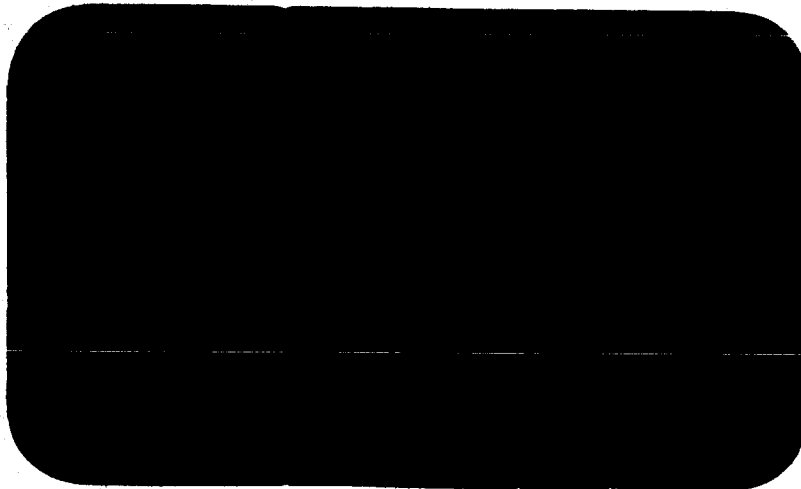
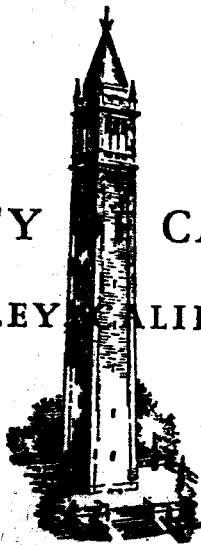


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THE ORGANIC GEOCHEMISTRY OF ANCIENT SEDIMENTS

PART II

by

R. B. Johns, T. Belsky, E. D. McCarthy, A.
L. Burlingame, Pat Haug, H. K. Schnoes, W.
Richter and M. Calvin

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February 15, 1966

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THE ORGANIC GEOCHEMISTRY OF ANCIENT SEDIMENTS*

PART II**

By R. B. Johns***, T. Belsky, E. D. McCarthy, A. L. Burlingame, Pat Haug,
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INTRODUCTION

Modern evolutionary theories presuppose a period of chemical evolution which continues gradually towards the point in time when the macromolecules previously abiotically formed reproduce themselves in a manner which we may consider characteristic of "living processes". Current extraterrestrial studies have intensified the need to establish the period during which life processes began on earth, as well as a method for unambiguous detection of their past or present existence. This paper presents evidence, deduced from chemical analysis of the hydrocarbon constituents of a representative spectrum of ancient sediments and oils, for the presence of life processes at least as early as 2.7 billion[†] years ago. The high level of development of the biological processes implied by our results calls for the inception of bio-organic evolution at a substantially earlier period.

The chemical approach involves the identification of "biological markers" from among the organic compounds present in ancient sediments. "Bio-

* A preliminary communication on part of this work has appeared: T. Belsky, R. B. Johns, E. D. McCarthy, A. L. Burlingame, W. Richter and M. Calvin (1965) Nature, 206, 446.

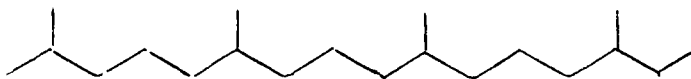
** Part I: Eglinton G., Scott P. M., Belsky T., Burlingame A. L., Richter W., and Calvin M. (in preparation) "Occurrence of Isoprenoid Alkanes in a Precambrian Sediment". Advances in Organic Geochemistry, Vol 2, Pergamon Press.

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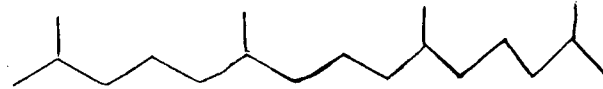
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† A billion is 1,000 million.

logical markers" are compounds which show reasonable chemical stability to diagenesis and possess a marked specificity of structure understandable in terms of known biosynthetic sequences and whose formation is of low probability by abiological synthesis. As Degens (1964a) points out, the synthetic origin of any particular compound viewed in isolation may be indeterminate and, therefore, the significance of the marker must be evaluated against the background of the accompanying extractables. Furthermore, the evaluation of the results from any one sediment is limited unless they can be placed in a time perspective provided by analysis of other samples of differing age. Isoprenoid hydrocarbons fulfill the requirement of structural specificity. Such considerations, as well as the availability of sensitive analytical techniques for isolation (gas chromatography) and characterization (mass spectrometry) made an initial analysis of hydrocarbon constituents particularly attractive. In order to secure an even firmer basis for conjecture as to the evidence of biological processes, it is desirable to identify as broad a spectrum of biologically significant molecules as possible (fatty acids and derivatives, etc.) and it is our intention to extend our analytical procedures. In this respect, the occurrence of steroid and triterpene hydrocarbons in the Soudan and Green River Shales have been reported from this laboratory (Burlingame et al., 1965). For the present we have sought to identify the isoprenoid alkanes, phytane (I) (2,6,10,14-tetramethylhexadecane) and pristane (II) (2,6,10,14-tetramethylpentadecane), as primary markers of biological origin.



I



II

Other homologies identified are considered to be collateral markers fitting the outlined requirements.

The carbonaceous shales examined in this report are the Soudan Shale from Minnesota ($\sim 2.7 \times 10^9$ years) and the Antrim Shale from Midland County, Michigan (about 265×10^6 years). A typical San Joaquin Valley Oil (about 30×10^6 years) and oil from the Abbott Mercury Mine in Lake County, California (about 3×10^6 years) have also been examined. These samples, together with a further study of the Nonesuch Oil (1×10^9 years) and a comparison with the Green River Shale (about 50×10^6 years) provide examples ranging up to the oldest carbonaceous Precambrian sediment known on the North American continent.

EXPERIMENTAL

An earlier paper (Eglinton et al., 1965) detailed the method of pulverization, extraction, isolation of the hydrocarbon fraction and its separation (by partition chromatography over TLC grade neutral alumina) into three major fractions: "Total", "Normal" and "Branched Cyclic" alkanes. The "Total" alkanes represent the first successive eluates in n-heptane from an alumina column which show minimal absorption in the ultraviolet at about 270 $m\mu$. The "Normal" alkanes are those straight chain molecules occluded in a 5 Å molecular sieve when the "Total" fraction and sieve are heated together in boiling benzene. The fraction remaining after treatment with the sieve represents the "Branched Cyclic" alkanes. From this last fraction the isoprenoids were isolated by gas-liquid chromatographic procedures and identified by mass spectrometric techniques. The procedures for isolation of iso-, anteiso- and cyclohexyl alkanes, the three new homologies reported in this paper, are illustrated in Figure I. The three successive GLC liquid phases used for purification of compounds were SE-30 silicone gum rubber, tetracyanoethylated pentaerythritol and seven-ring-meta-polyphenyl ether. All gas-liquid chromatograms reproduced in this paper, with the exception of Figure I, were run with column conditions as follows: 10 ft. x 1/16 in.; 3% SE-30 on 100-120 mesh Gaschrom Z; 20 ml/min. nitrogen at 50 psi; temp. programmed at 6°/min.; detector 250°, injector 300° (Aerograph Model 665-1). The gas-liquid chromatogram in Figure I, showing the separation and purification procedures of the Nonesuch Shale Oil, were run with the following column conditions: 10 ft. x 1/4 in.; 3% SE-30 on 80-100 mesh Chromosorb W(DMCS); 60 ml/min. helium; detector 245°, injector 280° (Aerograph Model, A-90-P2).

Mass spectra were determined on a modified C.E.C. 21-103C mass spectrometer (Walls and Burlingame, 1965) equipped with heated glass inlet system operated

at 200°. All spectra were determined at ionizing voltage of 70 e.v., ionizing current of 10-50 μ amp. and 160 to 180 volts per stage on the multiplier. Representative samples were examined in a direct inlet system (Burlingame, 1965). In the case of more volatile fractions like those containing the C₁₆ isoprenoid alkane, the probe used for introduction of the sample was cooled with liquid nitrogen to avoid pumping off small samples. Capillaries were cleaned prior to introduction with a 4:1 mixture of freshly distilled benzene and methanol, and then handled only with "Kimwipes". Fast scanning (30 seconds for the mass range m/e 90-600) was employed for samples of 15 μ g or less with prior background scanning. Representative samples of normal alkanes were isolated and identified mass spectrometrically; the remainder were characterized on the basis of gas chromatographic retention times. Colorado Green River Shale "normals" collected from an SE-30 phase contained components having one, two and three degrees of unsaturation, as evidenced from the mass spectrum. C₁₉ and C₂₀ isoprenoid were identified by direct comparison of the mass spectra with those of authentic pristane and phytane. The C₁₆-, C₁₈-, C₂₁- isoprenoid alkanes, along with the n-alkyl cyclohexanes, iso- and anteiso-alkanes, were deduced from the mass spectral fragmentation patterns. The mass spectra are given for all isolated samples and the intensities of mass spectral peaks, which are off-scale due to the choice of the reference peak, are given in Table III.

RESULTS

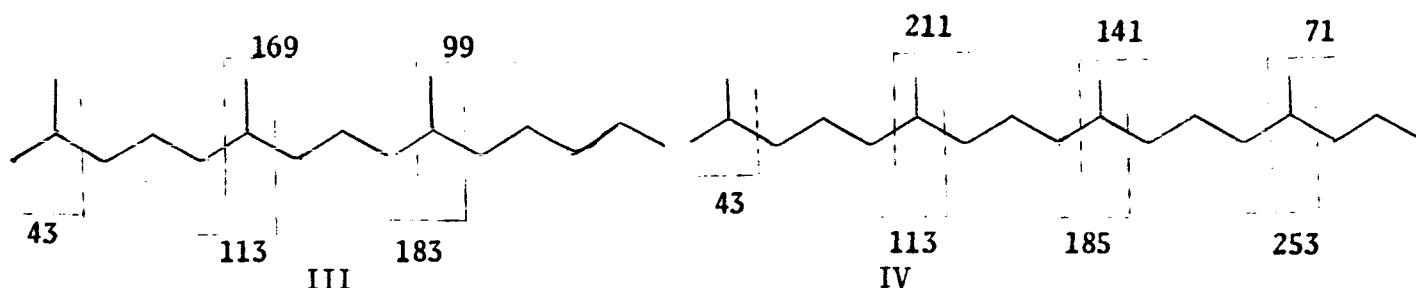
Soudan Shale

The oldest carbonaceous Precambrian sediment known on the North American continent is the Soudan Shale of northeastern Minnesota in the Lake Superior region (Cloud, Jr., et al., 1965). This shale has an age of deposition approaching 3 billion years, determined by the age of a 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 (1961). The two samples of shale analyzed were provided by Professor P. E. Cloud, Jr., of the University of California at Los Angeles; Sample (I) of the Soudan Iron Formation was cut from a surface exposure north of Tower, Minnesota, and is stratigraphically related to Sample (II) from the 21st Level of the Soudan Iron Mine, Soudan, Minnesota, at a depth of 1,800 feet below ground.

Sample (I) was prepared by cutting off the outer 1/4 inch of surface material; the inner core was taken and crushed to a particle size of less than 2 mm, sonicated in solvent (benzene-methanol, 4:1) and only then pulverized for extraction. Great care was taken to avoid contamination (Eglinton et al., 1965). In a typical experiment 600 gm of powdered shale yielded, on sonication in benzene-methanol (4:1) as solvent, 0.286 gm of soluble material. The *n*-heptane solubles (0.28 gm) after alumina chromatography yielded a "Total" alkane fraction of 0.24 gm.

Sample (II) was sonicated to wash off any surface contamination and then pulverized for extraction in the same manner. Powdered shale (570 gm) on sonication in benzene-methanol (4:1) yielded 2.2 gm of soluble material which appeared to contain a considerable amount of sulfur. The *n*-heptane solubles were passed down a colloidal copper column to remove sulfur (Blumer, 1957) and yielded a "Total" alkane fraction of 0.005 gm.

The GLC patterns of the "Total" alkanes of Samples (I) and (II) are compared in Figure II. In Figure III the gas chromatograms of the "Total", "Branched Cyclic" and "Normal" alkane fractions of the surface Sample (I) are shown. Samples of C_{18} - (III), C_{19} - (pristane, I), C_{20} - (phytane, II), and C_{21} -isoprenoid (IV) alkanes were isolated from the "Branched Cyclic" fraction (.22 gm) of Sample (I) and identified by their mass spectrometric fragmentation patterns. Figures IV-VII give the mass spectra of the C_{18} to C_{21} isoprenoids isolated from this shale, together with those of authentic pristane and phytane, Figures VIII and IX. For the C_{21} isoprenoid we suggest structure IV, 2,6,10,14-tetramethyl heptadecane, the regular isoprenoid. The peak at m/e 239 is somewhat more intense than expected for this structure but might be a contribution from the C_{20} iso-alkane or C_{19} anteiso-alkane; the C_{20} iso-alkane should have a GLC retention/^{time} similar to that of this C_{21} isoprenoid compound.

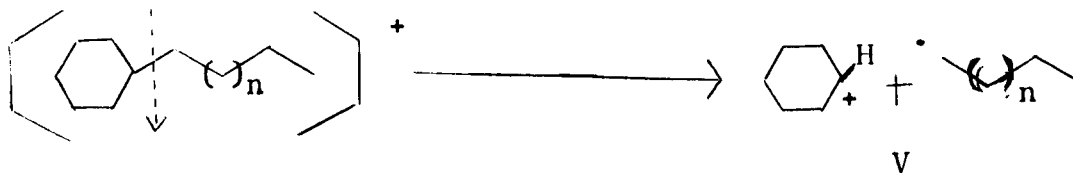


Although a pure sample of the C_{16} isoprenoid was not isolated, mass spectrometric data indicates its presence in a mixture. The C_{16} , C_{18} , C_{19} , C_{20} and C_{21} isoprenoids appear to occur in the ratios of 1:3:5:3:1 and constitute about 3.3% (as determined from the gas chromatogram) of the "Branched Cyclic" alkane fraction. Although the normal alkanes nominally run from C_{13} to C_{32} , 98% of the normal hydrocarbons are represented by the n - C_{15} to n - C_{20} alkanes, inclusively. There is also tentative mass spectrometric evidence for the presence of C_{30} saturated and unsaturated isoprenoid hydrocarbons. Mass spectra of several fractions

collected from the squalane region of the vapour phase chromatogram show similarities to the spectrum of authentic squalane.

Nonesuch Seep Oil

The Nonesuch Seep Oil has been analyzed previously in detail for isoprenoids and C_{15} , C_{16} , C_{19} and C_{20} isoprenoids have been reported (Eglinton et al., 1965). Figure XI gives the mass spectrum of the recently identified C_{21} isoprenoid (most probably the regular C_{21} isoprenoid structure with an impurity contributing to the m/e 239 peak, cf. Soudan). Evidence has also been found for the existence of a C_{18} isoprenoid. Further analysis has yielded three new homologous series: the C_{16} , C_{17} , C_{18} iso-alkanes, the C_{16} , C_{17} and C_{18} anteiso-alkanes, and the C_{16} to C_{19} n-alkyl cyclohexanes. The "Branched Cyclic" alkane pattern suggests that the Nonesuch Oil is composed of a series of homologues and analysis of several successive doublets has confirmed this assumption. Figure (I) illustrates the procedure adopted to analyze a representative GLC "cut". The pattern of elution of the iso- and anteiso-alkanes, as well as that for the cyclohexyl-normal alkanes, is predictable up to about carbon number C_{19} . A striking feature of Figure X is the similarity of the patterns for the Antrim Shale, Nonesuch Oil and Soudan Shale, which has made possible the prediction of the molecular compositions of maxima related to one another by their order of elution from the GLC column. The new series of C_{16} - C_{19} n-alkyl cyclohexanes isolated from the Nonesuch Shale were identified by their highly characteristic mass spectral fragmentation patterns. All exhibit an extremely abundant ion at m/e 83, corresponding to the highly favorable cleavage of the alkyl chain leaving the cyclohexyl carbonium ion (V).

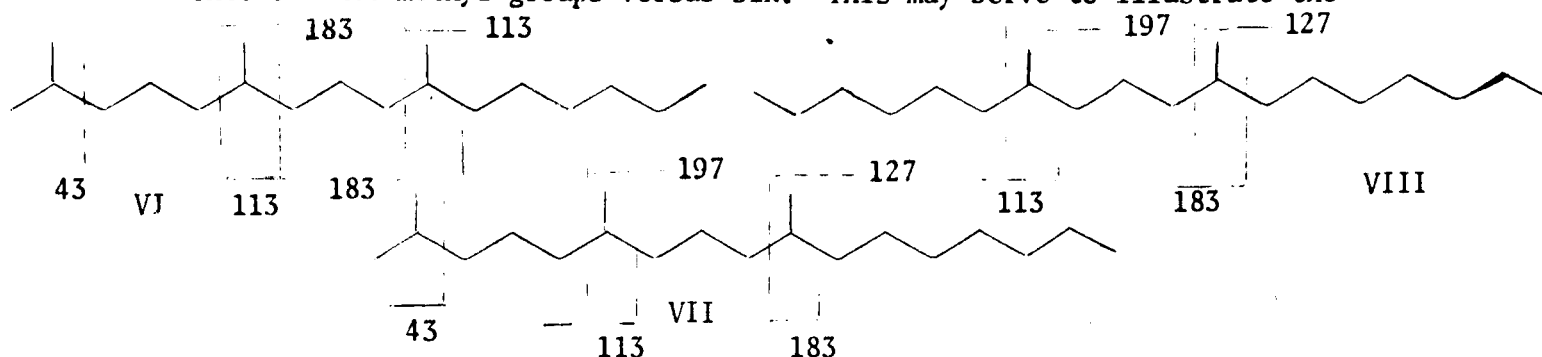


The mass spectrum of authentic C_{16} n-decyl cyclohexane (API #1009) is given in Figure XII, together with the mass spectra of the C_{16} - C_{19} n-alkyl cyclohexanes isolated from the Nonesuch Shale (Figures XIII-XVI). Fragmentation of iso-alkanes proceeds with some loss of methyl radical and a strong loss of the isopropyl radical. The mass spectra of the isolated iso-alkanes are given in Figures XVII-XX, together with that of authentic (API #983) C_{16} iso-alkane (2-methylheptadecane, Figure XVII). The anteiso-alkanes can be identified mass spectrometrically by a very pronounced loss of an ethyl radical. See Figures XXI-XXIV where Figure XXIV (API #1472) is authentic C_{21} anteiso-alkane (3-methyleicosane).

Antrim Shale

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 years (Matthews) and is the northern part of a large deposit which extends to the South and is referred to there as the Chattanooga Shale (Breger and Brown, 1963). The carbonaceous Antrim Shale is rich both in total carbon content and in extractable organic material (Table I). The core sample, prepared for extraction by the procedure described for the Soudan sample, yielded 2.2 gm extractable material from 480 gm of the powdered shale. The n-heptane solubles were separated into a "Total" alkane fraction (0.54 gm) in the usual way. The GLC patterns are shown in Figure XXV. Samples of C_{16} , C_{18} , C_{19} , C_{20} and C_{21} isoprenoid alkanes were isolated from the "Branched Cyclic" alkane fraction (0.26 gm) and identified by their characteristic mass spectrometric fragmentation patterns (Figures XXVI-XXX). The mass spectra of the C_{19} and C_{20} isoprenoids isolated from this shale are different enough from those of

pristane and phytane that 2,6,10-trimethyl hexadecane, structure VI, and 2,6,10-trimethyl heptadecane, structure VII, or 7,11-dimethyl octadecane, structure VIII, would seem to fit the data better. It should be noted that in the case of a slightly impure sample, distinction between structures II and VI would be particularly difficult because one is only dealing with a difference of five methyl groups versus six. This may serve to illustrate the



caution that must be used in assigning the structure of pristane or phytane to a sample on the basis of mass spectral peaks at m/e 113 and 183, or 113, 127, 183 and 197. It is interesting to note that the C_{18} , C_{19} and C_{20} isoprenoids to which we assign a five, six or seven membered chain all show a very similar fragmentation pattern. Since the C_{21} isoprenoid is obviously impure, an unambiguous definition of the structure is not possible. If the peak at m/e 239 is considered to be contributed by an impurity, then the remaining fragmentation pattern could be interpreted in terms of a regular C_{21} isoprenoid. One should note also that the spectrum is very similar to that of the Nonesuch C_{21} isoprenoid (Figure XI). Cyclohexyl- n -alkanes are present in the Antrim extracts; Figure XXXI gives the mass spectrum of a mixture of C_{18} and C_{19} compounds. One member of the iso-series, the C_{16} iso-alkane (Figure XXXII) was also identified.

San Joaquin Oil

A sample of a typical San Joaquin Valley Oil was provided by the courtesy of Dr. L. Lindemann of the California Research Corporation, Richmond. The oil

is considered to be about 30×10^6 years old (Lindemann) and the source rocks are thought to be of marine deposition (Lindemann). A sample (3.1 gm) was dissolved in n-heptane and chromatographed over TLC grade neutral alumina in the usual way (Eglinton et al., 1965). The "Total" alkane fraction separated weighed 1.21 gm. The GLC patterns are shown in ^{XXXIII.} Isoprenoids C_{16} , C_{18} , C_{19} (pristane) and C_{20} (phytane) were isolated and identified mass spectrometrically (Figures XXXIV-XXXVII) and were found to be present in approximately equal proportions.

Abbott Rock Oil

Abbott Mercury Mine Seep Oil and samples of frothy, siliceous Source Rock from Lake county in northern California were provided by Dr. E. Bailey of the U. S. Geological Survey, Menlo Park. The oil, which is occluded in siliceous froth veins deposited in surrounding Lower Cretaceous shale, is considered to have been deposited contemporaneously with the mercury deposit and cannot, therefore, be greater in age of deposition than about 3×10^6 years (Bailey et al., 1961). The siliceous Source Rock (49 gm) yielded, after pulverization and sonic extraction, 6.1 gm of benzene-methanol solubles. A sample of this extracted oil (1.7 gm) was dissolved in n-heptane, centrifuged and the soluble fraction separated on a neutral TLC grade alumina column (Eglinton et al., 1965) into a "Total" alkane fraction (0.65 gm). The GLC patterns of the "Total", "Branched Cyclic" and "Normal" alkane fractions are shown in Figure XXXVIII. Samples of C_{18} , C_{19} (pristane), C_{20} (phytane) and C_{21} (probably regular) isoprenoids were isolated and identified by mass spectrometry (Figures XXXIX-XLII). Our attention was first drawn to this oil by a paper suggesting that the mode of deposition in silica required an "abiogenic" origin (Sylvester-Bradley, 1964). Our results suggest that this is a normal, biologically formed oil in agreement with its known geological history (Bailey, 1959), whose GLC pattern differs from that of an abiogenic oil (Eglinton et al., 1965).

DISCUSSION

Migration

Before implications can be drawn from the hydrocarbons identified in the samples reported here, one must consider the problem of whether or not the hydrocarbons and their precursors extracted from the rock samples are contemporary with the sedimentary deposit or result from later migration from some outside source. In both the Abbott Source Rock and the Calcite Vein inclusion in the Nonesuch Marker Bed, the entrapped oil can be seen encased in the surrounding matrix and the problem of seepage does not arise. In the shale samples, however, the problem is less easily resolved. One approach to this difficulty has been to determine the C^{13}/C^{12} ratios for the extractable hydrocarbons and the non-extractable kerogen. The assumption is made that the kerogen, because of its chemical nature, has not migrated from the sediment with which it was originally formed. While this is an attractive hypothesis, it must not be forgotten that geochemists have little detailed knowledge of the true nature of kerogen or the conditions of its formation (Degens, 1965). Dr. T. Hoering of the Geophysical Laboratory of the Carnegie Institution of Washington has recently made a study of the C^{13}/C^{12} ratios for a variety of sediments ranging in age up to the Precambrian (Hoering; Cloud, Jr. et al., 1965). For the majority of sediments the isotope ratios for the extractable hydrocarbons agree reasonably well with the values obtained for the kerogen, but with Precambrian samples inconsistencies are more numerous. The Nonesuch Shale (1×10^9 years) (Hoering; Barghoorn, Meinschein and Schopf, 1965) and the Gunflint Chert from Ontario, Canada (2×10^9 years) (Hoering; Barghoorn and Tyler, 1965), show a suitable measure of agreement for the two types of carbon, but the Soudan Shale from the Soudan Mine does not (Hoering).

A value for δC^{13} of -34.54 ppm was obtained for the methanol insoluble "kerogen" to be compared with a δC^{13} value of -25.06 to -25.99 ppm for the

methanol soluble carbon of the Soudan Mine sample. No analysis has been done on the surface sample (I). The difference between the δC^{13} value of -22 to -27 ppm determined for extractable organic carbon in both ancient and recent sediments compared with values of about -13 ppm from contemporary carbonates is accounted for in terms of photosynthetic fractionation (Park and Epstein, 1960; Abelson and Hoering, 1961), leading to a lower C^{13} isotope composition and, correspondingly, a more negative δC^{13} value. The significant difference between the C^{13}/C^{12} ratios of the extractable and non-extractable carbon might be interpreted as an indication of their separate origins. Compaction of a sediment after initial deposition is known to occur very rapidly in the initial stages (Pettijohn, 1957; Jones, 1944; 1946), but even an initial deposition period of hundreds of millions of years is small in comparison to the age of the Soudan. Migration of hydrocarbons from outside sources into compacted sediments may be possible, but only in oil-producing areas (Degens, 1964b)--which is not the physical environment of the Soudan formation.

Migration could imply a lack of homogeneity in the sample and, accordingly, we have made a preliminary examination of the hydrocarbons released from our powdered surface Sample (I) by digestion in hydrofluoric acid, after previous exhaustive solvent-extraction of the powder with benzene-methanol. The results are in accord with those of Meinschein (1965), who, on the basis of stepwise dissolutions of the shale from the Soudan Mine, has also concluded from the distribution patterns that the alkanes originated within the sediment.

The Soudan Shale is metamorphosed and may have been subjected to a temperature as high as 350° or 400°C (Cloud, Gruner and Hagen, 1965). Some modification of the deposited organic materials may have been brought about by this heat treatment. This does not explain the low C^{13} content of the Soudan kero-gen, however, since simple thermodynamic arguments as well as the pyrolysis

experiments of Abelson and Hoering (1962/1963) demonstrate that degradation of organic compounds leads to the preferential release of C_{12} in the volatile low molecular weight fragments and to the retention of C_{13} in the non-volatile high molecular weight residue.

The δC^{13} value found for the extractable carbon from the Soudan Shale is comparable with that found in other ancient sediments, and this emphasizes the experimental result that the δC^{13} value found for the kerogen is abnormally high. This high δC^{13} value for the Soudan and most other Precambrian sediments is amenable to rationalization if we assume that the kerogen derives from specific source materials within the sediment. For contemporary biological systems it has been shown that metabolic processes not only result in a general enrichment of C^{12} over C^{13} , but also lead to isotopic fractionation among different constituents of the cell. For example, the plant lipid fraction is enriched in C^{12} as compared with the C^{12} level of the plant as a whole (Park and Epstein, 1961; Park and Dunning, 1961). From the evidence available on the nature of kerogen (Degens, 1965), it is clear that a difference does exist between Precambrian and Recent or Tertiary kerogen. It is particularly significant that Hoering (1965), by oxidizing kerogen from a Precambrian sediment of over 3.2×10^9 years, isolated an abundant yield of fatty acids (C_{16} to C_{24}). It is known that lignin-type materials, commonly thought to be the normal precursors of recent kerogen, came rather late in the evolution of biochemicals (Degens, 1965) and hence the higher δC^{13} value observed for the early Precambrian samples, arising presumably from lipids, is not unreasonable. The δC^{13} values of -19 to -33 ppm reported (Sackett et al., 1965) for contemporary phyto- and zooplankton as a reflection of temperature variation in their distribution over the Atlantic Ocean emphasizes the care necessary in assigning significance to the δC^{13} determination.

While, at present, it seems that C^{13}/C^{12} ratios cannot be used as evidence in favor of migration of the extractables into the Soudan Iron Formation, one must bear in mind that our arguments for the indigenous nature of the organic matter are tentative. The thermal history of the Soudan Shale extractables may be taken as an argument against the indigenous nature of the extractables. The conflicting evidence of Meinschein leaves this problem unresolved.

Genesis of Bio-organic Compounds

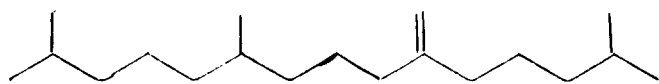
Isoprenoid hydrocarbons represent a significant proportion of the alkanes isolated from the sediments and oils which we have examined and, in fact, tend to proportionately increase with the age of the source. Their presence in the Cambrian and later periods presents no problem. From our examination of the Nonesuch Shale (Eglinton et al., 1965), we earlier suggested that the presence of the isoprenoid homology, in particular C_{19} and C_{20} isoprenoids, was at least presumptive evidence for life processes at the time of deposition one billion years ago. This conclusion has been substantiated both by the comprehensive investigation by Barghoorn, Meinschein and Schopf (1965) of the Nonesuch Shale and by Barghoorn's identification of acceptable morphological remains* in the Gunflint Chert (Barghoorn and Tyler, 1965), which is dated at 1.9 billion years. The report by Oró and co-workers (1965/6) of the recognition of pristane and phytane among the hydrocarbon extractables of the Gunflint Chert further strengthens the chemical approach. The identification of the isoprenoid homology in all three samples shown in Figure X is strongly indicative of a biological origin for the extractable hydrocarbons from the Soudan Shale and, therefore, [assuming them to be indigenous] of the existence of life processes at the time of its deposition. The indication in the Soudan extractives of the homologous steranes (Burlingame et al., 1965), themselves derived from isoprenoid precursors in contemporary sources, is further confirmation of our thesis.

* Very recently, Prof. Barghoorn also reported similar morphological remnants in the Fig Tree Formation from Swaziland, South Africa; this shale is considered to be 3.2×10^9 years old. (Reported in The Times (London) November 6, 1965 and The New York Times, November 5, 1965.)

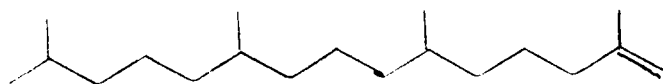
The similarities exemplified in Figure X suggest that the high level of development of the biosynthetic sequences which give rise to the isoprenoid hydrocarbons did not greatly differ over the nearly two billion years separating the deposition of the Nonesuch from the Soudan Shales and, further, that their inception and development must have occurred at an appreciably earlier time than 2.7 billion years ago.

Any discussion of the origin of the hydrocarbons isolated from ancient sediments must recognize at least two major determinants: the ecology at the time of deposition and the subsequent diagenetic changes which occur in ^{the} rock. This latter aspect includes bacterial degradation as well as non-biological transformation, which is extensive upon initial deposition. These factors are emphasized by comparison of Figure XXV of the Antrim Shale, of marine deposition (Cloud, Jr.), and Figure XLIII of the Green River Shale, of non-marine deposition (Eglinton et al., 1965; Robinson et al., 1965; Cloud, Jr.).

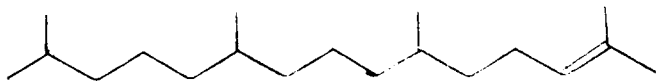
The isoprenoid pristane is commonly isolated from animal and especially marine sources (Sorenson and Mehlum, 1948; Bendoraitis et al., 1962; Blumer, Mullin and Thomas, 1963; Mold et al., 1963a), but it is noticeably lacking in contemporary plants. Especially pertinent is the recent isolation of pristane and mono-olefins (IX), (X) and (XI) with the pristane skeleton from zooplankton (Blumer and Thomas, 1965b). Diagenetic hydrogenation of these mono-olefins would yield a form of pristane.



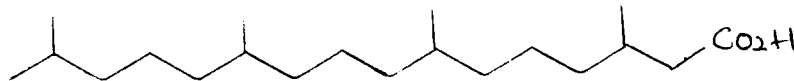
IX



X



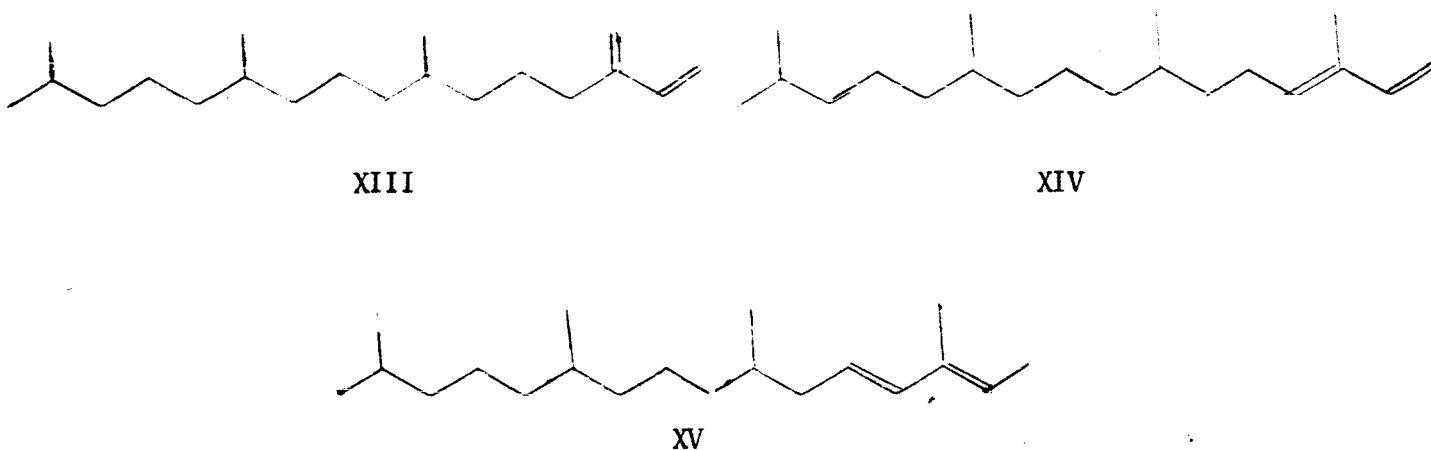
XI



XII

Hence, a substantial proportion of the geological pristane, at least from marine sources, could be derived from precursors of the same carbon number (Blumer and Thomas, 1965b). The isoprenoid acid (XII), which has been isolated from natural sources such as butterfat (Hansen and Shorland, 1953) and ox blood (Lough, 1963), as well as from petroleum (Cason and Graham, 1965), is a further possible precursor if subsequent diagenetic decarboxylation occurs.

Phytane presents a different problem, there being a paucity of reports of its isolation from living organisms (Ciereszko et al., 1963) and none from marine sources. Yet phytane is an important constituent of petroleum oils and sedimental extracts. It has been suggested that phytol (Bendoraitis et al., 1962) derived from chlorophyll in the source materials may be the precursor for phytane, and the recent report (Blumer and Thomas, 1965a) of the isolation of three isomeric phytadienes (XIII), XIV) and (XV) apparently derived from phytol in the diet of the zooplankton adds strength to this thesis.



A major contribution by chlorophyll-containing material in the Green River Shale (Eglinton et al., 1965; Robinson et al., 1965) may be inferred from the patterns in Figure XLIII; hence, a study of the changes in the relative abundance of the paraffins in the Green River Oil-Shale, as a function of depth (Robinson et al., 1965), is pertinent to a discussion of the origin of the phytane. That

study has shown the phytane content decreases with increasing depth of the samples, concurrent with a proportional increase of the C_{19} , C_{18} and C_{16} isoprenoids. This result is in excellent agreement with the assumption that phytol is the common precursor.

The increase in the proportion of C_{19} isoprenoid relative to C_{20} isoprenoid in the marine sediments, such as the Antrim, Nonesuch and Soudan, could be accounted for, at least in part, by the difference in their ecologies, that is, the predominance of marine organisms results in a consequent increase in the proportion of pristane.

The isoprenoid homology C_{16} , C_{18} , C_{19} and C_{20} has been identified in the Green River, Antrim and Soudan Shale extractives, and in the Nonesuch Oil. From the GLC patterns there is little doubt that this homology exists in other samples we have examined but have not analyzed in detail. The eluates corresponding to GLC maxima in the Green River Shale Branched Cyclic alkane pattern between the C_{16} and C_{18} isoprenoids have been isolated but do not contain any C_{17} isoprenoid. Similarly, we have taken small, successive GLC cuts of the Branched Cyclic alkanes from the Antrim and Soudan Shales and the Nonesuch Oil in the regions between the C_{16} and C_{18} isoprenoids. With the possible exception of the Antrim, a mass spectrometric survey of these fractions has produced no evidence for the presence of a C_{17} isoprenoid.

If phytol is regarded as the precursor of phytane, this conversion may be considered to occur by several, but related, pathways. By a sequence of abiological diagenetic processes, such as saturation and dehydration reactions, it is possible to envisage the conversion of phytol to phytane. Again, oxidation, decarboxylation and saturation could lead to pristane. Alternatively, or parallel with the above, we suggest another sequence which is more general in its scope. If phytenes of the type (XVI) to (XVIII) are formed during compaction of the sediments and are cleaved diagenetically at the preferred positions for chemical

manner, lower members of the series below C₁₅ may be considered as derived from farnesol or farnesene. There is no current biological parallel for the synthesis of the C₂₀ isoprenoid, 2,6,10-trimethyl heptadecane, structure (VII).

Table II lists the normal alkanes and two further homologies, the iso- and anteiso-alkanes which have been identified in our samples. Members of the three series have been recognized among extractives of contemporary plants and animals, and these homologies may be regarded as "biological markers" in their own right. In an abiogenic oil kindly supplied by Dr. C. Ponnamperna (Eglinton et al., 1965) and made by a corona discharge in an atmosphere of methane, we have been unable to detect the presence of any normal alkanes, suggesting their formation is not a preferred abiotic process, at least under these conditions. On the other hand, analysis of the hydrocarbons formed by the Fischer-Tropsch process* indicates that a homologous series of normal alkanes seems to be the major constituent of this mixture.

Normal alkanes isolated from contemporary plant sources commonly occur with carbon numbers greater than C₂₀ (Eglinton and Hamilton, 1963) and show an odd over even carbon number predominance (Eglinton and Hamilton, 1963; Oro, Nooner and Wikstrom, 1965). Normal alkanes from marine sources, however, do not show any special odd to even preferences (Koons et al., 1965) and in that sense show an agreement with the results obtained from analysis of extractives of ancient sediments (Bray and Evans, 1965). Our own results reported here and summarized in Figure XLIV clearly indicate that odd over even preferences in ancient oils is unsatisfactory as a mark of biogenecity in older sediments. No alternation was observed in the Gunflint Chert extractives (Oro, Nooner, Zlatkis, Wikstrom and Barghoorn, 1965).

* We thank Dr. Sharkey of the United States Bureau of Mines, Pittsburgh, for giving us this sample of the Fischer-Tropsch oil.

To account for the range in distribution, it is commonly suggested that the alkanes are formed by diagenetic modification of the fatty acids present in the biological source materials. From land sources fatty acids have been isolated in chain lengths of C_{14} to C_{20} and in marine sources from C_{20} to C_{26} . In the latter case, C_{20} to C_{22} make up 1/3 to 1/2 of the total composition (Eglinton and Hamilton, 1963). For Precambrian sediments, where the organisms recognized are of a simpler structure (Barghoorn, Meinschein and Schopf, 1965; Barghoorn and Tyler, 1965), contemporary forms such as blue-green algae might be expected to reflect more accurately the type of hydrocarbon and fatty acid precursors suggested by the hydrocarbon extractives. The identification of fatty acids with major constituents of carbon number C_{12} to C_{18} from blue-green algal mats (Parker and Leo, 1965) and our own isolation of $n-C_{17}$ as the major, normal hydrocarbon constituent of the Nostoc variety of blue-green algae are pertinent with respect to the maximization in the distribution of the normals. The normal alkane distribution shown for the Green River Shale (Figures XLIII and XLIV) emphasizes its non-marine origin (Robinson et al., 1965) and can be interpreted as due to a significant contribution by land plants to the organic source materials (Eglinton et al., 1965).

The branched chain fatty acids derived from lipids are a possible source for the iso- and anteiso-alkane homologies. The C_{10} to C_{26} iso-branched acids and the C_9 to C_{31} anteiso-branched fatty acids are constituents of hair (Deuel, Jr.), 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, i.e., major component of Micrococcus lysodeikticus (Shorland, 1963). Iso-paraffinic hydrocarbons have been isolated from rose petal wax (Waldron et al., 1961) and certain plants (Eglinton and Hamilton, 1963). Iso- and anteiso-alkanes have been isolated from a California naphtha (Lindemann and Annis, 1960), tobacco

leaf wax (Carruthers and Johnston, 1959), wool wax (Mold et al., 1963b, 1964; Downing et al., 1960), and Cuban sugar cane wax (Sorm et al., 1964). An ante-iso-paraffin has also been isolated from the American cockroach (Baker et al., 1963).

The isoprenoids, normal alkanes and iso- and anteiso-alkanes represent four homologies which are common to both extractives of Precambrian sediments and contemporary plants and animals. Their synthesis may be understood in terms of known biological mechanisms.

The fifth homology, the cyclohexyl-normal-alkanes, reported in Table II, is of interest because there is one tentative identification only of members of this series from contemporary plant sources (Kuksis, 1964), although the series has been reported in the Athabasca petroleum deposit (Nagy and Gagnon, 1961), and in paraffin wax (Levy et al., 1961). Elsewhere in nature (Meinschein, 1963), they have been reported on the basis of a broad mass spectral analysis, but no specific member has been isolated. We suggest that 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 to 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 (Weitkamp et al., 1947). 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.

ACKNOWLEDGEMENTS

We appreciate the generosity of Mr. R. D. Matthews for his sample of the Antrim shale, Dr. L. P. Lindemann for his sample of the San Joaquin oil, and Dr. E. H. Bailey and Prof. Preston E. Cloud, Jr. for their samples of the Abbott Rock oil and the Soudan shale, respectively, and for invaluable discussions about these samples. All mass spectra were determined by Miss Sherri Firth. One of the authors (R. B. J.) thanks the University of Melbourne, Australia, for the Leave of Absence during which this work was carried out (1964-65). This work was supported, in part, by the National Aeronautics & Space Administration (NsG-101-61) and by the U. S. Atomic Energy Commission.

SUMMARY

Several shales and oils ranging in age from 3 million to 2.7 billion years have been investigated for their hydrocarbon content using gas chromatography and mass spectrometry as primary analytical tools. From the Soudan Shale from Minnesota (2.7×10^9 years) the C_{18} , C_{19} , C_{20} and C_{21} isoprenoid-alkanes were obtained. The Antrim Shale from Michigan (about 265×10^6 years) yielded the C_{16} , C_{18} , C_{19} , C_{20} and C_{21} isoprenoids, as well as a C_{16} iso-alkane and the C_{18} and C_{19} cyclohexyl n-alkanes. The San Joaquin Oil (30×10^6 years) and the Abbot Rock Oil (3×10^6 years) contained the C_{16} , C_{18} , C_{19} , C_{20} and the C_{18} , C_{19} , C_{20} and C_{21} isoprenoids respectively. In addition, a series of iso-alkanes (C_{16} - C_{18}), anteiso-alkanes (C_{16} - C_{18}) and n-alkyl-cyclohexanes (C_{16} - C_{19}) as well as a C_{21} isoprenoid were obtained from the Nonesuch Seep Oil (1×10^9 years). This analysis provides a comprehensive picture of the types of biogenic hydrocarbons found in oils and shales of widely differing ages, and in particular, the finding of isoprenoid alkanes in the Soudan Shale furnishes evidence for life processes at that period of geological time.

TABLE I
PERCENTAGE COMPOSITION

SAMPLE	ANTRIM	NONESUCH		SOUDAN (I)	SOUDAN (II)
		OIL	SHALE		
Carbon Content	8.8		0.4	3.2	5.2
Sulphur Content	<0.1		<0.1	<0.1	25.3
Solvent Extractable Content of Rock	~0.46		0.03-0.1	~0.05	~0.39
Extractables Soluble in n-Heptane	~35		~90	~99	~0.25
Identified Isoprenoids in Branched-Cyclic Alkanes	~1.63	~1.8		~3.3	
Normal Alkanes	25-33	~37		~7.5	

ISOPRENOID CONTENT OF BRANCHED-CYCLIC ALKANES

C ₁₆	0.45	0.32	0.25
C ₁₈	0.46	0.40	0.75
C ₁₉	0.27	0.54	1.25
C ₂₀	0.23	0.30	0.75
C ₂₁	0.19	0.20	0.25

TABLE II

HYDROCARBONS IDENTIFIED

Sample	Age	Normals		Isoprenoids	Steranes	Iso & Anteiso Alkanes		Cyclohexyl Normal Alkanes
		Range	Max.					
Soudan Shale	2.7×10^9	C ₁₁ to C ₃₂	C ₁₇	C ₁₈ , C ₁₉ , C ₂₀ , C ₂₁	C ₂₇ , C ₂₈ , C ₂₉			
Nonesuch Seep Oil	1×10^9	C ₁₂ to C ₃₄	C ₁₇	C ₁₅ , C ₁₆ , C ₁₉ , C ₂₀ , C ₂₁		iso C ₁₆ , C ₁₇ , C ₁₈ anteiso C ₁₆ , C ₁₇ , C ₁₈		C ₁₆ , C ₁₇ , C ₁₈ , C ₁₉
Nonesuch Shale	1×10^9	C ₁₀ to C ₃₅	C ₁₃	C ₁₆ , C ₁₉				
Antrim Shale	265×10^6	C ₁₀ to C ₂₅	C ₁₃	C ₁₆ , C ₁₈ , C ₁₉ , C ₂₀ , C ₂₁		iso C ₁₆		C ₁₈ -nC ₁₉ mixture
Green River Shale	50×10^6	C ₁₃ to C ₃₃	C ₁₇ ⁺ , C ₂₉	C ₁₆ , C ₁₈ , C ₁₉ , C ₂₀	C ₂₇ , C ₂₈ , C ₂₉ , C ₃₀ , triterpane			
San Joaquin Oil	30×10^6	C ₁₀ to C ₃₃	C ₁₃	C ₁₆ , C ₁₈ , C ₁₉ , C ₂₀				
Abbott Seep Oil	3×10^6	C ₁₂ to C ₃₀	C ₁₇ ⁺ , C ₂₇	C ₁₉				
Abbott Rock	3×10^6	C ₁₄ to C ₂₈	C ₁₉	C ₁₈ , C ₁₉ , C ₂₀ , C ₂₁				

TABLE III
OFF SCALE MASS SPECTRAL INTENSITIES

M/E																	M/E
	IV	V	VI	VII	VIII	IX	XI	XII	XVII	XIX	XX	XXI	XXII	XXIII	XXIV	XXVI	
40			260				46		670					97			40
41	630	183	1160	950	163	700	520		878	374	1820	363	425	310	122	1000	41
42	190		340	240		176	140		398	140	750	356	216	86		290	42
43	1350	380	1900	2300	209	1240	*	165	*	908	440		*	601	184	2050	43
44	*		690	110					430		243	182	108	100		2090	44
55	360	144	720	720	119	440	430		418	278	920	147	230	162		245	55
56	340	144	580	500	115	380	380		377	184	755	228	336	218		340	56
57	1550	620	2250	2300	230	1500	*		1730	742	348	605	932	621	247	1150	57
58			120	170					101		307	57	433	48			58
69	160		290	340		172		250	189	260	419	62	102	66		245	69
70	150		260	340		176		250	213	300	419	56	89	60		334	70
71	890	520	1160	1400	200	1060	*		878	453	1948	249	669	225		1140	71
83			106	120					101	90	224						83
84				120					120		153						84
85	410	203	540	590		440	550		504	245	1044	169	230	164		690	85
97				105					108	120	153						97
98	109																98
99	110			120			120		121		224						99
113											139						113
127																	127

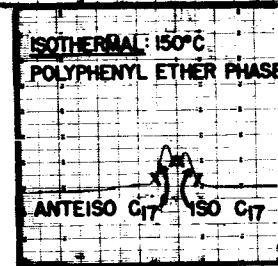
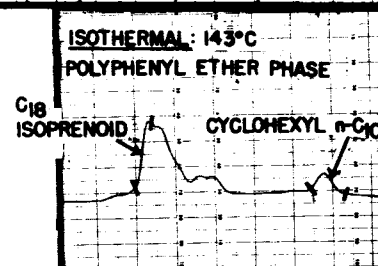
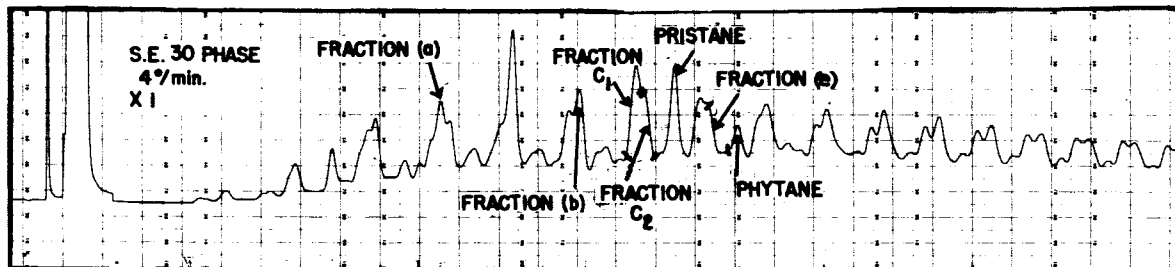
* Intensities off scale on original spectrum

TABLE III
OFF SCALE MASS SPECTRAL INTENSITIES

M/E	XXVII	XXVIII	XXIX	XXX	XXXI	XXXII	XXXIV	XXXV	XXXVI	XXXVII	XXXIX	XXL	XLI	XLII	M/E
40						117								336	40
41	711	500	860	550		1210	450	327	579	*		*	570	*	41
42	447	150	250	160		400	138	115	162	117		104	140	190	42
43	*	1070	2000	1150	140	2900	*	266	865	*	144	*	1310	*	43
44	221	110	590	140		140		286	286	*				200	44
55	342	290	500	600		580	283	234	309	*		240	410	480	55
56	300	270	360	350		445	217	110	251	*		250	340	360	56
57	1630	1300	1600	1620		2100	*	148	1028	*	193	*	1700	*	57
58	1350		240			105									58
69	194	150	280	380		300	105	108	192	*		120	200	260	69
70	160	109	220	280		400	212			*			170	240	70
71	810	760	960	1140		1550	730		660	*		*	1025	*	71
83		110	140	200		175								102	83
84		140	110	139		160								110	84
85	408	500	550	690		950	320	*	219	*		*	520	600	85
97			125	141		140								101	97
98	117														98
99	123	130	115	138		180								130	99
113															113
127										143					127

* Intensities off scale on Original Spectrum

- Figure I
- a) Gas Chromatogram of Nonesuch Shale
Branched-Cyclic Alkane Fraction
 - b) Gas Chromatogram of Fraction C₁
 - c) Gas Chromatogram of Fraction C₂



- Figure II a) Gas Chromatogram of Soudan Surface
Sample (I)
- b) Gas Chromatogram of Soudan Mine
Sample (II)

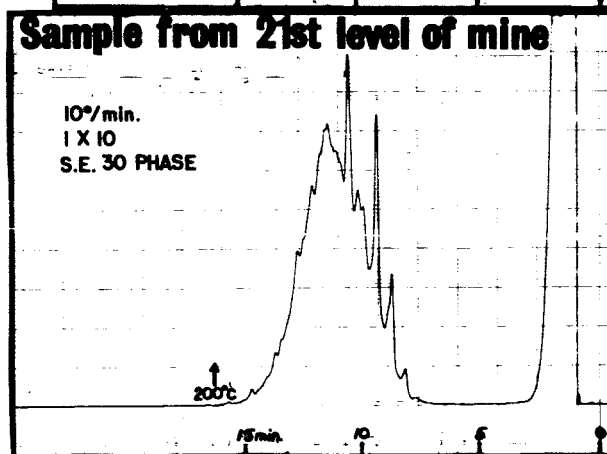
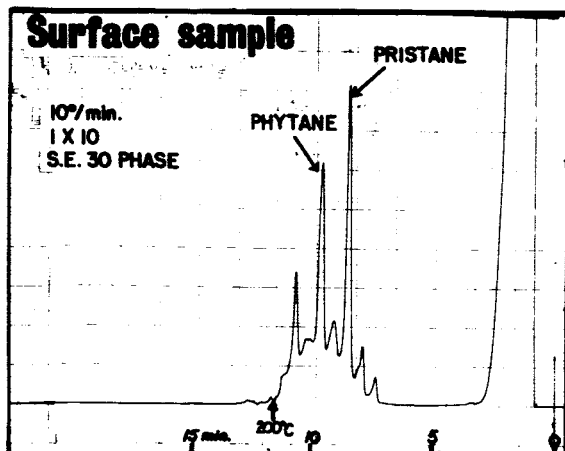
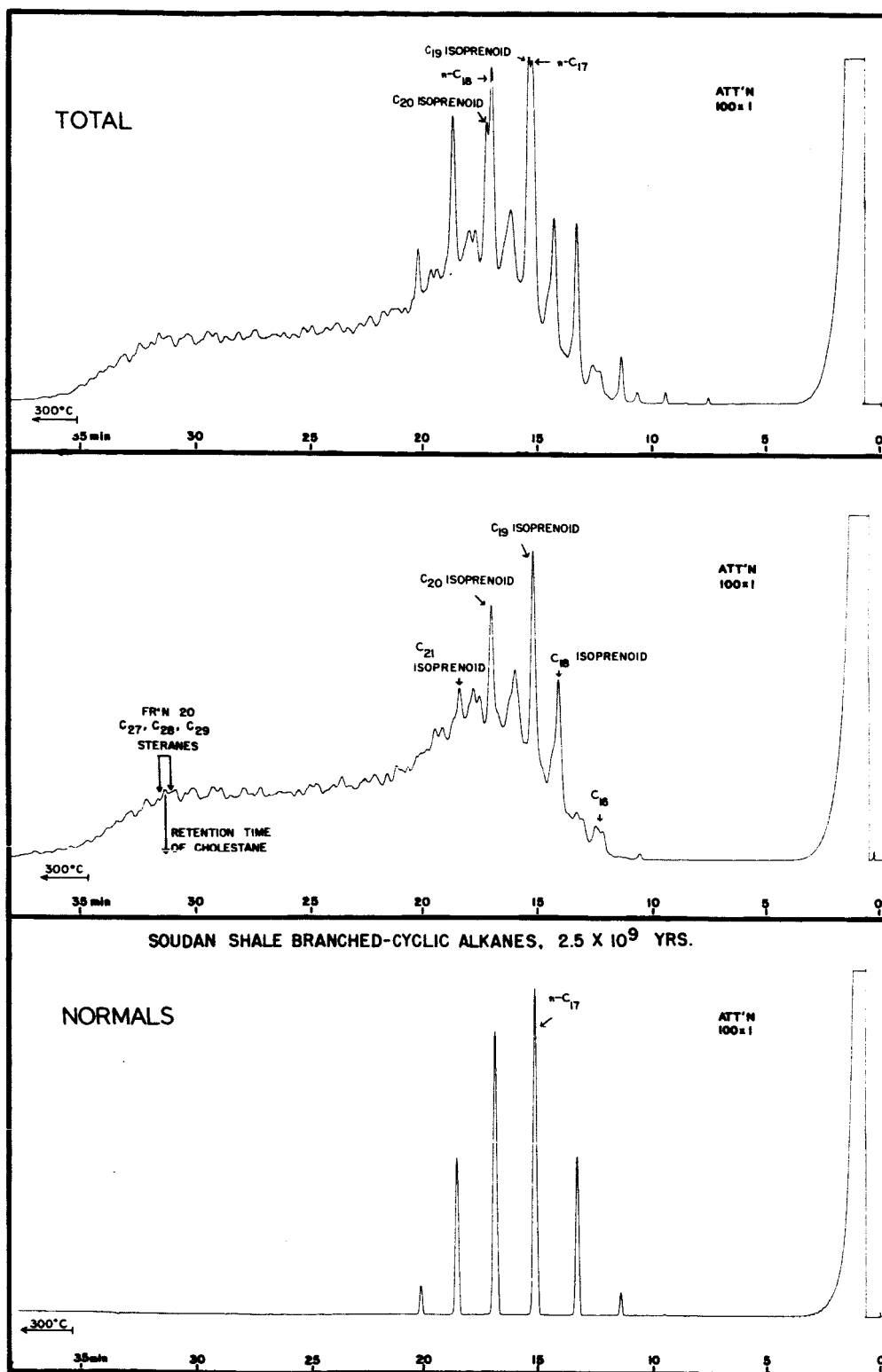


Figure III Gas Chromatograms of Surface Soudan Shale
Sample (I)



SUDAN SHALE ALKANE FRACTIONS

Figure IV Mass Spectrum of Soudan Shale C₁₈ Isoprenoid

Figure V Mass Spectrum of Soudan Shale C₁₉ Isoprenoid

Figure VI Mass Spectrum of Soudan Shale C₂₀ Isoprenoid

Figure VII Mass Spectrum of Soudan Shale C₂₁ Isoprenoid

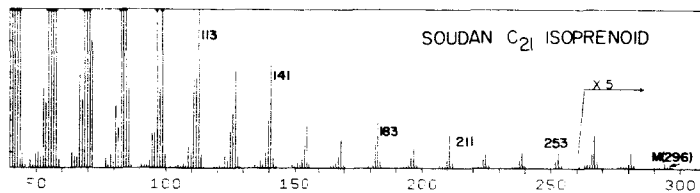
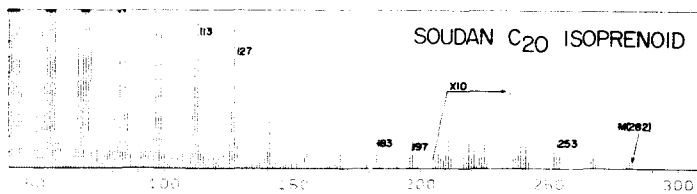
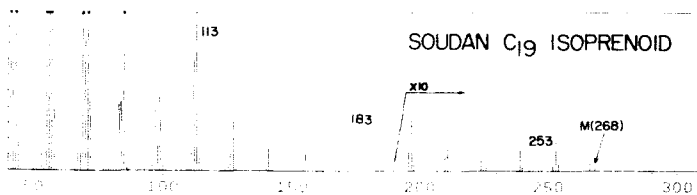
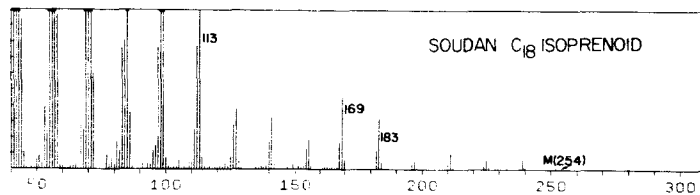
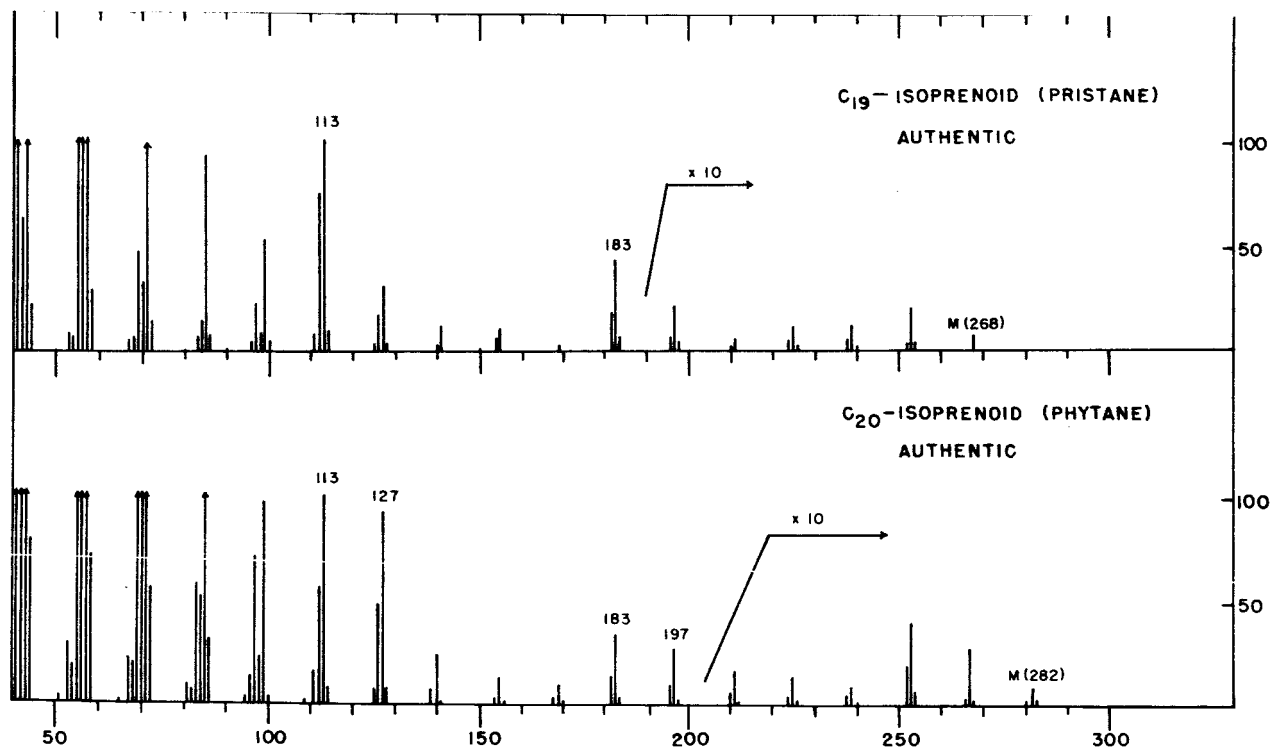


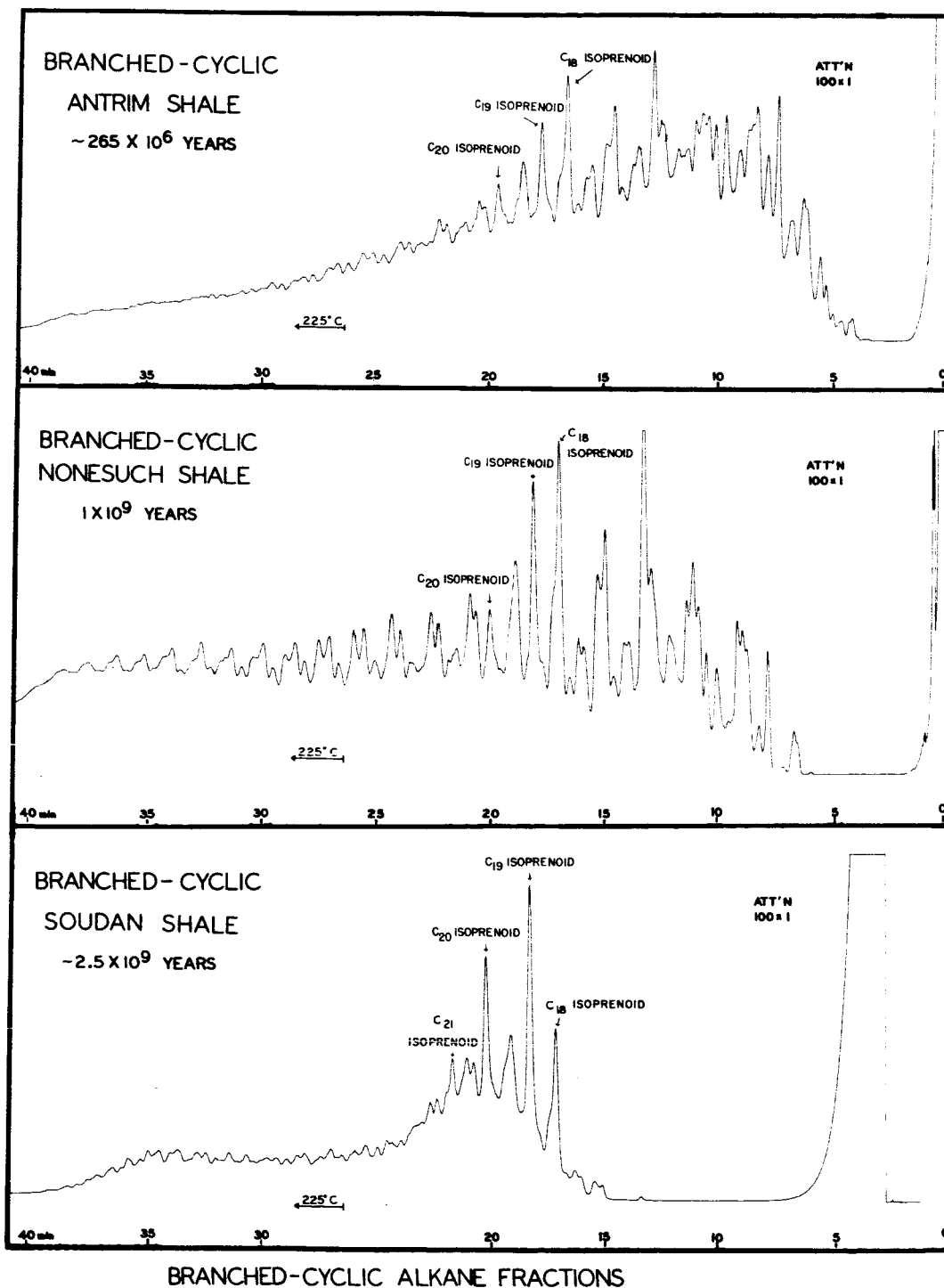
Figure VIII Mass Spectrum of Authentic Pristane

Figure IX Mass Spectrum of Authentic Phytane



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Figure X Comparison of Gas Chromatograms of the Branched-Cyclic Alkane Fractions of Antrim, Nonesuch and Soudan Shales.



- Figure XI Mass Spectrum of Nonesuch Seep Oil C_{21} Isoprenoid
- Figure XII Mass Spectrum of Authentic C_{16} n-Decyl Cyclohexane
- Figure XIII Mass Spectrum of Nonesuch Seep Oil C_{16} n-Alkyl Cyclohexane
- Figure XIV Mass Spectrum of Nonesuch Seep Oil C_{17} n-Alkyl Cyclohexane
- Figure XV Mass Spectrum of Nonesuch Seep Oil C_{18} n-Alkyl Cyclohexane
- Figure XVI Mass Spectrum of Nonesuch Seep Oil C_{19} n-Alkyl Cyclohexane

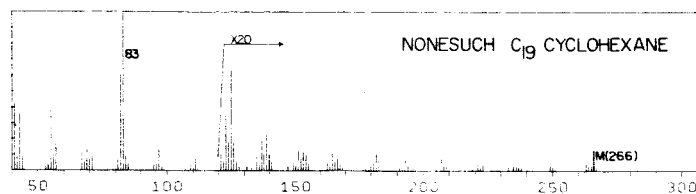
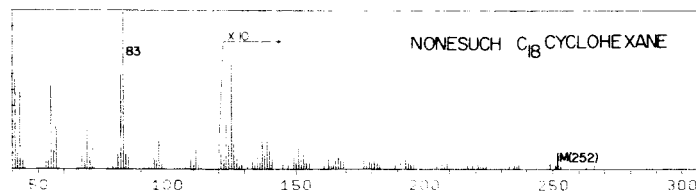
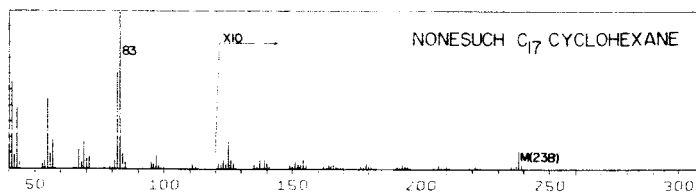
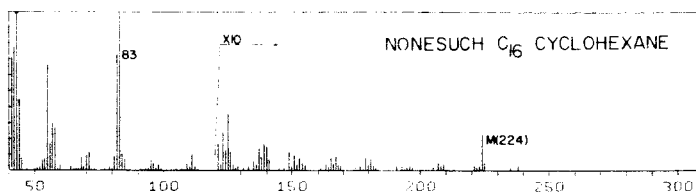
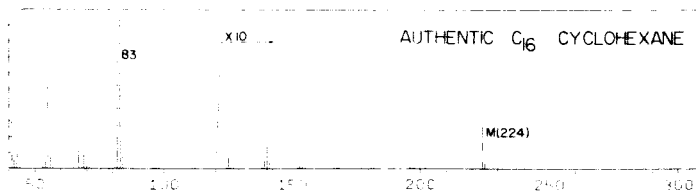
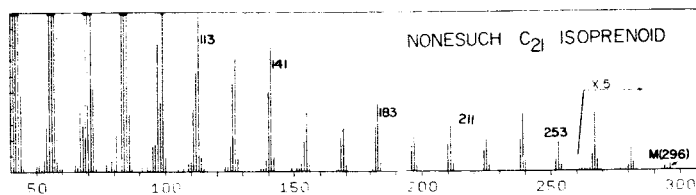


Figure XVII Mass Spectrum of Authentic C₁₆ Iso-Alkane

Figure XVIII Mass Spectrum of Nonesuch Seep Oil C₁₆ Iso-Alkane

Figure XIX Mass Spectrum of Nonesuch Seep Oil C₁₇ Iso-Alkane

Figure XX Mass Spectrum of Nonesuch Seep Oil C₁₈ Iso-Alkane

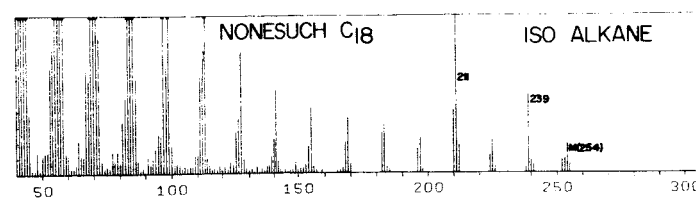
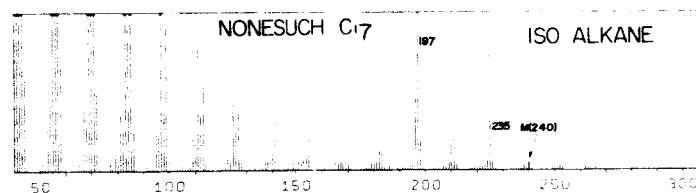
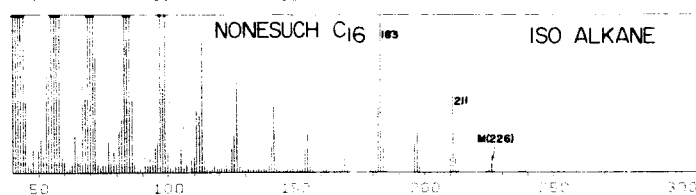
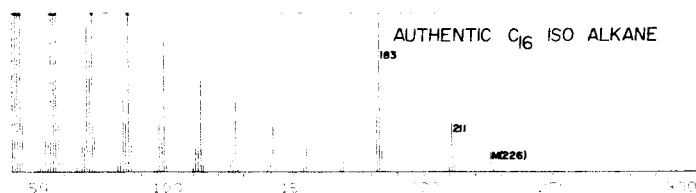


Figure XXI Mass Spectrum of Nonesuch Seep Oil C₁₆ Anteiso-Alkane

Figure XXII Mass Spectrum of Nonesuch Seep Oil C₁₇ Anteiso-Alkane

Figure XXIII Mass Spectrum of Nonesuch Seep Oil C₁₈ Anteiso-Alkane

Figure XXIV Mass Spectrum of Authentic C₂₁ Anteiso-Alkane

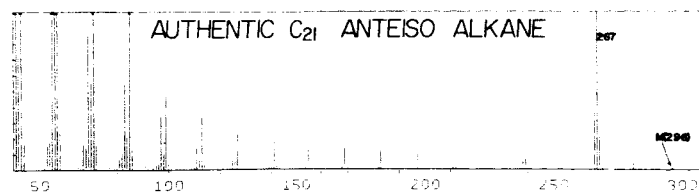
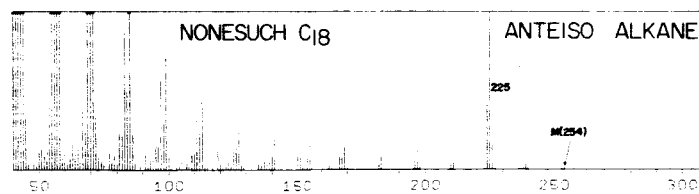
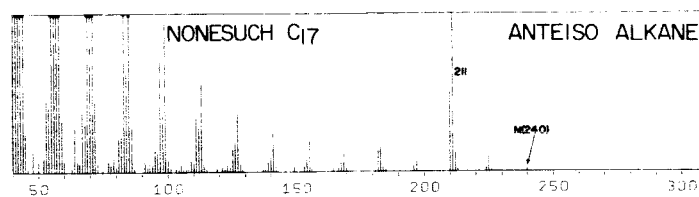
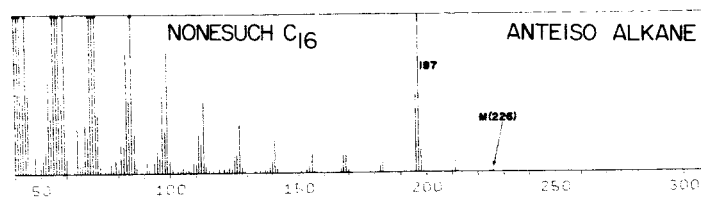
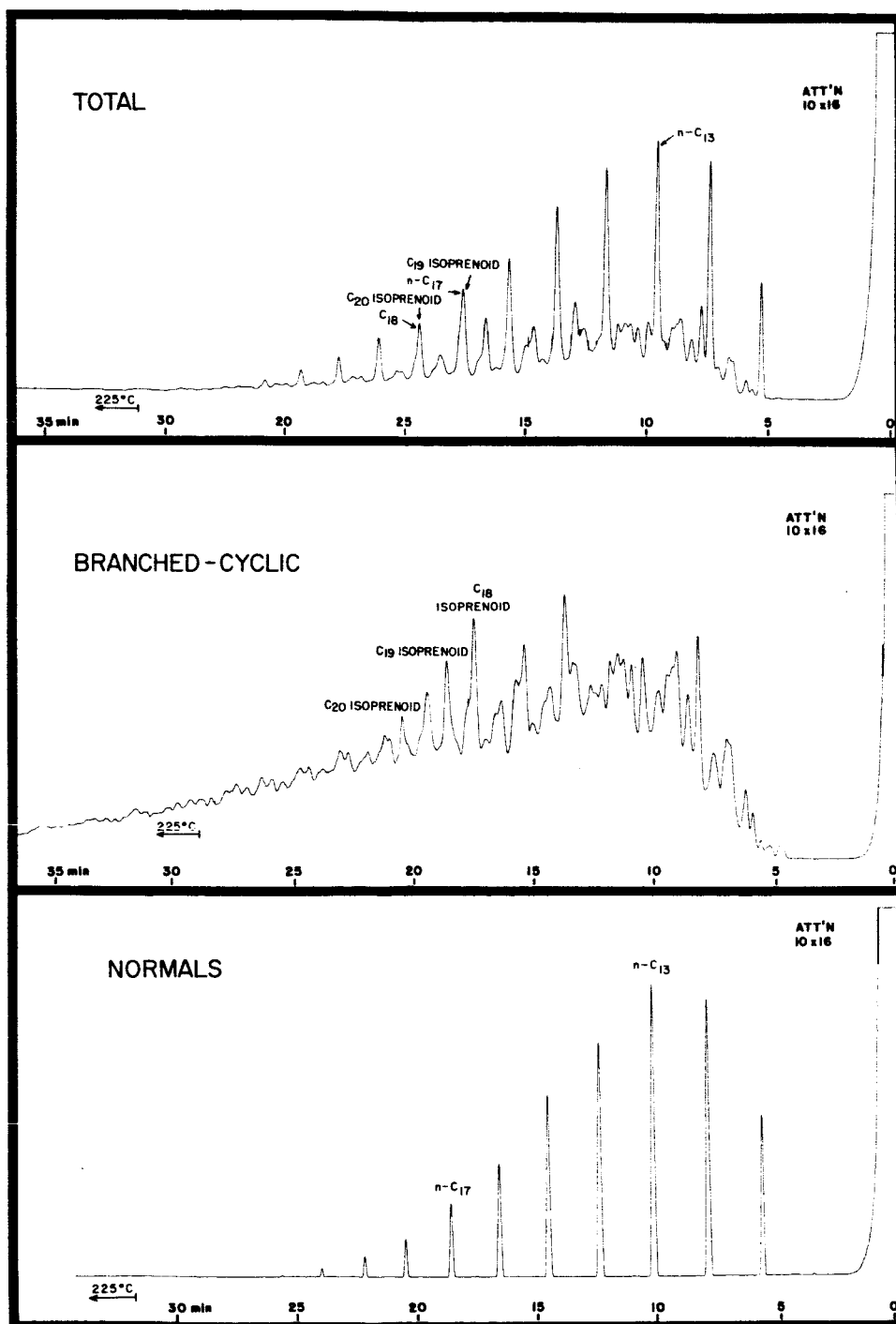


Figure XXV Gas Chromatograms of Antrim Shale



ANTRIM SHALE ALKANE FRACTIONS

Figure XXVI Mass Spectrum of Antrim Shale C₁₆ Isoprenoid

Figure XXVII Mass Spectrum of Antrim Shale C₁₈ Isoprenoid

Figure XXVIII Mass Spectrum of Antrim Shale C₁₉ Isoprenoid

Figure XXIX Mass Spectrum of Antrim Shale C₂₀ Isoprenoid

Figure XXX Mass Spectrum of Antrim Shale C₂₁ Isoprenoid

Figure XXXI Mass Spectrum of Antrim Shale Mixture of C₁₈
and C₁₉ Alkyl Cyclohexanes

Figure XXXII Mass Spectrum of Antrim Shale C₁₆ Iso-Alkane

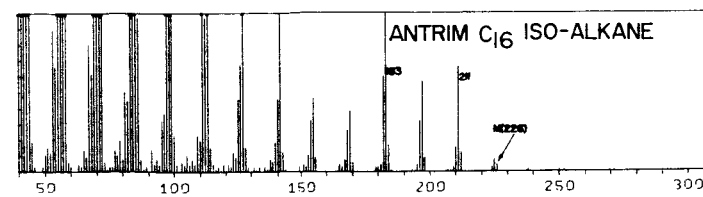
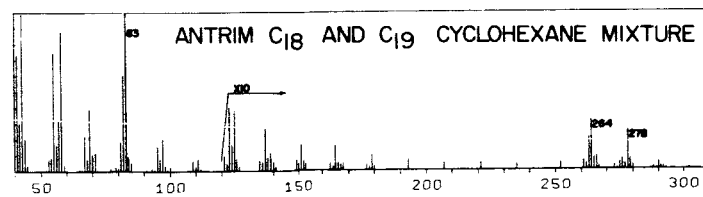
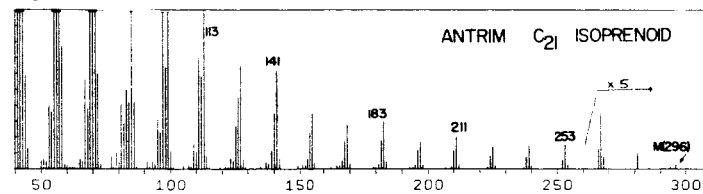
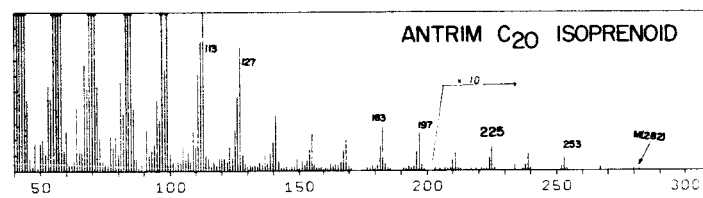
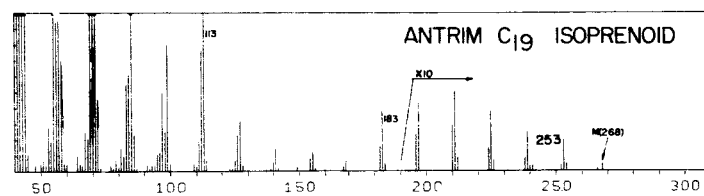
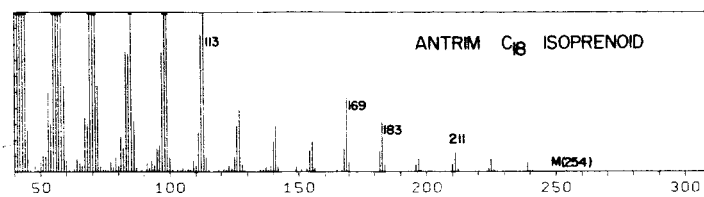
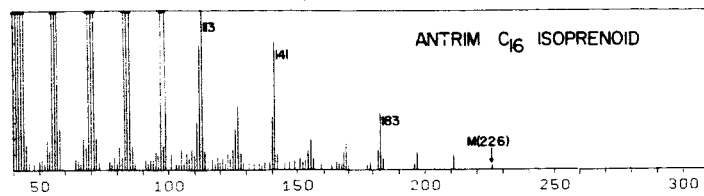


Figure XXXIII Gas Chromatograms of San Joaquin Oil

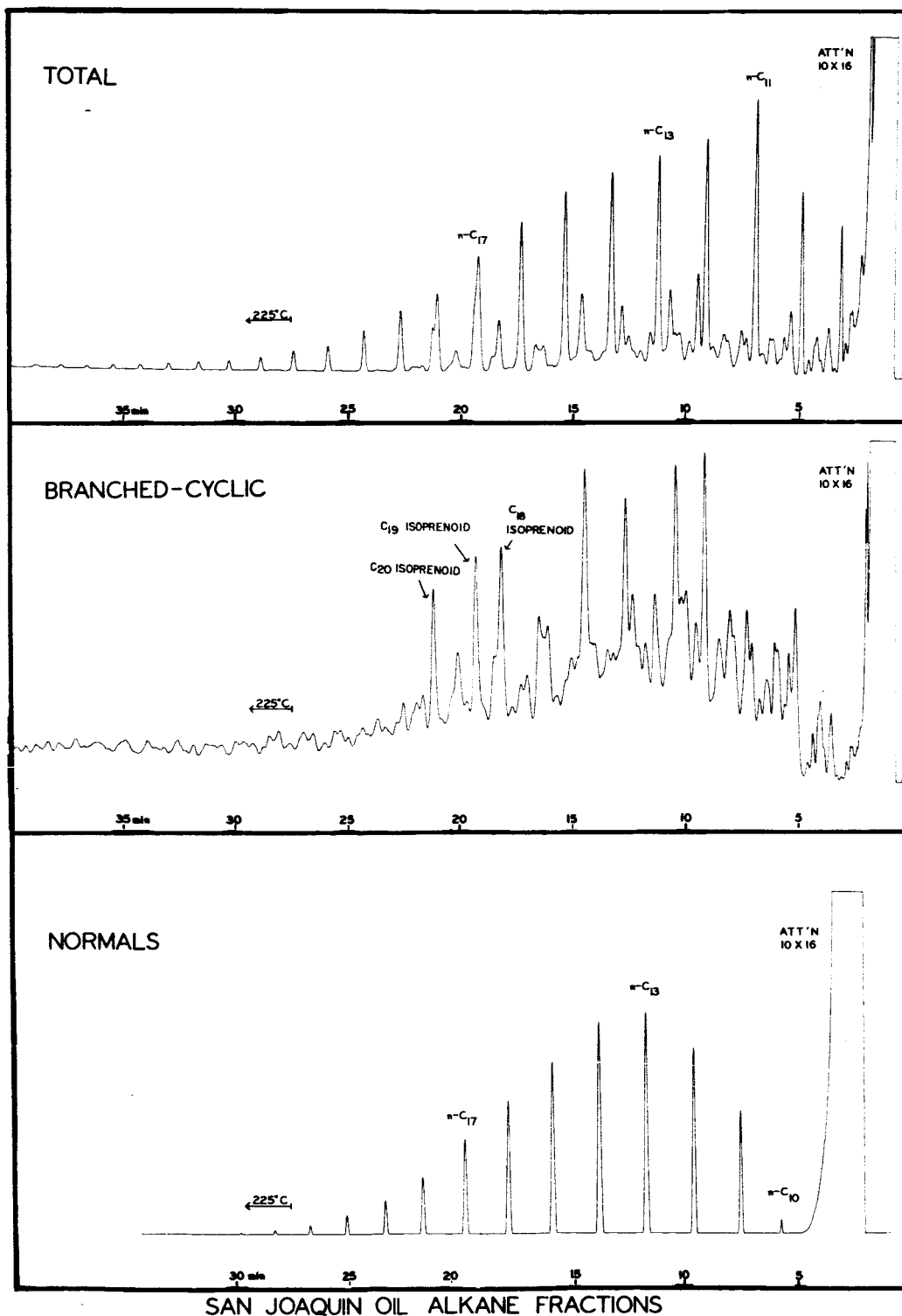


Figure XXXIV Mass Spectrum of San Joaquin Oil C₁₆ Isoprenoid

Figure XXXV Mass Spectrum of San Joaquin Oil C₁₈ Isoprenoid

Figure XXXVI Mass Spectrum of San Joaquin Oil C₁₉ Isoprenoid

Figure XXXVII Mass Spectrum of San Joaquin Oil C₂₀ Isoprenoid

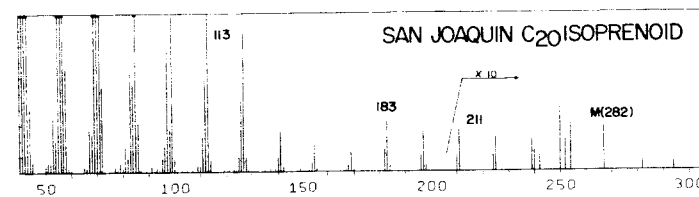
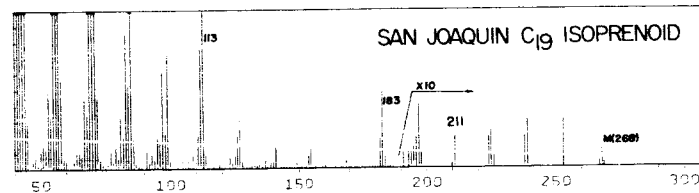
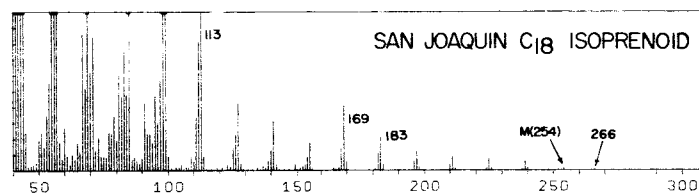
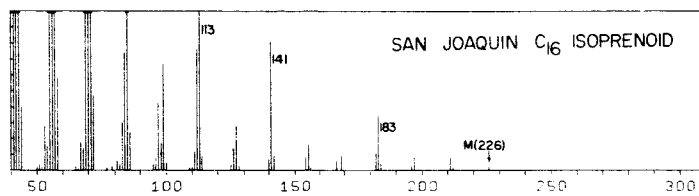
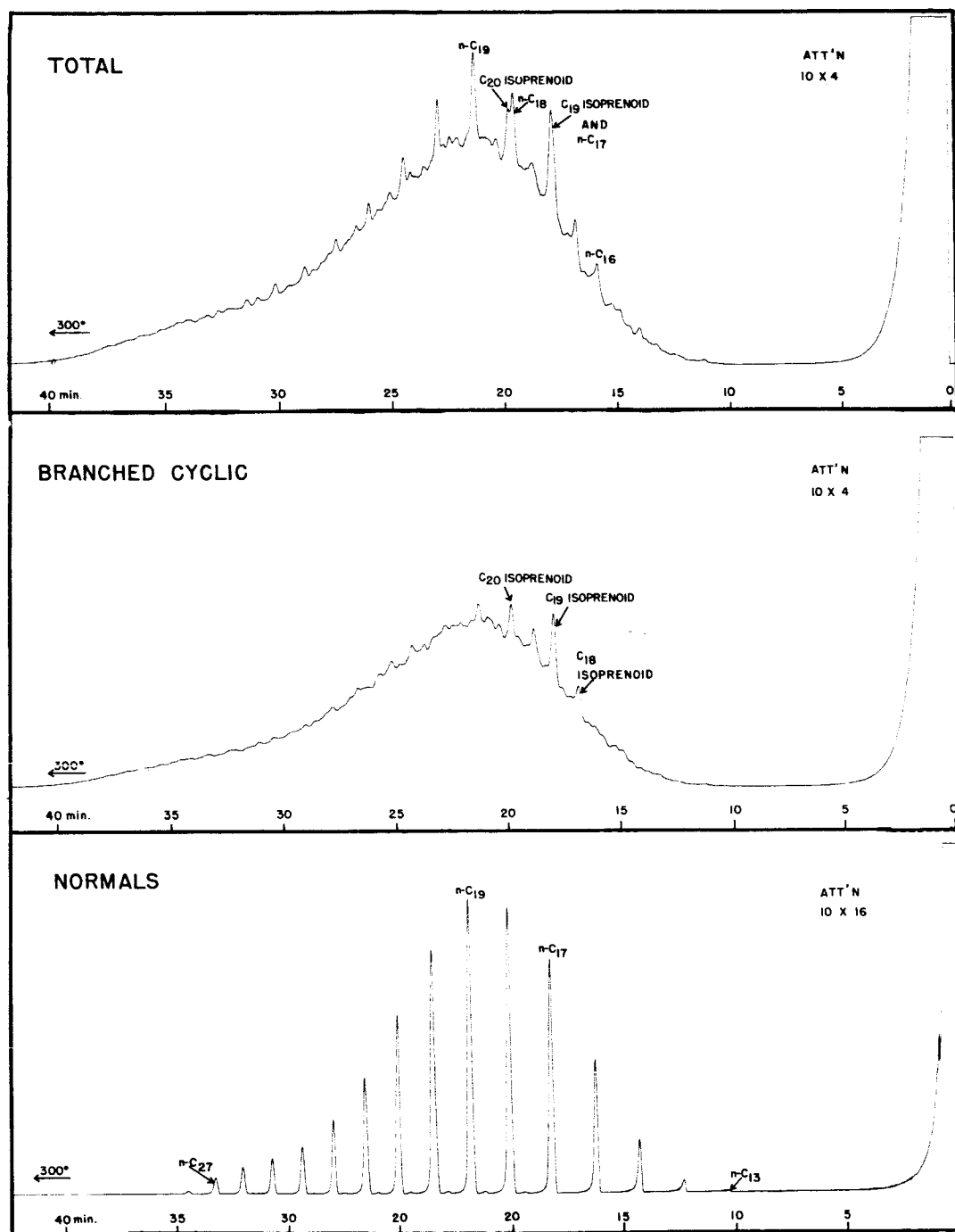


Figure XXXVIII Gas Chromatograms of Abbott Rock Oil



ABBOTT ROCK OIL, ALKANE FRACTIONS

Figure XXXIX Mass Spectrum of Abbott Rock Oil C₁₈ Isoprenoid

Figure XL Mass Spectrum of Abbott Rock Oil C₁₉ Isoprenoid

Figure XLI Mass Spectrum of Abbott Rock Oil C₂₀ Isoprenoid

Figure XLII Mass Spectrum of Abbott Rock Oil C₂₁ Isoprenoid

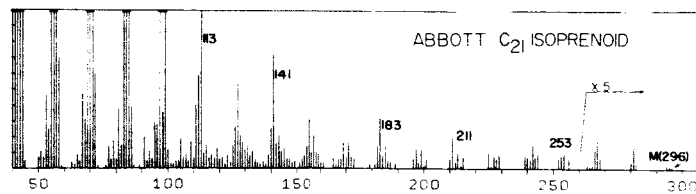
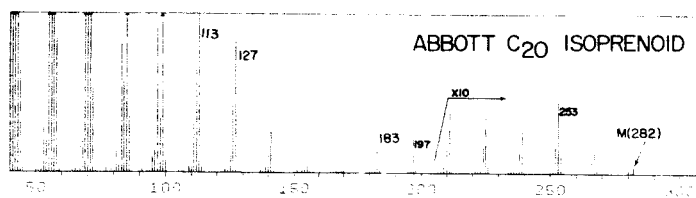
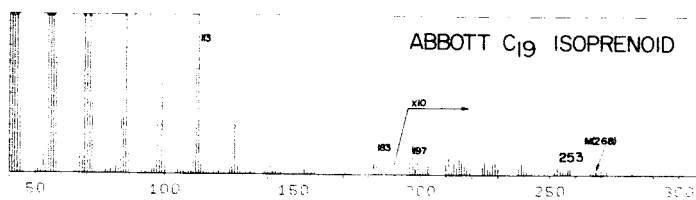
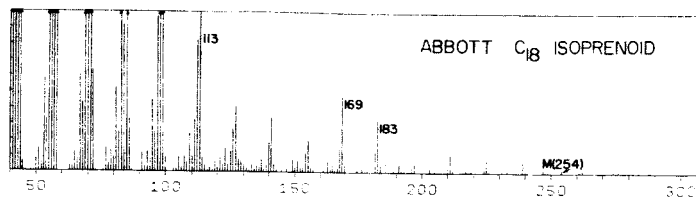
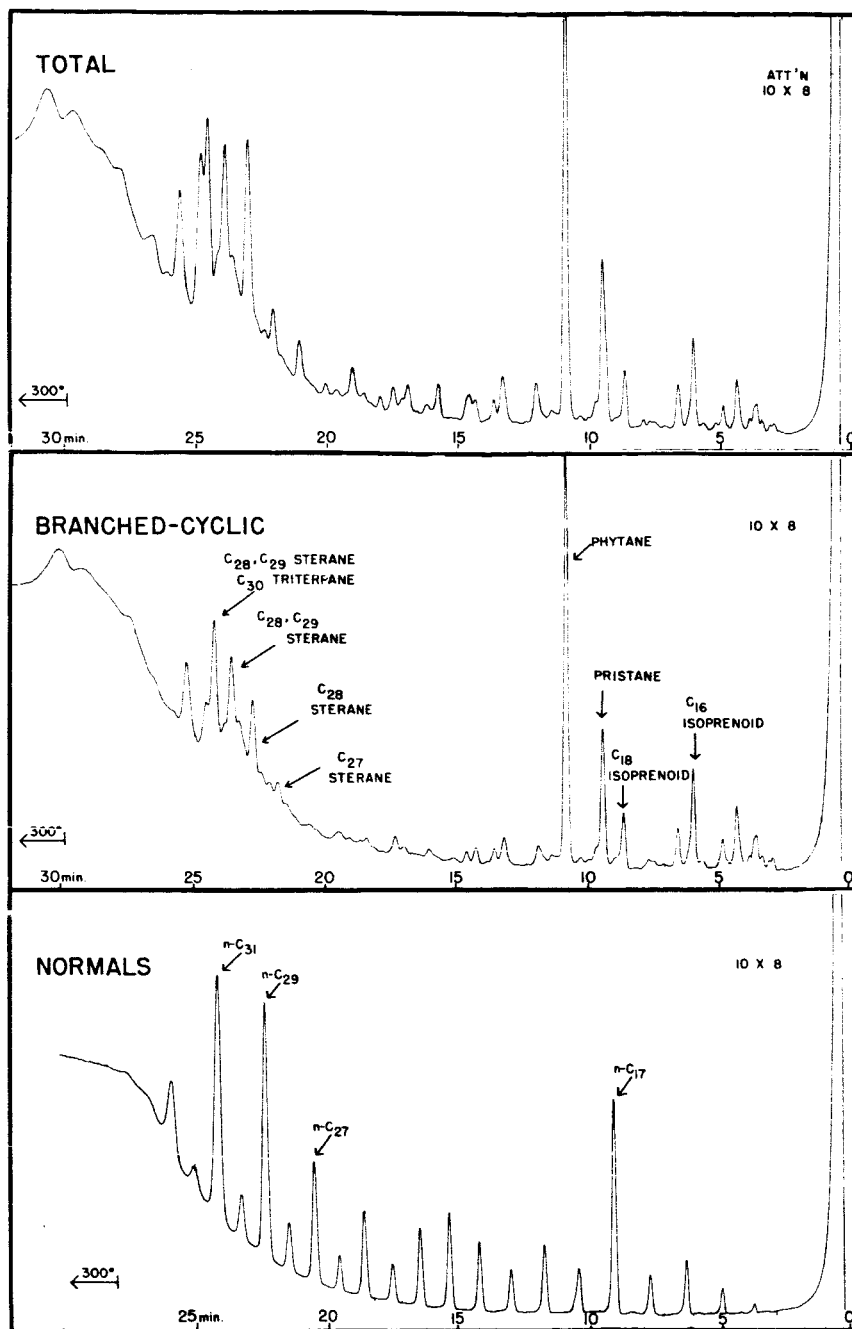


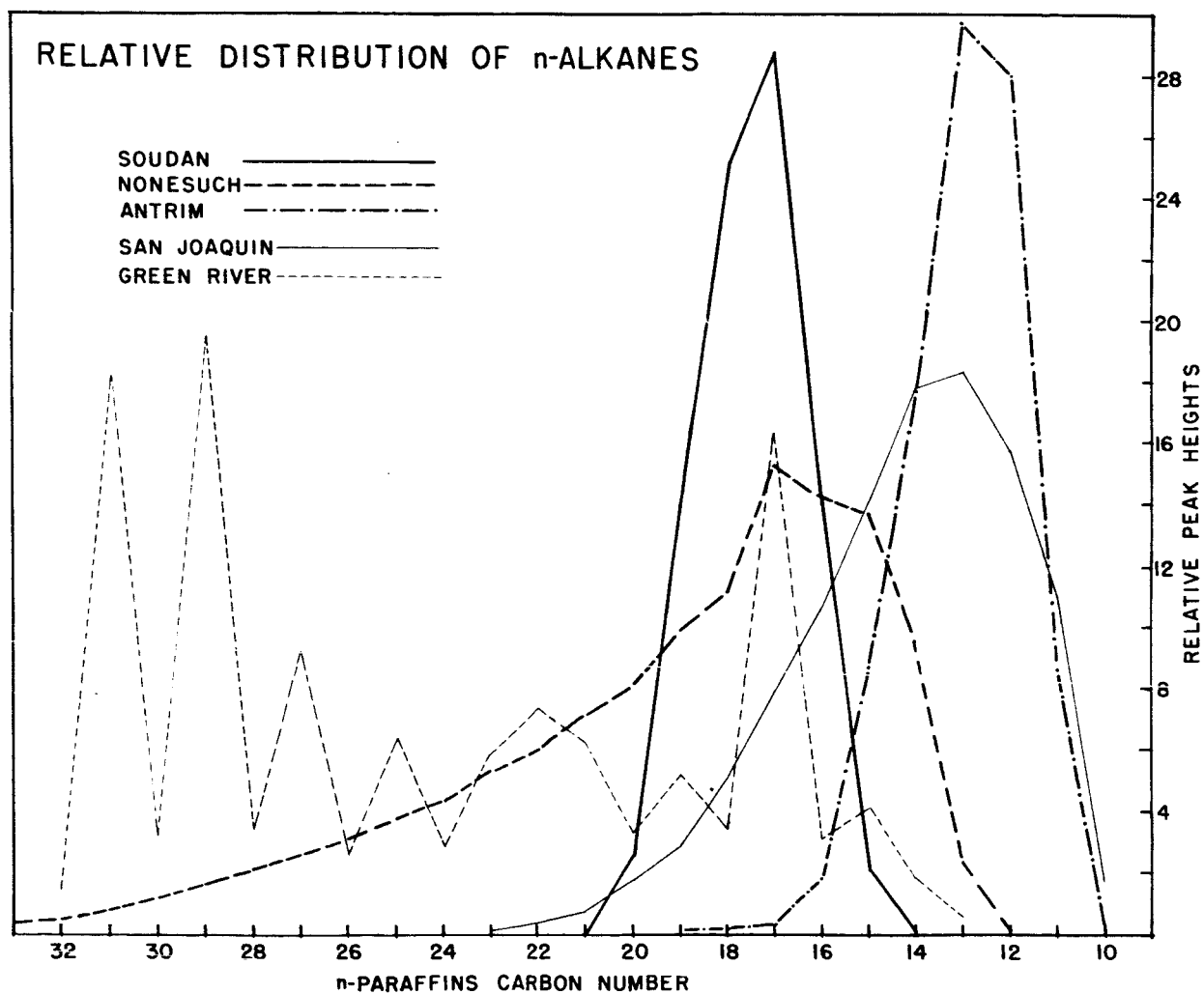
Figure XLIII Gas Chromatogram of Colorado Green River Shale



GREEN RIVER SHALE (COLORADO), ~60 X 10⁶ YRS. ALKANE FRACTIONS

MUB-3954

Figure XLIV Relative Distribution of n-Alkanes



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