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To my wife and parents.

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PART I

THERMAL AND CATALYTIC ALTERATION OF ORGANIC MATTER

INTRODUCTION

It is now widely accepted that sediments of all ages, including those from the Precambrian era as old at 3000 million years, contain organic matter. The organic matter exists in finely disseminated forms in the sedimentary basins of the earth's crust and is the most common form of organic carbon in the earth. It is mostly insoluble in common organic solvents and reagents, and is termed kerogen, but a variable proportion is soluble and has been shown to contain many different types of organic compounds. In 1956, Wickman[1] estimated the total mass of organic material present in sediments, petroleums, coal and living organisms to be of the order of 6×10^{15} metric tons. Hunt[2] has estimated that about 3.2×10^{15} metric tons of organic matter are distributed throughout the 30 million cubic miles of sediment in the basins and on the Continental Shelves of the world.[3] The origin and composition of such a vast accumulation of organic matter has not been examined in any detail until recently. Over the last 10 to 15 years it has become of increasing interest and importance to isolate and identify constituents of the sedimented organic matter. Their distributions can then be used, among other things, to rationalise the possible methods of petroleum origin and migration and also to correlate their distributional patterns

with those of identical or related compounds in present day living things.

The origin of petroleum has long been a topic of research interest. As early as 1888, Engler[4] had proposed probably the first biogenic theory of petroleum genesis. Since then there have been many theories proposed, ranging from purely abiogenic[5] and biogenic[6] to bimodal.[7] It is now generally accepted that a biogenic origin, with subsequent alteration of the organic debris which accumulates in sediments, is the most likely explanation for the occurrence of petroleum hydrocarbons. Nevertheless, some contribution from an abiogenic source cannot be excluded, as will be discussed later.

The remains of plants and animals incorporated in sediments contain a conglomeration of biologically derived organic compounds. Since this thesis is concerned with the origin of hydrocarbons in sediments, the ensuing discussion is limited only to the most likely biological precursors for the hydrocarbons. However, before discussing and correlating the compounds found in sediments with those of present day living organisms, a review of the known occurrence and carbon number distributions of these precursors is pertinent. Normal and branched alkanes are widespread in the plant kingdom, both in leaf, petal and fruit cuticular waxes and in the whole plant. Stransky et al,[8] Eglinton and Hamilton,[9] and Douglas and

Eglinton[10] have reviewed the distribution of wax hydrocarbons in plants at different evolutionary levels. These reveal that lower order plants, e.g. mosses, algae and lichens have carbon number distribution ranges usually from C_{10}-C_{23}, while higher plants are characterised by the predominance of the C_{23}-C_{35} alkane range. Stransky et al,[8] Oro et al[11] and Han et al[12] have shown that in lower plants such as some bacteria, some mosses, some algae and some lichens the ratio of odd to even numbered carbon chains of the n-alkane content approached unity, whereas in most high plants the carbon preference index (c.p.i.) varies from 10 to 30. In contrast, these authors have found that the n-C_{17} alkane predominates in algae and to a lesser extent in bacteria. The odd/even predominance of biologically derived n-alkanes has always been assumed to indicate a biological origin when such a distribution has been found in sediments. In view of these recent findings,[8,11,12] the absence of such a distribution does not necessarily mean that the sample has an abiogenic origin. This point will be discussed further with respect to alteration of the sedimented organic matter.

Apart from the n-alkanes, however, the proportion of branched alkanes found in plants is generally very low. Isoprenoid alkanes have been found in many sediments[13,14] and

petroleums.[15,16,17] The isoprenoid or terpenoid alkanes have branched chains comprised of 5-carbon units assembled mostly in a 'head to tail' fashion in a regular sequence so that the methyl groups are situated on every fifth carbon atom. It is widely held that chlorophyll a, having an isoprenoid sidechain, is one of the likely precursors of pristane and phytane.[18,19] Further confirmation of this is the widespread occurrence of vanadyl porphyrin in petroleums and shales which has chlorophyll a as its probable precursor. However other likely sources for pristane and phytane have been reported, e.g. zooplankton,[20] bacteria.[19]

Apart from the naturally abundant alkanes, other precursors for alkanes are possible. Martin et al,[21] Cooper,[22] Lawlor and Robinson[23] and Mair[24] have suggested that naturally occurring saturated normal fatty acids are suitable precursors for n-alkanes in the range C_{11}-C_{19}. The naturally occurring fatty acids are generally, predominantly, even carbon numbered and unbranched. Unsaturated fatty acids have also been suggested as possible precursors especially for the C_{18}-C_{20} n-alkanes.[21] Similarly, plant waxes, consisting of fatty acid esters and complex alcohols could be precursors for the C_{23}-C_{35} n-alkanes.[21,23] n-Fatty acids were chosen as likely precursors because of their similarity in structure to the n-alkanes, and because of their widespread occurrence in nature. Saturated and unsaturated

n-fatty acids are to be found in oils and fats as glyceride lipids, generally in the n-C_{12}-C_{22} range.[25] Palmitic acid (n-C_{16}) is the most abundant saturated fatty acid while the n-C_{18} unsaturated fatty acids are predominent. The n-fatty acids from waxes of fauna and flora usually exist as esters in the C_{24}-C_{36} range.[25] The plant waxes also contain long chain alcohols both in the free state and as esters with the fatty acids and are generally straight chain, monohydric and predominantly even carbon numbered in the C_{14}-C_{18} and C_{28}-C_{34} ranges. Ackman and Sipos[26] reported that marine lipids from phyto- and zooplankton, squids, fish, seals and whales contain n-fatty acids in the C_{12}-C_{20} range and are predominantly even carbon numbered. Ample evidence that normal fatty acids are to be found in sediments is supplied by the findings of Abelson et al,[27,28] Parker and Leo,[29] Cooper,[22] Kvenvolden,[30,31] Meinschein and Kenny.[32] Isoprenoid acids have been found in sediments by Douglas et al,[33] Eglinton et al,[34] Ramsay,[35] and Haug et al.[36]

Erdman has suggested that the amino acids, hydrolysis products of proteins, may be precursors for both normal and isoalkanes in the C_2-C_8 range. These amino acids, by processes such as decarboxylation and reductive deamination, could yield non-isoprenoid branched alkanes.

The mono cycloalkanes, such as cyclobutanes, pentanes

hexanes and heptanes, the bicycloalkanes such as the decalins and the tri-, tetra- and pentacycloalkanes, are postulated to have the terpenoids as their precursors.[24] The distribution and occurrence of steroids and triterpenoids is fully discussed in Part II and therefore little more need be said about this here. It has also been proposed that the unsaturated fatty acids could, by a Diels-Alder reaction between a diene and a mono-ene, give rise to some cycloalkanes.[38,39] The occurrence in petroleums and shales of oxygenated compounds identical with, or closely similar to, the naturally occurring terpenoids is well known.[40,41] Petroleums[24,42-45] and sediments[46-48] also contain hydrocarbons which have carbon skeletons or parts of carbon skeletons corresponding to those of the terpenoids.

The aromatic constituents found in petroleum so far range from monocyclic to tetracyclic compounds and the latter are the highest boiling compounds identified in crude oil. The proposed precursors for the aromatic constituents are the unsaturated fatty acids with three to six double bonds,[37,39] the terpenoids (through acyclic to pentacyclic),[24,37] the carotenoids[49-51] and finally the polyhydroxyquinones.[52-54]

If all the categories of compounds described above, together with the other biological materials such as the biopolymers, cellulose, sugars, lignin etc, were to form accumulations in sediments, coals, and as petroleums of a primary

type, i.e. largely unaltered chemically, then one would expect to find largely unaltered biological distribution patterns of alkanes, fatty acids and alcohols in sediments and petroleums of all ages. That is to say, the alkanes would always exhibit a high odd/even c.p.i., the fatty acids and alcohols would show a high even/odd c.p.i. The relative concentrations of these would be more or less the same as in the original organisms, and finally, the low proportion of branched chain carbon compounds would be maintained. However, the practical findings do not support this but point to a combination of primary and secondary accumulations, i.e. the original biological material has been altered in some way in the sediment to produce new constituents and thus new distribution patterns. The evidence in support of alteration may be summarised as follows:-

(i) The concentration of fatty acids in Recent sediments is lower than expected from their abundance in nature.[55]

(ii) The polyunsaturated fatty acids, abundant in most organisms, are not found in sediments. Parker and Leo[29] showed that, while blue-green algal mats contain substantial quantities of unsaturated fatty acids, the underlying old mats became progressively depleted in unsaturated molecules.

(iii) The even/odd c.p.i. values for n-fatty acids are higher in modern than ancient sediments and petroleums.[22,31]

(iv) The relative proportion of branched chain fatty acids

increases with increasing age.[55]

(v) The concentrations of alkanes in modern sediments are usually lower than in ancient sediments and petroleums.[2,56]

(vi) The odd/even c.p.i. values for n-alkanes are higher in modern than in ancient sediments.[57]

(vii) The proportion of branched chain alkanes increases with increasing age.[7]

(viii) The low molecular weight hydrocarbons in the range C_2-C_9 are generally not formed by organisms and are usually not found in Recent sediments. However, they are quite abundant in ancient sediments and petroleums.[58-60] The usual carbon number range to be found in biological systems is C_{10}-C_{40}.

(ix) The optical activity exhibited by petroleums decreases with increasing age.[61]

(x) The C^{13}/C^{12} stable carbon isotope ratios for modern marine and terrestrial organisms are almost the same as those for ancient marine and non-marine sediments.[62] This is an important finding which bears on the abiogenic/biogenic controversy on the origin of petroleum and will be discussed more fully in the appropriate section.

Some of these generalisations have been used to support both an abiogenic and a biogenic origin for petroleum. But the overwhelming evidence for a largely biological origin, provided by the presence of terpenoid or related structures,

the dominance of palmitic acid in sediments, the dominance of the $\underline{n}$-C_{27}, $\underline{n}$-C_{29} and $\underline{n}$-C_{31} alkanes in many sediments and finally the optical rotation exhibited by certain fractions of crude oil, seems to the author to be incontrovertable. In particular, the highly specific structures necessary for the occurrence of optical activity are produced solely by biological systems. It is true that experimental laboratory syntheses can produce stereospecific structures, but the reaction products are usually complex and are accompanied by low yields of individual compounds, in contrast to the specificity of biological synthesis. The optical activity of a petroleum was first detected by Walden[63] who related the rotatory power with a biological origin. Fenske et al,[64] Carnahan et al[65] and Rosenfeld,[61] among others, continued this work, but it was Oakwood et al[66] who first attributed optical rotation in petroleum fractions to hydrocarbons of the tetra- or pentacycloalkane categories. Many workers have since investigated the identities of compounds like these in both sediments and petroleums and have attempted to establish chemotaxonomic relationships.[6,40,48] In the second part of this thesis, an Eocene oil shale is shown to contain, of the identified structures, exclusively cycloalkanes of definite biological origin.

As stated above, a different interpretation may be placed on the above experimental data. In particular, the apparent

non-correlation of the distribution patterns of the alkanes and fatty acids between modern organisms, Recent sediments, ancient sediments and petroleums. The proponents of an abiotic origin for petroleum theory argue that chemical alteration of primary biologically derived deposits cannot adequately explain the differences in distribution patterns. The abiogenic theories proposed include a Fischer-Tropsch type of origin[7,67-71] and a degasification process of the interior of the earth.[5]

The Fischer-Tropsch reaction is a catalytic reaction involving carbon monoxide and hydrogen at temperatures between 200°C and 300°C and at atmospheric or higher pressures. Friedel and Sharkey[68] point out the close similarity between all the low molecular weight alkane isomers up to C_8 in certain crude oils and the Fischer-Tropsch products. These authors were also successful in correlating the observed abundance of these isomers in crude oil, the Fischer-Tropsch product and a predicted composition (based on the probabilities of chain lengthening and chain branching during the synthesis). Studier et al[71] have extended this postulate to explain the formation of hydrocarbons in the early solar system. They have provided some evidence for the presence of isoprenoid hydrocarbons in the reaction products in the range C_9 to C_{14}, but their claims must remain tenuous at the present time because of a lack of unambiguous evidence and, more important, the low yields of

these compounds. However if their claims are confirmed, then the accepted theory of the uniqueness of isoprenoid structures, being solely confined to a biogenic synthesis may no longer be watertight and their usefulness as biological markers must be questioned. Support for a non-biogenic synthesis of polyisoprenoid structures is afforded by the work of Natta[72] who showed that using a highly stereospecific catalyst, almost 100% stereospecific polymerisations of 1,3-pentadiene can be effected. However these abiogenic theories have not yet been able to account for the initial formation of the isoprene unit and the reactions are extremely sensitive to impurities. Nor can these theories account for the higher molecular weight isoprenoid material like pristane, phytane, steranes, triterpanes or perhydro-carotenes which occur in sediments and petroleums in high proportions.

Another factor which seriously weakens the case for a Fischer-Tropsch origin is the generalisation that (see above) the C^{13}/C^{12} stable carbon isotope ratios for modern marine and terrestrial organisms are almost the same as those for ancient marine and non-marine sediments.[62] The basis for this approach is that photosynthetic organisms discriminate against C^{13} in preference for C^{12}.[74,75] It has been shown that the stable carbon isotope data for modern and ancient carbonaceous materials show consistently low C^{13}/C^{12} ratios compared to the

ratios for inorganic carbonate minerals deposited in marine environments.[62] In a similar way it has been concluded that many petroleum distillate fractions, from low through to high molecular weight, have been formed originally from biogenic material, i.e. the lipids, and that subsequent alteration of some kind has altered the primary material to form the low molecular weight hydrocarbons found in natural gas and petroleums.[62]

Despite the abundance of the evidence pointing to a biogenic origin for petroleum, it is not possible to completely exclude some contribution from an abiogenic source. Rudakov in his review of the abiogenic theories,[5] has pointed out that degasification of the interior of the earth could lead to solution of the sedimented biological material in these gases, thus affording a bimodal origin for petroleum like that proposed by Robinson.[7]

If the largely biogenic source for hydrocarbon in sediments and petroleum is accepted, then the same differences in distribution patterns of the fatty acids and alkanes used by the proponents of an abiogenic origin have to be explained satisfactorily so that correlations may be established between the constituents of modern organisms, Recent and ancient sediments and petroleums.

The n-alkane distributions in sediments and petroleums have been related to those of biologically produced mixtures of fatty acids[21,28,30,31,76-80] and alcohols,[21,76] on the assumption that the latter are precursors. The main obstacle to the theory of simple decarboxylation of n-fatty acids is that by this means predominantly even carbon numbered molecules would produce predominantly odd carbon numbered alkanes. Thus if this process occurred to a significant extent, one would expect to find ancient sediments and petroleums exhibiting alkane distribution patterns with a high odd/even c.p.i. and that with time, the fatty acids would disappear. The evidence put forward again shows very clearly that the n-alkane distributions show c.p.i. values tending to unity with increasing age. The proportion of branched alkanes and fatty acids increases and that normal fatty acids have been found in significant proportions in Precambrian sediments. Therefore such a simple explanation is inadequate. Kvenvolden has proposed a slightly more complex theory which requires the operation of concurrent processes of decarboxylation and reduction of carboxyl groups which he claims would provide a slight even/odd c.p.i, value for the n-alkanes.[81] However, the work of Jurg and Eisma[77-79] showed experimentally that relatively mild thermal treatment of n-C_{22} fatty acid generated low molecular weight alkanes in the range C_3-C_6, n-alkanes in the

range C_{14}-C_{34} and normal fatty acids in the range C_{15}-C_{24}. Thus the alteration theories have to be widened to account for these data. Kvenvolden and Weiser[82] have recently postulated a mathematical model which relates the n-alkane distributions found in ancient sediments with n-fatty acids, which as precursors, could be altered by decarboxylation and reduction. The weaknesses in this approach appear to be that (i) at the final stage of the model the fatty acids disappear; (ii) the model cannot account for the formation of alkanes and fatty acids of higher carbon number than the original fatty acid (cf. Jurg and Eisma); (iii) it does not account for the formation of any branched carbon compounds; and finally, (iv) the reduction treatment requires the initial fatty acid distribution to have a high odd/even c.p.i. value which is in complete disagreement with every piece of information reported on Recent sediments and modern organisms.

Nevertheless, it is quite obvious that alteration of some kind must occur to the primary biogenic organic material during the accumulation, diagenesis and maturation of sediments, thus causing the secondary distributions of the alkanes to appear as they do in ancient sediments. The principal alteration processes occurring in a sediment are usually held to be bacterial action, radioactive bombardment and thermal and catalytic alteration.

(i) Bacterial action. The initial accumulation of biological organic debris is usually assumed to occur in marine or brackish water deposits. Bacterial activity is to be expected wherever organic matter is deposited, regardless of environmental influences such as salt or fresh water, aerobic or anaerobic atmospheres, warm or cold climatic conditions.[83] Typical marine bottom muds may have up to 10^8 organisms per gram for the first few centimetres of depth; with increasing depth the numbers fall off rapidly, but bacteria are claimed to have been found in ancient sediments and petroleum reservoir fluids at depths of up to several thousand feet.[83,84] Aerobic bacteria in the upper zones of a deposit may alter and destroy up to 50% of the organic debris.[85] Under anaerobic conditions, Desulfovibria bacteria are active and presumably play a part in preventing oxidative destruction and in facilitating the reductive conversion of some of the organic debris, e.g. alcohols and fatty acids, to hydrocarbons.[86]

Bacteria are known to produce methane,[83-86] but whether they can produce significant quantities of other hydrocarbons using the organic debris as feedstock is unanswerable at the present time. Obviously, investigations designed to discover the nature and distributions of bacteria in sediments and also the type of activity and alteration effected by them would cast some light on this largely obscure topic.

(ii) Radioactive bombardment. Radioactive bombardment of the organic debris by alpha particles emitted by elements such as thorium and uranium associated with the sediments may alter the organic debris and produce hydrocarbons.[38,87-89] However, it seems likely that this process does not occur to a significant extent, since the action of alpha particles on aliphatic material produces unsaturated compounds, helium and hydrogen. But natural gases containing the highest proportions of helium (ca. 4%) contain no hydrogen. It has also been shown that certain oil shales having unusually high levels of alpha radiation are not related to any petroleum reservoir,[38] but are rich in kerogen which may result from polymerisation of the unsaturated material.

(iii) Thermal and catalytic alteration. Laboratory experiments have shown that slow thermal alteration can effect some conversion of isoprenoid compounds to hydrocarbons commonly found in petroleums. The reaction of sulphur with cholesterol and farnesol at 150°C and 135°C respectively, produces, in the former case, benzenes, naphthalenes and phenanthrenes, in the latter case, a substituted naphthalene is one of the products.[90] Treatment of β-carotene at 188°C produced 2,6-dimethylnaphthalene, toluene, m-xylene and ionene.[37,51] With normal and branched chain alkanes, the products of "flash" pyrolysis at temperatures in the range 500-600°C,[91-97] have been investigated

and the mechanisms of thermal and catalytic cracking examined. Much of the early work at these temperatures was done without the aid of modern analytical techniques such as gas-liquid chromatography (g.l.c.) and mass spectrometry. Thermal alteration experiments have been carried out on a Recent sediment,[98,99] the kerogen from an Eocene sediment[100,101] and on a fatty acid.[77-79] The work reported in Part I of this thesis was started in late 1965, and a preliminary report on the findings was presented at the Third International Organic Geochemistry Meeting in London, September 1966. These results and those of other workers[98-101] have shown that n-alkanes can be generated from a sediment by relatively mild thermal treatment.

The differences in distribution patterns found by several workers[100-104] for n-alkanes obtained from similar sediments at increasing depths of burial in a single formation, are probably caused largely by thermal alteration. Temperatures of around 200°C have been reported for deeply buried sediments.[104]

In an attempt to further unravel the problems surrounding the origin of hydrocarbons in sediments and, in particular, in petroleum, a series of experiments were carried out involving thermal and catalytic alteration of an n-alkane, and the effects of thermal alteration at many different temperatures for varying periods of time on Eocene and Carboniferous sediments were studied.

SECTION 1

THERMAL AND CATALYTIC ALTERATION OF n-OCTACOSANE

Introduction

In this section the thermal and catalytic alteration of n-octacosane is studied at different temperatures for varying periods of time. From the results, an attempt is made to assess the importance of the related geochemical processes which may bring about the alteration of alkanes and other biologically derived lipids in a sediment. The n-octacosane was obtained from Applied Science Laboratories, Inc, and was 99.9% pure by g.l.c.

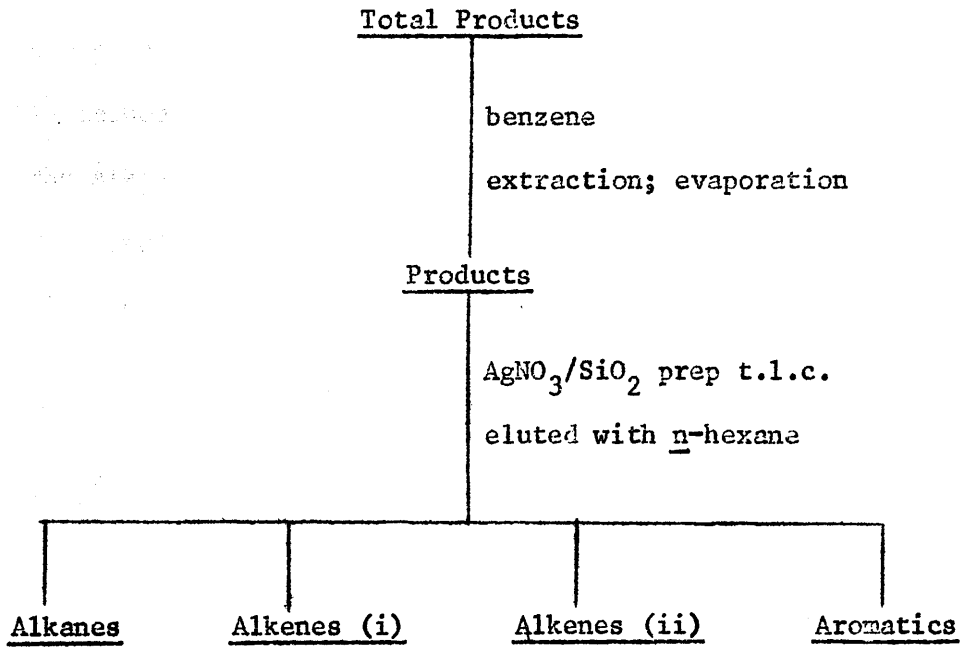
Discussion

Thermal Cracking of n-Octacosane

The alteration experiments were carried out at 375°C for varying periods of time (75, 100, 150 hr) in sealed Pyrex glass tubes under vacuum. The appearance of the contents changed markedly with increasing time; initially the n-octacosane was colourless but as the heat treatment progressed the products became liquid and increasingly yellow coloured and carbonised films were formed on the inside surface of the glass. Accompanying the deepening colour were marked pressure rises due to the formation of increasing quantities of gaseous products, probably low molecular weight hydrocarbons such as methane, ethane, propane etc. The products were analysed (Fig. 1) by analytical silver nitrate impregnated SiO_2 thin layer chromatography (t.l.c.) followed by separation of the hydrocarbon fractions using preparative t.l.c. on the same adsorbant mixture. Chromatographic retention data and infrared spectroscopy indicated the presence of two different classes of alkenes: (i) mainly trans-disubstituted (ν_{max}, 965 cm^{-1}); (ii) mainly terminal alkenes (ν_{max}, 990 and 910 cm^{-1}). The alkane and alkene fractions from the three experiments were analysed by capillary g.l.c. on a 175' x 0.01" stainless steel column coated with Apiezon L grease. A typical g.l.c.

Figure 1

The Separation and Analytical Procedure for the Analysis of the Pyrolysis Products of n-Octacosane at 375°C



analysis of three of the four fractions isolated from the 75 hr pyrolysis is illustrated in Figure 2. The alkane fraction consists predominantly of n-alkanes with a relatively smooth carbon number distribution centered around n-C_{21}. A striking feature is the complexity of the two alkene fractions. Each carbon position is a complex mixture of peaks with as many as seven components distinguishable, ranging from C_{28} to C_{14} in carbon number. The carbon number distribution patterns of the alkenes are relatively smooth with maxima at C_{22} and C_{18}. The complexity is due to the presence of geometric and positional double bond isomers and possibly, to a small extent, the presence of branched chain or cyclic isomers.

The n-alkane distributions are shown in bargraph form in Figure 3. These do not show the lower molecular weight hydrocarbons formed by the pyrolysis and subsequently lost in the work-up procedure. Certain apparently preferential fragmentations of the n-octacosane carbon chain occur, e.g. the higher abundance of the n-C_{26} alkane in the 75 hr pyrolysis seems to indicate that the loss of a two carbon fragment is slightly preferred. Similarly the initial abundance of the n-C_{14} alkane indicates a slight preferential cleavage at the midpoint, i.e. between carbons 14 and 15 in the n-C_{28} carbon chain. The loss of a methyl radical has a low probability. The overall trend towards generation of lower molecular

Figure 2

G.l.c. records of the total alkane, alkene (i) and alkene (ii) fromations from the 75 hour pyrolysis of n-octacosane.

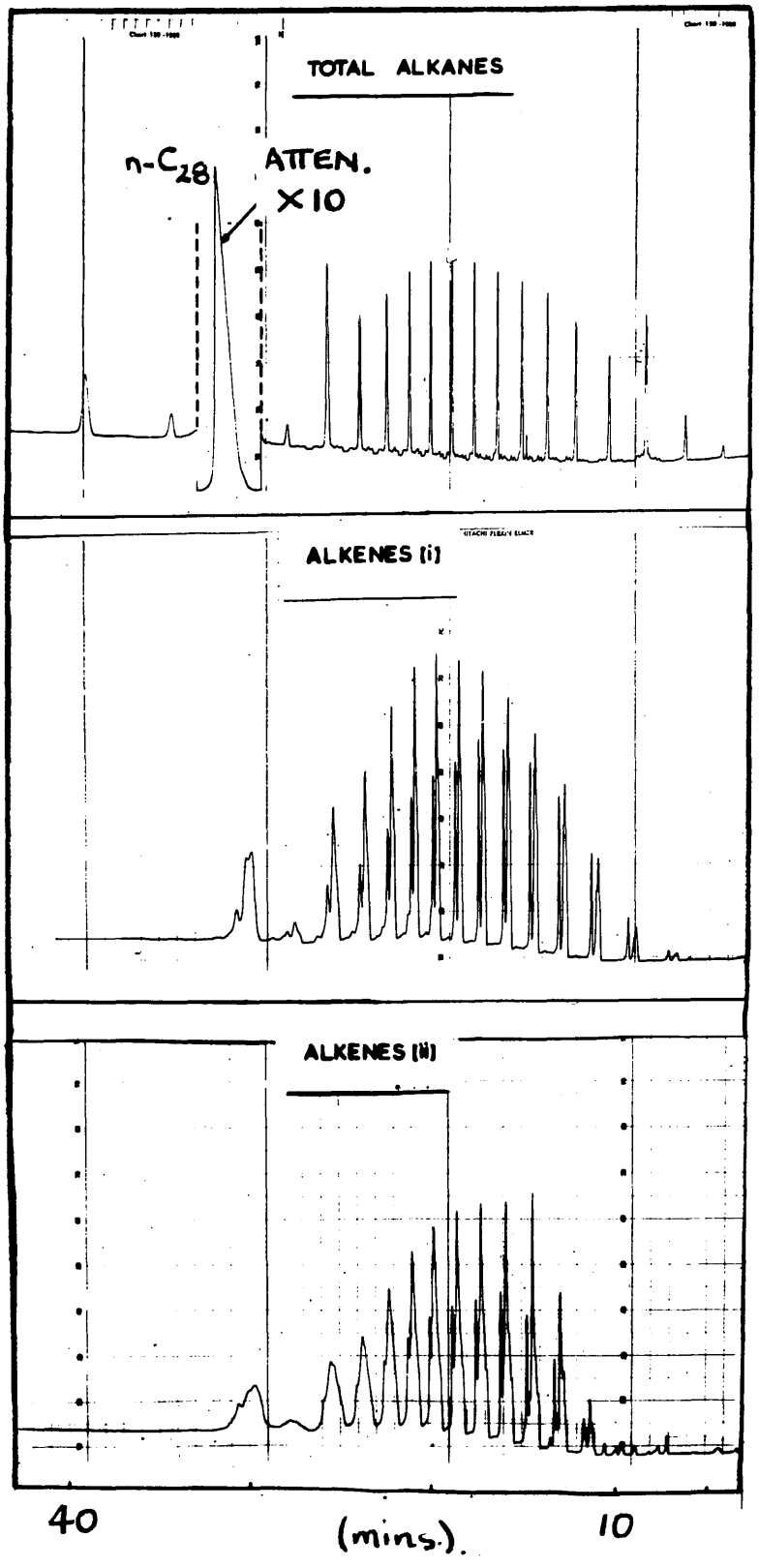
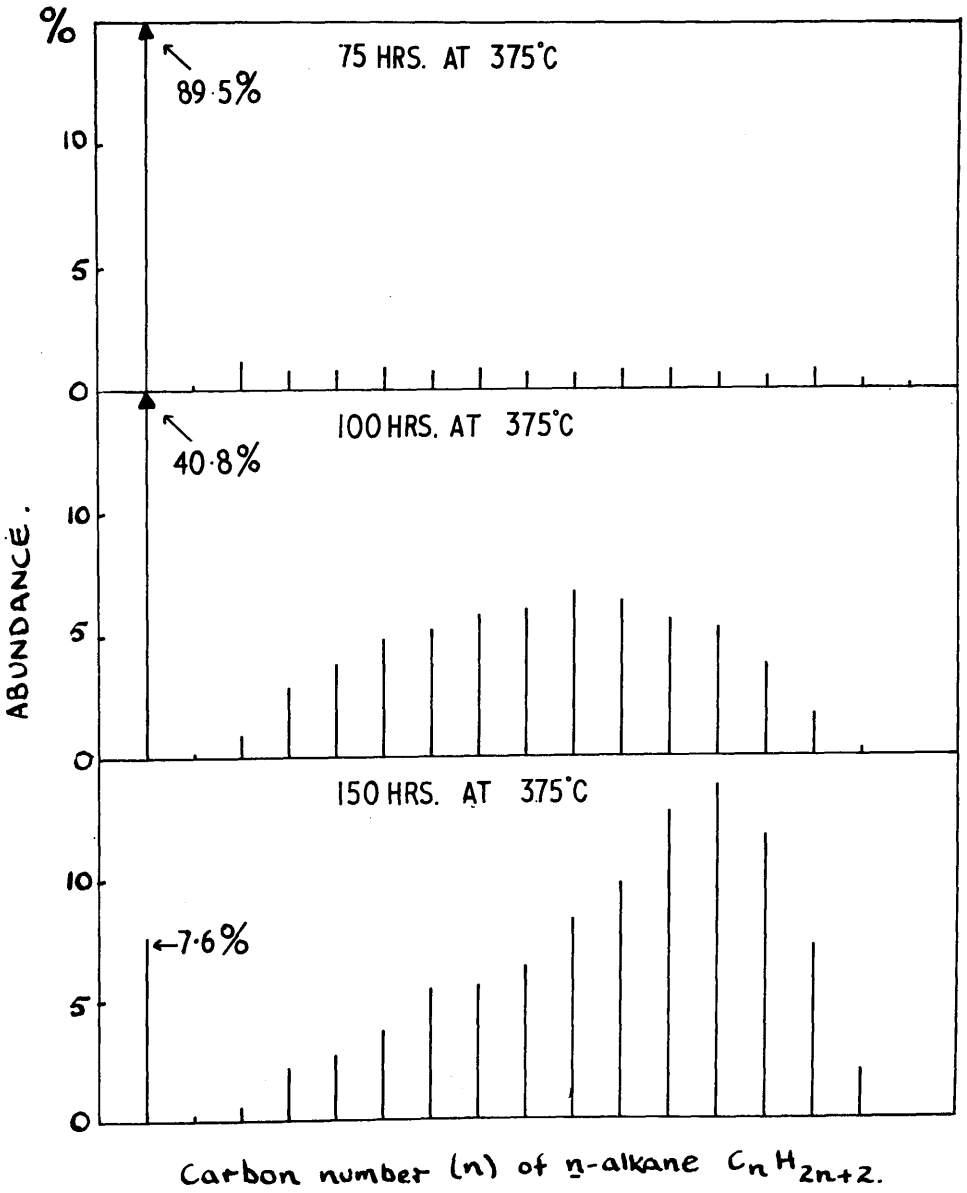

TOTAL ALKANES
n-C28
ATTEN.
X10
ALKENES (i)
ALKENES (ii)
40
(mins.)
10

Figure 3

The percentage abundance of the individual n-alkanes in the pyrolysis products from the n-C_{28} alkane, calculated by measuring g.l.c. peak areas.


%
75 HRS. AT 375°C
89·5%
100 HRS. AT 375°C
40·8%
150 HRS. AT 375°C
7·6%
10
5
0
ABUNDANCE.
Carbon number (n) of n-alkane C_nH_{2n+2}.

weight alkanes gives the n-alkane carbon number distribution patterns an increasingly skew appearance with time, but the 100 and 150 hr patterns resemble those for certain crude petroleums.

The yields and relative abundances of the hydrocarbons obtained are shown in Table 1. The general trends with increasing time are:- (i) the yield of alkanes decreases, (ii) the other n-alkanes are formed in increasing amounts relative to the n-C_{28} alkane, (iii) the proportion of branched and cyclic alkanes increases, (iv) the yield of alkenes and aromatics increases, (v) the carbon number maxima for the alkanes (Fig. 3) and the alkenes shift to lower values.

The above experiments conducted at 375°C have thus revealed considerable conversion of n-octacosane into other hydrocarbons but lower temperatures would be more acceptable as a parallel for geological experience. Therefore the n-octacosane was heated at 200°C for 1000 hr and under vacuum as before. The products were examined by capillary g.l.c. and it was found that less than 0.1% alteration of the n-octacosane had occurred. The activation energy for the fission of carbon-carbon bonds is high (ca. 75-80 Kcal/mole for an n-alkane), and the reaction consequently slow. These results show that the use of C^{14} labelled compounds are essential to follow and identify < 0.1% conversions of this type.

Table 1

The Percentage Yields of the Products after 75, 100 and 150 Hours Pyrolysis of n-C_{28} Alkane at 375°C

Time (hr)	Yield[a] of Products[b] (%)	Alkanes[c] / Products[b] (%)	n-C_{28} Alkane[c] / n-Alkanes (%)	n-Alkanes[c] / Alkanes (%)	Branched Cyclic[c] / Alkanes (%)	Alkenes[a] / Products[b] (%)	Aromatic[a] / Products[b] (%)
0	-	-	99.9	-	-	-	-
75	90	80	89	95	5	5	1
100	82	76	41	77	23	8	2
150	15	70	8	73	27	12	12

[a] Yields calculated by weighing (accuracy ± 0.1 mg). [b] Products soluble in benzene. [c] Yields calculated by measuring peak areas.

One of the most useful techniques developed in recent years in this field has been automatic scanning of radio t.l.c. plates. The mixture containing the radioactive material is placed on a t.l.c. plate as usual and eluted with the required solvent. The positions of the spots or layers are detected by a detector designed to count the disintegrations of the radioactive atoms. An automatic scanner was designed by Professor A. T. James (Unilever Research) and, with his help, a slightly modified instrument was constructed at Bristol for this work. However, due to the other work on hand, the author did not have time to pursue this topic any further. Future work in this and other fields should be greatly facilitated by this technique, especially when it is used in conjunction with a radio g.l.c. (Pye Instruments Ltd) which has recently become available in the Department. Because of the great sensitivity of these techniques, minute conversions in alteration experiments may be followed more easily and thus much lower temperatures may be used.

Possible experiments which could be conducted would be the addition of a small quantity of a C^{14} labelled n-alkane to a powdered sediment or suitable catalyst which could then be heated at around 100°C for long periods of time. Analysis of the products using radiochemical techniques would allow quantitation of tiny conversions, thus leading to calculations

of the Arrhenius factors, which could be extrapolated to predict the spans of geological time necessary to bring about the observed changes at lower temperatures. The Arrhenius equation is as follows:-

$$k = A\, e^{-E_a/RT}$$

where k = the rate constant

A = constant

E_a = Arrhenius activation energy

R = constant

T = temperature (^{o}A)

By studying the alteration achieved at several different temperatures, and substitution of the appropriate kinetic data in the Arrhenius equation, it should be possible to draw a graph of temperature against time to achieve a constant degree of alteration for a given compound. Thus by extrapolation one could then estimate the time required for a given diagenetic reaction to proceed to a given extent at temperatures which are geologically feasible, e.g. at 100^{o}C or lower. In this way, both thermal and catalytic cracking rates of reaction could be studied.

Thermal and Catalytic Cracking of n-Octacosane

Thermally induced cracking is accelerated by numerous catalytic materials.[92,93] Indeed, the carbonised films formed in the previous experiments may have been acting as catalysts

for the alterations.[92] Therefore the n-octacosane was heated at different temperatures for different periods of time with catalysts such as bentonite and water. With bentonite alone, at 200°C for 170 hr, approximately 1% of the n-octacosane had been converted into other alkanes and olefins and aromatic materials (Table 2), as indicated by their behaviour on thin layer chromatography and gas-liquid chromatography. Thus the bentonite had brought about a more than tenfold increase in the extent of alteration compared with the previous experiment, which had been conducted over a much longer period.

Two experiments, one with added water and one without, have been conducted at 375°C. The effect of the bentonite at this temperature was to bring about the conversion of more than 90% of the n-octacosane to insoluble black carbonaceous material. Only a small proportion of the n-octacosane remained in the solvent-soluble products (Table 2), which contained much larger proportions of alkenes and aromatics than in the previous experiments (Table 1). Perhaps the most interesting result is the high concentration of branched/cyclic alkanes apparent from the gas chromatographic record obtained for the alkane fraction from the treatment involving bentonite alone (Fig. 4). The repeating pattern of peaks is very reminiscent of that found for the alkane fractions of crude petroleums. Gas chromatograms for the alkane fractions derived from two

Table 2

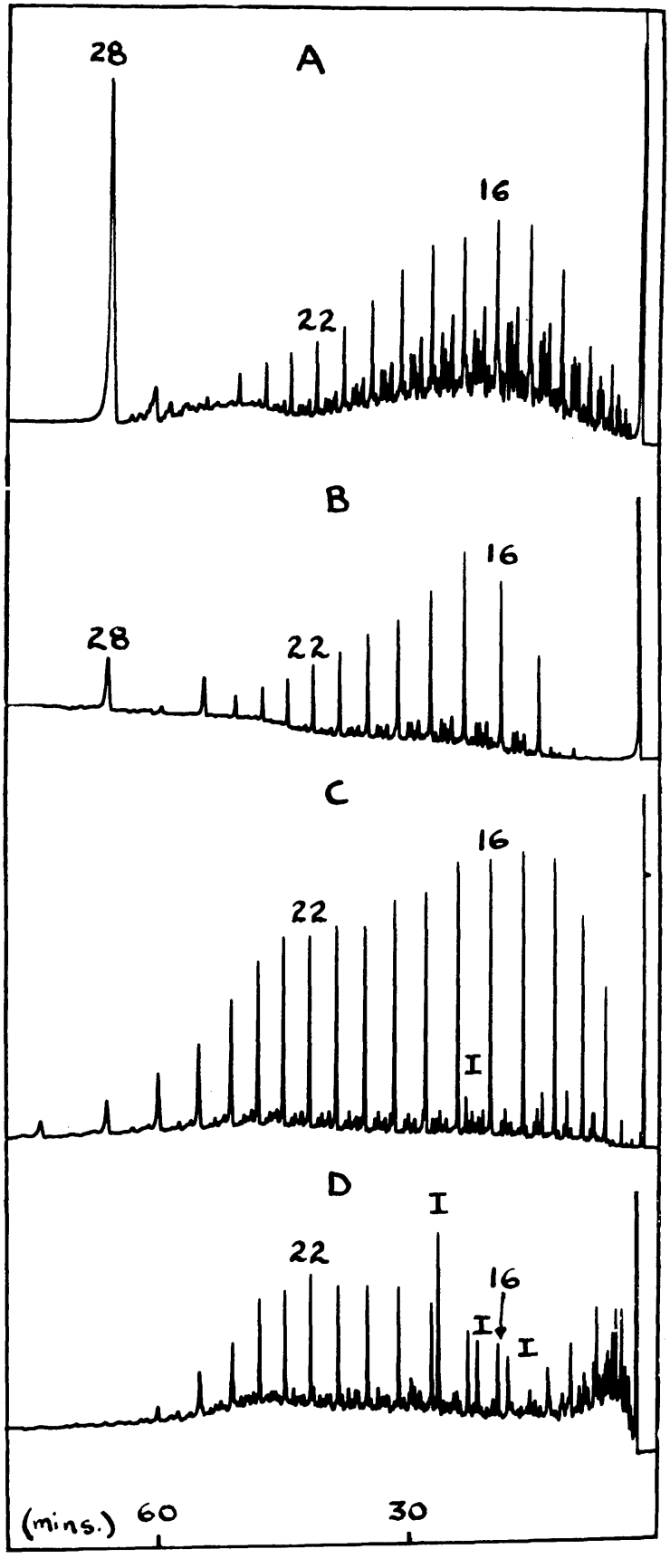
The Percentage Yields of the Products after Pyrolysis of $\underline{n}$-C_{28} Alkane in the Presence of Bentonite

Starting Material	Temp. (°C)	Time (hr)	Yield[a] of Products[b] (%)	Alkanes Products (%)	Alkenes Products (%)	Aromatics Products (%)
$\underline{n}$-C_{28} alkane/bentonite[c]	200	170	98	98	1	1
$\underline{n}$-C_{28} alkane/bentonite[c]	375	60	8	55	10	35
$\underline{n}$-C_{28} alkane/bentonite[c]/H_2O (1:10:1)	375	60	2	35	20	45

[a] Yields calculated by weighing (accuracy $\pm$ 0.1 mg). [b] Products soluble in benzene/methanol (3:1).

Figure 4

G.l.c. records of the alkane fractions from the heat treatment of n-octacosane and from certain crude oils: A and B, fractions formed by the heat treatment of n-octacosane (375°C for 60 hr) in the presence of:- A. bentonite and B. bentonite and water, alkane fractions from C. Nonesuch seep oil, and D. Boscan crude oil.


28
A
16
22
B
16
28
22
C
16
22
I
D
I
22
16
I
I
(mins.) 60
30

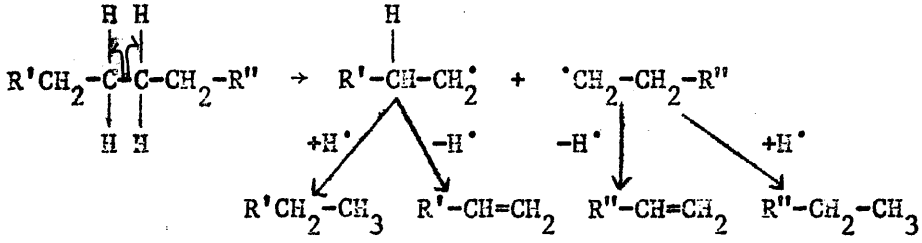
crude oils of very different ages are shown in Figure 4C and 4D. The Nonesuch seep oil exudes in very small quantities from fissures in the Parting shale stratum of the Nonesuch formation which is of Late Precambrian age (ca. 1 x 10^9 years). This shale has been shown to contain various biogenic residues including compounds of isoprenoid type and porphyrins.[105,106] The Boscan crude oil is produced commercially from Zulia, Venezuela (Cretaceous, ca. 1 x 10^8 years). The much older Nonesuch oil gives a chromatogram closely resembling in certain aspects that of the n-octacosane pyrolysis products. The Boscan oil, on the other hand, shows many divergencies from a regularly repeating pattern in accordance with, in our view, less extensive alteration. The slight predominance of even carbon number n-alkanes in this and other oils may be the result of hydrogenation in the sediment of corresponding long chain acids and alcohols.[31,81,99] Bimodal carbon number distributions could conceivably arise by thermal alteration of biological source material of two narrow, rather different carbon number groupings, e.g. plant waxes (ca. C_{30}) and glyceride lipids (ca. C_{16}).

These experiments indicate that an n-alkane (C_nH_{2n+2}), degrades thermally to the complete homologous series of n-alkanes, C_mH_{2m+2} (where $m < n$), and the corresponding n-alkenes, $C_{n-m}H_{2(n-m)}$ (Fig. 5). Other products are formed but we believe

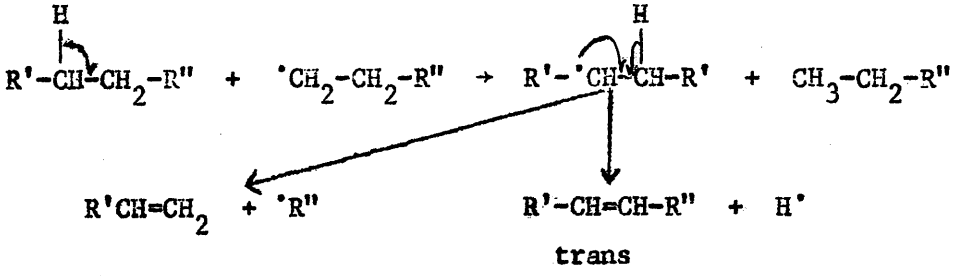
Figure 5

Mechanisms Involved in the Thermal Cracking of Alkanes

Disproportionation by homolytic fission



Hydrogen radical abstraction and disproportionation



that the significant point which bears on the observed distribution of n-alkanes in crude petroleums and sediments is the formation of the homologous series of n-alkanes. Each n-alkane in the mixture undergoing thermal alteration (maturation) will itself be generating a new series of n-alkanes of lower carbon number. This situation merits mathematical treatment. The pattern of abundance of the carbon numbers is affected by the laboratory work-up procedure, since the lower molecular weight alkanes are preferantially lost by volatilisation, resulting in a maximum. Under geological conditions similar losses could become apparent as natural gas accumulations. Branched/cyclic alkanes, such as the isoprenoid hydrocarbon squalane, undergo more rapid thermal cracking but only very small amounts of n-alkane are formed. We hold, therefore, that straight chain lipids must be the main source of the n-alkanes found in petroleum and other ancient sediments.

Experimental

Thermal Cracking of n-Octacosane

Samples of n-octacosane (0.078 g each, 99.9% pure by g.l.c.) were sealed in clean Pyrex glass tubes (4" x 0.75" thick wall) under vacuum and heated (at 375°C for 75, 100 and 150 hr and at 200°C for 1000 hr) in a muffle furnace. After opening the sealed glass tubes, a standard quantity of benzene (2 ml) was added and sonication carried out to ensure complete solution of the soluble organic material. The resulting solution containing fine pieces of suspended carbonised material was centrifuged at 2,500 r.p.m. and the supernatant liquid removed by pipette. The extraction was repeated three times.

Evaporation of the solvent on a rotary evaporator (Buchi) gave the organic extract. The products were examined (Fig. 1) by analytical thin layer chromatography using a silver nitrate impregnated silicic acid layer eluted with n-hexane. This indicated by R_f values and comparison with standard compounds (n-C_{17} alkane, n-C_{17} alk-1-ene and anthracene), that the products contained alkanes, two different classes of alkenes (i and ii) and aromatic hydrocarbons. Infrared spectroscopy confirmed the presence of the two types of olefinic double bond (i) mainly trans-disubstituted (ν_{max}, 965 cm^{-1}), (ii) mainly terminal alkenes (ν_{max}, 990 and 910 cm^{-1}). Separation

into the alkane and aromatic categories was effected by preparative t.l.c., again with a silver nitrate impregnated silicic acid layer eluted with n-hexane. The four fractions were collected and extracted with diethyl ether through a short column of neutral alumina. The yields and relative abundances of the products are given in Table 1. The fractions were analysed by capillary g.l.c. on Apiezon L grease (pre-treated to remove the polar material by passing a hexane solution of the grease through a neutral alumina column), coated on a 175' x 0.01" stainless steel capillary column. The carbon numbers of the fractions were determined by coinjection of standard n-alkanes, the peak areas for each alkane measured and the relative abundances calculated. The resulting bargraph for the n-alkanes is shown in Figure 3.

The low yields obtained for the olefinic and aromatic fractions precluded further investigation, and in fact the yields should be taken as approximations because the balance used had an accuracy of $\pm$ 0.1 mg and the products varied from 0 - 2.0 mg.

Thermal and Catalytic Cracking of n-Octacosane

The catalyst used was bentonite (B.D.H., Technical Grade). It was exhaustively soxhlet extracted with benzene/methanol (3:1), dried and then heated at 500°C for four hours and finally stored in a desiccator over blue silica gel.

n-Octacosane/bentonite/200°C. n-Octacosane (0.075 g) was dissolved in hexane (5 ml) and bentonite (0.75 g) added to the solution. The solution was then evaporated under a stream of N_2 in a glass tube as before, and placed under vacuum for 30 min before sealing. This was heated at 200°C for 170 hr.

The contents of the tube had darkened a little after the treatment and some pressure was noticed on opening the tube. The contents was ultrasonically extracted with benzene/methanol (3:1, twice), centrifuged at 2,500 r.p.m., and the supernatant liquid transferred by pipette, evaporated on a Buchi, and the residue weighed. The extract was dissolved in hexane and analysed by g.l.c. as above. There appeared to have been > 0.01% alteration of the n-C_{28}. The extract was separated by $AgNO_3/SiO_2$ preparative t.l.c., eluting with n-hexane, and three fractions collected. The yields are given in Table 2. The alkanes were analysed by capillary g.l.c. as before. The product alkanes were approximately 1% of the total alkanes, and were mostly in the carbon number C_{26}-C_{28} range.

n-Octacosane/bentonite/375°C. The n-octacosane (0.08 g) was mixed with bentonite clay (0.80 g) as before and the mixture sealed in a glass tube under vacuum and heated to 375°C for 60 hr in a muffle furnace. The contents of the tube after the treatment were completely black and considerable pressure was noticed on opening the tube. The contents were extracted

ultrasonically with benzene/methanol (3:1, three times) centrifuged and evaporated as before. The extract was dissolved in hexane and analysed by g.l.c. on a 200' x 0.01" 7-PPE capillary column. A large conversion had been achieved, i.e. ca. 90%. The products were extremely complex and mostly lower molecular weight material. The extract was separated by $AgNO_3/SiO_2$ preparative t.l.c. as before. The yields and relative abundances of the three fractions are shown in Table 2.

The alkanes were analysed as before using the 7-PPE capillary column (Fig. 4A). The g.l.c. trace is extremely complex with a high proportion of branched/cyclic alkanes. The $\underline{n}$-C_{28} alkane peak represents approximately 5-10% of the total alkane fraction.

n-Octacosane/bentonite/water/375°C. The n-octacosane (0.08 g) was mixed with bentonite (0.80 g) and distilled water (1 ml) and the mixture sealed in a glass tube under vacuum and heated at 375°C for 60 hr in a muffle furnace. The contents of the tube again were black after the treatment and a considerable pressure was noticed on opening the tube. The contents were extracted as before and the extract was separated by preparative $AgNO_3/SiO_2$ t.l.c. and the three fractions collected. The yields and relative abundances are shown in Table 2. The alkanes were analysed on the 7-PPE capillary column (Fig. 4B). The distribution pattern again was very com-

plex and the n-C_{28} alkane peak represented approximately 5% of the total alkanes.

Monesuch and Boscan Crude Oils

The sample of the Monesuch crude oil was supplied by Dr. P. E. Cloud, University of Minnesota, and the Boscan crude oil by Dr. P. A. Schenck, Koninklijke/Shell, Exploratie en Produktie Laboratorium, Rijswijk, The Netherlands.

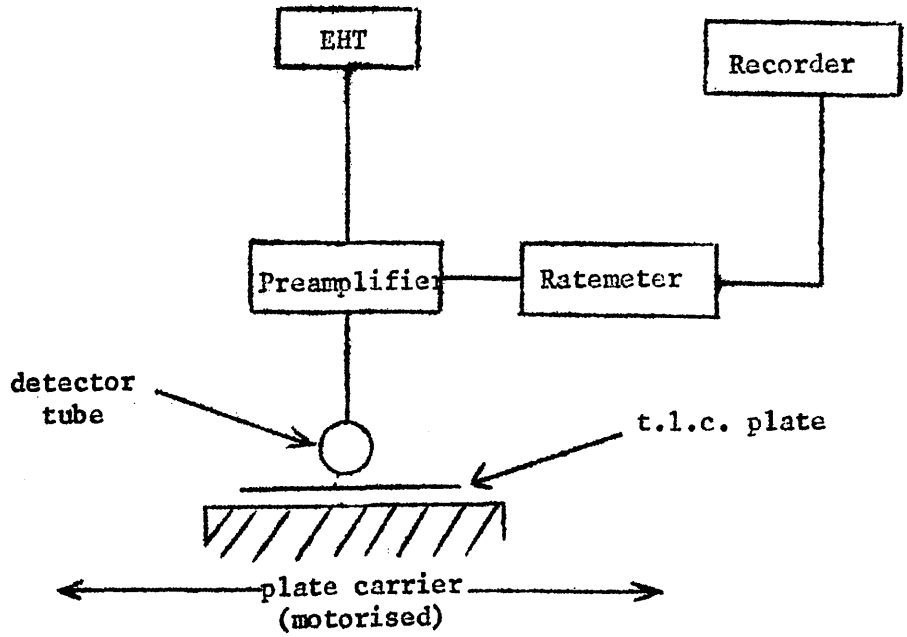
The alkane fractions from these crude oils were obtained by column chromatography on neutral alumina, eluting with n-hexane. These fractions were used for comparison with the product alkanes from the catalytic alteration experiments above and were analysed by capillary g.l.c. on the same column as before (Fig. 4C and 4D).

Radio Scanning of Thin Layer Chromatograms

One of the projects originally planned was the use of C^{14} labelled tracer compounds to follow the course and mechanism of the thermal alteration experiments. None of this work was actually started, but an instrument has been constructed to the original design of Professor A. T. James[107] (Biosynthesis Unit, Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford) with some modifications to count the disintegrations produced by labelled material on t.l.c. plates. The construction was carried out by the staff of the Chemistry workshop (Bristol University). Figure 6 shows a schematic diagram of the scanner

Figure 6

Schematic Diagram of Radio T.L.C. Scanner



components. The motorised plate carrier can be used for t.l.c. plates up to 20 cm x 20 cm in size. The dimensions of the slit in the detector tube are 1 cm x 0.1 cm. The drive mechanism for the plate carrier is linked directly to the drive shaft of a Beckman strip chart recorder, so that the trace produced during a scan can be exactly superimposed on the t.l.c. plate and thus locate the spots or layers containing C^{14} labelled material.

SECTION 2

THERMAL ALTERATION OF THE ORGANIC MATTER IN SEDIMENTS

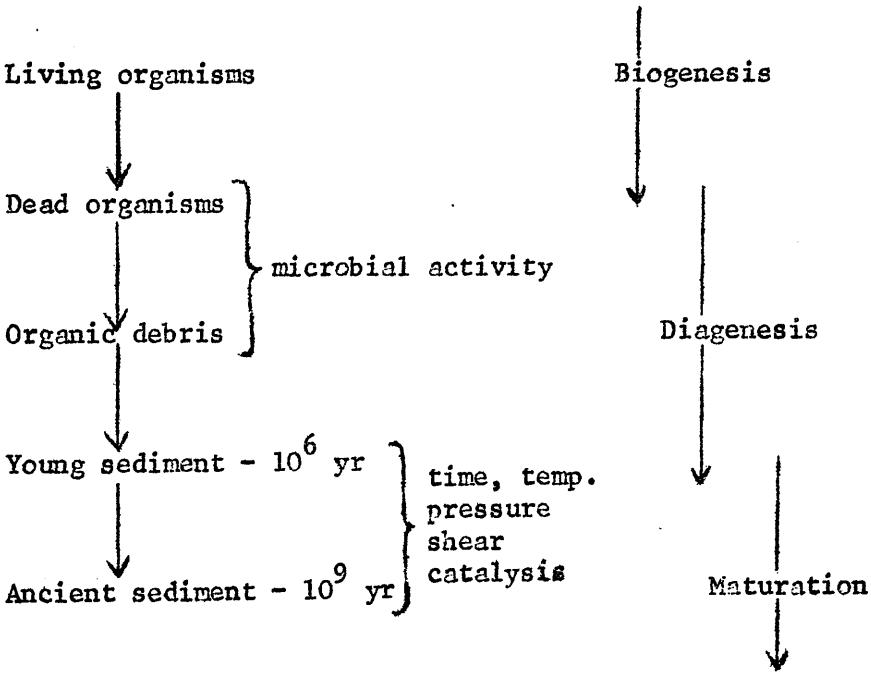
Introduction

This section is devoted to an investigation of the chemical changes undergone by the chemical constituents of organisms and sediments under the influence of elevated temperatures during diagenesis. The formation of compounds during diagenesis has been called "geogenesis" (Fig. 1). This is acceptable to the organic chemist for it implies the formation of compounds under earth conditions, thereby paralleling "biogenesis" in living things.

Our aim is to establish the pattern of change undergone by individual organic compounds, classes of organic compounds and types of organic debris, when present in a sediment exposed to raised temperatures. This has relevance to the geological experience of the higher temperatures occasioned by great depth of burial, earth movements and igneous intrusions. Definite results in turn should lead to the use of this approach as a guide to the past biological, physical and chemical history of a sediment. Commercially, shales are heated to distil out "shale oil".[108,109] In the realm of the space sciences, certain lunar and planetary probes are planned to operate by automated

Figure 1

Geogenesis of Organic Compounds



controlled pyrolysis of surface samples, followed by instrumental analysis and telemetry.[110] Three different geological materials were chosen for this investigation - the Eocene non-marine Green River shale from Colorado, the Carboniferous Torbanite from the Lothians of Scotland, and the hydrocarbon mineral Hatchettite from Wales.

The Green River formation is held on geological and paleontological grounds to be the accumulated sediments from very extensive shallow inland lakes. It is believed that at no time has this formation been deeply buried or subjected to high temperatures;[111] Jones and Vallentyne[112] suggest, from its alanine content, that it has been subjected to a maximum temperature of 74°C. It therefore provides a model for thermal alteration experiments since the compounds identifiable in the unaltered shale can be compared with those present in the same shale after laboratory thermal treatment. Torbanite, from the Carboniferous Limestone series of the Scottish Lothians, was described as a boghead or cannel coal by Macgregor[113] but more recently Dulhunty[114] has stated that Torbanite and cannel coal form distinct classes, as their respective peat stages resulted from the accumulation of unlike vegetable debris. Its microscopic structure reveals a large amount of alveolar "yellow bodies" which resemble the now living alga Botryococcus braunii,[115] this in turn is regarded as the precursor of the

Quaternary rubbery deposit Coorongite, and related materials.[115,116] Pyrolysis of Torbanite gives a very high yield of oil (90-130 gal/ton) which reflects its high organic content.

Chemical studies on the Green River shale have resulted in the identification of biologically significant alkanes such as a range of isoprenoids including farnesane (C_{15}), pristane (C_{19}) and phytane (C_{20})[13,14] and a number of steranes and triterpanes[47,48] and in Part II of this thesis. The n-alkanes show the appropriate marked dominance of odd numbered molecules, particularly n-C_{31}, C_{29}, C_{27}, and C_{17}, the n-carboxylic acids are dominately even numbered, and the presence of the C_{14}, C_{15}, C_{16}, C_{17}, C_{19}, C_{20} and C_{21} isoprenoid acids has also been shown[33,35] while Leo and Parker[117] have reported the presence of iso-acids in this shale. In summary, the alkanes and fatty acids already reported reflect the biological history of the shale to an impressive extent.

Similar chemical studies with Torbanite have resulted in the identification of phytane and pristane; there were however no steranes or triterpanes apparent. The n-alkanes showed no marked predominance of odd numbered molecules; in fact, the c.p.i. value was almost unity.[118] The fatty acids ranged from n-C_{10} to n-C_{28} with a marked dominance of the n-C_{16} and n-C_{18} acids.[33,35] The remaining acids showed no even/odd predominance.

The composition of a room temperature solvent extract of

shale will serve as a basis for comparison with those of the pyrolysates obtained by heating it to elevated temperatures. We can conduct comparable experiments by using a single batch of pulverised shale. Several types of heating techniques were used; (i) heating in a closed vessel in an atmosphere of nitrogen; (ii) heating in a sublimation apparatus under vacuum; and (iii) heating in a glass pyrolysis unit (Fig. 2).

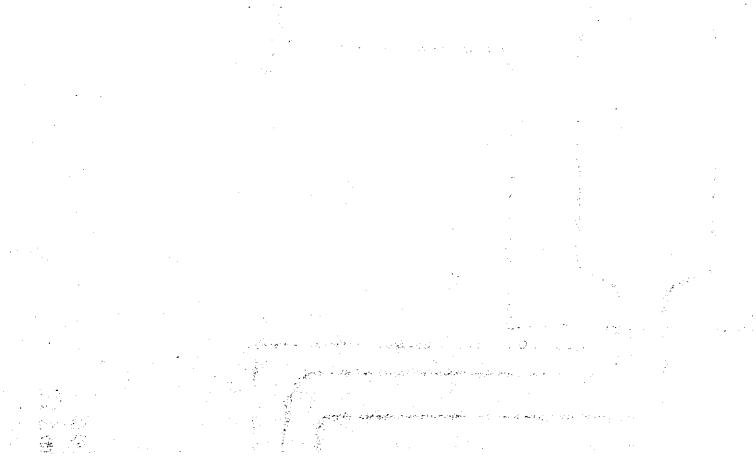
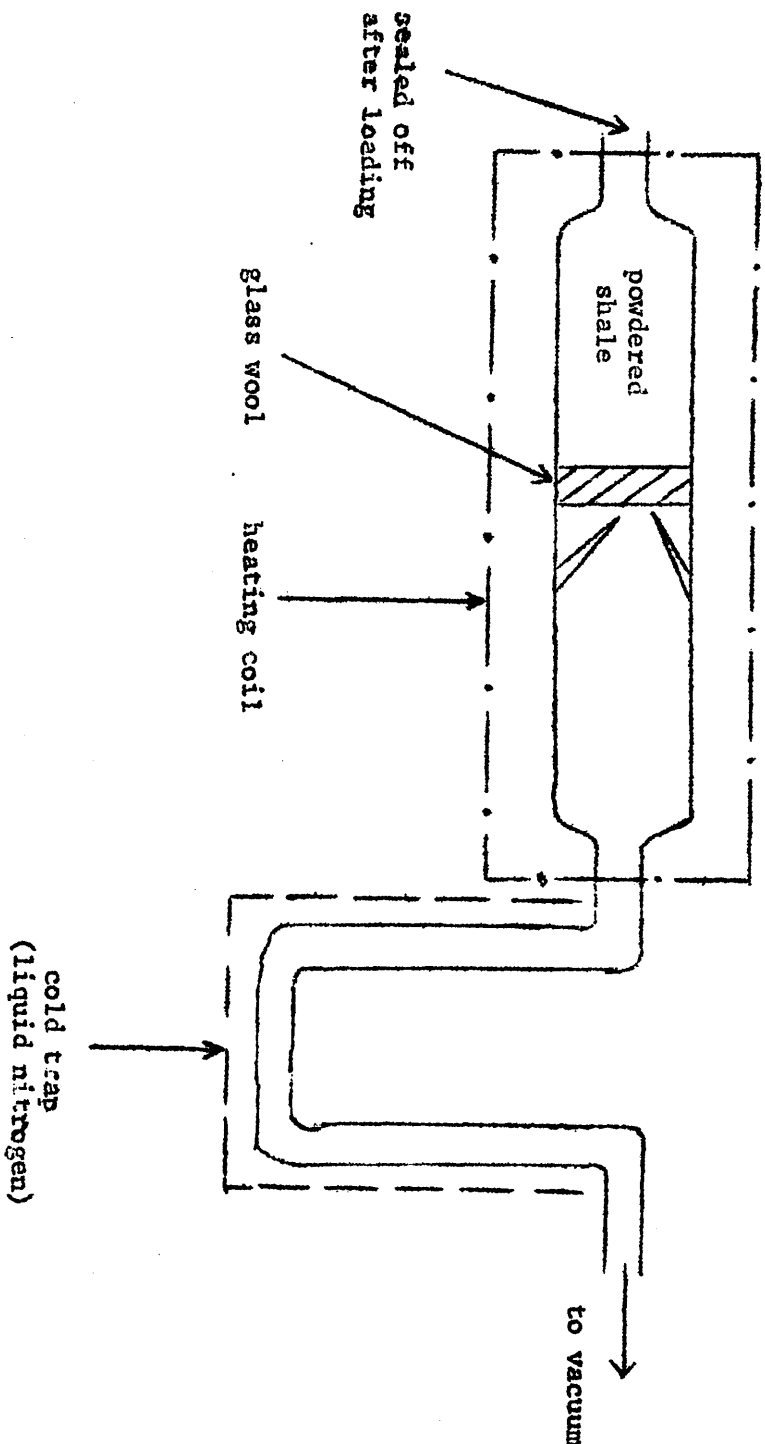


Figure 2

Glass Apparatus for Vacuum Distillation of Shale Samples



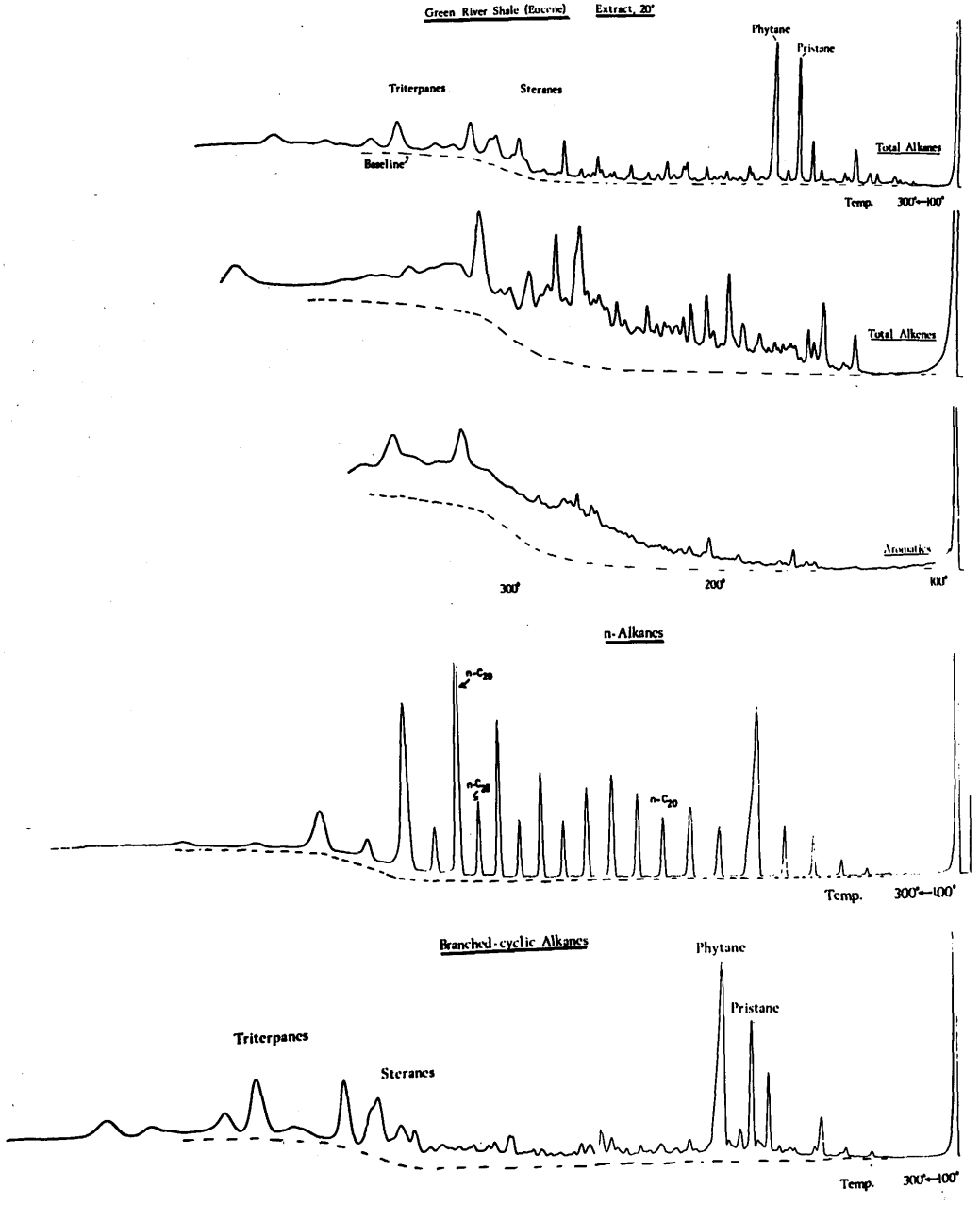
Discussion

The Green River Shale - Control Analysis

The room temperature solvent extraction of Green River shale gave a total alkane fraction, the gas chromatogram of which is shown in Figure 3; the results generally match those found by other workers.[13,14] Unexpectedly, however, in addition to the alkanes, alkenes were also found in this extract, the yield being 0.085% which is approximately one third of the yield of alkanes. The gas chromatogram of these alkenes is shown in Figure 3 and the pattern suggests the presence of a limited number of alkenes in the C_{30} region. Examination of the infrared spectrum of the mixture of alkenes showed not only the presence of trans-disubstituted and vinyl double bonds but also tri-substituted double bonds, and also a high percentage of methyl groups, some being present as gem-dimethyl groups. Since some of these alkenes resist hydrogenation, it would seem reasonable to suspect that some of the higher molecular weight components of this fraction are unsaturated triterpenes. If this suggestion is confirmed then other sediments and also crude oils should be examined for these compounds. Again it would be interesting to see what structural relationships exist between the oxygenated, unsaturated and saturated triterpenes in the Green River shale. Part II of this thesis discusses the

Figure 3

G.l.c. record of the hydrocarbon fractions isolated from the Green River shale at room temperature. The n-alkanes and branched/cyclic alkanes are also shown.


Green River Shale (Eocene)
Extract, 20°
Phytane
Pristane
Triterpanes
Steranes
Baseline
Total Alkanes
Temp. 300°←100°
Total Alkenes
Aromatics
300°
200°
n-Alkanes
n-C29
n-C28
n-C20
Temp. 300°←100°
Branched-cyclic Alkanes
Phytane
Pristane
Triterpanes
Steranes
Temp. 300°←100°

natural distribution and significance of triterpenoids.

The yield of aromatic hydrocarbons (as with the alkenes this is based on a 'cut' taken from a plate coated with silver nitrate impregnated silica gel and does not necessarily mean that all are aromatic hydrocarbons, or alkenes, since the observed R_f value depends on the functional groups, degree of substitution, hindrance due to alkyl substitution, etc) is 0.067% which is approximately $\frac{1}{4}$ that of the alkanes. The peaks of long retention time in the gas chromatogram, Figure 3, may be due to partially aromatic polycyclic compounds of high molecular weight. The most likely origin of such compounds would be dehydrogenation of the triterpenoid or steroid system. The yields of the alkane, alkene and aromatic fractions are shown in Table 1.

Green River Shale - Pyrolysis Distillation at 300°C and 500°C

Comparison of the results obtained from the pyrolysis products of the Green River shale with those from the room temperature solvent extract reveals many differences. Pyrolysis (even at 300°C) produced more volatile organic material than has been obtained by extraction, and the percentage yield increased rapidly with temperature (up to 500°C, Table 1). Secondly, the percentage yields of alkanes, alkenes and aromatic hydrocarbons were greater by a factor of about 10 for the 500°C pyrolysates compared with the room temperature ex-

Table 1

Yields from Pyrolyses of the Green River Shale and the Scottish Torbanite

Shale	Pyrolysis Temp. (°C)	Yield (Total) (%)	Alkane Yield* (%)	Alkene Yield* (%)	Aromatic Yield* (%)
Green River shale	R.T. extract	1.7	56 (0.2)	26 (0.1)	19 (0.07)
"	500	9.2	36 (1.4)	26 (1.0)	24 (0.9)
"	400	5.9	-	-	-
"	300	2.0	67 (0.9)	16 (0.2)	8 (0.1)
Torbanite	R.T.	0.6	0.3	-	-
	500	37	2.7 (8.0)	5.7 (16)	23 (64)

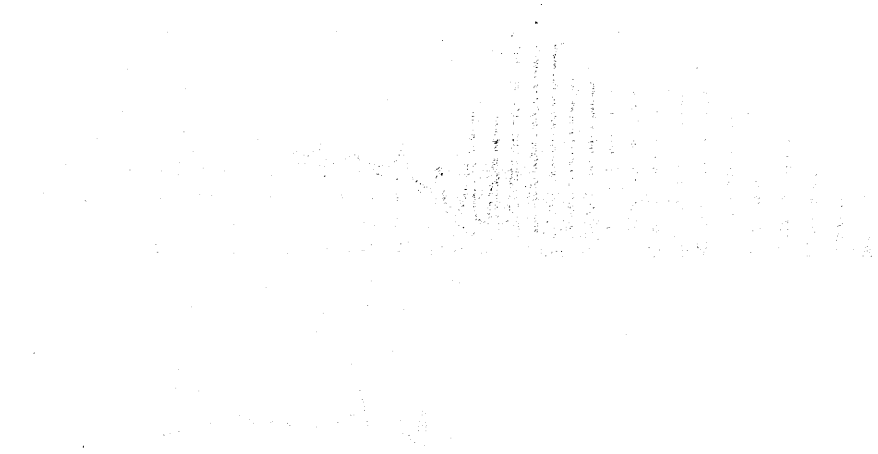
* The yields in parentheses represent the percentage yields by weight of shale used. The other yield figures represent the percentage yield from the solvent-soluble products.

tracts. The general trends observed for the products of pyrolysis at 500°C hold true for the products of pyrolysis at 300°C, but differ only in extent. Since the effects are most apparent in the 500°C experiment, the discussion will be based on these results. Where any divergences may occur from the general trends in the other experiments, these will be specifically mentioned.

When the gas chromatographic patterns for the total alkanes derived from the room temperature extraction (Fig. 3) and the 500°C pyrolysis (Fig. 4), were compared, it was apparent that the proportion of n-alkanes was greater in the pyrolysate. This was confirmed by direct comparison of the chromatographic data for the n-alkane fraction obtained from the total alkanes by the molecular sieve process (Fig. 5). Even numbered n-alkanes appear to have been generated and the c.p.i. value for the pyrolysate was approximately 1.0 compared with 3.6 in the room temperature extract. More n-alkanes appear to have been generated around the C_{30} region which could correspond to simple decarboxylation of the prominent long chain fatty acids in the Green River shale.[33,35] Certain isoprenoids, e.g. phytane and pristane were recognised in the branched/cyclic fraction from the pyrolysate and were identified by combined gas chromatography - mass spectrometry. There were also high molecular weight alkanes in the pyrolysate which could be triterpenoid though there was a marked "background" due to many unresolved

Figure 4

G.l.c. record of the hydrocarbon fractions isolated from the pyrolysate of the Green River shale at 500°C. The n-alkanes and branched/cyclic alkanes are also shown.



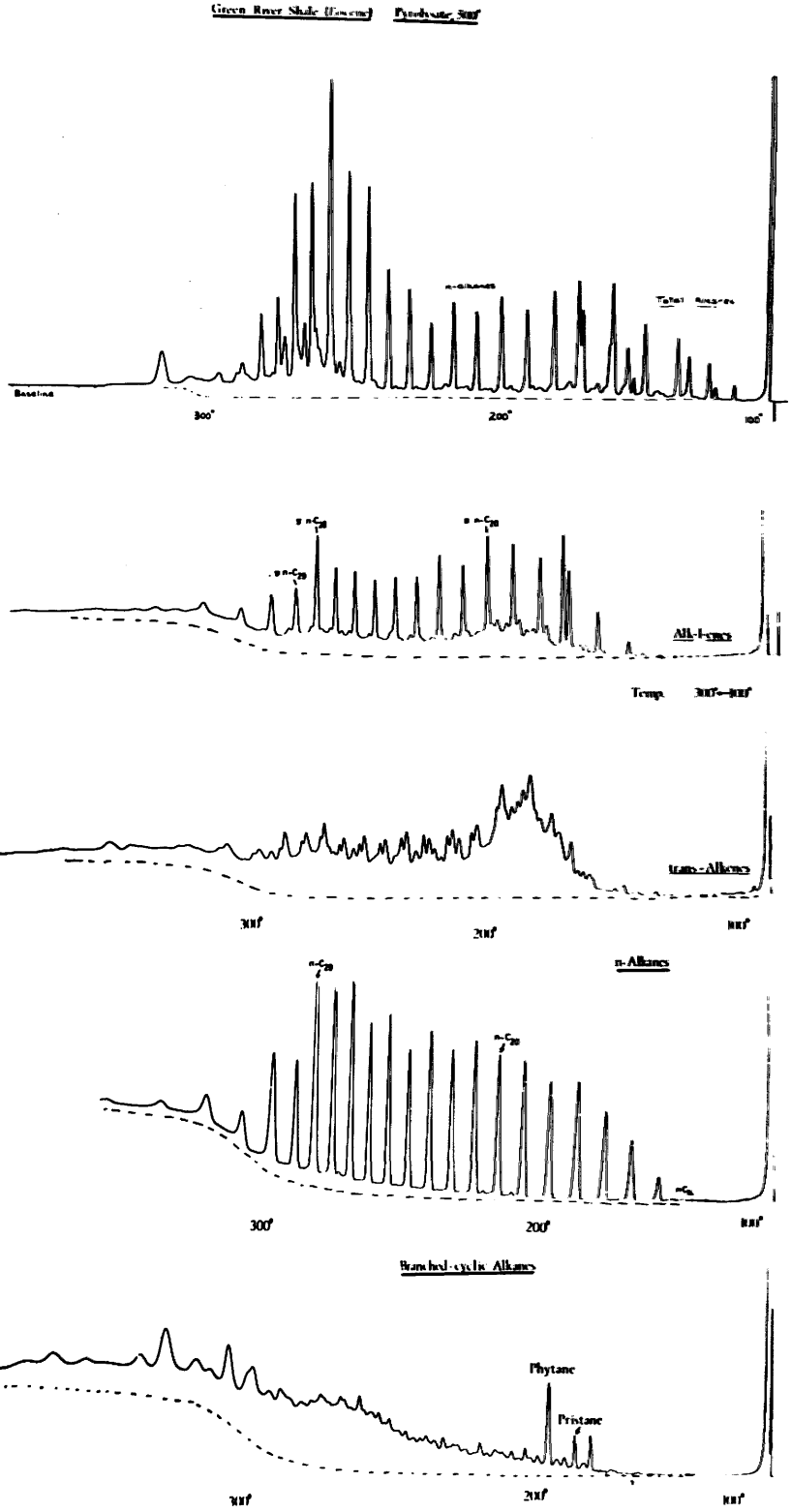
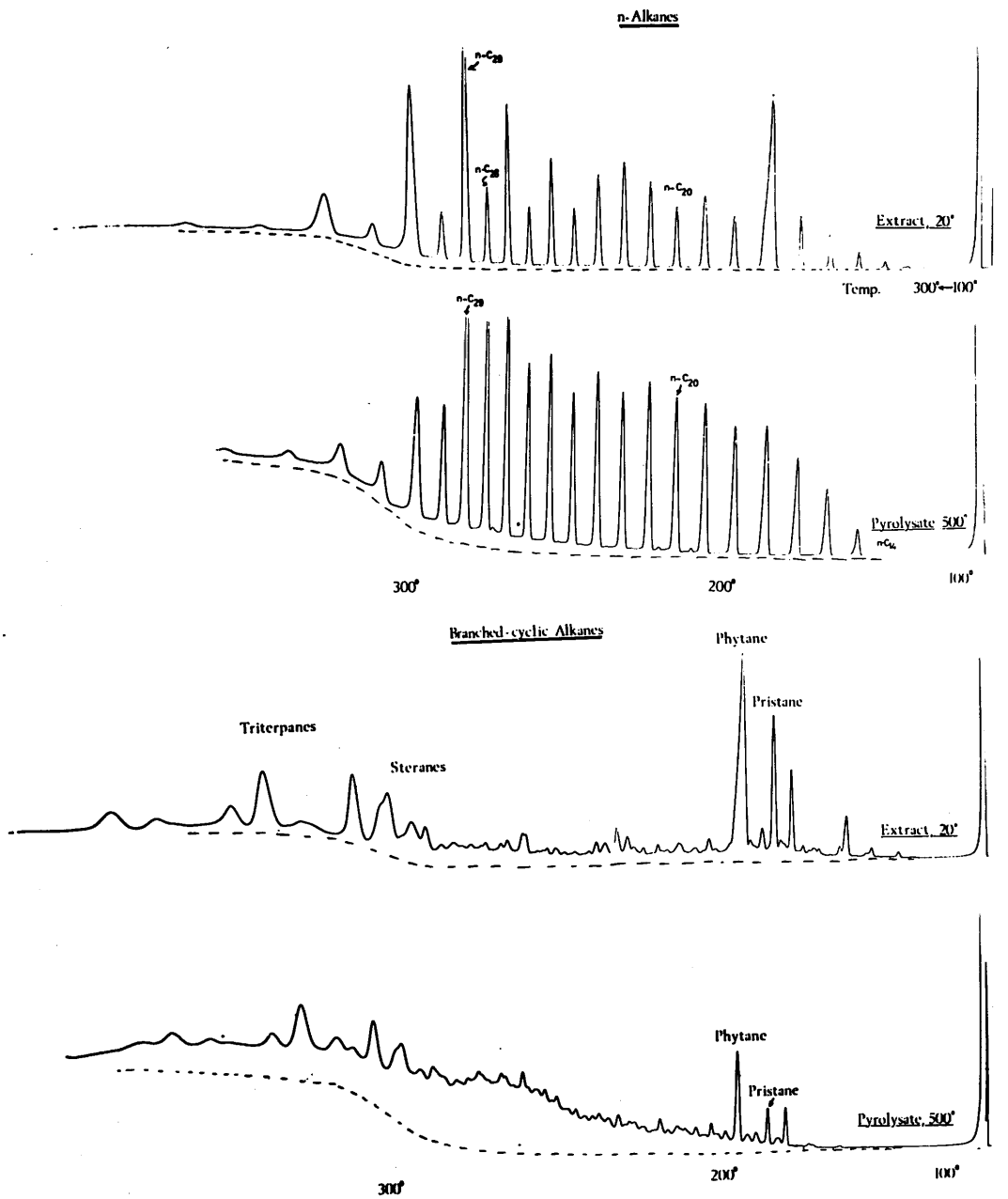

Green River Shale (Eocene) Pyrolysate, 300°
Baseline
300°
200°
100°
All-trans
Temp. 300°—800°
trans-Alkanes
300°
200°
800°
n-Alkanes
300°
200°
800°
Branched-cyclic Alkanes
Phytane
Pristane
300°
200°
800°

Figure 5

Comparison of the n-alkanes and branched/cyclic alkanes from the room temperature extract of the Green River shale with those from the pyrolysate at 500°C.


n-Alkanes
n-C29
n-C28
n-C20
Extract, 20°
Temp. 300°←100°
n-C29
n-C20
Pyrolysate 500°
nC14
300°
200°
100°
Branched-cyclic Alkanes
Phytane
Pristane
Triterpanes
Steranes
Extract, 20°
Phytane
Pristane
Pyrolysate, 500°
300°
200°
100°

peaks in this region of the gas chromatogram. Although the overall amount of the branched/cyclic fraction of the pyrolysate increased it was not possible to state whether the concentrations of individual components had increased or decreased during pyrolysis.

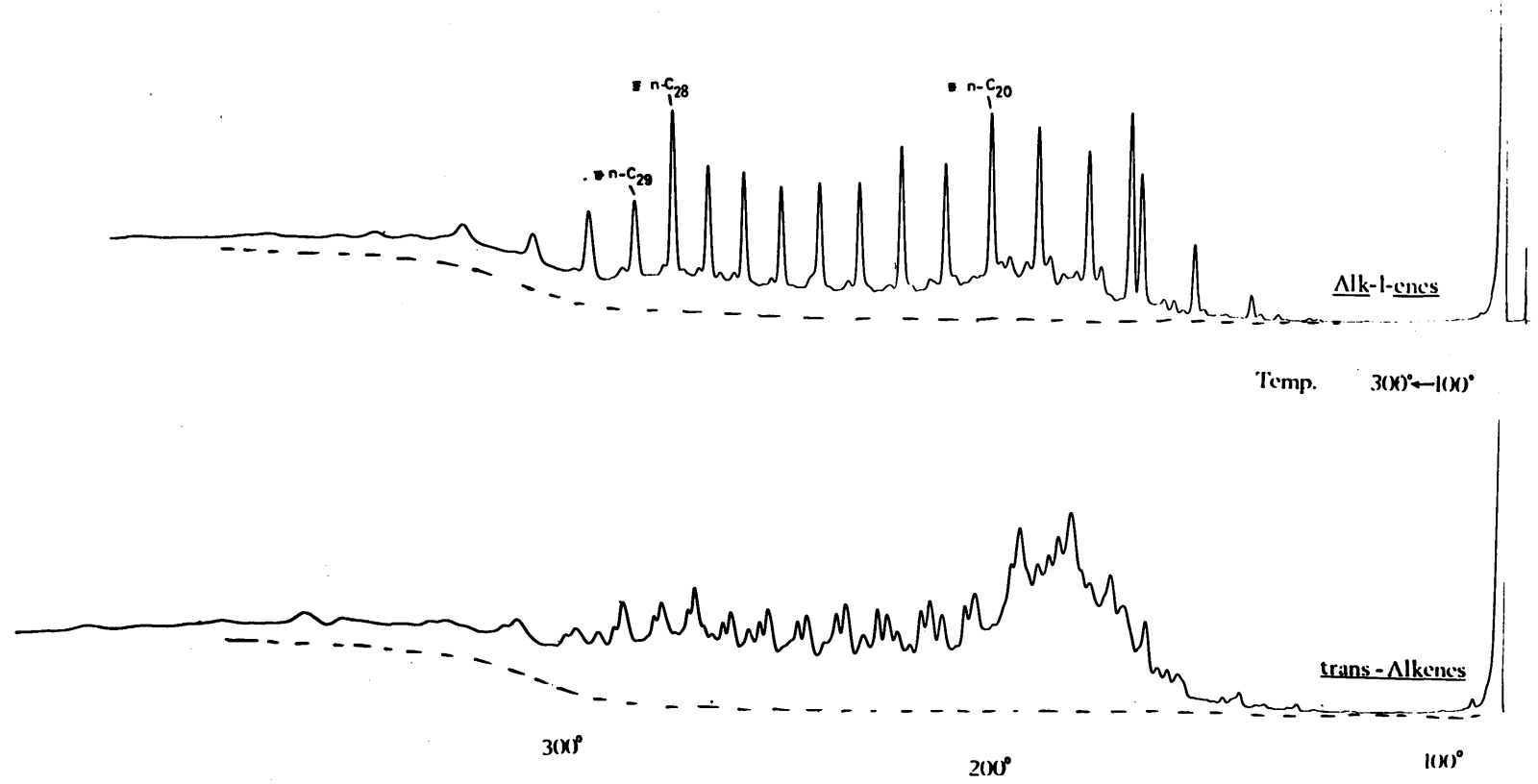
In the pyrolysis experiment, alkanes, alk-1-enes, trans-alkenes and aromatic hydrocarbons were obtained (Fig. 4, Table 1). Similar findings were reported by Iida et al,[120] in a paper published after the above work was carried out. These workers reported the occurrence of n-alkanes, alk-1-enes and trans-alkenes in the range C_{13}-C_{18} in a 'cut' from a Colorado Oil Shale distillate (b.p. 280-305°C), obtained by distilling the shale in a retort and thus effecting thermal alteration.

The gas chromatograms of the two alkene fractions obtained from the pyrolysate are shown in Figures 4 and 6. As with the alkane fractions, the proportion of straight chain compounds was greatly increased in the pyrolysate compared with the room temperature extract (Fig. 3). In the alkene fraction (ii), containing predominately alk-1-enes, it was the even numbered carbon chains, especially n-C_{20} and n-C_{28}, which were predominant. This even number dominance is significant in view of the odd number dominance found for most biologically-originated alkane mixtures; thermally induced generation of hydrocarbons in deeply buried sediments might thus explain the disappearance

Figure 6

The g.l.c. records of the two alkene fractions isolated from the products of pyrolysis of the Green River shale at 500°C.

Green River Shale (Eocene) Pyrolysate, 500° Alkenes



of the odd number dominance. The generation of the alk-1-enes described above might take place by one or more of the following pathways:-

(1) Dehydration of even numbered long chain alcohols could give rise to terminal alkenes of even number. Alcohols vary in the ease with which they undergo dehydration to alkenes, the decreasing order of reactivity being tertiary > secondary > primary. However, the primary alcohols are known to dehydrate fairly readily under certain conditions: thus Roberti *et al*[121] found that cetyl alcohol ($\underline{n}$-$C_{16}H_{33}OH$) gives a 65% conversion to hexadec-1-ene on passing the alcohol over alumina at 350°C.

$$CH_3(CH_2)_{13}CH_2CH_2OH \xrightarrow[350^oC]{Al_2O_3} CH_3(CH_2)_{13}CH{=}CH_2 + H_2O$$

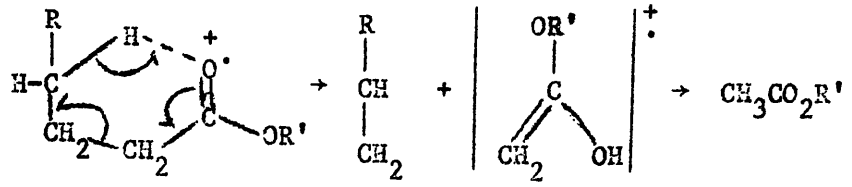
(2) A reaction can be written, formally at least, whereby an even numbered acid or ester would eliminate acetic acid to give an even numbered alk-1-ene

$$R(CH_2)_2CH_2CO_2R^1 \rightarrow R\text{-}CH{=}CH_2 + CH_3CO_2R^1$$

where R^1 = H or metal, or kerogen matrix, or alkyl,

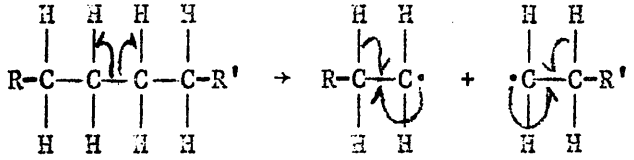
e.g. long chain wax esters.

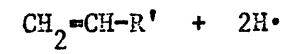
An analogy for this reaction is provided by the classic McLafferty rearrangement[122] undergone by carbonyl compounds (esters, acids, aldehydes, ketones, amides and carbonates) and other functional groups in the ionisation chamber of a mass spectrometer.



(3) Decarboxylation of an even numbered n-fatty acid furnishes principally the corresponding odd numbered alkane, but Jurg and Eisma[77-79] found that behenic acid (C_{22}) when heated with bentonite clay generates, in addition to the C_{21} alkane, a homologous series of n-alkanes and fatty acids with carbon chains both longer and shorter than that of the original acid. They suggest radical processes to account for the formation of these products.

(4) Thermally induced direct scission of carbon-carbon bonds in hydrocarbons furnishes alk-1-enes.





The laboratory experiments of Holman et al[95] demonstrate that heating an n-alkane in helium at 600°C results in a smooth distribution of n-alkenes of steadily diminishing carbon number. Pyrolysis of a predominately straight chain kerogen matrix might be expected to give similar results, and evidence for such a matrix is discussed by Forsman,[123] while oxidative studies of Green River shale by Robinson et al[124] established

the presence of large amounts of open chain acids with no evidence for aromatic acids.

(5) Polymerisation of ethylene, formed during the pyrolytic reaction from long chain material, could give rise to even numbered long chain alk-1-enes. However, there are two objections to this scheme which are difficult to rationalise, (i) the presence of odd numbered alk-1-enes and (ii), the hydrocarbons formed by such a process are usually of a very high molecular weight.

Polymerisation (radical propagation)

$$CH_2{=}CH_2 \rightarrow CH_3(CH_2{-}CH_2)_nCH_2^{\bullet}$$

Termination (radical coupling)

$$CH_3(CH_2{-}CH_2)_nCH_2^{\bullet} + {\cdot}CH_2(CH_2{-}CH_2)_nCH_3 \rightarrow CH_3(CH_2{-}CH_2)_{2n+1}CH_3$$

Termination (radical abstraction)

$$CH_3(CH_2{-}CH_2)_nCH_2^{\bullet} + {\cdot}CH_2HCH(CH_2{-}CH_2)_nCH_3 \rightarrow$$

$$CH_3(CH_2{-}CH_2)_nCH_3 + CH_2{=}CH(CH_2{-}CH_2)_nCH_3$$

(6) The Fischer-Tropsch reaction,[67-71] involving carbon monoxide and water at elevated temperatures and pressures, in the presence of a catalyst, produces as the major products, a smooth carbon number pattern of alk-1-enes and n-alkanes. One can conceive of some form of Fischer-Tropsch reaction taking place in the pyrolytic experiment but this leaves unexplained the dominance of the even numbered alk-1-enes.

The situation in an 'organic rich' sediment like the Green

River shale is complex, when attempts are made to rationalise the formation of the alk-1-enes. It has already been shown that thermal and catalytic cracking of n-alkanes can produce n-alk-1-enes (Section 1), but no particular carbon number dominance was noticed. Obviously all the different types of straight chain aliphatic material present in the shale and in the kerogen matrix can be contributing, but insufficient evidence is available to be specific. Nevertheless, this is an important point, because if generation of alk-1-enes is a possible geochemical reaction, then a dominance of even carbon numbered n-alk-1-enes could on subsequent reduction afford even carbon numbered n-alkanes thus accounting for the c.p.i. value tending to unity with increasing age and depth of burial of a sediment.

It is likely that the trans-alkenes from the pyrolysate are mainly straight chain with the double bond varying in position over the length of the chain and that their generation is probably related to that of the alk-1-enes. Isomerisation of the latter is one possibility, another would involve generation by hydrogen abstraction processes, e.g.

$$R\cdot + -CH_2-CH_2-CH_2-CH_2- \rightarrow RH + -CH_2-\cdot CH-CH_2-CH_2-$$

$$R\cdot + -CH_2-\cdot CH-CH_2-CH_2- \rightarrow RH + \underset{\text{trans}}{-CH_2-CH=CH-CH_2}$$

The alkanes, alkenes and aromatic hydrocarbons may have to be considered together as products of pyrolysis. For example,

alkanes formed by pyrolysis might, in the presence of hydro-aromatic systems, give rise to alkanes and aromatic hydrocarbons by hydrogen abstraction. The aromatic hydrocarbons could arise by cyclisation of unsaturated intermediates,[125] by hydrogenolysis and by dehydrogenation of cycloalkanes; this latter process may be the more important. Radical processes are the obvious choice to explain these hydrogen transfers and bond breakages.

It is tempting to propose that the alkenes, generated much more slowly under geological conditions, might suffer reduction, thereby providing an explanation for the smooth carbon number distributions of the n-alkanes in ancient sediments. If these pyrolytic experiments are meaningful and the results at least partially transferable to reactions at much lower temperatures over long periods of geological time, then alkanes, alkenes and aromatic hydrocarbons are in all probability geogenetically related. This is indicated by the observed trend of the c.p.i. value of the alkanes of Green River shale from 3.6 to about 1.0 on pyrolysis, and the parallel appearance of the alk-1-enes with slight even carbon number dominance.

The Scottish Torbanite - Pyrolysis Distillation at 500°C

The room temperature extraction results for the Scottish Torbanite were supplied by Dr. J. R. Maxwell.[118] The solvent-soluble extract represented 0.6% by weight of the shale (Table 1), and this afforded 0.3% by weight of alkanes. The

n-alkanes showed a smooth distribution with an odd/even c.p.i. of ca. 1. The isoprenoid alkanes farnesane, pristane and phytane were present, but the branched and cyclic alkanes were dominated by the n-alkanes. The high molecular weight n-alkanes (C_{27}, C_{29}, C_{31}) typical of contemporary plants and Recent sediments were not present in significant quantities. The n-alkane distribution had a carbon number maximum at the n-C_{20} position as indicated by g.l.c., and indeed, was typical of that shown by most crude oil alkane fractions. This implies that the primary organic material had undergone extensive alteration in the sediment, in contrast to the Green River shale.

Pyrolysis of the Torbanite at 500°C, afforded products representing 37% by weight of the shale, i.e. approximately 60 times greater than the solvent-soluble extract. After separation of the products by preparative $AgNO_3/SiO_2$ t.l.c. in the usual way, the yield of alkanes was 2.7% by weight of the shale, i.e. 10 times greater than the solvent-soluble alkane fraction. On analysis by g.l.c., the alkane distribution was very similar to the room temperature extract alkanes, the same carbon number range, but the carbon number maximum had shifted from the n-C_{20} to the n-C_{18} position which is not really a significant change. The alkene fraction represented 5.7% by weight of the shale and infrared analysis indicated the presence of both trans and vinyl double bonds in this fraction.

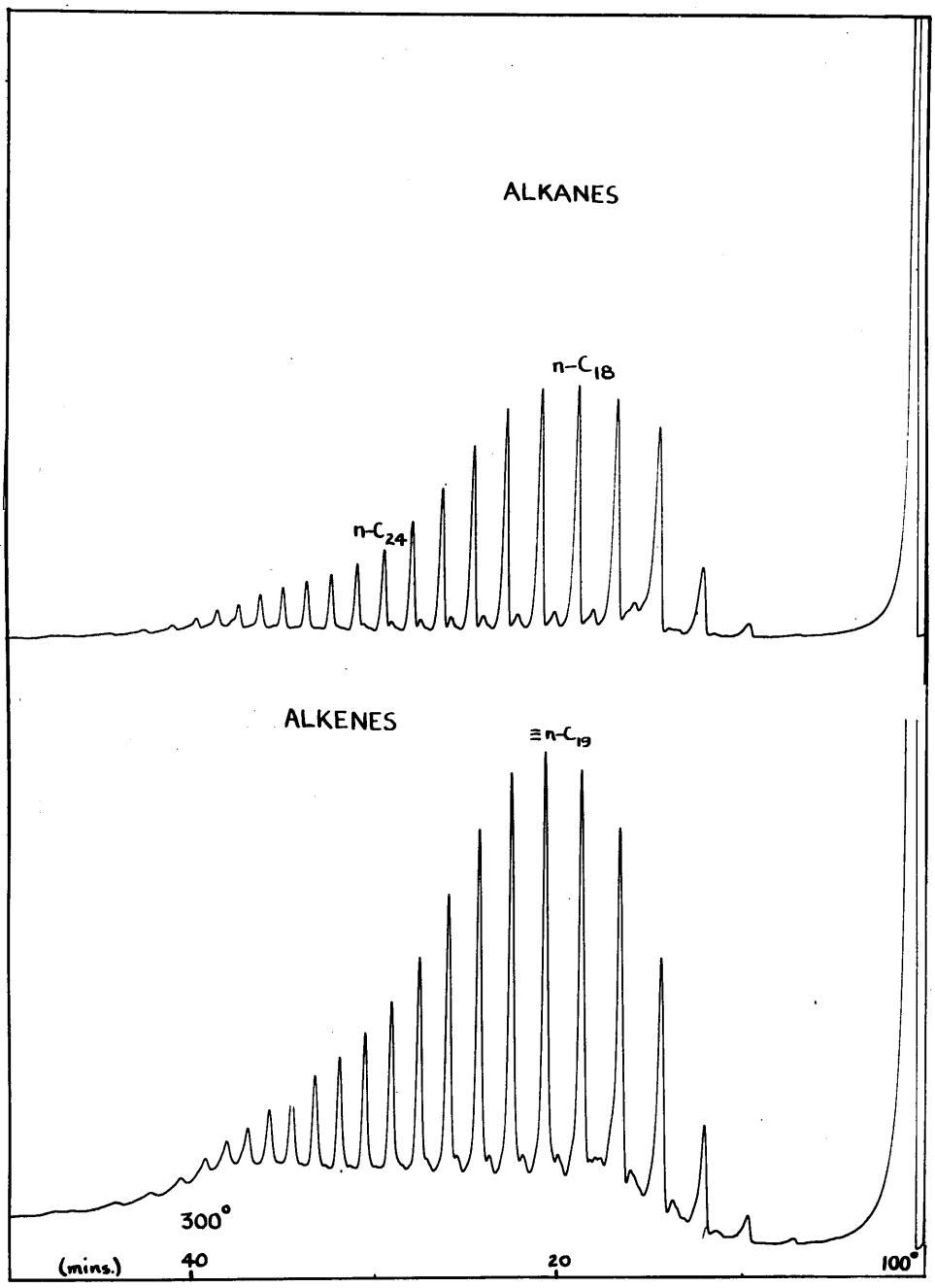
The aromatic fraction represented 23% by weight of the shale. The gas chromatographic records for the alkanes and alkenes are shown in Figure 7. The carbon number positions labelled have been determined by coinjection of standard n-alkanes and n-alkenes. The yields are shown in Table 1.

The general trends noted for the Green River pyrolysis products are continued for this sediment. The yields differ in that the Torbanite releases greater quantities of organic material on pyrolysis. This is further proof of the very high organic content of the Scottish Torbanite and also shows that the major portion of the organic material is contained in the solvent-insoluble kerogen matrix. The high yields of n-alkenes are further evidence in support of the theory that the kerogen is largely composed of polymerised lipid material, containing mostly straight chain material, which can be decomposed by thermal and catalytic cracking to produce straight chain alkanes and alkenes.

The pyrolysis distillation experiments on the Green River shale and the Scottish Torbanite pose an interesting problem. Where the complex mixture of hydrocarbons liberated can distill out of the shale, the yield of distillate (shale oil), is high (500°C, ca. 10% by weight of our sample of the Green River shale and up to 40% by weight for the Scottish Torbanite), the geological parallel would be an igneous intrustion into or near

Figure 7

The g.l.c. record of the alkane and alkene fractions isolated from the products of pyrolysis of the Scottish Torbanite at 500°C.


ALKANES
n-C18
n-C24
ALKENES
≡n-C19
300°
(mins.)
40
20
100°

to a sedimentary formation, the liberated hydrocarbons possibly appearing as veins of hydrocarbon minerals.[126,127] Hunt[128] has shown that igneous intrusion into sedimentary formations leads to thermal decomposition of the organic matter, yielding low molecular weight hydrocarbons. However when the laboratory pyrolysis is conducted in a closed system, the hydrocarbons initially formed suffer further breakdown and eventually carbonisation, as has been shown in Section 1 by the pyrolysis of the n-octacosane at 375°C, and later in this Section by the pyrolysis of the Green River shale at 375°C.

The mineral wax, Hatchettite, to be discussed later in this Section, is a probable example of a vein of hydrocarbon material formed by geological distillation.

Thermal Alteration for Prolonged Periods in Sealed Vessels

In contrast to the previous experiments where the pyrolyses were carried out over a period of three hours, thermal alteration of the Green River shale was conducted for long periods of time at different temperatures in sealed vessels. To maintain strict control, another analysis was conducted on a smaller sample (4 g) of the Green River shale. Since lower temperatures were to be used, resulting in correspondingly lower yields, it was felt that comparable results could only be obtained in these cases from comparable sample sizes. The extraction and analysis of the 4 g sample was conducted in

exactly the same way as before, but a lower yield for the solvent-soluble extract was obtained (0.6% vs 1.7%). The reason for this is not clear and it can only be explained by losses in the work-up procedure becoming more apparent on small samples. This should be investigated for future work and possible amendments to the techniques tried. Here again, the use of tracer (C^{14} labelled) compounds would be extremely useful. By the addition of a known quantity of the tracer (e.g. an n-alkane Uniformly - C^{14} labelled) to the powdered sediment, estimates of losses during the extraction procedure could be made and the sources of loss of material found by monitoring all the solvents after use, the sediment residue and the chromatography adsorbants (both column and thin layer). The yields for the alkane, alkene and aromatic fractions are shown in Table 2. The distribution patterns were the same as those obtained previously.

Green River Shale - Pyrolysis in a Sealed Tube at 375°C

After 150 hours at 375°C the tube was opened to the accompaniment of a violent explosion. Fortunately the tube had been carefully wrapped in clean aluminium foil, so only ca. 15% of the powdered shale was lost. The pressure was probably caused by the formation of low molecular weight gaseous hydrocarbons, as found in the pyrolysis of the n-octacosane. This yielded only a small quantity of solvent-soluble material

Table 2

The Percentage Yields of the Products After Thermal Alteration of the Green River Shale

Starting Material	Temp. (°C)	Time (hr)	Yield[a] of Products[b] (%)	Alkanes Products (%)	Alkenes[c] Products (%)	Aromatics[c] Products (%)
Green River shale (3 g)	375	150	0.07	5	55	40
Green River shale (10 g)	200	250	0.7	70	24	5
Green River shale (10 g)	200	1000	1.0	53	17	30
Green River shale (4 g)	ambient	--	0.6	56	26	17

[a] Yields calculated by weighing (accuracy ± 0.1 mg). [b] Products soluble in benzene/methanol (3:1). [c] Class of compound identified by behaviour on $AgNO_3/SiO_2$ t.l.c. and infrared spectroscopy.

(Table 2), which contained major proportions of fractions resembling olefinic and aromatic hydrocarbons in their chromatographic behaviour. These conditions, presumably involving both thermal and catalytic processes, had extensively altered the organic content of the shale and had resulted in almost total destruction of the alkane fraction, including the steranes and triterpanes present in the untreated shale. The shale residue was completely black in appearance, thus testifying to the extent of carbonisation affected at this temperature in a closed system. The very low yields (Table 2), e.g. ca. 0.1 mg for the alkanes from a 3 g sample of shale, make the figures in Table 2 approximations only, because of the inaccuracies involved at this level. Nevertheless, the general trends noted above are still recognisable and the olefinic and aromatic character of the products had increased. The alkenes were not analysed by infrared spectroscopy, but by their retention data on t.l.c., they were tentatively identified as trans and vinyl alkenes as before.

Green River Shale - Low Temperature Thermal Alteration

By contrast, relatively mild thermal treatments at 200°C for 250 and 1000 hr in closed systems brought about an increase in the solvent-soluble extract compared with unheated shale (Table 2). The increased quantity of lower molecular weight compounds must have arisen as a result of thermal and catalytic

cracking of the kerogen. No striking differences were found in the respective alkane fractions compared to the room temperature alkane fraction. However, alight increases in the relative proportions of the higher molecular weight n-alkanes were noticed in the carbon number range C_{20}-C_{26}, accompanied by increases in the proportion of alkanes in the lower molecular weight region, around C_{12}-C_{15} range. The isoprenoid alkanes, farnesane, pristane and phytane were still apparent, the latter two being dominant. When this is considered together with the evidence that even during the 500°C pyrolytic distillations of the Green River and the Torbanite shales, pristane and phytane were not completely destroyed, an interesting point is raised. As stated in Section 1, branched alkanes have a higher thermal cracking rate than n-alkanes because of the reduced activation energy required to cleave a bond adjacent to the carbon atoms bearing the alkyl sidechain. Therefore, one would expect the isoprenoid alkanes to disappear due to fragmentation at a higher rate than the n-alkanes. Since experimental data shows that pristane and phytane do not disappear, one is led to the implication that the kerogen can crack to produce branched chain compounds together with straight chain material. Therefore the kerogen is not composed solely of straight chain polymerised lipid material. This is an important point because of the identifications of pristane and phytane from Precambrian sediments and

many petroleums since the presence of these isoprenoid alkanes is usually accepted as proof of a biological origin for the sedimented organic matter as has been discussed in the Introduction. However, if a sediment does not show in its solvent-soluble extract significant proportions of pristane and phytane, this should not be taken as evidence of an abiogenic origin, until the kerogen of the sediment has been examined, possibly by pyrolysis, for the presence of isoprenoid material.

Hatchettite

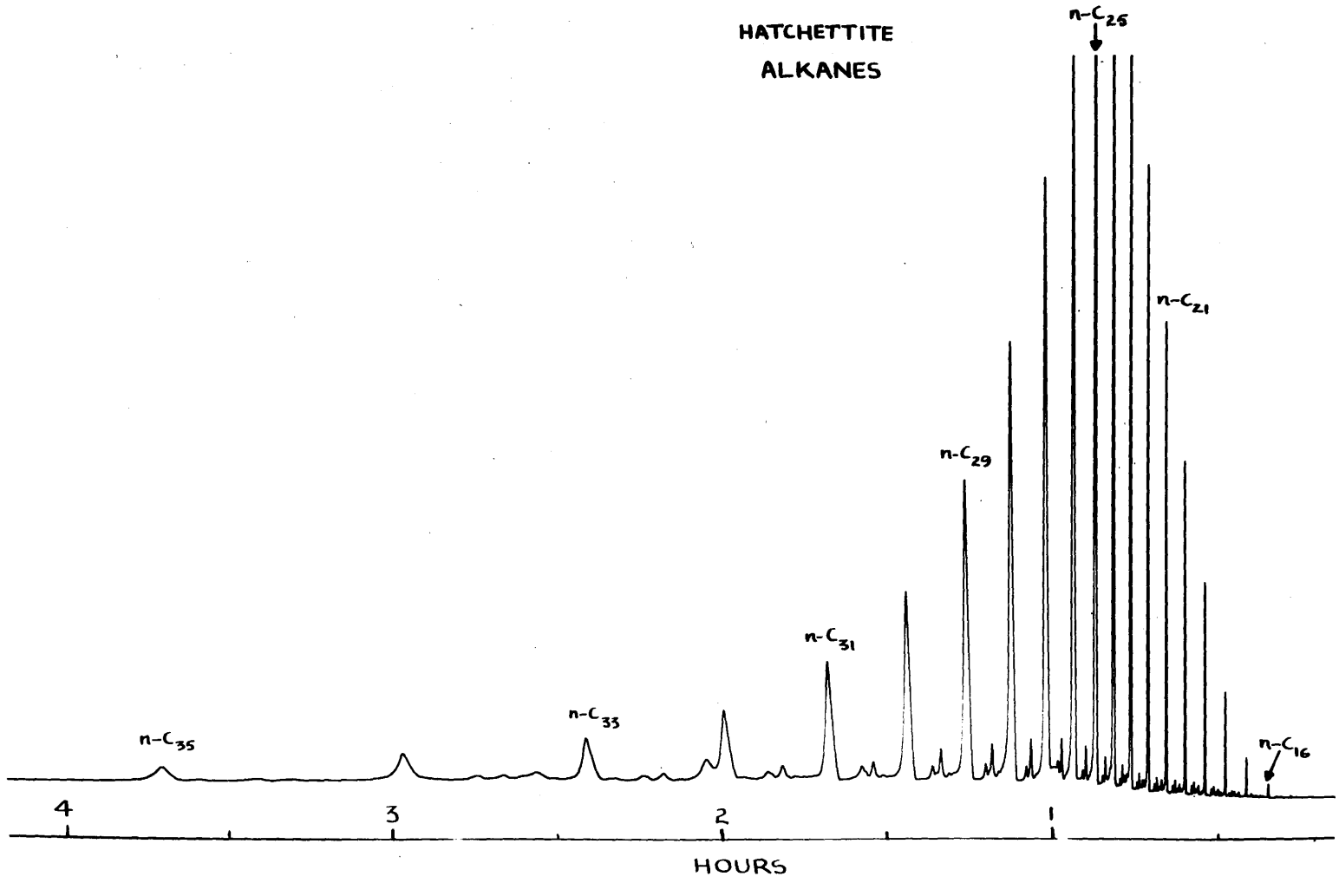
This is a typical hydrocarbon mineral wax which has been classified by Hunt et al,[129] together with Ozokerite, Montan wax and scheerite, as constituents of bitumen. Hatchettite was first described by Coneybeare[130] who found it in clay-ironstone nodules from Merthyr, Glamorgan. The Hatchettite used in this examination was found by Mr. N. J. Firth[131] in clay-ironstone nodules from the Rhondda Fach, Glamorgan, in mine tips from a Lower Coal series formation. The mineral occurs as a yellow-green waxy mass bridging the fissures inside the nodules and is associated with iron and nickel minerals, e.g. siderite and millerite ($NiSO_4$). Firth has proposed that these catalytic minerals probably acted as catalysts for the polymerisation of low molecular weight alkenes to form the Hatchettite deposit (largely saturated alkanes of high molecular weight). However, in view of the preceeding results and discussion of thermal alteration

processes, the author proposes that the Hatchettite deposit is probably a natural example of thermal alteration caused by igneous intrusion into a sedimentary formation. Thus, the sedimented organic material would distil off and crystallise as veins of hydrocarbon mineral wax. Some catalytic polymerisation could occur, but low molecular weight alkenes would probably react to form a random distribution pattern of alkanes with a higher proportion of branched and cyclic material, than is found in the laboratory experiments involving thermal alteration.

The Hatchettite was examined by analytical and preparative silver nitrate impregnated silica t.l.c. and on separation of the fractions, afforded an alkane fraction which was 85% by weight of the wax. The alkane fraction was examined by capillary g.l.c. and a carbon number range of $\underline{n}$-C_{16} - $\underline{n}$-C_{38} was found, with a carbon number maximum at $\underline{n}$-C_{25}, as shown in Figure 8. The distribution pattern was smooth with a small proportion of branched and cyclic alkanes. The carbon number range found for the Hatchettite is similar to that found in nature, i.e. C_{10} - C_{40}. There was no evidence for the presence of cycloalkanes such as steranes or triterpanes. Thus these results seem to fit the experimental laboratory data in that thermal alteration of sedimented organic material at temperatures up to 500°C generate mostly $\underline{n}$-alkanes both from the kerogen and non-kerogen material. Also, the biological distribution patterns for the $\underline{n}$-alkanes,

Figure 8

The g.l.c. record of the alkane fraction isolated from the mineral wax, Hatchettite, analysed by capillary g.l.c.


HATCHETTITE
ALKANES
n-C25
n-C21
n-C29
n-C31
n-C33
n-C35
n-C16
4
3
2
1
HOURS

are readily transformed to smooth distribution patterns similar to those found for ancient sediments and petroleums.

EXPERIMENTAL

General

Preparation of Shales

The Green River shale and the Torbanite (provided by the Hunterian Museum, University of Glasgow) were prepared for examination by first washing the exterior of the pieces of rock with a mixture of benzene/methanol (1:1) and then pulverising in a disc mill for 15 min to give a powder which passed through a 100 mesh sieve.

The thermal alteration experiments with powdered shale samples were carried out using four different procedures. To ensure representative samples of the shale, 2 g portions from a large quantity of sieved and thoroughly mixed powdered shale were used. These aliquots were then compressed using a hydraulic press at a pressure of 8 tons/sq.in. in an infrared sodium chloride die (13 mm). These compacted pellets were then placed in small Parr pressure bombs fitted with Teflon seals and sealed under nitrogen and the bombs placed in an oven at the required temperature.

Thermal Alteration Techniques

Secondly, a small insulated heating coil was constructed, consisting of asbestos paper wet-moulded around a glass tube of

the required diameter and left to dry. Then 11 ft of 2.82ohm per ft resistance wire was wound carefully onto the asbestos tube. The insulation was provided by further layers of wet-moulded asbestos paper on top of the resistance wire and finally completed by two layers of asbestos rope. The temperature was controlled by a Variac and the coil and the Variac carefully calibrated. The dimensions of the heating coil were 6" x 1" i.d. Glass vacuum distillation units were constructed from Pyrex glass with a cold trap (Fig. 2). The powdered shale was loaded into the charge space with clean glass wool and the end sealed. A vacuum was applied with shaking of the tube using an electric oil pump and finally the unit placed inside the heating coil and the open ends of the coil insulated with glass wool packing. The vacuum distillation was then carried out at the required temperature using the Variac control.

Thirdly, the powdered shale was placed in clean Pyrex glass tubes under vacuum (oil pump) with shaking and the open end sealed. The sealed tubes were then placed in a muffle furnace at the required temperature. Finally, sublimation of the organic material from the powdered shales was carried out using a sublimation unit and clean sublimation tubes under vacuum (oil pump).

Extraction of Pyrolysis Products

The pellets used in the prolonged thermal treatment of

the shales and the powdered shales were afterwards extracted ultrasonically with a mixture of benzene/methanol (3:1). The suspension was centrifuged and the supernatant solution was evaporated to give a crude extract. The pyrolysates which collected in a cold trap from the pyrolysis of the shales, were recovered using a solvent mixture of hexane, benzene and ethyl acetate, and then evaporated to furnish the crude extract.

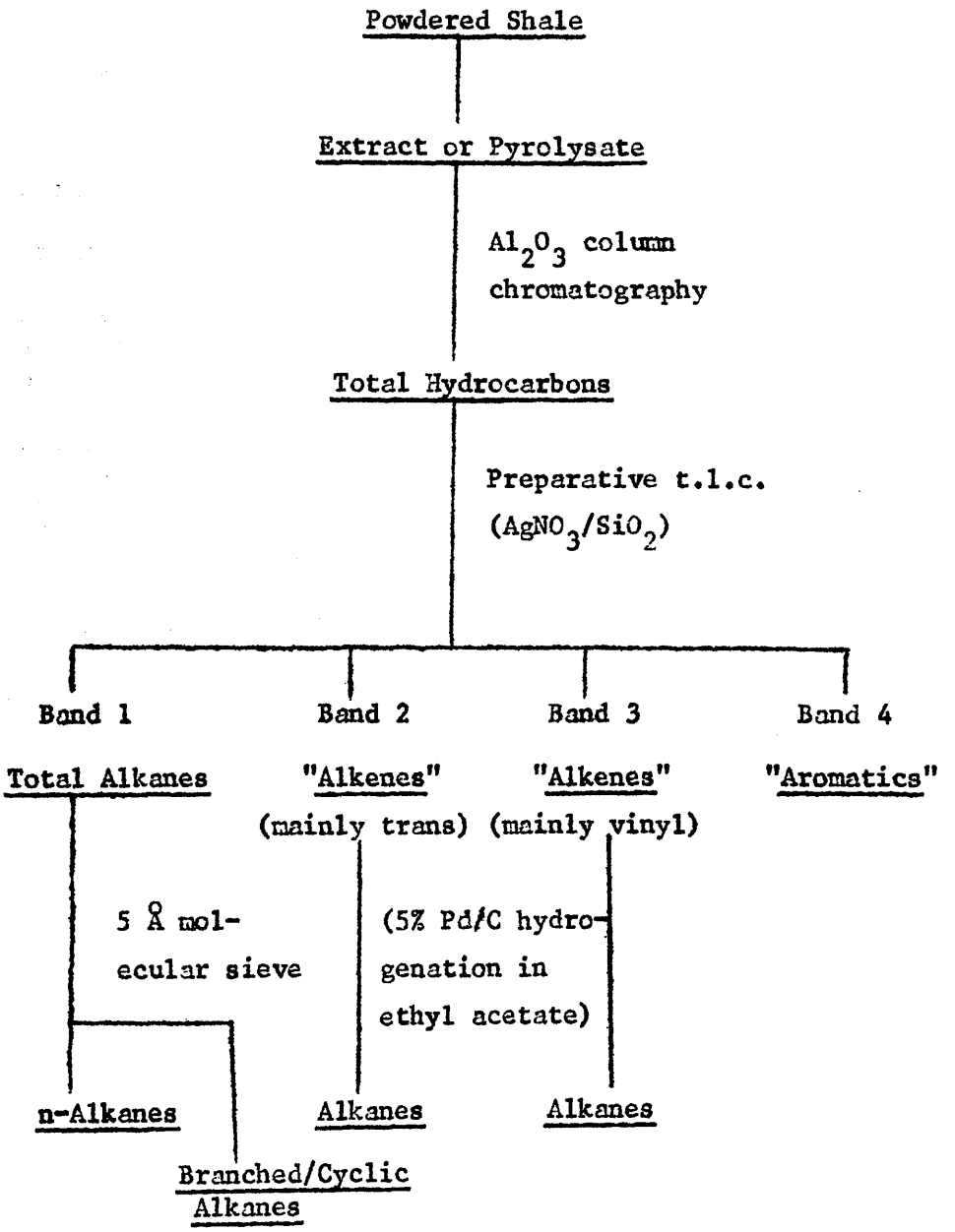
The analytical techniques used in the separation and work-up of the different classes of compounds included column chromatography with neutral alumina, thin layer chromatography (t.l.c.) using silica gel impregnated with silver nitrate, both on analytical and preparative scales, and finally gas-liquid chromatography (g.l.c.). The spectroscopic techniques included ultraviolet (u.v.) and infrared (i.r.) spectroscopy and mass spectrometry. The final identification step usually involved combined gas chromatography - mass spectrometry (g.c.-m.s.). Full details of these techniques are provided in the General Experimental for Part II of this thesis.

The separation and analytical procedure for the hydrocarbon fractions from the shale extracts and pyrolysates is shown in Figure 9.

The alkanes were sieved using $\frac{1}{8}$" pellets of 5 Å molecular sieve activated to 375°C for 24 hr. Separation of n-alkanes from branched and cyclic alkanes could still be achieved with

Figure 9

Flow Diagram Showing the General Separatory and Analytical Techniques Used for the Isolation and Analysis of Hydrocarbons



quantities of mixed alkanes of less than 1 mg when the apparatus was scaled down to an appropriate size. A detailed description of the sieving procedure is given in the General Experimental section for Part II of this thesis.

This work was mostly conducted in the early part of the three year period covered by the thesis and some of the techniques have now been improved so that better results are possible. In particular, the use of g.l.c. has been greatly enhanced by the advent of capillary columns. All but one of the g.l.c. Figures shown in this Section were obtained on packed columns and therefore were low resolution analyses. The analysis of the Hatchettite was conducted in 1968 and therefore capillary columns were used, thus providing a comparison of the advantages of using high resolution g.l.c. when Figure 8 is compared with Figures 3, 4, 6 and 7 in which packed columns were used.

EXPERIMENTAL

Room Temperature Solvent Extraction of Green River Shale as Control

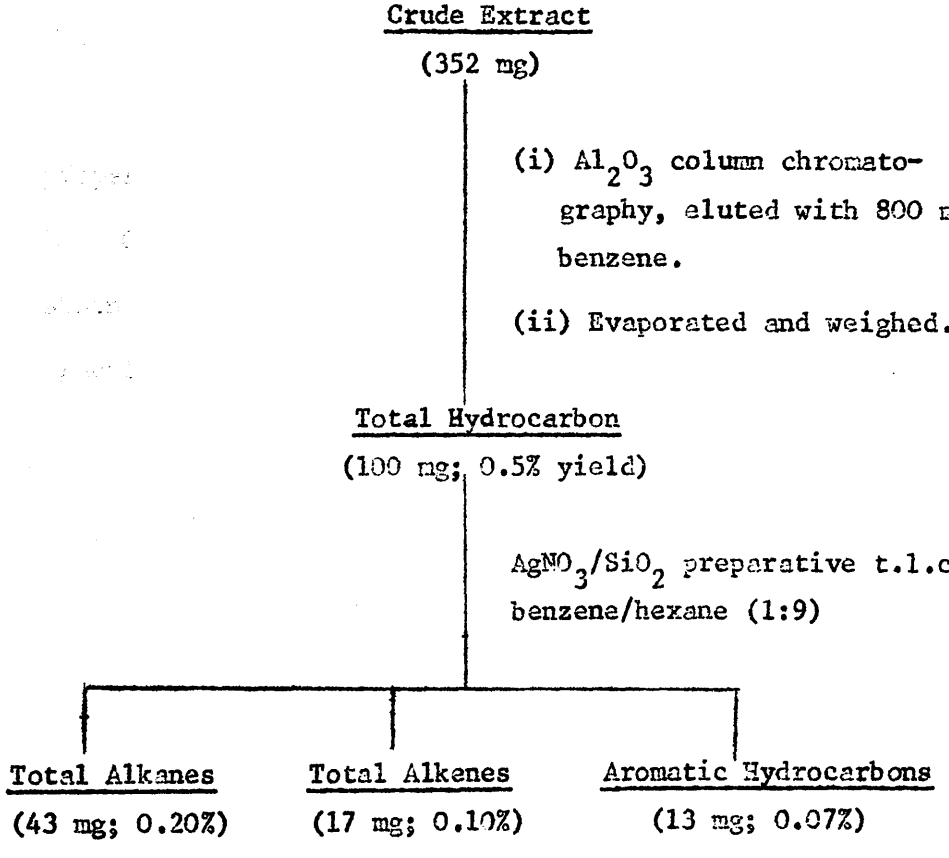
Powdered Green River shale (20 g) was ultrasonically extracted with benzene/methanol (3:1) for 5 hr with 5 changes of solvent. The total extract was then evaporated to give a gum (0.352 g, 1.76%) which was treated as summarised in Figure 10.

Column chromatography was carried out using neutral alumina (100:1) activated at 120°C for 30 min and the eluents were hexane and benzene. Evaporation of the eluate furnished the total hydrocarbon fraction, which was then separated into alkane, alkene and aromatic hydrocarbons by preparative t.l.c. on 1 mm layers of silica gel containing 10% silver nitrate. Development with hexane/benzene (9:1) afforded four bands which were 'cut-out' and eluted with diethyl ether to extract the fractions. The purity of each fraction was checked by analytical t.l.c., i.r. and g.l.c. (The yields are shown in Table 1).

The total alkane fraction was separated into normal and branched/cyclic fractions by boiling a solution of the alkanes (1 part) in iso-octane with 5 Å molecular sieve (60 parts) for three days. This gave 12% n-alkanes and 78% branched/cyclic alkanes, with 10% loss. The overall yield per 20 g of shale was, 0.02% n-alkanes and 0.17% branched and cyclic alkanes.

Figure 10

Flow Diagram for the Separation of the Total Alkane, Alkene and Aromatic Fractions from a Room Temperature Solvent Extract of Green River Shale



Infrared examination of the alkenes indicated the presence of trans-disubstituted and tri-substituted double bonds at 965 cm^{-1} and 815 cm^{-1} respectively. The alkanes were hydrogenated using 5% palladium on charcoal as catalyst and ethyl acetate as solvent. Hydrogenations were carried out in a micro-scale apparatus which permitted the efficient hydrogenation of less than 1 mg of material. The hydrogenated products were isolated in the normal way (filtration through alumina, evaporation, preparative t.l.c.) and then examined by analytical g.l.c.

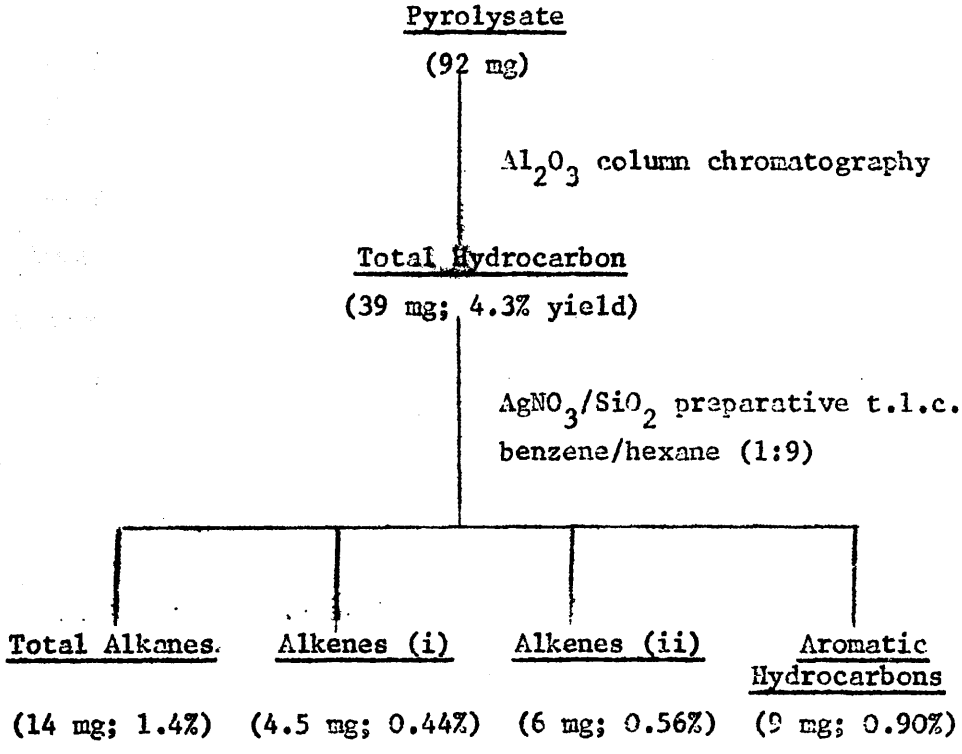
Gas chromatograms of the above hydrocarbon fractions are shown in Figure 3. The branched/cyclic and normal hydrocarbons, specifically or generally indicated have been identified by previous workers[12-14] and in Part II of this thesis. The identities of most of these peaks have been confirmed by combined g.c.-m.s. at Glasgow University on the LKB 9000.

Pyrolysis of Green River Shale at 300°C and 500°C

Powdered Green River shale from the same batch as above (1 g) was pyrolysed under vacuum (at 300 and 500°C, 0.3 mm Hg) for three hours and the distillate (e.g. at 500°C, 92 mg, 9.2%) was collected in a cold trap. This was then separated as shown in Figure 11 and the yields are shown in Table 1. An i.r. examination of the alkene fraction (i) showed absorption due to trans double bonds (965 cm^{-1}) with possibly some tri-substituted double bonds (weak absorption near 820 cm^{-1}), while alkene

Figure 11

Flow Diagram for the Separation of the Alkane, Alkene and Aromatic Fractions from the 500°C Pyrolysate of Green River Shale



fraction (ii) showed absorption due to vinyl double bonds (990 and 910 cm^{-1}) only. The total alkanes were sieved as before which gave the n-alkanes (45% of the total alkanes, 0.60% by weight of shale) and the branched/cyclic alkanes (55% of the total alkanes, 0.70% by weight of shale). The normal and the branched/cyclic alkanes were then examined by combined g.c.-m.s.; the C_{17}, C_{20} and C_{29} n-alkanes, pristane and phytane were identified (Fig. 4). The alkene fraction (i) was hydrogenated with 5% palladium/charcoal in ethyl acetate. However, as before, incomplete hydrogenation occurred, again, probably due to the known difficulty in reducing sterically hindered, tri- and tetra-substituted, double bonds.

The alkene fraction (ii) was hydrogenated as above and the alkanes obtained were examined by combined g.c.-m.s. This showed that the prominent peaks correspond to the normal alkanes ranging from C_{15}-C_{32}. No attempts were made to identify the small peaks.

Pyrolysis of the Scottish Torbanite at 500°C

The yields and composition of the alkane and alkene fractions are summarised in Table 1. Some data for the room temperature solvent extract are given for comparison purposes.[113] Pristane and phytane were present in the branched and cyclic fraction; there were no alkenes apparent and the aromatic fraction was not examined. After pyrolysis at 500°C the alkane fraction had

increased to 2.7% with a c.p.i. value of about 1.0. There were now 5.7% of alkenes present, mainly n-alk-1-enes and trans-n-alkenes with a c.p.i. value of about 1.0, and an aromatic fraction of about 22%. These results are similar to those found for a distilled oil supplied by industry,[113] obtained by retorting the shale. The difference in the percentage of alkanes at 20°C and 500°C is not likely to be due to the differences in the isolation technique, but rather to the formation of hydrocarbons as discussed earlier.

Room Temperature Extraction of a 4 g Sample of Green River Shale

Two pellets (2 g each) of powdered Green River shale from the same batch as before were extracted with benzene/methanol (3:1, 3x1/2 hr), affording a 0.6% yield of extract. Column chromatography on neutral alumina with hexane as eluent resulted in a hydrocarbon fraction whose yield was 0.2% by weight of shale. Preparative t.l.c. on silver nitrate impregnated silica separated the hydrocarbon fraction into the alkane (0.11% by weight of shale), alkene (0.05% by weight of shale), and aromatic (0.04% by weight of shale) categories (yields are shown in Table 2), using n-hexane as eluent. G.l.c. analysis of the alkane fraction revealed the same distribution pattern as before.

Pyrolysis of the Green River Shale in a Sealed Tube

Powdered Green River shale (3 g) from the same batch as before, was sealed under vacuum in a clean glass tube and heated

at 375°C for 150 hr. The tube exploded on opening with a resulting loss of ca. 15% of the contents. The explosion was probably caused by the formation of large quantities of low molecular weight hydrocarbons which placed the glass tube under great pressure. After extraction as usual with benzene/methanol, preparative t.l.c. on silver nitrate impregnated silica was used to separate the extract into the alkane, alkene and aromatic categories. The yields are shown in Table 2. The most notable feature was the almost total destruction of the alkane content of the shale. Only 5% of the extract was of alkane character (cf. 56% in the control experiment). The alkane fraction was examined by capillary g.l.c., revealing no outstanding peaks.

Prolonged Thermal Alteration of the Green River Shale in Sealed Vessels

Four pellets (2 g each) of the powdered Green River shale were placed in Parr bombs (fitted with Teflon seals) under an atmosphere of nitrogen and heated at 200°C for periods of 250 and 1000 hr. After the heat treatment the pellets were extracted as usual with benzene/methanol ultrasonically. The extracts were separated by analytical and preparative t.l.c. on silver nitrate impregnated silica, using n-hexane as eluent. The yields for the alkane, alkene and aromatic fractions are shown in Table 2. The alkane distribution patterns were examined by analytical g.l.c. using a packed column and a capillary column and on two

different g.l.c. instruments to be reasonably sure that any minor differences observed were reproducible under different analytical conditions. The general trends appeared to be that there were slight increases in the proportions of the n-alkanes in the carbon number range C_{20}-C_{26}, accompanied by similar increases in the lower range C_{12}-C_{15}. However it is not possible to make detailed comparisons between the control experiment, the 250 hr thermal treatment and the 1000 hr thermal treatment, because the first two were conducted at Glasgow before the large scale extraction of the Green River shale was carried out in collaboration with Mr. B. Urquhart. The analysis of the large scale extract revealed an interesting result. The total extract was carefully separated using column chromatography (neutral alumina) with n-hexane as eluent. Unexpectedly, this did not elute all the saturated hydrocarbons from the extract. On subsequent elution of the column with benzene, a fraction was obtained which was solely long chain n-alkanes, predominately n-C_{25} and upwards. Previously, it had been assumed that the elution with n-hexane would furnish all the alkanes from an extract, and this had been the procedure used for the control experiment and the first 250 hr thermal treatment of the Green River shale. The last experiment, the 1000 hr thermal treatment, was carried out with this knowledge, and the hexane and benzene eluates were combined before further separation with preparat-

ive t.l.c. Thus the alkane fractions from these three experiments are not strictly comparable. Nevertheless, the above general trends are still largely valid.

However, because of the differences in the separation techniques, the prolonged thermal alteration experiments on the Green River shale should be repeated before definite conclusions can be drawn from the results. The revised separation technique should be used and the analysis of the alkane and alkene fractions should be conducted using capillary columns so that small differences in the distribution patterns of these fractions are more easily observed. Since these experiments have to be repeated, it would be worthwhile conducting additional experiments using C^{14} labelled compounds such as an alkane and a fatty acid, added to the sediment and treated to the same conditions as before so that further insight into the mechanisms may be obtained, since the radio t.l.c. scanner and radio g.l.c. instruments should be available shortly.

Hatchettite

The mineral wax was dissolved in methylene chloride/hexane (1:1) and the alkane fraction obtained by separation using preparative t.l.c. on silver nitrate impregnated silica, eluting with n-hexane. The alkane fraction was extracted from the adsorbent as usual and represented 85% by weight of the wax. Analytical capillary column g.l.c. afforded the alkane distribution

pattern shown in Figure 8. The column used was a 200' x 0.01" stainless steel column coated with 7 ring metapolyphenylether. Peak identification was carried out by coinjection of authentic n-alkanes.

CONCLUDING REMARKS

The experiments with n-octacosane, with the Green River shale, and with the Scottish Torbanite show that even brief exposures, in geological terms, to temperatures of 200°C or over must result in extensive alteration of sediments containing organic debris. The absence of odd/even carbon number preference in the n-alkane fractions isolated from crude oils derived from deep wells,[101] where temperatures can reach 200°C is thus readily explained. Certainly a lack of odd/even predominance cannot be taken as evidence of either abiotic synthesis[67-69] or an unusual origin (in the sense of a smooth carbon number distribution for the constituent n-alkanes, n-fatty acids, etc),[8,11,12] since thermal alteration will quickly eliminate any carbon number alternation. Considering the extremely complex nature of the organic debris in a sediment, it is obvious that no one class of organic compound such as the alkanes can be solely responsible for the production of petroleum. Experimental evidence is available which bears on the thermal and catalytic alteration of other types of compound, such as the fatty acids,[77-79] alcohols[21,76,99] and isoprenoid hydrocarbons.[37,51,90]

A catalytic clay such as bentonite can only be a poor approximation to the complex mineral matrix comprising most sediments. It is commonly assumed that such matrices possess

marked catalytic properties and to some extent these have been demonstrated by laboratory experiments involving heat treatment of sediments.[98-102] In effect, thermal alteration in the laboratory of a complete sediment such as the Green River shale can provide information on the alteration of a very wide range of compounds, but the situation is experimentally complex. The Green River shale itself has had a mild thermal history[112] and the extractable alkanes have carbon numbers and carbon skeletons very suggestive of a plant origin,[13,47,48] typified by the cuticular waxes of land plants.[9] Most carbon skeletons formed biologically range in carbon number from C_{10}-C_{40} and Mair[24] has pointed out that probably well over 95% of the hydrocarbons in petroleum have carbon numbers below 40.

Welte[100,101] and Mitterer and Hoering[99] using kerogen alone and in the original sediment, have arrived at similar conclusions that thermal alteration is an important diagenetic process, while Brooks and Smith[102] have related the n-alkane patterns of different coals to their rank and the presumed extent of thermal alteration. In an earlier survey, Mueller[126] related the composition of hydrocarbon minerals to the presence of hydrothermal veins. Recent studies,[132,133] involving the oxidative and hydrolytic degradation of the Green River kerogen demonstrate the abundance of long alkyl sidechains. The kerogen is presumably formed during early diagenesis and represents an accumulation

of many types of compounds interlinked and cross-linked by a variety of covalent bonds.

The relatively high abundance of the straight chain material in the pyrolysates of Green River shale and Scottish Torbanite represents pre-existing straight chain material 'cracked' from the kerogen matrix. Welte[100,101] found that pre-extracted samples of a non-marine Eocene oil shale when heated gave fresh extractable material, the c.p.i. value of which decreased with increasing temperature, becoming unity at temperatures above 300°C. It seems unlikely that the straight chain hydrocarbons derive from pre-existing branched chain materials[95] since high temperature pyrolysis of n-alkanes yields a homologous series of n-olefins while branched alkanes yield mainly branched olefins. If this inference is correct, then pyrolysis experiments will give useful results about the nature of kerogen. This type of experiment will be useful in the laboratory and, possibly, in automated planetary probes.

Isoprenoid hydrocarbons such as pristane and phytane are major constituents of some crude oils and sediments of Precambrian age. These compounds can be generated from kerogen by thermal cracking, their production thereby countering their more rapid destruction.[99]

The thermal cracking processes require the formation of equal amounts of alkanes and alkenes. Alkenes are normally trace

constituents of petroleums and sediments, so if these mechanisms apply under geological conditions, then the initially formed alkenes must be removed, possibly by conversion to other products such as cyclic and aromatic hydrocarbons.[93,102,125] Other processes which may operate in a sediment include, hydrogenation,[99] oxygenation and polymerisation.[38] Olefins and alkanes undergo thermal cracking at about the same rate, but in the presence of an acidic catalyst such as bentonite the olefins form carbonium ions more readily and crack at a much high rate.[92] Olefins formed by thermal cracking might well undergo isomerisation, rearrangement, cyclisation and hydrogen transfer in the presence of such catalysts.[38,99] While thermal cracking produces high concentrations of n-alkanes, catalytic cracking therefore produces mostly branched and cyclic alkanes.[92] A combination of both processes could explain the relative proportions of normal, branched and cyclic alkanes found in sediments, particularly when the supposed biological source material is deficient in cyclic and branched carbon compounds. Water is present in sedimentary deposits during diagenesis but it is evident from the laboratory experiments that water does not inhibit these alteration processes, though it does affect the composition of the products.[77-79]

Thermal alteration of most sediments and crude oils does not appear to have proceeded to anywhere near completion, which

is in accord with the observation that sedimentary formations generally experience temperatures in the range 20-200°C. For this range we have no laboratory data. But, it seems reasonable that processes bringing about alteration of organic materials at 200°C and above in the laboratory may also be effective in sedimentary formations at much lower temperatures, over geological time measured in millions of years. The organic debris is trapped in close contact with the silicate matrix and the varying proportions and type of the two materials will affect the extent of the thermal and catalytic alteration. Furthermore, extensive physical and chemical data from the Los Angeles and Ventura basins have led Philippi[104] to conclude that the bulk of oil generated in these basins takes place at temperatures above 115°C. Estimates of the extent of alteration which could conceivably take place during a given period of geological time and within a given range of temperature have been made and are said to preclude extensive thermal (radical) cracking. Kinetic data are not available for the catalytic cracking which may occur under sedimentary conditions. Nevertheless, it appears from the foregoing results and the work of other researchers that thermal and catalytic alteration of biological source material in sediments occurs to an appreciable extent and must be, at the least, a contributory factor in the genesis of petroleum, becoming of increasing importance with increasing age, depth of

burial and temperature.

Future work which could be done in this area is varied and extensive. The thermal alteration of sediments, e.g. the Green River shale, at temperatures of 200°C and lower, needs to be repeated with particular attention being paid to the consistency of the extraction and separation procedures. The use of capillary column g.l.c. will allow small differences in distribution patterns of the alkanes to become more readily apparent. Experimental data obtained for several different temperatures will allow the use of the Arrhenius equation and therefore will produce kinetic data which may then be used to predict the extent of alteration possible under different sedimentary conditions.

The use of C^{14} labelled alkanes should give further insight to the mechanisms involved in these alteration processes. A particularly interesting and crucial experiment would be the addition of a C^{14} labelled isoprenoid alkane such as phytane to a sediment, before the thermal treatment. In this way some kinetic data could be obtained about the rate of destruction of isoprenoid alkanes in a sediment, not bound up in the kerogen matrix, which could then be compared with the rate of generation of the isoprenoid alkanes from the kerogen under the same conditions.

In a similar way, the results of Mitterer et al[99] could be further investigated. The addition of labelled alkanes to the

sediment would allow their rate of reduction to be established quantitatively and could also be used to quantitate the dehydration of alcohols.

Further work needs to be done on the thermal and catalytic alteration of individual organic compounds, like the n-alkanes, the branched and cyclic alkanes, the fatty acids and alcohols. The use of labelled compounds would be an invaluable aid in these experiments. The effects of alteration require to be studied at many different temperatures with many different catalysts, including the use of powdered shales (previously having had the organic content removed).

Work should be started in the areas disclosed by Part I of this thesis. The effects of igneous intrusions into sedimentary formations has to be studied on authenticated specimens of metamorphosed sediments and coals, and attempts made to relate the differences in distribution patterns of the alkanes found for different degrees of metamorphosis and thus thermal alteration.

Finally, a search for geogenetic markers should be started. This would mean attempting to isolate and identify compounds from untreated shales which could be related to some degree of thermal and/or catalytic alteration, or any of the other probable diagenetic processes, e.g. dehydrogenation of steranes and triterpanes to produce partially aromatised compounds.

This type of approach would be exactly analogous to the long-standing search for biological markers. In this work, a considerable contribution could be made by laboratory experimentation involving simulated environments. A typical example of a useful experiment could be: a plant of known steroid and triterpenoid content could be subjected to simulated conditions for a sedimentary deposit, i.e. suspension under water with powdered minerals and a reducing atmosphere provided; after varying periods of time, the effects of this traatment on the steroid and triterpenoid content could be examined. In this way, data might be obtained which could help to explain how, and when, cycloalkanes are formed in a sediment. The variety and scope of possible experiments appears to be unlimited, when one considers the variety of organic compounds in nature which must eventually be deposited on the earth's surface and then become incorporated in a sedimentary formation, and thus, sometime, experiance diagenetic alteration.

REFERENCES

1 F. E. Wickman, Geochim. Cosmochim. Acta, 9, 136 (1956).

2 J. M. Hunt, Geochim. Cosmochim. Acta, 22, 37 (1961).

3 L. G. Weeks, Habitat of Oil, Amer. Assoc. Petrol. Geol. Meeting, Tulsa, Oklahoma, p.58, 1958.

4 C. Engler, Ber., 21, 1816 (1888).

5 G. Rudakov, Chem. Geol., 2, 179 (1967).

6 W. G. Meinschein, Bull. Amer. Assoc. Petrol. Geol., 43, 925 (1959).

7 R. Robinson, Nature, 199, 113 (1963).

8 K. Sramsky, M. Streibl and V. Herout, Coll. Czech. Chem. Comm., 32, 3213 (1967).

9 G. Eglinton and R. J. Hamilton, in "Chemical Plant Taxonomy", ed. T. Swain, p.187 (1963), Academic Press, London and New York.

10 A. G. Douglas and G. Eglinton, in "Comparative Phytochemistry", ed. T. Swain, p.57 (1966), Academic Press, London and New York.

11 J. Oro, T. G. Tornabene, D. W. Nooner and E. Gelpi, J. Bacteriol., 93, 1811 (1967).

12 J. Han, E. D. McCarthy, W. van Hoeven, M. Calvin and W. H. Bradley, Proc. Nat. Acad. Sci., 59, 29 (1968).

13 J. J. Cummins and W. E. Robinson, J. Chem. Eng. Data, 9,

304 (1964).

14 G. Eglinton, P. M. Scott, T. Belsky, A. L. Burlingame, W. Richter and M. Calvin, in "Advances in Organic Geochemistry 1964", ed. G. D. Hobson and M. C. Louis, p.41 (1966), Pergamon Press, London.

15 R. A. Dean and E. V. Whitehead, Tetrahedron Let., 21, 768 (1961).

16 J. G. Bendoraitis, B. L. Brown and L. S. Hepner, Anal. Chem., 34, 49 (1962).

17 B. J. Mair, N. C. Krouskop and T. J. Mayer, J. Chem. Eng. Data, 7, 420 (1962).

18 G. Eglinton, Geol. Rund., 55, 551 (1965).

19 G. Eglinton and M. Calvin, Scientific American, 216, 32 (1967).

20 M. Blumer, M. M. Mullin and D. W. Thomas, Science, 140, 974 (1963).

21 R. L. Martin, J. C. Winters and J. A. Williams, Nature, 199, 110 (1963).

22 J. E. Cooper, Nature, 193, 744 (1962).

23 D. L. Lawlor and W. E. Robinson, Div. Petrol. Chem., Amer. Chem. Soc. Preprints, 10, 5 (1965).

24 B. J. Mair, Geochim. Cosmochim. Acta, 28, 1303 (1964).

25 T. P. Hilditch, "The Chemical Constitution of Natural Fats" (1956), John Wiley, New York.

26 R. G. Ackman and J. C. Sipos, Comp. Biochem. Physiol., 15, 445 (1965).

27 P. H. Abelson and P. L. Parker, Carnegie Inst. Washington Year Book, 61, 181 (1962).

28 P. H. Abelson, T. C. Hoering and P. L. Parker, in "Advances in Organic Geochemistry 1962", ed. U. Colombo and G. D. Hobson, p.169 (1964), MacMillan, New York.

29 P. L. Parker and R. F. Leo, Science, 148, 373 (1965).

30 K. A. Kvenvolden, Annual Meeting Geol. Soc. Amer. Abstracts, p.91, November 1965.

31 K. A. Kvenvolden, Nature, 209, 573 (1966).

32 W. G. Meinschein and G. S. Kenny, Anal. Chem., 29, 1153 (1957).

33 A. G. Douglas, K. Douraghi-Zadeh, G. Eglinton, J. R. Maxwell and J. N. Ramsay, in "Advances in Organic Geochemistry 1966", ed. G. D. Hobson and G. C. Speers, in press (1968), Pergamon Press, New York.

34 G. Eglinton, A. G. Douglas, J. R. Maxwell, J. N. Ramsay and S. Stallberg-Stenhagen, Science, 153, 1133 (1966).

35 J. N. Ramsay, M.Sc. Thesis, Glasgow University (1966).

36 P. Haug, H. K. Schnoes and A. L. Burlingame, Science, 158, 772 (1967).

37 J. G. Erdman, Geochim. Cosmochim. Acta, 22, 16 (1961).

38 B. T. Brooks, Ind. Eng. Chem., 44, 2570 (1952).

39 I. A. Breger, Geochim. Cosmochim. Acta, 19, 297 (1960).

40 D. H. R. Barton, W. Carruthers and K. H. Overton, J. Chem. Soc., 788 (1956).

41 P. Haug, H. K. Schnoes and A. L. Burlingame, Geochim. Cosmochim. Acta, 32, 358 (1968).

42 W. Carruthers and A. G. Douglas, Nature, 192, 256 (1961).

43 B. J. Mair and J. M. Barnewell, J. Chem. Eng. Data, 9, 282 (1964).

44 I. R. Hills and E. V. Whitehead, Nature, 209, 977 (1966).

45 N. Danieli, E. Gil-Av and M. Louis, Nature, 217, 730 (1968).

46 R. B. Johns, T. Belsky, E. D. McCarthy, A. L. Burlingame, P. Haug, H. K. Schnoes, W. Richter and M. Calvin, Geochim. Cosmochim. Acta, 30, 191 (1966).

47 I. R. Hills, E. V. Whitehead, D. E. Anders, J. J. Cummins and W. E. Robinson, Chem. Comm., 20, 752 (1966).

48 W. Henderson, this Thesis, Part II; and W. Henderson, V. Wollrab and G. Eglinton, Chem. Comm., in press (1968).

49 J. D. Mulik and J. G. Erdman, Science, 141, 806 (1963).

50 I. Mader, Science, 144, 533 (1964).

51 W. C. Day and J. G. Erdman, Science, 141, 808 (1963).

52 M. Blumer, Geochim. Cosmochim. Acta, 26, 225 (1962).

53 M. Blumer, Science, 149, 722 (1965).

54 D. W. Thomas and M. Blumer, Geochim. Cosmochim. Acta, 28, 1467 (1964).

55 P. L. Parker, Contrib. in Marine Science, IV, 135 (1967).

56 E. E. Bray and E. D. Evans, Geochim. Cosmochim. Acta, 22, 2 (1961).

57 E. E. Bray and E. D. Evans, Erdole u. Kohle, 18, 421 (1965); Chem. Abs., 63, 8043h.

58 K. O. Emery and D. Hoggan, Bull. Amer. Assoc. Petrol. Geol., 42, 2174 (1958).

59 J. G. Erdman, E. M. Marlett and W. E. Hanson, Div. Petrol Chem., Amer. Chem. Soc. Preprints, 3, 639 (1958).

60 M. L. Dunton and J. M. Hunt, Bull. Amer. Assoc. Petrol. Geol., 46, 2246 (1962).

61 W. D. Rosenfeld, J. Amer. Oil Chem. Soc., 44, 703 (1967).

62 S. R. Silverman, J. Amer. Oil Chem. Soc., 44, 691 (1967).

63 P. Walden, Chem. Z., 30, 891 (1906).

64 M. R. Fenske, F. L. Carnahan, J. N. Breston, A. H. Caser, and A. R. Rescorla, Ind. Eng. Chem., 34, 638 (1942).

65 F. L. Carnahan, R. E. Hersh and M. R. Fenske, Ind. Eng. Chem., 36, 383 (1944).

66 T. S. Oakwood, D. S. Schriver, H. H. Fall, W. J. McAleer and P. R. Wunz, Ind. Eng. Chem., 44, 2568 (1952).

67 W. G. Meinschein, Space Sci. Rev., 2, 665 (1963).

68 R. A. Friedel and A. G. Sharkey, Science, 139, 1203 (1963).

69 J. E. Lovelock, Nature, 207, 568 (1965).

70 E. D. McCarthy and M. Calvin, Nature, 216, 642 (1967).

71 M. H. Studier, R. Hayatsu and E. Anders, Geochim. Cosmochim. Acta, 32, 151 (1968).

72 G. Natta, L. Porri, P. Corradini and D. Morero, Chim. e Ind., Milan, 40, 362 (1958).

73 G. Natta, Scientific American, 205, 33 (1961).

74 R. Park and S. Epstein, Geochim. Cosmochim. Acta, 21, 110 (1960).

75 R. Park and S. Epstein, Plant Physiol., 36, 133 (1961).

76 H. M. Smith, J. Amer. Oil Chem. Soc., 44, 680 (1967).

77 J. W. Jurg and E. Eisma, Science, 144, 1451 (1964); and in "Advances in Organic Geochemistry 1966", ed. G. D. Hobson and G. C. Speers, in press (1968), Pergamon Press, London.

78 J. W. Jurg, Ph.D. Thesis, Eindhoven University (1967).

79 J. W. Jurg and E. Eisma, in "Organic Geochemistry: Methods and Results", ed G. Eglinton and Sister M. T. J. Murphy in press (1968), Springer-Verlag, New York.

80 J. E. Cooper and E. E. Bray, Geochim. Cosmochim. Acta, 27, 1113 (1963).

81 K. A. Kvenvolden, J. Amer. Oil Chem. Soc., 44, 628 (1967).

82 K. A. Kvenvolden and D. Weiser, Geochim. Cosmochim. Acta, 31, 1281 (1967).

83 R. W. Stone and C. E. Zobell, Ind. Eng. Chem., 44, 2564 (1952).

84 S. I. Kusnetsov, M. U. Ivanov and N. N. Lyalikova, "Introduction to Geological Microbiology" (1968), McGraw-Hill, New York.

85 J. M. Hunt, reprinted from "Carbonate Rocks", ed. G. V. Chilingar, H. J. Bissell and R. W. Fairbridge, p.225 (1967), Elsevier, Amsterdam.

86 J. B. Davis, Meeting Geol. Soc. Amer., New Orleans, 1967.

87 S. C. Lind and D. C. Bardwell, J. Amer. Chem. Soc., 48, 2335 (1926).

88 C. W. Sheppard and V. L. Burton, J. Amer. Chem. Soc., 68, 1636 (1946).

89 U. Colombo, E. Denti and G. Sironi, J. Inst. Petrol., 50, 228 (1964).

90 A. G. Douglas and B. J. Mair, Science, 147, 499 (1965).

91 A. Kossiakoff and F. O. Rice, J. Amer. Chem. Soc., 65, 590 (1943).

92 B. S. Greensfelder, H. H. Voge and G. M. Good, Ind. Eng. Chem., 41, 2573 (1949).

93 C. L. Thomas, Ind. Eng. Chem., 41, 2564 (1949).

94 J. M. Tedder, Quart. Rev., 14, 336 (1960).

95 R. T. Holman, M. Deubig and H. Hayes, Lipids, 1, 247 (1966).

96 A. I. M. Keulemans and S. G. Perry, "Gas Chromatography", p. 336 (1963), Butterworths, London.

97 J. Knotnerus, I. and E. C. Product Research and Develop-

ment, 6, 43 (1967).

98 T. G. Hoering and R. M. Mitterer, Meeting Geol. Soc. Amer., New Orleans, 1967.

99 R. M. Mitterer and T. G. Hoering, Carnegie Inst. Washington Year Book (1966-67).

100 D. H. Welte, Geol. Rund., 55, 131 (1965).

101 D. H. Welte, Bull. Amer. Assoc. Petrol. Geol., 49, 2246 (1965).

102 J. D. Brooks and J. W. Smith, Geochim. Cosmochim. Acta, 31, 2389 (1967).

103 W. E. Robinson, J. J. Cummins and G. U. Dineen, Geochim. Cosmochim. Acta, 29, 249 (1965).

104 G. T. Philippi, Geochim. Cosmochim. Acta, 29, 1021 (1965).

105 G. Eglinton, P. M. Scott, T. Belsky, A. L. Burlingame and M. Calvin, Science, 145, 263 (1964).

106 E. S. Barghoorn, W. G. Meinschein and J. W. Schopf, Science, 148, 461 (1965).

107 J. R. Ravenhill, and A. T. James, J. Chromatog., 26, 89 (1967).

108 D. Stewart and C. E. Forbes, in "Oil Shale and Cannel Coal", p.96 (1938), Inst. of Petrol., London.

109 N. de Nevers, Scientific American, 214, 21 (1966).

110 D. G. Rea, in "Biology and the Exploration of Mars", ed. C. S. Pittendrigh, W. Vishniac and J. P. T. Rearman (1966),

Nat. Acad. Sci., Washington.

111 C. Milton and H. P. Eugster, in "Researches in Geochemistry", ed. P. H. Abelson, p. 118 (1959), John Wiley, New York.

112 J. D. Jones and J. R. Valentyne, Geochim. Cosmochim. Acta, 21, 1 (1960).

113 M. Mcgregor, in "Oil Shale and Cannel Coal", p.6 (1938), Inst. Petrol., London.

114 J. A. Dulhunty, D.Sc. Thesis, Sidney University (1943).

115 K. B. Blackburn and B. N. Temperley, Trans. Roy. Soc. Edinburgh, 58, 841 (1936).

116 J. H. Belcher, Ph.D. Thesis, University College London (1958).

117 R. F. Leo and P. L. Parker, Science, 152, 649 (1966).

118 J. R. Maxwell, Ph.D. Thesis, Glasgow University (1967).

119 B. Urquhart, M.Sc. Thesis, Glasgow University, in preparation.

120 T. Iida, E. Yoshii and E. Ktatsuji, Anal. Chem., 38, 1224 (1966).

121 G. Roberti, C. Minervini and V. Berti, Energia Termica, 582 (1941); Chem. Abs., 36, 5767c.

122 F. W. McLafferty, in "Mass Spectrometry of Organic Ions", ed. F. W. McLafferty, p.309 (1963), Academic Press, London.

123 J. P. Forsman, in "Organic Geochemistry", ed. I. A. Breger, p.184 (1963), Pergamon Press, London.

124 W. E. Robinson, J. J. Cummins and K. E. Stanfield, Ind. Eng. Chem., 48, 1134 (1956).

125 G. M. Badger, K. Donnelly and T. M. Spotswood, Aust. J. Chem., 19, 1023 (1966).

126 G. Mueller, Congr. Geol. Internat., Compt. Rend., 19th Session, Algiers, 1952, No.12, 279 (1964).

127 T. A. Geissman, K. Y. Sun and J. Murdoch, Experentia, 23, 793 (1967).

128 J. M. Hunt, Proc. Internat. Scientific Oil Conf., Budapest, October 1962.

129 J. M. Hunt, F. Stewart and P. A. Dickie, Bull. Amer. Assoc. Petrol. Geol., 38, 1671 (1954).

130 J. J. Coneybeare, Ann. Philos., 1, 136 (1822).

131 N. J. Firth, Geochemistry Unit, School of Chemistry, Bristol University, personal communication.

132 W. E. Robinson and D. E. Lawlor, Fuel, 40, 375 (1961).

133 A. L. Burlingame and B. R. Simoneit, Science, 160, 531 (1968).

PART II

ISOLATION AND CHARACTERISATION OF CYCLOALKANES FROM A SEDIMENT

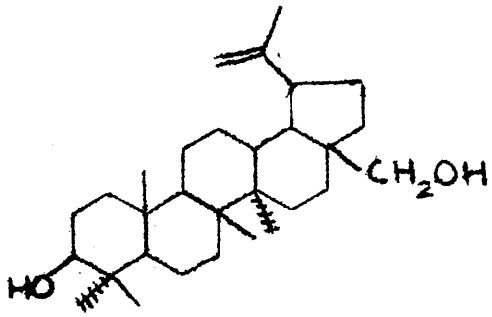
INTRODUCTION

Organic Geochemistry is concerned with the search for molecular carbonaceous remnants from sediments, petroleums and coals. The origin of these remnants is of extreme importance. It is now widely accepted that most of the organic compounds found in geological samples had a biological origin. The organic constituents of a shale or other geological sample are fossil natural products in the respect that the original compounds were synthesised by living systems and became incorporated in the shale by the normal sedimentary processes. To substantiate this theory, we have to relate any compound identified from a geological source to a known precursor in the plant or animal kingdom. Thus the term biological marker was coined, indicating that the structure of a compound isolated from a geological source was identical to, or could be closely related to, a compound known to be produced by a living system. For biological markers to be of value, they should have good chemical stability to diagenesis and maturation, they should not be synthesised in significant quantities by abiogenic processes and they should possess a high degree of specificity in their skeletal features.

Frequently used biological markers include the normal alkanes,[1-6] the 2- and 3-methyl alkanes,[3] the isoprenoid

alkanes (2,6,10-trimethylundecane, farnesane, pristane and phytane),[7-12] the corresponding isoprenoid acids,[13] the porphyrins,[14-17] the steroids,[18-24] the triterpenoids[20-32] and the tetraterpenoids.[33]

The steroids and triterpenoids possess a high degree of specificity in their structures which make them ideal biological markers. The optical activity exhibited by many petroleums[37] has been associated with polycyclic hydrocarbons and it has been postulated that these may be steranes and triterpanes derived from naturally occurring steroids and triterpenoids.[18,19,37] The work of Hills and Whitehead[28,29] and Danieli et al[27] on crude petroleums has corroborated this theory. Tentative evidence for the presence of steranes and triterpanes in sediments has been afforded by the work of Burlingame et al[21] and Murphy et al.[33] Ruhemann and Rand[38] found betulin (1), allobetulin (2) and oxyallobetulin (3) in brown coal. Sorm et al[30-32] found betulin and related compounds together with friedelin (4) and ursolic acid (5) in Czech. brown coal. Barton et al[23] identified oxyallobetulin which Carruthers and Cook[24] had isolated from an American crude petroleum. Hills et al[26] isolated and identified the saturated triterpane, gammacerane, from the Green River shale. These findings, together with the knowledge that the occurrence of steroids and triterpenoids is widespread throughout the plant



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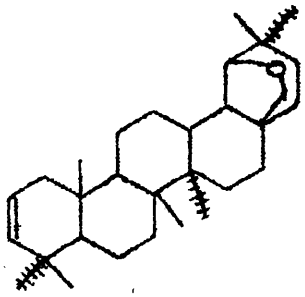

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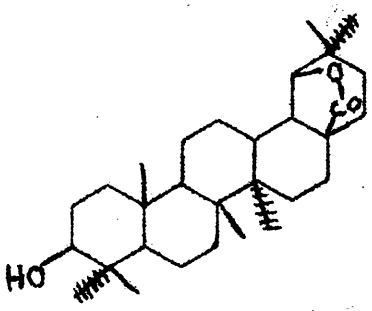

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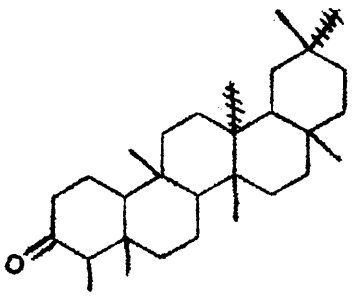

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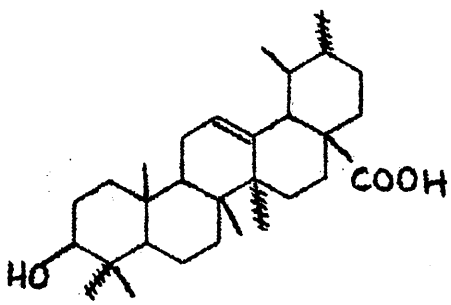

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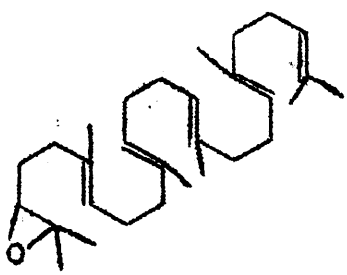

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and animal kingdoms, seem to indicate that these compounds are ideal biological markers.

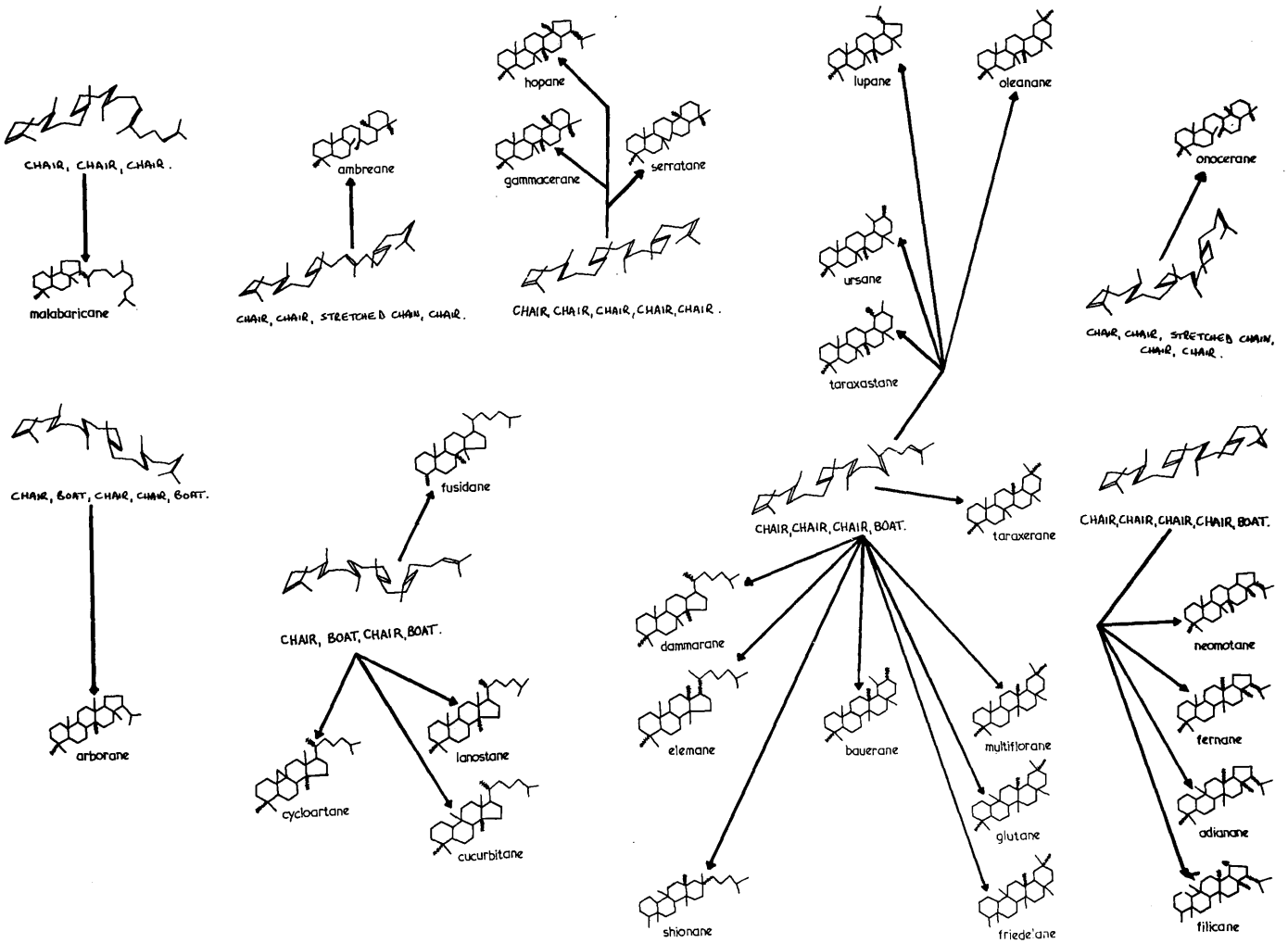
Biogenetic Classification of Terpenoids

Little is known at the present time about the biosynthesis of steroids and triterpenoids. It is widely accepted that the precursor of steroids and triterpenoids is squalene epoxide[39-41] (6) and that enzymes act as templates to hold the substrate in single rigidly folded conformations with the olefinic double bonds appropriately juxtaposed for cyclisation[42] (Figure 1). This would account for the high degree of stereo-specificity found in steroids and triterpenoids, e.g. β-amyrin (7) has eight asymmetric centres and therefore theoretically 256 stereoisomers could be derived but only a single isomer has been found in living things. However, it is possible that the paleobiogenesis of ancient organisms differed from modern plant biogenesis in that more than one stereoisomer was formed. Another possibility could be that, even if the paleobiogenetic system produced only one of the possible stereoisomers, rearrangement has occurred in the sediment, producing more than one stereoisomer. Therefore, in the study of fossil cycloalkanes from sediments or petroleums, stereoisomeric mixtures may be found which could complicate chemotaxonomic and paleochemotaxonomic correlations.

Theoretically at least, the formation of all the known

Figure 1

The possible conformations of squalene epoxide during the biosynthesis of triterpenes, and the cycloalkane structures derived from them.

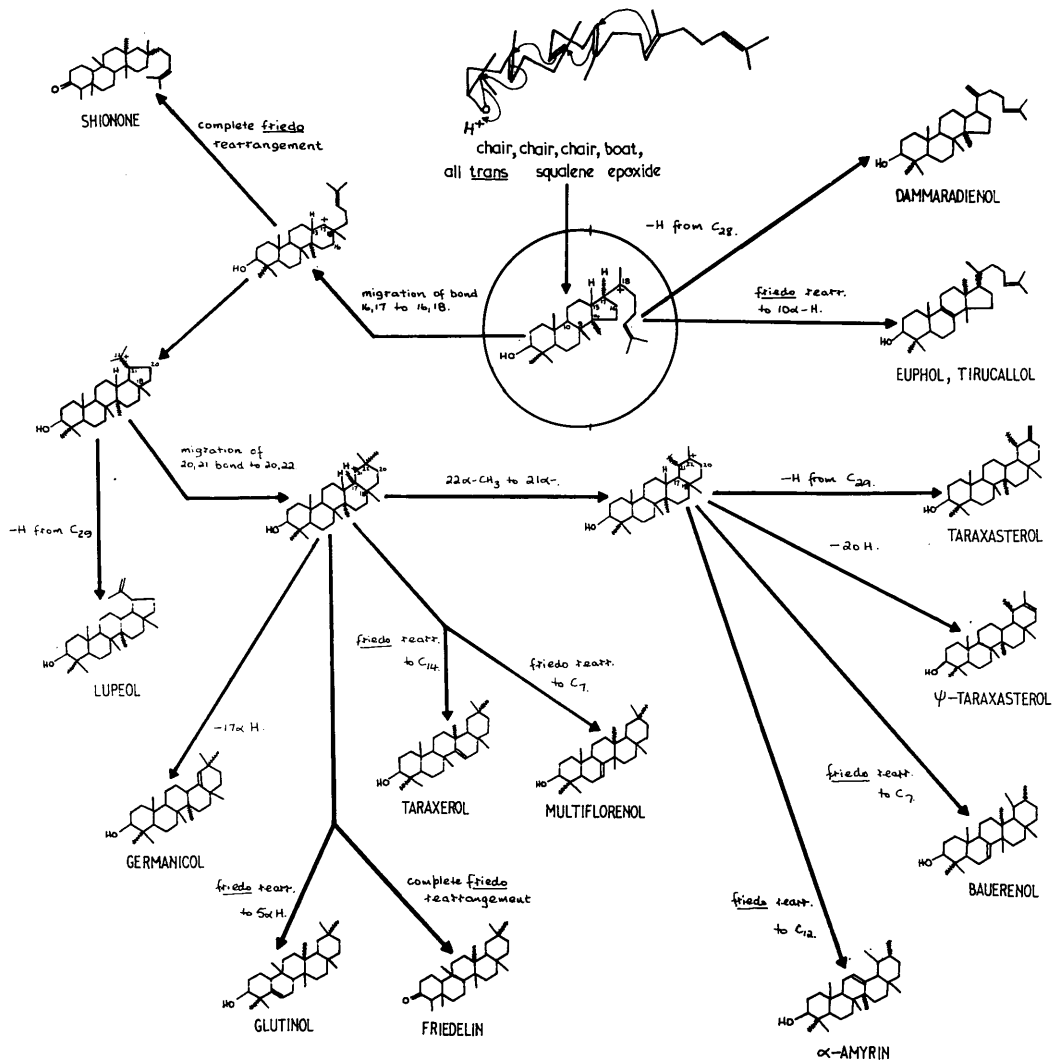

CHAIR, CHAIR, CHAIR.
malabaricane
ambreane
CHAIR, CHAIR, STRETCHED CHAIN, CHAIR.
hopane
gammacerane
serratane
CHAIR, CHAIR, CHAIR, CHAIR, CHAIR.
lupane
oleanane
ursane
taraxastane
onocerane
CHAIR, CHAIR, STRETCHED CHAIN, CHAIR, CHAIR.
CHAIR, BOAT, CHAIR, CHAIR, BOAT.
arborane
fusidane
CHAIR, BOAT, CHAIR, BOAT.
cycloartane
lanostane
cucurbitane
CHAIR, CHAIR, CHAIR, BOAT.
taraxerane
dammarane
elemane
bauerane
multiflorane
glutane
shionane
friedelane
CHAIR, CHAIR, CHAIR, CHAIR, BOAT.
neomotane
fernane
adianane
filicane

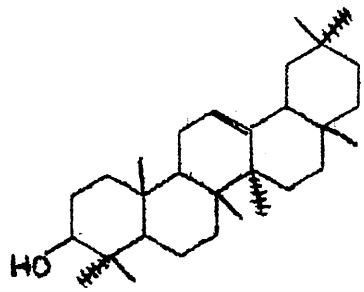
steroids and triterpenoids can be explained on the basis of the biogenetic isoprene rule.[40] Furthermore, the isoprene rule suggests new triterpenoid structures which may yet be discovered in nature.[43] The proposed mechanism for the cyclisation of all trans squalene epoxide,[41] involves acid attack on the epoxide ring which on opening, is followed by cyclisation in a well defined sequence of quasi chair and boat conformations. This may be followed in some cases by friedo rearrangements[44] (1,2 backbone skeletal rearrangements with a multiple sequence) and 1,2 eliminations, e.g. Figure 2. This assumes that the cyclisation steps are synchronous, leading to a common ion, which by rearrangements and eliminations, may form many different structures. However, there are other possibilities; the cyclisation and rearrangements may be synchronous, but forming all the different carbonium ions necessary; the cyclisation may follow a stepwise mechanism whereby each carbonium ion formed takes part in the subsequent steps, forming other carbonium ions.

Figure 1 illustrates all the categories of biologically derived triterpenes (as saturated hydrocarbons) known at the present time and the proposed squalene epoxide conformations from which they may be derived. The structures have been arranged so that the striking structural similarities are immediately apparent. For clarity, structures having a common

Figure 2

The proposed cyclisation of quasi chair, chair, chair, boat, all trans squalene epoxide and the triterpenes derived from it.


SHIONONE
complete Friedo rearrangement
H+
chair, chair, chair, boat, all trans squalene epoxide
DAMMARADIENOL
-H from C28.
migration of bond 16,17 to 16,18.
Friedo rearr. to 10α-H.
EUPHOL, TIRUCALLOL
migration of 20,21 bond to 20,22
22α-CH3 to 21α-.
-H from C29.
TARAXASTEROL
-H from C29
-2oH.
LUPEOL
Friedo rearr. to C14.
Friedo rearr. to C7.
Ψ-TARAXASTEROL
-17α H.
TARAXEROL
MULTIFLORENOL
Friedo rearr. to C7.
BAUERENOL
GERMANICOL
Friedo rearr. to 5α H.
complete Friedo rearrangement
Friedo rearr. to C12.
GLUTINOL
FRIEDELIN
α-AMYRIN



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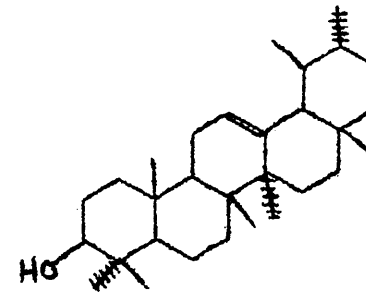
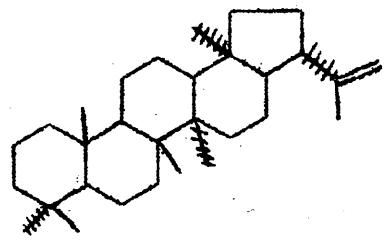


7a (α-amytin)



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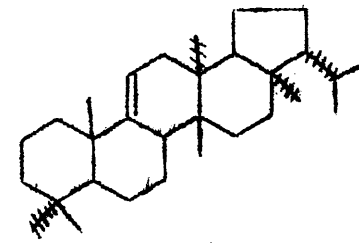

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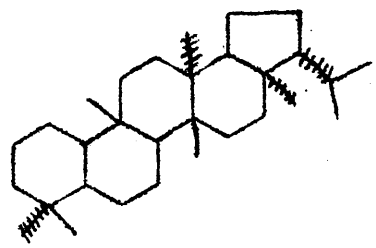

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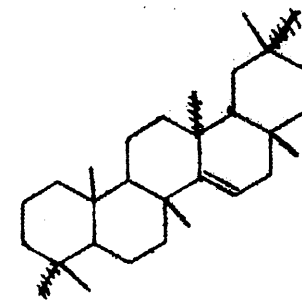

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proposed conformation of squalene as precursor are connected by arrows. The different carbon skeletons can be accounted for on the basis of different enzyme templates enforcing different squalene conformations prior to cyclisation. Even so, on examination horizontally across the lines of structures in Figure 1, some partial similarities in structure are to be found, e.g. adianane and glutane or filicane and friedelane. It may well be that the enzyme templates differ only partially in some cases and that these different structures may well be derived from the same conformer of squalene epoxide.

However, the biosynthesis of steroids and triterpenoids via the biogenetic isoprene rule from squalene epoxide cannot alone lead to a cycloalkane. (Most tricyclic, tetracyclic and pentacyclic terpenes found in geological samples are present as saturated hydrocarbons, although oxygenated compounds have been found as detailed above and there is some evidence for the presence of unsaturated hydrocarbon triterpenes in the Green River shale.) If these compounds were to be formed by plants and animals, a reductive enzyme system would have to be invoked. However, no saturated steroid or triterpenoid hydrocarbons have been found in nature up to the present time. The only hydrocarbon triterpenoids found in nature so far are the unsaturated triterpenoids fernene (8),[45] adianene (9),[45] diploptene (10)[46] and taraxerene (11).[47]

The Geological Environment

The presence of steranes and triterpanes in geological material may be explained in two ways. Firstly, they may have been formed from the naturally occurring steroids and triterpenoids during diagenesis and maturation of the sediments. The various alteration processes which may occur have been thoroughly discussed in Part I of this thesis; they are thermal, catalytic, radioactive bombardment and bacterial activity.[48,49] By one or more than one of these processes it is possible that reduction of olefinic double bonds and oxygenated functions[29] and also decarboxylation by bacterial or thermal activity could take place. Decarboxylation by bacteria could explain the presence of nortriterpanes in a crude petroleum.[29] This is not an unreasonable postulate since most sediments exist in anaerobic conditions and certain bacteria operate under these conditions,[50] liberating methane and thus contributing to the existing reducing environment.

The second hypothesis could be that the steranes and triterpanes have been formed by organisms, now extinct, with an enzymatic system capable of reducing steroids and triterpenoids to the respective saturated hydrocarbons, unlike contemporary organisms which, so far as is known, do not possess such a system.

The diagenetic and maturation processes, while providing

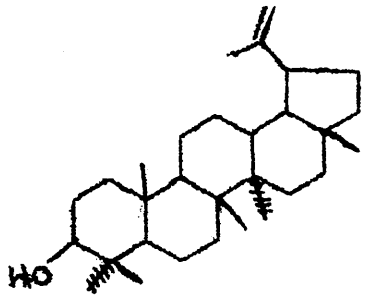
the facilities for reduction and decarboxylation, may also cause skeletal rearrangements. So that not only do we have to isolate and identify reduced steroids and triterpenoids of known carbon skeletons, but we also have to consider and look for new types of structures. In fact, in attempting to identify fossil products of biological origin, there is the possibility that new structures may be found which have not been encountered before in organic chemistry. This could create serious problems on the analytical side, since a new 'unknown' structure might not follow any of the general or predicted patterns of behaviour, and no authentic material would be available for comparison with it, and if stereoisomerisation has occurred, e.g. in the sediment, the behaviour of the isomers would overlap.

Chemotaxonomic Correlations

The occurrence of steroids and triterpenoids in contemporary plants and animals may be briefly summarised as follows: steroid nuclei with no substituent in the C_{24} position in the sidechain occur widely in all types of organisms, but only to a small extent in plants; 24-methyl or methylene steroids occur in plants (mostly fungi and algae) as well as in some sea animals, e.g. oysters; 24-ethyl or ethylene steroids are mainly found in algae and higher plants; squalene occurs widely in plants and also in some animals (in large quantities

in fish liver oil); only tetracyclic triterpenes of the lanostane and norlanostane type are found in animals; the only pentacyclic triterpene found in animals so far is tetrahymanol (12) (gammacerane skeleton) in Protozoan tetrahymena;[32b] tetracyclic and pentacyclic triterpenes occur mostly in higher plants and also to some extent in lower plants.

In order to make chemotaxonomic or paleochemotaxonomic observations on the distribution of steroids and triterpenoids, it is necessary to have much more accurate generalisations than those just given above. Using an excellent review paper by Halsall and Aplin[51] as a basis, the distributions of steroids, tricyclic, tetracyclic and pentacyclic triterpenes in the plant kingdom were examined. Steroids occur throughout the plant kingdom as free sterols and their esters. Triterpenes occur widely in nature as hydrocarbons, ketones, alcohols and glycosides and as acids. Both steroids and triterpenes form saponins, i.e. as glycosides. These complex structures have an important use, they are powerful surface active agents, their water solutions foam when shaken. Also because of the high yields obtained from some plants, certain saponins are used as starting material for the synthesis of steroid hormones to be used in medicine. Certain pentacyclic triterpenes have a widespread distribution, e.g. α- and β-amyrin,[1,2] lupeol(13), moretenol(14), friedelinol(15), betulin(1), ursolic acid(5)



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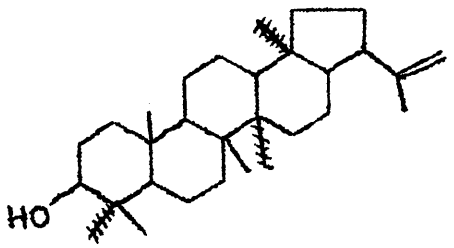

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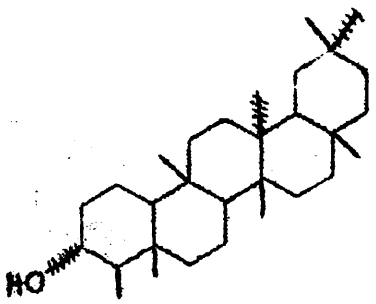

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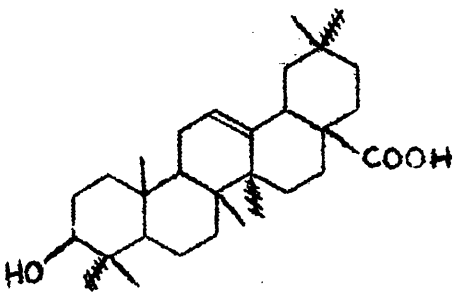

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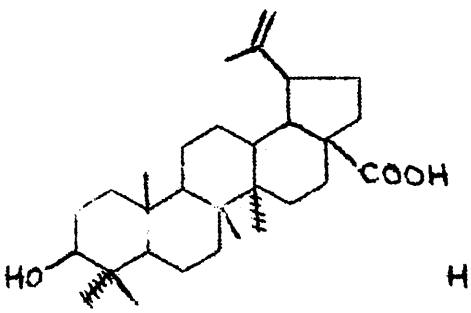

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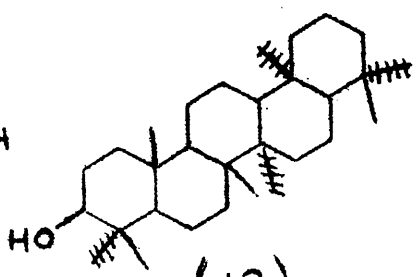
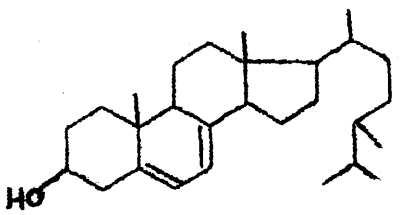


(12)

oleanolic acid(16) and betulinic acid(17). The most commonly encountered plant sterols are ergosterol(18),stigmasterol(19) β-sitosterol(20),stigmastanol(21) and spinasterol (22).

A chemotaxonomic survey of the occurrence of triterpenes is attempted in Table 1. This has been assembled by examining the occurrence of steroids and triterpenoids in the plant kingdom. These are represented by the cycloalkane structures which may be derived from the naturally occurring biological precursors by the effects of diagenesis, bacteria, thermal alteration etc.in a sediment, provided we can disentangle these effects. It can only be a preliminary survey, because, although many triterpenes and steroids have been isolated and characterised, it is by no means certain that those reported are the only structures present in a particular family or order. It is entirely possible that they are the major constituents of the triterpene and steroid category and that minor constituents remain unidentified. Moreover, many investigations of the distributions of steroids and triterpenoids in plants have been conducted without the aid of the more sophisticated techniques used today. It is entirely possible that some of the methods used, e.g. to isolate triterpenes, could have excluded the steroids, or vice versa. The published occurrences and distributions of steroids and triterpenoids are probably by no means complete and that major, as well as



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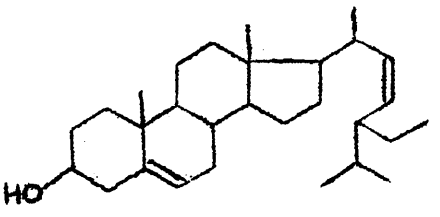

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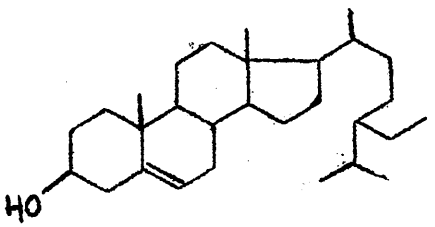

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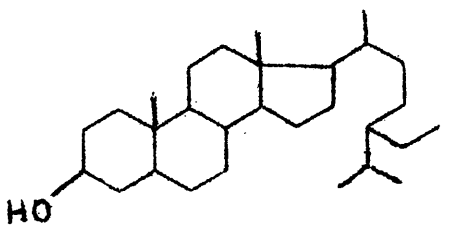

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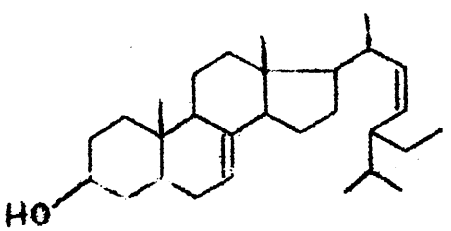

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Table 1

A preliminary chemotaxonomic survey of the occurrence of steroids and triterpenes is attempted. The steroids and triterpenes are represented as the cycloalkane compounds which could be derived from the former two classes of compounds.

Division	Class	Order	Cycloalkane distribution
Angiosperms	Monocotyledonae	Graminales Herb. plants, rice, wheat, oats, etc.	Multiflorane (D:C-friedo-O) Taraxerane (D-friedo-O) Stigmastane
	Dicotyledonae	Sapindales	Oleanane
		Ebenales	Oleanane
		Primulales	Oleanane
		Saxifrageles	Oleanane
		Caryophyllales	Oleanane
		Meliales	Oleanane
		Personales	Oleanane
		Verbenales	Oleanane
		Ariales	Oleanane Ursane
		Myrtales	Oleanane Lupane
		Hamamelidales	Oleanane Lupane
		Cactales	Oleanane Lupane
		Rubiales	Oleanane Ursane
		Rosales	Oleanane Lupane
		Geraniales	Oleanane Ursane Lupane

Table 1 continued.

Division	Class	Order	Cycloalkane distribution
Angiosperms (continued)	Dicotylodonae (continued)	Euphorbiales	Oleanane Ursane Multiflorane (D:C-friedo-O) Bauerane (D:C-friedo-U) Elemane Dammarane
		Fagales	Oleanane 3,4-seco-Oleanane Ursane Lupane Glutane (D:B-friedo-O) Friedelane (D:A-friedo-O)
		Loganiales	Oleanane 3,4-seco-Oleanane
		Ericales	Oleanane Glutane (D:B-friedo-O) Friedelane (D:A-friedo-O)
		Celastrales	Friedelane (D:A-friedo-O)
		Utricales	Lupane
		Rhamnales	Lupane abeo-Lupane (Ceanothane)
		Asterales	Taraxerane (D-friedo-O) Taraxastane (rearr. lupane)
		Myricales	Taraxerane (D-friedo-O)
		Umbellales	Ursane

Table 1 continued.

Division	Class	Order	Cycloalkane distribution
Angiosperms (continued)	Dicotylodonae (continued)	Rutales	Ursane Arborane
		Leguminales	Oleanane Onocerane Ergostane Stigmastane
		Cruciales	Ergostane
		Chenopodiales	Stigmastane
Gymnosperms		Coniferales	Hopane Ursane
Pterophyta	True ferns	Filicales	Fernane (E:C-friedo-Hopane) Hopane Adiantane (30-nor-Hopane) β-Sitostane
Bryophyta	Musci (mosses)	Sphagnidae	Ursane Taraxerane
Chlorophyta (green algae)	Chlorophycaea		Ergostane Stigmastane Sitostane
Xanthophyta (yellow-green algae)	Xanthophyceae		Sitostane
Euglanophyta (Euglenids)	Euglenophyceae		Ergostane
Rhodophyta (red algae)	Rhodophyceae		Cholestane Stigmastane Sitostane
Phaeophyta (brown algae)	Phaeophyceae		Stigmastane
Chrysophyta (golden algae and diatoms)	Chrysophyceae		Stigmastane

Table 1 continued.

Division	Class	Order	Cycloalkane distribution
Lichens			Taraxerane (D-friedo-O) Hopane
Eumycota (true fungi)	Basidiomycetes		C_{31} Lanostane Lanostane Ergostane Cholestane
	Ascomycetes (yeasts)		Lanostane Cholestane Ergostane

O = oleanane
U = ursane

N.B. Other steroids have been reported for the Algal divisions, but too few algae examined to make generalisations. It is significant to note that all the algae contain carotenes (α, β, γ, and ε)in view of the high proportion of perhydro-carotene found in the Green River shale.

minor constituents of these categories may remain unidentified.

Nevertheless, some generalisations are possible. In the Thallophyta Division, the algae, only steroids have been found. Since the algae have, at the present time, the longest authenticated history, from the Precambrian era and that the higher plants are postulated to have evolved from them, it would be reasonable to predict that plant orders containing both steroids and triterpenes would be the next stage of evolution, e.g. Eumycota, the Angiosperms, Chenopodiales and Leguminales. Another striking feature is the number of Angiosperm orders which contain only triterpene compounds which belong to the triterpane categories, i.e. oleanane, lupane, hopane and arborane (> 50%). The remainder contain one or more of the basic structures together with rearranged structures, or only rearranged structures. Two different points of view may be taken of the last two statements. Firstly, that as plant evolution proceeded, the plants became more sophisticated and correspondingly their triterpene constituents became more complex, i.e. a biochemical evolution from the oleanane, lupane, ursane, arborane and hopane types to the friedo rearranged compounds, e.g. multiflorane, bauerane, friedelane, glutane, taraxastane and taraxerane types. Secondly, the reverse argument could be that as the plants evolved, the triterpene categories above became dominant. In the case of

the sterols, considerable diversification is apparent in the higher plant sterols. Cholesterol occurs in primitive invertebrates only as one member of complex mixtures of sterols, whereas at higher levels of evolution, animals and man, cholesterol has become essentially the only sterol. If this were to hold for the triterpenes, then the second argument would be the more probable, i.e. that diversification of structural type occurred initially followed by the basic triterpenes becoming dominant. However, paleobotanists are still perplexed by many aspects of plant evolution and it may be that by more thorough examinations of steroid and triterpenoid occurrence, paying more attention to minor as well as major constituents, elucidation of this problem may be somewhat facilitated. The best solution to the problem would be a complete list and knowledge of the exact functions of the enzymes involved. Every plant probably has either all the enzymes or the ability to synthesise all the enzymes necessary to form these compounds. The DNA contains the information which determines which enzymes are manufactured. The instructions to release this information from the DNA are probably provided largely by protein molecules. The situation is undoubtedly complex and still only partially understood, but it is almost certain that the DNA contains far more information than is used, i.e. it is coded for the synthesis of more enzymes than

are commonly found in a particular cell. Thus our chemotaxonomy may represent only the final stage of the process and the situation would be different if we could read all the instructions of the DNA of that species.

Figure 3 shows in condensed form the chemical taxonomy with reference to steroids and triterpenoids for the plant kingdom, so that comparisons between different plant divisions may be made more easily. It is apparent from this that the triterpenes occur overwhelmingly in the higher plants, i.e. the Angiosperms. Figure 4 shows in graph form the authenticated histories of the various plant divisions with reference to the geological periods of the Earth's history. It is perhaps pertinent to note that estimates of the age of the Earth are of the order of 5×10^9 years and that only one division of the plant kingdom has reasonably authenticated fossils dating from the Precambrian period, i.e. the Blue-green Algae, earliest authenticated report ca. 1.5×10^9 years, some claims for 3.2×10^9 years. The authenticated occurrences of fossil plants do not imply that these species could not have existed at an earlier age. Fossilisation of organisms depends on the environment and the organisms, e.g. soft organisms may have left no fossil record at all in the older rocks. Thus the boundaries for any particular plant division must of necessity remain tenuous at the present time.

Figure 3

Chemotaxonomic Correlations for the Steroid and Triterpene Cycloalkanes in the Plant Kingdom

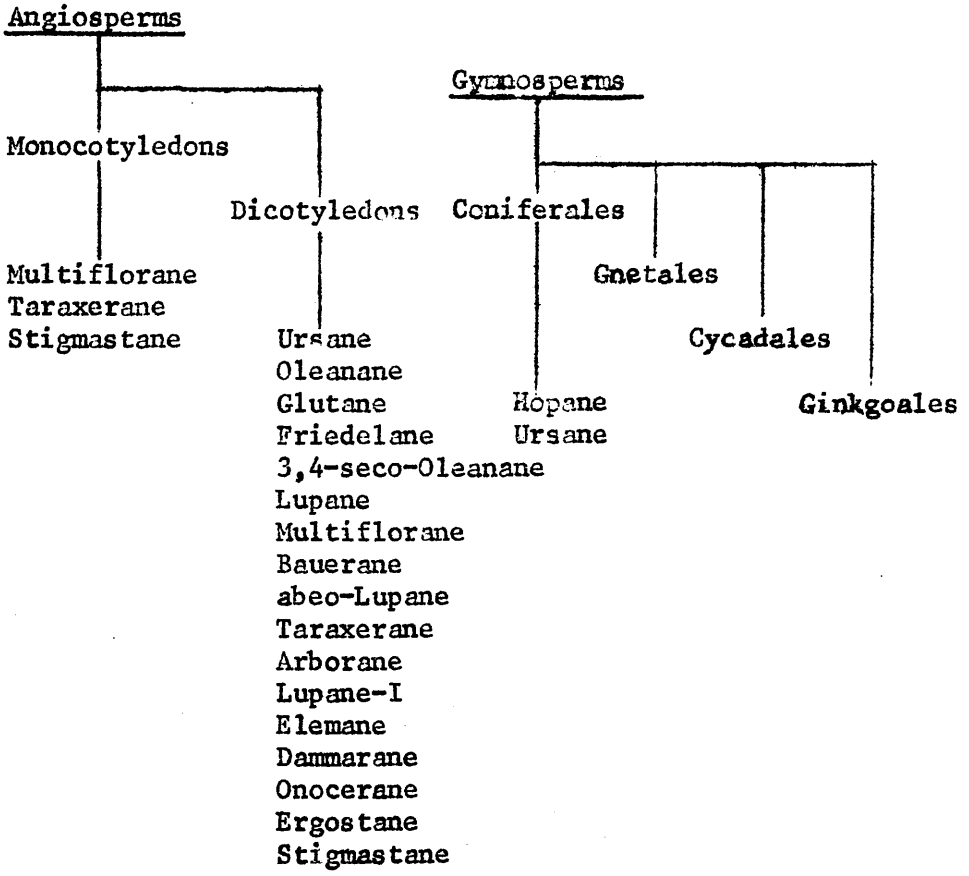


Figure 3 continued.

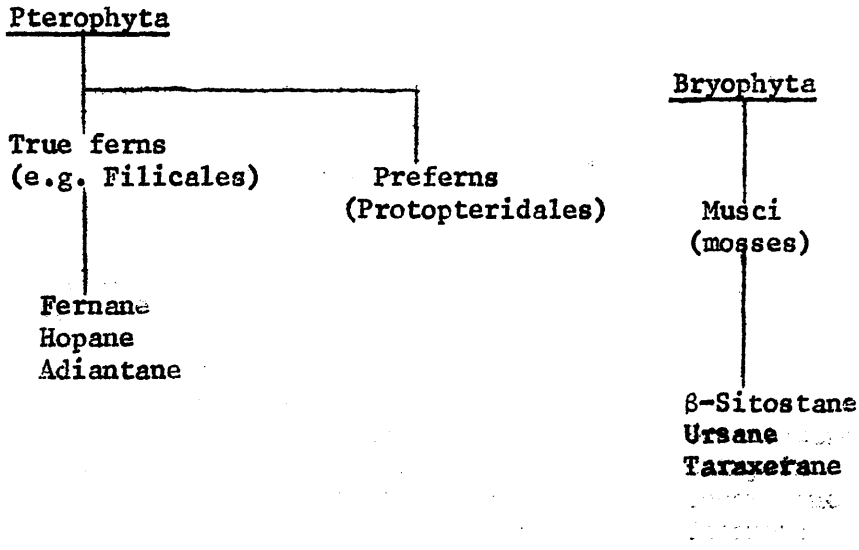


Figure 3 continued.

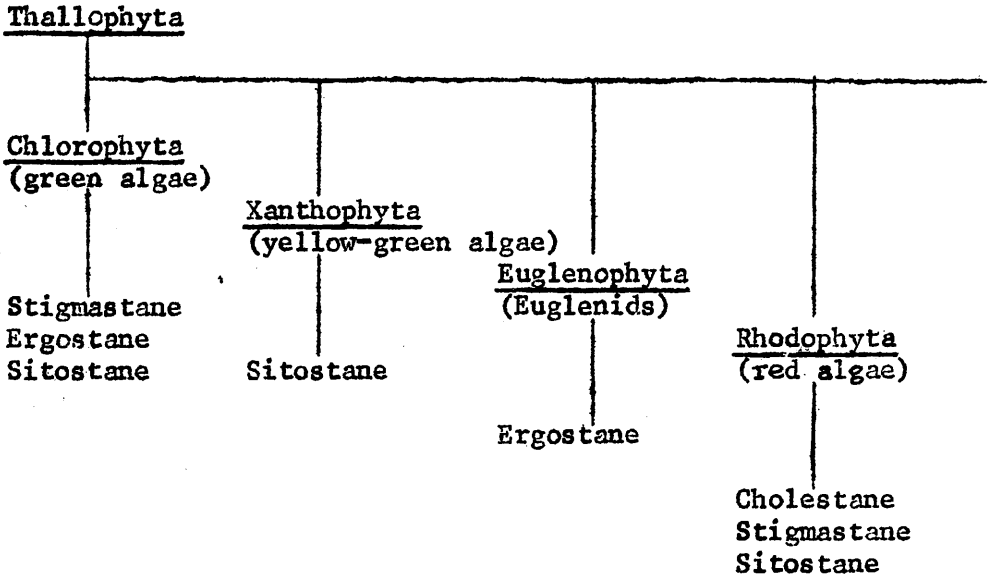


Figure 3 continued.

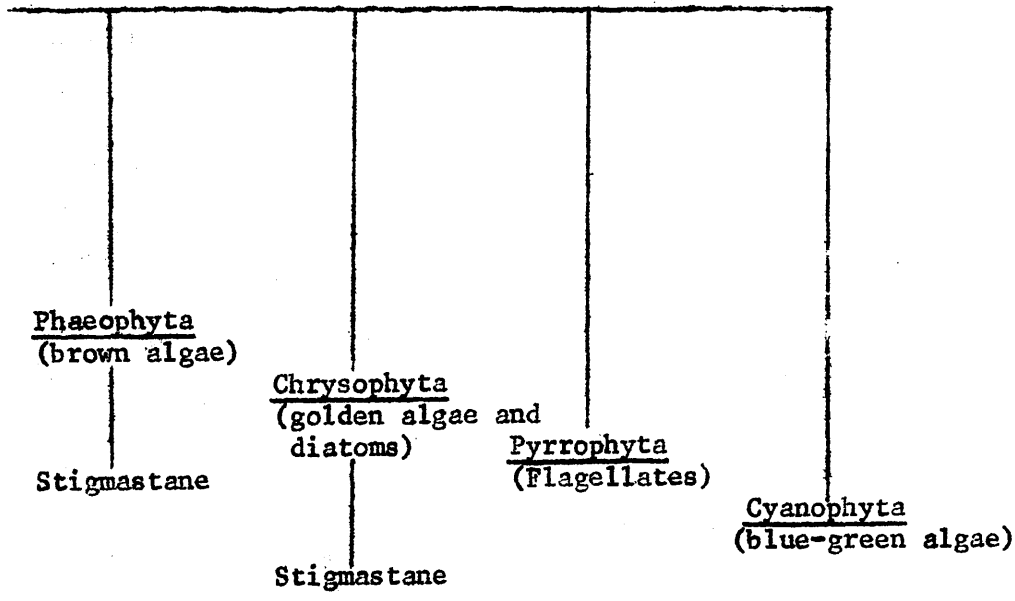


Figure 3 continued.

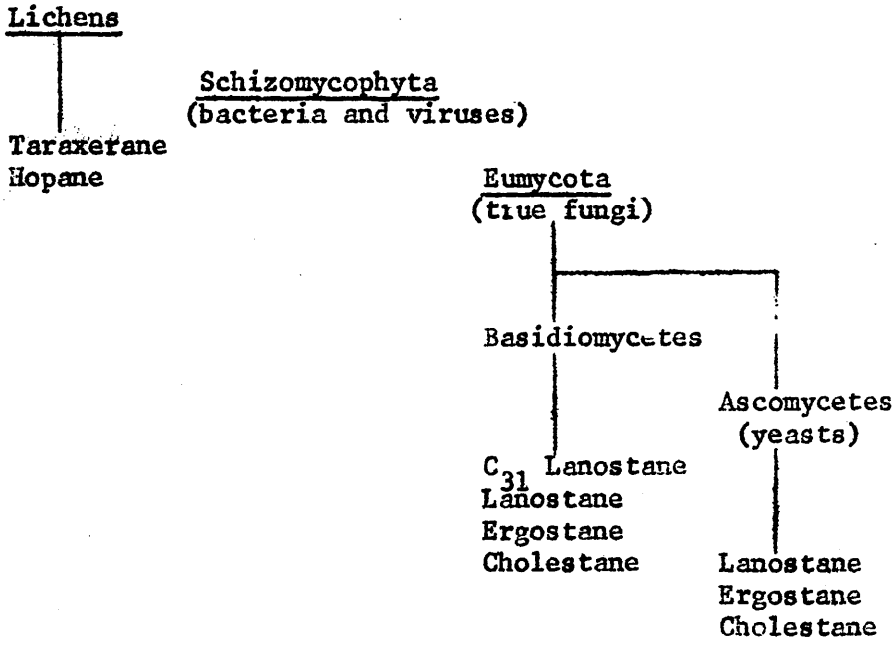
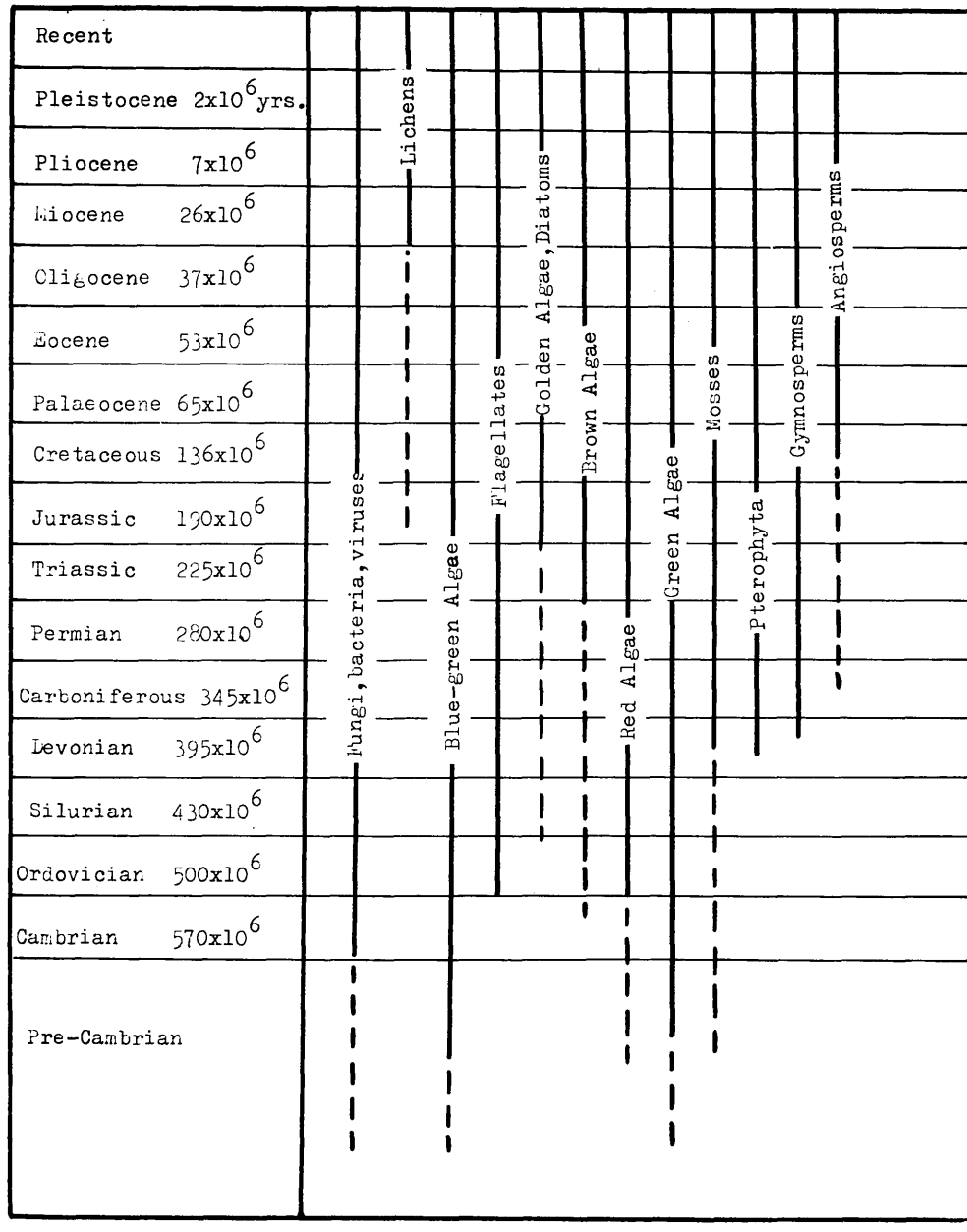


Figure 4

The distribution of the plant divisions with reference to the geological periods of the Earth's history.


Recent
Pleistocene 2x10^6 yrs.
Pliocene 7x10^6
Miocene 26x10^6
Oligocene 37x10^6
Eocene 53x10^6
Palaeocene 65x10^6
Cretaceous 136x10^6
Jurassic 190x10^6
Triassic 225x10^6
Permian 280x10^6
Carboniferous 345x10^6
Devonian 395x10^6
Silurian 430x10^6
Ordovician 500x10^6
Cambrian 570x10^6
Pre-Cambrian
Fungi, bacteria, viruses
Lichens
Blue-green Algae
Flagellates
Golden Algae, Diatoms
Brown Algae
Red Algae
Green Algae
Mosses
Pterophyta
Gymnosperms
Angiosperms

Chemotaxonomic studies could be usefully applied to algae and lower and higher plants, to determine more accurately the distributions of steroids and triterpenoids in these species. These plants are probably the largest source of cycloalkanes in sediments and petroleums.

Before the biological origin of a particular fossil natural product can be postulated and compared with contemporary plants and animals, i.e. paleochemotaxonomy, it is important that the structure of the compound be conclusively identified. In fact, paleochemotaxonomically, it is equally important to establish the absence of a particular type of sterane or triterpane as it is to establish its presence. Both pieces of evidence are equally informative.

Thus, in a study of this kind, it is of prime importance to establish conclusive identity and of secondary importance to establish the distributions of these compounds. The occurrence of these compounds may also provide information on the environment of the Earth at the time of deposition of the sediment and on the past history of the sediment. For example, the presence of a particular compound may indicate that the maximum temperature encountered by the sediment has never been as high as the decomposition point of the compound. On the other hand, the presence of a compound whose structure may be assumed to be altered from its original structure

provides information on the conditions and environmental influences which may have caused these changes.

The Green River Shale

The sediment chosen for this work was the Green River oil shale from Colorado, U.S.A. (Eocene, $\sim 60 \times 10^6$ years old). There were two main reasons for this choice. Firstly, a great deal of background information, both chemical and geological, was available through the work of the author and other researchers. Secondly, it is rich in organic material, thus providing a convenient sample on which to test techniques which can then be applied, once proven successful, to older, less rich ancient sediments and perhaps, extraterrestrial samples.

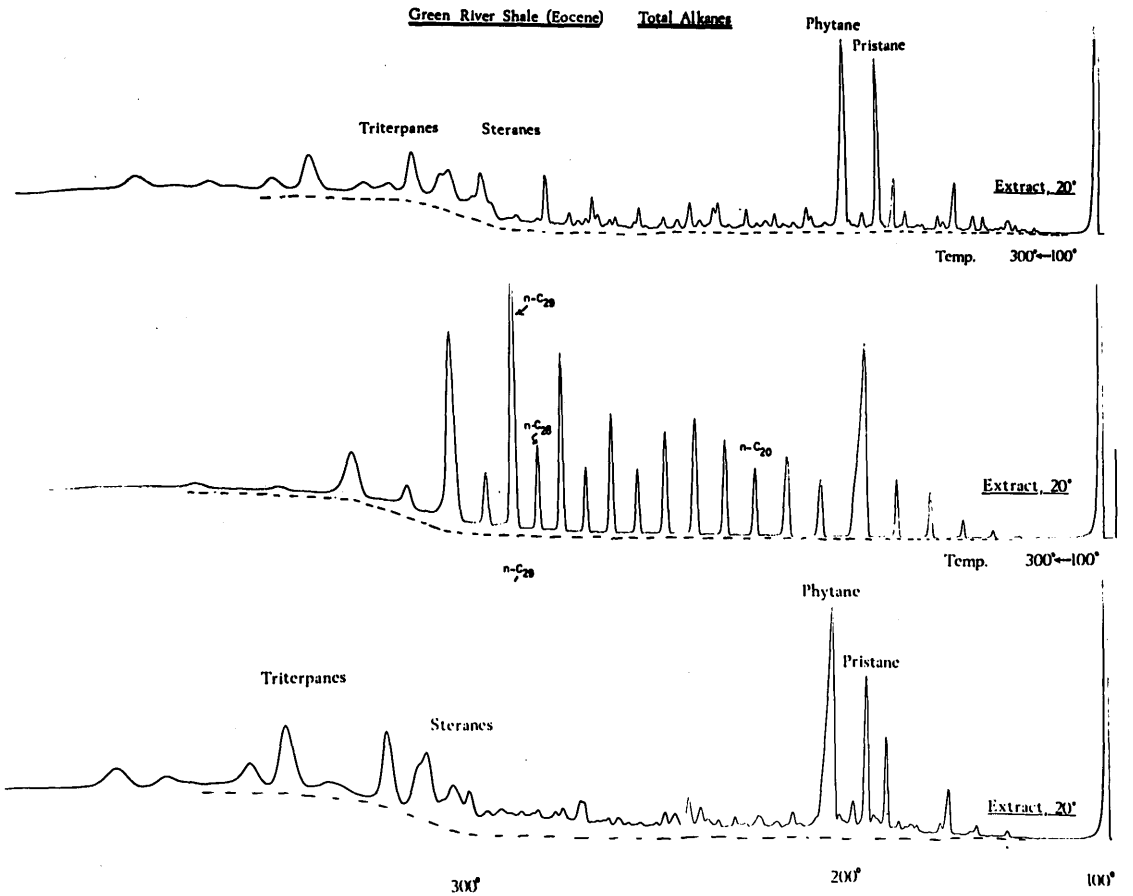
The geological history of this sediment is fairly well documented[51] in that it seems to have suffered no major upheavals, igneous intrusions and therefore, no greatly elevated temperatures throughout its history. It was deposited under a shallow inland lake covering several hundred square miles in Colorado, U.S.A. The organic material found in the shale was probably derived predominantly from aquatic organisms, such as microscopic algae and protozoa, rather than land plants, pollens and spores. Bradley[51] has stated that the nearest contemporary environment to that of the Green River shale is the Florida lake muds where the contribution from algae is

overwhelming. The temperature of the deposit has certainly never been higher than 74°C and therefore no excessive alteration of the organic material should have been effected.

The Green River shale may contain as much as 30% by weight of organic material. However, ultrasonic extraction yielded a geolipid fraction of only 1.8% by weight of the sediment. Therefore a high proportion of the organic matter is present as kerogen. This is corroborated in Part I where pyrolysis of the shale has been shown to lead to an increased yield of organic material. On separation, the geolipid fraction afforded the alkane fraction which was found to comprise 0.28% by weight of the sediment. The gas chromatogram of the alkane fraction (Fig. 5) reveals a complex mixture with the isoprenoid alkanes farnesane, pristane and phytane, identified by previous workers,[8] outstanding. The gas chromatogram of the n-alkane fraction, obtained by molecular sieving of the alkane fraction, shows a marked dominance of odd carbon numbered alkanes (Fig. 5) especially n-C_{31}, C_{29}, C_{27} and C_{17}, thus illustrating the biological history of the sediment to an impressive extent (as in the Introduction to Part I). The n-carboxylic acids have been reported as being dominantly even carbon numbered[8a,b] and the isoprenoid fatty acids, phytanic and pristanic acids identified.[8a] The gas chromatogram of the branched and cyclic alkane

Figure 5

G.l.c. record of the total alkane, the n-alkane, and the branched and cyclic alkane fractions isolated from the Green River shale; analysed on low resolution packed g.l.c. columns.


Green River Shale (Eocene)
Total Alkanes
Phytane
Pristane
Triterpanes
Steranes
Extract, 20°
Temp. 300°←100°
n-C29
n-C28
n-C20
Extract, 20°
Temp. 300°←100°
n-C29
Phytane
Pristane
Triterpanes
Steranes
Extract, 20°
300°
200°
100°

fraction, obtained from the molecular sieving of the alkane fraction, 0.22% by weight of the sediment, illustrates the complexity of the mixture, even in the high molecular weight region of the cycloalkanes (Fig. 5). This fraction was then subjected to a thorough analysis as detailed in the subsequent sections.

SECTION 1

CAPILLARY COLUMN GAS CHROMATOGRAPHIC ANALYSES OF AUTHENTIC STERANES AND TRITERPANES, AND THE BRANCHED AND CYCLIC ALKANE FRACTION FROM THE GREEN RIVER SHALE

Discussion

Open tubular coated capillary columns have high efficiencies which allow the separation of closely related compounds. These separations result from the special properties of capillary columns. The carrier gas flow is not restricted as it passes through the column since the liquid phase is evenly distributed as a thin film on the walls of the capillary tubing. This results in a small pressure drop throughout the column, enabling the use of long high performance columns with relatively short retention times.

The relatively high molecular weight range of steranes and triterpanes necessitates the use of higher temperatures, e.g. 250°C, therefore only liquid phases which are of the highest thermal stability may be used. This eliminates many of the polyester type of polar liquid phases. The hydrocarbon phase Apiezon L is generally the best liquid phase for the analysis of saturated hydrocarbons. On the non-polar Apiezon L, the hydrocarbons are mainly separated according to their

boiling points.[52] More polar phases cause changes in retention times because of different interactions between the components and the phase due to slight differences in the polarities of the molecules. Satisfactory results have been achieved with 7 ring metapolyphenyl ether, SE 52, SE 30 and dimethyl polysiloxane monodisperse gum. SE 30 and SE 52 are silicone gums with different proportions of alkyl and phenyl substituents in the siloxane chains. SE 30 has predominantly methyl substituents, while SE 52 has predominantly phenyl substituents. However, even with thermally stable liquid phases, there is a notable decrease in efficiency when operating at higher temperatures. The normal range of efficiencies produced by columns coated with these liquid phases is within the range of 25-50,000 theoretical plates measured at 250°C (100-200 ft long columns). The resolution under these conditions is still sufficient to separate some mixtures of components containing stereoisomers.

By the judicious use of polar and non-polar liquid phases coated on long capillary columns, satisfactory separations of sterane and triterpane mixtures should be accomplished. In the present work, pretreated Apiezon L (AP-L) grease and 7 ring polyphenyl ether (7-PPE) were the non-polar and polar phases used.

The retention data and Kovats indices[53] (Table 2) were

Table 2

The carbon number retention data for the available authentic steranes and triterpanes are listed, on two different capillary columns.

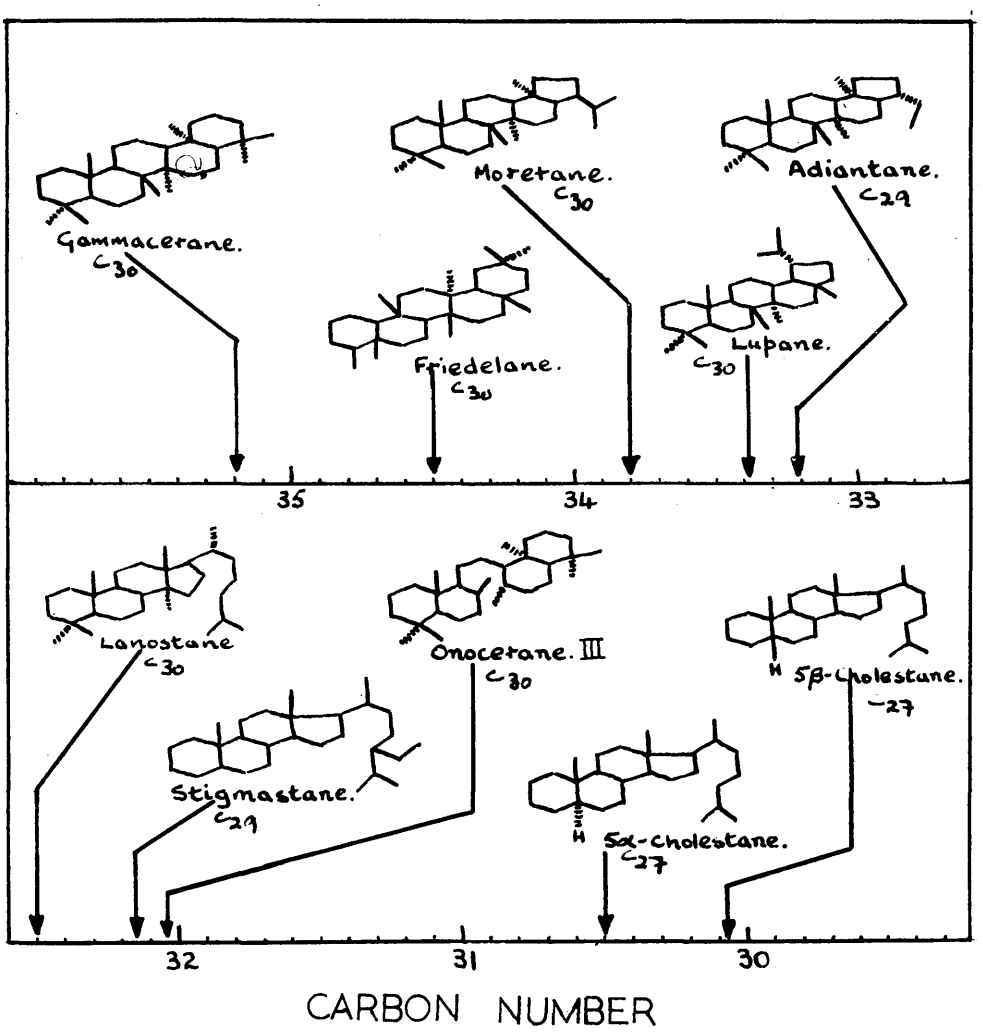
Authentic standard	Carbon number (AP-L)	Carbon number (7-PPE)
5β-cholestane	29.58	30.07
5α-cholestane	29.90	30.49
Onocerane III	31.23	32.05
Onocerane II	31.35	32.36
Lupane	31.42	33.38
Stigmastane	31.53	32.15
Onocerane I	31.59	32.68
Moretane	31.98	33.81
Lanostane	32.05	32.50
Adiantane	32.43	33.22
Friedelane	33.72	34.46
Gammacerane	34.06	35.20

calculated for each authentic standard by coinjection of each one with a normal alkane mixture as reference (n-C_{28}, C_{30} and C_{32}). The retention behaviour of some of the standards used is illustrated in Figure 6. The column used in this case was 150 ft x 0.01 in and coated with 7-PPE. The structures are shown so that the differences in retention behaviour may be more easily correlated with differences in structure. It is interesting to note that adiantane, which has only 29 carbon atoms, has a longer retention time than lanostane and onocerane III (both C_{30} compounds). It is apparent from the carbon number sequence that onocerane III with an AB-DE fused tetracyclic system has a shorter retention time than AB-CD fused tetracyclic triterpanes with a steroid skeleton, followed by pentacyclic triterpanes with ring E being 5 membered and finally pentacyclic triterpanes with only 6 membered rings. From these results the following relationships arise: for molecules with the same molecular weight the retention time increases with increasing numbers of fused rings; molecules with only 6 membered rings have longer retention times than those with one 5 membered ring; the shape and symmetry of a molecule may contribute to the retention time, e.g. gammacerane is a very symmetrical molecule but it has the longest retention time of all these standards.

However, before definite patterns of behaviour can be

Figure 6

The retention behaviour of some authentic sterane and triterpane structure types on a 150' x 0.01" stainless steel capillary column coated with 7 ring metapolyphenylether.


Gammacerane.
C30
Moretane.
C30
Adiantane.
C29
Friedelane.
C30
Lupane.
C30
35
34
33
Lanostane
C30
Onocetane. III
C30
5β-Cholestane.
C27
Stigmastane.
C29
5α-Cholestane.
C27
32
31
30
CARBON NUMBER

firmly established many more authentic compounds have to be examined. Nevertheless, comparisons of this kind may yield useful information about the branched and cyclic alkane fraction from the Green River shale, as will be seen in Section 3.

The branched and cyclic alkane fraction from the Green River shale was analysed on both AP-L and 7-PPE capillary columns. The g.l.c. records are shown in Figure 7 for comparison purposes. The reference alkanes n-C_{28}, C_{30} and C_{32} were coinjected with the mixture and their position indicated. The peaks in the chromatogram are numbered to facilitate discussion of peak identity. The retention data and Kovats indices were calculated and are shown in Table 3 together with the corresponding data for the authentic standard compounds. The percentage abundance of each peak in the chromatogram was calculated by measurement of peak areas from Fig. 7. Where coinjection of an authentic compound produced peak enhancement of one of the peaks in both the AP-L g.c. record and the 7-PPE g.c. record, then this was taken as one proof of identity and the standard compound was entered in Table 3 opposite the peak number it enhanced.

Thus from Table 3, it is immediately seen that the following peak enhancements occur: 5β-cholestane and peak 1a; 5α-cholestane and peak 1; onocerane III and peak 12; onocerane II and peak 13; stigmastane and peak 14; gammacerane and peak 26.

Figure 7

The g.l.c. records of the branched and cyclic alkane fraction isolated from the Green River shale on two different capillary columns:

A. 200' x 0.01" stainless steel column coated with Apiezon L grease.

B. 150' x 0.01" stainless steel column coated with 7 ring meta-polyphenylether.

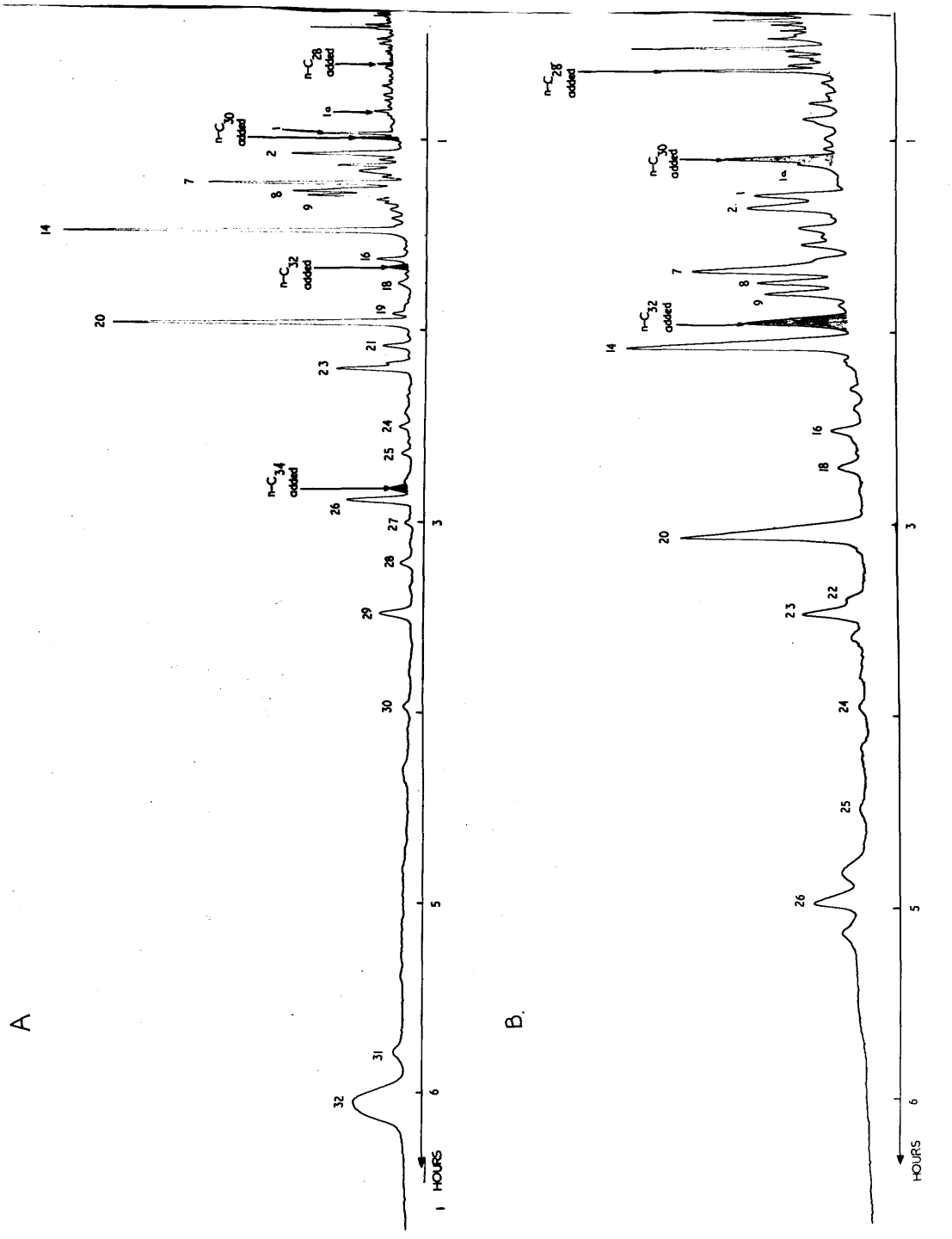

A
B.
n-C28 added
n-C30 added
n-C32 added
n-C34 added
HOURS
HOURS

Table 3

The carbon number retention data for the cycloalkanes present in the branched and cyclic alkane fraction of the Green River shale, and are compared with the carbon number data for the authentic steranes and triterpanes.

Peak no. (Fig. 4)	Abundance (%)	Carbon number (AP-L)	Carbon number (7-PPE)	Coinjected standards	Molecular* formula
1a	0.7	29.58	30.07	5β-cholestane	$C_{27}H_{48}$
1	2.3	29.90	30.49	5α-cholestane	$C_{27}H_{48}$
2	5.1	30.30	30.73	-	$C_{28}H_{50}$ and $C_{30}H_{56}$
3	1.3	30.40	30.75	-	-
4	2.5	30.44	31.07	-	$C_{29}H_{52}$ and $C_{33}H_{64}$
5	2.7	30.61	31.15	-	-
6	1.3	30.72	31.36	-	-
7	6.6	30.82	31.42	-	$C_{28}H_{50}$
8	4.8	30.94	31.52	-	$C_{30}H_{56}$
9	3.7	31.00	31.62	-	$C_{29}H_{52}$
10	1.5	31.06	31.79	-	-
11	1.1	31.12	-	-	-
12	1.2	31.23	32.05	Onocerane III	$C_{30}H_{54}$
13	1.2	31.35	32.36	Onocerane II	$C_{30}H_{54}$
-	-	31.42	33.38	Lupane	$C_{30}H_{52}$
14	11.6	31.53	32.15	Stigmastane	$C_{29}H_{52}$
-	-	31.59	32.68	Onocerane I	$C_{30}H_{54}$
15	0.8	31.76	-	-	-

(Table 3 continued)

Peak no. (Fig. 4)	Abundance (%)	Carbon number (AP-L)	Carbon number (7-PPE)	Coinjected standards	Molecular* formula
16	1.5	31.92	32.81	-	$C_{29}H_{50}$
-	-	31.98	33.81	Moretane	$C_{30}H_{52}$
-	-	32.05	32.50	Lanostane	$C_{30}H_{54}$
17	0.7	32.08	-	-	-
18	1.2	32.14	33.08	-	-
-	-	32.43	33.22	Adiantane	$C_{29}H_{50}$
19	1.1	32.44	33.25	-	-
20	11.2	32.60	33.51	-	$C_{30}H_{52}$
21	1.6	32.84	-	-	-
22	1.2	32.97	33.86	-	-
23	3.2	33.02	33.94	-	$C_{30}H_{52}$
24	0.9	33.52	34.41	-	$C_{31}H_{54}$
-	-	33.72	34.46	Friedelane	$C_{30}H_{52}$
25	1.0	33.74	35.10	-	$C_{31}H_{54}$
26	3.6	34.06	35.20	Gammacerane	$C_{30}H_{52}$
27	0.6	34.22	-	-	$C_{30}H_{52}$
28	1.1	34.46	-	-	-
29	2.4	34.76	-	-	-
30	1.0	35.27	-	-	-
31	3.4	36.66	-	-	-
32	16.0	36.82	-	β-Carotane	$C_{40}H_{78}$

* From mass spectrometric data, Section 3, Table 5.

MASS SPECTROMETRY OF AUTHENTIC STERANES AND TRITERPANES

Discussion

It is not proposed to discuss individual fragmentation patterns in detail, except where necessary, rather to compare and contrast the fragmentation patterns of as many authentic compounds as are available. By this means some general rules and some structural classifications become apparent.

Figure 1 shows all the known basic carbon skeletons of triterpenes as the corresponding saturated hydrocarbons. A careful examination of these reveals the fact, that for mass spectral purposes, these 26 structures may be broadly classified into three categories, according to their structural features: (i) pentacyclic with one 5 membered ring, e.g. lupane, hopane (Fig. 9); (ii) tricyclic, tetracyclic and pentacyclic with all 6 membered rings, e.g. gammacerane, onocerane (Fig.10); (iii) tetracyclic with one 5 membered ring, e.g. lanostane, dammarane and all the steranes (Fig. 11).

A complete comparison would require the mass spectrometric analysis of each of these 26 cycloalkanes. However, at the present time the author had only eight at his disposal. Therefore, any general rules or classifications which may appear, can only be verified on $\sim$ 25% of the possible structures. However useful information will still result from

a survey of this kind. This is a very necessary part of the project to analyse the branched and cyclic alkanes from the Green River shale because very little mass spectral studies on saturated hydrocarbon triterpenes have been either done or published.

Pentacyclic triterpanes are generally accepted to produce a major ion at m/e 191, attributed to one of the three species shown in Figure 8(b). However, not all pentacyclic triterpanes give rise to such fragments. Before examining the mass spectra of the authentic compounds, it is well worth while examining the possible reasons for bond cleavages to occur first of all, and secondly what causes a fragment to give an intense peak in a mass spectrum.

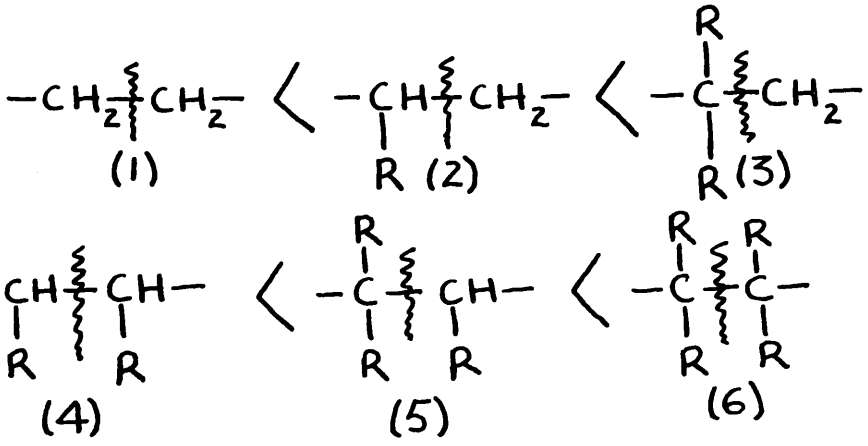
In cyclic systems, for fragmentation to occur, more than one carbon-to-carbon bond has to be broken. Normally, steroids and triterpenoids contain functional groups which give rise to high intensity ion fragments (e.g. McLafferty Rearrangement in keto triterpenes[54] and steroids[55]; Retro Diels-Alder decomposition in unsaturated steroids and triterpenes[54]). Nevertheless, in cycloalkanes, some characteristic high intensity ion fragments are still formed and it is these which have to be examined. Since at least two bonds have to be broken to fragment a cyclic system, some stability is conferred on a cyclic molecule and therefore the molecular ion

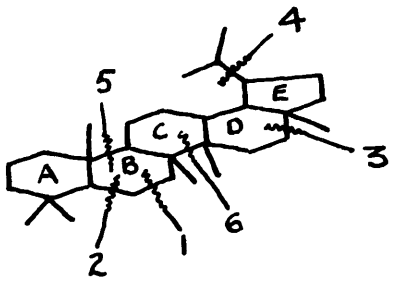
Figure 8

A. The relative probabilities for bond cleavage in cycloalkanes.

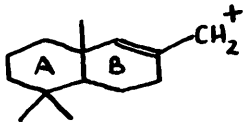
B. The ion fragments attributed to the m/e 191 peak in the mass spectra of many triterpanes.

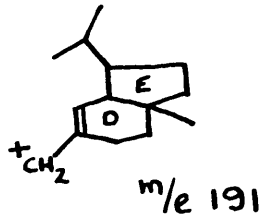
A.

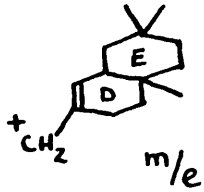




B.







(caused by the loss of one electron) of a triterpane should always be at least reasonably intense, the abundance depending on the ease of fragmentation of the remainder of the molecule.

The factors which govern the fragmentation of the positive molecular ions are the relative labilities of the bonds in the ion, the relative stabilities of the potential fragment ions and the neutral fragments formed by competing fragmentation processes.

The fragmentation of bonds depends upon the activation energy of the bond and the stability of the positive ions and neutral fragments follows the same general rules as carbonium ion solution chemistry. The order of probability of bond cleavage is shown, and applied to a typical triterpane, in Figure 8 (a). Fragmentations (2) and (3) are possibly of similar probability. Obviously, fragmentations (4), (5) and (6) are the most likely to produce high intensity fragment ions because of both their carbon-carbon bond lability and the stability of the fragment ions produced. If the structures and the mass spectral fragmentation patterns of the three broad categories of triterpanes are examined on the basis of the factors outlined above, the comparisons to be made may be facilitated.

(i) Pentacyclic with one 5 membered ring (Fig. 9). The seven structures have few common features other than being

Figure 9A

The mass spectral line diagrams for lupane, hopane and adiantane.

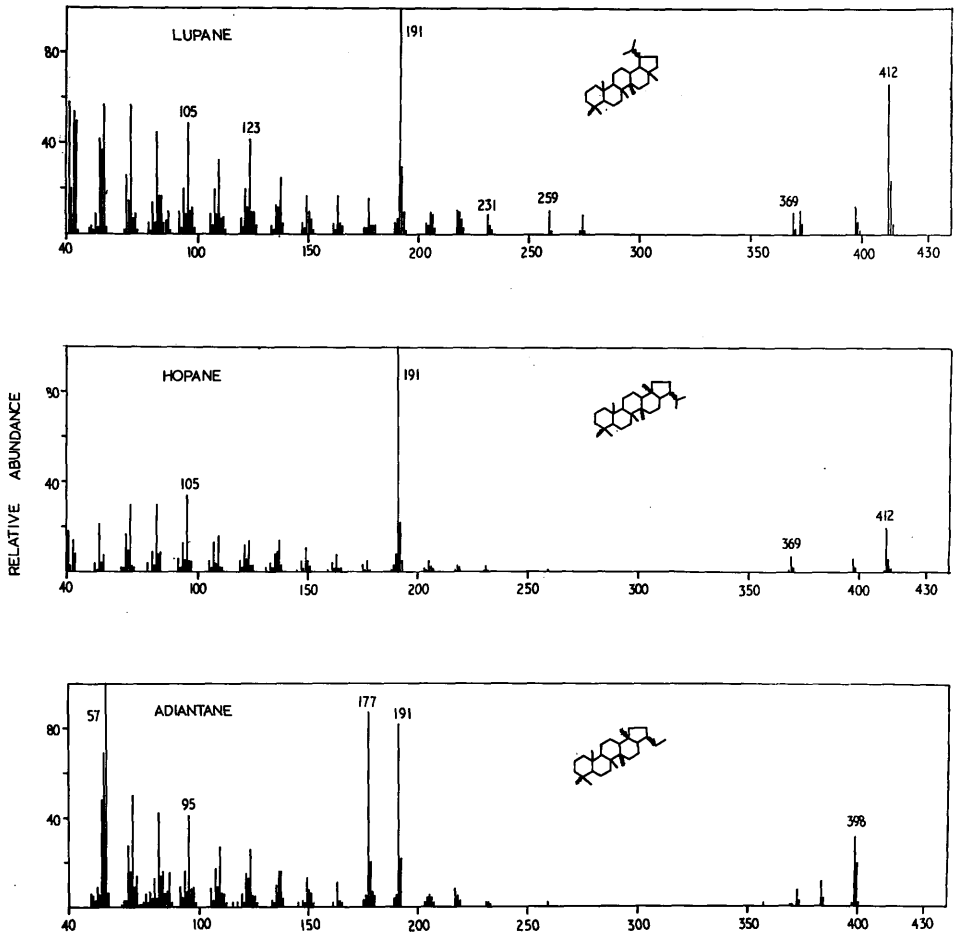
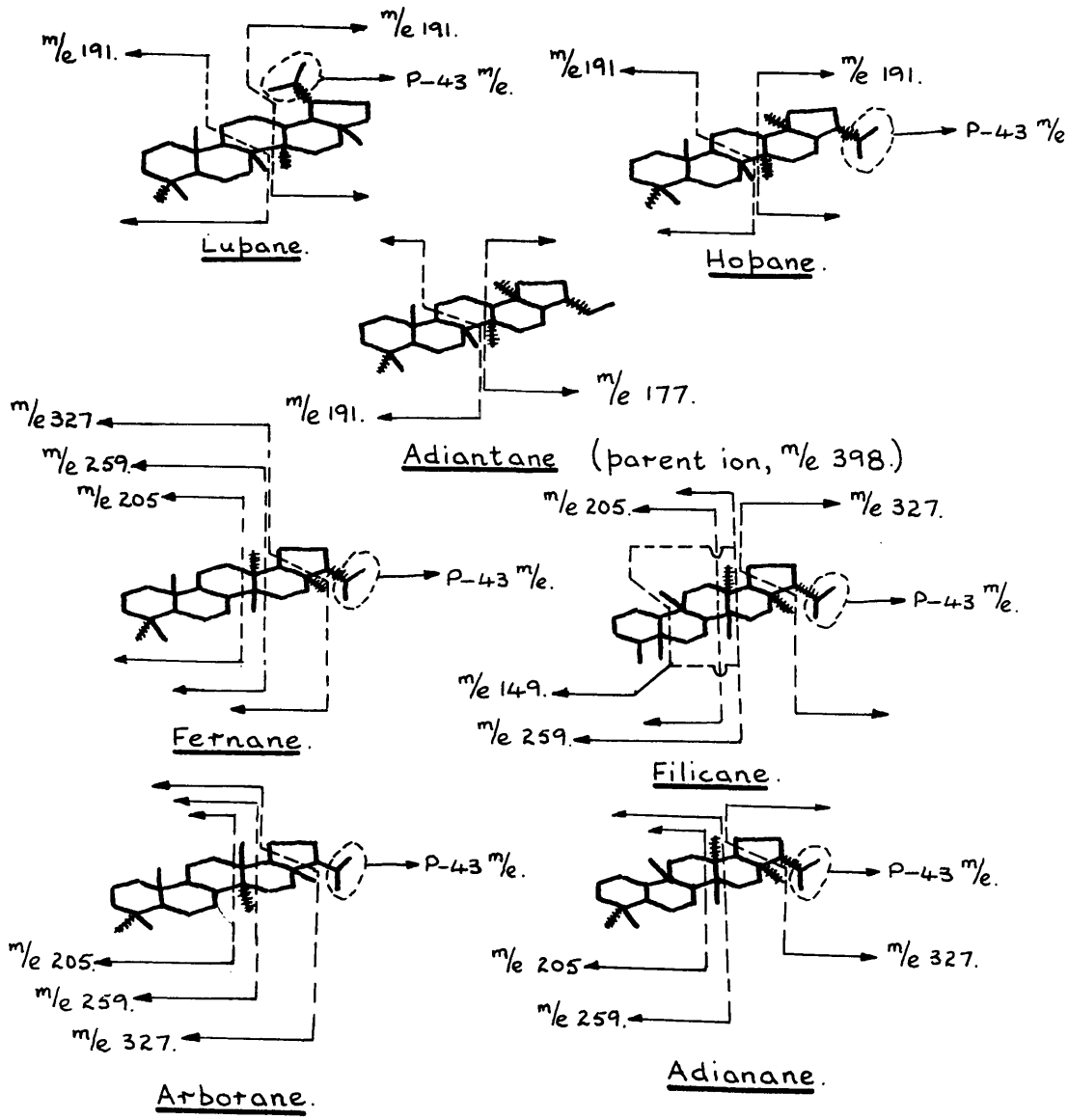

LUPANE
191
412
105
123
231
259
369
HOPANE
191
105
412
369
RELATIVE ABUNDANCE
ADIANTANE
57
177
191
95
398
80
40
40
100
150
200
250
300
350
400
430

Figure 9B

Observed and proposed mass spectral fragmentations for lupane, hopane, adiantane, fernane, filicane, arborane and adianane.


m/e 191.
m/e 191.
P-43 m/e.
Lupane.
m/e 191
m/e 191.
P-43 m/e.
Hopane.
m/e 191.
m/e 177.
Adiantane (parent ion, m/e 398.)
m/e 327
m/e 259.
m/e 205
P-43 m/e.
Fernane.
m/e 205.
m/e 327.
P-43 m/e.
m/e 149.
m/e 259.
Filicane.
P-43 m/e.
m/e 205.
m/e 259.
m/e 327.
Arbotane.
P-43 m/e.
m/e 205
m/e 259.
m/e 327.
Adianane.

pentacyclic. However, six of the seven have an isopropyl sidechain attached to ring E (the exception being adiantane). This substituent will always give rise to a (P-43) ion, e.g. in lupane, the loss of the isopropyl grouping produces an ion at m/e 369. The molecular ion for six of the seven structures will be m/e 412 corresponding to a molecular formula of $C_{30}H_{52}$, the exception again being adiantane whose molecular ion is m/e 398, corresponding to $C_{29}H_{50}$.

Within this broad category, it is obvious that there are two subsections, i.e. lupane, hopane and adiantane, the remainder being fernane, filicane, arborane and adianane.

The lupane, hopane, adiantane group has a characteristic feature of two adjacent quaternary carbon atoms at positions 9 and 14 at the junctions of rings B and C and rings C and D respectively. This type of carbon-carbon bond, as discussed above is the most labile one possible in a saturated hydrocarbon. Therefore, this should give rise to characteristic high intensity ion fragments. Lupane and hopane should have a very intense m/e 191 peak and this is indeed the case when the mass spectra of these compounds are examined (Fig. 9). Adiantane, having one carbon atom less than lupane will give rise to two approximately equally intense peaks at m/e 191 and m/e 177 (because of the methyl group missing from the isopropyl group in the fragment containing rings D and E).

This is verified on examination of the mass spectrum of adiantane (Fig. 9).

On this basis it is possible to differentiate between the spectra of lupane and hopane and that of adiantane, but it is difficult to differentiate between lupane and hopane. There are variations in their spectra, but mostly of small differences in intensity and these are not reliable. The conditions used when running mass spectra of this sort are extremely critical, slight differences may occur from one analysis to the next and one can even find intensity differences in consecutive mass spectral analyses of the same compound, e.g. slight temperature variation in the ion source or probe. Therefore, caution should be exercise before placing too much reliance on fine differences observable in routine low resolution mass spectra.

The lack of any authentic samples of the fernane, filicane, arborane and adianane group, precluded the examination of any of their mass spectra. However, their possible fragmentation behaviour may be at least partially deduced from the above discussion. They do not have two adjacent quaternary carbon atoms like the first group and therefore should not give rise to characteristic high intensity ions. In fact their parent ions will possibly be among the most intense in their spectra because there is no predominant cleavage which can

occur to expend the energy contained in the excited state of the molecular ion. Their spectra should exhibit a number of reasonably intense peaks, among which could be m/e 327, 259 and 205 as well as the (P-43) ion. Filicane, having two quaternary carbon atoms at positions 6 and 10 could produce a more intense m/e 149 than normal. Adianane may also have this, but to a lesser extent since the C_6 quaternary centre is missing. Apart from these points it will be difficult to differentiate between the spectra of these four compounds because the only other differences present are stereochemical with respect to the angular substituent groups. It is possible of course that steric and stereochemical factors may inhibit or promote preferred cleavages, thus giving rise to some characteristically intense peaks. This could only be verified by very careful analysis of the spectra of as many isomers as possible. A preliminary study of this kind was carried out and will be discussed later in this Section.

(ii) Tricyclic, tetracyclic and pentacyclic with all 6 membered rings (Fig.10). The eleven structures have no common features other than containing only 6 membered rings and the absence of an isopropyl group, so there will be no (P-43) ion in any of their spectra. Again, this broad classification may be split into three smaller categories: firstly gammacerane, oleanane, taraxastane and ursane; secondly onocerane

Figure 10A

The mass spectral line diagrams for gammacerane, friedelane and onocerane.

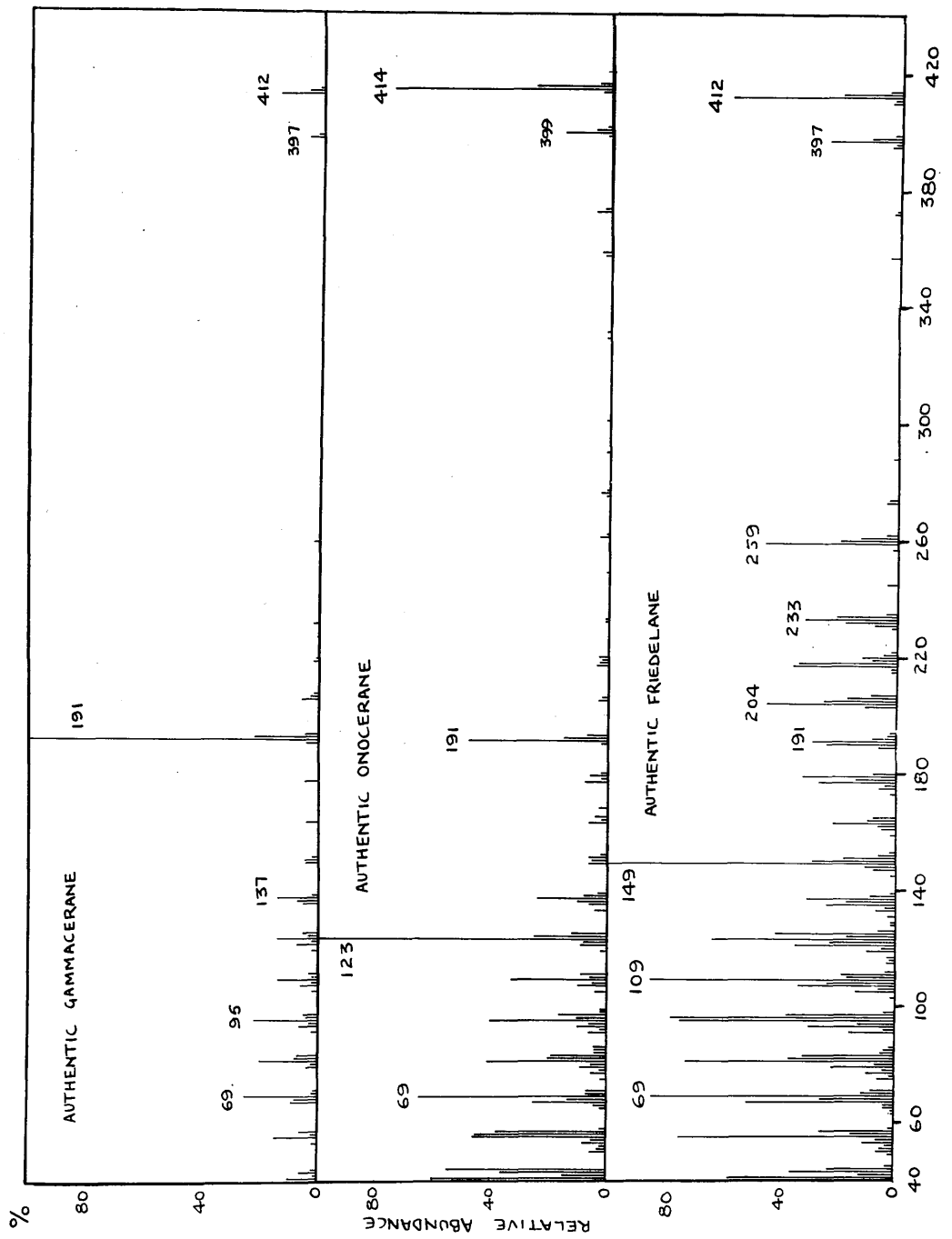
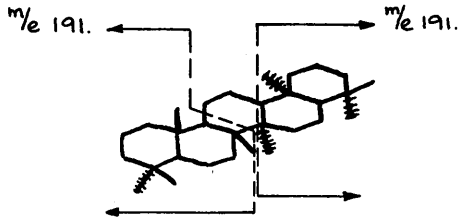
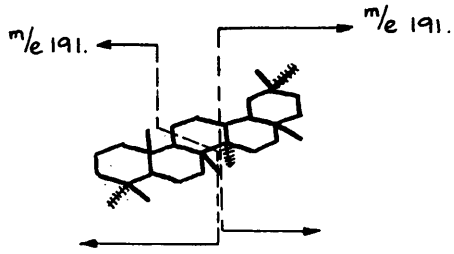

AUTHENTIC GAMMACERANE
AUTHENTIC ONOCERANE
AUTHENTIC FRIEDELANE
RELATIVE ABUNDANCE
%
191
412
397
137
96
69
123
414
399
149
109
204
233
259
420
380
340
300
260
220
180
140
100
60
40
80
0

Figure 10B

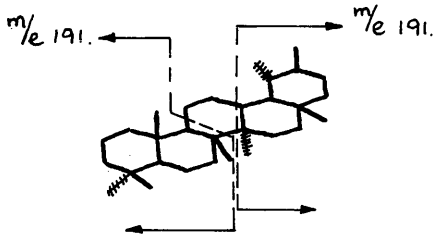
Observed and proposed mass spectral fragmentations for gamma-cerane, oleanane, taraxastane, ursane, onocerane, ambreane.



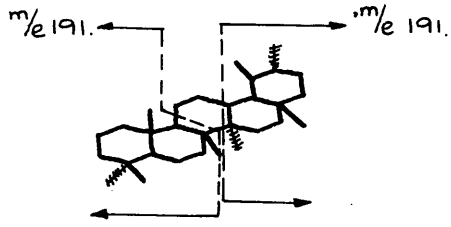
Gammacetane.



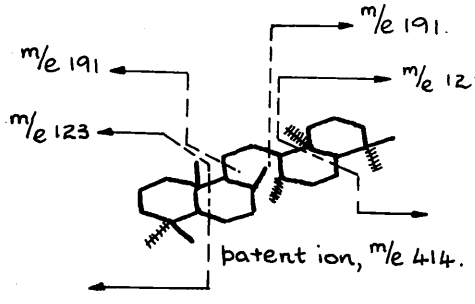
Oleanane.



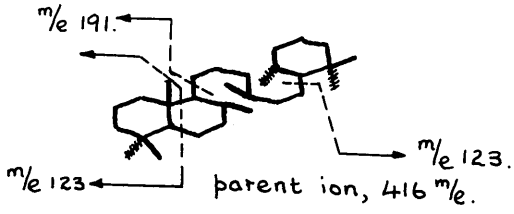
Taraxastane.



Ursane.



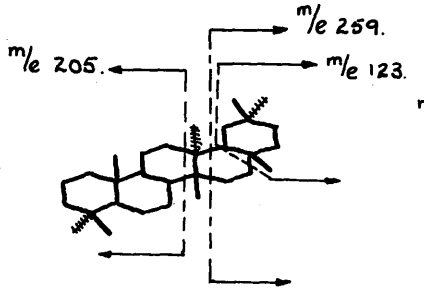
Onocetane.



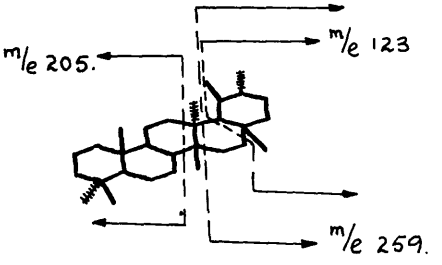
Ambreane.

Figure 10C

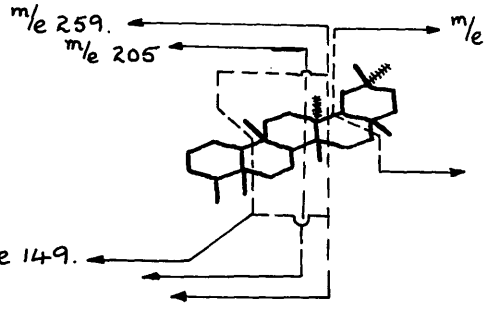
Observed and proposed mass spectral fragmentations for multiflorane, bauerane, friedelane, glutane and taraxerane.



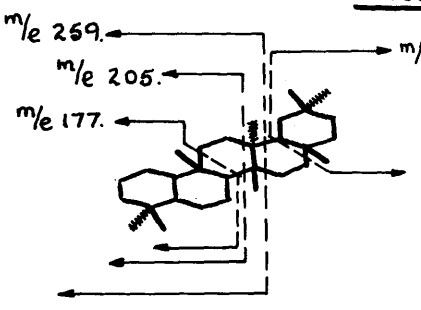
Multiflorane.



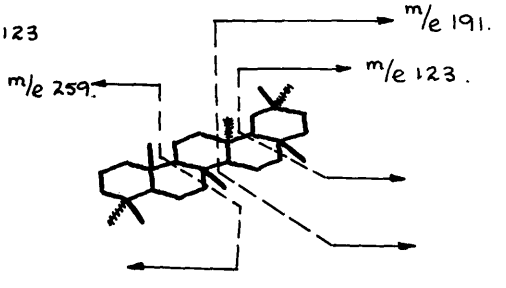
Bauerane.



Friedelane.



Glutane.



Taraxerane.

and ambreane; and thirdly multiflorane, bauerane, friedelane, glutane and taraxerane.

The first category of gammacerane, oleanane, taraxastane and ursane all contain two adjacent quaternary carbon atoms at positions 9 and 14 at the ring junctions between B and C and rings C and D respectively. As in section (i) above, this gives rise to characteristically intense fragments at m/e 191 (Fig.10b).There are no great differences in structure which could give rise to other intense peaks in their spectra, so all of their spectra will look alike and difficulty will be encountered when trying to differentiate between compounds of this structural type by their mass spectra.

The second category of onocerane and ambreane are tetracyclic and tricyclic compounds respectively. Therefore, their parent ions will both differ from those of the other triterpanes (normally parent ion is m/e 412), and are different from each other, i.e. onocerane will have a parent ion at m/e 414 and ambreane at m/e 416. Both these structures can produce an ion fragment of m/e 191, but it is interesting to examine each separately because the m/e 191 fragment is produced by a simple fission process by cleaving a single carbon-carbon bond.

In onocerane, the m/e 191 fragment is produced by cleavage of a bond linking a secondary carbon to a tertiary carbon (Fig.10 a,b) and the resulting ion may not be so well stabilised

as for instance, the m/e 191 fragments in gammacerane which has two adjacent quaternary carbon centres. Moreover, by complex fission, i.e. cleavage of two or more carbon-carbon bonds, fragments of m/e 123 can be formed by cleavage of two carbon-carbon bonds. As a general rule fragments produced from cyclic systems in this way have a high stability. Therefore, since two m/e 123 fragments are possible and also two m/e 191 fragments, the mass spectrum of onocerane should contain two characteristic peaks with the m/e 123 being the more intense because of the other minor cleavages which may occur, also giving rise to m/e 123 fragments. On examination of the mass spectra of onocerane (Fig.10a) this is found to be the case, with the m/e 123 being the base peak, i.e. 100%. The molecular ion is also very intense and is indicative of the stability of the molecule and the absence of the high probability cleavages between quaternary centres.

In ambreane, by the same reasoning, there should be two characteristic fragments of m/e 191 and m/e 123. However, this time there are fewer fragmentations possible which can give rise to m/e 123 fragments and therefore the m/e 191 peak should be more intense than the m/e 123 peak. Unfortunately, no authentic sample of ambreane was available, so the deduced partial fragmentation cannot be verified. Nevertheless, it will still be useful as a guideline when unknown spectra are

examined.

The third category of multiflorane, bauerane, friedelane, glutane and taraxerane (Fig.10c) do not have two adjacent quaternary carbon atoms and therefore will not produce any characteristically intense peaks like those of the first category. They are all pentacyclic compounds containing only 6 membered rings and their molecular ions will all be given by m/e 412 and should be of high intensity.

Multiflorane and bauerane should have characteristic peaks at m/e 259 and m/e 205 and will be difficult to differentiate because their structures are so alike. However, their spectra will be different from any of the other triterpanes in this classification.

Friedelane has two angular methyl groups at positions 6 and 10, which are different from any of the other structures so far, and this should produce some characteristic peaks. These characteristic fragmentations should give rise to fragments at m/e 259, m/e 205 and m/e 149 (Fig.10c). The m/e 149 fragment is the most intense in the spectrum (Fig.10a), probably because minor fragmentations can also produce fragments of m/e 149. The mass spectrum of friedelane should be typical of these structures which do not possess high probability possible carbon-carbon bond cleavages.

Glutane and taraxerane again have different angular methyl

substitution patterns. Glutane has an angular methyl group at position 10 and this should produce a reasonably intense peak at m/e 177, as well as the m/e 259 and 205 peaks discussed above. Taraxerane has an angular methyl substituent at position 9 and this could give rise to a reasonably intense peak at m/e 191 as well as the m/e 259 peak as before. No authentic samples were available of these two compounds so these deductions cannot be verified at the present time.

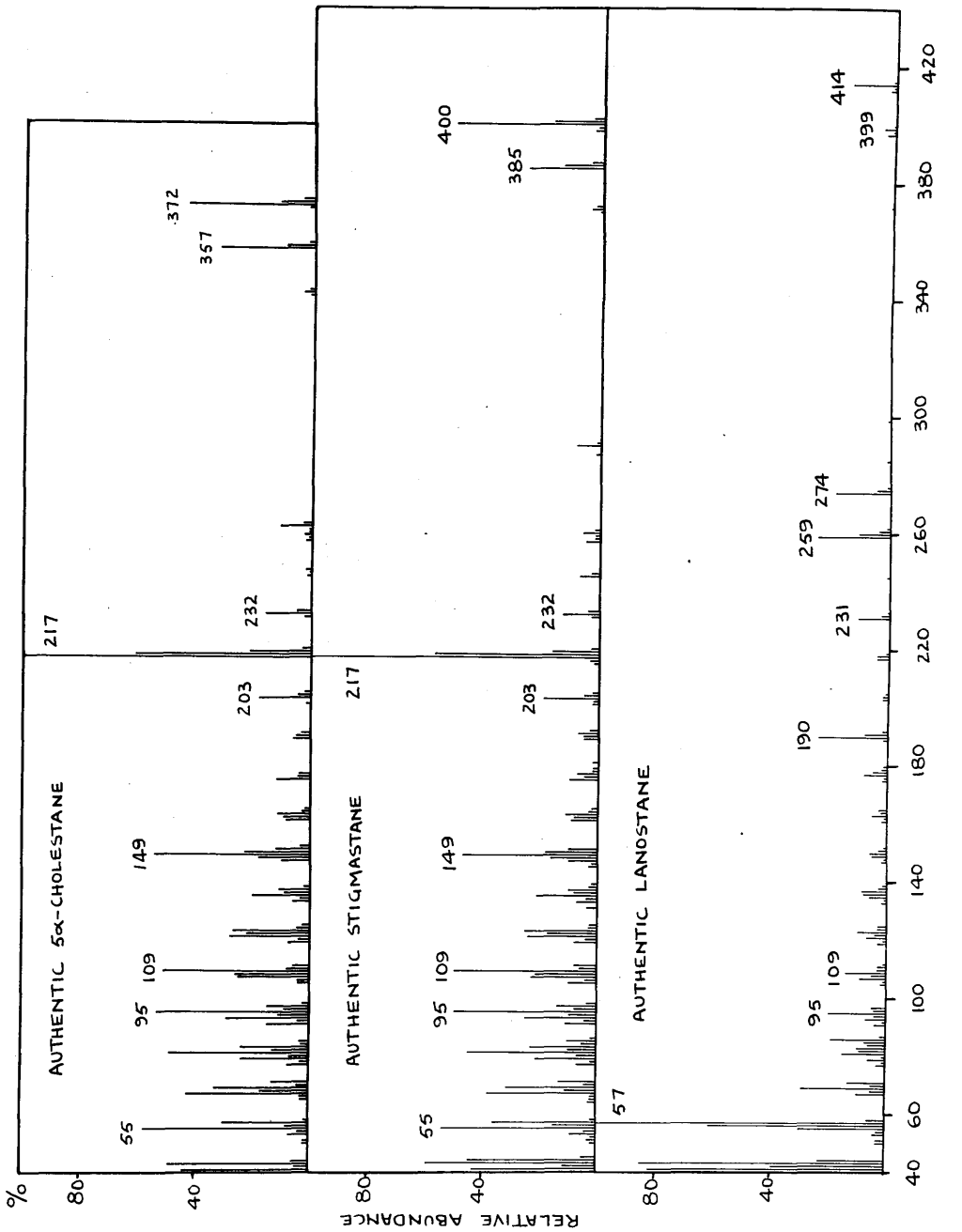
In summary, within, this group of eleven structures it is possible to differentiate between the observed and deduced mass spectra of some structures, with the exceptions of gammacerane, oleanane, taraxastane and ursane, and multiflorane and bauerane. Even if discrete structure identification cannot be made in these cases, at least the structure could be assigned to the particular type.

(iii) Tetracyclic with one 5 or all 6 membered rings (Fig. 11). The eight structures in this category include the tetracyclic triterpanes of the lanostane type and all the steranes. Once again, there are few features common to the whole group, but within the group there are features shared by some of the structures.

The structure of lanostane contains no adjacent quaternary centres which are likely cleavage points, therefore, the fragmentation would have been expected to produce ions of m/e

Figure 11A

The mass spectral line diagrams for lanostane, cholestane and stigmastane.


AUTHENTIC 5α-CHOLESTANE
AUTHENTIC STIGMASTANE
AUTHENTIC LANOSTANE
RELATIVE ABUNDANCE
%
80
40
55
95
109
149
203
217
232
357
372
400
385
57
190
231
259
274
399
414
60
100
140
180
220
260
300
340
380
420

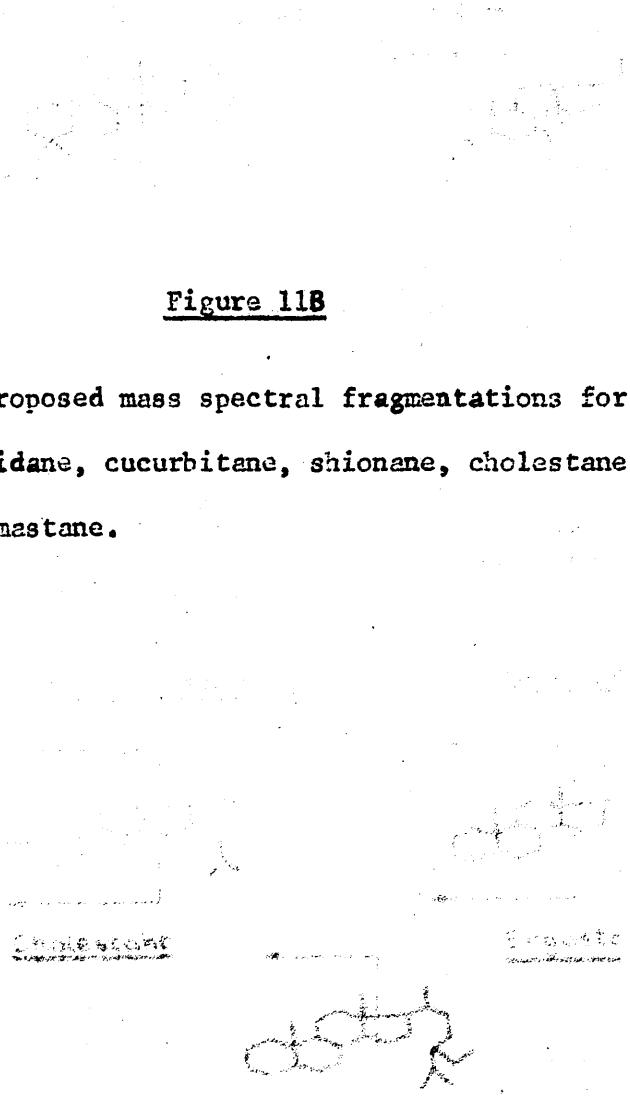
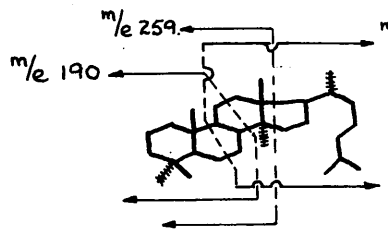
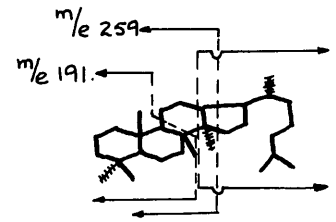


Figure 11B

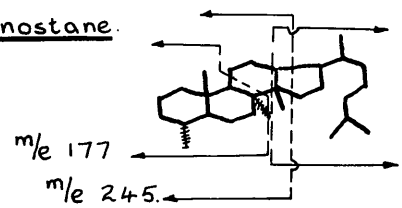
Observed and proposed mass spectral fragmentations for lanostane, dammarane, fusidane, cucurbitane, shionane, cholestane, ergostane and stigmastane.



Lanostane.

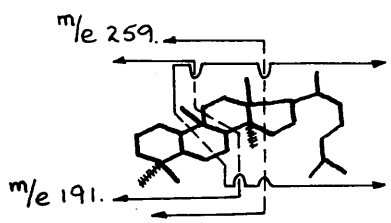


Dammarane.

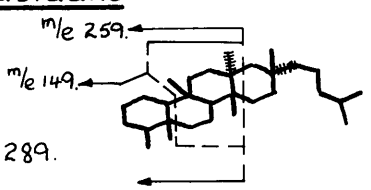


(Parent ion, m/e 400).

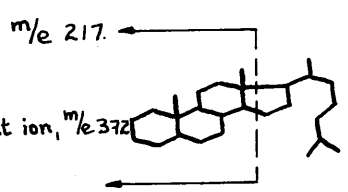
Fusidane.



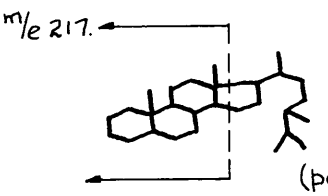
Cucurbitane.



Shionane

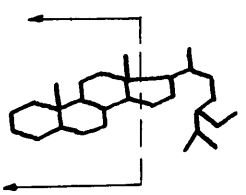


Cholestane.



(parent ion, m/e 386).

Ergostane.



(parent ion, m/e 400).

Stigmastane.

259 and 205, rather like those proposed for structures like fernane. However, examination of the mass spectrum of an authentic sample of lanostane (Fig.11a), reveals a somewhat different fragmentation pattern, i.e. peaks at m/e 274, 259 and 190 being among the most intense. The m/e 274 and 190 peaks may be explained by the proposed fragmentation in Figure 11b, where the normal two bond cleavages occur, probably followed by a rearrangement in which a neutral molecule and a hydrogen atom are eliminated. Cucurbitane has a similar structure except that the angular methyl group at position 6 has moved to position 10 and therefore may also be expected to produce ions of either m/e 289 or 288 and m/e 191 or 190.

Dammarane and fusidane are very similar in structure, the main difference being that fusidane has one less angular methyl than dammarane and will therefore have a parent ion of m/e 400 instead of m/e 414 for lanostane and dammarane. They both have two adjacent quaternary carbon atoms and should produce the same fragmentations as gammacerane, lupane etc. to give ions of m/e 193 and 191 for dammarane and m/e 193 and 177 for fusidane. The m/e 193 peak is caused by the ring E of pentacyclic triterpanes being missing in tetracyclic triterpanes of the lanostane type. Therefore, dammarane should be characterised by intense fragments of m/e 259, 193 and 191, and fusidane by intense ions of m/e 245, 193 and 177.

Shionane is a tetracyclic compound with only 6 membered ring and its angular methyl substitution pattern is virtually identical to that of friedelane in (ii) cf. Figure 10. Therefore shionane should produce the same fragmentation and give rise to high intensity ions of m/e 259 and 149.

Again, no authentic samples of dammarane, cucurbitane, fusidane or shionane were available so the deduced partial fragmentations given above cannot be checked at the present time. However, if the fragmentations do produce ions as described above, it should be possible to differentiate between all five of these structures.

The steranes contain only two quaternary carbon atoms and they are not adjacent, and since they only have two angular methyl substituents, their fragmentation patterns should be easier to interpret. In general, sterane mass spectra are recognised by the characteristic very intense peak of m/e 217 caused by the fragmentation shown in Figure **11b**. This is illustrated by the mass spectra of cholestane, and stigmastane (Fig.**11a**). The addition of an extra methyl group, or ethyl group is readily detected by the parent ion, i.e. m/e 372 or 386 or 400 for cholestane or ergostane or stigmastane respectively. The position of any additional methyl groups may also be detected in general terms. If an additional methyl group is attached to the sidechain, then the base peak, i.e. 100%

peak, will still be m/e 217. However, if the methyl group is attached to the cyclic nucleus, then the base peak will be given by m/e 231. In a similar way, loss of a methyl group will produce no change in the base peak if it was lost from the sidechain, but if it was lost from the cyclic nucleus then the base peak will be given by m/e 203 and so on. The characteristic peak of m/e 217 allows easy differentiation between steranes and triterpanes in general.

To compare, contrast and summarise the information to be gained from a mass spectrometric study of saturated hydrocarbon steroids and triterpenoids as has been discussed as briefly as possible above, the information has been tabulated (Table 4). Only the major characteristic ion fragment m/e values are listed with the respective parent ion values and the differences should be readily apparent. From this Table it is clear that, provided the molecules do fragment as postulated, most of these compounds may be identified and differentiated from the others by their mass spectra. The compounds which have almost identical spectra cannot be differentiated, but they may be readily assigned to a structure type on the basis of the Table.

However, to achieve individual identifications, e.g. between lupane and hopane some other method has to be used. Their structures are sufficiently different, allowing their

Table 4

The Characteristic Observed and Predicted Ion Fragments Produced in a Mass Spectrometer for Some Authentic Steranes and Triterpanes are listed

Compound	Parent ion	Observed and predicted characteristic ion fragments							
Lupane*	412	369				191			
Hopane*	412	369				191			
Adiantane*	398					191	177		
Fernane	412	369	327	259	205				
Filicane	412	369	327	259	205			149	
Arborane	412	369	327	259	205				
Adianane	412	369	327	259	205				
Gammacerane*	412					191			
Oleanane	412					191			
Taraxastane	412					191			
Ursane†	412					191			
Onocerane*	414					191			123
Ambreane	416					191			123
Multiflorane	412			259	205				123
Bauerane	412			259	205				123
Friedelane*	412			259	205			149	123

(Table 4 continued)

Compound	Parent ion	Observed and predicted characteristic ion fragments												
Glutane	412			259			205					177		123
Taraxerane	412			259					191					123
Lanostane*	414		274	259							190			
Cucurbitane	414	289		259					191					
Dammarane	414			259				193	191					
Fusidane	400				245			193				177		
Shionane	414			259									149	
Cholestane*	372					217								
Ergostane†	386					217								
Stigmastane*	400					217								

* Indicates that authentic samples were analysed by mass spectrometry and the fragmentation patterns studied.

† Mass spectra kindly provided by Mr. E. V. Whitehead (B.P. Research Centre, Sunbury-on-Thames).

separation by gas-liquid chromatography using long capillary columns. If the capillary column effluent is led into a mass spectrometer source, then mass spectra can be obtained of each separated component in the g.c. effluent. Thus combining the information gained by high resolution g.l.c. with the mass spectra obtained under combined g.c.-m.s. conditions, compounds like lupane and hopane may be separated and identified. This is an ideal example showing the usefulness of a combined g.c.-m.s. instrument.

Preliminary Investigation of the Low Voltage Mass Spectrometry of Cycloalkanes

Many authors[21,27,33] have published mass spectrometric data on sterane and triterpane mixtures without first separating them. The mass spectra are very complicated because of the many constituents in the mixture and because of the complex fragmentation patterns of the individual constituents. Reed and de Mayo[56] and Reed[57] carried out experiments on the thermal fragmentation, at 300-400°C at low voltages (10-15 eV), of steroids in an attempt to determine molecular weights and the mass of the steroid sidechain. The present investigation examines the fragmentations produced at voltages ranging from 12-70 eV at much lower temperatures (140-200°C) in collaboration with Dr. R. Binks using an MS-9 (A.E.I.). The reasons for attempting this were threefold: firstly, that a simple

method could be designed which could analyse a given mixture of steranes and triterpanes with respect to molecular weight ranges; secondly, to examine the fragmentations which persist even at the lower voltages in relation to sterane and triterpane structures;and thirdly, to examine possible differences produced by stereoisomers of steranes and triterpanes.

The formation of a molecular ion has been discussed previously and involves removal of one electron. The degree of excitation and the energy applied to the molecular ion govern the direction of fragmentation. If the applied energy is reduced then the molecular ion will not fragment so readily. If the applied energy is reduced so that no fragmentation occurs at all, then only the parent molecular ion will appear in the spectrum.

Firstly, it was found that at an applied voltage of 12 eV, steranes and triterpanes produce solely molecular ions. Thus if an unknown mixture is analysed by mass spectrometry at 12 eV, the molecular weight of each constituent may be established easily without interference from any other fragmentations.

Secondly, it was found that at 16 eV, steranes and triterpanes fragment, but the fragmentations produced are obviously those requiring the least applied energy. Therefore the ions obtained from steranes were the m/e 217 and the (M - 15) ion. By this means the mass of the sidechain could readily be

established. The triterpanes, as expected from the foregoing discussion, behaved in different ways, e.g. the ion at m/e 191 caused by the preferential cleavage between two quaternary centres is prominant at 16 eV. However, this project has not been finished at the present time so it is difficult to make general statements and so far we have no specific information on the differential fragmentation of stereoisomers. Nevertheless, the first two objectives were largely attained in that it has been proved possible to determine the range of molecular weight structures and many of the types of structures in a given mixture. These procedures may be valuable if a combined g.c.-m.s. instrument is not available and also could be used to some effect on a combined g.c.-m.s. if each eluted peak was subjected to high and low voltage scans, more information would be available and perhaps facilitate structure identification.

SECTION 3

COMBINED CAPILLARY COLUMN G.C.-M.S. OF THE BRANCHED AND CYCLIC ALKANE FRACTION FROM THE GREEN RIVER SHALE

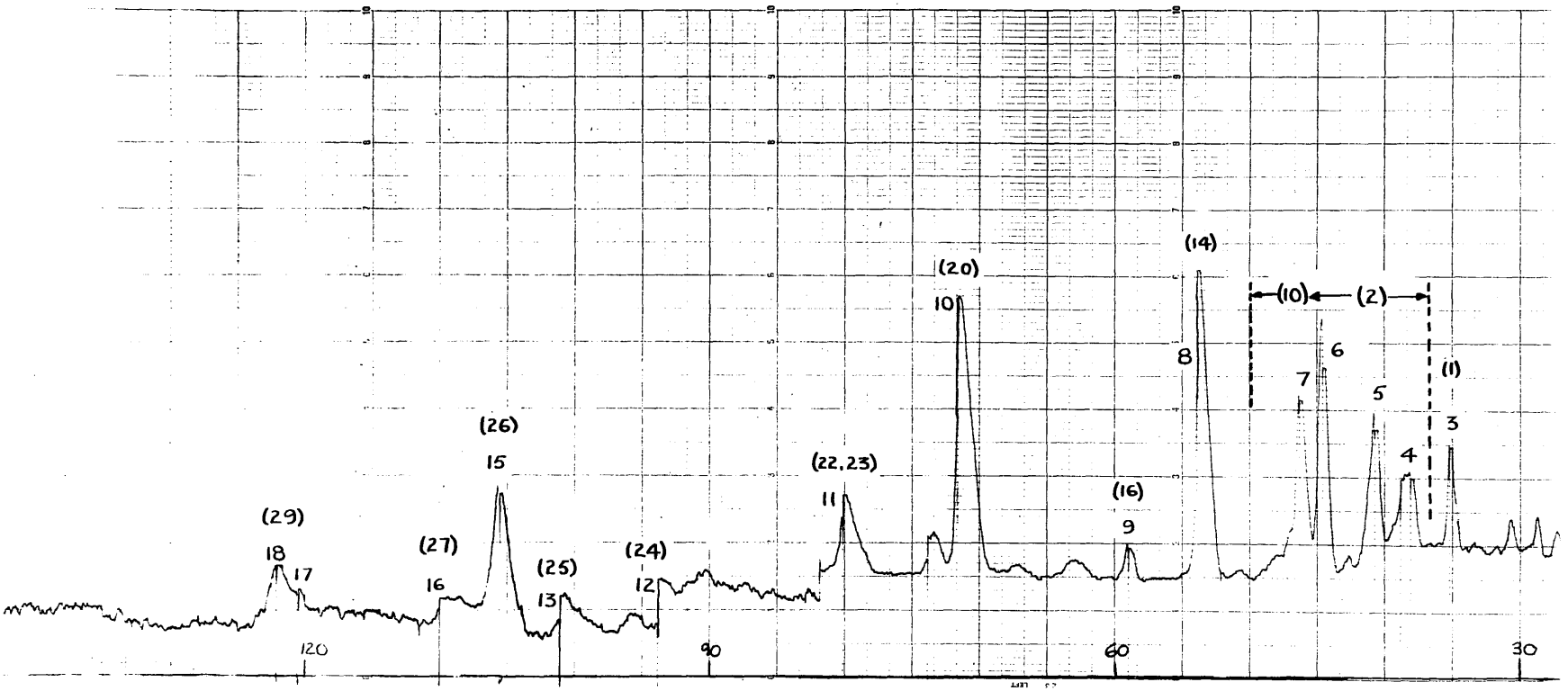
Discussion

The procedure outlined in the Experimental section (General) was used with an LKB 9000 g.c.-m.s. instrument fitted with a 200 ft x 0.01 in capillary column coated with AP-L grease (the same column as was used for the initial capillary g.l.c. analyses). Since the g.l.c. conditions had to be modified to fulfil the mass spectrometric requirements, i.e. the concentration of each component in the g.c. effluent reaching the ion source, the g.l.c. resolution was seriously affected. This is illustrated in Figure 12 which shows the g.l.c. record obtained from the g.c.-m.s. total ion current (t.i.c.) using the AP-L capillary column (cf. Fig. 7).

Two major effects are noticed, the first being that the region containing g.l.c. peaks 1-9 has not been well resolved in the g.c.-m.s. analysis. Secondly, because of the reduced sensitivity, the minor components observed in the g.l.c. record, e.g. peaks 10, 11, 12, 13, 18, 19, 24 and 25 are difficult to separate from the baseline noise. The baseline instability was caused by having to operate at the maxi-

Figure 12

The total ion current record for the branched and cyclic alkane fraction, isolated from the Green River shale, obtained by capillary column g.c.-m.s. using a 200' x 0.01" AP-L column.


(29)
18
17
120
(27)
16
(26)
15
(25)
13
(24)
12
90
(22,23)
11
(20)
10
(16)
9
60
(14)
8
(10)
(2)
7
6
5
4
(1)
3
30

mum t.i.c. sensitivity setting and thus factors like column bleeding and fluctuation in the helium concentration in the ion source produce an unstable baseline which are not observed at lower sensitivity settings. The mass spectrometric scan number with the corresponding g.l.c. peak number in parentheses are also shown in Figure 12. The parent ions, molecular formulae and compound type information obtained from these mass spectrometric scans are summarised in Table 5. This serves to illustrate the complexity and composition of the mixture.

In this Section, structure identifications will be discussed by combining the information obtained from the analytical capillary g.l.c. analyses with that from the g.c.-m.s. analyses. By this discussion the presence (or absence) of particular compounds will be established. The simplest way to present the discussion on possible structure identifications is to discuss each mass spectrometric scan in turn and assign the corresponding g.l.c. peak or peaks to it (Table 5).

Identification of 5α-Cholestane

Scan 3 corresponds to g.c. peak 1 and its mass spectrum shows a parent ion at m/e 372 giving a molecular formula of $C_{27}H_{48}$. Therefore, it has a tetracyclic structure and the characteristic base peak of m/e 217 identifies it as a sterane. Coinjection of authentic 5α-cholestane produced enhancement of peak 1 on two different capillary columns, i.e. the AP-L

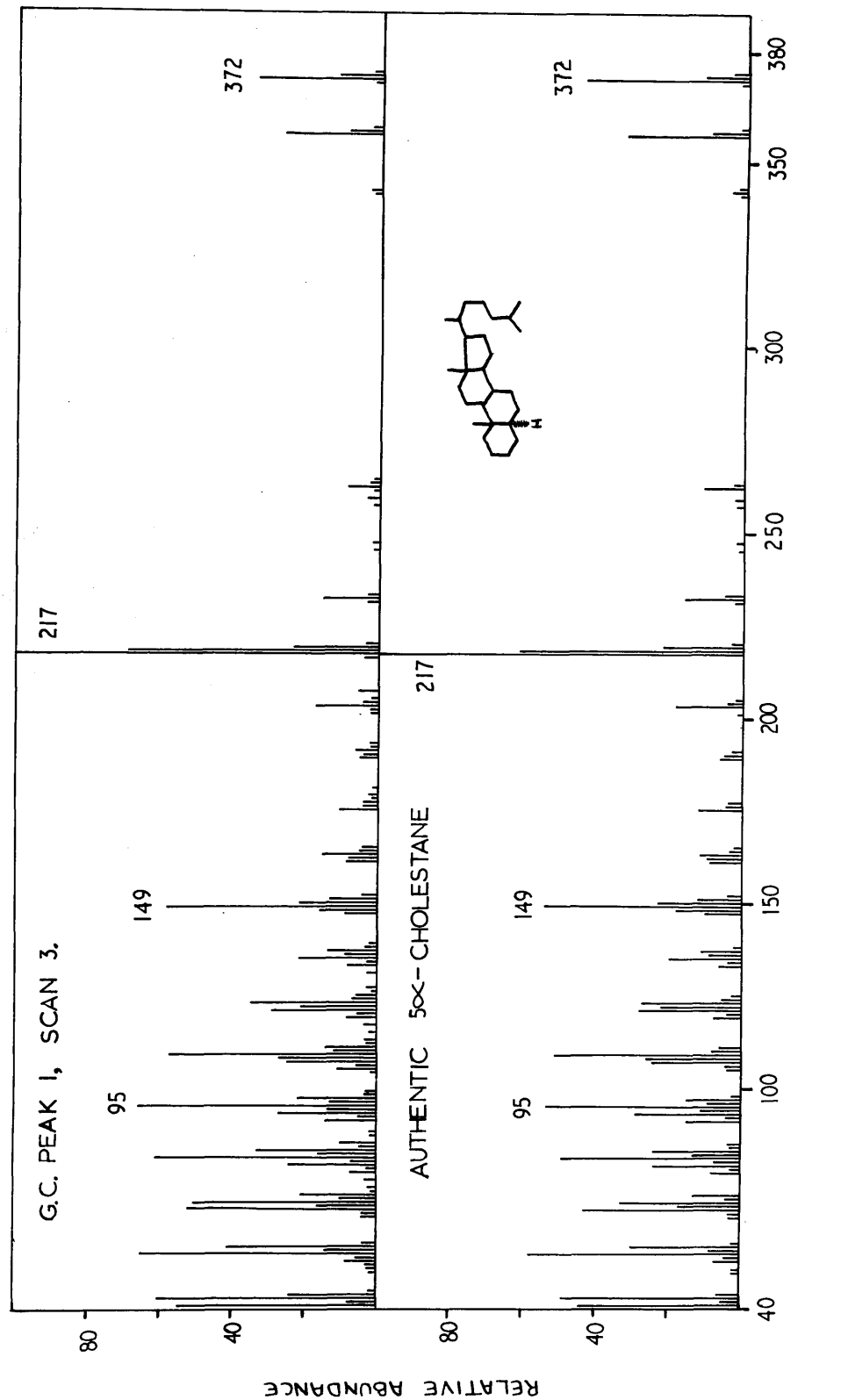
Table 5

Mass Spectrometric Data for the Cycloalkanes from the Green River Shale and the Deduced Structure Types

G.l.c. peak no. (Fig.7,12) (AP-L)	G.c.-m.s. scan no. (Fig.12) (AP-L)	Parent ion (m/e)	Molecular formula	Structure type
1	3	372	$C_{27}H_{48}$	Sterane (C_{27})
2	4	386	$C_{28}H_{50}$	Tetracyclic triterpane
		416	$C_{30}H_{56}$	Tricyclic triterpane
4	5	400	$C_{29}H_{52}$	Tetracyclic triterpane
5		460	$C_{33}H_{64}$	-
7		386	$C_{28}H_{50}$	Sterane (C_{28})
8	6	416	$C_{30}H_{56}$	Tricyclic triterpane
9		400	$C_{29}H_{52}$	Sterane (C_{29})
14	8	400	$C_{29}H_{52}$	Sterane (C_{29})
16	9	398	$C_{29}H_{50}$	Pentacyclic triterpane
20	10	412	$C_{30}H_{52}$	Pentacyclic triterpane
23	11	412	$C_{30}H_{52}$	Pentacyclic triterpane
24	12	426	$C_{31}H_{54}$	Pentacyclic triterpane
-	13	426	$C_{31}H_{54}$	Pentacyclic triterpane
26	15	412	$C_{30}H_{52}$	Pentacyclic triterpane
27	16	412	$C_{30}H_{52}$	Pentacyclic triterpane
28	17	476	$C_{34}H_{68}$	-
29	18	476	$C_{34}H_{68}$	-

Figure 13

Mass spectral line diagrams of g.c. peak 1a, scan 3 and authentic 5α-cholestane.


G.C. PEAK 1, SCAN 3.
AUTHENTIC 5α-CHOLESTANE
217
372
149
95
RELATIVE ABUNDANCE
80
40
100
150
200
250
300
350
380

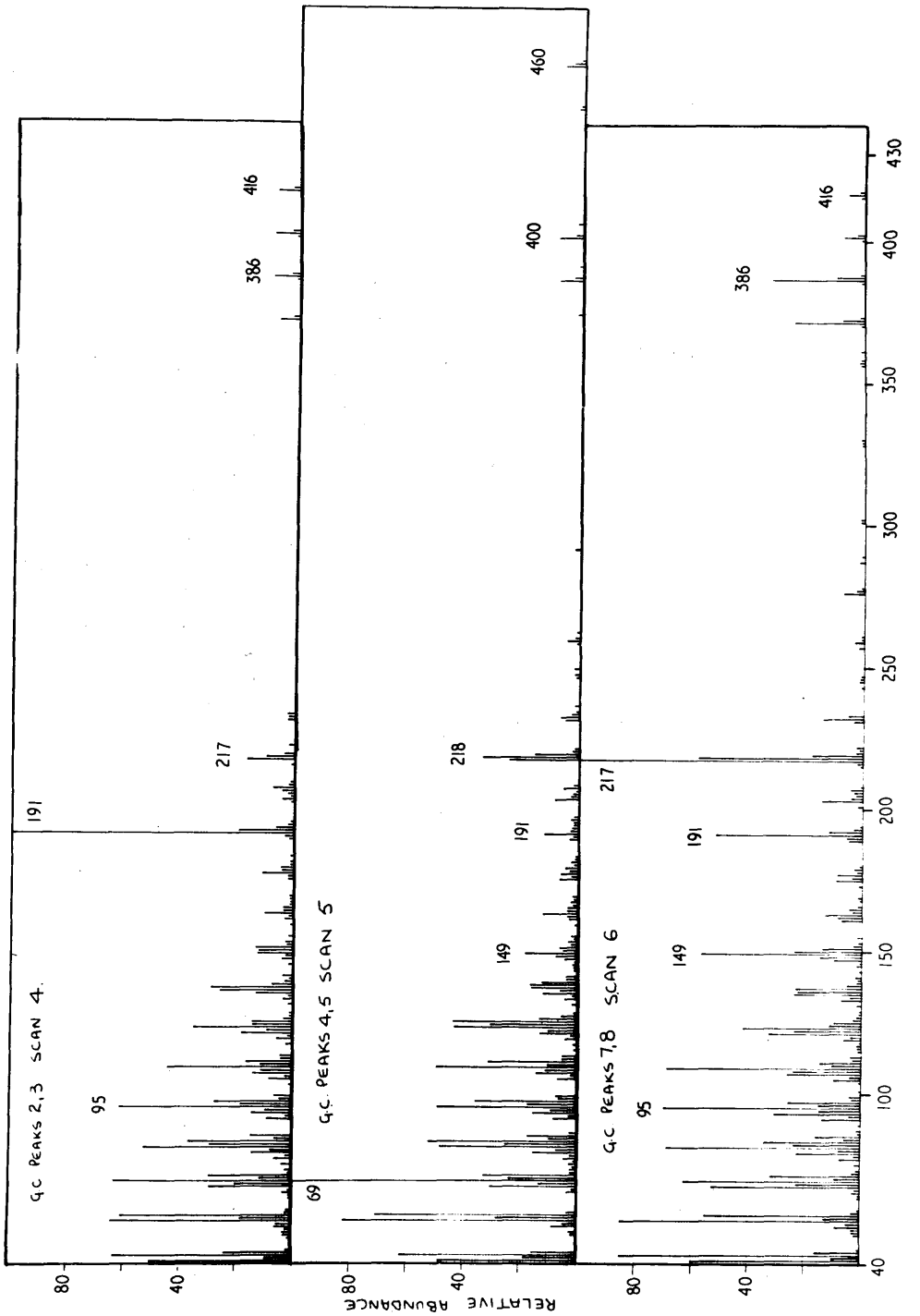
and 7-PPE coated columns. Comparison of the mass spectra of scan 3 and authentic 5α-cholestane shows they are identical (Fig. 13). Therefore peak 1 may be assigned to 5α-cholestane. Peak 1a was not observed in the g.c.-m.s. analysis, but on coinjection of authentic 5β-cholestane, enhancement of peak 1a occurred on both liquid phases. Thus peak 1a may probably be assigned to 5β-cholestane.

Identification of a Bis-Nor-Triterpane and a Tricyclic Triterpane

Scan 4 corresponds to g.c. peak 2 and probably 3. The mass spectrum (Fig. 14) shows that a mixture of two components is present, i.e. parent ions m/e 386 and 416, giving molecular formulae of $C_{28}H_{50}$ and $C_{30}H_{56}$ respectively. Considering the $C_{28}H_{50}$ component first, it can either be a sterane or a tetracyclic bis-nor-triterpane. Since there is no major peak at m/e 217, it cannot be a sterane. The presence of the m/e 191 peak as base peak indicates a triterpane structure for both components. The absence of a (P - 43) m/e peak shows that the structure contains no isopropyl group attached to the cyclic nucleus. A few of the possible structures are shown in Figure 14 The $C_{30}H_{56}$ component must be a tricyclic triterpane, confirmed by the m/e 191 base peak. It has no isopropyl group (P - 43 absent). Therefore ambreane or malabaricane, being the only tricyclic triterpanes known at the present time, are

Figure 14A

Mass spectral line diagram of g.c. peak 2 and 3, scan 4; mass spectral line diagram of g.c. peaks 4 and 5, scan 5; mass spectral line diagram of g.c. peaks 7 and 8, scan 6.


GC PEAKS 2,3 SCAN 4
GC PEAKS 4,5 SCAN 5
GC PEAKS 7,8 SCAN 6
RELATIVE ABUNDANCE
80
40
95
69
149
191
217
386
416
218
400
460
40
100
150
200
250
300
350
400
430

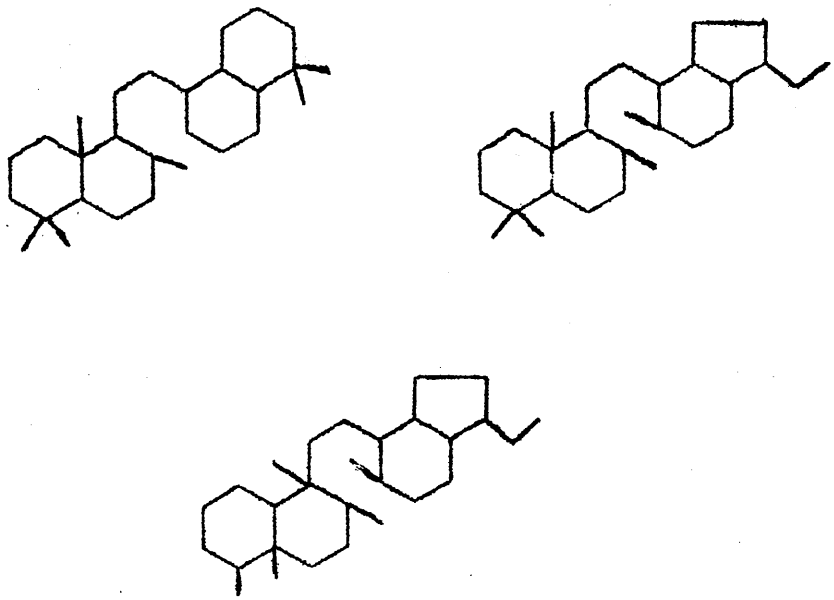


Figure 14B

Possible structures for g.c. peaks 2 and 3, scan 4.

possible structures. The mass spectrum is similar to the predicted fragmentation for ambreane and malabaricane. Coinjection of all the standard compounds produced no peak enhancement on either liquid phase. The carbon number calculated by g.l.c. does not exclude these structures.

Identification of a Friedelane-Type of Tetracyclic Triterpane

Scan 5 again is a mixture of two components, parent ions m/e 400 and 460. They may be assigned to g.c. peaks 4 and 5. Their molecular formulae calculated from the parent ions are $C_{29}H_{52}$ and $C_{33}H_{64}$. No more can be said about the $C_{33}H_{64}$ compound other than it contains two rings. The mass spectrum (Fig. 14) cannot be used in too much detail because of the unknown component ($C_{33}H_{64}$) but the m/e 218 peak is important. If the $C_{29}H_{52}$ component was a sterane, then it would exhibit an intense m/e 217 peak and also have a large m/e 149. There is no large m/e 149 and the m/e 217 peak is smaller than the m/e 218 peak. This is reminiscent of friedelane which also has a fairly intense m/e 218. There is no intense m/e 191, therefore there are no two adjacent quaternary carbon atoms which can give rise to the usual m/e 191 ions. Coinjection of the authentic standards produced no peak enhancement. It would appear to have a methyl substitution pattern somewhat like

friedelane and, from its retention data, a cyclic structure somewhat like the onoceranes. With the present data no structure may be assigned to this peak which fulfills all the requirements. It is possible that during diagenesis the carbon skeleton has been rearranged thus accounting for the difficulty in assigning the fragmentation pattern to known structures which obey the biogenetic isoprene rule.

Identification of Ergostane and a Tricyclic Triterpane

Scan 6 is also a mixture of two components and may be assigned to g.c. peaks 7 and 8. The parent ions are m/e 386 and 416, corresponding to molecular formulae of $C_{28}H_{50}$ and $C_{30}H_{56}$ respectively (Fig. 14). Peak 7 may probably be assigned to the $C_{28}H_{50}$ component and peak 8 to the $C_{30}H_{56}$ component because the relative intensity of the parent ion of $C_{28}H_{50}$ is five times greater than that of the $C_{30}H_{56}$ compound since peak 7 is larger than peak 8 in the g.l.c. record. The m/e 217 peak is the base peak of the spectrum and with the m/e 149 and 232 peaks indicates that the $C_{28}H_{50}$ component is a sterane like ergostane, i.e. a 24-methyl cholestane. Comparison of the spectrum of scan 6 with authentic ergostane (mass spectrum supplied by Mr. E. V. Whitehead) confirms the similarity. Unfortunately no authentic ergostane was available for coinjection purposes but the carbon number data is consistent with that of a $C_{28}H_{50}$ sterane, i.e. approximately mid-way

between 5α-cholestane and stigmastane. Peak 8 has an m/e 191 fragment indicating a triterpane structure. As for scan 4, the only tricyclic triterpanes known are ambreane and malabaricane, peak 8 possibly being isomeric with peak 2.

Identification of Stigmastane

Scan 7 shows that g.c. peak 9 is a single pure component,* i.e. parent ion m/e 400, corresponding to a molecular formula of $C_{29}H_{52}$. The fragmentation pattern (Figure 15) is characteristic of a sterane, i.e. m/e 232, 217 (100%) and 149. Comparison of this spectrum with that of authentic stigmastane (Fig. 15) shows a distinct similarity, but in scan 7 peaks m/e 163 and 191 are more intense than m/e 203, whereas in authentic stigmastane peaks m/e 163, 175 and 191 are less intense than m/e 203. Also the relative intensities of m/e 123 and 149 are different. Coinjection of authentic stigmastane did not enhance peak 9 on either liquid phase, but did enhance peak 14 on both phases. Peak 9 cannot be stigmastane, but it is probably related to it, because of this it will be advantageous to discuss scan 8, g.c. peak 14, in conjunction with scan 7 since some interesting conclusions appear.

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* It is possible that a peak, which, by mass spectrometry appears to be pure, could be a mixture of stereoisomers, inseparable by this technique. Development of optically active g.c. phases could solve this particular problem.

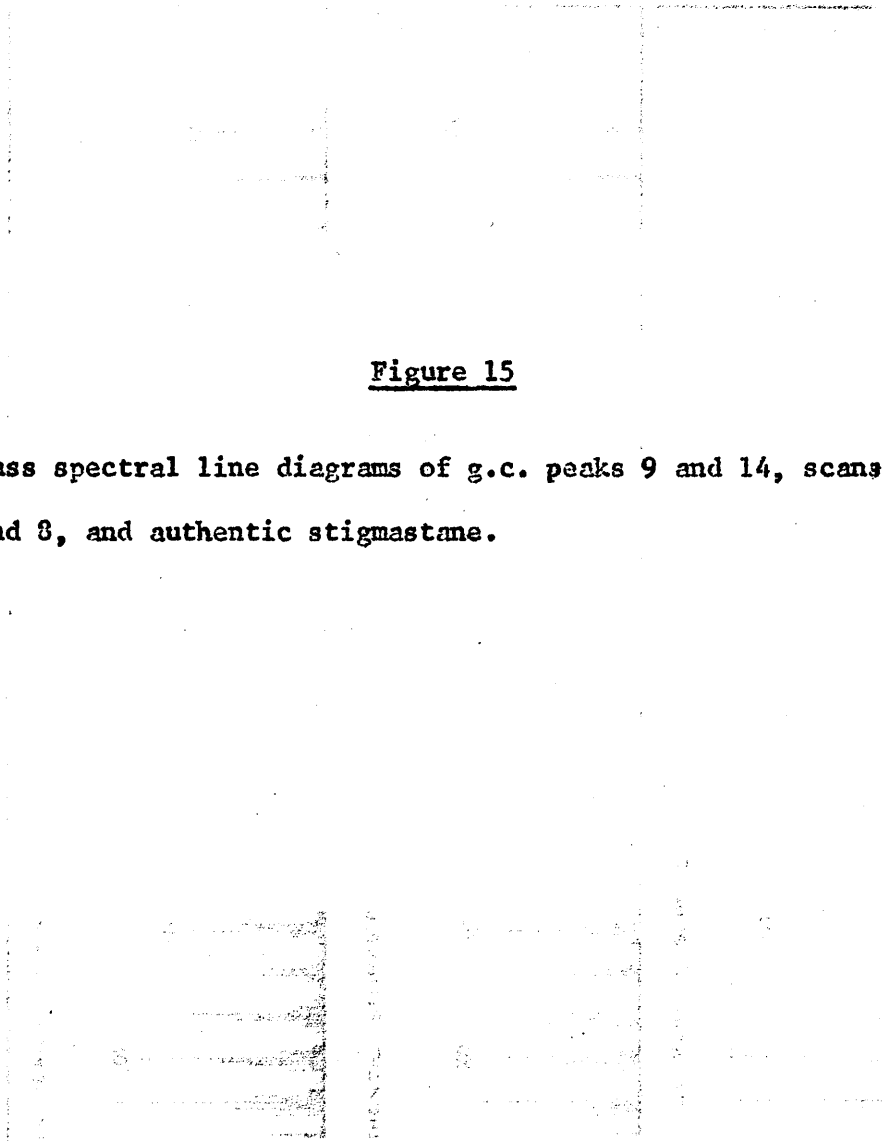
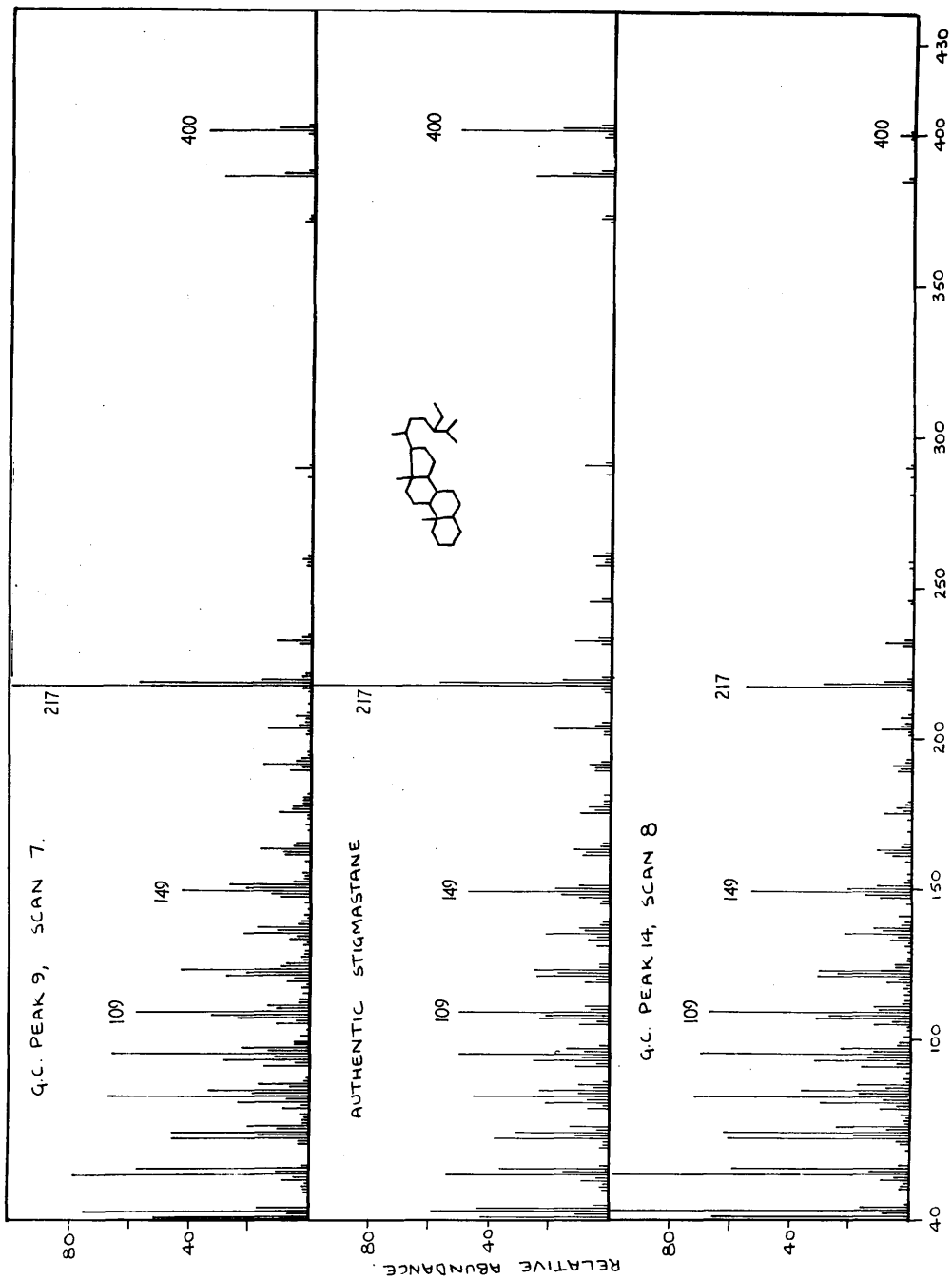


Figure 15

Mass spectral line diagrams of g.c. peaks 9 and 14, scans 7 and 8, and authentic stigmastane.


G.C. PEAK 9, SCAN 7.
AUTHENTIC STIGMASTANE
G.C. PEAK 14, SCAN 8
RELATIVE ABUNDANCE
80
40
109
149
217
400
40
100
150
200
250
300
350
400
430

Scan 8 shows that peak 14 is a single pure component of molecular formula $C_{29}H_{52}$ given by the parent ion m/e 400. The fragmentation pattern (Fig. 15) is identical to that of authentic stigmastane except for the intensities from m/e 200 → 400. They are lower than expected and the decrease is caused by the mass spectrometric scan having been taken too late when the maximum concentration of molecules in the source had been reached and was decreasing, so the peaks of higher m/e values showed a false intensity (since the LKB magnet scans from low to high mass). As stated before, coinjection of authentic stigmastane enhanced peak 14 on both liquid phases. Therefore peak 14 is stigmastane. A comparison of retention data for all the peaks now identified as being steranes provides evidence of the relationship between peaks 1a, 1, 7, 9, and 14 on both liquid phases (Table 6). By subtraction of the carbon number value of each peak from the other peaks, e.g. 1-1a, 7-1, 9-7, 14-9 followed by 7-1a, 9-1, 14-7 and finally 9-1a and 14-1, and the differences tabulated, identical or near identical values are obtained, the only exceptions being the values involving peak 1a. An error in the measurement of the carbon number of peak 1a of only 0.10 on the AP-L column or on the 7-PPE column would account for the erroneous value. Nevertheless, because the same differences are found between the same peaks on two different phases, these indicate that

Table 6

Tentative Correlation of Carbon Number Data for the Five Steranes Found in the Green River Cycloalkane Fraction

Peak Number (7-PPE)	Carbon Number	1 - 2	1 - 3	1 - 4	
1a (1)	30.07				5β-Cholestane
		0.42			
1 (2)	30.49		1.35		5α-Cholestane
		0.93		1.55	
7 (3)	31.42		1.13		Ergostane
		0.20		1.66	
9 (4)	31.62		0.73		Stigmastane type
		0.53			
14 (5)	32.15				Stigmastane

Peak Number (AP-L)	Carbon Number			
1a (1)	29.58			
		0.32		
1 (2)	29.90		1.24	
		0.92		1.42
7 (3)	30.82		1.10	
		0.18		1.63
9 (4)	31.00		0.71	
		0.53		
14 (5)	31.53			

their structures are all related and behave in a similar fashion towards the column substrates. This is an important finding because it illustrates another aspect of the power of combined capillary g.c.-m.s. A knowledge of the molecular formula and compound type combined with quantitative retention data allows correlations of the fractional retention changes accompanying the introduction of substituents or changes in stereochemistry in a series of compounds which are structurally related. In this way group retention factors[57a] may be assigned for changes like 5α converting to 5β in rings A and B of a sterane, or for hopane - iso-hopane isomerism in triterpanes. However for this to be a useful and meaningful technique, many authentic compounds will have to be analysed on several different liquid phases. Thus it appears likely that the compounds of molecular formula $C_{29}H_{52}$ representing peaks 9 and 14 are stereoisomers. Peak 14 has been identified as stigmastane so peak 9 probably is an isomer of stigmastane, although not necessarily of the same type of isomerism as exhibited by 5α and 5β-cholestane.

Identification of a Pentacyclic Nor-Triterpane

Scan 9 shows that g.c. peak 16 is a single pure component, its molecular formula of $C_{29}H_{50}$ being given by a parent peak of m/e 398. This corresponds to a pentacyclic nor-triterpane. The fragmentation pattern is interesting (Fig. 16). There is

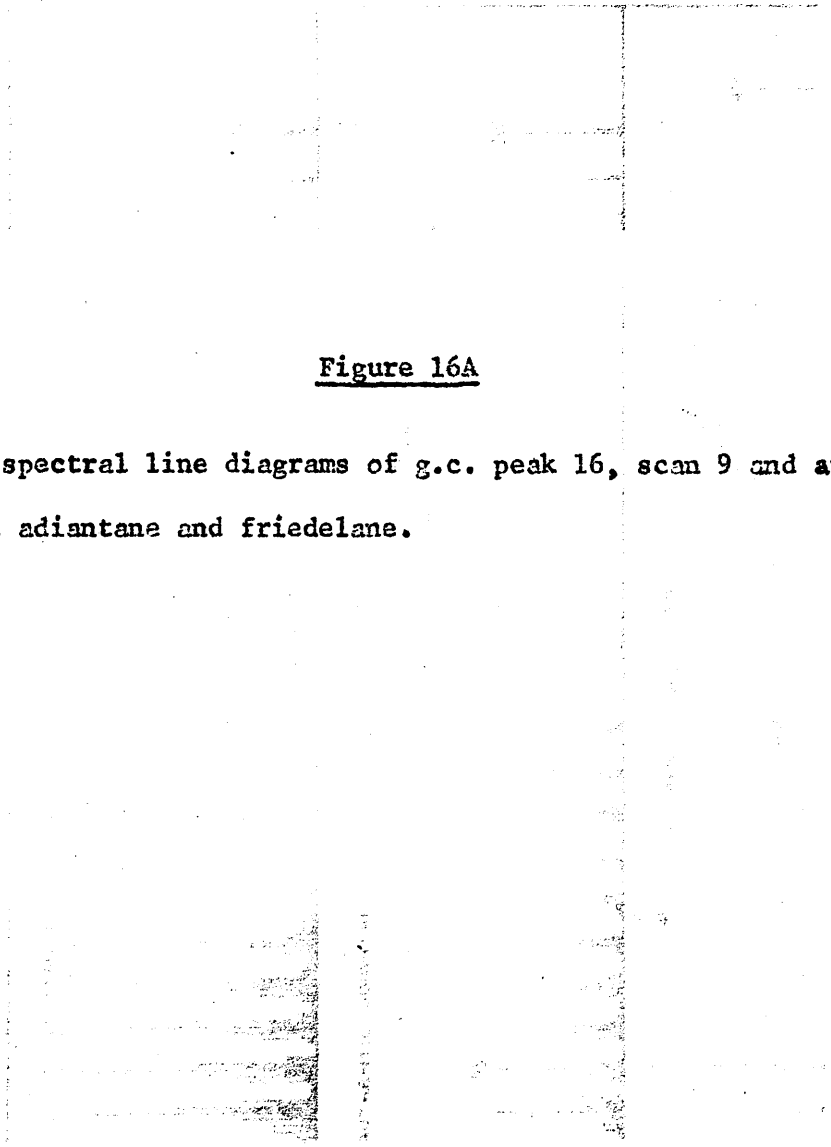


Figure 16A

Mass spectral line diagrams of g.c. peak 16, scan 9 and authentic adiantane and friedelane.

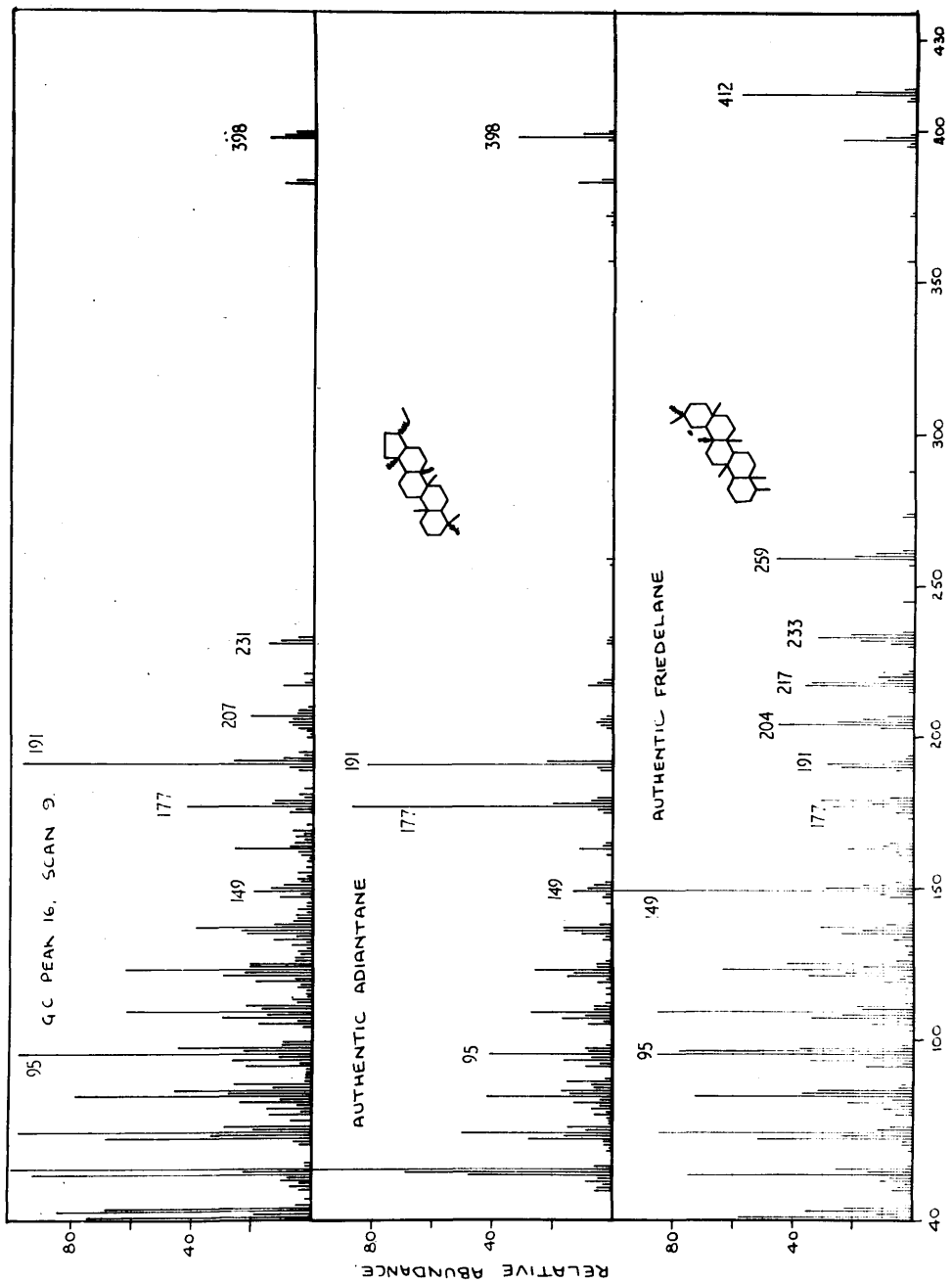
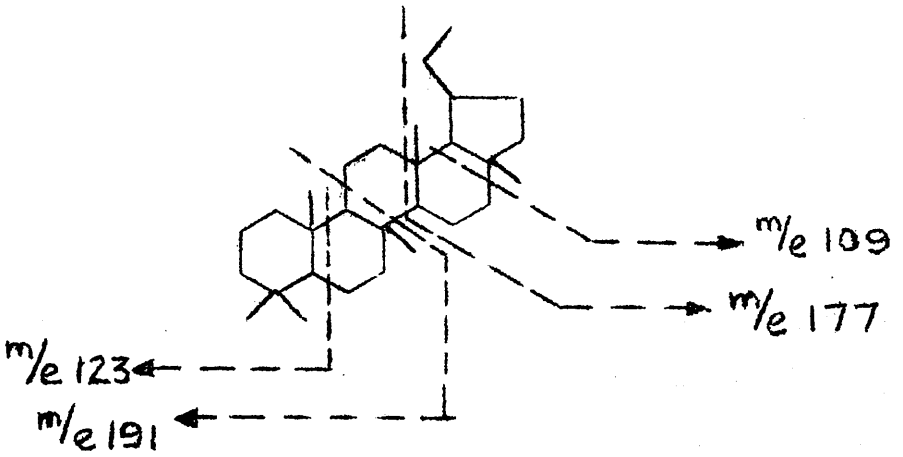
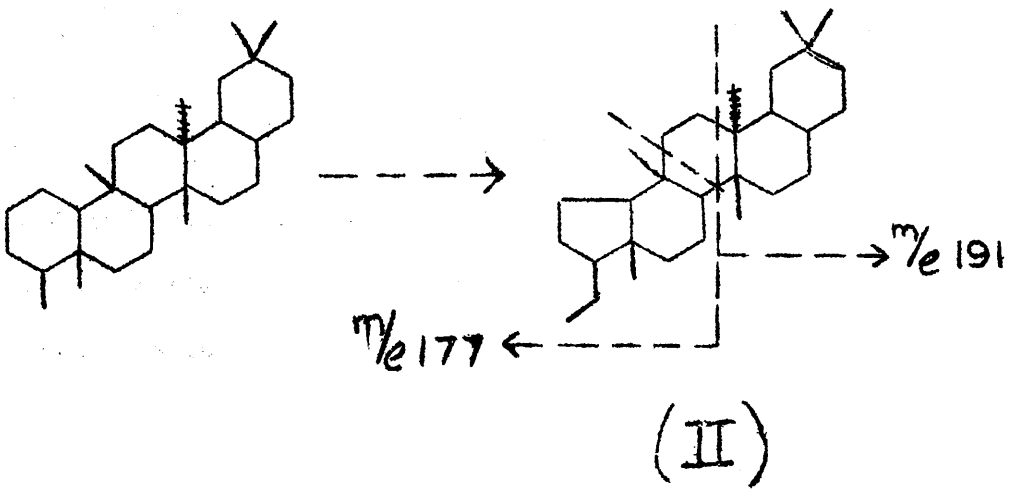

GC PEAK 16, SCAN 9.
AUTHENTIC ADIANTANE
AUTHENTIC FRIEDELANE
RELATIVE ABUNDANCE
95
149
177
191
207
231
398
412
259
233
217
204
40
80
100
150
200
250
300
350
400
430

Figure 16B

Two Possible Structures which Fit the Data for G.C. Peak 16.



(I).



(II)

no (P - 43) peak, therefore there is no isopropyl group in the structure. The m/e 191 peak is not the base peak, and the m/e 177 is of medium intensity. Comparison of scan 9 with the mass spectrum of authentic adiantane (a 30-nor-triterpane) shows that they are not similar. Adiantane has m/e 177 = m/e 191 because of the fragmentation at the two adjacent quaternary carbon atoms. Coinjection of authentic adiantane produced no enhancement of peak 16 on either of the two liquid phases, so peak 16 cannot be adiantane. No other authentic compound produced enhancement of peak 16 on coinjection. On the basis of the retention data and the mass spectrum, only a tentative structure can be postulated for peak 16. From the retention data, since it is pentacyclic, it must have one 5 membered ring because the evidence from the authentic triterpanes indicates that a pentacyclic structure with only 6 membered rings would have a much longer retention time. The low intensity of the m/e 149 peak and the absence of the m/e 259 peak indicates that it cannot have the friedelane type of methyl substitution pattern (cf. mass spectra of friedelane and scan 9 in Figure 16). The carbon number difference between moretane and lupane (both pentacyclic C_{30} triterpanes with ring E 5 membered with an isopropyl sidechain) is 0.43 with lupane having the lower value. The carbon number difference between adiantane and peak 16 is 0.41 with peak 16 having

the lower value. Therefore it could be that the structure of ring E in peak 16 is similar to that of lupane, the former being a 30-nor structure. If that was the only difference, the mass spectra of adiantane and scan 9 would be very similar, but the differences in the relative intensities of m/e 191 and 177 show that some other difference is present. Similarly, the carbon number difference between adiantane and peak 16 is somewhat higher than expected on comparison with the value for moretane and lupane, so possibly a single methyl rearrangement has occurred during diagenesis to produce a new angular methyl substitution pattern which could give rise to a mass spectrum similar to that of scan 9. Structure I is a possible example where the methyl group normally at the C_{14} position has migrated to the C_{13} position. This structure could produce ions of value m/e 191 and 177, m/e 123 and 109 of probably equal intensity and the m/e 149 would be small, by the fragmentations shown (Fig. 16). However, there would appear to be no reason why m/e 177 should be much less intense than m/e 191. Another possibility may be that peak 16 was originally derived from a friedelane type of structure which had lost a methyl group from the C_{28} position, i.e. a 28-nor-triterpane which then rearranged, possibly during diagenesis, to give structure II (Fig. 16). This structure could be expected to meet the retention data requirements and also the mass spectrometric re-

quirements. The possible fragmentation is shown and the m/e 191 peak would be expected to be of higher intensity than the m/e 177 peak. Of course there may be many more structures which are possible and meet the requirements, but until more quantitative g.l.c. data is available, the structure of the component represented by peak 16 must remain in doubt.

Identification of Hopane

Scan 10 shows that peak 20 is a single pure component of molecular formula $C_{30}H_{52}$ given by the parent ion m/e 412, corresponding to a pentacyclic triterpane. The fragmentation pattern (Fig. 17) is typical of structures containing the two adjacent quaternary carbon atoms at C_9 and C_{14} positions in that the m/e 191 peak is the base peak of the spectrum. The presence of a (P - 43) peak at m/e 369 indicates an isopropyl sidechain, i.e. it must be a pentacyclic structure with one 5 membered ring and an isopropyl substituent group. The combination of m/e 369 and 191 (100%) limits the possible structures to the lupane, moretane and hopane types. Coinjection of authentic lupane and moretane did not cause any enhancement of peak 20 on either of the liquid phases. Coinjection of authentic hopane was not possible because of lack of material. Comparison of the spectra of authentic lupane,

and hopane with that of scan 10 (Fig. 17), shows that scan 10 is similar to each authentic spectrum, but is

Figure 17A

Mass spectral line diagrams of g.c. peak 20, scan 10, and authentic lupane and hopane, and g.c. peak 23, scan 11.

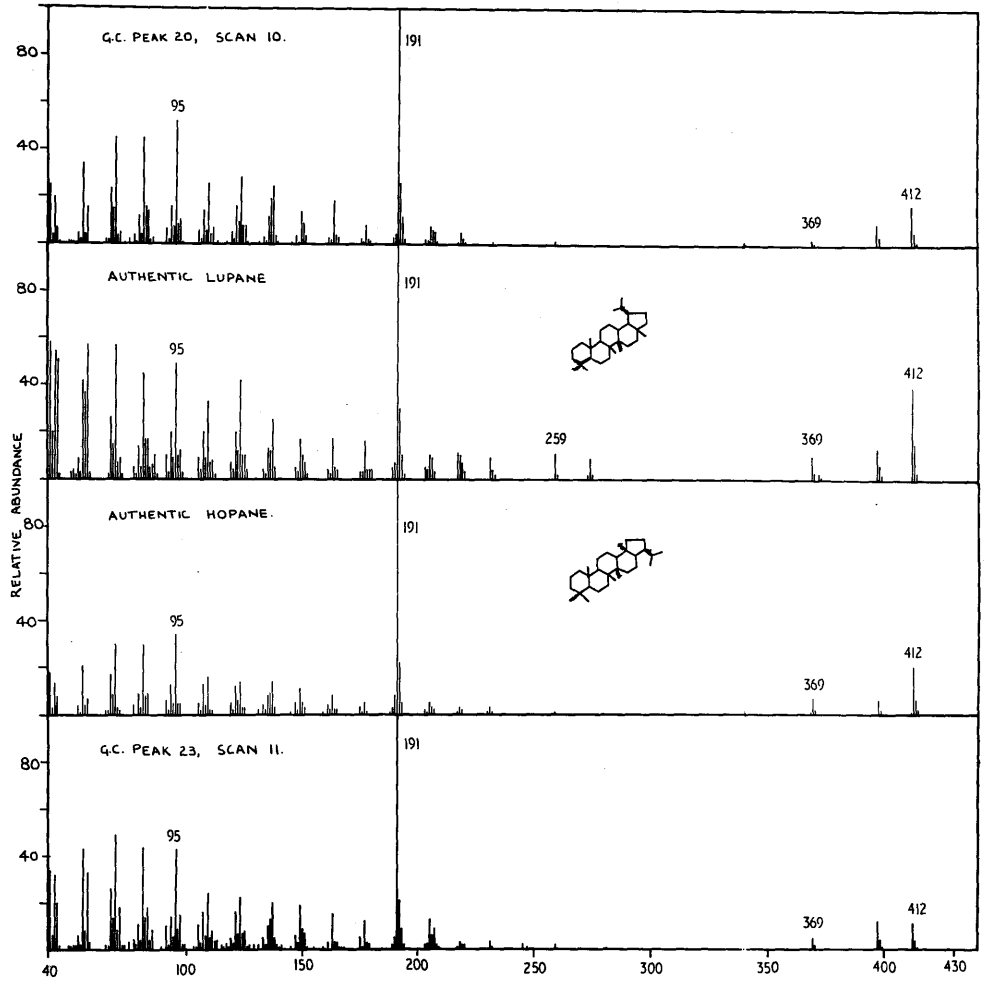
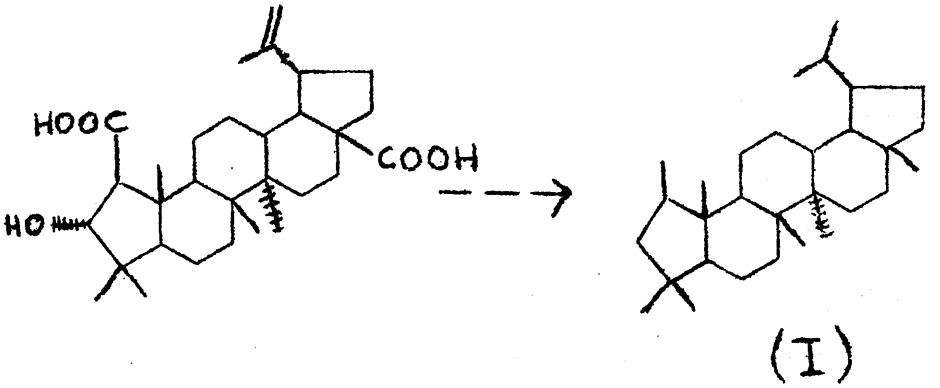
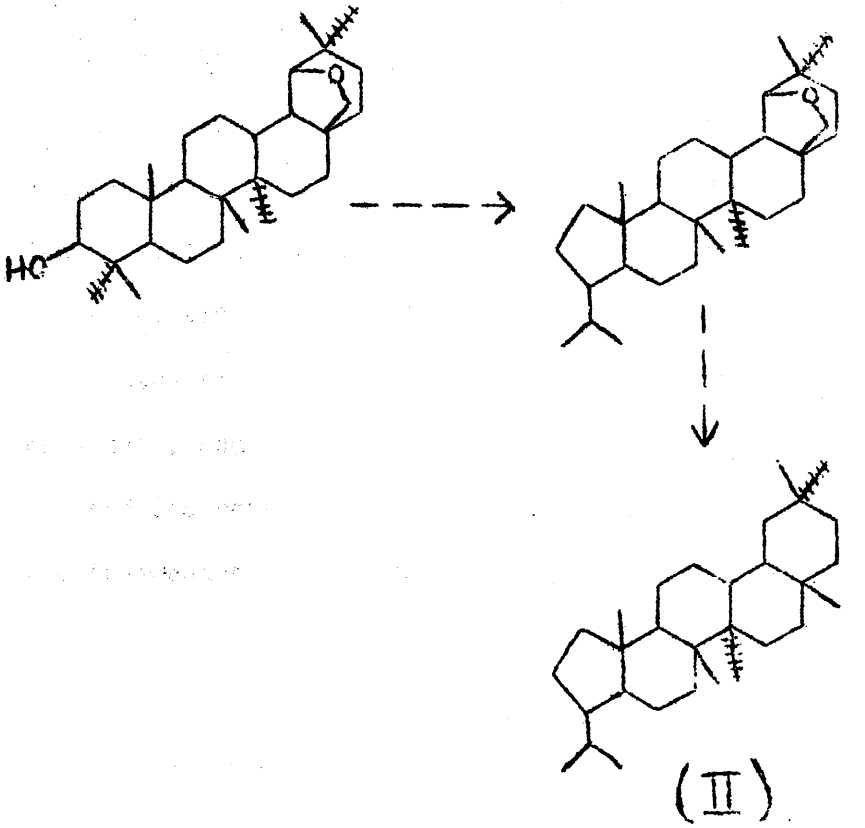

G.C. PEAK 20, SCAN 10.
AUTHENTIC LUPANE
AUTHENTIC HOPANE.
G.C. PEAK 23, SCAN 11.
191
95
259
369
412
RELATIVE ABUNDANCE
80
40
40
100
150
200
250
300
350
400
430

Figure 17B

Two Possible Structures for the G.C. Peak 23



(I)



(II)

identical with the authentic hopane spectrum. Therefore peak 20 is hopane.

Possible Identification of a Rearranged Pentacyclic Triterpane

Scan 11 shows that peak 23 is a single pure component of molecular formula $C_{30}H_{52}$ given by the parent ion m/e 412, corresponding to a pentacyclic triterpane. The fragmentation pattern (Fig. 17) is virtually identical to those of lupane and hopane with the exception of a few minor intensity differences. The m/e 369 indicates the presence of an isopropyl group and the m/e 191, the base peak, the two adjacent quaternary carbon atoms. Coinjection of authentic lupane and moretane produced no enhancement of peak 23 which had a higher carbon number value than both authentic samples (Table 2). Since the spectra of lupane, hopane and peak 23 are so similar, the structure of peak 23 must be clearly related to the authentic compounds, but by the retention data, some rearrangement may have occurred which increased its retention time, without causing a change in the fragmentation pattern. On the other hand, peak 23 may have been derived from a different class of triterpane and undergone rearrangement to produce a structure closely similar to the lupane, moretane and hopane types. Again, without the aid of more quantitative g.l.c. data, it is impossible to deduce a definite structure from the available facts, but some

possible structures may be postulated which meet the requirements of the present data. An example of a rearrangement which could occur in a lupane type structure is shown in Figure 17. Ceanothic[58] acid on reduction in a reducing environment during diagenesis could give abeo-lupane(I). The mass spectrum of abeo-lupane would not be very different from lupane, but its retention time would probably be shorter because of the more planar shape of the molecule. On the other hand, allobetulin (oleanane structure) can rearrange as shown in Figure 17 to produce α-apoallobetulin.[59] A hydrocarbon of this skeleton (II) would probably have a very similar fragmentation pattern to lupane, moretane and hopane, but its retention time would either be of the same order or slightly longer. It would appear that the latter example is the most likely kind of structure to meet the requirements of the available data for peak 23.

Identification of C_{31} Pentacyclic Triterpanes

Scan 12 shows that g.c. peak 24 is a single pure component of molecular formula $C_{31}H_{54}$ as given by the parent ion m/e 426. This corresponds to a pentacyclic structure with no isopropyl group since the (P - 43) peak is absent. The only characteristic peak in the mass spectrum is at m/e 191 (Fig. 18), but it is not the base peak. None of the authentic standard compounds afforded any peak enhancement on coinjection.

Figure 18A

Mass spectral line diagrams of g.c. peak 24, scan 12, scan 13, authentic lanostane.

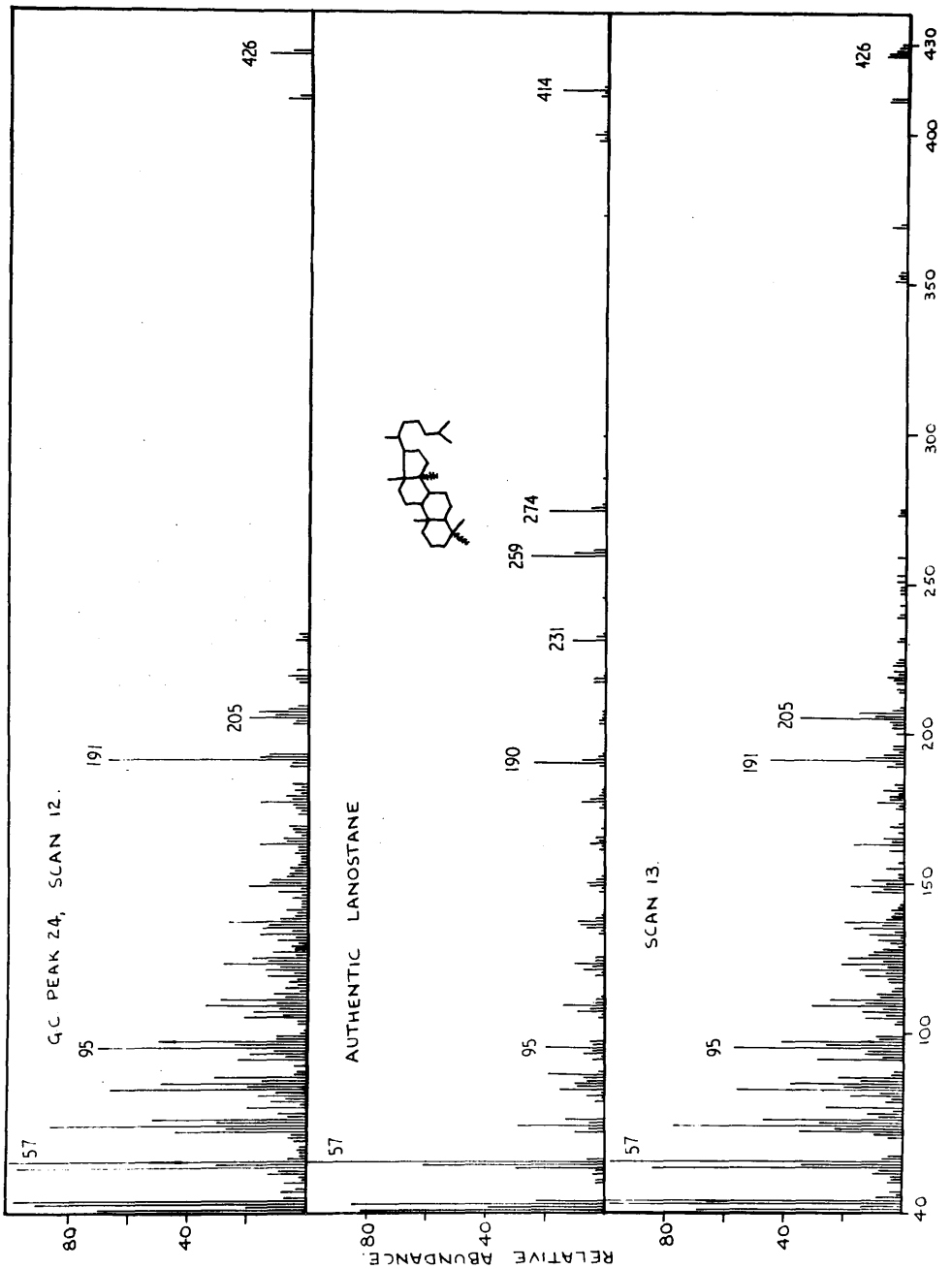
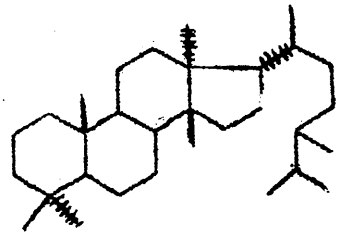
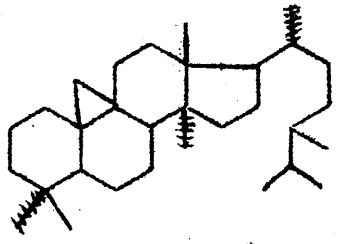

GC PEAK 24, SCAN 12.
AUTHENTIC LANOSTANE
SCAN 13.
RELATIVE ABUNDANCE
57
95
191
205
426
190
231
259
274
414
40
80
100
150
200
250
300
350
400
430

Figure 13B

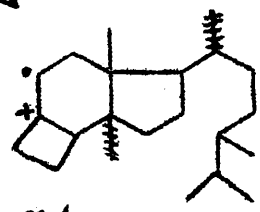
The structures of euphorbane and 24-methyl cycloartane and the characteristic fragmentation of a cycloartane type structure.



(I)



(II)



m/e 303

Since the molecular formula indicates a C_{31} pentacyclic structure, skeletons like those of euphorbane (I) and 24-methyl lanostane can be ruled out because they are both tetracyclic C_{31} compounds. Moreover, they would be expected to fragment like lanostane and would therefore not give rise to an intense m/e 191 peak. Again 24-methyl cycloartane (II) meets the molecular formula requirements, but will not give rise to an intense m/e 191 peak. The fragmentation of a cycloartane ring system is dominated by the cleavage centred on the 3 membered ring (cf. Fig. 18). In view of the available information, no tentative structure can be assigned to peak 24.

Scan 13 indicates a compound of molecular formula $C_{31}H_{54}$ (Fig. 18), given by a parent ion of m/e 426, corresponding to a pentacyclic structure. It was impossible to assign this scan to the corresponding g.c. peak number, so no retention data is available. Both scan 12 and 13 are of very low concentration peaks and the mass spectra had to be recorded at high electron multiplier settings. The fragmentation patterns therefore became more complex because of the background peaks (which had been negligible on previous scans) and possibly variations in the total ion current. Nevertheless, the parent peaks at m/e 426 and the peaks at m/e 191 were very clear in both cases, however the structure of these two components must remain unidentified at the present time.

Identification of Gammacerane

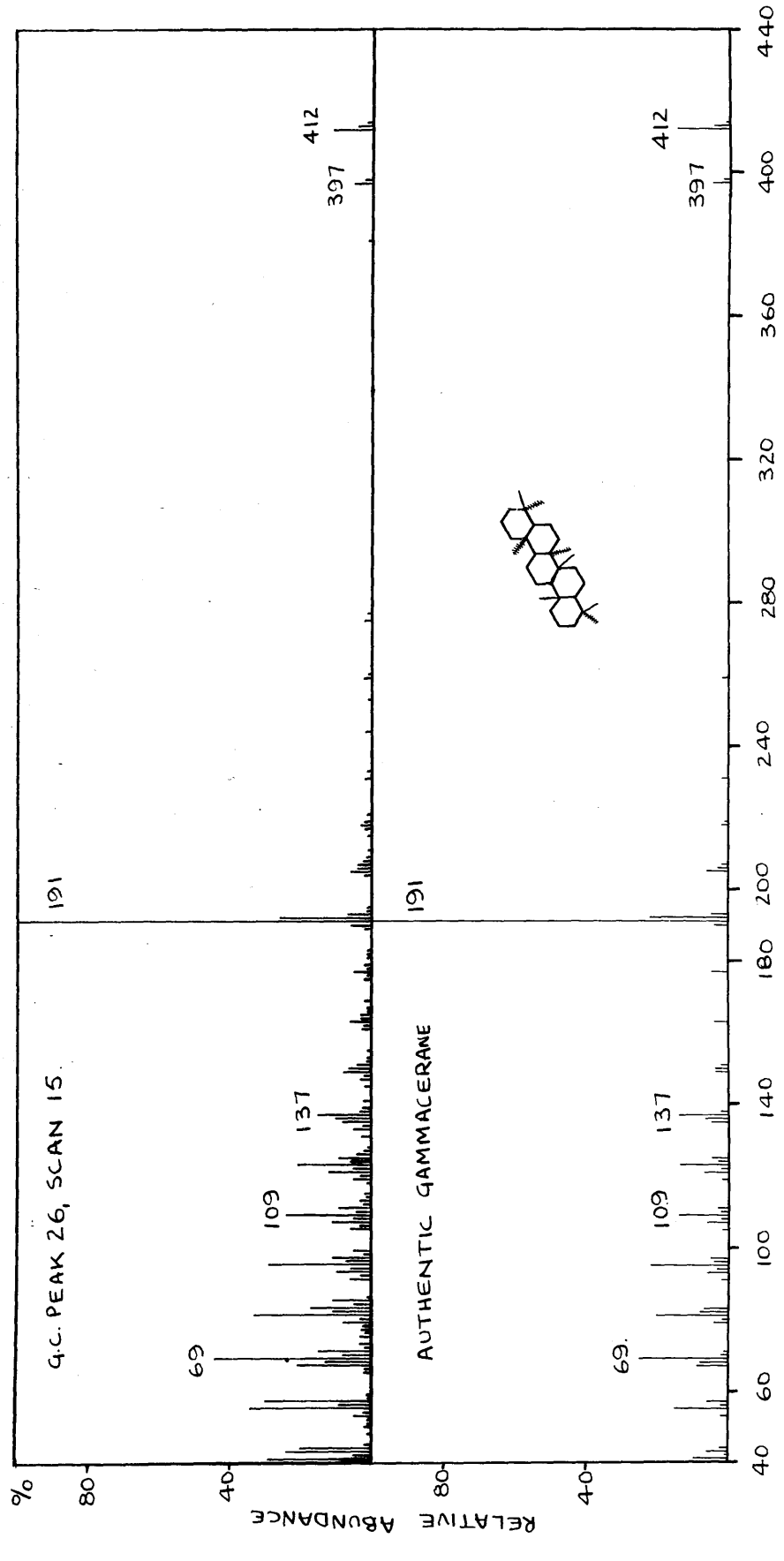
Scan 15 shows that peak 26 is a single pure component of molecular formula $C_{30}H_{52}$ given by the parent ion at m/e 412 (Fig. 19), corresponding to a pentacyclic triterpane. The absence of a (P - 43) peak indicates that the structure does not contain an isopropyl group and the base peak is at m/e 191. The mass spectrum is typical of a structure containing two adjacent quaternary carbon centres, the m/e 191 peak being the only characteristically intense peak in the spectrum. Co-injection of authentic gammacerane resulted in enhancement of peak 26 on both liquid phases. Comparison of the mass spectra of scan 15 and authentic gammacerane confirms the identity of peak 26 as being gammacerane (Fig. 19).

The mass spectra of scans 16, 17 and 18 of peaks 27, 28 and 29 respectively, were very weak and little conclusive information was derived from them. Scan 16 had a parent ion of m/e 412 corresponding to a molecular formula of $C_{30}H_{52}$, a pentacyclic triterpane possibly with an isopropyl group. Scans 17 and 18 appeared to have parent ions at m/e 476 corresponding to a molecular formula of $C_{34}H_{68}$. No peak enhancement occurred on coinjection of any of the authentic compounds. The identity of these three structures must remain unknown until more information becomes available.

G.c. peak 32 had previously been identified as a perhydro-

Figure 19

Mass spectral line diagrams of g.c. peak 26, scan 15 and authentic gammacerane.


G.C. PEAK 26, SCAN 15
AUTHENTIC GAMMACERANE
RELATIVE ABUNDANCE
%
80
40
69
109
137
191
397
412
40
60
100
140
180
200
240
280
320
360
400
440

carotene by other workers in the group.[33] It is possible that peak 31 is related to 32 and that peaks 28 and 29 are diagenetically degraded perhydro-carotenes, but no mass spectrometric scans were obtained of these peaks.

CONCLUDING REMARKS

The Green River shale has a well documented history with regard to environmental and paleobotanical aspects. The environment has been shown to be that of a shallow inland lake covering several hundred square miles. The geological history of the sediment has been reasonably uneventful, i.e. there have been no major geological movements such as uplift of the formation, and the maximum temperature suffered by the sediment has been claimed to be 74°C. The paleobotany of this sediment has been shown to be very similar to the Florida Lake mud deposits in that a largely algal source for the organic debris has been found.[51] Therefore the Green River shale provides an excellent test as to the compatibility of the chemical and paleochemotaxonomic information derived from the shale by the above techniques. In this way the premises and techniques are tested and if the results are compatible with previous knowledge, then they may be applied with some confidence to other geological samples such as discrete fossils and more ancient sediments and perhaps even extraterrestrial samples.

The chemical information now available on the organic content of the Green River shale is extensive. The alkane fraction is particularly impressive, in that the branched and cyclic alkanes are predominant over the n-alkanes. The n-alkanes

show a typical distribution of plant waxes, i.e. the $\underline{n}$-C_{27}, C_{29} and C_{31} alkanes are dominant, but the distribution appears to be bimodal with another carbon number maximum at $\underline{n}$-C_{17} alkane, typical of algal sources.[8,8a,8b]

The origin of the hydrocarbons in this sediment can be in no doubt when the branched and cyclic alkanes are considered. The identifications of the isoprenoid alkanes farnesane, pristane and phytane[8] and the impressive predominance of tricyclic, tetracyclic and pentacyclic cycloalkanes and perhydro-carotenes, must surely represent a biological origin.[21,26,33] This is a vindication of the aspirations of organic geochemistry which are in general to isolate and identify chemical fossils on a molecular level and then to establish chemotaxonomic and paleochemotaxonomic correlations with past and present biological source material.

The characterisation of ergostane and stigmastane isomers are compatible with a largely algal source for the organic material in the sediment. Figure 2 and Table 1 provide substantial evidence that sterols are abundant in algae and that ergostane and stigmastane isomers are dominant. Indeed the proportion of stigmastane present in the Green River shale is one of the highest, second only to the perhydro-carotene. The carotenes themselves are significant constituents of algae. The low concentration of the cholestanes bears out the prob-

ability of a small contribution from animals to the shale. Again, the high proportion of hopane found could be related to the occurrence of hopane in the Pterophyta division which might be expected to thrive around the shores of an inland lake and on islands. The gammacerane, derived from tetrahymanol, is the only pentacyclic triterpene so far found in animals, in Protozoan tetrahymena[26] and this again is compatible with the authentic data.

An important question which immediately arises from this investigation is when do the cycloalkanes first appear in the geological history of a sediment. Cycloalkanes have not been found in any of the present-day biological source materials investigated so far. It would seem reasonable to assume that they represent the products of diagenetic alteration of steroids and triterpenoids in sediments. Therefore a knowledge of when this occurs in the history of a sediment should illuminate further the problem of petroleum genesis. An obvious way to examine this question would be to analyse a geological core with an authenticated time scale from the present-day to perhaps Eocene or earlier geological periods.

Other sediments which could be examined for their cycloalkane content are marine and non-marine shales which should exhibit some characteristic differences because of differing biological source materials. The Scottish Westwood oil shale

(Carboniferous), reported by Maxwell[60] to contain some triterpanes, is another obvious choice. Similarly, the work of Whitehead et al[25,28,29] and Danieli et al[27] on crude oils of different origin should be correlated with the results from petroleum source rocks, thus perhaps establishing new techniques which could be used in the exploration for crude oil.

Future successful work in this area will depend on the compilation of more reference mass spectra of authentic and rearranged cycloalkanes, more high resolution g.l.c. data on the different behaviour of these compounds, and chemotaxonomic surveys of other significant biological sources for the cycloalkanes which can then be related to the geological environments.

In summary, with the important proviso that the chemotaxonomic data may not be completely correct, with the evidence available based on the above structure identifications, it is possible to generalise paleochemotaxonomically, that the sources of the organic material in the Green River shale were largely Thallophyta, Pterophyta and Gymnosperms. Small contributions could be expected from animals and the Angiosperms. In conclusion the technique appears to have been substantially successful, but it will have to be further tested on a more geologically complex sediment.

GENERAL EXPERIMENTAL

General Procedures used for the Isolation and Identification of Hydrocarbons

Where specific details or deviations require to be mentioned, these will be described in the appropriate experimental sections.

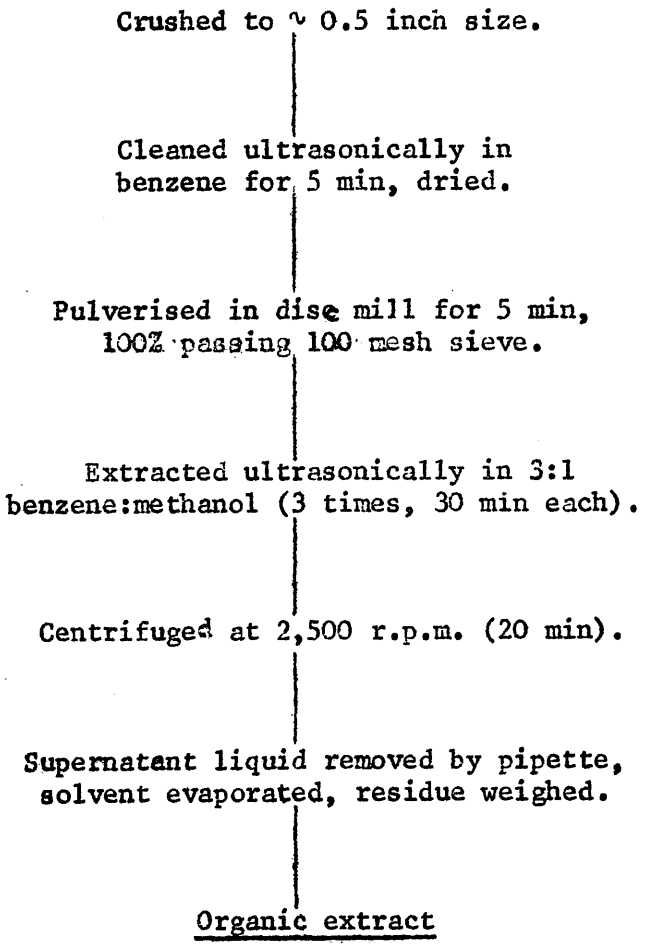
Preparation of Geological Samples (Fig. 20)

Pieces of rock having only freshly exposed surfaces were used. These fragments were broken on a clean metal surface into smaller fragments of approximately 0.5" in diameter using a hammer whose head was covered by several layers of aluminium foil. The resulting small fragments were carefully washed by sonication in benzene for 5 min in an ultrasonic tank (Dawe Instruments Ltd, type 1165/H60X, frequency 25.83 kcs, fitted with a 300/150 watt Soniclean generator). The dried fragments were powdered in a clean vibratory disc mill (Tema Machinery Ltd, Banbury) for 5 min. The period of milling was kept to a minimum since a considerable amount of heat was generated if the operation was carried on for 15 min or more; in such cases temperatures of 60°C were recorded.[60] For small sample sizes, i.e. 10 g or less, the powdering was carried out using a steel capsule ball mill (Glen Creston).

The resulting powder was sieved and only that portion

Figure 20

Preparation and Extraction of Geological Samples



passing through a 100 mesh sieve was used.

The moveable parts of both mills in contact with the rock were cleaned before and after the operation by sonication in a detergent solution (Lissapol NDB, I.C.I. Ltd), rinsed thoroughly in distilled water, acetone and finally in chloroform. While not in use the clean moveable parts were stored in desiccators over blue silica gel.

To minimise contamination, disposable polythene gloves were worn throughout these operations and also where further handling of the samples was required.

All solvents used were of the "Analar" grade and were further purified by distillation through an 18" column packed with glass helices, the first 100 ml of the distillate being discarded. Glassware was cleaned by sonication in a detergent solution (Lissapol) in the ultrasonic tank for 30 min, followed by thorough rinsing in distilled water, acetone and chloroform, stored in closed dust-free jars, and rinsed with solvent before use. Flasks, centrifuge tubes and other sample containers were stoppered or covered with aluminium foil between operations and if necessary samples were stored in a refrigerator. The polythene gloves and the aluminium foil were checked as sources of contamination by Mr. J. N. Ramsay.[8a]

Extraction of Organic Material from a Shale

Ultrasonic extraction was used to extract the organic

material from shale samples.[61] The powdered shales were placed in glass centrifuge tubes (100 ml capacity) with solvent (3:1 mixture of benzene:methanol) and the tube placed in the ultrasonic tank (Dawe Instruments Ltd, type 1165/H60X, frequency 25.83 kcs, fitted with a 300/150 watt Soniclean generator), with the water level in the tank the same as the solvent level in the tube. Sonication was allowed to proceed for 30 min. The suspension was centrifuged at 2,500 r.p.m. (M.S.E., Multex Mk III) for 20 min and the clear supernatant liquid removed using disposable glass pipettes. The extraction procedure was repeated three times.

Analytical Procedures

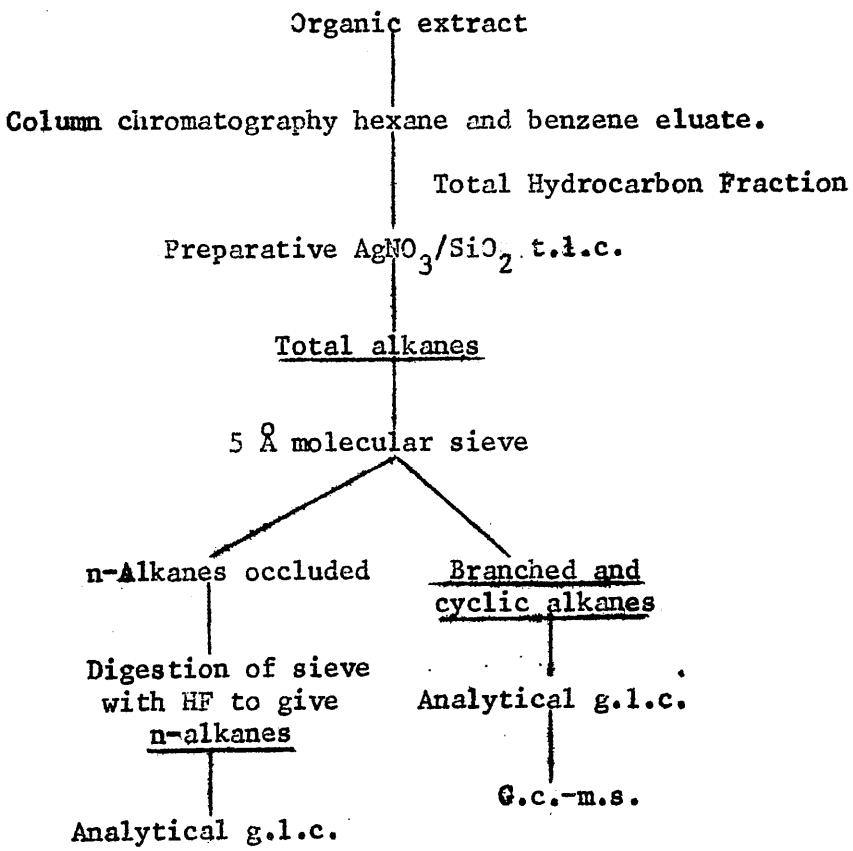
An outline of the separation and analytical procedures is given in Figure 21.

Column chromatography of the organic extract. Neutral alumina (Woelm Grade I) was used for all column chromatography and was pre-washed with n-hexane before use. Elution with n-hexane and then benzene allowed the separation of the hydrocarbon fraction from the total organic extract. The hexane eluate normally contained the alkane, alkene, and some aromatic constituents. The elution was monitored by thin layer chromatography (t.l.c.) .

Thin layer chromatography of the hydrocarbon fractions. Thin layer chromatography was carried out on silica gel (G.

Figure 21

The Separation and Analytical Procedures used to Isolate and Identify the Hydrocarbon Components from the Organic Extract



Merck) impregnated with 10% silver nitrate[62] (by weight). This was found to give an efficient separation of the alkanes from the alkenes and aromatics. The solvent used for elution was n-hexane for the alkanes and sterically hindered alkenes, while n-hexane/benzene mixtures were used for unhindered alkenes and aromatics.

The plates were prepared by coating with a slurry of the adsorbent in distilled water on a motorised t.l.c. spreader (Baird and Tatlock, London), allowed to air dry in a dark cupboard, activated at 120°C in an oven and finally stored in a desiccator ready for use.

Thin layer plates had a layer 0.25 mm thick and preparative thin layer plates were 1 mm thick. Detection was achieved by spraying with a 0.001% solution of fluorescein or Rhodamine 6G and viewed under a u.v. lamp (254 and/or 350 mμ). Occasionally when the quantities of material were particularly small, preparative t.l.c. was carried out on thin layer plates, i.e. 0.25 mm layers.

Molecular sieving of the alkanes. 5 Å molecular sieve (Linde Co, Division of Union Carbide Corporation) was used to separate the normal alkanes from the branched and cyclic alkanes. The method of O'Connor et al[63] was used. The sieve was activated at 240°C under reduced pressure (0.1 mm) for 24 hr before use. The alkanes were dissolved in dry benzene

or iso-octane and 1/16" pellets of 5 Å molecular sieve added in 50:1 ratio. The sieving was carried out under reflux, with a blue silica gel drying tube fitted to the top of the condenser for 72 hr. The solution containing the branched and cyclic alkanes was removed by pipette and was passed through a short column of alumina to remove any traces of powdered sieve. The sieve was thoroughly washed with benzene or iso-octane in a soxhlet apparatus and the washings added to the branched and cyclic alkane fraction which was then evaporated.

The washed sieve, containing the normal alkanes, was then dissolved using 40% hydrofluoric acid which was carefully added to the flask containing the sieve, distilled water and benzene or iso-octane. The sieve was stirred magnetically (Teflon coated stirring bar) until solution was complete. After separation of the layers, the organic solution was passed through a short column of alumina and one of anhydrous sodium carbonate (pre-washed with diethyl ether) and the solvent evaporated.

Infrared absorption spectroscopy (i.r.). The fractions obtained by t.l.c. were examined by infrared absorption spectroscopy. Spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer (accuracy $\pm$ 5 cm^{-1} above 2000 cm^{-1} and $\pm$ 2 cm^{-1} below 2000 cm^{-1}). When required, quantitative spectra were recorded on a Unicam SP-100 double beam spectro-

photometer, equipped with an SP-130 sodium chloride prism-grating double monochromator and operated under vacuum conditions (accuracy $\pm$ 1 cm^{-1}).

Gas-liquid chromatography (g.l.c.). The pure hydrocarbon fractions obtained by t.l.c. and molecular sieving were analysed by gas-liquid chromatography. Peak identity was established by coinjection of authentic standards and peak enhancement. Low resolution and high resolution g.l.c. columns were used.

Low resolution g.l.c. (packed columns). Low resolution analyses were carried out using Perkin-Elmer F-11 Mk I instruments, equipped with hydrogen flame ionisation detectors and linear temperature programmers. Stainless steel columns, ⅛" o.d. x 10' length and 1/16" diameter x 10' length, were used. The carrier gas was nitrogen at flow rates of 15-30 ml/min. The liquid phases were: 1%, 2%, and 3% SE30 (Applied Science Laboratories, Inc); 1% and 2% seven ring polyphenyl-ether (Applied Science Laboratories, Inc); 4.6% JXR (Applied Science); 3% OV-1 and 3% OV-17 (Applied Science). The solid inert supports usually used were Gas Chrom P (100-120 mesh, acid-washed and silanised; Applied Science); Gas Chrom Q (100-120 mesh, acid-washed and silanised; Applied Science); and Chromosorb G (100-120 mesh, acid-washed and silanised; Johns-Manville). The columns were prepared by thoroughly cleaning the

stainless steel tubing with a detergent solution (1.0% Lissapol in distilled water), then thoroughly rinsing with hot water, cold distilled water, ethanol, acetone, and chloroform (three times, 100 ml each). The column was dried in a nitrogen stream for 3 hr. The column was then packed with the liquid phase coated support under vacuum with vibration (Electric Engraver, Model VT-62, Burgess Products Co Ltd). The ends of the column were packed with metal gauze and the appropriate $\frac{1}{8}$" or 1/16" Swagelock fittings attached to the column ends for mounting in the g.l.c. instrument. The columns were conditioned by temperature programming to the maximum temperature of the liquid phase (mostly 300°C) at a rate of 1°/min with a slow nitrogen gas flow. The maximum temperature was maintained for 24 hr. Normal theoretical plateages achieved by this method were within the range of 250-500 plates/foot.

Preparative g.l.c. was carried out using Wilkens Aerograph A90P-3 instruments equipped with linear temperature programmers and thermal conductivity detectors. Copper or stainless steel columns (10' or 20' in length) $\frac{1}{4}$" or $\frac{1}{8}$" in diameter were used. The columns were packed by the same procedure as the analytical low resolution columns, the phases used were 1% and 3% SE30 on Gas Chrom P (100-120 mesh, acid-washed and silanised). The carrier gas was helium and the flow rates varied from 30 to 60 ml/min. The injector, detector, and

collector temperatures were maintained at 20°C above the maximum column temperature (usually approximately 320°C). Collection of fractions was achieved using glass melting point capillary tubes (10 cm x 1 mm), both ends being sealed in a micro-bunsen flame after collection.

Low resolution g.l.c. is adequate and extremely useful for analysis of reasonably simple mixtures of components, for example, simple normal alkane distribution patterns covering a wide range of carbon chain lengths. However, complex mixtures are more difficult to analyse, especially when a mixture contains isomers and different components where the differences are very slight. In such cases, short packed column g.l.c. becomes inadequate and for complete analysis of a mixture, high resolution g.l.c. conditions are necessary. This was found to be the case on analysis of the hydrocarbons formed by the thermal alteration experiments. The extremely complex g.l.c. distribution patterns necessitated the use of high resolution g.l.c. Similarly, the analysis of the branched and cyclic alkanes from the Green River shale, especially the cycloalkane constituents of sterane and triterpane structures, made high resolution g.l.c. an absolute necessity. The high resolving power was not only necessary to separate the constituents of the mixture, but, as a function of this power, peak identity could be more conclusively established by coinjection of

authentic compounds on two or more different liquid phases.

High resolution g.l.c. (capillary columns). High resolution g.l.c. may be achieved by two different methods. Firstly, long narrow packed columns, such as proposed by Halasz[64] may be employed. However, the difficulty in preparing these columns with high theoretical efficiencies and their long retention times, places a severe restriction on their use at the present time. Secondly, long open tubular coated capillary columns may be used. These columns are commonly 0.01", 0.02", and 0.03" in diameter and are used in lengths from 50' to as long as 1000'. The very high efficiencies and reasonable retention times achieved using these columns has made them increasingly important in the field of gas-liquid chromatography. The theories and uses of open tubular capillary g.l.c. have now been well established by the work of Keulemans,[65] Golay,[66,67] Desty,[68] Lipsky et al,[69] Scott,[70] Zlatkis et al,[71] and Ettre[72] among others. The main obstacle to the use of open tubular coated capillary columns has been the lack of a reproducible and reliable method of production of a thin film of liquid phase coated uniformly through the long narrow tubes. Indeed, even when this has been achieved, it is necessary that the thin film be stable to carrier gas flow and elevated temperatures, otherwise the whole exercise would be nearly pointless. Before describing the preparation and coating techniques

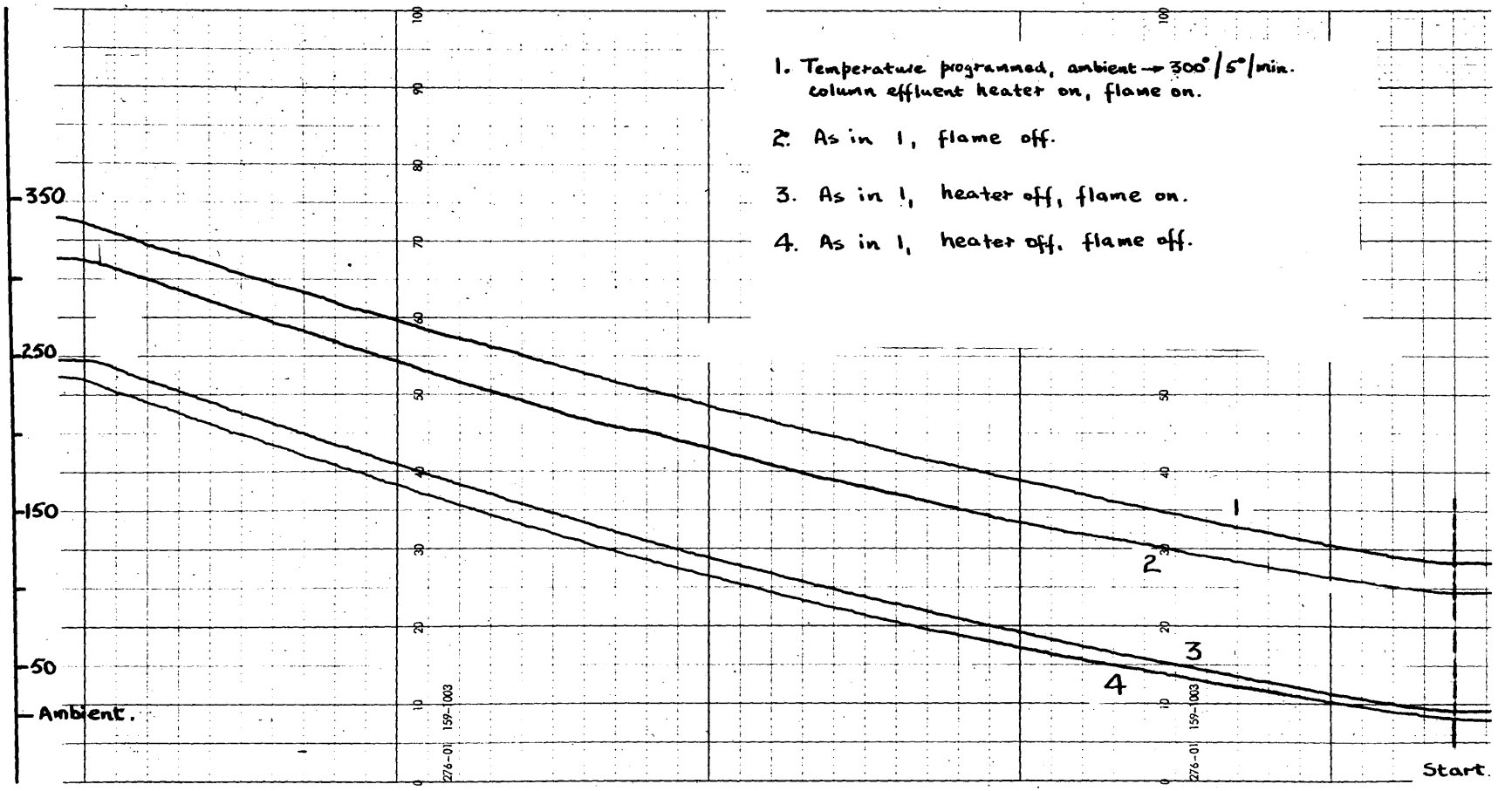
employed in making capillary columns, it is necessary to discuss briefly the g.l.c. instruments in which these columns were to be used.

High resolution g.l.c. instrumentation. A Perkin-Elmer F-11 Mk II was obtained on loan from Perkin-Elmer for a period of one year so that it could be adapted and developed for use with capillary columns. It was equipped with a stainless steel hydrogen flame detector, a pre-column splitting assembly, a linear temperature programmer and a temperature readout accessory.

Previous work carried out on the F-11 Mk I indicated that there was a temperature gradient between the column in the g.c. oven and the detector (outside the oven, but connected to the column by a 1/16" o.d. stainless steel pipe). This was tested on the new F-11 by attaching thermocouples to the oven interior, the connecting pipe and the detector. The temperatures were recorded on a strip chart recorder, the results of which are shown in Figure 22. From these curves it can be clearly seen that there could be up to 75°C difference between the oven and the connecting pipe during a temperature programme. This lag produces condensation of the column effluent and consequently broadens the appearance of the recorded peaks and most important produces tailing peaks thus resulting in a loss of resolution. To eradicate this fault,

Figure 22

Illustration of the temperature gradient between the g.l.c. column and the effluent tube leading to the flame ionisation detector of an F-11 g.l.c. instrument and the effect of a small heating coil fitted to the tube on the performance.


1. Temperature programmed, ambient → 300°/5°/min.
column effluent heater on, flame on.
2. As in 1, flame off.
3. As in 1, heater off, flame on.
4. As in 1, heater off, flame off.
350
250
150
50
Ambient.
Start.
1
2
3
4
276-01 159-1003
276-01 159-1003

a small coil heater was designed and fitted to the connecting pipe. The improved performance showed that the temperature gradient had been eliminated (Fig. 22).

Secondly, attempts were made to reduce the "dead volume" capacity in the F-11. This was achieved by drilling out all the Swagelock connections to 1/16" i.d. so that the column ends could be "butt-ended" with the connecting tubes.

Finally, a simple splitting valve and pre-column splitter was constructed which served two purposes. Firstly, when using helium as carrier gas, it is expensive and uneconomical to have a continuous split throughout a complete analysis of, for example, 100-200 ml/min of helium escaping to the atmosphere. Since having the split assembly open only during the actual splitting operation, i.e. at sample injection, and closed throughout the remainder of the analysis makes no appreciable difference to the gas flow through the column, the fitting of a simple on/off valve to the sample splitter should not reduce efficiency or resolution. Secondly, because of the small quantities of hydrocarbons available for analysis, it was wasteful to inject large samples (e.g. 5 µl of a concentrated solution) and then use a split ratio of 100-200/1 to introduce the tiny sample required to the capillary column. Instead an easily interchangeable splitter was constructed from 0.01" capillary tubing, the split ratio being varied by varying the

length of tubing used, thus allowing the use of smaller sample sizes, e.g. 0.1-1.0 µl. These minor modifications resulted in the low cost F-11 being an adequate g.l.c. instrument for use with high resolution capillary columns.

Latterly, a Varian Aerograph Model 1200 hydrogen flame instrument was obtained on a short term loan for evaluation purposes as to its capabilities as a g.l.c. instrument suitable for use with high resolution capillary columns.

Reports on the performances and suggested improvements to the instruments were sent to both Perkin-Elmer (Beaconsfield) and Varian Aerograph (Manchester).

Open Tubular Capillary Column Preparation and Coating Thin Films of Liquid Phases

Glass or metal capillary tubing were the only types feasible for use as g.l.c. columns because of the elevated temperatures necessary for the analysis, i.e. maximum temperature used was 300°C.

Attempts were first made to use glass capillary columns (obtained from Mr. E. V. Whitehead of B.P. Co Ltd, Sunbury-on Thames, Middlesex) since Hills et al[25] had used them in their analyses of the cycloalkanes from crude petroleums. A supporting bracket and holder was constructed to support these columns and attempts made to coat them. However, the fragility of these glass columns was such that they

could only be handled with extreme care and preferably, once installed safely in the g.l.c. oven, not at all, until their performance deteriorated and they had to be replaced. Even in the oven and securely mounted, breakages occurred which could only be explained by differential coefficients of expansion between glass and metal. Confirmation of this difficulty was received from other workers in this field.[73] One advantage of using glass capillary tubing is that one can watch the behaviour of the liquid phase solutions during the coating procedure which is impossible with metal tubing.

However, in view of the difficulties encountered with glass capillary tubing, stainless steel capillary tubing was chosen as the only other alternative available. The tubing was obtained (Handy-Harman Tube Co, Inc, Norristown, Pennsylvania, U.S.A.) in lengths of 200, 300 and 500' of 0.01 and 0.02" in diameter of a special g.l.c. quality stainless steel which was soft, easily malleable and the internal dimensions very uniform throughout each length (accuracy $\pm$ 0.001").

The column tubing must first be thoroughly cleaned in order to remove any lubricating material which may remain after the manufacturing process. There are many procedures published in the literature both for cleaning and for coating.[66,68,72] The cleaning procedure of König[74] and Upde Grove[75] were combined with the procedure used by the author and

resulted in a thorough cleaning process which will not only clean the inside surface of new tubing but will also clean used tubing prior to recoating a capillary column. This procedure is given below (all steps carried out at 500 p.s.i. N_2):-

(1) Flush tubing with water, acetone and chloroform (three times, each 20 ml).

(2) Flush tubing with 20 ml of 5% by weight of 40% hydrofluoric acid plus 40% by weight nitric acid in distilled water (twice).

(3) Flush tubing with distilled water (five times, 20 ml).

(4) Followed by concentrated ammonia (twice, 20 ml).

(5) Flush column with distilled water until the washings are neutral to litmus paper.

(6) Flush column with acetone and chloroform (three times, 20 ml each).

(7) The tube is dried in a N_2 stream prior to coating.

The reservoir for these steps was a ¼" i.d. stainless steel tube, 15" long. For coating purposes a Teflon tube (¼" i.d. x 15") was used so allowing examination of the level of the coating solution. This was possible since the gas pressures used in coating a column are of the order of 2-30 p.s.i., varying with the internal diameter and length of the column.

There are two basic methods for coating a thin film of

liquid phase in open tubular capillary columns.

The dynamic coating method.[72] Briefly, this method involves forcing a solution of the liquid phase through the capillary column by an inert gas such as nitrogen, helium or argon. There are two variations of this method, distinguished by whether the volume of the coating solution is greater or less than the volume of the tubing. When the volume of the solution is greater, the whole solution is forced through at a uniform rate, maintained by adjusting the inlet pressure. When the volume of coating solution is less, approximately 15% of the total length is filled with the solution and then this plug is forced through the tubing again at a uniform rate. Scott[76] maintains a uniform rate by connecting a second length of capillary tubing of the same internal diameter to the first, thus maintaining a resistence to the passage of the solution throughout the length of the first column. Whatever means are employed, it is vital to the efficiency of a capillary column that there is no sudden increase in the rate of movement of the solution as it nears the end of the column, because this causes the formation of drops of solution which, when evaporated, leave a non-uniform layer of phase on the walls of the tubing. The rate of movement of the solution should be in the range 0.1-1.0 ml/min for 0.01" and 0.02" columns.

The static coating method.[67] Briefly, the column is com-

pletely filled with the coating solution, then one end is sealed and by applying reduced pressure to the open end, either in an oven or at room temperature, the solvent is slowly evaporated leaving the liquid phase behind.

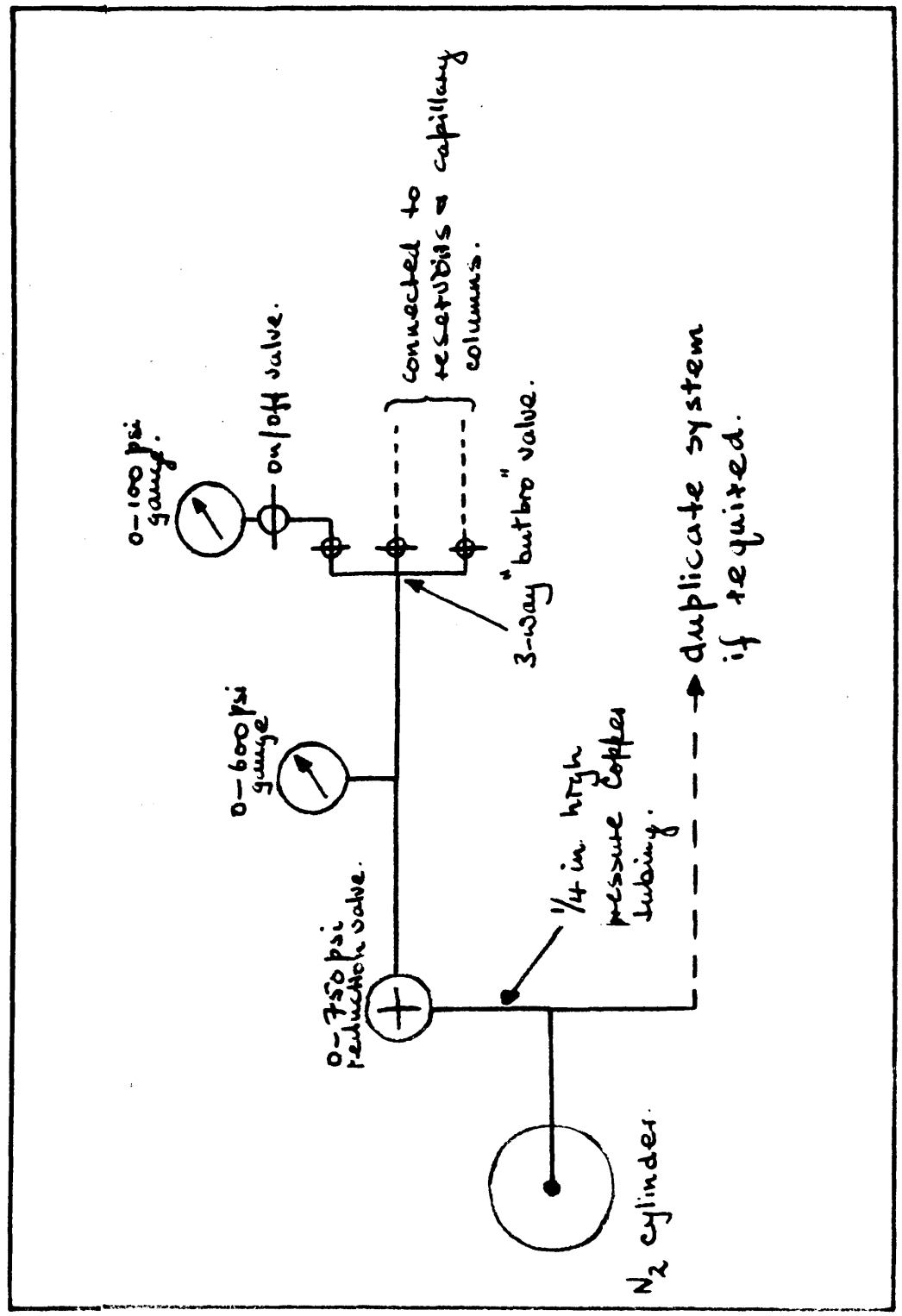
Both these methods were tried and some success was achieved using each of the methods. However, the reproducibility of consecutive columns coated with the same phase in both cases was low.

In an attempt to eliminate the human element from the causes of failures, a cleaning and coating apparatus was designed and constructed (Fig. 23). Not only did this help to standardise the procedure, but it allowed the cleaning and coating of more than one column at once, thus permitting a faster throughput when checking the various procedures.

Following the work of Averill,[77] in which he recommended the use of various surfactants in small quantities as additives to the coating solution to eliminate the adsorption effects of the column walls and also to stabilise the thin film, a commercial surfactant (Igepal Co880, Perkin-Elmer Ltd) was used. The columns prepared in this way with a 0.01% (by weight) Igepal Co880 added to the solution, did not show any appreciable improvement. Moreover, their performance appeared to deteriorate faster than before, probably caused by the decomposition of the surfactant at the elevated temperatures

Figure 23

The cleaning and coating apparatus used in the preparation and coating of open tubular capillary columns.


0-100 psi gauge.
on/off valve.
connected to reservoirs & capillary columns.
3-way "butbro" valve.
0-600 psi gauge
0-750 psi pressure valve.
1/4 in. high pressure copper tubing.
duplicate system if required.
N_2 cylinder.

necessary for the analyses.

Metcalfe et al[78] reported the successful use of an additive, trioctadecylmethylammonium bromide (Gas Quat L) in the preparation of open tubular capillary columns with a variety of liquid phases. On request they sent 100 mg of this material to be evaluated and indeed the results obtained using Gas Quat L indicate an increase in the reproducibility and stability of coated capillary columns. The procedure employed for coating is as follows:-

(1) A solution containing 10% by weight of liquid phase, 0.2% by weight of Gas Quat L is made up in filtered chloroform ("Analar" grade).

(2) The solution is measured and the required volume (for a 200' x 0.01" column 5 ml is necessary) transferred to the clean Teflon reservoir.

(3) Pressure is applied to the top of the reservoir using nitrogen and a uniform flow through the column is maintained. The pressure required to do this varies with the viscosity of the solution which varies from phase to phase.

(4) When the solution has passed through, the column is left for 24 hr with the same flow of nitrogen ($\sim$ 1 ml/min) to evaporate the solvent.

(5) The column is conditioned very carefully, i.e.

temperature programmed from room temperature to the maximum temperature of the column in 50°C stages at a rate of 1°/min over a period of two days with a carrier gas flow of 2 ml/min (for 0.01" columns).

(6) The column is then tested with a standard mixture for both efficiency and resolution at the temperature the column has to be used, e.g. for the cycloalkane analyses the temperature to be used was 250°C.

Most columns prepared in this way yielded theoretical plateages measured at 250°C in the range 30,000-50,000, and maintained their performance for a minimum of 2-3 months. (Subsequent attempts by other workers in the group have been unsuccessful, probably due to inadequate attention to detail.) The liquid phases coated on capillary columns successfully using this procedure were Apiezon L grease (pre-treated by passing a hexane solution of the grease through a neutral alumina column[79])a silicone gum SE52 (Applied Science Laboratories, Inc), seven ring polyphenylether (Applied Science) and dimethylpolysiloxane, monodisperse gum (Midland Silicones Ltd, Wales).

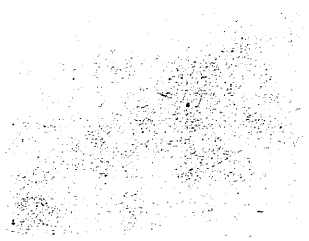
Combined gas chromatography - mass spectrometry (g.c.-m.s.). Complete mixtures and also fractions of mixtures

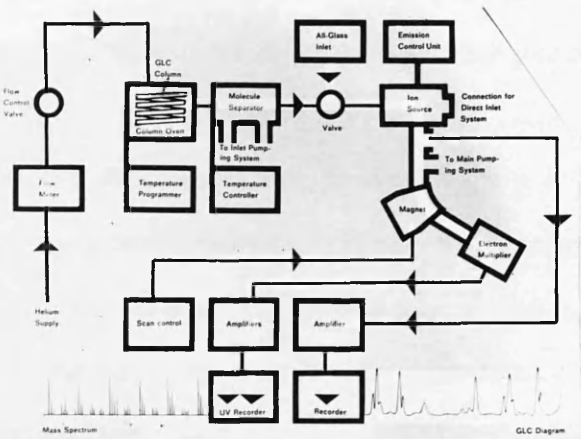
(collected by preparative g.l.c.) were analysed using the LKB 9000 gas chromatograph - mass spectrometer (at the Chemistry Department, University of Glasgow). Initially, packed columns were used (10' x 3 mm), normally with 1% SE30 as liquid phase and a helium flow rate of 30 ml/min. However, as the requirements of g.l.c. separations increased with respect to resolution and column efficiencies, and capillary column g.l.c. became feasible, it was obviously desirable to improve the quality of the g.c.-m.s. analysis by using capillary columns in the g.c.-m.s. This was not such a simple step as it at first seemed.

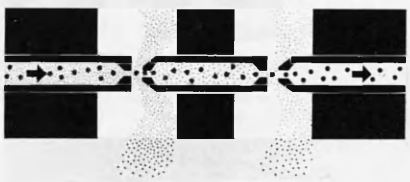
The main problem was that even with the low helium flows used in capillary columns, i.e. $\sim$ 2 ml/min, the LKB ion source could not accept the total g.c. effluent (maximum flow rate into source is 0.25 ml/min). Therefore some sort of splitting or preferential sample to carrier gas enrichment process must be introduced to the g.c. effluent. On the LKB, this is accomplished using a Becker-Ryhage jet type separator assembly[80] (Fig. 24). The first chamber is evacuated by the Fore vacuum pump and the second chamber by the oil diffusion pump. However the sample sizes used on capillary columns are so small that the separated components in the g.c. effluent were mostly lost while passing through the Ryhage separators. Single components or simple mixtures could be successfully analysed, but high

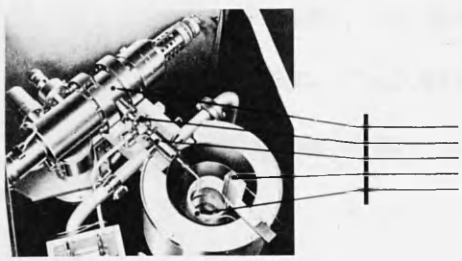
Figure 24

Schematic representation of the LKB 9000 combined g.c.-m.s. instrument and the principle of the molecular separator and the g.l.c.-m.s. interface.




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




Ion source
Inlet valve
Molecule separator
Flash heater with injection port
Column

molecular weight complex mixtures (such as the branched and cyclic alkane fraction from the Green River shale) were impossible to analyse. By a systematic process of trial and error, suitable modifications to the g.c. and m.s. interface were arrived at, which allowed the satisfactory analyses of complex mixtures using capillary columns. The procedure was as follows:-

(1) The LKB 9000 oven was replaced by a F-11 oven and analyser unit (without flame ionisation detector). This was done so as to reproduce the g.l.c. conditions used as exactly as possible.

(2) The effluent end of the capillary column was connected to the separator assembly by a 0.01" capillary tube (ca. 18" long) wound with resistance wire and insulated with asbestos. The effluent line could be heated to 300°C.

(3) The first separator jet was removed and the rotary pump line isolated but still "backing" the oil and mercury diffusion pumps connected to the second separator.

(4) When the separator assembly and the source had pumped down to a satisfactory vacuum, i.e. 10^{-6} to 10^{-7} mm Hg, the oil diffusion pump and the mercury diffusion pump were switched off. This

left the Fore vacuum pump to maintain the vacuum.

(5) The helium g.c. inlet pressure was increased to 80 p.s.i., thus increasing the pressure in the source by 20%, but still within the safe working limits of the source.

This system resulted in a higher proportion of the g.l.c. effluent reaching the source and thus higher sensitivity achieved. By this means the complex mixture of steranes and triterpanes from the Green River shale were successfully analysed by capillary column g.c.-m.s., the first time such a high molecular weight analysis had been achieved using a capillary column.

EXPERIMENTAL

Green River Shale

The sample of Green River shale (Rifle, Colorado, U.S.A.) used in this study was part of a large sample of shale kindly provided by Dr. W. E. Robinson (U.S. Department of the Interior, Laramie, Wyoming).

Treatment of Rock and Extraction of Organic Matter

A piece of Green River shale having only freshly exposed surfaces was broken into small pieces about 0.5" in diameter. These were carefully washed in benzene by sonication (Sonitank) for 5 min to minimise contamination. The pieces were then powdered as described above using the disc mill for 5 min. Only that portion of the powder passing a 100 mesh sieve was used. A sample (20 g) of the powdered Green River shale was extracted ultrasonically in the Sonitank with benzene/methanol (3:1, 100 ml) for 30 min, the resulting suspension centrifuged, and the supernatant liquid removed by pipette. The extraction procedure was carried out five times. The supernatant liquid, having been combined, was evaporated (Buchi) and the resulting dark brown gum weighed (0.35 g, 1.8%).

Isolation of the Hydrocarbon Fraction

The organic extract was chromatographed on neutral alumina (100 g, activated at 120°C for 1 hr). The column was eluted

with n-hexane (150 ml) followed by benzene (600 ml), each elution being monitored by $AgNO_3$/Si t.l.c. The combined eluates, after evaporation, were weighed (0.100 g, 0.5%). Finally the column was stripped using ethyl acetate and methanol (0.22 g, 1.1%).

Isolation of Alkane Fraction

Analytical $AgNO_3$ impregnated silica t.l.c. of the hydrocarbon fraction with the reference compounds, n-heptadecane, n-heptadec-1-ene and anthtacene, indicated the presence of alkane, alkene and aromatic fractions. The solvent system used was 10% benzene/hexane. Preparative $AgNO_3$/Si t.l.c. allowed the separation of these three fractions with the same eluent. This afforded the alkane fraction (0.055 g, 56% by weight of the hydrocarbon fraction and 0.28% by weight of the shale), free from olefinic and aromatic material. The infrared spectrum (thin film) was typical of a saturated hydrocarbon mixture, having absorption bands at 1465 (ν CH_2, CH_3), 1380 (ν CH_3, sym.) and 720 cm^{-1} ($-(CH_2)_n-$ 'rock'), the 720 cm^{-1} band indicating that long straight chain constituents were present.

Isolation of the Branched and Cyclic Alkane Fraction

A temperature programmed gas chromatogram using a packed column (10' x 1/16", packed with 2% 7-PPE on Gas Chrom Q) of the alkane fraction showed it to be a complex mixture (Fig. 5). The dominant peaks were the isoprenoid alkanes, pristane and

and phytane, previously identified by other workers.[8,12]

The alkane fraction (0.055 g) was dissolved in iso-octane (20 ml) and heated under reflux with 5 Å molecular sieve ($\frac{1}{8}$" pellets, 3.0 g) for 100 hr. The sieve was washed in an all-glass Soxhlet (6 hr) and the washings evaporated to give the branched and cyclic alkane fraction (0.044 g, 80% by weight of the alkane fraction, 0.22% by weight of the shale). Dissolution of the sieve, containing the n-alkanes, was achieved using hydrofluoric acid (40%) as described above, affording the n-alkane fraction (0.007 g, 12% by weight of the alkane fraction, 0.035% by weight of the shale). There was an overall loss of 8.0% by weight of the alkane fraction incurred in this separation. The n-alkanes were examined by g.l.c. (Fig. 5) and the positions of the C_{18} and C_{28} alkanes determined by coinjection. The branched and cyclic alkanes were examined by g.l.c., initially by temperature programming (Fig. 5). The mixture was shown to be very complex, especially around the higher molecular weight region of the tetra- and pentacycloalkanes.

Capillary G.L.C. Analysis of Authentic Cycloalkanes and the Cycloalkane Region of the Branched and Cyclic Alkane Fraction

The analyses of the behaviour of authentic steranes and triterpanes and the unknown mixture were conducted using an F-11 Mk II (Perkin-Elmer Ltd). Two different polarity liquid

phases were used; Apiezon L grease pre-treated as described above and seven ring polyphenylether (7-PPE), coated on 200' x 0.01" and 150' x 0.01" stainless steel columns respectively. The former column had a theoretical plateage of ca. 50,000 and the latter column a plateage of ca. 30,000, measured at 250°C with 5α-cholestane. The efficiencies of the columns were monitored throughout the analyses to ensure maintenance of performance. All samples of authentic and unknown materials were examined at an isothermal temperature of 250°C with a constant pre-column flash heater temperature of 320°C. The helium carrier gas flow was maintained at 2 ml/min, with a pre-column split ratio of 30:1 achieved as described above. The procedure followed was as follows:-

(1) The reference n-alkanes (n-C_{28}, n-C_{30}, and n-C_{32}) were run as a reference mixture.

(2) Each authentic compound was coinjected with the reference alkane mixture to determine their Kovats retention indices and carbon numbers (Fig. 6, Table 1).

(3) The branched and cyclic alkane fraction from the Green River shale was examined at 250°C isothermally so that the region containing the tetra- and pentacyclic cycloalkanes was well resolved.

(4) The branched and cyclic alkane fraction was co-injected with the reference n-alkane mixture and the Kovats retention indices and carbon numbers calculated for each peak, i.e. peaks 1-32, Fig. 7.

(5) Finally, each authentic compound was coinjected with the reference n-alkane mixture and the branched and cyclic alkane fraction and the g.l.c. record examined for the appearance of any peak enhancement.

This procedure was carried out on both capillary columns and the resultant data tabulated (Table 2).

Combined Capillary Column G.C.-M.S. of the Branched and Cyclic Alkane Fraction from the Green River Shale

The instrument used was an LKB 9000 combined g.c.-m.s., modified to accept the effluent from a capillary column as described above. The branched and cyclic alkane fraction was examined and the total ion current at 20 eV is shown (Figure 12; cf. the analytical g.l.c. record in Figure 7). Mass spectra were recorded at 70 eV for the peaks indicated and the scan (2 sec for a mass decade) points marked. In order to facilitate counting of the mass spectra and also to act as a check, polyfluorokerosene (PFK) was added to the effluent between the molecular separator and the ion sourse, at a constant rate and a second analysis of the branched and cyclic alkanes carried out. Then by comparison of the corresponding spectra

from the first and second analysis, each mass spectrum was counted and normalised line diagrams drawn for each scan (cf. Figs. 9-18). Unfortunately, due to limited time allotted for this work on the LKB 9000 (the author had moved to the School of Chemistry at Bristol with Dr. G. Eglinton, and had to visit Glasgow to use the LKB 9000, made available by Dr. C. J. W. Brooks), the author was unable to analyse the authentic compounds and obtain mass spectra by combined g.c.-m.s.

Mass Spectrometry of Authentic Steranes and Triterpanes

Mass spectra of the authentic compounds were obtained using an MS-9 (A.E.I.) instrument via the direct insertion probe at an applied voltage of 70 eV. These were used as reference spectra for the spectra obtained using the combined g.c.-m.s. instrument. Mass spectra were also recorded at 12, 16, 20, 35, 45 and 70 eV for each of 12 of the authentic steranes and triterpanes for a comparative analysis of the fragmentations produced at the different voltages. The mass spectrometric conditions were maintained as constant as possible throughout, e.g. source and insertion probe temperatures. This work was carried out jointly by the author and Dr. R. Binks.

REFERENCES

1 G. Eglinton and R. J. Hamilton, in "Chemical Plant Taxonomy", ed. T. Swain, p.187 (1963), Academic Press, London.

2 V. Wollrab, M. Streibl and F. Sorm, Coll. Czech. Chem. Comm., 28, 1904 (1963).

3 P. Jarolimek, V. Wollrab, M. Streibl and F. Sorm, Coll. Czech. Chem. Comm., 30, 880 (1965).

4 W. G. Meinschein and G. S. Kenny, Anal. Chem., 29, 1153 (1957).

5 E. E. Bray and E. D. Evans, Geochim. Cosmochim. Acta, 22, 2 (1961).

6 W. E. Robinson and J. J. Cummins, J. Chem. Eng. Data, 5, 74 (1960).

7 R. A. Dean and E. V. Whitehead, Tetrahedron Let., 768 (1961).

8 G. Eglinton, P. M. Scott, T. Belsky, A. L. Burlingame and M. Calvin, Science, 145, 263 (1964); and in "Advances in Organic Geochemistry 1964", ed. G. D. Hobson and M. C. Louis, pp.41-74 (1966), Pergamon Press, London.

8a A. G. Douglas, K. Douraghi-Zadeh, G. Eglinton, J. R. Maxwell and J. N. Ramsay, in "Advances in Geochemistry 1966", ed. G. D. Hobson and G. C. Speers, in press, Pergamon Press, London.

8b J. N. Ramsay, M.Sc. Thesis, Glasgow University (1966).

9 J. G. Bendoraitis, B. L. Brown and L. S. Hepner, Anal. Chem., 34, 49 (1962).

10 J. G. Bendoraitis, B. L. Brown and L. S. Hepner, Sixth World Petroleum Congress, Frankfurt, June 1963.

11 B. J. Mair, N. C. Krouskop and T. J. Mayer, J. Chem. Eng. Data, 7, 420 (1962).

12 J. J. Cummins and W. E. Robinson, J. Chem. Eng. Data, 9, 304 (1964).

13 J. Cason and D. W. Graham, Tetrahedron, 21, 471 (1965).

14 A. Treibs, Angew. Chem., 49, 682 (1936).

15 J. W. Moore and H. N. Dunning, Ind. Eng. Chem., 47, 1440 (1955).

16 E. W. Baker, Teh Fu Yen, J. P. Dickie, R. E. Rhodes and L. F. Clark, J. Chem. Soc., 89, 3631 (1967).

17 M. Blumer and W. D. Snyder, Chem. Geol., 2, 35 (1967).

18 W. G. Meinschein, Bull. Amer. Assoc. Petrol. Geol., 43, 925 (1955).

19 M. Louis, Rev. Inst. Francais du Petrole, 19, 277 (1964).

20 B. J. Mair and J. L. Martinez-Pico, Proc. Amer. Petrol. Inst., 42, 173 (1962).

21 A. L. Burlingame, P. Haug, T. Belsky and M. Calvin, Proc. Nat. Acad. Sciences, 54, 1706 (1965).

22 W. Henderson, V. Wollrab and G. Eglinton, Chem. Comm., in press (1968).

23 D. H. R. Barton, K. H. Overton and W. Carruthers, J. Chem. Soc., 788 (1956).

24 W. Carruthers and T. W. Cook, J. Chem. Soc., 2047 (1954).

25 I. R. Hills and E. V. Whitehead, Nature, 209, 977 (1966).

26 I. R. Hills, E. V. Whitehead, D. E. Anders, J. J. Cummins and W. E. Robinson, Chem. Comm., 752 (1966).

27 N. Danieli, E. Gil-Av and M. Louis, Nature, 217, 731 (1968).

28 I. R. Hills and E. V. Whitehead, Summer Meeting of the American Petroleum Institute's Research, Laramie, July 1966.

29 I. R. Hills and E. V. Whitehead, Third International Meeting in Organic Geochemistry, London, September 1966.

30 V. Jarolim, M. Streibl, K. Hejno and F. Sorm, Coll. Czech. Chem. Comm., 26, 451 (1961).

31 V. Jarolim, K. Hejno, M. Streibl, M. Horak and F. Sorm, Coll. Czech. Chem. Comm., 26, 459 (1961).

32a V. Jarolim, K. Hejno and F. Sorm, Coll. Czech. Chem. Comm., 28, 2318 (1963).

32b V. Jarolim, K. Hejno and F. Sorm, Coll. Czech. Chem. Comm., 28, 2443 (1963).

33 Sister M. T. J. Murphy, A. McCormick and G. Eglinton, Science, 157, 1040 (1967).

34a M. R. Fenske, F. L. Carnahan, J. N. Breston, A. H. Caser and A. R. Rescoria, Ind. Eng. Chem., 34, 633 (1942).

34b F. L. Carnahan, R. E. Hersh and M. R. Fenske, Ind. Eng. Chem., 36, 333 (1944).

35 T. S. Oakwood, D. S. Shriver, H. H. Hall, W. J. McAleer and P. R. Wunz, Ind. Eng. Chem., 44, 2568 (1952).

36 B. J. Mair, Geochim. Cosmochim. Acta, 28, 1303 (1964).

37 W. G. Meinschein Geochim. Cosmochim. Acta, 22, 58 (1961).

38 S. Ruhemann and H. Raud, Brunstoff-Chem., 13, 341 (1932).

39 A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, Helv. Chim. Acta, 38, 1890 (1955).

40a L. Ruzicka, Proc. Chem. Soc., 341 (1959).
40b R. B. Clayton, Quart. Rev., 19, 168 (1965).

41 E. E. van Tamelin, J. D. Willet, R. B. Clayton and K. E. Lord, J. Amer. Chem. Soc., 88, 4752 (1966).

42 W. S. Johnson, Accounts of Chem. Research, 1, 1 (1968).

43 J. Martin-Smith (Department of Pharmaceutical Chemistry, Strathclyde University), personal communication.

44 S. Allard and G. Ourisson, Tetrahedron, 1, 277 (1957).

45 H. Ageta K. Iwata and S. Natori, Tetrahedron Let., 1447 (1963).

46 H. Ageta, K. Iwata and K. Yonezawa, Chem. Pharm. Bull., Japan, 11, 407 (1963).

47 T. Bruun, Acta Chem. Scand., 8, 1291 (1954).

48 W. Henderson, G. Eglinton, P. Simmonds and J. E. Lovelock, Nature, in press (1968).

49 A. G. Douglas, G. Eglinton and W. Henderson, Third Inter-

national Meeting in Organic Geochemistry, London, September 1966; and in "Advances in Organic Geochemistry 1966", ed. G. D. Hobson and G. C. Speers, in press, Pergamon Press, London.

50 J. B. Davis, Meeting of the Geological Society of America, New Orleans, 1967.

51 W. H. Bradley, Geol. Soc. Amer. Bull., 77, 1333 (1966).

52 M. Streibl, P. Jarolimek and V. Wollrab, Coll. Czech. Chem. Comm., 29, 2855 (1964).

53 J. Jonas, J. Janak and M. Kratochvil, J. Gas Chrom., September, 332 (1966).

54 H. Budzikicuitz, J. M. Wilson and C. Djerassi, J. Amer. Chem. Soc., 85, 3688 (1963) (and the references therein).

55 D. H. Williams, J. M. Wilson, H, Budzikiewicz and C. Djerassi, J. Amer. Chem. Soc., 85, 2091 (1963), and the references therein.

56 P. de Mayo and R. I. Reed, Chem. Ind., 1481 (1956).

57 R. I. Reed, J. Chem. Soc., 3432 (1958).

57a R. B. Clayton, Biochemistry, 1, 357 (1962).

58 P. de Mayo and A. N. Starratt, Tetrahedron Let., 259 (1961).

59 V. Wollrab, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague, personal communic.

60 J. R. Maxwell, Ph.D. Thesis, Glasgow University (1967).

61 R. D. McIver, Geochim. Cosmochim. Acta, 26, 343 (1962).

62 A. T. James and L. J. Morris, "New Biochemical Separations", Chap.14 (1964), Van Nostrand, London.

63 J. G. O'Connor, F. H. Burrow and M. S. Norris, Anal. Chem., 34, 82 (1962).

64 I. Halasz and E. Heine, Nature, 194, 971 (1962).

65 A. I. M. Keulemans, "Gas Chromatography" (1957), Reinhold,

66 M. J. E. Golay, "Theory and Practice of Gas Liquid Partition Chromatography with Coated Capillaries", in "Gas Chromatography", ed. V. J. Coates, H. J. Noebels and I. S. Fagerson (1958), Academic Press, New York.

67 M. J. E. Golay, "Theory of Chromatography in Open and Coated Tubular Columns with Round and Rectangular Cross-Sections", in "Gas Chromatography", ed. D. H. Desty (1958), Butterworths, London.

68 D. H. Desty, "Coated Capillary Columns", in "Gas Chromatographie", ed. H. P. Angele (1959), Akademie Verlag, Berlin (Ost).

69 S. R. Lipsky, R. A. Landowne and J. E. Lovelock, Anal. Chem., 31, 852 (1959).

70 R. P. W. Scott, Nature, 183, 1753 (1959).

71 A. Zlatkis and H. R. Kaufman, Nature, 183, 2010 (1959).

72 L. S. Ettre, "Open Tubular Columns in Gas Chromatography" (1965), Plenum Press, New York.

73 R. Stirton (I.C.I., Ardeer, Scotland), personal communication.

74 W. Konig, personal communication.

75 C. Up de Grove (University of Houston, Texas), personal communication.

76 R. P. W. Scott (Unilever, Bedford, Middlesex), personal communication.

77 W. Averill, "Columns with Minimum Liquid Phase Concentration for Use in Gas Liquid Chromatography", in "Gas Chromatography", ed. N. Brenner, J. E. Callen and M. D. Weiss (1962), Academic Press, New York.

78 L. D. Metcalfe and R. J. Martin, Anal. Chem., 39, 1204 (1967).

79 W. Averill, Perkin-Elmer Instrument News, 17, No.4, 13 (1967).

80 R. Ryhage, Arkiv Kemi., 26, 305 (1967).