A Thesis

entitled

"STUDIES IN ORGANIC GEOCHEMISTRY"

submitted to the

UNIVERSITY OF GLASGOW

in part fulfilment of the requirements

for admittance to the degree of

DOCTOR OF PHILOSOPHY

in the Faculty of Science

by JAMES RANKIN MAXWELL, B.Sc.

Chemistry Department

April, 1967

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

To my parents, wife and daughter.

I wish to express my gratitude to Drs. G.Eglinton and J.D. Loudon for their close interest and guidance throughout the work of this thesis.

My appreciation is also due to Dr. A.G. Douglas, whose assistance at all times proved to be invaluable.

I would also like to thank Miss F. Greene and Miss T.Devitt for technical assistance, my colleagues of the Organic Geochemistry Unit and Dr. A. McCormick for their advice and help, Mr. J.M.L. Cameron and his staff for the micro-analyses, Mrs. F. Lawrie and Miss A. Roberts for the infra-red spectra, Mr. J. Gall for the nuclear magnetic resonance spectra, Drs. McCormick and J. Martin and their staffs for the mass spectra and Mrs. M Benzie for her typing.

The work was supported by a maintenance grant from the Science Research Council.

A B S T R A C T.

Organic geochemistry is the study of organic matter in geological situations, including contemporary environments. One facet of the subject is the isolation and identification of organic compounds from fossils and sediments. Correlations are then sought between such compounds and the biological compounds present in contemporary organisms, bearing in mind the chemical changes which could have taken place. In this thesis an attempt has been made to relate chemically a living alga, Botryococcus braunii, a derived rubbery deposit called Coorongite and Torbanite which is a sediment 300 million years in age.

The thesis can be divided into six parts, five of which are concerned with organic geochemistry.

The Introduction reviews the more significant contributions to organic geochemistry and describes the types of compounds found in sediments, with special reference to alkanes. Some of the various facets of the subject and the possible derivations of geological isoprenoid alkanes are also discussed.

Section I is concerned with the alkanes present in two samples of a young sediment (ca. 30 x 10^6 yrs.) from N.W. Bohemia, Czechoslovakia. The distributions of the normal alkanes are reminiscent of those of the normal alkanes of the surface waxes of most contemporary plants. Furthermore the predominance of triterpene

hydrocarbons in the branched-cyclic alkane fraction is in accordance with the fact that the plant species identified in the sediment are mainly angiosperms. The diterpene hydrocarbon fichtelite was identified in one of the samples.

Section II deals with the alkanes of a number of samples from the Scottish Carboniferous Formation (ca. 300 x 10^6 yrs.). The normal alkanes of these samples have smooth distributions in contrast to those found for the young sediment examined (Section I). A number of acyclic isoprenoid hydrocarbons were identified, indicating that the samples have a biological origin. Triterpene hydrocarbons were isolated from one of the samples, viz. the Westwood Shale and their occurrence reflects the difference in source material between this and a closely related sediment called Torbanite. The Westwood Shale is the oldest geological sample from which triterpanes have been isolated in a pure state.

Section III describes the hydrocarbons of a rubbery deposit called Coorongite (ca. 40 yrs.) which is the presumed precursor of Torbanite. The unusual hydrocarbon distributions found in Coorongite are thought by the author to be the result of bacterial activity. Three acyclic isoprenoid hydrocarbons were identified in the sample examined.

Section IV deals with the hydrocarbons of a living alga, Botryococcus braunii, which gives rise to Coorongite. No

saturated hydrocarbons were detected in the alga and the hydrocarbon fraction was found to consist almost entirely of two novel hydrocarbons. Approaches to the structural elucidation of these hydrocarbons are described.

The Appendix is concerned with the interactions which can take place between a nitro-group and a side chain in ortho-substituted nitrobenzenes. The types of interaction which have been observed are reviewed and two new examples are described. A mass spectral method for the identification of the N-oxide function in aromatic N-oxides is discussed.

CONTENTS

INTRODUCTION

Organic Geochemistry.

Organic geochemistry may be broadly defined as the study of the organic matter occurring in geological situations. The greatest activity in this field has been directed towards the isolation and identification of organic compounds from sediments, fossils and crude oils with a view to elucidating the geological and biochemical implications of the occurrence of these compounds. The most-studied classes of compounds have been the amino acids, fatty acids, alkanes, alkenes, aromatic hydrocarbons, porphyrins and carbohydrates.

In recent years organic geochemistry has been investigated as a possible tool for the detection of remnants of former life-processes, both on Earth and certain other planets of the solar system and their satellites, notably the Moon. At present this involves the isolation and identification of biologically-derived compounds from ancient sediments for which no adequate fossil record exists. Such experiments, when applied to samples from other planets, could only detect life-processes if the life were based on the carbon atom and a terrestrial biochemistry. This might not necessarily be the case.

The two main prerequisites for these investigations are that these compounds must be stable over geological time and cannot be synthesised in significant concentrations by simple abiogenic

means[1] such as the treatment of mixtures of methane, water vapour and ammonia (the presumed primitive atmosphere of the Earth[2]) with ionising radiation or an electric discharge. The latter restriction excludes, for example, the biologically-important amino acids, certain of which may be synthesised by the action of an electric discharge[3,4,5,6] or ionising radiation[7] on mixtures of the gases presumed to be the major constituents of the Earth's prebiotic atmosphere. In fact, Harada and Fox[8] have synthesised all the amino acids common to protein, except cystine (1) and methionine (2), simply by heating a mixture of methane, water vapour and ammonia to 1000°C in the presence of silica. In other experiments simulating prebiological conditions a wide array of biologically-significant molecules have been prepared, notably sugars[7], nucleic-acid bases (adenine (3)[9,10] and guanine (4)[10]), adenosine (5)[11], mono-nucleotides[12], adenosine diphosphate (ADP)(6) and adenosine triphosphate (ATP)(7)[13], alkanes[14,15], and carboxylic acids[16].

However certain alkanes and fatty acids are still valid as evidence of life-processes in the past since they exhibit great specificity of structure and have not been synthesised in primitive atmosphere experiments. Thus the acyclic isoprenoid hydrocarbons, steroid and triterpene hydrocarbons (see below), and the isoprenoid fatty acids are suitable as "biological markers". Isoprenoid fatty acids have been isolated from the Green River Shale of Wyoming and Colorado (Eocene, 60 x 10^6 yrs.)[17,18], and from a California petroleum[19].

Porphyrins are also valid as "biological markers" because of their stability and specificity of structure and have been isolated from a large number of sediments and crude oils, the oldest source at present being the Nonesuch Shale of Michigan[20] (Precambrian, 1 x 10^9 yrs.). The porphyrins are thought to be derived mainly from chlorophyll and their occurrence in this ancient sediment, along with the occurrence of isoprenoid hydrocarbons (see below) has been taken as evidence for the existence of photosynthetic organisms one billion (1 x 10^9) years ago.

However, life detection experiments are not the only biochemical applications of organic geochemistry. It has been suggested that comparisons between the organic constituents of discrete fossils (as opposed to sediments) and the equivalent contemporary organisms will yield much useful biochemical information[21]. Some of these examples are given below.

Tarlo and Tarlo[22] have demonstrated the presence of dentine in Pycnosteus tuberculatus, Rohon, a fish-like vertebrate which inhabited the seas more than 400 million years ago. This animal had neither teeth nor jaw bones but was covered with a hard dermal armour containing dentine. These authors concluded that the dentine covering the body of this animal was very similar to modern dentine and its presence and function in Pycnosteus reveals much about the origin of bone and teeth and accounts for many of their properties.

A fossil crinoid (sea-lily) of Jurassic age (135-180 x 10^6 yrs.)

has been shown to contain a number of pigments, termed fringelites[23,24] (8,R=H or OH). These pigments are very similar in their properties and structure to pigments of the aphin[25](9) and hypericin[26](10) types. The parent aromatic hydrocarbons were also isolated and identified[27] and, from a consideration of the structures of the fringelites and the hydrocarbons, Thomas and Blumer[27] have postulated a complex geochemical reaction sequence which converted the oxygen-rich organic pigments to their parent hydrocarbons. In 1933 Fikentscher[28] reported the isolation and identification of coproporphyrin in a crocodile coprolyte (Tertiary, 12-70 x 10^6 yrs.). There is some doubt regarding the validity of this claim because of the analytical techniques used. However, discrete fossils have as yet been little studied, as opposed to sediments - a fact which is surprising since the relative homogeneity of the source material makes a single fossil species particularly attractive for study[27]. Although the occurrence of the fringelite pigments in a fossil crinoid is per se interesting in a paleobiochemical sense, the reconstruction by the authors of the possible fate of these pigments exemplifies another important facet of organic geochemistry. This involves the investigation of the chemical changes undergone by organic matter after deposition, i.e. during diagenesis and maturation of the sediment or fossil. This problem has been tackled in two widely differing ways.

The "indirect" method is to compare the structures of certain compounds or the distributions of classes of compounds in a sediment or

fossil with the structures and distributions of contemporary biological compounds. Also, compounds in the sediment or fossil may be compared with related compounds in the sediment or fossil. The latter comparison applies to the geochemical sequence postulated for the fate of the fringelite pigments.

Thomas and Blumer[29] have also postulated a sequence of geochemical reactions demonstrating the possible derivation from chlorophyll and haemin of the various porphyrin pigments present in a Triassic (180-230 x 10^6 yrs.) sediment. For example, chlorophyll a, after loss of the magnesium atom and hydrolysis would give rise to the intermediate (11). This compound could subsequently give the porphyrins (12), (13) and (14) by a series of geochemically-feasible reactions involving reduction, hydrogenation, oxidative cleavage, and decarboxylation with radical intermediates. Mair[30] has postulated that the geochemical alteration of terpenoids could account for most of the hydrocarbons, containing benzene and cyclohexane rings, found in crude petroleums (and sediments). These compounds could have arisen from terpenoids by loss or addition of hydrogen or partial fragmentation during diagenesis.

The "direct" method (geogenesis) is the simulation of diagenesis under laboratory conditions. Jurg and Eisma[31,32] have found that, by heating behenic acid with bentonite clay, they could generate a homologous series of both n-alkanes and fatty acids with carbon chains both shorter and longer than that of the original acid. They suggest that a radical process could account for the formation of these products.

Douglas et al[33] have recently found that when a sample of the Green River Shale (Eocene, 60 x 10^6 yrs.) was pyrolysed at 500°C, the large odd/even carbon number preference* (C.P.I.[34] ~3) of the normal alkanes present in the sediment was markedly reduced (C.P.I. slightly greater than unity). This result is significant since ancient sediments do not show any marked odd/even carbon number preference of the normal alkanes (see later). Furthermore, a homologous series of normal vinyl alkenes was generated and showed a slight even/odd preference. The authors infer that the alkenes, generated more slowly under geological conditions, could suffer reduction by hydrogen transfer, thereby providing an explanation for the smooth carbon number distributions of the n-alkanes in ancient sediments. That is to say, the alkenes and alkanes are in all probability geogenetically related if the results are transferable to reactions at much lower temperatures over long periods of geological time.

However, at present any conclusions from both of the above geochemical methods for studying diagenesis must remain speculative.

Organic geochemistry has been applied to a number of other geological problems. For example, Grandsch and Eisma[35] have shown that the West Venezuelan crude oils must have originated in the nearby Cretaceous (~ 70 x 10^6 yrs.) La Luna formation by an examination of their respective contents of porphyrin/vanadium complexes.

A number of other classes of biological compounds have been studied geochemically. Carotenoids[36,37] have been isolated from a

* C.P.I. = $\frac{\text{sum of concentrations of odd C no. n-alkanes}}{\text{sum of concentrations of even C no. n-alkanes}}$

number of recent sediments up to 100,000 years old[38]. As yet carotenoids have not been isolated from sedimentary rocks or crude petroleum. This is explicable in terms of the relative instability of these compounds and presumably their identity is lost during diagenesis and maturation. Schwendinger and Erdman[39] have examined the carotenoid content of a number of freshwater and marine sediments. It was found that the carotenoid concentration was higher in the marine sediments. According to those authors this result was expected since there is a higher carotenoid content in algae than in higher plants.

The presence of sugars[40,41] and amino acids[41] in sediments has also been reported.

Hydrocarbons as Evidence of Life-Processes in the Past.

According to modern theories of evolution there was a period of chemical evolution[42] developing gradually to a period in time when biologically-significant macromolecules (e.g. proteins), formed abiotically, reproduced in a manner characteristic of living processes. Accordingly one branch of organic geochemistry has been directed towards finding chemical evidence for life processes in sediments for which no adequate fossil record exists.

Eglinton et al.[43] first suggested that the occurrence of the acyclic isoprenoid hydrocarbons, pristane (15) and phytane (16) in the Green River Shale (60×10^6 yrs.) of Colorado and the Nonesuch Shale (Precambrian, 1×10^9 yrs.) of Michigan, might be considered as evidence of a biological origin for these sediments. Although the Green River

Shale has well-characterised fossil remains[44], this is not necessarily the case for more ancient sediments, notably those of the Precambrian ($>1 \times 10^9$ yrs.) where microstructures[20,45,46,47] are often the only geological evidence for a biological origin. Biological markers, such as pristane and phytane, are compounds whose occurrence in geological samples is explicable in terms of known biosynthetic pathways and which show chemical stability under geological conditions. Obviously other isoprenoid hydrocarbons, such as the steroid and triterpene hydrocarbons, also qualify as biological markers because of their structural specifity and stability under geological time.

Such considerations must depend on a number of other factors, notably that these hydrocarbons cannot be synthesised abiotically in reasonable quantities and that the hydrocarbons themselves or their precursors have not migrated from a younger source to the sediment(s) under examination. Furthermore the occurrence of biologically-derived hydrocarbons should not, per se, be taken as proof of a biological origin and the presence of other "biological markers", such as the porphyrins and the isoprenoid fatty acids, should be sought after in any one sample under examination.

Ponnamperuma and his co-workers[14,15] have examined the hydrocarbons produced by methane spark discharge experiments. Gas-liquid chromatography of the hydrocarbon fraction thus produced showed no resolution of the mixture even when capillary columns were used. This

is in marked contrast to the hydrocarbon distributions in sedimentary rocks. Furthermore molecular sieving failed to isolate any normal alkanes from the total hydrocarbon fraction.

In the Fischer Tropsch conversion of carbon monoxide and hydrogen into alkanes and alkenes etc. the distribution of the alkanes differs from the distributions of the alkanes in sediments[48]. Also there is no evidence for the formation of isoprenoid hydrocarbons.

Therefore the postulation that certain hydrocarbons are useful as "biological markers" appears to be a valid one, especially since it is unlikely that simple abiological synthesis could account for the relative predominance of the acyclic isoprenoid hydrocarbons found in ancient sediments.

There is a strong presumption that pristane, phytane and the lower molecular weight isoprenoid hydrocarbons isolated from geological samples are derived in the first instance from the phytyl side chain of chlorophyll [49]. Another possibility is that they arise from the lipids of halophylic bacteria[50]. However the C_{21} isoprenoid hydrocarbon (17) isolated from a number of sediments (see below) cannot be derived directly from chlorophyll and may derive from a larger isoprenoid skeleton such as a carotenoid[51].

Johns *et al.*[51] have also suggested that phytenes of types (18), (19),(20) could be formed during compaction of the sediments and if they were cleaved at the double bonds the C_{19}(15), C_{18}(21),C_{16}(22), isoprenoid hydrocarbons might result. However, they point out that in

the case of the C_{19} and C_{16} isoprenoids this would involve conversion of a carboxyl group to a methyl group, which is an unlikely process in their opinion. In contrast, the C_{18} isoprenoid could be obtained simply by decarboxylation of the C_{19} isoprenoid acid formed from (18). These authors also state that the isoprenoid hydrocarbons may derive from saturation of the phytenes followed by thermal cracking.

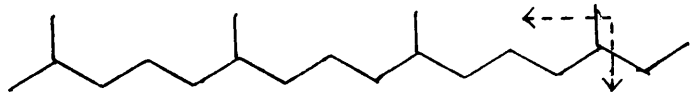
It is also possible that the pristane skeleton was present at the time of deposition since pristane (15) has been isolated from a number of marine sources[52]. However, a number of C_{19} mono-olefins (23), (24) and (25) have also been isolated from zooplankton and the liver oils of marine fishes and mammals[52]. Hydrogenation of these zamenes could, under geological conditions, also give rise to stereoisomers of pristane. It is possible, therefore, that for marine sediments the pristane isolated could derive in part from a C_{19} isoprenoid skeleton. However, it is still thought that the zamenes derive from the phytyl side chain of chlorophyll in the first instance.

The geological pristane could also arise in part by decarboxylation of phytanic acid (26) which has been isolated from natural sources such as butterfat[53], ox blood[54,55], ox fat[56] and sheep fat[57]. Similarly norphytanic acid (27) isolated from butterfat[58] could give rise in part to the C_{18} geological isoprenoid (21). This

postulation appears attractive since these acids may be more widely distributed than was previously thought.

Although phytane (16) itself is not a common constituent of living organisms the phytadienes (28), (29) and (30) have been isolated from zooplankton[59] and these could give rise to phytane by geological reduction. However, geological reduction of unsaturated bonds has yet to be conclusively demonstrated although it is widely postulated. The phytadienes themselves, like the zamenes, are thought to arise from chlorophyll. Therefore although there appear to be a number of possible pathways to the geological acyclic isoprenoid hydrocarbons, chlorophyll seems to be a likely common precursor, except for the C_{21} isoprenoid (see above) and perhaps to some extent the C_{15} isoprenoid farnesane (31) which has been found in some plants[60].

It is noteworthy that there is no record of the isolation of the C_{17} isoprenoid hydrocarbon (32) from a geological sample. A possible explanation for this has been suggested by Calvin and his co-workers[51], viz. that if the geological acyclic isoprenoid alkanes were formed by saturation of the phytenes (18), (19) and (20) (see above), followed by thermal cracking, then formation of the C_{17} isoprenoid would require two cleavage points as in (I)



(I)

As yet the stereochemistry of these presumed biologically-derived acyclic hydrocarbons is not known. There are four possible reasons for this:-

(a) these compounds have very small specific rotations

(b) the quantities involved would be minute

(c) a small optically active impurity would upset any measurements

(d) it would be difficult to separate the possible stereoisomers present.

Weedon and his colleagues[61] have recently elucidated the stereochemistry of phytol (33) derived from chlorophyll. The stereochemistry of the geological pristane and phytane must be compared to that of the pristane and phytane derived from phytol. If there is a direct relationship these compounds can truly be considered as "biological markers".

It should be stated at this point that these geolipids - are "biological markers" since they are presumably derived from biolipids and are not themselves biolipids (with one or two exceptions, see above).

Normal alkanes isolated from living plants usually show an odd/even carbon number preference[62] and this preference has been observed, for example, in the normal alkanes isolated from the Green River Shale[63,64]. Normal alkanes isolated from a few contemporary marine organisms do not show any special odd/even preference[65].

However, this finding requires re-investigation since there exists the possibility of oil contamination from the sea.

In any case the normal hydrocarbons isolated from sediments older than the Green River Shale (60 x 10^6 yrs.) do not in general show this preference. These factors indicate, according to Johns et al.[51], that the odd/even preference of normal alkanes is unsatisfactory as a mark of biogenicity in older sediments.

Alkanes in Sediments.

The Green River Shale of Colorado (Eocene, 60 x 10^6 yrs.) in particular has proved to be the source of a large number of presumed biogenetically-derived hydrocarbons. Since the isolation of farnesane (31), pristane (15), phytane (16), the C_{16} (22) and C_{18} (21) isoprenoid hydrocarbons[64], there has been widespread activity directed towards the isolation and identification of alkanes in this and other sediments. Eglinton et al.[43,63] subsequently isolated the above alkanes from the Green River Shale. The identifications were made by mass spectrometry after preparative-scale gas-liquid chromatography of the branched-cyclic fraction obtained by molecular sieving (5Å sieve) of the total hydrocarbon fraction. The normal alkanes[63,64] obtained by molecular sieving show the marked predominance of the odd carbon numbers, especially C_{27}, C_{29} and C_{31}, so characteristic of most plants[62]. The higher molecular weight branched-cyclic hydrocarbons were shown to contain steroid and triterpenoid hydrocarbons but the gas-liquid chromatographic equipment available did not allow the isolation of pure

samples except for a C_{28} sterane and a C_{27} sterane which were not characterised[66]. However, Whitehead et al.[67] have subsequently isolated the fully saturated triterpene hydrocarbon, gammacerane (34) from this shale. The stereochemistry was defined by comparison of the hydrocarbon with an authentic sample of gammacerane. The fully saturated carotenoid hydrocarbon, β-carotane (35) has also been isolated from the Green River Shale and identified by mass spectrometry[68].

The isolation of the acyclic isoprenoid hydrocarbons described above prompted Eglinton et al. to examine the hydrocarbon fraction of the older Nonesuch Shale of Michigan (Precambrian, 1×10^9 yrs.)[63]. Farnesane (31), pristane (15), phytane (16), and the C_{16} isoprenoid hydrocarbon (22) were isolated and identified by mass spectrometry. These results showed the shale to have a biological history and were significant because, earlier than about 600 million years ago, well-defined morphological remains are commonly scanty and of microscopic dimensions[20,45,47,69,70]. The normal hydrocarbons isolated showed no odd/even carbon number preference.

An even older sediment, the Gunflint Chert (2×10^9 yrs.) also contains pristane and phytane, but no odd/even carbon number preference of the normal hydrocarbons[71]. The identification of the hydrocarbons in this sediment was made using a combined gas chromatography-mass spectrometry instrument which proved to be

extremely useful for obtaining mass spectra of specific hydrocarbons in the very complicated total hydrocarbon fraction.

The oldest shale from which alkanes have been isolated is the Soudan Iron Formation of Minnesota which has been dated isotopically at greater than 2.7 x 10^9 years[72]. Pristane, phytane, the C_{16} isoprenoid, the C_{18} isoprenoid and the C_{21} isoprenoid were all characterised in the total alkane fraction[51]. A fraction of the higher molecular weight branched-cyclic hydrocarbons which was trapped from a preparative scale g.l.c. chromatogram was shown to contain a C_{27} sterane, a C_{28} sterane, and a C_{29} sterane[66] but as yet these have not been separated and identified. The authors state that the steranes and triterpanes constitute a small proportion of the total alkanes of the Soudan Iron Formation in contrast to those of the Green River Shale where there is a striking predominance of steranes and triterpanes in the total alkane fraction. According to the authors this is explicable in terms of the Green River Shale being a nonmarine sediment and the Soudan Shale being of marine origin and much greater age. In the former the steranes are presumably derived from plant sterols. However, there remains the possibility that the hydrocarbons are not indigenous to the Soudan Shale since there is some discrepancy in the isotope ratios of the stable carbon isotopes of the organic matter extracted by solvent and the kerogen (insoluble organic matter)[73]. The organic extract fractions had δC^{13}* values of -25.06 to -25.99 per mil whereas the δC^{13} value for the kerogen

* $\delta C^{13} = \frac{(C^{13}/C^{12})_x - (C^{13}/C^{12})_s}{(C^{13}/C^{12})_s} \times 1000$ where x = sample
s = standard reference sample

was -34.54 per mil. For the majority of sediments the isotope ratios for the extractable fractions agree reasonably well with the values obtained for the insoluble organic matter. According to Johns et al.[51] and Meinschein[74] migration of the hydrocarbons into the shale from an outside source could imply a lack of homogeneity in the samples examined. Accordingly, Meinschein compared the gas-liquid chromatographic records of the solvent extracted hydrocarbons with those of the hydrocarbons obtained by solvent extraction after digestion of the sample in hydrofluoric acid. Hydrofluoric acid dissolves the silicates and releases trapped organic matter. The comparison between the two sets of hydrocarbon distributions indicated to the authors that the hydrocarbons are indigenous to the shale. It was found that the highest molecular weight alkanes were present in the silicate phases and were only accessible to solvents after the shale had been treated with hydrofluoric acid. If migration had occurred, it would have been expected that the lower molecular weight alkanes would have been most widely distributed through the shale and the less mobile higher molecular weight alkanes would have been concentrated within the larger, more accessible pores and on the carbon surfaces. The above results indicate that there were photosynthetic organisms on the Earth 2.7×10^9 years ago since the acyclic isoprenoid hydrocarbons are thought to be derived from chlorophyll.

In contrast with the very old sediments mentioned above, recent sediments have, in general, quite different alkane distributions.

For example, the nonmarine montan wax of Germany ($\sim 30 \times 10^6$ yrs.) has a preponderance of higher molecular weight normal alkanes and the total alkane fraction shows a large odd/even carbon number predominance[75] as in most contemporary plants. This is because the brown waxy coal containing montan wax is a relatively unaltered deposit and consists of plant debris. Significantly, no pristane and phytane were isolated.

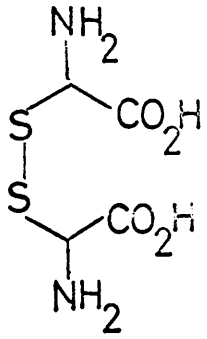
The normal hydrocarbon fraction of a modern sediment from the San Nicholas Basin off the California coast similarly has the large odd/even carbon number preference and preponderance of the C_{27}, C_{29}, C_{31}, and C_{33} normal alkanes[76] so characteristic of most plants[62]. Two Cretaceous sediments ($\sim 70 \times 10^6$ yrs.) viz. the Mowry Shale of Wyoming and the Thermopolis Shale of Wyoming, examined by the same author, also show the same distribution of normal alkanes for most the samples examined but this was not true in all cases.

Bray and Evans[77] examined the n-alkane distributions of a large number of sediments, ranging from Miocene ($\sim 25 \times 10^6$ yrs.) to Mississippian (250×10^6 yrs.) in age. The Carbon Preference Indices[34] (C.P.I.) of the normal alkanes were shown to be in the range 2.4-5.5 with a predominance of the higher molecular weight normals. Also a few of the ancient sediments had indices comparable to some of the recent sediments. Some of the former samples were as old as Mississippian.

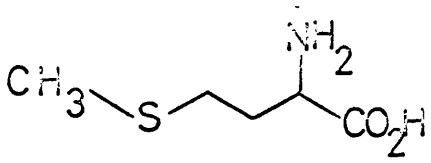
It would appear, therefore, from the above results that recent

sediments almost always have a C.P.I. greater than unity but this is rarely true of ancient sediments. Also there appears to be a lack of acyclic isoprenoid hydrocarbons in recent sediments in contrast to ancient sediments but this may be due to the fact that few recent sediments have been examined in such a way that these compounds would have been isolated and identified.

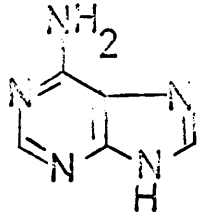
It is possible that the hydrocarbons in ancient sediments are partly non-biological in origin or that the normal alkanes contributed by the originating organisms already had a C.P.I. of unity. Alternatively the marked odd/even pattern could have been destroyed by migration, selective elimination or de novo synthesis during diagenesis[31]. The latter postulation appears to be very attractive since it has recently been found that thermal treatment of the Green River Shale (which has a C.P.I. greater than unity, see above) causes the C.P.I. to become almost unity[33]. There is only one reference in the literature to the occurrence of an acyclic isoprenoid hydrocarbon in recent sediments. Pristane, but not phytane, was isolated from a sediment from the Wilkinson Basin, Massachusetts, and one from Volden Fjord, Norway[78]. This is explicable in terms of the water column in both regions being rich in pristane-bearing copepods[79]. The authors therefore conclude that phytane and the other lower molecular weight hydrocarbons of ancient sediments are postdepositional geochemical products, which agrees with the theory that the phytyl side chain of chlorophyll is the common precursor.



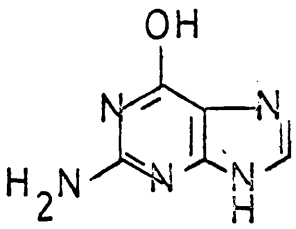
(1)



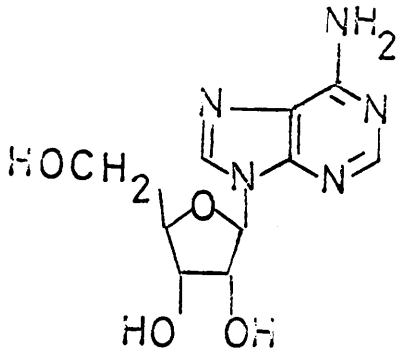
(2)



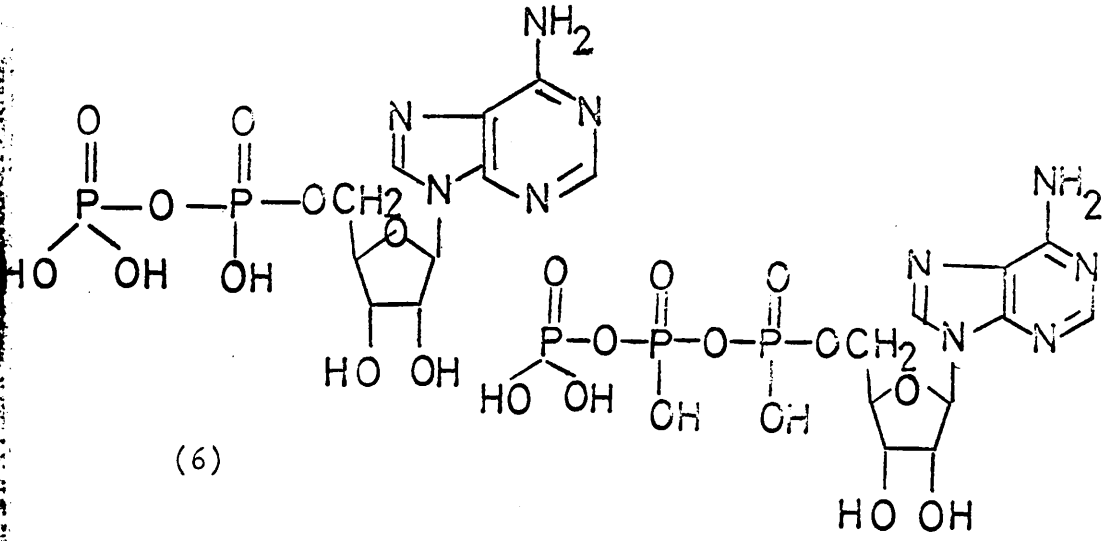
(3)



(4)

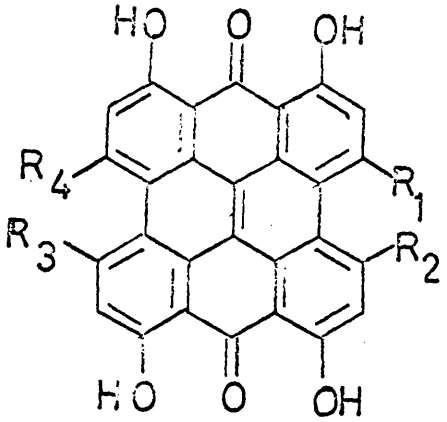


(5)

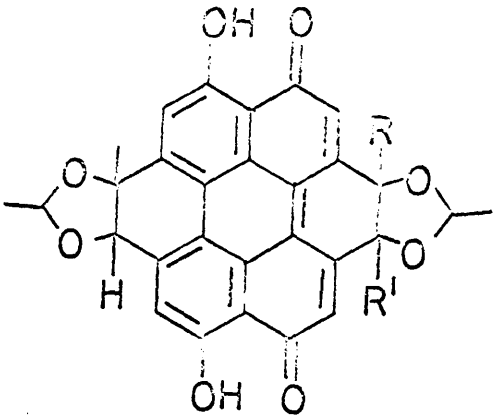


(6)

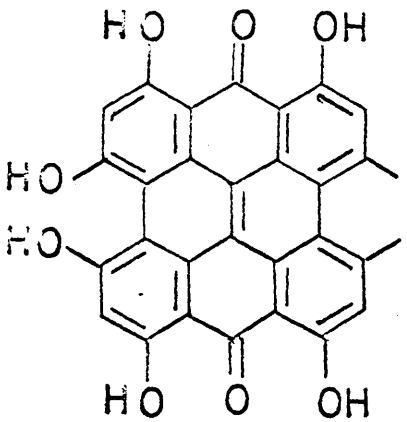
(7)


HO
O
OH
R4
R1
R3
R2
HO
O
OH

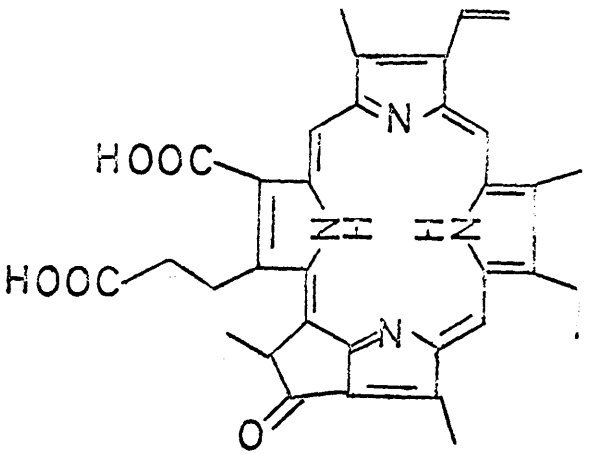
(8)


OH
O
O
O
H
O
R'
OH
O

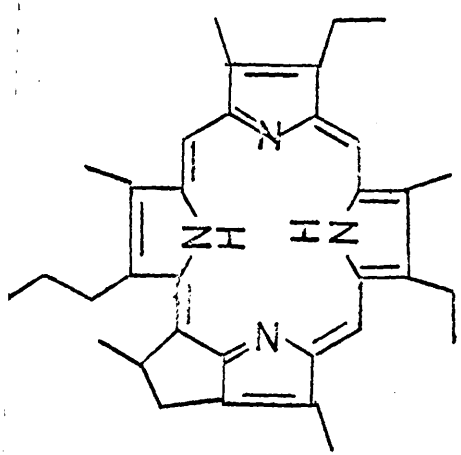
(9)


HO
O
OH
HO
HO
HO
O
OH

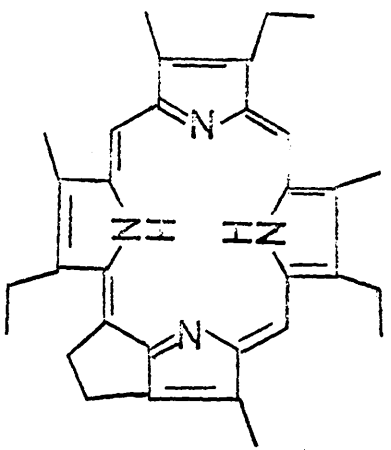
(10)


HOOC
HOOC
N
N
O

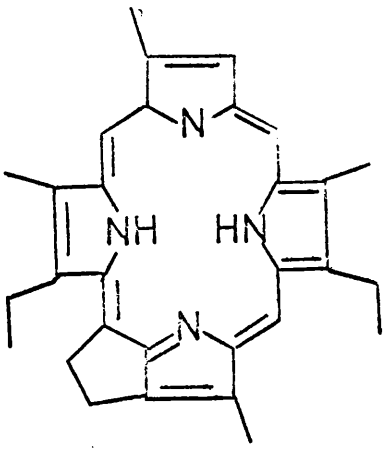
(11)


N
N

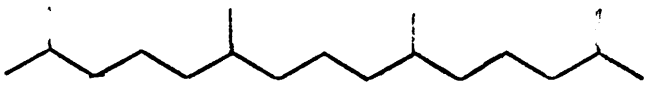
(12)


N
N

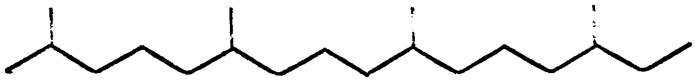
(13)



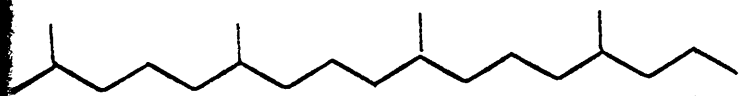
(14)



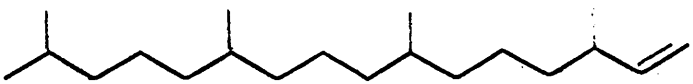
(15)



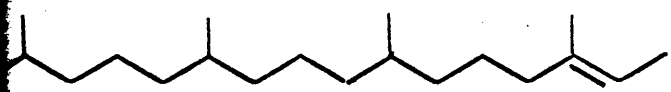
(16)



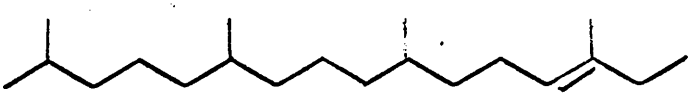
(17)



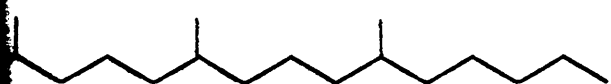
(18)



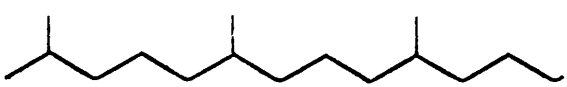
(19)



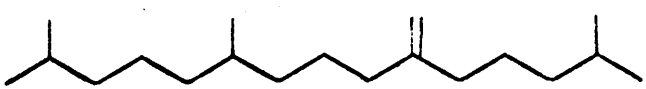
(20)



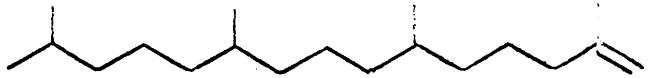
(21)



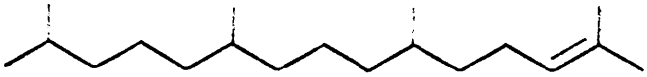
(22)



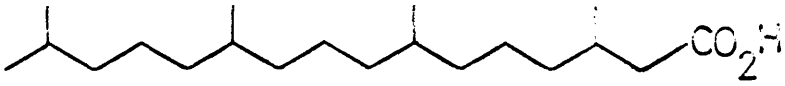
(23)



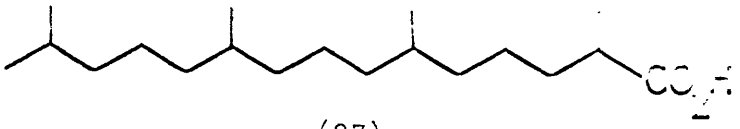
(24)



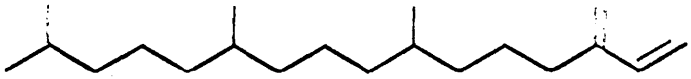
(25)



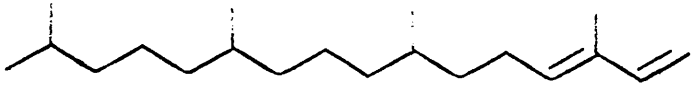
(26)



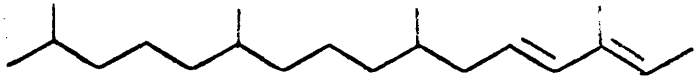
(27)



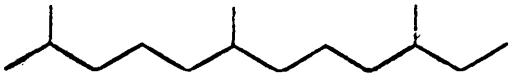
(28)



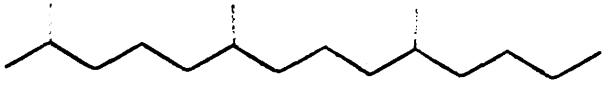
(29)



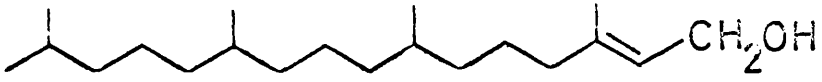
(30)



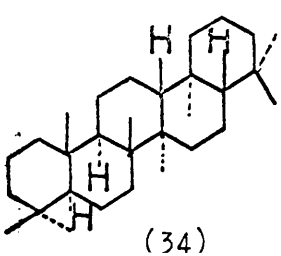
(31)



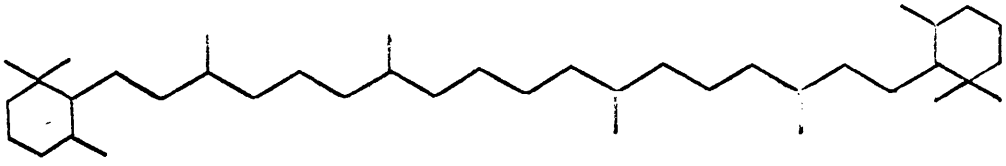
(32)



(33)



(34)



(35)

GENERAL EXPERIMENTAL

General procedures used for the isolation and identification of alkanes from geological samples.

Since the methods used were essentially identical for all of the geological samples examined the general outline is mentioned here. Further details or deviations applicable to any one sample are given in the appropriate experimental sections.

Treatment of rocks (Fig. 1).

The outer surface of the rock was removed in order to minimise contamination and only fragments having freshly exposed surfaces were used. The fragments were broken up on a clean metal surface into smaller fragments 1-1½ inches in diameter with a hammer whose head was covered with several layers of aluminium foil. The resulting chips of rock were carefully washed 3 times (5 min. each time) by sonication in benzene/methanol (1:1), using a titanium probe (Dawe Instruments Ltd., Model S75).

The dried chips were then powdered in a cleaned rotary hammer mill (Glen Creston, Star Beater Mill). In order to again minimise contamination the hammer mill was modified to accommodate lead gaskets rather than the standard rubber gaskets.

Final powdering was effected in a clean vibratory disc mill (Tema Machinery Ltd., Banbury), the milling operation lasting 15 min. The moveable parts of both mills in contact with the rock were cleaned by tank sonication in detergent (R.B.S.26, Medical Pharmaceutical Developments Ltd.), rinsed thoroughly with distilled water washed with distilled acetone and finally with chloroform.

It was found that, after pulverisation with the hammer mill, approximately 70% of the resulting powdered rock passed through a 200 mesh sieve. After the disc mill operation 100% of the powdered rock passed 200 mesh. A considerable amount of heat was evolved if the milling time for the disc mill was greater than 15 min.; in these cases the temperature rose to about 60°C.

Polythene gloves were worn during all of the above operations.

For smaller samples, chips about $\frac{1}{2}$ inch or less in diameter were pulverised in one operation using a ball mill (Glen Creston) equipped with a steel capsule (capacity $\sim$ 10g.).

Extraction of organic matter.

Ultrasonic extraction was used to extract the organic matter from the finely powdered shale since it is known to be a rapid, effective and convenient method for removing soluble organic matter from sediments[80]. The powdered rock was placed in a glass centrifuge tube (100ml. capacity) or bottle (250ml. capacity) with solvent (3:1 benzene/methanol) and the tube or bottle adjusted in the ultrasonic tank (Dawe Instruments Ltd., type 1165/H60X; frequency 25.83kcs.; fitted with a 300/150 watt Soniclean generator) such that the solvent level was at the same level as the water in the tank. The extraction was found to be most efficient when both the solvent and the water were at the same level. Sonication was allowed to proceed for 30 min. The resulting suspension was centrifuged at 2500 r.p.m. for 30 min. and the clear supernatant solution removed by pipette. For smaller samples of shale an M.S.E. "Super Multex" centrifuge (operating at 3000 r.p.m.) was used. The extraction was repeated 3-6 times. Evaporation of the

solvent on a rotary evaporator (Buchi) gave the total organic extract. Wherever possible the necks of the centrifuge tubes and bottles were covered with aluminium foil.

Column chromatography of the total organic extract (Fig.2).

Woelm Grade 1 neutral alumina was used for all column chromatography and was always pre-washed with n-hexane prior to use. Alumina/sample ratios were generally in the range 40-80:1. The total fraction containing the alkanes was obtained by eluting with n-hexane. The hexane eluate was monitored by thin-layer chromatography in order to ensure that all of the alkanes were eluted from the column.

Thin-layer chromatography of the total n-hexane eluate.

Thin-layer chromatography on silica impregnated with 10% silver nitrate[81] (see below) was found to give the most efficient separation of the alkanes from alkenes (if present) and aromatics (alkylbenzenes, etc.). The alkanes were obtained pure by preparative-scale thin-layer chromatography of the total n-hexane eluate using n-hexane or iso-octane as developer.

Molecular sieving of the total alkanes.

The normal alkanes were separated from the branched and cyclic alkanes by molecular sieving. The method of O'Connor et al[82] was used. Sieving was carried out by heating under reflux (30-48 hrs.) a solution of the alkanes in dry benzene or iso-octane with pellets (1/16 in.) of 5Å molecular sieve (Linde Co., Division of Union Carbide Corporation). The ratio of sieve to sample was about 50:1. The reflux condenser was always equipped with a drying tube containing blue silica gel. The solution, containing the branched and cyclic alkanes

was removed by pipette and passed through a short column of alumina to remove any traces of powdered sieve. The sieve was thoroughly washed with hot benzene or iso-octane (20-30 hrs.) in an all-glass Soxhlet apparatus and the washings added to the solution of branched-cyclic alkanes, which was evaporated.

The washed sieve, containing the normal alkanes, was treated with 24% hydrofluoric acid and benzene or iso-octane, and the mixture stirred magnetically (Teflon-coated stirring bar) until the sieve was dissolved. After separation of the layers, the solution of n-alkanes was passed through a short column containing a layer of alumina and one of anhydrous sodium carbonate, and the solvent evaporated. As a contamination check, a sample (10g.) of the sieve used was treated in the above manner. The "normal" and "branched-cyclic" fractions were examined by analytical gas-liquid chromatography. No peaks were observable in the gas chromatograms run under the usual analytical conditions. The sieve was activated prior to use by heating at 190-240°C under reduced pressure (0.005-0.1mm.) and stored in a desiccator in the presence of phosphorus pentoxide and blue silica gel.

Identification of individual alkanes.

The n-alkanes and branched-cyclic alkanes were subjected to analytical gas-liquid chromatography. Identification of the n-alkanes was made by co-injection of the n-alkane fraction with authentic samples. The branched-cyclic alkanes were separated by preparative-scale gas chromatography and examined by combined gas chromatography-mass spectrometry (see below). Identification of individual branched or

cyclic alkanes was made by means of their mass spectra and also, in some cases, by their infra-red spectra and co-injection with standards.

Analytical Procedures

General

Precautions were taken, wherever possible, to minimise contamination and polythene gloves were worn where contamination could arise through handling. Flasks were stoppered or covered with aluminium foil between operations and the time between these kept short. All solvents were of "Analar" grade and were distilled through an 18 in. column packed with glass helices. Before distillation the solvents were heated under reflux (30 min.). In a 2 l. sample of solvent the first 100 ml. of the distillate were discarded and only 1.8 l. allowed to distil with partial reflux. The n-hexane used contained approximately 0.1% of aromatics (estimated as benzene) before distillation. Glassware was cleaned ultrasonically (20 min.) in an ultrasonic tank (Dawe Instruments Ltd., type 1165/H60X; frequency 25.83 kcs.; 150 watts) thoroughly rinsed with distilled water, stored in closed dust-free jars, and rinsed with solvent before use. The detergent used for cleaning glassware was R.B.S.26 (Medical Pharmaceutical Developments Ltd.).

Teflon stopcocks were always used (chromatography columns, separating funnels, etc.). Solutions were transferred by means of disposable glass pipettes. Solvents were evaporated in a rotary evaporator (Buchi) under water-pump vacuum or in a vacuum oven (Thomson and Mercer Ltd., Croydon) under water pump vacuum when the quantity of solvent was small.

Thin-layer chromatography (t.l.c.)

Silica gel (Kieselgel G-E.Merck) impregnated with 10% (by weight) silver nitrate[81] was used as adsorbent unless otherwise stated. The plates were coated with a slurry of the adsorbent (in distilled water) on a motorised t.l.c. spreader (Baird and Tatlock, London). After air drying, the plates were activated at 120°C, for approximately 1 hr. and stored in a desiccator. Preparative plates were always developed with ethyl acetate prior to activation and were loaded by means of a 50 or 100µl. Hamilton syringe.

n-Hexane or iso-octane was used as eluent for the alkanes and benzene (or benzene/n-hexane mixtures) for the alkenes. Detection was achieved by spraying with 50% polyphosphoric acid followed by charring at 200°C. Preparative plates (20x20 mm., coating 1 mm. thick) were sprayed with a 0.2% solution of dichlorofluorescein in ethanol and viewed under a u.v. lamp (254 mµ.).

Infra-red absorption spectroscopy (i.r.).

Routine spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer (accuracy ± 5 $cm.^{-1}$ above 2000 $cm.^{-1}$ and ± 2 $cm.^{-1}$ below 2000 $cm.^{-1}$). Quantitative and high resolution spectra were recorded on a Unicam S.P.100 double-beam spectrophotometer, equipped with an S.P.130 sodium chloride prism-grating double monochromator and operated under vacuum conditions (accuracy ± 1 $cm.^{-1}$), or on a Perkin-Elmer 225 grating spectrophotometer (accuracy ± 1 $cm.^{-1}$). Fractions trapped by preparative g.l.c. were dissolved in CCl_4 (~4µl.) and the spectra recorded (on ca.40µg.) on the P.E.-257 instrument using a micro-cell

(0.5 mm.; capacity ~ 2.5µl.). The beam condenser was used in these cases.

Gas-liquid chromatography (g.l.c.)

Analytical Perkin-Elmer F-11 instruments, each equipped with a hydrogen flame ionisation detector, were used for analytical g.l.c. Stainless steel columns, 1/8 in. (6 or 10 ft. in length) or 1/16 in. (10 ft. in length) in diameter were employed unless otherwise stated. Nitrogen was used as carrier gas at flow rates of 20-30 ml./min. The liquid phases were: 1% and 3% SE-30 (Applied Science Labs. Inc.); 4.6% JXR (Applied Science); 2% seven ring polyphenyl ether (Applied Science); 3% OV-1 (Applied Science). Temperature programming was usually from 100-300°C. at rates of 4° to 8°/min. The supports used were Gas Chrom P (100-120 mesh, acid-washed and silanized, Applied Science); Chromosorb G (100-120 mesh, acid-washed and silanized, Johns Manville); Gas Chrom Q (60-80 mesh, acid-washed and silanized, Applied Science) unless otherwise stated. Columns were conditioned by being programmed from room temperature to 300°C. at 1°/min. and maintained at 300°C. for 24 hours.

Preparative Wilkens Aerograph A90P-3 instruments, each equipped with a thermal conductivity detector, were used for preparative g.l.c. Copper or stainless steel (20 ft.) columns, 1/4 or 1/8 in. in diameter, were utilised unless otherwise stated. The columns were packed with 3% SE-30 on Gas Chrom P (100-120 mesh, acid-washed and silanized) or 3% SE-52 on Chromosorb W (100-120 mesh, acid-washed and

silanized, Wilkens Aerograph). The carrier gas was helium with flow rates of 60-100 ml./min. at a pressure of 60 p.s.i. Injector and detector temperatures were 275 and 320°C respectively.

Collection of a requisite fraction was effected by trapping the eluate in a glass melting-point capillary (10cm. x 1 mm.). After collection both ends of the capillary were sealed with a small flame. The efficiency of collection was about 60-70%.

The capillaries were pre-cleaned by sonication in detergent solution, rinsing thoroughly with distilled water and acetone, and sonication in chloroform. Both ends of each capillary were flame polished to prevent contamination from the silicone rubber septa.

Combined gas chromatography-mass spectrometry (G.C.-M.S.).

The fractions trapped by preparative g.l.c. were examined by G.C.-M.S. using the LKB.9000 gas chromatograph-mass spectrometer. The columns used were 10 ft. x 3 mm. i.d. glass columns packed with 1% SE-30 on Gas Chrom P. The carrier gas was helium with a flow rate of 30 ml./min. The scanning time used for the mass spectrometer was approximately 4 secs.

The fractions in the glass capillaries were washed to one end with solvent (5 µl.) and aliquots removed for examination by G.C.-M.S.

FIG. 1.

Preparation of rock samples prior to solvent extraction; extraction of the powdered rock.

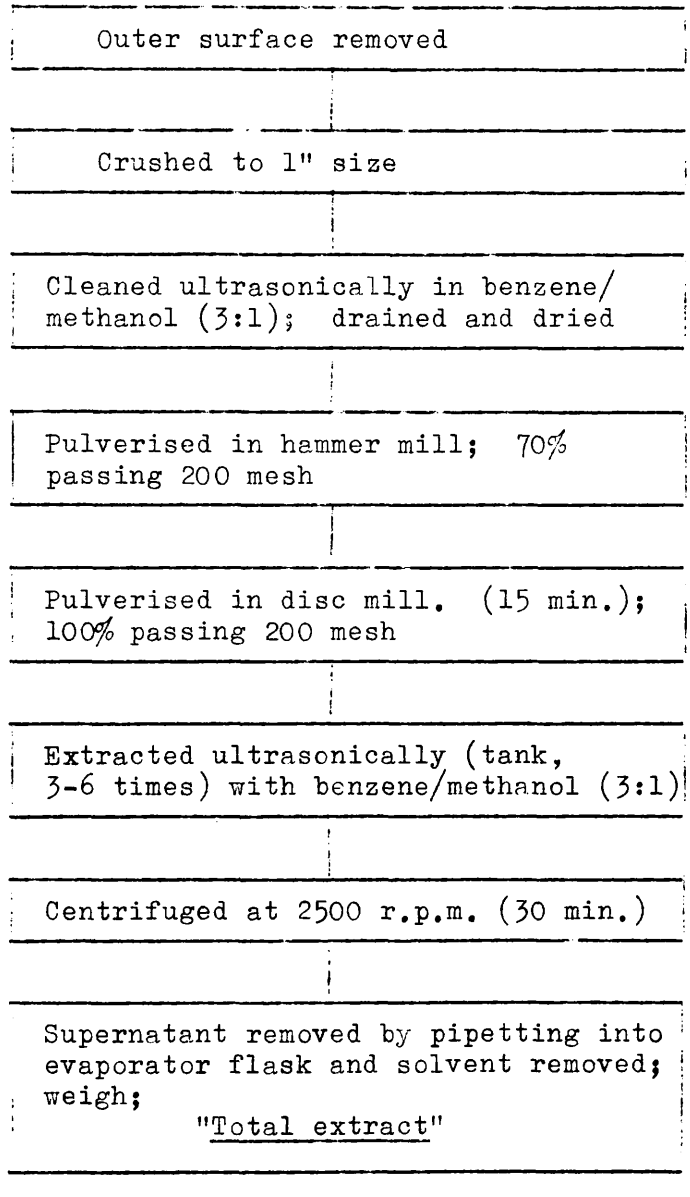
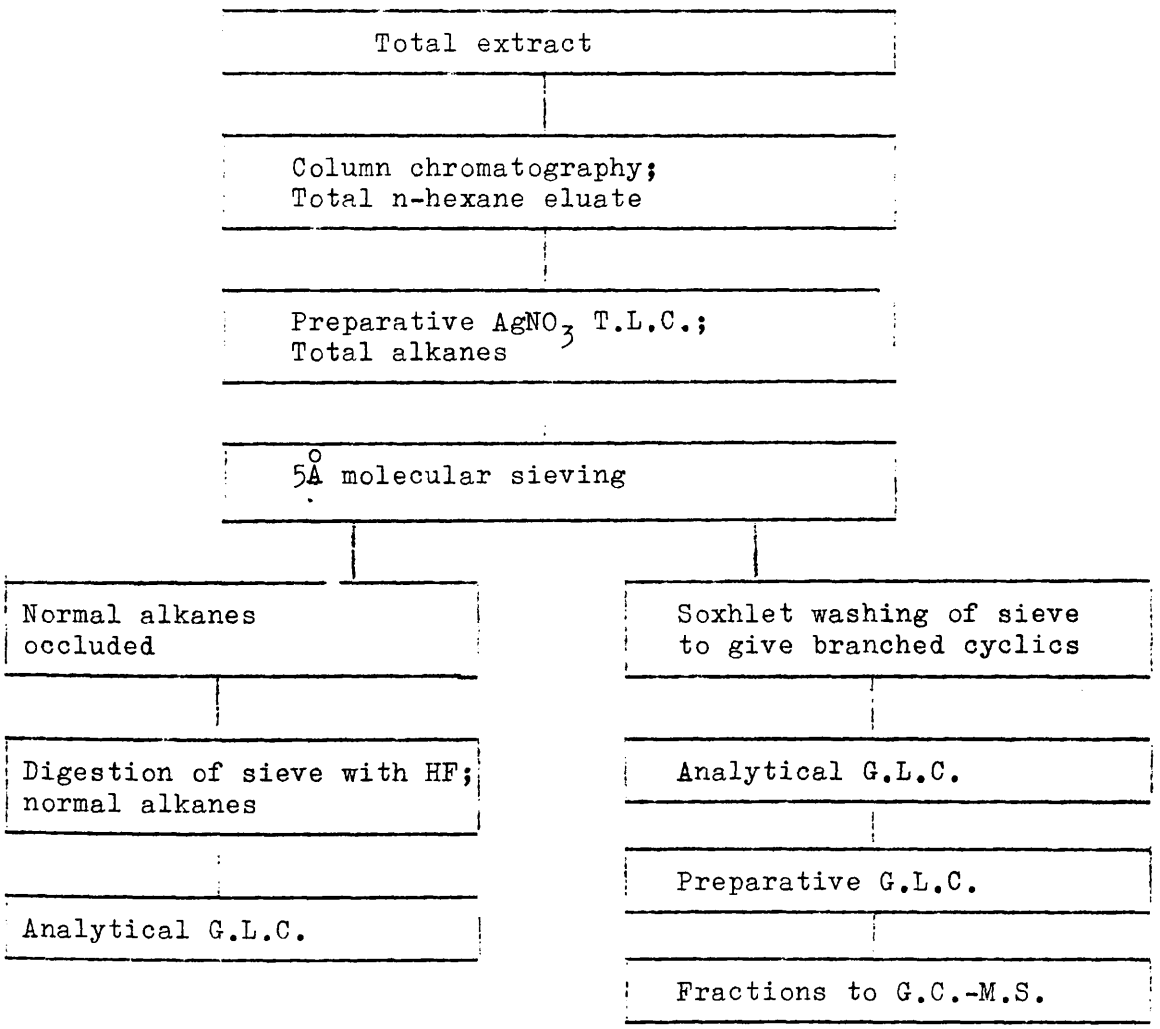


FIG. 2.

Isolation and identification of the alkanes from the total organic extract.



S E C T I O N I

THE HYDROCARBONS OF A LOWER MIOCENE (ca. 25 x 10^6 yrs.) LIGNITE.

INTRODUCTION

Lignitous coals (lignites) comprise a series of immature coals intermediate in composition between peat and bituminous coals. They are of comparatively recent origin, being almost entirely confined to the Tertiary (ca. 7 - 65 x 10^6 yrs.) and late Cretaceous (ca. 70 x 10^6 yrs.). They are almost invariably brown or brown-black in colour and vary in appearance from products resembling mature peats to products resembling bituminous coals. Occasionally they may be yellow in colour. The European brown coals are lignites which contain a great variety and quantity of plant debris, and are often waxy in appearance.

Thomson[83] has given an account of the rhythmic formation of typical brown coal deposits. The following steps are involved:

(a)"A basin of peat growing on a clay soil during a period of reduced subsidence, so that the character of the plant life gradually changed from moisture-loving vegetation to massive trees, favouring dry conditions. The latter were developed particularly during periods of no subsidence."

(b)"The preservation as layers of dark peat, and ultimately as dark brown lignite, of the angiosperms growing in the moister portions of the basin, or during conditions of gradual subsidence, during which moist conditions prevailed, with the partial destruction of the forests growing under dry conditions by fire."

(c)"Sudden flooding of the basin, due to marked subsidence, causing destruction of massive trees, the trunks breaking off and falling into a

horizontal position, leaving the shortened stumps upright. Layers of various thicknesses of mud or sand were deposited, in which the growth of aquatic plants commenced as soon as conditions became stable."

(d)"Reeds, grasses and aquatic vegetation grew in the open water, in which accumulated debris from swamp-loving plants with drift material, including leaves, branches and pollen from vegetation growing on drier land outside or inside the basin. These reeds and grasses, together with the accumulation of pollen, leaves and miscellaneous debris forming drift deposits in the open water, have been preserved as light layers in the lignite deposits. Accumulation of these light layers persisted during conditions of continued subsidence."

(e)"In the absence of further subsidence, the swamp gradually dried out to give conditions for the development of brushwood and forest trees, again forming lignite."

The Czechoslovakian brown coals are commercially important because they yield, on benzene extraction, a wax called Montan Wax. This wax is not peculiar to the Czechoslovakian brown coals and is also obtained from, for example, American, German and Nigerian lignites[84]. It is used extensively in the paper, plastics and rubber industries and is a constituent of some leather dressing materials and wood polishes. Some electric cable waxes, battery insulators, condenser insulators and electric line insulators also contain varying proportions of Montan Wax.

Since European lignite is a relatively unaltered deposit containing a wide variety of identifiable plant remains it is hardly surprising

that a number of biological and biologically-derived compounds have been isolated from it. Czechoslovakian Montan Wax (Bohemia) has been examined by Šorm and his colleagues. The normal alkanes, alcohols and acids[75, 85, 86] of the wax show a carbon number distribution clearly biological in origin. The n-alkanes range from C_{22} to C_{33} and show a marked odd/even preference with a maximum at C_{29}. Likewise the n-acids (both free and bound) range from C_{22} to C_{34} and show an even/odd predominance with a maximum at C_{28}. The normal primary alcohols also have an even/odd predominance and range from C_{22} to C_{32} with a maximum at C_{28}. These distributions are reminiscent of most living plants[62]. Later the range of n-alkanes was shown to be from C_{12} to C_{37}, the n-acids from C_{10} to C_{36} and the n-alcohols from C_{10} to C_{36}[87]. Three other homologous series, viz. n-alk-1-enes (C_{11} to C_{36}) and n-trans disubstituted alkenes (C_{14} to C_{31} and C_{11} to C_{40}) were also identified[88].

The above workers, by examining large quantities of wax have also succeeded in isolating and identifying a number of triterpenes[89,90], including friedelin (36); friedelin-3β-ol (37); R^1=OH, R=H, friedelin-3α-ol (37); R=OH, R^1=H, betulin (38), oxyallobetulin (39), allobetul-2-ene (40); R=H_2, oxyallobetul-2-ene (40); R=O, α-apoallobetulin (41); R=H_2, apo-oxyallobetulin (41); R=O. A number of aromatic compounds, including two unidentified chrysenes, an unidentified phenanthrene[89] and two compounds (42),(43) derived from allobetulin[90] have also been isolated. In addition, the wax contains a number of pentacyclic aromatic hydrocarbons[91] presumably derived from triterpenes.

Small amounts of dimethyl alkanes have been tentatively identified in Bohemian Montan Wax from their retention data which were obtained from a gas chromatographic examination of the total alkane fraction of the wax[92]. Later, the range of dimethyl alkanes was shown to extend from C_{16} to C_{35}[87]. In this instance the total alkane fraction was separated into a normal and a branched-cyclic fraction by means of 5Å molecular sieve. Tentative identification was again made from a study of their gas chromatographic retention data. Similar homologous series of dimethyl acids and alcohols appear to be present in the wax[87]. At present the exact points of branching in these compounds are not known since the identifications are tentative, but the authors maintain that the two methyl groups are near the ends of the chain in each case. It is possible, however, that they are monomethyl alkanes in which the methyl group is near the centre of the chain.

Hydrogenation at room temperature of the small alkene fraction present in Czechoslovakian Montan Wax also afforded a series of dimethyl paraffins and alcohols ranging from C_{10} to C_{35} and a series of dimethyl acids ranging from C_{13} to C_{35}. Normal and iso-paraffins, alcohols and acids were also obtained upon hydrogenation.

It is not the author's intention to review the chemistry of the other Montan Waxes since the topic is the subject of an excellent review by Wollrab and Streibl[93].

DISCUSSION

The two samples of Czechoslovakian lignite* examined herein were obtained from an exposed seam near Karlsbad, N.W.Bohemia. This zone is situated about seven miles from the main brown coal deposit of N.W. Bohemia and has been dated from an examination of the faunal content of the two different stratigraphic horizons associated with it. The lower is a sequence bearing volcanic rocks and, according to Wenz[94] is of Chattian age(Upper Oligocene). The other is a higher succession of upper Aquitanian-lower Burdigalic age[95] (lower Miocene). The coal deposit is therefore of upper Oligocene - lower Miocene age (ca. 25-30 x 10^6 yrs.). The following flora have been identified in the seam: Abacopteris styriaca; Libocedrus salicornioides; Magnolia sp.; Salix cf. angusta; Alnus sp.; cf. Brasenia sp.; Cinnamomophyllum lanceolatum; Lauraceae; Ulmus cf. fischeri; Zelkova vel Ulmus sp.; Carpinus grandis; Ostrya atlantides; cf. Rhus; Juglandaceae; Stratiotes cf. neglectus; cf. Typha latissima[96].

The Brown and Yellow (so-called "pyropissite") coal samples were pulverised and extracted ultrasonically with a mixture of benzene and methanol in order to isolate their geolipid fractions. It was found that the weight of the geolipid fraction of the Yellow Coal was approximately three times that of the corresponding fraction of the Brown Coal. This is in accordance with the fact that "pyropissite"

* Kindly supplied by Dr.V.Wollrab, Czechoslovak Academy of Science, Prague.

lignites contain a greater proportion of plant remains than brown lignites[84]. The alkane fraction of each lignite sample was obtained from the geolipid fraction by column and preparative-scale thin-layer chromatography. The gas chromatographic records (Figs.1 and 2) of both alkane fractions are similar and show a marked odd/even preference of the normal alkanes and predominance of the higher molecular weight normals, both of which are typical of contemporary plant waxes[62].

Treatment of each alkane fraction with molecular sieve afforded a normal and a branched-cyclic fraction. The distributions of both n-alkane fractions (Figs.1 and 2) are again similar and agree with the n-alkane distribution reported by Šorm et al for Bohemian Montan Wax. Once again there can be seen a marked odd/even preference and a dominance of the higher molecular weight components, the dominant alkanes being n-C_{27}, n-C_{29} and n-C_{31} in both cases. In fact the C.P.I. values are approximately 6 and 3 for the Yellow Coal and Brown Coal n-alkane fractions respectively in contrast to those of ancient sediments which generally have a C.P.I. of unity. The approximate percentage of each n-alkane in the n-alkane fractions is given in Table 1. The marked tailing apparent in both chromatograms at the high molecular weight end is probably indicative of incomplete sieving, and it is likely that there is some cyclic material present. The gas chromatographic records of the branched-cyclic fractions are also shown in Figures 1 and 2. Both distributions are similar and are not as complex as those of the branched-cyclic hydrocarbon fractions of most

ancient sediments. Co-injection of each fraction with pristane and phytane indicated that these hydrocarbons are either absent or present in very small amounts. This finding is again in contrast to the fact that pristane and phytane appear to be ubiquitous, and often abundant, components of ancient sediments. In the case of the Yellow and Brown Coals it appears that diagenesis has not degraded the chlorophyll present at the time of deposition to any great extent. The branched-cyclic fractions were subjected to preparative-scale gas chromatography and the major components of each fraction were collected. Figure 3A shows the preparative gas chromatogram of the Yellow Coal branched-cyclic fraction and fractions were collected as shown, whilst Figure 3B shows the gas chromatographic records of fractions A and B upon injection into the gas chromatograph-mass spectrometer. Mass spectra were recorded at the points indicated and the mass spectra of these two components are shown in Figure 4. The mass spectra recorded thus of the major components of both branched-cyclic fractions showed them to be cyclic terpene hydrocarbons.

In the gas chromatogram (Fig.1) of the Yellow Coal branched-cyclic alkane fraction the fractions labelled A, B and C are diterpene hydrocarbons. The mass spectra of fractions A and B (Fig.4) show that they are saturated tricyclic diterpenes of formula $C_{19}H_{34}$. The mass spectrum of fraction B has an abundant ion at m/e 219 indicating the loss of a C_3 fragment which was thought to be due to an isopropyl group. Accordingly the mass spectrum (Fig.4) of a sample of fichtelite*(44)

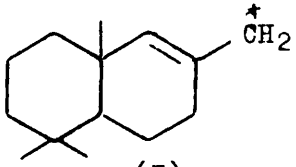
* Kindly supplied by Prof. A.W.Burgstahler, Chemistry Department, University of Kansas, Lawrence, Kansas.

was recorded under the same conditions in the gas chromatograph-mass spectrometer. The two spectra are identical except for a small impurity at m/e 276 (corresponding to $C_{20}H_{36}$) in the mass spectrum of fraction B. The micro-scale infra-red spectra of fichtelite and fraction B (Fig.5) are also identical. Unfortunately the spectra are somewhat low in intensity since only about 20µg of fraction B were available and the spectrum of fichtelite was run at approximately the same concentration for comparison purposes. Also co-injection of a standard solution of fichtelite and n-C_{18} alkane with fraction B on a capillary column coated with 7 ring m-polyphenyl ether showed enhancement of the fichtelite peak in the gas chromatogram. Fichtelite is a common component of fossil tree resins[93]. The mass spectrum of fraction A (Fig.4) has a very weak ion at m/e 219, unlike that of fichtelite, but has instead an abundant ion at m/e 233 indicating a loss of a C_2 fragment from the parent ion. It appears, therefore, that fraction A, although isomeric with fichtelite, has an ethyl and a methyl group in the molecule in place of an isopropyl group. Fraction C was shown to consist of two components, namely $C_{20}H_{34}$ and $C_{20}H_{36}$, which could not be further separated. The latter appears to be a tricyclic saturated diterpene whereas the former is most likely a tetracyclic saturated diterpene.

Comparing the Yellow and Brown Coal branched-cyclic fractions in the diterpene region, fraction A' in the gas chromatogram of the Brown Coal branched-cyclic alkane fraction has a mass spectrum which shows the parent ion at m/e 260, corresponding to $C_{19}H_{32}$. This compound also appears to be a tetracyclic diterpane.

The mass spectrum of fraction C' is identical to that of fraction C in the gas chromatogram of the Yellow Coal branched-cyclic alkane fraction and again contains two components, namely $C_{20}H_{34}$ and $C_{20}H_{36}$.

Turning to the higher molecular weight end of the chromatograms of the branched-cyclic fractions, the mass (Fig.6) and infra-red spectra of fraction D in the Yellow Coal alkane fraction are identical to those of fraction D' in the Brown Coal alkane fraction and the mass spectra are of the pentacyclic triterpane type with the molecular ion at m/e 370, corresponding to $C_{27}H_{46}$. Likewise fraction E in the Yellow Coal alkane fraction has mass (Fig.6) and infra-red spectra identical to those of fraction E' in the Brown Coal branched-cyclic alkane fraction and the mass spectra show the molecular ion to be at m/e 426, corresponding to $C_{31}H_{54}$. The mass spectra were also recorded using the direct inlet system of the mass spectrometer and perfluorokerosene was added to confirm that the parent ions are indeed at m/e 426. All of the triterpane spectra exhibit abundant ions at m/e 191 and in fact this is the base peak in the spectra of the C_{31} compound. This ion is an extremely ubiquitous one in the spectra of pentacyclic triterpanes and corresponds to (I)[97,98]. It is the most characteristic mass spectral fragmentation product of such compounds although it is not always the base peak.



m/e 191

(I)

C_{31} pentacyclic triterpenes are at present unknown in the plant world although C_{31} tetracyclic triterpenes [e.g. eburicoic acid (45)], are known. There remains the possibility that the above presumed C_{31} pentacyclic triterpane is in fact tetracyclic with a highly substituted or hindered double bond although there is no evidence for this in the infra-red spectra and both total alkane fractions were purified by silver ion thin-layer chromatography. If this compound is indeed a C_{31} pentacyclic triterpane, as seems likely from its mass spectrum, it is possible that this skeleton existed in the plants at the time of deposition. Alternatively, a carbon addition reaction could have taken place during diagenesis. Dr. Max Blumer* has recently reported the isolation of geological porphyrins which appear to have been formed by carbon addition reactions.

The C_{27} pentacyclic triterpane in both lignite samples possibly represents an intermediate (where the angular methyl groups are lost) in the formation of an aromatic pentacyclic hydrocarbon since polycyclic aromatic hydrocarbons presumably derived from both diterpenes and triterpenes are common constituents of sediments and crude oils. Alternatively carbon atoms could be lost during diagenesis from an oxygenated C_{30} triterpene by, for example, decarboxylation.

Šorm and his colleagues have isolated an unidentified C_{30} triterpane with melting point 227°C from Czechoslovakian Brown Coal[89]. A number of pentacyclic triterpanes, including four with molecular formulae $C_{27}H_{46}$, $C_{29}H_{50}$ and two with molecular formula $C_{30}H_{52}$ have been

* Personal communication

isolated from a crude oil by Hills and Whitehead[99]. These workers have also identified the optically active triterpane gammacerane (45) in the Green River Oil Shale bitumen[67] and this is the only recorded, complete identification of a pentacyclic triterpane from a geological sample. In addition, other unidentified triterpanes are present in the Green River Shale[68]. The presence of triterpanes in the Soudan Iron Formation has also been reported[66]. The difficulty in identifying these compounds on the micro-scale is mainly due to the fact that most of them are not naturally occurring since they presumably derive from oxygenated plant triterpenes. There is, therefore, at present a lack of standards available for comparison. On the micro-scale it is also difficult to purify and characterise triterpanes from geological samples.

Hills and Whitehead have briefly reviewed the occurrence of terpenes in the plant kingdom and state:- "Bacteria do not appear to contain pentacyclic triterpenes and only rarely steroids. The algae, however, which dominate marine flora and form part of the division thallophyta, contain an abundance of phytosterols but apparently few pentacyclic triterpenes. The remaining two classes, fungi and lichens, in the division thallophyta are sometimes regarded as transition stages, in adaption of plant forms to a terrestrial environment. The preferred phytosterols in fungi appear to be based on lanosterol and no pentacyclic triterpenes appear to have been isolated from this class. Lichens on the other hand carry both sterols and pentacyclic triterpenes, and examples based on ursane (47), hopane (48) and rearranged oleanane systems have

been reported. Triterpenes based on gammacerane (46) and hopane along with phytosterols have been identified in the plant kingdom divisions bryophyta and pteridophyta. The gymnosperms, which dominated the mesozoic era (65-225 x 10^6yrs.) with the pteridophytes, contain phytosterols with an abundance of diterpenes; pentacyclic triterpenes, however, have been found infrequently in this division. The angiosperms appeared around the Cretaceous period (70-136 x 10^6 yrs.) of geological time and rapidly achieved the dominance that they maintain to the present day. This vast division generates phytosterols, simple and complex triterpenes in abundance and considerable variety."

The great similarity in the gas chromatograms of the branched-cyclic and normal alkane fractions of the Yellow and Brown Coals provides chemical evidence that the plant types contributing to both coal samples were the same or very similar. Also the high terpene content of both branched-cyclic hydrocarbon fractions agrees with the fact that the plant types identified in the seam are almost entirely angiosperms which have a high terpene content in comparison to other plant types. The dimethyl alkanes, presumed to be present in the sample of Montan Wax examined by Sorm and his co-workers can only be present in very small amounts in the above two coal samples. Table 2 summarises the compounds identified in the samples of the Yellow and Brown Coals examined herein and work is proceeding with the structural elucidation of these and the other hydrocarbons present.

EXPERIMENTAL

Examination of the Yellow "pyropissitic" Coal and Brown Current Wax Coal. Extraction of organic matter and isolation of alkanes.

Two samples (30g. each) of the waxy coals were broken into small pieces (ca 1/4 in. or less in diameter) and pulverised in the ball mill in 10g. batches (2 min. each batch). The resulting powdered coals were extracted ultrasonically in n-hexane (80ml.) for 30 min. The extraction was repeated six times. Evaporation of the hexane (Buchi) gave the two extracts (1.3g. from the Yellow Coal and 0.4g. from the Brown Coal) as yellow gums. The Brown Coal extract was lighter in colour than the Yellow Coal extract. The i.r. spectrum (film) of the Yellow Coal extract had absorption at 1710 (ν C=O), 1463 (δCH_2, CH_3), 1375 (δCH_3), and 720cm.$^{-1}$ ($-(CH_2)_n$-rock). The Brown and Yellow Coal extracts were chromatographed on alumina (30g. and 8g. respectively) and both total n-hexane eluates collected. The eluates were monitored by t.l.c. on silica/silver nitrate (iso-octane developer) with a standard n-alkane mixture to ensure that all of the alkanes had been eluted. The remainder of the extracts were eluted with ether. Preparative-scale t.l.c. (conditions as for analytical t.l.c.) gave the two total alkane fractions (22mg. and 6mg.) respectively.

Examination of the alkanes.

G.l.c. on 3% SE-30 (Fig.1 and 2) showed both alkane fractions to be composed almost entirely of n-alkanes with a maximum at n-C_{29} in each case. The g.l.c. records of both fractions are almost identical

and have a predominance of the higher m.wt. alkanes with the odd/even preference characteristic of most living plants[62]. The positions in both traces of the n-C_{32} and n-C_{34} alkanes were checked by co-injection of the alkane fractions with these standards. As a further check certain of the Brown Coal alkanes were examined by GC-MS (column 10 ft. x 3mm.; 1% SE-30 on Gas Chrom P. 100-120 mesh, acid-washed and silanized; programmed from 100-250°C at 6°/min.) and the n-C_{25}, n-C_{26}, n-C_{27} and n-C_{28} alkanes identified by their mass spectra on comparison with standard spectra. The Yellow Coal alkanes (21mg.) were sieved by heating under reflux with 5Å molecular sieve (0.8g.) in iso-octane (8ml.) for 18 hours. The normal alkanes (15mg.) and the branched-cyclic alkanes (3mg.) were obtained by working up the sieve in the usual manner. Likewise, after heating under reflux with 5Å molecular sieve (0.4g.) the Brown Coal alkanes (6mg.) afforded an n-alkane fraction (2mg.) and a branched-cyclic fraction (1.5mg.). The g.l.c. records of the n-alkane fractions are very similar, but not identical (Figs.1 and 2). Although both chromatograms have a pronounced odd/even preference of the higher m.wt. alkanes and a maximum at n-C_{29}, the Yellow Coal alkanes have a slightly greater proportion of lower m.wt.normals. The n-alkanes of the Brown Coal were seen to extend from about C_{17} to C_{33} and the Yellow Coal alkanes from about C_{14} to C_{33}. The g.l.c. records of the branched-cyclic alkane fractions are also similar (Figs.1 and 2), but there is more of a bias towards the higher m.wt. alkanes in the Brown Coal branched-cyclic fraction. The fractions labelled in both chromatograms

were collected in the usual manner and examined in the gas chromatograph-mass spectrometer. The mass spectra showed all of the fractions labelled in both chromatograms to be cyclic terpene hydrocarbons. The fraction labelled fichtelite in Figure 1 C. had an identical mass spectrum and infra-red spectrum to a sample of authentic fichtelite. The infra-red spectra (Fig.5) of the isolated and authentic fichtelite were run in the micro-cell (0.5mm.) using solutions in CCl_4 (ca. 20µg. in 2.5µl.) and show absorption at 1385 (s, δCH_3; sym.) and 1375cm.$^{-1}$(m, δCH_3; sym.) from the gem dimethyl group present. Co-injection of a standard solution of fichtelite and n-C_{18} alkane with the isolated fichtelite showed enhancement of the peak due to the former (column 50m. x 0.25mm. capillary; 26,000 plates (checked by injecting n-C_{18} alkane); coated with 7 ring m-polyphenylether; programmed from 150° to 200°C at 4% min.).

TABLE 1

n-Alkanes of the Brown and Yellow Coals

Carbon Number	% (calculated as peak areas in the gas chromatogram[a])	
	Yellow Coal	Brown Coal
14	< 1	
15	< 1	
16	< 1	
17	< 1	
18	< 1	
19	2	< 1
20	1	< 1
21	3	< 1
22	3	< 1
23	7	4
24	5	2
25	8	7
26	6	3
27	15	18
28	5	4
29	27	30
30	5	3
31	11	21
32	< 1	2
33	2	7

TABLE 2

Molecular Species Identified in the Branched-Cyclic Alkane Fractions of the Yellow and Brown Coals.

G.l.c. Peak	Molecular Formula	Probable Structural Type	Comments
A	$C_{19}H_{34}$	Tricyclic diterpane	Contains an an ethyl group
A'	$C_{19}H_{32}$	Tetracyclic diterpane	
B	$C_{19}H_{34}$	Tricyclic diterpane	Fichtelite
C, C'	$C_{20}H_{34}$, $C_{20}H_{36}$	Tetracyclic, tricyclic diterpane	
D, D'	$C_{27}H_{46}$	Pentacyclic triterpane	
E, E'	$C_{31}H_{54}$	Pentacyclic triterpane	

Fig.1

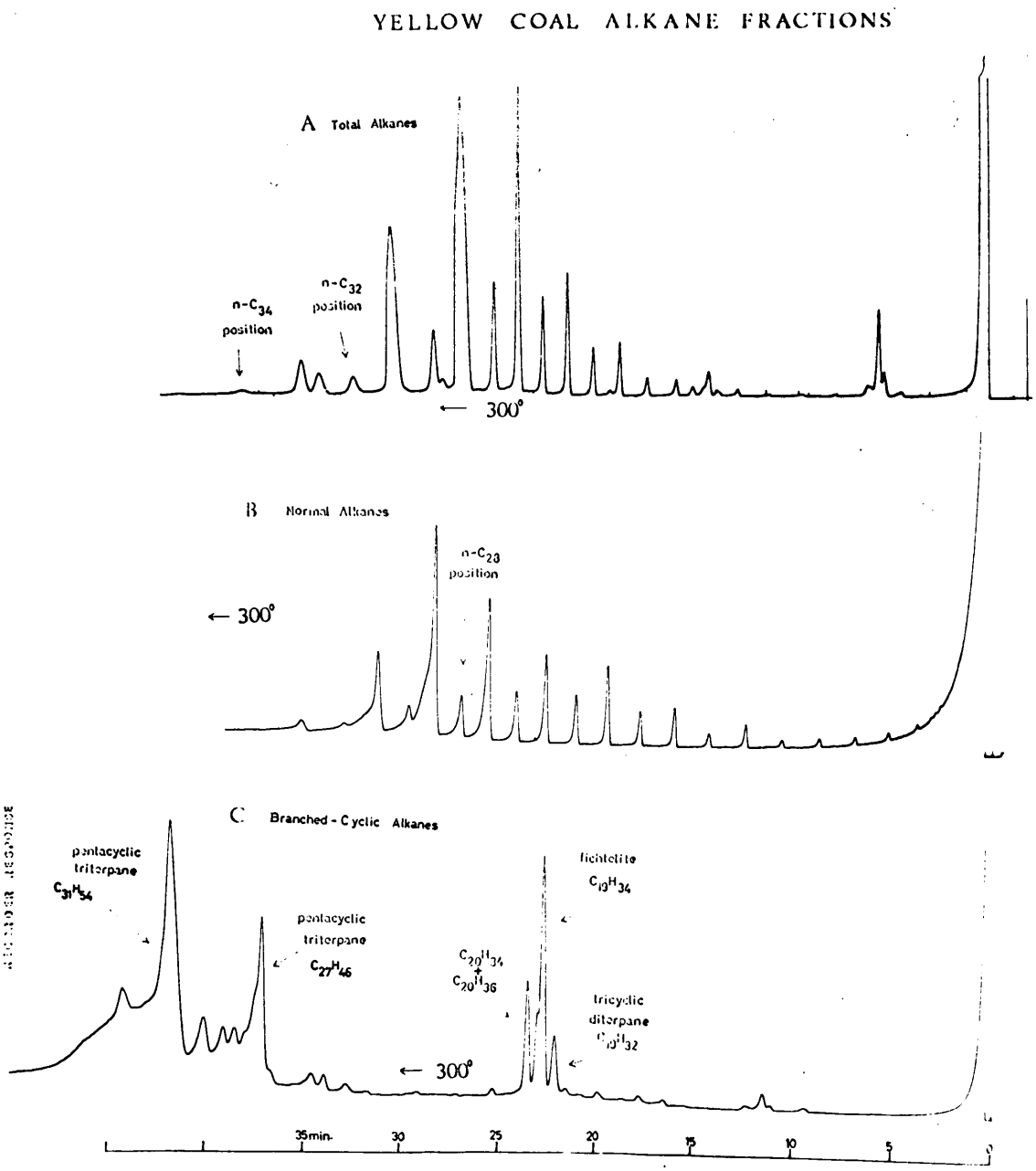

YELLOW COAL ALKANE FRACTIONS
A Total Alkanes
n-C34 position
n-C32 position
← 300°
B Normal Alkanes
n-C28 position
← 300°
C Branched - Cyclic Alkanes
pentacyclic triterpane C31H54
pentacyclic triterpane C27H46
C20H34
C20H36
fichtelite C19H34
tricyclic diterpane C19H32
← 300°
35min.
30
25
20
15
10
5

Fig.1 Legend

Gas chromatograms of Yellow Coal alkanes. Conditions:

A. Column 10 ft. x 1/16 in; 3% SE-30 on Gas Chrom P; approximately 20ml./min. nitrogen at 16 p.s.i.; temperature programmed from 140° to 300°C at 6°/min.; sample size 0.3µl. of a solution in hexane; attentuation 1×10^2.

B. Column 10 ft. x 1/16 in; 3% OV-1 on Gas Chrom Q; 20 ml./min. nitrogen; temperature programmed from 100° to 300°C at 5°/min; sample size 0.3µl. of a solution in iso-octane; attenuation 5×10^2.

C. Column 10 ft. x 1/16 in.; 3% SE-30 on Gas Chrom P; 20 ml./min. nitrogen; temperature programmed from 150° to 300°C at 5°/min.; sample size 9µl. (split) of a solution in benzene; attenuation 1×10^2.

Fig.2 Legend

Gas chromatograms of Brown Coal alkanes. Column conditions:-

A. as for Fig.1A; sample size 0.2µl. of a solution in n-hexane

B. as for Fig.1B.

C. as for Fig.1C; sample size 8µl. of a solution in benzene; attenuation $5 \times 10^2 \dashrightarrow 2 \times 10^2$.

Fig.2

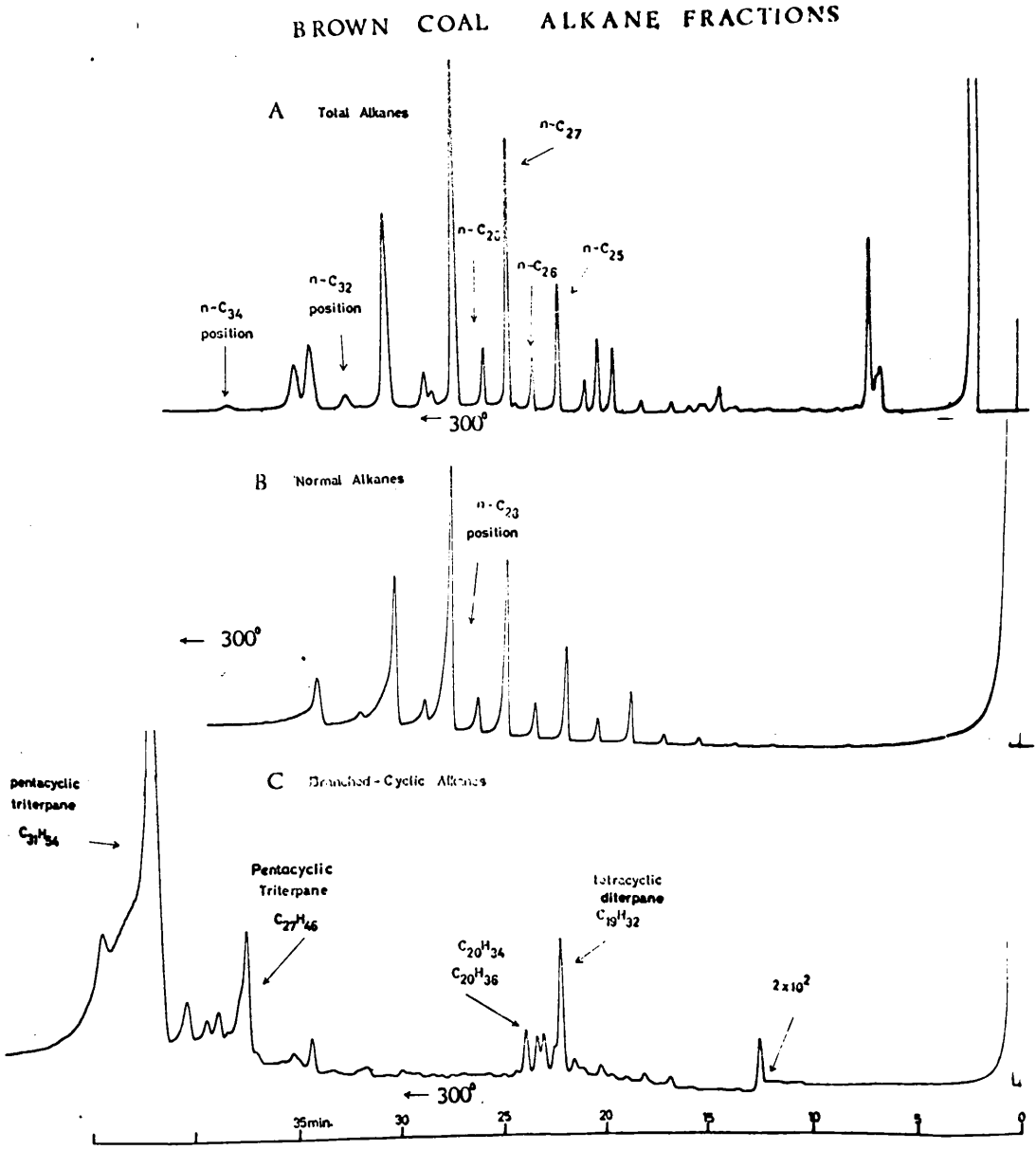

BROWN COAL ALKANE FRACTIONS
A Total Alkanes
n-C27
n-C29
n-C26
n-C25
n-C34 position
n-C32 position
← 300°
B Normal Alkanes
n-C23 position
← 300°
C Branched-Cyclic Alkanes
pentacyclic triterpane
C31H54
Pentacyclic Triterpane
C27H46
C20H34
C20H36
tetracyclic diterpane
C19H32
2x10²
← 300°
35min 30 25 20 15 10 5 0

Fig.3 Legend.

A. Preparative gas chromatogram of Yellow Coal branched-cyclic alkane fraction. Fractions collected at the points shown.

Conditions: column 20ft. x 1/8in.; 3% SE-52 on Chromosorb W, 80-100 mesh; flow rate 60 ml./min. helium; temperature programmed from 100° to 300°C at 6°/min., sample size 10 µl. of a solution in benzene; attenuation 1 x 1.

B. G.C.-M.S. runs of fractions A and B from Yellow Coal branched-cyclic alkane fraction.

Conditions: column 10ft. x 3 mm.; 1% SE-30 on Gas Chrom P, 100-120 mesh; flow rate 30 ml./min. helium; sample size 5 µl. of a solution in n-hexane; mass spectra recorded at the points shown.

Fig.3.

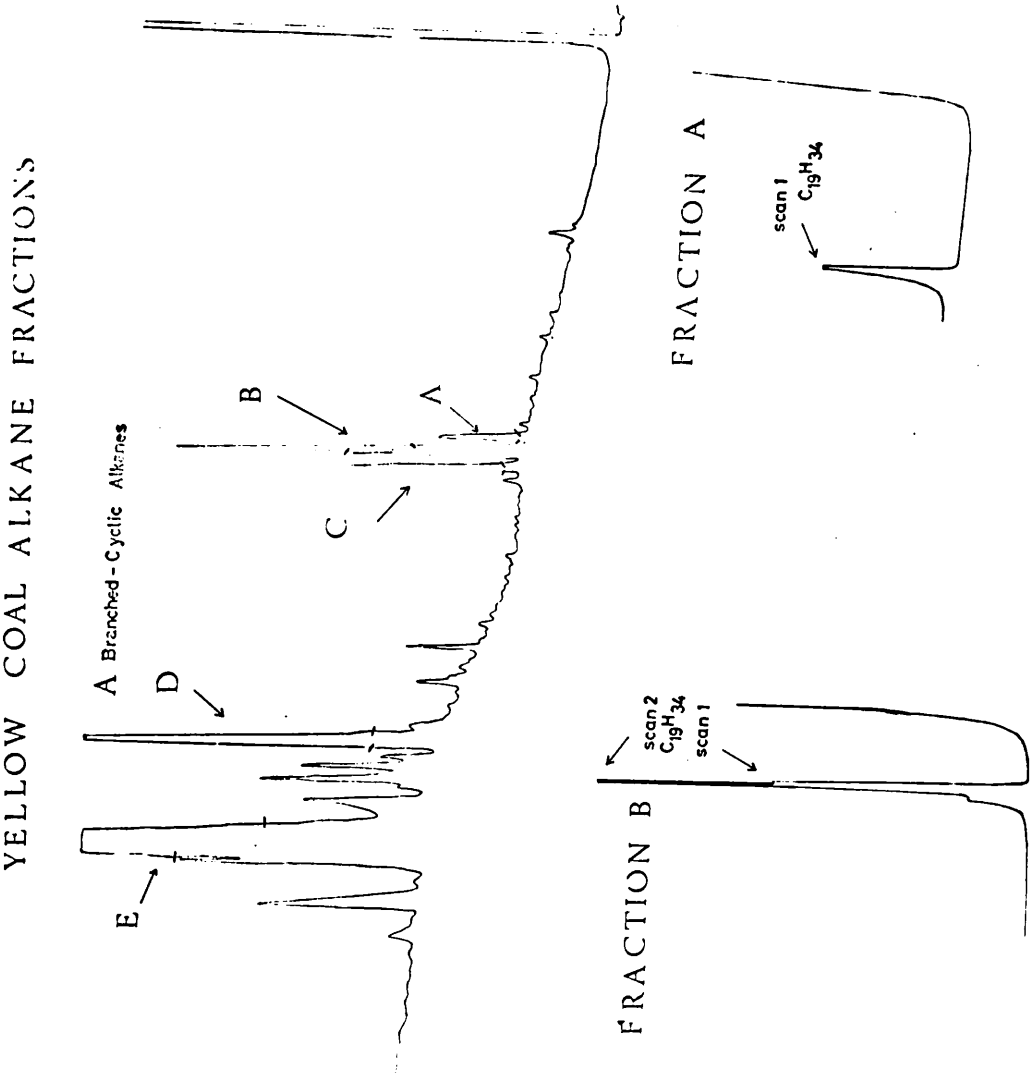
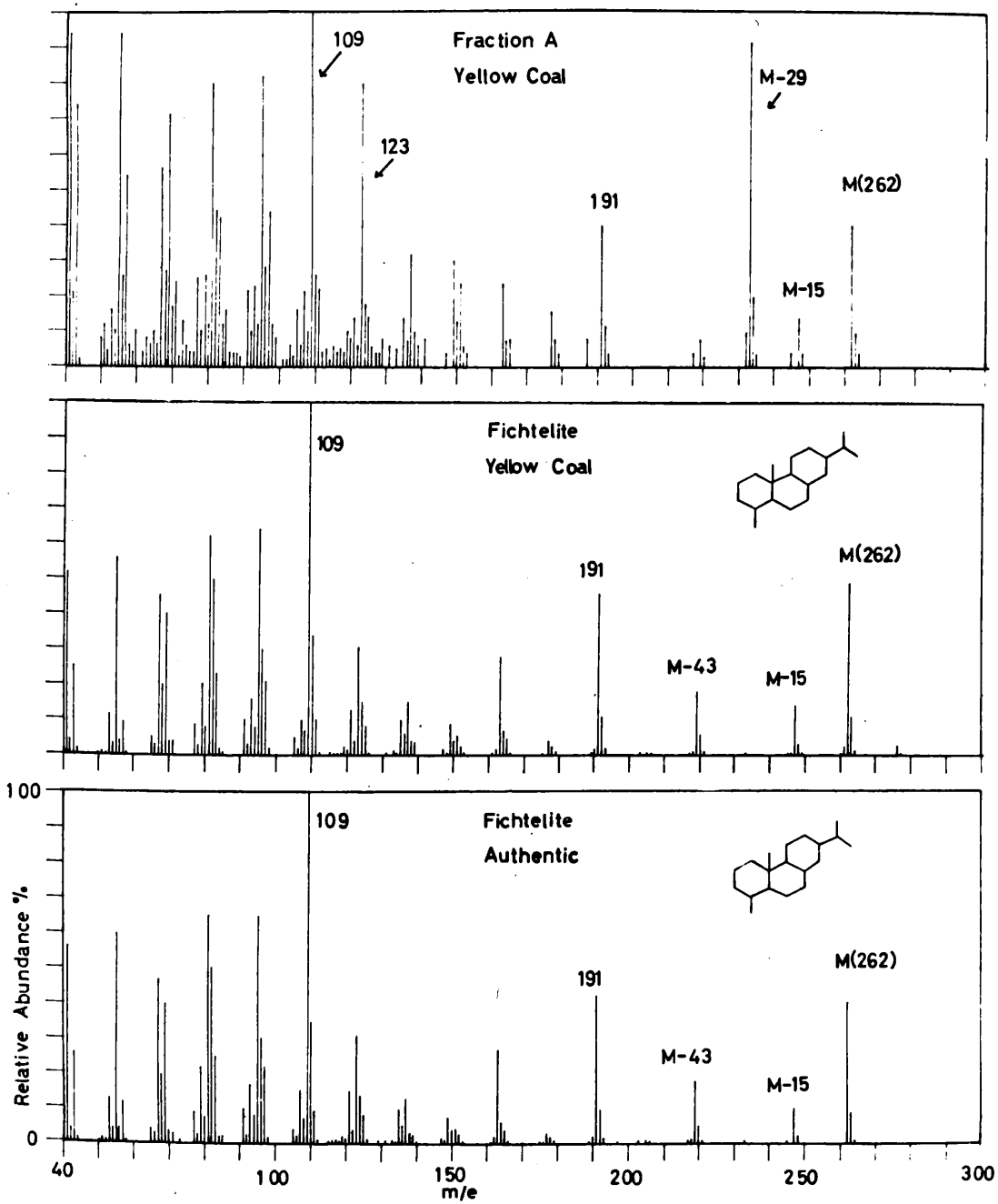

YELLOW COAL ALKANE FRACTIONS
A Branched-Cyclic Alkanes
A
B
C
D
E
FRACTION A
scan 1
$C_{19}H_{34}$
FRACTION B
scan 2
$C_{19}H_{34}$
scan 1

Fig.4.

Mass Spectra of Diterpene Hydrocarbons



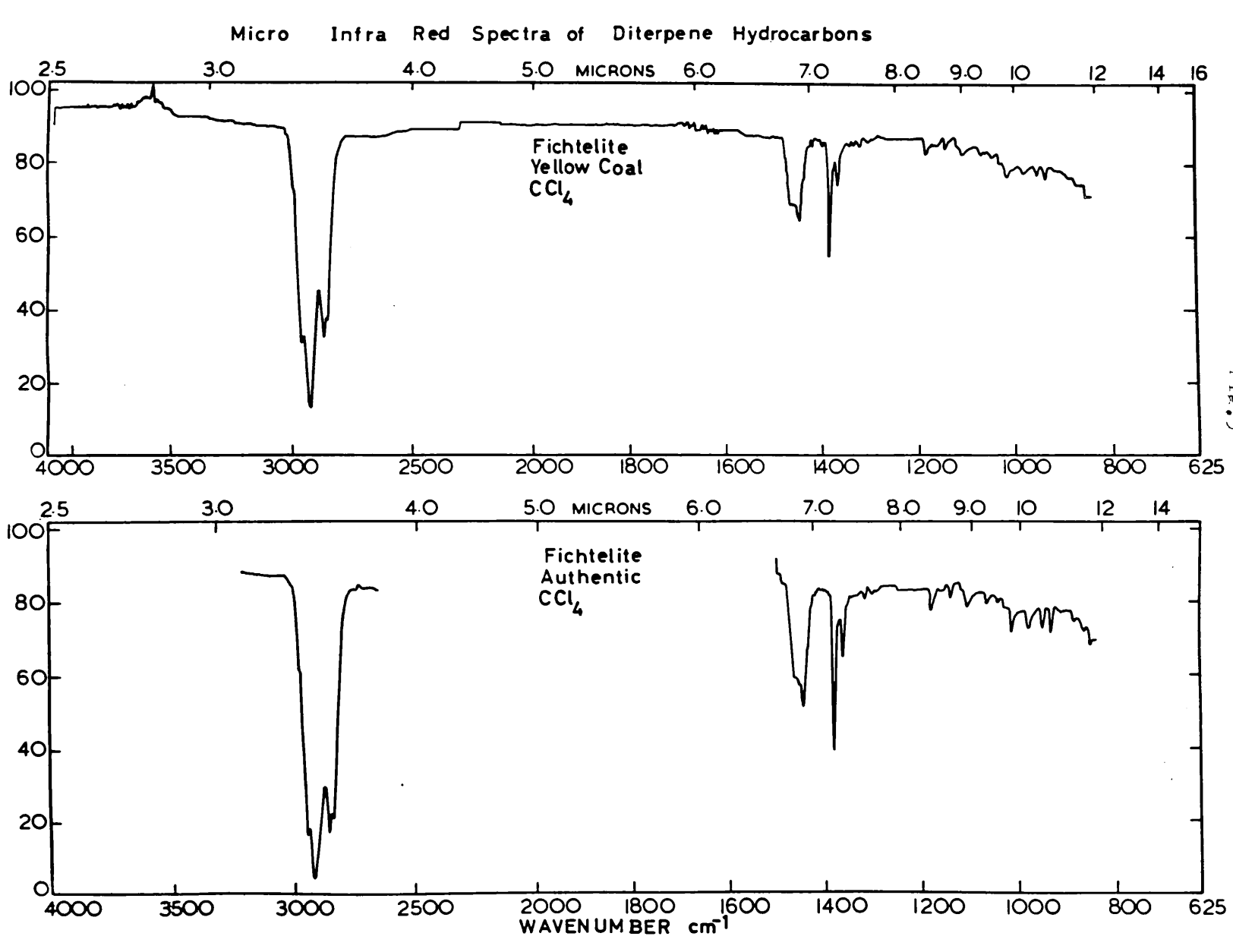
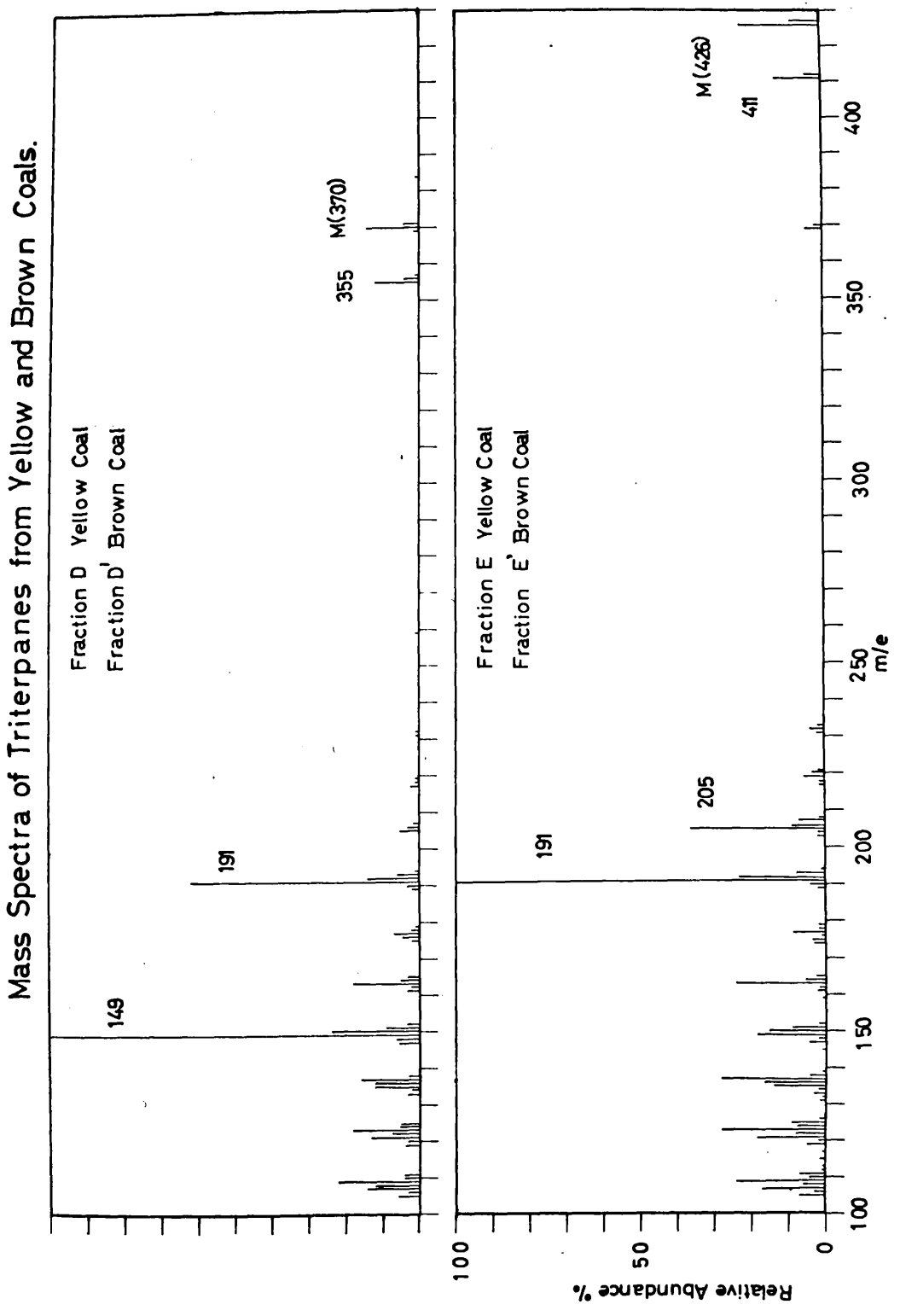
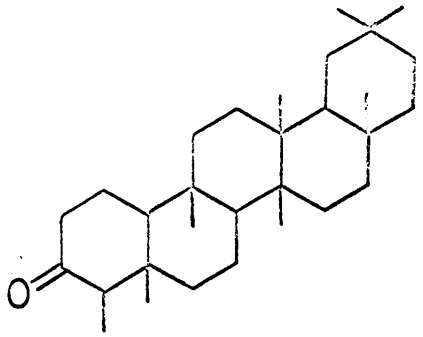

Micro Infra Red Spectra of Diterpene Hydrocarbons
2.5 3.0 4.0 5.0 MICRONS 6.0 7.0 8.0 9.0 10 12 14 16
100 80 60 40 20 0
Fichtelite
Yellow Coal
CCl4
4000 3500 3000 2500 2000 1800 1600 1400 1200 1000 800 625
2.5 3.0 4.0 5.0 MICRONS 6.0 7.0 8.0 9.0 10 12 14
Fichtelite
Authentic
CCl4
4000 3500 3000 2500 2000 1800 1600 1400 1200 1000 800 625
WAVENUMBER cm-1

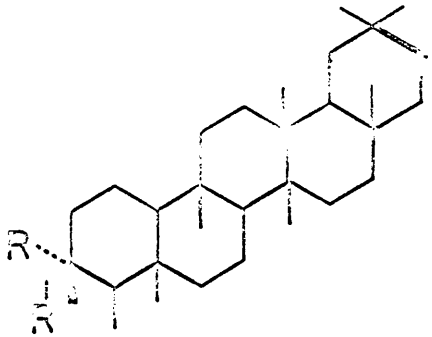
Fig. 5

Fig.6.

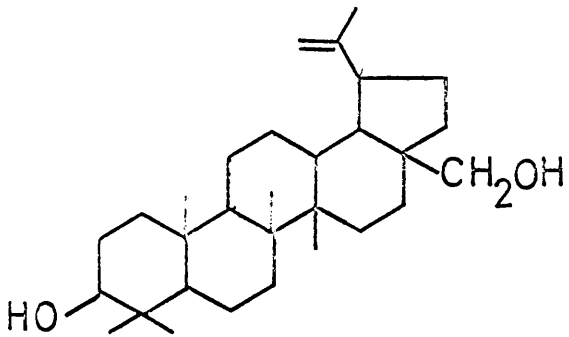

Mass Spectra of Triterpanes from Yellow and Brown Coals.
Fraction D Yellow Coal
Fraction D' Brown Coal
149
191
355
M(370)
Fraction E Yellow Coal
Fraction E' Brown Coal
191
205
411
M(426)
Relative Abundance %
100
50
0
100
150
200
250
300
350
400
m/e



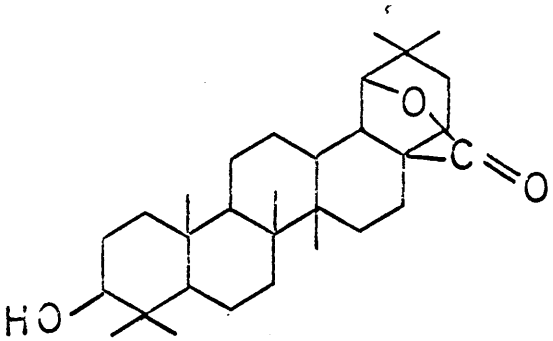
(36)



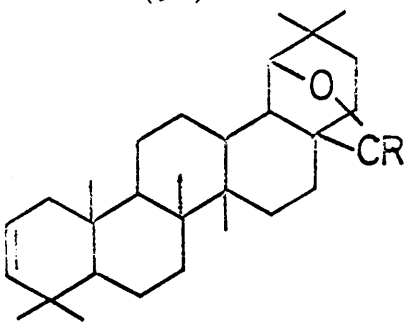
(37)



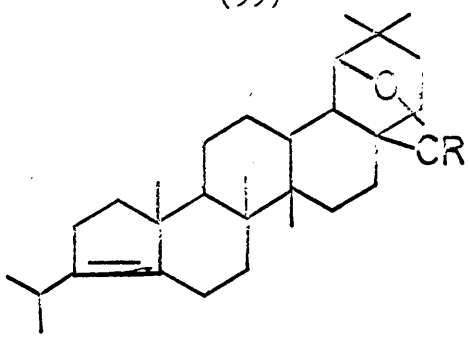
(38)



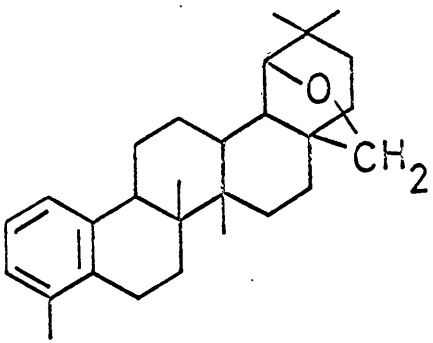
(39)



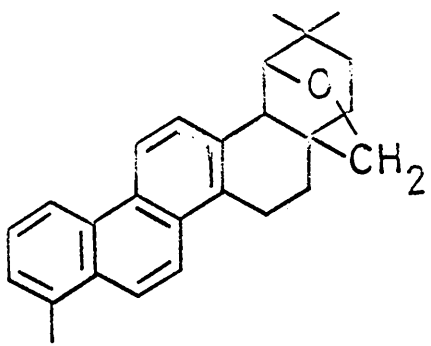
(40)



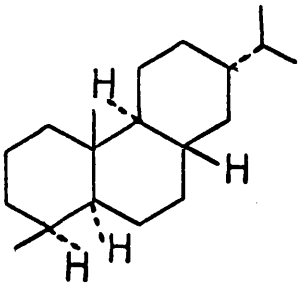
(41)



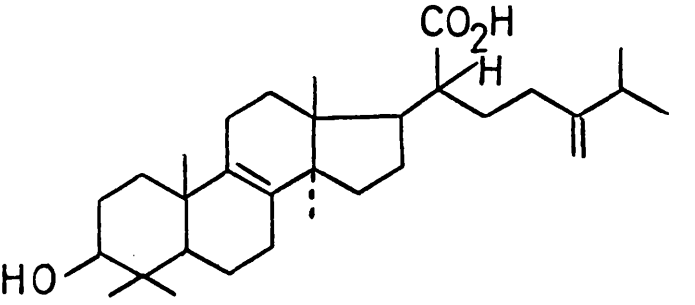
(42)



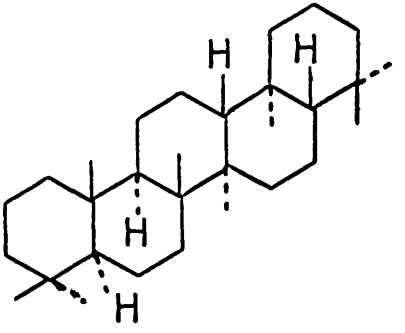
(43)



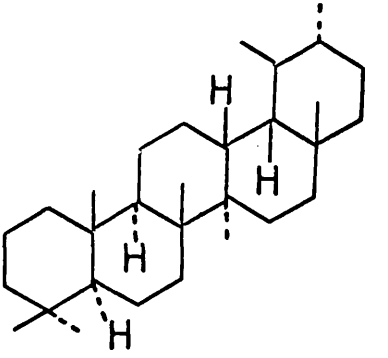
(44)



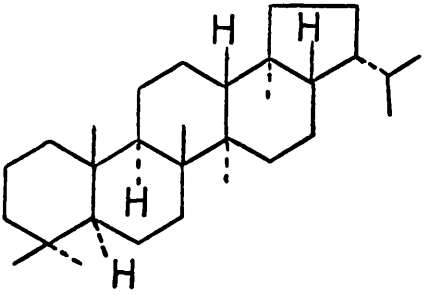
(45)



(46)



(47)



(48)

S E C T I O N I I

THE HYDROCARBONS OF CERTAIN SAMPLES FROM THE SCOTTISH CARBONIFEROUS FORMATION (250-300 x 10^6 YRS.).

INTRODUCTION

Torbanite was first discovered at Torbanehill, near Bathgate, Scotland. When viewed under the microscope, Torbanite is seen to consist principally of minute yellow globules, called "yellow bodies" (Fig.1). Torbanite has evinced widespread geological interest because there has been great controversy about the origin of these "yellow bodies" (See Section IV); most geologists and botanists now believe that the "yellow bodies" are the remains of the alga Botryococcus Braunii. Markedly similar sediments having the same "yellow bodies" are also found in New South Wales, Australia and at Autun, France.

In 1851 James Young ("Paraffin" Young) erected a works at Bathgate for the commercial distillation of Torbanite since it was known to be very rich in organic matter. Lubricants, naphtha (solvent in rubber and paint manufacture) and burning oil were soon put on the market. The deposit was exhausted in 1862. However, from these beginnings emerged the Scottish oil-shale industry which continued until a few years ago.

Although consisting principally of the "yellow bodies", Torbanite also contains a very small proportion of plant debris. According to McGregor[100] the sediment was formed in lakes or pools among the swamp forests of Carboniferous times. These lakes or pools were presumably

surrounded in many cases by a fringe of swamp vegetation which prevented the deposition of all but the finest of sediment. The sediment thus deposited would have consisted almost entirely of the remains of aquatic plants (presumably the above-mentioned algæ) which grew in the lakes or pools. Dulhunty[101] has given a similar but more detailed account of the deposition of the New South Wales Torbanite. He concludes that the algæ was preserved because, "after burial, polymerisation of the algal substance and the weight of overlying beds, changed the deposits to relatively hard, compact material". After deposition, the sediment became buried under a pressure of up to 9,000 lb. per square inch and temperatures of 38 to 65°C.

The cannel coals, which are similar to Torbanite, are thought to have been formed in a likewise fashion but in these cases there were rivers nearby which carried some plant debris into the lakes.

The Scottish Oil-Shale Group occurs in the lower part of the Carboniferous Formation and was laid down at an earlier period than Torbanite and the cannel coals. With reference to the deposition of the oil shales, McGregor[100] has stated that "The finely laminated structure of the oil shales themselves, their faunal and floral content and the nature of the sediments associated with them all indicate deposition in the waters of shallow inland lagoons. The fine sediment which was swept into these lagoons was laid down as thin layers of mud, and brought with it varying amounts of comminuted plant debris (fragments of woody tissue, microspores, parts of leaves, etc.). To this vegetable content

were added the remains of the algal colonies of the lagoons themselves". This is in keeping with the fact that the Oil-Shale Group does not contain "yellow bodies" to the same extent as Torbanite and the cannel coals. Also present in the oil shales are minute carbonised fragments of plants, occasional microspores, shells of minute crustaceans, and fish remains[102], but there is no evidence that animal matter played any appreciable part in the deposition of the Oil-Shale Group.

Torbanite is richer in organic matter than the oil shales, the former yielding about 120 gallons of crude oil per ton of sediment pyrolised. According to McGregor[100], the yield of oil has a direct relation to the content of "yellow bodies".

Until recently, the Oil-Shale Group was worked extensively as a source of shale oil. The shale was heated in vertical retorts about 30 feet in height and superheated steam passed through the retort, the distillation temperature being about 480°C [103]. The resulting shale oil has been examined by the U.S.Bureau of Mines, and a large number of low molecular weight (up to $\sim C_{12}$) alkenes, alkanes and aromatic hydrocarbons were isolated and identified[104].

Small quantities of crude oil were also found as seeps in the Scottish shale mines and there is evidence that some, or possibly all, of this oil is a product of the natural distillation of oil-shale bands by igneous intrusions[105]. However the crude oil from the shale mines was found to contain only about 5-7% of unsaturated hydrocarbons whereas that obtained by distillation of the shale had a high proportion of unsaturated

hydrocarbons[106]. To account for this difference it was postulated that the natural distillate would have been equivalent to a steam distillation. Under certain conditions steam distillation in the laboratory is claimed[103] to produce a distillate containing little or only a small proportion of unsaturated hydrocarbons.

Oil from a well drilled at D'Arcy, near Dalkeith, Midlothian, was worked in commercial amounts, and an analysis carried out on this oil and two other oils, viz. oil from the Dunnet shale mine and the Breich pit, showed all three to be chemically very similar[106]. According to Wyllie[106] oil from the Dunnet mine oozes from an igneous sill in contact with a burnt seam. By analogy, therefore, it was thought that perhaps D'Arcy oil was produced in the same way although there is little evidence for igneous intrusions in that area.

DISCUSSION

The geological samples from the Carboniferous Formation of East Scotland were chosen for examination because it was hoped that a comparison of their respective alkane contents would be interesting from a geochemical point of view. Scottish Torbanite was also deemed worthy of attention because of the long-standing controversy over the origin of the extensive "yellow bodies" present in it and their relationship to the alga Botryococcus braunii (Section IV) and the derived rubbery deposit called Coorongite (Section III).

Torbanite and the Westwood Shale.

Figure 1 depicts the "yellow bodies" present in the sample of Torbanite examined herein. The infra-red spectrum of a finely powdered sample of Torbanite indicated the presence of organic matter since there were bands at 2922, 2850 (ν C-H), 1706 (ν C=O), 1456 ($\delta CH_2, CH_3$) and 1379cm.$^{-1}$ (δCH_3). That there were silicates present was shown by the presence of the bands at 1100, 1017, 1005, 908 and 694cm.$^{-1}$ These bands correspond remarkably well with those recorded by Flaig and Beutelspacher[107] for the mineral Dickite ($Al_4[(OH)_8Si_4O_{10}]$). The high organic content is also reflected in the analytical figures which gave a figure of 58%for carbon. However, ultrasonic extraction of Torbanite afforded a geolipid fraction which was only 0.6% by weight of the sediment. It therefore seems likely

that a high proportion of the organic matter is present as kerogen (insoluble organic matter) or is otherwise trapped in the Torbanite. This is further substantiated by the fact that the yield of organic matter rises when the shale is pyrolised at 500°C[33].

The geolipid fraction was examined in the usual manner in order to isolate the alkane fraction which was found to comprise 0.3% of the sediment. The alkane content is about eight times the fatty acid content[18] of the organic matter obtained by solvent extraction. The gas chromatogram of the total alkane fraction (Fig.2) shows a predominance of the n-alkanes and the n-alkane fraction itself, obtained by molecular sieving of the total alkane fraction, has a smooth distribution, ranging from C_{12} to C_{37}, with a maximum at C_{20}. The marked odd/even preference and abundance of the higher molecular weight n-alkanes, characteristic of contemporary plants and recent sediments, is absent. The fatty acids obtained by solvent extraction of Torbanite have a smooth distribution ranging from n-C_{10} to n-C_{28} but there is a marked dominance of the n-C_{16} and n-C_{18} fatty acids[18]. There is no obvious relationship between the n-alkanoic acids and the n-alkanes to support the hypothesis that the hydrocarbons derive from the fatty acids by a radical decarboxylation process[34].

The gas chromatogram of the total hydrocarbon fraction of the Westwood Shale (Fig.3) also shows a smooth distribution similar to Torbanite with the marked exception that there are a number of prominent

peaks in the C_{30} region which are almost absent from the chromatogram of the total alkanes of the latter. The n-alkane fraction (Fig.3) of the Westwood Shale again shows a smooth envelope of peaks ranging from n-C_{12} to n-C_{30} with a maximum at n-C_{15}. The total fatty acid fraction of the sediment ranges from n-C_{10} to n-C_{29} with n-C_{12}, C_{16} and C_{18} the dominant acids[18]. As in the case of Torbanite any genetic relationship between the n-alkanes and n-alkanoic acids cannot arise solely through a mechanism involving loss of carbon dioxide.

The distribution of the branched-cyclic fraction of Torbanite, obtained by molecular sieving, is shown in Fig.2. The peaks enhanced by co-injection with pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane) were trapped from a preparative-scale gas chromatogram and run isothermally in the combined gas chromatograph-mass spectrometer. The mass spectra (Fig.4) of these components confirm that they are indeed pristane and phytane, pristane being the most abundant branched alkane present. The mass spectra of authentic pristane and phytane, run under the same conditions in the gas chromatograph-mass spectrometer are shown in Figure 5. Mass spectrometry is ideally suited for the identification of geological acyclic hydrocarbons since branched hydrocarbons fragment at the points of branching on electron impact and the ion intensities in the mass spectrum are very sensitive to the location of branching. The occurrence of pristane and phytane in Torbanite is in direct contrast to the fact that isoprenoid acids[18, 108] have not been detected in this sediment. The

latter finding seems rather surprising since B. braunii, the presumed precursor of Torbanite, is an alga which contains chlorophyll and has been assigned to the order Chlorophyceae[109].

The gas chromatogram of the branched-cyclic fraction of the Westwood Shale is shown in Figure 3 and contrasts with that of the corresponding fraction of Torbanite in that there are a number of peaks in the C_{30} region which are very abundant. A preparative gas chromatogram of the fraction is shown in Figure 6A and the fractions which were collected are indicated. Figure 6B shows the gas chromatographic record of fraction A when run in the gas chromatograph-mass spectrometer and indicates the points at which mass spectra were recorded. The mass spectra of the peaks enhanced by co-injection of the branched-cyclic fraction with pristane and phytane (fractions B and C) are shown in Figure 7 and the identities of these two components were established by comparison of the mass spectra with those of pristane and phytane (Fig.5). In addition, the major component of fraction A (Fig.6B) has a mass spectrum (Fig.7) corresponding to the C_{16} isoprenoid alkane (2,6,10-trimethyltridecane). The mass spectrum is exactly as would be predicted for this structure and is almost identical to that of the C_{16} acyclic isoprenoid alkane isolated by Eglinton *et al.*[63] from the Green River and Nonesuch Shales with the exception that the ion at m/e 169 is more intense in Fig.7. In addition, fraction A was found to contain two other compenents which could not be separated with the column conditions used in the GC-MS instrument. One

component has a molecular weight of 206 (corresponding to $C_{15}H_{26}$) and is probably a saturated tricyclic sesquiterpene hydrocarbon. A few C_{15} cyclic terpene hydrocarbons have been isolated from lignites but have not been identified.[110,111,112] Also Streibl and Sorm have recently reported the occurrence of two unidentified C_{15} tricyclic saturated sesquiterpene hydrocarbons in Bohemian Montan Wax[88]. There is no record of the isolation of such compounds from an ancient sediment. Fractions F and G (Fig.6A) trapped in a similar manner were also examined by GC-MS. The mass spectra of the dominant components (triterpanes B and C respectively) are shown in Fig.8. The spectra show both compounds to be pentacyclic triterpanes. Triterpane B has a molecular weight of 398, corresponding to a nortriterpane of molecular formula $C_{29}H_{50}$ whereas triterpane C has a molecular weight of 412 corresponding to $C_{30}H_{52}$. As in the case of most pentacyclic triterpanes the base peak occurs at m/e 191 in both spectra. Fractions F and G collected from five similarly temperature programmed gas chromatographic analyses were purified by further gas chromatography under isothermal conditions. The infra-red spectra of the resulting triterpanes B and C are shown in Fig.9. The mass spectrum of triterpane C is very similar to that of lupane (Fig.10) but its infra-red spectrum is somewhat different. Triterpane C has also been isolated from the much younger Green River Shale but again was not identified[68].

Fraction E shown in the gas chromatogram of the branched-cyclic fraction was found to contain a C_{27} pentacyclic tris-nortriterpane (triterpane A, Fig.8) and in addition a number of

branched chain compounds and possibly a small proportion of a C_{28} pentacyclic bis-nortriterpane.

It is conceivable that the biologically-derived alkanes present in Torbanite and the Westwood Shale migrated into the sediments at a later date. This is unlikely, however, in view of the very low permeability of these sediments.

The occurrence of acyclic isoprenoid hydrocarbonsin Torbanite and cyclic and acyclic isoprenoid hydrocarbons in the Westwood Shale provides chemical evidence for their presumed biological origin. Current theories on the origin of the acyclic isoprenoids are discussed above (Introduction). The dominance of triterpanes in the Westwood Shale constitutes chemical evidence for the presumed difference in the mode of deposition of these two sediments (see above). Both sediments contain extensive "yellow bodies" (presumably derived from the alga B. braunii) but the proportion of these varies in each. Torbanite is composed of "yellow bodies" with traces of plant debris whereas the Westwood Shale contains a higher proportion of identifiable plant remains. The triterpane content of the Westwood Shale reflects the plant contribution at the time of deposition for the reasons discussed in Section I.

Scottish Oil Shale Distillate.

The sample of crude oil, formed by commercial distillation of the Scottish Oil Shale, was chromatographed on alumina and the total hexane eluate was separated into three fractions by means of preparative thin-layer chromatography. The gas chromatogram of the alkane fraction thus obtained

is shown in Figure 11 and is somewhat similar in appearance to that of the alkane fraction of the Westwood Shale itself with the exception that the abundant triterpane peaks are absent. The n-alkane fraction (Fig.11) exhibits, as would be expected, a smooth distribution. This is again in contrast to the fatty acid distribution in which the n-C_{14}, C_{16} and C_{18} acids are dominant[18]. Figure 11 also shows the gas chromatographic record of the branched-cyclic fraction obtained from the total alkane fraction. The abundant triterpane peaks of the Westwood Shale branched-cyclic fraction are again absent, although the complexity of the gas chromatogram in the C_{28}-C_{30} region suggests the possible presence of such compounds. It is difficult to offer a rational explanation for the apparent sparsity of these compounds. However, it is possible that their decrease in relative abundance has been paralleled by an increase in the abundance of normal and branched-chain hydrocarbons derived from thermal cracking of the long chain material in the kerogen matrix of the shale. Pristane and phytane were identified in the usual manner by co-injection with authentic samples and by means of their mass spectra (Fig.12). The C_{16} isoprenoid hydrocarbon (Fig.12) was also identified, and its mass spectrum was almost identical to that of the C_{16} isoprenoid isolated from the Westwood Shale, with the exception that the ion at m/e 169 is almost as intense as that at m/e 183, indicating that the sample is not completely pure. Co-injection of the branched-cyclic fraction with authentic farnesane (C_{15} isoprenoid) indicated that there is very little if any of this component present. The relative abundance of these acyclic isoprenoid hydrocarbons is reduced in comparison to their abundance in the shale itself (Fig.3). It is

interesting, however, that the biological origin of this oil is still evident even after pyrolysis of the shale at high temperatures.

An alkene fraction, whose infra-red spectrum showed that the unsaturation present was of the trans disubstituted type, was also obtained from the total hexane eluate. The gas chromatographic record of this complicated fraction is shown in Fig.13. Although the trans-alkenes were not reduced to alkanes and examined by gas chromatography, it is probable that the former, generated by pyrolysis of the shale, are mainly straight chain with the double bond varying in position over the molecule.

In addition, a series of vinyl alkenes was isolated. This fraction has a smooth distribution of what appear to be n-alk-1-enes, ranging from C_{11} to C_{31} with a maximum at n-pentadec-1-ene (Fig.13). Douglas et al.[33] have performed pyrolysis experiments on the Green River Shale under carefully controlled laboratory conditions. A series of vinyl alkenes (in addition to a trans alkene fraction) was generated. It was observed that the C.P.I. of the n-alkanes dropped from about 3.5 to 1 on pyrolysis. The n-alk-1-enes formed on pyrolysis had a slight even/odd preference. The authors therefore maintain that these two compound classes are geogenetically related and might be considered together (in geological situations) as products of geogenetic maturation processes. It is difficult to corroborate these findings in the case of the Westwood Shale and its distillate since the n-alkanes of the shale already have a C.P.I. of unity before pyrolysis.

D'Arcy Oil.

The sample of oil examined was purported to have been collected from the D'Arcy well, Midlothian. However, there remains some dubiety about its origin because of the high alkene content and the fact that a second sample of D'Arcy Oil obtained from the Royal Scottish Museum, Edinburgh, had a very small alkene content. It is therefore possible that the sample is in fact a commercially distilled oil although it is undoubtedly of Carboniferous age. As in the case of the commercially distilled oil, the oil was chromatographed on alumina and the total hexane eluate collected. Preparative thin-layer chromatography of the total hexane eluate afforded three fractions and the alkane fraction thus obtained has a distribution (Fig.14) typical of the other Carboniferous samples examined herein. The n-alkane fraction, obtained from the total alkane fraction, also has the smooth distribution (Fig.14) typical of the above Carboniferous samples and extends from C_{11} to C_{32} with a maximum around C_{16}, C_{17}. Once again there is no obvious genetic relationship to the fatty acids[18] isolated from the same sample.

The distribution of the branched-cyclic alkane fraction is also shown in Figure 14. The peaks corresponding in retention time to farnesane, pristane and phytane were trapped from a preparative gas chromatogram and run isothermally in the gas chromatograph-mass spectrometer. The resulting mass spectra (Figs.16,17) confirm their assignment. The mass spectra of authentic farnesane, pristane and phytane are shown in Figure 5 for comparison. Furthermore the C_{16} and

C_{18} isoprenoid hydrocarbons (2,6,10-trimethyltridecane and 2,6,10-trimethylpentadecane respectively were identified from their mass spectral fragmentation patterns (Fig.16).

The trans alkene fraction obtained from the total hexane eluate is not as complex (Fig.17) as that obtained from the commercially distilled oil (Fig.13) and comprises 13% of the oil compared with 8% for the commercially distilled oil. This fraction was hydrogenated and the distribution of the resulting alkane fraction is shown in Fig.18. Hydrogenation simplified the distribution of the trans alkenes (Fig.17) somewhat, indicating that there are homologous series of isomeric n-trans-alkenes present in the trans alkene fraction. The hydrogenated alkanes were separated into a normal fraction and a branched-cyclic fraction with molecular sieve. The normal fraction (Fig.18) has a distribution markedly similar to that of the normal fraction (Fig.14) of the total alkane fraction. The branched-cyclic fraction distribution (Fig.18) is also very similar to that of the total alkane branched-cyclic fraction (Fig.14) and is again dominated by pristane and phytane. These two components and the C_{18} isoprenoid were again identified by their mass spectra (Fig.19). It appears, therefore, that the branched trans alkenes may derive from, or be related in origin to, the branched alkanes.

The vinyl alkene fraction is dominated by the n-alk-1-enes and extends from n-C_{10} to n-C_{31} with a maximum at n-C_{15} (Fig.17). The distribution is almost identical to that of the vinyl alkenes (Fig. 13) of the

commercially distilled oil. The vinyl alkene fraction of the D'Arcy Oil comprises about 15% of the oil whereas that of the distilled oil comprises about 13% of the oil. Although the distributions of branched-cyclic alkane fractions of both oils are somewhat different in appearance, there is no doubt that the D'Arcy oil sample is a distilled oil, especially since both oils contain abundant quantities of trans and vinyl alkenes. Furthermore, alkenes have not been isolated from any crude petroleum to an appreciable extent.[113] If, therefore, the sample of D'Arcy Oil examined herein is genuine, it seems likely that it arose via a natural distillation through the effect of an igneous intrusion on the shale. Alternatively, if the sample is not genuine it is most likely a commercially distilled oil derived from one of the Lothian Oil Shales. Although the sample was stored in a corked bottle in a museum for a number of years it is unlikely that bacterial action could solely give rise to the large proportion of alkenes present.

Table 1 summarises the hydrocarbons which have been identified in the samples from the East Scottish Carboniferous Formation. All of the normal alkane fractions have similar smooth distributions, as stated above, with maxima in the n-C_{15} to n-C_{20} regions. This contrasts with the n-alkane distributions of most younger geological samples which normally exhibit marked odd/even preferences and is in keeping with the distributions reported for ancient sediments. The isoprenoids identified are also listed in Table 1 and their overall

relative abundance in the various branched-cyclic fraction indicates a probable biological origin for all of the samples examined. Table 2 summarises the proportions of the various fractions of the Carboniferous samples. It can be seen that Torbanite contains three times as much extractable organic matter as the Westwood Shale. This is in accordance with the fact that Torbanite gives a higher yield of shale oil than any of the members of the Scottish Oil Shale Group.

EXPERIMENTAL

Torbanite.

The sample of Torbanite used in the present study was kindly provided by Dr. W.D.I. Rolfe, Assistant Curator of the Geological Collection of the Hunterian Museum, Glasgow University. Dr. W.D.I. Rolfe also provided a microsection which clearly shows the "yellow bodies" (Fig.1). An analysis performed by A. Bernhardt, Max-Planck Institut, Germany, on a finely powdered (passing 200 mesh) sample is as follows:- C, 58.0; H, 7.4; N,0; S,0; ppt., 27.2%. An i.r. spectrum (KCl disc) had absorption at 3620 (w, νO-H of H_2O), 2922 (m, νC-H), 2850 (m,ν C-H), 1706 (w,ν C=O?), 1457 (w, δCH_2,CH_3), 1379cm.$^{-1}$ (w, δCH_3). There were also intense bands at 1100, 1017, 1005, 908 and 694cm.$^{-1}$ corresponding remarkably well with the silicate bands of the mineral Dickite ($Al_4[(OH)_8Si_4O_{10}]$)[107] (DMS ref.card no. A.0230).

Treatment of rock and extraction of organic matter.

A piece of Torbanite, with the outer surface removed, was broken into pieces about 1 in. in diameter. These were sonicated (soniprobe) in benzene/methanol (1:1, 3x5 min.) to minimise contamination. The chips were then powdered, as described above, using the hammer and disc mills, until the resultant powder passed through a 200 mesh sieve. A sample (250g.) of the powdered Torbanite was extracted ultrasonically in the sonitank with benzene/methanol (3:1, 500 ml.) for 30 min., the resulting suspension centrifuged, and the clear solvent layer removed by pipette. The extraction was

repeated four times. Evaporation of the solvent gave the total organic extract as a dark brown gum (1.5g; 0.6%).

Isolation of the alkane fraction.

The total organic extract was chromatographed on alumina (50g.) and the total n-hexane eluate collected by taking 15x100ml. fractions. The fractions were monitored by t.l.c. on silica, with a standard n-alkane mixture to ensure that all of the alkanes had been eluted from the column. T.l.c. of the total hexane eluate on silica showed fluorescent spots under u.v. light (254mμ.) indicating the presence of aromatic compounds. However preparative-scale t.l.c. on silica impregnated with silver nitrate (n-hexane developer) afforded the alkane fraction (0.63g.) completely free of aromatics. The u.v. spectrum of the alkanes (n-hexane, 12.8mg./10ml., 1cm. cell) showed no absorption due to aromatics. The i.r. spectrum was of the alkane type with bands at 1465 ($\delta CH_2, CH_3$) 1378 (δCH_3; sym.) and 720cm.$^{-1}$ ($-(CH_2)n-$"rock"), the 720cm.$^{-1}$ band indicating that long unbranched chains were present.

Examination of the alkane fraction.

A temperature-programmed gas chromatogram of the total alkane fraction (Fig.2) showed it to consist of a very complicated mixture, with the n-alkane peaks dominating the chromatogram.

An aliquot (238mg.) of the total alkane fraction in dry benzene (60ml.) was heated under reflux with 5Å molecular sieve (23g.) for 80 hrs. The sieve was washed in an all-glass Soxhlet (6 hrs.) and the washings evaporated to give the branched-cyclic alkane

fraction (118mg.). The n-alkane fraction (101mg.) was obtained by dissolving the sieve in HF as described above. G.l.c. of the n-alkane fraction (Fig.2) showed a smooth distribution with a maximum at C_{19}, C_{20}. The positions of the C_{16} and C_{24} n-alkanes were established by co-injection. A temperature-programmed gas chromatogram of the branched-cyclic fraction showed it to consist of an extremely complicated mixture. Co-injection with farnesane, pristane and phytane (Fig.2) indicated that peaks corresponding to all three hydrocarbons were present and that the largest peak in the chromatogram had a retention time identical to that of pristane. The peaks thought to be pristane and phytane were trapped in the manner described above from a preparative-scale gas chromatogram (column 20ft. x 1/4 in.; 3% SE-30 on Gas Chrom P, 100-120 mesh, acid washed and silanized; programmed from 100-300°C at 6°/min.). The appropriate fractions were then run isothermally in the GC-MS and mass spectra recorded. The presence of pristane and phytane in the total branched-cyclic fraction was then established by a comparison of their mass spectra (Fig.4) with those of standards (Fig.5).

Control Experiment.

The extraction procedure was repeated on a small sample of Torbanite (20g.). The total extract (51.3mg.) was chromatographed as before to obtain the alkane fraction (25mg.) which was separated into a normal (12mg.) and a branched-cyclic (9mg.) fraction. The g.l.c.

records of the total, normal and branched-cyclic alkane fractionswere identical to those of the corresponding fractions derived from the large scale extraction. In addition, a sample (20g.) of finely ground glass beads, as a contamination control, was treated in an identical manner, but the solvents gave no peaks in the g.l.c. chromatogram under the usual analytical conditions.

Westwood Shale (Scottish Oil Shale).

A large (ca. 2 cu.ft.) unfractured piece of the shale was collected for the present study by Dr. W.D.I. Rolfe, Mr. J.N. Ramsay and the author from the stock-pile at the Westwood Works of Scottish Oils Ltd., West Calder, Midlothian.

The fatty acids were first removed by Mr. J.N. Ramsay[18] from the sample (600g.) examined. This was achieved by treating the powdered shale with mixed hydrofluoric acid/hydrochloric acid (3:1), extracting the residue with benzene/methanol and passing an ethereal solution of the extract down a silicic acid/potassium hydroxide column. The acids were adsorbed on the column. The remainder of the extract (0.84g.) dissolved in n-hexane (10ml.) was chromatographed on alumina (50g.; 50ml. fractions) and the total n-hexane eluate (0.41g.) collected. The fractions were monitored by t.l.c. (iso-octane developer) as usual.

An aliquot (136 mg.) of the n-hexane eluate was examined by preparative-scale t.l.c. on silica/silver nitrate (iso-octane developer) and the alkane fraction (102 mg.) recovered. G.l.c. of the alkane fraction (Fig.3) showed the distribution to be similar to the Torbanite

alkanes but there were several prominent peaks in the n-C_{28} to n-C_{31} region. An aliquot (23 mg.) of the alkane fraction was sieved in the usual manner. A normal (5mg.) and a branched-cyclic fraction (15 mg.) were thereby obtained. G.l.c. (Fig.3) showed the normal alkanes to have a smooth distribution, ranging from C_{12} to C_{30}, with a maximum at C_{15} (established by co-injection with n-C_{14}).

The gas chromatogram of the branched-cyclic fraction is shown in Fig.3 and the peaks corresponding in retention time to pristane and phytane were found to be dominant. These two compounds were identified by their mass spectra (Fig.7) in the usual manner by trapping from a preparative gas chromatogram (Fig.5) and by examination by GC-MS. The C_{16} acyclic isoprenoid hydrocarbon (Fraction A) was also identified by means of its mass spectrum (Fig.7). The mass spectra of fractions F and G were also recorded (Fig.8) and had spectra of the pentacyclic triterpane type. The infra-red spectra of triterpanes B and C obtained from five temperature-programmed gas chromatograms (Fig.6) of the branched-cyclic alkane fraction were recorded (Fig.8) after the resulting fractions F and G were further purified by g.l.c. under isothermal conditions (column 20 ft. x 1/8 in.; 3% SE-52 on Chrom. W; temperature 275°C.).

Scottish oil shale distillate.

The crude unrefined sample of oil used in the present study was provided by Mr. Thomson of Scottish Oils Ltd., who obtained the sample from the Pumpherston Refinery, Midlothian. The sample was manufactured about 5 years ago at the Westwood Works, West Calder.

An aliquot (6.8g.) of the crude oil, suspended in n-hexane, was chromatographed on alumina (350g.) and the total hexane eluate collected. As before, the fractions were monitored by t.l.c. to ensure the elution of all of the alkane fraction. The total n-hexane eluate was dark brown in colour. The eluate was therefore re-chromatographed on alumina (350g.) and the total n-hexane eluate (4.1g.) collected. T.l.c. of the hexane eluate on silica impregnated with silver nitrate showed it to consist of three spots which were separated by preparative scale t.l.c. Three fractions (a)-(c) were thereby obtained from an aliquot (310 mg.) of the hexane eluate.

Fraction (a). (203mg.) had R_f identical to a standard n-alkane mixture and the i.r. spectrum showed typical alkane absorption with bands at 1465 (δCH_2, CH_3), 1375 (δCH_3) and 720cm.$^{-1}$ ($-(CH_2)_n-$"rock"). G.l.c. (Fig.11) showed the fraction to have the same general appearance as the alkane fraction derived from Torbanite. An aliquot (150 mg.) of the total alkane fraction was sieved by heating it under reflux (82 hrs.) in benzene (20 ml.) in the present of 5Å molecular sieve (1.2g.). A normal fraction (62 mg.) and a branched-cyclic fraction (79 mg.) were obtained in the usual manner. G.l.c. showed the n-alkane fraction (Fig.11) to extend from C_{11} to C_{34} with a maximum at C_{16}, C_{17}. The identification of the C_{20} and C_{28} n-alkanes was established by co-injection of the n-alkane fraction with the two standard alkanes. The branched-cyclic fraction was shown to consist of a very complicated mixture with no special dominance of any one hydrocarbon (Fig.11). The farnesane, pristane and phytane positions in the g.l.c. trace were

established by co-injection and the pristane and phytane identified by their mass spectra (Fig.12) after a preparative gas chromatogram.

Fraction (b). (63 mg.). The i.r. spectrum had absorption at 965 (m, γC-H trans disubstituted) and 720cm.$^{-1}$(m,-$(CH_2)_n$-rock). G.l.c. showed fraction (b) to be very complex with several homologous series present and the distribution was a smooth one (Fig.13).

Fraction (c). (34 mg.). The i.r. spectrum (film) had absorption at 3070 (m, ν C-H), 1640 (m, ν C=C), 990 and 910 (m, s, γC-H vinyl) and 720cm.$^{-1}$(m,-$(CH_2)_n$-rock). G.l.c. showed the fraction to consist mainly of n-alkenes with small amounts of branched or cyclic alkenes present (Fig.13). Co-injection with n-C_{17}-1-ene showed that the maximum in the smooth distribution was at n-C_{15}-1-ene.

D'Arcy oil.

The sample examined was provided by Dr. W.D.I. Rolfe, Assistant Curator, Hunterian Museum, Glasgow University. According to the label, the sample had been obtained by the late Professor Gregory of the Geology Department, Glasgow University, from a boring made around 1936 by the D'Arcy Exploration Company at a site 2½ miles south-east of Dalkeith, by Edinburgh.

An aliquot (4.47g.) of the crude oil was sonicated in n-hexane (ca.20ml.) to aid solution. The resulting suspension was chromatographed on alumina (150g.; 50ml. fractions) and the total n-hexane eluate (2.37g.) collected. The fractions were monitored by t.l.c. on silica (n-hexane developer) in the usual manner. The

hexane eluate was brown in colour and was re-chromatographed on alumina (160g.; activated at 200°C. for 36 hrs.; 50ml. fractions) and the n-hexane eluate (2.08g.) again collected. The i.r. spectrum (film) of the 4th fraction showed absorption at 1645 (w, νC=C), 1610(w, νC=C aromatic), 1500 (w, νC=C aromatic), 990 and 910 (w, m, γC-H vinyl), 970 (m, γC-H trans disubstituted), 810(m), 750(m), 720(m, $-(CH_2)_n-$rock), and 700cm.$^{-1}$(m,γC-H aromatic). The i.r. spectrum (film) of the total hexane eluate had absorption at 3080 (w, νC-H), 1820(w, overtone γC-H), 1643(m, ν C=C), 990 and 910 (w,m,γC-H vinyl) 968 m,γC-H trans disubstituted), 890(w), 760(w) and 720cm.$^{-1}$(s,$-(CH_2)_n-$rock). T.l.c. of the hexane eluate on silica impregnated with silver nitrate (n-hexane developer) showed it to consist of three major spots which were separated by preparative scale t.l.c. of an aliquot. Three fractions (a)-(c) were thereby obtained.

Fraction (a) [85mg.] had R_f identical to a standard n-alkane mixture. The i.r. spectrum (film) showed no carbon-carbon double bond absorption and had absorption at 1465 (s, $\delta CH_2, CH_3$), 1380(m,δCH_3) and 720cm.$^{-1}$(m,$-(CH_2)_n-$rock). G.l.c. (Fig.14) showed the fraction to have a distribution similar to Torbanite (Fig.2) and the Scottish Oil Shale distillate (Fig.11) with a maximum at n-C_{15}, and the fraction comprised about 19% of the crude oil. Sieving was carried out in the usual manner by heating an aliquot (88mg.) of the alkane fraction under reflux (120 hrs.) in dry benzene with 5Å molecular sieve (8g.). A normal (39mg.) and a branched-cyclic fraction (34mg.) were thus obtained.

G.l.c. showed the n-alkanes to extend from C_{11} to C_{32} (Fig.14) with a maximum at C_{16}, C_{17} in the smooth distribution (established by co-injection with n-C_{20} and n-C_{28}). The branched-cyclic fraction was shown to consist of a complicated mixture (Fig.15) with prominent peaks corresponding in retention time to farnesane, pristane and phytane. The latter were identified by their mass spectra (Fig.16) in the usual manner as were the C_{16} and C_{18} isoprenoid alkanes (Fig.16).

Fraction (b). The i.r. spectrum (film) had absorption at 965(m, γC-H trans disubstituted) and 720cm.$^{-1}$ (m, -$(CH_2)_n$-rock). The fraction comprised about 13% of the crude oil. The n.m.r. spectrum (60m/c) exhibited a broad singlet at τ4.6 for the trans double bond protons. A gas-liquid chromatogram of the fraction is shown in Fig.17.

An aliquot (20mg.) of fraction (b) was reduced at room temperature in ethyl acetate (10ml.) in the presence of 10% palladium on charcoal (5mg.) as catalyst. When no more hydrogen was being absorbed the mixture was passed through a small column of alumina to remove the catalyst. T.l.c. of the product (12mg.) on silica/silver nitrate (n-hexane developer) showed it to consist almost entirely of alkanes since it had an R_f identical to a standard alkane mixture. The product was purified by preparative-scale t.l.c. and g.l.c. (Fig.18) showed it to have a more simple distribution than fraction (b) itself (Fig.17), indicating that there was a homologous series of double bond isomers present in fraction (b). The

distribution showed a predominance of n-alkanes with a maximum at n-C_{17}.

An aliquot (9mg.) of the product of hydrogenation was treated with 5Å molecular sieve in the usual manner and a branched-cyclic fraction (5mg.) and a normal fraction (2mg.) were thus obtained. The most abundant peaks in the gas chromatogram of the former corresponded in retention time to pristane and phytane (Fig.18). This was established by co-injection of the branched-cyclic fraction with the appropriate standards. Pristane and phytane were identified in the usual manner by comparison of their mass spectra (Fig.19) with those of the authentic isoprenoids (Fig.5). The C_{18} isoprenoid was also identified from its fragmentation pattern in the gas chromatograph-mass spectrometer. The n-alkane fraction (Fig.18) had a smooth distribution ranging from n-C_{14} to n-C_{30} with a maximum at n-C_{19} (established by co-injection with n-C_{14} and n-C_{20}).

Fraction (c). The i.r. spectrum (film) had absorption at 1640(m, νC=C), 990 and 910 (w,m, γC-H vinyl) and 720cm.$^{-1}$(m, -$(CH_2)_n$-rock) with bands low in intensity at 965 (sh. , γC-H trans disubstituted) and 885cm.$^{-1}$(sh, γC-H exomethylene). The n.m.r. spectrum (60 m/c.) showed a number of signals in the range 4.2-5.4τ, confirming the unsaturation present. Fraction (c) comprised about 15% of the sample of oil examined. G.l.c. (Fig.17) showed the fraction to have a smooth distribution of vinyl alkenes, with a maximum at n-C_{15} and the distribution ranged from C_{11} to C_{31} with the n-alkenes predominating.

TABLE 1.

A. Hydrocarbons identified in samples from the Scottish Carboniferous Formation.

Sample	Normals		Acyclic Isoprenoids	Tri-terpanes
	Range	Max.		
Torbanite	C_{12} to C_{37}	C_{20}	C_{19},C_{20}	
Westwood Shale	C_{12} to C_{30}	C_{15}	C_{16},C_{19},C_{20}	C_{27},C_{29},C_{30}
Oil Shale Distillate	C_{11} to C_{34}	$C_{16,17}$	C_{16},C_{19},C_{20}	
D'Arcy Oil	C_{11} to C_{32}	$C_{16,17}$	C_{15},C_{16},C_{18}, C_{19},C_{20}.	
Alkane fraction obtained by reduction of the <u>trans</u> alkene fraction of the D'Arcy Oil.	C_{14} to C_{30}	C_{19}	C_{18},C_{19},C_{20}	

B. Proportions of Fractions present in the Carboniferous Samples.

Sample	Geolipid fraction % (total extract)	Alkane fraction %	Normal fraction % *	Branched-cyclic fraction %
Torbanite	0.6	0.3	0.1	0.13
Westwood Shale	0.2 †	0.05	0.01	0.04
Oil Shale Distillate	-	39.3	16.3	21.1
D'Arcy Oil	-	19.0	7.4	8.5

* The sum of the normal and branched-cyclic fractions does not correspond exactly to the alkane fraction % since recovery from the molecular sieving operations was never 100%.

† Extract obtained after treatment of shale with HF/HCl.

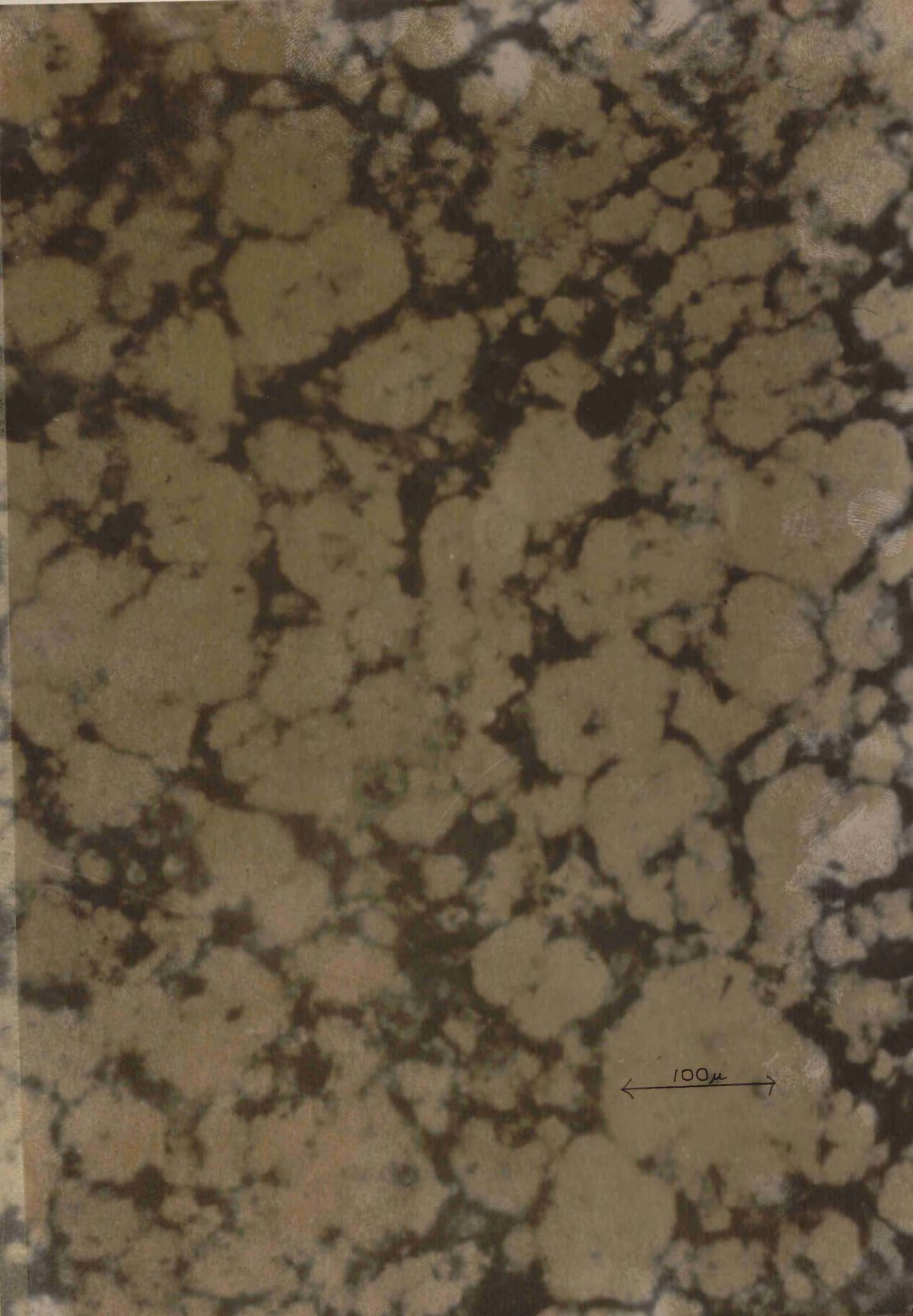

100μ

Fig. 1 legend.

Photograph of a polished microsection of Torbanite.

Fig. 2 legend.

Gas chromatograms of Torbanite alkane fractions.

Conditions:

A. Column 6 ft. x 1/8 in.; 1% JXR on Gas Chrom P, 100-120 mesh; flow rate approximately 28 ml./min. nitrogen at 16 p.s.i.; temperature programmed from 100° to 300°C at 6°/min.; sample size 0.3µl. of a solution in n-hexane; attenuation 50 x 1.

B. As for Fig. 2A; sample size 0.5µl. of a solution in n-hexane.

C. Column 6 ft. x 1/8 in.; 3% SE-30 on Gas Chrom P, 100-120 mesh; flow rate approximately 28 ml./min. nitrogen at 16 p.s.i.; temperature programmed from 150° to 310°C at 8°/min.; sample size 1 µl. of a solution in n-hexane; attenuation 5 x 10^2.

Fig.2.

TORBANITE ALKANE FRACTIONS

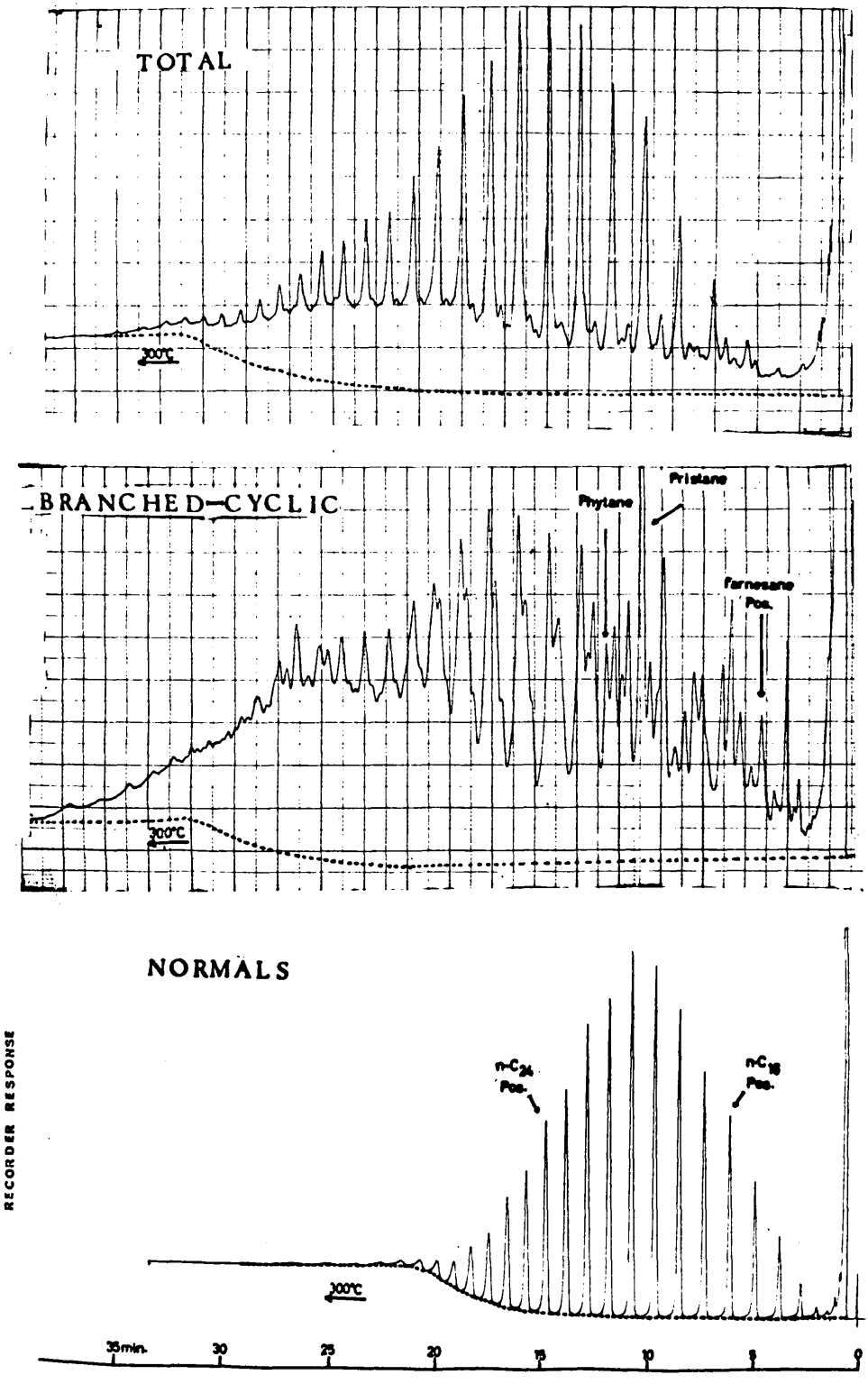


Fig. 3 legend.

Gas Chromatograms of Westwood Shale alkane fractions.

Conditions:

A. Column 10 ft. x 1/16 in.; 3% SE-30 on Gas Chrom P, 100-120 mesh; flow rate 20 ml./min. nitrogen; temperature programmed from 100° to 300°C at 5°/min; sample size 5µl.(split) of a solution in n-hexane; attenuation 1 x 10^2,

B. Column 10 ft. x 1/16 in.; 3% OV-1 on Gas Chrom Q 60-80 mesh; flow rate 20ml./min. nitrogen; temperature programmed from 100° to 300°C at 5°/min.; sample size 4µl.(split) of a solution in n-hexane; attenuation 2 x 10^2.

C. As for Fig. 3A; sample size 8µl.(split) of a solution in n-hexane; attenuation 1 x 10^2.

Fig.3

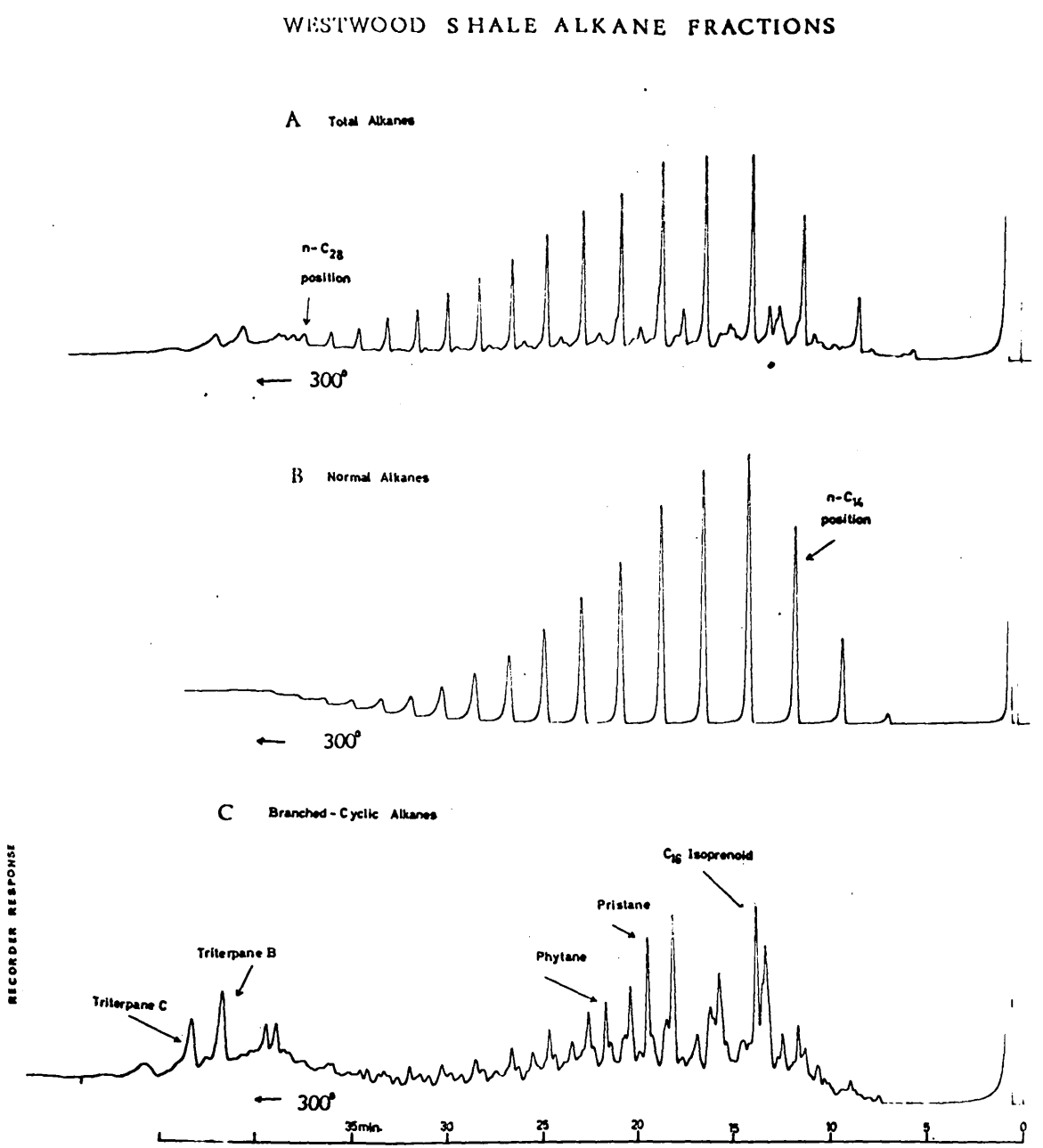

WESTWOOD SHALE ALKANE FRACTIONS
A Total Alkanes
n-C28 position
300°
B Normal Alkanes
n-C14 position
300°
C Branched - Cyclic Alkanes
C16 Isoprenoid
Pristane
Phytane
Triterpane B
Triterpane C
300°
35min.
30
25
20
15
10
5
0
RECORDER RESPONSE

Fig.4.

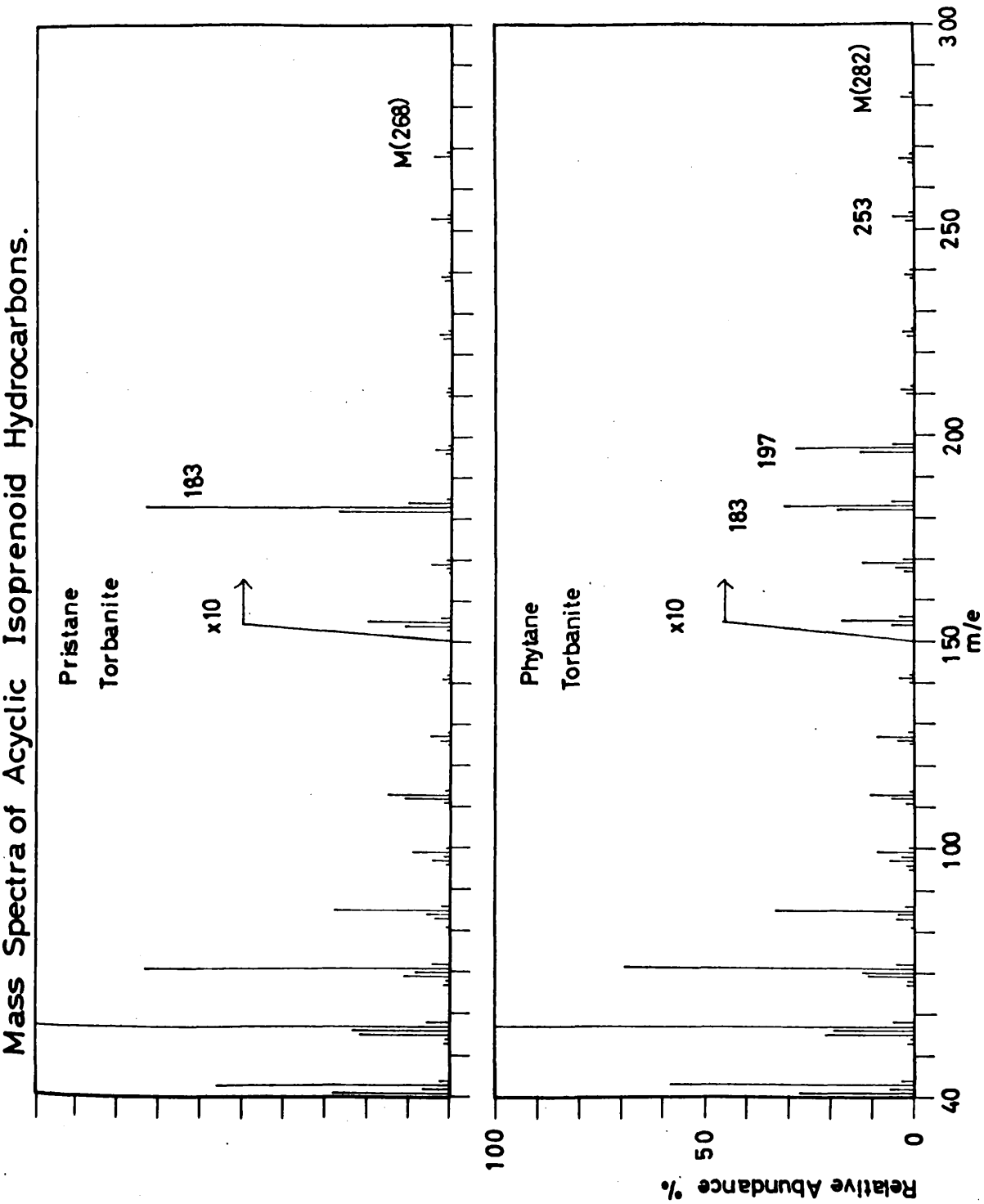

Mass Spectra of Acyclic Isoprenoid Hydrocarbons.
Pristane
Torbanite
183
x10
M(268)
Phytane
Torbanite
183
197
253
M(282)
x10
Relative Abundance %
100
50
0
40
100
150
200
250
300
m/e

Fig.5.

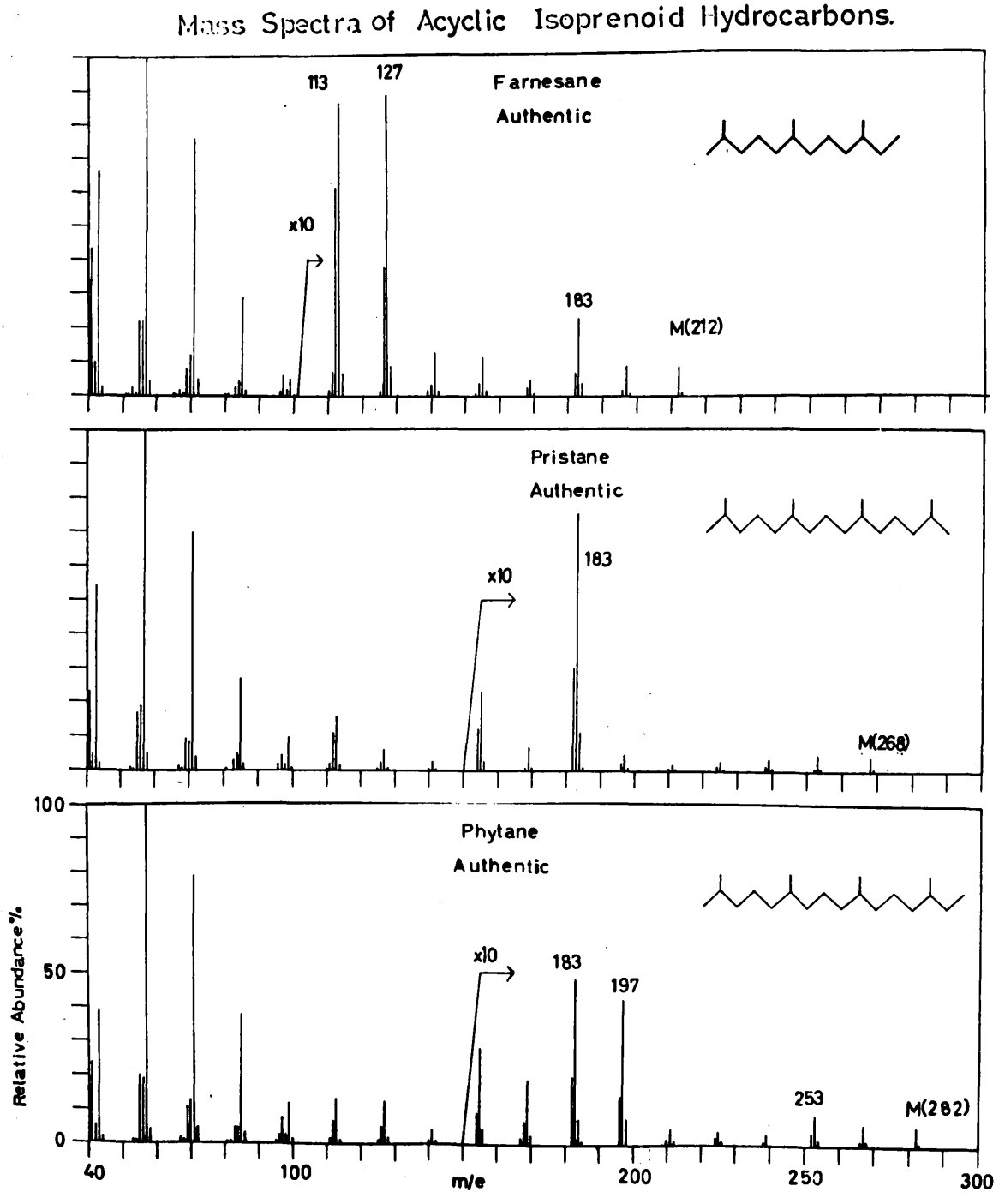

Mass Spectra of Acyclic Isoprenoid Hydrocarbons.
Farnesane
Authentic
113
127
x10
183
M(212)
Pristane
Authentic
183
x10
M(268)
Phytane
Authentic
x10
183
197
253
M(282)
100
50
0
Relative Abundance%
40
100
m/e
200
250
300

Fig.6 legend.

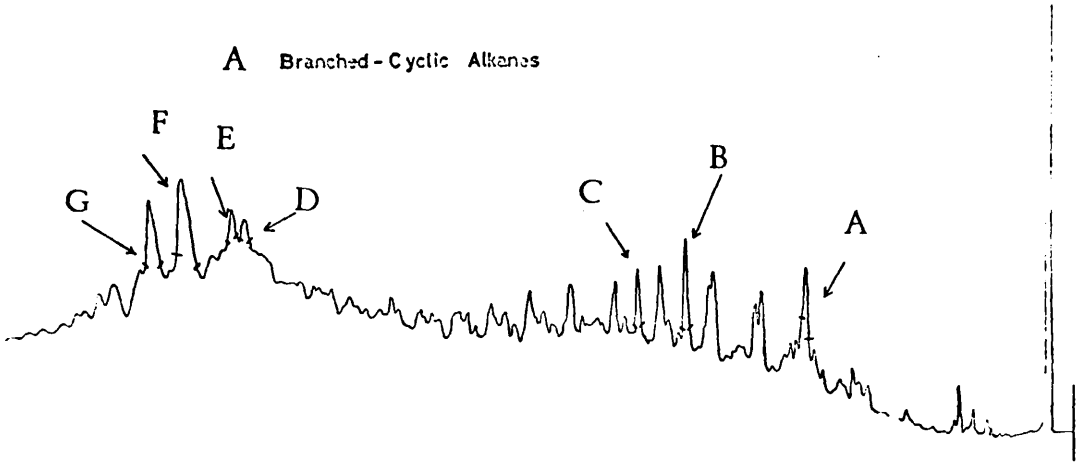
Gas Chromatograms of Westwood Shale branched-cyclic alkane fractions.

Conditions:

A. Column 20 ft. x 1/8 in.; 3% SE-52 on Chromosorb W, 80-100 mesh; flow rate 60ml./min. helium; temperature programmed from 100° to 300°C at 6°/min.; sample size 8µl. of a solution in n-hexane; attenuation 1 x 4.

B. Column 10 ft. x 3mm.; 1% SE-30 on Gas Chrom P, 100-120 mesh; flow rate 30ml./min. helium; temperature programmed from 60° to 100°C at 4°/min.; sample size 5µl. of a solution in n-hexane; mass spectra recorded at the points shown.

WESTWOOD SHALE ALKANE FRACTIONS



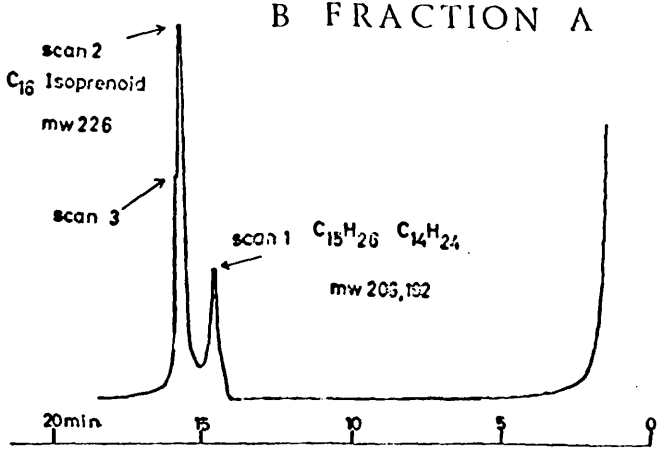


Fig.6.

Fig.7.

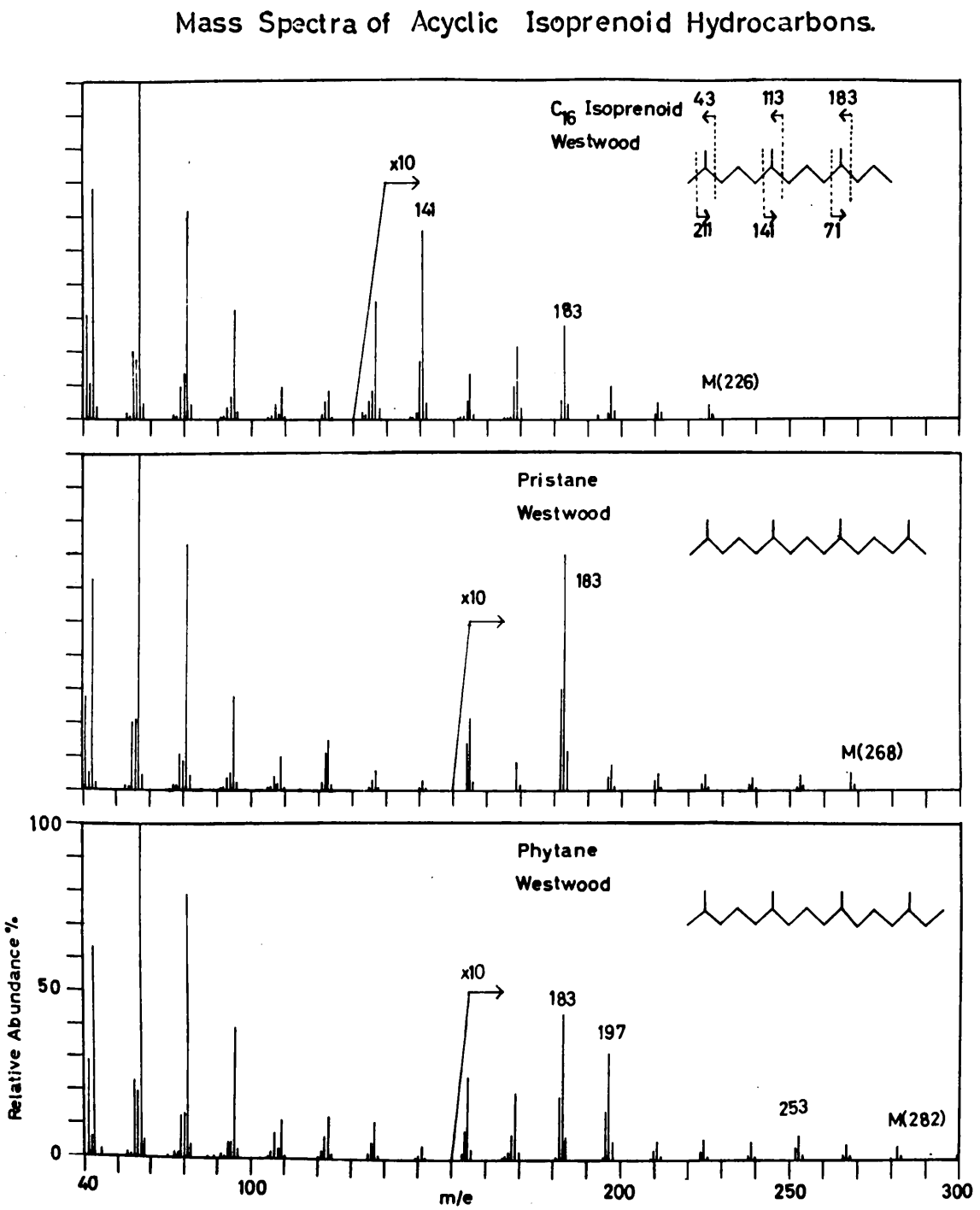

Mass Spectra of Acyclic Isoprenoid Hydrocarbons.
C16 Isoprenoid
Westwood
43
113
183
211
141
71
x10
141
183
M(226)
Pristane
Westwood
x10
183
M(268)
Phytane
Westwood
x10
183
197
253
M(282)
100
50
0
Relative Abundance %
40
100
m/e
200
250
300

Fig.8.

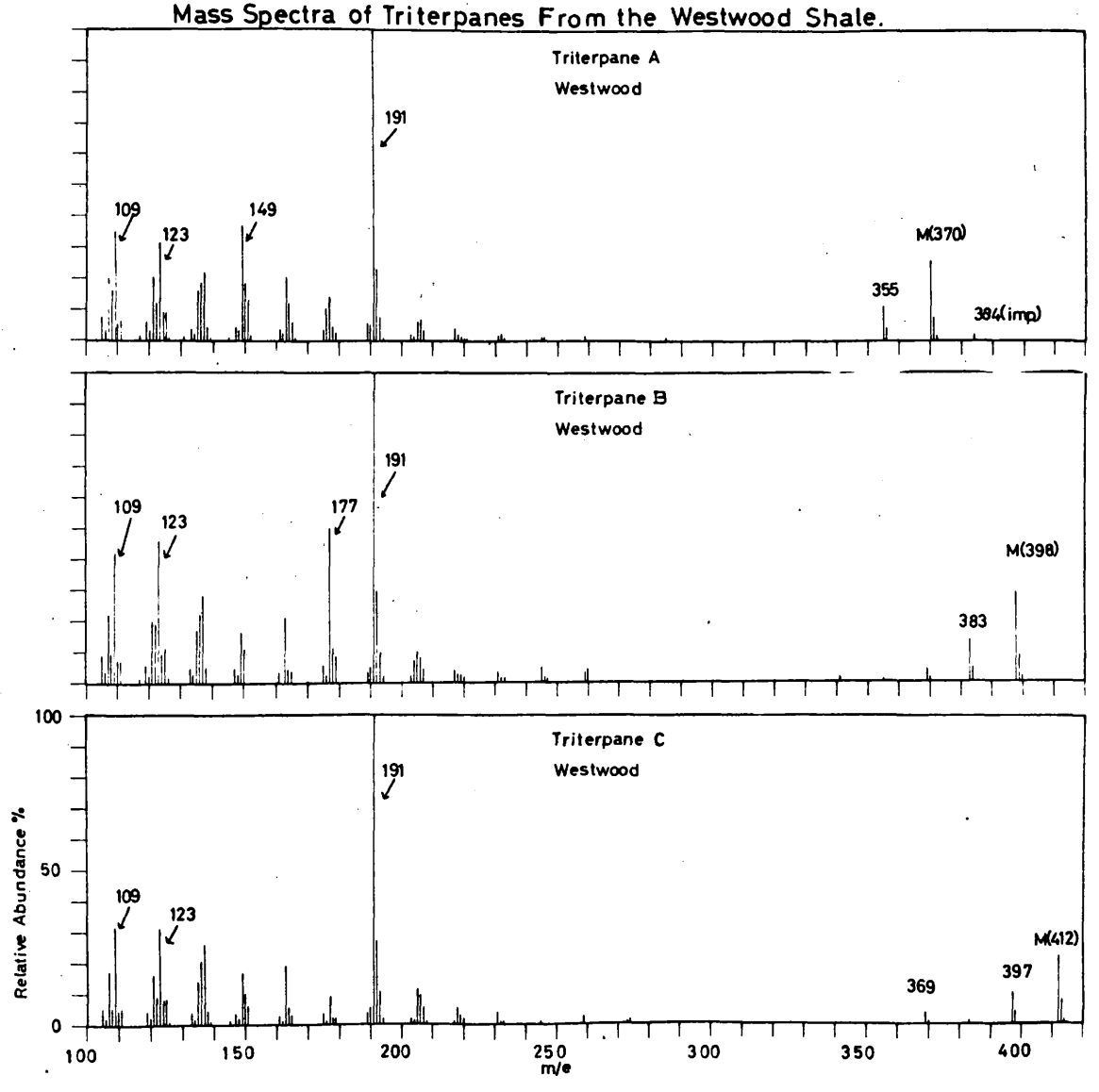
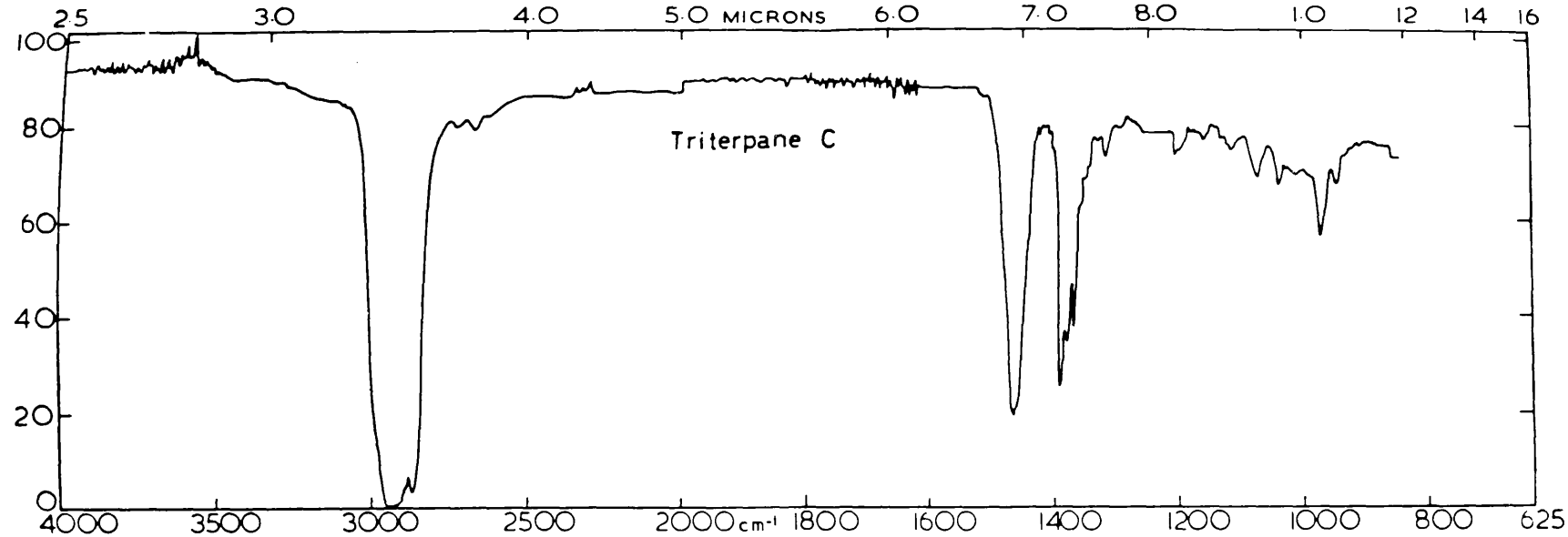

Mass Spectra of Triterpanes From the Westwood Shale.
Triterpane A
Westwood
191
109
123
149
M(370)
355
384(imp)
Triterpane B
Westwood
191
109
123
177
M(398)
383
Triterpane C
Westwood
191
109
123
M(412)
397
369
100
50
0
Relative Abundance %
100
150
200
250
300
350
400
m/e

Fig. 9 Legend

A. Infra-red spectrum of triterpene 1 from Wort and Chale; solution of ca. 40μg. in CCl_4; cell path 0.5mm.; Perkin-Elmer 257.

B. Infra-red spectrum of triterpene 2, conditions as for Fig. 9A.


2.5
3.0
4.0
5.0 MICRONS
6.0
7.0
8.0
1.0
12
14
16
100
80
60
40
20
0
Triterpane C
4000
3500
3000
2500
2000 cm-1
1800
1600
1400
1200
1000
800
625

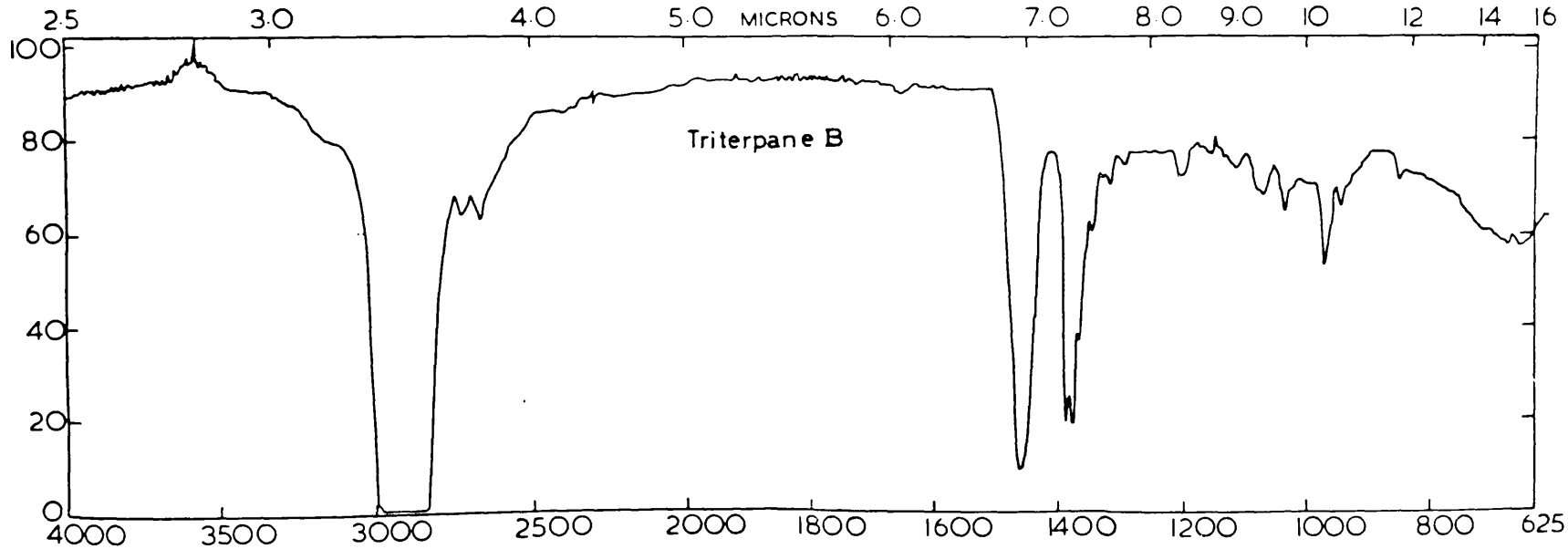

2.5
3.0
4.0
5.0 MICRONS
6.0
7.0
8.0
9.0
10
12
14
16
100
80
60
40
20
0
Triterpane B
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
625

Fig.10.

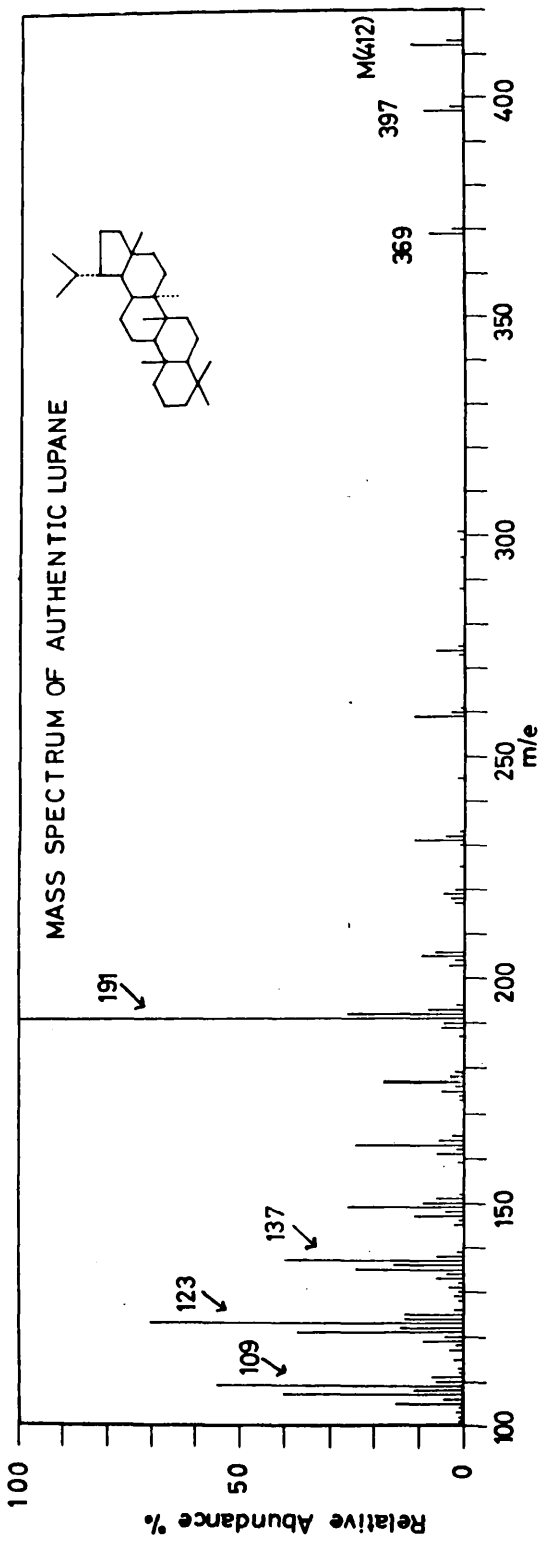

MASS SPECTRUM OF AUTHENTIC LUPANE
Relative Abundance %
100
50
0
100
150
200
250
300
350
400
m/e
191
123
109
137
369
397
M(412)

Fig.11 legend.

Gas Chromatograms of Scottish Oil Shale Distillate alkane fractions.

Conditions:

A. Column 10 ft. x 1/8 in.; 1% SE-30 on Chromosorb W, 100-120 mesh; temperature programmed from 100° to 300°C at 6°/min.; flow rate approximately 30ml./min. nitrogen at 20 p.s.i.; sample size 1µl. of a solution in n-hexane; attenuation 1×10^{2}.

B. As for Fig. 11a; sample size 0.7µl. of a solution in n-hexane.

C. As for Fig. 11a; sample size 0.4µl. of a solution in n-hexane.

Fig.11

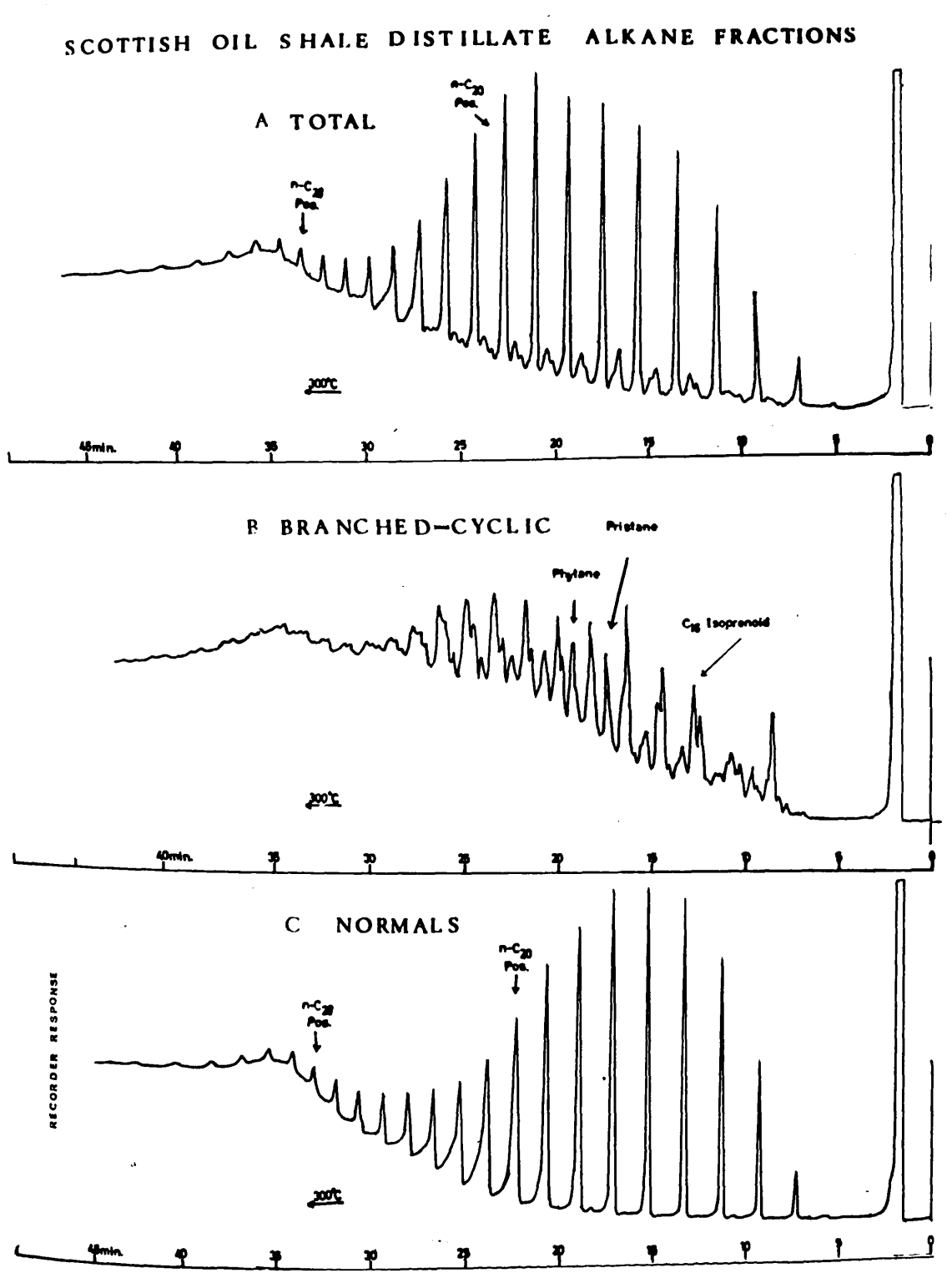

SCOTTISH OIL SHALE DISTILLATE ALKANE FRACTIONS
A TOTAL
n-C20 Pos.
n-C18 Pos.
300°C
45min.
40
35
30
25
20
15
10
5
0
B BRANCHED-CYCLIC
Pristane
Phytane
C18 Isoprenoid
300°C
40min.
35
30
25
20
15
10
5
0
C NORMALS
n-C20 Pos.
n-C18 Pos.
RECORDER RESPONSE
300°C
45min.
40
35
30
25
20
15
10
5
0

Fig.12.

Mass Spectra of Acyclic Isoprenoid Hydrocarbons.

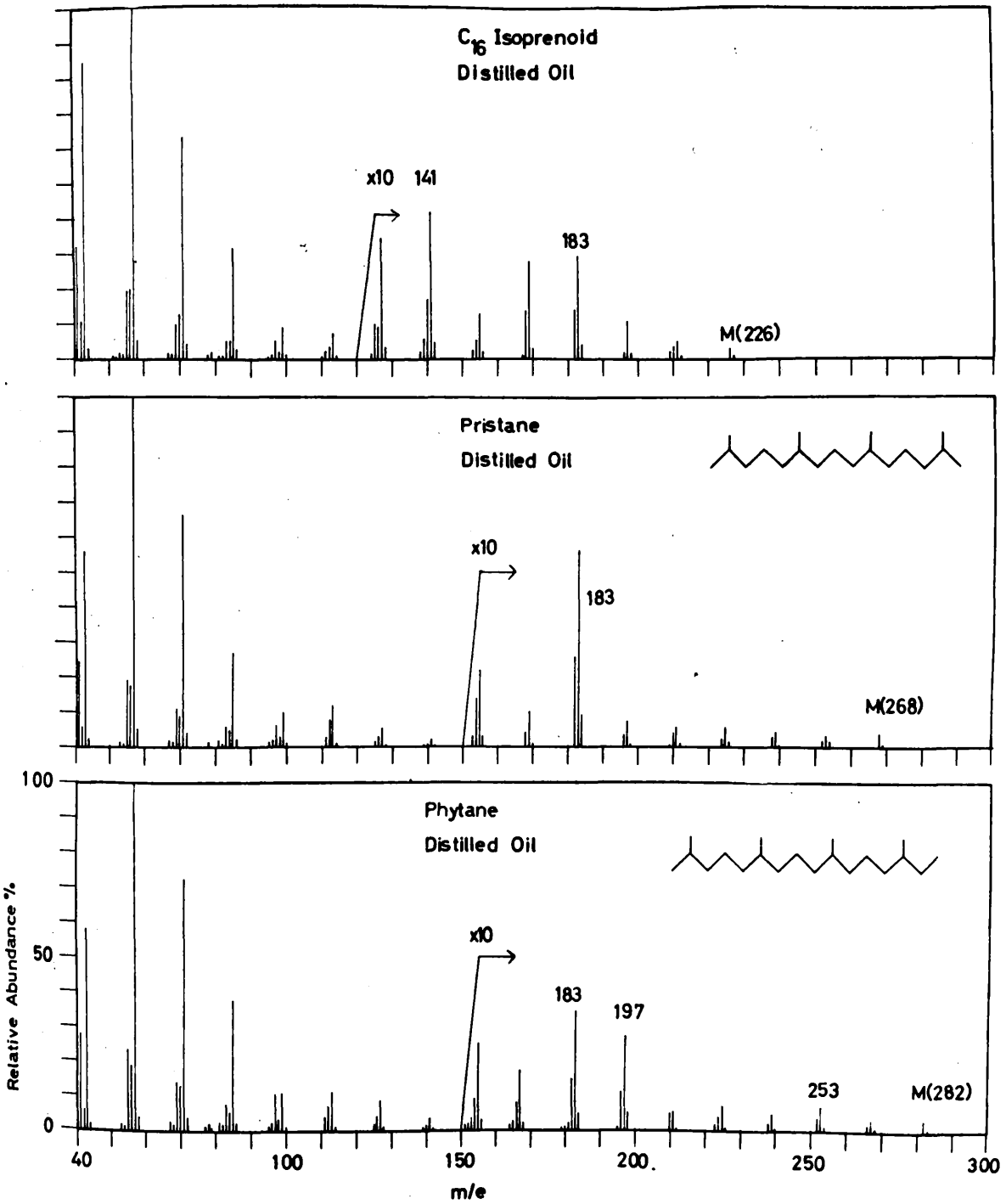


Fig. 13 legend.

Gas Chromatograms of the Scottish Oil Shale Distillate alkene fractions.

Conditions:

A. Column 10 ft. x 1/16 in.; 3% SE-30 on Gas Chrom P, 100-120 mesh; temperature programmed from 100° to 300°C at 6°/min.; flow rate approximately 20ml./min. nitrogen at 18 p.s.i.; sample size 0.4µl. of a solution in benzene; attenuation 50 x 1.

B. As for Fig.13A; sample size 0.36µl. of a solution in n-hexane.

Fig.13.

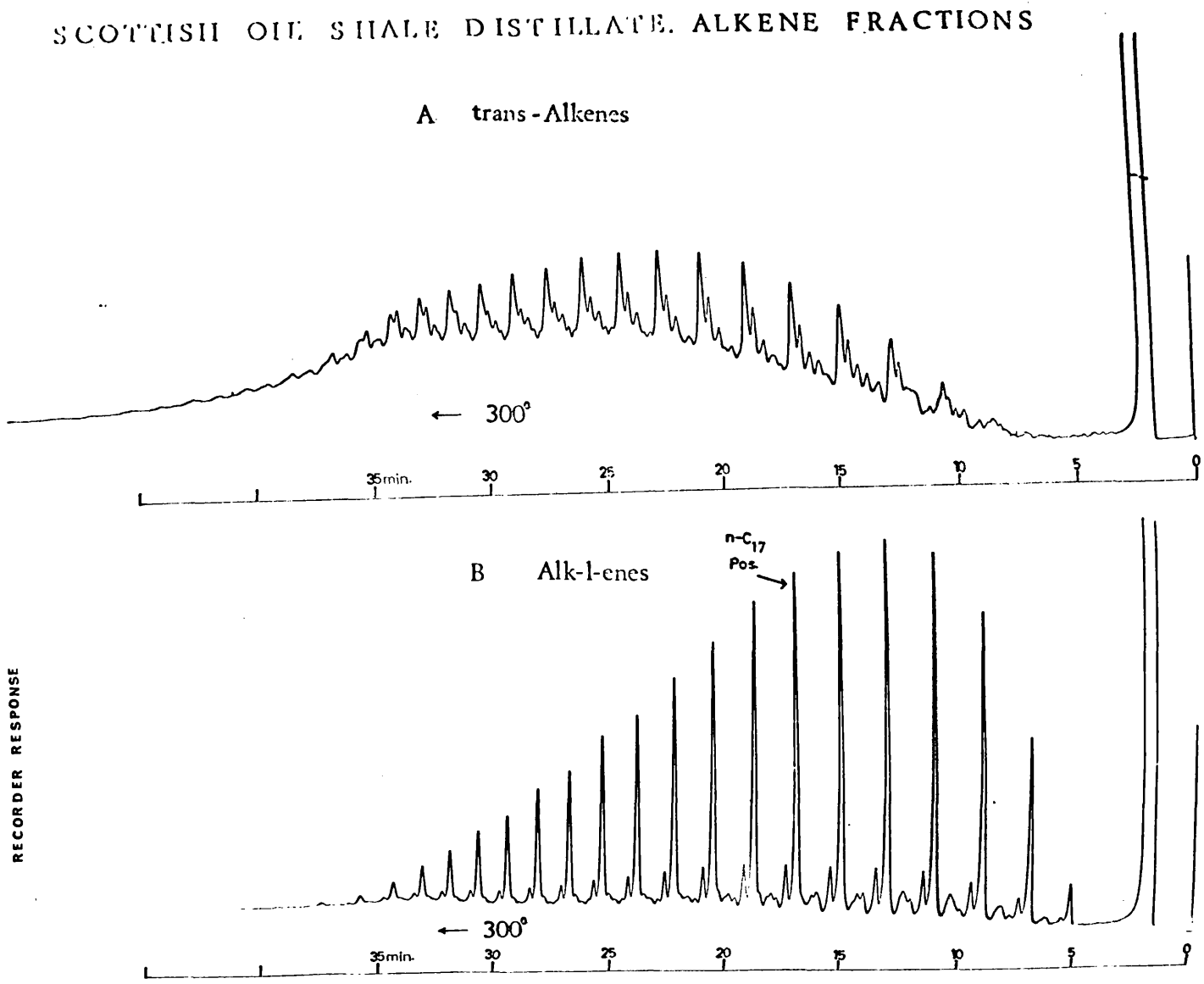

SCOTTISH OIL SHALE DISTILLATE. ALKENE FRACTIONS
A trans-Alkenes
← 300°
35min.
30
25
20
15
10
5
0
B Alk-1-enes
n-C17
Pos.
RECORDER RESPONSE
← 300°
35min.
30
25
20
15
10
5
0

Fig.14 Legend.

Gas Chromatograms of D'Arcy Oil alkane fractions.

Conditions:

A. Column 10 ft. x 1/8 in.; 1% SE-30 on Chromosorb W, 100-120 mesh; temperature programmed from 100° to 300°C at 6°/min.; flow rate approximately 25ml./min. nitrogen at 20 p.s.i.; sample size 0.2µl. of a solution in n-hexane: attenuation 1×10^2.

B. As for Fig. 14A; sample size 1µl. of a solution in iso-octane.

C. As for Fig.14A; temperature programmed from 100° to 300°C at 8°/min.; sample size 0.3µl. of a solution in iso-octane.

Fig.14.

CRUDE OIL: D'ARCY WELL ALKANE FRACTIONS

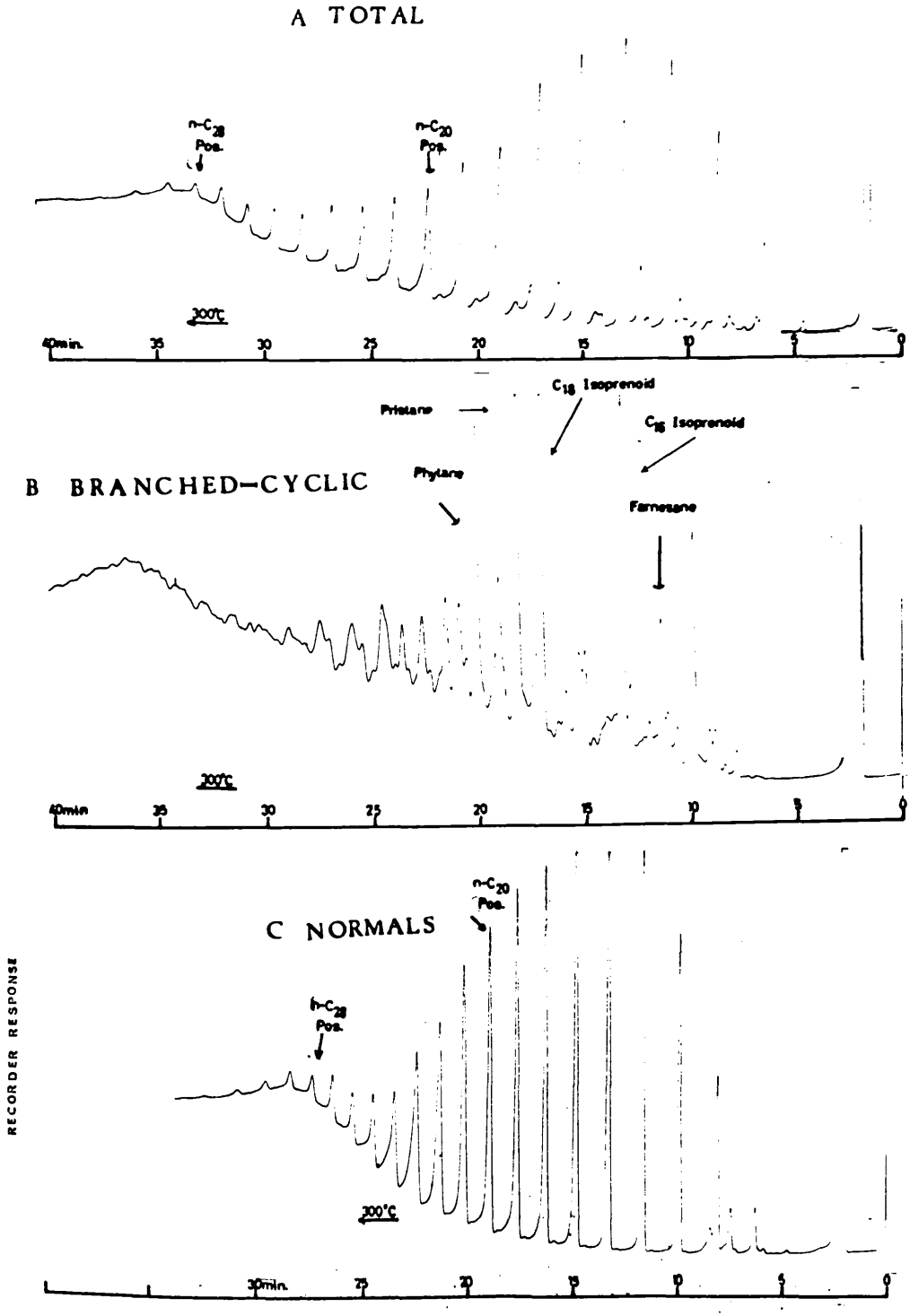


Fig.15.

Mass Spectra of Acyclic Isoprenoid Hydrocarbons.

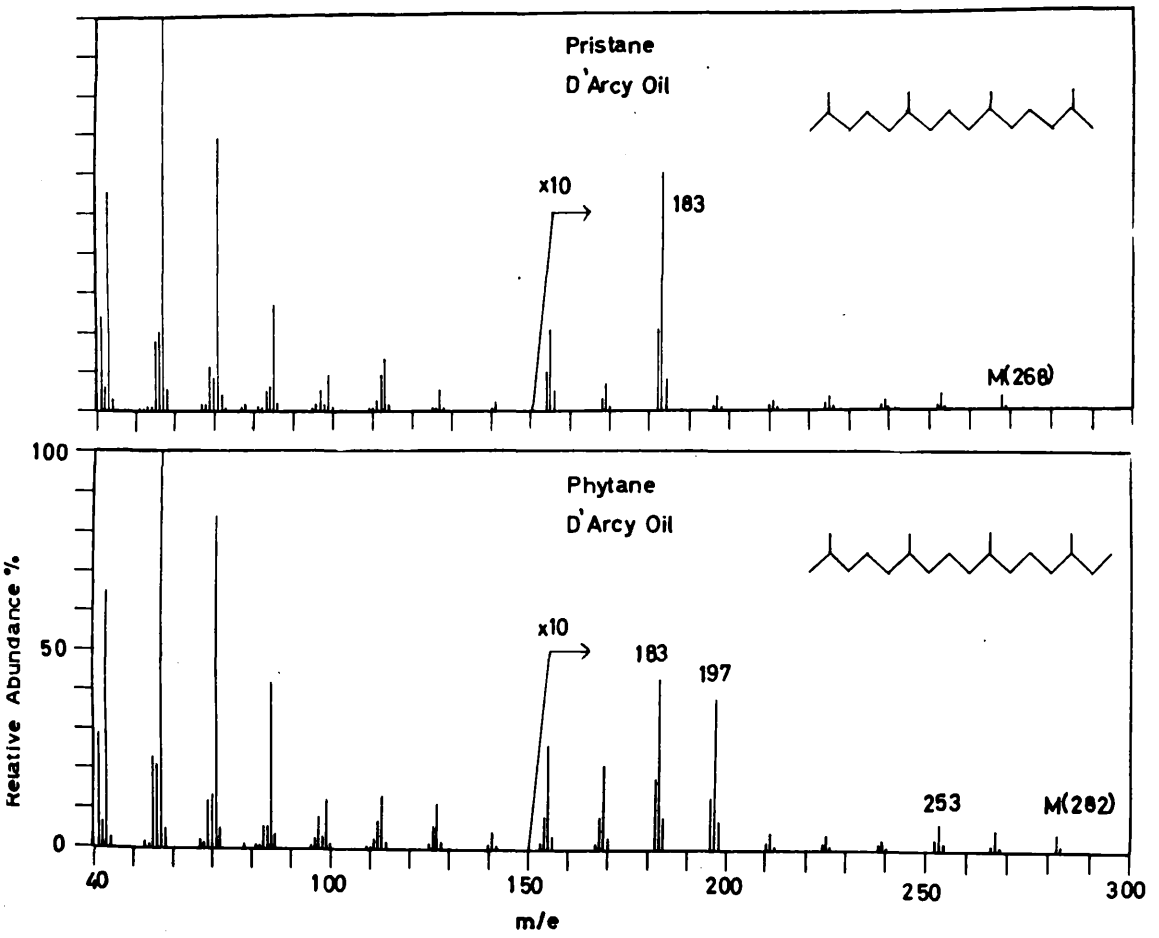
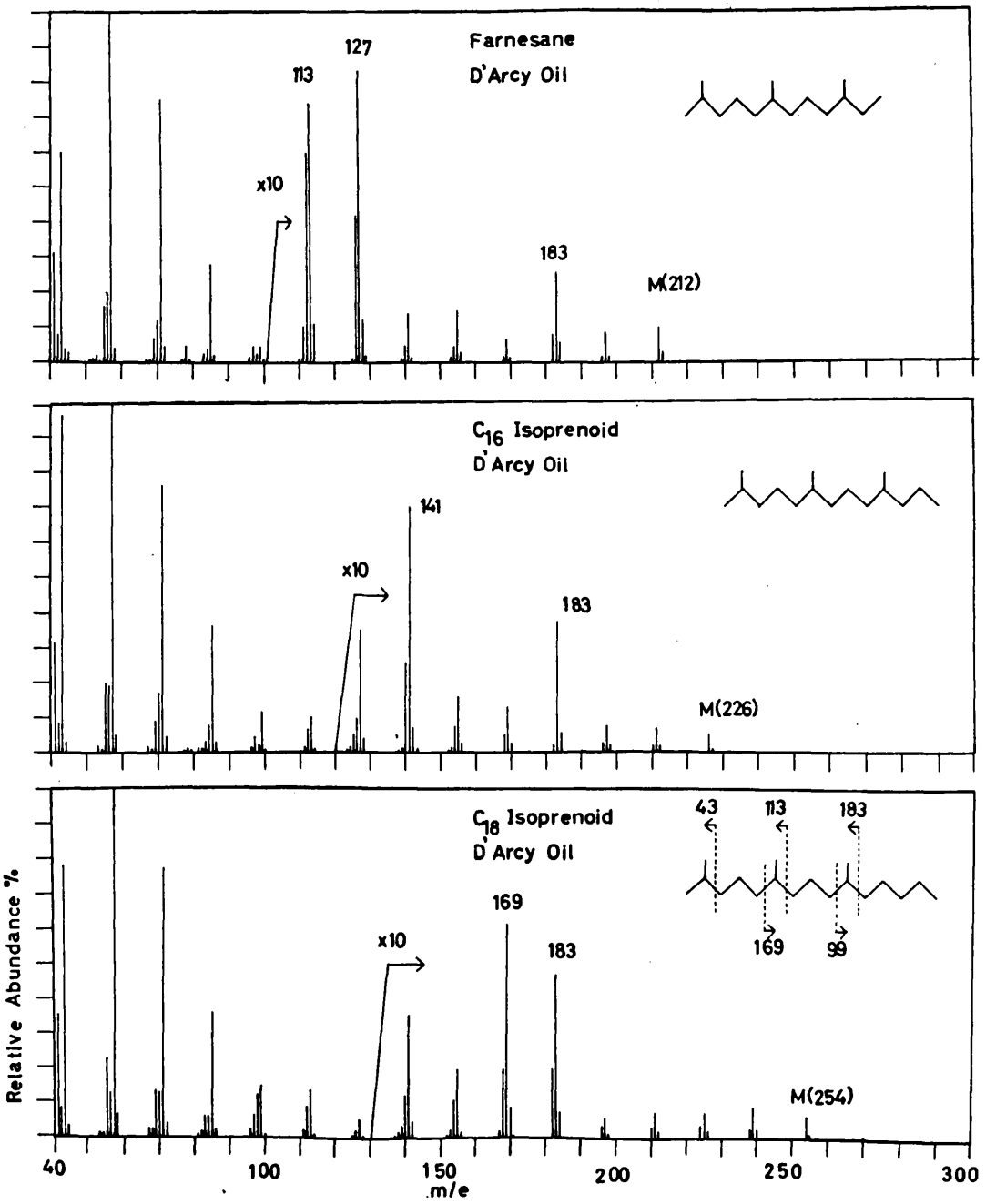


Fig.16.

Mass Spectra of Acyclic Isoprenoid Hydrocarbons.



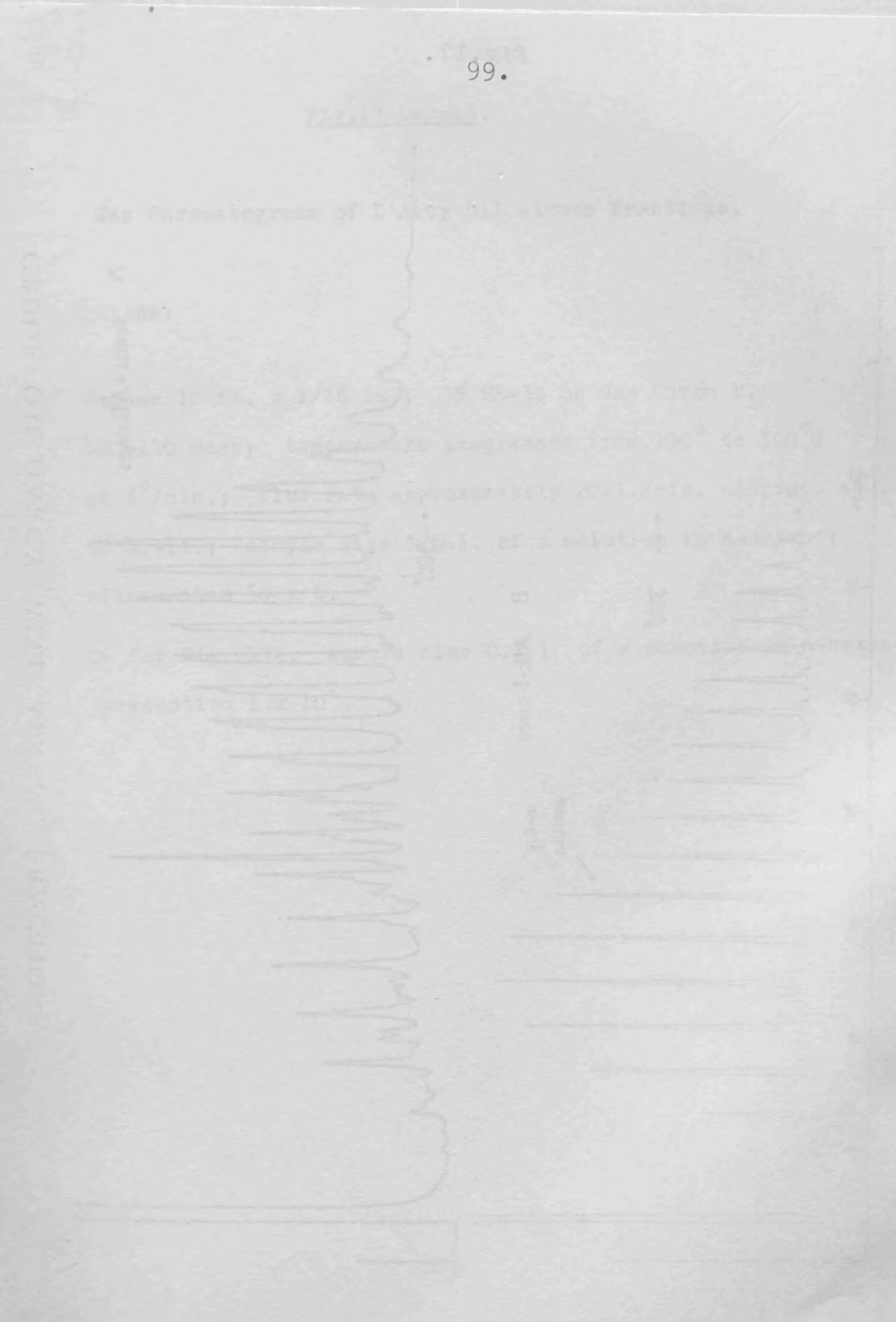


Fig.17 Legend.

Gas Chromatograms of D'Arcy Oil alkene fractions.

Conditions:

A. Column 10 ft. x 1/16 in.; 3% SE-30 on Gas Chrom P, 100-120 mesh; temperature programmed from 100° to 300°C at 6°/min.; flow rate approximately 20ml./min. nitrogen at 16 p.s.i.; sample size 0.5µl. of a solution in n-hexane; attenuation 50 x 1.

B. As for Fig. 17A; sample size 0.1µl. of a solution in n-hexane; attenuation 1 x 10^{2}.

CRUDE OIL: D'ARCY WELL. ALKENE FRACTIONS

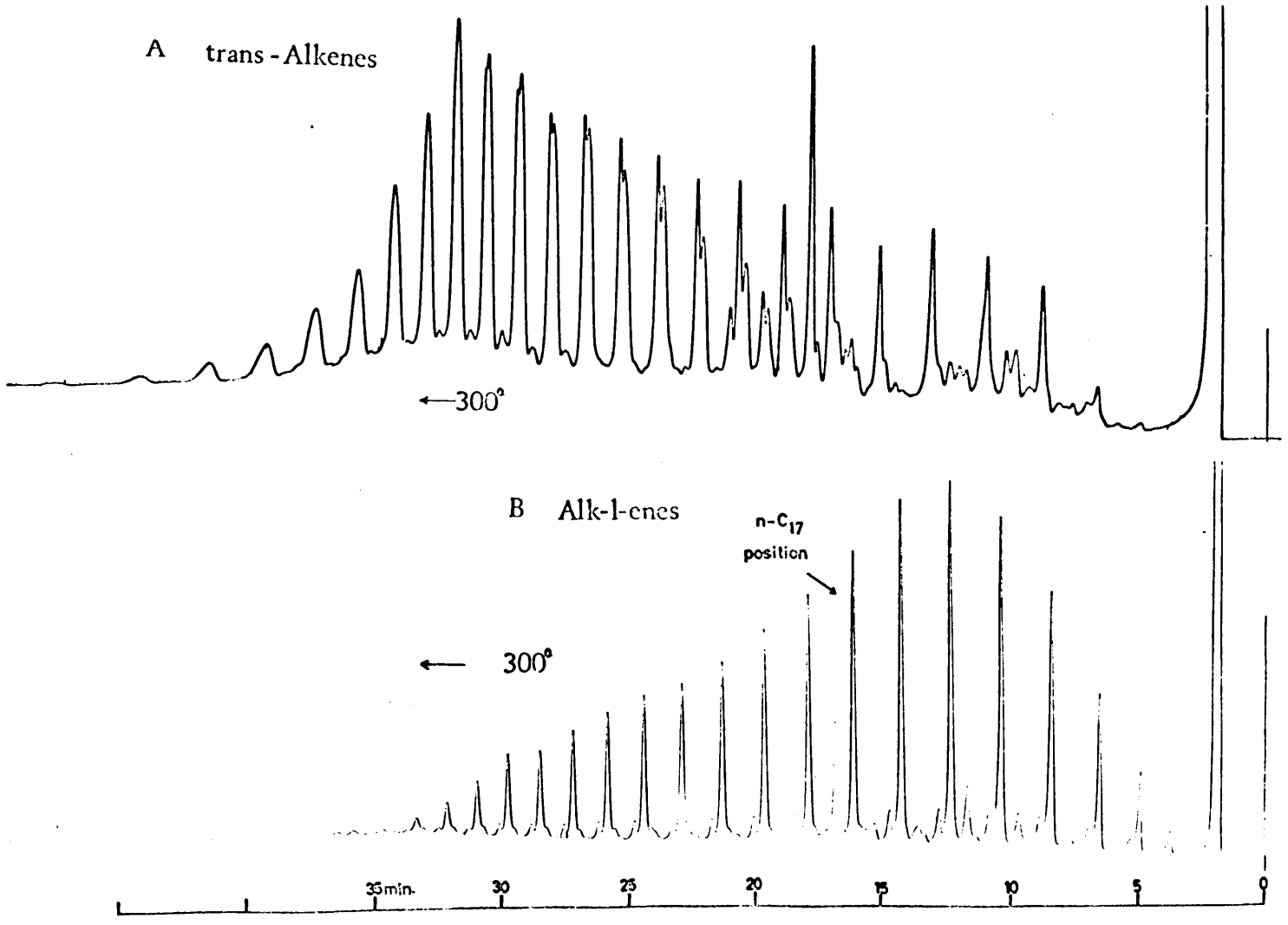


Fig.17.

Fig.18 Legend.

Gas Chromatograms of the alkane fractions obtained by reduction of the trans alkene fraction of D'Arcy Oil.

Conditions:

A. Column 10 ft. x 1/8 in.; 1% SE-30 on Chromosorb W, 100-120 mesh; 25 ml./min. nitrogen; temperature programmed from 100° to 300°C at 6°/min.; sample size of a solution in n-hexane 0.3µl.; attenuation 1 x 10^2.

B. Column 10 ft. x 1/16 in.; 3% OV-1 on Gas Chrom Q, 60-80 mesh; 20 ml./min. nitrogen; temperature programmed from 100° to 300°C at 3°/min.; sample size 20µl. (split) of a solution in n-hexane; attenuation 1 x 10^2.

C. Column 10 ft. x 1/16 in.; 3% SE-30 on Gas Chrom P, 100-120 mesh; 20 ml./min. nitrogen; temperature programmed from 100° to 300°C at 6°/min.; sample size 0.18µl. of a solution in n-hexane; attenuation 50 x 1.

Fig.18

ALKANE FRACTIONS OBTAINED BY REDUCTION OF THE TRANS ALKENES OF THE D'ARCY OIL

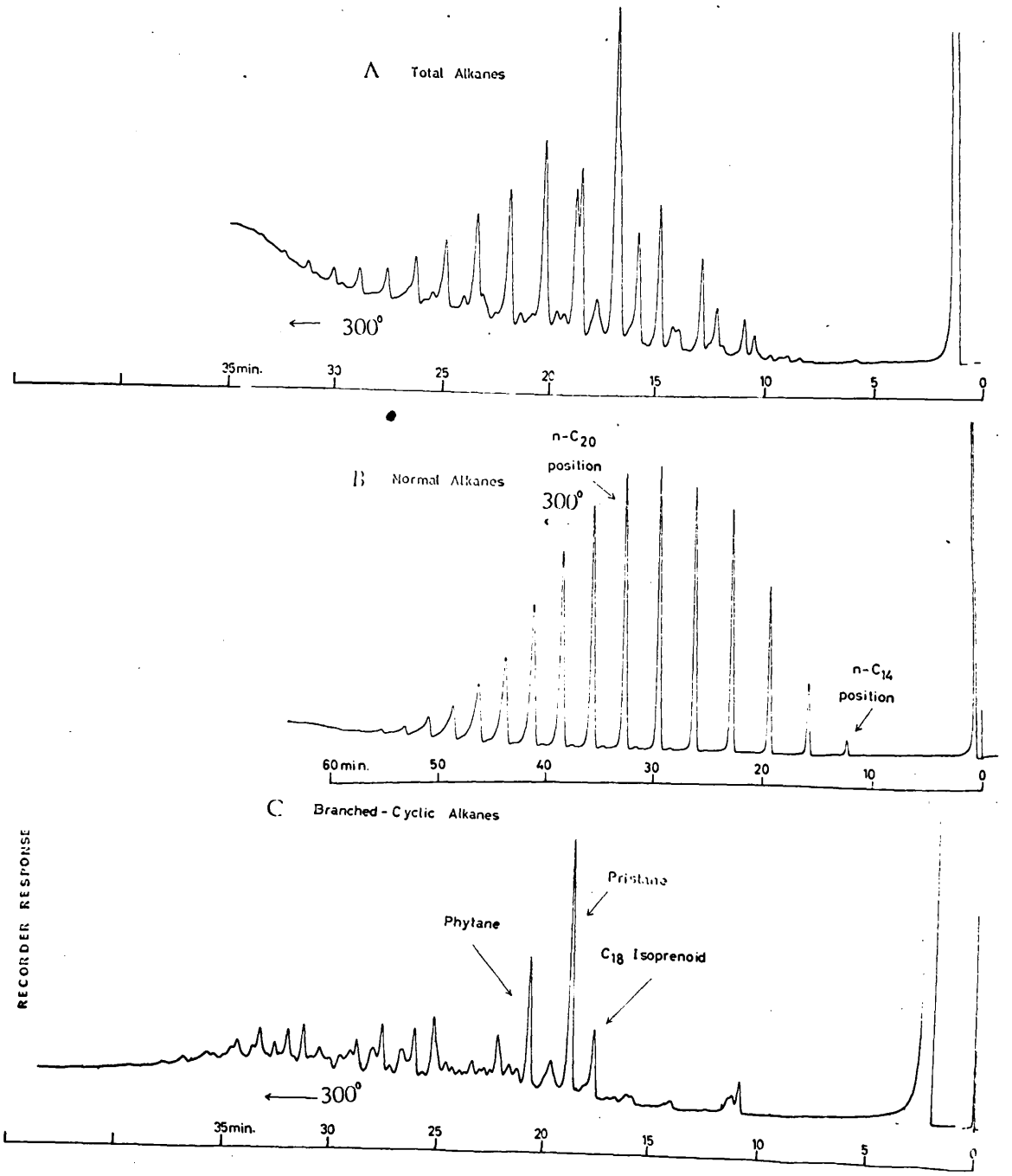
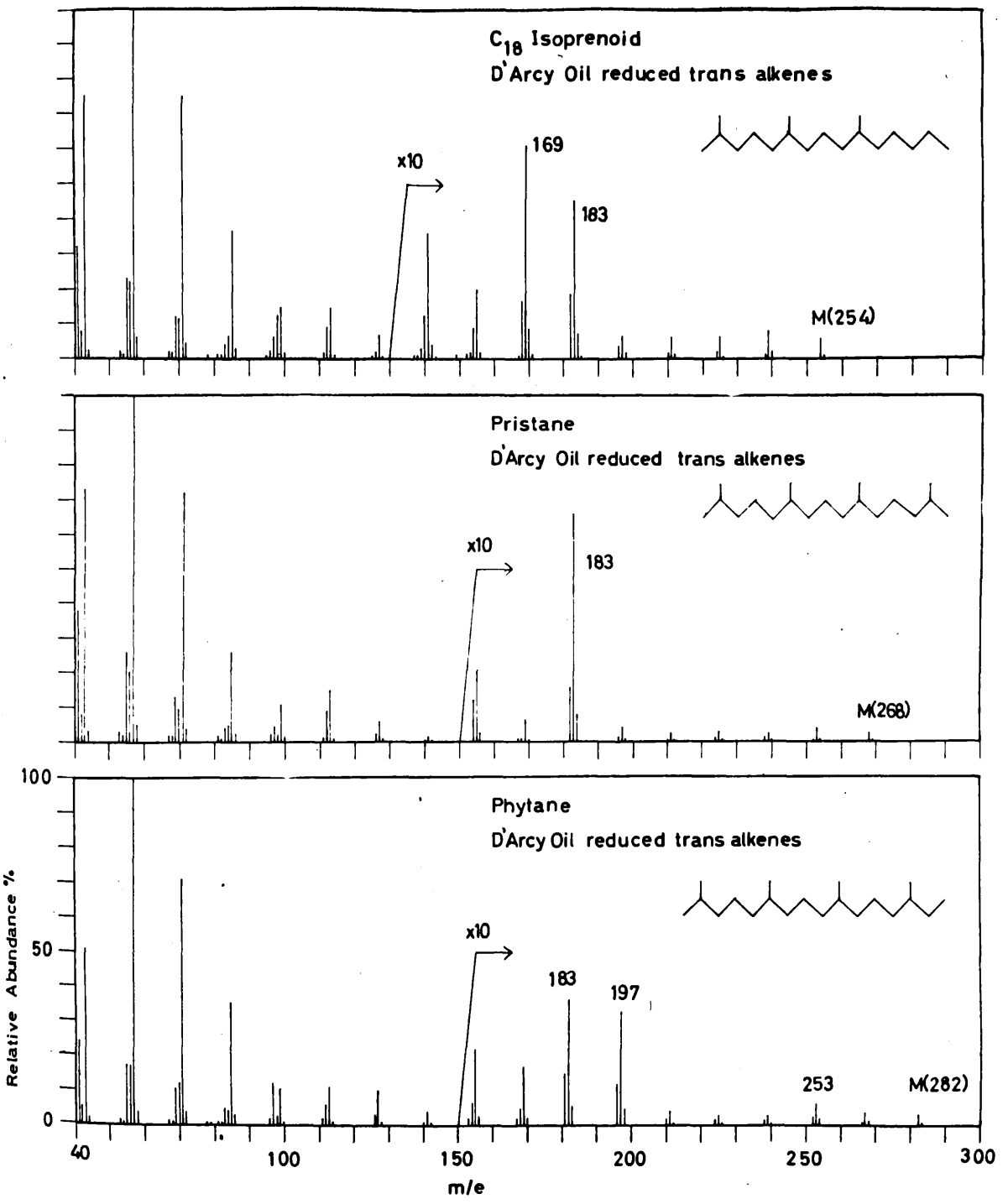


Fig.19.

Mass Spectra of Acyclic Isoprenoid Hydrocarbons.



SECTION III

THE HYDROCARBONS OF COORONGITE

INTRODUCTION

Coorongite is a dark-coloured inflammable material whose occurrence was first reported in 1852 in the Coorong district of South Australia. It resembles india rubber in appearance and texture; when ignited it melts and burns with a smoky flame. Its mode of occurrence was first described by Dyer[114] in 1872, who states "It consists of sheet-like masses somewhat more than an inch in thickness..... and is confined to a depressed portion of the district, the bottom of which is sandy and grass-covered; it occurs on the banks forming the margin of the depression and also on the sides of island-like elevations which are scattered about it." He concluded that it was of mineral origin.

However the actual formation of Coorongite was observed in 1920 by Broughton[115] who reported the accumulation of a green scum on lagoons in the Coorong district. As the scum dried it formed a skin which was driven by changing winds to the shore and was deposited there as fresh Coorongite. The formation was also reported by de Hautpick[116] who reported that a green material, rising to the surface of the lagoons, was driven by the wind to be deposited in strips on the shore. The green scum giving rise to Coorongite was later identified as the alga Botryococcus Braunii[117]. This alga was also identified in material (Balkhashite) similar to Coorongite found on Lake Balkhash[118]. Materials closely related to Coorongite also occur in Portuguese East

Africa (N'hangellite), Brazil (Marahunite) and Fiji[119]. B. braunii and Coorongite are regarded as the precursors of Torbanite[117,119] (Section IV).

It was early found that oil resembling mineral oils could be distilled from Coorongite. However, to date, almost all of the analyses carried out have been very crude. According to Cummings[120] analysis there were two constituents present in Coorongite besides the ash. One of these was an unsaponifiable wax-like solid which could be extracted with carbon disulphide and which constituted about one third the weight of Coorongite. The other was insoluble in carbon disulphide but could be saponified by ethanolic potassium hydroxide, forming a soap. Coorongite has been shown to contain unsaturated centres, as the ether extract of a sample had an iodine value of 71.6[117]. The insoluble residue, left after the solvent extraction of Coorongite was also examined with the following findings "The substance was treated with fuming nitric acid at 100°C. and then steam distilled. No nitrophenols appeared in the distillate; from this one can deduce that no considerable amounts of aromatic compounds were present. On the other hand, fatty acids were discovered which, on further treatment, were found to boil in the region of 100°-180°C. The residue from the steam distillation was filtered and yielded oxalic acid on evaporation. The fatty acids were about 20% of the original weight."

DISCUSSION

The examination of the hydrocarbon content of Coorongite was undertaken because of the presumed derivation of Torbanite from Coorongite and the alga Botryococcus braunii[117]. There is no doubt that Coorongite arises from B. braunii since its formation has actually been observed in the natural environment of the latter. Although there is no proof that Coorongite and B. braunii are the precursors of Torbanite there is very good evidence for this from the striking morphological similarities between the "yellow bodies" of Torbanite and the colonies of B. braunii[117].

Ultrasonic extraction of Coorongite with a mixture of benzene and methanol afforded the total lipid fraction as a brown gum whose infra-red spectrum indicated the presence of aromatic compounds, fatty acids and vinyl double bonds. The presence of unsaturation confirms the findings of earlier investigators[117].

Separation of the total lipid fraction by means of column and thin-layer chromatography gave a number of hydrocarbon fractions, the alkane fraction comprising 0.16% by weight of the sample of Coorongite examined (0.32% of the total lipid fraction). The gas chromatogram of the total alkane fraction is shown in Fig.1 and unexpectedly shows no odd/even predominance of the normal alkanes although the two Coorongite samples examined are only about forty years old. The hydrocarbon distribution ranges from n-C_{14} to n-C_{27} and contrasts markedly with the total fatty acid distribution which ranges from n-C_{14} to n-C_{28} but which shows a marked even/odd

preference with n-C_{16} and n-C_{18} the most abundant acids[18]. The normal alkane fraction of Coorongite obtained by treatment with 5Å molecular sieve shows a smooth distribution ranging from about n-C_{14} to about n-C_{25} with a maximum at n-C_{17}. The distributions of the total alkanes and n-alkanes bear no resemblance to the fatty acids of B. braunii itself since the hydrogenated fatty acids of the latter, comprising 0.01% of the dry weight of the alga, show a very marked even/odd preference. In addition, the saturated fatty acids obtained by silver ion thin layer chromatography of the total fatty acid fraction of B. braunii show a marked even/odd preference with the n-C_{16} and n-C_{18} acids the most abundant[121]. The gas chromatograms of the alkane fractions derived from both Coorongite samples examined show similar distributions. It is unlikely, therefore, that the alkanes could have arisen from contamination of the samples through handling prior to the analysis. Contamination during the analysis is unlikely, since a blank experiment carried out in parallel with one of the extractions showed no peaks in the gas chromatogram under identical analytical conditions.

The gas chromatogram of the branched-cyclic fraction obtained by treating the total alkane fraction with 5Å molecular sieve is shown in Fig.2. The major components of peaks B and C in the gas chromatogram have mass spectra (Fig.3) markedly similar to those of pristane and phytane respectively (Section II, Fig.5). However, both spectra are somewhat reduced in intensity, indicating that the samples contain minor impurities. Co-injection of these two fractions with pristane and phytane showed enhancement of Peaks B and C

in the gas chromatograms. Peak A in the gas chromatogram has a mass spectrum corresponding to that of the C_{18} isoprenoid hydrocarbon but unfortunately no standard was available for comparison.

The infra-red spectrum of the second fraction obtained from the preparative thin-layer chromatogram indicated the presence of long chain aromatic compounds. This fraction was not examined further.

In addition, a fraction whose infra-red spectrum indicated vinyl absorption at 910 and 990cm.$^{-1}$ was isolated from the total hydrocarbon fraction. The gas chromatogram of this fraction is shown in Figure 2. Co-injection with n-heptadec-1-ene suggested that there was a homologous series of normal vinyl alkenes present, ranging from n-C_{16} to about n-C_{28}, with n-nonadec-1-ene the most abundant component. It is interesting to note that a series of n-monoenoic acids, each having a vinyl double bond, has also been isolated from Coorongite[18]. In this instance, however, the acids range from n-C_{16} to n-C_{20} and exhibit a marked even/odd preference. In the gas chromatogram shown in Figure 2 small amounts of branched vinyl alkenes can also be seen.

The infra-red spectrum of the fraction remaining at the origin of the preparative thin-layer chromatogram indicated vinyl and trans disubstituted absorption. The presence of a band at 720cm.$^{-1}$ indicated the presence of long chain hydrocarbons. Although this fraction was not examined further by thin-layer or gas-liquid

chromatography the absence of any exomethylene absorption in the infra-red spectrum indicates that the botryococcenes (Section IV) are either absent or present in very small amounts. Table I summarises the proportions of the various hydrocarbon fractions present in the sample of Coorongite examined.

The origin of the alkanes and the vinyl alkenes present in Coorongite raises some interesting points, especially as the n-alkanes show no odd/even predominance and there are no straight chain aliphatic hydrocarbons in the sample of B. braunii examined (Section IV). Also the geolipid fraction of Torbanite contains n-alkanes which comprise about 0.1% of the sediment.

It is noteworthy that Schwartz[122] has isolated desulphurising bacteria from Coorongite. Although very little information is available on the composition, nature and distribution of the simpler aliphatic hydrocarbons of bacterial lipid extracts, Albro and Huston[123] have examined the hydrocarbon content of Sarcina Lutea. This bacterium, found in air, soil and water all over the Earth, contains n-alkanes which show no odd/even preference, and in which n-C_{28} is the most abundant hydrocarbon. The total fatty acids of this bacterium range from C_8 to C_{22} in carbon number with n-C_{14} and n-C_{16} the most abundant acids[123].

It seems probable, therefore, that the alkane and vinyl alkene fractions arise from the bacteria present. Schwartz also

maintains that the formation of Coorongite is connected with microbiological processes. It follows from Section IV that the formation of Coorongite from B. braunii is almost certainly due to polymerisation of the botryococcene hydrocarbons present in the cell matrix but the bacteria present may play a part in the polymerisation.

The presence of bacteria in Coorongite may also explain the occurrence of isoprenoid hydrocarbons in the branched-cyclic alkane fraction since no saturated hydrocarbons were observed in the lipid fraction of B. braunii (Section IV). It appears that, in this instance, the formation of isoprenoid hydrocarbons from the chlorophyll of B. braunii is a consequence of bacterial action. This result is unusual since the occurrence of phytane in recent sediments is unknown and the Coorongite sample examined is only about forty years old.

If Coorongite is the precursor of Torbanite and the alkanes and vinyl alkenes are indeed bacterial lipids then it seems likely that the alkanes of Torbanite are in part derived from these. The n-alkanes may also arise in part from the fatty acids of Coorongite and B. braunii. Jurg and Eisma[31,32] have shown that thermal treatment of behenic acid gives rise to a homologous series of n-alkanes and suggest that a radical process accounts for this. Very little is known at present about the role played by bacterial action in the diagenesis of sediments or the contribution of bacterial lipids to geolipids. Leo and Parker[124] suggest that bacterial lipid is a likely source for the

high proportion of iso- and anteiso-acids in the Green River Shale. The C_{20} isoprenoid di-ether isolated from the lipids of certain halophilic bacteria[50] may also partly account for the phytane found in sediments. Douglas et al[108] have recently isolated a homologous series of α, ω -dicarboxylic acids from Torbanite and conclude that these may have arisen from bacterial action. Bacterial oxidation of hydrocarbons and mono-carboxylic acids is known[125] and the above authors state that the appearance of these acids in Torbanite may not reflect the organic matter indigenous to the original deposit but rather microbial oxidation of the appropriate substrate. ZoBell[126, 127] has suggested that bacteria play a considerable part in the diagenesis of plant remains; this is clearly a field worthy of study by the organic geochemist.

EXPERIMENTAL

Two separate samples of Coorongite were examined in the present investigation. One sample was collected about 1926 by Dr. K. Washington from the Coorong district, South Australia, and was obtained through the courtesy of Professor M.F. Glaessner, Geology Department, University of Adelaide, via the good offices of Dr.W.D.I. Rolfe. The second sample was obtained from Dr. W.E. Robinson, U.S. Bureau of Mines, Laramie, U.S.A. and was also from the Coorong district.

Treatment of Coorongite and lipid extraction procedure.

A piece(6.2g.) of Coorongite (first sample) was cut into thin sections and extracted with benzene/methanol (3:1; 50ml.) for 60 min. It was noted that the ultrasonic energy and the solvent did not break up the sections. The solvent was removed and the sections were macerated with a little benzene/methanol in a homogeniser (M.S.E. Micro Emulsifier) for 15 min. until the Coorongite became an oily sludge. The suspension was then decanted into a centrifuge bottle (100 ml.) and extracted ultrasonically (sonitank) four times (30 min. each) with benzene/methanol (3:1; total 200 ml.). The clear solution obtained after centrifuging was decanted and evaporated. The i.r. spectrum (film) of the resulting brown gum (3.1g.) showed absorption at 3300 (m, νO-H), 1710 (m, νC=O), 1645 (m, νC=C), 1595 (s, νC=C). 990 and 910 (w, m, γC-H vinyl) $725cm.^{-1}$(m. $-(CH_2)_n$-rock).

Isolation of Alkanes and Alkenes.

The extract was chromatographed on alumina (150g.) and the total n-hexane eluate (34mg.) was collected. The i.r. spectrum (film) showed absorption at 1645 (w, νC=C), 968 (m, γC-H trans disubstituted), 990 and 910 (m, w, γC-H vinyl) and 720 cm.$^{-1}$ (m, -$(CH_2)_n$-rock). T.l.c. on silica/silver nitrate (iso-octane developer) showed that the hexane eluate was comprised of four fairly discrete compound types. Four fractions (a)-(d) were subsequently obtained by preparative-scale t.l.c.

Fraction (a) (10mg.) had an R_f value identical to that of a standard n-alkane mixture. The i.r. spectrum (film) showed only saturated hydrocarbon bonds which absorbed at 1460 (s, $\delta CH_2, CH_3$), 1375(m, δCH_3), 720cm.$^{-1}$(m, -$(CH_2)_n$-rock). G.l.c. (Fig.1) showed that the distribution of the alkane fraction was mainly between n-C_{15} and n-C_{21} (established by co-injection with n-C_{16} and n-C_{18}). The same alkane distribution was also obtained from the second sample of Coorongite.

An aliquot (5mg.) of the alkane fraction was treated with 5Å molecular sieve in the usual manner. A normal (1mg.) and a branched-cyclic (3mg.) fraction were thus obtained. G.l.c. (Fig.1) showed the n-alkane fraction as a smooth envelope of peaks, ranging from C_{14} to about C_{25} with a maximum at n-C_{17} (established by co-injection with n-C_{14}). The branched-cyclic fraction (Fig.2) had a distribution somewhat similar to that of the total alkane fraction.

The fractions containing the three most abundant components were collected in the usual manner by preparative-scale g.l.c. An examination of these two fractions in the gas chromatograph-mass spectrometer indicated that each fraction was comprised of a number of components and the best separation was achieved by using a column containing 10% carbowax on Gas Chrom P. The mass spectrum of the major component of each fraction is shown in Fig.3. The major component of fractions B and C had mass spectra corresponding to those of pristane and phytane respectively. Co-injection of fractions B and C with pristane and phytane showed enhancement of the peaks due to the major components (column 50m. x 1/4 m.m., 7 ring m-polyphenyl ether; isothermally at 158°C). The major component of fraction A had a mass spectrum very similar to that of the C_{18} isoprenoid hydrocarbon isolated from some of the Carboniferous samples (Section II).

Fraction (b) [6mg.] was not a single discrete spot under conditions of analytical t.l.c. and the i.r. spectrum (film) showed absorption at 1605 (w, νC=C), 1460 (m, $\delta CH_2, CH_3$), 1375 (m, δCH_3), 820(w), 745(w), 720(w, $-(CH_2)_n$-rock), 700cm.$^{-1}$ (w, γC-H) indicating that the fraction probably contained alkylbenzenes.

Fraction (c) [7mg.]. The i.r. spectrum (film) showed absorption at 3070 (w, νC-H), 1640(w, νC=C), 990 and 910(w, m, γC-H vinyl), 720cm.$^{-1}$ (m, $-(CH_2)_n$-rock). G.l.c. (Fig.2) showed that the fraction had a fairly smooth distribution of vinyl alkenes, ranging from C_{16} to

about C_{29}, with a maximum at n-C_{19}.

Fraction (d) [10mg.]. The i.r. spectrum (film) showed absorption at 1640 (w, νC=C), 990 and 910 (w, m, γC-H vinyl), 965 (m, γC-H trans disubstituted), 720cm.$^{-1}$(m, -$(CH_2)_n$-rock), indicating that there was unsaturation present in addition to long carbon-carbon chains.

TABLE 1

Proportion of Total Lipid Fraction and Hydrocarbon Fractions of Coorongite.

Fraction	% (by weight)
Total Extract	50
Alkanes	0.002
Alkylbenzenes	0.001
Vinyl Alkenes	0.001
Fraction (d)	0.002

Fig. 1 legend.

Gas chromatograms of Coorongite alkane fractions.

Conditions:

A. Column 30m. x 1/4 mm. SE-30 capillary; temperature programmed from 100° to 250°C at 6°/min.; sample size 2.2µl (split) of a solution in iso-octane; attenuation 50 x 1.

B. Column 50m. x 1/4 mm. 7 ring m-Polyphenylether capillary; temperature programmed from 150° to 210°C at 4°/min.; sample size 2.5µl. (split) of a solution in iso-octane; attenuation 20 x 1.

COORONGITE ALKANE FRACTIONS

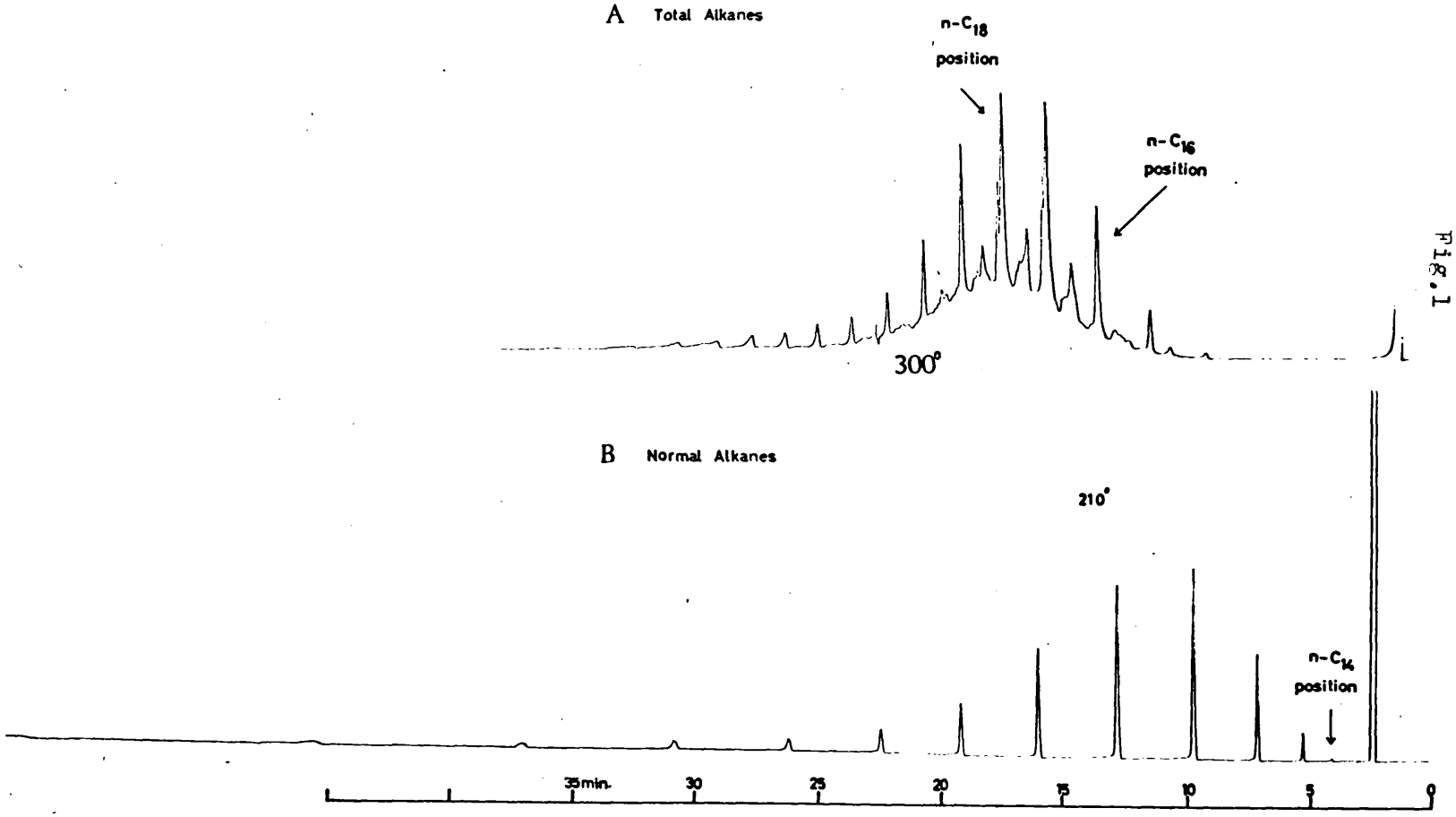


Fig. 1

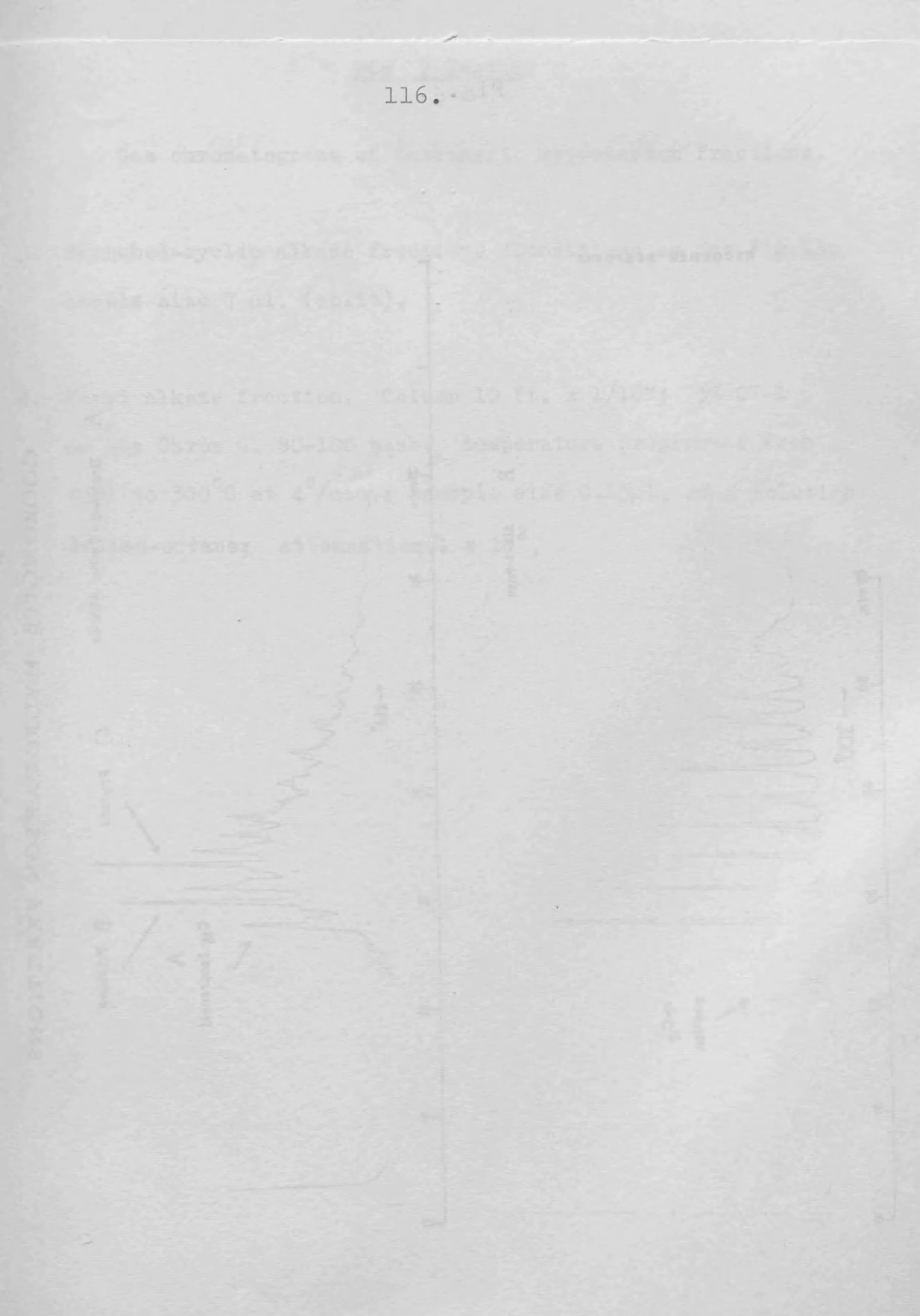


Fig 2 legend.

Gas chromatograms of Coorongite hydrocarbon fractions.

A. Branched-cyclic alkane fraction. Conditions as for Fig.1A; sample size 7 µl. (split).

B. Vinyl alkene fraction. Column 10 ft. x 1/16"; 3% OV-1 on Gas Chrom Q, 80-100 mesh; temperature programmed from 100° to 300°C at 4°/min.; sample size 0.15µl. of a solution in iso-octane; attenuation 1 x 10^2.

Fig.2.

COORONGITE HYDROCARBON FRACTIONS

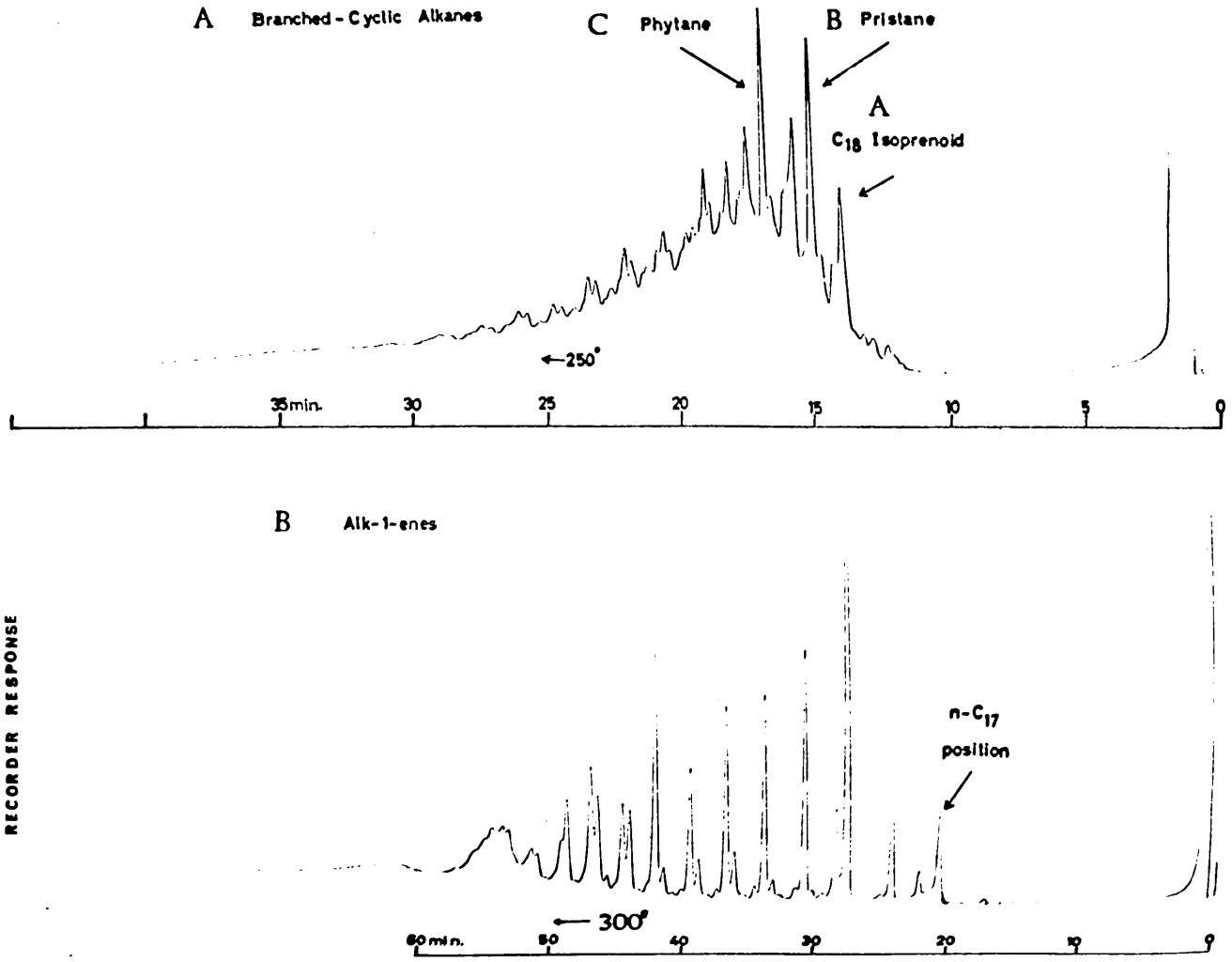
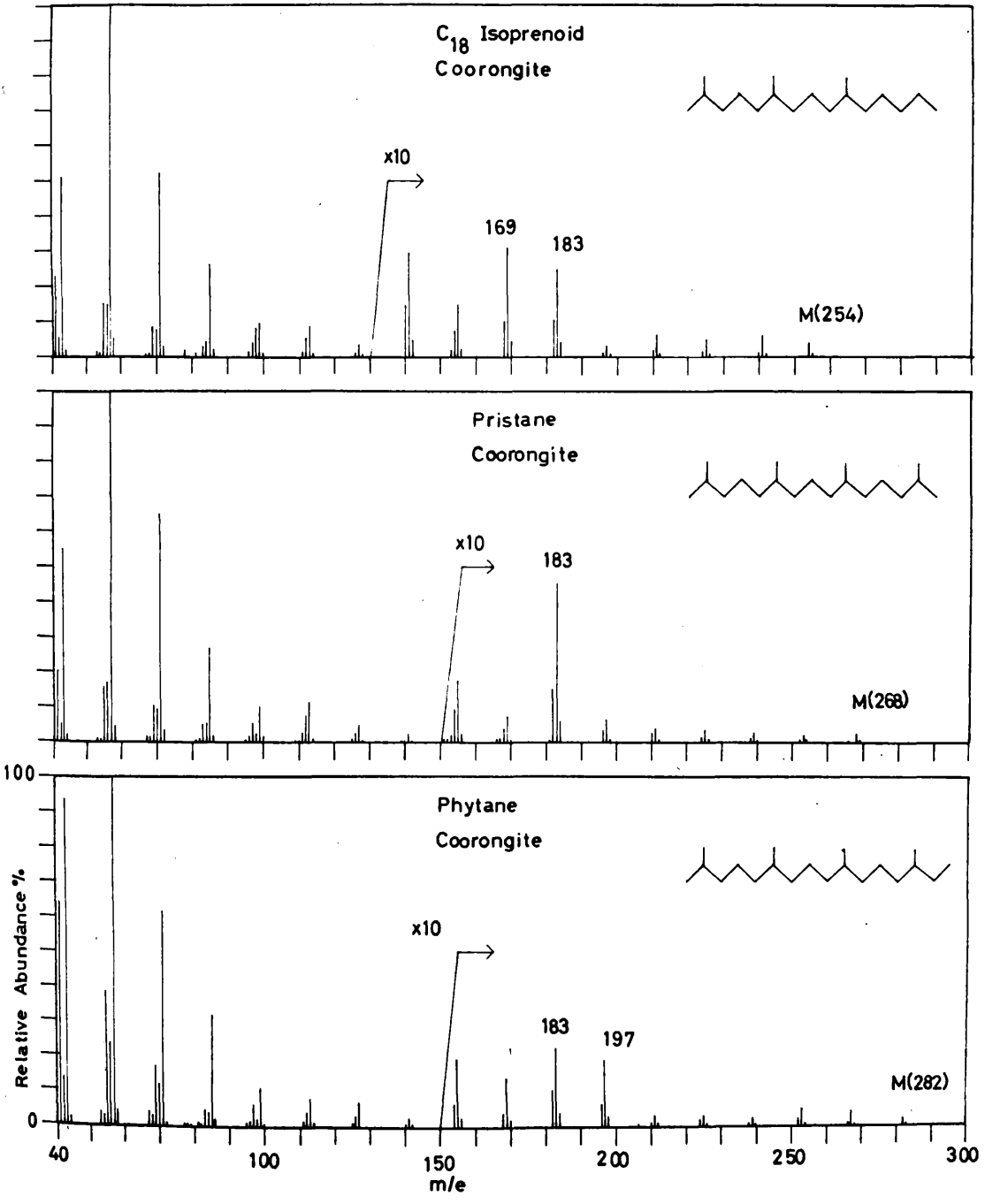


Fig.3.

Mass Spectra of Acyclic Isoprenoid Hydrocarbons.



SECTION IV

THE BOTRYOCOCCENES - HYDROCARBONS OF NOVEL STRUCTURE FROM THE ALGA BOTRYOCOCCUS BRAUNII, KUTZING

INTRODUCTION

Botryococcus Braunii Kutzing is the most widespread of the British Botryococcus algae, of which there are four[128]. It is common but not abundant in Britain and occurs in Scandinavia, Africa, Asia, America, Australia and New Zealand in a variety of climates. In Australia it gives rise to rubbery deposits called Coorongite. Usually the alga is found in fresh water but according to Blackburn[117] it occurs also in the salt water of Lake Balkhash. Blackburn[117] assigned B. braunii to the Chlorophyceae because of the presence of green chloroplasts and starch in the cells. This finding was later confirmed by Belcher and Fogg[109] who isolated chlorophylls a and b from the alga. Prior to this B. braunii had been assigned to the Xanthophyceae by some authors and to the Chlorophyceae by others.

The unusual morphology, behaviour and chemical composition have been extensively studied by Blackburn[117] and Belcher[129]. These findings prompted Belcher to record that "Though there is probably no organism which resembles Botryococcus as a whole, parallels may be found in other algae to single aspects of its structure or behaviour".

B. braunii forms approximately spherical colonies which may be attached by threads to form larger compound colonies. According to Belcher[129], the colonies vary in size, texture and structure, with the

result that some authors have assumed the existence of a number of different races. The colonies are green or orange, the orange colour being apparent under conditions of nutrient starvation, although in culture they are usually green. After reaching a certain size the colonies increase in number by forming daughter colonies. The actual cells are oval or pear-shaped and radially arranged in the peripheral part of the colony. The most conspicuous part of the cell contents is the green parietal chloroplast which is full of starch grains. The nucleus is small and lies in the middle of the widest part of the cell, surrounded by a large number of large globules whose exact constitution is unknown.

Each cell is embedded in a cup of "fatty material" and when a cell divides the two daughter cells secrete "fatty" cups, while remaining inside the cup of the mother cell. Thus the matrix of the colony is built up of the cups of the daughter cells. The colonies have a rubbery texture and can be flattened out under the pressure of a cover slip so that drops of oil from the colony matrix are exuded. The colonies recover their shape when the pressure is removed.

The chemical constitution of the colony matrix has been the subject of several investigations. Blackburn[117] found that B. braunii, when placed in iodine solution, absorbed so much iodine that the colonies sank to the bottom. The high degree of unsaturation present in the living alga was also implied by the high iodine value of 71.6 for the ether-soluble extract of Coorongite. Earlier investigators[118, 130,131], also examined the chemical constitution of Coorongite and quoted

a number of high iodine values for various fractions.

From these and her own results Blackburn came to the conclusion that the readily-soluble fraction of the alga contained "oils and fatty acids", whereas the less soluble fraction probably consisted of "more complicated derivatives of fatty acids which, by condensation or anhydride formation became quite insoluble and inert".

Belcher[129], working with the living alga, found B. braunii to contain 7 - 12% saponifiable lipid and 16 - 23% unsaponifiable lipid. However, Colleyer and Fogg[132] examined a number of algae and found the unsaponifiable lipid content to be usually less than 1%. The unsaponifiable lipid from B. braunii was an oil at room temperature but Belcher was unable to obtain it free from β-carotene by using various solvents and by chromatography over calcium hydroxide.

It was also highly unsaturated, as shown by the increase in viscosity on exposure to the air. Although he quoted no iodine value he showed that the algal colonies strongly absorbed iodine from an aqueous solution. Further attempts to separate and characterise the components of this lipid fraction were unsuccessful. The chemical composition of B. braunii has been summarised by Belcher[129] (Table 1). β-carotene and α-carotene were also identified from their u.v. spectra but could not be obtained in the crystalline state because of contamination by the unsaponifiable lipid fraction. B. braunii is very unusual in producing large quantities of β-carotene, but not unique in this respect.

Swain and Gilby[133] identified the alga floating on Lakes Nicaragua and Managua in Nicaragua as Eloeophyton coorongiana Thiessen

and found it to be composed of "more than 90% oils and other hydrocarbons". This alga was incorrectly named since Thiessen[131] failed to identify E. coorongiana as B. braunii and pronounced the alga to be a new species. Incidentally, particles of the alga were observed in the stomach and gut of the living ostracods of the two lakes and Swain and Gilby believe it furnishes an important food supply for the ostracods.

B. braunii has also featured in the boghead coal controversy. From microscopic examination boghead coals are known to consist mainly of minute yellow globules called "yellow bodies". Balfour[134] and Redfern[135] thought that the globules were cells in a plant tissue whereas Quekett[136] and Bennett[137] claimed they were globules of bitumen. David[138] suggested that the "yellow bodies" were of algal origin but the idea did not receive general acceptance because it was difficult to see how unmineralised algae, which were thought to be perishable, could have survived - apparently intact - in the formation of the boghead coals. This idea was contested by Jeffrey[139], who thought the "yellow bodies" were spores, by Conacher[102], who believed them to be composed of resin, and by Cunningham-Craig[140], who regarded them as globules of oil solidified on an inorganic or other nucleus.

In 1914 Zalessky[130] first suggested that there was a correlation between the "yellow bodies" and B. braunii and Thiessen[131] was the first to make a direct comparison of the "yellow bodies" with a living alga but named the alga Eloeophyton coorongiana. C.E. Bertrand[141, 142, 143, 144], C.E. Bertrand and B.Renault[145, 146, 147, 148] and P. Bertrand[149, 150] also believed in the algal theory and gave an extensive description of the

"yellow bodies" but could not explain the apparent preservation of the alga. Zalessky[118] was the first to show how the alga could remain preserved in the boghead coals. He observed that the B. braunii colonies on the shores of Lake Balkhash coalesced to form rubbery deposits of "Coorongite" which were decay resistant. If these deposits were formed without coalescence on the lake floor in a matrix of mud then a mass similar to the boghead coals would form. Finally, in 1936 Blackburn and Temperley[117], after an extremely detailed microscopic examination of the alga and the boghead coals, maintained that the "yellow bodies" were formed from B. braunii.

DISCUSSION

The present study of this unusual alga was undertaken because of the supposed, above-mentioned derivation of Torbanite from B. braunii. Furthermore the description of the actually observed formation of Coorongite from the growth of B. braunii suggested that the chemical composition of the lipid fraction would be worthy of thorough study by modern chemical methods.

The expected difficulties in obtaining a large batch of this alga - which normally grows in very small quantities at any one time - were suddenly obviated by the appearance of an extensive "bloom" on Oakmere, Cheshire, in November 1965.

Fig.1 shows a number of colonies in the Botryococcus sample and Fig.2 represents a single colony under high magnification and clearly shows the single cells.

Solvent extraction of a freeze dried sample of B. braunii afforded the lipid fraction as a brownish-green oil whose infra-red spectrum had carbon-carbon double bond absorption at 892, 917, 979 and 1002cm^{-1}., confirming the high degree of unsaturation present in the colony matrix implied by earlier investigators (see above). However there was no carbonyl absorption in the infra-red, which indicated that the colony matrix was not composed of fatty acids as Blackburn[117] had suggested. This has been confirmed in this laboratory by Douglas, Eglinton and Douraghi-Zadeh[121] who have found the fatty acid content of B. braunii to be very small. Column chromatography of the crude lipid

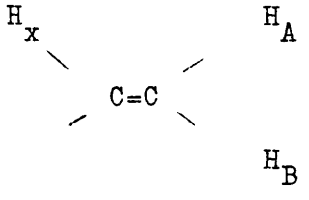
fraction gave a hydrocarbon fraction which comprised 76% of the dry weight of the alga. This remarkable result shows B. braunii to contain the highest concentration of hydrocarbon known in nature and contrasts with Belcher's figure of a maximum of 23% for the unsaponifiable lipid content of B. braunii[129] However the difference may be due to a seasonal variation or to the fact that Belcher's sample of B. braunii had been cultivated in the laboratory. That the hydrocarbon fraction is highly unsaturated is shown by the infra-red spectrum (Fig.3) which shows carbon-carbon double bond absorption at 892, 917, 980 and 1002cm^{-1}. Gas-liquid chromatography (Fig.4) showed the fraction to consist of two major components, termed botryococcene and isobotryococene, which were present in the ratio of about 90% to 10% respectively. Blecher's finding of β-carotene in the alga was also confirmed since the fraction immediately succeeding the hydrocarbon fraction eluted from the chromatographic column had an ultraviolet spectrum identical to that of β-carotene.

Separation of botryococcene and isobotryococcene was achieved by t.l.c. on silica impregnated with silver nitrate and a molecular formula of $C_{34}H_{58}$ was assigned to them from their mass spectra (Fig.5), which show the molecular ion to be at m/e 466 in each case. The infra-red spectra (Fig.3) of the two hydrocarbons were almost superposable on each other and on the infra-red spectrum (Fig.3) of the total hydrocarbon fraction of B. braunii. The bands at 891(2), 916 and 1002, and 979cm.$^{-1}$ were assigned to exomethylene, vinyl and trans disubstituted carbon-carbon double bond absorption respectively.

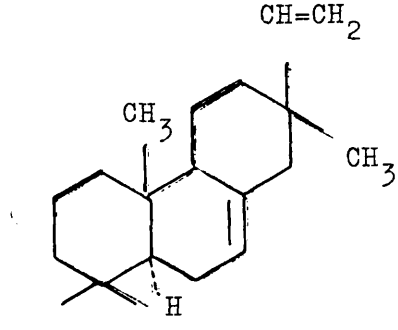
Intensity measurements of these bands in the spectrum (Table 2) of botryococcene were compatible with one vinyl double bond, one trans disubstituted double bond and four exomethylene double bonds in the hydrocarbon, on comparison with relevant standards. The normal intensity (εa) for γ(C-H) of a trans disubstituted double bond is ~100 and the intensities recorded for this absorption in botryococcene and isobotryococcene (116 and 112 respectively) therefore indicate that there is one trans double bond in each hydrocarbon. The intensities quoted by Eglinton et al[151] for the vinyl double bond of rosololactone (1) and deoxyrosenolactone (2) also compare favourably with those recorded for both hydrocarbons. The intensity measurements measured from the spectrum of isobotryococcene are almost identical to the corresponding values for botryococcene except that the value for the γ(C-H) absorption of the exomethylene band of the former is compatible with there being one fewer exomethylene bond present. The frequencies recorded for the trans and vinyl γ(C-H) absorptions are within the range of frequencies normally quoted for these bands. For olefins a trans disubstituted double bond absorbs between 964 and 979cm.$^{-1}$ and a vinyl double bond between 909 and 916cm.$^{-1}$ and 991 and 1004cm.$^{-1}$[152]. The high frequency of 1002cm.$^{-1}$ for the γ(C-H) absorption of the vinyl double bond in the botryococcenes provides evidence that this bond is attached to a fully substituted carbon atom (see below). When the vinyl double bond of an olefin is attached to a fully substituted carbon atom the frequency of this absorption rises to the region 995-1005cm.$^{-1}$[153]. The absence of

any $-(CH_2)n-$absorption at ~720cm.$^{-1}$ in both spectra indicates that both hydrocarbons are highly branched. This was confirmed by gas-liquid chromatography since the fully saturated hydrocarbon, botryococcane had a retention time of 1.0 relative to n-C_{28} alkane. That the double bonds of both compounds are unconjugated was demonstrated by their ultra-violet spectra which show end absorption only.

The nuclear nagnetic resonance spectra (Fig.6) of both compounds show a sharp singlet at 5.4 τ, which was assigned to exomethylene protons and integrated on scale expanded spectra at high sensitivity for about eight protons for botryococcene and about six for isobotryococcene. The vinyl group (Fig.6) thought to be present from the infra-red evidence was assigned as an ABX(ABC) system (1), corresponding remarkably well with that described by Carman[154] for the hydrocarbon rimuene (II).

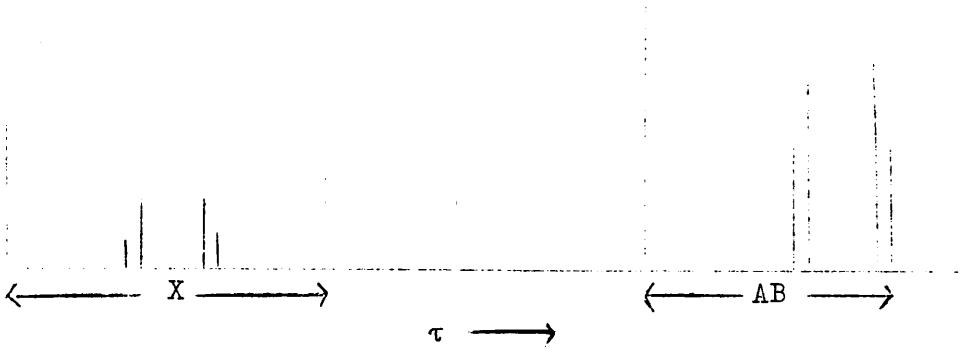


(I)



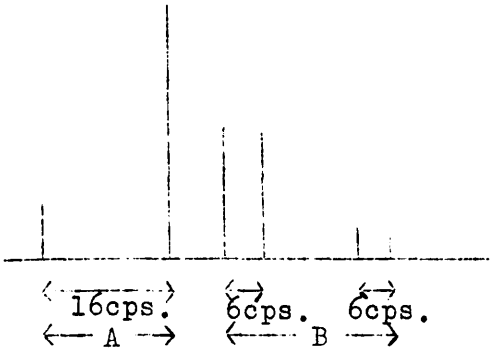
(II)

On the basis of theoretical calculation he explained successfully the multiplicity of the ABX system (III) of rimuene.

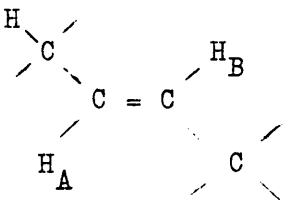


(III)

The two doublets and the singlet in the 5.37 - 5.1τ region (rimuene 5.35 - 5.04τ) of both spectra (Fig.6) were assigned to the AB protons of the vinyl double bond thought to be present in both botryococcene and isobotryococcene. Likewise the singlet, two doublets, and singlet in the 4.48 - 4.2τ region of the spectrum of botryococcene and 4.46 - 4.18τ region of the spectrum of isobotryococcene (rimuene 4.4 - 4.1τ) were assigned to the X proton of the vinyl double bond. These data indicate that in both hydrocarbons the vinyl group is attached to a fully substituted carbon atom as in rimuene. Double resonance experiments confirmed this conclusion since irradiation of the AB part produced a change in the X part and vice versa. The trans di-substituted bond protons thought to be present in both spectra were assigned as an AB quartet ($J_{A,B}$= 16 cps.) with the A part further split by an allylic proton to give the multiplicity shown (IV).



(IV)



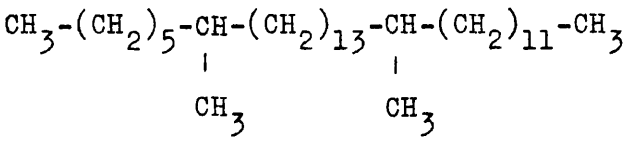
(V)

Therefore the trans disubstituted double bond in both hydrocarbons was thought to be attached to a fully substituted carbon atom on one side and to a disubstituted carbon on the other, as in (V). On irradiation of the saturated CH, CH_2 region of the spectrum the A part of the AB system collapsed to a doublet as expected.

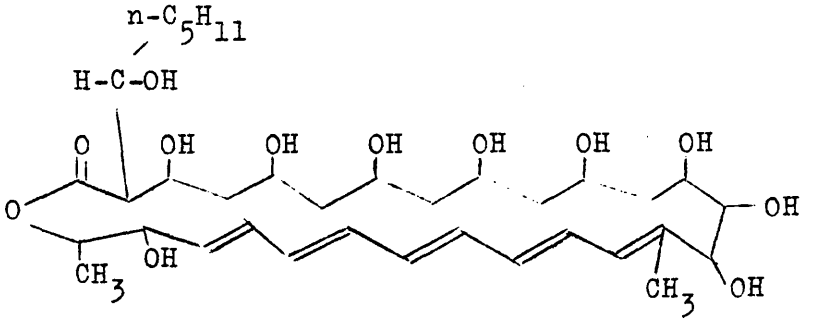
Although almost identical in the region of absorption of protons on double bonds, the spectra differ slightly. The spectrum of botryococcene shows no absorption between 7.5 and 7.7τ whereas that of isobotryococcene shows a broad allylic proton absorption at 7.55τ integrating for one or two protons. There are also differences in the 7.7 - 9.2τ region (Fig.6). The spectrum of botryococcene exhibits a singlet at 8.38τ whereas that of isobotryococcene has a narrow doublet at the same position. The integrations for these signals are compatible with there being two methyl groups on double bonds in each compound. The methyl region ($\tau > 9$) in both spectra integrates for five or six methyl groups.

Hydrogenation of botryococcene (and isobotryococcene) afforded the fully saturated hydrocarbon botryococcane whose molecular weight was assigned as 478 and molecular formula as $C_{34}H_{70}$ after some difficulty

(see Experimental section). The molecular ion is not visible in the mass spectrum (Fig.5) but this behaviour is not unusual for high molecular weight multi-branched saturated hydrocarbons. The mass spectrum of 7, 21-dimethyltritriacontane[155] (VI) prepared from the antibiotic fungichromine[156] (VII) by removal of all the oxygen functions and hydrogenation shows no parent ion but has a very small ("M-2")$^+$ ion due to contaminating olefin.



(VI)



(VII)

A small ("M-2")$^+$ ion was also observed in the mass spectrum of botryococcane and is also probably due to a tiny trace of olefin present.

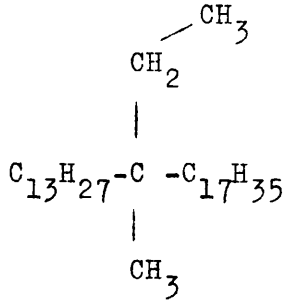
On electron impact saturated hydrocarbons fragment at the branching points with preferential elimination of the longest chain. The absence of an (M-15)$^+$ ion in the mass spectrum of botryococcane is not surprising although there are twelve or thirteen methyl groups present.

The methyl radical is the least stable of the alkyl radicals and will not be eliminated readily if other fragmentations are facile.

In the spectrum of squalane (Fig.7) the points of branching are readily inferred from the series of peaks at m/e 113, 183, 267 and 337 which are much more intense than those at the neighbouring carbon numbers and correspond to secondary carbonium ions. Another series at m/e 85, 155, 239 and 309 corresponds to fission at the branching positions with formation of primary ions. The peaks at m/e 239 and 309 are more intense than their neighbours but much less intense than those due to the secondary ions produced by fission of the same bonds. The lower mass fragments m/e 85 and m/e 155 are not so readily picked out as arising from cleavage at chain branches. Because of the usual accumulation of fragments of smaller mass the lower part of the spectrum of a branched hydrocarbon tends to look very similar to that of a straight chain hydrocarbon. The more highly branched the chain, the more difficult it is to pick out the points of substitution on the carbon chain, particularly if the substituents themselves are branched hydrocarbon chains[155].

In the mass spectrum of botryococcane (Fig.5) the ions at m/e 449 and 448 ($M-C_2H_5$) are probably due to loss of the ethyl group formed by hydrogenation of the vinyl group of botryococcene. The ions at m/e 449 and 448 are very intense in the mass spectrum, indicating that the ethyl group is attached to a tetrasubstituted carbon atom (the n.m.r. spectrum of botryococcene shows that the vinyl group is attached to a tetrasubstituted carbon atom, (see above). Likewise the

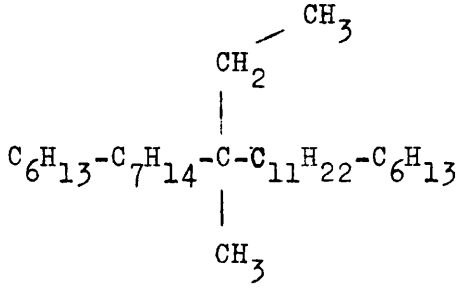
ions at m/e 295 and 294 ($M-C_{13}H_{27}$) and 239 and 238 ($M-C_{17}H_{35}$) are very much more intense than their neighbours. On this basis (VIII) is a possible schematic structure for botryococcane.



(VIII)

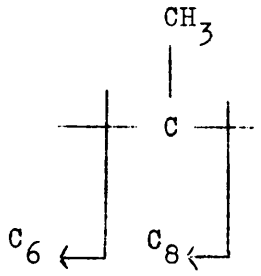
Although the ion at 435 ($M-C_3H_7$) is very small in intensity this does not exclude isopropyl groups from the structure of botryococcane since the mass spectrum of squalane (Fig.7) also shows a negligible loss of a C_3 fragment.

There is no observable loss of a C_4 or a C_5 fragment in the mass spectrum of botryococcane, so it is unlikely that there is a branch situated on the fifth or sixth carbon atoms from either end of the hydrocarbon chain. This indicates that there is at least a six carbon chain before branching occurs, as in (IX).



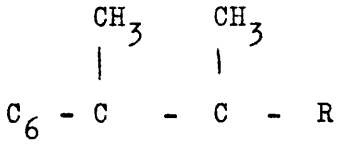
(IX)

In (IX) a methyl branch on C_7 would give an $(M-C_6)$ secondary ion at m/e 393 (see Fig.5) on electron impact. Such a fragmentation would also give an $(M-C_8)$ primary ion as in (X).



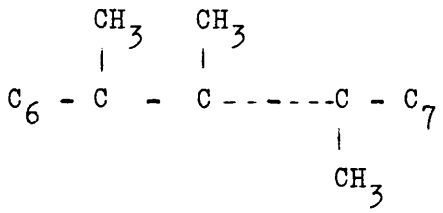
(X)

However, in the mass spectrum of botryococcane the $(M-C_8H_{17})$ ion is much more intense than its neighbours. It therefore seems more likely that this ion is a secondary ion, as would be expected from (XI).



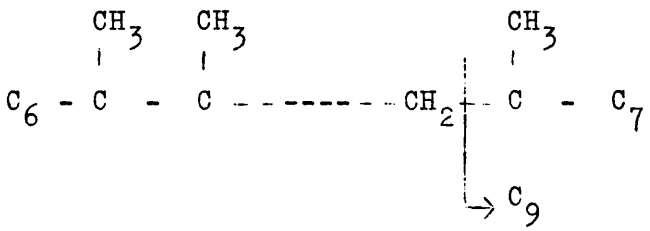
(XI)

This partial structure explains the presence of the large $(M-C_8H_{17})$ ion since it would produce a secondary carbonium ion as well as a secondary radical on electron impact. Structure (XI) also explains the $(M-C_6H_{13})$ ion in the mass spectrum of botryococcane, but not the $(M-C_7H_{15})$ ion. Botryococcane therefore probably has another chain with seven carbon atoms to a branching point, as in (XII).



(XII)

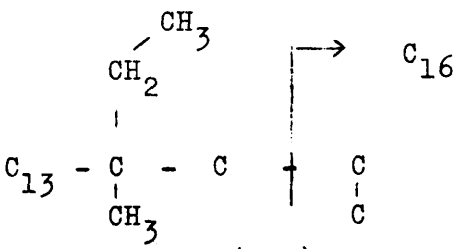
The ion at m/e 351 ($M-C_9$) in the mass spectrum of botryococcane is slightly more intense than expected and may be a primary ion arising from fission at a branching point as in (XIII).



(XIII)

It is difficult to draw any further conclusions from the mass spectrum since the ions in the C_nH_{2n+1} series below m/e 300 follow the smooth curve typical of the mass spectra of normal hydrocarbons except for the ions at m/e 295, 239, 225 and 211. The ions at m/e 295 and 239 are discussed above.

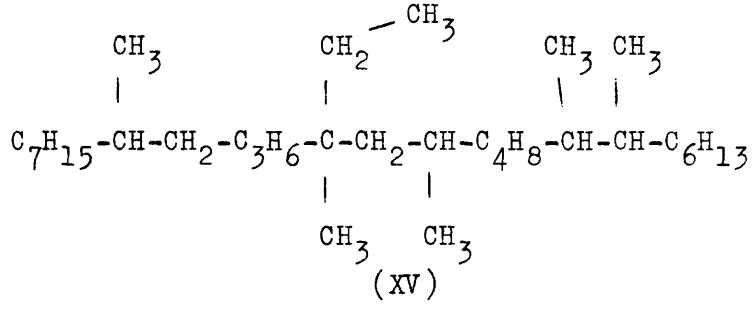
The ion at m/e 225 corresponds to an $M-C_{18}$ ion (i.e. a C_{16} fragment). Fission of (XIV) is a possible explanation for the presence of this ion in the mass spectrum.



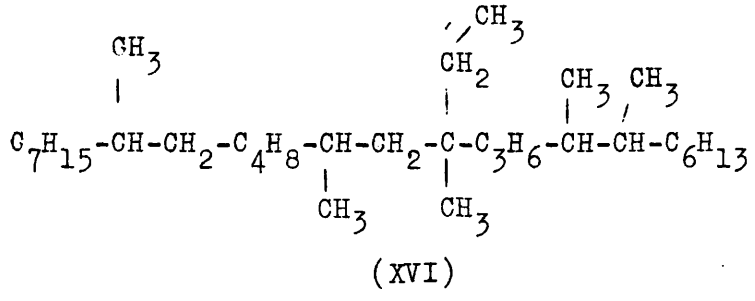
(XIV)

The ion at m/e 211 (i.e. a C_{15} fragment) cannot be accounted for at present in the partial structures proposed above.

From the mass spectral evidence presented above (XV) and (XVI) are possible partial structures for botryococcane.



(XV)



(XVI)

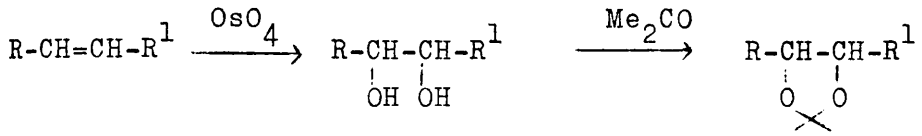
The infra-red spectrum of botryococcane shows no double bond absorption and under conditions of high resolution the methyl bending region shows absorption at 1386, 1378 and 1366cm.$^{-1}$ (Fig.8). The same region for squalane (3) is shown on Fig.8 for comparison. In squalane (3) the bands at 1386 and 1365cm.$^{-1}$ arise from the four geminal methyls of the two isopropyl groups present. For squalane (3) the 1365cm.$^{-1}$ has $\varepsilon a \sim 143$ (Table 2) whereas the 1366 cm.$^{-1}$ band in the spectrum of botryococcane has $\varepsilon a \sim 99$ (Table 1), so it is probable that botryococcane has one or two gem dimethyl groups. The infra-red spectrum also shows that botryococcane is more highly branched than squalane since the ratio

of the integrated area of the δ CH_2, CH_3 band to the area of the δ CH_3 bands is 2:1 compared with 1.57:1 for botryococcane.

The nuclear nagnetic resonance spectrum (Fig.9) shows that no double bond protons are present and the methyl region of a scale expanded spectrum at higher sensitivity integrates for twelve or thirteen methyl groups. The nuclear nagnetic resonance spectrum of squalane is also shown on Fig.9 as a comparison to show the greater proportion of methyl groups to CH and CH_2 groups in botryococcane. These data suggest that botryococcene does not have a simple isoprenoid skeleton. Corroboratory evidence for this is given by the fact that botryococcene did not form an adduct with thiourea, unlike squalene.

Attempts to partially hydrogenate the hydrocarbon fraction of B. braunii with palladium on charcoal were unsatisfactory since isomerisation of the double bonds appeared to be taking place in addition to the hydrogenation. The intention was to see if trans mono-olefins derived from botryococcene and isobotryococcene could be obtained by thin-layer chromatographic separation of the products. Gas-liquid chromatography of the fractions with trans disubstituted absorption in the infra-red showed them to consist of complicated mixtures of products. However, it may be possible to obtain the required trans mono-olefins from these fractions by careful preparative scale gas chromatography and to locate the trans double bond by mass spectrometry according to the method of McCloskey[157].

This involves treating the unsaturated compound first with osmium tetroxide and then with acetone:-



The position of the double bond originally present may then be located from the mass spectral fragmentation pattern of the acetonide. The method has the advantage that it can be carried out on a very small quantity of the unsaturated compound.

Partial reduction of the total hydrocarbon fraction of B. braunii with P-2 nickel boride[158], which inhibits isomerisation, was more successful. Preparative t.l.c. on silica impregnated with silver nitrate allowed the isolation of two partially reduced products, viz. dihydrobotryococcene and dihydroisobotryococcene, whose infra-red spectra showed that the vinyl double bond of each hydrocarbon had been reduced. This was confirmed by the mass spectra, which showed the parent ions to be at m/e 468 in each case. These compounds offer better prospects for oxidative cleavage of the double bonds since the number of double bonds is reduced in each case. This implies a lessening in the number of side reactions and a reduction in the complexity of the products.

The best method of attacking the problem of the structural elucidation of the botryococcenes would seem to be to first cleave the double bonds of the partially reduced dihydrobotryococcene (and dihydroisobotryococcene) to form ketone and acid groups. Identification of the resulting keto acids or acids should then allow formulation of the

structure of dihydrobotryococcene (and dihydroisobotryococcene). Subsequent cleavage of the double bonds of the more highly unsaturated botryococcene and isobotryococcene should then allow formulation of the structures of botryococcene and isobotryococcene.

The ozonolysis procedure employed for oxidation of the double bonds of botryococcene and isobotryococcene gave rise to a very complicated mixture of products (see Experimental section), even allowing for the fact that the total hydrocarbon fraction (comprising both compounds) was used in the oxidation experiment.

Oxidation with potassium permanganate/potassium periodate[159] or osmium tetroxide/potassium periodate[160] may be more useful. The method of ozonolysis successfully employed by Donniger and Popják[161] for cleavage of the double bonds of squalene may also be suitable for oxidative cleavage of botryococcene and isobotryococcene (and dihydrobotryococcene and dihydroisobotryococcene). In this reaction ozone is passed into a solution of the compound in ethyl chloride at -70°C and lithium aluminium hydride added. After the addition of acetic anhydride the solution is warmed to 120°C for one hour. This method of double bond cleavage gives rise to a mixture of acetates (XVII) which may then be identified.

$$R\text{-}CH = CH - R^1 \longrightarrow R\text{-}CH_2OA_c + R^1\text{-}CH_2OAc$$

(XVII)

It would be extremely difficult to make a heavy atom derivative of botryococcene or isobotryococcene which would be suitable for X-ray

structural analysis. Ideally an X-ray derivative should contain only one (or at the most two) heavy atoms per molecule. Botryococcene and isobotryococcene are both highly unsaturated and it is unlikely that a heavy atom reagent would selectively attack only one (or two) of the double bonds.

Work on the structural elucidation of botryococcene and isobotryococcene is proceeding in this department.

In addition there remains the problem of why this unique alga synthesises an extensive colony matrix almost entirely composed of these highly unsaturated hydrocarbons. This problem requires extensive biological study but in the naive opinion of the author there are three possible reasons for this high hydrocarbon content:-

(a) The oil serves as a protection for the alga

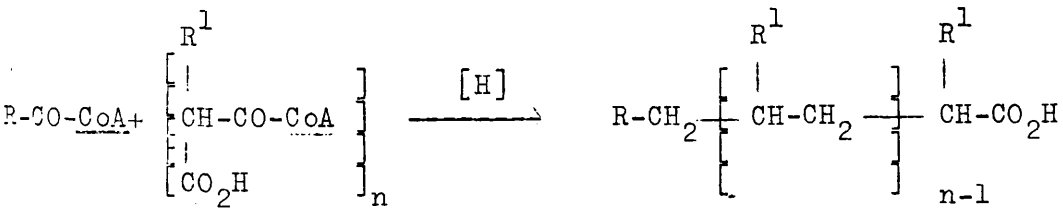
(b) The alga uses the oil as a food storage which may then be subsequently metabolised.

(c) The oil allows the alga to rise to, and remain at, the surface. This would then allow the alga to obtain carbon dioxide more easily for photosynthesis.

Two biosynthetic pathways are known which could lead to a highly branched long chain hydrocarbon, viz. the isoprenoid pathway and the polyketide pathway. In the isoprenoid pathway, the original unit is acetyl coenzyme A which is converted into isopentenyl pyrophosphate as shown in Fig.10(a). By proton rearrangement this yields 3,3-dimethylallyl pyrophosphate (4) which is the active alkylating

agent in isoprenoid biosynthesis. Thus polyisoprenoid units are built up from isopentenyl pyrophosphate ("starter") and 3,3-dimethylallyl pyrophosphate ("extender") as shown in Fig. 10(b). Squalene (5) is biosynthesised in this way, but with a central "tail to tail" linkage of isoprenoid units between two C_{15} "head to tail" chains (farnesyl pyrophosphate and nerolidyl pyrophosphate).

The polyketide pathway gives rise to fatty acids and a hydrocarbon could be obtained by decarboxylation of the corresponding carboxylic acid. There are a number of possible starter units here, including acetyl CoA, propionyl CoA, isobutyryl CoA, and α-methylbutyryl CoA. The pathway may be summarised as follows:-



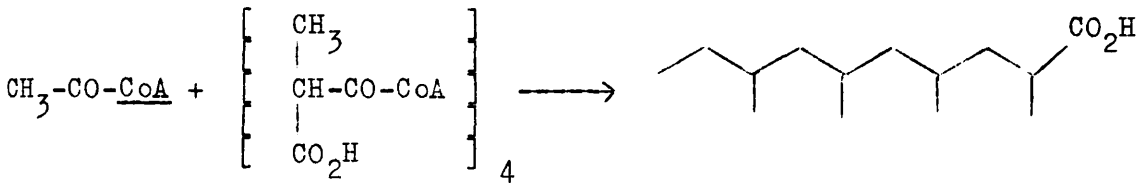
starter extender

R = alkyl
R^1 = H or alkyl

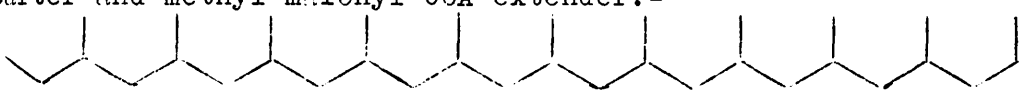
In the common biosynthesis of the straight chain, even-numbered fatty acids (n-alkanoic acids) the starter unit is acetyl CoA($R = CH_3$) and the extender is malonyl CoA($R^1 = H$). The starter unit is propionyl CoA ($R=CH_3CH_2$) in the biosynthesis of most straight chain, odd-numbered fatty acids[162].

Highly branched systems are found where one of the extenders is methyl malonyl CoA($R^1=CH_3$) an example being 2, 4, 6, 8-tetramethyl-decanoic acid isolated from the preen gland of the mute swan[163].

The biosynthesis of this preen gland fatty acid has still to be proved but almost certainly proceeds as follows:



It is easy to envisage a highly branched C_{34} hydrocarbon which could be synthesised by the simple polyketide pathway using acetyl CoA as starter and methyl malonyl CoA extender:-

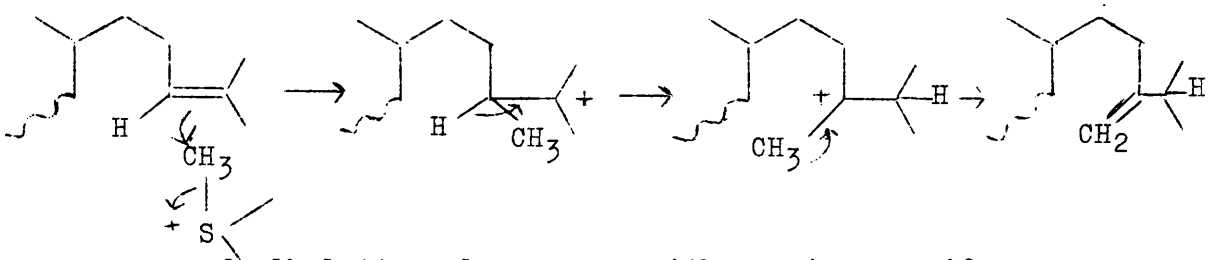


This structure has the requisite number of methyl groups (botryococcene has twelve or thirteen) but obviously does not satisfy the other evidence obtained for the structures of botryococcene and isobotryococcene (see summary on p.141)

In the isoprenoid pathway although it is difficult to achieve a suitable skeleton, there is no difficulty about accommodating the three types of double bonds present in botryococcene and isobotryococcene. Examples of these types of double bonds are well known in the terpene and steroid series.

Kates[162] has reviewed the methods whereby unsaturation is introduced in the polyketide pathway. Normally unsaturation is introduced after synthesis of a saturated structure by the polyketide route. In the case of the botryococcenes it would appear to be extremely difficult for the alga to introduce the large number of double bonds found. However such a consideration still does not completely

rule out the polyketide pathway. In the isoprenoid pathway further branching can be achieved by methylation with methionine. It is known that in plant sterols the isoprenoid side chain can be methylated with methionine[164]. The mechanism for the methylation is thought to proceed as follows[165]:-



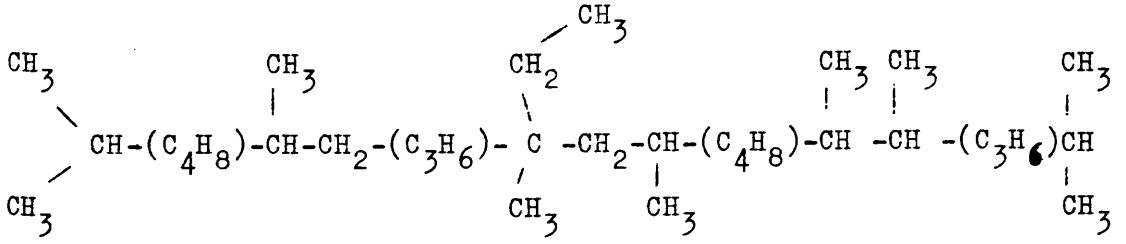
The same process of alkylation also occurs with non isoprenoid structures.

There appears to be no reason why methylation cannot occur with an acyclic isoprenoid to give a more highly branched structure although there is no instance of this as yet. If botryococcene is an alkylated isoprenoid alkylation with a C_2 unit could account for the vinyl group as an alternative to its being a portion of a pre-existing isoprenoid unit.

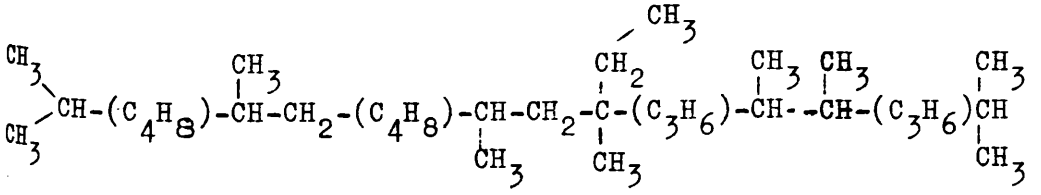
The evidence accumulated so far for the structure of botryococcene can be summarised as follows. Botryococcene is a hydrocarbon with molecular formula $C_{34}H_{58}$ (mass spectrum). It is acyclic and contains six double bonds (hydrogenation to botryococcane, $C_{34}H_{70}$) none of which are in conjugation (u.v. spectrum). There are four exomethylene double bonds present (i.r. spectrum and n.m.r. spectrum), one vinyl double bond attached to a fully substituted carbon atom

(i.r. spectrum; n.m.r. spectrum; marked loss of aC_2 fragment in the mass spectrum of botryococcane; selective hydrogenation with P-2 nickel boride to dihydrobotryococcene), and one <u>trans</u>-disubstituted double bond attached to a fully substituted carbon on one side and a trisubstituted carbon on the other (i.r. spectrum and n.m.r. spectrum). It contains five or six methyl groups on saturated carbon atoms (n.m.r.) and two methyl groups on double bonds (n.m.r.). The evidence above, by inference, requires these methyls to be on exomethylene double bonds (also the i.r. spectrum of botryococcane suggests one, possibly two geminal dimethyl groups in botryococcane). Long methylene chains [$-(CH_2)_n-$ $n>4$] are absent (i.r.). Botryococcene contains no methylene or methine groups which are di-allylic, i.e. no $C=C-CH_2-C=C$ or $C=C-\underset{|}{CH}-C=C$ (n.m.r.).

The fully saturated hydrocarbon botryococcane has a molecular formula of $C_{34}H_{70}$ (mass spectrum) and is highly branched (i.r. spectrum) with twelve or thirteen methyl groups (n.m.r. spectrum). There appear to be one or perhaps two geminal dimethyl groups (i.r. spectrum). Possible partial structures for botryococcane are:



and



if there is a gem dimethyl group present at each end of the chain.

Isobotryococcene is isomeric with botryococcene and, although it has not been as extensively studied as botryococcene, is very similar in structure - almost all of the information given above for botryococcene applies to isobotryococcene. It appears to have one exomethylene double bond fewer than botryococcene (i.r. spectrum, n.m.r. spectrum). Since reduction of isobotryococcene affords botryococcane it seems likely that it has a double bond (possibly trisubstituted or tetrasubstituted) which is not seen in either the infra-red or n.m.r. spectra. Unlike the n.m.r. spectrum of botryococcene, the n.m.r. spectrum of isobotryococcene exhibits a band at 7.55τ which may be due to di-allylic protons (Fig.6).

From the evidence at present available it is very unlikely that the botryococcenes have a simple acetate-methyl malonate biogenesis. Likewise they do not appear to have a simple isoprenoid biogenesis. If either the polyketide or isoprenoid pathway is involved in their biosynthesis, the above data require some modification of these pathways.

It is also possible that they have a mixed biogenesis or are synthesised via another biogenetic pathway still to be discovered.

EXPERIMENTAL

General.

Nuclear magnetic resonance spectra were determined on a Varian HA 100 (100 megacycles) spectrophotometer and mass spectra on an A.E.I. MS9 double focussing mass spectrometer using the direct inlet system. Osmometric determinations were performed on a Mechrolab vapour pressure osmometer (model 301A).

Gas-liquid chromatography (g.l.c.) was carried out on a Perkin Elmer F-11 gas chromatograph equipped with a flame ionisation detector. Retention times were measured on a 10 ft. x 1/16 in. column operating at 240°C and a flow rate of 25 ml./min., unless otherwise stated. The column was packed with "Chromosorb G" (100-120 mesh, acid washed and silanized) coated with 2% seven-ring polyphenyl ether (Applied Science).

The unsaturated hydrocarbons, both prepared and isolated, were stored in solvent under nitrogen in the refrigerator. An Edwards High Vacuum Ltd., freeze dryer (Model 10P) was used for all freeze drying operations.

Botryococcus Braunii Kutzing

The sample of B. Braunii used in the present study was observed by Mr. J. Osborne, warden of Rostherne N.N.R., on 3rd November, 1965, as an orange oily patch (50-60 yds. square) floating on the surface of Oakmere, Cheshire (area 50 acres, average depth 9 ft. map reference SJ (33) 575677 [1 inch sheet no. 109]). Mr. Osborne collected about nine gallons of the algal suspension on November 4th in new polythene containers (freshly rinsed with Oakmere water). This was achieved by making a small

depression (with a lip $\frac{1}{2}$ in. under the water) in the bank and allowing the oily supernatant suspension to flow over the lip and under a small barrier. The sample was sent per passenger train to the author on the day of collection. On arrival half of the containers were stored in the refrigerator at 0°C and half in the deep freeze at -20°C. Only samples from the deep freeze were subsequently used for the isolation of the hydrocarbons (botryococcenes) reported herein. The mode of of storage was necessitated by the fact that a small sample of B. braunii became rubbery in texture after exposure (48 hours) to the atmosphere at room temperature. However, the hydrocarbons could still be recovered from the exposed B. braunii, albeit in smaller quantities. The authenticity and purity of the sample of alga were vouched for by Dr. J.W.G. Lund of the Freshwater Biological Association, Windermere, and Dr. E.Conway of the Botany Department, Glasgow University, from a microscopic examination of the characteristic yellow colonies. Dr. Lund also kindly arranged for the collection of the B. braunii examined herein. In addition, colour photomicrographs were kindly made by Dr. J.S.Gillespie of the Physiology Department, Glasgow University.

Isolation of the Lipid content of B. Braunii

Preliminary experiments.

A sample (~ 80 ml.) of the algal suspension was decanted free from most of the water and shaken with hexane (60 ml., three times). Removal of the yellow hexane layer and evaporation gave a brownish oil whose i.r. spectrum (film) was very similar to the hydrocarbon fraction later isolated. The procedure was repeated with ether and benzene.

In both cases the i.r. spectra were identical to that of the hexane extract. Since difficulties were encountered with solvent removal because of the dispersal of the algal suspension between the solvent and the water layers it was decided to freeze dry the alga prior to solvent extraction.

Isolation of the total lipid content of B. braunii.

An aliquot (800 ml.) of the greeny-orange algal suspension was freeze dried in two 1 l. flasks (12 hours). The dry orange powder (12 g.) was then extracted ultrasonically (30 min.) with acetone (400 ml.) in two 250 ml. centrifuge tubes placed in the ultrasonic tank (water). The suspension was centrifuged for 20 min. and the yellow extract was decanted. The extraction was repeated five times, whereupon the last extract was colourless. Evaporation of the combined extracts (Buchi evaporator) afforded a brownish-green oil (10 g., 83% of dry wt. of B. braunii).

Isolation of the total hydrocarbon fraction of B. braunii.

The combined acetone extracts were dissolved in the minimum volume of n-hexane and chromatographed on alumina (70 g.). Elution with n-hexane (800 ml.) gave the total hydrocarbon fraction as a colourless oil (9.14 g., 76% of dry weight of B. braunii) on evaporation. Subsequent elution with benzene (300 ml.) gave a fraction whose u.v. spectrum (n-hexane) showed typical carotenoid absorption (λ_{max} 449, 472 mμ. Belcher[129] quotes λ_{max} 449, 470mμ for β-carotene, the principal carotenoid of B. braunii). The column was eluted with ethyl acetate (300 ml.) until

the eluate became colourless; this eluate was stored.

Isolation of the botryococcenes from the total hydrocarbon fraction.

T.l.c. of the hexane eluate (benzene developer) showed three spots [Fig.11(A)]. The i.r. spectrum (Fig. 3, film) shows absorption at 1788 (w, overtone γ C-H), 1646 (s, ν C=C), 1000 (w) and 917 (m) (γ C-H vinyl), 980 (m, γ C-H trans disubstituted), 892 $cm.^{-1}$ (s, γ C-H exomethylene). The u.v. spectrum (n-hexane) showed end absorption only (λ_{max} 215 mμ). G.l.c. (under the standard conditions) showed the presence of six components (Fig.4), two components comprising over 97% of the total hydrocarbon fraction. There was no resolution at all on a 10 ft. x 1/16 in. 3% SE-30 column under the same conditions. An aliquot of the total hydrocarbon fraction (51.5mg.) gave two compounds, which were termed botryococcene (45 mg.) and isobotryococcene (4.3 mg.), after preparative t.l.c (twice) under the conditions described for the analytical t.l.c.

(a) Botryococcene

The botryococcene was shown to be over 96% pure by g.l.c. (t_R = 0.81 relative to n-C_{28} alkane) with three very small peaks immediately following the main one. The purity was checked by GC-MS. by recording mass spectra (scanning time 4 seconds) at six points on the g.l.c. peak. There was no difference in the mass spectra obtained except for the expected small differences in the relative abundances of a few of the ions. G.l.c. (Pye Argon chromatograph) on 4 ft. x 3/8 in. columns packed with "Gas Chrom. P", 100-120 mesh, coated with 1% CHDMS/2% P.V.P., 1% D.C.-710, and

1% "Polymer Z" failed to reveal any further separation. Although the hydrocarbon appeared pure by g.l.c. there must remain the possibility of stereoisomers or isomers being present. The hydrocarbon was shown to polymerise readily on exposure to the air, whereupon small droplets of polymer appeared in the oil. T.l.c. of an exposed pure sample (benzene developer) showed the presence of polymer at the origin. The hydrocarbon was therefore always stored under solvent in the refrigerator and all experiments and spectroscopic determinations were performed as soon as possible after purification by preparative t.l.c The mass spectrum (Fig.5) shows the molecular ion at m/e 466 (corresponding to $C_{34}H_{58}$) and prominent ions at m/e 369, 300, 299, 287, 285, 259 and 257. The u.v. spectrum had end absorption only (λ_{max} 211.5 mμ., ε600). The i.r. spectrum (film, Fig.3) shows double bond absorption at 3080 (m, ν C-H), 1786 (overtone γ C-H), 1644 (s, ν C=C), 1002 (w) and 915 (m) (γ C-H vinyl), 979 (m, γ C-H trans disubstituted), 891 $cm.^{-1}$(s, γ C-H exomethylene), and methyl absorption at 1374 $cm.^{-1}$ (m, δ CH_3; sym.) but no $-(CH_2)_n$ -rock absorption in the 720$cm.^{-1}$ region. The relevant information from a quantitative i.r. spectrum (CS_2, 4.8mg/ml.) is shown in Table 2. The n.m.r. spectrum (Fig.6) shows a singlet at 5.4τ (about 8 exomethylene protons), two doublets and a singlet between 5.2-5.1τ (AB part of ABX system, CH_2 protons of vinyl double bond), an AB quartet (J=16 cps.) centred at 4.9τ with the A part further split (J=6cps.) into two doublets (2 protons of trans double bond with one further split by a CH proton), and a multiplet centred on 4.3τ (X part of ABX system, CH proton of vinyl double bond).

(b) Isobotryococcene

The isobotryococcene was shown to be over 98% pure by g.l.c. (t_R= 0.76 relative to n-C_{28} alkane) with two very small peaks immediately following the main one. The purity was checked by GC-MS by recording mass spectra at six points on the g.l.c. peak under the same conditions as for botryococcene. There was no difference in the mass spectra except for the expected small differences in the relative abundances of a few of the ions. The other g.l.c. columns used for botryococcene also failed to give any further separation of isobotryococcene. Although the hydrocarbon appeared pure by g.l.c. there must remain the possibility of isomers or stereoisomers being present. The mass spectrum (Fig.5) shows the molecular ion to be at m/e 466 (corresponding to $C_{34}H_{58}$) and prominent ions at m/e 397, 369, 341, 287, 259 and 257. The i.r. spectrum (film, Fig.3) shows double bond absorption at 3080 (m, νC-H), 1788 (overtone γ C-H), 1646 (s, νC=C), 1002 (w) and 916 (m) (γ C-H vinyl), 980 (m, γ C-H *trans* disubstituted), 892 $cm.^{-1}$ (s, γ C-H exomethylene) and methyl absorption at 1376$cm.^{-1}$ (m, δCH_3; sym.) but no $-(CH_2)_n$-rock absorption in the 720$cm.^{-1}$ region. The relevant information from a quantitative i.r. spectrum (CS_2, 4.15mg./0.5ml.) is shown in Table 2. The n.m.r. spectrum (Fig.6) is identical to that of botryococcene in the τ4-5.5 region (protons on double bonds) except that the exomethylene singlet at τ5.4 integrates for about six protons. There are also differences in the CH_3 and CH_2,CH regions (see Fig.6 and Discussion).

Hydrogenation of botryococcene

Botryococcane

Botryococcene (75mg.), from a preparative silver nitrate t.l.c. plate, was hydrogenated with stirring for 4 hours in ethyl acetate (20 ml.) in the presence of 10% palladium charcoal (10mg.) whereupon no more hydrogen was being absorbed. Removal of the catalyst, evaporation of the solvent, and preparative t.l.c. on silver nitrate (n-hexane developer) gave the fully saturated hydrocarbon, which was termed botryococcane (60 mg. R_f = 1.0 relative to n-C_{28} alkane, and t_R=1.0 relative to n-C_{28} by g.l.c.). The mass spectrum (Fig.5) shows what appeared to be the molecular ion at m/e 476 (corresponding to $C_{34}H_{68}$) and prominent ions at m/e 450, 449, 448, 435, 393, 379, 365, 351, 337, 323, 309, 295, 294, 239 and 238). Two independent osmometric determinations in CCl_4 gave the m.w. as 627 and 623 respectively. The botryococcene was distilled under reduced pressure (160°C at 0.4mm.Hg) to remove the suspected polymeric material arising from polymerisation of the sample of botryococcene used and the m.w. was redetermined (using a solution of 5.875mg. in CCl_4 [0.7922g.]). The m.w. was 469 (calculated for $C_{34}H_{70}$ m.w. 478) and was within the experimental error ($\pm$ approximately 1%) of the calculated m.w. The m.w. of squalane measured at the same time as a control was 423 (correct m.w. 422). The hydrocarbon was again subjected to preparative t.l.c. as a purity check and two cuts taken across the band on the plate. The mass spectra of the two cuts were identical to each other and to the first mass spectrum of botryococcane. Perfluorokerosene was added

as a marker to check the mass of the prominent ion at m/e 449 since it was extremely difficult to explain this ion as a $(P-27)^+$ ion from a saturated hydrocarbon of m.w.476. The marker showed that this ion was indeed located at m/e 449 and so other mass spectra of botryococcane were again recorded, after the sample had been exposed to the air for 48 hours. It was observed that the intensity of the ion at m/e 476, previously interpreted as the parent ion, was very substantially reduced in intensity and in fact was completely absent in one spectrum. Therefore the ion at m/e 476 was caused by a small unsaturated impurity which had been oxidised and the true m.w. of botryococcane was 478, corresponding to $C_{34}H_{70}$, the parent ion being absent from all of the spectra. Also the mass spectrum is of the type expected for a completely saturated hydrocarbon with no double bond equivalents since there were no prominent ions with formulae corresponding to one double bond equivalent as would be expected for a m.w. of 476. Hydrogenation of isobotryococcene also afforded botryococcane; the mass spectrum was identical to that of the botryococcane obtained by hydrogenation of botryococcene. The i.r. spectrum (film) showed no double bond absorption and had absorption at 1380 (s, δCH_3; sym.), 1368 (m, δCH_3; sym.), 1155 and 1125 $cm.^{-1}$ (possibly skeletal modes of gem dimethyl group). The relevant information from a quantitative i.r. spectrum (CCl_4, 2mg./20µl.) is shown in Table 2. The n.m.r. spectrum (Fig.9) shows no signals from protons on double bonds, and has a ratio of CH_3 protons ($\tau > 9$) to CH, CH_2 protons ($\tau < 9$) of ~1.3:1.

Attempted partial reduction of the total hydrocarbon fraction of B. braunii.

An aliquot (389mg.) of the total hydrocarbon fraction was hydrogenated in the presence of 5% palladium-charcoal catalyst (50mg.) until 3.5 mole equivalents of hydrogen were absorbed. Removal of the catalyst gave a crude product (370mg.) whose i.r. spectrum (film) showed absorption at 975 (s, γ C-H trans disubstituted double bond) and 880cm.$^{-1}$ (w). T.l.c. on 10% silver nitrate/silica gel (hexane/10% benzene developer) showed four fairly discrete spots (a)-(d) [Fig. 11(b)] which were separated by preparative scale t.l.c.

Fraction (a). The i.r. spectrum (film) showed absorption at 975cm.$^{-1}$ (m, γ C-H trans) and no vinyl or exomethylene absorption but g.l.c. (Fig.12, conditions as for botryococcene) showed six closely spaced peaks with t_R = 22.4, 23.9, 26.6, 29.9, 33.5 and 37.3 min. respectively.

Fraction (b). The i.r. spectrum (film) showed weak absorption at 875cm.$^{-1}$ but no vinyl, trans or exomethylene absorption. The band could not have been fully saturated from its R_f value and possibly had tetrasubstituted and trisubstituted double bonds present.

Fraction (c). The i.r. spectrum (film) showed absorption at 975cm.$^{-1}$ (m, γ C-H trans) and no vinyl or exomethylene absorption but g.l.c. [Fig.12(c)], conditions as for botryococcene) showed seven peaks with t_R = 22.5, 25.5, 27.7, 30.7, 34.2, 38.4 and 43 min. respectively.

Fraction (d). The i.r. spectrum (film) showed absorption at 1660 (w, broad, νC=C), 975 (m, γ C-H trans disubstituted), 880 (w), 835 (w), and 780cm.$^{-1}$(w). It appeared that there were several types of double bond present.

The experiment was repeated on an aliquot (129mg.) of the hydrocarbon where absorption of four mole equivalents of hydrogen was permitted. The i.r. spectrum (film) of the crude product (121 mg.) showed no double bond absorption and t.l.c. (hexane developer) showed three spots (a)-(c) [Fig.11(c)] which were separated by preparative scale t.l.c.

Fraction (a). The i.r. spectrum (film) and R_f were identical to those of botryococcane.

Fraction (b). The i.r. spectrum (film) showed absorption at 875cm.$^{-1}$(w) and no vinyl, trans disubstituted, or exomethylene absorption.

Fraction (c). The i.r. spectrum (film) showed a few ripples in the 1000-800cm.$^{-1}$ region.

It was apparent from these hydrogenation experiments that isomerisation to trisubstituted and perhaps tetrasubstituted double bonds was taking place to a large extent.

Reduction of botryococcenes by the general method of Brown and Brown[158]. Dihydrobotryococcene and dihydroisobotryococcene.

Nickel acetate (0.62g, 2.5m. moles) was dissolved in benzene-free ethanol (20ml., 95%) in the hydrogenation flask. The system was flushed out with hydrogen and sodium borohydride (2.5ml. of a 1.0 M solution) in ethanol was added. An aliquot of the B. braunii total hydrocarbon fraction (200mg.) was slowly added via a side arm in the flask and the hydrogenation allowed to proceed for three hours at atmospheric pressure. The solution was allowed to stand overnight and was then evaporated to dryness. The residue was rinsed with n-hexane

and the resulting solution was passed through a small column of alumina (5g.). Evaporation of the solvent and azeotroping with benzene gave the crude product as a colourless oil (170mg.) whose i.r. spectrum (film) was similar to that of the B. braunii total hydrocarbon fraction. T.l.c. (benzene developer) showed the presence of four spots [Fig.11(d)] which were separated by preparative t.l.c. Four fractions (a)-(d) were thereby obtained. The compositions of these fractions were then investigated:-

Fraction (a) Dihydroisobotryococcene

Dihydroisobotryococcene (9 mg.) from fraction (a) was shown to be substantially pure by g.l.c. (t_R= 0.82 relative to n-C_{28} alkane) and the i.r. spectrum (film) showed absorption at 3080 (m, ν C-H), 1646 (s, ν C=C), 978 (m, γC-H trans disubstituted), and 890cm.$^{-1}$ (s, γC-H exomethylene). The mass spectrum showed the molecular ion at m/e 468 (corresponding to $C_{34}H_{60}$). The relevant information from a quantitative i.r. spectrum (CS_2, 3.1mg./0.5ml.) is shown in Table 2).

Fraction (b) Dihydrobotryococcene

Dihydrobotryococcene (55mg.) from fraction (b) was shown to be almost pure by g.l.c. (t_R= 0.79 relative to n-C_{28} alkane) and the i.r. spectrum (film) showed absorption at 3080 (m, ν C-H), 1646 (s, ν C=C), 978 (m, γC-H trans disubstituted), and 890cm.$^{-1}$ (s, γC-H exomethylene). The mass spectrum showed the molecular ion at m/e 468 (corresponding to $C_{34}H_{60}$) and prominent ions at m/e 440, 439, 398, 371, 369, 302, 301, 290, 289, 287, 273. The n.m.r. spectrum (Fig. 6) shows the

disappearance of the ABX protons of the vinyl group of botryococcene. The relevant information from a quantitative i.r. spectrum (CS_2, 8.85mg./ml) is shown in Table 2. In addition, the spectrum showed very weak absorption at 1001 and 916cm.$^{-1}$ probably caused by the small impurities seen by g.l.c. This was most likely due to isomers arising from the hydrogenation.

Fraction (c). Fraction (c) had i.r. spectrum (film) and t_R identical to botryococcene.

Fraction (d). Fraction (d) had i.r. spectrum (film) and t_R identical to isobotryococcene.

Attempted preparation of botryococcene dodecabromide.

Bromine (90mg., 0.0006 moles) in glacial acetic acid (1 ml.) was added dropwise to botryococcene (52 mg., 0.0001 moles) in glacial acetic acid (1 ml.) until the bromine colour persisted in the solution. No crystals appeared on concentration of the solvent. Evaporation gave the product as a brown gum which could not be crystallised.

Ozonolysis of the total hydrocarbon fraction from B. braunii.

Ozone was passed through an aliquot (301 mg.) of the total hydrocarbon fraction dissolved in ethyl acetate (15 ml.) at -70°C until the characteristic blue colour appeared in the solution. The solution was allowed to warm slowly to ambient temperature and zinc (200 mg. "Analar" grade) and glacial acetic acid (10 ml.) added. The mixture was stirred overnight at room temperature, filtered, washed with ferrous sulphate solution followed by water and dried by

azeotroping with benzene. The crude product (205 mg.) was shown by g.l.c. on JXR (4.6%; column 7 ft. x 1/16 in.; programmed from 100-300°C at 4°/min.) to consist of a complex mixture of at least seventeen components. T.l.c. on silica gel (ether developer) showed at least eight spots [Fig. 11(e)]. The i.r. spectrum (film) showed absorption at ~ 3500 (m, ν O-H), and 1720cm.$^{-1}$ (s, ν C=O) but no absorption due to C=C double bonds. The overall complexity of the ozonolysis product was such that the experiment was abandoned at this point.

Attempted acid catalysed isomerisation of the total hydrocarbon fraction of B. braunii with p-toluene sulphonic acid.

An aliquot (50 mg.) of the total hydrocarbon fraction was heated under reflux (16 hrs.) in benzene containing a small quantity (one crystal) of p-toluenesulphonic acid. The resulting red solution was passed through a small column containing a layer of alumina and one of sodium bicarbonate (anhydrous). The solution was further dried (sodium sulphate) and on evaporation afforded a yellow oil whose i.r. spectrum (CS_2) had absorption at 3530 (w, ν O-H?), 1700cm.$^{-1}$ (m, ν C=O acid?) and very broad absorption between 900 and 1200cm.$^{-1}$. No bands due to carbon-carbon double bond absorption were observed. T.l.c. (benzene developer) showed the product to consist of at least three spots [Fig.11(f)]. After preparative t.l.c. the main band was re-examined by analytical t.l.c. Three spots were again observed, indicating that oxidation was taking place. When the yellow oil was allowed to stand in a sealed test tube polymerisation to a yellow gum took place. The

experiment was abandoned at this point and further experiments to cause isomerisation are required.

Attempted thiourea adduction of botryococcene

(Experiment performed by Sister M.T.J. Murphy, R.S.M.).

Botryococcene (~ 20mg.) in chloroform (0.5ml.) was added to a solution of thiourea (~270mg.) in methanol (1 ml.). The mixture was allowed to stand in the refrigerator for four hours. Only crystals of thiourea appeared. The solvent was allowed to evaporate slowly by exposure to the air and methanol (1ml.) and chloroform (0.5ml.) were again added. Long chunky needles formed which had the appearance of adduct crystals under the polarising microscope. However, after an X-ray examination kindly performed by Dr. A. Kerr of the Chemistry Department, Cambridge University, the crystals were shown to be thiourea itself and not an adduct.

TABLE 1

Gross chemical composition of B. braunii (Belcher[129])

	% (dry weight)
Nitrogen	
Total	2.2 - 3.4
Total soluble	0.5 - 0.6
Ammonia - N	0.2
Amide - N	0.02
α - amino - N	0.06 - 0.07
Carbohydrate	
Total	14 - 16
Reducing sugar	6.0 - 6.5
Non-reducing sugar	6.0 - 6.5
Hydrolysable polysaccharide	2 - 3
Lipid	
Saponifiable	7 - 12
Unsaponifiable	16 - 23
Inorganic Ash	0.4 - 1.6

TABLE 2

Significant infra-red bands

Compound	Solvent	ν cm.$^{-1}$	$\Delta\nu_{1/2}$ cm.$^{-1}$ [a]	O.D.	εa
Botryococcene	CS_2	1001	(15)	0.055	60
		977	16	0.098	116
		915	13	0.152	170
		891	8	0.65	757
Isobotryococcene	CS_2	1001	15	0.06	67
		978	17	0.10	112
		916	12	0.15	166
		892	8	0.51	573
Botryococcane	CCl_4	1378	*	0.44	(209)
		1365	*	0.21	(99 sh.)
Squalane	CCl_4	1376	*	0.28	(182)
		1365	*	0.22	(143 sh.)
Dihydrobotryococcene	CS_2	978	12	0.12	127
		890	8	0.63	666
Dihydroisobotryococcene	CS_2	975	11	0.10	151
		888	8	0.35	536
Rosololactone[151](1)	CCl_4	996	11	-	110
		910	10	-	180
Deoxyrosenolactone[151](2)	CCl_4	999	8	-	90
		913	9	-	170

sh. = shoulder
Figures in parentheses are only approximate. * Not measured

Fig.1 Legend

Photograph of a culture of *B. braunii* in water

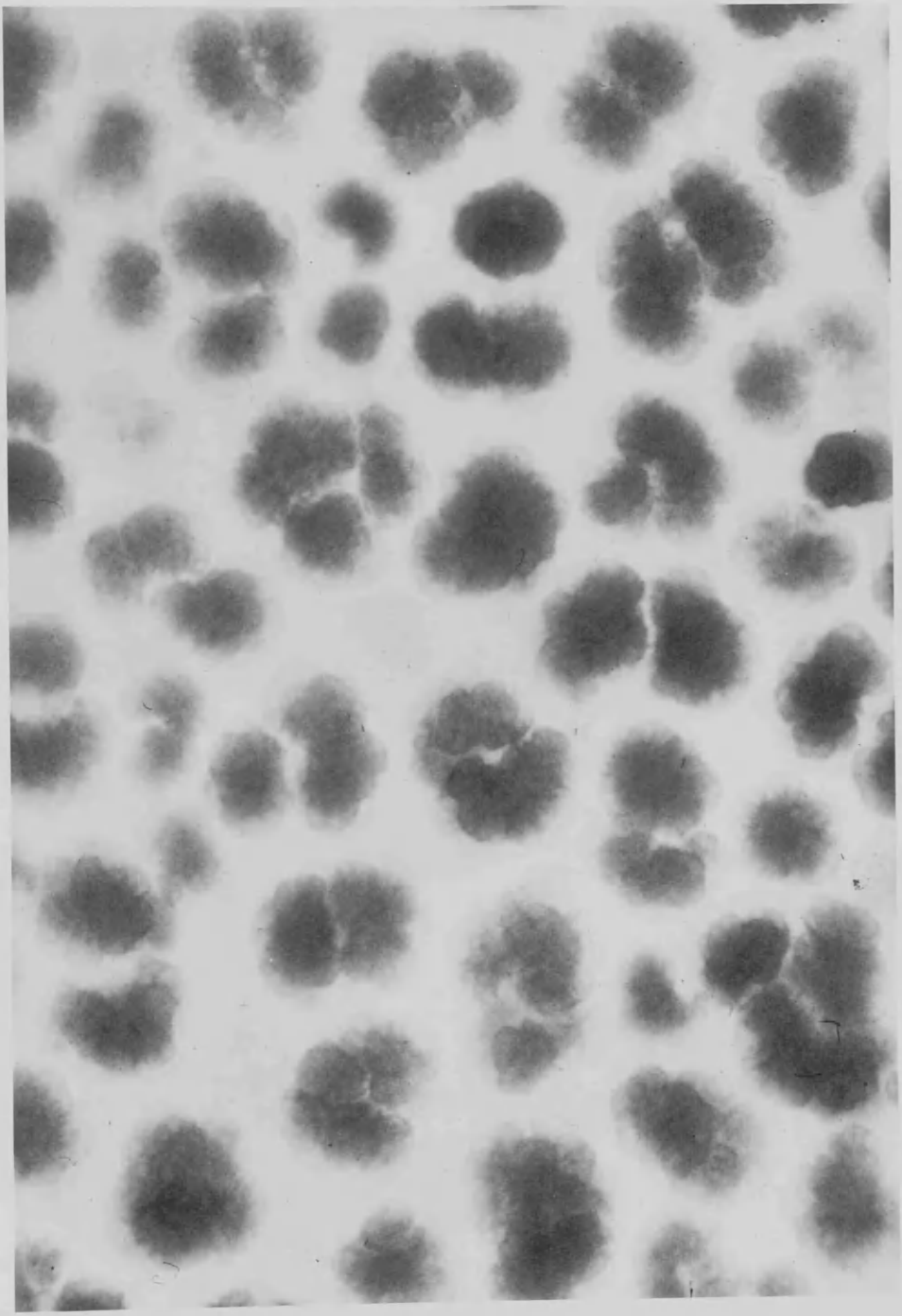


Fig. 2 Legend

Photograph of a single colony of a culture of *B. braunii* in water under conditions of high magnification.

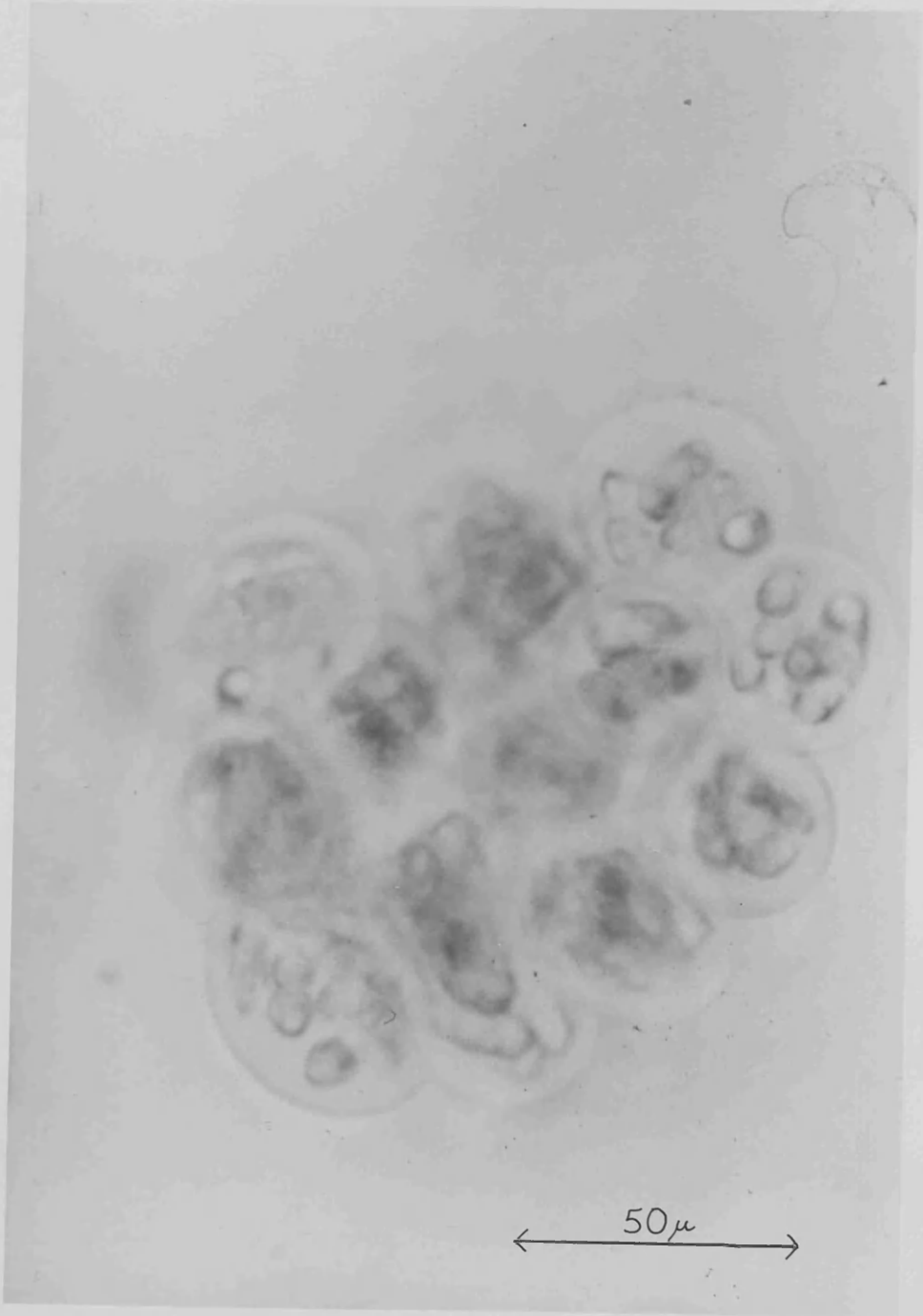

50μ

Fig.3.

I.R. SPECTRA OF B. BRAUNII HYDROCARBON FRACTION AND THE BOTRYOCOCCENES.

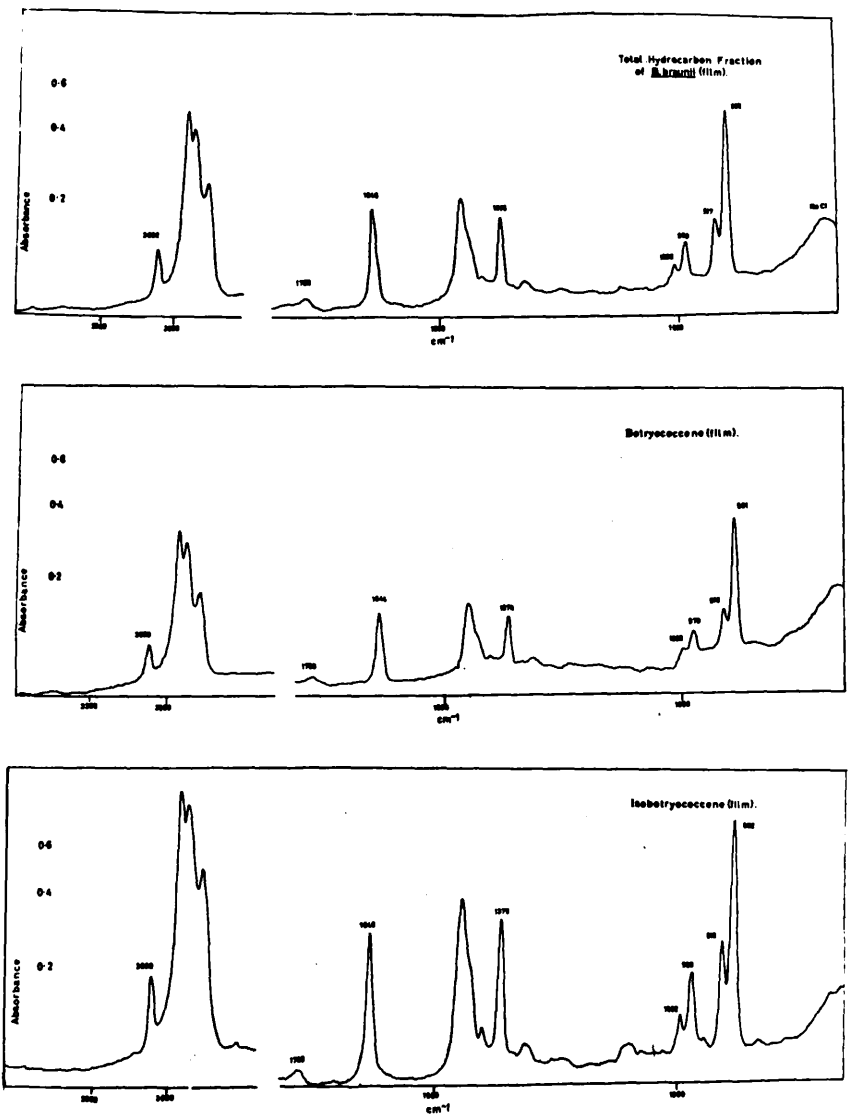


Fig. 4 Legend

Gas chromatogram of the total hydrocarbon fraction of *B. braunii*.

Conditions:

Column 10 ft. x 1/16 in.; 2% 7 ring *m*-polyphenyl ether on Chromsorb G, 100-120 mesh; isothermal at 240°C; sample size 0.1µl of a solution in iso-octane, attenuation 1 x 10^2.

TOTAL HYDROCARBON FRACTION OF B. BRAUNII.

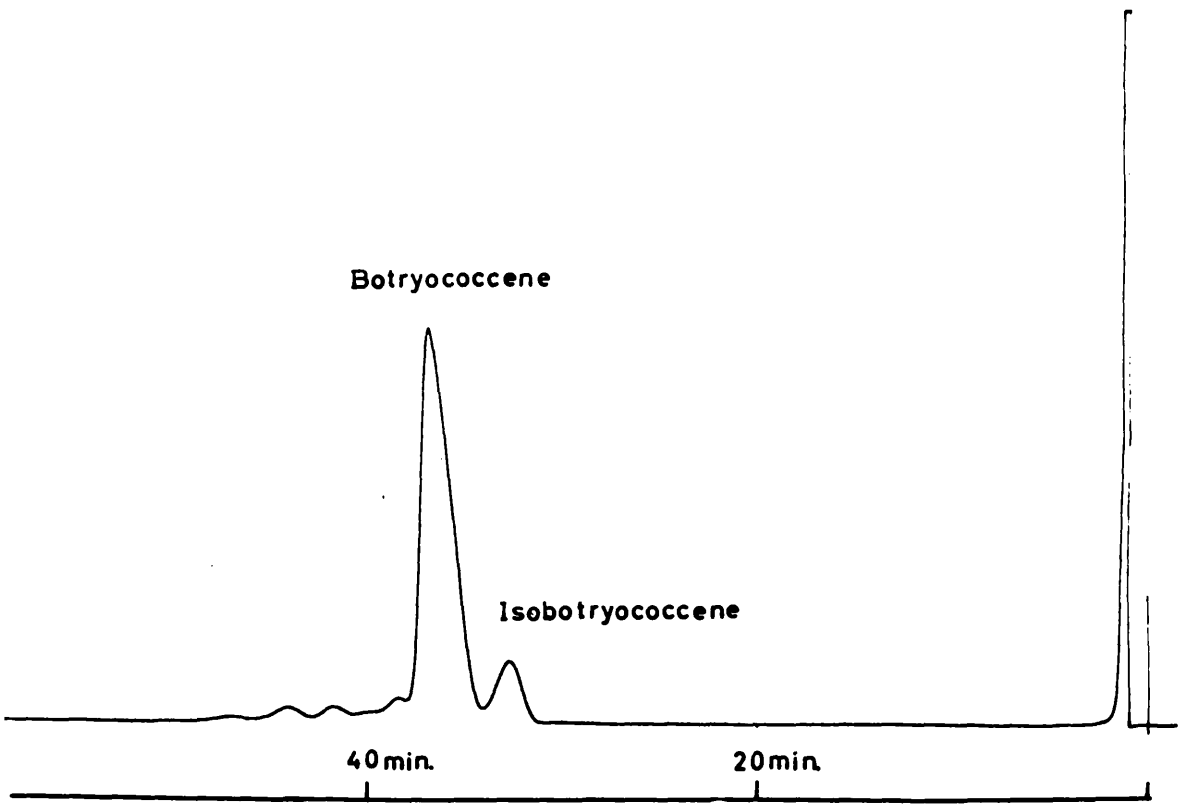


Fig.5.

MASS SPECTRA OF THE BOTRYOCOCCENES AND BOTRYOCOCCANE.

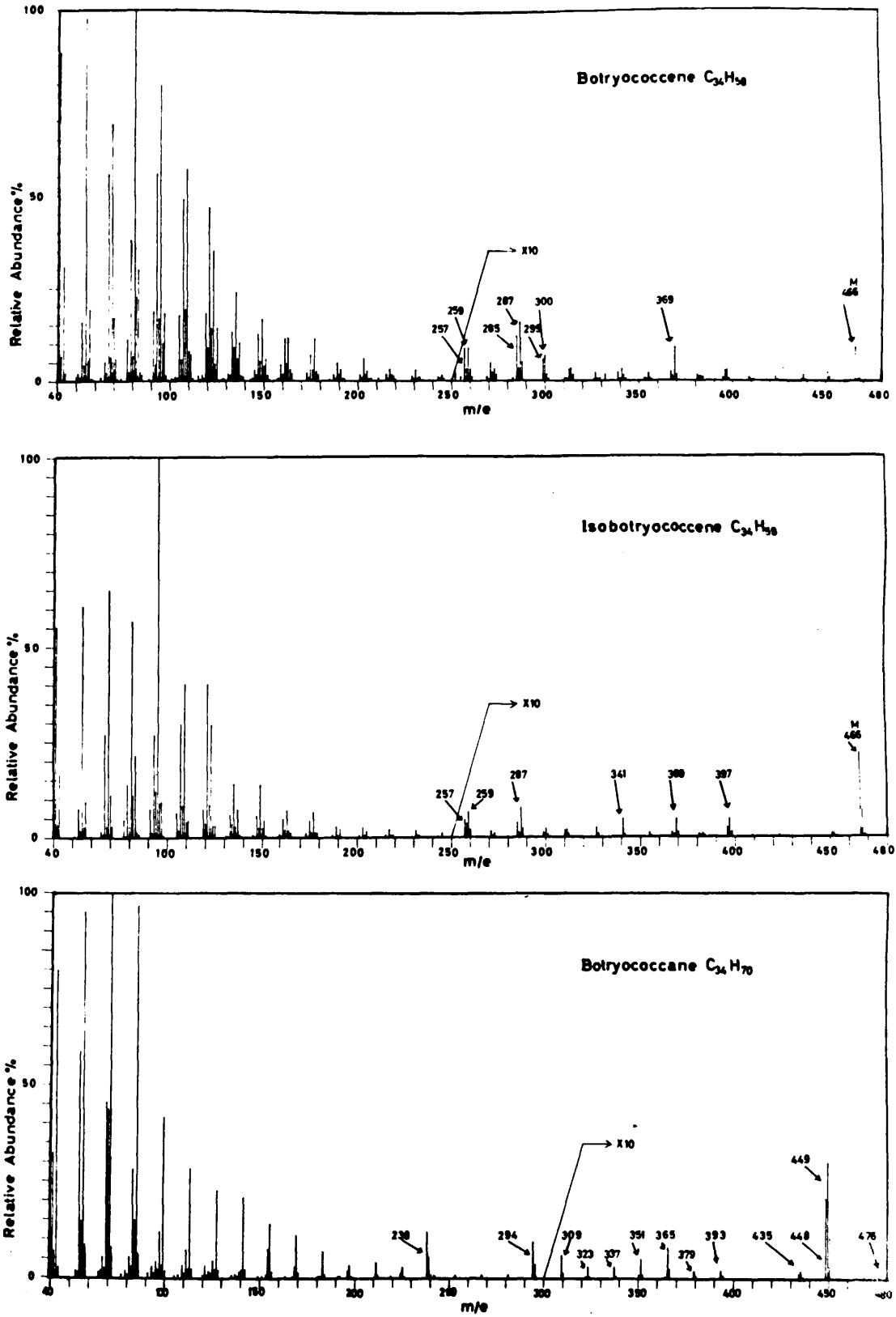


Fig.6.

NMR SPECTRA OF THE BOTRYOCOCCENES AND DIHYDROBOTRYOCOCCENE.

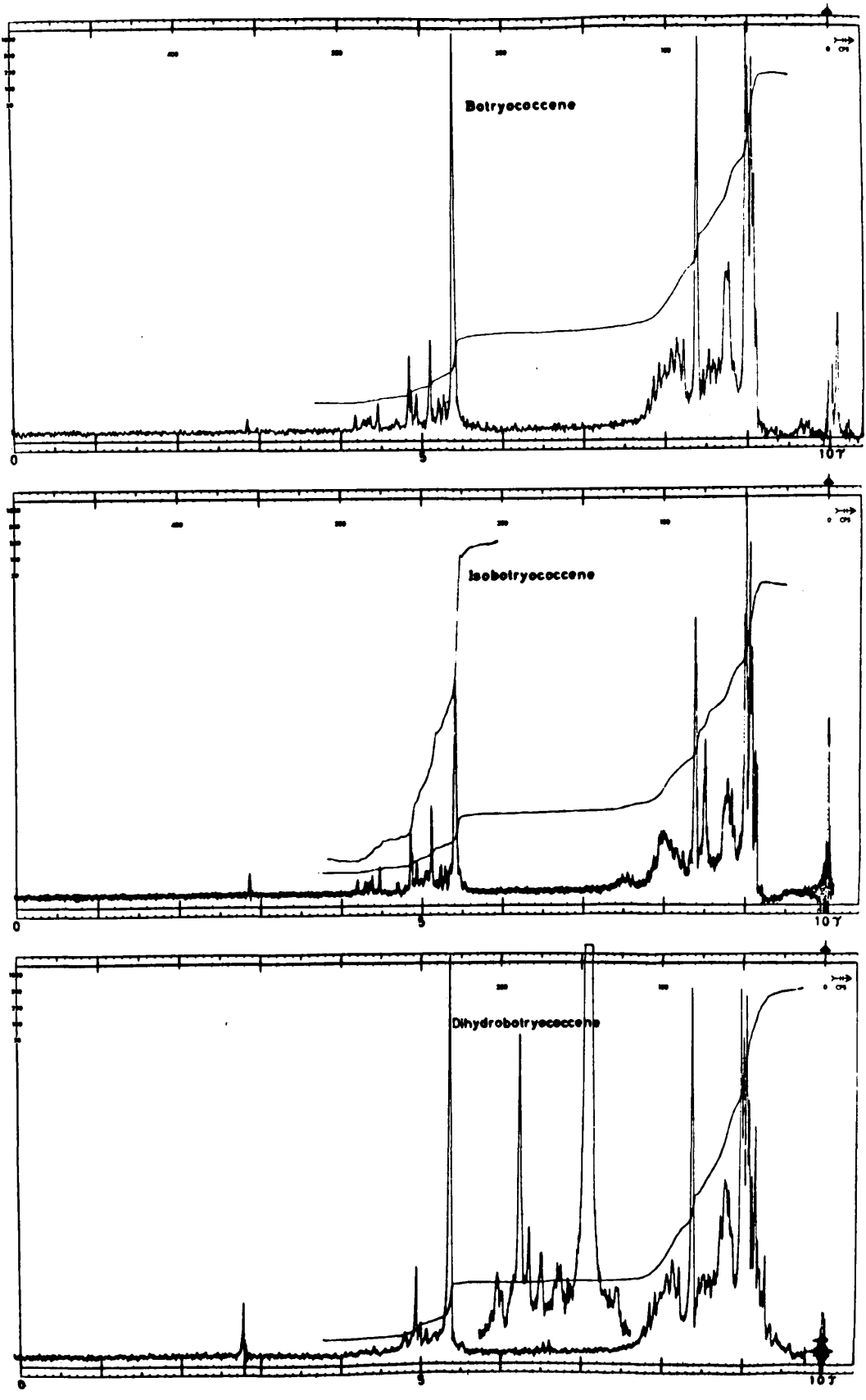


Fig.7.

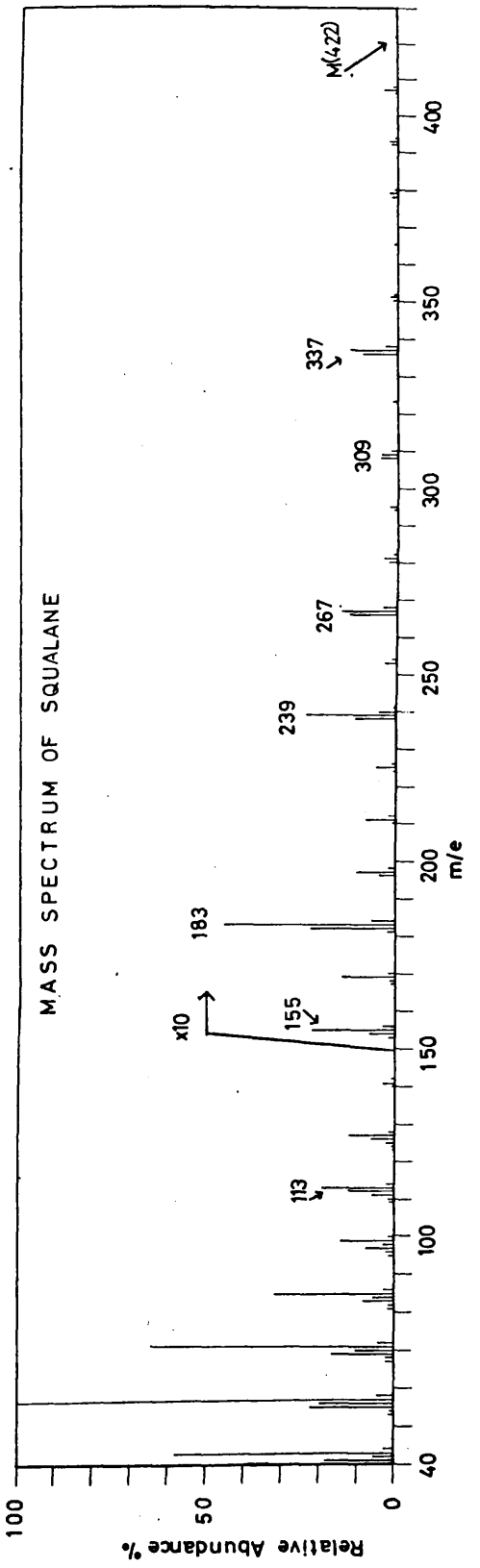
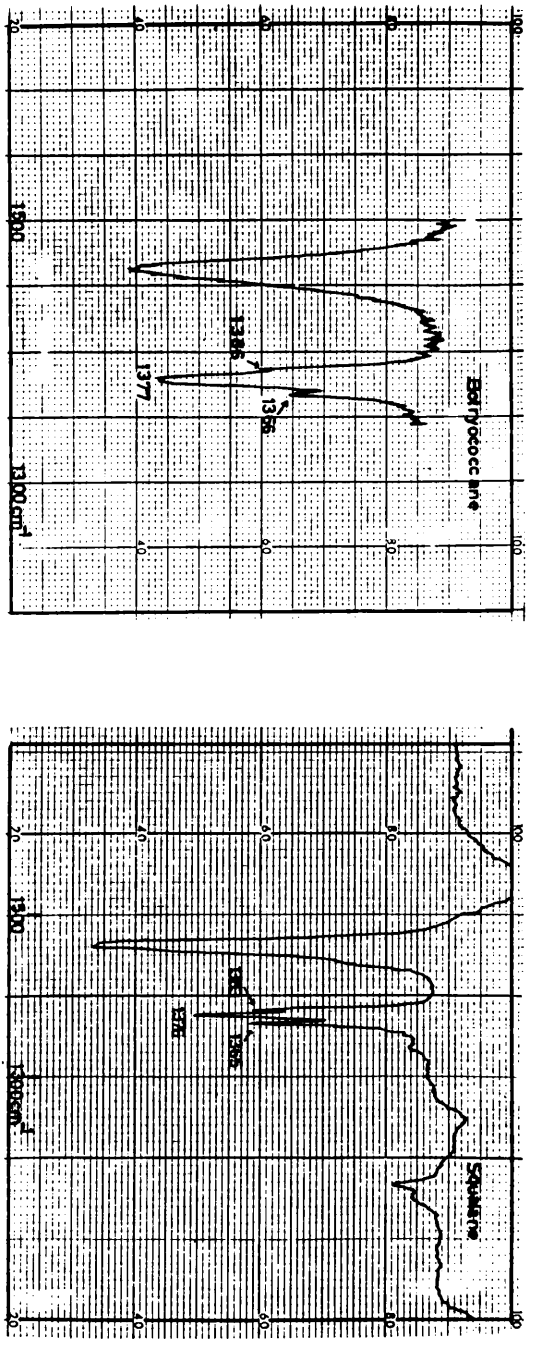
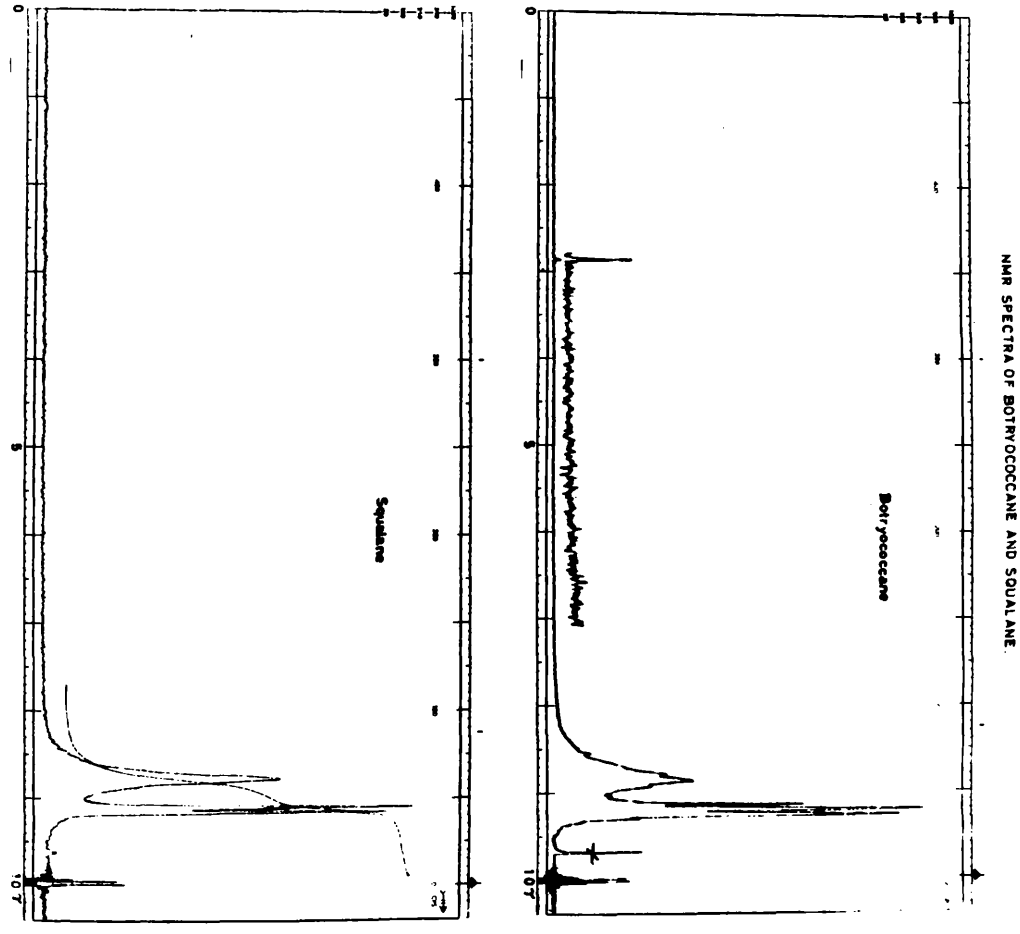

MASS SPECTRUM OF SQUALANE
Relative Abundance %
100
50
0
40
100
150
200
250
300
350
400
m/e
M(422)
337
309
267
239
183
155
x10
113

Fig.8.



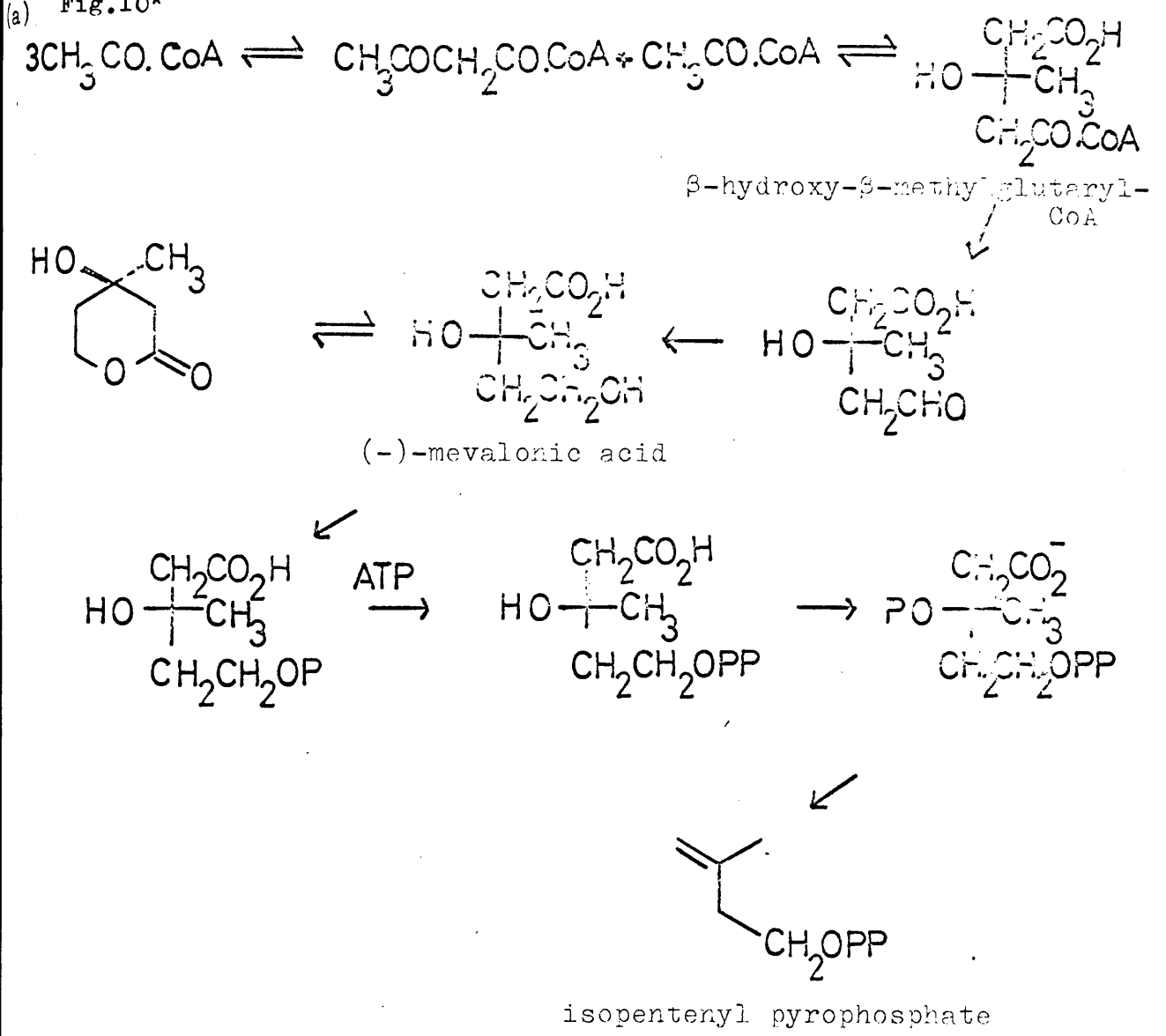
I.R. SPECTRA OF BOTRYOCOCCANE AND SQUALANE - δC-H REGION.

Fig.9.

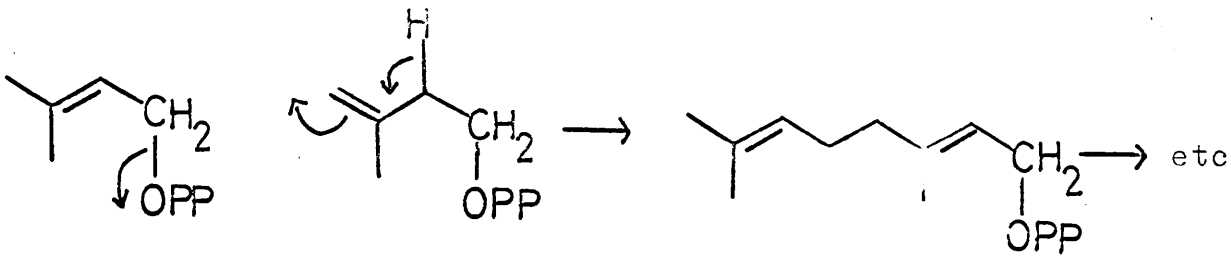


NMR SPECTRA OF BOTRYOCOCCANE AND SQUALANE.

(a) Fig.10*



(b)



* Abstracted from "The Biosynthesis of Natural Products" by J.D. Bu'Lock, McGraw-Hill (1965).

Fig.11 Legend

Thin layer chromatograms of fractions pertaining to *B. braunii*.

(a) *B. braunii* total hydrocarbon and n-C_{28} alkane as reference; benzene developer.

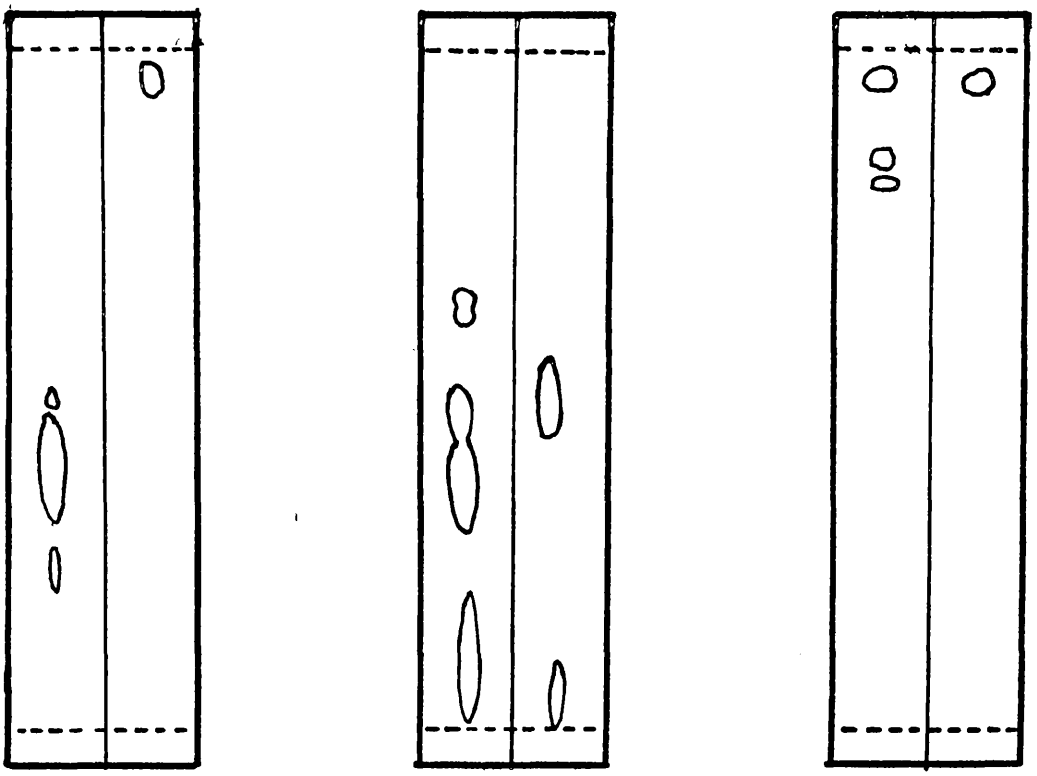
(b) Fraction resulting from the total hydrocarbon fraction of *B. braunii* after absorption of 3.5 mole equivalents of hydrogen with *trans* and vinyl alkenes of D'Arcy oil as reference; n-hexane/10% benzene developer.

(c) Fraction resulting from the total hydrocarbon fraction of *B. braunii* after absorption of 4 mole equivalents of hydrogen with n-C_{28} alkane as reference; n-hexane developer.

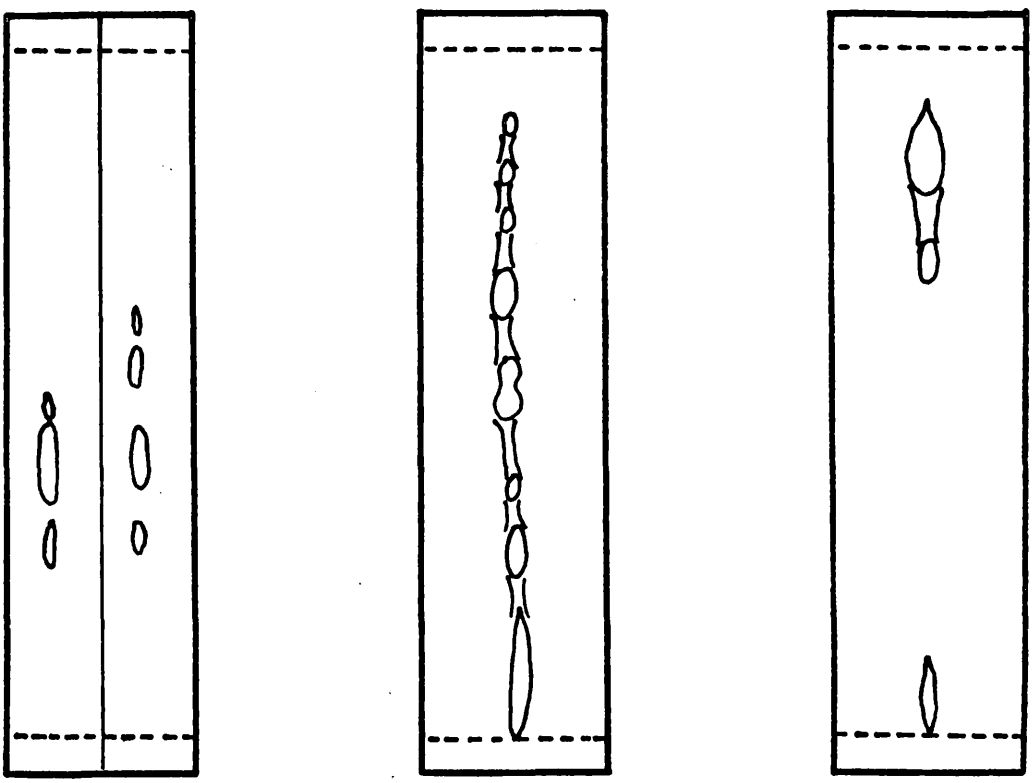
(d) *B. braunii* total hydrocarbon fraction and the fraction arising from treatment of this fraction with hydrogen in the presence of P-2 nickel boride; benzene developer.

(e) Ozonolysis product of *B. braunii* total hydrocarbon fraction; ether developer.

(f) Fraction arising from treatment of the total hydrocarbon fraction with p-toluene sulphonic acid; benzene developer.



(a) (b) (c)



(d) (e) (f)

Fig.12 Legend

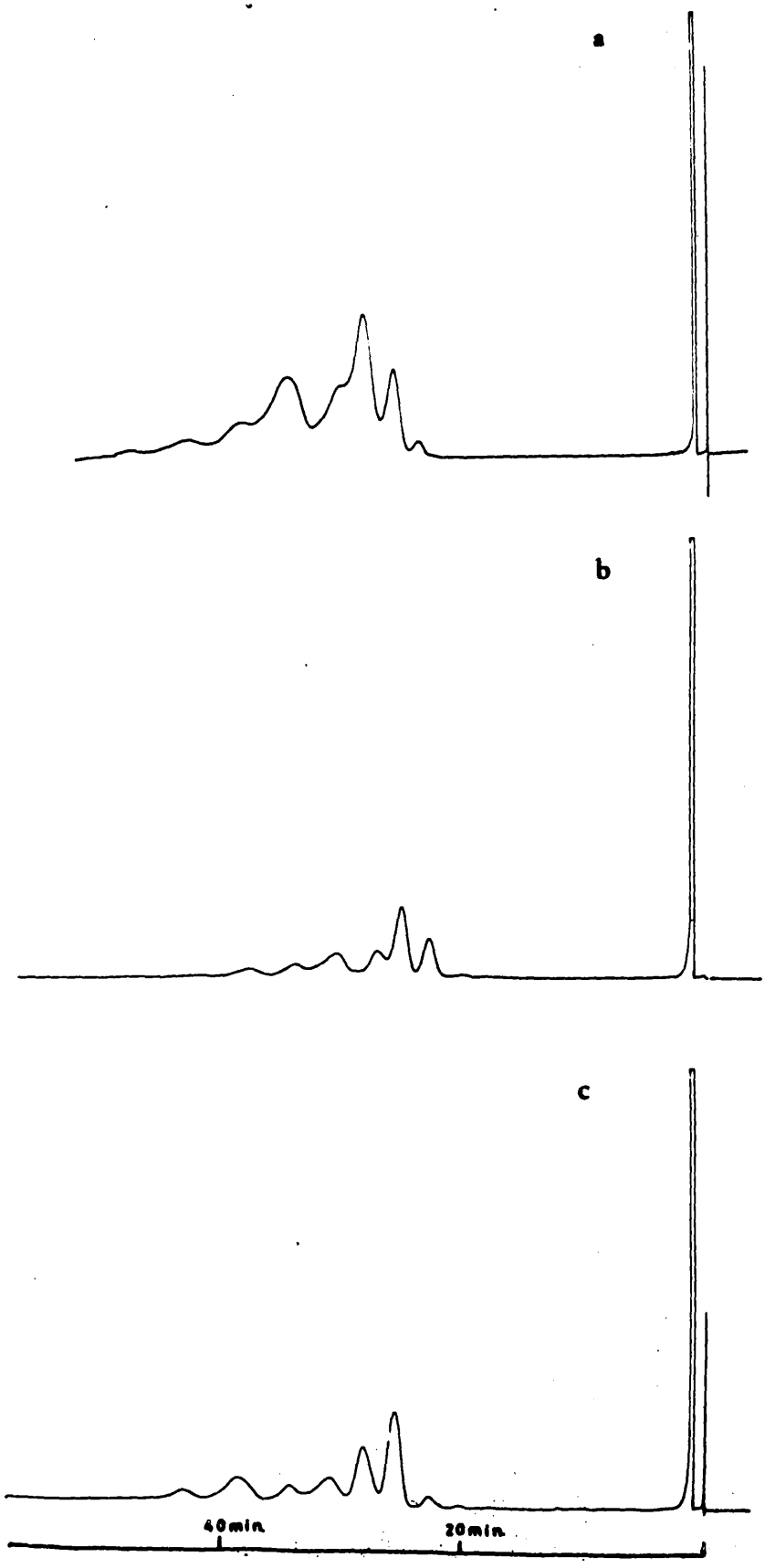
Gas Chromatograms of fractions arising from the hydrocarbon fraction of *B. braunii* after absorption of 3.5 mole equivalents of hydrogen.

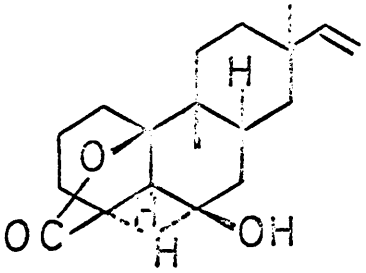
Conditions:

(a) Total product; Column 10 ft. x 1/16 in. 2% 7 ring *m*-polyphenyl ether on Chromosorb G, 100-120 mesh; isothermal at 240°C; sample size 0.1µl. of a solution in iso-octane; attenuation 1 x 10^2.

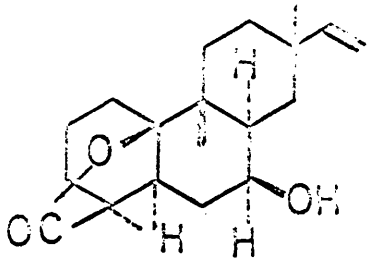
(b) Fraction of total product showing *trans* absorption in the infra-red; conditions as for Fig.12(a); sample size 0.15µl. of a solution in iso-octane.

(c) Second fraction of total product showing *trans* absorption in the infra-red; conditions as for Fig.12(a); sample size 0.1µl. of a solution in iso-octane.

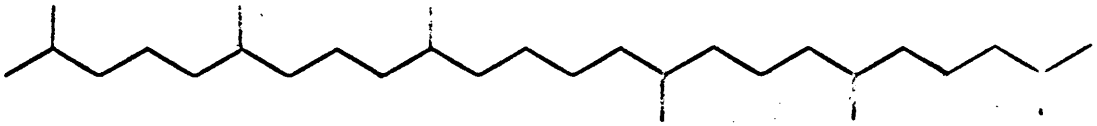

a
b
c
40min.
20min.



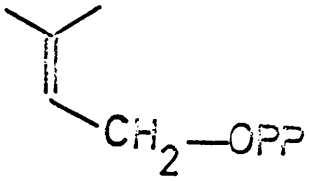
(1)



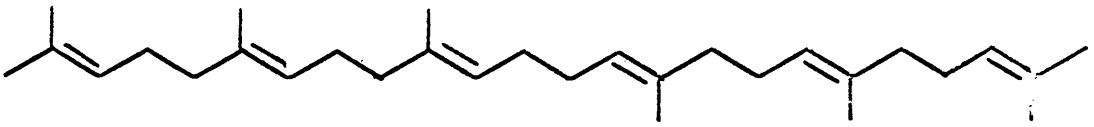
(2)



(3)



(4)



(5)

CONCLUDING REMARKS

This thesis describes the hydrocarbons from a number of geochemically important sources: an unusual living alga, B. braunii; a rubbery deposit of Quaternary age called Coorongite held to be derived from B. braunii; a Carboniferous sediment, Torbanite, composed almost entirely of algal remains, probably B. braunii; a Carboniferous sediment, the Westwood Shale, containing remains of what is apparently B. braunii in addition to some plant remains; a crude oil of Carboniferous age from the same formation as Torbanite and the Westwood Shale; and finally, a shale oil obtained by commercial pyrolysis of Westwood Shale. The connecting link between these samples is the alga B. braunii as the originating biological material, However the Westwood Shale has a marked contribution of plant remains and one further geological sample, a Czechoslovakian lignite, was chosen for examination. This lignite, although much younger and much less consolidated than the Westwood Shale is very largely of flowering-plant origin.

The differences in the alkane distributions of the relatively young lignite (Brown and Yellow Coals) and the older Carboniferous samples emphasise what appear to be major differences in the organic geochemistry of young and ancient sediments. The n-alkanes of the lignite have a marked odd/even preference which is not apparent in the smooth distributions of the corresponding

fractions in Torbanite and the Westwood Shale. Furthermore the branched-cyclic hydrocarbon fractions of the lignite are dominated by cyclic diterpene and triterpene hydrocarbons whereas those of Torbanite and the Westwood Shale, although dominated by isoprenoid hydrocarbons (mainly acyclic), have large proportions of components which are most likely non-isoprenoid branched chain hydrocarbons. In common with the few other young ($< 50 \times 10^6$ yrs.) sediments which have been examined, the Czechoslovakian lignite contains little or no acyclic isoprenoid hydrocarbons. Although possible explanations for the lowering of the C.P.I. of the n-alkanes with time have been discussed, many of the species present 300 million years ago in the Carboniferous period of geological time may have had a smooth distribution of the n-alkanes. The flora contributing to the Westwood Shale comprised the divisions Thallophyta, Bryophyta and Pteridophyta. A few of the contemporary species in these plant divisions have been examined and some members of the Thallophyta and Pteridophyta contain simple triterpenes. The triterpanes present in the Westwood Shale are likely to be derived from plants in these two divisions. In contrast, the lignite has a very different botanical origin and contains plant debris which indicates that the Angiosperms contributed to these sediments to a large extent. The dominance of triterpanes in the branched-cyclic alkane fractions of the lignite possibly reflects the extensive contribution of the Angiosperms since

this division contains simple and complex triterpenes in abundance. The Angiosperms had not yet evolved at the time of deposition of the Westwood Shale. The identification of individual triterpanes from geological sources may therefore provide chemical evidence for the types of flora contributing to different sediments, even after diagenesis of the organic matter.

Although there is no evidence for the presence of saturated hydrocarbons in B. braunii, their occurrence in Coorongite, possibly due to the metabolic activity of bacteria, may in part account for the alkanes present in Torbanite. The isolation of phytane from Coorongite shows that this isoprenoid hydrocarbon could have been present at the time of deposition of Torbanite. This postulate contrasts with the fact that there is no record of the isolation of phytane from a young sediment. Therefore, although current theories of organic geochemistry state that the acyclic isoprenoid hydrocarbons are in the main formed after a long period of diagenesis, such compounds may be rapidly formed by, for example, bacterial action or the chemical changes occurring under special conditions of diagenesis.

The fact that the colony matrix of B. braunii is almost entirely composed of two highly unsaturated hydrocarbons accounts for the formation of Coorongite and presumably Torbanite. Polymerisation of the botryococcenes, perhaps as a result of bacterial action, sunlight, heat or atmospheric oxidation, could result in the formation

of Coorongite which is rubbery in texture. After deposition of the Coorongite compaction of the polymeric material could subsequently result in the formation of Torbanite where the morphology of the alga was preserved. Unfortunately the author has as yet been unable to trace a younger "Torbanite", intermediate in age between Coorongite and Torbanite itself, for examination.

In future the molecular approach to organic geochemistry will require further refinement of the methods for the isolation, purification and identification of geolipids. Also other methods of structural elucidation, especially micro-scale nuclear magnetic resonance spectroscopy and micro-scale optical rotation measurements, must be utilised to define beyond all doubt the structures of the compounds isolated. This is exemplified by the recent finding of McCarthy and Calvin[166] who have shown that the mass spectral fragmentation pattern of pristane is somewhat similar to those of two of its isomers, namely 2,6,10-trimethyl hexadecane and 2,6,10,13-tetramethyl pentadecane, although all three isomers are readily separated by means of high resolution gas chromatography. However the mass spectra of the pristane fractions isolated in the present work compare more favourably with that of authentic pristane than with those of the two isomers.

REFERENCES

1. P.C. Sylvester-Bradley and R.J.King, Nature, 198, 728 (1963).
2. C. Ponnamperuma, address given to Third International Radiation Research Congress, Cortina, Italy (1966).
3. S.L.Miller, Science, 117, 528 (1953).
4. S.L.Miller, J.Amer.Chem.Soc., 77, 2351 (1955).
5. S.L.Miller and H.C.Urey, Science, 130, 245 (1959).
6. S.L.Miller, Ann.N.Y.Acad.Sci., 69, 260 (1957).
7. C.Palm and M.Calvin, J.Amer.Chem.Soc., 84, 2115 (1962).
8. K.Harada and S.W.Fox, Nature, 201, 305 (1964).
9. C.Ponnamperuma, R.M.Lemon, R. Mariner and M. Calvin, Proc. Nat.Acad.Sci., 49, 737 (1963).
10. C. Ponnamperuma, Icarus, 5, 450 (1966).
11. C. Ponnamperuma, R. Mariner and C.Sagan, Nature, 198, 1199 (1963).
12. C. Ponnamperuma and R. Mack, Science, 148, 1221 (1965)
13. C. Ponnamperuma, C.Sagan and R. Mariner, Nature, 199, 222 (1963).
14. C. Ponnamperuma and F. Woeller, Nature, 203, 272 (1964).
15. C. Ponnamperuma and K. Pering, Nature, 209, 979 (1966).
16. C. Ponnamperuma, personal communication.
17. G. Eglinton, A.G. Douglas, J.R. Maxwell and J.N. Ramsay, Science, 153, 1133 (1966).
18. J.N. Ramsay, M.Sc. Thesis, Glasgow (1966).
19. J. Cason and D.W. Graham, Tetrahedron, 21, 471 (1965).
20. E.S. Barghoorn, W.G. Meinschein and J.W. Schopf, Science 148, 461 (1965).

21. P.H.Abelson, Ann.N.Y.Acad.Sci., 69, 276 (1957).

22. B.J.Tarlo and L.B.H. Tarlo, Discovery, 20, September (1965).

23. M.Blumer, Nature, 188, 1100 (1960).

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

25. B.R.Brown, A.Calderbank, A.W.Johnson, B.S.Joshi, J.R.Quayle and A.R.Todd, J.Chem.Soc., 959 (1955).

26. H.Brockmann, Fortschr..Chem.Org.Naturstoffe, 14, 141 (1957).

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

28. R.Fikentscher, Zool.Anz., 103, 289 (1933).

29. D.W.Thomas and M.Blumer, Geochim.et Cosmochim.Acta, 28,1147 (1964).

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

31. J.W.Jurg and E.Eisma, Science, 144, 1451 (1964).

32. J.W.Jurg and E.Eisma, in "Advances in Organic Geochemistry", vol.3, Pergamon Press, in press.

33. A.G.Douglas, G.Eglinton and W.Henderson in "Advances in Organic Geochemistry", vol.3, Pergamon Press, in press.

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

35. J.A.Gransch and E.Eisma in "Advances in Organic Geochemistry", vol.3, Pergamon Press, in press.

36. J.R.Vallentyne, Limnology and Oceanography, 1, 252 (1956).

37. J.R.Vallentyne,Arch.Biochem.Biophys., 70,29 (1957).

38. S.T.Andersen and K.Gundersen, Experientia, 11,345 (1955).

39. R.B.Schwendinger and J.G.Erdman, Science, 141, 808 (1963).

40. J.R.Whittaker and J.R.Vallentyne, Limnology and Oceanography, 2, 98 (1957).

41. S.C.Rittenberg, K.O.Emery, J.Hulsemann, E.T.Degens, R.C.Fay, J.H.Reuter, J.R.Grady, S.H.Richardson and E.E.Bray, J.Sed.Petrol, 33, 140 (1963).

42. M.Calvin, Proc.Tenth International Bot.Cong., (1964).

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

44. W.H.Bradley, Amer.J.Sci., 262, 413 (1964) and references therein.

45. E.S.Barghoorn and S.A.Tyler, Science, 147, 563 (1965).

46. P.E.Cloud, J.W.Gruner and H.Hagen, Science, 148, 1713 (1965).

47. M.Schlidlowski, Nature, 205, 895 (1965).

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

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

50. M.Kates, L.S.Yengoyan and P.S.Sastry, Biochim.Biophys.Acta, 98, 252 (1965).

51. R.B.Johns, T.Belsky, E.D.McCarthy, A.L.Burlingame, P.Haug, H.K.Schnoes, W.Richter and M.Calvin, Technical Report N.S.G.101-61, Series 7, Issue No. 8, (1966) and Geochim.et Cosmochim.Acta, 30, 1191 (1966).

52. M.Blumer and D.W.Thomas, Science, 148, 370 (1965).

53. R.P.Hansen, F.B.Shorland and J.D.Morrison, J.Dairy Res., 32, 21 (1965) and references therein.

54. W.R.H. Duncan and G.A.Garton, Biochem.J., 89, 414 (1963).

55. A.K.Lough, Biochem.J., 86, 14P (1963).

56. R.P.Hansen, F.B.Shorland and N.J. Cooke, J.Sci.Food Agr., 9,391 (1958

57. R.P.Hansen, New Zealand J.Sci., 8, 158 (1965).

58. R.P.Hansen and J.D.Morrison, Biochem.J., 93, 225 (1964)

59. M.Blumer and D.W.Thomas, Science, 147, 1148 (1965).

60. G.Ourisson, personal communication to A.G.Douglas and G.Eglinton, 1965.

61. J.W.K.Burrell, R.F.Garwood, L.M.Jackman, E.Oskay and B.C.L.Weedon, J.Chem.Soc., (C), 2144, 1966.

62. G.Eglinton and R.J.Hamilton, "The Distribution of Alkanes", in Chemical Plant Taxonomy, Academic Press, London (1963).

63. G.Eglinton, P.M.Scott, T.Belsky, A.L.Burlingame, W.Richter and M.Calvin in "Advances in Organic Geochemistry", vol.2, Pergamon Press, London (1965).

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

65. C.B.Koons, G.W.Jamieson and L.S.Ciereszko, Amer.Assoc.Petrol. Geo.Bull., 49, 301 (1965).

66. A.L.Burlingame, P.Haug, T.Belsky and M.Calvin, Proc.Nat.Acad.Sci. U.S.A., 54, 1406 (1965).

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

68. Sister M.T.J. Murphy and A.McCormick, personal communication.

69. J.S.Harrington and A.D.Toens, Nature, 200, 947 (1963).

70. C.G.A. Marshall, J.W.May and C.J.Perret, Science, 144, 290 (1964).

71. J.Oro, D.W.Nooner and A.Zlatkis, Science, 148, 77 (1965).

72. P.E.Cloud, J.W.Gruner and H.Hagen, Science, 148, 1713 (1965).

73. T.C.Hoering, personal communication to W.G.Meinschein (1965). T.C.Hoering and P.H.Abelson, Ann.Rep.Carnegie Inst. of Washington, 64, 218 (1965).

74. W.G. Meinschein, Science, 150, 601 (1965).

75. V.Wollrab, M. Streibl and M. Šorm, Coll.Czech. Chem. Comm., 28 1904 (1963).

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

77. E.E. Bray and E.D. Evans, Bull.Amer.Assoc.Petrol.Geol., 49, 248 (1965).

78. M.Blumer and W.D.Snyder, Science, 150, 1588 (1965).

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

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

81. A.T.James and L.J. Morris, "New Biochemical Separations" chapt.14, D. Van Nostrand Co. Ltd., London (1964).

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

83. P.W.Thomson "Braunkohle, Wärme und Energie", no. 3/4, 39(1950); cited by W.Francis "Coal" E.Arnold Ltd. (1954).

84. W.Francis "Coal", E. Arnold Ltd. (1954).

85. V.Wollrab, M.Streibl and F.Šorm, Chem.and Ind., 1762 (1962).

86. V.Wollrab and M.Streibl, Coll.Czech.Chem.Comm., 28, 1895 (1963).

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

88. M.Streibl and F.Šorm, Coll.Czech.Chem.Comm. 31, 1585 (1966).

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

90. V.Jarolim, K.Hejno and F.Šorm, Coll.Czech.Chem.Comm., 28, 2443 (1963)

91. V.Jarolim, K.Hejno, F.Hemmert and F.Šorm, Coll.Czech.Chem.Comm. 30, 873 (1965).

92. P.Jarolimek, V.Wollrab, M.Streibl and F.Šorm, Chem.and Ind., 237 (1964).

93. V.Wollrab and M.Streibl, in "Organic Geochemistry. Methods and Results" ed. G.Eglinton and Sister M.T.J.Murphy, Springer-Verlag, N.Y. Inc., in press.

94. W.Wenz, Jb. Nassauish, Ver.Naturk.Wiesbaden, 7, 39 (1917); cited by Čtyroky et al. ref. (95).

95. P.Čtyroky, O.Fejfar and F.Holy, N.Jb. Geol.Palaont.Abh., 119, 134 (1964).

96. V.Wollrab, personal communication.

97. H.Budzikiewicz, J.M.Wilson and C.Djerassi, J.Amer.Chem.Soc., 85, 3688 (1963).

98. J.Karliner and C.Djerassi, J.Org.Chem., 31, 1945 (1966).

99. I.R.Hills and E.V.Whitehead, in "Advances in Organic Geochemistry", vol.3, Pergamon Press. in press.

100. M.McGregor in "Oil Shale and Cannel Coal", 6, The Institute of Petroleum (1938).

101. J.A.Dulhunty,D.Sc.Thesis, Sydney (1943).

102. H.R.J.Conacher, Trans.Geol.Soc. Glasgow, 16, 164 (1917).

103. D.Stewart and C.E.Forbes in "Oil Shale and Cannel Coal", 96, The Institute of Petroleum (1938).

104. H.M.Thorne and J.S. Ball, in "The Chemistry of Petroleum Hydrocarbons", 63, Reinhold Publishing Corp. (1954).

105. H.R.J. Conacher, Trans.Geol.Soc. Edin., 11, 319 (1925).

106. B.K.N. Wyllie in "Oil Shale and Cannel Coal", 19, The Institute of Petroleum (1938).

107. W.Flaig and H.Beutelspacher, Leitz. Mitt.Wiss.u Tech., 7, 199(1961).

108. A.G.Douglas, K.Douraghi-Zadeh, G.Eglinton, J.R.Maxwell and J.N.Ramsay in "Advances in Organic Geochemistry", vol.3, Pergamon Press, in press.

109. J.H.Belcher and G.E.Fogg, New Phytologist, 54, 81 (1955).

110. R.Ciusa and A.Galizzi, Gazz.Chim.Ital., 51, 55 (1921).

111. R.Ciusa and M.Croce, Gazz.Chim.Ital., 52, 125 (1922).

112. R.Ciusa and A.Galizzi, Ann.Chim.Appl., 15, 209 (1925).

113. W.L. Whitehead and I.A.Breger, in "Organic Geochemistry" Pergamon Press (1963).

114. T.Dyer, J.Bot., 103 (1872).

115. A.C.Broughton, personal communication to H.R.J.Conacher, ref.119.

116. E. de Hautpick, Bull.Soc.Geol.France, Ser.4, 26 (1926).

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

118. M.D.Zalessky, Rev.Gén.Bot., 38, 31 (1926).

119. H.R.J.Conacher in "Oil Shale and Cannel Coal", Institute of Petroleum (1938).

120. A.C.Cumming, Proc.Roy.Soc.Victoria, 15, 134 (1902).

121. A.G.Douglas, K.Douraghi-Zadeh and G.Eglinton, personal communication.

** cited by Conacher, ref. 119

122. W.Schwartz, Florida State University, Talahassee, Florida, personal communication.

123. P.W.Albro and C.K.Huston, J.Bacteriol., 88, 981 (1964).
C.K.Huston and P.W.Albro, J.Bacteriol., 88, 425 (1964).

124. R.F.Lee and P.L. Parker, Science, 152, 649 (1966).

125. A.S.Kester and J.W.Foster, J.Bacteriol., 85, 859 (1963).

126. C.E. Zobell, Science, 102, 364 (1945).

127. C.E. Zobell, Bact.Revs., 10, 1 (1946).

128. F.E.Fritsch, "Structure and Reproduction of the Algae", Vol.1, Cambridge University Press (1935).

129. J.H. Belcher, Ph.D. Thesis, London (1958).

130. M.D.Zalessky, Bull. Comité Géol.Pétersbourg, 33, no.248, 495 (1914).

† 131. R.Thiessen, U.S.Geol.Surv.Prof.Papers, 1321, 121 (1925).

132. D.M.Colleyer and G.E.Fogg, J.Exp.Bot., 6,256 (1955).

133. F.M.Swain and J.M.Gilby, Pubbl.staz. zool.Napoli, 33 suppl., 361 (1964).

134. J.H.Balfour, Trans.Roy.Soc.Edin., 21, 187 (1854).

** 135. P.Redfern, Quart.Journ.Micr.Soc., 106 (1855).

** 136. J.Quekett, Trans.Micr.Soc.London, 2, 34 (1853).

137. J.H.Bennett, Trans. Roy.Soc.Edin. 21, 173 (1854).

** 138. T.W.E. David, Proc.Linn.Soc. N.S.W., Ser. 2, 4. 483 (1889).

** 139. E.C.Jeffrey, Rhodora, 9, 61, Boston (1909).

140. E.H.Cunningham-Craig, J.Inst.Pet.Tech., 2, 238 (1916).

† cited from reference 133.

** cited from reference 117.

** 141. C.E. Bertrand, Bull.Soc.Géol.Paleént., 7, 45 (1894).

** 142. C.E. Bertrand, Bull.Soc.Hist.Nat.Autun. 9, 193 (1896).

** 143. C.E. Bertrand, Bull.Soc.Industr.Min., ser. 3, 6, 453 (1892).

** 144. C.E. Bertrand, Proc.Linn.Soc. N.S.W., 25, 617 (1901).

** 145. C.E. Bertrand and B.Renault, Ann.Soc.Géol.Nord., 20, 213 (1892).

** 146. C.E. Bertrand and B.Renault, Bull.Soc.Hist.Nat. Autun, 5, 159 (1892).

** 147. C.E. Bertrand and B.Renault, Bull.Soc.Industr.Min., ser.3, 7, 499 (1893).

** 148. C.E. Bertrand and B.Renault, Bull.Soc.Hist.Nat.Autun, 7, 321 (1893)

149. P. Bertrand, Comt.Rend.Soc.Biol., 96, 695 (1927).

** 150. P. Bertrand, Congrès Internat.des Mines, Metallurgie et Geol. appliquée, Sect. de Géol. 6th Session, 159 (1930).

151. T.Cairns, G.Eglinton, A.I.Scott and D.W. Young, J.Chem.Soc., (B), 654 (1966).

152. H.L. McMurray and V.Thornton, Anal.Chem., 24, 318 (1952).

153. D.Barnard, L.Bateman, A.J.Harding, H.P.Koch, N.Sheppard and G.B.B.M. Sutherland, J.Chem.Soc., 915 (1950).

154. R.M.Carman, Aust.J.Chem., 16, 1104 (1963).

155. K.Biemann, "Mass Spectrometry", McGraw-Hill (1962).

156. A.C. Cope, R.K.Bly, E.P. Burrows, O.J.Ceder, E.Ciganek, B.T.Gillis, R.F. Porter and H.E. Johnson, J.Amer.Chem.Soc. 84, 2170 (1962).

** cited from reference 117.

157. J.A. McCloskey and M.J.McClelland, J.Amer.Chem.Soc., 87 5090 (1965)

158. H.C.Brown and C.A. Brown, J.Amer.Chem.Soc., 85, 1005 (1963).

159. E.Von Rudlhoff, Can.J.Chem., 33, 1714 (1955).

160. R.Pappo, D.S.Allen, R.V.Lemieux and W.S.Johnson, J.Org.Chem., 21, 478 (1956).

161. C.Donniger and G. Popjàk, Proc.Roy.Soc., 163, 465 (1966).

162. M.Kates, Ann. Rev. Microbiol., 20, 13 (1966).

163. G.Ödham, Arkiv För Kemi, 23, nr. 35, 431 (1965).

164. L.J. Goad and T.W. Goodwin, Biochem.J., 99, 735 (1966).

165. M.Akhtor, P.F.Hunt and M.A. Parvez, Chem.Comm., 16, 565 (1966).

166. E. McCarthy and M. Calvin, Meeting of Amer.Chem.Soc., New York Oct. (1966).

A P P E N D I X.

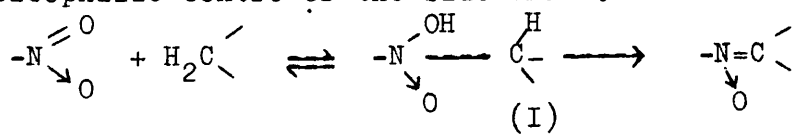
SUBSTITUENT INTERACTIONS IN ORTHO-SUBSTITUTED NITROBENZENES.

INTRODUCTION

For a number of years there has been considerable interest in this department in substituent interactions in ortho-substituted nitrobenzenes. In these compounds, interaction can occur between the nitro-group and an ortho side chain to produce a bewildering array of heterocyclic products, depending on the nature of the side chain and on the reaction conditions. Such reactions are most often, but not always, base or acid catalysed.

Loudon and Tennant[1] have gathered together examples scattered throughout the literature and their review includes examples of all of the known types of interaction, excluding photochemical transformations. It has been postulated by these workers that the nitro-group may be intact, in the aci-nitro form or the nitroso form in the ring-forming step.

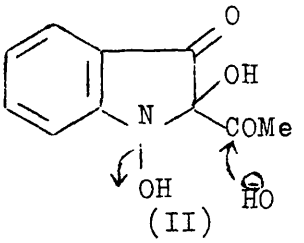
Treatment of certain 2'-substituted 2-nitrodiphenyls [(1);R=CO_2Me,$CONH_2$,CN,COPh,SO_2Ph] with sodium hydroxide in methanol gives phenanthridine derivatives[2,3] of type (2) R'=CO_2Me or CO_2H, $CONH_2$,CN,H,OH respectively. Here the nitro-group cannot be influenced by the ortho side chain as there is no hydrogen atom in the side chain in the position α to the nitro-substituted benzene ring. Therefore it appears that the nitro-group is intact in the ring-forming step and an aldol type of mechanism (I) takes place with the nucleophilic centre of the side chain.



(I)

It is noteworthy that cyclodehydration does not occur for (1);R=H,OH,Br,Ph,CO_2H since presumably these substituents are inadequate for carbanion formation in the side chain. Also 2'-amino-2-nitrodiphenyl (3) undergoes cyclodehydration to give the N-oxide (4).

If methylene reactivity at the position ω to the benzene ring is enhanced (as above), o-nitroacetophenone derivatives (5) should undergo this type of cyclodehydration to give isatogens of type (6). Here the nitro-group should also be intact in the ring-forming step as there is no α hydrogen atom available. When o-nitrobenzoylacetone (5);R=COMe is treated with potassium hydroxide in aqueous ethanol, isatin (7) is formed[4]. According to Loudon and Tennant, who recorded this reaction, the isatin may be formed by hydroxide attack on the hydrated form of the expected 2-acetyl isatogen as in (II).



(II)

Since the completion of the work described in the discussion below, Ahmad and Shamsi [5] have reported that the nitro compound (8) affords the quinoline N-oxide (9) when heated with potassium hydroxide. Here again the nitro-group appears to be intact in the ring-forming step, as the hydrogen atom in the position α to the benzene ring is not available to it.

Although there are α protons in the side chains of o-nitrobenzylmalonic acid (10) and ethyl o-nitrobenzylacetoacetate (11) it is possible that the nitro-group is intact in the ring-forming step for the formation of 1-hydroxyindole-2-carboxylic acid[6,7](12) since proton removal is more likely at the β carbon atom to give a carbanion. If this is the course of the reaction an intermediate such as (13) would be expected. In the related diethyl-α-cyano-2-nitrobenzylmalonate (14) and diethyl-α-carbamoyl-α-2-nitrobenzylmalonate (15) the α position is activated both by the α substituent and by the nitro-group. When treated with sodium carbonate these compounds give rise to the expected indole derivatives[8] (16);R=CN or $CONH_2$. Here an intermediate similar to (13) is a possibility, where proton removal at the position α to the benzene ring occurs after the cyclisation step. Alternatively the α proton may be removed first to give the aci-nitro form (17). Removal of the β hydrogen followed by condensation would also give the indole (16);R=CN or $CONH_2$. There is also a third possibility for the course of this reaction i.e. that a nitroso intermediate (18) is involved in the ring-forming step for indole formation. The nitrile (14) also reacts with potassium hydroxide to give ethyl-4-cyano -1,2-dihydro-1-hydroxy-2-oxoquinoline-3-carboxylate[8](19) instead of the indole (16); R=CN. The authors state that for formation of this product a strongly alkaline environment and reactivity in the benzylic hydrogen atom of (14) are required and in a weakly alkaline medium or in absence of the activating cyano group the predominant product is the 1-hydroxyindole

(16),R=CN. Product (19) would normally require a precursor of the hydroxylamine type but compound (14) by intramolecular oxidation/reduction can only provide a nitroso intermediate (18), so it appears that reduction, presumably by the reaction medium, is involved. Direct reduction of (14) with zinc and ammonium chloride gives the 3,4-dihydro derivative[8](20) of the quinolone (19). Loudon and Tennant conclude that if the dihydride can be rejected as an intermediate, then the course depicted via the nitroso compound and the hydroxylamine is a likely one. The 1-hydroxyquinolone can also be obtained by treating o-nitrobenzylidenemalonate (21) with potassium cyanide.

In a similar fashion 2-nitro-α-phenylcinnamonitrile (22) and α-o-nitrophenylcinnamonitrile (23) form 2-amino-4-cyano-3-phenylquinoline-1-oxide (24) and 3-cyano-1-hydroxy-2-phenylindole (25) on treatment with potassium cyanide[9]. The expected intermediate adduct (26) was not isolated in these two reactions.

The α-benzyl-o-nitrobenzyl cyanides (25);R=Ph and $C_6H_2(OMe)_3$ both yield indoles on treatment with potassium hydroxide despite the feeble activation of the β methylene protons.

Consideration of the reactions of the above nitro compounds each with an acidic α (and perhaps β) hydrogen atom led Loudon and Tennant[1] to record that "there are too many uncontrolled variables to warrant extensive discussion of these reactions. Broadly, a highly reactive α-hydrogen atom in the side-chain of the nitro compound

appears to be a facilitating factor even for indole formation, but whether it operates before or after the ring-forming step is uncertain".

Only a few of the substituent interactions in ortho-substituted nitrobenzenes have been recorded here in order to emphasise the difficulties encountered in considering the course of such reactions. In the examples described it has been assumed that polar mechanisms operate, but this might not necessarily be the case since nitro-group interactions may take place under photochemical conditions[10].

DISCUSSION.

Whilst examining the action of phosphorus pentachloride on non-symmetrically substituted acetones Zaki and Iskander[11] reported that treatment of ethyl-α-2,4-dinitrophenyl-γ-phenylacetoacetate (28) with sodium ethoxide gave a yellow crystalline compound, which they formulated as 3-hydroxy-1-ethoxy-2-2',4'-dinitrophenyl-napthalene (29) solely from its carbon, hydrogen and nitrogen analysis. They reported that the compound was unchanged by dilute acid but was resinified with concentrated sulphuric acid and on treatment with sodium hydroxide gave 1,3-dihydroxy-2-2',4'-dinitrophenylnapthalene (29);OH for OEt.

Loudon and co-workers[12], from a knowledge of interactions in o-substituted nitrobenzenes, considered that the yellow crystalline compound might be the isomeric 2-phenyl-3-hydroxy-4-carbethoxy-7-nitroquinoline-1-oxide (30). This appeared to be the case since the infra-red spectrum of the compound had absorption at 1650cm.$^{-1}$, indicative of an ester of the salicylic type. The corresponding acid (isomeric with the above dihydroxy napthalene) was not obtained pure but the infra-red spectrum again showed salicylic-type carbonyl absorption.

With a view to proving conclusively that the product of cyclisation was indeed the quinoline-1-oxide and not the napthalene, ethyl-α-2,4-dinitrophenyl-γ-phenylacetoacetate (28) was treated with sodium ethoxide and the resulting yellow crystalline product isolated.

The infra-red spectrum had absorption at 1645cm.$^{-1}$, indicating the presence of a bonded ester carbonyl. The nuclear magnetic

resonance spectrum was also in accord with the N-oxide structure (30) (Table 1). The nuclear magnetic resonance spectrum expected for the napthalene structure (29) would be very similar to that recorded but it is very unlikely that the protons at positions 4,5,6,7 and 8 in (29) would give the singlet observed at τ2.30 for the five protons of the monosubstituted benzene ring of the N-oxide (30). Also the mass spectrum showed an abundant $(M-16)^+$ ion characteristic of aromatic N-oxides (see below). Hydrogenation of the product of cyclisation in acetic anhydride and acetic acid gave 2-phenyl-3-hydroxy-4-carbethoxy-7-acetaminoquinoline (31) in which the N-oxide function had been lost through hydrogenolysis. The infra-red spectrum of (31) had absorption at 3330 and 1665cm.$^{-1}$ for the acetamino group. Both the nuclear magnetic resonance spectrum (Table 1) and the molecular weight, from the mass spectrum, were in accordance with (31).

If compound (30) is in fact the product of cyclodehydration by way of a nitro-group - side-chain interaction in (28), then the compound obtained by Zaki and Iskander by treatment of the "ethoxy-napthalene" with sodium hydroxide should have been 2-phenyl-3-hydroxy-4-carboxy-7-nitroquinoline-1-oxide (30); CO_2H for CO_2Et. However, when (30) was treated with sodium hydroxide, the product had no carbonyl absorption in the infra-red. This result would explain why Loudon and his co-workers[12] did not obtain the carboxylic acid pure since it appears that decarboxylation takes place extremely readily during the work-up or the hydrolysis itself.

The infra-red spectrum and the mass spectrum were in agreement with (32) as the main product of hydrolysis. Sublimation of the N-oxide (32) under reduced pressure afforded a mixture of two compounds which were separated with sodium bicarbonate. The bicarbonate-soluble fraction was unchanged 2-phenyl-3-hydroxy-7-nitroquinoline-1-oxide (32) whereas the bicarbonate-insoluble fraction proved to be the corresponding quinoline, 2-phenyl-3-hydroxyquinoline (33) whose analysis, infra-red spectrum, and molecular weight (mass spectrum) agreed with the proposed structure.

The above results show that the compound formulated by Zaki and Iskander was in fact 2-phenyl-3-hydroxy-4-carbethoxy-7-nitroquinoline-1-oxide (30).

In order to expand the scope of this particular type of interaction between an o-substituted side-chain and a nitro-group it was decided to examine the effect of sodium ethoxide on the di-ester(28);CO_2Et for Ph . The ω hydrogen atoms in this compound should be more activated than those in (28) itself. Indeed,treatment of acetone dicarboxylic ester and 1-Chloro-2,4-dinitrobenzene with sodium ethoxide to obtain (26);CO_2Et for Ph resulted in the direct formation of 2,4-dicarbethoxy-3-hydroxy-7-nitroquinoline-1-oxide (34). The expected 2,4-dinitrophenyl acetoacetic ester derivative (28);CO_2Et for Ph was not isolated. The structure of the cyclodehydration product of this reaction was proved in an analogous

manner to that for 2-phenyl-3-hydroxy-4-carbethoxy-7-nitroquinoline-1-oxide (30). The compound was soluble in base and gave a red colour with ferric chloride. The infra-red spectrum, mass spectrum and nuclear magnetic resonance spectrum (Table 1) were in accord with the proposed N-oxide structure. The phenol proton disappeared from the nuclear magnetic resonance spectrum when dimethyl sulphoxide, containing a little water, was used as solvent.

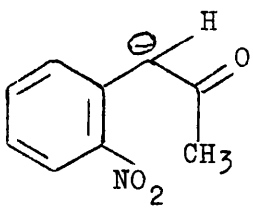
Hydrogenation of (34) in acetic anhydride and acetic acid again afforded a quinoline, viz. 2,4-dicarbethoxy-3-hydroxy-7-acetaminoquinoline (35) in an analogous fashion to the hydrogenation of (30).

The di-ester (34) was not hydrolysed by sodium carbonate, dilute acid or a mixture of concentrated sulphuric acid and acetic acid. However, hydrolysis with sodium hydroxide gave a crude product which was separated into two components. One of the components had an infra-red spectrum indicative of an acid and the nuclear magnetic resonance spectrum showed the appearance of a new aromatic proton, indicating that decarboxylation had taken place at either the 2 or 4-position of (34) after hydrolysis. This compound was therefore probably either 3-hydroxy-4 carboxy-7-nitroquinoline-1-oxide (36) or 2-carboxy-3-hydroxy-7-nitroquinoline-1-oxide (37). The other product of hydrolysis showed no carbonyl absorption in the infra-red and the spectrum was very similar to that of 3-hydroxy-7-nitroquinoline-1-oxide (38) (see below). These results indicated

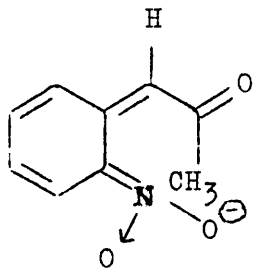
that decarboxylation was again readily taking place either during hydrolysis or during the work-up itself.

The total product of hydrolysis was sublimed under reduced pressure and the resulting two products separated by column chromatography on silica. The first compound eluted from the column was shown to be 3-hydroxy-7-nitroquinoline (39) and all the spectral evidence was in accord with this structure. Compound (39) afforded a crystalline benzoate (40) on treatment with benzoyl chloride. The second compound eluted proved to be the N-oxide of (39), 3-hydroxy-7-nitroquinoline-1-oxide (38).

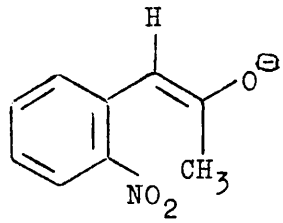
Consideration of the course of the above two nitro-group interactions raises some interesting mechanistic problems. There is no record of the cyclisation of o-nitrophenyl acetone (41) to a quinoline N-oxide, presumably because anion formation is more likely to occur at the activated α position than at the terminal methyl group and a condition such as (III) ⟷ (IV) ⟷ (V) operates



(III)



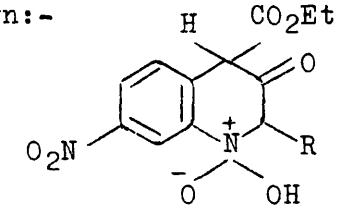
(IV)



(V)

However, the same situation should apply in (28) and (28; CO_2Et for Ph) where the ω hydrogens are less activated than the α hydrogen but cyclodehydration occurs in these cases. It is possible that for

some reason anion formation occurs at the less activated ω centre and interaction takes place with an intact nitro-group to give an intermediate as shown:-



Alternatively, for the situation corresponding to (IV) where the nitro-group is reduced in electrophilicity as an aci-nitro group, cyclisation could still occur if the unfavourable situation corresponding to (V) were overcome. Since there are not protons in both the α and β positions, it is unlikely that there is a nitroso intermediate in these two cyclisation reactions.

An examination of the mass spectra of the above quinolines and quinoline N-oxides showed that there was always an abundant $(M-16)^+$ ion in the mass spectra of the N-oxides but this ion was either absent or of very small intensity in the spectra of the quinolines themselves. That this ion corresponded to the loss of a single oxygen atom from the N-oxide group was shown by the fact that the spectra of (32) and (38) were identical to the spectra of (33) and (39) respectively except for the presence of the parent ions at $^m/_e$ 282 and 206 in the spectra of (32) and (38) respectively. Also since (32) and (38) give rise to (33) and (39) respectively on sublimation at reduced pressure (see above) it was not surprising that, when the spectra of the N-oxides were

recorded using the heated inlet system maintained at 250°C, only the spectra of the corresponding amines were recorded. Therefore a direct inlet system was used to observe the $(M-16)^+$ ions in the mass spectra.

The abundance of the $(M-16)^+$ ion in the spectra of the quinoline N-oxides ranged from 15-40% of the base peak intensity whereas the abundance of this ion in the spectra of the quinolines ranged from 0-0.4% of the base peak intensity. The small $(M-16)^+$ ion in the spectra of the latter compounds was due to fission within the nitro-group present in the 7 position since it is known that small $(M-16)^+$ ions have been observed in the spectra of nitro compounds[13]. The abundances of the $(M-16)^+$ ions in the spectra recorded are shown in Table 2.

The mass spectra of a number of other compounds were determined to see if the abundant $(M-16)^+$ ion was present in the spectra of N-oxides other than the quinoline type. The $(M-16)^+$ ion was the base peak in the spectra of phenazine di-N-oxide*(42) and 2,6-dichlorophenazine di-N-oxide*(43). In addition, the spectra of these di-N-oxides showed abundant $(M-32)^+$ ions of 75% and 63% respectively, corresponding to the loss of both oxygen atoms from the parent ions.

Azoxybenzene (44) and 4-nitropyridine N-oxide (45) had $(M-16)^+$ ions which were 38% and 68% respectively of the base peak

* Kindly supplied by Dr. M.F.Grundon, Chemistry Department, Queen's University, Belfast.

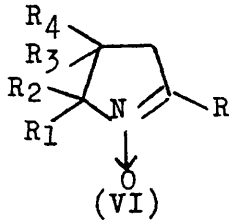
intensity.

Also the spectra of 2-hydroxy-2,3 dihydro-6,7-dimethoxy-3-oxoisoquinoline**(46) and 1-hydroxy-2-methyl-3-acetyl-1,4-dihydro-4-oxoquinoline (47) had $(M-16)^+$ ions with abundances of 44% and 42% respectively. Each of the latter compounds can tautomerise to an N-oxide.

Loss of a single oxygen atom has been observed for nitro compounds (see above), anthraquinones[14], and epoxides[15] but the losses reported are less than 3% of the base peak intensity except in the case of p-nitrophenol[13] (6%) and 1,5-dihydroxyanthraquinone[14] (8%).

Mass spectrometry, therefore, can indicate the presence of an N-oxide group in a molecule very simply.

Grigg and Odell[16] have since observed $(M-16)^+$ ions of 87%, 40% and 17% for pyridine N-oxide, 2-methylpyridine N-oxide, and 2-ethylpyridine N-oxide respectively. According to these workers the alkyl substitution at the 2-position accounts for the lowering of the abundance of the $(M-16)^+$ ions in the spectra of 2-methylpyridine N-oxide and 2-ethylpyridine N-oxide. The base peaks in the spectra of the latter two compounds were the $(M-17)^+$ ions. i.e. (M-OH). The base peak in the spectrum of (47) was also the $(M-17)^+$ ion but the $(M-16)^+$ ion abundance was 42% (Table 2). Grigg and Odell also recorded the spectra of a number of Δ^1-pyrroline N-oxides (VI) and found much smaller $(M-16)^+$ ions (1-10%). They therefore concluded that an abundant $(M-16)^+$ ion is not characteristic of the N-oxide group of nitrones (VI).



(VI)

** Kindly supplied by Dr.A.McCulloch of this department.

However, Delpierre and Lamchen[17] have discussed fully the difference between nitrones and aromatic N-oxides and conclude that these two types of N-oxides have completely different properties. Most of the differences arise because there is considerable delocalisation of the positive charge of the nitrogen or aromatic N-oxides.

Coats and Katrizky[18] have reported a colour test for N-oxides. When a mixture of the N-oxide, dimethyl aniline, and concentrated sulphuric acid is boiled for one minute and ethanol subsequently added, a blue colour develops. However, this test also applies to nitro compounds and therefore would not detect an N-oxide group in the presence of a nitro-group.

The N-O stretching frequency of an aromatic N-oxide group occurs in the infra-red in the "fingerprint" region between 1300 and 1200cm.$^{-1}$ [19], and is therefore not always easy to identify. Addition of methanol lowers this band by 20-40cm.$^{-1}$ and causes a new band to appear at ~ 3360cm.$^{-1}$ due to the association (VII). This fact is of some use for N-oxide identification.

(VII)

Abundant $(M-16)^+$ ions in the mass spectra of aromatic N-oxides are characteristic of the N-oxide function and allow the identification of an N-oxide function in the presence of a nitro-group. The mass spectral method has also the further advantage that only microgram

quantities of the N-oxide are required for identification of the N-oxide function. However, the full scope and limitations of this method are not yet known and further work is required in this direction.

EXPERIMENTAL

General. Melting points were recorded on a Kofler microscope hot-stage and are uncorrected. Light petroleum refers to the fraction boiling between 60°C and 80°C.

Infra-red absorption (i.r.) spectra were recorded on Unicam S.P.200 prism spectrophotometers and a Perkin-Elmer 237 grating spectrophotometer. Nuclear magnetic resonance spectra (n.m.r.) were determined on a Perkin-Elmer R.S.10 (60 megacycles) spectrometer and mass spectra on an A.E.I. M.S.9 spectrometer. Thin-layer chromatography (t.l.c.) was carried out with silica (Kieselgel G - E.Merck).

Preparation of ethyl-α-2,4-dinitrophenyl-γ-phenylacetoacetate (28).

1-Chloro-2,4-dinitrobenzene (1g.) dissolved in the minimum volume of dry ethanol was added to ethyl-γ-phenylacetoacetate (1.015g.). Sodium (0.11g.) dissolved in dry ethanol (10ml.) was added and the mixture allowed to stand in a stoppered flask for 48 hours. Water was added and the reaction mixture acidified and extracted with dilute sodium hydroxide. The alkali washings were acidified (dilute sulphuric acid) and extracted with chloroform. The chloroform extracts were washed with water and dried over sodium sulphate. Evaporation of the chloroform gave the required product (28) (500mg. - 18%) as yellow needles, m.p. 128°C. (Zaki and Iskander[11] give m.p. 127°C.) from ethanol, $\nu_{max.}$ (mull) 1665cm.$^{-1}$ (s, ν C=O ester) 1540cm^{-1}. (s, ν NO_2), 1355cm.$^{-1}$ (s, ν NO_2), 710cm.$^{-1}$(s, νPh). The n.m.r. spectral data are shown in Table 1.

Preparation of 2-Phenyl-3-hydroxy-4-carbethoxy-7-nitroquinoline-1-oxide (30)

Ethyl-α-2,4-dinitrophenyl-γ-phenylacetoacetate (28) (348 mg.) was heated under reflux for 90 min. with the equivalent quantity of sodium ethoxide. Water was added and the reaction mixture acidified (dilute sulphuric acid). The reaction mixture was extracted with chloroform and the chloroform extracts washed with water and dried over sodium sulphate. Evaporation of the chloroform gave the required product (30) (140 mg. - 42%) as yellow needles, m.p. 179°C. (Zaki and Iskander[11] give m.p. 179°C.) from ethanol $\nu_{max.}$ (mull) 1645 $cm.^{-1}$ (s, ν C=O bonded ester) 1530$cm.^{-1}$ (s, ν NO_2), 1350$cm.^{-1}$, (s, ν NO_2), 710$cm.^{-1}$ (s, νPh). The molecular weight was 354 (mass spectrum, $C_{18}H_{14}O_6N_2$ requires M.W.354). The n.m.r. spectral data are shown on Table 1.

Hydrogenation of 2-Phenyl-3-hydroxy-4-carbethoxy-7-nitroquinoline -1-oxide (30): 2-phenyl-3-hydroxy-4-carbethoxy-7-acetaminoquinoline (31).

2-Phenyl-3-hydroxy-4-carbethoxy-7-nitroquinoline-1-oxide (30) (100mg.) in a mixture of glacial acetic acid (15 ml.) and acetic anhydride (15ml.) was hydrogenated with 5% palladium on charcoal (50 mg.) as catalyst. After no more hydrogen was absorbed the catalyst was filtered off and the yellow solution concentrated (5ml.). Water (25 ml.) was added and the mixture shaken (2 hours). The crystals which appeared were filtered off, dissolved in chloroform, and the chloroform solution dried over magnesium sulphate. Evaporation of the chloroform gave 2-phenyl-3-hydroxy-4-carbethoxy-7-acetaminoquinoline (31) (25mg.-27%) as yellow needles, m.p. 186-187°C. from ethanol. (Found: C, 68.4;

H, 5.07; N, 8.15. $C_{20}H_{18}N_2O_4$ requires C,68.6; H, 5.1; N, 8.15). The i.r. spectrum (mull) showed absorption at 3330cm.$^{-1}$ (m,νN-H), 1665cm.$^{-1}$(s,νC=O ester and amide), 720cm.$^{-1}$ (s,νPh). The mass spectrum showed the molecular ion at m/e 350 ($C_{20}H_{18}N_2O_4$ requires M.W.350). The n.m.r. spectral data are shown on Table 1.

Preparation of 2-Phenyl-3-hydroxy-7-nitroquinoline-1-oxide (32).

The N-oxide (30) (150mg.) was heated under reflux in dilute sodium hydroxide (4 ml. of a 5% solution) for five minutes. The reaction mixture was acidified (dilute sulphuric acid) and the yellow precipitate formed crystallised from methanol,yielding yellow needles (135mg.) m.p. 256-260°C. ν_{max} (mull) 3100cm.$^{-1}$ (w,νC-H), 1505cm.$^{-1}$ (s,νNO_2), 1345cm.$^{-1}$ (s,νNO_2), 710cm.$^{-1}$ (s,νPh.). The mass spectrum had the molecular ion at m/e 282 ($C_{15}H_{10}N_2O_4$ requires M.W.282). The compound was most likely 2-phenyl-3-hydroxy-7-nitroquinoline-1-oxide (32).

Preparation of 2-phenyl-3-hydroxy-7-nitroquinoline (33).

The N-oxide (32) (130 mg.) was sublimed under reduced pressure (0.03 mm. at 200°C.) and the pale yellow crystalline product (60 mg.) dissolved in ether (200ml.). The ether solution was washed with saturated sodium bicarbonate, water and dried over sodium sulphate. The dried ether solution was chromatographed on silica (2g.) and the chloroform eluate evaported to give 2-phenyl-3-hydroxy-7-nitroquinoline (33) (20mg. - 16%) as yellow needles, m.p. 235 - 240°C. from ethyl acetate/light petroleum (Found: C,67.8;

H, 3.9; N, 10.1. $C_{15}H_{10}N_2O_3$ requires C, 67.7; H, 3.8; N, 10.5%). The i.r. spectrum (KCl disc) showed absorption at 3080cm.$^{-1}$ (w, νC-H), 1525cm.$^{-1}$ (s,νNO_2), 1340cm.$^{-1}$ (s, νNO_2), 700cm.$^{-1}$ (s, νPh). The mass spectrum had the molecular ion at m/e 266 ($C_{15}H_{10}N_2O_3$ requires M.W.266). The bicarbonate washings were acidified (dilute sulphuric acid), extracted with chloroform and dried over sodium sulphate. Evaporation of the chloroform solution gave yellow needles (from ethanol) whose mass spectrum and i.r. spectrum (mull) were identical to those of 2-phenyl-3-hydroxy-7-nitroquinoline-1-oxide (32).

Preparation of 2,4-dicarbethoxy-3-hydroxy-7-nitroquinoline-1-oxide (34)

1-Chloro-2,4-dinitrobenzene (1g.) dissolved in the minimum volume of dry ethanol was added to acetone dicarboxylic ester (1g.). Sodium (0.11g.) in dry ethanol (10ml.) was added and the mixture allowed to stand in a stoppered flask for 48 hours. Water was added and the mixture acidified with dilute sulphuric acid and extracted with chloroform. The chloroform extracts were washed with dilute sodium hydroxide, acidified and re-extracted with chloroform. The chloroform extracts were dried over sodium sulphate. Evaporation of the chloroform gave the N-oxide (34) (350mg.-15%) as yellow needles, m.p. 170-172°C. from ethanol (Found: C, 51.4; H, 4.0; N, 8.0. $C_{15}H_{14}N_2O_8$ requires C, 51.4; H, 4.1; N, 8.0%). The i.r. spectrum (mull) showed absorption at 1740cm.$^{-1}$ (s, νC=O ester), 1660cm.$^{-1}$ (s, νC=O bonded ester), 1535cm.$^{-1}$ (s, νNO_2), 1350cm.$^{-1}$ (s, νNO_2). The mass spectrum showed the molecular ion at m/e 350 ($C_{15}H_{14}N_2O_8$ requires M.W.350). The N-oxide (34) was soluble in base and gave a red colour in ethanol with

ferric chloride. The n.m.r. data (Table 1) were in complete agreement with (34) and the phenol proton disappeared when the n.m.r. spectrum was run in dimethyl sulphoxide containing a little water, confirming its assignment. When ether was used as solvent instead of ethanol the yield of (34) fell to 11%.

Hydrogenation of 2,4-dicarbethoxy-3-hydroxy-7-nitroquinoline-1-oxide (34): 2,4-dicarbethoxy-3-hydroxy-7-acetaminoquinoline (35).

To a solution of the N-oxide (34) (100mg.) dissolved in glacial acetic acid (15 ml.) and acetic anhydride (15ml.) was added 5% Palladium on charcoal (100mg.). The mixture was shaken in a hydrogen atmosphere until no more hydrogen was taken up. The catalyst was filtered off, the solution concentrated (5ml.), and water (25ml.) added. The mixture was shaken (2 hrs.), during which time a brown oil appeared. The total mixture was neutralised with sodium bicarbonate, extracted with chloroform, and the chloroform extracts washed with water and dried (sodium sulphate). T.l.c. (chloroform developer) showed the extract to consist of two components, one being present in much greater proportion than the other. The components could not be separated by column chromatography on silica but were separated by preparative t.l.c. The major component, (35), was obtained as a yellow oil which crystallised as tiny yellow needles (23mg.- 22%, m.p. 183-185°C.) from ethyl acetate/light petroleum (Found: C, 58.6; H, 5.2; N, 8.3. $C_{17}H_{18}N_2O_6$ requires C, 58.9; H, 5.2; N, 8.1%). The i.r. spectrum (mull) showed absorption at 3350cm.$^{-1}$ (m, νN-H amide), 1730cm.$^{-1}$ (s, νC=O free ester), 1690cm.$^{-1}$ (s, νC=O amide and bonded ester). The mass spectrum showed

the molecular ion at m/e 346 ($C_{17}H_{18}N_2O_6$ requires M.W.346).

Attempted hydrolysis of 2,4-dicarbethoxy-3-hydroxy-7-nitroquinoline-1-oxide (34) with sodium carbonate.

The N-oxide (34) (50mg.) was heated under reflux with a saturated solution of sodium carbonate (5ml.). Aliquots (1ml.) were removed at intervals of 10, 30, 60 and 90 minutes. Each aliquot was acidified (dilute hydrochloric acid) and the resulting precipitate washed with water and dried. The i.r. spectrum of each aliquot was identical to that of the starting material.

Hydrolysis of 2,4-dicarbethoxy-3-hydroxy-7-nitroquinoline-1-oxide (34).

The N-oxide (34) (250mg.) was heated under reflux (5 min.) in dilute sodium hydroxide (10 ml. of a 5% solution). The reaction mixture was acidified (dilute sulphuric acid), extracted with ethyl acetate, and the ethyl acetate extracts washed with water and dried (sodium sulphate). Evaporation of the ethyl acetate gave brownish-yellow needles from ethanol. The product was not obtained pure enough for analysis. However, the i.r. spectrum showed absorption at 3500-2500cm.$^{-1}$ (w, νO-H bonded hydroxyl), 1660cm.$^{-1}$ (s, νC=O acid), 1560cm.$^{-1}$ (s, νNO_2), 1350cm.$^{-1}$ (s, νNO_2). The n.m.r. spectrum (Table 1) showed a singlet at τ1.50 indicating that decarboxylation of one of the two acid groups had taken place. The crude product was probably either (36) or (37). The ethanol mother liquors yielded brownish-yellow needles whose i.r. spectrum showed no carbonyl absorption. The spectrum was similar to that of 3-hydroxy-7-nitroquinoline-1-oxide (38) (see below). It appeared that either

hydrolysis or the work-up itself was causing decarboxylation at both the 2 and the 4 positions of (34).

Preparation of 3-hydroxy-7-nitroquinoline-1-oxide (38) and 3-hydroxy-7-nitroquinoline (39).

The N-oxide (34) (450mg.) was heated under reflux (10 min.) in dilute sodium hydroxide (10ml. of a 5% solution). The reaction mixture was acidified (dilute sulphuric acid), extracted with ethyl acetate and the extracts washed with water. The extracts were evaporated to dryness and the crude product sublimed under reduced pressure (200-250°C. at 0.4mm.). T.l.c. (chloroform developer) of the resulting yellow crystalline powder (130mg.) indicated the presence of two components. Column chromatography on silica afforded two fractions, one on elution with chloroform, and the other on elution with a 50% chloroform/50% ethyl acetate mixture. The first compound eluted was resublimed under reduced pressure (160°C at 0.1mm.). Crystallisation from ethyl acetate or acetonitrile afforded 3-hydroxy-7-nitroquinoline (39) (50mg.) as yellow needles, mp. 246-252°C. (Found: C, 56.7; H, 3.2; N, 14.7. $C_9H_6N_2O_3$ requires C, 56.8; H, 3.2; N, 14.7%). The mass spectrum showed the molecular ion at m/e 190 ($C_9H_6N_2O_3$ requires M.W.190). The i.r. spectrum (mull) had absorption at 3550-2700cm.$^{-1}$ (w, νO-H bonded), 1610cm.$^{-1}$ (s, νC=C aromatic), 1540cm.$^{-1}$ (s, νNO_2), 1350cm.$^{-1}$ (s, νNO_2). The n.m.r. spectrum was also compatible with structure (39) and the data are given in Table 1.

The second fraction eluted from the column afforded 3-hydroxy-7-nitroquinoline-1-oxide (38) (30mg.) as yellow needles, m.p.260-262°C.

from ethanol (Found: C,52.6; H,3.1; N, 13.5. $C_9H_6N_2O_4$ requires C, 52.4; H, 3.0; N, 13.6%). The mass spectrum showed the molecular ion at m/e 206 ($C_9H_6N_2O_4$ requires M.W. 206). The i.r. spectrum (mull) had absorption at 3500-2600cm.$^{-1}$ (w, νO-H bonded), 3100cm.$^{-1}$ (w, νC-H), 1620cm.$^{-1}$ (m, νC=C aromatic), 1540cm.$^{-1}$ (s, νNO_2), 1345cm.$^{-1}$ (s, νNO_2).

Preparation of the benzoate (40) of 3-hydroxy-7-nitroquinoline (39).

Benzoyl chloride (10 mg.) was added to a solution of 3-hydroxy-7-nitroquinoline (13.5mg.) in dry pyridine (3ml.) and the reaction mixture allowed to stand overnight at room temperature. Dilution with water and crystallisation of the resultant precipitate from benzene afforded 3-hydroxy-7-nitroquinoline benzoate (40) (16 mg.-80%) as white silky needles, m.p. 189-190°C. (Found: C, 65.6; H, 3.7; N, 9.9. $C_{16}H_{10}N_2O_4$ requires C, 65.3; H, 3.4; N, 9.5%). The i.r. spectrum (mull) had absorption at 1715 cm.$^{-1}$ (s, ν C=O), 1540cm.$^{-1}$ (s, νNO_2), 1345cm.$^{-1}$ (s, νNO_2), 710cm.$^{-1}$ (s, νPh).

Attempted hydrolysis of 2,4-dicarbethoxy-3-hydroxy-7-nitroquinoline-1-oxide (34) under acid conditions.

The N-oxide (34) (50mg.) was heated (60 min.) in 50% dilute sulphuric acid/50% acetic acid in a water bath but was recovered unchanged. The N-oxide (34) (44mg.) dissolved in concentrated sulphuric acid and a little acetic acid to aid solution was allowed to stand overnight at room temperature. The reaction mixture was poured into water and extracted with chloroform. Evaporation of the chloroform extracts and crystallisation from ethanol afforded the starting material.

		multiplet	~2.04	5		phenyl protons
		doublet	1.40	1	9	H_5
		singlet	-2.33	1		O-H proton
(34)	$CDCl_3$	triplet	8.51	3	7	methyl protons of ester group
		triplet	8.39	3	7	methyl protons of ester group
		quartet	5.36	2	7	methylene protons of ester group
		quartet	5.25	2	7	methylene protons of ester group
		2 doublets	1.49	1	10,3	H_6
		doublet	0.90	1	10	H_5
		doublet	0.45	1	3	H_8
		singlet	-2.98	1		O-H proton
(36) or (37)**	dimethyl sulph-oxide	2 doublets	1.65	1	10,3	H_6
		singlet	1.50	1		H_2 or H_4
		doublet	1.08	1	10	H_5
		doublet	0.86	1	3	H_8
(39)**	deuter-ated dimethyl sulph-oxide	doublet	2.43	1	2	H_2 or H_4
		doublet	2.07	1	9	H_5
		2 doublets	1.83	1	9,2	H_6
		doublet	1.38	1	2	H_2, H_4 or H_8
		doublet	1.29	1	2	H_2, H_4 or H_8

** O-H protons exchanged with water present in dimethyl sulphoxide

n.m.r. spectral data at 60 m/c.

Compound	Solvent	Multiplicity	Centred at τ	No. of protons	J cps.	Assignment
(28)	$CDCl_3$	triplet	8.90	3	7	methyl protons of ester group
		A B quartet	6.52	2	16	methylene protons adjacent to OH
		multiplet	~5.85	2		methylene protons of ester group
		multiplet	~2.88	5		phenyl protons
		doublet	2.53	1	9	H_6
		2 doublets	1.61	1	9,3	H_5
		doublet	1.15	1	3	H_3
		singlet	-3.25	1		OH proton
(30)	$CDCl_3$	triplet	8.36	3	7	methyl protons of ester group
		quartet	5.24	2	7	methylene protons of ester group
		singlet	2.30	5		phenyl protons
		2 doublets	1.46	1	10,3	H_6
		doublet	0.85	1	10	H_5
		doublet	0.29	1	3	H_8
		singlet	-3.40	1		OH proton
(31)	$CDCl_3$	triplet	8.48	3	7	methyl protons of ester group
		singlet	7.82	3		methyl protons of amide group
		quartet	5.46	2	7	methylene protons of ester group
		multiplet	~2.52	2		H_6 and H_8

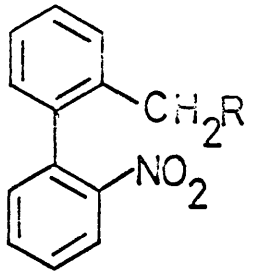
TABLE 2

Intensities of N-oxide $(M-16)^+$ ions

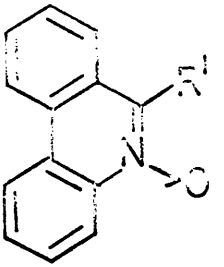
Compound	Molecular Weight	$(M-16)^+$ ion % of base peak
(30)	354	40
(32)	282	38
(34)	350	15
(38)	206	22
(42)*	212	100
(43)**	280	100
(44)	198	38
(45)	140	68
(46)	221	44
(47)	217	42

* $(M-32)^+$ ion 75% of base peak intensity

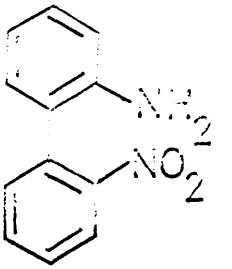
** $(M-32)^+$ ion 63% of base peak intensity



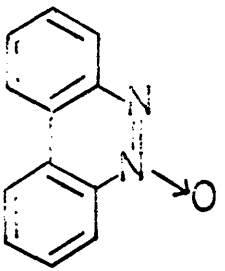
(1)



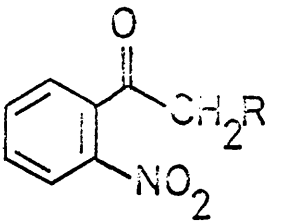
(2)



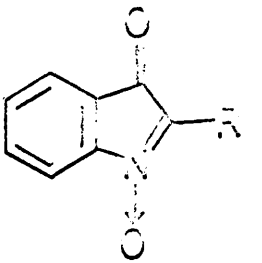
(3)



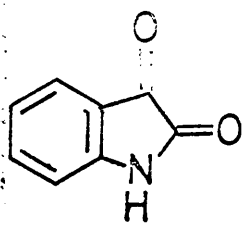
(4)



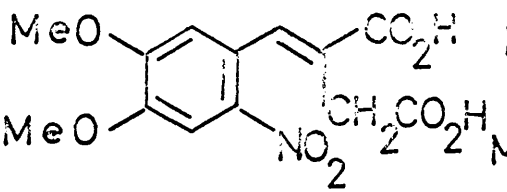
(5)



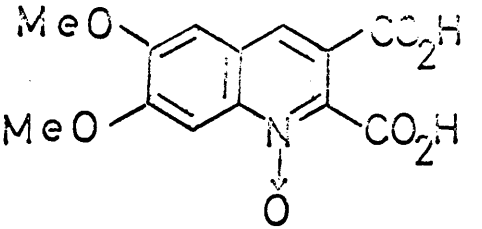
(6)



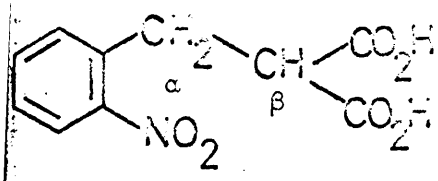
(7)



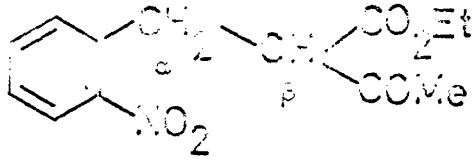
(8)



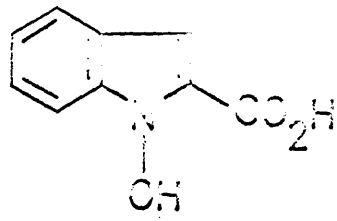
(9)



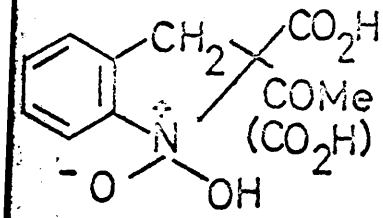
(10)



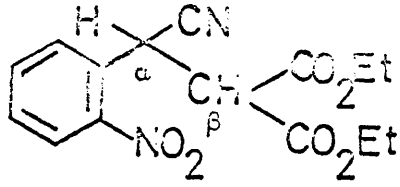
(11)



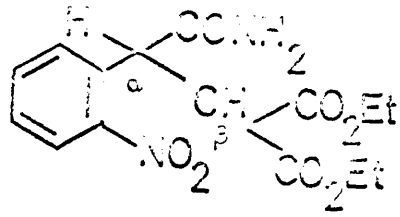
(12)



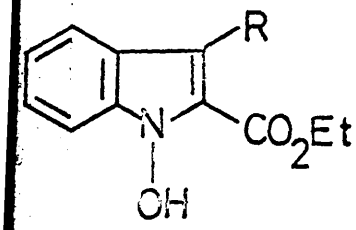
(13)



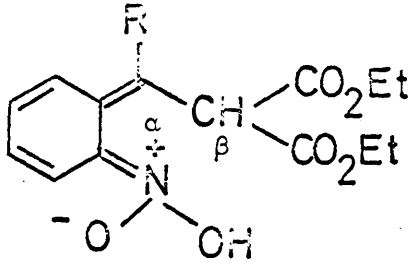
(14)



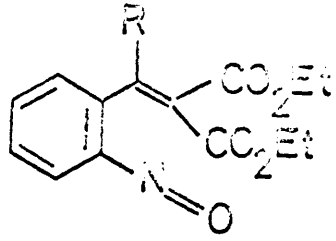
(15)



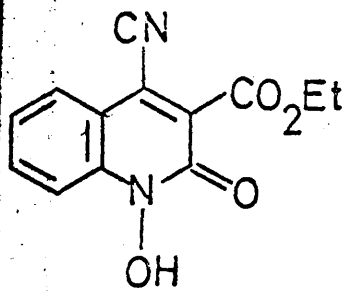
(16)



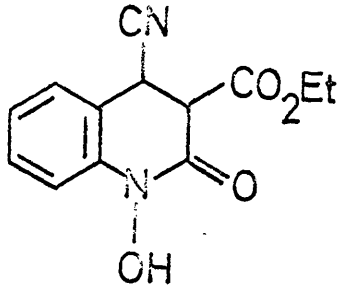
(17)



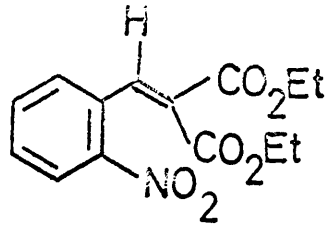
(18)



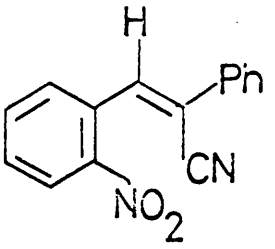
(19)



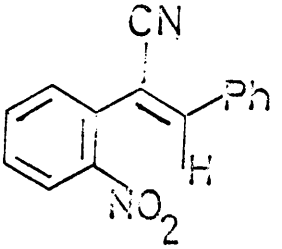
(20)



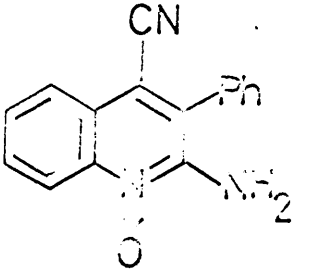
(21)



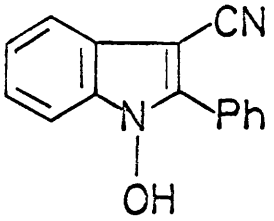
(22)



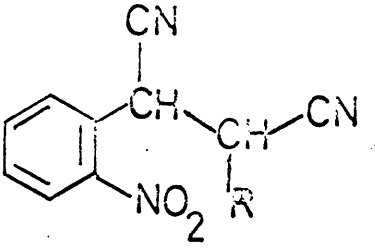
(23)



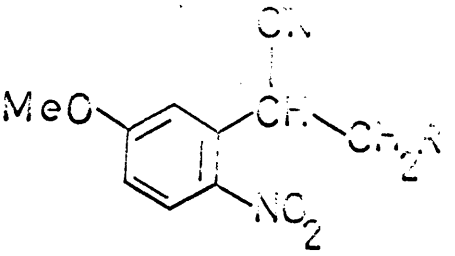
(24)



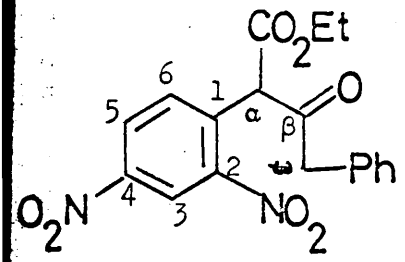
(25)



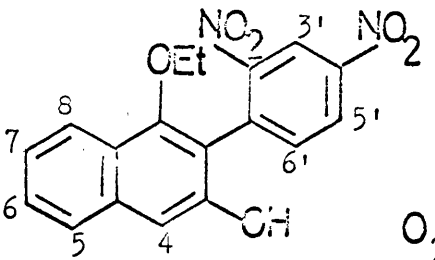
(26)



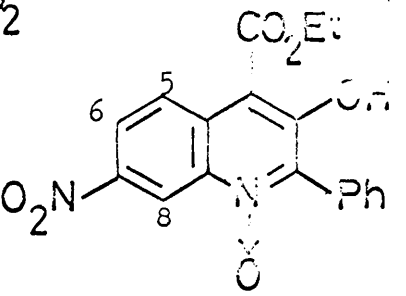
(27)



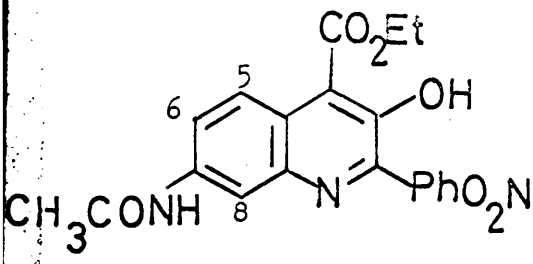
(28)



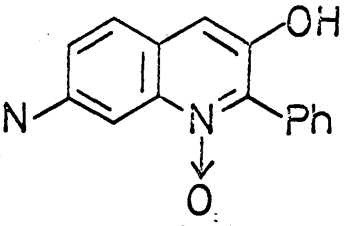
(29)



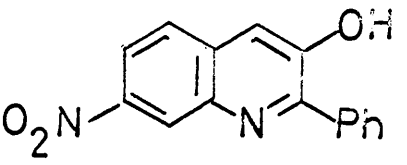
(30)



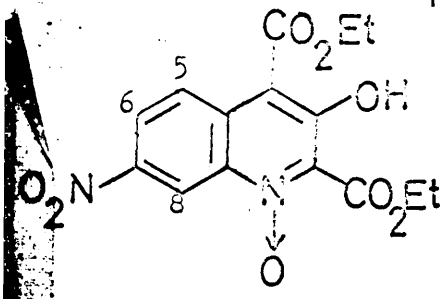
(31)



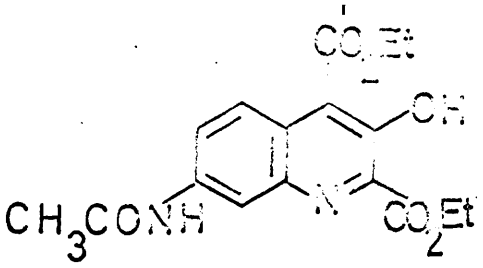
(32)



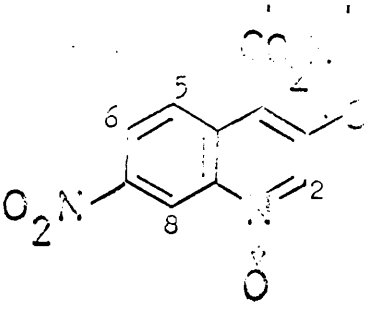
(33)



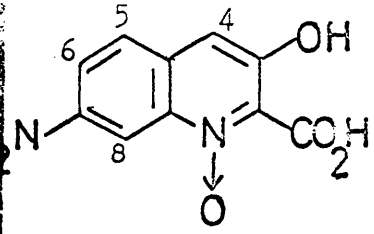
(34)



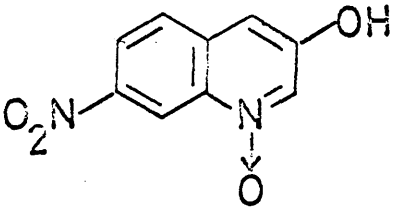
(35)



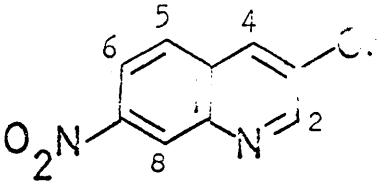
(36)



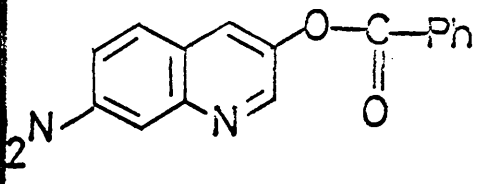
(37)



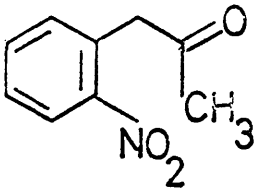
(38)



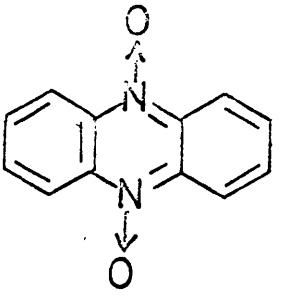
(39)



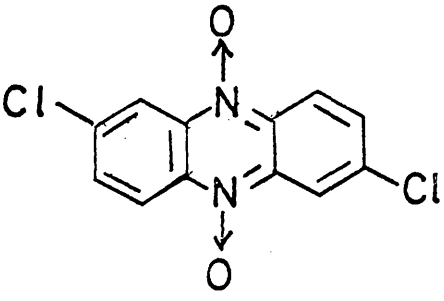
(40)



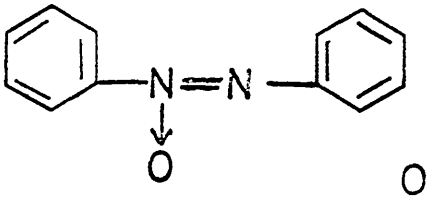
(41)



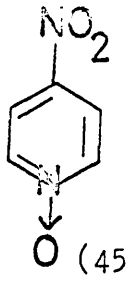
(42)



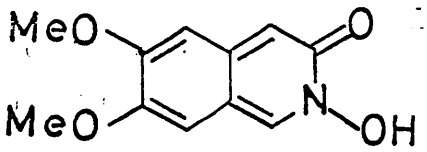
(43)



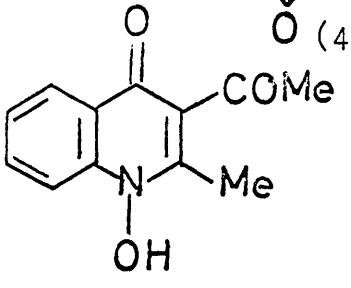
(44)



(45)



(46)



(47)

REFERENCES

(1) J.D.Loudon and G.Tennant, Quart.Revs., 18, 389 (1964).

(2) C.W.Muth, J.C.Ellers, and O.F.Folmer, J.Amer.Chem.Soc., 79, 6500 (1957).

(3) C.W. Muth, N. Abraham, M.L. Linfield, R.B. Wotring and E.A. Pacofsky, J.Org.Chem., 25, 736 (1960).

(4) J.D. Loudon and G.Tennant, J.Chem.Soc., 4268 (1963).

(5) Y.Ahmad and S.A. Shamsi, Bull.Soc.Japan, 39, 195 (1966).

(6) A. Reissert, Ber., 29, 639 (1896).

(7) S. Gabriel, W.Gerhard and R. Wolter, Ber., 56, 1024 (1923).

(8) J.D. Loudon and I. Wellings, J.Chem.Soc., 3462 (1960).

(9) J.D. Loudon and G.Tennant, J.Chem.Soc., 3466 (1960).

(10) P. de Mayo and S.T.Reid, Quart.Revs., 15, 393 (1961).

(11) A. Zaki and Y. Iskander, J.Chem.Soc., 68 (1943).

(12) J.P. Cairns, J.D. Loudon and A.S. Wylie, unpublished work.

(13) J.H. Beynon, R.A. Saunders and A.E. Williams, Ind.Chem.Belg., 29, 311 (1964).

(14) J.H. Beynon and A.E.Williams, Appl. Spectroscopy, 14, 156 (1960).

(15) A. McCormick, unpublished results.

(16) R. Grigg and B.G. Odell, J.Chem.Soc., (B), 218 (1966).

(17) G.R. Delpier and M. Lamchen, Quart.Revs., 19, 329 (1965).

(18) W.A.Coats and A.R. Katrizky, J.Org.Chem., 24, 1836 (1959).

(19) K. Nakanishi, "Infra-red Absorption Spectroscopy", 51, Holden-Day (1962).