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OCCURRENCE OF ISOPRENOID ALKANES  
IN A PRECAMBRIAN SEDIMENT

by

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## INTRODUCTION

Two experimental approaches currently bear on the origin of terrestrial life and the time of its first appearance. The "primitive atmosphere" experiments (reviewed by Horowitz and Miller, 1962) demonstrate that a wide variety of small molecules of biological significance can be formed in the laboratory from mixtures of extremely simple substances, such as methane, ammonia and water. The geological approach utilizes the record in the ancient sediments which is written both in the form of shapes attributable to fossil organisms and in the chemical nature of the imprisoned organic matter (Abelson, 1959; Cloud and Abelson, 1961; Breger, 1963; Colombo and Hobson, 1964). Earlier than about 600 million years ago, well-defined morphological remains are commonly scanty and of microscopic dimensions, and are generally difficult to relate conclusively to living things. (See inter alia, Barghoorn and Tyler, 1963; Harrington and Toens, 1963; Marshall et al, 1964; Vologdin, 1962).

A firm correlation between the morphological evidence and the organic matter present in the same rock would permit a systematic search for chemical evidence of early life in the ancient sediments. Certain classes of compound, such as the alkanes (Meinschein, 1963), the long-chain fatty acids (Abelson et al, 1964; Cooper and Bray, 1963), and the porphyrin pigments (Dunning, 1963), show promise as biological markers since they are evidently stable for long periods of time under geologic conditions. These compounds are truly valid as biological markers only insofar as they cannot be synthesized in significant proportions by abiogenic means. For this reason, therefore, "primitive atmosphere" experiments play an important role.

The range of compounds based on the isoprenoid subunit is particularly attractive, for here we have a high degree of structural specificity coupled with a widespread distribution in nature. Thus pristane (2,6,10,14 - tetramethylpentadecane, IV) and other isoprenoid hydrocarbons (e.g. I-III, V) have been isolated from, or identified in, crude petroleums of moderate ages (Mesozoic and Paleozoic) in concentrations vastly greater than those anticipated for individual branched alkanes in a thermally-derived mixture (Dean and Whitehead, 1961; Bendoraitis et al, 1962; for other papers, see Cummins and Robinson, 1964). Pristane is a known constituent of living things [inter alia zooplankton (Blumer et al, 1963), fish and whale oils (Hallgren and Larsson, 1963; Lambertsen and Holman, 1963, and references cited therein), wool wax (Mold et al, 1963 b, 1964) and marine sponges (Bergmann, 1963)], but the original source of the mineralized material may be the phytol portion of chlorophyll degraded either biogenically or abiogenically (Curphey, 1952; Bendoraitis et al, 1962). There is every prospect, therefore, that the isoprenoid hydrocarbons, and the related alcohols and acids, will be useful biological markers (see Mair, 1964).

In the present work we have studied the alkane fractions derived from a Precambrian sediment and an associated oil seepage. Small-scale adaptations of established separation and identification procedures have revealed the presence of inter alia, farnesane, pristane and phytane (2,6,10,14 - tetramethylhexadecane) in these fractions. We consider this presumptive evidence for biological activity at the time of deposition and compaction of the sediment (ca.  $1 \times 10^9$  years ago). Older formations containing accepted morphological fossils are, of course, known but the way seems open for the extension of

this particular aspect of the organic chemical approach to morphologically-barren rocks of great age.

The necessary experimental conditions for this study were established by processing suitable model mixtures and reference materials. These included the alkane fractions of known composition derived from tobacco leaf wax (Mole et al, 1963a) and a Colorado oil shale (Cummins and Robinson, 1964). Some preliminary findings with a supposedly abiogenic oil and with a synthetically-derived hydrocarbon fraction are also reported.

## EXPERIMENTAL

### Materials

All solvents were of analytical reagent grade and were distilled through an 18 inch column packed half-full of glass helices. Light petroleum (b. p. 66-75<sup>0</sup>), n-hexane and n-heptane (Phillips Petroleum Company, pure grade) contained approximately 0.9%, 3% and 0.03% respectively, of aromatics (estimated spectroscopically as benzene). Iso-octane was percolated through a column of powdered Linde molecular sieve (5 <sup>0</sup> Å); this solvent and cyclohexane were virtually free of aromatic compounds. Glassware was cleaned in detergent (Labtone), often ultrasonically, thoroughly rinsed with distilled water and solvents, kept in dust-free containers, and always rinsed with distilled benzene immediately before use. Teflon stopcocks were used.

Tobacco wax (Nicotiana tabacum; green leaves) was provided by Dr. W. Carruthers, University of Exeter, England; further reference samples of tobacco wax and high molecular weight alkanes were provided by Dr. J. D. Mold, Liggett and Myers Tobacco Company, Durham, North Carolina. Colorado oil shale of Eocene age from the Green River Shale, Rifle, Colorado, was kindly supplied in powdered form and as rock chunks by Dr. W. E. Robinson, Bureau of Mines, Laramie, Wyoming. The Nonesuch Oil occurred coated on rock from the upper part of the Parting Shale, White Pine Copper Mine, White Pine, Ontonagan County, Michigan. It was collected at a depth of about 900 feet, near drill hole 35 F. The oil-bearing calcite vein was from the dark gray massive bed of the Parting Shale member, and filled a joint or minor fault about an inch thick. The Nonesuch "Marker Bed" of the Upper Shale member of the White Pine Shale

was located in Block 34 D of the White Pine Mine at a depth of about 500 feet. We thank the owners of the mine for allowing collection of these samples, Mr. J. Trammell of the White Pine Copper Company, for their collection, and Dr. P. E. Cloud, Jr., of the University of Minnesota, for sending them to us. The Nonesuch formation is of Keweenawan age (Precambrian) and has been described by White and Wright (1954) and White (1960). Rose quartz from Colorado was bought from a Berkeley supplier. The oil from the Abbott mercury mine, Lake Country, California, was from a fissure at the 400 foot level, below the Bogess ore body. It was collected by E. H. Bailey and D. E. White, U. S. Geological Survey, Menlo Park, California, and kindly supplied by Dr. Bailey, together with a sample of the opal froth vein from the mine (Bailey, 1959).

2, 6, 10, 14-Tetramethylpentadecane (pristane, IV) was obtained from the Eastman Kodak Company. Phytane (V) was synthesized by hydrogenation (in hexane and 10% Pd-C at room temperature and pressure) of phytene and purified by preparative gas-liquid chromatography (tetracyanoethylated pentaerythritol phase). Farnesane (I) was similarly purified. This compound and the phytene were synthesized by Dr. D. W. Graham (Cason and Graham, 1964). Squalene, cholestane and  $C_{10}$ - $C_{18}$  even carbon numbered n-alkanes were from commercial sources, and  $C_{17}$  and  $C_{27}$  n-alkanes were kindly provided by Dr's A. G. Polgar and D. P. Stevenson, Shell Development Company, Emeryville, California.

### General

Precautions were taken to minimize contamination. Care was

taken in handling glassware and apparatus, and forceps were used where possible. Flasks were stoppered or covered with aluminum foil between operations; the time between these was kept short. Solutions were transferred by means of disposable glass pipettes. Solvents were evaporated in a rotary evaporator under water pump vacuum, with a silicone-greased rotary evaporator and a splash head; the air was carefully let in at the flask, so as to avoid contamination from the rubber tubing. A final evacuation on the oil pump was for a short time only (about 1 minute) to reduce loss of low-boiling compounds, which was appreciable with small samples. Concentration of solutions in small vials warmed on an aluminum heating block was achieved by evaporation in a fine stream of nitrogen (filtered through alumina). Vials were covered with screw caps lined with aluminum foil or Teflon.

#### Treatment of rocks

In general, after breaking up the rock on a clean surface with a hammer, pieces about 2 - 3 inches in size, all surfaces being fresh exposures, were crushed to small chips (not more than 1/2 inch) in a steel jaw crusher (Braun Ore Crusher, Braun Corporation, Los Angeles, California) and sieved to remove fine material. In certain cases of smaller samples (e. g. Nonesuch calcite vein) crushing was achieved with a heavy, cylindrical, hardened-steel pestle and close-fitting steel mortar. Small chips were carefully washed with HF and/or organic solvents (sonication was sometimes used) before powdering. This was best accomplished by a carefully cleaned hammer mill (mikropulverizer, Pulverizing Machinery Company, Summit, New Jersey), modified by using lead gaskets (no rubber washers). On a



very small scale (about 100 mg) it was possible to powder rock chips by means of a wig-1-bug (Crescent Dental Mfg. Company), but close fitting of the parts of the capsule was essential. Ball milling (Burundum tubular grinding media) of the Nonesuch shale in a roller-type jar mill was incomplete after several days.

### Extraction

The simplest extraction procedure was to heat a suspension of the powdered rock in a solvent for several hours, but the use of pre-extracted all-glass Soxhlet (with a sintered disc sealed to the bottom of a cylinder in which the powder was placed) was more efficient. The use of ultrasonics (McIver, 1962) to hasten extraction was explored. The powdered rock and solvent could be placed (in a suitable container such as a conical flask or centrifuge tube) in an ultrasonic tank (Disintegrator System Forty, Ultrasonic Industries Inc., Albertson, New York) partially filled with out-gassed water up to the level of the solvent in the flask, and sonicated at 90 kc/s and 40 watts for 5-20 minutes. An alternative and very convenient method was to use a titanium ultrasonic probe (Branson Sonifier Model S-75, Branson Instruments, Inc., Stanford, Connecticut), the tip of which was placed about 1 cm below the surface of a suspension of powdered rock and organic solvent in a centrifuge tube or bottle (250 ml), stirred magnetically (Teflon-coated stirring bar) if necessary. Sonication was for not more than 20 - 30 minutes and cooling was sometimes needed. On the scale of a few grams of rock and with solvent-sample ratios of about 10:1 efficiencies of the two methods of sonication were comparable. On a larger scale (up to 100 grams of powdered rock per probe sonication, repeated several times if necessary) with solvent/sample ratios

of only about 2:1, repeated extraction of the rock was essential to recover most of the organic material. In all extraction procedures, the resultant suspension was centrifuged, and the supernatant solution removed and evaporated.

#### Alumina column chromatography

Superior column performance results (Duncan, 1962) from the use of adsorbents specially prepared for thin-layer chromatography (TLC). BIO-RAD AG 7 neutral aluminum (2 - 44  $\mu$ , containing 15%  $\text{CaSO}_4$ ; BIO-RAD Laboratories, Richmond, California) was activated at 200° for 2 days and the column prewashed with n-hexane before chromatography. Also used was the same alumina prewashed with hot methanolbenzene (1:1) in an all-glass Soxhlet prior to activation, where polar solvents were to be used in the chromatography. Alumina/sample ratios were generally in the range of 20 - 100:1, and the column diameter was either 2.5 cm or 1 cm (for less than 10 g alumina). Chromatography was usually carried out with slight air pressure (air filtered through alumina) to obtain a drip rate of approximately one drop/sec. Saturated alkanes were eluted with n-hexane or n-heptane. A control run (without any added sample) produced no detectable eluate at the concentrations and sensitivity levels used for analytical gas-liquid chromatography (GLC).

#### Sieving

Pellets (1/16 inch) of 5 Å<sup>o</sup> molecular sieve (Linde Company, Division of Union Carbide Corporation) were dried for 12 hours at 200° in vacuo (mercury pump) in the presence of  $\text{P}_2\text{O}_5$ , and stored

in a desiccator. The ratio of sieve to sample varied from 20:1 to 100:1 (O'Connor et al, 1962). Dry benzene was usually used as solvent, and the mixture refluxed in a round-bottomed flask, fitted with a reflux condenser and drying tube (Drierite), for 1 to 3 days. The solution was removed (pipette) and centrifuged. The sieve was thoroughly washed with hot benzene (all-glass Soxhlet) for several hours and the washings added to the solution of the branched-cyclic alkanes, which was then evaporated.

The benzene-washed sieve, containing the normal alkanes, was treated with 24% HF and benzene, and the mixture stirred magnetically (Teflon-coated stirring bar) with cooling until the sieve dissolved (approximately 1 hour). After separation of the layers, the benzene solution of n-alkanes was filtered through a short column of anhydrous  $\text{Na}_2\text{CO}_3$  (prewashed with benzene) and the solvent evaporated. Gas-liquid chromatographic analyses showed the n-alkane peaks only, with no isomerization to branched alkanes. A blank run on 10.5 g sieve, 70 ml 24% HF and 25 ml benzene treated in the above manner gave no peaks in the gas chromatogram under the usual analytical conditions. An alternative method for recovery of n-alkanes was tried with the Nonesuch Oil: Soxhlet extraction with refluxing n-hexane was slow (recovery was still incomplete after 2 days) and the distribution of the n-alkanes was noticeably weighted to lower molecular weight compared to that of the n-alkanes isolated by the HF treatment.

The effectiveness of the method in completely removing n-paraffins from branched and cyclic alkanes is illustrated by the treatment of cholestane (7.0 mg), squalane (719 mg), pristane (6.2 mg) and n-C<sub>27</sub> (9.3 mg) with benzene (4 ml) and 5 Å<sup>o</sup> molecular sieve (1.1 g).

Refluxing for 3 hours did not completely remove the  $\underline{n}\text{-C}_{27}$  from the solution, but after 22 hours, the  $\underline{n}\text{-C}_{27}$  was not detectable by GLC (present in less than 0.1% of its original amount). None of the branched or cyclic hydrocarbons were found in the  $\underline{n}\text{-C}_{27}$  recovered from the sieve with HF, showing the effectiveness of the washing with hot benzene (which did not remove  $\underline{n}\text{-C}_{27}$  -- GLC analysis of the washings).

### Thin layer chromatography

Though not actually used for the separation of individual hydrocarbons from rock extracts, TLC was useful for testing them and determining the relative proportions of the alkanes and aromatic compounds present. In one case, a thicker layer (1 mm) was used for small scale isolation of saturated hydrocarbons. Adsorbents used were BIO-RAD alumina (2 - 44  $\mu$ , neutral AG-7 and acid AG-4, with 15% and 5%  $\text{CaSO}_4$  respectively) and Woelm neutral TLC alumina (Alupharm Chemicals, New Orleans, Louisiana). Coated plates (0.25 mm) were usually prewashed with methanol/benzene (1:1) or hydrocarbon solvent, activated at 200° and stored in a desiccator. Samples were applied as solutions in a 1  $\mu$ l micropipette (Drummond "Microcaps"). Detection was by iodine vapour, or by fine sprays of 7% phosphomolybdic acid in methanol or of alcoholic  $\alpha$ -cyclodextrin followed by iodine. Prior to spraying, fluorescence under UV light (254 m $\mu$ ) was used to detect the presence of aromatic compounds. Spots detected by iodine could be sucked up onto a small glass sinter, eluted and analyzed by GLC.

The separation of individual alkanes by TLC might be anticipated from the findings of Evans et al (1957) who used alumina columns with very high alumina/sample ratios for the separation of  $\underline{n}$ -paraffins

and naphthenes and also n-paraffins of different molecular weights. Table 1 gives  $R_c$  values (ratio of the  $R_f$  of the sample to the  $R_f$  of cholestane under the same conditions) for various hydrocarbons\* (5  $\mu$ g samples) when run on neutral TLC alumina plates (BIO-RAD) with cyclohexane.

TABLE I

<u>Compound</u>	<u><math>R_c</math> value</u>
<u>n</u> -C <sub>27</sub>	0.86
<u>n</u> -C <sub>31</sub>	0.59
<u>iso</u> -C <sub>31</sub>	0.73
<u>anteiso</u> -C <sub>30</sub>	0.85
<u>anteiso</u> -C <sub>32</sub>	0.71
squalane	1.09

The  $R_f$  of cholestane was 0.82 under these conditions, which we recommend for the separation of a high carbon-numbered alkane mixture. Development of a plate of Woelm neutral TLC alumina with n-heptane gave an  $R_f$  value of 0.60 for cholestane, and  $R_c$  values of 1.10 for both pristane and squalane.

Hydrocarbons and esters have been separated from each other by thin-layer chromatography on alumina (Haahti and Nikkari, 1963). On acid BIO-RAD alumina, with development by n-hexane, we found the following  $R_c$  values ( $R_f$  for cholestane = 0.86): cholesteryl stearate 0.01, methyl stearate 0.08, Nonesuch Oil Alkanes 1.05 (approximate

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\* The terms iso- and anteiso- refer to 2-methyl and 3-methyl substituted n-alkanes, respectively e. g. iso-C<sub>31</sub> is 2-methyltriacontane.

center),  $\underline{n}\text{-C}_{31}$  0.71 and the Colorado oil shale alkanes 0.65 - 1.1. Carbon tetrachloride moves the esters (e. g. methyl stearate,  $R_f = 0.19$  compared to the Nonesuch hydrocarbons at  $R_f = 0.95$ ). The order of polarity (assuming no effect of the different aluminum oxides used) is  $\underline{n}\text{-hexane}$  (3% benzene) > cyclohexane >  $\underline{n}\text{-heptane}$  (0.03% benzene), deduced from the  $R_f$  values of cholestane in the different systems.

### Preparative Gas-Liquid Chromatography

The instrument used was the Aerography A90 P-2, equipped with thermal conductivity detector and 5 ft. x 1/4 in. stainless steel columns. The solid supports were 60-80 mesh Chromosorb W, acid washed (DMCS), 100-120 mesh Gas Chrom Z, or 100-120 mesh Gas Chrom RA. Helium flow rates were 50 to 70 ml/min at 50 p. s. i. Injector and detector temperatures of 200° to 300° and 185° to 280°, respectively, were used depending on the boiling range of the material to be chromatographed. Silicone gum (3% SE-30) was used as stationary phase in the first purification step. The selection of the stationary phase for the second step was made empirically by using as criterion the separation of  $\underline{n}\text{-C}_{17}$  and pristane (which coincide on SE-30). These tests were made on analytical columns (5 ft. x 1/8 in. except for a 10 ft. x 1/8 in. column of Apiezon L). On 1% Apiezon L there was no separation ( $R_T$ , 15 min); on 5% Carbowax 20 M the two peaks were partially resolved; on 1% QF-1 and 3% Triton X the peaks still overlapped at their bases; and on 5% TCEPE (tetracyanoethylated pentaerythritol) there was an excellent separation of the peaks. For preparative purposes, the latter phase was deposited on 100-200 mesh Gas Chrom RA by evaporation from  $\text{CH}_2\text{Cl}_2$  solution. The column was

conditioned at 130° in a slow flow of nitrogen for 2 days; the bleed at 100° was then acceptable (after one period of tests at 100° over a period of weeks this column became much less efficient).

The branched-cyclic alkane fractions derived from the various alkane mixtures by the sieving procedures were subjected to an initial fractionation on 3% SE-30. In some instances this fractionation was confined to the separation of the alkanes into two parts: low boilers (from 100° to 200°) and high boilers (from 200° to 300°). In the initial exploratory investigations central cuts of peaks from this column were taken for analysis by mass spectrometry (MS), but in later studies these cuts were injected onto a second column loaded with 5% TCEPE. For instance fraction (d) from the Nonesuch oil fractionation was resolved into three or four partially separated peaks with pristane (IV) the largest and last peak to emerge (Fig. 7F). However, a 2°/min temperature-programmed GLC of fraction (d) on a 150 ft. x 0.010 in. polyphenyl ether (OS-138) column (run on a Perkin-Elmer Model 226 by Dr. Warren Averill of the Perkin-Elmer Corporation, Norwalk, Conn.) showed about twenty-three minor constituents when the pristane peak was well off-scale. Also, the distribution was different, with pristane eluting first.

A central cut of the pertinent peak from the TCEPE column was collected (by methods described below) for analysis by MS, IR, and analytical GLC (1/8 in. packed and 0.010 in. capillary columns).

The collection of the GLC fractions of molecular weight greater than  $n\text{-C}_{13}$  was made by two methods. The first was to collect the eluate in a straight glass capillary (1 mm bore by 10 cm long) that had been cleaned with chromic acid and rinsed with distilled water. One end was flame-polished to prevent contamination by silicone rubber

septum material. After collection the end was then sealed, the condensate centrifuged and the other end sealed to prevent loss by evaporation. For further GLC, the condensate was either washed into a pear-shaped vial (1 ml) with solvent (20  $\mu$  ) and then reinjected by microsyringe, or, dissolved in solvent (10  $\mu$  ) and withdrawn from the capillary directly by syringe. Recoveries were better by the latter method and averages 80%. The second method, which was more direct and very convenient for repetitive collection and reinjection of GLC eluates, had maximum recoveries of around 90%. This technique consisted of collecting the eluates in 2-in. long hypodermic needles (No. 23 gauge) whose tips had been bent at an angle so that plugging by injector septa did not occur. Reinjection was done by filling a 50  $\mu$ l Hamilton microsyringe (minus the standard needle) with about 40  $\mu$ l of solvent, fitting on the hypodermic needle, and injecting onto another column. Most of the solvent remains in the needle hub and does not affect the performance of 1/4 in. columns. A No. 16 needle was prepared as a borer and used to cut holes in the rubber septa used at the exit port. The GLC cuts were made under either isothermal or temperature programmed conditions and carrier gas flows were about 50 ml/min. to minimize aerosol formation and consequent sample loss. For storage purposes aluminum foil was placed over the hub of the needle (Teflon plugs may also be used), and the tip of the needle inserted into a silicone rubber septum. However, for final collection of the GLC eluates for analytical studies (MS, IR and capillary GLC) we preferred collection in glass capillaries by the first method.



## Analytical Gas-Liquid Chromatography

An Aerography 665-1 (Wilkins Instr. and Research, Inc.) with 5 ft or 10 ft by 1/8 in. packed columns was used to display the general patterns of hydrocarbon distribution (temperature programmed) and for retention time studies for the identification of individual hydrocarbons (isothermal conditions). An Aerograph Hy-F1 was also used for the latter purpose. Both instruments had hydrogen flame detectors and nitrogen as the carrier gas with flow rates of 30 ml/min. Temperature programming of the columns was usually from 100° to 300° at rates of from 3°/min to 10°/min. The injector and detector temperatures were set appropriately at 220° - 300° and 150° - 240°, respectively. We noted that occasional batches of silicone rubber septa exuded high molecular weight volatiles, which were still evident after sonication in solvent and two days under vacuum at 220°. Another effect noticed was the apparent shifting of the maximum of the envelope of the normal alkanes with different temperature programming rates on the Aerograph 665-1. The shift was small (usually only one carbon number) and the relative peak areas did not seem to change.

The Aerography Hy. F1 was modified to take a 150 ft x 0.010 in. capillary column. A sample splitter embodying concentric sample and effluent tubes was installed, as well as a detector base that allowed the hydrogen gas to enter behind the end of the capillary tubing. A scavenger flow of 20 ml/min of nitrogen was added to the hydrogen gas inlet and this modification further increased the resolution by a factor of two. Splitting ratios were from about 500:1 to about 5000:1 and were obtained by using various sizes of hypodermic needles for the splitter effluent. When a mixture of pristane and  $n\text{-C}_{17}$  alkane was injected onto a 150 ft x 0.010 in. Apiezon L column with a carrier gas

velocity of 10 cm/sec. , the retention times were around 25 min. , the theoretical plateages were about 135,000 and the separation factor 4.22.

A Barber-Colman Model 20, equipped with a 200 ft. x 0.010 in. Apiezon L column, was made available to us through the courtesy of Prof. W. G. Dauben. This instrument was used with an argon ionization detector, a carrier gas velocity of 15 cm/sec and a splitting ratio of 100 : 1. Both the Hy-F1 and the Model 20 were used to test the equivalency in retention times of our collected samples and standard compounds (by injection of mixed solutions) and to test for purity.

### Spectrophotometry

Ultraviolet spectra were recorded on a Perkin-Elmer Model 202 and infrared survey spectra on Perkin-Elmer Models 137 and 237. For high-resolution infrared spectra of micro samples the Beckman IR-7 was used with a Beckman beam condenser and a Type D micro-cavity cell (1 mm path, 4  $\mu$ l vol.) made by Barnes Engineering Company of Stamford, Conn. Use of the beam condenser resulted in dispersion-type bands in the water-vapor region, but these could be reduced, though not eliminated, by careful adjustment. The GLC condensate in carbon tetrachloride (10  $\mu$  ), was transferred from the collection capillary to the micro cell with a Hamilton micro syringe. Contamination was minimized by handling micro apparatus with forceps and reasonable spectra in the  $1380\text{ cm}^{-1}$  region could be obtained with 25  $\mu$ g of pristane ( $\text{C}_{19}\text{H}_{40}$ , IV).

### Mass Spectrometry

Mass spectra were determined on a modified CEC 21-103C mass spectrometer (for details of modifications and performance, see 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  $\mu$  amps and 160 to 180 volts per stage on the multiplier. Representative samples were examined in a direct inlet system (Burlingame, 1964). In the case of more volatile fraction like those containing a C<sub>15</sub>-isoprenoid (farnesane, I) the probe used for introduction of the sample into the glass inlet system or via the vacuum lock had to be precooled with liquid nitrogen for successful operation. Without this precaution C<sub>15</sub> samples in the range of 15  $\mu$ g or less were rapidly pumped off and sometimes lost during introduction. Great care was exercised not to contaminate the outside of the capillaries containing minute samples. The capillaries were cleaned prior to introduction with a 3 : 1 mixture of freshly distilled benzene and methanol, and after this operation they were handled only with forceps or nylon gloves. Fast scanning (30 seconds for the mass range m/e 90-600) was advantageous for small samples (15  $\mu$ g or less). The background was always scanned before a sample was introduced.

## RESULTS

### Tobacco Wax

Tobacco wax (Nicotiana tabacum) was used to test the efficacy of the sieving and gas chromatographic procedures.

Tobacco wax (19.7 mg) in benzene (8 ml) was heated with molecular sieve (1.6 g) for 3 days. The branched alkane fraction showed no normal hydrocarbons and the normals no branched (GLC; cf Fig. 1). The distribution of alkanes was in good agreement with published data (Mold, et al, 1963a). Representative GLC fractions taken from the normal and branched series were subjected to mass spectrometric analysis and were identified as follows: n-C<sub>27</sub>, n-C<sub>31</sub>, iso-C<sub>31</sub>, iso-C<sub>33</sub>, anteiso-C<sub>30</sub>, and anteiso-C<sub>32</sub> hydrocarbons. As previously reported by Mold, et al (1963a), the odd and even-numbered alkanes found to be predominately 2 - methyl (iso-) and 3 - methyl (anteiso-) substituted, respectively, did contain small amounts of the other isomer, as determined from the abnormally intense M - (C<sub>2</sub>H<sub>5</sub>) fragment in the mass spectrum (Fig. 2B) of the C<sub>33</sub> branched alkane fraction. As shown in the spectrum (Fig. 2A) of pure 2 - methyl dotriacontane, M - (C<sub>3</sub>H<sub>7</sub>) is a very intense fragment in the high mass region, in contrast to the very low intensity of the M - 29 peak. However, loss of the ethyl radical becomes predominant in the mass spectral fragmentation of the anteiso - paraffins (Fig. 2C).

An initial attempt to apply the sieving process with iso-octane as solvent (5 hr. reflux) did not result in complete removal of the 6-alkanes.

Thin layer chromatography of tobacco wax (Bio-Rad AG-7

neutral alumina, development with n-hexane) and examination of 4 regions by GLC (1% SE-30) showed that partial separation of a plant alkane mixture could be achieved. The lower molecular weight alkanes of a homologous series moved faster than those of higher molecular weight, as expected. The first cut comprised n-C<sub>27</sub>, anteiso-C<sub>30</sub>, iso-C<sub>29</sub>, and some of the n-C<sub>28</sub>; the second cut contained n-C<sub>28</sub>, n-C<sub>29</sub>, iso-C<sub>31</sub>, and anteiso-C<sub>32</sub>, while the third cut was mainly the n-C<sub>31</sub> alkane. The fourth cut contained an unidentified compound, with the same GLC retention time (on Apiezon L) as n-C<sub>28</sub>. There is thus a difference in the order of elution of the alkanes between TLC (alumina) and GLC (SE-30 and Apiezon L), and this may yet prove useful in handling mixtures of alkanes.

#### Green River Shale (Colorado)

(i) The powdered shale (21 g, 78% of a test sample passed through a 200 mesh sieve) was extracted with n-hexane in an all-glass Soxhlet-type apparatus for several hours. Evaporation of the solvent gave a brown gum (400 mg). Alumina chromatography (20 gm neutral TLC alumina) with elution by first n-hexane (95 ml) and second, CCl<sub>4</sub> (40 ml) gave a saturated hydrocarbon fraction (125 mg, only a trace of C=O absorption near 1700 cm<sup>-1</sup> in the infrared spectrum). The gas-liquid chromatogram is shown in Figure 3A. This fraction was sieved (8 g of 5 Å<sup>o</sup> molecular sieve, 20 ml benzene) for 63 hours and the branched-cyclic alkane fraction (72 mg) obtained. The GLC analysis (Fig. 3B) showed the presence of large proportions of hydrocarbons with the retention times of phytane and pristane, together with compounds in the region delineated by the retention times of n-C<sub>30</sub> to n-C<sub>37</sub> alkanes. The n-alkanes (10 mg; recovered by HF treatment of

the powdered sieve) showed a marked predominance of odd carbon numbers (Fig. 3C).

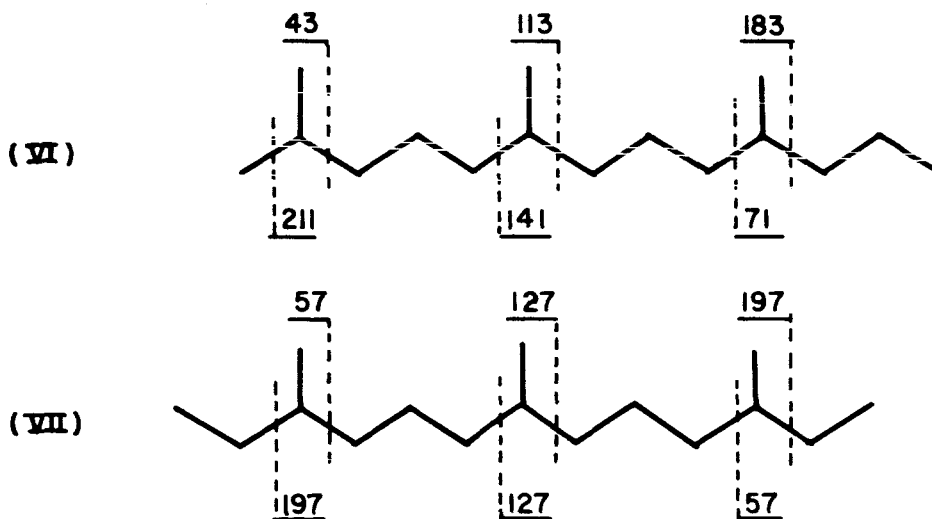
(ii) A sample of the powdered shale (12 g) was heated in n-hexane (with glass wool to prevent bumping) for about 5 hours, the mixture centrifuged and the solvent evaporated to give a dark brown oil (170 mg). This was chromatographed on a short column of alumina (TLC grade), and the n-hexane eluate (30 ml) evaporated. The saturated hydrocarbon fraction (53 mg) was sieved (100:1 sieve/sample ratio, 10 ml benzene) for 45 hours and the branched-cyclic fraction obtained (42 mg). The n-alkanes (ca. , 10 mg) were recovered from the benzene-washed, powdered sieve.

(iii) On a larger scale, the powdered shale (100 g) was extracted three times ultrasonically (Branson probe, 20 kc/s) with n-heptane (total: 330 ml) for 1/2 hours, the solvent removed after centrifuging to give a pale brown gum (1.34 g recovered). This showed a band about  $1700\text{ cm}^{-1}$  in the infrared spectrum together with hydrocarbon absorption. Chromatography on neutral TLC alumina (43 g) and elution with 75 ml n-heptane yielded the saturated hydrocarbons (339 mg). Sieving this fraction (339 mg) with 14 g of 5 Å<sup>o</sup> molecular sieve 20 ml benzene (67 hours), removed the normal alkanes. The branched-cyclic fraction (267) was a mobile colorless oil.

Samples of this fraction corresponding in retention times to the  $C_{16}$ ,  $C_{18}$ ,  $C_{19}$  and  $C_{20}$  isoprenoids (1/4" column 3% SE - 30) were subjected to mass spectrometric analysis. The highly characteristic spectra (Fig. 4A, 4B, 4C, and 4D, respectively) not only established the anticipated number of carbon atoms, but also characterized the various types of branching and hence led to the confirmation of the complete structures (II - V). These findings reaffirm the

results of Cummins and Robinson (1964).

Branched hydrocarbons fragment predominantly at the sites of branching, giving energetically favoured tertiary or secondary carbonium ions, and thus intense signals at certain mass numbers. This type of compound is ideally suited for mass spectrometric structure determination. The spectra of all four compounds display the usual hydrocarbon spectrum with peak groups at an equal spacing of 14 mass numbers (differences of  $-\text{CH}_2-$  groups). The intensities of these are, however, very sensitive to the location of branching. The intense  $\text{C}_n\text{H}_{2n+1}$  peaks at  $m/e$  183, 141, 113 in the spectrum of the  $\text{C}_{16}$  compound (Fig. 4A) demand the familiar isoprenoid branching of 2,6,10-trimethyltridecane (II) with a fragmentation pattern as indicated in (VI).



Other non-isoprenoid structures are obviously ruled out as is the isoprenoid 3,7,11-trimethyltridecane (VII), where a simpler fragmentation pattern would be required by the molecular symmetry. The

structures of the  $C_{18}$ ,  $C_{19}$ , and  $C_{20}$  hydrocarbons were determined similarly and identified as 2,6,10-trimethylpentadecane (III; Fig. 4B), 2,6,10,14-tetramethylpentadecane (pristane; IV), and 2,6,10,14-tetramethylhexadecane (phytane; V). The spectra (Figs. 4C and 4D) of the two latter isoprenoids were compared with those of authentic pristane and phytane (Figs. 4E and 4F).

Minor impurities in the isoprenoids isolated which were frequently encountered are indicated by peaks occurring at M-2, M-8, and M-10. These necessarily cyclic constituents provide an easily traceable fragmentation pattern since the mass numbers do not always coincide with those of the saturated isoprenoid. Of course, in some cases fragments from these impurities do coincide with those from the acyclic isoprenoids, resulting in small variations in fragment abundances as compared with the spectra of the authentic pure compounds. Contributions by the impurities to the peaks characteristic of the saturated isoprenoid (i. e.  $m/e$  113, 183), were minimized by choosing  $m/3$  113 as the reference peak in all cases. These minor constituents are the subject of further investigation.

(iv) On a small scale, a chip of the shale (261 mg) was twice washed (1 hr.) in boiling benzene/methanol (3:1) and powdered ("Wig-L-Bug"). The powder (118 mg) was refluxed twice with n-heptane (2 ml) for a total of 19 hours, the extracts combined after centrifuging and the solvent removed. The extract (3 mg) was chromatographed on a plate of Woelm neutral TLC alumina (1 mm thick), with n-heptane as developing solvent. The saturated alkanes were scraped off, eluted with benzene, and the eluate sieved (110 mg of 5  $\overset{\text{O}}{\text{\AA}}$  sieve) in iso-octane (2 ml) for 55 hours. The GLC patterns for the branched-cyclic and normal alkane fractions closely resembled those obtained in the other



experiments (i) - (iii).

#### Nonesuch Shale Formation - Oil Seep

The oil-coated parting shale was washed with benzene and the solvent evaporated to yield a black oil, which showed only hydrocarbon absorption (and a trace of water) in the infrared. The oil was chromatographed on a column of neutral TLC alumina (90 g), and eluted with n-hexane (250 ml), yielding ca. 4.0 g of a hydrocarbon fraction (GLC, Fig. 5A) which contained only small amounts of aromatic hydrocarbons (UV and IR spectra, TLC and fluorescence under UV light). Treatment with 5 Å molecular sieve (81 g sieve, 100 ml benzene, 88 hours) gave 2.5 g of the branched-cyclic alkane fraction as an almost colourless, mobile oil. The distribution of the alkanes in this fraction was examined by packed-column GLC (Fig. 5B) and by capillary-column GLC (Fig. 6A), which revealed its great complexity. Complete removal of normal alkanes was indicated by the constancy of the pattern even after a further 97 hours treatment with fresh molecular sieve. Normal paraffins which were recovered from the sieve by treatment with 24% HF, showed a distribution with a maximum at C<sub>17</sub> decreasing smoothly to at least C<sub>35</sub> (Fig. 5C) with a marginal odd/even predominance.

Thiourea adduction of the branched-cyclic fraction was not successful. A small sample (25 µl) in benzene (0.5 ml), was poured into a saturated solution of thiourea in methanol (5 ml), and the mixture left for 4 days at 0°. After centrifuging, the liquid layer was withdrawn, diluted with water, centrifuged with n-heptane (3 ml), washed with water and concentrated to about 45 µl. The GLC pattern of this fraction was very similar to that obtained from the n-heptane extract

after decomposition of the crystalline adduct with warm water.

The total branched-cyclic alkane fraction, or a portion of it ( $a_0$  - g), was now gas chromatographed (3% SE-30) and certain cuts ( $a_0$ , a, d, e and f) collected (Fig. 7A). Capillary column GLC (Apiezon L, Fig. 6B) had shown the presence of peaks in the regions ( $a_0$ ), (d) and (f) coinciding in retention time with co-injected farnesane (I), pristane (IV), and phytane (V) respectively. Examination of the individual fractions by mass spectrometry and by further GLC (1% QF-1 and 1% Apiezon L) indicated that these fractions contained isoprenoids of the appropriate molecular weight. As it was not possible to resolve lettered fractions by TLC (neutral alumina), they were further purified (Fig. 7B - E) by GLC over a highly polar phase, 5% tetracyanoethylated pentaerythritol (TCEPE). The cuts from this latter column (Fig. 7B - 7E) were found to be substantially pure and to have the retention times of the appropriate isoprenoids by isothermal analytical GLC as follows: 5 ft. x 1/8 in. columns packed with 1% QF-1 ( $100^\circ$ ), 3% SE-30 ( $144^\circ$ ) 5% Carbowax 20M ( $140^\circ$ ), and 5% TCEPE ( $125^\circ$ ); 10 ft. x 1/8 in. column packed with 1% Apiezon L ( $162^\circ$ ); 150 ft. x 0.01 in. and 200 ft. x 0.01 in. capillary columns coated with Apiezon L and with Carbowax 20M.

Infrared spectra over the region  $1150 - 1500 \text{ cm}^{-1}$  were obtained on the micro fractions of pristane (65 g) and phytane (25  $\mu\text{g}$ ) isolated as above. The spectra over the methyl bending region  $[8(\text{CH}), \text{sym. } 1350 - 1420 \text{ cm}^{-1}]$  for these fractions agree well with those for the reference alkanes, as shown in Fig. 8. Finally, the mass spectral data on the above, purified fractions, showed that the spectra obtained from the  $\text{C}_{15}$  (Fig. 9A),  $\text{C}_{19}$  (Fig. 9C) and  $\text{C}_{20}$  (Fig. 9D) fractions are in good agreement with the reference spectra of authentic farnesane (Fig. 9E), pristane (Fig. 4E) and phytane (Fig. 4F), respectively. The

spectrum (Fig. 9B) of the  $C_{16}$  branched compound permits its identification with the  $C_{16}$  isoprenoid hydrocarbon (II), 2, 6, 10-trimethyltridecane, (Fig. 4A) from the Green River Shale.

Pristane and phytane were estimated to be present in the branched-cyclic fraction to the extent of some 1.2 and 0.6% respectively (GLC area measurements).

#### Nonesuch Shale Formation — Marker Bed

(i) In a preliminary small-scale experiment, chips of rocks were washed with dilute HF, water, methanol, benzene/methanol (3 : 1) and n-pentane. The chips were ball-milled (90 hours) and the powder (18 g, smaller than 80 mesh) was extracted ultrasonically (disintegrator) with n-heptane (40 ml) for 20 min. The slurry was centrifuged and evaporation of the extract yielded approximately 5 mg of an oil. The extract was chromatographed (TLC alumina) and eluted with n-heptane (10 ml). The saturated alkanes so obtained (ca. 3 mg) were heated with 5 Å (molecular sieve (70 mg) and benzene (0.5 ml) for 23 hours. The GLC pattern of the total, branched-cyclic, and normal alkane fractions were similar to those (Fig. 5) of the Oil seep.

(ii) A large chunk of the shale was hammered into 2 in. pieces, crushed and washed ultrasonically (benzene/methanol, 3 : 1). The cleaned chips were pulverized (87% of the powder was smaller than 200 mesh) and the powder (1140 g) was extracted ultrasonically (Sonifier) in 100 gm batches in 250 ml centrifuge bottles with 160 ml benzene/methanol (4 : 1) per batch (1.5 liters total, including washings; solvent from one run was usually used for the subsequent one). The combined extracts were centrifuged and evaporated to dryness to yield a brown fluorescent gum (340 mg 0.03%). It was estimated that the

solvent extractable material could be raised to around 0.1% of the rock if sufficient solvent were to be used. The oil showed a small band at about  $1700\text{ cm}^{-1}$  in the IR spectrum. The heptane-soluble material was chromatographed (TLC neutral alumina, 35 g) and the alkane fraction (236 mg) eluted with n-heptane (40 ml). Treatment (67 hour) with molecular sieve (50:1 sieve/sample) gave the branched-cyclic and normal alkane fractions. The GLC patterns for these fractions (Fig. 10B and C, respectively) were similar to those (Fig. 5B and C) of the Nonesuch Oil Seep. Cuts corresponding in retention times to the isoprenoid alkanes were collected and purified by GLC as already described for the Oil Seep and examined (MS and IR). Mass spectral data for the  $C_{16}$  and  $C_{19}$  isoprenoid cuts, which are shown in Figs. 11A and 11B compare well with the reference data (Figs. 4A and 4E respectively), although the pristane clearly contains cyclic impurities.

#### Nonesuch Shale Formation - Calcite Vein

The vein (about 1 in. thick) was removed from the shale and crushed. The chips (147 g) were washed successively with benzene/methanol, benzene, and n-pentane and dissolved in hydrochloric acid (10%, 1.2 litres). The benzene extract (200 ml) was washed with water, dried ( $\text{MgSO}_4$ ) and the solvent evaporated, yielding a brown oil (121 mg). After chromatography (neutral TLC alumina) as before, the total alkane fraction (76 mg) was sieved and yielded 55 mg and 12 mg of the branched-cyclic and normal alkane fractions, respectively. The GLC patterns were again similar to those of the Oil Seep (Fig. 5), although the distribution of n-alkanes was centred around  $C_{20}$  rather than  $C_{17}$ , and the relative proportions of the peaks containing the isoprenoids were also somewhat different. See Figures 5: D, E, F.

Abbot Mercury Mine — Oil Seep

The oil (3.18 g), which was almost completely soluble in n-heptane, was chromatographed (TLC alumina, 68 g) in the usual way. The first eluate (1.87 g) contained only about 12 mg of aromatic compounds (estimated at 271 and 277 m $\mu$ ) but later fractions contained more. The first fraction was treated with molecular sieve but was recovered practically unchanged. The n-alkane fraction, which comprised only about 0.1% of the total oil and ranged from about C<sub>13</sub> to C<sub>30</sub>, centered on C<sub>17</sub>, will be discussed in a subsequent publication. The GLC of the recovered branched-cyclic fraction (Fig. 12A) reveals a much more complex mixture of alkanes than do the other materials examined. However, the standard GLC isolation procedure already described afforded a fraction corresponding in retention time to pristane. The mass spectrum obtained (Fig. 11C) on this fraction establishes the presence of pristane (IV), although other alkanes are present in small amounts. An estimate based on GLC peak areas gives the percentage of pristane as about 0.25% of the branched-cyclic alkane fraction.

Similar GLC fractionations and mass spectrometric studies indicate the presence of other isoprenoids, but they are minor constituents. Indeed, branched alkanes, seem to be largely absent, whereas monocyclic alkanes C<sub>n</sub>H<sub>2n</sub> appear to be major constituents. This complex mixture is being examined further. (Dr. R. B. Johns, personal communication).

Fluorescence and electron spin resonance (ESR) measurements of certain concentrates of the crude oil indicate the presence of trace quantities of metal porphyrins or chlorins.

### Control Experiments

(1) Rose quartz. Chips (1/2 in.) were first washed ultrasonically with benzen/methanol (3:1) and benzene, and then pulverized (steel mortar, followed by the Mikro pulverizer). The powder (232 g, 80% smaller than 200 mesh) was extracted ultrasonically by probe for 2 hours in benzene/methanol (3:1, 300 ml) and the slurry centrifuged and the extract evaporated. There was no weighable residue but alumina chromatography followed by GLC analysis in the usual way showed the presence of small quantities of alkanes in the region normally examined (of the order of 10  $\mu$ g per Kg rock). The level of this contamination, which probably derives from the mill, is adjudged too low to affect the analyses reported in this paper.

(2) Dandruff. Ultrasonic extraction of dandruff (4.3 mg) with n-heptane gave material with a single GLC peak (equivalent to 0.11 mg, total) in the region n-C<sub>28</sub> to n-C<sub>29</sub>. The compound was eluted from alumina by n-heptane, and destroyed by bromine treatment, and was presumed to be squalene, which has been reported to be the principal hydrocarbon of the human scalp.

(3) Finger grease. An n-heptane extract of human skin grease was obtained by brief immersion of the fingers in this solvent. This extract exhibited a characteristic GLC pattern, with few peaks below the n-C<sub>20</sub> position and several large peaks above the n-C<sub>25</sub> position.

## DISCUSSION

The work reported in this paper is part of a general program of organic geochemistry which is concerned with the development of life-detection methods for terrestrial and extraterrestrial samples. Our present concern is with the Precambrian era ( $> 0.6 \times 10^9$  years ago), and it is our hope that chemical data will augment the rather sparse morphological evidence now available. The alkane fractions were chosen for study on account of the ubiquitous distribution of hydrocarbons in geologic materials, their stability, and the existence of powerful methods for their isolation, separation, collection and identification. Further, the validity as biological markers of alkanes having an isoprenoid skeleton, for example pristane (IV), phytane (V), squalane, and cholestane, has yet to be questioned though this point will be reassessed later in the discussion.

The procedures employed in the isolation and identification of the individual alkanes have been drawn from the literature and modified where appropriate. They may be summarized as follows: rock samples were crushed into small chips, thoroughly washed with organic solvent, and then pulverized to a fine powder. Hot extraction or, alternatively, ultrasonic extraction with organic solvent, followed by concentration in vacuo, furnished an extract which was then chromatographed in hexane over activated alumina. The alkane fraction emerged in the first eluates. Some idea of the carbon number distribution over the approximate range  $C_{11}$  to  $C_{37}$  could be conveniently obtained for this fraction by gas-liquid chromatography (GLC); lower and higher molecular weight hydrocarbons do not fall readily within the scope of the analytical method and, further, they may be lost preferentially in

the isolation procedure. Linear temperature programming ( $100^{\circ} - 300^{\circ}$ ) of GLC analyses on short packed columns permits the rapid estimation of n-alkane distribution over a wide range of carbon numbers (e. g. Fig. 3C) but the complex mixtures of branched-cyclic alkanes are only partially resolved, though the resulting chromatograms are still very useful (Fig. 5B). In this latter situation the much higher plate efficiencies of long capillary columns are desirable for analytical purposes (Fig. 6). Treatment with 5  $\overset{\circ}{\text{A}}$  sieve in benzene effected a clean removal of the normal alkanes, which were analysed separately (GLC); the alternative mass spectrometric approach (MS), based upon the parent ion intensities, was not used in this instance (Hood and O'Neal, 1959 and Carlson et al, 1963). The branched-cyclic alkane fraction remaining in solution could then be studied by taking cuts at selected times after injection of the sample into the gas chromatograph. Further fractionation on a second GLC column with different characteristics provided samples of individual alkanes suitable for mass spectrometric examination. All procedures were checked, singly and together, for the introduction of alkane contamination, and in every case the level of contamination was well below 1% of the quantity of alkanes normally handled. The examination of some specific sources of contamination is discussed under "Control Experiments" in the preceding section.

Experiments carried out with model alkanes and with the mixture (Figs. 1 and 2) from tobacco wax demonstrate that, where suitable precautions are taken, the 5  $\overset{\circ}{\text{A}}$  molecular sieves operate in a highly selective fashion. The n-alkanes are retained within the sieve and resist extraction with branched or cyclic hydrocarbon solvents. Conversely, even those long-chain alkanes bearing a single penultimate methyl substituent (iso-alkanes; 2-methyl alkanes) are rejected and the small



quantities adsorbed on the sieve surface may be removed completely by solvent extraction. Hence there is no problem in completely removing and studying the normal isomers from the highly complex mixture of alkanes derived from a rock or an oil. This batch-wise approach would appear to be more efficient and troublefree, though admittedly more time consuming, than that employing direct gas-liquid chromatography over powdered molecular sieve (Schenk and Eisma, 1964).

As a preliminary assay with geologic material we chose to repeat the recent and highly significant work of Cummins and Robinson (1964) on the Green River Shale from Rifle, Colorado. This shale formation is Eocene in age (ca.  $60 \times 10^6$  yr.) and represents the main oil shale reserve of the United States. Using samples of shale and powdered shale, kindly supplied by Dr. Robinson, we found hydrocarbon distributions (Fig. 3) closely paralleling those reported by this worker and his colleague. The normal alkanes showed the marked dominance of the odd-carbon numbers, especially  $C_{27}$ ,  $C_{29}$ , and  $C_{31}$ , so characteristic of most plants (Eglinton and Hamilton, 1963) and relatively young sediments (Meinschein, 1963). The previous workers showed that the lower molecular weight range of the branched and cyclic alkane fraction is mainly composed of phytane (V) pristane (IV) and other terpenoids. We have confirmed their findings; thus, chromatographic fractions collected in capillary tubes from 1/4 in. columns displayed the appropriate mass spectral characteristics (Fig. 4). These data firmly establish the assigned structures but small amounts of impurities are often apparent in the MS records for such GLC fractions. The combined, GLC fast scanning MS instrument (see, for example, Hellstrom and Ryhage, 1964) should greatly facilitate the analysis of such difficultly-separable mixtures.

The biological history of this Cenozoic rock is therefore evident from both the very uneven distribution of the n-alkanes and from the presence of large proportions of isoprenoid alkanes, some of which almost certainly derive from the phytol portion of the chlorophyll content of the plants of that time and geographic location. Again, the farnesane (I) may derive from the farnesyl side chain of certain bacteriochlorophylls, for example the pigment of the obligate anaerobe, Chlorobium.

We have begun our study of Precambrian rocks with the Nonesuch Shale Formation. Dr. Preston E. Cloud, Jr., first drew our attention to this dense, greyish-black shale, which is of Keweenawan age and is dated stratigraphically at ca.  $1 \times 10^9$  years old. It is now exposed at the drift-mining operation for native copper presently under way at the White Pine Mine in northern Michigan. The formation is rendered particularly attractive for study by the presence of small amounts of crude oil which exude from cavities and fractures in the shale and which have been collected by the mine geologist, Mr. John Trammell.

We treated a small sample of the black, viscous Oil Seep using the procedures outlined above and found that the members of the n-alkane series range from  $C_{11}$  to about  $C_{35}$ . Low-boilers are not present to any extent in the original Oil Seep, for this also was examined directly, without any pre-treatment. The distribution reaches a maximum around  $C_{19}$  and there is a very slight, but quite definite, predominance of odd-numbered members (Fig. 5C). The branched-cyclic fraction, which still contains small amounts of aromatic hydrocarbon, is very complex; a repeating pattern of peaks, poorly resolved on the packed column (Fig. 5B) and much better resolved with the capillary column (Fig. 6A) is observed and may correspond to a series of

homologues which repeat at every carbon number. Such multiple series of homologues probably relate to the maturation undergone by the original source material. However, superimposed upon this general pattern are several prominent peaks in the region corresponding in retention time to that between  $n\text{-C}_{14}$  and  $n\text{-C}_{18}$  on the silicone gum column (Figs. 5B and 6). Our immediate supposition was that these peaks might correspond to the isoprenoids already observed and identified in the Green River Shale. We therefore collected amounts of the order of a milligram for each of these peaks by successive sampling and then rechromatographed these upon a strongly polar substrate (Fig. 7). Two of the fractions so obtained gave single peaks of the same retention times as pristane (IV) and phytane (V) when rechromatographed on a variety of substrates. These identifications were confirmed by direct infrared (IR; Fig. 8) and mass spectral (Fig. 9) comparisons. Mass spectral data show that other compounds with isoprenoid skeletons are present. Thus, farnesane (I) and the  $\text{C}_{16}$  alkane (II) have been identified and work is continuing with other fractions which may represent, in part, geologic breakdown products from the more abundant phytane and pristane. Capillary column studies of cuts taken from the packed columns show that the branched fraction is an extremely complex mixture (Fig. 6B); even so, the phytane and pristane comprise approximately 0.6% and 1.2% respectively, of the branched-cyclic fraction. No attempt has been made to make optical activity measurements on the very small quantities of isoprenoids isolated in the present work. Sir Robert Robinson (1964) has suggested that isoprenoids in oils might be derived abiotically by the polymerization of isoprene but there seems little likelihood that such a polymerization would give substantial proportions of alkanes within the observed narrow molecular weight range.

If one accepts the presence of these hydrocarbons as evidence of former life, there remains the question of the relationship between the oil and the rock. We believe that the oil is indigenous to the rock since we have found very similar GLC patterns for the normal and the branched-cyclic alkane fractions isolated from the Marker Bed of the Nonesuch Shale (Figs. 10 and 11) and from the imprisoned alkanes from a calcite vein within the Nonesuch Shale Formation. There is admittedly some variation in the relative proportions of the isoprenoids and of the range of n-alkanes. However, such variations are conceivably to be expected in view of the possible fractionation effects inherent in a large rock formation. No method for the dating of ancient organic matter exists as yet, so that some doubt must remain as to the precise age of the hydrocarbons; however, the geologic evidence (White, 1960) favours the viewpoint that the organic matter and the associated copper are sedimentary in origin, and contra indicates derivatior of the oil from any other formation. We rule out the possibility that the isoprenoids derive from contemporary contamination, as in handling or isolation procedures, and the pore-sizes in the extremely compact Marker Bed Shale are inadequate for microbial activity which might otherwise provide a means for their generation in situ. Further evidence in favour of the early diagenetic origin of the alkanes comes from the independent work of Meinschein and his collaborators which was reported (Meinschein et al, 1964) simultaneously with our own preliminary announcement (Eglinton et al, 1964). These workers also identified pristane and phytane in certain fractions of the oil, using capillary GLC and MS, though their identifications were based mainly on coincidence of GLC retention times of certain peaks, rather than on MS data for single hydrocarbons, as in our own case.

However, they correlated the petroliferous content of the shale with the relative abundance of discrete 'organic fragments'. "Micro-paleontological study revealed the presence of abundant, triturated plant-tissue, largely of unorganized morphology, but exhibiting occasional filaments and spherical spore-like bodies." In addition Meinschein, et al quote significant optical activity in the alkane fraction and report spectroscopic evidence for the presence of vanadyl porphyrins in the shale, which they regard as "constituting evidence of the existence of photosynthetic organisms in Precambrian times and of a mild thermal history for the Nonesuch sediments". We have made a preliminary study of two further situations. These relate to the present approach, wherein the isoprenoid alkanes are utilized as biological marker substances. In the first situation we have examined a supposedly abiogenic oil, which occurs trapped within opal froth veins at the Abbott Mercury Mine, Lake County, California. Sylvester-Bradley (1964) suggests a non-biological origin for this oil, but Dr. E. H. Bailey, a local geologist, is of the opinion that the oil derives from the hydrothermal extraction of sedimentary formations and subsequent deposition in the present formation ( $1 - 10 \times 10^6$  yr). We find that the oil is almost entirely hydrocarbon in nature, with a substantial proportion of aromatics, and that the n-alkanes surprisingly constitute only about 0.1% of the total oil. The main bulk of the alkane fraction appears to be an extremely complex mixture of cyclic alkanes (Fig. 12A). However, we have been able to isolate and identify pristane (Fig. 11C) from the alkane fraction, of which it makes up some 0.25%. It might be argued that this pristane represents subsequent dilution of an abiogenic oil with biologically derived material; in our view the results support the hypothesis that the Abbott oil is a thermally degraded oil of

sedimental origin.

Finally, we have examined the products from a methane spark-discharge experiment conducted by Dr. Cyril Ponnampereuma and Fritz Woeller at the Ames Research Center. The particular conditions used (Corona discharge in one atmosphere of methane at ambient temperature) lead to a colourless mobile oil in high yield consisting mainly of alkanes, ranging in molecular size up to about  $n\text{-C}_{23}$  with a maximum corresponding to the  $n\text{-C}_{14}$  position (GLC, Fig. 12B). The mixture is evidently much more complex than even the Abbott oil, for there is no indication of any individual peak above the  $n\text{-C}_{15}$  position. This is in accord with expectation for a synthetic process involving more or less random bond fission and formation and contrasts strongly with the total alkane patterns for the Green River and Nonesuch Shales (Fig. 3A and 5A). Work is continuing with this material and to date it has been shown that  $n$ -alkanes are undetectable by the sieving process (Dr. R. B. Johns, personal communication).

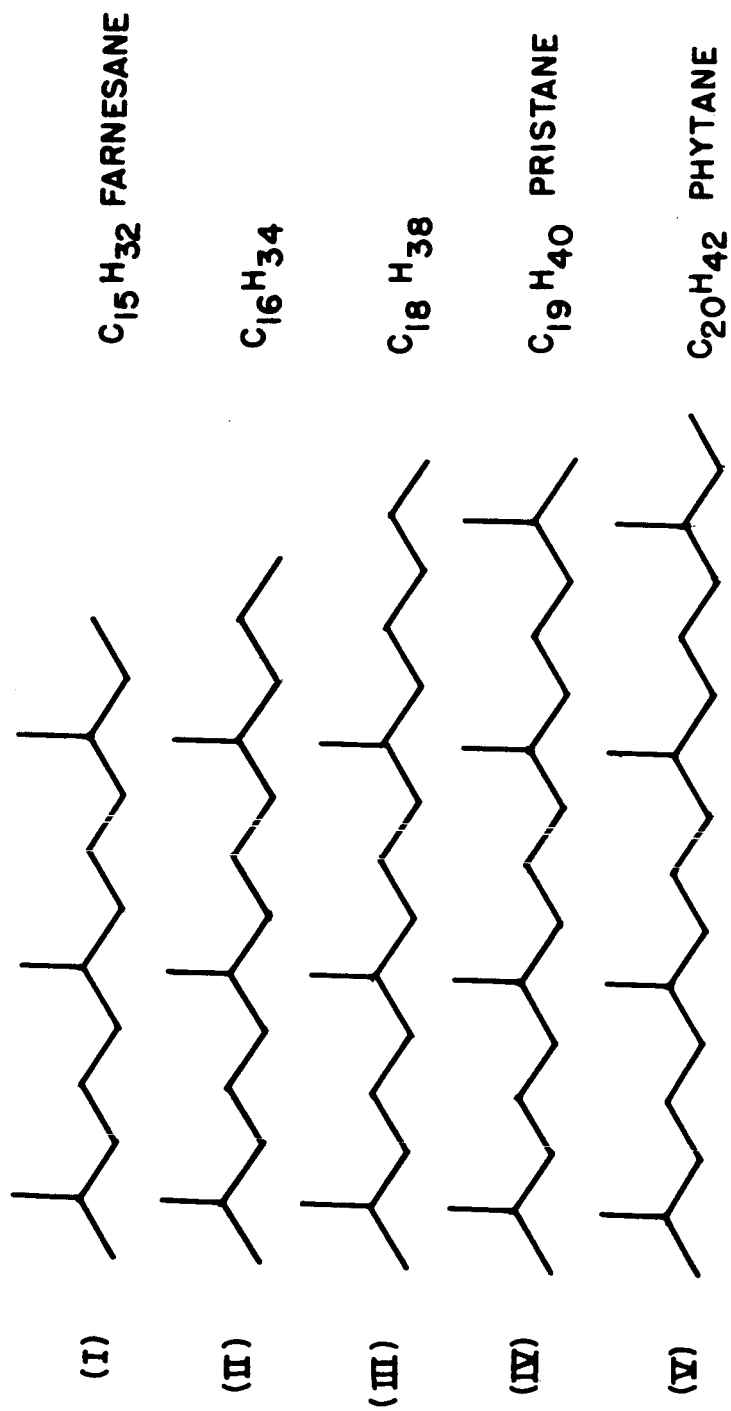
### SUMMARY

Isoprenoid hydrocarbons, including farnesane ( $C_{15}H_{32}$ ), pristane ( $C_{19}H_{40}$ ), and phytane ( $C_{20}H_{42}$ ), are present in the Marker Bed of the Precambrian Nonesuch Shale Formation at the White Pine Mine, Michigan, and in the oil seep associated with it. Column chromatography over activated alumina, molecular sieve ( $5 \text{ \AA}$ ) treatment, and gas-liquid chromatography provided the isolation procedures for the individual hydrocarbons, which were identified by retention time on gas-liquid chromatography, and by comparison of infrared and mass spectra.

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## FIGURE LEGENDS

### Figure 1

Gas-liquid chromatogram of Leaf Wax Total Alkanes; Nicotiana tabacum (Solanaceae). Column Conditions: 5 ft. x 1/8 in.; 3% SE-30 on 80 - 100 mesh Chromosorb W (DMCS); 30 ml/min. nitrogen at 50 psi; temp. programmed at 7.5°/min; initial temperatures-column 100°, detector 225°, injector 230° (Aerograph Model 665-1).

### Figure 2

Mass spectra of 2-methyldotriacontane (iso-C<sub>33</sub>), (A) synthetic and (b) from tobacco wax, and of 3-methyl hentriacontane (anteiso-C<sub>32</sub>), (C) from tobacco wax.

### Figure 3

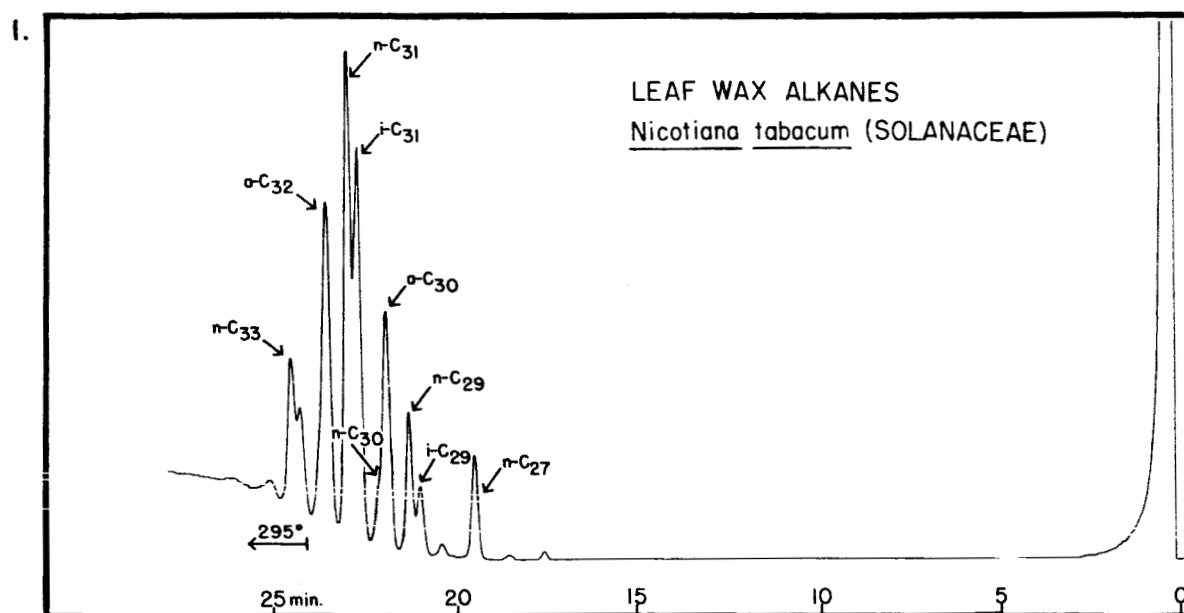
Gas-liquid chromatograms of alkane fractions from the Green River Shale, Colorado. (A) Total, (B) Branched-cyclic and (C) Normal alkanes column conditions as for Fig. 1.

### Figure 4

Mass spectra of isoprenoid alkanes isolated from the Green River Shale, Colorado, and of authentic pristane (IV) and phytane (V).

### Figure 5

Gas-liquid chromatograms of alkane fractions from the None-such Shale Formation - Oil Seep, (A) Total, (B) Branched-cyclic and (C) Normal alkanes; Calcite Vein, (D) Total, (E) Branched-cyclic and (F) Normal Alkanes. Column conditions as for Fig. 1.

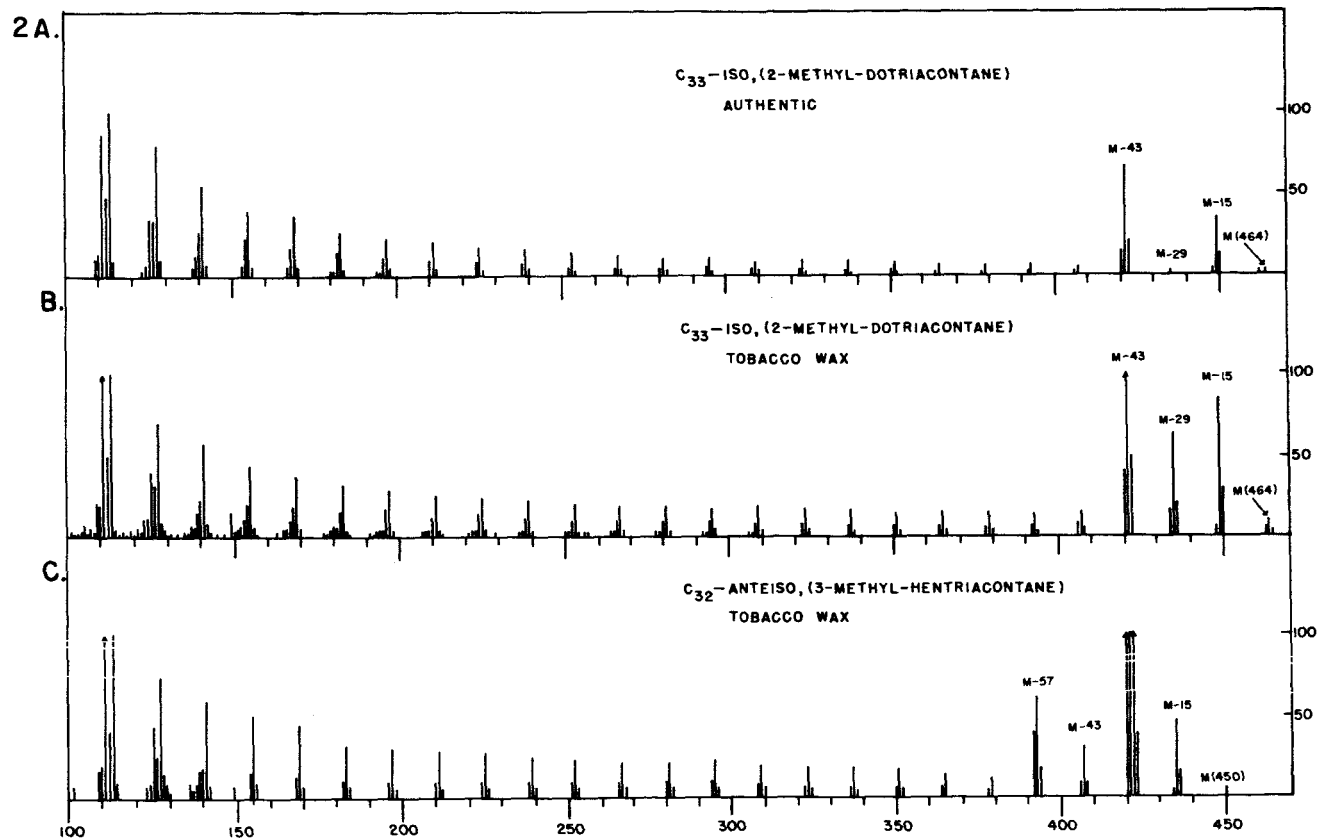


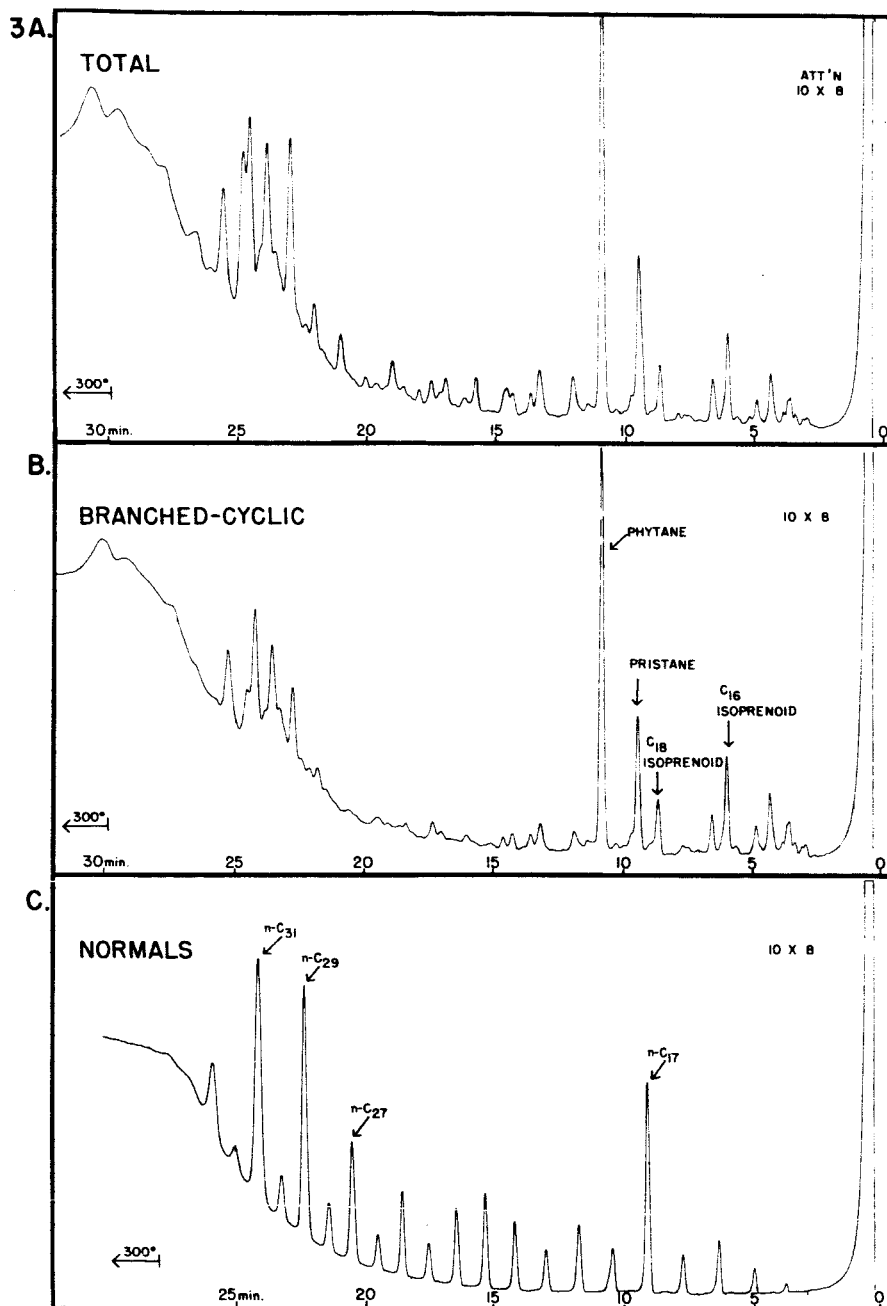
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FIGURE 2

Supplemental intensity data for the relative intensities of peaks which are either off-scale (indicated by arrows in the drawings) due to  $m/e$  113 being chosen as reference intensity ( $=100$ ), or have  $m/e$  less than 100.

<u>m/e</u>	<u>2A</u>	<u>m/e</u>	<u>2B</u>	<u>m/e</u>	<u>2B</u>	<u>m/e</u>	<u>2C</u>
41	900	40	23	72	42	41	430
42	410	41	410	73	13	42	116
43	3900	42	160	74	2	43	1050
44	120	43	1210	75	2	44	45
53	35	44	63	77	6	45	10
54	70	45	9	78	3	53	19
55	1000	48	6	79	11	54	45
56	700	50	5	80	6	55	470
57	3000	51	7	81	43	56	650
58	80	52	4	82	61	57	1500
67	75	53	23	83	250	58	70
68	90	54	49	84	34	60	11
69	520	55	430	85	530	61	5
70	300	56	340	86	43	67	58
71	920	57	1300	87	4	68	60
72	72	58	60	91	9	69	310
81	40	59	5	92	4	70	200
82	70	60	11	93	7	71	800
83	300	61	3	94	5	72	44
84	115	63	2	95	31	73	15
85	700	64	9	96	36	77	5
86	50	65	5	97	190	78	8
95	15	66	6	98	59	81	39
96	35	67	61	99	150	82	57
97	205	68	61	100	14	83	270
98	70	69	310	-----		84	90
99	172	70	170	422	155	85	590
100	14	71	750			86	36
						95	24
						96	33
						97	200
						98	67
						99	129
						100	129
						-----	
						420	200
						421	800
						422	250





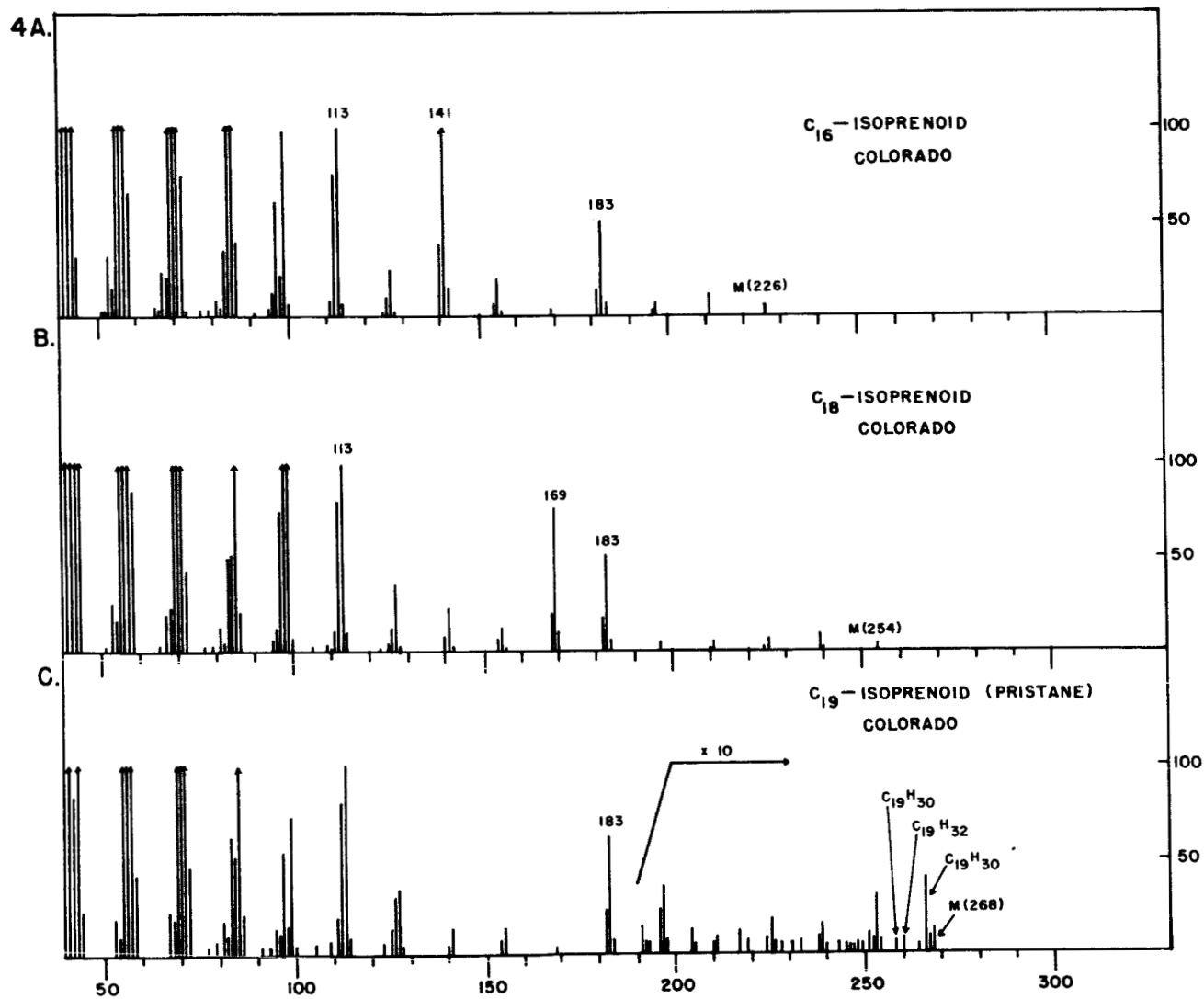
GREEN RIVER SHALE (COLORADO), ~60 X 10<sup>6</sup> YRS. ALKANE FRACTIONS

MUB-3954

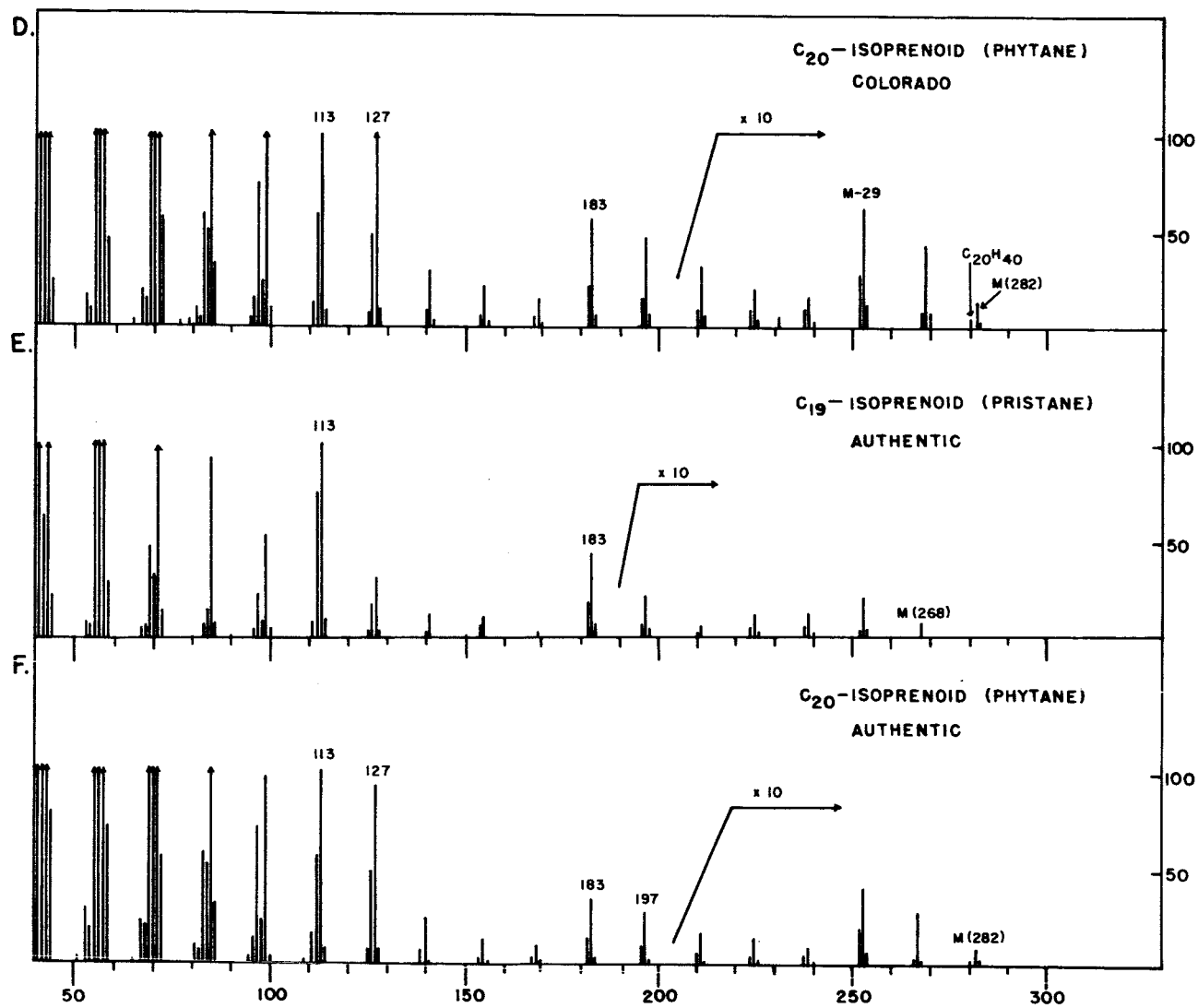
FIGURE 4

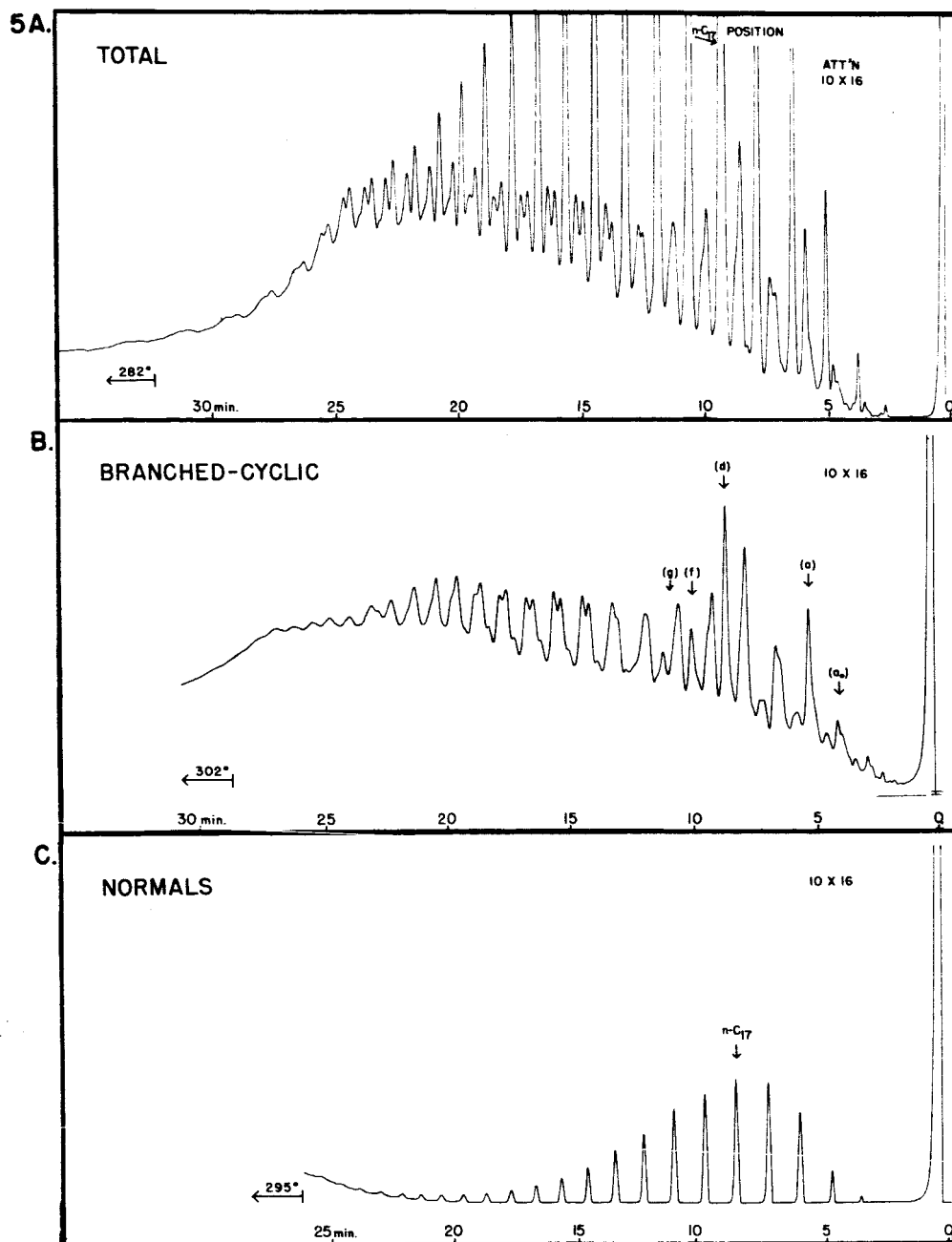
Supplemental intensity data for the relative intensities of peaks which are off-scale (indicated by arrows in the drawings) due to m/e 113 being chosen as reference intensity (= 100).

<u>m/e</u>	<u>4A</u>	<u>4B</u>	<u>4C</u>	<u>4D</u>	<u>4E</u>	<u>4F</u>
41	550	500	380	450	163	700
42	160	130				176
43	1200	960	680	800	209	1240
44		120				
55	360	540	220	260	119	440
56	300	480	210	210	115	380
57	1430	1880	920	1020	230	1580
63	160		160			
69				150		172
70	320	130	110	140		176
71	1380	1000	820	940	200	1060
84	120					
85	570	360	240	530		440
98		140				
99				102		
113	100	100	100	100	100	100
127				105		
141	117					



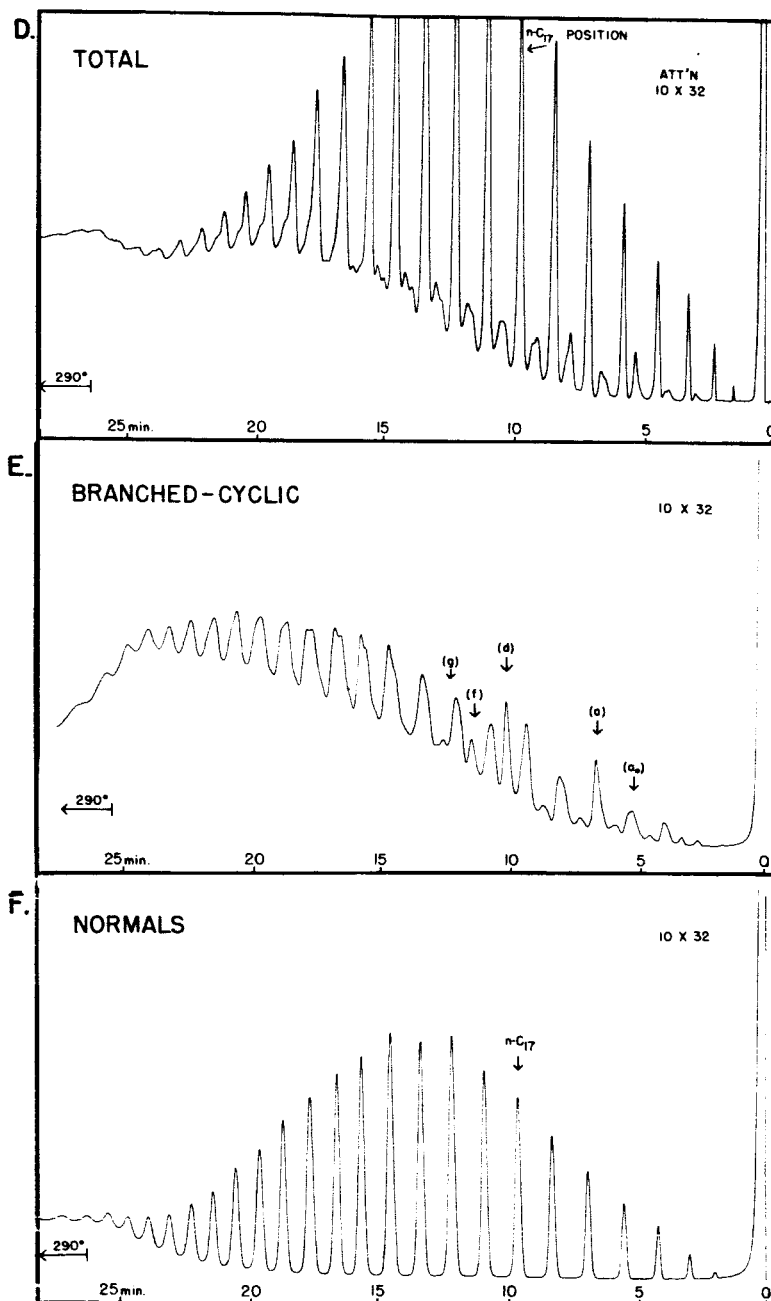






NONESUCH OIL. ALKANE FRACTIONS

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NONESUCH CALCITE VEIN. ALKANE FRACTIONS

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Figure 6

Gas-liquid chromatograms of branched-cyclic alkanes from the Nonesuch Shale Formation - Oil Seep. Column conditions. (A) 150 ft. x 0.01 in. s/s; Apiezon L; nitrogen, 20 psi; temp. programmed at  $2.5^{\circ}/\text{min}$ ; initial temperatures - column  $100^{\circ}$ , detector  $185^{\circ}$ , injector  $310^{\circ}$ . Split ratio, 100 : 1 (Perkin-Elmer Model 226).

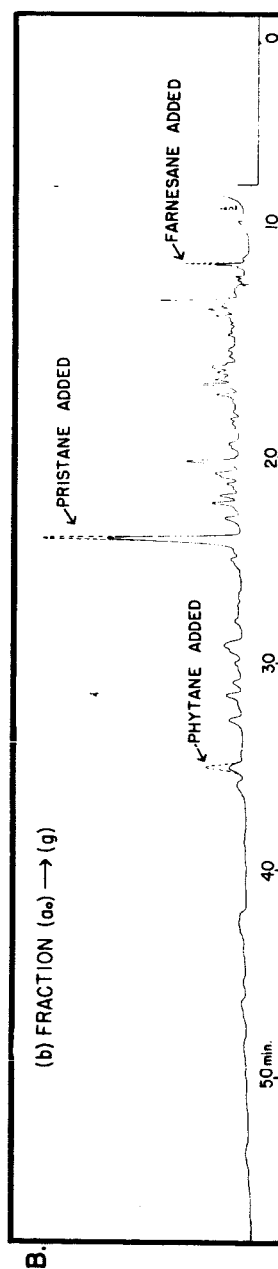
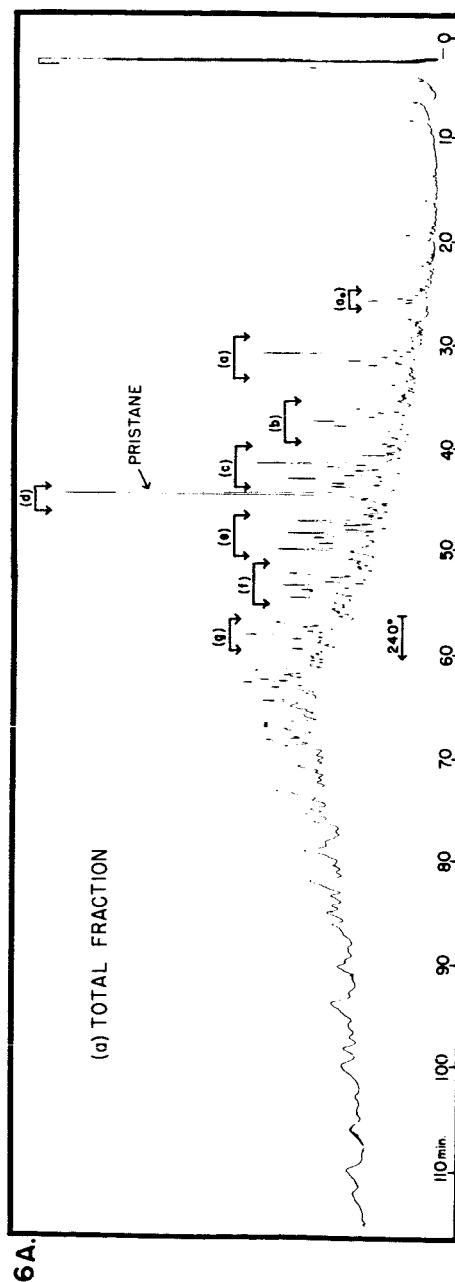
(B) 150 ft. x 0.01 in. s/s; Apiezon L; nitrogen, 8 psi, column temp.  $179^{\circ}$  and injector temp.  $307^{\circ}$ . Split ratio, 500 : 1 (Modified Aerograph Hy-F1).

Figure 7

Gas-liquid chromatograms of branched-cyclic alkanes from the Nonesuch Shale Formation - Oil Seep. Separation and purification of isoprenoids. Column conditions: (A) 5 ft. x  $1/4$  in. 3% SE-30 on 80 - 100 mesh Chromosorb W (DMCS); 70 ml/min. helium; temp. programmed at  $3^{\circ}/\text{min}$ ; initial temperatures - column  $100^{\circ}$ , detector  $245^{\circ}$ , injector  $220^{\circ}$  (Aerograph Model A. 90-P2).

(B) to (E) 5 ft. x  $1/4$  in. 5% TCEPE on 80 - 100 mesh Gas-Chrom. RA; 60 ml/min. helium; column temp.  $66^{\circ}$ ,  $68^{\circ}$ ,  $93^{\circ}$ , and  $100^{\circ}$  for (B), (C), (D), and (E), respectively; detector  $225^{\circ}$  and injector  $200^{\circ}$  (Aerograph Model A90-P2).

(F) 5 ft. x  $1/8$  in. 5% TCEPE on 80 - 100 mesh Gas-Chrom. RA; 30 ml/min. nitrogen; column temp.  $129^{\circ}$ , detector  $225^{\circ}$ , injector  $239^{\circ}$  (Aerograph Model 665-1).



NONESUCH OIL, BRANCHED-CYCLOC ALKANE FRACTION

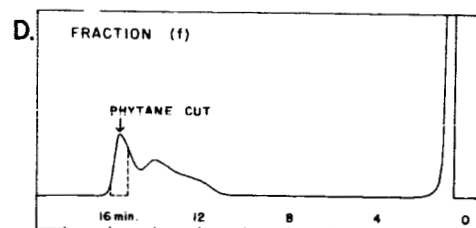
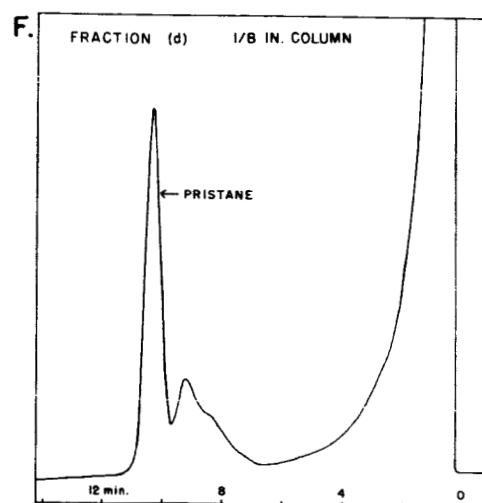
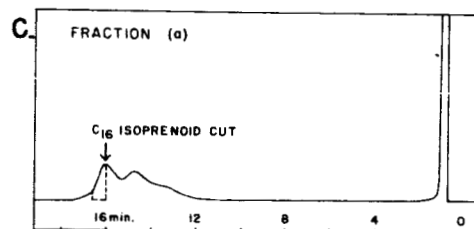
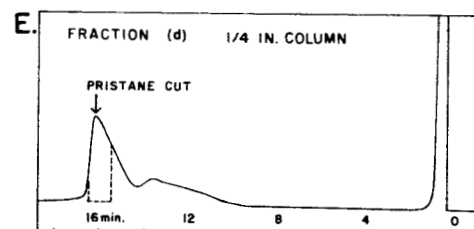
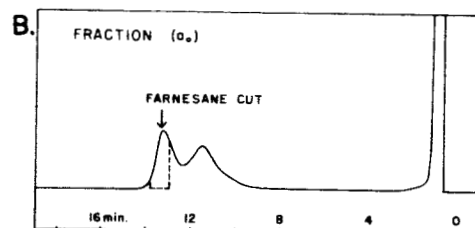
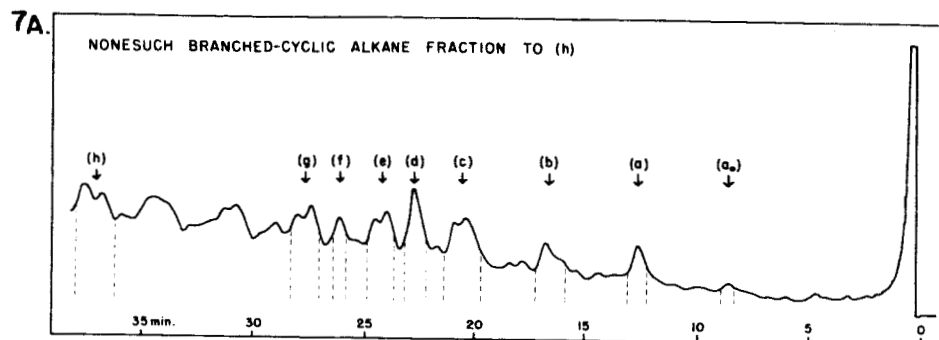


Figure 8

Infrared spectra (in  $\text{CCl}_4$ ) over the methyl symmetrical bending region of pristane (IV) and phytane (V): A, B, and D, authentic samples; C and E, isolated from the Nonesuch Shale Formation - Oil Seep. Resolution  $2\text{ cm}^{-1}$  at  $1350\text{ cm}^{-1}$ . Cells: A, 0.54 mm standard; B to E, 1.0 mm micro cell - for E, the background absorption is the same as D but has been subtracted because of the small sample size ( $\sim 20\text{ }\mu\text{g}$ ). For A to D, the solutions are about 1%; quantity of hydrocarbon for B to D approximates to  $50\text{ }\mu\text{g}$ .

Figure 9

Mass spectra of isoprenoid alkanes isolated from the Nonesuch Shale Formation - Oil Seep, and of authentic farnesane (I).

Figure 10

Gas liquid chromatograms of alkane fractions from the Nonesuch Shale Formation - Marker Bed. A Total; B, Branched-cyclic; and C, Normal alkanes. Column conditions as for Fig. 1.

Figure 11

Mass spectra of isoprenoid alkanes isolated from the Nonesuch Shale Formation - Marker Bed, and from the Abbott-Mercury Mine - Oil Seep.

Figure 12

Gas liquid chromatograms of A, the alkane fractions from the Abbott Mercury Mine - Oil Seep, and B, the total product from a methane spark - discharge experiment (cf., Ponnampuruma and Woeller, 1964). Column conditions as in Fig. 1, except for the column packing (5% SE-30 on 60 - 80 mesh Chromosorb W) and in B, the programming rate ( $6^\circ/\text{min}$ ).

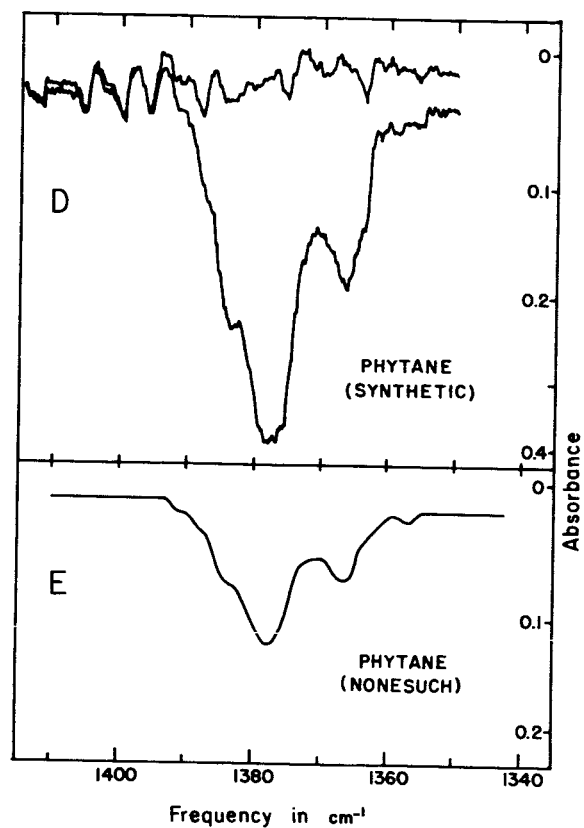
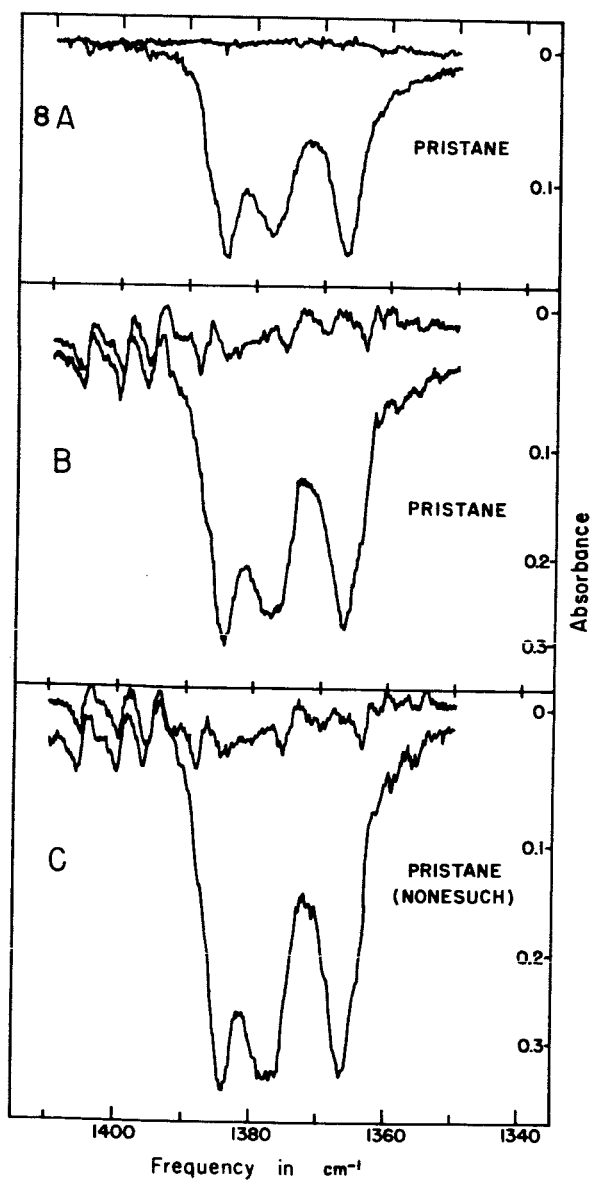
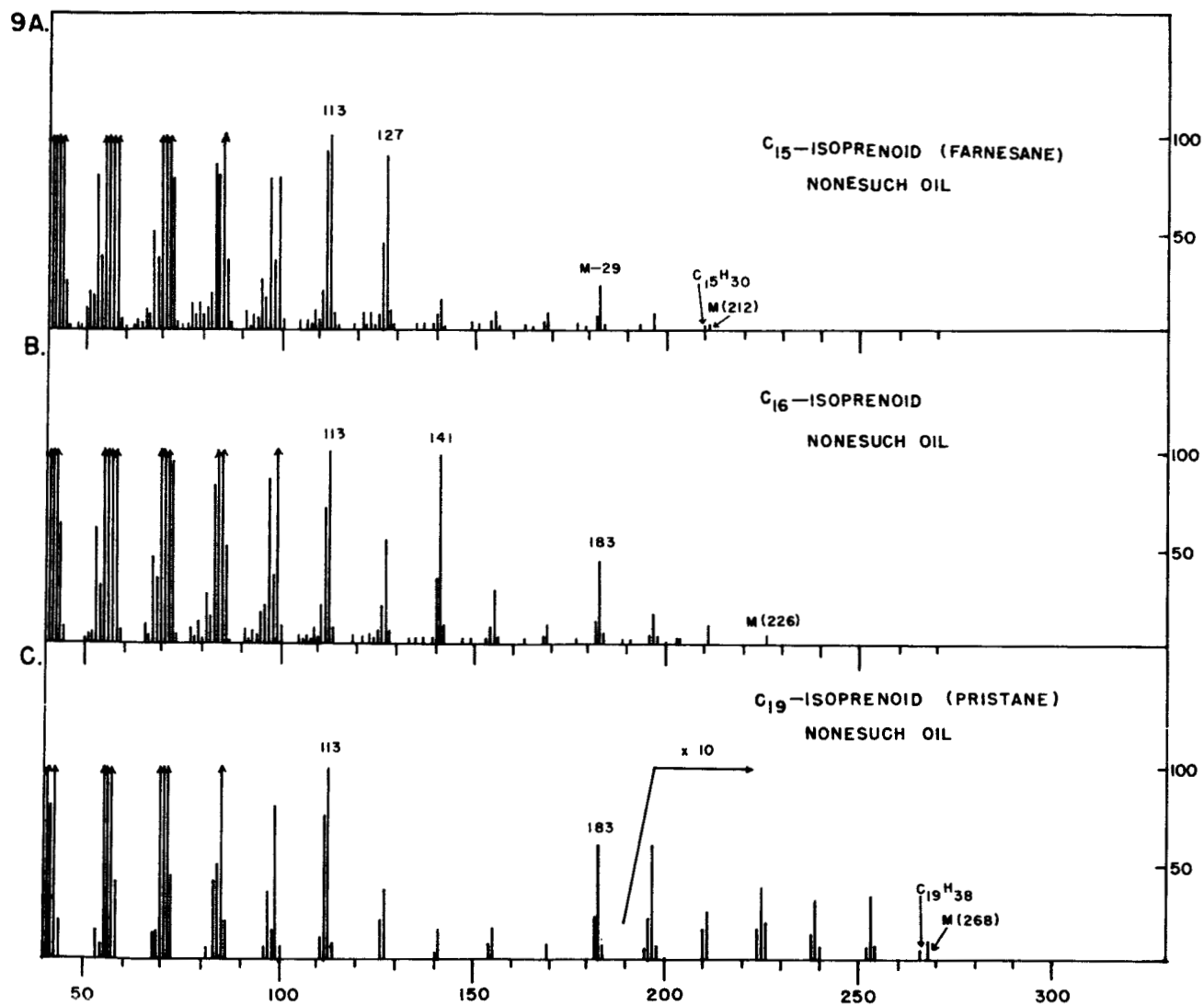


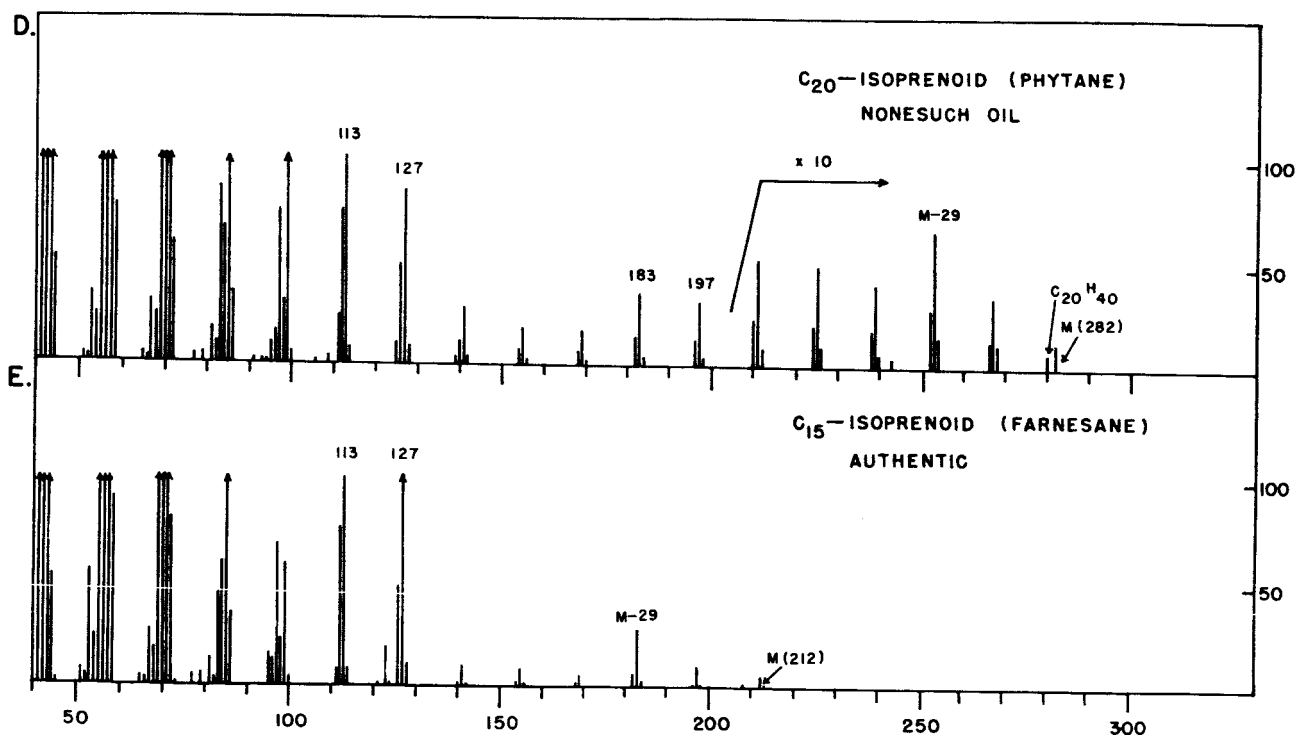


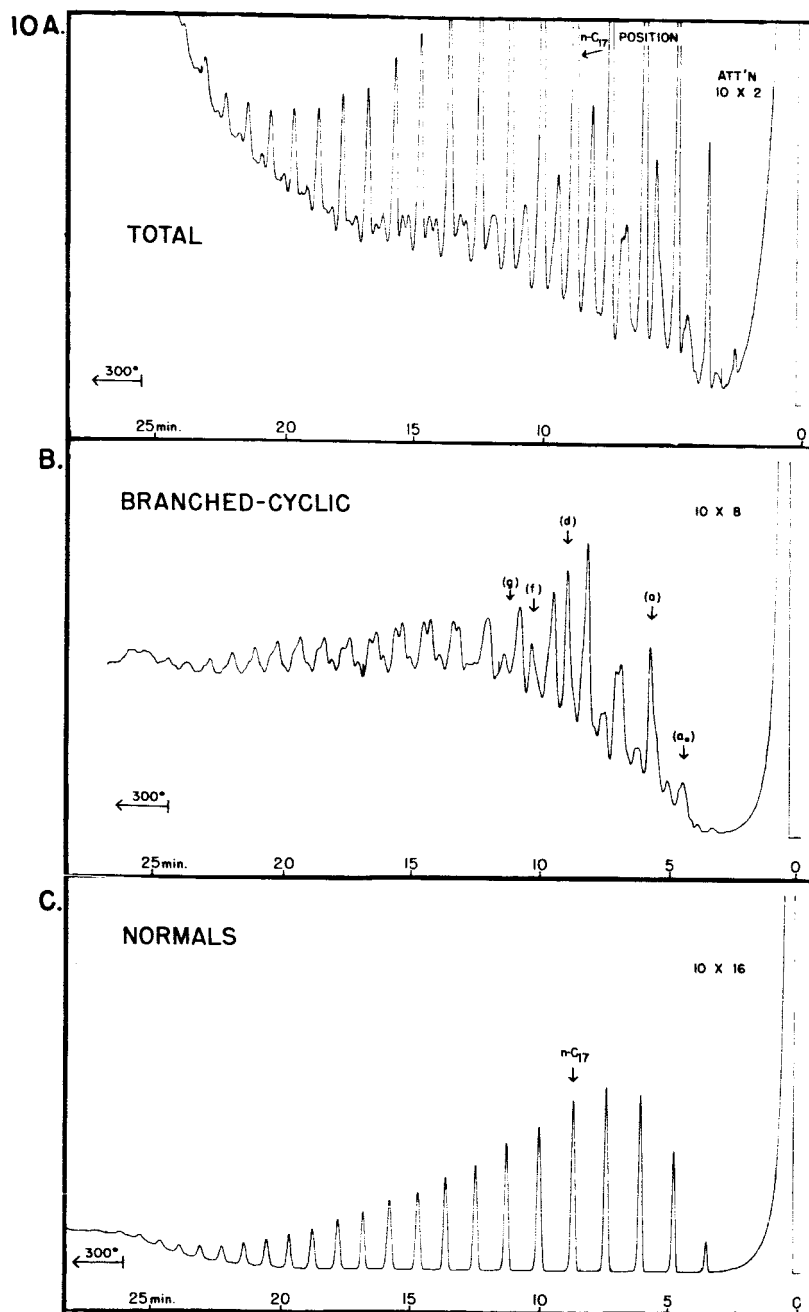
FIGURE 9

Supplemental intensity data for the relative intensities of peaks which are off-scale (indicated by arrows in the drawings) due to m/e 113 being chosen as reference intensity ( = 100 ).

<u>m/e</u>	<u>9A</u>	<u>9B</u>	<u>9C</u>	<u>9D</u>	<u>9E</u>
41	800	730	340	750	1040
42	260	200	700	160	240
43	1600	1500			1580
44	270				
53				1350	
55	480	550	210	470	450
56	510	450	200	380	500
57	2100	1320	925	1880	1950
58		104			
63		250			
69	210		130	215	170
70	240	400		200	230
71	900	1500	820	1130	1430
84		140			
85	380	700	325	520	530
99		140		100	
113	100	100	100	100	100
127					114







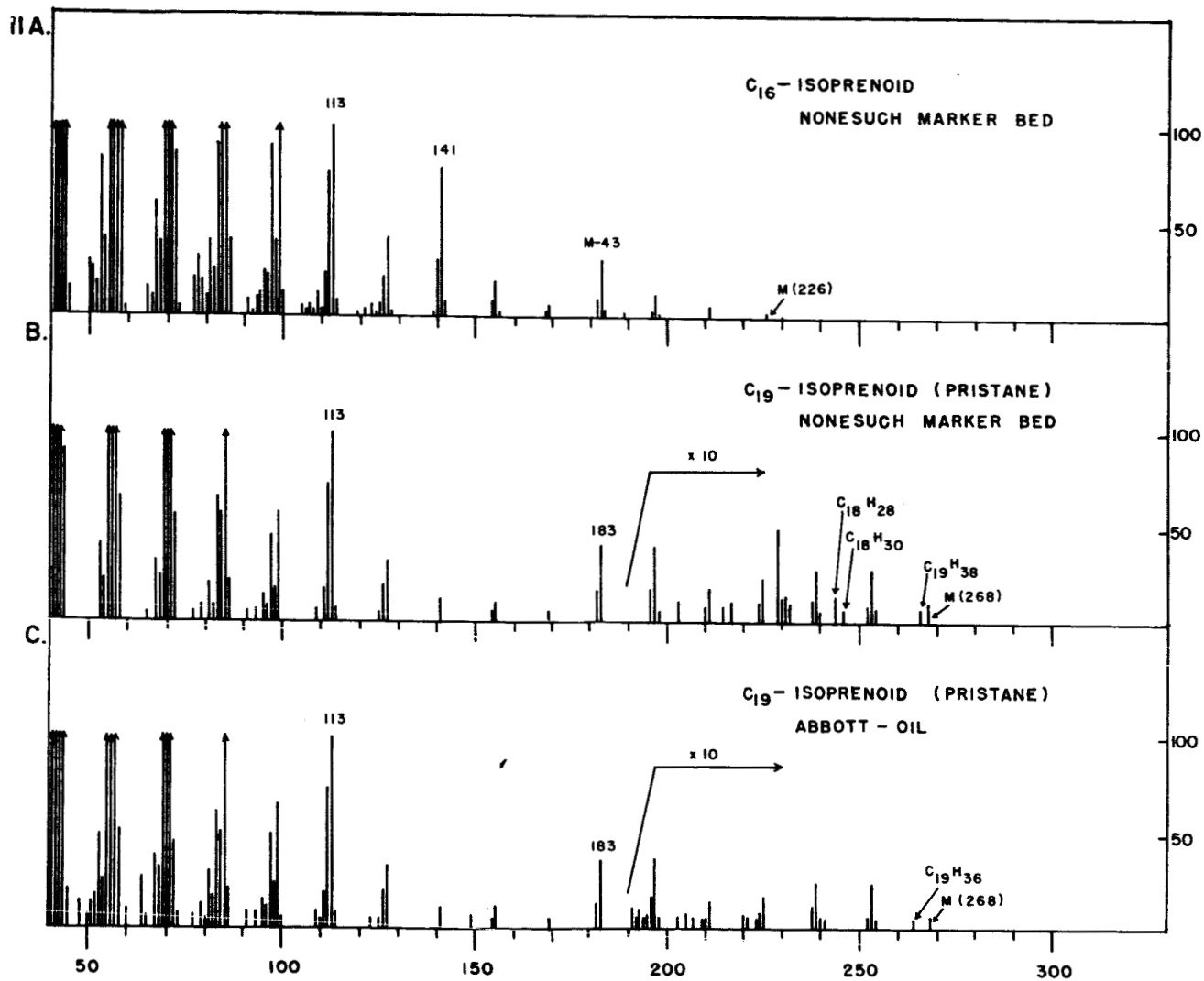
NONESUCH MARKER BED. ALKANE FRACTIONS

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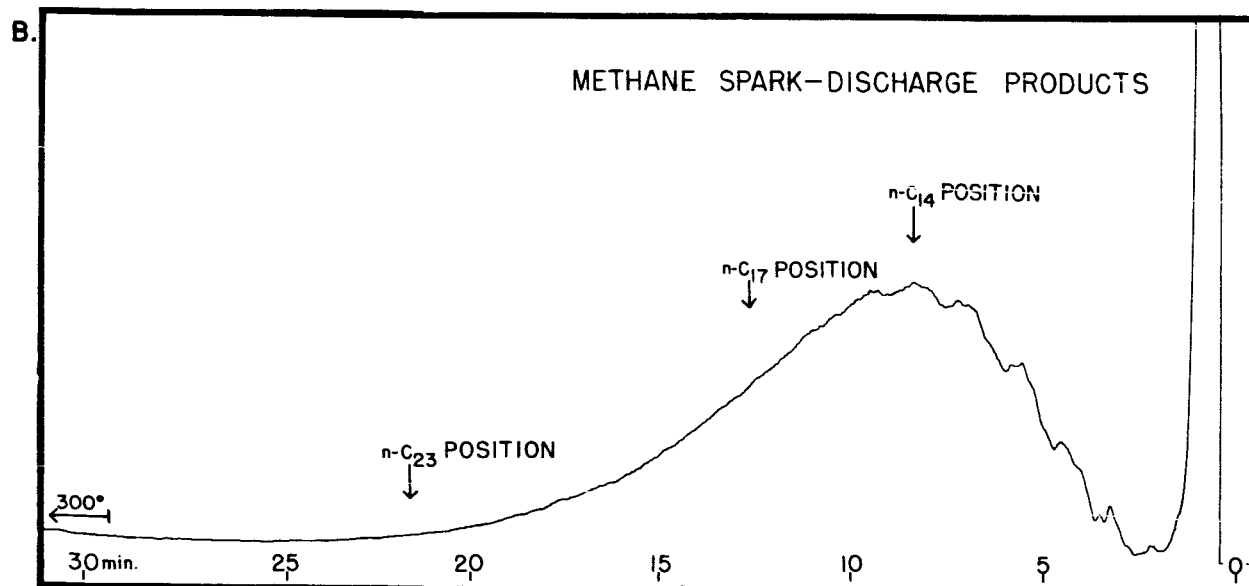
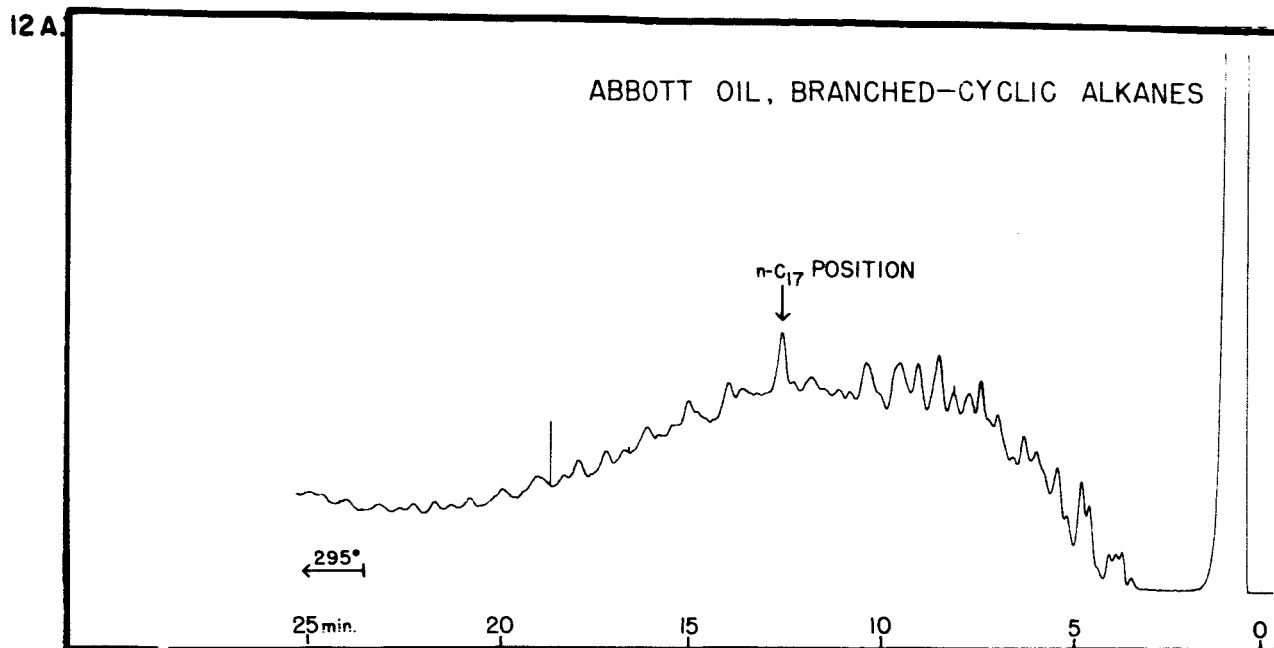
FIGURE 11

Supplemental intensity data for the relative intensities of peaks which are off-scale (indicated by arrows in the drawings) due to m/e 113 being chosen as reference intensity (= 100).

<u>m/e</u>	<u>11A</u>	<u>11B</u>	<u>11C</u>
41	120	630	440
42	330	170	120
43	2400	1230	810
44	600		130
55	700	420	290
56	550	380	260
57	2400	1560	1150
58	110		
69	280	205	160
70	390	130	103
71	1500	870	730
84	140		
85	650	310	280
99	112		
113	100	100	100



MUB-4637



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