PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Lipids of Aquatic Sediments, Recent and Ancient

G. Eglinton, S. K. HajIbrahim, J. R. Maxwell, J. M. E. Quirke, G. J. Shaw, J. K. Volkman and A. M. K. Wardroper

Phil. Trans. R. Soc. Lond. A 1979 **293**, 69-91
doi: 10.1098/rsta.1979.0080

Phil. Trans. R. Soc. Lond. A **293**, 69–91 (1979) [69]
Printed in Great Britain

Lipids of aquatic sediments, Recent and ancient

By G. Eglinton, F.R.S., S. K. HajIbrahim, J. R. Maxwell, J. M. E. Quirke, G. J. Shaw, J. K. Volkman and A. M. K. Wardroper
Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol BS8 1TS, U.K.

Computerized gas chromatography – mass spectrometry (g.c.–m.s.) is now an essential tool in the analysis of the complex mixtures of lipids (geolipids) encountered in aquatic sediments, both Recent (less than 1 Ma (10^6 years) old) and ancient. Most geolipid studies have been performed in the e.i. mode at low resolution but the techniques now being applied include c.i. and h.r.m.s. The large quantities of data acquired from capillary g.c.–m.s. runs necessitate fast data acquisition and data processing, including the capability for the automatic selection and refinement of key spectra. Even so, the chemist is faced with the identification and/or recognition of at least several hundred good quality spectra from a single run. Fast routine search procedures are useful here, especially for known compounds, while classification routines based on established rules for manual interpretation can be of assistance even with novel compounds. Examples from recent studies (at Bristol) of contemporary, Recent and ancient sediments, are presented. Geolipids show abundance patterns of homologous series which, while related to those of known organisms, display many novel features, including extensive carbon number ranges and stereospecific distributions. Additionally, certain carbon skeleton types, commonly thought to be rare as natural products, are major components of geolipid fractions, presumably reflecting the composite inputs and early microbial diagenetic activity.

Introduction

Organic compounds are now known to be components of nearly all sedimentary rocks, including those as old as the earliest investigated, from the Precambrian times over 3000 Ma ago. Most research has concentrated on the solvent-extractable lipid fractions (geolipids) which are both readily obtainable and amenable to analysis by modern chromatographic and spectrometric techniques. Mass spectrometry in particular has proved to be an essential tool in such analyses, and its use, especially in combination with gas chromatography, has led to the realization that many of the geolipids contained in ancient sediments have retained at least some portion of the carbon skeletons of the presumed original, biologically derived precursor lipids. This has led to the use of hydrocarbons, fatty acids and other compounds as 'biological markers' or 'chemical fossils' which can, in some cases, reflect their palaeoenvironment of deposition and also reflect the subsequent geological processes to which they have been subjected. This article attempts to illustrate some of the ways in which mass spectrometry is being used to study these complex mixtures of lipids from Recent and ancient aquatic sediments.

Many research areas which rely on extensive analytical data exhibit an interactive relation between the analytical techniques and the problems tackled and solved. Thus, the information which can be obtained by mass spectrometric analysis of complex mixtures is conditioned by the techniques employed and the consequent limitations in mixture separation, vaporization, ionization and fragmentation in the source of the mass spectrometer, resolution and quantitation of the ions and, finally, in the speed and capability of data processing for compound

identification. The complex mixtures present in geolipid and environmental lipid fractions are still being explored. An almost limitless range of structures may be possible, in view of the unknown nature of the original inputs and the effects of the environmental and geological processes. Thus it is not surprising that application of each new technique to sedimentary lipid studies has led to a further appreciation of their complexity. Hence, implementation of developments in mass spectrometry is immediately opening up important new vistas in organic geochemistry and environmental organic chemistry. However, the converse must be that we remain frequently unaware of hidden complexities: hence, appropriate provisos have to be entered against the identifications and quantitations obtained. Examples described in this paper illustrate these points.

Another aspect of this interrelation between the analytical techniques employed and the scientific problems addressed concerns the nature of the compound classes analysed. Here, as in other fields, the compounds found are highly dependant on those sought. Thus, Recent sediments must contain a multitude of multifunctional components of high molecular mass such as nucleic acids, proteins, polysaccharides and other complex biological molecules and their degradation products. However, these are not readily amenable to analysis involving the techniques commonly used in organic geochemistry and, in consequence, they are generally not looked for, or are not reported. The introduction of new techniques should lead to reports of these compounds in Recent and, possibly, some ancient sediments.

Organic geochemistry

As a broad generalization, organic geochemistry is concerned with the fate of carbon compounds both in the short and the long term. This continuum of time can be divided into two sections:

(i) the present and the recent past, i.e. the fate of organic compounds in contemporary environments, involving the water column and the underlying Recent sediments (environmental organic geochemistry); and

(ii) the remainder of the geological record, i.e. their fate in the underlying sediments and in ancient sediments.

Figure 1 indicates the flow of organic materials and the terminology involved. To be able to understand and rationalize the fate of the organic compounds through such a great span of time and variety of situations, much future research will be needed. Studies of the compounds present in the aquatic environments, the underlying Recent sediments and in the ancient sediments are complementary and mutually supportive; examples are given below.

Biologically formed organic compounds passing through the geological sequence (figure 1) carry information relating to their origin and history in the form of their precise molecular structure, relative abundances and their isotopic composition. Four general cases can be discerned: (1) molecule unchanged; (2) molecule partly altered, rearranged or degraded; (3) molecule extensively altered (scrambled); (4) molecule completely degraded to CO_2, etc. (mineralized). In the first case, an unchanged molecule may provide a direct indication of the original organism that contributed it to the environment or the palaeoenvironment. In ancient sediments only those molecules that are difficult to degrade by biochemical or chemical means would be encountered, an example being the C_{29} *n*-alkane, nonacosane. The presence or absence of unchanged compounds can therefore be informative about the original sources

and the subsequent conditions. The same applies to cases 2 and 3 where the emphasis lies in the changes brought about in the original structures and processes that may be inferred to have given rise to these changes. In the fourth case, the complete breakdown of the molecule may still give some information, e.g. in the form of its isotopic contributions localized perhaps in carbonates formed from the evolved CO_2 or in biogenic matter formed by organisms utilizing the evolved small molecules such as CO_2 or methane.

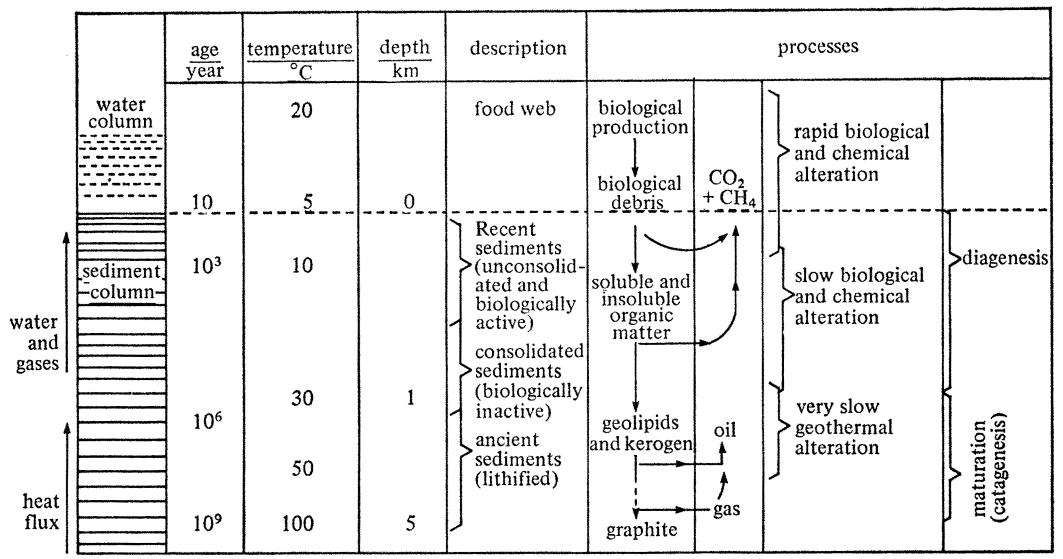


FIGURE 1. Fate of carbon compounds in aquatic environments and associated sediments. Most organic compounds entering natural aquatic environments are biogenic. Their fate, involving partial or complete mineralization to CO_2, may range from the short term (hours–years) in the water body to the long term (millions of years) in the deposited sediments. Note: ages, temperatures, depths and zones are generalized for this schematic, idealized system.

Considerable progress is being made along the lines indicated above but there remain major problems. Thus, an individual geolipid isolated from a sediment may contain molecules derived from a variety of sources, and thereby represent the summation of the operation of different chemical and physical pathways. In this case, multiple sources have contributed the compound or its derived products in disparate and varying amounts.

Organic geochemical studies of aquatic environments and related sediments

Fortunately, the mixtures of geolipids, while complex, have been found to comprise mainly compounds whose structures are recognizably related to those of major groups of contemporary natural products, typically of acetate and mevalonate derivation. However, the bulk of the sedimentary organic matter must have been produced and modified in the water column and in the underlying sediments, i.e. in the primary production of the phytoplankton and its subsequent fate through microbial action (figure 2). Comparatively little is known of the chemistry of the organisms involved, and the detailed information covering the particular compounds contributed or attacked by specific organisms is not available. Indeed, the microorganisms operating in aquatic sediments have received little study and are inadequately documented. In any case, they are certain to operate interactively in the sedimentary ecosystems. Faced with this situation, organic geochemists have documented the lipid components of a number

of individual aquatic organisms, but there are two categories of source material which seem especially relevant and which illustrate useful concepts at this present stage of development of the subject. These are the limited, 'mini'-laboratory ecosystems and the bottom sediments beneath well characterized water columns.

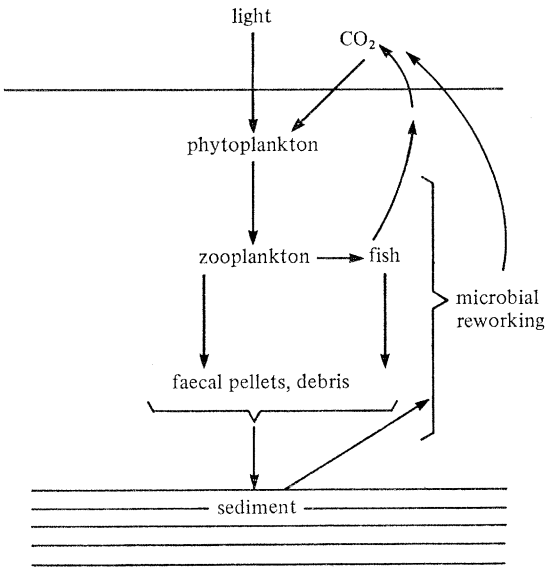


FIGURE 2. Aquatic ecosystem. A highly stylized and simplistic representation of the flow of carbon in an aquatic environment.

Laboratory ecosystems offer the opportunity to select a manageable portion of the aquatic environment and follow the generation and fate of individual lipids within it. For example, a joint project with E. D. S. Corner of the Marine Biological Association Laboratory at Plymouth is designed to study the role of marine zooplankton faecal pellets in the removal of planktonic lipids from the euphotic zone to marine bottom sediments. It involves analysis of the lipids of the phytoplankton food, copepods, and copepod faecal pellets for most classes of hydrocarbon, fatty acid, alcohol, sterol, etc. This study has already led to interesting findings concerning the origin of the wax esters found in Recent marine sediments, and will be discussed further in the text.

Bottom sediments must reflect, at least in part, the conditions and biological activity in the overlying water column. Correlation of sediment composition and water column characteristics for important types of water column should provide a firm basis for organic geochemical and palaeoenvironmental assessment of ancient sediments. One such site under active study by several groups, including that at Bristol, is on the continental shelf off Walvis Bay, SW Africa, in water depths of less than 200 m. Upwelling of nutrient-rich (Si, N, P), oxygen-poor, cold (5 °C) water mixing with oxygen-rich, warm surface waters brings about periodic blooms of phytoplankton. The algal material is deposited relatively rapidly, forming an organic-rich, highly reducing diatomaceous ooze, consisting largely of microbiologically reworked algal matter and bacterial debris. Land input from the adjacent Namib desert is known to be minor and the site, therefore, provides a useful type case for organic geochemical study.

Applications of mass spectrometry

Analyses for lipids extracted from environmental samples and from aquatic sediments have much in common with those conducted for lipids from biological systems. The same techniques may be used, though often the environmental and geological mixtures are more complex. The applications have been reviewed by Burlingame *et al.* (1976), and with the exception of computerized gas chromatography – mass spectrometry, will not be referred to in detail here. Recent developments include the application of field desorption methods to chlorophylls and other sensitive molecules: major advances can be expected in analyses of this range of moleuclar masses (to m/z 1000). Pyrolysis mass spectrometry and pyrolysis gas chromatography – mass spectrometry employ Curie point pyrolysis with or without gas chromatography before the mass spectrometry, followed by on-line data processing. Pyrolysis has been shown to afford a rapid means of evaluation of the general type of insoluble organic debris that makes up a major portion of the total organic matter in nearly all sediments (Weyman 1977). As in other fields, chemical ionization procedures should be of value for processing complex mixtures, with or without prior g.c. separation. High resolution mass spectrometry can be applied to mixtures and to single substances and affords greater reliability in the detection of individual compounds in that the elemental composition of the ions can be determined.

Combined gas chromatography – mass spectrometry plays a key role in the analysis of lipid fractions isolated from aquatic environments and from sediments. Most work is conducted with low resolution instruments employing fast scanning and glass capillary columns. Wall-coated glass capillary columns are now becoming widely adopted as they offer excellent separatory power at loadings that provide sufficient compound for the mass spectrometer. Careful attention to coating procedures and the nature of the connecting sections result in adequate control of adsorption and catalytic decomposition. There is still a need for improved test mixtures to search out the weaknesses of combined g.c.–m.s. systems employed in the analysis of lipid fractions extending to high molecular mass. However, hydrocarbon fractions extracted from ancient sediments are of a complexity that still defies these techniques. Prior separation, e.g. by adsorption or partition chromatography, can ensure that the complexity is reduced before analysis by g.c.–m.s.

Appropriate phases and high resolving power can result in the separation of stereoisomers of certain types of sedimentary lipids. For example, butanediol succinate or diethylene glycol succinate coated capillary columns afford separations of the diastereoisomers of certain sedimentary acyclic isoprenoid alkanes, ketones, alcohols (as acetates), and carboxylic acids (as methyl and menthyl esters) (Maxwell *et al.* 1972; Maxwell *et al.* 1973; Brooks *et al.* 1978). It should therefore be possible to determine routinely the stereoisomeric composition of these sedimentary compounds by g.c.–m.s., even though the stereoisomers do not differ markedly in molecular shape.

In relation to data acquisition and processing, the computerized g.c.–m.s. systems are required to accept scans of fractional second duration, detect over a wide dynamic range and to be capable of storage of several thousand scans from a single run. Commercial systems are now capable of processing data in such a way that background may be removed routinely and the spectra of partly separated components provided. Several procedures are available for automatic and semi-automatic recognition of common components of mixtures by file search and other techniques (see, for example, Kwok *et al.* 1973). Specialized libraries of components

frequently encountered in environmental and geological samples are desirable. Some methods take account of various features of the scans to achieve partial characterization of unknown and novel structures. These can be very useful in assessing geolipid fractions. Finally, it is advantageous to have the ability to profile classes of compounds either by following a limited number of ions during the analysis or by selecting ions from the full scan data after analysis. Computerized retrieval after analysis is extensively used in geolipid studies and is invaluable in providing fingerprints for correlation studies. When detection of the components at very low levels or with maximum reproducibility is required, then single or multiple ion monitoring is valuable.

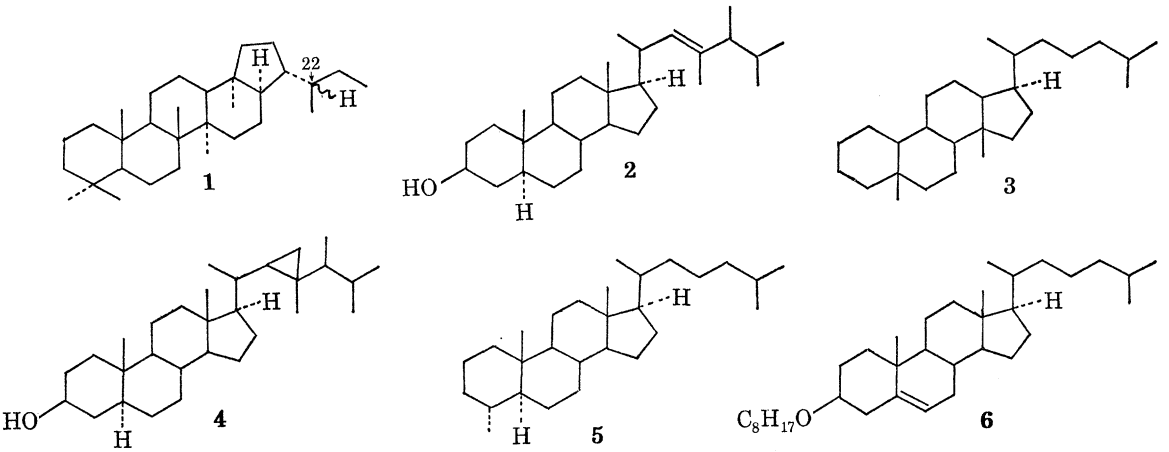
TABLE 1. EXAMPLES OF UNUSUAL 'NATURAL PRODUCTS' ISOLATED FROM SEDIMENTS

compound	occurrence	reference
1. 17αH-homohopane	mature ancient sediments and crude oils (C-22, *R* and *S*) Recent sediments (C-22 single isomer)	e.g. Brooks *et al.* (1977*a*)
2. 23,24-dimethyl-5α-cholest-22-en-3β-ol	Recent diatomaceous ooze, Walvis Bay	Wardroper *et al.* (1978)
3. diacholestane	mature ancient sediments and crude oils	Ensminger *et al.* (1978)
4. gorgostanol	Recent diatomaceous ooze, Walvis Bay	Wardroper *et al.* (1978)
5. 4α-methyl-5α-cholestane	mature ancient sediments	Rubinstein *et al.* (1975)
6. octylcholesteryl ether	Recent diatomaceous ooze, Walvis Bay	Boon (1978)
7. perylene	Recent reducing sediments, immature ancient sediments and petroleums?	e.g. Aizenshstat (1973)
8. dehydroabietic acid	Recent and immature ancient sediments	Simoneit (1977)
9. aetioporphyrin-III	ancient sediments and crude oils	Baker *et al.* (1967)
10. deoxophylloerythroaetioporphyrin	ancient sediments and crude oils	Baker *et al.* (1967)
11. 2,6,10,14,18,22,26,30-octamethyl-dotriacontane	crude oil	Albaiges *et al.* (1978)
12. 2,6,10,14,19,23,27,31-octamethyl-dotriacontane	ancient sediments	Kimble *et al.* (1974)
13. dihydrophytol	Recent sediments and immature ancient sediments	e.g. Maxwell *et al.* (1973)
14. 4,8,12,16-tetramethylheptadecanoic acid	ancient sediments	Maxwell *et al.* (1973)

When the components of large numbers of sedimentary samples require recognition for comparison purposes, computer classification of g.c.–m.s. mass spectra can be useful. Although file search methods are the most widely used (see, for example, Heller *et al.* 1974), another approach for spectrum recognition uses simple algorithms based on empirical rules derived from the fragmentations of known members of the compound class under study (see, for example, Gray & Gronneberg 1975). The latter include steroidal and triterpenoidal alkanes, steroidal monoenes, sterols and stanols (as trimethylsilyl ethers) (Wardroper *et al.* 1977; Gaskell 1974; Wardroper 1979). This 'Interpreter' approach is advantageous in that it can be implemented on a small laboratory computer, allows distinction between closely related spectra, and can provide structural information about unknowns if the fragmentation pattern follows that of the available standards of the compound class (Wardroper *et al.* 1977). Problems with this approach to spectral interpretation include the level of background, reproducibility of spectra and insufficient g.c. resolution.

One of the most remarkable features of the distribution of pentacyclic triterpanes in ancient sediments and petroleums is that these distributions are dominated by members of the hopane and closely related skeletons (Van Dorsselaer *et al.* 1974). Thus, mature sedimentary rocks and petroleums contain mainly mixtures of the 17αH-hopane series (e.g. **1**, table 1) extending

from C_{27} to C_{35} (excluding the C_{28} member), the side chain at C-21 varying from H to $-CH(CH_3)(CH_2)_5CH_3$ and occurring as virtually equimolar mixtures of C-22 diastereoisomers. In Recent sediments the C_{31} compound (**1**) appears to occur only as one of the two C-22 diastereoisomers, unless the sediment is polluted by petroleum products. In these cases an unequal distribution of the isomers is observed (Dastillung & Albrecht 1976). It is believed that the precursors of these sedimentary compounds are functionalized hopane triterpenoids biosynthesized by blue-green algae and bacteria, which are altered and degraded in the water column and the bottom sediments (e.g. Rohmer & Ourisson 1976). Identifications by g.c.–m.s. and comparison with synthetic standards are now routine, since the stereoisomers found (17βH,21βH; 17αH,21βH; 17βH,21αH) show distinct differences in the relative abundances of the ions m/z 191 and (148 + R) where R = alkyl side chain. The wide occurrence and the apparent resistance to biodegradation of the hopanes has led to the application of the distribution as fingerprint parameters in oil exploration and pollution studies (Seifert & Moldowan 1978; Dastillung & Albrecht 1976; Brassell *et al.* 1978). Although the absolute amounts are often low, mass fragmentogram or multiple ion detection (m.i.d.) techniques with the use of m/z 191 and (148 + R) readily provide usable fingerprints.



The most investigated tetracyclic molecules to date in the geosphere are based on the steroid skeleton (e.g. **5**). Most research has centred on the alkane and alkene species, together with the sterols and stanols from which the former are presumed to be derived. The steranes appeared to be late stage products found only in ancient sediments and petroleums. The mixtures are complex but the high structural specificity allows the distributions to be used as fingerprint parameters, as with the hopanes. Multiple fingerprints can be provided by mass fragmentography or m.i.d. with the use of the ion m/z 217 (most steranes), 218 (14βH-steranes), 231 (4-methyl steranes). The relative intensities of minor ions in the spectra of regular steranes, such as m/z 259 (loss of side chain) and m/z 262 + 14n (loss of ring A, C-6 and 19) allow differentiation of the 8β,14α(H), the 8β,14β(H) and the 8α,14β(H) configurations (Mulheirn & Ryback 1977).

In some sedimentary rocks, the relative abundances of 4-methyl steranes (e.g. **5**) with respect to steranes are surprisingly high in view of the low abundances of 4-methyl sterols in commonly studied organisms. The methane-utilizing bacterium *Methylococcus capsulatus* is a noticeable exception (Bird *et al.* 1971). It seems likely that there are abundant organisms that contribute

organic matter containing 4-methyl sterols to forming sediments. This situation typifies the interesting aspects of natural product chemistry revealed by sediment analysis.

Rearranged steroidal skeletons also occur in mature samples in the form of rearranged steranes ((**3**) diasteranes); these compounds are thought to arise from precursor sterols via acid catalysed rearrangement of Δ^2-sterenes and stanols (Rubinstein *et al.* 1975). Non-stereospecific reduction in the sediment of the $\Delta^{13,17}$ rearranged sterenes thus formed would afford mixtures of stereoisomers at C-13 and C-17 (e.g. **3**).

Mass spectra of both the 13,17-βα- and αβ-diasteranes are characterized by a significant contribution of m/z 259 (loss of side chain). However, the two series can be distinguished by the presence of m/z 232 in the αβ series. 4-Methyl substituted diasteranes are characterized by $M^{+\cdot} - 71$ (ring A fragmentation) (Ensminger 1977).

The precursor biolipids of the geological steranes, the sterols, occur widely in organisms and therefore are not unexpected as components of Recent sediments. However, unlike in organisms, the corresponding Δ^5 saturated derivatives, the stanols, occur in much higher relative abundances compared with sterols. There are believed to be two reasons for this; reduction of the Δ^5 double bond by microorganisms in the sediments and selective degradation of the sterols relative to stanols. This would lead to an enhancement of the initially low concentrations of contributed stanols. The mass spectral fragmentations of the trimethylsilyl ethers of such compounds have been widely discussed (see, for example, Brooks *et al.* 1968). G.c.–m.s. studies are routinely used to identify constituents of sedimentary stanol mixtures: a recent study (Wardroper *et al.* 1978) of a marine sediment from Walvis Bay (see below) has shown that the enhanced separation afforded by glass capillaries over packed columns in g.c.–m.s. is necessary for analysis of sedimentary mixtures. Two unusual reduction products (**2** and **4**) of marine sterols were identified by mass spectral interpretation and comparison of the mass spectrum of the corresponding sterol and by mass spectral interpretation and comparison with an authentic standard, respectively. Another novel series of sterol derivative, exemplified by **6**, has been reported in the same sediment (Boon 1978); this finding must now be followed by a search for the originating organism since such ethers seem unlikely to be geochemical products.

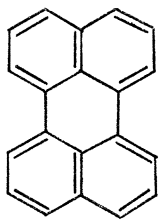
Alkylated polynuclear aromatic hydrocarbons occur widely in mature geological sample and the carbon skeletons of many of these can be related directly to precursor steroidal and triterpenoidal compounds. Recent sediments have been found to contain mixtures of unsubstituted polynuclear aromatic hydrocarbons (see, for example, Laflamme & Hites 1978). However, there are several reports of high relative abundances of perylene (**7**) in Recent and immature ancient sediments. This compound is thought to derive from reduction of hydroxyquinone precursor(s), possibly contributed by fungi and other microorganisms (Aizenshstat 1973). Like the other sedimentary polynuclear aromatics, perylene is readily identified by glass capillary g.c.–m.s.

A tricyclic aromatic compound (**8**), found distributed in Recent and immature ancient sediments, almost certainly has its origin in conifer diterpenoids: it has therefore been suggested as an indicator of terrestrial forest input to sediments (Simoneit 1977).

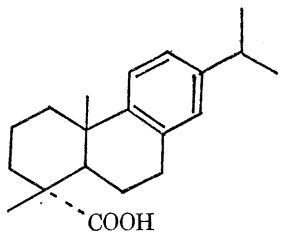
The petroporphyrins are red pigments found in ancient sediments that are believed to be derived from chlorophyll *a* and related biological pigments via intermediary chlorins as a result of diagenetic and maturational changes in the sediments. The petroporphyrins occur both as the free and metallated species, the latter being mainly the nickel and vanadyl complexes. Light absorption spectrophotometry can be used to distinguish between the aetioporphyrin

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

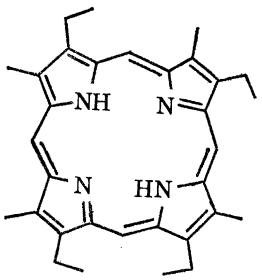
and deoxophylloerythroaetioporphyrin systems (**9** and **10**) and to provide a sensitive measure of the quantities of petroporphyrins present. However, mass spectrometry is required to estimate the range of 'homologues' composing the mixtures. Electron impact mass spectrometry affords intense molecular ions and has traditionally been used to determine these mixtures. The problem of distinguishing the structural and positional isomers is formidable, however, and presents a considerable challenge. This problem is discussed further below.



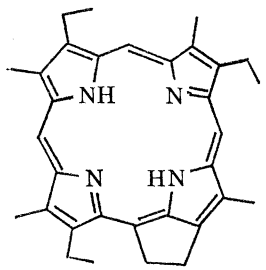
7



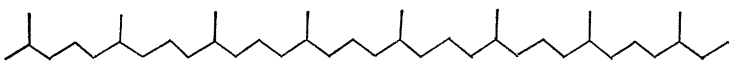
8



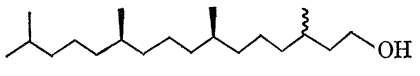
9



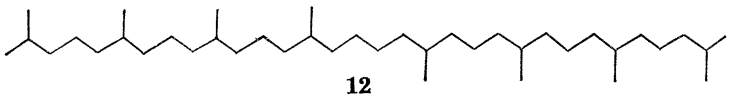
10



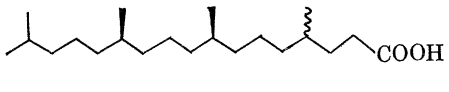
11



13



12



14

The acyclic isoprenoids, represented by **11** and **12**, are major components of the branched/cyclic alkane fraction of ancient sediments. Compound **11** is the C_{40} regular isoprenoid and is, to date, found to be less widely distributed than **12**, the tail-to-tail linked C_{40} perhydrocarotenoid. The precursors of these alkanes are believed to be the corresponding unsaturated and oxygenated tetraterpenoids, but as these alkanes, as with the steranes, are not known components of living organisms, it is presumed that they have arisen by reduction and defunctionalization during diagenesis and maturation. Of prime importance here is the determination of stereochemistry, but as yet there are no methods available. Capillary g.c.–m.s. does afford

recognition of these gross structures by coinjection and by the fragmentation patterns corresponding to the methyl branches. The fragmentations are, in some cases, not sufficiently discriminatory owing to the low intensity of the corresponding ions.

Compounds **13** and **14** are examples of a functionalized diterpenoid and a homoditerpenoid, respectively. Once again, the stereochemistries would provide important information. For **13** the relative, but not absolute, stereochemistry can be ascertained by careful capillary g.c. of the acetates. In **14**, the interest lies in the additional carbon represented by the extended chain. The series extends at least to C_{22}. Stereochemistry, once again, can be ascertained by the use of the methyl and menthyl esters. Capillary g.c.–m.s. provides identification of the branch points but, as before, standards are important as the fragmentation patterns are dominated by the structures closest to the carboxyl group.

The preceding examples have indicated some of the compound types that have been found in Recent and ancient sediments. The discovery of these and other compounds has opened up new areas of work, particularly the search for the precursors and the organisms that have generated them. Some of these compounds may serve as useful indicators of palaeoenvironment and of the generation of geological processes.

In each of the following three sections, a single class of lipid is discussed in detail to exemplify further the way in which mass spectrometry is being used to explore the fate of lipids in the environment and geosphere. The three groups selected are the wax esters, the steroids and the chlorophyll-derived pigments.

TABLE 2. EXAMPLES OF NATURAL DISTRIBUTIONS OF WAX ESTERS

type	chain length range	features	reference
higher plant			
maize	38–58	saturated	Tulloch (1975)
carnauba palm	44–62	saturated	Tonani & Bianchi (1976)
insect			
beeswax	40–52	saturated	Holloway (1969)
marine			
copepods	32–44	highly unsaturated	Sargent *et al.* (1977)
spermaceti	26–38	even-chain saturated	
phytoplankton	—	absent?	Lee *et al.* (1971)
aquatic sediments			
North Sea	?	present	Sargent *et al.* (1977)
Walvis Bay	32–44	saturated?	Boon (1978)
Corner Inlet†	28–36	saturated and unsaturated	Volkman (1977)
Lonnekermeer‡	40–51	saturated?	Boon (1978)

† Intertidal flats, Australia. ‡ Lacustrine, Holland.

FATE OF WAX ESTERS

Long-chain alkyl esters (wax esters, RCOOR′) are widely distributed in nature, particularly in the leaf waxes of higher plants and in marine organisms. Table 2 lists some of the wax ester distributions characteristic of higher plants, insects, marine organisms and sediments. In general, the wax esters of higher plants consist almost entirely of even carbon number straight chain saturated esters, extending from C_{30} to C_{68} total chain lengths (Tulloch 1975). This is in sharp contrast to the wax esters found in the marine environment where the chain length distributions are considerably more restricted (C_{26} to C_{42}) and the wax esters contain up to

six double bonds (Nevenzel 1970). There are relatively few reports of wax esters in Recent sediments, although this is almost certainly a reflexion of the difficulties associated with their analysis rather than their actual abundance. Wax esters of concentrations of 0.2 mg/100 g of sediment in sediments from the North and Irish seas have been reported (Sargent *et al.* 1977), indicating a considerable input of esters, at least in these marine environments. Montan wax, commercially extracted from lignite, contains saturated waxes, C_{44} to C_{68}.

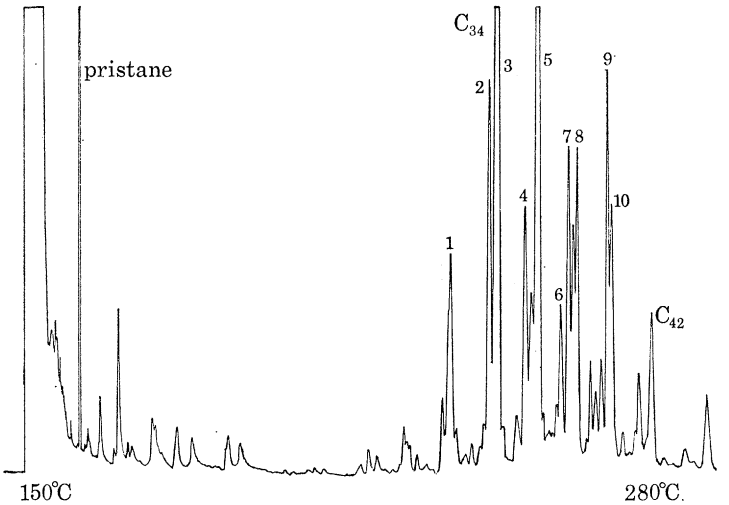


FIGURE 3. Gas chromatogram of total volatile lipids of *Calanus finmarchicus* (sample provided by J. R. Sargent, Institute of Marine Biochemistry, Aberdeen). Conditions: 8 m × 0.3 mm i.d. OV-1 glass WCOT capillary column, temperature programmed from 150 to 280 °C at 4 °C/min; injector and detector held at 350 °C; helium carrier gas at 5 cm^3/min. Peak 1, 32:3; peak 2, 34:4; peak 3, mainly 34:1, plus 34:2, 34:3; peak 4, 36:5; peak 5, 36:1; peak 6, 38:5; peak 7, 38:3; peak 8, 38:1; peak 9, 40:4; peak 10, 40:2. Wax esters are designated by number of carbon atoms : number of double bonds.

The involatility of wax esters presents serious analytical difficulties associated with their analysis. One approach has been to obtain a mass spectrum of the total wax ester distribution (Tonani & Bianchi 1976) and from the molecular ions present deduce the chain length distribution in the mixture. It is difficult to obtain quantitative data with this approach and it also provides no information concerning the specific combinations of alcohols and acids present.

A more desirable approach is to gas chromatograph the wax esters and thus directly obtain the chain length distribution. Some success has been obtained in this area with packed columns (Tulloch 1975), but the low resolution and requirement of high elution temperatures present a barrier to the use of these columns to study more complex mixtures. Furthermore, few studies have used mass spectrometry in conjunction with g.c. to characterize the wax ester distribution.

In this paper we report the use of short (10 m or less), moderate bore (0.3 mm i.d.) glass capillary columns directly coupled to the mass spectrometer to study wax esters extending up to C_{50} in chain length. These columns offer both good resolution and low adsorption combined with the advantage of much lower elution temperatures, thereby facilitating g.c.–m.s. analysis.

This approach is illustrated in figure 3, which shows a gas chromatogram of the total lipids of the copepod *Calanus finmarchicus*. This lipid extract was not prefractionated before g.c. since

wax esters are the major lipid class present and involatile lipids such as phospholipids and triacylglycerols remain on the glass liner in the injector. Esterification and silylation of the lipid extract makes it possible to analyse all of the volatile lipids present in the one g.c.–m.s. analysis, particularly if the mass fragmentography approach is used. Other techniques such as Ag^+–SiO_2 t.l.c. would allow prefractionation of wax ester fractions according to unsaturation.

In the sample of *Calanus finmarchicus*, the wax esters range from C_{30} to C_{44} and are mainly of even carbon number. The chain length distribution calculated from the g.c. areas is C_{30} (0.3%), C_{32} (3.8%), C_{34} (24.6%), C_{36} (28.0%), C_{38} (20.1%), C_{40} (15.5%), C_{42} (5.5%) and C_{44} (2.2%).

It is evident from figure 3 that the capillary column achieves a partial separation of wax esters differing in the degree of unsaturation. It thus becomes possible to determine the specific combination of fatty alcohols and fatty acids from the mass spectrum of each g.c. peak using the molecular ion $(RCOOR')^{+\cdot}$ and ions characteristic of the fatty acid $(RCO_2H_2)^+$ and fatty alcohol $(R'–H)^+$ (Aasen *et al.* 1971). Thus, the major wax ester 36:1 is found to be a mixture of 14:0/22:1 and 16:0/20:1 (acid/alcohol) in the ratio of 8:3. It is interesting that despite the presence of C_{18} fatty acids and alcohols in the hydrolysis products, there appears to be no significant contribution of these chain lengths to the 36:1 ester. Of course, double bond positions and stereochemistry remain to be determined: techniques are available for the individual acid and alcohol moieties.

To date there is only one detailed examination of the wax esters occurring in a marine sediment. Boon (1978) found that the wax esters in a sediment from Walvis Bay, Namibia, ranged from C_{32} to C_{44} and were exclusively saturated. This chain length distribution is suggestive of an origin from zooplankton (copepods) but the absence of unsaturation is very unlike the distributions commonly found in zooplankton (table 2). Hydrogenation of the deposited wax esters is one possibility but bacterial syntheses must also be considered. It is hoped that now that analytical techniques are available to study the isomeric composition of wax ester distributions, it will be possible to assign the origin of wax esters in sediments with more certainty. Feeding experiments involving the laboratory system (phytoplankton, copepod, faecal pellet) already mentioned, are in progress. Preliminary results indicate that the copepod faecal pellets contain only traces of the wax esters so dominant in the copepods themselves.

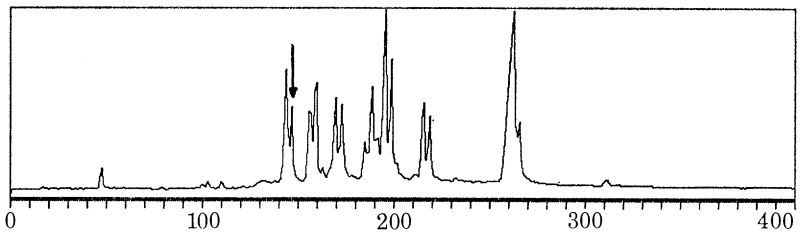
Fate of sterols

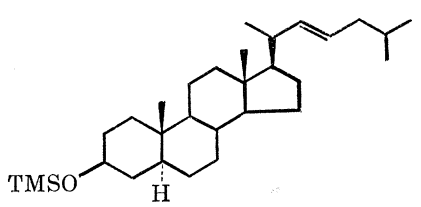
Sterol distributions have been proposed as indicators of the input to sediments. The autochthonous and allochthonous components in marine and lacustrine systems can be monitored by comparing the relative concentrations of sterols characteristic of plankton and terrigenous plants, respectively (Huang & Meinschein 1976; Nishimura 1978). The ratio of 5α- to 5β-stanols in sediments may be attributable to E_h conditions prevailing at the time of deposition (Reed 1977).

An example of a contemporary environment being examined is Walvis Bay, where green diatomaceous oozes are found. Extraction of a sample of bottom sediment and t.l.c. separation of the neutral fraction (SiO_2 gel G; CH_2Cl_2) yielded a Δ^5-sterol and 5α-stanol fraction. The computer-reconstructed total ion current (t.i.c.) of the g.c.–m.s. analysis of this fraction is shown in figure 4, together with the mass spectrum, structure and Interpreter output for one of the components, 5α-cholest-22-en-3β-ol. This particular analysis revealed novel sedimentary lipids; the two major sterols are 23,24-dimethylcholesta-5,22-dien-3β-ol and 22,23-methylene-23,24-dimethylcholest-5-en-3β-ol (gorgosterol), as shown by g.c.–m.s. and by coinjection on

glass capillary columns (Wardroper *et al.* 1978), perhaps contributed by coelenterates and/or their symbiotic algae.

Every sterol co-occurs with its 5α-saturated analogue. The ratio of sterol to stanol varies from 1:1 to 7:1, thus indicating a direct contribution of stanols to the sediment, unless microbial hydrogenation occurs preferentially for certain compounds. Relatively simple distributions such as these are ideal for the Interpreter approach.





spectrum of Walvis Bay sterols
spectrum code number: 147
sum of ion currents: 22967
number of ions: 336
*** suggested structure is D22 C27 sterol
sum of ion currents accounted for: 6047
number of ions accounted for: 8
molec. mass = 458
derivation of structure was
D22 sterols
sterol TMSi

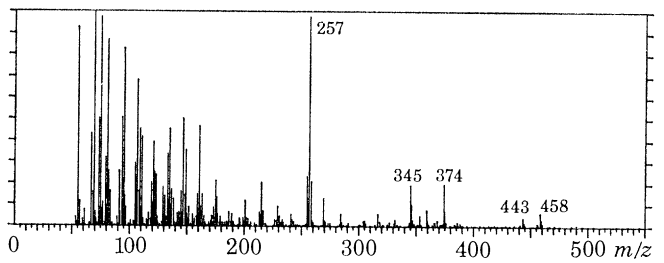


FIGURE 4. Computer reconstructed t.i.c. (top) for Walvis Bay 5α-stanols and sterols, plus mass spectrum (bottom), structure and Interpreter output for scan number 147. Experimental conditions: OV-1 25 m × 0.25 mm programmed from 50 to 260 °C at 6 °C/min; data acquisition from 220 °C; scan time 2.5 s; ion source temperature 250 °C; filament current 400 μA and electron energy of 35 eV.

As the maturity of geological samples increases, so does the complexity of the steroidal derivatives in the extracts. For example, the sterane distributions of crude oils are particularly complex owing to scrambling of original stereochemistry; it has been shown that epimerization at C-24, 20 and 14 occurs (Mulheirn & Ryback 1975). Such mixtures present severe problems for analysis by g.c.–m.s., and pre-analysis simplification procedures are useful.

Figure 5 shows the distribution of thiourea adduct and non-adduct of the Rozel Point (a crude oil) branched and cyclic alkane fraction, which is remarkable in consisting almost exclusively of steranes, with no detectable diasteranes and only minor amounts of triterpanes. Coinjection with authentic standards on high resolution g.c. is essential, owing to the similarity of sterane mass spectra.

Another example of the complexity of geological mixtures is shown in figure 6, which shows the t.i.c. of a g.c.–m.s. analysis of branched and cyclic alkanes from a mature sample of the Toarcian shales (Lower Jurassic; 180 Ma) of the Paris Basin. Also included are mass fragmentograms for m/z 217 and m/z 191, characteristic of steranes and triterpanes respectively.

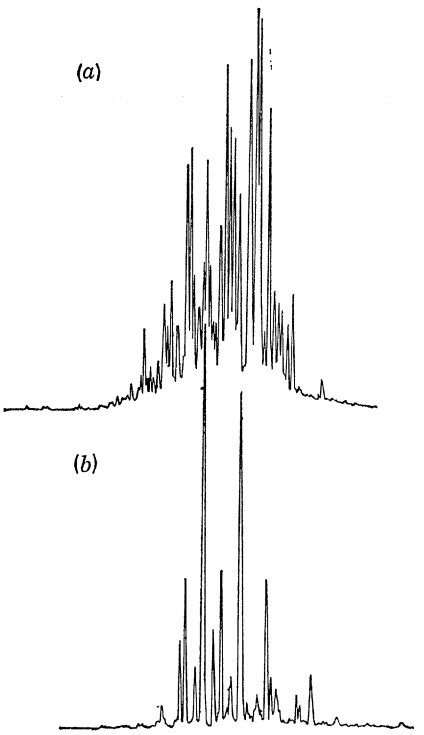


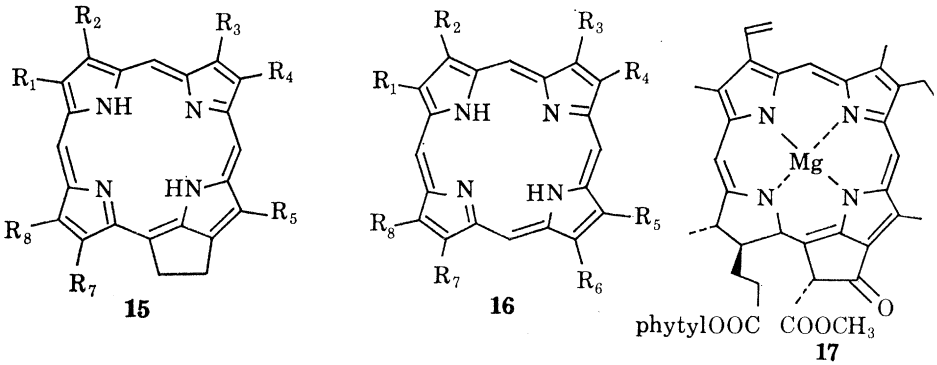
FIGURE 5. Partial g.c. traces of the non-adduct (*a*) and thiourea adduct (*b*) of the branched and cyclic alkanes from Rozel Point crude oil. Experimental conditions: OV-1 20 m × 0.25 mm, programmed from 130 to 260 °C at 2.5 °C/min. Portions shown above 240 °C.

This demonstrates the necessity of such a fingerprinting technique for monitoring relatively small amounts of specific compound classes in such complex mixtures. The inset on figure 6 shows the molecular ion region for scan 184. The Interpreter method is unable to cope with such spectra. Developments such as higher resolution g.c. and faster scanning, combined with computer techniques for 'cleaning up' data (Biller & Biemann 1974) are certainly required.

The final fate of sterols in the geosphere is unknown. Steranes are not present in the most mature oils and oldest sediments examined, maybe owing to degradation or possibly because sterols were not biosynthesized by ancient organisms (Hollerbach & Welte 1977).

FATE OF CHLOROPHYLL *a*

Petroporphyrins were first isolated by Treibs (1934). He recognized two major series: the deoxophylloerythroaetioporphyrin (DPEP) series (**15**) and the aetioporphyrin series (**16**). Corwin (1959) proposed that both series of petrophyrins with carbon skeletons up to C_{32} were mainly produced through loss of functional groups and decarboxylation of the two carboxyl moieties of chlorophyll *a* (**17**), by far the most abundant of the naturally occurring chlorophylls, by slow low temperature thermal processes. Three other naturally occurring chlorophylls,



(**15**), (**16**): R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 = alkyl or H.
(**16***a*): $R_1 = R_4 = R_5 = R_8 = CH_3$;
$R_2 = R_3 = R_6 = R_7 = C_2H_5$.

chlorophyll *b*, chlorophyll *c*, chlorophyll *d*, together with bacteriochlorophylls a_p, a_g and *b*, have the same chlorin skeleton and might also be expected to behave similarly. After the discovery of petroporphyrins with carbon skeletons greater than C_{32} it was proposed that the *Chlorobium* chlorophylls could be additional precursors (Baker *et al.* 1967), producing petroporphyrins with carbon skeletons up to C_{37}. As each carbon number skeleton has many possible isomers, elucidation of the precise structures would lead to a better understanding of the fate of chlorophylls, thus providing information about the mode of degradation of vinyl, carboxyl and, possibly, formyl groups of the precursor compounds, together with an insight into the manner in which petroporphyrins undergo thermal maturation.

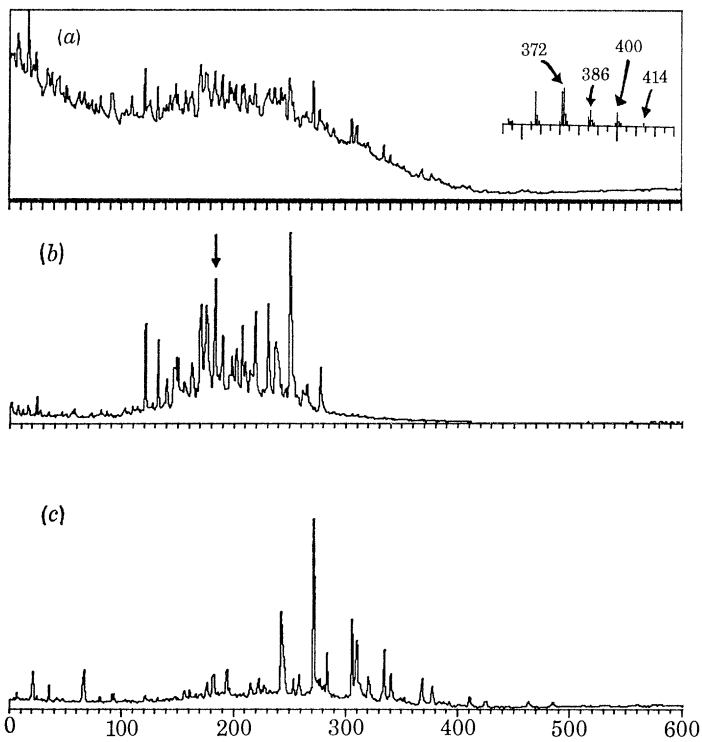


FIGURE 6. Computer reconstructed t.i.c. (*a*) for Cesarville branched and cyclic alkanes, together with mass fragmentograms for *m/z* 217 (*b*) and 191 (*c*); multiplication factor for (*b*), ×6.7; for (*c*), ×4.4. The inset shows the molecular ion region for scan number 184. Experimental conditions: as for figure 4 except programmed from 50 to 260 °C at 4 °C/min; data acquisition from 200 °C; scan time 2.0 s.

Analysis of petroporphyrin mixtures

Electron impact mass spectrometry (e.i.m.s. by direct insertion probe) has provided the principal tool for the analysis of petroporphyrin mixtures. The interpretation of e.i. mass spectra needs much care as the volatility of individual porphyrins varies, presumably owing to differing alkyl substitution patterns. For example, the relative intensities of the ions at m/z 476 and 462 (figure 7) show that the C_{32} DPEP porphyrin is slightly more volatile than the C_{31} DPEP porphyrin.

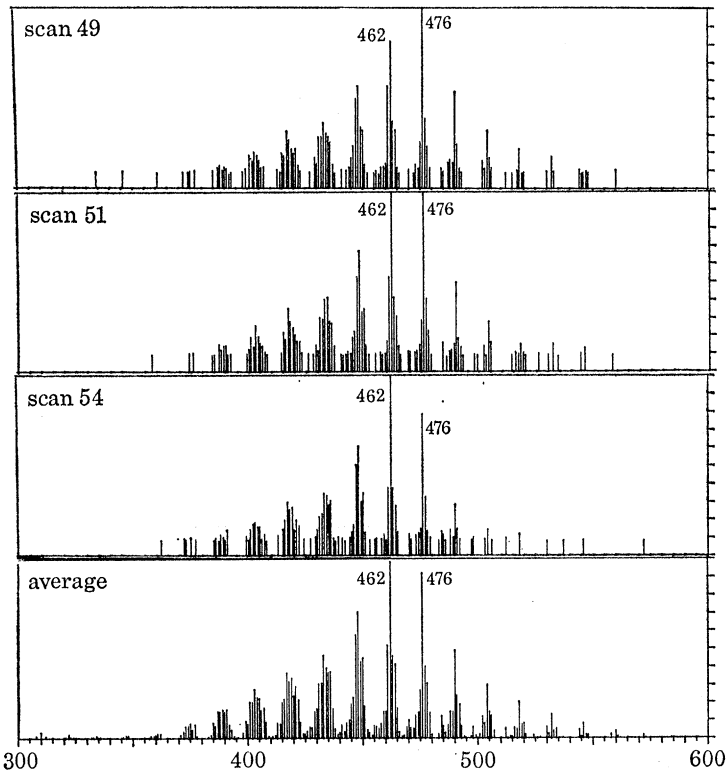


FIGURE 7. Sequential and averaged e.i. mass spectra (m/z 300–600) of the total petroporphyrin fraction of Boscan BN23 oil. Mass spectra were obtained with a Varian MAT CH-7 mass spectrometer. Operating conditions consisted of a source temperature of *ca.* 230 °C, electron energy of 70 eV and emission current of 350 μA. The direct insertion probe was temperature programmed from ambient to 300 °C at *ca.* 20 °C/min. All of the porphyrin-containing spectra were averaged.

To quantitate accurately the individual carbon number components in a mixture by using such e.i.m.s. analyses, it is necessary to operate the mass spectrometer in a temperature-programmed cyclical scanning mode, and to average the respective ion intensities of the scans covering the entire range of porphyrin volatilities.

Literature concerning petroporphyrin distributions in which only a single scan mass spectrum is used for quantitation has to be treated with caution. Usually the aetioporphyrin and DPEP petroporphyrin distributions resemble two apparently Gaussian envelopes (see, for example, Baker *et al.* 1967). Thus, each series appears to be homologous by analogy with the distributions of alkanes in oils and shales. This analogy is rather misleading, since neither the aetioporphyrin nor the DPEP petroporphyrins are homologous series as there are notable differences in substitution patterns.

The technique of g.c.–m.s. has been applied with limited success to the porphyrins. Although the involatility of free base porphyrins precludes their analysis by g.c., the silicon complexes are sufficiently volatile to permit such analyses, provided the two hydroxyl ligands complexed to the silicon are silylated. Mixtures of standard porphyrins have been successfully analysed by using OV-1 packed g.c. columns (Boylan *et al.* 1969). The technique has much untapped potential, particularly with the development of capillary g.c., as it may be possible to separate porphyrin type (i.e. positional) isomers.

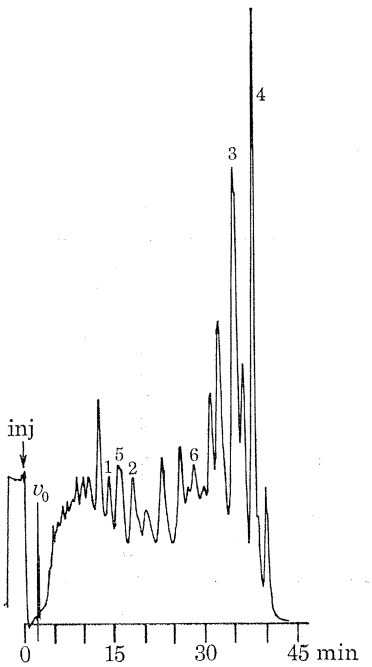


FIGURE 8. H.p.l.c. of petroporphyrins of Boscan BN23 oil. Conditions: 25 cm × 4.6 mm i.d. stainless steel columns were packed with Partisil (5 μm Whatman). Gradient elution was performed with the following solvent mixtures: system A (hexane:toluene 9:1 by volume) and system B (toluene:chloroform 1:1 by volume). The programme started at 20 % B, reaching 100 % B after 40 min. The flow rate was 1.5 ml/min with a concave gradient, monitoring at 400 nm. Peak 1, C_{32} aetioporphyrin; peak 2, C_{31} aetioporphyrin; peak 3, C_{32} DPEP porphyrin; peak 4, C_{31} DPEP porphyrin; peak 5, C_{29} aetioporphyrin; peak 6, C_{29} aetioporphyrin.

Recently, the application of high pressure liquid chromatography (h.p.l.c.) has added a new dimension to the analysis of petroporphyrin mixtures (HajIbrahim *et al.* 1978), whereby it is possible to resolve petroporphyrin mixtures into more than 17 components (figure 8). The trapping of the individual peaks followed by e.i.m.s. analysis has shown that petroporphyrins exist in isomeric forms. In Boscan BN23 oil there are at least two C_{29} aetiopetroporphyrins (peaks 5 and 6, figure 8). The large difference in retention time between the two components indicates that they are structural rather than positional isomers. The interfacing of h.p.l.c. and a mass spectrometer provides an ideal tool for the analysis of a total petroporphyrin mixture, and preliminary studies (W. H. McFadden 1978, personal communication) have enabled a partial analysis of a petroporphyrin mixture; the mass fragmentograms of some components are shown in figure 9. Although h.p.l.c.–m.s. yields general information on the number and species of petroporphyrins in a mixture, it provides very little information on the structure of individual carbon number components. Porphyrins have relatively simple e.i. mass spectra, usually showing molecular ions as base peak, and the principal fragmentation

ions involve alkyl losses owing to benzylic cleavage of side chains (Smith 1975). By examination of a petroporphyrin mass spectrum it is possible to confirm the presence of ethyl or propyl groups, but it is not possible to determine either the number or disposition of these groups about the macrocycle.

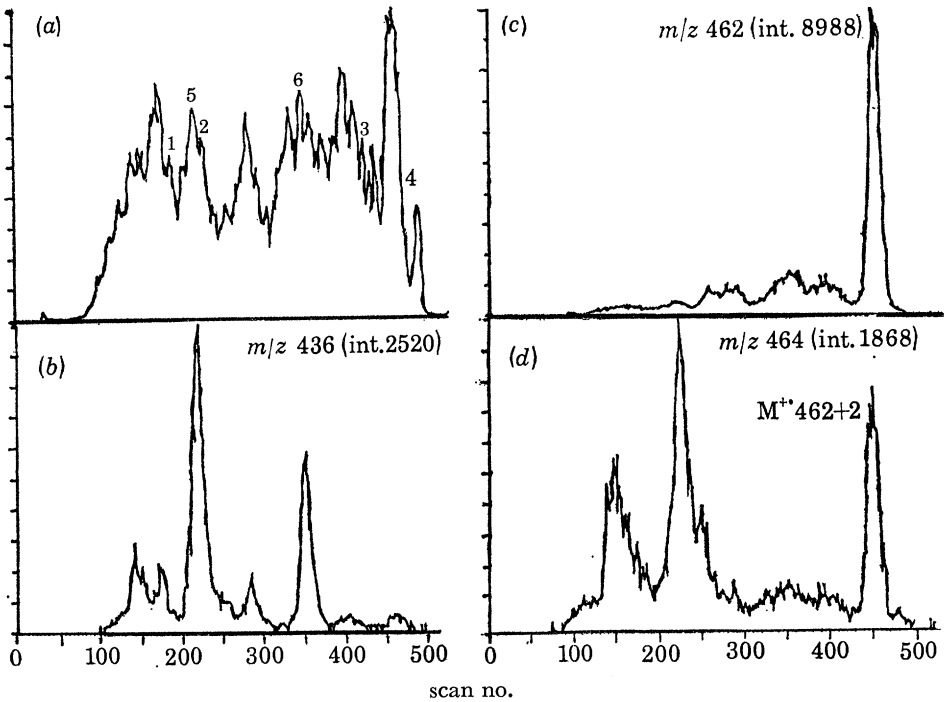


FIGURE 9. Total ion current (*a*) and mass fragmentograms of C_{29} aetioporphyrin (*b*), C_{31} DPEP (*c*) and C_{31} aetioporphyrin (*d*) petroporphyrins of Boscan BN23 oil. The h.p.l.c.–m.s. interface has been described elsewhere (McFadden *et al.* 1976). The vaporizer–ion source temperature was 310 °C. A Kapton belt (Dupont polyimide) was used for solute transfer. The clean-up heater was set at 350 °C. The h.p.l.c. programme is the same as that described in figure 8. The solvent flow was split 1:10 through a zero dead volume splitter before introduction to the interface belt. Data were acquired and processed by using a Finnigan 6110 data system. Mass spectra were scanned from *m/z* 400–650 and scan points collected at 10 s intervals. Peak 1, C_{32} aetioporphyrin; peak 2, C_{31} aetioporphyrin; peak 3, C_{32} DPEP porphyrin; peak 4, C_{31} DPEP porphyrin; peak 5, C_{29} aetioporphyrin; peak 6, C_{29} aetioporphyrin.

By using chemical ionization mass spectrometry (c.i.m.s.), a milder form of ionization, it is usually possible to obtain a much less complex mass spectrum than would be obtained by e.i.m.s. C.i. has been used for the quantitation of partially separated petroporphyrin mixtures (Shaw *et al.* 1978). However, its use as a quantitative tool is limited owing to the generation of a cluster of quasi-molecular ions $[M-1]^+$ to $[M+7]^+$ which prevents the quantitative analysis of mixtures containing DPEP and aetiopetroporphyrins of the same carbon number because of overlap between the clusters, e.g. a C_{31} DPEP petroporphyrin would yield a quasi-molecular ion cluster of *m/z* 461–469 which overlaps with the cluster produced by a C_{31} aetiopetroporphyrin (*m/z* 463–471). However, if separations of DPEP and aetiopetroporphyrins can be achieved by thin layer chromatography, carbon number distributions can be estimated by c.i.

Recently we have found that the c.i. mass spectrum of a standard porphyrin, aetioporphyrin-II (**16*a***), with methane as reagent gas, is dependent on the ion source temperature. Thus, at

300 °C, the quasi-molecular ion $[M+1]^+$, m/z 479, is the base peak, and there is little fragmentation as might be expected, but on lowering the source temperature to 200 °C, it becomes relatively minor (*ca.* 15% of base peak) and several species of fragments appear (figure 10). This effect may be attributable to the reductive degradation of the *meso* positions of the porphyrin macrocycle to yield pyrroles, in a manner analogous to the reductive degradation of porphyrins with the use of hydrogen iodide and acetic acid (Chapman *et al.* 1971). For aetioporphyrin-II, four pyrroles would be produced by such a process, two of which would have the same molecular ion (figure 10). It has previously been observed that such reduction of porphyrins may occur within the source of a mass spectrometer, as the quasi-molecular ion cluster at a 300 °C source temperature (CH_4 c.i.) reveals ions at m/z (M+2), (M+3) ..., of an intensity that cannot be accounted for by ions containing ^{13}C (Shaw *et al.* 1978).

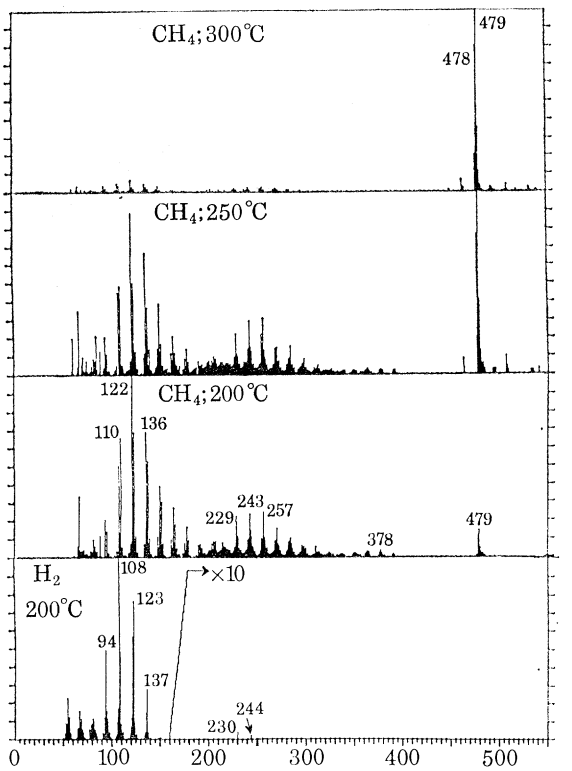


FIGURE 10. C.i.m.s. of aetioporphyrin-II obtained at different source temperatures (sample provided by G. W. Kenner, F.R.S., and K. M. Smith, University of Liverpool). Mass spectra were obtained by using a Finnigan 4000 quadrupole mass spectrometer. The scan time was 5 s and the scan range m/z 50–550. Operating conditions consisted of an indicated source temperature of 300 °C, electron energy 35 eV, emission current 350 μA for both methane and hydrogen reagent gases, the source pressure was *ca.* 13.3 Pa.

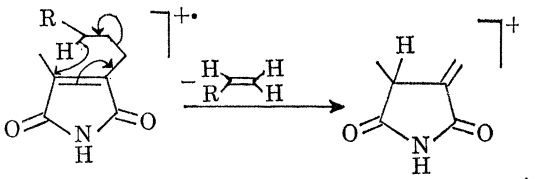
As well as the monopyrrolic fragments, ions which can be attributed to dipyrrolic and tripyrrolic species are observed. These ions are potentially of great significance as they could be used to infer the partial alkyl substitution pattern of the porphyrin macrocycle. However, the possibility that the dipyrrolic and tripyrrolic units are formed by recombination of monopyrrolic species cannot be discounted and further studies are in progress.

The low-temperature CH_4 c.i. mass spectra of porphyrins are complex, probably because of the attachment of alkyl ions in the gas plasma to the pyrrolic species. However, by using H_2 as a reagent gas, the mass spectra are considerably simplified, being effectively limited to ions

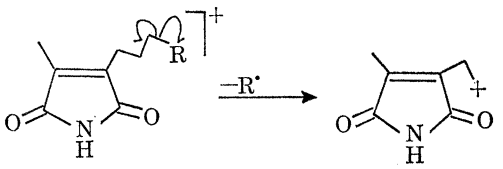
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

due to the monopyrrolic species and benzylic loss of Me$^{\cdot}$ from them, together with small amounts of dipyrrolic species.

The reductive degradation processes that are occurring within the source volume must involve several collisions with plasma species. Thus, it is reasonable to suggest that for this to occur, a long residence time is necessary and it may not be a coincidence that these processes are occurring at a lower temperature, i.e. one that is close to the boiling point of porphyrins at the pressures used. Whether the processes occur within the ion volume, on the probe tip or catalytically on the metal surfaces of the source is debatable.



18 **19** (m/z 125)



18 **20** (m/z 138)

The field desorption mass spectra of porphyrins and chlorins have been described by Evans *et al.* (1975) and give analogous results to c.i.m.s. at 300 °C. The bulk of the ion current is carried in the molecular ion region, but the envelope of quasi-molecular ions, observed in the c.i., is not apparent, the major ion being the molecular ion and higher peaks being due to isotopic contributions.

Apart from the novel method of degradation outlined above, there are other, more classical, methods of obtaining structural information of porphyrins by chemical degradation techniques. The reductive conversion to pyrroles has already been mentioned (Chapman *et al.* 1971) but the products are unstable, so the alternative of oxidative cleavage to the respective maleimides (Ellsworth & Aronoff 1968) is preferred, despite the loss of information about the *meso* positions on the macrocycle. The mass spectra of methyl alkyl maleimides are very characteristic, showing abundant ions at m/z 125 due to a McLafferty type rearrangement, and m/z 138 due to β-cleavage (Budzikiewicz *et al.* 1967).

Thus, the use of high resolution capillary gas chromatography, coupled with high sensitivity mass spectrometry, has shown the existence of several isomeric series of long-chain methyl maleimides (Soper 1977) derived from the degradation of Boscan BN23 petroporphyrins. Problems can arise, though, owing to the nature of the imido group, which can lead to poor g.c. peak shape, but this may be partially overcome by formation of the trimethylsilyl derivatives, although complete silylation is difficult to achieve. The mass spectrum of the trimethylsilyl derivative is dominated by the abundant ion due to the loss of Me$^{\cdot}$ from the silyl group, which enables the detection of trace quantities of these compounds by mass fragmentography or, better still, m.i.d. Trials with octaethylporphyrin and aetioporphyrin-II have shown that the maleimides from 100 μg of these porphyrins are readily detectable. It is likely that structural information will be obtainable from a few micrograms of individual petroporphyrin samples obtained by the trapping of h.p.l.c. peaks of crude fractions.

To obtain more detailed information about the nature of the petroporphyrins, it was necessary to isolate some single carbon number petroporphyrin fractions and attempt to elucidate their structure by classical chemical techniques. The bitumen Gilsonite (Eocene, *ca.* 50 Ma) from the Uinta Basin was chosen as it had a relatively simple petroporphyrin distribution in fairly high abundance, 100 parts/10^6 (see, for example, Baker *et al.* 1967).

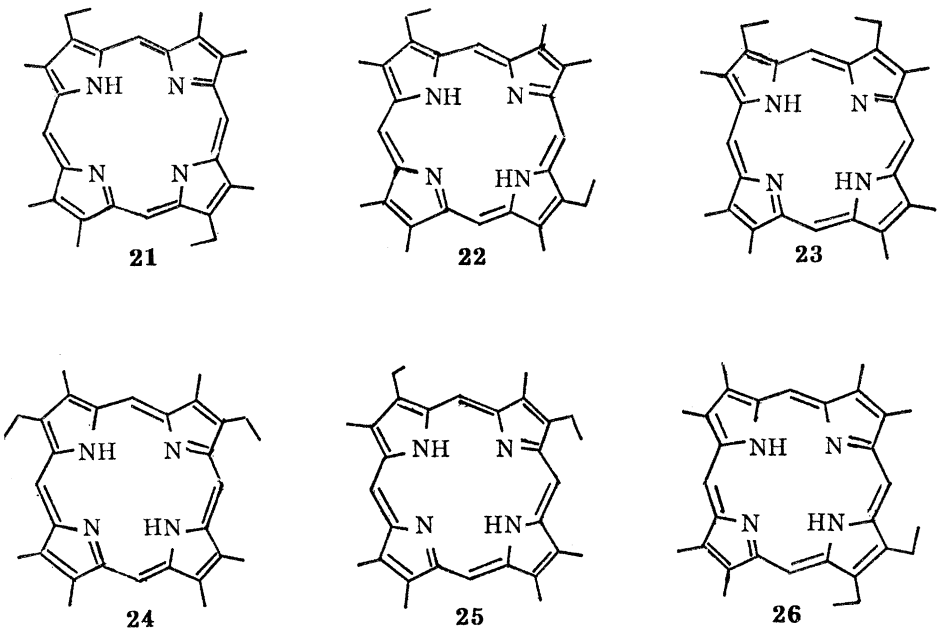


FIGURE 11. Possible structures of a C_{30} aetiopetroporphyrin isolated from Gilsonite.

A C_{30} aetiopetroporphyrin has been isolated from Gilsonite in sufficient quantity (*ca.* 1 mg) for ^1H Fourier transform n.m.r. analysis to be performed. The spectrum indicates that the compound can be one, or perhaps a combination, of the six isomers shown in figure 11, and the maleimide degradation and c.i.m.s. techniques may then be used to eliminate some of the possibilities. Thus, the absence of 3,4-diethylmaleimide in the oxidation products would eliminate structure **26**. Also, if the dipyrrolic units observed by c.i.m.s. at 200 °C source temperature are derived directly from the porphyrin, as opposed to being formed by recombination of the pyrroles, then structures **21** and **22** may be distinguished from **23**, **24** and **25** by comparison of the relative intensities of the dipyrrolic components of the mass spectrum. Studies are in progress that will, we hope, eliminate at least some of the possible isomers.

These results indicate that the aetiopetroporphyrins contain only alkyl substituents and exist as a number of structurally isomeric forms. The quantity of the different carbon number aetioporphyrin and DPEP petroporphyrin components varies from one oil to another, presumably due to differences in both palaeoenvironmental and maturational histories. It is essential that the structures of at least some of the petroporphyrin components be elucidated to determine the degradative pathway from chlorophylls, and it seems likely that a combination of e.i.m.s. and c.i.m.s., n.m.r. spectroscopy and possibly g.c.–m.s. of the silicon derivatives will be needed to achieve this.

Conclusion

The application of mass spectrometry, and particularly g.c.–m.s., has been instrumental in the rapid development of organic geochemistry and environmental organic chemistry in recent years. The techniques used have resulted in the identification of numerous compounds of a variety of types in sediments. Most attention has been concentrated on molecules of limited size, chiefly below 500 molecular mass, and of limited functionality, for example, hydrocarbons, fatty acids and alcohols.

The future holds many possibilities for extending the range of compounds studied: humic acids, carbohydrates, bases, peptides and, of course, kerogen degradation products. The complexity of these mixtures will necessitate improvements in both qualitative and quantitative procedures.

A wide range of environments and ecosystems awaits study. They present a major challenge for instrumentation and its application.

We thank Dr G. Ryback and Dr L. J. Mulheirn for providing the Rozel Point samples. We wish to thank the National Aeronautics and Space Administration (subcontract from NGL 05-003-003) for financial support. The computing and mass spectrometric facilities were provided by the Natural Environment Research Council (GR3/2951) and the Nuffield Foundation. We are grateful to Dr W. K. Seifert and the Chevron Oil Field Research Company for providing financial support for S.K.H., and also to the Natural Environment Research Council for the support of J.K.V. (GR3/3419). We thank Dr W. H. McFadden (Finnigan Instruments, Sunnyvale, California) for providing the l.c.–m.s. spectra of porphyrin samples, and Mrs A. Gowar for obtaining the e.i. mass spectra.

One of us (G.J.S.) wishes to thank the Science Research Council for a Studentship.

References (Eglinton *et al.*)

Aasen, A. J., Hofstetter, H. H., Iyengar, B. T. R. & Holman, R. T. 1971 *Lipids* **6**, 502–507.
Aizenshstat, Z. 1973 *Geochim. cosmochim. Acta* **37**, 559–567.
Albaiges, J., Borbon, J. & Salagre, P. 1978 *Tetrahedron Lett.*, pp. 595–598.
Baker, E. W., Yen, T. F., Dickie, J. P., Rhodes, R. E. & Clark, L. F. 1967 *J. Am. chem. Soc.* **89**, 3631–3639.
Biller, J. E. & Biemann, K. E. 1974 *Analyt. Lett.* **7**, 515–528.
Bird, C. W., Lynch, J. M., Pirt, S. J., Reid, W. W., Brooks, C. J. W. & Middleditch, E. S. 1971 *Nature, Lond.* **230**, 473–474.
Boon, J. J. 1978 Ph.D. thesis, University of Delft.
Boylan, D. B., Alturki, Y. I. & Eglinton, G. 1969 In *Advances in organic geochemistry 1968* (ed. P. A. Schenck), pp. 227–240. Oxford: Pergamon Press.
Brassell, S. C., Eglinton, G., Maxwell, J. R. & Philp, R. P. 1978 In *Aquatic pollutants, transformation and biological effects* (ed. O. Hutzinger, L. H. van Lelyveld & B. C. J. Zoeteman), vol. 1, pp. 69–86. Oxford: Pergamon Press.
Brooks, C. J. W., Horning, E. C. & Young, J. S. 1968 *Lipids* **3**, 391–402.
Brooks, P. W., Cardoso, J. N., Didyk, B., Eglinton, G., Humberston, M. J. & Maxwell, J. R. 1977*a* In *Advances in organic geochemistry 1975* (ed. R. Campos & J. Goni), pp. 433–453. Madrid: Enadimsa.
Brooks, P. W., Eglinton, G., Gaskell, S. J., McHugh, D. J., Maxwell, J. R. & Philp, P. 1977*b* *Chem. Geol.* **20**, 189–204.
Brooks, P. W., Maxwell, J. R. & Patience, R. L. 1978 *Geochim. cosmochim. Acta* **42**, 1175–1180.
Budzikiewcz, H., Djerassi, C. & Williams, D. H. 1967 *Mass spectrometry of organic compounds.* (690 pages.) San Francisco: Holden Day.
Burlingame, A. L., Kimble, B. J. & Derrick, P. J. 1976 *Analyt. Chem.* **48**, 368R–403R.
Calvert, S. E. & Price, N. B. 1971 *Deep Sea Res.* **18**, 505–523.
Chapman, R. A., Roomi, M. W., Morton, T. C., Krajcraski, D. T. & MacDonald, S. F. 1971 *Can. J. Chem.* **49**, 3544–3564.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Corwin, A. H. 1959 Petroporphyrins. *Proc. 5th World Petrol. Cong.* New York, vol. 5, pp. 119–129.
Dastillung, M. & Albrecht, P. 1976 *Mar. Pollut. Bull.* **7**, 13–15.
Ellsworth, R. A. & Aronoff, S. 1968 *Arch. Biochem. Biophys.* **124**, 358–364.
Ensminger, A. 1977 Ph.D. thesis, University of Strasbourg.
Ensminger, A., Joly, G. & Albrecht, P. 1978 *Tetrahedron Lett.*, pp. 1575–1578.
Evans, N., Games, D. E., Jackson, A. H. & Matlin, S. E. 1975 *J. Chromat.* **115**, 325–333.
Gaskell, S. J. 1974 Ph.D. thesis, University of Bristol.
Gray, N. A. B. & Gronneberg, T. O. 1975 *Analyt. Chem.* **47**, 419–424.
HajIbrahim, S. K., Tibbetts, P. J. C., Watts, C. D., Maxwell, J. R., Eglinton, G., Colin, H. & Guichon, G. 1978 *Analyt. Chem.* **50**, 549–553.
Heller, S. R., Koniver, D. A., Fales, H. M. & Milne, C. W. A. 1974 *Analyt. Chem.* **46**, 947–950.
Hollerbach, A. & Welte, D. H. 1977 *Naturwissenschaften* **645**, 381–382.
Holloway, P. J. 1969 *J. Am. Oil Chem. Soc.* **46**, 189–190.
Huang, W. & Meinschein, W. G. 1976 *Geochim. cosmochim. Acta* **40**, 323–330.
Kimble, B. J., Maxwell, J. R., Philp, R. P., Eglinton, G., Albrecht, P., Ensminger, A., Arpino, P. & Ourisson, G. 1974 *Geochim. cosmochim. Acta* **38**, 1165–1181.
Kwok, K.-S., Venkataraghavan, R. & McLafferty, F. W. 1973 *J. Am. chem. Soc.* **95**, 4185–4194.
Laflamme, R. A. & Hites, R. E. 1978 *Geochim. cosmochim. Acta* **42**, 289–303.
Lee, R. F., Nevenzel, J. C. & Paffenhofer, G. A. 1971 *Mar. Biol.* **9**, 99–108.
Maxwell, J. R., Cox, R. E., Ackman, R. G. & Hooper, S. N. 1972 In *Advances in organic geochemistry 1971* (ed H. R. v. Gaertner & H. Wehner), pp. 277–291. Oxford: Pergamon Press.
Maxwell, J. R., Cox, R. E., Eglinton, G., Pillinger, C. T., Ackman, R. G. & Hooper, S. N. 1973 *Geochim. cosmochim. Acta* **37**, 297–313.
McFadden, W. H., Schwartz, H. L. & Evans, S. 1976 *J. Chromat.* **122**, 389–396.
Mulheirn, L. J. & Ryback, G. 1975 *Nature, Lond.* **256**, 301–302.
Mulheirn, L. J. & Ryback, G. 1977 In *Advances in organic geochemistry 1975* (ed. R. Campos & J. Goni), pp. 173–192. Madrid: Enadimsa.
Nevenzel, J. C. 1970 *Lipids* **5**, 308–319.
Nishimura, M. 1978 *Geochim. cosmochim. Acta* **42**, 349–357.
Reed, W. E. 1977 *Geochim. cosmochim. Acta* **41**, 237–247.
Rohmer, M. & Ourisson, G. 1976 *Tetrahedron Lett.*, pp. 3633–3636.
Rubinstein, I., Seiskind, O. & Albrecht, P. 1975 *J. chem. Soc. Perkin* I, pp. 1833–1836.
Sargent, J. R., Gatten, R. R. & McIntosh, R. 1977 *Mar. Chem.* **5**, 573–584.
Seifert, W. K. & Moldowan, J. M. 1978 *Geochim. cosmochim. Acta* **42**, 77–95.
Shaw, G. J., Quirke, J. M. E. & Eglinton, G. 1978 *J. chem. Soc. Perkin* I, pp. 1655–1659.
Simoneit, B. R. T. 1977 *Geochim. cosmochim. Acta* **41**, 463–476.
Smith, K. M. 1975 In *Porphyrins and metalloporphyrins* (ed. K. M. Smith), pp. 381–398. Amsterdam: Elsevier.
Soper, P. D. 1977 B.Sc. thesis, University of Bristol.
Tonani, R. & Bianchi, G. 1976 *Maydica* **21**, 89–95.
Treibs, A. 1934 *Justus Leibigs Annln Chem.* **510**, 42–62.
Tulloch, A. P. 1975 *J. chromat. Sci.* **13**, 403–407.
Van Dorsselaer, A., Ensminger, A., Spyckerrelle, C., Dastillung, M., Seiskind, O., Arpino, P., Albrecht, P., Ourisson, G., Brooks, P. W., Gaskell, S. J., Kimble, B. J., Philp, R., Maxwell, J. R. & Eglinton, G. 1974 *Tetrahedron Lett.*, pp. 1349–1352.
Van Dorsselaer, A., Albrecht, P. & Ourisson, G. 1977 *Bull. Soc. Chim. Fr.*, pp. 165–170.
Volkman, J. K. 1977 Ph.D. thesis, University of Melbourne.
Wardroper, A. M. K., Brooks, P. W., Humberston, J. M. & Maxwell, J. R. 1977 *Geochim. cosmochim. Acta* **41**, 499–510.
Wardroper, A. M. K. 1979 Ph.D. thesis, University of Bristol.
Wardroper, A. M. K., Maxwell, J. R. & Morris, R. J. 1978 *Steroids* **33**, 203–221.
Weyman, A. C. M. 1977 In *Analytical pyrolysis* (ed. C. E. R. Jones & C. A. Cramers), pp. 225–232. Amsterdam: Elsevier.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES