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GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC INVESTIGATION OF SEEP OIL ISOPRENOID ALKANES

by

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Thesis Director's signature

Pat Haug

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ABSTRACT

A Costa Rican seep oil known to have a high isoprenoid content was analyzed for the presence and relative amounts of branched alkanes, especially isoprenoid alkanes in the range of C_{16}-C_{30}. The analysis was conducted by means of gas chromatography and mass spectrometry and is an effort to gain an insight into possible precursor or precursors of the isoprenoids. The isoprenoid content of various geological samples can possibly then be used to indicate the presence of living organisms and, the diagenetic mechanisms which alter buried organic matter.

The oil was fractionally distilled, and gas chromatography used to separate and purify the constituent compounds. Approximate measurements of the relative amounts of the constituents, and rough correlations of unknowns with standard alkanes were also obtained by gas chromatography. The purified samples were then analyzed by low-resolution mass spectrometry.

The analysis revealed a complete set of regular isoprenoids from C_{16} to C_{25}, except for the C_{17} and C_{22} regular isoprenoids. The largest component was the C_{19} isoprenoid (pristane) and the second largest component was the C_{20} isoprenoid (phytane). Though the C_{17} regular

isoprenoid was absent, three-, seven-, eleven-trimethyl tetradecane was tentatively identified and the possible presence of three-, seven-, eleven-trimethyl hexadecane noted. There was no evidence of irregular branched isomers that would arise from squalane degradation, and the evidence strongly indicates phytol, rather than squalene, as the major precursor for isoprenoids below C_{21}.

The C_{23}, C_{24}, and C_{25} regular isoprenoids were also identified. These have been rarely reported by other researchers, and the amount present was considerably less than that of the C_{15}-C_{20} isoprenoids. No C_{22} regular isoprenoid, and no irregularly branched C_{24} and C_{25} isomers were found, suggesting that a regular isoprenoid structure, rather than lycopene or a similar compound, is the precursor. Squalane was not found. The C_{29} and C_{30} regular isoprenoids were tentatively identified, by mass spectrometry. The existence of these two compounds suggest possible precursors such as regular isoprenyl alcohols of the C_{30} size or greater.

INTRODUCTION TO GEOCHEMISTRY

The origin of Life on Earth is one of the most difficult of scientific questions. When and how life began and what the nature of life was are problems which several different but inter-related branches Science are seeking to solve. Paleontology, especially micro-Paleontology, investigates physical evidence, such as fossilized micro-organisms, in regard to age and morphological evolution. Chemical evolution studies seek to duplicate hypothesized primordial conditions to elucidate the progression from large organic molecules and coacervates to functioning, living organisms. Organic Geochemistry examines the organic chemical residues ("chemical fossils") present in ancient samples in order to find answers to questions about the origin of Life.

These organic remains are widely distributed in nature. The vast bulk of the organic material deposited geologically is in the form of organic deposits in sedimentary rock, with the petroleum, natural gas, and other sources constituting only a minor portion of the total amount of geological organic carbon. Although the amount of carbon in sediments is in the parts per million range, there is an estimated 0.75×10^{12} tons[5] of organic carbon in marine sediments alone; with lesser amounts in other sedimentary types.

Several factors make geochemical analyses difficult, and results sometimes controversial. Although the total amount of geological organic carbon is large, the concentrations in sediments is low: ancient shale sediments, for example, contain about 21,000 ppm of organic material, ancient carbonates about 2900 ppm.[23] Of this amount, the greatest part is bound in the rock matrix as insoluble, cross-linked,

high molecular weight polymers. These polymers, called kerogen, are not extractable from the rock matrix by ordinary means and can only be removed by degradation. The soluble organic material in sediment is considerably smaller (~200 ppm in ancient sediments[23]), thus, the available amount of usable organic material is small. Due to the large number of compounds present and the many possible isomers in the small amounts of material, separation, purification, and unequivocal identification are frequently difficult.

The utilization of the organic remains as reliable indicators of previous life processes ("biological markers") is hampered by the fact that an organic residue in a sediment can be the end product of any one or more of several processes. Hence, it is difficult to determine whether or not a residue is an authentic organic remnant, and what its precursor was.

These processes which produce and alter organic molecules are abiogenesis, biogenesis, diagenesis, and microbial attack. Abiogenesis is the synthesis of organic molecules by strictly chemical means. An example of this is the Fischer-Tropsch reactions, where alkanes, other hydrocarbons, and water are produced from $CO + H_2$ with a catalyst. Abiogenic synthesis is especially important in determining when life originated, for abiogenic synthesis would leave organic residues in sediments deposited before life began. It is thought that the amount of abiogenic synthesis (after the origin of life) is small compared to biogenic synthesis, due to the relative scarcity of abiotic reagents and catalysts and to the higher efficiency of biogenic processes.[23] However, the presence of detectable amounts of hydrocarbons in meteorites[9] serves to indicate that abiogenic organic material,

though not substantial, may be present in various samples.

Biogenesis is the synthesis of organic matter by living organisms. It is biogenic organic carbon that most interests geochemistry, since by studying the remaining products of life, it may be possible to trace the development of life back through time. There are several criteria for separating biotic from abiotic organic material: molecular structure, including stereochemistry and optical activity; abundance of positional and stereo-isomers of each compound; presence of homologous series and the relative abundances of the components; and the types and classes of compounds present[6]

Diagenesis is the term covering the alterations due to heat, pressure, water, catalysis, etc. that occur in sediments (and thus in the organic material) after deposition. The recognition and utilization of chemical fossils is hampered by diagenetic action since the many organic reactions which occur during diagenesis frequently so alters the original carbon skeleton of a compound that recognition of its precursor type is difficult and unreliable.

Organic matter deposited in sediment muds has been shown to be susceptible to attack by bacteria and other micro-organisms soon after deposition. These micro-organisms are both aerobic and anaerobic, and can be active for long periods of time after deposition (up to 100,000 years) under rather extreme ranges in temperature. Microbial action not only alters the original composition and quantity of the organic matter by catabolic action, but also leaves behind its own metabolic residues, which also undergo diagenetic alteration.

From the above discussion, it can be discerned that, in order for a class of compounds to be of use as reliable biological markers in

studying ancient life, several criteria must be fulfilled. These criteria include: 1) not synthesized abiotically in significant amounts;[29] 2) have good chemical stability;[29] 3) skeletal structure be obviously and significantly related to biosynthetic sequences;[29] 4) not subject to extensive microbial attack. The compounds should also be as simple as possible to extract, isolate, and characterize.

These criteria are most nearly filled by terpenoid hydrocarbons. Terpenoids in general consist of molecules formed from one or more isoprene units arranged according to the isoprene rule. Rarely are terpenes of one isoprene unit (hemiterpenes). More commonly, they are to two (monoterpenes), and three (sesquiterpenes),four (diterpenes), six (triterpenes), eight (tetraterpenes or carotenoids), or more 5-carbon units. Terpenoids in living material can be either acyclic or cyclic, and occur as hydrocarbons (rarely), alcohols, acids, esters, ketones, aldehydes, oxides, and lactones. With the exception of steroid, and occurrences of squalene, terpenoids appear to be of plant, rather than animal, origin. Most terpenes, especially of low molecular weight, are unsaturated, this unsaturated character causing these compounds to be unstable. If in the course of diagenetic action the molecules become saturated, cyclized or aromatized, the resultant compounds are extremely stable and long-lived.

Due to their highly specific molecular structure and stereochemistry and the general resistance of most cyclic hydrocarbons to microbial attack, the steranes and triterpanes are usually accepted as reliable biological markers. Because of their complex and highly specific structure, random abiotic synthesis is unlikely, especially since geochemical terpenoids exhibit significant optical activity, which abioti-

cally synthesized mixtures do not show. Their basic carbon skeletons (the cyclic structures) also appear to be more or less intact and little changed by diagenetic action from their precursors.

The basic criteria for biological markers are also well met by the isoprenoid terpenoids. Isoprenoids consist of isoprene units linked together in chains rather than cyclic or bi-cyclic fashion, and almost always occur geochemically as acids or hydrocarbons. The frequency and regularity of the methyl branching, as well as the lack of larger side chains, makes undirected abiogenic synthesis unlikely. Synthesis of isoprenoid hydrocarbons by a closed-system Fischer-Tropsch reaction has been reported;[30] however, the small quantities of isoprenoids synthesized the relative distribution of molecular sizes, and the other compounds also synthesized all have little relation to terrestrial situations. In general, it is safe to assume that isoprenoids are biogenic. They are posessed of a carbon skeleton which is quite stable diagenetically and which can be related to biosynthetic products. Isoprenoids, especially the alkanes and acids, are also present in substantially larger amounts and in a wider distribution than other terpenoids. However, due to the above mentioned possible alterations to buried organic material, the exact nature of the precursors of the isoprenoids, especially the alkanes, has not been determined from among several possibilities. If the precursors of the isoprenoids can be identified with a degree of certainty, then knowledge of the nature and characteristics of the originating life forms can be inferred from the isoprenoid content by relating the types and quantities of isoprenoids in the geochemical sample to the occurrence of their precursors in nature today. The isoprenoid alkanes were analyzed with respect to rela-

tive quantities and presence or absence of certain members of the isoprenoid homologous series and related compounds in an attempt to gain new insight into the probable precursors of the isoprenoids.

ISOPRENOID ALKANES

Isoprenoid alkanes, the most ubiquitous of the terpene hydrocarbons, generally constitute from 1.5 to 3 percent of the soluble organic matter in sediment and oils.[23] Isoprenoids are composed of isoprene units linked together at the ends to form long hydrocarbon

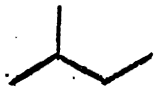


Fig. I (a)

chains. While almost all the isoprenoid compounds (i.e. isoprenoid alcohols, acids, carotenoids, and hydrocarbons) found in living organisms are unsaturated (frequently highly unsaturated), unsaturated fossil isoprenoid alkanes are almost unknown. This is the result of the instability of all olefinic compounds as shown by the paucity of olefinic components in sedimented organic matter and in oils.

Because of the methyl branching of the isoprene unit, isoprenoids are long-chain hydrocarbons with recurring methyl branches. The asymmetry of the isoprene units due to the methyl branches on the four-member carbon chains results in two types of isoprenoids: "regular" and "irregular". In regular isoprenoids, all the isoprene units are linked "head to tail", the "head" being the one-position carbon atom (with the methyl branch at the two-position carbon, as in two-methyl butane) and the "tail" at the four-position. Regular

Fig. II (a)
["head to tail linkage]

isoprenoid alkanes therefore have methyl groups at the two-, six-, ten-, fourteen-, eighteen-, etc., positions. (See Figure I.)

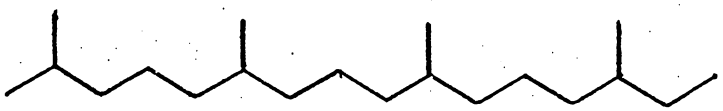
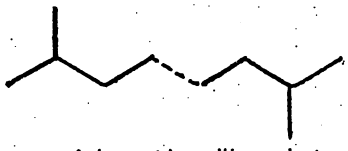


Fig. I

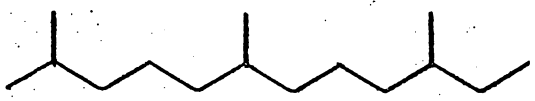
2-,6-,10-,14-tetramethyl hexadecane
Phytane

Irregular isoprenoids, on the other hand, contain one or more "head-to-head", or "tail-to-tail" linkages of isoprene units, resulting in methyl group branching at irregular intervals. In fossil



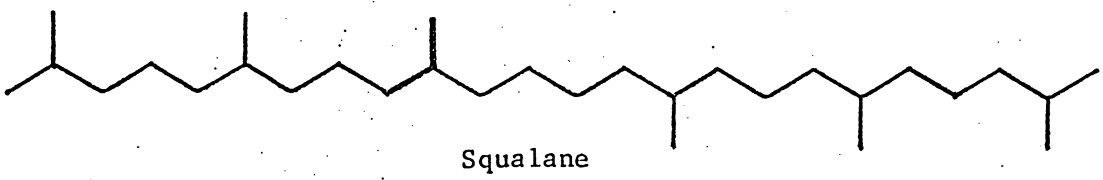
"head-to-tail" linkage

isoprenoids, the "head-to-head" irregularity has not been found. However, the "tail-to-tail" linkage is found in many isoprenoid molecules and is quite important geochemically in the formation of isomers that differ only by a four carbon versus a three carbon bridge. In most irregular isoprenoids there is only one tail to tail linkage, generally joining two smaller regular isoprenoid units (usually the same group) by their "ends".



Farnesane

Fig. II



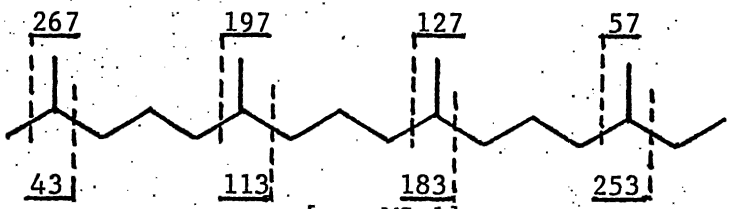
Squalane

In living organisms regular isoprenoids most frequently occur as acids, alcohols, esters, and only rarely as hydrocarbons, while irregular isoprenoids are frequently found as highly unsaturated hydrocarbons, or as highly unsaturated hydrocarbon chains with cyclic groups (with or without functional groups) at the ends. Both regular and irregular isoprenoids are commonly present in living orga-

nisms today.

Because of the nature of the carbon-carbon bond and their regular branched structure, isoprenoid hydrocarbons (and acids) lend themselves readily to analysis by mass spectrometry. This is especially beneficial because, in general, the amount of organic matter preparable for analysis is so small that other means of characterization are difficult or impossible to employ. Isoprenoid mass spectra show a distinct pattern due to the methyl branching of the chain. Since a bond between a tertiary carbon atom and a primary or secondary carbon atom will cleave preferentially to a bond between two secondary carbon atoms or a secondary and a primary carbon atom, electron impact induced cleavage of an isoprenoid ion will occur primarily at the branching sites. Because a secondary carbonium ion can stabilize a positive charge more easily than a primary carbonium ion, when an isoprenoid cleaves, the fragment with the methyl branch will preferentially carry the positive charge from the cleavage, since it can form a secondary ion, while the other (primary) fragment goes off as a neutral free radical. Hence, the mass spectra of isoprenoid hydrocarbons will show the most prominent peaks at the sites of the methyl group branches (smaller peaks from primary cleavages will also be present). Since cleavage can occur on either side of the branching site, two possible ions can result from cleavage at each branching site. As M/e increases, the relative intensities of the peaks decreases due to decrease in stability of the larger fragments as opposed to fragments of lower M/e. The typical isoprenoid mass spectrum will therefore exhibit a series of peaks at M/e C_nH_{2n+1}, spaced fourteen mass units apart, with more prominent peaks for the fragments

where cleavage at a branch occurs, with a general decrease in intensity as the M/e increases. In a regular isoprenoid, the branch cleavages are five carbons apart (including the methyl group), hence the branched peaks will form one or two series (depending upon the configuration of the parent molecule) with the peaks of each series seventy mass units apart.

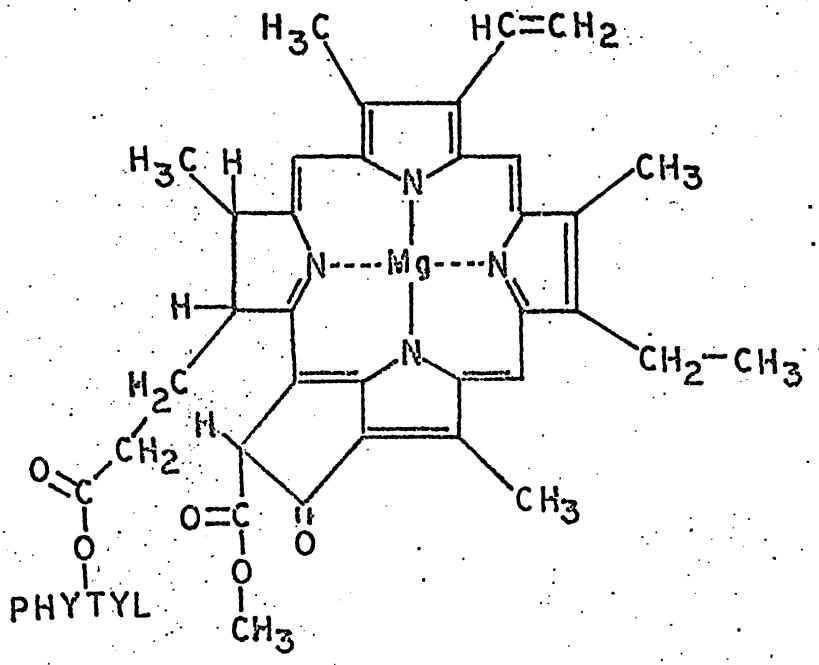


[see MS 1]

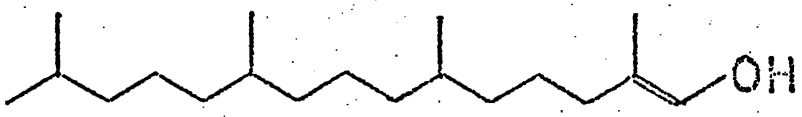
Fig. III
Phytane

There are several proposed precursors for fossil isoprenoid alkanes. The first and most frequently mentioned isoprenoid precursor is phytol. Phytol is twenty-carbon, singly unsaturated isoprenoid alcohol which occurs in nature as an ester side chain of cholrophylls. (See Figure IV next page.) Diagenetic action, it is postulated, will hydrolyze the phytol side chain from the chlorophyll nucleus. Diagenetic reduction and oxidation and de-carboxylation will result in pristane (two-, six-, ten-, fourteen-tetramethyl pentadecane), which can then be degraded to smaller isoprenoid molecules. Alternatively, complete reduction can occur, resulting in phytane (two-, six-, ten-, fourteen-tetramethyl hexadecane), which can then be degraded to smaller molecules. Evidence for this is the large amount of pristane and phytane present as compared with other isoprenoids, and the scarcity of the C_{17} analogue. Since the formation of the C_{17} isoprenoid would require cleavage of two bonds, it is not as likely to be formed as molecules requiring

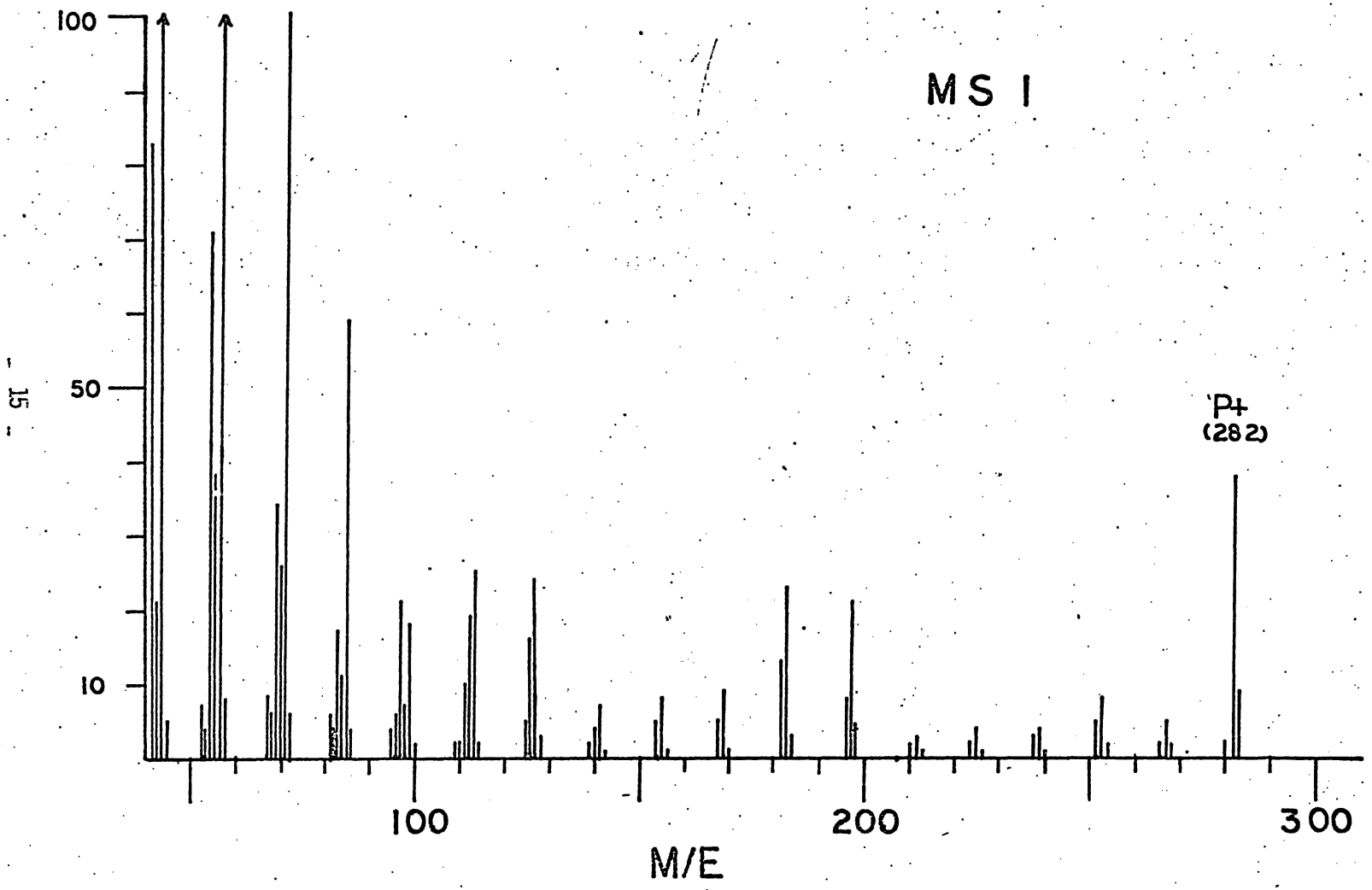
FIGURE IV



CHLOROPHYLL A



PHYTOL


MS I
100
50
10
- 15 -
P+
(282)
100
200
300
M/E

only a single cleavage.[22]

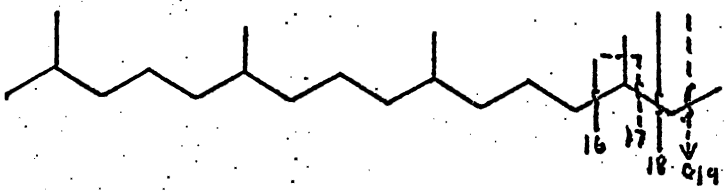
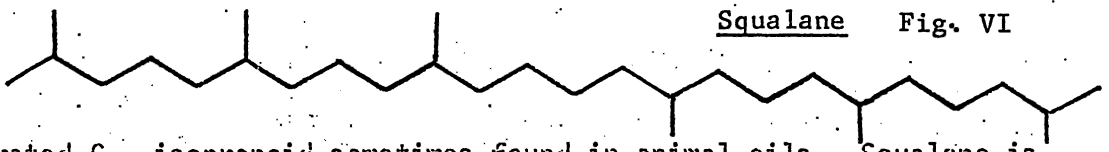


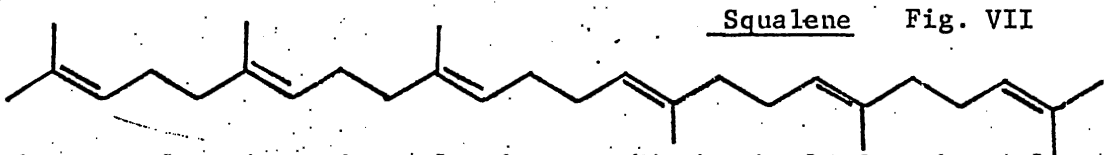
Fig. V

Recently squalane has been suggested[22] as a possible isoprenoid precursor. Squalane is a thirty-carbon, irregular isoprenoid composed of two regular C_{15} isoprenoid units (Farnesane) linked tail to tail. Squalane is a derivative of squalene, which is an unsatu-



Squalane Fig. VI

rated C_{30} isoprenoid sometimes found in animal oils. Squalene is also the compound from which sterols are synthesized by animals.



Squalene Fig. VII

Since squalane is an irregular isoprenoid, it should form irregular isoprenoids in the C_{19} range (two-, six-, ten-trimethyl hexadecane) and above, and should occur in the organic deposits. Though squalane has been reported in oils,[8,13,18,19] the expected irregular isoprenoids have not been found. However, the regular C_{17} isoprenoid, which could be formed from squalane has been reported.[12,13,22]

Lycopene, a C_{40} carotenoid, has also been mentioned as a possible forerunner of the isoprenoids. Lycopene is a heavily unsaturated irregular molecule composed of two regular unsaturated C_{20} isoprenoid units linked tail to tail. By saturation and degradation, lycopene can give rise to all the isoprenoids up to C_{22} except the C_{17}. It would also give rise to an irregular C_{24} isoprenoid

analogous to two-, six-, ten-trimethyl hexadecane possible from squalane. Regular C_{23} isoprenoid molecules would be difficult to form from lycopene, while the C_{22} isoprenoid, which is unlikely to form from a regular isoprenoid, could be formed (as C_{17} is formed from squalane).

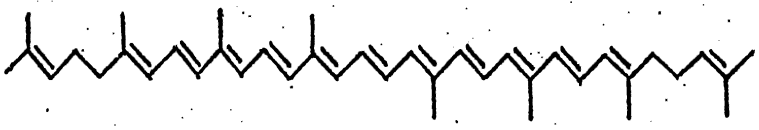


Fig. VIII

Lycopene

Another possible precursor of the isoprenoids in sediments and petroleum is a series of C_{25}, regular, isoprenoidal alcohols which have recently been identified in living organisms[25,26] and from which the lower isoprenoids could be formed by the same diagenetic pathways, which can alter phytol.

Other possible precursors are solanesol (a C_{45} alcohol), and some C_{30} to C_{60} isoprenoid alcohols, which have recently been discovered in living organisms.[5,15,33]

Pristane is the only regular isoprenoid hydrocarbon reported in living organisms in significant quantities.[3] It has been found in sizable quantities in the zooplankton Calanus, in which it acts as a buoyancy control. Since Calanus occupies a major place in the cold-water marine food chain, this may be a significant source of pristane in the cold-water marine environment.

SAMPLE DESCRIPTION

The subject of analysis was a sample of petroleum obtained from Esso Research Corporation by Geoffrey Bayliss (now of Geochem Company). The seep oil sample is from an area of Costa Rica.

The sample is a seep oil, i.e., is found on the surface in pools, rather than extracted from underground reservoirs. This particular sample was selected because preliminary studies by Esso Research indicate that the isoprenoid content is much higher than the 1.5 to 3.0 percent isoprenoid constituents normally reported for crude oils and shales.[1,23]

The oil is a naturally occurring seep oil and not a man-made product that was inadvertantly collected. This is shown by the wide range of compound classes and molecular weights found in the sample. If the oil were a product of a refining or other artificial process, then only certain types of compounds and only a portion of the molecular weight range would be present. The broad distribution of compounds and molecular weights present are those of a naturally occurring petroleum.

The small amount of normal alkanes present in the sample (see Table I) indicates extensive bacterial alteration. Oil-metabolizing bacteria usually attack normal alkanes,[23] only very infrequently attacking the branched or cyclic alkanes, thus depleting the normal alkane content to the low level seen in this sample. Although natural sieving could account for the small amounts of normal alkanes found here, bacterial action, which is quite common, is a more likely explanation. Since hydrocarbon-metabolizing bacteria are not known to synthesize isoprenoid compounds, the enrichment of the isoprenoid

content does not appear to be a result of this bacterial action.

The only maximum in the normal alkane distribution appears at heptadecane (see GC III). The single maximum indicates one formational process and the C_{17} maximum is characteristic of oils from marine sources. There appears to be a slight, but distinct, odd-over-even carbon number preference, but the possible presence of iso- and anteiso-isomers makes this uncertain.

The oil itself is a medium-brown, moderately viscous liquid with a greenish cast to it and a slight odor resembling machine oil.

EXPERIMENTAL METHODS

Sample Handling

The sample was obtained from Esso Research Corporation in two forms: a jar of untreated crude and in the form of fractional distillation cuts of the hydrocarbon fraction.

The fractional distillation yielded thirty distillation cuts and the still residue. Cuts No. 1 to No. 23 were at 5° Centigrade intervals from 50° to 165° at atmospheric pressure. Cuts No. 24 to No. 30 were done under .0005 mm. vacuum distillation. The still residue had been separated by silica gel chromatography into alkane-cycloalkane, aromatic, and polar fractions.

In order to obtain a reliable chromatograph of the saturated portion (Chromatograph 1), some of the crude oil was fractionated by alumina and silica chromatography. One part crude oil was placed on twenty-five parts of alumina (Alcoa, F-20, 80-200 mesh, activated at 400° C for two hours) on a 1 cm x 38 cm column. Exhaustive elution with hexane stripped off the hydrocarbon fraction. One part of the hydrocarbon fraction was placed on twenty-five parts of silica gel (200 mesh, extracted with hexane and dried at 110° for two hours) on a 1 cm x 37 cm column. Exhaustive elution with iso-octane stripped off the alkane and cycloalkane components. The normal alkanes were then separated from the branched alkanes and cycloalkanes by molecular sieving in iso-octane.

Separation of the normal (straight-chain) from branched fractions was accomplished by dissolving each distillation fraction in benzene and refluxing for eighteen to twenty-four hours with Linde

5A pore Molecular Sieves. The Molecular Sieves were activated by heating at 300° C for 24 hours under helium in the presence of P_2O_5. A large excess above the 100:1 ratio of sieve to mixture was used to insure good separation. The benzene was poured off and the sieves washed with benzene four times and the washings added to the original benzene. The mixture was then filtered, evaporated, weighed, and re-dissolved in n-hexane for preparative gas chromatography. Good separation of normals from branched components was obtained throughout.

Gas Chromatography

Separation and purification of the constituent compounds of each branched fraction for mass spectral analysis was accomplished by means of preparative gas chromatography. Fractions No. 24 through No. 30 were separated and purified by chromatography on a sequence of three columns:

1) 10 foot x 1/4 inch 3% Polysev (7-ring meta polyphenyl ether) coated on 80/100 mesh Chromasorb W-Acid-Washed (DMCS). Polysev is an intermediate-polarity phase which separates on the basis of boiling point and polarity. It was selected as the first column of the sequence because it produced the best initial separation for each distillation cut, and because it was able to separate the saturated components from cyclic, olefinic (if any) and aromatics (if any). In the Polysev chromatogram, the saturated components eluted first, followed by cyclic components, and then the aromatics. (Note: distillation cut No. 24 was separated on a 5-ring meta-polyphenyl ether column, which gave the same general results, but with less resolution.)

2) 10 foot x 1/4 inch 3% SE-30 (silicone gum rubber) phase,

80/100 mesh on Chromasorb W-Acid-Washed (DMCS).

SE-30 is a non-polar phase which separates on the basis of boiling point.

3) 10 foot x 1/4 inch 5% FFAP on 80/100 mesh Varaport 30. FFAP is a polar phase and separates on a variety of properties (polarity, boiling point, solubility). It is a specific for fatty acids and esters, but works quite well for hydrocarbons.

The still residue was initially chromatographed on an SE-30 column and the various cuts purified isothermally on FFAP. Certain cuts were further purified on a third column: 4% Apiezon-L coated on 80/100 mesh Chromasorb-W Acid-Washed (DMCS), 12 foot x 1/4 inch. This column was also used in the separation of possible C_{19} and higher weight isomers.[22]

Other phases such as Tetracyanoethyl Pentaerythritol, Carbowax 20, and OV-17 were tried, but were found to be of no greater utility than the columns used.

The Gas Chromatography was done on a Varian Aerograph Auto-prep A-700 gas chromatograph with programmer accessory and thermal conductivity detectors. Samples were collected in thin-walled pyrex capillary tubes which had been washed in chromic-sulfuric acid. Collection efficiency was approximately 60 to 75 percent, depending upon the condition of the silicone rubber gaskets and the adeptness of the operator.

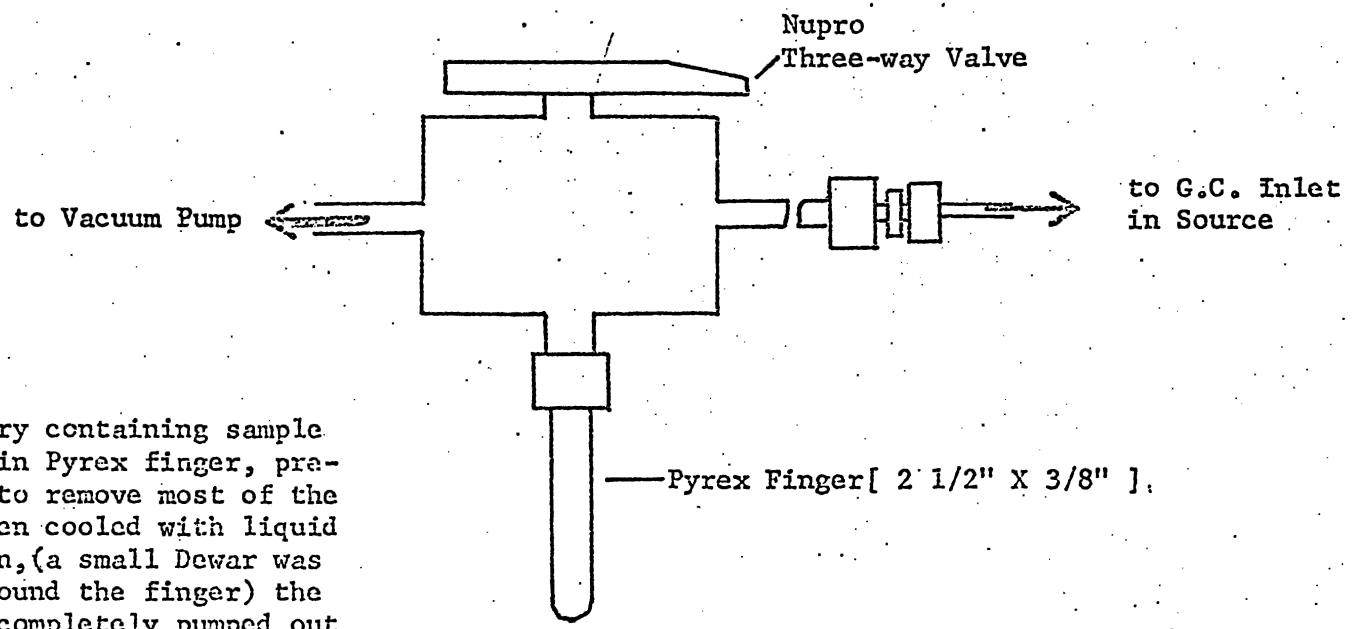
Mass Spectrometry

Identification of the compounds was by means of low-resolution mass spectrometry. Mass spectra were run on a CEC 21-110B double-focusing, high-resolution mass spectrometer at ordinary (~3000) low resolution. The CEC 21-110B is equipped with a heated-inlet-gold

leak system, and a direct introduction probe (on which most samples were run), and uses a 17 dynode, 200 volt per stage (maximum) electron multiplier detector. The recorder is a galvanometer deflection visicorder. Mass spectra were obtained with $.5 \times 10^{-12}$ amperes of ion beam. Sample conditions were ionizing voltage: 70 ev; accelerating voltage: 6000 volts; filament emission current: 100 uamperes. Source temperatures were 100° to 120° C for samples No. 24 through No. 30. Samples from the still residue were run at 150° to 200° C.

Due to the high pumping speed (115 1/sec) of the source diffusion pump and the low source pressure (2×10^{-5} torr maximum), many small (0.2 ul) and/or volatile samples were pumped away before spectra could be taken. A liquid-nitrogen cooled, heatable introduction system was installed by Dr. James E. Hudson, which made it possible to run very small samples (See Drawing A). The lower limit to sample size is approximately 0.1 ul, depending upon volatility. The presence of the glass insulator and metal capillary in the source made it necessary to lower accelerating voltage from 8 kv to 6 kv to avoid arcing in the source, however no significant loss of sensitivity was noted.

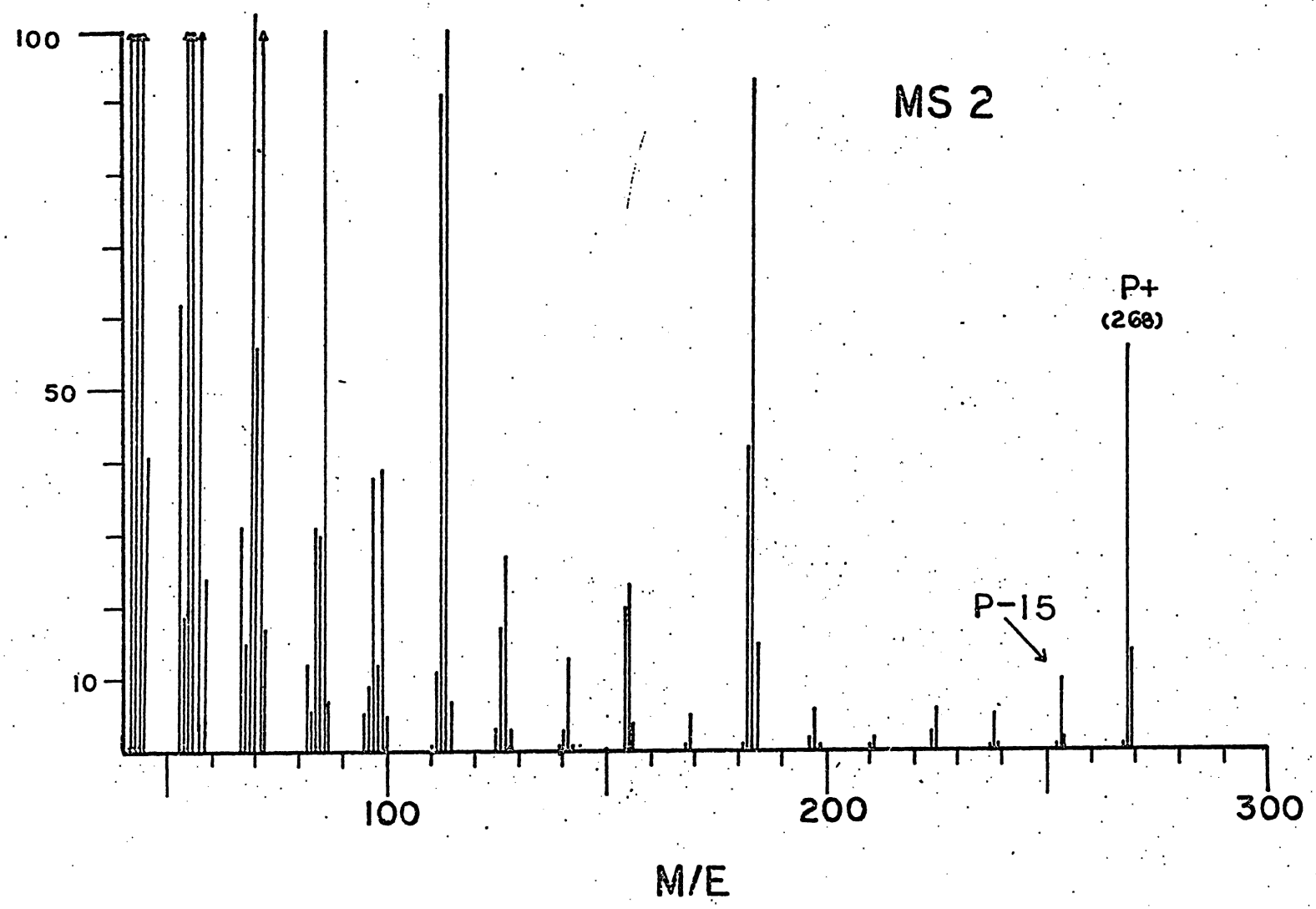
A salient characteristic of spectra taken on this 21-110B is the size of the parent ion. As can be seen from MS 2 (authentic pristane) and MS 3 (pristane from the sample), the parent ion is quite large. In most spectra cited in the literature, however, the parent ion of hydrocarbons is usually small. (See MS 4: authentic pristane from the dissertation of Dr. P. L. Haug.) As can also be noted from the spectra, the P-15 ion, corresponding to the loss of a methyl group, is smaller than expected. These phenomena rendered the structural identification of some spectra difficult until they were taken into account, though

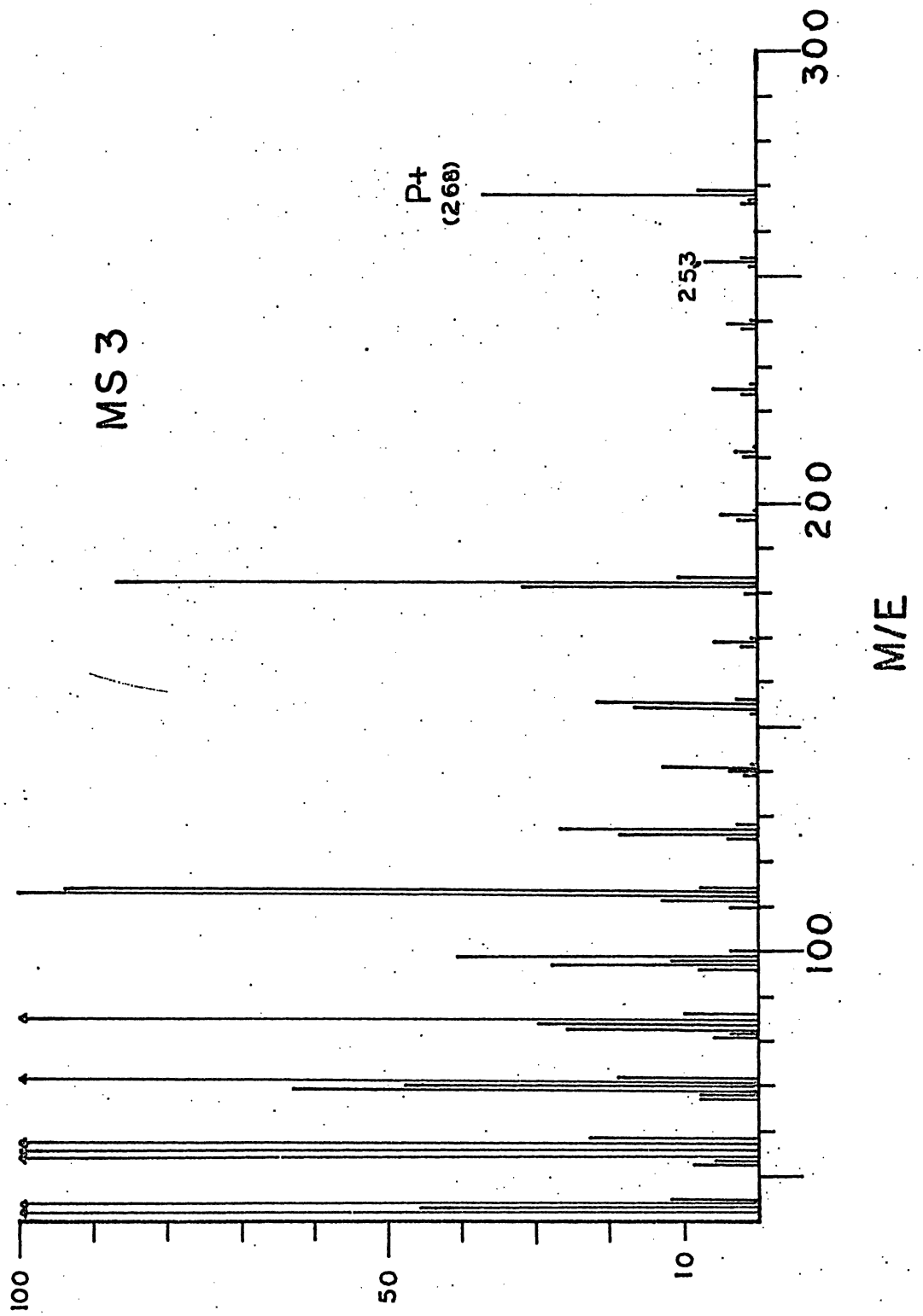


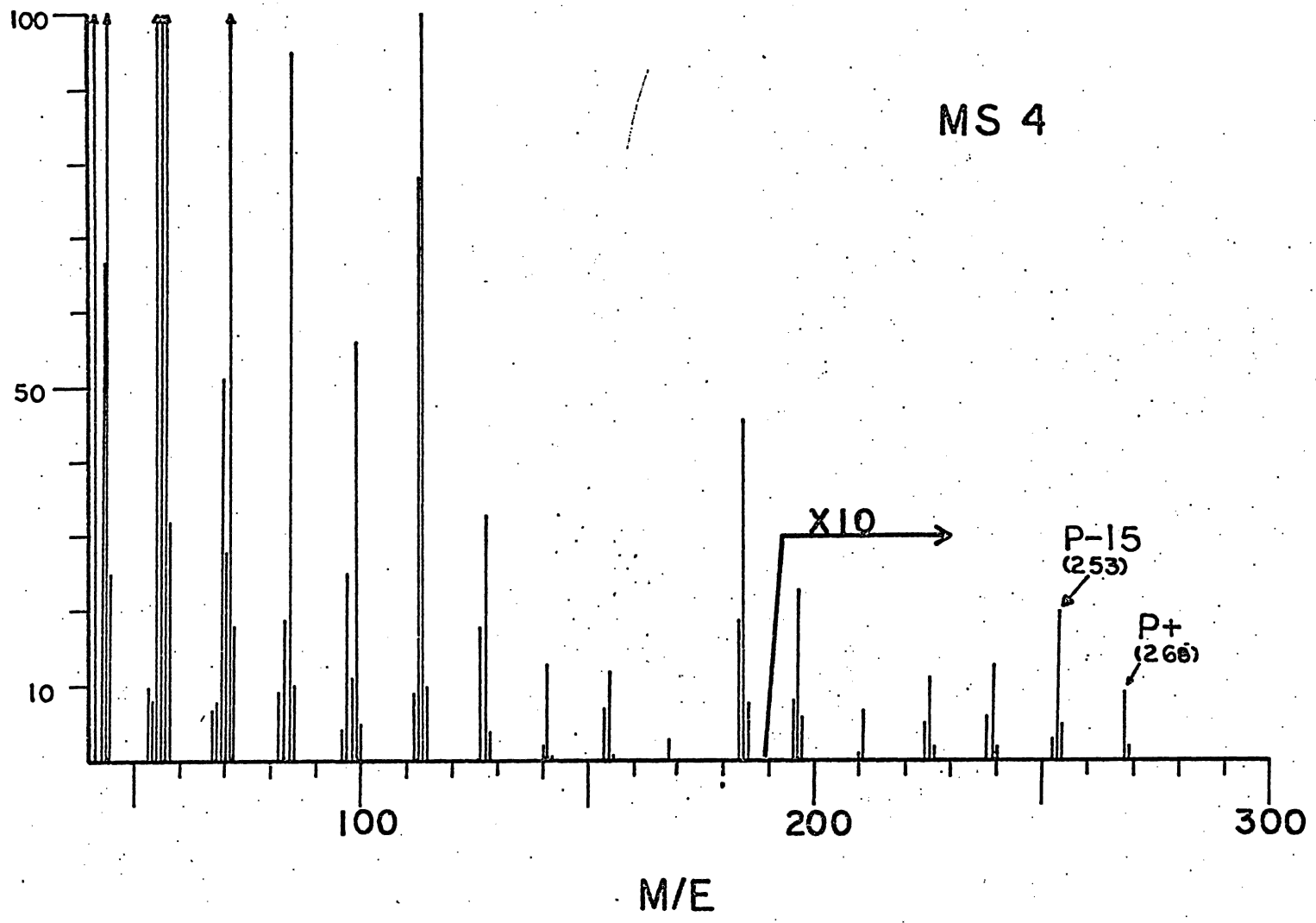
[Capillary containing sample placed in Pyrex finger, pre-pumped to remove most of the air, then cooled with liquid nitrogen,(a small Dewar was held around the finger) the finger completely pumped out and then openned to the source]

DRAWING A

COOLED INTRODUCTION SYSTEM


MS 2
100
50
10
P+
(268)
P-15
100
200
300
M/E


MS 3
P+
(268)
253
300
200
100
M/E
100
50
10


MS 4
100
50
10
X10
P-15
(253)
P+
(266)
100
200
300
M/E

the identification of the molecular ion was simplified. The effects of these phenomena on spectra identification will be discussed in more detail in the next part.

These phenomena were observed for most samples and standards and are probably due to the reduced ion source temperature.[2] They were not linked to the change in accelerating voltage.

All glassware was dipped in chromic-sulfuric acid solution after washing and was oven-dried to minimize contamination. All solvents used, with the exception of n-hexane, were ACS reagent grade and were re-distilled for purity. Reagent grade n-hexane was not available in sufficient quantities, therefore Practical Grade hexane was re-distilled fractionally, discarding the first 500 ml and last 750 ml of each two Kg. bottle.

INTERPRETATION AND DISCUSSION OF RESULTS

The analysis of the seep oil yielded several dozen branched and cyclic alkanes, the chromatographic techniques detailed above having made it possible to by-pass the aromatic and most napthenic components. Because this program was directed primarily to isoprenoids and related compounds, the other classes of compounds will not be discussed here in detail. Large numbers and quantities of compounds that were either cyclo-alkanes or olefins were detected. The scarcity of fossil olefins,[23] plus the general mass spectral patterns (i. e., very high M/e 83, 97, and 111 peaks; and a regular progression of C_nH_{2n-1} fragments) seem to indicate a cyclic nature for most of the unsaturated compounds. Though most napthenes were by-passed, some, especially in the higher molecular weight ranges, were detected.

The analysis of the saturated compounds yielded a complete set of isoprenoids from C_{16} to C_{25}, with the significant exceptions of the C_{17} and C_{22} isoprenoids. In addition, several other interesting compounds have been tentatively identified.

In many of the isoprenoid mass spectra the unsaturated M/e 97 peak is as large or larger than the expected saturated fragment at M.e 99 (MS 1, 2, 6, 10, 11, 14, 15). Likewise, the M/e 98 peak is also higher than the expected M/e 99 (MS 7,8) and the M/e 112 is higher than the expected saturated fragment at M/e 113 (MS 3, 5, and 10). It should especially be noted that the spectrum (MS 2) of authentic pristane (obtained through Dr. Julia Sever of Gulf Coast Research Labs from Applied Science Lab.), exhibits very large peaks at M/e 97, 112, and 154 when compared with MS 4 (authentic pristane by P. L. Haug). These effects, especially the appearance of large C_nH_{2n}

peaks, may be due to the low ion source temperature (120° C), which could give rise to secondary fragmentation processes, and which would also increase the molecular ion peak size.[2] Han, McCarthy, and Calvin have noted the loss of a hydrogen atom and formation of a C_nH_{2n} fragment of M/e 98 or 112 in isomers with a long straight chain (4 or 5 carbon atoms) on one side of a branch.[21,28] It has been established that column bleed from the SE-30, Polysev, and FFAP phases did not contribute to these peaks. However, contribution from cyclic and olefinic compounds is a possibility since several spectra (MS 1, 3, 6, 7, 8, 10, 11, 14) show sizable M-2 peaks. This contribution, however, would not explain the noted anomalies in the authentic pristane scan, in which no cyclic or olefinic peaks were noted.

The mass spectrum of the C_{16} isoprenoid is shown as MS 5. The C_{16} isoprenoid was found in small amounts in distillation cut 24 and is probably present in large amounts in cut 23. The spectrum exhibits major peaks at M/e 113, 141, 183, and 226 which, as can be seen from the predicted cleavage pattern of the C_{16} isoprenoid (Fig. IX), are

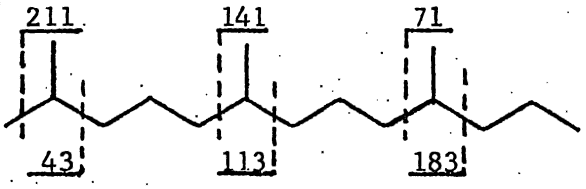
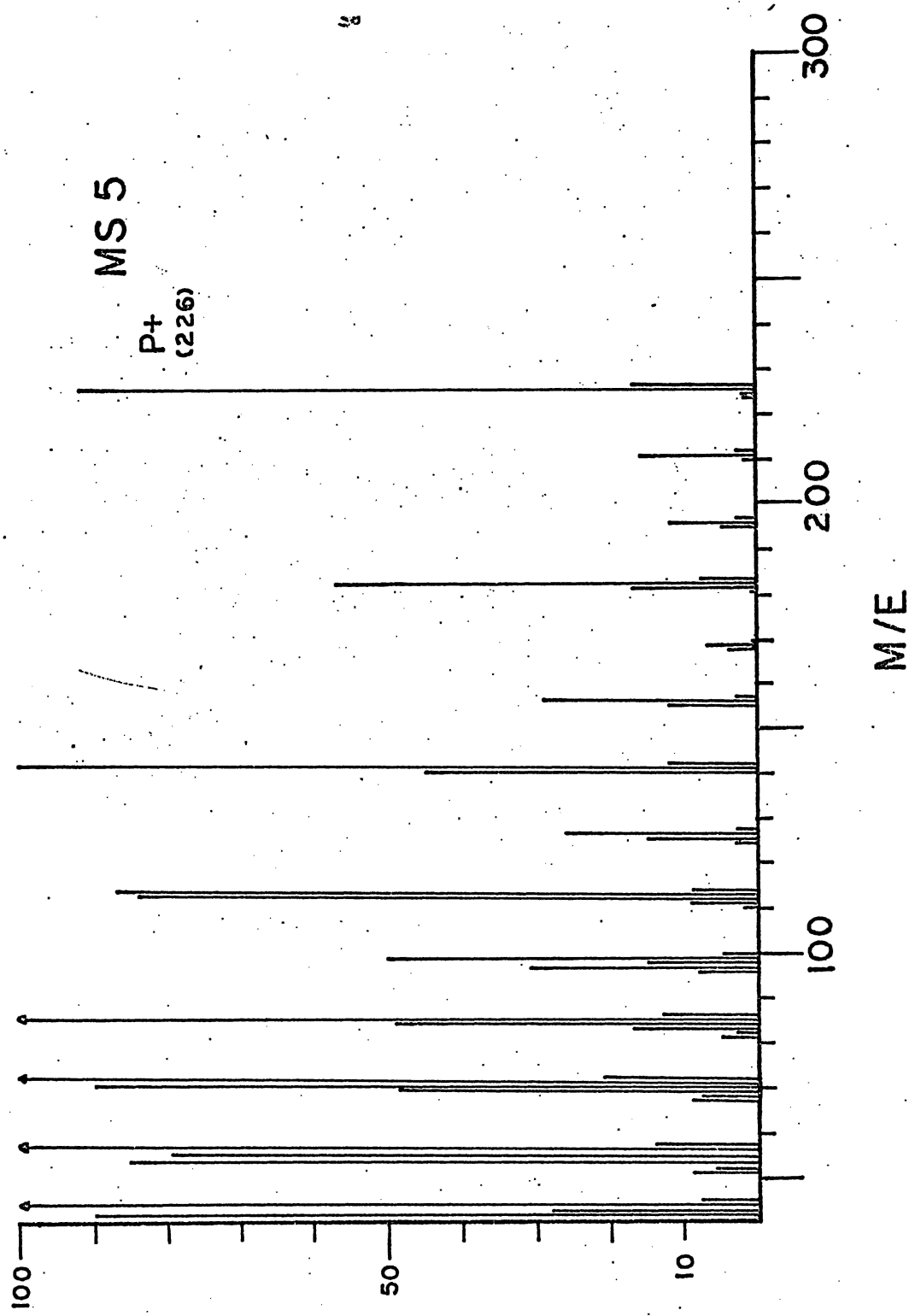


Fig. IX

the major peaks to be expected from the fragmentation of the molecule. The M/e 211 peak is somewhat small but still larger than the M/e 197 or 169, while the parent ion is quite large (92% of the base peak at M/e 141). These are characteristics which were noted in all the spectra run at low temperatures. (See the discussion in the Experimental Methods section.)


MS 5
P+
(226)
100
50
10
100
200
300
M/E

A very careful search was conducted for the regular C_{17} isoprenoid. Though C_{17} isoprenoids have been reported elsewhere (though very rarely)[12,13,22] in terrestrial samples, no detectable trace of the C_{17} regular isoprenoid was found in this sample. However, several branched C_{17} saturates were found in distillation cut 24. The mass spectrum of one of these is shown by MS 6. The tentative identification of this compound, based on the mass spectrum, is three-, seven-, eleven-trimethyl tetradecane:

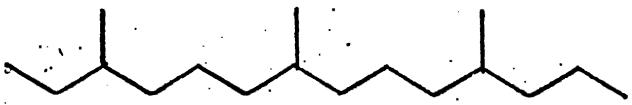


Fig. X

3-,7-,11-trimethyl tetradecane

This assignment is indicated by the prominent peaks at 127, 141, 197, and 211, all of which are noticeably larger than their neighboring peaks. The M/e 57-127-197 sequence of prominent peaks indicates a C_4-C_9-C_{14} progression of major fragments, and the M/e 71-141-211 sequence indicates a C_5-C_{10}-C_{15} fragment sequence. The small M/e 113 and 183 peaks would seem to indicate either irregular spaced branching or the absence of a terminal isopropyl group. As seen from Fig. XI, the fragmentation of three-, seven-, eleven-trimethyl tetradecane would yield the mass spectrum shown is MS 6.

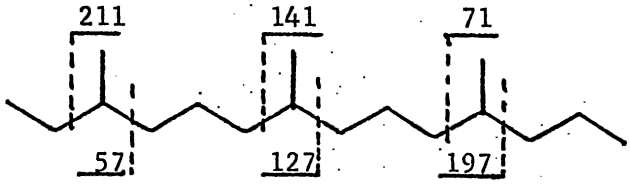
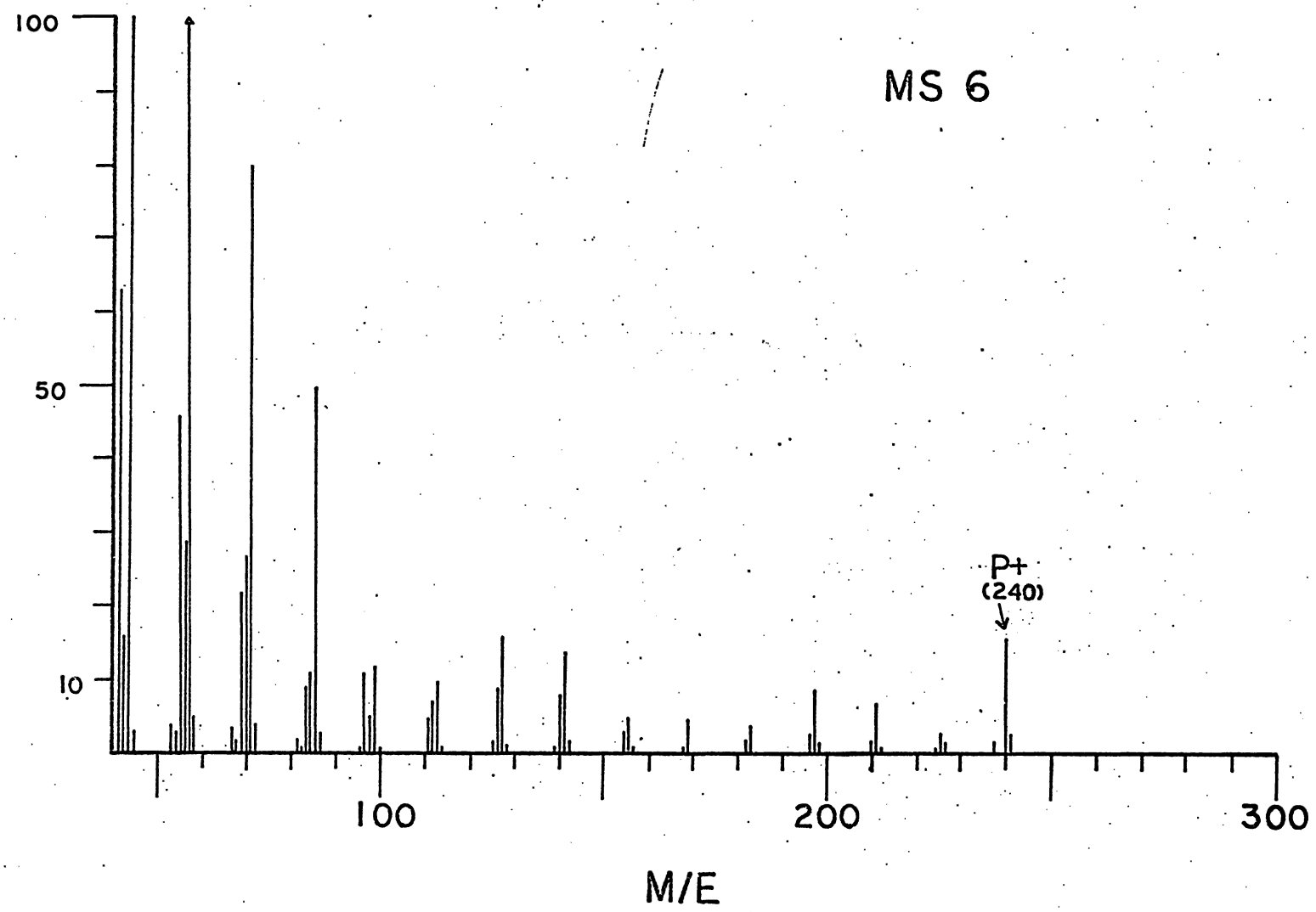


Fig. XI

The presence of this "semi-regular" isoprenoid would be of significance since it could be formed from phytane by the loss of the terminal isopropyl group.

An unknown, saturated C_{17} component is shown in MS 7. Because of


MS 6
100
50
10
P+
(240)
100
200
300
M/E

the small amount of compound that was isolatable, the sample was pumped off before the spectrum could be completely scanned. Hence the peaks below M/e 100 have reduced intensities. The spectrum is still interesting because of the relative sizes of the M/e 155 and 169 peaks as compared to 113 and 183. As can be seen from the size of the 169 and 155 peaks, there are large C_{12} and C_{11} ions. The size of M/e 225 peak (the P-15) is too small to definitely indicate a fragment resulting from cleavage at a branching site, though, due to the decrease of P-15 peak intensities, it could be such a fragment. It is not really possible to rationalize this spectrum definitely, though it may be a mixture of two C_{17} isomers, such as the mixture of 7-methyl hexadecane and 8-methyl hexadecane found in the Nostoc algae by Calvin et al.[21] The M/e 155-169 doublet was seen in several other spectra. The samples in which this doublet was seen were all quite small.

The C_{18} regular isoprenoid was isolated from distillation fractions 25 and 26. The mass spectrum of the isoprenoid from 26 is shown as MS 8 (the mass spectrum for the isoprenoid from fraction 25 is essentially identical). Although no standard was available for comparison, identification was possible due to the characteristic fragmentation (Fig. XII) and comparison with the literature.[13] As in the

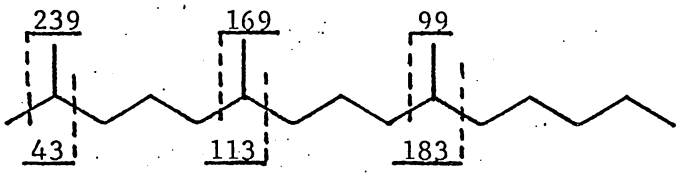
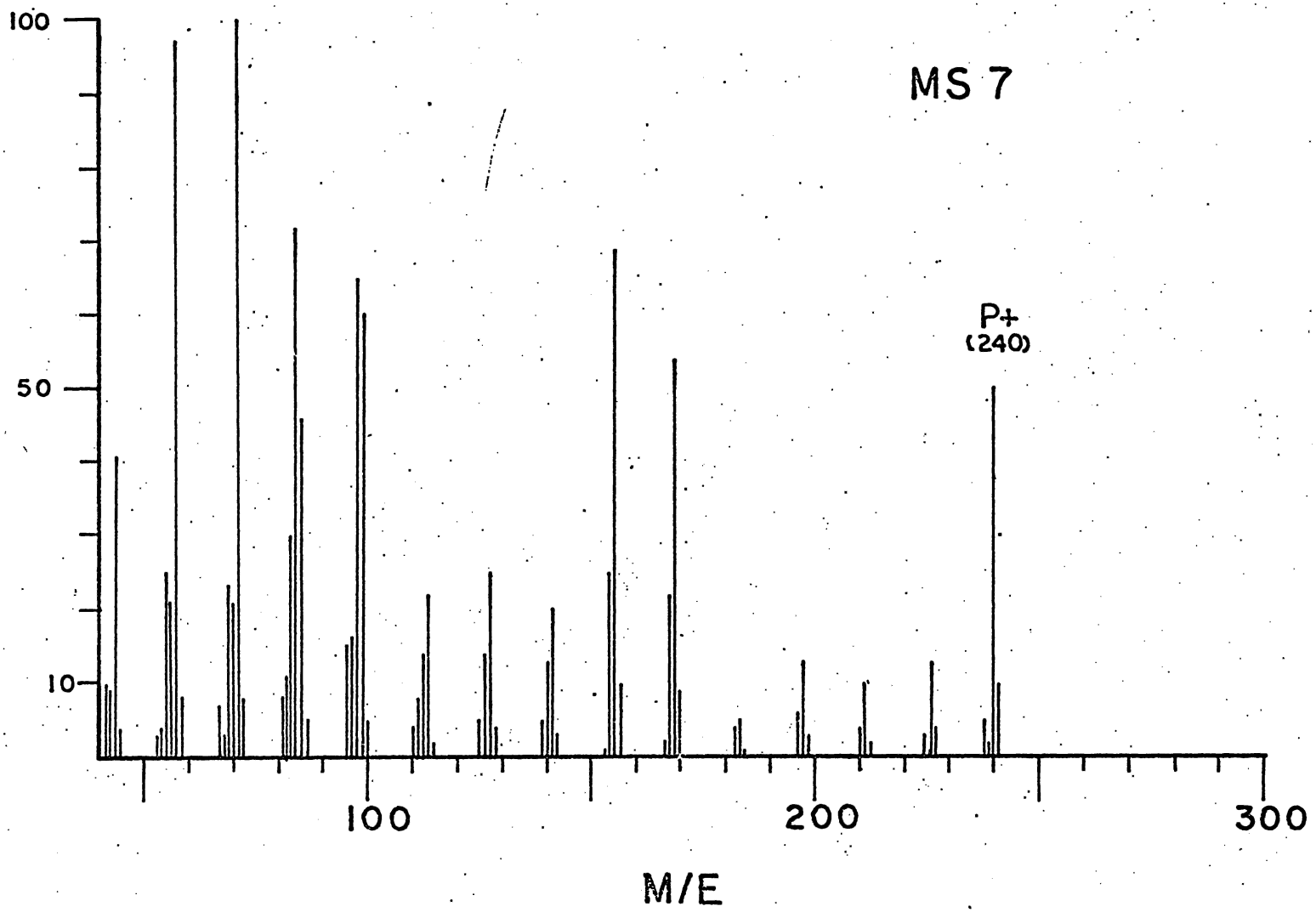
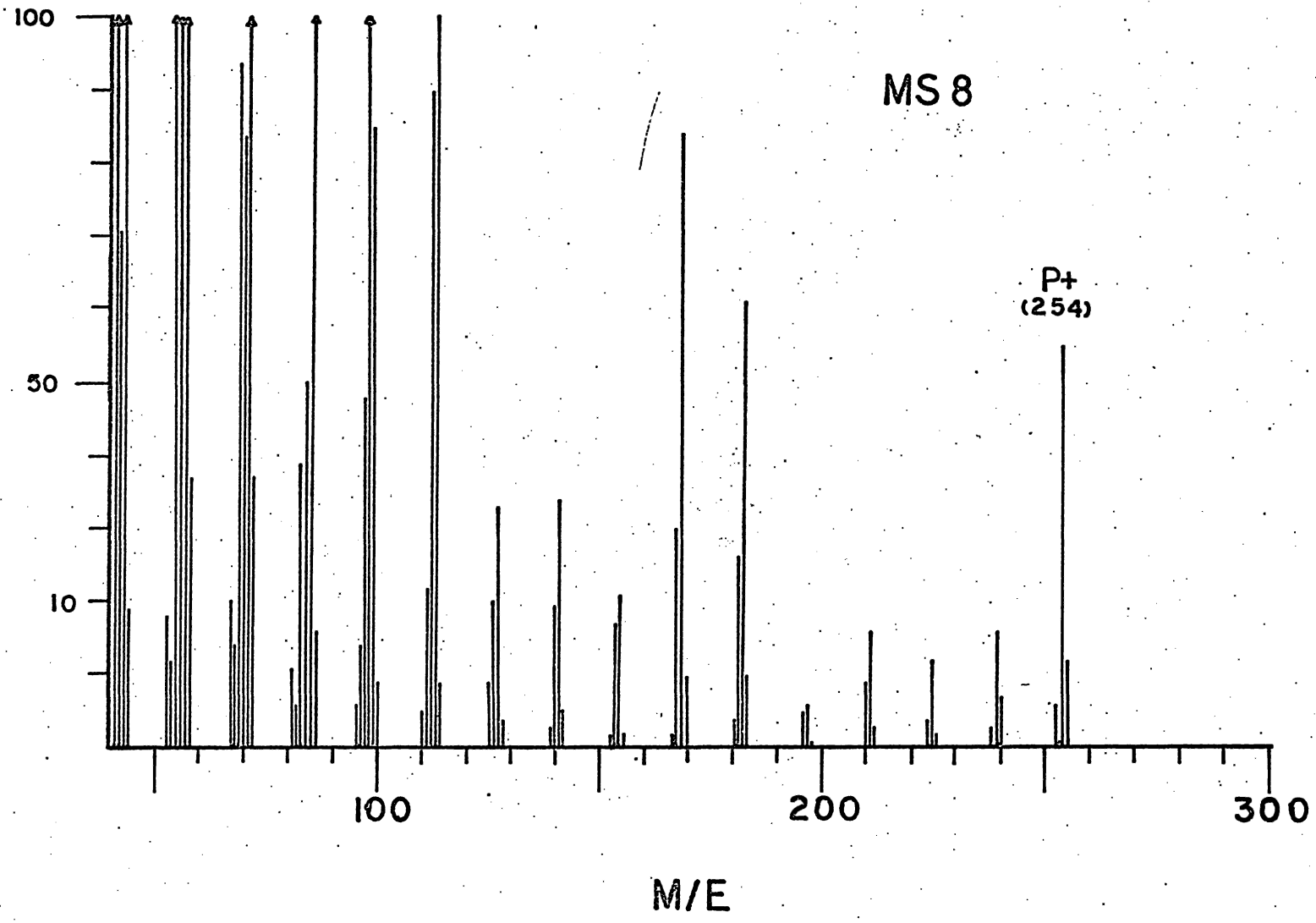


Fig. XII

C_{16} isoprenoid spectrum, the M/e 112 peak is quite large (almost the size of the 113 fragment), and the M/e 98 peak is larger than the 99 fragment (121% of the base peak as opposed to 85%).


MS 7
100
50
10
P+
(240)
100
200
300
M/E


MS 8
100
50
10
P+
(254)
100
200
300
M/E

Pristane, the regular C_{19} isoprenoid, comprised the largest individual constituent of both the isoprenoids and the branched alkane fraction as a whole (Chromatogram 1), and was isolated from distillation cuts 26, 27, and 28. The spectrum shown here (MS 9) is from distillation cut 28. Comparison of MS 9 (the crude oil pristane) with MS 2 (authentic pristane, courtesy of Dr. Julia Sever) shows an excellent agreement between the two. The close correspondence between the sample and standard spectra, identical gas chromatographic retention times when sample and standard were co-injected, and the simplicity of the pristane fragmentation pattern (Fig. XIII) all provide conclusive evidence for the identification of pristane in the sample.

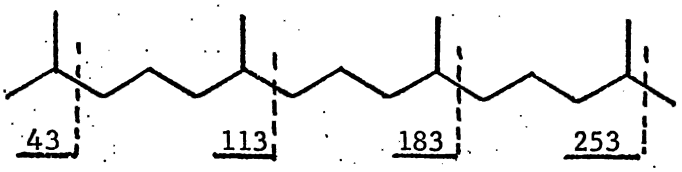


Fig. XIII

Some care, however, must be taken in identifying C_{19} unknowns as pristane solely on the basis of mass spectral data. There are several possible isomers whose structures and mass spectra closely resemble pristane. One of these, two-, six-, ten-trimethyl hexadecane, can be formed from squalane and differs from pristane only in one methyl group (there are five methyl groups as opposed to six methyl groups in pristane). Consequently, as can be seen from Fig. XIV, the mass spectra of pristane and two-, six-, ten-trimethyl hexadecane will be virtually identical, differing only in the height of the M/e 253 peak,

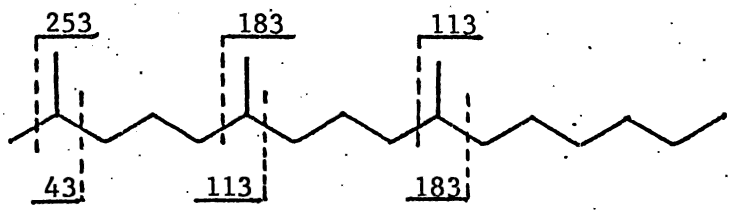
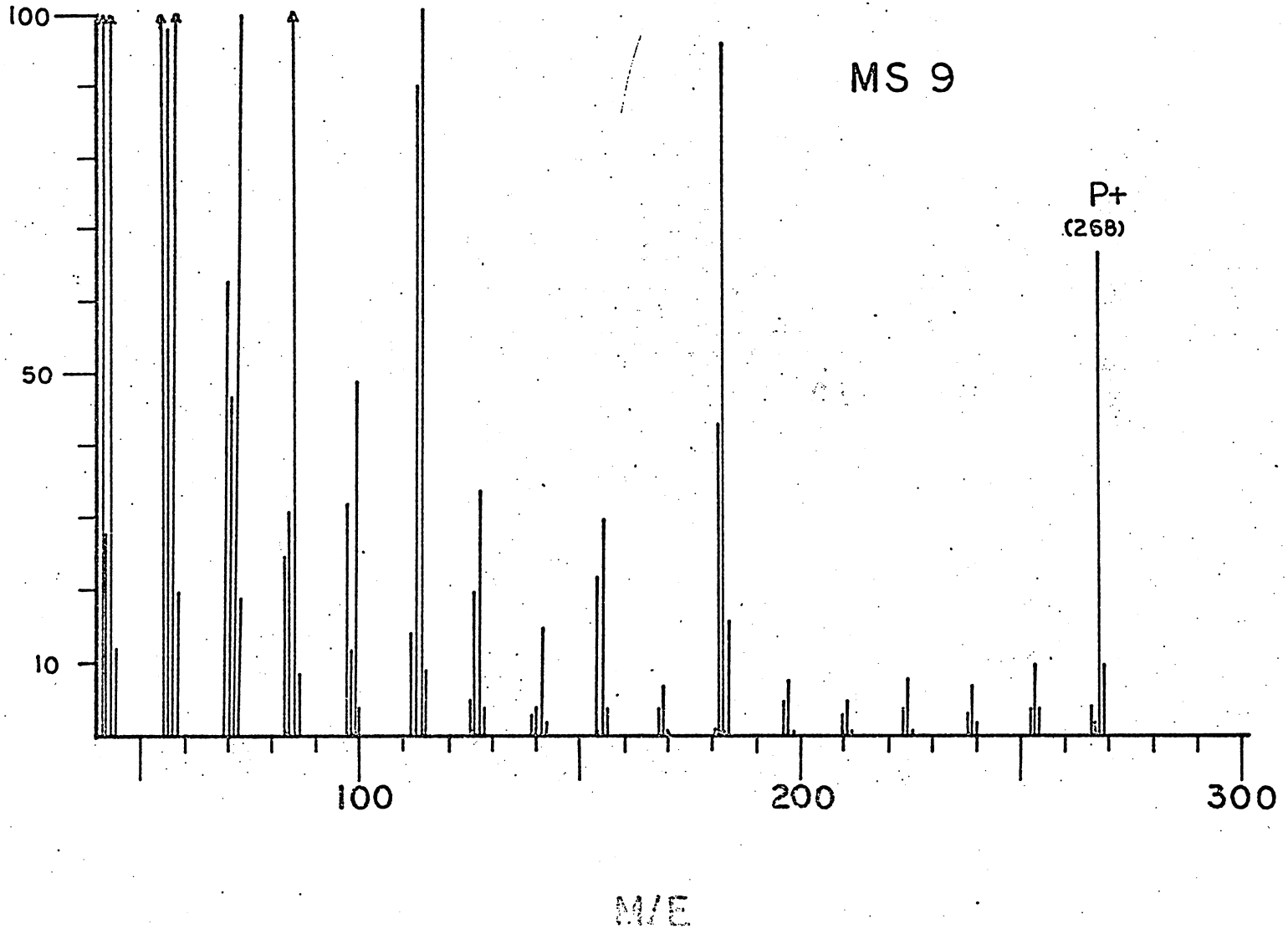


Fig. XIV


MS 9
P+
(268)
100
50
10
100
200
300
M/E

a difference which is generally indistinguishable. Furthermore, the two compounds have been shown to have indistinguishable gas chromatographic retention times on SE-30.[22] Since it has been shown that the two isomers have different retention times on an Apiezon-L phase,[11,22] an Apiezon-L column (12' x 1/4") was prepared and the unknown and authentic pristane co-injected. The two compounds had identical retention times under a variety of conditions. Though the Apiezon-L column was not a capillary column, the separation between the two C_{19} isomers on a capillary column[11,22] is such that they could probably be separated on a packed column. This would indicate that the MS 3 (the spectrum of the component compared with authentic pristane on the Apiezon column) is pristane.

The mass spectrum of an unknown branched C_{19} component isolated from distillation cut 27 is shown by MS 10. The most interesting aspect of this spectrum is what appears to be three regular fragmentation sequences: M/e 99-169-239; M/e 113-183-253; and M/e 57-127-197. These peaks, as can be seen from the spectrum, appear to be the most prominent peaks. An interesting rationalization of this spectrum is possible. The spectrum can be rationalized as a mixture of pristane (also isolated from cut 27), which would yield peaks at M/e 113, 183, 253, and 268, and three-, seven-, eleven-trimethyl hexadecane, which would yield peaks at M/e 99, 169, 239, 127, 197, and 268. (Fig. XV) Three-, seven-, eleven-trimethyl hexadecane is the C_{19} homologue of

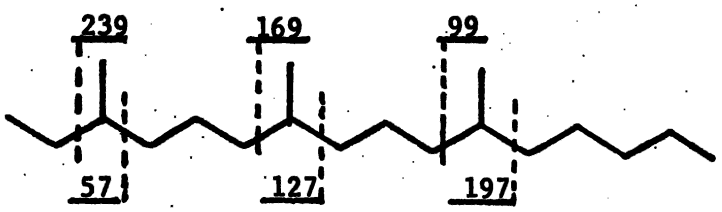
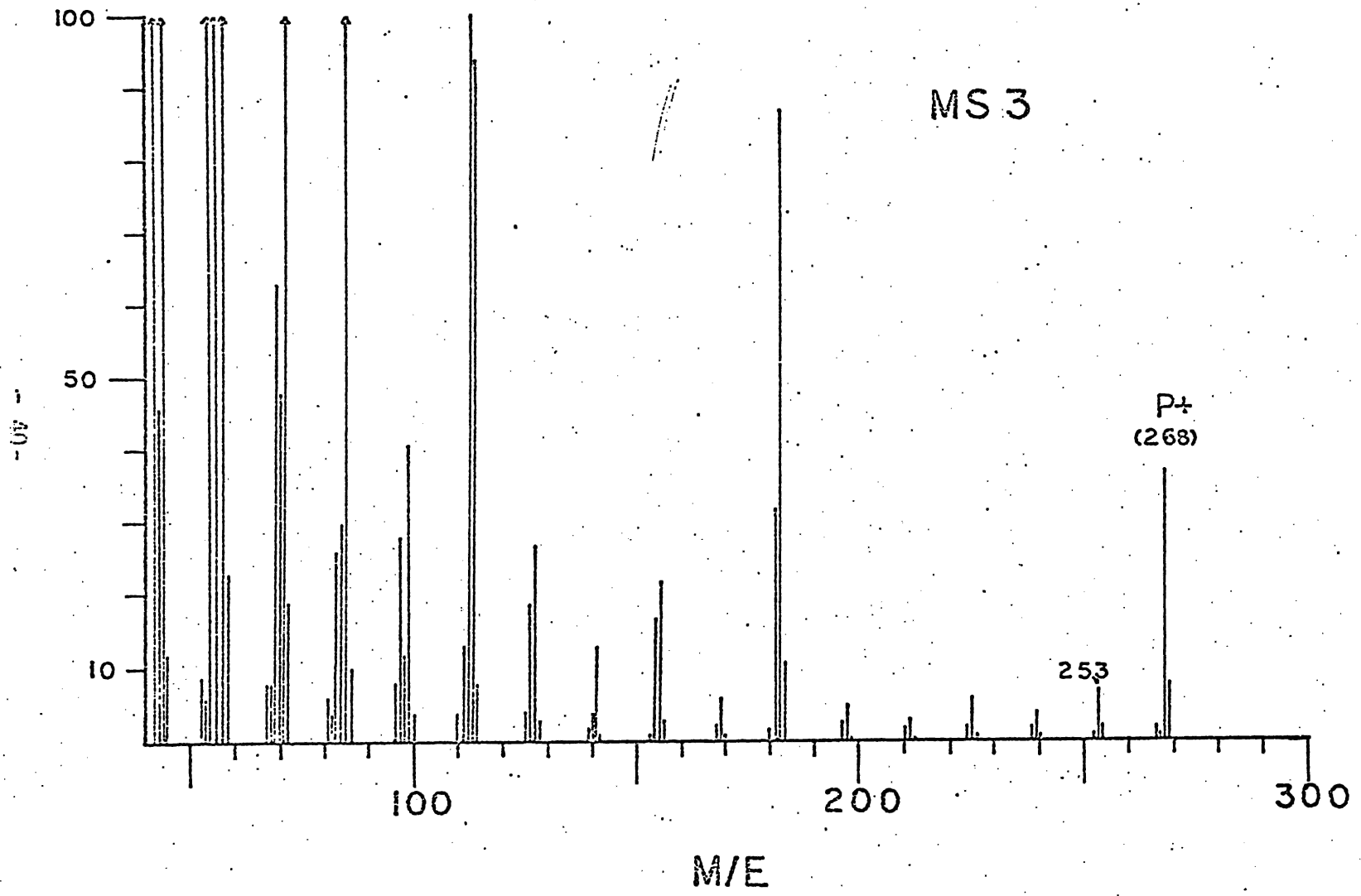
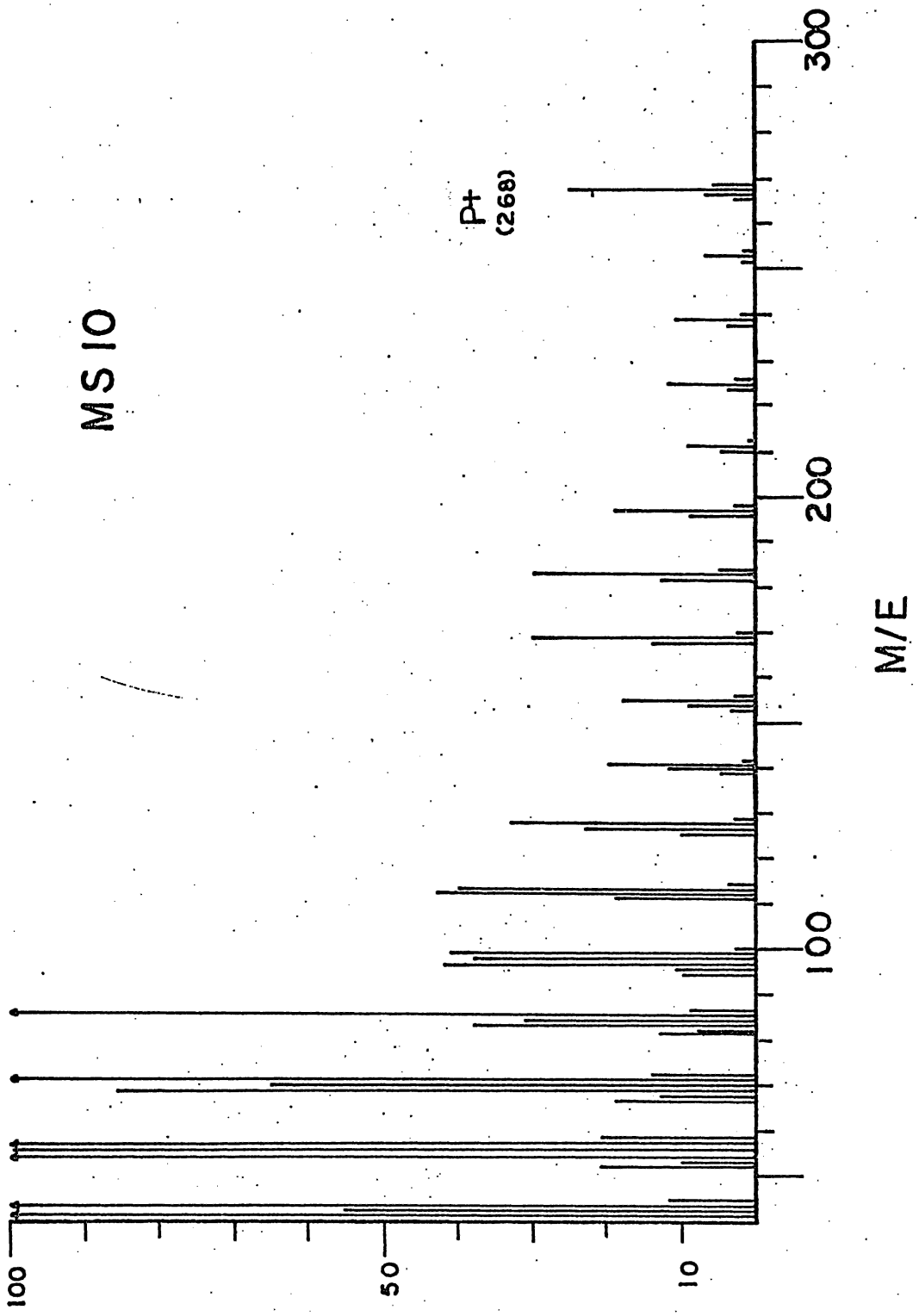


Fig. XV


MS 3
100
50
10
P+
(268)
253
100
200
300
M/E


MS 10
P+
(268)
100
50
10
100
200
300
M/E

three-, seven-, eleven-trimethyl tetradecane (see above). The C_{19} homologue could be formed from phytane by the loss of a terminal methyl group (in much the same manner that three-, seven-, eleven-trimethyl tetradecane could be formed by loss of an isopropyl group. This unknown could also contain one or more C_{19} isomers formed by the loss of one of the other methyl groups of the phytane molecule.

MS 1 exhibits the spectrum of the phytane component isolated from distillation cut 27. Phytane, also isolated in sizable quantities from fraction 28, was the second most abundant isoprenoid isolated and the second most abundant component of the total branched alkane fraction. The spectrum, as can be seen from MS 1, exhibits the prominent peaks at M/e 113, 127, 183, 197, 253, 267, and 282 that would be expected from the predicted fragmentation pattern (Fig. XVI). Phytane,

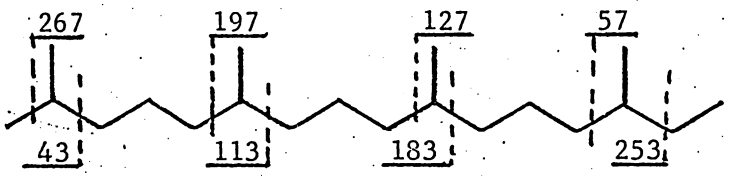


Fig. XVI

a regular isoprenoid, lends support for phytol as a precursor and tends to exclude squalane.

MS 11 shows the spectrum of the C_{21} saturated compound from cut 29 identified as the C_{21} regular isoprenoid. As with the other isoprenoids (except pristane), no standard was available. However, the spectrum exhibits the predominant peaks at M/e 113, 183, 253, and M/e 141, 211 and 281 that would be expected from the predicted fragmentation pattern of the C_{21} isoprenoid (Fig XVII):

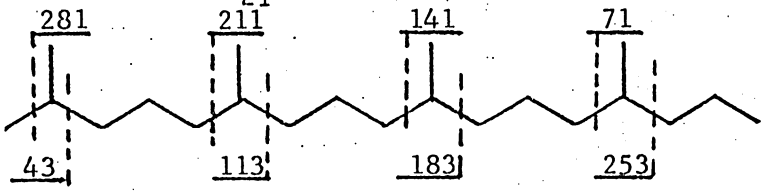
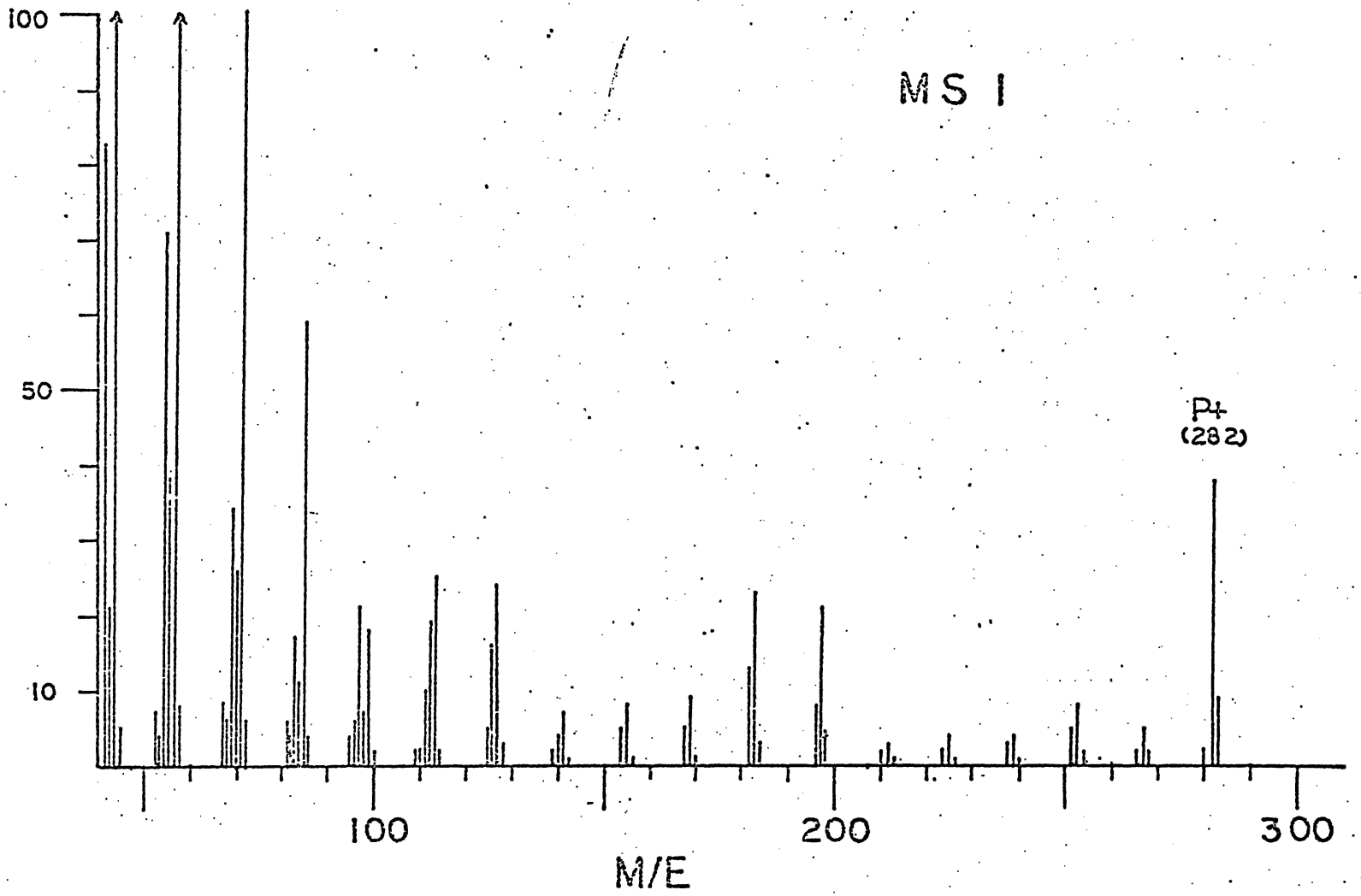
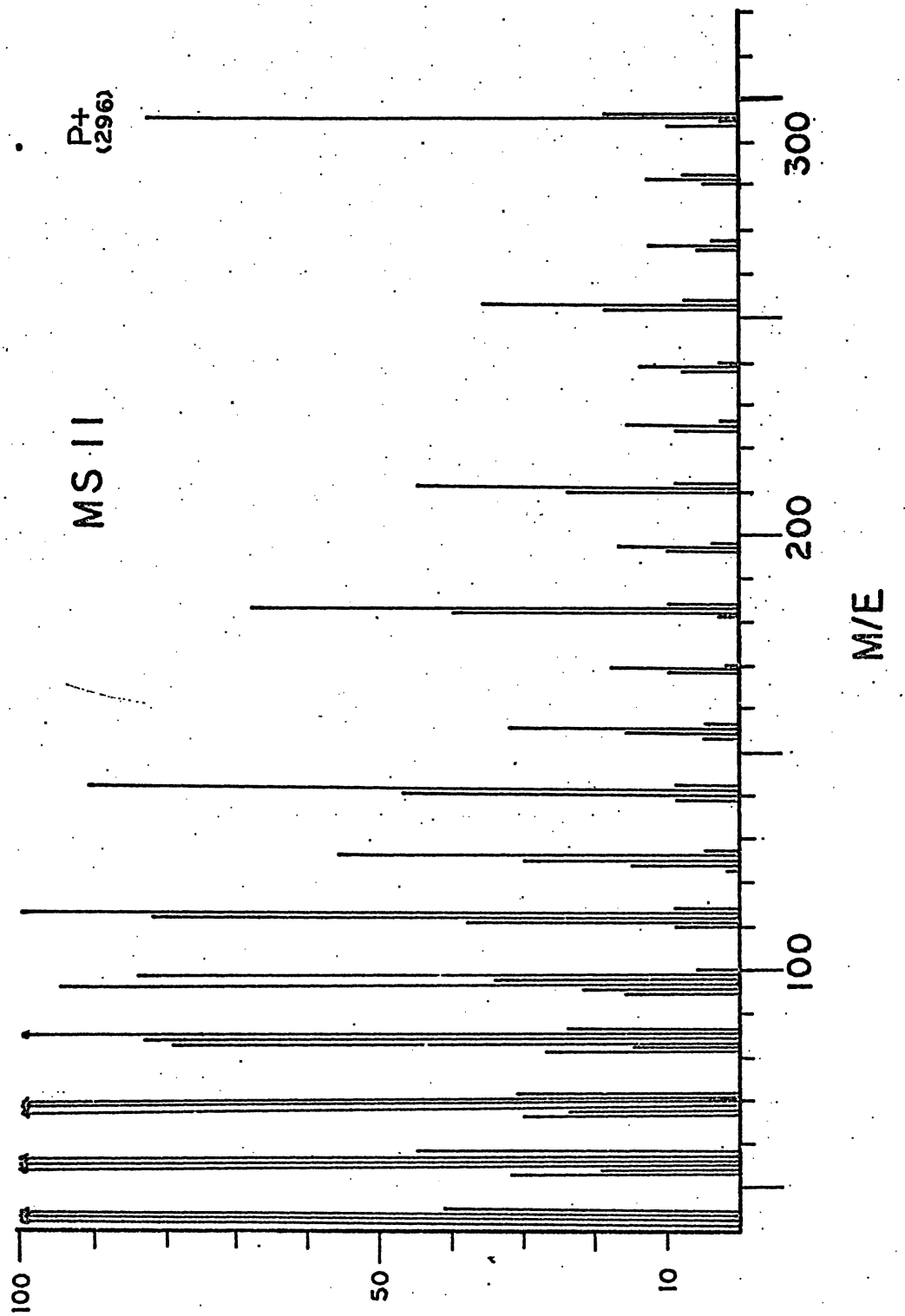


Fig.XVII


MS I
100
50
10
P+
(282)
100
200
300
M/E


MS II
P+
(296)
100
50
10
100
200
300
M/E

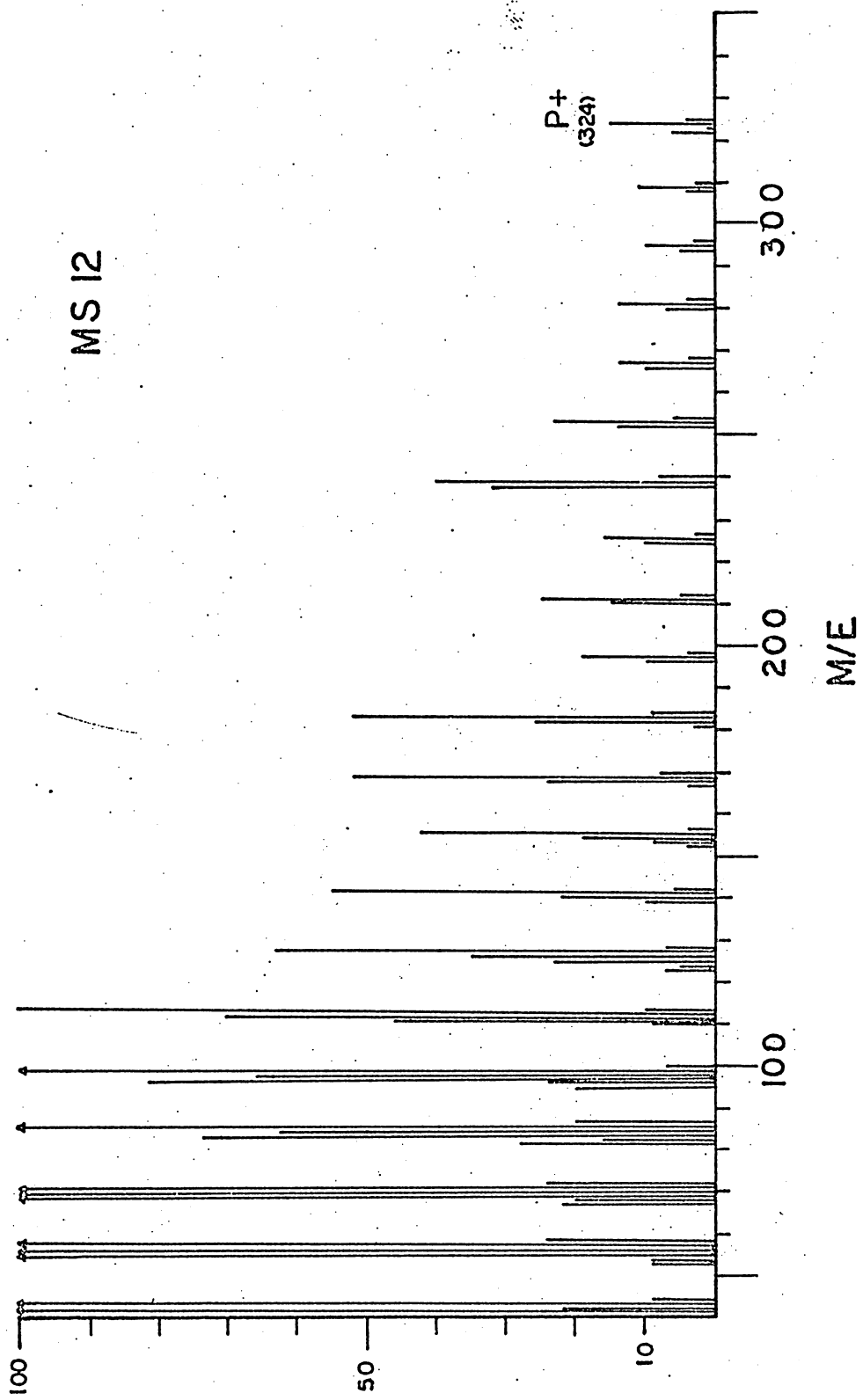
The intensities of the peaks in the spectrum agree well with spectra cited in the literature.[12,13]

(All the following compounds were isolated from the branched-cyclic fraction of the still residue by gas chromatography on SE-30 and Polyphenyl Ether columns, with some further purification of FFAP or Apiezon-L.)

A careful search of the still residue peaks was conducted for evidence of higher weight isoprenoids. These isoprenoids, ranging in size from 22 carbon atoms on up, have only rarely been reported,[12,32] and then in very small amounts. The proof of their presence is important in establishing the tenability of various compounds, such as squalane and lycopene, as possible important isoprenoid precursors.

No C_{22} isoprenoid was found in this sample, though Han and Calvin have reported it in the Bell Creek crude oil.[12] The apparent absence of the C_{22} isoprenoid could be interpreted in much the same way as the absence of the C_{17} isoprenoid: a regular isoprenoid precursor, from which the C_{17} and C_{22} regular isoprenoids could be formed only by cleavage of two bonds to the same carbon atom.

Even though no C_{22} isoprenoid was detected, the C_{23} isoprenoid (two-, six-, ten-, fourteen-tetramethyl nonadecane) was isolated from the distillation residue. No standard was available, but the gas chromatographic retention time on SE-30 correlates with the positions of the other identified isoprenoid peaks (see Chromatogram I), and the mass spectrum (MS 12) shows the prominent peaks predicted by the fragmentation pattern of the C_{23} isoprenoid (Fig XVIII), and resembles fairly closely the spectrum of the C_{23} isoprenoid presented by Han and Calvin.[12] It is interesting to note the rather sizable C_nH_{2n} peaks


MS 12
P+
(324)
100
200
300
M/E
100
50
10

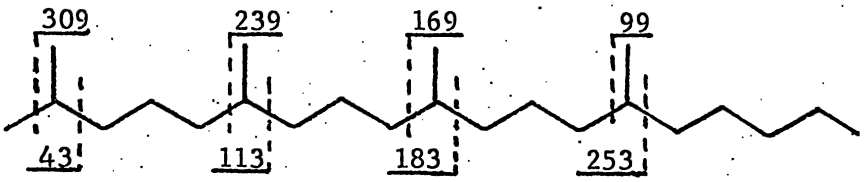


Fig. XVIII

at M/e 238 and 252. Han also accounts for these by loss of hydrogen from long chain fragments.[12]

MS 13 shows the spectrum of the C_{24} regular isoprenoid isolated from the still residue. This spectrum, due to the very simple fragmentation pattern of C_{24} isoprenoid (homologous with pristane) predicted in Fig. XIV:

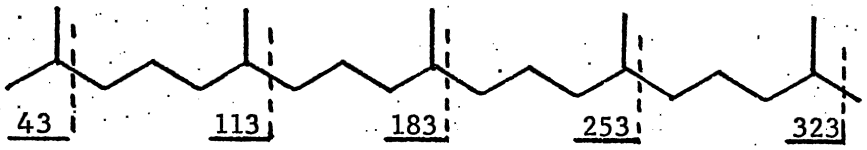
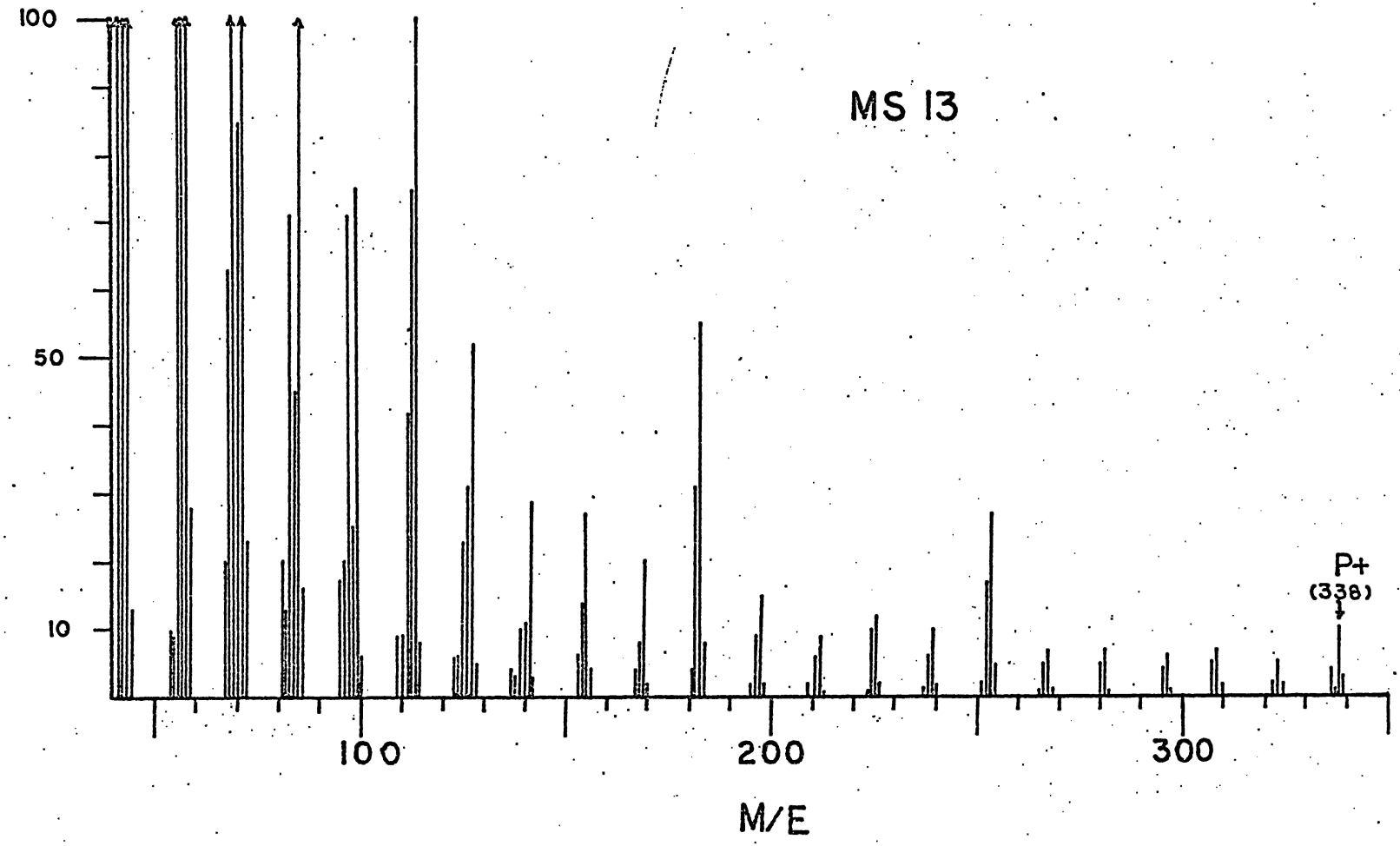


Fig. XIX

is easily, and with some certainty, identified as the C_{24} isoprenoid. The predicted peaks are easily the most prominent peaks in the spectrum, with decreased intensity only at the M/e 323 peak (P-15). The C_{24} regular isoprenoid was found to elute just before an n-C_{22} standard on SE-30 (see Chromatogram II).

There exists the possibility of formation of similar C_{24} isomers from lycopene which would yield mass spectra virtually identical to the C_{24} isoprenoid. For this reason the collection cuts were purified on Apiezon-L, just as with the cuts with possible C_{19} isomers. Only one major gas chromatographic peak was obtained. Although the M/e 323 fragment (P-15) shows decreased intensities, this compound (MS 13) has been identified as the regular C_{24} isoprenoid by comparison with the spectrum of Han and Calvin, comparative gas chromatographic retention times, and predicted fragmentation patterns.[12]


MS 13
100
50
10
100
200
300
M/E
P+
(338)

The regular C_{25} isoprenoid was also isolated from the still residue, the spectrum of which is shown as MS 14. This assignment is made on the basis of the correlation between the predicted fragmentation pattern (Fig. XX) and the predominant peaks of the spectrum, and on

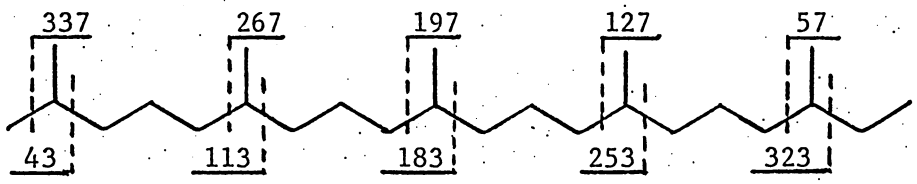
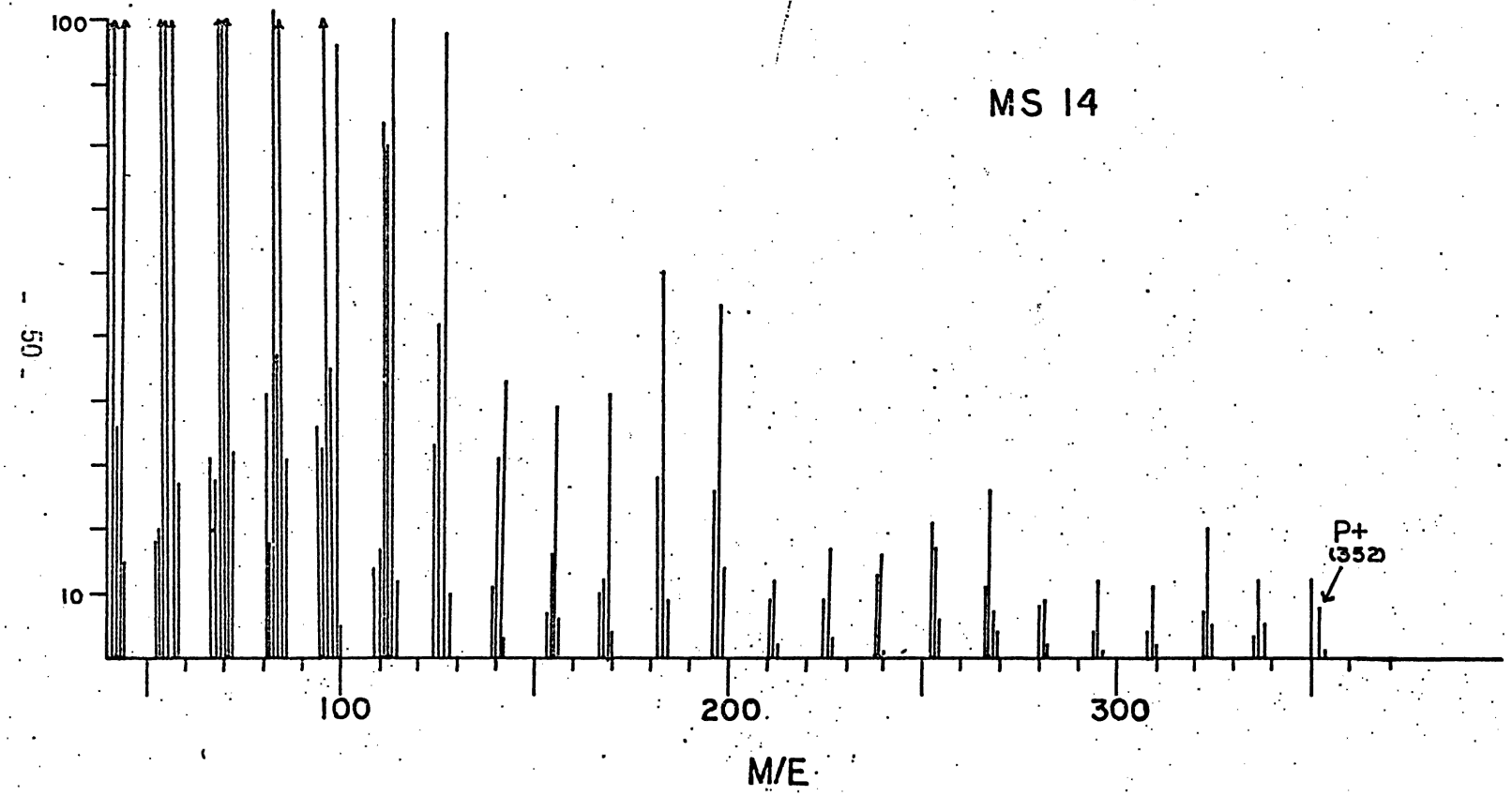
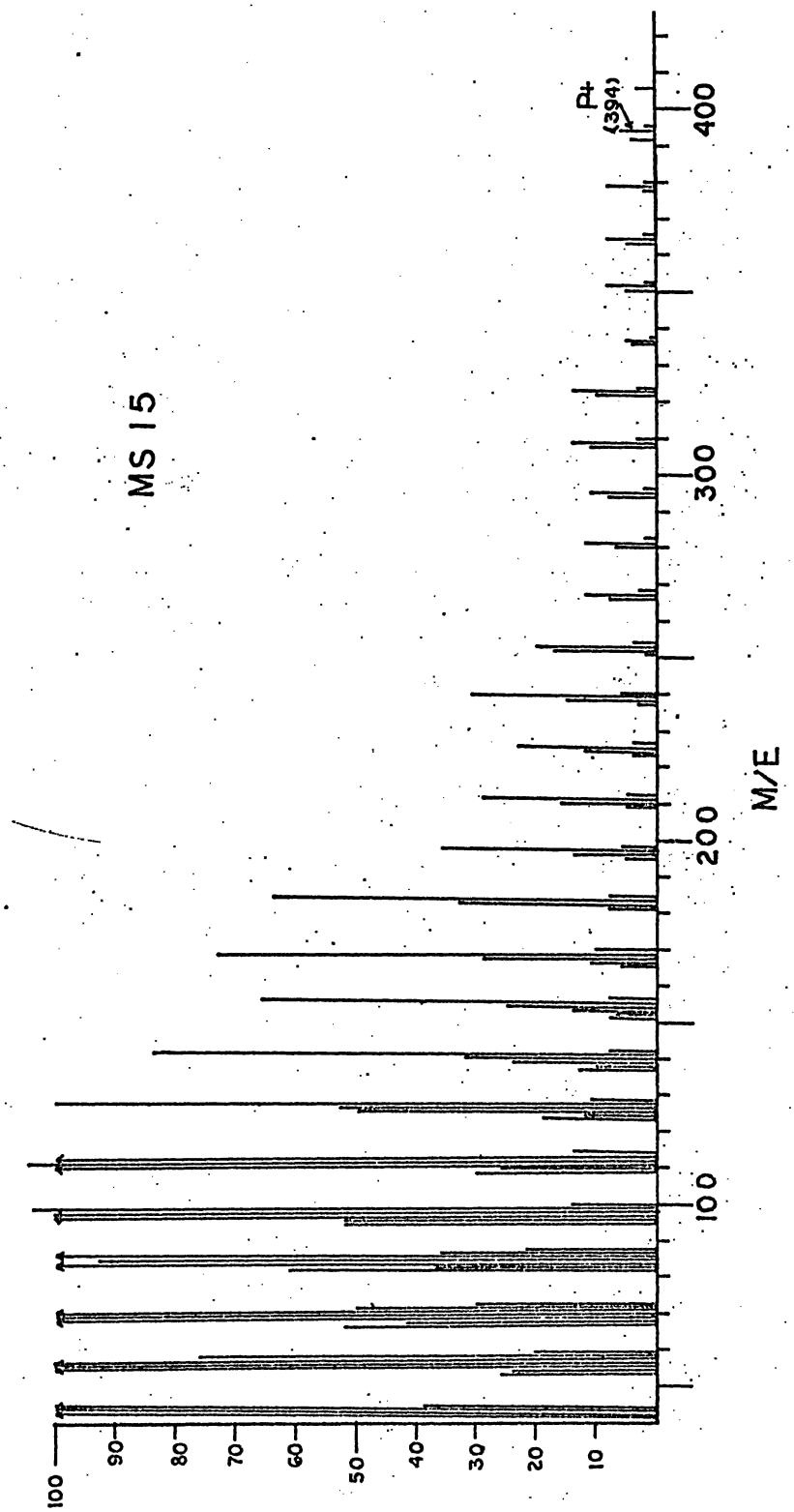


Fig. XX

the basis of comparison with spectra of the C_{25} regular isoprenoid isolated by Han and Calvin[12] and by Maples, et al.[32] Since any isomer formed from a possible irregular precursor (lycopene) would have a methyl branch at the 19-position, rather than the 18-position, its spectrum would differ appreciably from the regular C_{25} isoprenoid spectrum. The C_{23}, C_{24}, and C_{25} regular isoprenoids are present in very small quantities compared to the amount of pristane and phytane present (see Table II). Since the regular C_{23}, C_{24}, and C_{25} isoprenoids are present while the regular C_{22} and irregular C_{23}, C_{24}, and C_{25} isoprenoids are absent suggests a large (i. e., C_{25} or greater) regular isoprenoid as a precursor, rather than an irregular compound.

Although squalane has been reported in some sediments and crude oils,[8,13,16,19] it was not detected in this seep oil. However, two rather interesting compounds were found. MS 15 shows the mass spectrum of one of these, a C_{28} saturate (see Chromatograms I and II). The prominent peaks of the spectrum appear to form two fragmentation sequences: M/e 99-169-239-309-379, and M/e 113-183-253-323, with the parent ion at M/e 394, although the fragments at M/e 253, 309, and 323 are rather small. This spectrum can be rationalized as a C_{28} isoprenoid structure on the basis of the predicted fragmentation pattern shown in Fig. XXI:


MS 14
100
50
10
100
200
300
M/E
P+
(352)


MS 15
Pt+
(394)
400
300
200
100
M/E
100
90
80
70
60
50
40
30
20
10

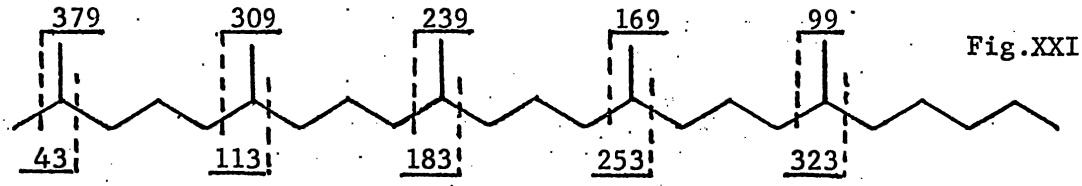
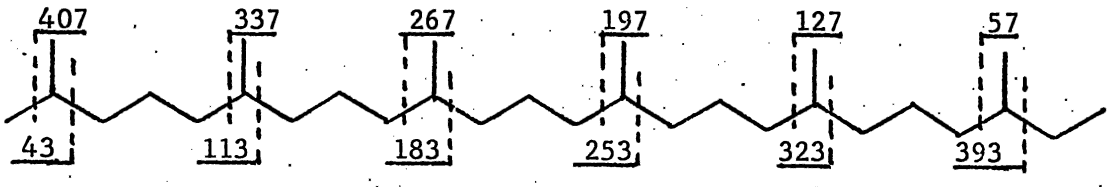


Fig.XXI

The presence of some impurities (as seen from the large 111 and 97 peaks), the formation of C_nH_{2n} ions, and the stabilization of the positive charge on the primary fragment when the fragments are very long all complicate rationalization of this spectrum.

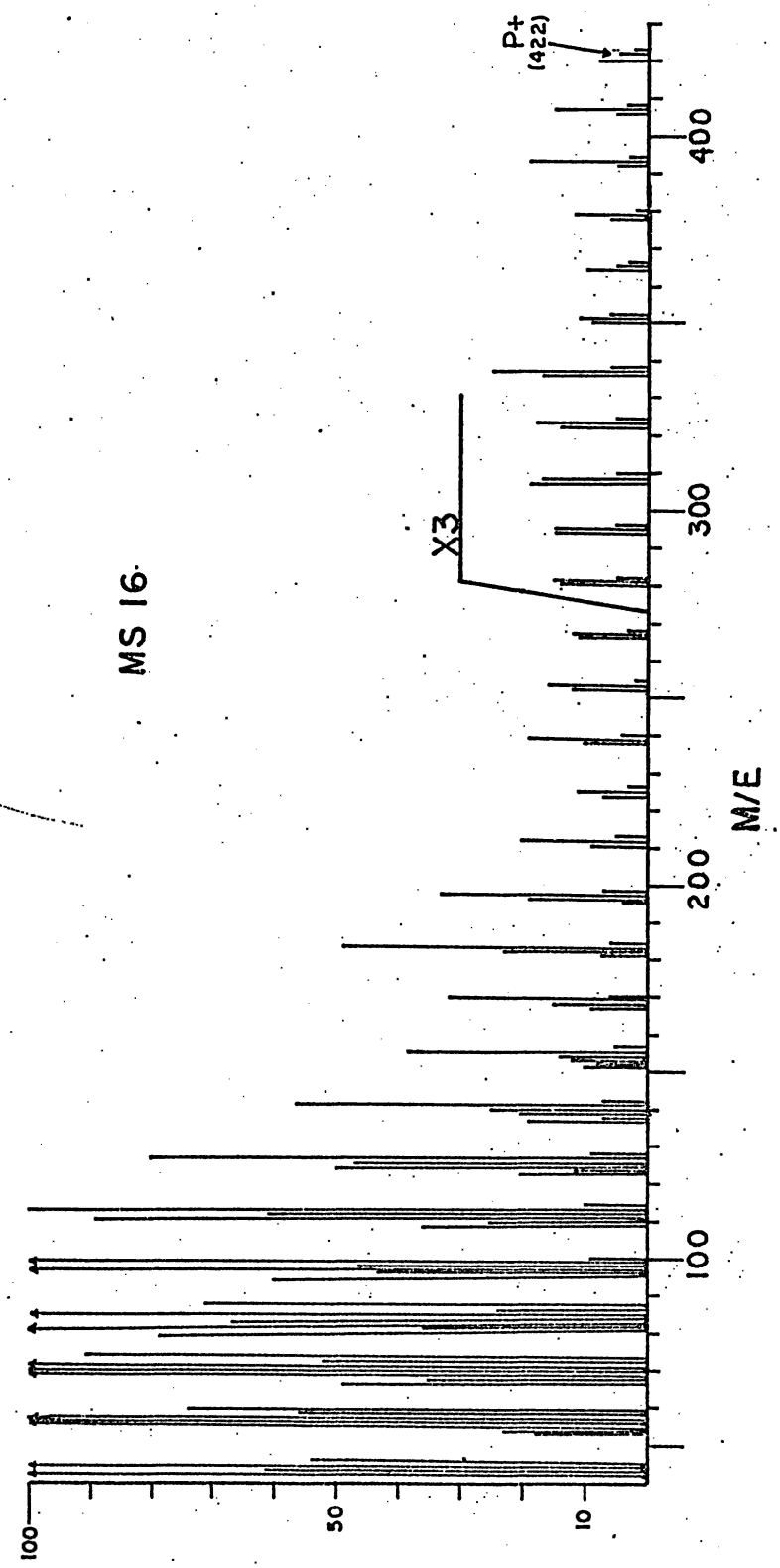
MS 16 is the spectrum of a C_{30} saturate also isolated from the still residue. The most striking feature is what appears to be a sequence of peaks at M/e 127-197-267-337-407 (the M/e 267 peak is fairly small, but the accompanying 266 peak is almost the same size). There is also what appears to be a sequence of M/e 113-183-253-323-393. Accepting these ions as secondary fragments resulting from cleavage at a methyl branch could lead to the rationalization of this spectrum as a C_{30} isoprenoid (Fig. XXII):

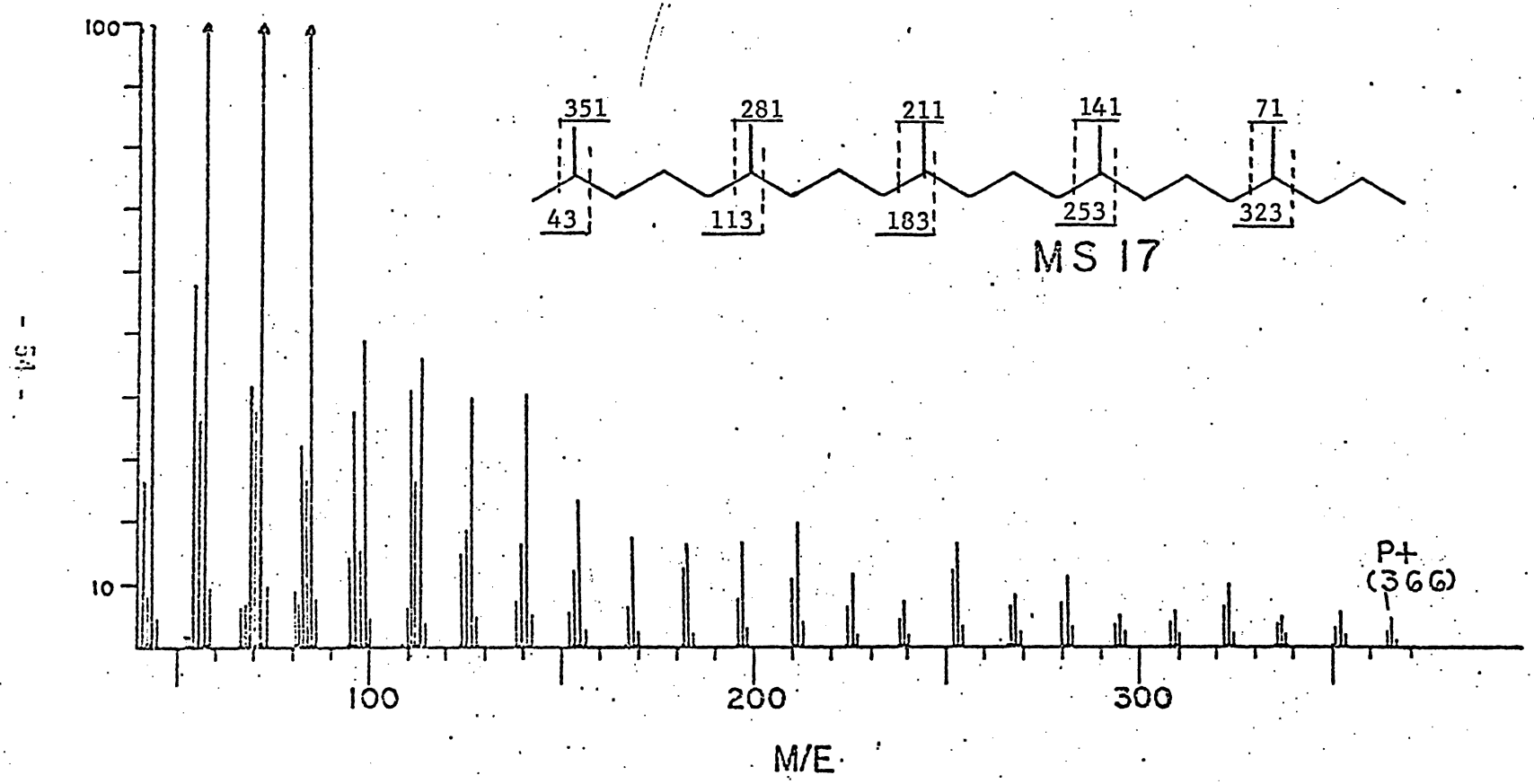
Fig. XXII



The large fragments at M/e 239 and 309 could be accounted for either as peaks from an impurity or as primary fragments which are carrying the positive charge instead of the secondary fragments (C_{13} and C_9).

A saturated C_{26} compound was also detected in the still residue that could possibly be the C_{26} isoprenoid. The mass spectrum is shown as MS 17. The only significant anomaly in this spectrum is the size of the M/e 183 peak. This fragment is an expected regular isoprenoid fragment and the intensity should be much higher than it is.


MS 16
P+
(422)
X3
100
50
10
100
200
300
400
M/E


100
10
351
281
211
141
71
43
113
183
253
323
MS 17
P+
(366)
100
200
300
M/E

However, the intensity of the M/e 183 fragment shown on the sample and PFK standard scan is what would be expected (the sample M/e 183 peak and PFK M/e 183 peak occurred as a discernible doublet). The lack of intensity may be due to the improper detector-response shown by the mass spectrometer on other parts of the scans.

The compounds that were detected and the quantities in which they were present suggest several interpretations. Pristane was the largest single component of the total Alkane Fraction (see Table II, Chromatogram I). Pristane could be formed from phytol (the suggested major precursor) by oxidation of phytol to phytanmic acid, followed by decarboxylation to the C_{19} olefin, which would then be reduced to the alkane. The ubiquity of phytol could account for the large amounts of pristane found in this and almost all other sediments. The large amounts of pristane detected argue very strongly against squalane as a significant precursor. As can be seen from Fig. XXIII, pristane

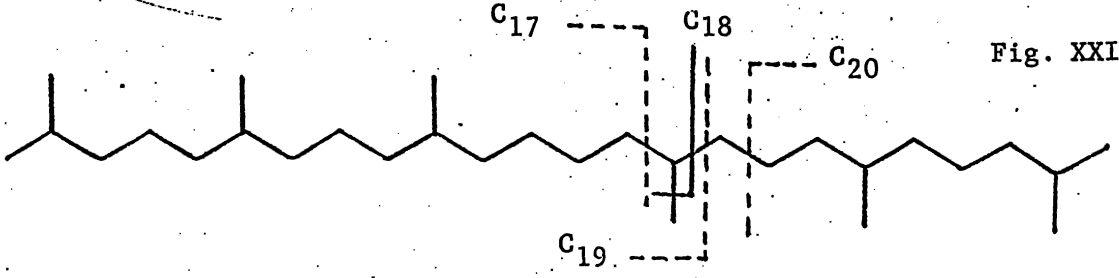


Fig. XXIII 13,22

could not be formed from squalane by a cracking pathway, hence some other precursor is necessary to explain the large amounts of fossil pristane. Pristane could be formed from lycopane (see below). However, the amount of lycopane in living organisms is too small to account for all the pristane; and no mechanism could account for the formation of pristane in preference to other compounds from lycopane.

The presence of sizable quantities of phytane also argue in favor of phytol and against squalane as a precursor. As can be seen from

Fig. XXVII (above) phytane could not be formed by degradation of squalane, an irregular C_{20} isomer (2-, 6-, 10-, 15-tetramethyl hexadecane) being formed instead. However, by a reduction-dehydration-reduction series of reactions under reducing conditions, phytol would be degraded to phytane. Nissenbaum et al.[24] have shown that if the initial oxidizing conditions are mild, sizable quantities of phytol would escape oxidation to pristane, and then could be reduced to phytane. Phytane could also be formed from lycopane. However, the same arguments applied to pristane could also be applied to phytane.

The large amount of phytane as compared to pristane (70% of the pristane amount) indicates that, if phytol is the precursor, then the seep oil has been subjected to relatively mild diagenetic conditions. If the initial reducing conditions had been severe, then most of the phytol would have been oxidized to pristane. The large amounts of phytane also suggest a relatively mild thermal history, since a substantial portion of the phytane has not been cracked to lower molecular weight compounds.

The presence of quantities of the C_{16} and C_{18} regular isoprenoids also suggest phytol as a precursor. These two isoprenoids are considered to be formed by cracking of larger isoprenoids since they are not found in any living organisms. As seen from Fig. XXIV, both isoprenoids could be formed by single bond cleavages at a branching site of phytane and pristane. From Fig. XXIII, above, it can be seen that

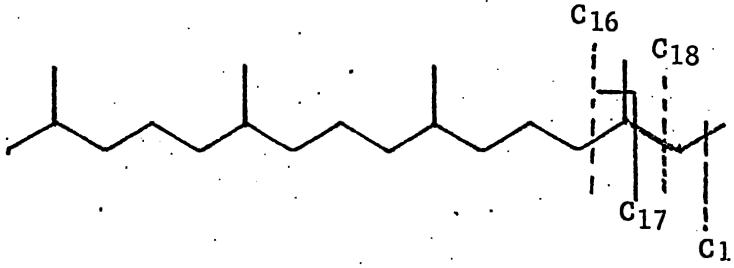


Fig. XXIV[13]

the C_{18} isoprenoid, the third most abundant branched alkane component, could not be formed from squalane by one bond cleavage. The absence of the C_{17} regular isoprenoid also argues in favor of phytol and against squalane as a precursor. If squalane were the precursor, the C_{17}, formed from one cleavage (Fig XXIII) should be present. If phytane (phytol) or another regular isoprenoid were the precursor, the amount of C_{17} isoprenoid would be very small since it could be formed only by cleavage of two bonds to the same carbon atom.

Besides its ubiquity in nature, the role of phytol, rather than a different regular isoprenoid (i. e., pristane) precursor is supported by the presence of the irregular C_{17} isoprenoid 3-, 7-, 11-trimethyl tetradecane. This compound would be formed by cracking of phytane at the end with the terminal isopropyl group rather than terminal ethyl group (Fig. XXV):

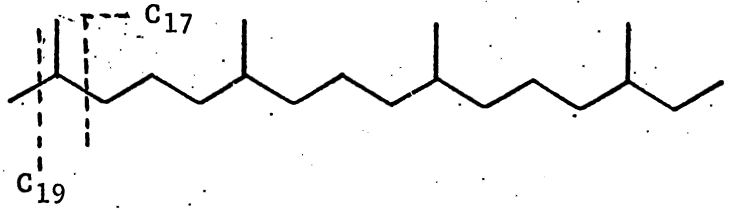


Fig. XXV

This mode of cracking would be expected for this oil because of the large amount of phytane present which could be cracked. Cracking of phytane at the ethyl group end would produce regular isoprenoids, while at the isopropyl group end cracking would produce these irregular isoprenoids. This would strongly indicate phytane (phytol) or possibly a C_{25} or higher regular isoprenoid ending in an ethyl group as a major precursor. The presence of three-, seven-, eleven-trimethyl tetradecane must be said to be only tentative since there is only the mass spectrum. Neither the C_{18} or C_{19} homologues of this

compound were definitely found. This is as expected, for the same reasons that would preclude regular C_{17} isoprenoid formation from phytane, while the C_{19} homologue is formed by loss of a methyl group, a process not nearly as favorable energetically as the loss of an isopropyl group (to form the C_{17}) or a tertiary butyl group (to form the C_{16} homologue: three-, seven-, eleven-trimethyl tridecane)[7]. The cracking of pristane, which would be as probable as the cracking of phytane, would produce regular isoprenoids no matter where the molecule cleaved.

The possible presence of the C_{19} analogue, 3-, 7-, 11-trimethyl hexane was also noted. This could be formed by the loss of one of the terminal methyl groups (see Fig XXV). The loss of the methyl group of phytane by diagenetic cracking would be less energetically favored than the loss of an isopropyl group,[7] hence the amount present would probably be quite small. Since pristane and three-, seven-, eleven-trimethyl hexadecane are so similar in structure, their retention times on the gas chromatographic columns used in the separation sequence would be similar. The proof of the presence of three-, seven-, eleven-trimethyl hexadecane would strongly indicate a precursor similar to phytane in its structure (i. e., a regular isoprenoid with multiples of complete 5-carbon units--C_{20}, C_{25}, etc.). The sample which may contain this C_{19} isomer may also contain one or more isomers resulting from the loss of a methyl group from the middle of the phytane chain. None of these compounds could be formed from squalane.

The absence of certain compounds would also argue against squalane as a precursor. As seen from Fig. XXIII, the cracking of squalane should produce, besides the regular C_{17} isoprenoid, the irregular iso-

mers 2-, 6-, 10-trimethyl hexadecane and 2-, 6-, 10-, 15-tetramethyl hexadecane rather than pristane and phytane. None of the other possible cracking products of squalane, and no squalane itself, were detected. Hence it is likely that phytol, rather than squalane, is the principle precursor of the isoprenoids below C_{20}.

It is possible that another regular isoprenoid may be the precursor. However, none of the possible precursors, except pristane (in plankton), is sufficiently widespread in nature to account for the amounts of pristane and phytane found. Additionally, no mechanism could account for the formation of pristane and phytane in preference to the other isoprenoids. Pristane could be a likely precursor except for the presence of large amounts of phytane and the presence of the irregular 3-, 7-, 11-methyl branched homologues.

The presence of some of the regular isoprenoids from C_{21} to C_{30} implies that there is another precursor for the isoprenoids. Because of the drastic decrease in the amount of isoprenoids above C_{20}, as compared to pristane and phytane, it is probable that the bulk of the pristane, phytane, C_{18}, and C_{16} isoprenoids came from phytol (see above). All the higher molecular weight isoprenoids (C_{21}--C_{30}) were regular. No trace was found of irregular isomers or of the C_{22} regular isoprenoid. As seen from Fig. XXVI:

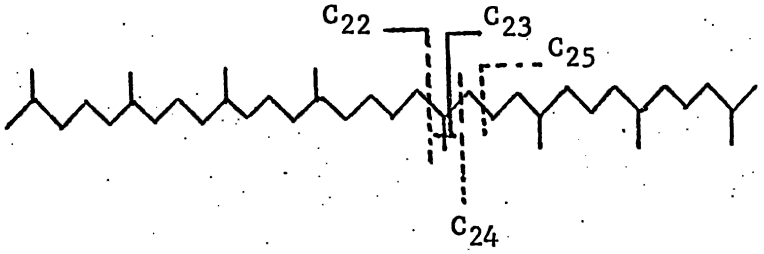


Fig. XXVI

if lycopane or a similar irregular isoprenoid were the precursor, the C_{22} regular isoprenoid and the irregular isomers 2-, 6-, 10-, 14-trimethyl eicosane and 2-, 6-, 10-, 14-, 19-pentamethyl eicosane should be formed by cracking of the molecule. The regular C_{23} isoprenoid could be formed only by cleavage of two bonds to the same carbon atom, and the C_{25} and larger regular isoprenoids could not be formed.

The presence of the regular isoprenoids (except C_{22}) and the absence of the irregular isomers, indicate that the precursor was a regular isoprenoid compound. There have been several reports of large isoprenyl alcohols occurring in living organisms (see below), including in bacterial cell walls.[5,15,23] Since isoprenyl alcohols are the most common regular isoprenoids above C_{20}, and since they could be degraded to isoprenoid hydrocarbons by the same processes as degrade phytol (oxidation-decarboxylation-reduction; oxidative cleavage; or reduction-dehydration-reduction).

SUMMARY

From the results discussed above, several interesting points are immediately discernible regarding the precursors of the isoprenoid alkanes in this sample.

From the data obtained, it appears quite probable that phytol is the precursor of the isoprenoids below C_{21}. Phytol is much more ubiquitous in nature (being found in most chlorophylls) than the other possible isoprenoid precursors. The absence of only the regular C_{17} isoprenoid, not only in this case, but in almost all other instances, would seem to indicate that the precursor has a regular isoprenoid structure. Since the C_{17} isoprenoid could be formed only by cleavage of two carbon-carbon bonds to the same atom in a regular isoprenoid, but by only one cleavage in an irregular structure (squalane), its absence, when the other isoprenoids are present in appreciable amounts would indicate a regular structure, i. e., phytol, as a precursor (see Fig. XXIII and XXIV above). That phytol is the precurser, and not a higher weight compound, is further supported by the drastic decrease in the amounts of isoprenoids above C_{20} as compared to the amounts of pristane and phytane. If a higher weight isoprenoid was the precursor, then a gradual change in the relative amounts of the various isoprenoids would be expected rather than the dramatic drop in the amount seen here. (See Table II). The tentative identification of three-, seven-, eleven-trimethyl tetradecane and the possible presence of three-, seven-, eleven-trimethyl hexadecane also argue for a phytol precursor. Since the considerable quantities of C_{16} and C_{15} isoprenoids present in this oil indicate significant hydrocarbon cracking after degradation of the alcohol group and reduction, molecules should be formed as a result of

cracking at the other end of the phytane molecule from the former location of the alcohol group. As seen from Fig. XXV, this is a possible synthesis of three-, seven-, eleven-trimethyl tetradecane and related compounds, and would indicate phytol as an original precursor.

If squalane was a significant precursor, some trace of it should be expected to apear in the sample since it is a hydrocarbon and hence geochemically stable. However, no detectable amount of squalane was found. Likewise, no detectable amounts of either the regular C_{17} isoprenoid, or the C_{19} isomer two-, six-, ten-trimethyl hexadecane were found. This also argues against a squalane precursor since, as seen from Fig. XXIII, they would be formed from squalane along with the other isoprenoids.

The determination of the nature of the precursors of these isoprenoids must account for the presence of the C_{23}, C_{24}, C_{25}, C_{28}, and C_{30} isoprenoids. The presence of these isoprenoids, though in small amounts, indicates a precursor of higher molecular weight than phytol was also present. This isoprenoid precursor, from the evidence available, appears to be a regular isoprenoid structure. This is indicated by the absence of the C_{22} regular isoprenoid and the C_{24} isomer two-, six-, ten-, fourteen-tetramethyl eicosane, and the presence of the regular C_{23} and C_{24} isoprenoids, which, just as in the discussion of regular-irregular precursors for C_{19}, indicates a regular structure to the isoprenoid precursor. (See Fig. XXVI above). This argues against lycopene and similar compounds as precursors.

There are several higher molecular weight isoprenyl alcohols which could act as precursors for the higher molecular weight isoprenoids. Nozoe et al. have isolated geranylnerolidol,[25] a C_{25} alcohol,

from a phytopathogenic fungus, and Rios and Perez have found geranylfarnesol in insect wax.[26] However, because it appears that significant diagenetic cracking occurred in this sample and because of the possible presence of isoprenoids larger than C_{25}, it is conceivable that an isoprenyl alcohol of C_{30} or larger was the precursor. Solanesol (a C_{45} alcohol) has been isolated from tobacco leaves,[27] a series of betulaprenols (C_{30}--C_{45} alcohols) have been found in birch wood,[29] a C_{55} isoprenyl alcohol in three species of Lactobacillus,[31] and several other large isoprenyl alcohols have also been detected in living organisms.[5,14,15,33] As in the case of phytol, these high molecular weight isoprenyl alcohols could form isoprenoid alkanes by initial degradation of the alcohol end, followed by reduction and cracking processes.

Hence, from the data, it appears that phytol is the primary precursor of the lower molecular weight isoprenoids (C_{15}--C_{20}) in this sample. There is also another precursor, for the higher molecular weight isoprenoid alkanes, which is of regular isoprenoid structure, probably one or more isoprenyl alcohols of C_{25} in size or greater.

POSSIBILITIES FOR FUTURE STUDY

The results of this program suggest several interesting and potentially important lines for further research.

The most important of these is the pursuit of the search for isoprenoid alkanes above C_{25}, including confirmation of the C_{28} and C_{30} structures. These compounds have not been examined (though squalane has been discovered), and their discovery would do much to shed light upon the role of high molecular weight regular isoprenyl alcohols (C_{25} and above) in the formation of isoprenoids. These alcohols, though heavily unsaturated, would still be expected to undergo much the same reactions as phytol, hence, if they do play a part in isoprenoid synthesis, isoprenoids of molecular size greater than C_{25} should be present.

Another line of potentially important inquiry is the confirmation of the existence of the isoprenoid-like molecules that terminate on one end with an ethyl, rather than a methyl group (i. e., 3-, 7-, 11-trimethyl tetradecane and its homologues.) This homologous series would be expected to be cracked from phytane in the same manner that the C_{18} and C_{19} isoprenoids are formed, though in lesser amounts. In this and in most other oils and sediments, the three-, seven-, eleven-trimethyl tridecane homologue, which would fall in the C_{16} isoprenoid range, would be more abundant (due to the loss of a tert-butyl group) than the C_{17} homologue already tentatively identified. The positive identification of two or more elements of this homologous series in several different samples would provide excellent evidence for phytane (phytol) as a major precursor as well as provide insight into the diagenetic mechanisms by which the lower weight isoprenoids are derived

from their precursors.

A third important line of search is the detection of the irregular isoprenoids (such as 2-, 6-, 10-trimethyl hexadecane; 2-, 6-, 10-, 15-tetramethyl hexadecane; and 2-, 6-, 10-, 14-tetramethyl eicosane). Their detection is of special importance (and possibility) in those samples in which the regular C_{17} isoprenoid (or the regular C_{22} isoprenoid) is found, since the detection of the irregular isomers with these two regular isoprenoids would strongly point to squalene and/or lycopene as isoprenoid precursors. However, the detection of the irregular isomers is difficult due to their similarities to the regular isoprenoids, especially the resemblance between pristane and two-, six-, ten-trimethyl hexadecane and between the regular C_{24} isoprenoid and two-, six-, ten-, fourteen-tetramethyl eicosane.

TABLE I

Composition of Distillation Fractions

Fraction	Color	B.P. (@.005mm Hg)	% Branched
24	colorless	70-75	79
25	pale yellow	75-80	88
26	pale yellow	80-85	80
27	colorless	85-90	74
28	yellow	90-95	76
29	yellow	95-100	75
30	yellow	102	85
still residue	colorless	---	82

Composition of Column

Chromatography Fractions
(from total sample)

% Hydrocarbons (from Alumina Column)	34
% "Parafin Concentrate"* (from Silica Gel Column)	26
% "Parafin Concentrate" of Hydrocarbon Fraction	76

* "Parafin Concentrate" is the alkane-cycloalkane fraction of the Hydrocarbon Fraction from Alumina chromatography.

TABLE II

Approximate Relative Amounts of Isoprenoid Components [Pristane = 1.00]

C_{16}	0.52
C_{18}	0.64
C_{19}	1.00
C_{20}	0.66
C_{21}	0.14
C_{23}	0.07
C_{24}	0.06
C_{25}	0.04
C_{26}	0.05

Approximate Amounts of Isoprenoid Components [% of Branched Alkane-Cyclo-alkane Fraction]

C_{16}	3.0%
C_{18}	3.6%
C_{19}	5.7%
C_{20}	3.8%
C_{21}	0.8%
C_{23}	0.5%
C_{24}	0.3%
C_{25}	0.2%
C_{26}	0.3%
Total	18.2%

TABLE II
(continued)

DISTILLATION DATA
[from Esso Research*]

Chromatographic Separation

Fraction	% of Oil
Benzene sol. asphaltenes	0.5
Benzene insol. asphaltenes	2.1
Saturate	37.4
Aromatic	18.8
Recovered NSO**	0.4
Non-recovered NSO	2.3

(** NSO is Nitrogen-Sulfur-Oxygen compounds)

Mass Spectrometry Data

Naphthenic Analysis

Para	1-R	2-R	3-R	4-R	5-R	6-R	Mono-aromatic
19.4	24.6	19.1	13.	13.6	6.2	3.2	0.0

Other Data

Seep oil sample was collected from near San Antonio, Costa Rica.

High temperature Distillation yielded three fractions:
50-200 , 37.2 %; 200-300 , 26.1%; 300-350 ,17.9%

Bulk of Isoprenoid Components were shown by analysis to be in Fraction 2, indicating that most of the sample has a carbon number approximating 20.

[* courtesy of Pat Monaghan of Esso Research Corporation]

TABLE III

OFF-SCALE INTENSITIES

MS	41	42	43	55	56	57	69	70	71	83	84	85	97	98	99	111	112	113
1	-	-	108	-	-	121	-	-	100	-	-	-	-	-	-	-	-	-
5	-	-	300	-	-	406	-	-	348	-	-	192	-	-	-	-	-	-
6	-	-	100	-	-	off	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-
8	210	-	445	172	160	625	-	-	496	-	-	200	-	121	-	-	-	100
9	119	-	307	104	-	386	-	-	325	-	-	114	-	-	-	-	-	100
10	142	-	283	115	100	376	-	-	266	-	-	161	-	-	-	-	-	-
11	388	109	1022	464	245	880	209	185	561	-	-	343	-	-	-	-	-	100
12	236	-	643	176	140	855	161	105	480	-	-	280	-	-	109	-	-	100
13	129	-	315	185	149	611	253	-	430	-	-	218	-	-	-	-	-	100
14	165	-	236	196	192	off	128	112	off	101	-	303	109	-	-	-	-	100
15	501	106	821	600	238	804	470	172	494	300	-	312	271	111	104	112	105	122
16	264	-	400	348	180	719	250	129	521	140	-	324	139	-	113	-	-	100

TABLE IV

GAS CHROMATOGRAPHIC CONDITIONS
INITIAL SEPARATIONS

FRACT.	COLUMN	FLOW RATE [ml/ min]	PRESSURE [PSIG]	INITIAL TEMP. [-min. isotherm.]	PROG. RATE [deg./min.]
24	PPE*	37.5	50	150-2.5 min	10
25	Polysev**	40	50	175- 3 min	6
26	Polysev	37.5	50	183-3.5 min	6
27	Polysev	46	50	187- 5 min	8
28	Polysev	37.5	50	210- 0	2
29	Polysev	37.5	50	214- 0	2
30	PPE	37.5	50	219- 0	2
Still Residue	SE-30	40	50	201- 0	6
Total Branched Alkanes	SE-30	43	50	180-5 min	12

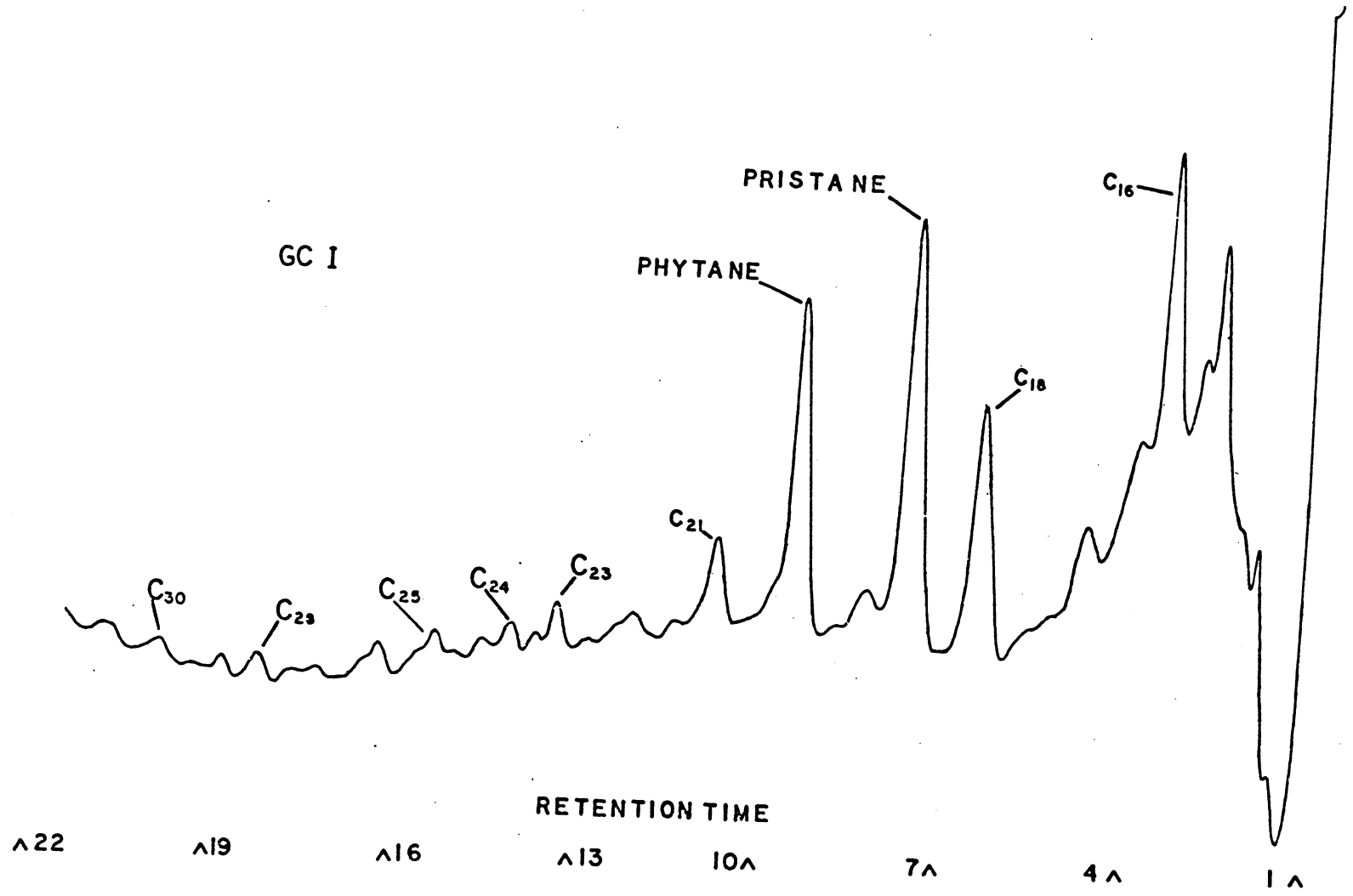
[* 6 ring meta-polyphenyl ether]

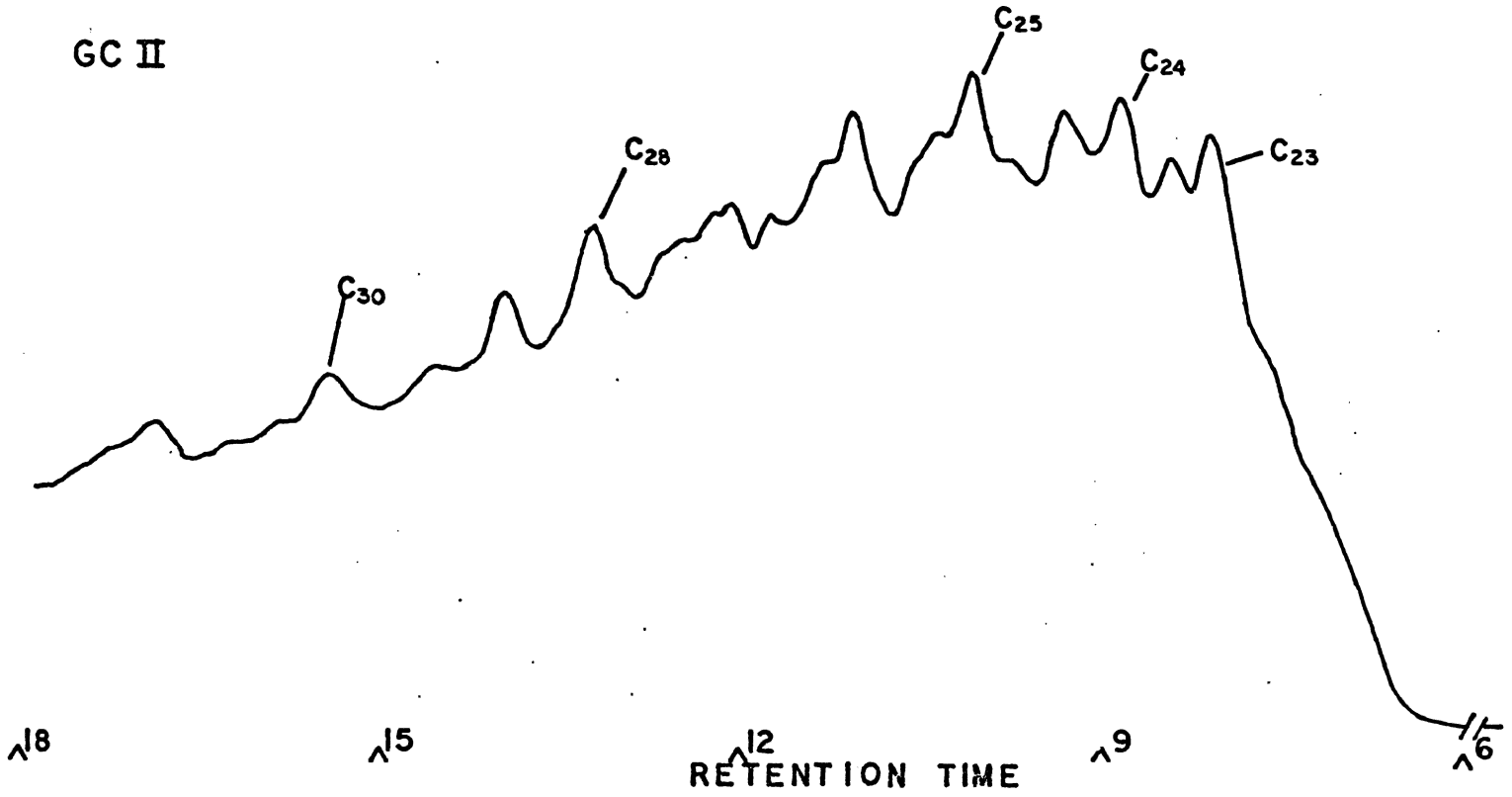
[** 7 ring meta-polyphenyl ether]

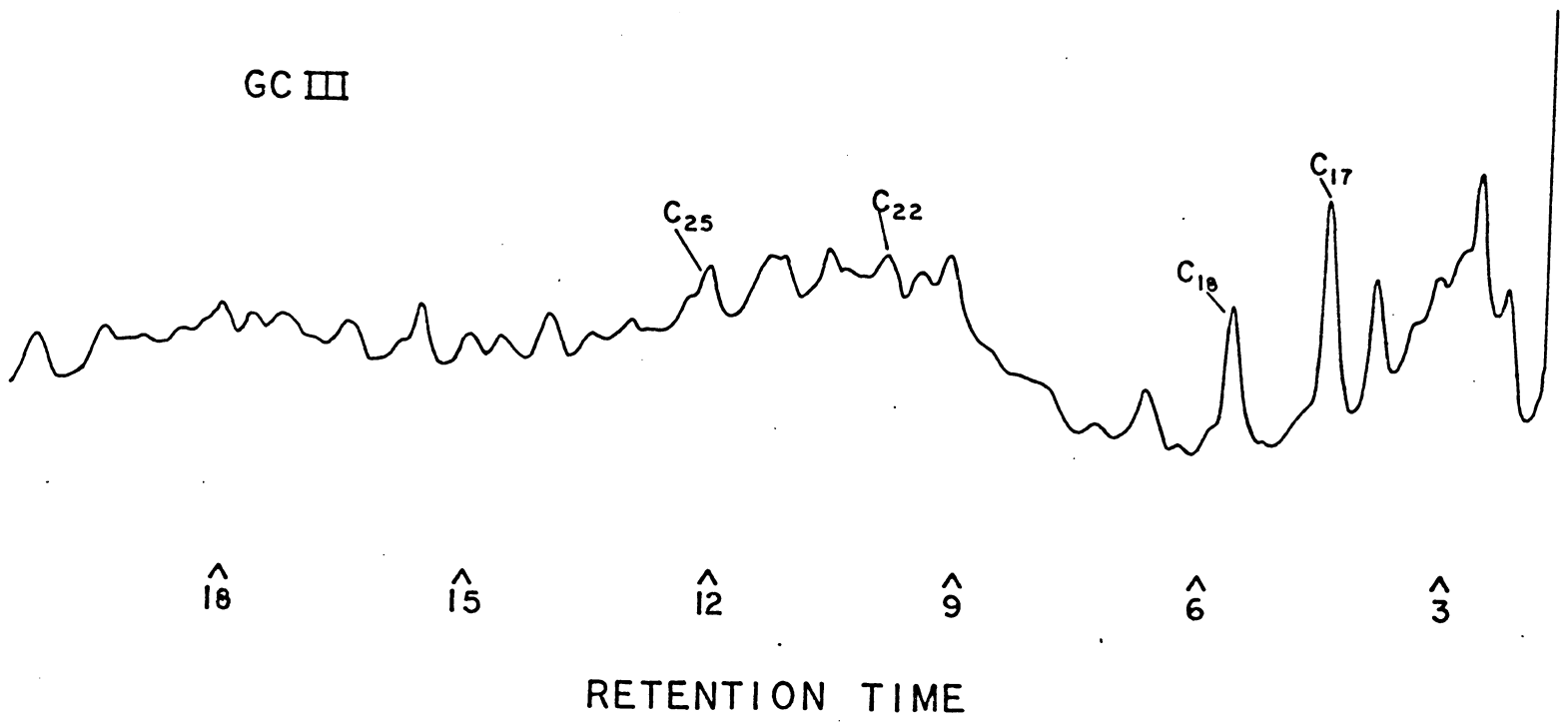
TABLE V
OTHER COMPOUNDS DETECTED IN THE SAMPLE

No. Carbon Atoms: No. Unsat. Sites (M/e)

Still Residue	30	29	28	27	26	25	24
26:2 (362)	20:1 (280)	20:1 (280)	19:2 (264)	20:1 (280)	19:1 (266)	19:2 (264)	17:0 (240)
30:1 (420)	21:2 (292)		20:2 (278)	19:0 (268)	18:1 (252)	18:1 (252)	17:0 (240)
31:1 (434)	21:1 (294)		20:1 (280)	18:2 (250)	18:1 (252)	19:1 (266)	18:1 (252)
31:2 (432)			22:1 (308)	17:2 (236)	18:0 (254)	18:2 (250)	19:1 (266)
22:1 (308)			21:1 (294)	16:1 (224)	18:1 (252)	18:3 (248)	19:3 (262)
23:1 (322)			20:1 (280)	18:0 (254)		17:2 (236)	18:2 (250)
23:2 (320)			20:3 (276)	18:1 (252)		17:3 (234)	18:1 (252)
22:2 (306)			20:2 (278)	19:2 (264)		19:3 (262)	17:1 (238)
22:3 (304)			19:1 (266)	20:1 (280)		19:4 (260)	18:1 (252)
25:1 (350)			19:2 (264)	19:1 (266)		18:3 (248)	24:6 (340)
24:1 (336)			18:1 (252)	17:1 (238)		18:4 (246)	18:3 (248)
			19:1 (266)	18:1 (252)		19:5 (258)	18:4 (246)
			20:2 (278)	17:0 (240)		16:2 (222)	19:2 (264)
			20:1 (280)	18:0 (254)		18:0 (254)	17:1 (238)
				20:1 (280)		17:0 (240)	18:3 (248)
				19:1 (266)		19:2 (264)	18:0 (252)
				18:1 (252)			17:0 (240)
				19:1 (266)			
				18:2 (250)			


GC I
PRISTANE
PHYTANE
C16
C18
C21
C23
C24
C25
C28
C30
RETENTION TIME
22
19
16
13
10
7
4
1


GC II
C25
C24
C28
C23
C30
18
15
12
9
6
RETENTION TIME


GC III
C25
C22
C18
C17
18
15
12
9
6
3
RETENTION TIME

FOOTNOTES

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