APPLICATION OF COMBINED GAS CHROMATOGRAPHY-MASS SPECTROMETRY TO THE ANALYSIS OF ORGANIC PRODUCTS OF BIOGEOCHEMICAL SIGNIFICANCE

A Dissertation

Presented to the

Faculty of the Department of Chemistry

College of Arts and Sciences

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

by

Emilio Gelpi-Monteys

August, 1968

a Esperanza

a mis padres

y a quien, hace años, tanto influyo en mi forma de pensar con la frase, "de aquellos que mucho recibieron mucho sera exigido."

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ABSTRACT

A large number of organic products of diverse origins have been studied by gas chromatography and by combined gas chromatography-mass spectrometry. This combination technique allows the rigorous structural characterization of the individual compounds present in multicomponent mixtures. Its application to the determination of bioorganic lipids provides data on the biological order at the molecular level. Thus important correlations, relevant to the distributions, origins and biosynthetic pathways of the compounds, can be established.

Among the lipids, the hydrocarbons and fatty acids have been the most extensively studied but in general the interest has been centered around the isoprenoid hydrocarbons because of their structural specificity and diagenetic stability. Also the characteristic bimodal distribution of the isoprenoids in natural products was found to be in line with their presumed biological origin.

The correlations and distributional criteria established as a result of this investigation are based on the following observations: Microorganisms contain significant amounts of aliphatic hydrocarbons and fatty acids. Branched paraffins belonging to the iso and anteiso series have been identified in a few cases. In general, isoprenoids are absent. All of the microscopic algae analyzed contain high amounts of n-C_{17} and in two instances, high molecular weight olefins (C_{17}-C_{33})

were found to be present in relatively high proportions. Several isomeric unsaturated isoprenoids were found in higher plants although their saturated counterparts are absent. High amounts of isoprenoids (pristane and squalene) were found in shark liver oil. The saponifiable fraction contains mainly normal unsaturated fatty acids. In general the hydrocarbons and fatty acids of all these products are unrelated in their distributions. Relatively large amounts of isoprenoids from the C_{14} to C_{21} as well as of n-alkanes were identified in a crude oil. The sample contains too, iso and anteiso alkanes and cycloalkanes. Paraffins, olefins, and methyl alkanes ranging from C_{10} to C_{33} were identified in a concentrate of tobacco smoke. The isoprenoids in this sample are mainly unsaturated. Terrestrial graphites contain n-alkanes, small amounts of methyl alkanes and isoprenoids from C_{14} to C_{22}. The outside parts of the graphite contain more hydrocarbons than the inner parts. The possibility of abiotic synthesis of isoprenoids has been investigated using the Fischer-Tropsch reaction between hydrogen and carbon monoxide. Although n-alkanes and methyl branched isomers have been obtained in the products of more than 50 synthesis experiments, no traces of isoprenoids are present.

A detailed study of a number of hydrocarbons observed in gas chromatographic analyses of six carbonaceous chondrites has resulted in the identification of nine homologous series

of isomeric alkanes in addition to the n-alkane series. Two of them show isoprenoid structures corresponding to 2,6,10-trimethyl and 2,6,10,14-tetramethyl alkanes, with members in the C_{14}-C_{19} and C_{19}-C_{21} ranges, respectively. The rest is made up of five series of monomethyl alkanes (6-,5-,4-,3-, 2-methyl alkanes) and two of monocycloalkanes (cyclohexyl and cyclopentyl). An identical study of the graphite-troilite nodules of three iron meteorites yielded results qualitatively very similar to the carbonaceous chondrites. Nine homologous series have been identified mass spectrometrically: the n-alkane series (C_{13} to C_{26}); the 2-,3-,4-,6-methyl alkane series; the two isoprenoid series, 2,6,10-trimethyl and 2,6,10,14-tetramethyl alkanes (C_{16} to C_{19} and C_{19} to C_{21}, respectively), and two series of monocycloalkanes (cyclohexyl and cyclopentyl). The internal parts of the nodules show (1) smaller amounts of hydrocarbons (from three to seven times less than the surface), and (2) an increase in the ratio of isoprenoids to normal alkanes.

Isoprenoids are present in all meteoritic samples analyzed. Usually they show a major maximum at C_{19} (pristane) and a secondary maximum at C_{16} (methyl farnesane) with a minimum at C_{17}. Comparative studies of isoprenoid distributions typical of meteorites show a much greater degree of correlation with their distributions in sediments and petroleum crudes than with those produced by continuous flow Fischer-Tropsch processes.

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I.

STATEMENT OF THE PROBLEM

STATEMENT OF THE PROBLEM

A. Gas Chromatography-Mass Spectrometry and Its Application to the Analysis of Natural and Synthetic Products

The combined performance of a technique such as gas chromatography and its highly selective separatory power, with a mass spectrometer which is at present the most sensitive source of structural information available to the organic chemist, has opened a new era in the field of lipid analysis.

In theory the applications of this new analytical technique should be as many and varied as those of gas chromatography. In a pure analytical sense the purpose of this study was twofold; (1) to apply the new gas chromatographic-mass spectrometric methods to the separation and determination of the organic compounds present in complex mixtures of natural and synthetic origin, and (2) to secure in all cases reliable mass spectrometric identifications of these components. To achieve this it is necessary to understand the mass spectral characteristics of the compounds under study, group them according to their common fragmentation patterns and establish the necessary empirical correlations that will later serve as guidelines in the interpretation of future data.

B. Organic Geochemistry of Terrestrial and Extraterrestrial Products

The knowledge gained in this way about the nature and

exact composition of the bio- and geochemical samples studied has been used in trying to develop our present understanding the organic geochemical problems, such as those concerning the origin of petroleum, and in general, the processes of chemical evolution within the geological environment. A rigorous structural characterization of the hydrocarbons in extraterrestrial samples was also carried out in order to clarify the uncertainties about their origin. In this context, the role of abiotic synthesis of organic matter has to be considered and experiments have been carried out in this direction.

C. The Role of the Isoprenoid Hydrocarbons in the Biological Record of Life Events

The search for the origin of life either of terrestrial or extraterrestrial origin has been based on the characterization of structural order at the molecular level by means of the combined technique of gas chromatography and mass spectrometry. In this connection the presence, distribution and unequivocal identity of the normal and isomeric alkanes, especially the isoprenoid hydrocarbons, has been thoroughly investigated since these compounds usually have been considered to be "biological markers" of earlier life processes. Their possible abiogenic origin has also been given a detailed consideration.

II.

INTRODUCTION

INTRODUCTION

A. Gas Chromatography-Mass Spectrometry Combination. Development and Applications

The operation of a gas liquid chromatograph with a mass spectrometer offers today a powerful tool for the identification of multicomponent mixtures of lipid materials. By connecting the output of a gas chromatograph to a mass spectrometer each one of the chromatographically-resolved components of the original mixture can be studied individually in the mass spectrometer which can provide the kind of structural information necessary for their characterization. Thus, the inherent limitations of gas chromatography in qualitative analyses are in this way effectively removed. These limitations arise from the fact that while gas chromatography can separate substances with a high degree of resolving power it can not give more than preliminary information on their structure. In a rigorous sense the gas chromatographic columns are not analytical instruments but only a means of separation. The detectors associated with them in general respond only to the quantity of the sample being eluted from the column. The use of gas chromatographic retention data may be adequate for the tentative identification of unknown mixtures of simple and unrelated structures but when this methodology is carried to the analysis of complex hydrocarbon mixtures of very

closely related isomers, the results obtained lack the necessary qualitative reliability.

The theoretical and experimental background to the gas chromatographic techniques is discussed in a number of books (274,390,437). The theory and practice of mass spectrometry has also been discussed in several books (40,42,76-78,206, 293,395) and reviews (41,43,57,127,213,327,346). Nevertheless a brief summary of the present knowledge of organic mass spectrometry will be appropriate in the context of this study.

The sample subjected to mass spectrometric analysis is positively ionized at low pressure (10^{-5}-10^{-7} mm Hg) by electron bombardment with low energy electrons (12-100 eV).

The positively charged molecular ions then undergo unimolecular dissociations and rearrangements to form a characteristic array of positively charged fragment ions. According to the statistical theory of mass spectra first proposed by Rosenstock et al. (405) the molecular ion does not dissociate immediately. It first undergoes a random distribution of the excitation energy (electronic and vibrational energy) among its internal degrees of freedom. This takes place by a series of rapid, radiationless transitions between the various electronic states. Then it dissociates into fragment ions which correspond to transition states where the energy concentrated is enough to cause decomposition at those particular sites, "weak points", in the molecule. These

fragment and molecular ions are accelerated in a narrow beam out the ionization chamber and under the influence of a sweeping magnetic field are sorted out into their mass to charge ratios (m/e) according to the relation:

$$\frac{m}{e} = \frac{r^2H^2}{2v}$$

where r is the radius of curvature of the magnetically deflected beam, H is the magnetic field strength and v the accelerating potential. The results, recorded either by an electron multiplier or by means of a photoplate, are presented as a "mass spectrum" in which the relative abundances of each m/e ion are plotted versus their respective m/e values. These results can be recalculated either as the ratios of the intensity of each m/e to the largest peak, which is given the arbitrary value of 100, or as the percentage of the total ion intensity. From the m/e of the molecular ion, the molecular weight can be obtained, and from the distribution of fragments, information on chain lengths, branching position, and ring structures can also be obtained. Since the mass spectrometer is capable of providing this kind of information on a particular sample even when it is only available in quantities of the order of 10^{-6} to 10^{-8} g, it is especially suited for the direct analysis of the effluents from a gas chromatographic column.

The combination technique is nowadays in a state of rapid development and there is a considerable amount of literature on the use of direct introduction of gas chromatographic eluates into a mass spectrometer (44,73, 83,84,115,116,129,142,143,145,146,152,169-171,176-178,191, 192,231,260,265,267,271,281,282,286,288,289,320,348,350,352, 358,363-369,371,391,411,413,418,424,436,437,462,472,474,476, 480,482,500,501,506,507) including several reviews of the subject (265,286,437).

In connection with this combination technique, it is important to realize that the very low pressure (10^{-6}-10^{-8} mm Hg) required for the optimum performance of a mass spectrometer imposes a restriction to the gas flow entering the ion source. For this reason the flow into these instruments is limited to 0.2 ml per minute at most or about 10% of the flow rate of a capillary column, if the pressure inside the ion source is to be maintained within the critical range. One way to achieve these conditions is by diverting approximately from 0.3 to 1% of the total effluent of packed chromatographic columns into a rapid scan Time-of-Flight mass spectrometer (135,176, 271), or alternatively, if capillary columns are used with a bore small enough to restrict the total gas flow to about 1% of that in larger bore packed columns, then the total effluent can be directly introduced into a TOF mass spectrometer (288). In any case the instrument sensitivity is considerably

reduced due to the "dilution effect" of the sample in the carrier gas within the ion source. Thus it is more desirable to pump away most of the carrier gas before it reaches the ion source. In other words, the higher the sample to carrier gas ratio, the higher the sensitivity. For this reason direct sampling can only be used effectively in connection with instruments of very high sensitivities. Fime-of-Flight mass spectrometers have often been used for the direct sampling of GLC eluates (135,176,177,217,288).

From a practical point of view the combination of GLC and MS is practically made possible by means of the preferential removal of the carrier gas before it reaches the ion source of the mass spectrometer, without disturbing the sample. There are at present five different techniques to perform this separation: (1) the jet-molecular diffusion system (411,412, 418), (2) the fritted glass tube (506), (3) the heated porous Teflon tube (273), (4) a heated thin organic film (269) and (5) porous metal membranes (111). Since the jet-molecular separators (411,412,418) served as the interphase between the gas chromatograph and the mass spectrometer used throughout this study a brief review of its operating principle will be given here.

The pressure reduction system developed by Ryhage is constructed according to the principles used by Becker (27) in the separation of isotopic species. In this system the

effluent from the gas chromatographic column passes through the small diameter holes of two jets in tandem. The exits of the two jets are under vacuum so that only the heavier molecules of the sample (light inert gases such as helium must be used as carrier gases) will keep a straight path on through and out both jet type separators. The carrier gas being lighter diffuses to the sides and it is pumped away.

The unit works in two stages and is able to provide a reduction in pressure from atmospheric to 10^{-4} mm Hg.

This system was initially tested on an Atlas CH4 mass spectrometer coupled to a gas liquid chromatographic column at Baylor College of Medicine, Houston, Texas (411). Now it is the standard equipment in all LKB 9000 Mass Spectrometers (LKB Produkter, Skockholm).

The sample to carrier gas ratio is claimed to be increased at least 100 times in this way (411). This ratio is a function of three important parameters such as (1) the diameter of the jet orifices, (2) the distance between the jets, and (3) the pressure drop in each of the two separators which is in turn a function of the pumping speed of the separator vacuum system and the speed of the gas stream. According to the latest work of Ryhage (412) the separation factors are virtually independent of temperature and the system has low memory effects. In a more realistic approach Ryhage (412) has redetermined the efficiency of this system and found that

more than 50% of the sample passes the separators to the ion source. The rest is pumped away with the carrier gas.

Studies of the efficiencies of molecular separators have also been reported by other authors (180) but unfortunately the jet-molecular diffusion system was not included in these studies and so its performance can not be compared to that reported for other systems.

The scan rate is another important requirement of the GLC-MS combination. In other words it is necessary to have fast scanning facilities (129) since the spectrum of the GC eluent has to be recorded while it is emerging from the column. In fact it must be recorded in a fraction of that time because of the changes in concentration of the sample in the source as reflected by the GC peak shape. If the spectrum is not scanned fast enough the ion distribution pattern will be biased and will not be representative of the compound under study. A study of the statistical limitations affecting the scan rate in combined gas chromatography-mass spectrometry has been reported by McFadden (287). It shows that scan rate is not determined by the spectrometer design but by the frequency limitations of the recording system and by the resolution of the mass spectrometer.

Temperature programming is used extensively in this kind of work because it results in peaks of comparable widths over a wide boiling range. This in turn gives more representative

ion distribution patterns for all the components.

The capillary column efficiencies and the limitations on the sensitivity imposed by the column bleeding in gas chromatographic-mass spectrometric analyses have been discussed by Terarnishi et al. (475).

B. Lipids in Living Systems and Products of Biological Origin

Lipid compounds, especially the hydrocarbons, have been commonly found in geological environments (4,5,21,60,62,65, 80,82,106,112,130-134,138,141,142,144,150,153,220-223,231, 243,253,254,262,266,305,316,321-323,365,369,372,402,421,442, 455,468,469), petroleum products (72,75,87,89,106,220,243, 258,262,278,296,298,300,304,305,316,407,408,421,422,442) and meteorites (317,318,324,339,341,348,350,352,357,364,368, see reference 201 for more information). Their presence in these types of samples is of particular importance because they are believed to represent records of past ecologies. Thus the study of the structural characteristics of the "fossil lipids" will permit, taking into consideration the possible diagenetic changes of the parent compound, the understanding of the kind and nature of the primitive organisms that deposited them and eventually the elucidation of their biosynthetic mechanisms. In any case, before drawing any conclusions on the distribution and occurrence of the geological lipids it is important to have a good knowledge of our present lipid geochemistry. This

can be gained only through the study of the occurrences and distributions of lipids in modern organisms and biological products.

1. Hydrocarbons

The inherent inertness of hydrocarbons in general, and in particular the alkanes, makes them the best candidates to study the biochemical evolution of life.

a) Occurrence in living organisms

The presence of hydrocarbons in the living tissues and cells of plant and animal products and microscopic organisms has been demonstrated repeatedly by several investigators. Most of the work done in this field has been reviewed by Eglington and Hamilton (140) and by Douglas and Eglington (130).

(1) n-alkanes

The first important contribution to the knowledge of the paraffinic content of plant waxes was apported by Chibnall et al. in 1934 (92,93). A great number of publications have appeared since then on this subject (88,130, 139,173,188,248,250,302,303,329,347,502,503,505).

Long chain n-alkanes have been shown to be common to the surfaces of roots, leaves, stems, flowers, fruits, seeds and pollen, and also in fungi (137,163) and fungal spores (363). Normal alkanes have also been reported in insects (20,45,58, 92,126,154,374,386), animals and animal products (318,341,

345,366), in human sebum (124), in washings of human skin surface lipids (185), in animal and insect waxes (132,133,505), in microscopic algae (92,99,171,231,348,371), in marine organisms such as "coral" reef organisms (96), plankton (455, 456), and in microorganisms (9,371,480,482).

(2) Branched chain alkanes

The presence of branched alkanes was first noted in plants by Carruthers (88) who found relatively high amounts of 2-methyl alkanes in tobacco leaves. Later Waldron et al. (502) confirmed this report and in addition showed mass spectrometric evidence for the isoparaffins in rose petal wax. Isoalkanes have also been found in tobacco leaf wax by Mold et al. (329), by Sorm et al. (448) in Cuban sugar cane and by Eglington et al. (139) in certain members of the family Crassulaceae. Later reports indicate the presence of branched alkanes in rose (518), Humulus and Populus species (228), Ruta species (36), some seed oils (252), Hypericum (309). Anteiso alkanes were reported for the first time in plants by Mold et al. (329) and were later confirmed by Eglington et al. (144). Other methyl alkanes have been found also in insect and animal species (20,26,133,330,331,456).

(3) Monocycloalkanes

Evidence for this type of compounds is incomplete and not much is known about their presence in living systems in general. They have been reported in plants

(252), paraffin wax (267), wool wax (330), and butter (284).

(4) Unsaturated hydrocarbons

Olefins usually occur in low amounts and they easily escape detection. Simple straight chain olefins have been reported for a number of plant species (173,188, 505,518). Sorm et al. (448) have shown the presence of long chain mono and diolefins in rose petals and sugar cane. Iwata and Sakurai (227) reported hexacos-1-ene in Chlorella. Olive oil contains normal di, tri and tetraolefins ranging from C_{13} to C_{28} (173). Horsetails (Equisetum species) contains unsaturated C_{21} hydrocarbons (401). Highly unsaturated polyacetylenic hydrocarbons have been also discovered in plants (173,444).

(5) Isoprenoids

Although as it will be pointed out later the isoprenoid hydrocarbons appear to be widely distributed in geological samples, their presence in living systems, especially in the higher plants and animals, is not as common.

Only one of the isoprenoid alkanes, farnesane, has been found in the resin of plants (130). Its unsaturated homologues, β-farnesene (338,449) and α-farnesene (219) have been reported in the natural coatings of apples and pears.

Pristane has been isolated from a number of marine products and organisms such as sharks, whales, herring, ambergris sponges and other fish (48,170,188,257,264,311,445,484,485,

489,491,493), from plankton (50) and from algal mat communities (348). Reports on its isolation from land sources are more scarce. So far it has only been found in wool wax (330,436) and in an extract of Anise fruit (66).

Phytane is present in some petroleum oils, sediment extracts and meteorites but there is only one report on its isolation from living organisms (95) and none from marine sources. A recent report claims its presence in beef brains (344).

Several isomeric isoprenoid olefins have been isolated from zooplankton and marine liver oils. These include: the isomeric C_{19} monoolefins (48,53,94,494) and the four isomeric phytadienes (C_{20} diolefins) isolated from mixed zooplankton (52).

The polyunsaturate isoprenoid squalene which is an intermediate in the synthesis of cholesterol is more commonly found among the lipids of living systems (90,91,173,185,202, 445,490,492). Its saturated counterpart squalane has been found recently in beef brains (344).

b) Distribution

In general most plants show a predominance of odd-carbon numbered n-alkanes ranging from C_{25} to C_{35} with maxima around C_{29} or C_{31}. As a result of the application of modern analytical techniques the earlier assumptions on the exclusive occurrence of odd-carbon numbered n-alkanes have been proved

incorrect since even numbered n-alkanes have been shown to be present in a number of plants and plant products (88,133,140, 248,367,459,502,503). It has also been observed that the paraffins in the medium molecular weight range (C_{15}-C_{25}) show a smaller odd over even ratio that in some cases approaches unity (252,459). The odd carbon preferences observed in the distributions of normal alkanes has usually been taken as proof of their biogenic origin, although it has been indicated that this may not be a necessary prerequisite of biogenicity (369). Marine organisms in general do not have the odd number carbon preference which is common in higher organisms. This is also the case in most microorganisms (480,482) and some microscopic algae (99).

2. Polar Compounds

a) Fatty acids

Among polar compounds the fatty acids have been the most extensively studied in living systems (392). Also they have been found in many substances of geological interest.

Fatty acids are not usually free in living organisms but associated with glycerol forming the triglycerides. Hydrolysis of the triglycerides permits the study of the distribution of fatty acids in nature. In general the biologically derived fatty acids have an even number of carbon atoms (401) and they are widely distributed in living systems.

(1) Normal fatty acids

Normal saturated, mono and polyunsaturated acids have been reported in natural fats and waxes (205), in plant lipids (103,434), in marine organisms (6,205,514), in algae (371,375,376), diatoms (239) and bacteria (224,238, 371,376,482).

The even numbered normal fatty acids found in nature range from C_2 to C_{38} (301). Natural fats usually contain fatty acids from C_4-C_{26} (205), while insect and plant waxes contain the higher fatty acids (301). Fatty acids with odd numbers of carbon atoms are also found in natural products (6,205,248,401,432) but in much lower concentrations than the even carbon numbered acids.

(2) Branched fatty acids

In relation to the methyl branched fatty acids, the even and odd iso and anteiso acids have been reported in natural fats (435), marine lipids (6,7), algal mat communities (371) and bacteria and protozoa (155,205,234, 290,301,433,482).

(3) Isoprenoid acids

Phytanic acid, the C_{20} acid presumably directly derived from phytol has been isolated from butterfat (194, 198,199,443), sheep and ox fat (193,195,197), ox blood (275), fish oil (425), Rumen bacteria (196) and marine lipids. (See also reference 141 for more information.) Pristanic acid on

the other hand appears to be less frequent. Some lipids also contain small amounts of isoprenoid acids, such as phytenic acid (25).

(4) Other polar compounds

Among the most common constituents of natural products are the ketones and alcohols which show odd and even carbon number predominances respectively. The ketones are usually associated with the aromas of plant products and essential oils (216) while long chain alcohols have been isolated from marine organisms (65), wool wax (133), and other waxes (65). (See also reference 216 for more details.)

C. Chemistry of the Environment

During the past few years man has become increasingly aware of his environment and has also begun to realize and experience the dangers of air and water pollution in his modern world. This has opened the way towards a scientific appraisal of this problem through chemical and physical analyses. Among the organic matter found to be present in aquatic environments and in airborne particles, the hydrocarbons have been the object of several investigations (15,33,209,229,230,268,336,393,420,438,512,515), although work in this area has been mainly concentrated on the polynuclear aromatics (15,268,420) because of their possible carcinogenic effects.

Hydrocarbons are known to exist on land (315,316,323,441) and marine sediments (134,442). Aliphatic hydrocarbons are also evolved from most combustion engines (33). Went et al. (512) reported that studeis carried out even in deserts and isolated mountain valleys show that there is organic matter in the atmosphere (predominantly terpenes) which is given off by living and dying plants. Moreover, volatile hydrocarbons, including paraffins, naphthenes and aromatics are liberated from coal during storage (423). Air samples from Montreal, Rochester and Houston were found to contain hydrocarbons ranging from C_{16} to C_{32}. Traces of isoprenoids were also reported (348) and in heavy traffic arteries up to 2.3 ppm of hydrocarbons have been found in the air (396). In this respect the hydrocarbon content of tobacco smoke has been reported, and also there are numerous publications on the hydrocarbons in petroleum (38,75,305,407,408,421) and paraffin waxes (267). Undoubtedly the total contribution of the hydrocarbons in these products to the terrestrial and aquatic environment must be significant. The accommodation of alkanes in natural water systems has been studied by Peake and Hodgson (377) in relation to the possible role of the water in the mobilization and accumulation of the hydrocarbons in petroleum (17,18). The results obtained by these investigators show that the hydrocarbons in the C_{20}-C_{30} range can be accommodated in distilled water in amounts much greater than would be expected by simple

solubility considerations. Normal hydrocarbons in the same range were also found in surface waters and oil field waters. The lipids of sea water have also been investigated by several authors (229,230,430,478,515), and in some instances mixtures of alkanes have been detected (229,230). It is interesting to note that Jeffrey et al. (230) have shown a gas chromatogram of a hydrocarbon fraction of a sea water sample where all the even carbon numbered alkanes clearly predominate over the odd alkanes. Dust, which is another product of ubiquitous nature has also been shown to contain relatively high amounts of alkanes in the range C_{18} to C_{33} (268,348).

D. Abiogenic Hydrocarbons

The present day knowledge about the abiotic formation and occurrence of presumed abiotic high molecular weight hydrocarbons will be reviewed in a chronological order, with the exception of the Fischer-Tropsch syntheses which will be given special attention because the present study is in part concerned with the production of hydrocarbons by this process. Also, since petroleum is, in at least 75%, a mixture of paraffins, naphthenes and aromatics (39), any review on the abiogenic origin of hydrocarbons is by extension a review on the possible abiogenic theories of the origin of petroleum. The first hypothesis on the abiotic origin of

hydrocarbons was advnaced by Berthelot (37) in 1866. It involved the reaction of the carbides, resulting from the reaction of alkali metals and carbonates, with water to produce acetylene which could generate petroleum like hydrocarbons. A year later Warren and Storer (504) obtained a hydrocarbon naphtha from the destructive distillation of lime-soap. In 1877 Mendelejeff (325) supported the carbide theory set forth by Berthelot. Low temperature cracking reactions of biological organic matter were advocated by Engler (147) as responsible for petroleum hydrocarbon generation.

The production of an oily mixture of liquid hydrocarbons by alpha irradiation of gaseous hydrocarbons was demonstrated by Lind in 1938 (270). Also in 1938, Berl (35) lended support to the low temperature cracking theory. Rice and Kossiakoff (398) presented evidence in 1943 on the formation of n-paraffins, α-β olefins and ethylene from the thermal decomposition of n-paraffins. According to the data of Getling and Frost (172), organic compounds such as alcohols, esters and ketones may undergo at moderate temperatures and in the presence of clay catalysts reactions of dehydrogenation, isomerization, disproportionation, polymerization, condensation and decomposition to give petroleum like hydrocarbons.

The effect of α-particle bombardment of fatty acids was studied by Sheppard and Burton (430,431) in 1946. Their results show that dehydrogenation and decarboxylation to

hydrocarbons does take place. In 1949 Thomas (477) and Greensfelder et al. (181) studied the formation of branched hydrocarbons upon catalytic cracking of n-paraffins. During the same year Brooks (70) discussed the role of "catalytic cracking" on the generation of hydrocarbons from sedimentary organic matter. Silicate clays, shales and sandstones were used as catalysts. In 1955 Zelinskii (520) in accordance with the free radical reaction mechanisms proposed earlier by Kassel (237) and Rice et al. (397), expressed the view that methane can be the source of higher paraffin hydrocarbons. The thermal cracking of methane leading to the formation of higher hydrocarbons may proceed in the following way

$$CH_4 \longrightarrow CH_3\cdot + H\cdot$$

$$CH_4 + H \longrightarrow CH_3\cdot + H_2$$

$$CH_3\cdot + CH_3\cdot \longrightarrow C_2H_6$$

$$C_2H_6 + CH_3\cdot \longrightarrow C_3H_8 + H$$

according to Kassel (237) and Rice (397). Petersil'ye (380) in a study of the hydrocarbon gases in bitumens reached the conclusion that they were syngenetic with the rocks, in other words that the gases were formed within the rock minerals and so they are to be considered as primary. Another abiogenic route to the hydrocarbons lies in decarboxylation and

deamination of amino acids (see reference 149 for more information). In 1958 and 1959 Meinschein reported (314, 315) that laboratory dehydrogenation of isoprenoids (an exothermic reaction) gives rise to aromatic compounds of the type found in sediments and crude oil. The abiotic stereospecific polymerization of isoprene was reported by Natta et al. (343). By changing the stereospecific nature of the catalyst they were able to obtain either trans or cis ruber polymers. Breger reported in 1960 (63) on the formation of C_4-C_6 gaseous hydrocarbons by heating organic extracts of marine mud, and also considered the possible role of Diels-Alder reactions in the conversion of fatty acids to hydrocarbons. In the same year Hoare (208) studied the formation of light paraffins, aromatics and tars produced at the expense of the olefins obtained by gamma irradiation of ligh hydrocarbons. Bogomolov et al. (54) reported that the thermo-catalysis of fatty acids (stearic acid) produces mainly iso paraffins. A year later Bogomolov and Panina (56) performed the same experiments with oleic acid and got similar results. The same authors (55) reported in 1962 that using an alumino silicate catalyst at 275°C they had obtained a C_{34} paraffin from stearic acid. Cycloparaffins were also obtained in this way (54,56). In 1963, Petersil'ye (381) showed evidence on the inorganic synthesis of gaseous hydrocarbons and bitumens in the Khibina Massif. Laboratory synthesis and thermal reactions of methane

were discussed. Also in 1962, Wilson (516) proposed his theory on the abiological synthesis of straight chain hydrocarbons via a free radical mechanism from methyl radicals. This hypothesis rested on the assumption that the methyl radicals produced by interaction of solar photons on methane attack molecules of fatty acids terminally when they are crowded in a monomolecular layer of water, so that only the ends can engage in the reaction. Hunt (220) has obtained paraffinic and naphthenic hydrocarbons by heating a sample of green River oil shale, and Bedov (28) reported on the preparation of petroleum like hydrocarbons by thermal catalytic conversion of fatty acids. Day and Erdman (117) studied in 1963 the thermal degradation products of carotenes and found some aromatic hydrocarbons among the reaction products. Sylvester-Bradley and King (470) discussed the existing evidence for abiogenic hydrocarbons in hydrothermal deposits, in thucholites and in igueous hydrocarbon mixtures. Marx and Breisacher (307) reported the formation of high molecular weight paraffins by the thermal reaction of graphite with hydrogen. In 1964 Wilson and Johnson (517) proved experimentally the mechanism proposed in 1962 by Wilson, by demonstrating that palmitic acid and can be built up to n-nonadecanoic acid by stepwise attack of methyl radicals.

The effect of clay numerals in hydrocarbon formation was studied by Klubova (244). A significant report on the

role of catalysts was that of Jurg and Eisma (232) which obtained long chain alkanes from behenic acid. The production of light paraffins from gaseous methane in electric discharges was reported by Ponnamperuma and Woeller (389). Davis and Libby (114) have shown that heavy hydrocarbons can be produced by the polymerization of solid methane under Co-60 γ rays. Marx (306) proposed in 1965 that the hydrogenation of graphite could be a source of petroleum hydrocarbons, and Douglas and Mair (131) showed that terpenoids and steroids can be transformed into aromatic hydrocarbons by the action of sulfur at 150°C. Kudryavtsev (251) in 1965 proposed that CH_3 radicals are formed by direct synthesis in deep seated zones of the earth. These radicals would combine with each other and with hydrogen to form petroleum like hydrocarbons in the cooler parts of the earth mantle. Ponnamperuma and Pering (388) have studied the similarities between the distribution of the hydrocarbons from the sparking of methane and some naturally occurring hydrocarbons and found indications of the possible abiogenic origin of the hydrocarbons in some formations (Trinidad Asphalt Lake and Mountsorrel). The mechanisms of the generation of petroleum hydrocarbons from fatty acids has been discussed in some detail by Jurg and Eisma (233). Holman et al. (211) have shown that upon pyrolysis, phytane produces a mixture of olefins ranging from C_{15} to C_{19} with the exception of the C_{17} which was not found. The

possible implications of high temperature graphite-hydrogen reactions in the formation of hydrocarbons were studied by Gulbransen (183) with the conclusions that the reaction takes place at 1300-1400°C under conditions were the reaction products are quenched. Oró and Han (358,359) reported that relatively large amounts of aromatic hydrocarbons and small amounts of of aliphatics are produced by passing methane through silica gel at 1000°C. Also Oró et al. (362) have shown that a great number of aromatic compounds can be obtained upon pyrolysis of isoprene.

In summary it can be concluded from the existing data that a great variety of hydrocarbons could be produced in abiological systems by means of several different mechanisms such as (1) the reaction of carbides with water, (2) thermal cracking reactions (via free radical mechanisms), (3) catalytic cracking (via carbonium ion mechanisms), (4) radioactivity, (5) thermo-catalytic processes and (6) electric discharges.

Fischer-Tropsch synthesis

Briefly, the Fischer-Tropsch synthesis could be defined as a process of catalytic hydrogenation of carbon monoxide that produces mainly straight chain hydrocarbons and oxygenated molecules. The first demonstration that carbon monoxide can be hydrogenated to methane in the presence of a nickel or cobalt catalyst is due to the experiments of Sabatier (419)

in 1902. Water is liberated from the reaction.

Later in 1923 Fischer and Tropsch (161,162) made the observation that this hydrogenation reaction could be modified (by changes in the total hydrogen pressure and by the nature of the catalyst) for the production of a complex mixture of aliphatic products in which the hydrocarbons were predominating (reference 460, p. 1). The overall reaction being:

$$nCO + 2nH_2 \longrightarrow C_nH_2 + nH_2O$$

$$nCO + (2n+1)H_2 \longrightarrow C_nH_{2n+n} + nH_2O$$

The product obtained in the initial experimental work which was carried out at 100-150 atm and 400-450°C was called "synthol" and contained mainly oxygenated compounds (alcohols, acids, aldehydes, ketones) plus a small amount of hydrocarbons. By reducing the pressure to 7 and later to 1 atm the yield was reversed in favor of the olefinic and paraffinic hydrocarbons as indicated by the reactions given above.

The first catalyst found to be active at atmospheric pressure was an iron-zinc oxide preparation which Fischer reported in 1925 (159). With this catalyst he obtained yields of the order of 100 g of liquid and solid hydrocarbons per cubic meter of water gas (H_2 + CO).

The use of finely divided hydrogen reduced iron at 305°-340°C produced only very small yields of gaseous hydrocarbons from a mixture of $2H_2$ + 1CO gas (460, p. 118). On

the other hand when water gas was passed at 270°C over pure iron oxide, a 20% contraction in the gas volume was observed, and 37.3 g of C_1 - C_4 hydrocarbons plus 11.6 g of C_5+ hydrocarbons were thus obtained. Iron-nickel in the ratio 1:1 did not yield any appreciable amounts of hydrocarbons. In 1928 Smith et al. (313,440) tested several catalytic materials but none was found to be commercially suitable for big scale operations. This was not achieved until the precipitation of nickel-thoria on Kieselguhr by Fischer in 1931 (160). Kieselguhr (a diatomacous earth) plays an important role in producing the proper dispersion and porosity of the active metal granules and it influences the yield and life of the catalysts. With the use of these precipitated catalysts, hydrocarbons ranging from C_1 to waxes of 2000 molecular weight were produced. The average distribution of liquid and solid products obtained in the German Rurchemie process is a follows, in weight per cent: gasoline 35-60; diesel oil 30-35; wax 10-30, depending upon the pressure. Intensive research on this method of synthesis was triggered in Germany because of the fuel and lubricant shortage that it experienced during World War II. The detailed development of the industrial Fischer-Tropsch synthesis has been adequately reviewed by Storch et al. (460).

It is known (460) that all of the active catalysts used in this type of synthesis are readily poisoned by sulfur with

the resultant losses in efficiency and yields.

The distribution of the hydrocarbon products obtained in the Fischer-Tropsch synthesis has been reviewed also by Storch et al. (460). The distribution curves of the hydrocarbons are usually characterized by i) high yields of methane, ii) a minima at C_2, and iii) an increase in the yield of hydrocarbons up to another maximum around C_5. After this second maxima the distribution curve shows steady decrease towards the high molecular weight hydrocarbons. The olefin formation tendency increases from nickel to cobalt to iron. Usually olefin formation is a function of the space velocity of the synthesis gas, the temperature, the length of the catalyst bed and the gas composition. In other words, a low H_2:CO ratio, high flow velocity and high temperature in a long catalyst bed will favor the production of unsaturated hydrocarbons.

Straight chain compounds predominate over their branched isomers. In general among the branched products only the monomethyl substituted paraffins appear to be synthesized in substantial amounts (12,165), while the dimethyl paraffins are present in trace amounts (12,74,510). Trimethyl substituted compounds have never been found in the Fischer-Tropsch products. Since the straight chain isomers are less stable thermodinamically than most of the corresponding branched isomers, it has been pointed out (460) that they must be produced by primary

processes rather than by subsequent isomerization of other isomers. Olefins are also thermodinamically unstable with respect to hydrogenation or hydro-cracking and are produced by primary processes. Although α-olefins are usually less stable thermodinamically than olefins with internal double bonds, the results obtained by Friedel and Anderson (165) and Bruner (74) showed higher amounts of α-olefins than it would have been expected for equilibrium with internal double bond isomers.

All these lead to the consideration of the mechanism of the synthesis, which will not be discussed here because the mechanistic problem was not considered relevant to, or within the scope of these studies. A detailed account on the mechanism of synthesis can be found in the publications by Storch et al. (460), Anderson et al. (12), Weitkamp and Frye (510), Gibson and Clarke (174), Polyakin (387), Krause (249) and by Friedel and Sharkey (166).

The distribution of the hydrocarbons obtained in this process is of special interest in the context of the present studies because of the possible relationship to the hydrocarbon distributions typical of extraterrestrial samples (350,357). Sharkey et al. (429) obtained carbon number distribution data from C_{11} to C_{38} and also calculated the ratio of branched to normal hydrocarbons in the same range. The distribution was found to follow a logarithmic decrease, which fits the

expression previously derived by Anderson et al. (12) to predict the values for the isomer distributions. The results also showed that the proportion of branched-chain isomers increases regularly with synthesis temperature. Friedel and Sharkey also studied the possible interrelationships of natural products such as petroleum, coal and natural gas with Fischer-Tropsch products (166,167) because of the possible significance of these non-biological syntheses in the origin of natural products. Similarities were found in the low molecular weight alkanes from crude oil and Fischer-Tropsch synthesis products, suggesting that the volatile components of crude oils could have originated from such syntheses.

Experiments using meteoritic iron as catalyst have been independently carried out by Studier et al. (467) and Oró et al. (361), in order to explore the possibility whether this type of catalytic synthesis could account for the observed distributions of hydrocarbons in meteorites. Both authors claimed the possible presence of isoprenoids in their products. Fischer-Tropsch processes have been suggested as probably playing a role in abiotic organic syntheses in general (259, 355,467) and have been specifically considered responsible for the formation of the hydrocarbons in carbonaceous chondrites (259,467). Recently Oró et al. (357) have presented preliminary evidence that appears to invalidate the earlier assumptions

about the involvement of Fischer-Tropsch reactions in the formation of meteorite hydrocarbons. High molecular weight alkanes from a Fischer-Tropsch product product at 300°C and under pressure have also been reported by Belsky (29). The distribution mode was at n-C_{23} and no indications of isoprenoids were obtained in this product.

E. Organic Geochemistry

The organic chemistry of our modern environment is of a particular importance to man, as it has just been indicated above, because it may have a direct effect on his way of life.

Another kind of environment, somewhat less apparent or more concealed, is the past geological environment whose organic chemistry may very well hold significant clues to the origin and first appearance of terrestrial life. The geological formations of the earth contain morphological (fossil structures of ancient organisms) and molecular (chemical fossils of these organisms) records of past life events. The study of these records can provide an insight into the evolutionary history of the living organisms on the earth.

Although fossils had been observed since Greek times, they were not studied in a systematic manner until the science of paleontology came into being around 1800. Paleontologists have been able to find well defined morphological remains (310) within a geological period that ends about 600 million

years ago, at the start of the Precambrian. Micropaleontologists have been able to extend this period into the Precambrian with the identification of microscopic sized remains of simple morphological entities (21,22,23) which are thought to represent the earliest forms of life on earth. On the other hand since the search for "chemical fossils" does not require the presence of an actual hard form on the rock (138), the organic geochemical approach (3,64,104,138,231,281,365) of searching for the evidence of life at the molecular level, is perhaps the only tool available today to penetrate deeper into the earlier geological eras and perhaps get a glimpse at the biological processes prevailing at the time. The experimental foundations of organic geochemistry were laid by Treibs in 1935 (488) who found metal complexed porphrins in mineral oils. The discovery of these first chemical fossils had a marked influence on the theories on the origin of petroleum since it fixed a maximum temperature for the environments in which oil is found. These porphyrins are not stable over 250^{o}C and so the theories that assumed the participation of a high temperature phase in the origin of the oil were invalidated. More work on the geochemistry of pigments has been carried out by Blumer (47). With the development of the modern chromatographic and highly sensitive mass spectrometric techniques, and especially with the combination of gas chromatography-mass spectrometry, it has been possible to

carry out organic geochemical studies on a number of samples of sediments from different geological epochs and thus gather a wealth of chemical evidence on ancient life.

The oldest sediment (3.2×10^9 yrs. old) that has been studied from a geochemical point of view is the Fig Tree sediment of the Swaziland system in South Africa (365). If the n-alkanes and isoprenoids found in this sediment are taken as biological markers, deposited at the time of compaction of the sediment, then it must be accepted that life appeared on earth more than three billion years ago. The literature on the paraffinic hydrocarbons of recent and ancient sediments has been reviewed by Nooner (348).

The evidence provided by the presence of organic matter in the Fig Tree sediment is supported by the discovery of bacterium like microfossils and biogenic organic filaments (22) in the same formation.

These kind of morphological and geochemical correlations have also been reported in other cases, such as the Gunflint Chert formation (23,369), the Nonesuch Shale (21,231,322) and in the Ketilidian sediments of SW Greenland (256,379). The detection of organic compounds in geological environments where morphological remains are also present strengthens the organic geochemical approach.

1. Diagenesis

It is reasonable to assume that the organic substances

deposited at the time of formation of the sediments would undergo chemical changes with time, the extent of which would be dependent upon the anaerobic biological activity, the pressure, the temperature and even the possible catalytic effect of the rock matrix which contains these compounds (122). The overall process of change is referred to as "diagenesis" (120) and the particular changes undergone by the trapped organic material, as diagenetic changes. For instance, the decarboxylation of the fatty acids, present in the earlier forms of life, to the corresponding hydrocarbons, would be a significant diagenetic change (2), and in fact it has been proposed as the mechanism responsible for occurrence of alkanes (preferentially odd carbon numbered) in sediments and oil shales (63,254,255,263). Distributions of even and odd carbon numbered fatty acids in which the even acids were predominating have been found in a number of sediments ranging in age from Precambrian to Recent (255).

Reduction is another important transformation that may take place within the geological time. Blumer has obtained geochemical evidence for reduction in his study of fossil porphyrins (47) and Abelson (1) and Bergmann (34) have discussed the reduction of carboxyl to methyl groups as a probable geochemical process. Hydrogenolysis has also been proposed as a geochemical diagenetic mechanism of transformation, but it is not considered a likely process (255) in view

of the severe conditions that it requires (catalysis, high temperature and pressure). Polymerizations and thermal degradation processes are likely to occur but our understanding of the diagenetic mechanisms in general is only superficial. Degens (121) has indicated that non microbial diagenetic transformations most likely go through i) polymerization and condensation of the deposited organic matter with the associated minerals; ii) a redistribution of the pre-existing organic molecules and iii) alterations due to temperature, and possibly pressure also, which may be catalyzed by clay minerals. He also states that the first step in the formation of fossil organic matter is the microbial degradation of biopolymers (122). Concerning the role of microorganisms in diagenesis it has been reported that bacteria are present in sediments (521); can create the anaerobic conditions necessary for reduction of the deposited organic matter (524) and also can reform in a significant manner the nature of the initially deposited organic debris (522,523). But it is also true that bacterial activity ceases a few feet below the surface of the sediment (281).

An important consideration in relation to the diagenetic changes suffered by organic matter is the thermal stabilities of the different classes of compounds (121). Among the compounds most stable for long periods of time under geological conditions are the alkanes (318) and the fatty acids (255).

2. Biological Markers

Living organisms and their organic constituents are extremely complex systems possessing a high degree of order in their molecular and cellular structures (30). In spite of this complexity there are many general resemblances between different kinds of life and biological remnants (455). Among the biological remnants or fossils, only the structures stable enough to resist diagenesis will retain the order which distinguishes animate from inanimate things. Since biological systems use templates to synthesize their vital organic compounds, specificity should be added to the list of biological characteristics. Thus the unique structural properties of biogenic molecules will help in setting them apart from the rather unselective abiotic distributions.

Thus the properties of any "biological marker" can be listed:

a) characteristic structure

b) diagenetic stability

c) structural specificity

d) low probability of abiotic formation

Of all the organic compounds which are constituents of living organisms, the lipids appear to fit best these requirements although their stability is highly variable (65). Breger in his review of the Geochemistry of Lipids (65) indicates that "many lipids while subject to some changes following

elimination from biological systems, are eigher preserved intact or converted into products that are stable and tend to be preserved." Among the lipids the hydrocarbons (316,318, 320) appear to provide the best means to study the biochemical evolution of life, but within this class compounds only the isoprenoids (21,30,31,112,143,318,320,322,369) meet the four requirements stated above. A number of hydrocarbons including the n-alkanes have been produced abiotically (see section D) but to date none of the isoprenoid hydrocarbons has been produced outside of a biological system. The presence of isoprenoid hydrocarbons in living systems and in their products has already been reviewed. Attention will be turned now to their occurrence in sediments, shales and petroleum products, all constituents of the geological environment. The regular isoprenoids (isoprene units linked in a head to tail fashion) isolated from these samples cover the range C_9 to C_{21}.

Isoprenoids in Precambrian sediments

Eglington et al. (142,143,144), Barghoorn et al. (21) and Meinschein et al. (322) isolated farnesane, pristane, and phytane from the Precambrian Nonesuch Shale formation ($\simeq 1 \times 10^9$ yrs. old). Oró et al. (369) reported the presence of pristane and phytane in the Gunflint iron formation (1.9×10^9 yrs. old).

Isoprenoids ranging from C_{18} to C_{21} were also detected

in the Soudan iron formation (2.7×10^9 yrs. old) by Belsky et al. (30), by Meinschein (321) and by Johns et al. (231). The oldest Precambrian sediment analyzed, the Fig Tree series of South Africa, 3.2×10^9 yrs. old, also showed the presence of the isoprenoids pristane and phytane plus norpristane, Oró and Nooner (365).

Recently Pedersen and Lam have reported the presence of pristane and phytane in a meter-thick coal graphite and dolomitic layers in the Ketilidian sediments of Greenland (256,379).

Isoprenoids in Recent sediments

From the Green River oil shale (≃ 50 million yrs. old) Robinson et al. (402) and Lawlor and Robinson (263) isolated the C_{15}, C_{16}, C_{18}, C_{19} and C_{20} isoprenoids. Cummins and Robinson (112) found an identical distribution of isoprenoids in the oil shale bitumen. Independently Eglington et al. (144) reported the presence of the C_{16}, C_{18}, C_{19} and C_{20} isoprenoids. Blumer and Snyder (51) reported the presence of pristane in a recent sediment but could not find phytane. Johns et al. (231) have reported the identification of the C_{16}, C_{18}, C_{19}, C_{20} and C_{21} isoprenoids in the Antrim Shale (265×10^6 yrs. old). Gohring et al. (178) have also reported on a series of isoprenoid alkanes ranging from C_9 to C_{14} in cretaceous bituminous shales. Isoprenoids have been also

found in coal-tar by Kochloefl et al. (245,246).

Isoprenoids in petroleum

Dean and Whitehead (119) first reported on the presence of phytane in a petroleum destilate. To this report followed those of Bendoraitis et al. (31,32) on the identification of the C_{14}, C_{15}, C_{16}, C_{18}, C_{19}, C_{20}, and C_{21} isoprenoids in an East Texas Crude. Norfarnesane (C_{14}) and farnesane (C_{15}) pristane (C_{19}), phytane (C_{20}) and a 2,6-dimethyl octane, were reported by Mair et al. (295,297,298).

Rossini (407) reported a 2,6-dimethyl heptane which constitutes the lowest dimethyl isoprenoid found in petroleum. Eglington et al. (143,144) found the C_{15}, C_{16}, C_{19} and C_{20} isoprenoids in oil seeps and in a later report Johns et al. (231) presented evidence for the C_{18} and C_{21} compounds in these oils. These same authors also analyzed samples of the San Joaquin and Abbot Rock oils and found isoprenoids ranging from C_{16} to C_{21}.

Gohring (178) found dimethyl substituted isoprenoids ranging from C_9 to C_{14}, as in bituminous shales.

Isoprenoid fatty acids have also been reported in recent sediments (49,141) and in petroleum (89).

In general the distributions of the isoprenoid hydrocarbons in all of these samples show a major maximum at C_{19}, a secondary maximum at C_{15} or C_{16} and a marked minimum at C_{17}

(357). In fact there is only one report of the isolation of the C_{17} isoprenoid in a sediment (282). This typical and widespread distribution of the isoprenoid hydrocarbons in nature can provide clues as to the nature of the parent isoprenoid (281).

Because of the small amounts of the fossil isoprenoids most of these identifications have required the use of the combined technique of gas chromatography and mass spectrometry.

3. Petroleum Hydrocarbons

Petroleum is a complex mixture of hydrocarbons, which account for more than a 75% of the total, plus a smaller percent of non hydrocarbon heteroaromatic, organometallic complexes, sulfur, etc.

It has been pointed out by Bestougeff (39) and Rossini (407) that the major classes of hydrocarbons in petroleum are:

a) normal paraffins
b) branched chain paraffins
c) cycloparaffins (naphthenes) (mono-, bi- and polycyclic)
d) aromatics (mono-, bi- and polycyclic naphtheno-aromatics or "hybrid hydrocarbons")

Of all these classes only the first three will be given consideration in this study. A brief presentation of the existing knowledge about their presence and distributions will be attempted taking as a basis the recent review on

petroleum hydrocarbons by Bestougeff (39).

When all the theoretically possible hydrocarbon isomers in the range C_1 to C_{60} are considered the numbers can be of astronomical size but actually all the experimental analytical evidence indicates that petroleum is by no means that complex. The total number of compounds present represents only a negligible amount of the theoretically possible structures (39). The characterization of the individual components in the mixture represents another example of the potentialities of modern fractionation and identification methods, among which gas liquid chromatography and mass spectrometry have proved to be highly efficient (38,75,215, 278,305). Even with the use of these techniques only 340 individual hydrocarbons have been identified in crude oils; thus our knowledge of the overall chemical composition of petroleum is still not complete but perhaps sufficient to understand its nature and to permit extrapolations into the not so well studied molecular weight ranges. Rossini and Mair (407,408) succeeded in identifying 159 different hydrocarbons in one crude oil and found that they represented about 50% of the total hydrocarbons.

a) Normal paraffins (C_1-C_{35})

Normal paraffins are common in petroleum (38,75, 123,226,299,305,407,408,421). In general the lighter members of this series (C_5-C_7) are predominant but Martin and Winters

have shown that, in four of the crude oils analyzed by them, the C_5-C_7 fraction was very low and in one case at least, the maxima was around C_{27} (305). Irregular n-paraffin distributions also have been reported by Brunnock (75) who found distribution maxima at C_{15} and C_{17} in Libyan and Nigerian crude oils, respectively. Some of the petroleum crudes were also found to exhibit predominances of certain odd-numbered members, being this effect concentrated within the C_{11}-C_{19} range (75,305) and within the C_{25}-C_{35} range (75). Also a predominance of even carbon numbered paraffins above C_{40} was observed for the first time in crude oils by Brunnock (75).

The n-paraffins comprised 15-20 percent of the total American crudes (40 in all) (305). A tabulation of the total n-paraffin content of European petroleum crudes compiled by Bestougeff (38) shows a wider range (between 3 and 49 percent), although it is indicated by the author (38) that usually the content in light crudes is within 20-25%, and that the smaller paraffinic content (less than 10%) of some of the crudes is associated with a high sulfur content. This peculiarity which has also been observed in American petroleum crudes, is further emphasized by the very low amounts of sulfur present in ancient petroleums which are rich in n-paraffins (38).

It appears that the predominance of the light paraffins is accentuated in the ancient crude oils without changing the

overall paraffinic content (38).

The distributions of normal hydrocarbons in petroleum have also been studied in our laboratory (348). Alkanes ranging from n-C_{14} through n-C_{18} were found in two East Texas crudes. No odd over even preference was reported.

b) Branched paraffins

Branched compounds are also common in petroleum. They are concentrated below C_{20} and are usually present in variable amounts relative to the n-paraffins (38,39). Their concentrations can be much lower than those of the n-alkanes, higher or they may be present in comparable amounts. Mono and dimethyl paraffins have been found in crude oils, kerosene and gas oil fractions (38,166,231,271,297,304,407,408). The 2- and 3-methyl compounds often predominate. The 4-methyl paraffins have also been reported but in smaller amounts. Martin and Winters (304), Desty et al. (123) and Friedel and Sharkey (166,167) have studied the relationships between mono and polysubstituted branched paraffins, and especially the predominance of the even carbon numbered 2-methyl paraffins and the predominance of the odd numbered 3-methyl paraffins. This, as it will be recalled, is just the opposite of the distribution observed by Mold et al. (329,330) in tobacco and wool wax. It is a general observation that the monomethyl substituted hydrocarbons are always present in higher concentrations than their dimethyl substituted isomers and the

trisubstituted compounds are found only in trace amounts (39, 407). The isoprenoids constitute an exceptional class of compounds because of their relatively high concentrations in petroleum products (32). Their occurrence in petroleum has been discussed in the preceeding section.

c) Cycloparaffin hydrocarbons

Cycloparaffins with one through nine rings in the molecule have also been found in petroleum. Usually their concentrations range between 30 and 60%, being commonly the principal constituents of crude oils. The monocyclic naphthenes (C_6-C_9), cyclohexyl and cyclopentyl alkanes, have been isolated in several crudes. The latter are in general more abundant. This class of compounds constitute a major interference in the gas chromatographic separations of the n-paraffins because they have retention times which coincide with those of the corresponding n-paraffins with one carbon atom more. This is especially so for the cyclopentyl alkanes. The limitation imposed by this coincidence was recognized by Levy et al. (267) in their studies of the composition of waxes and by Bestougeff (39) in his studies of the paraffins in petroleum.

4. Petrogenesis

Ever since Berthelot proposed in 1866 his carbide theory for the origin of petroleum, this has been a subject of many controversies as it is well indicated by the proliferation of theories that have appeared on this subject. In

spite of all the long and intensive research efforts lead by many geologists, chemists, physicists, and microbiologists, there is still no complete agreement on how petroleum hydrocarbons are produced or whether they have an inorganic or organic origin. In general, it appears that the organic theories are perhaps better established than those resting on a purely inorganic origin. Also, the failures inherent to some of the arguments in favor of the organic speculations have been complemented by the proposed dual origin hypotheses. Since the mechanisms and theories of abiotic production of hydrocarbons (see section D) have already been reviewed, they will not be repeated here.

Before giving a brief presentation on the proposed organic and dual origin hypotheses it will be appropriate to review the physical conditions surrounding the present oil deposits (454) and the factors which are thought to affect its formation.

It is known that most of the petroleum pools are associated with marine sediments (442). Oil is found within a time span of 500 million years and must have had a low temperature history ($<200^{o}C$) as indicated by its thermolabile constituents (porphyrins, carboxylic acids, etc.). The formation process must be slow as evidenced by the rare occurrence of oil in rocks younger than 1×10^{6} years. The formation process is thought to be affected by physicochemical factors such as: temperature, pressure and catalysts, rate of sedimentation,

depth of oxygen content, kind and abundances of contributing organisms and finally rate of migration.

These factors constitute the basis for most of the present organic and dual origin hypotheses on the formation and origin of petroleum.

In 1893 the theory of destructive destillation of plant and animal products proposed by Engler (148) replaced inorganic reactions as the most widely accepted provider of hydrocarbons. In 1916 Clark (97) discussed the transformation of organic matter in sediments and proposed his "source rock" theory. Whitmore (513) indicated that crude oils result from the selective accumulation of naturally occurring hydrocarbons. This view was supported by Smith (442), Swain (468) and Meinschein (315), Baker (16) and Stevens (454). Taking into account the physical conditions prevailing in sediments, in 1946 Cox (109) placed a "geological fence" around the theories of petroleum formation which helped to centralize all speculations around a "most likely environment". Hunt (220) stated that oil formation is a result of the degradation of raw organic matter and the preservation and concentration of hydrocarbons from the remains of plants and animals. In 1956 Stevens, Bray and Evans (455,456) observed that while the paraffins in recent marine sediments showed a strong odd over even predominance, the n-paraffins in oil did not show such a preponderance. According to Stevens (454) petroleum

arises from slow physiochemical processes in sediments and selective accumulation of organic matter of marine and non marine origin rather than from direct biogenic processes. He also indicated that there is no evidence for the biochemical synthesis of oil, although there may be direct contributions of hydrocarbons from plants and animals.

To explain the process of selective accumulation of hydrocarbon components in petroleum reservoirs Baker (16,17) proposed a mechanism of accumulation by a ground water solution followed by a release process.

In 1956 Hunt and Jamieson (223) proposed that the hydrocarbons in petroleum have a dual origin. According to these authors nearly all shales and carbonates contain soluble hydrocarbons, soluble asphalts and insoluble kerogens. Ancient sediments have higher contents than Recent sediments which they took as evidence that the hydrocarbons in oil were made both by organisms and by the slow reduction of organic non hydrocarbon material in sediments. Robinson (399,400) also believes that petroleum hydrocarbons have both a biogenic and abiogenic origin, the latter being much older.

Kvenvolden (254) believes that paraffin formation involves a maturation process in which the distributions approach equal abundances of odd and even carbon numbered molecules as a result of degradations of biogenic fatty acids.

The leveling of the odd carbon number preference can also be accounted for by taking into consideration Baker's theory (16,17) and assuming that the content of even numbered paraffins in sediments is enough to saturate the interstitial waters. The role of bacteria in petroleum genesis has been actively investigated by Zobell and coworkers (459,521,522). In general it is felt (454) that bacteria play a major role in the initial degradation (122) of organic debris but do not enter in later stages of the genesis of oil. It has been reported that the activity of anaerobic bacteria ceases at a depth of about 25 feet below the sea bottom.

An important point of view on the problem of petroleum genesis, dealing with the ubiquity of hydrocarbons on earth and the role of water systems on their transport and accumulation processes, was expressed in 1965 by Hodgson and Hitchon (210). Because of its many implications to the problem of the origin of hydrocarbons in petroleum and also in extraterrestrial matter it will be discussed later in some detail.

F. Hydrocarbons in Extraterrestrial Organic Matter

Meteorites are the only cosmic material available for direct study of samples of our planetary system. Meteoritics as a science faces three major problems and at the same time

shares a common characteristic with the science of petrochemistry. These problems are all based on the explanation of:

1. the origin of meteorites
2. their history
3. their role in the origin and development of our planetary system.

And as in petrochemistry the matter of their origin is, as that of the petroleum on earth, surrounded by many hypotheses and arguments. None of them, though, has yet been conclusively proven to be right. It has been recognized for a long time that meteorites usually contain a wide spectrum of simple and polymeric organic compounds (201,350) whose origin may have a relation with the theories of meteorite origin (201). These organic compounds have been the object of many investigations on their nature and possible mode of formation. All nineteenth century work is rather limited to superficial and gross identifications, mainly because of lack of suitable analytical techniques. Certainly last century organic chemistry could not offer the highly sensitive methods and high purity solvents that are available today. On the other hand the application of modern methods of analysis such as gas chromatography and mass spectrometry has yielded a wealth of information on the organic material present in meteorite specimens. A 30% of this organic material in carbonaceous

chondrites is extractable with water and organic solvents while the remaining 70% is most likely polymeric. Iron meteorites also contain carbon but mainly in the form of graphite (350,357).

It is generally felt that the presence of the extractable organic material, if indigenous, limits the thermal history of the meteorite bodies to low temperatures. Also the presence of gases such as helium and Argon, of water and of strained glass fragments seem to place the limit at around 200°C or less (201).

The first work on meteorites published in this century was the elemental analysis of the cold Bokkeveld carried out by Muller (335). After this work the presence of extractable organic compounds (hydrocarbons and other compounds) in meteorites was established by Calvin and Vaughan (85,86), by Briggs (67) and Sztrokay et al. (471). In 1961, Nagy, Meinschein and Hennessy (341) applied a standard method developed during analyses of terrestrial sediments, soils and crude oils (323), to the hydrocarbons extracted from the Orgueil meteorite and concluded that the meteoritic hydrocarbons were very similar to the biological material found in terrestrial fossils. They implied with these results that that hydrocarbons were remnants of extraterrestrial biological activity and thus raised a controversy which is still not definitely settled. Urey (496) in his 1966 review of biological

material in meteorites expresses his doubts about the extra-terrestrial existance of some forms of life, able to produce terrestrial like biological material in the absence of water.

The possibility of extraterrestrial biological activity was also studied from a micropaleontological approach and supposedly fossilized elements were detected in meteorites by Claus and Nagy (100) and Staplin (451,452). In particular, Claus and Nagy announced the presence of "microscopic-sized" particles, resembling fossil algae, in relatively large quantities within the Orgueil and Ivuna carbonaceous chondrites. This added to the controversy for several authors showed that most of these "organized elements" were either contaminants or inorganic artifacts (11,68,334). Nagy et al. (340) concluded from later work that the organized elements, in spite of every criticism, still resembled biological forms and were not contaminants, though no proof of their biogenic origin had been obtained.

Anders (10) critisized the conclusions reached by Nagy et al. (341) on the extraterrestrial biogenic nature of the hydrocarbons in Orgueil on grounds that i) there was enough lack of resemblance between the biogenic materials so as to invalidate their use as standards for evaluating meteorite data ii) contamination from different sources could not be excluded iii) the distribution observed could be several steps removed from the original one and iv) the compounds observed

could have been produced abiotically. Nagy, Meinschein and Hennessy (10) discussed the criticisms raised by Anders and defended their thesis demonstrating the strong similarities between the organic compounds found in the Orgueil and those found in ancient sediments.

The initial suggestion by Anders (10) about the possible abiogenic nature of the hydrocarbons in meteorites was further developed by Studier, Hayatsu and Anders (465). Their hypothesis rested on the assumption that the organic matter in carbonaceous chondrites formed in the solar nebula under conditions of thermodynamic equilibrium, after most of the hydrogen had been lost. The observed distribution of the volatile components, particularly the high methane-ethane ratio (1000:1), together with the preponderance of aromatic hydrocarbons up to at least C_{10}, was found to be in agreement with the distribution of compounds calculated by Dayhoff et al. (118) for conditions of thermodynamic equilibrium in an ideal gas mixture of carbon, hydrogen, oxygen and nitrogen at $500^{o}K$ and 1 atm of pressure. In order to explain the presence of aliphatic hydrocarbons the initial hypothesis was extended to include also near-equilibrium processes.

Urey and Lewis (497) objected to this hypothesis in view of the absence of graphite which under the postulated conditions is very stable thermodynamically. According to their concluding remark these authors believe that the carbon

compounds detected by Studier et al. (465) and other investigators could have been produced only by high energy radiations, or, in some instances, by living organisms of either terrestrial or possibly extraterrestrial origins. Studier, Hayatsu and Anders (466) answered Urey's criticisms and also tried to support their hypothesis experimentally (464). An expanded version of their work has recently been published (467).

Studier et al. worked on the assumption that although elemental carbon should be the stable equilibrium product in the asphalt region of the C-H-O phase diagram (118) as contended by Urey (497), large amounts of aromatic hydrocarbons could form as metastable products if the formation of free carbon would be kinetically inhibited long enough. Experimentally they observed that mixtures of CO and H_2, in the presence of meteoritic iron as catalyst, reacted according to the familiar Fischer-Tropsch type process and produced hydrocarbons via oxygenated intermediates. The possible participation of Fischer-Tropsch processes in the abiotic synthesis of hydrocarbons in the early solar system had been previously proposed by Oró (355). Work along these lines carried out in our laboratory had been equally successful (360). Metastability was also considered a necessary prerequisite for the formation of n-paraffins under these conditions. More theoretical and experimental support on the formation of organic compounds under conditions of metastable limited

equilibrium was later presented by Eck et al. (136).

Abiogenic processes in the synthesis of the organic compounds in meteorites were also advocated by Kaplan et al. (235) and Briggs and Mamikunian (69). An important point to consider in this respect is the failure to synthesize "biogenic" compounds such as pristane and phytane by methods purely inorganic, although there have been tentative claims of their synthesis by Fischer-Tropsch processes (360,467).

The results of a systematic search for the aliphatic and aromatic hydrocarbons in meteorites which was undertaken in our laboratory by Nooner and Oró (348,350,364,368) and by Olson et al. (352) resulted in the most complete study available of the distributions of these hydrocarbons. This study, however, did not answer the question of their origin, even though preliminary results on the hydrocarbons found in nodules of iron meteorites suggested that the possibility of a general contamination process was worth investigating. In fact Nooner and Oró (350) stated that their results could be interpreted as supporting either of the following possibilities i) that the hydrocarbons may be fossil terrestrial hydrocarbons introduced after arrival of the meteorites on earth ii) that they may be remnants of life that existed on the earth moon system billions of years ago and were subsequently returned to earth by lunar meteoritic impacts in accordance to the hypotheses of Urey (496) and iii) that they may be abiogenic hydrocarbons produced during genesis of the meteorites and/or

during their flight in space. In summary no support for extraterrestrial life as a source of these hydrocarbons could be justified.

The presence of pristane and phytane, both considered to be biological markers was also detected in the gas chromatograms of several meteorite extracts. In a few instances mass spectra of these compounds had been obtained but the identifications were only considered tentative in view of the slight discrepancies between these spectra and those of the corresponding standards, and also because of possible contributions of closely related isomeric structures, a fact emphasized by Hayes in his recent review on the organic constituents of meteorites (201). As a continuation of the work in our laboratory (350,352,364,368) a new series of analyses were undertaken emphasizing the mass spectrometric identification of all the compounds previously detected in meteorites by gas chromatographic techniques. Some preliminary results on the gas chromatographic-mass spectrometric identification of n-alkanes, methyl branched paraffins, cycloalkanes and isoprenoids have already been reported for the first time (357). In a recent article Mason (308) states that

> the present situation has been reviewed by Hayes (201) and can perhaps be summarized as follows. Hydrocarbons, alkanes, cycloalkanes and aromatic-phenolic compounds, and fatty acids have been identified in one or more carbonaceous chondrites. Amino acids have been reported in minute amounts, but are probably terrestrial contaminants.

Heterocyclic nitrogen compounds of no biological significance (melamine and ammeline) have been identified. Minute amounts of porphyrins have been found. Evidence for optical activity or the presence of extraterrestrial organisms is still inconclusive. The isoprenoid hydrocarbons pristane, phytane, possible degradation products of chlorophyll have been identified by Meinschein and independently confirmed by Oró and his coworkers. The evidence for nonbiological origin of meteoritic organic compounds is easily summarized: every compound found in meteorites, except pristane and phytane, has been made in some type of abiogenic synthesis.

III.

EXPERIMENTAL

EXPERIMENTAL

A. List of Samples Analyzed

1. Organisms

Bacteria: (a) *Sarcina lutea*, (b) *Bacillus cereus*, (c) *Staphilococcus aureus*, (d) *Halobacterium cutirubrum*, (e) *Vibrio marinus*.

Algae: (a) *Algal mat populations*, (b) *Anacystis nidulans*, (c) *Chlorella pyronoidosa*, (d) *Anacystis montana*, (e) *Botryococcus braunii*, (f) *Fucales*, (g) *N. putrida*.

Plants: (a) Cabbage, (b) clover, (c) mistletoe, (d) seeds, (e) fungal spores.

2. Products of Biological Origin

(a) Shark liver oil, (b) Robuoy pristane, (c) sheep manure, (d) crude oil.

3. Other Terrestrial Products

(a) Tobacco smoke, (b) dust, (c) graphite.

4. Products of Abiological Syntheses

(a) Amino acids, (b) Fischer-Tropsch hydrocarbons.

5. Meteorites

Carbonaceous chondrites: (a) Essebi, (b) Grosnaja, (c) Mokoia, (d) Murray, (e) Orgueil, (f) Vigarano.

Nodules from iron meteorites: (a) Odessa, (b) Cosby's Creek, (c) Canyon Diablo.

B. Analytical Procedure

The present analyses of meteorites, terrestrial products and products of biological as well as abiotic origin are based on the extraction and fractionation of their lipids (320,323), followed by the gas chromatographic-mass spectrometric study of their individual components. Essentially these same methods have been used repeatedly in our laboratory for the past four years (350,364,365,366,367,369,371) and only slight modifications have been introduced in the course of this investigation. The bench set up of the apparatus is shown in Figure 1.

1. Extraction

The extractions were carried out in all-glass Soxhlet-type extractors shown in Figure 2 (320). The extraction solvent, 50 ml of a 3:1 benzene-methanol mixture, was heated to moderate reflux by means of an electric heating mantle, the output of which was controlled by a powerstat. The condensed solvent drained from the condenser through the pulverized sample which was supported by a discoidal filter made of coarse powder fritted or sintered glass. Preextracted glass beads were used to prevent bumping. All power to the apparatus was controlled by an automatic timer. Extractions were carried out for a minimum of eight hours. When meteorites were extracted 0.5 g of colloidal copper was added to the system to quantitatively remove the elemental sulfur (46)

FIGURE 1

PHOTOGRAPH OF EQUIPMENT USED IN THE ANALYSES OF TERRESTRIAL AND EXTRATERRESTRIAL ORGANIC MATTER

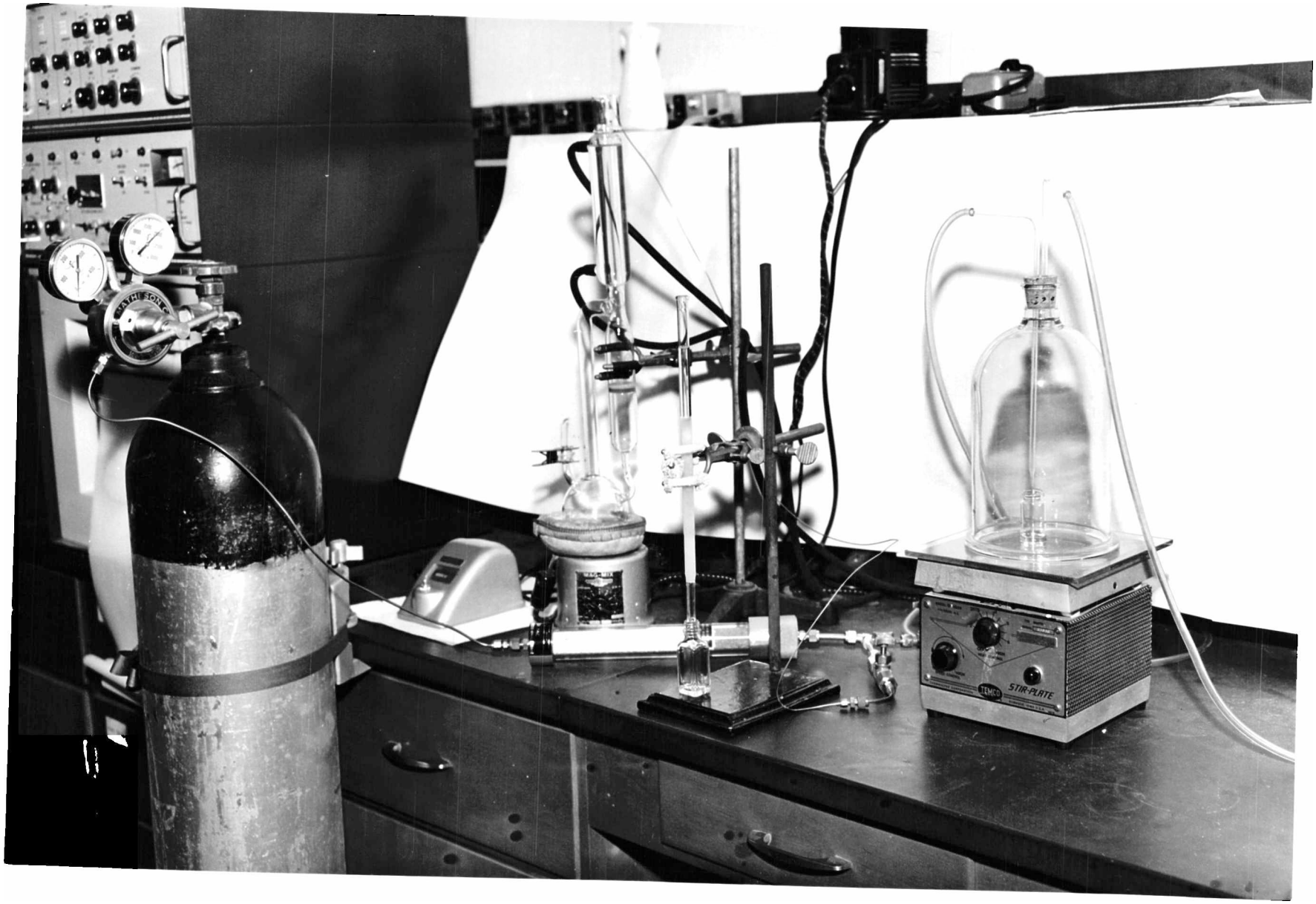
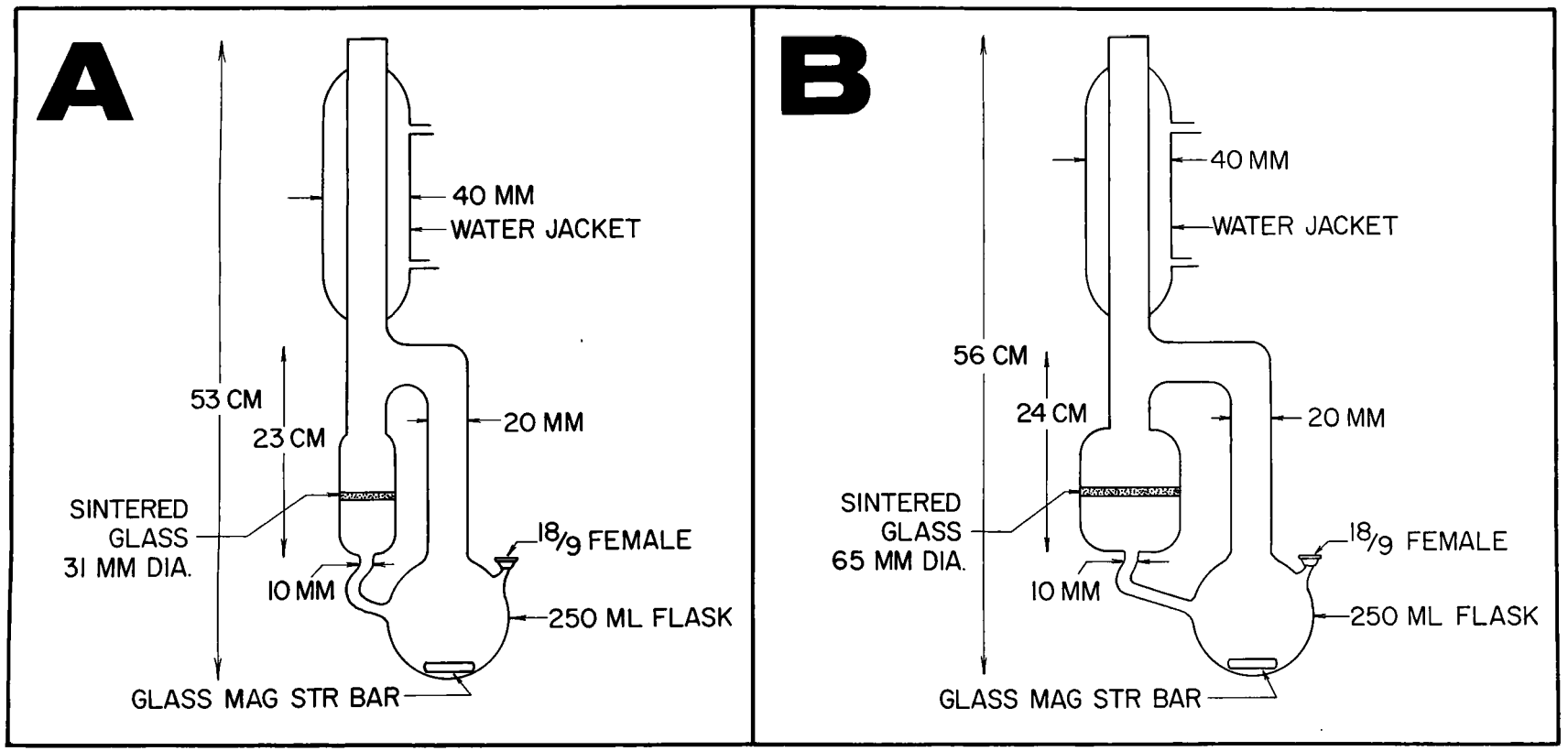

MATHESON
TEMCO
STIR-PLATE

FIGURE 2

DRAWING OF SOXHLET-TYPE EXTRACTORS



SOXHLET-TYPE EXTRACTORS

present in the meteorite extracts.

After extraction, the solvent containing the liposoluble material extracted from the samples was carefully poured through the 9/18 ground glass opening (Figure 2) of the soxhlet into a 100 ml beaker. The solvent was removed by evaporation at 40°C (see inverted bell jar in Figure 1) under a stream of water-pumped nitrogen, which was purified by passing it through a Dri-Pac column (Illinois Instrument Group, Des Plaines, Illinois) containing molecular sieves and humidity-indicating silica gel (see Figure 1). Some samples that did not require the soxhlet extraction procedure were extracted in eigher a liquid-liquid or liquid-solid phase using pentane as the extracting solvent. The pentane extracts were decanted into a beaker and evaporated to dryness or to a small volume under a forced stream of nitrogen as previously described.

2. Fractionation

All the extracted organic residue was separated on a 1x30 cm glass column provided with a sintered-glass filter disc and filled to a depth of 16-18 cm with silica gel that had been heat activated 24 hours at 410°C (see Figure 1). The column was washed with 20-40 ml of n-pentane or occasionally n-heptane. Three 3 ml aliquots of n-pentane was then agitated with a glass stirring rod and transferred to the top of the column. After the residue was added to the column it was

separated into 3 fractions. Each eluate was collected in 57x28 mm (20 ml) glass vials. The first fraction containing the aliphatic hydrocarbons was eluted from the column with either n-pentane or n-heptane. The second fraction was obtained by elution with benzene and contained the aromatic compounds as well as the ketones and other related constituents. The third fraction was obtained by elution with methanol; this fraction contained the glycerides and other lipids.

The eluates to be studied in each particular case were evaporated to the desired volume under a stream of purified nitrogen, as previously described. They were not in any case taken to dryness in order to try to avoid any changes in the original distribution of the low molecular weight compounds in the sample. Further fractionation of the alkane eluates can be achieved by sieving.

Molecular sieving

It constitutes a convenient way to achieve the physical separation of the branched and cyclic alkanes from their normal counterparts. Pellets of 5Å molecular sieve were dried for 19 hours at 400°C under vacuum. The samples to be sieved were dissolved in 20 ml of isooctane (Phillips Petroleum Company, spectroquality) and refluxed, with about 2 g of sieves for each sample, in a round bottomed flask fitted with a reflux condenser, for 1-2 hours (143). The ratio of sieve

to sample was between 20:1 to 100:1 (351). The solution containing branched-cyclic alkanes was then centrifuged. The remaining sieve was refluxed with benzene in a soxhlet extractor for eithg hours and the washings added to the solution of the branched-cyclic alkanes which was evaporated and analyzed.

3. Chemical pretreatment of the samples

It generally involves the use of simple chemical reactions which by changing some of the physical properties of a molecule, without disturbing its structural identity, allow a wider application of the gas chromatographic-mass spectrometric techniques.

Fatty acid methyl esters

Fatty acids were liberated from the methanol fraction by alkaline hydrolysis. The residue from the methanol fraction was placed in a 20 ml apparatus containing 10% NaOH and 50% methanol for three hours at 75°C. The hydrolysate was cooled and adjusted to a Ph of 1 with hydrochloric acid. It was then extracted three times with 5 ml of n-heptane. The extract was taken to dryness under a stream of nitrogen. The fatty acids were prepared for gas chromatography by putting the residual in a refluxing condenser to which was added methanol containing 0.5% (by weight) concentrated sulfuric acid and 5% (by weight) 2,2-dimethoxypropane (9).

After two hours of refluxing, the reaction was stopped with water and the methyl-acid-esters were extracted three times with n-heptane. The n-heptane extracts were prepared for analysis as described above.

Hydrogenation

The n-heptane eluate from a silica gel column (from Sarcina lutea) was placed in a 500 ml glass reaction vessel with 20 ml of diethyl ether and approximately 0.1 g of Palladium on powdered charcoal (10%) (Sargent Laboratory and Supply Co., Houston, Texas). Care was taken when adding the palladium to the mixture in displacing the air in the vessel with nitrogen, to avoid solvent-vapor combustion by the dry catalyst. The reaction vessel was placed in a Parr low-pressure rocker type hydrogenator (Parr Instrument Co., Moline, Ill.) for 30 hours at room temperature and a hydrogen pressure of 10 psi. The vessel was removed and the solvent evaporated off by a stream of nitrogen at 40^{o}C. The catalyst was separated from the reduced product by transferring the residue with n-heptane to a silica gel column and eluting the hydrocarbons with n-heptane as before.

4. Gas chromatographic and gas chromatographic-mass spectrometric analyses

The eluates obtained in the fractionation step were prepared for GC and GC-MS analysis by evaporation of the

solution to the desired volume (usually from five to fifty microliters, depending on the kind of sample). Aliquots of this solution containing, either the hydrocarbons, the aromatics, ketones, etc., or the fatty acids, were injected in the gas chromatograph by means of a Hamilton 10 microliter syringe (No. 701-A). From 0.5 to 3 microliters of solution were injected in capillary columns and up to 8 ml in packed columns. A description of the gas chromatographic columns used in the various phases of this work is given in Table I.

Almost all of the gas chromatographic analyses presented in the thesis were recorded on a F&M Model 810 Gas Chromatograph, equipped with a flame ionization detector, F&M Scientific Corp., Avondale, Pennsylvania. Because gas chromatography by itself is not an unequivocal way to the structural identification of organic compounds it was used mainly as a very efficient separation tool without placing too much significance on the chromatographic position of any particular compound. Thus the validity of all of the identifications reported in this work rests on the gas chromatographic-mass spectrometric analysis of the samples. The theory behind the combination of a gas chromatograph with a mass spectrometer and the wide range of applications of the technique has been discussed in some detail in the previous chapter. It will be briefly reviewed here in connection with the experimental description of the LKB-9000 Gas Chromatograph-Mass Spectrometer,

shown in Figures 3 and 4.

The block diagram of the instrument in Figure 4 illustrates its mode of operation. The model 9000 is a 60° angle, single focusing mass spectrometer with a 20 cm radius magnetic analyzer. The sample entering the ion source is subjected to bombardment by the electrons (0-100 eV) emitted by a Rhenium filament. The ions produced in this form are (1) accelerated out of the source by a variable maximum accelerating voltage of 3.5 KV and (2) magnetically scanned in the analyzer region by the action of a sweep generator (0-13 Kilogauss). Ion detection is accomplished by a 14-stage electron multiplier, followed by an electrometer and a wide band amplifier that feeds a direct-writing UV recording oscillograph for the display of the mass spectrum.

The chromatographic patterns are obtained simultaneously by the continuous recording of the signal from a total ion monitor placed at the exit of the ion source.

The critical point in the system, as discussed before, lies in the pressure reduction system or sample enricher. Its basic operating principle (Figure 5) has already been discussed in some detail and it will not be repeated here (see Chapter IIA).

The mass spectra of the components of a given mixture were taken as each of the corresponding individual compounds emerged from the gas chromatographic column.

FIGURE 3

PHOTOGRAPH OF THE LKB TYPE 9000 GAS CHROMATOGRAPH-MASS SPECTROMETER COMBINATION

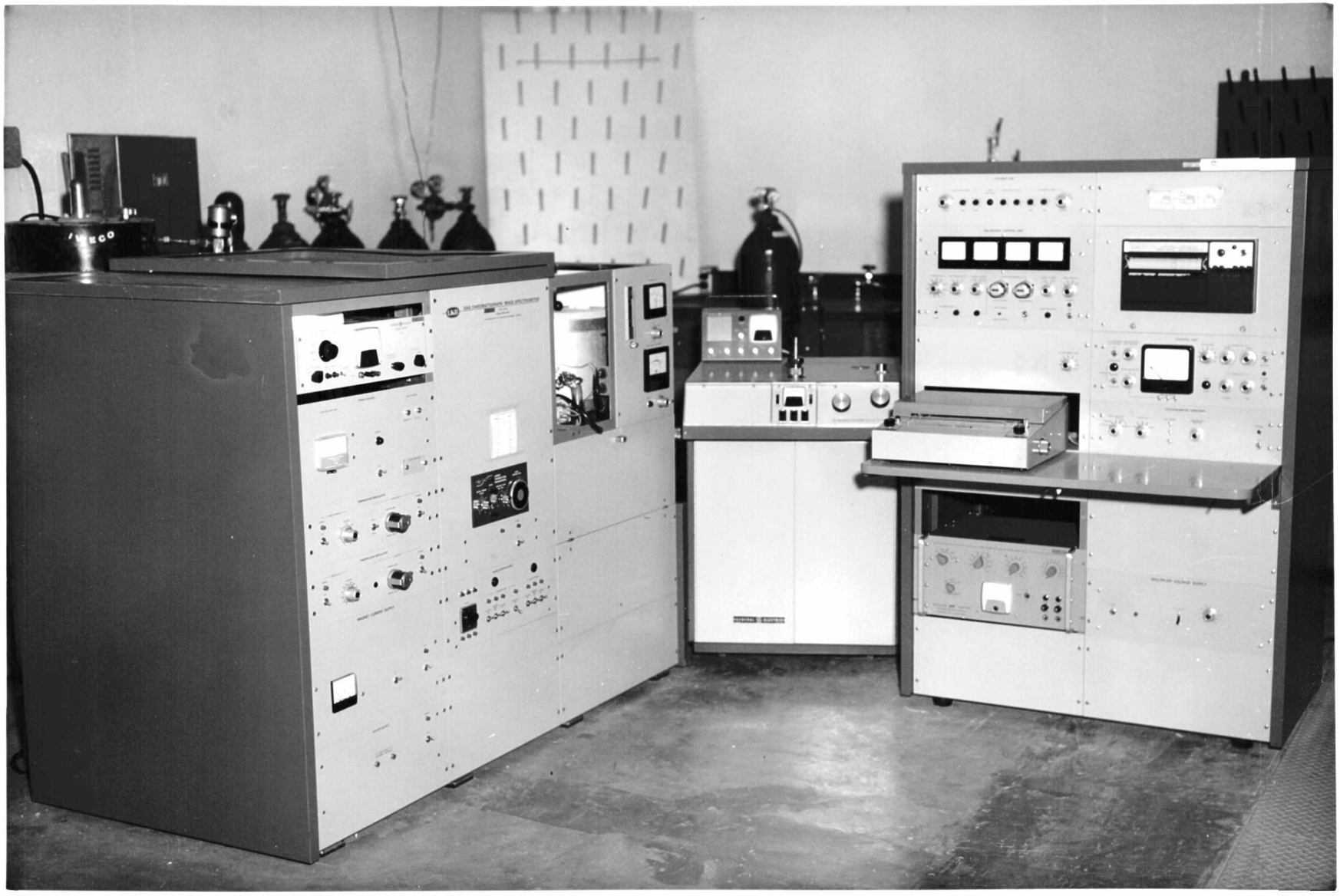


FIGURE 4

BLOCK DIAGRAM OF THE LKB TYPE 9000 GAS CHROMATOGRAPH-MASS SPECTROMETER COMBINATION

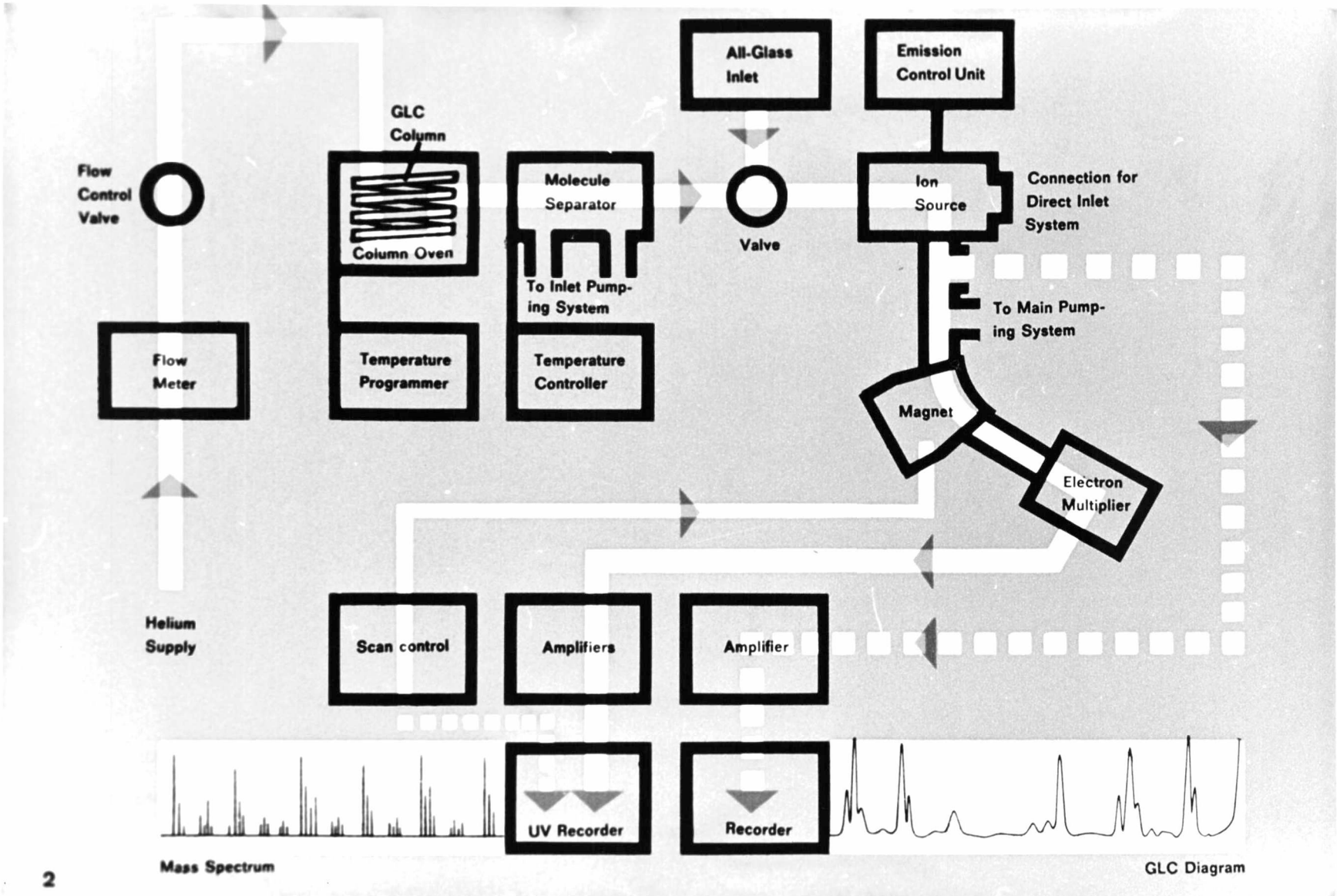
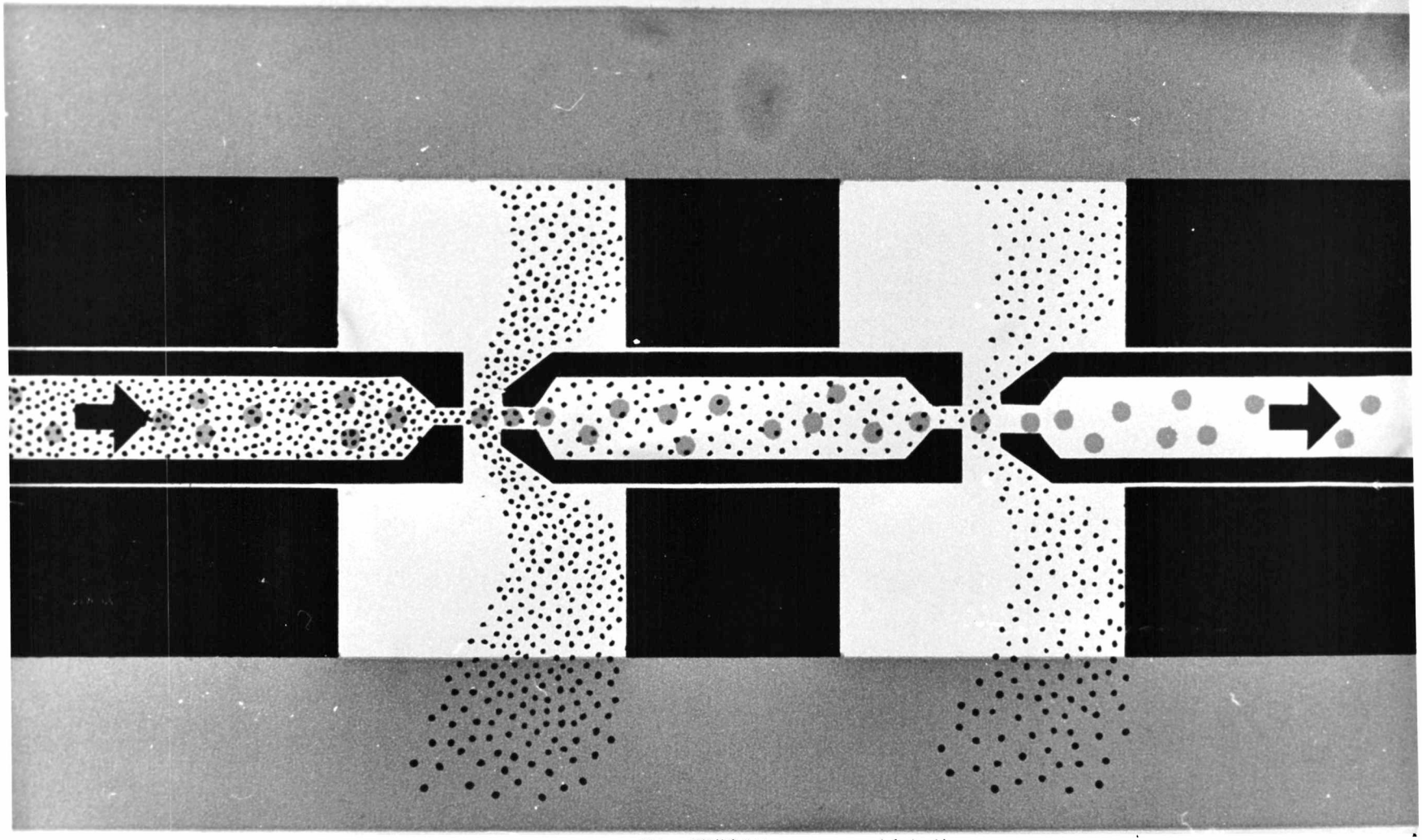

All-Glass Inlet
Emission Control Unit
GLC Column
Flow Control Valve
Molecule Separator
Ion Source
Connection for Direct Inlet System
Column Oven
Valve
To Inlet Pumping System
To Main Pumping System
Flow Meter
Temperature Programmer
Temperature Controller
Magnet
Electron Multiplier
Helium Supply
Scan control
Amplifiers
Amplifier
UV Recorder
Recorder
Mass Spectrum
GLC Diagram

FIGURE 5

SKETCH SHOWING PRINCIPLE OF MOLECULAR SEPARATOR

Sketch showing principle of molecular separator



Typical operating conditions were:

Electron energy:	20 or 70 eV
Ionizing current:	60-125 μA
Accelerating voltage:	3.5 KV
Temp. of ion source:	≈250-290°C
Analyzer pressure:	$5X10^{-6}$-10^{-7} mm Hg
Electron multiplier voltage:	1.7-2.5 KV according to the size of each peak
Scanning speed:	25 millisec / amu

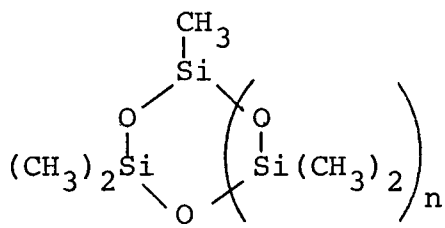
The specific conditions used in different analyses will be found in each of their corresponding sections.

Bleeding of the gas chromatographic columns

The thermal stability of the liquid phase constitutes a very important factor to consider in this type of work because it may reduce considerably the sensitivity of the instrument towards the sample if it comes off the column together with a relative large proportion of the phase. In other words since the molecular separators can not be selective towards sample vs "bleeding" the best performance of the instrument will be dependent upon the highest ratio of sample to liquid phase "bleeding". This is not easy to achieve and in most cases columns considered to be low "bleeders"

when taken to the mass spectrometer give rise to very populated backgrounds. In any case, of all the columns used (see Table I) the silicone and the polyphenyl ether phases gave the best results and among them the Polysev columns were rated as the best in terms of the quality and quantity of their "bleed".

In fact a moderate amount of background peaks is in a sense desirable if they can be used as internal mass markers. In general silicone oils give a very characteristic array of peaks at m/e 133,147,207,221,267,281,295,341,355,415,and 429 (reference 42, p. 70,171). The major peaks observed are the ones at m/e 207,281,and 355 which arise from the ions:



where n=0,1,2,3 ...

These fragments in general do not usually interfere too much with the samples studied because with the exception of the m/e 267,281, and 295 they do not belong to the alkyl ion series.

5. Other methods of analysis

Thin layer chromatography and infrared analysis were used in some instances. But since none of these methods was

TABLE I

GAS CHROMATOGRAPHIC COLUMNS AND STATIONARY PHASES

Stainless Steel Columns*	Stationary Phase	Literature References**
1. 91 m x 0.076 cm	Polysev (m-bis m-(phenoxyphenoxy)-phenoxy benzene), Applied Sci. Lab. Inc., State College, Penna.	170, 348, 350, 359, 362 357, 364, 365, 369, 368 371, 480
2. 155 m x 0.076 cm	Same as 1	
3. 310 m x 0.076 cm	Same as 1	
4. 31 m x 0.026 cm	10% Apiezon L (a high temperature grease), Applied Sci. Lab., Inc., State College, Penna.	234, 246, 363, 170, 482 350
5. 150 m x 0.05 cm	OV-17 (fluid methyl phenyl silicone) Applied Sci. Lab., Inc., State College, Penna.	170, 171, 483
6. 155 m x 0.076 cm	Igepal Co-990 (nonyl phenoxy poly-oxyethylene ethanol), General Aniline and Film Corp., New York, New York.	170, 371, 348, 480, 481 482
7. 155 m x 0.05 cm	Same as 6	

TABLE I (CONTINUED)

GAS CHROMATOGRAPHIC COLUMNS AND STATIONARY PHASES

	Glass Columns	Stationary Phase	Literature References**
8.	1.7 m x 0.3 cm	1% SE 30 on 80-100 Mesh Chromosorb W (Aw-DMCS), Curtin Chemical Co., Houston, Texas.	329, 170, 366, 367
9.	1.7 m x 0.3 cm	1% OV-1 (Methyl silicone gum), Applied Sci. Lab., Inc., State College, Penna.	170

* Stainless Steel tubing was obtained from Handy and Harmon Tube Co., Inc., Norristown, Penna.

** Taken in part from Analabs' Guide to Stationary Phases for Gas Chromatography, 1968 edition and from work carried out at this laboratory.

used extensively, the details of these analysis will be given in relation to each individual case where they were applied. In general none of these methods is sensitive enough for the determination of the trace amounts of organic matter frequently encountered in most of the samples analyzed.

6. Quantification of results

It has been mentioned (348,350) that in general the largest error in obtaining quantitative results for the microanalysis of organic compounds lies, in part in the difficulty in dissolving the sample and obtaining a properly measured aliquot and in part in the uncertainty involved in measuring the width of narrow peaks for the purposes of area computation.

With this in mind, and in order to obtain the most precise quantitative results possible, the total volume of the solutions ready for analysis was accurately measured with a Hamilton syringe (50-100 microliters depending on the volume of the solution). Then the proper aliquots were taken from it with a smaller Hamilton syringe (10 μl) and were injected in the gas chromatograph. Care was taken in making the necessary corrections in the cases where some of the sample was left in the syringe after the injection. For instance after the injection of 1 μl sometimes as much as 0.2 μl may remain in the syringe (depending on the positive pressure in the injector system against which the needle plunger is driven).

The areas under the peaks were obtained in this case by means of an electronic integrator, Infotronics CRS-llAB/H/41. This is an all-transistorized printing electronic digital integrator which takes the output signal from the GC detector and filters and amplifies it independently of the recorder. Digitizing and integration are accomplished by converting input signals to a proportional high frequency pulse train. The time integral (peak area) of the input signal represents the number of pulses accumulated in its electronic counter during a given time interval. The count rate can be as high as 50000 per second.

An automatic peak sensor detects peak onset and end, as well as retention time. The areas and retention times are printed digitally in columnar form on adding machine tape. The recorder is connected to the integrator analog output terminals and this set up does not interfere with its normal operation.

7. Contamination

a) During analysis

One of the most important analytical factors on this kind of investigations is the adequate control of the contamination levels introduced during the analysis. While it perhaps would not be realistic to assume that even with due care no contamination at all can be introduced during sample manipulation it is of utmost importance to keep the amount

of contamination regardless of its source, very close to or below thr instrumental detection limits. The three most direct sources of contamination during analysis are solvents, chromatographic materials, (silica gel), and equipment.

Several different organic solvents had been tested for purity by Dr. Nooner in our laboratory (348). It was shown that of the various commercial solvents tested the Spectral Quality reagents from Matheson Coleman and Bell were of the highest quality (348,350). Later it was also realized that blank correction values were necessary in cases where spectroscopic grade benzene had been used due to its trace amounts of aromatics (352). Consequently higher purity Nanograde benzene was used in all the analysis presented here.

A similar situation exists between the Spectroquality grade methanol and the higher purity Pesticide grade methanol, although differences are not that pronounced in this case because methanol is the solvent used in smaller volume (12.5 ml per extraction). A description of the solvent evaluation procedures used in connection with this work can be condensed as follows:

1a. n-Heptane

Matheson Coleman and Bell, Spectroquality grade Cat. No. HX 77. Quality evaluated by gas chromatography by either of the two following methods:

a) Twenty to twenty-five ml passed through a silica gel column, evaporated just to dryness under a stream of N_2 and then diluted with benzene. About 1/2 of the dilute sample injected in the chromatograph.

b) Same amount evaporated to a total volume of 1 or 2 µl and injected directly in the gas chromatograph. In both cases the amount of impurities in the C_{12} - C_{20} range was extremely low (below the nanogram level). In spite of this it was discontinued because the concentrations of trace components were found to be variable on different solvent batches.

1b. n-Pentane*

Matheson Coleman and Bell, Spectroquality grade Cat. No. PX 165, SG 2466. Evaluated by gas chromatography (same way as n-heptane). Found free of impurities at the levels of detection sensitivity used.

2. Benzene

Matheson Coleman and Bell, Spectroquality Cat. No. BX 215, SG 2738. Quality evaluated by gas chromatography. Found relatively free of trace amounts of alkanes (same procedure as for the n-heptane) but discontinued in favor of the nanograde quality, for reasons stated before.

Benzene*

Mallinckrodt Chemical Works, Nanograde Cat. No. 1043. Evaluated by gas chromatography by either of the two following

methods:

a) 50 ml of solvent evaporated under a stream of N_2 to a final volume of approximately .01 ml. Of this 1 µl taken for injection into the gas chromatograph.

b) Same as heptane. Found free of impurities at the levels of detection sensitivity used.

3a. Methanol*

Matheson Coleman and Bell, Spectroquality Cat. No. MX 475, SG 7859. Evaluated by gas chromatography (same way as n-heptane). Found free of impurities at the levels of detection sensitivity used.

3b. Methanol*

Matheson Coleman and Bell, Pesticide grade Cat. No. MX 484. Evaluated by gas chromatography (same way as n-heptane). Found free of impurities at the levels of detection sensitivity used.

*Solvents most commonly used.

The standard gas chromatographic method of direct solvent evaluation used in our laboratories although perhaps far from ideal has proved repeatedly its practical value.

In summary the overall procedure consists in checking the quality of the solvent in question under the same instrumental conditions that those used for the samples. The difference being that while usually an aliquot of 1 part out of 5-50 parts is taken from a sample for analysis, the solvents are

checked by injection of either the total or half of the solvent concentrate volume. (See Figure 6). The solvent concentrate volume refers to the volume left after evaporation of an amount of solvent (25-50 ml) equivalent to that introduced in the preparation of the samples. Thus, if the gas chromatogram of the solvent concentrate obtained with the same column and instrument used for the analysis of the samples appears to be free of trace components, the solvent is considered suitable for its use in sample preparations.

At this point it may be appropriate to bring to the attention of all the investigators in this field the use of the low temperature zone melting technique for the production of ultrapure solvents (Huckle, M. T. Chemistry and Industry, 1490 (1966); Beynon, J. H., Mass Spectrometry, Elsevier (1960) p. 191).

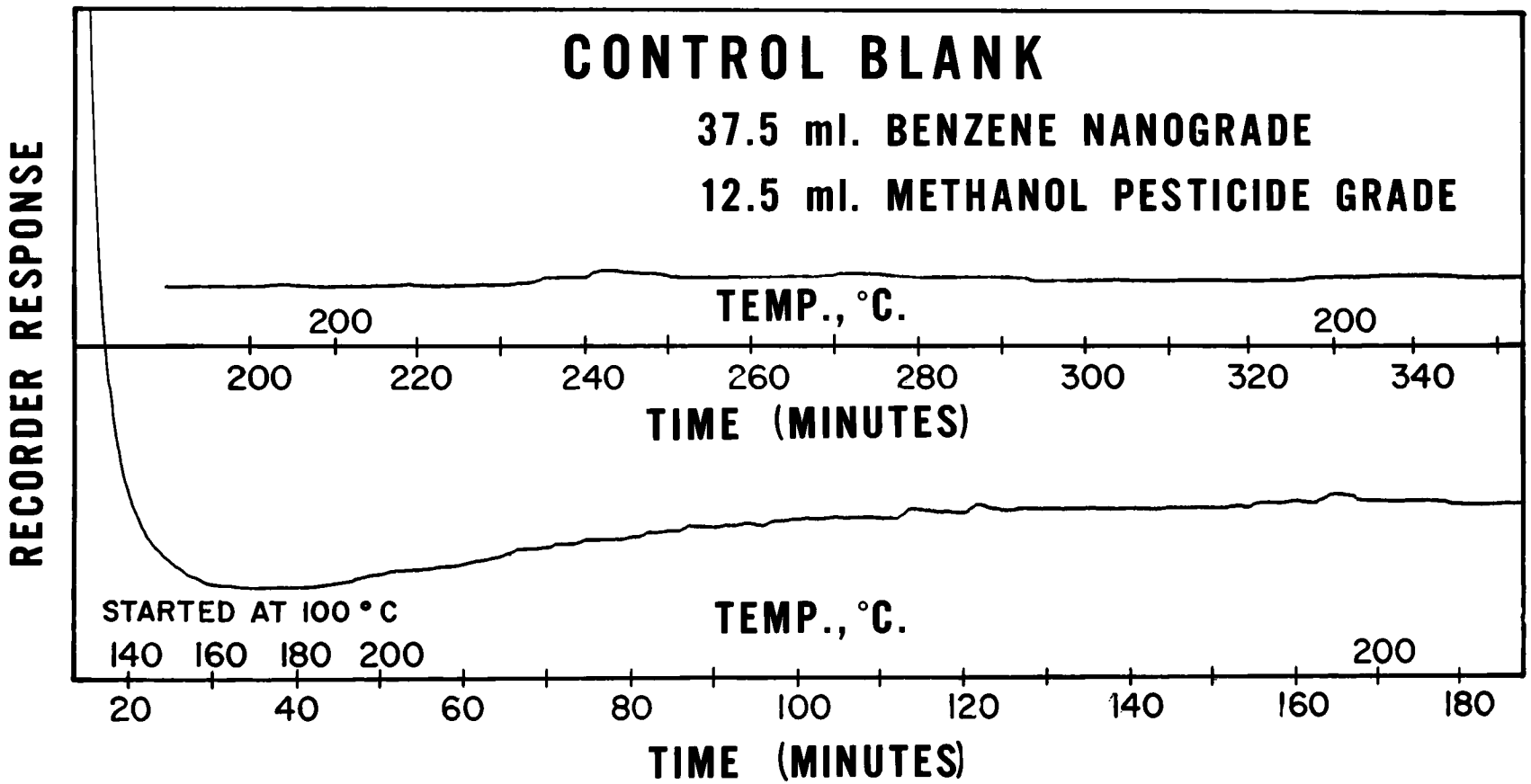
The silica gel (Baker Analyzed Reagent) used in column chromatographic separations was purchased from W. H. Curtin and Co., Houston, Texas, and was found for the most part to be relatively free of contamination (348). The column washings were also collected for later analysis. In a few instances they were found to contain small amounts of alkanes. For this reason the columns were washed twice with 20 ml of solvent before charging the samples. The glassware was cleaned with hot fresh chromic acid, washed with distilled water and dried. This method has previously been proved to give good

FIGURE 6

EXTRACTION SOLVENT BLANK

Total injection (1/1) of a volume of 50 ml of extraction solven (Benzene-methanol, 3:1) evaporated to a final volume of about 1 µl.

Stainless steel capillary column (195 m long by 0.076 cm i.d.) coated with Polysev (see Table I). 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Range 10; attenuation, 1. Nitrogen pressure, 1053 g/cm^2. No split. Temperature programmed from 100°C to 200°C at approximately 2°C/min.


CONTROL BLANK
37.5 ml. BENZENE NANOGRADE
12.5 ml. METHANOL PESTICIDE GRADE
RECORDER RESPONSE
TEMP., °C.
200
200
200
220
240
260
280
300
320
340
TIME (MINUTES)
STARTED AT 100 ° C
TEMP., °C.
140 160 180 200
200
20
40
60
80
100
120
140
160
180
TIME (MINUTES)

results (350). A more detailed discussion about the handling of the equipment is given in the section pertaining to the meteorite nodules.

b) Before the analysis

There is no way in which this can be prevented since most of the samples (especially the extraterrestrial samples) have had very uncertain histories before reaching the laboratory. Perhaps the only approach in this case is to attempt to establish correlations if any between the type and distribution of the organic matter found in the samples analyzed and the environment to which they have been exposed.

IV.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF NATURAL PRODUCTS

GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF NATURAL PRODUCTS

Capillary gas chromatography is today one of the most powerful tools for the analysis of wide boiling mixtures or difficulty separable isomers, but in general the chromatographic separation process alone does not lead by itself to an unequivocal determination of the identity of the components present in the particular mixture under study. Thus the combination in series of a gas chromatograph with a mass spectrometer allows the use of the latter in the molecular structure determination of each individual component as it is isolated by the gas chromatographic column.

The growing importance of mass spectrometry in the field of molecular structure identification (42,76,77,78) is reflected in the large number of recent reviews on the subject (19,43,57,127,128,294,342,450).

The initial work of Stenhagen and Ryhage on fatty acid derivatives (415,417) and of Biemann on alkaloids (42) has been expanded in many laboratories throughout the world which are engaged in research problems requiring the structural identification of natural products. In the course of the present work the combined technique of gas chromatography-mass spectrometry has been applied repeatedly to the following classes of compounds:

A. n-alkanes

B. isoalkanes

C. isoprenoids

D. alkenes

E. unsaturated isoprenoids

F. naphthenes

G. deuterated hydrocarbons

H. ketones

I. fatty acids

J. amino acid derivatives

K. steroids

A discussion on the characteristic fragmentation phenomena observed under electron bombardment for each of these type of compounds as well as a description of their corresponding gas chromatographic properties will be attempted in this chapter to provide a background for the later discussions on the actual applications of gas chromatographic-mass spectrometric techniques in the analysis of natural products.

A. n-alkanes

The mass spectra of the normal saturated hydrocarbons can be considered among the simplest of the fragmentation patterns exhibited by natural organic products (214). The typical spectra of an alkane shows a smooth-curve distribution of "fragment peaks" corresponding mainly to ions of the following

stoichiometry; C_nH_{2n-1} (alkenyl), C_nH_{2n} (alkene or olefin) and C_nH_{2n+1} (alkyl), in order of their ascending m/e values. The highest mass peak (C_nH_{2n+1}) in each carbon number region, known as the "head peak" (184), is also the highest in intensity (Figure 16). The distribution curve has a maxima around the C_4, C_5 fragments with a rapidly decreasing slope that terminates at the "parent peak" (C_nH_{2n+2}) or molecular ion, which is the unfragmented molecule minus one electron.

A convenient presentation of the mass spectral data other than the common graphic plot of atomic mass units vs relative peak heights is represented by the matrix or grid form (102,353). In this type of data presentation, the masses of each homologous series of peaks are arranged in fourteen columns characterized by the "z-number" which is the value obtained from the formula C_nH_{2n+z}. Since the mass difference between two consecutive carbon numbers amounts to 14 amu (the CH_2 group) there will be 14 z-numbers ranging in value from -11 to +2 and representing all fragment peaks of integral mass allowed between each n-paraffin carbon number. Thus all mass numbers corresponding to a given z-number fall in one column and all mass numbers corresponding to a given C number fall in one row. The m/e values increase consecutively by a value of one in going from left to right along a horizontal row and by increments of fourteen units when going down a

column. Table II illustrates this point and at the same time provides an example of a typical n-alkane mass spectrum. In this table the z=+1 column contains all the C_nH_{2n+1} fragments ("head peaks") which are the highest peaks in each C number region, except in the parent peak region where the z=+2 column contains the relative intensity of the molecular ion peak. The molecular ion in normal hydrocarbon spectra is always higher in intensity than the immediately preceeding alkyl ions. For handier reference and easier cross checking with the mass spectra presented throughout this work the masses of the alkyl ions (z=+1) have also been included (in brackets) together with the carbon number, n, (Table II).

In general the alkyl fragments are higher in intensity than the olefin fragments and these in turn are higher than the alkenyl ions (see Figure 16 masses 125,126, and 127) except in the low molecular weight region where the alkenyl ions usually predominate up to a certain C number; in this case up to C_7. The contributions of the isotope atoms present in the molecule (H^2,C^{13}) will be felt in columns corresponding to z=+2 and z=-11 for the alkyl ions, and z=-11 and z=-12 for the molecular ions.

The single cleavage of the terminal methyl groups, to yield CH_3 radicals and retention of the positive charge in the larger M-15 alkyl fragment, is not a favorable process as indicated by the characteristic absence of a fragment of mass

TABLE II

PARTIAL MASS SPECTRUM OF n-TETRACOSANE*

		Mass Series (Z in C_nH_{2n+Z})													
n	(m/e)	Z	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	+1	+2
6	(85)						.74	.45	1.12	.43	3.33	56.7	27.5	16.8	100	6.67
7	(99)		.23				.77	.17	.24	.17	1.49	2.74	17.4	11.2	22	1.75
8	(113)		.16		.08		.24	.09	.14		.60	1.37	7.45	8.02	14.9	1.38
9	(127)		.16				.14				.27	.76	3.26	7.02	11.3	1.18
10	(141)		.11				.19				.17	.50	1.31	5.12	9.05	1.02
11	(155)		.09								.16	.38	.76	4.29	7.62	.93
12	(169)		.08								.15	.29	.54	3.75	7.67	.94
13	(183)		.08						.11		.13	.26	.44	3.20	6.06	.86
14	(197)		.08						.19		.15	.18	.28	2.68	5.49	.85
15	(211)		.08										.21	2.36	5.13	.89
16	(225)		.12										.15	1.97	4.75	.84
17	(239)		.08											1.73	4.41	.83
18	(253)		.08											1.46	4.09	.83
19	(267)		.11											1.25	3.94	.81
20	(281)		.09											1.05	3.87	.89

TABLE II CONTINUED

PARTIAL MASS SPECTRUM OF n-TETRACOSANE*

		Mass Series (Z in C_nH_{2n+Z})													
n	(m/e)	Z	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	+1	+2
21	(295)		.23											.59	2.24	.52
22	(309)		.07											.38	1.42	.26
23	(313)															
24	(327)													.16	.18	7.44
25	(331)		1.99	.26												

*American Petroleum Institute Research Project 44 Serial N. 30-m

(m/e) corresponds to the alkyl ion mass series (Z=+1)

M-15 (M being the mass corresponding to the parent ion). Table III gives the spectrum of n-pentadecane. Columns corresponding to z-numbers -10 to -4 are not shown since n-alkanes do not give fragments of appreciable intensity in this range. The paraffin peaks are concentrated prevalently at the z=+1 column and to some extent in the 0 and -1 columns. A note should be made to the fact that the relative intensity of the parent ion peak in this spectrum (Table III) taken at an electron energy of 20 eV is a 10.5% of the intensity of the C_6 fragment compared to a value of 2.9% at 70 eV (API spectra #1004). Low electron energy mass spectrometry (this chapter, C) of heavy hydrocarbons results in an increased parent peak intensity. This helps in the location of this peak in the mass spectrum, especially in the case of the heavier paraffins where the molecular ion decreases with increasing molecular weight (reference 40, pp. 241,327).

The simplicity shown by the spectra of heavy saturated hydrocarbons does not have a mechanistic parallel and in this sense it can be said that n-alkanes and heavy hydrocarbons in general give very complex mass spectra (326). Attempts to understand the complete ionic breakdown process under electron impact have not been very successful. Summarizing the current knowledge on this field Meyerson (326) states that "n-alkanes appear to break down mainly by initial non selective cleavage of carbon-carbon bonds, followed by further

TABLE III

PARTIAL MASS SPECTRUM OF PENTADECANE

n (m/e) \ Z	Mass series (Z in C_nH_{2n+2})						
	-11	-3	-2	-1	0	+1	+2
4 (57)				2.3	11	128	6
5 (71)		0.3	1	5	15	100	6
6 (85)			2.5	5.5	12	69	4.6
7 (99)			1	4.5	11	21	1.6
8 (113)			0.7	2.1	8.4	13.1	1.2
9 (127)			0.2	0.9	6.5	10.4	1.0
10 (141)				0.2	4.4	8.5	0.9
11 (155)					2.9	6.6	0.8
12 (169)					1.9	9.1	0.5
13 (183)					0.7	2	0.3
14 (197)						--	--
15 (211)							21.2
16 (225)	1.7						

LKB 9000 Gas Chromatograph-Mass Spectrometer

Ionizing current 60 μA

Electron energy 20 ev

Multiplier voltage 1.7 Kv

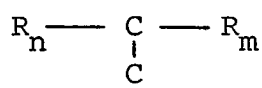
Ion source temperature 270°C

m/e: values of the alkyl ion mass series.

stepwise decomposition of the primary products, with intervening energy releasing rearrangements to give a characteristic product distribution." Examples of the gas chromatographic separations of n-alkanes in different kinds of samples will be given later. All of the stationary phases employed, polar or non polar, (Table I) have given good results in these separations. Silicon phases are particularly useful for compounds of relatively high molecular weight and for the useful background that their bleeding introduces in the spectra (see Experimental, section B4).

B. Isoalkanes

This section refers to the medium and high molecular weight monomethyl substituted alkanes represented by the structure



where n can have any value ranging from 4 to 30 and m may take values from 1 to 7, being always smaller than n.

The general characteristics of the n-alkane spectra, just discussed, apply equally well to the isoalkanes with the following special considerations:

1. The relative intensities of molecular ions are much lower.

2. Contrary to the n-alkane spectra the M-15 peak is usually higher than the parent peak.

3. The location of the tertiary or quaternary carbon atoms in the molecule is indicated by the large C_nH_{2n+1} peaks corresponding to the loss of the smaller alkyl group (R_m) attached to that carbon atom. The loss of the larger alkyl group (R_n) also takes place but at a much lower extent since retention of the positive charge in the secondary (or tertiary) C atom seems to be more favored the longer the alkyl groups attached to this carbon (with the exception of structures where m=1). This behavior can be predicted by taking into consideration the so-called "Stevenson's rule" (457) which states that upon cleavage of a bond the positive charge will reside on the particle having the lower ionization potential. The inductive effect of electron supplying substituents such as the alkyl groups (57) will lower the ionization potential of the tertiary carbon atom thus favoring the cracking at this site with the resultant formation of a stable secondary carbonium ion (Figure 7). This will be more so the larger the alkyl group.

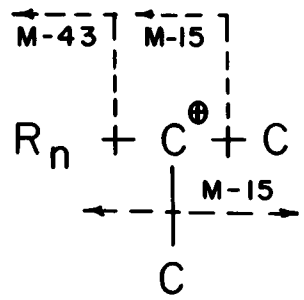
4. The two C_nH_{2n+1} fragments arising from cleavage of either of the two C-C bonds adjacent to the alkyl substituent are accompanied by a relatively high olefin ion peak which indicates the secondary loss or transfer of a hydrogen atom.

FIGURE 7

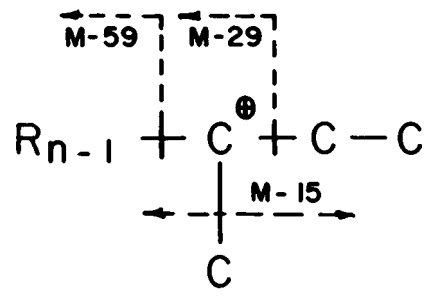
ISOALKANES. PREDICTED EFFECT OF THE METHYL SUBSTITUENT ON THE MASS SPECTRAL FRAGMENTATION PATTERNS

ISO ALKANES

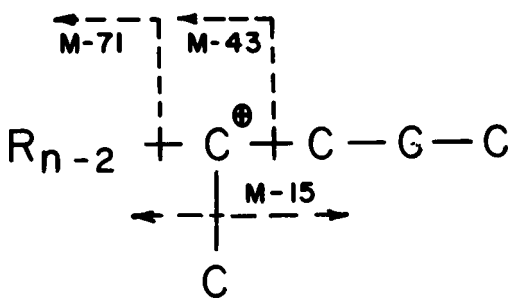
PREDICTED EFFECT OF THE METHYL SUBSTITUENT ON THE MASS SPECTRAL FRAGMENTATION PATTERNS



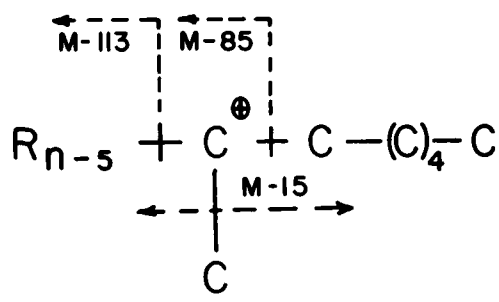
a) 2-METHYL ALKANE



b) 3-METHYL ALKANE



c) 4-METHYL ALKANE



d) 7-METHYL ALKANE

TABLE IV

PARTIAL MASS SPECTRA OF FOUR METHYL ALKANES

		Relative Intensities (Z in C_nH_{2n+Z})														
		2MeC$_{16}$			3MeC$_{16}$			4MeC$_{16}$			7MeC$_{16}$			nC$_{17}$		
	C_n \ Z	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2
	C_4	56	349	16	170	329	15	14	244	11	10	100	5	8.5	100	5
	C_5	26	256	15	35	237	14	273	409	22	15	97	6.5	10	82	5
	C_6	21	195	15	15	166	12	23	179	12	15	44	4	10	58	4
	C_7	16	100	9	10	75	7	15	76	7	7	19	2	8.5	21.5	2
	C_8	13	65	7	8	56	6	11	57	5	46	25	2.5	7	14.5	1.5
M-113	C_9	10	51	6	5	41	5	9	45	5	7.5	4.5	0.5	5.5	10	1
M-99	C_{10}	7	38	6	4	31	4	7	31	4	1.7	2.5		4.5	8.2	0.9
M-85	C_{11}	6	24	4	4	21	3	4	16		28	21	2.5	3.3	6.7	0.9
M-71	C_{12}	6	14	3	3	10	2	22	18	3	1.7	2		2.3	5.7	0.7
M-57	C_{13}	4	9		12	14	3	3	4		1.5	1.5		1.8	4.9	0.7

TABLE IV CONTINUED

PARTIAL MASS SPECTRA OF FOUR METHYL ALKANES

		Relative Intensities ($Z in C_nH_{2n} + Z$)														
		$2MeC_{16}$			$3MeC_{16}$			$4MeC_{16}$			$7MeC_{16}$			nC_{17}		
	C_n \ Z	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2
M-43	C_{14}	36	100	17	3	6	1	63	100	16	1.8	2		0.9	2.7	0.4
M-29	C_{15}		5		46	100	17	6	9		1.7	3	0.5	0.5	1.4	0.3
M-15	C_{16}	4	29	6	2	6	2	3	9		2	4.5	0.7	-	-	-
M	C_{17}			10			5			6			1.5			8.7

For experimental conditions, see Table III

All this is clearly illustrated in Table IV by the partial spectra of 2-,3-,4-, and 7-methyl hexadecane standards.

The features of the isoalkane spectra will appear superimposed on the smooth-curve distribution typical of the corresponding unsubstituted alkane; in this case heptadecane (see Table IV). The results expected for these four isoalkanes are summarized in Figure 7.

The four main characteristics for the isoalkane mass spectra listed above can be correlated with the data shown on Table IV and Figure 7 in the following way:

1. While the intensity of the molecular ion for n-heptadecane relative to m/e 71 (C_5 fragment) has a value of 10%, the intensities of the molecular ions of 2-MeC_{16}, 3-MeC_{16} and 4-MeC_{16} relative to the same peak are only 3.9, 2.1 and 1.4% respectively. This reduction in the parent ion stability towards fragmentation under electron impact can certainly be expected as a consequence of the introduction of a preferred fragmentation mode in the cracking pattern. The addition of a substituent group such as the CH_3 will lead to the stabilization of the substituted carbonium ion and the molecule will show an increase tendency to fragment at this site as indicated before.

2. The loss of the methyl substituent is reflected by the intensity of the C_{16} alkyl fragment (M-15) which is in all three cases higher than the parent ion (M). The n-heptadecane

does not show any appreciable M-15 peak.

3. The higher stability of the secondary carbonium ions (Figure 7) results in the high M-43 and M-29 peaks which are diagnostic of a methyl group very close to one of the ends of the molecule. In the case of 3-methyl hexadecane (n=14 in Figure 7b), the loss of the terminal ethyl radical would leave a relatively quite stable secondary carbonium ion fragment with 15 carbon atoms, (Table IV), while cleavage at the other side of the methyl substituent would yield a less stable primary carbonium ion of mass equivalent to M-57. This M-57 fragment is reflected in the slightly higher intensity of the C_{13} ion compared to the C_{12}. On this type of cleavage the positive charge will have a greater relative tendency to remain with the smaller fragment containing the secondary C_4 carbonium ion. That means that in the low molecular weight region the ion distribution curve will most likely have its maxima at C_4 (Table IV).

Along the same lines the 4-methyl hexadecane (Figure 7c) should exhibit a very high M-43 peak, a smaller M-71 peak and in this case the ion distribution curve should have a maxima at C_5. Likewise the 7-methyl hexadecane would be expected to show a high M-85 peak together with a C_8 fragment. Both cases are confirmed by the standard spectra shown in Table IV. In connection with this last case attention should be drawn to the height of the C_8 alkyl fragment relative to the C_7

fragment which clearly indicates, as compared to the C_{11} and C_{10} fragments, that the positive charge is still more stable in the left hand side fragment because of its larger number of carbon atoms (C_{11} vs C_8). The 2-methyl hexadecane being itself a extreme case (the substituent is in the last position available) deviates to a certain extent from these general empirical rules, according to which this compound would be expected to show a very high M-15 peak and a smaller M-43 (Figure 7a). On the other hand it shows an M-43 peak similar to that of the 4-methyl hexadecane, and a smaller M-15. The fact that the formation of a primary carbonium with 14 C atoms (R_n, n=14, Figure 7a) appears to be preferred over the secondary carbonium ion with 16 carbon atoms may be due to the relative stabilities of the two resulting radicals; $\cdot C_3$ vs $\cdot C_1$. It has been pointed out in the section about n-alkanes that the loss of the terminal CH_3 groups as radicals to give the fragment at M-15 does not appear to be an energetically favorable process.

With this in mind caution must be taken in attempting to identify the spectra of the two later compounds since it will be very easy to mistake one for the other as it will be demonstrated later.

By a close inspection of the spectra of these two compounds, given in Table IV, it can be seen that there are enough differences between the two to assist in their

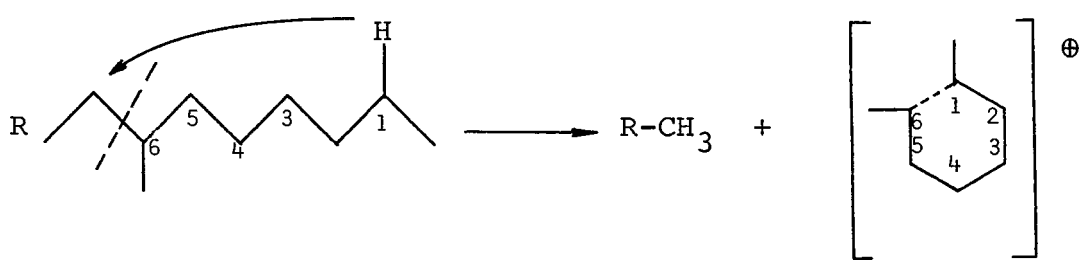
molecular structure determinations. Namely, the 4-methyl alkane has a lower M-15 peak, it shows a definite M-71 ion, maxima at C_5 and a very high olefin ion in the M-43 region. On the other hand the isoalkane shows a relatively high M-15 peak, does not have an M-71 ion, its maxima should be at C_3 and has a relatively lower olefin ion in the M-43 region. The C_3 or lower fragments will not be included in any of the spectra considered throughout this thesis because of the discriminating effect of the mass spectrometer used toward these low masses.

4. The olefin ions associated with the large alkyl fragments originated at the points of branching, can also be seen in Table IV. Usually as R_m grows (m increases) the intensity of the olefin ion relative to the alkyl ion (at the branching points) increases, too. This trend can be seen clearly in Table IV. The value goes from 36% for 2-meC_{16} (m=1) to 46% for 3-meC_{16} (m=2), to 63% for 4-meC_{16} (m=3), and eventually it surpasses the respective alkyl fragments (C_8 and C_{11}) in the 7-meC_{16} (m=5).

Although the occurrence of these secondary olefin ions in the fragmentation patterns of branched hydrocarbons has been previously observed by Biemann (reference 42, p. 80) the mechanism responsible for their formation had not been studied. Recently McCarthy et al. (283) recognizing the importance of these ions in relation to the information they may

provide in assisting the interpretation of a mass spectrum tried to establish a mechanism based on their empirical correlations.

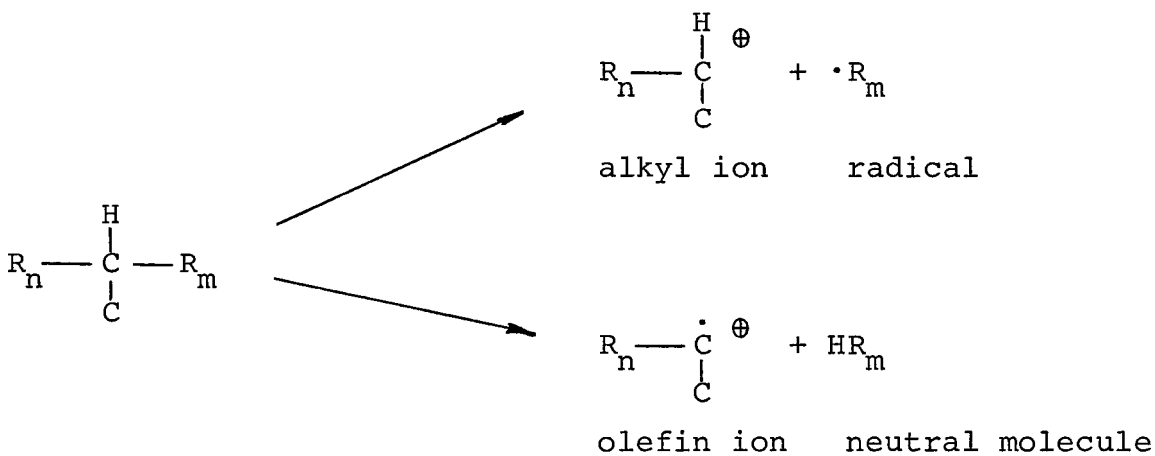
Concurrent with their efforts similar work had also been in progress in this laboratory, and the results obtained do not seem to agree with the conclusions reached by these authors. Namely, i) that the olefin peak (C_nH_{2n}) will be more intense than the corresponding alkyl peak (C_nH_{2n+1}) when the fragment ion contains seven or more carbons in the chain and ii) that the mechanism involves the transfer of hydrogen from a secondary position and takes place forming a <u>cis</u> 1,2-dialkylcyclohexane ion and an alkane, in the following way



For one thing this mechanism does not readily account for the observation that in dialkyl substituted hydrocarbons when both substituents are located on the same carbon the olefin ion is not observed.

On the other hand, in accordance with the empirical correlations established in the present work it appears that i) it is not the length of the fragment retaining the positive charge that determines the intensity of the olefin ion

associated with it, but the length of the leaving R_mH (using the nomenclature established before). In other words the larger the value of m, the larger the olefin ion formed relative to its corresponding odd fragment peak and ii) while there is not enough evidence to propose a reasonable mechanism it seems that the lost hydrogen probably belongs to the substituted carbon atom



As it has been previously pointed out, the data in Table IV for the isomeric C_{17} hydrocarbons, shows that the intensity of the olefin ions (C_nH_{2n}) increases with the value of m. Each increment in m must be necessarily accompanied by a corresponding reduction in the value of n when isomeric structures of this type are considered and while in all cases n is greater than seven, the olefin ions in the 2-,3-, and 4-methyl hexadecanes are not larger than its corresponding alkyl fragment ion. These observations have been made for the whole 2-,3-,4-,5-, and 6-methyl alkanes in the range

C_{12} to C_{20} and they appear to be very consistent. McCarthy et al. (283) have tried to substantiate their generalizations by examining other alkyl branched hydrocarbons in the mass spectral tables of the API project 44. The tabulated intensities of the even and odd branched fragments were given as shown in Table V (283). A detailed look at this table shows that the data appears to fit better when the length of the neutral molecule formed is considered instead of the length of the fragment ion.

For instance, spectrum #491 corresponding to the structure

$$C-C_5-\underset{\underset{C_3}{|}}{C}-C_5-C$$

would be expected to show prominent C_{10} and C_{13} fragments. The olefin ion would be expected to be higher in the C_{10} than in the C_{13} fragment, which is what can be observed in Table V. The loss of the C_6 molecule results in a larger olefin ion relative to the alkyl ion than that produced by the loss of propane. The same is true in numbers 1469, 1470, 1320, 593 to mention a few.

Perhaps the best example can be found in the mass spectra of the isomeric butyl docosanes given in Table VI. In spectrum #861 it can be seen that the loss of 17 carbons (heptadecane) gives a C_9 olefin which is twice as large (24.7) as that of the corresponding alkyl ion (10.7), while

TABLE V

BRANCHED ALKANE MASS SPECTRA

A.P.I.#	Compound	Even (m/e)	Intensity	Odd (m/e)	Intensity	Base Peak
2018	5-n-butylnonane	126	42.8	127	18.8	43
	5,8-diethyldodecane	98	31.27	99	21.45	71
2019	4,9-di-n-propyldodecane	98	50.4	99	24.9	71
704	7-methyltridecane	112	29.4	113	14.3	57
491	7-n-propyltridecane	140	23.1	141	12.9	57
		182	9.18	183	11.7	
592	7-n-hexyltridecane	182	29.1	183	23.9	57
1469	8-n-hexylpentadecane	196	26.20	197	23.31	71
		210	12.31	211	12.62	
1470	5-n-butylhexadecane	126	21.91	127	12.68	71
		224	15.94	225	20.72	
578	6,11-di-n-pentylhexadecane	154	24.0	155	11.1	57
1320	9-n-hexylheptadecane	210	18.48	211	15.89	71
		238	11.80	239	14.46	
983	9-n-octylheptadecane	238	19.30	239	20.91	71
593	4-n-propylheptadecane	98	19.5	99	9.84	57
		238	9.40	239	13.8	
1032	5,14-di-n-butyloctadecane	126	23.3	127	13.8	71
1257	7-n-hexyleicosane	182	12.56	183	11.01	71
		280	8.29	281	11.31	
1472	10-methyleicosane	154	16.15	155	10.75	71
		168	13.76	169	10.56	
1474	9-n-octyleicosane	238	12.87	239	13.51	71

TABLE V CONTINUED

BRANCHED ALKANE MASS SPECTRA

A.P.I.#	Compound	Even (m/e)	Intensity	Odd (m/e)	Intensity	Base Peak
864	11-n-pentylheneicosane	224 294	22.7 7.86	225 295	19.9 12.3	43
579	11-n-decylheneicosane	294	18.7	295	24.2	57
1256	11-(3-pentyl)-heneicosane	294	4.30	295	4.57	71
861	5-n-butyldocosane	126 308	24.7 11.2	127 309	10.7 18.2	43
706	7-n-butyldocosane	154 280	14.1 4.30	155 281	8.13 5.42	57
862	9-n-butyldocosane	182 252 308	15.6 8.83 7.11	183 253 309	10.9 9.06 13.0	43
863	11-n-butyldocosane	210 224 208	12.9 11.6 7.29	211 225 309	10.5 10.0 13.6	43
865	7-n-hexyldocosane	182 308	19.1 15.8	183 309	12.9 12.5	43
866	9-n-octyldocosane	238 308	11.1 13.3	239 309	10.4 17.1	43
867	11-n-decyldocosane	294 308	10.1 17.7	295 309	11.6 21.3	43
1259	11-n-decyltetracosane	294 336	6.87 8.92	295 337	8.17 12.65	71
1475	9-n-octyltetracosane	238 336	15.43 14.57	239 337	15.38 22.36	71
1355	13-n-undecylpentacosane	336	16.79	337	23.17	71

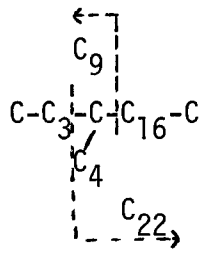
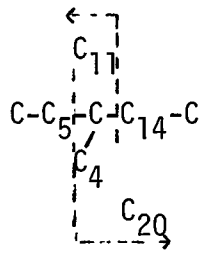
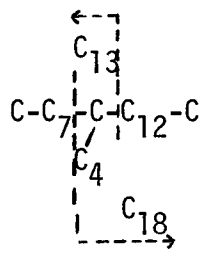
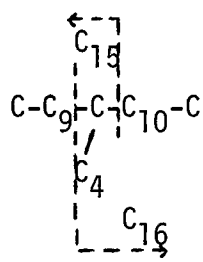
TABLE V CONTINUED

BRANCHED ALKANE MASS SPECTRA

A.P.I.#	Compound	Even (m/e)	Intensity	Odd (m/e)	Intensity	Base Peak
1476	13-n-dodecylhexacosane	350 364	7.67 13.15	351 365	10.72 19.25	71
1322	9-n-octylhexacosane	238 364	13.28 8.89	239 365	12.91 14.63	71
1357	11,20-di-n-decyltricontane	294	35.70	295	26.34	85

TABLE VI

MASS SPECTRA OF BUTYL DOCOSANES

A.P.I.		C_{n+5}	C_{m+5}
861		$\frac{C_9}{24.7/10.7}$	$\frac{C_{22}}{11.2/18.2}$
706		$\frac{C_{11}}{14.1/8.13}$	$\frac{C_{20}}{4.3/5.42}$
862		$\frac{C_{13}}{15.6/10.9}$	$\frac{C_{18}}{8.83/9.06}$
863		$\frac{C_{15}}{12.9/10.5}$	$\frac{C_{16}}{11.6/10.0}$

Values to the left in each carbon number represent the intensities of the corresponding olefin ions, those to the right represent the intensities of the alkyl ions.

the loss of 4 carbons (butane) to give the C_{22} fragment produces an alkyl ion larger than the olefin. As "m" decreases from 17 to 11 the olefin associated with the branched fragment also decreases until it almost equals the alkyl ion. At the same time since n is increasing at the same rate from C_4 to C_{10} the olefin associated with the remaining larger fragments also increases until it surpasses the intensity of the branched alkyl fragment. These correlations can also be extended to dimethyl substituted hydrocarbons and other multibranched structures such as the isoprenoids. For instance, the mass spectrum of pristane (Figure10) shows a higher C_8 olefin relative to the corresponding alkyl fragment than the C_{13} olefin.

Before applying any of these correlations to structural studies it must taken into account that the heights of the olefin ions appears to be dependent on the electron energy of the bombarding electrons. Compare Figure 48 and Figure 10. Most likely it will also be dependent on temperature. Although the proposed generalization fits well the experimental data it does not completely rule out the assumptions of McCarthy et al. (283). In fact in some cases a combination of both ideas seems to be more appropriate. Work with deuterated standards must be completed before a final word can be issued on the formation and stabilities of these olefin ions.

The isomeric methyl branched hydrocarbons are very difficult to separate from each other by gas chromatographic methods because of their close boiling points and similar low polarities. This applies even to the analyses carried out in very efficient columns and is especially accentuated for all isomers other than the 2-,3-,4-, and 5-methyl alkanes. That is, it is practically impossible to achieve a satisfactory resolution between compounds with the methyl groups in the 7 and 8 positions or higher. A gas chromatogram of a standard mixture is shown in Figure 8. Long capillary columns coated with Polysev (Table I) have been found to be the most effective in this kind of separations.

C. Isoprenoids

Although the isoprenoids have been the object of many mass spectrometric studies (31,32,112,169,178,231,282,357) few reference standards are readily available. This lack of reference material does not prove to be too much of a problem in the identification of these type of compounds by mass spectrometry because their highly ordered structures allow the development of a gross theoretical treatment of their fragmentation patterns. In general the predicted pattern fits very well the actual spectra of the few isoprenoids synthesized in the laboratory. By making use of the inherent instability of the two C-C bonds connecting a methyl substituted carbon to

FIGURE 8

GAS CHROMATOGRAPHIC SEPARATION OF A STANDARD MIXTURE OF ALKANES

Stainless steel capillary column, 310 m long by 0.076 cm i.d., coated with Polysev. Range 10; attenuation, 2. Nitrogen pressure, 1050 g/cm^2. No split. Temperature started at 80°C and programmed at 1°C per minute to 200°C.

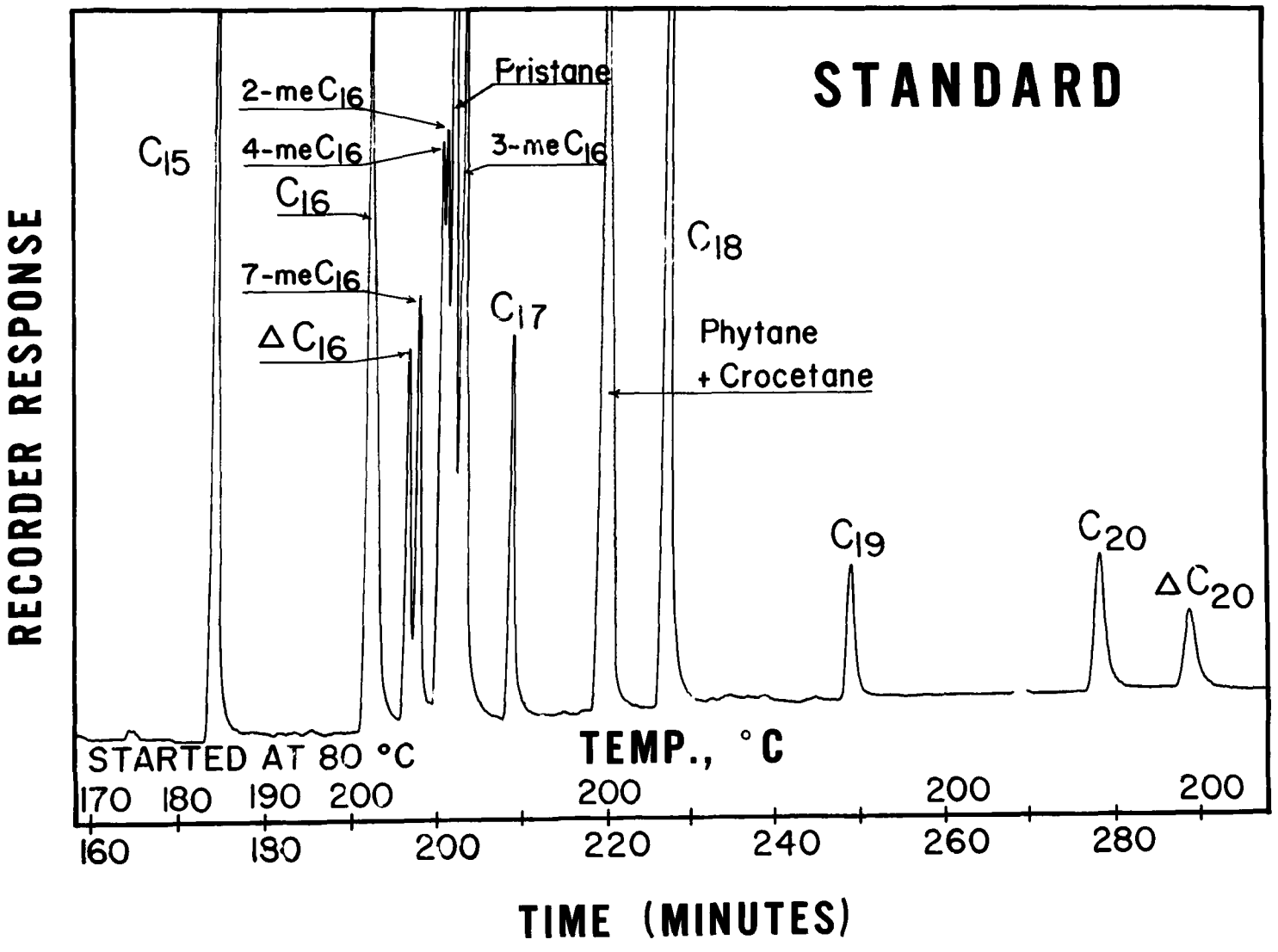
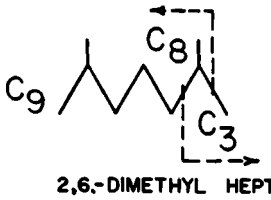

STANDARD
RECORDER RESPONSE
C15
2-meC16
4-meC16
C16
7-meC16
Δ C16
Pristane
3-meC16
C17
C18
Phytane
+ Crocetane
C19
C20
Δ C20
STARTED AT 80 °C
TEMP., °C
170 180 190 200
200
200
200
160 180 200 220 240 260 280
TIME (MINUTES)

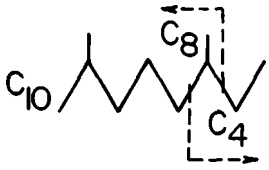
FIGURE 9

ISOPRENOID FRAGMENTATION PATTERNS

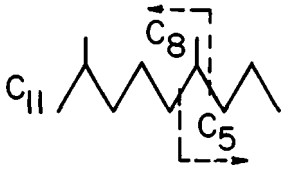
REGULAR ISOPRENOID FRAGMENTATION PATTERNS



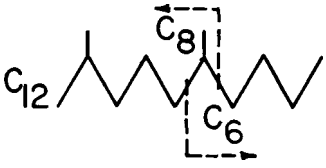
2,6,-DIMETHYL HEPTANE



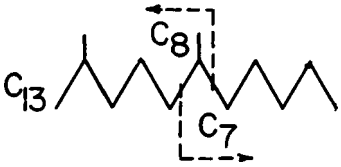
2,6,-DIMETHYL OCTANE



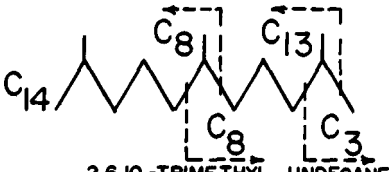
2,6,-DIMETHYL NONANE



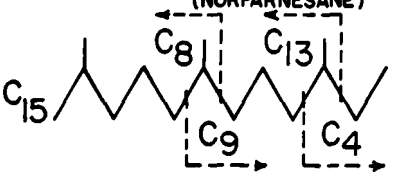
2,6,-DIMETHYL UNDECANE



2,6,-DIMETHYL DECANE

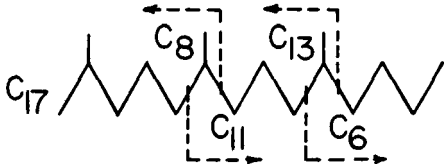


2,6,10,-TRIMETHYL UNDECANE
(NORFARNESANE)

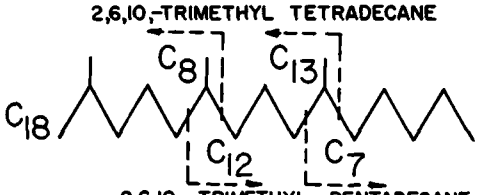


2,6,10,-TRIMETHYL DODECANE
(FARNESANE)

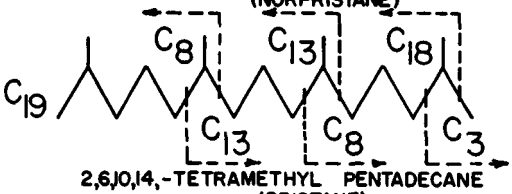
2,6,10,-TRIMETHYL TRIDECANE



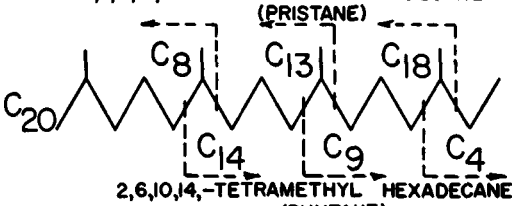
2,6,10,-TRIMETHYL TETRADECANE



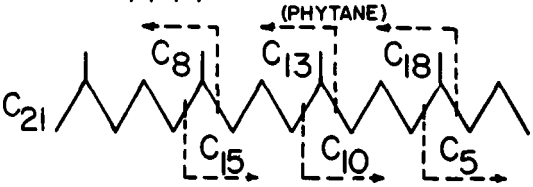
2,6,10,-TRIMETHYL PENTADECANE
(NORPRISTANE)



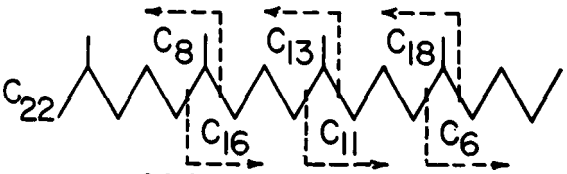
2,6,10,14,-TETRAMETHYL PENTADECANE
(PRISTANE)



2,6,10,14,-TETRAMETHYL HEXADECANE
(PHYTANE)



2,6,10,14,-TETRAMETHYL HEPTADECANE

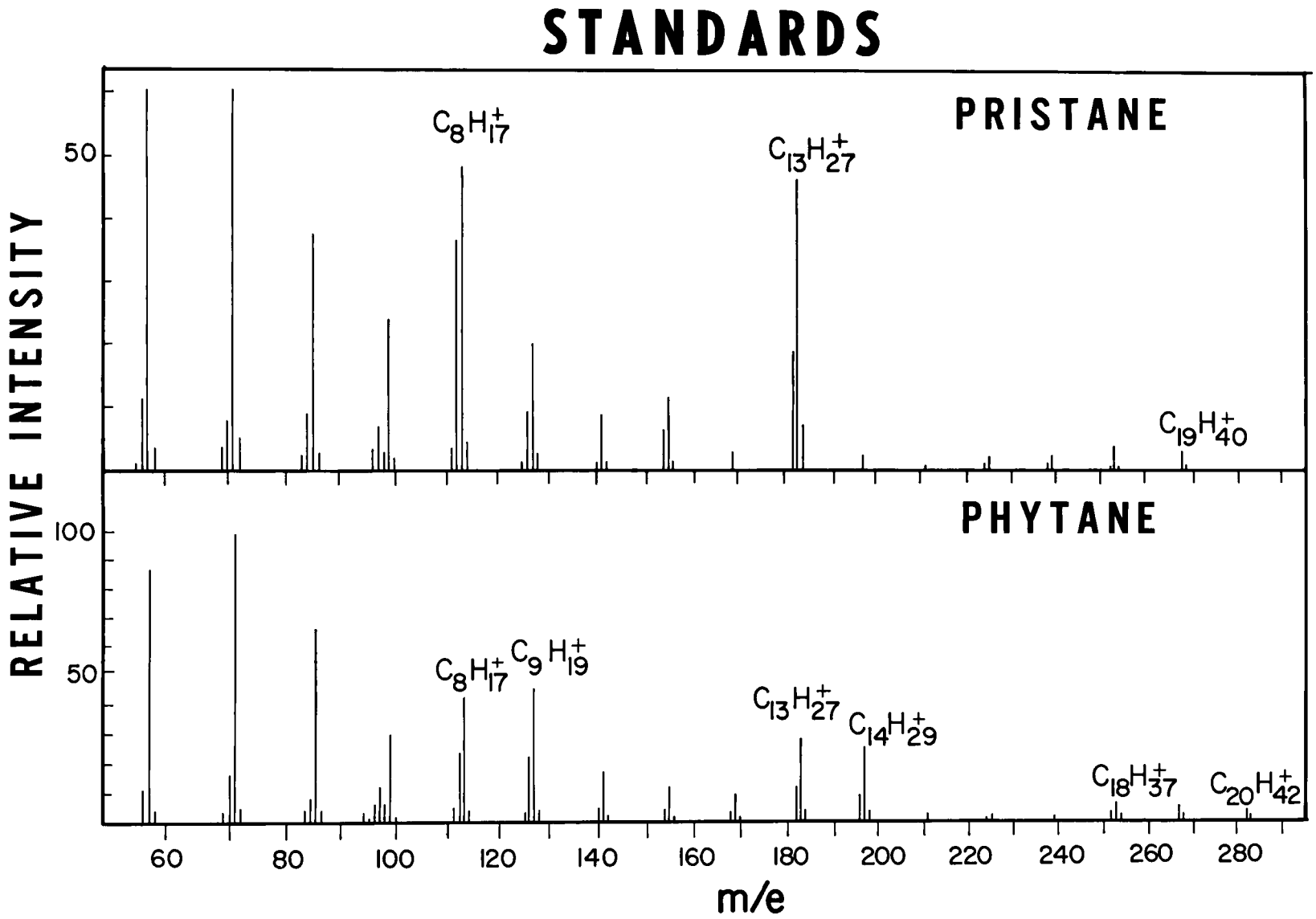


2,6,10,14,- TETRAMETHYL OCTADECANE

FIGURE 10

STANDARD MASS SPECTRA OF PRISTANE AND PHYTANE

The mass spectra were taken as the two components were eluted from a stainless steel capillary column (91 m long by 0.076 cm i.d.) coated with Polysev, and entered the ion source of the LKB 9000. They were ionized by electron impact at 70 eV.


STANDARDS
PRISTANE
PHYTANE
RELATIVE INTENSITY
50
100
50
$C_8H_{17}^+$
$C_{13}H_{27}^+$
$C_{19}H_{40}^+$
$C_8H_{17}^+$
$C_9H_{19}^+$
$C_{13}H_{27}^+$
$C_{14}H_{29}^+$
$C_{18}H_{37}^+$
$C_{20}H_{42}^+$
60
80
100
120
140
160
180
200
220
240
260
280
m/e

the two larger alkyl groups on either side, as discussed in the section dealing with the isoalkanes, a relatively accurate picture of the major breakdown products of these structures can be obtained as shown in Figure 9. It becomes immediately apparent that the high degree of structural order existing along the whole sequence of regular C_9 to C_{22} isoprenoids gives in turn rise to a highly regular fragmentation scheme as shown in Table VII. The combination of Figures 9, 10 and Table VII suggests that the appearance of relatively intense C_8 fragments (at m/e 113) in a mass spectrum can be regarded as an indication of the possible presence of an isoprenoid type structure. As the molecular weight increases along the isoprenoid homologous series the appearance of the C_{13} fragment (m/e 183) adds the second most important characteristic of the isoprenoid mass spectra (Table VII). Although these two fragments can be regarded as the basic isoprenoid characteristics (Figure 10) they can not be used by themselves as "the way" to structure identification. Compounds such as 7-methyl octadecane, 5,6-dimethyl pentadecane, 7,10-dimethylheneicosane, to name a few, would also give fragment peaks at m/e 113 and m/e 183, without having any relationship with the isoprenoid compounds built from isoprene units. For this reason the spectrum of farnesane (C_{15} isoprenoid), to take an example, in addition to the two characteristic fragment peaks at C_8 and C_{13} would also be expected to show a relatively intense

TABLE VII

MASS SPECTROMETRIC FRAGMENTATION SEQUENCES OF ISOPRENOID HYDROCARBONS

C atoms in molecules \ Fragment Mass	43	57	71	85	99	113	127	141	155	169	183	197	211	275	239	253
9	C_3					C_8										
10		C_4				C_8										
11			C_5			C_8										
12				C_6		C_8										
13					C_7	C_8										
14	C_3					$2C_8$					C_{13}					
15		C_4				C_8	C_9				C_{13}					
16			C_5			C_8		C_{10}			C_{13}					
17				C_6		C_8			C_{11}		C_{13}					
18					C_7	C_8				C_{12}	C_{13}					
19	C_3					$2C_8$					$2C_{13}$					C_{18}
20		C_4				C_8	C_9				C_{13}	C_{14}				C_{18}
21			C_5			C_8		C_{10}			C_{13}		C_{15}			C_{18}
22				C_6		C_8			C_{11}		C_{13}			C_{16}		C_{18}
23					C_7	C_8				C_{12}	C_{13}				C_{17}	C_{18}

ion at m/e 127 corresponding to the C_9 fragment and a C_{15} molecular ion. No other isoprenoid in this series would show these same features. These predictions have been fully substantiated by the mass spectra of a few synthetic isoprenoids. Table VIII represents a collection of the published mass spectral data on authentic reference compounds. All the characteristic isoprenoid fragments given in Table VIII are marked by an asterisk. As can be seen there is a perfect correlation between Tables VII and VIII. In other words between theory and practice.

As it is the case with the isoalkanes the fragment ions associated with the secondary carbonium ion are also accompanied by the corresponding olefin ions. See Figure 10 for the standard of pristane and phytane. Note the relatively high intensities of the olefin ions (z=0) at each one of the two major branching points (C_8, m/e 112 and C_{13}, m/e 182). The presence of these olefinic fragments is especially "valuable" in cases where the molecule fragments extensively to give several peaks at different m/e values. Compare for instance the number of fragments of the C_{13} isoprenoid and those of the C_{20} isoprenoid in Table VII. In those cases the percentage of the total ionization for each of the individual ion will be less than if ionization gives rise mainly to one or two fragments. As an example, computing the percentage of the total ionization, $\%\Sigma_{57}$ for the ions of mass 113 and 183

TABLE VIII

PARTIAL MASS SPECTRA OF SYNTHETIC ISOPRENOIDS

		Alkyl Ion Series: (Z = +1)					
m/e	Ion	C_{15}[1]	C_{17}[2]	C_{18}[3]	C_{19}[1]	C_{20}[1]	C_{21}[2]
57	$C_4H_9^+$	100.0		100.0	100.0	100.0	
71	$C_5H_{11}^+$	68.1		44.3	69.6	65.3	
83	$C_6H_{13}^+$	28.7		18.5	26.1	34.6	
99	$C_7H_{15}^+$	4.0	72	7.6	7.6	7.6	
113	$C_8H_{17}^+$	9.5*	100*	4.9	16.2*	10.4*	100*
127	$C_9H_{19}^+$	10.6*	31	1.7	5.2	10.2*	58
141	$C_{10}H_{21}^+$	1.3	10	1.0	2.2	3.1	71*
155	$C_{11}H_{23}^+$	1.0	57*	0.6	2.1	2.0	32
169	$C_{12}H_{25}^+$	0.6	4	2.7*	0.5	1.5	21
183	$C_{13}H_{27}^+$	2.9*	30*	1.8*	9.6*	5.0*	44*
197	$C_{14}H_{29}^+$	0.9	4	0.1	0.5	4.3*	23
211	$C_{15}H_{31}^+$		4	0.2	0.1	0.4	29*

TABLE VIII (CONTINUED)

PARTIAL MASS SPECTRA OF SYNTHETIC ISOPRENOIDS

		Alkyl Ion Series: (Z = +1)					
m/e	Ion	C_{15}[1]	C_{17}[2]	C_{18}[3]	C_{19}[1]	C_{20}[1]	C_{21}[2]
212	$C_{15}H_{32}^+$	0.6(M)					
225	$C_{16}H_{33}^+$		7	0.3	0.3	0.2	20
239	$C_{17}H_{35}^+$			0.4	0.3	0.2	8
240	$C_{17}H_{36}^+$		4(M)				
253	$C_{18}H_{37}^+$				0.6*	0.8*	17*
267	$C_{19}H_{39}^+$					0.5	5
268	$C_{19}H_{40}^+$				0.2(M)		
281	$C_{20}H_{41}^+$						4
282	$C_{20}H_{42}^+$					0.2(M)	
296	$C_{21}H_{44}^+$						1(M)

* Characteristic isoprenoid fragments

[1] Bendoraitis, J. G., et al.[31,32], [2] McCarthy, E. D.[281], [3] Lawlor, D. I and Robinson, W. E.[263]

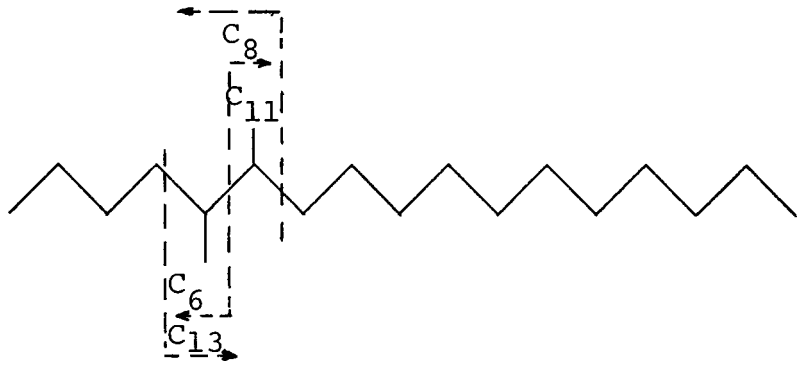
in the spectra of pristane (Table VIII) the results are 6.7 and 3.9% respectively. On the other hand the same kind of calculation, $\%\Sigma_{57}$, for the ions at m/e 113, 127, 183 and 197 in the spectrum of phytane (Table VIII) gives a 4.2, 4.1, 2 and 1.7% respectively. Thus in all cases where the fragment ions produced at a branched point are not prominent over the rest of the ions in the spectra, the height of their olefin counterparts adds reliability to the interpretation. This will become clear later with the presentation of the spectra of the isoprenoid homologies obtained from meteorites, petroleum crudes, graphite and other samples.

In general it may be expected that the $\%\Sigma$ for the C_8 ion and C_{13} ions will reach its maximum values with the C_{14} and C_{19} isoprenoids (Table VII).

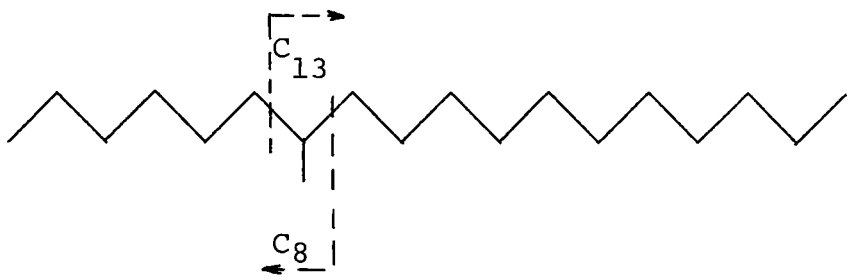
The problems related to the molecular structure identification of isomeric isoprenoids and other mass spectrometrically related compounds.

Mass spectrometry by itself, without the proper high purity standards, can not provide in many cases an unequivocal identification of the structure of isoprenoid hydrocarbons. Furthermore, the significance assigned to the isoprenoids in the field of organic geochemistry (231,365,369) makes the exact determination of the positions of their methyl branched an imperative. In this respect three extreme examples of

non isoprenoid structures have already been given above where the mass spectra would be expected to exhibit the characteristic isoprenoid peaks at C_8 and C_{13}. One of them in particular, the 5,6-dimethylpentadecane (a C_{17} compound)



would give the same distribution of fragment peaks as the C_{17} isoprenoid. (See Table VII). Likewise the 7-methyl octadecane (a C_{19} compound)



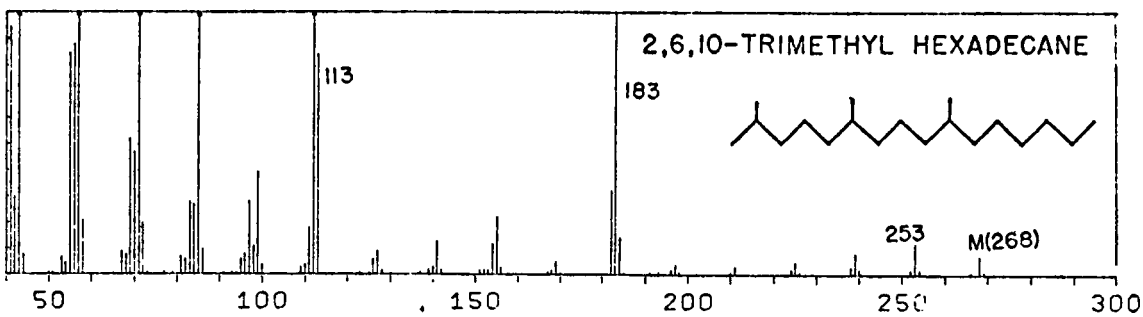
would give the same two fragments characteristic of pristane, the C_{19} isoprenoid (Figure 10 and Table VII). While it is true that a careful analysis of the relative intensities of the fragment and parent ions of these two spectra will most likely succeed in differentiating them from the true isoprenoids, a more difficult problem is posed by structurally related isomeric isoprenoids. A good example of this has been

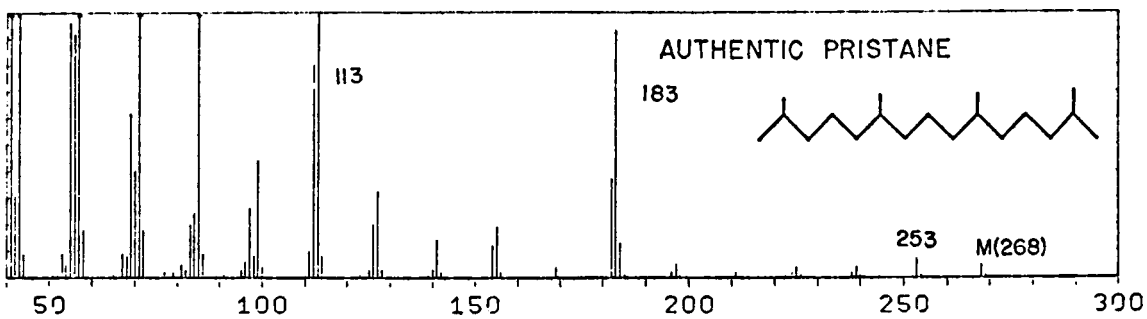
presented by McCarthy and Calvin (282) and by McCarthy (281) for three C_{19} isomeric isoprenoid alkanes (Figure 11).

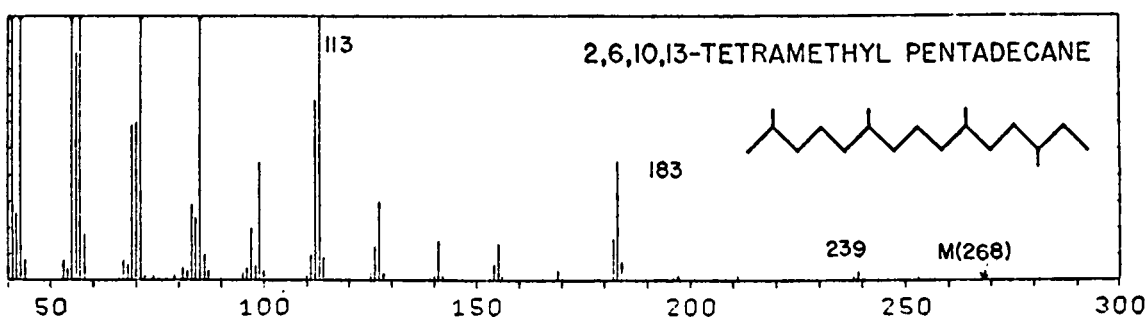
Here is where gas chromatography plays a critical role since the differences in the order of elution of the isomeric isoprenoids, even if small, can provide a qualitative differentiation between the otherwise almost identical structures as indicated by their individual mass spectra (Figure 11). Figure 12 is also a reproduction taken from the work of McCarthy (281) on the actual gas chromatographic separation of these three structures on a capillary column coated with Apiezon L. The shaded peaks have been added to show the approximate relative positions of the n-C_{16} and n-C_{17} alkanes. As it will be pointed out later the 2,6,10-trimethyl alkane has been identified in a few meteorite samples and although its mass spectrum is very similar to that of pristane (Figure 11) its corresponding GC peak is eluted immediately after the n-C_{17} alkanes (Figure 12), while pristane appears before and close to the n-C_{17} alkane in the Apiezon columns, and about halfway in between the n-C_{16} and n-C_{17} GC peaks if chromatographed on Polysev columns. None of the products investigated in this laboratory appears to contain any amounts of 2,6,10,13-tetramethyl pentadecane. Finally going back to the two mono and dimethyl substituted compounds mentioned above, it is important to consider that in spite of giving spectra similar to the C_{17} and C_{19} isoprenoids they

FIGURE 11

MASS SPECTRA OF THREE ISOMERIC C_{19} ISOPRENOIDS



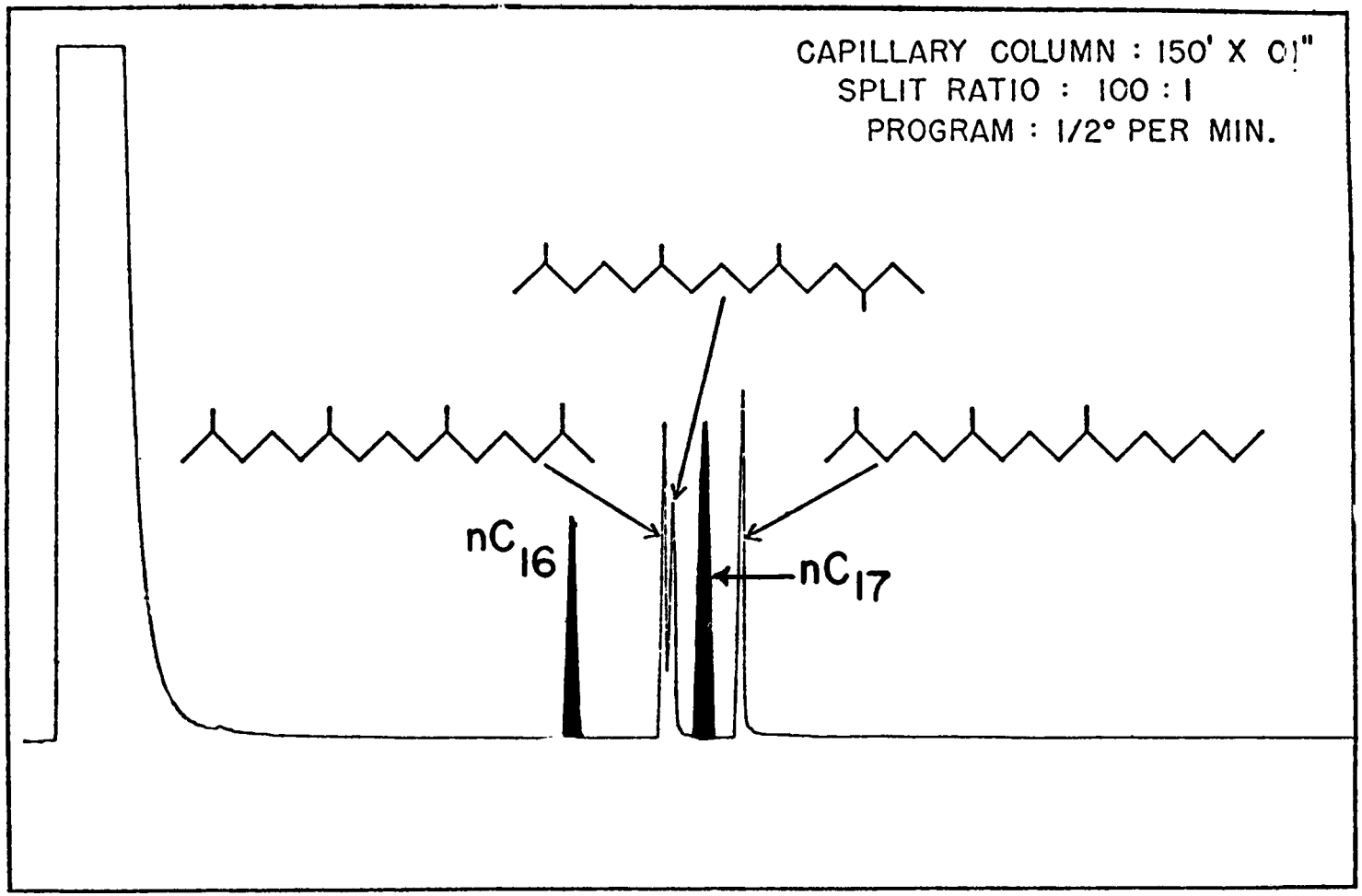




McCARTHY, E.D. (1967)

FIGURE 12

GAS CHROMATOGRAPHIC SEPARATION OF ISOMERIC ISOPRENOIDS



ORDER OF ELUTION OF C_{19} ISOPRENOID ISOMERS. APIEZON L. PHASE

McCARTHY, E.D.(1967)

MUB 11871

would be fully characterized by their relative gas chromatographic retention times. In the GC columns usually employed in this work the dimethyl substituted C_{19} compound would appear soon after the n-C_{18} alkane while pristane has an elution time shorter than that of the n-C_{17} alkane. Also the monomethyl substituted compound would have an elution time greater than the n-C_{18} and so it will not be easily confused with pristane. Thus the advantages of the combination of gas chromatography and mass spectrometry are demonstrated in a practical manner. Although gas chromatography does not provide the same degree of detailed structural information as does the mass spectrometer it usually can provide a good separation between similar compounds, which facilitates the final assignment of structures from their corresponding mass spectra.

Gas chromatography of n-alkanes, isoalkanes and isoprenoids.

In some instances, for example, when dealing with complex hydrocarbon mixtures of very closely boiling isomers it is necessary to make use of highly efficient columns. This point will be best illustrated with a discussion of a practical application which will prove of a great significance later on. In Figure 8 it is shown the gas chromatographic separation of a standard mixture of hydrocarbons in a high resolution column, 310 m long by 0.076 cm i.d. coated with Polysev (Table I) (its efficiency has been calculated at 226,054 plates).

Only a very slight degree of separation between compounds such as the 2-, and 4-methyl hexadecane can be achieved and also pristane can not be completely resolved from its surrounding methyl branched alkanes. This is an important factor to keep in mind when interpreting GC-MS data since the contributions of these structures to the mass spectrum of pristane may be significant depending on their concentrations relative to the latter compound. No resolution at all has been achieved under these conditions for the two isomeric C_{20} isoprenoids, phytane and crocetane.

While some degree of resolution can be obtained between pristane and the isoalkanes, the situation is still more problematic in connection with phytane and the 3-methyl heptadecane. These two compounds have been found to be very difficult to separate under any conditions. If the efficiencies of the columns are reduced by diminishing their lengths, the iso and anteiso C_{17} alkanes may collapse into one single peak with pristane and the iso and anteiso C_{18} may also be buried underneath the phytane peak.

This point will repeatedly be brought up in later discussions.

1. Other mass spectrometric techniques for the determination of isoprenoid structures.

The determination of isoprenoid structures by electron impact mass spectrometry is usually made difficult by the fact that the fragmentation processes are so extensive

that the intensity of the parent ion peak is greatly reduced or even nondetectable (Table VIII). This especially the case for pristane (31,188), which being a compound of salient bio-geochemical significance (142,322,369) often requires a more reliable technique for its determination. Among the mass spectrometric techniques that have ann effect on the parent ion intensity the following will be mentioned.

a) Chemical ionization

The theory of chemical ionization as a new technique in mass spectrometry (155), recently developed by M. S. B. Munson and F. H. Field (337), is based on the ionization of the sample being studied in a mass spectrometer by means of ionic reactions instead of by the conventional process of electron impact ionization.

A small amount of the sample investigated is introduced together with a reaction gas into the ion source of a mass spectrometer. The pressure of the reaction gas is 1 torr while the partial pressure of the sample is kept within 10^{-3} to 10^{-4} torr. By keeping this ratio of pressures ($P_{sample}/P_{reacting\ gas} \simeq 10^{-3}$), the amount of sample ionized by electron impact compared with the ionization produced in the reaction gas will be practically negligible.

Also at these high pressures (1 torr), an ion can make about 25 collisions within the ion source of the spectrometer and so the probability of a collision induced reaction with very small amounts of any other material will be relatively

high.

Methane (157) has been extensively used in chemical ionization processes as the reaction gas since once it is ionized, the primary ions produced (CH_4^+, CH_3^+, CH_2^+, CH^+ ...) can undergo ion molecule reactions which result in several product or secondary ions capable of serving as the reactant ions.

Other gases can be used, but methane seems to be preferred due to energetic considerations. The use of H_2 for instance would result in H_3^+ as a stable product ion and since its proton transfer reactions are more exothermic than those corresponding to the product ions of methane, more fragmentation of the molecule can be expected.

The major stable product ions in methane are CH_5^+ (48%), $C_2H_5^+$ (40%) and $C_3H_5^+$ (6%). Other ions are predominantly $C_2H_4^+$, $C_3H_7^+$ and some $C_2H_2^+$, $C_3H_4^+$, $C_3H_3^+$ and $C_4H_9^+$.

The analytical application of ion-molecule reactions has leadto a mass spectrometric technique which provides a better identification of the molecular mass by means of the quasi-parent ions formed (155,157,158,337).

The advantages of this technique over the electron impact method become apparent by inspection of the spectra which consist almost exclusively of alkyl ions of comparable intensity.

In hydrocarbons, no m/e = M is found, but the relative intensity of the m/e = M-1 ion is about 32% in the normal

paraffins, being independent of the size of the molecule.

For branched hydrocarbons, the intensity may range from 0 to 50% being usually higher than the 32% found for n-hydrocarbons. This indicates that branching affects the stabilities of the ions formed to a lesser extent in chemical ionization than in electron impact.

For these reasons this method appeared especially suitable for isoprenoid analysis where the intensities of the parent ions are very low. Pristane was the obvious choice as a test of the practical applications of the theory (169).

The experimental procedure is briefly summarized as follows: A standard of pristane obtained from Eastman Organic Chemicals had a purity greater than 99% as estimated by gas chromatographic analysis. It was examined using chemical ionization techniques in the Esso Research and Engineering Chemical Physics mass spectrometer (337). Methane was used as reactant gas, and the technique was that described previously (337). The pressure of methane in the ion source was kept at 1 torr.

As it has already been mentioned the molecule of pristane (Figure 9) shows by electron impact a spectrum (Figures 10, 11 and 13A) whose most characteristic features are the peaks at m/e 183 and m/e 113, and a parent ion which most of the time is not seen due to a very low relative intensity. By chemical ionization, this difficulty is overcome

FIGURE 13

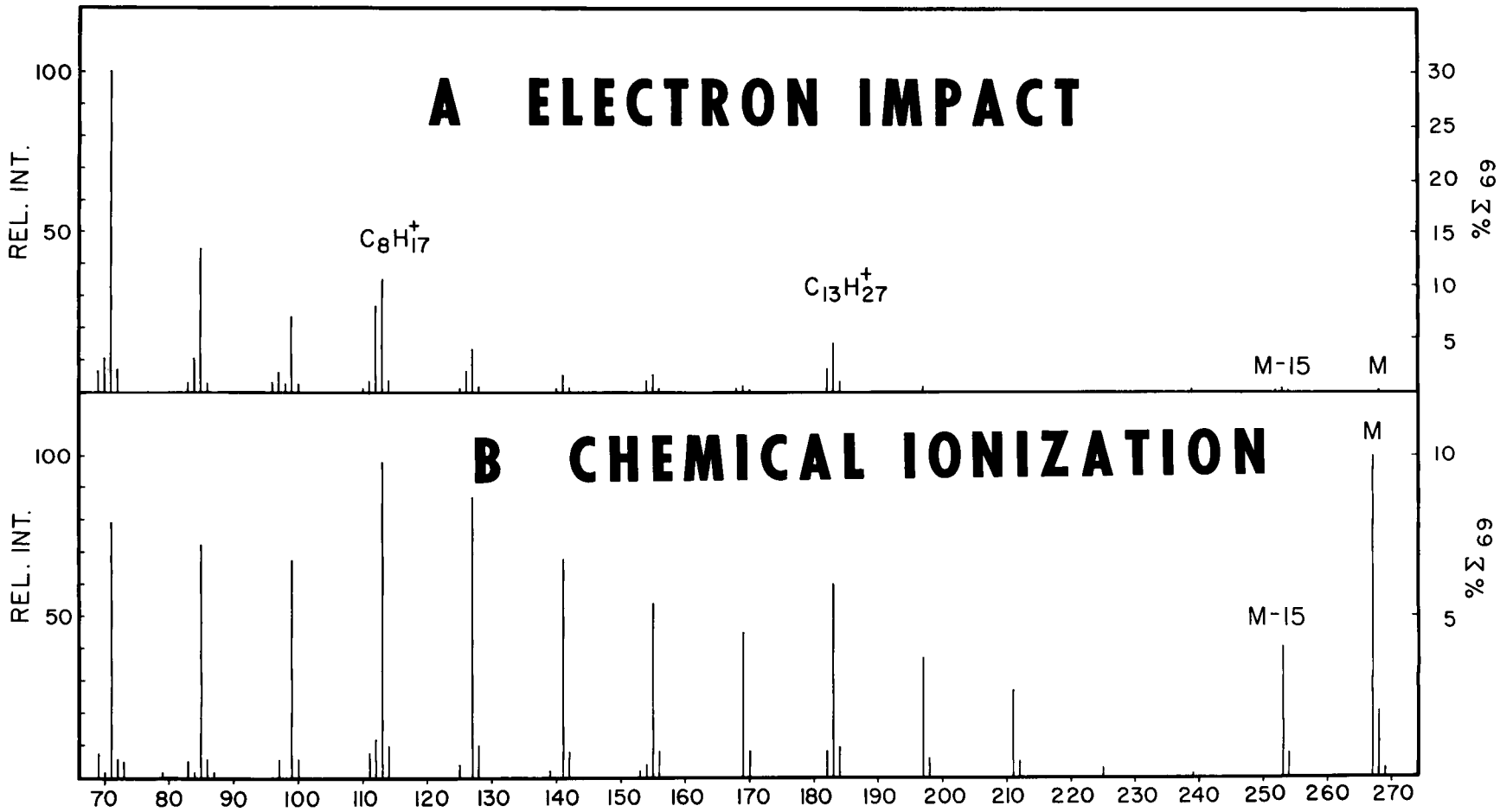
CHEMICAL IONIZATION AND ELECTRON IMPACT

MASS SPECTRA OF PRISTANE

A. Electron impact. Pristane Standard: 2 μl injected in a 180 cm X 3 mm column packed with 1% SE-30 on Gas Chrom-P. Mass spectrum taken immediately after elution from the column on a gas chromatograph-Atlas CH4 mass spectrometer combination.

B. Chemical ionization. Same pristane standard: 1.78 μl directly introduced in the Esso Research and Chemical Physics mass spectrometer (reference 337).

MASS SPECTRA OF PRISTANE



since the quasi parent ion (M-1) shows a relative intensity as high as 10% of the total ionization (see Figure 13B). The points of branching are easily distinguishable here too. The chemical ionization process originated by the reactant ions takes place either by dissociative proton transfer or hydride ion transfer to the molecules of the sample followed by fragmentation (alkyl ion displacement).

Considering now the two major product ions derived from methane ($CH_5^{\oplus}$ and $C_2H_5^{\oplus}$), the chemical ionization and fragmentation of pristane can be accounted for by the following processes:

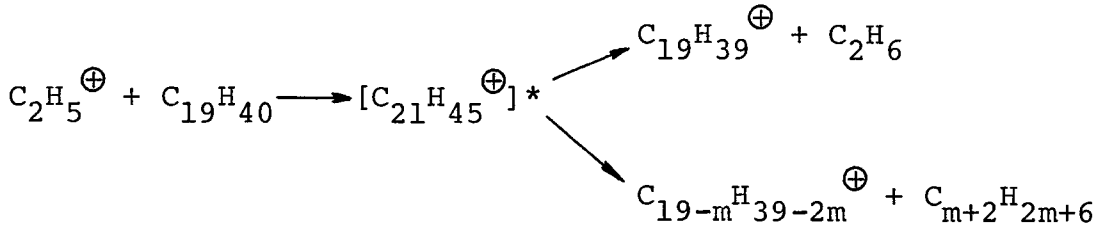
(1)

$$CH_5^{\oplus} + C_{19}H_{40} \longrightarrow [C_{19}H_{41}^{\oplus}]^* \begin{cases} \longrightarrow C_{19}H_{39}^{\oplus} + H_2 \\ + CH_4 \longrightarrow C_{19-n}H_{39-2n}^{\oplus} + C_nH_{2n+2} \end{cases}$$

Since $CH_5^{\oplus}$ is a strong Bronsted acid, it can transfer a proton to pristane to form the starred activated complex (there is no evidence for its existence here except that they are known to occur in low energy ion molecule reactions) leading to the (M-1) ion by H_2 removal if the C-H bonds are involved. However, C-C bond fission leads to alkyl ion

displacements (fragmentation):

(2)



Here, since $C_2H_5^{\oplus}$ is a Lewis acid, a hydride ion abstraction takes place resulting in the (M-1) ion. If the attack involves a C-C bond, then alkyl ion displacement occurs.

The attack of the methane ions on the pristane molecule involves a random electrophilic attack followed by localized reaction, and as discussed, whenever the attack is on a C-H bond, the M-1 ion results, while if on a C-C bond, the results are the different m/e alkyl ions.

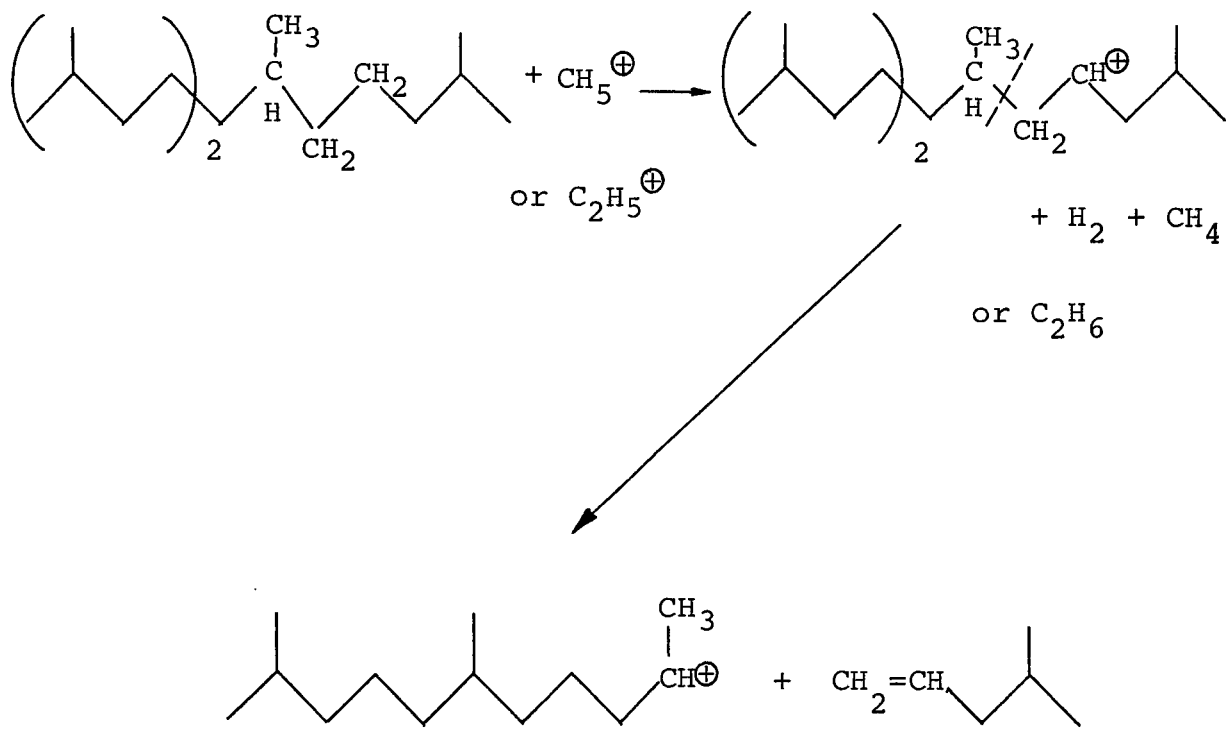
From the relative intensities of the M-1 ion (32%) and the alkyl ions (68%) in normal hydrocarbons, it can be assumed that C-C fission takes place twice as often as C-H fission.

Now if a branching point is present in the molecule, the relative number of C-C bonds is greater around the branching point and so C-C bond rupture is enhanced as compared to C-H fission.

In conclusion, the M-1 ion results from the removal of hydrogen from the molecule, being the relative intensity of the resulting peak function of the number of hydrogens

available (non-primary hydrogens not β to a branching point).

The M-1 ion can undergo exothermic fission at the branched point which is an additional fragmentation process enhancing the intensity of the corresponding alkyl ion.



m/e 183

The high M-15 peak in the spectrum (m/e 253) indicates methyl branching in the molecule. Peaks at m/e 268 and 269 are isotope peaks corresponding to the ion $C_{19}H_{39}^{+}$.

The peak at m/e 239 (M-29) seems to have a relative intensity higher than expected since for most of the branched compounds it is absent. Its absence should be expected in the pristane

spectrum because no M-29 ion can be formed by single C-C cleavage. The pristane sample run had a maximum of 0.1% of impurities present as determined by gas chromatography, which in lieu of a better explanation could account for the intensity value of the M-29 peak.

Alkenyl ions ($C_2H_{2n-1}^+$) and olefin ions ($C_nH_{2n}^+$) are also found on the spectra. The alkenyl ions are concentrated on the lower end of the chemical ionization spectra while the olefin ions appear only at masses corresponding to branching points with the exception of methyl side chains. (No olefin ion is found with the M-15 peak). Although the mechanism of formation of these olefin ions is not known at present, it may be very similar in nature to the corresponding process under electron impact.

The low intensity of the M-43 indicates that the retention of the positive charge on the large fragment is not the most favorable process, which is the only indication in this case of the isopropyl branching in the molecule, since no olefin ion is found with the M-15 peak as an indication of branching.

Finally, it must be kept in mind that up to mass 73, we can have contributions to the relative intensities from the methane ionization. For instance, most of the contribution to m/e 57 by the ion $C_4H_9^+$ comes from the methane. Once the

TABLE IX

CHEMICAL IONIZATION

MASS SPECTRA OF PRISTANE ($C_{19}H_{40}$)

Methane Reactant P_{CH_4} = 1.0 Torr

Additive volume: 1.78µl $C_{19}H_{40}$

m/e	Ion	Rel. Intensity[a]	m/e	Ion	Rel. Intensity
69	$C_5H_9^+$	7.6[b]	141	$C_{10}H_{21}^+$	67.2
70		1.9	142		7.6
71	$C_5H_{11}^+$	78.7	153	$C_{11}H_{21}^+$	1.9[b]
72		5.7	154		3.8
73		4.8	155	$C_{11}H_{23}^+$	53.7
79		1.9	156		7.6
83	$C_6H_{11}^+$	4.8[b]	169	$C_{12}H_{25}^+$	44.1
84		1.9	170		7.6
85	$C_6H_{13}^+$	72.0	182	$C_{13}H_{26}^+$	7.6[c]
86		5.7	183	$C_{13}H_{27}^+$	59.5[d]
87		1.9	184		8.6
97	$C_7H_{13}^+$	5.7[b]	197	$C_{14}H_{29}^+$	36.4
99	C_7H_{15}	67.2	198		5.7
100		5.7	211	$C_{15}H_{31}^+$	26.8
111	$C_8H_{15}^+$	6.7[b]	212		4.8
112	$C_8H_{16}^+$	11.5[c]	225	$C_{16}H_{33}^+$	2.8
113	C_8H_{17}	97.9[d]	239	$C_{17}H_{35}^+$	0.9
114		9.6	253	$C_{18}H_{37}$	40.3[e]
125	$C_9H_{17}^+$	3.8[b]	254		7.6
127	C_9H_{19}	86.4	267	$C_{19}H_{39}^+$	100
128		9.6	268		21.1
139	$C_{10}H_{19}^+$	1.9[b]	269		2.8

[a] Excluding masses of ions lower than 69 m/e.

[b] Alkenyl ions, $C_2H_{2n-1}^+$.

[c] Olefin ions $C_nH_{2n}^+$: $C_8H_{16}^+$, $C_{13}H_{26}^+$, indicate branching.

[d] Similarities with electron impact mass spectrometry.

e Major differences with electron impact mass spectrometry.

relative intensities due only to methane are subtracted, it can be shown that the most intense peak in the spectra is that of the M-1 ion.

Since the contribution of methane to the lower masses has not been subtracted here, the intensities (Table IX) are reported starting at m/e 69, where the contribution of the methane ions begins to be negligible.

A comparison between the spectra of pristane obtained by the conventional electron impact ionization (Figure 13A) and chemical ionization mass spectrometry (Figure 13B) demonstrates the potential usefulness of the latter approach.

It is hoped that with the eventual improvement on the sensitivity, this method may become an important analytical tool for the determination of the masses of labile molecules and highly branched structures of natural products.

b) Low energy mass spectrometry

The method is based on decreasing the total fragmentation probability by the use of low energy electrons. In the commonly used electron energy range of 50-100 eV, the energy transferred to the molecule by the bombarding electron is enough not only to cause its ionization but to produce an extensive fragmentation that results in complex cracking patterns and reduced parent ion intensities. On the other hand, the reduction of the electron energy to values much closer to the ionization potential of the molecule (12-20 eV)

brings about an enhancement of the parent ion intensity. Table X gives a comparison of farnesane spectra taken at 20 eV and at 70 eV electron energies, where the intensity of the parent ion at 20 eV is 6.6 times greater than at 70 eV. A practical application of this point can also be found in Table XI. When applying this technique it has to be considered that due to the overall decrease in the probability of ion formation, the increase in parent ion intensity is achieved at the expense of instrumental sensitivity. Although this method has proved its value in the analysis of olefins and aromatic hydrocarbons (156,279) it is not particularly suitable for isoprenoid analysis where due to the low concentrations in which these compounds are present in natural products, maximum instrument sensitivities are generally required.

D. Alkenes and Other Unsaturated Compounds

1. Monoolefins

The mass spectral fragmentation pattern of this type of compounds shows great similarity to that of the n-alkanes in its smooth-curve distribution of ions, which after passing through a maximum in the low molecular weight region decreases rapidly towards the parent ion peak. As in the case of their saturated counterparts the molecular ion is larger than the immedicately preceeding peaks. Likewise the M-15 peak is practically absent in the spectra of straight chain

TABLE X

PARTIAL MASS SPECTRA OF FARNESANE

AT TWO DIFFERENT ELECTRON ENERGIES

m/e	Ion	Electron Energy 20 ev	70 ev[1]
57	$C_4H_9^+$	100	100
71	$C_5H_{11}^+$	114	68.1
83	$C_6H_{13}^+$	60	28.7
99	$C_7H_{15}^+$	14	4
113	$C_8H_{17}^+$	32	9.5
127	$C_9H_{19}^+$	36	10.6
141	$C_{10}H_{21}^+$	4.3	1.3
155	$C_{11}H_{23}^+$	4.0	1.0
169	$C_{12}H_{25}^+$	2	0.6
183	$C_{13}H_{27}^+$	10	2.9
197	$C_{14}H_{29}^+$	3	0.9
212	$C_{15}H_{32}^+$	4(M)	0.6(M)

[1] Bendoraitis, J. G., et al.[32].

TABLE XI

PARTIAL MASS SPECTRA OF BASKING SHARK SQUALENE AT DIFFERENT ELECTRON ENERGIES

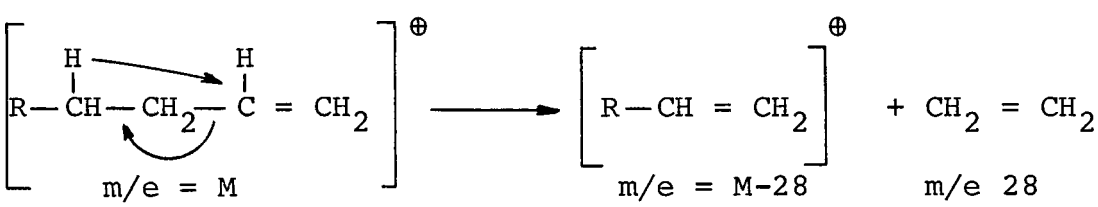
m/e	70 ev	20 ev
41	312	
55	72	1
69	1110	48
81	405	60
95	90	32
109	40	45
123	43	75
136	55	368
137	100	100
149	23	50
163	8	19
177	5	26
191	9	54
192	6.4	78
703	6	43
231	3.5	26
273	2.8	21
299	1.7	15
341	7	60
367	2.5	17
395	0.6	2
410	4.2(M)	32(M)

olefins. On the other hand, in spite of the overall similarities in the ion distribution curves between alkanes and alkenes the effect of the presence of a double bond on these type of spectra is not just confined to the reduction in the mass of the parent peak by two units. It has been shown that double bond isomers give almost identical mass spectra (42), thus indicating that the double bond does not play a passive role during fragmentation under electron impact but rather that it migrates easily along the molecule. This outstanding mobility completely counteracts the expected resonance stabilization effect characteristic of an allyl cation, and thus the fragmentation of the C-C bond β to the double bond can not take place to the extent that it would if the mobility of the unsaturation center were completely suppressed. Several methods have been proposed to accomplish the immobilization of the double bond (14,125,242). The two that appear most promising involve the oxidation of the double bond with OsO_4 to form a diol followed either by condensation with acetone to form o-isopropylidene derivatives (285) or treatment with silane derivatives to form the corresponding sylylethers.

It is hoped that these techniques will allow in the near future to substantiate some of the empirical correlations observed among the spectra of unsaturated hydrocarbons. Working with the spectra of unsaturated hydrocarbons from

natural and synthetic products, it was observed that in several cases the spectra of straight chain olefins exhibit an M-28 peak which is higher than the preceeding fragment. This is not observed in any of the spectra of saturated hydrocarbons. A search through the olefin spectra in the API tables shows that this is indeed the case for ten olefins with more than 13 C atoms as indicated by the underlined figures in the z=0 series of Table XII. The same thing can be seen in Tables XIII and XIV that show the partial spectra of the monoolefins identified in two microscopic algae, and also in Figure 14.

This peak (M-28) corresponds to the loss of 28 amu which may be taken as an indication that terminal olefins split off ethylene as a neutral molecule and retain the positive charge in the larger fragment.



A similar process could possibly account for the prominent olefin ions observed in the branched fragments of isoalkane mass spectra.

On this basis it may be tentatively generalized that the presence of a high M-28 ion in the mass spectra of straight

TABLE XII

PARTIAL SPECTRA OF 1-OLEFINS

	$\Delta'C_{11}$		$\Delta'C_{12}$		$\Delta'C_{13}$		$\Delta'C_{14}$		$\Delta'C_{15}$		$\Delta'C_{16}$	
C_n \ Z*	-1	0	-1	0	-1	0	-1	0	-1	0	-1	0
C_8	6.5	5.8	5.8	3.3	22.9	15.1	25.5	16.4	29.7	16.5	32.4	17.5
C_9	2.4	3.2*	1.7	2.1*	7.2	5.6	10.1	9.0	12.0	10.2	13.2	9.7
C_{10}			0.4	1.5	2.2	4.2*	2.7	3.5*	3.8	5.9*	4.5	6.1*
C_{11}		5.7			0.9	4.0	1.2	2.9	1.6	2.4	2.2	4.0
C_{12}				2.1	0.1		0.6	3.3	0.9	2.1	1.1	1.8
C_{13}						4.7			0.3	2.9	0.5	1.5
C_{14}								4.0			0.3	2.5
C_{15}										3.5		
C_{16}												3.7
API Nos.	988		485		1010		1011		1012		1013	

*values of Z in $C_nH_{2n} + Z$

TABLE XIII

BOTRYOCOCCUS BRAUNII HEPTADECENE

PARTIAL MASS SPECTRUM

C_n (m/e) \ Z	Mass Series (Z in C_nH_{2n+Z}) -3	-2	-1	0	+1	+2
C_5 (69)	6	9.5	54	62.5	45.5	3
C_6 (83)	6.8	29.5	96	53	21.5	2.5
C_7 (97)	5	21	100	38	10	
C_8 (111)	3	12	64	30	8	
C_9 (125)	2	7.5	32.5	19	4	
C_{10} (139)	1.8	5	13	13 *	3	
C_{11} (153)		3.5	7	8.5	2.1	
C_{12} (167)		2.2	10	6	2	
C_{13} (181)		1.5	2.5	3	1.5	
C_{14} (195)			1.5	2		
C_{15} (209)			1.8	5.5		
C_{16} (223)						
C_{17} (237)				55(M)	11.2	2.1

* Most characteristic ions

m/e: value of alkenyl ion mass series

TABLE XIV

PARTIAL MASS SPECTRA OF TWO HIGH MOLECULAR WEIGHT OLEFINS FROM ANACYSTIS MONTANA AND THEIR RESPECTIVE ALKANE STANDARDS

A.	n-Pentacosene					n-Pentacosane			
	Mass Series (Z in $C_nH_{2n}+Z$)								
C_n \ Z	-3	-2	-1	0	+1	-1	0	+1	+2
C_7	7	35	100	25	12	11.9	7.0	15.5	1.2
C_8	5	16	60	20	9	5.0	4.9	10.2	0.9
C_9	4.5	11	38	15	6.5 (1)	2.2	3.7	7.7	0.8 (1)
C_{10}	3.5	8.5	20	11.5	5	0.8	2.9	6.0	0.6
C_{11}	1.5	6	14	9	4	0.4	2.4	4.9	0.6
C_{12}	1	4.6	9.5	7	3	0.3	2.0	4.1	0.5
C_{13}	0.8	3.9	7.1	6.2	2.5	0.2	1.6	3.5	0.5
C_{14}	0.6	3.5	5.6	4.1	1.9	0.1	1.4	3.1	0.4
C_{15}	(3)	3.7	4.7	4.4	1.5	0.1	1.1	2.7	0.4
C_{16}	0.6	3.3	3.7	3.8*	1.4	-	1.0	2.5	0.4
C_{17}	0.5	2.8	2.9	3.2	1.1	-	0.8	2.2	0.4

TABLE XIV CONTINUED

PARTIAL MASS SPECTRA OF TWO HIGH MOLECULAR WEIGHT OLEFINS FROM ANACYSTIS AND THEIR RESPECTIVE ALKANE STANDARDS

	n-Pentacosene					n-Pentacosane			
	Mass Series (Z in $C_nH_{2n} + Z$)								
C_n \ Z	-3	-2	-1	0	+1	-1	0	+1	+2
C_{18}	0.5	2.6	2.5	2.7	0.9	-	0.7	2.0	0.4
C_{19}	0.3	2.1	2	2.3	0.8	-	0.6	1.9	0.4
C_{20}	0.2	1.3	1.5	2.1	(1.3) (1)	-	0.5	1.7	0.3 (1)
C_{21}	-	0.9	1.0	1.6	0.6	-	0.4	1.5	0.3
C_{22}	-	0.7	0.8	1.8	0.7	-	0.2	1.0	0.2
C_{23}	-	0.4	0.6	4.7	1.4	-	0.1	0.6	0.1
C_{24}	-	-	-	-	-	-	-	-	-
C_{25}	-	3.5	1.7	21.5	6.6	-	-	-	1.6

n-Pentacosene : anacystis montana

n-Pentacosane : API # 1313

(1) See Figure 16

( ) Background peaks

TABLE XIV CONTINUED

PARTIAL MASS SPECTRA OF TWO HIGH MOLECULAR WEIGHT OLEFINS FROM ANACYSTIS MONTANA AND THEIR RESPECTIVE ALKANE STANDARDS

B.	n-Heptacosene					n-Heptacosane			
	Mass Series (Z in $C_nH_{2n}+Z$)								
C_n \ Z	-3	-2	-1	0	+1	-1	0	+1	+2
C_7	3.5	27	100	23	12	13.3	7.0	15.8	1.2
C_8	2	11	57	18	8	5.7	4.9	10.5	0.9
C_9	2.1	8	34	13.5	6.5	2.4	3.7	7.7	0.8
C_{10}	2	6.5	18.5	10	5.0	0.9	2.9	6.1	0.7
C_{11}	-	5	12	8	3.5	0.5	2.4	4.9	0.6
C_{12}	0.5	4	9	6.4	2.9	0.3	2.0	4.2	0.5
C_{13}	0.4	3.2	6.6	5.4	2.3	0.2	1.7	3.6	0.5
C_{14}	0.3	2.9	5.2	4.5	1.8	0.1	1.4	3.1	0.4
C_{15}	(0.9)	2.6	4.1	3.8	1.5	0.1	1.2	2.7	0.4
C_{16}	-	2.3	3.3	3.2	1.3	-	1.0	2.4	0.4
C_{17}	-	2.1	2.7	2.8*	1.1	-	0.9	2.2	0.4

TABLE XIV CONTINUED

PARTIAL MASS SPECTRA OF TWO HIGH MOLECULAR WEIGHT OLEFINS FROM ANACYSTIS MONTANA AND THEIR RESPECTIVE ALKANE STANDARDS

	n-Heptacosene					n-Heptacosane			
	Mass Series (Z in $C_nH_{2n} + Z$)								
C_n \ Z	-3	-2	-1	0	+1	-1	0	+1	+2
C_{18}	-	2.0	2.3	2.5	1.0	-	0.7	2	0.4
C_{19}	-	1.7	1.8	2.2	0.8	-	0.6	1.8	0.4
C_{20}	-	1.5	1.5	1.9	0.7	-	0.5	1.7	0.3
C_{21}	-	1.3	1.3	1.6	0.6	-	0.4	1.6	0.3
C_{22}	-	0.9	1	1.5	0.5	-	0.4	1.4	0.3
C_{23}	-	0.6	0.8	1.2	0.5	-	0.3	1.2	0.3
C_{24}	-	0.5	0.6	1.2	0.4	-	0.1	0.8	0.2
C_{25}	-	0.3	0.5	3.5	1.1	-	0.1	0.5	0.1
C_{26}	-	-	-	-	-	-	-	-	-
C_{27}	-	0.7	-	15.6	5	-	-	-	1.2

n-Heptacosene : anacystis montana

n-Heptacosane : API # 1314

FIGURE 14

MASS SPECTRUM OF ALGAL MAT HEPTADECENE

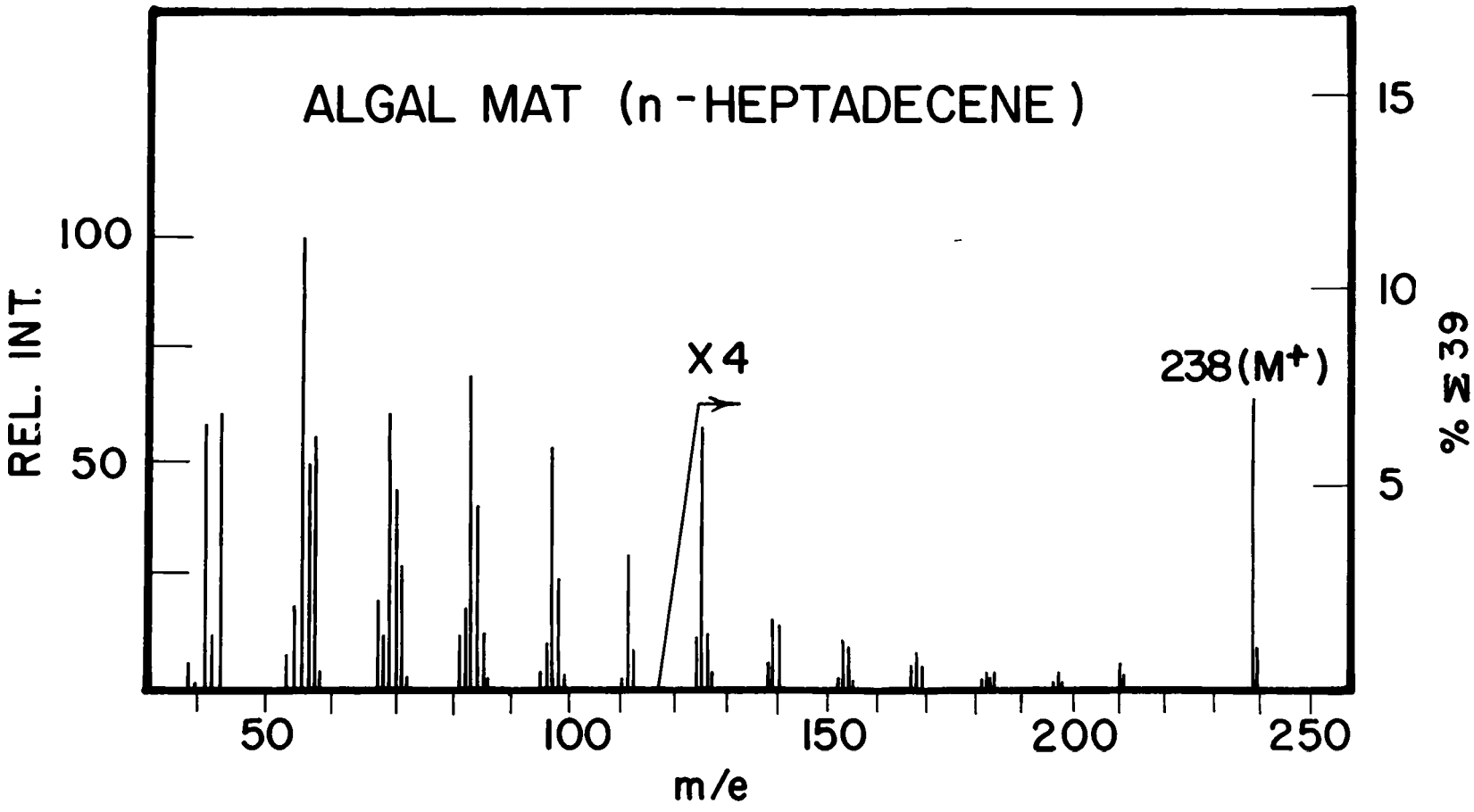
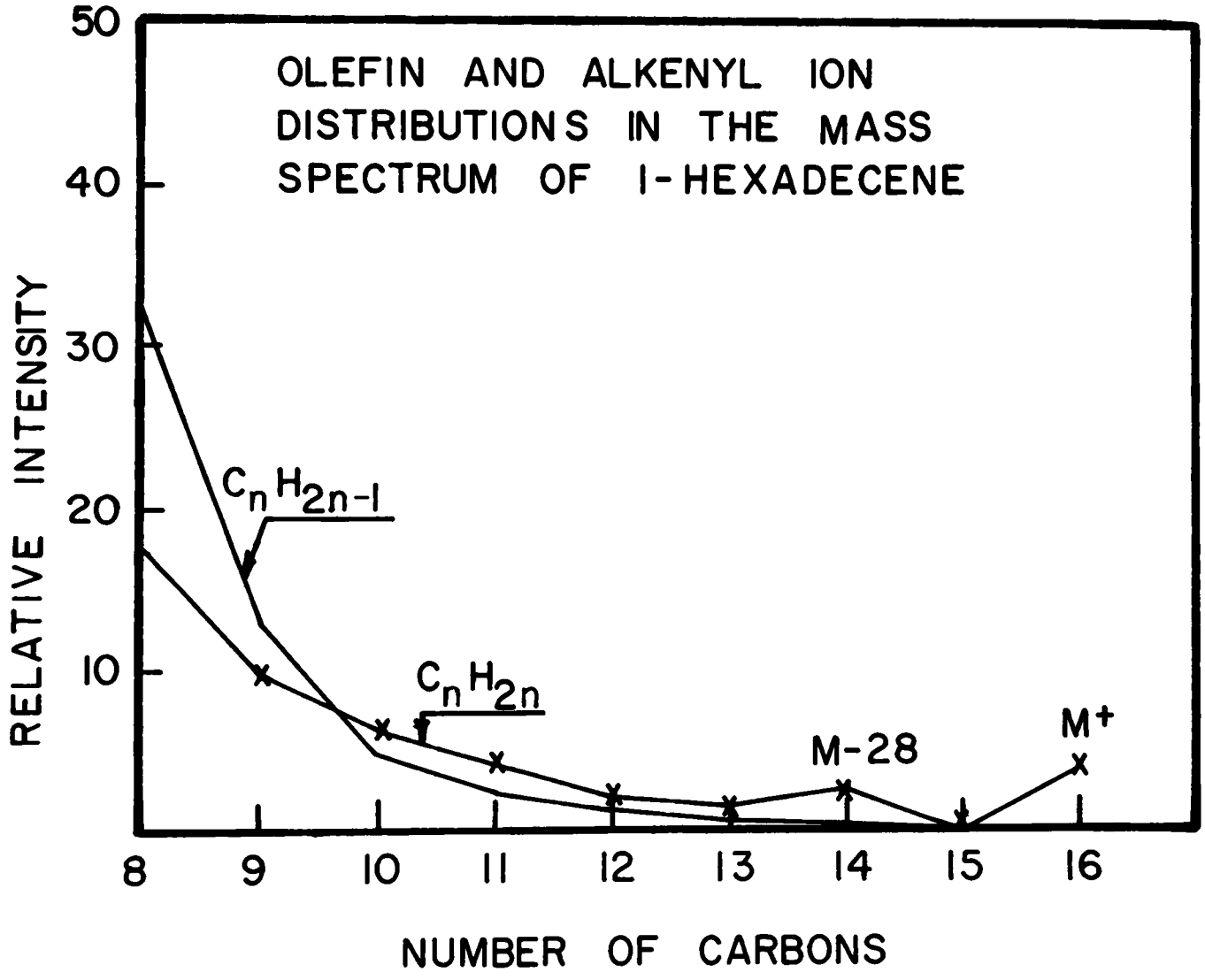

ALGAL MAT (n-HEPTADECENE)
REL. INT.
100
50
X4
238(M+)
15
10
5
% Σ 39
50
100
150
200
250
m/e

FIGURE 15

ION DISTRIBUTIONS IN THE MASS SPECTRUM OF 1-HEXADECENE


OLEFIN AND ALKENYL ION
DISTRIBUTIONS IN THE MASS
SPECTRUM OF 1-HEXADECENE
RELATIVE INTENSITY
50
40
30
20
10
C_nH_{2n-1}
C_nH_{2n}
M-28
M+
8
9
10
11
12
13
14
15
16
NUMBER OF CARBONS

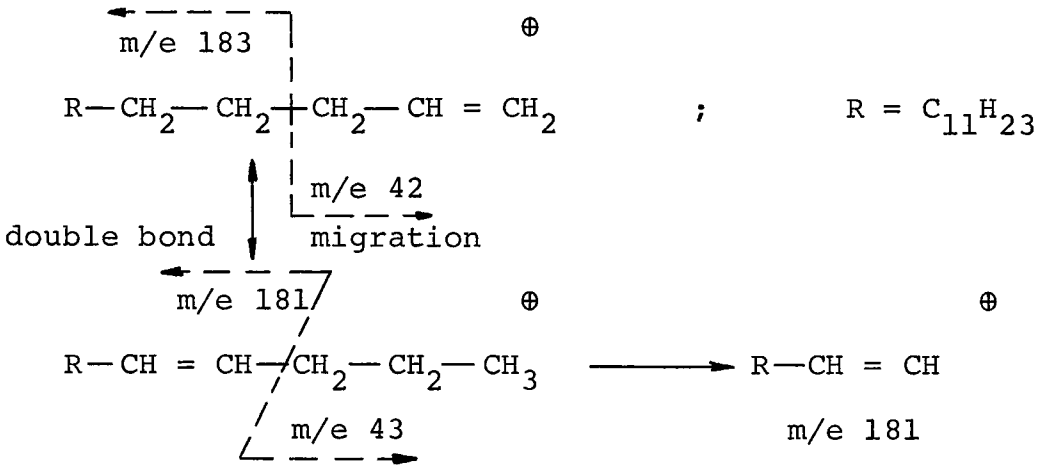
chain olefins indicates that the double bond is in a terminal position.

The mass spectrometry of 1-olefins labelled in different positions should confirm or invalidate such an assumption.

Another rather peculiar characteristic of these olefins is indicated in Figure 15. Simple cleavage of carbon-carbon bonds would produce a spectrum in which the ions of the series C_nH_{2n-1} (alkenyl ions) would predominate. Only the mass of the molecular ion would fall in the olefinic series (C_nH_{2n}). But as can be seen in Figure 15, there are two main ion distribution curves corresponding to the two series (C_nH_{2n-1} and C_nH_{2n}) with a well defined predominance of the olefin ion series above C_9. This has been observed to be a general case in the mass spectrometry of alkenes.

The C number where the intensity of the olefin ions takes over is indicated in Tables XII, XIII, and XIV by a star next to the reported intensity of the olefin ion. This takes place at C_9 for the C_{11} and C_{12} olefins (Table XII), at C_{10} for the C_{13} through the C_{17} olefins (Tables XII and XIII) and at C_{16} and C_{17} for the C_{25} and C_{27} olefins (Table XIV). The presence of these olefin ions and their relative intensities appears to indicate that i) the positive charge resides

always in the fragment which contains the double bond



ii) abstraction of hydrogen by the C_nH_{2n-1} ions to give the olefin or C_nH_{2n} ions seems to take place with increasing probability the higher the mass of the C_nH_{2n-1} fragment. Finally a comparison between the mass spectra of two high molecular weight olefins and their corresponding saturated alkanes (Table XIV) will serve to summarize everything said about the characteristics of the spectra of n-alkanes and n-alkenes. In both cases the ion distribution curve decreases smoothly towards the parent ion, after it has gone through its maxima in the low molecular weight region. Usually the unsaturated hydrocarbons have maxima displaced a few C atoms toward higher mass numbers (C_7, Table XIV) as compared to the saturated hydrocarbons. (Both pentacosane and heptacosane show maxima at C_3; not included in Table XIV). While the

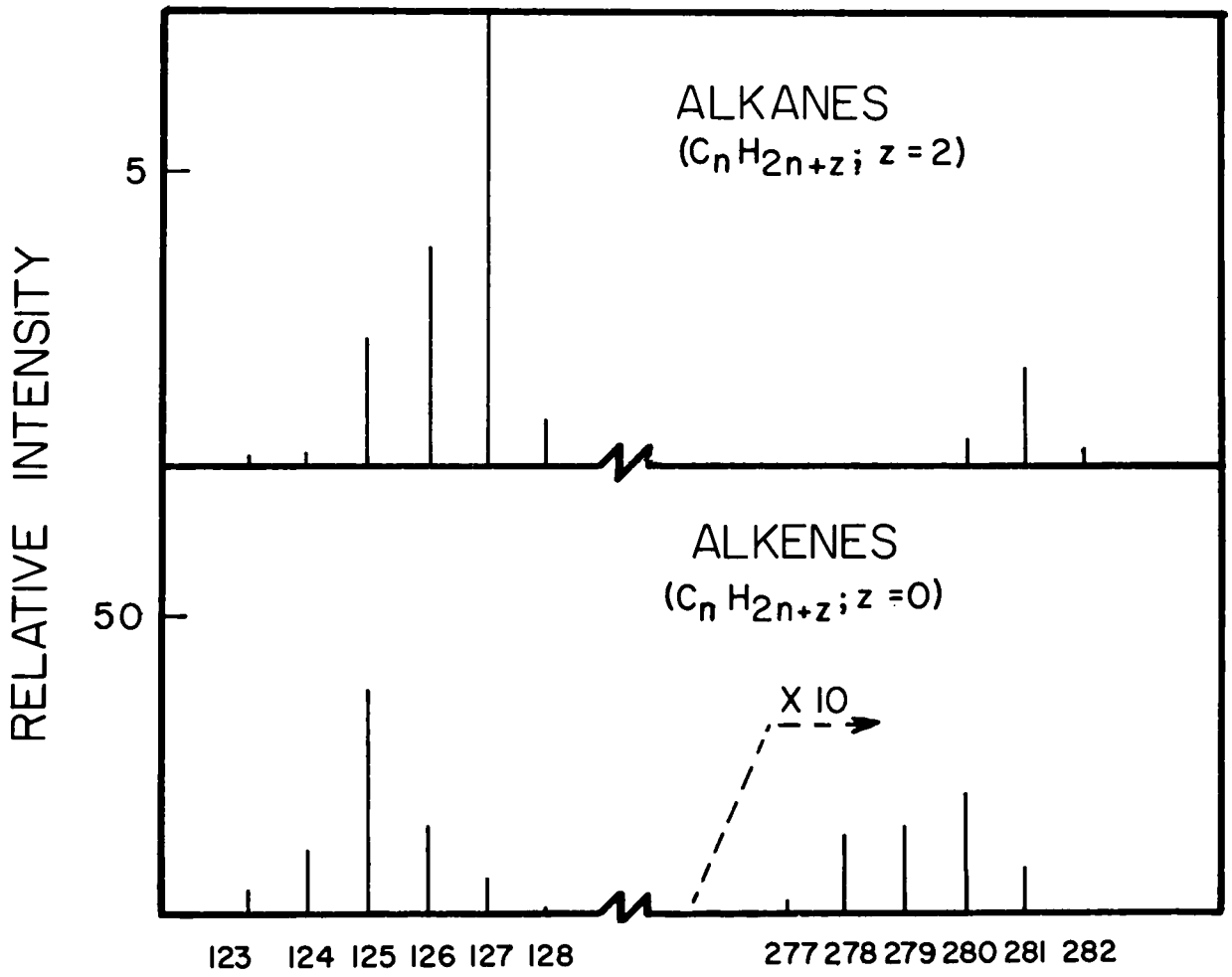
n-alkanes have one well defined series of prominent peaks ("head peaks") in the z=+1 mass series (m/e 127, 281, in Figure 16), there is no such parallel in the spectra of monoolefins, which show two series of prominent peaks. One of them important in the low molecular weight range, the C_nH_{2n-1} or alkenyl series (m/e 125, 279 in Figure 16) and the other, the C_nH_{2n} or olefin series (m/e 126, 280 in Figure 16) which takes over in the high molecular weight range of the spectra (from C_{16} and C_{17} on, Table XIVA,B). Data shown inside the boxes in Table XIVA is represented graphically in Figure 16. The M-28 peak (underlined) is quite apparent in the spectra of n-pentacosene and n-heptacosane. The M-15 peak is practically non existent in the four examples indicating the straight chain structure of the compounds and the parent ion, although it decreases with increasing molecular weight, is always much higher when the molecule contains at least one double bond. In fact its intensity seems to increase proportionally to the number of double bonds present. This observation is also supported by the spectra of compounds with a more highly unsaturated character, such as diolefins and polyisoprenoid olefins. In general it appears that unsaturation brings stability to the molecule. Delocalization of the positive charge over the entire molecule will be more favored the greater the number of double bonds present. This charge distribution will make it more difficult to

FIGURE 16

CHARACTERISTIC FRAGMENTATION PATTERNS OF HIGH MOLECULAR WEIGHT HYDROCARBONS

Graphical representation of the actual ion distributions corresponding to two carbon numbers (n = 9 and 20) in the spectra of high molecular weight hydrocarbons (see Table XIV).

CHARACTERISTIC FRAGMENTATION PATTERNS OF HIGH MOLECULAR WEIGHT HYDROCARBONS



fragment the molecule at any given place and thus the resulting stability will be reflected in the higher intensities of the parent ions. The gas chromatography of these type of olefins (with one double bond) does not present any particular problems (see Figure 8). Although non polar phases such as silicones can be used, the best results will be obtained using polar phases that will show a selective retention towards the unsaturated molecules. For instance on a column coated with Polysev (Table I) the olefins are eluted after the corresponding n-alkanes. Satisfactory resolution can be achieved even with the low resolution columns.

2. Diolefins

Since standards of high molecular weight olefins are not available, this section will be represented by the highest odd carbon numbered diolefins (C_{27} through C_{33}) identified in Botryococcus braunii (a microscopic algae, see Chapter V). Their partial mass spectra is given in Table XV. These compounds belong to the mass series C_nH_{2n-2} and upon simple fragmentation of C-C bonds the most intense fragments should appear in the C_nH_{2n-3} mass series. But as in the case of the monoolefins the double bonds will engage in extensive migration processes along the molecule and hydrogen atom abstractions by the fragments of the C_nH_{2n-3} series will also take place. That this abstraction of hydrogen is even more important here is demonstrated by the absolute

TABLE XV

HIGH MOLECULAR WEIGHT DIOLEFINS FROM BOTRYOCOCCUS BRAUNII

Partial Mass Spectra

	C_{27} Diolefin					C_{29} Diolefin				
	Mass Series (Z in C_nH_{2n-Z})									
C_n \ Z	-3	-2	-1	0	+1	-3	-2	-1	0	+1
C_5	14.5	43	20	15	14	15	37	17	13.5	14.5
C_6	29	98	50	10	9.5	32	95	47	10	9
C_7	34	100	52	8	4	38	100	55	7	4
C_8	25	42	27	6	4	27	41	27.5	6.5	4
C_9	20	31	13.5	3.5	2	21	31	15	4	2
C_{10}	15	24.5	8	3	1.5	15	25	7.5	3.5	1.5
C_{11}	8	16	5	3	1.5	8.5	16	6	3.5	1.5
C_{12}	6	11	5.5	3	1.6	6	11	6	3	1.5
C_{13}	4.5	7	2.5	2.9	1.4	4.5	8	3	3	1
C_{14}	5.6	6.8	1.8	1.4	0.6	4.1	7	2.1	1.8	0.7
C_{15}	(7.7)	7	2.2	0.9	0.4	(9.2)	7.2	2.6	1.3	0.8

TABLE XV CONTINUED

HIGH MOLECULAR WEIGHT DIOLEFINS FROM BOTRYOCOCCUS BRAUNII

Partial Mass Spectra

	C_{27} Diolefin					C_{29} Diolefin				
	Mass Series (Z in C_nH_{2n-Z})									
C_n \ Z	-3	-2	-1	0	+1	-3	-2	-1	0	+1
C_{16}	3.2	6.8	1.8	0.7		3.5	5.7	1.8	0.7	0.4
C_{17}	2.3	11.1	2.5			2.2	6.1	1.7	0.8	
C_{18}	3.4	14.4	3.3			2.4	6.8	1.8	0.7	0.6
C_{19}	5.2	11.6	2.7			2.7	12.6	3.1	0.7	
C_{20}	3.7	11.1	4.2	0.8	(3)	4.5	16.6	5.5	1.1	(3.6)
C_{21}	2	5	1.4			6.4	13.5	3.5	0.7	
C_{22}	1.3	2.6	0.7			4.1	12.7	3.6	0.7	
C_{23}	1	2.1	0.6			2.7	6	1.7		
C_{24}	1	2.3	0.9			1.7	3.5	1.1		
C_{25}	1.6	1.5				1.5	2.8	1		

TABLE XV CONTINUED

HIGH MOLECULAR WEIGHT DIOLEFINS FROM BOTRYOCOCCUS BRAUNII

Partial Mass Spectra

	C_{27} Diolefin					C_{29} Diolefin				
	Mass Series (Z in C_nH_{2n-Z})									
C_n \ Z	-3	-2	-1	0	+1	-3	-2	-1	0	+1
C_{26}	0.3	0.2				2	4.3	1.4		
C_{27}		43	14	3.5		2.3	3	1		
C_{28}										
C_{29}						7	55	23	5	

( ) Background peaks

TABLE XV CONTINUED

HIGH MOLECULAR WEIGHT DIOLEFINS FROM BOTRYOCOCCUS BRAUNII

	Partial Mass Spectra									
	C_{31} Diolefin					C_{33} Diolefin				
	Mass Series (Z in C_nH_{2n-Z})									
C_n \ Z	-3	-2	-1	0	+1	-3	-2	-1	0	+1
C_5	13	41	18	14	15	14	40	18	17.8	18
C_6	28	97	48.5	10	10	28	96	47	12	12
C_7	32	100	54	8	4	32	100	56	10	5
C_8	25	43	28	5.5	3	24	44	30	9	6
C_9	21	32	14.5	4	2	20	34	17	5	3
C_{10}	15	27	9	3	1.5	16	28	11	4	2
C_{11}	8	17	6	3.5	1.8	9	18	7	4.7	2
C_{12}	5.5	11.5	4.5	3	1.2	6	13.2	9	3.7	1.8
C_{13}	4	6.8	2.2	2.5	0.9	5	8.5	3.1	3.4	1.5
C_{14}	3.3	5.9	1.7	1.7	0.7	0.4	7.5	2.5	2.5	1.2
C_{15}	(4)	5	1.5	0.9	0.4	(24.5)	9.6	0.4	0.6	1

TABLE XV CONTINUED

HIGH MOLECULAR WEIGHT DIOLEFINS FROM BOTRYOCOCCUS BRAUNII

Partial Mass Spectra

	C_{31} Diolefin					C_{33} Diolefin				
	Mass Series (Z in C_nH_{2n-Z})									
C_n \ Z	-3	-2	-1	0	+1	-3	-2	-1	0	+1
C_{16}	2.1	3.9	1.2	0.6	0.3	5	5.6	2.5	1.2	
C_{17}	1.6	4	1.4	0.7	0.4	2	5.2	1.9	1	
C_{18}	1.5	4.1	1.1	0.4	0.3	2	5.5	1.6		
C_{19}	1.7	5	1.4	0.3	0.2	2.5	7.5	2.2		
C_{20}	1.9	5.9	1.9			2.7	9	5	1.5	(11)
C_{21}	2.3	11.2	2.8	0.5		3.4	10	2.5		
C_{22}	3.7	14.2	3.9	0.6		3.1	10.5	3.1		
C_{23}	5.3	11.7	3.1	0.6		3.4	10.5	3		
C_{24}	2.8	10.5	3.1	0.6		4	11.5	3.4		
C_{25}	1.7	4.3	1.3	0.3		3.7	8.5	2.5		

TABLE XV CONTINUED

HIGH MOLECULAR WEIGHT DIOLEFINS FROM BOTRYOCOCCUS BRAUNII

Partial Mass Spectra

	C_{31} Diolefin					C_{33} Diolefin				
	Mass Series (Z in C_nH_{2n-Z})									
C_n \ Z	-3	-2	-1	0	+1	-3	-2	-1	0	+1
C_{26}	1.1	2.2	0.7	0.1		2.5	7	2.2		
C_{27}	1	2.1	0.8	0.2		1.6	5.5	1.7		
C_{28}	0.9	3.3	1.1	0.2		1	2.5			
C_{29}	1.7	3.5	1.2	0.3		2	(18.5)	7		
C_{30}						0.7	2	0.6		
C_{31}	3	60	22.5	5		2.5	(19.5)	7.5	2	
C_{32}										
C_{33}							28	12		

( ) Background peaks

predominance of ions in the C_nH_{2n-2} or molecular ion series. All of the four ion distributions show maxima at C_7 and the most intense fragments in the z=-2 column.

A peculiar feature of these four spectra not observed in compounds with only one double bond is the second minor maxima at C_{18}, C_{20}, C_{22} and C_{24} respectively. This corresponds to an M-126 fragment in each case. There is no evidence of a higher M-28 ion in these spectra. The introduction of another double bond as illustrated by comparison of Table XIV and XV results in a slightly greater complexity of the mass spectral pattern. On the other hand the presence of this second double results in a predictable greater retention time in the gas chromatographic columns relative to that of the mono unsaturated homologs and also in higher parent ions.

3. Isoalkenes

A discussion on the mass spectrometric characteristics of methyl branched olefins can be found in the section dealing with bacteria (Chapter V, S. lutea). In principle there are no other more characteristic features in the mass spectra of these compounds than those resulting from the combination of a classical isoalkane spectrum with an olefin spectrum.

E. Unsaturated Isoprenoid Compounds

At present not much is known about the mass spectrometry

of this class of compounds, mainly because their occurrence in natural products is not as widespread as in the case of their saturated counterparts or because our methods of analysis are in some way discriminatory against them. This discussion will be limited to the few isoprenoid mono and polyenes that have been identified in this work. That includes an isomeric C_{19} monoolefin, two isomeric C_{20} monoolefins, a few isomeric phytadienes and squalene. All of them may be separated by GLC techniques for their mass spectrometric study but very little published data is available (170,216,218).

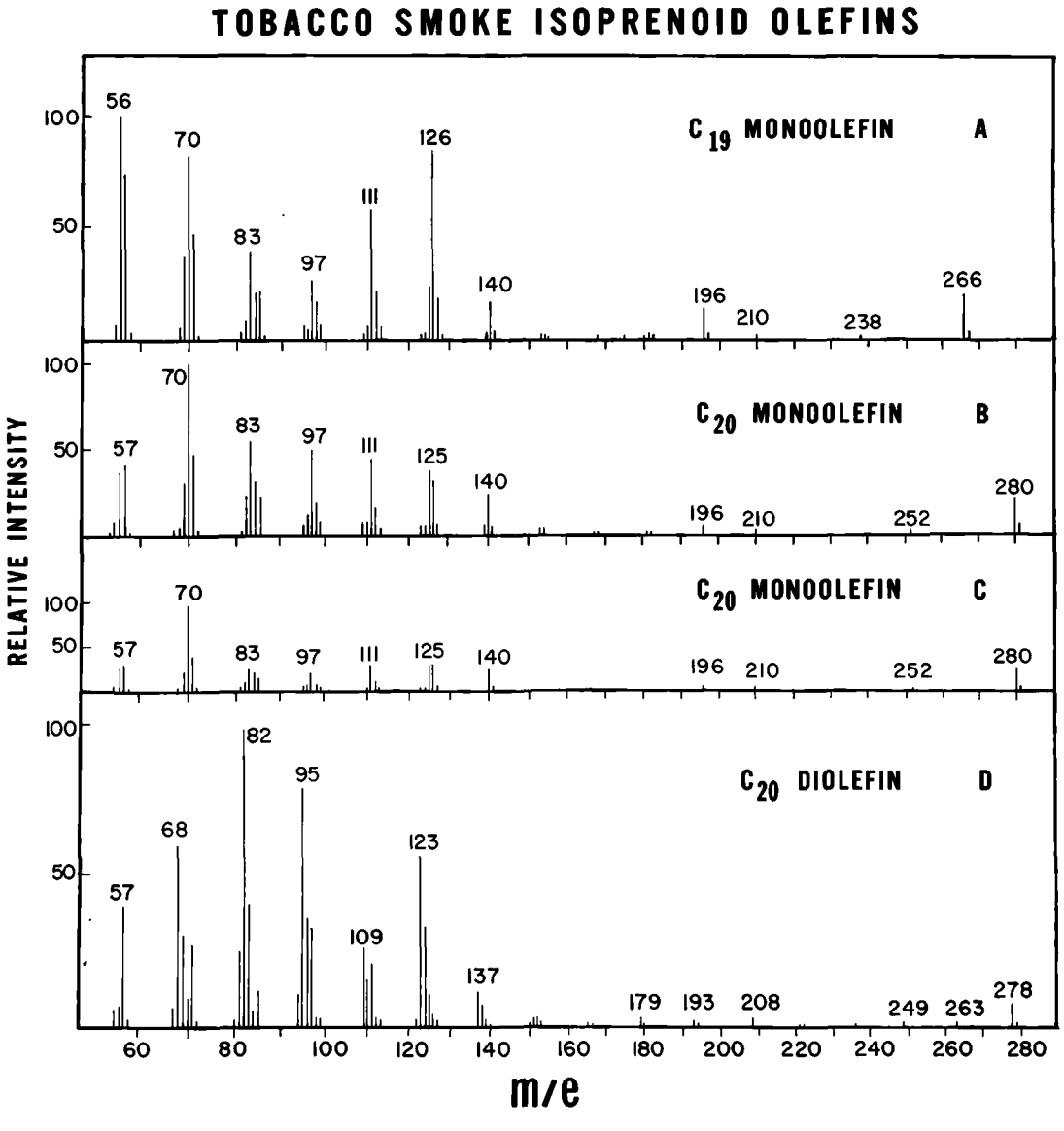
1. C_{19} isoprenoid alkene

The mass spectrum of a C_{19} isoprenoid alkene identified in the aliphatic hydrocarbon fraction of tobacco smoke is given in Figure 17A. The almost perfect match with the spectrum of a synthetic 2,6,10-trimethylhexadecene reported by McCarthy (281), could be indicative that this compound does not have the regular isoprenoid structure of pristane (53). On the other hand there might not be appreciable differences between the spectrum of 2,6,10-trimethylhexadecene and that of 2,6,10, 14-tetramethylpentadecene. Although this assumption can not be proved because there is no mass spectral data on the latter compound, this has been found to be the case with the two saturated homologs (Figure 11) (281,282) which give very similar mass spectra, thus indicating that the terminal methyl group does not have a great effect on the overall

FIGURE 17

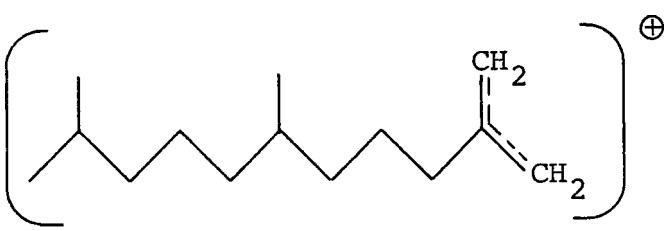
MASS SPECTRA OF ISOPRENOID OLEFINS FROM TOBACCO SMOKE

The spectra were taken as the compounds were eluted from a stainless steel column (310 m long by 0.076 cm i.d.) coated with Polysev. LKB 9000 gas chromatograph-mass spectromet combination. Electron energy, 20 eV. Ionizing current 60 μA.


TOBACCO SMOKE ISOPRENOID OLEFINS
RELATIVE INTENSITY
100
50
56
70
83
97
111
126
140
196
210
238
266
C19 MONOOLEFIN
A
70
57
83
97
111
125
140
196
210
252
280
C20 MONOOLEFIN
B
70
57
83
97
111
125
140
196
210
252
280
C20 MONOOLEFIN
C
82
95
68
123
57
109
137
179
193
208
249
263
278
C20 DIOLEFIN
D
60
80
100
120
140
160
180
200
220
240
260
280
m/e

mass spectrometric pattern. Once again the mass spectrometric information must be complemented by the gas chromatographic data in order to achieve a reliable identification. In this case no standards were available for this purpose and so the problem can not be definitely settled. Nevertheless, assuming that the increase in the retention time of the n-C_{16} hydrocarbon by the addition of one double bond to form the n-Δ-C_{16} will be similar to that experienced by pristane when one double bond is added to its structure, an interesting extrapolation can be made on Figure 54. That is, the 2,6,10, 14-tetramethylpentadecene should appear just before the nC_{17} alkane. Now, as it has already been mentioned (see Figure 12) the 2,6,10-trimethyl hexadecane is eluted after the n-C_{17} on Polysev columns and so close to it that under the chromatographic conditions prevailing in Figure 54 it would not be resolved from this n-alkane. Then the presence of a double bond in this trimethyl alkane would displace it to the position in which the C_{19} isoprenoid monoene appears. On this basis and in accordance to the MS data the most probably structure of this peak is:

The great stabilizing influence of the double bond is reflected in the relatively high intensity of the molecular ion (m/e 266). Compare it with the relative intensity of the corresponding molecular ion (m/e 268) in the fully saturated C_{19} structure (see Figures 10, 11, 13A). Also, the effect of the double bond can be clearly seen in the fragmentation pattern. The spectrum of pristane as well as that of 2,6,10 trimethyl hexadecane exhibit two major fragments at m/e 113 and m/e 183, and it could be expected that the presence of a double bond in the molecule with its migrating ability would produce the correspondingly unsaturated major fragments at m/e 111 and m/e 181. The fact that the major fragments appear at m/e 126 and m/e 196 (Figure 17A) suggests that a process of allylic cleavage is somehow involved. Observe that there is not an M-15 ion. The loss of the $-CH_3$ groups is most likely prevented by the combined effects of the migration of the double bond along the molecule and the allylic resonance stabilizations such as:



m/e 196

Also while there is no M-15 ion a small M-28 peak can be seen in Figure 17A. This appears to support the above extrapolation on the gas chromatographic data since it would be hard to visualize the loss of 28 units from the regular norphytene structure.

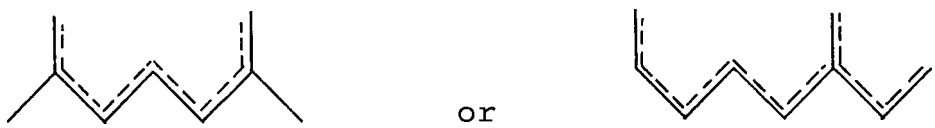
2. C_{20} isoprenoid monoenes

Two of the C_{20} monoenes identified in tobacco smoke (Figure 17B and 17C) appear to have a minimum of three or four methyl branches because of their reduced elution times. Although the position of the methyl substituents can not be readily determined from the mass spectral patterns they show a striking similarity to the patterns of the C_{19} and C_{20} unsaturated isoprenoids (Figure 17A and 17D). On these basis they have been tentatively identified as C_{20} isoprenoid monoolefins.

3. C_{20} isoprenoid dienes

While the C_{19} isoprenoid alkene has been found only once, these type of compounds appear to be more common. Four isomeric phytadienes have been isolated from zooplankton by Blumer (52). During this investigation the presence of four isomeric peaks, the mass spectra of which appears consistent with that of an unsaturated phytane structure, was detected in at least two different occasions (Mistletoe, Clover, see Chapter V). Only one major isomer was found in a tobacco smoke condensate. In all cases the spectra do not

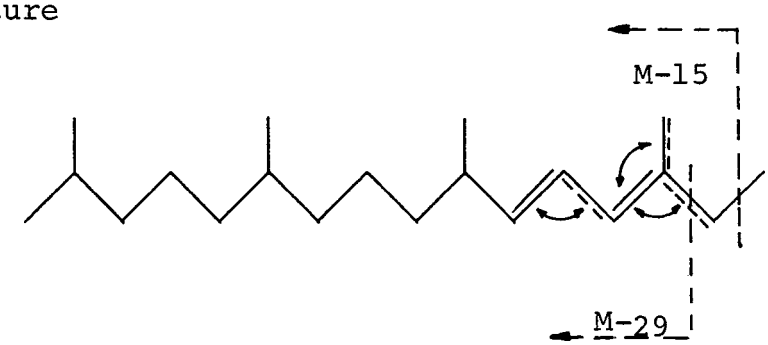
differ much from the one shown in Figure 17D as an example. The molecular mass of 278 corresponds to a C_{20} diolefin and its chromatographic position, just before nonadecane (see Figure 54) indicates that it must be branched. Although no synthetic standard is available in this case for comparison of the spectra, both its fragmentation pattern and chromatographic data suggest that it is an isoprenoid diolefin or phytadiene (52). As in the spectrum of the isoprenoid monoolefin Figure 17A there is a maximum at C_9 (m/e 123 in this case) (Figure 17D). It would correspond to either of these two fragments



m/e 123 m/e 123

where resonance stabilization due to double bond migration is indicated by the dotted lines. The C_{13} and C_{14} fragments characteristic in the spectra of phytane (Tables VII, VIII) can also be seen here at m/e 179 and 193. Allylic cleavage gives rise to the C_{15} fragment (m/e 208). A major difference with the spectrum of the C_{19} compound (Figure 17A) is the

presence in this case of relatively high M-15 and M-29 ions. This appears to make sense now because the participation of an allylic resonance process of the kind shown in this structure



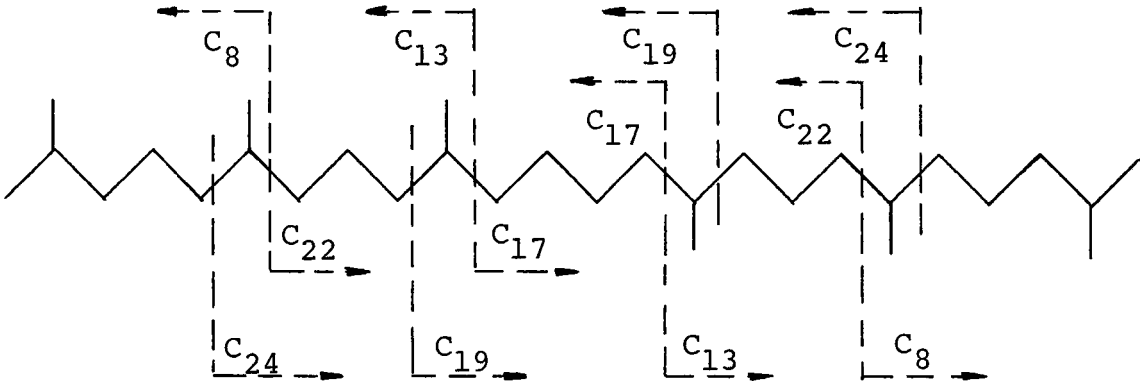
would tend to cause fragmentation at the sites indicated, thus giving rise to the M-15 and M-29 fragments. Note that it is the last methyl group of the backbone chain the one that is lost not the methyl substituent. These two processes can not take place in the C_{19} structure considered before. The enhanced intensity of the parent ion as a consequence of the two double bonds can also be seen in this case.

4. Squalene

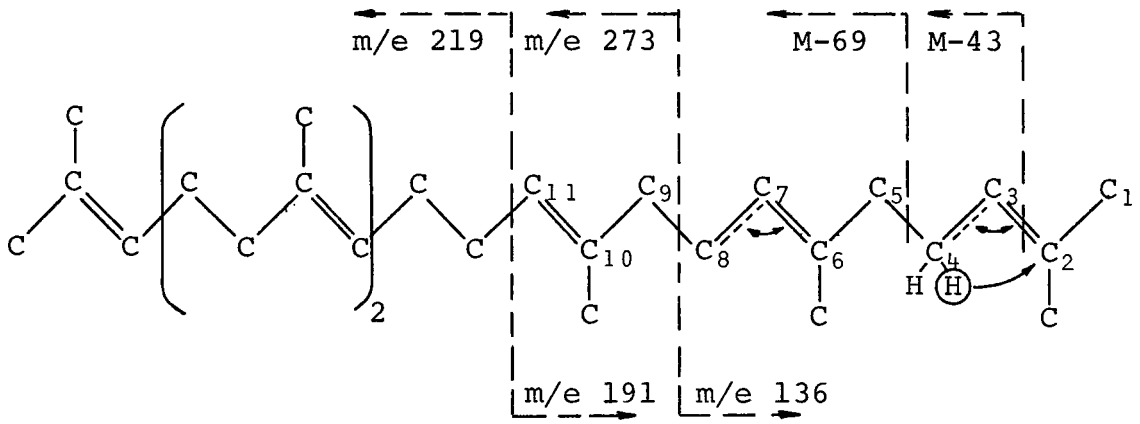
Although the mass spectra of squalene has appeared once in the literature (170) no discussion was presented at that time concerning its major fragmentation modes. In line with the results presented for the C_{19} and C_{20} isoprenoid alkenes (Figure 17) it would be expected that the spectra of squalene (2,6,10,15,19,23 hexamethyl tetracosa -2,6,10,14,18, 22-hexaene) would exhibit major differences in their respective fragmentation modes. Indeed this has been found to be true

demonstrating once more the considerable influence that double bonds may have on a mass spectrometric pattern.

The spectrum of squalane ($C_{30}H_{62}$)



shows as expected, major fragments corresponding to those indicated in the above scheme (C_8, C_{13}, C_{19}, C_{24}), and minor peaks at C_{17} and C_{22}. The parent ion is only 0.07% of the intensity of the C_8 fragment (see API spectrum No. 126-m) and there is a rather large M-15 peak. The spectra of its unsaturated counterpart squalene are given in Figure 49 and in Talbes XI and XXIV. According to the observed pattern it appears that the spectrum can be rationalized in the following way:



Abstraction of a hydrogen atom by the terminal C_3H_6 group with the resultant formation of a terminal olefin bond between carbons 3 and 4 would result in an M-43 ion.

The formation of the M-69 fragment would involve the simple allylic cleavage of carbons 4 and 5. In this respect it must be pointed out that a mistake was introduced in the spectra previously reported (170) where an M-68 ion is indicated instead of the M-69 (see Figure 49).

Allylic cleavage of the bond between carbons 8 and 9 with resonance stabilization of the positive charge on carbons 6 and 8 and subsequent loss of a hydrogen atom would produce the major fragment at m/e 136. The intensity of the parent peak in the standard of Figure 49 is as high as 13% of the major peak. A comparison of these features with those of the saturated C_{30} isoprenoid will immediately reveal the great changes introduced by the unsaturation of the molecule.

F. Naphthenes

To report on the olefinic content of any kind of sample it is important to be able to differentiate between the olefins and their isomeric cyclo-alkanes. This can not be done only on a molecular weight basis because both kinds of compounds belong to the same mass series, the C_nH_{2n} or olefin series. Table XVI shows the partial spectra of an olefin together with the corresponding isomeric alkylcyclohexane. An alkylcyclopentane with one carbon atom less is also included.

TABLE XVI

MASS SPECTRAL CHARACTERISTICS OF UNSATURATED AND CYCLIC HYDROCARBONS

	Relative Peak Height[a]								
	1-Hexadecene[b]			n-Decylcyclohexane[c]			n-Decylcyclopentane[d]		
C_n \ Z	-2	-1	0	-2	-1	0	-2	-1	0
C_4	24.6	148	88.6	5.8	46.6	8.2	13.1	73.1	33.2
C_5	21.2	97.5	75.4	3.9	11.2	4.4	92	100	21.6
C_6	25	100	47.9	70.6	100	8.4	26.3	61.6	19.1
C_7	10.9	73.8	27.7	1.2	5.65	1.5	4.8	26	11.2
C_8	4.6	31.8	16	0.2	1.66	0.9	1.9	11.9	6.3
C_9	2.1	12.3	8.5		0.47	0.2	1.0	5.1	3.8
C_{10}	1.3	4.03	5.1*		0.30	1.1*	0.6	1.9	2.7*
C_{11}	0.5	1.88	3.3		0.10	0.09	0.3	0.9	1.4
C_{12}	0.3	0.92	1.4		0.06	0.05	0.1	0.5	0.6
C_{13}	0.1	0.47	1.1		0.03	0.02	0.1	0.2	2.7
C_{14}		0.20	1.9		---				---
C_{15}		0.02			0.01				7.11 (M)
C_{16}			2.41 (M)			2.81 (M)			

a) For series C_nH_{2n-1} peaks relative to m/e 83 peak

b) A.P.I. Research Project 44 No. 1013

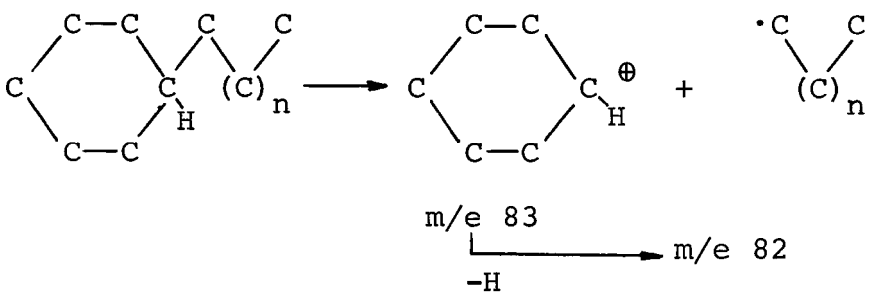
c) A.P.I. Research Project 44 No. 948

d) A.P.I. Research Project 44 No. 889

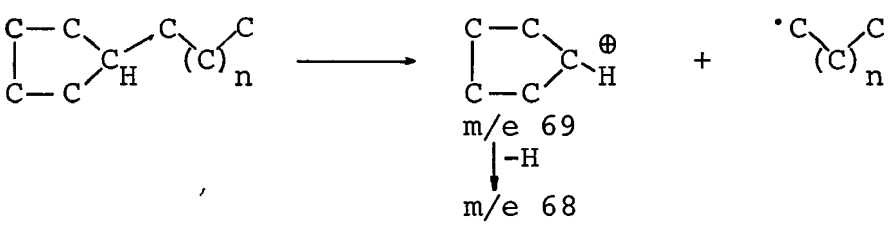
The most important feature in this table is the high relative intensity of the C_6 and C_5 fragments in the C_nH_{2n-2} mass series (underlined in the Table XVI). In other words the base peak in the spectra of cyclic hydrocarbons is always accompanied by a high peak at one mass unit less.

The base peak itself arises by cleavage of the alkyl chain, leaving a very stable cyclic carbonium ion.

1. Alkyl cyclohexanes



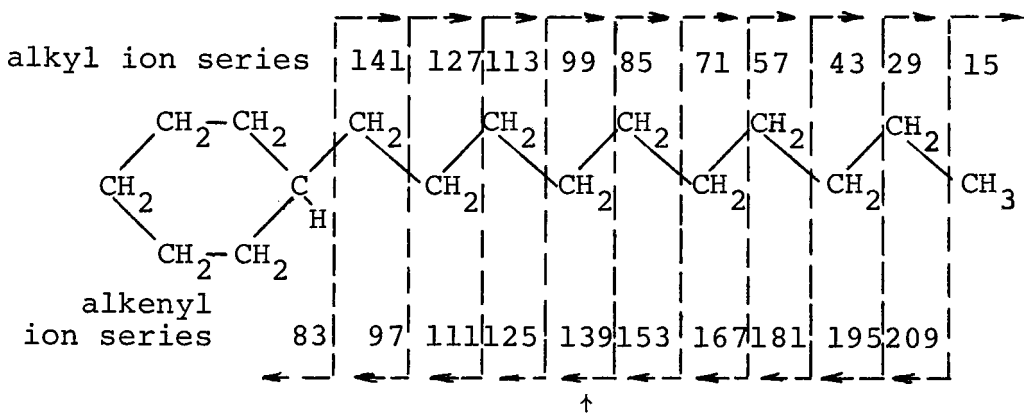
2. Alkyl cyclopentanes



The C_6 fragment (m/e 83) of n-decylcyclohexane (Table XVI) is the highest peak of the spectrum. The peak at m/e 82 shows a relative intensity (70.6%) which is almost three times as high as that of the corresponding ion in the spectra of n-hexadecene (25%). Note also that the base peak (highest peak) in the spectra of n-hexadecene is not the C_6 fragment (m/e 83) but the C_4 (m/e 55). The spectra of decylcyclopentane

shows a base peak at m/e 69 (cyclic C_5 fragment) and also a very high peak at m/e 68 (92%) as indicated above.

In general the parent ion peaks of cyclic hydrocarbons are larger than in their corresponding straight chain saturated or unsaturated compounds. The predominant ions in the spectra of cycloalkanes belong to the C_nH_{2n}, C_nH_{2n-1} and C_nH_{2n-2} series, and in parallel to the ion distribution curves observed in the case of alkenes, the C_nH_{2n-1} or alkenyl ion series is the predominant series up to a certain carbon number (marked with a star in Table XVI) where the C_nH_{2n} or olefin series takes over. In particular the general feature observed in the majority of the spectra obtained from different samples, including the standards, is the predominance of the olefin ion series from the M-84 fragment to the molecular ion, M. In Table XVI an asterisk marks the M-84 peak. This effect is well illustrated in the series of alkyl cyclohexanes identified in the Murray meteorite (see Tables LXIII and LXIV). In alkyl cyclopentanes the same appears to be true above the M-70 fragment (Table XVI). Considering as an example a molecule such as n-decylcyclohexane



All fragments containing the cyclohexyl ring will generate the series of alkenyl ions (C_nH_{2n-1}).

Since the alkyl ion series (C_nH_{2n+1}) is of very low importance in these kind of spectra it must be concluded that single bond cleavage with retention of the positive charge on the straight chain alkyl fragment is not a favorable process.

In accordance to the experimental evidence all of the ring containing fragments to the right of the arrow have a great tendency to pick up an extra hydrogen to give a relatively high even mass ion.

Chromatographically the cyclic hydrocarbons are very hard to separate from the n-alkanes with one carbon atom more. They are selectively retained in the polar columns (Polysev) with a resultant increase in their elution time of about one whole carbon unit. The gas chromatographic separation of the hydrocarbons from the Murray meteorite (Figure 80) in a high resolution column, shows that some of the cycloparaffins have been resolved from the corresponding n-alkanes plus one C atom. These compounds, however, had not been detected before on lower resolution GC columns. It is important to keep this in mind since this lack of resolution may affect to some extent the quantitative distribution curves of the n-alkanes. Although in general this is not believed to have introduced any serious error in the samples

analyzed in this work, due to the low concentrations in which the cyclic alkanes have been detected relative to the n-alkanes, nevertheless it must be taken into account for cycloparaffins have been found in concentrations of up to 30% in petroleum. A convenient method of separation prior to gas chromatographic analysis of the n-alkanes consists on the treatment of the mixture of cycloparaffins and n-paraffins with molecular sieves.

G. Deuterated Hydrocarbons

A great number of fully deuterated hydrocarbons was obtained from the Fischer-Tropsch synthesis (see Chapter VI). These synthetic hydrocarbon products were examined repeatedly using the combined technique of gas chromatography and mass spectrometry and the results obtained will be briefly summarized here.

In general there are no major differences between the overall mass spectrometric ion distribution pattern of an alkane and that of its fully deuterated isomer. Also as it would be expected the substitution of hydrogen atoms by deuterium results in a displacement of the mass scale a number of mass units proportional to the number of deuterium atoms in the molecule. Thus a fully deuterated C_{10} n-alkane for instance would have a molecular weight 22 mass units higher than the non deuterated decane and all of the fragment ions would be expected to appear at even mass numbers only. This

is illustrated in Table XVII in the matrix or grid form that has been introduced earlier in this chapter. The masses corresponding to the fully deuterated series of fragment and molecular ions have been arranged according to the z number. While the basic format is still the same as that used for the MS data tabulation of non deuterated molecules, the z numbers range in this case only from +2 to -5 and so only eight series of ions of different masses are allowed within each carbon number.

In the same way as before, the z=+2 series is that of the molecular ions, z=+1 represents the alkyl ion series and z=0 and z=-1 the olefinic and alkenyl series, respectively. The mass spectra are represented in this form by plugging the relative intensities of each of the masses in the corresponding slot in the grid. It will be recalled from Table II that the masses belonging to the z=+1 or alkyl ion series in non deuterated hydrocarbons are 15,29,43,57,71,85,99,113,127 .... Compare them with those in the z=+1 column in Table XVII.

Typical examples of fully deuterated methyl branched paraffins obtained by Fischer-Tropsch synthesis are given in Table XVIII. Everything said before for the isoalkanes applies equally well here. Compare the features in Table XVIII to those in Table IV and Figure 7.

In regard to the gas chromatographic properties of this type of compounds a striking characteristic was observed.

TABLE XVII

MASS SERIES OF PERDEUTERATED ORGANIC COMPOUNDS

Z-Number Table (C_nD_{2n+Z})

C_n \ Z	-5	-4	-3	-2	-1	0	+1	+2
C_1				12	14	16	18	20
C_2	22	24	26	28	30	32	34	36
C_3	38	40	42	44	46	48	50	52
C_4	54	56	58	60	62	64	66	68
C_5	70	72	74	76	78	80	82	84
C_6	86	88	90	92	94	96	98	100
C_7	102	104	106	108	110	112	114	116
C_8	118	120	122	124	126	128	130	132
C_9	134	136	138	140	142	144	146	148
C_{10}	150	152	154	156	158	160	162	164
C_{11}	166	168	170	172	174	176	178	180
C_{12}	182	184	186	188	190	192	194	196
C_{13}	198	200	202	204	206	208	210	212

Mass numbers are substituted by their respective intensities to report mass spectrometric data[102,353].

TABLE XVIII

DEUTERATED METHYL ALKANES

2-METHYL HEXADECANE

C_n (m/e) \ Z	Mass Series (Z in C_nD_{2n+Z}) -1	0	+1	+2
4 (66)	2.8	18	96	
5 (82)	11	32	100	
6 (98)	16	17	78	
7 (114)	23	13	46	
8 (130)	16	11	33	
9 (146)	9	9.5	27	
10 (162)	5	8	22	
11 (178)	3	6	15	
12 (194)	2	8	12.5	
13 (210)	1.5	5	9	
14 (226)	2	29	73	
15 (242)		2	4.5	
16 (258)		6	27.5	
17 (276)		3.8		10

TABLE XVIII (CONTINUED)

DEUTERATED METHYL ALKANES

3-METHYL HEXADECANE

C_n (m/e) \ Z	Mass Series (Z in C_nD_{2n-Z}) -1	0	+1	+2
4 (66)	2	50	100	
5 (82)	13.5	25	92	
6 (98)	18	21	81	
7 (114)	23	15	43	
8 (130)	20	13	35	
9 (146)	12	11	29	
10 (162)	6.5	9.5	27.5	
11 (178)	4.5	7	20	
12 (194)	2	7	14	
13 (210)	2.5	12	17	
14 (226)	2	7.5	14	
15 (242)	2	36.5	109	
16 (258)		6	13	
17 (276)				5

(m/e) corresponds to alkyl ion series (Z = +1)

Namely that the fully deuterated molecules are less retained by the chromatographic columns than their undeuterated counterparts. It had been anticipated that being heavier the deuterated molecules would have slightly greater retention times than their undeuterated isomers but, as it was demonstrated by coinjection techniques, the reverse is true.

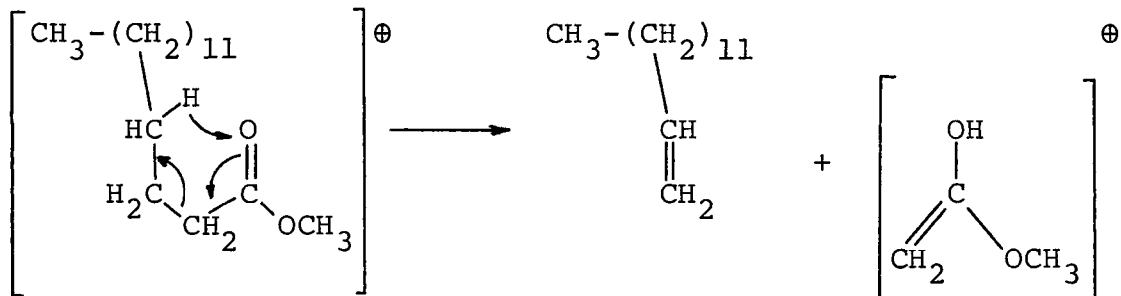
H. Fatty Acid Esters

The fragmentation mechanisms that the fatty acid esters follow in their breakdown under electron impact have been the object of detailed investigations by Ryhage and Stenhagen (416,417). The results of these investigations have been applied repeatedly in the course of this work to the mass spectrometric identification of fatty acids in plants and microorganisms. The following discussion on the general mass spectral characteristics of fatty acid esters will be divided into three main subsections: (a) the saturated straight chain esters, (b) the methyl branched esters and (c) the unsaturated esters. The saturated straight chain and branched esters of the bacteria Sarcina lutea will be used as model compounds and for the discussion on the unsaturated acids some of the ones found in algae (371) will also be used as typical examples.

As a consequence of the experimental methods applied to the recovery and analysis of the fatty acids, all of their esters discussed here will be methyl esters.

a) Saturated straight chain esters

The interpretation of the long chain esters by mass spectrometry is given in the characteristic fragmentation pattern for iso-C_{15}, anteiso-C_{15} and n-C_{16} (Figure 18), (40,417,482). The base peak in spectra of this type is usually formed at m/e = 74 (unless substituted at the α-C). It is produced by a cyclic rearrangement [McLafferty rearrangement (292)] involving the migration of a γ-hydrogen atom to the carboxyl oxygen of the ester group with a concerted β-cleavage (42,292). This results in the formation of a neutral olefin molecule and a charged enol. Hexadecanoic acid methyl ester (Figure 18) will be used as an illustration of the mechanism involved



The next intense peak found at m/e = 87, is formed by simple β-cleavage of the chain with considerable exchange of hydrogen atoms between carbon 2 and carbon 5,6 and 7. The majority of the remaining intense peaks are oxygen containing fragments formed also by simple cleavage of the chain (m/e

FIGURE 18

MASS SPECTRA OF FATTY ACID METHYL ESTERS

FROM SARCINA LUTEA

The mass spectra was taken as the components were eluted from a stainless steel capillary column coated with Polysev (91 m long by 0.076 cm i.d.). The components were ionized by electron impact at 70 eV as they entered the ion source of the LKB 9000 Gas Chromatograph-Mass Spectrometer.

FATTY ACID METHYL ESTERS

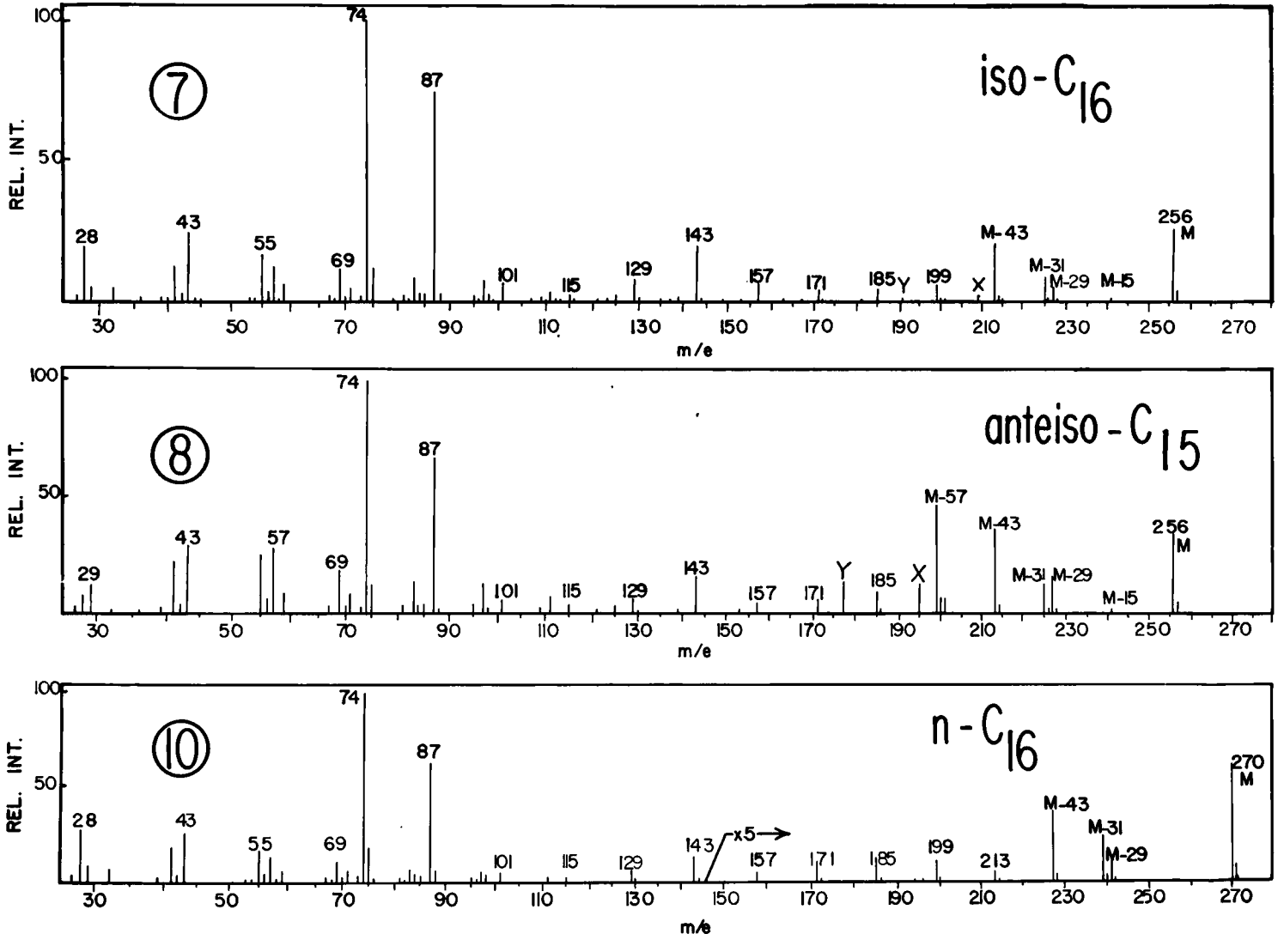
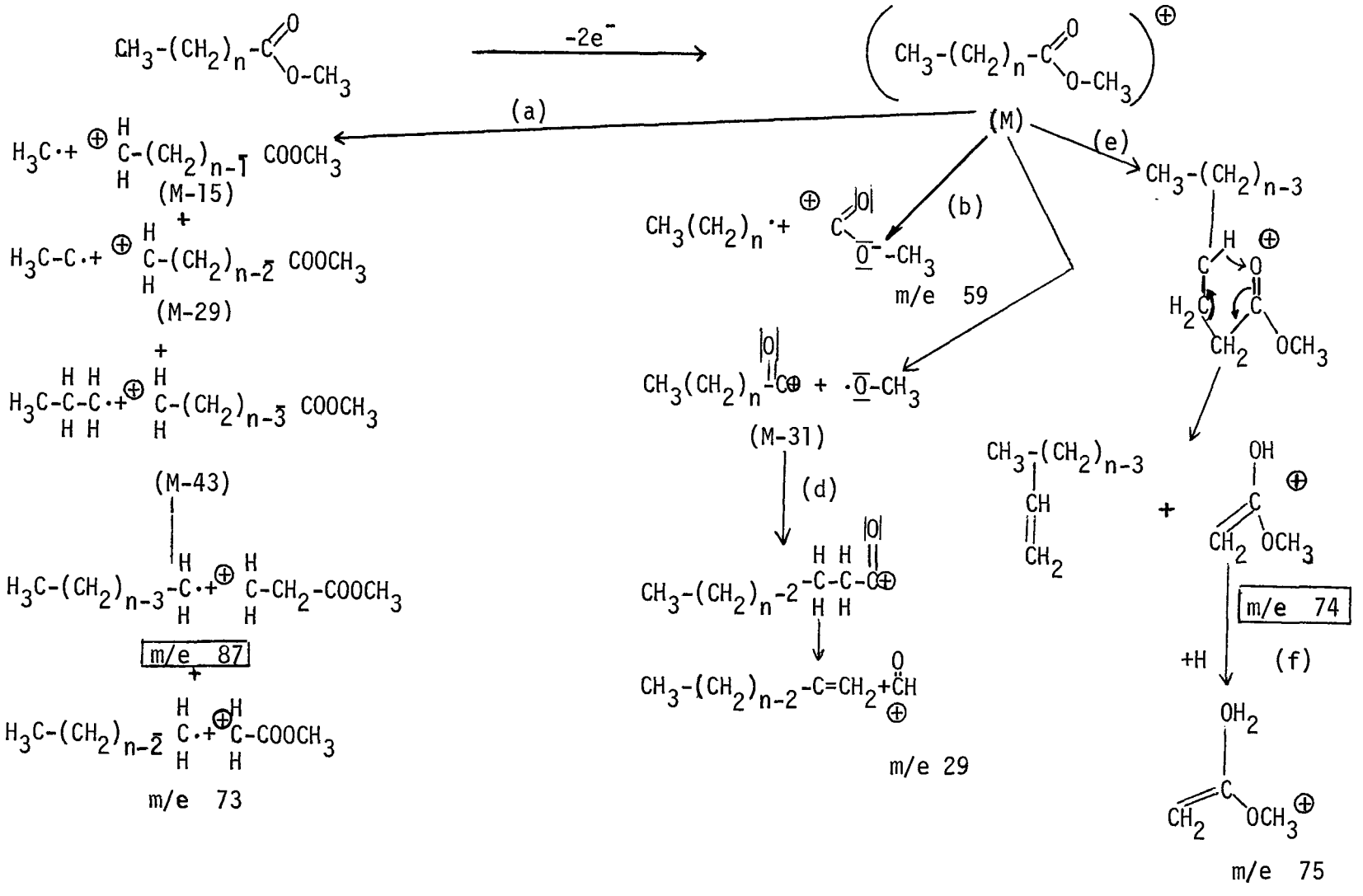


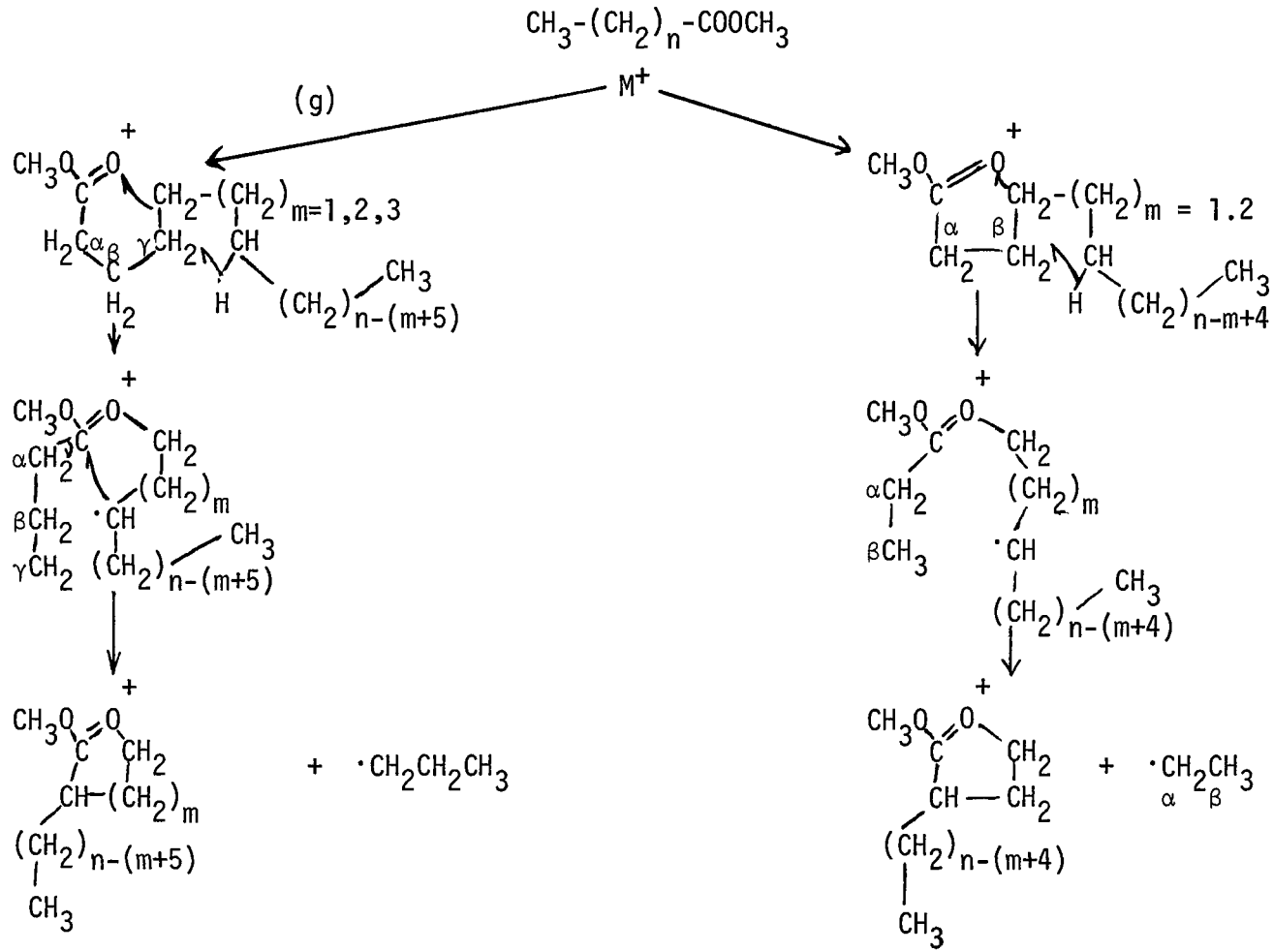
FIGURE 19

FATTY ACID ESTER FRAGMENTATION SCHEME

FRAGMENTATION SCHEME OF FATTY ACID ESTERS



FRAGMENTATION SCHEME OF FATTY ACID ESTERS



101, 129, 143, 157, etc.). The ion at m/e 143 which gives a relatively high intensity in the mass spectra of long chain fatty acid esters originates through simple 7,8-cleavage. To account for its higher probability, Beynon suggested a rearranged dicyclic structure (40). All these breakdown pathways are summarized in Figure 19 (pathways "a" and "e"). The m/e values of the two major ions (see Figure 19) are enclosed in boxes. An interesting observation can be made to the effect that while the m/e 87 has a relatively high intensity the m/e 73 peak is practically absent. Comparing the structures of both ions, shown in Figure 19, the absence of the m/e 73 fragment can be explained taking into consideration that the positive charge next to the carbonyl carbon atom will be destabilized by polarization of the carbonyl bond (42). On the other hand the same positive charge on a β carbon atom seems to result in a very stable fragment. No explanation has been given or is readily apparent for this behavior.

In going over the finer details of the spectra one of the features most readily noticed is the relative intensity of the ion at m/e 75, which is much higher than it would correspond to the expected isotope for the base peak at m/e 74. Its intensity that increases with chain length (414) certainly reflects a protonation of the enol ion as shown in Figure 19 (pathway "f"). The source of the second hydrogen is

not known. A close inspection of the mass spectral patterns shown in Figure 18 reveals the presence of a peak at m/e 59 and another more prominent at M-31. Both arising via fragmentation "b" in Figure 19. That is, excluding other factors the resonance form of the carbonyl ion resulting from an α cleavage (on either side of the CO group) would tend to lower the ionization potential of the ion enough to make both processes highly probable. This assumption is borne out in the case of the M-31 fragment as can be deduced by its high relative intensity in the spectra. On the other hand the m/e 59 fragment is not highly significant. There are two reinforcing effects to be considered here that offer a plausible explanation of the facts. Firstly, the inductive electron releasing effect of the long pentadecyl substituent in the stabilization of the positive charge on the carbonyl group will be by far superior to that exerted by the methoxy group, and secondly the stability of the $CH_3(CH_2)_{14}\overset{\oplus}{C}=O$ ion together with the stability of the $\cdot\underline{\overline{O}} - CH_3$ as a radical due to the oxygen's negative inductive effect, will lead to dissociation favoring the M-31 ion over the m/e 59 fragment. This fragment (m/e 59) really belongs in the (a) breakdown series but because of the resonance effect involved in its formation it has been set apart in Figure 19.

Finally a rearrangement involving the transfer of a hydrogen atom to the carbon attached to the oxygen [Bieman's,

type F (42)] in the M-31 fragment will give rise to the small peak at m/e 29. (Figure 19 pathway "d"). In the fragmentation scheme depicted in Figure 19 it is shown how the ions at M-29 and M-43 could arise via simple C-C bond cleavages (pathway "a") but their relative intensity, especially in the case of the M-43 suggests that some other mechanism must be involved in their formation. As established by deuterium labelling experiments (417) the M-43 peak as well as the M-29 originate through an expulsion mechanism rather than by loss of the terminal propyl and ethyl groups respectively.

The expulsion of ($-\overset{\alpha}{C}H_2 - \overset{\beta}{C}H_2 - \overset{\gamma}{C}H_2 - +H$) gives rise to the M-43 peak while the M-29 is formed by expulsion of the group ($-\overset{\alpha}{C}H_2 - \overset{\beta}{C}H_2 - +H$). The mechanism proposed (reference 76, p. 14) for such processes most likely involves the collapse of the molecular ion through a cyclic radical transfer process as illustrated in Figure 19 (pathways "g" and "h").

b) Branched fatty acids

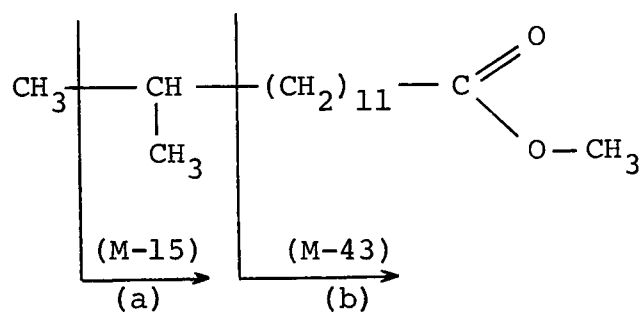
Concerning the branched isomers of the normal fatty acids, it is important to emphasize that the introduction of an alkyl side chain at a carbon atom other than C-2 does not have any great effect on the overall appearance of the spectrum, except for an increased tendency of the alkyl chain to fragment at the carbon atom bearing the additional substituent. The presence of a methyl side chain is readily determined by means of the peak formed at M-15, which is absent in the normal

compounds (Figure 18). End branching of the iso and anteiso type would not be expected to change the fragmentation pattern significantly except at both ends of the spectrum.

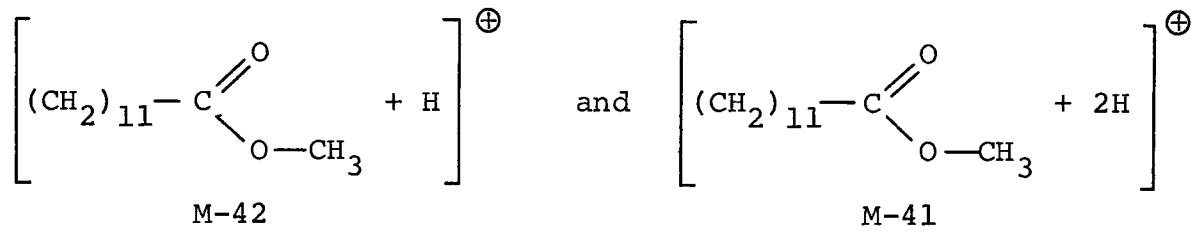
This is indeed the case in the branched fatty acid esters of Sarcina lutea shown in Figure 18 Nos. 7 & 8, where there was no evidence of any appreciable change in the general fragmentation pattern between the normal and the methyl branched compounds. This, as said before, can be accounted for by considering that the methyl substituent is in an iso or an anteiso position so that only the lower and higher end of the spectra will show any changes, while the region between m/e 74 and M-57 remains largely undisturbed.

The characterization of the iso branching in those molecules is at first unclear because the peak originated by the loss of the isopropyl radical with retention of the positive charge on the large oxygen containing fragment coincides with the M-43 peak originated by the expulsion mechanism mentioned above (see Figures 18, 19).

The extra fragmentation process due to the presence of the methyl substituent in the iso position, can be described as follows:

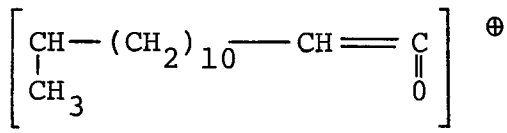


Ion (b) is accompanied by rearrangement ions of structures:



M-42 and M-41

The best clue to the position of the methyl branch is obtained from the presence of a ketene ion (m/e 209) (415) which is derived from the branched structure through the loss of methanol (peak (X) in Figure 18).

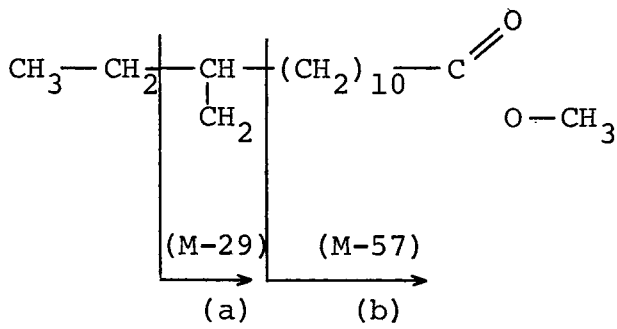


m/e 209

The loss of water from the ketene ion gives a peak at m/e 209-m/e 18 = m/e 191 [(Y) in Figure 18]. Although of very low intensity, both peaks (X & Y) are reliable since they are completely absent in the spectra of normal fatty acids.

In the case of anteiso long chain methyl esters, the problem is somewhat simpler since there is a peak at M-57 (due to loss of the butyl radical) which is much higher than the corresponding peak in a normal fatty acid ester. The anteiso-C_{15} (Figure 18) can be accounted for in terms of the

following characteristic peaks:



As in the case of the iso structure the ion (b) is accompanied by the rearrangement ions at M-56 and M-55. The ketene ion appears here at m/e 195 [(X) in Figure 18] and by loss of water gives rise to a peak m/e 177 [(Y) in Figure 18]. A pronounced feature is the higher intensity of the M-29 peak relative to that of the M-31 (loss of the methoxy radical). This is expected since there are now two different processes contributing to the formation of the M-29 ion, (the expulsion process mentioned before and a single bond cleavage next to the methyl substituent).

c) Unsaturated esters

Mass spectra showing the M-32, M-74 and M-116 maxima were observed for the methyl oleate and other monoenoic fatty acid methyl esters. (Figure 46). In general, the mass spectra of the positional isomers of monounsaturated carboxylic acids are very much alike (190) when the double bonds are at position 6,7 or higher in the chain. Usually a peak

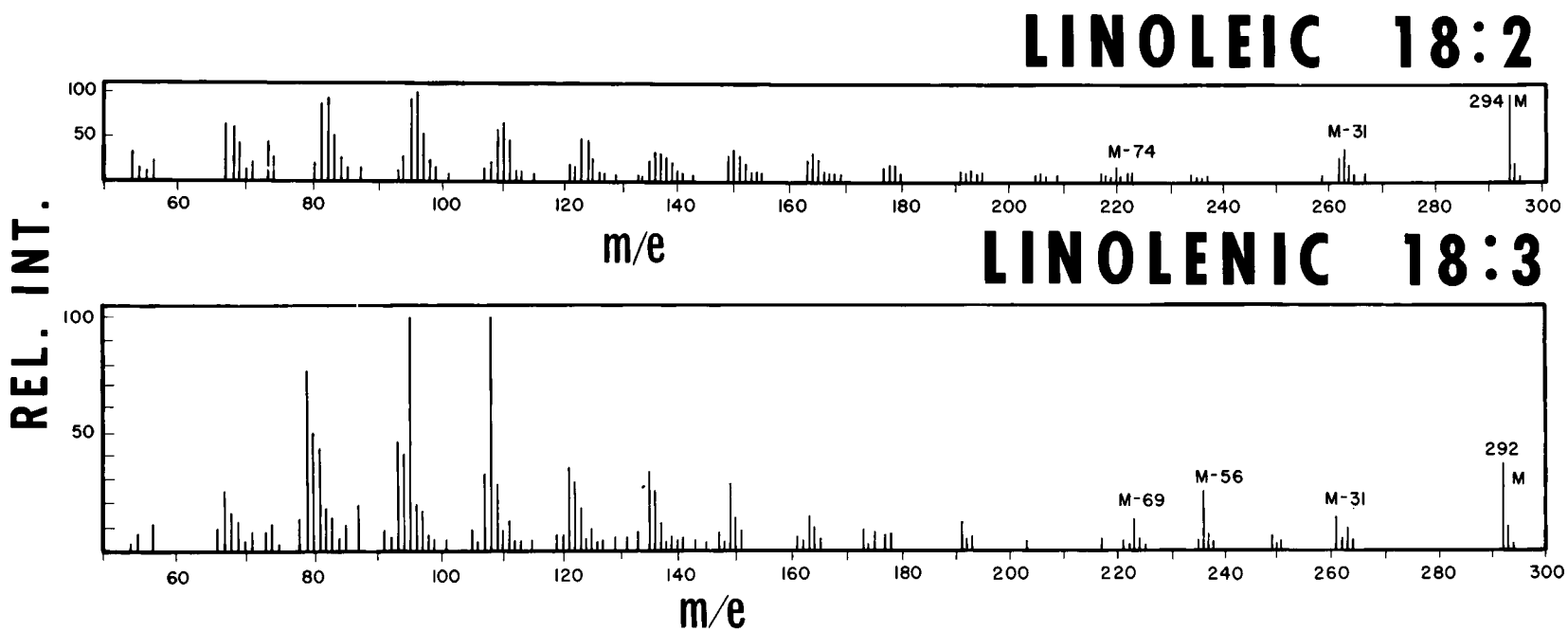
corresponding to the loss of methanol (m/e = M-32) is found in their spectra together with other characteristic peaks at m/e = M-74 and m/e = M-116. The peak at M-74 is produced by β cleavage with concerted rearrangement of a γ-hydrogen atom to the carbonyl oxygen of the ester group (42,292), in the same way in which the base peak (at m/e 74) in the spectra of saturated esters is produced. The only difference being that in the case of monounsaturated acids the resulting diolefin apparently has a more marked tendency towards stabilization of the positive charge and so not only the m/e 74 fragment is seen but also the diolefin ion at M-74. The peak at M-116 arises from a 5,6-cleavage. Observations in line with these fragmentation mechanisms were made in the case of the monoenoic fatty acid methyl esters.

Slightly different characteristic fragmentation patterns were observed with the C_{18} di- and trienoic fatty acid methyl esters (Figure 20). Since these two important acids, linoleic and linolenic, have only been analyzed a few times before, (190,418) their mass spectra are shown in Figure 20 as confirmation of this work and as a representative example of our analyses. It should be noted that in the case of the methyl linoleate and methyl linolenate (Figure 20) a peak appears at m/e = M-31 instead of the M-32 peak corresponding to the monoenoic esters. Two other peaks appear for linolenate at m/e = M-56 and m/e = M-69. It is possible that these peaks

FIGURE 20

MASS SPECTRA OF OLEFINIC ACIDS FROM ALGAL MATS

The mass spectra was taken as the components were eluted from a stainless steel capillary column coated with Polysev (91 m long by 0.076 cm i.d.). The components were ionized by electron impact at 70 eV as they entered the ion source of the LKB 9000 Gas Chromatograph-Mass Spectrometer.


LINOLEIC 18:2
100
50
294
M
M-31
M-74
60
80
100
120
140
160
180
200
220
240
260
280
300
m/e
REL. INT.
LINOLENIC 18:3
100
50
292
M
M-56
M-69
M-31
60
80
100
120
140
160
180
200
220
240
260
280
300
m/e

originate through single bond cleavage favored by resonance stabilization of the alkyl cation and concerted rearrangement of hydrogen atoms.

It has been pointed out that unsaturated hydrocarbons have more intense molecular ion peaks than their saturated counterparts. This has also been found to be true too for the molecular ion peaks of unsaturated methyl esters. This effect has been noted too by other investigators (428).

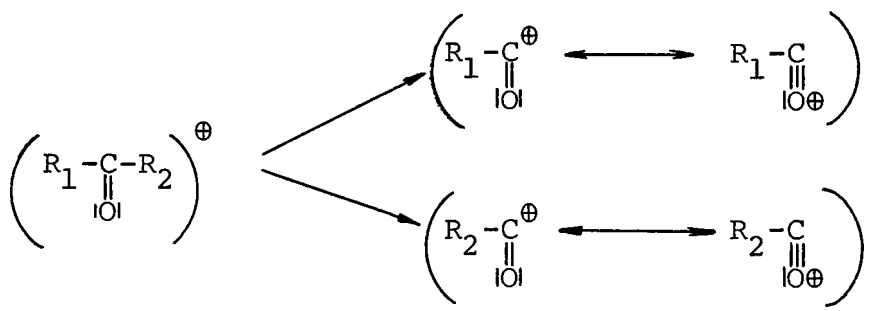
The gas chromatographic separation of the types of fatty acid esters described above require their esterification prior injection in the GC column. The high polarity of the carboxylic group gives rise to strong interaction effects with the chromatographic phase which make difficult their elution from the columns. On the other hand once the acidic group has been tied up by the methyl group, they can be easily chromatographed on most liquid phases. The best results in terms of resolution between branched and normal esters and their unsaturated homologs have been achieved on polar phases such as the Igepal Co 990 and Co 880. Non polar phases such as Apiezon L also give satisfactory resolution with exception of the methy linoleate and linolenate which can not be resolved in this phase, and the same is true for the Polysev columns. In the GC-MS combination, however, due to the limitations on the sensitivity imposed by the bleeding

rates from the GC columns the choice was restricted to Polysev columns, with the resultant loss of resolution between the n-fatty acids and their corresponding monounsaturated acids of the same carbon number. Polyenoic acid esters were better resolved by the Polysev columns. See Experimental section and Table I for a detailed description of these liquid phases. Finally it may be pointed out that the isomeric branched olefinic fatty acid esters, iso, anteiso, normal olefin and normal saturated fatty acid esters are eluted in this same order in Apiezon L columns. Polysev columns give similar results at least for the iso, anteiso and normal isomers, but the olefinic fatty acids are eluted after their corresponding saturated homologs which is also the case in the Co 990 and Co 880 columns.

I. Ketones

The mass spectrometry of ketones has been discussed in the literature (42,76,427) in some detail but as generally encountered in this investigation most discussions and correlations deal only with the lowest members in the homologous series of each particular group of compounds. Application of current ideas on the fragmentation mechanisms assumed to take place for these type of compounds has been made in the elucidation of the structure of a high molecular weight ketone found to be present in cabbage leaves (261).

In general the mass spectra of long straight chain ketones will be dominated by the two fragments resulting from cleavage of the two single bonds adjacent to the carbonyl group (42,76).

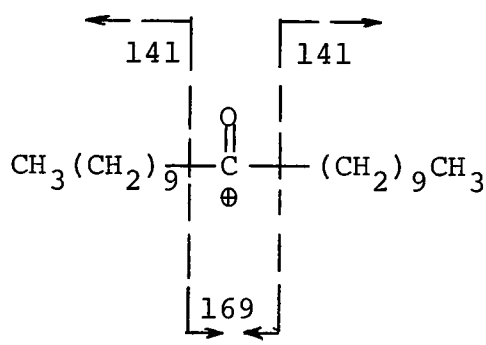


The non allylic resonance stabilization (42) of both ions, which will reduce considerably their ionization potential, will be the major driving force towards fragmentation. Furthermore the inductive electron releasing effect of the two R groups will also play a secondary part in the overall stabilization of the positive charge on the carbonyl group. This will be felt on the relative intensities of both ions. In other words, the larger the R group attached to the carbonyl the higher its intensity. Whenever the R groups contain more than three carbon atoms the molecule can also participate in the well known McLafferty rearrangement (292) since it will have an available hydrogen atom γ to the carbonyl group. This has been discussed in more detail in the section on fatty acid mass spectrometry (this chapter, section H).

This type of rearrangement will be in competition with the resonance process of charge delocalization. As shown experimentally the latter will predominate to an extent measured by the relative intensity of the corresponding ions.

The resulting enol ion (this chapter, H) can undergo again the same type of rearrangement providing that the alkyl group is larger than C_3 (that is, it contains a γ hydrogen). A very characteristic and often diagnostic ketone peak at m/e 58 is the result of such "double rearrangement" process. All this will be best illustrated by the spectra of 11-heneicosanone for example (Table XIX).

This compound would be expected to produce the following fragmentation pattern:



It represents a specially simple case where $R_1 = R_2$ and so the two fragments containing the carbonyl group will have the same mass (m/e 169, intensity underlined in Table XIX). Retention of the positive charge on the alkyl group, R, will lead to a fragment of mass 141, but this will not be an important event as indicated by the low intensity of the alkyl

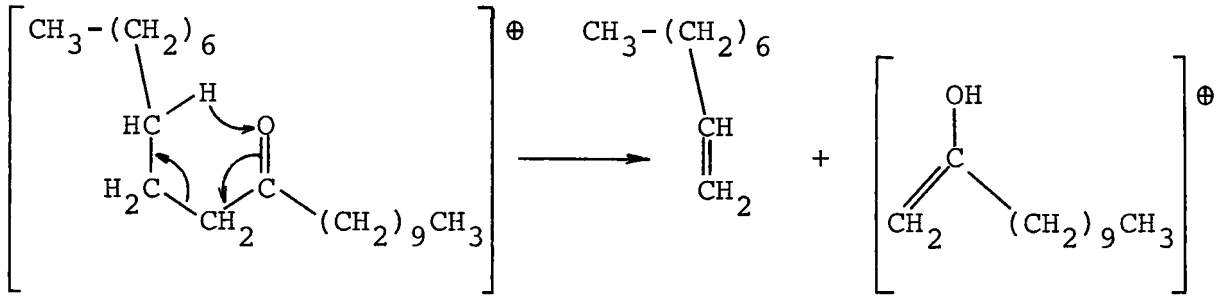
TABLE XIX

PARTIAL MASS SPECTRA OF 11-HENEICOSANONE(1)

n (m/e) \ Z	Mass series (Z in C_nH_{2n+Z}) -3	-2	-1	0	+1	+2
C_4 (57)	7.7	9.9	77.5	21.3	100	85.3 (dr)
C_5 (71)	13.9	7.6	29.5	12.6	88.6	6.3
C_6 (85)	12.3	15.0	21.3	8.5	35.9	3
C_7 (99)	15.7	11.2	11.1	6.1	3.6	5
C_8 (113)	6.2	5.8	4.1	1.7	3.6	1.9
C_9 (127)	1.2	25.7	3.9	9.5	6.2	1.8
C_{10} (141)	0.9	0.7	0.6	0.8	4.1	2.3
C_{11} (155)	1.2	0.5	0.3	0.3	1.5	0.8
C_{12} (169)	0.1	5.6	1.2	0.7	75.6	9.2
C_{13} (183)	---	0.1	0.1	0.2	0.1	27.6 (sr)
C_{14} (197)	---	0.1	0.3	0.1	15.4	2.1
C_{15} (211)	---	0.3	0.1	0.1	3	0.5
C_{16} (225)	---	0.1	0.1	---	0.5	0.5
C_{17} (239)	---	---	---	---	0.6	0.2
C_{18} (253)	---	---	---	---	0.8	0.2
C_{19} (267)	---	---	---	0.1	0.7	0.2
C_{20} (281)	---	---	---	---	0.5	0.1
C_{21} (295)	---	0.4	0.1	0.9	0.3	---
C_{22} (309)	---	---	---	---	0.4	2.88

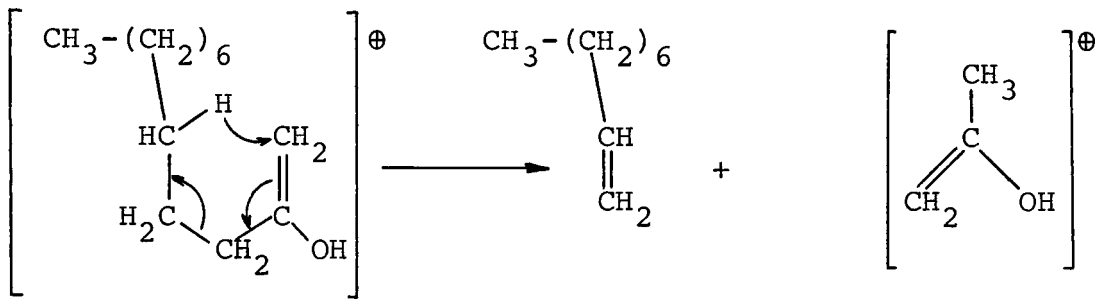
(1) Intensities taken from A.P.I. spectra #1395, 70 ev spectra
(sr) single rearrangement; (dr) double rearrangement
(m/e) masses corresponding to alkyl ion mass series (Z = +1)

fragment with 10 carbon atoms. The fragment of m/e 184 (C_{13}, column +2) will arise through the McLafferty's rearrangement in the following way:



m/e 126 m/e 184

and upon a second rearrangement

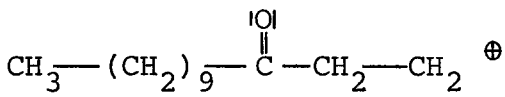


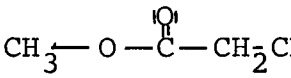
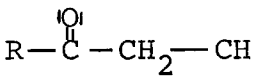
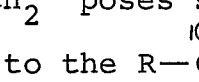
m/e 184 m/e 126 m/e 58

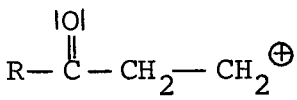
the m/e 58 fragment is formed (dr; double rearrangement, in Table XIX). There is also a strong peak at m/e 185 (rel. int 25.8%) not given in Table XIX because it corresponds to the z=-11 mass series. It arises in the same manner as the m/e 75 fragment in the spectra of long chain fatty acid esters; by

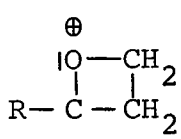
abstraction of another H atom to form a stable protonated enol (see section on fatty acids). The intensities of the fragments in columns -3, -2, and -1 suggest that many olefin ions are formed, most likely through processes involving the abstraction of hydrogen atoms. For instance the C_9 olefin resulting from the McLafferty rearrangements appears to lose two more hydrogens to give the C_9 diolefin (column, -2).

The relatively high fragment appearing at m/e 197 (C_{14}) corresponds to the structural formula:



The stability factors leading to its formation are most likely similar, if not identical to those responsible for the formation of the  ion (m/e 87) in the mass spectra of esters. In general it appears that ions of the type  poses some degree of structural stability as opposed to the  ions. Perhaps the polarization effect of the carbonyl bond would lead to a four membered cyclic intermediate in which the positive charge would be stabilized on the oxygen atom. Something such as:

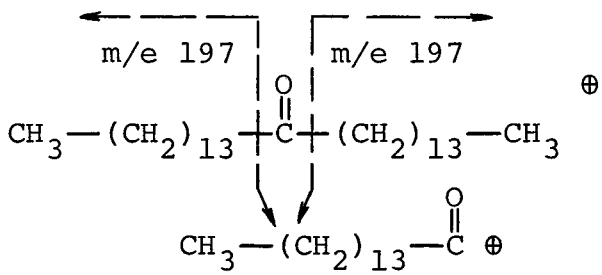




As a practical application of the usefulness of these mechanisms in the structural elucidation of ketones, the mass spectra of a symmetrical C_{29} ketone found in cabbage leaves is shown in Figure 21 (261).

The elucidation of its structure is based on the following reasons. The spectrum shows a parent peak at m/e 422 which would correspond either to $C_{30}H_{62}$ or $C_{29}H_{58}O$. The isotope peaks at m/e 423 and 424 are higher than expected for a compound made up only of C and H atoms. The major peak at m/e 225 corresponds to a fragment of empirical formula, $C_{16}H_{33}$ or $C_{15}H_{29}O$. Its great intensity indicates that a driving force other than just the relatively poor inductive effect of the alkyl groups must be involved in its formation.

Its appearance can be rationalized in accordance to the following scheme:



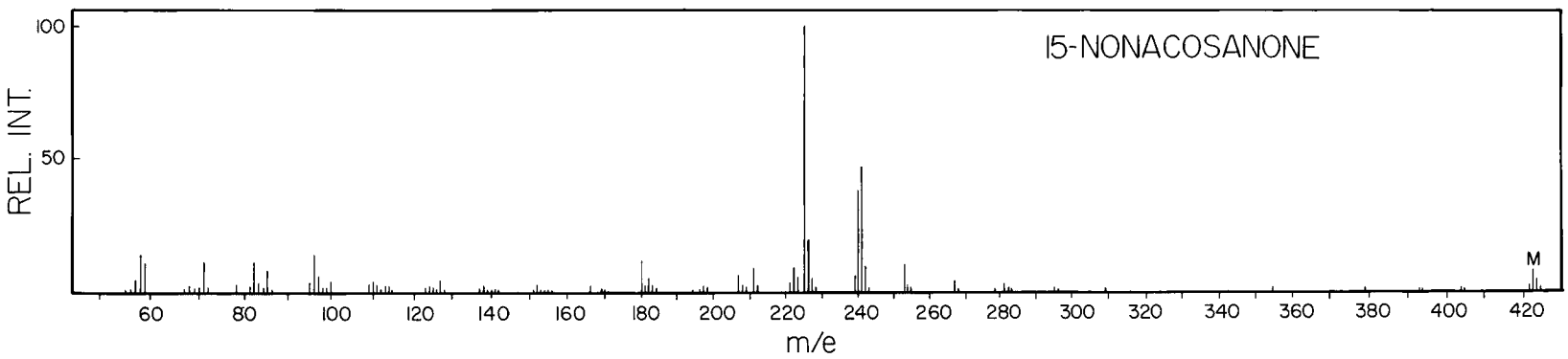
m/e 225

Because of the resonance stabilization of the positive charge on the carbonyl fragment its intensity should be very much greater than that of the alkyl fragment (m/e 197) as seen in the spectrum.

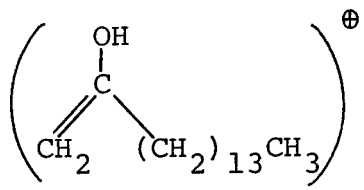
FIGURE 21

MASS SPECTRUM OF 15-NONACOSANONE

The spectrum was taken as the compound was eluted from a glass column (1.8 m long by 0.64 cm i.d.) packed with SE-30. It was ionized at 20 eV. Further details can be found in reference (261).

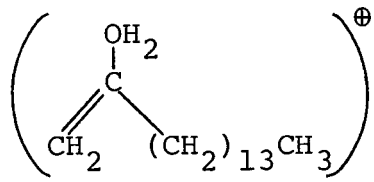

15-NONACOSANONE
REL. INT.
100
50
M
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
m/e

The ions at m/e 240 and 241 arise through the McLafferty rearrangement as discussed above.



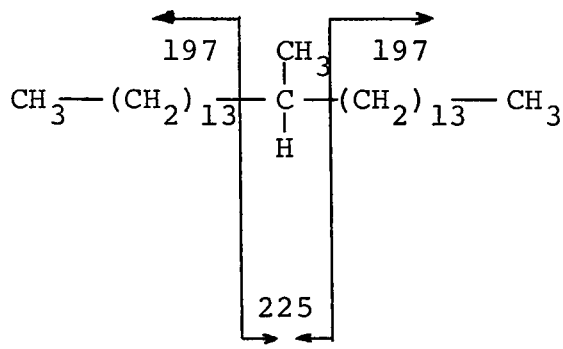
m/e 240

and



m/e 241

It has been mentioned that the intensity of the protonated enol ion increases with the molecular weight (414). In the C_{21} ketone the protonated enol form at m/e 185 represents about a 92% of the intensity of the simple enol, while in this case the m/e 241 ion represents about 120% of the enol (m/e 240). A structure like



would also account for the peak at m/e 225 but not for its high intensity nor for the fragments at m/e 240 and m/e 241.

On the other hand there are some other peaks present at masses not corresponding to fragments derived from a

$C_{30}H_{62}$ hydrocarbon. These can be seen (Figure 21) at m/e 58, m/e 82, m/e 96, m/e 138, m/e 180, m/e 182.

The fragment at m/e 58 is in itself a characteristic of the mass spectra of ketones as mentioned above. The peculiar stability of the ion originated by single cleavage of the β C-C bond discussed above is represented in this spectrum by the peak at m/e 253 ($CH_3—(CH_2)_{13}—\overset{O}{\overset{\|}{C}}—CH_2—CH_2^{\oplus}$). Taking all this into account the structure that best fits the observed pattern is that of a symmetrical C_{29} straight chain ketone. At a low electron energy (20 eV) the spectrum of this nonacosanone is clearly dominated by the fragment ions involved in resonance or rearrangement processes while most of other fragmentation possibilities are considerably reduced (Figure 21). This constitutes another example of the simplification introduced in spectra taken at low electron energies. A comparison of the pattern in Figure 21 to that of Table XIX clearly illustrates the point once more.

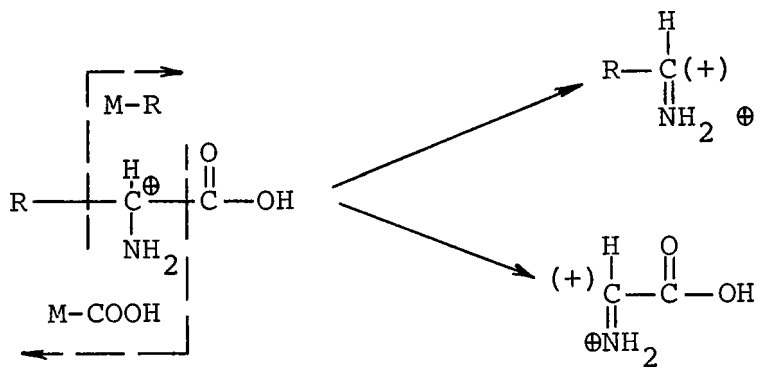
Gas chromatography of long alkyl chain ketones can be carried out effectively either in polar or non polar phases. A non polar phase, such as SE 30, (see Table I) relatively stable to high temperatures had to be used in this case due to the requirements imposed on the GC conditions by the high molecular weight of the sample.

J. Amino Acids

As a result of certain abiotic syntheses carried out in this laboratory under carefully controlled conditions, some amino acids were detected in the reaction mixtures (356). The reactants used in all cases were considered to be possible prebiotic precursors of the amino acids. Because of the implications of these results to current theories on evolution it becomes necessary to establish in an unequivocal manner the identities of the compounds produced. The combination of gas chromatography-mass spectrometry with its high specificity and sensitivity affords once more the most reliable analytical tool for this purpose. But because of their low vapor pressure and high polarity, the amino acids can not be directly chromatographed on either polar or non polar phases. In order to achieve their gas chromatographic separation it is necessary to destroy the acidity of the carboxyl group and the basicity of the amino group. This can be accomplished by any of the following methods: conversion to the methyl, ethyl, isopropyl or butyl esters with the corresponding alcohol-hydrochloric acid; formation of the doubly substituted N-alkyl and carboxyl esters; the N-acetyl, n-amyl esters; the trimethyl-silyl ethers esters; the trifluoroacetyl derivatives and the trifluoroacetyl, methyl, ethyl, isopropyl or butyl esters. The method employed in our analysis involves the conversion to

the N-trifluoroacetyl, n-butyl esters according to the procedure outlined elsewhere [Gehrke, C. W. and Stalling, D. L., Sep. Sci. 2, 101 (1967)].

Before attempting to rationalize the fragmentation patterns of the spectra shown in Figures 22-24 according to definite structural characteristics attention must be drawn to the basic characteristics of amino acid mass spectrometry. In general the spectra of amino acids will be dominated by the fragments resulting from cleavage of the bonds adjacent to the amino substituted carbon.



Of the two possibilities the M-CO_2H fragment $(R—CH—NH_2)^{\oplus}$ or "amine fragment" will predominate because in the M-R fragment the resonance stabilization effect set up by the nitrogen will be counteracted by the polarizability of the carbonyl group. Thus the M-R fragment will not be as stable.

Figures 22-24 show the spectra of the n-butyl N-trifluoroacetyl derivatives of glycine, alanine and aspartic acid standards on top of the spectra of three of the compounds

FIGURE 22

MASS SPECTRUM OF n-BUTYL N-TRIFLUORO

ACETYL GLYCINE

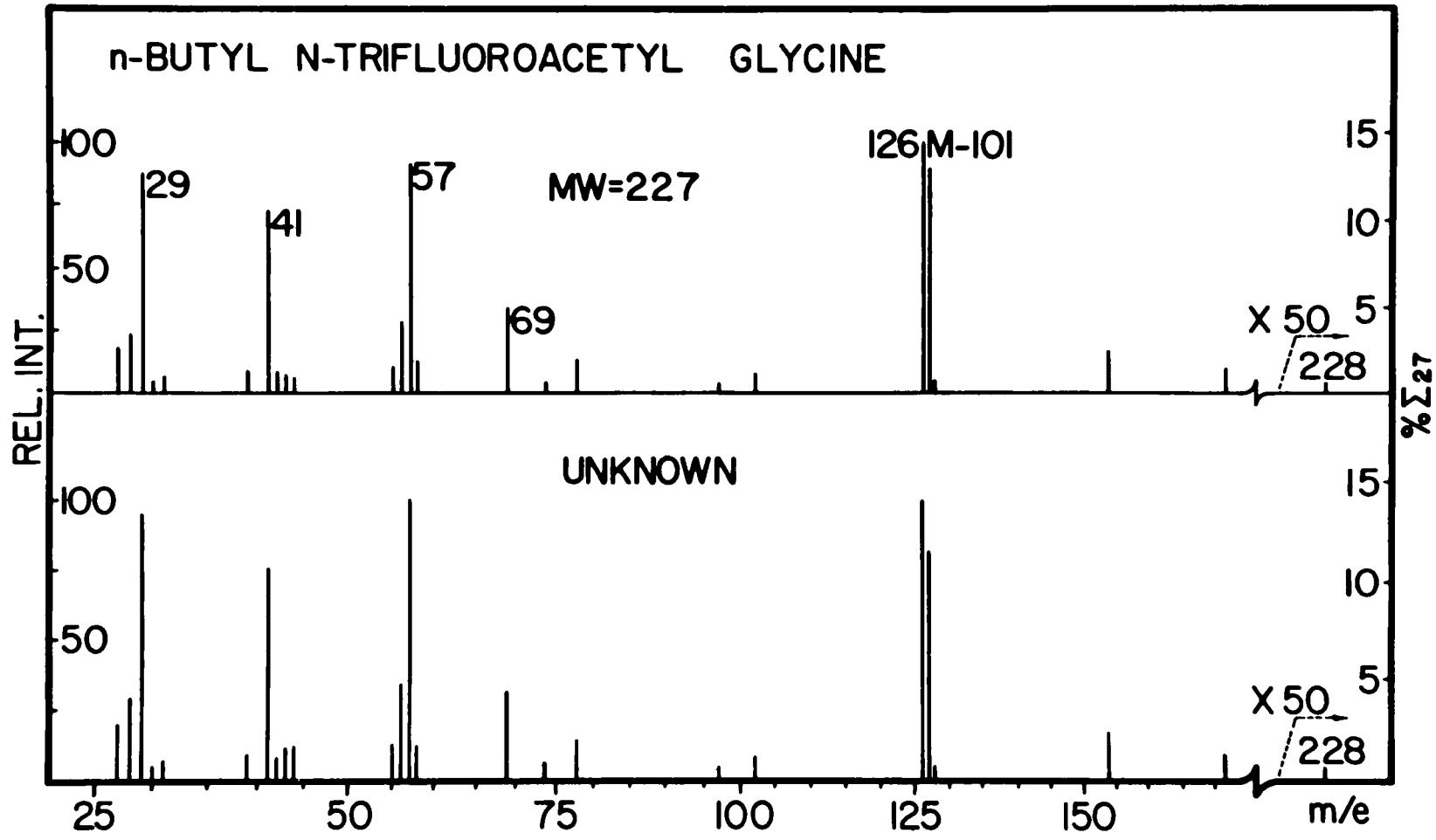

n-BUTYL N-TRIFLUOROACETYL GLYCINE
MW=227
29
41
57
69
126
M-101
X 50
228
UNKNOWN
X 50
228
REL. INT.
100
50
15
10
5
%Σ27
25
50
75
100
125
150
m/e

FIGURE 23

MASS SPECTRUM OF n-BUTYL N-TRIFLUORO

ACETYL ALANINE

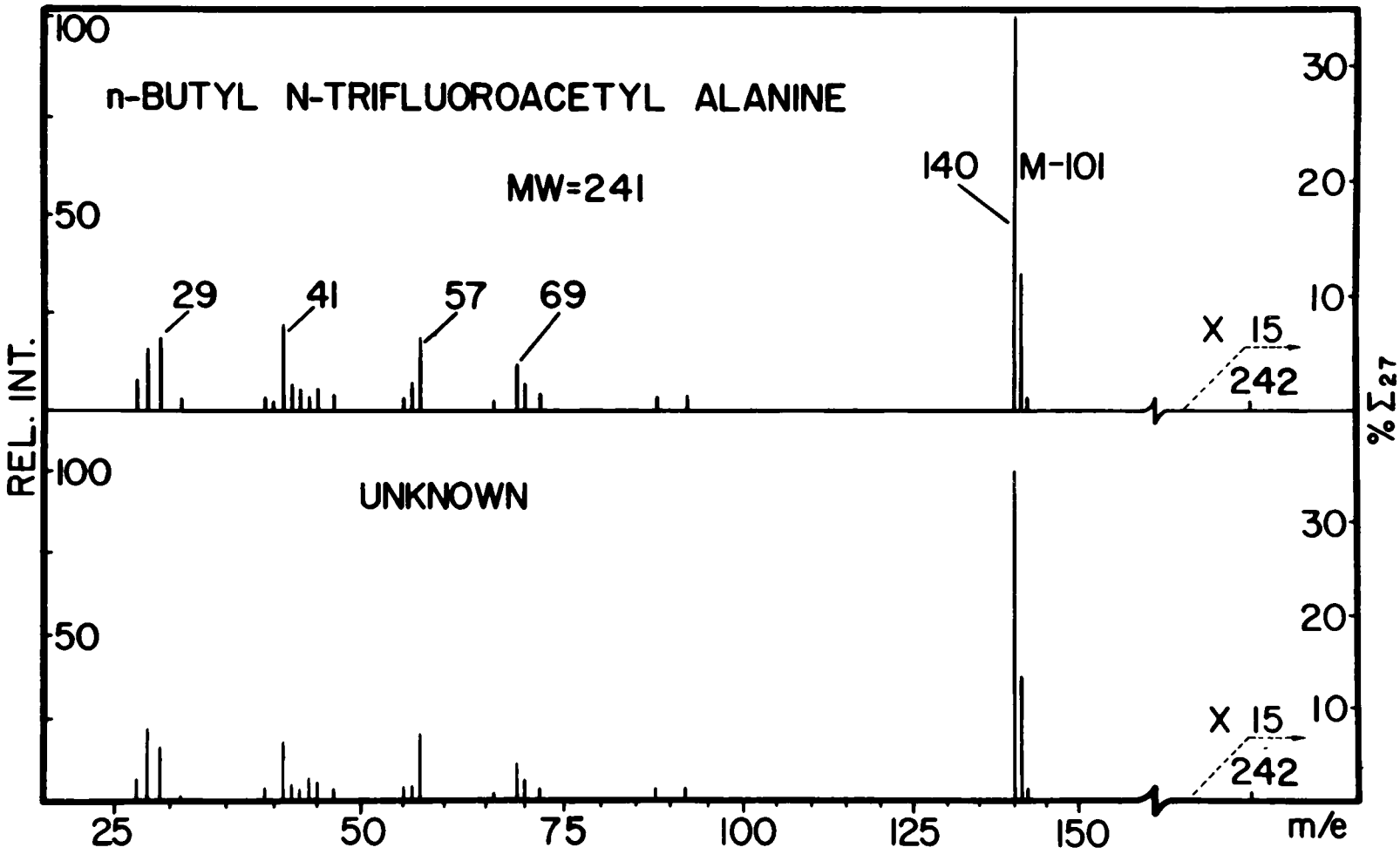
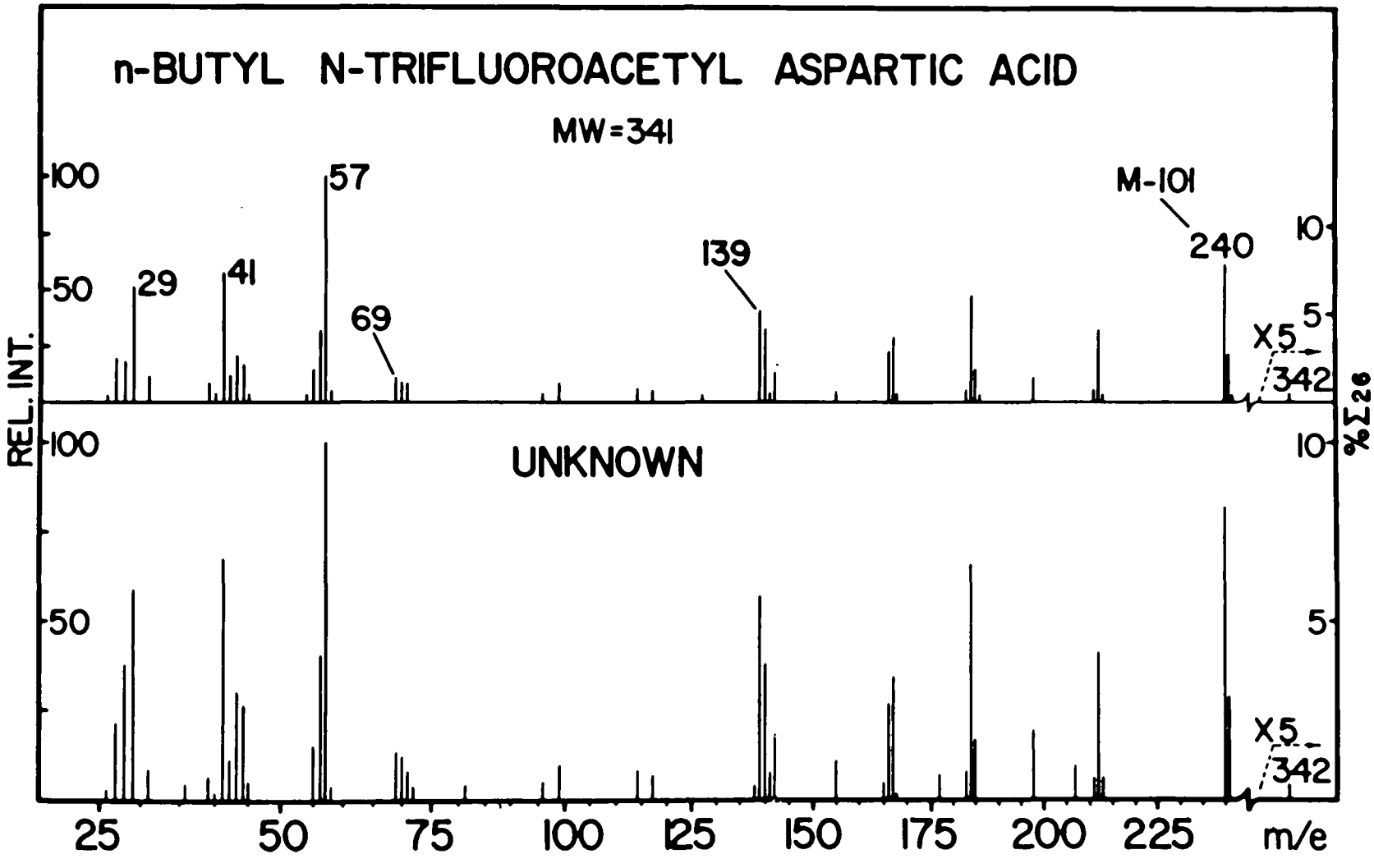

100
n-BUTYL N-TRIFLUOROACETYL ALANINE
MW=241
140
M-101
50
29
41
57
69
30
20
10
X 15
242
REL. INT.
% Σ27
100
UNKNOWN
50
30
20
10
X 15
242
25
50
75
100
125
150
m/e

FIGURE 24

MASS SPECTRUM OF n-BUTYL N-TRIFLUORO

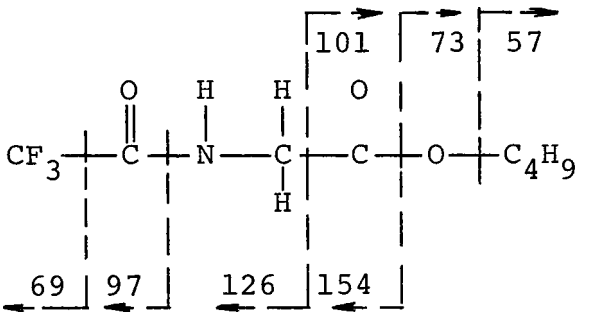
ACETYL ASPARTIC ACID


n-BUTYL N-TRIFLUOROACETYL ASPARTIC ACID
MW=341
100
50
57
29
41
69
139
M-101
240
10
5
X5
342
REL. INT.
100
50
UNKNOWN
10
5
X5
342
%Σ20
25
50
75
100
125
150
175
200
225
m/e

produced by synthesis.

The correlation of the standards and unknown structures with the mass spectral fragmentation patterns can be established as follows:

1. n-Butyl N-Trifluoroacetyl Glycine



Molecular weight; M = 227. The spectrum (Figure 22) shows an $(M+1)^+$ ion (m/e 228) instead of the M^+ ion. This has been observed before for this type of molecules (42), when the pressure inside the ion source is high. It reflects the higher stability of the protonated molecule. In summary the spectrum shows:

Significant Peaks	Fragment
m/e 29	$CH_2 = NH^{\oplus}$ or, $HC \overset{\oplus}{=} O$
m/e 41	$C_3H_5^{\oplus}$
m/e 57	$C_4H_9^{\oplus}$
m/e 69	$CF_3^{\oplus}$

m/e 97 $CF_3-\overset{\oplus}{C}=\underline{O}$

m/e 102 $\oplus C(=O)-O-C_4H_9 + H$

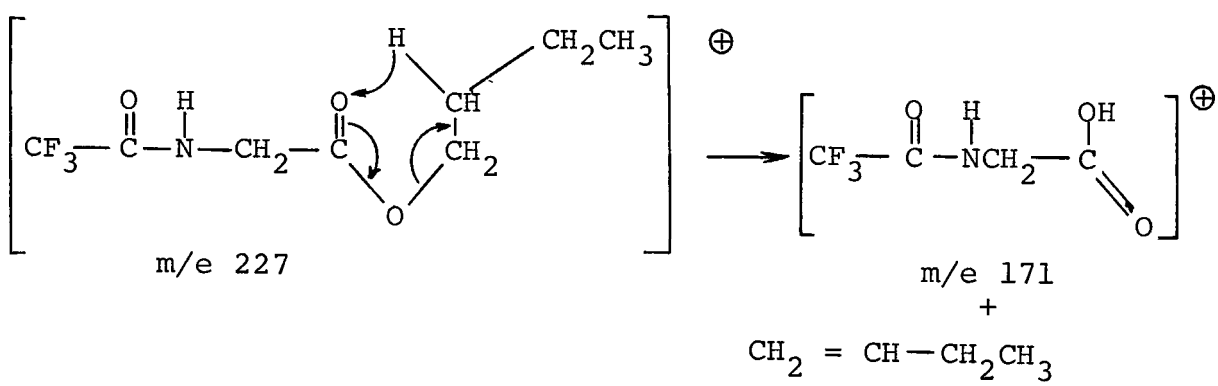
m/e 126 $CF_3-C(=O)-\underline{N}(H)-C(H)\oplus$ (base peak)

m/e 127 same + H

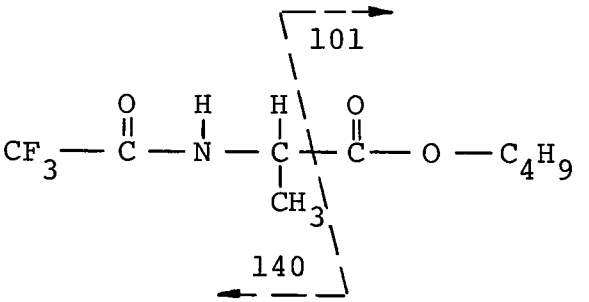
m/e 154 $CF_3-C(=O)-\underline{N}(H)-C(H)-C(=O)\oplus$

m/e 171 $CF_3-C(=O)-\underline{N}(H)-C(H)-C(OH)=O^{\oplus}$ rearrang. ion

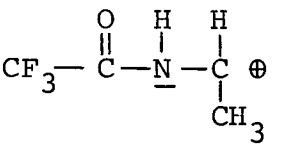
The m/e 29 fragment may be the result of either one of the two structures shown. Because it is also present in the other two spectra (Figures 23,24) the $H\overset{\oplus}{C}=O$ structure appears a more likely choice since the same type of cleavages would give an $R-CH=NH^{\oplus}$ ion in the other two cases. On the other hand its relative intensity (over 13% of Σ_{27}) indicates that the two structures may play a significant role in the spectrum of the glycine derivative. The height of the m/e 127 fragment indicates that this part of the molecule has a high tendency towards protonation. The rearrangement ion (m/e 171) could arise through the following mechanism.



2. n-butyl N-trifluoroacetyl alanine; M = 241



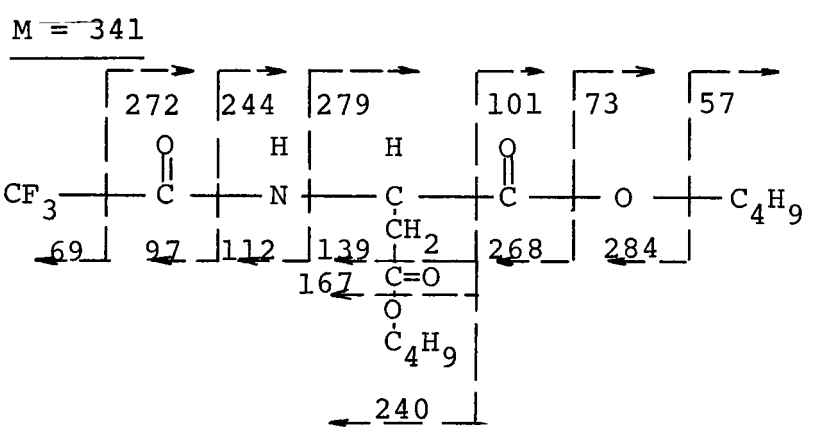
The overall spectral pattern (Figure 23) is similar to the one of Figure 22. The major difference is the higher probability of formation of the ion



m/e 140

The stabilizing effect of the electron donating methyl group will add to the resonance stabilization effect of the nitrogen. As a consequence the spectra is dominated by this fragment to a greater extent than in the case of the glycine derivative.

3. n-butyl N-triflouroacetyl aspartic acid



The peaks at m/e 29, 41, 57, 69 are common to the three spectra and with the exception of m/e 29 they originate in the substituent groups. The peaks at m/e 189 and 212 (Figure 24) are rearrangement peaks formed in the same manner as the m/e 171 peak in the spectra of the glycine derivative (Figure 22).

The most characteristic feature common to the three spectra is the base peak at an m/e corresponding to M-101 or $M-\left[\overset{O}{\overset{\|}{C}}-O-C_4H_9\right]$. This can be generalized in the sense that most of the other amino acid derivatives of this sort will give spectra with strong M-101 peaks.

K. Steroids

This class of compounds have not been extensively studied in the course of this work, which has been concerned mainly with aliphatic structures. Nevertheless to further emphasize the versatility of the gas chromatographic-mass spectrometric combination, the general characteristics of steroid mass spectrometry, which led to the identification of cholestane in a diatom for the first time, will be briefly discussed here.

It is known that the presence of a tetracyclic steroid skeleton as well as the length of its side chain can be determined from the mass of the fragment [M - (R + 42)] arising from the elimination of the side chain (R) plus 42 mass units (see reference 42, page 339 for details).

The main features in the spectrum shown in Table XX are: a base peak at m/e 217 (C_{16} fragment), a high m/e 149 ion (C_{11} fragment), a relatively very high parent ion at m/e 372 and also a high M-15 fragment (m/e 367).

The base peak at that high mass indicates that a cyclic structure is most likely involved in the formation of this fragment. Rearrangement of a hydrogen atom is also indicated by the high m/e 218 peak (394). Substituting M in M-(R+42)=217 by its value of 372 in this case yields an R equal to 113 which would be equivalent to a C_8 fragment. The fragment at m/e 217 is characteristic of the dimethyl substituted perhydrophenanthrene moiety in the structure of normal tetracyclic steroids minus one hydrogen. The fragment at m/e 149 would correspond to the mass of the rings A and B minus one hydrogen (see reference 40, p. 336). On the basis of all these indications and also upon comparison of the spectrum in Table XX with the reference API spectrum No. 1000, it was concluded that the spectrum under study was that of cholestane.

TABLE XX

MASS SPECTRUM OF CHOLESTANE FROM N. PUTRIDA

n \ Z	Mass Series (Z in C_nH_{2n+Z})								
	-7	-6	-5	-4	-3	-2	-1	0	+1
7	-	-	2.5	4	10.5	8	12	6.8	4.5
8	3.5	2	3.5	9	13	5.5	9	5	5
9	-	-	-	11	12	4.5	-	-	-
10	-	-	9.5	7	8	-	-	-	-
11	6	9	38	17	9	-	-	-	-
12	3.5	5	6	-	-	-	-	-	-
13	5.2	2.8	2.5	-	-	-	-	-	-
14	3.5	4	3	-	-	-	-	-	-
15	11	-	-	-	-	-	-	-	-
16	100	76	24	4	-	-	-	-	-
17	-	20	5.5	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-

TABLE XX CONTINUED

MASS SPECTRUM OF CHOLESTANE FROM N. PUTRIDA

		Mass Series (Z in C_nH_{n2+Z})								
n	Z	-7	-6	-5	-4	-3	-2	-1	0	+1
19		-	-	-	11.5	3.5	-	-	-	-
20-23		-	-	-	-	-	-	-	-	-
24		-	-	4.5	-	-	-	-	-	-
25		5	-	-	-	-	-	-	-	-
26		29.5	10	2.5	-	-	-	-	-	-
27		-	53(M)	17.5	-	-	-	-	-	-

V.

TERRESTRIAL ORGANIC MATTER

TERRESTRIAL ORGANIC MATTER

A. Organisms

1. Bacteria

Cultures of three terrestrial bacteria: a) Sarcina lutea (482), b) Bacillus cereus (370), c) Staphylococcus aureus (480), d) Halobacterium cutirubrum, a halophilic bacterium (483), e) Vibrio marinus, a marine bacterium (371) were extracted, fractionated and analyzed by the methods previously described (see Chapter III) in order to study their lipid content. All cultures were grown in the laboratory by Dr. Tornabene who also obtained all the gas chromatographic and quantitative data (480).

a) Sarcina lutea

The hydrocarbons and fatty acids of Sarcina lutea have been investigated by Huston and Albro (9,224,225). The lipid extractable material was analyzed by thin-layer and gas liquid chromatography and by infrared spectrophotometry. The following presents new evidence on the identification of the fatty acids and hydrocarbons of S. lutea from the data obtained by gas chromatographic and mass spectrometric analysis (482). Although the identity of some of the fatty acids was confirmed in this work, the nature of some of the fatty acids and of most of the hydrocarbons was found to be significantly different from that previously reported.

The Sarcina lutea was purchased from American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. Culture number ATCC 533. It was cultivated in Erlenmeyer flasks containing Trypticase Soy Broth, pH 7.0 (BBL). The cultures were placed in water baths adjusted to 25 C and were continuously aertaed for 48 hr. The cells were collected in plastic tubes in an RC-2 Servall centrifuge, washed three times in a 0.15 M NaCl solution, frozen, and then dried over P_2O_5 under vacuum.

Extractions. The method used to extract, fractionate, and analyze the hydrocarbons and fatty acids from bacteria has been described in the experimental section.

A 1.5-g amount of dried cells was placed in an all-glass Soxhlet-type apparatus and extracted with 50 ml of a benzene-methanol mixture (3:1) for 8 hr. The extract was transferred to a beaker, and the solvent was removed by evaporation at 40 C under a stream of purified nitrogen.

Column fractionation. The extracts were fractionated on silica gel columns into three fractions: the n-heptane fraction, containing the aliphatic hydrocarbons (alkanes, olefins, etc.); the benzene fraction, saved for future analysis; and the methanol fraction, containing the fatty acids and glycerides, among other lipids. The n-heptane eluate collected from the silica gel column was divided into two subfractions. One subfraction was saved for pigment analysis. The other subfraction was transferred to a glass column (1 by 30 cm).

The column was provided with a sintered-glass disc and was filled to a depth of 10 cm with alumina that had been previously activated at 340 C for 24 hr, and washed with 10 ml of n-heptane. The aliphatic hydrocarbons, relatively free from pigments, were eluted with n-heptane.

Preparation of derivatives. The fatty acids were liberated from the glycerides of the methanol fraction by alkaline hydrolysis. Methyl esters of the fatty acids were prepared for gas chromatography and mass spectrometry as previously described (Chapter III).

The cells of S. lutea dried over P_2O_5 were found to contain on the average about 1.45% of total lipid extractable material. The amount of aliphatic hydrocarbons in the n-heptane eluate from the alumina column (this excludes the pigment) was found about 0.25% of the dried cell mass. These values are in close agreement with those reported (1.30% and 0.27%, respectively) by Huston and Albro (224).

Gas chromatography-mass spectrometry of the fatty acids.

Figure 25 shows a typical gas chromatographic pattern of the fatty acid methyl esters from S. lutea. Fifteen of the peaks (numbered from 1 to 15) were identified by both gas chromatography and mass spectrometry. Their identities are listed in Table XXI. With the exception of oleic and linoleic acids all the other analyzed fatty acids were found to be saturated. Typical mass spectra for the compounds corresponding

FIGURE 25

GAS CHROMATOGRAPHIC SEPARATION OF FATTY ACID METHYL ESTERS FROM SARCINA LUTEA

Stainless steel capillary column (155 m long by 0.076 cm i.d.) coated with Igepal Co 990. Nitrogen pressure, 933 g/cm^2; no split. 'F and M Model 810" Gas Chromatograph equipped with a flame ionization detector. Range 10^2; attenuation, 1. Temperature started at 120°C and programmed at about 6°C per minute to 200°C and held isothermally. From 1.5 gms of extracted cells, 1/100 of the sample was injected.

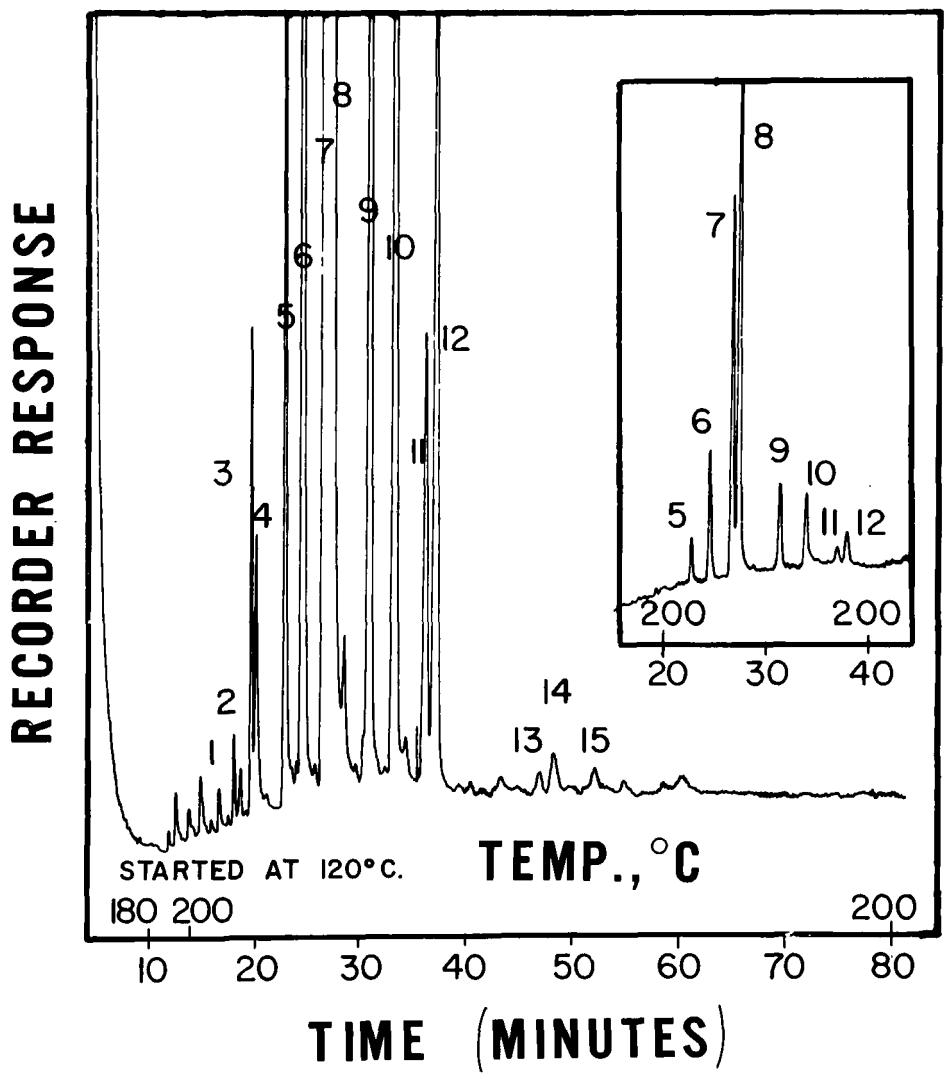

RECORDER RESPONSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
STARTED AT 120°C.
180 200
TEMP., °C
200
10
20
30
40
50
60
70
80
TIME (MINUTES)
5
6
7
8
9
10
11
12
200
200
20
30
40

TABLE XXI

FATTY ACIDS OF *SARCINA LUTEA*

1. i 12:0	9. i 16:0
2. 12:0	10. 16:0
3. i 13:0	11. i 17:0
4. ai 13:0	12. ai 17:0
5. i 14:0	13. 18:0
6. 14:0	14. 18:1
7. i 15:0	15. 18:2
8. ai 15:0	

Symbols: i = iso
ai = anteiso

The first number resresents the chain length, the second number resresents the number of unsaturations.

to peaks 7, 8 and 10 are shown in Figure 18. A discussion of their mass spectrometric characteristics has been given in Chapter IV, section H.

The fatty acids found in S. lutea follow a distribution pattern having neither a predominance of even-carbon nor odd-carbon numbered chains. However, careful inspection of the peaks in the chromatogram of Figure 25 and their identity, as listed in Table XXI, will show the existence of pairs of dyads of fatty acids grouped in two distinct families. One of these families is made of fatty acids with an even-number of carbon atoms, the pairs being iso and normal C_{12}, C_{14} and C_{16} saturated acids; the other family is made of fatty acids with an odd number of carbon atoms, the pairs being iso and anteiso C_{13}, C_{15} and C_{17} saturated acids. Some of the C_{18} and other fatty acids were in too small amounts to be properly characterized. As indicated earlier, of all the fatty acids identified the only unsaturates found were the common oleic and linoleic acids.

As seen from Figure 25, the two predominant fatty acids of S. lutea are iso and anteiso pentadecanoic acids which are probably related to the sarcinic acid (branched-C_{15}) reported by Akashi and Saito (8). When comparing various fatty acid methyl ester chromatograms and mass spectra it was found that the iso and anteiso-C_{15} reported here were identical with the C_{15} fatty acids of Bacillus cereus and Staphylococcus

aureus (see below, b and c).

Control analytical runs in which all the steps of the procedure were followed in the absence of sample as well as analyses of the media and solvents, showed either negligible or no measurable quantities of saponifiable material.

Gas chromatography and mass spectrometry of the aliphatic hydrocarbons.

The gas chromatographic pattern for the aliphatic hydrocarbons is shown in Figure 26 and 27. The identities are given in Table XXII (typical mass spectra are shown in Figure 28). The two patterns agree well with each other, although they are from different columns of unrelated phases. The range of hydrocarbons is from C_{22} to C_{29} with the major component being an anteiso-Δ-C_{25}. In Figure 26, peaks a, b and c were relatively unstable components that periodically appear in freshly prepared samples. Conclusive mass spectra was not obtained for these components; however, indications were obtained that they are polyunsaturates.

As in the case of the fatty acids the aliphatic hydrocarbons in S. lutea show a distribution having neither a predominance of even- nor of odd-carbon numbered chains. However examination of the two chromatograms (Figures 26 and 27) and Table XXII will also show, with certian parallelism with the fatty acids, the existence of tetrads (instead of dyads) of hydrocarbons grouped in two distinct families. One of these

FIGURE 26

GAS CHROMATOGRAPHIC SEPARATION OF ALKANES

FROM SARCINA LUTEA

Stainless steel capillary column (31 m long by 0.025 cm i.d.) coated with 10% Apiezon L. Nitrogen pressure, 1430 g/cm^2; no split. 'F and M' Model 810 Gas Chromatograph. Range 10^2; attenuation, 1. Temperature started at $125^{\circ}C$ and programmed at $6^{\circ}C$ per minute to $300^{\circ}C$. From 1.5 gms of extracted cells 1.5/100 of the sample was injected.

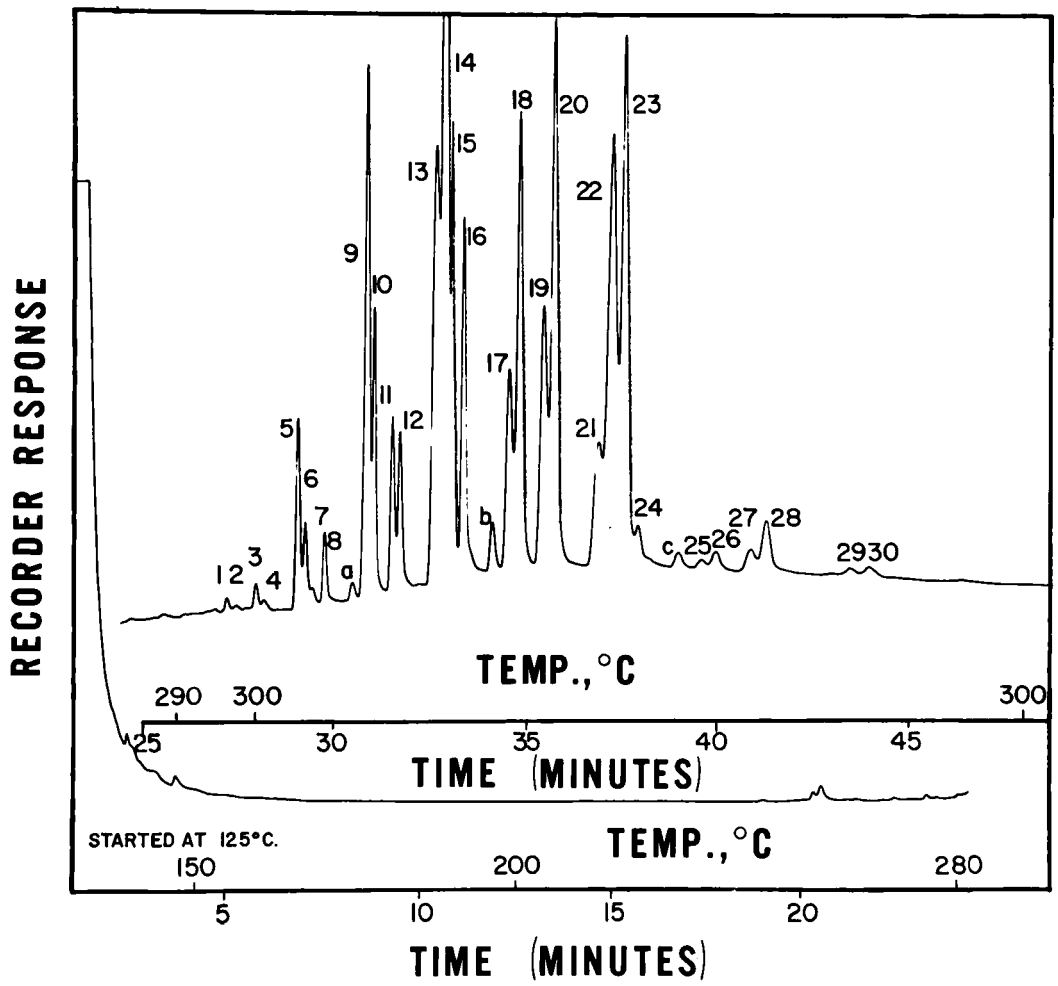

RECORDER RESPONSE
1
2
3
4
5
6
7
8
a
9
10
11
12
13
14
15
16
b
17
18
19
20
21
22
23
24
c
25
26
27
28
29
30
TEMP., °C
290
300
300
25
30
35
40
45
TIME (MINUTES)
STARTED AT 125°C.
TEMP., °C
150
200
280
5
10
15
20
TIME (MINUTES)

FIGURE 27

GAS CHROMATOGRAPHIC SEPARATION OF ALKANES

FROM SARCINA LUTEA

Stainless steel capillary column (155 m long by 0.076 cm i.d.) coated with Igepal Co 990. Nitrogen pressure, 933 g/cm^2; no split. 'F and M Model 810' Gas Chromatograph equipped with a flame ionization detector. Range 10^2; attenuation, 1. Isothermal at $200^{\circ}C$. From 1.5 gms of extracted cells about 1/75 of the sample was injected.

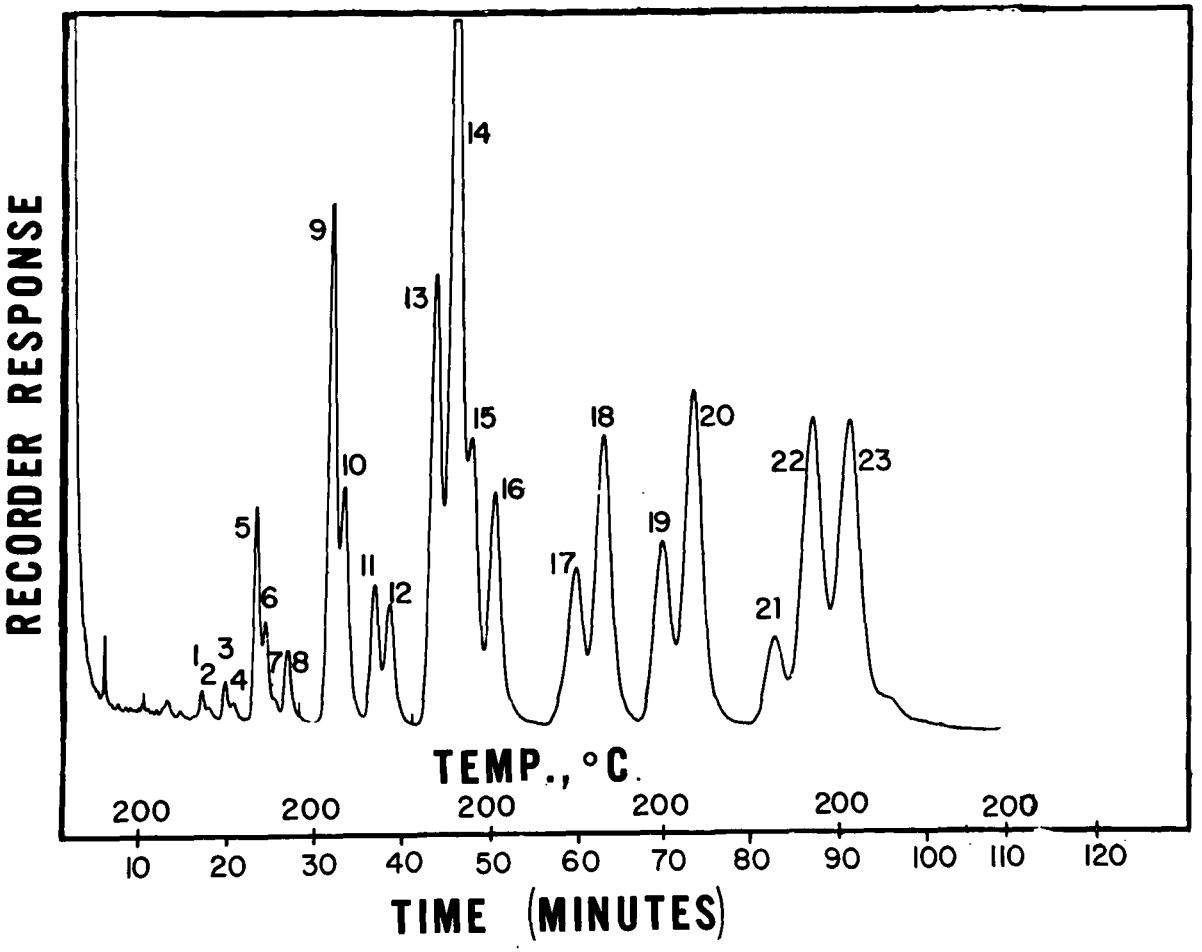

RECORDER RESPONSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
TEMP., °C.
200
200
200
200
200
200
10
20
30
40
50
60
70
80
90
100
110
120
TIME (MINUTES)

TABLE XXII

HYDROCARBONS OF SARCINA LUTEA

C_{22}

1. iso-Δ-C_{22}
2. anteiso-Δ-C_{22}
3. iso-Δ-C_{22}
4. anteiso-Δ-C_{22}

C_{23}

5. iso-Δ-C_{23}
6. anteiso-Δ-C_{23}
7. anteiso-Δ-C_{23}
8. iso-Δ-C_{23}

C_{23}

9. iso-Δ-C_{23}
10. anteiso-Δ-C_{23}
11. iso-Δ-C_{24}
12. anteiso-Δ-C_{24}

C_{24}

13. iso-Δ-C_{25}
14. anteiso-Δ-C_{25}
15. anteiso-Δ-C_{25}
16. iso-Δ-C_{25}

C_{26}

17. iso-Δ-C_{26}
18. anteiso-Δ-C_{26}
19. iso-Δ-C_{26}
20. anteiso-Δ-C_{26}

C_{27}

21. iso-Δ-C_{27}
22. anteiso-Δ-C_{27}
23. anteiso-Δ-C_{27}
24. iso-Δ-C_{27}

C_{28}

25. iso-Δ-C_{28}
26. anteiso-Δ-C_{28}
27. iso-Δ-C_{28}
28. anteiso-Δ-C_{28}

C_{29}

29. iso-Δ-C_{29}
30. anteiso-Δ-C_{29}

SYMBOLS: Δ = double bond

families is made of hydrocarbons with an even number of carbon atoms, the tetrads being four isomers in the following order: iso, anteiso, iso, anteiso of each of the C_{22}, C_{24}, C_{26} and C_{28} olefins. The other family is made of hydrocarbons with an odd number of carbon atoms, the tetrads being four isomers in the following order: iso, anteiso, anteiso and iso of each of the C_{23}, C_{25} and C_{27} olefins. Only traces of two C_{29} alkenes (iso and anteiso) were found although it is possible that with increased sensitivity the four isomers could have been detected.

This constitutes a remarkable distribution, with a unique order, of hydrocarbons. The following characteristic features were found: 1) all the components were identified as branched hydrocarbons with substituents at either iso or anteiso positions 2) all were identified as monounsaturated olefins (monoenes), 3) the components appeared in groups of four, with all four components of the same group (tetrad) having the same molecular mass, therefore, being isomers, 4) the four isomers in the groups with an odd number of carbon atoms were found to emerge from the gas chromatographic column very close together, whereas the four isomers in the groups with an even number of carbon atoms were better resolved and separated clearly into two pairs.

Of the eighteen times that S. lutea was grown by Dr. Tornabene

to study lipid formation, culturing conditions (481), and biosynthetic pathways, the lipid fractions were analyzed eight times by gas chromatography-mass spectrometry yielding essentially identical results.

The outstanding feature revealed by the mass spectral data was the same molecular mass for each of the four gas chromatographic peaks corresponding to a tetrad. The four components of the tetrad were also found to be olefins. This may be seen in Figure 28 which exemplifies the mass spectra of the four C_{25} olefins corresponding to the four gas chromatographic peaks 13 (iso), 14 (anteiso), 15 (anteiso), and 16 (iso). Very similar mass spectral data was obtained for the isomers of the tetrads. For that reason, the mass spectra corresponding to the C_{25} group, given in Figure 28, should be considered as representative of the fragmentation patterns found within the other tetrads of four isomers as well.

The first and second components show, in all of the groups, spectra which are virtually identical to the mass spectra of peaks 13 (iso) and 14 (anteiso) (Figure 28). The only difference between the groups was found in the relative retention times of the last two components in each group. While their spectra are also practically undistinguishable from those corresponding to peak 15 (anteiso) and 16 (iso) (Figure 28), they reverse positions in the olefin chains

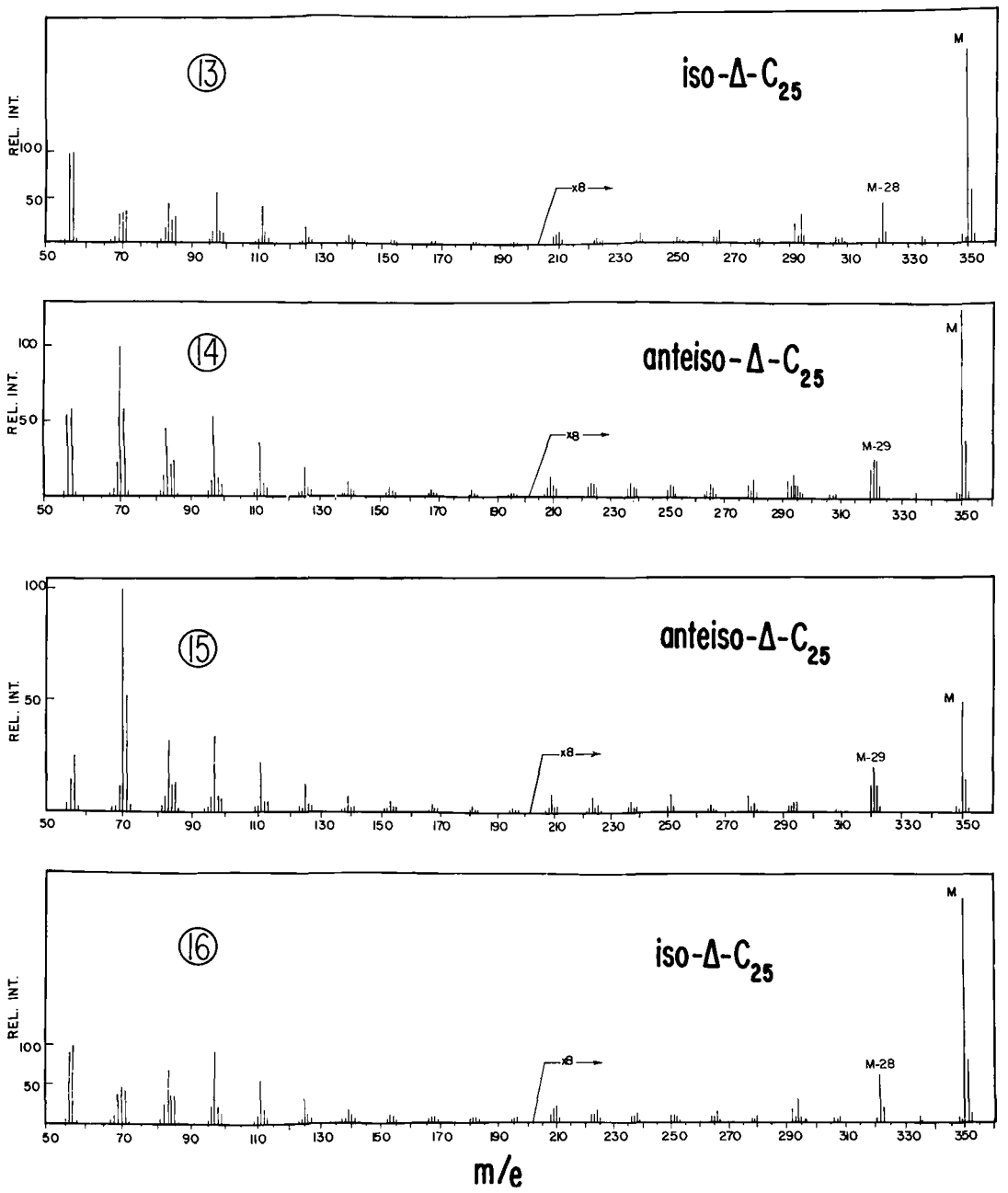
FIGURE 28

MASS SPECTRA OF OLEFINIC HYDROCARBONS

FROM SARCINA LUTEA

The mass spectra was taken as the components were eluted from a stainless steel capillary column coated with Polysev (91 m long by 0.076 i.d.). The components were ionized by electron impact at 20 eV as they entered the ion source of the LKB 9000 Gas Chromatograph-Mass Spectrometer.

OLEFINIC HYDROCARBONS



of even carbon number.

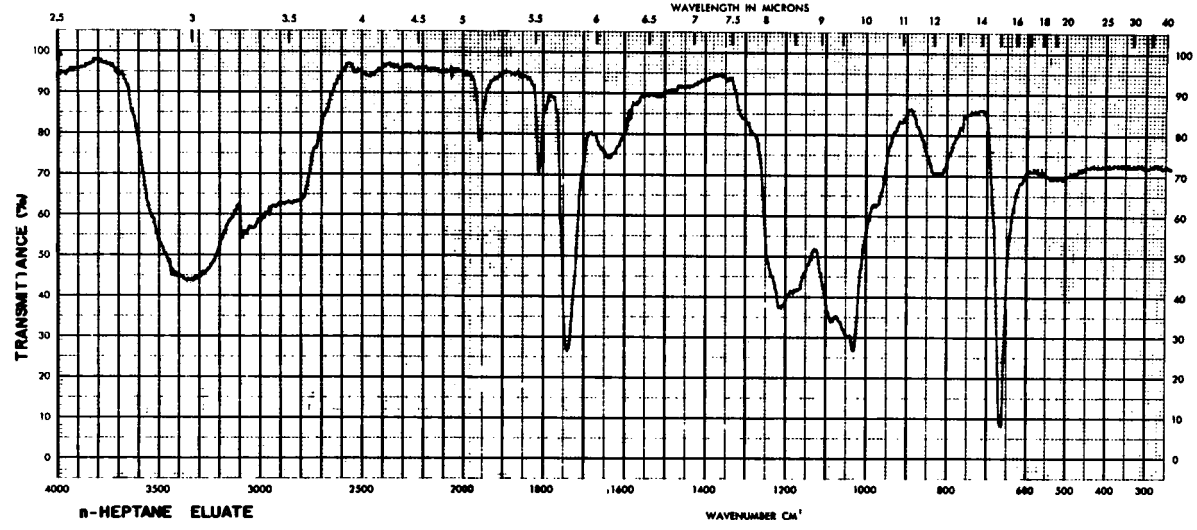
Since the double bond is rather mobile in this type of compounds and migrates easily via successive shifts of hydride ions and hydrogen radicals, as indicated before (Chapter IV, D), it was difficult to locate its exact position although it was assumed to be close to one of the ends of the molecule. The eveness of the central portion of the spectra indicated an iso or anteiso type of branching. In order to remove the possible interaction of the double bond with the side chain and thus obtain unequivocal data on the exact position of the branching on the chain, a portion of the n-heptane eluate from a silica gel column was hydrogenated catalytically (Pt) to reduce the pigment and any unsaturated hydrocarbons present. After reduction the hydrogenated compounds were removed from the catalyst by passing the product through a silica gel column as before and eluting with n-heptane.

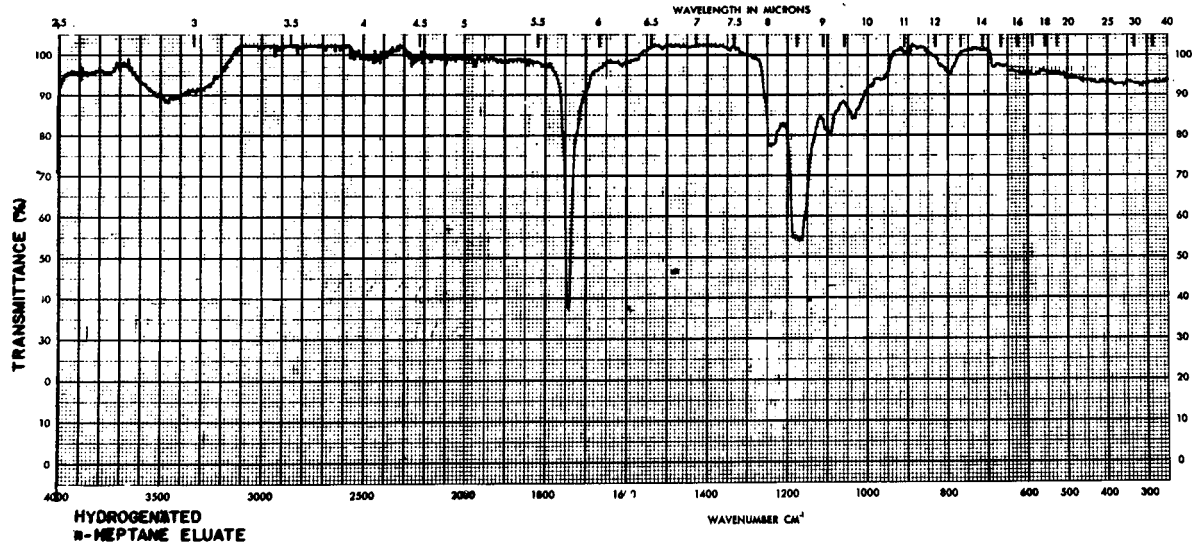
Infrared spectra of the n-heptane eluates

An infrared spectral analysis of the n-heptane eluate and the hydrogenated n-heptane eluate was recorded (Figure 29) with an IR 10 Infrared Spectrophotometer. The absorption for the n-heptane eluate between 3650-2600 cm^{-1} and at 1740 cm^{-1} owing to stretching vibration of OH and C=O respectively, was attributed to the carotenoid pigments that were present. The absorption at other wave numbers show that olefins are present

FIGURE 29

INFRARED SPECTRA OF THE OLEFINIC AND HYDROGENATED HYDROCARBONS OF SARCINA LUTEA RECORDED ON AN IR 10 INFRARED SPECTROPHOTOMETER


WAVELENGTH IN MICRONS
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 9 10 11 12 14 16 18 20 25 30 40
TRANSMITTANCE (%)
100 90 80 70 60 50 40 30 20 10 0
4000 3500 3000 2500 2000 1800 1600 1400 1200 1000 800 600 500 400 300
WAVENUMBER CM⁻¹
n-HEPTANE ELUATE


WAVELENGTH IN MICRONS
2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 9 10 11 12 14 16 18 20 25 30 40
TRANSMITTANCE (%)
100 90 80 70 60 50 40 30 20 10 0
4000 3500 3000 2500 2000 1800 1600 1400 1200 1000 800 600 500 400 300
WAVENUMBER CM⁻¹
HYDROGENATED
n-HEPTANE ELUATE

as indicated by the double bond stretching frequency around 1640 cm^{-1} and the absorptions from 600 to 1000 cm^{-1}. The bands around 650, 820 and 975 cm^{-1} are indications of cis olefins, tri-substituted olefins and trans-disubstituted olefins, most of which belong to the carotenoid pigments. The IR spectra of the hydrogenated n-heptane eluent (Figure 29) confirms the hydrogenation of the olefins by the deletion of the absorptions in question.

The mass spectral patterns resulting from the hydrogenated n-heptane eluate are shown in Figure 30 for the C_{25} tetrad. They demonstrate that: 1) all of the components within each of the isomer groups are methyl branched paraffins 2) the methyl side chain occupies the iso position in two of the four isomers and the anteiso position in the other two 3) the mass spectral patterns corresponding to the iso and anteiso paraffins are not only essentially identical within each group (Figure 30), but also when compared to those of the other groups of isomers. As in the case of the olefins, the spectra of the C_{25} paraffins presented in Figure 30 are representative of the spectra found for all of the other groups.

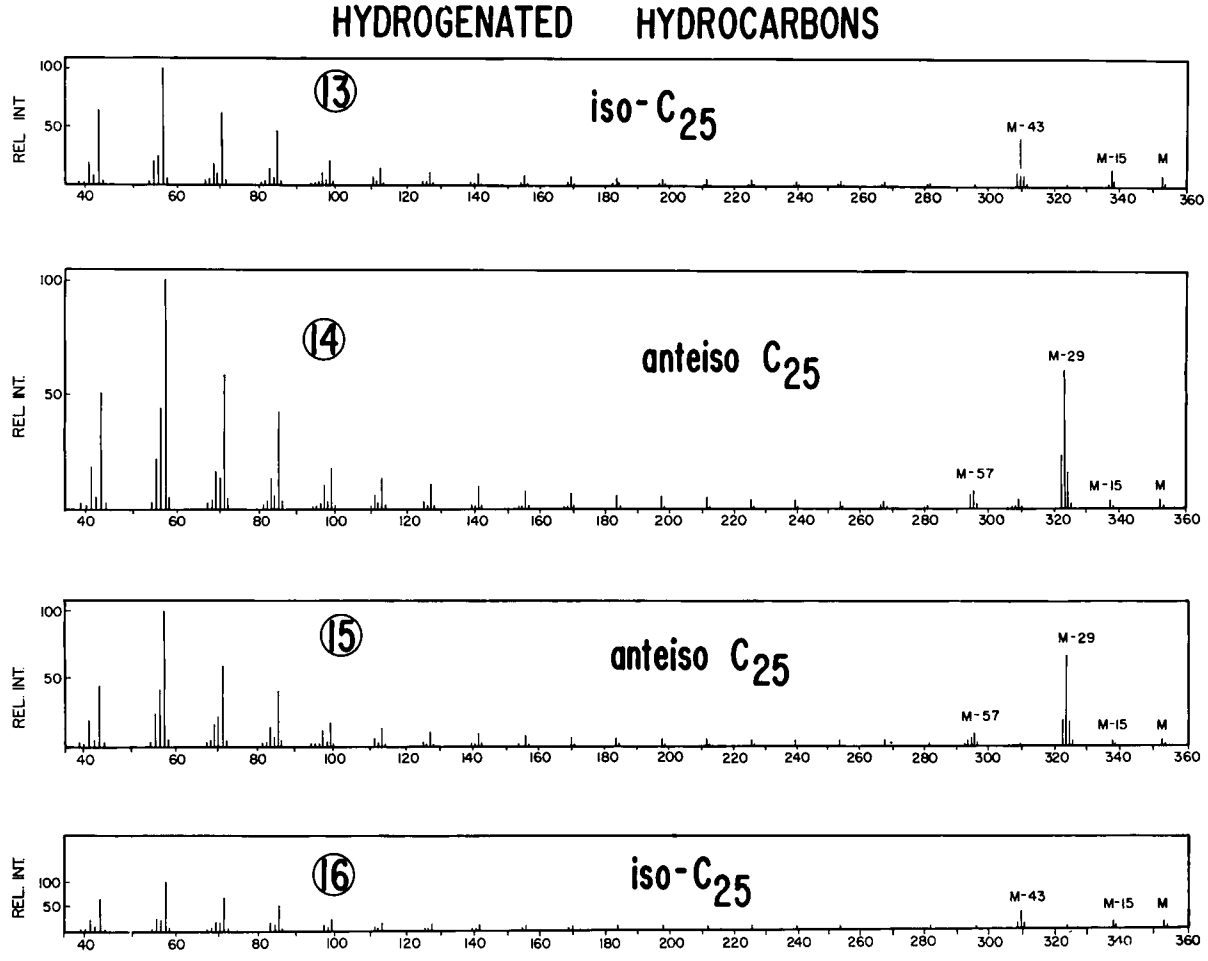
From this data, it can be shown that the first component is always an iso-olefin and the second an anteiso-olefin while the third is an iso-olefin in the even carbon numbered chains, but it corresponds to an anteiso-olefin in the odd carbon numbered chains (see Table XXII). In accordance with

FIGURE 30

MASS SPECTRA OF HYDROGENATED HYDROCARBONS

FROM SARCINA LUTEA

All conditions are the same as described in Figure 28.


HYDROGENATED HYDROCARBONS
REL INT
100
50
⑬
iso-C25
M-43
M-15
M
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
⑭
anteiso C25
M-29
M-57
M-15
M
⑮
anteiso C25
M-29
M-57
M-15
M
⑯
iso-C25
M-43
M-15
M

these results, upon hydrogenation of the sample the four peaks within each group of olefin isomers would be expected to collapse into two when analyzed by the gas chromatograph. The fact that this does not take place and that there is an almost identical reproducibility of the gas chromatographic pattern after hydrogenation of the sample indicates, 1) that the double bond does not play a significant role in the resolution of each of the isomers as it was first thought and 2) that it is necessary to provide for some configurational differences between each of the iso and anteiso isomers, in order to explain the unusual resolution encountered. Although the spectra seem to indicate only the presence of a methyl substituent on each of the components, the possibility of having iso or anteiso configurations on both ends of the molecule can not be excluded since this would not change the overall mass spectral pattern. On the other hand, the clear differentiation of iso and anteiso fragmentation patterns indicates that the coexistence of iso and anteiso methyl branching in any one of the molecules is not possible. Therefore, any near-terminal methyl disubstitutions on the olefins must be of the same type, either both iso or both anteiso.

More work will have to be done to locate the position of the double bond and to determine the possible presence of some doubly near-terminal methyl-substituted isomers. The possibility of having a disubstituted molecule is attractive,

since it would explain the variation in retention times of the isomers through diastereoisomeric forms.

b) Bacillus cereus

This organism was isolated from a sample of the Murray meteorite which had been received from E. P. Henderson. It is one of the three types of bacteria isolated from two carbonaceous chondrites during a study of the possible bacterial contamination of carbonaceous meteorites undertaken in this laboratory (370). The isolation, identification and cultivation procedures have been described in the literature by Oró and Tornabene (370).

To complement the microbiological observations, the lipid composition of this bacterium was studied by gas chromatography and mass spectrometry according to the usual techniques.

The gas chromatographic patterns of the aliphatic hydrocarbons and fatty acids are shown in Figure 31. The identification of the hydrocarbon components given in Figure 31A have been substantiated by gas chromatographic elution data and the corresponding mass spectrometric patterns. Figure 31C shows the fatty acid methyl ester distribution obtained from the same cells that produced the hydrocarbon pattern given on top. The mass spectrometric analysis showed a rather complex distribution of fatty acids with a high proportion of branched and unsaturated chains. No correlations are apparent between the hydrocarbons and fatty acids of this bacterium.

FIGURE 31

GAS CHROMATOGRAPHIC SEPARATION OF ALKANES AND FATTY ACID METHYL ESTERS FROM BACILLUS CEREUS

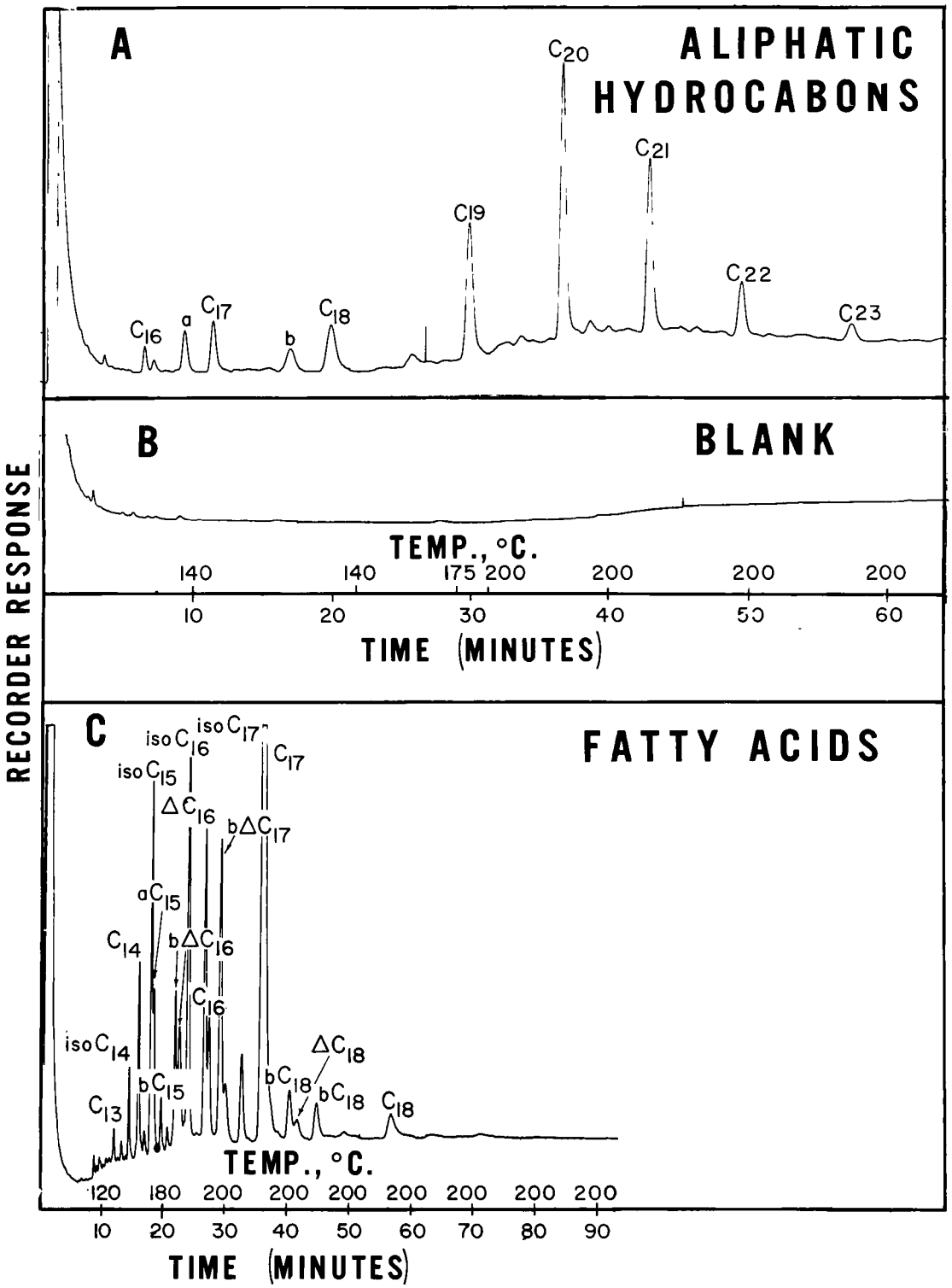
(A) Stainless steel capillary column coated with Polysev (91 m long by 0.076 cm i.d.). 0.23 g extracted, about 1/4 of the n-heptane eluate was injected. Range, 10^2; attenuation, 1. Isothermal at 140°C for 22 minutes, then programmed 6°C per minute to 200°C.

(B) Extraction and gas chromatographic analysis blank. All of the sample was injected. Conditions same as A.

(C) Stainless steel capillary column coated with Igepal Co 990 (155 m long by 0.076 cm i.d.). From 0.23 g of the cells extracted, about 1/25 of the sample was injected. Range, 10^2; attenuation, 1. Isothermal at 120°C for 10 minutes, then programmed 6°C perminute to 200°C.

Symbols: b = branching, a = anteiso and Δ = double bond.

BACILLUS CEREUS



Although the similarities between the B. cereus hydrocarbon pattern and the hydrocarbon patterns of meteorites are remarkable (see references 350 and 364). This data (Figure 31A) appears to be somewhat controversial since it does not seem to be readily reproducible (480).

c) Staphylococcus aureus

A culture of Staphylococcus aureus was obtained from the stock culture collection of the Department of Biology, University of Houston, Houston, Texas. It was grown and extracted as described by T. G. Tornabene (480).

The gas chromatographic-mass spectrometric analysis of the fatty acid methyl esters of S. aureus was found to give results similar to the fatty acid preparation of B. cereus (Figure 31C) in that it also has a high proportion of branched and unsaturated fatty acids. The individual components identified by their mass spectra are listed in Table XXIII. No hydrocarbon pattern could be obtained until after the storage of the cells for two months in a desiccator (480).

d) Halobacterium cutirubrum

The lipid components of this halophilic microorganism have been the object of an extense study by M. Kates and coworkers (240) who have found that a phytyl-

TABLE XXIII

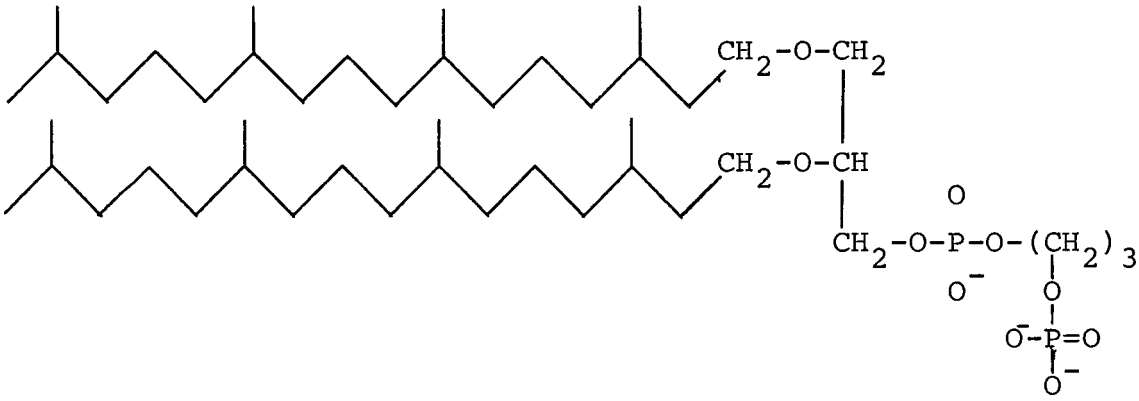
FATTY ACID METHYL ESTERS FROM

STAPHILOCOCCUS AUREUS

br 12:0
i 13:0
ai 13:0
i 14:0
14:0
i 15:0
ai 15:0
15:0
br 16:1
16:0
16:1
i 17:0
ai 17:0
17:0
18:0
18:1
18:2
18:3
19:0
20:0
21:0

Symbols: Same as in Table XXI

containing lipid (diphytyl phospholipid)



is commonly present in this salt bacterium. These type of lipids can be readily separated from the total extractable lipids by their insolubility in cold acetone. Since not much was known on the acetone soluble fraction of the cell extract, which contains the "neutral lipids", it was further fractionated on a heat activated silica gel column into the heptane, benzene, chloroform and methanol fractions. A preliminary analysis of the eluates showed that the bulk of the components are in the benzene fraction.

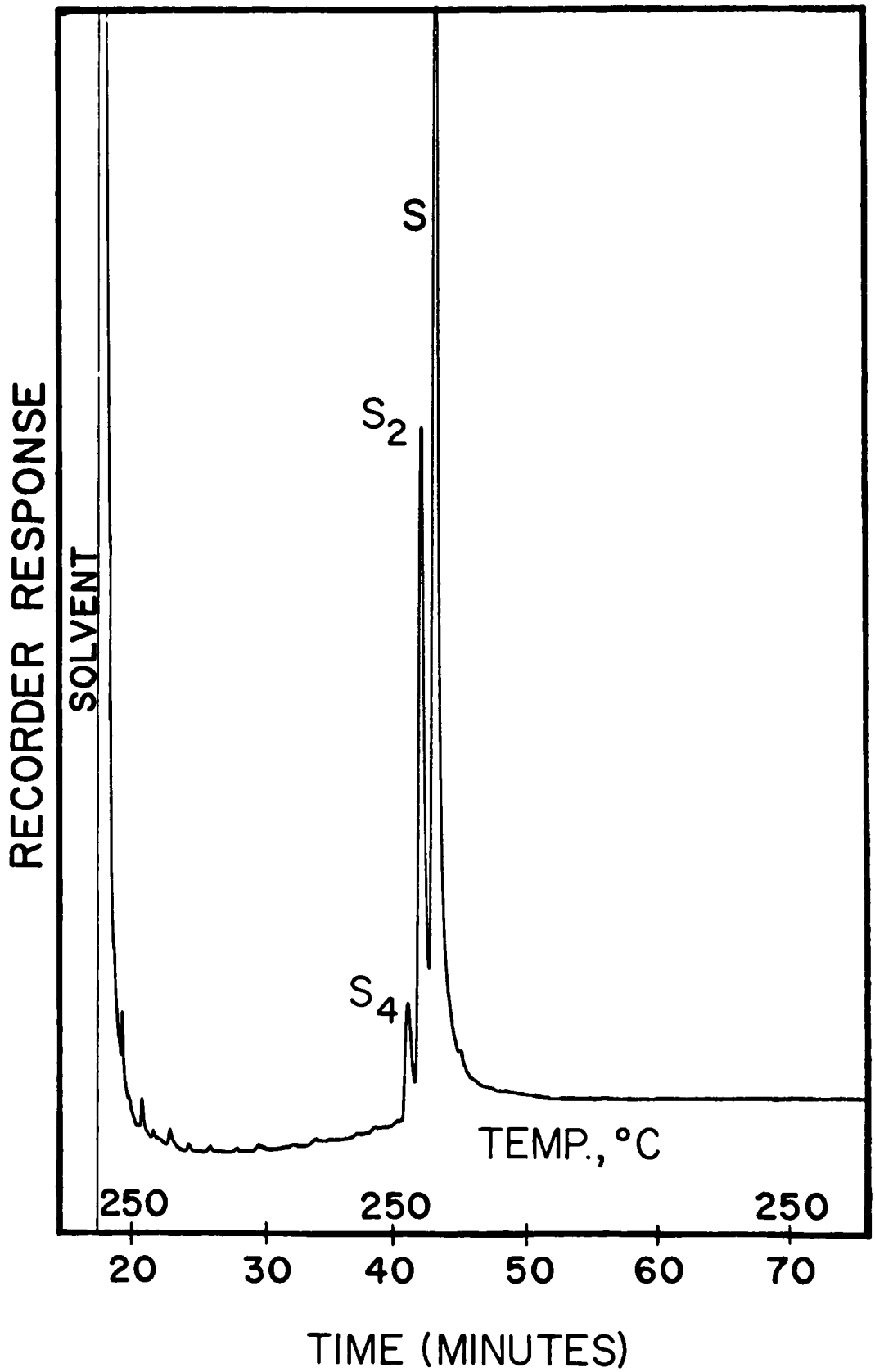
Samples of the n-heptane and benzene eluates of the acetone soluble extract of H. cutirubrum were sent to this laboratory by Drs. Kates and Tornabene. The mass spectrometric study of the heptane eluate showed that it had been contaminated with mineral oil hydrocarbons and no more work could be done on it. The benzene eluate was chromatographed on a short glass column packed with OV-1 (methyl phenyl silicone) (Table I). Three major components were obtained (Figure 32) which were

FIGURE 32

GAS CHROMATOGRAPHIC SEPARATION OF SQUALENES

FROM HALOBACTERIUM CUTIRUBRUM

Glass packed column (1.7 m by 0.3 cm i.d.). Stationary phase, OV-1 (1%). Nitrogen pressure 703 g/cm^2, no split. 'F and M Model 810'Gas Chromatograph equipped with a flame ionization detector. Range, 10^2; attenuation, 2. Temperature programmed $2^{o}C$ per minute from 200^{o} to $250^{o}C$. About 0.2/500 of the sample was injected.


RECORDER RESPONSE
SOLVENT
S
S2
S4
TEMP., °C
250
250
250
20
30
40
50
60
70
TIME (MINUTES)

shown to be squalene (the major component, S) and two other analogs of squalene (S_2 and S_4) differing only in their degree unsaturation, according to their respective mass spectra. The mass spectrum of peak S in H. cutirubrum (Figure 32) is given in Table XXIV (see also Table XI and Figure 49) together with that corresponding to a sample of authentic squalene. The most prominent and characteristic features exhibited by these two mass spectra are: the base peak at m/e 81, the relatively high doublet at m/e 136 and 137, the M-69, M-43 and M-15 ions which appear at m/e 341, 367 and 395, respectively, and finally the relatively high intensity of the ion at m/e 410 (the molecular ion). The middle component (S_2 in Figure 32) shows similar fragmentation pattern (Table XXIV) but the M-69, M-43, M-15 and M ions are in this case two mass units higher, indicating that this compound while retaining the same structural characteristics as squalene has one double bond less. The smaller component (S_4 in Figure 32) also shows a similar spectrum but the molecular ion at m/e 414 indicates that another double bond has been saturated. The mass spectral fragmentation of squalene has been discussed in Chapter IV. Dr. Tornabene has also recently confirmed these identifications by thin layer chromatography and Nuclear Magnetic Resonance (483). The amounts of the squalenes S_4 and S_2 relative to the squalene S are about 10 and 44% respectively.

TABLE XXIV

MASS SPECTRA OF SQUALENES FROM HALOBACTERIUM CUTIRUBRUM

m/e	Standard	S	S_2	S_4
69	79	88	81	34
81	100	100	100	45
83	6	10	73	100
95	24	2	31	27
109	19	16	20	17
123	24	21	23	13
136	48	40	36	-
137	44	42	35	-
149	18	17	17	8.5
163	5	4	6	4.3
177	5	3.8	5	3.2
191	7.8	6.6	8	3.4
192	8.1	6.5	7.6	3.7
193	1.5	-	-	9
203	6.1	5.1	4.5	-
205	4.7	-	7	3.8
207	-	-	-	-
218	3.4	2.8	3.4	-
220	-	-	4.2	11.3
231	3.6	2.9	3.4	1.5
233	-	-	-	-
273	2.9	2.5	3	-
275	-	-	2.2	4.4
299	1.9	1.7	3	-
301	-	-	2.2	8
328	1.8	1.5	1.1	-
330	-	-	1.5	2.8
341	7.5	6.3	-	-
343	-	-	7.2	2
367	2.2	1.9	-	-
369	-	-	2.5	10
395	0.4	0.4	-	-
397	-	-	0.5	-
399	-	-	-	0.6
410	4.4	4.4	-	-
411	1.4	1.3	-	-
412	0.4	0.5	7.8	-
413	-	-	2.6	-
414	-	-	0.7	8.7
415	-	-	-	3.3
415	-	-	-	0.8
416	-	-	-	-

The smaller compounds eluted before the squalenes give spectra which do not differ appreciably from the characteristic silicone phase background (see Chapter III). This goes in line with the silicone absorption band seen in the NMR spectrum of squalene-S. Its appearance has been traced back to the silicone anti-foaming agent added during the cultivation of large quantities of cells (483). The presence of these polyisoprenoid structures in halophilic microorganisms may well be related to the isoprenoid content of marine plankton (50,52) and higher organisms such as the shark (48,170).

e) Vibrio marinus

A marine bacterium isolated from the Pacific Ocean (105) was cultivated at 15°C with heavy aeration to the late exponential phase of growth in a S. D. B. medium previously described (186). The cells were sent to us in the frozen state, immediately after harvesting, as a gift from Dr. R. Morita, Oregon State University, Corvalis, Oregon. The frozen culture was dried over P_2O_5 under a vacuum. The cell lipid extract was prepared for analysis according to the methods already described. A more detailed account of the analytical procedures can be found in the literature (371).

The gas chromatographic pattern for the hydrocarbons of Vibrio marinus is given in Figure 33A. The gas chromatographic analysis allowed the identification of the individual hydrocarbons as alkanes and alkenes ranging from C_{15} to C_{18};

FIGURE 33

GAS CHROMATOGRAPHIC SEPARATION OF HYDROCARBONS AND FATTY ACID METHYL ESTERS FROM VIBRIO MARINUS WITH A CAPILLARY COLUMN (IGEPAL CO 990)

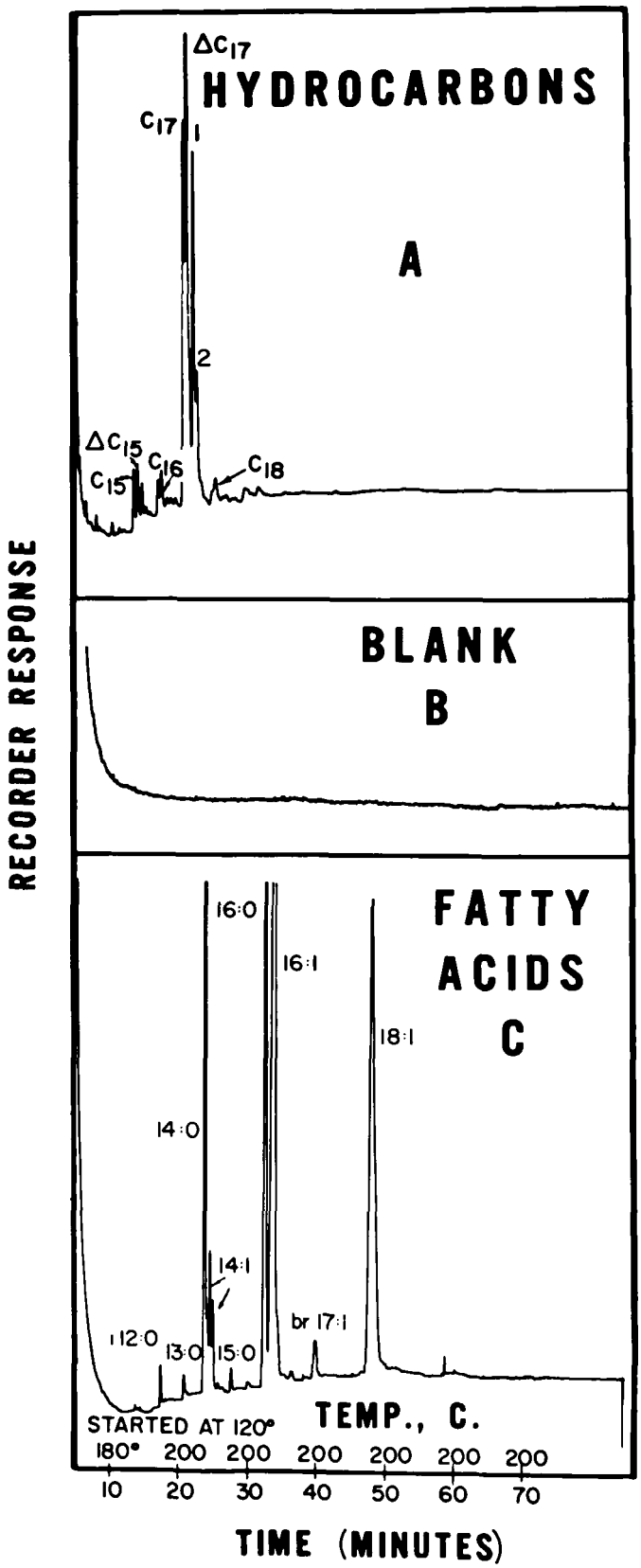
Stainless steel capillary column (155 m long by 0.076 cm i.d.) coated with Igepal Co 990. No split. Range, 10^2, attenuation, 1. Temperature programmed at approximately 6 degree per minute from 120^{o} to 200^{0} and held isothermally at 200^{o}C. From 3 grams of extracted cells:

(A) 1/10 of the sample was injected.

(B) 1/1 of the sample was injected.

(C) 1/1200 of the sample was injected.

VIBRIO MARINUS



$n\text{-}C_{17}$ and $n\text{-}\Delta\text{-}C_{17}$ being the two major components. The other two major peaks were identified as another n-heptadecene (peak 1) and a n-heptadecadiene (peak 2). It is possible that there is a higher quantity of the lower molecular weight hydrocarbons since small amounts may be expected to be lost by the drying procedure and the evaporation of the extract under a stream of nitrogen. The results can not be due to any artifact or contamination during our studies since no measurable hydrocarbons were found in the procedure as shown by the controls and solvent blanks (see Figure 33B). Judging from the pattern shown in Figure 33A, predominance of odd carbon numbered hydrocarbons is obvious.

The gas chromatographic pattern for the fatty acids is given in Figure 33C. The components were identified by their gas chromatographic relative retention time and by mass spectrometric analysis mainly as even-carbon numbered fatty acids, ranging from C_{12} to C_{18}, the major components being palmitoleic and oleic acids.

2. Algae

Because of the possible geochemical significance of the algal lipids (138,171,192,376) in relation to the organic matter of ancient and recent sediments, several species of algae have been analyzed for their lipid content and the results are described in this section.

a) Algal mats

The algal mats, collected on the Texas Gulf Coast, were secured in a dried form by Mr. C. C. Smith, Tenneco Oil Corporation, Houston, Texas. The algal mat sample SM-7 was collected February 23, 1964, southwest of San Luis Pass on the southeast of Oyster Bay. Sample SM-8 was collected August 1, 1964, west of Horse Triangulation Station and south of Harlingen Channel through Laguna Atascosa National Wildlife Refuge. Sample SM-9 was collected August 1, 1964, from the Laguna mud flat on the Lagoonal side of Padre Island. The algal species found in Sample SM-7 were identified as Microcoleus chthonoplastes (Mert.) Zanard, Lyngbya aestaurii (Mert.) Lyngb. and in lesser amounts Schizothrix calcicola (ag.) Gom. Four to five grams of the algal mats were analyzed.

The gas chromatographic analyses of the three different algal mat samples are shown in Figure 34 (371). Although a resemblance with V. marinus is apparent, the analyses show a larger number of hydrocarbons ranging from n-C_{15} through n-C_{31}, although in small concentrations above n-C_{23}. The small amounts of hydrocarbons above n-C_{23} were separated on a stainless steel capillary column coated with Apiezon L and are not shown here. It could not be determined whether the isoprenoid hydrocarbons pristane and phytane which are shown as peaks a and b respectively in Figure 34 were produced by the algae or were derived from other sources.

FIGURE 34

GAS CHROMATOGRAPHIC SEPARATION OF HYDROCARBONS FROM ALGAL MATS

Stainless steel capillary column coated with Polysev (91 m long by 0.076 cm i.d.). No split. Isothermal at 135°C for 18 minutes, then programmed at approximately 6 degrees per minute to 200°C.

(A) Algal mat (SM-7) extracted, 4.7 g. About 1/15 of the n-heptane sample eluate was injected. Range, 10^2, attenuation, 2.

(B) Algal mat (SM-8) extracted, 5.1 g. About 1/20 of the n-heptane eluate was injected. Range, 10^2, attenuation, 4.

(C) Algal mat (SM-9) extracted, 4.1 g. About 1/12 of the n-heptane eluate was injected. Range, 10^2, attenuation, 1.

Symbols: Δ = double bond

HYDROCARBONS OF ALGAL MATS

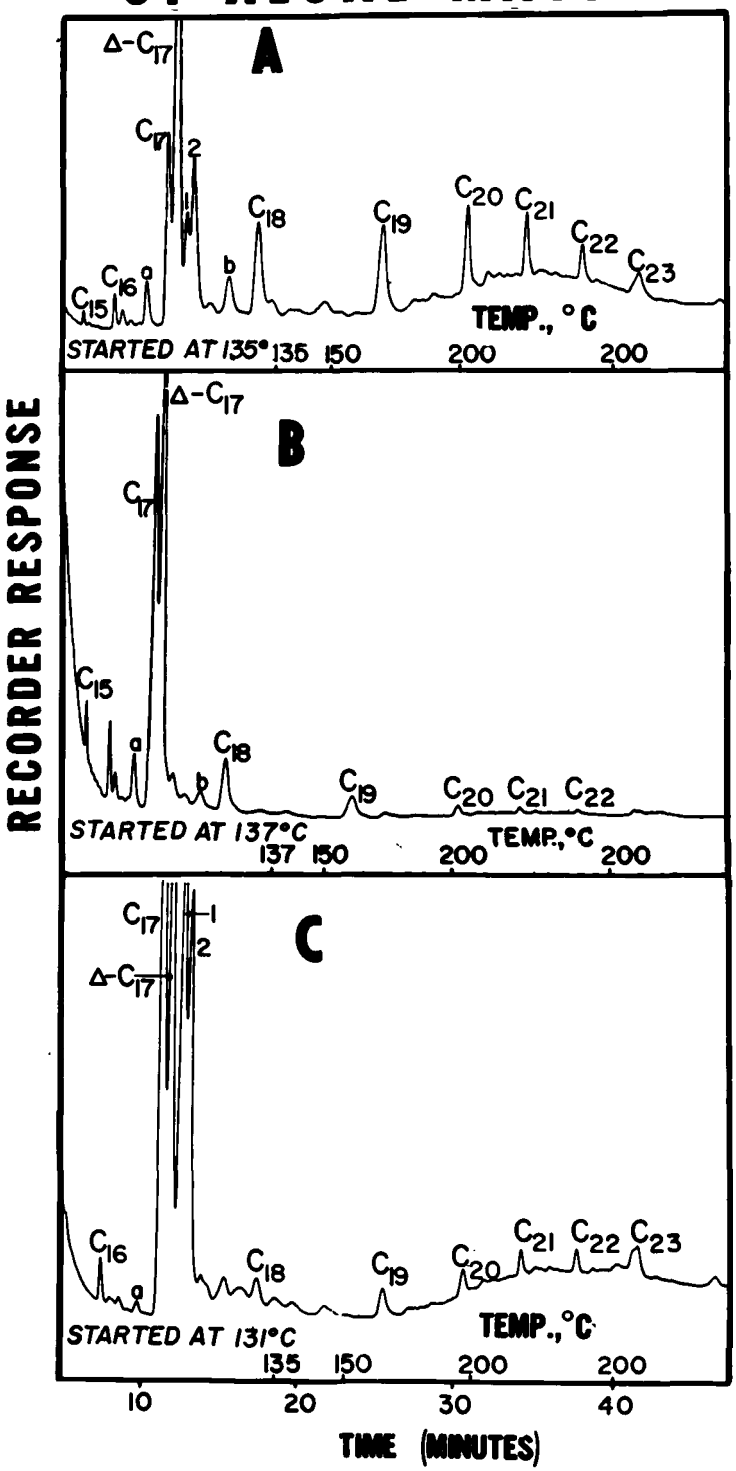


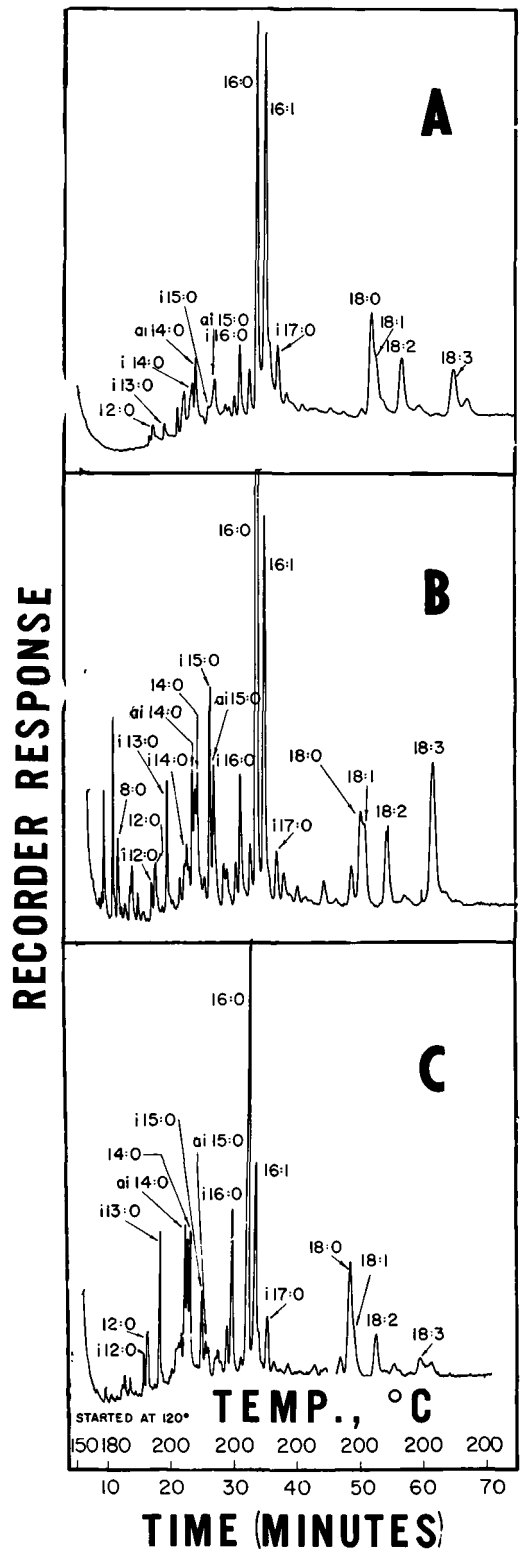
FIGURE 35

GAS CHROMATOGRAPHIC SEPARATION OF FATTY ACID METHYL ESTERS FROM ALGAL MATS

All conditions are the same as in Figure 33. About 1/200 of each sample was injected. The first numbers indicate the number of carbon atoms in the chain of the fatty acid; the second number indicates the number of double bonds.

Symbols: i = iso; ai = anteiso

FATTY ACIDS OF ALGAL MATS



The major components have been identified by their respective mass spectra as a n-C_{17}, n-Δ-C_{17} (Figure 34) plus br-ΔC_{18} (peak 1) and anteiso-C_{18} (peak 2). On the other hand the gas chromatographic data indicates that in this particular instance the mass spectrum of peak 2 was misinterpreted. It has been indicated before (Chapter IVB) that the anteiso C_{18} is eluted together with phytane in the Polysev columns. This will become more clear after the discussion on the identification of isoalkanes in meteorites. It can be seen in Figure 34 that phytane (peak b) is eluted after the compound taken as an anteiso C_{18} (peak 2). This illustrates the way in which both techniques, gas chromatography and mass spectrometry complement each other in problems of structure determination of organic compounds.

The fatty acids of these algal mats (Figure 35), also identified by the combination gas chromatography-mass spectrometry, were found to be in fair agreement with the fatty acids found in algae by Parker and associates (375,376). Typical mass spectra of two unsaturated C_{18} acid esters of the algal mats have been given (Figure 20) together with the discussion on fatty acid mass spectrometry.

b) Anacystis nidulans

A blue-green alga species, Anacystis nidulans, was obtained from Dr. D. S. Hoare, Department of Microbiology, University of Texas, Austin, Texas. The organism was grown

autotrophically (by Dr. Tornabene) in the light at about 28°C on medium D_M of Van Baalen (498). Details on the culturing conditions can be found elsewhere (371,480).

The fatty acids and the hydrocarbons of several species of blue-green algae have been reported by Parker and associates (375,376), by Holton et al. (212) and recently by Oró et al. (371) and Gelpi et al. (171).

The gas chromatographic pattern showing the fatty acid methyl esters and hydrocarbons of Anacystis nidulans (371) is given in Figure 36. The hydrocarbon distribution pattern appears to be related to the ones just shown for the other algae and marine bacterium.

Mass spectrometric analysis made on the hydrocarbon peaks (Figure 36A) as they were eluted from the columns identified them as alkanes and alkenes ranging from C_{14} to C_{19} with the major peaks being nC_{15}, nC_{16}, nC_{17} and n-Δ-C_{17}.

The fatty acids have a distribution similar to that of the bacterium Vibro marinus (Figure 33) and the algal mat populations (Figure 35). Their identities are given in Figure 36. The mass spectrometric patterns have been discussed before.

c) Anacystis montana

A culture of this blue-green alga (171) was obtained from M. Rodriguez-Lopez, Centro de Investigaciones Biologicas, Instituto Marañon, Madrid, Spain. It was grown

FIGURE 36

GAS CHROMATOGRAPHIC SEPARATION OF HYDROCARBONS AND FATTY ACID METHYL ESTERS FROM ANACYSTIS NIDULANS

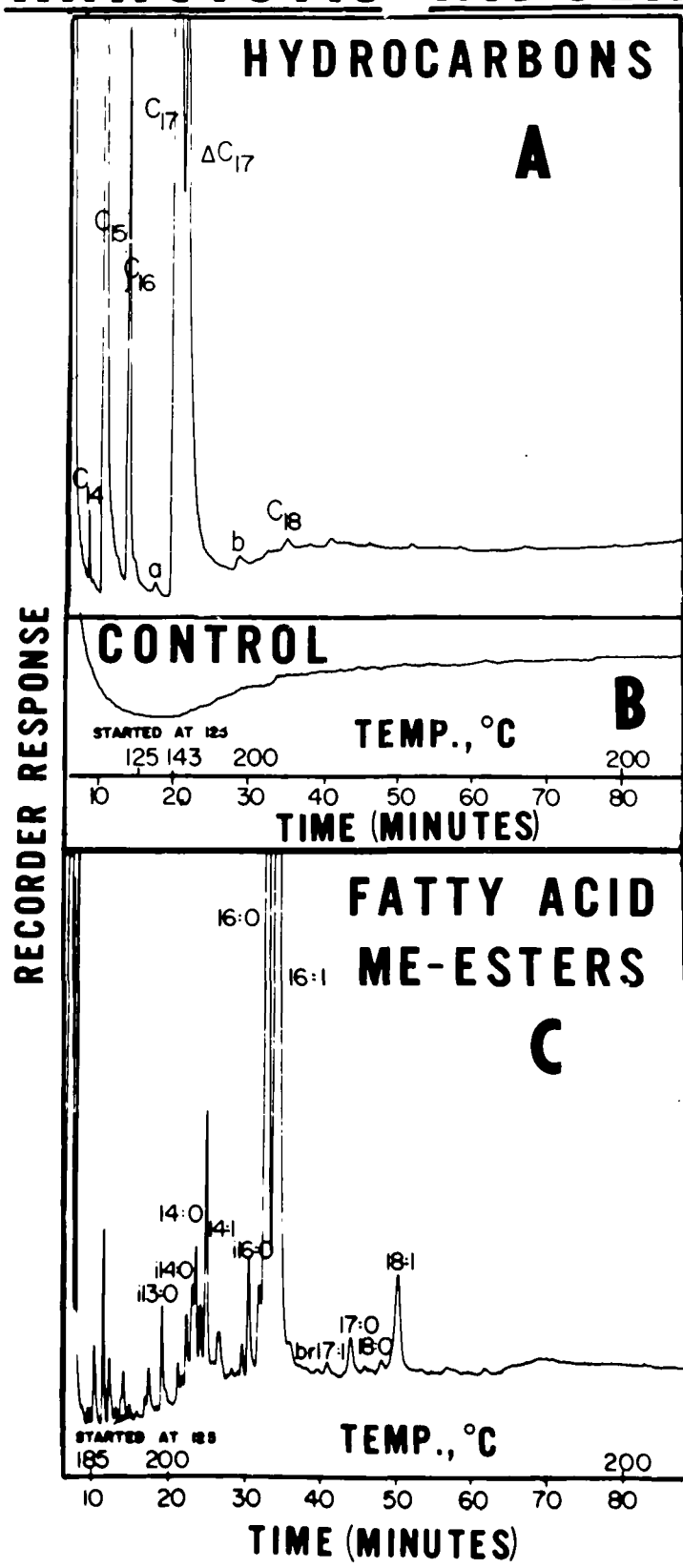
All conditions are the same as in Figure 33, except for the starting temperature at 125°C. From 0.26 g of cells extracted:

(A) Approximately 1/5 of the sample was injected.

(B) 1/1 of the sample was injected.

(C) 1/100 of the sample was injected.

ANACYSTIS NIDULANS



autrotrophically in the light at 28°C by Mr. H. Schneider. Three liters of D medium (498) were used to support adequate growth and the culture was aerated continuously with filtered air. Cells were harvested by centrifugation, washed with a saline solution and dried over P_2O_5 under vacuum.

Methods of extraction and fractionation have been described in Chapter III.

The aliphatic hydrocarbons of A. montana (Figure 37) were identified by their respective mass spectra as mono and diolefins (171) in the C_{19} to C_{29} range (Table XXV). The major peak is the C_{27} monoolefin. Heptadecane is the only paraffin present. Partial mass spectra of the C_{25} and C_{27} monoolefins have been given in Table XIV. The mass spectral patterns do not show any indication of cycloalkyl compounds. Two major characteristics of this distribution are: the presence of large amounts of high molecular weight aliphatic hydrocarbons, and their marked odd over even predominance, both of which are considered typical of the higher plants (92,140, 367,502). In general the major part of the high molecular weight hydrocarbons bio-synthesized by the higher plants are saturated while in A. montana all of them with the exception of the C_{17} paraffin are unsaturated.

Although not as common, the occurrence of high molecular weight olefins in plants has been reported by Šorm et al. (448) and very recently Stransky et al. have reported in

FIGURE 37

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS FROM ANACYSTIS MONTANA

Stainless steel capillary tubing (30 m by 0.025 cm i.d.) coated with 10% Apiezon L. 'F and M Model 810' gas chromatograph with flame ionization detector. Nitrogen pressure 1406 g/cm^2. Range, 10^2; attenuation, 1.

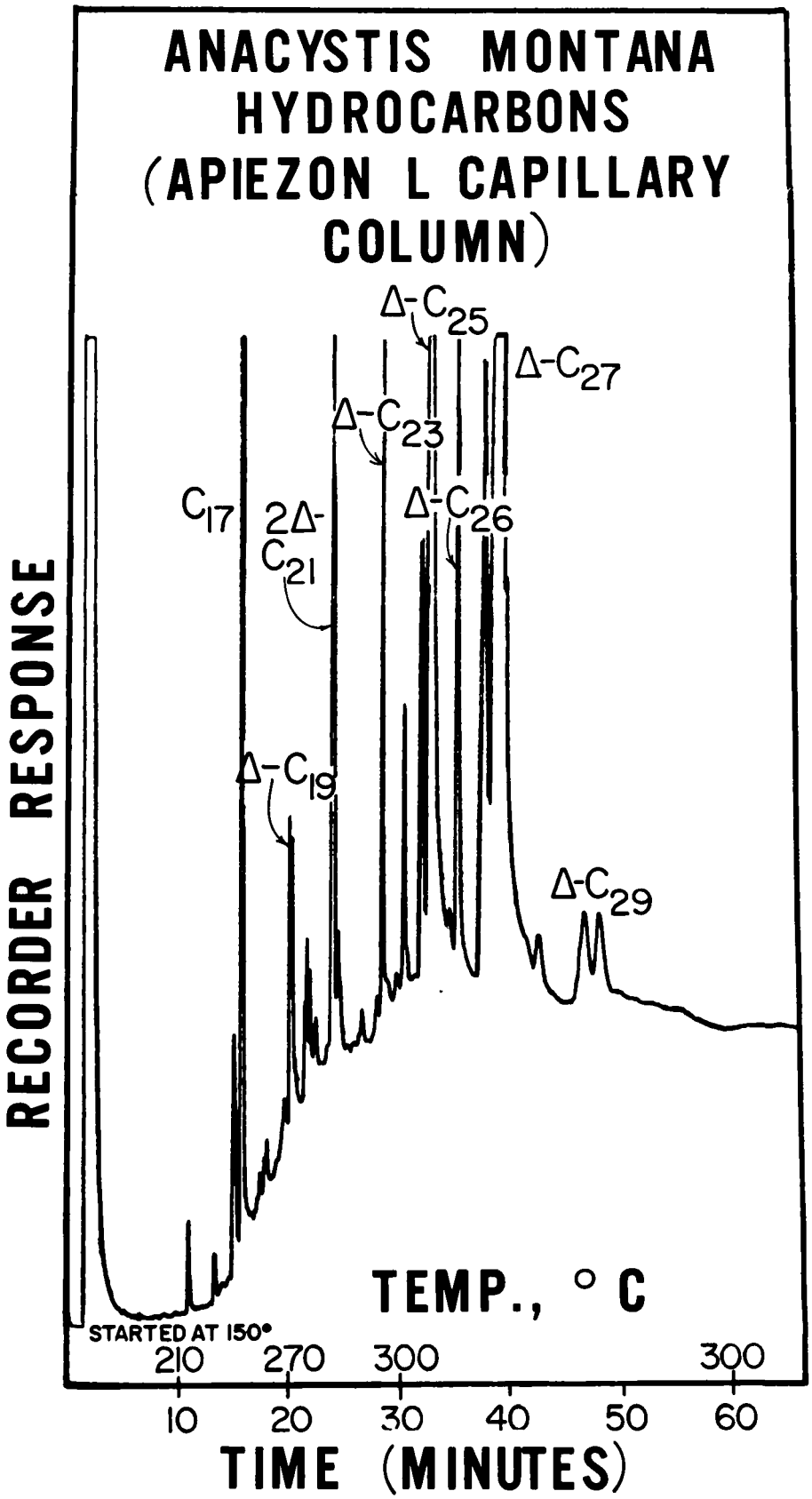

ANACYSTIS MONTANA
HYDROCARBONS
(APIEZON L CAPILLARY
COLUMN)
Δ-C25
Δ-C27
Δ-C23
C17
2Δ-C21
Δ-C26
Δ-C19
Δ-C29
RECORDER RESPONSE
TEMP., ° C
STARTED AT 150°
210
270
300
300
10
20
30
40
50
60
TIME (MINUTES)

TABLE XXV

RELATIVE PERCENT CONTENT OF HYDROCARBONS

	Anacystis	Botryococcus
$n\text{-}C_{17}$	11.5	-
$\Delta\text{-}C_{17}$	-	1.52
$\Delta\text{-}C_{19}$	0.2	-
$\Delta\text{-}C_{20}$	0.1	-
$2\Delta\text{-}C_{21}$	8.9	-
$\Delta\text{-}C_{23}$	8.0	0.14
$\Delta\text{-}C_{24}$	0.2	-
$\Delta\text{-}C_{25}$	14.6	-
$2\Delta\text{-}C_{25}$	0.2	0.65
$3\Delta\text{-}C_{25}$	-	0.10
$\Delta\text{-}C_{26}$	3.8	-
$\Delta\text{-}C_{27}$	34.7	-
$2\Delta\text{-}C_{27}$	2.8	11.10
$\Delta\text{-}C_{28}$	0.1	-
$2\Delta\text{-}C_{28}$	-	0.65
$\Delta\text{-}C_{29}$	0.2	-
$2\Delta\text{-}C_{29}$	-	50.40
$3\Delta\text{-}C_{29}$	-	5.54
$2\Delta\text{-}C_{31}$	-	27.90
$2\Delta\text{-}C_{33}$	-	2.00

agreement with our results the presence of high molecular weight alkanes and alkenes in Scenedesmus quadricanda a green freshwater alga (462).

d) Botryococcus braunii

A culture of B. braunii, a golden-brown alga was obtained from Dr. R. C. Starr, Department of Botany, Indiana University. It was also grown by H. Schneider using the same methodology described before (171).

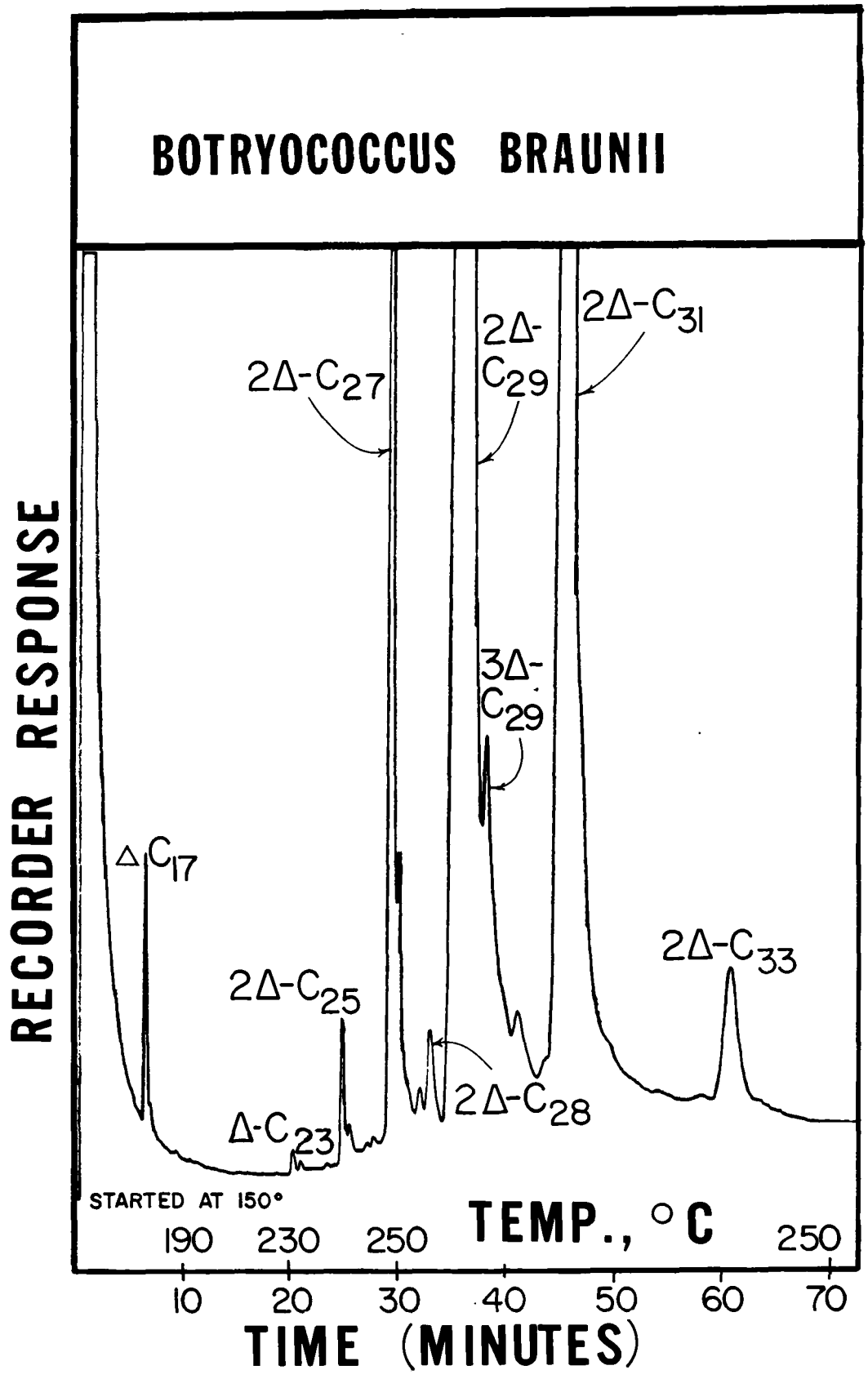
In this case the aliphatic hydrocarbons (Figure 38) were identified by gas chromatography-mass spectrometry as alkenes, mainly with one and two double bonds, ranging from C_{17} to C_{33} (Table XXV). The C_{27}, C_{29} and C_{31} diolefins were predominant, the major component being the C_{29} diolefin (171). Partial mass spectra of this three diolefins are given in Table XV. The mass spectra of the C_{17} olefin can be found in Table XIII. No evidence of cycloalkyl structures could be found in this sample either.

The hydrocarbon distribution of B. braunii (Figure 38) shows basically the same characteristics as the blue-green alga, Anacystis montana; high molecular weight unsaturated hydrocarbons and predominance of odd carbon numbers. The differences here are the higher degree of unsaturation (mass spectra with the characteristics of C_{25} and C_{29} triolefins were obtained) and the larger range of high molecular weight hydrocarbons, up to C_{33}. Proper controls were run and

FIGURE 38

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS FROM BOTRYOCOCCUS BRAUNII

Glass column (1.7 m by 0.3 cm i.d.), packed with OV-1. Nitrogen pressure, 703 g/cm^2. Range, 10^2; attenuation, 2. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector.


BOTRYOCOCCUS BRAUNII
RECORDER RESPONSE
2Δ-C27
2Δ-C29
2Δ-C31
3Δ-C29
ΔC17
2Δ-C25
2Δ-C33
Δ-C23
2Δ-C28
STARTED AT 150°
TEMP., °C
190 230 250
250
10 20 30 40 50 60 70
TIME (MINUTES)

necessary precautions taken to exclude any possible source of contamination. Moreover, the unique nature of this two patterns (A. montana and B. braunii) tends to minimize the possible contribution of extraneous material.

This microscopic alga has been found in shales (241) and sediments (486). Its geochemical significance will be discussed in some detail later.

e) Chlorella pyronoidosa

This green algae was obtained from Carolina Biological Supply. It was also grown and analyzed in the same way as the other algae by H. Schneider. The hydrocarbon distribution is shown in Figure 39. Heptadecene was identified as the major component in the cells. It represents a 76.9% of the total hydrocarbon fraction. This is in contrast with the report of Iwata and coworkers who had identified it as a n-hexadecene (227), according to the melting point and elementary analysis. In agreement with the results presented here, Stransky et al. (462) have also found a large amount of n-heptadecane in the lipid fraction of chlorella. The identifications were supported by their respective mass spectral patterns. In its simplicity, this pattern is closely comparable to the ones shown in Figures 33 and 36.

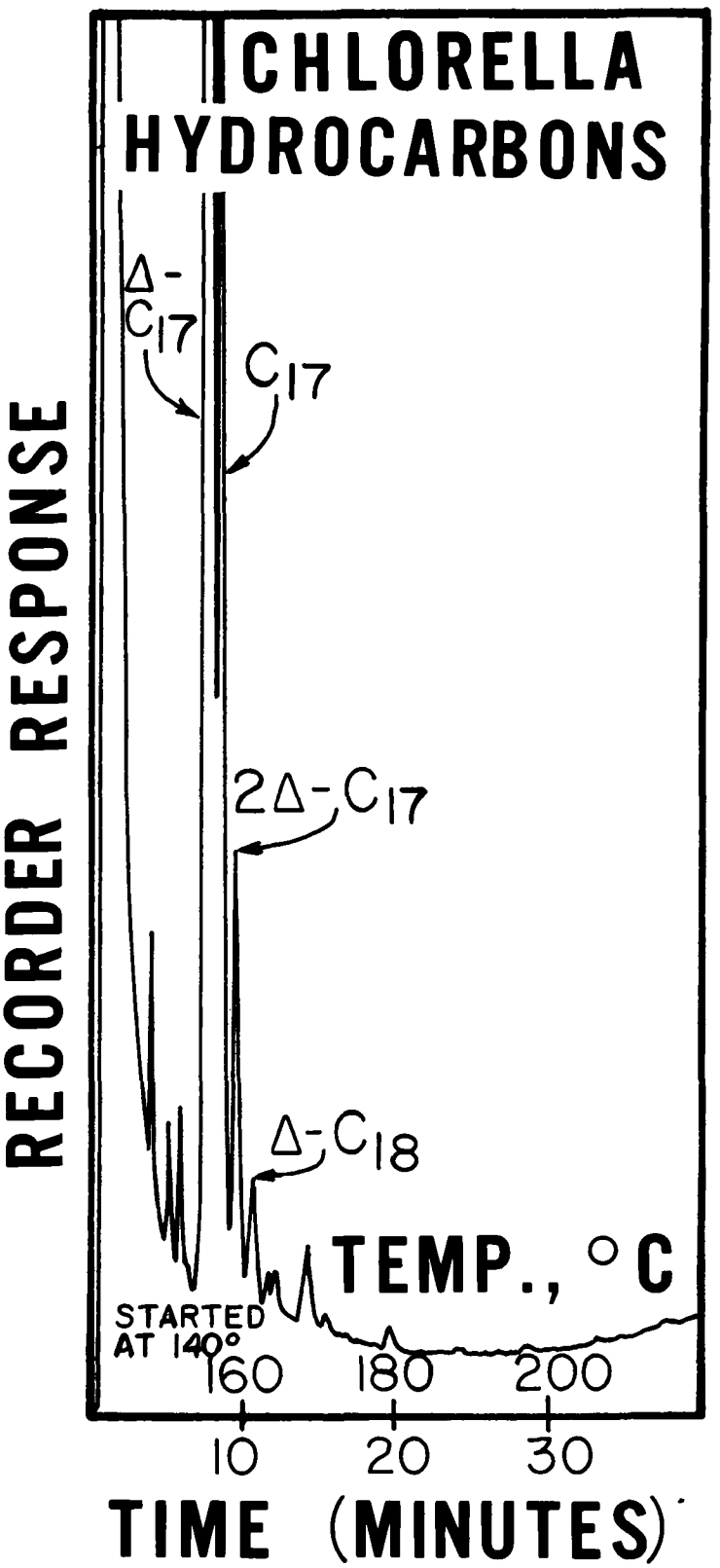
f) Fucales

This marine alga collected by Dr. Oró in Hawaii was also grown and analyzed for its hydrocarbon content.

FIGURE 39

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS FROM CHLORELLA PYRONOIDOSA

Glass column (1.7 m long by 0.3 cm i.d.) packed with OV-1. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Nitrogen pressure, 910 g/cm^2. Range, 10; attenuation, 4. Temperature programmed from 140°C to 240°C at 2°C/min.


CHLORELLA
HYDROCARBONS
Δ-C17
C17
2Δ-C17
Δ-C18
TEMP., °C
STARTED AT 140°
160
180
200
10
20
30
TIME (MINUTES)
RECORDER RESPONSE

In this case the gas chromatographic-mass spectrometric analysis showed a major peak identified as heptadecane in a relative amount of 92%. An 8% of heptadecene was also detected.

3. Plants

The same gas chromatographic-mass spectrometric techniques were applied to the study of plant hydrocarbons and fatty acids with the following results:

a) Cabbage leaf wax (261)

Seeds of Brassica oleracea leaves (Var. Flat Dutch) were obtained commercially from Mandenville and King Co., Rochester, New York. The seedlings were grown under the appropriate experimental conditions and after 2-3 weeks the leaves were collected, extracted and analyzed gas chromatographically by Dr. Laseter (260).

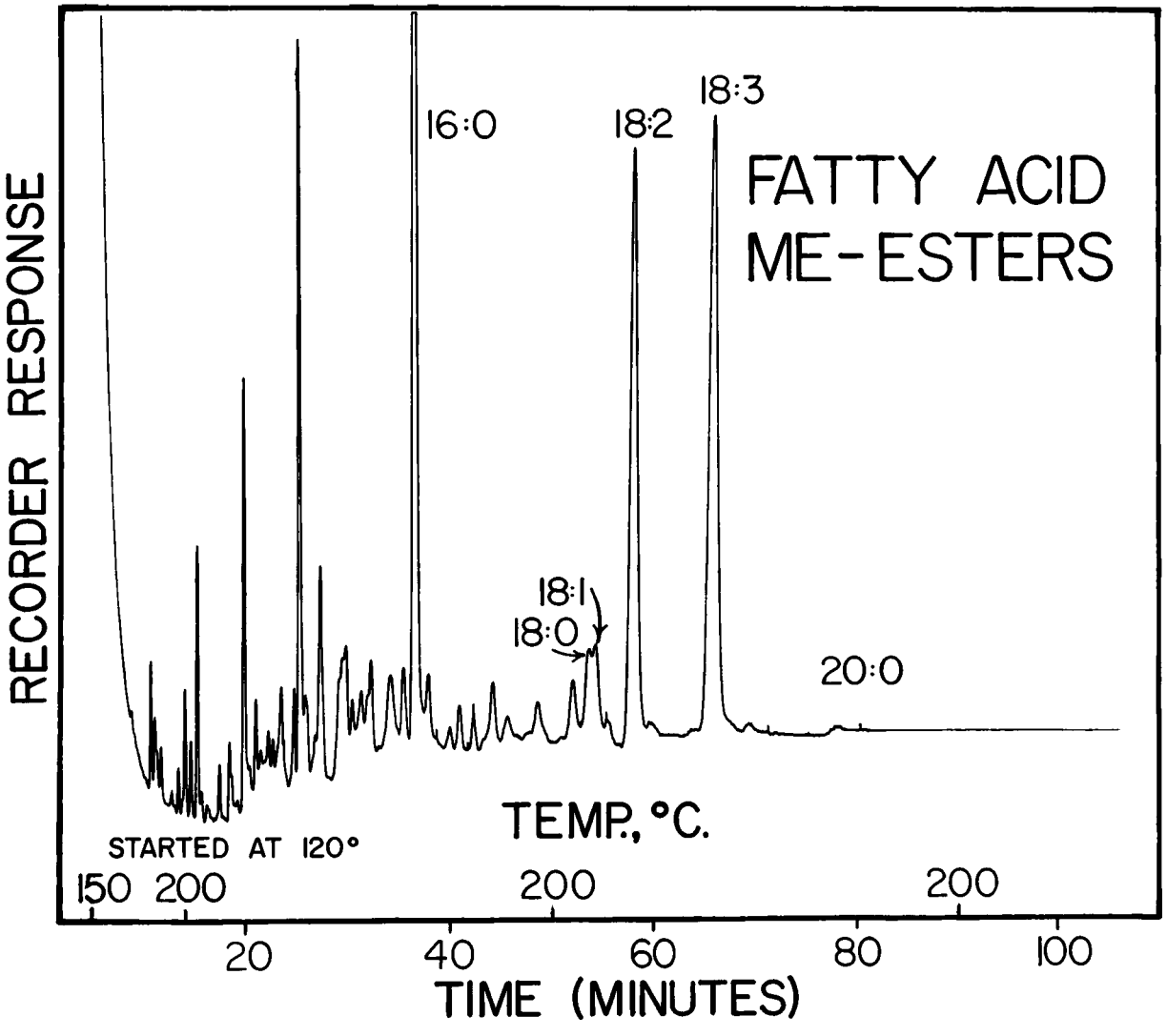
The gas chromatographic-mass spectrometric analysis of the benzene fraction showed a major component in high concentration which was shown to have the structure of 15-nonacosanone (see Chapter IV, section I, and Figure 21). A mechanism for the biosynthesis of this compound has been proposed by Laseter (260).

A similar analysis of the esterified methanol fraction using a 91 m x 0.076 m Polysev column resulted in the mass spectrometric identification of the methyl esters of palmitic, stearic, oleic, linoleic, linolenic and arachidonic acids as shown in Figure 40. Although the stearate and oleate compounds

FIGURE 40

GAS CHROMATOGRAPHIC SEPARATION OF THE METHYL ESTERS FROM YOUNG CABBAGE LEAVES (BRASSICA OLERACEA)

Stainless steel capillary column (155 m long by 0.076 cm i.d.) coated with Igepal Co-990. Nitrogen carrier gas at 2430 g/cm^2. Programmed at approximately 6° per minute from 120° to $200^{\circ}C$, and held isothermally at $200^{\circ}C$. (See references 260 and 261).


RECORDER RESPONSE
16:0
18:2
18:3
FATTY ACID
ME-ESTERS
18:1
18:0
20:0
TEMP., °C.
STARTED AT 120°
150 200
200
200
20
40
60
80
100
TIME (MINUTES)

are partially resolved in this chromatogram (Figure 40) the Polysev column does not give any separation at all and as it has been mentioned it was used in conjunction with the mass spectrometer because of the limitations imposed on the sensitivity of the system by the high bleeding rates of the Igepal columns.

The hydrocarbons from cabbage were analyzed by Dr. Nooner (348) using a packed column (SE-30) attached to an Atlas CH_4 mass spectrometer (411).

b) Spotted bur clover (Medicago arabica) (367)

The saturated hydrocarbons in the n-heptane eluate of clover have been reported by Oró, Nooner and Wikstrom (367).

The gas chromatographic-mass spectrometric analysis of the benzene fraction showed the presence of at least four isomeric phytadienes. Their spectra were practically identical to the spectra of phytadienes A, C, D and E of mistletoe in Figure 41. Their relative ratios were 80, 71, 100 and 51% respectively. The mass spectrometric characteristics of this type of compound have been discussed in Chapter IV.

c) Mistletoe (Phoradendron flavescens)

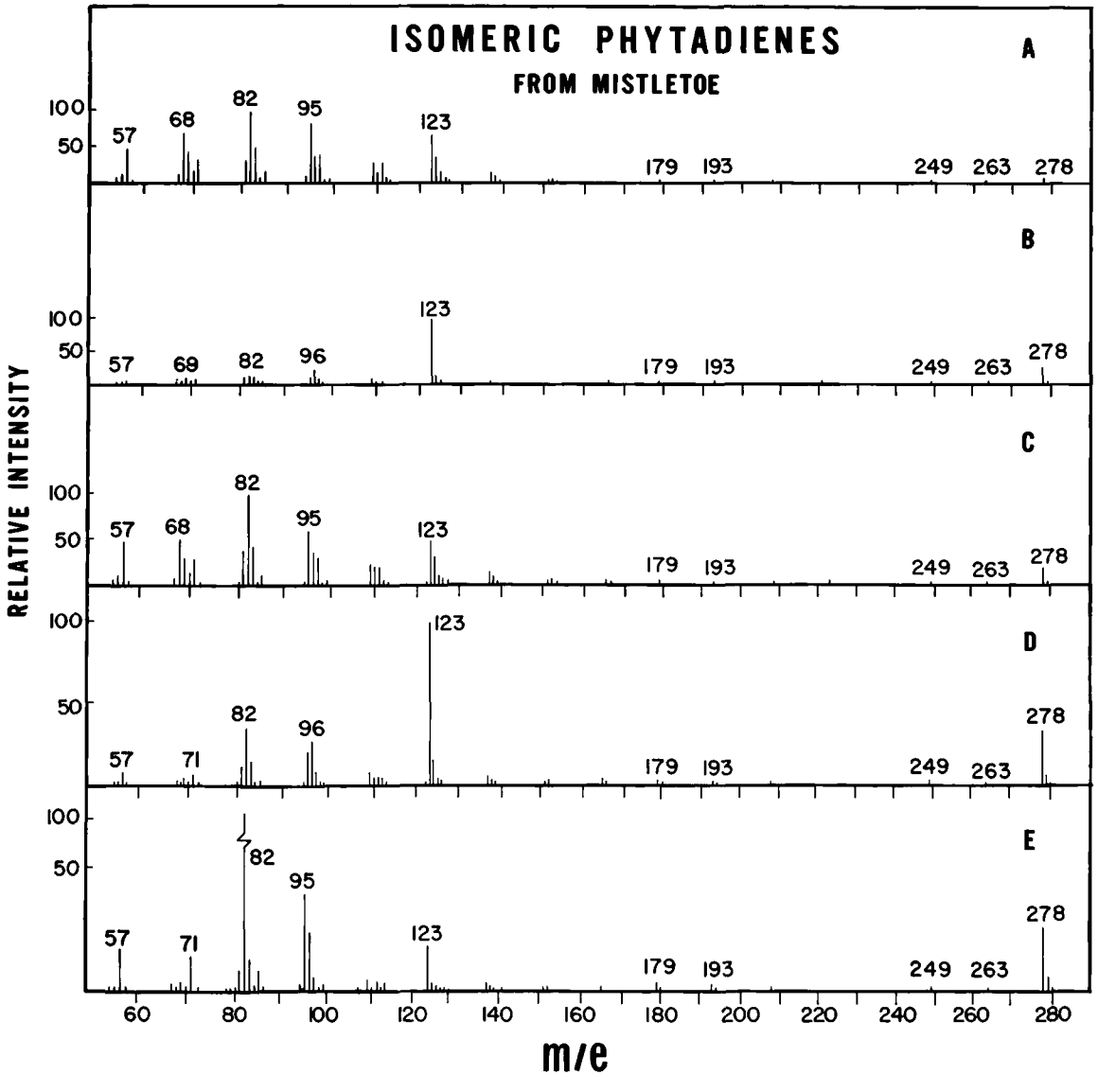
Nooner has also reported on the saturated hydrocarbons of mistletoe (348). The gas chromatographic pattern of the benzene eluate of this sample was found to be qualitatively identical to that obtained for the clover

FIGURE 41

MASS SPECTRA OF ISOMERIC PHYTADIENES

FROM MISTLETOE

Mass spectra taken as the compounds were eluted from a stainless steel capillary column coated with Polysev. LKB 9000 gas chromatograph-mass spectrometer. Electron energy, 70 eV.


ISOMERIC PHYTADIENES
FROM MISTLETOE
A
B
C
D
E
RELATIVE INTENSITY
100
50
57
68
82
95
123
179
193
249
263
278
69
96
71
60
80
100
120
140
160
180
200
220
240
260
280
m/e

phytadienes. The approximate relative ratios of the chromatographic peaks for the phytadienes A, B, C, D and E are 72, 1.9, 63, 100 and 80% respectively. The mass spectra of the five isomeric compounds are given in Figure 41 (see also Chapter IV).

d) Seeds

A number of important correlations were reported by Hilditch and Lovern (203) while comparing the composition of fat from various species against the phylogenetic scale based on morphological considerations. The occurrence of erucic acid was confined to the seeds of Cruciferae and that of petroselinic acid to the seeds of Umbelliferae (203). The data on the distribution of fatty acids in plant lipids, including a detailed review on seed fats, have appeared (434). Based on the occurrence of fatty acids in the seed fats, these have been classified into four groups (434). According to this classification, the seeds of Plantaginaceae belong to Group Ib, which includes seeds with linoleic-rich seed fats (434). In line with the previous reports, Mazliak has also shown that the seeds of the avocado are characterized by their high amounts of linoleic acid (313).

This laboratory has been interested in studying the distribution and biogenesis of hydrocarbons (365,367,371,482). Classical procedures of organic chemistry have proved to be

quite inadequate for the separation and analysis of these compounds and it is only during the last five years that methods of sufficient sensitivity such as gas chromatography and mass spectrometry have been used for this purpose. Therefore, our knowledge of their distribution in plants is limited. Although it is commonly accepted that one direct route to the plant hydrocarbons involves the decarboxylation of the corresponding alcohols or fatty acids with one carbon atom more (92), the results obtained from studies on lipid distributions from this (371,482) and other (313, p. 122) laboratories do not, in general, find the necessary correlations to support such mechanisms. As an extension of this work, the present report is concerned with the study of the distributions of fatty acids and alkanes in seeds of one species of plantage belonging to the family Plantaginaceae using the technique of gas chromatography and combined gas chromatography-mass spectrometry.

The husks of Plantarum ovata were obtained from a plantation in Phoenix, Arizona through the courtesy of Mr. M. C. Rosenthal, P. O. Box 6157, Phoenix, Arizona 85005.

The distribution patterns of hydrocarbons in the seed and the seed coat of P. ovata are shown in Figure 42A and 42B, respectively, and the relative percentages are reported in Table XXVI. The various peaks were identified by gas

FIGURE 42

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS FROM PLANTARUM OVATA

(A) Stainless steel capillary column (198 meters x 0.076 cm) coated with OV 17. Nitrogen pressure 1050 g/cm^2. No split. 'Barber Colman series 5000' gas chromatograph equipped with a flame ionization detector. Range XI; attenuation, 10. Temperature 160°C isothermal. Symbols: n=normal; i=iso; ai=anteiso. Relative percentage of hydrocarbons in each peak is reported in Table XXVI.

(B) Same conditions as above.

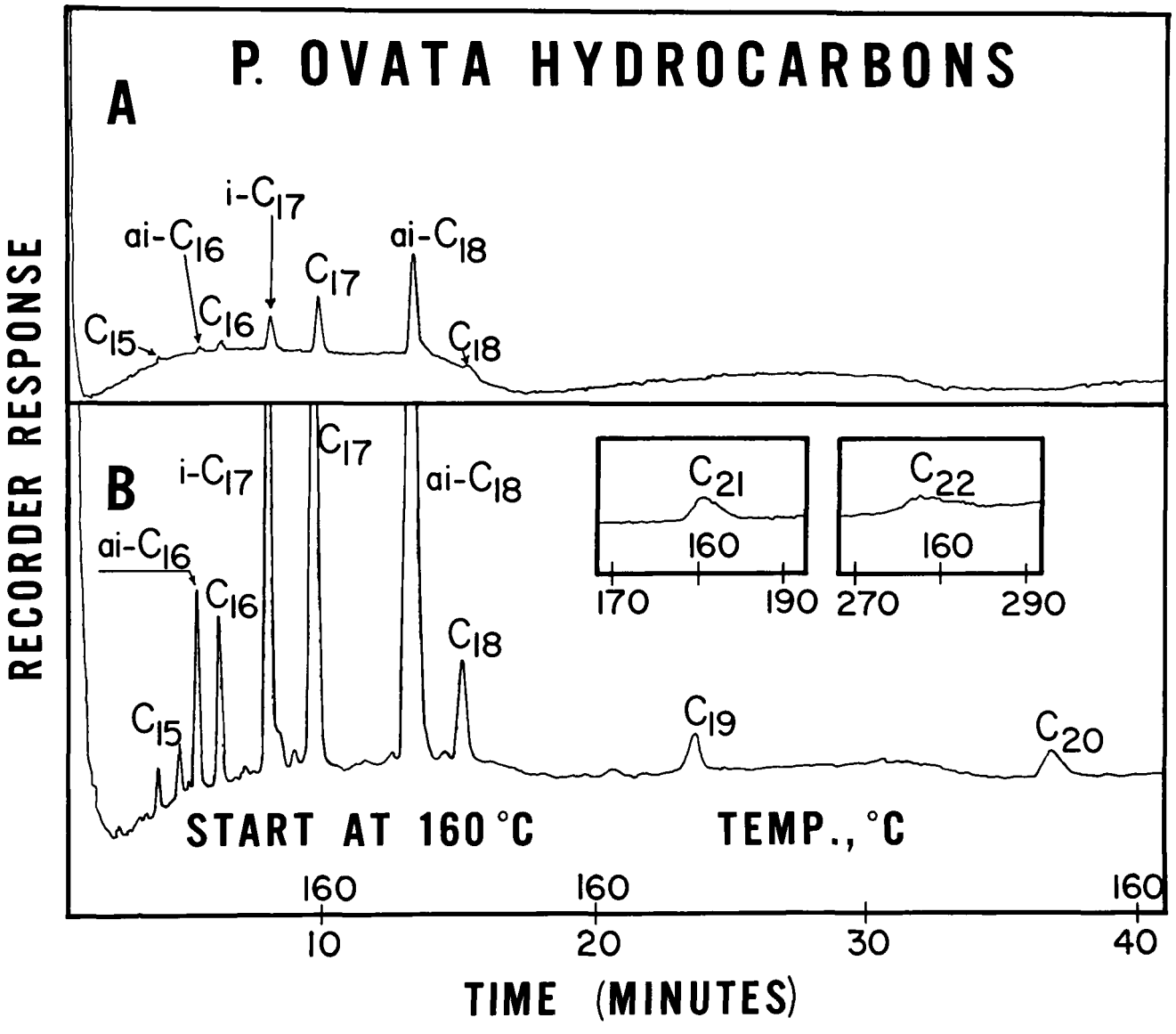

P. OVATA HYDROCARBONS
A
i-C17
ai-C16
C17
ai-C18
C15
C16
C18
B
i-C17
C17
ai-C18
C21
C22
160
160
170
190
270
290
ai-C16
C16
C18
C15
C19
C20
START AT 160 °C
TEMP., °C
160
160
160
10
20
30
40
TIME (MINUTES)
RECORDER RESPONSE

TABLE XXVI

RELATIVE PERCENTAGE COMPOSITION OF HYDROCARBONS

IN THE SEED COAT OF PLANTARUM OVATA

Hydrocarbon*	Content %
$i\text{-}C_{16}$	<1
$ai\text{-}C_{16}$	2.9
$n\text{-}C_{16}$	2.7
$i\text{-}C_{17}$	9.9
$n\text{-}C_{17}$	25.9
$i\text{-}C_{18}$	1
$ai\text{-}C_{18}$	42.8
$n\text{-}C_{18}$	<1
$n\text{-}C_{19}$	<1

* The hydrocarbons were identified by their gas chromatographic retention times and by their respective mass spectrometric fragmentation patterns. Symbols: n = normal; i = iso; ai = anteiso. The percent composition of the hydrocarbons was calculated on the basis of their gas chromatographic area, which was obtained by multiplying the peak heights by the widths at half peak heights. The total hydrocarbon content was 6.7 ppm.

chromatography and mass spectrometry (Figure 43). The results are significant in that previous reports on the nature of hydrocarbons in most plant sources show the presence of alkanes with carbon numbers between C-25 and C-35 (140), while the seed coat and the seeds of P. ovata showed the presence of C-16 to C-22 hydrocarbons. Anteiso-C_{18} was the major component (42.8%, followed in decreasing amounts by n-C_{17} (25.9%); iso C_{17} (9.9%); anteiso-C_{16} (2.9%); and n-C_{16} (2.7%). Smaller amounts of n-C_{18} through n-C_{22} hydrocarbons and traces of iso C_{16} and iso C_{18} were also detected. The mass spectrometric patterns of the iso C_{17} and anteiso C_{18} alkanes are shown in Figure 43.

The seeds contain a much smaller amount of alkanes as compared to the seed coat (Figures 42A and 42B) but the pattern is essentially the same. The distribution pattern of fatty acids in the seed coat of P. ovata are shown in Figure 44, and its relative percentages are reported in Table XXVII. The total hydrocarbon content of this sample (6.7 ppm) is extremely low as compared to the amount of fatty acids (2610 ppm).

Plantago ovata is an annual caulescent herb native to Asia and the Mediterranean countries. This plant is extensively cultivated in India and of late in Arizona, U.S.A.

Although, in general, the overall distribution pattern of its seed hydrocarbons (see Figure 42) agrees with the

FIGURE 43

MASS SPECTRA OF ISO AND ANTEISO

ALKANES FROM PLANTARUM OVATA

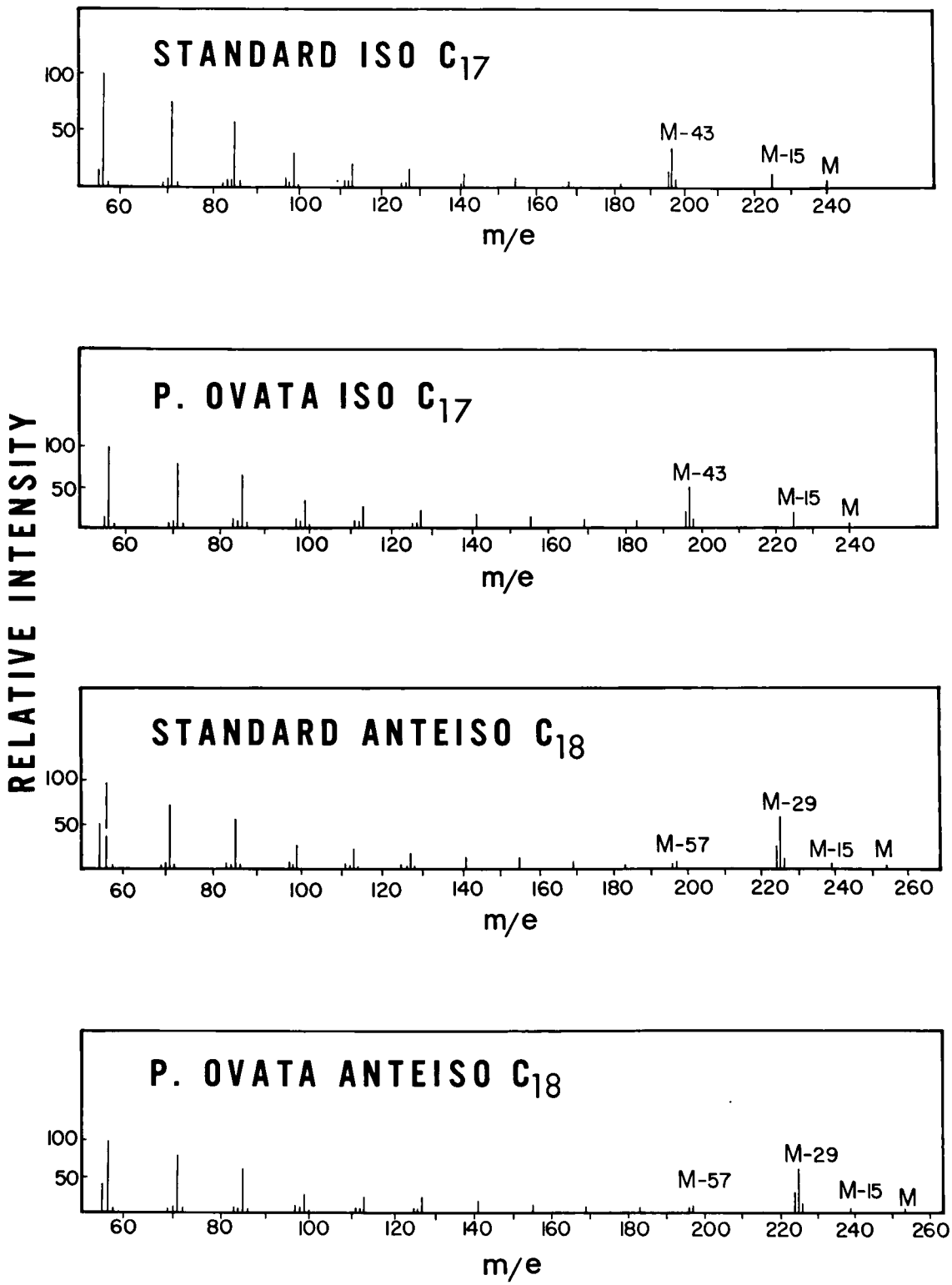

STANDARD ISO C_{17}
P. OVATA ISO C_{17}
STANDARD ANTEISO C_{18}
P. OVATA ANTEISO C_{18}
RELATIVE INTENSITY
100
50
M-43
M-15
M
M-29
M-57
60
80
100
120
140
160
180
200
220
240
260
m/e

FIGURE 44

GAS CHROMATOGRAPHIC SEPARATION OF THE FATTY ACIDS FROM PLANTARUM OVATA

Stainless steel capillary column (195 m long by 0.076 cm i.d.) coated with Igepal Co-880. 'Barber Colman series 5000' gas chromatograph. Nitrogen pressure 733 g/cm^2. No split. Range X5; attenuation, 10. Temperature 200^{o}C isothermal. Relative percentage of fatty acid methyl ester in each peak is reported in Table XXVII.

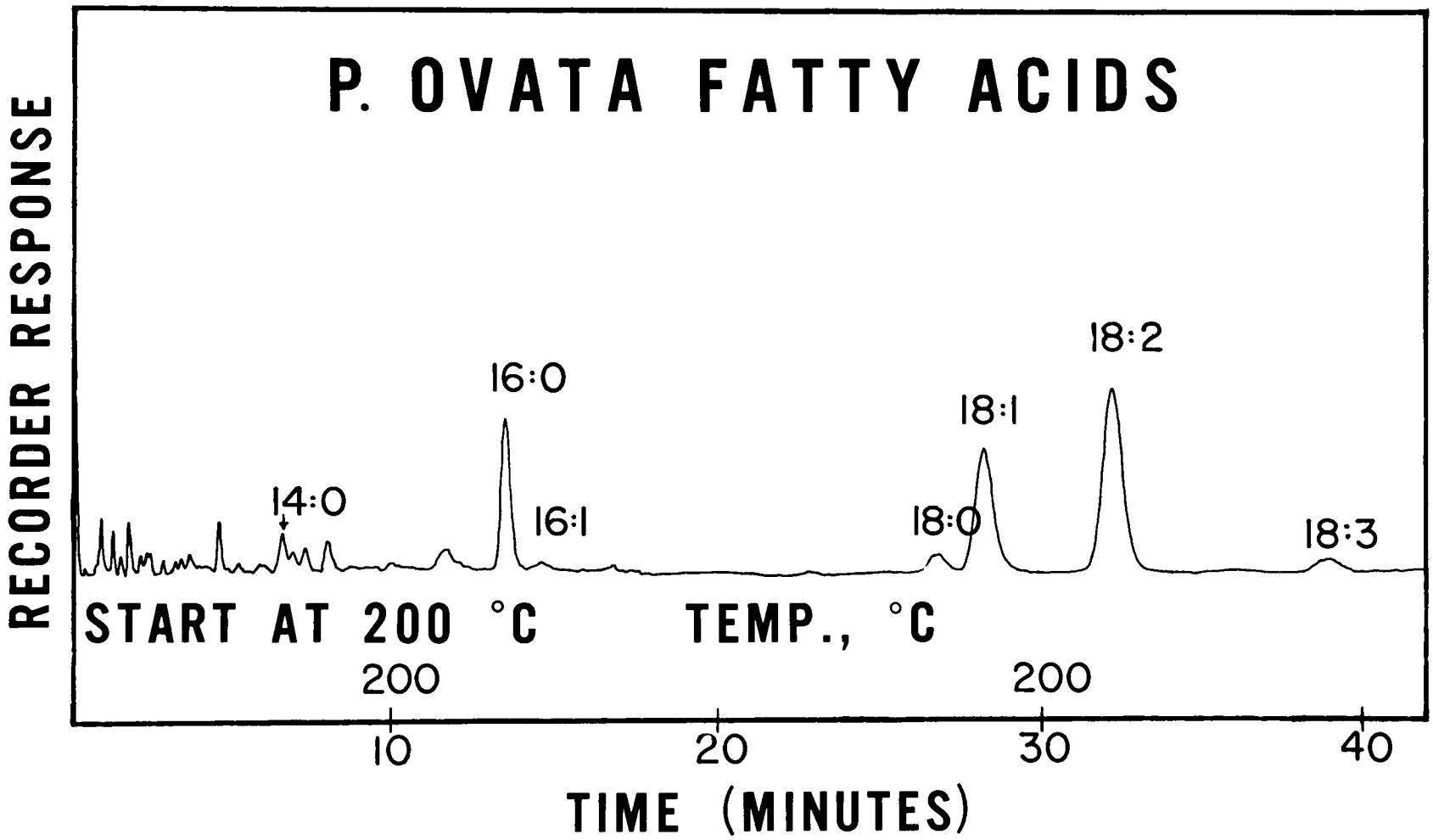

P. OVATA FATTY ACIDS
RECORDER RESPONSE
14:0
16:0
16:1
18:0
18:1
18:2
18:3
START AT 200 °C
TEMP., °C
200
200
10
20
30
40
TIME (MINUTES)

TABLE XXVII

RELATIVE PERCENTAGE COMPOSITION OF FATTY ACIDS

IN THE SEED COAT OF PLANTARUM OVATA

Fatty Acid*	Content %
Myristic	2.2
Palmitic	10.9
Stearic	2.5
Oleic	16.0
Linoleic	26.3
Linolenic	3.0

* The fatty acids were identified as methyl esters by mass spectrometry and by comparing their retention time with that of authentic samples. The percent composition of the fatty acids was calculated on the basis of their gas chromatographic areas which were obtained by multiplying the peak heights by the widths at half peak heights. The total lipid content of the seed coat was 2.5% while the methyl esters of fatty acids were 2610 ppm.

expected predominance of the odd numbered n-alkanes which is common in plant sources (140), it is remarkable that while higher plants contain only traces of low molecular weight hydrocarbons the bulk of these P. ovata seed alkanes is concentrated around heptadecane. This particular distribution mode has been found to be a characteristic of lower plants such as green algae (99,171,192,371). An alternating pattern in the concentrations of the iso and anteiso components has been reported by Mold, et al. (329,330) in wool and tobacco wax. According to their results they found that the iso homologs with the odd number of carbon atoms predominated over the corresponding anteiso compounds and that the anteiso homologs with even number of carbons predominated over the corresponding isoalkanes; this is also the case here as indicated in Table XXVI. Perhaps the most unusual feature of the pattern shown in Figure 42 is the high amount of the anteiso C_{18} (almost twice as much as the amount of n-C_{17} alkane). Although a similar situation was reported by Eglington, et al. for the high molecular weight alkanes (C_{25}-C_{35}) in the surface wax of the leaves of certain species of Aconium (139), this appears to be the first case in which such a predominance has been found i) in the low molecular weight (C_{16}-C_{19}) range and ii) for an anteiso alkane.

It is most probable that one route to the biosynthesis of plant hydrocarbons involves decarboxylation of the corresponding

long chain fatty acids or their immediate precursors (140). Mazliak (312) reported that non-crystalline fraction of the apple cuticle wax contains fatty acids ranging from C_{16} to C_{30}, and alkanes ranging from C_{15} to C_{29}. In the present study the results show that one of the pathways for the hydrocarbon synthesis may be the decarboxylation route because C_{18} fatty acids and C_{17} hydrocarbons are both present in significant concentrations. On the other hand, the presence of anteiso-C_{18} alkane (42.8%) can not be explained on this assumption because the P. ovata seed coat does not contain branched C_{19} fatty acids. Presumably other mechanisms, viz., condensation on the elongation route, which have been discussed by Eglington and Hamilton, or some other possible mechanism may be involved. This aspect of the study deserves further investigations using tracer metabolites as precursors of hydrocarbon biogenesis.

e) Fungal spores

As a part of the studies by Laseter (260,363) on the paraffinic hydrocarbon content of chlamydospores, the n-heptane eluate of Sphacelotheca reiliana (see references 260 and 363 for experimental details) was analyzed by gas chromatography-mass spectrometry using the same system as for the hydrocarbons of cabbage. The range of hydrocarbons identified from their mass spectra goes from the n-C_{23} to a branched C_{36}. The high mass end portion of the mass spectrum

of the major peak is presented in Table XXVIII. The peaks immediately preceeding each of the n-alkanes identified in this sample have been found to be the a mixture of the corresponding mono- and di-olefins (each with the same carbon number as the alkane that they precede). After the n-C_{29} peak the branched alkanes predominate up to C_{36}. Two of these branched compounds were identified as the anteiso C_{30} and anteiso C_{32}. Others show a high degree of branching which difficulted the determination of their molecular masses. No definite identifications were obtained in these cases.

B. Products of Biological Origin

1. Hydrocarbons and Fatty Acids in Shark Liver Oil

In general, fish liver oils have a very low content of unsaponifiable matter (1-2%) and relatively large amounts of unsaturated fatty acids (≈50%) of which the main components are highly unsaturated eicosenoic and docosenoic acids (205).

On the other hand the unsaponifiable fraction in the marine animal oil extracted from the liver fats of Elasmobranchii species ranges from 0.3 up to 80% of the lipid content being in this case of marked monoethenoid character. The higher content of unsaponifiable matter is found within the squalidae family where it ranges from about 33 to 80% (203,236).

Although not always present (90,202) and randomly distributed among the shark families, squalene, whenever found,

TABLE XXVIII

PARTIAL MASS SPECTRUM OF A HIGH MOLECULAR WEIGHT ALKANE

	Mass Series (Z in C_nH_{2n+Z})		
n \ Z	0	+1	+2
21	11	29	8
22	10	27	7
23	8.5	25	7
24	7	23	6
25	6	20	6
26	4	12.5	4
27	2	8	3
28	-	-	-
29	-	-	27(M)

seems to be the major component of the hydrocarbon fraction (90,91,200,202,490,492). It has been reported that the liver oil of a Formosan shark contains as much as 87.5% of unsaponifiable matter, of which 84% was mainly squalene (200). Smaller proportions of pristane (48,445,446,447,484,491) and zamene (48,53,94,203,446,447,494) have also been reported.

Because of current interest in the distribution and formation of isoprenoid hydrocarbons and since there are no mass spectrometric data on the composition of shark liver oil, the hydrocarbons from the unsaponifiable fraction of a common species of shark have been analyzed by the new technique of combined gas capillary chromatography-mass spectrometry (see Chapter III). At the same time, in order to gain some understanding of the formation of these hydrocarbons the fatty acid fraction has also been analyzed with the same technique.

One gallon of Basking shark liver oil from the South American coast was obtained from Aristo Oil Products Co., and a 45.2 mg sample was taken for analysis. The sample was fractionated by means of silica gel column chromatography following the techniques described before (Chapter III). Three fractions were collected of which the methanol fraction containing the fatty acids and other lipids and the n-pentane fraction containing the aliphatic hydrocarbons were analyzed by both gas chromatography and gas chromatography-mass spectrometry. The methyl esters of the total fatty acids

liberated by alkaline hydrolysis were prepared for gas chromatographic analysis according to the method described previously (see also Chapter III). The benzene fraction was analyzed by gas chromatography for its aromatic content but no compounds other than squalene were found in appreciable concentrations.

Because it was not possible to collect all of the unsaponifiable matter in only one fraction, and in order to show all of the aliphatic hydrocarbons in one single chromatogram, a portion of the shark oil was analyzed without any previous fractionation.

The sample of squalene used as a standard was obtained from Eastman Organic Chemicals.

Monoethenoid fatty acids of even carbon number from C_{18} to C_{22} together with the saturated n-C_{16} fatty acid were observed as the major components of the lipid fraction (see Figure 45). The identities of these fatty acids are based on their GC retention times and on the GC mass spectrometric analysis (Figure 46). Their spectra were identical to those described in the literature (417,482). All components of the methanol fraction identified by these techniques are normal unbranched fatty acids. The total fatty acid content in the sample analyzed is found to be of the order of 15.3 mg which represents a 33.8% of the shark liver oil.

FIGURE 45

GAS CHROMATOGRAPHIC SEPARATION OF FATTY ACIDS

OF BASKING SHARK LIVER OIL

Stainless steel tubing (150 m x 0.076 cm) coated with Igepal Co 990. Nitrogen pressure 1,400 g/cm^2, no split. 'F and M Model 810' Gas Chromatograph equipped with a flame ionization detector. Range, 10^2; attenuation, 1. Temperature programmed at $6^{\circ}C$ per minute from 125° to $200^{\circ}C$. About 1.5/30 of the sample was injected.

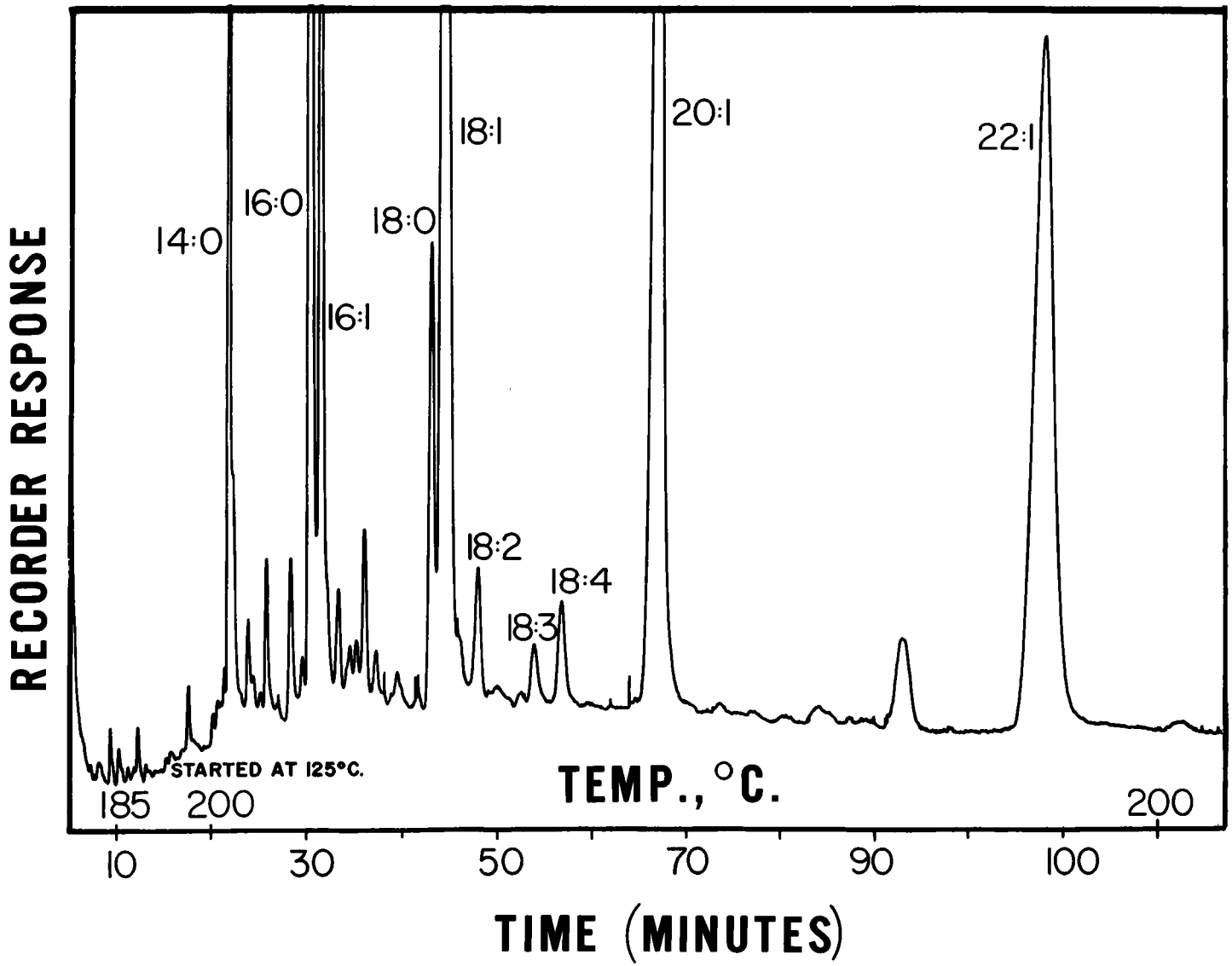
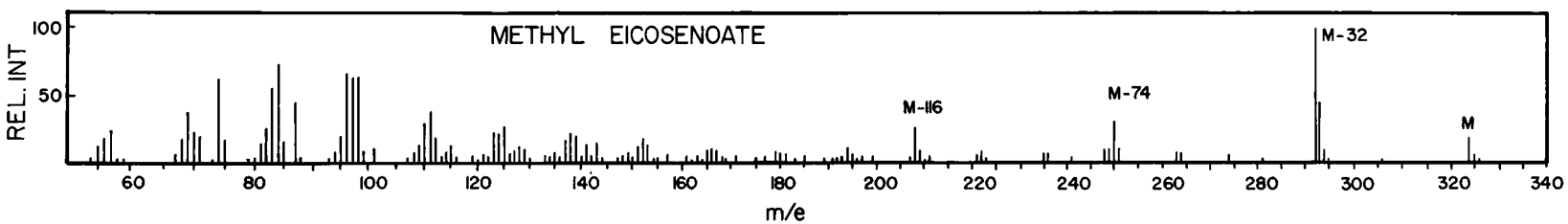

RECORDER RESPONSE
14:0
16:0
16:1
18:0
18:1
18:2
18:3
18:4
20:1
22:1
STARTED AT 125°C.
185 200
TEMP., °C.
200
10
30
50
70
90
100
TIME (MINUTES)

FIGURE 46

MASS SPECTRUM OF METHYL EICOSENOATE

IN BASKING SHARK LIVER OIL

Taken as it was eluted from a 90 m x long 0.076 cm i.d. stainless steel capillary tubing coated with Polysev. The energy of ionization was set at 20 eV and the accelerating voltage at 3.5 KV. The electron multiplier voltage was 2.5 KV. The temperature of the ion source 290^{o}C.


METHYL EICOSENOATE
REL. INT
100
50
M-116
M-74
M-32
M
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
m/e

The qualitative and quantitative results given in Table XXIX agree quite well that those previously reported for this shark species (see Table XXX) except for the fact that in the present work, the C_{20} saturated and the C_{24} unsaturated fatty acids have not been found and that there seems to be a considerable deviation on the concentration of the unsaturated C_{18} fatty acid. It may be pointed out that the distribution found closely resembles also that reported for the small spotted dogfish (277).

The non-saponifiable hydrocarbon fraction consists of only two major peaks which account for about 38.6% of the total oil. Quantitative data on this fraction was obtained from 18.0 mg of whole Basking shark liver oil by analyzing directly a small aliquot on a stainless steel column coated with OV-17 (Figure 47). The first peak in the chromatogram which represents a 7.6% of the total amount of oil has the same retention time as pristane. Its identity was verified by means of the retention data on four different stationary phases (Polysev, Apiezon L, OV-17 and OV-1) and by use of an internal standard of known concentration. Final proof of the structure was obtained from its mass spectrum which corresponds to that of pristane as shown on Figure 48 (31,32, 169).

It is important to mention at this point that the application of low electron energy mass spectrometry (12-20 eV)

TABLE XXIX

RELATIVE PERCENT COMPOSITION OF THE BASKING SHARK LIVER OIL FATTY ACIDS

14:0	5.5
16:0	20.3
16:1	6.8
18:0	3.1
18:1	18.73
18:2	0.6
18:3	0.3
18:4	0.8
20:1	18.76
22:1	18.4

TABLE XXX

BASKING SHARK FATTY ACIDS

Previously Reported		(277)[a]	Found[b]
Saturated	C_{14}	2.1	5.5
	C_{16}	13.6	20.3
	C_{18}	3.2	3.1
	C_{20}	3.6	
Unsaturated	C_{14}	0.5	
	C_{16}	11.9	6.8
	C_{18}	12.8	20.43
	C_{20}	23.2	18.76
	C_{22}	20.0	18.4
	C_{24}	5.6	

[a] Oils from South African coast

[b] Oils from South American coast

FIGURE 47

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS OF BASKING SHARK LIVER OIL

Stainless steel tubing (150 m x 0.05 cm i.d.) coated with OV-17. Nitrogen pressure 1050 g/cm^2, no split. Barber Colman series 5000 gas chromatograph equipped with a flame ionization detector. Range x 5; attenuation, 10. Temperature programmed at $2^{\circ}C$ per minute from 150° to $250^{\circ}C$. 1/1000 of the sample injected. Pristane: 2,6,10,14-Tetramethyl pentadecane Squalene: 2,6,10,15,19,23-Hexamethyl tetracosa-2,6,10,14,18,22-hexaene.

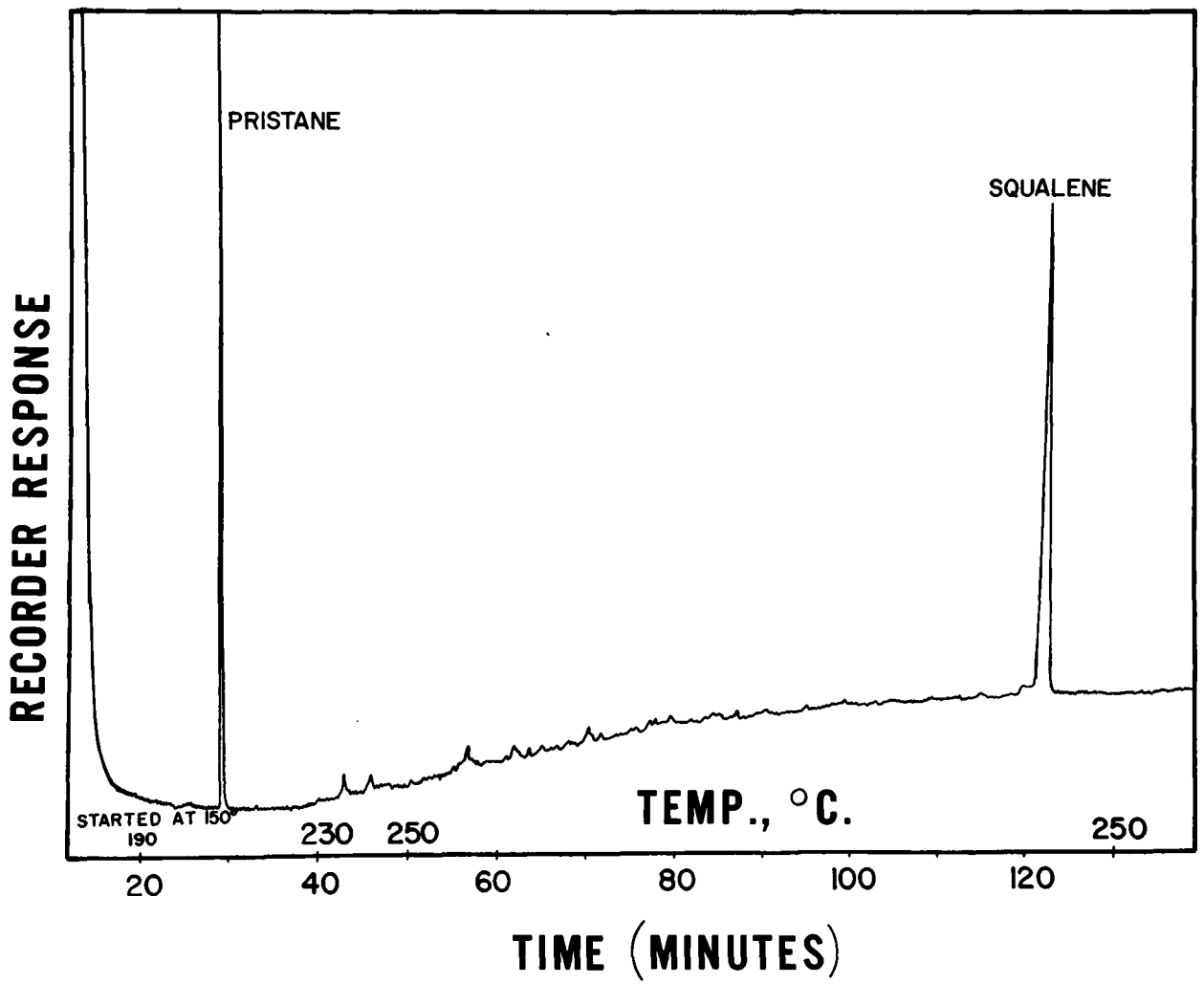
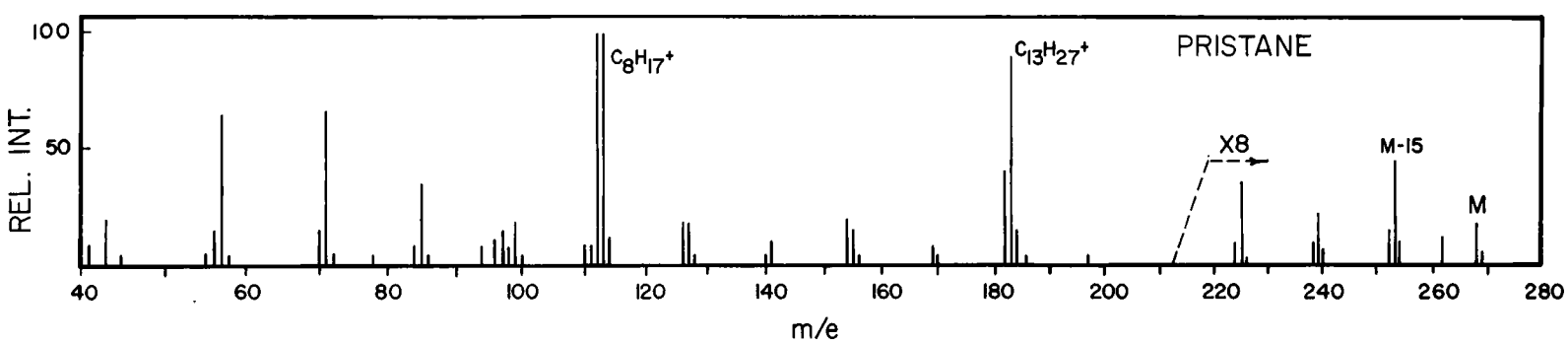

PRISTANE
SQUALENE
RECORDER RESPONSE
STARTED AT 150°
190
230
250
TEMP., °C.
250
20
40
60
80
100
120
TIME (MINUTES)

FIGURE 48

MASS SPECTRUM OF BASKING SHARK LIVER OIL PRISTANE

It was taken as it was eluted from a 2 m x 2.5 mm i.d. glass column coated with SE-30 and it was ionized by electron impact at 20 eV when it entered the ion source of the LKB 9000 gas chromatograph-mass spectrometer.


PRISTANE
$C_8H_{17}^+$
$C_{13}H_{27}^+$
X8
M-15
M
REL. INT.
100
50
40
60
80
100
120
140
160
180
200
220
240
260
280
m/e

provides a remarkable enhancement in the intensities of the characteristic fragment ions at m/e 113 (base peak) and m/e 183, as well as for that of the parent ion (m/e 268). While at high electron energies the parent ion of pristane can have a relative intensity as low as 0.2% of the base peak (m/e 57) (31,169), it is shown here with an intensity close to 2.5% of the base peak at 20 eV. For a more detailed discussion on the effect of low energy mass spectrometry see Chapter IV.

The first analysis that was carried out on this fraction showed a small peak eluted after pristane which appeared to be one of the isomeric C_{19} olefins but the mass spectrum is somewhat unreliable in this case due to the very small amount in which it was present. Its presence here would not seem surprising especially since the isomeric C_{19} olefins, known by the collective name of "Zamene" have been found in several marine organisms including the Basking shark (48,53). The fact that upon standing this peak disappeared would also be in favor of the tentative mass spectrometric identification, since by reduction, it would be transformed into pristane (484,494). It is known that this kind of oils have a great tendency towards saturation or hydrogenation (277).

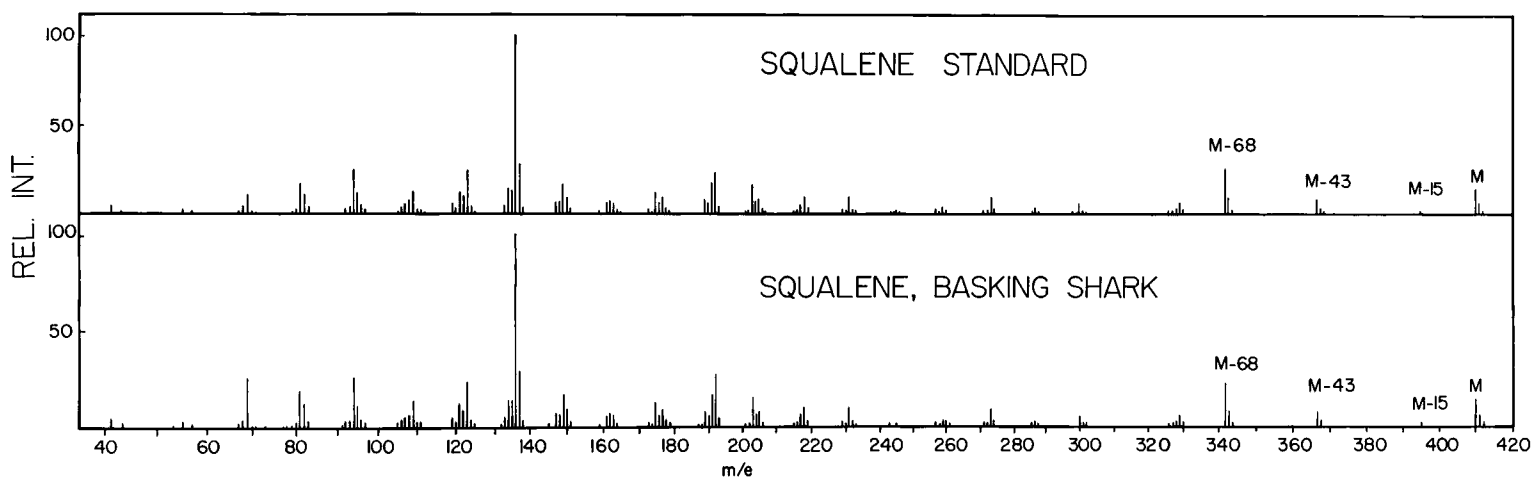
The gas chromatographic retention data (Figure 47) together with the mass spectrum (Figure 49) obtained from the second major component in Figure 47 confirmed the presence of squalene in Basking shark liver oil. This component represents

FIGURE 49

MASS SPECTRA OF SQUALENE

Squalene standard: Spectrum taken as the sample was eluted at 270°C from a short glass column packed with SE-30. It was ionized by electron impact in the ion source of an LKB 9000 gas chromatograph-mass spectrometer at 70 eV. The ionizing current was set at 65 μA and the accelerating voltage at 3.5 KV. The electron multiplier voltage was 2.5 KV.

Basking shark liver oil squalene: Spectrum taken under exactly the same conditions as those described for the standard. About 2/1000 of the sample at a concentration of 14.1 mg/ml, was injected.


SQUALENE STANDARD
SQUALENE, BASKING SHARK
REL. INT.
100
50
M-68
M-43
M-15
M
m/e
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420

31.5% of the oil.

In line with these results, the thin-layer chromatograms show compounds with R_F values corresponding to hydrocarbons and glycerides (the sample does not contain free fatty acids). In addition, they indicate the possible presence of cholesterol in low amounts and some fatty alcohols as previously reported (203).

Phytane was not detected in this oil. This apparent absence is in contrast with the fact that when the C_{19} isoprenoid is found in sediments (112,231,365,369,402) and petroleum (31,32) it is usually accompanied by the C_{20} and other isoprenoids. There is only one report of the presence of pristane in a sediment and the absence of phytane (51). Therefore it becomes of interest to analyze commercial pristane derived by distillation from Basking shark liver oil in order to determine the possible presence and the nature of other components existing in trace amounts, even though it was realized that the thermal treatment (distillation) may have partially modified the initial composition of the oil. The results showing the presence of phytane among other components are presented here.

2. "Robuoy" Pristane

A sample of pristane isolated as a by-product from Basking shark liver oil (406) with the commercial name of Robuoy was obtained from Robeco Chemicals, Inc., New York.

Direct gas chromatographic analysis indicated the presence of phytane in a concentration of the order of 1% of the total amount of pristane in the sample (Figure 50), which in turn accounts for almost 99% of the whole product.

In order to increase the concentration of phytane and the other minor components in the sample (see Figure 50), a fractionation by distillation was carried out. A spinning band column was used for the distillation of about 600 ml of the original product and a total of nine cuts were collected within the temperature range of the initial boiling point and 171.5^{o}C (Table XXXI).

The first cuts represented fractions enriched in the light weight materials with pristane as the major component as shown by gas chromatographic analysis. For instance cut No. 1 was run on a 300 m x 0.076 cm i.d. stainless steel column coated with SF-96 (a methyl silicone oil), at 202^{o}C. The gas chromatograph showed only two peaks with retention times corresponding to pentadecane and pristane.

The cuts of most interest were the last fractions. Both cuts Nos. 7 and 8 show a significant increase in the relative concentration of phytane, which allowed its mass spectrometric identification as well as a preliminary measurement of its optical activity.

To get a complete picture of the composition of the phytane containing sample, one of these distillation cuts (No. 8) was

FIGURE 50

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS OF "ROBUOY PRISTANE"

Stainless steel capillary column (300 m x 0.076 cm i.d.) coated with Polysev. Barber Colman 5000 apparatus equipped with a flame ionization detector. Hydrogen pressure 1400 g/cm^2. Isothermal at 170°C. About 0.2 µl of the original sample was injected.

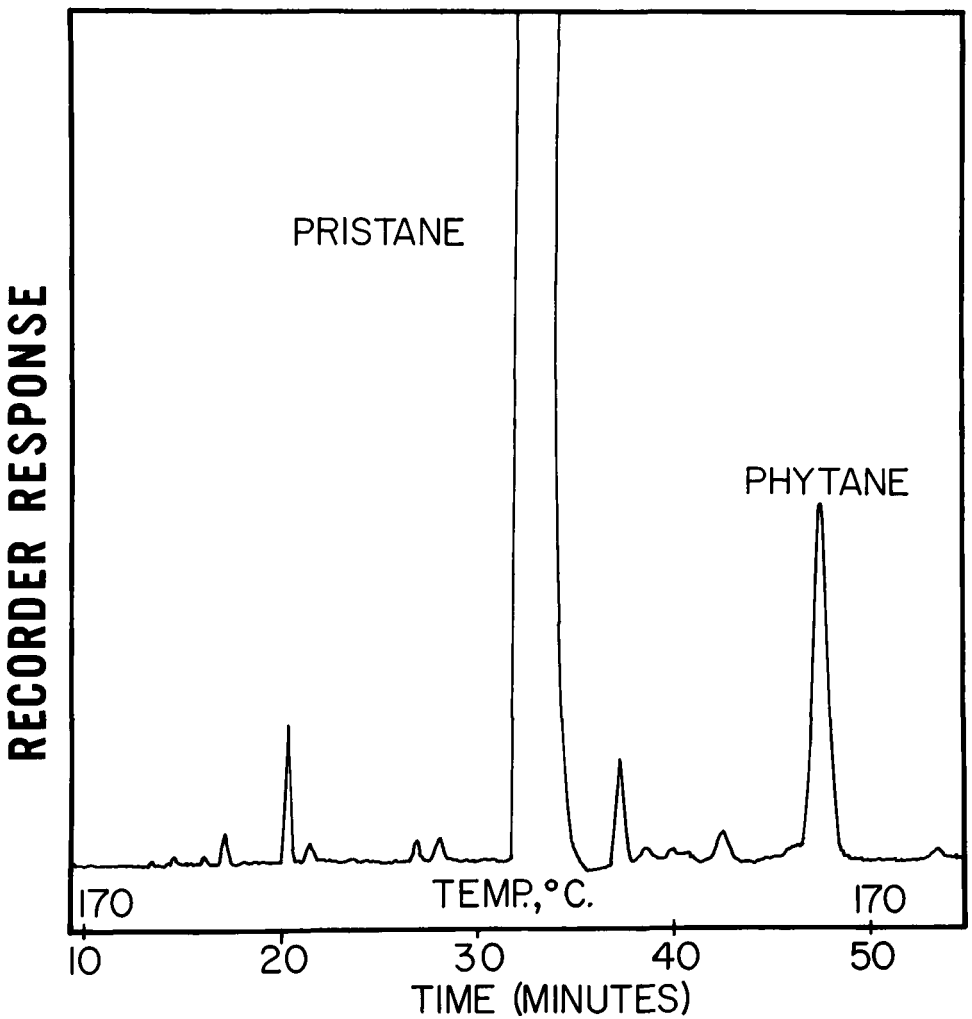

RECORDER RESPONSE
PRISTANE
PHYTANE
170
TEMP.,°C.
170
10
20
30
40
50
TIME (MINUTES)

TABLE XXXI

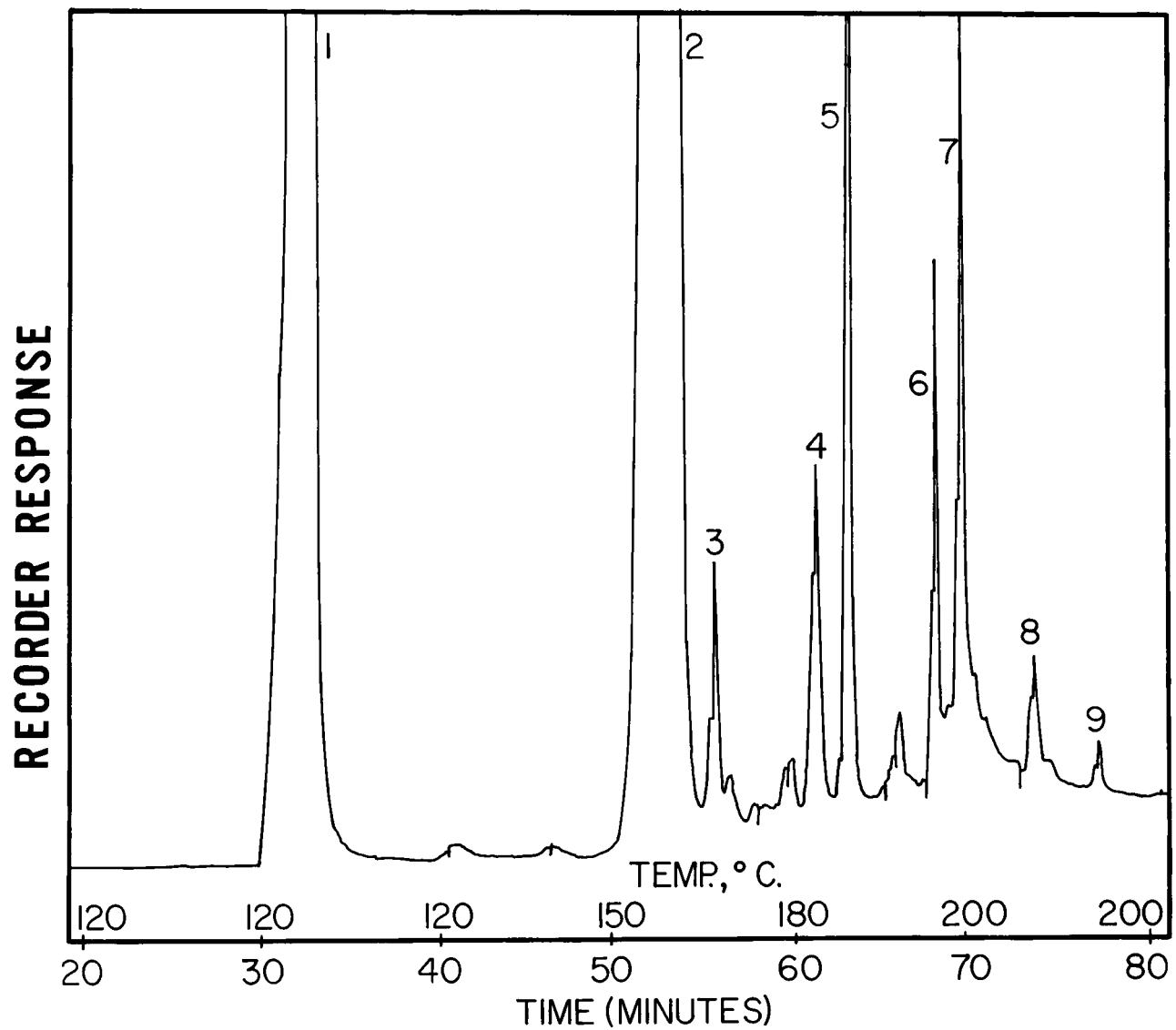
DISTILLATION CUTS FROM A COMMERCIAL SAMPLE OF PRISTANE

Cut No.	Ml. Off	Vapor Temperature @ 10 mm Hg	Liq. Vol. %
1	1.4	IBP - 162.7°C	.24
2	3.6	162.7 - 165°C	.26
3	17.4	165 - 167.1°C	2.98
4A	156.0	167.1 - 168.7°C	26.77
4B	274.0	168.7 - 168.7°C	47.03
4C	115.0	168.7 - 168.7°C	19.73
5	5.2	No temp. reading	.89
6	2.7	No temp. reading	.46
7	2.7	No temp. reading	.46
8	1.8	No temp. reading	.31
9	3.0	171 - 171.5°C	.51

FIGURE 51

GAS CHROMATOGRAPHIC SEPARATION OF THE COMPONENTS OF A DISTILLATION CUT OF ROBUOY PRISTANE

Stainless steel capillary column (300 m x 0.076 cm i.d.) coated with Polysev (m-bis-m-(phenoxy-phenoxy)-phenoxy benzene) using an LKB 9000 gas chromatograph-mass spectrometer. Helium pressure 1050 g/cm^2. Isothermal at 120°C for 40 minutes then programmed at 3°C per minute to 200°C. The steps or lines indicate the points where mass spectra were taken. The chemical nature of the numbered peaks is given in Table XXXII.


RECORDER RESPONSE
1
2
3
4
5
6
7
8
9
TEMP., ° C.
120
120
120
150
180
200
200
20
30
40
50
60
70
80
TIME (MINUTES)

analyzed by gas chromatography-mass spectrometry (Figure 51). The identities of each of the components according to their mass spectral pattern are given on Table XXXII.

In this cut, pristane (peak 1) represents a 28.6% of the total amount collected, while phytane (peak 2) amounts to about 55.4%. The mass spectrum of phytane is given in Figure 52 (32). According to this figure, a 0.17% of the total amount of phytane in the commercial product was present in this particular distillation cut.

The spectra of the peaks labelled as 6 and 7 in the gas chromatogram each gave a complex pattern corresponding to a mixture of at least two different compounds. Peak 7 is most likely a mixture of a fatty acid ester, possibly a C_{14} fatty acid and a C_{20} hydrocarbon. Their complete identification has not been pursued further.

The optical activity of cut No. 7 was measured by means of a Rudolf Polarimeter. Two blanks of n-heptane were used as reference. Only a very weak specific rotation of about $+1.1^{\circ}$ was observed at 200-220 mμ and $20^{\circ}C$.

There seems to be no direct correlation between the fatty acids of the Basking shark liver oil and the hydrocarbons. The identities, nature and concentrations of the fatty acids as well as those of the unsaponifiable matter are well within the range expected for the natural abundance of those compounds in the liver oils of this marine organism.

TABLE XXXII

IDENTITIES AND RELATIVE PERCENTAGE OF THE COMPONENTS OF DISTILLATION CUT #8

1.	Pristane	28.4
2.	Phytane	55.4
3.	Octadecane	0.9
4.	3,6,10,13-Tetramethyl heptadecane	1.6
5.	Nonadecane	3.2
6.	C_{20} Hydrocarbon*	1.4
7.	C_{20} Hydrocarbon and fatty acid ester*	2.8
8.	Methyl n-hexadecanoate	0.4
9.	Ethyl n-hexadecanoate	0.1

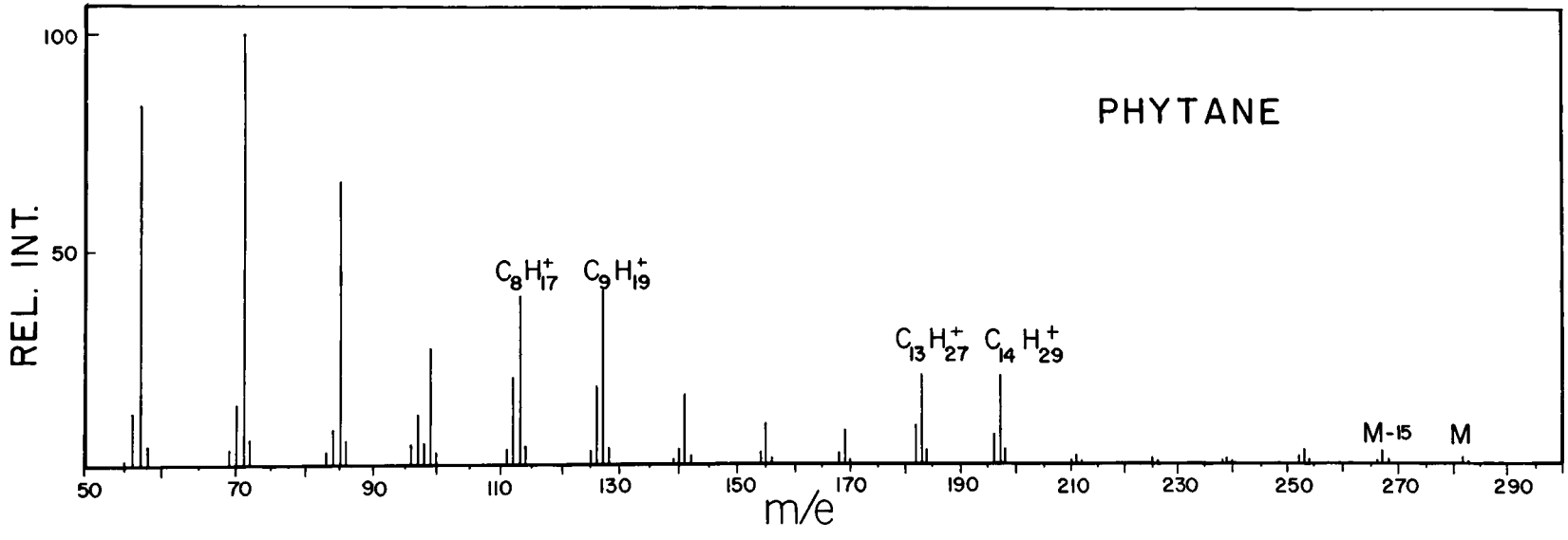
* Tentative identification

FIGURE 52

MASS SPECTRUM OF PHYTANE FROM

ROBUOY PRISTANE

Taken as the component was eluted from the GC column under the conditions described in Figure 51. (See peak 2 in Figure 51). It was ionized by electron impact at 20 electron volts. The ion source temperature was 290^{o}C. The ionizing current 70 μA and the accelerating voltage 3.5 KV.


PHYTANE
REL. INT.
100
50
m/e
50
70
90
110
130
150
170
190
210
230
250
270
290
$C_8H_{17}^+$
$C_9H_{19}^+$
$C_{13}H_{27}^+$
$C_{14}H_{29}^+$
M-15
M

The relatively small differences found in the concentrations of the fatty acid and unsaponifiable components when compared to other reported data can be explained by natural variations between species and by different diets and environmental conditions which affect the formation of these oils.

Phytanic acid, a possible biogenic precursor of pristane, has not been found among the fatty acids--supporting the view that sharks do not synthesize but rather accumulate this hydrocarbon (48,50,203).

It appears that pristane together with the small amounts of the unsaturated isoprenoid C_{19} isomer comes from the marine organisms on which the sharks feed (50,53,203), such as the zoo-plankton. Indeed, it has been reported that copepods are the immediate source of pristane in the liver oils of sharks and whales (48,50). The isomeric monoolefins with the skeleton of pristane are present too in marine zoo-plankton, fishes and mammals (53).

The concentration of both isoprenoids, pristane and phytane, in the shark oil products analyzed is remarkably high in both cases. Pristane has been found before only as a minor constituent in this type of liver oils (48,203,484), and phytane is considered to be absent both in zooplankton (48,50, 53) and in Basking shark oil (48, this report).

The primary source of pristane is presumed to be the phytyl group of the chlorophyl from marine photo-synthetic

microorganisms (48,50,53,203). Furthermore, it is known that phytol can be easily dehydrated to phytenes and phytadienes, both of which by hydrogenation could yield phytane (32,48). For this reason the presence of phytane as a minor component in samples obtained from marine animals should not be totally unexpected.

Taking into consideration that the search for phytane has yielded negative results in raw oils, its presence in a commercial distillation product of Basking shark liver oil could be accounted for by different sources of the oils or by the possibility that phytane is generated from certain precursors in the shark oil during the distillation process used for the preparation of commercial pristane.

Although the necessary precautions were taken, the quantitative data presented here should be considered as a lower limit in all cases due to the possible losses by volatilization suffered during the preparation and analysis of the sample.

The non-saponifiable matter and the total fatty acid content of the oil analyzed represent a 72.7% of the total. This leaves a maximum 27.3% to be accounted for which most likely is made up by cholesterol, fatty alcohols and glyceryl ethers.

These compounds are known to be minor constituents of Elasmobranchii liver oils (203,205).

No attempts were made towards a positive identification of all these components by the techniques described herein. Their presence in the oil is considered irrelevant to the main purpose of this work, which, as stated, was directed towards the unequivocal characterization of isoprenoid hydrocarbons and the search for their possible biogenic precursors in shark liver oil.

3. Sheep (Ovis, breed unknown) manure

It was obtained as commercial fertilizer (powder) from Midwest Feed Yards, San Angelo, Texas. The presence of normal paraffins in sheep wool was first reported by Dowing et al. (133), by Mold et al. (330,331) and recently by Simmonds et al. (436). Sheep manure has also been the object of a recent gas chromatographic study of its paraffinic hydrocarbons made by Nooner (348). He reported two distributions of alkanes with maxima at C_{21} and C_{31}. On the basis of their retention times on an Apiezon L and a Polysev capillary columns, pristane and phytane were also identified in the sample. This appeared to confirm the reports on wool wax and sheep wool (330,436). The results obtained from the gas chromatographic-mass spectrometric analysis of a sample of sheep manure provided by Dr. Nooner fully support the earlier gas chromatographic identifications of n-alkanes and isoprenoids, and at the same time complement them with MS data on norpristane and several unsaturated compounds. The GC-MS analysis by

their mass spectra can be listed, according to their retention time in a Polysev column as follows:

$n\text{-}C_{15}$	Phytane
$\Delta\text{-}C_{15}$	nC_{18}
$n\text{-}C_{16}$	ΔC_{20}
Norpristane	ΔC_{20}
ΔC_{16}	$2\Delta C_{20}$
Pristane	$2\Delta C_{20}$
nC_{17}	nC_{19}
ΔC_{17}	$2\Delta C_{21}$

The mass spectrometric patterns of the C_{20} monoolefins and their gas chromatographic position indicate that they may be related to the polyisoprenoid structure of phytane (phytamonoenes), and likewise the C_{20} diolefins appear to give spectra consistent with a phytadiene structure.

4. Crude oil

The gas chromatographic analyses of crude oils have also been reported in our laboratory by Nooner (348). He studied two Texas crude oils and found them to contain alkanes ranging from $n\text{-}C_{14}$ to $n\text{-}C_{31}$. Norpristane, pristane and phytane were also present according to the GC identifications. The remarkable similarity of the patterns thus obtained to those found in Type II and Type III-A meteorites (348,350) was also noted.

For this reason it becomes of interest to confirm and if possible extend this data by means of the GC-MS analysis. Since the crudes studied by Nooner (348) show identical patterns in terms of the number of components present, only one of them was analyzed by mass spectrometry.

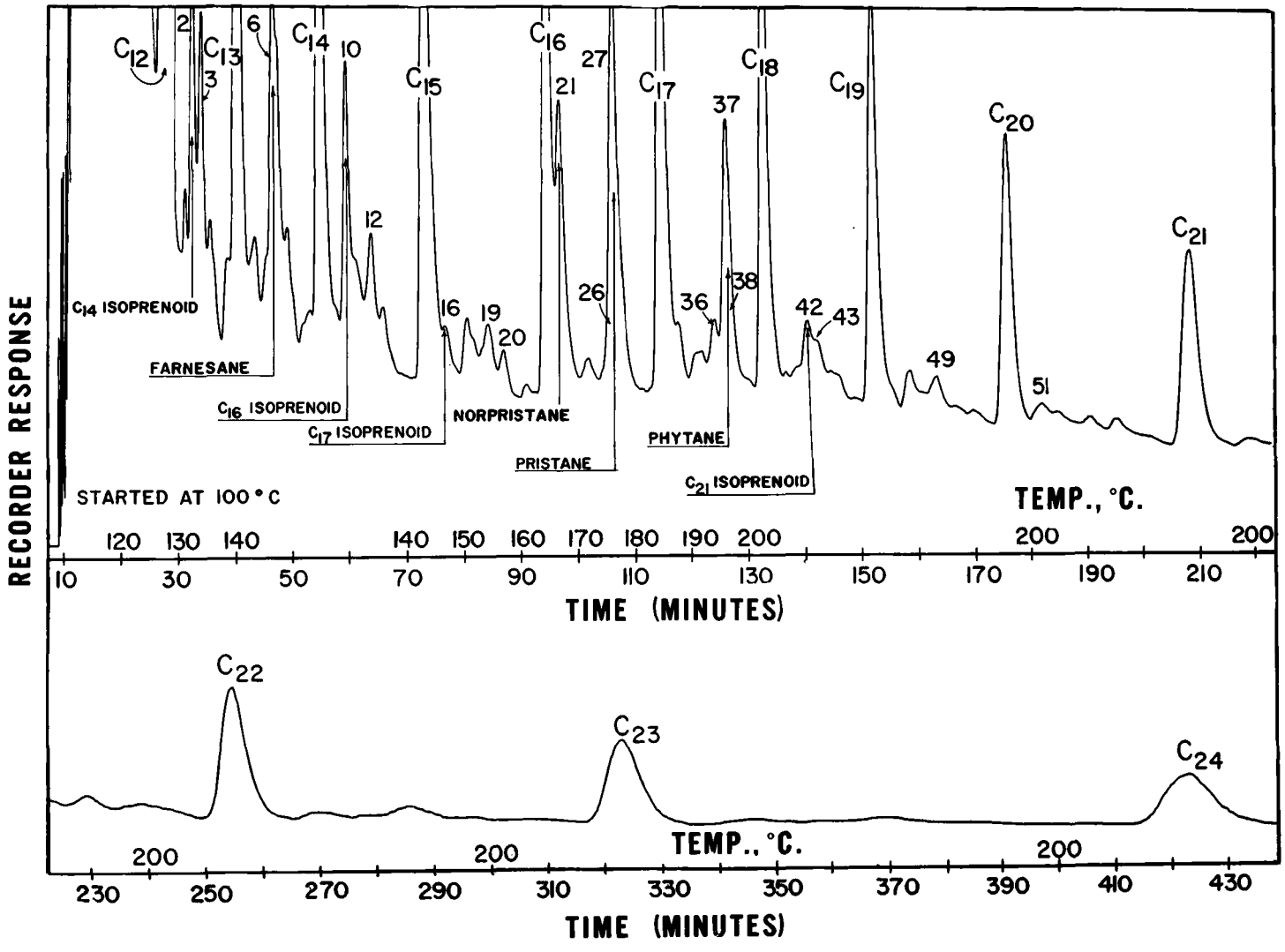
This crude oil, a sweet crude from East Texas, had been obtained by Dr. Nooner (348) from Dr. R. D. Schwartz, Shell Development Company, Houston, Texas. The gas chromatographic analysis can be seen in Figure 53. The resulting hydrocarbon distribution, although qualitatively identical to that reported previously (reference 348, p. 172), shows a major difference in the position of its maxima, which is not at C_{17} as previously reported (348). It has been pointed out by Nooner and Oró (350) that taking the samples to dryness, at any point during the recovery procedure, usually results in the loss of the light molecular weight compounds up to the C_{18}. Thus the true distribution mode is drastically altered. As a practical example a comparison can be made between this pattern (Figure 53) and that obtained earlier with the same sample (reference 348, p. 172), where the alkane distribution starts with a very low n-C_{12} and gradually increases towards its maxima at C_{17}, then declines toward nC_{29}. As can be seen here (Figure 53), the same sample not taken to dryness gives from C_{12} on a continuously decreasing pattern. Although the highest peak is the nC_{12} alkane, it is not known where the

FIGURE 53

GAS CHROMATOGRAPHIC SEPARATION OF THE ALKANES FROM A PETROLEUM CRUDE

Stainless steel capillary column (195 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Range, 10; attenuation, 1. Nitrogen pressure 1050 g/cm^2. No split. Temperature started at 100°C and programmed to 190°C at about 1°C/min. 0.0146 g extracted. About 0.9/35 of the sample was injected.

EAST TEXAS PETROLEUM CRUDE



real maxima is in this case, because all the lower compounds came together with the solvent front. Nevertheless the size of this peak gives an idea of the high concentration of compounds below n-dodecane. In line with other reports on the distribution of paraffins in petroleum it is assumed that the maxima probably lies around C_5 and C_7 (38).

Losses during evaporation of the solvents are also reflected in the overall quantitative results. A total of 50000 ppm was previously reported (348) for the alkane content of this crude oil. This figure has been recalculated and appears to be close to 75000 ppm. A difference of 25000 ppm of volatile paraffins lost.

While this is not meant as a criticism to the work of Nooner (348), it is emphasized here to offer a word of caution in future work and also to demonstrate the advantages of introducing this minor modification (samples are not taken to dryness) in the otherwise highly efficient analytical procedures.

The mass spectrometric identifications are indicated in Figure 53. Because of the correlations to be established later the same identification number system used throughout the meteorite analyses is also used here. Refer to Table XLIX for the compound identification. Although the isoprenoids are identified in the figure by their corresponding numbers in Table XLIX their names are also given underneath each one of them for the sake of clarity.

C. Other Terrestrial Products

Because of the possible influence that the hydrocarbons in the surrounding environment may have on the distribution patterns of the hydrocarbons present at the ppm level in extraterrestrial samples, the alkane content and nature of rather ubiquitous materials such as tobacco smoke, and dust was investigated. The results obtained on the analysis of the hydrocarbons of terrestrial graphite samples are also included in this section.

1. Tobacco smoke

Cigarette smoke has been the object of a relatively large number of analyses. The low molecular weight range (C_1-C_{10}) has been the most extensively studied (385). Philippe reports (384) that "the complete series of alkanes from C_1 to C_6 have been identified with the exception of neopentane, neohexane and 2,3-dimethylbutane. The full alkene series is present from C_2 to C_5 and 9 of the 17 possible C_6 isomers have been found. All cycloalkanes and cycloalkenes with 5 or 6 carbon rings have been reported." He goes on describing several substituted aromatics that have also been detected in small amounts and concludes by saying that "the bulk of the hydrocarbon fraction consists of low molecular weight gases (C_1 to C_3) and of isoprene, a decomposition product of polyisoprenoid compounds present in tobacco." There are also some reports on higher molecular weight alkanes (88,101,

247,404). Members of the series of normal and branched saturated aliphatic hydrocarbons have been reported (88,247). As stated by Philippe (384), these compounds are most likely transferred directly from tobacco waxes, which contains appreciable amounts of such hydrocarbons (88,93,144,329,453).

Among other compounds neophytadiene (175) and several phytadienes (403) have also been detected in cigarette smoke, but only neophytadiene has been reported as a constituent of tobacco plants (175,354,409). It is believed that the isomeric phytadienes arise from thermal isomerization of neophytadiene. Temperatures of up to 850^{o}C have been measured in some parts of the tip of a burning cigarette. In view of all this it was desirable to study the smoke composition using the same techniques applied to the analysis of meteorites, sediments and biological products in order to be able to establish distributional correlations, if any, that would indicate a possible source of contaminating products in the cigarette smoke.

The smoking operation was carried out on a vacuum system. The smoke was condensed in a trap cooled with liquid nitrogen. An approximate flow rate of 20 ml/sec was maintained during each 3 seconds puff. A total of 10 cigarettes (non filter) were thus smoked to approximately 23 mm of butt length. The smoke condensate was collected in n-pentane by washing the

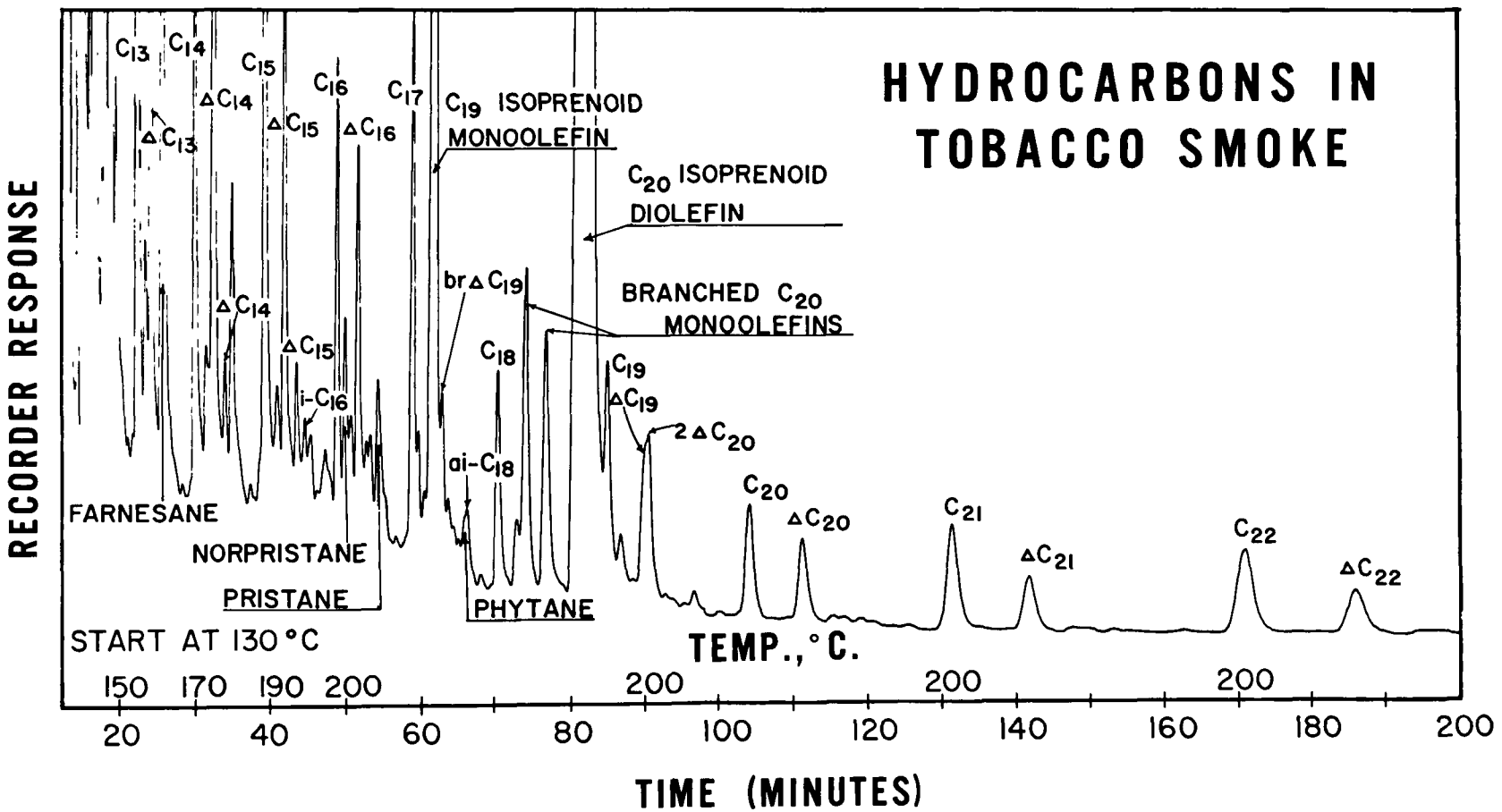
trap several times with a few milliliters of this solvent. The pentane soluble condensate was fractionated on a silica gel column and treated for analysis according to the methods described in Chapter III.

Two separate gas chromatographic-mass spectrometric analysis were performed on the n-pentane eluate. In one of them, the range C_{10}-C_{23} (Fraction I) was studied by means of a capillary column (195 m x 0.076 cm) coated with Polysev and in the other, the range C_{20}-C_{33} (Fraction II) was studied with a short glass column packed with OV-1 (Table I). This fractionation of the sample was necessary due to the limitations of the columns in covering such a wide molecular weight range (C_{10}-C_{33}). The gas chromatographic analysis of Fraction I is shown in Figure 54. There is a clear predominance of odd over even hydrocarbons for the n-alkanes while the contrary appears to be true for the corresponding olefins in the low molecular weight range. In general the area ratio, odd alkane / odd olefin with the same carbon number is about 2 while in the case of the even alkanes and their corresponding olefins this ratio is about 1. According to the GC-MS analysis, the isoprenoids, farnesane, norpristane, pristane and phytane, are present in decreasing concentrations. The dominating feature in this pattern is the presence of very high amounts of a C_{19} isoprenoid olefin, identified as 2,6,10-trimethyl-hexadecene (281) and a C_{20} isoprenoid diolefin identified as

FIGURE 54

GAS CHROMATOGRAPHIC SEPARATION OF THE LOW MOLECULAR WEIGHT HYDROCARBONS IN TOBACCO SMOKE

Same conditions as described in Figure 53, except for attenuation, 2. The temperature of injection was held at 130°C for 10 minutes and then programmed to 200°C at 2°C/min.


HYDROCARBONS IN TOBACCO SMOKE
RECORDER RESPONSE
C13
C14
C15
C16
C17
ΔC14
ΔC13
ΔC15
ΔC16
C19 ISOPRENOID MONOOLEFIN
C20 ISOPRENOID DIOLEFIN
br ΔC19
BRANCHED C20 MONOOLEFINS
ΔC14
C18
C19
ΔC15
i-C16
ΔC19
2 ΔC20
ai-C18
C20
ΔC20
C21
ΔC21
C22
ΔC22
FARNESANE
NORPRISTANE
PRISTANE
PHYTANE
START AT 130 °C
TEMP., °C.
150 170 190 200
200
200
200
20 40 60 80 100 120 140 160 180 200
TIME (MINUTES)

2,6,10,14-tetramethyl pentadecadiene (phytadiene). Their corresponding mass spectra are given in Figure 17 and have been discussed in Chapter IV. The mass spectra of the two compounds identified as branched C_{20} monoolefins in Figure 54 are also included in Figure 17. While there is no positive evidence of their isoprenoid character their mass spectra show many resemblances to the spectra of the isoprenoid C_{19} and C_{20} mono and diolefins. The chromatographic retention times seems to support their identification as multibranched olefins, which enhances the confidence in the mass spectrometric interpretations of the data. In line with previous reports (88) small amounts of methyl alkanes were also detected. The gas chromatographic-mass spectrometric analysis of Fraction II (C_{20}-C_{33}) produced identical results as those reported by Carruthers (88) for the cigarette smoke and by Mold (329) and Eglington (144) for the leaf wax alkanes of Nicotiana tabacum. Hentriacontane (88,101,144,247,329) was found to be the most abundant component in this fraction. Also, as in tobacco leaves (144,329), two series of methyl alkanes were found, the isoalkane series is composed mainly by compounds with odd numbers of carbon atoms while the anteiso series consists principally of homologs with even numbers of carbon atoms. The alkanes in Fraction II (C_{20}-C_{33}) identified by mass spectrometry are listed in Table XXXIII in order of their elution times from an OV-1 column (see Table I). The total alkane content was calculated to be

TABLE XXXIII

HIGH MOLECULAR WEIGHT HYDROCARBONS

IN TOBACCO SMOKE

	Rel %
n-C_{22}	8
n-C_{23}	2
n-C_{24}	3
n-C_{25}	6
n-C_{26}	3
n-C_{27}	32
n-C_{28}	3
iso C_{29}	6
n-C_{29}	32
anteiso C_{30}	57
iso C_{31}	28
n-C_{31}	100
anteiso C_{32}	75
iso C_{33}	22
n-C_{33}	33

over 0.115 mg per cigarette.

The results obtained in general seem to confirm the logical assumption that the paraffins in tobacco smoke are directly distilled from the tobacco leaf waxes (403). In addition to all the compounds previously reported, a series of alkenes (with the doulbe bond in a terminal position as indicated by the mass spectra), and a new isoprenoid compound (2,6,10-trimethyl hexadecene) have also been found. Thermally induced rearrangements and pyrolitic reactions are most likely responsible for their formation. It is interesting to recall that the even carbon numbered olefins appear to be present in higher relative amounts.

2. Dust

Because it can come easily in contact with meteorites and other samples where the determination of the indigenous amount of hydrocarbons is critical, it has been analyzed according to our general techniques. The gas chromatographic pattern of a dust sample received from the Paris Museum is shown in Figure 55. The hydrocarbons in this sample range from C_{17} to C_{34} and show maxima at C_{27}. Also there is a clear predominance of odd over even alkanes above C_{23}. A similar distribution has been reported by Nooner (348) for laboratory dust (see Figure 56). The total hydrocarbon content for this museum dust is about 1700 ppm which is also very close to the value found (1695 ppm) by Nooner (348) in his sample of

FIGURE 55

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS FROM MUSEUM DUST

Stainless steel column (150 m long by 0.076 cm i.d.) coated with OV-17 (see Table I). 'F and M Model 810' with flame ionization detector. Range, 10^2; attenuation, 1. Nitrogen pressure, 1050 g/cm^2. No split. Temperature started at 150°C and programmed to 240°C at 2°C/min.

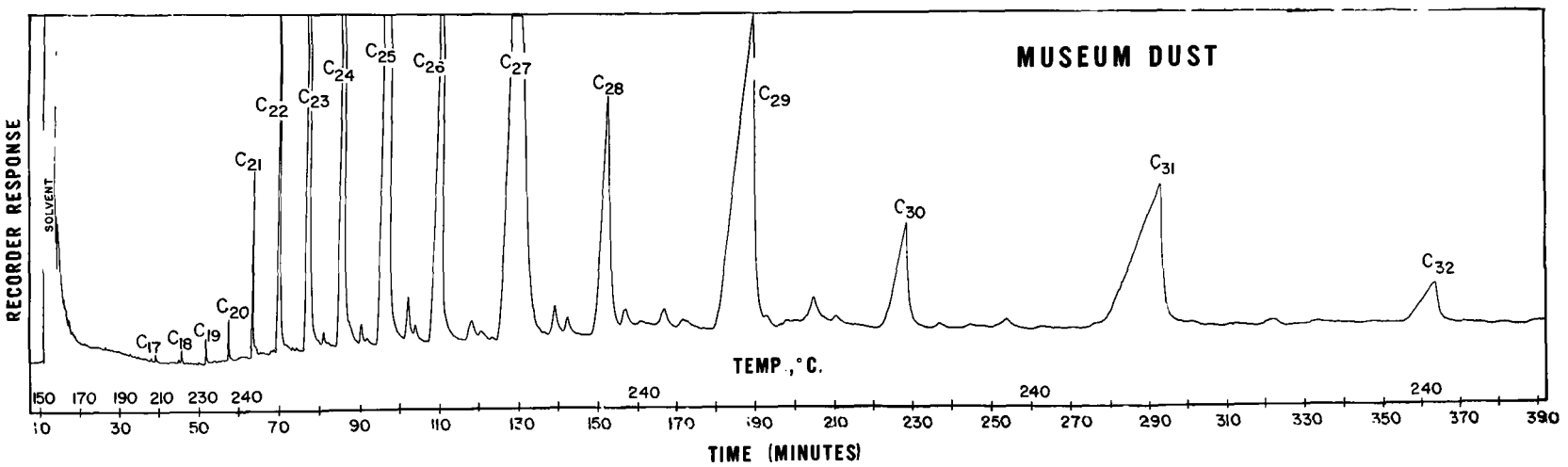
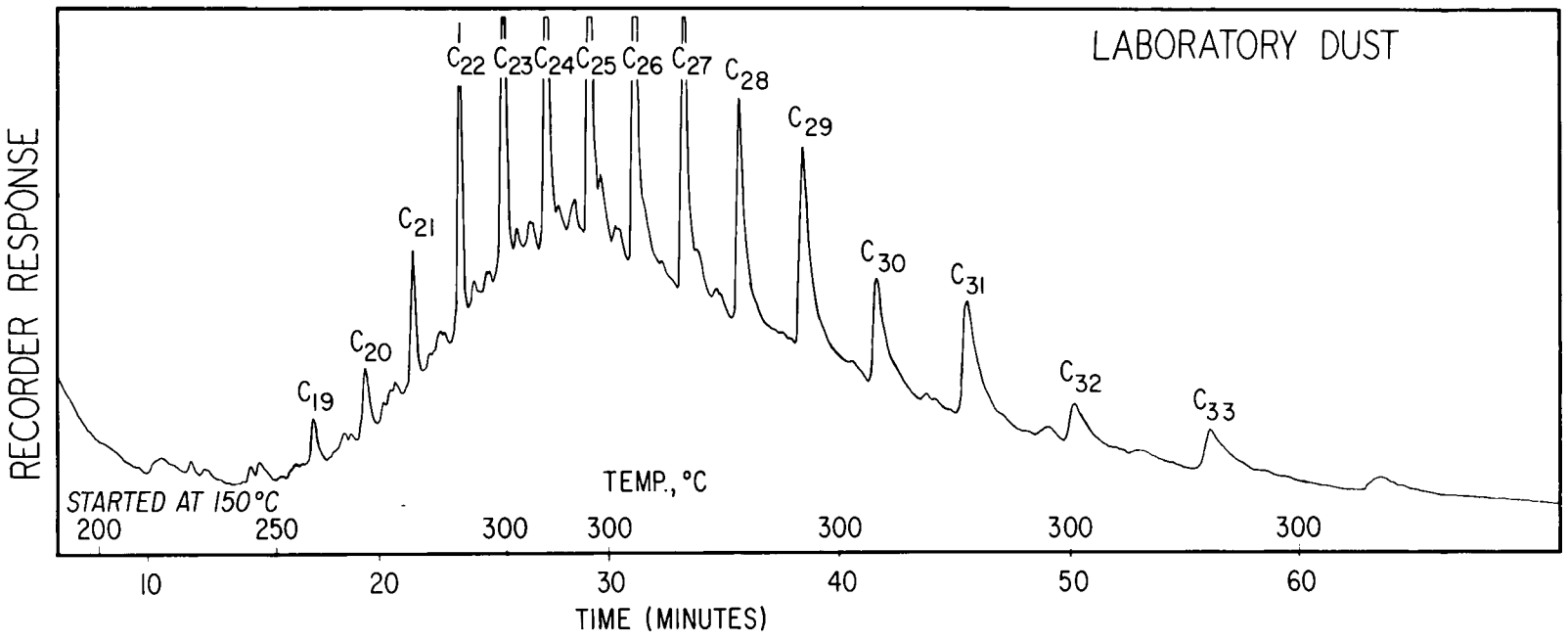

MUSEUM DUST
RECORDER RESPONSE
SOLVENT
C17
C18
C19
C20
C21
C22
C23
C24
C25
C26
C27
C28
C29
C30
C31
C32
TEMP., °C.
150 170 190 210 230 240 240 240 240
10 30 50 70 90 110 130 150 170 190 210 230 250 270 290 310 330 350 370 390
TIME (MINUTES)

FIGURE 56

GAS CHROMATOGRAPHIC SEPARATION OF ALKANES

FROM LABORATORY DUST

Stainless steel tubing (46 m x 0.025 cm) coated with Apiezon L. Barber Colman Series 5000 equipped with a flame ionization detector. See reference 348 for further details.


LABORATORY DUST
RECORDER RESPONSE
C22 C23 C24 C25 C26 C27 C28
C29
C21
C20
C19
C30
C31
C32
C33
STARTED AT 150°C
TEMP., °C
200
250
300
300
300
300
300
10
20
30
40
50
60
TIME (MINUTES)

laboratory dust. No evidence of isoprenoids was found in this case either, although the small peaks preceeding the n-C_{17} and nC_{18} in Figure 55 would correspond to pristane and phytane respectively. Also two dust samples collected in a room of the Chemistry Department, University of Houston, isolated from human contamination were analyzed for their hydrocarbon content. The room was exposed to the outside air through a mesh screen. Two fractions were collected: one of them, the "light fraction" consisted of fine loose dust particles from the tops of several boxes, recently deposited there. The other, a "heavy fraction" with a much coarser appearance, was taken from the bench top and obviously it had had a longer deposition history.

The gas chromatographic analysis of the "light fraction" is presented in Figure 57. Since no mass spectrometric data was obtained in this case only the n-alkane peaks are identified in the figure. It is interesting though, that the distribution of these hydrocarbons in the range C_{14}-C_{19} is identical to that found in most meteorite samples. Peaks in the chromatographic positions corresponding to norpristane, pristane and phytane are also present in this chromatogram (Figure 57). As in most dust samples analyzed, this one also shows odd over even predominance and it contains a relative high amount of high molecular weight alkanes as illustrated in Table XXXIV. The total hydrocarbon content was calculated to be only about

FIGURE 57

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS IN DUST FROM AN ISOLATED ROOM

Stainless steel capillary column (195 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' Gas Chromatograph. Range, 10; attenuation, 1. Nitrogen pressure 1050 g/cm^2. No split. Temperature held at 130°C for 10 minutes and then programmed to 200°C at 2°C/min. 0.5758 g extracted. About 1.9/3 of the n-pentane eluate was injected.

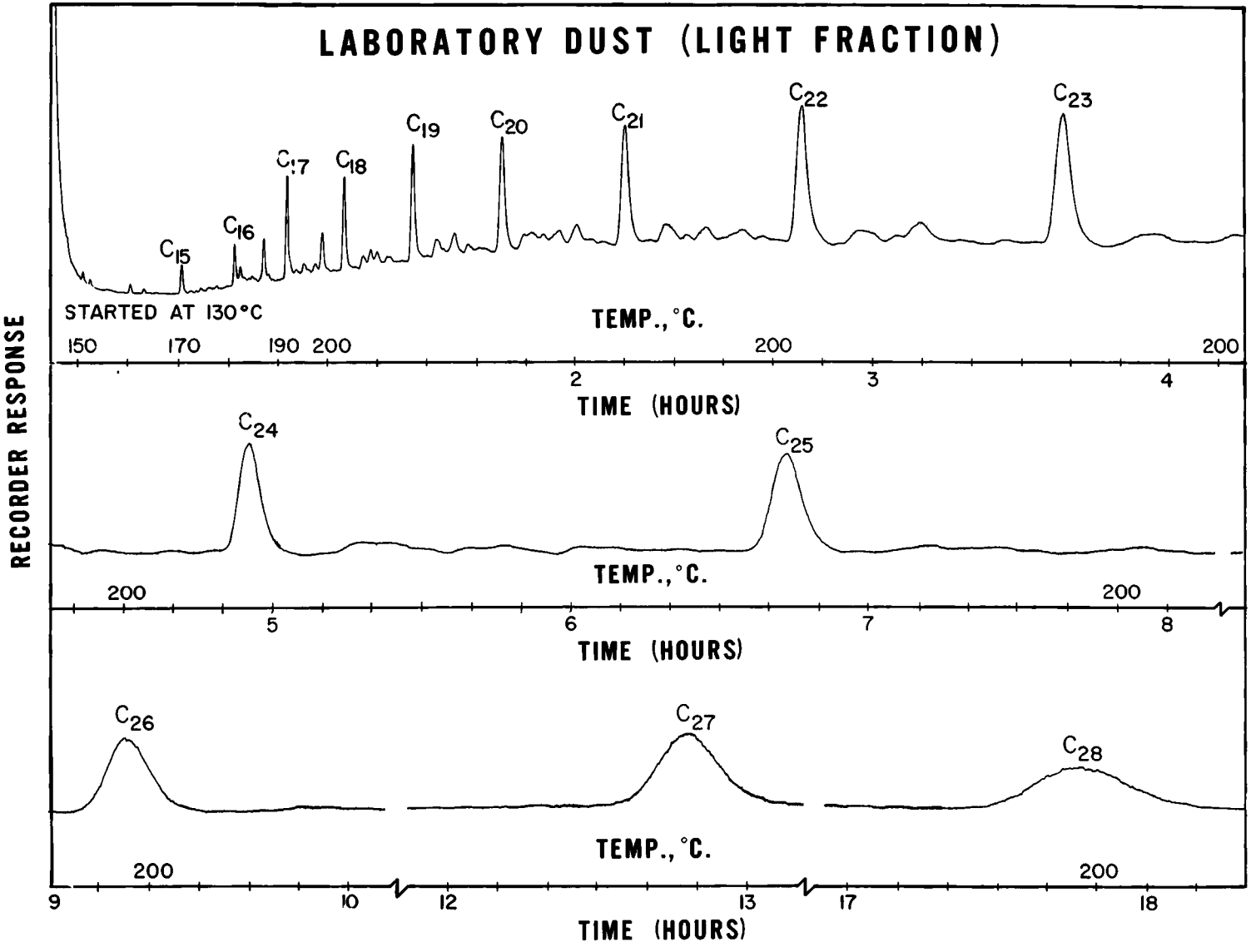

LABORATORY DUST (LIGHT FRACTION)
RECORDER RESPONSE
C15
C16
C17
C18
C19
C20
C21
C22
C23
STARTED AT 130°C
TEMP., °C.
150
170
190
200
200
200
2
3
4
TIME (HOURS)
C24
C25
TEMP., °C.
200
200
5
6
7
8
TIME (HOURS)
C26
C27
C28
TEMP., °C.
200
200
9
10
12
13
17
18
TIME (HOURS)

TABLE XXXIV

HYDROCARBONS IDENTIFIED IN A SAMPLE OF ROOM DUST

C_{13}	0.0052 ppm
C_{14}	0.0117 ppm
C_{15}	0.0415 ppm
C_{16}	0.0570 ppm
C_{17}	0.1613 ppm
C_{18}	0.1715 ppm
C_{19}	0.2705 ppm
C_{20}	0.3723 ppm
C_{21}	0.5374 ppm
C_{22}	0.4502 ppm
C_{23}	1.1744 ppm
C_{24}	0.7835 ppm
C_{25}	0.8939 ppm
C_{26}	0.8158 ppm
C_{27} - C_{30}	0.7300 ppm
TOTAL	6.4762 ppm

7.4 ppm in the range detected by gas chromatography (nC_{13} to nC_{30}). The n-alkanes account for 6.4 ppm which leaves a maximum of 1.0 ppm for the other isomeric alkanes. The gas chromatographic analysis of the heavier fraction of the dust showed an identical distribution of hydrocarbons with a higher relative amount of isoprenoids and more marked predominance of the odd numbered hydrocarbons in the low molecular weight range.

3. Terrestrial Graphite

Although the hydrocarbon content of some samples of graphite of extraterrestrial origin have been analyzed in some detail recently (350,357), only a couple of reports are available on the presence of these compounds in terrestrial coal-graphite deposits (256,379). For this reason it became of interest to analyze some of the naturally occurring terrestrial graphites, hoping that the results obtained would lead to fruitful correlations between the hydrocarbons from meteoritic samples, petroleum and sediments. These studies were carried out in cooperation with Dr. D. W. Nooner (349).

a) Aliphatic hydrocarbons

Graphite from Ceylon

The origin of this graphite which occurs in veins and fissures has not been adequately explained (24,98). The geological evidence appears to indicate a possible inorganic origin or at least that the graphite is not a product of

metamorphosed organic matter.

Gas chromatograms of the aliphatic hydrocarbons extracted from the outside and inside of a sample of this graphite are shown in Figures 58 and 59 respectively. The hydrocarbons in the outside and inside samples are qualitatively and quantitatively similar (Table XXXV). A salient feature which has also been observed in meteoritic graphite extracts (357) is the relatively high amount of the isoprenoid hydrocarbons in the inside sample (see Table XXXV). All of the hydrocarbons identified by mass spectrometry are listed in Table XXXVI. In addition to normal hydrocarbons, which range from C_{13} through C_{20}, the graphite contains the isoprenoid (C_{16}, C_{18}, C_{19} and C_{20}), isoalkane (C_{14}-C_{19}), and anteiso alkane (C_{18} and C_{19}) homologies. An n-alkylcyclohexane, tridecylcyclohexane, was also identified in the n-heptane eluate of the extract. The alkanes in Table XXXVI are given in order of their gas chromatographic elution times from capillary columns coated with Polysev.

Partial mass spectra of all of the four isoprenoid hydrocarbons identified in an inside sample of this Ceylon ore are given in Tables XXXVII, XXXVIII and XXXIX. These spectra are displayed in the grid type pattern proposed by Clerck et al. (102) and by O'Neal et al. (353). The different mass series are arranged in columns under their corresponding z value which is taken from the hydrocarbon formula C_nH_{2n+z}.

FIGURE 58

GAS CHROMATOGRAMS OF PARAFFINIC HYDROCARBONS EXTRACTED FROM OUTSIDE OF GRAPHITE ORE

Stainless steel tubing, 152.4 m long x 0.076 cm inside diameter, coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Nitrogen 1054 g/cm^2. No split. Isothermal at 140°C for 20 minutes, then programmed at approximately 2°C per minute to 200°C.

(A) 61.2 g extracted. About 1/40 injected.
Range, 10^2; attenuation, 4.

(B) 26.4 g extracted. About 1/1050 injected.
Range, 10^2; attenuation, 4.

(C) 42.0 g extracted. About 1/10 injected.
Range, 10^2; attenuation, 1.

Identification numbers correspond to those given in Table XXXV. Numbers circled indicate the isoprenoid compounds.

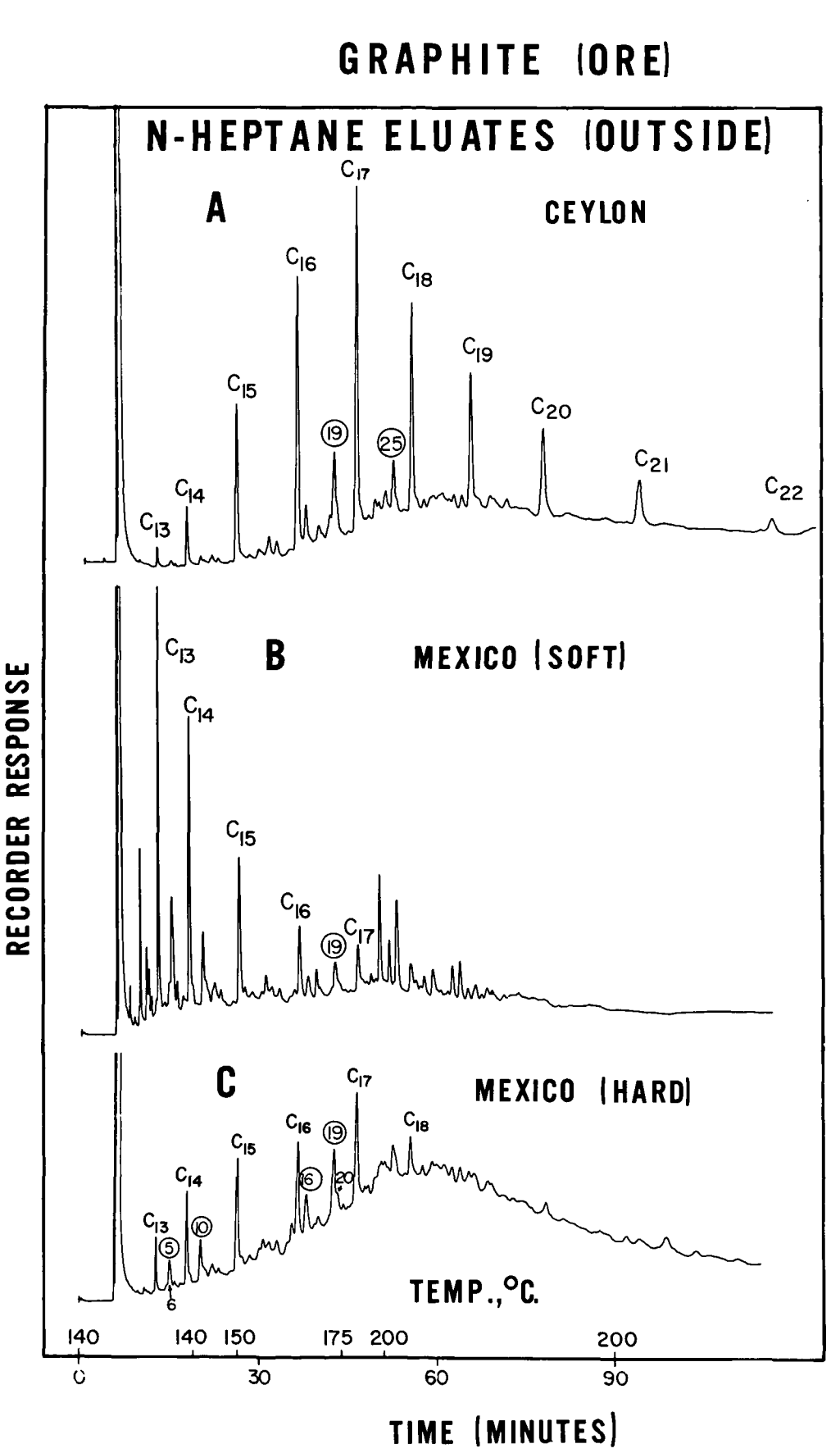

GRAPHITE (ORE)
N-HEPTANE ELUATES (OUTSIDE)
A
CEYLON
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
19
25
B
MEXICO (SOFT)
C13
C14
C15
C16
C17
19
C
MEXICO (HARD)
C13
C14
C15
C16
C17
C18
19
5
10
16
20
6
RECORDER RESPONSE
TEMP., °C.
140
140
150
175
200
200
0
30
60
90
TIME (MINUTES)

FIGURE 59

GAS CHROMATOGRAMS OF PARAFFINIC HYDROCARBONS EXTRACTED FROM INSIDE OF GRAPHITE ORE

Stainless steel tubing, 152.4 m long x 0.076 cm inside diameter, coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Nitrogen, 1054 g/cm^2. No split. Isothermal at 140°C for 20 minutes, then programmed at approximately 2°C per minute to 200°C.

(A) 41.6 g extracted. About 1/6 injected.
Range, 10^2; attenustion, 32.

(B) 17.3 g extracted. About 1/50 injected.
Range, 10^2; attenuation, 32.

(C) 21.1 g extracted. About 1/3 injected.
Range, 10^2; attenuation, 1.

Identification numbers correspond to those given in Table XXXV
Numbers circled indicate the isoprenoid compounds.

Symbols: mn: monomethyl naphthadene
dn: dimethyl naphthalene
tn: trimethyl naphthalene

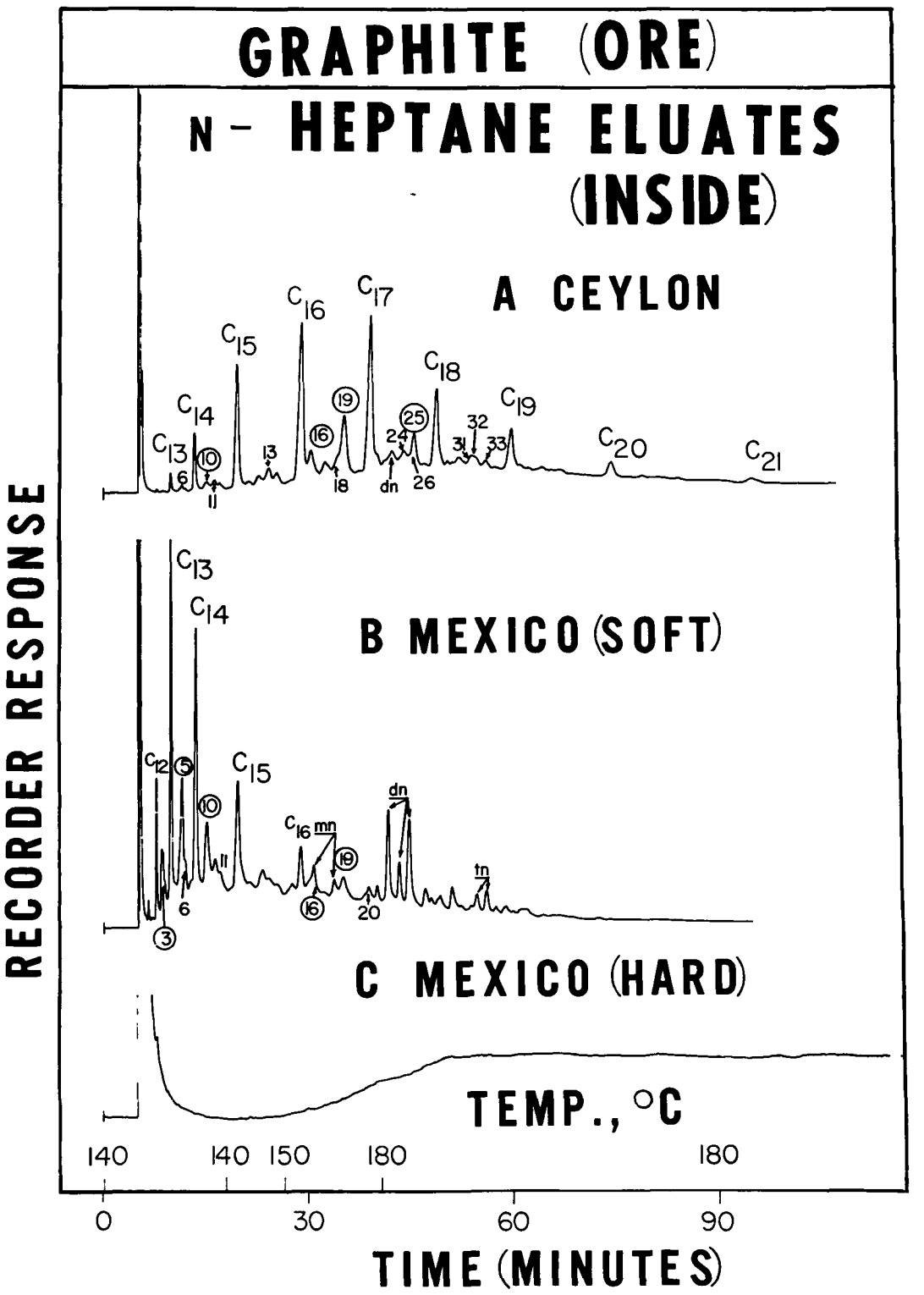

GRAPHITE (ORE)
N- HEPTANE ELUATES (INSIDE)
A CEYLON
B MEXICO (SOFT)
C MEXICO (HARD)
TEMP., °C
RECORDER RESPONSE
TIME (MINUTES)
0
30
60
90
140
140
150
180
180

TABLE XXXV

ESTIMATION OF HYDROCARBONS IN TERRESTRIAL GRAPHITE

Graphite	Origin	Sample	Amount Analyzed, grams	Isoprenoids, ppm (C_{18}, C_{19}, & C_{20}) A	Alkanes, ppm B	Paraffins, ppm A + B	Aromatics, ppm
Ore	Ceylon	Outside	61.2	1.45	11.75	13.2	1.62
		Inside	41.6	1.75	10.95	12.7	1.86
Ore (Soft)	Mexico	Outside	26.4	27	671	698	96.8
		Inside	17.3	-	-	316	21.7
Ore (Hard)	Mexico	Outside	42.0	0.42	1.58	2.0	0.13
		Inside	21.1	0.0	<0.01	<0.01	<0.01
Commercial (Powder)	Ceylon	-	4.0	0.80	0.80	1.6	
Commercial (Powder)	Madagascar	-	4.0	3.16	15.4	18.6	

TABLE XXXVI

ISOPRENOIDS AND ALKANES IDENTIFIED IN TERRESTRIAL GRAPHITES BY GC-MS COMBINATION

		CI	CC	MH	MS			CI	CC	MH	MS
1	nC_{11}				*	20	$2\Delta C_{16}$			*	*
2	nC_{12}				*	21	nC_{17}	*	*	*	
3	2,6,10-trimeC_{11}				*	22	2,6,10-trimeC_{16}		*		
4	nC_{13}	*		*	*	23	brΔC_{19}			*	
5	2,6,10-trimeC_{12}		*	*	*	24	2-meC_{17}	*	*		
6	2-meC_{13}	*		*	*	25	2,6,10,14-tetrameC_{16}	*	*		
7	3-meC_{13} + ΔC_{14}				*	26	3-meC_{17}	*			
8	4,8-dimeC_{13}				*	27	nC_{18}	*	*	*	
9	nC_{14}	*	*	*	*	28	undecylcyclohexane		*		
10	2,6,10-trimeC_{13}	*	*	*	*	29	brΔC_{20}			*	
11	2-meC_{14}	*	*	*	*	30	2,6,10,14-tetrameC_{17}		*		
12	nC15	*	*	*	*	31	4-meC_{18}	*			
13	2-meC_{15}	*	*			32	2-meC_{18}	*	*		
14	3-meC_{15}		*			33	3-meC_{18}	*	*		
15	nC_{16}	*	*	*	*	34	nC_{19}		*	*	
16	2,6,10-trimeC_{15}	*	*	*	*	35	2,6,10,14-tetrameC_{18}		*		

TABLE XXXVI CONTINUED

ISOPRENOIDS AND ALKANES IDENTIFIED IN TERRESTRIAL GRAPHITES BY GC-MS COMBINATION

		CI	CC	MH	MS			CI	CC	MH	MS
17	5-meC_{16}		*			36	2-meC_{19}		*		
18	2-meC_{16}	*				37	nC_{20}	*			
19	2,6,10,14-tetrameC_{15}	*	*	*	*	38	tridecylcyclohexane	*			

CI: Ceylon ore, inside sample

CC: Ceylon, commercial sample

MH: Mexican graphite, Hard (outside sample)

MS: Mexican graphite, Soft (inside sample)

TABLE XXXVII

ISOPRENOIDS IN AN INSIDE SAMPLE OF A CEYLON GRAPHITE

C_n \ Z*	C_{16} isoprenoid				C_{18} isoprenoid			
	-1	0	+1	+2	-1	0	+1	+2
C_4	13	41	285	14	8	36	282	13
C_5	28	86	292	13	20	46	215	14
C_6	35	57	181	12	43	37	147	11
C_7	46	28	70	9	42	77	89	11
C_8	26	93	100	10	28	72	100	10
C_9	15	30	49	7	17	22	46	7
C_{10}	9	46	103	11	8	19	40	5
C_{11}	6	19	31		4	18	30	4
C_{12}		10	15	6	5	36	76	11
C_{13}	5	29	64	15	2	25	59	9
C_{14}		15	16	7		8	12	
C_{15}			14			15	20	
C_{16}				22	5	11	18	
C_{17}							12	
C_{18}								15

*Z, value in C_nH_{2n+Z}

TABLE XXXVIII

ISOPRENOIDS IN GRAPHITE

PRISTANE

	Std			Mexico hard			Mexico soft			Ceylon			2-meC_{16} std.		
C_n \ Z	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2
C_4	33	180	10	33	174	10	29	173	9	30	177	10	15	100	5
C_5	25	185	12	33	180	11	34	170	10	33	175	12	8	75	5
C_6	19	77	5	39	95	6	33	88	7	29	112	9	7	56	4
C_7	7	39	4	31	48	7	22	52	4	19	66	5	5	29	2
C_8	75	100	9	93	100	9	88	100	11	78	100	10	4	19	1.7
C_9	18	40	5	30	38	6	24	40	4	22	35	7	2	15	1.5
C_{10}	2	18	2	13	22	4	10	20	5	11	27	10	1.6	11	1.3
C_{11}	13	23	3	23	29	4	20	29	4	20	32	4	1.4	7	0.9
C_{12}	1	7		8	11	3	7	9		9	15		1.5	4	0.6
C_{13}	38	94	14	44	89	15	40	90	13	39	85	12	0.6	2	0.4
C_{14}	1.5	5		9	11	3	9	10		23	47*	8	12	33	6
C_{15}		1		7	8		8	9		8	9			0.4	
C_{16}	1	4		17	12	3	12	12		10	20*	4	1.2	9.5	1.6
C_{17}	2	4		12	10	4	9	7		8	7	9*			4
C_{18}	1	8			9			8			10				
C_{19}			6			12			13			13			

*Major contributions of 2-methyl hexadecane to the mass spectrum of Ceylon pristane.
Ions formed at the branch sites are underlined.

TABLE XXXIX

ISOPRENOIDS IN GRAPHITE

PHYTANE

C_n \ Z	Phytane Std. 0	Phytane Std. +1	Phytane Std. +2	Ceylon 0	Ceylon +1	Ceylon +2	$3MeC_{17}$ Std. 0	$3MeC_{17}$ Std. +1	$3MeC_{17}$ Std. +2
C_4	26	196	7	55	205	12	55	100	5
C_5	38	226	15	50	225	12	7	74	5
C_6	20	151	10	32	160	12	4	57	
C_7	14	70	5	25	77	7		27	
C_8	55	100	10	65	100	11		21	
C_9	52	105	9	62	105	8		18	
C_{10}	10	40	4	19	45	4		14	
C_{11}	9	27	4	19	40	16		12	
C_{12}	8	22	3.8	18	36	6		9	
C_{13}	27	65	9	31	65	12		5	
C_{14}	20	58	9	43*	67*	10	5	8	
C_{15}	2	6	1	12	16	2		1	
C_{16}	1.5	4	1	25	55*	9	26	60	12
C_{17}	2	3		12	11			3	
C_{18}	5	12	3	15	18	5			3
C_{19}	1.5	9	2	8	12	3			
C_{20}			6			15			

*Contributions of 3-methyl heptadecane to the spectrum of Ceylon phytane. Ions formed at the branch sites are underlined.

Each row is characterized by a constant carbon number, n. The alkenyl (z=-1), olefin (z=0), alkyl (z=+1) and alkane (z=+2) ion series in the mass spectra of the C_{16} and C_{18} isoprenoid hydrocarbons are shown in Table XXXVII, where the most characteristic fragments have been underlined. Both fragmentation patterns appear to fit very closely the structures given in Figure 9 for the C_{16} and C_{18} isoprenoids. They also agree with the spectra of C_{16} and C_{18} isoprenoid compounds reported by Lawlor and Robinson (263). See Table VIII. These identifications are supported too by their respective gas chromatographic retention data (357).

Likewise mass spectrometric evidence of the presence of pristane and phytane in this sample can be found in Tables XXXVIII and XXXIX, respectively. The apparent major deviations (marked by asterisks in Tables XXXVIII and XXXIX) of these spectra from their respective standards can be readily explained if it is taken into consideration that the gas chromatographic stationary phase and the length of the columns used in these analyses were not adequate to achieve satisfactory resolution of pristane from the iso-C_{17} and of phytane from the anteiso-C_{18} (357), see also Figure 8. Thus since these two isoprenoid peaks contain substantial amounts of monomethyl branched alkanes (see shape of these peaks in Figures 58 and 59), it may be expected that their fragmentation patterns would be influenced by the fragmentation of the iso (Table XXXVIII)

and anteiso (Table XXXIX) compounds, to an extent dependent upon the concentration of these two latter compounds. The presence of the iso-C_{17} (2-methyl hexadecane) will result in an increased intensity of the C_{16} and C_{14} alkyl ions in the spectrum of pristane and also in a higher intensity of the C_{17} ion in the z=+2 or parent ion series (see Table XXXVIII). The C_{16} and C_{14} alkyl ions correspond to the M-15 and M-43 fragments in the spectrum of iso C_{17}. By the same reasoning the higher relative intensity of the C_{16} alkyl ion (marked with an asterisk in Table XXXIX) in the mass spectrum of phytane can be accounted for by considering the fragmentation pattern of the anteiso C_{18} hydrocarbon (or 3-methyl heptadecane) which is given in Table XXXIX. This peak is in fact the M-29 peak of the unresolved anteiso C_{18}. Qualitatively, the aliphatic hydrocarbons in the Ceylon graphite are quite similar to those found in the Precambrian Nonesuch Seep oil (231), and other more recent petroleum crudes in general (39). They also resemble quite closely the hydrocarbon distributions typical of meteoritic extracts (350,357). The hydrocarbons in a sample of commercial graphite derived from Ceylon are shown in Figure 60B. Identifications are based on the results of the gas chromatographic-mass spectrometric analysis. With the possible exception of the C_{17} isoprenoid, all of the isoprenoid hydrocarbons in the range C_{15} to C_{22} have been

FIGURE 60

GAS CHROMATOGRAMS OF PARAFFINIC HYDROCARBONS

EXTRACTED FROM COMMERCIAL GRAPHITE

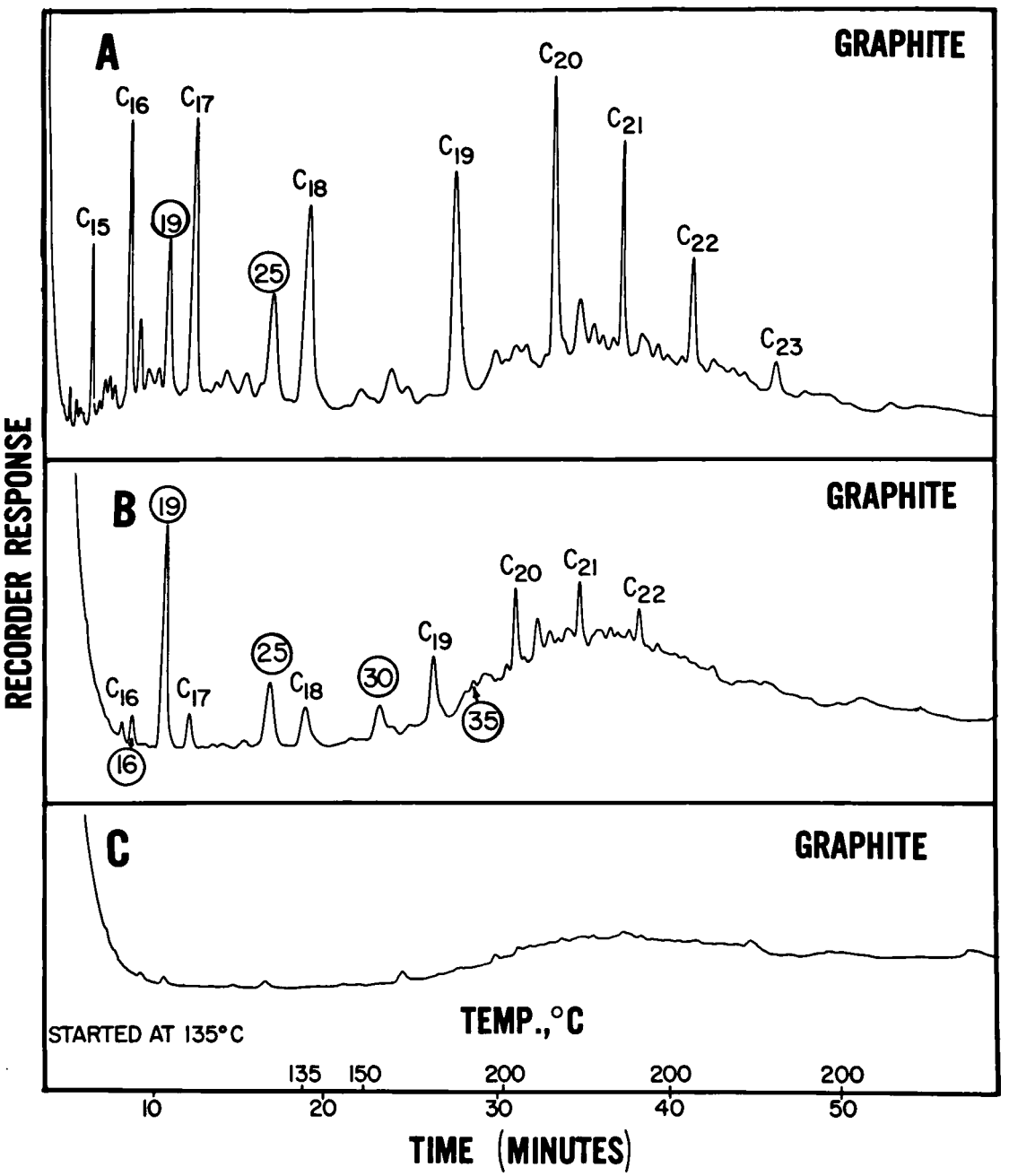
Stainless steel tubing, 91.5 m long x 0.076 cm inside diameter, coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Nitrogen, 700 g/cm^2. No split. Isothermal at 135°C for 18 minutes, then programmed at approximately 6°C per minute to 200°C.

(A) 4.0 g estracted (from Madagascar). About 1/10 injected. Range, 10^2; attenuation, 2.

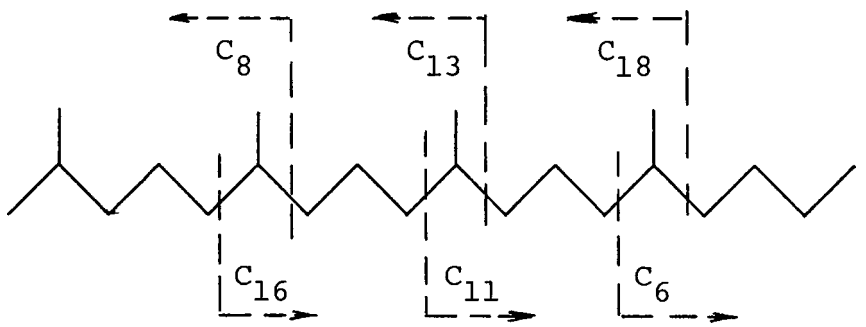
(B) 4.0 g extracted (from Ceylon). About 1/5 injected. Range, 10^2; attenuation, 1.

(C) 4.0 g extracted (from Mexico). About 1/5 injected. Range, 10^2; attenuation, 1.

Circled numbers indicate isoprenoid hydrocarbons. See Table XXXVI for identifications.


A
GRAPHITE
C15
C16
C17
19
25
C18
C19
C20
C21
C22
C23
B
GRAPHITE
19
C16
16
C17
25
C18
30
C19
35
C20
C21
C22
C
GRAPHITE
RECORDER RESPONSE
STARTED AT 135°C
TEMP.,°C
135
150
200
200
200
10
20
30
40
50
TIME (MINUTES)

identified by mass spectrometry (see Table XXXVI). Their partial mass spectral fragmentation patterns are given in Table XL. With the identification of the C_{22} isoprenoid (2,6,10,14-tetramethyl octadecane) a new member has been added to the known isoprenoid homology. This compound has a retention time (between the C_{19} and C_{20} hydrocarbons) which is consistent with such a structure and shows a mass spectrometric fragmentation pattern with maxima at C_6, C_8, C_{10}, C_{11}, C_{13}, C_{16} and C_{18}, also indicative of an isoprenoid type arrangement (Figure 9).



The molecular weight at m/e 310 shows that the molecule has 22 carbons.

The identification of this new isoprenoid compound may help to clarify the possible role that the carotenoids play as precursors of the isoprenoid hydrocarbons in general. The possible participation of squalene (282) in the diagenetic schemes leading to the formation of the isoprenoid homology within the geological environment is not supported by the existence of this C_{22} isoprenoid (see Figure 108). However,

TABLE XL

ISOPRENOID HYDROCARBONS IN A COMMERCIAL GRAPHITE

C_n \ Z(1)	C_{15}			C_{16}			C_{18}			C_{19}			C_{20}			C_{21}			C_{22}		
	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2
C_4	22	82		20	95		13	100		17	97		16	79		17	88		20	94	
C_5	33	100		38	100		15	74		12	100		19	100		39	100		20	100	
C_6	14	48		34	59		15	42		14	47		14	66		33	92		41	88	*
C_7	20	20		15	27		50	49		5	33		12	35		24	50		22	37	
C_8	19	23	*	28	36	*	30	40	*	53	68	*	29	48	*	41	59	*	34	41	*
C_9	13	31	*	8	21		9	16		12	25		26	51	*	20	36		19	27	
C_{10}	6	9		12	34	*	7	12		2	10		6	18		23	50	*	11	21	
C_{11}	9	14		10	10		6	10		8	14		5	14		13	23		17	33	*
C_{12}	2.5	4		3	4		8	25	*	1	4		4	11		7	13		9	15	*
C_{13}	6	11	*	9	14	*	7	20	*	20	50	*	13	28	*	20	37	*	14	21	*
C_{14}	3	6			4		2	4		2	4		11	24	*	5	8		8	12	
C_{15}			5		10		4	7		1	1.5		-	4		14	28	*	-	8	
C_{16}						4	2	5		1.5	3		3	5		6	7		7	12	*
C_{17}							2	7		1.3	2.5		1.5	3		4	4		5	5	
C_{18}									3	1	4		3.5	5.3		10	12	*	6	8	*

TABLE XL CONTINUED

ISOPRENOID HYDROCARBONS IN A COMMERCIAL GRAPHITE

C_n \ $Z^{(1)}$	C_{15}			C_{16}			C_{18}			C_{19}			C_{20}			C_{21}			C_{22}		
	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2	0	+1	+2
C_{19}												4		4.5		5	6		4	8	
C_{20}															-	-	-		-	-	
C_{21}																		4		6	
C_{22}																					1

$^{(1)}Z$ in C_nH_{2n+Z}

* Most characteristic ions

higher polyenes like lycopene (32) appear to be the most likely precursors.

Graphite from Mexico

The soft graphite ore from Mexico resembled the ore from Ceylon. It was either vein graphite or graphite from coal that had been almost 100 percent graphitized. The high hydrocarbon content of the sample indicated that it probably did not come from the same location as the hard graphite sample, or it could simply be related to a higher absorptivity of the soft (more porous) material towards hydrocarbons in general.

The distribution of hydrocarbons from the soft graphite ore differs from that of the ore from Ceylon in that it is skewed toward lower molecular weight hydrocarbons. Similar distributions have been reported too in coal graphite layers from South-West Greenland (370), and have also been observed in most inside samples from the graphitic nodules of iron meteorites (reference 155 and Chapter VII).

Hydrocarbon series in which two or more members were found (Table XXXVI) include normal alkanes (C_{11} through C_{18}), isoprenoids (C_{14}, C_{15}, C_{16}, C_{18} and C_{19}) and isoalkanes (C_{14} and C_{15}). The sharp peaks following C_{17} (Figure 58B) represent methyl substituted naphthalenes. The large amount of these hydrocarbons in the extract apparently overloaded the silica

gel columns and a clean separation between aliphatic and aromatic hydrocarbons was not obtained. Hydrocarbons from the soft ore that were identified by mass spectrometry are given in Table XXXVI. Mass spectra of the C_{14}, C_{15} and C_{16} isoprenoids in this graphite can be found in Table XLI. The partial mass spectrum of the C_{19} isoprenoid has already been given in Table XXXVIII. The hydrocarbons in the outside and inside samples of the soft graphite ore differ quantitatively (698 vs 316 ppm) but are similar qualitatively.

The hard graphite ore from Mexico is of organic origin being derived from coal. The graphite produced from this ore is classified as amorphous graphite. The outside sample of the hard ore contained a small amount of hydrocarbons (2.0 ppm) with a distribution truncated near C_{18} (Figure 58C). Hydrocarbons found to be predominant in the outside sample include normal alkanes (C_{13} through C_{18}) and isoprenoids (C_{15}, C_{16}, C_{18} and C_{19}). Hydrocarbons in the outside sample that have been identified by mass spectrometry are presented in Table XXXVI. Although the position of the branches in the C_{19} and C_{20} olefins identified in this sample can not be readily determined from the spectra due to the low amounts of these two compounds, their chromatographic retention times suggest that they may be related to the corresponding isoprenoid C_{19} and C_{20} olefins.

TABLE XLI

PARTIAL MASS SPECTRA OF ISOPRENOID HYDROCARBONS IN AN INSIDE SAMPLE OF A GRAPHITE ORE FROM MEXICO

C \ Z(1)	C_{14} isoprenoid				C_{15} isoprenoid				C_{16} isoprenoid			
	-1	0	+1	+2	-1	0	+1	+2	-1	0	+1	+2
C_4	5	51	260	13	9	65	390	16	11	45	257	13
C_5	13	30	224	13	27	87	385	21	27	95	284	12
C_6	9.5	43	70	6	28	39	202	14	39	62	75	13
C_7	13	22	28	3	57	33	68	7	63	26	70	7
C_8	6	92	100	10	29	74	100	8.5	42	86	100	10
C_9	3.5	20	23.5	3.5	15	60	104	11	33	23	37	5
C_{10}	-	7	9	2	7.5	19	25	4	14	50	103	13
C_{11}	-	9	18	2	5	47	68*	9.5	9	16	30	3
C_{12}	-	6	9	2.5	3	10.5	18	3.5	9	11	10	3
C_{13}	-	3	9	6.5*	3	15	46	7.5	6.5	25	63	11
C_{14}	-	-	-	12	2.5	7	13	12.5*	6	16	13	6
C_{15}	* contribution of iso C_{13}				-	-	-	18	2.5	11	15	4
C_{16}					* contribution of iso C_{14}				-	-	-	20

(1) Z in C_nH_{2n+Z}

The corresponding inside sample of the hard graphite ore contained only traces of hydrocarbons (0.01 ppm) (Figure 59). Similarly a commercial sample of amorphous graphite from Mexico contained only a total of 0.1 ppm of hydrocarbons (Figure 60C).

b) Aromatic hydrocarbons

The benzene eluates of the extracts of graphite ores were found contain relatively large amounts of aromatic hydrocarbons, except for the hard graphite from Mexico (Figures 61 and 62).

As with the aliphatic hydrocarbons, the aromatic hydrocarbons in the inside and outside samples of each ore were identical except for the hard ore. The hard graphite ore from Mexico contained aromatic hydrocarbons only on the outside, also the same as with the aliphatic hydrocarbons which were found on the outside only. The aromatic hydrocarbons in the ores were analyzed by gas chromatography-mass spectrometry and most appeared to be representative of a single class of compounds; methyl substituted naphthalenes. The ore from Ceylon contained trimethyl and tetramethyl naphthalene, the outside of the hard ore from Mexico contained dimethyl naphthalene and others that were not identified. The soft ore from Mexico contained di-, tri- and tetramethyl naphthalenes as well as diphenyl propane and diphenyl methane.

FIGURE 61

GAS CHROMATOGRAMS OF AROMATIC HYDROCARBONS EXTRACTED FROM OUTSIDE OF GRAPHITE ORES

Stainless steel tubing, 198.1 m x 0.076 cm inside diameter coated with OV-17. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Nitrogen, 1406 g/cm^2. No split. Range, 10^2; attenuation, 2. Temperature, $180^{\circ}C$.

(A) 61.2 g extracted. About 1/20 injected.

(B) 26.4 g extracted. About 1/290 injected.

(C) 42.0 g extracted. About 1/3 injected.

GRAPHITE (ORE)

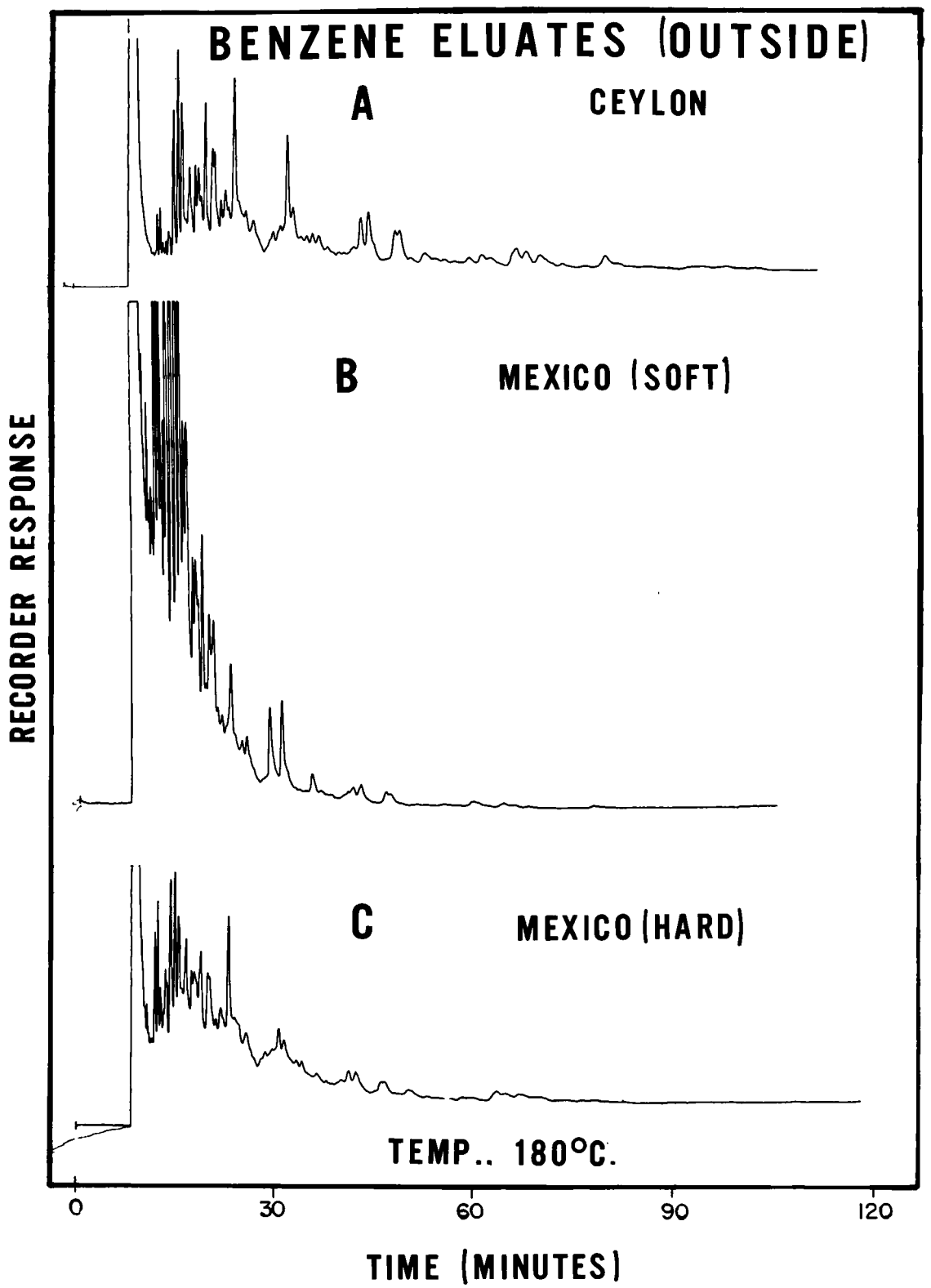


FIGURE 62

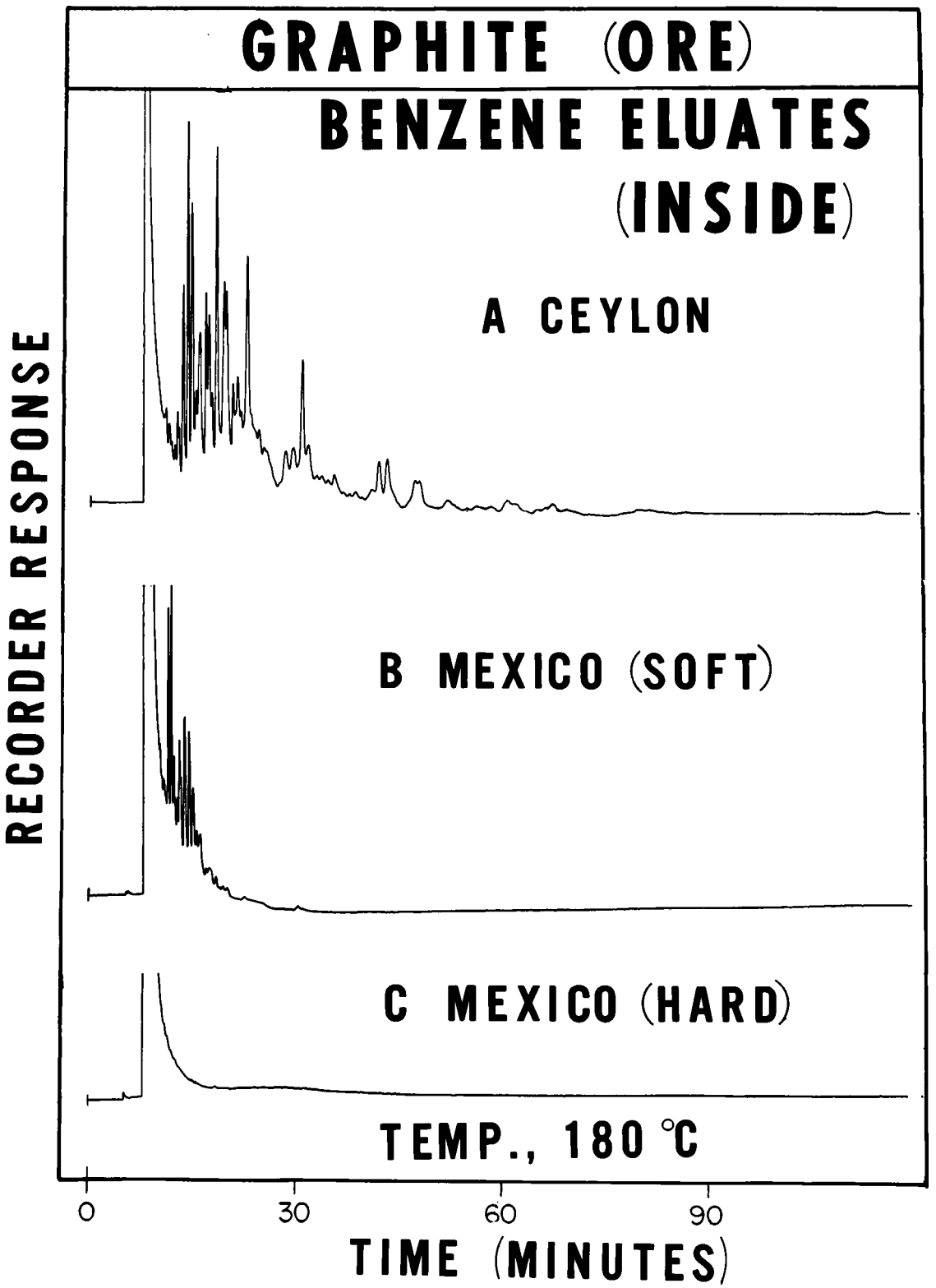
GAS CHROMATOGRAMS OF AROMATIC HYDROCARBONS EXTRACTED FROM INSIDE OF GRAPHITE ORES

Stainless steel tubing, 198.1 m x 0.076 cm inside diameter coated with OV-17. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Nitrogen, 1406 g/cm^2. No split. Temperature, 180°C.

(A) 41.6 g extracted. About 1/10 injected.
Range, 10^2; attenuation, 2.

(B) 17.3 g extracted. About 1/500 injected.
Range, 10^2; attenuation, 1.

(C) 21.1 g extracted. About 1/5 injected.
Range, 10^2; attenuation, 1.


GRAPHITE (ORE)
BENZENE ELUATES
(INSIDE)
A CEYLON
B MEXICO (SOFT)
C MEXICO (HARD)
TEMP., 180 °C
RECORDER RESPONSE
0
30
60
90
TIME (MINUTES)

Methyl naphthalenes are also among the predominant compounds identified by Pedersen and Lamb (379) in coal-graphite. The aliphatic hydrocarbons in terrestrial graphite may be assumed to be (1) products of reaction whereby petroleum is formed from graphite and hydrogen, (2) residues from biogenic organic matter that eventually became graphite, (3) hydrocarbons that have entered the graphite from external sources and/or (4) diagenetic products of biological activity associated with the graphite deposits. However, in relation to the first possibility, the different homologies detected, especially that of the isoprenoids, certainly indicate a biogenic origin (231,357). It would be hard to imagine how an abiotic process could be capable of producing the kind and selective distribution of the hydrocarbons found in graphite. Such a regularity is usually associated with biological processes. (This will be considered in a more detailed manner in the general discussion, Chapter VIII). Thus the assumption that hydrocarbons in graphite could be the result of reactions involving graphite and hydrogen (306,307) is not considered likely.

The difficulties inherent to case (2) are that hydrocarbons may not survive the conditions necessary to change organic matter to graphite and that all graphite is not of organic origin.

Because of the porosity of graphite ore and the high absorbance of graphite for hydrocarbons (182,382), the possibility of an external origin of the hydrocarbons in graphite is a real one. Also, in view of the ubiquity of hydrocarbons on the earth (130,173,365,367,371) this mode of origin probably presents fewer problems than any of the others. Fossil hydrocarbons may migrate into the graphite from surrounding formations; microorganisms and other living systems such as algae, higher plants and some species of animal life can contribute either directly or via the air and water systems to the particular hydrocarbon patterns observed in the graphite samples examined. Air and water, especially the latter (377,378) are involved in the mobilization and transport of hydrocarbons from living things, fossil hydrocarbons and human activity.

The adsorbance of these hydrocarbons by the graphite, initiates an accumulation process that may eventually lead with time to the distributions observed (Figures 58-62).

Apparently the lack of correlation observed between the hydrocarbon content and the presumed origin of the graphite ores and also the differences in the distributions (Figures 58A and 58B) could be ascribed to differences in the adsortivity of each individual sample.

Furthermore the aromatic hydrocarbons found in graphite ore are similar to those found in kerosene (72) and are

further evidence of contamination by the more volatile components of petroleum. On the other hand the distribution of the observed overall isoprenoid homology (C_{14} to C_{22}) appears to indicate a process involving diagenetic changes of the organic matter initially accumulated in the graphite formations as a result of biological activity. Contemporary living organisms are not known to produce such an array of isoprenoids. At this point it may be advanced that the nature and characteristic bimodal distribution of the isoprenoid hydrocarbons can be taken as a strong indication of the bioorganic origin of the extractable material in these graphites regardless of their actual source.

VI.

PRODUCTS OF ABIOLOGICAL SYNTHESES

FISCHER-TROPSCH SYNTHESES

Introduction

It has been suggested (355) that the Fischer-Tropsch process might have been involved in the abiological synthesis of biochemical compounds. This suggestion was based on the fact that 2-methylbutane has been demonstrated to be formed in substantial amounts by the above catalytic process (166) and that this C_5-branched alkane (isoprane) could be considered as a precursor of the side chain of the amino acids leucine and isoleucine (355) and also probably as the monomeric unit out of which saturated polyisoprenoid hydrocarbons could be synthesized. In order to explore this possibility two series of experiments have been conducted under conditions leading to Fischer-Tropsch type syntheses. Iron and chondritic meteorites were used as catalysts on one phase of the work. Industrial metals, mainly iron-nickel alloys, were used in another parallel series of syntheses. The products obtained from these experiments have been compared to those from a Fischer-Tropsch sample received from the U. S. Bureau of Mines. The distribution patterns of hydrocarbons in both types of synthetic products appear to be certainly related in a qualitative sense.

Data collected from this work clearly demonstrates first, that certain types of meteorites are capable, when acting as

catalysts in Fischer-Tropsch processes, of producing a distribution of hydrocarbons which bears a close resemblance to that produced by other metallic catalysts of industrial origin. Second, that there is no conclusive evidence thus far on the presence of polyisoprenoid hydrocarbons in the synthetic products thus obtained.

Experimental

The experimental procedure is similar to that used in our laboratory for the synthesis of other hydrocarbons (359) and may be briefly summarized as follows: A Pyrex U tube, 20 cm long with an inside diameter from 3 to 11 mm was filled with catalyst. A description of the catalysts used can be found in Table XLII. Before placing the finely divided catalyst in the tube it was extracted to remove any hydrocarbons which could be present from any source. Prior to carrying out the actual experiment of synthesis the tube was heated to temperatures of about 300^{o}C for two to five hours and deuterium or hydrogen was allowed to pass through the catalyst. This was followed by a similar heating period during which carbon monoxide was passed through the same catalyst. These preparatory treatments were performed to remove any possible traces of residual hydrocarbons from any source which might have been present in the catalyst or the apparatus, being for that reason good control blanks.

TABLE XLII

FISCHER-TROPSCH CATALYSTS

IRON METAL	99.5%, electrolytic; annealed -100 mesh (Consolidated Astronautics, Inc. 41-45 Crescent St. Long Island, New.York 11101)
NICKEL METAL	99.7%, H_2 reduced -100 mesh (Consolidated Astronautics, Inc.)
NICKEL-IRON	58%Ni - 42%Fe, -325 mesh (Consolidated Astronautics, Inc.)
NICKEL METAL	58%Ni on Kieselguhr (Harshaw Chemical Co. 1945 East 97th Street Cleveland, Ohio 44106)
NICKEL METAL	Powder 85% Ni-15% Carbon (Electronic Space Products, Inc. 854 So. Robertson Blvd., Los Angeles, California 90035)
METEORITIC IRON	Canyon Diablo Iron #34.6050 (American Meteorite Laboratory Denver, Colorado)

Gas chromatographic analysis of these blanks, carried out in an identical manner as in the case of the actual experiments, showed that the overall level of contamination was practically negligible.

After these pretreatments, a mixture of carbon monoxide and deuterium was allowed to flow through the catalyst at temperatures between 250 and 500°C for periods of 11 to a maximum of 19 hours (Table XLIII). After several experiments, 300°C was found to be the optimum synthesis temperature at these conditions. In most of the experiments the ratio CO/D_2 was 1/2. The flow rate in a typical experiment was 3.3 cm^3/min for CO and 6.6 cm^3/min for D_2. More details are given in Table XLIII. All syntheses were carried out at atmospheric pressure. The volatile products coming out of the heated tube were trapped in a cold-finger tube cooled with ice water. In general the system was very similar to that used by J. Han (361) in his preliminary experiments.

The cold-finger tube contained about 5 ml of n-heptane. At the end of the heating period, the n-heptane from the tube was transferred to a beaker together with rinsings (about 5 ml of n-heptane) of the cold-finger tube. The n-heptane was evaporated to almost dryness (0.1-0.5 ml) and the residue then transferred quantitatively to the top of a silica gel tube (1 x 18 cm) for separation into three fractions (n-pentane,

TABLE XLIII

FISHER TROPSCH SYNTHESES

EXPERIMENTAL DATA AND RESULTS

FT No.	Catalyst	Amount (gr)	Temp. (°C)	Time[1] (Hrs)	Range	Yield (ppm)	Ratio n/br[3]	Fig[2]
106	Ni-Fe121	9.85	300	11:30	C_{11}-C_{19}	9.3	3.5	
110	Ni-Fe2110	10.46	300	15:00	C_{11}-C_{23}	5.2	4.1	
114	Ni-Fe2110	11.56	295	19:00	C_{11}-C_{23}	22	4.5	67
115	Ni-Fe2110	10.64	200	15:45	C_{9} -C_{14}	2.2	5.0	
116	Ni-Fe2110	9.34	300	15:45	C_{12}-C_{22}	28.5	4.1	
117	Ni-Fe2110	11.29	300	16:25	C_{9} -C_{20}	3.9	11.0	
118	C.D.[4]	8.8	300	17:10	C_{11}-C_{21}	14.9	4.0	67
119	C.D.[4]	6.04	285-300	17:00	C_{9} -C_{26}	233.6	1.7	71
120	C.D.[4]	7.76	300	18:00	C_{10}-C_{24}	19.7	2.2	69
121	C.D.[4]	8.58	300	17:55	C_{10}-C_{26}	13.5	2.4	
124	Ni-Fe2110+S	11.07	288-305	15:30	-	-	-	67
127	Ni-Fe2110	13.03	298	12:30	C_{10}-C_{24}	12.2	4.3	68

TABLE XLV CONTINUED

FISHER TROPSCH SYNTHESES

EXPERIMENTAL DATA AND RESULTS

FT No.	Catalyst	Amount (gr)	Temp. (°C)	Time[1] (Hrs)	Range	Yield (ppm)	Ratio n/br[3]	Fig[2]
128	Ni-Fe2110/SiO_2	9.01	295-305	17:50	C_{10}-C_{22}	21.2	3.4	72
129	Fe	11.6	290-307	16:15	C_{10}-C_{23}	5.4	3.0	66
131	Ni	3.68	300	15:15	C_{10}-C_{15}	0.6	-	65
132	C.D.+Graphite	4.56	300	19:00	C_{10}-C_{19}	10.4	-	73
133	Ni50/SiO_2	6.56	298-315	15:40	-	0.07	-	65
135	Ni-Fe062	8.89	300-325	16:30	C_{11}-C_{17}	0.2	-	
136	Fe	10.5	292-310	18:30	-	6.5	-	

[1]Total synthesis time with the mixture of CO + D_2, except for the 106 and 110 FT where H_2 was used instead of D_2

[2]The corresponding gas chromatographic pattern will be found in the Figure indicated

[3]Ratio of n-alkanes/branched alkanes

[4]C.D.; short for Canyon Diablo iron

benzene, and methanol eluates) as described earlier. The purity of all solvents used in the processing of the samples was checked by gas chromatography as it has already been described in Chapter III. In general only the n-pentane eluate from the silica gel column was analyzed for aliphatic hydrocarbons. For this, two gas chromatographs (an F and M Model 810 and a Barber Colman Series 5000) equipped with temperature programmers, capillary columns, and flame ionization detectors were used. The features of the open tubular columns and stationary phases are given in Table I. Other details of the gas chromatographic analysis may be also found in Chapter III.

The Fischer-Tropsch product from the U. S. Bureau of Mines in Pittsburgh, Pennsylvania, was supplied by Dr. A. G. Sharkey, Jr. The sample was labelled "C_5 + Products from F-T Cat. D 3001. Tested in 1963 J. F. Schultz." A preliminary analysis of this sample by gas chromatography indicated the presence of a large number of components, mainly between C_9 and C_{32} (Figure 63).

The combined gas chromatographic-mass spectrometric analysis were performed using an LKB 9000 Gas Chromatograph-Mass Spectrometer.

1. Analysis of Fischer-Tropsch products

A partial gas chromatogram of the n-pentane eluate of the Fischer-Tropsch product obtained from the U. S. Bureau of

Mines is shown in Figure 63. The two major peaks in each carbon number correspond to the n-alkane and the α-olefin of the same number of carbon atoms as demonstrated by their respective mass spectra. These two peaks are followed by another two olefins and an anteiso alkane (see Figure 63) in a very repetitive fashion as it would be expected from a regular chain build up synthesis (12). Also the gas chromatographic-mass spectrometric analysis of the same sample using a higher resolution Polysev capillary column showed the presence of a greater number of minor components (about 15 partially resolved peaks within each n-alkane). Good mass spectra were obtained for the 6-methyl C_{14}, 2-methyl C_{14}, 7-methyl C_{16}, 5-methyl C_{16} and 2-methyl C_{16}. The ratio of branched to normal hydrocarbons could not be determined since the product was not hydrogenated before its GC analysis (429). As it can be seen in Figure 64 the olefins are clearly predominating in this particular case up to the C_{16} hydrocarbon. It has been shown that olefins are produced by Fischer-Tropsch processes, and that olefin production is a function of (1) the ratio H_2:CO, (2) the space velocity within the catalyst bed, (3) temperature, (4) nature of the catalyst and (5) pressure (460). The high amount of olefins in this sample would be consistent with a low H_2:CO ratio, high space velocity and high temperature, although the actual experimental data is not available. The analysis of the "raw" catalyst used to synthesize these

FIGURE 63

GAS CHROMATOGRAPHIC SEPARATION OF ALKANES

FROM A FISCHER-TROPSCH PRODUCT

Stainless steel capillary column (150 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph. Range, 10^2; attenuation, 1. Nitrogen pressure 1050 g/cm^2. No split. Isothermal at 120°C for 10 minutes, then programmed to 200°C at 2°C/min.

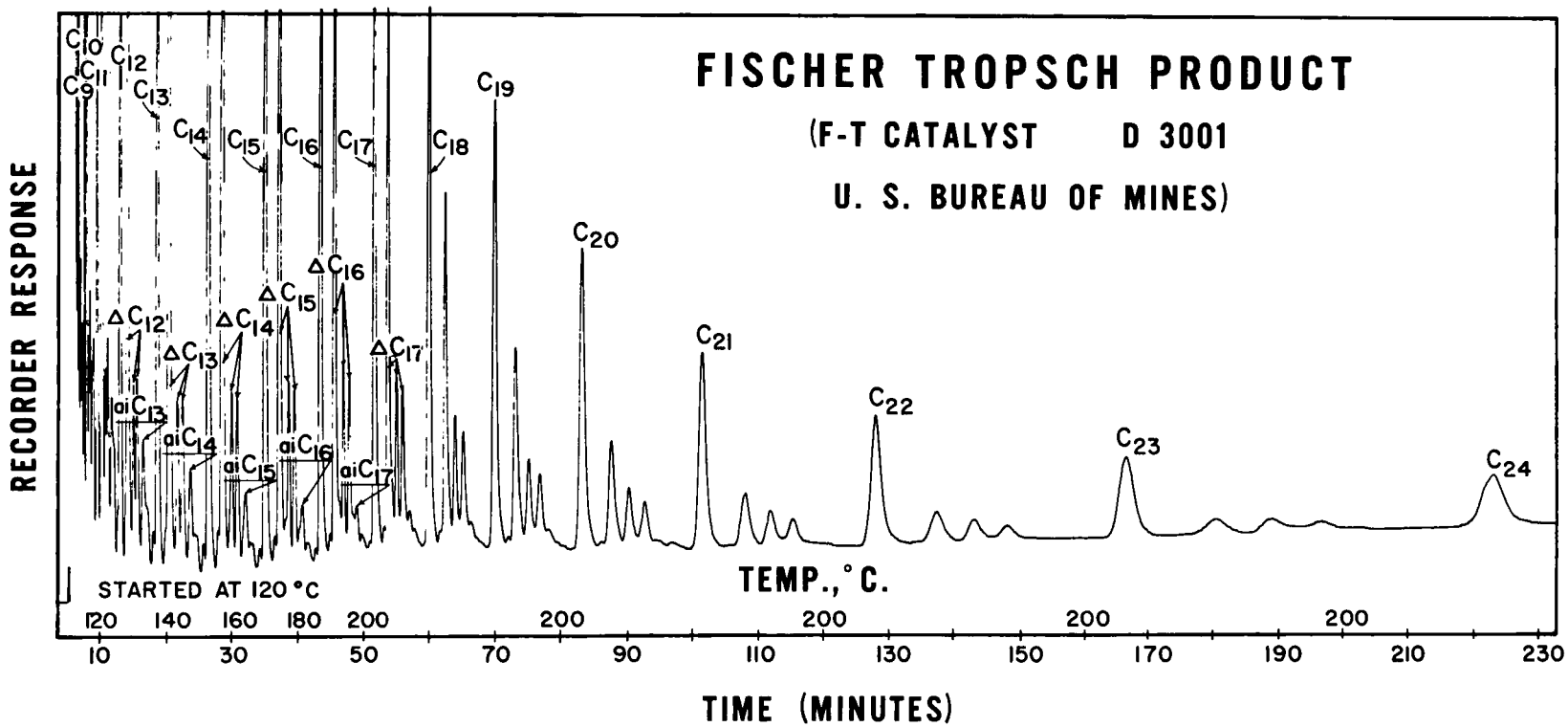
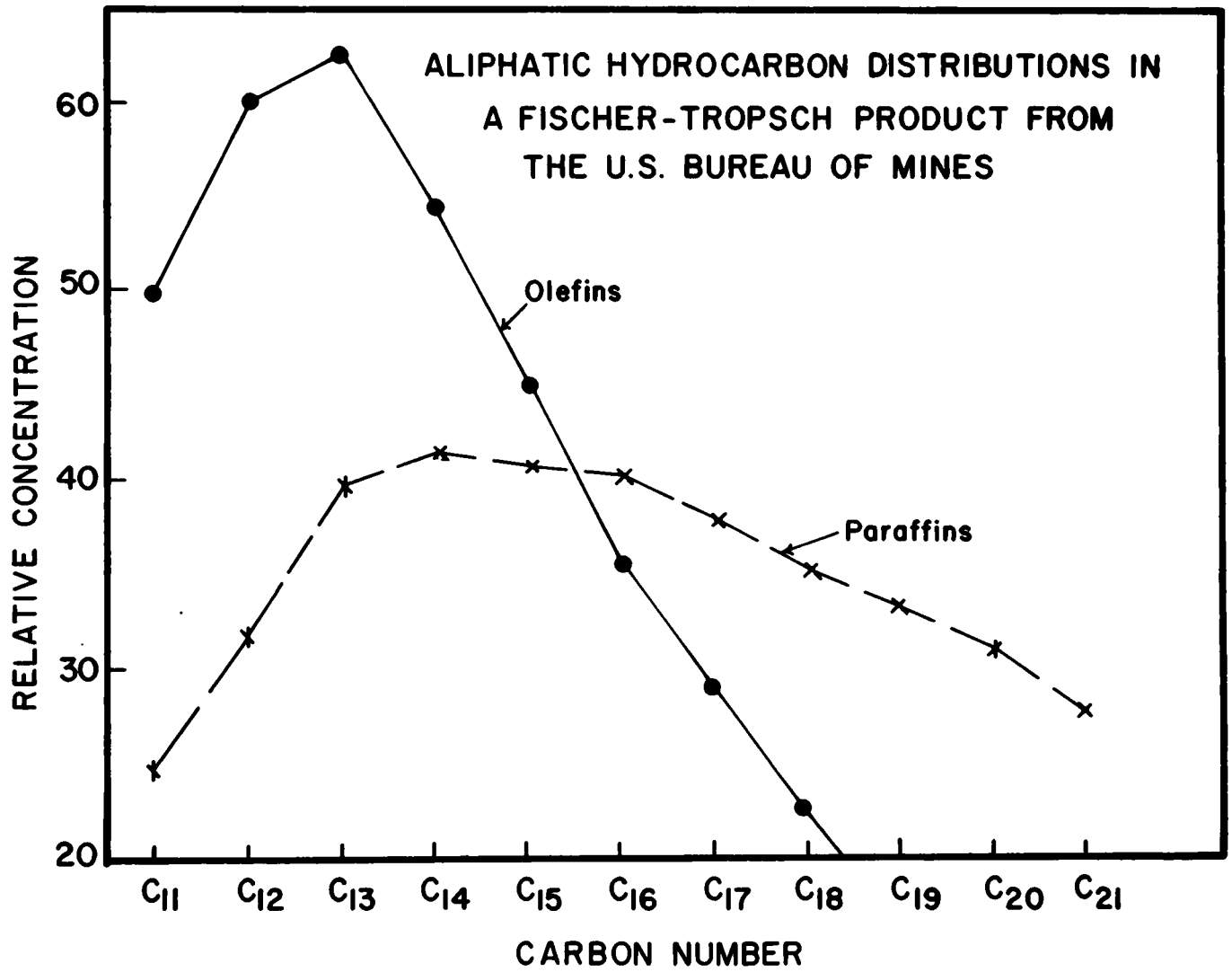

FISCHER TROPSCH PRODUCT
(F-T CATALYST D 3001
U. S. BUREAU OF MINES)
RECORDER RESPONSE
C9
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
C23
C24
ΔC12
ΔC13
ΔC14
ΔC15
ΔC16
ΔC17
aiC13
aiC14
aiC15
aiC16
aiC17
STARTED AT 120 °C
TEMP., °C.
120 140 160 180 200 200 200 200 200
10 30 50 70 90 110 130 150 170 190 210 230
TIME (MINUTES)

FIGURE 64

ALIPHATIC HYDROCARBON DISTRIBUTIONS IN A FISCHER-TROPSCH PRODUCT FROM THE U.S. BUREAU OF MINES

Graphical representation of the areas under the olefin and paraffin peaks shown in Figure 63.


ALIPHATIC HYDROCARBON DISTRIBUTIONS IN
A FISCHER-TROPSCH PRODUCT FROM
THE U.S. BUREAU OF MINES
Olefins
Paraffins
RELATIVE CONCENTRATION
60
50
40
30
20
C11
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
CARBON NUMBER

hydrocarbons yielded a 67.4% of iron (426). It has been pointed out that olefin formation increases from nickel to cobalt to iron catalysts (460). The overall distribution pattern agrees with that expected from a Fischer-Tropsch synthesis. It has been shown that the distributions of hydrocarbon products from hydrogen and carbon monoxide are characterized by their continuously decreasing concentration curves from the C_5 to the C_{32} alkane. The fact that the distribution shown in Figure 63 shows maxima at C_{13} and C_{14}, more clearly indicated in Figure 64, is most likely a consequence of the losses in volatile alkanes suffered in the process of sample preparation.

The rapid decrease in the α-olefin content of this sample appears to support the mass spectrometric identification, since it has been reported that with iron catalysts α-olefins predominate over other internal double bond isomers and that they decrease with molecular weight (165). Although earlier work (360) on this same sample had shown the presence of peaks with the retention times of the isoprenoids pristane and phytane in the chromatograms obtained in four different stationary phases, a thorough mass spectrometric study of the sample has not yielded any detectable amounts of these isoprenoids. This is in itself a good example that illustrates the dangers of using gas chromatography alone for the identification of

a particular compound in a complex mixture of isomers with very close boiling points as it has been emphasized several times already. The peak that was assumed to be pristane (360), on the basis of its retention time in four different columns, has been shown to be the 3-methyl hexadecane, according to its mass spectral pattern.

2. Fischer-Tropsch laboratory syntheses

In order to check on the possibility of isoprenoid synthesis by the Fischer-Tropsch reaction, a total of 53 runs involving the reaction of either H_2 or D_2 and CO in a continuous flow system were carried in the laboratory. In about 25 of these runs deuterium was used, instead of hydrogen, to minimize the possibilities of contamination. The presence of contaminants in the system is easily spotted in the mass spectra because of the differences in the masses of the deuterated vs the undeuterated compounds (see Chapter IV).

Since different kinds of catalysts were used, the results will be grouped into three different categories: (a) Fischer-Tropsch synthesis with industrial catalysts, (b) with meteoritic catalyst, (c) with other combination catalysts. Special emphasis was placed on the detection of the possible presence of isoprenoid structures.

a) Fischer-Tropsch synthesis with industrial catalysts

In the series of experiments involving industrial catalysts, iron, nickel and alloys of nickel-iron were used

in the syntheses. A description of the characteristics of these catalysts can be found in Table XLII.

(1) Nickel catalysts

Nickel (Table XLII) was not found to be an efficient catalyst under the conditions prevailing in these experiments. The maximum yield of hydrocarbons obtained with this catalyst was 0.6 ppm (see Figure 65A).

(2) Iron catalysts

Iron, on the other hand, gave a typical Fischer-Tropsch distribution of hydrocarbons in the range C_{10}-C_{23} (Figure 66 and Table XLIII). The carbon number distribution of the n-paraffins is in this case similar to that reported by Sharkey et al. (429) for a hydrogenated Fischer-Tropsch product in that it also appears to have a second distribution mode. In this case it is centered around C_{16}; ten carbon atoms lower than in the distribution curve reported by Sharkey et al. (429). The branched paraffin distributions between each carbon number are very closely related to those usually encountered with nickel-iron catalysts (Figures 67,68), with the exception that in this case the olefin content is much higher as evidenced by the height of the first peaks after each n-alkane. This is in agreement with the expected effect of the iron as a catalyst in these reactions (460).

(3) Nickel-iron catalysts

When iron and nickel were used together in

FIGURE 65

GAS CHROMATOGRAPHIC SEPARATIONS OF PERDEUTERO ALKANES IN FISCHER-TROPSCH PRODUCTS FROM NICKEL CATALYSTS

Stainless steel capillary column (195 m long x 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Range, 10; attenuation, 2. Nitrogen pressure, 1050 g/cm^2. No split. Temperature of injection was held at 90°C for 10 minutes and then programmed to 190°C at 1°C/min.

(A) Synthesis experiment 131 FT (Table XLIII).

(B) Blank run before 131 FT.

(C) Synthesis experiment 133 FT (Table XLIII).

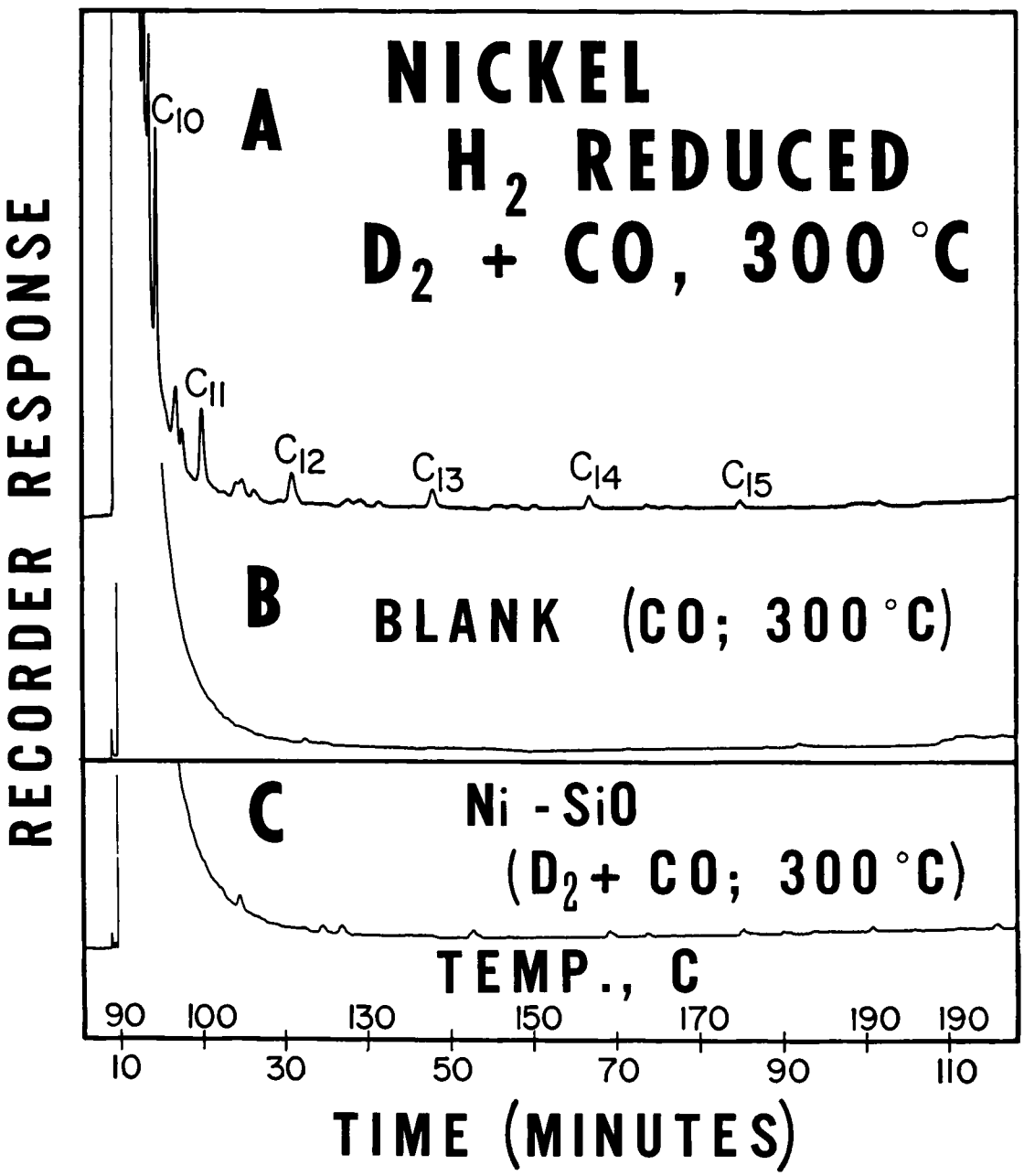
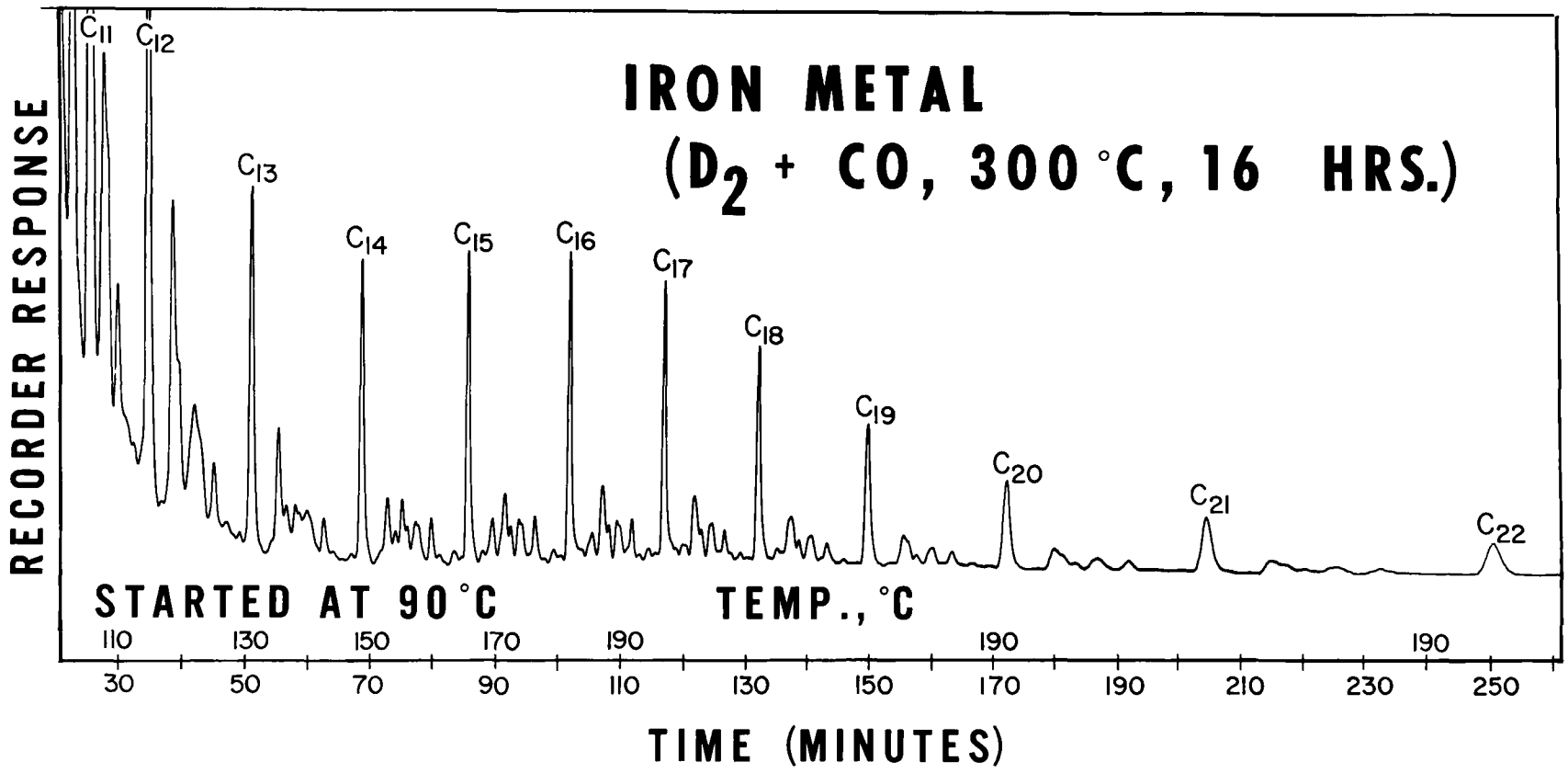

NICKEL
H2 REDUCED
D2 + CO, 300 °C
A
C10
C11
C12
C13
C14
C15
B
BLANK (CO; 300 °C)
C
Ni - SiO
(D2 + CO; 300 °C)
RECORDER RESPONSE
TEMP., C
90 100 130 150 170 190 190
10 30 50 70 90 110
TIME (MINUTES)

FIGURE 66

GAS CHROMATOGRAPHIC SEPARATIONS OF PERDEUTERO ALKANES IN A FISCHER-TROPSCH PRODUCT FROM AN IRON CATALYST

Same conditions as described in Figure 65.

Experiment 129 FT. (Table XLIII)


IRON METAL
(D_2 + CO, 300 °C, 16 HRS.)
RECORDER RESPONSE
C_{11}
C_{12}
C_{13}
C_{14}
C_{15}
C_{16}
C_{17}
C_{18}
C_{19}
C_{20}
C_{21}
C_{22}
STARTED AT 90 °C
TEMP., °C
110
130
150
170
190
190
190
30
50
70
90
110
130
150
170
190
210
230
250
TIME (MINUTES)

prealloyed mixtures (Table XLII), moderate amounts of hydrocarbons were produced.

A summary of the most characteristic runs can be found in Table XLIII. Typical gas chromatographic patterns are given in Figures 67 and 68. Normal alkanes are the major components in the products which is a fact well established by previous work (166,460,461). In all cases the overall hydrocarbon distribution in these synthetic products was found to decrease in an exponential mode towards the high molecular weight paraffins. The true maxima of such distributions lies most likely around the C_5 paraffin as mentioned above (495). Because of the interference of the solvent front, the low molecular weight paraffins can not be studied. Their chromatographic determination would have required the use of a stationary phase more suitable for the separation of the more volatile compounds, such as squalene. But since this region has been the most extensively studied (166,460) and the isoprenoid compounds of interest in this investigation lie in the higher C_{10}-C_{20} range, no work was undertaken in that direction.

The samples were carefully stripped off their solvent by forced evaporation under a stream of purified nitrogen (see Chapter III) and by not allowing them to go to "dryness" at any moment losses of the volatile components were effectively prevented.

FIGURE 67

GAS CHROMATOGRAPHIC SEPARATION OF PERDEUTERO ALKANES IN THREE FISCHER-TROPSCH PRODUCTS

Stainless steel column (90 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Range, 10; attenuation, 4. Nitrogen pressure 1050 g/cm^2. No split. Temperature of injection held at 120°C for 10 minutes and then programmed to 200°C at 2°C/min.

Canyon Diablo	Experiment 118	(see Table XLII
Nickel-Iron Alloy	Experiment 114	(see Table XLII
Nickel-Iron + Sulfur	Experiment 124	(see Table XLII

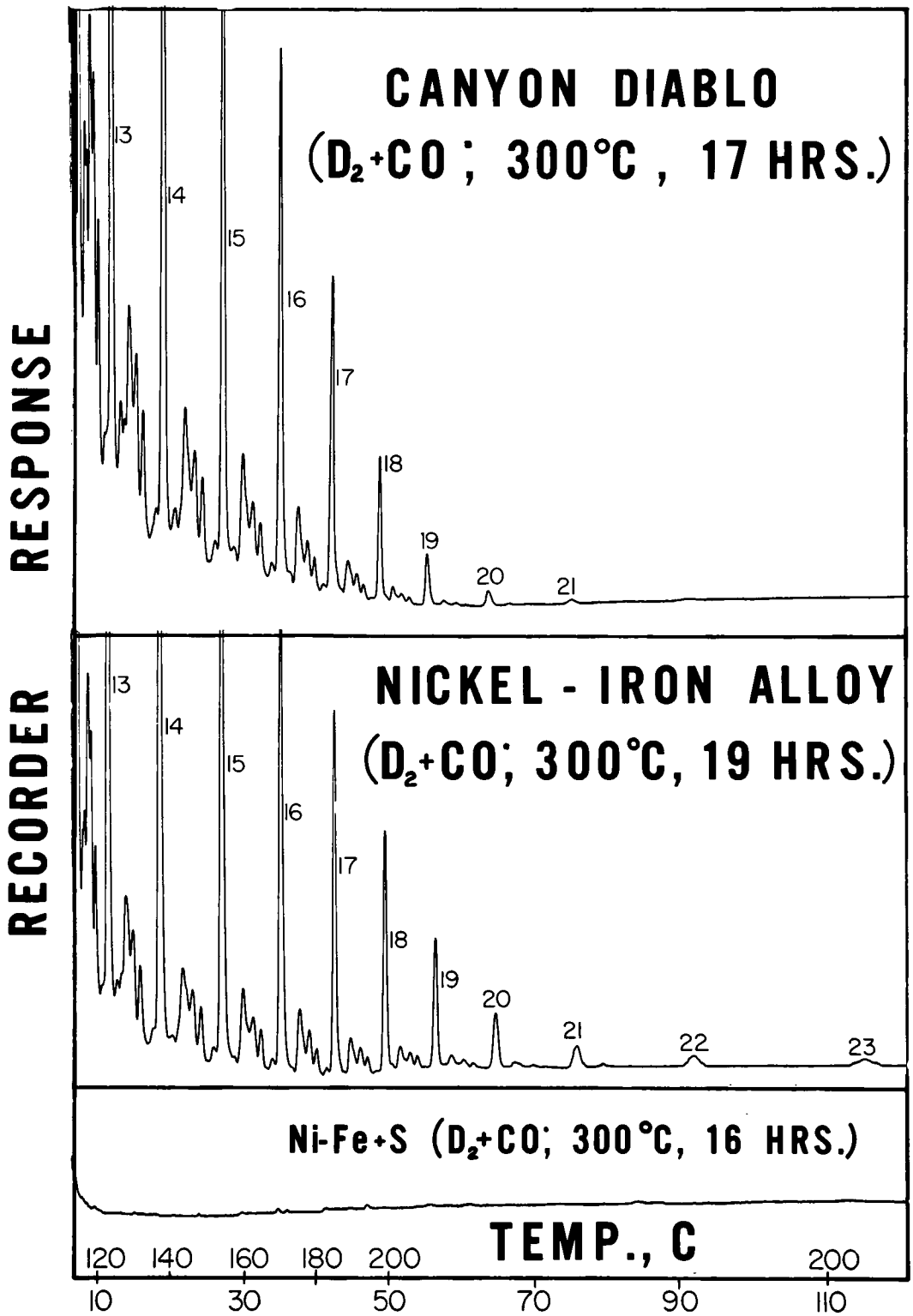
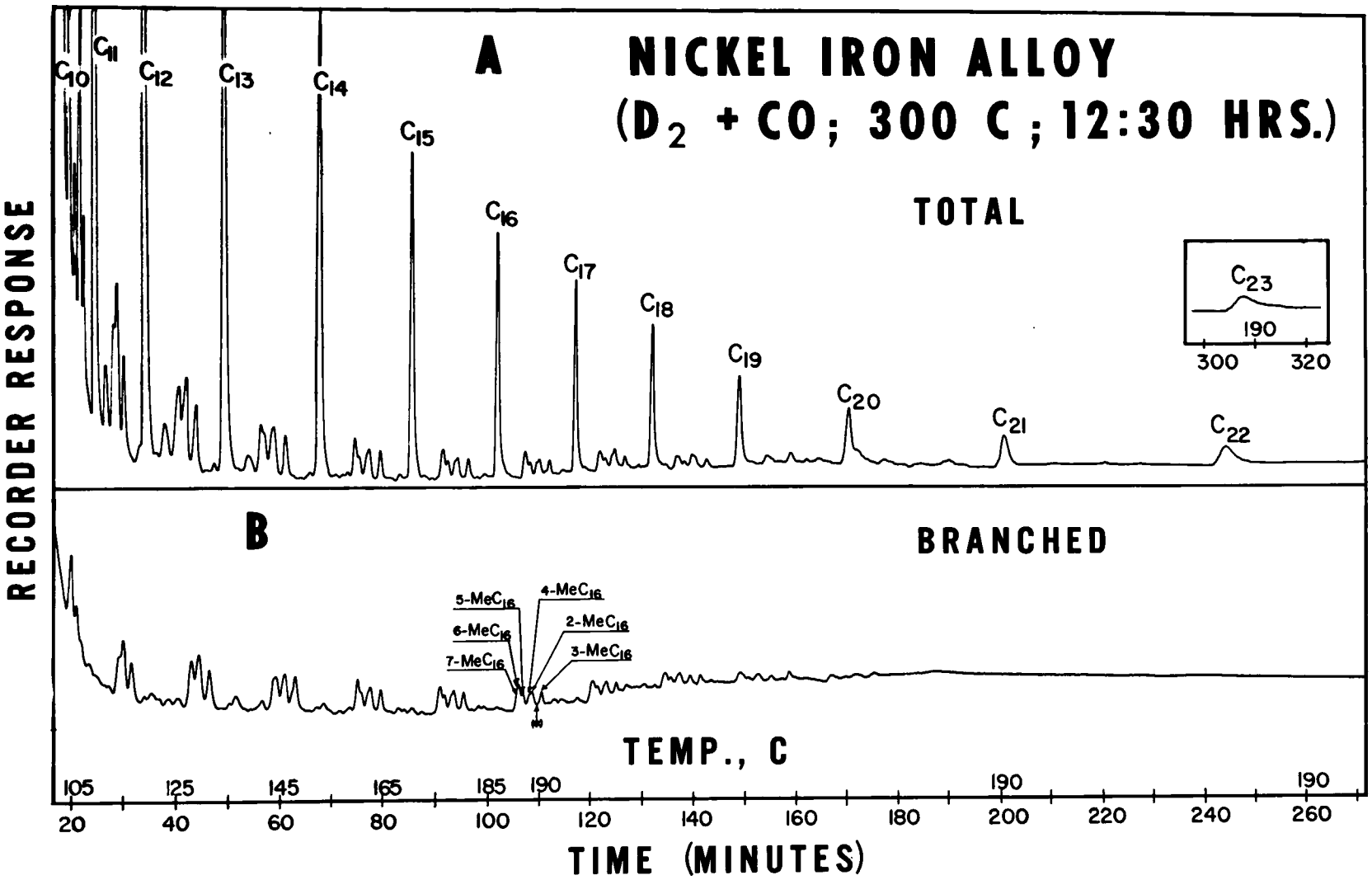

CANYON DIABLO
(D2+CO ; 300°C , 17 HRS.)
RESPONSE
13
14
15
16
17
18
19
20
21
NICKEL - IRON ALLOY
(D2+CO; 300°C, 19 HRS.)
RECORDER
13
14
15
16
17
18
19
20
21
22
23
Ni-Fe+S (D2+CO; 300°C, 16 HRS.)
TEMP., C
120 140 160 180 200
200
10 30 50 70 90 110

FIGURE 68

GAS CHROMATOGRAPHIC SEPARATION OF PERDEUTERO ALKANES IN A FISCHER-TROPSCH PRODUCT FROM A NICKEL-IRON CATALYST

Conditions, the same as described in Figure 65.

Experiment 127 (see Table XLIII).


A
NICKEL IRON ALLOY
(D2 + CO; 300 C ; 12:30 HRS.)
TOTAL
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
C23
190
300
320
B
BRANCHED
5-MeC16
4-MeC16
6-MeC16
2-MeC16
7-MeC16
3-MeC16
TEMP., C
105
125
145
165
185
190
190
190
RECORDER RESPONSE
20
40
60
80
100
120
140
160
180
200
220
240
260
TIME (MINUTES)

The gas chromatographic analysis of these samples, Figures 67 center and 68, shows a regularly repeating sequence of isomeric paraffins and olefins between each of the major n-alkane peaks. In some instances this sequence was found to match quite closely the hydrocarbon pattern of the product from the U.S. Bureau of Mines (Figure 64).

The great similarities between the patterns in Figures 67 and 68 are readily apparent at first glance but upon a closer inspection it can be seen that some of the small single peaks in Figure 67 are split into partially resolved doublets in Figure 68. The increase in resolution was achieved with the use of a more efficient gas chromatographic column but as it was demonstrated with standard mixtures (Figure 8) and also in the case of the branched isomers in meteorites this type of mixtures of isomeric hydrocarbons can not be perfectly resolved, even in the more efficient columns available.

Table XLIV gives a summary of the paraffins and olefins observed in most of the Fischer-Tropsch products analyzed. The positions of the C_{17} methyl branched paraffins are given in Figure 68B. Removal of the n-paraffin peaks from this sample was accomplished by treatment with molecular sieves as described before (Chapter III). Since the compounds between the n-paraffin peaks are of identical nature throughout the entire range and differ only in the number of carbons they contain it is not necessary to label all of them in the

TABLE XLIV

DEUTERATED HYDROCARBONS PRODUCED BY FISCHER-TROPSCH SYNTHESIS AND DETECTED BY THE GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS

n-paraffins,	C_8 to C_{26}
methyl paraffins,	2-meC_{10} to 2-meC_{19}
	3-meC_{10} to 3-meC_{19}
	4-meC_{12} to 4-meC_{19}
	5-meC_{11} to 5-meC_{19}
	6-meC_{12} to 6-meC_{18}
	7-meC_{15} to 7-meC_{17}
	8-meC_{17}
dimethyl paraffins,	2,3-di meC_{14} and C_{15}
	2,4-di meC_{14}
	3,4-di meC_{13}
	3,6-di meC_{11} and C_{14}
	3,7-di meC_{13}, C_{14} and C_{15}
olefins,	C_8 to C_{19}

chromatogram. As indicated in Figure 68B the 7- and 6-methyl alkanes are the most difficult to separate. Only slight resolution is obtained for the 5- and 4-methyl alkanes. The anteiso structure or 3-methyl C_{16}, in this case, is always the best resolved compound. By optimizing all the chromatographic parameters it was possible to achieve a 20-30% improvement in the overall resolution and thus good mass spectra could be obtained for each one of these components. Even then, the first major peak in the group of isomeric methyl alkanes had to be scanned at different points to obtain evidence for the presence of the 8-, 7- and 6-methyl alkanes. No isoprenoids were detected in any of the Fischer-Tropsch syntheses with industrial catalysts. The chromatographic position of pristane as determined with standards (see Figure 8) is marked by an asterisk in Figure 68B.

This type of synthesis process shows a remarkably high degree of reproducibility in terms of the number and nature of the compounds produced though this is not so in a quantitative sense. It is very difficult to control experimental parameters such as localized temperature effects inside the catalyst, packing of the catalyst and the flow rate of the gases through the catalyst bed. For these reasons the great variations in the quantitative yield of the reaction should not be totally unexpected. See for instance the yields reported in Table XLIII for the syntheses 110-117 and 127 in which the same

catalyst was used in comparable amounts but the yields vary from 2.2 ppm to 28.5 ppm.

The ratio of the n-alkanes to the branched fraction in these runs with the Ni-Fe 2110 (Table XLIII) averages 4.4, excluding the one case where n-alkanes were found to predominate over the branched paraffins by a factor of 11. With this exception the value of 4.4 agrees with the reported ratio of 4:1 for the straight chain alkanes over the branched chain hydrocarbons (166). The fact that it is somewhat higher may be explained by considering the contribution of the olefins since in these work the products were not hydrogenated (429).

A different Nickel-iron alloy (see FT 135 in Table XLIII) produced only traces of hydrocarbons.

The effect of the temperature of the catalyst in the production of paraffins was also studied. Several runs were made at different temperatures from 200°C to 450°C. The optimum temperature on the scale of these experiments was found at 300°C. A 50°C increase, produced a very rapid exponential decrease in the paraffin distribution curve which terminated around dodecane. A similar situation was encountered when the temperature of the catalyst was decreased to 200°C (see Table XLIII).

Also an increase in the amount of the catalyst from 10 to 80 g resulted in negligible yields of hydrocarbons and

very rapidly decreasing curves. This effect was almost expected since an increase in the amount of catalyst will result in an increase in the heat generated by the exothermic reaction and if no adequate cooling system (thermostatic control) is provided (440), this will cause excessive local overheating of the catalyst with the resulting decrease in its activity (440).

b) Fischer-Tropsch syntheses with meteoritic iron

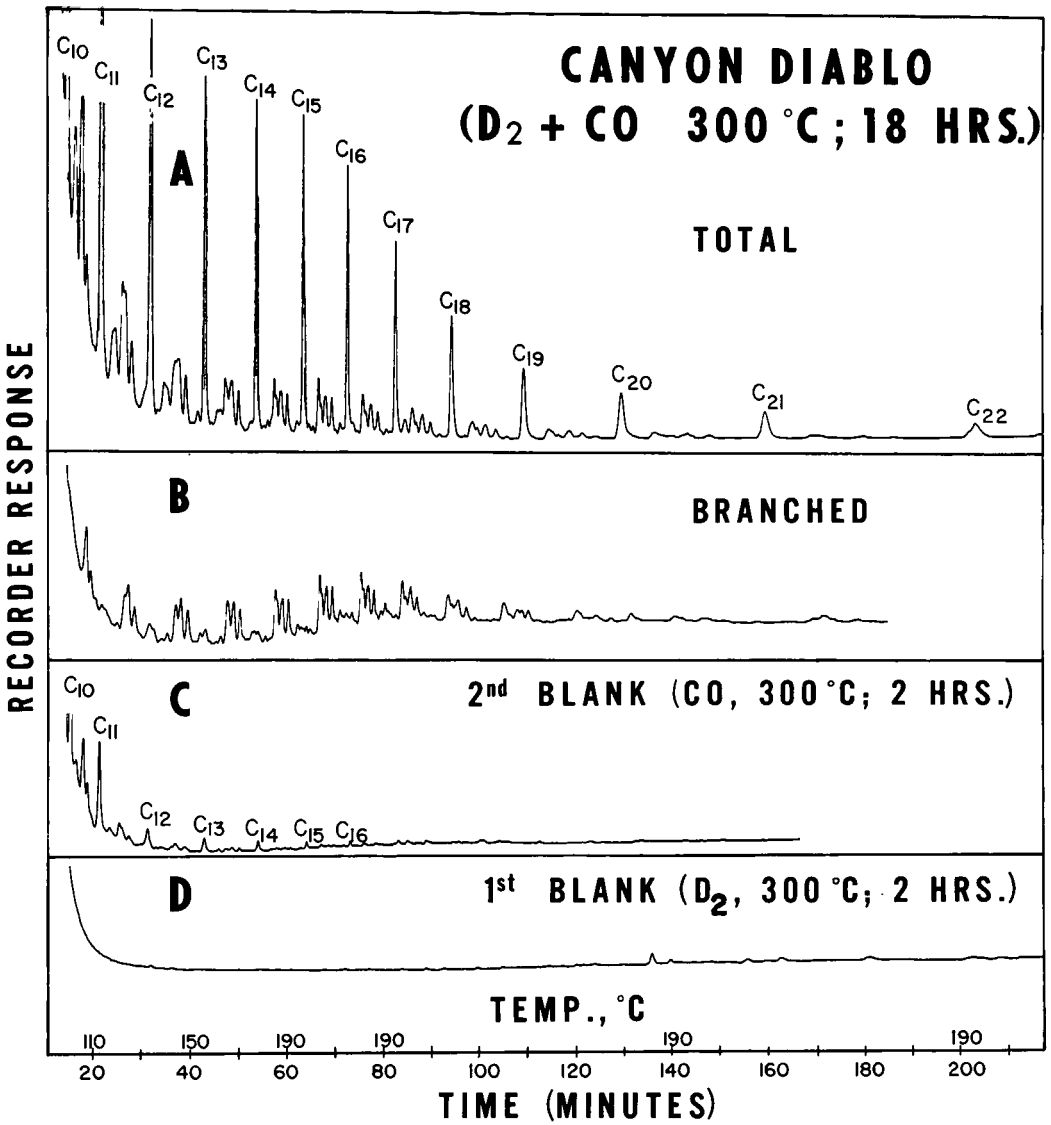
Since the Canyon Diablo iron meteorite also contains a 7.1% of nickel (333), results similar to those obtained with the industrial nickel-iron alloys could be expected. This point is best illustrated by the comparison of the chromatograms given in Figure 67. Another chromatogram of a synthesis product with meteoritic iron is shown in Figure 69A. The n-alkanes of the "total" fraction have been removed by molecular sieving as before and the resulting "branched" fraction is shown on Figure 69B. The gas chromatographic and mass spectrometric analysis of this fraction and of all the Canyon Diablo products in general yielded the same series of monomethyl substituted alkanes (Table XLIV) found in all the other cases involving the use of commercial catalysts for the syntheses. The gas chromatographic patterns of the control blanks are given in Figure 69C and D. In general the D_2 blanks which are collected after deuterium gas is allowed to pass through the

FIGURE 69

GAS CHROMATOGRAPHIC SEPARATION OF PERDEUTERO ALKANES IN A FISCHER-TROPSCH PRODUCT FROM A METEORITIC IRON CATALYST

Same conditions as described in Figure 65.

Experiment 120 (Table XLIII).


CANYON DIABLO
(D2 + CO 300 °C ; 18 HRS.)
A
TOTAL
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
B
BRANCHED
C
2nd BLANK (CO, 300 °C; 2 HRS.)
C10
C11
C12
C13
C14
C15
C16
D
1st BLANK (D2, 300 °C; 2 HRS.)
TEMP., °C
110
150
190
190
190
190
20
40
60
80
100
120
140
160
180
200
TIME (MINUTES)
RECORDER RESPONSE

catalyst for a certain period of time, gave no appreciable amounts of hydrocarbons (Figure 69D). On the other hand in some of the CO blanks, collected after the subsequent passing of CO gas alone, there is a pattern of peaks that resembles the Fischer-Tropsch distributions. The amounts though are much smaller. Their mass spectrometric analysis showed all of them to be deuterated hydrocarbons as deduced by the gas chromatographic elution times (Figure 69C). Since the CO blanks are started right after the D_2 gas flow is cut off, it appears that the residual amount of deuterium adsorbed on the catalyst surface reacts with the CO to produce minor yields of synthetic hydrocarbons. In a short time when all the D_2 has been consumed the reaction stops.

The partial mass spectra of the 2-, 3-, 4- and 5-perdeutero methyl alkanes can be found on Tables XLV, XLVI, XLVII, and XLVIII. A discussion of the characteristic fragmentation patterns of these deuterated isomeric alkanes has been given in Chapter IV (see Table XVIII). The spectra as mentioned there do not differ in any appreciable way from the spectra of the corresponding undeuterated alkanes (see Table IV and Figure 7).

The hydrocarbon patterns obtained by J. Han (361) during the first phase of these synthesis are shown in Figure 70, where the results obtained at different temperatures are given. The distributions in this figure clearly show that any increase in the temperature of the catalyst over the optimum range

TABLE XLV

PARTIAL MASS SPECTRA OF DEUTERATED 2-METHYL ALKANES

m/e	M	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}
50		41	26	24	25	22	20	17	18	12
66		126	79	81	97	86	90	81.5	85	89
82		100	100	100	100	100	100	100	100	100
98		58	61	77	78	75	75	76	76	74
114		19	13	24	39	40	39	40	43	40
130		37	8	10	21	25	28.5	28	33	31
146		7	34	9	13	18	21.5	24	25	27
162		13.5	3.5	43	9	11	15.5	18.5	22	24
178	(180)	- (8)	12	5	48	9	10	13	20	20
194	(196)		- (4)	13	3.5	60	8.5	10	15	18
210	(212)			- (4)	21	4.5	64	8.5	12	15
226	(228)				- (4.5)	20	4.5	65	11	12
242	(244)					- (5.5)	21	4.5	61	10
258	(260)						- (7)	24	6	58
274	(276)							- (6.5)	26	6
290	(292)								- (7.5)	26
	(308)									(6.5)

Molecular ions shown in parentheses.

TABLE XLVI

PARTIAL MASS SPECTRA OF DEUTERATED 3-METHYL ALKANES

m/e	M	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}
50		25	29	12.5	10	7	7	50	7	6
66		100	100	100	100	100	100	100	100	100
82		107	83.5	79	83.5	85	83	98	93	88
98		58.5	89	72.5	67.5	68	79	83	85	83
114		5	20.5	27.5	18	23	34	39	40	42
130		6	6.5	15	24	27	28.5	30	30	34
146		46	7	6.5	12	16	22	27	30	27
162		4.5	50.5	6	8	11	18	24	22	26
178	(180)	- (3.5)	4.5	53.5	7	11	13	20	23	22
194	(196)		- (3)	7	67	7	14.5	14	16	20
210	(212)			- (3)	7	72	9	15	11	16
226	(228)				- (3)	7	81	12	14	10
242	(244)					- (3)	10.5	102	8	13
258	(260)						- (3)	11	112	10
274	(276)							- (4)	11	120
290	(292)								- (3)	12
	(308)									- (3)

Molecular ions shown in parentheses.

TABLE XLVII

PARTIAL MASS SPECTRA OF DEUTERATED 4-METHYL ALKANES

m/e	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}
50	19	13.5	8	10	9	9
66	79	56	60	61	58.5	58
82	100	100	100	100	100	100
98	69.5	57	76	70	67	63
114	37.5	15	27.5	30	26	28
130	18.5	11	22	23	23	22
146	9	6.5	16	21	20	22.5
162	8	8	10	13	15	18
178	49	9.5	8	9	11	16
194	6	49.5	9	11	9	13
210	26	6	43	11	10	9
226 (228)	- (5)	9	6.5	62	9	11.5
242 (244)		- (3)	9.5	6	65.5	10
258 (260)			- (3)	11	8	69
274 (276)				- (3.5)	11	8
290 (292)					- (3)	13
(308)						- (3)

Molecular ions shown in parentheses.

TABLE XLVIII

PARTIAL MASS SPECTRA OF DEUTERATED 5-METHYL ALKANES

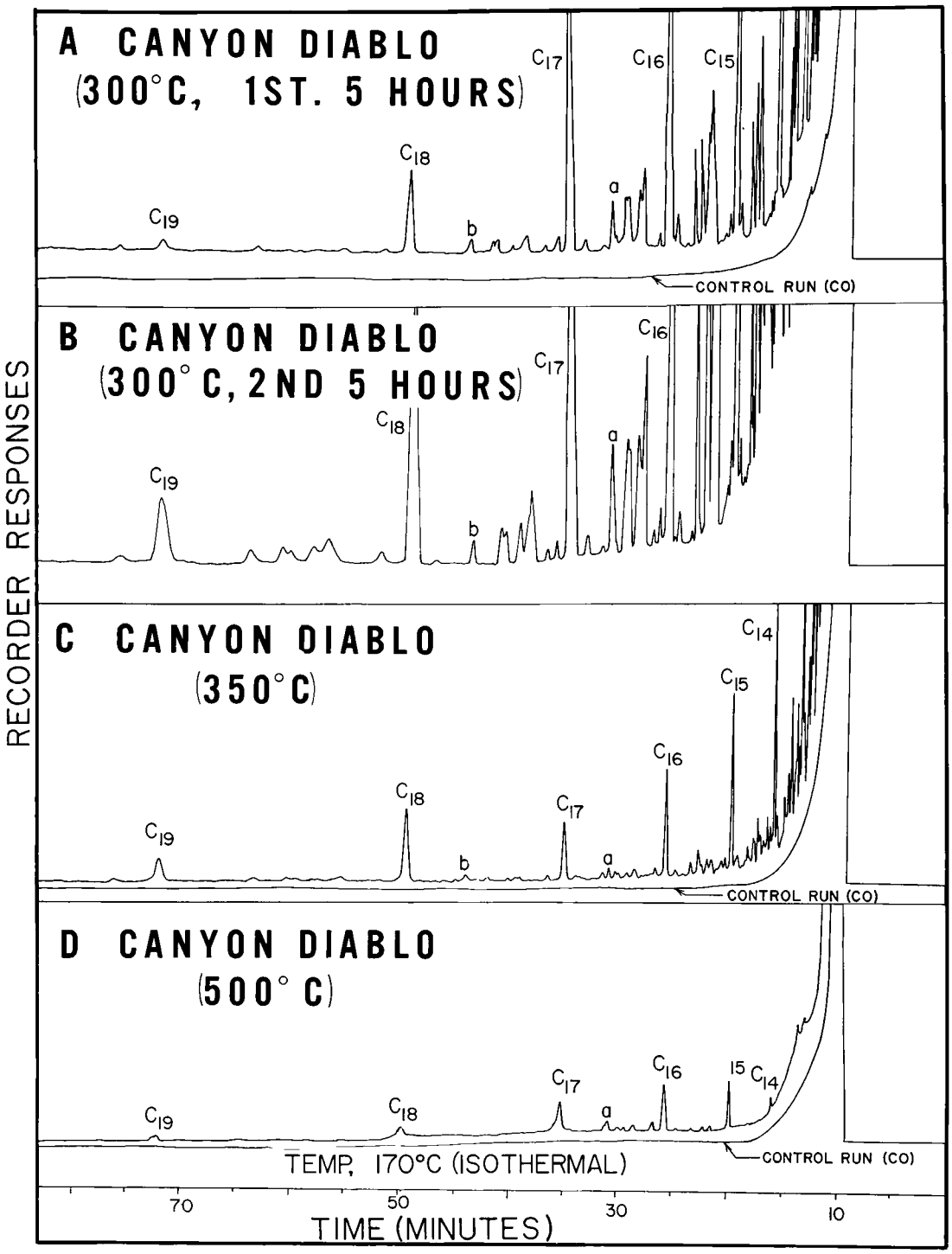
m/e	C_{12}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}
50	46.5	26	15	11	11	8	9
66	185	78	81.5	78.5	73.5	60	50.5
82	101	71	66	68	67.5	57	50.5
98	100	100	100	100	100	100	100
114	25	19.5	25	30	30	28	16
130	40	10	14.5	20	21.5	21	20
146	8	9.5	11	13.5	18.5	18	18
162	11	27	10	10	12	14	19
178	10	4	24.5	12	11	11.5	13
194 (196)	- (3)	6	5	34	10.5	9.5	9
210 (212)		7.5	8	5	40	10	10
226 (228)		- (3.5)	7.5	9	7	40	9
242 (244)			- (3)	9.5	9.5	6	40.5
258 (260)				- (3)	11	10	7
274 (276)					- (3.5)	10.5	8
290 (292)						- (3.5)	11.5
(308)							(2.5)

Molecular ions shown in parentheses.

FIGURE 70

GAS CHROMATOGRAPHIC SEPARATION OF ALKANES PRODUCED AT DIFFERENT TEMPERATURES BY FISCHER-TROPSCH SYNTHESIS ON METEORITIC IRON

Aliphatic hydrocarbons produced at 300°C, 350°C, and 500°C when $CO:H_2$ = 1:2 were passed through 5 g of Canyon Diablo. Gas chromatograms were obtained on Polysev stainless steel 305 m long and 0.76 mm inside diameter. Isothermal at 170°C and hydrogen (1400 g/cm^2) as carrier gas were used for this purpose.


A CANYON DIABLO
(300°C, 1ST. 5 HOURS)
B CANYON DIABLO
(300° C, 2ND 5 HOURS)
C CANYON DIABLO
(350°C)
D CANYON DIABLO
(500° C)
RECORDER RESPONSES
C19
C18
C17
C16
C15
C14
a
b
CONTROL RUN (CO)
TEMP. 170°C (ISOTHERMAL)
70
50
30
10
TIME (MINUTES)

and conditions of the reaction, decreases the yield of the synthesis significantly as mentioned above.

At that time it was claimed that the peaks labelled a and b in Figure 70 corresponded to pristane and phytane respectively. This appeared to be rather well established by the comparison of the retention times of those two peaks and the corresponding standards in four different columns coated with different stationary phases (Igepal, Apiezon L, SF-96 and Polysev). On the other hand it has already been shown (Figure 8) that pristane is usually eluted together with the iso and anteiso C_{17} and phytane is eluted together with the anteiso C_{18}. The dangers inherent in the gas chromatographic identification of these type of compounds have also been demonstrated in numerous occasions lately in this laboratory. Unfortunately no mass spectral data is available to support the gas chromatographic identifications made by Han (361). The present gas chromatographic and mass spectrometric investigation on the possible occurrence of isoprenoids in Fischer-Tropsch products seems to invalidate the earlier assumptions on the identities of peaks a and b (Figure 70) on the grounds that their gas chromatographic elution times, their mass spectra and also their relative abundances in the synthetic hydrocarbon mixture correspond to those of the 3-methyl hexadecane and 3-methyl heptadecane respectively.

Of course, it can be argued that the exact conditions prevailing in that particular experiment have not been reproduced in this work, in spite of having used the same catalyst, (Canyon Diablo iron), same flow rates (6.6 cc/min H_2 or D_2 and 3.3 cc/min CO) and same temperature (300°C). This may be true, but as observed in more than 50 different experiments the variations usually lie in the overall quantitative yields of the reaction, not in the qualitative nature of the distributions. Even when the quantitative yields change from one experiment to the other as pointed out above, the relative ratios of the methyl alkanes are always the same; that is, if the anteiso alkanes produced in experiment 119, which resulted in an unusual yield of 233 ppm, are present in the same amounts as the corresponding isoalkanes this is also the case in run 121 which only gave 13.5 ppm. The ratio of anteiso to n-alkane may also change from experiment to experiment depending on many parameters but the ratio iso: anteiso will be constant.

With all these in mind compare now the pattern in Figure 70 to those presented in Figures 68,69. Since the chromatogram in Figure 70 was obtained on a high resolution column it can only be compared to those obtained in columns of similar efficiencies.

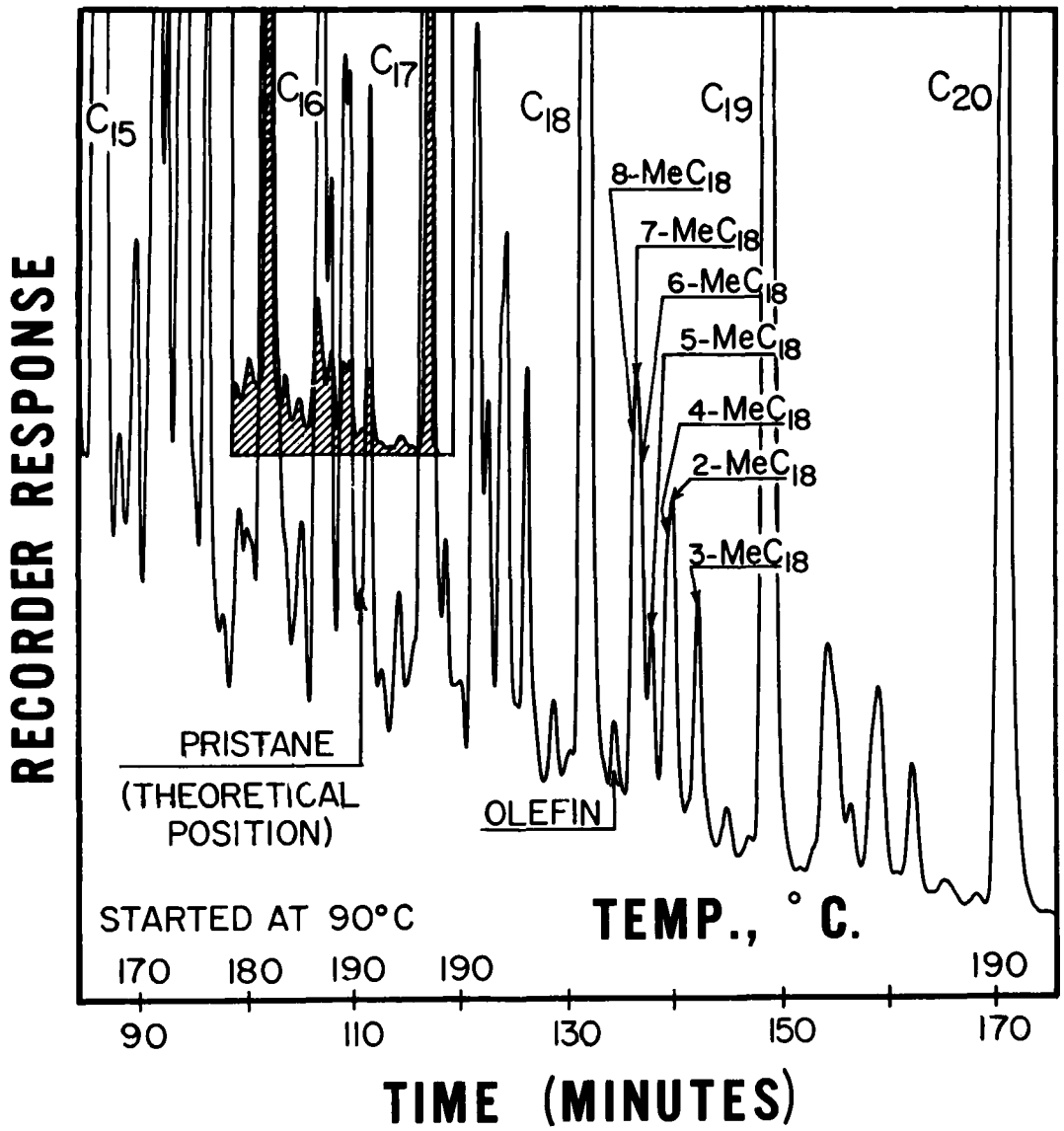
A close inspection of the patterns will reveal a perfect one to one correlation in all cases and this includes the

relative peak size. Such a perfect match is also demonstrated on Figure 71 where one of the gas chromatograms containing the supposed pristane and phytane peaks has been partially superimposed in the C_{16} to C_{17} range (shaded area) to the chromatogram corresponding to experiment 119 in Table XLIII. While the two chromatograms are identical even in the minor details, no evidence could be obtained from the mass spectrometric study of experiment 119 or any other, on the presence of the isoprenoids, pristane and phytane. The theoretical position of pristane in Figure 71 is marked by the arrow. This has been verified with the proper standards and with the corresponding mass spectra. Even the spectra of the small peak at the valley between the 2-methyl and 3-methyl C_{16} was studied in search of pristane but with negative results. The same is true for phytane. In any case, these arguments could be disregarded, assuming that in spite of everything they do not apply to the very same sample shown in Figure 70, but rather they refer to experiments performed at a later date on other equivalent samples. It must be pointed out in this connection that the mass spectrometric analysis of the same Fischer-Tropsch product received from the U.S. Bureau of Mines, which had been demonstrated to contain pristane and phytane by the same method of gas chromatographic analysis, showed that this product does not contain such isoprenoids in detectable amounts, but instead it does contain the anteiso C_{17} and

FIGURE 71

TYPICAL ALKANE GAS CHROMATOGRAPHIC PATTERN PRODUCED BY FISCHER-TROPSCH REACTIONS

(See pages 362-364).


RECORDER RESPONSE
C15
C16
C17
C18
C19
C20
8-MeC18
7-MeC18
6-MeC18
5-MeC18
4-MeC18
2-MeC18
3-MeC18
PRISTANE
(THEORETICAL
POSITION)
OLEFIN
STARTED AT 90°C
TEMP., °C.
170 180 190 190 190
90 110 130 150 170
TIME (MINUTES)

anteiso C_{18} alkanes and that these two compounds had been misidentified before.

c) Fischer-Tropsch synthesis with other combination catalysts

The activity and possible selectivity of the catalysts shown in Table XLIII was also tested in the presence of sulfur, silica gel and graphite.

The effect of the addition of sulfur (about 13%) to a catalyst which had proved in several occasions its activity towards Fischer-Tropsch syntheses can be seen in Figure 67 bottom. The poisoning of the catalyst by sulfur is significant in relation to the question on the possible abiotic origin of the hydrocarbons in meteorites where relatively high amounts of sulfur and sulfides are present (357).

The effect of silica and graphite on the synthesis products was investigated mainly because it was thought that by serving as templates they could possibly favor the formation of isoprenoids. Figure 72 illustrates the results obtained with the addition of SiO_2 to a commercial nickel-iron alloy in the ratio 1:8. The pattern of branched alkanes did not change in any extent. The only difference noticed was perhaps the much higher relative amounts of light hydrocarbons.

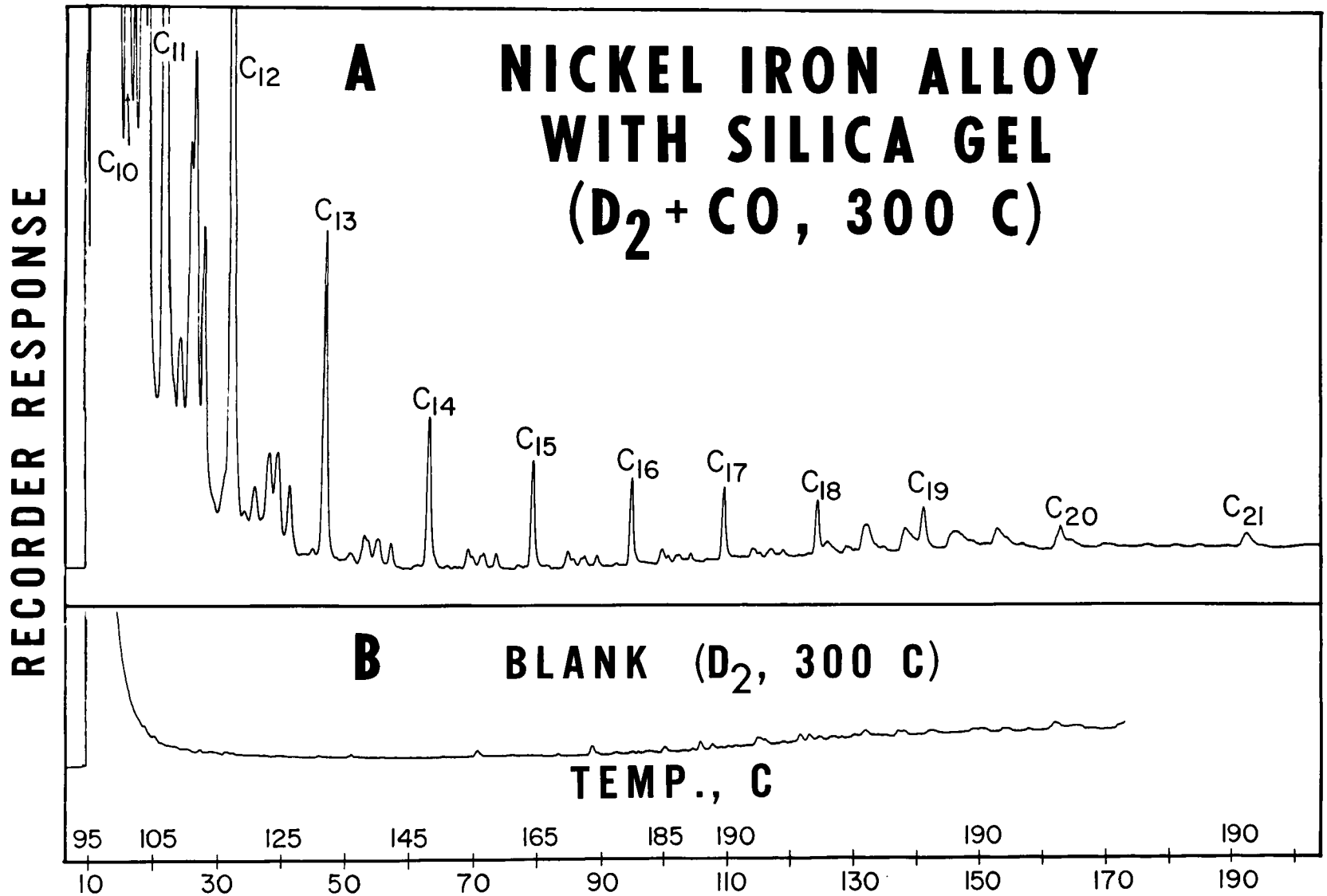
The addition of SiO_2 to a commercial nickel catalyst (133 FT, Figure 65C) did not bring about any major changes in

FIGURE 72

GAS CHROMATOGRAPHIC SEPARATION OF PERDEUTERO ALKANES IN A FISCHER-TROPSCH PRODUCT FROM A NICKEL-IRON-SILICA CATALYST

Same conditions as described in Figure 65.

Experiment 128 (Table XLIII).


A
NICKEL IRON ALLOY
WITH SILICA GEL
(D_2 + CO, 300 C)
C_{10}
C_{11}
C_{12}
C_{13}
C_{14}
C_{15}
C_{16}
C_{17}
C_{18}
C_{19}
C_{20}
C_{21}
RECORDER RESPONSE
B
BLANK (D_2, 300 C)
TEMP., C
95
105
125
145
165
185
190
190
190
10
30
50
70
90
110
130
150
170
190

the observed low activity of this catalyst (Figure 65A).

Likewise the presence of graphite in a meteoritic iron catalyst (Figure 73) did not result in the formation of any new structures, other than those formed when the meteorite was used by itself. Incidentally, the fine powdered graphite mixed with the meteoritic iron was also of meteoritic origin. Briefly summarized, the data collected indicates that iron either by itself or in combination with nickel is a good Fischer-Tropsch catalyst. On the other hand, nickel is not efficient unless alloyed with iron. Meteoritic iron (a combination of nickel-iron in which iron makes an 80-90% of the total) does support Fischer-Tropsch syntheses in a similar extent as the commercial iron-nickel alloys.

Typical n-alkane distributions obtained with commercial Fe and Ni-Fe catalysts, and with meteoritic iron as catalyst are given in Figure 74. Aside from the rapid decrease in the concentrations of the individual members as the hydrocarbon chains grow, an interesting observation can be made in the sense that there is a marked predominance of the C_{12} and C_{14} alkanes in most of the curves shown in the figure. Also a slight predominance of the C_{16}, C_{18} and C_{20} hydrocarbons can be observed in the curves corresponding to experiments 120 and 129 FT. This had been also observed by J. Han in some of his experiments (361). It is possible that under certain conditions the condensation of two carbon units, such as

FIGURE 73

GAS CHROMATOGRAPHIC SEPARATION OF PERDEUTERO ALKANES IN A FISCHER-TROPSCH PRODUCT FROM A METEORITIC IRON-GRAPHITE CATALYST

Same conditions as described in Figure 65.

Experiment 132 (Table XLIII).

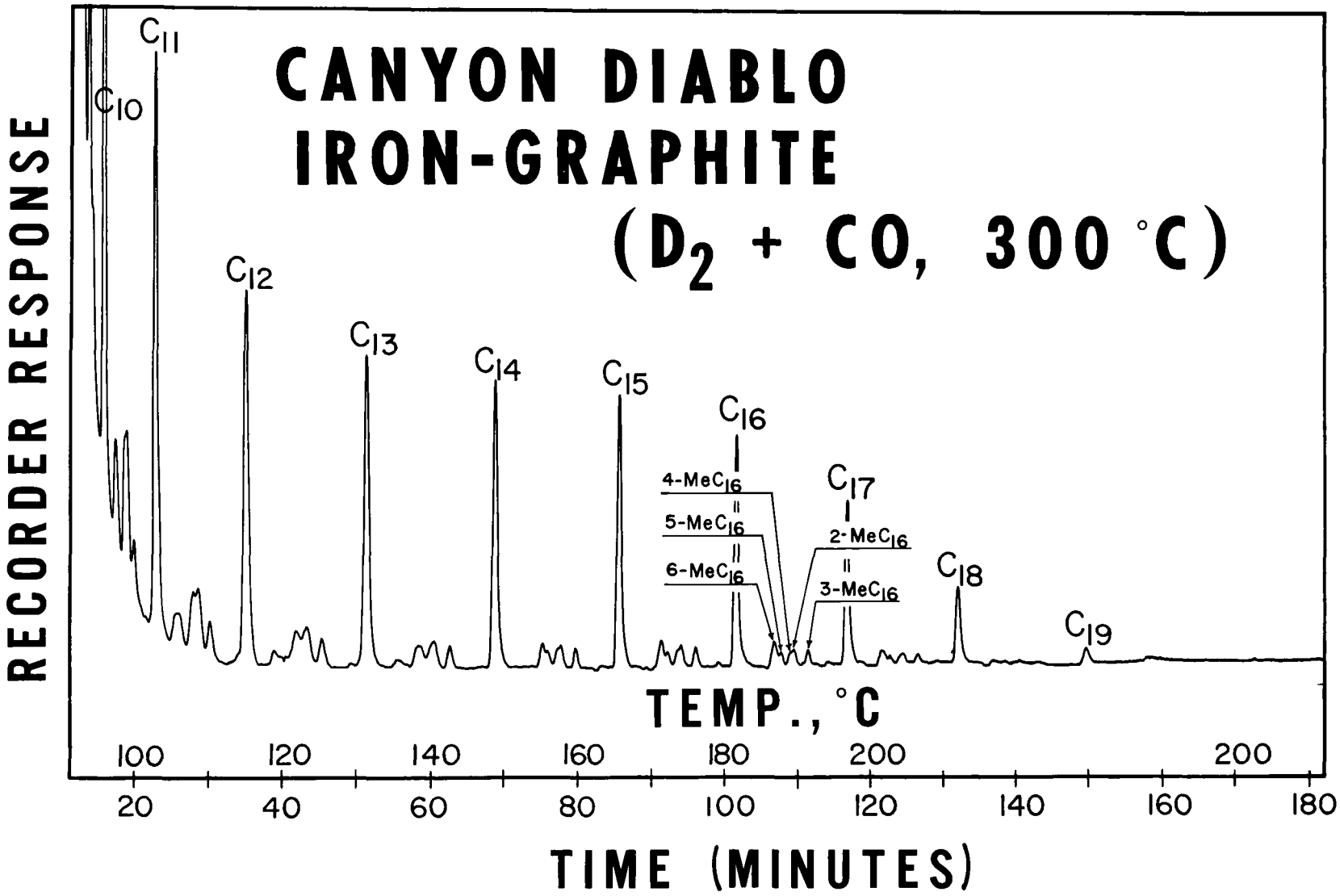
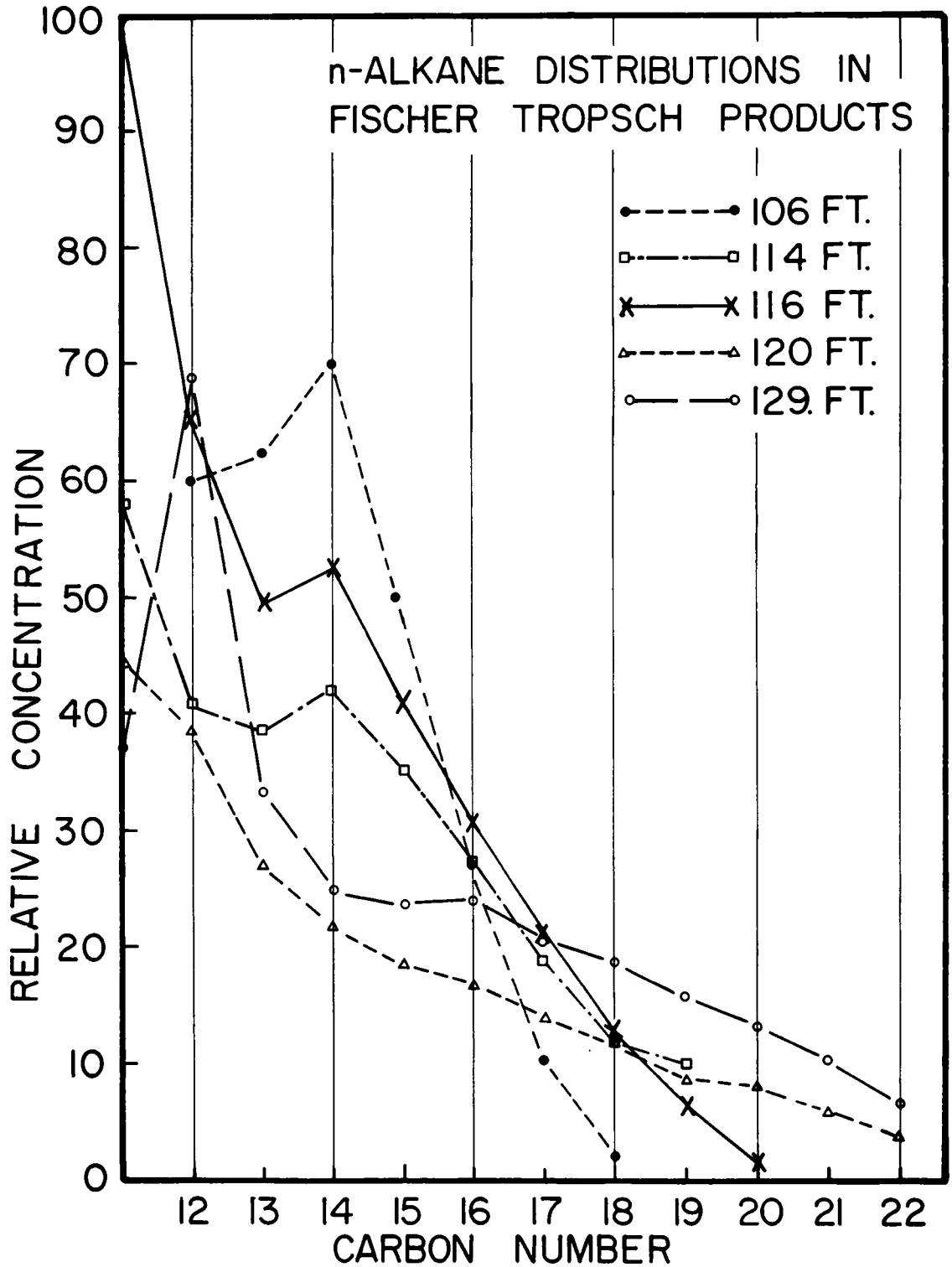

CANYON DIABLO
IRON-GRAPHITE
(D2 + CO, 300 °C)
RECORDER RESPONSE
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
4-MeC16
5-MeC16
6-MeC16
2-MeC16
3-MeC16
TEMP., °C
100
120
140
160
180
200
200
20
40
60
80
100
120
140
160
180
TIME (MINUTES)

FIGURE 74

ALKANE DISTRIBUTIONS IN FISCHER-TROPSCH PRODUCTS


n-ALKANE DISTRIBUTIONS IN
FISCHER TROPSCH PRODUCTS
106 FT.
114 FT.
116 FT.
120 FT.
129. FT.
RELATIVE CONCENTRATION
100
90
80
70
60
50
40
30
20
10
0
12
13
14
15
16
17
18
19
20
21
22
CARBON NUMBER

ethylene, may be favored leading to the formation of even carbon numbered olefins, which due to the higher content of H_2 over CO in the synthesis gas will be reduced to alkanes.

In any case the proposed reaction mechanisms (12,166, 249,387,460,510) may have to be revised to explain this new observation. It may be pointed out here that the effect of the predominance of even carbon numbered chains is in general very hard to observe directly from the peak heights of the gas chromatographic patterns because it is not too pronounced. This is especially the case in the C_{16}-C_{20} range, where practically the only way to detect it is by plotting the actual areas under the peaks vs carbon number as shown in Figure 74.

Although at present the reaction mechanism of the Fischer-Tropsch synthesis has not been completely elucidated, it appears that a stepwise chain growth mechanism involving one-carbon additions to the first or second carbon atom at the end of the growing chain (12,13) is widely accepted nowadays because it is capable of predicting the isomer and carbon-number distributions from iron and cobalt catalysts. On the other hand, Eidus and coworkers (cited in reference 13) have carried out experiments on the incorporation of tagged ethylene on synthesis products and reported that 20 percent of the hydrocarbon chains were initiated by ethylene. Hall et al. (187) also reached similar results. In view of the results presented

here perhaps more serious consideration needs to be given to the possible participation of ethylene in the chain growth mechanisms. The repeating pattern of the methyl branched hydrocarbon sequences (n-C_x,7-MeC_x to 3-MeC_x,n-C_{x+1}) is also in line with the stepwise chain growth hypotheses. No definite predominances were observed in this case but even if they occur they will not be easily detectable at these low levels of concentrations. Concerning the isoprenoids, either the proposed linear growth mechanism (12) or a mechanism of growth involving the polymerization of isoprane (2 methyl butane) which is known to be formed in substantial amounts in the Fischer-Tropsch process (166), should be both rather unselective processes which would lead to the formation of all of the members of the regular isoprenoid series. In other words if the prevailing mechanism is that of isoprane polymerization, compounds such as the 2,6-dimethyl octane, 2,6,10-trimethyl dodecane (farnesane), 2,6,10,14 tetramethyl hexadecane (phytane) and so forth, should predominate over other possibilities. Note the absence of pristane by this mechanism. On the other hand if the regular stepwise chain growth (one C at a time) is predominant then the product should include compounds such as 2,6-dimethyl octane, 2,6-dimethyl-nonane, 2,6-dimethyldecane...and so forth. Although there are some indications of the presence of dimethyl substituted compounds (see Table XLIV) among the products analyzed,

none of them does conform to an isoprenoid structure. This is not totally unexpected if the probabilities of chain branching are considered (13).

Dimethyl substituted alkanes have also been reported recently by Studier et al. (463,467). A careful inspection of the partial chromatograms presented by these authors reveals the possibility that these dimethyl substituted compounds may actually be a mixture of monomethyl alkanes of the type reported here. It has already been discussed how easy it would be to misinterpret a combination spectrum of two methyl alkane structures (192). It is remarkable that in spite of having identified the 2- and 3-methyl alkanes among the products of their reaction mixtures no mention is made of other monomethyl alkanes. However, the chromatographic elution times of some of the dimethyl alkanes (2,3-,3,4-, and 4,5-dimethyl alkanes) seem to correspond to those of the 4- through 7-methyl alkanes. According to these authors, isoprenoid compounds from C_9 to C_{19} have also been produced in many of the runs. In terms of the amount produced they claim that the dimeric 2,6-dimethyl alkanes were predominating. All these claims though have not been substantiated with adequate data and should be considered as rather speculative at the moment.

VII.

EXTRATERRESTRIAL ORGANIC MATTER

EXTRATERRESTRIAL ORGANIC MATTER

A. Carbonaceous Chondrites

A substantial amount of data on the aliphatic and aromatic hydrocarbons in carbonaceous chondrites has already been presented (201,350,352,364,368). The initial work had been directed mainly towards the gas chromatographic identification of these compounds, and in a few instances attempts were made with success to confirm part of the results by mass spectrometry. Mass spectra with the general characteristics of pristane and phytane were obtained for the Boriskino, Santa Cruz, Mighei, Mokoia, and Murray meteorites. Mass spectra corresponding to norpristanewere also obtained for the Boriskino, Mokoia and Murray (350).

The present work (357) represents an extension of the earlier investigations by Nooner and Oró (350,364,368). All efforts have been now concentrated in the positive mass spectrometric identification of most of the paraffinic compounds previously detected by gas chromatography. Emphasis is placed in particular on the unequivocal characterization of isoprenoid structures.

Qualitative analysis

The new results obtained on the gas chromatographic-mass spectrometric analysis of the alkane fraction from six carbonaceous chondrites (Essebi, Grosnaja, Mokoia, Murray,

TABLE XLIX

ISOPRENOIDS AND ALIPHATIC HYDROCARBONS IN METEORITES - GC-MS IDENTIFICATION

		Mo.	Mu.	O.	V.
	nC_{11}				1
	nC_{12}	0.1	1		1
1	Decahydronaphthalene	1			1
2	2,6,10 $TriMeC_{11}$			1	0.1
3	$\underline{2MeC_{12}}$			1	1
4	$3MeC_{12}$			1	1
5	4,8 $DiMeC_{12}$			1	
	nC_{13}	1	1		1
6	2,6,10 $TriMeC_{12}$	1	1		1
7	$\underline{2MeC_{13}}$	1	1		1
8	$3MeC_{13}$	1	1		1
9	4,8 $DiMeC_{15}$		1		1
	nC_{14}	1	1		3
(10)	2,6,10 $TriMeC_{13}$	1	1		1
11	$\underline{4MeC_{14}}$		1		
(12)	$2MeC_{14}$	1	1		3
(13)	$3MeC_{14}$	1	1		3
14	4,8 $DiMeC_{14}$		1		
	nC_{15}	2	2	1	4
(15)	2,6,10 $TriMeC_{14}$	1	1		0.1
17	$\underline{5MeC_{15}}$		1		1
18	$4MeC_{15}$	1	1		

		E.	Gr.	Mo.	Mu.	O.	V.
(19)	$2MeC_{15}$		1	1	1	1	1
(20)	$3MeC_{15}$		1	1	1		1
	nC_{16}	1	1	4	6	1	4
(21)	2,6,10 $TriMeC_{15}$	1	1	2	2		2
(23)	$\underline{6MeC_{16}}$			1	1		
24	$5MeC_{16}$			0.1	1		1
(25)	$4MeC_{16}$			1	1		
(26)	$2MeC_{16}$	1	1	1	1	1	1
(27)	2,6,10,14 $TetraMeC_{15}$	2	1	6	4	1	3
(28)	$\underline{3MeC_{16}}$	1		1	1		1
	nC_{17}	3	1	8	14	3	8
(30)	2,6,10 $TriMeC_{16}$			1			1
(31)	$\underline{4,7,11\ TriMeC_{16}}$			0.1	1		1
32	Decylcyclohexane		1	1	1		
(33)	$6MeC_{17}$				2		1
(34)	$5MeC_{17}$			1	1		
(35)	$4MeC_{17}$	1		1	1		
(36)	$2MeC_{17}$	1	1	2	1	1	1
(37)	2,6,10,14 $TetraMeC_{16}$	3	1	4	3	1	2
(38)	$\underline{3MeC_{17}}$	1	1	1	1	1	1
	nC_{18}	6	1	7	9	3	5
(39)	Dodecylcyclopentane				1	0.1	
(40)	Undecylcyclohexane			3	1		1

		E.	Gr.	Mo.	Mu.	O.	V.
41	$5MeC_{18}$			1			1
(42)	2,6,10,14 $TetraMeC_{17}$	1	0.1	1	1		1
43	$\underline{4MeC_{18}}$			1	1		
(44)	$2MeC_{18}$	1		2	1		1
(45)	$3MeC_{18}$	1		1	1		1
	nC_{19}	5	2	6	8	2	3
(46)	Tridecylcyclopentane						0.1
(47)	Dodecylcyclohexane			1	1		1
48	$4MeC_{19}$			1	1		1
(49)	$2MeC_{19}$			1	1		1
(50)	$3MeC_{19}$			1	1		1
	nC_{20}	1	2	3	5	1	2
(51)	Tridecylcyclohexane			1	1		1
52	$2MeC_{20}$				1		
(53)	$3MeC_{20}$				1		
	nC_{21}	1	2	1	6	1	1
54	Tetradecylcyclohexane				1		
55	$2MeC_{21}$				1		
	nC_{22}			1	4	1	1
	nC_{23}				2		1
	nC_{24}				2		1
	nC_{25}						1
	nC_{26}						1

E.(Essebi); Gr. (Grosnaja); Mo. (Mokoia); Mu. (Murray); O. (Orgueil); V. (Vigarano). The amounts given (in ppm) have been obtained by rounding the actual values to the next higher number. Blanks in the columns of the Table do not necessarily indicate the absence of certain alkanes, but rather that no useful mass spectra were obtained. Compounds other than n-alkanes have an identification number corresponding to that given in the gas chromatograms. When this number is placed in parentheses, it indicates that the compound has also been identified in graphite nodules from iron meteorites.

Orgueil and Vigarano) are summarized in Table XLIX. Typical gas chromatograms are shown in Figures 75 and 76. The alkanes in Table XLIX are given in order of their gas chromatographic elution times from a capillary column (310 m long x 0.076 cm i.d.) coated with Polysev (see Table I). The amounts of each component are given in parts per million, and they represent an upper limit in their concentrations in each of the six meteorites. Numbers to the left of most of the compounds listed in Table XLIX correspond to the identification numbers in the GC patterns (Figures 75,79,80). The normal alkanes are not given an identification number because they are explicit in the figures.

Each assignment of structural identity for a compound has been confirmed by mass spectral data. Blanks in the columns of the table do not necessarily indicate the absence of certain alkanes from a given meteorite but rather that no useful mass spectra were obtained, because either the components were in too low concentration or the sample had been treated in such a way that some of its components were probably lost. For instance, by blowing the samples completely to dryness the light molecular weight hydrocarbons of the Essebi and the Orgueil, if present at all, were lost by evaporation. These light components have been found in other samples of Orgueil (350).

FIGURE 75

GAS CHROMATOGRAPHIC SEPARATION OF THE ALKANES FROM THE MOKOIA AND VIGARANO METEORITES

Stainless steel capillary tubing (155 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Nitrogen pressure 1050 g/cm^2. No split. Programmed from 122° to 175°C at approximately 20°C/min. Range, 10^2; attenuation, 2. Vigarano; 0.5 g extracted, 1/5 injected. Mokoia; 0.5 g extracted, 1/3 injected. (See Table XLIX for peak number identification).

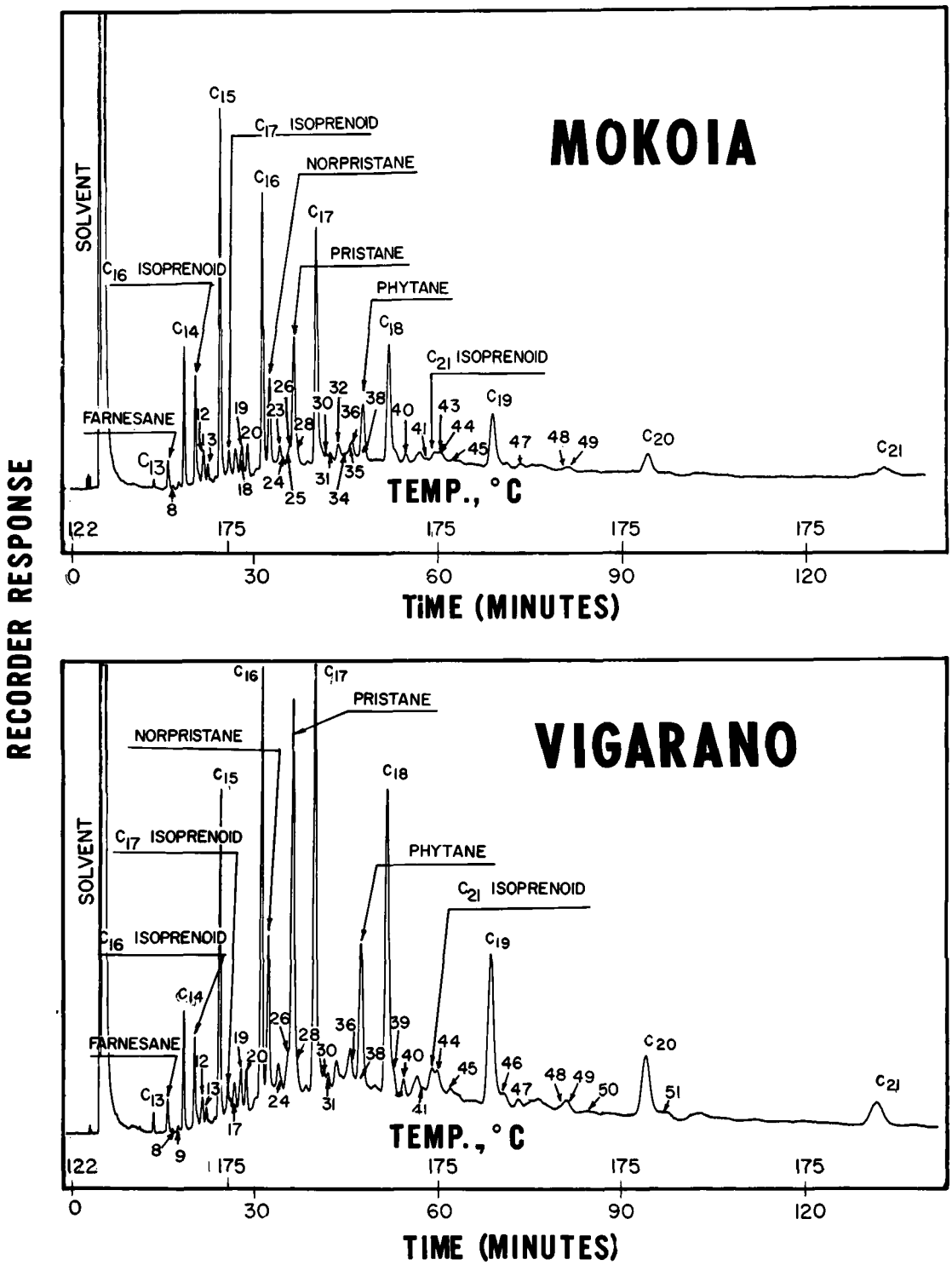
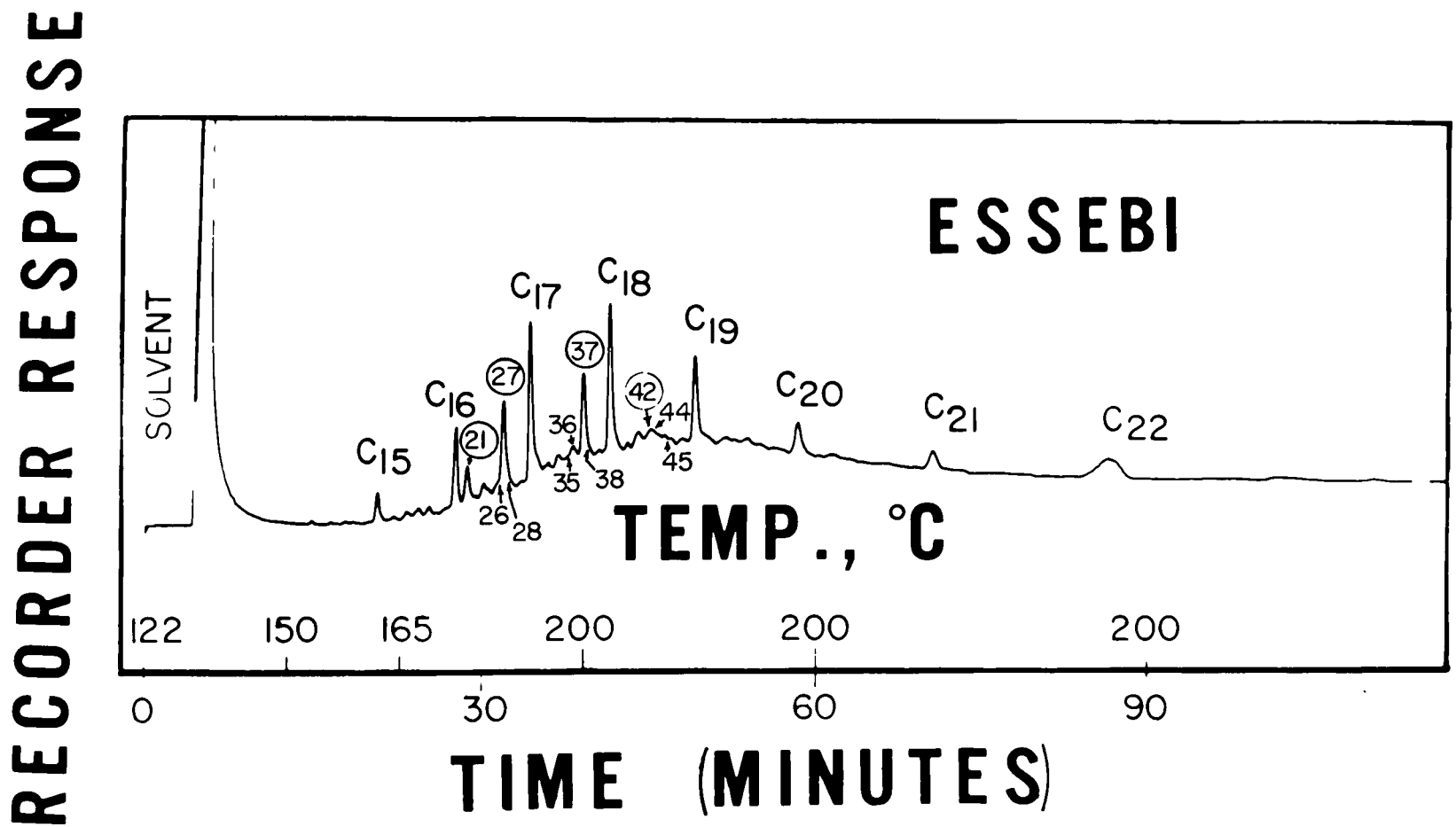

MOKOIA
SOLVENT
C15
C17 ISOPRENOID
NORPRISTANE
C16
C17
PRISTANE
C16 ISOPRENOID
PHYTANE
C18
C14
C21 ISOPRENOID
FARNESANE
C19
C13
C20
C21
TEMP., °C
122
175
175
175
175
0
30
60
90
120
TIME (MINUTES)
VIGARANO
C16
C17
PRISTANE
NORPRISTANE
C18
C15
C17 ISOPRENOID
PHYTANE
C21 ISOPRENOID
C16 ISOPRENOID
C19
C14
FARNESANE
C20
C13
C21
TEMP., °C
122
175
175
175
175
0
30
60
90
120
TIME (MINUTES)
RECORDER RESPONSE

FIGURE 76

GAS CHROMATOGRAPHIC SEPARATION OF THE ALKANES FROM THE ESSEBI METEORITE

Same conditions as in Figure 75.


RECORDER RESPONSE
ESSEBI
SOLVENT
C15
C16
C17
C18
C19
C20
C21
C22
21
26
27
28
35
36
37
38
42
44
45
TEMP., °C
122
150
165
200
200
200
0
30
60
90
TIME (MINUTES)

Ten well defined homologous series of alkanes can be selected from the data presented in Table XLIX. These are: the normal alkane series (in the C_{11} to C_{26} range), the five monomethyl alkane series (6-,5-,4-,3-,2-methyl alkanes) with members in the C_{13} to C_{22} range, the two isoprenoid homologies formed by the 2,6,10-trimethyl alkane or farnesane series (C_{14}-C_{19}) and the 2,6,10,14-tetramethyl alkane or phytane series (C_{19}-C_{21}), and finally the two monocycloalkane homologous series (cyclohexyl and cyclopentyl) with members in the C_{16}-C_{20} range. The presence of a dimethyl alkane series (4,8-dimethyl alkanes from C_{14} to C_{16}) would bring the total to eleven different series but the mass spectrometric data should be considered tentative in this case.

Two new isoprenoid compounds, the C_{19} member of the 2,6,10-trimethyl alkane series (2,6,10-trimethylhexadecane) and an irregular C_{19} isoprenoid structure (4,7,11-trimethyl-hexadecane) have been identified in some of the meteorites. Although all the other isoprenoids have been found in a large variety of terrestrial samples (31,39,119,178,231,263,365), the presence of these two compounds apparently has gone undetected until now. This is most likely due not to their absence from terrestrial samples but rather to the difficulty in separating them from the n-C_{17} alkane. Their possible significance in the diagenetic degradation schemes of a parent isoprenoid compound will be discussed later.

Quantitative analysis

Detailed quantitative evaluations of the hydrocarbons detected in four of the six meteorite samples are given in Table L and LI. The members of the ten homologous series indicated above have been grouped into four major classes of related structures, namely the cycloalkanes (A), methylalkanes (B), isoprenoids (C), and n-alkanes (D). The total values given in Table L correspond to the sum A+B+C+D+E, where "E" is made up by the contribution of components (1) not included under any of the four major groups, and (2) of components present in the gas chromatograms but not identified by mass spectrometry. Its value is given on a relative percent basis in Table LI. In the case of the Murray and Grosnaja carbonaceous chondrites, where most of the components of the alkane fraction have been identified, "E", it has a very small value, but not in Mokoia and Vigarano (36.16 and 17.89% respectively), which contain a considerable amount of unidentified high molecular weight material. The position of some of the peaks in the gas chromatograms together with their mass spectral patterns seem to indicate the presence of sterane like structures. Definite identifications have not been attempted because the sterane fraction does not chromatograph well on the long high resolution columns employed for optimum results within the range C_{10}-C_{23}.

TABLE L

GAS CHROMATOGRAPHIC ESTIMATION OF HYDROCARBONS IN METEORITES

		Paraffinic Hydrocarbons									
Meteorite	Type	Cycloalkanes (A) ppm	range	me-Alkanes (B) ppm	range	Isoprenoids (C) ppm	range	n-Alkanes (D) ppm	range	Maximum	Total (A+B+C+D+E)
Murray	II	1.2	(C_{16}-C_{20})	10.5	(C_{13}-C_{21})	8.9	(C_{14}-C_{21})	55.1	(C_{12}-C_{24})	n-C_{17}	77.8
Grosnaja	III	0.4	(C_{16})	0.8	(C_{16}-C_{18})	0.9	(C_{18}-C_{20})	5.8	(C_{16}-C_{21})	n-C_{21}	6.7
Mokoia	III	—	—	—	—	10.4	(C_{16}-C_{21})	35.6	(C_{12}-C_{21})	n-C_{19}	85.1
		1.8	(C_{16}-C_{18})	6.6	(C_{14}-C_{20})	11.4	(C_{15}-C_{21})	33.4	(C_{12}-C_{22})	n-C_{17}	82.8
Vigarano	III	0.5	(C_{17}-C_{19})	3.8	(C_{13}-C_{20})	5.5	(C_{14}-C_{21})	29.3	(C_{11}-C_{26})	n-C_{17}	45.6

E - Represents the value of compounds not included in these four categories plus the value of the unidentified compounds.

TABLE LI

RELATIVE PERCENT COMPOSITION OF PARAFFINIC

HYDROCARBONS IN THE TOTAL n-PENTANE EXTRACT OF METEORITES

Meteorite	Cycloalkanes	me-Alkanes	Isoprenoids	n-Alkanes	Total	E
Murray	1.54	13.44	11.39	70.52	96.89	3.11
Grosnaja	5.00	10.00	11.25	72.50	98.75	1.25
Mokoia	2.16	7.92	13.68	40.08	63.84	36.16
Vigarano	1.05	7.98	11.55	61.53	82.11	17.89

E - Total percentage of compounds unidentified or not considered in this table.

In Table LII a comparison is made between the total values of isoprenoids plus n-alkanes obtained in this work and those previously reported (350). Taking into consideration the different methods employed for the quantitation of the results, peak areas from an electronic digital integrator versus manual computation, there appears to be a close correlation between the values obtained in both investigations for the samples that have a common source, such as the Murray and Mokoia specimens. The values obtained with the digital integrator appear to be higher than the ones obtained by Nooner and Oró (350) by a factor of about 1.5. This could indicate that the manual integration of the peak areas resulted in an underestimation of the real values, but it could also be taken as an indication that the organic material is constantly being absorbed from the environment by the meteorite because of the time elapsed between analyses. In any case the deviation observed by any of the two methods is not great as demonstrated by the results of the replicate runs.

It is interesting that great differences in the amounts of alkanes are obtained when the samples are from different donors, and so they probably have somewhat different stories. See the values for Vigarano and Grosnaja in Table LII. The differences observed between the two samples of the Grosnaja meteorite are particularly striking. They differ by a factor of 61 (see Figures 77 and 78).

TABLE LII

TOTAL ALIPHATIC HYDROCARBON CONTENT (IN ppm)

IN FOUR CARBONACEOUS CHONDRITES

Meteorite	Type	Total (C+D)[1]	Total (C+D)[2]
Murray	II	64.0 (Henderson)	35.3 (Henderson)
Grosnaja	III	6.7 (Vdovykin)	415.0 (Mueller)
Mokoia	III	44.8 (Moore) 46.0	29.3 (Moore) Replicate 33.3 runs
Vigarano	III	34.8 (Hey)	223.0 (Olsen) 108.3 (Nagy)

C - isoprenoids, D - n-alkanes

[1] This work

[2] Nooner and Oro[350]

FIGURE 77

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS FROM THE GROSNAJA METEORITE

Same conditions as described in Figure 75 except for the temperature (Isothermal at 100°C for 10 minutes and then programmed to 200°C at 1°C/min.) and the Range (10^2).

(A) 0.9062 g extracted. About 1.9/40 of the n-pentane eluate was injected.

(B) Second extraction blank. About 1.9/40 of the n-pentane eluate was injected. Range, 10; attenuation, 1.

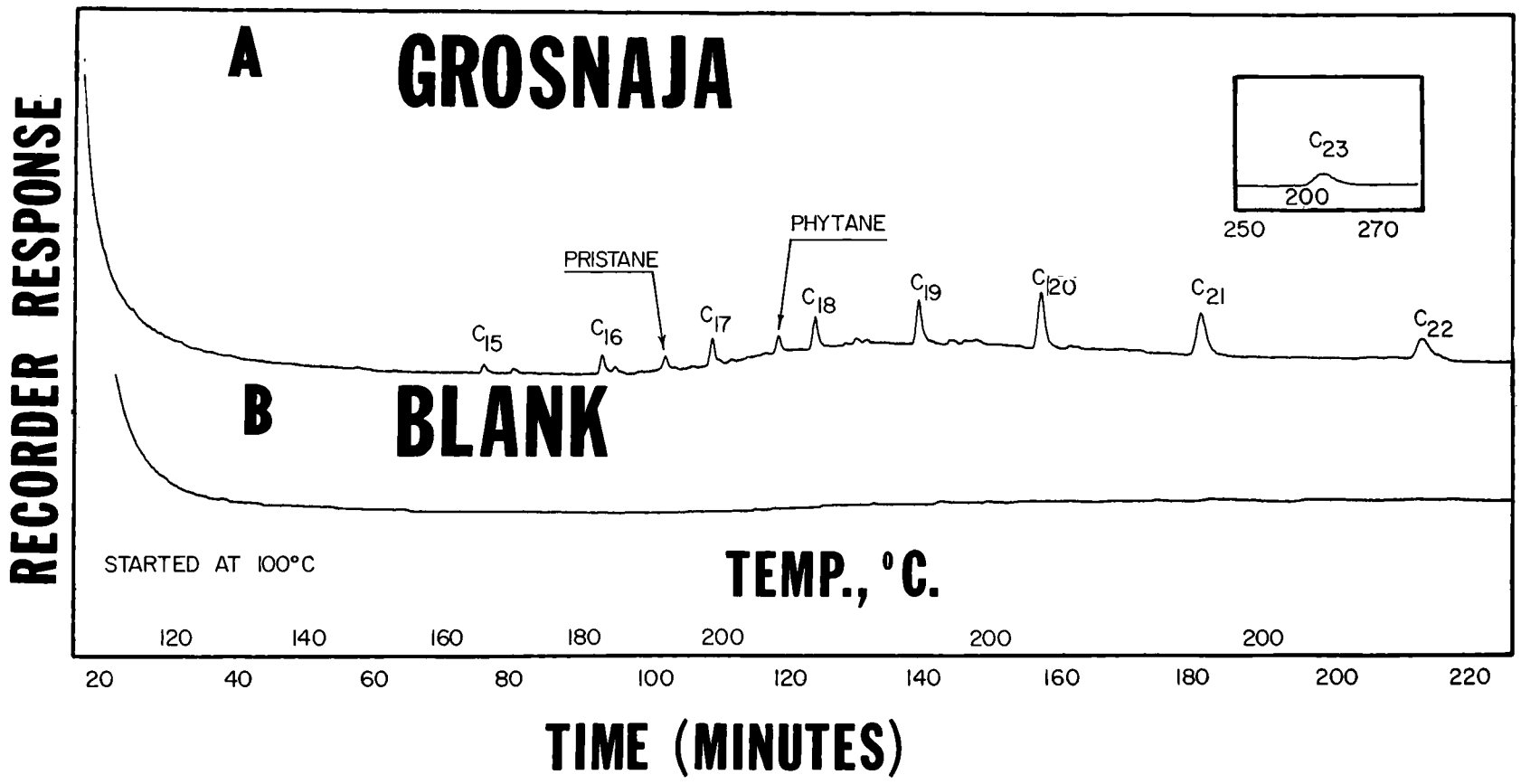
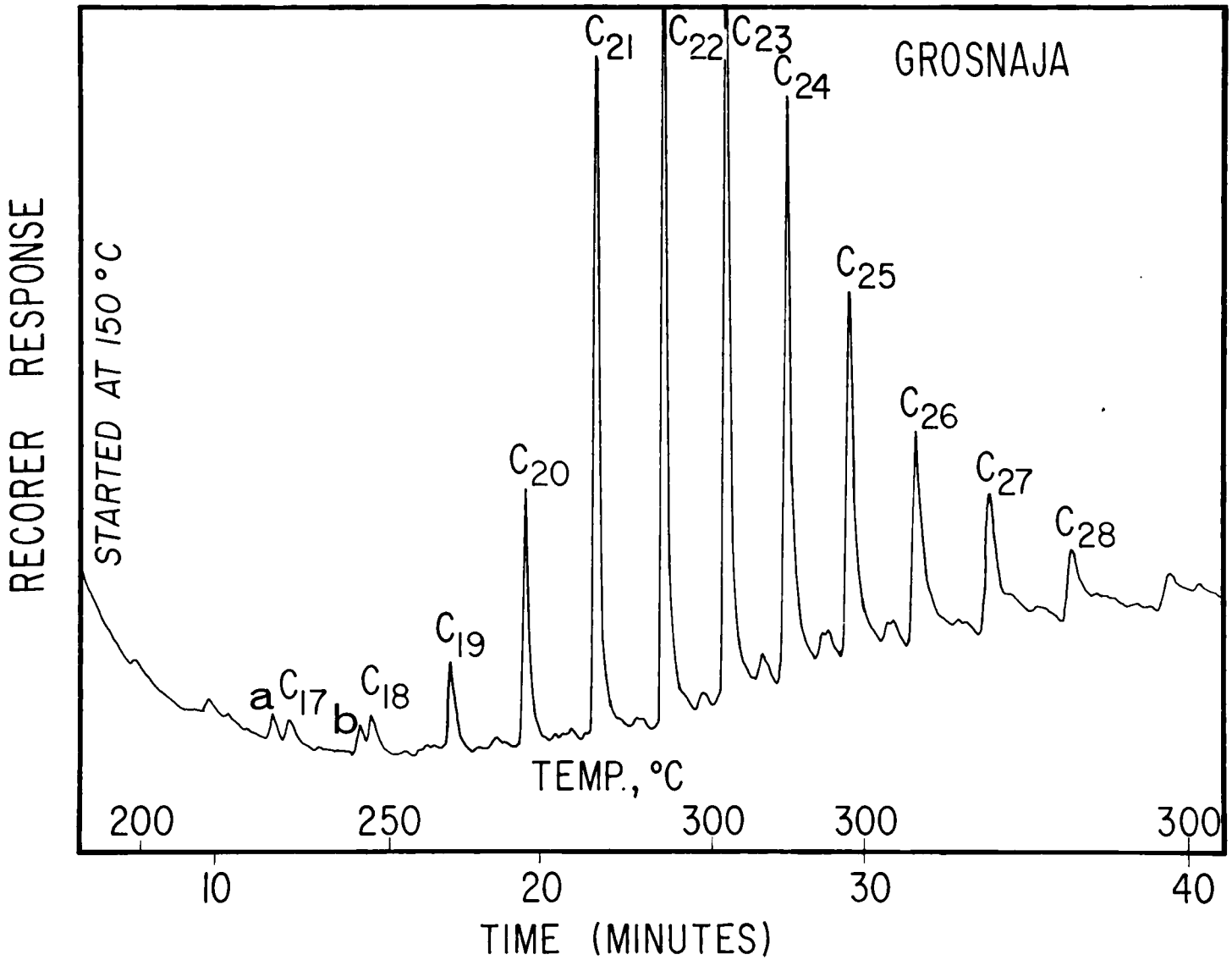

A
GROSNAJA
B
BLANK
RECORDER RESPONSE
PRISTANE
PHYTANE
C15
C16
C17
C18
C19
C20
C21
C22
C23
200
250
270
STARTED AT 100°C
TEMP., °C.
120
140
160
180
200
200
200
20
40
60
80
100
120
140
160
180
200
220
TIME (MINUTES)

FIGURE 78

GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS FROM THE GROSNAJA METEORITE

Stainless steel capillary column (46 m by 0.025 cm i.d.) coated with Apiezon L. Barber Colman Series 5000 gas chromatograph equipped with a flame ionization detector. Nitrogen pressure, 2813 g/cm^2. Small split. Meteorite extracted (sample from Mueller), 0.4 g. About 1/50 of the n-heptane eluate was injected. Attenuation, 10. Programmed from 150° to 300°C at 6.0°C per minute. (Data taken from reference 348)


RECORER RESPONSE
STARTED AT 150 °C
GROSNAJA
C21
C22
C23
C24
C25
C26
C27
C28
C20
C19
a
C17
b
C18
TEMP., °C
200
250
300
300
300
10
20
30
40
TIME (MINUTES)

The very high value (415 ppm) obtained in the first analysis (350), together with the small amount of pristane and phytane, was taken as a possible indication that the sample had undergone a process of contamination by commercial waxes at some earlier stage of its history. On the other hand the extremely low values (6.7 ppm) obtained in the more recent analysis of a sample of the same meteorite which was taken from a well preserved inside piece*, seem to strengthen the earlier assumption about the source of the hydrocarbon pattern found in this meteorite (Figure 78).

It may be pointed out here the analysis of the paper which the meteorite was wrapped when received, yielded only less than 1.5 ppm of hydrocarbons and that the two distributions were not identical. So any contribution to the Grosnaja hydrocarbon content by the paper must have had a very minor effect.

The Vigarano hydrocarbons although differing also considerably in the total content of the two specimens, show in both cases a completely identical gas chromatographic pattern (Figure 75, bottom). The data shown on Table L , recalculated on a relative percent basis is given in this form in Table LI. It becomes

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*Vdovykin, G. P., Private communication to Dr. Oró. The sample, an inside piece, had been recently cut and it was analyzed shortly after its arrival to the laboratory.

apparent that the cycloalkanes are always present in low amounts; the methyl alkanes are in general lower than the isoprenoids and the ratio of n-alkanes to isoprenoids goes from 6.4 for the Grosnaja to 2.9 for Mokoia.

The relative isoprenoid distribution is given in Table LIII. It is worthy of notice that (1) in all cases pristane is the major isoprenoid compound, (2) the concentration of the C_{17} is always the lowest among all the isoprenoids, and (3) there is a second maxima at the C_{15} or C_{16} isoprenoids. The significance of all these observations will become clear later.

High resolution gas chromatography-mass spectrometry

The utility of the mass spectral data obtained from very complex mixtures of alkanes containing many isomeric forms is limited by the resolving power of the gas chromatographic columns used. In other words if two or more components of the mixture under analysis are eluted together in one peak, the corresponding mass spectra will represent a combination of their individual fragmentation patterns. This point has already been covered in some detail in the section dealing with the gas chromatographic-mass spectrometric analysis of the isoalkanes.

The identification of a mixture of two compounds giving characteristic ions belonging to different mass series, such as fatty acids and alkylbenzenes for instance, may not present a specially difficult problem, but on the other hand, it will

TABLE LIII

RELATIVE ISOPRENOID DISTRIBUTION IN CARBONACEOUS CHONDRITES

	Relative Percent[1]			
	Murray	Grosnaja	Mokoia	Vigarano
2,6,10-Tri meC_{11}	0.01	---	---	0.06
2,6,10-Tri meC_{12}	0.51	---	0.06	0.31
2,6,10-Tri meC_{13}	0.30	---	0.14	0.42
2,6,10-Tri meC_{14}	0.12	---	0.01	0.02
2,6,10-Tri meC_{15}	1.62	<2.12	1.65	2.47
2,6,10,14-Tetra meC_{15}	4.92	4.50	6.12	4.93
2,6,10-Tri meC_{16}	---	---	0.24	---
2,6,10,14-Tetra meC_{16}	3.68	4.25	4.41	2.77
2,6,10,14-Tetra meC_{17}	0.25	0.37	1.02	0.27

[1]Of total isoprenoid content as given in Table LI

be very hard to tell the difference between a dimethyl substituted alkane and the equivalent mixture of two monoethyl substituted alkanes. A specific practical example has been recently discussed in the literature (192). It involves the spectra of 7,9-dimethylhexadecane vs the combination of 7-methyl and 8-methylheptadecanes.

In an effort to obtain more representative mass spectra of each individual component present in the alkane fraction of the meteorite extracts, part of the work carried out on low resolution columns (90 m and 195 m long x 0.076 cm i.d.) was repeated using higher efficiency columns (310 m long x 0.076 cm i.d.). Some of the results are shown in Figures 79 and 80. The partial gas chromatographic pattern in Figure 79 can be directly compared to that shown in Figure 5 (bottom part), since both represent different analyses of the same meteorite, the Vigarano. The identification numbers in the chromatograms correspond to those given in Table XLIX. The effects of the higher efficiency column on this pattern can be clearly seen in Figure 79, where the 2,6,10-Trimethyl C_{12} or farnesane peak has now been partially resolved into three peaks. Likewise the C_{16} isoprenoid peak (2,6,10 Trimethyl C_{13}) in Figure 75 bottom has been shown to be a mixture of three distinct peaks; the isoprenoid plus the two unresolved smaller components preceeding peak 11 in Figure 79. The pattern in Figure 80 illustrates how difficult it is to separate certain isomeric

FIGURE 79

PARTIAL HIGH RESOLUTION GAS CHROMATOGRAM OF THE HYDROCARBONS FROM VIGARANO

Stainless steel capillary column (195 m long by 0.076 cm i.d. coated with Polysev. 'F and M Model 810' gas chromatograph with flame ionization detector. Range, 10; attenuation, 2. Nitrogen pressure 1050 g/cm^2. No split. Temperature held isothermally at $105^{o}C$ for 20 minutes and then programmed to $195^{o}C$ at $2^{o}C$/min. (See Table XLIX for peak number identifications).

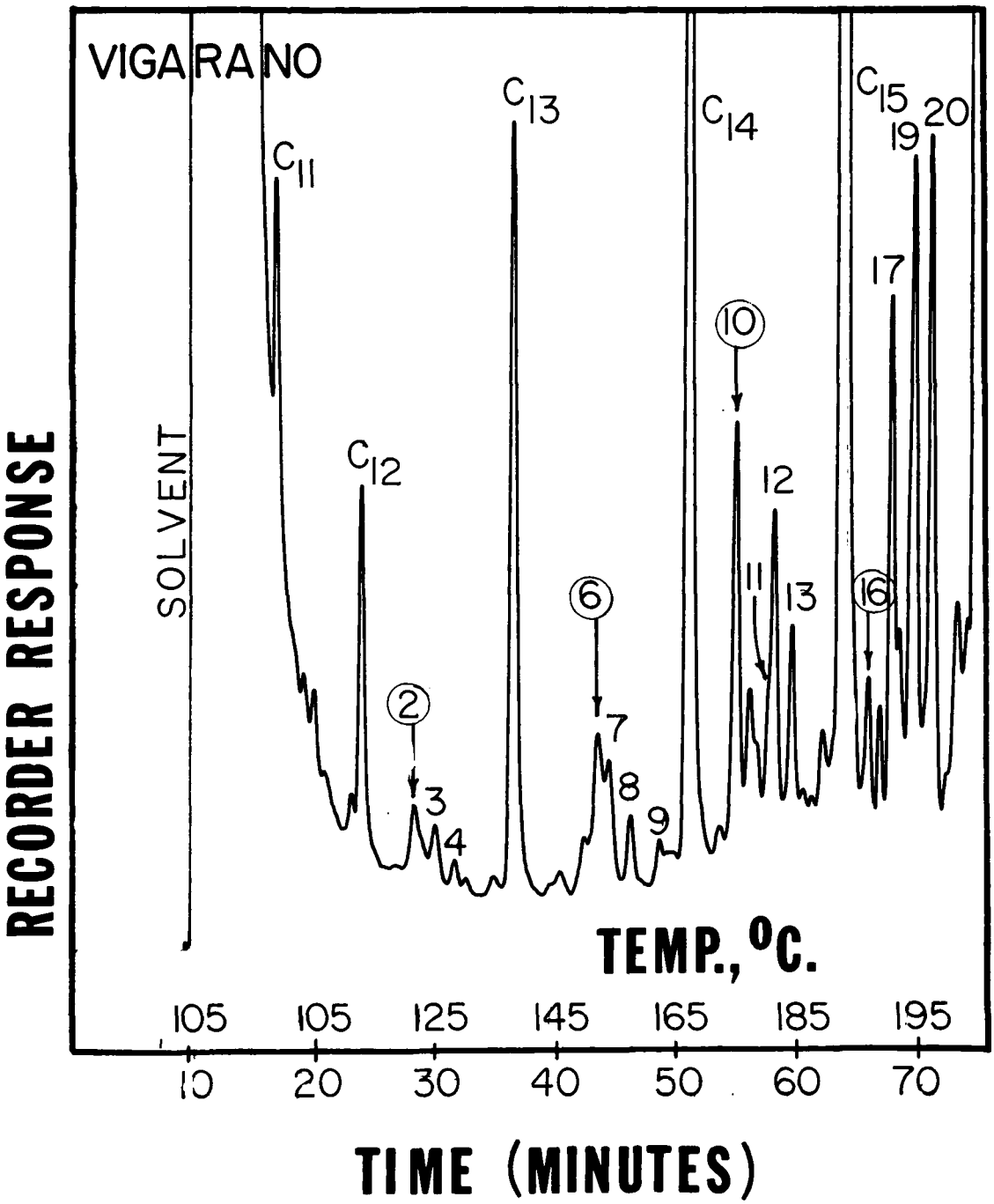
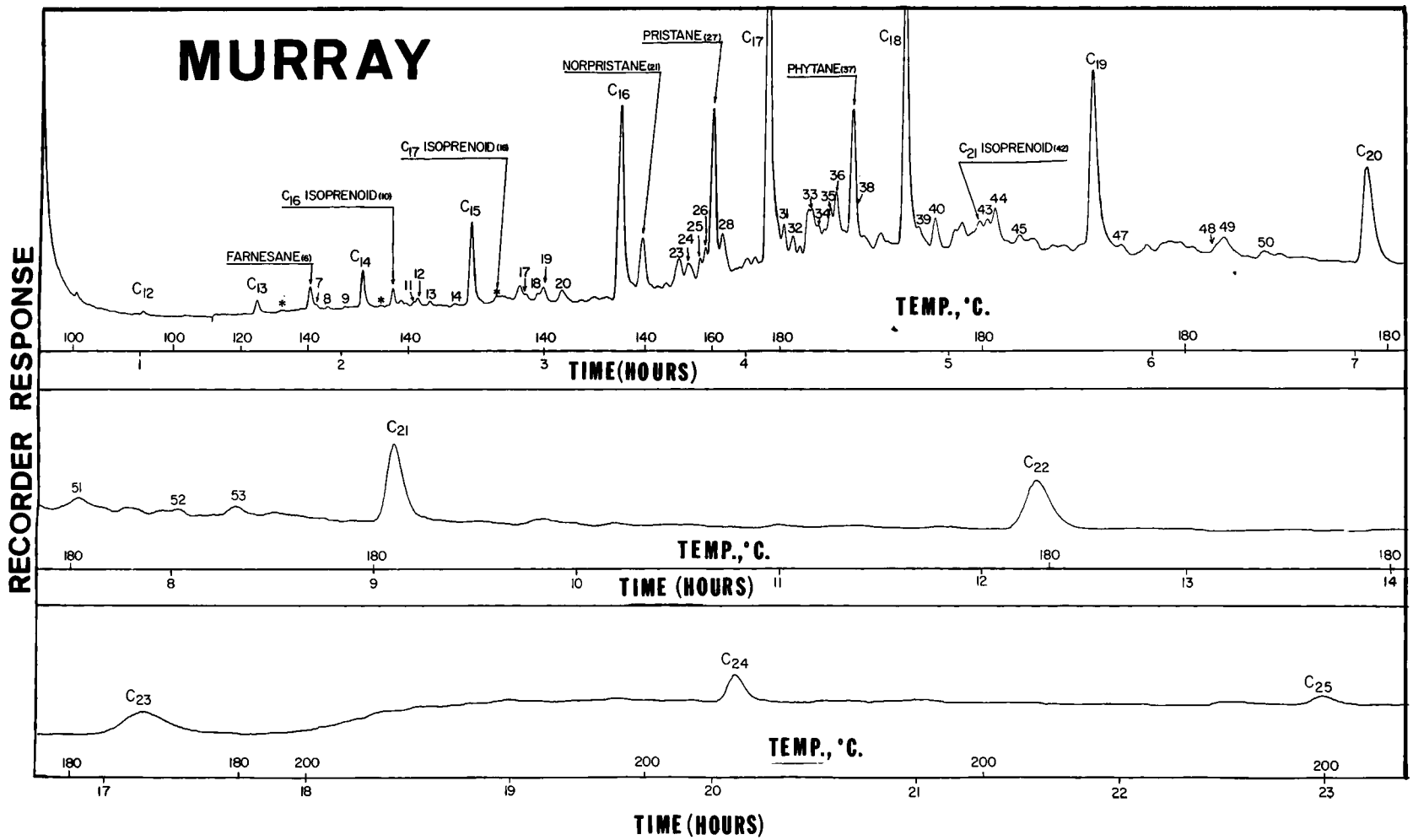

VIGARANO
C11
C12
C13
C14
C15
19
20
17
10
12
11
13
16
6
7
8
9
2
3
4
SOLVENT
RECORDER RESPONSE
TEMP., °C.
105
105
125
145
165
185
195
10
20
30
40
50
60
70
TIME (MINUTES)

FIGURE 80

HIGH RESOLUTION GAS CHROMATOGRAM OF THE HYDROCARBONS FROM THE MURRAY METEORITE

Stainless steel capillary column (310 m long x 0.076 cm i.d.) coated with Polysev. Same conditions as in Figure 79. Temperature held isothermally at 100°C for about 1 hr. and 10 min., then programmed to 140°C at 1°C/min. and held at this temperature for 1 hr. 40 min. after which it was programı at 1°C/min. to 180°C. After about 17 hrs. the temperature was pushed to 200°C to elute the last components. (See Table XLIX for peak number identifications).

A total of 4.1373 g of the meteorite were extracted. One hal: of this extract was taken for this analysis and about 1.9/2.9 of the n-pentane eluate was injected.


MURRAY
RECORDER RESPONSE
PRISTANE(27)
NORPRISTANE(21)
PHYTANE(37)
C16 ISOPRENOID(10)
C17 ISOPRENOID(13)
C21 ISOPRENOID(42)
FARNESANE(6)
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
C23
C24
C25
TEMP., °C.
TIME(HOURS)
TIME (HOURS)

alkanes. See for example peaks 11 and 12, also 18 and 19. In the high resolution column, although the partial separation achieved is enough to obtain better mass spectra of each compound, it is far from being ideal but this is the best that can be achieved.

Table LIV shows an example of the mass spectrometric approach to the identification of the components present in an unresolved peak. It represents an actual case encountered in the analysis of the alkane fraction from the Mokoia meteorite. It is always difficult to get three separate peaks out of the mixture of iso, anteiso C_{17} and pristane (see Figure 75, peaks 26, pristane and 28), but the mass spectra taken at some points along the upward slope, at the top of the peak and at some points along the downward slope can usually tell each component apart from the others. A careful inspection of Table LIV shows that the contribution of pristane to the 2-methyl C_{16} spectra is very low, the spectra being in this case fully representative of the isoalkane structure as indicated by the parent ion at m/e 240, the M-15 peak at m/e 225 and the high M-43 peak at m/e 197. In the next scan pristane starts to dominate the spectrum as shown by the increase of the intensity of the peak at the m/e 268, the parent ion of pristane, and also by the increase in the intensities of the M-15 peak (m/e 253) and that of the characteristic fragment at m/e 183. Two more things to consider are the decrease of the m/e 99

TABLE LIV

PARTIAL SPECTRA REPRESENTING FIVE SUCCESSIVE SCANNINGS OF A THREE COMPONENT GAS CHROMATOGRAPHIC PEAK

m/e	ion	Relative Intensities				
		2-meC_{16}	Pristane +2-meC_{16}	Pristane	Pristane +3-meC_{16}	Pristane +3-meC_{16}
99	$C_7H_{15}^+$	127 *	62	57	71	106
113	$C_8H_{17}^+$	100	100	100	100	100
127	$C_9H_{19}^+$	80.5	41	38	42	71
141	$C_{10}H_{21}^+$	60	20	18	45	55.5
155	$C_{11}H_{23}$	42 *	.5	20	24	42
169	$C_{12}H_{25}^+$	32.5	8	6.5	12	31
183	$C_{13}H_{27}^+$	25	63	61	55	43
197	$C_{14}H_{29}^+$	138 *	26	8.5	10	24
211	$C_{15}H_{31}^+$	13.5 *	4	2.5	12	42.5
225	$C_{16}H_{33}^+$	42.5	7.5	5.1	8	18
239	$C_{17}H_{35}^+$	7.5	4	3.5	4	--
240	$C_{17}H_{36}^+$	13.5	2	1	1.5	15.5
253	$C_{18}H_{37}^+$	3	5.5	5	4.3	4
267	$C_{19}H_{39}^+$	4	--	--	--	--
268	$C_{19}H_{40}^+$	2	5	4.8	3.8	--

peak and the intensity of the C_{11} fragment (see Pristane std., Figures 10,81). In the next mass spectrum there is practically no contribution of the iso structure as indicated by the intensity of the iso C_{17} parent ion. Then, on the last two spectra taken on the descent of the peak, the anteiso C_{17} features start to appear on top of the spectrum of pristane. The m/e 99 peak rises, the C_{11} fragment is no longer higher than the preceeding C_{10} fragment, the intensity of the m/e 240 ion begins to grow again and the characteristic M-29 peak at m/e 211 starts to appear. All this is more pronounced in the last spectra which has not been labelled as 3-methyl C_{16} only with the purpose to emphasize that the spectrum does still show a relatively high 183 fragment.

All this illustrates how the identification problems can be approached in these cases which are often encountered in these type of analyses.

Mass spectrometric analysis of isoprenoids in meteorites

Typical mass spectra for pristane and phytane from five of the carbonaceous chondrites are shown in Figures 81 and 82. In connection with the major fragment ions of these compounds it is important to remember that the enhanced intensities of their corresponding olefin ions are also good indication of the branching site. This was discussed in detail in the sections dealing with the mass spectrometry of isoalkanes and

FIGURE 81

MASS SPECTRA OF PRISTANE STANDARD AND PRISTANE FROM FIVE DIFFERENT METEORITES

The spectra were taken as the corresponding pristane peak in each sample was eluted from a 190 m by 0.076 cm i.d. stainles steel capillary column coated with Polysev. LKB 9000 Mass Spectrometer combination. Electron energy, 70 eV. Ionizing current, 120 μA. Accelerating voltage, 3.5 KV.

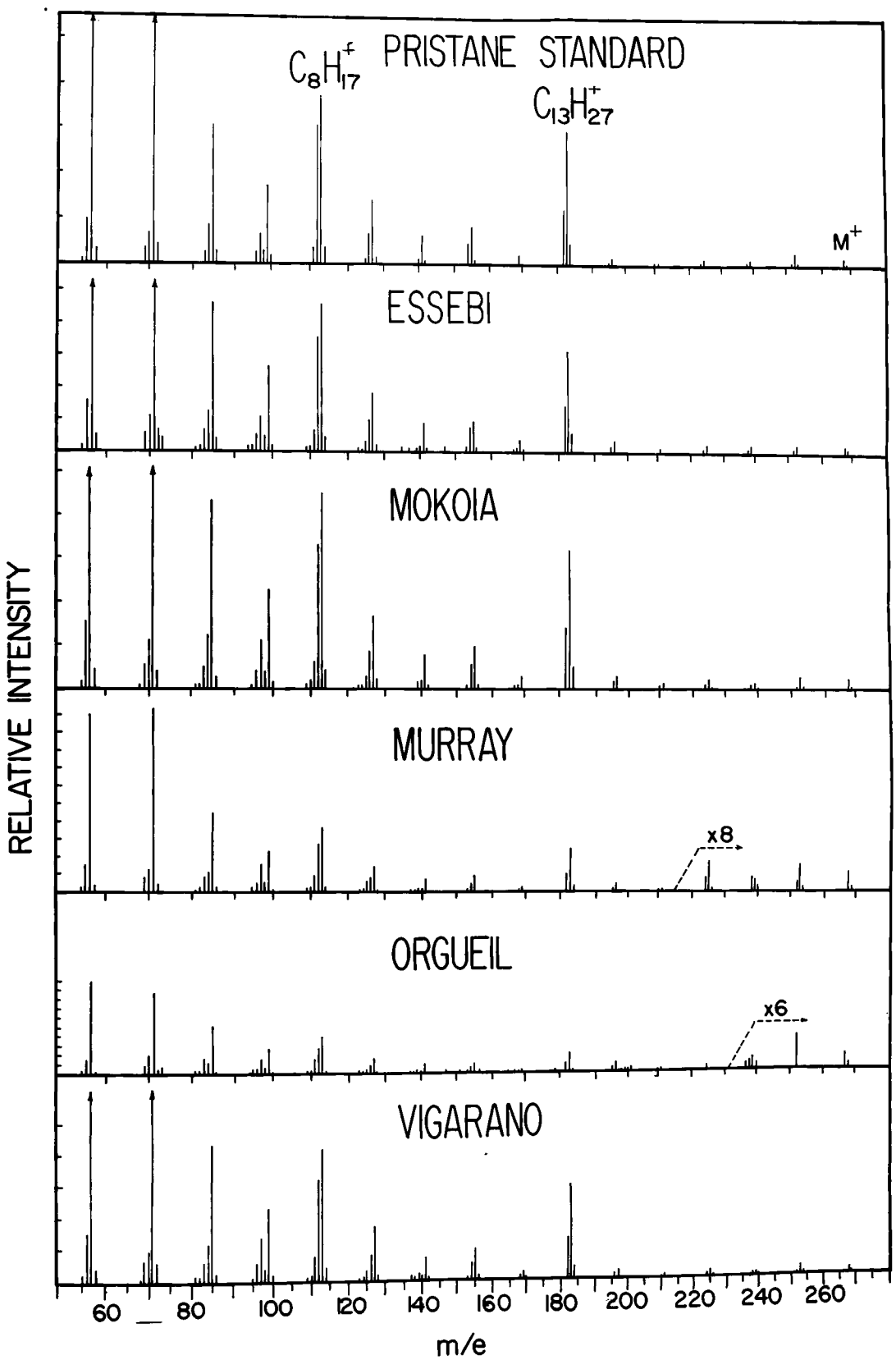
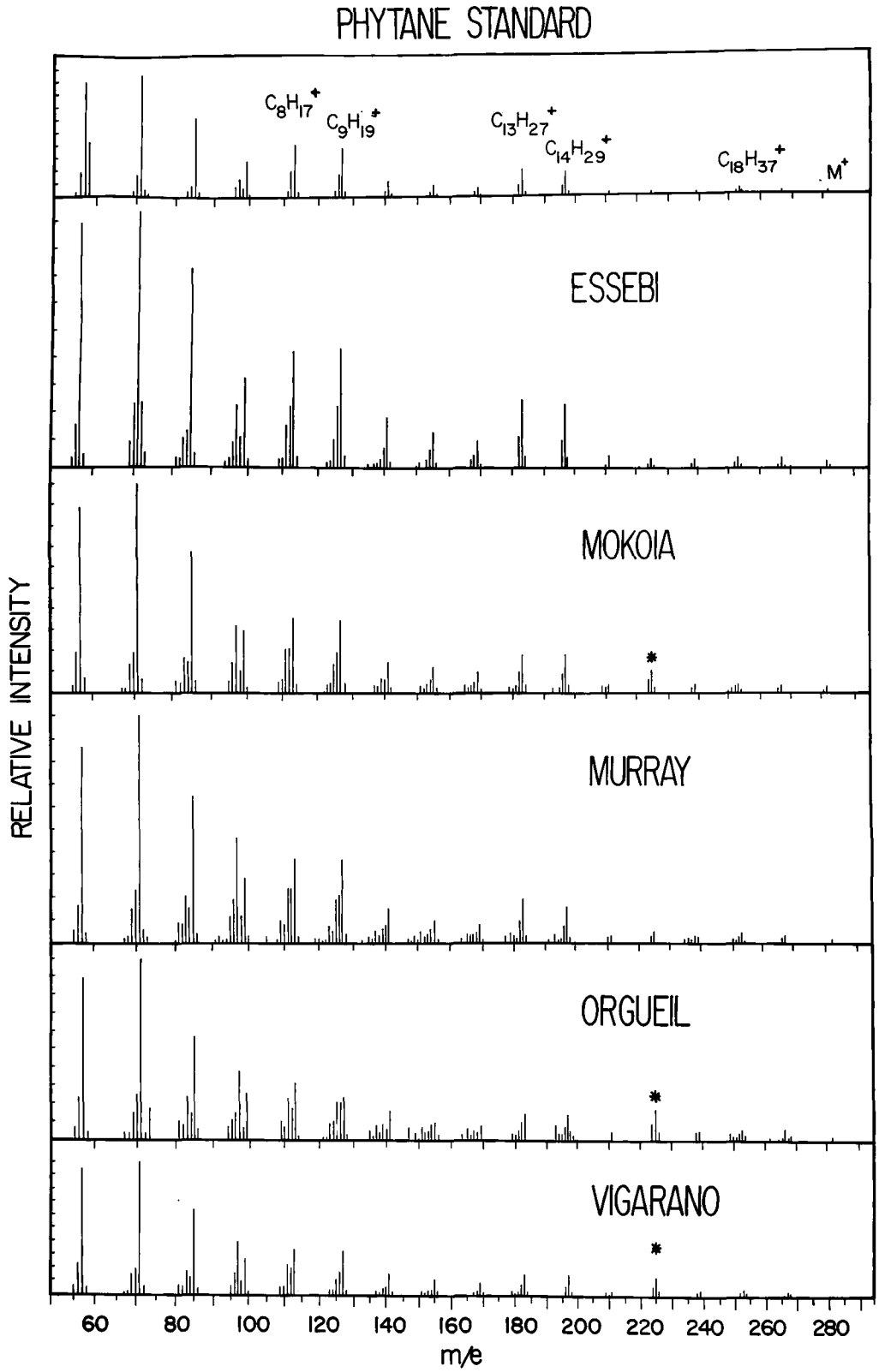

PRISTANE STANDARD
$C_8H_{17}^+$
$C_{13}H_{27}^+$
M^+
ESSEBI
MOKOIA
MURRAY
x8
ORGUEIL
x6
VIGARANO
RELATIVE INTENSITY
60
80
100
120
140
160
180
200
220
240
260
m/e

FIGURE 82

MASS SPECTRA OF PHYTANE STANDARD AND PHYTANE

FROM FIVE DIFFERENT METEORITES

Same conditions as described in Figure 81.


PHYTANE STANDARD
$C_8H_{17}^+$
$C_9H_{19}^+$
$C_{13}H_{27}^+$
$C_{14}H_{29}^+$
$C_{18}H_{37}^+$
M^+
ESSEBI
MOKOIA
MURRAY
ORGUEIL
VIGARANO
*
RELATIVE INTENSITY
60
80
100
120
140
160
180
200
220
240
260
280
m/e

isoprenoids (see Chapter IV). Although the stationary phase used (Polysev) afforded excellent separation of pristane and phytane from the C_{17} and C_{18} normal alkanes, respectively, the length of the columns and the range of temperatures used for these analyses were not adequate to achieve satisfactory resolution of pristane from the iso-C_{17} and of phytane from the anteiso-C_{18} (see Figure 8 and Figures 75,76).

This relative lack of resolution does not interfere with the obtaining of good mass spectra in the case of pristane as can be seen in Figure 81, but it does to a certain extent for phytane because this compound is present in smaller amounts (Table L) and the total percent contribution of the anteiso-C_{18} to the phytane peak is greater.

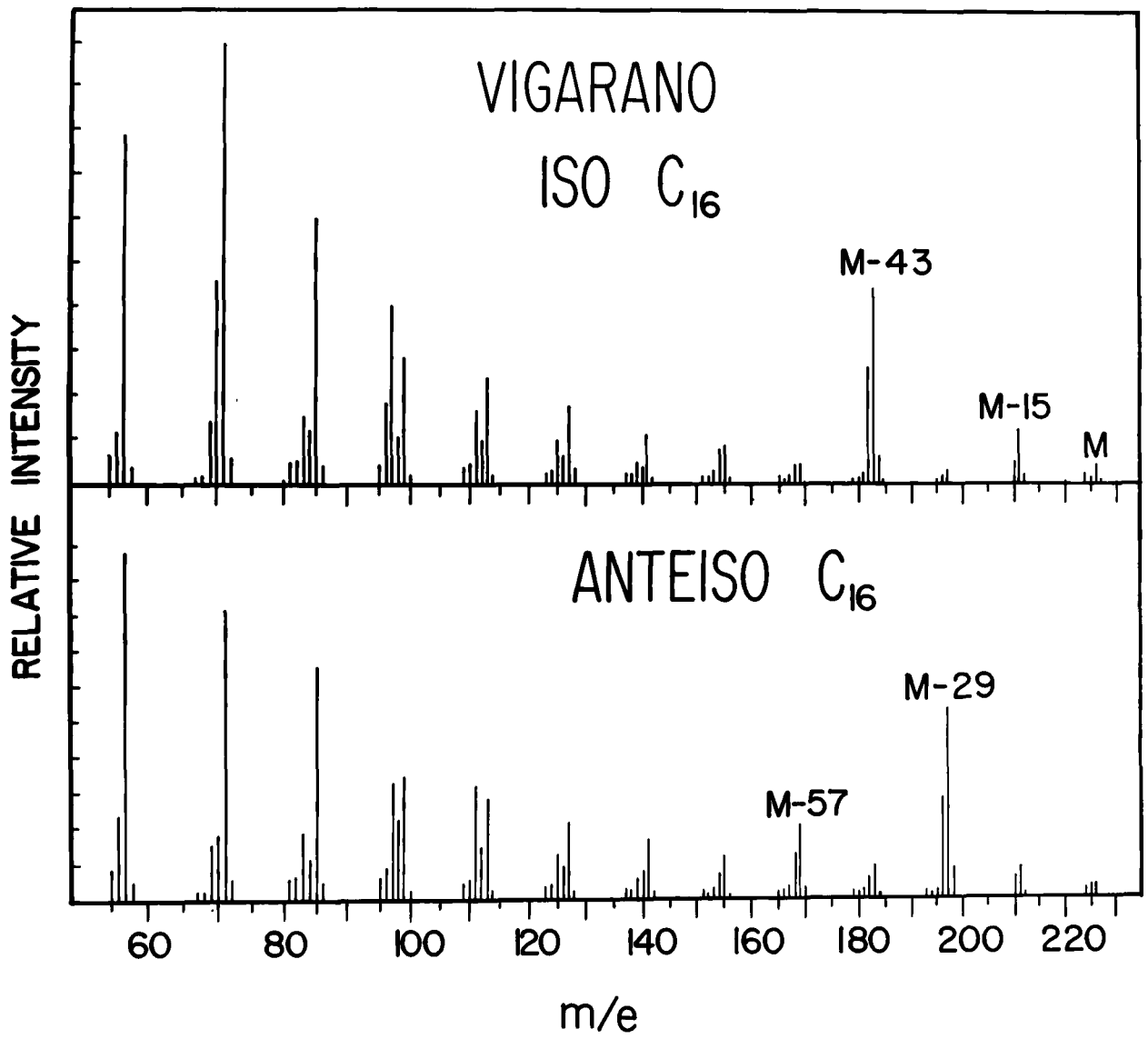
However, as shown in Figure 82, there is a good general agreement between the mass spectrum of the phytane standard and the spectra of the gas chromatographic peak corresponding to phytane in the meteorites studied. The higher relative intensity of the ion at m/e 225 (marked with an asterisk in Figure 82) can be accounted for by taking into consideration the fragmentation pattern of the anteiso-C_{18} hydrocarbon (see Figure 43 and Table XXXIX). The anteiso or 3-methyl C_{18} which is eluted together with phytane will produce a high M-29 peak (Figures 43,83) at m/e 225, and its characteristic fragmentation pattern will be superimposed on that of phytane. The coincidence of their respective elution

FIGURE 83

MASS SPECTRA OF ISOALKANES FROM

THE VIGARANO METEORITE

Same conditions as described in Figure 81.


VIGARANO
ISO C_{16}
M-43
M-15
M
ANTEISO C_{16}
M-29
M-57
RELATIVE INTENSITY
60
80
100
120
140
160
180
200
220
m/e

times has been proved experimentally with the proper standards. A similar situation has been presented in Table XXXIX.

In line with the data presented in Table XLIX and Figure 75 the members of the isoprenoid series (other than pristane and phytane) identified in the extracts from Vigarano have been arranged according to their molecular weights in Figure 84. The C_{14} isoprenoid, not shown in this figure was also identified. If the pristane and phytane fragmentation patterns in Figures 81 and 82 are included in this series in their respective places, between the C_{18} and C_{21} isoprenoids, the regularity and order of the fragmentation patterns of these isoprenoid structures becomes apparent.

As it has been already discussed in the section devoted to the mass spectrometry of isoprenoids, the C_8 and C_{13} fragments are the common characteristic exhibited by all isoprenoid spectra (see Figure 9 and Table VII). This can be expected to be so since the process of chain buildup by successive addition of carbon atoms to the right end side of the chain will not change the left to right carbon count, but it will progressively increase by one carbon the length of the right to left fragments associated with the C_8 and C_{13} ions. This effect is demonstrated by the two series of increasing fragments that start at C_4 and C_9 in the spectra of the C_{15} isoprenoid, Figure 84 (top spectrum). The presence and

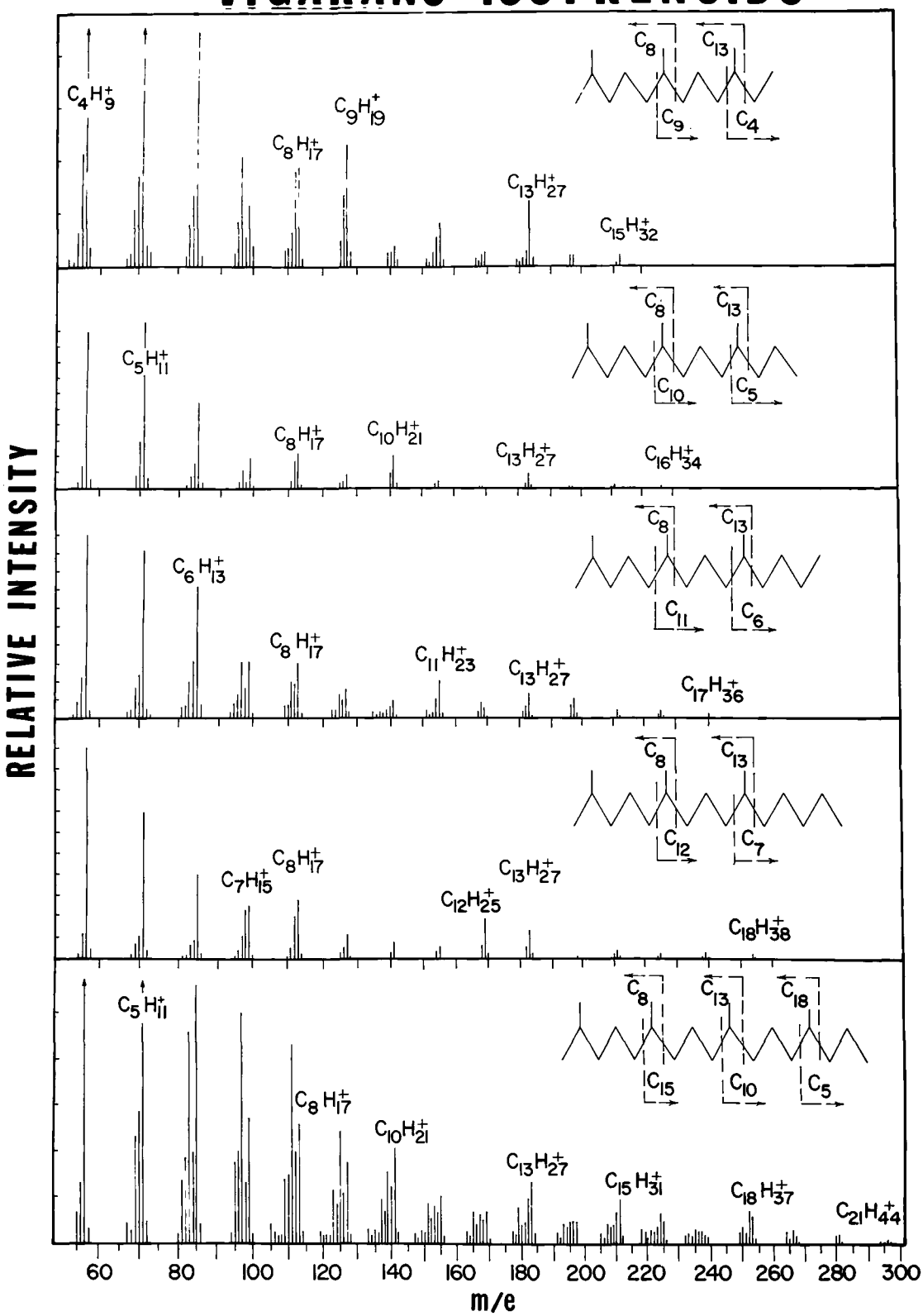
FIGURE 84

MASS SPECTRA OF FIVE MEMBERS OF THE

VIGARANO ISOPRENOID SERIES

Same conditions as described in Figure 81.

VIGARANO ISOPRENOIDS



relative intensity of the olefin ions is especially useful in the low mass region of the spectra where due to the high concentration of ions it is more difficult to detect the fragments originated at a branching center.

The isoprenoids from the Murray meteorite, from the C_{14} to the C_{21}, are also displayed in the same manner in Figures 85 and 86. See Table XLIX for numbers in the figures. The mass spectrum of the C_{14} isoprenoid (2,6,10-Trimethyl undecane) not shown before in the Vigarano isoprenoid series is included in this case in Figure 85. In both instances the C_8 fragment was found to dominate the spectrum with its high intensity. This is in line with the effect of the symmetry of this molecule on the predicted pattern.

The C_{11} fragment, of smaller size, corresponds to the loss of the terminal propyl group (M-43). The peak at C_{13} corresponds to the loss of a methyl group, in general not a preferred mode of fragmentation.

The contribution of the anteiso-C_{18} to the m/e 225 peak in the spectra of phytane can be seen again in Figure 86.

The mass spectrum of the C_{17} isoprenoid shows all the features expected from its structural characteristics plus a peak at C_{14} that does not seem to fit into the pattern. It can be seen in both cases Figures 84 and 85. The characterization of this isoprenoid is of particular importance since it is not usually found in detectable amounts in

FIGURE 85

MASS SPECTRA OF MURRAY ISOPRENOIDS

The spectra were taken as each individual component was eluted from a stainless steel capillary column (310 m long by 0.076 cm i.d.) coated with Polysev. Ionized by electron impact at 20 eV. LKB 9000 gas chromatograph-mass spectromete combination.

Circled numbers to the right hand side correspond to identification numbers in Table XLIX.

MURRAY ISOPRENOIDS

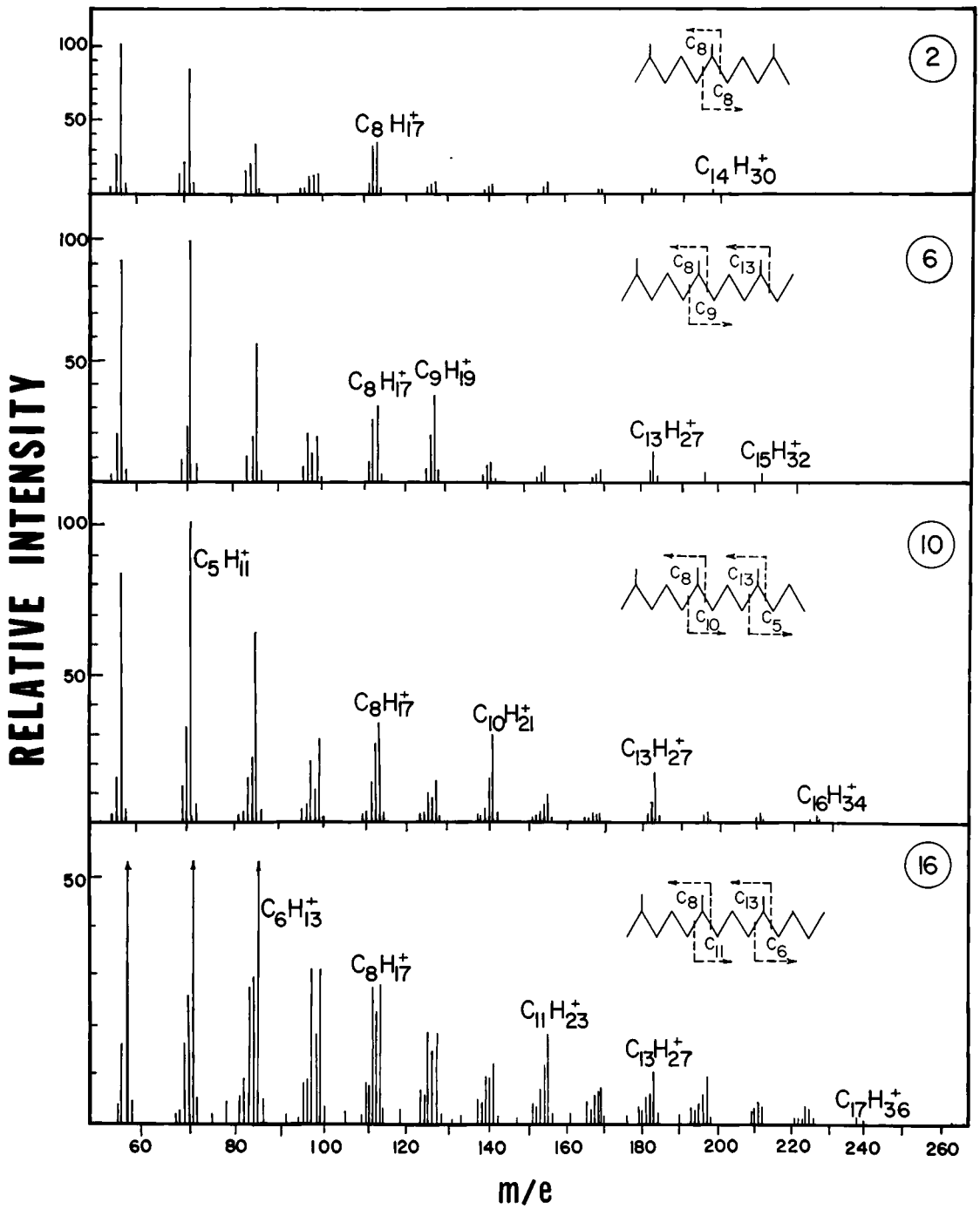
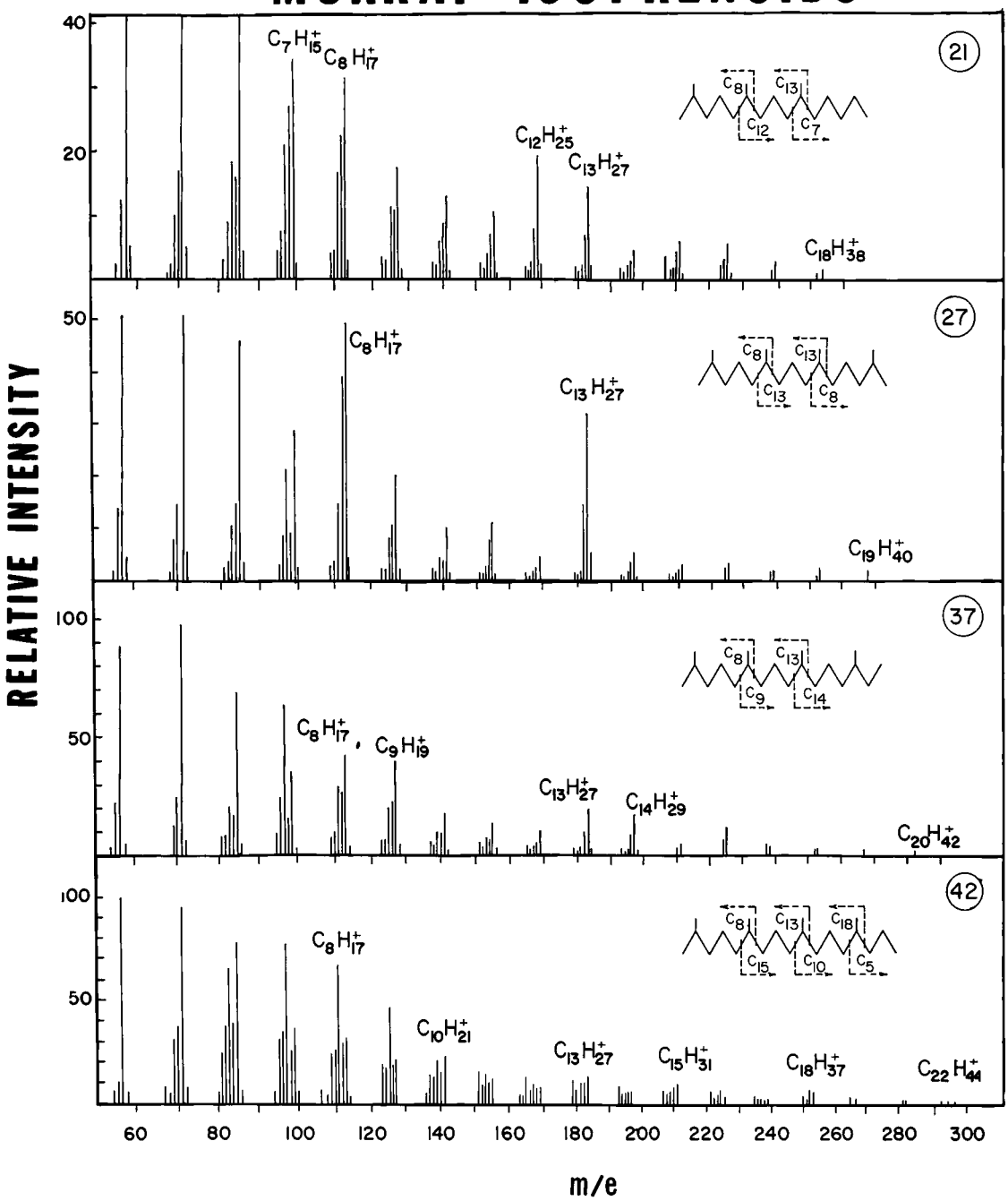


FIGURE 86

MASS SPECTRA OF MURRAY ISOPRENOIDS

Same conditions as described in previous figure.

MURRAY ISOPRENOIDS



natural products. There is only one report in the literature of its isolation and mass spectrometric identification (282). Because of the relatively high intensity of the fragment at C_{14}, it may be argued that the spectrum may not be that of a C_{17} isoprenoid. On the other hand the gas chromatographic retention data supports the mass spectrometric identification. A close look at the chromatogram in Figures 79 and 80 will reveal that the trimethyl substituted isoprenoids get progressively closer to the n-alkanes that preceed them as their molecular weight increases. The position of the C_{17} isoprenoid is exactly that corresponding to such a structure. In connection with this problem it must be taken into account that there is an unidentified homologous series whose chromatographic positions are marked by an asterisk in Figure 80. These components show relatively large fragments at C_{12}, C_{13} and C_{14} respectively. The isoprenoid homologous series and this unknown series seem to converge at the same point just after the n-C_{15}. This most likely could account for the relatively higher intensity of the C_{14} fragment in the spectrum of the C_{17} isoprenoid, see Figures 84 and 85.

Although the positive identification of these compounds has not been accomplished, in view of the data presented in Table XLIX, it seems logical that they may be lowest members of the cyclohexyl homologous series. Also in the same way that the unknown compound with the maxima at C_{14} interacts

with the spectra of the C_{17} isoprenoid, the spectra of the C_{18} isoprenoid would be expected to show a high C_{15} peak which would represent the continuation of this unknown series. In fact it does, as shown in Table LV.

The relative intensities of the fragments at m/e 82 and m/e 83 corresponding to the cleavage of the ring appear to support the assumption that these compounds are indeed members of the cyclohexyl series. It has already been shown that this class of compounds would be eluted after their corresponding n-alkanes with one more carbon atom. Thus the cyclohexyl series shown in Table XLIX for the Murray meteorite may be tentatively expanded to include the lower members down to heptyl cyclohexane.

The partial spectra of all of the isoprenoids identified in each one of the carbonaceous chondrites analyzed by the combination gas chromatography-mass spectrometry are given in Table LVI. The most characteristic isoprenoid fragments are marked by an asterisk to the left of their position in the reference spectrum. The peak at m/e 113 corresponding to the C_8 fragment has been selected as the base peak of all the spectra to facilitate comparisons. The published isoprenoid standards have also been recalculated on this basis.

In general there is a very close correlation between the spectra of the standards and those of the identified compounds.

TABLE LV

PARTIAL MASS SPECTRUM OF A MIXTURE OF NORPRISTANE AND NONYLCYCLOHEXANE IN THE MURRAY METEORITE

m/e	ion	Rel. Int.	
82	$C_6H_{10}^+$	32	}nonylcyclohexane
83	$C_6H_{11}^+$	44	
84	$C_6H_{12}^+$	28	
85	$C_6H_{13}^+$	100	
99	$C_7H_{15}^+$	58	
113	$C_8H_{17}^+$	56	
127	$C_9H_{19}^+$	28	
141	$C_{10}H_{21}^+$	19	
155	$C_{11}H_{23}^+$	14	
169	$C_{12}H_{25}^+$	34	
183	$C_{13}H_{27}^+$	25	
197	$C_{14}H_{29}^+$	5	
210	$C_{15}H_{30}^+$	8	M (nonylcyclohexane)
211	$C_{15}H_{31}^+$	9	
225	$C_{16}H_{33}^+$	5.2	
239	$C_{17}H_{35}^+$	5.2	
254	$C_{18}H_{38}^+$	3	M (norpristane)

M = molecular ion

TABLE LVI

PARTIAL MASS SPECTRA OF ISOPRENOIDS IN METEORITES

m/e	Ion	C_{14} (Norfarnesane)			C_{15} (Farnesane)		
		Murray	Vigarano	Mokoia	Murray	Vigarano	Published[1]
57	$C_4H_9^+$	295	339	345	289	400	1052
71	$C_5H_{11}^+$	247	380	375	314	525	716
85	$C_6H_{13}^+$	98	90	200	179	235	302
99	$C_7H_{15}^+$	45.5	30	65	60	61	42
113	$C_8H_{17}^+$	100	100*	100	100	100	100*
127	$C_9H_{19}^+$	25	25	115.5	113	122	111*
141	$C_{10}H_{21}^+$	21.5	7.5	30	26	21	13
155	$C_{11}H_{23}^+$	28	14*	22	21	43.5[2]	10
169	$C_{12}H_{25}^+$	10.5	9	15	17	15	6
183	$C_{13}H_{27}^+$	8	6.5	39	40	65[2]	30*
197	$C_{14}H_{29}^+$			11	15	11	9

TABLE LVI (CONTINUED)

PARTIAL MASS SPECTRA OF ISOPRENOIDS IN METEORITES

m/e	Ion	C_{14} (Norfarnesane)		C_{15} (Farnesane)			
		Murray	Vigarano	Mokoia	Murray	Vigarano	Published[1]
198	$C_{14}H_{30}^{+}$	7	6				
212	$C_{15}H_{32}^{+}$			15	12	10	6

[1] From reference 32

[2] Contributions of the iso C_{14} alkane

* Most characteristic ions

TABLE LVI (CONTINUED)

PARTIAL MASS SPECTRA OF ISOPRENOIDS IN METEORITES

		C_{16} Isoprenoid				C_{17} Isoprenoid			
m/e	Ion	Mokoia	Murray	Vigarano	Published[3]	Mokoia	Murray	Vigarano	Published[4]
57	$C_4H_9^+$	295	257	462	810	277	401	342	
71	$C_5H_{11}^+$	302	309	490	669	286	370	310	
85	$C_6H_{13}^+$	185	196	250	314	219	278	248	
99	$C_7H_{15}^+$	84.5	86	85	76	104	111.5	105	72
113	$C_8H_{17}^+$	100	100	100	100*	100	100	100	100*
127	$C_9H_{19}^+$	40	41	38	27	58	64	52	31
141	$C_{10}H_{21}^+$	91.5	92	91	93*	33	43.5	33	10
155	$C_{11}H_{23}^+$	25	28	18	17*	76	65	69	57*
169	$C_{12}H_{25}^+$	8	11	3.5	5	18	26	20	4
183	$C_{13}H_{27}^+$	62	49	46	44*	42	37.5	45	30*
197	$C_{14}H_{29}^+$	14.5	12	6	7	28	34	35	4

TABLE LVI (CONTINUED)

PARTIAL MASS SPECTRA OF ISOPRENOIDS IN METEORITES

		C_{16} Isoprenoid				C_{17} Isoprenoid			
m/e	Ion	Mokoia	Murray	Vigarano	Published[3]	Mokoia	Murray	Vigarano	Published[4]
211	$C_{15}H_{31}^{+}$	13	11	8	11	14	13	14	4
225	$C_{16}H_{33}^{+}$					10	9	13	7
226	$C_{17}H_{36}^{+}$	11	10	6.5					
240	$C_{18}H_{38}^{+}$					6	4	7	4

[3] From reference 263

[4] From reference 281

* Most characteristic ions

TABLE LVI (CONTINUED)

PARTIAL MASS SPECTRA OF ISOPRENOIDS IN METEORITES

		C_{18} (Norpristane)						C_{19} (Pristane)						
m/e	Ion	E.	Gr.	Mo.	Mv.	V.	Pub.[5]	E.	Gr.	Mo.	Mv.	O.	V.	Pub.[6]
57	$C_4H_9^+$	308	310	370	400	358	2048		205	230	215	250		
71	$C_5H_{11}^+$	228	226	270	300	247	906		200	210	203	220		
85	$C_6H_{13}^+$	145	166	170	216	142	377	99	102	98	105	130	103	90
99	$C_7H_{15}^+$	89.5	95	91	112	89	155	58	61	59	60.2	70	54	50
113	$C_8H_{17}^+$	100	100	100	100	100	100*	100	100	100	100	100	100	100*
127	$C_9H_{19}^+$	42.5	43	45.5	56	41	34.5	39	46	37.5	40.5	45	40	37
141	$C_{10}H_{21}^+$	30	31	33	40	27	20.5	19	18	18	20	27	17.5	16
155	$C_{11}H_{23}^+$	23	30	25	32	20	12.2	20.5	22	20	23	26.5	22.5	21
169	$C_{12}H_{25}^+$	53	49	63	58	67	55.1*	7	8.5	6.5	8.8	9	6	4.5
183	$C_{13}H_{27}^+$	44.5	40	49	44	48	36.7*	69	59	61	65.8	56	71	79*
197	$C_{14}H_{29}^+$	14	13	9	14	4	2	7	9	8.3	11	30	5.8	4

TABLE LVI (CONTINUED)

PARTIAL MASS SPECTRA OF ISOPRENOIDS IN METEORITES

		C_{18} (Norpristane)						C_{19} (Pristane)						
m/e	Ion	E.	Gr.	Mo.	Mu.	V.	Pub.[5]	E.	Gr.	Mo.	Mu.	O.	V.	Pub.[6]
211	$C_{15}H_{31}^+$	13	18	13.5	18.4	12	4	2.5	5	2	6.2	13.5	2.9	1.5
225	$C_{16}H_{33}^+$	8	9	7	17.2	7	6	4.5	5.5	4.7	7	11	4.8	3.2
239	$C_{17}H_{35}^+$	12	10	10.5	8	10	8	4	3.5	2.7	4	4	3.4	3.4
253	$C_{18}H_{37}^+$							5	7.8	4.5	5	10	5.5	6.2
254	$C_{19}H_{40}^+$	7	9	7.2	4.4	6								
268	$C_{20}H_{42}^+$							4.5	6	4.3	4	6	4.5	4.3

[5] From reference 263

[6] From reference 169

* Most characteristic ions

E. (Essebi); Gr. (Grosnaja); Mo. (Mokoia); Mu. (Murray); V. (Vigarano)

TABLE LVI (CONTINUED)

PARTIAL MASS SPECTRA OF ISOPRENOIDS IN METEORITES

		C_{20} (Phytane)							C_{21}				
m/e	Ion	E.	Gr.	Mo.	Mu.	O.	V.	Pub.[7]	E.	Mo.	Mu.	V.	Pub.[8]
57	$C_4H_9^+$	207	217	251	236	288	282	242	248	300	313	310	
71	$C_5H_{11}^+$	221	242	281	274	326	290	265	232	285	292	295	
85	$C_6H_{13}^+$	171	210	189	176	174	189	170	245	215	237	214	
99	$C_7H_{15}^+$	77	79	82	76	82	81	76	93	95	112	103	
113	$C_8H_{17}^+$	100	100	100	100	100	100	100*	100	100	100	100	100*
127	$C_9H_{19}^+$	102	95	96	88.5	71	96	103*	65	68	69.5	66	58
141	$C_{10}H_{21}^+$	43	40	41	41	47	43	32	80	80	76	79	71*
155	$C_{11}H_{23}^+$	32	35	33	28	31	33	23	44	39.5	43	39	32
169	$C_{12}H_{25}^+$	22	25.5	26	21	24	26	19	27	26	29	26	21
183	$C_{13}H_{27}^+$	58.5	51.5	50	51	44	49	57*	51	51	48.5	51	44*
197	$C_{14}H_{29}^+$	53.5	48.5	49	42	46	45	49*	18	18.5	23	18	23

TABLE LVI (CONTINUED)

PARTIAL MASS SPECTRA OF ISOPRENOIDS IN METEORITES

		C_{20} (Phytane)							C_{21}				
m/e	Ion	E.	Gr.	Mo.	Mu.	O.	V.	Pub.[7]	E.	Mo.	Mu.	V.	Pub.[8]
211	$C_{15}H_{31}^+$	9.5	11	9	8	17	8	4.2	9	39	38.8	36	29*
225	$C_{16}H_{33}^+$	8	13	30*	12	57*	47*	4	12	13.5	17	18	20
239	$C_{17}H_{35}^+$	7	7	9	4.5	15	8.5	3	13	8	15	5.5	8
253	$C_{18}H_{37}^+$	13.5		12	11.5	17	12	14.5	19	23	24.5	22.5	17*
267	$C_{19}H_{39}^+$	7		7	7.5		8	7.5	8.5	8	9	6	5
281	$C_{20}H_{41}^+$									18	8	7	4
282	$C_{21}H_{42}^+$	5		6	3	4	5	4					
296	$C_{22}H_{44}^+$									4	2.8	3	1

[7] From reference 31

[8] From reference 32

* Most characteristic ions

E. (Essebi); Gr. (Grosnaja); Mo. (Mokoia); Mu. (Murray); O. (Orgueil); V. (Vigarano)

None of the isoprenoid fragments has a lower intensity than what it should: if anything, intensities are higher than expected. This may be due to contributions of other gas chromatographically unresolved compounds, although in most cases this is not a likely explanation. The real reason probably lies in the use of low energy electrons (20 eV) to bombard the sample with. This as it has been discussed before (170) enhances the intensity of the fragment peaks as well as that of the molecular ions (see Chapter IV).

It must be kept in mind also that, as mentioned above, the degree of resolution obtained, even with the high efficiency columns, is far from ideal and so all of these spectra may show contributions of other compounds in different degrees. For instance, the case of phytane and the anteiso-C_{18} has already been mentioned.

The two other isoprenoid-like structures not yet reported in any terrestrial sample have been tentatively identified in three of the carbonaceous chondrites. These are the 2,6,10-trimethyl hexadecane which has been discussed in the literature (281) in relation to the problem of structural mass spectrometric identification of pristane, but has not been isolated or identified, and the 4,7,11-trimethyl hexadecane, an irregular isoprenoid-like structure that may have a bearing on the proposed schemes of diagenetic degradation of the parent isoprenoid compound.

Although the mass spectra obtained for both compounds seem to fit rather well the breakdown pattern predicted for these two structures, a final word on both identifications can not be said until the proper standards are synthesized and the mass spectral results compared. A mass spectral pattern consistent with the 2,6,10-trimethyl hexadecane structure (Table LVII) is that of compound 30 in Table XLIX which shows the characteristic C_8 and C_{13} fragments typical of the regular isoprenoid structure. Besides, its chromatographic retention time also seems to have the right value. As it has been demonstrated by McCarthy (281) and by Eglington and Calvin (138) by means of a synthetic compound with this same structure, those are practically the only features exhibited by its spectrum. In fact it is so similar to the mass spectrum of pristane that these authors consider the possibility of confusing one with the other, although this has been shown here to be chromatographically unlikely (Figure 12). In attempting to analyze this spectrum and before drawing any conclusions it must be kept in mind that this compound is not perfectly resolved from the 4,7,11-trimethyl C_{16} and that both appear in a region strongly dominated by the C_{16} cycloalkanes. In fact there are indications of the presence of peaks corresponding to the C_{16} cyclopentyl structure, like the relative intensities of the C_5H_8 and C_5H_9 fragments, the intensity of the ions in the olefin series

TABLE LVII

ISOPRENOID RELATED STRUCTURES IN MOKOIA

PARTIAL MASS SPECTRA

	2,6,10-Trimethyl C_{16} (+ undecylcyclopentane)[2]					4,7,11-Trimethyl C_{16}					4,7,11-Trimethyl C_{16} (+ decylcyclohexane)[2]				
	Mass Series (Z in C_nH_{2n+Z})														
C_n \ Z	-2	-1	0	+1	+2[1]	-2	-1	0	+1	+2[1]	-2	-1	0	+1	+2[1]
C_5	12	26	28.5	100		5	20	23	100		5	20	35	100	
C_6	11	32	25	66		11	30	23	78		67	73	24	74	
C_7			17	27				29	39*				32	36*	
C_8			31	34*				22	30				20	28	
C_9			18	21				20	29*				19	27*	
C_{10}			10.5	16				11	16				13	17	
C_{11}			10	14				8.5	12				7	10	
C_{12}			12.5	13				10	18*				11	18*	
C_{13}			12	17.5*				7	9				7	8	
C_{14}			6	6				8	12.5*				8	11*	
C_{15}			4.3	5.5				4.7	7.5				4.5	5.5	
C_{16}			10.5	11				6.5	11*				12	11*	

TABLE LVII(CONTINUED)

ISOPRENOID RELATED STRUCTURES IN MOKOIA

PARTIAL MASS SPECTRA

	2,6,10-Trimethyl C_{16} (+ undecylcyclopentane)[2]					4,7,11-Trimethyl C_{16}					4,7,11-Trimethyl C_{16} (+ decylcyclohexane)[2]				
	Mass Series (Z in C_nH_{2n+Z})														
C_n \ Z	-2	-1	0	+1	+2	-2	-1	0	+1	+2	-2	-1	0	+1	+2[1]
C_{17}			4	4.3				4.5	7.5				4	5.5	
C_{18}			4	3.3				3.5	3.8				3	4	
C_{19}				0.6	0.9(M)					1.6(M)					1.2(M)

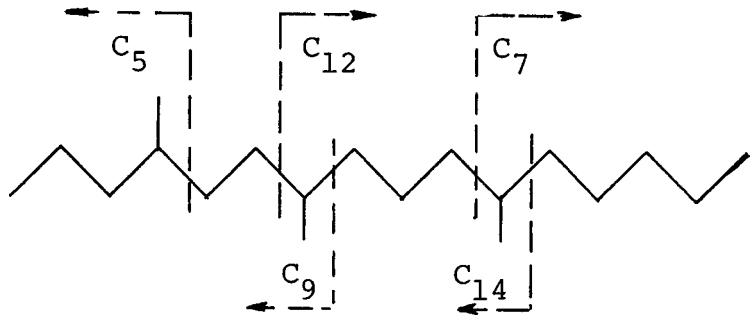
[1] Isotopic peaks (Z = +2) not included

[2] Tentative identification

* Most characteristic ions indicated by a star

(Z=0) and the small maxima at C_{16} (see Table LVII).

The mass spectrum of 4,7,11-trimethyl hexadecane would be expected to exhibit



prominent C_5, C_7, C_9, C_{12}, C_{14} and C_{16} peaks. The fragmentation at C_{12} and C_{14} being perhaps the most likely events. This is in full agreement with the second spectrum shown in Table LVII.

The three spectra were taken consecutively in a very narrow range of partially resolved gas chromatographic peaks and as said before the contributions of one spectra to the other must be considered whenever possible. The spectrum in the middle appears to be the one less influenced by the other compounds as indicated by the intensities of the fragments in the mass series z=-2 and -1 and the C_{16} olefin ion. The later appearance in a very close scan of a decylcylohexane is indicated by the raise in the C_6H_{10} and C_6H_{11} ions plus the C_{16} olefin ion. The same reasoning applies to the spectrum shown in Table LVIII.

Isoalkanes in meteorites

The mass spectrometric characteristics of isoalkanes have

TABLE LVIII

PARTIAL MASS SPECTRUM OF 4,7,11-TRIMETHYL HEXADECANE FROM VIGARANO

	Mass Series (Z in C_nH_{2n+2})					
C_n \ Z	-2	-1	0	+1	+2	
C_6	26	45	20	100		
C_7			42	42		*
C_8			20	32		
C_9			21.5	36		*
C_{10}			12	20		
C_{11}			6.5	13		
C_{12}			12	25.5		*
C_{13}			6	7		
C_{14}			10.5	16		*
C_{15}			4	5		
C_{16}			15	18		*
C_{17}			5.5	7		
C_{18}			3.5	3.8		
C_{19}					1.3 (M)	

Isotope peaks not included in Z = +2 mass series.

Most characteristic ions indicated by a star (*)

been described in Chapter IV. The theoretical and empirical correlations described there have been put into practice in the identification of these type of compounds in meteorite extracts. The results are presented in Figure 83 and Tables LIX, LX, LXI and LXII.

Figure 83 gives an example of the typical spectra exhibited by the 2-, and 3-methyl alkanes (iso and anteiso alkanes). The M-43 fragment ocrresponding to the loss of the terminal isopropyl group and the M-29 in the anteiso spectrum corresponding to the loss of the terminal ethyl group (see Chapter IV). The M-43 peak has been selected as the base peak in Table LIX to best show its progressive displacement along the isoalkane series. The only salient features of these Murray isoalkanes are the M-15 and M-43 fragments which are diagnostic of the iso structure. Table LX shows the partial spectra of the anteiso alkanes from the same meteorite. Here the most characteristic peak, the M-29, has also been selected as the base peak for the same reasons stated in connection with the previous table of isoalkanes. In this case due to the lack of resolution between pristane and anteiso-C_{17}, phytane and anteiso-C_{18}, these two methyl alkanes are not included in the table because no individual mass spectrum was obtained for them.

The smaller M-57 fragment that accompanies the M-29 fragment in these spectra, although not readily apparent in

TABLE LIX

PARTIAL MASS SPECTRA OF THE 2-METHYL ALKANES

FROM THE MURRAY METEORITE

m/e		C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}
71		408	670	340	300	310	300	605	395	348	315
85		198	450	250	240	216	249	450	330	276	240
99		125	170	120	110	113	136	235	175	152	140
113		88.5	160	91	82	87	101	188	138	126	111
127		50	130	86	63	69	85	140	127	102	88
141		100	52	45	45	55	75	123	108	79	75
155		43	100	28	33	38	55	101	89	79	69
169	M	48	42	100	22.5	31	39	66	68	80	68
183	184	7	183*	20	100	24	34	73	55	60	62
197	198		8	39	20	100	26	44	50	46	52
211	212			10	34	17	100	48	40	35	40
225	226				9	34	17	100	29	32	35
239	240					9.5	40	21	100	31	31
253	254						10	55	18	100	28
267	268							11.5	40	18	100
281	282								10	40	27
295	296									9	38
	310										9

M - molecular ion mass series

* Contribution of Farnesane (see Figure 80).

TABLE LX

PARTIAL MASS SPECTRA OF THE 3-METHYL ALKANES

FROM THE MURRAY METEORITE

m/e		C_{13}	C_{14}	C_{15}	C_{16}	C_{19}	C_{20}	C_{21}
71		395	440	324	290	410	372	325
85		325	320	264	237	335	325	268
99		139	154	127	137	161	180	158
113		114	125	105	102	126.5	148	153
127		80	90	75	75	104	123.5	109
141		57	55	47	57	88	105	81
155		100	45	40	35	75	89	70
169	M	54	100	34	58	56	87	65
183	184	7	43	100	34	47	71	90
197	198		7	29	100	34	47	45
211	212			8	29	33	37	31
225	226				6	33.5	34	29
239	240					100	33.5	28
253	254					30	100	33
267	268					7.5	32	100
281	282						9.5	28
	296							5

The C_{17} and C_{18} anteiso alkanes are not shown here because they were not resolved from the C_{19} and C_{20} isoprenoids (see Figure 80).

M - molecular ion mass series

TABLE LXI

PARTIAL MASS SPECTRA FOR THE 4-METHYL ALKANES

FROM THE MURRAY METEORITE

m/e		C_{15}	C_{16}	C_{17}	C_{18}	C_{20}
57		455	420	475	315	530
71		495	430	495	461	580
85		362	300	410	352	475
99		169	131	207	172	245
113		133	104	165	143	200
127		90	82	118	122	169
141		62	58	120	92	136
155		49	44	78.5	63	141
169		100	35	79	53	100
183		43	100	80	52	72
197	M	39	30	100	43	63
211	212	8	33	45	100	58
225	226		7.5	47	31	57
239	240			9	45	100
253	254				7	37
267	268					47
281	282					12

M - molecular ion mass series

TABLE LXII

PARTIAL MASS SPECTRA OF THE 5-METHYL AND

6-METHYL ALKANES FROM THE MURRAY METEORITE

m/e		5Me C_{15}	5Me C_{16}	5Me C_{17}	6Me C_{16}	6Me C_{17}
85		580	470	570	380	540
99		222	195	250	206	266
113		165	160	194	178	202
127		155	110	167	110	156
141		105	80	130	82	122
155		76	68	87	71	88
169		100	70	80	100	84
183		39	100	71	50	100
197		57	35	100	39	43
211	M	47	43	44	43	45
225	226	9	42	53	38	67
239	240		10.5	61	11	45
	254			12		12

M - molecular ion mass series

the table, does show in all cases a high olefin ion which helps to emphasize its association with the branching center. The 4-methyl alkanes which have also been discussed before are shown in Table LXI. Again the M-43 ion has been selected as the base peak to show its progressive displacement along the chain with each addition of a terminal C atom to the unbranched end of the chain.

These spectra are generally difficult to distinguish from those of their isomeric 2-methyl alkanes. This point has been discussed in some detail in Chapter IV section B and it will not be repeated here, except to say that upon a careful study of both patterns enough differences can be detected to allow a rather reliable identification. For instance, note the higher intensity of the M-29 ion in the spectra of 4-methyl alkanes (Table LXI) in comparison to the intensity in the spectra of the 2-methyl isomers (Table LIX) (see also Figure 7). The M-71 fragment (Figure 7) was also found to be accompanied always by a high olefin ion.

Finally the partial spectra of 5-methyl and 6-methyl alkanes can be found in Table LXII. By extension of the emperical correlations presented in Chapter IV, the 5-methyl alkanes would be expected to show a high M-57 peak together with an almost equally high M-58 peak (olefin ion). The mass series corresponding to the olefin ions is not given in the table, but the M-57 peak has been taken as the base peak

and can be easily seen. The same characteristics would be expected for the 6-methyl alkane spectra, with the exception that in this case the fragment produced by cleavage at the tertiary carbon atom with the loss of the small alkyl group will result in an M-71 fragment. See peaks at m/e 169 and m/e 183 for the 6-methyl C_{16} and 6-methyl C_{17} respectively, in Table LXII.

Although only mass spectrometric data from the Murray has been chosen to be presented here, all this information can also be made extensive to the other carbonaceous chondrites as well. The presentation of all the data obtained from the other five meteorites would be repetitious and has been omitted because it would add no significant information.

Cycloalkanes

The mass spectrometric fragmentation pattern of this type of structures has also been discussed in Chapter IV. A typical example is given in Figure 87. The partial mass spectra of other cycloalkanes identified in the alkane fraction from the Murray, Mokoia and Vigarano meteorites can be found in Tables LXIII through LXVI. The seemingly general tendency of the olefin ion series to dominate over the alkyl ions above the M-84 peak is indicated by the asterisks placed in Tables LXIII and LXIV. The greater deviations of this empirical observation are found in the alkyl cyclohexane

FIGURE 87

MOKOIA CYCLOALKANES

Same conditions described in Figure 85.

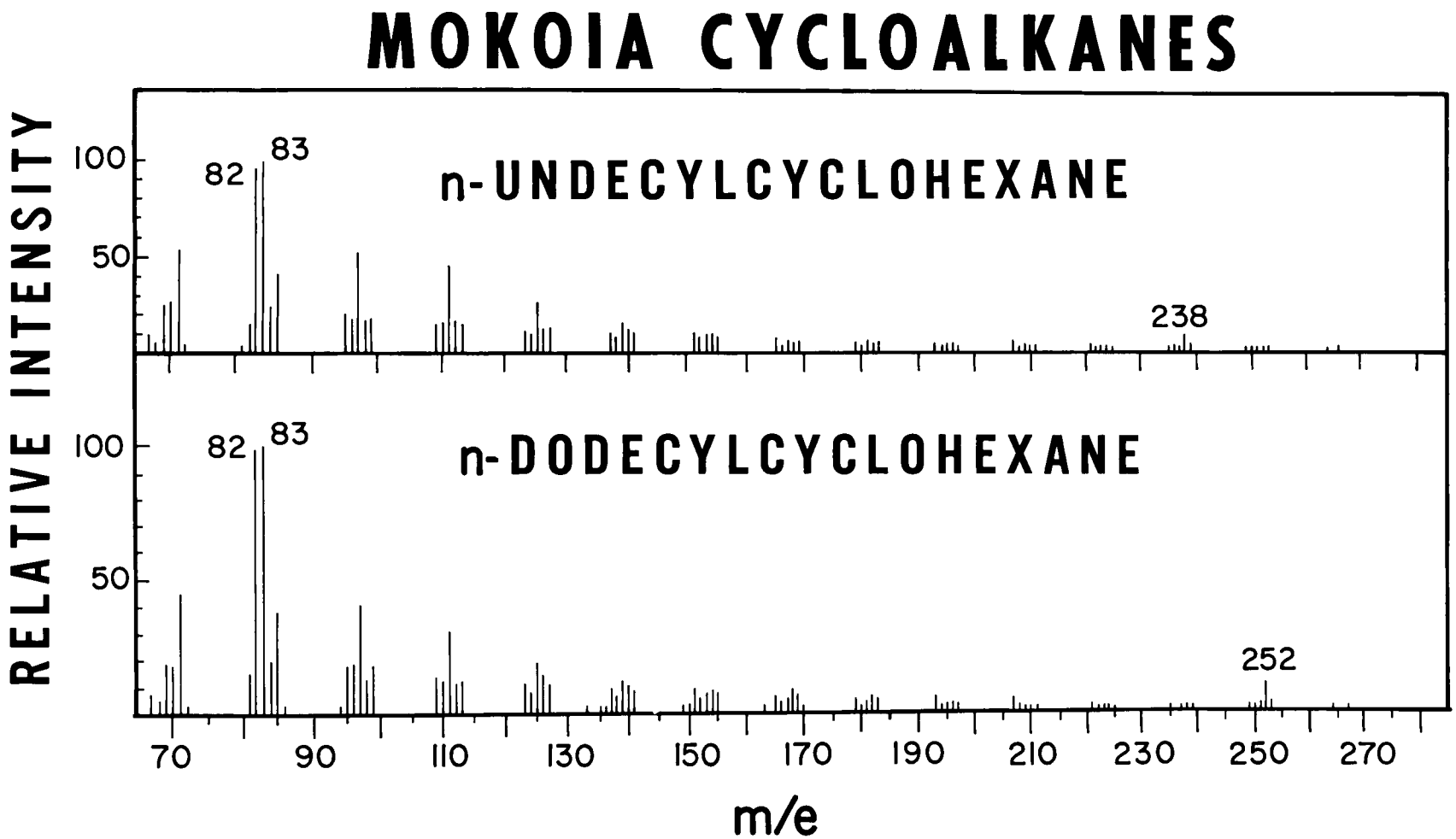

MOKOIA CYCLOALKANES
RELATIVE INTENSITY
n-UNDECYLCYCLOHEXANE
n-DODECYLCYCLOHEXANE
82
83
238
252
100
50
70
90
110
130
150
170
190
210
230
250
270
m/e

TABLE LXIII

PARTIAL MASS SPECTRA FOR ALKYLCYCLOHEXANES IN THE MURRAY METEORITE

Relative Peak Height

m/e	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}
82	86.5	94	82	79	104
83	100	100	100	100	100
84	20	20	37	38	33.5
97	46	47	104	124	82
98	12	14	30	41	23
111	38	42	95	107	77
112	13	15	36	45	20
125	20.5	25	68	85	55
126	13.5	14	33	38.5	17.5
139	11	14	37	41	27.5
140	13 *	13	24	29	12
153	7	7	22	28	18
154	8.5	12 *	20	23	9
167	4.8	5	17	22	14
168	3.5	7	19 *	23.5	8
181	4	5	12	15	12
182	4	6	15	24 *	7
195	3.5	4	9	13	10
196	3.5	4	10	16	13.5 *

TABLE LXIII CONTINUED

PARTIAL MASS SPECTRA FOR ALKYLCYCLOHEXANES IN THE MURRAY METEORITE

Relative Peak Height

m/e	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}
209			9	9	7.5
210			7.5	13	9
223				7	
224	10 (M)		7	8.5	
237				7	
238		12 (M)		8	
252			10.5 (M)		
266				10 (M)	
280					9.8 (M)

See Table LV for spectra of the C_{15} compound

(M) Molecular ion

* points to M-84 fragment (see Chapter IV, F for discussion)

TABLE LXIV

PARTIAL MASS SPECTRA FOR ALKYL CYCLOHEXANES IN THE MOKOIA METEORITE

Relative Peak Height

m/e	C_{17}	C_{18}	C_{19}
82	96	98	99
83	100	100	100
84	24	18	20
97	52	39	56
98	16.5	12	18
111	45	30	42
112	15	10	13
125	25	18.5	31
126	12	13	10
139	15	11	17
140	10	10	7
153	9	7	11
154	10 *	7.5	6
167	5.5	5	9
168	5	8 *	6.5
181	6	4	5
182	4.5	6	7 *
195	4	3	5
196	5	4	5.5
210	4	2.5	4
224	3.5	2.5	
238	9.5 (M)		
252		11 (M)	
266			8.5 (M)

(M) Molecular ion

* M-84 fragment

series of the Vigarano meteorite (Table LXV). In this connection it must be pointed out that due to the lower resolving power of the gas chromatographic column employed in this analysis, contributions to these spectra from other structures may be important enough to disrupt the pattern to that extent. The same may be true in the case of the alkyl cyclopentanes (Table LXVI). An interesting observation that can be made here is the presence of a high M-28 peak in the spectra of these alkyl cyclopentanes. This has also been observed in the standards (Table XVI) but not in the alkylcyclohexanes.

Alkenes

Although they have not been reported in Table XLIX because no single isolated peaks could be identified with an olefin structure, there is some mass spectrometric evidence of their presence in these meteorites. For instance a relatively high intensity peak at m/e 266 that would be the molecular mass of a C_{19} monoolefin is found among the spectra of the gas chromatographic peaks immediately following the n-C_{17} in the Mokoia and Vigarano meteorites. Its chromatographic position indicates some branching and besides it corresponds roughly to the position in which C_{19} isoprenoid alkenes would be eluted. (See discussion of the branched olefins of tobacco smoke, Chapter V).

Likewise, a high m/e 280 corresponding to a C_{20}

TABLE LXV

PARTIAL MASS SPECTRA OF ALKYL CYCLOHEXANES FROM VIGARANO

	UNDECYL CYCLOHEXANE			DODECYL CYCLOHEXANE			TRIDECYL CYCLOHEXANE		
	Mass Series (Z in C_nH_{2n+Z})								
C_n \ Z	-2	-1	0	-2	-1	0	-2	-1	0
C_6	89	100	20	102	100	19	92	100	17
C_7		45	12.5		38	10		57	16
C_8		35	11		29	12		42	11.5
C_9		20	9		19	15		31	8.5
C_{10}		11	6		11	10		16	6.5
C_{11}		7	6.5		6	7		11	5.5
C_{12}		5	4		5	8		9.5	6
C_{13}		5	3		3	6		8	6.5
C_{14}		2.5	3			4		5	4.5
C_{15}			2			2.5			3.5
C_{16}									5
C_{17}			10 (M)						
C_{18}						9 (M)			
C_{19}									9 (M)

M: Molecular ion

TABLE LXVI

PARTIAL MASS SPECTRA OF ALKYL CYCLOPENTANES FROM VIGARANO

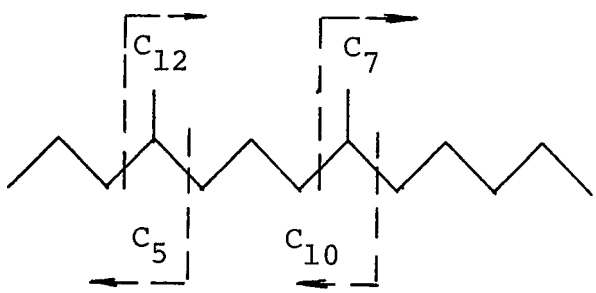
	Dodecylcyclopentane			Tridecylcyclopentane		
	Mass Series (Z in C_nH_{2n+Z})					
C_n \ Z	-2	-1	0	-2	-1	0
C_5	96.5	100	66.5	91	100	42
C_6		147	55		138	46.5
C_7		160	41		169	41
C_8		122	40		128	34
C_9		86.5	35		102	30
C_{10}		47	26		49	23
C_{11}		25	18		30	17
C_{12}		18	15.5		22	15
C_{13}		14.5	14		17.5	13
C_{14}		16	13		14	10
C_{15}		10	15			7.5
C_{16}						14
C_{17}			27.5 (M)			
C_{18}						27 (M)

M : Molecular ion

monoolefin was found in some of the spectra following that of the n-C_{19} in the same meteorites. Its chromatographic elution time also suggests a branched structure and it is found in a position where the phytenes would be eluted. Some other olefin parent ions were found but in general all the olefins are present in small amounts when compared to the other saturated compounds.

Dimethyl substituted alkanes

The search for this type of compounds has not been particularly successful since only one possible homologous series, the 4,8-dimethyl alkane series, has been tentatively identified. For instance, the mass spectrum of the compound number 9, in Table XLIX, found in the Vigarano meteorite and which is given in Table LXVII fits this structure



The prominent fragments are indicated by the asterisks in the table.

The gas chromatographic data also suggests a dimethyl substituted structure since this would be the only way in which a compound with fifteen carbon atoms could be eluted shortly before the n-C_{14} alkane (see also Figure 80).

TABLE LXVII

PARTIAL MASS SPECTRUM OF A DIMETHYL ALKANE FROM VIGARANO

4,8-Dimethyl C_{13}

C_n \ Z	Mass Series (Z in C_nH_{2n+Z}) 0	+1	+2(1)
C_4	13	100	
C_5	28 *	77	
C_6	13	57	
C_7	26 *	25	
C_8	10	15	
C_9	8	8	
C_{10}	8 *	18	
C_{11}	4	5	
C_{12}	5 *	11 *	
C_{13}	2	4	
C_{14}	3	4	(M-15)
C_{15}			3 (M)

(1) Isotopic ions in the mass series C_nH_{2n+Z} are not included in this table

* Most characteristic ions

TABLE LXVIII

DIMETHYL SUBSTITUTED ALKANES IN THE MURRAY METEORITE

PARTIAL SPECTRA

m/e	ion	4,8-di meC_{12}	4,8-di meC_{13}
85	$C_6H_{13}^+$	100	100
99	$C_7H_{15}^+$	38	48 *
113	$C_8H_{17}^+$	36	32
127	$C_9H_{19}^+$	26	26
141	$C_{10}H_{21}^+$	19 *	28 *
155	$C_{11}H_{23}^+$	19 *	15 *
169	$C_{12}H_{25}^+$	13	20 *
183	$C_{13}H_{27}^+$	6 (M-15)	10
197	$C_{14}H_{29}^+$	--	7 (M-15)
198	$C_{15}H_{32}^+$	1.5 (M)	--
212	$C_{16}H_{34}^+$	--	2 (M)

* Most characteristic ion

M :Molecular ion

Also two other compounds in the Murray meteorite give gas chromatographic data and spectra consistent with such a structural arrangement (see Table LXVIII). There are also some indications of the presence of compounds such as the 3,5-dimethyl alkanes among others but in general the identification of the dimethyl alkanes has proved to be very difficult, in part because they are present in very low amounts and in part because of the practical limitations on the resolution of the gas chromatographic columns towards complex mixtures of alkanes.

B. Nodules from Iron Meteorites

The socalled iron meteorites are combinations of nickel-iron in which the nickel content may range from 5 to 15%. They sometimes contain small embedded nodules of a graphitic like material. Carbon is in general the predominant element, (graphite nodules) (179) and relatively high amounts of sulfur are also present in the form of SFe (graphite-troilite nodules) (179). Table LXIX gives the elemental composition of a number of nodules from three different meteorites. The sulfur content is in all the cases rather high. According to these values all of the nodules involved in this work can be classified as graphite-troilite nodules. One of them (Cosby's Creek) was further analyzed for its oxygen and nitrogen content which were found to be both very low. Not

TABLE LXIX

GRAPHITIC NODULES - ELEMENTAL ANALYSIS

	G	H	S	Fe	O	N		Source
2 Canyon Diablo*	40.18	<0.1	21.08	35.44			Lewis	Ariz. St. U. April 1967
3 Canyon Diablo*	58.60	<0.1	12.39	22.21			Huss	Am. Met. Lab Jan 1967
1 Odessa*	31.36	<0.1	22.53	42.04				Am. Met. Lab
Canyon Diablo Shallow embedded nod. SURFACE	52.11	<0.2	13.45	28.34			Huss	Am. Met. Lab April 1967
Shallow embedded nod. BOTTOM	52.49	<0.2	11.91	28.27				
Canyon Diablo Weathered nod. INSIDE+OUTSIDE	45.69	<0.2	0.75	34.21			Huss	Am. Met. Lab April 1967
Cosby's Creek	33.27	<0.2	19.90	38.85	2.23	0.06	Fondel	Harvard June 1967
Odessa	37.48	<0.2	0.36	31.44			Fondel	Harvard June 1967

* Analyzed by D. W. Nooner[348](Gas chromatographic analysis)

low enough though in the case of the oxygen, to rule out the presence of detectable amounts of oxygenated compounds. The analytical techniques used in this case are essentially the same as those applied to the analysis of organic matter in the carbonaceous chondrites and have been described in some detail in the experimental section (see Chapter III). In essence the method consists in crushing the nodules to about a 100 particle size and extraction of the samples with organic solvents, fractionation by silica gel chromatography and analysis by gas chromatography and combined gas chromatography-mass spectrometry. No further mention will be made of these techniques except where new or different methods need to be introduced. A condensed description of the kind of nodules analyzed, their donors, date received in the laboratory, the weights taken for the analysis and the extraction data is given in Table LXX.

The gas chromatographic-mass spectrometric analysis of the alkane fractions extracted from these nodules yielded a total of 49 compounds identified. Of course, there are many more than 49 compounds present in the nodules, as is indicated by their gas chromatograms, but as in the case of carbonaceous chondrites only the gas chromatographic identifications which have been supported by mass spectrometric evidence are considered of any value. Table LXXI contains a

TABLE LXX

EXPERIMENTAL DATA ON GRAPHITIC NODULES

Canyon Diablo Graphitic Nodules	Source and Date	Cuts Analyzed: Surface	Inside-Center	Bottom	No.	Hrs/Blk	Extraction Time (Hrs)
Shallow embedded nodule	Huss Am. Met. Lab. April, 1967	0.8098	0.7966	0.7966	2	2	15
Deep embedded nodule	Huss Am. Met. Lab. April, 1967	0.2901	2.4316		2	8	8
Embedded nodule	Huss Am. Met. Lab. Jan. 1967	2.2846	2.2846		2	2	8
Weathered nodule	Huss Am. Met. Lab. April, 1967	1.5126	1.5915		2	8	8
		1.6130			2	8	8
OTHER NODULES		WHOLE NODULE					
Cosby's Creek	Frondel Harvard June 1967	1.5295			2	2	8
Odessa	Frondel Harvard June 1967	2.1433			2	2	8

display of the qualitative and quantitative data organized in a similar form as in Table XLIX. The same numbering system is used in both tables to facilitate the cross-checking of data between carbonaceous chondrites and nodules of iron meteorites. In other words a compound such as the 2-methyl C_{16} alkane will have the same identification number in all chromatograms and tables throughout this chapter, that is number 26. The skipping of a number in Table LXXI, for instance the number 24, means that there is a compound eluted between compound numbers 23 and 25, namely the 5 methyl hexadecane, which although not "detected" in any nodule it has been found in carbonaceous chondrites or vice versa. It should be kept in mind that compounds in Tables XLIX and LXXI are arranged according to their gas chromatographic elution times. Under this system the isoprenoids from the graphitic nodules have the numbers 10,16,21,27,30,37, and 42 and in the figures they are circled for quicker identification.

From the data presented in Table LXXI nine different homologous series can be sorted out. Namely, the n-alkane series (C_{13} to C_{26}); the 2-,3-,4-,6-methyl alkane series; the two isoprenoid homologies made up by the 2,6,10-trimethyl alkanes (C_{19}-C_{21}) and finally the two series of monocylo-alkanes (cyclohexyl and cyclopentyl). The possible presence of a 5-methyl alkane series is suggested by the identification of the 5-methyl C_{17}.

TABLE LXXI

ISOPRENOIDS AND ALIPHATIC HYDROCARBONS IN GRAPHITE NODULES FROM IRON METEORITES - GC-MS IDENTIFICATIONS

	Canyon Diablo Nodules						Odessa	Cosby's C.	
	Shallow		Embedded		Weathered		whole nodule	whole nodule	
	S	C	S	C	I	I+O			
nC_{13}							.01	.19	
nC_{14}	.28	.2				.03	.01	.07	
(10) 2,6,10 $TriMeC_{13}$	.09	.08						.19	
(12) $2MeC_{14}$								.19	
(13) $3MeC_{14}$						.03	.01		
nC_{15}	.96	.2	.09		.04	.11	.03	1.71	
(15) n-octylcyclohexane									(*)
(16) 2,6,10 $TriMeC_{14}$	.03		.03			.03	.01		
(19) $2MeC_{15}$	.09	.08	.03			.03	.01	.19	
(20) $3MeC_{15}$	.09	.08				.03	.01	.19	
nC_{16}	.96	.2	.28			.29	.07	.57	
(21) 2,6,10 $TriMeC_{15}$	.28	.08	.03			.29	.01	.19	
(22) n-nonylcyclohexane									(*)
(23) $6MeC_{16}$	.03								
(25) $4MeC_{16}$							.01		
(26) $2MeC_{16}$	.03		.03			.03	.01		
(27) 2,6,10,14 $TetraMeC_{15}$	.96	.2	.28	.5	.06	.5	.03	.19	
(28) $3MeC_{16}$			.03			.011	.01		
(29) 3,5 $DiMeC_{16}$							.01		
nC_{17}	.96	.2	.8	.5		.5	.14	1.71	
(30) 2,6,10 $TriMeC_{16}$			.03			.03			
(31) 4,7,11 $TriMeC_{16}$			.03			.03			
(32) n-decylcyclohexane	.03		.03				.1		
(33) $6MeC_{17}$							.01		

TABLE LXXI CONTINUED

ISOPRENOIDS AND ALIPHATIC HYDROCARBONS IN GRAPHITE NODULES FROM IRON METEORITES - GC-MS IDENTIFICATIONS

	Canyon Diablo Nodules						Odessa	Cosby's C.
	Shallow		Embedded		Weathered		whole nodule	whole nodule
	S	C	S	C	I	I+O		
(34) $5MeC_{17}$			.03					
(35) $4MeC_{17}$			.03				.01	
(36) $2MeC_{17}$	.09		.03			.03	.01	
(37) 2,6,10,14 $TetraMeC_{16}$	.28	.08	.09	.5	.02	.29	.07	.19
(38) $3MeC_{17}$	.03		.03			.03	.01	
nC_{18}	.96	.08	.8	.1	.2	.5	.28	1.71
(39) n-dodecylcyclopentane			.03					
(40) n-undecylcyclohexane	.03		.03			.03	.01	
(42) 2,6,10,14 $TetraMeC_{17}$	.03		.03			.03	.01	
(44) $2MeC_{18}$	.03		.03				.01	
(45) $3MeC_{18}$			.03		.02	.03	.01	.19
nC_{19}	.28		.28	.05	.04	.29	.28	1.71
(46) n-tridecylcyclopentane						.03		
(47) n-dodecylcyclohexane			.03			.03	.03	
(49) $2MeC_{19}$						.03	.03	
(50) $3MeC_{19}$						.03		
nC_{20}	.09		.03			.11	.28	1.71
(51) n-tridecylcyclohexane			.03					
(53) $3MeC_{20}$						.03		
nC_{21}						.11	.28	1.71
nC_{22}								1.71
nC_{23}								1.71
nC_{24}								
nC_{25}								
nC_{26}								

TABLE LXXI CONTINUED

ISOPRENOIDS AND ALIPHATIC HYDROCARBONS IN GRAPHITE NODULES FROM IRON METEORITES - GC-MS IDENTIFICATIONS

Notes:

Blanks in the columns of the table do not necessarily indicate the absence of certain alkanes from a given meteorite but rather that no useful mass spectra were obtained in these cases

(*) compounds identified in a sample of a Canyon Diablo graphitic nodule which is not included in this table

S: Surface cut

I: Inside cut

C: Center cut

O: Outside cut

These results are practically identical to those obtained for the carbonaceous chondrites.

1. Odessa graphitic nodule

The whole nodule weighing 2.1433 g (Table LXX) was crushed to pass a 100 mesh test screen and treated according to the methods described earlier. Figure 88 shows the gas chromatographic distribution of the alkanes in this nodule. It is interesting to note the strong resemblance with the GC patterns of the carbonaceous chondrites in general. These results are comparable to those obtained previously in this laboratory (350) with a specimen from the same meteorite but different precedence. The earlier sample had been received from Dr. Huss at the American Meteorite Laboratory in Denver, Colorado. The results shown here were obtained with a nodule sent by Dr. Frondel (Table LXX). Quantitatively the correlation between the two analysis is not so good. The first determination carried out by Nooner yielded a total hydrocarbon content of 15.5 ppm for an outside piece of the nodule and 3.0 ppm for an inside piece. On the other hand the total alkane content of this whole nodule was not found to be greater than 1.32 ppm according to the later analysis.

No predominance of odd carbon numbered alkanes has been found here or on the earlier analysis by Nooner and Oró (350).

This particular sample was studied by mass spectrometry in order to obtain data on the nature of the alkanes present,

FIGURE 88

GAS CHROMATOGRAPHIC SEPARATION OF THE ALKANES FROM THE ODESSA GRAPHITIC NODULES

Stainless steel capillary column (195 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph with a flame ionization detector. Range, 10; attenuation, 2. Nitrogen pressure, 1050 g/cm^2. No split. Temperature programmed from 160°C to 200°C at 2°C/min.

(A) Benzene nanograde solvent. Instrumental control

(B) About 2/8 of the n-heptane eluate was injected. 2.1433 g extracted (see Table LXX).

See Table LXXI for identification.

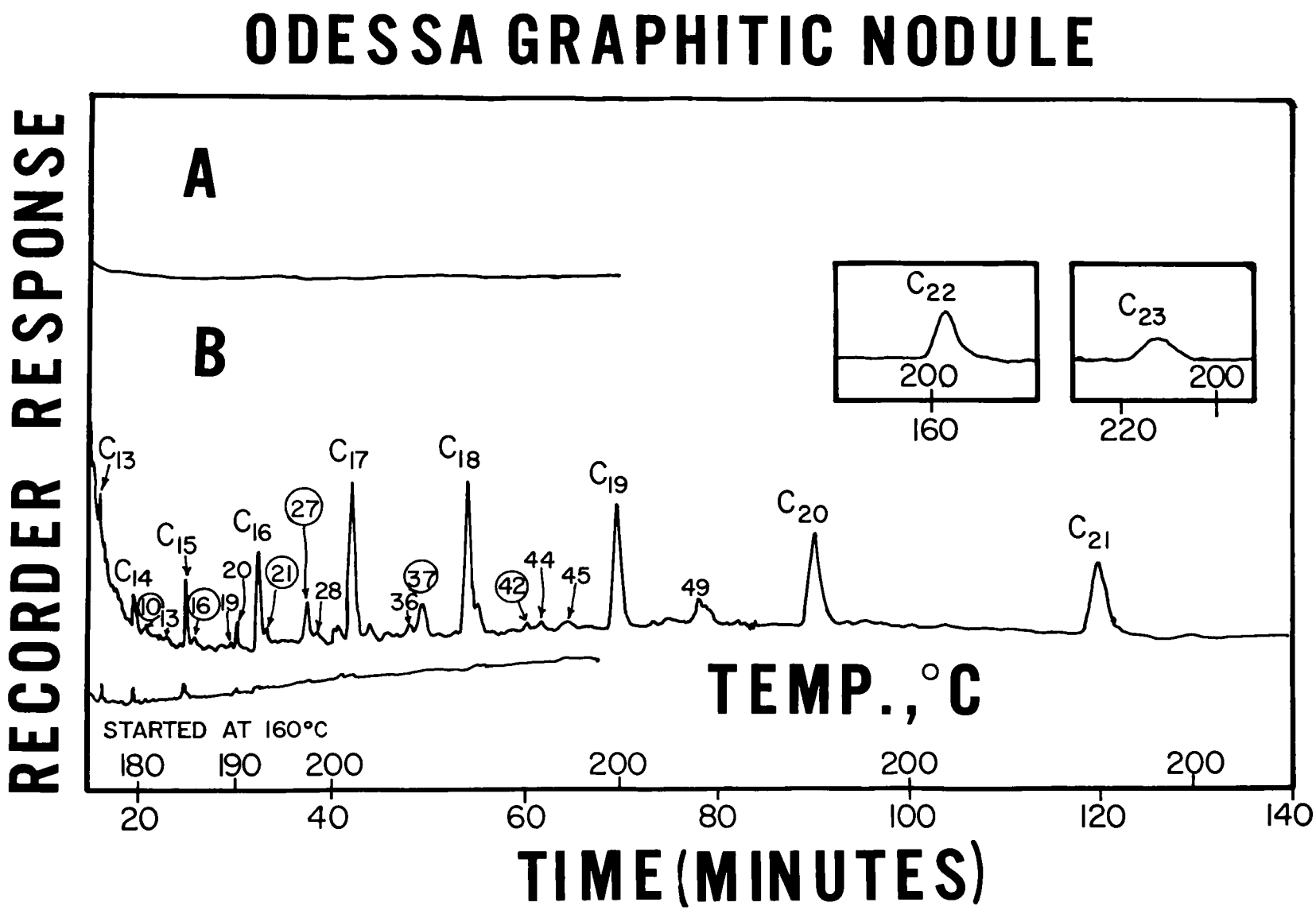
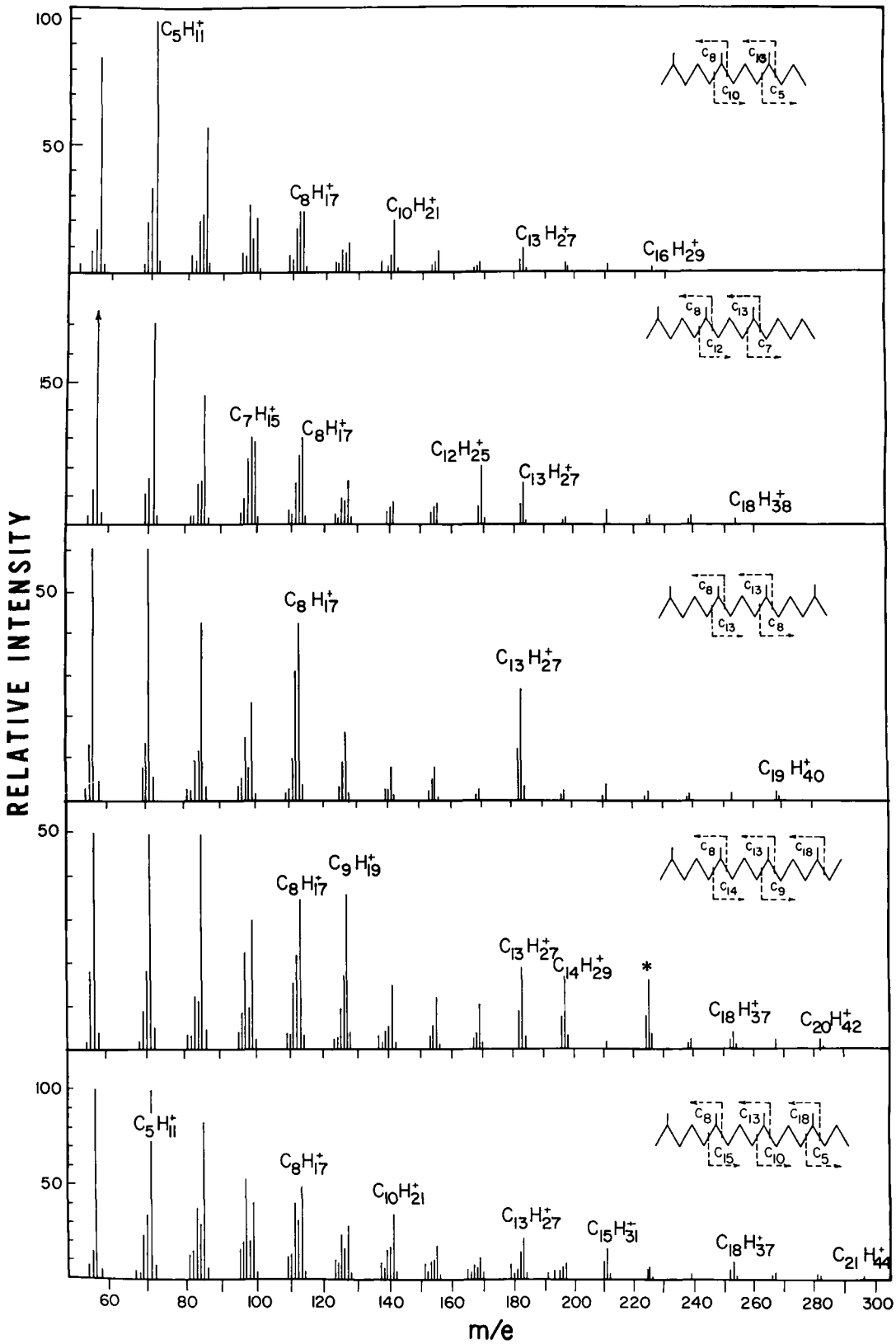

ODESSA GRAPHITIC NODULE
RECORDER RESPONSE
A
B
C22
200
160
C23
200
220
C13
C14
C15
C16
C17
C18
C19
C20
C21
10
13
16
19
20
21
27
28
36
37
42
44
45
49
STARTED AT 160°C
180
190
200
200
200
200
TEMP., °C
20
40
60
80
100
120
140
TIME (MINUTES)

FIGURE 89

MASS SPECTRA OF THE ISOPRENOIDS FROM THE ODESSA GRAPHITIC NODULE

The spectra were taken as each individual component was eluted from a stainless steel capillary column (150 m long by 0.076 cm i.d.) coated with Polysev. Energy of ionization 70 eV. Ionizing current, 120 μA.

ODESSA ISOPRENOIDS



especially on the isoprenoids, which were found to range from the C_{16} to the C_{21} isoprenoid. Their mass spectrometric patterns are given in Figure 89. Compare the mass spectra in this figure to those shown in Figures 85 and 86. The significant contribution of the anteiso C_{18} to the mass spectrum of phytane (see peak marked by an asterisk in Figure 89) has also been discussed previously.

2. Cosby's Creek

This second whole graphitic nodule weighing 1.5295 g (Table LXX) was treated in the same manner as the one from the Odessa meteorite.

The interesting distribution mode of the hydrocarbons in this sample is shown in Figure 90. There is in this case a marked odd carbon number predominance. The isoprenoids though show the same constant distribution found in all the previous meteorite analysis, with maxima at C_{19} and C_{16} and minimum at C_{17}. The range goes also from the one with 16 carbons to the one with 21 carbon atoms. The spectra of the C_{16}, C_{19} and C_{20} isoprenoids is shown in Figure 91.

A graphical representation of these rather unique distribution is found in Figure 92, where the values of the areas under each n-alkane peak have been plotted vs their respective carbon numbers. Although the significance of such a pattern will be discussed later it may be added at this point that it bears a strong resemblance to the

FIGURE 90

GAS CHROMATOGRAPHIC SEPARATION OF THE ALKANES FROM THE COSBY'S CREEK GRAPHITIC NODULE

Same conditions discribed in Figure 88 except for the programming rate which was set at $2^{\circ}C$/min.
1.5295 g extracted. About 1.2/35 was injected (see Table LXX).
Identification numbers given in Table LXXI.

COSBY'S CREEK GRAPHITIC NODULE

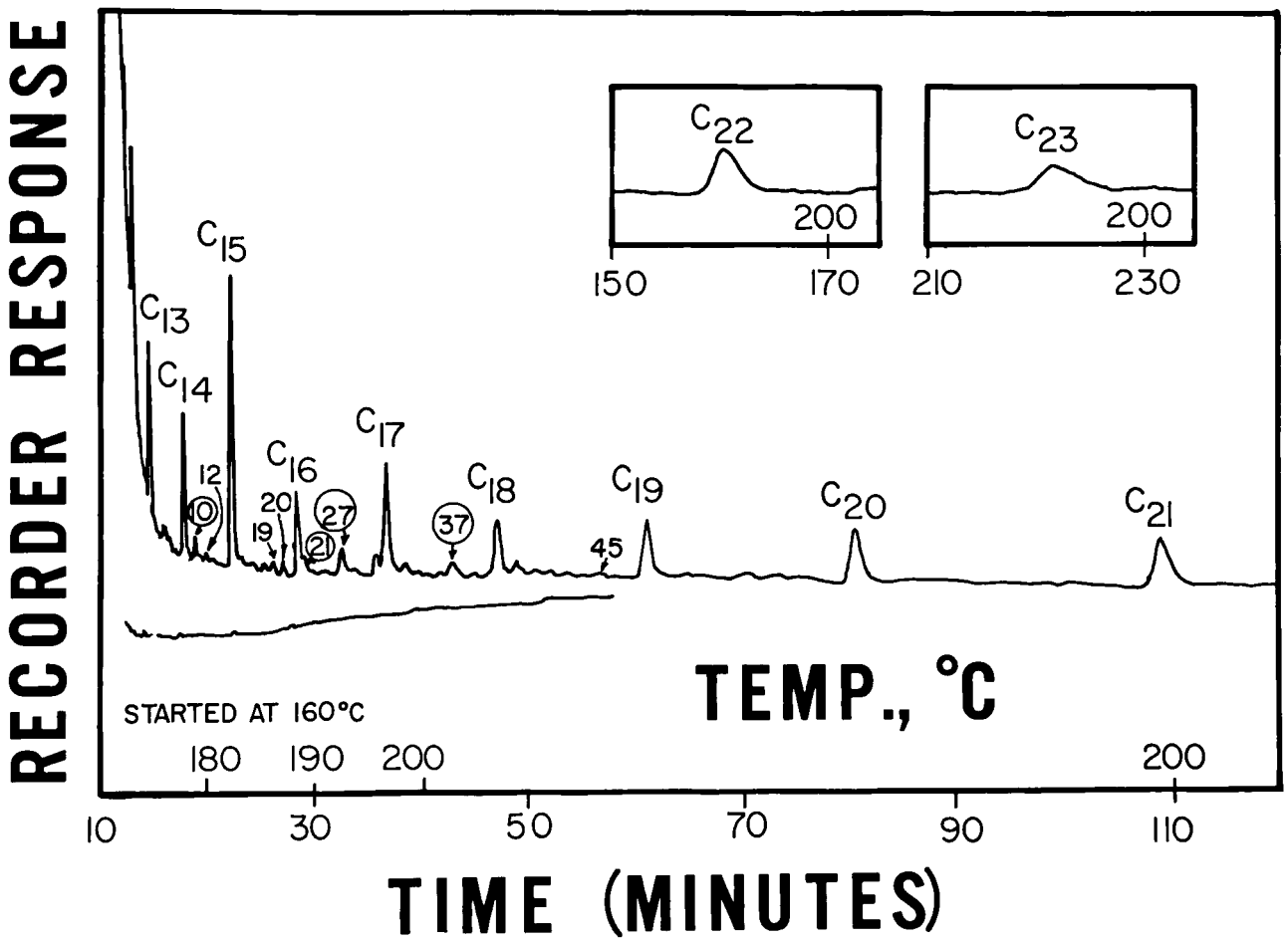


FIGURE 91

MASS SPECTRA OF COSBY'S CREEK ISOPRENOIDS

Same conditions as described in Figure 89.

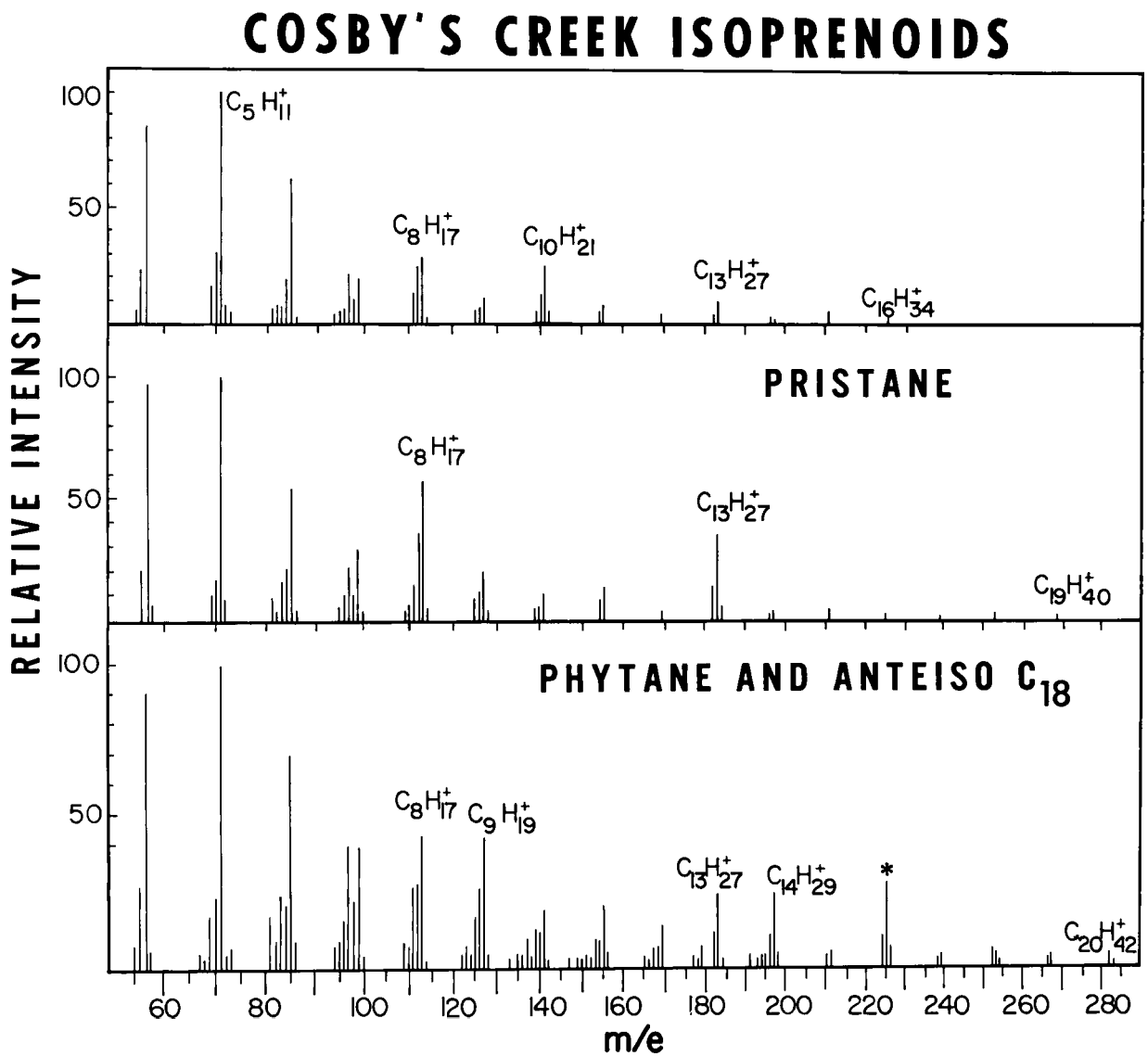
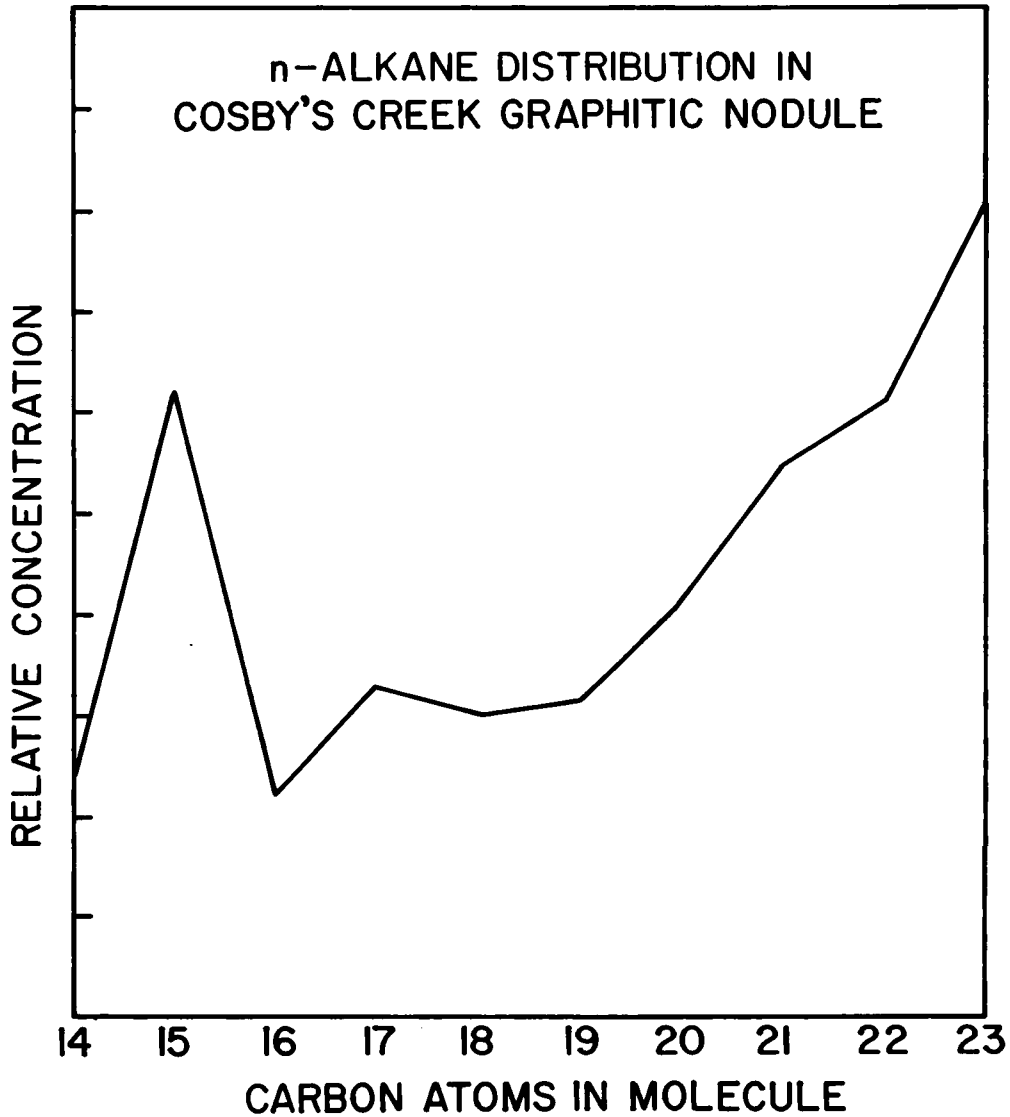

COSBY'S CREEK ISOPRENOIDS
RELATIVE INTENSITY
100
50
$C_5H_{11}^+$
$C_8H_{17}^+$
$C_{10}H_{21}^+$
$C_{13}H_{27}^+$
$C_{16}H_{34}^+$
PRISTANE
$C_8H_{17}^+$
$C_{13}H_{27}^+$
$C_{19}H_{40}^+$
PHYTANE AND ANTEISO C_{18}
$C_8H_{17}^+$
$C_9H_{19}^+$
$C_{13}H_{27}^+$
$C_{14}H_{29}^+$
*
$C_{20}H_{42}^+$
60
80
100
120
140
160
180
200
220
240
260
280
m/e

FIGURE 92

DISTRIBUTION OF n-ALKANES IN COSBY'S CREEK GRAPHITIC NODULE


n-ALKANE DISTRIBUTION IN
COSBY'S CREEK GRAPHITIC NODULE
RELATIVE CONCENTRATION
14
15
16
17
18
19
20
21
22
23
CARBON ATOMS IN MOLECULE

hydrocarbon pattern of some marine algae (348). The Cosby's Creek sample was received in a wax like material which was analyzed by gas-liquid and thin layer chromatography and was found to be free of interfering hydrocarbons.

3. Canyon Diablo graphite-troilite nodules

These nodules were not treated in the same way as the ones from the Odessa and Cosby's Creek meteorites. Because in these two cases, the whole nodules were taken for the analysis, the results thus obtained give an idea only of the overall content and distribution of hydrocarbons in the nodules. In order to check whether there was any uniformity in the observed hydrocarbon distribution within the entire nodule, the Canyon Diablo samples were divided into "surface" and an "inside" cuts and then each one of these cuts was treated as an individual nodule. A more detailed account of the procedure will be given in the next paragraphs.

a) Weathered nodule

A heavily weathered graphite nodule which contained rust and graphitized carbon was obtained from Dr. G. I. Huss (Table LXX) and was analyzed for its hydrocarbon content. The outside and inside parts of this nodule were removed with a hacksaw blade, taking the necessary precautions to avoid contamination. Figure 93 shows the results obtained for each one of the fractions. The inside and the outside cuts give approximately the same pattern of alkanes. The

FIGURE 93

GAS CHROMATOGRAPHIC SEPARATION OF THE ALKANES FROM A WEATHERED CANYON DIABLO GRAPHITIC NODULE

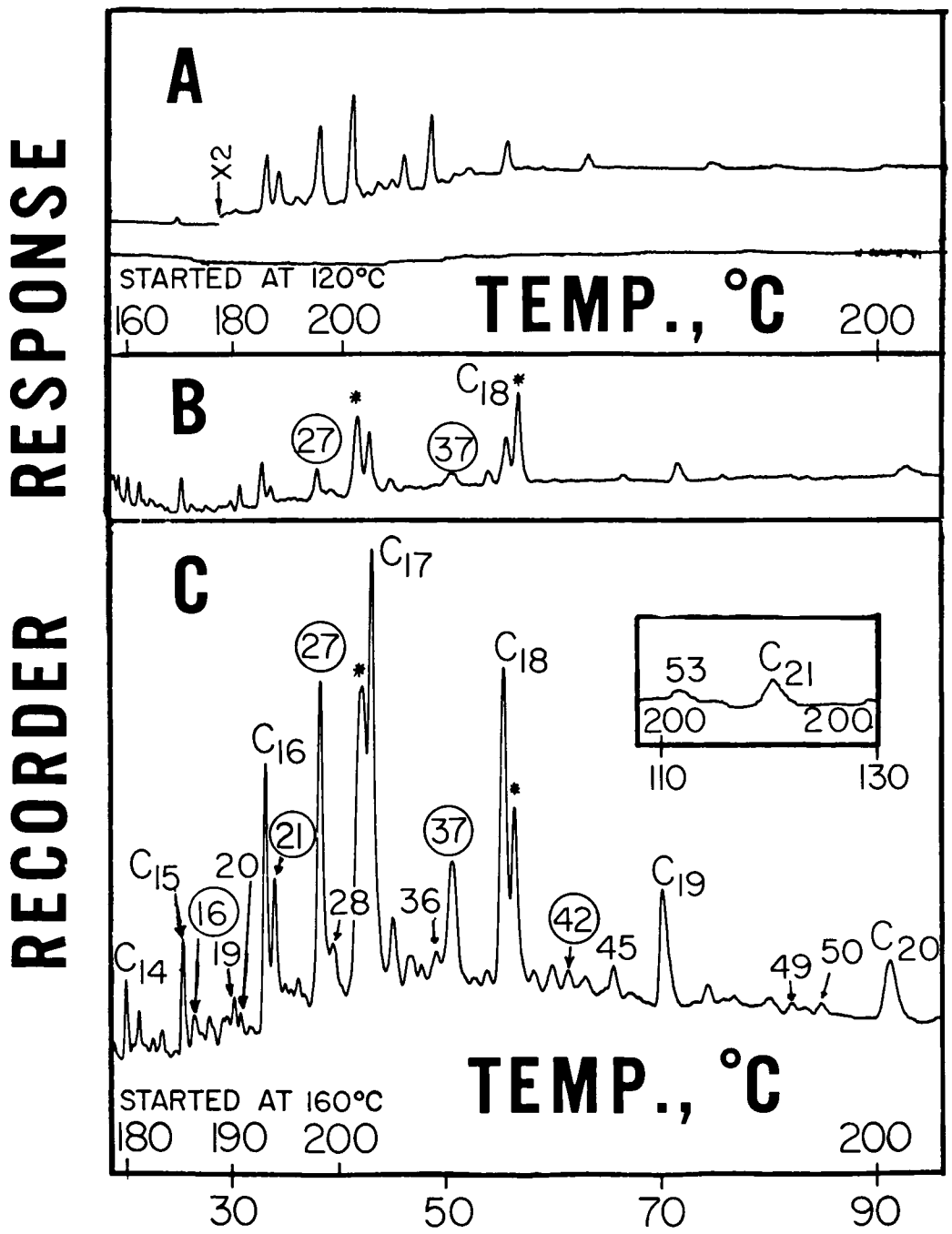
Stainless steel capillary column (150 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph wi a flame ionization detector. Range, 10; attenuation, 2. Nitrogen pressure, 1050 g/cm^2. No split. (See Table LXX for experimental data).

(A) Surface cut and extraction blank. 1.5126 g extracted. About 1/8 of the n-pentane eluate was injected in both cases. Temperature held at 120°C for 10 min., then programmed to 200°C at 2°C/min.

(B) Inside cut. 1.5915 g extracted. About 1/8 of the n-pentane eluate was injected. Temperature programmed from 160°C to 200°C at 1°C/min.

(C) Inside and surface cuts. 1.6130 g extracted. About 1.1/4 was injected. Temperature programming, same as in B. See Table LXXI for identification numbers.

CANYON DIABLO WEATHERED GRAPHITIC NODULE



inside piece which contains about four times less hydrocarbons than the sample taken from the portion of the nodule in contact with the atmosphere also shows a few peaks (marked by an asterisk in Figure 93) of aromatic character as induced from their mass spectrometric patterns. Their molecular ions belong to the phenanthrene or anthracene mass series (z=-18) and all of them must be branched to some extent (with alkyl groups) because of their appearance in the pentane fractions of the extracts. The results obtained with a mixture of the inside and outside cuts are given at the bottom of Figure 93. In general and with the exception of the "aromatic peaks" the patterns look very much the same as those from other nodules and meteorites. The mass spectra of three of the five isoprenoids found in these sample are shown in Figure 94. The two isoprenoid structures, 2,6,10-trimethyl and 4,7,11 trimethyl hexadecane found occasionally in carbonaceous chondrites (Table XLIX) have also been identified here (Table LXXI).

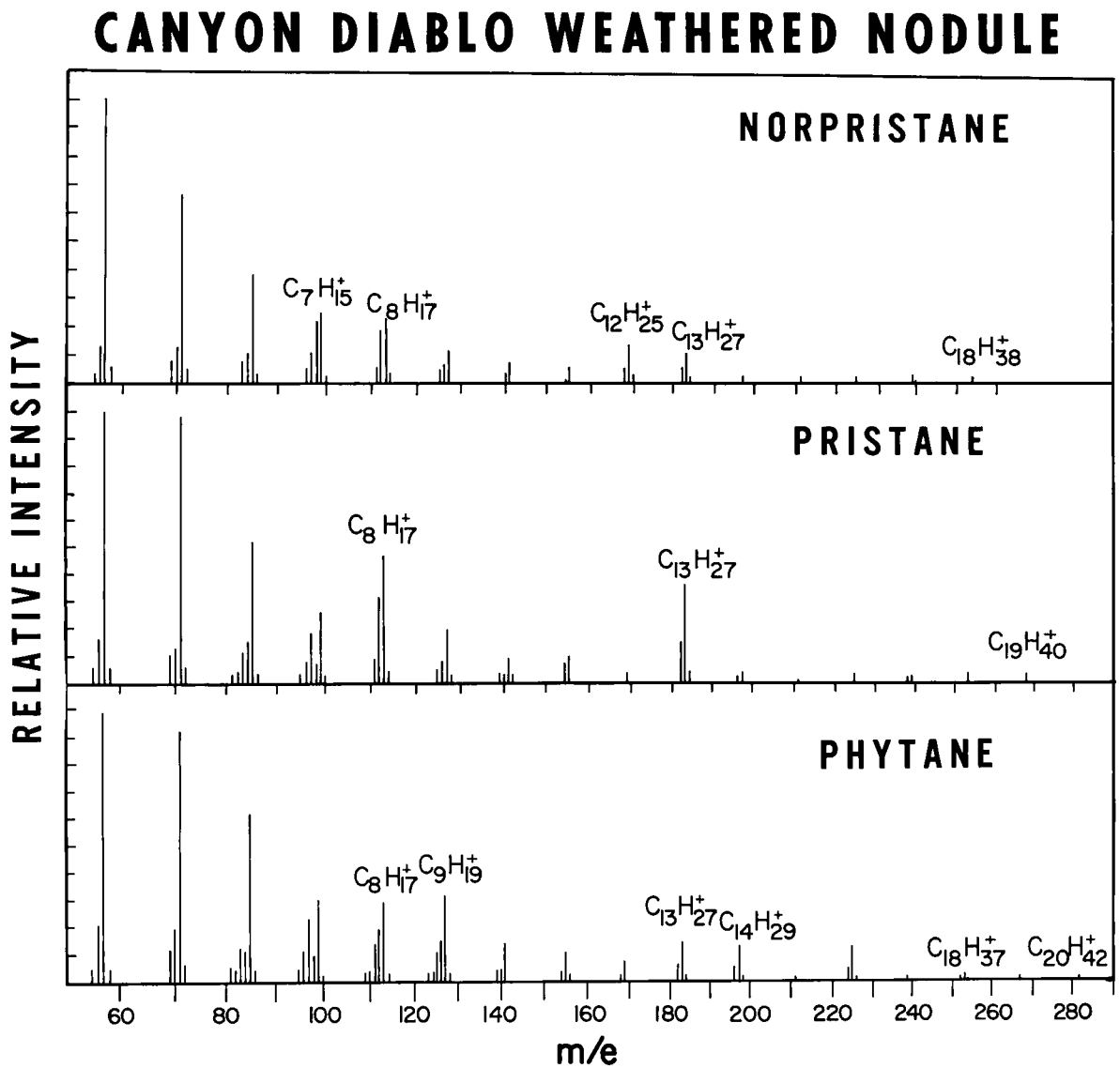
b) Embedded nodule

In this particular case the nodule was completely embedded in the iron meteorite. After cutting several iron pieces with a carborundum saw (cutting wheels #CW-14, Bethlehem Apparatus Company, Inc., Hillerton, Va.) a successful cut was made which showed a relatively large nodule divided by the saw in approxiamtely two halves, each one embedded in the corresponding half of the iron meteorite.

FIGURE 94

MASS SPECTRA OF ISOPRENOIDS IN THE WEATHERED CANYON DIABLO NODULE

Same conditions as in Figure 89.


CANYON DIABLO WEATHERED NODULE
NORPRISTANE
PRISTANE
PHYTANE
RELATIVE INTENSITY
m/e
60
80
100
120
140
160
180
200
220
240
260
280
C7H15+
C8H17+
C12H25+
C13H27+
C18H38+
C8H17+
C13H27+
C19H40+
C8H17+
C9H19+
C13H27+
C14H29+
C18H37+
C20H42+

This operation was carried out at the American Meteorite Laboratory. Water was used as a coolant and all precautions were taken to avoid contamination.

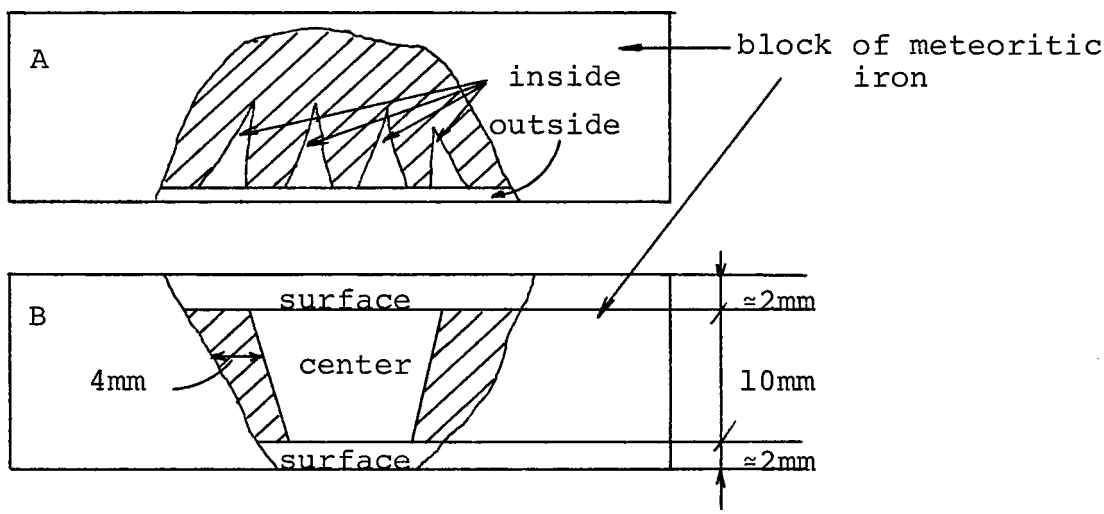
The graphitic material of one of the two halves (A) had been analyzed earlier (348,357). With the following results for the total alkane content:

Outside, at surface of saw cut (0.3 g)	1.7 ppm
Inside, embedded in meteoritic iron (3.1 g)	0.07 ppm

Ratio Outside / Inside ≈24

The other half (B) which was kept in a tightly closed jar was also analyzed and the results are described here.

Since this half nodule was exposed on both sides,



the material on both sides was scrapped with a clean knife to a depth of about 1-2 mm. This left the center part exposed which then was removed in the same way and in the manner indicated in the diagram (Block B), taking care of avoiding the inner surfaces. Exactly equal weights were

taken for the analysis (Table LXX). This had not been done in previous analyses (see above) in which the amount taken off the surface was between 3 and 5 times less than that of the inside. Since the amount of hydrocarbons found is divided by the weight of the sample it could be argued that a constant level of contamination introduced during the analysis, when divided by smaller weights of the surface cut would give larger values than when divided by the larger weight of the inside portion and this in turn could account for the differences in the results reported. The total alkane values obtained here using equal weights (Figure 95) are:

Surface	2.18 ppm
Center	0.32 ppm

Ratio surface / center = 6.8

These results go in line with those obtained before for the other half (A) of the nodule, in the sense that the amount of hydrocarbons is greater on the surfaces of the cuts than inside the nodules. On the other hand these new values are greater in this case by a factor of 1.2 for the surface cuts and 4.5 for the inner cuts. While the first factor can be considered to be within the experimental error, the relative increase of the inner concentration of alkanes with time (more than six months elapsed between the two analyses) could be indicative of a diffusion process of the surface hydrocarbons towards the interior of the nodule.

FIGURE 95

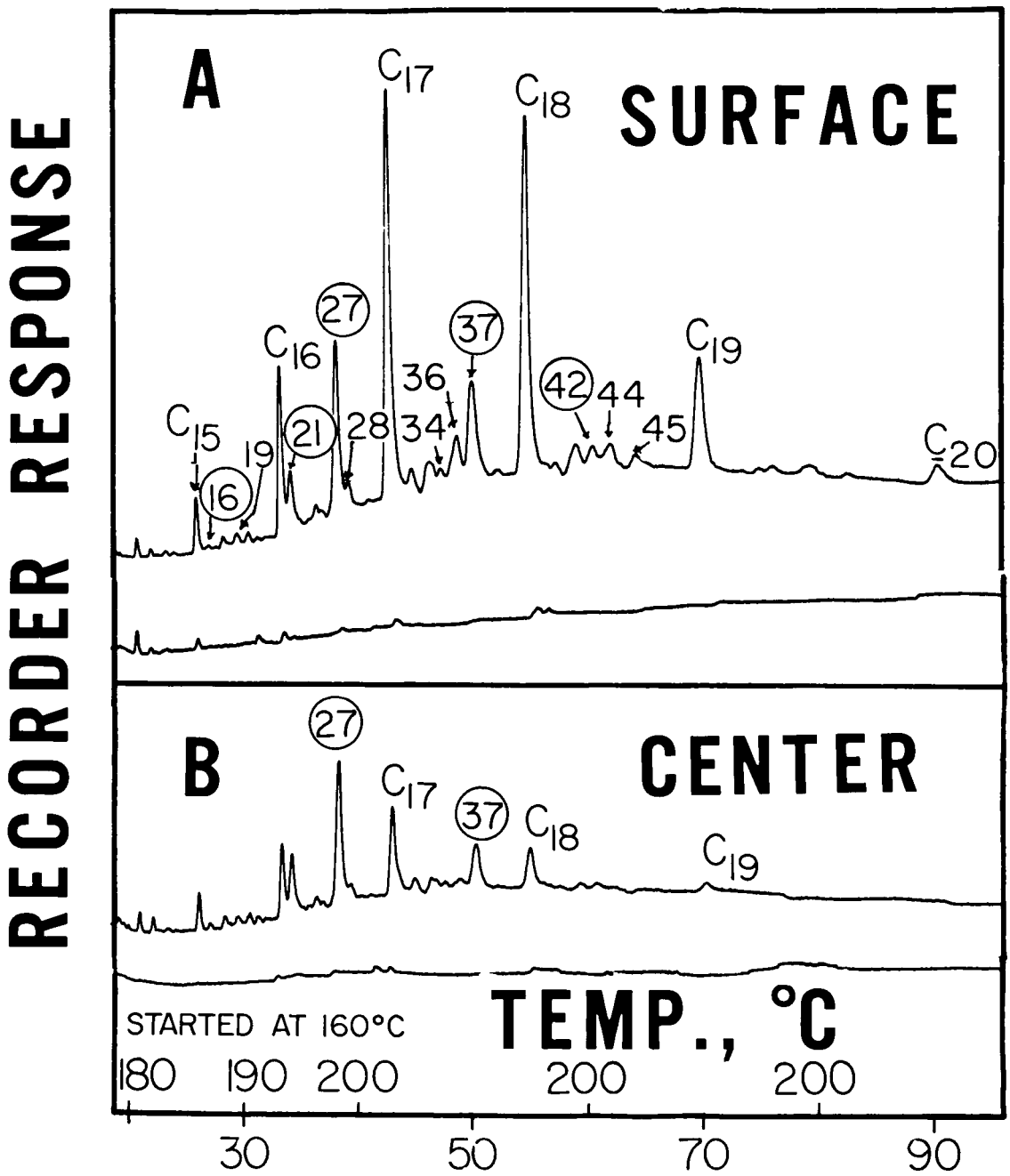
GAS CHROMATOGRAPHIC SEPARATION OF THE ALKANES FROM SURFACE AND CENTER CUTS OF AN EMBEDDED CANYON DIABLO GRAPHITIC NODULE

Stainless steel capillary column (195 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810 gas chromatograph equipped with a flame ionization detector. Nitrogen pressure 1050 g/cm^2. No split. Range, 10; attenuation, 2. Temperature programmed from 160°C to 200°C at 1°C/min.

(A) Surface cut and extraction blank. 2.2846 g extracted. About 1/8 of the n-pentane eluate was injected in both cases. (See Table LXX).

(B) Inside cut and extraction blank. 2.2846 g extracted. About 1/8 of the n-pentane eluate was injected in both cases. (See Table LXX). See Table LXXI for identification numbers.

CANYON DIABLO GRAPHITIC NODULE



RECORDER RESPONSE

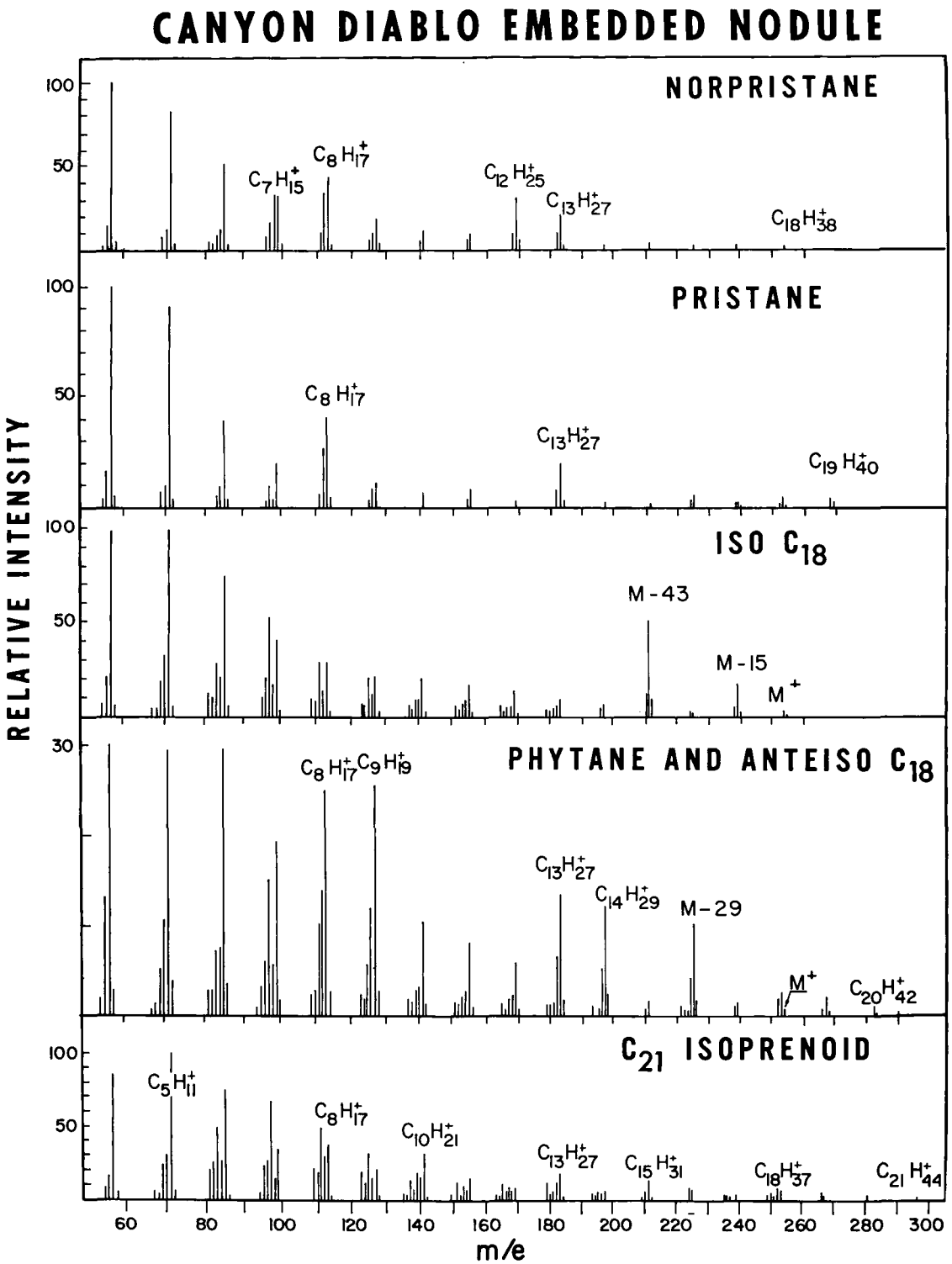
TIME (MINUTES)

Three important points to consider are: first, the strong resemblance of this distribution with its overall maxima at nC_{17} and the isoprenoid maxima at C_{19} (Figure 95) to the distributions typical of carbonaceous chondrites and the other nodules in general. Second, an interesting observation can be made in regard to the distribution differences within the graphitic nodule; that is, the ratio of the isoprenoids to the normal alkanes increases towards the center of the nodule. This effect is so pronounced as to shift the distribution mode from its maxima at $n\text{-}C_{17}$ in the surface cut to the pristane peak (#27 in Figure 95, bottom). And third, the concentration of low molecular weight hydrocarbons is relatively higher in the center cut than at the surface. All peaks numbered in Figure 95 have been identified by mass spectrometry as in all the other samples and typical spectra of all of the isoprenoids (except the C_{17} isoprenoid) identified in the surface cut are given in Figure 96. The mass spectra of the iso and the anteiso C_{18} compounds are included in the figure according to their elution times. This has been done to strengthen once again the point of the contribution of the anteiso C_{18} spectra to that of pristane which is most pronounced at the m/e 255 fragment. Among the other compounds of interest identified in this sample it is important to mention the presence of 2,6,10-trimethyl hexadecane and 4,7,11-trimethyl hexadecane in the surface

FIGURE 96

MASS SPECTRA OF BRANCHED ALKANES FROM A CANYON DIABLO GRAPHITIC NODULE

The spectra were obtained in an LKB 9000 gas chromatograph-mass spectrometer. They were taken as the corresponding peaks were eluted from a stainless steel capillary column (150 m long by 0.076 cm i.d.) coated with Polysev. Electron energy, 70 eV. Ionizing current, 120 μA. Accelerating voltage, 3.5 KV.


CANYON DIABLO EMBEDDED NODULE
NORPRISTANE
C7H15+
C8H17+
C12H25+
C13H27+
C18H38+
PRISTANE
C8H17+
C13H27+
C19H40+
ISO C18
M-43
M-15
M+
PHYTANE AND ANTEISO C18
C8H17+C9H19+
C13H27+
C14H29+
M-29
M+
C20H42+
C21 ISOPRENOID
C5H11+
C8H17+
C10H21+
C13H27+
C15H31+
C18H37+
C21H44+
RELATIVE INTENSITY
100
50
30
60
80
100
120
140
160
180
200
220
240
260
280
300
m/e

cut. Both spectra are given in Tables LXXII and LXXIII. Table LXXII contains the partial spectrum of the 2,6,10 trimethyl compound which has been already discussed in connection with the Mokoia meteorite (Table LVII). This spectrum shows very similar characteristics with relatively high fragments at C_8 and C_{13}. The possible contributions of decylcyclohexane to this spectrum has been also considered earlier in this chapter.

Table LXXIII shows the complete spectrum of the irregular isoprenoid structure identified as a 4,7,11 trimethyl hexadecane. The details of this spectrum have been also described in relation to its identification in carbonaceous chondrites (Tables LVII and LVIII). The spectrum is given here in full to substantiate the identification of this compound in graphitic nodules and support that description. The most characteristic alkyl and olefin ion fragments are underlined in the table for faster reference. Typical spectra of some of the methyl alkanes, cyclohexanes and a cyclopentyl-dodecane identified in this nodule (see Table LXXI) are given in Tables LXXIV and LXXV. The C_{16} cycloalkane is not included in Table LXXV because it could not be separated from the 4,7,11-trimethyl hexadecane. In this table (LXXV) the high intensity of the C_6 fragments in the mass series -2 and -1 is diagnostic of the cyclohexyl ring.

TABLE LXXII

PARTIAL MASS SPECTRUM OF 2,6,10-TRIMETHYL HEXADECANE FROM THE SURFACE OF A CANYON DIABLO EMBEDDED NODULE

	Mass Series (Z in C_nH_{2n+2})				
C_n \ Z	-2	-1	0	+1	+2
C_5	25	34	29	100	
C_6	19	46	31	69	
C_7			17	28	
C_8			36	30 *	
C_9			20	18	
C_{10}			9	16	
C_{11}			14	13	
C_{12}			9	9.5	
C_{13}			10	18 *	
C_{14}			6.5	6	
C_{15}			6	6.2	
C_{16}			11	12	
C_{17}			4.5	3.5	
C_{18}			4	3.5	
C_{19}					2

* Most characteristic ions

TABLE LXXIII

MASS SPECTRUM OF 4,7,11-TRIMETHYL HEXADECANE FROM THE SURFACE OF A CANYON DIABLO EMBEDDED NODULE

	Mass Series (Z in C_nH_{2n+Z})							
C_n \ Z	-5	-4	-3	-2	-1	0	+1	+2
C_5				6.5	22	30	90	6
C_6	12.5	6	16	93	100	23	80	5
C_7		4	15	20	39	<u>37.5</u>	<u>41</u>	3
C_8	3		13	12	28	18	31	3.5
C_9	3.1	3.1	9	9	26	<u>19</u>	<u>35</u>	
C_{10}	5	3	7.5	5	13	13	18.5	
C_{11}			7.5	4	8	7	11	
C_{12}	4	3	6	3.5	6	<u>14.5</u>	<u>28</u>	5
C_{13}	4.8		6	4.5	5	5	8	
C_{14}			6	4.8	5	<u>7.8</u>	<u>17</u>	3
C_{15}			22	6.5	7	6	5	
C_{16}			4	3	3	<u>20</u>	<u>17</u>	4
C_{17}						4.5	6.8	
C_{18}				3		4	5	
C_{19}						3		3

The characteristic fragments originated around the methyl branches are underlined

TABLE LXXIV

PARTIAL SPECTRA OF ISOMERIC ISOALKANES FROM A CANYON DIABLO EMBEDDED NODULE (SURFACE CUT)

m/e	5-meC$_{17}$	4-meC$_{17}$	2-meC$_{17}$
57	94	76	99
71	72.5	100	100
85	100	62	76
99	29	30	42
113	21	26	30
127	17.5	22	22
141	14	17.5	20.5
155	9	11	17
169	7.5	9	14
183	6.5	11 M-71	10
197	<u>25</u> M-57	65	7
211	6	<u>39</u> M-43	<u>53</u> M-43
225	6	4	3
239	9.5 M-15	7 M-15	18 M-15
240	4.5 (M)	3.5 (M)	4.5 (M)

Most characteristic ion is underlined

TABLE LXXV

CYCLO ALKANES IN THE CANYON DIABLO EMBEDDED NODULE

PARTIAL MASS SPECTRA

CYCLOHEXYL ALKANES

	C_{17}			C_{18}			C_{19}		
	Mass Series (Z in C_nH_{2n+Z})								
C_n \ Z	-2	-1	0	-2	-1	0	-2	-1	0
C_6	103	100		113	100		112	100	
C_7		46			51			60	
C_8		37.5			41			49	
C_9		21.5			25			36.5	
C_{10}		12			15.5			15	
C_{11}		12.5			7			12	
C_{12}		5.5			7.5			9	
C_{13}		5.5			4.5			6	
C_{14}		5			3.5			5	
C_{15}		4			5.5			9	
C_{16}		5			3.5			3	
C_{17}		- 13			3			-	
C_{18}					- 14			-	
C_{19}								9	

CYCLOPENTADODECANE

C_n \ Z	-2	-1	0
C_5	94	100	
C_{17}			20

Ions other than the molecular ion belonging to the Z = 0 mass series not included in the table

c) Deep embedded nodule (357)

Gas chromatographic analysis of the alkanes from the external and internal parts of another graphite-troilite nodule from the Canyon Diablo iron meteorite are shown in Figure 97. The name selected for identification purposes is not entirely artificial but it is meant to imply that this particular half nodule (the other half will be discussed next) appears to extend deeper into the iron than the other half. The block of meteoritic iron was cut at the American Institute Laboratory, Denver, Colorado, and it was sampled according to the method depicted in the diagram shown before (Block A). As indicated in Table LXX unequal weights were taken for analysis and so this particular sample may be subjected to the criticisms exposed earlier. The two analysis (inside and surface cuts) are qualitatively practically identical as shown by the respective chromatograms and mass spectral data (Figures 97,98, and 99). Quantitatively though, two outstanding facts are apparent in comparing these two analysis: the relatively larger concentration of low molecular weight hydrocarbons and the large amount of isoprenoids relative to the normal alkanes in the inside sample as compared to the surface sample.

The mass spectrometric analysis of this sample has extended the homologous series of cycloalkanes in meteorites down to cyclohexaoctane, the lowest member identified in any

FIGURE 97

GAS CHROMATOGRAPHIC SEPARATION OF THE ALKANES FROM SURFACE AND INSIDE CUTS OF A DEEP EMBEDDED CANYON DIABLO GRAPHITIC NODULE

Stainless steel capillary column (90 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Nitrogen pressure, 1053 g/cm^2. No split. Temperature was held at 120°C for 10 min. and then programmed from 120°C to 200°C at approximately 2°C/min.

(A) Surface cut and extraction blank. 0.2901 g extracted (see Table LXX). About 1.8/7 of the n-pentane eluate was i injected in both cases. Range, 10; attenuation, 4.

(B) Inside cut and extraction blank. 2.4316 g extracted (see Table LXX). About 2/8 of the n-pentane eluate was injected in both cases. Range, 10; attenuation, 4. See Table LXXI for identifications.

CANYON DIABLO GRAPHITIC NODULE

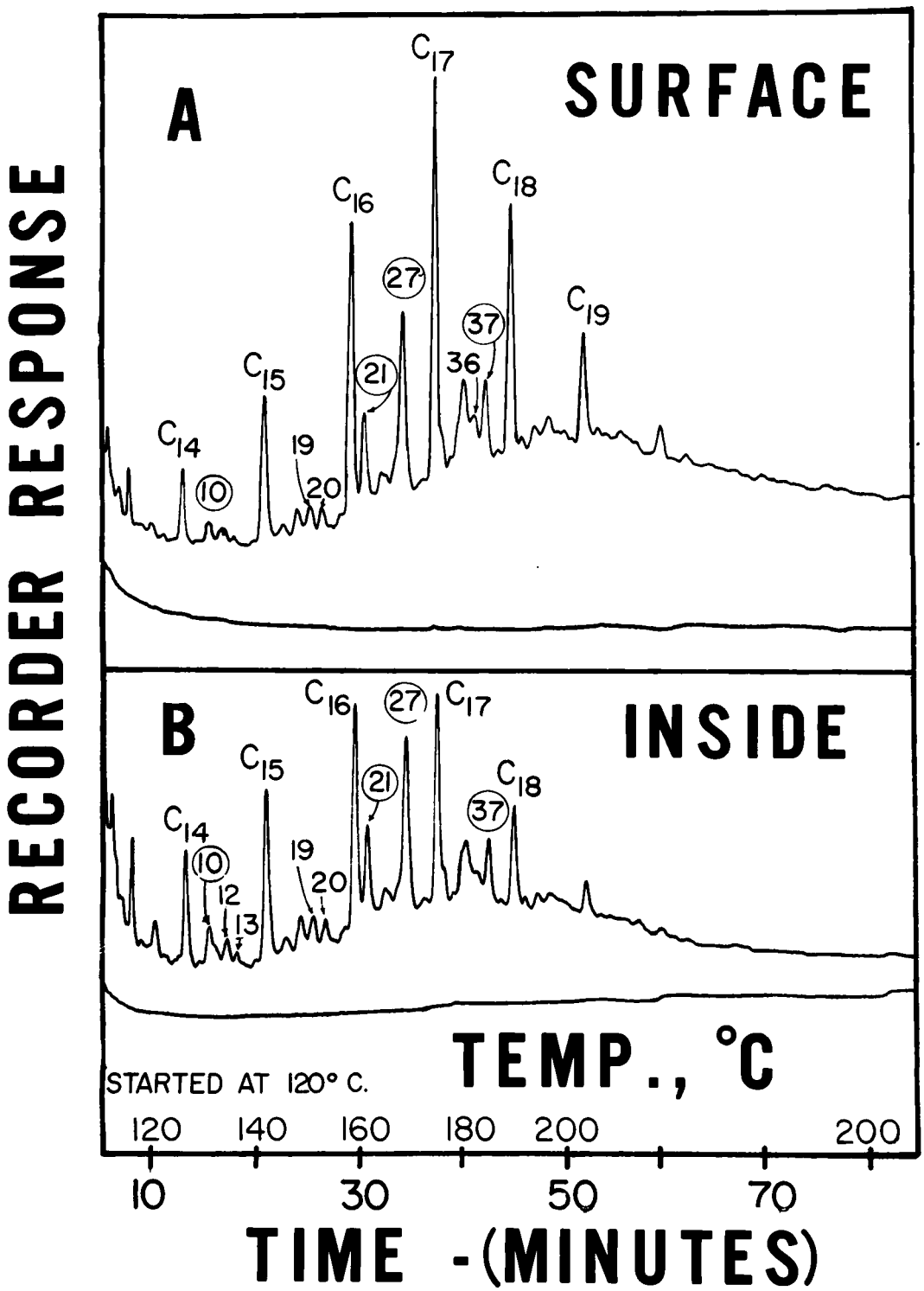


FIGURE 98

MASS SPECTRA OF ISOPRENOIDS IN THE SURFACE CUT OF A CANYON DIABLO DEEP EMBEDDED GRAPHITIC NODULE

Same conditions as in Figure 96.

CANYON DIABLO DEEP EMBEDDED NODULE

(SURFACE CUT)

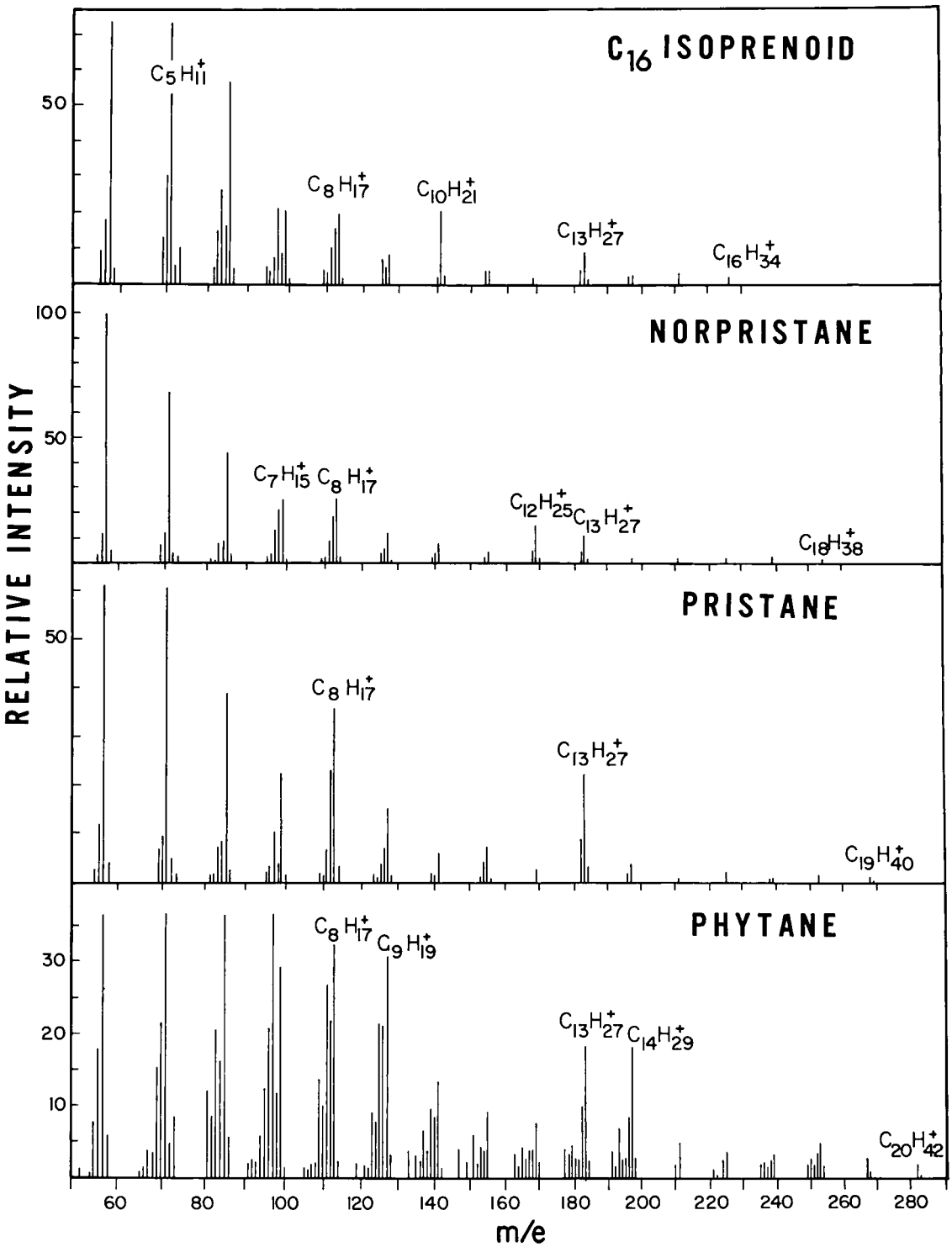


FIGURE 99

MASS SPECTRA OF ISOPRENOIDS IN THE INSIDE CUT OF A CANYON DIABLO DEEP EMBEDDED GRAPHITIC NODULE

Same conditions as in Figure 96.

CANYON DIABLO
DEEP EMBEDDED NODULE
(INSIDE CUT)

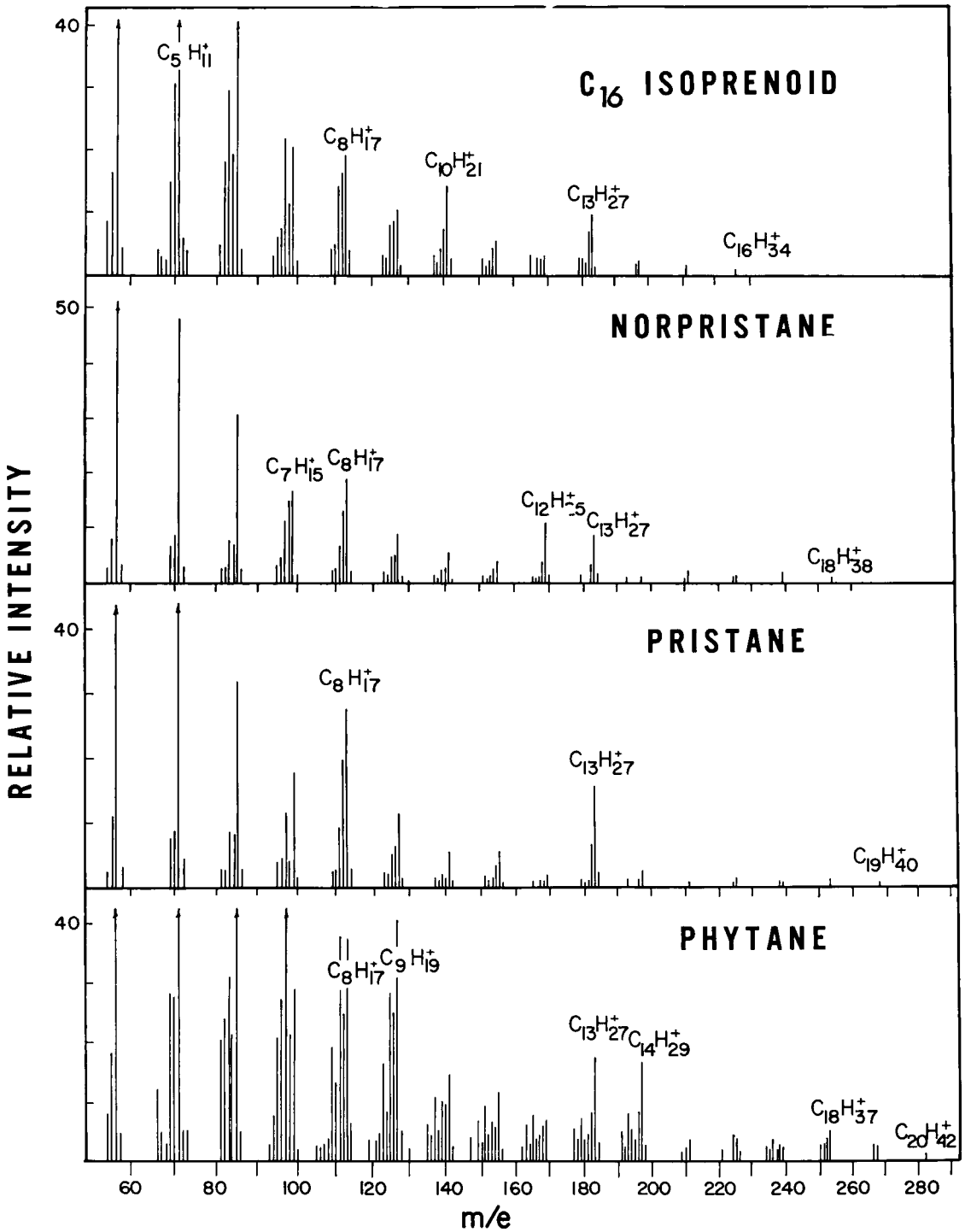


TABLE LXXVI

PARTIAL MASS SPECTRA OF CYCLOALKANES IN A DEEP EMBEDDED CANYON DIABLO NODULE (INSIDE CUT)

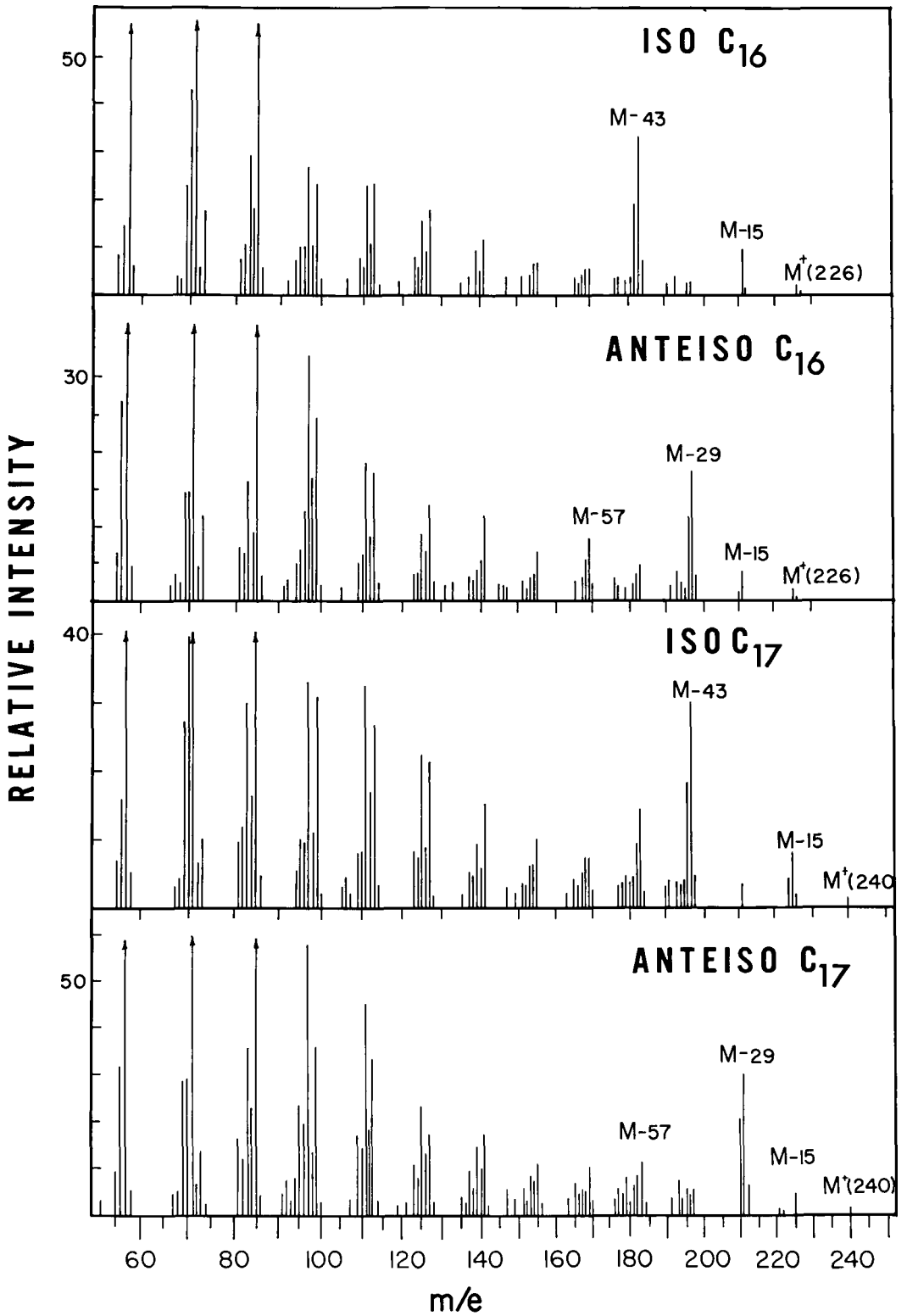
	Cyclohexaoctane			Cyclohexanonane			Cyclohexadecane		
	Mass Series (Z in C_nH_{2n+Z})								
C_n \ Z	-2	-1	0	-2	-1	0	-2	-1	0
C_6	71	100		85	100		92	100	
C_7		55	19		52	17		40	14
C_8		45	19		40	24		29	12
C_9		24	28		20	20		15	8.5
C_{10}		12	20		9	13		7.5	6.5
C_{11}		6	8		6.5	10		5	4
C_{12}		5	6		4	8		4	4.3
C_{13}		5	6		3.5	4		3	5
C_{14}		-	14 (M)		4	4.1		-	-
C_{15}					-	14 (M)			-
C_{16}									8 (M)

FIGURE 100

MASS SPECTRA OF METHYL BRANCHED ALKANES IN A DEEP EMBEDDED CANYON DIABLO GRAPHITIC NODULE

Same conditions as in Figure 96.

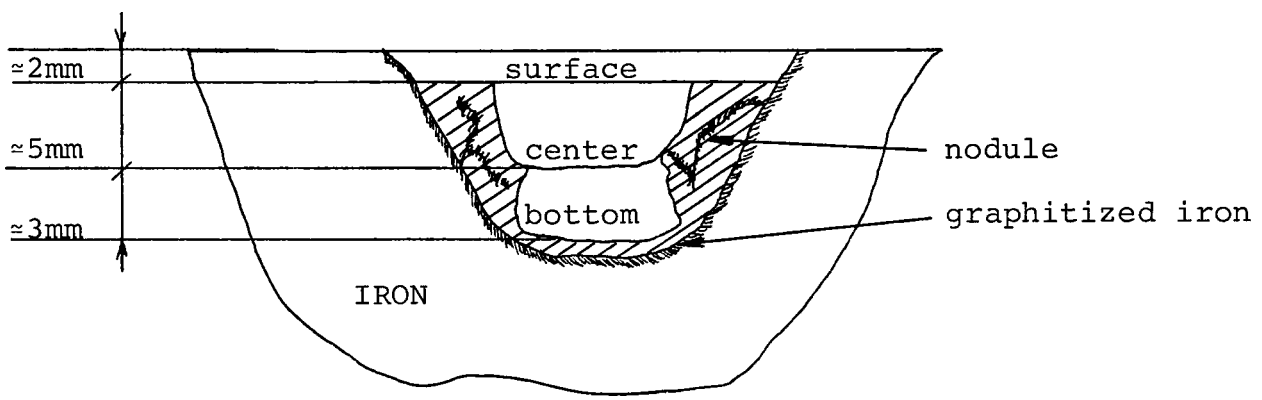
CANYON DIABLO DEEP EMBEDDED NODULE



extraterrestrial sample. The partial mass spectra of the three cyclic alkanes identified in the inside piece of the nodule are given in Table LXXVI. Figure 100 gives the spectra of four of the isoalkanes identified in this particular specimen.

d) Shallow embedded nodule

This name is given to the shallower half of the deep embedded nodule just described. A summary of the experimental data can be found in Table LXX. It was prepared for analysis in a similar way as that described for the embedded nodule (section c), the only major difference being that in this case a third portion of the nodule was taken. This portion was considerably more removed from the surface than in any of the previous cases. The three cuts can be represented in cross section as follows



The bottom part of this nodule was found to be well protected from the environment by the upper layers of graphitic carbon

and by the meteoritic iron itself. A very hard interphase of graphitized iron was found between the nodule and the iron block, with some veins extending into the nodule (darker lines in shaded area). Equal weights of the three fractions were taken and treated according to the techniques already described. The results of the gas chromatographic analysis are given in Figure 101. The surface cut (Figure 101) shows again an alkane and isoprenoid distribution which is qualitatively identical and quantitatively similar to the other Canyon Diablo nodules. A very clear concentration gradient from top to bottom is apparent in these chromatograms. In other words, the total hydrocarbon content decreases as one goes away from the surface. On the other hand, as observed with the other nodules too, the lighter components seem to predominate in the inner samples and also the relative isoprenoid content is higher below the surface. Mass spectra were obtained for several of the compounds in the surface and center cuts but not for the bottom cut for obvious reasons. In any way the GC pattern of this fraction is superinposable on the other two. Typical mass spectra of isoprenoids and C_{16} isoalkanes are given in Figures 102 and 103. A summary of the most outstanding features revealed by the gas chromatographic-mass spectrometric analysis of these Canyon Diablo nodules can be given in the following points.

FIGURE 101

GAS CHROMATOGRAPHIC SEPARATION OF THE ALKANES FROM DIFFERENT PARTS OF A CANYON DIABLO SHALLOW EMBEDDED GRAPHITIC NODULE

Same conditions as in Figure 95. (See Table LXX for experimental data).

(A) Benzene nanograde solvent. Instrumental control.

(B) Surface cut and extraction blank, 0.8098 g extracted. About 2/7.7 of the n-pentane eluate was injected in both cases

(C) Center cut and extraction blank. 0.7966 g extracted. About 2/8 of the n-pentane eluate was injected in both cases.

(D) Bottom cut and extraction blank. Same as in C.

(See Table LXXI for identification numbers).

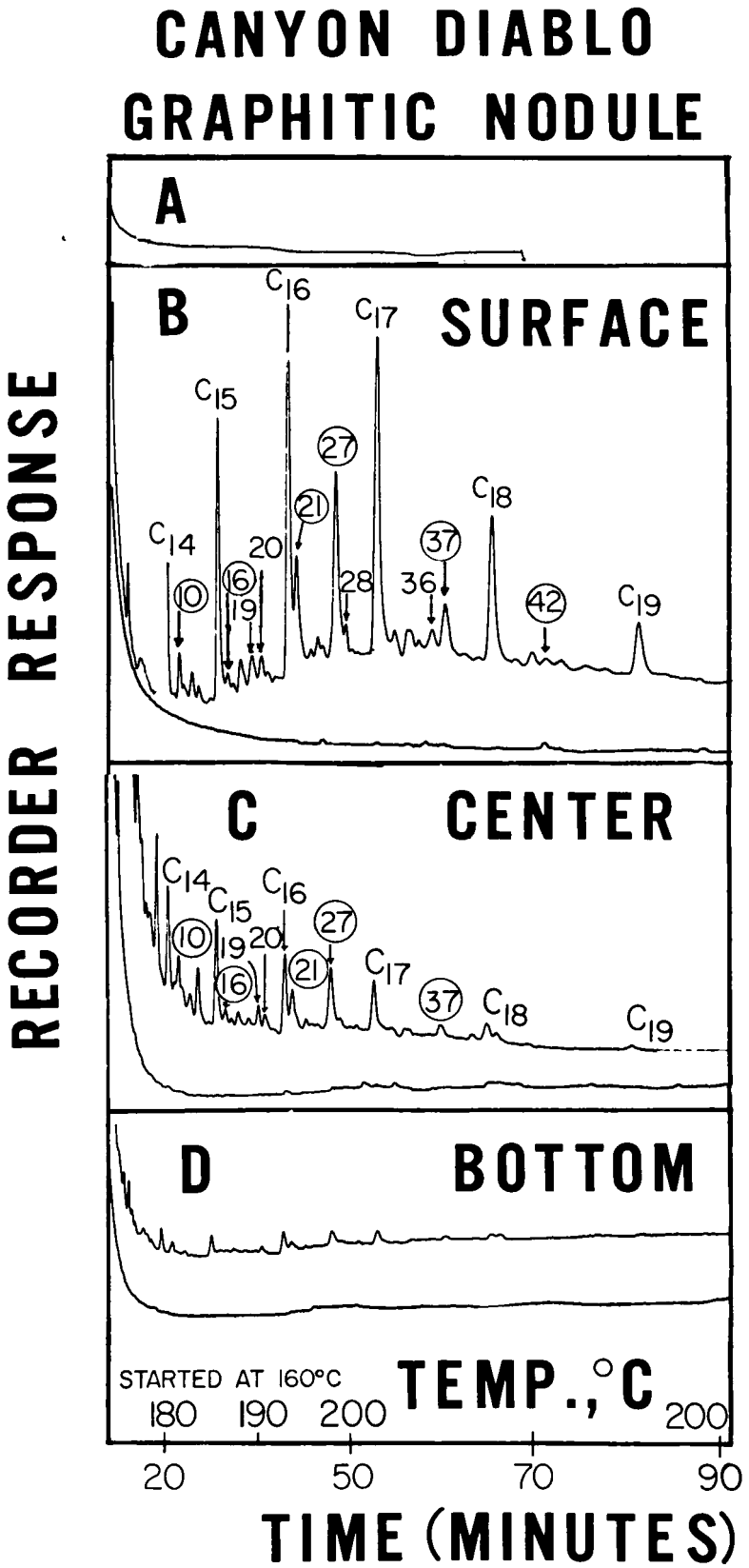

CANYON DIABLO
GRAPHITIC NODULE
A
B
SURFACE
C16
C17
C15
27
21
C18
C14
20
37
16
28
36
10
19
42
C19
C
CENTER
C14
C15
C16
10
19
20
27
16
21
C17
37
C18
C19
D
BOTTOM
STARTED AT 160°C
180
190
200
TEMP.,°C
200
20
50
70
90
TIME (MINUTES)
RECORDER RESPONSE

FIGURE 102

MASS SPECTRA OF ISOPRENOIDS FROM A CANYON DIABLO SHALLOW EMBEDDED GRAPHITIC NODULE

Same conditions as in Figure 96.

CANYON DIABLO
SHALLOW EMBEDDED NODULE

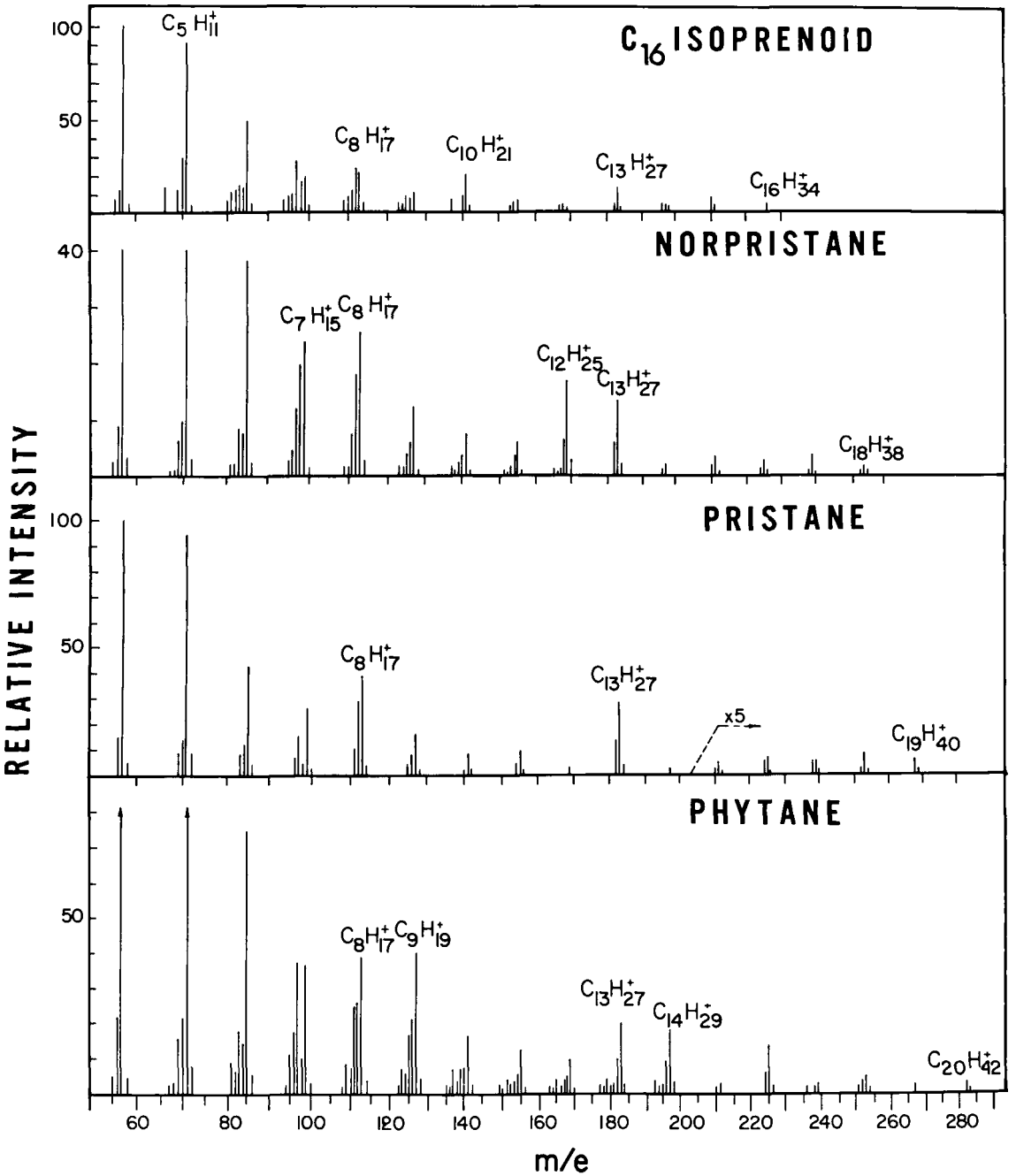
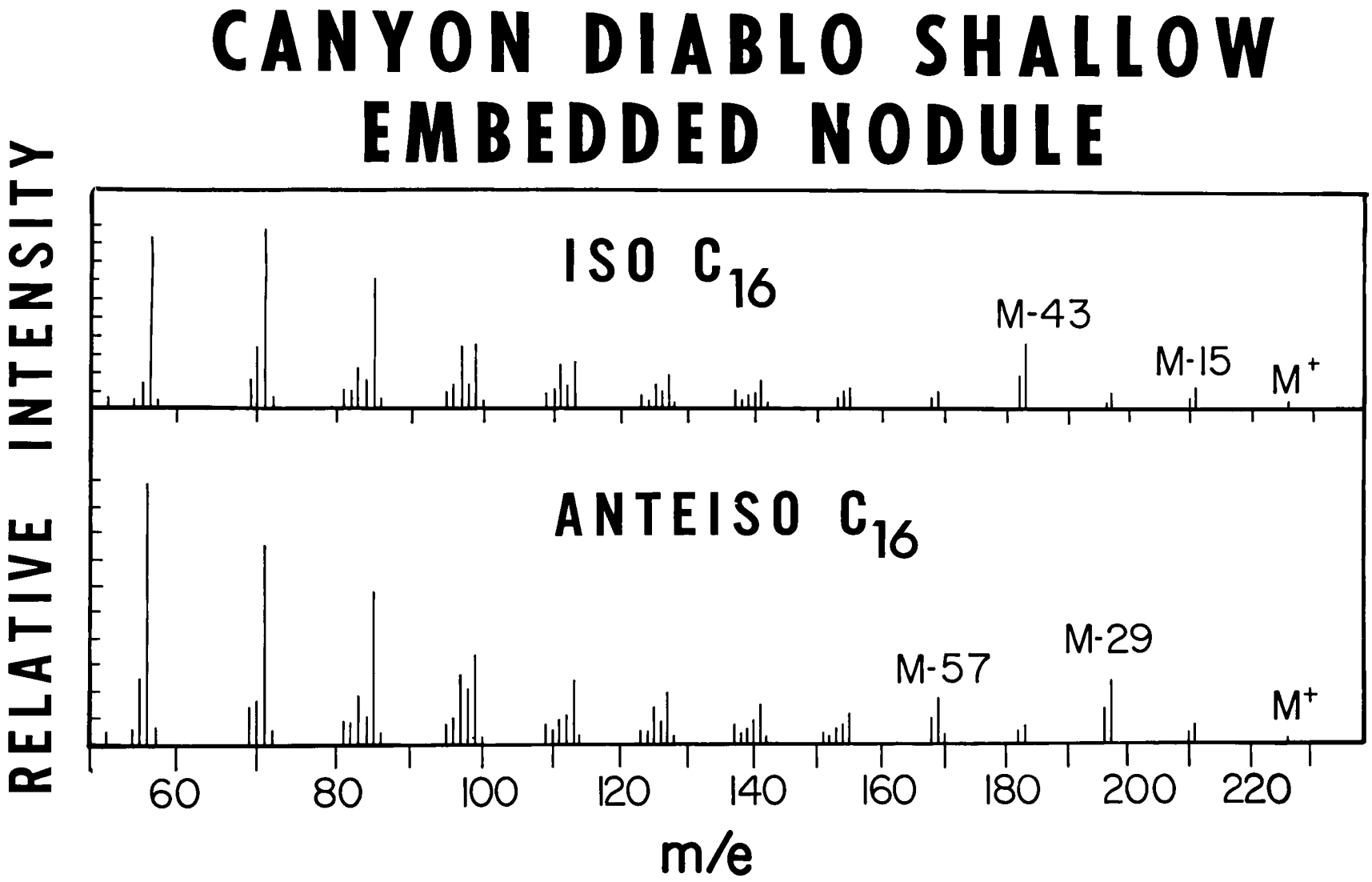


FIGURE 103

MASS SPECTRA OF METHYL BRANCHED ALKANES FROM A CANYON DIABLO SHALLOW EMBEDDED NODULE

Same conditions as in Figure 96.


CANYON DIABLO SHALLOW EMBEDDED NODULE
RELATIVE INTENSITY
ISO C16
M-43
M-15
M+
ANTEISO C16
M-57
M-29
M+
60
80
100
120
140
160
180
200
220
m/e

(1) Their hydrocarbon distributions are very similar to those of carbonaceous chondrites. The distribution mode for the n-alkanes usually peaks at C_{17} and at C_{19} for the isoprenoids.

(2) Most of the compounds identified in carbonaceous chondrites have also been found in these nodules (compare Tables XLIX and LXXI).

(3) The more exposed parts of the nodules contain larger amounts of hydrocarbons.

(4) The relative concentration of light molecular weight alkanes increases in the inner cuts.

(5) The relative isoprenoid content also increases in the same fashion.

Quantitative analysis of graphitic nodules

Quantitative data obtained from all the nodules under consideration in this chapter can be found in Table LXXVII. In all cases the total alkane concentration is lower in the inside or center cuts. This effect is especially marked in the values obtained for the three cuts from the shallow embedded nodule. The same thing is true of the isoprenoids with exception perhaps of the deep embedded nodule where their concentration is greater in the center cut. In general the surface content of the total alkane fraction is from 3 to 7 times higher than that of the inner samples, and the total

TABLE LXXVII

GRAPHITIC NODULES

(CANYON DIABLO, ODESSA AND COSBY'S CREEK)

Quantitative Data in ppm

		Total Alkane	Total Isoprenoid	Isoprenoids C_{16}	C_{18}	C_{19}	C_{20}
Shallow embedded nodule,	SURFACE	4.17	0.95	0.05	0.29	0.5	0.11
	CENTER	1.38	0.29	0.05	0.08	0.14	0.02
	BOTTOM	0.76	0.048	0.01	<0.009	0.02	<0.009
Deep embedded nodule	SURFACE	3.45	0.67		0.21	0.40	0.06
	INSIDE	3.19	0.86		0.25	0.45	0.06
Embedded nodule	SURFACE	2.18	0.40	0.01	0.05	0.22	0.12
	CENTER	0.32	0.14		0.02	0.07	0.05
Weathered nodule	OUTSIDE	3.9	0.87			0.5	0.37
	INSIDE	0.93	0.08			0.06	0.02
	OUTSIDE+ INSIDE	3.46	0.62	0.02	0.13	0.32	0.15
ODESSA		1.32	0.093		0.003	0.03	0.06
COSBY'S CREEK		9.36	0.3	0.06	0.05	0.14	0.05

surface isoprenoid content is from 3 to 10 times higher.

The C_{17} and C_{21} isoprenoids are not included in this table because due to their extremely small concentrations it was not possible to obtain reliable quantitative data on them from the GC patterns. Total injection of the sample in the gas chromatograph-mass spectrometer was necessary to detect their presence.

Contamination

Before attempting any discussions on the meaning of these results a few statements need to be made to support the validity of this unique piece of data. The experimental section contains a detailed discussion and evaluation of the problem of contamination which applies to this part of the work as well, but because of the extremely low values reported in Table LXXVII a few more words on the subject are warranted. All glassware was cleaned at least twice with hot chromic acid solution and rinsed in distilled water. Table LXX contains the information on the blanks run. In all cases a minimum of two 2 hr. blanks were run before extracting the nodule; this does not include the preliminary washing of the dry soxhlet extractor with the same benzene-methanol solution used for the blanks and extractions. These washings were discarded and then the first blank started. Details of the procedure from this point on are given in the

experimental section. All of the corresponding blanks are shown underneath each chromatogram in Figures 88,90,93,95 and 101. Heptane was used in some cases (Figures 88,90) for the column chromatographic fractionation of the extract but it was soon discarded because the blanks contained small amounts of low molecular weight impurities. In any case since usually twice the same amount of blank than of sample was injected in the gas chromatograph the contamination level observed is not enough to disturb the quantitative results. Results on the purity check of the pentane used as solvent in the other cases are given in Figure 104A. On the other hand the patterns themselves seem to exclude the possibility of laboratory contamination. How could patterns like those of Figures 95B and 101C be explained on the basis of contamination?

Before injecting <u>any</u> of the meteorite samples in the gas chromatograph an amount of nanograde benzene equivalent to the sample volume to be injected was introduced under the same conditions as those to be later used in the actual GC analysis. This was done to insure that no traces of hydrocarbons were present in the clean syringe or were trapped at any point in the gas chromatographic apparatus (for instance in the injection system). An example of the results thus obtained is shown at the top of Figures 88 and 101. Although not indicated before this was done in all cases including the

FIGURE 104

GAS CHROMATOGRAMS OF SOLVENT

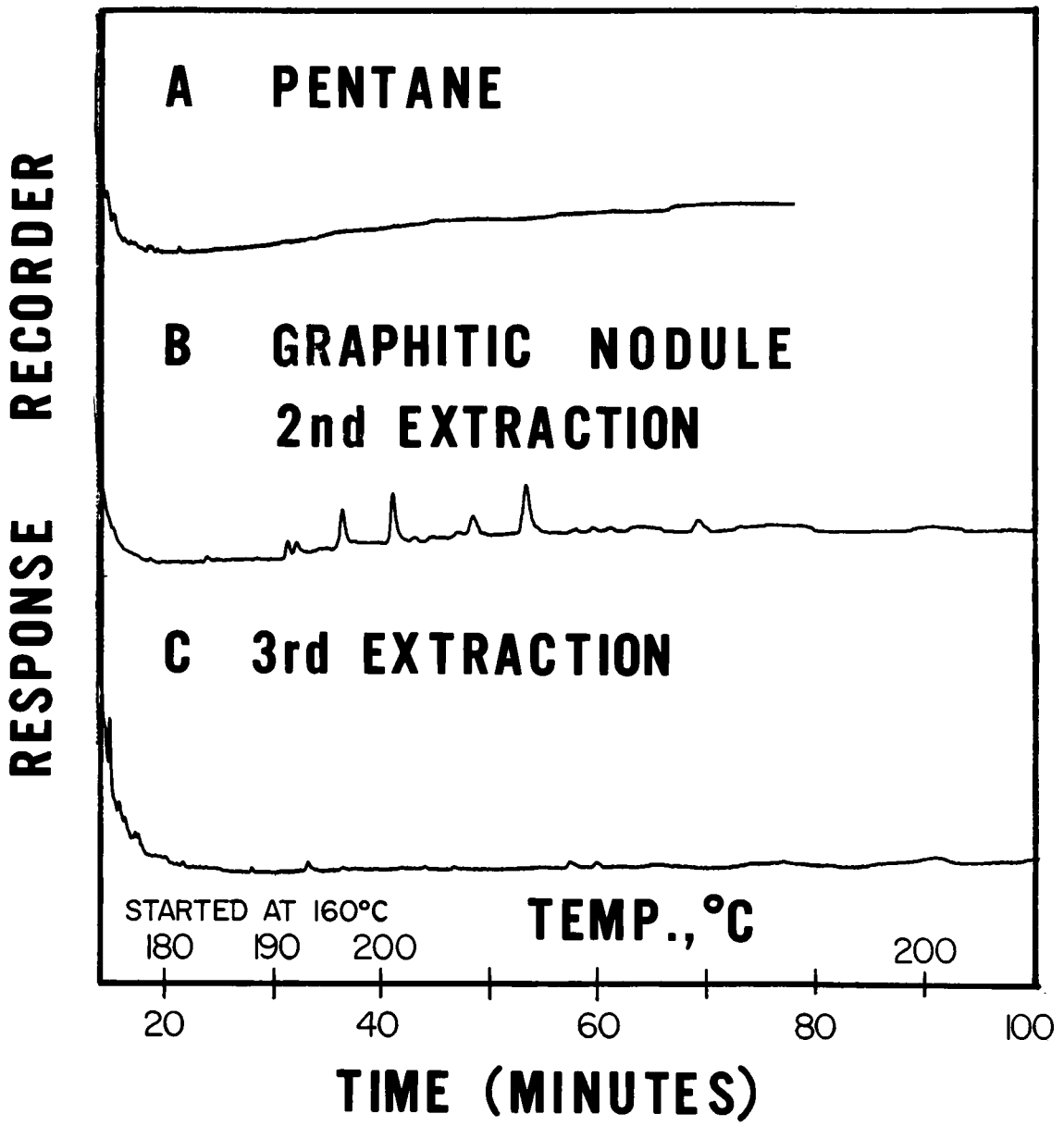
AND EXTRACTION CONTROLS

Stainless steel capillary column (195 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Nitrogen pressure 1050 g/cm^2. No split. Range, 10; attenuation, 2. Temperature programmed from 160°C to 200°C at 1°C/min.

(A) n-Pentane solvent. About 20 ml evaporated to 1.4 ml and injected.

(B) Reextraction of the inside cut of the Canyon Diablo embedded nodule used as control blank

(C) Same as B.


A PENTANE
B GRAPHITIC NODULE
2nd EXTRACTION
C 3rd EXTRACTION
RESPONSE RECORDER
STARTED AT 160°C
180 190 200
TEMP., °C
200
20 40 60 80 100
TIME (MINUTES)

carbonaceous chondrites.

The possible contributions to these patterns by the tissues used to wrap these samples (shallow embedded nodule) was also checked by subjecting 1 g of the paper to the same extraction and recovery procedures used for the meteorites. The results were negative. The GC patterns look like one of the blanks shown in the previous figures. Likewise it has also been indicated above that there was no appreciable contribution to the pattern exhibited by the Cosby's Creek by the waxy material on which it had been mounted.

Finally as an overall check of the whole procedure a control run was made with the center cut of the Canyon Diablo embedded nodule. It was crushed again in the Carver press test cylinder like if it were an untreated nodule and soxhlet extracted for 8 hrs. The results of this second extraction are shown in Figure 104B. The mass spectrometer confirmed the presence of pristane, n-C_{17}, phytane and n-C_{18}. Later a third extraction performed under the same experimental conditions gave negative results Figure 104C. This rather than contamination (especially considering that the blanks run prior to the second extraction look very clean) appears to indicate the high retentive properties of the graphitic nodule towards hydrocarbons. This same observation was also made in connection with the analysis of some commercial

graphites (see Chapter V). One extraction is usually not enough to remove all the material entrapped in the graphitic matrix.

n-Alkane and isoprenoid distributions in graphite-troilite nodules from the Canyon Diablo iron meteorite

A plot of the concentration of n-alkanes found in each of the three sections of the shallow nodule versus the average distance of the cuts from the surface shows very clearly the logarithmic decrease in the total alkane concentration that takes place with depth (Figure 105). This constitutes a graphical representation of the general trend encountered in all of the different graphitic nodules. The next figure (106) shows an equivalent plot of the changes in the isoprenoid concentrations between the internal and external part of three of the graphitic nodules. As for the n-alkanes the overall concentration of the isoprenoids is greater at the surface.

It has been pointed out that the concentration of the isoprenoid hydrocarbons relative to the n-alkanes seems to increase towards the internal parts of the nodules. This is best demonstrated by the plot of Figure 107. The upper family of lines identified by the number 1 in the figure are joining the values of the ratio, pristane/nC_{17}, obtained for the embedded, deep embedded and shallow nodules. Those

identified by the number 2 are joining the values obtained for the ratio norpristane/nC_{16} in the same specimens. Without exception both ratios show higher values in the inside cuts of the nodules indicating that although the overall isoprenoid concentration decreases with depth within the nodule, as well as that of the n-alkanes, the concentration of isoprenoids relative to the latter increases.

In the case of the shallow embedded nodule where the ratio pristane/nC_{17} reaches a plateau this may indicate an even distribution of the isoprenoids within the inner parts of the nodule. However, this cannot be substantiated by more analytical data at the moment and it should not be overemphasized.

FIGURE 105

TOTAL ALKANE DISTRIBUTION IN A CANYON

DIABLO GRAPHITIC NODULE

See Table LXXVII, shallow embedded nodule.

TOTAL ALKANE DISTRIBUTION
CANYON DIABLO GRAPHITIC NODULE

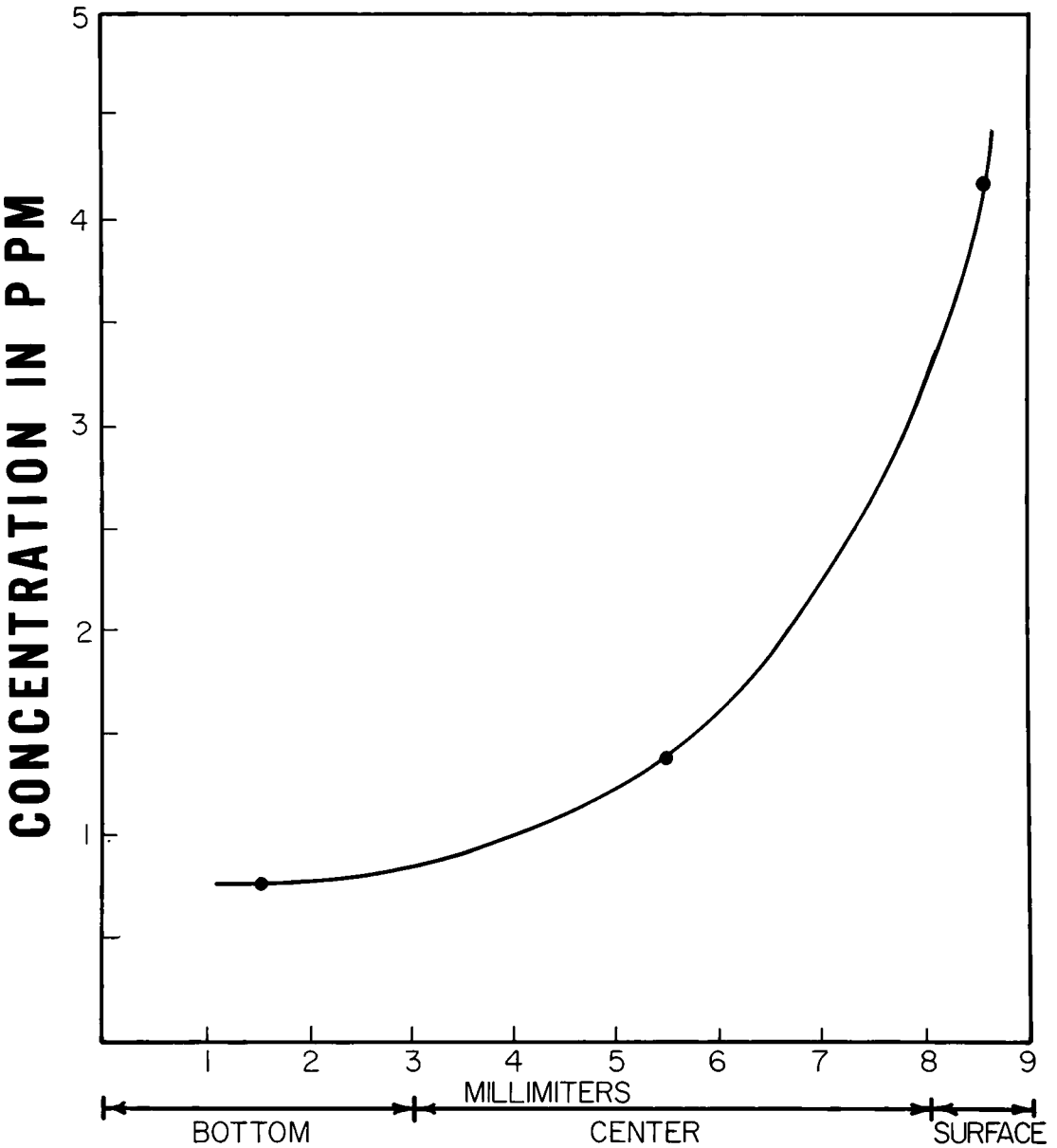
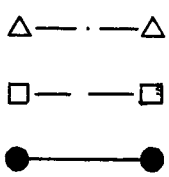


FIGURE 106

TOTAL ISOPRENOID DISTRIBUTION IN

THREE GRAPHITIC NODULES



Canyon Diablo embedded nodule

Canyon Diablo weathered nodule

Canyon Diablo shallow embedded nodule

TOTAL ISOPRENOID DISTRIBUTION
IN THREE GRAPHITIC NODULES

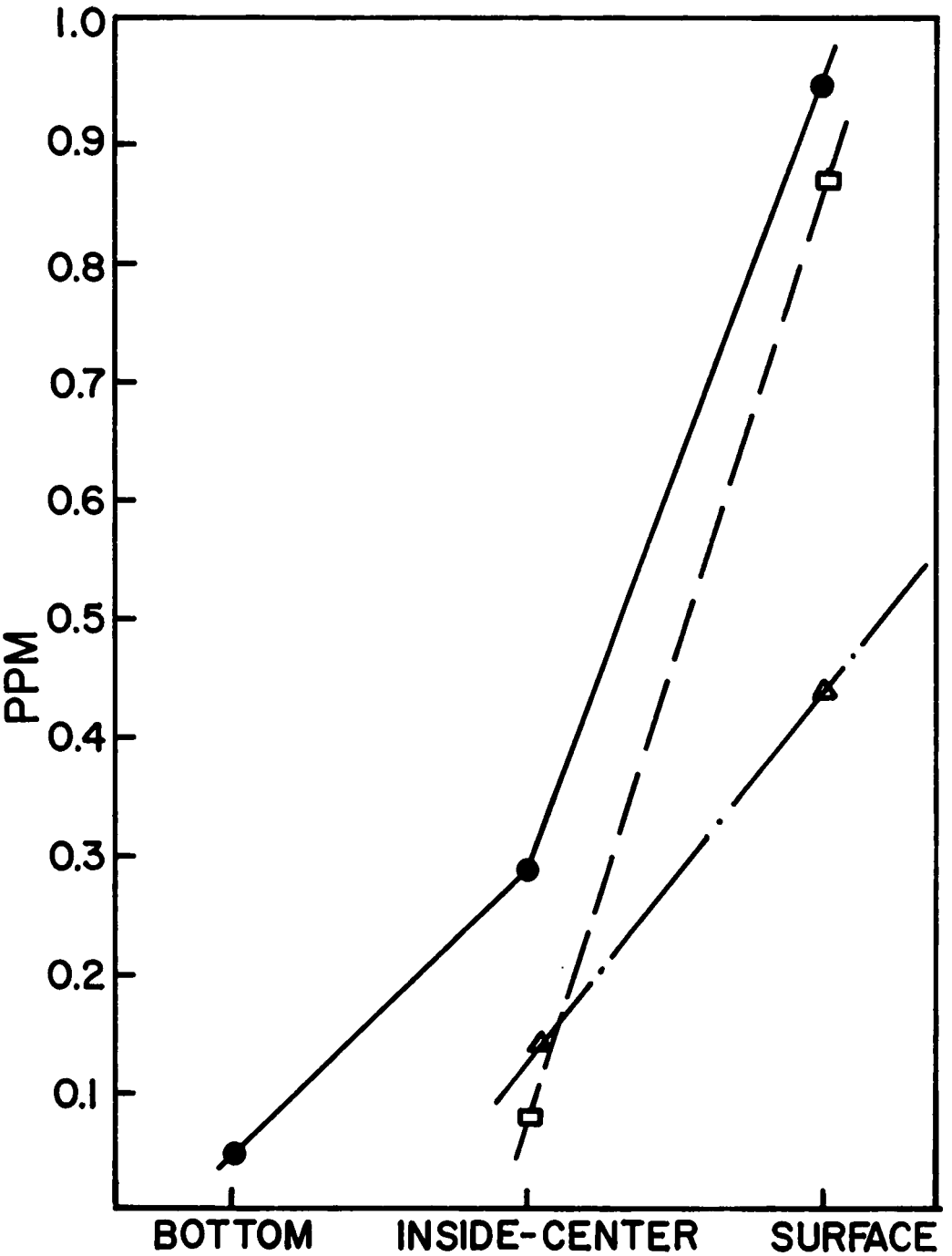


FIGURE 107

ISOPRENOID DISTRIBUTION IN THREE

GRAPHITIC NODULES

1 Pristane / nC_{17} ratio

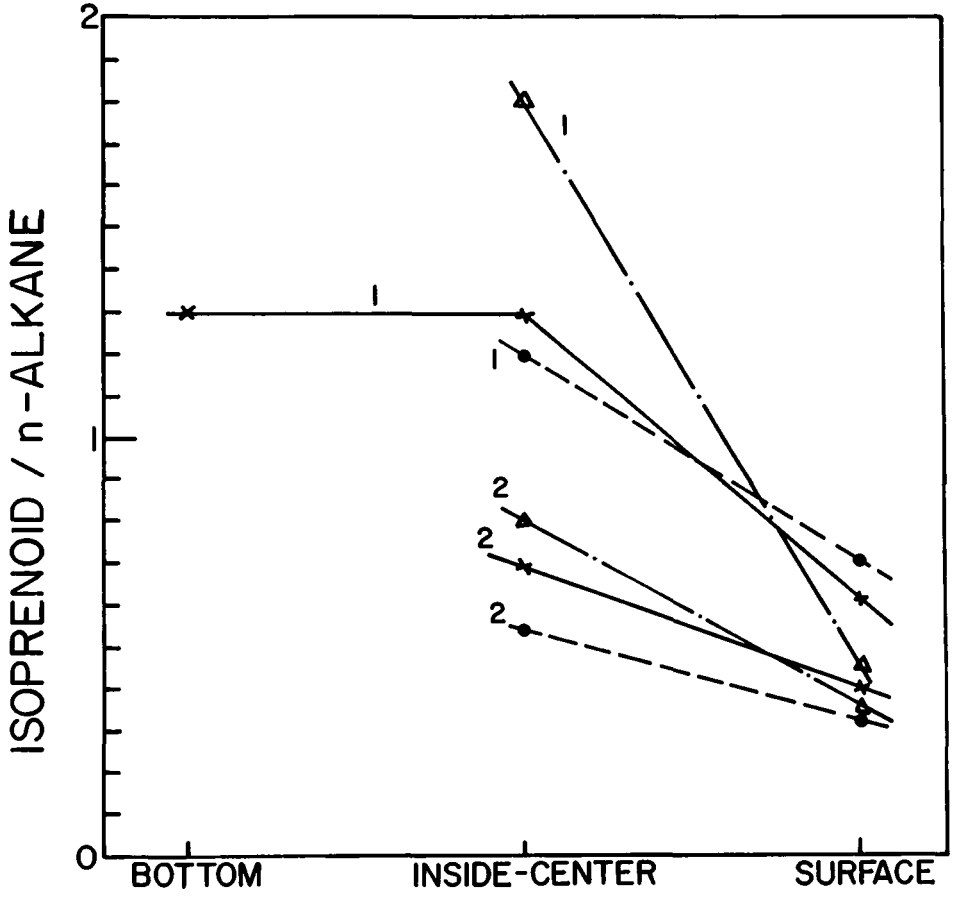
2 Norpristane / nC_{16} ratio

△—·—△ Canyon Diablo embedded nodule

×——× Canyon Diablo shallow embedded nodule

○---○ Canyon Diablo deep embedded nodule

ISOPRENOID DISTRIBUTION IN THREE METEORITE NODULES



VIII.

DISCUSSION

DISCUSSION

Partial discussions and evaluations of the data presented here have already been given for most individual samples whenever found pertinent. The following discussion will not be centered on the results obtained with any specific sample or on isolated observations by themselves but rather will attempt to unify within the context of organic geochemistry all the information gathered throughout this investigation. The major goals to be pursued will be i) the evaluation of the isoprenoids as biological markers, ii) their implications in the detection of terrestrial or extraterrestrial life and, iii) the determination of the origin of the hydrocarbons detected in meteorites. In addition it is also felt that these results may add supporting evidence to current theories on the organic origin or petroleum.

A. The Application of "biological markers" in Geo- and Cosmochemistry as a Means of Life Detection.

The chemical approach to the determination of the presence of life processes on earth or in any other place in the solar system, involves the structural identification of "biological markers" among the organic compounds either present in living systems or in products suspected of bearing or having borne any particular form of life.

The reasons for selecting the isoprenoid hydrocarbons as the compounds best suited to be regarded as biological markers, which have been reviewed earlier, are based mainly on their chemical and structural properties. Their occurrence in natural products and in geochemical as well as in extraterrestrial samples has also been considered in some detail. Two of the most significant observations that can be made after a careful consideration of the results presented here and the existing reports on the occurrence of isoprenoids are: their ubiquity in nature and their complete absence from abiogenic mixtures of hydrocarbons.

Both observations are important factors for the evaluation of the biogenicity of this class of organic compounds. It needs not to be emphasized that such an evaluation is of utmost importance in the establishment of methods of detection of present and past life processes.

In this context, the abiogenic synthesis of any isoprenoid hydrocarbon would seriously undermine the present theories about the chemical record of Precambrian life. Many attempts have been made recently in this direction without success. (See Chapter II for a review of this work.) But to date none of the claims to the laboratory synthesis of isoprenoids has been adequately substantiated with the proper analytical data. For example, it has already been discussed and demonstrated here how the anteiso C_{17} and the anteiso C_{18}

alkanes had been initially misidentified in Fischer-Tropsch products (360,361) as pristane and phytane respectively, on the basis of a rather convincing gas chromatographic analysis on four different stationary phases (see Chapter VI). The gas chromatographic-mass spectrometric analysis of one of these same samples and also of products obtained duplicating identically the experimental conditions used previously (361) proved the earlier identifications to be incorrect in spite of all the gas chromatographic data supporting them.

Also the apparent inconsistencies of the data reported by Studier et al. (463,467) on the Fischer-Tropsch synthesis of low molecular weight isoprenoids have been discussed in some detail (Chapter VI). In tacit support of the criticisms expressed here on the identifications and analytical procedures of these authors, it may be added that while the identities of most of the n-alkanes and branched alkanes are reinstated (463), in their latest publication (463) no mention is given to the isoprenoid structures previously claimed to be among other components in their synthesis products. A consideration of the results obtained in the present study on the possible abiotic synthesis of isoprenoid hydrocarbons coupled to the results obtained by other investigators (see Chapter II,D) clearly indicate that no conclusive positive evidence on this matter has been or can be presented yet.

On the other hand, a possible route to the abiotic formation of isoprenoids that has proved to be feasible process as well as a great advance in the rubber technology involves the manufacture of isoprene from isobutilene and methanol. Isobutilene is derived from isobutane. The isoprene product can be stereospecifically polymerized to polyisoprene polymers with the regular head to tail linkages (343) by means of highly stereospecific catalysts. The work of Natta and coworkers showed that the 1,4 trans as well as the 1,4 cis configuration of biopolymers such as gutta and rubber could be reproduced in a non biological system. This fact which has had a tremendous influence on the synthetic rubber industries could also represent a serious challenge to the validity of isoprenoid hydrocarbons as biological markers. Nevertheless the industrial process of synthesis of isoprene would not be expected to be operative in the primordial atmosphere of the earth, where reactions were forced to take place under the prevailing conditions. That is, in a reducing atmosphere containing C, O and N in their reduced form, methane, water, and ammonia (356). On the other hand Dayhoff et al. (118) have postulated that isoprene would be one of the products in their hypothetical equilibrium processes taking place in such an atmosphere, and McCarthy (281) concludes from thermodynamic considerations that isoprene is the most stable of the C_5H_8 isomers and that

above certain temperatures it can be derived from the saturated analogues of the same carbon number. From these considerations and from the work of Natta (343) it is possible to assume that after its formation isoprene could have been polymerized stereospecifically during the early history of the earth. Nevertheless it has been mentioned by McCarthy (281) that the characteristic tail-to-tail linkage in biosynthesized isoprenoid compounds seems to take place exclusively at the C_{15} carbon to give C_{30} compounds such as squalene, and at C_{20} to give C_{40} compounds such as lycopene. He suggests the possibility that the criterium of biogenicity may lay in the non branched 4-carbon unit within the tail-to-tail linkage (281).

Even though the abiotic synthesis of isoprenoids such as pristane and phytane seems a remote possibility in spite of the stereospecific synthesis process of Natta just mentioned, it can not be discarded a priori and so it becomes necessary to develop a reliable criterium to differenciate between the possible abiogenic and biogenic isoprenoids.

Some of the criteria proposed involve i) the determination of the absolute configuration of the optical centers in the isoprenoid molecules. But any measurement of optical activity requires amounts of material far in excess of what can be obtained from geo- or cosmochemical samples. A promising possibility to overcome such a limitation lies in the use of

the more sensitive gas chromatographic conditions to separate the diastereoisomeric forms of these compounds. The absolute configuration of phytol has been determined by Burrell et al. (81) and by Crabbe et al. (110) and shown to be a 3,D-7, D-11,15-tetramethylhexadec-trans-2-ene-1 ol. Carbons 7 and 11 have the meso configuration. The biologically derived pristane isolated from living systems has also this configurations and so it could be used as a standard. This approach to the problem although sound in theory has met serious difficulties in practice due to the lack of suitable chromatographic phases capable of achieving a satisfactory resolution of the isomers (281); ii) a study of the carbon isotope ratios of the individual isoprenoid molecules will also provide information about the biological or abiological origin of the molecule. This has not been put into practice due to the difficulties in the isolation and crystallization procedures. Although both approaches could provide in theory the most relaible answers to the question of the validity of isoprenoid hydrocarbons as "biological markers", their practical limitations have to be solved before they can be of any use.

For this reason the search for an acceptable criterium of biogenicity has been oriented in another direction during the course of this investigation, namely it has been approached from a distributional point of view (357).

A close inspection of the relative concentrations of the isoprenoid hydrocarbons in all of the samples analyzed in this work indicates that all possess similar distribution patterns. These patterns in general agree quite well with the resulting distributions expected from the diagenetic changes of a parent substance such as phytol or higher biopolymers (squalene, lycopene, etc.).

In order to substantiate these observations all of the quantitative analysis of isoprenoid hydrocarbons reported in the literature (32,112,143,231,281,348,402) were compiled into a single table and then compared with the data gathered during the present work. With some explainable variations all of the distributions appear to fit a unique and common "biological pattern". Almost without exception pristane (the C_{19} isoprenoid) is the major component among the isoprenoids and the C_{17} isoprenoid is always in such low concentrations that excluding some of the results obtained here it has been detected only once before by other investigators (282). Among the lower isoprenoids there appears to be another small maximum at the C_{16}. All this is depicted in Table LXXVIII. Each star in the table has been assigned a value of 10. Pristane was selected as the reference compound and its concentration in all the samples considered was assigned a value of 100. The overall relative values of the other isoprenoids were

TABLE LXXVIII

RELATIVE DISTRIBUTION OF THE ISOPRENOID HYDROCARBONS IN NATURE

Norfarnesane (C_{14})	*
Farnesane (C_{15})	**
C_{16} isoprenoid	*****
C_{17} isoprenoid	
Norpristane (C_{18})	******
Pristane (C_{19})	**********
Phytane (C_{20})	******
C_{21} isoprenoid	*****

obtained according to the following relationship

$$\left(\sum_{1}^{m} \frac{\text{concentration of } C_n}{\text{concentr. of } C_{19}} \times 100 \right) \Big/ m =$$

$$= \text{Average relative concentration of } C_n (\%)$$

where m ranged from 3 to 11 depending on the number of values of the concentration reported for a given isoprenoid hydrocarbon, and n is the isoprenoid carbon number, which ranged from 14 to 21. It is clear that the two maxima at C_{16} and C_{19} are well separated by a pronounced minima at C_{17} which imparts a marked bimodal character to the overall distribution.

There is certainly an order and a degree of selectivity in this pattern that can not be defined from an inorganic point of view. Of course, it is true that most likely we are dealing with <u>products</u> rather than <u>survivors</u> of diagenesis (122) and so this distribution does not reflect in a direct way the nature of the originally deposited bioorganic matter.

Indications that such a distribution of isoprenoid hydrocarbons is actually the product of diagenetic changes over geologic periods of time come from the consideration that i) living organisms do not produce such a range of isoprenoids (only pristane is found in relatively high amounts in marine animals, see Chapter II); ii) pristane and phytane are more abundant in a 500 than a 100 million year old crude

oil (319), and iii) Robinson et al. (402) have shown that the phytane content of the Green River Oil-Shale decreases with increasing depth while the amount of C_{19}, C_{18} and C_{16} isoprenoids increases proportionally.

All this suggests, on one hand that the lower isoprenoids are degradation products of a larger parent(s) isoprenoid molecule(s) and on the other, going back to the characteristic and widespread distribution in geological samples, that such a selective pattern can not be the product of random inorganic syntheses.

There is in theory more than one possible way to fit the observed distribution into a likely diagenetic degradation scheme, considering that the different thermal and kinetic stabilities of the bonds and functional groups at different sites within the molecule, may lead under various conditions to diverse degradation pathways. In any case, because of the mentioned differences in bond stabilities there will be, in a certain environment, a preferred mode of degradation which will impart selective character to the final distribution of products.

Moreover, it would be hard to visualize how a synthetic inorganic process could be so discriminating against a specific compound such as the C_{17} isoprenoid and could favor the C_{19} isoprenoid over the rest to such a significant extent.

It could be argued at this point that while the isoprenoids being recovered today from geological samples are not the product of synthetic abiotic reactions their parent molecules could have arisen abiogenically either before, during or shortly after the deposition of the sediments or the formation of petroleum. This argument can also be answered in the same grounds; that is, why only one parent molecule should be specifically produced in an abiotic system?, and if all of them were produced in similar concentrations, then diagenesis of such a mixture of isoprenoids can not account for the distribution observed today.

The criterium proposed here as a measure of the validity of the isoprenoids as biological records although perhaps not as theoretically elegant as the criteria based on their individual isotopic ratios or absolute stereochemical configurations appears to be practical and of immediate application, as it will be demonstrated later on.

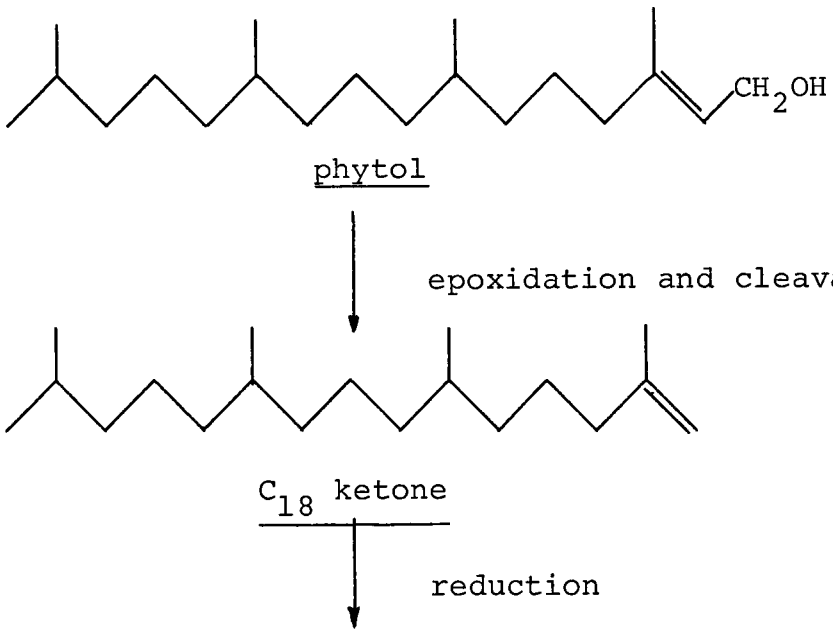
It must be emphasized at this point that if the role of the isoprenoids as biological markers is accepted then living systems date at least as far back as 3.2×10^9 years (365).

Questions arising now are, what kind of life, how about its biochemistry, was it comparable to the biochemistry of modern organisms? A knowledge of the metabolic products may provide a clue to the answers. This, in the context of the

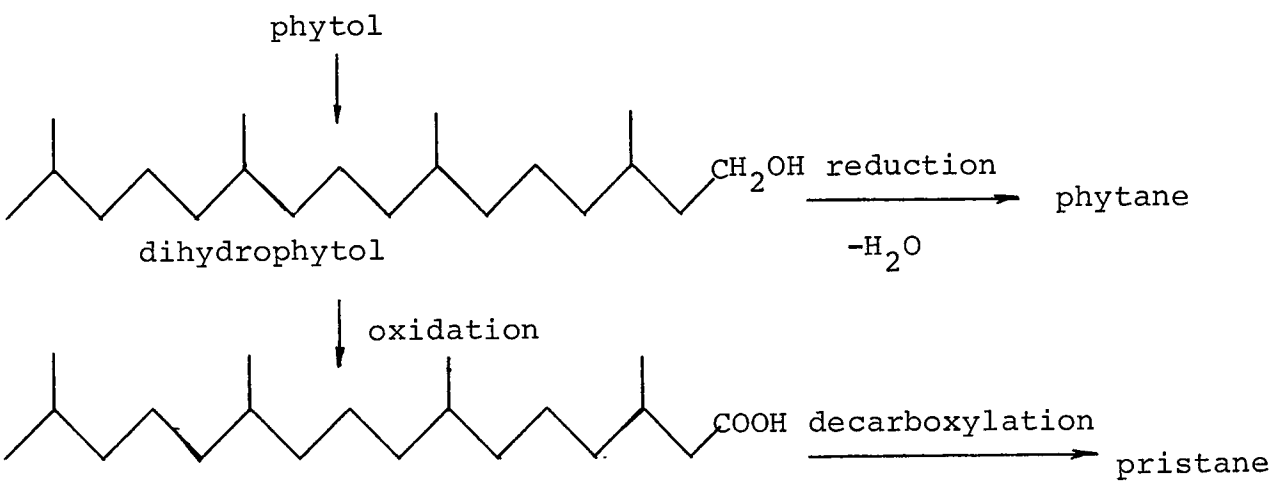
present discussion, would imply a knowledge of the identity of the parent isoprenoid compound.

While it is accepted that the phytol portion of the chlorophyll molecule may be the precursor of the isoprenoids, pristane and phytane (32), there is still much speculation about the exact mechanisms prevailing under the particular geological conditions that lead to their formation. Besides, phytol is by no means the only possibility since several other biological compounds can act as precursors, according to several likely diagenetic pathways. Among these compounds, farnesol (32), squalene (281,282),carotenoids such as lycopene (32) and recently the diphytyl phospholipids of halobacteria (240) (see Chapter V) have been considered as attractive alternatives. Either of these precursor compounds can undergo diagenesis through more than one possible pathway and as a result their relative contributions to the final distribution are not clear.

Among the postulated theories on the diagenetic processes suffered by the precursor compound during the geological time that of Curphey (113) was the first to propose that phytol acts as a source of isoprenoids through epoxidation and thermal cracking mechanisms.



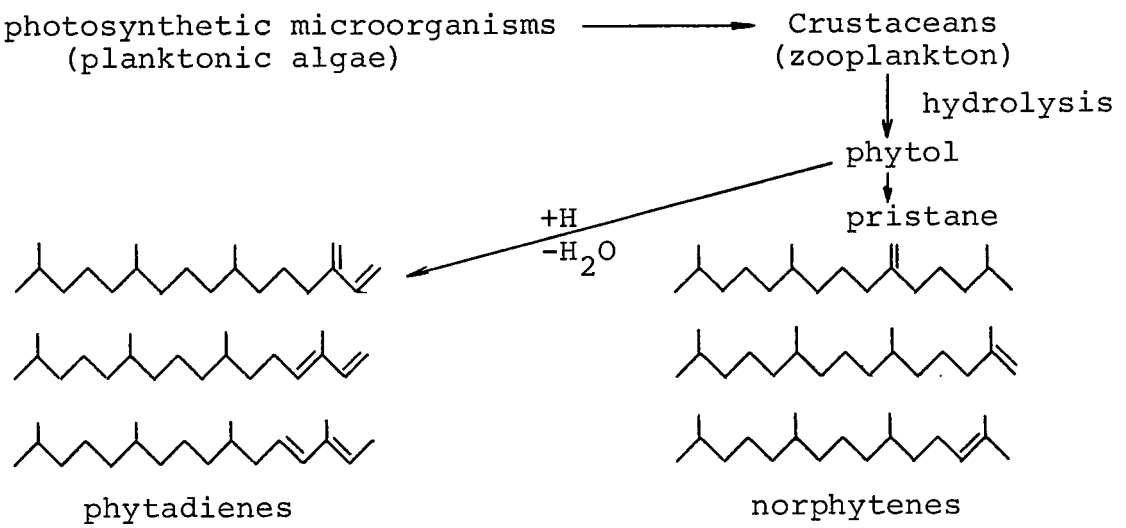
Once the C_{18} isoprenoid alkane is formed, thermal cracking mechanisms give rise to the lower members of the isoprenoid homology. This scheme although plausible does not account for the two major isoprenoid compounds, pristane and phytane. For this, Bendoraitis (32) proposed a specific sequence of reactions starting with the enzymatic cleavage of the phytol from the chlorophyll, and then;



According to this scheme phytane can be produced without oxidation. This mechanism in turn does not account for the lower isoprenoids unless thermal degradation reactions are considered.

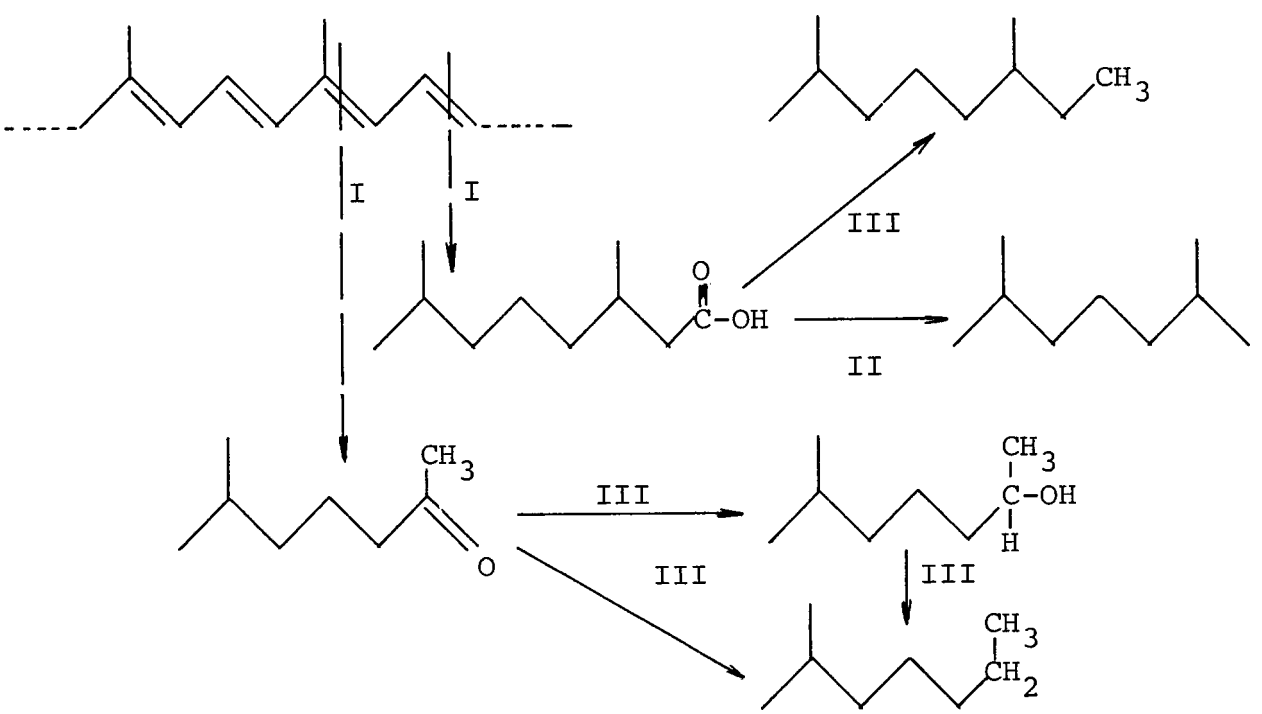
Robinson et al. (402) indicated that oxidative cleavage of phytol would produce a C_{17} acid and a C_{18} ketone. The C_{17} acid could be decarboxylated to the C_{16} isoprenoid and the ketone could be reduced to the corresponding hydrocarbon.

Blumer (47,48,50-53) has gathered supporting evidence for the following scheme



Pristane and the monoenes (norphytenes) and dienes (phytadienes) are found in marine animals (copepod species, Basking shark, etc.). But of these compounds, only pristane is found in crude oils and sediments as a consequence of the reduction processes that take place within the geological environment.

Phytane can be formed from the diagenetic reduction of the phytadienes produced from phytol either by the action of organisms or the dehydration on clay minerals. This process according to Blumer (47) is most likely responsible for the absence of free phytol in recent sediments. The diagenetic reactions of the lower C_{15} homolog (farnesane) will not be considered because they would result in a range of isoprenoids outside of that studied here. In any case the processes should be very similar to those described for phytol. Polyene systems, such as the carotenoids and squalene, are very labile to oxidative cleavage, being the acids and ketones the main products of these processes. In turn those acids and ketones can, under geological conditions, give rise to isoprenoid hydrocarbons according to the following mechanisms:



I. Oxidative cleavage, giving either an acid or if next to a methyl branch a ketone. This can be followed by:

II. Decarboxylation (2). It has been calculated by Abelson (2) that a significant production of low molecular weight hydrocarbons from fatty acid decarboxylation would require at temperatures of about 100°C relatively short times; of the order of one million years. Thus decarboxylation either catalytic or bacterial of the isoprenoid acid intermediates could follow an oxidative stage.

III. Reduction of the functional group in one or more steps. Abelson (1) and Bergman (34) have endorsed the feasibility of reduction of carboxyl to methyl groups as a geochemical process.

It must be pointed out that III, although considered a likely process, involves a drastic change in the environment from a highly oxidizing stage to a highly reducing one since they can not coexist. Thus a separation of both processes either in time or place is necessary. All of these reactions can be applied to the postulated isoprenoid precursors in order to verify whether the observed distributions of these compounds in geochemical samples can also be explained in this more general way. This approach was first used by Bendoraitis et al. (32) who developed an hypothetical oxidative cleavage

using lycopene as a model. The scheme proposed by these authors was not found to be entirely general for in spite of accounting for most of the isoprenoids isolated in petroleum it still has certain limitations. Namely, it does not account for the presence of C_{16} and C_{17} isoprenoids. Squalene (32,281) was offered as an alternative to the formation of both of these isoprenoids. Both model schemes have been developed here to include reduction as well as decarboxylation of the acids. (Figure 108)

The resulting products indicate that either these two precursors are not the right choice or the mechanisms are not correct. While it is true that the combination of both diagenetic schemes accounts for the whole isoprenoid series between C_{14} and C_{22}, their predicted distributions do not conform to the pattern indicated in Table LXXVIII. In this sense it does not really matter, in the case of lycopene for instance, whether the probabilities of formation of each one of the degradation products are assigned on the basis of a faster reaction rate for reduction as opposed to decarboxylation or vice versa. Besides the extremely low concentration of the C_{17} isoprenoid does not appear to be adequately explained by the oxidative cleavage of squalene, even assuming a lower probability for reaction III than for II (see Figure 108) which does appear likely. Similar results are obtained when

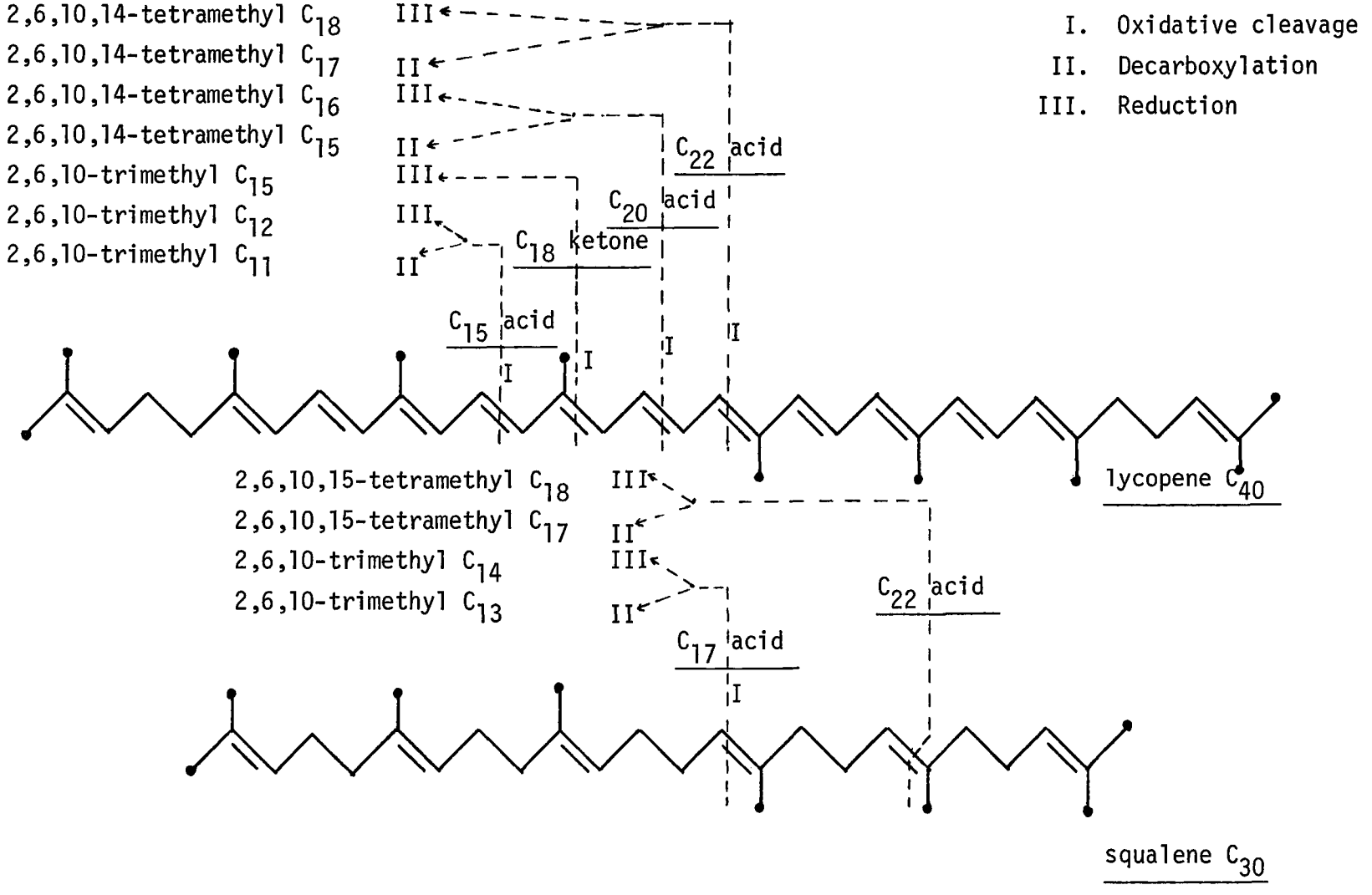
FIGURE 108

ISOPRENOID HYDROCARBONS FROM

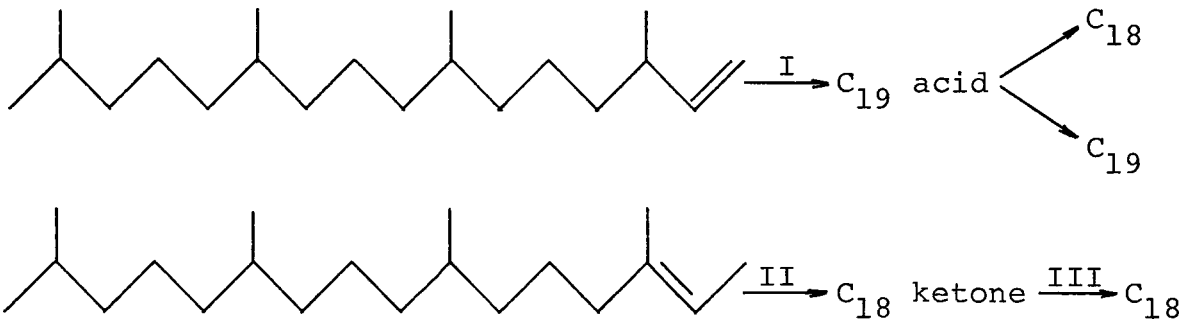
LYCOPENE AND SQUALENE

FIGURE 108

ISOPRENOID HYDROCARBONS FROM LYCOPENE AND SQUALENE



the oxidative cleavage hypothesis is applied to isoprenoid structures such as the phytenes and norphytenes. For instance, in the case of the phytenes



A possibility not considered before is that isomerization of the double bond would produce the whole range of isoprenoids up to C_{20} but even in this case the C_{17} would be entirely missing. Thus the participation of squalene is still necessary to explain the presence, if not the distribution of this isoprenoid. On the other hand the isolation of a C_{22} isoprenoid from graphite (see Chapter V) with the structure 2,6,10,14-tetramethyl octadecane appears to oppose the role of squalene as a precursor (see Figure 108). Another hypothesis which appears to explain these anomalies is based on the thermal cracking of the fully reduced isoprenoid precursor. Although the source of hydrogen is not well understood it has been suggested that hydrogenation reactions could be coupled with dehydrogenation reactions, such as those giving rise to aromatic compounds (asphaltenes, kerogen). The thermal

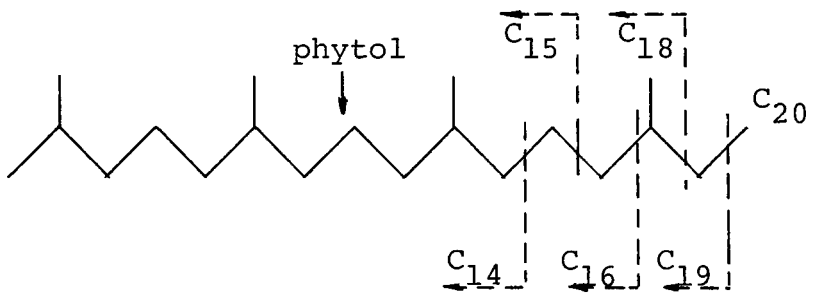
cracking mechanism has usually been disregarded because the temperatures involved in the process are usually much higher than those prevailing in geological environments (109,315). This process has been recently "rediscovered" once it was realized that given enough time, cracking reactions can take place even in relatively mild thermal environments (2). Welte (511) advocates this possibility taking as a basis the calculations of Abelson (2), who, considering these reactions as first order, applied the Arrhenius equation to calculate the time required for the cracking processes to produce low molecular weight hydrocarbons. From the calculated time / temperature curve it appears that cracking would take 100 million years (a still relatively short geological time) at 100°C to produce an amount of products similar to that produced in one million years by decarboxylation processes.

Also according to Degens and Matheja (122) thermal degradation or organic matter is the number one diagenetic factor.

Calvin and coworkers are also strong advocates of this theory (138,231,281) which recently has been experimentally supported by the work of Holman (211) and Bayliss (26). Holman and coworkers subjected phytane to pyrolisis for short periods of time and found among the products several monoolefins ranging from C_{15} to C_{19}. A significant observation

was the absence of the C_{17} olefin. The possible geothermal cleavage of the phytol components of chlorophylls, preserved in the organic kerogen of sediments, to isoprenoid hydrocarbons was studied by Bayliss (26). It was found that the thermal degradation (hydrogenolysis) of alfalfa and algal chlorophylls. and of oil shale kerogen and bitumen yielded significant amounts of pristane, phytane and farnesane. Trace amounts of C_{16} and C_{18} isoprenoids were also detected.

These findings tend to emphasize the role of phytol as a likely common precursor. For example, the thermal diagenetic pathway of phytol has been represented as follows (231)



It is clear that the thermal degradation of phytol requires the cleavage of two bonds, in order to produce the C_{17} isoprenoid, which is considered a low probability process. The phytenes and phytadienes that may be formed during compaction of sediments, besides the possibility of undergoing oxidative cleavage at the double bond sites can also be reduced and cracked to lower molecular weight isoprenoids. In this later case, the C_{17} isoprenoid could be formed but the low probability of the process is consistent with the

observed distributions.

Thermal cracking may also explain the possible participation of the diphityl phospholipids discovered by Kates (240) in salt bacteria, as a source of sedimentary isoprenoids. The diagenetic scheme prevailing in this case will be similar to those indicated above for phytol.

However, the presence of the regular C_{21} and C_{22} isoprenoids (2,6,10,14 tetramethyl C_{17} and C_{18}) requires an isoprenoid precursor higher than phytol. It has been indicated in Figure 108 how they could arise from the degradation of lycopene but not from squalene. Several other biological precursors have been proposed (281). Among them the isopropenyl alcohols, undecaiso-propenol-1 and dodecaisopropenol-2 isolated by Fukawa et al. (168) from silkworm feces, a series of C_{30}-C_{45} terpenols from birch wood (272), the C_{55} isoprenoid alcohol from Lactobacilli (281), the C_{45} isoprenoid alcohol (solanesol) isolated from silkworm feces (168) and from flue-cured tobacco (410). Also the long side chains of ubiquinone, Vitamin K_1 and K_2 and chlorobium chlorophylls could be precursors of the higher isoprenoids.

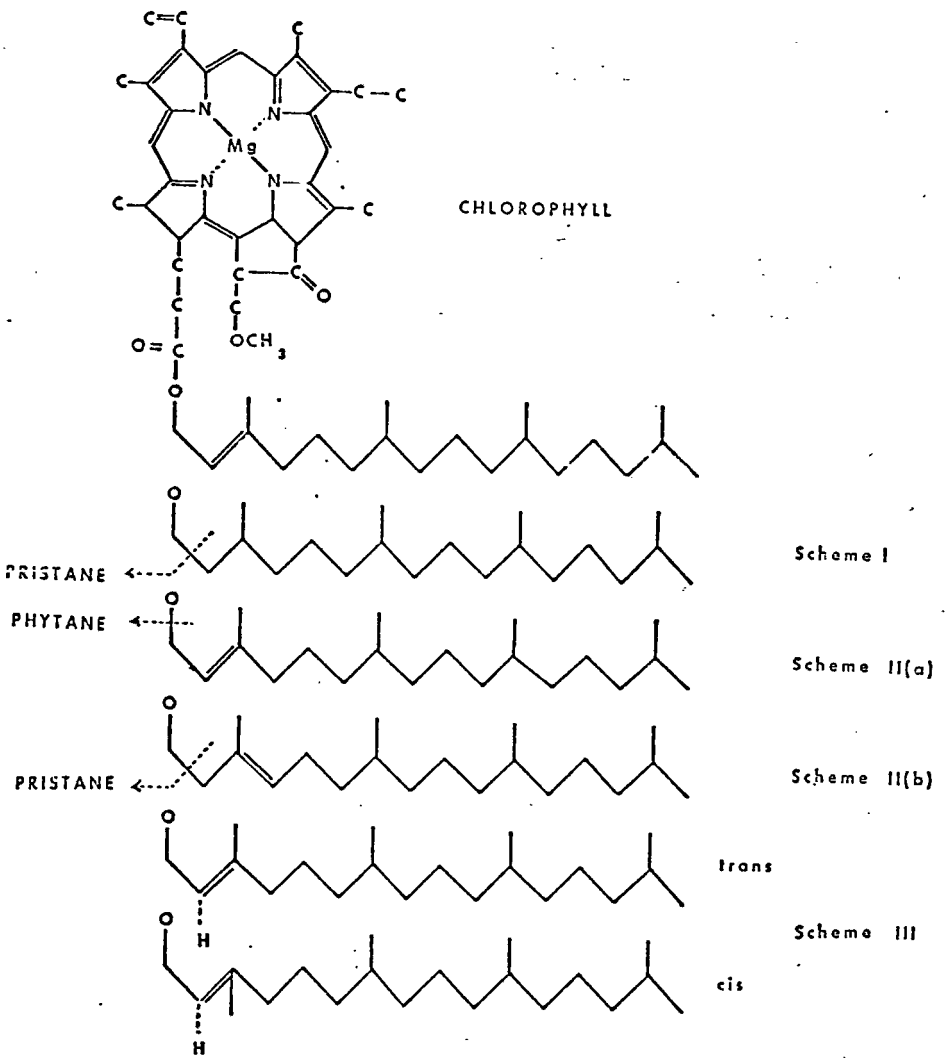
A feature that it is somewhat hard to understand by any of the mechanisms discussed here is the relatively high concentration of pristane (31,32,231,281) and occasionally

phytane (402) in geological samples and petroleum. This has been explained by Blumer (51) by considering that they actually are, for the most part, post depositional products mostly of marine origin. The rather high content of pristane in marine organisms has already been discussed (see Chapter II and Chapter V, Basking Shark liver oil). Thus the possibility exists that pristane and phytane may enter the sediments or other terrestrial samples directly.

Recently Bayliss (26) in his studies on the thermal degradation of chlorophyll came across some interesting and quite pertinent observations. Briefly, the hydrogenolysis products of alfalfa chlorophyll and Green River oil-shale kerogen showed a predominance of pristane over phytane while the reverse was true in the case of the algal chlorophyll and the Green River oil shale bitumen. Such differences in the thermal degradation of chlorophylls have been explained by the author by assuming that differences in the molecular structure of the phytyl component would influence the point of preferential cleavage. This would involve the position of the double bond and also the stereochemistry of the molecule. He proposes the schemes shown in Figure 109 to explain the results. Scheme I which involves the cleavage of a non olefinic hydrocarbon chain has been proposed to account for the predominance of pristane in the hydrogenolysis product

FIGURE 109

ISOPRENOID HYDROCARBONS FROM CHLOROPHYLL



Bayliss (1968)

of oil-shale kerogen where the apparent absence of olefinic compounds indicates that it may have been hydrogenated under geological conditions. The results for the bitumen and chlorophylls are explained by schemes II(a) and II(b). The possible isomerization processes postulated above in relation to the thermal cracking of phytenes is also reflected here. To summarize it has to be concluded that after a careful appraisal of all the information gathered at present on this problem, it seems certain that there is neither a unique precursor nor a single diagenetic scheme but rather that the interplay of various likely diagenetic processes on several possible precursors is the most realistic approach. Among these possible precursors phytol seems to play a major role and among all the degradation processes considered the geothermal maduration of the initially deposited precursors seems to fit best the actual isoprenoid distributions found in sediments and petroleum products. It must also be realized that because of the experimental limitations and the small amounts in which they are found in nature not all of the isoprenoid structures have yet been fully characterized. An example is the recent identification of the C_{17} isoprenoid (281,357) which previously had been considered to be absent, and the recent identification in this laboratory of a C_{22} isoprenoid in graphite. It is expected that further

refinements in the analytical techniques and instrumental sensitivities will help to complete the sedimentary isoprenoid homology in the very near future. In conclusion, the presence and distributions of these isoprenoid hydrocarbons in Precambrian sediments and the possibility of relating them to precursor substances not different from those common to the biosynthetic processes of living organisms constitutes evidence of the early appearance of photosynthetic organisms on earth.

B. Petrogenesis

Although the problem of the origin of petroleum has not been approached here in a direct way, the overall results of the studies on the distribution of hydrocarbons in natural products may provide an indirect answer to this problem.

The biogenic origin of petroleum appears to be a rather well established fact based on the following observations: i) the presence of porphyrins (487) in all mineral hydrocarbon oils, ii) the optical activity of these oils (295), and iii) the predominance of polycyclic hydrocarbons which may be derived from naturally occurring steroids and triterpenoids (207,316). The presence of isoprenoid hydrocarbons has also been taken as a sign of biological origin but because of the possible dual origin of petroleum proposed by Robinson (400) and in lieu of a convincing criterium of biogenicity for these compounds the evidence was regarded as somewhat inconclusive.

In fact Robinson has presented rather strong arguments in favor of the partial contributions of abiogenic materials to a biological admixture. According to his observations the composition of petroleum crudes, especially the more ancient appears to be consistent in part with a hydroformed mixture of n-alkanes and some of its constituents, such as neopentane and adamantane are cited as examples of typical abiological hydrocarbons. It also may be added here that in support of this theory, it has been mentioned that laboratory experiments seem to indicate that the aromatics and even perhaps the asphaltenes could be derived from catalytic synthesis of the Fischer-Tropsch type (167). Furthermore while the biogenetic theory appears to be fully substantiated by the observations stated above it has also been pointed out that these "biological" indications fade with time and that they are not particularly strong in the most ancient "crude oils" (400). This can possibly be explained by taking into consideration the more extensive role of diagenesis with time. In other words the more ancient crude oils may have lost a greater part of their primary biological marks because they have been subjected to changes in their geological environment for longer periods of time. A pertinent observation is the higher content of light paraffins in the older crudes (38) and also in older sediments (134). Certainly the application of the distributional

criterium of biogenicity just developed to the isoprenoid hydrocarbons found in petroleum crudes strengthens considerable the foundations of the biogenic theory. A detailed account of the reasons why an isoprenoid distribution such as that presented in Figure 53 can not have been produced abiotically has been given in the preceeding section. Furthermore after the comparison of this pattern to the reported distributions and concentrations of isoprenoids in petroleum (31,32,112,143, 231,295,297,348), it becomes apparent that this distribution (Figure 53) is general in petroleum products. While this evidence does not exclude or invalidate the dual theory proposed by Robinson (400) it does provide a stronger support to the biological origin of petroleum. What it is still not possible, is to determine the extent of the contribution of the abiotic products, if any. Nevertheless the bulk of the hydrocarbons appears to be biological in nature.

Having established their biological origin, at least in part, attention will be given now to the actual source of the n-paraffin hydrocarbons in petroleum. Among the most likely precursors (210) the fatty acids (63,71,72,107,255) have been given much support because i) the even carbon numbered fatty acids are ubiquitous in nature being widely distributed in both plants and animals and ii) they are structurally similar to most of the paraffin hydrocarbons of petroleum. The relationship between the fatty acids in living systems

and the hydrocarbons in petroleum however, is not readily apparent. While the paraffins in biological systems are mainly odd numbered (93,130) in accordance to the proposed mechanisms of formation from the corresponding even-carbon numbered fatty acids (63,92,108,232,233), such a predominance is absent in crude oils. This lack of correlation has cast doubts on the role of fatty acids in the formation of petroleum hydrocarbons although mechanisms have been proposed to account for it (18,108,402).

On the other hand Stevens et al. (455,456) observed the predominance of nC_{29} and nC_{31} alkanes in a recent sediment. Other authors have also reported on the predominance of odd carbon number paraffins in recent sediments (60,61,112,253, 254,263,421). With time however the distribution tends toward the equalization of carbon numbers (108,255,402) and so the biological character of the n-alkane distributions is lost. It is precisely this gradual disappearance of the typical biological preponderance of odd numbered alkanes which requires the adoption of other criteria to be able to establish whether the hydrocarbons in ancient sediments and petroleum are biological or non biological. A similar process appears to take place with the normal fatty acids. That is in ancient sediments and crude oils the ratio of odd to even carbon acids increases (206,254). It may be indicated at this point that although decarboxylation of fatty acids appears to be the most

likely mode of formation of n-paraffins within the geological environment, this process does not appear to be so general in living systems. Most of the work presented here, on the analysis of biological products, gives evidence of the apparent lack of correlation between hydrocarbons and fatty acids. See for example the analyses of bacteria, algae, plants and other products of biological origin in Chapter V. The work done by Dr. Tornabene on the hydrocarbon and fatty acid distributions in microorganisms (480) also indicates a general lack of relation between both distributions. Considering now the hydrocarbon distributions of certain common photosynthetic microscopic algae and their immediate relation to the hydrocarbon distribution of recent sediments the possibility arises that algal populations may constitute an important contribution of primary hydrocarbons. For example it has already been discussed (see B. braunii, Chapter V) how the distribution of a contemporary golden-brown algae (171) bears almost a one to one correlation with the paraffin distribution reported for the Green River shale (a relatively recent sediment) (231). A similar situation also has been found to exist between these algal hydrocarbons and the hydrocarbon distributions of two more recent Ventura Basin oil shales (Saticoy and Ventura) (383).

Another interesting and more general observation of a possible taxonomic value is the ubiquity of the C_{17} alkane in

most forms of algae (99,171,192,371) which can be considered directly associated to the usual predominance of this odd numbered alkane in recent sediments (59,153,254,282,402).

Thus algae could directly contribute in a significant amount to the alkane content of sediments without being necessary to invoke the participation of diagenetic changes of fatty acids to explain the distributions observed. Likewise the observed changes in the concentration of the C_{17} alkane with the depth of burial within the Green River oil-shale (402) could be explained by seasonal changes in the algal population of the lake that produced the Green River Formation.

A predominance of the nC_{17} alkane is also found in the Nigerian and Libyan crudes analyzed by Brunnock (75). In the same way, their possible contributions to petroleum should be considered and, in relation to this discussion, the work of Martin, Winters and Williams (305) deserves special attention. In their studies of the distributions of n-paraffins in crude oils they found two "atypical" cenozoic crudes (Uinta Basin, eocene, and State Line, paleocene) that contained high molecular weight paraffins in the range C_{20} to C_{30} with odd carbon numbered predominances. These distributions would be comparable to that of the Green River oil shale which incidentally belongs to the same epoch (Lower Eocene). Thus this particular distribution of petroleum hydrocarbons could also be explained in terms of a flourishing algal population

during the early Cenozoic.

In this way also, the hydrocarbons of petroleum may be in some instances the direct products of living organisms.

The participation of algal populations as a primary source of sediment and petroleum hydrocarbons is certainly an attractive possibility. Their importance stems from the fact that being relatively simple organisms, it could be assumed that they may very well be the contemporary representatives of earlier forms of life and in fact organic remains with the morphology of algae have been discovered in Precambrian sediments (23). Thus the hydrocarbons in Precambrian sediments could be derived from the algae. Time, a reductive environment and geothermal maturation would tend to yield an equalized distribution of saturated n-paraffins from an original distribution such as those in Table XXV. The isoprenoid hydrocarbons would also be explained from the thermal degradation of the phytol from the algal chlorophylls. A good correlation in time can be established between the presumed original distribution (that of B. braunii, Table XXV, for example), the distribution of alkanes observed in the Green River oil shale (60×10^6 yrs.), and those typical of older sediments (365,369). A comparison of the n-hydrocarbons of B. braunii with those in the Green River shows that, in the relatively short geological time span of 60 million years, only reduction has taken place (the hydrocarbons in the Shale show an identical distribution to

that of the algal hydrocarbons, the only difference being that they are all saturated). The isoprenoids in Green River show a definite predominance of phytane and smaller amounts of pristane, C_{16} and C_{18} isoprenoids, which could be reasoned in terms of scheme IIa (Figure 109). Their presence in this eocene shale also indicates that a maximum of 60×10^6 yrs. is necessary to release them from the phytyl group of the chlorophyll. On the other hand it has been suggested (192) that the isoprenoids are absent from a Post Pleistocene Mud Lake which is a Recent Analog of the Green River oil shale because the phytyl group has not been released yet from the chlorophyll. Now considering the observed smooth distributions of hydrocarbons in the Precambrian Sediments the following rationalizations can be made: i) the increase in time of $\simeq 2.0 \times 10^9$ years would allow the development of thermal cracking processes which would end up equalizing (402) the initial bimodal distribution of odd numbered paraffins and ii) the amounts of the lower isoprenoids would increase at the expense of phytane (402). Similar reasoning would also account for the nature and distributions of hydrocarbons in petroleum. It must be remembered that this is only a seemingly plausible speculation designed to emphasize the role of algae in the organic geochemical approach to petrogenesis and that other contributing sources can not be discarded a priori.

Before leaving this subject another important fact needs to be considered. That is the ubiquitous distribution of hydrocarbons on the earth. (See Chapter II) Attenuation to this fact has been called upon before by other authors (173, 210) and it has been definitively vindicated by the results obtained in connection with the present work, as well as with other work carried out in our laboratory in general (348,350, 352,357,363,364,365,366,367,369,371,480). It is the consideration of this world wide distribution of hydrocarbons that prompted Hodgson and Hitchon to point out that "it may not really matter what is their actual source", to answer the question on the origin of petroleum. According to these authors, once it is realized that hydrocarbons are present in large, small or even trace amounts in almost every conceivable product on earth, the vital processes in the genesis of oil fields lie in the mobilization and accumulation of these hydrocarbons. Thus a new perspective is given to the origin of petroleum.

C. Environmental contributions to the observed hydrocarbon distributions in terrestrial and extraterrestrial organic matter

Because of the ubiquity of hydrocarbons on earth, serious consideration needs to be given to the very real possibility that some of the hydrocarbon distributions observed may have been influenced to a certain degree by their environment.

It has already been discussed how contamination in the laboratory can be efficiently prevented and so there is no need to consider it anymore. However, the kind and degree of contamination that a sample has suffered in its contact with its surroundings can not be assessed with certainity, but only estimated.

Again it must be realized that because of the widespread distribution of hydrocarbon compounds their actual sources may not be as important as the mechanisms whereby these compounds can be incorporated into terrestrial or extra-terrestrial products such as the meteorites, some which are known to have had uncertain histories after their arrival on the earth. In this respect the same processes that play a role in the formation of oil fields have to be regarded as the major causes of the presence of non indigenous hydrocarbons in the samples analyzed. It is known that water is present everywhere and recently the misconceptions about the solubility of hydrocarbons in water systems have been corrected by the work of McAuliffe (280), Baker (16,17,18), Franks (164), Tobolsky (479) and Peake and Hodgson (377,378), who showed that hydrocarbons can be accommodated in water by a variety of mechanisms.

The results obtained by Peake and Hodgson will be briefly discussed here because of their significant implications to the study of hydrocarbon distributions. When their initial

observations on the accommodation of single and binary alkane systems in water, were extended to fused n-alkane mixtures, results such as those shown on Figures 110,111 were obtained. In spite that the presence of liquid hydrocarbons was found to increase the accommodation of the solid alkanes in binary systems well above their normal values in single systems, this enhancement effect is not enough in multicomponent mixtures such as the one shown on Figure 110, to prevent the systematic decrease in the accommodation of the alkanes higher than C_{20}. On the other hand, the disaccommodation effect is preferentially felt in the lower molecular weight alkanes. Note the decrease in the concentration of the C_{12} upon standing (Figure 111). The net effect being that of a surprising preferential accommodation of alkanes in the C_{16}-C_{20} range. This water may be considered as a major contaminating agent especially when dealing with porous or adsorbent materials.

It has been calculated that assuming a content of 100 ppb. of hydrocarbons in the present oceans of the world, this would amount to 33 cubic miles of hydrocarbons at this time, or approximately one fifth of the known oil reserves of the world which have been generated and collected over geologic time (Weeks (508), cited in reference 210).

Air is also another major factor to be considered in any studies of our environment. As mentioned before we are just becoming increasingly aware of the emissions into the air of

FIGURE 110

GAS CHROMATOGRAM OF ALKANES ACCOMMODATED IN DISTILLED WATER

Peake, E. and Hodgson, G. W. (377)

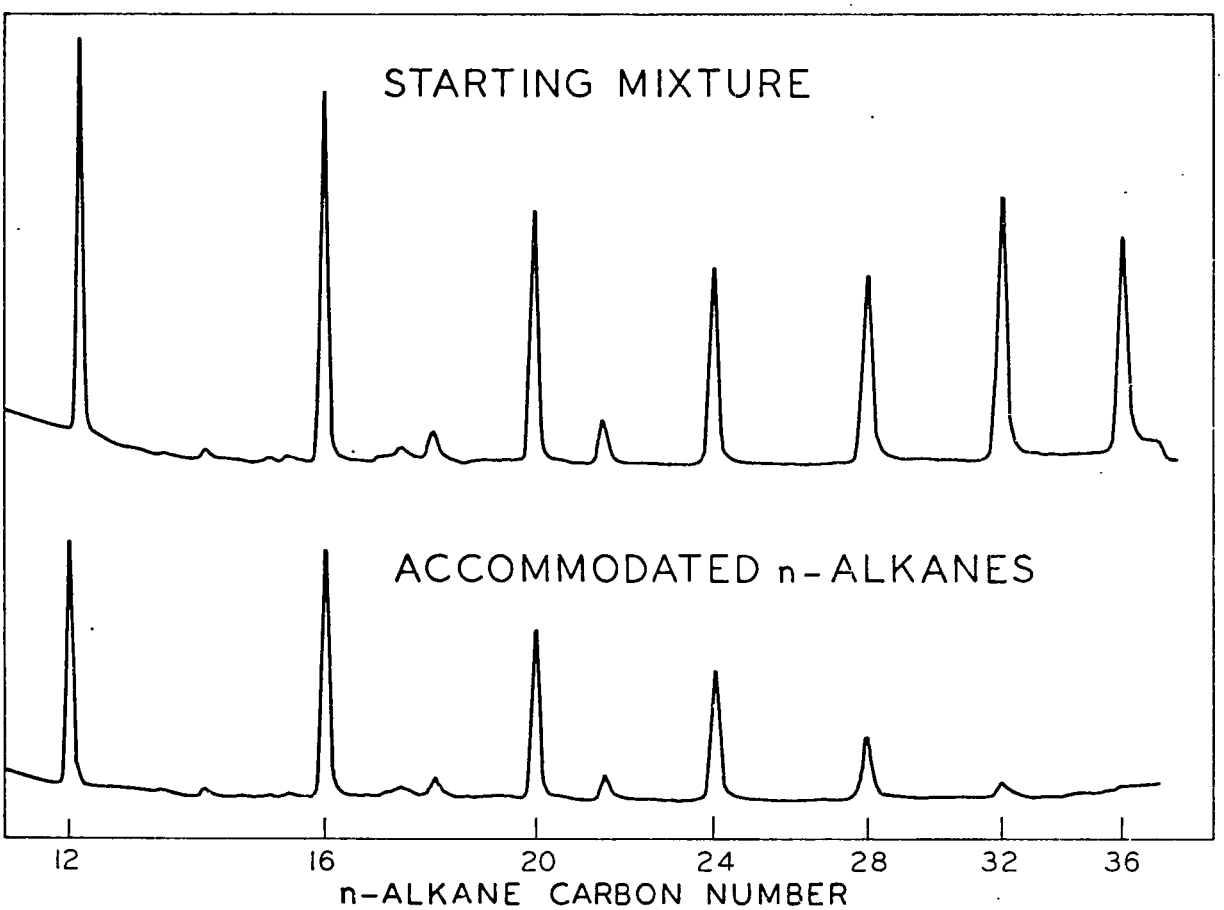
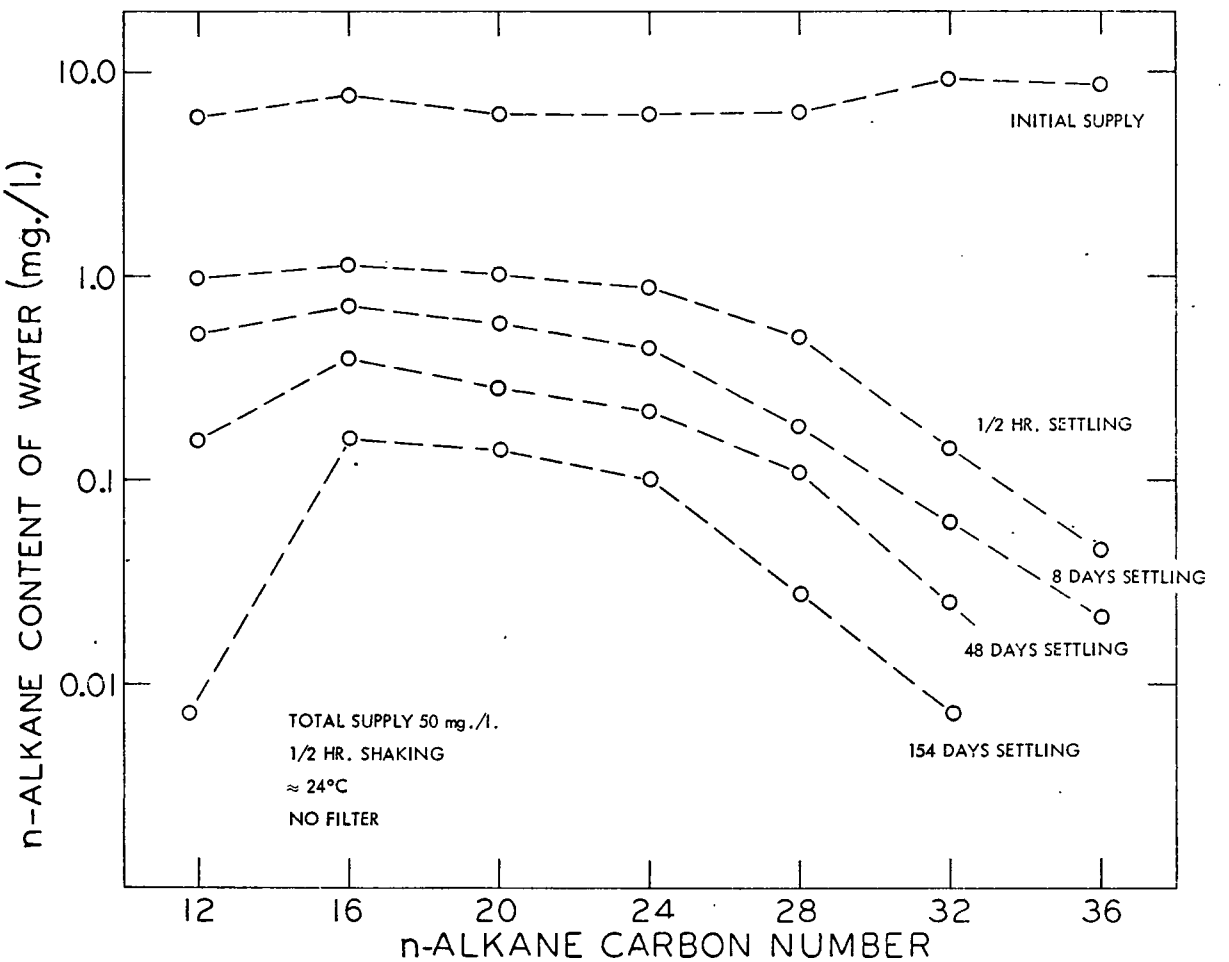

STARTING MIXTURE
ACCOMMODATED n-ALKANES
12
16
20
24
28
32
36
n-ALKANE CARBON NUMBER

FIGURE 111

DISTRIBUTION PATTERN AFTER ACCOMMODATION AND SETTLING

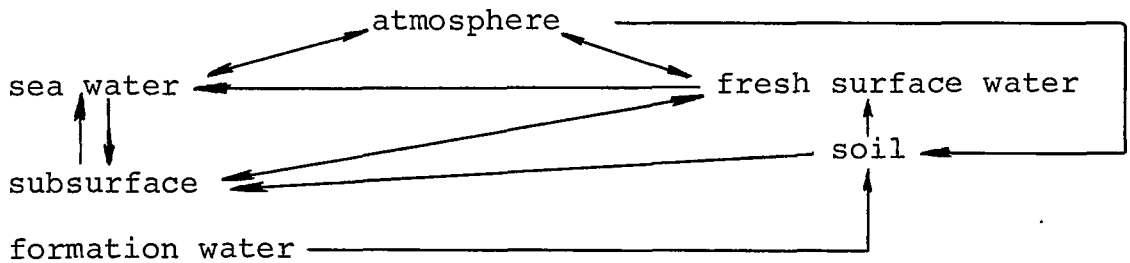
Peake, E. and Hodgson, G. W. (377)


10.0
1.0
0.1
0.01
n-ALKANE CONTENT OF WATER (mg./l.)
INITIAL SUPPLY
1/2 HR. SETTLING
8 DAYS SETTLING
48 DAYS SETTLING
154 DAYS SETTLING
TOTAL SUPPLY 50 mg./l.
1/2 HR. SHAKING
≈ 24°C
NO FILTER
12
16
20
24
28
32
36
n-ALKANE CARBON NUMBER

effluents from domestic heating, industrial plants, oil refineries and automobiles. These effluents have been shown to contain great quantities of unburned hydrocarbons, which undergo photochemical reactions that are believed to be related to the production of "smog". Although this effects of air and water pollution tend to be concentrated in heavily populated areas, pollution in general needs not to be confined to these areas as is best illustrated by the discovery of organochlorine pesticides in Antartica (473) and in seals and porpoises from different localities (209). The suggestion of a world wide pattern of pesticide distribution (473) can certainly be made extensive to the hydrocarbons, which because of their ubiquity will be highly dispersed throughout our modern environment.

Another ubiquitous carrier of hydrocarbons studied here is dust, which has been found to contain medium and high molecular weight hydrocarbons(Figures 55,56,57) in substantial amounts. The distribution patterns especially that of Figure 57 are similar to patterns found in different products.

The interrelationships between the hydrocarbons of these elements, water, air, oil deposits and soils, led to the concept of a major hydrocarbon cycle (210) identical to the water cycle



The hydrocarbons taken up by the water cycle are transported all over the entire cycle and can be released at any point, and so it would be hard to find an environment completely free of these compounds.

D. The Origin of the Organic Compounds in Meteorites

This problem can be approached in a more realistic way now that the exact distribution and nature of most of the aliphatic hydrocarbons found in these samples are known. The distribution of the hydrocarbon patterns in both carbonaceous chondrites and graphite nodules certainly suggests a common origin, which is also supported by the similar nature of the alkane components in these type of samples. Compare Tables XLIX and LXXI.

It has been suggested especially in the case of the iron meteorites that these organic compounds could have been formed by reaction of trapped gases after the high temperature phase had passed (519). Evidence of metamorphism was obtained by Wood (519) from his studies of the nickel distribution patterns in metal grains of nine carbonaceous chondrites. It was determined that the meteorite body had been subjected to temperatures of the order of 1400-1535^{o}C before the accretion of the chondritic material, and that cooling from this temperature was very rapid.

On this basis, such an assumption could even explain the

organic compounds found in the graphitic nodules. An attractive possibility of reaction in the gas phase would be the Fischer-Tropsch process (355). (See Chapter VI). At moderate pressures (10^{-1} atm) and temperatures above 800°C, CO is predominant in a cosmic gas (276,519). Thus during the high temperature stage all carbon compounds would be present in the form of CO. When the temperature decreases, graphite becomes stable but the high activation energy required for its formation leads to the appearance of metastable products (118,464) which will eventually end up as graphite if the temperature interval from 800 to 500°K is not traversed rapidly. But the rapid cooling experienced by the carbonaceous chondrites, according to the data of Wood (519) would favor the persistance of the initial distribution of organic compounds.

That the Fischer-Tropsch reaction is an effective means of hydrocarbon synthesis under primitive conditions has been rather well demonstrated by the data presented here in Chapter VI and independently by Studier et al. (464,467). It thus can be argued that by allowing CO and H_2 to be among the trapped gases during the cooling stage, the presence of the nickel-iron surfaces would provide an ideal system for such a reaction to proceed. Typical results of this type of process using a meteoritic iron catalyst have been shown (Figures 67,69-71,73 and 74).

In any case it still remains to be explained how this high temperature crop of hydrocarbons could survive (259). Larimer and Anders propose as an explanation that lightning discharges may heat the gas over 800°C reforming CO by reaction of the CH_4 with water. This CO would start a second generation of Fischer-Tropsch hydrocarbons. But this, at present, is not more than an unproven conjecture.

On the other hand since the time required for the formation of the hydrocarbon chains is of the order of a few seconds, it would be possible, at least on a time scale basis, that the hydrocarbon patterns could have been formed upon the entry on the earth atmosphere. However on this respect it has been shown that the effects of atmospheric heating extend to a depth of only 10-15 mm (218). Also the energy of impact can not be a significant source of heat unless the meteoritic body has retained part of its cosmic velocity and for that it must have a preatmospheric mass of more than one ton and must traverse the atmosphere without fragmenting.

Having considered the existing evidence surrounding the formation of an abiotic mixture of organic compounds in meteorites, the next step would be to consider the evidence for the biological origin of such compounds, and then evaluate both possibilities.

Evidence of the biological origin of these hydrocarbons (Tables XLIX and LXXI) will be obtained through the distributional criterium proposed earlier for the isoprenoid hydrocarbons. The already existing tentative mass spectrometric evidence of the presence of the C_{18}, C_{19} and C_{20} isoprenoids in the Mokoia and Murray meteorites (350,364,368) has been unequivocally confirmed by the data presented here (Figures 81,82,84,85,and 86) (357). Additional evidence of their presence has also been obtained for the meteorites Essebi (Figures 81,82), Orgueil (Figures 81,82), Vigarano (Figures 81,82,84) and Grosnaja (Table LVI). This together with the previous report from this laboratory (350) on the identification of isoprenoids in Boriskino, Santa Cruz y Mighei, extends the count of carbonaceous chondrites containing pristane and phytane to nine. Furthermore, gas chromatographic and mass spectrometric evidence has also been obtained for other isoprenoids such as the C_{15} (farnesane), C_{16}, C_{17}, C_{18}, C_{19} (trimethyl substituted) and C_{21} (Figures 84-86). Likewise the C_{16}, C_{17}, C_{18}, C_{19}, C_{20} and C_{21} isoprenoids have also been identified mass spectrometrically in several samples of graphitic nodules or iron meteorites (Table LXXI).

These identifications have extended the known isoprenoid homology into a more favorable range for comparative studies between meteoritic, fossil and synthetically produced hydrocarbons (Figure 112). The application of the criterium

FIGURE 112

ALKANE AND ISOPRENOID DISTRIBUTIONS

Typical normal alkane and isoprenoid distribution observed in sediments, meteorites, petroleum crudes and Fischer-Tropsch products. Isoprenoids are shown in black. The position of the components in the graph corresponds approximately to their relative retention times on gas chromatographic columns coated with Polysev.

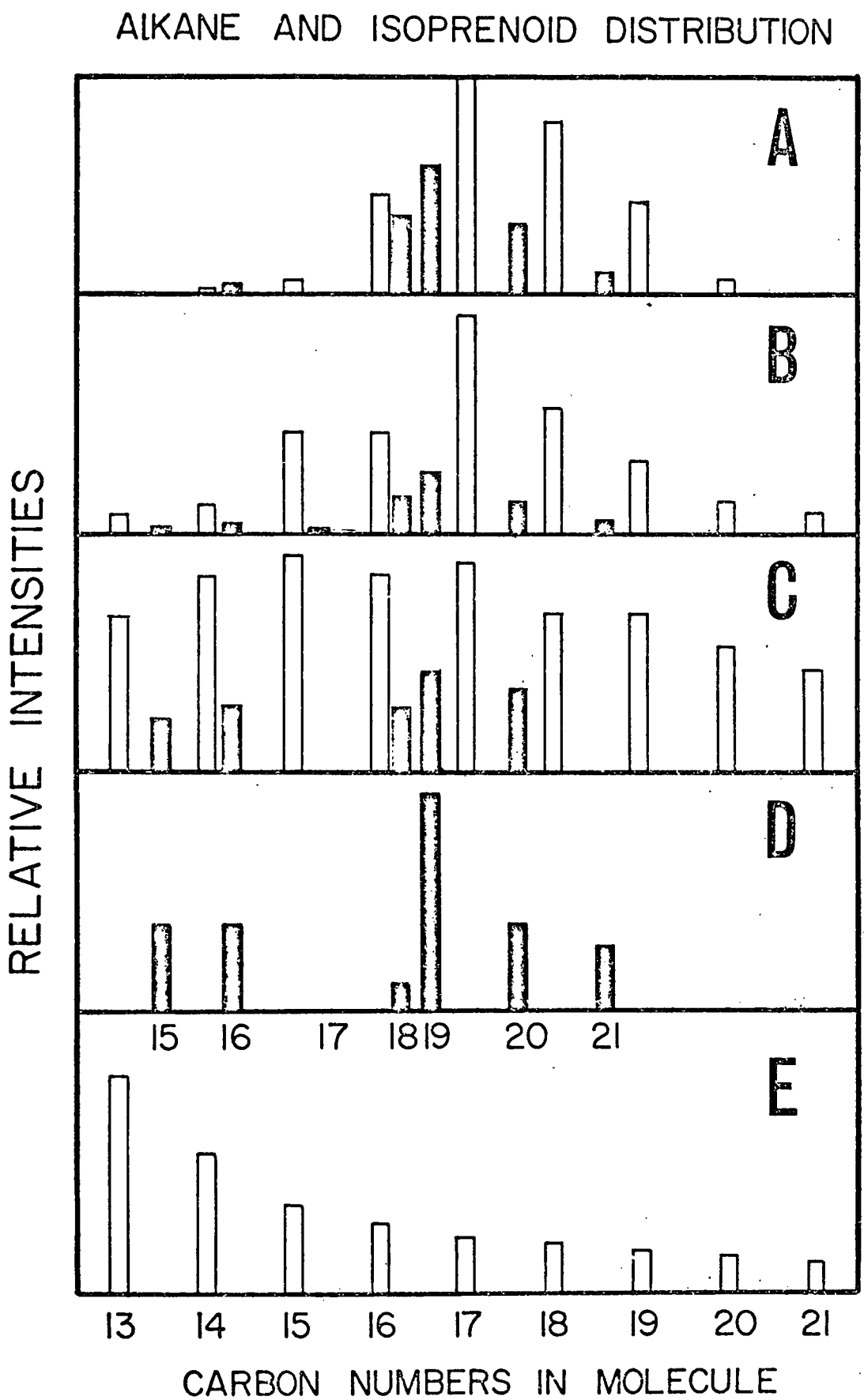
(A) Soudan shale hydrocarbons (231)

(B) Vigarano meteorite hydrocarbons (350)

(C) West Texas petroleum crude sampled at Houston Refinery Shell Oil Company, Inc. (348)

(D) Isoprenoids in petroleum (32)

(E) Fischer-Tropsch hydrocarbons (see Chapter VI)


ALKANE AND ISOPRENOID DISTRIBUTION
A
B
C
D
E
RELATIVE INTENSITIES
15 16 17 18 19 20 21
13 14 15 16 17 18 19 20 21
CARBON NUMBERS IN MOLECULE

developed at the beginning of this discussion clearly demonstrates that the order and selectivity exhibited by the distribution of isoprenoids in meteorites is certainly identical to that which is characteristic of terrestrial products (see Table LXXVIII and Figure 112) and according to the ideas stated before can not be the result of a primary abiogenic distribution of isoprenoid precursors. The reason for referring to the distribution (singular) rather than the distributions of isoprenoids in meteorites becomes clear after inspection of Tables XLIX and LIII and Figures 75-80.

A comparative study of the unique isoprenoid distribution in sediments (A, Figure 112) and in petroleum crude (C and D, Figure 112) with that of the Vigarano meteorite (B, Figure 112), which can be considered representative of most carbonaceous chondrites, strongly suggests a biogenic origin for the hydrocarbons in meteorites. Supporting evidence can be found in i) the presence of the homologous series of methyl branched alkanes, among which the iso and anteiso compounds predominate, ii) the two cycloalkane homologies (cyclopentyl and cyclohexyl)(Figure 113), and iii) in the predominance of normal alkanes with odd numbers of carbon atoms (C_{17} and C_{15}). It should be pointed out here that the predominance of the C_{17} and C_{15} alkanes has also been observed in the Orgueil and in other analyses of carbonaceous chondrites and nodules of iron meteorites.

The iso and anteiso-alkanes are common to Precambrian sediments petroleum and contemporary plants and animals (see Chapter V and Figure 113). They may be derived from the corresponding branched chain fatty acids.

Although reports on the occurrence of cyclic n-alkanes are scarce, they appear to be present in contemporary plant sources, petroleum and paraffin wax (Chapter V). It has been suggested (231) that this homology is derived from the unsaturated fatty acids which become saturated by intramolecular cyclization.

Part of the data available on the occurrence of hydrocarbons in terrestrial and extraterrestrial products is summarized in Figure 113.

Aside from the above correlations, it still may seem more realistic to assume an abiological origin for the hydrocarbons in extraterrestrial samples.

Nevertheless the results presented here, besides demonstrating for the first time in a rather convincing manner the biological origin of these hydrocarbons, show that the nature and distribution of such compounds resembles very closely that of any terrestrial biological product. From this, it is only natural to assume that any kind of extraterrestrial life processes giving rise to such a distribution of bioproducts should be very similar in their biosynthetic mechanisms to terrestrial type biochemistry.

FIGURE 113

OCCURRENCE OF HYDROCARBONS IN THE C_{10}-C_{25} RANGE IN TERRESTRIAL AND EXTRATERRESTRIAL SAMPLES

FIGURE 113

OCCURRENCE OF HYDROCARBONS IN THE C_{10}-C_{25} RANGE

IN TERRESTRIAL AND EXTRATERRESTRIAL SAMPLES

	Crudes	Graphite	Fischer-Tropsch	Carb. Cond.[1]	Nodules
n-Hydrocarbons					
alkanes	*	*	*	*	*
alkenes	-	-	*	-	-
Methyl Hydrocarbons					
iso	*	*	*	*	*
anteiso	*	*	*	*	*
others	*	*	*	*	*
Isoprenoids	*	*	-	*	*
Cycloalkanes					
cyclopentyl	-	-	-	*	*
cyclohexyl	*	*	-	*	*

* Detected by gas chromatographic-mass spectrometric methods.

[1] Carbonaceous Chondrites

In this case it has been pointed out that this kind of terrestrial life can only exist in an aqueous environment (496). However all the evidence gathered from rocket photography of the Moon and Mars, the absence of sedimentary material (496) in meteorites and other cosmological indicators, indicates that the lack of free water has kept the conditions on the meteorite parent bodies below the "minimum" for the evolution of organisms. For these reasons abiotic processes, like the Fischer-Tropsch synthesis, which requires starting materials readily available in the solar nebula appear to offer attractive possibilities to explain the origin of the organic compounds in meteorites (357). On the other hand the data obtained so far do not seem to support the possibility of abiotic synthesis. A condensation of the typical results obtained with the single pass of CO, D_2 mixtures over a Ni-Fe catalyst (industrial or meteoritic) at 300^{o}C is given in Figure 112E. There is a proportional decrease of the aliphatic hydrocarbons (mainly normals) as their molecular weight increases. There are no maxima at odd carbon numbered alkanes; on the contrary, in a few cases maxima at even carbon numbered alkanes have been observed. No mass spectrometric evidence for any isoprenoid hydrocarbon has been obtained so far, even though small peaks at the corresponding places for pristane and phytane appear at the gas chromatograms. These peaks have been unequivocally identified as the corresponding

iso and anteiso alkanes. However the identification of the homologous series of the iso and anteiso alkanes and other methyl branched alkanes (Figures 66-74 and Table XLIV) among the Fischer-Tropsch products casts some doubts on the validity of these compounds as biogenic indicators. Their simple structures can easily be understood in terms of random non biological syntheses. On the other hand the complete failure to produce any isoprenoid structure of the kind found in meteorites (C_{14}-C_{21}) does not support the idea that this process could have been involved in the formation of meteoritic hydrocarbons. It is also questionable whether variations of this process may produce the desired result. One may argue that phytol could be produced first by Fischer-Tropsch catalysis and that isoprenoid hydrocarbons were derived later from this compound by diagenetic processes. However, the limitations of this type of synthesis become obvious when one considers that i) a random synthetic process based on a mechanism of stepwise growth of the hydrocarbon chains can not selectively produce only one precursor compound but its whole homologous series, ii) even if this were so, diagenetic changes of this series could not give rise to the observed distribution in natural products (Figure 112 A,B,C,D and Table LXXVIII) and iii) both the carbonaceous chondrites and the nodules from iron meteorites contain substantial amounts of sulfur or sulfides which are known to be very

effective poisons of this catalytic process. In fact if the Fischer-Tropsch synthesis is performed in the presence of sulfur, no aliphatic hydrocarbons of any kind are formed (Figure 67, bottom).

It may eventually be possible to obtain by abiotic synthetic processes distributions of normal, and particularly isoprenoid and isomeric alkanes which correlate well with those in carbonaceous chondrites and nodules from iron meteorites. However, at the present time all the evidence points to a biological origin.

Once this is accepted the most important question is-- What is the source or procedence of these hydrocarbons? Is it extraterrestrial? A partial answer to these questions has already been advanced when the evidence of the absence of free water in parent meteorite bodies was discussed above. However the real and definitive clue to this problem can be found in the analysis of the graphitic nodules of iron meteorites. As can be seen in Table LXXI and LXXVII the hydrocarbon distributions of the graphitic nodules are extremely similar to those of carbonaceous chondrites, the only major difference being the smaller overall content of these hydrocarbons in the nodules. Like for the carbonaceous chondrites, it can be stated that in accordance with all the criteria exposed above, the hydrocarbons found in the graphitic nodules are also products biological origin. However it would certainly be

hard to imagine any kind of biological activity within these nodules.

Their procedence is pinpointed by the fact that the internal portions of the exposed nodules contain much smaller amounts of hydrocarbons than their external portions, which indicates that the alkanes have been acquired from the outside. Such a concentration gradient (Figure 105) is just the reverse of what would be expected from a synthetic process of the Fischer-Tropsch type. It must be taken into consideration that once the embedded nodules are cut in half the exposed surfaces represent the center portion of these nodules, and that this is the part which is more removed from the inter-phase between the graphite and the iron, where the synthesis would be most active. Thus once a nodule is exposed to the environment it picks up minute amounts of hydrocarbons which then diffuse towards the inner parts, establishing the observed gradient of concentrations. This is dramatically confirmed by the almost total absence of these hydrocarbons from the inside of nodules which were still embedded in the iron meteorite and therefore protected from the terrestrial environment. Likewise this view is also supported by similar observations made on the practical absence of these hydrocarbons from the internal parts of certain samples of carbonaceous chondrites. Additional arguments in favor of this interpretation are: (1) one can not expect to find any hydrocarbons inside

iron meteorites which have been heated at temperatures much above 500°C for millions of years (519); (2) nor can one expect the formation of hydrocarbons by Fischer-Tropsch processes in nodules of iron meteorites and carbonaceous chondrites which contain substantial amounts of elemental sulfur or sulfides (see Table LXIX); (3) if any hydrocarbons were left from the graphitization process, they would not show such typically biogenic distributions and they would be most likely aromatic hydrocarbons which are by genetic and stability considerations closer to graphite. Yet the evidence essentially indicates total absence of these hydrocarbons from the internal nodules analyzed (350,357).

All the results combined point to processes of contamination by the terrestrial hydrocarbons of the hydrocarbon-water cycle. Again it is not necessary to specify a definite source but rather to realize the ubiquity of this type of hydrocarbons in the terrestrial environment. The possibility of contamination would not seem remote if it is considered that we are actually breathing and drinking traces of hydrocarbons everyday.

Concerning the actual mechanisms of contamination the work carried out by Peake and Hodgson (377) on the accommodation of hydrocarbons in water systems is especially relevant to this question. It has been discussed before how a complex mixture of hydrocarbons with a wide molecular weight range can be modified by water to retain preferentially the components

between C_{16} and C_{20}, a range that can be called the meteoritic range. Even though all of the meteorites analyzed were recovered immediately after their fall, and thus had no real chance of becoming in contact with surface waters for any prolonged length of time, this still does not exempt them from being in contact with the water which is present in the atmosphere mainly in the form of aerosols. These water particles, suspended in the air, may carry within their boundaries the hydrocarbon constituents of the meteoritic range, being their distribution very similar to that depicted in Figure 111. The slow accumulation of the hydrocarbons of the water cycle by the meteorites may very well explain the fact that different samples of meteorites (from different parts of the world) give a very similar overall distribution pattern. Although water offers a mechanism of support whereby substantial amounts of hydrocarbons may be present in the atmosphere the contributions of air by itself should not be overlooked. Hydrocarbons are continuously released into the air because of their appreciable volatilities which decrease in proportion to their molecular weights. Eventually they condense upon a receptor surface which may be far removed from their original site of evolution into the air. In doing so and depending on the characteristics (degree of adsortibity) of the condensation surface they may become trapped or selectively retained.

All this can be illustrated by the following experiments. Different samples of meteorites and terrestrial graphite were placed next to an equivalent amount of a petroleum crude (East Texas Crude, see Figure 53) inside of a bell jar for 10 to 15 days at room temperature. All of the samples had been preextracted according to the methods described earlier. After the two weeks of exposure to the crude oil vapours, they were reextracted and analyzed using the same standard procedures applied to all other samples. A typical gas chromatogram is shown in Figure 114A. This is the distribution observed after exposing to the petroleum crude a preextracted Canyon Diablo graphite nodule (extracted 3 times, see Figure 104), which is in line with what could have been expected i) from the relative volatilities of the hydrocarbons in this oil (Figure 53) and ii) from the apparent selectivity of the graphite itself, that tends to enhance the concentration of the lighter hydrocarbons (Figures 95B, 97B and 101C). The same thing was done with a preextracted sample of a commercial graphite with identical results, but in this case a second gas chromatographic analysis was performed (Figure 114B) after having taken the sample to dryness by forced evaporation of the solvent under purified nitrogen (Chapter III). As mentioned earlier this will produce a marked depletion of the more volatile hydrocarbons and thus the resulting distribution (Figure 114B) will look more like the characteristic

FIGURE 114

ADSORPTION OF HYDROCARBONS ON

POROUS AND METALLIC SURFACES

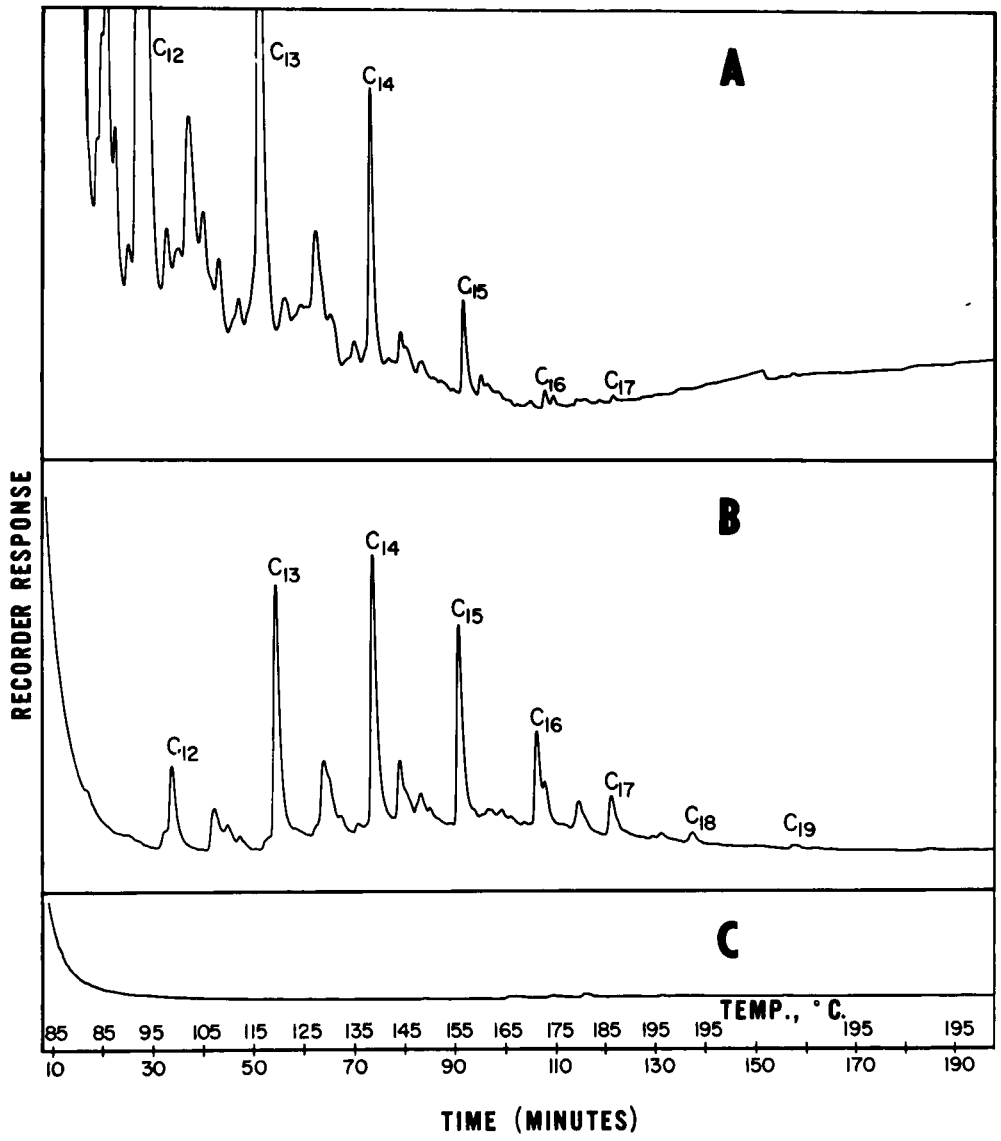
Stainless steel capillary column (195 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Range, 10; attenuation, 2. Nitrogen pressure 1050 g/cm^2. No split. Temperature started at 85^{o}C and held isothermally for 20 min. then programmed to 195^{o}C at the rate of 1^{o}C/min.

(A) Canyon Diablo embedded nodule. Center cut, 1.7655 g. See Figure 104. About 1/9.2 of the n-pentane eluate was injected.

(B) Sample of commercial graphite. 1.7655 g. Taken three times to dryness. All injected.

(C) Canyon Diablo iron. 17739 g. About 1/9 of the sample was injected.

The three samples were exposed to 1.7655 g of an East Texas Petroleum crude for 10-15 days.


A
B
C
C_{12}
C_{13}
C_{14}
C_{15}
C_{16}
C_{17}
C_{18}
C_{19}
RECORDER RESPONSE
TEMP., °C.
85 85 95 105 115 125 135 145 155 165 175 185 195 195 195 195
10 30 50 70 90 110 130 150 170 190
TIME (MINUTES)

hydrocarbon patterns of meteorites. Although the maxima lies at C_{14} in this case it could have been displaced towards the C_{17} alkane by a more rigorous evaporation of the lighter alkanes. This is illustrated in Figure 115 which represents the gas chromatographic pattern of a typical Fischer-Tropsch product subjected to forced evaporation of the solvent. Finally, negative results (Figure 114C), in terms of adsorption of the alkanes in the crude oil, were obtained with a preextracted sample of meteoritic iron, which demonstrates that the porosity or adsortivity characteristics of a substance play an important role in its slow build up of environmental hydrocarbons.

In this way, irregular and porous surfaces will tend to become more contaminated with hydrocarbons than the flat non porous surfaces and also, as in the case of the accommodation of hydrocarbons in water, the relatively low volatilities of the high molecular weight alkanes together with the high volatilities of the light alkane fraction may result in a preferred accumulation of the alkanes in the C_{16} to C_{20} range or "meteoritic range".

FIGURE 115

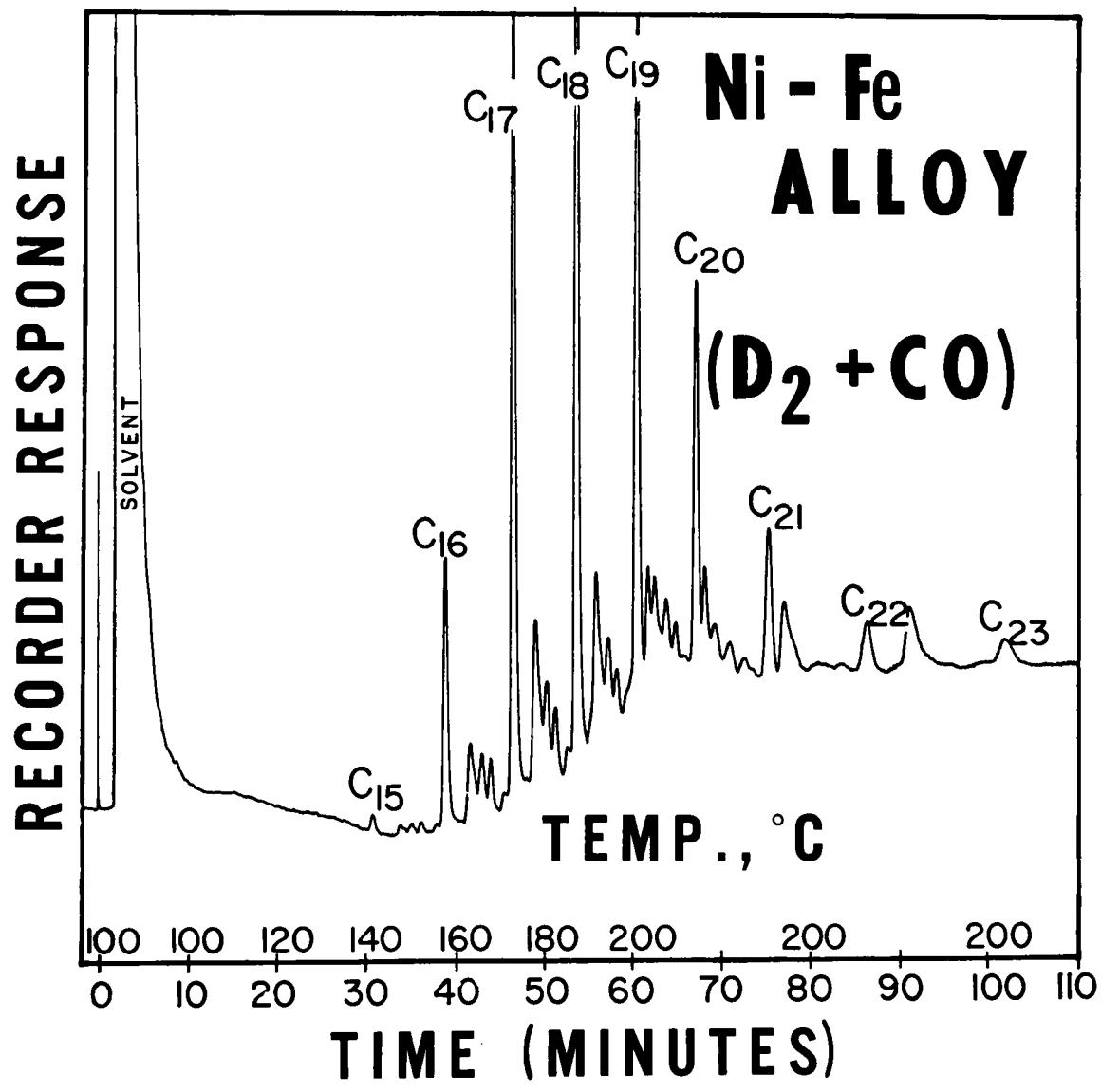
FINAL DISTRIBUTION OF A FISCHER-TROPSCH PRODUCT

AFTER EVAPORATION OF THE SOLVENT

Stainless steel capillary column (195 m long by 0.076 cm i.d.) coated with Polysev. 'F and M Model 810' gas chromatograph equipped with a flame ionization detector. Range, 10; attenuat 1. Nitrogen pressure 1050 g/cm^2. No split. Temperature held at 100°C for 10 minutes, then programmed to 200°C at 2°C/min.

Experiment 113 FT. Nickel-iron catalyst.

About 2/48 of the sample was injected.


RECORDER RESPONSE
SOLVENT
C15
C16
C17
C18
C19
C20
C21
C22
C23
Ni - Fe
ALLOY
(D2 + CO)
TEMP., °C
100 100 120 140 160 180 200 200 200
0 10 20 30 40 50 60 70 80 90 100 110
TIME (MINUTES)

IX.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present work can be divided into three main parts dealing with i) the theory and practice of the modern analytical combination technique of gas chromatography-mass spectrometry, ii) its application to the analysis of terrestrial biotic and abiotic products and iii) to the analysis of extraterrestrial samples. The combination of a gas chromatograph and a mass spectrometer has made possible the detailed study of the individual components of complex organic mixtures regardless of their origin. Several different classes of organic compounds have been studied by means of this technique, with the resultant development of significant mass spectrometric structural correlations (see Chapter IV), especially in the case of the isoprenoid and methyl branched hydrocarbons. The process of preferential abstraction of hydrogen from the molecular fragments associated with a branching site results in the formation of the corresponding olefin ions. These ions have been shown to be important clues to the position of the branch in the molecule. Most of the mass spectra shown here have been reported for the first time and thus a set of standards has been established for future reference. This mass spectrometric study has also revealed much of the biological order which prevails at the molecular level, and as a consequence useful criteria of

biogenicity have been developed on the basis of the results obtained throughout this investigation.

The results obtained on the gas chromatographic-mass spectrometric analyses of (1) organisms, (2) products of biological origin, (3) other terrestrial products, (4) products of abiological syntheses and, (5) extraterrestrial matter (meteorites) can be summarized as follows.

1. Organisms

Bacteria:

a) Sarcina lutea was found to contain three major homologies of fatty acids in the C_{12}-C_{18} range; the normal, iso and anteiso acids, distributed in two families of pairs (iso-normal for the C_{14} and C_{16} and iso-anteiso for C_{13}, C_{15} and C_{17}). The hydrocarbons showed two families of tetrads of unsaturated aliphatic hydrocarbons in the range C_{22}-C_{29}, all with methyl branching. The four isomeric components of each tetrad were identified as two iso and two anteiso olefins. The only difference between the odd and even tetrads was found in the relative gas chromatographic retention times of the last two components of each group.

b) Bacillus cereus

It was found to contain iso, anteiso, normal and unsaturated fatty acids in the C_{13}-C_{18} range, being the C_{17} fatty acid the major component. The aliphatic hydrocarbons (C_{16}-C_{23}), including the isoprenoids were qualitatively and

quantitatively similar to the distributions found in meteoritic specimens.

c) Staphilococcus aureus

The fatty acids (C_{12}-C_{21}) extracted from this bacterium were qualitatively similar to those found in Sarcina lutea.

d) Halobacterium cutirubrum

This halophilic bacterium was found to contain relatively high amounts of squalene with different degrees of unsaturation. Namely, tetrahydrosqualene, dehydrosqualene and squalene itself.

e) Vibrio marinus

This marine bacterium showed relatively simple hydrocarbon and fatty acid patterns. The bulk of the hydrocarbons in these cells was made up by the C_{17} hydrocarbon. The fatty acids ranged from C_{14} to C_{18} and exhibited a high degree of unsaturation.

Algae:

All of the algae analyzed (algal mats, Anacystis nidulans, Chlorella pyronoidosa, Anacystis montana, Botryococcus braunii, Fucales) contain high amounts of the C_{17} hydrocarbon either saturated or unsaturated, which may be of taxonomical falue. In addition two of these algae (A. montana and B. braunii) contain high molecular weight hydrocarbons with maxima at C_{27} and C_{29} respectively. The fatty acids of algal

mats and A. nidulans range from C_{12} to C_{18} with maxima at C_{16}. The analysis of the sterol fraction of the diatom, Nitschia Putrida shows the presence of cholestane.

Plants

a) Cabbage leaves

A symmetrical straight chain C_{29} ketone was identified in cabbage leaves. A number of unsaturated and saturated fatty acids (C_{16}-C_{20}) were also identified.

b,c) Mistletoe and Clover were found to contain several isomeric phytadiene structures.

d) Seeds

The seeds of Plantarum ovata showed small amounts of saturated hydrocarbons ranging from C_{15} to C_{22}. The iso and anteiso alkanes are predominant, being the anteiso C_{18} the major component in the seeds (42% of the total). The seed coat contains a larger amount of hydrocarbons than the seeds. The fatty acids range from C_{14} to C_{18} with high amounts of palmitic oleic and linoleic.

e) Fungal Spores

The fungal spores analyzed contained high molecular weight hydrocarbons ranging from C_{23} to C_{36}.

2. Products of biological origin

a) Shark liver oil and related products

The liver oil from the Basking sharks has a relatively high amount of the polyisoprenoids pristane and

squalene. Although phytane was not detected in this oil, a sample of commercial pristane derived from it had a 1% of phytane. The nature of the fatty acids of the Basking shark oil (C_{14}-C_{22}) is typical of marine oils in general, due to its high content of unsaturated acids.

b) Sheep manure was found to contain a wide range of normal, olefinic and isoprenoid hydrocarbons but only those in the C_{15}-C_{19} range were identified. The possible presence of phytadienes was noted.

c) Crude oil

The hydrocarbons in the range C_{12} to C_{24} contained in a sample of East Texas petroleum crude were identified as mainly n-alkanes and isoprenoids. Mass spectra of the entire series of C_{14} to C_{21} isoprenoid hydrocarbons were obtained. Methyl alkanes (iso and anteiso) and a cyclohexyltridecane were also identified in this sample.

3. Other terrestrial products

a) Tobacco smoke

A relatively high amount of paraffins and olefins ranging from C_{10} to C_{33} were identified in a concentrate of cigarette smoke. The hydrocarbons show a predominance of the odd carbon numbered chains but the contrary is true for the olefins (C_{13}-C_{22}). The paraffins show a bimodal distribution with maxima centered below C_{12} and at C_{31}. Two series of methyl alkanes were also detected. In the high molecular

weight region (C_{29}-C_{33}) the anteiso alkanes with odd number of carbon atoms predominate over the even numbered isoalkanes. The smoke also contained high amounts of C_{19} and C_{20} isoprenoid olefins. The C_{15}, C_{18}, C_{19} and C_{20} saturated isoprenoids are present in decreasing concentrations.

b) Dust

Hydrocarbons ranging from C_{15} to C_{33} were identified in several dust samples. Isoprenoids and other branched alkanes also appear to be present in a few of the samples.

c) Graphite

Different samples of terrestrial graphite were found to contain extractable aliphatic and aromatic hydrocarbons. The aliphatic hydrocarbon fraction was made up mainly by straight chain alkanes (C_{11}-C_{22}) with small amounts of methyl branched alkanes (C_{14}-C_{20}). The C_{14} to C_{22} isoprenoids were also identified. The C_{22} isoprenoid for the first time. The aromatic fraction was composed almost exclusively of alkyl substituted naphthalenes. In general the outside parts of the graphite samples contain a larger amount of hydrocarbons than the inner parts.

4. Products of abiological origin

Fischer-Tropsch synthesis of hydrocarbons

This type of synthesis produced small amounts of saturated and unsaturated hydrocarbons ranging from C_8 to about

C_{26}. The saturated hydrocarbons were found to be predominantly n-paraffins plus smaller amounts of methyl branched isomers (6-,5-,4-,3-,2-methyl alkanes). Several metal catalysts either industrial or meteoritic were tested, but no trace of isoprenoids was produced in any of the experiments. The individual concentrations of these synthetic hydrocarbons decrease with molecular weight in a continuous manner. Sulfur is an effective poison of the catalyst.

6. Meteorites

a) Carbonaceous chondrites

All of the carbonaceous chondrites were found to contain n-alkanes (C_{11}-C_{26}), iso, anteiso and other methyl branched alkanes (C_{13}-C_{22}) plus two isoprenoid homologies (the 2,6,10-trimethyl alkane or farnesane series (C_{14}-C_{19}) and the 2,6,10,14-tetramethyl alkane of phytane series (C_{19}-C_{21})) and cyclohexyl (C_{16}-C_{20}) and cyclopentyl alkanes (C_{17}-C_{18}). The n-alkane distribution modes show maxima at C_{17} as usually found in this type of samples. The methyl alkanes are present in very low amounts, their individual concentrations being not higher than 1 ppm. The iso and anteiso alkanes appear to be present in the same relative amounts. The high molecular weight alkyl cyclohexanes clearly predominate over the shorter alkyl chain cyclohexanes. The isoprenoids show a consistent pattern in all the samples investigated, with a major maximum at C_{19}, a second maximum at C_{15} and a

very pronounced minimum at C_{17}.

b) Graphitic nodules from iron meteorites

The results of their GC-MS analysis show that the hydrocarbons in the nodules are very similar in nature and distribution to those of carbonaceous chondrites. The distribution mode for the n-alkanes peaks at C_{17} and at C_{19} for the isoprenoids. The more exposed parts of these nodules contain larger amounts of hydrocarbons. However, the inner cuts have a larger relative concentration of light molecular weight alkanes and a larger proportion of isoprenoids.

From the comparative and critical evaluation of all of these results, the following general conclusions can be drawn:

1. The mass spectra of methyl substituted alkanes are characterized by i) the low molecular ion intensity, ii) the relatively high M-15 fragment, iii) the major alkyl ions corresponding to the loss of the samller alkyl group attached to the tertiary (or quaternary) carbon atoms and iv) the high olefin fragment which accompanies these alkyl ions.

2. The olefin ions in the spectra of methyl substituted alkanes are diagnostic of the positions of the methyl substituents. Their intensity appears to be dependent on the length of the leaving neutral molecule.

3. The degree of structural order existing along the whole sequence of regular C_9 to C_{22} polyisoprenoids gives rise to a highly regular fragmentation scheme, which is

characterized by the C_8 and C_{13} fragments.

4. The identification of complex hydrocarbon mixtures of very closely boiling isomers should not rely solely upon gas chromatographic techniques because of the many coincidences of the retention times of a large number of components.

5. The low molecular ion intensities of heavily substituted hydrocarbons can be increased considerably by techniques such as low energy and chemical ionization mass spectrometry. Both result in the overall decrease of instrumental sensitivities.

6. The mass spectra of straight chain olefins show a relatively high M-28 ion when the double bond is in a terminal position.

7. Unsaturation in hydrocarbons results in higher stabilities towards fragmentation by electron impact. This apparently is related to the delocalization of the positive charge over the molecule.

8. The identities of iso and anteiso fatty acids can be easily deduced mass spectrometrically from the presence of fragments corresponding to the formation of a ketene ion, derived from the branched structure through the loss of methanol and the loss of water from the ketene ion.

9. The application of mass spectrometry to the analysis of complex biological products is possible only after these products have been resolved into their individual components

by gas chromatography. The same is true for any mixture of organic compounds regardless of their nature and origin. The present investigation has demonstrated the exceedingly wide range of applications of the GC-MS combination technique.

10. Hydrocarbons are widely distributed and occur in significant amounts in biotic as well as in abiotic products.

11. Isoprenoid hydrocarbons are also present in most biological products but to the present it has not been possible to synthesize in the laboratory any of the common isoprenoid structures.

12. The common and almost exclusive occurrence of the C_{17} hydrocarbon in algae may prove of taxonomical value.

13. This algal C_{17} may be directly involved in shifting the maxima of many hydrocarbon distributions towards the C_{16}-C_{18} region.

14. The high molecular weight hydrocarbons found in two contemporary algae may explain the hydrocarbon distributions of some recent and ancient sediments, where the chemical criteria is complemented by morphological criteria, as well as atypical distributions such as that of the petroleum of Uinta Basin.

15. In general there seems to be no direct correlation between the fatty acids and the hydrocarbons in most of the samples analyzed. As a consequence the biosynthetic pathways of the hydrocarbons can not be understood in terms of simple

direct decarboxylation mechanisms.

16. Apparently with the isolation and identification of squalene and dehydrophytyl phospholipids in halophilic bacteria (H. cutirubrum) the initial link in the isoprenoid food chain has been established (Bacteria ⟶ marine photosynthetic organisms (phytoplankton) ⟶ zooplankton ⟶ higher organisms).

17. A relatively high amount of isoprenoid structures are present in tobacco smoke condensates but their distribution does not resemble that usually found in bioproducts.

18. The predominance of even carbon numbered alkanes in Fischer-Tropsch products may suggest the participation of ethylene units in the mechanisms of chain growth.

19. Iron meteorites are capable, when acting as catalysts in Fischer-Tropsch processes, of producing a distribution of hydrocarbons which bears a close resemblance to that produced by other commercial metallic catalysts.

20. The failure to produce isoprenoid hydrocarbons by purely abiotic reactions coupled to the marked bimodal character of the overall distribution of these compounds in nature certainly encourages their use as "biological markers." Since the order and degree of selectivity inherent in the isoprenoid pattern can not be defined from an inorganic point of view, a criterium of biogenicity based on the observed distribution can be established.

21. Consideration of the possible isoprenoid precursors and the diagenetic schemes associated with their formation, leads to the conclusion that there is neither a unique precursor compound nor a single diagenetic scheme but rather an interplay of various likely diagenetic processes acting on several possible precursors, under a not well defined set of conditions.

22. The recent identification of a C_{22} isoprenoid suggests that the higher members of the isoprenoid series may be also present. If this is the case, the relative concentration of the C_{23} and C_{24} isoprenoids would help to prove or discredit the participation of thermal cracking processes in the formation of the common isoprenoid distribution.

23. The application of the distributional criteria of biogenicity to the isoprenoids in petroleum strengthens considerably the foundations of the biogenic theory of formation.

24. As a consequence of the world wide pattern of hydrocarbon products, the contributions of highly mobile environmental agents such as air and water to the observed hydrocarbon distribution in terrestrial as well as in extraterrestrial samples, may be more important than what is generally felt.

25. The distributions of the isoprenoid hydrocarbons in extraterrestrial samples can not be explained by abiotic

processes, but they fit very closely the proposed distributional criterium of biogenicity.

26. The nature, distribution and concentration gradient of the hydrocarbons found in different parts of the graphitic nodules of iron meteorites, indicates that these compounds have been adquired from the external terrestrial environment.

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