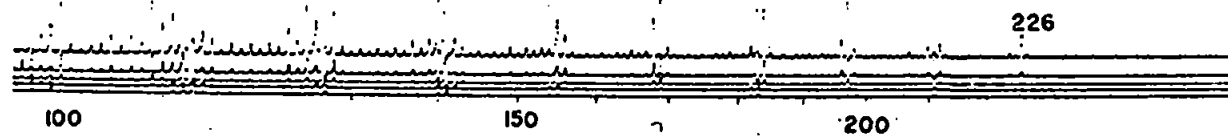
A 17 stage pi type electron multiplier with a current gain of 2×10^6 was incorporated. This permitted the amplifier input impedance to be reduced resulting in a higher frequency response, allowing reduction in scan time to 1 1/2 minutes instead of the previously required 15 minutes. These modifications increase the effective sensitivity of the instrument by a factor of 10 both in electronic amplification and in the shorter sample residence time required to determine a mass spectrum.

Figure VII. Original Mass Spectrum of Moonie Oil
C₁₆ Isoprenoid

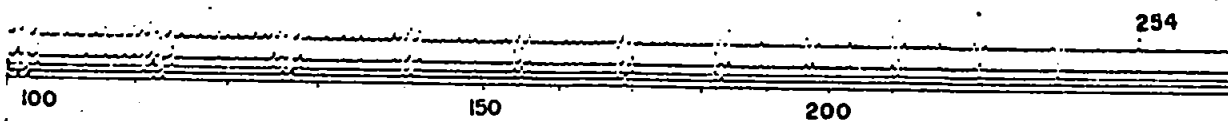
Figure VIII. Original Mass Spectrum of Moonie Oil
C₁₈ Isoprenoid

Figure IX. Original Mass Spectrum of Moonie Oil
C₁₉ Isoprenoid

MOONIE C₁₆ ISOPRENOID



MOONIE C₁₈ ISOPRENOID



MOONIE C₁₉ ISOPRENOID

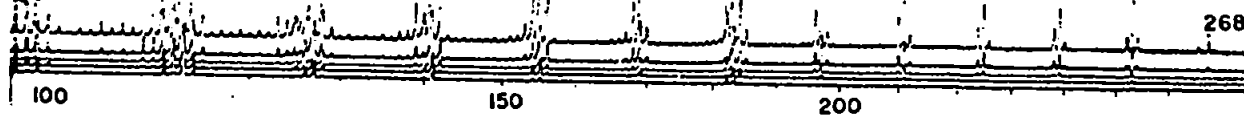


Figure X. The Direct Inlet System

- A To Isatron
- B Tungsten Heater Wire
- C Inside Surface Silvered
- D All Glass Valve
- E To Diffusion Pump
- F Gold Leak
- G Inlet System
- H Kovar Seal
- I 4 mm Constriction
- J In Line Valve
- K Teflon Sample Holder and Seal
- L Stop
- M To Diffusion Pump
- N To Rough Pump
- O Quick Disconnect Nut
- P Teflon Chevron Seal
- Q Sample Handle

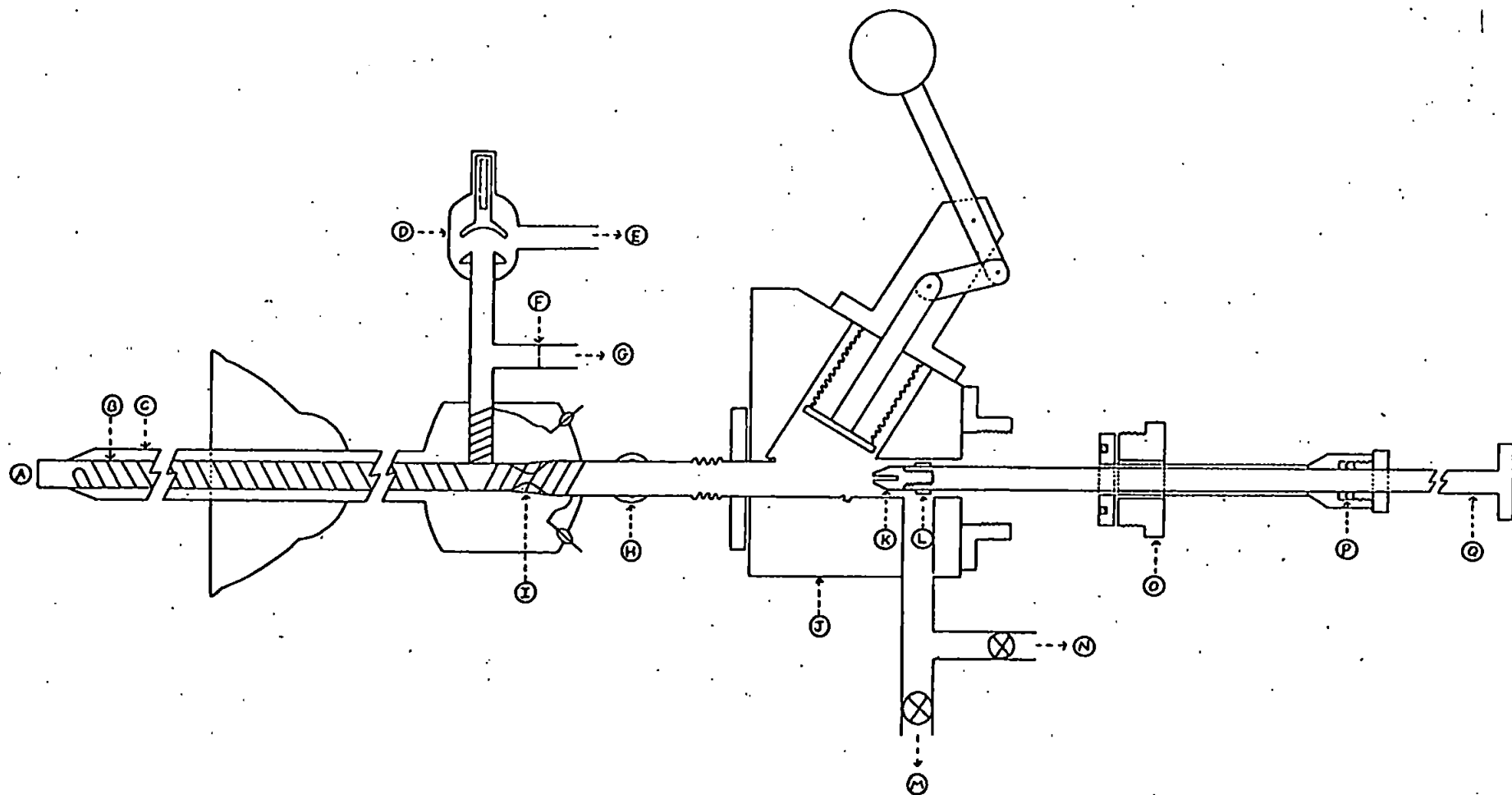
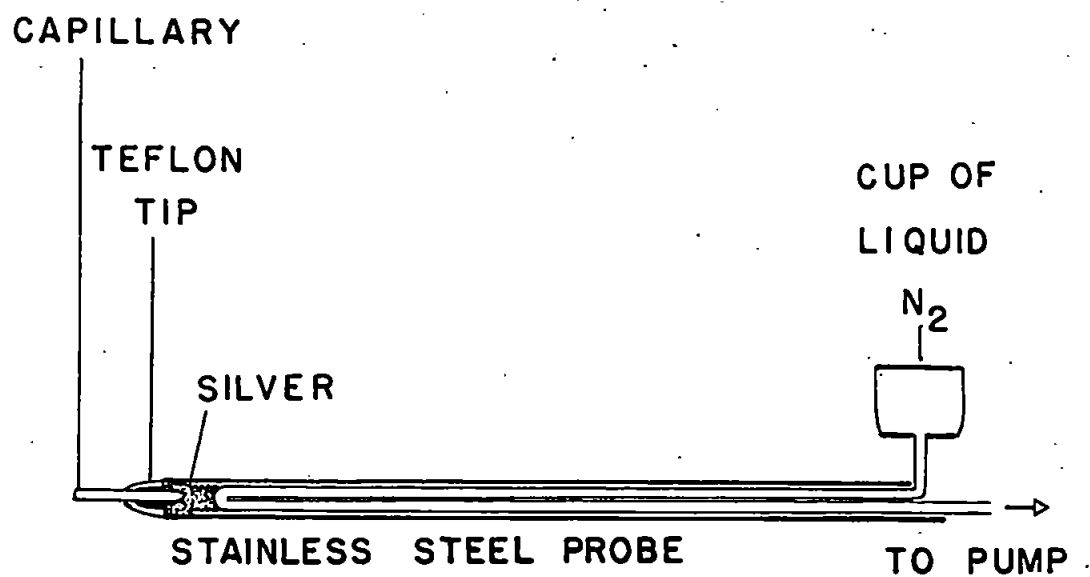


Figure XI. The Cooled Probe

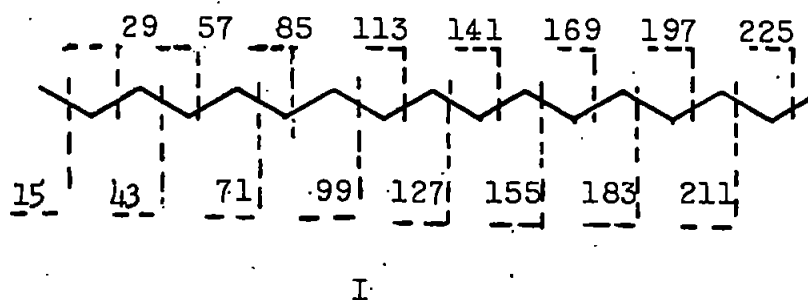


COOLED PROBE

MUB13086

Straight Chain Hydrocarbons

From the Fischer-Tropsch product the normal C_{17} , C_{18} , and C_{19} alkanes were isolated. The normal C_{17} alkane was also isolated from the Nostoc algae. Numerous normal alkanes in the range of C_{11} - C_{30} have been found in the sediments investigated. (See Table V.) The mass spectra are characterized by a smooth envelope of peaks fourteen mass units apart (m/e 43, 57, 71, 85, etc.) which maximize at C_4 and decrease in intensity with increasing mass. The example cited in Structure I may suffice to convey a picture of the typical mass spectral pattern.



I

Figures XIII-XV give the mass spectra of three different n - C_{19} mono olefins also isolated from the Fisher-Tropsch mixture. The degree of unsaturation is indicated by the parent peak mass and further confirmed by the even mass peaks formed by hydrogen rearrangement. The mass spectra of these types of compounds makes it difficult to determine the positions of the double bonds without corresponding standards because the double bond is delocalized by electron bombardment. Isomeric olefins, thus, exhibit very similar fragmentation patterns.²¹ An extensive

review of unique ways of converting microgram quantities of geochemical olefins to compounds whose mass spectra unambiguously determine the position of the double bond is given by Schnoes and Burlingame.²²

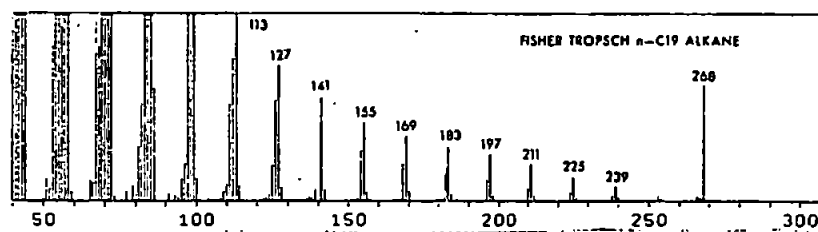
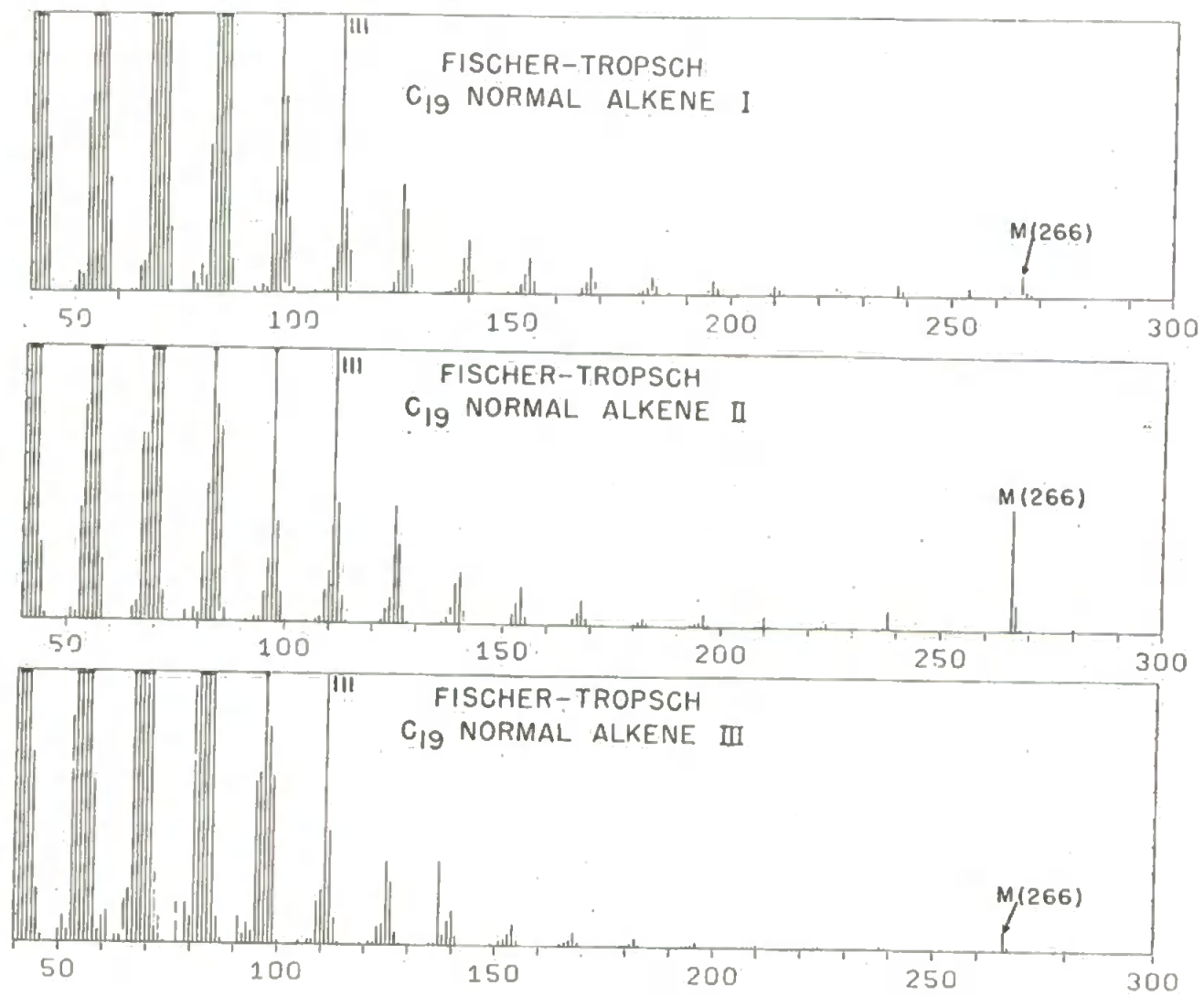


Figure XII. Mass Spectrum of Fischer Tropsch $n\text{-C}_{19}$ Alkane

Figure XIII. Mass Spectrum of Fischer Tropsch $\underline{n}$ -C₁₉ Alkene I

Figure XIV. Mass Spectrum of Fischer Tropsch $\underline{n}$ -C₁₉ Alkene II

Figure XV. Mass Spectrum of Fischer Tropsch $\underline{n}$ -C₁₉ Alkene III



MUB-33726

Iso and Anteiso Alkanes

Figure XVI gives the mass spectrum of C_{15} iso-alkane (2-methyltetradecane) isolated from the Moonie Oil; Figure XVIII, the mass spectrum of the C_{16} iso-alkane isolated from the Antrim Shale; Figure XX, that of the C_{17} iso-alkane isolated from the Abbott Seep Oil; Figures XIX, XXI, and XXII, the C_{16} , C_{17} and C_{18} iso-alkanes isolated from the Nonesuch Oil. These should be compared with the mass spectrum of authentic C_{16} iso-alkane in Figure XVII. The mass spectra of 2-methyl alkanes are quite characteristic. Fragmentation proceeds with loss of methyl radical and strong loss of the isopropyl radical.

Figure XXIII gives mass spectral evidence for a C_{16} anteiso-alkane in a mixture (with a component of molecular weight 238) obtained from the Antrim; Figures XXIV-XXVI give the mass spectra of the C_{16} , C_{17} and C_{18} anteiso alkanes from the Nonesuch Oil; and Figure XXVII gives the mass spectrum of the C_{18} anteiso-alkane from the Moonie Oil. These can be compared with authentic C_{21} anteiso-alkane in Figure XXVIII. The anteiso-alkanes can be identified mass spectrometrically by their very pronounced loss of ethyl radical.

The branched chain fatty acids derived from lipids are a possible source for the iso- and anteiso-alkanes. The C_{10} to C_{26} branched acids and the C_9 to C_{31} anteiso-branched fatty acids are constituents of hair,²³ while in bacteria the C_{15} and C_{17} iso-acids are the major constituents of the lipid fraction isolated from Bacillus subtilis and the C_{15}

anteiso-acid is the major component of Micrococcus
lysodeikticus.²⁴ Iso-paraffinic hydrocarbons have been
isolated from rose petal wax²⁵ and certain plants.²⁶ Iso-
and anteiso-alkanes have been isolated from a California
naphtha,²⁷ tobacco leaf wax,²⁸ wool wax²⁹⁻³¹ and Cuban sugar
cane wax.³² An anteiso-paraffin has also been isolated from
the American cockroach.³³

- Figure XVI. Mass Spectrum of Moonie C₁₅ Iso-alkane
- Figure XVII. Mass Spectrum of Authentic C₁₆ Iso-alkane
- Figure XVIII. Mass Spectrum of Antrim C₁₆ Iso-alkane
- Figure XIX. Mass Spectrum of Nonesuch C₁₆ Iso-alkane
- Figure XX. Mass Spectrum of Abbott C₁₇ Iso-alkane
- Figure XXI. Mass Spectrum of Nonesuch C₁₇ Iso-alkane
- Figure XXII. Mass Spectrum of Nonesuch C₁₈ Iso-alkane

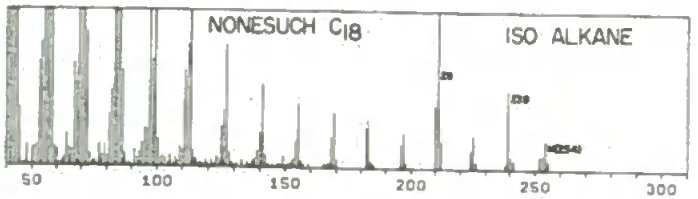
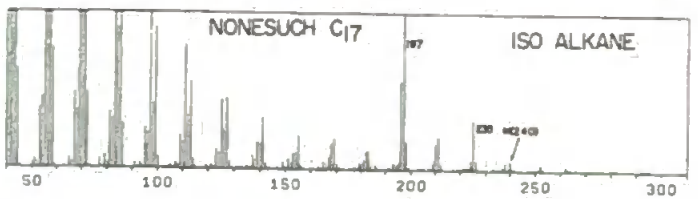
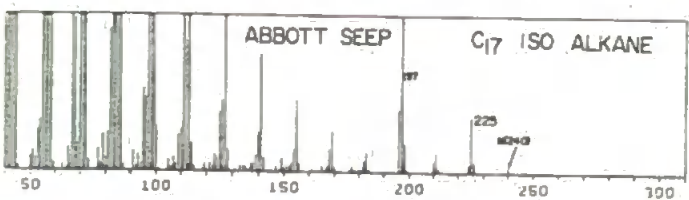
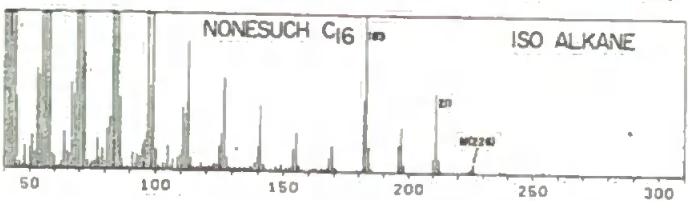
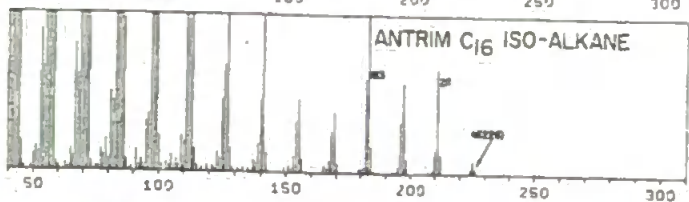
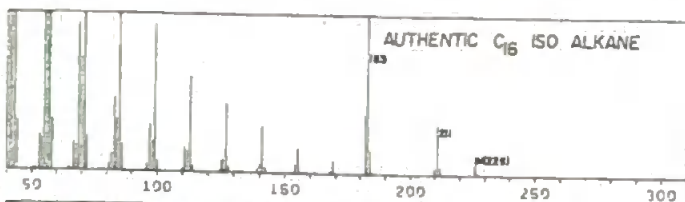
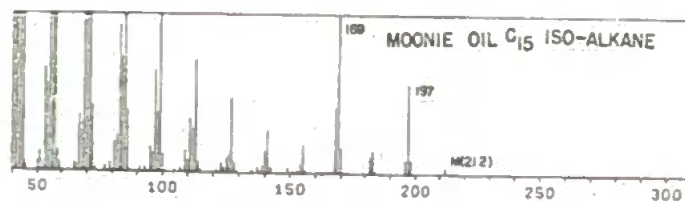


Figure XXIII. Mass Spectral Evidence of Antrim C₁₆
Anteiso-alkane

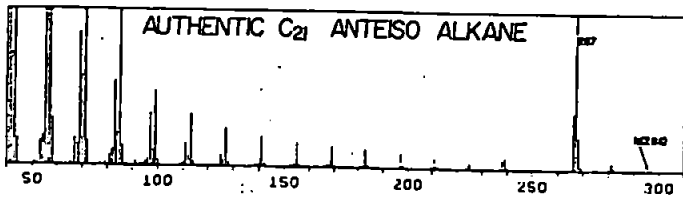
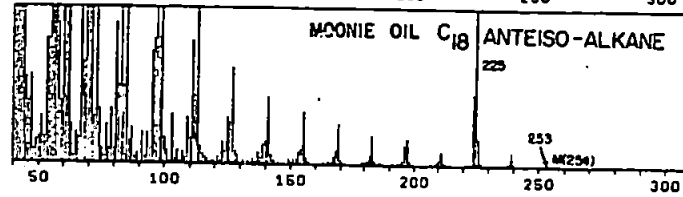
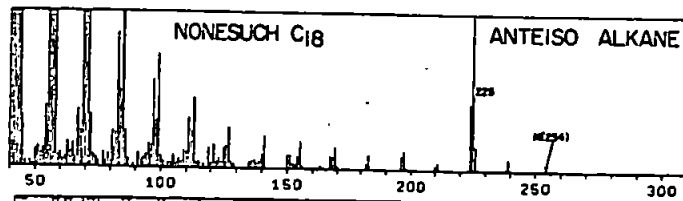
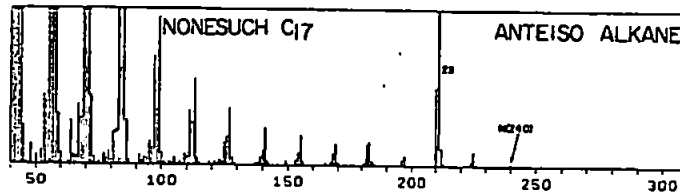
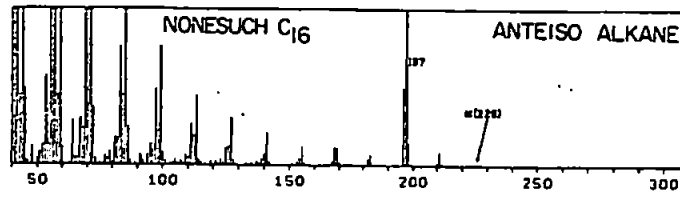
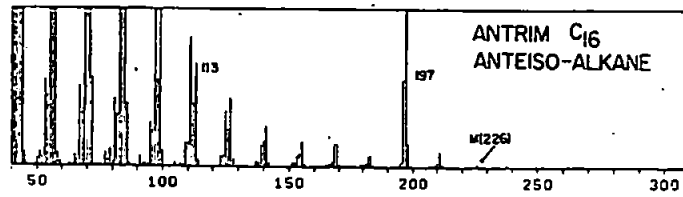
Figure XXIV. Mass Spectrum of Nonesuch C₁₆ Anteiso-alkane

Figure XXV. Mass Spectrum of Nonesuch C₁₇ Anteiso-alkane

Figure XXVI. Mass Spectrum of Nonesuch C₁₈ Anteiso-alkane

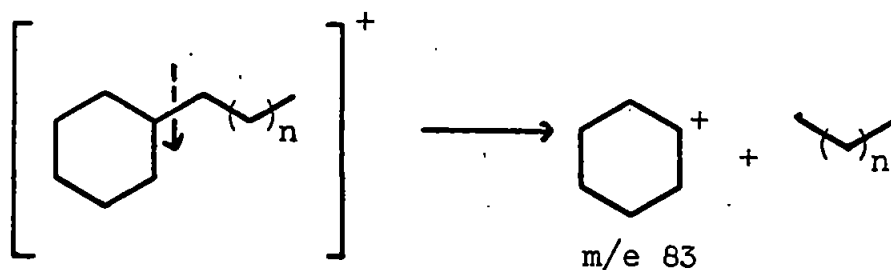
Figure XXVII. Mass Spectrum of Moonie C₁₈ Anteiso-alkane

Figure XXVIII. Mass Spectrum of Authentic C₂₁ Anteiso-alkane



n-Alkyl-Cyclohexanes

The mass spectra of the C_{15} and C_{16} cyclohexanes from the Moonie Oil are given in Figures XXIX-XXX, those of the C_{16} - C_{19} cyclohexanes from the Nonesuch Oil in Figures XXXII-XXXV, and a C_{18} cyclohexane (impure) from the Antrim Shale in Figure XXXVI.¹⁸ The mass spectrum of authentic C_{16} cyclohexane is shown in Figure XXXI. This series is characterized by an extremely abundant ion at m/e 83 resulting from the highly favorable cleavage of the alkyl chain leaving the cyclohexyl carbonium ion:



It is interesting to note here, that while the compounds can be identified as n-alkyl-cycloalkanes quite unambiguously (and are, for instance, easily distinguished from isomeric olefins - see page 17), the determination of ring size is not as straight forward. The ion at m/e 83 can be accounted for both by a cyclohexyl carbonium ion and a methylcyclopentyl ion - a distinction would be possible only if known standards for both series were available. For the methylcyclopentyl n-alkane five structural possibilities would still have to be considered: 1,1; 1,2(cis or trans); and 1,3(cis or trans).

The cyclohexyl-normal alkanes are of interest because there is only one tentative identification of this series from contemporary plant sources,³⁴ although the series has been reported in the Athabasca petroleum deposit,³⁵ and in paraffin wax.³⁶ Elsewhere in nature, they have been reported on the basis of a broad mass spectral analysis, but no specific member has been isolated.³⁷ Possibly this homology derives from the unsaturated fatty acid components of the original lipids, becoming saturated by an intramolecular cyclization. Mono-olefinic fatty acids are known in nature ranging from C_7 - C_{22} in chain length, and more commonly the double bond is found in the C_6 to C_7 position, although some are known where it is located in the C_8 to C_9 position.³⁸ Terminal mono-olefinic fatty acids are also known, for example, 9-decenoic and 10-undecenoic acids, but this series is neither well distributed nor conveniently converted to the end hydrocarbons by rational processes. A possible sequence, explaining the genesis of cyclic alkanes from unsaturated acids is outlined on the next page. A much more detailed study of other cyclic alkanes would be necessary before any conclusions can be drawn as to the merit of this scheme.

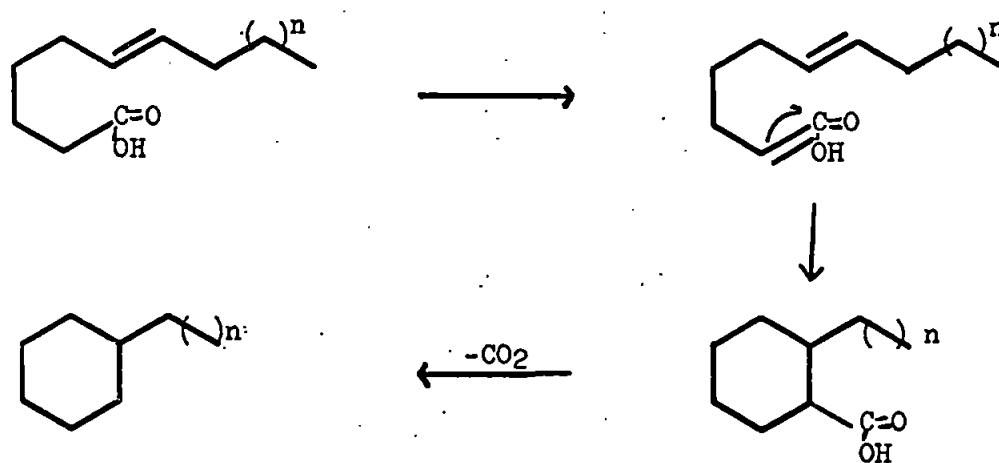


Figure XXIX. Mass Spectrum of Moonie Oil C_{15} Cyclohexane

Figure XXX. Mass spectrum of Moonie Oil C_{16} Cyclohexane

Figure XXXI. Mass Spectrum of Authentic C_{16} Cyclohexane

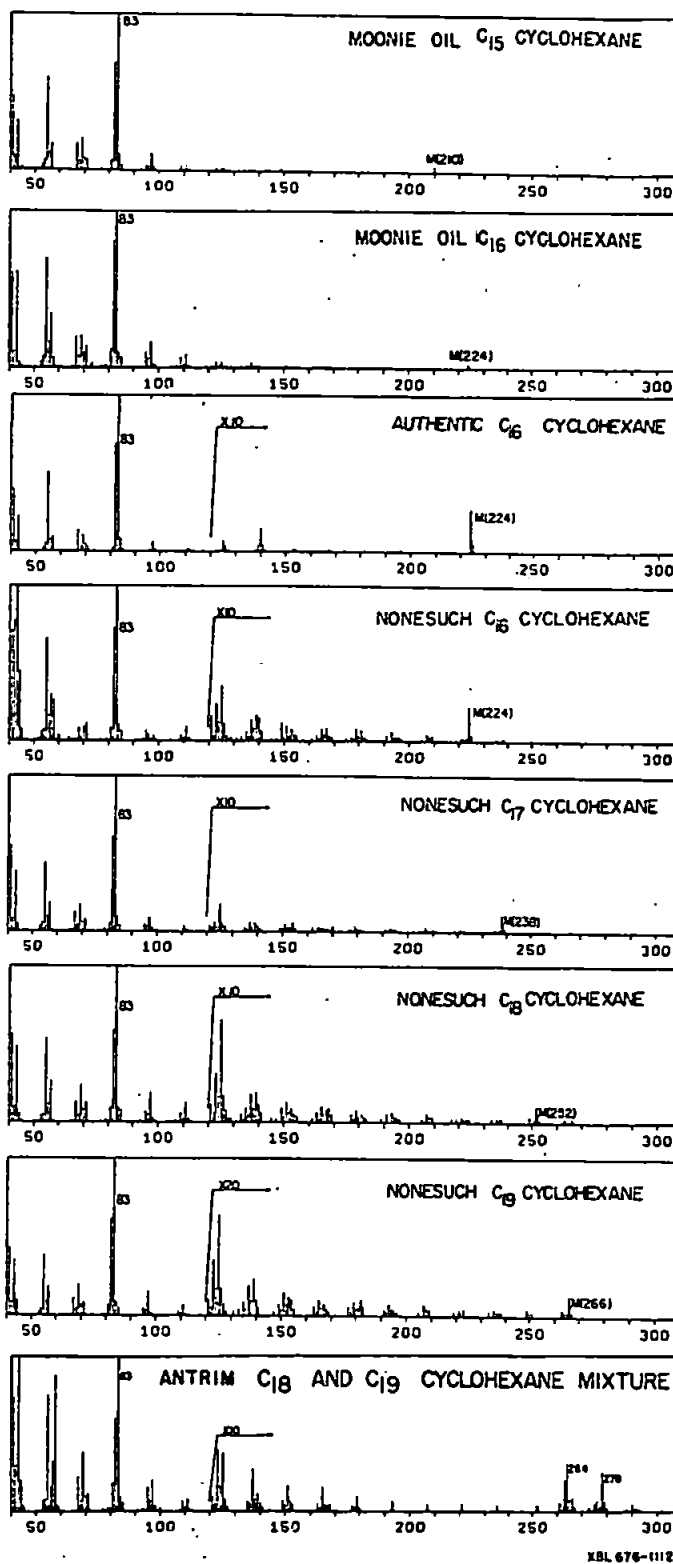
Figure XXXII. Mass Spectrum of Nonesuch C_{16} Cyclohexane

Figure XXXIII. Mass Spectrum of Nonesuch C_{17} Cyclohexane

Figure XXXIV. Mass Spectrum of Nonesuch C_{18} Cyclohexane

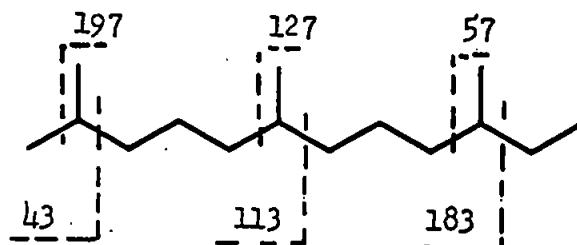
Figure XXXV. Mass Spectrum of Nonesuch C_{19} Cyclohexane

Figure XXXVI. Mass Spectral Evidence for Antrim C_{18} Cyclohexane



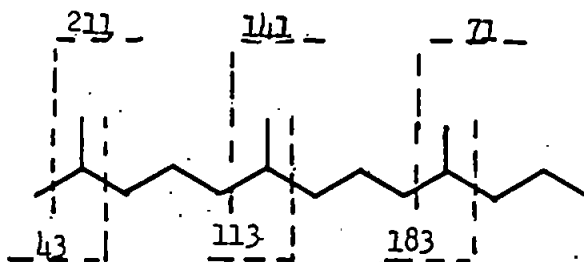
Isoprenoids

Isoprenoid alkanes are one of the most interesting classes of acyclic branched hydrocarbons occurring in geological environments since their structure implies very definite biological origin. Their unambiguous identification is thus of great importance. The mass spectrum of C_{15} isoprenoid isolated from the Moonie oil (2,6,10-trimethyldodecane) is shown in Figure XXXVII. Fragmentation proceeds by carbon-carbon cleavage, where the most abundant ions are the preferred secondary carbonium ions, as illustrated in Structure II.



II

Although a sample of authentic C_{16} isoprenoid was not available, it was easily identified mass spectrometrically by the highly characteristic predominant fragments illustrated in Structure III.



III

Examples of this typical pattern are shown in Figures XXXVIII-XLI, the mass spectra of the C_{16} isoprenoids isolated from the San Joaquin Oil, the Moonie Oil, the Antrim Shale, and the Nonesuch Oil, respectively. A pure sample of the C_{16} isoprenoid was not obtained from the Soudan but mass spectrometric data indicate its presence in a mixture.

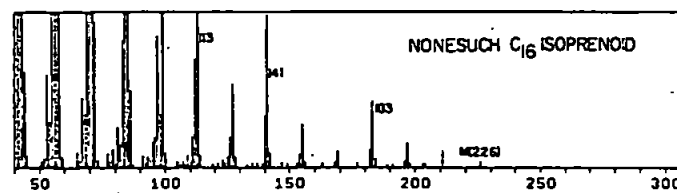
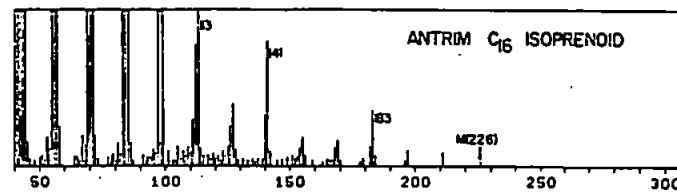
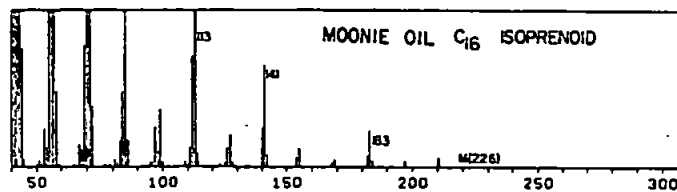
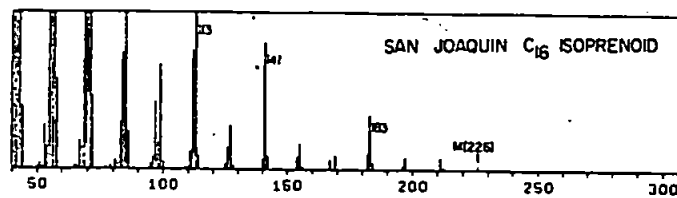
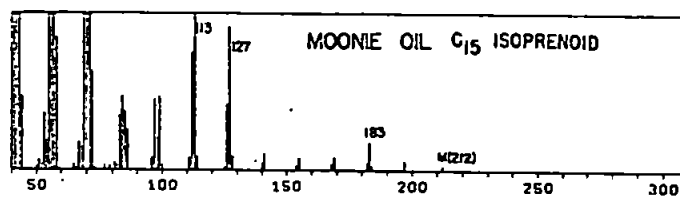
Figure XXXVII. Mass Spectrum of Moonie Oil C_{15} Isoprenoid

Figure XXXVIII. Mass Spectrum of San Joaquin C_{16} Isoprenoid

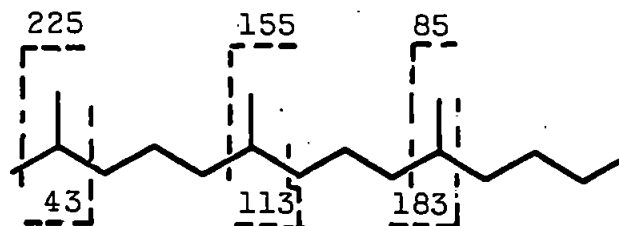
Figure XXXIX. Mass Spectrum of Moonie Oil C_{16} Isoprenoid

Figure XL. Mass Spectrum of Antrim C_{16} Isoprenoid

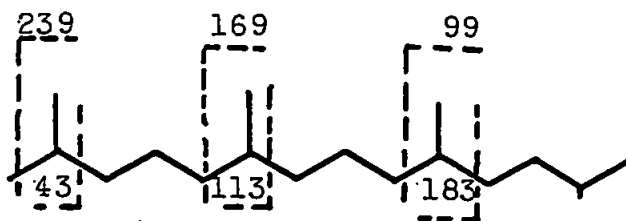
Figure XLI. Mass Spectrum of Nonesuch C_{16} Isoprenoid



A very careful search for the C_{17} isoprenoid (Structure IV) was made. The only indication of this compound was found in the Antrim Shale; the evidence is given in Figure XLIII together with the mass spectrum of the synthetic 2,6,10-trimethyltetradecane made by Eugene McCarthy (Figure XLII).⁸



IV



V

The mass spectra of the C_{18} isoprenoid isolated from the Abbott Oil, San Joaquin Oil, Moonie Oil, Antrim Shale, and Soudan Shale are given in Figures XLIV-XLVIII. Here again, although there was no synthetic standard, the compound could be unequivocally identified by its highly characteristic fragment ions indicated in Structure V.

Figure XLII. Mass Spectrum of Authentic C₁₇ Isoprenoid

Figure XLIII. Mass Spectrum of Antrim C₁₇ Isoprenoid

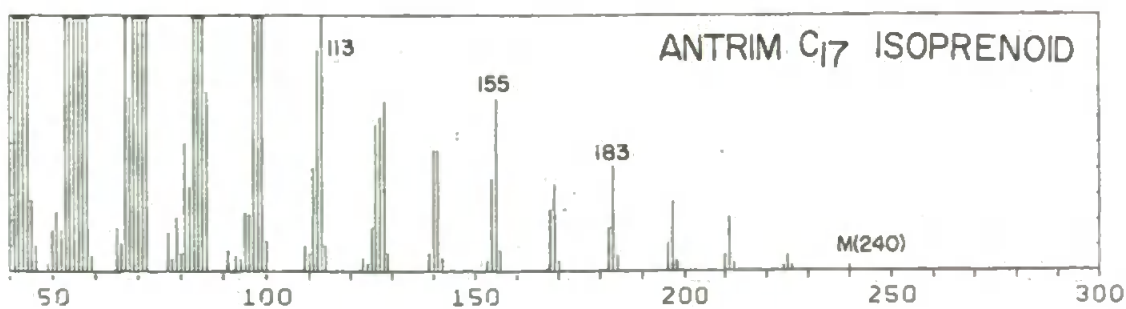
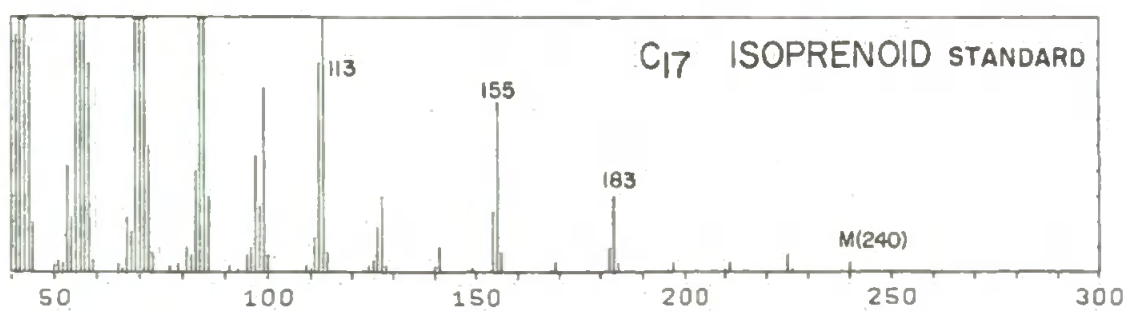


Figure XLIV. Mass Spectrum of Abbott Oil C₁₈ Isoprenoid

Figure XLV. Mass Spectrum of San Joaquin C₁₈ Isoprenoid

Figure XLVI. Mass Spectrum of Moonie Oil C₁₈ Isoprenoid

Figure XLVII. Mass Spectrum of Antrim C₁₈ Isoprenoid

Figure XLVIII. Mass Spectrum of Soudan C₁₈ Isoprenoid

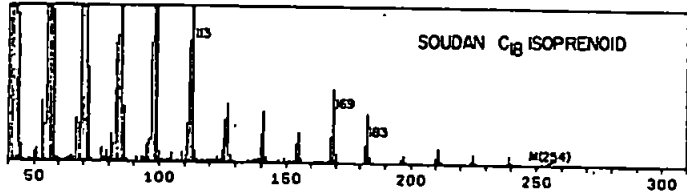
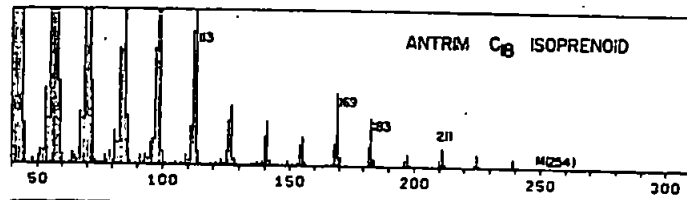
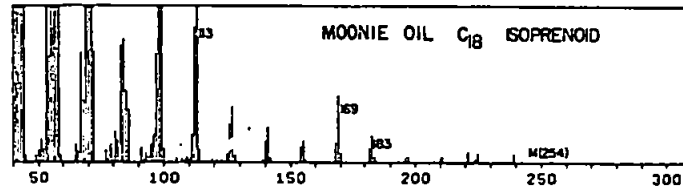
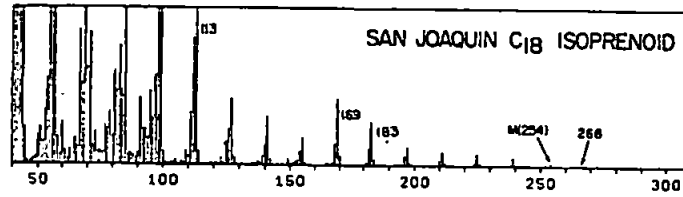
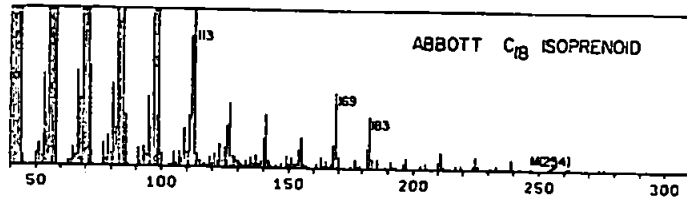
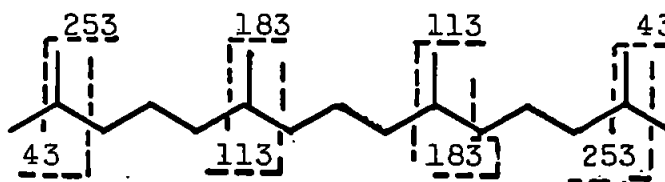
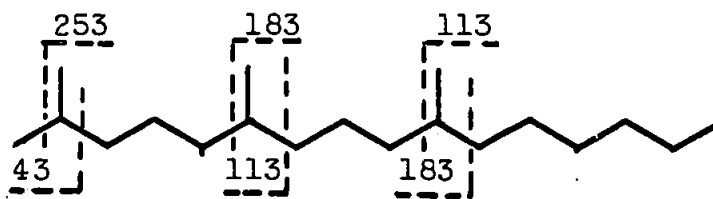


Figure XLIX gives the mass spectrum of authentic pristane, the regular C_{19} isoprenoid, (Structure VI). Figures L-LIV give the mass spectra of C_{19} isoprenoids isolated from the Abbott Oil, the San Joaquin Oil, the Moonie Oil, the Antrim Shale, and the Soudan Shale. However, caution must be used in assigning the structure of pristane to these compounds, particularly in the case of the Antrim Shale, where the mass spectrum is sufficiently different enough from that of pristane that 2,6,10-trimethylhexadecane, Structure VII, would seem to fit the data also.

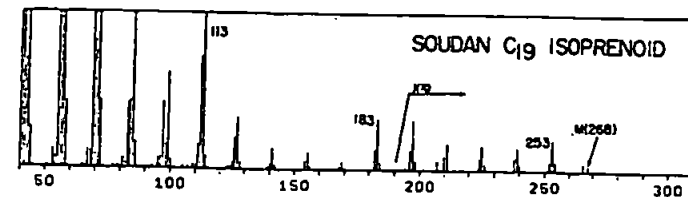
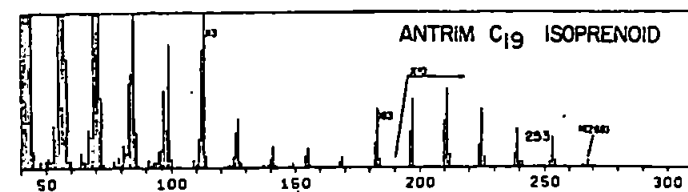
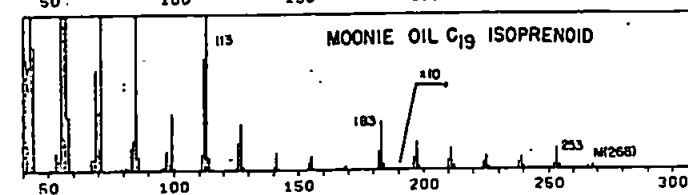
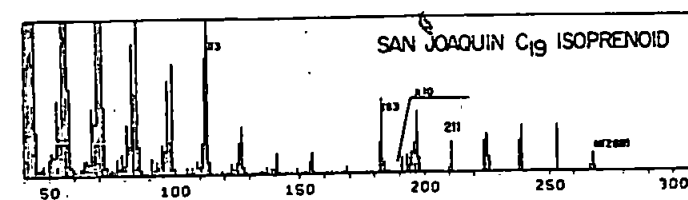
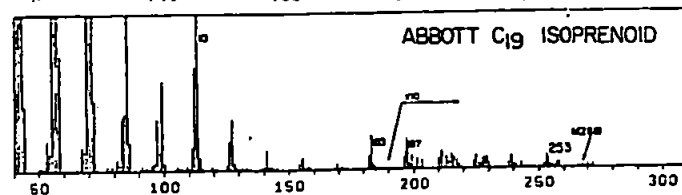
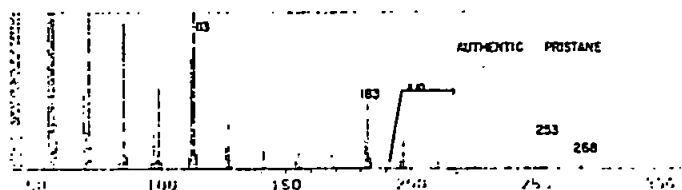


VI



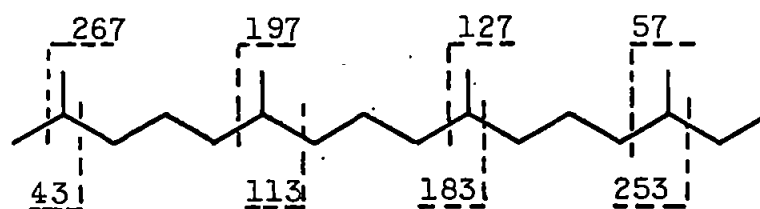
VII

It should be noted that in the case of a slightly impure sample, distinction between Structures VI and VII would be particularly difficult because one is dealing only with a difference of five methyl groups versus six, and almost identical fragmentation patterns as the structures above indicate. A recent synthesis of compound VII has shown that the gas chromatographic retention times are considerably different, it being possible to identify the isolated compounds conclusively.⁸

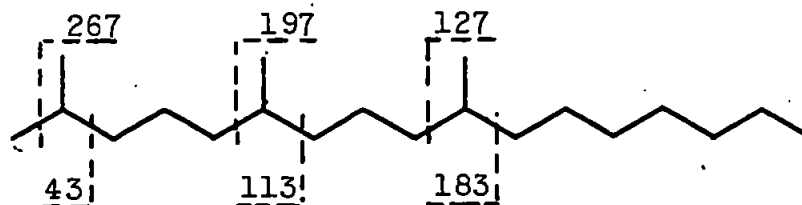


XBL 676-1093

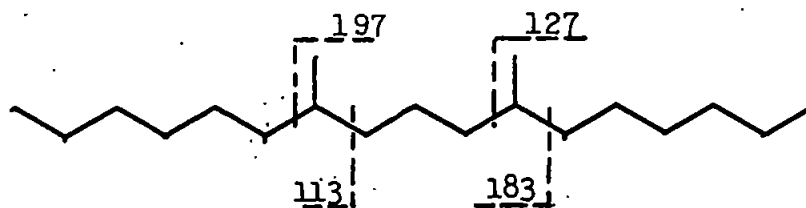
Figure LV gives the mass spectrum of authentic phytane, the regular C_{20} isoprenoid (Structure VIII); Figures LVI-LX the mass spectra of the C_{20} isoprenoids isolated from the Abbott Oil, the San Joaquin Oil, the Moonie Oil, the Antrim Shale and the Soudan Shale. The structure of the compound isolated from the Antrim Shale is ambiguous since 2,6,10-trimethylheptadecane, Structure IX, or 7,11-dimethyloctadecane, Structure X would also seem to fit the mass spectral data.



VIII



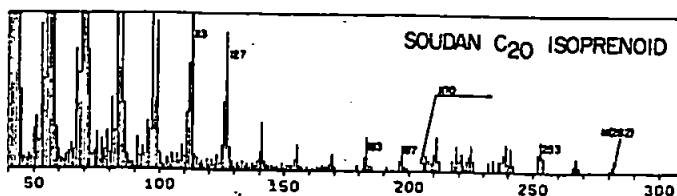
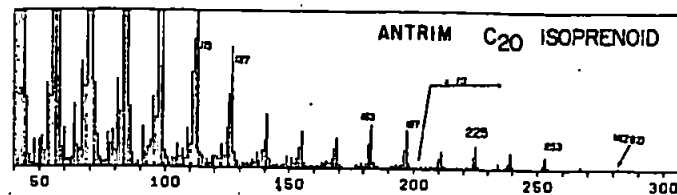
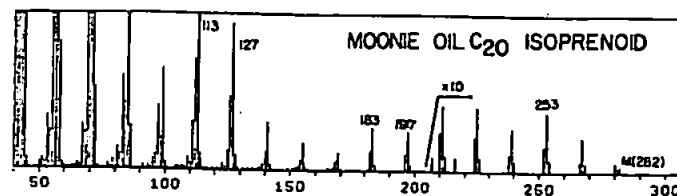
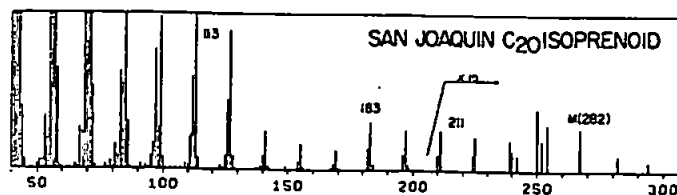
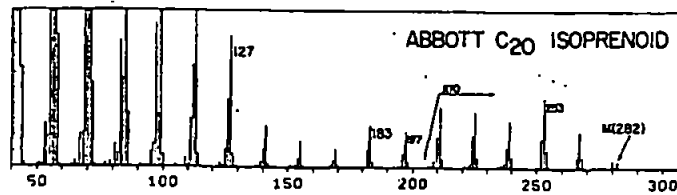
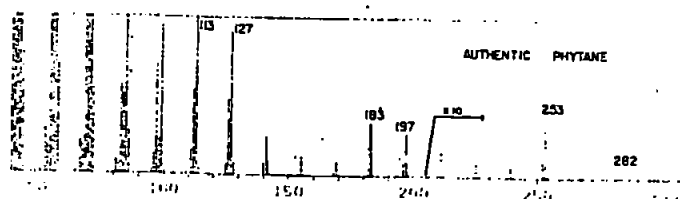
IX



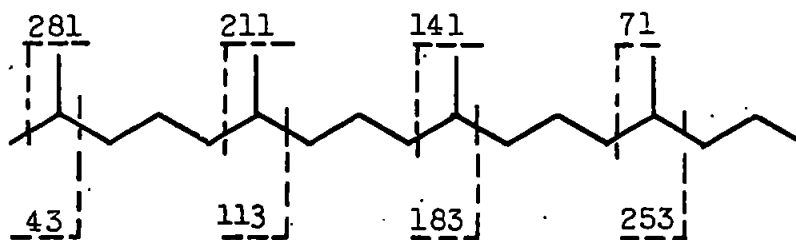
X

The compounds are distinguishable on the basis of their gas chromatographic retention times.⁸ Nevertheless, the difficulty in deriving definite structures from mass spectral data alone, is well illustrated here.

- Figure LV. Mass Spectrum of Authentic C₂₀ Isoprenoid
 (Phytane)
- Figure LVI. Mass Spectrum of Abbott C₂₀ Isoprenoid
- Figure LVII. Mass Spectrum of San Joaquin C₂₀ Isoprenoid
- Figure LVIII. Mass Spectrum of Moonie Oil C₂₀ Isoprenoid
- Figure LIX. Mass Spectrum of Antrim C₂₀ Isoprenoid
- Figure LX. Mass Spectrum of Soudan C₂₀ Isoprenoid



The mass spectrum of the regular C_{21} isoprenoid, 2,6,10,14-tetramethyl heptadecane, which was synthesized by William Van Hoewan, is given in Figure LXI. Figures LXII-LXV give the mass spectra of the C_{21} isoprenoid isolated from the Abbott Oil, the Antrim Shale, the Nonesuch Oil, and the Soudan Shale. Since they are obviously impure an unambiguous definition of structure is difficult. If the peak at m/e 239 particularly in the case of the Nonesuch, but also to some extent in the Antrim and Soudan, is considered to have a contribution from the C_{20} iso-alkane or the C_{19} anteiso-alkane, (the C_{20} iso-alkane has a GLC retention time similar to that of the C_{21} iso-prenoid compound), then the remaining fragmentation pattern could be interpreted in terms of a regular C_{21} isoprenoid, Structure XI.



XI

In the case of the Soudan, there is tentative mass spectrometric evidence for the presence of C_{30} saturated and unsaturated isoprenoid hydrocarbons. Mass spectra of a fraction collected from the squalane region of the vapor phase chromatogram show similarities to the spectrum of authentic squalane. Figure LXVIII exhibits the mass spectrum of authentic squalane; Figures LXVI and LXVII that of two successive mass

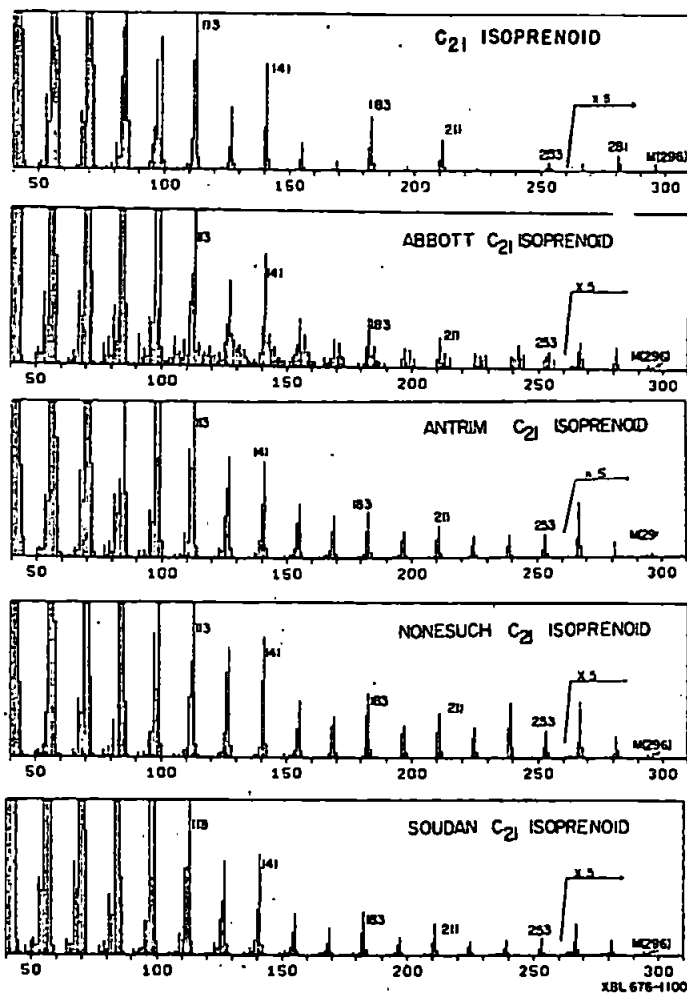
Figure LXI. Mass Spectrum of Authentic C₂₁ Isoprenoid

Figure LXII. Mass Spectrum of Abbott C₂₁ Isoprenoid

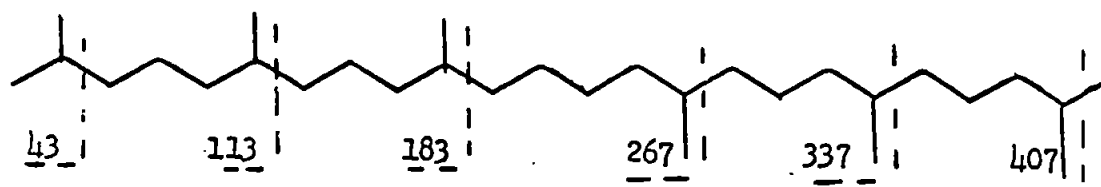
Figure LXIII. Mass Spectrum of Antrim C₂₁ Isoprenoid

Figure LXIV. Mass Spectrum of Nonesuch C₂₁ Isoprenoid

Figure LXV. Mass Spectrum of Soudan C₂₁ Isoprenoid



spectral scans. Scan 1 (Figure LVI) shows a molecular ion at m/e 422 expected for squalane but this pattern is clearly that of a mixture of compounds. By contrast scan 2 (Figure LVII) gives a mass spectrum which shows great similarity in its fragmentation pattern to that of authentic squalane, although a molecular ion is not observed - probably due to the rapid evaporation and pump off of the sample in the mass spectrometer. The expected fragmentation of squalane is indicated in Structure XII.



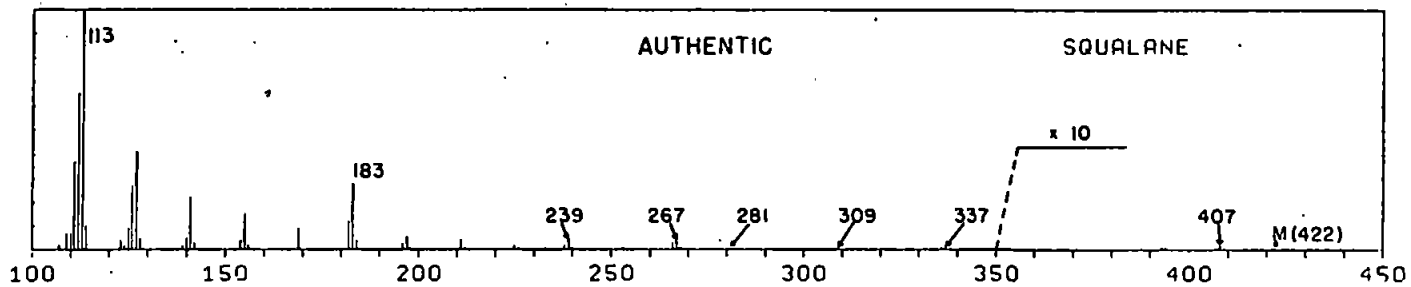
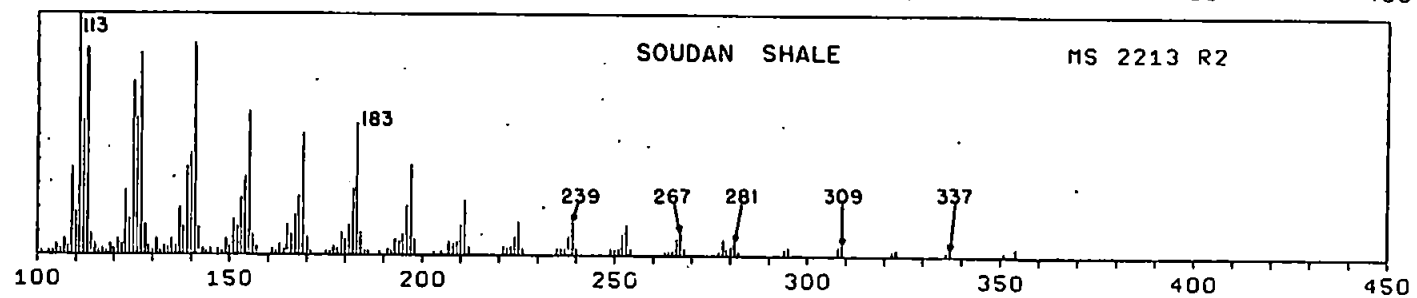
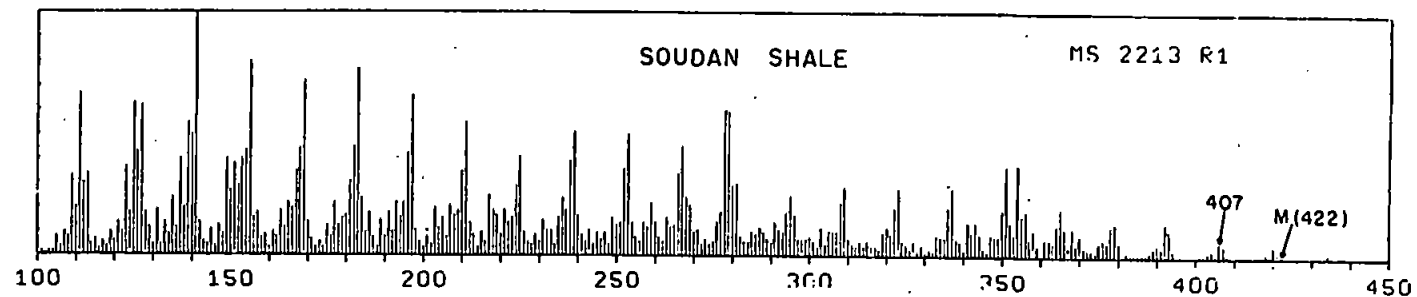
XII

The isoprenoid alkanes are assumed to be definite indications of life processes because of their high degree of structural specificity. This view should be maintained with some caution since Anders and Coworkers have recently shown that isoprenoid alkanes are formed in small quantities in abiogenic synthesis.⁵⁰ Pristane is found in animal and marine sources³⁹⁻⁴² although it is apparently lacking in contemporary plants. Recently several mono-olefins of pristane have also been isolated from zooplankton,⁴³ the diagenetic hydrogenation of which would yield pristane. The C_{20} isoprenoid

Figure LVI. Mass Spectrum of Soudan Shale Squalane Fraction Scan 1

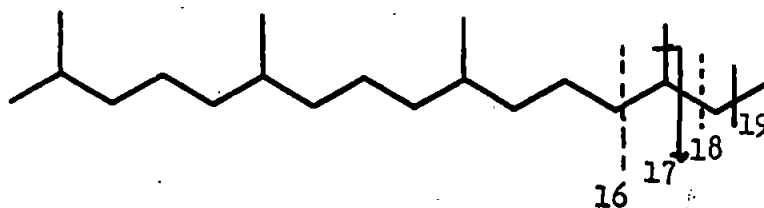
Figure LVII. Mass Spectrum of Soudan Shale Squalane Fraction Scan 2

Figure LVIII. Mass Spectrum of Authentic Squalane



NO 11727

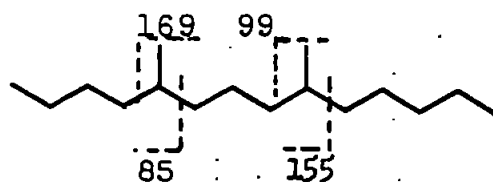
acid has been isolated from butter-fat,⁴⁴ ox blood,⁴⁵ and petroleum,⁴⁶ and could conceivably undergo diagenetic decarboxylation to form pristane. There are a few reports of the isolation of phytane from living organisms but none from marine sources. Phytol could be converted to phytane by a sequence of abiobiological diagenetic processes, such as saturation and dehydration reactions. Oxidation, decarboxylation and saturation of phytol could also lead to pristane. The diagenetic cleavage of the double bonds of phytenes could account for the formation of the C_{16} and C_{18} isoprenoids. If one assumes that phytol is the precursor of the isoprenoid alkanes and acids then (Structure XIII) formation of the C_{17} isoprenoid alkane or acid would require cleavage of two bonds to the same carbon atom. The absence in most sediments of the C_{17} isoprenoid is complimented by the work of Cason and Graham, who have found in petroleum the C_{11} , C_{14} , C_{15} , C_{19} , and C_{20} isoprenoid acids^{46,47,48} but no C_{17} or C_{18} acids. On the basis of this argument (Structure XIII) one would not expect a C_{17} or C_{18} acid, but rather a C_{18} ketone. The possible presence of the C_{17} isoprenoid in the Antrim may be rationalized in terms of the cracking of squalene.



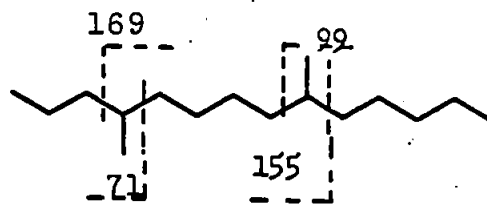
XIII

Unknowns

Figures LXIX-LXXII give the mass spectra of four C_{16} unknown branched hydrocarbons isolated, Figures LXXIII-LXXIV the mass spectra of two C_{17} unknown branched hydrocarbons, and Figures LXXV-LXXVII the mass spectra of two C_{18} unknown branched hydrocarbons. Previously,⁶ Structure XIV (5,9-dimethyltetradecane) was suggested as a possibility for the Moonie Oil Branched C_{16} X_1 (Figure LXIX) and Structure XV (4,9-dimethyltetradecane) for the Moonie Oil Branched C_{16} X_2 (Figure LXX). It is interesting to note that the formation of such compounds might be possible by cracking of squalane. The relative gas chromatographic retention times of the four compounds isolated from the Moonie Oil are given in Table IV and the extreme complexity of the regions from which they were isolated is illustrated by the gas chromatogram in Figure LXXXI. High resolution mass spectra run by Dennis Smith verify that these are saturated branched hydrocarbons. It is of particular interest that the Branched C_{16} compounds isolated from the Antrim Shale and the Nonesuch Oil are apparently identical. The general reproducibility of the isolation procedure and mass spectral analysis is indicated by comparison of Figures LXXV and LXXVI which appear to be the same compound isolated on two different occasions. Rationalizations for the mass

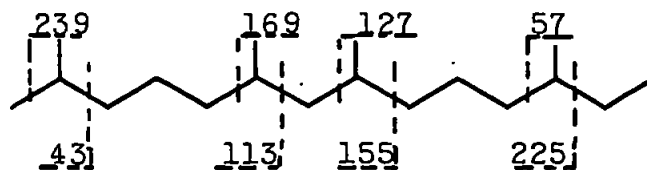


XIV



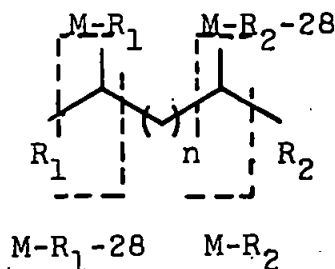
XV

spectra of these peculiar compounds are particularly difficult in the absence of adequate standards. Even the C_{18} branched alkane isolated from the Nostoc blue-green algae does not appear to be isoprenoidal and, in fact, structures capable of explaining the mass spectrum such as Structure XVI, would not usually be considered particularly biogenic.

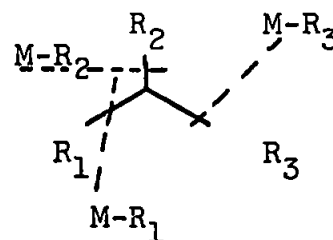


XVI

Therefore, beyond indicating the types of structures which might give rise to such spectra, little can be said:



Type I



Type II

Figures LXXVIII-LXXX give the mass spectrum of a component isolated from the Thucolite (an oil which was thought to be abiogenic, kindly supplied by Professor Clifford Frandel of Harvard and fractionated on a 3% SE-30 gas chromatographic column by Bill Van Hoeven), the Soudan, and the Nostoc blue-green algae. The mass spectra all bear some resemblance

to each other (ions at m/e 284, 256, 149, 129, and 111) but are difficult to interpret in the absence of other data. The difficulty experienced here in identifying hydrocarbons from a known living organism emphasizes the magnitude of the problem of interpreting the results of sediment analysis. Other peculiarly branched compounds reported in the literature are 2-methyl-3-ethyl-heptane from petroleum⁵¹ and 4- and 5-methyl alkanes in paraffin wax.⁵² It is to be hoped that in the future the identities of such minor components may be of value.

Figure LXIX. Moonie Oil Branched C_{16} Alkane (X_1)

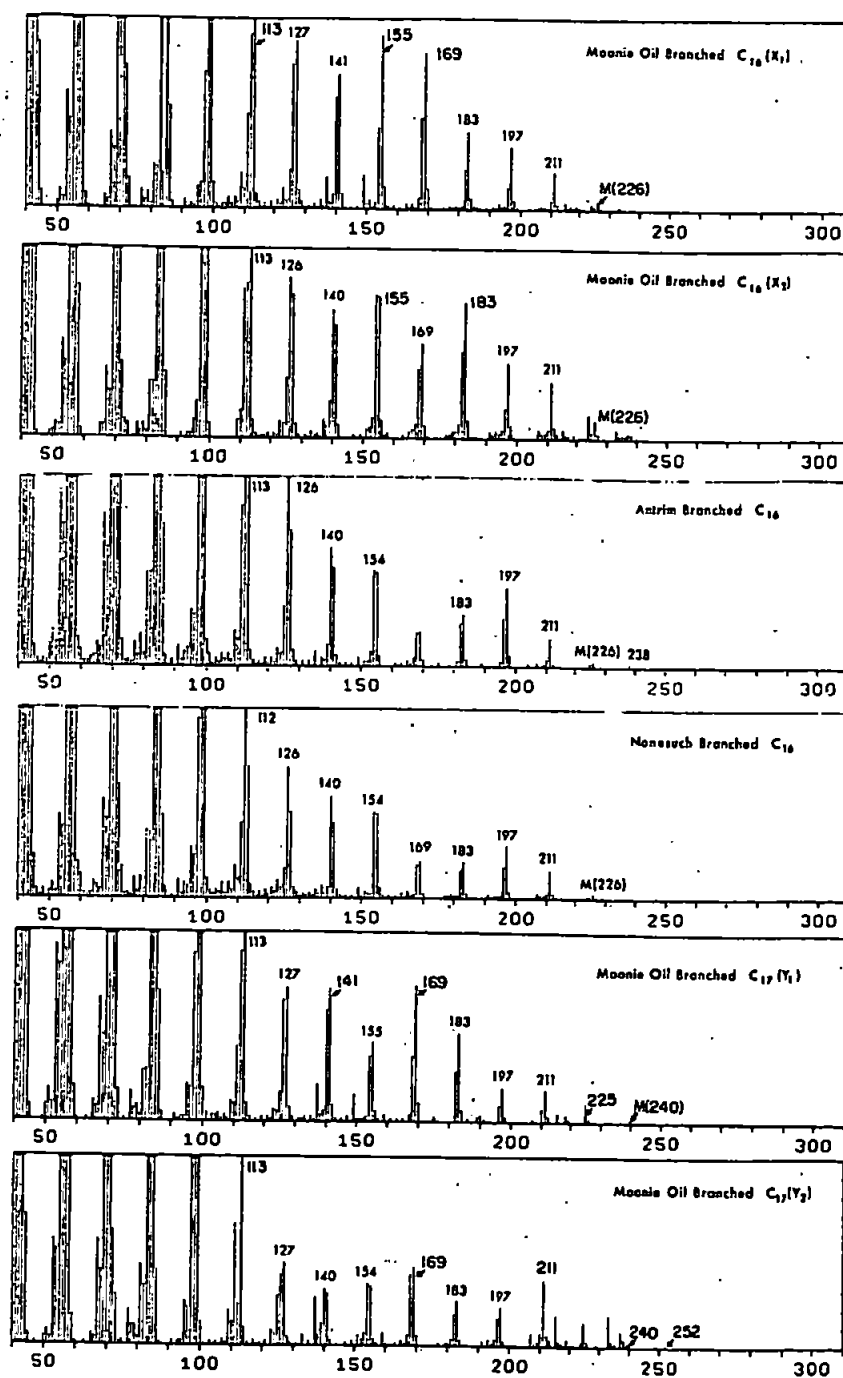
Figure LXX. Moonie Oil Branched C_{16} Alkane (X_2)

Figure LXXI. Antrim Branched C_{16} Alkane

Figure LXXII. Nonesuch Branched C_{16} Alkane

Figure LXXIII. Moonie Oil Branched C_{17} Alkane (Y_1)

Figure LXXIV. Moonie Oil Branched C_{17} Alkane (Y_2)

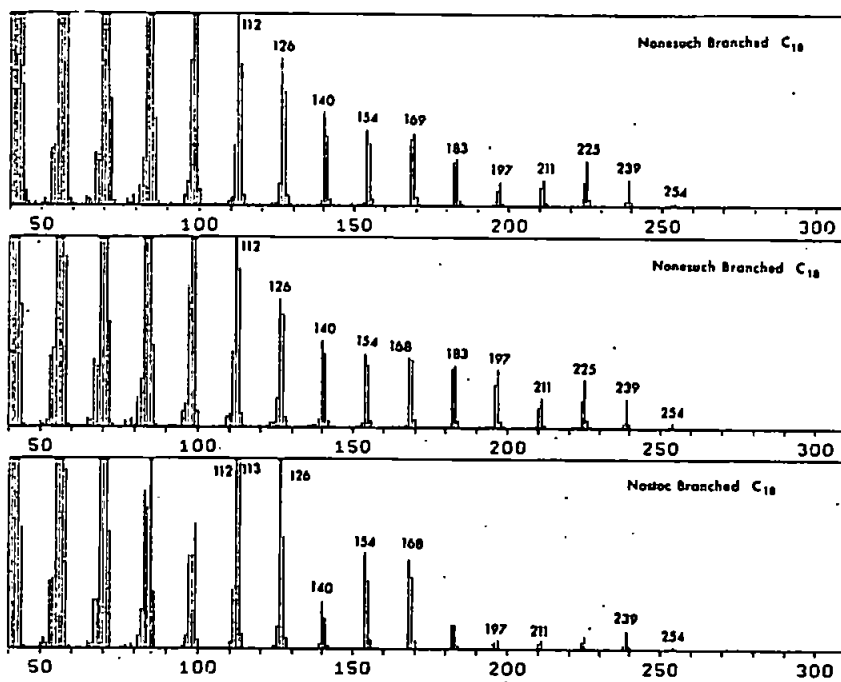


XBL 678-6104

Figure LXXV. Nonesuch Branched C_{18} Alkane

Figure LXXVI. Nonesuch Branched C_{18} Alkane (Procedure
Reproducibility)

Figure LXXVII. Nostoc Branched C_{18} Alkane



XBL 678-6106

Figure LXXVIII. Mass Spectrum of Unknown Fraction from
Thucolite

Figure LXXIX. Mass Spectrum of Unknown Fraction from
Soudan

Figure LXXX. Mass Spectrum of Unknown Fraction from
Nostoc

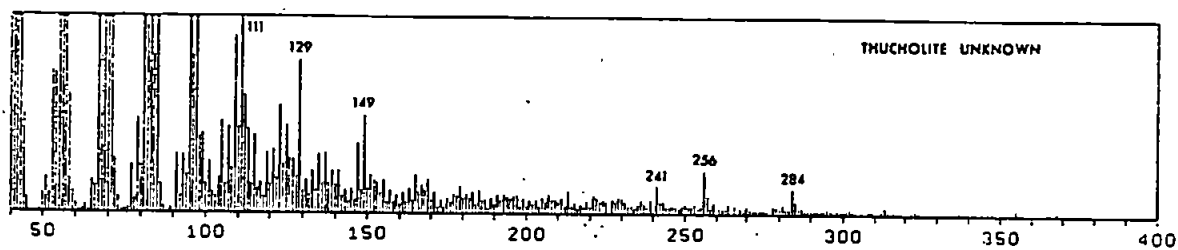
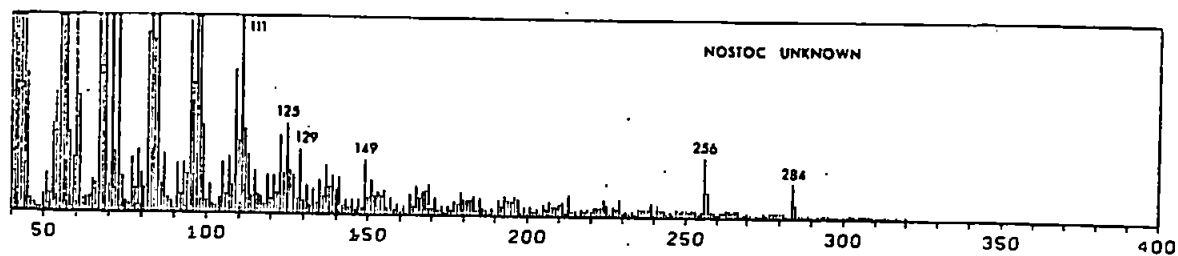
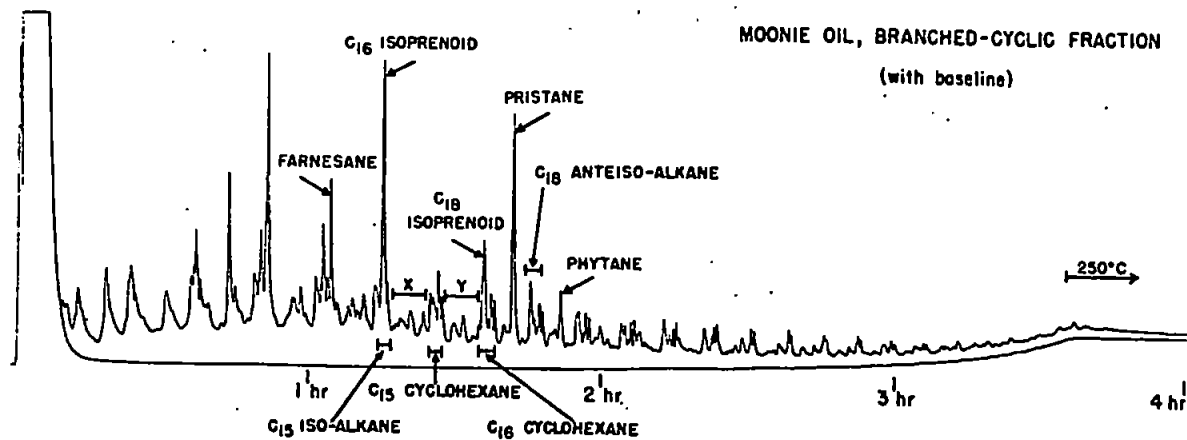


Figure LXXXI. Capillary Column Gas Chromatograph of
Moonie Oil "Branched-Cyclic" Hydrocarbon
Fraction



Data Correlations

From the sediments examined, isoprenoid alkane, iso-alkane, anteiso-alkane, n-alkylcyclohexane, and normal alkane series have been isolated (See Table V). It is becoming evident that among biogenic sediments, variations due to individual sediment history and ecology are to be found primarily in the relative amounts of these compounds, and possibly in the hydrocarbons present only in small quantities, rather than in the types of hydrocarbons. It is unfortunate that only one definitely non-marine sediment was examined since this does not permit much extrapolation and limits the conclusions that can be drawn. However, it is possible at this stage to define a few "ecology indices" which may prove useful in evaluating future sediment analyses.

Table I shows a remarkable increase in the percent of hydrocarbons for the Soudan shale. This might simply reflect the greater stability of these compounds or else a more marked degree of reduction which the compounds of the older sediments have undergone. A decrease in the percent of normals with age is also notable. Robinson et al.⁵³ have found increasing amounts of the lower molecular weight isoprenoids with increasing depth suggesting that cracking is occurring in the diagenetic process.

Normal alkanes isolated from contemporary plant sources commonly occur with carbon numbers greater than C₂₀ and show an odd over even carbon number predominance. The extreme odd

over even predominance of normal alkanes in the Green River Shale may be related to the non-marine nature of the sediment. Geologists have for a long time plotted the carbon number of normal alkanes on one axis and their relative concentration on the other. Dotted lines have been drawn through these points, the jagged character of odd over even predominance suggesting biogenic origin. The use of the odd/even ratio defined as

$$\text{odd/even ratio} = \frac{\text{concentration of odd normal alkanes}}{\text{concentration of even normal alkanes}}$$

has also been used and is tabulated in Table VI for all sediments analyzed.

The normal alkane present in greatest concentration is of interest particularly since algae, bacteria, and other biological organisms exhibit very marked maxima. For example, 98% of the normal paraffins of Ascomyllum nodosum and Fucus algae are $n\text{-C}_{15}$ and 99% of apple skin wax is $n\text{-C}_{29}$. Therefore, the normal alkane maximum of all sediments analyzed is also tabulated in Table VI. Non-marine sources often have two maxima.

The very high degree of structural specificity possessed by steranes and triterpanes in terms of their exact stereochemistry or precise structure would be expected to provide considerable information about the local ecology. (This concept will be further discussed in Part II.) The optical activity of specific compounds would be of interest since certain of the acids isolated from petroleum have been found to be devoid of the expected optical activity. Isotope ratios of individual

compounds could represent another series of ecology indices; a biologically formed molecule has undergone many very specific biosynthetic reactions possessing well defined and distinct isotopic discriminations.⁵⁴

In conclusion, let it be stated that three new hydrocarbon sediments are reported here: the iso-alkanes, anteriso-alkanes and n-alkylcyclohexanes. Also presented is the isolation of C_{15} - C_{21} isoprenoids from a number of geological sources. This work fits in quite nicely with that of other investigators who have found C_{14} - C_{20} isoprenoids in various geological sources: (C_{14} - C_{21}) has been reported in a Texas gas oil,^{55,56} pristane in a Midcontinent oil;⁵⁶ phytane⁵⁷ and the C_{14} and C_{15} isoprenoids⁵⁸ in a light gas oil fraction; C_{15} - C_{20} isoprenoids in the Colorado Green River Shale; pristane and phytane in the Gunflint Chert⁵⁹ and Fig Tree Shale⁶⁰ (both Precambrian); normal and isoprenoid alkanes in the Soudan and Nonesuch Shales;^{61,62} and a few other isoprenoid alkane identifications.⁶³⁻⁶⁶ Aside from isoprenoids, petroleum research has yielded a considerable number of other hydrocarbons which are partially or completely characterized.⁶⁷

TABLE I
Percentage Composition

Sample	Abbott Oil	San Joaquin	Green River	Moonie Oil	Antrim Shale	Nonesuch Shale	Soudan (I)	Soudan (II)
Carbon Content	87.33	85.2	20.1	86.3	8.8	0.4	3.2	5.2
Hydrogen Content	11.9	12.9	2.3	13.3				
Nitrogen Content	0.76	0.69	.57	.57				
Sulfur Content	<0.1	1.2	.08	<0.1	<0.1	<0.1	<0.1	25.3
Solvent Extractable Content of Sediment	12.4	100	1.3	100	~0.46	0.03-0.1	~0.05	~0.39
Extractables Soluble in <u>n</u> -Heptane	28	100	~80		~35	~90	~99	~0.25
"Total" Hydrocarbon Fraction in Extract- ables	38		31	38	25		86	
Normal Alkanes in "Total" Hydro- carbon Fraction			53		25-33		~7.5	

TABLE II

Gas Chromatogram Conditions

Figure VI

150 feet x .01 inch ID Apiezon L column temperature--
programed at 1°/minute.

Figures I, II, V

10 feet x 1/16 inch 3% SE-30 on 100-120 mesh Gaschrom Z,
20 ml/minute, nitrogen at 50 psi, temperature programed
at 6°/minute detector 250°, injector 300° (Aerograph
Model 665-1).

Figure LXXXI

150 feet x 0.010 inch 3% SE-30 on 100-120 mesh
Gaschrom Z temperature-programed at 1°/minute.

Figures II, IV

10 feet x 1/4 inch 3% SE-30 on 80-100 Mesh Chromosorb
WCDMCS, 60 ml/minute helium detector 245°, injector
280° (Aerograph Model A-90-P2).

TABLE III

Off Scale Mass Spectral Intensities

M/E	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI
40	1260			121		117	670		
41	290	670	1230	2140	483	1210	878	off	374
42	1690	156	255	820	176	400	398	864	140
43		750	1430	3520	1000	2900	off	off	908
44			160	157		140	430	191	
54	150		1200						
55	1010	650	590	515	223	580	418	1000	278
56	530	290	1000	393	172	445	377	900	184
57	1020	480		1790	786	2100	1730	off	742
58						105	101	200	
67	130		152					113	
68	120		140						
69	520	276	530	179		300	189		260
70	380	189	360	150		400	213	436	300
71	400	177	365	800	422	1550	878	off	453
72								140	
82	120								
83	430	177				175	101	360	90
84	180		174			160	120	211	
85	190		173	672	282	950	504	1582	245
86								107	
97	270	123	267	143		140	108	236	120
98								107	
99						180		358	
111								110	
113						100		182	
127								115	

TABLE III
Off Scale Mass Spectral Intensities

M/E	XXII	XXIII	XXIV	XXV	XXVI	XXVII	XXVIII	XXXII	XXXVI
40		210	138		214	670			
41	1820	1100	817	767	682	1000	122		
42	750	260	804	389	191	800			
43	440	1650	off	off	1318	6000	184	145	off
44	243	150	411	194	218	3300			
55	920	570	330	417	355	600			
56	755	650	513	606	477	260			
57	348	2000	1366	1683	1364	500	247		
58	307		off	783	105				
60						400			
61						3500			
62						150			
69	419	320	138	183	145	250			
70	419	220	125	161	132	110			
71	1948	950	563	667	545	250			
83	224	200		100		130			
84	153								
85	1044	600	379	417	359	113			
97	153	240				210			
99	224	120				190			
113	139								

TABLE III
Off Scale Mass Spectral Intensities

M/E	XXXVII	XXXVIII	XXXIX	XL	XLI	XLII	XLIII	XLIV	XLV
40				1300					
41	770	450	420	390	730		off		327
42	220	138	134		200	300	650		115
43	off	off	1070	580	1500	610	off	144	266
44				880			450		286
53							110		
55	335	283	230	141	550	220	off		234
56	400	217	194	113	450	200	810		110
57	off	off	950		1920	780	off	193	148
58					100				
69	120	105			250	110	500		108
70	180	212	147		400	180	720		
71	off	730	820	400	1500	500	off		
72							130		
83							195		
84					140	140	235		
85		320	250	690	700	390	off		
97							130		
99					140		175		

TABLE III
Off Scale Mass Spectral Intensities

M/E	XLVI	XLVII	XLVIII	XLIX	L	LI	LII	LIII	LIV	LV
40	210									
41	2000	711	630	163	off	579	250	500	183	700
42	500	447	190		104	162		150		176
43	3300	110	1350	209	off	865	157	1070	380	1240
44	131	221				286		110		
53	off									
54				119						
55	900	342	360	115	240	309	145	290	144	440
56	660	300	340	230	250	251	160	270	144	380
57	3400	1630	1550		off	1028	off	1300	620	1500
58	170	1350								
69	285	194	190		120	192		150		172
70	230	160	150			113		109		176
71	1300	810	890	200	off	660	435	760	520	1060
85		408	410		off	219	133	330	230	440
98	140	117	109							
99	130	123	110							

TABLE III

Off Scale Mass Spectral Intensities

M/E	LVI	LVII	LVIII	LIX	LX	LXI	LXII	LXIII	LXIV	LXV
40			180		260		336			
41	570	462	710	860	1160	609	off	650	520	950
42	140	111	170	250	340	182	190	160	140	240
43	1310	off	1400	2000	1900	1545	off	off	off	2300
44			150	590	690		200			110
55	410	312	420	500	720	482	490	480	430	720
56	340	264	320	360	580	364	360	290	390	500
57	1700	off	1720	1600	2250	1727	off	off	off	2300
58				240	120					170
69	200	157	270	280	290	209	260	280		340
70	170	150	280	220	260	218	240	200		340
71	1025	off	910	960	1160	1091	off	920	off	1400
83				140	106		102	140	100	120
84				110			110	120	100	120
85	520	417	410	550	540	409	600	520	550	590
97				125			off	110		105
99	100			115			130	130	120	120

TABLE IV
Relative Retention Times

Compound	10' x 1/4", SE-30 Program Rate 4°/min.	25' x 1/4", 2-1/2% PPE Isothermal 136°C.
C ₁₆ Isoprenoid	22.7 min.	10.8 min.
X ₁	23.5 "	11.9 "
X ₂	24.2 "	12.8 "
Y ₁	26.7 "	19.0 "
Y ₂	28.2 "	20.8 "
C ₁₈ Isoprenoid	28.6 "	23.1 "

TABLE V
Approximate Relative Abundance of Alkanes*

Sample	Normal Alkanes												
	C ₉	C ₁₀	C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₂₀	C ₂₁
Soudan Shale						0.01	0.07	0.5	1	0.9	0.5	0.9	
Nonesuch Oil				0.01	0.05	0.3	0.7	0.95	1	0.9	0.75	0.55	0.4
Nonesuch Calcite Vein			0.2	0.1	0.3	0.4	0.6	0.8	1	1.2	1.35	1.35	1.30
Antrim Shale		0.005	3.0	5.0	5.0	4.0	3.0	1.5	1	0.5	0.2	0.005	
Moonie Oil	0.03	0.3	0.3	0.4	0.3	0.5	0.7	0.8	1	1.04	1.03	0.9	0.8
Green River Shale					0.01	0.02	0.05	0.04	1	0.06	0.1	0.02	0.08
San Joaquin Oil		0.2	1.3	2.0	2.4	2.2	1.8	1.4	1	0.6	0.4	0.2	0.1
Abbott Seep Oil							0.1	0.7	1	0.4	0.2	0.08	0.06
Abbott Rock Oil					0.01	0.05	0.2	0.6	1	1.2	1.3	1.0	0.8

TABLE V
Approximate Relative Abundance of Alkanes*

Sample	Normal Alkanes												
	C ₂₂	C ₂₃	C ₂₄	C ₂₅	C ₂₆	C ₂₇	C ₂₈	C ₂₉	C ₃₀	C ₃₁	C ₃₂	C ₃₃	C ₃₄
Soudan Shale													
Nonesuch Oil	0.3	0.2	0.1	0.09	0.07	0.6	0.5	0.04	0.03	0.02	0.01	0.01	0.01
Nonesuch Calcite Vein	1.2	1.1	1.0	0.8	0.7	0.6	0.4	0.3	0.2	0.1	0.08	0.05	0.03
Antrim Shale													
Moonie Oil	0.7	0.65	0.4	0.3	0.1	0.06	0.01	0.005					
Green River Shale	0.25	0.1	0.01	0.15	0.01	0.25	0.2	1.1	0.02	1.1	0.01	0.09	
San Joaquin Oil	0.05												
Abbott Seep Oil	0.05	0.04	0.025	0.025	0.025	0.06	0.04	0.07	0.08	0.3	0.1	0.05	0.03
Abbott Rock Oil	0.5	0.3	0.2	0.15	0.1	0.08	0.01						

TABLE V
Approximate Relative Abundance of Alkanes*

Sample	Age	<u>Iso-Alkane</u>				<u>Anteiso-Alkane</u>		
		C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₆	C ₁₇	C ₁₈
Nonesuch Oil	1 x 10 ⁹		<0.1	0.2	0.07	<0.1	0.2	0.05
Moonie Oil	200 x 10 ⁶	0.1						0.1

TABLE V

Approximate Relative Abundance of Alkanes*

Sample	Age	<u>n</u> -Alkyl-Cyclohexanes					X ₁	X ₂	Y ₁	Y ₂
		C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₁₆	C ₁₆	C ₁₇	C ₁₇
Nonesuch Oil	1 x 10 ⁹		0.1	0.1	<0.1	0.1				
Moonie Oil	200 x 10 ⁶	0.2	0.3				0.1	<0.1	0.05	0.1

TABLE V
Approximate Relative Abundance of Alkanes*

Sample	Age	Isoprenoids						
		C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₂₀	C ₂₁
Soudan Shale	2.7 x 10 ⁹		0.08		0.6	1	0.6	0.1
Nonesuch Oil	1 x 10 ⁹	0.4	1.2			1	0.3	0.1
Nonesuch Calcite Vein	1 x 10 ⁹	0.2	0.8			1	0.3	0.1
Antrim Shale	265 x 10 ⁶			0.2	1.3	1	0.6	
Moonie Oil	200 x 10 ⁶	0.3	0.9			1	0.3	<0.05
Green River Shale	50 x 10 ⁶		0.4	<0.1	0.2	1	5.5	
San Joaquin Oil	30 x 10 ⁶				0.9	1	0.9	
Abbott Seep Oil	3 x 10 ⁶					1	0.3	
Abbott Rock Oil	3 x 10 ⁶				0.4	1	0.5	

*Determined from gas chromatographic peak heights.

TABLE V
Approximate Relative Abundance of Alkanes*

Sample	Age	Steranes			Triterpanes
		C ₂₇	C ₂₈	C ₂₉	C ₃₀
Soudan Shale	2.7 x 10 ⁹	0.03			1.2
Green River Shale	50 x 10 ⁶	0.05	0.5	0.8	1.2

TABLE VI
Ecology Indices

Sample	Age	Origin	O/E	Normal Maximum	Phytane/Pristane
Soudan Shale	2.7×10^9	Marine	.68	C ₁₇	.6
Nonesuch Oil	1×10^9	Marine ?	1.01	C ₁₇	.3
Antrim Shale	265×10^6	Marine	1.11	C ₁₃	.3
Moonie Oil	200×10^6	?	.97	C ₁₂ , C ₁₈	.3
Green River Shale	50×10^6	Non-Marine	8.75	C ₁₇ , C ₂₉	5.5
San Joaquin Oil	30×10^6	?	1.05	C ₁₃	.9
Abbott Rock Oil	3×10^6	Non-Marine	1.05	C ₁₉	.5
Apple Skin Wax		Non-Marine	99*, 12 ⁺	C ₂₉ * ⁺	--
Marigold Flowers		Non-Marine	.8	<u>n</u> C ₃₀	--
Algae, <u>Ascophyllum</u> <u>nodosum</u>		Marine	19.8	<u>n</u> C ₁₅	--
Nostoc Blue-Green Algae		Marine		C ₁₇	--

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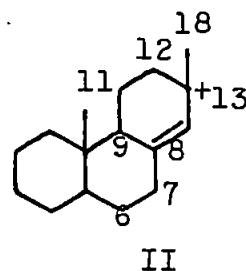
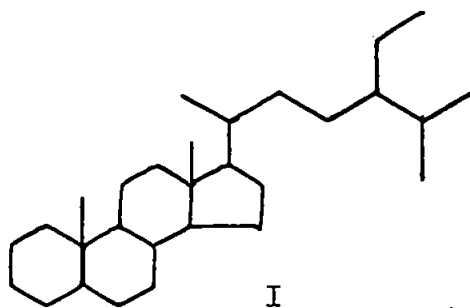
Part II
Steranes and Triterpanes from The
Colorado Green River Shale

The Colorado Green River Shale

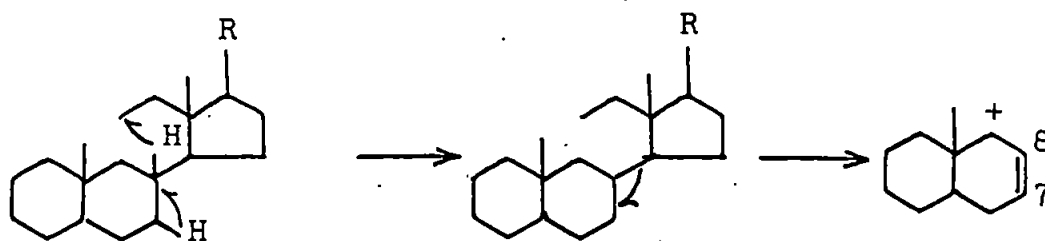
The Colorado Green River Shale; a carbon-rich sediment of Eocene Age (52×10^6 years), is perhaps the most extensively investigated sediment.¹⁻²⁵ Isoprenoid hydrocarbons and a series of normal alkanes have been identified,^{7,12} and more recently¹³⁻¹⁸ various classes of acids have also been isolated (See Part III). The abundance of the isoprenoids pristane and phytane is a striking feature of the hydrocarbon content of this rock (See Figure I). Earlier investigations in these laboratories,¹² have dealt with the identification of isoprenoid and normal hydrocarbons. Gas chromatographic fractions from the high molecular weight region of the gas chromatogram obtained were investigated by mass spectrometry leading to the discovery of a series of sterane and triterpane substances.¹⁹ The mass spectra of these compounds are quite characteristic and permit definite skeletal assignments.

Steranes and Triterpanes Isolated from Green River Shale

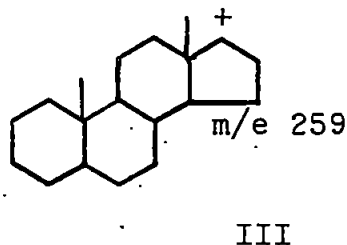
Fractions were collected from the Green River (Mahogany-Zone bed of the Green River formation near Rifle, Colorado) Shale (provided by Dr. Robinson)⁷ extract¹² whose gas chromatogram is given in Figure I. Mass spectra of these fractions led to the discovery of three steranes, the C₂₇, C₂₈ and C₂₉ hydrocarbon skeletons of steroids. The three mass spectra in Figures II-IV were obtained by collecting peaks on a 10 ft x 1/4 inch, 3% SE-30 (Gaschrom Z, 100-120 mesh) with a program rate of 4°/min. and then rechromatographed isothermally at about 270°C. For purposes of comparison the mass spectrum of authentic sitostane (Structure I) is given in Figure V.²⁶



The very intense peak at m/e 217 may be rationalized by the ion represented in Structure II. However, deuterium labeling studies are ambiguous with respect to which hydrogen is lost so that the location of the double bond is uncertain.²⁷ The peaks at m/e 149 may arise by the pathway:²⁸

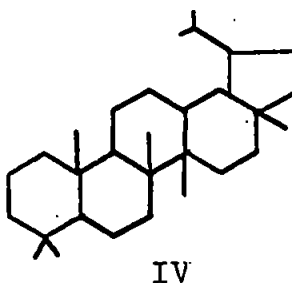


The small peak at m/e 259 results from simple loss of the side chain to give Structure III



The peaks at m/e 191, 203, 231 probably arise from small amounts of triterpane impurities.

A C_{30} triterpane was also isolated and its mass spectrum (Figure VI) is quite similar to that of the lupane (Structure IV), Figure VII, a sample of which was provided by Professor Carl Djerassi and Dr. H. Budzikiewicz of Stanford University.²⁹



The intense peak at m/e 191 is formed by two different ruptures of the central ring to give fragments V and VI.

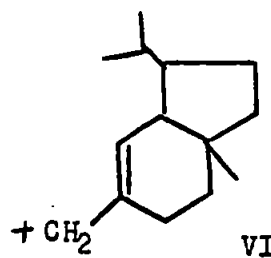
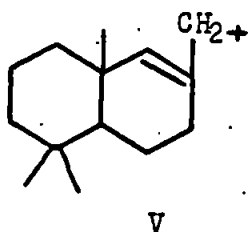
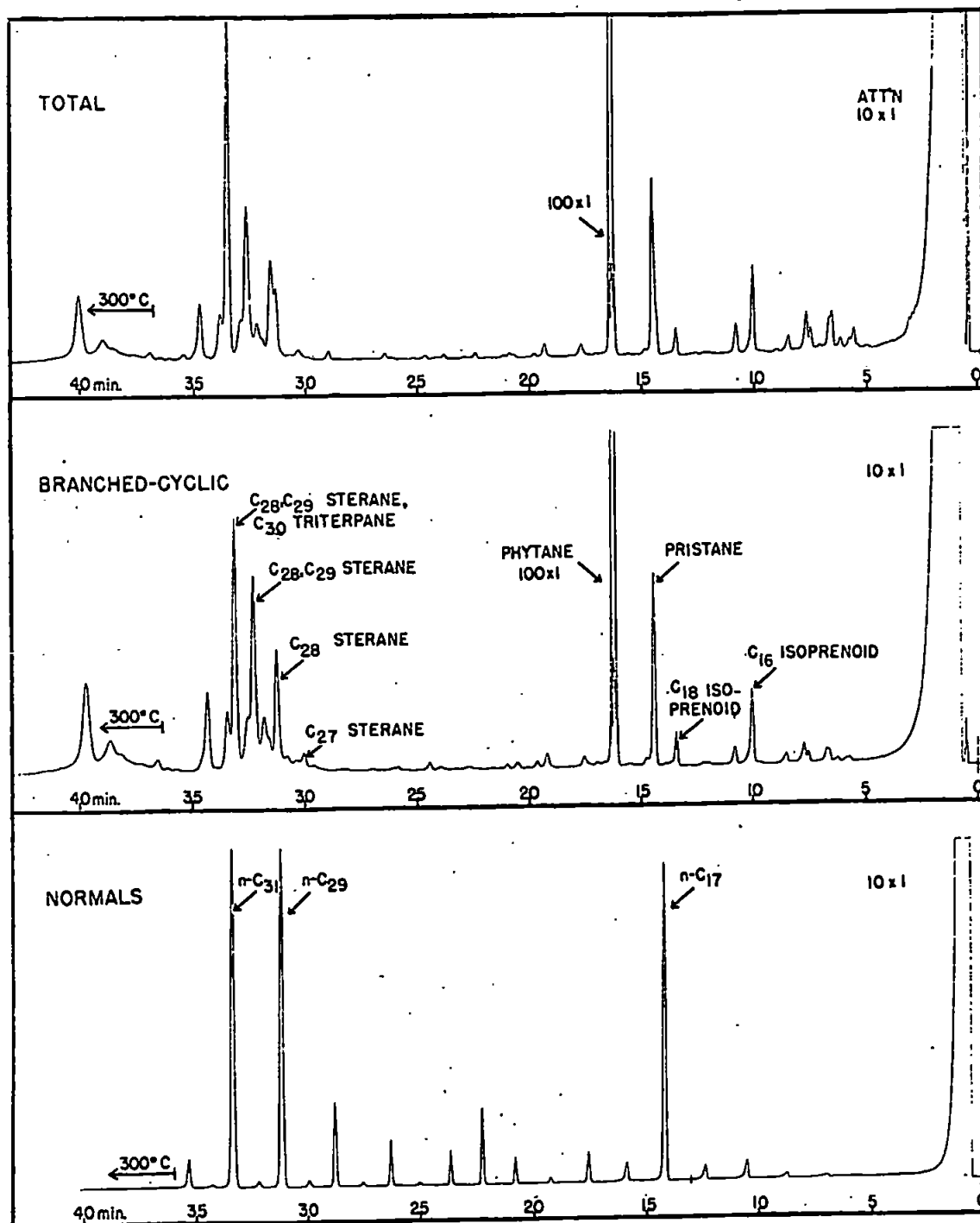


Figure I. Gas Chromatogram of the Branched-Cyclic Fraction
of The Colorado Green River Shale

GREEN RIVER SHALE (COLORADO), ~60X10⁶ YRS. ALKANE FRACTIONS

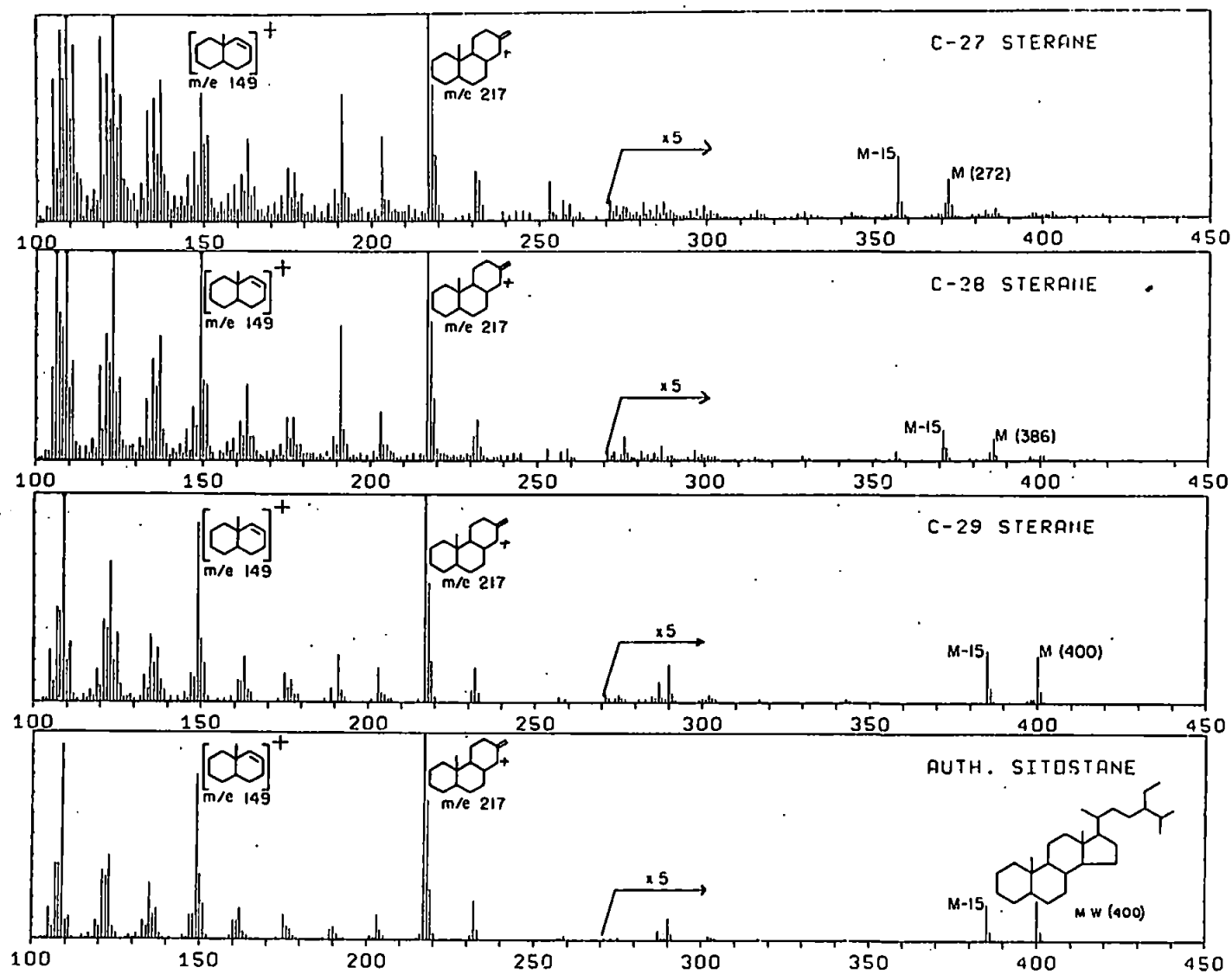
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Figure II. Mass Spectrum of Colorado Green River
Shale C₂₇ Sterane

Figure III. Mass Spectrum of Colorado Green River
Shale C₂₈ Sterane

Figure IV. Mass Spectrum of Colorado Green River
Shale C₂₉ Sterane

Figure V. Mass Spectrum of Authentic Sitostane



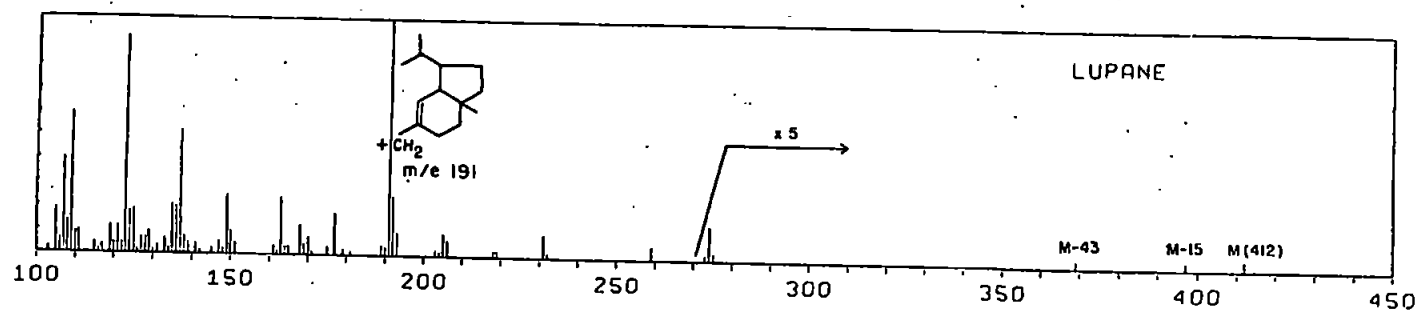
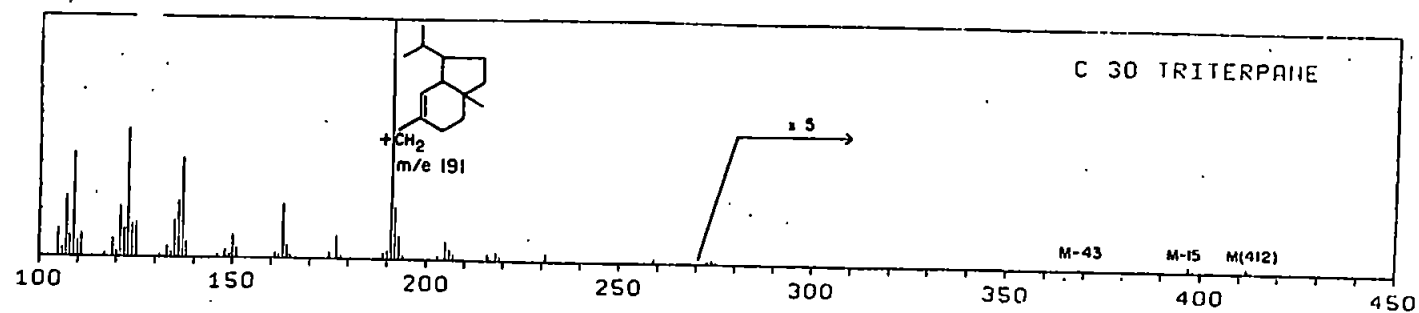


Figure VI. Mass Spectrum of the Colorado Green River Shale C₃₀ Triterpane

Figure VII. Mass Spectrum of Authentic Lupane

Extraction

Seventy pounds of Colorado Green River Shale were collected* from the side of a cliff in Parachute Creek, 8 miles northwest of Grand Valley, Colorado, latitude N 39° 37', longitude W 108° 7', elevation 7300 ft. The white stratifications in the photograph of Figure VIII show the general area. The outer surface of the rock was removed with hammer and chisel.** Large pieces were then crushed in a rock-crusher*** Rock chips between 3 and 20 mesh were sonicated with 4:1 benzene/methanol (redistilled*** ACS reagent grade Baker) for at least seven minutes (in 200 ml batches) to remove the outer organic material. The collected residue represents the "washings." The rock was then pulverized and sonicated twice (in 500g batches) with two liters of 4:1 benzene/methanol (redistilled ACS reagent grade Baker) for not less than twenty minutes with mechanical stirring. Since it was found that as much organic extract could be removed without sonicating, the rock for columns IV and V (See below) was not sonicated but only stirred with solvent containing a slightly higher benzene/methanol ratio. It should be noted

*By Martin Senn and Bernd Simoneit.

**With the help of Dr. G. Eglinton, Dr. Heinrich Schnoes, Bernd Simoneit and Ted Belsky.

***With the assistance of Bernd Simoneit.

that the second sonication gave approximately half the amount of extract given by the first sonication and that the third sonication gave approximately half the amount of extract obtained from the second. Yields of extract were proportional to the amount of solvent used. A total of 17.5 kg of rock were extracted yielding 181g of crude extract.



Figure VIII. Photograph of Colorado Green River Shale
Sample Location*

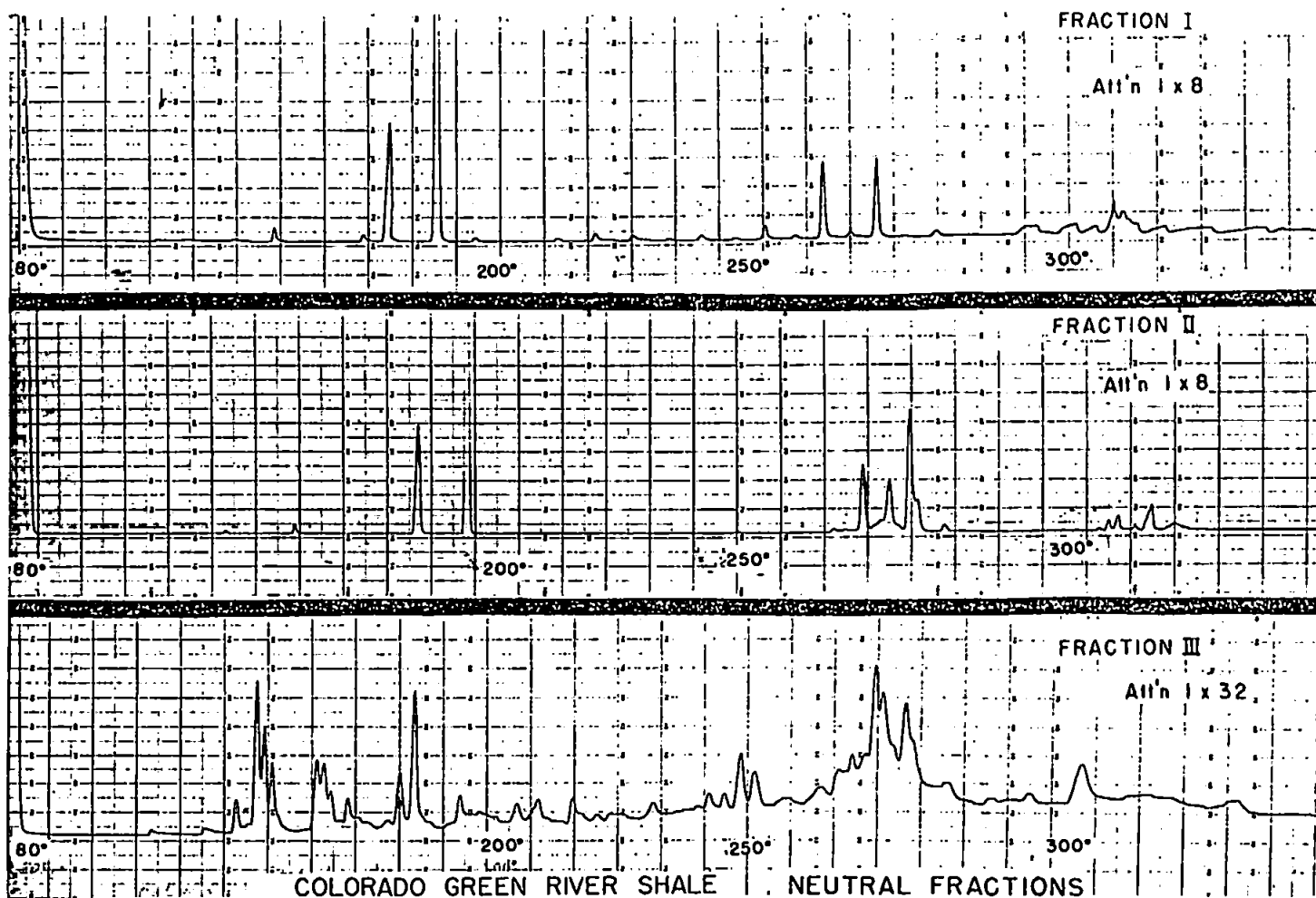
*Taken by Bernd Simoneit.

Alumina Column Chromatography

The limiting factor in a uniform treatment of the extract proved to be its passage down an alumina column. For the fractionation of the extracted material by column chromatography the total extract was divided into five portions of 30-40 grams each (See Table I). Each portion was chromatographed separately on 2 kg of aluminum oxide. All columns were 10 cm in diameter and 20-30 cm in height depending on the type of alumina used and the mode of packing (See Table I). All columns were washed with two gallons of methanol (redistilled ACS reagent grade Merk), two gallons of benzene (redistilled ACS reagent grade Baker), and two gallons of hexane (redistilled ACS reagent grade Matheson, Coleman, and Bell). The most efficient method of packing a column was to make an alumina slurry by swirling equal volumes of alumina and hexane which was then poured into a column containing one and a half gallons of hexane running at a rapid rate; a column could thus be obtained with a flow rate of at least two liters per hour. Extract was added to the top of each column in hexane, and eluted in order with hexane, benzene, and methanol. Fractions of 1 liter each were collected. The first hydrocarbon fractions were almost colorless; later ones were a pale yellow. After the brown and yellow bands an interesting pink to rose colored band was eluted by benzene. Table I gives the details on the column preparation and chromatography of the five columns.

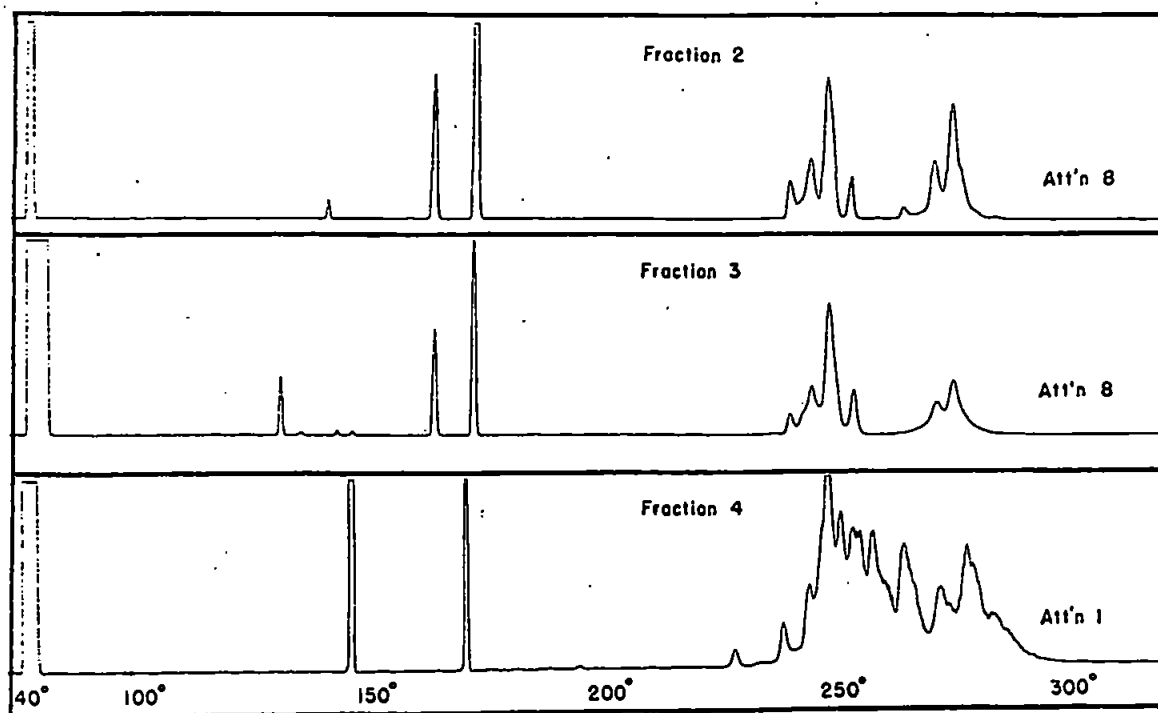
All fractions eluted with hexane were checked for absorption by ultraviolet spectroscopy and those exhibiting only end absorption and a gas chromatographic pattern similar to that in the preliminary experiments were taken as the total saturated hydrocarbon fraction for further processing (See Table II). Gas chromatograms of the first fractions eluted with hexane from these columns are shown in Figures IX-XII. They give the typical "total hydrocarbon" pattern expected from the Green River Shale from previous experiments.

Figure IX. Gas Chromatograms of Column I Fractions .



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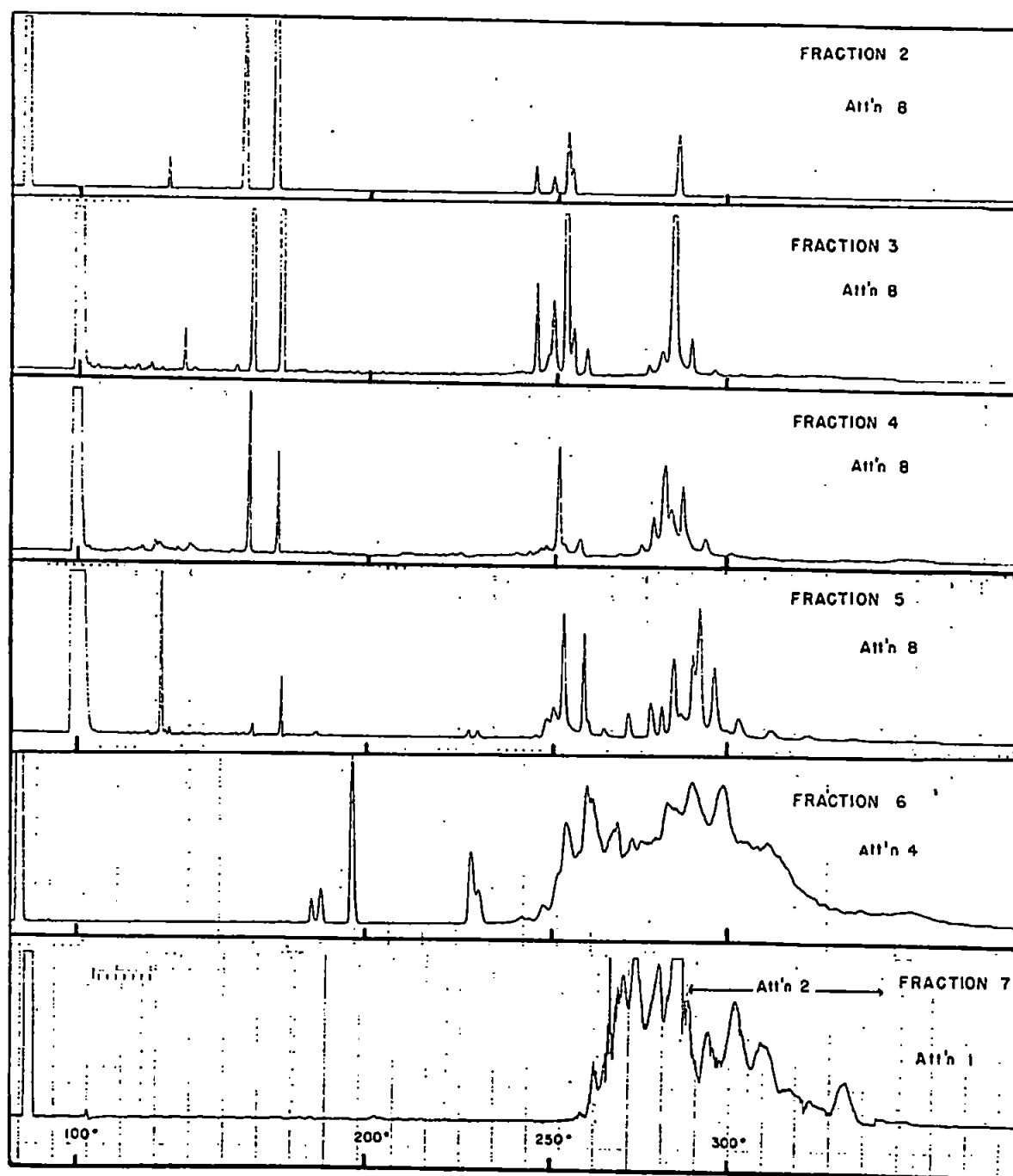
Figure X. Gas Chromatograms of Column III Fractions



COLORADO GREEN RIVER SHALE. COLUMN III

XBL 673-1007

Figure XI. Gas Chromatograms of Column IV Fractions

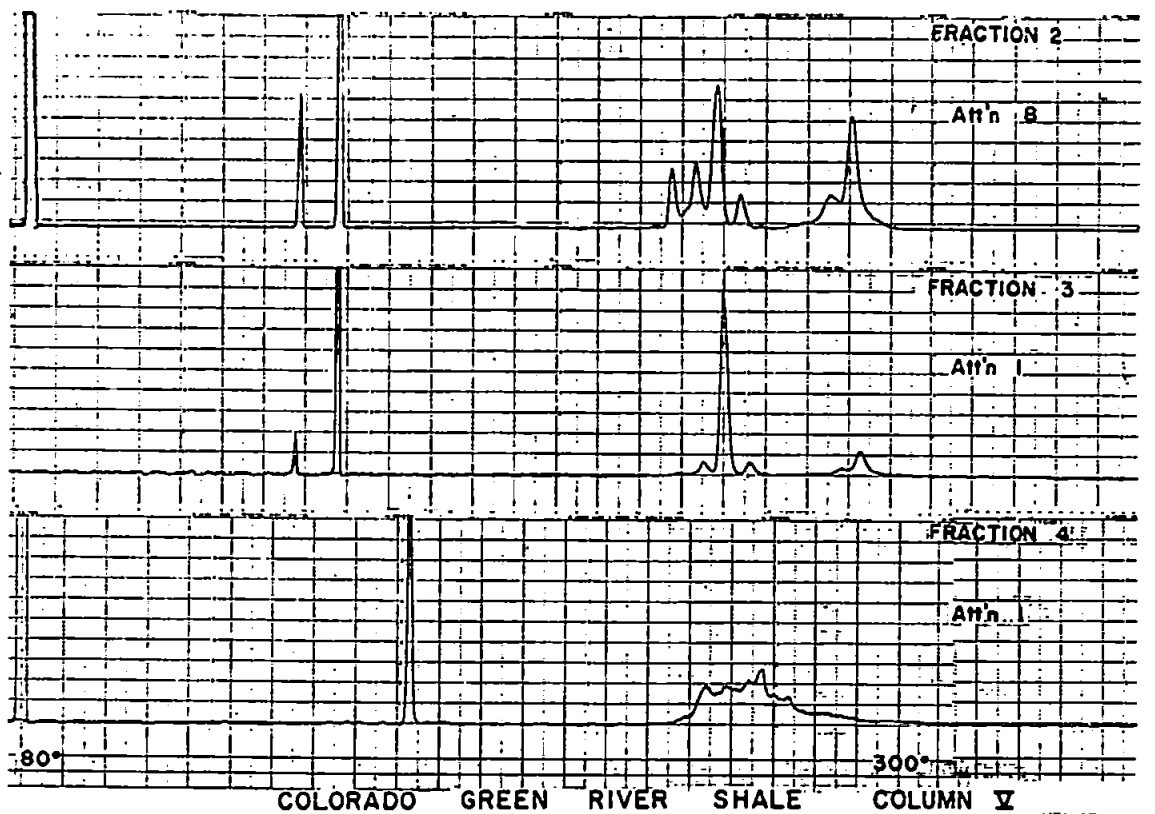


COLORADO GREEN RIVER SHALE

COLUMN IV

XBL 672-624

Figure XII. Gas Chromatograms of Column V Fractions



XBL 674-1018

TABLE I
Alumina Column Description

<u>Column</u>	<u>Alumina</u>	<u>Extract</u>
I	2,000g Bio. Rad Aluminum Oxide for thin layer chromatography. Neutral Alumina AG 7, 2-44 microns with binder (packed in hexane).	31g of extract minus 5g hexane insoluble .18g hexane soluble acids and .11g hexane soluble bases.
II	2,000g Bio. Rad Aluminum Oxide for thin layer chromatography. Neutral Alumina AG 7, 2-44 microns without binder (packed in methanol).	33g of extract (from 5826g rock including extract of III) minus 5g hexane insoluble material.
III	2,000g Bio. Rad Aluminum Oxide for thin layer chromatography. Neutral Alumina AG 7, 2-44 microns without binder (packed in hexane).	26g of extract minus 7g hexane insoluble, .20 hexane soluble acids, .10g hexane soluble bases.
IV	2 kg. E. Merck Ag. Darmstadt Aluminum Oxide active neutral for chromatography activity 1. (packed in hexane and reactivated after methanol wash).	48g of extract (from 6, 7507g rock together with extract of V, division being carried out with hexane) minus 14g hexane insoluble material.
V	2 kg. Bio. Rad Aluminum Oxide for Column Chromatography. Neutral Alumina AG 7, minus 200 mesh (packed in hexane but not reactivated after methanol wash).	43g of extract minus 5g hexane insoluble material.

TABLE II

Hydrocarbon Fractions Eluted from Alumina Column
and Sieving Treatment

<u>Fraction</u>		<u>Sieving Treatment</u>
I	1	0.27g. (normal hydrocarbons) unprocessed
I	2	5.65g sieved with 500g 1/16" 5 Å molecular sieve in benzene yielded 4.06g branched-cyclic hydrocarbons sieved with 250g 1/8" 10 Å molecular sieve in hexane, 2.33g recovered from outside and 2.38g adsorbed, 1.59g of which was sieved with 155.5g 1/16" 5 Å molecular sieve in benzene and yielded 1.14g.
I	3	0.52g (sieved with 50g 1/16" 5 Å molecular sieve in benzene yielded 0.35g.
I	4	0.21g (high molecular weight) unprocessed.
II	1	7.23g sieved with 637g 1/16" 5 Å molecular sieve in benzene yielded 4.82g branched-cyclic hydrocarbons sieved with 488g 1/8" 8 Å molecular sieve in hexane, 2.21g recovered from outside and 1.01g (some lost) adsorbed, which was sieved with 57g 1/8" 5 Å molecular sieve in benzene and gave .71g.
II	2	3.95g (sieved with 300g 1/8" 5 Å molecular sieve in benzene yielded 3 g branched-cyclic hydrocarbons.
II	3	0.65g (high molecular weight material) unprocessed.
III	1	No material eluted.
III	2	5.79g sieved with 287g 1/16" 5 Å molecular sieve in benzene yielded 4.09g branched-cyclic hydrocarbons.
III	3	4.22g sieved with 123g 1/16" 5 Å molecular sieve in benzene yielded 3.85g branched-cyclic hydrocarbons.
III	4	0.68g (high molecular weight) unprocessed.

TABLE II (Cont'd.)

Hydrocarbon Fractions Eluted from Alumina Column
and Sieving Treatment

<u>Fraction</u>		<u>Sieving Treatment</u>
IV	1	No material eluted.
IV	2	2 g (normal and low molecular weight branched) unprocessed.
IV	3	5.81g sieved with 265g 5 Å molecular sieve and vacuum distilled at 1.2 cm pressure and 200°C left undistilled 4.0g only 1.8g of which was used.
IV	4	1.38g vacuum distilled at .5 cm of mercury undistilled .08g.
IV	5	0.32g.
IV	6	
IV	7	
V	1	No material eluted.
V	2	8.28g sieved with 490g 1/16" 5 Å molecular sieve in benzene yielded 6g branched-cyclic hydrocarbons.
V	3	11.1g sieved with 601g 1/8" 5 Å molecular sieve in benzene yielded 10.8g branched-cyclic hydrocarbons.
V	4	1.70g.
V	5	0.43g.

Molecular Sieving

Hydrocarbon fractions collected from all five alumina columns (See Table III) were subjected to sieving experiments. All molecular sieves were either heated to 350°C for 24 hours or to 200°C under vacuum (5 mm Hg) in the presence of P_2O_5 . For the removal of normal alkanes, all fractions used in the large scale sterane and triterpane isolation were sieved with 1/8" or 1/16" 5 Å molecular sieve in a ratio greater than 50/1 molecular sieve extract.⁴ In an attempt to simplify the purification procedure by separating out the steranes and triterpanes, Fraction I-2 (which consisted of 4.06g branched-cyclic hydrocarbons after being refluxed in benzene with 500g 1/16" 5 Å molecular sieve) was refluxed with 250g of 1/8" 10 Å molecular sieve in hexane. Figure XIII shows the gas chromatogram resulting after sieving for 3 days with the 5 Å molecular sieve; Figure XIV shows the gas chromatogram resulting after sieving for 3 days with the 10 Å sieve, while Figure XV shows the gas chromatogram of the organic extract obtained by dissolving the 10 Å sieve with 24% HF and extracting with benzene. It is of interest that the sieve adsorbs predominantly the triterpanes, as is evident from a comparison of the mass spectra of those compounds in the sterane region adsorbed by the 10 Å molecular sieve (Figures XVII, XIX, XX, XXII, XXIV) with those not adsorbed (Figures XVI, XVIII, XXI, and XXIII).

Fraction II-1, therefore, was sieved with 1/8" 8 Å molecular sieve (after sieving with 5 Å molecular sieve); the

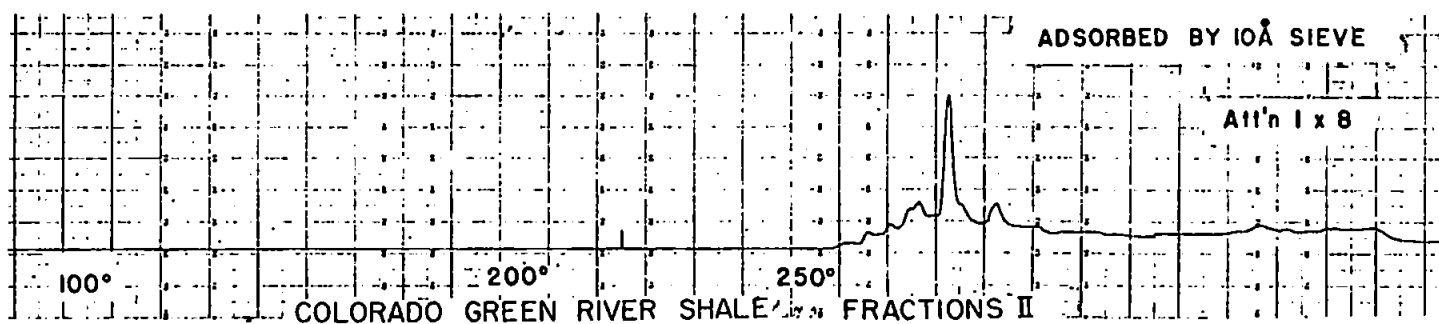
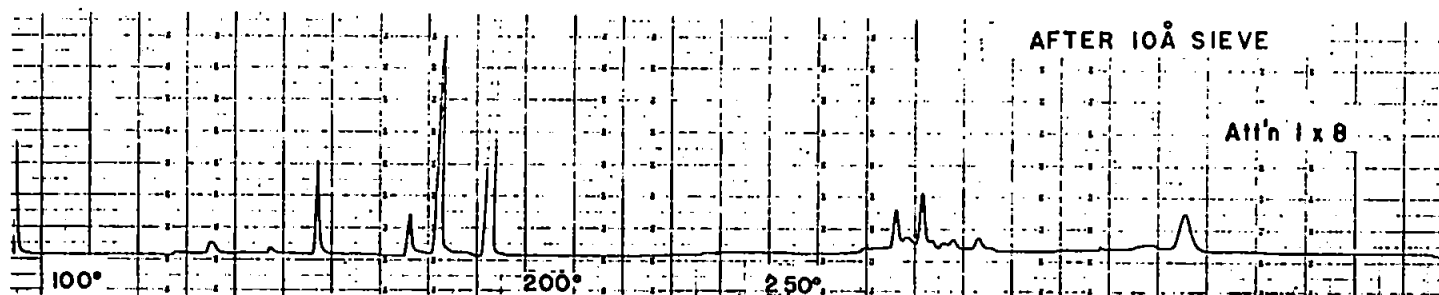
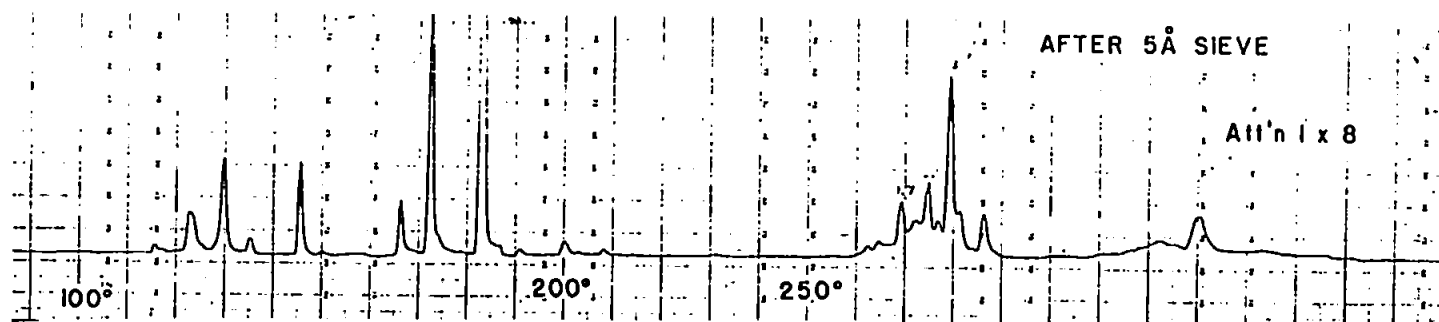
resulting gas chromatograms are given in Figures XXV-XXVII. It should be noted that in both cases (Fraction I-2 and II-1) the material recovered from the sieve was a dark brown gum, while the extract remaining outside was a colorless liquid. Material from the inside of the sieve was then resieved with 5 Å molecular sieve although it had little effect on the color. All sieving data is summarized in Table II.

At this stage it was already apparent that this sterane-triterpane mixture was quite different and indeed far more complex than the original mixture. Compare Figures XVI-XXIV with Figures II-V.

Figure XIII. Gas Chromatogram of Fraction I-2 After 5 Å Molecular Sieve

Figure XIV. Gas Chromatogram of Fraction I-2 After 10 Å Molecular Sieve

Figure XV. Gas Chromatogram of Fraction I-2 Adsorbed by 10 Å Molecular Sieve



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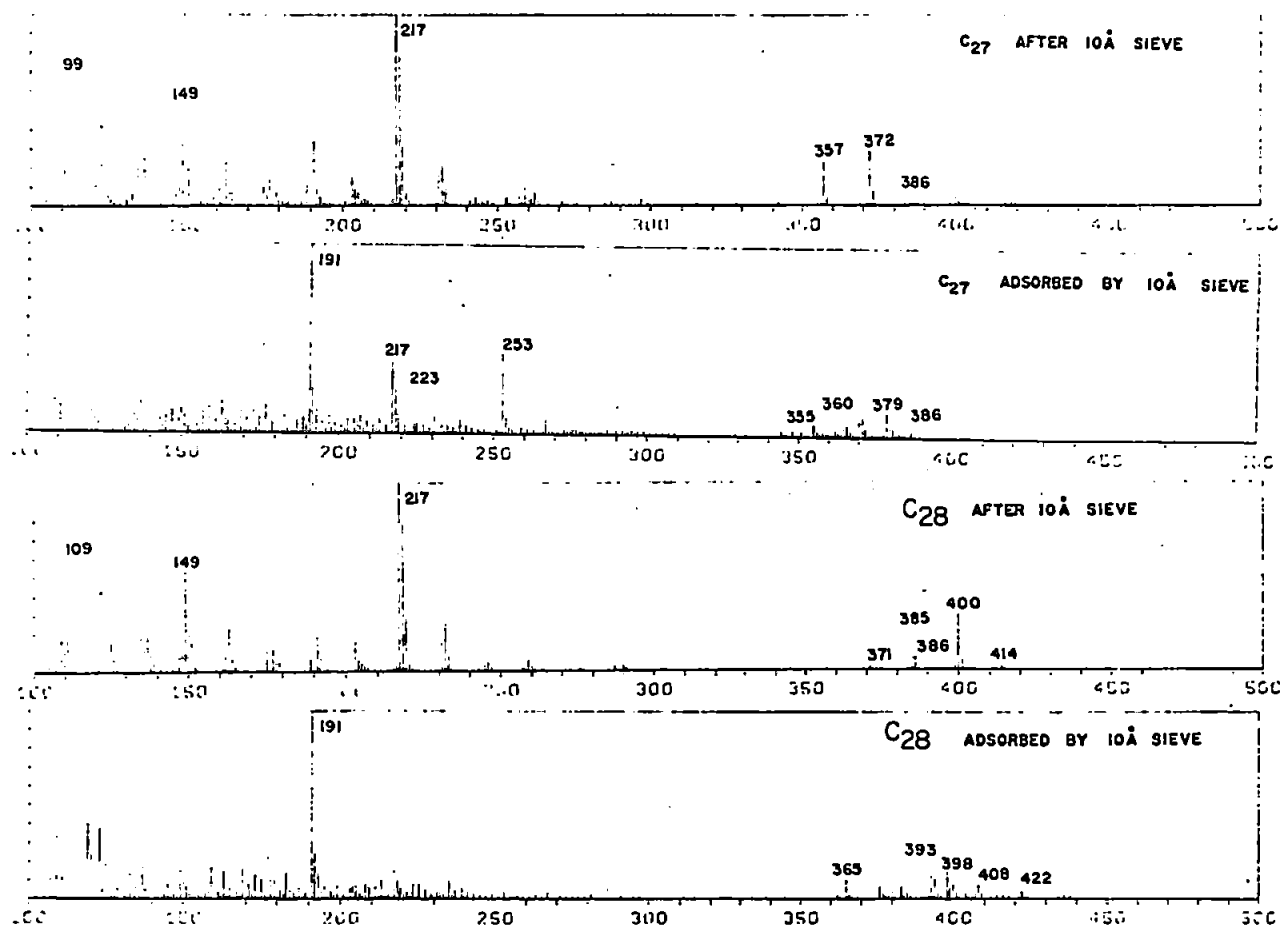
Figure XVI. Mass Spectrum of C_{27} After 10 Å Molecular Sieve

Figure XVII. Mass Spectrum of C_{27} Adsorbed by 10 Å Molecular Sieve

Figure XVII. Mass Spectrum of C_{28} After 10 Å Molecular Sieve

Figure XIX. Mass Spectrum of C_{28} Adsorbed by 10 Å Molecular Sieve

Figure XX. Mass Spectrum of C_{29} Adsorbed by 10 Å Molecular Sieve



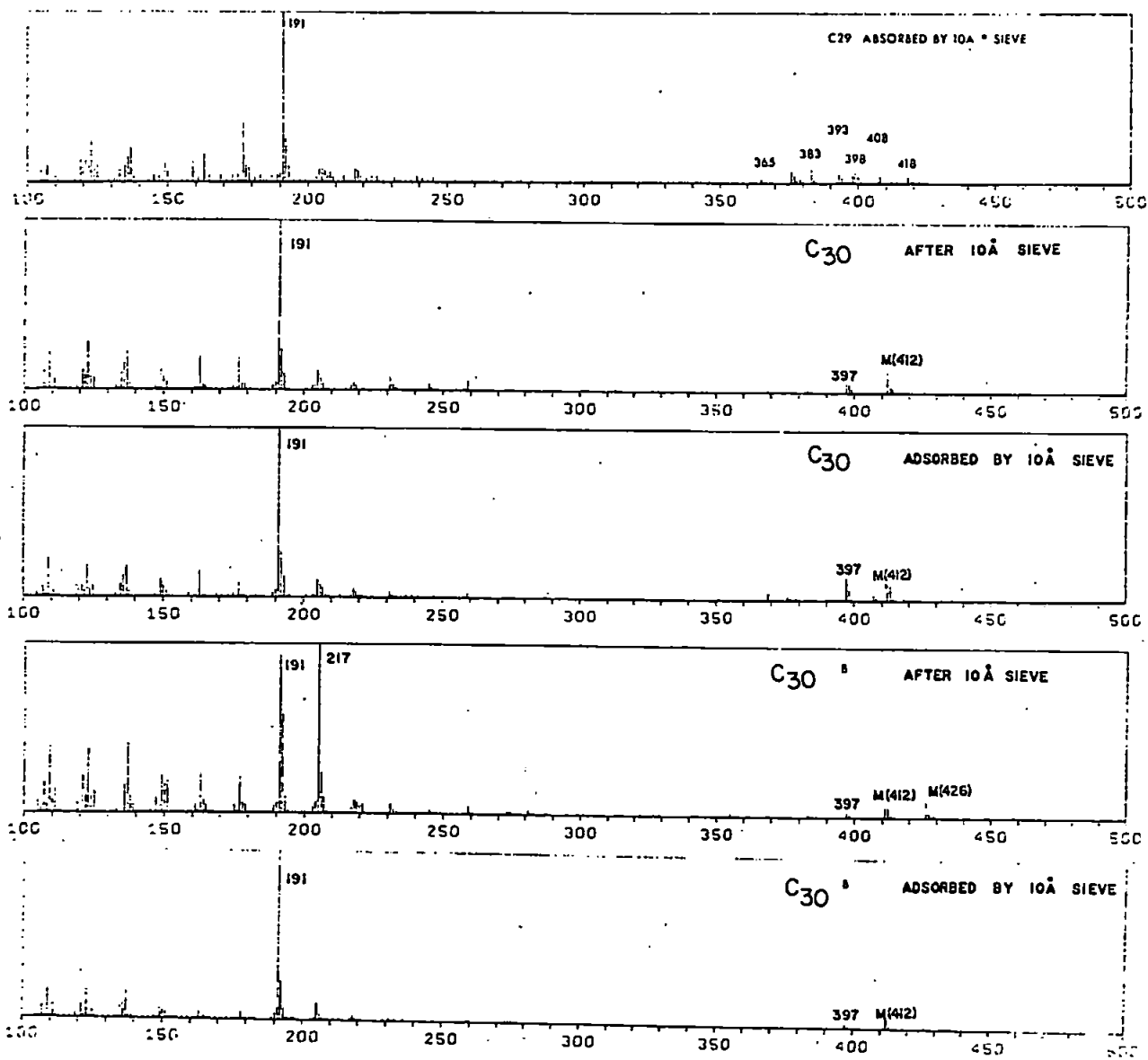
XBL 678-6105

Figure XXI. Mass Spectrum of C_{30} After 10 Å Molecular Sieve

Figure XXII. Mass Spectrum of C_{30} Adsorbed by 10 Å Molecular Sieve

Figure XXIII. Mass Spectrum of $C_{30}B$ After 10 Å Molecular Sieve

Figure XIV. Mass Spectrum of $C_{30}B$ Adsorbed by 10 Å Molecular Sieve

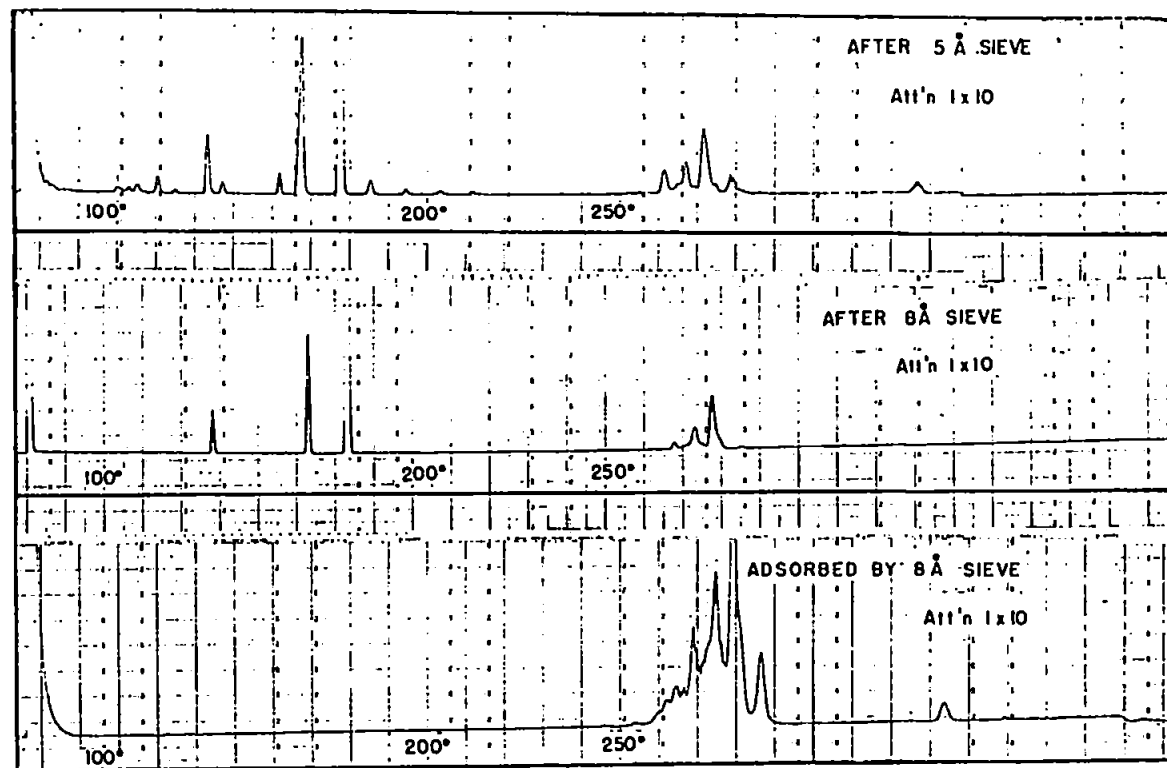


XBL 678-6103

Figure XXV. Gas Chromatogram of Fraction II-1 after 5 Å Molecular Sieve

Figure XXVI. Gas Chromatogram of Fraction II-1 after 8 Å Molecular Sieve

Figure XXVII. Gas Chromatogram of Fraction II-1 Adsorbed by 8 Å Molecular Sieve



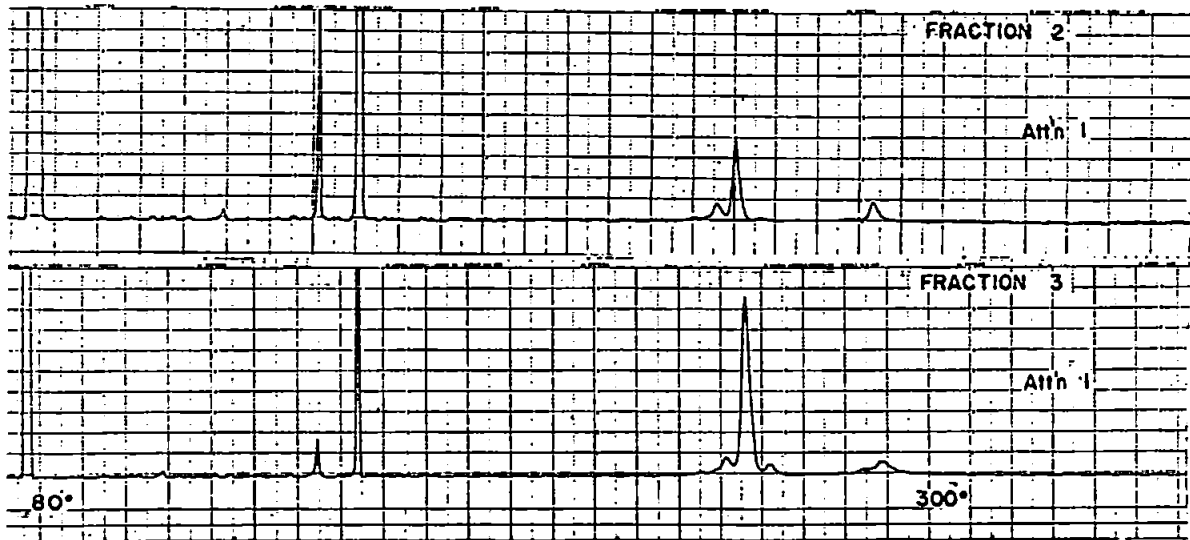
COLORADO GREEN RIVER SHALE

COLUMN II FRACTION I

XBL 672-62

Figure XXVIII. Gas Chromatogram of Fraction III-2 after 5 Å^o
Molecular Sieve

Figure XXIX. Gas Chromatogram of Fraction III-3 after 5 Å^o
Molecular Sieve



COLORADO GREEN RIVER SHALE COLUMN III AFTER 5 Å SIEVE

XBL 674-1019

Gas Chromatography

The only adequate method of separating the sterane and triterpane fractions from the complex mixture of hydrocarbons appeared to be the use of vapor phase gas chromatography. Accordingly fractions were first injected on an Areograph A-90 P-3 and programmed from 200°-300° at 10°/minute. Fractions collected were the low molecular weight branched cyclics, the C₂₇, C₂₈, C₂₉, C₃₀, C₃₀B and high molecular weight fractions (based on original sterane and triterpane collections as indicated in Figure I). This was an extremely time consuming method since the septum and injector were only capable of holding injections of 1/3 cc at a time (this 1/3 cc was necessarily half solvent to permit the injection syringe to be filled). However, resolution was good enough to permit (even with maximum load) collection of crystalline (solid) C₃₀ and C₃₀B fractions. In this manner, Fractions I-2 Non-adsorbed* and II-1 Non-adsorbed were separated using 1/4" x 10' columns coated with 3% SE-30 on 100-120 mesh areopak, and Fraction I-2 Adsorbed was separated using a 1/2" x 10' column of 5% SE-30 on 80-100 mesh areopak.

For the fractionation of large quantities, a Hewlett Packard F and M Model 775 Preparative Gas Chromatograph was used. In the beginning the manifold trap collection system was utilized and a column of 3/4" x 6' coated with 20% UCW 98 coated on Cromosorb P (60-80 mesh) followed by a 1/4" x 4' connector filled with uncoated support. The column

*"Non-adsorbed" here and in the future refers to the mixture not adsorbed by molecular sieves; "Adsorbed" refers to the mixture occluded in the sieve and recovered by HF treatment.

was operated isothermally at 290°C, each run requiring about three hours for completion. Fraction I-2 Adsorbed was separated in this manner, and triterpane C₃₀B could be obtained as solid white material.

However when it was observed that extensive decomposition was taking place in the manifold, the manifold was removed and fractions collected manually directly from the detector outlet. The 4' x 1/4" connector was discovered to be the cause for the requirement of abnormally high head pressure and subsequently removed even though Fraction C₃₀B could no longer be isolated in solid form. A special collection system was constructed consisting of glass U tube traps filled with glass wool (heated to 350°C for 24 hours and washed with distilled ACS benzene) which were connected directly to the detector outlet via a glass/metal (copper) joint, and a teflon sleeve. The peaks were then collected manually. Unfortunately resolution was quite poor and the peaks very broad. An attempt was made to use a larger column (4" x 6', 20% W 98 on cromosorb P, 10-60 mesh). However, difficulties were encountered with the head pressure as well as the extremely large sample size required. Alumina column Fractions II-1 Adsorbed, I-3, III-2, III-3, V-2 and V-3 (See Table II) were separated in this manner. Five fractions were collected, which, are labeled as C₂₇, C₂₈, C₂₉ steranes, and C₃₀ and C₃₀B triterpanes since they corresponded in retention times to the compounds isolated in the preliminary experiments. About 0.5g of each was obtained.

Recrystallization

Attempts were made to crystallize compounds of these fractions from hexane, benzene, and dichloromethane. While a white solid could usually be precipitated from hexane, no pure substances could be obtained in this way. Co-crystallization might of course, be expected for these compounds, since they all possess very similar properties.

Fluorosil Columns

Fluorosil (Sargent SC-21176, 60-100 mesh) was washed with redistilled methanol, benzene, and hexane until 200 cc of the latter evaporated to dryness gave no perceptible peaks on the gas chromatograph. All five fractions obtained from preparative GLC (C_{27} , C_{28} , C_{29} , C_{30} , $C_{30}B$) were purified on columns about 1 cm x 10 cm. In all cases the first fraction eluted was the only one which was pure white and gave the cleanest mass spectra. Only this fraction was, therefore, subjected to further purification. Typical fluorosil fractionation data are given for Fraction $C_{30}B$ in Table III.

Sublimation

Fractions C_{28} and $C_{30}B$ were further purified by sublimation under high vacuum (mercury diffusion pump). For Fraction C_{28} , 0.0953g of sublimate could be collected at 140°C. The data for the sublimation of Fraction $C_{30}B$ are given in Table IV.

TABLE III
Fluorosil Fractionation of $C_{30}B$

<u>Fraction</u>	<u>Solvent</u>	<u>Weight</u>	<u>Color</u>
1	hexane	0.44g	white
2	hexane	0.10g	off white
3	benzene	0.01g	pale yellow
4	benzene	0.01g	pale yellow
5	methanol	0.01g	pale brown
6	methanol	0.01g	pale brown

TABLE IV
Sublimation of Fraction $C_{30}B$

<u>Fraction</u>	<u>Temperature</u>	<u>Weight</u>	<u>Description</u>
1	20°C-100°C	0.007g	yellow syrup
2	100°C-120°C	0.088g	pale yellow solid
3	100°C-120°C	0.083g	pale yellow solid
4	120°C-160°C	0.035g	pale yellow solid
5	Residue	0.054g	yellow solid

TABLE V
Silica Fractionation of $C_{30}B$

<u>Fraction</u>	<u>Solvent</u>	<u>Weight</u>	<u>Description</u>
1	Hexane	0.030g	white crystalline solid
2	Benzene	0.050g	white solid
3	10% DiClmethane	0.082g	pale yellow solid
4	DiClmethane	0.018g	light yellow solid

Fraction C₂₈

A GLC of the sublimate of Fraction C₂₈ (0.095g) exhibited two major peaks. The mass spectra of both indicated the presence of a C₂₈ sterane and a C₃₀ triterpane. No further separation of these compounds was attempted. The total sublimation fraction of C₂₈ exhibited an optical activity of $[\alpha]_D = 20 \pm 1$.

Fraction C₃₀^B

Sublimation Fractions 2 and 3 (See Table IV) were combined and chromatographed on a silica gel column (10 cm x 1 cm) after first washing with distilled methanol, benzene, and hexane. Data for this fractionation are given in Table V. This first silica fraction was further purified by gas chromatography (1/4" x 10' column of 3% SE-30 on aeropak 30, 80-100 mesh). Fractions were collected the mass spectra of which are exhibited in Figures XXX and XXXIV. The spectra in Figures XXX-XXXII are from three distinct GLC peaks and are apparently three different triterpanes of molecular weight 412. Figure XXXIII is a later scan of the same fraction given in Figure XXX and reveals a very interesting component of molecular weight 426 which is of particular interest since it could represent either a C₃₁ triterpane or a C₃₀ keto triterpane. Figure XXXIV gives the mass spectrum of a very minor component with a base peak at m/e 272 and molecular ion at m/e 412. The major GLC fraction gave solid white material with an apparent melting point of 285-290°. The optical rotation of Sublimation 2 (See Table IV) was measured and a value of $[\alpha]_D = +16.6 \pm .1$ was obtained. For comparison it may be noted that cholestane has a rotation of $[\alpha]_D = +26$ and lupane $[\alpha]_D = -7.5$.

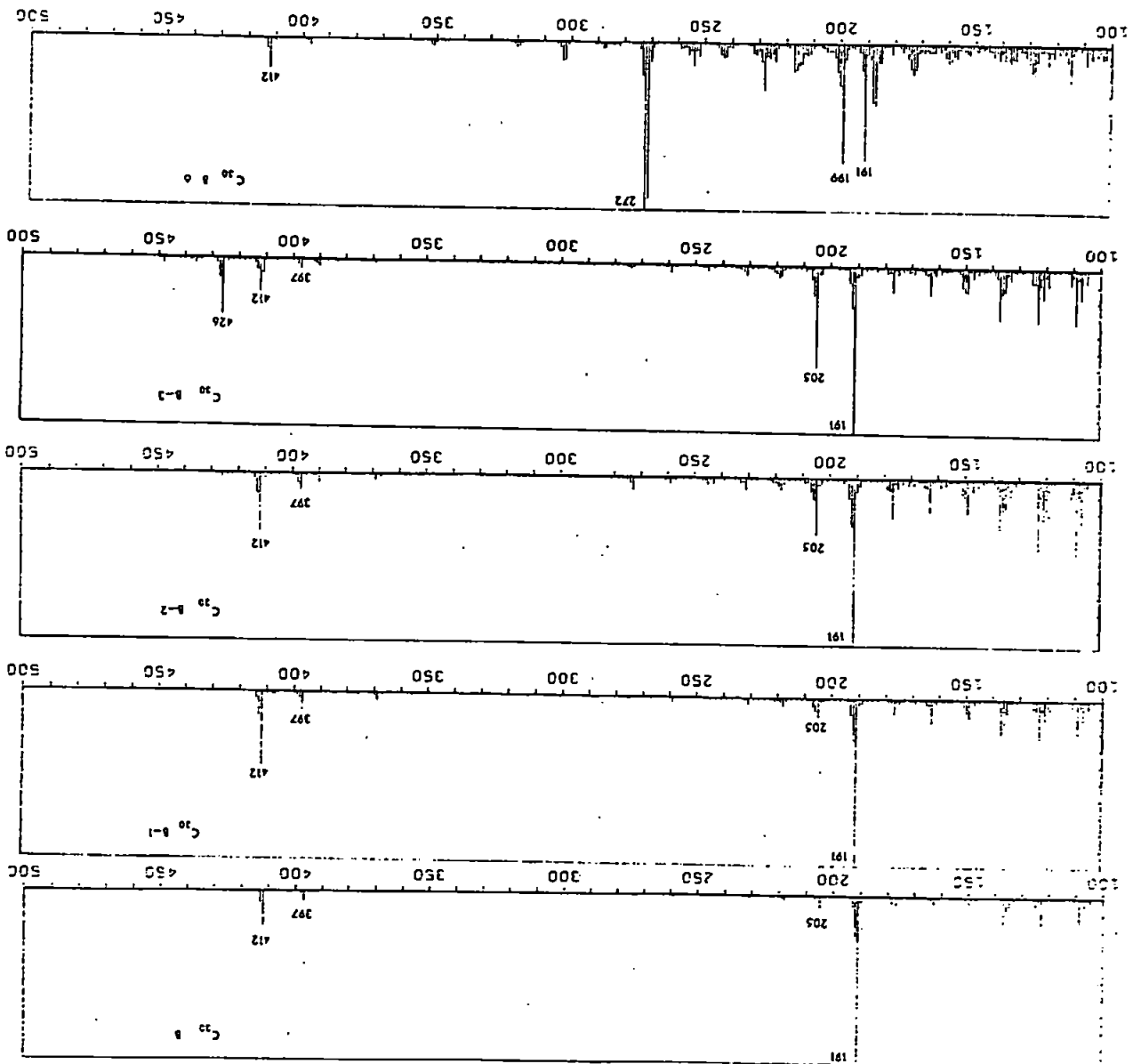
Figure XXX. Mass Spectrum of $C_{30}B$

Figure XXXI. Mass Spectrum of $C_{30}B$ (1)

Figure XXXII. Mass Spectrum of $C_{30}B$ (2)

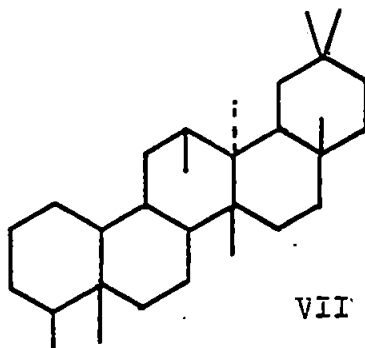
Figure XXXIII. Mass Spectrum of $C_{30}B$ (3)

Figure XXXIV. Mass Spectrum of $C_{30}B$ (6)



Polycyclic Hydrocarbons from the Soudan

The isolation* of a fraction from the 2.7 billion year old Soudan Shale whose mass spectrum (Figure XXXV) bore strong resemblance to those of steranes and triterpanes (in particular the ions at m/e 149, 217, 218, 257, 272, 286 and 500)¹⁹ aroused considerable excitement and resulted in an attempt to purify the fraction further. The fraction (collected from a 3% SE-30, 1/4"x 10' column) was rechromatographed successively on columns of 2.5% 7-ring-meta-polyphenyl ether (1/4"x 25') and 5% XE-60 cyanoethymethyl-silicone (1/4" x 10') isothermally at 250°C. Figures XXXVI, XXXVII XXXIX -XLI give the mass spectra of some of the fractions thus isolated. Figure XXXVII shows a fairly clean pattern, exhibiting essentially one molecular ion at m/e 372. The intensity of the peak at m/e 218 deserves comment since a sterane structure would be expected to have a more intense peak at m/e 217; it would seem to require a non-steroidal skeleton for this compound. The tetracyclic hydrocarbons (molecular weights 372, 386, 400) of Figure XXXVIII display the same feature. It is possible that they bear some structural relationship to Friedelane, Structure VII, whose mass



*By Ted Belsky.

spectrum is displayed in Figure XXXIX. (Note the intensity of m/e 218 as compared with m/e 217). The tetracyclic compounds of Figures XL and XLI (where m/e 217 is more intense than m/e 218) lack dominant ions at m/e 217. In the absence of adequate standards for comparison it is difficult to draw very definite conclusions about the compounds.

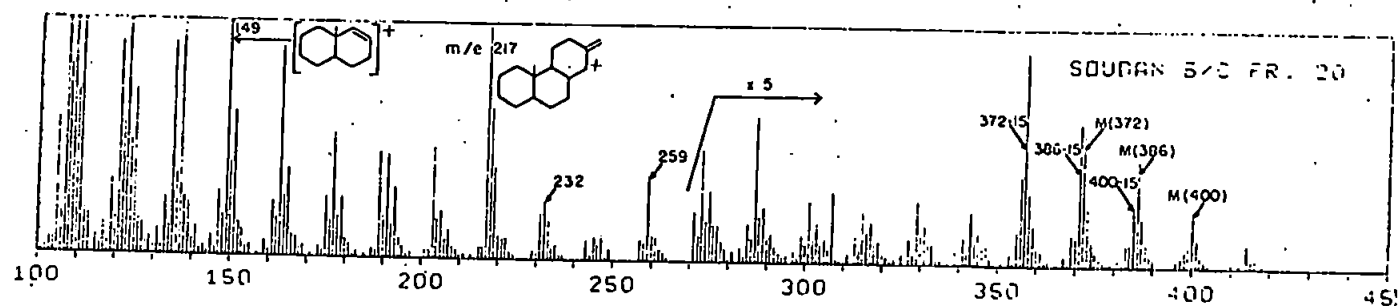


Figure XXXV Mass Spectrum of Soudan Crude Tetracyclic Hydrocarbons

- Figure XXXVI. Mass Spectrum of Soudan C_{27} Tetracyclic Hydrocarbon (MS 2985)
- Figure XXXVII. Mass Spectrum of Soudan C_{27} - C_{29} Tetracyclic Hydrocarbons (MS 2998)
- Figure XXXVIII. Mass Spectrum of Friedelane
- Figure XXXI. Mass Spectrum of C_{27} - C_{29} Tetracyclic Hydrocarbons (MS 2224)
- Figure XL. Mass Spectrum of C_{27} - C_{29} Tetracyclic Hydrocarbons (MS 2230)
- Figure XLI. Mass Spectrum of C_{29} Tetraunsaturated Alkene (MS 2223)

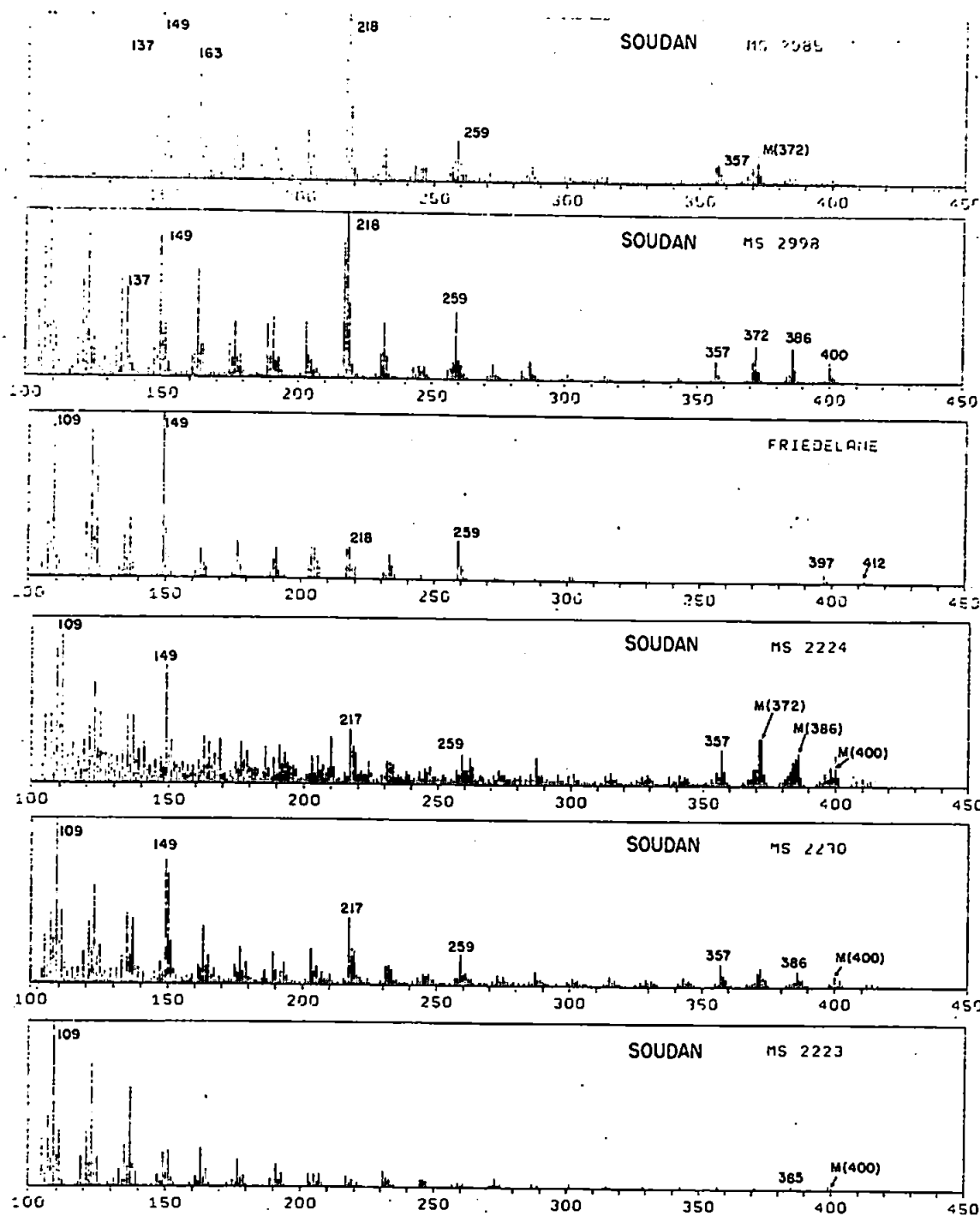
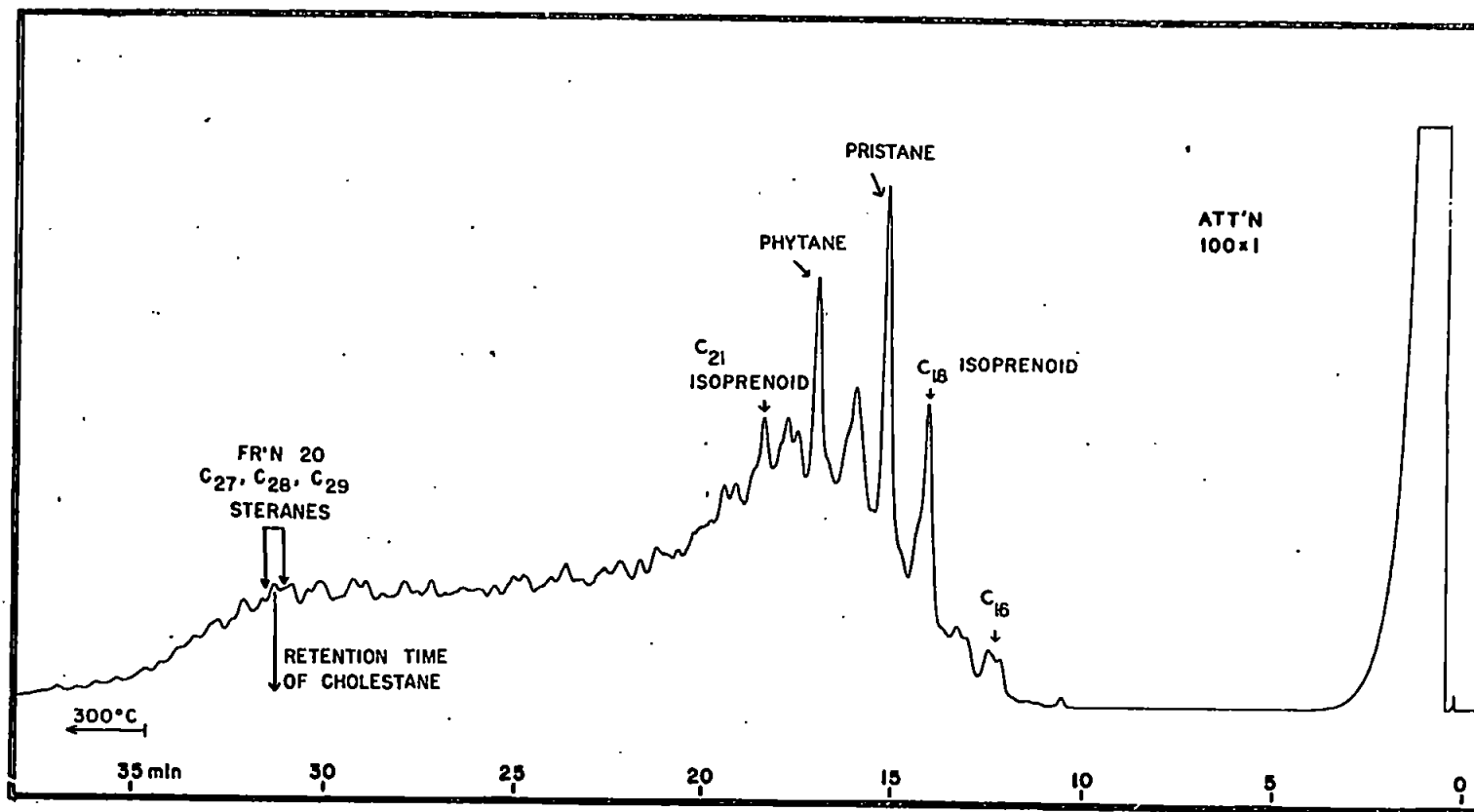


Figure XLII. Gas Chromatograph of Soudan Extract.



SUDAN SHALE BRANCHED-CYCLIC ALKANES, 2.5×10^9 YRS.

MUB-6664

TABLE III

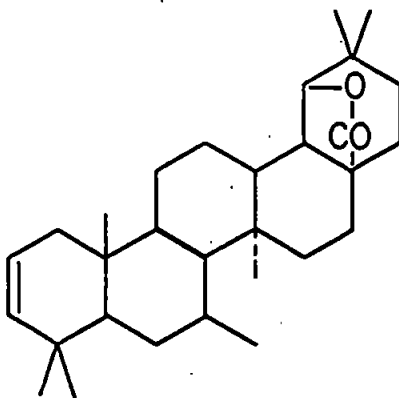
Off Scale Mass Spectral Intensities

M/e	C-27 Sterane II	C-28 Sterane III	C-29 Sterane IV	Soudan B/C Frac. 20 XXXV	Soudan MS 2985 XXVI	Soudan MS 2998 XXXVII
106		116				
107				120	120	
109	off	187	120	off	272	190
111				130		
123	off	127		off	143	113
149		103		131	125	

Significance of Steranes, Triterpanes, and Optical Activity

Steranes and triterpanes are one of the most complex classes of compounds isolated from geological sources. They seem to occur, however, in a relatively wide range of geological sediments. Indications of steranes have been reported in petroleum^{30,31} and in recent sediments³² on the basis of the mass spectra of complex hydrocarbon mixtures. Meinschein has indicated the presence of a C₂₇ sterane in the Nonesuch Shale on the basis of large peaks at 372, 218, 217, and 149 in the mass spectrum of a carbon tetrachloride eluant fraction from an alumina column.³³

Barton and Carruthers³⁴ have reported the isolation and identification of oxyallobetul-2-ene, a derivative of a plant terpenoid, from high boiling petroleum distillates (it sublimes at 345° and has an $[\alpha]_D = +75^\circ$ where the authentic sample sublimes at 345° and has an $[\alpha]_D = +79^\circ$).



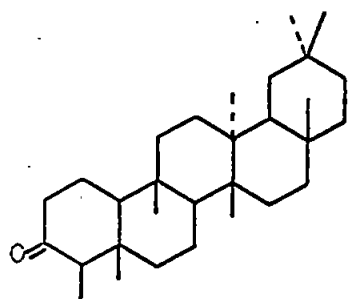
VIII

Schorm and co-workers have reported a series of triterpanes in Bohemian brown coal (the age of which is estimated to be tens

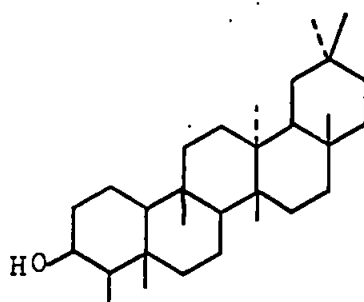
TABLE III

Compound Isolated from Bohemian Brown
Coal by Scherm

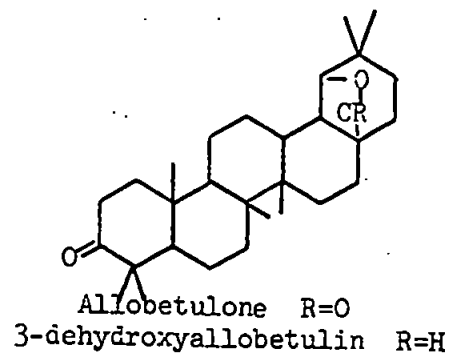
Compound	Melting Point °C	$[\alpha]_D^{20}$ in CHCl_3
Friedelin	253-255°	-21.6°
Friedelan-3 β -ol	285-286°	20.7°
<u>Allo</u> Betulone	228-229°	84.4°
Friedelan-3 α -ol	310°	24.2°
3-Dehydroxy <u>allo</u> betulin	338°	91.3°
<u>Allo</u> Betulin	266°	50.7°
Oxy <u>allo</u> Betulin	347°	46°
Betulin	254-255°	26.4°
α -Apo <u>allo</u> Betulin	218-222°	65.5°
Δ^2 - <u>Allo</u> Betulin	250-250.5°	72.6°
Apooxy <u>allo</u> Betulin	289-291°	70.3°
Δ^2 -oxy <u>allo</u> Betulene	369°	75.2°
IX	261°	-
X	250°	-
1,2,3,4,4a,5,6,14b-oct. a. hydro- 2,2,4, 4a -tetramethylpicene	233-235°	-34.2°
1,2,3,4-Tetrahydro-2,2,9- trimethylpicene	251-252	0
2,9-Dimethylpicene	230-231.5°	-
1,2,3,4-Tetrahydro-1,2,9- trimethylpicene		50°



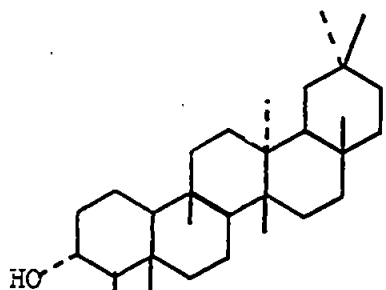
Friedelin



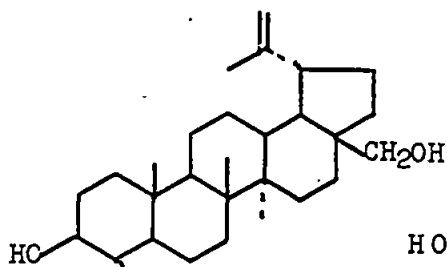
Friedelan-3-ol



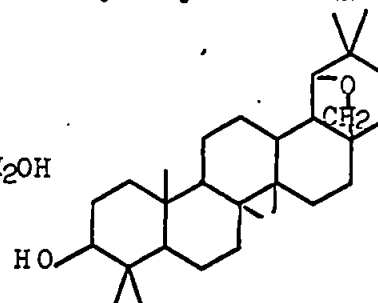
3-dehydroxyallobetulin R=H



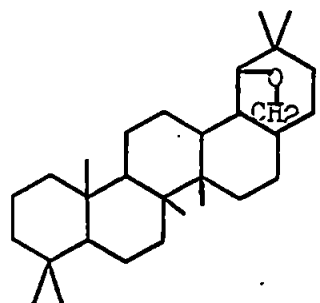
Friedelan-3-ol



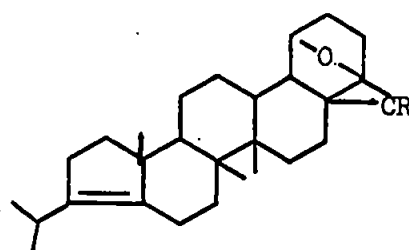
Betulin



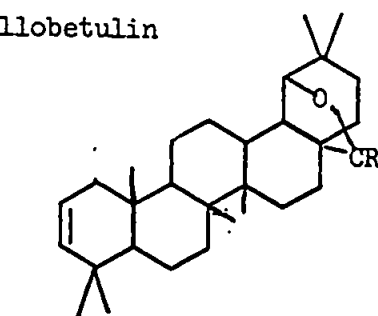
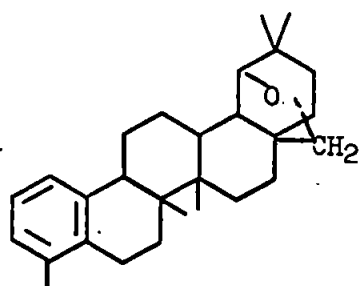
Allobetulin



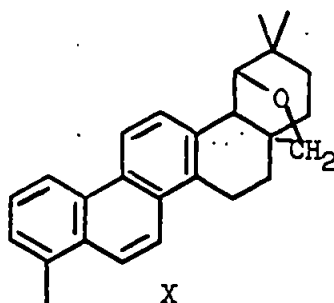
Oxyallobetulin



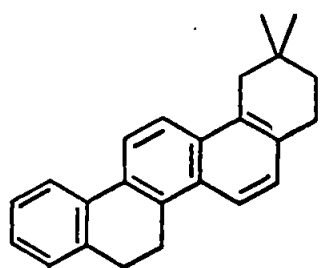
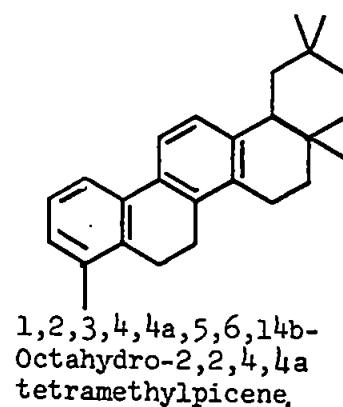
Apooxyallobetulin R=O


 Δ^2 Allobetulin R=H₂
 Δ^2 oxyallobetulin R=O


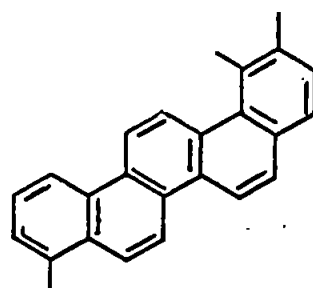
IX



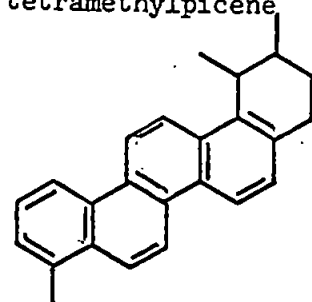
X



1,2,3,4-tetrahydro-2,2,9-trimethylpicene

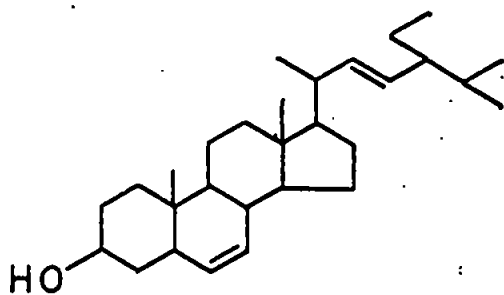


1,2,9-trimethylpicene

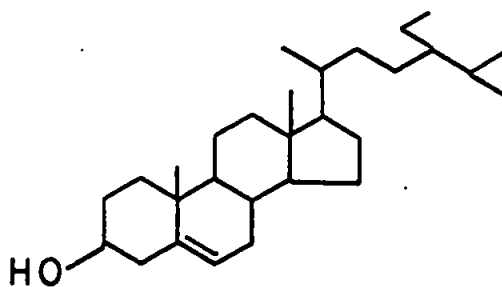
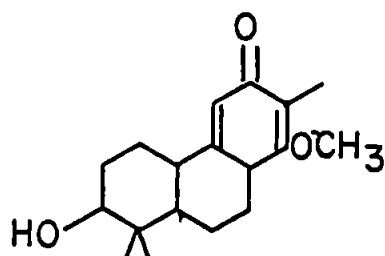
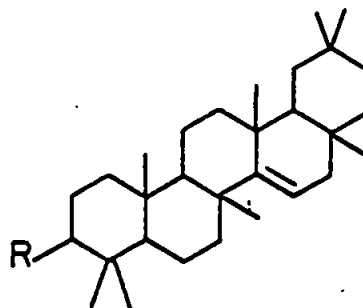


1,2,3,4-tetrahydro-2,2,9-trimethylpicene

of millions of years based on geological strata),³⁵⁻⁴⁴ some of which are summarized in Table III. In this connection it is interesting to note the earlier work on peat by McLean, Rettie, and Spring,⁴⁶ who attempted to ascertain the chemical changes associated with humification. They have identified friedelan-3 β -ol, (mp 280-383°, $[\alpha]_D = -21.9^\circ$) as well as a sterol (mp 135-136°, $[\alpha]_D = -15.8^\circ$) believed to be a mixture of β -sitosterol and stigmastanol. Ives and O'Neal had even earlier identified a mixture (mp 135-137°, $[\alpha]_D = -12^\circ - -24^\circ$ with average -18°) of β -sitostanol and β -sitosterol as well as α -amyrin, taraxerol, and taraxerone from peat moss (Sphagnum).⁴⁶⁻⁴⁷



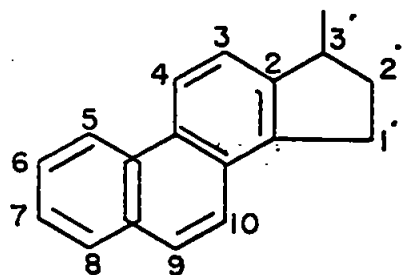
Stigmasterol

 β -Sitosterol α -Amyrin

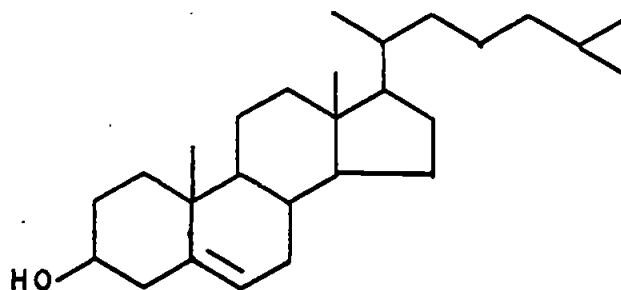
Taraxerol R = OH

Taraxerone R = O

Mair⁴⁸ has noted the similarity between the skeletal structures of hydrocarbons isolated from petroleum and those of steroids and triterpenoids from plants. In particular he cites the relationship of 3⁶-methyl-1,2-cyclopentanophenanthrene, XI, (isolated from petroleum along with phenanthrene, 1-methylphenanthrene, 2-methylphenanthrene, 3-methylphenanthrene, 9-methylphenanthrene, 1,8-dimethylphenanthrene, and trimethylphenanthrene) to cholesterol, XII, (which yields 3⁶-methyl-1,2-cyclopentanophenanthrene among other cyclopentanophenanthrenes upon dehydrogenation with selenium).⁴⁹

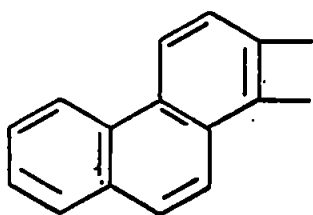


XI

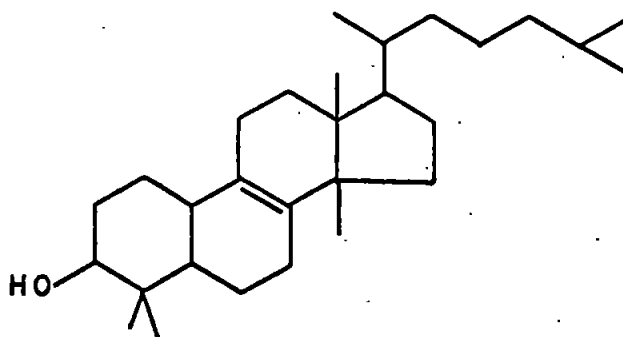


XII

Similarly dehydrogenation of lanosterol, XIV, yields 1,2,8-trimethylphenanthrene, XIII, which has been isolated from petroleum by Carruthers and Douglas.⁵⁰

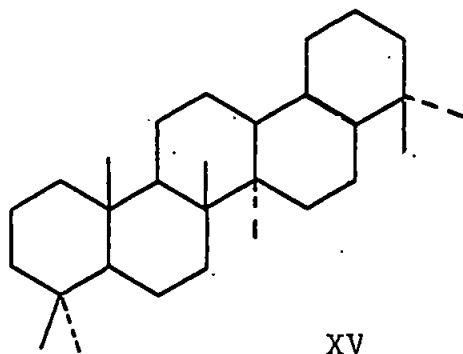


XIII

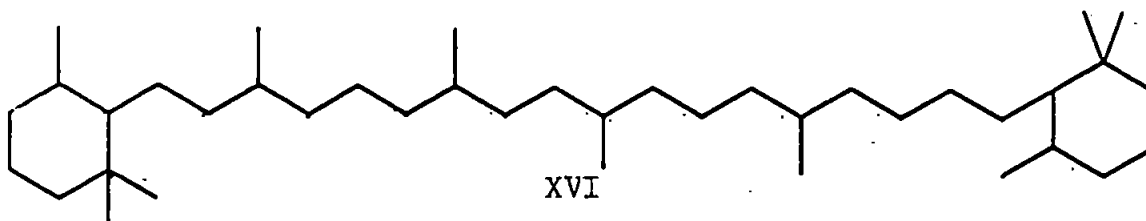


XIV

After our initial report²² of the C₂₇, C₂₈, C₂₉ steranes and C₃₀ triterpanes in the Colorado Green River Shale (which are now known to be optically active if not in the desired state of optical purity), Hills and Whitehead found several unidentified pentacyclic triterpanes (two of molecular weight 412 and one of molecular weight 398) from an optically active distillate from a Nigerian crude oil.⁵¹ Recently a hydrocarbon of melting point 285-286° (uncorrected) and optical rotation $[\alpha]_D = +31.9^\circ \pm 0.4^\circ$ isolated by Cummings, Anders, and Robinson⁷ from the Colorado Green River Shale was identified by Hills and Whitehead²⁵ as gammacerane, XV, of $[\alpha]_D^{21} = 29.4^\circ$



$\pm 0.3^\circ$ and melting point 301°.⁵⁴ Much more recently Murphy, McCormick and Eglinton²³ reported the identification of perhydro- β -carotene, XVI, from the Colorado Green River Shale.



They also verify the assignment of the three steranes (C₂₇, C₂₈, and C₂₉) as cholestane, ergostane, and sitostane using

a combination of micro-infrared spectroscopy and a combined gas chromatograph-mass spectrometer.

In most of these investigations mass spectrometry has provided important structural information. Indeed with the quantities of material isolated, mass spectrometry was often the only method which permitted the assignment of carbon skeletons. It should be stressed that mass spectrometry alone is insufficient for the unambiguous structural determination of compounds of this complexity unless direct comparison with known substances is possible.

The complexity of these steranes and triterpanes suggest biological origin and as such would represent definitive indications of life processes. Nevertheless, such molecules are not unambiguous particularly when derived from very old sediments. The non-enzymatic cyclizations of polyisoprenoids to give tetra or pentacyclic compounds are known.⁵² Of course such abiological syntheses are expected to be much less selective than biological syntheses.

Optical activity has been considered to be an indication of life processes, the ordered structures (a result of optical purity) of life, then, fit into the thermodynamics of irreversible processes and open steady state systems where order or information is equivalent to negative entropy. Indeed, optical asymmetry or optical purity of metabolites is absolutely essential to life. T. L. V. Ulbricht,⁵³ in an excellent review, has noted that unwanted isomers are eliminated by excretion and optically specific destruction by oxidases, that some organisms

can convert one optically active amino acid into the other, and that in some cases the presence of both isomers is essential for playing two different and distinct chemical roles. Since there is a finite probability that a D-amino acid will be built into an enzyme (a very small probability because normally none are present) and since enzymes have high rate constants but do not effect equilibrium (racemization is thermodynamically irreversible and therefore bound to occur eventually) it might be possible to find in very ancient sediments differences in optical purity reflecting enzymes less efficient than those of today, which have necessarily evolved to a high degree of efficiency. (Of course the effects of diagenesis would be difficult to take into account.)

In any case, optical activity is an intricate part of life and as such has been taken as an indication of life past or present. The observation of optical activity in a meteorite, for example, led to the immediate assumption of contamination.⁵⁴ Other experiments have pointed out some of the difficulties involved in the measurement of optical activity in sedimentary components.⁵⁵⁻⁵⁶ Optically active fractions in petroleum have been known for a long time⁵⁷⁻⁶⁰ and are quite well characterized.⁶¹ A systematic decrease in the optical activity of sediments with age has been reported in the literature,⁶² although the experimental mode of calculation is both ambiguous and questionable.

The origin of optical activity is quite unknown. One of the older and more popular evolutionary theories postulates that at some stage of life evolution, one optical macromolecule

gained a selective advantage over the other. Quite recently, a number of theories have been advanced in an attempt to explain optical activity as a natural consequence inherent in the chemical reactions composing life. It has been postulated, for example, that light traveling through the earth's atmosphere could be converted via sea refraction and the earth's magnetic field to circularly polarized light, which has been shown to effect one optical isomer more strongly than the other.⁶³ With regard to the theory that graphite may have given rise to the organic matter in sediments (mentioned in the Introduction) it has been noted that the spiral growth patterns found in natural graphite⁶⁴ provide a possible explanation for their optical activity.⁶⁵ One of the most intriguing ideas was conceived with the discovery of the non-conservation of parity in weak interactions (electrons from radioactive β -decay and from meson decay in cosmic rays are predominantly left-handed) and basically suggests that optical asymmetry is a reflection of the asymmetry of this part of the universe.⁶⁶⁻⁶⁸

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Part III

Acids from the Colorado Green River Shale

Acids and Bases from Geological Sediments

The hydrocarbons of a sediment are usually assumed to have been formed through chemical change by the process of both biological (bacterial) and abiological (due to geological pressure, heat, and time exposure) diagenesis. The minute quantity of reactive organic material possessing reactive heteroatoms such as oxygen in the case of acids and nitrogen in the case of bases is of geochemical interest particularly since it might be expected to be more closely related to the original organic debris deposited with the sediment (although bacterial oxidation must also be kept in mind). As in the case of hydrocarbons it would be of particular value if the acids or bases from a sediment bore a predictable relationship to the acidic and basic constituents of the living organisms from which they are presumably derived. Comparison of the acids isolated from geological sources with the known distribution of acids present in present-day (primitive) organisms might be expected to lead to a better comprehension of their biological origin and the important diagenetic factors. An extensive review of the fatty acids isolated from geological sediments has been given by Ramsay.¹ A considerable number of basic compounds have been isolated from petroleum, but no individual compounds have been identified in sediments.^{2,3,4,5}

The Colorado Green River Shale is thought to be the end product of aquatic organisms such as algae and protozoa sedimented in a series of fresh water lakes. Several reports

have dealt with the fatty acids obtained from this sediment. Abelson and Parker⁶ reported the presence of normal fatty acids in the range C_{12} - C_{13} , Lawlor and Robinson⁷ found normal C_{10} - C_{34} acids, and Leo and Parker⁸ reported the occurrence of iso and anteiso acids as well as normal acids (C_{12} - C_{18}). Eglington and collaborators⁹ have reported the presence of isoprenoid fatty acids from the shale ranging from C_{14} - C_{21} (including both phytanic and nor-phytanic) with the exception of C_{18} and noted that they parallel the distribution of corresponding hydrocarbons. The investigation described here was intended as a complete mass spectral survey of the types of acids present in the extracts of the Green River Shale. Individual acids were isolated and identified using mass spectrometry.¹⁰⁻¹³

Acid and Base Extraction of Shale Extracts

Before passing the extracts down Columns I and III (26g) and (29g), -respectively- (refer to Part II), they were each dissolved in 500 ml hexane and extracted three times with 100 ml 1 N sodium hydroxide solution and once with 50 ml saturated sodium chloride solution. These aqueous solutions were combined and back-extracted three times with 100 ml of hexane (this hexane solution being returned to the neutral hexane solution), filtered and acidified to a pH of 1. This solution was extracted three times with 50 cc of hexane and three times with 50 cc of dichloromethane. These hexane soluble acids and dichloromethane soluble acids were dried over magnesium sulfate and evaporated. The results are given in Table I.

The filtered neutral solution was extracted twice with 100 ml 1 N sulfuric acid and once with saturated sodium chloride solution. The combined extracts were then back-extracted three times with 50 cc of hexane (which was added to the neutral hexane solution). The aqueous solution was brought to a pH of 10 and extracted three times with 50 ml of hexane and three times with 50 ml of dichloromethane, which was dried with magnesium sulfate and the solvent evaporated. Table I gives the results of this extraction.

Half of the hexane soluble acids were dissolved in 10 ml of hexane and extracted three times with 3 ml of sodium bicarbonate (saturated) and 3 ml of distilled water. The material which remained in the hexane solution constitutes

the phenol fraction. The bicarbonate solution was then acidified to a pH of 2 and re-extracted three times with 25 ml of hexane. This hexane fraction constitutes the acid fraction.

The acid fraction was treated with methanol/boron-trifluoride reagent and refluxed for an hour. The solution was then concentrated, 3 ml of water added and extracted three times with 5 ml of hexane. The hexane extracts were combined and washed with dilute base and water, concentrated, and the hexane evaporated. Figure I shows a gas chromatogram of the esters using a 1/16" x 10' column of 3% SE-30 on 80-100 mesh aeropack and a flow rate of 30 cc/min. programmed at 2°/min. from 50° to 280° C. Esters were collected from a 5% SE-30 on 80-100 mesh aeropack, 10' x 1/4" column with a flow rate of 50 cc/min. programmed from 50° to 280° in two separate runs. The first was programmed at 2°/min. and fractions collected were not purified but immediately analyzed by mass spectrometry. The second was programmed at 4°/min. and fractions collected were further purified by injection onto a 3% HIEPF 8 BP on 80/100 gas chrom Q (Applied Science) 6' x 1/4" column programmed at 6°/min. with a flow rate of 50 ml/min. A third collection, similar in conditions to the first, was made to collect fractions for high resolution mass spectrometric analysis.

Figure I. Gas Chromatogram of Colorado Green River Shale Esters

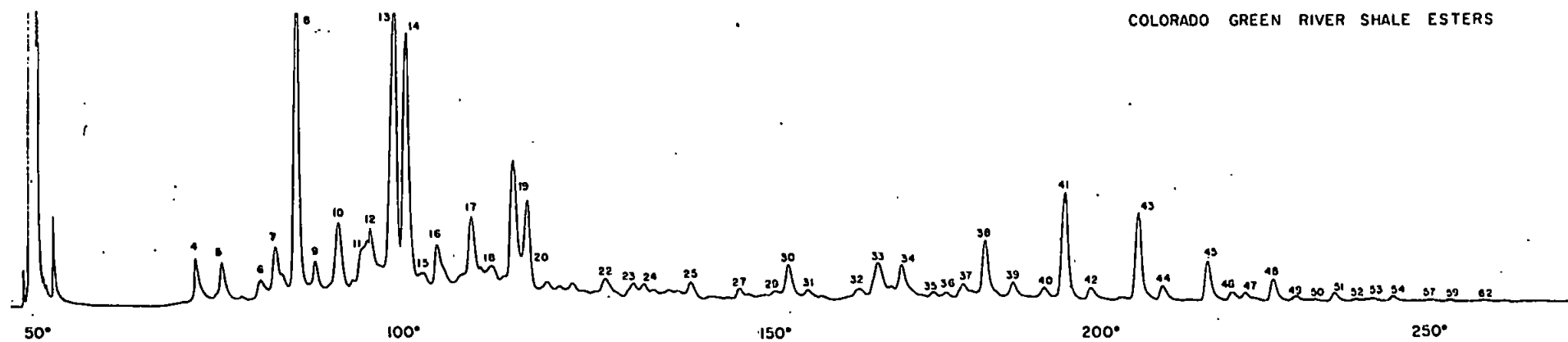


TABLE I

Acid and Base Extraction Data

Column	Neutral Components	Acids		Bases	
		Hexane	Dichlormethane	Hexane	Dichlormethane
I	31g	.1837g	.0965g	.1076g	.0167g
II	36g	.1005g	.1094g	.0979g	.0272g
Washings	6.75g		.04g		.007g

High Resolution Mass Spectrometric Instrumentation

A Consolidated Electrodynamics Corporation Model 21-110 (employing Mattauch-Herzog geometry) high resolution mass spectrometer, capable of distinction between three millimass units, was used to determine the empirical compositions of all fragments formed upon electron impact. A photoplate was used to collect all ions simultaneously with subsequent data transmission of the digitized line positions and plate blackening (in percent transmission) by a Jarrell-Ash model 23-500, high precision microphotometer. These data were recorded by magnetic tape and fed into a 7090 computer together with the calibration masses of perflurokerosene. The output consists of the accurate mass together with the empirical composition of all peaks in the spectrum (found by comparison with a self generated mass table). Further computer programs permit automated graphical presentation of this data.¹⁴

Low resolution mass spectra of individual esters were also run on this instrument.* All mass spectral intensities which are off scale due to the choice of the reference peak are tabulated in Table II.

* By Professor Heinrich Schnoes. The spectra were measured by Garry Zellweger and Gene Tobias.

TABLE II

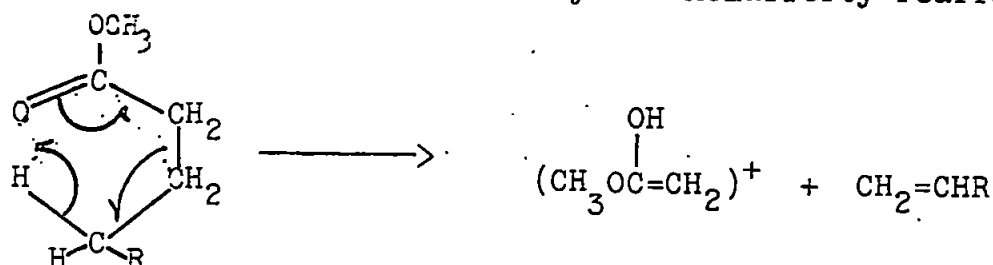
Off Scale Mass Spectral Intensities

M/E	XVIII	XXIII	XXIX	XXX	XXXIII	XXXV	LII	LXXX	LXXXII	LXXXVIII	LXXXIX
41		off	100	200	110	115			102		
43							202	off		240	198
44	750										
55						102			off		
69									off		
74									off		
84									103		
87									103		
98									off		

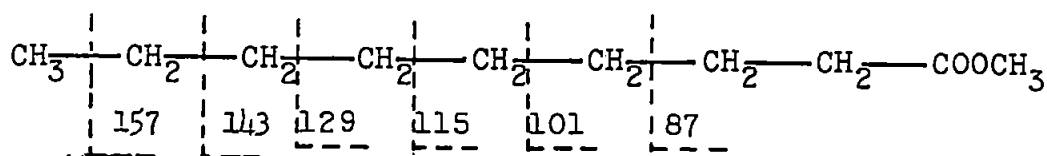
Normal Esters

Normal esters have been extensively studied and their fragmentation patterns permit unambiguous identification. Figures II-VII are the C_7 - C_{11} normal methyl esters isolated from the Colorado Green River Shale together with that of authentic methyl laurate.

The major ion is formed by the McLafferty rearrangement



to give a peak at m/e 74. An important series arises by simple carbon carbon bond cleavage as illustrated in I, where the first member of this series is 87, followed by 101, 115, 129, etc. The molecular ion is also a reasonably



I

prominent ion. Fragments corresponding to M-29 (in Figures II - VII these are 129, 143, 157, and 171, respectively) and M-43 (115, 129, 143, and 157) ions are due to expulsion of the α and β methylene groups and expulsion of the α , β and γ methylene groups plus a hydrogen. The loss of 31 mass units (127, 141, 155, and 169) is, of course, due to loss of the methoxy radical (OCH_3).

It is interesting to note that the C_6 - C_9 normal acids have also been isolated from petroleum.¹⁵ In biological systems palmitic acid (normal C_{16}) is usually the predominant species (15-50% of total acid content) among the saturated acids, and is almost never absent. Accompanying it is often oleic acid. Ramsay has categorized the saturated normal acids as: n-even found in the range of C_2 - C_{26} with n- C_{16} predominating in natural fats and in the range C_{14} - C_{34} with n- C_{28} and n- C_{30} predominating in insect and plant wax; n-odd in the range C_3 - C_{25} in the fats of ruminants.¹

Figure II. Mass Spectrum of Ester 4

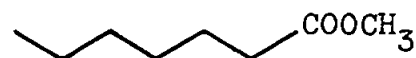


Figure III. Mass Spectrum of Ester 8 Fraction 2

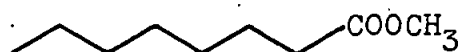


Figure IV. Mass Spectrum of Ester 14 Fraction 2

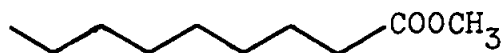


Figure V. Mass Spectrum of Ester 19 Fraction 1



Figure VI. Mass Spectrum of Ester 22 Fraction 1

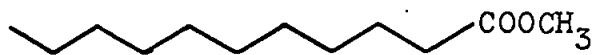
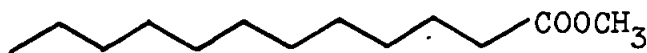
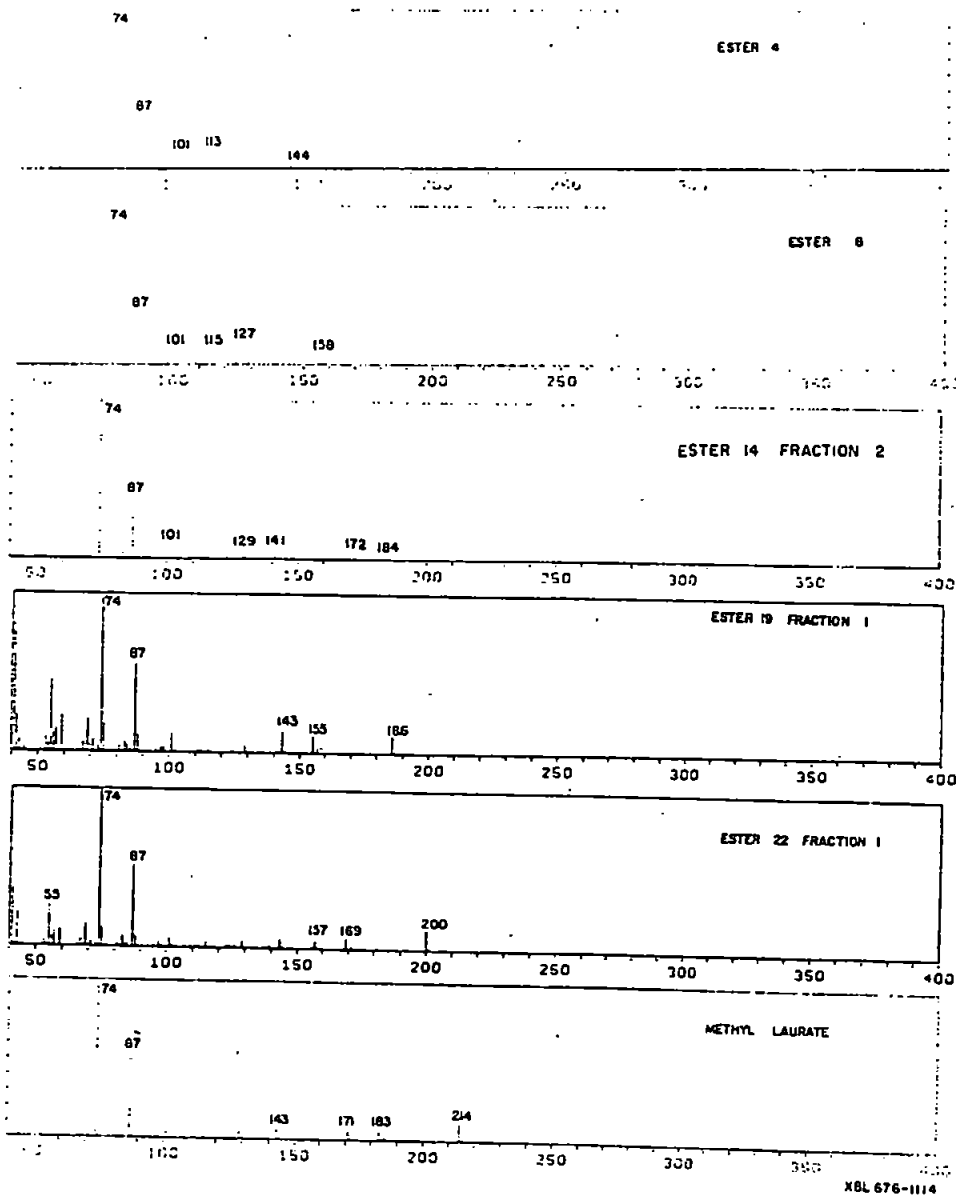


Figure VII. Mass Spectrum of Methyl Laurate

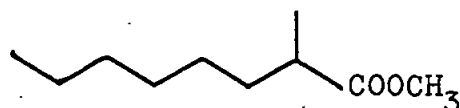


GREEN RIVER SHALE NORMAL ESTERS



Branched Esters

The mass spectral pattern of branched esters is quite sensitive to the site of branching, and structures are usually determined by the fragmentation pattern. For example, the mass spectrum of Ester 10 Fraction 1 (Figure VIII) establishes the structure as methyl 2-methyl octanoate (II). The 2 methyl group shifts the typical carboxyl fragment from m/e 74 ($\text{CH}_2\overset{\text{OH}+}{\text{COCH}_3}$) to m/e 88 ($\text{CHCH}_3\overset{\text{OH}+}{\text{COCH}_3}$). As would be expected the expulsion



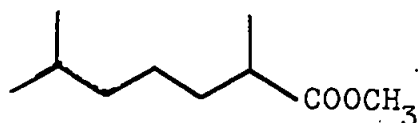
II

of the α and β methylene carbons results in the loss of 43 mass units (m/e 129) while the expulsion of the α , β , and γ carbons produces the peak at m/e 115. The presence of an α methyl substituent and the lack of further branching completes the identification.

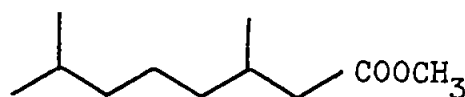
It is worthy of note that 2-methyl pentanoic, and 2-methyl hexanoic acids have been isolated from petroleum and identified by mixed melting points of the p-toluidides.¹⁶

The isoprenoidal Structure III is assigned to Ester 8 Fraction 1 (Figure IX). The rearrangement peak at m/e 88 requires an α methyl substituent while the loss of 15 and 43 mass units (m/e 157 and 159) are consistent with a

terminal isopropyl group. The combination of these structural groups requires Structure III. The gas chromatographic data support this conclusion because the mono-



III



IV

methyl substituted ester (Ester 10 Fraction 1) would be expected to have a longer retention time than the isomeric dimethyl substituted compound (III).

Ester 14 Fraction 1 appears to be an isoprenoidal homologue of the isoprenoid Ester 8 Fraction 1 and was identified as 3,7-dimethyloctanoate, Structure IV. Its mass spectrum, exhibiting an intense peak at m/e 101 (Figure X), is typical for esters possessing a methyl branch at C_2 . The identity is confirmed by comparison with a synthetic sample of methyl 3,7-dimethyloctanoate,* the mass spectrum of which is shown in Figure XI.

There were indications of small quantities of other saturated esters that could not be isolated in pure enough state to make even tentative structural suggestions.

* Kindly provided by Professor James Cason.

Eglinton has found the C_{13} - C_{20} isoprenoid acids with the exception of the C_{18} in Colorado Green River Shale digested with hydrofluoric acid.^{1,9} Cason has noted the presence of the isoprenoidal C_{14} , C_{15} , C_{19} , and C_{20} acids in petroleum. He searched for the C_{10} isoprenoid but was unable to find it.^{17, 18, 19}

Figure VIII. Mass Spectrum of Ester 10 Fraction 1

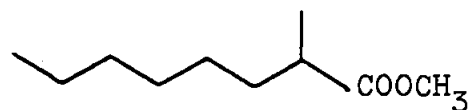


Figure IX. Mass Spectrum of Ester 8 Fraction 1

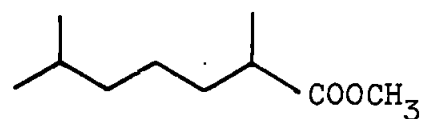


Figure X. Mass Spectrum of Ester 14 Fraction 1

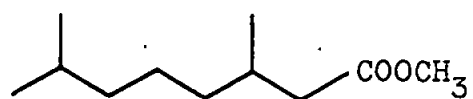
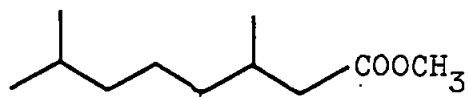
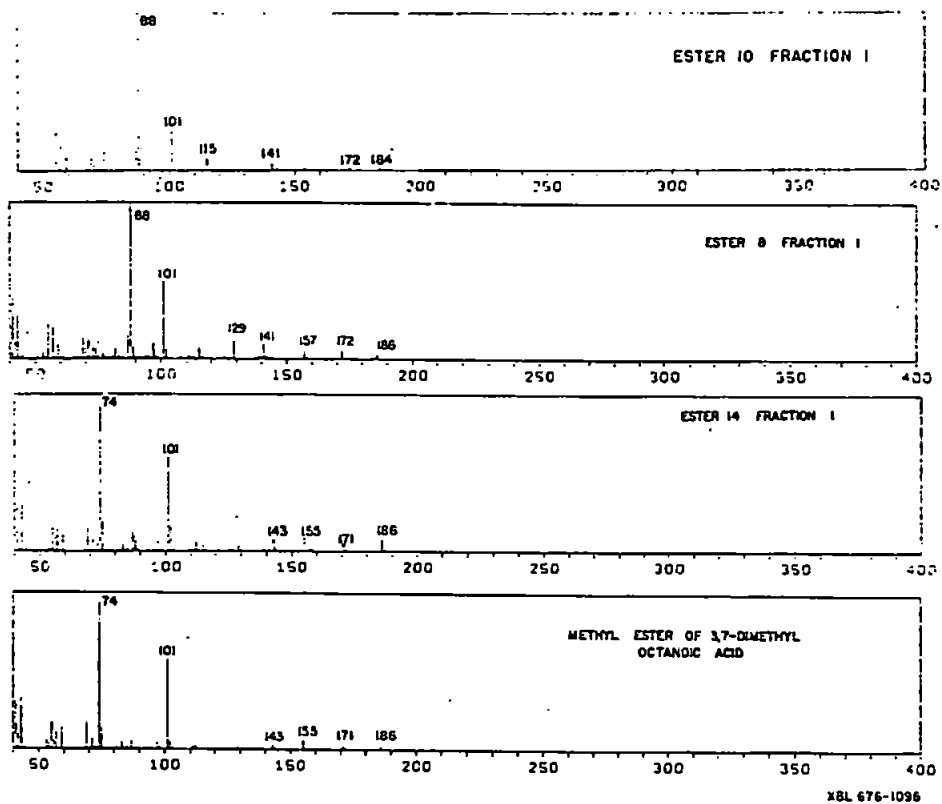


Figure XI. Mass Spectrum of Methyl Ester of 3,7-Dimethyloctanoic Acid

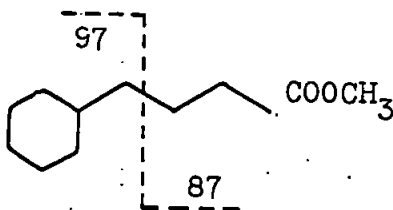


GREEN RIVER SHALE BRANCHED ESTERS



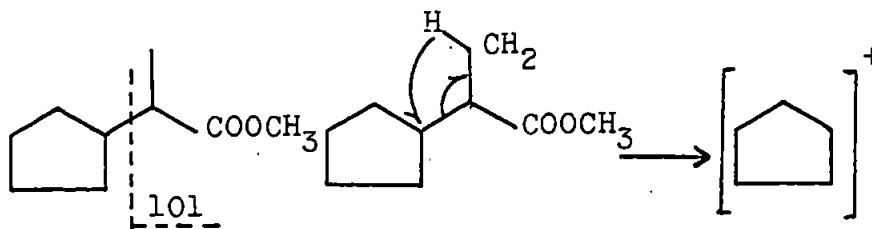
Cyclic Esters

Cyclic acids have long been known to exist in petroleum. Lochte has reported cyclopentanoic carboxylic, 2-methylcyclopentanoic, 3-methylcyclopentanoic, cyclopentylacetic, 3-methylcyclopentylacetic, 2,3-dimethylcyclopentylacetic and cyclohexylacetic acids.²⁰ Cason has identified trans 2,2,6 trimethylcyclohexylacetic acid²¹ and 3-ethyl-4-methylcyclopentylacetic acid²² from petroleum. Ramsay mentions having observed cyclic acids in the Green River Shale but gives no data about them.¹ Cyclic acids are known to be present in many and diverse biological systems.^{23,24} Unfortunately their mass spectrometric fragmentation patterns have received very little attention.²⁵ Figure XII gives the mass spectrum of methyl cyclopentylacetate while Figures XIII-XVII give the mass spectra of the series of methyl cyclohexyl esters from the acetate through caproate. It is to be noted first of all that there is no way of distinguishing a cyclopentyl from a cyclohexyl ester as the peaks at m/e 69 and m/e 83 are neither distinct nor characteristic. The spectra of all of these standards have intense peaks at m/e 74. All show reasonable loss of 31 mass units. Methyl cyclohexyl propanoate shows loss of 28 mass units as well as intense ions at m/e 87 and m/e 97. The fact that



methyl cyclohexylbutyrate loses 32 mass units in preference to 31 mass units was somewhat unexpected. Equally unexplained is the loss of 76 mass units from methyl cyclohexylvalerate. Since little is understood about the known cyclic esters it is difficult indeed to interpret the mass spectra of those isolated from the sediment (Figures XVIII-XXXV) particularly since many of them are obviously impure.

The mass spectrum of Ester 6 Fraction 1 (Figure XVIII) and Ester 7 Fraction 2 (Figure XIX) containing molecular ions at m/e 156 and 170 could be explained by structures such as methyl cyclopentylacetate and methyl 2-cyclopentylpropionate. The peak at m/e 101 could then be explained by loss of the cyclopentyl ring from the latter and the m/e 70 by a hydrogen rearrangement:



Ester 10 Fraction 3 (Figure XXII) could be interpreted as either methyl trimethylcyclopentyl carboxylate or methyl dimethylcyclohexylcarboxylate, whereas Ester 12 Fraction 2 (Figure XXIII) could be envisioned as a methyl methylcyclohexylacetate.

At least one cyclic ester of molecular weight 184 appears to have an α substituent as evinced by the m/e 88 peak of Ester 15 Fraction 3 (Figure XXV). Comparison of Ester 15 Fraction 4 (Figure XXVI) with methyl cyclohexylpropionate

(Figure XIV) suggests that it contains methyl cyclohexylpropionate and methyl methylcyclohexylpropionate (although the possibility of methyl methylcyclopentylpropionate and methyl dimethylcyclopentylpropionate cannot be discarded, as indicated previously.

The intense m/e 88 peak and the lack of an m/e 87 ion in the spectrum of Ester 17 Fraction 2 (Figure XXVII) combined with the loss of 15 mass units suggests a possible β methyl branch. However, it is evident that at least five isomers of molecular weight 198 are present. Note the presence of the mono-unsaturated cyclic esters (or possibly bicyclic esters) at m/e 196 and 210 in Ester 21 Fraction 3 (Figure XXX).

Esters 23 Fraction 2 and Ester 24 Fraction 2 (Figures XXXIV and XXXV) appear to be a mixture of a C_{12} cyclic and a C_{12} bicyclic ester.

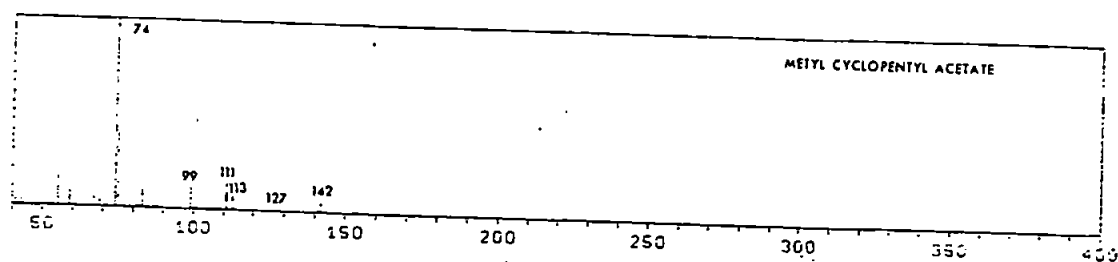


Figure XII. Mass Spectrum of Methyl Cyclopentylacetate

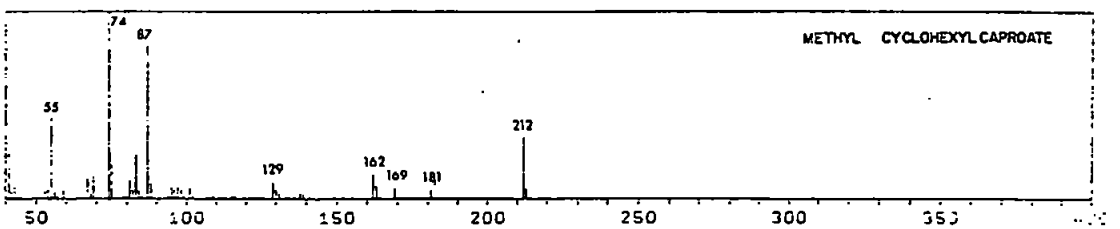
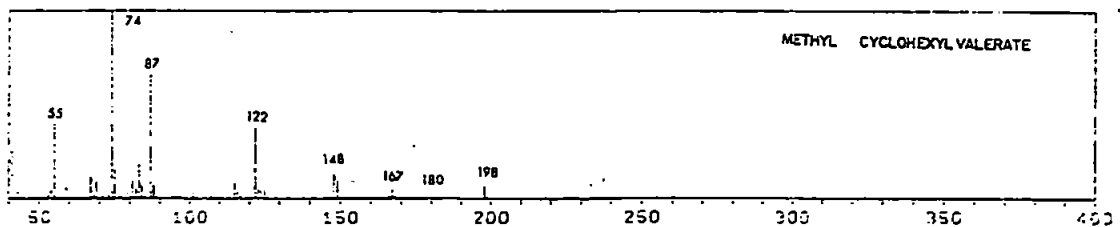
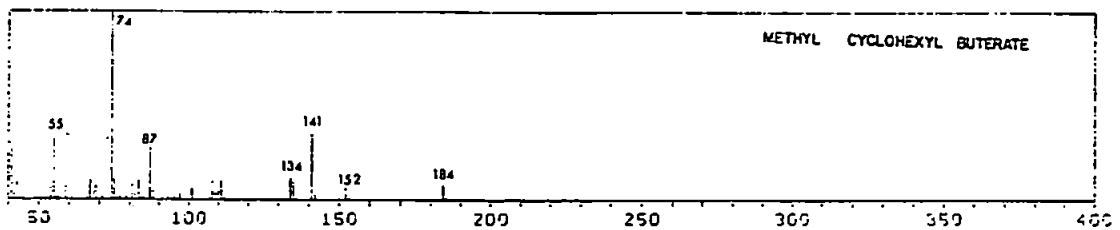
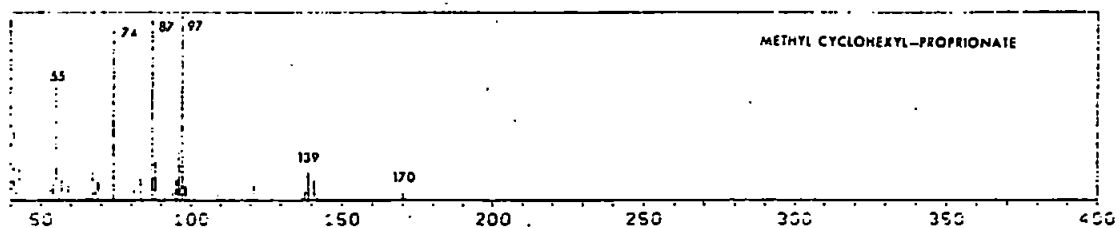
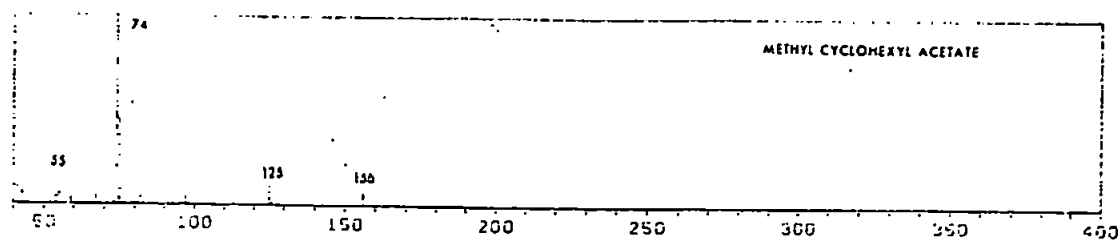
Figure XIII. Mass Spectrum of Methyl Cyclohexylacetate

Figure XIV. Mass Spectrum of Methyl Cyclohexylpropionate

Figure XV. Mass Spectrum of Methyl Cyclohexylbutyrate

Figure XVI. Mass Spectrum of Methyl Cyclohexylvalerate

Figure XVII. Mass Spectrum of Methyl Cyclohexylcaproate



XBL 678-4594

Figure XVIII. Mass Spectrum of Ester 6 Fraction 1

Figure XIX. Mass Spectrum of Ester 7 Fraction 2

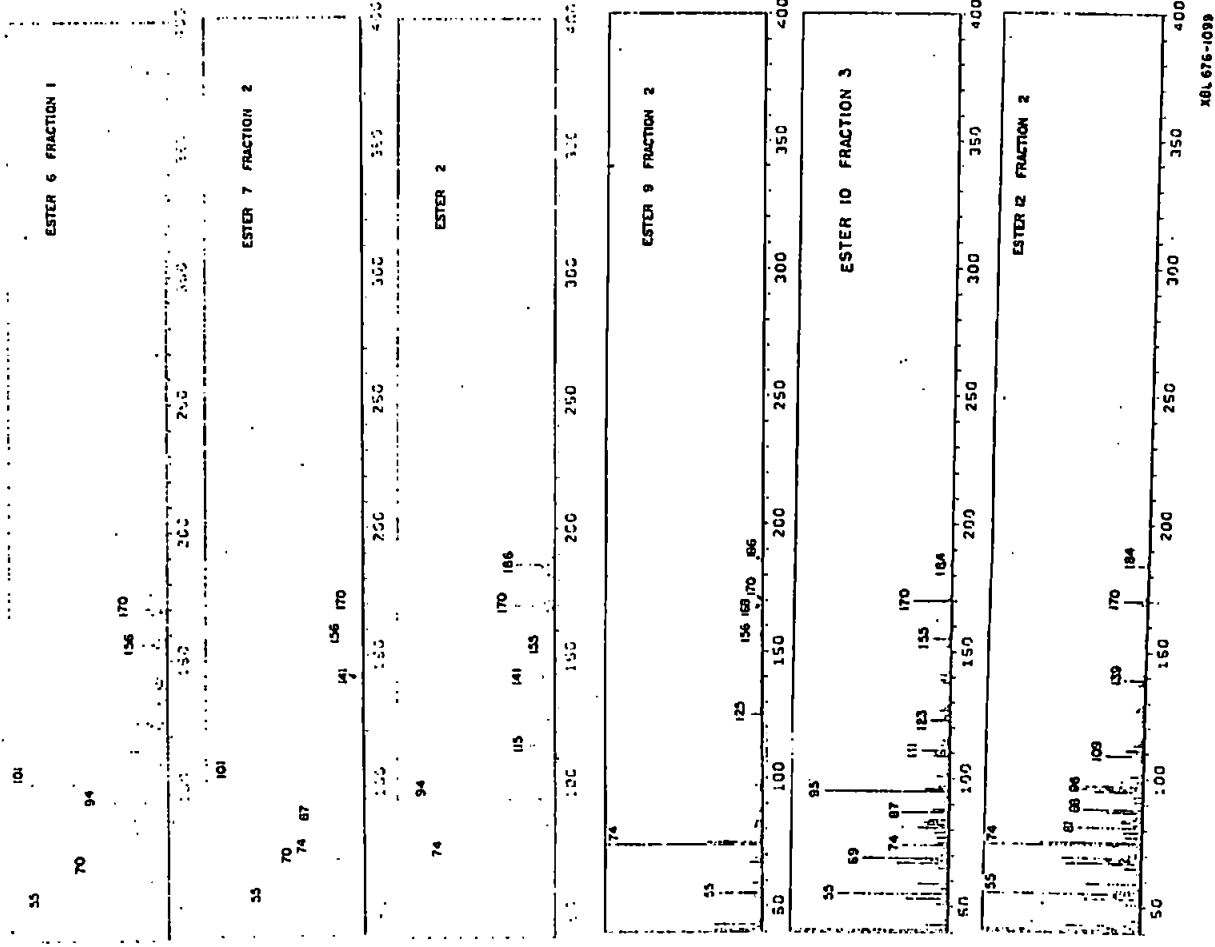
Figure XX. Mass Spectrum of Ester 2

Figure XXI. Mass Spectrum of Ester 9 Fraction 2

Figure XXII. Mass Spectrum of Ester 10 Fraction 3

Figure XXIII. Mass Spectrum of Ester 12 Fraction 2

GREEN RIVER SHALE CYCLIC ESTERS



XOL 676-1099

Figure XXIV. Mass Spectrum of Ester 13 Fraction 2

Figure XXV. Mass Spectrum of Ester 15 Fraction 3

Figure XXVI. Mass Spectrum of Ester 15 Fraction 4

GREEN RIVER SHALE CYCLIC

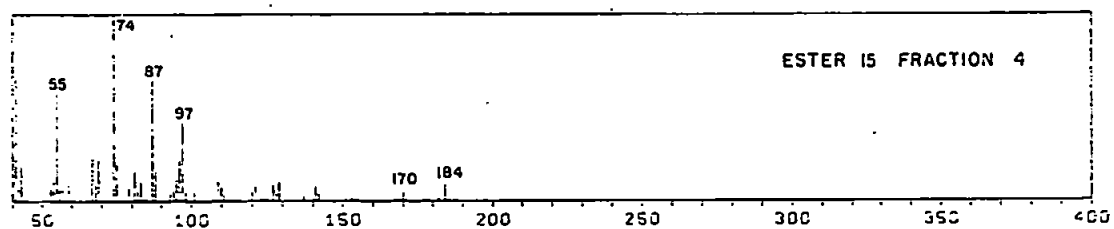
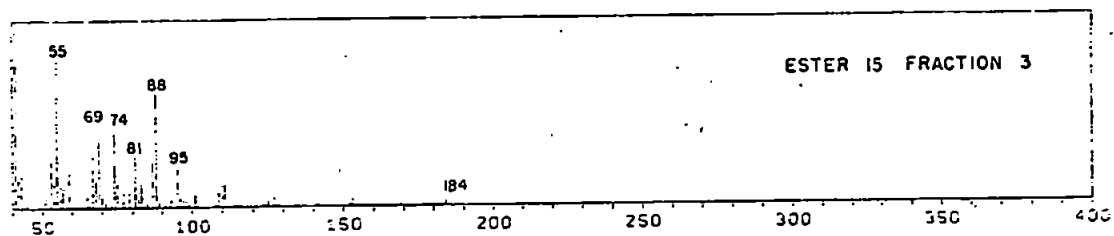
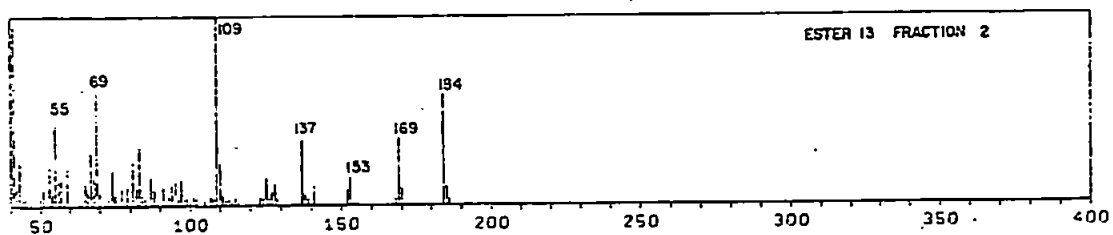


Figure XXVII. Mass Spectrum of Ester 17 Fraction 2

Figure XXVIII. Mass Spectrum of Ester 18 Fraction 1

Figure XXIX. Mass Spectrum of Ester 19 Fraction 2

Figure XXX. Mass Spectrum of Ester 21 Fraction 3

XBL 676-1109

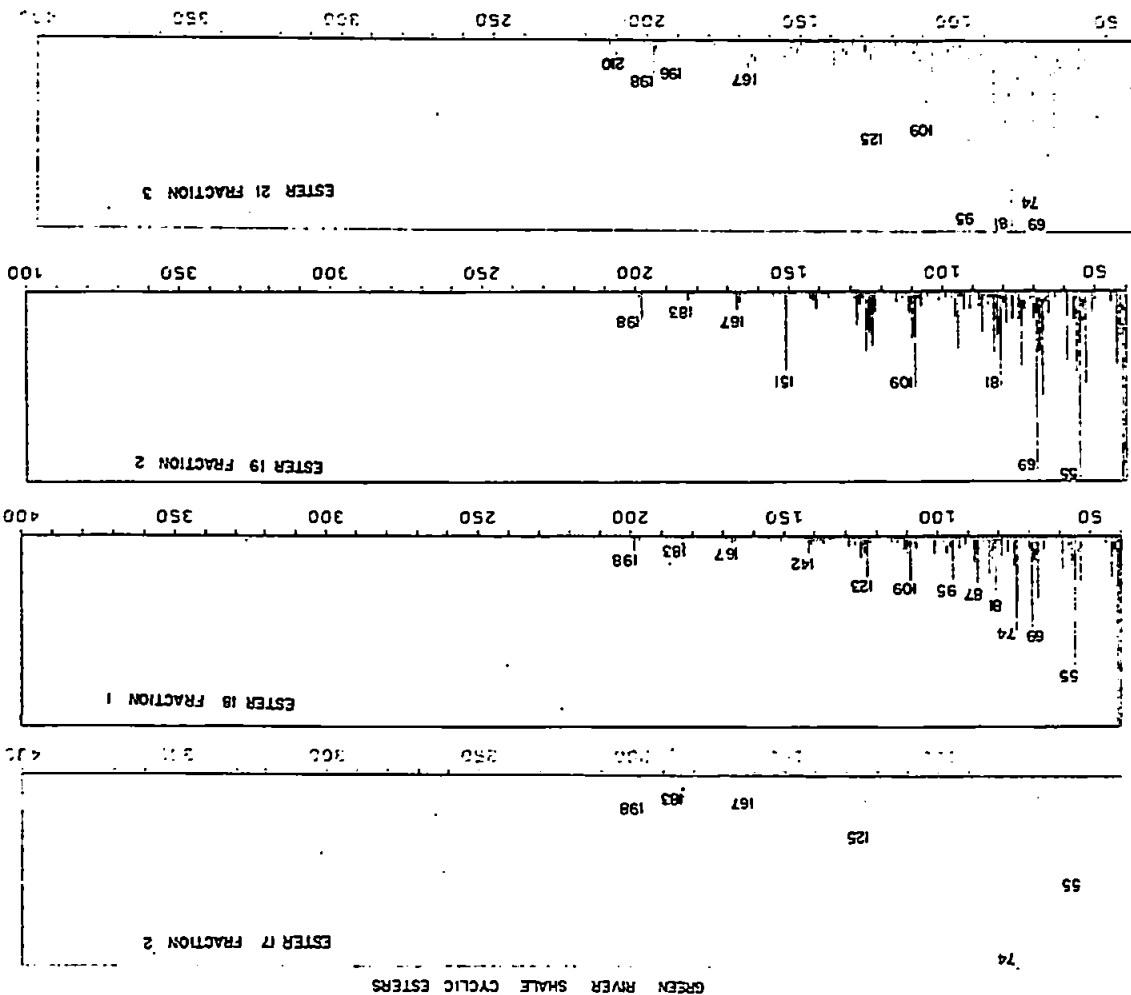
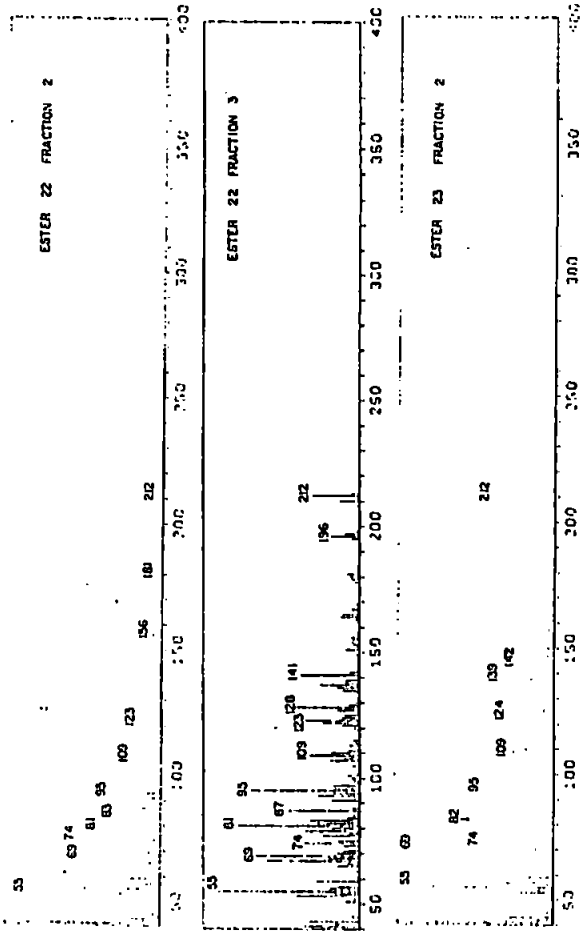


Figure XXXI. Mass Spectrum of Ester 22 Fraction 2

Figure XXXII. Mass Spectrum of Ester 22 Fraction 3

Figure XXXIII. Mass Spectrum of Ester 23 Fraction 2

GREEN RIVER SHALE CYCLIC ESTERS



XBL 676-1108

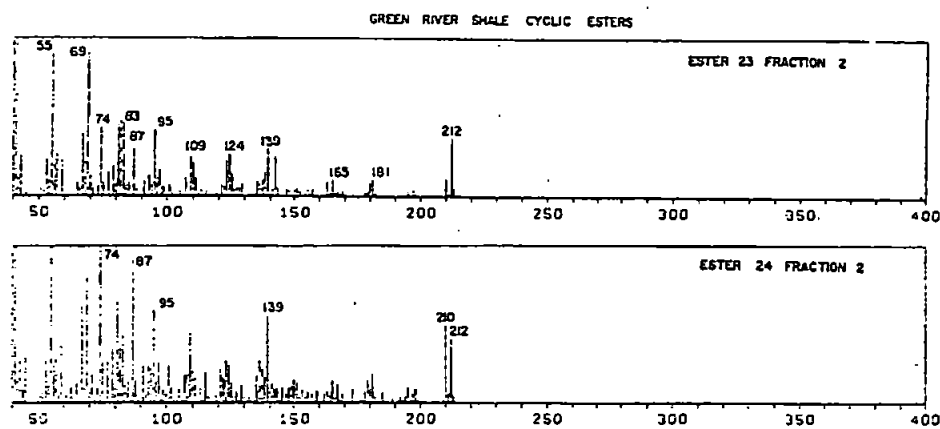


Figure XXXIV. Mass Spectrum of Ester 23 Fraction 2

Figure XXXV. Mass Spectrum of Ester 24 Fraction 2

Unsaturated Esters

Figures XXXVI-XXXIX give the mass spectra of authentic methyl 10-undecenoate and three unsaturated esters which were isolated. Assignment to the class of unsaturated esters is based primarily on the molecular weight; differentiation from the cyclic esters is based on the strong loss of 32 mass units (OHCH_2). Such fragmentation is characteristic for unsaturated esters and would not be expected for monocyclic esters (see cyclic esters). Unfortunately no single compound can be identified, since all of them are apparently impure. Comparison of the mass spectra of the three unknowns with that of the known undecenoate shows quite clearly the similarities between them, i.e. the loss of 32 and 74 mass units and major ions at 55, 69, 74, and 87. Nevertheless such prominent peaks as m/e 111, 109, and 95 in the spectra of Ester 9 Fraction 1, Ester 11 Fraction 1 and Ester 14 Fraction 3 seem to indicate contribution from cyclic isomers. A detailed interpretation of the spectra is not possible since representative unsaturated acids were not available for study and the data from the literature do not prove particularly helpful in this case.

Another complicating factor in the theoretical interpretation of unsaturated esters is the delocalization of the double bond upon electron impact. It might be possible to gain more information about these esters using chemi-ionization in a high pressure mass spectrometer at very low

ionizing voltages. Since ionization is accomplished by excited simple molecules, small samples could theoretically be observed; the low energy transferred would not be expected to disturb the double bond. However, this laboratory was not equipped for such studies.

Unsaturated esters have so far not been reported from geological sources with the exception of the work of Ramsay,¹ who has reported normal C_{16} - C_{20} terminal monoenoic acids in a recent sediment and an ancient mineral oil. He has theorized that recent bacterial action is the source of such compounds; in the case of the oil an igneous intrusion is invoked to cause cracking. Oleic (cis-octade-9-enoic acid) is the most common constituent of all natural fats contributing at least 30% to the total fatty acids.²⁶ Even unsaturated acids range from C_{16} - C_{24} maximizing at C_{18} in aquatic oils, while they range from C_{27} - C_{34} in waxes and seed fats. Odd unsaturated acids are found in human hair fat.¹

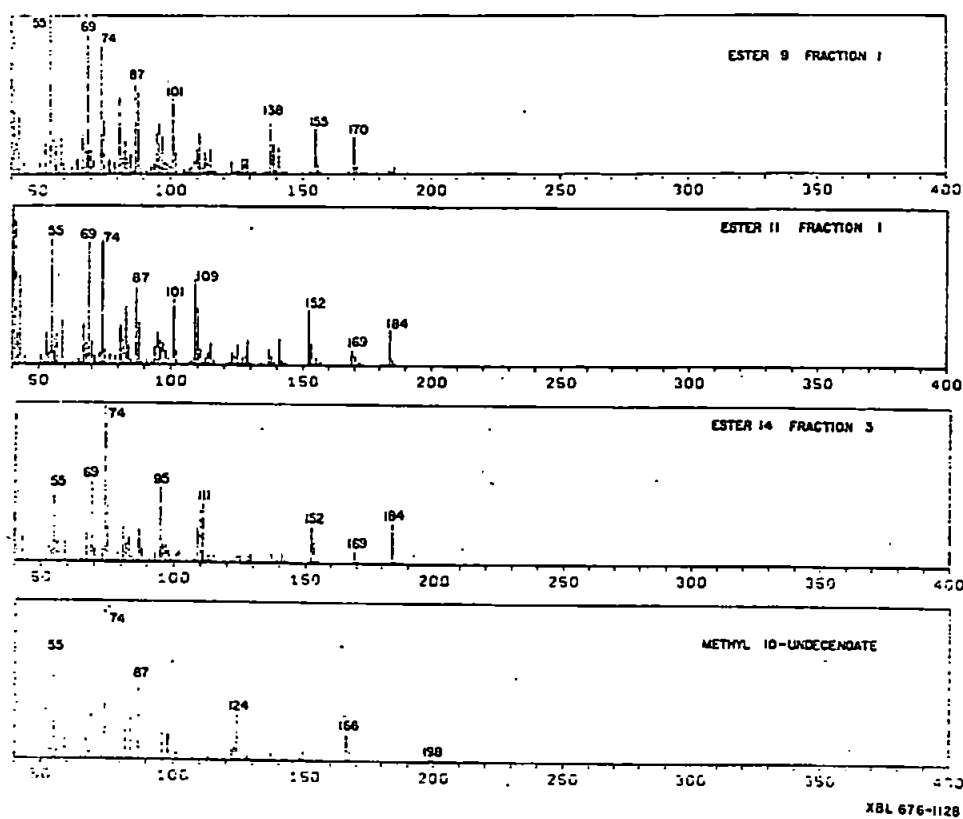
Figure XXXVI. Mass Spectrum of Ester 9 Fraction 1

Figure XXXVII. Mass Spectrum of Ester 11 Fraction 1

Figure XXXVIII. Mass Spectrum of Ester 14 Fraction 3

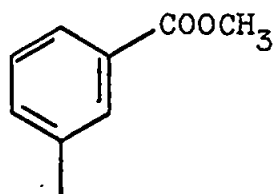
Figure XXXIX. Mass Spectrum of Methyl 10-Undecenoate

GREEN RIVER SHALE UNSATURATED ESTERS

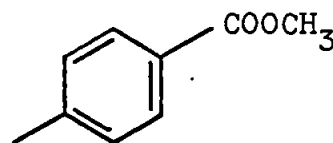


Methyl Benzoates

Several series of aromatic esters were isolated, the first of which is the methyl substituted methyl benzoate series. Two isomers of mono-methyl substituted methyl benzoate were isolated and identified on the basis of their mass spectra (Figures XL and XLI) and their GLC properties. The major ion at m/e 119 is the characteristic ion in methyl substituted methyl benzoate esters, resulting from the loss of 31 mass units (OCH_3). Ester 12 Fraction 4 is assigned the structure of methyl m-toluate, Structure V, and Ester 13 Fraction 3 that of methyl p-toluate, Structure VI.. Although the mass spectra are practically identical, they show minor differences such as the ion at m/e 118 corresponding to M-32. (Refer to Table III) It should be noted that one can definitely eliminate the ortho isomer; distinction between meta and para compounds is made primarily on the basis of the GLC retention time.

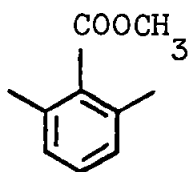


V

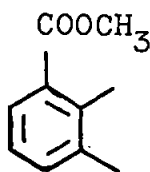


VI

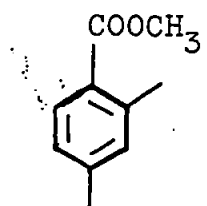
Four methyl demethylbenzoates of molecular weight 164 were found; their mass spectra are given in Figures XLII-XLV. The alternative tolylacetates are ruled out by the fact that these esters all have strong peaks at M-31 and not at M-59 as would be expected from tolylacetates. The lack of an intense ion at M-15 eliminates the possibility of methyl ethylbenzoates. The intensity of the M-32 peak indicates that all have at least one ortho substituent which reduces the structural possibilities to four, Structures VII-X.



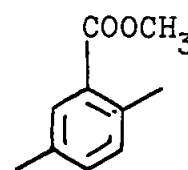
VII



VIII



IX



X

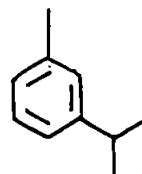
Ester 17 Fraction 4 and Ester 16 Fraction 4 have very similar spectra; presumably they are different isomers although GLC tailing could result from a single compound. (See Table IV)

The next homologues of this series (Figures XLVI-XLVII) have molecular weight 178; two isomers were isolated. Both are identified as substituted methyl benzoates by the base peak at m/e 147. The lack of an intense M-15 peak suggests that all substituents are methyl groups. However, definite assignments are not possible without standards. It is interesting that Ester 23 Fraction 7 bears a close similarity to methyl 2,4,5-trimethylbenzoate. (Table V)

It would be premature to speculate extensively on the origin of these benzoic acid derivatives. Obviously they could represent degradation products of terpenoids such as the p-cymene and m-cymene class of monoterpenes. Both of the aromatic parent compounds occur in nature (the p-cymene class is much more abundant) and of course many saturated



p-cymene

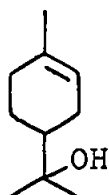


m-cymene

compounds of this type are known:



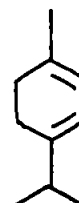
limonene



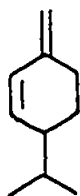
α-terpineol



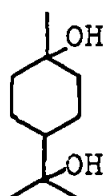
terpinolene



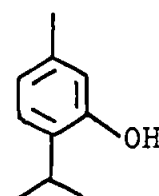
terpinene



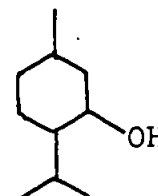
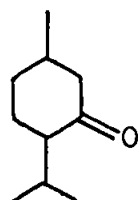
phellandrene



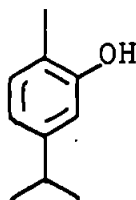
terpin



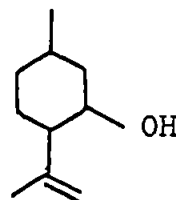
thymol

menthol
O=CH₃

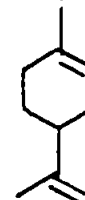
menthane



carvacrol

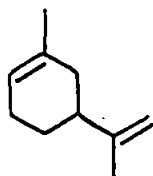


iso-pulegol



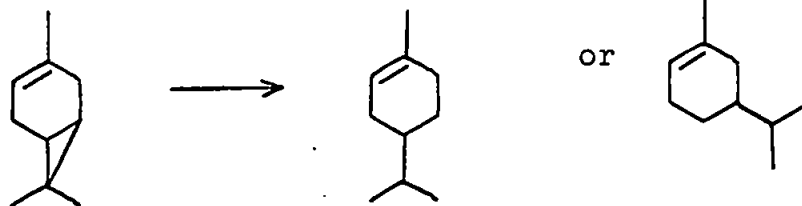
perillaldehyde

Only a few representatives of the m-cymene class occur in nature, for example:



carvestrene

Bicyclic compounds, mono terpenoids, could yield aromatic derivatives upon diagenesis, for example:



car-3-ene

Figure XL. Mass Spectrum of Ester 12 Fraction 4

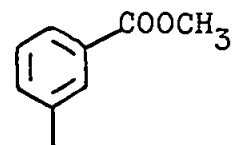


Figure XLI. Mass Spectrum of Ester 13 Fraction 3

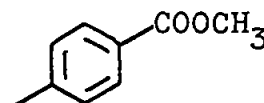


Figure XLII. Mass Spectrum of Ester 16 Fraction 4

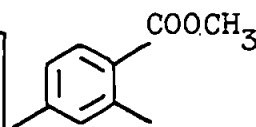


Figure XLIII. Mass Spectrum of Ester 17 Fraction 4

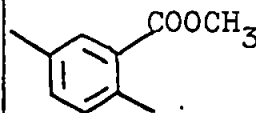


Figure XLIV. Mass Spectrum of Ester 18 Fraction 3

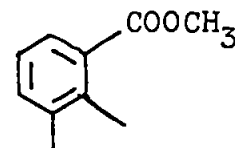
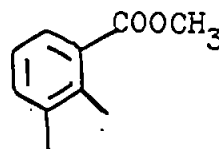
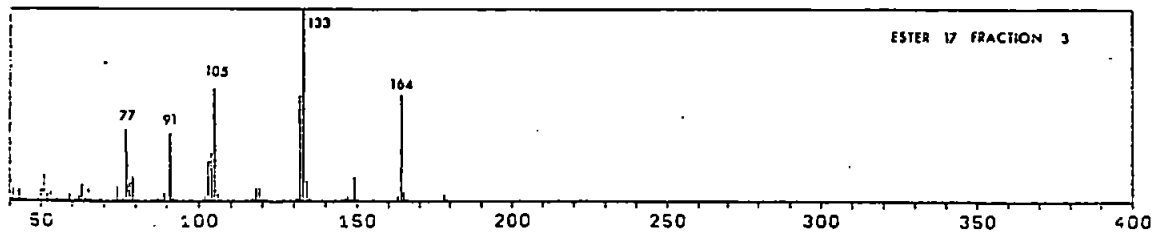
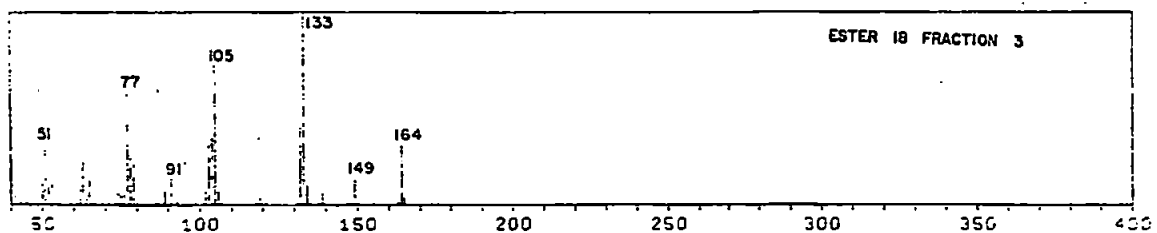
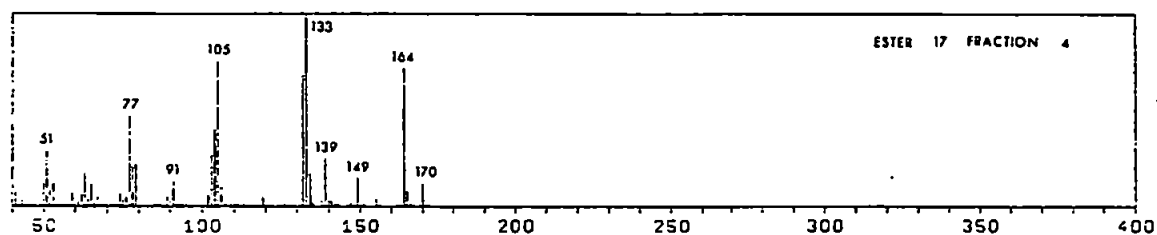
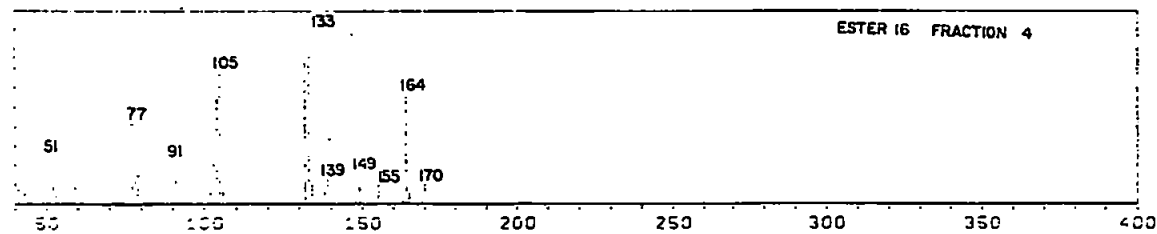
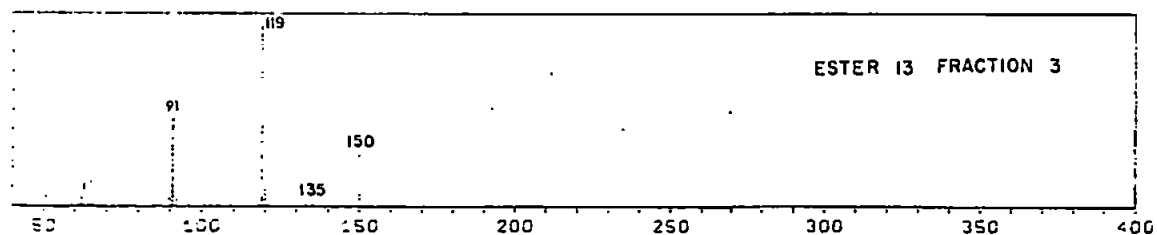
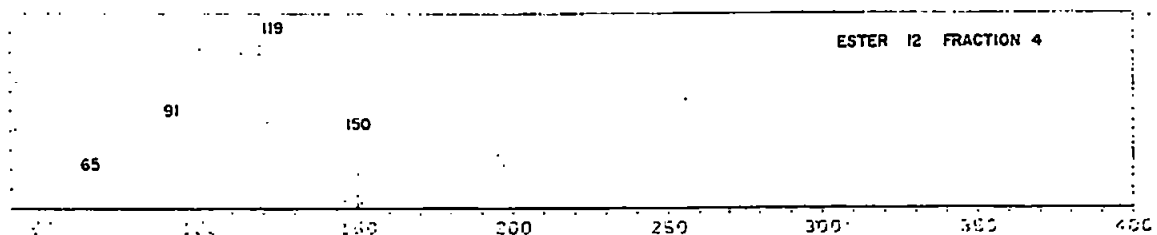


Figure XLV. Mass Spectrum of Ester 17 Fraction 3



GREEN RIVER SHALE AROMATIC ESTERS



XBL 676-1121

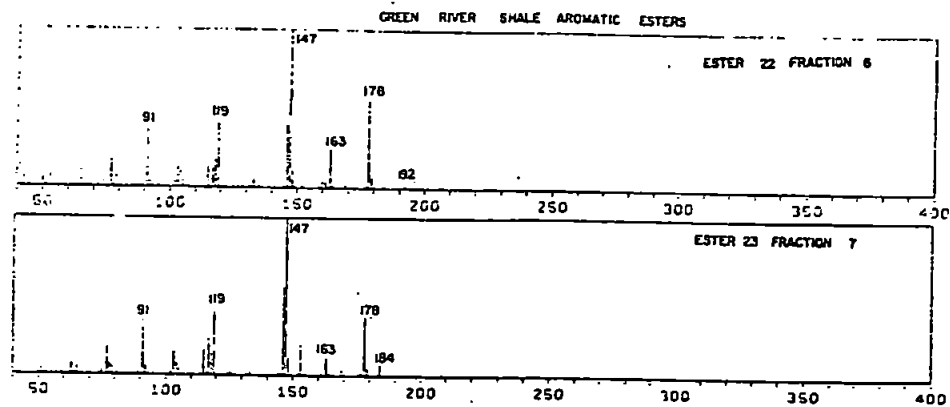


Figure XLVI. Mass Spectrum of Ester 22 Fraction 6

Figure XLVII. Mass Spectrum of Ester 23 Fraction 7

TABLE III

A Comparison of the Mass Spectrometric Fragments
of Methyl Toluates

		ortho*	meta*	para*	12-4	13-3
M-31	119	100	100	100	100	100
M-32	118	62	1	2	1	2
M-33	117		7	18		
M-34	116		4	10		
	105	6	2	1	2	2
	92	5	4	3	4	4
	91	67	51	43	46	46
M-60	90	18	5	8	5	7

*McLafferty, Fred W. and Gohlke, Roland S., "Mass Spectrometric Analysis of Aromatic Acids and Esters," Analytical Chemistry, 31, no. 12, p. 2076 (December 1959).

TABLE IV
Comparison of the Relative Intensities
of Methyl Dimethylbenzoates

		16-4	17-3	17-4	18-3	2,5*	2,4*	3,5*
P	164	56	46	72	31	56	38	40
P-1	163	2	3	3	2	2	2	2
P-15	149	15	13	15	13	15	6	2
P-17	147	2	3	2	1	1	1	0
P-18	146	1	2	1	0	0	0	0
P-31	133	100	100	100	100	100	100	100
P-32	132	73	39	68	40	77	40	1
P-44	120	1	1	1	1	0	0	0
P-45	119	3	7	5	4	2	2	0
P-59	105	68	41	75	73	65	51	39
P-60	104	56	17	40	35	41	14	5
	91	22	24	13	14	2	5	3
	77	42	26	47	58	40	26	18

*T. Acazel, H. E. Lumpkin, Analytical Chemistry, 34, 33 (1962).

TABLE V
Comparison of the Relative Intensities of
Methyl Trimethylbenzoates

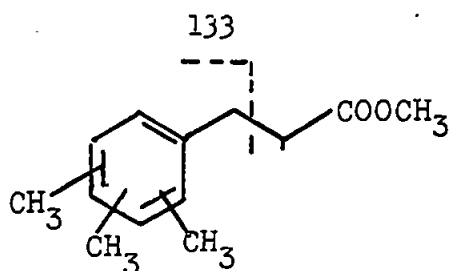
	22-6	23-7	2,4,5*
P 178	55	38	50
P-1 177	4	2	2
P-15 163	25	12	12
P-17 161	4	2	1
P-18 160	5	0	0
P-31 147	100	100	100
P-32 146	40	58	59
P-44 134	1	1	1
P-45 133	7	3	4
P-59 119	1	42	39
P-60 118	21	14	26
91	38	35	30
77	20	18	18
105	6	5	4
104	10	8	7

* T. Acazel, H. E. Lumpkin, Analytical Chemistry,
34, 33 (1962).

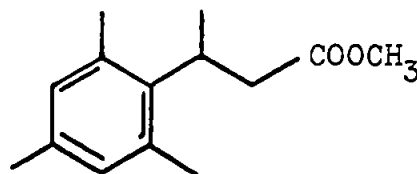
Phenyl Alkyl Esters

Another series is represented by one isomer of molecular weight 192 (Figure XLVIII), five isomers of molecular weight 206 (Figures XLIX-LIII), seven isomers of molecular weight 220 (Figures LIV-LX), and three isomers of molecular weight 234 (Figures LXI-LXIII). Since many of them are apparently mixtures, structural assignments are not possible aside from recognition of the class. Figures LXIV-LXVIII give the mass spectra of several reference standards. Ester 27 Fraction 7 (Figure LII) represents a mixture containing an aromatic component of molecular weight 206 (to which are assigned the fragments at m/e 175, 132, and 105): a cycloaromatic compound would account for the molecular ion at m/e 218 (fragments at m/e 189 and 145) and a keto ester of molecular weight 214 might explain the fragments at 183, 157, and 125.

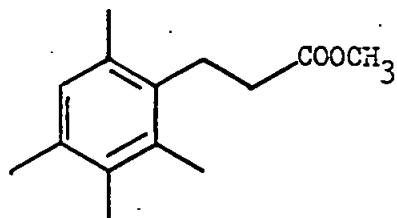
The fact that Ester 29 Fraction 4 (Figure LIII) loses 73 in preference to 74 mass units (m/e 133) might be explained by a structure such as Structure XI where there is no transferable hydrogen. The dominance of the ion of m/e 147 in the



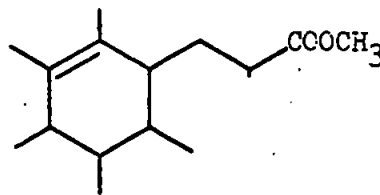
XI



XII



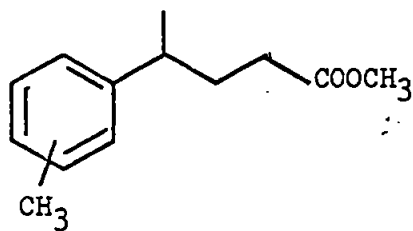
XIII



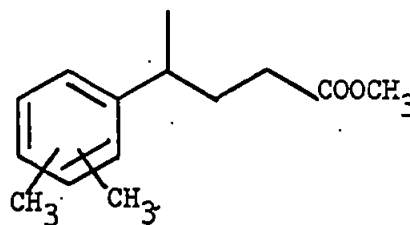
XIV

mass spectrum of Ester 27 Fraction 6 suggests a structure such as Structure XII or XIII. Isomers of phenyl alkyl acids of molecular weight 234 appear to be present. Most of these could not be obtained in pure enough form to permit structural deductions to be made. However the mass spectrum of Ester 32 Fraction 4 (Figure LXII) suggests a methyl substituted phenylpropionate (XIV). Possible biological precursors might include substances like β -ionone or long chain isoprenoids with a ring such as vitamin A (C_{20}).

Ester 25 Fraction 6 (Figure XLIX) appears to be a methyl monomethyl substituted 4 phenylvalerate (compare with 4 phenylvalerate in Figure LXVII) as indicated in Structure XV. A possible structure for Ester 28 Fraction 2 (Figure LVII) would appear to be methyl 4-dimethylphenylvalerate, Structure XVI. Aromatic acids to which a side chain is assigned with a branch next to the ring, could

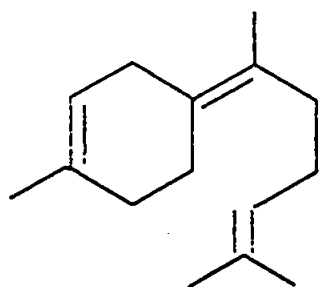


XV

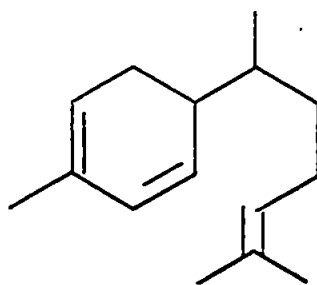


XVI

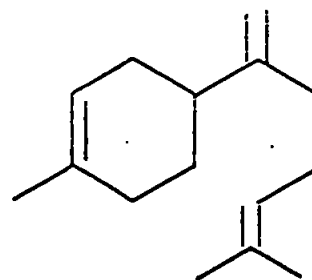
be derived from sesquiterpenes like the following:



bisabolene

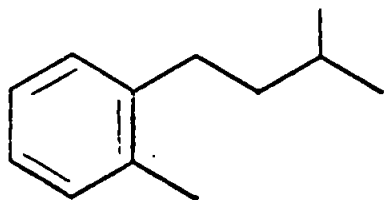


aingiberene

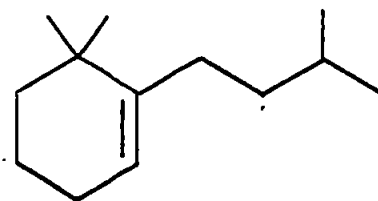


lanceol

Ester 30 Fraction 1 is identified as methyl 2-methyl 4-dimethylphenyl butyrate, Structure XXVII, on the basis of the intense peak at m/e 88 indicative of the α branched rearrangement ion $(CH_3CH_2COOCH_3)^+$ and by the intense peak at m/e 119 representing the dimethyl tropyllium ion. (It should be noted that the composition of the latter peak has been verified by high resolution mass spectrometry to be indeed C_9H_{11}). A plausible diortho substituted precursor would be a hydrocarbon of the β carotene-type, Structure XVIII, where one methyl group could be lost in aromazation during diagenesis. Of course, the mass spectral data do not permit assignment of methyl groups to specific positions on the aromatic ring.



XVII



XVIII

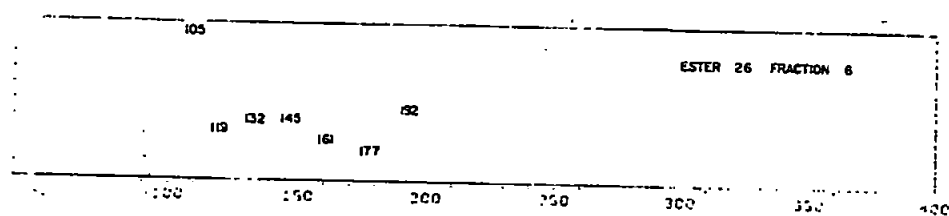


Figure XLVIII. Mass Spectrum of Ester 26 Fraction 6

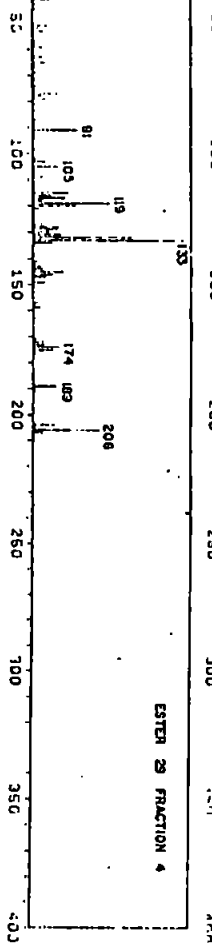
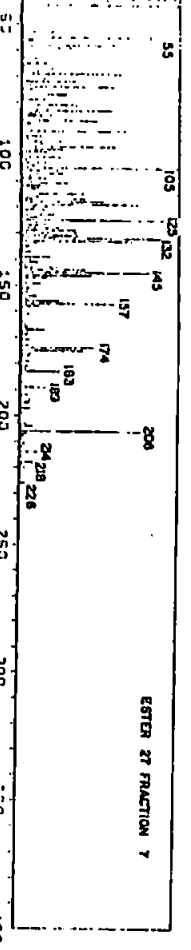
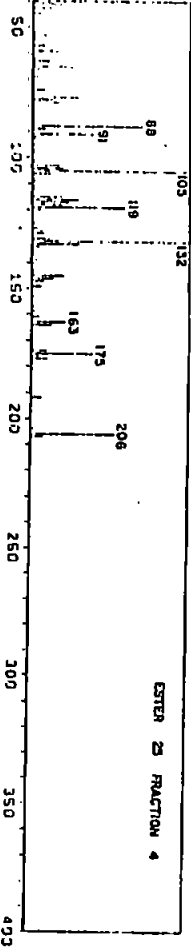
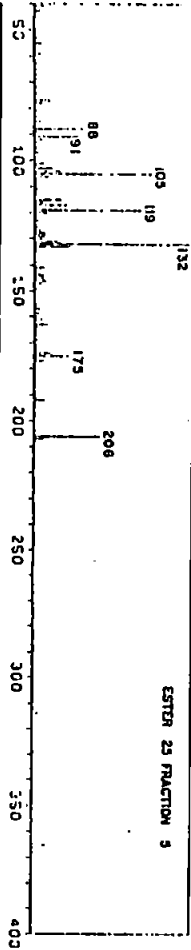
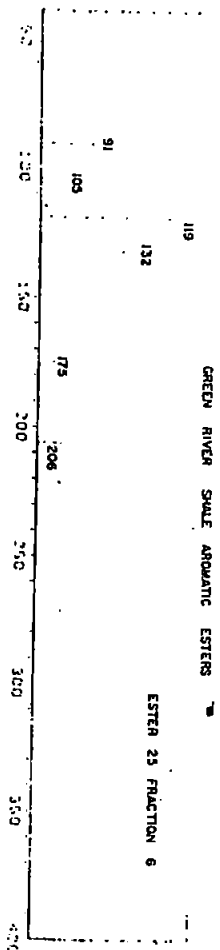
Figure XLIX. Mass Spectrum of Ester 25 Fraction 6

Figure L. Mass Spectrum of Ester 25 Fraction 5

Figure LI. Mass Spectrum of Ester 25 Fraction 4

Figure LII. Mass Spectrum of Ester 27 Fraction 7

Figure LIII. Mass Spectrum of Ester 29 Fraction 4.



XL 676-1126

Figure LIV. Mass Spectrum of Ester 26 Fraction 5

Figure LV. Mass Spectrum of Ester 27 Fraction 5

Figure LVI. Mass Spectrum of Ester 27 Fraction 6

Figure LVII. Mass Spectrum of Ester 28 Fraction 2

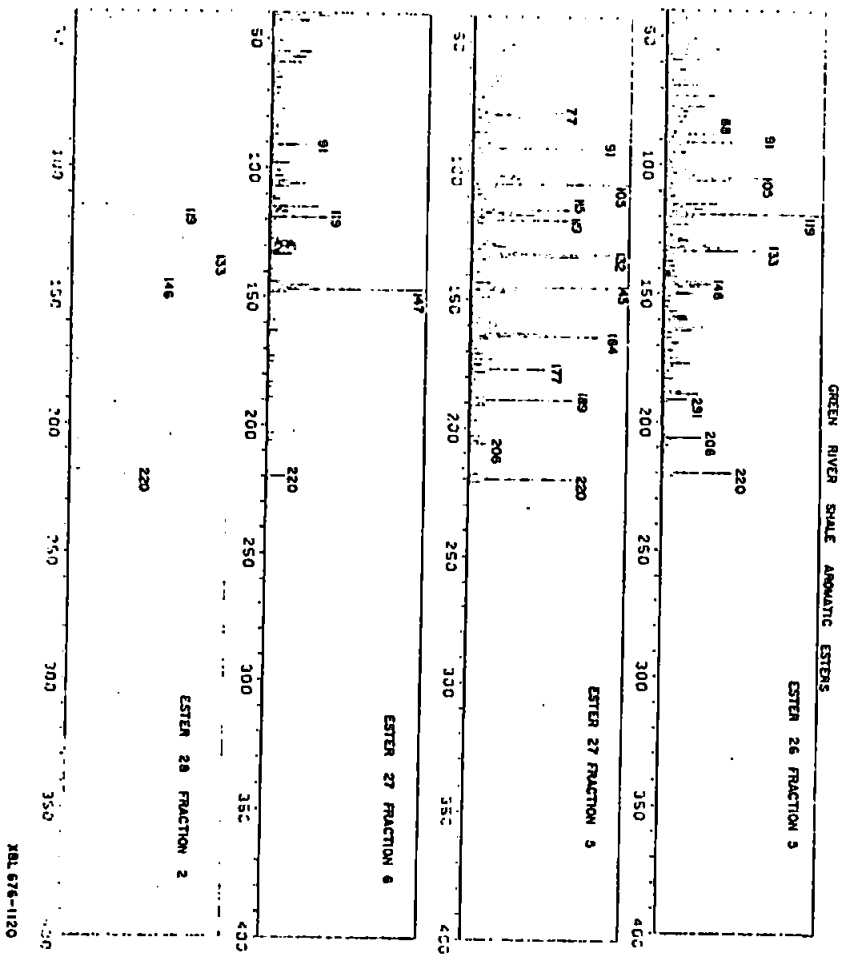


Figure LVIII. Mass Spectrum of Ester 29 Fraction 3

Figure LIX. Mass Spectrum of Ester 30 Fraction 1

Figure LX. Mass Spectrum of Ester 31 Fraction 3

GREEN RIVER SHALE AROMATIC ESTERS

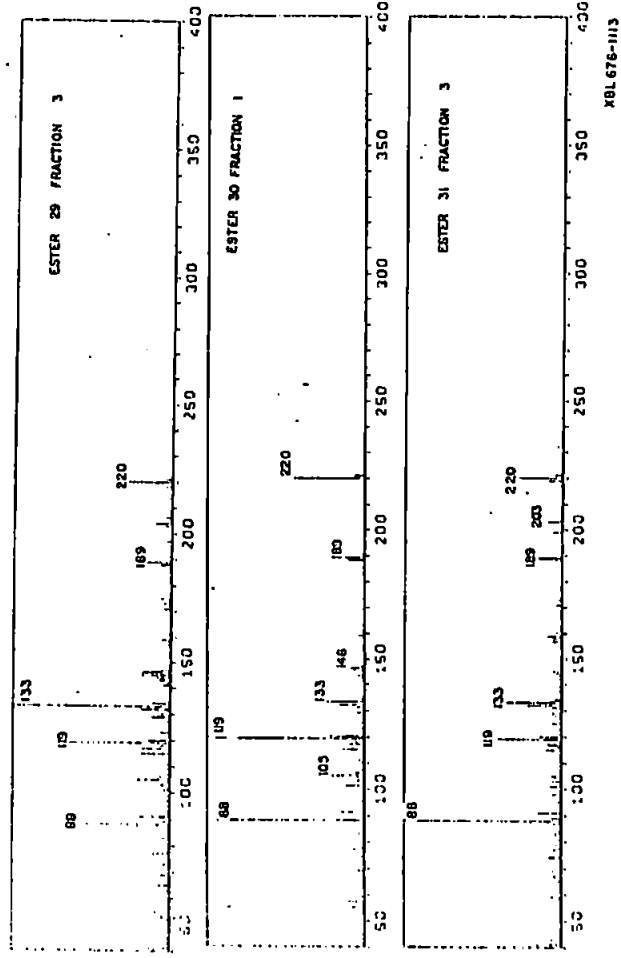


Figure LXI. Mass Spectrum of Ester 32 Fraction 2

Figure LXII. Mass Spectrum of Ester 32 Fraction 4

Figure LXIII. Mass Spectrum of Ester 34 Fraction 4

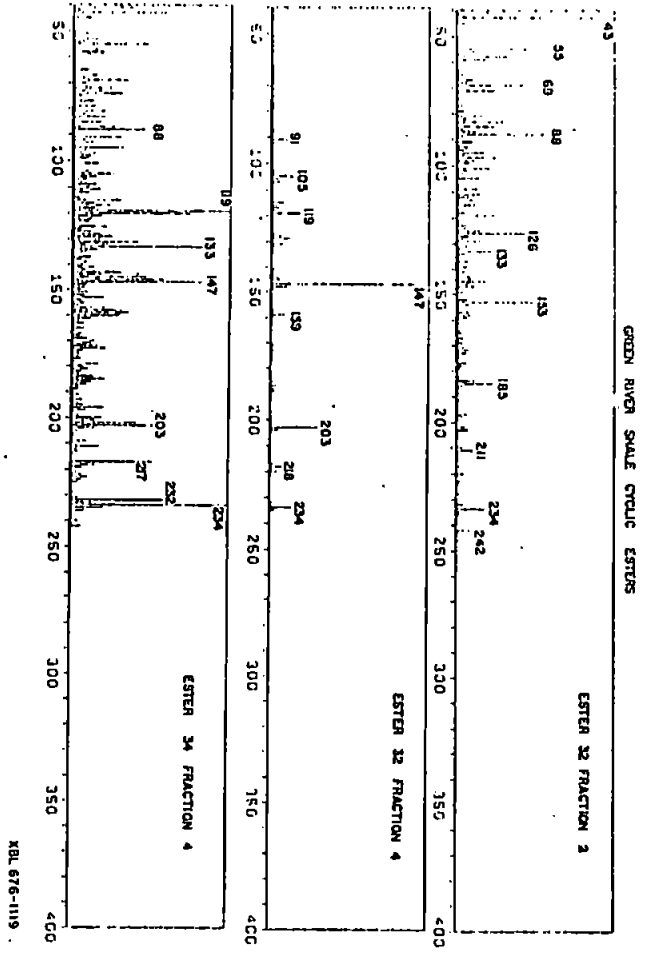


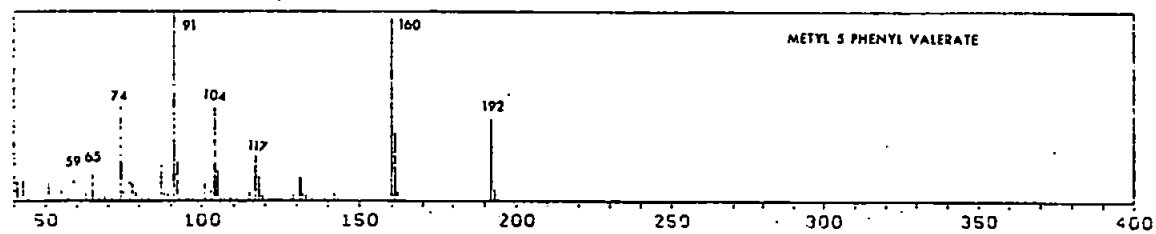
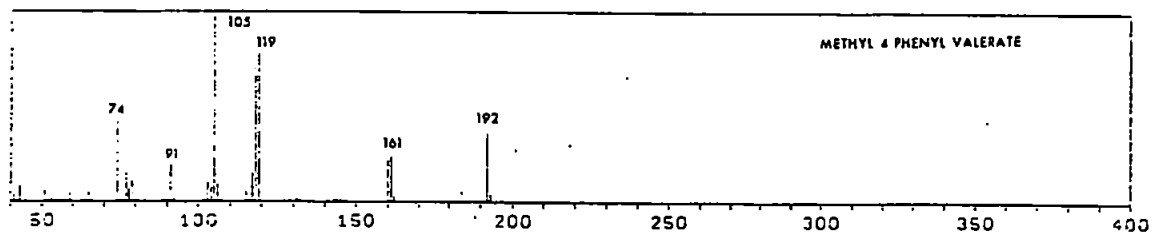
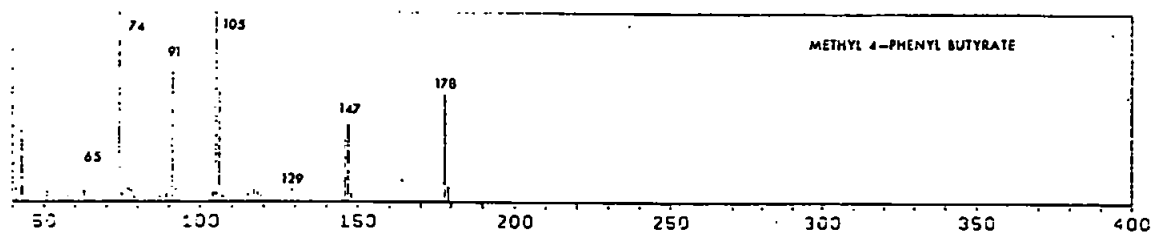
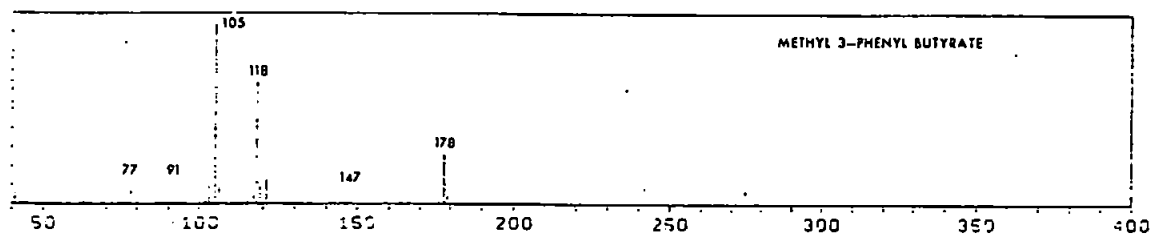
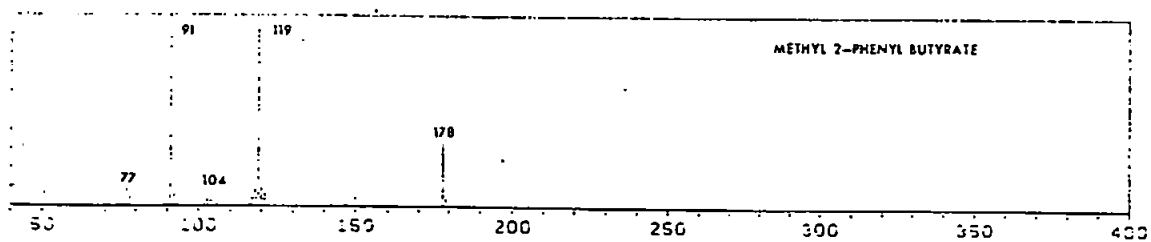
Figure LXIV. Mass Spectrum of Methyl 2-Phenylbutyrate

Figure LXV. Mass Spectrum of Methyl 3-Phenylbutyrate

Figure LXVI. Mass Spectrum of Methyl 4-Phenylbutyrate

Figure LXVII. Mass Spectrum of Methyl 4-Phenylvalerate

Figure LXVIII. Mass Spectrum of Methyl 5-Phenylvalerate



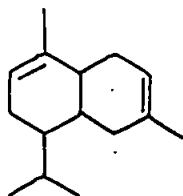
XBL 678-6109

Methyl Methyl Substituted Napthoate Esters and Cyclo-Aromatic Esters

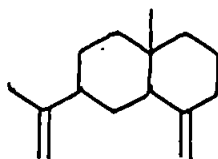
Two methyl methyl substituted napthoate esters of molecular weight 200 were isolated; their mass spectra are given in Figures LXXI and LXXII. High resolution mass spectrometric data verify that the composition of m/e 200 is $C_{13}H_{12}O_2$, 169 is $C_{12}H_9O$, and 141 is $C_{11}H_7$. Unfortunately the mass spectra do not permit the points of substitution to be determined. The spectrum of authentic methyl 1-napthoate is given for comparison in Figure LXX. Note that methyl napthylacetate is ruled out by comparison with Figure LXIX. Other members of this series were found with molecular weights of 214, 228, and 242 in very small quantities.

Also found was a series of cyclo-aromatic acid esters of molecular weight 204, 218, and 232 (Figures LXXIV-LXXVI). The mass spectra of Esters 31 Fraction 4 and 33 Fraction 5 are characterized by intense peaks at $M-15$. Ester 31 Fraction 5 consists of a mixture of compounds of molecular weight 218 and 204. These substances may possess structures of the tetrahydronaphthalene-type, but the spectra do not permit more definite assignments.

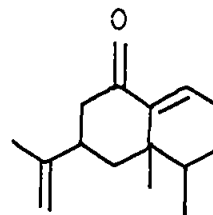
Many bicyclic compounds also occur in nature which could conceivably produce aromatics or cyclo aromatics, among them are:



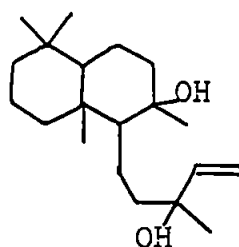
cadinene



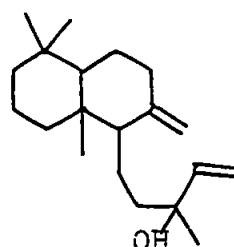
selinene



eremophilone



sclareol



manool

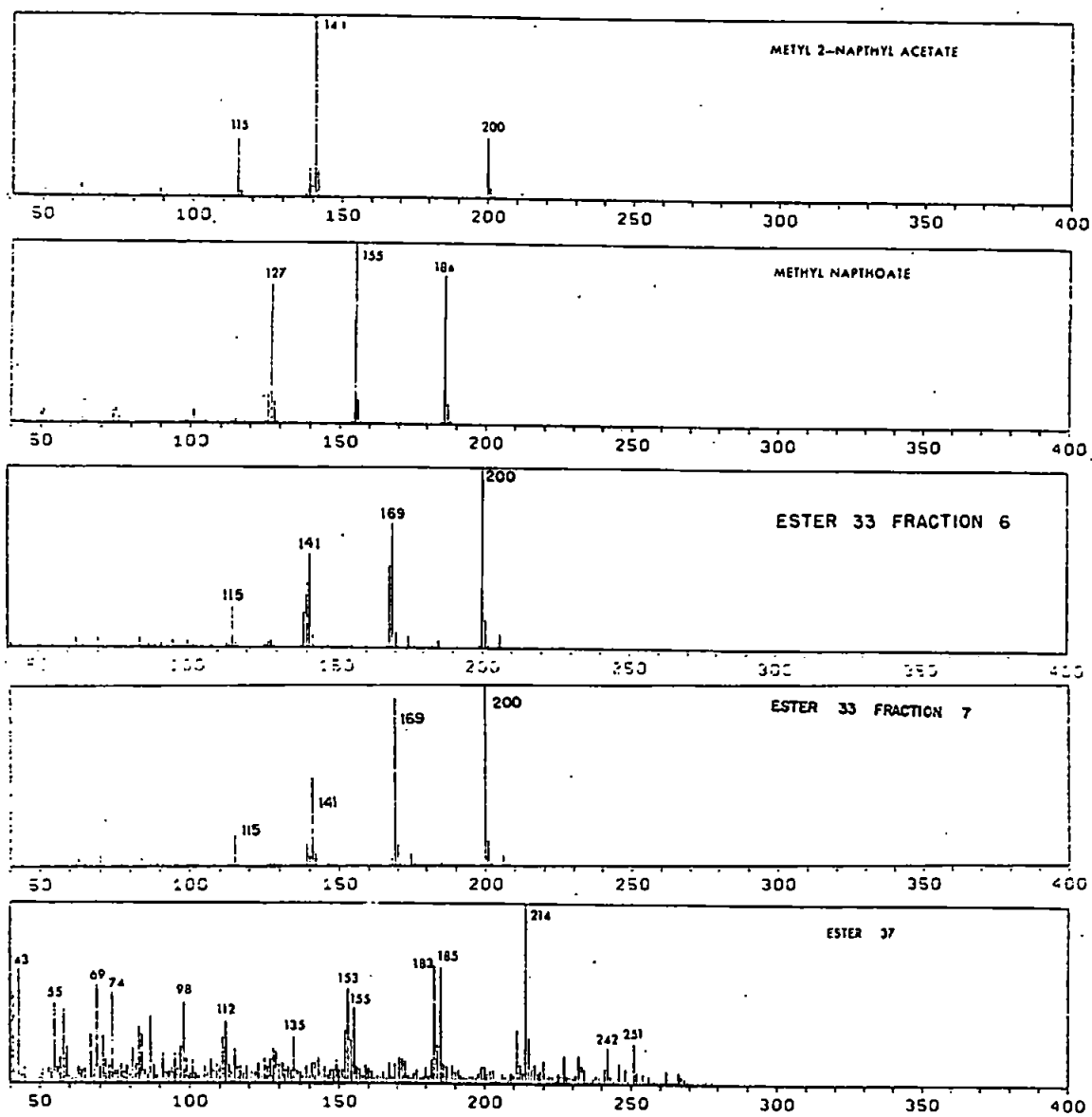
Figure LXIX. Mass Spectrum of Methyl 2-Naphthylacetate

Figure LXX. Mass Spectrum of Methyl Napthoate

Figure LXXI. Mass Spectrum of Ester 33 Fraction 6

Figure LXXII. Mass Spectrum of Ester 33 Fraction 7

Figure LXXIII. Mass Spectrum of Ester 37



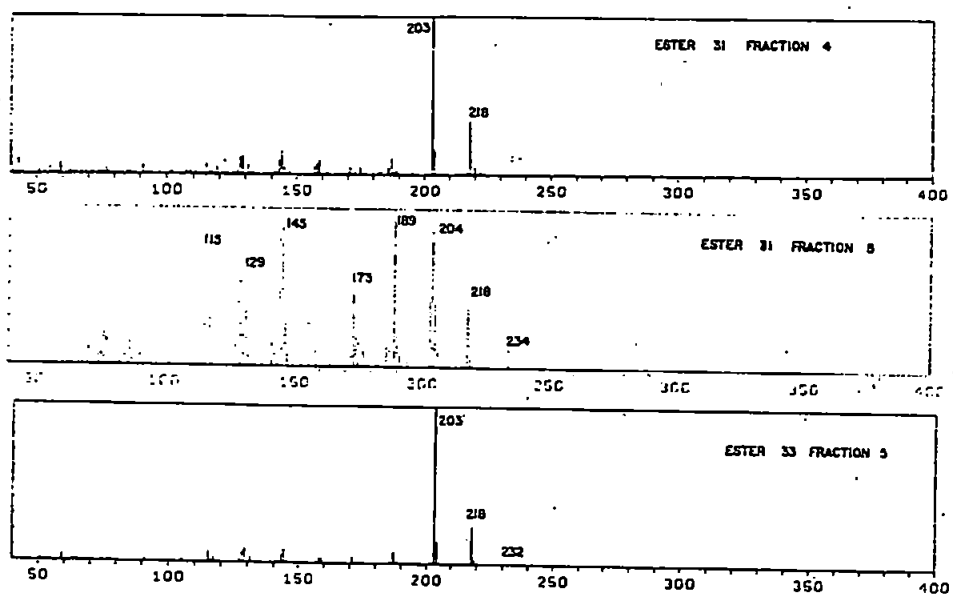
XBL 678-4593

Figure LXXIV. Mass Spectrum of Ester 31 Fraction 4

Figure LXXV. Mass Spectrum of Ester 31 Fraction 5

Figure LXXVI. Mass Spectrum of Ester 33 Fraction 5

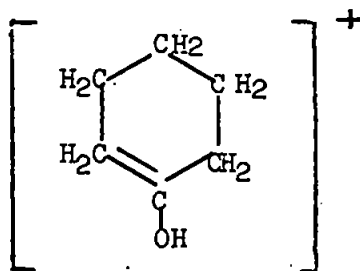
GREEN RIVER SHALE CYCLO AROMATIC ESTERS



XBL 676-1117

Dicarboxylic Acid Esters

An interesting series of compounds isolated from the shale is represented by the GLC peaks 38, 41, 43, 45, 48, 51, and 54 (See Figure I). Mass spectrometric analysis of these compounds revealed a homologous series of straight chain C_{11} - C_{16} α,ω -dicarboxylic acid esters. The mass spectra of these compounds are quite characteristic and permit unambiguous identification. (See Figures LXXVII-LXXXIV.) The high mass region is characterized by a small molecular ion peak and strong loss of both of 31 mass units (OCH_3) and of 73 mass units (CH_3OCCH_2). The base peak of the spectra is the ion of m/e 98 (except when the ester is substituted in which case the base peak is a higher homologue). Structure XIX has been suggested for this ion. The fact that

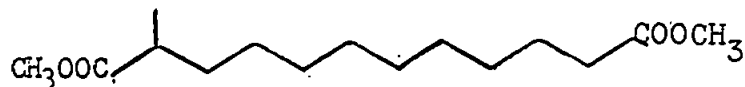


XIX

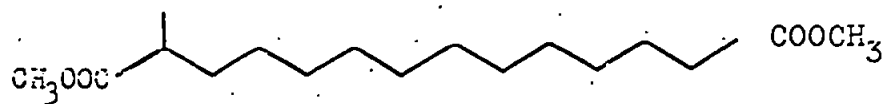
in all spectra ions of m/e 98 represent the base peak and the presence of ions at m/e 74 and 87, quite conclusively indicates that they are straight chain. It should be noted that Ester 38 (Figure LXXVIII) has an impurity at m/e 214 which may be a dimethyl substituted naphthalic ester, the peak at m/e 183 representing loss of methoxy radical, and 155,

loss of carbomethoxy radical, or a methoxy radical and carbon monoxide. High resolution mass spectrometric data verify that the composition of m/e 98 is $C_6H_{10}O$, 112 is $C_7H_{12}O$, 126 is $C_8H_{14}O$, 153 is $C_9H_{13}O$, 185 is $C_{11}H_{21}O_2$, and 199 is $C_{12}H_{23}O_2$.

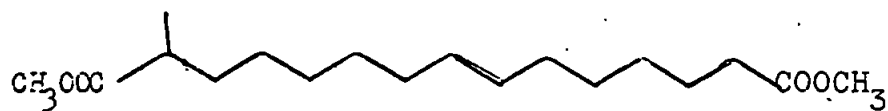
Three α methyl branched dicarboxylic acid esters were also found. Figures LXXXV-LXXXVII give their mass spectra. The assignment of Structures XXI-XXIII (dimethyl 2-methyl-1,12-dioate; dimethyl 2-methyl-1,14-dioate; and dimethyl 2-methyl-1,15-dioate) is based on the prominent loss of m/e 87 (CH_3OCHCH_3) and m/e 73 (CH_3OCCH_2) as indicated by the ions at m/e 185 and 199 in Ester 39, m/e 213 and 227 in Ester 44 and m/e 217 and 241 in Ester 46. In the low mass region there are intense peaks at both 74 and 88, and 98 and 112. This combination of these peaks makes it imperative that one α -carbon be substituted and the other unsubstituted by a methyl group. Note that the impurity at m/e 214 in Ester 39 accounts for the peak at m/e 183 (loss of OCH_3) but does not appear to contribute significantly to the rest of the spectrum.



XX



XXI



XXII

Although the absence of dicarboxylic acids in petroleum naphthenic acid fractions has been noted by Lochte,² Douglas et al. have recently reported the identification of C₈-C₂₂ α,ω-dicarboxylic acids from carboniferous Scottish Torbanite.²⁸

Figure LXXVII. Mass Spectrum of Authentic 1,12 Dimethyl
Dodecanedioate

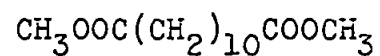


Figure LXXVIII. Mass Spectrum of Ester 38

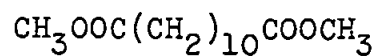


Figure LXXIX. Mass Spectrum of Ester 41

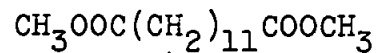


Figure LXXX. Mass Spectrum of Ester 43

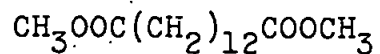
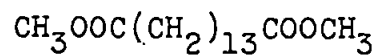
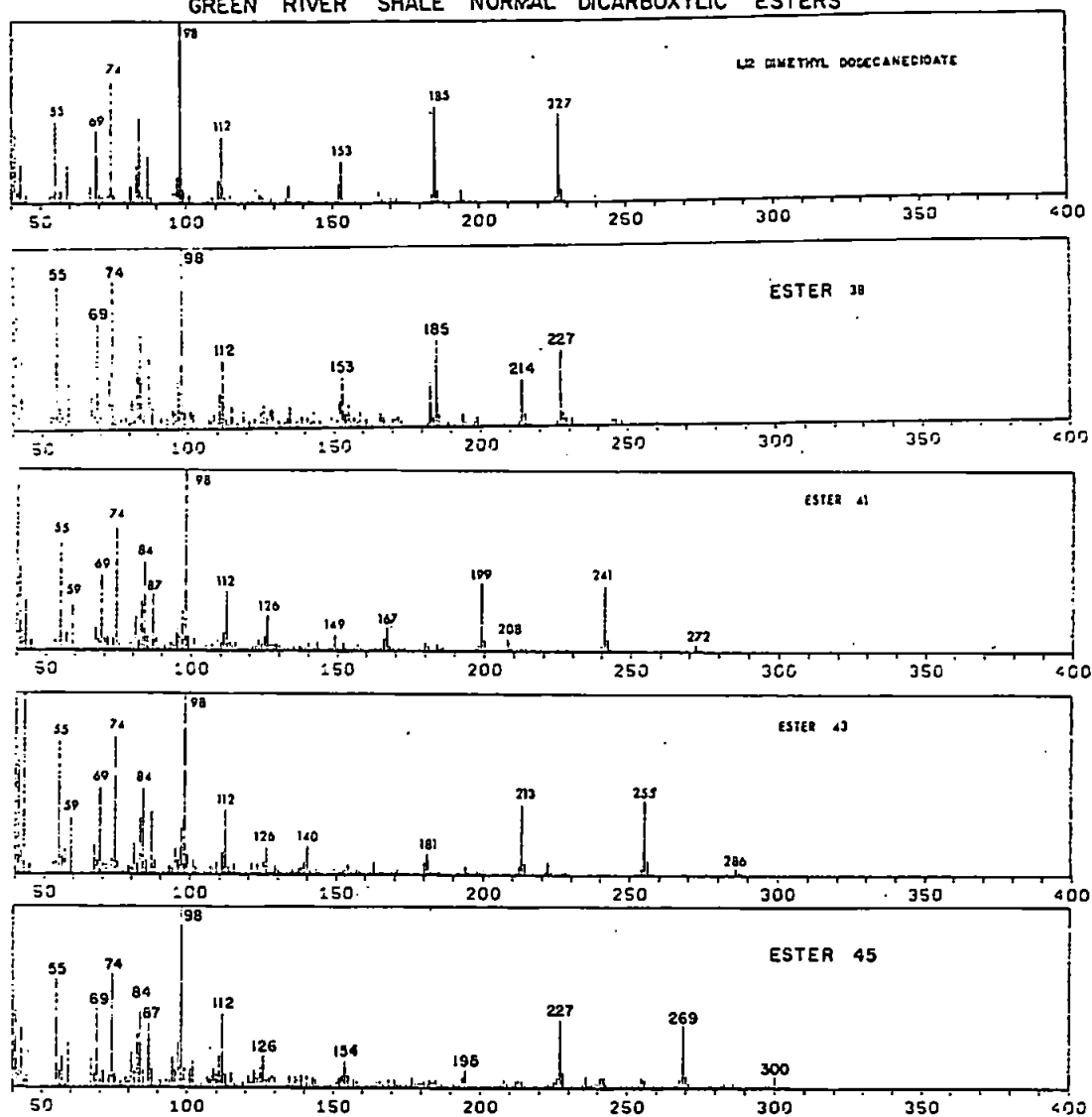


Figure LXXXI. Mass Spectrum of Ester 45



GREEN RIVER SHALE NORMAL DICARBOXYLIC ESTERS



XBL 678-6127

Figure LXXXII. Mass Spectrum of Ester 48

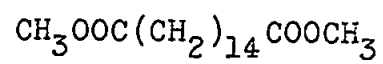


Figure LXXXIII. Mass Spectrum of Ester 51

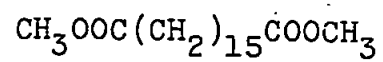
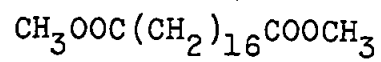
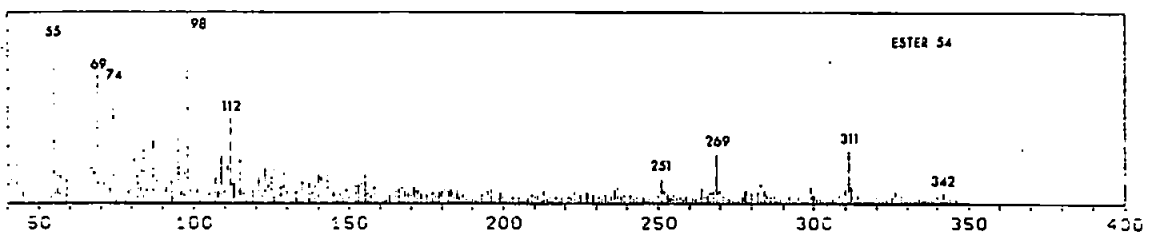
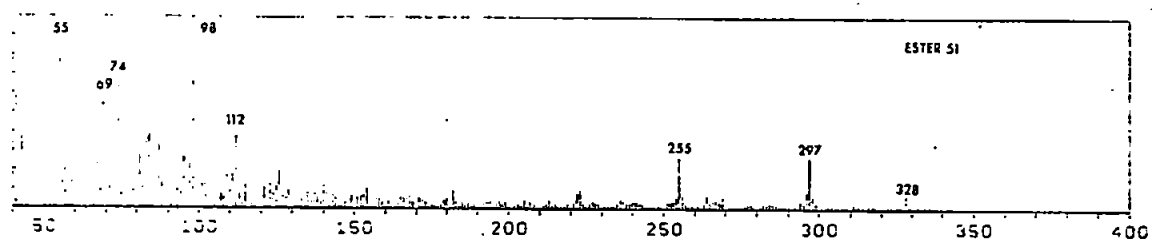
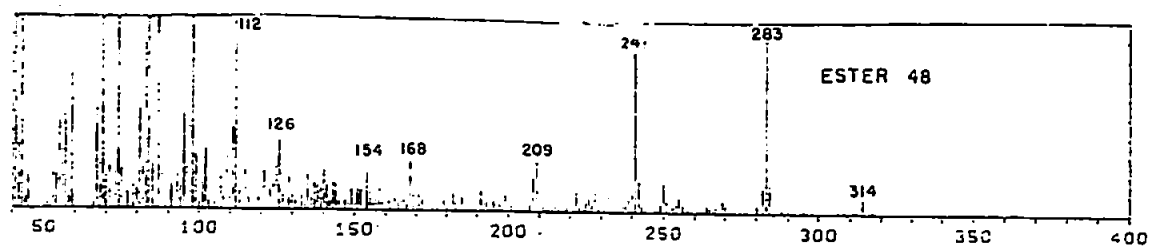


Figure LXXXIV. Mass Spectrum of Ester 54





XBL 676-1124

Figure LXXXV Mass Spectrum of Ester 39

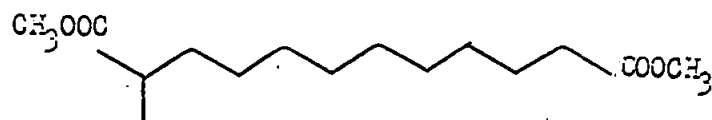


Figure LXXXVI. Mass Spectrum of Ester 44

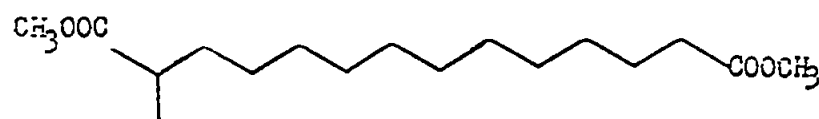
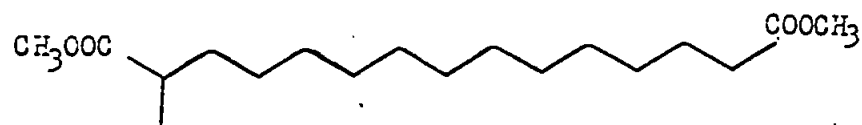
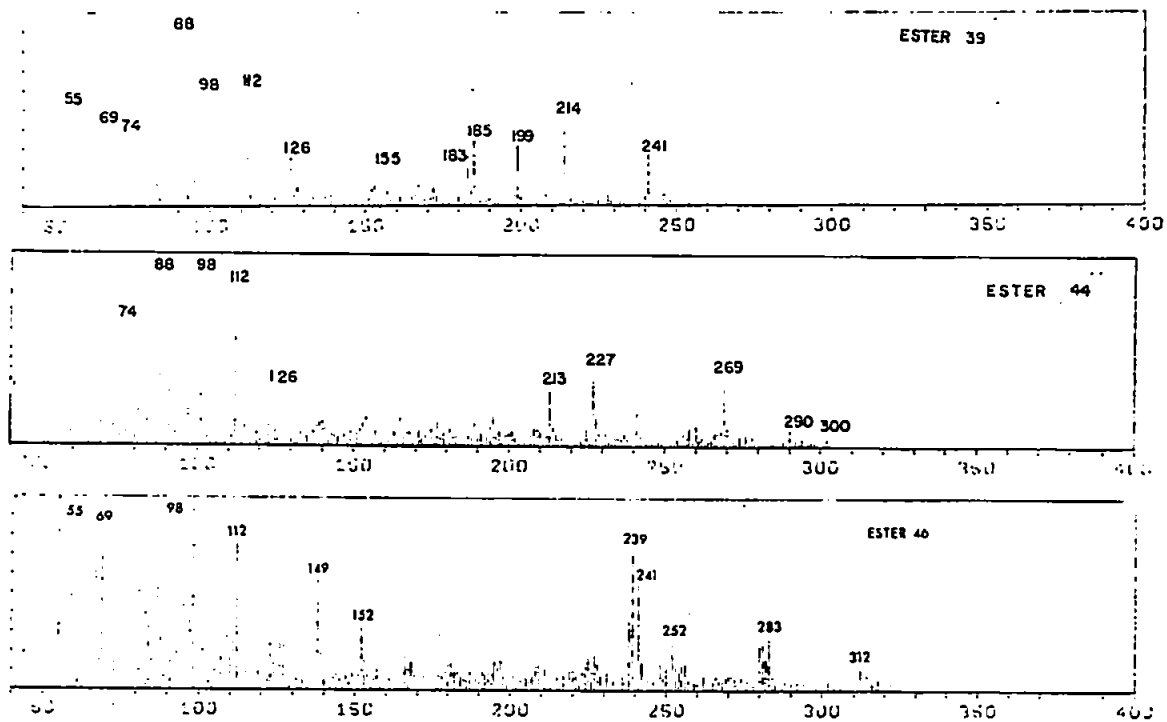


Figure LXXXVII. Mass Spectrum of Ester 46



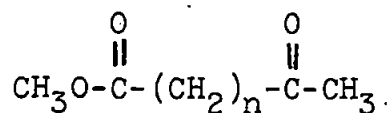
GREEN RIVER SHALE BRANCHED DICARBOXYLIC ESTERS



XBL 678-6128

Keto Acids

Two fractions were obtained which were identified as saturated keto esters by their mass spectra (Figures LXXXVIII-LXXXIX). The first (Figure LXXXVIII) has a molecular ion at m/e 214 and strong peaks at m/e 183 (M-31), 157 (M-57), 125 (M-57-32), 97, 87, 74, 69, and 58 (base peak). The second compound (Figure LXXXIX) has a molecular ion at m/e 256 and peaks at 225 (M-31), 199 (M-57), 167 (M-57-32), 149 (167-18), 87, 74, and 58 (base peak). These data clearly identify the compound of molecular weight 214 as methyl 11-oxydodecanoate (XXIII) and the compound of molecular weight 256 as methyl 13-oxytetradecanoate (XXIV).



XXIII $n = 8$

XXIV $n = 10$

The mass spectra of keto-esters have been studied and their fragmentation patterns are characteristic of aliphatic diketo-functionalization; in particular, the sequence M-31, M-57 and M-57-32, together with the intense peak at m/e 58 (resulting from the McLafferty rearrangement involving the keto grouping), identify these compounds as methylketo-esters. Confirmation of this interpretation is provided by the high resolution spectra* of several fractions which that for the

*Run by Bernd Simoneit.

214 keto ester the peaks at m/e 183, 157, 125, and 58 had compositions $C_{11}H_{19}O_2$, $C_9H_{17}O_2$, $C_8H_{13}O$ and C_3H_6O respectively. The 256 keto ester had peaks at m/e 199, 167, 149, and 58 with the compositions of $C_{12}H_{23}O_2$, $C_{11}H_{19}O$, $C_{11}H_{17}$, and C_3H_6O . An impure sample of the keto acid corresponding to molecular weight 242 was obtained and the presence of the remaining isomer of molecular weight 228 is indicated in a mixture.

The finding of methyl keto acids in an ancient sediment is of some interest, since these long chain terminal keto acids are relatively rare in nature.³¹ Long chain methyl ketones (C_{17} - C_{37}) have been isolated from soil.³² Since it is known that certain micro-organisms such as Penicillium, can metabolize fatty acids up to C_{14} to methyl ketones,^{33,34} it is possible that keto acids represent intermediates of microbial degradation of n-fatty acids. The isolation of such minor components from ancient sediments is thus of some significance with respect to any speculations or deductions on the history of a given sediment and the original diagenetic transformation of organic material.

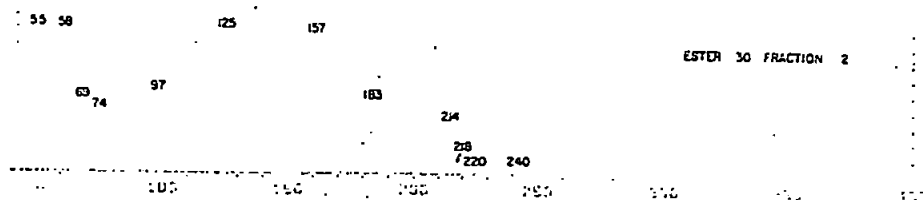


Figure LXXXVIII. Mass Spectrum of Ester 30 Fraction 2

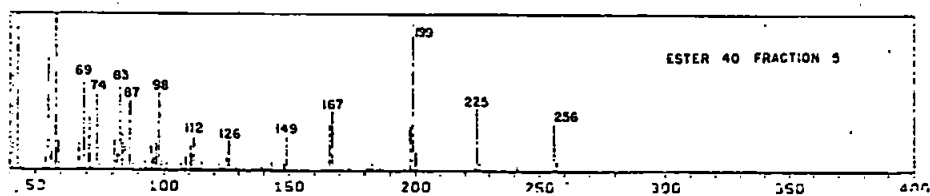


Figure LXXXIX. Mass Spectrum of Ester 40 Fraction 5

Discussion of Esters

For the first time a comprehensive study has been made which has provided interesting and important information for an understanding of the total acid constituents of a shale. Table V summarizes the methyl esters which were isolated and identified (C_7 - C_{12} normal carboxylic acids; C_9 and C_{10} isoprenoid carboxylic acids; C_9 - C_{10} mono-unsaturated acids; C_9 - C_{12} cyclic acids; C_8 (two), C_9 (four), and C_{10} (two) benzoic acids; C_{11} , C_{12} (five), C_{13} (seven), and C_{14} (three) phenylalkyl acids; C_{12} (two), C_{13} , and C_{14} naphthoic acids; C_{11} - C_{13} cyclo-aromatic acids; C_{12} - C_{18} normal α,ω -dicarboxylic acids; C_{13} , C_{15} , and C_{16} mono- α -methyl branched α,ω -dicarboxylic acids; and C_{10} and C_{12} keto acids) in order of GLC elution for comparison of relative abundance as approximated by the gas chromatogram in Figure I.

The data presented permit definite structural assignments only in several instances, where either the mass spectrum itself is unambiguous and conclusive, or where known standard compounds were available for comparison. Nevertheless, in all other cases, at least the type of ester could be clearly identified. It is of particular value, since previous work has concentrated exclusively on the saturated normal or branched acids in the sediment and also from other geological sources. Indeed, at present no aromatic acids, for example, have been reported from either petroleum or sediments.

The identification of series of phenylalkyl, dicarboxylic, naphthoic, cyclic, and keto acids, as well as normal and iso-

prenoid acids is of significance in connection with any theories on the origin of these compounds and their geologic history. Eventually, of course, detailed identification of these acids will have to be undertaken, but the study was intended primarily to investigate the range of acids present. It may thus serve as a basis for structural studies on individual compounds. The normal and isoprenoid acids found are all in the low molecular weight range, and insignificant quantities only of the higher fatty acids appear to be present in our extracts. This contrasts somewhat with the results of Eglinton and collaborators on the same shale, who found that pristanic and phytanic acids (C_{19} and C_{20} isoprenoid acids) are major constituents and isolated normal fatty acids up to C_{20} . Parker and Leo reported the presence of significant quantities of iso-acids, which were not found in this study or by Eglinton's group. This discrepancy in results may be due either to methods of extraction and work up or the inhomogeneous nature of the sediment. Corroboration of the latter idea is born out by the steranes and triterpanes where it was also noted (See Part II) that the two samples examined varied considerably in the types and concentrations of the steranes and triterpanes present. Nevertheless, such discrepancies deserve further attention, and studies utilizing different experimental approaches are urgently needed to define the true acid content of a given shale sample. At the same time, shales from different locations and different depths of deposition should be studied, since such data might shed much light on the origin of the organic material

and the ancient ecologies which may have given rise to it. This was meant as a preliminary study toward future investigations and as such results are not quite final. However, it does serve to illustrate the value of such surveys utilizing high resolution mass spectrometry on the total fraction as well as low and high resolution mass spectrometry on isolated fractions. It has revealed an intriguing distribution of organic acids, but any conclusions as to the significance of the various compound types must await further studies on other shales as well as the detailed definition of the molecular structure of the acids found here.

TABLE VI

Tabulation of Esters Identified from the
Colorado Green River Shale

Ester	Fraction	Identification
Ester 2		C ₉ Cyclic
Ester 4		C ₇ Normal
Ester 6	Fraction 1	C ₈ -C ₉ Cyclic
Ester 7	Fraction 2	C ₈ -C ₉ Cyclic
Ester 8	Fraction 1	C ₉ Isoprenoid
Ester 8	Fraction 2	C ₈ Normal
Ester 9	Fraction 1	C ₉ Mono-unsaturated
Ester 9	Fraction 2	C ₉ Cyclic
Ester 10	Fraction 1	C ₉ Methyl Branched
Ester 10	Fraction 3	C ₉ -C ₁₀ Cyclic
Ester 11	Fraction 1	C ₁₀ Mono-unsaturated
Ester 12	Fraction 2	C ₉ -C ₁₀ Cyclic
Ester 12	Fraction 4	C ₈ Benzoic
Ester 13	Fraction 2	C ₁₀ Cyclic
Ester 13	Fraction 3	C ₈ Benzoic
Ester 14	Fraction 1	C ₁₀ Isoprenoid
Ester 14	Fraction 2	C ₉ Normal
Ester 14	Fraction 3	C ₁₀ Mono-unsaturated
Ester 15	Fraction 4	C ₁₀ Cyclic
Ester 15	Fraction 3	C ₁₀ Cyclic
Ester 16	Fraction 4	C ₉ Benzoic
Ester 17	Fraction 2	C ₁₁ Cyclic
Ester 17	Fraction 3	C ₉ Benzoic
Ester 17	Fraction 4	C ₉ Benzoic

TABLE VI

Tabulation of Esters Identified from the
Colorado Green River Shale

Ester	Fraction	Identification
Ester 18	Fraction 1	C ₁₁ Cyclic
Ester 18	Fraction 3	C ₉ Benzoic
Ester 19	Fraction 1	C ₁₀ Normal
Ester 19	Fraction 2	C ₁₁ Cyclic
Ester 21	Fraction 3	C ₁₁ Cyclic
Ester 22	Fraction 1	C ₁₁ Normal
Ester 22	Fraction 2	C ₁₂ Cyclic
Ester 22	Fraction 3	C ₁₂ Cyclic
Ester 22	Fraction 6	C ₁₀ Benzoic
Ester 23	Fraction 2	C ₁₂ Cyclic
Ester 23	Fraction 5	C ₁₀ Benzoic
Ester 23	Fraction 7	C ₁₀ Benzoic
Ester 24	Fraction 2	C ₁₂ Cyclic
Ester 25	Fraction 4	C ₁₂ Phenylalkyl
Ester 25	Fraction 5	C ₁₂ Phenylalkyl
Ester 25	Fraction 6	C ₁₂ Phenylalkyl
Ester 25	Fraction 7	C ₁₂ Phenylalkyl
Ester 26	Fraction 5	C ₁₃ Phenylalkyl
Ester 26	Fraction 6	C ₁₁ Benzoic
Ester 27	Fraction 5	C ₁₃ Phenylalkyl
Ester 27	Fraction 6	C ₁₃ Phenylalkyl
Ester 27	Fraction 7	C ₁₂ Phenylalkyl

TABLE VI

Tabulation of Esters Identified from the
Colorado Green River Shale

Ester	Fraction	Identification
Ester 28	Fraction 2	C ₁₃ Phenylalkyl
Ester 29	Fraction 3	C ₁₃ Phenylalkyl
Ester 29	Fraction 4	C ₁₂ Phenylalkyl
Ester 30	Fraction 1	C ₁₃ Phenylalkyl
Ester 30	Fraction 2	C ₁₀ Keto
Ester 31	Fraction 3	C ₁₃ Phenylalkyl
Ester 31	Fraction 4	C ₁₄ Cyclo-aromatic
Ester 31	Fraction 5	C ₁₃ -C ₁₅ Cyclo-aromatic
Ester 32	Fraction 2	C ₁₄ Phenylalkyl
Ester 32	Fraction 4	C ₁₄ Phenylalkyl
Ester 33	Fraction 5	C ₁₄ Cyclo-aromatic
Ester 33	Fraction 6	C ₁₂ Naphthalic
Ester 33	Fraction 7	C ₁₂ Naphthalic
Ester 34	Fraction 4	C ₁₄ Phenylalkyl
Ester 37		C ₁₃ Naphthalic
Ester 38		C ₁₂ Normal dicarboxylic
Ester 39		C ₁₄ Branched dicarboxylic
Ester 40	Fraction 5	C ₁₂ Keto
Ester 41		C ₁₃ Normal dicarboxylic
Ester 43		C ₁₄ Normal dicarboxylic
Ester 44		C ₁₆ Branched dicarboxylic
Ester 45		C ₁₅ Normal dicarboxylic

TABLE VI

Tabulation of Esters Identified from the
Colorado Green River Shale

Ester	Fraction	Identification
Ester 46		C ₁₇ Branched dicarboxylic
Ester 48		C ₁₆ Normal dicarboxylic
Ester 51		C ₁₇ Normal dicarboxylic
Ester 54		C ₁₈ Normal dicarboxylic

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Part IV

High Resolution Mass Spectrometry: A Study of Shale Extracts

High Resolution Mass Spectrometry

Single focusing mass spectrometers, capable of resolution $M/\Delta M$ of the order of 1000-2000, have been employed in organic chemical and geochemical research for some time. These instruments permit the determination of the nominal mass of the molecular ion and recognition of the fragmentation pattern. Resolution and accuracy of mass measurement is, however, insufficient to permit the determination of elemental composition of either molecular or fragment ions. Double focusing instruments (high resolution mass spectrometers), first introduced to organic chemical applications by Beynon¹ and since then exploited successfully in various structural and mechanistic studies in this laboratory as well as those of Biemann, Lederer, and Djerassi to name a few, provides this capability. Commercially available instruments are capable of resolution in the order of 20-30,000, and grosses an accuracy of mass measurement (in the order of a few parts per million) which permits the calculation of elemental composition of any mass peak in the high resolution mass spectrum. The advantages of such instruments to geochemical studies are evident in particular when investigations of the heteroatomic species of a geochemical sample are considered. For example, in a high resolution mass spectrum the molecular ion peaks of methyl methylnapthoate, methyl undecanoate, and a C_{10} keto methyl ester--all possessing a nominal molecular weight of 200, would be clearly separated and their elemental composition could be determined accurately. The capability of

determining elemental composition of molecular and fragment ions, facilitates, of course, the interpretation of the spectrum of pure compounds, and permits the recognition of compound types present in a mixture.

For these reasons, high resolution mass spectrometry has found early applications to geochemical studies. Carlson et al.² first introduced the technique in their study of aromatic compounds in petroleum, and since then the work of Lumpkin³, Reid⁴ and Johnson and Aczel⁵ is illustrative of the application to various studies of petroleum fractions. Concurrently, this exploitation of computer methods^{6,7} to the reduction of high-resolution mass spectral data has advanced considerably, and fairly sophisticated approaches to data reduction, handling and presentation have been worked out.^{6,8,9,10} For a detailed discussion of high resolution mass spectrometry and computer applications to chemical problems the work of Smith¹¹ should be consulted, and recent geochemical studies utilizing high resolution instrumentation in conjunction with sophisticated computer methods have been presented by Hayes.¹² A recent review of the status of high resolution mass spectrometry in organic geochemistry is available also,¹³ so that an extensive discussion of the methodology and technology of this research tool is not required here.

In this work high resolution mass spectrometry was applied to the study of several total shale extracts and of acidic and basic mixtures, in an attempt to gain preliminary information on the heteroatomic content of the sediments, and to deduce,

whenever possible, some structural features of capacity present in these sample mixtures. In Part III high resolution mass spectral data obtained on individual compounds or GLC fractions have been cited in support of various structural proposals. In this section concentration will be on the high resolution experiments performed on total extracts, and the interpretation will be concerned with the recognition of compound types and their distribution, rather than with structural assignments to individual components.

Instrumentation

A high resolution mass spectrometer employing the Mattauch-Herzog geometry (Model 21-110B, Consolidated Electrodynamics Corporation) was used in these experiments. The instrument had a resolution of about 20,000, and mass measurement accuracy of ± 3 mmu could be achieved. An electron bombardment source was used to ionize the sample (70 eV). The complete high resolution mass spectrum of sample and calibration compound was recorded on a photoplate. Positions of all lines and their intensity (as percent transmission) on the plate were subsequently measured via a precision microphotometer (Jarrell-Ash, Model 23-500). Digitized data from the microphotometer were recorded on magnetic tape, from which accurate mass and elemental composition of each ion were calculated by computer (IBM 7090). The computer output was in the form of a listing of accurate mass of each peak together with empirical

composition. From these listings, high resolution spectra (as shown in Figure IV, for example) were computer-plotted whenever desired. Smith¹¹ gives a detailed description of the instrument and computer techniques utilized in this work.

Sediments Examined

High resolution mass spectra of total organic extracts of the Pierre, Nonesuch, and Soudan Shale were obtained. For a description of these shales see Part I. Spectra were also measured of the neutral, basic and acidic fraction obtained from the extract designated "Washings" (See Part III) from the Green River Shale, as well as two individual components isolated from the acidic fraction.

Samples were introduced into the mass spectrometer via a direct introduction system (similar to the probe described in Part I). Several plate exposures were obtained for each sample as the source temperature was slowly raised to a maximum of about 250°. Perflorokerosene, contained in an auxiliary reservoir was used as the mass calibration compound. The data are presented here as element plots. All ions were sorted by computer according to their heteroatomic content, i.e. CH, CHO, CHO₂, CHN, etc. The individual species were then plotted against mass number. This method is no longer in use in these laboratories and has been replaced by heteroatomic plotting techniques which permit the direct reading of the elemental composition of each ion.^{10,11}

The two individual acids obtained from the Green River Shale acidic fraction were isolated by GLC methods. The conditions were: 10' x 1/4" column, of 3% SE-30 on gas-chrom

Q, helium flow rate of 45 ml/min., programed at 4°/min., from 75°-300°. The acids were identified by the temperature at which they were eluted from the column, i.e. "105° Acid" and "150° Acid." Esters were prepared by refluxing the total mixture with BF_3 /Methanol reagent (1 hr.), followed by partial removal of solvent, dilution with water, and extraction of the esters with petroleum ether.

Green River Shale Neutral Fraction

As expected the high resolution spectrum of the Neutral Fraction showed a preponderance of C/H ions, corresponding mainly to saturated alkyl fragments, in agreement with the high hydrocarbon content of this shale. Two interesting O_2 -containing species are notable which could correspond to the methyl esters of C_{18} and C_{19} acids respectively (note $\text{C}_3\text{H}_6\text{O}_2$ peak at 74 typical for methyl esters).

Green River Shale Basic Fraction

The basic fraction of the Green River Shale shows particularly intense peaks in the C/H N plot of the high resolution mass spectrum (Figure I). (It should be noted that the C/H O_3 and C/H N plots are very similar; this is due to the fact that distinction between combinations of C/H O_3 and C/H N is difficult a priori since such peaks fall within the error limit of the mass spectrometer - the computer therefore plots both combinations.) Peaks at m/e 107, 121, 135 in the C/H N plot could correspond to molecular ions of simple alkyl pyridines, and fragment ions at even mass of m/e 106, 120,

134, 162 would be a further indication of this group of compounds.

A series of alkyl indoles seems to be indicated by the fragment peaks of m/e 130, 144, 158, 172, 186, 200, and 210, and the series at m/e 143, 157, 171, 185, 199, and 213 would represent the molecular ions of C_1 - C_6 alkyl quinolines or iso-quinolines. Fragment ions at even mass corresponding to this series can be noted also. The C_3 , C_4 , and C_6 alkyl quinoline (m/e 171, 185, 213) appear to be present in greatest abundance. A fourth series is apparent from the spectrum, commencing with the peak of m/e 133, and containing m/e 147, 161, 175, 189, 203, 217 to which the structure of tetrahydroquinolines (from C_0 to C_6) could be ascribed, but, of course, other cyclopyridines would also give rise to this series. Even mass peaks, such as the strong ions at m/e 146, 160, 174 could represent fragments. Alkyl pyrroles may be present, since the peaks at m/e 207 and 193 to the C_{10} and C_9 alkyl pyrrole molecular ions. The interpretation of other plots of the base fraction is difficult since many possibilities must now be considered, and no firm conclusion could be drawn.

Acidic Fraction

The high resolution mass spectrum (Figure II) is dominated by abundant ions in the C/H , $C/H O$ and $C/H O_2$ category, as might be expected for a fraction containing predominantly saturated acids. The intense peaks at m/e 60 and 74 ($C_2H_4O_2$ and $C_3H_6O_2$) correspond to the expected rearrangement ions of saturated

acids, whereby the intensity of m/e 74 peak suggest contribution from branched acids. The ions at m/e 73, 87, 101, 115, 129, 143, 157, 171, 185, and 199 are the expected fragment ions of the general composition $C_nH_{2n-1}O_2$, typical for saturated alkyl acids. The corresponding molecular ion of these acids can be found at mass 102, 116, 130, 144, 158, 172, 186, and 200, whereby the C_{11} acid at m/e 186 seems to be a major component. The most intense molecular ions on the C/H C_2 plot are due to cyclic and aromatic species. This may of course not reflect their greater abundance, since cyclic and aromatic esters would be expected to show more prominent molecular ions. Thus the series at m/e 114, 128, 142, 156, 170, 184, and 198 could correspond to monocyclic saturated acids (C_6 - C_{12}), the C_{10} -acid at m/e 184 ($C_{11}H_{20}O_2$) being the major component. The C_6 -acid could correspond to cyclopentyl carboxylic acid, but a definite structure can, of course, not be assigned. Some intense odd-mass ions, arising probably by fragmentation of these cyclic acids should be noted: m/e 113, 127, 141, and particularly 155 (170-15?) are the most prominent.

A further homologous series may be recognized: phenyl alkyl acids give rise to the series of peaks at m/e 122 (benzoic acid), 136 (methylbenzoic), 150 (dimethylbenzoic ?) 164, 178, 192, and 220; the acid of molecular weight 178 (C_5 alkyl phenyl) appears to be the major constituent of this group. Indications of cyclo-aromatic compounds are present: the peaks at m/e 218, 204, and 190 belong to this category, and the peaks at m/e 189 and 175 could be explained as $M-CH_3$ fragments from m/e 204 and 190 respectively.

Green River Shale Esters

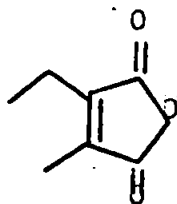
It is interesting to compare the results obtained for the free acids with those revealed by the high resolution spectrum (Figure III) of the corresponding methyl esters. It is seen that the pattern is quite similar. The C/H O_2 plot again shows intense rearrangement ions at m/e 74 and 88 (for methyl esters) and the corresponding series of $C_nH_{2n-1}O_2$ peaks (87, 101, 115 ...). The aromatic group is evident with molecular ion peaks at m/e 150, 164, 178, 192, 206, 220, and 234 (shifted by 14 mass units since methyl esters). Likewise the cyclic series can be easily recognized; it suffices here to point out the dominate C_{10} compound at m/e 184 and the corresponding fragment peak (M- CH_3) at m/e 169, which relates to the peaks of m/e 170 and 169 seen in the acid spectrum.

Notable also are two components evident in the C/H O_2 -aromatic plot. Molecular ions at m/e 200 and 214 correspond to $C_{13}H_{12}O_2$ and $C_{14}H_{14}O_2$ and thus identify these compounds as the methyl C_1 -alkylnapthoate and a methyl C_2 -alkylnapthoate.

This comparison of acids and esters, shows that our results are consistent and in this sense, the interpretation is put on a firmer basis. To confirm some of our assignments and to test the results several free acids were isolated by GLC methods and separately analyzed. Results for two of them are presented.

105° Acid. The high resolution mass spectrum (Figure IV) exhibits a molecular ion at m/e 140 of composition $C_7H_8O_3$. It is to be noted that this same ion appeared in the C/H O_3 plots

of the acids (Figure II). The spectrum shows some loss of methyl group (m/e 125) and prominent loss of carbon monoxide (m/e 112 in C/H O_2 plot). A peak due to loss of H_2O should also be noted (m/e 122). The composition requires a fairly unsaturated system. These data do not permit definite structure assignment. A trihydroxytoluene derivative would be a possibility, but carbonyl absorption in the infrared spectrum does not agree with such a postulate. Ethyl methyl maleic anhydride (I) would fit the composition also, and seem to agree reasonably well with all data.

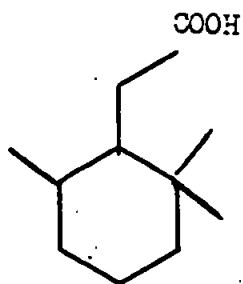


I

Such a compound could be expected since dimethyl maleic anhydride has been found in California petroleum.¹⁴

150° Acid. The high resolution mass spectrum of this acid (Figure v) clearly indicates the compound to be the major cyclic acid already noted in the mixture spectrum (Figure II). The molecular ion at m/e 184 ($C_{11}H_{20}O_2$) requires one degree of unsaturation or one ring. The cyclic nature of this substance is evident from its fragmentation pattern: Loss of methyl is very prominent (m/e 169) and some loss of ethyl is also observed (m/e 155). The peak at m/e 125 in the C/H plot, could correspond to the elimination of 59 mass units and would thus indicate an acetic acid side chain. The

peak at m/e 109 (C_8H_{13}) could be rationalized as resulting from the sequence $M-C_2H_4O-CH_3$. The spectrum would thus suggest a structure of type II, in which the position of substituents is of course speculative at this time. This acid has however been isolated from petroleum fractions.¹⁵



II

Nonesuch Shale

The striking feature of the spectrum (Figure VI of the Nonesuch Shale is the almost total absence of heteroatomic species. Hydrocarbon ions predominate. From the C/H plot the typical alkyl ions are readily discernable, but detailed interpretation would be fruitless, since the spectrum is extremely complex. This example illustrates, however, very nicely the value of preliminary high resolution data, in estimating the distribution of various component types; extraction of the Nonesuch Shale would be expected to yield very little basic and acidic material according to these data.

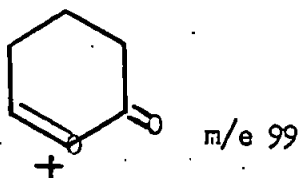
Soudan Shale

The second Precambrian sediment examined gave very similar results: the spectrum (Figure VII reveals almost exclusively

hydrocarbon species. The C/H plot, as in the case of the Nonesuch, is complex and shows peaks at almost all masses. Ions containing heteroatoms are negligible. Those apparent in the C/H O₃ and C/H O₄ plots are probably contributed by the calibration compound.

Pierre Shale

The results for the Pierre Shale (Cretaceous) contrast sharply with those obtained for the Precambrian rocks and resemble more closely the Green River data. Again hydrocarbon ions are most abundant (in the C/H plot of Figure VIII note that the intensity of the peaks above 100 in the C/H plot have been arbitrarily increased - X 10 - to permit reading of the plot) but the contribution of heteroatomic species (note in particular the C/H O₂ and C/H N plots) is quite notable. The C/H O₂ plot shows a strong rearrangement peak at m/e 60, expected for fatty acids. The peaks at high mass are too small to permit definite assignments, but the ion of m/e 186, corresponding to a C₁₁-saturated acid is noteworthy. Furthermore, odd-mass peaks at m/e 87, 101, 115, 129 are present. The ion at m/e 99 is intriguing, since it might correspond to a lactone fragment, i.e.



The C/H N plot also exhibits an interesting pattern. The ion at m/e 129 (C₉H₇N) could correspond to quinoline (or iso-

quinoline). A higher homologue of m/e 143 appears to be present. A C_1 -tetrahydroquinoline (or equivalent structure) is evidenced by the peak at m/e 147, and the intense ion of m/e 219 and that at m/e 163 could represent C_{10} - and C_6 -alkyl pyridines respectively. A more detailed interpretation of other plots seems futile at present since the structural possibilities become enormous. The peak at m/e 87 in the C/H NO plot might represent, for example, a rearrangement ion of amides.

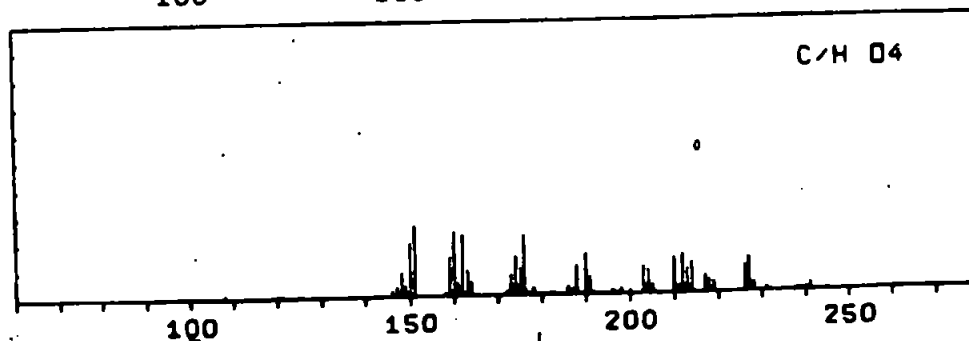
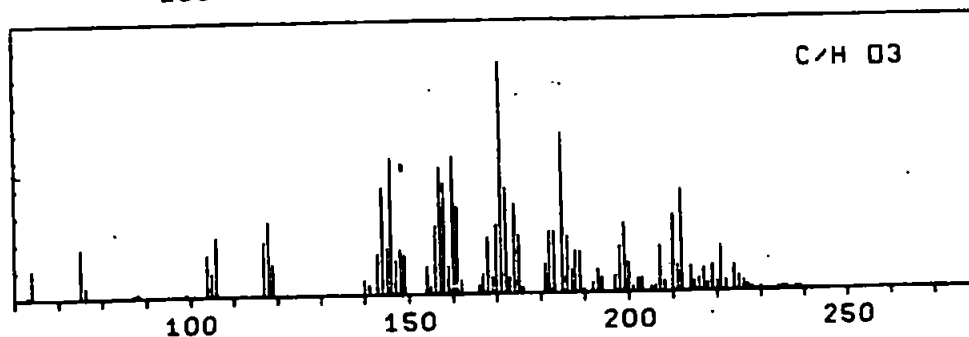
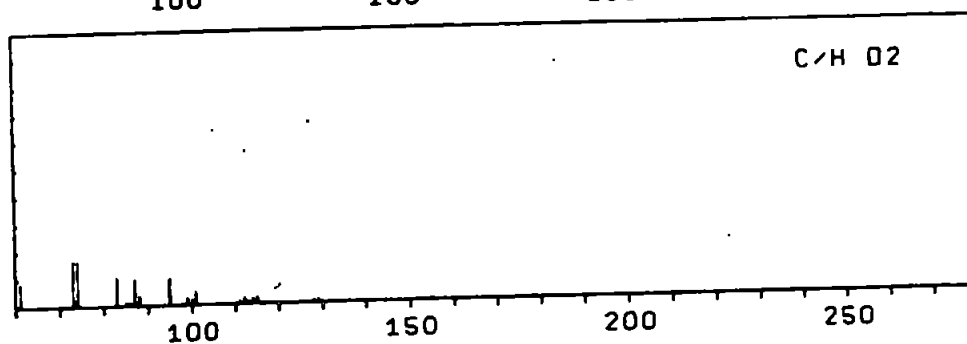
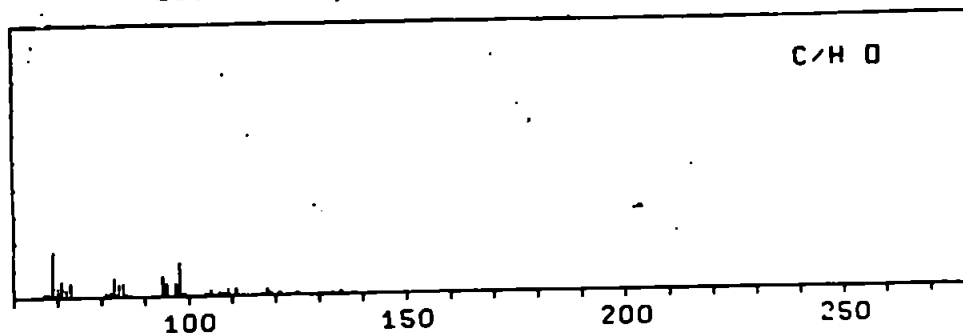
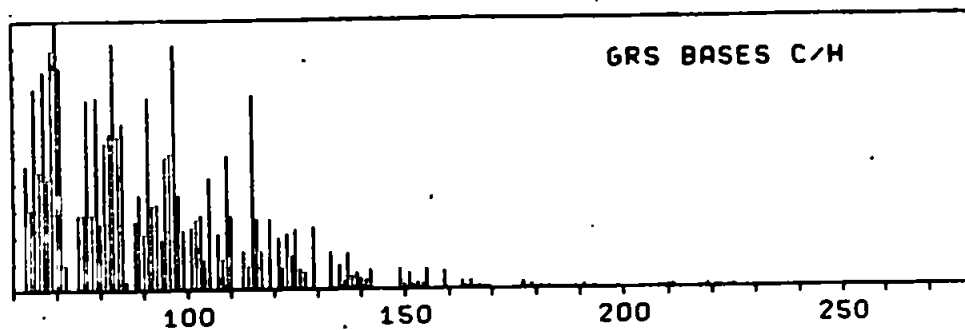
Discussion

The preliminary nature of these results and of the experimental method should be stressed. No detailed information was sought, rather a study into the possibilities of using high resolution mass spectrometry for the analysis of complex geochemical samples was intended. The preceding interpretation illustrates, however, the potential application of the technique. The special advantages of the method are its rapidity and the basic nature of the information obtained. Much information on the distribution of heteroatomic species and their structural type is readily made available by these spectra, and more detailed experiments can then be planned with such data at hand. Furthermore the results of detailed analysis can then be checked against those of the preliminary investigation. A good example of this, is provided by the

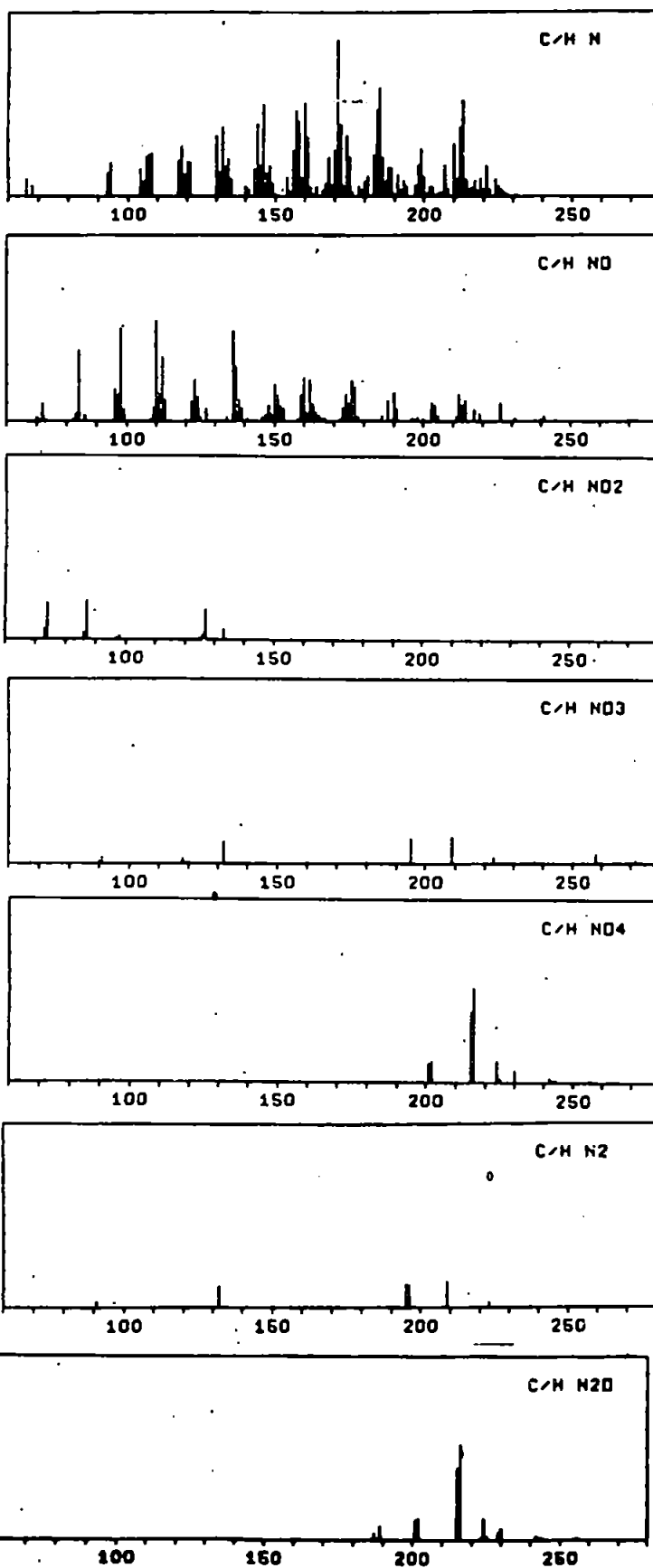
comprehensive analysis of Green River Shale acids, presented in Part III. A comparison of the high resolution data discussed above, and the results of Part III, makes evident immediately that many conclusions drawn solely from the high resolution spectrum of a complex mixture, were indeed born out by the detailed experiments described in Part III.

It should also be obvious that the methods and techniques used in these experiments were relatively crude. Much refinement is possible; in particular the use of low energy electrons to avoid fragment peaks, or the use of field ionization for the same purpose, would permit much more detailed analysis. Computer methods could be refined, to the extent that preliminary sorting and interpretation according to compound type must not be done by the operator. Finally chemical fractionation according to compound types would allow a much more definitive interpretation of the resulting spectra. With these possibilities in mind, it is felt that the data presented above demonstrate quite convincingly the extraordinarily powerful data high resolution mass spectrometry can furnish in organic geochemical investigations.

Figure I. High Resolution Mass Spectrum of Colorado
Green River Shale Bases (from Washings)

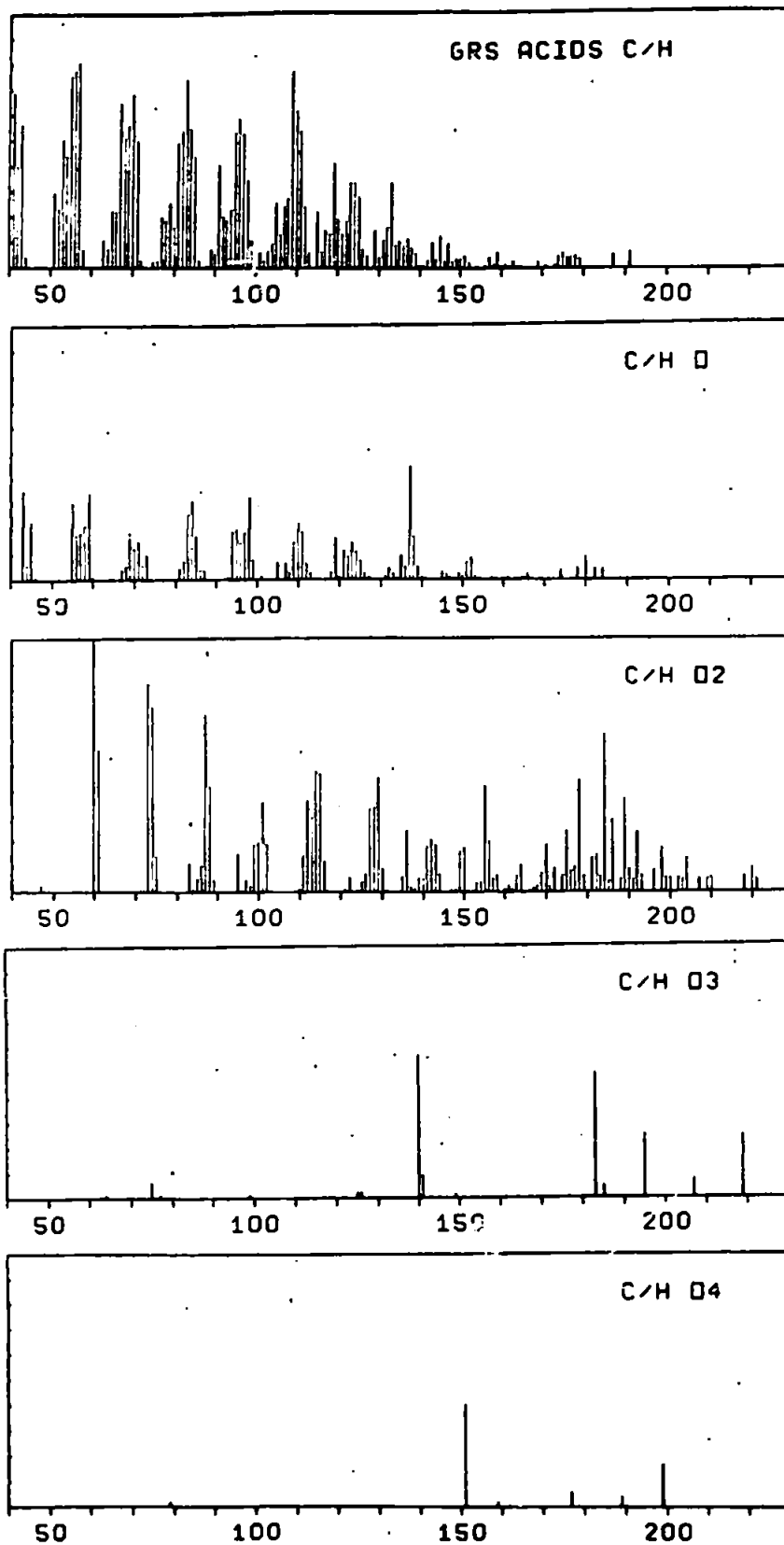


GREEN RIVER SHALE BASIC FRACTION
(ALL SPECTRA X5 ABOVE 208)

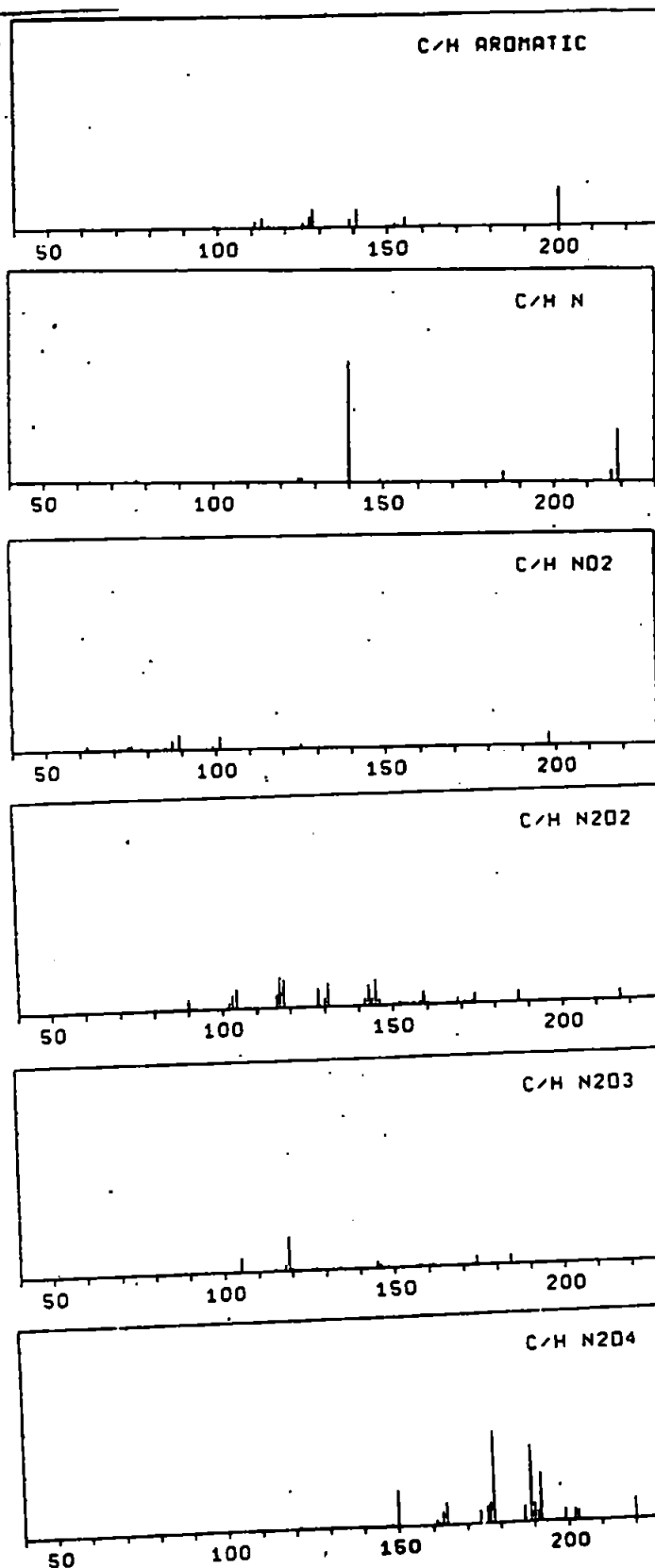


COLORADO GREEN RIVER SHALE BASIC FRACTION
(ALL SPECTRA X5 ABOVE 200)

Figure II. High Resolution Mass Spectrum of Colorado
Green River Shale Acids (from Washings)

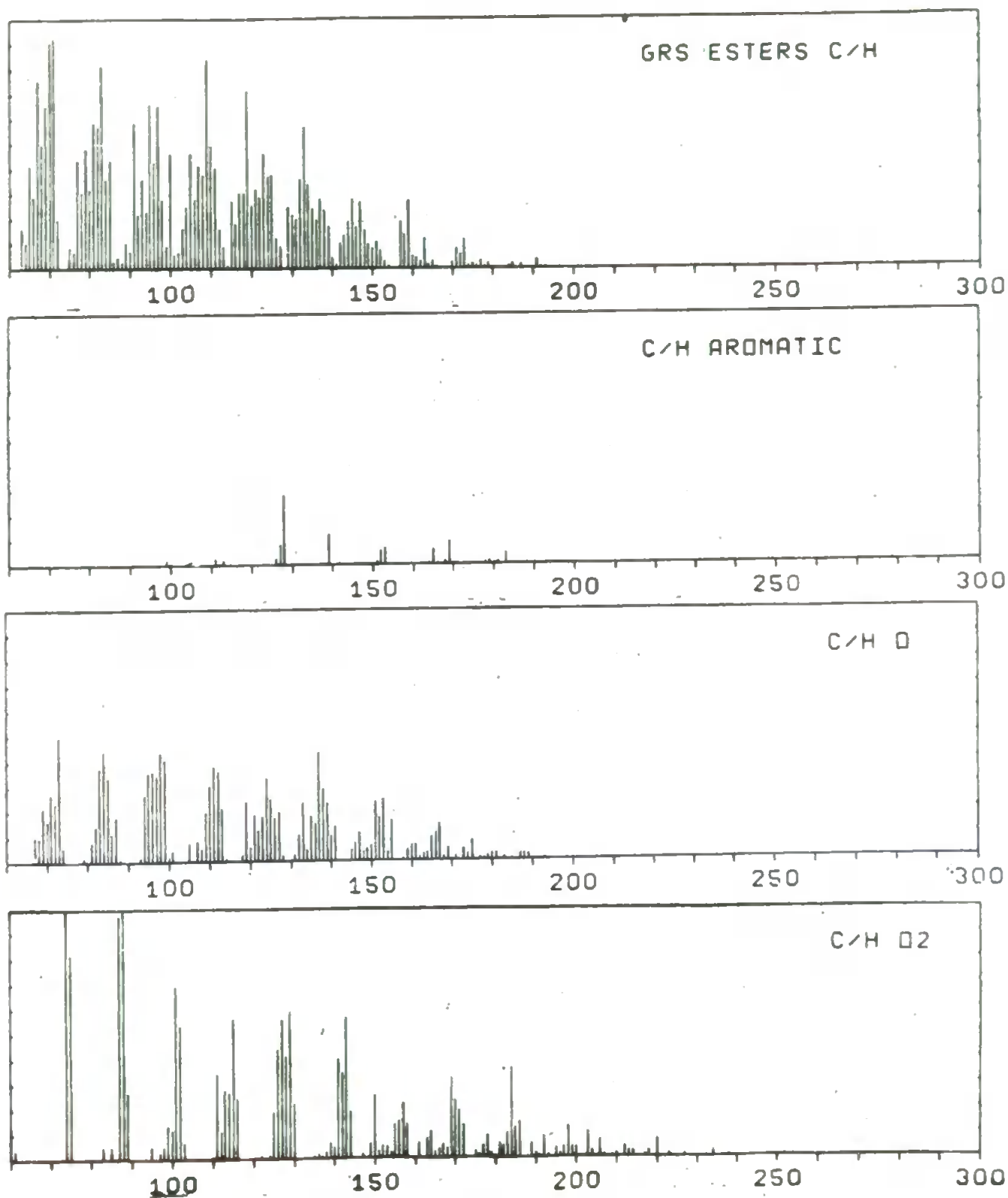


COLORADO GREEN RIVER ACID FRACTION
(ALL SPECTRA X10 ABOVE 173)

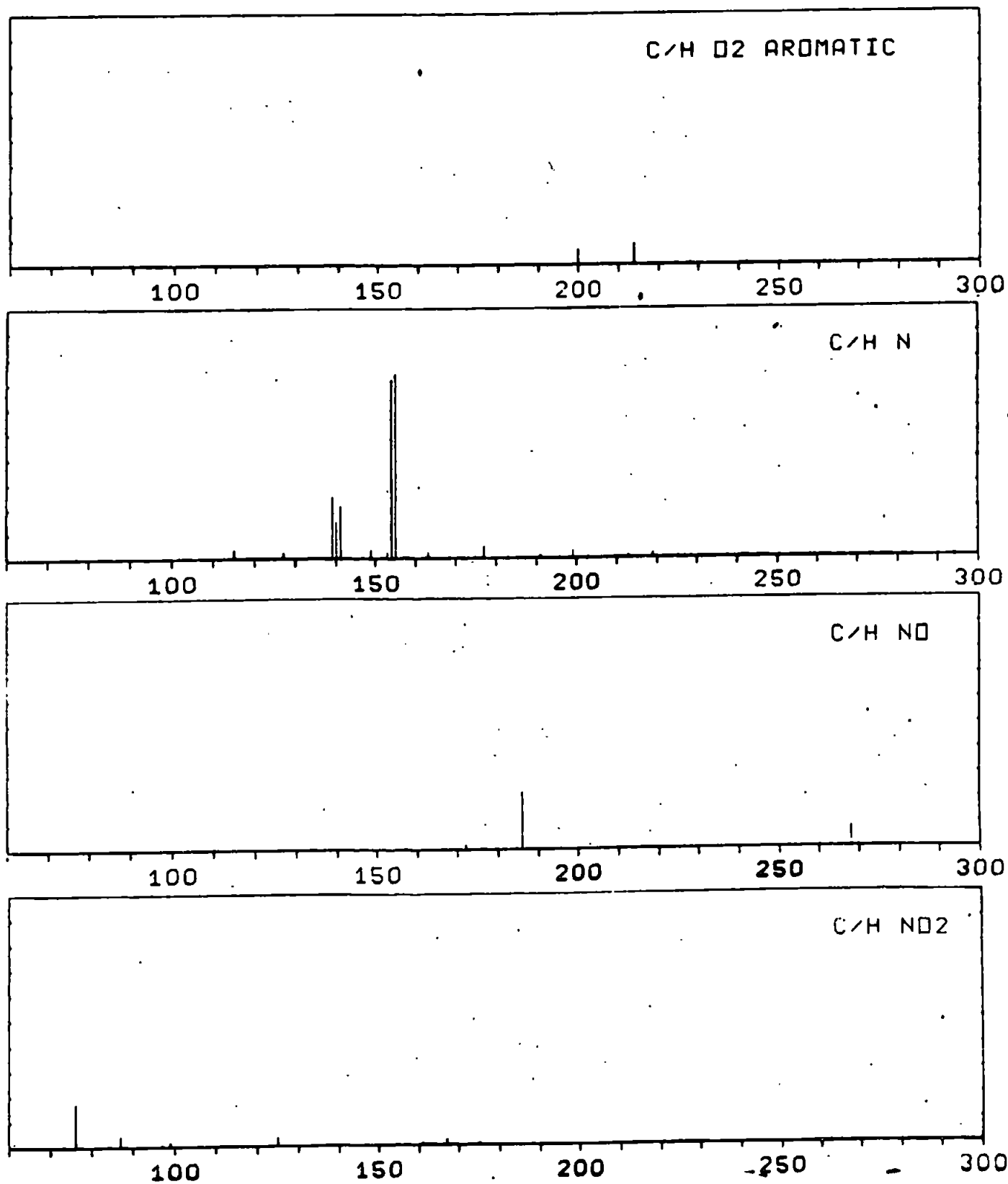


GREEN RIVER SHALE ACIDIC FRACTION
(ALL SPECTRA X10 ABOVE 173)

Figure III. High Resolution Mass Spectrum of Colorado
Green River Shale Esters (from Washings)

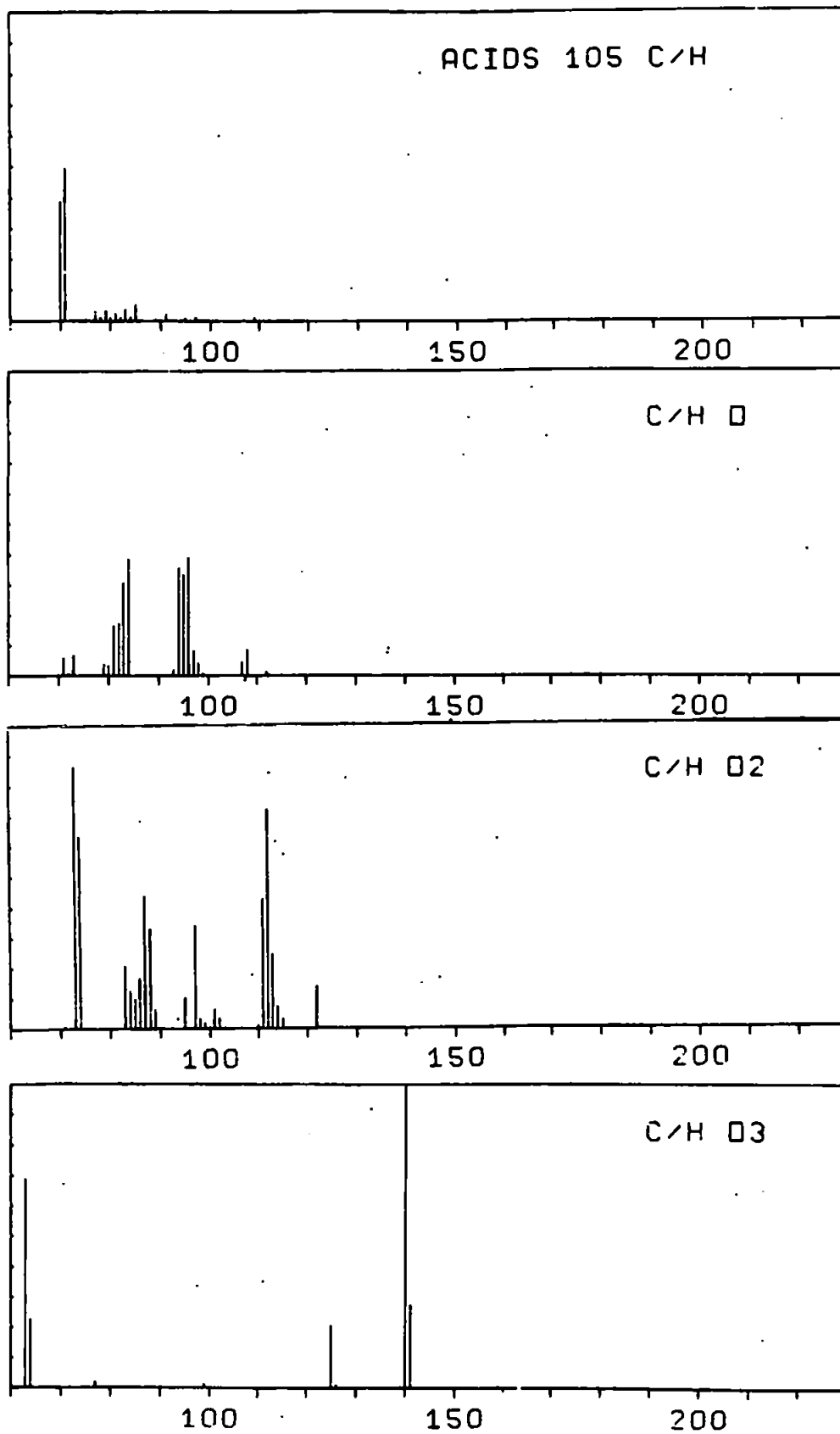


COLORADO GREEN RIVER SHALE ESTERS



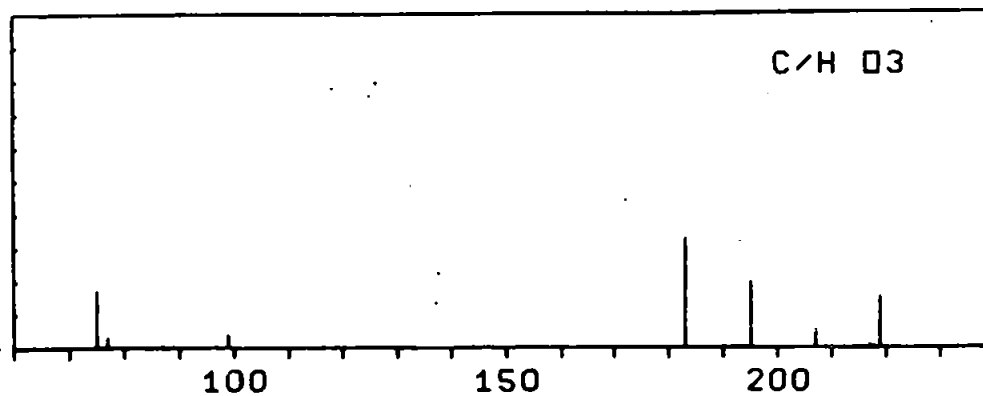
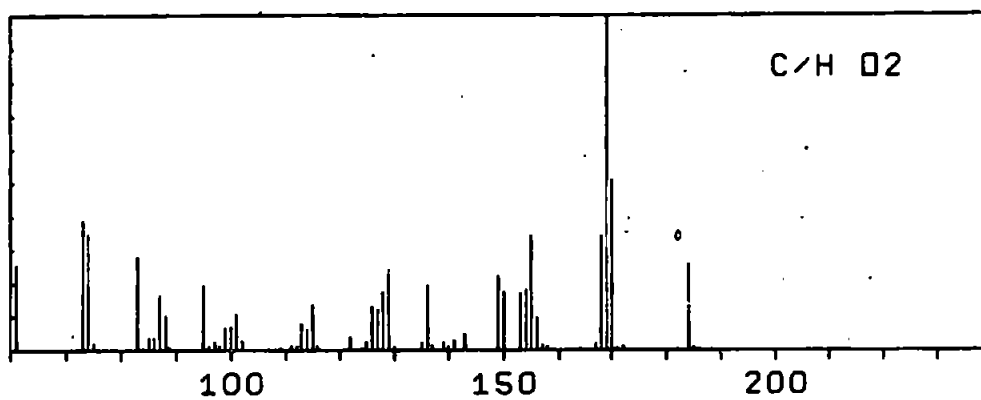
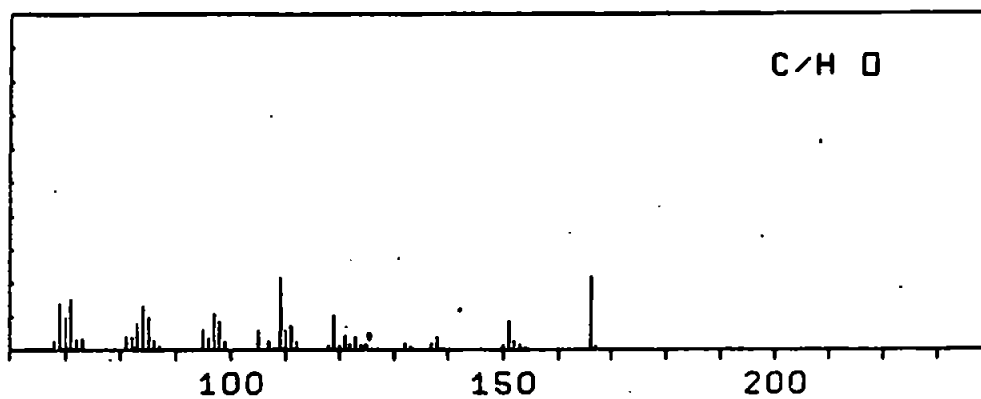
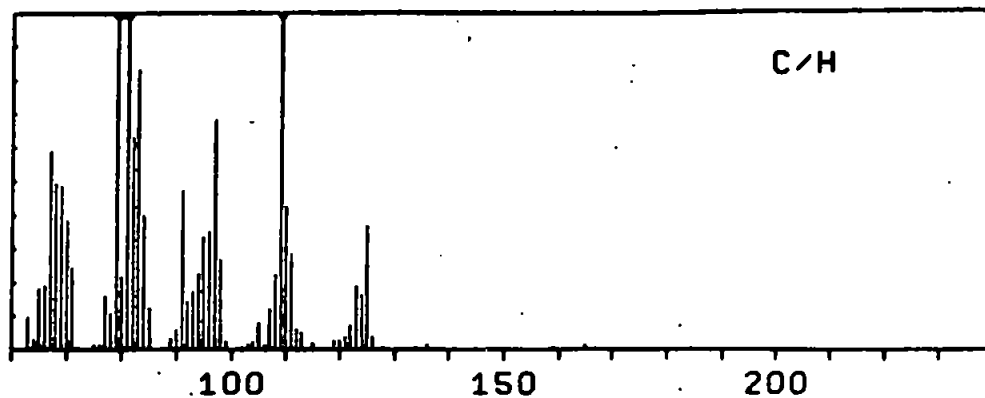
COLORADO GREEN RIVER SHALE ESTERS

Figure IV. High Resolution Mass Spectrum of Colorado
Green River Shale 105° Acid (from Washings)



COLORADO GREEN RIVER SHALE 105° ACID

Figure V. High Resolution Mass Spectrum of Colorado
Green River Shale 150° Acid (from Washings)



COLORADO GREEN RIVER SHALE 150° ACID
(ALL SPECTRA X10 ABOVE 152)

Figure VI. High Resolution Mass Spectrum of Nonesuch

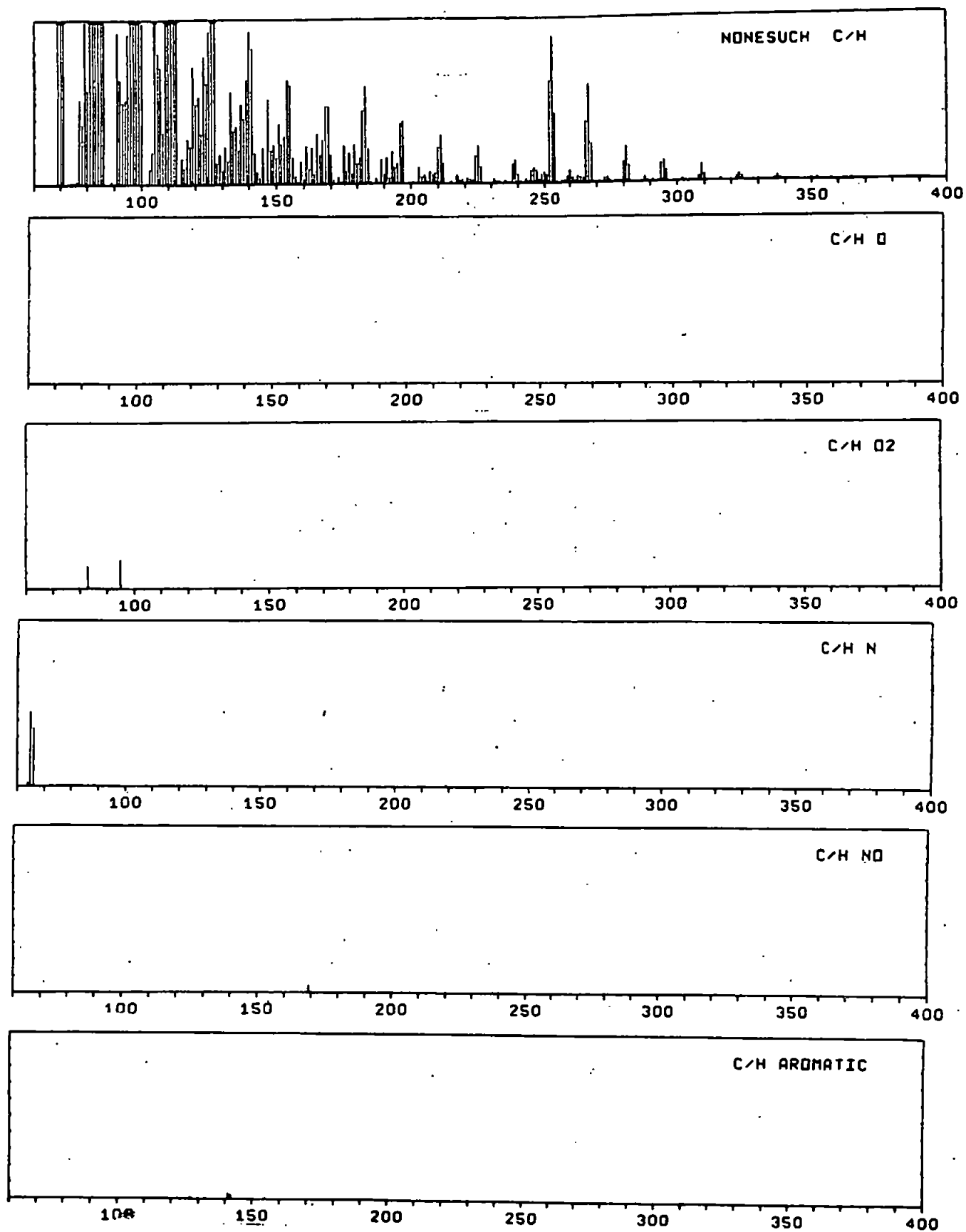
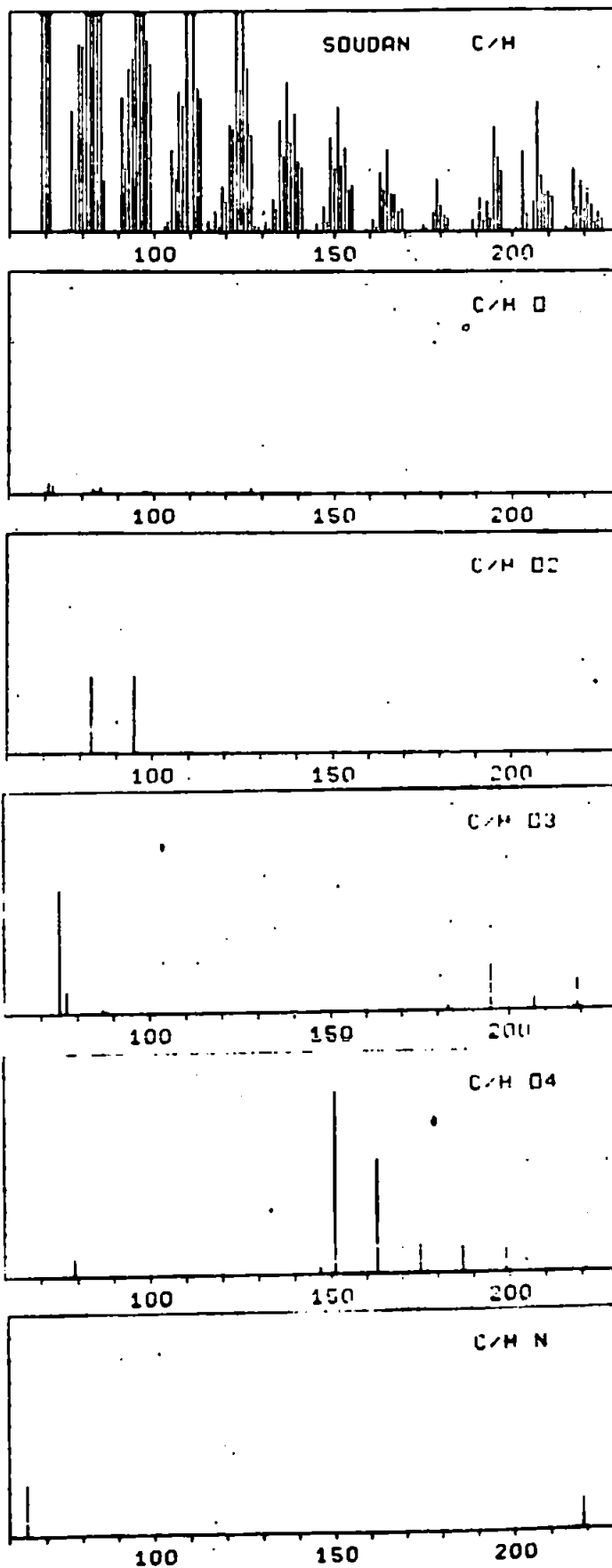
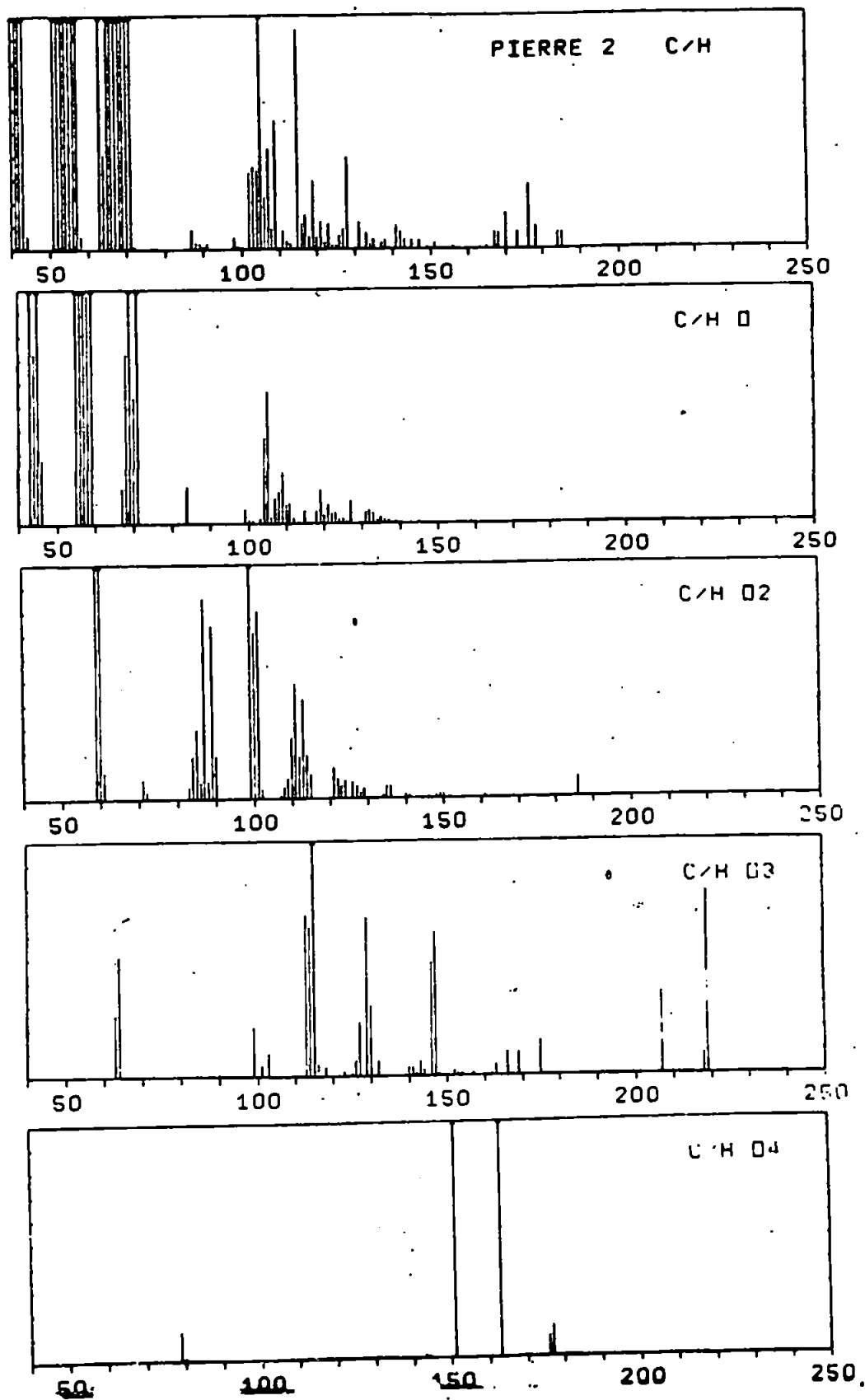


Figure VII. High Resolution Mass Spectrum of Soudan

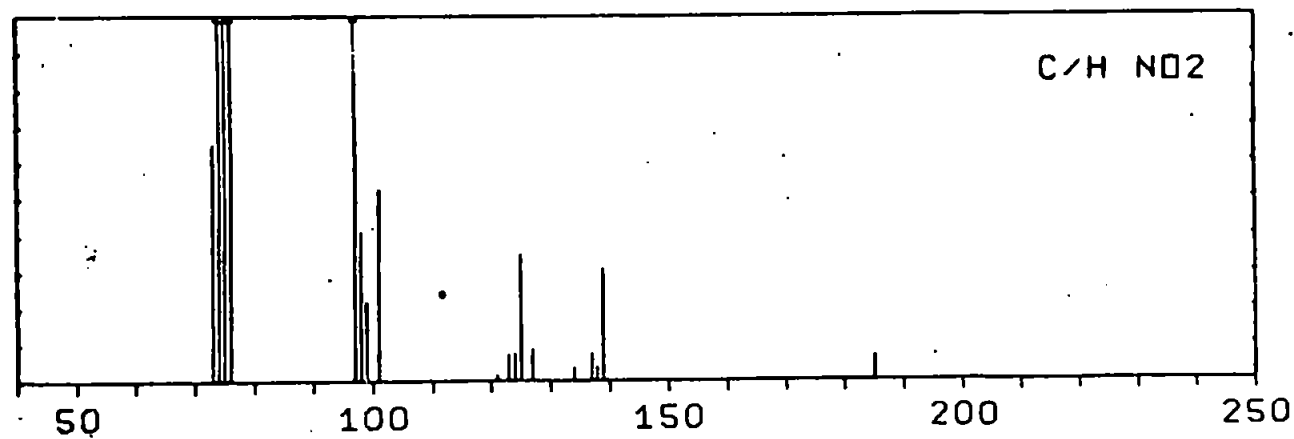
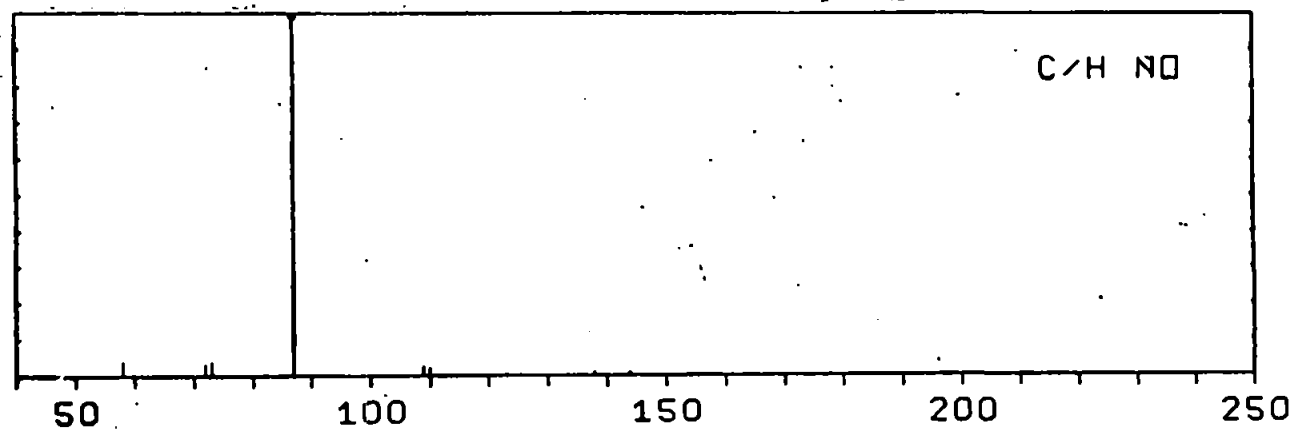
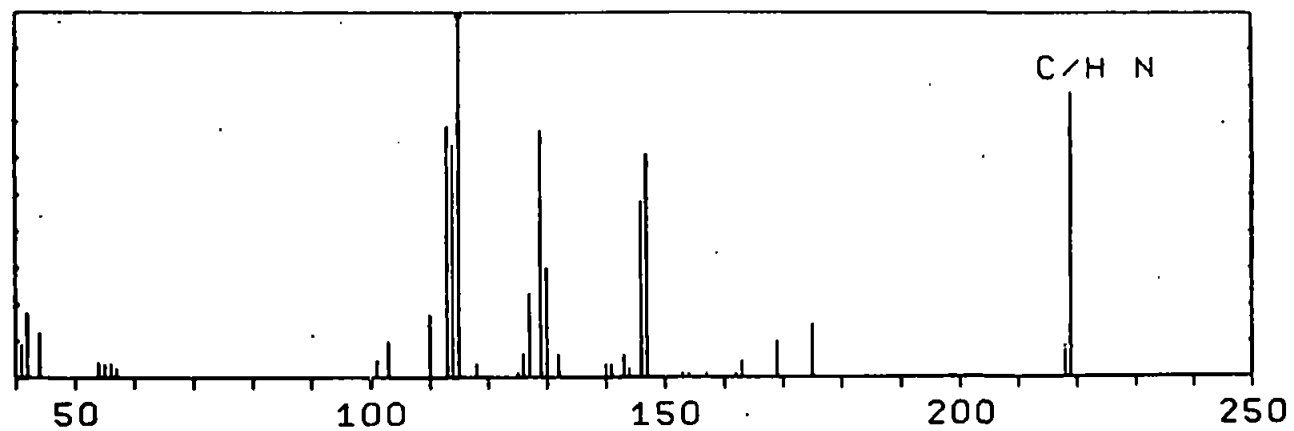


SUDAN SHALE (ALL SPECTRA $\times 10$ ABOVE 194)

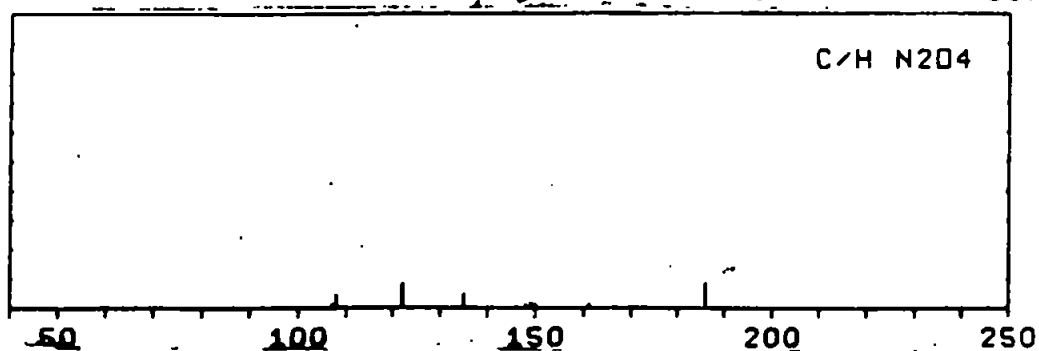
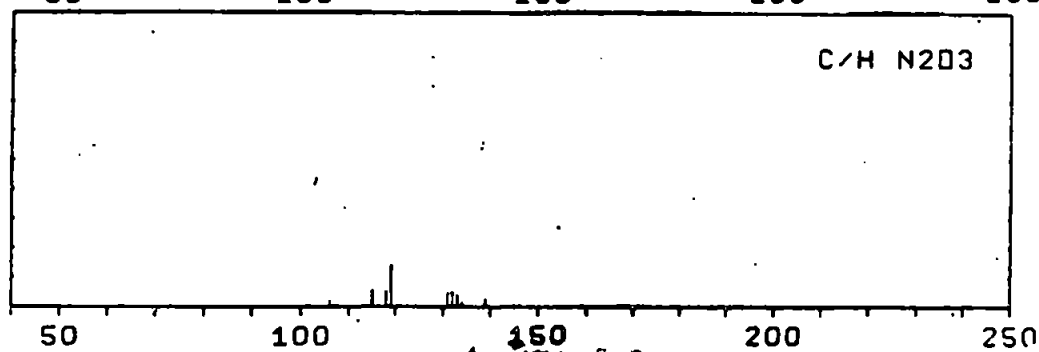
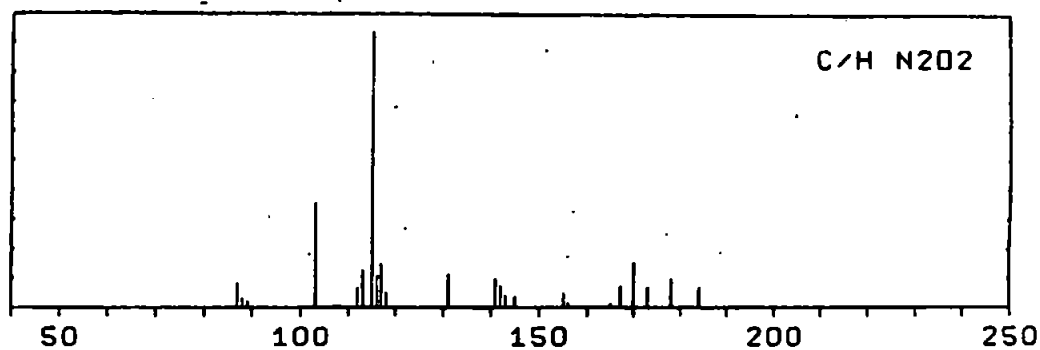
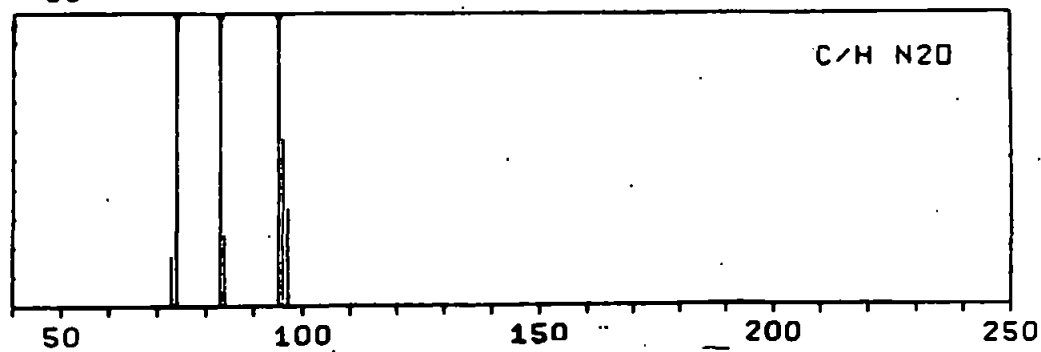
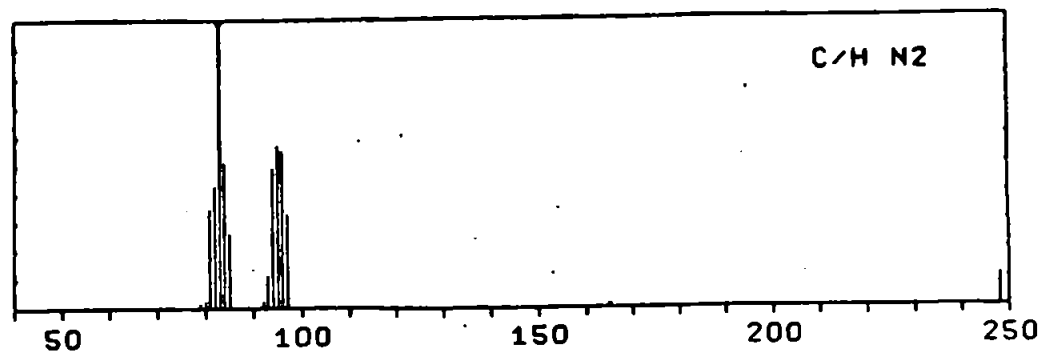
Figure VIII.. High Resolution Mass Spectrum of Pierre Shale



PIERRE SHALE (ALL SPECTRA x10 ABOVE 165)



PIERRE SHALE (ALL SPECTRA x 10 ABOVE 165)



PIERRE SHALE (ALL SPECTRA x 10 ABOVE 165)

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