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ORGANIC GEOCHEMISTRY OF FATTY ACIDS

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A B S T R A C T

The aims of organic geochemistry included the investigation of the distribution of specific classes of organic compounds and the rigorous identification of the individual components interred in geological materials. Assuming that the organic matter present in a rock is of biological origin, it is of particular interest to investigate classes of compounds which are present in biological systems and which have been preserved in sediments. A number of workers have isolated and identified alkanes and fatty acids in sediments; this thesis is concerned with the examination of sedimentary fatty acids.

The methods currently employed for the isolation and identification of fatty acids are adapted for geological samples. The object is to discover whether or not the fatty acids entombed in a rock bear any relationship to the probable fatty acid distribution of the original debris derived from the living organisms existing during a particular epoch.

When components and metabolites of organisms are incorporated into a sediment they might be expected to vary in their resistance to change. Fatty acids are relatively stable compounds. However, there is some evidence that they do decompose when exposed to thermal, reducing or oxidizing conditions of the sedimentary environment. It might therefore be expected that the quantity of fatty acids found in a rock would be considerably less than that originally deposited. Accordingly, methods are developed in this thesis to deal with the presence of minute quantities of fatty acids isolated from geological specimens.

The way is now open for the examination of a wide range of geological materials and it seems likely that the approach in which the individual acids are rigorously identified and the distribution qualitatively established will be very fruitful.

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The isolation of the fatty acids from powdered rock was effected with and without dissolving the inorganic matrix with mineral acids. The fatty acids were esterified and separated by column, thin-layer and gas-liquid chromatography, examined by infrared spectroscopy and identified in certain cases by mass spectrometry and the recent development of combined gas chromatography-mass spectrometry. The individual procedures for the isolation of the fatty acids were examined with standard fatty acids and full controls developed to establish the levels of probable contamination.

The proved methods have been used to analyse free fatty acids extracted from several geological materials. The results show that the normal fatty acids tend to exhibit an even/odd predominance, as do contemporary biological systems (Shorland, 1954).

One of the gratifying findings is that isoprenoid acids are quite abundant in some materials. For example, phytanic, nor-phytanic, and farnesanic acids are major constituents of Green River Shale and parallel the distribution of the corresponding hydrocarbons. Presumably, these compounds are derived from the phytyl side chain of chlorophyll (Bendoraitis et al, 1962). Whatever their source, there is a need to correlate the stereochemistry of isoprenoid acids from geological samples with those obtained by biological and laboratory synthesis.

A further interesting find was the presence of monoenoic acids in a Recent sediment and an ancient mineral oil. Recent bacterial action is probably the source of such compounds, though an igneous intrusion may have induced cracking in the latter case.

The way is now open for the examination of a wide range of geological materials and it seems likely that the approach in which the individual acids are rigorously identified and the distribution qualitatively established will be very fruitful.

INTRODUCTION

The word geochemistry was first adopted in 1838, by the German chemist C.F. Schonbein who "considered the task of geochemistry to consist of the investigation of the chemical and physical properties of all geological formations and their age relations" (Manten, 1966). Later, continues Manten, Schonbein emphasised that a study of the chemical nature and origin of the masses composing the earth is of equal value to geology as the determination of the relative age and fossil content of geological formations. Latter workers narrowed the definition somewhat, to "the study of the history of the chemical elements in the Earth's crust and their behaviour under different thermodynamic and physico-chemical natural conditions" (Fersman, 1922, quoted by Manten). Manten finally quotes Mason's opinion that "in the simplest terms, geochemistry may be defined as the science concerned with the chemistry of the Earth as a whole and of its component parts. At one and the same time it is both more restricted and also more extensive in scope than geology."

Although the origins of geochemistry in general are well documented, those of organic geochemistry in particular are obscure. The name itself is a recent addition to the many fields of scientific endeavour and only over the last 20 years has interest been growing fast. Manten (1966) defines this branch of geochemistry as, "the study of all naturally occurring carbonaceous substances," interred in sediments. Current developments, especially recent advances in analytical techniques and new instrumentation, have established organic geochemistry as a progressive and comprehensive sphere of investigation embracing the biological, geological and physical sciences.

Three major surveys on progress made in organic geochemistry are those of Breger (1963), Colombo and Hobson (1964) and Hobson and Louis (1966).

Hobson (1965) has discussed the aims of organic geochemistry. He points out that workers are concerned with the identification of organic matter in the lithosphere and the explanation of the origin and subsequent history of such organic deposits. He writes, "the early work was on the composition of coals and crude oils as well as the nature of the organic matter of soils. Interest had developed from the determination of bulk elemental composition to attempts to identify types of compounds and even specific compounds." The field would not have been so active to-day if it were not for petroleum and coal geochemists (Manten, 1966). An outstanding example of the above aim is the isolation and characterisation of lipids in Precambrian rocks (Eglinton et al, 1964, Oro et al, 1965, Belsky et al, 1965).

The explanations required for specific problems are numerous, ranging from the nature of kerogen (insoluble organic matter, Breger and Brown, 1962) the origin and evolution of petroleum, to organic matter in carbonaceous chondrites (meteorites).

The results of these studies are not merely of academic interest but have practical applications. For example, the Green River Formation, Colorado, constitutes the largest known oil-shale reserve in the United States and has been studied extensively both geologically (Bradley, 1964) and chemically (Robinson et al, 1963; Cummins and Robinson, 1964; Eglinton et al, 1964; Robinson et al, 1965).

Speculation about the existence of extra-terrestrial life has intensified the efforts to penetrate the problems surrounding the origin of

terrestrial life. One of the approaches currently bearing on the origin of life involves a utilisation of the record in sediments which is written both in the form of shapes due to fossil organisms and in the chemical nature of the interred organic matter (Cloud and Abelson, 1961; Breger, 1963; Colombo and Hobson, 1964; Hobson and Louis, 1966).

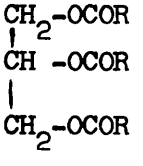
A strict correlation between the morphological evidence and the organic matter present in the same rock would allow a systematic search for chemical evidence of early life in the ancient sediments. Certain classes of compounds such as alkanes (Meinschein, 1963), alkanoic acids (Cooper and Bray, 1963) and porphyrins (Dunning, 1963) show promise as biological markers since they are evidently stable for long periods of time under geologic conditions. These compounds are truly valid as biological markers only insofar as they cannot be synthesised in significant amounts by abiogenic means (Sylvester-Bradley and King, 1963). A successful attempt has been made to produce hydrocarbons abiogenically (Ponnamperuma and Woeller, 1964; Ponnamperuma and Pering, 1966) by spark discharges on methane. The hydrocarbons appeared to contain no normal or branched alkanes but only highly unsaturated compounds. The distributions differed markedly, therefore, from those obtained for ancient sediments.

Clearly, in attempting to interpret the geological data it is essential to work with a background knowledge of the distribution of lipids in present day organisms and of fatty acids in particular with which this thesis is concerned. In the following pages a brief survey of the occurrence and distribution of fatty acids in nature, is given.

1. Fatty acids in plant and animal lipids

The word lipid is "the collective title for the whole group of natural products in which the higher fatty acids are present as essential components." This definition is taken from the major account of fatty acids in lipids by Hilditch and Williams (1964). The fatty acids described therein were isolated and identified mainly by classical methods. Gunstone (1958) gives more detailed attention to analytical techniques but again from the classical standpoint. Progress in the Chemistry of Fats and other Lipids, (Editor, Holman) inaugurated in 1952, brings the study of fatty acids up-to-date. Included is an account of gas chromatography (Horning et al, 1964), and a detailed study of higher saturated branched-chain fatty acids which reports the use of such techniques as gas chromatography, infrared spectroscopy and mass spectrometry (Abrahamsson et al, 1963).

Hilditch and Williams (1964) point out that in different groups of organisms, both vegetable and animal, certain fatty acids predominate:
(i) oleic acid (cis - octadec -9- enoic acid) is the most common constituent of all natural fats contributing at least 30% to the total fatty acids. A fat is a natural tri-glyceride,



(ii) Palmitic acid (n- C_{16}) is predominant among the saturated acids and there are few if any natural fats in which it is completely absent. It may contribute from 15% - 50% of the total fatty acid content.

The naturally occurring fatty acids may be divided into the following: (results are taken from Shorland, 1962).

(a) n-saturated acids (odd- and even-numbered).

	Occurrence	Range	Predominant Acid
n - even	e.g.		
	All natural fats	C_2 - C_{26}	n - C_{16}
	insect and plant waxes	C_{14} - C_{34}	n - C_{28} & n - C_{30}
n - odd	fats of ruminants	C_3 - C_{25}	—

(b) n - unsaturated acids (Monoenoic)

	Occurrence	Range	Predominant Acid
n - even	e.g. Oils of aquatic species	C_{16} - C_{24}	n - C_{18}
	Waxes and seed fats	C_{24} - C_{34}	-
n - odd	human hair fat	C_{11} - C_{17}	-

(Waxes are usually defined as the higher fatty acid esters of the higher fatty alcohols $R \cdot CO \cdot O \cdot CH_2R^1$).

Di-, tri-, and polyethenoid acids are predominant in seed and algal fats, especially C_{20} and C_{22} in the latter.

(c) Branched-chain fatty acids.

These include,

(i) <u>iso</u> acids, $CH_3CH(CH_3)(CH_2)nCO_2H$

(ii)(+)- <u>anteiso</u> acids, $CH_3CH_2CH(CH_3)(CH_2)_nCO_2H$

(iii) isoprenoid acids, $CH_3CH(CH_3)(CH_2)_3CH(CH_3)(CH_2)_3CH(CH_3)(CH_2)_3CHCH_2CO_2H$

e.g. phytanic acid

	Occurrence	Range
iso acids		
(a) odd and even series	ruminant fats	C_{13} - C_{18}
(b) even series only	wool grease	C_{10} - C_{28}
anteiso acids		
(a) odd series	ruminant fats	C_{13} - C_{17}
(b) odd series	wool grease	C_{9} - C_{31}
isoprenoid acids	butterfat, ox blood and serum, Refsum's disease, ruminant bacteria (for references, see Discussion)	C_{19}, C_{20}

The branched-chain fatty acids contribute about 30% to the fatty acid content of wool wax (Abrahamsson et al, 1963). Certain bacteria which are anaerobically grown produce iso and anteiso, C_{15} and C_{17} acids (Shorland, 1963) while certain protozoa produce considerable quantities of iso and anteiso acids (quoted by Leo and Parker, 1966).

(iv) Specific branched acids such as those isolated from tubercle bacilli and other acid fast bacteria include tuberculostearic, phthiocic and mycolic acids.

Hilditch and Williams (1964) have conveniently divided fats into:

(i) aquatic

(ii) animal (terrestrial)

(iii) plant (terrestrial)

(i) aquatic fats contain a wide range of mainly unsaturated fatty acids ranging from C_{14} to C_{24}. Palmitic acid is present to the extent of

15% while stearic acid is a minor constituent (3%). The fats of freshwater plants and animals are relatively rich in di-unsaturated C_{18} and monounsaturated C_{16} acids ($\sim$30%) with a low content of polyunsaturated C_{20} and C_{22} acids. The fats of marine plants and animals show differences in the relative proportions of the various unsaturated members. For example, algae are rich in C_{20} and C_{22} polyunsaturated acids which are present in amounts equivalent to those in marine animals.

(ii) animal (terrestrial) fats have a simpler composition than aquatic fats. Oleic and palmitic acids predominate, the latter occurring in larger proportions ($\sim$30%) than in aquatic animal fats.

(iii) land plant fats contain oleic and linoleic (octadeca -9, 12-dienoic) acids as the main constituents while palmitic acid is normally a minor constituent ($\sim$10%).

Hilditch and Williams point out that there exists the tendency for the more complex and highly developed forms of life to elaborate the simplest fatty acid pattern.

In summary:

(i) fatty acids containing an even number of carbon atoms make up the overwhelming proportion of those present in natural fats.

(ii) branched-chain fatty acids are present in bacteria and protozoa in appreciable amounts.

(iii) algae contain considerable quantities of polyunsaturated acids, especially the C_{20} and C_{22} acids.

2. Fatty Acids in Sediments.

Fatty acids have been isolated from soils (Schreiner and Shorey, 1910), peats and lignites (Cawley and King, 1945), Montan wax (Hewett et al, 1961, Wollrab et al 1962), and Recent and ancient sediments (Cooper, 1962). In addition the presence of fatty acids has been demonstrated in petroleum (Lochte and Littman, 1955), ocean waters (Williams, 1961), fresh water (Goryunova, 1952), fossil brines (Cooper, 1962), and meteorites (Nagy and Bitz, 1963). Since the present concern is with the fatty acid content of sediments, the presence of fatty acids in petroleum and the hydrosphere will be discussed last.

Most noteworthy is the observation that acids with odd numbers of carbon atoms are found along with those having even numbers of carbon atoms in sediments. This is in contrast to nearly all biological systems where fatty acids are even numbered (Shorland, 1954). However, before attempting to explain the odd/even distribution in sediments a few items of pertinent information on the nature and abundance of fatty acids in various geological materials will be presented. The techniques used by various authors for the isolation, separation and identification of fatty acids determine to some extent the interpretation placed on their findings. These will be discussed prior to the results.

The only comprehensive study of soil waxes is that of Meinschein and Kenny (1957) who based the identification of the principal constituents of the waxes on mass spectrometric and infrared analysis of the hydrogenolysis products of wax esters. The normal aliphatic acids closely resembled those found in beeswax with the even-numbered acids overwhelmingly predominant; acids as large as n-C_{35} were found, the predominant acid was palmitic.

Montan wax is the benzene extract of Yellow Pyropissic Coal and of Brown Current Wax Coal and has been investigated by a number of authors (e.g. Edwards et al, 1963). It contains acids, esters, asphalt and resins. Wollrab et al (1962) have detected the presence of fatty acids ranging from C_{16} to C_{35} by gas chromatography of the hydrogenolysis products of the esters and have reported the predominance of even-numbered fatty acids. The major constituent was triacontanoic acid (n-C_{30}); Edwards et al (1963) have reported similar findings.

Fatty acid fractions have been isolated from Recent sediments by a number of workers (Cooper, 1962; Abelson and Parker, 1962; Nagy and Bitz, 1963; Parker and Leo, 1965; Leo and Parker, 1966). Fatty acids have also been detected in ancient sediments (Cooper, 1962; Abelson and Parker, 1962; Nagy and Bitz, 1963; Lawlor and Robinson, 1965; Kvenvolden, 1966; Leo and Parker, 1966). While the general aim of the above authors has been the same, namely, an attempt to relate n-alkanes to n-alkanoic acids, the methods used for the isolation of the fatty acids have differed somewhat.

Abelson and Parker (1962), for example, employed Soxhlet extraction to isolate fatty acids from powdered rock samples. The fatty acid fraction was purified by what they termed "chemical refining and solvent extraction". This consisted of oxidizing (alkaline permanganate) and reducing the tars present in the crude ester fraction. They examined the fatty acids (as the methyl esters) by gas chromatography in the range n-C_{12} to n-C_{18} (isothermal run on a polar phase) and reported the following results:

(i) In Recent and ancient sediments the even/odd ratio was considerably greater than one.

(ii) Palmitic acid was the predominant n-alkanoic acid in Recent sediments and stearic acid in ancient sediments.

A variation of the isolation procedure described above, in which Soxhlet extraction of the powdered sample was followed by formation of the potassium salts of the isolated fatty acids in the extraction flask, was used by Nagy and Bitz (1963). This enabled an analytical step to be eliminated and thus reduced the possibility of contamination during analysis. Methyl esterification was followed by urea adduction of the ester fraction. The adducted esters were examined by infrared spectroscopy and temperature programmed gas-chromatography. The presence of odd-numbered acids was reported in both Recent and ancient sediments.

Several workers (Cooper, 1962; Lawlor and Robinson, 1965; Kvenvolden, 1966) isolated the fatty acids by alkaline digestion of the powdered rock sample followed by acidification and esterification of the freed fatty acids. The normal acids were separated from the branched and cyclic acids by urea adduction. Lawlor and Robinson carried out a preliminary extraction of the powdered material (Green River Shale, Mahogany Zone) with benzene to effect removal of non-polar lipids. This would also remove free fatty acids and free esters present in the rock. Although the method of urea adduction (Baron, 1961) concentrates the normal esters with respect to branched and cyclic esters, with the small quantities of saturated material available in geological samples it tends to lead to relatively large losses (Abelson and Parker, 1962). This has also been the author's experience and will be discussed in the Results Section. Kvenvolden (1966) reported that the fractionation of esters containing less than sixteen carbon atoms was severe as a result of the unfavourable equilibrium for urea adduct formation with lower

esters.

All of the above workers employed mass spectral analysis of the total ester fraction in order to determine the relative amounts of esters of different chain length. This is unsatisfactory since it is known (Ryhage and Stenhagen, 1959) that the relative intensities of parent molecule ions vary with carbon number, increasing from n-C_{14} to n-C_{21}. A separate gas chromatographic analysis is required, otherwise the results from the use of one particular technique can be misleading. Cooper (1962) and Kvenvolden (1966) used both gas chromatography and mass spectrometry in the course of their study. The former author reported poor qualitative agreement between a mass spectroscopic and gas chromatographic analysis of the fatty acid distribution in a Mississippian shale. Once again the presence of odd-numbered acids was reported by each group but in certain instances the ratio of even-numbered acids to odd-numbered acids was near unity.

Parker and Leo (1965) extracted the organic material from Recent sediments ultrasonically and isolated the fatty acids as their potassium salts. The fatty acids (as their methyl esters) were identified by gas chromatography.

The range of fatty acids detected in sediments by the various groups mentioned above depended on the gas chromatographic conditions employed. Some authors (Cooper, Nagy and Bitz, Kvenvolden) reported temperature programmed gas chromatogram while others used isothermal conditions (Abelson and Parker, Parker and Leo). No direct comparison, therefore, can be made between the findings of the groups in question. The method of urea adduction separates the normal from the branched-chain esters; the latter might contain iso and anteiso acids which are known constituents of protozoa and bacteria (Shorland

1963). These have been ignored in all but one report (Leo and Parker, 1966), which will be discussed shortly.

The Recent sediments examined so far, show a decided preference of even-numbered over odd-numbered n-saturated acids, the predominant one being palmitic acid. Parker and Leo (1965) examined Recent sediments with a view to estimating the relative times of survival of different fatty acids with depth of deposition. The core samples examined by these authors were part of a blue-green algal mat community from a Texas lagoon and the findings were that the relative times of survival of the various acids depended on the degree of unsaturation. The living mats were especially rich in palmitoleic (cis - hexadec-9-enoic) acid and linolenic (octadec-9:12-dienoic) acid as well as palmitic acid. The underlying mats showed the early disappearance of oleic, linoleic and palmitoleic acids which may be the result of chemical interaction (polymerization) or biological activity. Abelson (1962) subjected the alga Chlorella pyrenoidosa to wet and dry incubation under anaerobic conditions at temperatures of 142° and 190°C, the purpose of the study being to test the thermal stability of fatty acids. The main feature was the relatively rapid disappearance of the more highly unsaturated fatty acids, for example, linolenic acid; the saturated and monounsaturated acids were about equally stable. Decomposition times of many billions of years have been estimated (Abelson, 1962) from the Arrhenius equation for n-alkanoic acids. However, the thermal tests agree only in part with what has been observed in nature, for it appears that organic detritus undergoes first aerobic and then anaerobic degradation when deposited. Abelson et al (1962) calculated that 10% - 20% of the original organic debris would consist of fatty acids.

Ancient and Recent sediments when examined by the above authors (for example, Green River Shale, $\sim 4 \times 10^7$ years old and Alum Shale, Sweden, $\sim 5 \times 10^8$ years old) yielded approximately 10^{-4} to 10^{-5} grams of fatty acids per gram of organic matter in the sediments. Other authors have reported similar findings (e.g. Cooper and Bray, 1963) and in general the fatty acids contribute about 0.1% of the total organic content of a rock.

The following table indicates the extent to which ancient sediments have been examined for fatty acids:

Epoch †	Location	Authors	No.
Cambrian	Alum Shale, Sweden	Abelson & Parker (1962)	1
Mississippian	Chattanooga Shale, Oklahoma	Cooper (1962)	2
Lower Cretaceous	Skull Creek Shale, Wyoming	"	3
"	Mowry Shale, Wyoming	"	4
"	Mowry Shale, Upton, Wyoming	Kvenvolden (1966)	5
"	Mowry Shale, Douglas, Wyoming	"	6
"	Thermopolis Shale, Casper, Wyoming	"	7
"	Thermopolis Shale, Kaycee, Wyoming	"	8
Upper Cretaceous	Eagle Ford Shale, Texas	Cooper (1962)	9
"	Navesink Shale N.J.	Nagy & Bitz (1963)	10
Eocene	Green River Shale (Mahogany Zone)	Abelson & Parker (1962)	11
"	"	Lawlor & Robinson (1965)	12
"	"	Leo & Parker (1966)	13

† See Fig. 22 on page 135 (taken from Whitcomb and Morris, 1963)

The results of the analysis of the above shales are summarized in the following table; the numbers in the above table indicate the samples in the following table:

No.	Methods used	Range of n-acids	Predominant acid	Even/odd † ratio
1	Soxhlet extr'n., gas chromat. (GC)	n-C_{12} - n-C_{18}	n-C_{18}	high
2	Alkaline digestion, urea adduction, G.L.C., Mass spec (M.S.)	n-C_8 - n-C_{28}	n-C_{16}	1.57
3	"	n-C_{11} - n-C_{34}	n-C_{16}	1.45
4	"	n-C_8 - n-C_{34}	n-C_{20}	1.42
5	" plus infrared (I.R.)	n-C_{12} - n-C_{33}	n-C_{28}	2.5
6	"	n-C_{10} - n-C_{32}	n-C_{18}	1.05
7	"	n-C_{12} - n-C_{33}	n-C_{18}, n-C_{20} and n-C_{22}	1.05
8	"	n-C_{11} - n-C_{34}	—	1.32
9	Alkaline digestion, urea adduction, G.L.C., M.S.	n-C_{11} - n-C_{34}	n-C_{16}	1.74
10	Soxhlet extr'n., urea adduction, G.L.C., I.R.	n-C_{14} - n-C_{30}	n-C_{16} and n-C_{18}	high
11	Soxhlet extr'n., G.L.C.	n-C_{12} - n-C_{18}	n-C_{18}	high
12	Alkaline digestion, urea adduction, M.S.	n-C_{10} - n-C_{34}	n-C_{18}	high
13	Ultrasonic extr'n., G.L.C., I.R.	n-C_{12} - n-C_{18} (branched acids reported)	n-C_{18}	high

† low indicates a value slightly greater than unity and less than 2 while high indicates a value greater than 2.

Independent of age, sediments normally show a larger proportion of even-numbered over odd-numbered fatty acids, the most common acids usually being palmitic and stearic. However, the relative abundance of odd-numbered

acids increases with geologic time. Kvenvolden (1966) has shown that some distributions of n-alkanoic acids are smooth, showing almost equal abundances of even-and odd-numbered molecules. Contamination of samples with naturally occurring fats and oils cannot account for the presence of odd-numbered molecules since this would result in the introduction of even-numbered acids only (Cooper & Bray, 1963). Cooper and Bray also report that oxidation during collection, storage of sample and work up during the analytical scheme cannot account for the appearance of odd-numbered acids.

Three possibilities have been suggested to explain the appearance of odd-numbered fatty acids. Each explanation has its own relative merit and must be considered along with the history of the sample .

(1) Selective solubility. Cooper and Bray (1963) have commented on the possibility that the concentration of odd-numbered acids, initially very small, could show an apparent increase resulting from the selective removal of even-numbered acids. The authors consider this an unsatisfactory explanation because the enrichment occurs in both waters and sediments (Cooper, 1962).

(2) Microbial activity. Under certain conditions microorganisms consume even - but not odd-numbered acids (Silliker and Rittenberg, 1952). A great number of microorganisms have been found in sediment of the deepest oceans and can live under aerobic or anaerobic conditions and some can exist simultaneously in reducing and oxidizing environments. (Degens, 1965). The number of microbes in soils and sediments sharply decreases with depth of burial but may be able to alter the pattern of fatty acids to increase the odd-numbered acids, even over a short period of time.

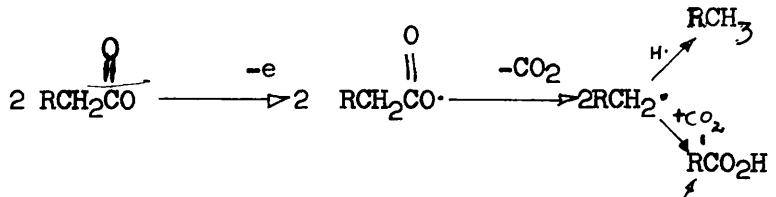
(3) Chemical action, involving the loss of carbon dioxide (decarboxylation) or the gain of carbon dioxide. Several authors have postulated mechanisms

to account for the appearance of odd-numbered acids in sediments by loss of carbon dioxide (Cooper and Bray, 1963; Lawlor and Robinson, 1965).

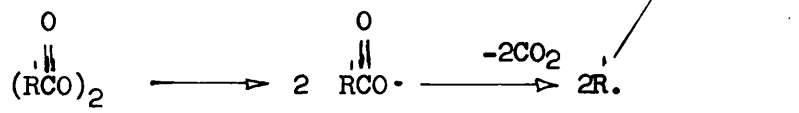
It is well known that fatty acids can be decarboxylated by bacterial decarboxylase under anaerobic conditions (Porter, 1946). It is possible that a similar mechanism operates during the diagenesis of organic matter in a sediment. Several reactions are encountered in living organisms in which carbon dioxide is added to a molecule, and are known as "CO_2 fixation" reactions (Conn and Stump, 1963). The enzymes catalyzing the reactions are found in animals, higher plants and micro-organisms.

Cooper and Bray (1963) proposed a mechanism involving the conversion of even-numbered fatty acids to alkyl radicals having one less carbon atom, by reactions similar to peroxide decomposition or Kolbe synthesis. The alkyl radicals might be further oxidized to fatty acids or add on hydrogen to form hydrocarbons.

Kolbe synthesis:



Peroxide decomposition:



Cooper and Bray point out that no specific reaction can be considered for the one electron oxidation of the fatty acid or its anion; however, oxidation by a metal ion, either free or chelated, is a possibility. Whatever the nature of the reaction, it must occur under generally reducing conditions.

Lawlor and Robinson (1965) proposed that the odd-numbered fatty acids

were related in one of two ways to the even-numbered fatty acids:

(a) in the range n-C_{23} to n-C_{34} the even-numbered acids gave rise to the odd-numbered with one carbon less. Nearly parallel curves resulted when the abundances of the even-numbered fatty acids (obtained from mass spectral analysis) were plotted to correspond to the abundance of the odd-numbered fatty acids having one carbon atom less.

(b) in the range n-C_{15} to n-C_{23} the even-numbered fatty acids gave rise to the odd-numbered fatty acids with one carbon more. Nearly parallel curves resulted when the abundances (obtained from mass spectra) of the even-numbered fatty acids were plotted to correspond to those for the odd-numbered fatty acids having one more carbon atom.

As mentioned above, the decarboxylation scheme is held to account for the presence of n-alkanes which occur in considerable quantities in sediments along with fatty acids. The n-alkanes are only minor constituents of biological systems and normally the odd-numbered molecules predominate (Douglas and Eglinton, 1966). In sediments the odd-numbered n-alkanes also predominate. Their generation from fatty acids would explain the disappearance of much of the fatty acid content of a sediment. Jurg and Emma (1964) heated behenic acid (n-C_{22}) with bentonite both in the presence and absence of water, at about 200°C. The object was to try to illuminate the above problem. As well as producing the n-C_{21} alkane, lower n-alkanes and n-alkanes with 22 to 34 carbon atoms were obtained in small yield ($\sim$10%). Although conditions were quite different from those occurring in sediments the results suggested that not all n-alkanes may be derived from fatty acids with longer chain lengths. If this is true for n-alkanes, it might be true for fatty acids.

The decarboxylation scheme has been used as one of the main arguments for the observed carbon-number distribution of n-alkanes in petroleum (Martin et al, 1963). It holds reasonably well for Paleozoic crude oils but difficulty is encountered when attempts are made to explain the smooth distributions of n-alkanes in older oils. Robinson (1963) points out that "the decarboxylation mechanism is not strongly supported by what is known of the metabolism of fatty acids. If it occurred as a major step in the generation of n-alkanes in the remote part there should surely be some evidence for its importance at the present time."

Kvenvolden (1966) presents the case for decarboxylation with more success than previous investigators and makes two important observations:

(i) the rule of parallelism of distributions of acids and n-alkanes can occasionally break down;

(ii) the distributions of fatty acids and n-alkanes can differ in similar lithologies of the same geological unit.

Degens (1965) points out that not only are the parallel distributions between the even-numbered fatty acids and n-alkanes minus one carbon atom most common, but also, the increase in the abundance of odd-numbered acids with time apparently matches the generation of even-numbered paraffins. This parallelism suggests related processes for the formation of odd-numbered acids and even-numbered paraffins.

Not all investigators have been concerned with the role of normal saturated fatty acids in petroleum formation. Leo and Parker (1966) have very recently reported the presence of branched-chain fatty acids in several Recent marine sediments and one Eocene sediment (Green River Shale, Mahogany Zone). They showed that the methyl branched acids gave two straight lines when the

logarithm of their retention values was plotted against carbon number, corresponding to the homologous series of iso - and anteiso - acids. Confirming evidence was supplied by infrared spectroscopy. The Recent sediments examined by these authors contained appreciable amounts of branched-chain acids ranging from C_{12} to C_{18}. For higher marine organisms (Ackman & Sipos, 1965) the ratio of straight chain to branched acids lies between 100:1 and 500:1, but for sediments this ratio was between 1:1 and 20:1. The presence of such high concentrations of branched-chain acids in some sediments requires explanation, considering that normal and branched-chain acids are equally stable. Leo and Parker suggested that since bacterial lipids are noted for being rich in branched-chain acids (Shorland, 1963), the presence of bacteria in the sediment could account for them. Furthermore, protozoa which derive food from the organic debris also contain significant amounts of branched-chain acids (Erwin and Bloch, 1963) which would enrich the sediment on the death of the organisms.

Fatty Acids in the hydrosphere.

The presence of fatty acids in petroleum reservoir waters (fossil brines) and ocean waters has also been reported (Cooper and Bray, 1963; Williams, 1961; Slowey et al, 1962; Jeffrey et al, 1964). Cooper and Bray (1963) established the presence of very small quantities of even-and odd-numbered fatty acids in fossil brines in which concentration differences between neighbouring odd-and even-numbered acids were very small. In addition, a nearly straight line decrease in relative abundance from n-C_{14} to n-C_{30} was shown. Williams (1961) has noted the presence of even-and odd-numbered fatty acids in Pacific ocean waters and detected a decrease in the

concentration of unsaturated acids with depth. The waters resembled biological systems insofar as they had palmitic and stearic acids as the predominant acids.

Slowey et al (1962) studied the fatty acid content in waters from the Gulf of Mexico. It was observed that fatty acids in shallow waters exhibited some similarity to the composition of plankton collected from a nearby location. Saturation seemed to increase and chain length decrease with increase in water depth. The major acid in a sample from the greatest depth at one sampling point appeared to be dodecanoic (n-C_{12}) acid (94%). However, the amount of fatty acid was more or less uniform within a water column of 2000 metres. It was suggested that the decrease in unsaturation and chain length with depth was caused by extended exposure of fatty acids to oxidizing conditions in deep water (Slowey et al, 1962).

Fatty Acids in petroleum.

An interesting find by Cason and Graham (1965) in a recent study is the occurrence of polyisoprenoid acids in a Californian petroleum. These authors have also isolated from the same petroleum a small fraction of normal saturated acids, which showed a slight even/odd predominance (Graham, 1965; quoted by Kvenvolden, 1966). Mention will be made of the isoprenoid acids in the discussion.

Fatty Acids in meteorites.

Nagy and Bitz (1963) reported the presence of fatty acids ranging from C_{12} to C_{28} in the Orgueil meteorite; these showed a slight predominance of even- over odd-numbered fatty acids. Three explanations were put forward by these authors for the presence of fatty acids in the meteorite:

(i) The result of terrestrial contamination by microorganisms or other biological matter.

(ii) Abiogenic production of the fatty acids by, for example, the oxidation of unsaturated hydrocarbons either through years of museum storage or on the meteorite parent body itself.

(iii) Biological activity on the meteorite parent body.

The authors concluded that the origin of the fatty acids was not known.

Wilson and Johnson (1964) have put forward an abiogenic scheme which would result in the formation of linear hydrocarbons and fatty acids. They proposed that if molecules were held crowded together so that the ends only were available for reaction this would prevent branching. They tested this scheme by forming methyl radicals over a monolayer of palmitic acid on water and recovered acids ranging from n-C_{16} to n-C_{19} in 3% overall yield with the production of negligible quantities of branched chain acids. This suggests itself as a possible reaction scheme for the Abiogenic synthesis of fatty acids isolated from the Orgueil meteorite.

The results afforded so far by the several investigators mentioned above, would lead one to anticipate one or more of the following observations in the fatty acid distributions in sediments yet to be examined:

(i) A definite decrease in the quantity of fatty acids in Recent and ancient sediments compared with the sediment when originally deposited. The observed decreases are due to diagenesis which is a form of low temperature metamorphism (Pettijohn 1957). It refers primarily to the reactions which take place within a sediment between one or several components (in this case organic metabolites) and is the beginning of the process of the internal reorganisation of compounds in a sediment.

(ii) The relative scarcity of unsaturated compounds in Recent sediments and their virtual absence in ancient sediments as a result of diagenesis.

(iii) The presence of n-C_{16} or n-C_{18} in both ancient and Recent sediments as predominant acids.

(iv) The existence of an even/odd preference in Recent and ancient sediments. There is usually a decrease in the even/odd preference with the ageing of sediment, sometimes a smooth distribution being encountered. This is said to be due to a maturation process (Kvenvolden, 1966) which is described by Pettijohn (1957) as "the extent to which a sediment approaches the ultimate end product to which it is driven by the formative processes that operate upon it".

(v) An increase in the n-alkane content of the sediment with age. The proposed diagenetic relationship between n-alkanes and fatty acids is said to give rise to this increase (Cooper, 1962), the n-alkanes being generated from the n-alkanoic acids by a decarboxylation scheme or other mechanism.

(vi) The presence of considerable quantities of branched-chain acids which might be of bacterial origin.

DISCUSSION

The work reported in this thesis is part of a general programme of organic geochemistry at present in progress in the Chemistry Department of Glasgow University. Initially, organic geochemistry will gain most from the study of geological situations where extractable organic compounds are indigenous to the facies, and where chemical correlation with biological matter originally incorporated in the sediment should be possible. Torbanite (Carboniferous, ca. 250 x 10^6 yr.), an algal coal from Bathgate, Scotland, Scottish Oil-Shale (Carboniferous, ca. 300 x 10^6 yr.) from the Lothian district of Scotland, and Green River Shale (Eocene, 60 x 10^6 yr.), from Colorado, U.S.A. are dense compacted materials of low permeability and are believed to fulfil this requirement to some extent.

The fatty acid fractions were chosen for study on account of their ubiquitous distribution in geologic materials, their relative stability, and the existence of powerful methods for their isolation, separation and identification on a micro scale. Although the n-alkanoic acids in geological materials may reasonably be used as evidence of a biological history the presence of the more structurally specific isoprenoid acids would be powerful tools as biological markers (Eglinton et al, 1966). Phytanic acid (3,7,11, 15- tetramethylhexadecanoic acid) and nor-phytanic acid (2,6,10,14- tetramethylpentadecanoic acid) have a fairly widespread distribution in nature albeit in small amounts and have been isolated from and identified in a Californian petroleum (Cason and Graham, 1965), butterfat (Hansen and Morrison, 1964; Hansen et al, 1965), sheep fat (Hansen, 1965), ox fat (Hansen, 1965), ox serum (Lough, 1963, 1964), rumen bacteria (Hansen, 1966) and the tissues, serum and urine of humans afflicted with Refsum's syndrome (Hansen, 1965).

Farnesanic acid (3,7,11- trimethyl dodecanoic acid) has been isolated from and identified in a Californian petroleum (Cason and Graham, 1965). The original source of these compounds may be the phytyl portion of chlorophyll, degraded either biogenically or abiogenically (Bendoraitis et al, 1962).

In this work the free fatty acid fractions were selected for examination because of the relative ease of isolating them from geological materials, but in one case (a sample of Torbanite) the total fatty acids were isolated by alkaline hydrolysis of the shale. The procedures employed in the isolation and identification of the fatty acids have been drawn from the literature and modified where appropriate. They may be summarised as follows:- rock samples were crushed into small pieces, thoroughly washed with organic solvent to remove surface contamination, and then pulverised to a fine powder. Digestion of the inorganic matrix in mixed hydrofluoric/hydrochloric acid was followed by ultrasonic extraction (McIver, 1962) with benzene/methanol. Concentration in vacuo furnished an extract which was then chromatographed from solution in ether over silicic acid containing potassium hydroxide (McCarthy and Duthie, 1962). The free fatty acid fraction retained on the column was eluted with 2% formic acid in ether, concentrated in vacuo and esterified (anhydrous methanolic hydrogen chloride). Chromatography on alumina from solution in benzene, followed by thin-layer chromatography (TLC) yielded a pure ester fraction (Mangold, 1961).

Some idea of the carbon number distribution over the approximate range C_{10} to C_{35} was conveniently obtained for this fraction by gas-liquid chromatography (GLC) of the fatty acid methyl esters (Horning et al, 1964). Linear temperature programming ($\sim$100-300°C.) of the gas chromatograph allowed analyses on short packed columns (6-ft. to 10-ft.) and thus provided a rapid

estimation of the fatty acid distribution over a wide range of carbon numbers (e.g. Fig. 5A, page 117). Although the complex mixtures of branched/cyclic fatty acids are only partially resolved (e.g. Fig. 7A, page 120) the resulting chromatograms are still very useful. Fractionation on a second GLC column containing a liquid phase having different characteristics from the above, followed by entrapment of single peaks or groups of peaks (e.g. Fig. 12C, page 125) provided samples of fatty acid esters suitable for mass spectrometric examination. Fractions considered (from GLC conditions) to contain a single component were inserted directly into the mass spectrometer. Fractions known to contain several components were subjected to combined gas chromatography-mass spectroscopy, whence the groups of peaks were cleanly resolved and their mass spectra recorded individually. The mass spectrometric approach (Cooper and Bray, 1963; Lawlor and Robinson, 1965; and Kvenvolden, 1966) which determines the relative abundance of components in an ester mixture from the parent molecule ion peak heights or the acylium ion (RCO) peak heights was not used in this instance for reasons stated previously in the Introduction.

Infrared spectroscopy (samples in solution) was used not only to establish the presence of fatty acid methyl esters (carbonyl absorption at 1740 cm^{-1}) but also to provide information about the extent of methyl branching possessed by fatty acid components. The ratio of optical densities (OD) for ν CO and νCH_{2850} for a particular ester fraction relative to the ratio obtained for pure methyl stearate (99.8%) provided an index of the number of methylene ($-CH_2-$) or methine ($-\underset{|}{C}H-$) groups present. An increase in the value of this ratio compared with that for methyl stearate (1.0) would indicate either the presence of shorter chain fatty acids or methyl branched-chain fatty acids.

The necessary experimental conditions (e.g. isolation technique, solvent systems, etc.) for obtaining organic matter from a rock were established by processing available Green River Shale (Mahogany Zone). The efficiency of the ultrasonic extraction procedure was found to be satisfactory by recovery of stearic acid from an intimate mixture of bentonite and stearic acid. The technique used for the isolation of free fatty acid fractions as described by McCarthy and Duthie (1962), gave results which were in close agreement with those of the authors.

The ability of 5Å molecular sieve to separate n-paraffins from branched cyclic paraffins is well known (Thomas and Mays, 1961). The remark by these authors that 5Å sieve has been used to separate normal from branched alcohols and aldehydes prompted us to try to separate normal from branched/cyclic fatty acid esters by this means. Using methyl stearate as a typical n-acid ester it was found that only 10% was entrapped in the sieve.

The use of molecular sieves will require a more thorough investigation in order to assess their real value for the above separations.

All procedures were checked, singly and together, for fatty acid contamination, and in every case the level of contamination was well below the quantity of fatty acids handled. The examination of some specific sources of contamination is discussed under "Control Experiments" in the Results section.

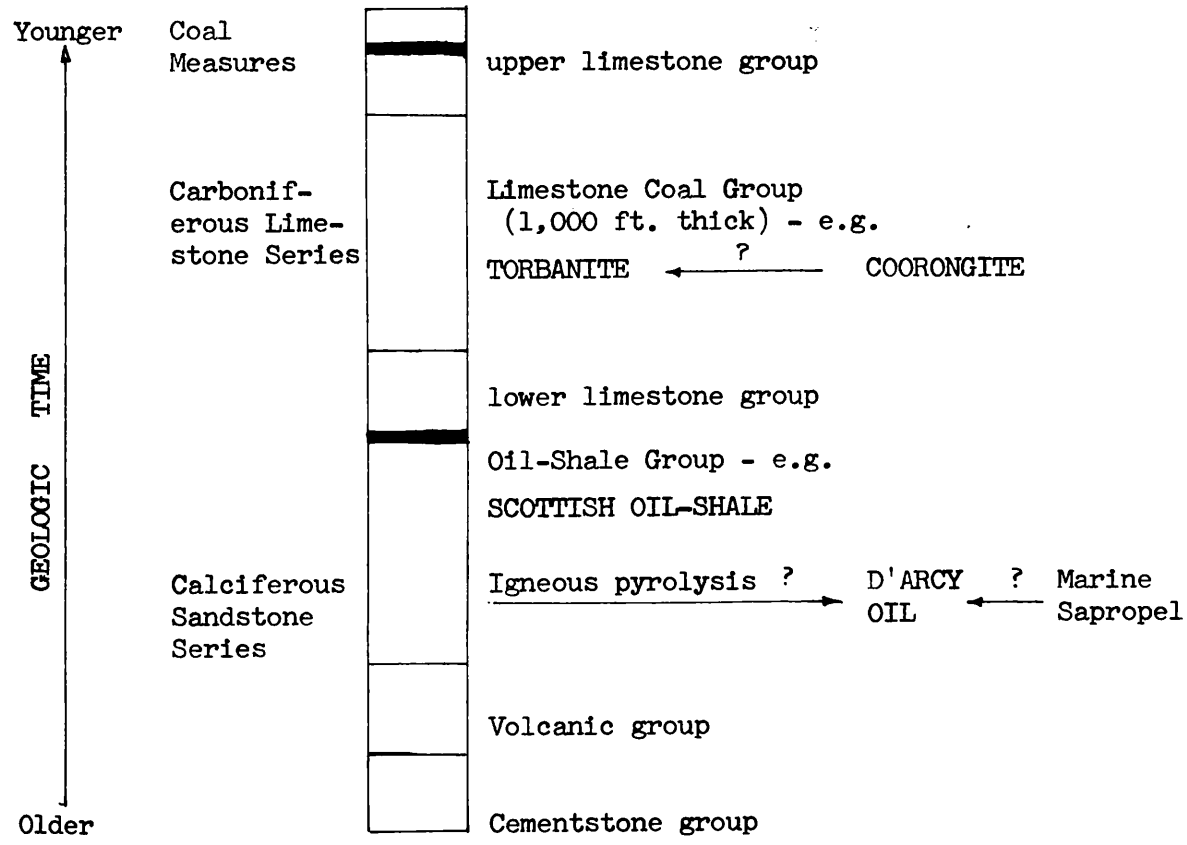
1. Examination of Carboniferous Materials

The geological materials from the Carboniferous Formation (250-300 x 10^6 yrs.) were chosen partly because of the long standing controversy over the relationship of boghead coals (e.g. Torbanite) to the alga Botryococcus braunii (Blackburn and Temperley, 1936). The boghead coals, formed from organic remains of algae and plant debris in pools in the swamp forests of Carboniferous times (Skilling, 1938) contain so-called "yellow-bodies" which are supposed to be the remains of colonies of an alga which does not differ in any material respect from the living alga, named above. The Scottish Oil-Shale, formed in a manner similar to Torbanite but at an earlier period, also contained the yellow-bodies although to a lesser extent. The mineral Torbanite is very rich in organic matter whereas the Scottish Oil Shale is lean.

The availability of a small quantity of the mineral Coorongite, sometimes regarded as the mother substance of boghead coals (Conacher, 1938), provided the means of correlating these supposedly-related substances by examining their fatty acid content. Coorongite is known to have been produced by the decomposition of Botryococcus Braunii in shallow coastal lagoons in South Australia (Conacher, 1938).

A further problem connected with the Carboniferous Formation is the presence of considerable quantities of oil in juxtaposition to the oil-shale. Most of the crude petroleum found in this area (Lothians of Scotland) is evidently a product of the distillation of shale-bands caused by igneous intrusive sheets and dikes, which are widespread in Scottish Carboniferous formations. The amount of oil-shale which has been "devolatilised" by this

agency is very large (Wyllie, 1938). However, there was some doubt as to the source of the D'Arcy oil examined in this study since igneous intrusions are comparatively rare in the part of the oil-shale area from which D'Arcy Oil was obtained. Wyllie suggested that D'Arcy oil might have arisen as a natural petroleum in the ordinary sense from a marine sapropel (decaying organic matter) by bacterial and chemical decomposition of the organic detritus without the intervention of heat. D'Arcy oil is found at approximately the same depth as the Scottish Oil-Shale. The relative stratigraphic positions are shown below:



Torbanite (or Torbanehill mineral; Carboniferous, $\sim$ 250 x 10^6 yrs.)

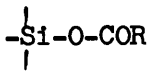
The powdered material was subjected to various extraction procedures in an attempt to recover the maximum quantity of fatty acids. The fatty acid fraction obtained by ultrasonic solvent extraction of the shale (untreated by mineral acids) showed acids ranging from n-C_{10} to n-C_{28} and the marked dominance of the even-numbered acids, n-C_{16} and n-C_{18} (Fig. 5A, page 117) characteristic of most plants (Shorland, 1962) and relatively young sediments (Cooper and Bray, 1963). The remaining acids from C_{10} to C_{28} form a pattern which not only shows that the relative abundance of fatty acids having odd numbers of carbon atoms is greater than in biological systems (Shorland, 1954), but also that the increase is such that there no longer exists an even/odd predominance. The smooth distribution depicted in Fig. 5A probably arose by a process of maturation as the sediment aged. The structures of the n-C_{16} and n-C_{18} fatty acid methyl esters were established by mass spectrometry (Table 1, I and II, page 136). It is instructive to point out that the n-alkanes, recently examined (Maxwell, 1965), range from n-C_{12} to n-C_{37} and show a smooth distribution (i.e. no odd/even predominance exists). The most abundant n-alkane appears to be n-C_{20}. In this case there is no obvious relationship between the n-alkanoic acids and the n-alkanes to support the thesis that the hydrocarbons derive from the acids by a decarboxylation process. The quantity of alkanes isolated from 250 g of Torbanite was approximately 800 mg and the quantity of fatty acids about 8 mg. Since alkanes are not major constituents of biological materials, the large quantity of alkanes isolated from Torbanite compared with the small quantity of acids isolated suggests that some relationship between the acids and paraffins may

exist. It is possible, of course, that the alkanes have been produced abiogenically in the sediment (Ponnamperuma and Pering, 1966), or that they have migrated into the rock at a later date. This latter explanation is unlikely considering the low permeability of Torbanite. It is evident from the distribution of the fatty acids that there is a maturation process operating, but it is not at all certain how the acids and paraffins are related.

The residue of Torbanite from the first extraction, described above, was further treated with hydrofluoric acid, the acid was removed and an attempt was made to recover the fatty acids by esterification <u>in situ</u>, using anhydrous methanolic hydrogen chloride.

$$\text{pre-extracted shale} \xrightarrow{HF} \text{residue} \xrightarrow[HCl]{MeOH} \text{esters}$$

A small quantity of fatty acid methyl esters ($\sim$2 mg) was obtained and the gas chromatogram (Fig. 6C, page 119) shows that the n-C_{16} acid is predominant. It also shows that there is a similar distribution of acids to that initially obtained from the same portion of Torbanite, except that the n-C_{12} acid is present in a slightly greater proportion than before. In other words, a slight even/odd predominance is evident. The object of this method was to determine to what extent kerogen esters (acids bonded to the kerogen matrix; Abelson and Parker, 1962) were present in Torbanite. These authors also thought that there might be the possibility of acids bonded to the silicate matrix. One might speculate on the form of these bonds in the following structure:



The strong hydrolysing conditions used were considered sufficient to free the acids esterified with the silica or kerogen matrices. However, the small quantity of acids isolated made the results inconclusive.

A further portion of powdered Torbanite (250 g.) was treated with hydrofluoric acid prior to extraction of the organic matter, and the fatty acid fraction obtained on extraction (12 mg) showed a marked increase in branched-chain and cyclic components (Fig. 7A, page 120). This is rather puzzling since the fatty acid fraction obtained from the hydrofluoric acid treated residue, described above, did not show this pattern. Several explanations may be proposed, but the first would seem to be the most feasible.

(i) It is possible that the branched and cyclic acids are entombed in the silicate matrix. The presence of organic solvent might prevent the hydrofluoric acid from effectively removing the inorganic matrix. (The residue which was treated with HF, described previously, was moist with solvent).

(ii) The possibility that the acids were isomerized during the work up is not confirmed by the results obtained by treating stearic acid with hydrofluoric acid.

(iii) The possibility of a non-homogeneous sample is unlikely since the sample was taken from one side of a large chunk ($\sim$12 cu.ft.).

(iv) A further explanation is that the treatment of rock by acid prior to the extraction of the organic matter might have resulted in the loss of fatty acids in the mineral acid and washings. The question arises as to why the normal acids would be removed in preference to the branched/cyclic acids.

A further batch of powdered Torbanite (400 g) was heated under reflux with methanolic potassium hydroxide to isolate the total fatty acids (Cooper and Bray, 1963). The quantity of acids recovered as esters ($\sim$11 mg) did

not show the expected increase over the quantity of free fatty acids isolated from Torbanite. This may have been due to losses incurred during the analytical scheme, or else to the possibility that only a small quantity of acids are bonded to the kerogen. It is also possible that a longer time is required for the alkaline hydrolysis of certain geological materials in which the inorganic matrix is predominately silicate as in the case of Torbanite. The distribution of esters is shown in the gas chromatogram in Fig. 10C (page 123).

Scottish Oil-Shale (Carboniferous, $\sim$ 250-300 x 10^6 yrs.).

The powdered rock, on treatment with hydrofluoric acid and subsequent extraction of the organic matter, yielded only a small quantity of fatty acids, (3.5 mg from 600 g shale). This might have been predicted from a knowledge of the carbon content and oil yield on pyrolysis (Gibson, 1922). A comparison of the carbon content etc. of the Scottish Oil-Shale with Torbanite is given below:

	Torbanite	Scottish Oil-Shale
% Carbon content	60	20
% Inorganic matter	20-30	60-80
Oil yield on pyrolysis (gallons/ton)	90-130	20-60

A gas chromatogram of the fatty acid methyl esters (Fig. 8A, page 121) shows the range of acids to be from n-C_{10} to n-C_{29}, with n-C_{12}, n-C_{16} and n-C_{18} as the predominant acids. The structures of the n-C_{12}, n-C_{16} and

$n\text{-}C_{18}$ acids were established from their mass spectra. Discounting the large peak at the $n\text{-}C_{12}$ position, the pattern is similar to that obtained from Torbanite (Fig. 5A). The appearance of the large peak due to the $n\text{-}C_{12}$ (lauric or dodecanoic) acid is enigmatic, since it does not appear to be a ubiquitous lipid constituent. However, as was noted in the Introduction, dodecanoic acid occurs in large proportions (94% of the fatty acid content) in deep ocean waters (Slowey et al, 1962). Further, Hilditch and Williams (1964) record that seed fats (endosperm) of the species Palmae (palm trees) which occur in tropical climates contain lauric acid (45-50% of the fatty acid content) and seed fats of Lauraceae (e.g. cinnamon plant) also contain lauric acid (80-90% of the fatty acid content). It thus appears that some species of plants contain large proportions of fatty acids not predominant in the majority of biological systems. It is possible that the plants of the swamp forests of Carboniferous times, e.g. seed ferns, scouring rushes, scale trees or cordaites (Dunbar, 1963) also possessed similar fatty acid patterns in the endosperm of their seeds, as the above two families.

Scottish Oil-Shale Distillate

This material, a dark viscous oil, was provided by Scottish Oils (B.P.) Ltd., and was a commercial distillate obtained by pyrolysis of Scottish Oil-Shale.

Gas chromatograms of the fatty acid methyl esters on two different phases (Figs. 9A and 9B, page 122) show an even/odd predominance. It is possible that these acids ranging from n-C_{11} to n-C_{26} were formed during the retorting process since a small percentage of oxygen and water was introduced into the retort when operating (Stewart and Forbes, 1938). If this explanation for the presence of fatty acids in the distillate is discounted, it must be accepted that fatty acids can withstand brief exposure to high temperatures ($\sim 600^{\circ}$C.), and the thermal cracking which occurs, in all probability at the top of the retorts. Studies which are currently being pursued in this laboratory by a colleague, Mr. W. Henderson, may help to provide an answer to this question. In these studies, shales are being pyrolysed under strictly controlled conditions and the products of pyrolysis are being compared with the constituents of the raw shale.

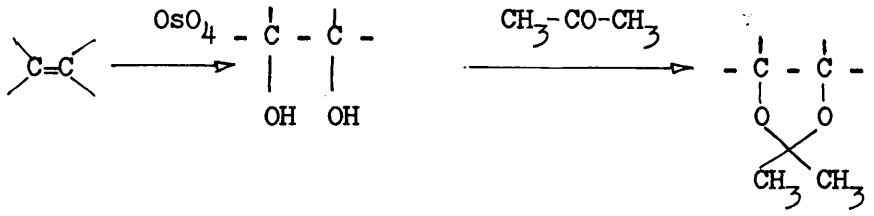
D'Arcy Oil

The methyl esters of the free fatty acids obtained from D'Arcy Oil gave the gas chromatogram shown in Fig. 10A (page 123). They ranged from n-C_{10} to n-C_{28} and the pattern is similar to that obtained from Torbanite (Fig. 5A, page 117) except that the n-C_{16} and n-C_{18} esters (identified from their mass spectra) constitute a larger proportion of the fatty acid fraction from D'Arcy Oil. In addition, the peak eluted prior to the n-C_{18}

acid (Fig. 10A) or subsequent to n-C_{18} (Fig. 11A, fraction 10, page 124) appeared to be a normal C_{18} monoenoic acid, tentatively identified as oleic acid (as its methyl ester). This identification, using gas chromatography-mass spectrometry (Fig. 14A, page 127) and infrared spectroscopy is not rigorous; for this it would be necessary to establish unequivocally the position of the double bond. On a small scale this can be effected,

(i) by deuterating the double bond or forming the ozonide followed in both cases by recording the mass spectra of the products (Hallgren et al, 1959).

(ii) or by forming the O-Isopropylidene derivatives of unsaturated fatty esters, followed by mass spectrometry (McCloskey and McClelland, 1965)



On a larger scale oxidative degradation, e.g. ozonisation, followed by an oxidative work up might be used (Gunstone, 1958).

Three possible explanations suggest themselves for the occurence of this unsaturated acid in D'Arcy Oil:

(i) The diagenesis of organic rich sediments under anaerobic conditions might favour the preservation of unsaturated compounds. Alkenes, however, have not been isolated from any crude petroleum to any extent (Whitehead and Breger, 1963) but have been isolated from Green River Shale in small amounts (Henderson, 1966):

(ii) The igneous distillate theory is a further possibility. There exists a small quantity, (ca. 4%) of alkenes in D'Arcy Oil, (Wyllie, 1938), which have also been examined (Maxwell, 1965), using TLC (silver nitrate), GLC,

hydrogenation and mass spectrometry. It is well known that the crude shale oil produced on retorting the shale contains a high proportion of unsaturated hydrocarbons, (Wyllie, 1938), and the effect of an igneous intrusion on the shale might produce unsaturated compounds. However, one would expect to find more than one unsaturated acid present in the acid fraction in substantial amounts if the above mechanism operated:

(iii) The acid might derive from bacteria present in the sample since it was stored in a corked bottle for many years in a museum. (However, a small quantity of a second sample of oil was obtained from the Royal Scottish Museum, Geology Department, Edinburgh, and afforded an identical fatty acid pattern, including the unsaturated acid.)

Zo Bell (1943, 1958) has proposed support for the hypothesis of the biogenic formation of oil in marine sediments on the basis of a study of bacteria capable of accelerating biochemical reactions. Although the individual aspects of Zo Bell's scheme are well founded scientifically his suppositions remain hypothesis only (Kuznetsov et al, 1963). The possibility that crude oils such as D'Arcy Oil were formed from marine sapropels such as that forming in the Black Sea (Kuznetsov et al, 1963; Wyllie, 1938) remains an unsolved problem in geochemistry.

The n-alkanes isolated from D'Arcy Oil and examined in this laboratory (Maxwell, 1966), showed a smooth distribution (no odd/even predominance) ranging from n-C_{11} to n-C_{32}. The predominant alkane was n-C_{16}. As in the case of Torbanite, there is no observed relationship between the n-acids and n-alkanes; i.e. it cannot be said that a particular alkane is derived by decarboxylation from the fatty acid with one more carbon atom.

Coorongite

The quantity of mineral available for examination was small (7g.). The fatty acid distribution, ranging from n-C_{14} to n-C_{28} is shown in the gas chromatogram of the methyl esters in Fig. 10B (page 123) and Fig. 11B (page 124). Fractions 3,5,7,8 and 9 (Fig. 11B) contained n-monoenoic acids ranging from n-C_{16} to n-C_{20} (compounds VI to X) tentatively identified by G.L.C., I.R. and mass spectrometry. The mass spectra are shown in Figs. 14B to 16B (pages 127 to 129). It appears from the infrared evidence (ν = 990,910 cm^{-1}) that the unsaturation is terminal (vinyl). These acids could be identified as described in the previous section on "D'Arcy Oil". The vinyl unsaturation is unusual though not altogether uncommon in fatty acids (Hilditch and Williams, 1964). Their existence could be explained as the product of biological activity. Professor Schwartz (1966) in a letter to Dr. Eglinton states that he has been working on coorongite for some time and has isolated desulphurizing bacteria from it; he maintains that the origin of coorongite is connected with microbiological processes.

Apart from the presence of unsaturated acids, coorongite contained n-C_{16} and n-C_{18} acids as the predominating saturated acids (Fig. 11B, peaks 2 and 6, respectively) characterised from the mass spectra of their methyl esters. Branched-chain acids were present in small amounts and the mass spectra of their esters showed them to be <u>iso</u> and <u>anteiso</u> acids. The bacterial origin of such acids in sediments (Leo and Parker, 1966) would seem to be supported by the presence of bacteria in coorongite, assuming the bacteria present contained <u>iso</u> and <u>anteiso</u> acids.

The hydrocarbon fraction (7 mg. from 7g.) has been isolated and

identified (Maxwell, 1966) and showed a smooth distribution ranging from $n\text{-}C_{14}$ to $n\text{-}C_{28}$ with the predominant alkane in the $n\text{-}C_{19}$ position.

2. Green River Shale

This shale is of Eocene age (ca. 60 x 10^6 yr.) and represents the main oil-shale reserve of the United States (Nevers, 1966). Samples of shale were kindly supplied by Dr. Robinson, U.S. Bureau of Mines, Laramie, and were taken from the 1100-ft. and 1900-ft. levels of the formation. They are not to be confused with samples described by Lawlor and Robinson (1965) and Leo and Parker (1966) taken from the Mahogany Zone (which is at the 800-ft. level and above).

The organic material in Green River Shale was presumably formed in an environment in which aquatic organisms such as microscopic algae and protozoa predominated over land plants, pollens and spores and is impregnated in clay (Schaeffer and Mangus, 1965; de Nevers, 1966). Cummins and Robinson (1964) examined samples of Green River Shale (Mahogany Zone) for the presence of hydrocarbons and described the isolation and identification of n-C_{13} to n-C_{33} alkanes and C_{15}, C_{16}, C_{18}, C_{19} and C_{20} isoprenoid hydrocarbons. Eglinton et al (1966) also examined the Green River Shale and found a distribution of alkaneswhich closely paralleled that found by the above authors. In a later paper Lawlor and Robinson (1965) have described the fatty acids of the Green River Shale from the Mahogany Zone (see Introduction).

The samples examined in this study were taken from the 1100-ft. and 1900-ft. levels of the Green River Formation Oil-Shale. The 1100-ft. sample afforded a complex mixture of fatty acids (Fig. 12A, page 125) which included a series of normal acids ranging from C_{10} to C_{29} and a series of isoprenoid acids ranging from C_{14} to C_{17} and C_{19} to C_{21}. The latter acids were found along with n-C_{12} to n-C_{18} acids, in fractions 3 to 9, respectively (Fig. 12C.

page 125) and are designated compounds XIA to XVIIA. The identification of the isoprenoid acids was provided by the mass spectra of their methyl esters (Figs. 18-21, pp. 131). The 1900-ft. sample afforded a similar fatty acid distribution (Fig. 13A, page 126) with unbranched acids ranging from n-C_{10} to n-C_{30}. The isoprenoid acids C_{14} to C_{17} and C_{19} to C_{21} were again present in the acid fraction, obtained from cuts 2 to 8, Fig. 13C (page 126). The mass spectra of their methyl esters were very similar to those obtained for the isoprenoid acid methyl esters from the 1100-ft. sample. (Tables 5 to 8, pp. 140).

Mr. J. Maxwell also obtained mass spectra of nor-phytanic (XV) and phytanic (XVI) acid from the ester fraction, 1100-ft. sample while visiting the laboratory of Professor S. Stallberg-Stenhagen, Department of Medical Biochemistry, University of Gothenburg, Sweden. The instrument used was an LKB 9000 gas chromatograph-mass spectrometer; the gas chromatographic runs were temperature programmed.

Robinson et al (1965) examined nine cores taken from stratigraphic positions of increasing depth. The uppermost and lowest samples were provided by Dr. Robinson, for this present study. The main results of the hydrocarbon analysis by Robinson et al were as follows:

(i) Benzene extracts of the shale showed that the bitumen (solvent-soluble organic matter) content of the core samples increased with depth.

(ii) The paraffinic (n-, iso, and cyclo alkane) content of the bitumens increased with depth. Nearly one-half of the bitumen at the lowest level consisted of normal, iso and cycloparaffins.

(iii) The n-alkanes increased from 4% to 14% and the isoprenoid compounds from 2% to 6% of the bitumens with depth.

(iv) The five isoprenoid hydrocarbons identified (G.L.C. and M.S.) were the C_{15} (farnesane), C_{16}, C_{18}, C_{19} (pristane) and C_{20} (phytane) compounds. (These were also present in the Mahogany Zone). The amount of phytane decreased with depth, the concentration of pristane remained fairly constant while the amount of farnesane, C_{16} and C_{18} compounds showed a tendency to increase with depth, suggesting degradation of a C_{20} precursor.

The authors proposed that the n-alkanes were derived from the fatty acids by decarboxylation. In the present study the n-C_{16} acid predominates slightly over the n-C_{18} acid (Fig. 12A, page 125) in the free fatty acid fraction from the 1100-ft. sample. Robinson et al reported that the amount of n-C_{17} alkane is considerably greater than n-C_{15} alkane (15% and 3%, respectively from the same level). If the decarboxylation mechanism was correct one would expect a much higher proportion of n-C_{15} alkane, if one assumes that palmitic acid would predominate in these times and that the fatty acids would lose carbon dioxide at about the same rate to produce the n-alkanes. It is possible, however, that stearic acid predominated in the organisms living then, or more likely, that oleic acid which is normally present in much larger quantities than stearic was reduced to stearic acid. This would account for the large quantity of n-C_{17} alkane assuming the validity of the decarboxylation scheme.

The problem is much less acute when the 1900-ft. sample is examined. Once again the n-C_{16} acid predominates slightly over n-C_{18} (Fig. 13A and B, page 126), while the amount of n-C_{17} alkane just exceeds the n-C_{15} alkane (4.6% and 3.3% respectively). Robinson et al suggest that the loss of one carbon atom from the fatty acids can explain high odd/even n-alkane ratios found in the Mahogany Zone, but the mechanism does not explain the much lower

values for n-paraffins at greater depth. The varying amounts of n-C_{17} alkane could be due to differences in the amount of available precursor C_{18} acid or in the amount of oxidation of unsaturated precursor acids. With increase in depth there may have been an increasing loss of oleic acid due to chemical degradation. The differences in distribution of the n-paraffins may reflect differences in environmental conditions, contributing to differences in oxidative cleavage of the fatty acids, and reduction processes (biological and chemical) and in the composition of the precursor material. It has been reported (Hunt et al, 1954, quoted by Robinson et al, 1965) that the salinity of the lake increased with time and this is an important factor controlling the composition of the hydrocarbons.

Two observations can be made with respect to the acids:

(i) The uneven distribution of the fatty acids at both levels. The ratio of even/odd acids is about the same for both levels, with respect to the lower acids (n-C_{12} - n-C_{18}) but shows a definite decrease in the lower level for the higher acids n-C_{19} - n-C_{29} (Fig. 12A and 13A). This latter fact is paralleled by a definite decrease in the odd/even ratio for the alkanes ranging from n-C_{19} to n-C_{29} in the 1900-ft. level.

(ii) A decrease with depth, in the quantity of acids isolated, paralleled by an increase in the n-paraffins with depth. It is significant that the concentration of n-alkanes from n-C_{19} to n-C_{29} is greater in the 1900-ft. level.

This can be summarised as follows:

ACIDS	QUANTITY Isolated	Even/odd ratio*	Smooth distributions
1100 ft.	10 mg.	decrease	-
1900 ft.	2 mg.	↓	n-C_{19} to n-C_{29}

* Values cannot be quoted since the n-acid esters were not separated from the

total ester fraction, and in the gas chromatograms overlapping of some of the peaks is evident.

n-ALKANES	% of bitumen	Odd/even ratio	Smooth distribution
1100 ft.	4	1.43	-
1900 ft.	14	1.25	$n\text{-}C_{18}$ to $n\text{-}C_{28}$

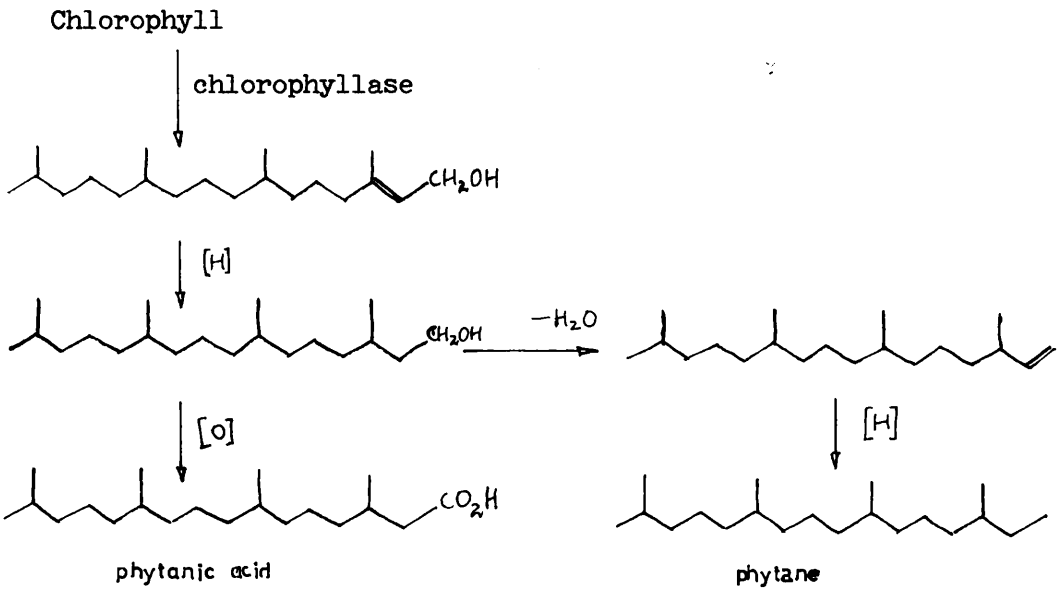
These results are in fair agreement with the view that the n-alkanoic acids are the precursors of the n-alkanes and that a maturation process operates with ageing of the sediment, displayed in the smoothing out of the distributions between $n\text{-}C_{19}$ and $n\text{-}C_{29}$ for the acids and alkanes.

The large proportion of $n\text{-}C_{12}$ acid which occurred in the Scottish Oil-Shale is again present in the 1900-ft. level of the Green River Shale (Fig.13A). Since controls showed that the presence of this acid was not due to contamination, it might be concluded that it occurs as a degradation product of unsaturated precursors or as the main component of the fatty acid fraction of seeds or spores present in abundant supplies at that particular period (see section on Scottish Oil Shale, page 32). One might speculate on the structure of the fatty acids of the algae at the time of deposition; the number of generations must be high and it is possible that there have been genetic changes. The $n\text{-}C_{11}$ alkane occurs only in a small amount in the 1100-ft. sample which suggests that either the decarboxylation mechanism is not valid or that the acid is a contamination product present in the sample before processing, or that loss of the lower n-alkanes either <u>in situ</u> or during the analytical scheme has occurred.

There exists a parallelism between the distribution of isoprenoid acids

isolated in this study and the isoprenoid alkanes isolated by Robinson et al (1965), with the addition of the C_{21} and C_{14} compounds to the acid series.

The dominant C_{20} isoprenoid acid (XVI) is paralleled, however, by the dominance of the C_{20} isoprenoid alkane. If the acids are precursors of the alkanes, then the decarboxylation scheme would not appear to hold in this case. If phytanic acid (XVI) is derived from the phytyl side chain of chlorophyll (Bendoraitis et al, 1962) then a reduction process would be necessary to explain the presence of the C_{20} hydrocarbon in substantial amounts (9% of the total paraffins in the 1100-ft. level)



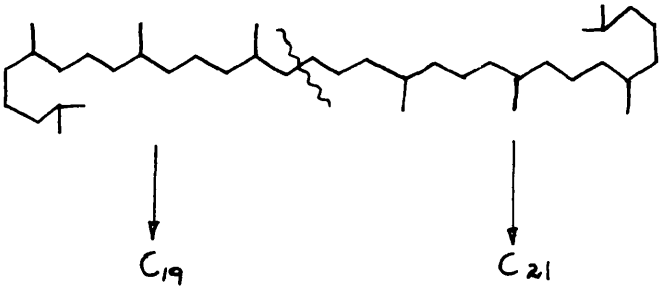
It may be, of course, that there is no connection between the two groups of compounds except in the original biosynthesis. However, the fact that reducing conditions and anaerobic organisms operate in marine and fresh-water sediments lends support to the argument that reduction of the isoprenoid acid leads to the hydrocarbon of the same carbon number.

If decarboxylation is a significant process, then one would expect to

find the C_{14} hydrocarbon since the C_{15} isoprenoid acid is present in appreciable amounts. The C_{14} isoprenoid alkane was not reported to have been isolated in any of the bitumens examined by Robinson et al (1965) or in the Mahogany Zone samples (Cummins and Robinson, 1964). As was mentioned earlier, Robinson et al reported a decrease in the proportion of C_{20} isoprenoid hydrocarbon with depth, an increase in the C_{16} and C_{18} isoprenoid alkanes with depth and a fairly constant concentration of the C_{19} compound with depth. In this study a marked decrease in the amount of C_{20} and C_{19} isoprenoid acids with depth was observed. Decarboxylation of the C_{20} isoprenoid acid would give rise to the C_{19} isoprenoid hydrocarbon. The fact that the concentration of the latter stays fairly constant with depth lends support to the decarboxylation scheme.

The presence of the C_{15} isoprenoid acid (XII) in substantial amounts (containing three complete isoprene units) could have derived from the farnesyl side chain of certain bacterio-chlorophylls, for example, the pigment of the obligate anaerobe, Chlorobium (Rapoport and Hamlow, 1961).

The presence of the C_{21} isoprenoid acid might lead one to infer an open chain tetraterpenoid hydrocarbon as precursor which by appropriate scission would give rise to the acid in question; for example, a suitable derivative of the following,



might conceivably give rise to nor-phytanic acid and C_{21} isoprenoid acid dihydrophytyl ethers

Kates et al (1965) have isolated and identified ^ from the lipid fraction of the halophilic bacillis, Halobacterium cutirubrum thus providing a further possible derivation of isoprenoid structures.

One last point concerns the description of the isoprenoid acids as a homologous series (Hansen and Morrison, 1964). The only compounds that can be truly classed as isoprenoid acids are the C_{15} and C_{20} compounds since they are composed of three and four complete isoprenoid units respectively. These will form part of a homologous series. The remaining compounds C_{14}, C_{16}, C_{17}, C_{19} and C_{21} represent portions of polyisoprenoid skeletons. These will also form part of an homologous series, the C_{14} and C_{19} acids and the C_{16} and C_{21} acids, since they differ by one isoprenoid unit.

The biological history of this Cenozoic rock is therefore evident from the uneven distribution of n-alkanoic acids and the presence of large proportions of isoprenoid acids. Difference in source materials such as the variation in the proportion of land plants to aquatic plants (algae and protozoa) as well as variations in the chemical composition of source materials (e.g. algae) no doubt occurred at different times during deposition and will partially account for the variation in the acids present.

3. Concluding Remarks

(1) Torbanite, Scottish Oil-Shale, D'Arcy Oil, Coorongite.

The following table summarizes the main features of the fatty acid patterns from the Carboniferous materials and from coorongite.

Material	Range of n-acids	Predominant n-acids	Range of branched acids
Torbanite	$n\text{-}C_{10}$ to $n\text{-}C_{28}$	$n\text{-}C_{16}$, $n\text{-}C_{18}$	C_{13} to C_{24} (GLC only)
Scottish Oil-Shale	$n\text{-}C_{10}$ to $n\text{-}C_{29}$	$n\text{-}C_{12}$, $n\text{-}C_{16}$ $n\text{-}C_{18}$	C_{13} to C_{18} (GLC only)
D'Arcy Oil	$n\text{-}C_{10}$ to $n\text{-}C_{28}$	$n\text{-}C_{16}$, $n\text{-}C_{18}$	C_{13} to C_{17} (GLC only)
Coorongite	$n\text{-}C_{14}$ to $n\text{-}C_{28}$	$n\text{-}C_{16}$, $n\text{-}C_{18}$	C_{14} to C_{18} (MS)

The fatty acid fractions isolated from the Carboniferous materials show an envelope or smooth distribution, if the $n\text{-}C_{16}$ and $n\text{-}C_{18}$ acids (and for Scottish Oil-Shale, the $n\text{-}C_{12}$ acid) are ignored. This apparent absence of even/odd predominance may be accounted for by a process of maturation operating in the sediment due to the physical, chemical and biological forces operating therein over the long period of time. It is conceivable that a unique combination of the foregoing factors operates in each geological formation. The similarity of the fatty acid patterns from the Carboniferous materials not only suggests common biological precursors but also similar geological

conditions, even over millions of years. It was noted in the Introduction that the Alun shale ($\sim 500 \times 10^6$ yrs) examined by Abelson and Parker (1962) afforded a fatty acid pattern with a high even/odd predominance. One of the factors which might eliminate such a high ratio could be a high silicate content as in the case of Torbanite, producing a catalytic action leading to the formation of a smooth fatty acid distribution. The n-alkanes from Torbanite and D'Arcy Oil also displayed the envelope or smooth distribution. The fact that neither the n-alkanes nor the n-alkanoic acids showed a decided odd/even or even/odd predominance, respectively, does not invalidate the proposed geogenetic relationship via decarboxylation. Factors operating during diagenesis might erase the effects of the above scheme.

Bearing in mind the presence of the unsaturated acids in coorongite it is conceivable that these acids were degraded underspecific conditions (eg. microbial activity) to give rise to the above fatty acid distributions.

The proportion of n-C_{18} saturated acid in most biological systems is relatively small (3-5%). However, the n-C_{18} acid occurs in considerable amounts in the Carboniferous fatty acid fractions and in coorongite, present in much the same proportions as the n-C_{16} acid which is predominant in biological systems. The marked increase in the n-C_{18} acid may be explained as follows:

(i) The n-C_{18} acid probably arose by reduction of n-C_{18} unsaturated acids, principally oleic acid which is a major constituent of present day lipids. The coorongite fatty acid fraction also contains a considerable quantity of n-C_{18} saturated acid. If Torbanite is derived from coorongite it is evident that this reduction process would have to take place as the organic matter is being deposited and not during the lithification and compaction of the sediment.

Coorongite is around 30 to 40 years old.

(ii) Biological systems contributing to the organic detritus in the sediments of the Carboniferous epoch might have contained a higher proportion of saturated n-C_{18} acid than present day systems.

The presence of branched-chain acids in the Carboniferous fatty acid fractions could be ascribed to microbial activity as the sediment was being laid down. There have been periodic claims to the isolation of viable bacteria from ancient geological materials, but it would be far from easy to substantiate these claims considering the difficulty in finding and using the best possible aseptic technique (Degens, 1965). One might assume that the branched-chain acids were either present in the dead organisms contributing to the sediment at the beginning of deposition or in the microorganisms acting on the sediment.

Contamination is a problem which must be taken into consideration at every stage in the analytical scheme. Risk of contamination outside of the laboratory is even greater. For example, the source of the n-C_{12} acid in the Scottish Oil-Shale, Green River Shale (1900 ft.) and the n-C_{18} monoenoic acid in D'Arcy Oil is uncertain. Contamination of a sample after removal from the ground or even in situ over a long period of time, cannot be excluded even though the sources of contamination do not readily suggest themselves. The n-C_{18} unsaturated acid from D'Arcy Oil could have arisen through the action of microorganisms producing specifically oleic acid. The same is more likely to be true of coorongite considering the reported evidence of desulphurising bacteria acting on the organic matter. In this case it would be advisable to examine a culture of bacteria grown on coorongite to determine the structure of their fatty acids.

Further progress in this study of fatty acids in geological materials will be effected by:

(i) procuring samples of Botryococcus braunii and examining the fatty acid content (now being conducted in this laboratory by Dr. K. Douraghi-Zadeh);

(ii) procuring material known to be derived from coorongite and of intermediate age between coorongite and Torbanite.

(2) Green River Shale.

The fatty acids procured from Green River Shale provide supporting evidence for the proposed geogenetic relationship between the n-acids and n-alkanes; the former decrease with depth, the latter increase with depth. The evidence is not nearly so certain when the isoprenoid acids and alkanes are considered.

It may be, of course, that there is no connection between the fatty acids and the alkanes. However the maturation effect displayed by the n-acids and n-alkanes in the smoothing out of the distributions between n-C_{19} and n-C_{29} does suggest a geogenetic relationship between the two classes of compounds. To be noted is the much decreased amount of acids in the lower level with a corresponding increase in the n-alkanes (Robinson et al, 1965), especially the higher molecular weight ones in the case of the alkanes.

As has been pointed out already, most workers in the field have been concerned with the normal saturated acids only and in only one case other than our own have the branched-chain acids been considered (Leo and Parker, 1966). The Green River Shale from the Mahogany Zone contained branched-chain acids ranging from C_{12} to C_{18}. Using GLC only they did not report the presence of isoprenoid acids which one would expect to be present, considering that the

corresponding hydrocarbons have been detected (Cummins and Robinson, 1964; Eglinton et al, 1966). The branched-chain acids require a more detailed study for it is evident that the role of microorganisms in the formation of a sediment is important and that the bacteria contribute considerably more to the lipids in a sediment than was previously imagined. There is a need to procure larger quantities of fatty acid fractions so that the branched acids, which are normally only a minor fraction of the total fatty acid fraction can be identified more rigorously. The most efficient method for effecting the removal of larger quantities of acids might consist in treatment of the rock sample, suitably powdered, with mineral acid, followed by alkaline hydrolysis of the residue for a considerable time (up to 100 hrs), removal of the alkali and application of the McCarthy and Duthie method for an efficient separation of the acids from other lipids.

Having isolated sufficient quantities of the fatty acids the individual acids would then be separated either by the use of preparative GLC or clathrate formation followed by preparative GLC. The individual acids could then be examined by IR (Hansen et al, 1965; Leo and Parker, 1966), nuclear magnetic resonance (Cason and Graham, 1965: Hopkins, 1963) and optical rotation studies which are especially useful for the determination of stereochemistry. The latter technique would involve the use of synthetic and biologically produced compounds to effect a correlation of the configurations. Good methods must be sought for the synthesis of the latter compounds.

There is a need to know more about the differences between the free fatty acid, total fatty acid, and free fatty acid ester fractions, in order to determine to what extent acids are bound either to the kerogen, the inorganic matrix or to both without actually destroying, for example, the kerogen matrix

by oxidation or reduction (Robinson et al, 1963). Further, the presence of hydroxy or keto acids has not been previously investigated and they are worthy of notice because of their biological significance (Hilditch and Williams, 1964). These have been isolated from Montan wax but not from older geological materials.

With regard to techniques for the isolation and identification of fatty acids other than the normals, the following points are worthy of consideration:

(i) Use should be made of thin-layer chromatography using silver-nitrate impregnated silica when young sediments are being examined. This would provide a rapid separation of unsaturated acids from other acids prior to the separation of branched acids from normals.

(ii) An alternative method to the formation of the hydrogenolysis of esters should be sought for the efficient separation of micro quantities of normal acids from branched acids from cyclic acids. GLC might then be used to study the patterns of the individual types of fatty acids.

(iii) There is a need for more stable phases for the GLC of high molecular weight fatty acids. Better resolution can be effected by the use of capillary columns or narrow bore packed columns but the relative stability of the liquid phase limits the technique.

(iv) Little is known about the mass spectra of cyclic esters.

EXPERIMENTAL

1. Materials

Samples

(1) Torbanite - Torbanehill Mineral. The mineral Torbanite is described by Macgregor (1938) as a boghead or cannel coal from the Carboniferous Limestone Series, Bathgate, Westlothian, Scotland. The presence of scales, teeth and spines of freshwater fish, as well as fossil algal and plant debris, indicate the formation of Torbanite in pools among the swamp forests of Carboniferous times.

Skilling (1938) had extensively examined the nature of Scottish cannels and reported the presence of spores and yellow bodies having an alveolar appearance. The concentration of yellow bodies is so high in the Torbanehill mineral that the presence of the reddish-brown matrix, presumably kerogen, which accompanies them in small and varying proportions, is barely distinguishable. The conclusion reached by Temperley (1936) that the yellow bodies might be the remains of colonies of an alga which does not differ in any material respect from the living alga Botryococcus Braunii is of considerable interest.

Gibson (1922) reported Torbanite to be very rich in organic matter, yielding from 90 - 130 gallons of crude oil per ton of shale pyrolysed. The oil yield, according to Macgregor (1938), appears to have a definite relation to the algal content. It is generally believed that the entombed oil is associated with the yellow bodies. The ash content is about 20%, consisting mainly of alumino-silicates.

The sample examined in the present investigation was part of a large piece of Torbanite which had been used as an exhibit in the law-suit,

successfully contested in the 1850's, by James Young, founder of the Scottish Oil-Shale Industry. Young proved the mineral to be essentially a coal (Murray, 1959). The Torbanite was generously provided by Dr. Ian Rolfe, Assistant Curator of the Geological Collection of the Hunterian Museum, Glasgow University, who also provided a microsection of it for examination.

(2) Scottish Oil-Shale. Gibson (1922) describes this particular rock as an oil-bearing shale occurring in the Calciferous Sandstone Series near the base of the Carboniferous system. Macgregor (1938) regards the Oil-Shale Group in general "as having been laid down in an irregular inland depression in which alternating lagoonal and estuarine conditions of sedimentation prevailed". He continues, "to the plant debris (fragments of woody tissue, microspores, parts of leaves) deposited in the shallow inland lagoons were added remains of algal colonies of the lagoons themselves."

Gibson (1922) reported the oil-shale to yield only a fraction of the crude oil obtained from the boghead coal (Torbanite) ranging from 19 - 60 gallons of crude oil per ton of shale pyrolysed. Since there exists a much smaller proportion of yellow bodies in the oil-shale to the reddish-brown ground-mass, the proposed relation between the yellow bodies and organic matter would account for the smaller yield of oil on pyrolysis of the oil-shale compared with the boghead coal. Although their structures and organic content are similar, the oil-shale often shows little or no structure at all on examination of a microsection. The latter contains 60-80% inorganic matter mainly in the form of alumino-silicates and iron compounds (Gibson, 1922).

In summary, the oil-shales occur in the lower part of the Carboniferous Formation, which is known as the Upper Calciferous Sandstone Group,

whereas the boghead coals such as Torbanite, occur in the Upper Carboniferous Formation, in the Carboniferous Limestone Series. The oil-shales have low oil yields compared with the boghead coals and this is explained by the relative scarcity of the yellow bodies in the former.

A large (~2 cu.ft.) unfractured piece of oil-shale was secured for the present study by Dr. Rolfe and the writer from the stock-pile at the Westwood Works of Scottish Oils, Ltd., West Calder, Midlothian.

(3) Scottish Oil-Shale Distillate. The crude oil distillate is described by Stewart and Forbes (1938) as having been obtained by commercial pyrolysis of the oil-shale at a temperature around 600°C, air and steam being sometimes used in the process. The crude, unrefined sample used in the present study was provided through the courtesy of Mr. Thomson of Scottish Oils, Ltd., who secured a sample from the Pumpherston Refinery, Midlothian. It had been manufactured some 5 or 10 years ago at the Westwood Works, West Calder.

(4) D'Arcy Oil. According to Gibson (1922), the naturally occurring mineral oil known as D'Arcy Oil, was associated with an anticline in the Lower Limestone Group of the Carboniferous System at the eastern end of the Midlothian Coalfield. Wyllie (1938) reported that oil found associated with oil-shales in various parts of the Carboniferous System would seem to have originated through the action of igneous intrusions on the oil-shale. For example, the oil found in the Dunnet Mine oozes from an igneous sill in contact with a burnt shale-seam. Wyllie quotes Conacher as reporting that the Dunnet Shale was known to have been pyrolysed over an area of 18 sq. miles. It seems probable to these authors that the same agency had been responsible for all of the free oil found in the shale-mines. However, the possibility that the D'Arcy Oil might be a natural

petroleum, was not excluded by Wyllie, since intrusive igneous rocks are comparatively rare in the region of the D'Arcy boring. Furthermore, marine sediments appear to form a much larger proportion of the total thickness of the eastern part of the oil-shale area. Wyllie concludes that this area might possibly have included a marine sapropel, in which the transformation of the initial deposits might have resulted in a free petroleum without the intervention of heat.

The sample of crude oil examined in the present work had been obtained by the late Professor Gregory, Geology Department, Glasgow University, from a boring made around 1936 by the D'Arcy Exploration Company, situated $2\frac{1}{2}$ miles south-east of Dalkeith, by Edinburgh. Dr. Rolfe kindly arranged for this sample to be made available from the Hunterian Collection. Wyllie had a sample submitted to a standard testing procedure developed by the then Anglo-Iranian Research Laboratory at Sunbury, consisting of vacuum distillation of the crude oil at 2 mm. pressure, the fraction b.p. 75° - 175°C being collected. Olefines were present in this fraction to the extent of about 4% and paraffins, 80%.

(5) Coorongite. The mineral coorongite is reported by Conacher (1938) to be of recent algal origin, occurring in the neighbourhood of a salt water lagoon known as the Coorong on the coast of South Australia. It has some resemblance to crude india-rubber and is sometimes regarded as the probable source of boghead coals and allied materials, and its origin has been ascribed by Blackburn (1936) to the alga Botryococcus Braunii. Professor Glaessner in a personal communication to the writer states that coorongite is formed as an algal scum on the surface of a lake in a condition of high productivity. Broughton (1920; quoted by Conacher, 1938) reported that as coorongite formed

the algal mass changed from a green paint-like material to a tough, elastic substance within a few minutes, when scooped with the hand from the surface of the lake. The green slime weathers to coorongite, the cell walls being full of oil and not composed of carbohydrates.

The present sample had been collected 30 years ago by Dr. K. Washington Gray and was obtained through the courtesy of Professor Glaessner, Geology Department, University of Adelaide, via the good offices of Dr. Rolfe. It had been stored without any special protection in the museum.

(6) Green River Shale. The Green River Formation originated in the Eocene epoch as limy muds, sand and organic matter (mainly algal remains) laid down in large, shallow, freshwater lakes in north west Colorado (Shaeffer and Mangus, 1965). These lacustrine sediments differ from most tertiary deposits in this area which are of flood plain origin. The shale is a marlstone consisting of carbonates and clays impregnated with kerogen and organic matter.

The samples of oil-shale, already pulverized, were provided by Dr. W.E. Robinson, U.S. Bureau of Mines, Laramie, Wyoming, from $3\frac{1}{2}$ in. core sections taken from the Green River Formation by the Equity Oil Company at the Surphur Creek Unit, Well No.10 in Rio Blanco County, Colorado (Robinson etal., 1965). Robinson etal reported that the two cores examined in the present study, taken from a depth of 1056-1080 feet and 1884-1923 feet from the surface, yielded 34 gallons and 20 gallons of oil per ton of shale respectively, on pyrolysis.

Reagents.

Only analytical reagent grade chemicals were used; i.e. all solvents, hydrofluoric acid (40% solution; Hopkins and Williams and British Drug House,) hydrochloric acid, potassium hydroxide, (pellet form). Solvents were distilled

through a glass column (15 in. x 1 in.), packed with glass helices, prior to use. Mallinckrodt silicic acid (100 mesh) was used as a support in the isolation of free fatty acids. Woelm neutral alumina Grade 1 was employed in column chromatography.

2. Analytical Procedures

General

Precautions were taken to minimise lipid contamination. Care was taken in handling glassware and apparatus, and polythene gloves were used where possible. Flasks were stoppered or covered with aluminium foil between operations; the time between these was kept short. Solutions were transferred by means of disposable glass pipettes. Solvents were evaporated in a rotary evaporator under water pump suction; the air was carefully let in at the flask so as to avoid contamination from the rubber tubing. Final evacuation on an oil pump was limited to about two minutes to reduce the loss of low boiling compounds. Teflon stopcocks were used.

Glassware was cleaned ultrasonically in a soni-tank (Dawe Instruments Ltd.,) with detergent solution (R.B.S.26, Medical Pharmaceutical Developments Ltd.,). Sonication was carried out at 25 Kc/S and 150 watts for twenty minutes. Thorough rinsing with distilled water followed, prior to oven drying.

Column Chromatography

Chromatography columns were prepared by slurrying a weighed amount of alumina with n-hexane and pouring into a glass column (1 cm. o.d). The column was lightly tapped to ensure uniform packing of the adsorbent which was then washed thoroughly with hexane. The sample was introduced on to the column in a minimal amount of hexane and washed into the column with hexane (1-2 ml).

Alumina/sample ratios were about 30:1. Care was taken to ensure that the column was covered with solvent. The fractions collected were monitored by thin-layer chromatography on microscope slides.

Thin-layer Chromatography (T.L.C.)

Use was made of T.L.C. in the course of this investigation. Plates were prepared according to Mangold (1961). The adsorbent, Kieselgel G Silica, was slurried with distilled water for 30 secs., the resulting slurry was poured into a Desaga Spreader, and rapidly spread over the plates. The plates were agitated for a few seconds to produce a more uniform layer. After air drying, the plates were activated in an oven at 120°C from one hour and stored in a desiccator. The coated plates were always pre-washed with ethylacetate followed by re-activation at 120°C for 30 minutes. Both thin and thick (or preparative) plates were treated in the above manner.

Plates were developed in 5% diethyl ether/hexane and the eluted esters monitored with a standard mixture containing n-C_{18}, n-C_{24} and n-C_{30} methyl esters of fatty acids. Esters were detected on thin-layer plates by spraying the developed plates with 50% sulphuric acid followed by charring in an oven at 200°C. Detection of the esters on a preparative plate consisted of spraying the developed plate with a 0.2% solution of fluorescein in ethanol and viewing under a U.V. lamp (λ max 254 mu).

Silver nitrate† impregnated thin-layer plates were used to detect unsaturated esters, and were made in an identical manner to the ordinary silica plates. Detection consisted of spraying the plates with 50% phosphoric acid and charring at 200°C. Methyl oleate was used to monitor the eluted esters.

† (10%)

Infrared Spectroscopy

Infrared spectra of the "total extracts" and powdered rocks were recorded on a Perkin Elmer 237 spectrophotometer, using thin films for the former and KCl discs for the latter.

Infrared spectra of the pure methyl esters were recorded on a Unicam S.P.100 double beam spectrophotometer equipped with an S P. NaCl prism grating double monochromator operated under vacuum conditions. Spectra were taken in solution (CCl_4 solvent in 0.5 mm. cells) employing a semi-micro technique. The regions 850-1500cm $^{-1}$, 1600-1800cm $^{-1}$, and 2600-3650cm $^{-1}$ were investigated.

Gas-Liquid Chromatography (GLC)

(1) Analytical Gas-Liquid Chromatography. A Perkin Elmer F11 instrument employing 6 or 10 ft by $\frac{1}{8}$ in. stainless steel packed columns was used to display the general pattern of the free fatty acid methyl ester distribution (temperature programmed). The instrument was equipped with a hydrogen flame ionisation detector with nitrogen as carrier gas at flow rates of 20-30 ml. per min. Temperature programming of the columns with silicone gum (1% and 3% SE-30) as liquid phase was usually from 100-300°C at rates of from 4° to 8° per min. and with ethylene glycol adipate polyester (10% PEGA) as liquid phase was from 150°-215°C at 4° or 5° per min. The injector temperature was set at about 300°. Occasional batches of silicone rubber septa exuded high molecular weight volatiles. A successful attempt was made to remove these volatiles by heating at 360° under high vacuum for 8 hours.

Column packings containing 1-3% SE-30 (Applied Science Labs. Inc.) were prepared by pouring the support (20g, 100-120 mesh, Gas Chrom P, acid-washed and silanized, Applied Science Labs. Inc.) previously screened to select the

correct size of particles, into a solution of liquid phase in chloroform (80 ml) with gentle swirling to minimise fracture of the support particles. The suspension of the support in the solution containing liquid phase was filtered on a Buchner funnel to remove the solution as rapidly and completely as possible. The coated support was freed from solvent in a vacuum oven (50°) under water pump suction. All columns were packed with coated support under oil pump suction assisted by vibration provided by a laboratory whirlmixer.

Column packings containing 10% PEGA (Applied Science Labs. Inc.) were prepared by suspending the support in a solution of liquid phase in chloroform. After thorough mixing, the solvent was removed on a rotary evaporator. The column using PEGA as liquid phase gave the required separation for a standard mixture containing the esters of stearic, oleic, and linoleic acids (James, 1959).

(2) Preparative Gas-Liquid Chromatography. The instrument used was a Wilkens Aerograph A90 P-3, equipped with thermal conductivity detectors, and 6 ft. by $\frac{1}{4}$ in. copper columns. Chromosorb W (100-120 mesh, Johns Manville) as the solid support was coated with 10% PEGA, as described above. The carrier gas was helium with a flow rate of 60 ml. per min. at 50 p.s.i. Injector and detector temperatures were around 250° and 270°C, respectively.

The collection of GLC fractions of molecular weight greater than that of the n-C_{11} fatty acid methyl ester was effected by trapping the eluate in a straight glass melting-point capillary (1mm. bore and 10 cm. long). These capillaries had been previously cleaned by sonication in detergent solution, thoroughly rinsed with distilled water, acetone and finally sonicated in chloroform. Both ends of the capillaries were flame polished to prevent contamination at the collection port, due to silicone rubber septum material, etc.

After collection both ends were sealed under a very small flame.

Mass Spectroscopy and Gas Chromatography-Mass Spectroscopy

Some mass spectra were determined by Dr. J. Martin on an A.E.1. MS9 double-focusing mass spectrometer at ionising voltage of 70 e.v. Samples were examined on a ceramic probe by direct insertion into the mass spectrometer and only those samples which were known to consist of a single peak by analytical GLC were treated this way.

With the subsequent availability of an LKB 9000 gas chromatograph-mass spectrometer, Dr. A.G. Douglas was able to examine fractions collected during preparative GLC and which were known to contain several components by analytical GLC. The column then used was a 10 ft. by $^{1}/_{8}$ in. packed column with 1% SE-30 as liquid phase. Carrier gas was helium with a flow rate of 30 ml. per min. All runs were isothermal, the temperatures being selected from the known analytical GLC conditions. The mass spectra were obtained with a rapid scanning, single-focusing magnetic analyser with ionising voltage of 70 e.v. Sample enrichment was achieved by preferential removal of the carrier gas from the effluent stream in a molecular separator. Interpretation of the recorded mass spectra was facilitated by an almost linear mass scale.

Where samples were liquids, the capillary tubes were centrifuged before opening, after which the sample was removed with solvent using a 10 μl Hamilton syringe. Where samples were solid, the walls of the capillary tube (broken near the seal) were carefully washed with solvent (1 μl portions) before injection into the mass spectrometer.

Several mass spectra were also recorded by Dr. G. Eglinton through the courtesy of LKB - Produkter AB at the factory in Stockholm on an LKB 9000 gas chromatograph-mass spectrometer.

3. General Procedure for the Extraction of Organic Matter and Isolation of Free Fatty Acids

Treatment of Rocks. (Fig. 1.)

(1) Powdering of the rock samples. In general, the rock was broken up on a clean metal surface with a hammer into pieces 1-1½ in. in size, which were carefully washed by probe sonication, using a titanium probe (Dawe Instruments Ltd.) in benzene:methanol (3:1) for ten minutes, prior to powdering. All surfaces selected were fresh exposures.

The initial powdering was accomplished by a carefully cleaned rotary hammer-mill (Glen Creston, Star Beater Mill) modified to take lead rather than rubber gaskets. Final powdering was effected in a carefully cleaned vibratory disc mill [Tema (Machinery) Ltd., Banbury], the milling operation lasting about fifteen minutes. Prior to use, the moveable parts of both mills which were in contact with the rock, were cleaned by tank sonication in detergent solution, rinsed thoroughly with distilled water, washed with acetone and finally with chloroform. The various moveable parts of the mills were handled with polythene gloves while reassembling.

In the case of the hammer-mill, 70% of the resulting powdered rock was found to pass through a 200 mesh sieve, while the disc mill operation resulted in 100% of the powdered rock passing through a 200 mesh sieve. A considerable amount of heat was evolved if the milling time for the disc mill was extended beyond fifteen minutes; in such cases the temperature rose to about 60°C.

The two stage pulverizing was employed when samples were obtained as rock chunks, otherwise pulverizing in the disc mill was sufficient.

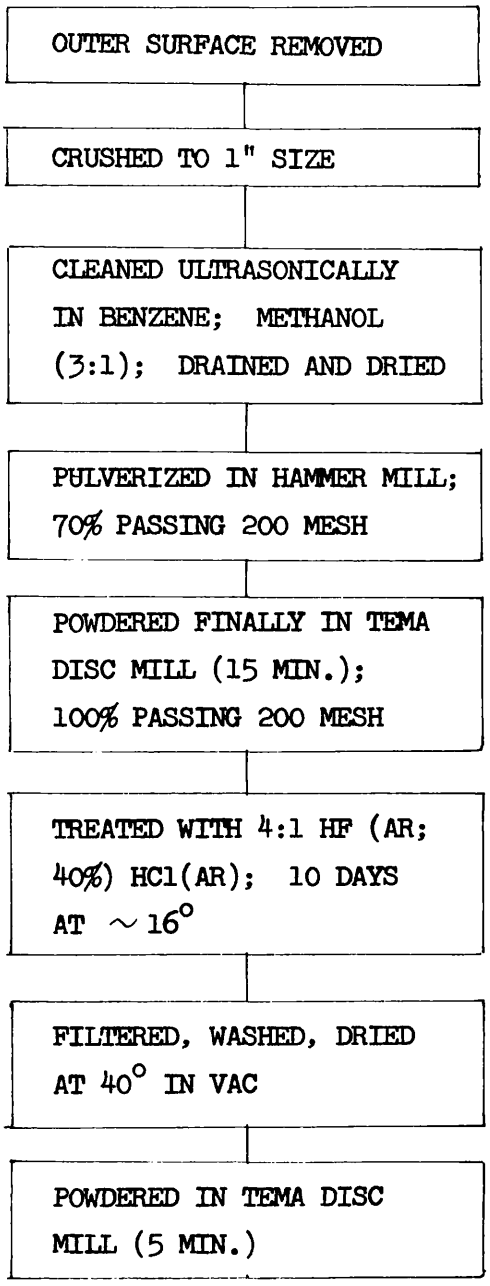


Fig. 1. Flow diagram for the preparation of rock samples prior to solvent extraction.

(2) Acid treatment of the powdered rock. To remove the mineral matter in the rock, present as silicates or carbonates or both, and at the same time to release the fatty acids from their calcium salts, portions of powdered rock (50g) were treated with hydrofluoric and hydrochloric acids (300 ml) in the ratio of 3:1 in polythene bottles (500 ml; hydrofluoric acid containers supplied by British Drug House). The bottles were stoppered and the suspension of powdered rock in acid solution was allowed to stand at room temperature for 7 days. The suspension was then filtered through a chloroform-pre-extracted, acid-resistant filter paper in a Buchner funnel, under water pump suction. The residue was then washed with distilled water until acid free. The filtrate was retained to be examined for free fatty acids. Drying of the residue was effected in a vacuum oven at 40°C with water pump suction for several hours, after which it was found that the cake of concentrate required further pulverizing.

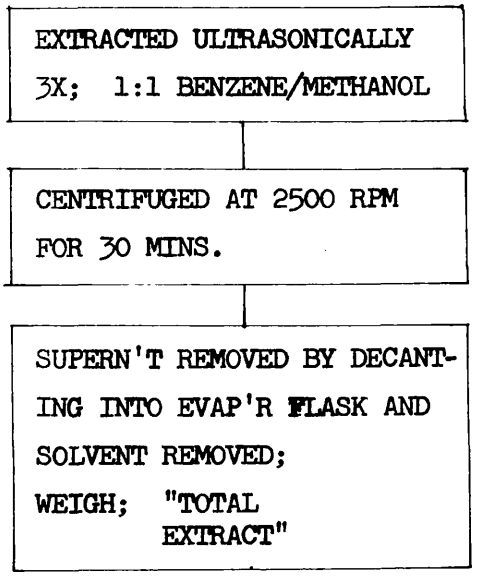


Fig. 2. Flow diagram for the extraction of solvent soluble organic matter, the "total extract."

Five minutes milling in the disc mill reduced the residue to a very fine powder before proceeding to the extraction of the organic matter.

A freeze-drying technique might have avoided this latter step.

Extraction of Organic Matter. (Fig. 2)

The simplest extraction procedure used was to heat a suspension of the powdered rock in a solvent for several hours, but the use of a pre-extracted all glass Soxhlet (with a sintered disc sealed to the bottom of the cylinder in which the powder was placed) was more convenient. The use of ultrasonics (McIver, 1962) was explored, to hasten extraction. The powdered rock or residue was placed in a glass centrifuge tube or bottle (250 ml.) with solvent in an ultrasonic tank, the level of solvent in the bottle being at the level of the water in the tank. The titanium ultrasonic probe was convenient for small scale experiments with a few grams of rock sample, but on a larger scale with up to 500g of powdered rock, tank sonication was by far the most convenient, and with solvent/sample ratios of 3:1, three successive extractions of thirty minutes duration recovered most of the organic matter soluble in organic solvents. The solvent system normally used was benzene:methanol (1:1).

In all extraction procedures the resultant suspension of solvent and powdered rock was centrifuged at 2500 r.p.m. for thirty minutes (MSE, R Magnum Centrifuge) and the clear supernatant solution was removed by decantation followed by evaporation of the solvent on a rotary evaporator. The organic matter recovered will hereinafter be called the "total extract".

Isolation of Free Fatty Acids as Methyl Esters from the "Total Extract" (Fig.3)

In the present study the free fatty acids were recovered from the "total extract" by passing the latter through a column containing potassium hydroxide supported on silicic acid. Prior to adopting this method (due to McCarthy and Duthie (1962)), the use of Amberlite IRA400 resin was explored as a means of isolating the free fatty acids (Hornstein, 1960). The attraction of the latter method lay in the possibility of isolating the free fatty acids as their methyl esters by forming the latter on the resin. Little success, however, was achieved since the resin required to be regenerated several times before it became reasonably active. This was one of the findings of McCarthy and Duthie. Furthermore, the resin contained organic contaminants which could not be readily eliminated. The method McCarthy and Duthie which provided a rapid quantitative separation of free fatty acids from other lipids was adopted in the present work.

(1) Preparation and Operation of the Silicic Acid/Potassium Hydroxide Column.

Potassium hydroxide pellets (25g) were dissolved in isopropanol (400 ml) by warming on a steam bath. The supernatant liquid was decanted from the small amount of aqueous KOH clinging to the bottom of the flask and the solution was cooled and stored in the refrigerator. It contained approximately 50 mg/ml of potassium hydroxide.

The flow of solvent through the silicic acid was improved by removing the fines, conveniently done by suspending silicic acid (100g) in methanol (400 ml), stirring, and decanting that which did not settle after 5 minutes. This procedure was repeated with methanol and once with acetone (400 ml), followed by rinsing with ether. The silicic acid was allowed to dry in air.

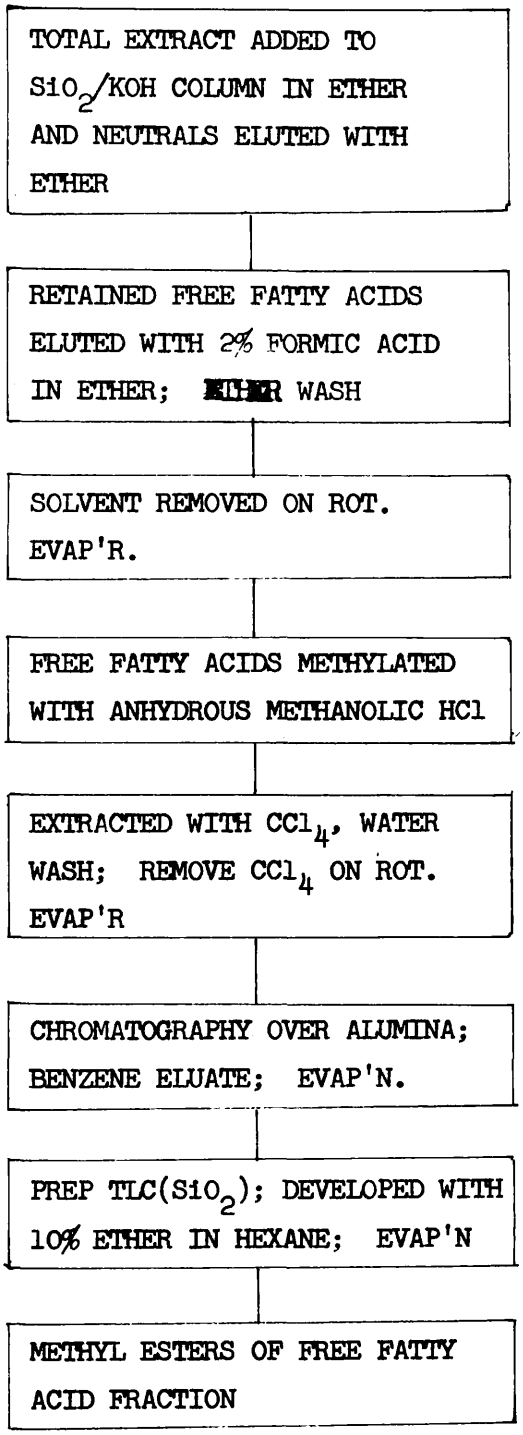


Fig. 3. Isolation and purification of the free fatty acid fraction (as their methyl esters) from the total extract.

Silicic acid (20g) was suspended in the standard isopropanol/potassium hydroxide solution (20 ml) with diethyl ether (60 ml) added. After standing for 5 minutes the suspension was slurried into a glass column (40 x 2.5 cm.), containing a pre-extracted cotton wool plug. The column was elutedwith diethyl ether (200 ml), after which the sample was placed on to the column in a small quantity of diethyl ether. Neutral lipids were removed by eluting the column with a further volume of diethyl ether (200 ml).

The free fatty acids retained on the column were then removed by formic acid in diethyl ether (2%, 150 ml) followed by a final elution with diethyl ether (200 ml). The solvent was removed from the eluate on a rotary evaporator and at the oil pump (55°, 10^{-1}mm).

(2) Preparation and Purification of the Methyl Esters of the Free Fatty Acids. The crude free fatty acids fraction was heated under reflux with anhydrous methanolic/hydrogen chloride (50 ml) for $2\frac{1}{2}$ hours. (The methylating agent was prepared by passing anhydrous hydrogen chloride into super-dry methanol (Vogel, 1956). A teflon stirring bar was used for mixing and thin walled teflon tubing employed for passing the hydrogen chloride from a cylinder into methanol). On cooling, distilled water (100 ml) and carbon tetrachloride (100 ml) were added. The organic layer was removed and combined with two further washings of carbon tetrachloride (100 ml). The solvent was washed with distilled water until neutral and finally removed on a rotary evaporator. Traces of water were removed by addition of methanol and evaporation.

The crude esters were initially purified by alumina chromatography, traces of neutral lipids being removed by elution with n-hexane, the esters themselves being eluted with benzene. Fractions collected were monitored by

thin-layer chromatography using microscope slides.

Final purification of the free fatty acid methyl esters were effected by preparative thin-layer chromatography, the development being monitored by a standard mixture consisting of n-C_{18}, n-C_{24} and n-C_{30} methyl esters.

RESULTS

1. Procedures for the Isolation of Free Fatty Acids

1. Choice of isolation technique.

Four methods were considered for the isolation of the total extract from a rock.

(a) Extraction of the total extract by heating under reflux a suspension of powdered rock in solvent.

(b) Soxhlet extraction using a glass thimble with a sintered glass disc sealed to the bottom of the thimble.

(c) Extraction by probe sonication of a suspension of powdered rock in solvent.

(d) Extraction by tank sonication of a suspension of powdered rock in solvent.

A single batch procedure was employed and the solvent system was benzene/methanol (3:1). A small quantity of Green River Shale from the Mahogany Zone of that formation was used as the test material (provided through the courtesy of Dr. W.E. Robinson, U.S. Bureau of Mines, Laramie) and portions of powdered rock (2g) were suspended in benzene/methanol (50 ml) in the case of methods (a), (c) and (d) and extracted accordingly. The results are tabulated below:

Method	Duration	Weight of total extract/g of powdered rock
(a) Reflux in solvent	10 mins.	38.7 mg
(b) Soxhlet extraction	24 hrs.	33.2 mg
(c) Probe sonication	15 mins.	33.5 mg
(d) Tank sonication	30 mins.	32.0 mg

Method (a) provided the largest amount of total extract. However, of the four methods examined it was the least convenient with respect to the recovery of the supernatant solution. Soxhlet extraction is time consuming and the thimble is an added source of contamination. Probe sonication is convenient for small samples only ($\sim$ 10g) while tank sonication proved to be the most convenient method when dealing with large batches (up to 500g). The latter method was selected for use in the present work.

2. Choice of Solvent system.

The suitability of several solvents was investigated for use in extraction procedure. Portions of powdered Green River Shale (2g) were extracted ultrasonically (sonitank) for 30 mins. in centrifuge tubes (100 ml) with 50 ml solvent. The resultant suspension was centrifuged and the clear supernatant solution was decanted and evaporated on a rotary evaporator to yield varying quantities of a brown gum. A second extraction using the same quantity of solvent isolated a further small quantity of total extract ($\sim$10% of the total weight of extract) while a third extraction yielded approximately 5% of the total weight of extract isolated. The infrared spectrum of each extract was recorded using thin film and the results are shown below:

Solvent	Wt. of total extract/g rock	Carbonyl absorption at 1700cm^{-1}
Light Petroleum	11.3 mg	weak band
Benzene	11.8 mg	weak band
Methanol	14.7 mg	medium band
Pyridine	21.2 mg	medium band
Benzene/Methanol (2:1)	32.0 mg	medium strong band

On the basis of the quantity of total extract isolated from powdered rock, the solvent system, benzene/methanol, was selected for use in the present investigation.

3. Investigation of the efficiency of the tank sonication procedure; recovery of stearic acid from finely powdered acid-washed sand.

The tank sonication method having been selected for large scale isolation of total extract from a powdered rock sample, the efficiency of this method with particular respect to fatty acids was further investigated.

Acid-washed sand (50g laboratory supply) was pre-extracted with chloroform and crushed in the disc mill for 15 mins. after which stearic acid (50mg, Hopkins and Williams) was added to the crushed sand and an intimate mixture produced by further milling for 10 mins. The mixture of stearic acid with sand was suspended in benzene/methanol (2:1, 100 ml) in a centrifuge bottle (250 ml) and extracted ultrasonically (sonitank) for 30 mins. After two further extractions using the same quantity of solvent the clear supernatant solution obtained by centrifuging the suspension was decanted and the solvent removed on a rotary evaporator. Final removal of solvent was effected at the oil pump and in the vacuum oven (55°).

The recovered fatty acid was esterified (methanolic/HCl) and examined by TLC, GLC, and infrared spectroscopy. TLC revealed a spot corresponding to methyl stearate, while GLC on 10% PEGA at 180° gave a retention time corresponding to methyl stearate (10 mins.), while the infrared spectrum recorded on a thin film showed intense carbonyl absorption at $1740cm^{-1}$.

The weight of ester recovered was 44 mg making the percentage recovery of stearic acid 84%.

4. Examination of the possible effect of hydrofluoric acid on fatty acids.

In order to determine whether fatty acids undergo rearrangement to branched or cyclic compounds during the treatment of rocks with hydrofluoric acid, stearic acid in powder form (1g, Hopkins and Williams) was added to hydrofluoric acid (100 ml) and left standing for 10 days. The stearic acid did not dissolve to any appreciable extent in HF even after sonication. The stearic acid was subsequently collected by filtration, esterified (methanolic/HCl) and a GLC trace recorded on the ester fraction (10% PEGA at 180°). Comparison of this trace with that recorded for authentic methyl stearate (prepared from the same sample of stearic acid) revealed no additional peaks and the retention times of the methyl stearate peaks were identical.

5. Investigation of the efficiency of the McCarthy and Duthie procedure [J. Lipid Res., 3, 117(1962)] for isolation of free fatty acids from the total extract.

On the basis of a comprehensive series of experiments, McCarthy and Duthie (1962) claimed a 98% recovery of free fatty acids from lipid extracts. It was not clear, however, to what extent a large quantity of neutral (non-polar) lipids would affect the retention of free fatty acids on a silicic acid/potassium hydroxide column. The possibility that the removal of the non-polar lipids by a large volume of solvent might tend to wash the potassium salts of the free fatty acids down the column could not be excluded.

Accordingly, stearic acid (1g, GPR) was added to a sample of mineral oil (Kuwait oil, 4g) and the resultant mixture made up to 50 ml with ether. A portion of this solution (10 ml) was placed on a silicic acid/potassium hydroxide column (20g SiO_2, 20 ml standard isopropanol/KOH solution) and the

stearic acid isolated and esterified by the usual method. The ester fraction was chromatographed on silica gel using 10% diethyl ether in n-hexane as developing agent and quantity recovered (195 mg) represented 93% recovery of the stearic acid used. It was concluded that the presence of a large quantity of non-polar lipids did not materially effect the isolation procedure for free fatty acids described by the above authors.

6. Treatment of methyl ester fractions with $5A^{\circ}$ molecular sieve.

An attempt, using molecular sieves, was made to provide an alternative to urea adduction for the separation of branched/cyclic esters from normal esters. Pellets ($^{1}/16$ in.) of $5A^{\circ}$ sieve (Linde Co., Division of Union Carbide Corporation) were dried for twelve hours at 200° in vacuo, and stored in a desiccator. A solution of methyl stearate (50 mg) in benzene (50 ml) was heated under reflux (24 hrs) with the dried sieve (5g). A drying tube (SiO_2) was provided. The solution was then removed and the sieve washed with hot benzene (all glass soxhlet) for 4 hours. Finally the benzene-washed sieve was heated under reflux (6 hrs) with methanol to recover entrapped methyl stearate.

The methyl stearate recovered from the sieve was dissolved in benzene (5 ml). A GLC trace recorded on 1 μl of this olution, indicated that a small quantity (5 mg) of methyl stearate had been entrapped in the sieve.

2. Control Experiments

Acid-washed sand.

A full control was developed to establish the probable level of contamination encountered in the present study. Accordingly, sand (50g laboratory supply) was washed with chloroform and then extracted ultrasonically with benzene/methanol (1:1, 80 ml). The solvent was decanted and removed on a rotary evaporator after which the flask was rinsed with ether and the contents added to a silicic acid/potassium hydroxide column. The free fatty acid fraction was recovered and esterified in the usual manner. Benzene (10 ul) was added to the 'ester' fraction and a GLC trace recorded on 1 ul of sulution. The absence of peaks indicated that the precautions taken to eliminate contamination in the above procedure were sufficient.

Parker† has pointed out that batches of commercial potassium hydroxide are often contaminated by fatty acids due to handling during manufacture. Fusion of the potassium hydroxide ensures the removal of the fatty acids. This procedure, however, was not considered necessary in view of the negative results obtained from the GLC analysis of the control in the present work. The same batch was employed throughout the work.

Finger Grease.

In order to trace possible sources of contamination, a benzene/methanol (1:1) extract of human sebum was obtained by brief immersion of the fingers of several individuals in this solvent system. The free fatty acid fraction was recovered, esterified, purified by TLC and the infrared spectrum recorded (intense carbonyl absorption at 1740cm^{-1}). The GLC pattern is shown in Fig.9, Tracing C, and the positions of the normal acid esters were located by

†(Personal communication)

coinjection of n-C_{14}, n-C_{16} and n-C_{18} fatty acid methyl esters obtained from the collection of samples synthesised by Professor Chibnall. The reference esters were found to be substantially pure and showed only traces of other components when examined individually by GLC.

MacDonald (1964) examined human sebum by GLC and identified the main components of the free fatty acid fraction as palmitic, palmitoleic and oleic acids on poly ethylene glycol succinate (EGS). Both palmitoleic and oleic acids as their methyl esters are known to be eluted after the normal acid ester of the same carbon number and appear to be present in the free fatty acid fraction isolated in the present work.

Lurie and Villee (1966) have recently reported sebum as a contaminant of samples for GLC and have demonstrated that one thumb print could significantly contaminate 500 ml of organic solvent. In the present work, however, the blank runs did not reveal the pattern shown in Fig. 9C, and it was concluded that the precautions taken to exclude contamination as much as possible, were satisfactory.

Hydrofluoric Acid.

In order to determine towhat extent any platicizer from the polythene containers is present in hydrofluoric acid (supplied in 500 ml polythene bottles), HF (300 ml) was evaporated on a rotary evaporator and the residue added in ether to a silicic acid/potassium hydroxide column. The 'free fatty acid' fraction was recovered and esterified in the usual manner and a GLC trace recorded by adding benzene (10 ul) to the 'ester' fraction and injecting 1 ul of this solution. The GLC analysis showed the presence of small quantities of compounds (three significant peaks) in the region normally examined (of the

order of 0.1 ug per 500 ml HF). The level of this contamination is adjudged too low to affect the analysis reported in this paper.

RBS 26 Detergent Solution. (Medical Pharmaceutical Dev. Ltd.)

A small portion (20 ml) of this detergent was dissolved in benzene/methanol (1:1) and the solvent removed on a rotary evaporator. The dry residue was added in ether solution to a silicic acid/potassium hydroxide column and the 'free fatty acid' fraction recovered and esterified in the usual manner. Examination of the 'ester' fraction by TLC and GLC revealed the presence of several compounds which on mass spectral analysis (LKB 9000 GC-MS) did not reveal the characteristic methyl ester fragmentation pattern. It is possible that these compounds are derivatives of sulphonic acid. Similar spectra were not encountered during the analysis of fractions by the combined GC-MS procedure and so it was concluded that the rinsing of glassware subsequent to sonication in detergent solution was effective.

3. Examination of the Geological Samples

Torbanite

1) Isolation of organic matter without acid treatment of the rock.

A large piece of rock was hammered into pieces, 1 in. in size, which were then washed ultrasonically (soniprobe) in benzene/methanol (3:1) and then pulverized as described previously. The powdered rock (250g) was extracted three times ultrasonically (sonitank) in batches (50g) in centrifuge bottles (250 ml), with 150 ml benzene/methanol (1:1) per batch (total 2250 ml) for 30 mins per extraction. The solvent-extracted shale was not washed with solvent after each extraction; the second and third extractions were considered sufficient to remove the solvent-soluble organic matter as completely as possible. The clear solution obtained on centrifuging the suspension of rock powder in solvent, was decanted and the solvent removed on a rotary evaporator to give a yellow gum (1.5g). The infrared spectrum recorded on a thin film revealed a carbonyl peak of low intensity at $1700cm^{-1}$.

The free fatty acids were isolated from the total extract on a column consisting of silicic acid (20g) and potassium hydroxide (20 ml of standard isopropanol/potassium hydroxide solution), the non-polar lipids being removed with ether (200 ml) and the free fatty acids with 2% formic acid in ether (150 ml). Esterification of the fatty acids was followed by chromatography over alumina (5g neutral alumina) with elution first by n-hexane (10 ml) to remove any traces of the neutral lipid fraction followed by benzene (100 ml) to recover the crude methyl ester fraction. (The neutral lipid fraction eluted from the silicic acid column was examined for free esters by removing the solvent and recording the infrared spectrum

(thin film). The absence of carbonyl absorption between 1750 and 1700cm^{-1} indicated that free esters did not exist in the original rock.

The crude methyl ester fraction (10mg) was further chromatographed on TLC silica (1 mm thick) with 5% diethyl ether in n-hexane as the developing agent. The elution was monitored by a mixture of n-C_{18}, n-C_{24} and n-C_{30} fatty acid methyl esters (Fig. 4A) and the plate was viewed under U.V. light (254 mμ) prior to spraying with fluorescein. The band corresponding to the position of the reference esters was scraped off and eluted with chloroform. The purified methyl ester fraction (8mg) afforded an infrared spectrum, recorded in solution (40 ug ester in CCl_4, semi-micro 0.5 mm cells), which revealed an intense carbonyl band at 1740cm^{-1}. The ratio of the optical densities (OD) for νCO and ν CH_{2850}, OD νCO/OD νCH_{2850}, was found to be 1.17. This ratio was 1.00 for methyl stearate (99.8%, Applied Science Labs) in solution (CCl_4), and provides an index of the number of methylene groups or methylene hydrogen atoms present. An increase in the value of this ratio would indicate either the presence of shorter chain fatty acids or methyl branching.

The GLC pattern of the pure methyl ester fraction is shown in Fig. 5, Tracings A and B. Internal standards used in the GLC runs, n-C_{14}, n-C_{16} and n-C_{18} fatty acid methyl esters, located the two major peaks with retention times corresponding to the n-C_{16} and n-C_{18} fatty acid methyl esters. Preparative GLC of the ester fraction gave the pattern shown in Fig. 5, Tracing C. Mass spectral examination of peaks 6 and 8 indicated the characteristic spectra possessed by saturated, normal fatty acid methyl esters (Table 1). The mass spectra of the isolated n-C_{16} and n-C_{18} esters were measured on the AEI instrument and found to be comparable with that for authentic methyl stearate

($n\text{-}C_{18}$) listed in Table 1. The mass spectra of the isolated $n\text{-}C_{16}$ and $n\text{-}C_{18}$ fatty acid methyl esters were also comparable with those reported by Ryhage and Stenhagen (1959).

The presence of a large number of minor peaks on gas chromatography as shown in Fig. 5, Tracing A, indicates the effectiveness of SE-30 as liquid phase for the resolution of the components of complex mixtures. The resolution of these minor components was not effected to any appreciable extent by PEGA (Fig. 5 Tracings B and C). The minor components will correspond to the branched and cyclic fatty acid methyl esters and although no attempt was made to establish their identity by mass spectrometry (e.g. on LKB 9000 GC-MS), plots of relative retention times against the number of carbon atoms constructed from Tracing A might indicate the presence of iso and anteiso acids (Woodford and Van Gent 1960). The temperature programming of the gas chromatograms was known not to be truly linear. However, a plot of the relative retention times for the n-alkanoic acids (relative to $n\text{-}C_{12}$ as unity) was almost linear (Fig. 5D). A near linear plot, parallel to that for the n-alkanoic esters, was obtained for the series of components eluted immediately ahead of the normal esters. According to Woodford and Van Gent (1960) this plot would correspond to the anteiso compounds. A further near linear plot which would correspond to the iso acids was obtained for the compounds eluted ahead of the anteiso compounds.

In conclusion, a series of n-alkanoic acids ranging from C_{10} to C_{27} along with minor quantities of other acids, possibly iso and anteiso, was isolated from Torbanite, and the methyl esters examined by TLC, GLC, and infrared spectroscopy. In addition, the two major components were examined by mass spectrometry and identified as the $n\text{-}C_{16}$ and $n\text{-}C_{18}$ fatty acid methyl esters.

2) Isolation of fatty acids by esterification in situ subsequent to acid treatment of the rock.

An attempt was made to isolate further quantities of fatty acids from the solvent-extracted residue described in the previous section. To this end, the residue (~200g) with the remains of solvent clinging to it, was treated with mixed hydrofluoric acid/hydrochloric acid (3:1, total 1200 ml) for 7 days at $\sim 18^{\circ}$ after which the suspension was filtered, washed, dried and repulverized. The residue (~180g) was then heated under reflux with anhydrous methanol (600 ml) for 6 hours while anhydrous hydrogen chloride was bubbled through the suspension, which was stirred continuously.

Following this treatment, the suspension was filtered and the residue washed with methanol (250 ml). Distilled water was added to the filtrate plus washings and the mixture was then extracted three times with carbon tetrachloride (total 1200 ml). The CCl_4 was washed until acid-free and then removed on a rotary evaporator. Chromatography over alumina (2g neutral alumina) with elution first by n-hexane (20 ml) to remove the non-polar lipids fraction followed by benzene (80 ml) yielded a crude methyl ester fraction (4 mg). Preparative TLC with 10% diethyl ether in n-hexane as developing agent, monitored by the standard mixture of esters (Fig. 4A), yielded a pure methyl ester fraction (2.4 mg). The infrared spectrum was recorded in solution (30 μg in CCl_4, 0.5 mm cells) and showed an intense carbonyl peak at 1740cm^{-1} (Spec 2). The ratio of optical densities, $\frac{OD\,\nu_{CO}}{OD\,\nu_{CH\ 2850}}$ was calculated to be 0.945, and the spectrum showed that the material was substantially straight chain. The spectrum of methyl stearate is shown in Spec. 1.

The GLC pattern is shown in Fig. 6, Tracings A, B and C. The ester fraction was subjected to combined gas chromatographic-mass spectrometric analysis (LKB 9000) at Stockholm. Peaks 1,2,5 and 6 (Tracing A) were unambiguously identified as the n-C_{13}, n-C_{14}, n-C_{17} and n-C_{24} fatty acid methyl esters,

In summary, the method described in this section effected the isolation of a small quantity of normal fatty acids ranging from C_{10} to C_{24} the presence of which was detected by TLC, GLC and infrared spectroscopy. Furthermore, four components of the fatty acid fraction were identified by mass spectrometry, as the normal fatty acid methyl esters, C_{13}, C_{14}, C_{17} and C_{24}.

3) Isolation of free fatty acids from the total extract after acid treatment of the rock.

Following the initial attempt to isolate free fatty acids from Torbanite as described in section 1., a further quantity of powdered rock (250g) was treated in batches (50g) with mixed hydrofluoric acid/hydrochloric acid (3:1, total 1500 ml) at $\sim 18^{\circ}$ for 10 days. The resultant suspension was filtered, washed, dried and repulverized. The powdered residue (160g) was then extracted three times ultrasonically (sonitank) in batches (50g) with benzene/methanol (1:1, total 2250 ml) for 30 mins. per extraction.

The clear supernatant solution, obtained on centrifuging the suspension, was decanted and the solvent removed on a rotary evaporator, affording a dark yellow gum (3.1g). (The amount of total extract (solvent-soluble organic matter) obtained by removing the inorganic matrix was $2\frac{1}{2}$ times greater than that obtained from the shale without previous treatment with acid

(Torbanite, Section 1)). This gum revealed a carbonyl band of low intensity at 1700cm^{-1}.

Isolation of the free fatty acid from the total extract was effected in the usual manner by passing the extract in ether through a silicic acid/potassium hydroxide column (25 g SiO_2, 25 ml isopropanol/KOH Solution) and removing the free fatty acids with 2% formic acid in ether subsequent to the elution of the non-polar lipids. Esterification was followed by chromatography over alumina (4g neutral alumina) with n-hexane (10 ml) to remove any traces of neutral lipids, followed by benzene (180 ml) to recover the ester fraction. (The non-polar eluate from the silicic acid column was evaporated and then examined for carbonyl absorption in the infrared. As there was no evidence of carbonyl absorption, it was concluded that free esters of fatty acids were not present in the Shale.)

The partially purified ester fraction (13.5 mg) was further chromatographed by preparative TLC (Kieselgel G silica, 1 mm) and after eluting with 5% diethyl ether in n-hexane, the band corresponding in position to the esters (Fig. 4A) was scraped off and elutedwith chloroform to yield a pure ester fraction (12.2 mg). The TLC elution was monitored with the standard ester mixture. The infrared spectrum recorded in solution (40 ug in CCl_4 0.5 mm cells) gave a value of 1.24 for the ratio of optical densities, $\frac{OD\ \gamma_{CO}}{OD\ \gamma_{CH2850}}$ (pure methyl stearate, 1.0), which would indicate the presence of a substantial amount of branched material.

The GLC pattern for the ester fraction is displayed in Fig. 7, Tracings A and B and shows a substantial amount of branched and cyclic

components. The distribution of fatty acid methyl esters is markedly different from that obtained without acid treatment of the rock (see Fig. 5, Tracings A and B).

An attempt was made to separate the branched/cyclic fraction from the normal ester fraction by the method of urea adduction (Dinerstein, quoted by Baron, 1961). The method consisted of adding the ester fraction (10 mg), to a urea solution (0.5 ml isooctane, 0.1 ml methanol, 100 mg urea) and mixing for 1 hour. This was followed by filtering and washing the adducted material with isooctane (0.2 ml) and decomposing the adduct with an aqueous solution of urea (0.3g in 0.2 ml water) at 90°C. The isolation was partially successful as can be seen from the GLC trace (Fig. 7, Tracing C) recorded on the branched cyclic fraction. Comparison of Tracing C with A shows that the normal esters have been removed to some extent. It was concluded that the quantity of esters used was insufficient for this particular separation technique.

A complex mixture of normal and branched fatty acids was isolated from Torbanite after acid treatment of the rock, and their methyl esters examined by TLC, GLC, and infrared spectroscopy.

4) Isolation of the total fatty acids by alkaline digestion of the Shale.

A comparison was made between the methods currently employed by other investigators (e.g. Lawlor and Robinson, 1965) for the isolation of fatty acids from rocks, and those adopted in the present work. Accordingly, powdered Torbanite (400g) was heated for 24 hours under reflux in 10% methanolic potassium hydroxide (600 ml). The suspension was filtered, the residue washed with methanol, and distilled water (600 ml) added to the filtrate.

The fatty acids were released from their potassium salts by acidifying the solution (to pH3) with hydrochloric acid (AR). Benzene (3 x 600 ml) was then added to the acidified solution to recover the fatty acids, was washed with small quantities of distilled water until acid-free and finally removed on a rotary evaporator. The fatty acid fraction was then esterified and chromatographed over alumina and finally on TLC silica (Fig. 4A) to give pure ester fraction (11 mg). The infrared spectrum recorded in solution was substantially the same as that obtained for the ester fractions in sections 1 & 2.

The GLC pattern is shown in Fig. 10, Tracing C. It appears that the higher acids (e.g. n-C_{18}) are not completely extracted from the shale, as compared with the ultrasonic extraction (sonitank) procedure. It is evident that the method of alkaline treatment of a rock for the isolation of the fatty acids, requires a much longer period than that specified by previous workers (Lawlor and Robinson, 1965, time reported was 18 hours) for the isolation of a larger quantity of fatty acids.

Scottish Oil-Shale.

A large chunk of the shale was hammered into 1 in. pieces, and the pieces washed ultrasonically (titanium soniprobe, Dawe Instruments) in benzene/methanol (3:1). The cleaned pieces were pulverised in the hammer mill (70% of a test sample passing a 200 mesh sieve) and finally in the disc mill. The powder (600g) was suspended in mixed hydrofluoric acid/hydrochloric acid (3:1, total 3.6 litres) for 10 days at $\sim 16^{\circ}$ with subsequent filtering, washing, drying and repulverizing.

The residue (200g) was extracted ultrasonically (sonitank) three times in batches (50g) in centrifuge bottles (250 ml) containing 160 ml benzene/methanol (1:1) per batch (total 2 litres). The suspension was centrifuged and the clear solution decanted and evaporated to yield a brown gum (1.2g) which revealed a carbonyl band of low intensity at 1700cm^{-1} when examined by infrared spectroscopy (thin film).

The free fatty acids were isolated from the total extract on a silicic acid/potassium hydroxide column (20g SiO_2, 20 ml standard isopropanol/KOH solution) followed by esterification and chromatography over alumina (3.5g neutral alumina). Elution first by n-hexane (15 ml) followed by benzene (150 ml) to recover the ester fraction yielded a crude ester fraction (4.5 mg). (The non-polar lipid eluate from the silicic acid column was examined for carbonyl absorption by removing the solvent and recording the infrared spectrum on a thin film. From the absence of carbonyl absorption around 1700-1740 cm^{-1} it was inferred that free esters were not present in the shale.)

Preparative TLC of the crude ester fraction (4.5mg) on a thick plate (1 mm) developed with 5% diethyl ether in n-hexane yielded a pure ester fraction (3 mg). The chromatogram is shown in Fig. 4A. The infrared spectrum recorded on 30/ug in solution (CCl_4, 0.5 mm cells) gave the ratio $\frac{OD\,\nu_{CO}}{OD\,\nu_{CH\ 2850}} = 0.8$, indicating that straight chain compounds were predominant in the ester fraction.

The GLC pattern for the esters is shown in Fig. 8, Tracings A and B. The internal standards used were n-C_{14}, n-C_{16} and n-C_{18} fatty acid methyl esters. The predominance of even numbered acids over odd numbered ones is evident. A rather striking predominance at the n-C_{12} position suggested

contamination at some point in the work up but this was subsequently rejected since none of the controls revealed a peak at this position.

The ester fraction was further gas chromatographed on a preparative scale (2mg) (Fig. 8, Tracing C). Fractions 1 to 14 were collected individually and fractions 2, 6 and 8 identified as the n-C_{12}, n-C_{16} and n-C_{18} fatty acid methyl esters respectively, by mass spectrometry (AEl).

The n-alkanoic acids isolated from a Scottish Oil Shale ranged from C_{10} to C_{29}. The methyl esters were examined by TLC, GLC and infrared spectroscopy. In addition the major components were identified by mass spectrometry as the n-C_{12}, n-C_{16} and n-C_{18} fatty acid methyl esters. Traces of branched acids were present (e.g. shoulder at n-C_{16}, Fig. 8A).

Scottish Oil-Shale Distillate.

The crude oil (15g), suspended in ether (30 ml) was added to a silicic acid column (40g SiO_2, 40 ml standard isopropanol/KOH solution) and the free fatty acids isolated by the usual method. Esterification was followed by chromatography over alumina (5g neutral alumina) with elution first by 20 ml n- hexane and then 100 ml benzene to recover the ester fraction. The crude ester fraction (8mg) was chromatographed on a thick plate (1 mm) with 5% diethyl ether in n-hexane as the developing agent. The purified ester fraction ($\sim$ 6mg) still contained a considerable quantity of yellow material, which further chromatography did not remove to any appreciable extent. The impurity fluoresced under U.V. light. It is possible that this fluorescent material was being oxidised by the air during chromatography on silica, forming a continuous streak on the developed plate (Fig. 4A). The ratio of optical densities $\frac{OD\,\nu_{CO}}{OD\,\nu_{CH2850}} = 0.6$ is rather low and can be explained by

the presence of impurities.

The GLC pattern for the esters, as shown in Fig. 9, Tracings A and B, is significant in that the major constituents had retention times corresponding to n-C_{16} and n-C_{18} fatty acid methyl esters when internal standards n-C_{14}, n-C_{16} and n-C_{18} esters were added. Branched acids were also present.

D'Arcy Oil.

The crude oil (20g) was added in ether (20 ml) to a silicic acid/potassium hydroxide column (30g SiO_2, 30 ml standard isopropanol/KOH solution) and the free fatty acids recovered in the usual manner. Esterification was followed by chromatography over alumina (5g, neutral alumina) with elution first by n-hexane (20 ml) followed by benzene (150 ml) to recover the ester fraction. The crude ester fraction (19 mg) was subsequently rechromatographed on a preparative scale (1 mm thick plate) with 5% diethyl ether in n-hexane as developing agent, to yield a purified ester fraction (16.7 mg). Fluorescence on the plate, when viewed under U.V. light, was negligible. The chromatogram is shown in Fig. 4A.

The infrared spectrum recorded in solution (40 ug in CCl_4, 0.5 mm cells) revealed an intense carbonyl absorption at 1740cm^{-1} and provided the value of 1.1 for the ratio of optical densities, $\frac{OD\gamma_{CO}}{OD\gamma_{CH2850}}$

The GLC pattern (Fig. 10, Tracing A) as well as displaying the usual fatty acid distribution with n-C_{16} and n-C_{18} fatty acid methyl esters as the major components (located by using the n-C_{14}, n-C_{16} and n-C_{18} fatty acids methyl esters as internal standards) also revealed a major peak eluted ahead of the n-C_{18} fatty acid methyl ester.

The ester fraction was further chromatographed on silver nitrate-impregnated silica to determine whether or not the unknown compound possessed unsaturation (Fig. 4B). The presence of a spot eluted behind the normal esters and with a position corresponding to methyl oleate indicated the presence of unsaturated material.

The ester fraction (6 mg) was subjected to preparative gas chromatography and fractions 1 to 13 were collected individually. Fractions 6 and 8 to 10 were examined by combined GC-MS (LKB 9000). The component in fraction 6 gave the anticipated fragmentation pattern for the n-C_{16} fatty acid methyl ester while fractions 8 and 9 consisted of the n-C_{18} fatty acid methyl ester. Fraction 10, however, gave the fragmentation characteristic of a n-C_{18} monoenoic acid methyl ester as reported by Hallgren, Ryhage and Stenhagen (1959). The mass spectrum is shown in Fig. 14A and the partial mass spectrum listed in Table 2. It compares favourably with the mass spectrum of methyl oleate (A.R., supplied by B.D.H.) which was also recorded on the LKB 9000 GC-MS. The partial mass spectrum for methyl oleate is given in Table 2.

The spectra show significant ionized fragments at m/e 264 (M-32), formally corresponding to the loss of methanol and at m/e 222(M-74) which Hallgren *et al* state to be due to the loss of methylene methoxycarbonyl, $-CH_2\ COO\ CH_3$ of mass 73, together with one hydrogen atom. The peak due to the parent molecule-ion at m/e 296 is relatively small. The mass spectra of mono-unsaturated normal fatty acids give no indication of the position of the double bond nor of the *cis* or *trans* nature of the isomers except in the case of *cis* and *trans* - $D^{2:3}$ isomers which differ from each other and those of esters in which the double bond is in the position 6:7 or beyond (Hallgren *et al*, 1959).

The infrared spectrum was examined more closely for information about the cis or trans nature of the double bond. A definite shoulder adjacent to the methyl stretching region between 3030 and 3010 cm^{-1} suggested the presence of unsaturation. However, the absence of significant absorption in the regions 1660-1620cm^{-1} and 1000-850cm^{-1} eliminated the possibility of a trans or vinyl double bond. Cis double bonds are known to possess very weak or no absorption in the region 1660-1620cm^{-1} (Eglinton, 1965), while the region 730-665cm^{-1} is masked by CCl_4. Absorption was detected using a thin film.

A plot of the relative retention times for the normal esters (relative to n-C_{14}) against carbon number from the gas chromatogram shown in Fig. 11, Tracing A, was found to be linear and the 'carbon number' for the n-C_{18} monoenoic methyl ester was estimated to be 18.20 from the graph. A similar linear plot was obtained for a mixture of n-C_{14}, n-C_{16} and n-C_{18} fatty acid methyl esters and methyl oleate, using identical GLC conditions and the value of the carbon number of methyl oleate was found to be 18.20.

On the basis of TLC (silver nitrate impregnated silica), infrared evidence, GLC retention time data and mass spectral analysis, the structure possessed by methyl oleate is tentatively assigned to the normal monoenoic fatty acid methyl ester isolated from D'Arcy Oil.

Also isolated from D'Arcy Oil was a series of n-alkanoic acids ranging from C_{10} to C_{24} along with minor quantities of branched acids. The methyl esters were examined by TLC, GLC and infrared spectroscopy. In addition, the major saturated normal acids were identified as n-C_{16} and n-C_{18} fatty acid methyl esters by mass spectral analysis.

Coorongite.

1) Isolation of the free fatty acids as methyl esters.

Coorongite (7.5g) was homogenised (MSE Micro Emulsifier) for 10 mins. on benzene/methanol (1:1, 50 ml). The suspension was then carefully transferred from the emulsifier to a centrifuge bottle (250 ml) by decantation, followed by several washings (3 x 30 ml, benzene/methanol, 1:1) which were added to the centrifuge bottle. The suspension was then extracted ultrasonically (sonitank) five times with benzene/methanol (1:1) total 750 ml. The clear solution obtained on centrifuging the suspension was decanted and removed on a rotary evaporator to give a brown gum (2g) which showed weak carbonyl absorption at $1700cm^{-1}$.

The free fatty acids were isolated on a silicic acid/potassium hydroxide column (30g SiO_2, 30 ml standard isopropanol/KOH solution) by the usual procedure. Esterification was followed by chromatography over alumina (5g, neutral alumina) with elution first by n-hexane (10 ml) followed by benzene (150 ml) to recover the methyl ester fraction. Preparative TLC of the crude ester fraction (9 mg) with 5% diethyl ether in n-hexane as developing agent afforded a purified ester fraction (7.5 mg). The chromatogram is shown in Fig. 4A. The infrared spectrum recorded in solution (40 ug in CCl_4, 0.5 mm cells) showed that the material was substantially straight chain. The ratio of optical densities $\frac{OD\,\nu_{CO}}{OD\,\nu_{CH2850}}$ was calculated to be 0.73. A closer examination of the spectrum revealed absorption at $1640cm^{-1}$ ($OD_{C=C}$ = 0.04) at $990cm^{-1}$ and $910cm^{-1}$ (OD_{CH_2} = 0.08) indicating the presence of vinyl double bonds.

Further TLC on silica impregnated with silver nitrate ($\sim$20 ug of ester fraction) with 5% diethyl ether in hexane as developing agent, monitored by methyl stearate, methyl oleate and n-C_{12} and n-C_{20} alkanes yielded a chromatogram which indicated the presence of unsaturated esters (Fig. 4B).

The GLC pattern for the ester fraction is shown in Fig. 10, Tracing B. Major peaks with retention times corresponding to n-C_{16}, n-C_{18} and n-C_{20} fatty acid methyl esters were located by coinjection of internal standards, n-C_{14}, n-C_{16}, n-C_{18} methyl esters. The ester fraction was further gas chromatographed on a preparative scale (4 mg) (Fig.11, Tracing B). Fractions 1 to 9 were collected individually and examined by combined GC-MS(LKB 9000). The marked difference between the GLC traces obtained on SE-30 and PEGA will be discussed later.

2) Identification of Individual Fatty Acid Methyl Esters.

(i) n-Alkanoic acids were identified by comparing the mass spectra so obtained with those reported in the literature (Ryhage and Stenhagen, 1959). Fractions 1,2,4,6,7,8 and 9, contained the n-C_{14}, n-C_{16}, n-C_{17}, n-C_{18}, n-C_{19}, n-C_{20} and n-C_{21} fatty acid methyl esters, respectively. The predominance of even-numbered acids over off numbered acids is evident, the n-C_{16} acid being the largest saturated constituent followed by the n-C_{18} acid. The other normal acids were present in relatively small amounts.

(ii) Branched acids. Small quantities of branched acids were found to be present in fractions 1,2,3,5 and 6, ranging from C_{14} to C_{18} and were identified from their mass spectra which were compared with the mass spectra reported by Ryhage and Stenhagen (1960) for iso and anteiso acids.

(iii) Monoenoic Acids. The most interesting constituents of the free fatty acid fraction isolated from coorongite as methyl esters, were mono-unsaturated acids ranging from C_{16} to C_{20}. These are designated compounds VI (C_{16}), VII (C_{17}), VIII (C_{18}), IX (C_{19}) and X (C_{20}) and were present in fractions 3,5,7,8 and 9, respectively. The mass spectra for compounds VI to X are shown in Figs. 14 to 16 and the partial mass spectra listed in Tables 3 and 4. The ionized fragments, which are characteristic of normal monoenoic acid methyl esters, are listed below. The spectra of the compounds VI to X are similar to that for methyl oleate (Table 2).

Compound	VI	VII	VIII	IX	X
Table No.	3	3	3	4	4
Fig. No.	14B	15A	15B	16A	16B
M - m/e	m/e	m/e	m/e	m/e	m/e
Base peak	74	74	74	55[1]	55[2]
Methylene Methoxy carbonyl ($CH_2\ CO_2\ CH_3$)	87	87	87	87	87
M-116	152	166	180	194	208
M-74 (loss of $CH_2CO_2CH_3$+H)	194	208	222	336	250
M-32 (loss of MeOH)	236	250	264	268	292
M, Parent Molecule-ion	268	282	296	310	324
No. of Carbon Atoms	16	17	18	19	20

1. m/e 74 is 75% of base peak.

2. m/e 74 is 60% of base peak.

The designation 'monoenoic' to compounds VI to X is consistent with the presence of the ionized fragments and parent molecule-ions listed above.

The 'carbon numbers' for compounds VI to X were obtained from the linear plot of relative retention time against carbon number for n-alkanoic acids (Fig. 17), (relative to the n-C_{14} fatty acid methyl ester) and are listed below:

Compound	VI	VII	VIII	IX	X
'Carbon No.'	16.6	17.6	18.7	19.7	20.7

since the carbon numbers are greater than for mono-unsaturated esters with the double bond in the middle of the carbon chain (e.g. methyl oleate, 'carbon number' 18.20, using the same GLC conditions described in the previous section, D'Arcy Oil), it was thought that the unsaturation might be located further away from the methoxy carbonyl group. In addition to the above finding, a plot of the actual number of carbon atoms for compounds VI to X (as determined from the parent molecule-ion in the mass spectrum) against the relative retention times (relative to the n-C_{14} ester) yielded a linear plot, parallel to that obtained for the n-alkanoic acids. This suggested a series of monoenoic acids of similar structure. The presence of vinyl absorption in the infrared spectrum (no trans absorption present) further suggested that the double bond in each of the monoenoic acids is located at the terminal position. Hallgren et al (1959) reported that a shift of double bond to the terminal position has little effect on the mass spectrum.

Several workers have studied the behaviour of positional isomers of methyl octadecenoate when subjected to gas chromatography on polar and non-polar phases (APL & PEGA). James (1959) found that the retention time increases as the double bond moves from the central 9- position towards the methoxy - carbonyl end. The positional isomers examined were methyl 9- octadecenoate, methyl

6 - octadecenoate and methyl 4- octadecenoate and the liquid phase was PEGA. Scholfield et al (1961), on the other hand, reported that as the double bond moves towards the methyl end, the retention time increases. The order of elution was methyl 9- octadecenoate, methyl 12- octadecenoate, methyl 15- octadecenoate and this order was preserved when the phase was charged from EGS polyester to APL. Ackman and Burgher (1963) reported the relative retention times (relative to methyl 9- octadecenoate) for the 9-, 12- and 15- positional isomers (received in a personal communication from Scholfield), and these are given below:

Compound	Relative Retention Time †
Methyl 9- Octadecenoate	1.00
Methyl 12- Octadecenoate	1.04
Methyl 15- Octadecenoate	1.10

† Liquid phase was EGS

On the basis of the findings of the above authors it is suggested that as the double bond moves to the terminal position the relative retention time would increase still further. The large relative retention times for the monoenoic acids isolated as the methyl esters from coorangite, coupled with vinyl absorption in the infrared spectrum, can be explained by the presence of a terminal double bond in each of the isolated acids.

The structures tentatively assigned to compounds VI to X on the basis of an examination of the methyl esters by TLC (silver nitrate), GLC, infrared spectroscopy and mass spectral analysis are shown below:

No.	Structure	Approx %
VI	$CH_3OCO(CH_2)_n CH = CH_2$ (n = 13)	1.6
VII	$CH_3OCO(CH_2)_n CH = CH_2$ (n = 14)	3.6
VIII	$CH_3OCO(CH_2)_n CH = CH_2$ (n = 15)	19.7
IX	$CH_3OCO(CH_2)_n CH = CH_2$ (n = 16	10.4
X	$CH_3OCO(CH_2)_n CH = CH_2$ (n = 17)	20.7

Green River Shale.

1) Isolation of the free fatty acids as methyl esters.

A. Sample from the 1100 ft. level.

The pre-powdered shale (200g, 80% of a test sample passing through a 200 mesh sieve) was further pulverized in the disc mill for 15 mins. The powder was then suspended in hydrofluoric acid/hydrochloric acid (3:1, total 1200 ml) for 24 hours at $\sim 16^{\circ}$, with subsequent filtering, washing, drying and repulverizing. The residue (90g) was extracted ultrasonically (sonitank) three times in batches (30g) in centrifuge bottles (250 ml) containing 160 ml benzene methanol (1:1) per batch (total 1500 ml). The suspension was centrifuged and the clear solution decanted and evaporated to yield a brown gum (5g), which showed carbonyl absorption in the infrared spectrum (thin film,

$$\frac{OD_{\nu\,CO}}{OD_{\nu\,CH2850}} = 0.3)$$

The free fatty acid fraction was then isolated from the total extract on a silicic acid/potassium hydroxide column (30g SiO_2, 30 ml standard isopropanol/KOH solution. Esterification and alumina chromatography (5g neutral alumina) with elution first by n-hexane (20 ml) and then benzene (180 ml), furnished the impure ester fraction (14.8 mg).

The non-polar fraction which had been eluted from the original silicic acid/KOH column showed a carbonyl peak of low intensity ($OD_{CO} = 0.1$, $OD_{CH2850} = 0.8$) indicating the presence of free esters in the shale.

Preparative TLC of the crude ester fraction (14.8 mg) on a thick plate (1 mm) developed with 5% diethyl ether in n-hexane yielded a pure ester fraction (12.5 mg). The TLC chromatogram is shown in Fig. 4A. The infrared

spectrum of the ester fraction was recorded on 60 ug in solution (CCl_4 0.5 mm cells) and revealed carbonyl absorption at 1740cm^{-1} $\left(\frac{OD\gamma_{CO}}{OD\gamma_{CH2850}} = 1.49\right)$. The spectrum (Spec 3) indicated a substantial amount of methyl branching.

The GLC pattern for the pure ester fraction is shown in Fig. 12, Tracings A and B. The internal standards used were the n-C_{14}, n-C_{16} and n-C_{18} fatty acid methyl esters. Tracing A (SE-30) reveals a more complex pattern than Tracing B(PEGA). Although in the present study the n-alkanoic acids have not been separated from the branched and cyclic acids, there is an obvious predominance (Tracing A) of the even numbered normal acids over the odd numbered normal acids as has been reported by Lawlor & Robinson (1965) for the total fatty acids extracted from a sample from the Mahogany Zone (less than 800 ft. from the surface) of the same formation.

Fig. 12, Tracing C, represents a further gas chromatogram of the ester fraction on a preparative scale (5 mg) on PEGA. Fractions 1 to 15 were collected individually and cuts 3 to 9 were subjected to combined gas chromatography-mass spectrometry (10ft. x 1/8 in., packed column with 1% SE-30 as liquid phase). Each fraction contained several components which were cleanly resolved on SE-30. The main component in cuts 3 to 6 and 9 was the n-alkanoic acid (n-C_{12}, n-C_{13}, n-C_{14}, n-C_{15}, and n-C_{18} fatty acid methyl esters in cuts 3 to 6 and 9 respectively), identified by the presence of the base peak at m/e = 74 and a peak due to the acylium ion (m/e = M-31, -OC(CH_2)n CH_3). Although the normal acids n-C_{16} and n-C_{17} were present in substantial amounts in cuts 7 and 8 respectively, the main component in cut 7 was a saturated C_{19} branched acid and in cut 8, a saturated C_{20} branched acid (designated XVA and XVIA respectively). Gas chromatography of the ester fraction on SE-30

(Fig. 12, Tracing A) gave the ester of the C_{19} branched acid as a partially resolved band ahead of the methyl ester of the n-C_{17} acid while the ester of the C_{20} branched acid appeared ahead of the ester of the n-C_{18} acid. Gas chromatography of the ester fraction on PEGA showed an earlier appearance of the C_{19} and C_{20} branched esters, the former being eluted prior to the n-C_{16} methyl ester while the latter coincided with the n-C_{17} methyl ester. These observations, combined with the high molecular weight obtained from the mass spectra, suggested that compounds XVA and XVIA might be the methyl esters of multibranched acids. In addition to the presence of the C_{19} and C_{20} multibranched acids in cuts 7 and 8, cuts 3 to 6 and 9, also showed the presence of fatty acid methyl esters of the multibranched variety on mass spectral examination of each cut (designated hereinafter as compounds XIA, XIIA, XIIIA, XIVA and XVIIA respectively).

B. Sample from the 1900 ft. Level.

The procedure for isolation of the free fatty acids from the sample obtained from the 1900 ft. level, was identical to that carried out on the 1100 ft. sample. The powdered shale (200g) on acid treatment yielded a residue (120g) which on solvent extraction afforded a brown gum (3g). The free fatty acids isolated from the total extract according to the method of McCarthy and Duthie (1962), followed by esterification, and column and preparative TLC afforded a pure methyl ester fraction (3.5 mg). A typical TLC chromatogram is shown in Fig. 4A. The infrared spectrum recorded in solution (40 ug in CCl_4, 0.5 mm cells) showed less methyl branching than the 1100 ft. level, the ratio of optical densities for ν CO and νCH2850 being 1.09.

Gas chromatography gave the representative traces shown in Fig. 13, Tracings A and B. The predominance of even numbered over odd numbered acids is again evident, but the quantity of branched acids of the isoprenoid type has been considerably reduced.

Preparative gas chromatography of the ester fraction (2 mg) yielded the trace shown in Fig. 13, C. Fractions 1 to 14 were collected individually and fractions 2 to 8 were subjected to the combined GC-MS procedure. Mass spectra of the individual components of each cut confirmed the presence of n-alkanoic acids ranging from C_{12} to C_{18} and also the series of isoprenoid acids already isolated from the 1100 ft. sample. The latter compounds exhibit the same behaviour on gas chromatography as described previously and are designated compounds XIB to XVIIB, respectively.

The compound constituting the major portion of cut 2 and thought to be a contamination product, was shown by mass spectral analysis to be the n-C_{12} fatty acid methyl ester. Contamination, however, was ruled out as an explanation for the appearance of this peak by GLC, since the control used during the work up of the samples did not exhibit a peak by GLC at the n-C_{12} ester position.

2). Identification of Individual Fatty Acid Methyl Esters from the 1100 ft. and 1900 ft. levels.

(i) The n-alkanoic acids present in both levels were identified by comparing the mass spectra so obtained with those reported in the literature (Ryhage & Stenhagen, 1959), thus establishing the anticipated number of carbon atoms inferred from coinjection of the authentic n-C_{14}, n-C_{16} and n-C_{18} fatty

acid methyl esters during gas chromatography. Cuts 3 to 9 from the 1100 ft. sample contained the n-C_{12} to n-C_{18} fatty acid methyl esters respectively, while cuts 2 to 8 from the 1900 ft. sample contained the n-C_{12} to n-C_{18} fatty acid methyl esters.

In addition to the normal acids examined by mass spectrometry, gas chromatography indicated the presence of n-alkanoic acid ranging from C_{10} to C_{29} in the 1100 ft. sample and from C_{11} to C_{30} in the 1900 ft. sample.

(ii) Isoprenoid Acids

(a) C_{14} isoprenoid acid methyl ester (XIA and XIB)

The structure assigned to compounds XIA and B is shown in Fig. 18A and the partial mass spectra are given in Table 5. The parent molecule-ion was found at m/e 242, indicating a saturated C_{14} methyl ester. The positions of the methyl branching were established by the presence of peaks due to the following ionized fragments:-

m/e	M-m/e	Assignment	Position of Methyl Substitution
88	M-138	$CH_3CH = C(OH)OCH_3$ rearrangement ion formed by 2, 3 cleavage	2-
129	M-113	$CH_3OCO\,CH(CH_2)_3\,\underset{CH_3}{\overset{}{]}}^{+\cdot}$	6-
157	M-85	$CH_3OCO\,CH(CH_2)_3\,\underset{CH_3}{\overset{CH_3}{]}}^{+\cdot}$ (bracketing a low intensity peak at m/e 143)	
125	M-117	Ketene fragment m/e (157-32) where 32 is formally the loss of methanol	6-
195	M-47	Ketene fragment	10-
152	M-90	$C_{11}H_{20}$ di-unsaturated hydrocarbon fragment	2- 6-

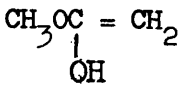
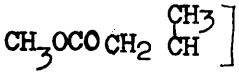
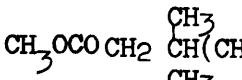
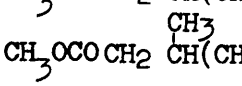
Ryhage and Stenhagen (1960) have reported that esters containing a methyl substituent in the 6- position and having no α- substituent have a prominent peak at M-76 (the loss of a rearranged ion and two hydrogen atoms) with methyl substituents in the 2- and 6- positions this peak would appear at m/e - M-90. A small M-65 ion supported the conclusion that the end of the chain was an isopropyl group. This peak has been reported in compounds possessing an end isopropyl group (e.g. Hansen and Morrison, 1964). The above are consistent with the presence of branching in the 2-, 6- and 10- positions. The compound has, therefore, been assigned the formula Methyl 2, 6, 10-trimethylundecanoate.

This acylic acid of isoprenoid structure has recently been isolated from a California petroleum by Cason and Graham (1965). Although the partial mass spectrum was not illustrated in their paper, it was reported that the complete mass spectrum of the isolated isoprenoid acid ester was very similar to that of an authentic sample synthesised by them. However, the significant ionized fragments were reported as follows: the base peak at m/e 88; m/e 129 (4.7%) and 157 (5.3%), bracketing a low intensity peak at m/e 143 (0.3%), indicating methyl branching at the 6- position; m/e 152 (3.3%); parent molecule-ion at m/e 242. The findings in the present study compare favourably with the findings of the above authors.

(b) C_{15} isoprenoid acid methyl ester (XIIA and XIIB); tetrahydro-farnesanic acid.

The structure assigned to compounds XIIA and B is shown in Fig. 18B and the partial mass spectra listed in Table 5. The parent molecule-ion at m/e 256 indicates a saturated C_{15} methyl ester. The positions of methyl

substitution were established as follows:

m/e	M-m/e	Assignments	Position of Methyl Substitution
74	M-182	rearrangement peak  due to 2,3 cleavage	2- absent
101 (basepeak)	M-155		3-
143	M-113		7-
171	M-85	 (bracketing a low intensity peak at m/e 157)	
139	M-117	ketene fragment m/e (171-32)	7-
191	M-65	loss of isopropyl group	11-
209	M-47	ketene fragment [(M-15) - 32]	11-

On the basis of the above assignments it is suggested that the formula for compounds XIIA and B is methyl 3,7,11 trimethyldodecanoate.

Cason and Graham (1965) in the course of investigating the naphthenic acids in the California petroleum, identified this C_{15} isoprenoid acid. The partial mass spectrum of the synthetic methyl ester is recorded in Table I of their report and includes the following significant peaks; base peak at m/e 101 (100%); m/e 143 (5%), and 171 (5.0%), bracketing a low intensity peak at 157 (0.2%), indicating methyl substitution at the 6- position; m/e 191 (M-65), loss of isopropyl group (0.4%); parent molecule-ion at m/e 256 (2.6%).

The near identity of the partial fragmentation patterns of the isolated esters and the synthetic sample quoted by Cason and Graham (1965) established the naturally occurring acids as having the C_{15} isoprenoid structure.

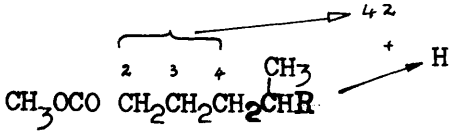
(c) C_{16} isoprenoid acid methyl ester (XIIIA and XIIIB)

The structure assigned to compounds XIIIA and B on the following basis of their mass spectra is given in Fig. 19A and the partial mass spectra recorded in Table 6. The parent molecule-ion was at m/e 270, the value for a saturated C_{16} methyl ester. Ryhage and Stenhagen (1960) report that in the mass spectrum of a 4- methyl substituent fatty acid ester base peak occurs at m/e 87 due to ions formed by simple 3, 4 cleavage. The fact that there is only one hydrogen on carbon atom four does not account for m/e 74 being only 50% of the base peak. The mass spectrum is produced by a set of competing decomposition reactions. The peak at m/e 87 occurs because of a more favoured process.

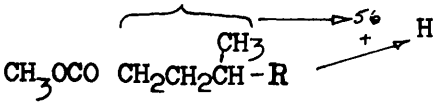
For this isoprenoid acid to possess branching at carbon atom 8, relatively intense peaks would be expected to occur at m/e 157 and 185 bracketing a low intensity peak at m/e 171. Ryhage and Stenhagen (1960) however, found that the mass spectrum of methyl 8- methyloctadecanoate had a larger peak at m/e 143 (in fact the base peak), corresponding to the ion formed by cleavage of the chain on the methoxycarbonyl side of carbon atom 8. The peak due to the ionized fragment, $CH_3OCO\,(CH_2)_6\,\overset{CH_3}{CH}\,]^{+}\cdot$ m/e 171, was unexpectedly small. The peak at m/e 139 (171-32), the ketene fragment produced by the formal loss of methanol, was of appreciable height (27%). This finding of the above authors supports the assignment of a methyl group to the 8- position in the C_{16} acid methyl ester isolated in the present study. The peak at m/e 157,

$CH_3OCO(CH_2)_2\overset{CH_3}{\underset{|}{C}}H(CH_2)_3$ is present ($\sim$ 14%), while the peak at m/e 185 is of low intensity (1.2%). On the other hand the ketene fragment $O{=}C{=}CH\ CH_2\overset{CH_3}{\underset{|}{C}}H(CH_2)_3\overset{CH_3}{\underset{|}{C}}H]^+$ is present at m/e 153 (7.5%). This is sufficient evidence for a methyl substituent in the 8- position.

Finally the peak at m/e 213 (M-57) almost eclipses m/e 227 (M-43) Ryhage and Stenhagen (1960) report that on the basis of the study of deuterium substituted esters, these ions are formed by a complex process involving double cleavage and recombination of fragments. The formation of the ion at m/e = M-43 involves the loss of methylene groups 2, 3 and 4 together with one hydrogen atom:



When the methyl substitution is in the 4- position a three-carbon fragment is not easily removed because carbon atom 4 will carry with it the methyl side chain. Thus in this case we observe m/e = M-57



Summarizing the assignments made (see Table 6).

m/e	M-m/e	Assignments	Position of Methyl Substitution
87	M-183	$CH_3OCO(CH_2)_2\overset{+}{\cdot}$ 3,4 cleavage	4-
115	M-155	$CH_3OCO(CH_2)_2\overset{CH_3}{\underset{|}{C}}H^{+\cdot}$ 4,5 cleavage	
213	M-57	loss of C_4H_9	4-
157	M-113	$CH_3OCO(CH_2)_2\overset{CH_3}{\underset{|}{C}}H(CH_2)_3]^{+\cdot}$	8-
185	M-85	$CH_3OCO(CH_2)_2\overset{CH_3}{\underset{|}{C}}H(CH_2)_3\overset{CH_3}{\underset{|}{C}}H]^{+\cdot}$	
153	M-117	ketene fragment 185-32	8-
219	M-65	end isopropyl	12-
223	M-47	ketene fragment [(M-15)-32]	12-

There is, in addition, a distinct C_{14} alkyl type peak at m/e 197(M-73) due to ions formed by 2,3 cleavage. This supports the presence of a 4- methyl group.

The occurrence of this acid, the formula of whose ester is proposed to be methyl 4, 8, 12- trimethyltridecanoate, has not been previously reported in the literature.

(c) C_{17} isoprenoid acid methyl ester (XIVA and XIVB)

The mass spectrum and the assigned structure for compounds XIVA and B are given in Fig. 19B and Table 6. The parent molecule-ion occurs at m/e 284 and the base peak at m/e 74. Methyl branching occurs at the following positions:

m/e	M-m/e	Assignments	Position of Methyl Substitution
101	M-183	$CH_3OCO(CH_2)_3]^+$	5-
129	M-155	$CH_3OCO(CH_2)_3\underset{\displaystyle CH_3}{CH}]^+$	
	bracketing a low intensity ion at m/e 115		
171	M-113	$CH_3OCO(CH_2)_3\underset{\displaystyle CH_3}{CH}(CH_2)_3]^+$	
199	M-85	$CH_3OCO(CH_2)_3\underset{\displaystyle CH_3}{CH}(CH_2)_3\underset{\displaystyle CH_3}{CH}]^+$	9-
167	M-117	ketene fragment 199-32	9-
237	M-47	ketene fragment [(M-15)-32]	13-
241	M-43	$CH_3OCO + [\underset{\displaystyle CH_3}{CH}(CH_2)_3\,2\underset{\displaystyle CH_3}{CH}\,CH_3]$ (loss of C_3H_7)	5-

This compound is designated 5, 9, 13- trimethyltetradecanoate and has not been reported in the literature as having been isolated from a natural source.

(e) C_{19} isoprenoid acid methyl ester (XVA and XVB); nor-phytanic acid methyl ester.

Fig. 20A displays the mass spectrum of compounds XVA and B, while the partial mass spectra are given in Table 7. The parent molecule-ion at m/e 312 indicates a saturated C_{19} methyl ester. The presence of the following ionized fragments establishes the position of the methyl branching:

m/e	M-m/e	Assignments	Position of Methyl Substitution
88	M-224	rearrangement ion formed by 2, 3 cleavage	2-
129	M-183	$CH_3OCO\left[\begin{matrix}CH_3\\ CH(CH_2)_3\end{matrix}\right]^+$	
157	M-155	$CH_3OCO\left[\begin{matrix}CH_3 & CH_3\\ CH(CH_2)_3 & CH\end{matrix}\right]^+$	6-
	(bracketing a low intensity peak at m/e 143)		
125	M-187	ketene fragment (157-32)	6-
199	M-113	$CH_3OCO\left[\left\{\begin{matrix}CH_3\\ CH(CH_2)_3\end{matrix}\right\}_2\right]^+$	
227	M-85	$CH_3OCO\left[\left\{\begin{matrix}CH_3\\ CH(CH_2)_3\end{matrix}\right\}_2\begin{matrix}CH_3\\ CH\end{matrix}\right]^+$	10-
195	M-117	ketene fragment (227-32)	10-
222	M-90	$C_{16}H_{30}$ di-unsaturated hydrocarbon fragment	2- 6- 10-
247	M-65	end isopropyl group	14-
265		ketene fragment [(M-15)-32]	14-

The mass spectral analysis would suggest the compound methyl 2, 6, 10, 14- tetramethylpentadecanoate. This compound has previously been isolated from butterfat by Hansen and Morrison (1964) and from a California petroleum by Cason and Graham (1965) and unambiguously identified in both cases. The mass spectra obtained in the present study compare well with that reported by Hansen and Morrison.

(f) C_{20} isoprenoid acid methyl ester (XVIA and XVIB); phytanic acid methyl ester.

The structure assigned to compounds XVIA and B is shown in Fig. 20B and the partial mass spectrum recorded in Table 7. The parent molecule-ion at m/e 326 indicates a saturated C_{20} methyl ester. The positions of methyl substitution were established by the presence of the following ionized fragments:

m/e	M-m/e	Assignments	Position of Methyl Substitutiom
74	M-252	rearrangement ion 73 +1	2- absent
101 base peak	M-225	ion formed by 3,4 cleavage	3-
143	M-183	$CH_3OCO\,CH_2\left[CH(CH_2)_3\right]^+$	7-
171	M-155	$CH_3OCO\,CH_2\left[CH(CH_2)_3CH\right]^+$	
		(bracketing a low intensity peak at m/e 157)	
139	M-187	ketene fragment (171-32)	7-
213	M-113	$CH_3OCO\,CH_2\left\{CH(CH_2)_3\right\}_2^+$	11-
241	M-85	$CH_3OCO\,CH_2\left\{CH(CH_2)_3\right\}_2CH^+$	
		(bracketing a low intensity peak at 227)	
209	M-117	ketene fragment 241-32	11-
279	M-47	ketene fragment [(M-15)-32]	15-

The compound appears to be methyl 3, 7, 11 15- tetramethylhexadecanoate and the mass spectrum is similar to that obtained from the C_{20} acid methyl ester isolated from butterfat and identified by Hansen, Shorland and Morrison

(1965). Cason and Graham (1965) also reported the isolation of this acid from a California petroleum and identification of the acid as its methyl ester by mass spectral analysis but do not report the actual values for peak intensities.

(g) C_{21} isoprenoid acid methyl ester (compounds XVIIA and XVIIB)

The structure assigned to the C_{21} acid ester is shown in Fig. 21 and the partial mass spectrum recorded in Table 8. Branching due to methyl substituents is tentatively established by the presence of the following ionized fragments:-

m/e	M-m/e	Assignments	Position of Methyl Substitution
87 base peak	M-253	$CH_3OCO\ (CH_2)_2\ ]^+$	4-
115	M-225	$CH_3OCO\ (CH_2)_2\overset{CH_3}{\underset{|}{CH}}\ ]^+$	
283	M-57	$CH_3OCO + \{(CH_2)_2\overset{CH_3}{\underset{|}{CH}}\}_3CH_3$ (M-43 peak was negligible)	4-
157	M-183	$CH_3OCO\ (CH_2)_2\overset{CH_3}{\underset{|}{CH}}(CH_2)_3\ ]^+$	8-
185+	M-155	$CH_3OCO\ (CH_2)_2\overset{CH_3}{\underset{|}{CH}}(CH_2)_3\ -\overset{CH_3}{\underset{|}{CH}}\ ]^+$	
153	M-187	ketene fragment (185-32)	
227	M-113	$CH_3OCO(CH_2)_2\{\overset{CH_3}{\underset{|}{CH}}(CH_2)_3\}_2\ ]^+$	12-
255	M-85	$CH_3OCO(CH_2)_2\ \overset{CH_3}{\underset{|}{CH}}(CH_2)_3\}_2\overset{CH_3}{\underset{|}{CH}}\ ]^+$ (bracketing a low intensity peak at 241)	
223	M-117	ketene fragment 255-32	12-
293	M-47	ketene fragment [(M-15)-32]	16-
267	M-73	C_{19} alkyl type due to 2,3 cleavage	4-

+ This is a low intensity peak (see 'C_{16} isoprenoid acid', section ii c).

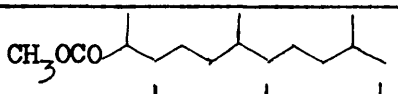
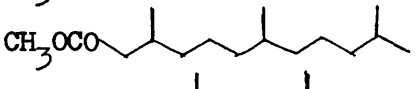
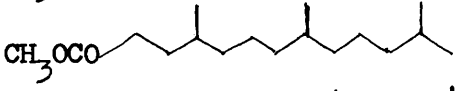
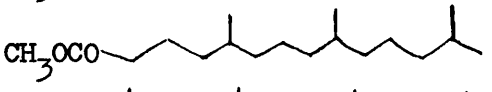
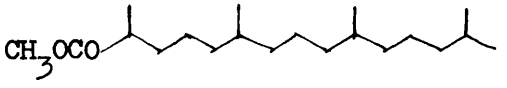
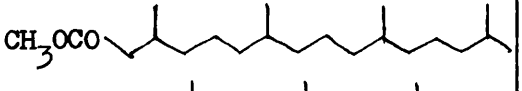
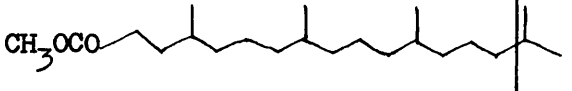
The compound appears to be methyl 4,8,12,16- tetramethylheptadecanoate and has not been reported previously.

Individual variations in the peak heights between the two spectra of the same compound requires explanation. The LKB 9000 GC-MS scans from the low mass to the high mass end in a relatively short time ($\sim$4-10 secs.); the time adopted in the present study was about 4.5 sec. This means that the concentration of a particular compound eluted from the column will have decreased considerably as the high mass end is being scanned. This might explain the difference betwen the peak heights for the parent molecule-ions of compounds XIVA and XIVB (Table 6) and for compounds XVIIA and XVIIB (Table 8). The temperature of the ion source is another variable affecting the relative intensity of the parent molecule-ion. Furthermore, since the concentration of a sample is constantly changing as a particular peak is being scanned it is impossible to obtain a constant sample pressure. Differences such as are observed in Table 5 for the M-29 and M-15 ion fragments of compounds XIA and B can be explained by the variation in the sample pressure, especially if the amount of the sample present is small. The short term gain of the electron multiplier plus the low sensitivity at fast scan will also contribute to the variation in the intensities of individual peaks.

Although the mass spectrometric identification is adequate for the recognition of the carbon skeletons of these isoprenoid acids it does not provide any information about the configurations at the carbon atoms containing the methyl branching.

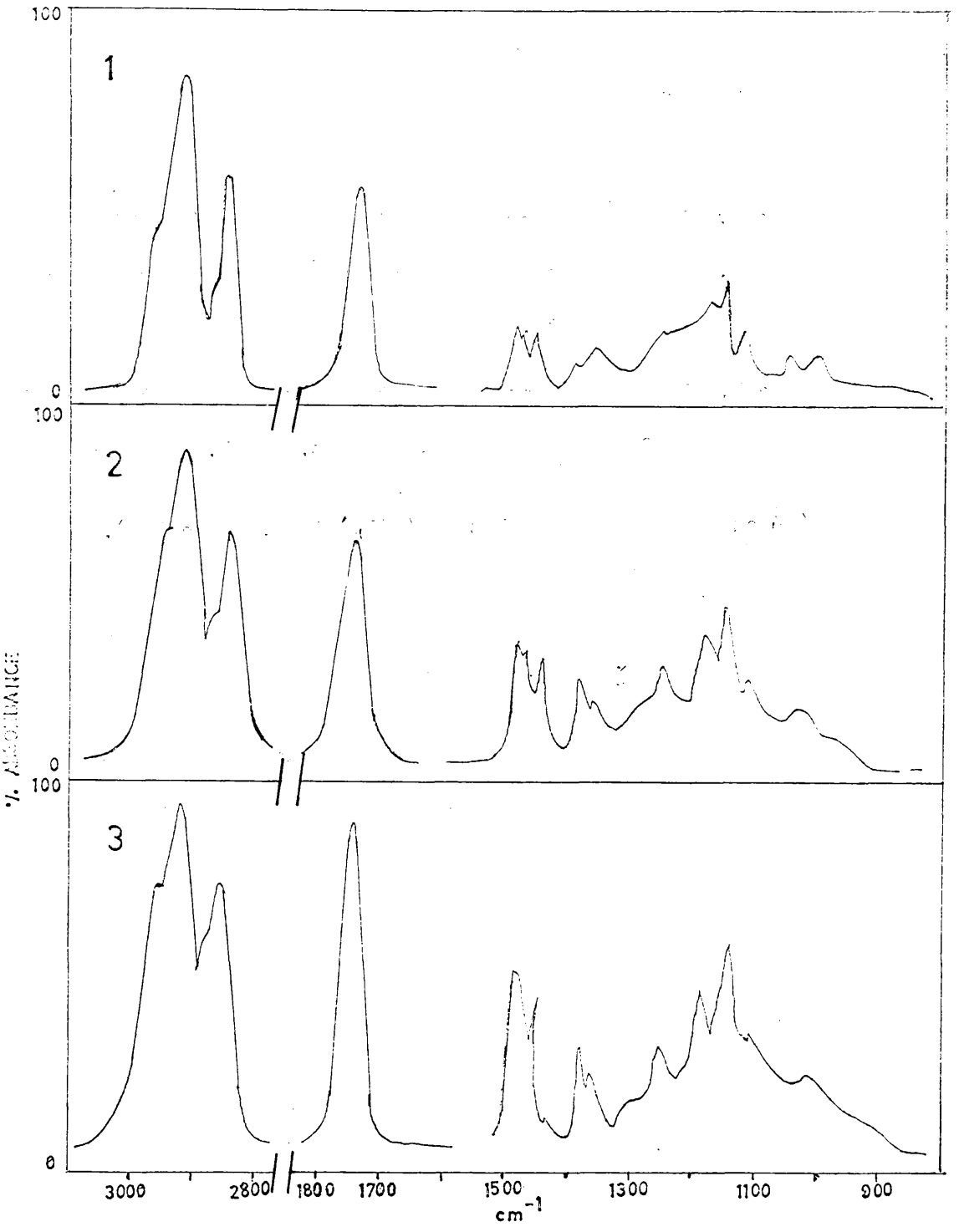
There was an insufficient quantity of raw shale from both the 1100 ft. and 1900 ft. levels to effect the isolation of the quantities of the individual isoprenoid acids needed for infrared and nuclear magnetic resonance

spectroscopy. This would have enabled a more rigorous analysis to be carried out on the isoprenoid acids in order to establish their structures unambiguously. The structures assigned to compounds XI to XVII are given below along with the approximate percentage of the fatty acid fraction.

Compound No.	Structure	Approx %	
		1100 ft. level	1900 ft. level
XI		2.8	2.0
XII		6.7	3.3
XIII		3.7	3.7
XIV		2.8	2.6
XV		7.9	3.7
XVI		9.6	4.0
XVII		2.0	1.6

(iii) Other branched and cyclic acids.

The mass spectra of other components of the fractions subjected to the GC-MS procedure suggested the presence of iso, anteiso and cyclic acids. The interpretation of the spectra was ambiguous as the concentration of these components was small.



Spectra 1, 2, 3. (For figure legends see reverse side of diagrams).

Spec 1. Infrared spectrum of methyl stearate (99.8%)

Spec 2. Infrared spectrum of fatty acid methyl ester fraction ex Torbanite.

Spec 3. Infrared spectrum of fatty acid methyl ester fraction ex Green River Shale, 1100 ft. level.

(Spectra recorded in solution (CCl_4) in 0.5 mm cells).

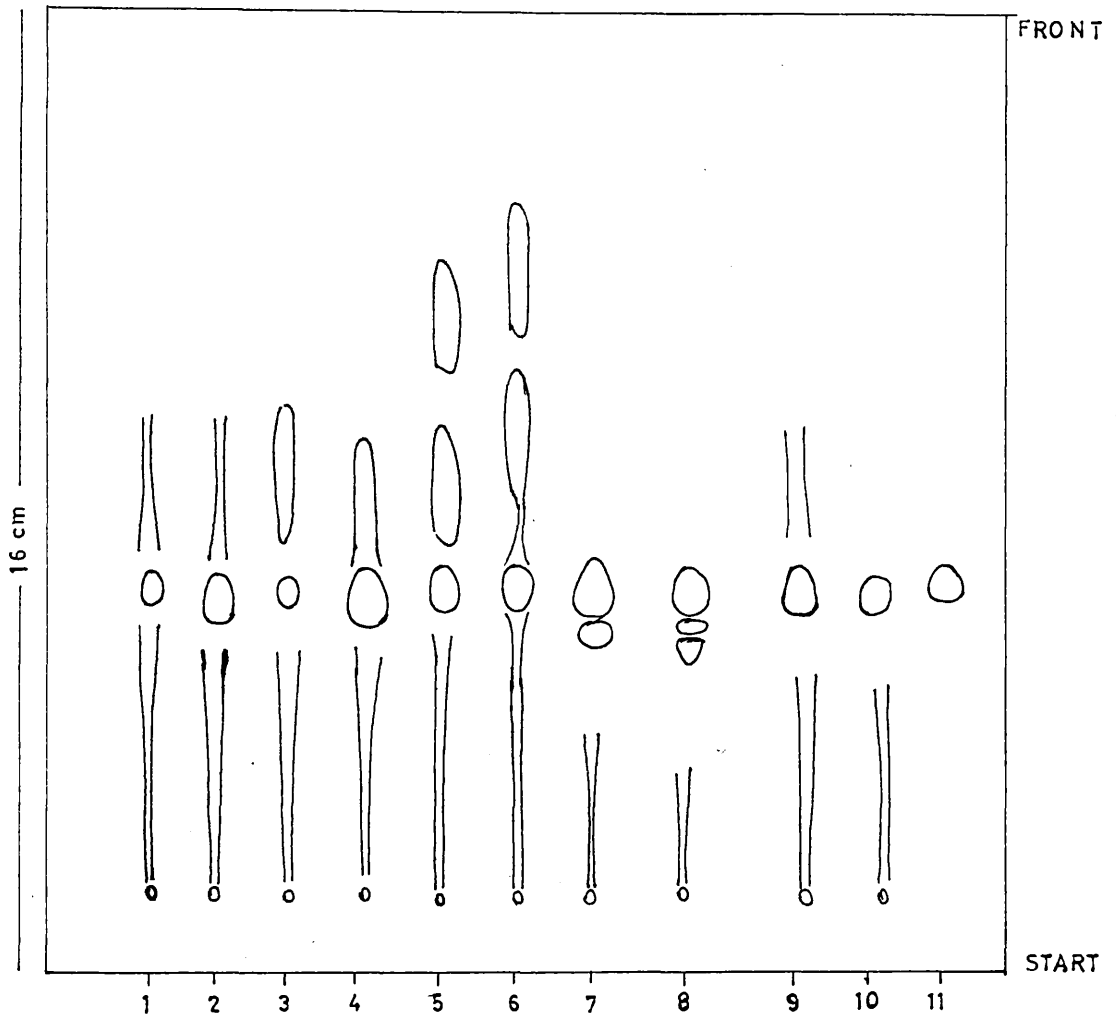


Fig. 4A. Chromatograms on silica (TLC) of methyl esters isolated from various geological samples. Solvent system: hexane (95%), diethyl ether (5%)

1. Methyl esters from Torbanite.
2. Methyl esters from Torbanite; ex HF, MeOH/HCl on shale.
3. Methyl esters from Torbanite; ex HF.
4. Methyl esters from Scottish Oil-Shale.
5. Methyl esters from Torbanite; ex MeOH/KOH on shale.
6. Methyl esters from Scottish Oil-Shale Distillate.
7. Methyl esters from D'Arcy Oil.
8. Methyl esters from Coorongite.
9. Methyl esters from Green River Shale, 1100 ft.
10. Methyl esters from Green River Shale, 1900 ft.
11. Standard methyl esters ($n\text{-}C_{18}$, $n\text{-}C_{24}$, $n\text{-}C_{30}$)

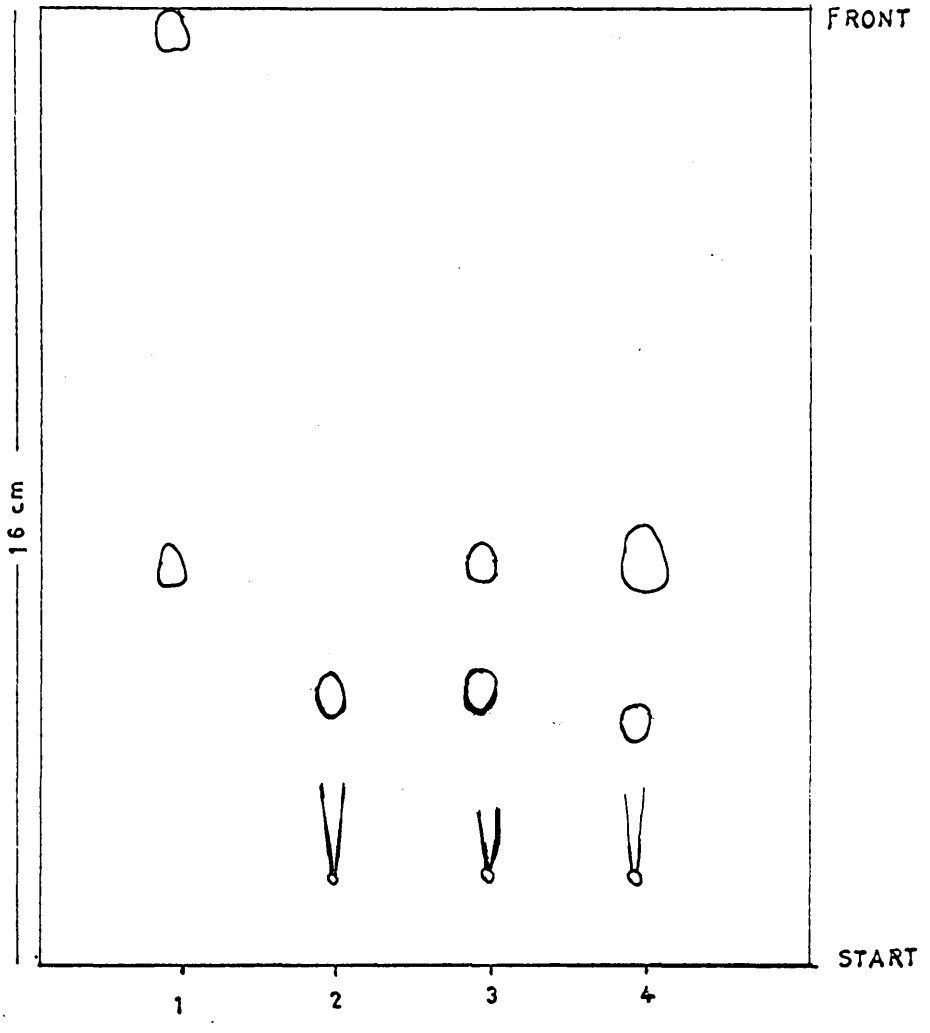
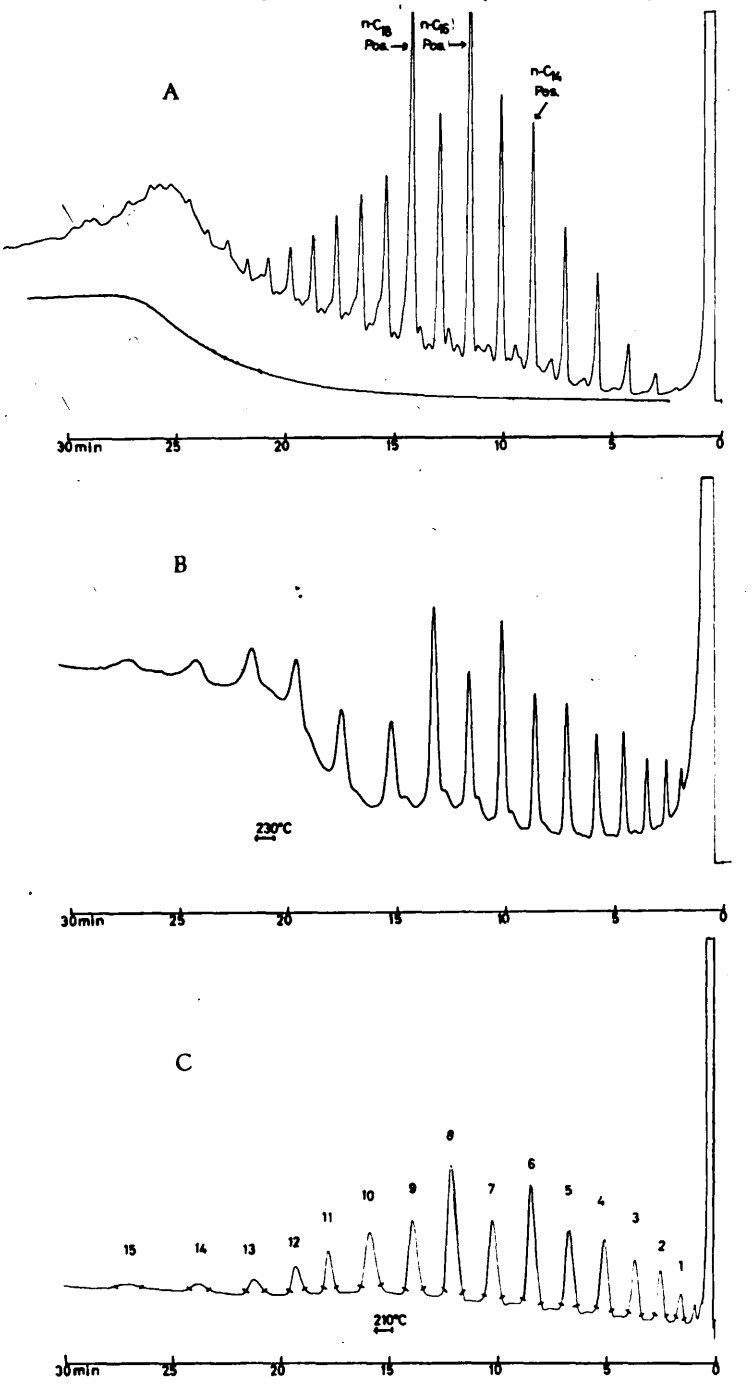


Fig. 4B. Chromatograms on silica (impregnated with silver nitrate) of methyl esters from several geological samples. Solvent system: hexane (95%), diethyl ether (5%).

1. Methyl stearate and n-C_{20} alkane.
2. Methyl oleate.
3. Methyl esters from D'Arcy Oil.
4. Methyl esters from Coorongite.

Torbanite-Torbane Hill (Carboniferous)-Fatty acids as methyl esters



Figs. 5 A,B,C (For figure legends see reverse side of diagrams)

Fig. 5

Gas Chromatograms of methyl esters of free fatty acids from Torbanite. Column conditions:-

(A) 6 ft. x $\frac{1}{8}$ in., 3% SE-30 on 100-120 mesh Gas Chrom P(DMCS); 30 ml/min. nitrogen; temperature programmed at 4°/min. from 125° to 300°, injector temperature 280°C.

(B) 6 ft. x $\frac{1}{8}$ in., 10% PEGA on 100-120 mesh Gas Chrom P(DCMS); 30 ml/min. nitrogen; temperature programmed at 5°/min. from 150° to 230°, injector temperature 280°C.

(C) 6 ft. x $\frac{1}{4}$ in., 10% PEGA on 100-120 mesh Chromosorb W(OCMS); 60 ml/min. helium; temperature programmed at 4°/min. from 140° to 210°, injector temperature 265°. Sample size was 3 mg of esters in 40 ul of benzene.

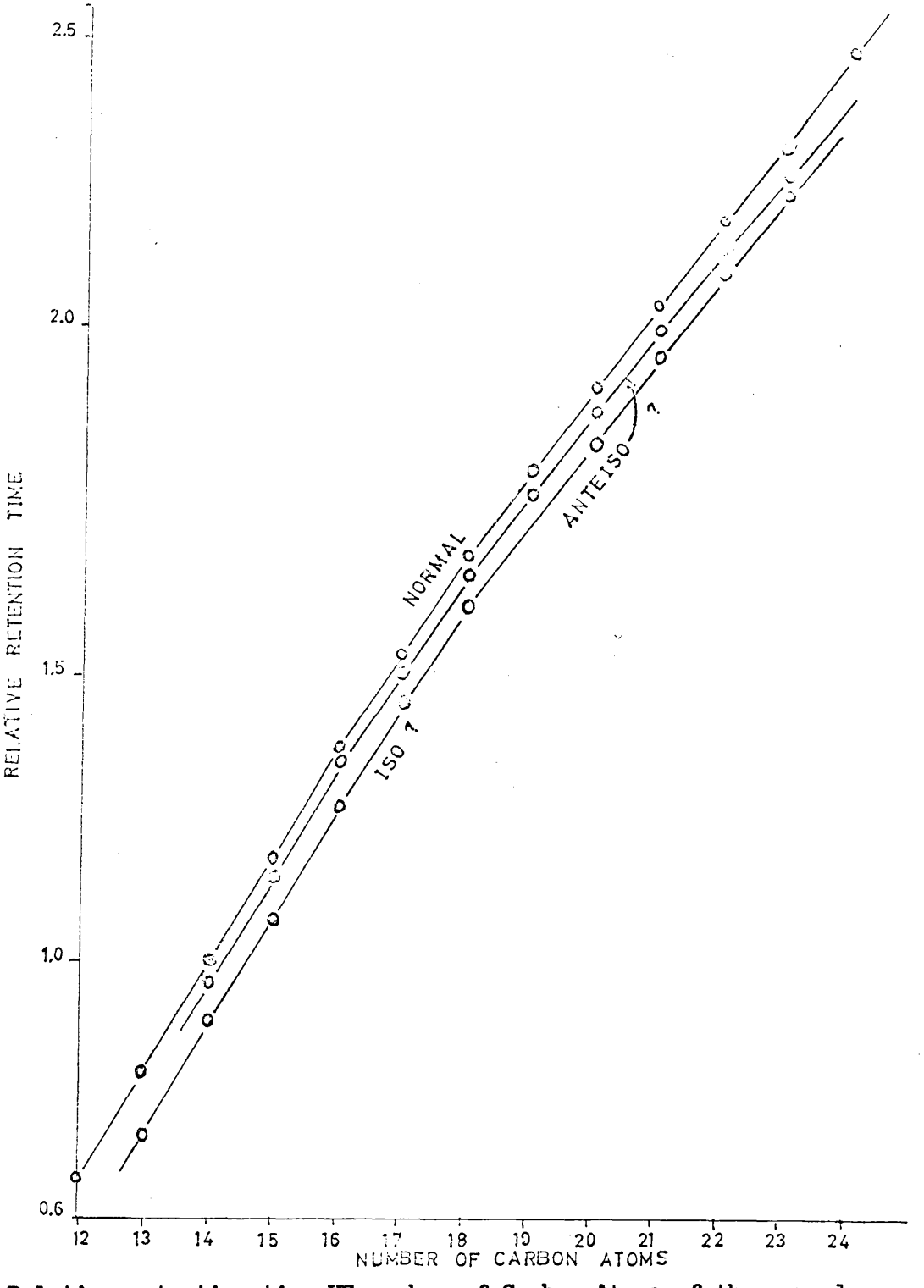
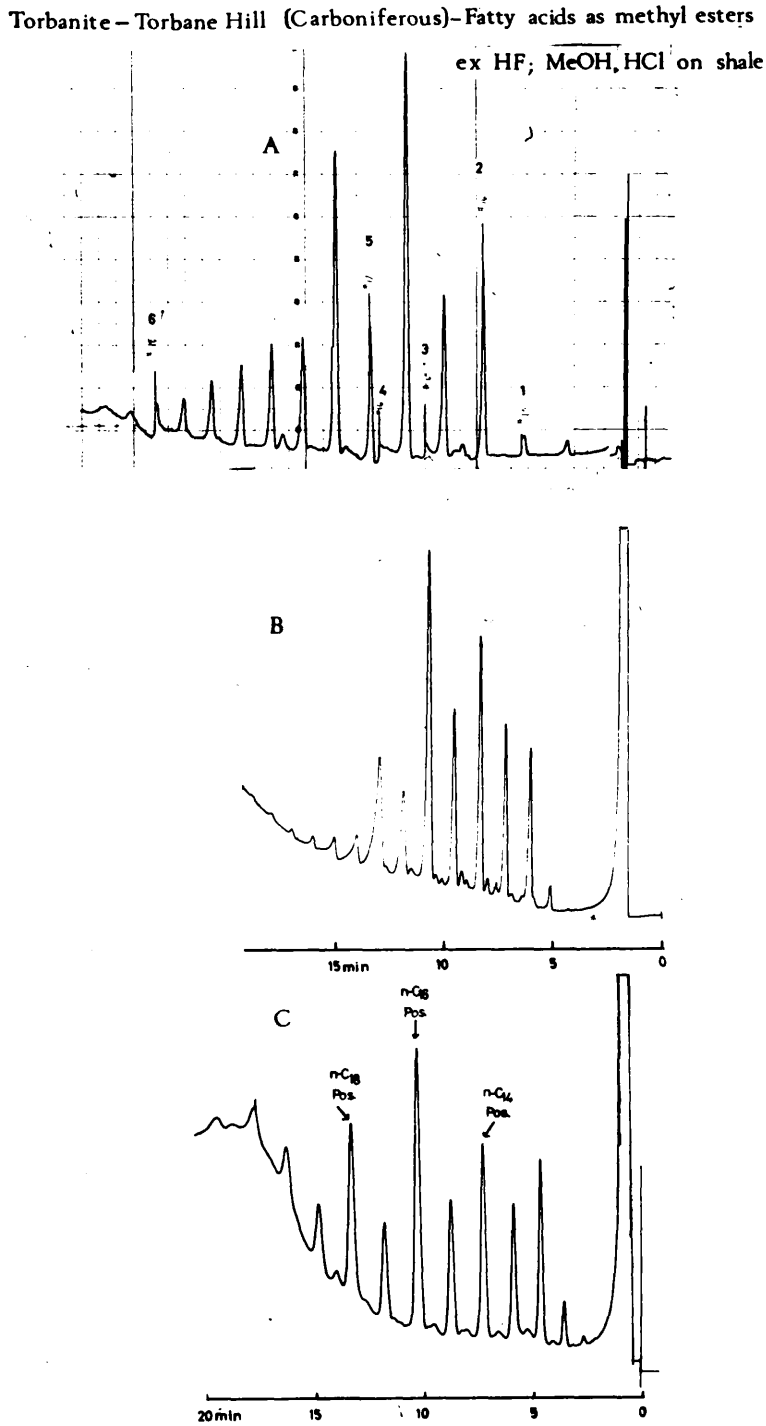


Fig. 5D Relative retention time VS number of Carbon Atoms of the normal, iso (?) and anteiso (?) acid esters isolated from Torbanite (temperature programmed chromatogram)



Figs. 6 A,B,C. (For figure legends see reverse side of diagrams).

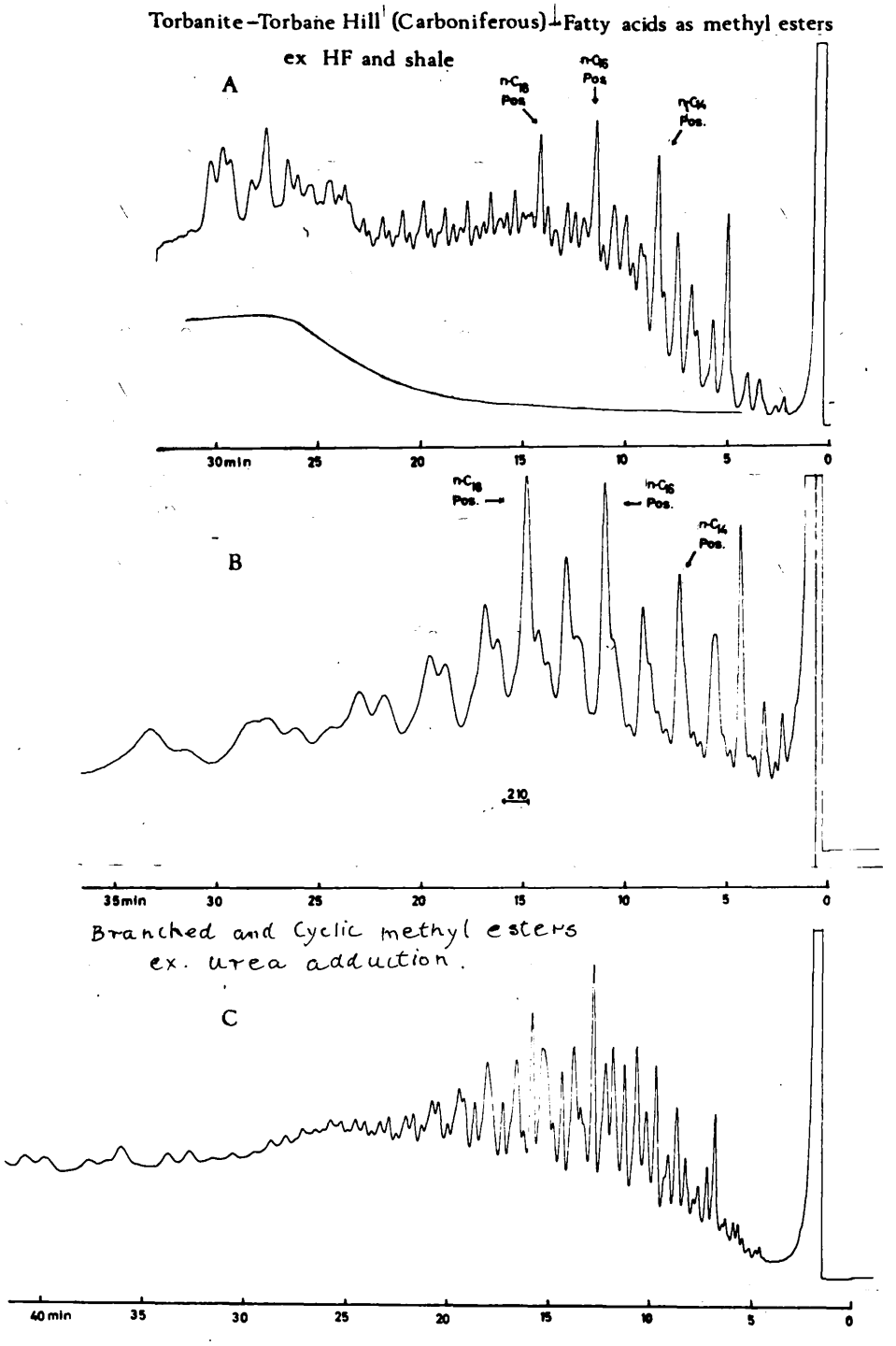
Fig. 6.

Gas chromatograms of methyl esters of fatty acids from Torbanite. Column conditions:-

(A) 6 ft. x $^{1}/_{16}$ in., 2% SE-30; 30 ml/min helium; temperature programmed at 5°/min. from 100° to 250° on LKB 9000, GC-MS.

(B) 10 ft. x $\frac{1}{8}$ in., 1% SE-30 on 100-120 mesh Gas Chrom P(DMCS); 20 ml/min. nitrogen; temperature programmed at 8°/min, from 150° to 300°, injector temperature 280°.

(C) 6 ft. x $\frac{1}{8}$ in., 10% PEGA on 100-120 mesh Gas Chrom P(DMCS); 30 ml/min., nitrogen; temperature programmed at 5°/min. from 150° to 230°, injector temperature 280°.



Figs. 7 A,B,C (For figure legends see reverse side of diagrams)

Fig. 7

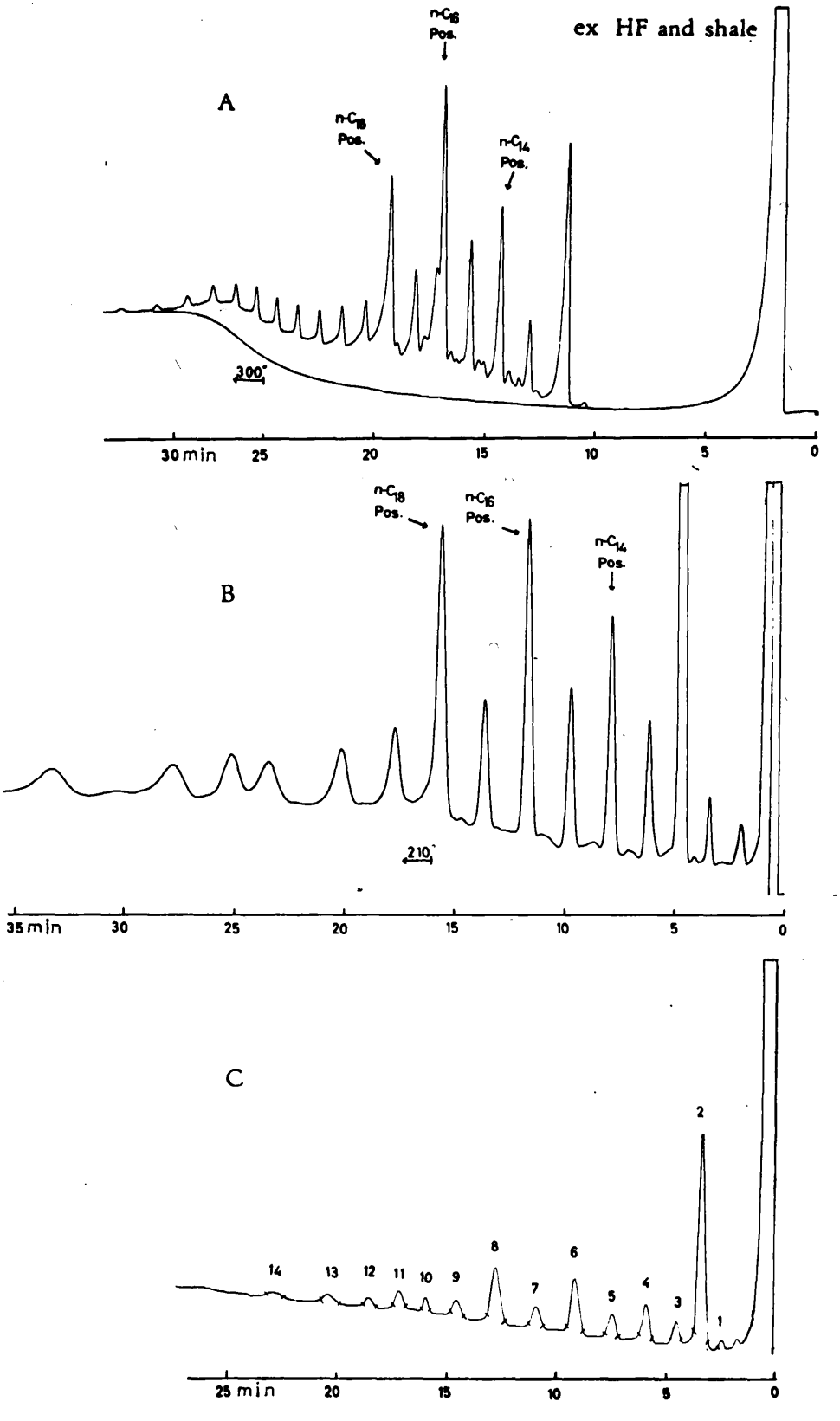
Gas Chromatograms of methyl esters of free fatty acids from Torbanite. Column conditions:-

(A) 6 ft. x $\frac{1}{8}$ in., 3% SE-30 on 100-120 mesh Gas Chrom P(DMCS); 30 ml/min. nitrogen; temperature programmed at 4°/min. from 150° to 300°, injector temperature 280°.

(B) 6 ft. x $\frac{1}{8}$ in., 10% PEGA on 100-120 mesh Gas Chrom P(DMCS); 30 ml/min. nitrogen; temperature programmed at 4°/min. from 150° to 210°, injector temperature 280°C.

(C) 10 ft. x $\frac{1}{8}$ in., 1% SE-30 on 100-120 mesh Gas Chrom P(DMCS); 20 ml/min. nitrogen; temperature programmed at 8°/min. from 100° to 300°, injector temperature 280°.

Scottish Oil-Shale (Carboniferous)–Fatty acids as methyl esters



Figs. 8 A,B,C (For figure legends see reverse side of diagrams)

Fig. 8

Gas Chromatograms of methyl esters of free fatty acids from Scottish Oil-Shale. Column conditions:-

(A) 10 ft. x $\frac{1}{8}$ in., 1% SE-30 on 100-120 mesh Gas Chrom P(DMCS); 20 ml/min. nitrogen; temperature programmed at 8°/min. from 100° to 300°, injector temperature 280°.

(B) 6 ft. x $\frac{1}{8}$in., 10% PEGA on 100-120 mesh Gas Chrom P(DMCS); 30 ml/min. nitrogen; temperature programmed at 4°/min. from 150° to 210°, injector temperature 280°.

(C) 6 ft. x $\frac{1}{4}$ in., 10% PEGA on 100-120 Chromosorb W; 60 ml/min. helium; temperature programmed at 4°/min. from 150° to 215°, injector temperature 265°. Sample size was 1 mg in 30 ul of solution.

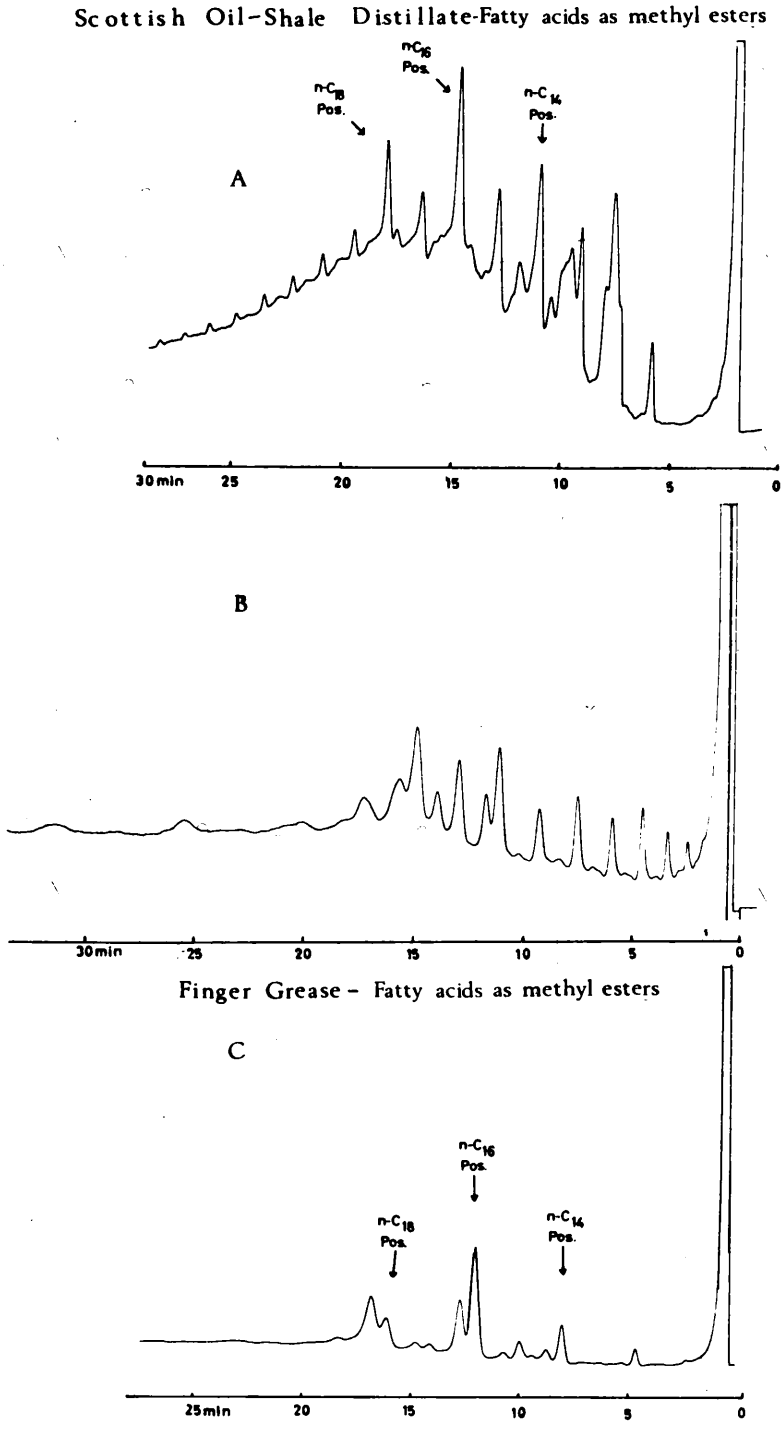


Fig. 9 A,B,C (For figure legends see reverse side of diagrams)

Figs. 9 A,B.

Gas Chromatograms of methyl esters of free fatty acids from Scottish Oil-Shale Distillate. Column conditions:-

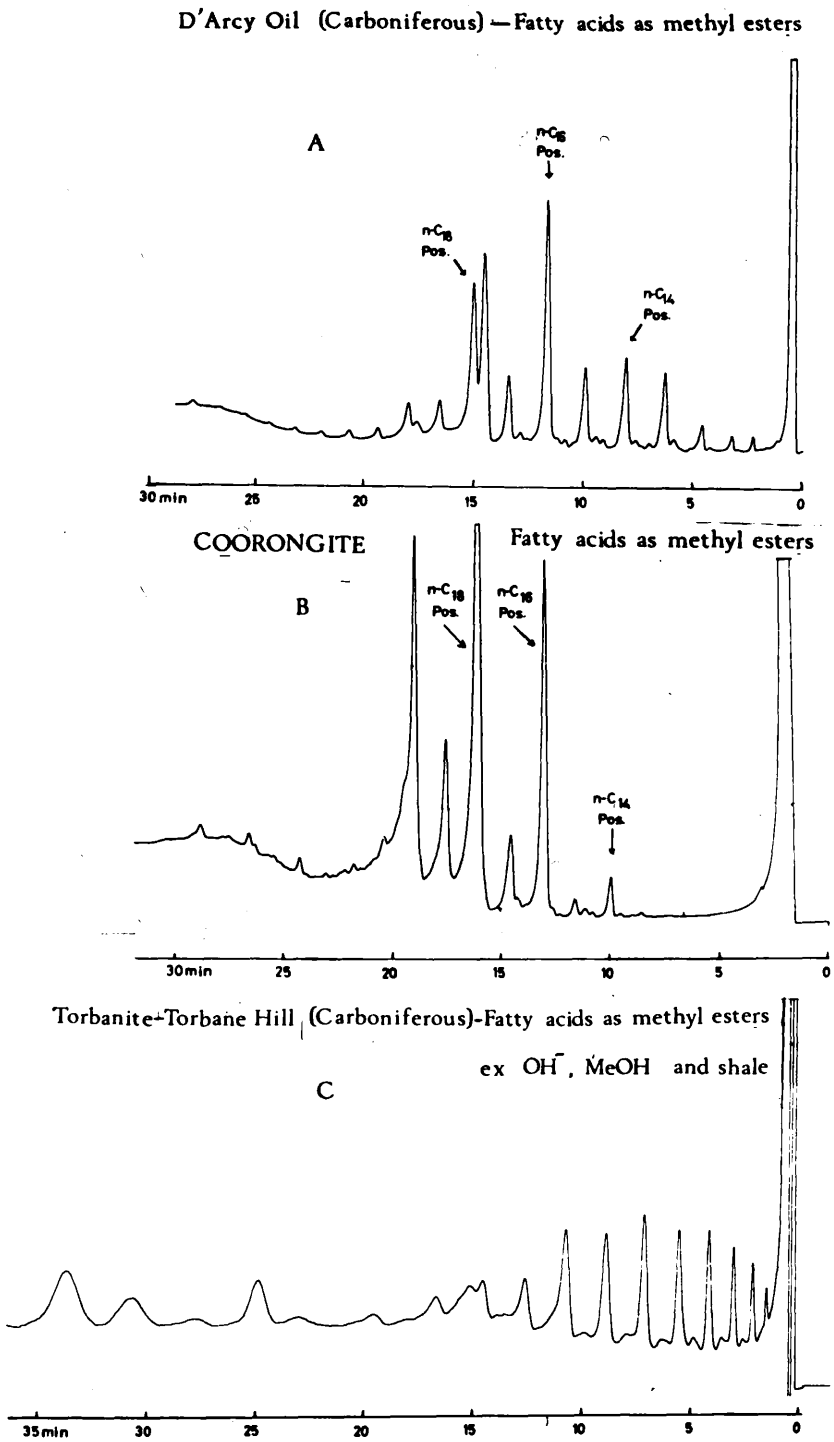
(A) 10 ft. $\frac{1}{8}$ in., 1% SE-30 on 100-120 mesh Gas Chrom P(DMCS); 20 ml/min. nitrogen; temperature programmed at 6°/min. from 150° to 300°, injector temperature 280°.

(B) 6 ft. x $\frac{1}{8}$ in., 10% PEGA on 100-120 mesh Gas Chrom P; 30 ml/min. nitrogen; temperature programmed at 4°/min. from 150° to 210°, injector temperature 280°.

Fig. 9 C

Gas Chromatogram of methyl esters of free fatty acids from finger grease. Column condition:-

(C) 6 ft. x $\frac{1}{8}$ in., 10% PEGA on 100-120 Gas Chrom P(DMCS); 30 ml/min. nitrogen; temperature programmed at 4°/min from 150° to 210°, injector temperature 280°.



Figs. 10 A,B,C (For figure legends see reverse side of diagrams)

Fig. 10

(A) Gas Chromatogram of methyl esters of free fatty acids from D'Arcy Oil. Column conditions:-

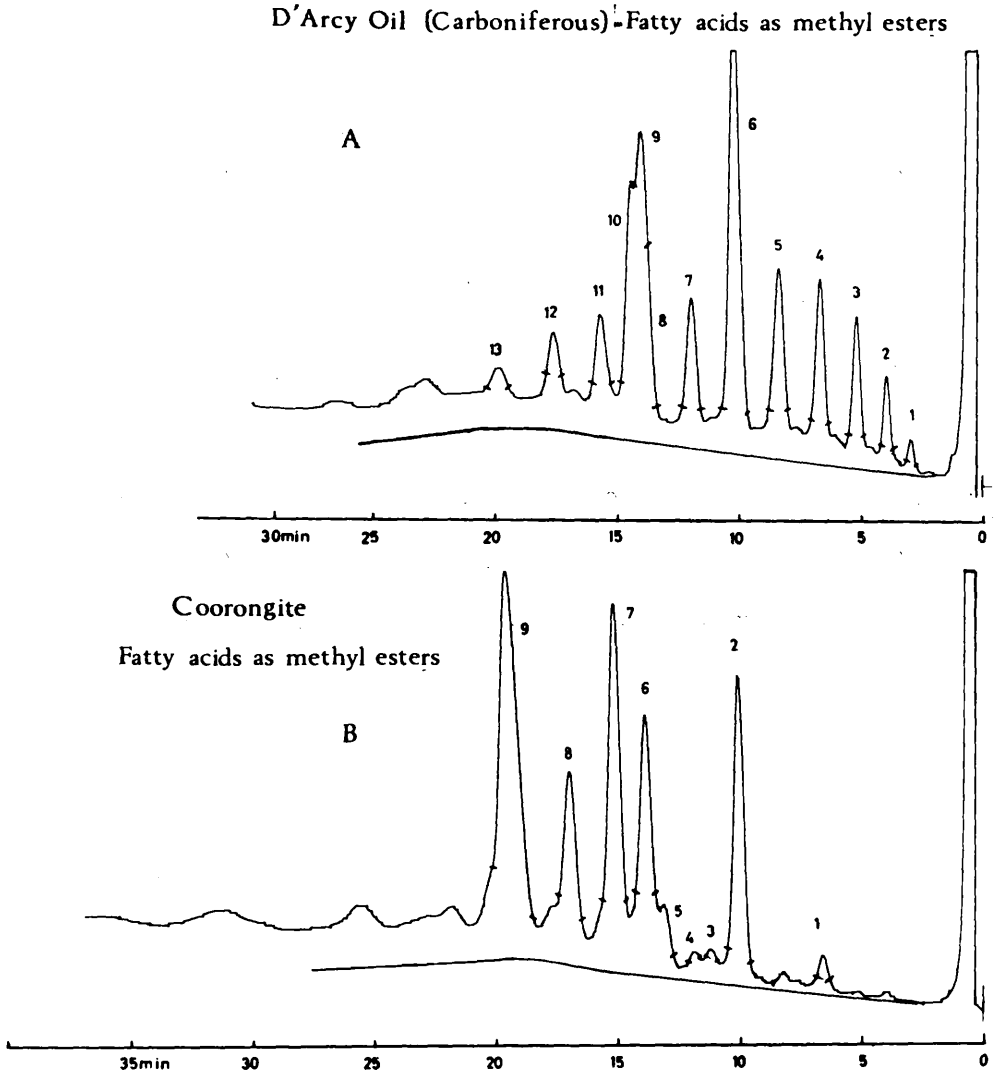
10 ft. x $\frac{1}{8}$ in., 1% SE-30 on 100-120 mesh gas Chrom P(DMCS); 20 ml/min. nitrogen; temperature programmed at 6°/min. from 150° to 300°, injector temperature 280°.

(B) Gas Chromatogram of methyl esters of free fatty acids from Coorongite. Column conditions:-

10 ft. x 8 in., 1% SE-30 on 100-120 mesh Gas Chrom P(DMCS); 20 ml/min. nitrogen; temperature programmed at 6°/min. from 150° to 300°, injector temperature 280°.

(C) Gas Chromatogram of methyl esters of total fatty acids from Torbanite. Column conditions:-

6 ft. x $\frac{1}{8}$ in., 10% PEGA on 100-120 mesh Gas Chrom P(DMCS); 30 ml/min. nitrogen; temperature programmed at 4°/min. from 150° to 210°, injector temperature 280°.



Figs. 11 A,B (For figure legends see reverse side of diagrams)

Fig. 11

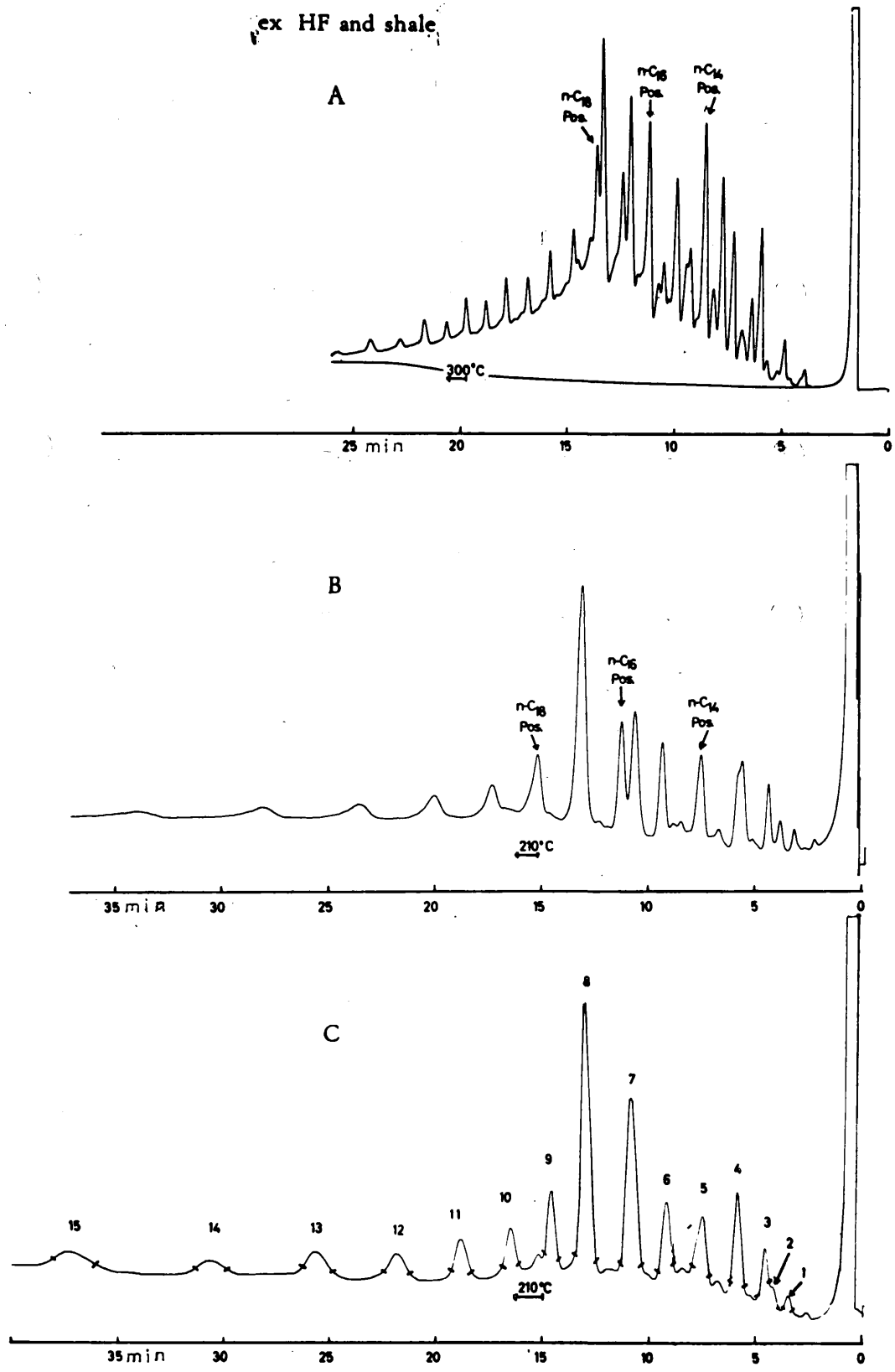
(A) Gas Chromatogram of methyl esters of free fatty acids from D'Arcy Oil. Column conditions:-

6 ft. x) in., 10% PEGA on 100-120 mesh Chromosorb W; 60 ml/min. helium; temperature programmed at 4°/min. from 145° to 215°C, injector temperature 265°. Sample size was 5 mg in 40 ul of benzene.

(B) Gas Chromatogram of methyl esters of free fatty acids from Coorangite. Column conditions:-

6 ft. x $\frac{1}{4}$ in., 10% PEGA on 100-120 mesh Chromosorb W; 60 ml/min. helium; temperature programmed at 4°/min. from 145° to 215°, injector temperature 265°. Sample size was 3 mg in 40 ul of benzene.

ex HF and shale



Figs. I2 A,B,C. (For figure legends see reverse side of diagrams)

Fig. 12

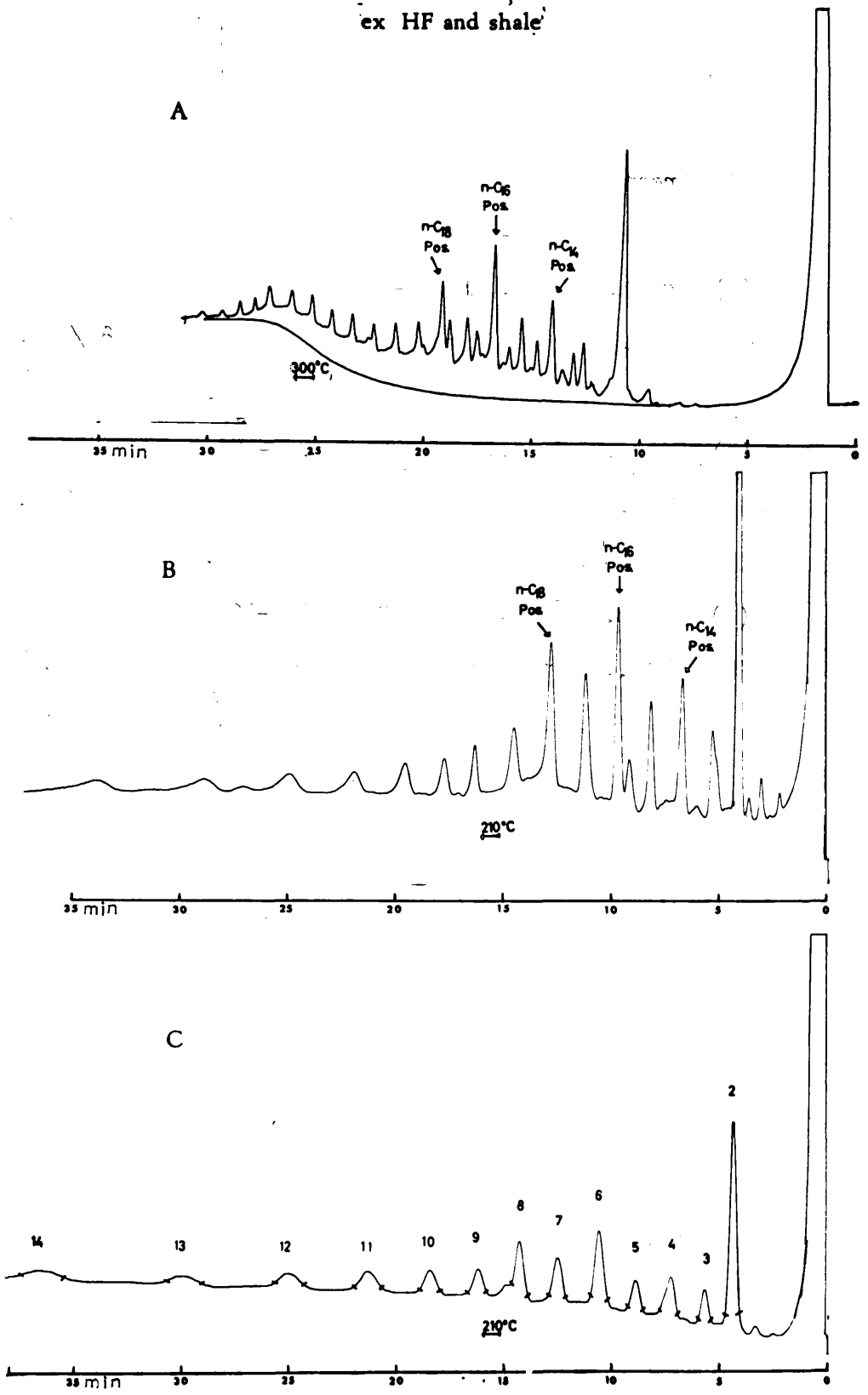
Gas Chromatograms of methyl esters of free fatty acids from Green River Shale, 1100 ft. level. Column conditions:-

(A) 10 ft. x $\frac{1}{8}$ in., 1% SE-30 on 100-120 mesh Gas Chrom P(DMCS); 20 ml/min. nitrogen; temperature programmed at 8°/min. from 150° to 300°, injector temperature 280°.

(B) 6 ft. x $\frac{1}{8}$ in., 10% PEGA on 100-120 mesh Gas Chrom P(DMCS); 30 ml/min. nitrogen; temperature programmed at 5°/min. from 150° to 210°, injector temperature 280°.

(C) 6 ft. x $\frac{1}{4}$ in., 10% PEGA on 100-120 mesh Chromosorb W; 60 ml/min. helium; temperature programmed at 4°/min. from 150° to 210°, injector temperature 265°. Sample size was 5 mg in 40 ul of benzene.

Green River Shale (Eocene) 1900 ft + Fatty acids as methyl esters ex HF and shale



Figs.13 A,B,C (for figure legends see reverse side of diagrams)

Fig. 13

Gas chromatograms of methyl esters of free fatty acids from Green River Shale, 1900 ft. level.

Column Conditions:-

(A) 10 ft. x $\frac{1}{8}$ in., 1% SE-30 on 100-120 mesh Gas Chrom P(DMCS); 20 ml/min. nitrogen; temperature programmed at 8°/min. from 125° to 300°, injector temperature 280°C.

(B) 6 ft. x $\frac{1}{8}$ in., 10% PEGA on 100-120 mesh Gas Chrom P(DCMS); 30 ml/min. nitrogen; temperature programmed at 5°/min. from 150° to 210°, injector temperature 280°C.

(C) 6 ft. x $\frac{1}{4}$ in., 10% PEGA on 100-120 mesh Chromosorb W(DCMS); 60 ml/min., helium; temperature programmed at 4°/min. from 140° to 210°, injector temperature 265°. Sample size was 2 mg of esters in 40 ul of benzene.

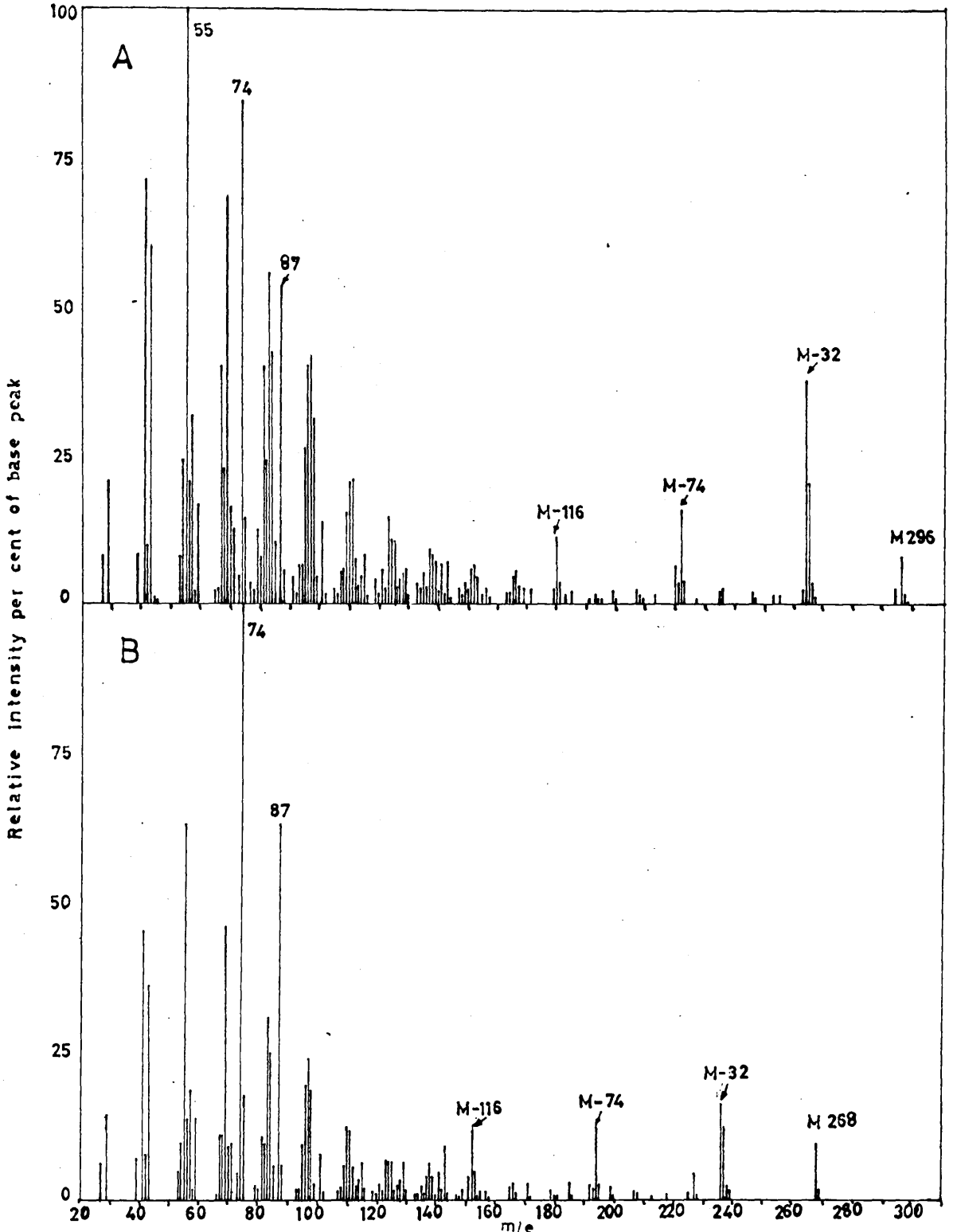


Fig. 14 A. Mass spectrum of methyl ester of C_{18} monoenoic acid (IV) isolated from D'Arcy Oil.

B. Mass spectrum of methyl ester of C_{16} monoenoic acid (VI) isolated from Coorongite.

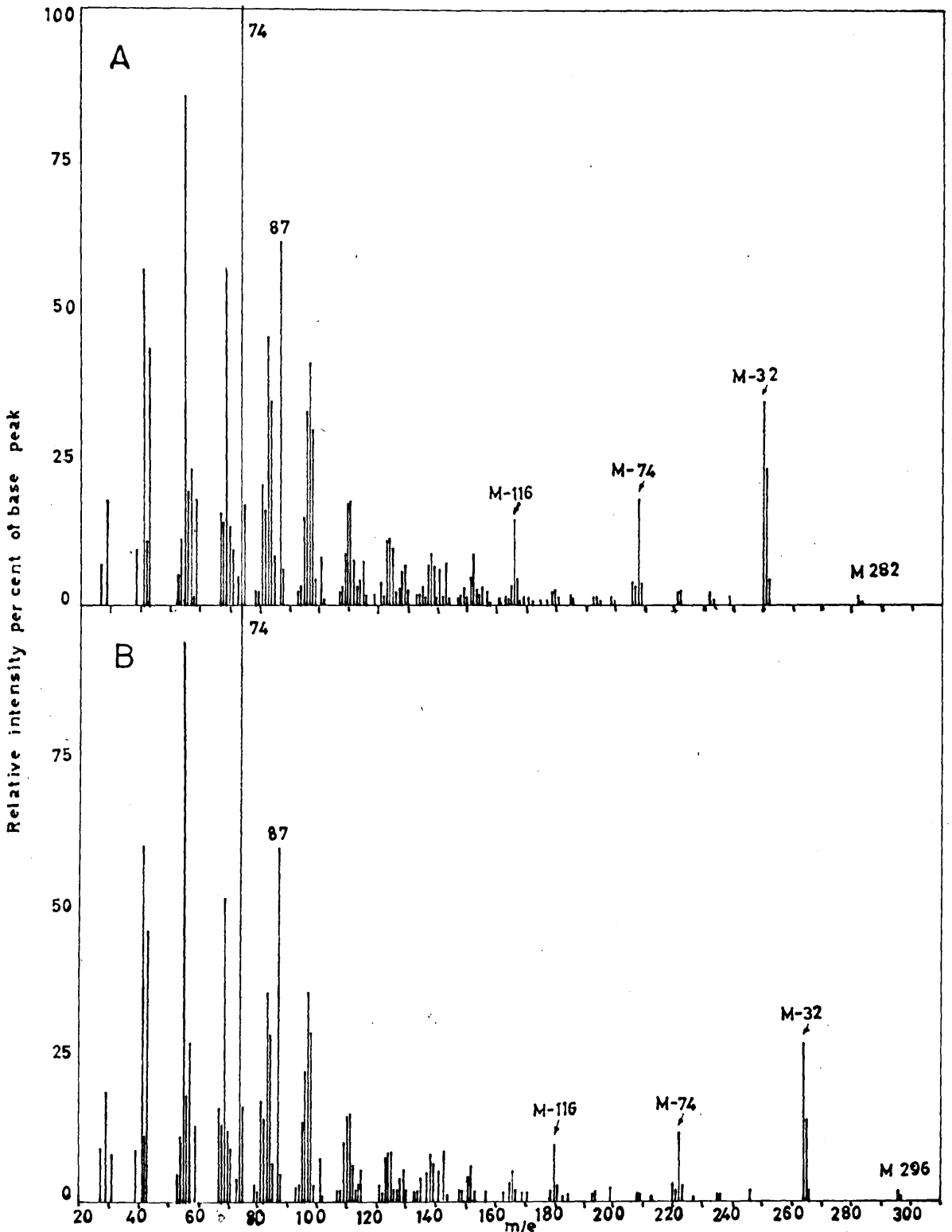


Fig. 15 A. Mass spectrum of methyl ester of C_{17} monoenoic acid (VII) isolated from Coorongite.

B. Mass spectrum of methyl ester of C_{18} monoenoic acid (VIII) isolated from Coorongite.

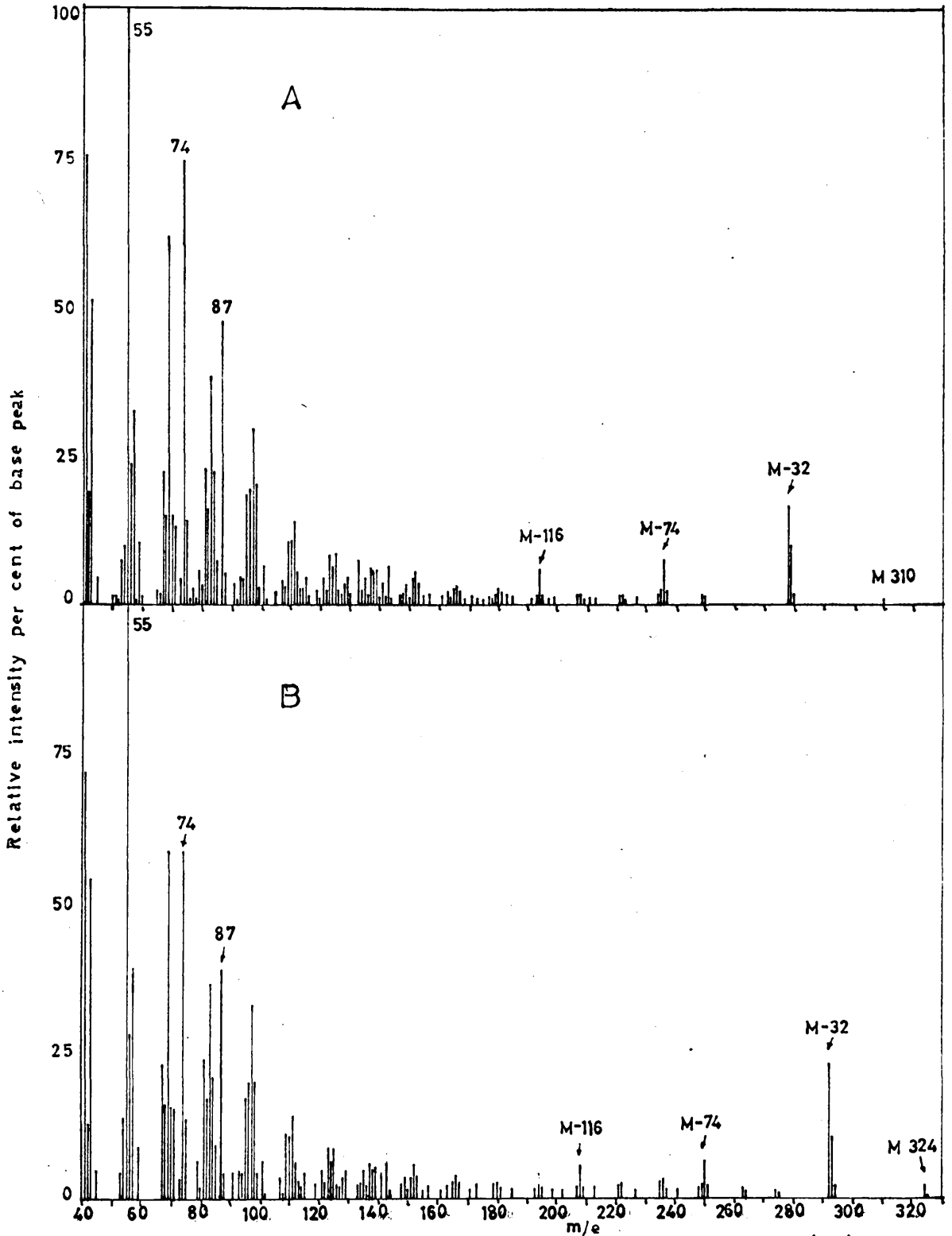


Fig. 16 A. Mass spectrum of methyl ester of C_{19} monoenoic acid (IX) isolated from Coorongite.

B. Mass spectrum of methyl ester of C_{20} monoenoic acid (X) isolated from Coorongite.

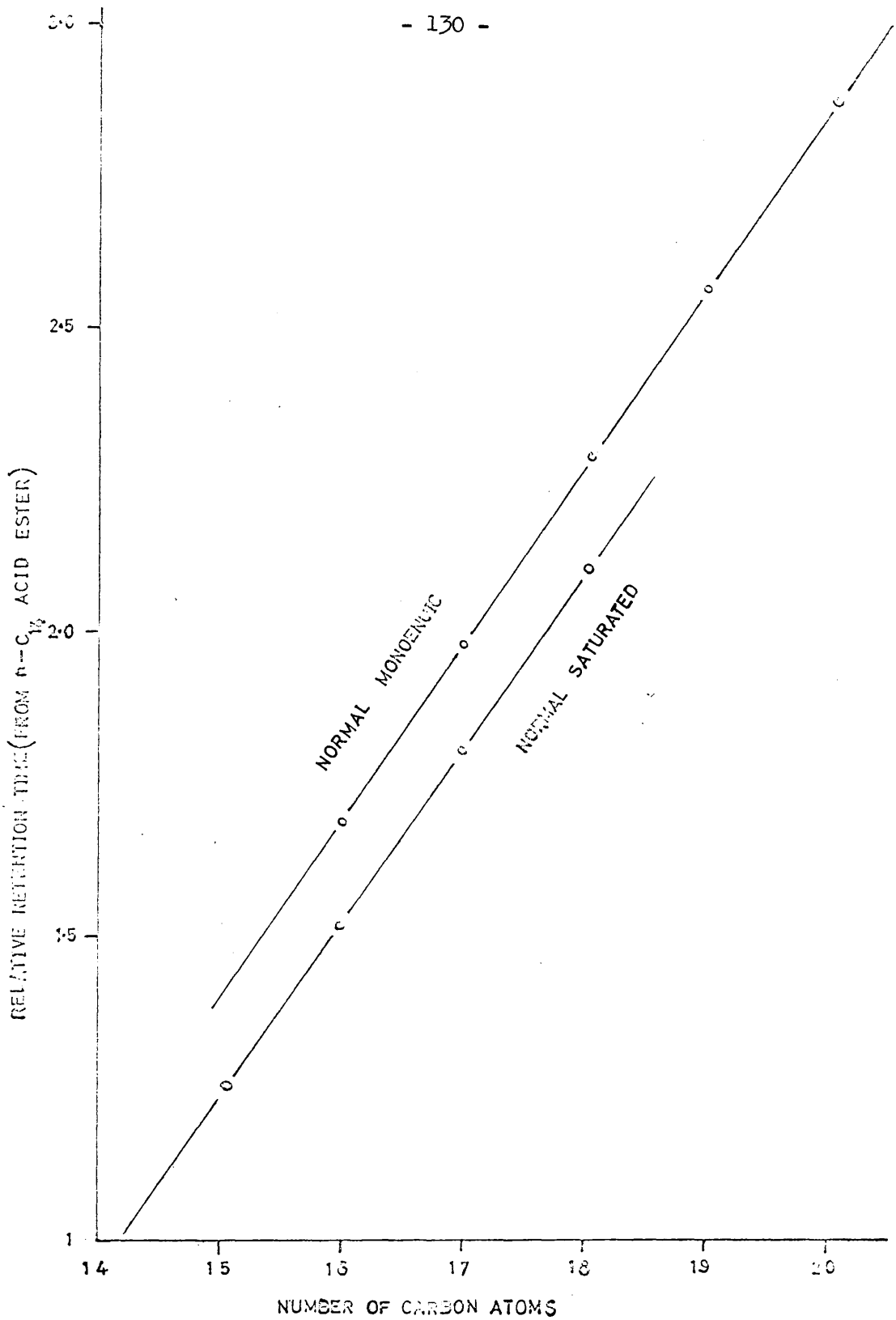


Fig. 17. Relative retention time VS number of Carbon Atoms of the normal saturated and normal monoenoic acid esters isolated from Coorongite (temperature programmed chromatogram).

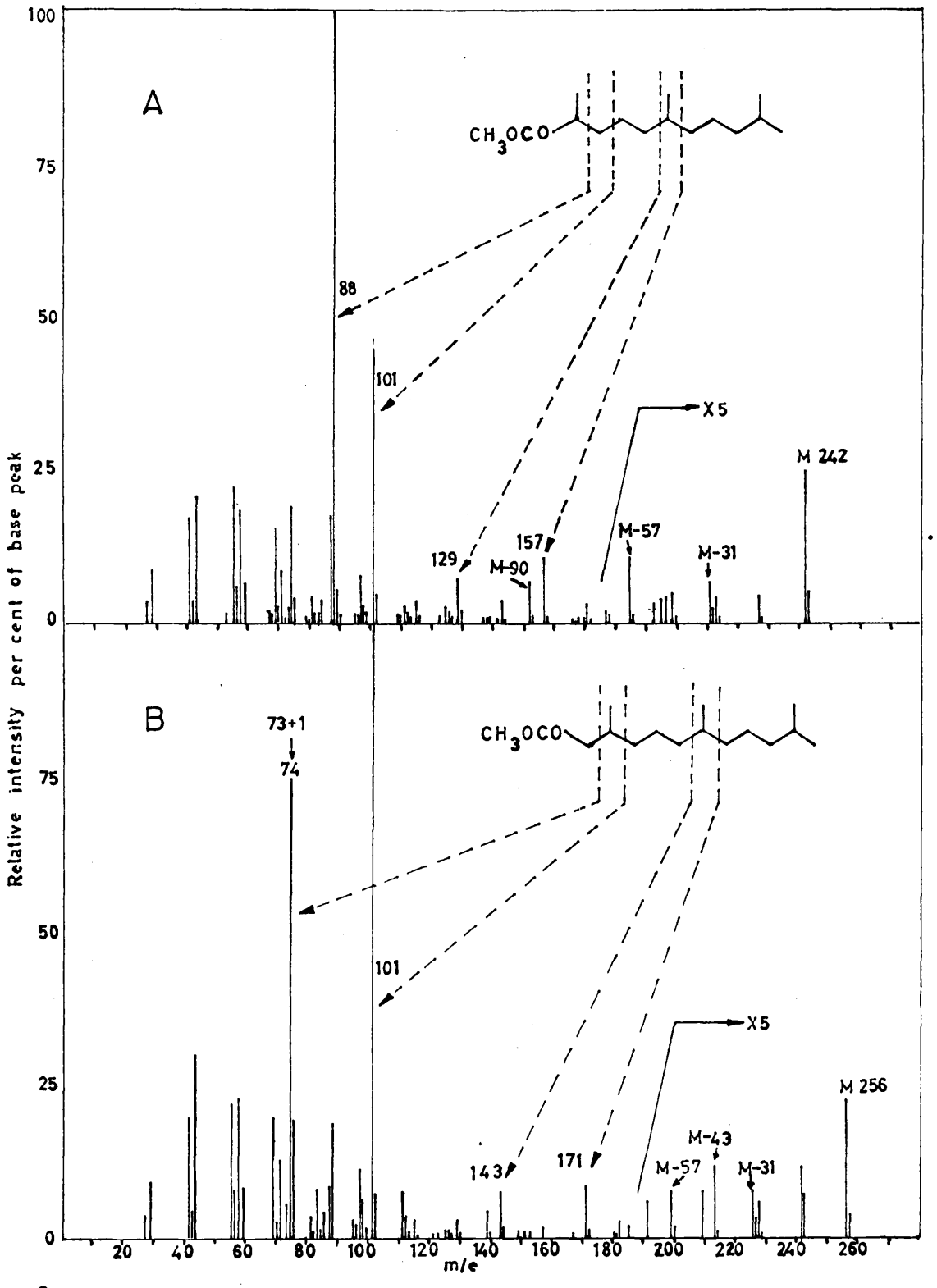


Fig. 18 A. Mass spectrum of methyl ester of C_{14} isoprenoid acid (XI) isolated from Green River Shale.

B. Mass spectrum of methyl ester C_{15} isoprenoid acid (XII) farnesanic acid, isolated from Green River Shale.

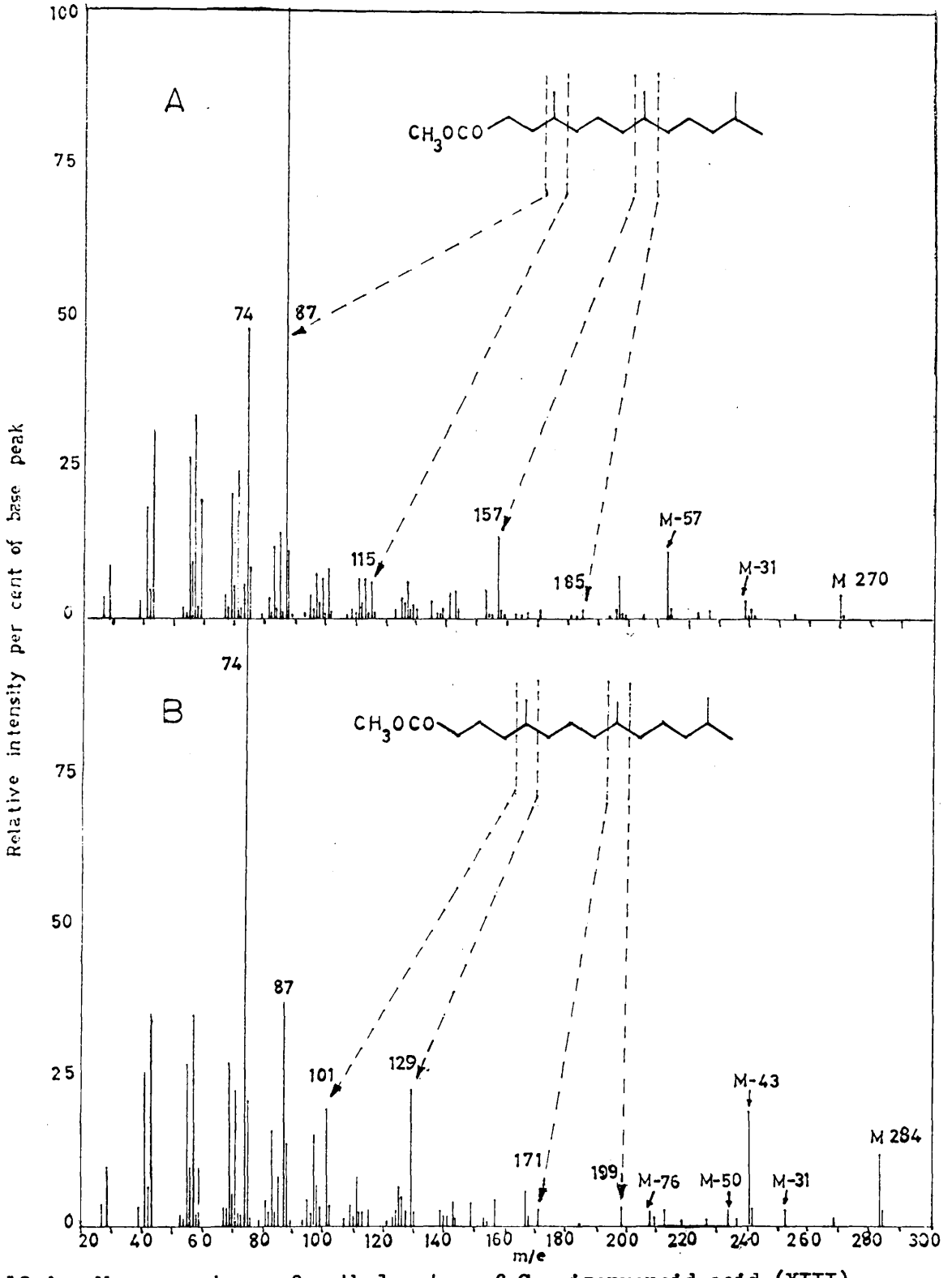


Fig. 19 A. Mass spectrum of methyl ester of C_{16} isoprenoid acid (XIII) isolated from Green River Shale.

B. Mass spectrum of methyl ester of C_{17} isoprenoid acid (XIV) isolated from Green River Shale.

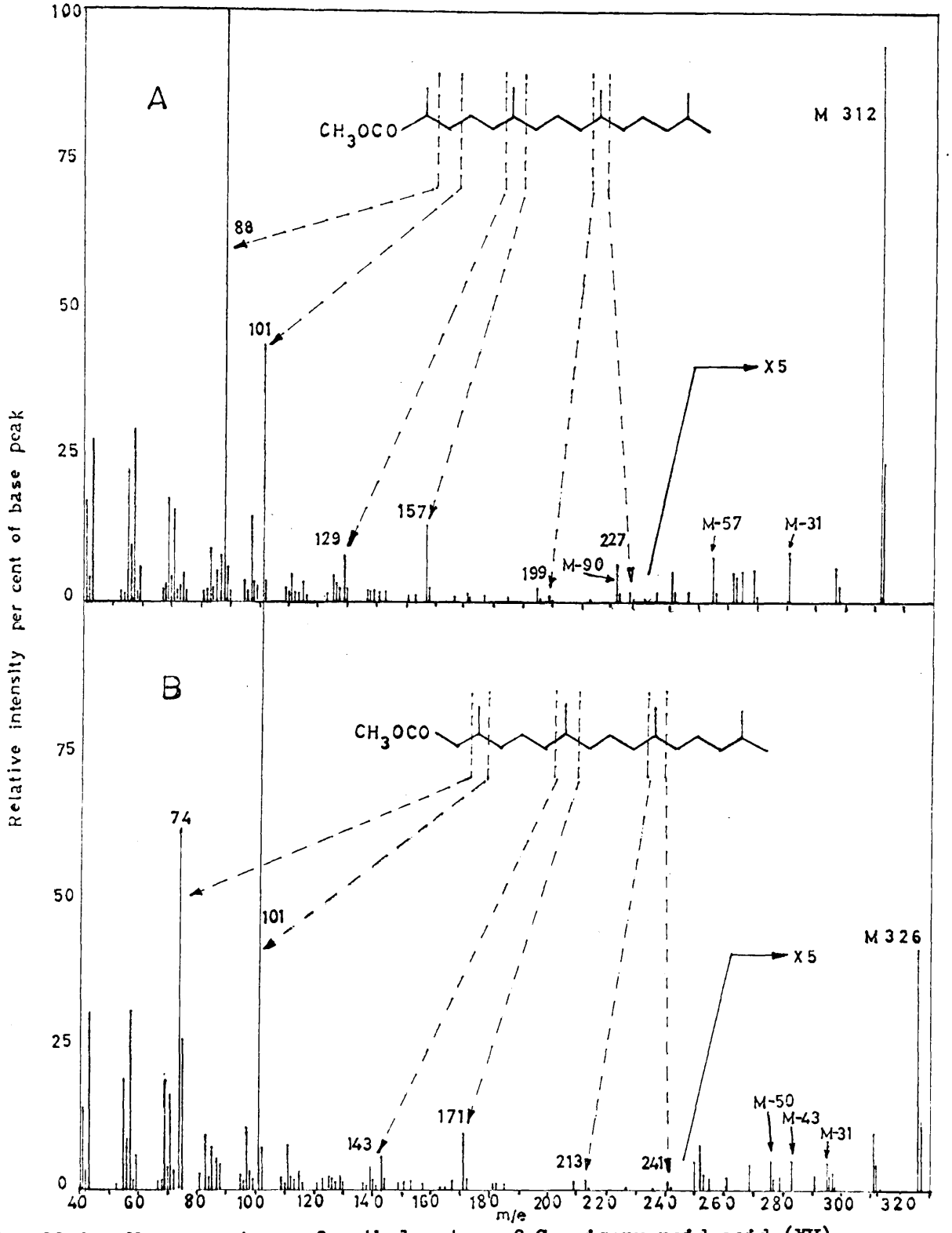


Fig. 20 A. Mass spectrum of methyl ester of C_{19} isoprenoid acid (XV) nor-phytanic acid, isolated from Green River Shale.

B. Mass spectrum of methyl ester of C_{20} isoprenoid acid (XVI) phytanic acid, isolated from Green River Shale.

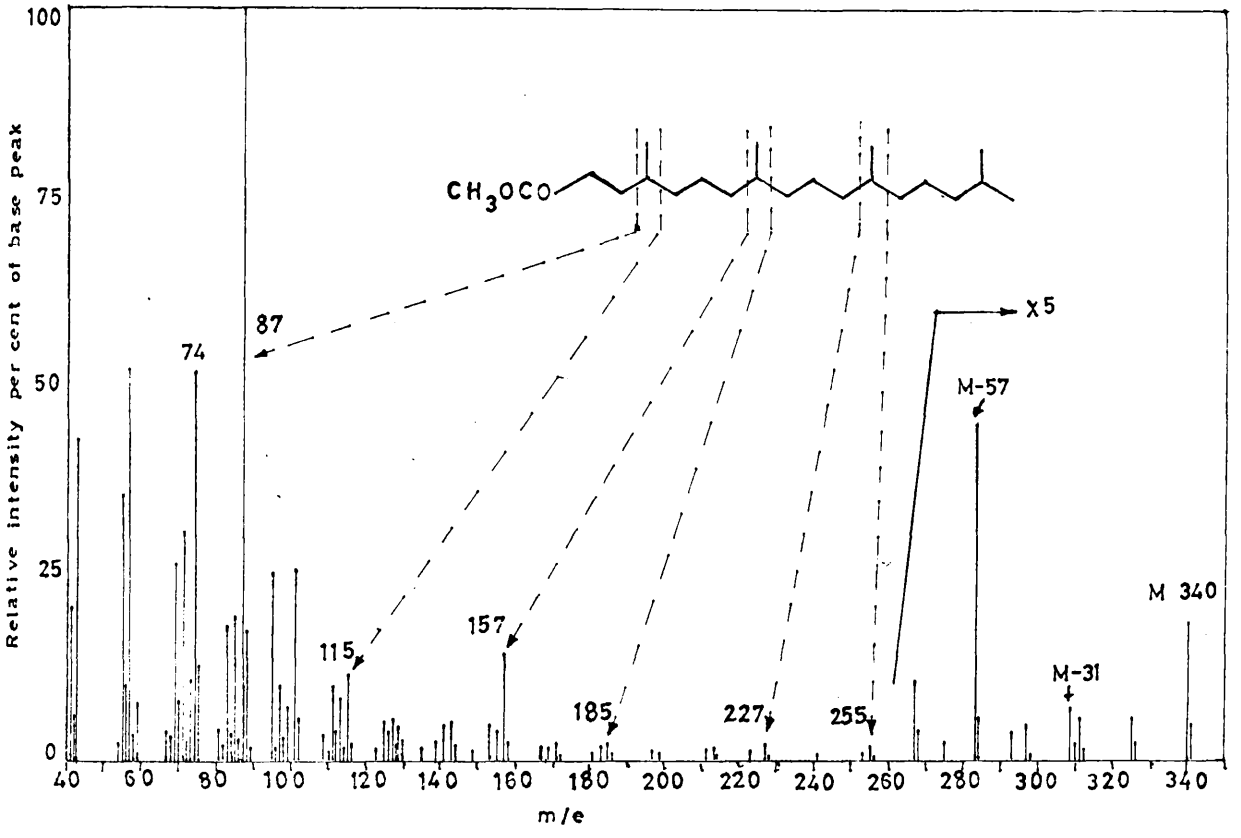


Fig. 21. Mass spectrum of methyl ester of C_{21} isoprenoid acid (XVII) isolated from Green River Shale.

Figure 22 GEOLOGIC TIME TABLE.

MAIN DIVISIONS AND EVENTS OF GEOLOGICAL TIME

ERAS	PERIODS	CHARACTERISTIC LIFE	ESTIMATED YEARS AGO
CENOZOIC	Quaternary: Recent Epoch Pleistocene Epoch	Rise of modern plants and animals, and man	25,000 975,000
	Tertiary: Pliocene Epoch Miocene " Oligocene " Eocene " Paleocene "	Rise of mammals and development of highest plants	12,000,000 25,000,000 35,000,000 60,000,000 70,000,000
MESOZOIC	Cretaceous	Modernized angiosperms and insects abundant. Foraminifers profuse. Extinction of dinosaurs, flying reptiles, and ammonites.	70,000,000 to 200,000,000
	Jurassic	First (reptilian) birds. First of highest forms of insects. First (primitive) angiosperms.	
	Triassic	Earliest dinosaurs, flying reptiles, marine reptiles, and primitive mammals. Cycads and conifers common. Modern corals common. Earliest ammonites.	
PALEOZOIC	Permian	Rise of primitive reptiles. Earliest cycads and conifers. Extinction of trilobites. First modern corals.	(Carboniferous)
	Pennsylvanian	Earliest known insects. Spore plants abundant.	
	Mississippian	Rise of amphibians. Culmination of crinoids.	
	Devonian	First known seed plants. Great variety of boneless fishes. First evidence of amphibians.	200,000,000 to 500,000,000
	Silurian	Earliest known land animals. Primitive land plants. Rise of fishes. Brachiopods, trilobites, and corals abundant.	
	Ordovician	Earliest known vertebrates. Graptolites, corals, brachiopods, cephalopods, and trilobites abundant. Oldest primitive land plants.	
	Cambrian	All subkingdoms of invertebrate animals represented. Brachiopods and trilobites common.	
PROTEROZOIC	Keweenawan	Primitive water-dwelling plants and animals.	500,000,000 to 1,000,000,000
	Huronian		
ARCHEOZOIC	Timiskaming	Oldest known life (mostly indirect evidence).	1,000,000,000 to 1,800,000,000
	Keewatin		

Table 1. Mass Spectra of Samples of Normal Long Chain Fatty Acid Methyl Esters

METHYL n-HEXADECANOATE			METHYL n-OCTADECANOATE			
m/e	n[a]	Isolated Sample I[b]	m/e	n[a]	Isolated Sample II[b]	Synthetic Sample III[c]
74		100	74		100	100
87	2	63.9	87	2	70	66.6
101	3	9.1	101	3	8.0	5.3
115	4	3.0	115	4	3.1	2.0
129	5	5.3	129	5	6.3	6.0
143	6	15.1	143	6	16.5	14.6
157	7	2.5	157	7	2.7	1.3
171	8	3.7	171	8	1.8	0.6
185	9	4.0	185	9	3.1	2.0
199	10	3.3	199	10	5.7	5.3
213	11	1.6	213	11	2.5	1.3
227(M-43)		8.6	227	12	0.8	0.5
239(M-31)		7.2	241	13	1.8	1.3
241(M-29)		2.5	255(M-43)		9.5	8.7
270	d	45.3	267(M-31)		6.8	4.7
			269(M-39)		2.5	2.0
			298	d	40.0	13.3

a. Value of n in formula, $(CH_2)_nCO_2CH_3$

b. Samples I and II isolated from Torbanite collected as shown in Fig. 5, Tracing C, cuts 6 and 8 respectively.

c. Synthetic sample supplied by Applied Science Labs. (99.8%).

d. The molecular ion.

Table 2. Mass Spectra of Samples of

Monoethenoid Fatty Acid Methyl Esters

Isolated Sample			Synthetic Sample
C_{18} Unsaturated Acid Methyl Ester			Methyl Oleate
m/e	n[a]	IV[b]	V[c]
55		100	100
74		84.3	85.0
87	2	53.4	84.0
101	3	13.8	10.0
115	4	8.6	8.0
129	5	6.0	5.5
143	6	7.1	6.0
157	7	2.7	2.0
171	8	2.7	1.5
180(M-116)		11.1	8.5
185	9	2.2	1.5
199	10	2.2	1.5
213	11	1.8	1.0
222(M-74)	d	15.8	11.0
227	12	1.0	-
241	13	-	-
255		1.3	-
264(M-32)	e	37.8	21.0
296	f	8.0	3.0

a. Value of n in formula, $(CH_2)_nCO_2CH_3$

b. Sample IV isolated from D'Arcy Oil: collected as shown in Fig. 11, Tracing A, cut 10.

c. Analytical Reagent supplied by B.D.H. Mass Spectrum recorded on LKB 9000 GC-MS.

d. Fragment formed by the loss of $-CH_2CO_2CH_3$

e. Fragment formed by the loss of methanol.

f. The molecular ion.

Table 3. Mass Spectra of Samples of Monoethenoid Fatty Acid Methyl Esters

Isolated Samples								
C_{16} Acid Methyl Ester			C_{17} Acid Methyl Ester			C_{18} Acid Methyl Ester		
m/e	n[a]	VI[b]	m/e	n[a]	VII[b]	m/e	n[a]	VIII[b]
74		100	74		100	74		100
87	2	63.0	87	2	60.8	87	2	59.5
101	3	7.8	101	3	8.5	101	3	7.4
115	4	5.2	115	4	7.5	115	4	5.1
129	5	6.2	129	5	6.8	129	5	8.4
143	6	9.0	143	6	7.0	143	6	8.7
152(M-116)		11.2	157	7	2.3	157	7	2.0
157	7	1.4	166(M-116)		14.0	171	8	1.7
171	8	2.4	171	8	1.5	180(M-116)		9.1
185	9	2.4	185	9	1.8	185	9	1.5
194(M-72)	c	12.0	199	10	1.8	199	10	2.3
199	10	2.0	208(M-72)	c	18.0	213	11	1.0
213	11	0.8	213	11	-	222(M-72)	c	11.4
227		4.4	227	12	-	227	12	1.0
236(M-32)	d	16.0	239		1.8	264(M-32)	d	26.8
268	e	9.2	250(M-32)	d	34.0	296	e	1.7
			282	e	1.8			

a. Value of n in formula, $(CH_2)_nCO_2CH_3$

b. Samples VI, VII and VIII isolated from Coorongite: collected as shown in Fig. 11, Tracing B, cuts 3, 5 and 7 respectively.

c. Fragments formed by the loss of $-CH_2CO_2CH_3$

d. Fragment formed by the loss of methanol.

e. The molecular ion.

Table 4. Mass Spectra of Samples of Monoethenoid Fatty Acid Methyl Esters

Isolated Samples					
C_{19} Acid Methyl Ester			C_{20} Acid Methyl Ester		
m/e	n[a]	IX[b]	m/e	n[a]	X[b]
55	b	100	55	b	100
74		74.5	74		58.5
87	2	47.6	87	2	38.3
101	3	6.4	101	3	6.3
115	4	4.7	115	4	4.3
129	5	4.9	129	5	4.8
143	6	6.6	143	6	6.0
157	7	1.8	157	7	2.0
171	8	1.5	171	8	1.5
185	9	1.6	185	9	1.8
194(M-116)		6.0	199	10	1.5
199	10	1.3	208(M-116)		5.5
213	11	1.0	213	11	2.0
227	12	1.0	227	12	1.3
236(M-74)	c	7.7	241	13	1.5
278(M-32)	d	16.8	250(M-74)	c	6.3
310	e	0.8	292(M-32)	d	22.5
			324	e	1.8

a. Value of n in formula, $(CH_2)_nCO_2CH_3$

b. Samples IX and X isolated from Coorongite: collected as shown in Fig. 11, Tracing B, cuts 8 and 9 respectively.

c. Fragments formed by the loss of $-CH_2CO_2CH_3$

d. Fragments formed by the loss of methanol.

e. The molecular ion.

Table 5. Mass Spectra of Samples of Isoprenoid Acid Methyl Esters

Isolated Samples							
METHYL 2,6,10-TRIMETHYLUNDECANOATE				METHYL 3,7,11-TRIMETHYLDODECANOATE			
m/e	n[c]	XIA[a]	XIB[b]	m/e	n[c]	XIIA[x]	XIIB[y]
88	d	100	100	74		75.0	73.5
101	3	39.6	44.5	101	3	100	100
115	4	3.0	3.2	115	4	3.0	2.4
125	e	2.9	2.6	129	5	3.0	1.4
129	5	4.0	6.8	139	f	4.5	3.0
143	6	3.1	3.8	143	6	7.6	7.6
152(M-90)		4.1	6.4	157	7	1.5	0.8
157	7	6.4	10.4	166(M-90)		-	-
166(M-76)		-	0.5	171	8	8.3	9.8
171	8	2.2	3.2	182(M-74)		2.3	1.8
177(M-65)		-	0.5	185	9	1.5	0.6
185(M-57)		2.2	2.1	191(M-65)		1.2	0.6
192(M-50)		-	-	199(M-57)		1.5	1.1
195	e	0.5	0.8	206(M-50)		-	0.8
199(M-43)		1.0	1.0	209	f	1.5	0.8
211(M-31)		1.4	1.3	213(M-43)		2.3	1.8
113(M-29)		1.2	0.8	225(M-31)		1.5	1.1
227(M-15)		2.4	0.8	227(M-29)		1.2	0.6
242	g	3.4	4.9	241(M-15)		2.3	1.8
				256	g	4.5	3.1

a. From the Green River Shale, 1100 ft. level (Fig.12, Tracing C, cut 3)
b. From the Green River Shale, 1900 ft. level (Fig.13, Tracing C, cut. 2)
c. Value of n in the formula $(CH_2)_nCO_2CH_3$
d. This is the rearrangement peak $CH_3CH = C(OH)O\ CH_3$
e. This is a ketene fragment of general formula,
$O=C=C(CH_3)\left((CH_2)n\ CH\ CH_3\right)m$ where n = 3 & m = 1, 2.
f. This is a ketene fragment of general formula,
$O=C=CH\ CH(CH_3)\left((CH_2)n\ CH\ CH_3\right)m$ where n = 3 & m = 1, 2.
g. Molecular ion.
x. From the Green River Shale, 1100 ft. level (Fig.12, Tracing C, cut 4)
y. From the Green River Shale, 1900 ft. level (Fig.13, Tracing C, cut 3)

Table 6. Mass Spectra of Samples of Isoprenoid Acid Methyl Esters

Isolated Samples							
METHYL 4,8,12-TRIMETHYLTRIDECANOATE				METHYL 5,9,13-TRIMETHYLTETRADECANOATE			
m/e	n[c]	XIIIA[a]	XIIIB[b]	m/e	n[c]	XIVA[x]	XIVB[y]
74		47.5	52.0	74		100	100
87	2	100	100	87	2	36.5	28.6
101	3	8.0	10.0	101	3	19.7	19.7
115	4	6.1	9.0	115	4	2.8	1.9
129	5	1.8	3.8	129	5	22.8	31.4
143	6	4.2	7.6	143	6	4.0	3.3
153	d	4.6	7.5	157	7	4.3	3.4
157	7	13.7	14.0	167	e	5.3	4.7
171	8	1.2	3.2	171	8	2.5	3.0
180(M-90)		-	1.6	185	9	0.3	0.5
185	9	1.3	1.2	194(M-90)		-	-
194(M-76)		0.5	-	199	10	3.0	2.3
199	10	0.9	1.5	208(M-76)		2.2	1.2
205(M-65)		0.6	0.8	213	11	2.5	1.3
213(M-57)		10.2	8.7	219(M-65)		1.0	0.5
220(M-50)		0.3	-	227(M-57)		1.3	0.5
223	d	0.8	0.8	234(M-50)		2.5	1.3
227(M-43)		1.2	1.5	237	e	0.5	0.6
239(M-31)		2.9	2.0	241(M-43)		18.8	14.5
241(M-29)		1.8	1.5	253(M-31)		2.8	1.2
255(M-15)		0.8	1.0	255(M-29)		-	-
270	f	3.7	2.5	269(M-15)		1.5	0.7
				284	f	12.0	4.1

a. From the Green River Shale, 1100 ft. level (Fig.12, Tracing C, cut 5)
b. From the Green River Shale, 1900 ft. level (Fig.13, Tracing C, cut 4)
c. Value of n in the formula $(CH_2)n\ CO_2\ CH_3$
d. This is a ketene fragment of general formula.

$$O{=}C{=}CH\ CH_2\ CH(CH_3)\left((CH_2)n\ CH\ CH_3\right)m \qquad \begin{matrix} m = 1, 2 \\ n = 3 \end{matrix}$$

e. This is a ketene fragment of general formula

$$O{=}C{=}CH(CH_2)_2\ CH(CH_3)\left((CH_2)n\ CH\ CH_3\right)_m \qquad \begin{matrix} m = 1, 2 \\ n = 3 \end{matrix}$$

f. Molecular ion.
x. From the Green River Shale, 1100 ft. level (Fig.12, Tracing C, cut 6)
y. From the Green River Shale, 1900 ft. level (Fig.13, Tracing C, cut 5)

Table 7. Mass Spectra of Samples of Isoprenoid Acid Methyl Esters

Isolated Samples							
METHYL 2,6,10,14 - TETRAMETHYL— PENTADECANOATE				METHYL 3,7,11,15 - TETRAMETHYL— HEXADECANOATE			
m/e	n[c]	XVA[a]	XVB[b]	m/e	n[c]	XVIA[x]	XVIB[y]
88	d	100	100	74		56.5	61.5
101	3	40.0	43.5	101	3	100	100
115	4	1.9	3.0	115	4	3.0	2.6
125	e	3.5	4.1	129	5	2.0	2.0
129	5	5.8	7.4	139	f	4.4	4.0
143	6	0.5	1.1	143	6	6.8	5.9
157	7	12.6	12.8	157	7	0.5	1.0
171	8	0.5	1.1	171	8	12.4	9.3
185	9	0.5	0.5	185	9	-	0.4
195	e	2.0	2.3	199	10	0.5	0.5
199	10	0.9	1.0	209	f	1.3	1.5
213	11	0.9	1.0	213	11	1.9	1.6
222(M-90)		5.3	6.1	227	12	-	0.3
227	12	1.0	1.5	236(M-90)		-	0.3
236(M-76)		-	0.3	241	13	1.2	1.3
241	13	0.5	1.0	250(M-76)		1.1	1.0
247(M-65)		0.2	0.3	255	14	0.5	0.4
255(M-57)		1.0	1.5	261(M-65)		-	0.3
262(M-80)		-	1.0	269(M-57)		0.7	0.8
265	e	0.9	1.0	276(M-80)		2.0	1.0
269(M-43		1.0	1.1	279	f	0.4	0.4
281(M-31)		1.3	1.7	283(M-43)		1.2	1.0
283(M-29)		-	-	294(M-31)		1.1	0.9
297(M-15)		0.8	1.1	297(M-29)		0.6	0.6
312	g	17.1	18.8	311(M-15)		2.1	2.0
				326	g	11.2	8.1

a. From the Green River Shale, 1100 ft. level (Fig.12, Tracing C, cut 7)
b. From the Green River Shale, 1900 ft. level (Fig.13, Tracing C, cut 6)
c. Values of n in the formula $(CH_2)n\ CO_2\ CH_3$
d. This is a rearrangement peak $CH_3\ CH = C(O\ H)OCH_3$
e. This is a ketene fragment of general formula,
$O{=}C{=}C(CH_3)\left((CH_2)n\ CH\ CH_3\right)m$ where m = 1,2,3
n = 3
f. This is a ketene fragment of general formula
$O{=}C{=}CH\ CH(CH_3)\left((CH_2)n\ CH\ CH_3\right)m$ m = 1,2,3
n = 3
g. Molecular ion
x. From the Green River Shale, 1100 ft. level (Fig.12, Tracing C, cut 8)
y. From the Green River Shale, 1900 ft. level (Fig.13, Tracing C, cut 7)

Table 8. Mass Spectra of Samples of Isoprenoid Acid Methyl Esters

Isolated Samples			
METHYL 4,8,12,16 - TETRAMETHYLHEPTADECANOATE			
m/e	n[c]	XVIIA[a]	XVIIB[b]
74		52.5	45.5
87	2	100	100
101	3	25.2	20.5
115	4	11.5	7.0
129	5	4.2	3.5
143	6	5.2	5.5
153	d	4.8	3.5
157	7	14.5	15.5
171	8	2.2	3.0
185	9	2.2	2.0
199	10	1.0	1.5
213	11	1.8	2.5
223	d	1.2	1.0
227	12	2.2	2.0
241	13	1.2	2.0
250(M-90)		-	-
255	14	2.0	2.0
264(M-76)		-	-
269	15	-	-
275(M-65)		0.5	-
283(M-57)		9.0	9.5
290(M-50)		-	0.5
293	d	0.8	0.5
297(M-43)		1.0	2.5
309(M-31)		1.5	2.0
311(M-29)		1.2	1.5
325(M-15)		1.2	1.5
340	e	3.8	9.0

a. From the Green River Shale, 1100 ft. level (Fig.12, Tracing C, cut. 9)
b. From the Green River Shale, 1900 ft. level (Fig.13, Tracing C, cut. 8)
c. Value of n in formula $(CH_2)n\ CO_2\ CH_3$
d. This is a ketene fragment of general formula

$$O{=}C{=}CH\ CH_2\ CH(CH_3)\left((CH_2)n\ CH\ CH_3\right)m \qquad m = 1,2,3;\ n = 3$$

e. Molecular ion

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