Organic carbon as a palaeoenvironmental indicator in the marine realm

J. W. DE LEEUW,[1] N. L. FREWIN,[1,2] P. F. VAN BERGEN,[1,2]
J. S. SINNINGHE DAMSTÉ[1] & M. E. COLLINSON[2]

[1]*NIOZ Division of Marine Biogeochemistry, Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB, den Burg, Texel, The Netherlands*

[2]*Department of Geology, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK*

Abstract: Recent developments in biogeochemistry have made accessible all information currently known to be present in sedimentary organic compounds, i.e. the carbon skeleton structure, the positions of functional groups and stable carbon isotope ratios. This combined information has strengthened the applications of sedimentary organic compounds as biomarkers and palaeoenvironmental indicators. This review summarizes the most important biomarkers and their applications in reconstructing marine palaeoenvironments and processes. The marine environment is characterized by the combined presence of specific compounds, such as organic sulphur compounds, dinosteroids, 24-*n*-propylsteroids (even in trace amounts), high amounts of C_{25} HBI hydrocarbons and the absence of long chain *n*-alkanes with odd over even predominance, bicadinanes and specific higher-plant triterpenoids (e.g. oleanane). Furthermore, palaeoenvironmental conditions, such as photic zone anoxia, hypersalinity, microbial sulphate-reducing activity, algal blooming, palaeo-upwelling and palaeo-surface seawater temperature are indicated by molecular and/or isotopic characteristics. Biomarkers from terrestrial sources are included because land-derived organic matter is an important contributor to coastal and shallow-marine environments. A short case study, within a Holocene terrestrial/marine setting (Florida Bay, USA), is also presented to illustrate the reconstruction of transgressive–regressive trends using selected organic compounds.

Depending on the conditions in the original depositional environment, e.g. production, preservation, 0.1–1% of biologically derived organic matter is not recycled in the biosphere or biogeosphere and 'escapes' into the geosphere (Fig. 1). The composition of this sedimentary organic matter is substantially different from that of living organisms because major differences exist in the degree of biodegradation of biochemicals. For example, polysaccharides and proteins, representing the bulk of the biomass in most organisms, are very efficiently mineralized by bacteria and are thus selectively removed during transport through the water column or in the top layers of sediments. As a consequence, other biochemicals, such as certain low- and high-molecular weight compounds occurring in cell walls and membranes, and aromatic biomacromolecules, such as lignin, are selectively preserved and thus enhanced in sedimentary organic matter. In fact, series of novel highly aliphatic, highly resistant biomacromolecules in cell walls of algae and plant structures (i.e. cuticles, periderm, seed coats) were discovered by detailed analyses of microscopically recognizable fossilized organic matter before they were identified as relatively minor constituents in their living counterparts (Nip *et al.* 1986; Tegelaar *et al.* 1993; Van Bergen *et al.* 1994*a*). In depositional environments where sulphate reduction prevails, lipids, which otherwise would be biodegraded, are preserved through reactions with inorganic sulphur species (H_2S and HS_x^-) as low- or high-molecular weight organic sulphur compounds (OSC; Sinninghe Damsté & De Leeuw 1990 and references therein).

Despite the above mentioned processes, whereby only a minute, highly biased, fraction of the original biochemicals is preserved as such, partly altered or as sulphur derivatives, sedimentary organic compounds can still be considered as highly specific information carriers or molecular fossils, applicable in palaeoenvironmental reconstruction, biochemical evolution and as maturity indicators (Fig. 2). This is because many organisms or groups of organisms biosynthesize numerous highly specific biomolecules. Furthermore, analytical chemical tools have been developed to such an extent that all the information known to be present in sedi-

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mentary organic molecules can, in principle, be retrieved.

Three modes of information are present in biochemicals: (1) the structure of the carbon skeleton (including its stereochemistry); (2) the position(s) and nature of functional groups; and (3) the stable carbon isotope ($^{13}C/^{12}C$) composition. The carbon skeleton structures and the position(s) and nature of functional groups are determined by species-specific biosynthetic pathways, whilst stable carbon isotope ratios in biochemicals are determined by biosynthesis and carbon source. Because the stable carbon isotope ratio of the carbon source is dependent on its nature, and on its position in the food web, stable carbon isotope ratios of biochemicals give clues to the niche of the organisms which biosynthesized these biochemicals. As already mentioned, retrieval of all three modes of information present in sedimentary organic compounds is now possible because of recently developed methods and technologies which are reviewed below.

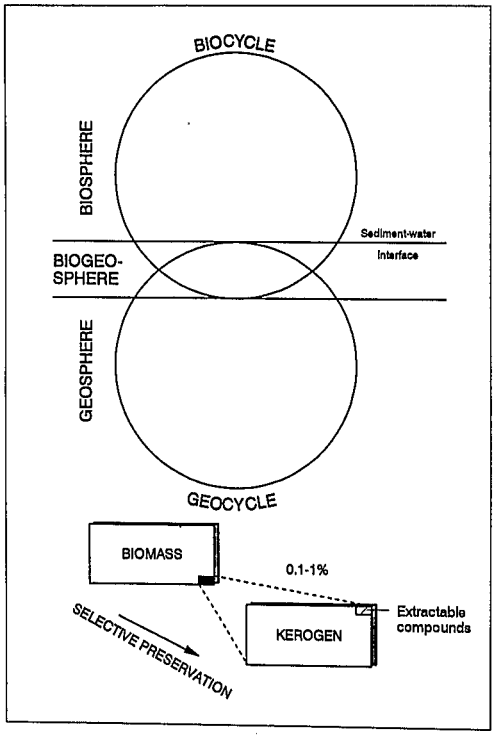

Fig. 1. Schematic diagrams showing the relationship between the biocycle and the geocycle and the selective preservation of the kerogen fraction.

How to obtain information from sedimentary organic molecules

Isolation and fractionation of solvent soluble organic compounds (loosely termed lipids) from fossils or whole sediments is generally performed by ultrasonic or Soxhlet extractions with common organic solvents, followed by column or thin-layer chromatography to obtain separation of classes of compounds according to their relative polarity (Fig. 3). These fractions are analysed using gas chromatography–mass spectrometry (GC–MS) or, if the compounds present in the fractions are thermolabile or too polar, by liquid chromatography–mass spectrometry (LC–MS). The residue after extraction generally contains the bulk of the organic matter which is macromolecular in nature. This residual insoluble organic matter, kerogen (Fig. 1), is analysed by spectroscopic methods (e.g. NMR and IR) and/or by chemolytic or pyrolytic methods. The latter analyses are indirect

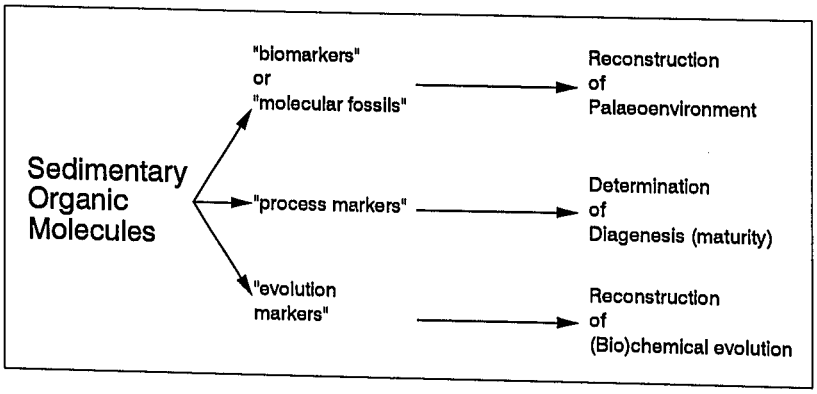

Fig. 2. Applications of sedimentary organic molecules.

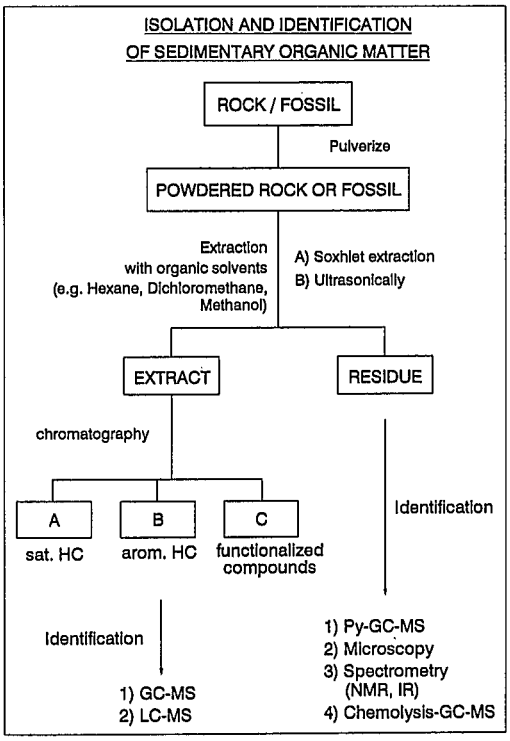

Fig. 3. A laboratory procedure for isolation and identification of sedimentary organic matter. Sat. HC, saturated hydrocarbons; arom. HC, aromatic hydrocarbons.

because characteristic moieties of the kerogen are released by specific chemical reagents (chemolysis) or by heating in an inert atmosphere (pyrolysis; Py). The compounds thus released are separated and identified by GC–MS or LC–MS. Reconstruction of the molecular structure of the macromolecular organic matter is a mental exercise based on the qualitative and quantitative analysis of the pyrolytic or chemolytic products obtained and on knowledge of chemolytic and pyrolytic mechanisms (Boon 1992; Hedges 1991 and references therein; Schouten *et al.* 1993*a* and references therein). Ultimately, the carbon skeletons as well as the position(s) of functional groups in sedimentary organic compounds are established in this way. It should be emphasized that in many cases the original functional groups, in particular double bonds and carbonyl groups in ketones and aldehydes, have reacted with hydrogen sulphides, intra- or intermolecularly, resulting in low- or high-molecular weight OSC. However, it should be stressed that the position(s) of the sulphurized carbon(s) in the carbon skeletons are the same as the position(s) of the original functional group(s) (Kohnen *et al.* 1992, 1993).

The determination of stable carbon isotope ratios in individual organic compounds present in complex mixtures is performed by isotope ratio monitoring–GC–MS (irm–GC–MS), a technique which has only recently become commercially available (Freeman *et al.* 1990). The compound mixture to be analysed is injected on to a gas chromatograph (Fig. 4) and separated, individual compounds eluting from the gas chromatograph are combusted on-line to CO_2 and H_2O in a combustion reactor coupled to the gas chromatograph. After removal of the water (by a water separator) the CO_2 peaks are introduced into an isotope mass spectrometer where $^{13}C/^{12}C$ ratios can be measured reliably if the compounds are separated well during GC. In this way $^{13}C/^{12}C$ ratios of GC amenable sedimentary organic compounds are determined. The optimal interpretation of these carbon isotope data requires some prerequisite knowledge of isotope fractionation in organisms. An overview of $\delta^{13}C$ fractionations, based on a recent paper by Hayes (1993), is given in Fig. 5. Different families of primary producers on land fix atmospheric CO_2 via different enzymatic pathways (C3, C4, CAM) resulting in clearly distinct $^{13}C/^{12}C$ values. Photosynthetic organisms in aqueous environments also differ in the pathway of CO_2 fixation using, for example, HCO_3^- pumping, the enzyme ribulose bisphosphate carboxylase (RuBisCo) or the reverse tricarboxylic acid cycle (TCA), again leading to major differences in $^{13}C/^{12}C$ ratios in their biomass. Methanogenic bacteria discriminate against ^{13}C even more significantly, leading to heavily depleted biomass and extremely negative $^{13}C/^{12}C$ values of CH_4. If methanotrophic bacteria, using this light CH_4, are present in the depositional environment further discrimination against ^{13}C occurs so that biomass of these bacteria may have $\delta^{13}C$ values of $-80‰$ or less. Apart from these biosynthetically induced isotope variations, the CO_2 concentration and its ^{13}C contents (which usually varies with water depth), the water temperature, the salinity, as well as many other physical and (bio)chemical parameters, ultimately determine the $^{13}C/^{12}C$ value of a biochemical. Furthermore, $\delta^{13}C$ values of different classes of compounds within an organism vary due to different biosynthetic pathways. Compared with the total biomass of an organism, polysaccharides and proteins are somewhat enriched in ^{13}C, leading to less negative values, whilst *n*-alkyl lipids and isoprenoid lipids are 4.5 and 3.5‰ lighter (more negative), respectively (Hayes 1993 and references therein).

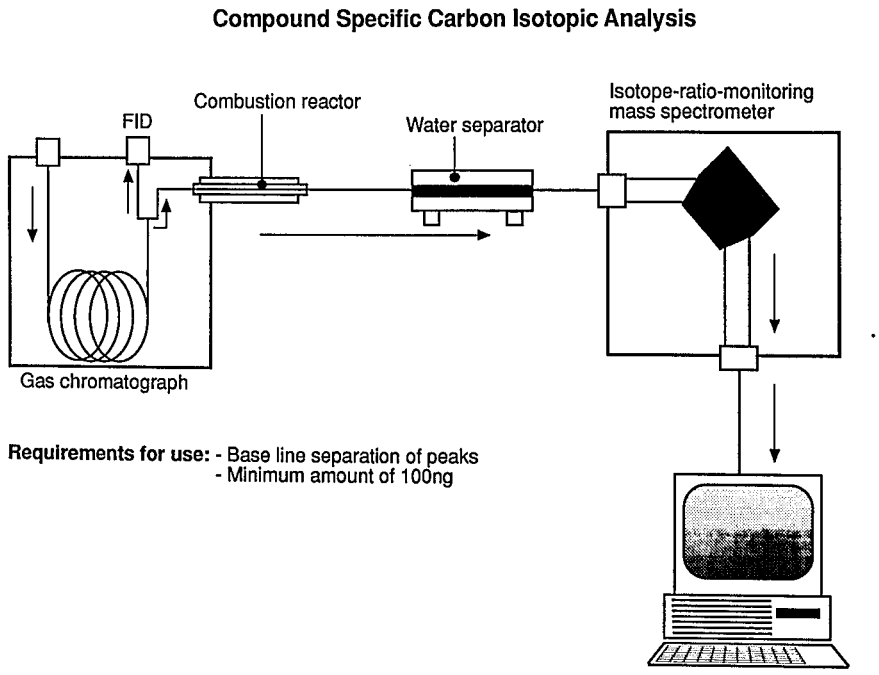

Fig. 4. Schematic diagram for isotope ratio monitoring–GC–MS set-up (after Hayes 1993).

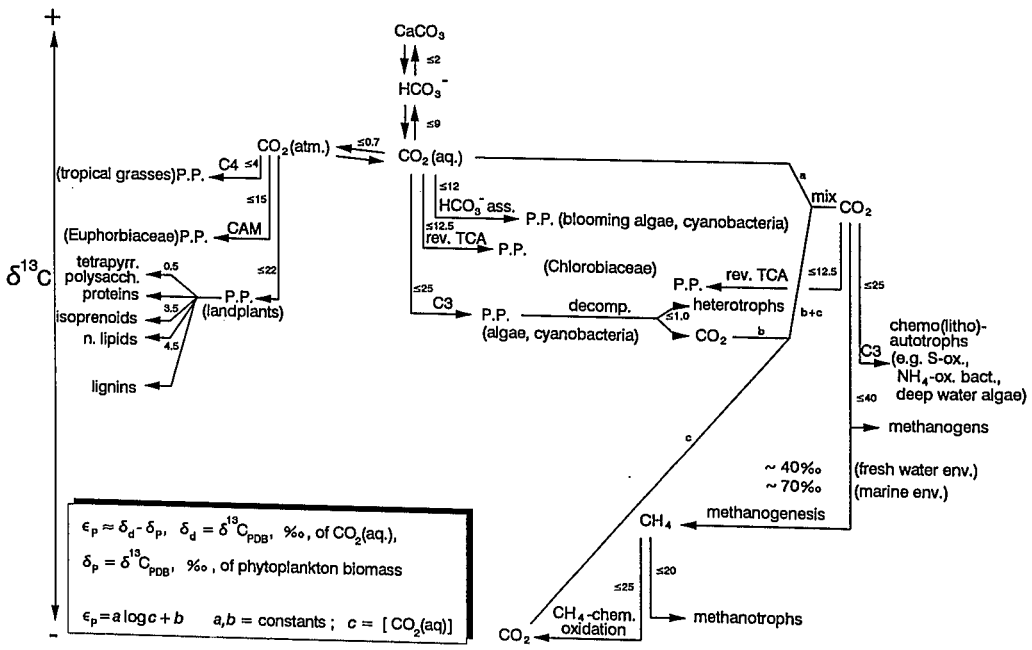

Fig. 5. Overview of $\delta^{13}C$ fractionation in the biosphere. P.P., primary producers; tetrapyrr, tetrapyrrols; polysacch, polysaccharides; n. lipids, normal linear lipids; decomp, decomposition; CH_4-chem. oxidation, CH_4 chemical oxidation; env., environment; S-ox, S-oxidation; NH_4-ox, NH_4 oxidation; atm, atmosphere; aq., aqueous. For additional information see Hayes (1993).

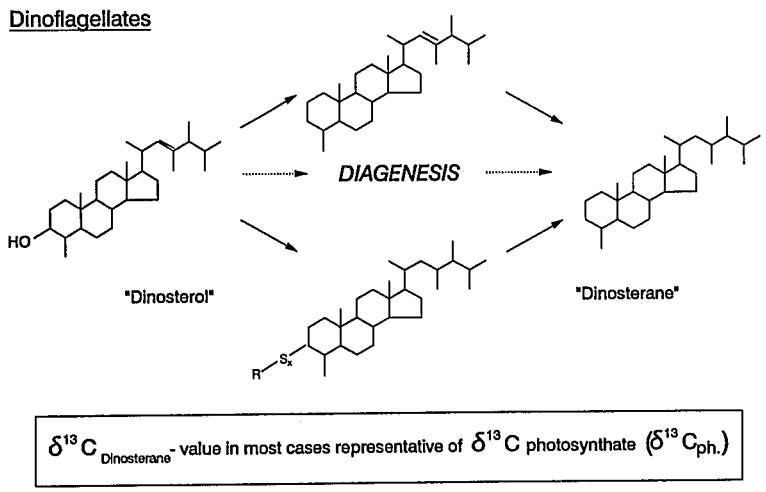

Fig. 6. Palaeoenvironmental indicators for dinoflagellates.

In the following section a number of examples of biochemicals specific for certain organisms have been selected to show the scope and limitations of this molecular palaeontological or molecular biogeochemical approach at its present stage; Appendix A provides some insight into the sometimes specialized nomenclature. Although the paper addresses palaeoenvironmental indicators in the marine realm a significant number of examples dealing with (palaeo)biochemicals derived from terrestrial higher plants are given. This is justified by the fact that terrestrially derived organic matter ending up in the seas and the oceans by riverine or aeolian transport mechanisms can contribute very significantly to sedimentary organic matter in marine sediments (Hedges 1992) and is important in the recognition of shoreline environments. Primary producers in the marine realm, the algae, are presented first, followed by the bacteria and finally the higher land plants.

Organic material derived from eukaryotic algae and higher plants (e.g. dinoflagellate cysts, cuticles, wood fragments, pollen and spores), as well as from certain animal sources, is often recognized in preparations of palynological organic matter from marine sediments. Coverage of these microscopically recognizable entities is outside the scope of this paper. The interested reader should consult Tappan (1980), Traverse (1988), Lipps (1993) and Jansonius & McGregor (in press) for an introduction to these topics.

Examples of palaeoenvironmental indicators

Dinoflagellates

The relatively stable saturated cyclic hydrocarbon 4,23,24-trimethylcholestane or 'dinosterane' (Fig. 6) is frequently encountered in crude oils or extracts of Late Triassic, or younger, relatively mature sediments (e.g. Summons *et al.* 1987; Thomas *et al.* 1993). It is thought to represent the diagenetic product of the biochemical 'dinosterol' or 4,23,24-trimethylcholesterol, a steroid present in relatively high amounts in many dinoflagellate algae (Nes & McKean 1977) and recently also encountered in the carnivorous fresh water plant *Utricularia neglecta* (Klink *et al.* 1992), and in very small amounts in a diatom (*Navicula* sp.; Volkman *et al.* 1993). It is, however, assumed that the presence of dinosterol in the carnivorous plant is of dietary dinoflagellate origin. To date, it is believed that dinosterol is mainly biosynthesized by dinoflagellate algae. Hence, the presence of dinosterol, dinosterane or intermediate diagenetic products in sediments, even in the absence of dinoflagellate cysts, is a strong palaeoindicator of dinoflagellate algae in the depositional environment (e.g. Boon *et al.* 1979). In some cases the dinosterane skeleton is only present as a sulphur bound moiety (carbon atom 3 is mostly involved in the C—S bond, indicating the original position of the hydroxy- or carboxy-group; see Appendix A for carbon numbering) in high-

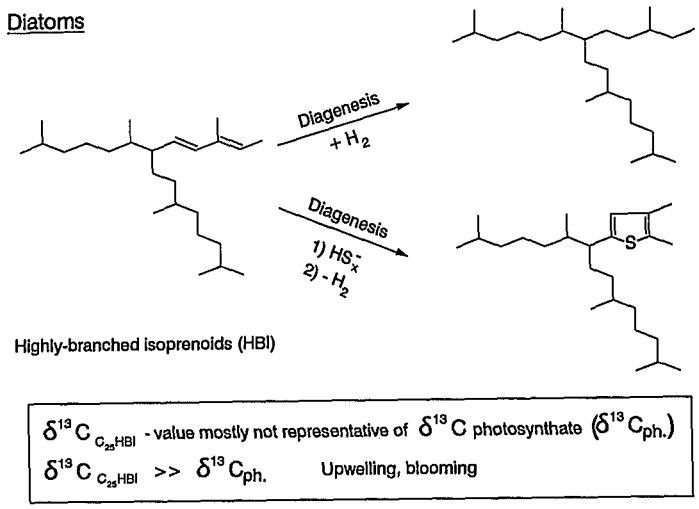

Fig. 7. Palaeoenvironmental indicators for diatoms.

molecular weight fractions of sediment extracts (Fig. 6). However, chemolysis of such a high-molecular weight fraction using Raney nickel releases dinosterane through selective cleavage of the C—S bond and the molecular fossil is retrieved (Kohnen *et al.* 1992). $\delta^{13}C$ values of dinosterol or its diagenetic products measured so far are in agreement with an origin from photosynthetic algae fixing CO_2 via the RuBisCo pathway (Kohnen *et al.* 1992).

Diatoms

Highly branched C_{25} and C_{30} isoprenoid (HBI) alkanes, alkenes and thiophenes (Fig. 7) have been encountered for some time in crude oils and sediment extracts (e.g. Robson & Rowland 1986; Sinninghe Damsté *et al.* 1989*a*). Only recently has firm proof emerged that these specific compounds are biosynthesized by several species of diatoms (Nichols *et al.* 1988; Volkman *et al.* 1992). In sediment of the Black Sea it was noticed by Kohnen *et al.* (1990*a*) that the HBI thiolanes contain two double bonds less than their C_{30} polyunsaturated HBI precursors, indicating that these biochemicals readily react with hydrogen sulphides in the top layers of sediments. These sulphurized C_{25} HBI compounds, as well as their fully hydrogenated counterparts, are thus considered to be diagenetic products of the corresponding unsaturated biochemicals with the HBI skeleton. They strongly indicate the presence of diatoms in the depositional environment. It should be mentioned that these HBI compounds play an important role as molecular fossils in sediments where the silica frustules of diatoms are absent, presumably through solution of the silica. $\delta^{13}C$ values have been measured for these compounds in several sediments (e.g. Kohnen *et al.* 1992; Summons *et al.* 1993; Freeman *et al.* 1994; Schouten *et al.* 1994). These values are less negative in comparison with those from compounds from other algal primary producers. This is usually due to seasonal blooming, triggered by upwelling, of certain diatoms biosynthesizing these HBI compounds. Less negative $\delta^{13}C$ values during blooming are caused by low CO_2-dissolved concentrations so that ^{13}C cannot be discriminated substantially (Deuser 1970). It should be noted, however, that there is evidence that non-blooming diatoms also use relatively enriched CO_2, probably by bicarbonate pumping (Summons *et al.* 1993). The phenomenon of less negative $\delta^{13}C$ values due to blooming may also apply to other algae.

Prymnesiophyte algae

Long chain di- and triunsaturated methyl- and ethylketones with 37, 38 and 39 carbon atoms (Fig. 8) are biosynthesized by the coccolithophorid (Prymnesiophyta) *Emiliania huxleyi* (Volkman *et al.* 1980) and some other present-day prymnesiophytes, like *Gephyrocapsa* sp. (Volkman *et al.* 1994). Although *E. huxleyi* is only present in the geological record for the last 150 000 years, these ketones have been encountered in sediments deposited since the Cretaceous (Marlowe *et al.* 1990). To date, it is believed

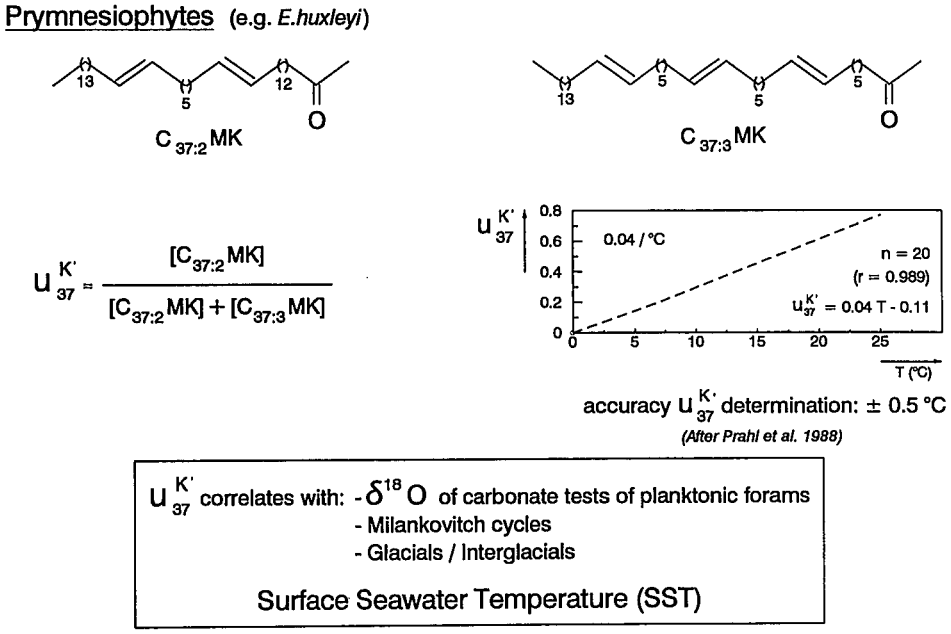

Fig. 8. Palaeoenvironmental indicators for prymnesiophytes. MK, methylketones.

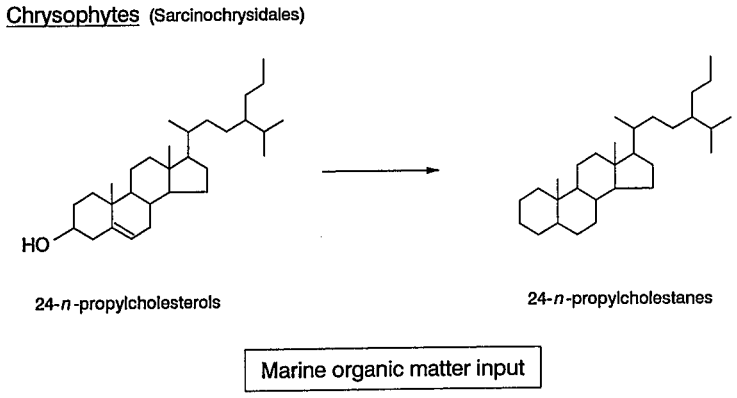

Fig. 9. Palaeoenvironmental indicators for chrysophytes.

that these long-chain ketones are exclusively biosynthesized by species belonging to the Prymnesiophyta (Jasper & Gagosian 1993). The importance of these compounds lies in the fact that they remain present in the sediments even though the carbonate remains of the coccoliths may be absent.

It is now well established that these ketones, when present in Recent sediments, can be used as palaeo-surface seawater temperature indicators (e.g. Brassell *et al.* 1986; Prahl *et al.* 1988) with an accuracy of 0.5°C. A relatively simple analysis of sediment extracts enables the determination of the so-called $U_{37}^{K'}$, a ratio of the C_{37} ketones with two and three double bonds. Calibration of this ratio using cultures of *E. huxleyi* grown under well-controlled temperature conditions enables the recognition of natural climatic changes in the past. The $U_{37}^{K'}$ is also correlated with other temperature or climate indicators, such as $\delta^{18}O$ values in carbonate tests of planktonic forams and/or

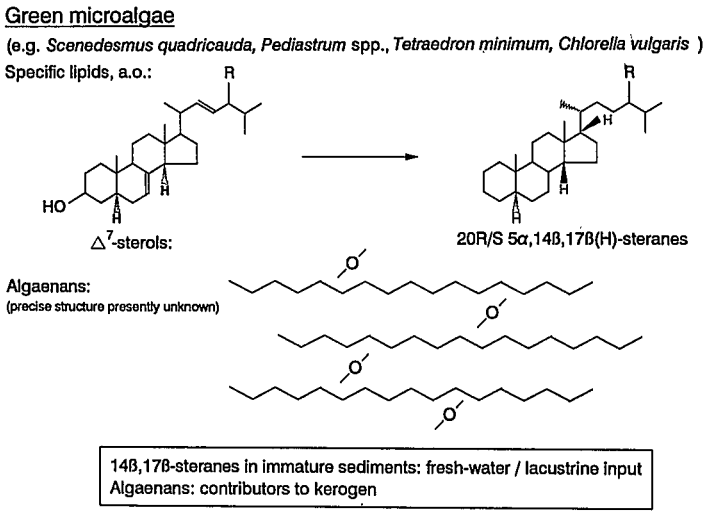

Fig. 10. Palaeoenvironmental indicators for green microalgae.

the Milankovitch cycles (Brassell *et al.* 1986). It is now well documented that these ketones can react with inorganic sulphur species during early diagenesis (Sinninghe Damsté *et al.* 1988, 1989*b*, 1990*b*). Reactions of carbonyl groups and double bonds in the original molecule with inorganic hydrogen polysulphides lead to the formation of (poly)sulphide bonds, in this case yielding sulphurized C_{37}–C_{39} carbon skeletons. Upon Raney nickel desulphurization of high-molecular weight fractions of sediment extracts these carbon skeletons are released as saturated hydrocarbons with 37–39 carbon atoms (Sinninghe Damsté *et al.* 1988, 1990*a*; Schouten *et al.* 1993*b*). Summarizing, it can be stated that these compounds fulfil two functions: (1) ketones or their diagenetic counterparts (e.g. sulphur bonded) reveal the presence of prymnesiophytes in the depositional environment; and (2) the ketones, when present unaltered, function as a palaeothermometer.

Chrysophyte algae

Recently, it has been shown by Moldowan *et al.* (1990) that the presence of 24-*n*-propylcholesterols or their diagenetically derived counterparts, 24-*n*-propylcholestanes (Fig. 9), in sediment extracts and oils can be considered as the most powerful indicators for the input of marine organic matter in the depositional environment. The sterols with this highly specific 24-*n*-propyl group are biosynthesized by the Sarcinochrysidales, a group of marine Chrysophyte algae.

Green microalgae

A number of very common freshwater, green microalgae (e.g. *Scenedesmus* spp., *Pediastrum* spp., *Tetraedron minimum* and *Chlorella vulgaris*) biosynthesize Δ^7 sterols (Fig. 10) instead of Δ^5 sterols (Nes & McKean 1977; De Leeuw *et al.* unpublished results). The diagenetic pathways of these compounds are different from Δ^5 sterols (which are produced by most eukaryotic plants) and, as a result of this, $5\alpha,14\beta,17\beta$(H) steranes with 20R and 20S stereochemistries are already present in relatively immature sediments (De Leeuw *et al.* 1989; Peakman *et al.* 1989). When present in more mature sediments, or crude oils, the $5\alpha,14\beta,17\beta$(H) steranes cannot be used as indicators of freshwater, green microalgae because these steranes may also be derived from Δ^5 sterols in later stages of maturation (Peters & Moldowan 1993).

The above mentioned algae, as well as the three races of *Botryococcus braunii* and a few marine microalgae (e.g. *Nannochlorum eucaryotum*), biosynthesize highly aliphatic, non-saponifiable biomacromolecules called algaenans (Largeau *et al.* 1986; Derenne *et al.* 1992; Fig. 10). These substances make up a substantial part of algal cell walls and have been recognized recently by spectroscopic and pyrolytic analysis

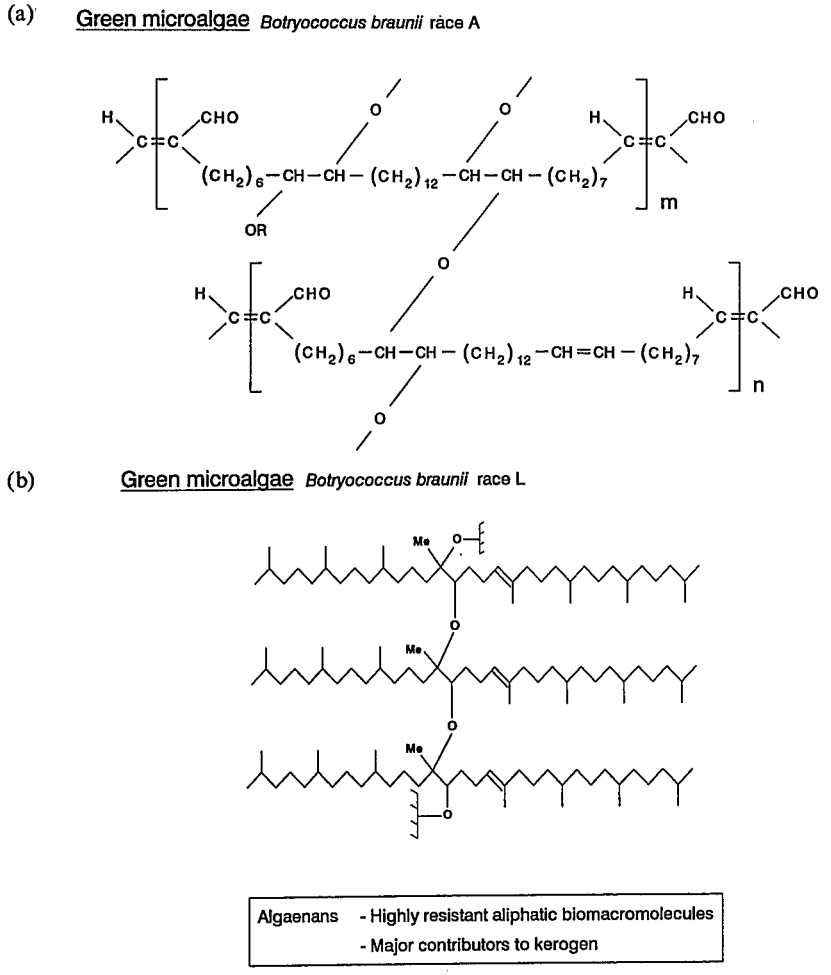

Fig. 11. Palaeoenvironmental indicators for *Botryococcus braunii*: (a) race A (modified after Metzger *et al.* 1991); (b) race L. Me, methyl (after Gelin *et al.* 1994*b*).

methods (Goth *et al.* 1988; De Leeuw *et al.* 1991; Gelin *et al.* 1994*b*). Although algaenans of different algae are all highly resistant, non-saponifiable and aliphatic in nature, very precise structures are not known except for *B. braunii* races A and L (Fig. 11 a & b; Derenne *et al.* 1989; Metzger *et al.* 1991; Gelin *et al.* 1993, in press). Detailed analyses of flash pyrolysates of algaenans of these different algae indicate quite substantial differences in their chemical structures although they are thought, with one exception, to possess a backbone of *n*-alkyl chains linked via ether bridges (Gelin *et al.* 1993). The exception is the algaenan of *B. braunii* race L, which consists of ether-linked isoprenoidal lycopadiene units (Fig. 11b; Derenne *et al.* 1990; Gelin *et al.* 1994*b*).

Because of their apparent resistance against (bio)degradation these algaenans are the major constituents of many kerogens and important precursors of *n*-alkanes in crude oils. For example, the widely studied kerogen of the Messel oil shale (Eocene, Germany) consists almost exclusively of algaenan of *T. minimum* (Goth *et al.* 1988), whilst many so-called torbanites and coorongites are made up almost exclusively of algaenans or comparable high-molecular weight substances originating from *B. braunii* species (Largeau *et al.* 1986). Hence, the presence of highly aliphatic macromolecules in kerogens indicate the selective preservation of green microalgae of terrestrial and/or marine origin. However, it should be noted that similar types of resistant insoluble highly aliphatic macromolecules also occur in a variety of tissues of higher plant (see below). Stable

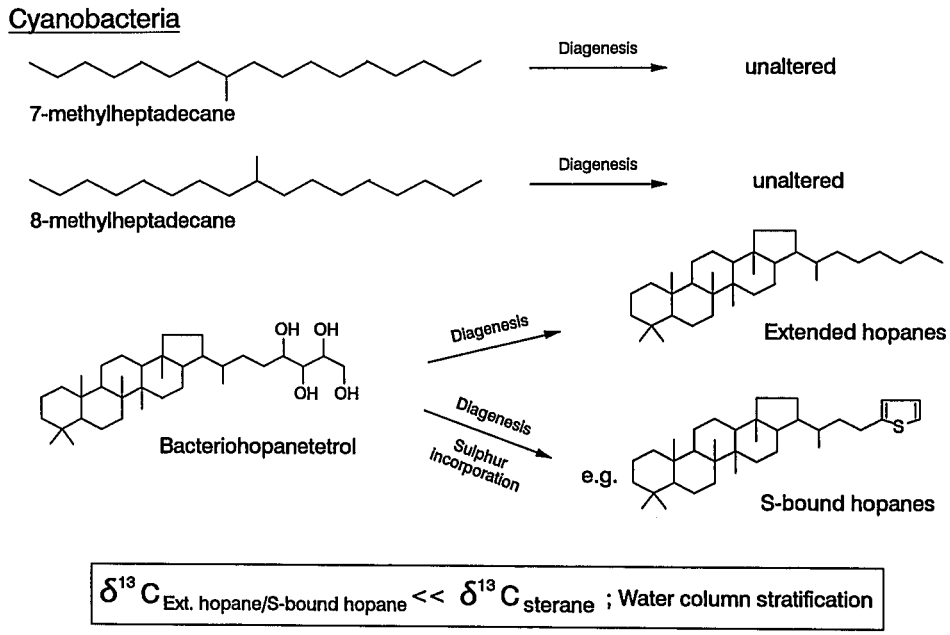

Fig. 12. Palaeoenvironmental indicators for cyanobacteria.

carbon isotope measurements of homologous series of *n*-alkanes and *n*-alk-1-enes released from kerogens upon pyrolysis are in accordance with an origin from primary producers (Eglinton *et al.* 1993).

Cyanobacteria

A number of monomethyl branched alkanes, in particular 7-methylheptadecane and 8-methylheptadecane (Fig. 12), have been encountered in several cyanobacteria (Sheia *et al.* 1990). Because these two compounds virtually coelute during GC and co-occur in about equal amounts their first identification in cyanobacteria was not straightforward (Han & Calvin 1970). These branched hydrocarbons, as well as many others encountered in cyanobacteria (Sheia *et al.* 1990), can survive unaltered and thus are considered specific biomarkers for cyanobacteria when encountered in sediments (Han 1970).

Like many other bacteria, several cyanobacteria use the pentacyclic isoprenoid bacteriohopanetetrol derivatives (Fig. 12) to rigidify their cell membranes (Rohmer *et al.* 1989). These hopane derivatives occur exclusively in bacteria and can be considered as the bacterial counterparts of steroids in the cell membranes of algae. Depending on depositional and burial conditions a large variety of extended (i.e. containing more than 30 carbon atoms) hopanes and functionalized hopanoids can be encountered in sediments (e.g. Peters & Moldowan 1993; Ourisson *et al.* 1979, 1984). In anaerobic marine sediments with an active population of sulphate-reducing bacteria a considerable part of the extended hopanoids is preserved as their sulphurized counterparts. In particular, C_{35} hopanoid thiophenes (Valisolalao *et al.* 1984), like the one indicated in Fig. 12, as well as macromolecularly sulphur-bound hopanoids, sometimes represent an important contribution to the extractable organic matter in sediments (Kohnen *et al.* 1990*b*; Köster *et al.* 1993). Although these hopanetetrols are not exclusively biosynthesized by cyanobacteria, circumstantial evidence is building up that in many cases, in particular if their concentrations are relatively high, extended hopanoids in sediments are predominantly derived from cyanobacteria and much less from heterotrophic bacteria (Schoell *et al.* 1994). At present, we therefore consider them as relatively unique indicators of cyanobacterial activity in the depositional environment and not as indicators of bacterial reworking. $\delta^{13}C$ values of extended hopanoids in sediments have been measured on a few occasions (Kohnen *et al.* 1992; Schoell *et al.* 1994; Schouten *et al.* 1994). These isotope data confirm an origin from cyanobacteria (Kohnen

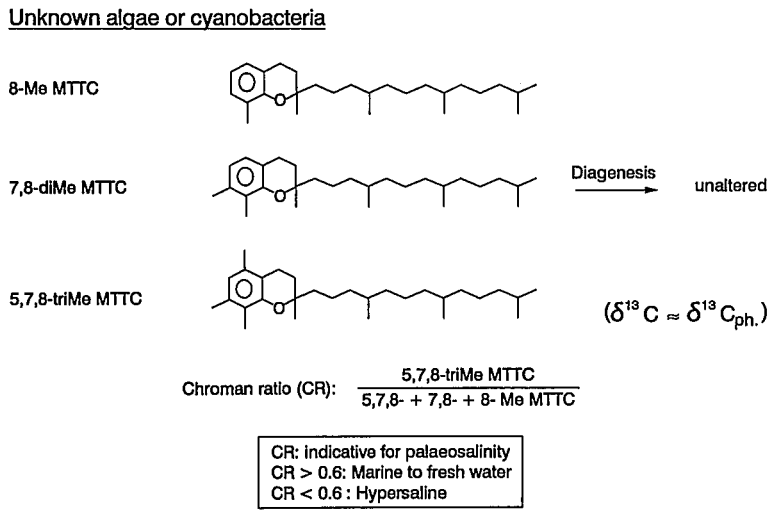

Fig. 13. Palaeoenvironmental indicators for palaeosalinity of unknown origin.

et al. 1992). In a recent paper Schoell *et al.* (1994) use differences between stable carbon isotopes of extended hopanes and steranes as a strong indication of water-column stratification, assuming that cyanobacteria live at a greater depth in the water column than eukaryotic algae. The underlying reason for this is that water stratification is normally associated with temperature change (deeper water being colder), CO_2 concentrations are higher in colder water leading to more discrimination against ^{13}C during CO_2 fixation.

Algae/cyanobacteria?

Several years ago series of so-called methylated chromans (Fig. 13) were unambiguously identified in aromatic hydrocarbon fractions of a variety of sediment extracts (Sinninghe Damsté *et al.* 1987). The relative amounts of the different methylated chromans can vary substantially. Based on circumstantial evidence it was assumed that changes in their relative amounts, as expressed by the chroman ratio (CR; Fig. 13), indicate salinity changes, between normal marine and hypersaline, in the depositional environment (Sinninghe Damsté *et al.* 1987). Recent investigations of organic matter in the Mulhouse Basin further supports this idea (Sinninghe Damsté *et al.* 1993*a*). From this latter study, it also became clear that these highly specific compounds are probably biosynthesized by algae or cyanobacteria because their distribution reflected the salinity (normal marine or hypersaline) of the upper part of the water column of Lake Mulhouse. In addition, their stable carbon isotope values were similar to those of molecules related to photosynthetic organisms living in the upper part of the water column. It is interesting to note that these chromans can thus be used as palaeosalinity indicators despite the fact that their biological origin is not (yet) known.

Photosynthetic sulphur bacteria

Another group of bacteria, the photosynthetic sulphur bacteria, belonging to the family Chlorobiaceae, biosynthesize a number of very specific diaromatic carotenoids, e.g. isorenieratene (Fig. 14) and chlorobactene. These carotenoids are preserved in many sediments varying in their mode of occurrence from: (1) unaltered (Repeta, 1993); (2) to diaryl isoprenoids in which the isoprenoid chain between the benzene moieties has been fully reduced (Schaefle *et al.* 1977; Requejo *et al.* 1992; Hartgers *et al.* 1994); (3) to macromolecularly bound sulphur moieties when hydrogen polysulphides were available in the anaerobic depositional environment (Kohnen *et al.* 1992; Sinninghe Damsté *et al.* 1993*b*; Hartgers *et al.* 1994); and/or (4) incorporated in kerogen (Douglas *et al.* 1991; Hartgers *et al.* 1991, 1994). In more mature sediments and crude oils C_{11}–C_{31} arylisoprenoids, with the characteristic aromatic substitution pattern of isorenieratene, have also been encountered (Summons & Powell 1987; Requejo *et al.* 1992; Hartgers *et al.* 1994). It is proposed that these arylisoprenoids are formed from macromolecu-

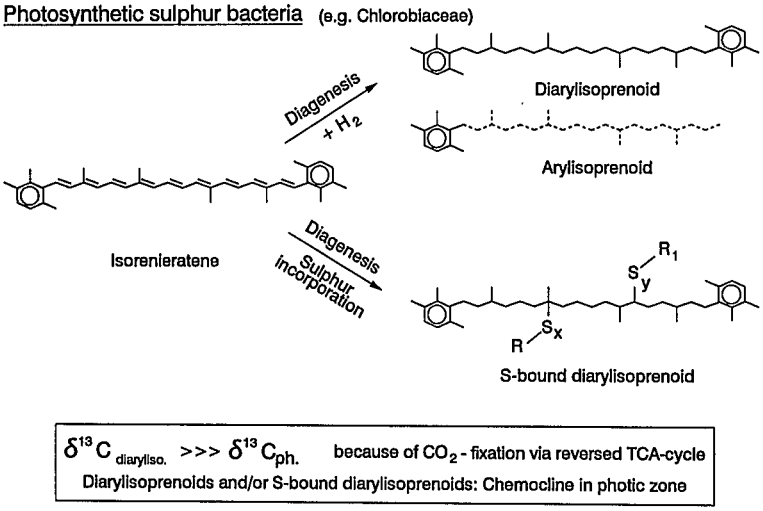

Fig. 14. Palaeoenvironmental indicators for photosynthetic sulphur bacteria.

larly bound diarylisoprenoids during diagenesis and catagenesis via carbon–carbon cleavages triggered by a sulphide- or ether-functionality at one of the carbon atoms (Hartgers *et al.* 1994). Because Chlorobiaceae fix CO_2 via the reversed TCA cycle (Quandt *et al.* 1977; Sirevag *et al.* 1977), isorenieratene, and hence their diagenetic products, are extremely enriched in ^{13}C. $\delta^{13}C$ values of -8 to $-18‰$ have been recorded for diarylisoprenoids, sulphur-bound isorenieratene and the specific arylisoprenoids (Summons & Powell 1987; Kohnen *et al.* 1992; Sinninghe Damsté *et al.* 1993*b*; Hartgers *et al.* 1994). Such values make these compounds unambiguous biomarkers for Chlorobiaceae. These bacteria live at a chemocline which must be present in the photic zone, indicating a very specific niche in the depositional environment. Therefore, the presence of their specific carotenoids or carotenoid derivatives with the extremely high (less negative) $\delta^{13}C$ values clearly act as environmental indicators for a chemocline in the photic zone. In a recent paper the presence/absence of sulphurized isorenieratene in a 6000 year old sediment sequence of the Black Sea was used to reconstruct the depth variation of the chemocline during this period (Sinninghe Damsté *et al.* 1993*b*).

Gram-negative bacteria

Gram-negative bacteria are characterized by the presence of an extracellular substance called lipopolysaccharides (LPS; Fig. 15; for a review see De Leeuw and Largeau 1993). LPS consists of a bacterium-specific polysaccharide moiety linked via phosphate groups and 2-keto-3-deoxyoctanic acid (KDO) to the so-called lipid A part. This latter moiety contains esterified and amide bound normal, iso- and anteiso β-hydroxy acid moieties with chain lengths of C_{12}–C_{18}. In particular, the amide-bound β-hydroxy fatty acids are preserved to some extent in recent sediments and can be released from them after subsequent extraction, saponification and acid hydrolysis (Goossens *et al.* 1989*a*, *b*). Bacterial β-hydroxy fatty acids can occur in more mature sediments and are thought to result from natural cleavage during diagenesis of β-hydroxyacid moieties of LPS (Matsumoto & Nagashima 1984). To the best of our knowledge $\delta^{13}C$ values of sedimentary β-hydroxyacids have not yet been reported. The occurrence of bacterial β-hydroxyacids in sediments indicate the presence of bacterial activity or reworking in the depositional environment.

Sulphate reducing bacteria

Boon *et al.* (1977) have reported the presence of iso- and anteiso C_{15} and C_{17} monounsaturated fatty acids in cultures of sulphate-reducing bacteria as well as in a few very recent marine sediments. Although these acids are thought to be highly specific for some sulphate-reducing bacteria, their significance as biomarkers for

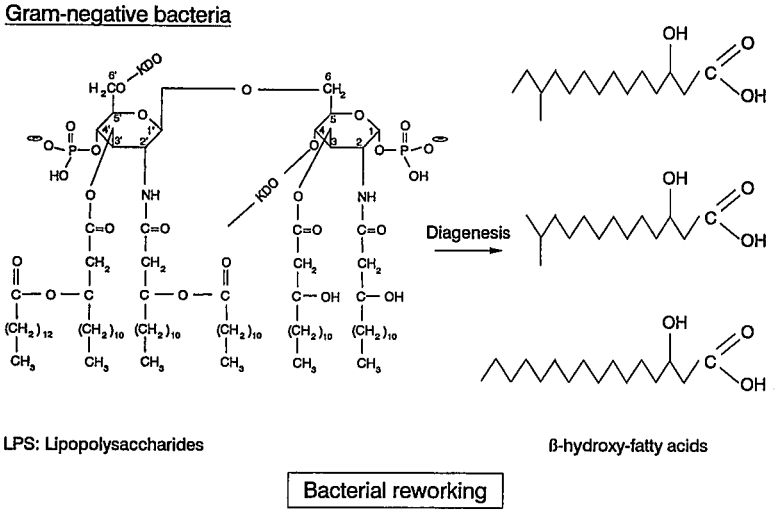

Fig. 15. Palaeoenvironmental indicators for gram-negative bacteria.

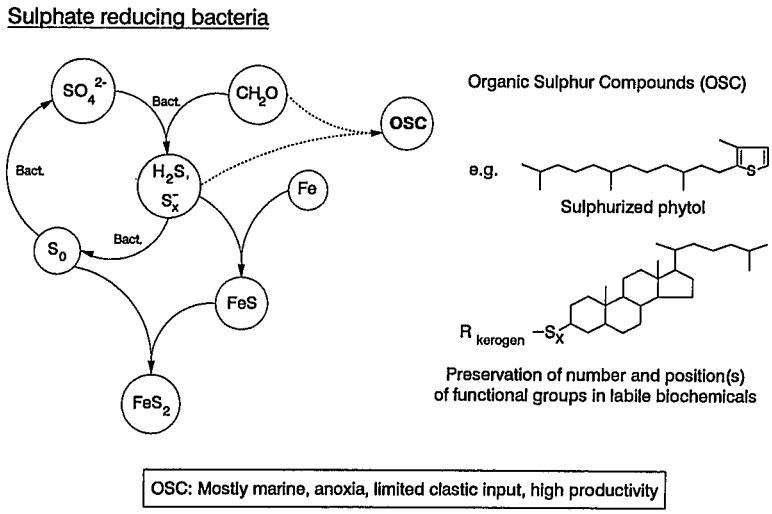

Fig. 16. Palaeoenvironmental indicators for sulphate-reducing bacteria. Bact, bacteria.

these organisms is limited because they are presumably mineralized at early stages of diagenesis. The presence of sulphate-reducing bacteria in anoxic, mostly marine, depositional environments can be deduced indirectly, but very clearly, from the presence of low- and high-molecular weight OSC in immature and mature sediments as well as in oils and coals. Over the last ten years it has become evident that organic compounds are sulphurized during the very early stages of diagenesis as a consequence of the reaction of inorganic polysulphides (H_2S and HS_x^-), produced by sulphate-reducing bacteria, with double bond, ketone or aldehyde functionalities (Fig. 16) (for a recent review see Sinninghe Damsté & De Leeuw 1990; Schouten *et al.* 1993*b*). These sulphur constituents, often present as very complex mixtures, act as excellent biomarkers since they are not suitable substrates for bacteria so that biochemicals are preserved which would otherwise have been mineralized. Moreover, the position of the sulphur moiety in the carbon skeleton reveals the position of the original functional group. It is clear that the presence of OSC in sediments reveals sulphate-reducing activity. Therefore,

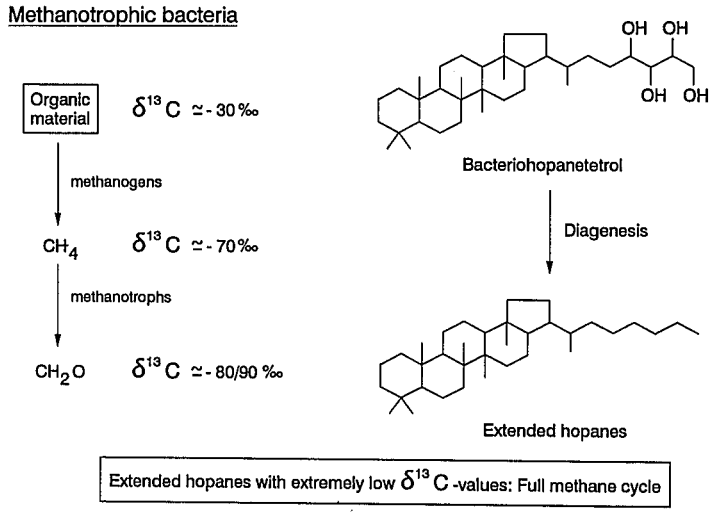

Fig. 17. Palaeoenvironmental indicators for methanotrophic bacteria.

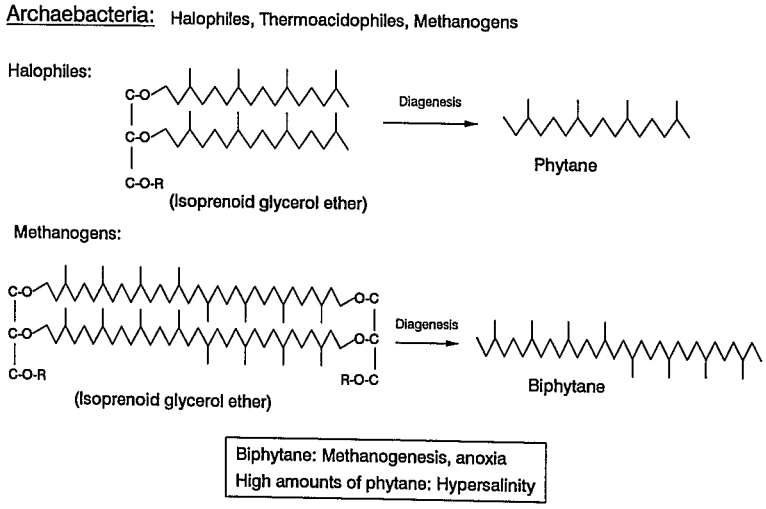

Fig. 18. Palaeoenvironmental indicators for archaebacteria.

their presence points to anoxia in mostly marine sediments with a limited clastic input and presumably a relatively high production of organic matter in the water column, since the sulphate-reducing bacteria require relatively fresh organic matter as a substrate. From the above it is evident that $\delta^{13}C$ values of OSC can be highly variable because sulphurization can occur with molecules derived from all kinds of organisms living in different habitats in the depositional environment (e.g. Kohnen *et al.* 1992).

Methanotrophic bacteria

Methanotrophic bacteria (methanotrophs) oxidize methane to biosynthesize their cell constituents. The methane consumed is produced by methanogens which induce a major isotopic shift between their biomass and the methane released (cf. Fig. 17; Hayes 1993). The isotopically very light methane (very negative $\delta^{13}C$ values) is further fractionated during assimilation so that lipids of methanotrophic bacteria are extremely light ($\delta^{13}C$ *c.* −80 to −90‰). Amongst others,

bacteriohopanetetrol derivatives are biosynthesized by methanotrophs. These triterpenoids are diagenetically altered to hopanes with similarly highly negative $\delta^{13}C$ values. The presence of such hopanes and their methylated counterparts in sediments clearly indicate that a full methane cycle existed in the palaeoenvironment (Freeman *et al.* 1990; Collister *et al.* 1992).

Archaebacteria

Archaebacteria are well known for their specific membrane lipids which consist of series of isoprenoid glycerol ethers (Fig. 18). It has been suggested by Albaiges (1980) that these isoprenoid ethers yield the corresponding isoprenoid hydrocarbons upon diagenesis. In particular, biphytane represents a highly specific carbon skeleton biosynthesized predominantly by methanogenic archaebacteria. The presence of this isoprenoid in oils or in sediments clearly indicates the presence of methanogens and, hence, anoxia in the depositional environment. Very high concentrations of phytane (or its sulphurized counterpart) may indicate palaeohypersalinity as a consequence of the presence of relatively high amounts of biphytanylglycerol ethers in halophylic archaebacteria (Volkman & Maxwell 1986). Methanogenic archaebacteria do not significantly discriminate against ^{13}C in their organic substrate (Kohnen *et al.* 1992).

Vascular higher plants

As mentioned in the beginning, the contribution of vascular higher plants to sedimentary organic matter in the marine realm can be very significant (Hedges 1992). Vascular higher plants biosynthesize different suites of resistant bio(macro)molecules. Accumulation of terrestrial-derived material may be due to transportation of bio(macro)molecules by aeolian and/or riverine transport, or to the *in situ* development and senescence of specific aquatic higher-plant communities, such as seagrasses. As vascular higher plants do not occur throughout the marine realm, but only in shallow shelves or lagoons, all of the vascular higher plant derived macromolecules are indicative of input from relatively nearshore material. In several cases, e.g. lignin from woody material, spores, pollen and resins, these inputs must be derived from paralic or fully terrestrial settings.

The following account describes biomolecules found in both aquatic and terrestrial higher plant tissues, followed by the assignation of biomolecular signals to particular terrestrial and submarine higher plant groups. Finally, a brief case study is presented where an attempt has been made to distinguish the depositional input of molecules from a terrestrial higher-plant source against molecules from a submarine higher-plant source.

General bio(macro)molecules The most well-known low-molecular weight markers for higher-land plant input in sediments are long chain *n*-alkanes (C_{25}, C_{27}, C_{29}, C_{31}) with a strong odd over even predominance. These compounds are derived from epicuticular waxes on leaf surfaces (Eglinton & Hamilton 1967). The $\delta^{13}C$ values for these long-chain alkanes are *c.* $-27‰$ for C3 plants, whereas those from C4 plants are relatively enriched in ^{13}C (Hayes 1993). In addition to the alkanes, numerous other low-molecular weight compounds have been encountered indicating higher-plant input (see Mangroves and Case study). However, most higher-plant material contributing to the organic matter in the marine environment is macromolecular and consequently is resistant against biodegradation. In the following sections the macromolecular compounds present in or produced by higher plants will be discussed.

(a) Cutin and cutan. Chemical analyses of the insoluble matrix of higher-plant cuticles of leaf and stem surfaces have shown that they are composed of the biopolyester cutin, or of an insoluble, non-hydrolysable biomacromolecule, named cutan, or, most commonly, a mixture of both (Nip *et al.* 1986; Tegelaar *et al.* 1991). Cutins are composed of esterified fatty acids, hydroxy fatty acids and dicarboxylic acids of C_{16} and C_{18} carbon chain lengths. These acids often possess a mid-chain functional group (hydroxyl or epoxy) (Holloway 1984). The cutan is thought to be composed exclusively of *n*-alkyl moieties linked together via relatively stable ether bonds (De Leeuw *et al.* 1991). Although most cuticles are thought to be composed of these aliphatic macromolecules (cutin/cutan), a recent paper by Kögel-Knabner *et al.* (1994) reports the presence of lignin (see below) as the resistant biomacromolecule in *Picea abies* needle cuticles. The contribution of aliphatic cuticle constituents to marine sedimentary organic matter is difficult to prove since several marine green microalgae also contain resistant highly aliphatic macromolecules. However, cuticle fragments could easily be transported into the marine realm by wind or fluvial transport and Tegelaar *et al.* (1989*b*) have suggested that the cutans may contribute significantly to kerogen.

(b) Suberin and suberan. Aliphatic macro-

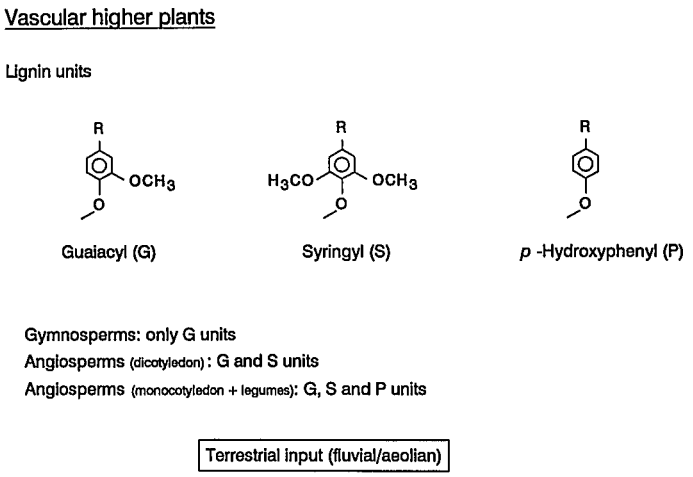

Fig. 19. Palaeoenvironmental indicators for vascular higher plants: lignins.

molecules, comparable with cutin and cutan, are also identified in periderm (bark of woody plants) tissues of fossil and extant higher plants (Holloway 1984; Collinson *et al.* 1994; Tegelaar *et al.* 1989*a*, 1994). These are named suberin and suberan, respectively. Suberin is a biopolyester similar to cutin, however, the main monomeric units are C_{18} monomers (Holloway 1984; Tegelaar *et al.* 1989*a*). The molecular structure of suberan is thought to be mainly composed of *n*-alkyl moieties, similar to cutan (Collinson *et al.* 1994; Tegelaar *et al.* 1994). Bark fragments could also be transported into the marine organic matter by fluvial or aeolian means.

(c) Sporopollenin. One distinctive group of higher-plant structures which often provide microscopically observable proof of their long distance transport into the marine realm are spores and pollen (Traverse 1988). The recognizable entities are the spore or pollen walls of which the resistant insoluble constituent is named sporopollenin. The molecular structure of this compound is still not completely clear despite numerous studies (for a review see De Leeuw & Largeau 1993). However, recent papers imply the presence of both aromatic and aliphatic moieties (Van Bergen *et al.* 1993; Hemsley *et al.* 1993) of which the aromatic moieties can be altered quite drastically upon diagenesis (Collinson *et al.* 1994). Nonetheless, the presence of benzaldehyde, acetophenone and acetophenol in combination with phenol in pyrolysates of marine kerogens may be used to determine input from spores and/or pollen.

Being microscopically recognizable, and identifiable to parent plant groups, spores and pollen in marine sediments provide evidence of the type of vegetation on the surrounding land and, hence, also some indication of regional climate. Furthermore, the overall proportions of land-derived spores and pollen vs. marine derived algal resting cysts (e.g. dinoflagellates) are indicative of distance from the land. Certain categories of spores and pollen are known to undergo very long distance transportation in air (e.g. bisaccate pollen of conifers such as *Pinus*) whilst others (e.g. certain fern spores) tend to undergo very little transport. Hence, a suite of pollen and spores not only documents terrestrial input but provides evidence for the proximity of source material as well as indicating climate and the vegetation from which it was derived. For further details consult Jansonius & McGregor (in press), Traverse (1988) and references therein (also see Plaziat, this volume).

(d) Lignin. Despite the importance of the mainly aliphatic biomacromolecules mentioned above, because of their selective enrichment upon diagenesis, the most abundant biomacromolecules in extant vascular higher plants are lignins. Three different types of lignins are recognized (Fig. 19; Sarkanen & Ludwig 1971). Gymno-sperm lignin contains only monomethoxyphenol units (guaiacyl; G), whereas angiosperm lignin contains dimethoxy (syringyl; S) as well as monomethoxyphenol units (e.g. Sarkanen & Ludwig 1971; Saiz-Jimenez & De Leeuw 1986; Ralph & Hatfield 1991). Within the angiosperms the lignin of the monocotyledons and legumes are composed of both methoxyphenol units (S and G) and *p*-hydroxyphenyl units (P) (cf. Fig. 19; e.g. Ralph & Hatfield

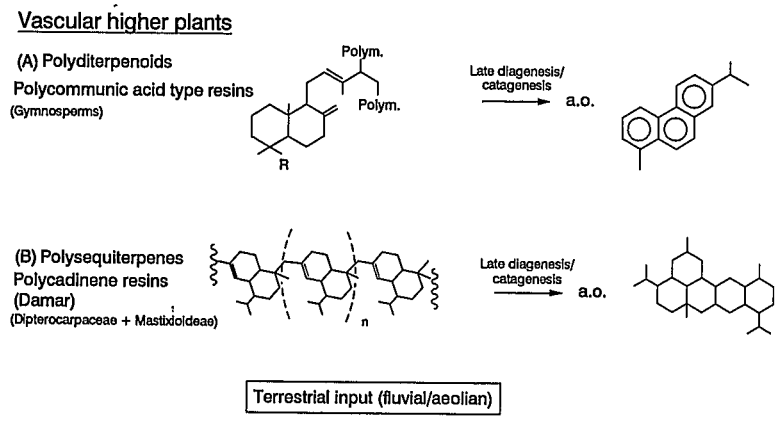

Fig. 20. Palaeoenvironmental indicators for vascular higher plants: resins.

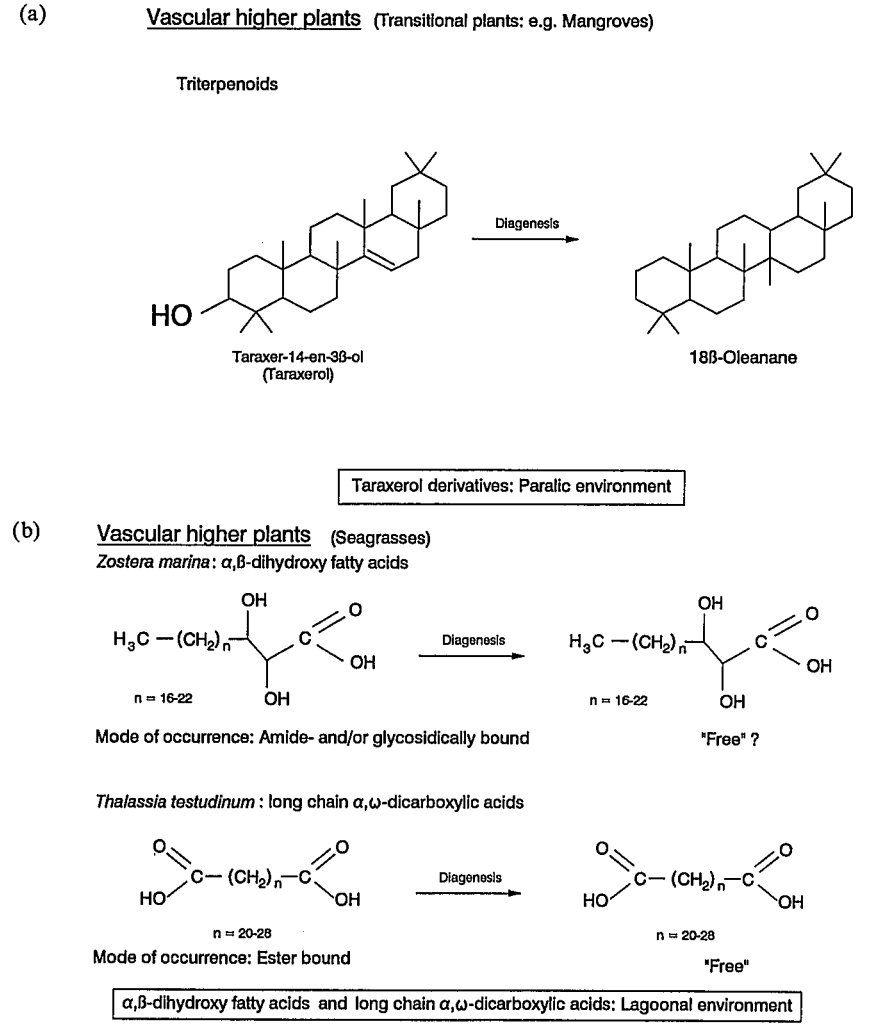

Fig. 21. Palaeoenvironmental indicators for: (a) transitional higher plants – mangroves; (b) lagoonal aquatic higher plants – seagrasses.

1991). The presence of these different specific lignin units in marine sediments can be used to determine the relative contribution of terrestrial material into the marine realm (Hedges & Parker 1976; Goñi & Hedges 1992 and references therein). However, in more ancient sediments, lignin macromolecules can be altered considerably (Hatcher *et al.* 1989; Van Bergen *et al.* 1994*b*) depending on overburden and lithology (Van Bergen *et al.* 1994*c*). Nevertheless, if present, the specific methoxyphenol units can still be recognized. Variations in the $\delta^{13}C$ values, due to different fixation pathway of atmospheric CO_2 of the C3 (*c.* −28‰) vs. C4 plants (*c.* −17‰), are also reflected in the lignin building blocks (Benner *et al.* 1987; Goni *et al.* 1993).

(e) Resins. The macromolecular substances in resins and resinites can be subdivided into three major structural types (Anderson *et al.* 1992). Polyditerpenoids are macromolecules of which the basic structural unit has the diterpenoid labdane carbon skeleton (Fig. 20a; Carman *et al.* 1970); these polymers are present in gymnosperm resins. The second type are polysesquiterpenes (Fig. 20b), recognized in resins from Dipterocarpaceae and Mastixioideae, which have as their basic structural unit a sesquiterpenoid cadinane skeleton (Van Aarssen *et al.* 1990, 1994). The third type is a natural polystyrene which is reported from *Liquidambar* (Grimaldi *et al.* 1989). During late diagenesis and catagenesis these macromolecules break down generating compounds which are frequently encountered in sediments and crude oils (e.g. Van Aarssen *et al.* 1990). Since, in most cases, these compounds still reveal some of their original structure they can be used to interpret the source plants and they indicate terrestrial input into the marine realm.

Mangroves One group of molecular markers commonly associated with higher-plant input in marginal marine sediments and oils are the non-hopanoid pentacyclic triterpenoids. Angiosperm input of oleananes and other pentacyclic triterpenoids (Fig. 21a) since the late Cretaceous (100 Ma) is well documented (Whitehead 1974; Ten Haven & Rullkötter 1988). For example, crude oils from the Tertiary Niger Delta have significant amounts of oleananes which are known to be sourced from terrestrial higher plants (Ekweozer & Udo 1988). In extant plant tissues the pentacyclic triterpenoids are secondary metabolites and have a major protective function within resins and waxes as they are secreted outside the cell or plant (Baas 1983).

The diagenetic transformation pathway from biosynthesized pentacyclic triterpenols (e.g. α- or β-amyrin and taraxerol) within angiosperm tissues to saturated products (triterpanes, e.g. oleanane) has recently been determined in greater detail by Killops & Frewin (1994) (Fig. 22). Comparative diagenetic transformation of sterol precursors to steranes is known to occur through dehydration at the alcohol functionality with sterene intermediates (De Leeuw *et al.* 1989; De Leeuw & Baas 1986). Until recently, such intermediates were rarely found in sediments, despite the frequent alcohol functionality at C3. However, work by Ten Haven *et al.* (1992) has shown, from Recent sediments, that dehydration of triterpenols to Δ^2-triterpenes does occur. Triterpadienes and A-ring contracted triterpadienes were identified in sediment extracts. The A-ring contracted homologues arise from 3β-OH alcohols, whilst Δ^2-triterpenes are known to derive from 3α-functionalities. This led Ten Haven *et al.* (1992) to suggest a degradational pathway based on competing microbial and chemical transformations, whereby microbial action produces the triterpadienes and chemical action produces the A-ring contracted homologues. This suggestion was confirmed in work by Frewin *et al.* (1993) and Killops & Frewin (1994) where transformation products of pentacyclic triterpenols were isolated from the external membrane of the plant tissue itself, thus indicating that microbial degradation can occur at an even earlier stage than was at first speculated.

A study of leaf cuticular membranes from the mangrove *Rhizophora mangle* was performed using a methodology combining solvent extraction and elucidation of insoluble constituents using pyrolytic techniques (Frewin 1993). Cuticle extracts yielded quantities of triterpadienes which could be directly related to their triterpenol precursors in the higher-plant tissue. Pyrolysis of external tissues enabled direct thermal dehydration of most pentacyclic triterpenols and demonstrated the fractionated distribution of triterpenoids within the different functional tissues. For example, in *R. mangle*, β-amyrin was mostly located in solvent soluble wax fractions, whilst taraxeroid-type compounds were found as bonded/occluded moieties within the matrix of the cuticle and were not directly extractable. This provided compelling evidence for the preservation of selected triterpenoidal structures during sedimentary diagenesis. Similar extract and pyrolytic analyses to those conducted on plant tissue were undertaken for < 5000 years BP sediment samples from Florida Bay, southeastern USA (Frewin 1993). Solvent extracts yielded substan-

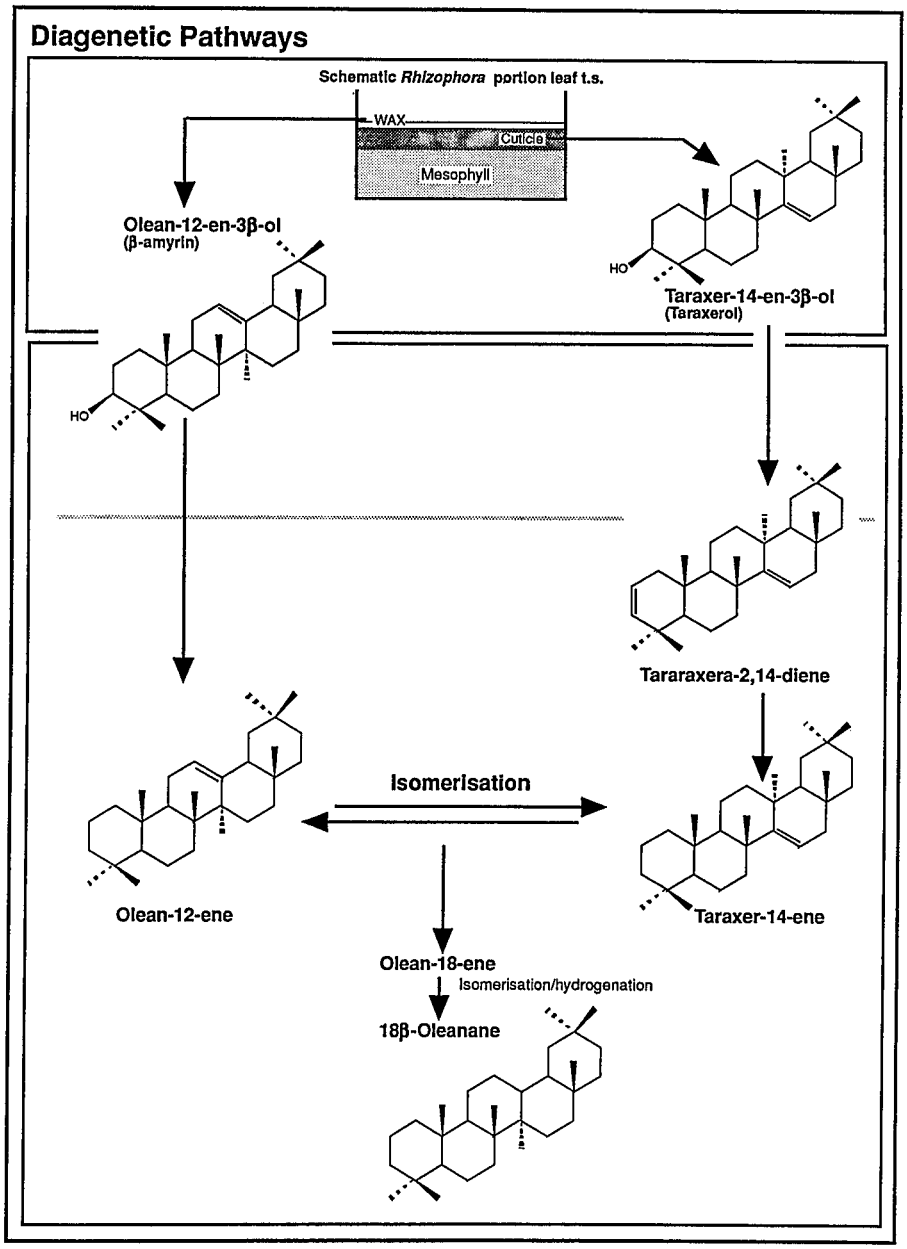

Fig. 22. Diagenetic pathway for pentacyclic triterpenoids from mangroves.

tial amounts of β-amyrin, whilst pyrolysis again demonstrated that taraxeroid structures were retained within the insoluble fraction. These data pointed to the selective release of taraxeroid structures into the geosphere and their rapid isomerization to oleanoid structures, thus joining the oleanoid degradational pathway, as was earlier speculated by Ten Haven & Rullkötter (1988).

Interestingly, these observations were true for the *Rhizophora* species and not for another mangrove species, *Avicennia germinans*. In the latter, all triterpenoids were directly extractable and none were retained in the pyrolysate (Frewin 1993).

The information presented above has several implications. Firstly, mangrove ecosystems have been described as one of the most productive

(Lugo & Snedaker 1974) and, in the case of Florida Bay, they are documented as producing 40% of the organic matter entering the marine system (Heald 1971). Amongst angiosperms, this places them in a position of particularly high preservation potential, with deposition of litter occurring directly at the water–sediment interface (compared with plants of the terrestrial hinterland which are deposited sub-aerially). Secondly, the unusual distribution of pentacyclic triterpenoids within some species of mangroves and not others means that the origin of these compounds within sediments and oils should be re-evaluated. For example, the high oleanane content of the southern Nigerian crude oils was not ascribed to mangrove vegetation (which has dominated the marine margins of the delta throughout Tertiary and Recent sediments; Ekweozer & Udo 1988). This reasoning was based on the observation that the endemic *Rhizophora* species did not contain amyrin constituents and that, therefore, fluviatile transport of allochthonous organic matter from other sources was considered a more likely explanation (Ekweozer & Udo 1988). *Rhizophora mangle*, which was examined by Frewin (1993), contained an unusual distribution of triterpenoids, as described above, and this may well be reflected in the Nigerian species with all structures joining the oleanoid degradational pathway. High amounts of taraxerol were isolated from anoxic, semi-restricted, lacustrine sediments of Kau Bay in Indonesia (Middelburg *et al.* 1993). The abundant presence of taraxerol in these sediments is thought to reflect the surrounding mangrove vegetation and its survival is a result of anoxic conditions during sedimentation.

Thus, the presence of specific non-hopanoid pentacyclic triterpenoids in ancient sediments may be more indicative of shoreline proximity than previously thought and may corroborate other methods of recognition (see Plaziat this volume).

Aquatic higher plants Aquatic higher plants (freshwater as well as marine) require a different physiology with which they control the effects of their local environment and this may be reflected in their biomolecular composition. Freshwater plant remains which might be transported into the marine realm include resistant parts such as seed material. The biomacromolecular composition of such seeds and the preservation potential in the marginal marine realm have recently been studied by Van Bergen *et al.* (1991, 1994*a, b, c*). They showed that these seeds had seed coats which were composed of two layers. The outer seed coat layer was composed of lignin which was dramatically altered during diagenesis (Van Bergen *et al.* 1994*b*), whereas the inner seed coat layer was composed of a cutan-like highly aliphatic macromolecule (Van Bergen *et al.* 1994*a*). The latter, in particular, could contribute to amorphous kerogen in marine sedimentary organic matter in a way similar to that of cutan (Van Bergen *et al.* 1994*a*).

One group of marine flowering plants are the seagrasses which occur in large areas of marginal shallow marine environments such as lagoons. This means that the blade material may form a large proportion of the photoautotrophically derived organic detritus. Pyrolysis–GC–MS of the thin external membrane covering the seagrass blade suggests that it is chemically different to that demonstrated in their terrestrial counterparts (Frewin 1993). The tissues are found to contain a high proportion of carbohydrates in association with a biopolymer which shows affinities to both the classical cutin chemistry and the suberin structure described by Holloway (1984). This may be a reflection of its submarine existence. The derivation of distinctive chemical markers for seagrass blade material is difficult to achieve as epibionts are generally found in close and inseparable association with blade tissues (Frewin 1993). However, extract analyses of a number of samples in different stages of senescence, from a number of different species, show that modified cutin-derived, long chain α,ω-dicarboxylic acids (C_{26}–C_{28}; Fig. 21b) are reliable markers for seagrass blade material within sediments (Nichols *et al.* 1982; Nichols & Johns 1985; Frewin 1993). Further acid treatment of temperate seagrass blades (*Zostera marina*) yields a distinctive series of α,β-dihydroxy fatty acids (Fig. 21b; De Leeuw *et al.* unpublished results). The high yields of these acids from extant tissues suggest that they are biosynthesized by the plants and incorporated by amide or glycosidic bonds and thus not directly extractable.

Estimation of shoreline proximity – a case study The distinction between biomolecules derived from terrestrial higher plants and those derived from submarine higher plants has been addressed in a study of the lagoonal carbonate sediments of Florida Bay, southeastern USA (Fig. 23), by Frewin (1993) where there are two main floral types being actively incorporated into the sediments. The paralic margins of the bay are dominated by mangrove swamps, whilst the subtidal mud-banks of the bay have dense carpets of seagrass which extend 30–40 km from the nearest terrestrial higher plant input.

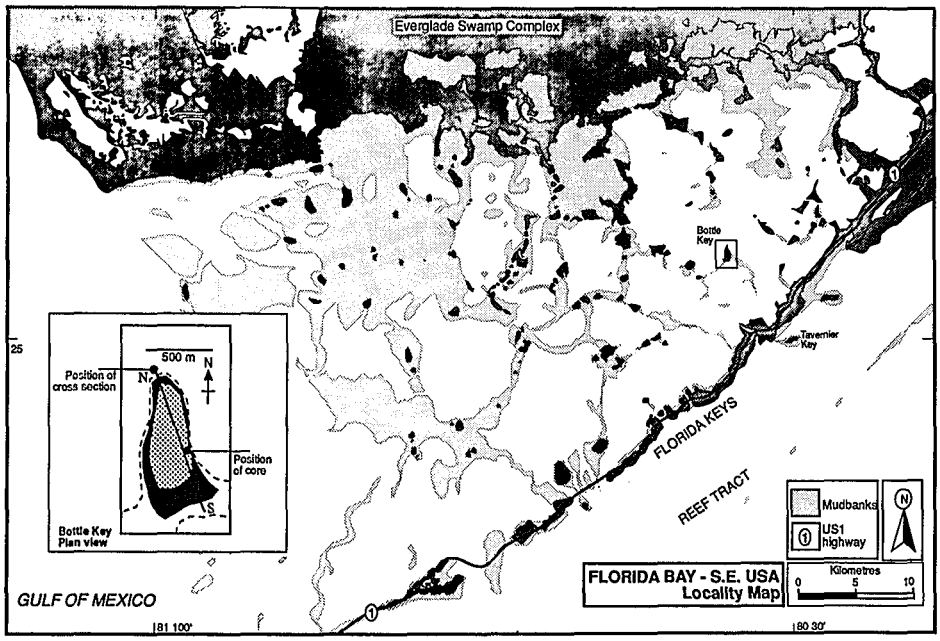

Fig. 23. Map of Florida Bay showing location of Bottle Key core.

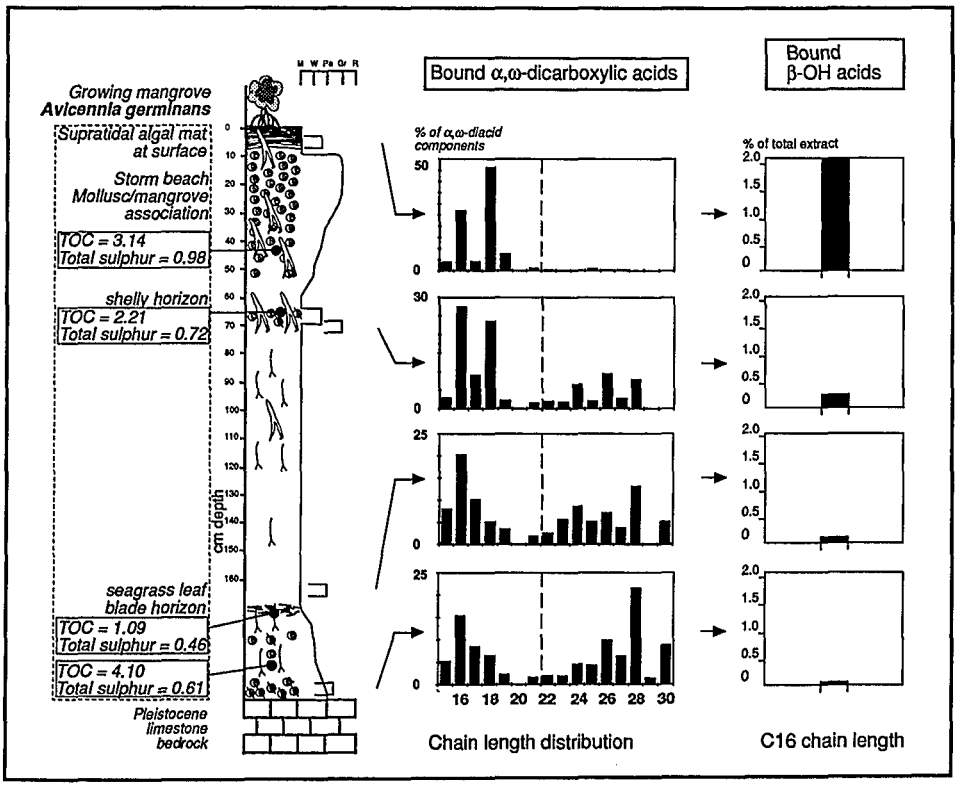

Fig. 24. Downcore distribution of two organic molecular components isolated from sediment extracts. Sediments taken from a core at Bottle Key, Florida Bay, southeast Florida. (Molluscs, half-filled circles; seagrass, thin roots; mangrove, thick roots.)

Table 1. *Overview of the relationship between organism, biomolecule, molecular fossil and palaeoenvironment*

Organisms	Biomolecule **Biomacromolecule**	Molecular fossil	Palaeoenvironmental parameter
PROKARYOTES			
Cyanobacteria	Mid-chain-methyl alkanes Bacteriohopanetetrol	Unaltered Extended hopanes, S-bound hopane	Water-column stratification
Archaebacteria (especially methanogens)	Isoprenoid ether lipids **Bound isoprenoid ether lipids**	Specific isoprenoid alkanes **Bound isoprenoid moieties**	Anoxia
Sulphate reducing bacteria	Functionalized compounds	Organic Sulphur Compounds (OSC)	Anoxia
Photosynthetic sulphur bacteria	Specific carotenoids	Diarylisoprenoids S-bound diarylisoprenoids	Chemocline in photic zone
Methanotrophic bacteria	Bacteriohopanetetrol	'Isotopic light' extended hopanes	Methane cycling
Gram-negative bacteria	LPS	β-OH fatty acids	Bacterial reworking
EUKARYOTES			
Dinoflagellates	Dinosterol	Dinosterane	Dinoflagellates even when cysts are absent
Diatoms	Highly branched isoprenoid alkanes/alkenes	Highly branched isoprenoid alkanes	Blooming through upwelling
Prymnesiophtyes	Long chain unsaturated ketones	Long chain unsaturated ketones	Surface sea-water temperatures
Chrysophytes	24-*n*-propylcholesterols	24-*n*-propylcholestanes	Marine setting
Green microalgae	Δ^7-Sterols **Algaenans**	5α,14β,17β(H)-steranes **Algaenans**	Freshwater/lacustine input Mainly freshwater/lacustrine
Algae/cyanobacteria	Chromans/chromane precursors	Chromans	Salinity
HIGHER PLANTS			
Terrigenous angiosperms	Long chain *n*-alkanes **Cutan** **Sporopollenin** **Angiosperm lignin** **Polycadinene resins**	Long chain *n*-alkanes **Transformed cutan** **Transformed sporopollenin** **Transformed angiosperm lignin** **Polycadinanes**	Terrestrial input (aeolian/fluvial)
Terrigenous gymnosperms	**Sporopollenin** **Gymnosperm lignin** **Polycommunic acid resins**	**Transformed sporopollenin** **Transformed gymnosperm lignin** **Transformed polycommunic acid resins**	Terrestrial input (aeolian/fluvial)
Transitional plants (e.g. Mangroves)	Specific triterpenoids	Specific triterpanes	Paralic input
Seagrasses	**Amide bound α,β-diOH-fatty acids** **Esterified α,ω-dicarboxylic acids**	**α,β-diOH-fatty acids** **α,ω-dicarboxylic acids**	Shallow marine input
Freshwater plants	**Cutan** **Lignin**	**Transformed cutans** **Transformed lignins**	Freshwater input

The Holocene marine inundation of the south Florida peninsula has been well documented (e.g. Scholl 1964) and the effects of a transgressive regime on coastal sedimentation have been studied in some detail (e.g. Parkinson 1989). The south Florida peninsula has an extremely low relief and sea-level changes are recorded by sediments containing organic matter derived from various coastal and shallow marine vegetation types. The submarine and terrestrial floras (in this case seagrass and mangrove) are sensitive to the changes in salinity, amongst other variables, existing between the freshwater of the Everglades swamps to the marine conditions of the lagoonal mud-banks. An examination of particulate organic matter within the marine sediments of the bay failed to locate evidence for such a vegetational stratigraphy, as structured organic matter was rapidly reworked to amorphous masses (Frewin 1993) and this hindered the recognition of the relative position of the south Florida coastline. Root material may form as secondary fabrics in sediments, and was thus discounted from identification of colonizing communities. Root material was removed from sediment samples by sieving. Organic geochemical studies of the bay sediments isolated several groups of molecular markers pertinent to the study of backstepping submarine and terrestrial higher-plant types in Florida Bay.

Two organic marker parameters were used in the distinction of the two higher-plant sources. The relative contribution of C_{16} and C_{26}^{+} α,ω-dicarboxylic acids were used to identify terrestrial higher-plant input and seagrass input, respectively. A typical mud-bank sequence is shown in Fig. 24. Using the markers described above, it can be concluded that seagrass colonization was occurring at the base of the sequence. The increased relative abundance of C_{16} chain lengths suggests that mangroves, or other terrestrial higher-plant material, were also located nearby, but colonization was not apparent at this particular point in the cored section. Further mangrove colonization was occurring higher in the sequence, in association with storm beach material. It is evident that seagrass input is very much reduced at this point in the core. In this way, the relative contribution of terrestrial and submarine organic colonizers existing in juxtaposition may be assessed.

Conclusions

As a result of recent developments in organic geochemistry, i.e. determination of OSC and analyses of $\delta^{13}C$ values of individual sedimentary compounds, it is possible to decode all information known to be present in sedimentary organic molecules, i.e: (1) carbon skeleton structures; (2) positions of functional groups; and (3) stable carbon isotope ratios. The information strengthens relationships between molecular fossils (or biomarkers) and (palaeo)-biochemicals (Table 1). Furthermore, biochemical assemblages give clues to their precursor organisms and also reveal palaeoenvironmental conditions (i.e. temperature, stratification, salinity, etc.). The combined presence of OSC, dinosteroids, 24-*n*-propylsteroids (even in trace amounts), high amounts of C_{25} HBI hydrocarbons and the absence of long chain *n*-alkanes with odd over even predominance, bicadinanes and specific higher-plant triterpenoids (e.g. oleanane) characterize a marine environment. Likewise, palaeoenvironmental conditions and diagenetic processes are indicated by molecular and/or isotopic characteristics. For example, the presence of isotopically very heavy diaromatic carotenoid derivatives (less negative $\delta^{13}C$ values) indicate photic zone anoxia; the chroman ratio indicates hypersalinity vs. marine to fresh water conditions (even though the source organism is unknown); OSC indicate the presence of microbial sulphate-reducing activity, i.e. anoxia; isotopically heavy C_{25} HBI hydrocarbons indicate diatom blooming normally associated with palaeo-upwelling; the relative concentrations of di- and triunsaturated long-chain ketones derived from certain prymnesiophyte algae provide palaeo-surface seawater temperatures. Hence, biomarkers (or molecular fossils) can be upgraded to (molecular) palaeoenvironmental indicators.

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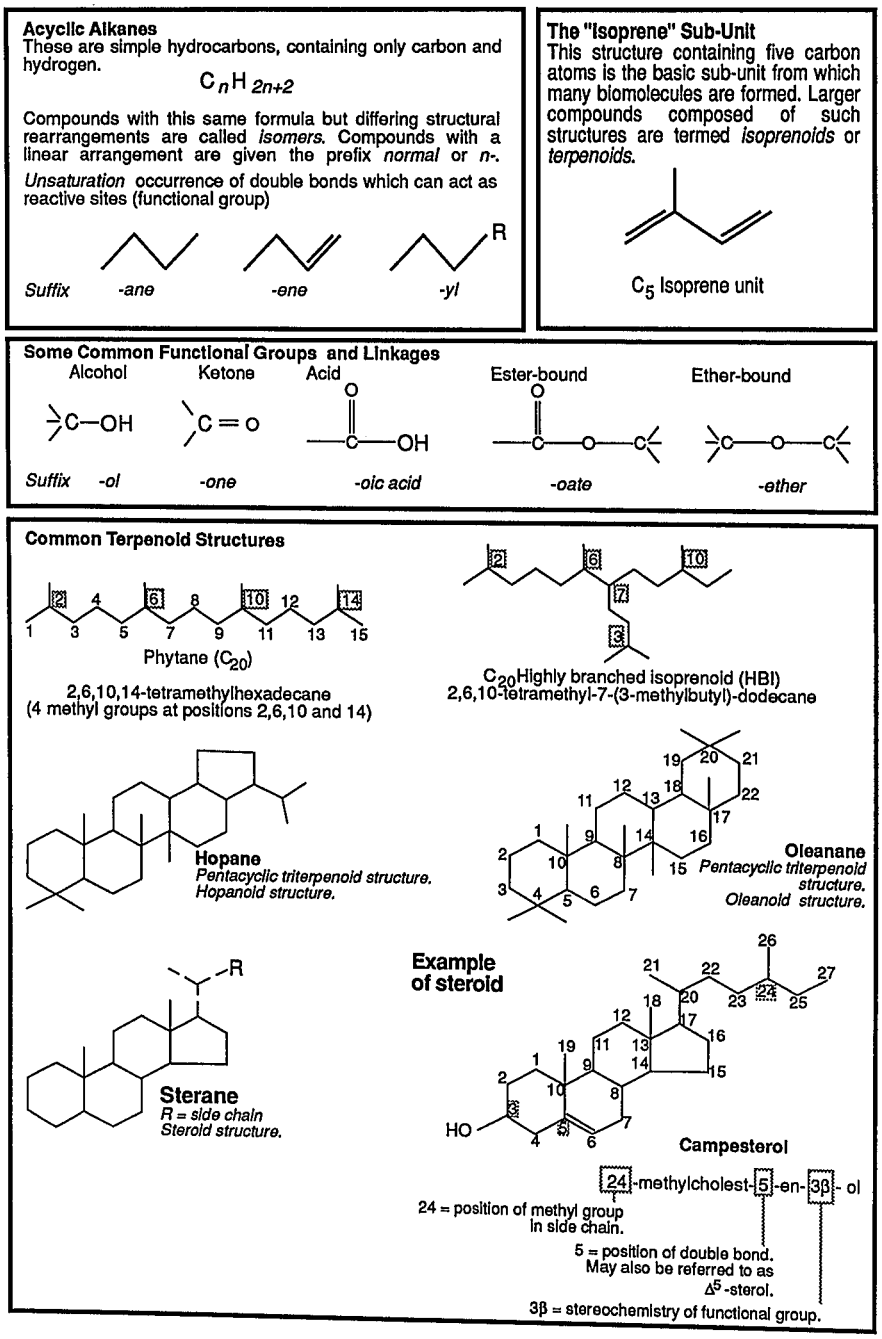
Appendix A

A simple guide to nomenclature in organic chemistry. For a more detailed appraisal of stereochemistry and chemical nomenclature in a geological context, the reader is referred to Peters & Moldowan (1993).



References

ALBAIGES, J. 1980. Identification and geochemical significance of long chain acyclic isoprenoid hydrocarbons in crude oils. *In:* DOUGLAS, A. G. & MAXWELL, J. R. (eds) *Advances in Organic Geochemistry 1979*. Pergamon Press, Oxford, 19–28.

ANDERSON, K. B., WINANS, R. E. & BOTTO, R. E. 1992. The nature and fate of natural resins in the geosphere – II. Identification, classification and nomenclature of resinites. *Organic Geochemistry*, **18**, 829–841.

BAAS, W. J. 1983. *Triterpenoids in latex and leaf wax of some* Hoya *species*. PhD thesis, University of Utrecht.

BENNER, R., FOGEL, M. L., SPRAGUE, E. K. & HODSON, R. E. 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. *Nature*, **329**, 708–710.

BOON, J. J. 1992. Analytical pyrolysis mass spectrometry: new vistas opened by temperature-resolved insource PYMS. *International Journal of Mass Spectrometry and Ion Processes*, **118/119**, 755–787.

———, DE LEEUW, J. W., HOEK, G. & VOSJAN, J. H. 1977. Significance and taxonomic value of iso- and anteiso monoenoic fatty acids and branched β-hydroxy acids in *Desulfovibrio desulfuricans*. *Journal of Bacteriology*, **129**, 1183–1191.

———, RIJPSTRA, W. I. C., DE LANGE, F., DE LEEUW, J. W., YOSHIOKA, H. & SHIMIZU, Y. 1979. Black sea sterol – a molecular fossil for dinoflagellate blooms. *Nature*, **277**, 125–127.

BRASSELL, S. C., EGLINTON, G., MARLOWE, I. T., SARNTHEIN, U. & PFLAUMANN, U. 1986. Molecular stratigraphy – a new tool for climatic assessment. *Nature*, **320**, 129–133.

CARMAN, R., COWLEY, D. E. & MARTY, R. A. 1970. Diterpenoids. V. Dundathic acid and polycommunic acid. *Australian Journal of Chemistry*, **23**, 1655–1665.

COLLINSON, M. E., VAN BERGEN, P. F., SCOTT, A. C. & DE LEEUW, J. W. 1994. The oil-generating potential of plants from coal and coal-bearing strata through time: A review with new evidence from Carboniferous plants. *In:* SCOTT, A. C. & FLEET, A. J. (eds) *Coal and Coal-bearing Strata as Oil-prone Source Rocks*? Geological Society, London, Special Publication, **77**, 31–70.

COLLISTER, J. W., SUMMONS, R. E., LICHTFOUSE, E. & HAYES, J. M. 1992. An isotopic biogeochemical study of the Green River oil shale. *Organic Geochemistry*, **19**, 265–276.

DE LEEUW, J. W. & BAAS, M. 1986. Early stage diagenesis of sterols. *In:* JOHNS, R. B. (ed.) *Biological Markers in the Sedimentary Record*. Elsevier, Amsterdam, 101–123.

———, COX, H. C., VAN GRAAS, G., VAN DER MEENT, F. W., PEAKMAN, T. M., BAAS, J. M. A. & VAN DER GRAAF, B. 1989. Limited double bond isomerisation and selective hydrogenation of sterenes during early diagenesis. *Geochimica et Cosmochimica Acta*, **53**, 903–909.

——— & LARGEAU, C. 1993. A review of the major classes of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal and petroleum formation. *In:* ENGELS, M. H. & MACKO, S. A. (eds) *Organic Geochemistry, Principles and Applications*. Plenum Publishing Corporation, New York, 23–72.

———, VAN BERGEN, P. F., VAN AARSSEN, B. G. K., GATELLIER, J-P. L. A., SINNINGHE DAMSTÉ, J. S. & COLLINSON, M. E. 1991. Resistant biomacromolecules as major contributors to kerogen. *Philosophical Transactions of the Royal Society of London. Series B*, **333**, 329–337.

DERENNE, S., BERRE, L. E. F., LARGEAU, C., HATCHER, P. G., CONNAN, J. & RAYNAUD, J. F. 1992. Formation of ultralaminae in marine kerogens via selective preservation of thin resistant outer walls of microalgae. *Organic Geochemistry*, **19**, 345–350.

———, LARGEAU, C., CASADEVALL, E. & BERKALOFF, C. 1989. Occurrence of a resistant biopolymer in the L race of *Botryococcus braunii*. *Phytochemistry*, **28**, 1137–1142.

———, ———, ——— & SELLIER, N. 1990. Direct relationship between the resistant biopolymer and tetraterpenic hydrocarbon in the lycopadiene race *Botryococcus braunii*. *Phytochemistry*, **29**, 2187–2192.

DEUSER, W. G. 1970. Isotopic evidence for diminishing supply of available carbon during diatom bloom in the Black Sea. *Nature*, **225**, 1069–1071.

DOUGLAS, A. G., SINNINGHE DAMSTÉ, J. S., FOWLER, M. G., EGLINTON, T. I. & DE LEEUW, J. W. 1991. Unique distributions of hydrocarbons and sulphur compounds released by flash pyrolysis from the fossilised alga *Gloeocapsomorpha prisca*, a major constituent in one of four Ordovician kerogens. *Geochimica et Cosmochimica Acta*, **55**, 275–291.

EGLINTON, G. & HAMILTON, R. J. 1967. Leaf epicuticular waxes. *Science*, **156**, 1322.

EGLINTON, T. I., MEUZELAAR, H. L. C., HUAI, H., SEELY, J., UDEN, P. C. & TEGELAAR, E. W. 1993. Chemical discrimination of organic sub-facies within the Ferron delta (Cretaceous), Utah. *In:* ØYGARD, K. (ed.) *Organic Geochemistry*. Poster sessions from the 16th International Meeting on Organic Geochemistry, Stavanger, 1993. Falch Hurtigtrykk, Oslo, 451–455.

EKWEOZER, C. M. & UDO, O. T. 1988. The oleananes: Origin, maturation and limits of occurrence in southern Nigeria sedimentary basins. *Organic Geochemistry*, **13**, 131–140.

FREEMAN, K. H., HAYES, J. M., TRENDEL, J. M. & ALBRECHT, P. 1990. Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons. *Nature*, **343**, 254–256.

———, WAKEHAM, S. G. & HAYES, J. M. 1994. Predictive isotopic biogeochemistry: hydrocarbons from anoxic basins. *Organic Geochemistry*, **21**, 629–644.

FREWIN, N. L. 1993. *The distribution and preservation of organic matter in recent lagoonal carbonate Sediments, Florida Bay, USA*. PhD Thesis,

University of London.

———, KILLOPS, S. D., VAN BERGEN, P. F., DE LEEUW, J. W. & COLLINSON, M. E. 1993. Preservation potential of biomacromolecules of higher plant cuticles in Florida Bay. *In:* ØYGARD, K. (ed.) *Organic Geochemistry*. Poster sessions from the 16th International Meeting on Organic Geochemistry, Stavanger, 1993. Falch Hurtigtrykk, Oslo, 566–569.

GELIN, F., DE LEEUW, J. W., SINNINGHE DAMSTÉ, J. S., DERENNE, S., LARGEAU, C. & METZGER, P. 1993. Contribution of the aliphatic polyaldehyde of *Botryococcus braunii* A race to the formation of algal kerogens. *In:* ØYGARD, K. (ed.) *Organic Geochemistry*. Poster sessions from the 16th International Meeting on Organic Geochemistry, Stavanger 1993. Falch Hurtigtrykk, Oslo, 509–512.

———, ———, ———, ——— & ——— 1994*a*. The similarity of chemical structures of soluble aliphatic polyaldehyde and insoluble algaenan in the green microalga *Botryococcus braunii* race A as revealed by pyrolysis. *Organic Geochemistry*, **21**, 423–435.

———, ———, ———, ——— & ——— in press. Scope and limitations of flash pyrolysis–gas chromatography as revealed by the thermal behaviour of high molecular weight lipids derived from the green algae *Botryococcus braunii*. *Journal of Analytical and Applied Pyrolysis*.

GOÑI, M. A. & HEDGES, J. I. 1992. Lignin dimers: Structures, distribution, and potential geochemical applications. *Geochimica et Cosmochimica Acta*, **56**, 4025–4043.

——, EGLINTON, T. I. & BOON, J. J. 1993. Compound specific isotope analysis of lignin derived phenols from plant tissues and sediments. *In:* ØYGARD, K. (ed.) *Organic Geochemistry*. Poster sessions from the 16th International Meeting on Organic Geochemistry, Stavanger, 1993. Falch Hurtigtrykk, Oslo, 539–543.

GOOSSENS, H., DUREN, R. R., DE LEEUW, J. W. & SCHENCK, P. A. 1989*b*. Lipids and their modes of occurrence in bacteria and sediments I: Lipids in the sediment of a freshwater lake. *Organic Geochemistry*, **41**, 27–41.

———, DE LEEUW, J. W., RIJPSTRA, W. I. C., MEYBURG, G. J. & SCHENCK, P. A. 1989*a*. Lipids and their modes of occurrence in bacteria and sediments I: A methodological study of the lipid composition of *Acinetobacter calcoacetices* LMD 79–41. *Organic Geochemistry*, **14**, 15–25.

GOTH, K., DE LEEUW, J. W., PÜTTMANN, W. & TEGELAAR, E. W. 1988. Origin of the Messel Oil Shale Kerogen. *Nature*, **336**, 759–761.

GRIMALDI, D., BECK, C. W. & BOON, J. J. 1989. Occurrence, chemical characteristics and palaeontology of the fossil resins from New Jersey. *American Museum Novitates*, **2948**, 1–28.

HAN, J. 1970. *Chemical studies of terrestrial and extraterrestrial life*. PhD Thesis, University of California.

——— & CALVIN, M. 1970. Branched alkanes from blue–green algae. *Chemical Communications*, 1490–1491.

HARTGERS, W. A., SINNINGHE DAMSTÉ, J. S. & DE LEEUW, J. W. 1991. Alkylbenzene distributions in kerogen pyrolysates: A comparative study. *In:* MANNING, D. A. C. (ed.) *Organic Geochemistry: Advances and Application in Energy and the Natural Environment*. Manchester University Press, Manchester, 420–423.

———, ———, REQUEJO, A. G., ALLAN, J., HAYES, J. M. & DE LEEUW, J. W. 1994. Evidence for a small bacterial contribution to sedimentary organic carbon. *Nature*, **369**, 224–227.

HATCHER, P. G., LERCH, H. E. & VERHEYEN, T. V. 1989. Organic geochemical studies of the transformation of gymnospermous xylem during peatification and coalification to subbituminous coal. *International Journal of Coal Geology*, **13**, 65–97.

HAYES, J. M. 1993. Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Marine Geology*, **113**, 111–125.

———, FREEMAN, K. H., POPP, B. N. & HOHAM, C. H. 1989. Compound specific isotope analysis: a novel tool for reconstruction of ancient palaeobiochemical processes. *Advances in Organic Geochemistry 1988. Organic Geochemistry*, **16**, 1115–1128.

HEALD, E. 1971. The production of mangrove detritus in a south Florida estuary. *Sea Grant Technical Bulletin*, **6**, 110 p.

HEDGES, J. I. 1991. Lignin, cutin, aminoacid and carbohydrate analyses of marine particulate organic matter. *In:* HURD, D. C. & SPENCER, D. W. (eds) *Marine Particles: Analysis and Characterization*. Geophysical Monograph 63. American Geophysical Union, 129–137.

——— 1992. Global biogeochemical cycles: progress and problems. *Marine Chemistry*, **39**, 67–94.

——— & PARKER, P. L. 1976. Land-derived organic matter in surface sediments from the Gulf of Mexico. *Geochimica et Cosmochimica Acta*, **40**, 1019–1029.

HEMSLEY, A. R., BARRIE, P. J., CHALONER, W. G. & SCOTT, A. C. 1993. The composition of sporopollenin: its contribution to living and fossil spore systematics. *Grana 1993*, Supplement 1, 2–11.

HOLLOWAY, P. J. 1984. Cutins and suberins, the polymeric plant lipids. *In:* MANGOLD, H. K. (ed.) *CRC Handbook of Chromatography*, Lipids. Volume 1. CRC Press Inc., Boca Raton, 321–346.

JANSONIUS, J. & MCGREGOR, C. in press. Palynology: principles and applications. *American Association of Stratigraphic Palynologists*, Houston.

JASPER, J. P. & GAGOSIAN, R. B. 1993. The relationship between sedimentary organic carbon isotopic composition and organic biomarker compound concentration. *Geochimica et Cosmochimica Acta*, **57**, 167–186.

KILLOPS, S. D. & FREWIN, N. L. (1994) Triterpenoid diagenesis and cuticular preservation. *Organic Geochemistry*, **21**, 1193–1209.

KLINK, G., DREIER, F., BUCHS, A. & GULACHAR, F. O. 1992. A new source for 4-methyl sterols in freshwater sediments: *Utricularia neglecta* L.

(Lentbuliaraceae). *Organic Geochemistry*, **18**, 757–764.

KÖGEL-KNABNER, I., DE LEEUW, J. W., TEGELAAR, E. W., HATCHER, P. G. & KERP, H. 1994. A lignin-like polymer in the cuticle of spruce needles: implications for the humification of spruce litter. *Organic Geochemistry*, **21**, 1219–1228.

KOHNEN, M. E. L., SCHOUTEN, S., SINNINGHE DAMSTÉ, J. S., DE LEEUW, J. W., MERRIT, D. A. & HAYES, J. M. 1992. Recognition of paleobiochemicals by a combined molecular sulphur and isotope geochemical approach. *Science*, **256**, 358–362.

———, ———, BAAS, M., KOCK-VAN DALEN, A. C. & DE LEEUW, J. W. 1993. Sulphur-bound steroid and phytane carbon skeletons in geomacromolecules: Implications for the mechanism of incorporation of sulphur into organic matter. *Geochimica et Cosmochimica Acta*, **57**, 2515–2528.

———, ———, RIJPSTRA, W. I. C. & DE LEEUW, J. W. 1990*b*. Alkylthiophenes as sensitive indicators of palaeoenvironmental changes: A study of a Cretaceous oil shale from Jordan. *In:* ORR, W. L. & WHITE, C. M. (eds) *Geochemistry of Sulphur in Fossil Fuels*, ACS Symposium Series 429. American Chemical Society, Washington DC, 444–485.

———, ———, RULLKÖTTER, J., TEN HAVEN, H. L. & DE LEEUW, J. W. 1990*a*. Origin and transformations of C_{25} and C_{30} highly branched isoprenoid sulphur compounds: Further evidence for the formation of organic sulphur compounds during early diagenesis. *Geochimica et Cosmochimica Acta*, **54**, 3053–3063.

KÖSTER, J., SCHOUTEN, S., DE LEEUW, J. W. & SINNINGHE DAMSTÉ, J. S. 1993. Palaeoenvironmental and maturity related variations in compostions of macromolecular organic matter and distribution of sulphur and non-sulphur biomarkers in Triassic organic-rich carbonate rocks. *In:* ØYGARD, K. (ed.) *Organic Geochemistry*. Poster sessions from the 16th International Meeting on Organic Geochemistry, Stavanger, 1993. Falch Hurtigtrykk, Oslo, 402–406.

LARGEAU, C., DERENNE, S., CASADEVALL, E., KADOURI, A. & SELLIER, N. 1986. Pyrolysis of immature torbanite and of the resistant biopolymer (PRB A) isolated from the extant alga *Botryococcus braunii*. Mechanism for the formation and structure of torbanite. *Organic Geochemistry*, **10**, 1023–1032.

LIPPS, J. H. 1993. *Fossil Prokaryotes and Protists*. Blackwell Scientific Publications, Boston.

LUGO, A. E. & SNEDAKER, S. C. 1974. The ecology of mangroves. *Annual Review of Ecology and Systematics*, **5**, 39–64.

MARLOWE, I. T., BRASSELL, S. C., EGLINTON, G. & GREEN, J. C. 1990. Long chain alkenones and alkyl alkenoates and the fossil record of marine sediments. *Chemical Geology*, **88**, 349–375.

MATSUMOTO, G. I. & NAGASHIMA, H. 1984. Occurrence of 3-hydroxy acids in microalgae and cyanobacteria and their geochemical significance. *Geochimica et Cosmochimica Acta*, **48**, 1683–1687.

METZGER, P., LARGEAU, C. & CASADEVALL, E. 1991. Lipids and macromolecular lipids of the hydrocarbon rich microalga *Botryococcus braunii*. *In:* HERZ, W. Y., KIRBY, G. W., STEGLICH, W. & TAMM, Ch. (eds) *Progress in the Chemistry of Organic Natural Products 57*. Springer Verlag, Berlin, 1–70.

MIDDELBURG, J. J., BAAS, M., TEN HAVEN, H. L. & DE LEEUW, J. W. 1993. Organic geochemical characteristics of sediments from Kau Bay. *In:* ØYGARD, K. (ed.) *Organic Geochemistry*. Poster sessions from the 16th International Meeting on Organic Geochemistry, Stavanger, 1993. Falch Hurtigtrykk, Oslo, 413–417.

MOLDOWAN, J. M., FAGO, F. J., LEE, C. Y. *ET AL.* 1990. Sedimentary 24-*n*-propylcholestanes, molecular fossils diagnostic of marine algae. *Science*, **247**, 309–312.

NES, W. R. & MCKEAN, M. L. 1977. *Biochemistry of Steroids and other Isopentenoids*. University Park Press, Baltimore.

NICHOLS, P. D. & JOHNS, R. B. 1985. Lipids of the seagrass *Thalassia hemiprichii*. *Phytochemistry*, **24**, 81–84.

———, KLUMPF, D. W. & JOHNS, R. B. 1982. Lipid components of the seagrasses, *Posidonia australis* and *Heterozostera tasmanica* as indicators of carbon source. *Phytochemistry*, **21**, 1613–1621.

———, VOLKMAN, J. K., PALMISSANO, A. C., SMITH, G. A. & WHITE, D. C. 1988. Occurrence of an isoprenoid C_{25} diunsaturated alkene and high neutral lipid content in Antarctic Sea Ice diatom communities. *Journal of Phycology*, **24**, 90–96.

NIP, M., TEGELAAR, E. W., BRINKHUIS, H., DE LEEUW, J. W., SCHENCK, P. A. & HOLLOWAY, P. J. 1986. Analysis of modern and fossil plant cuticles by Curie point Py–GC and Curie point Py–GC–MS: Recognition of a new, highly aliphatic and resistant biopolymer. *Organic Geochemistry*, **10**, 769–778.

OURISSON, G., ALBRECHT, P. & ROHMER, M. 1979. The hopanoids. Palaeochemistry and biochemistry of a group of natural products. *Pure and Applied Chemistry*, **51**, 709–729.

———, ——— & ——— 1984. The microbial origin of fossil fuels. *Scientific American*, **251**, 44–51.

PARKINSON, R. W. 1989. Decelerating sea level rise and its influence on southwest Florida coastal evolution: a transgressive/regressive stratigraphy. *Journal of Sedimentary Petrology*, **59**, 960–972.

PEAKMAN, T. M., RULLKÖTTER, J., TEN HAVEN, H. L., DE LEEUW, J. W. & BAAS, J. M. A. 1989. Occurrence of (20 R)- and (20 S) $\Delta^{8,14}$- and Δ^{14} 5α(H)-sterenes and the origin of 5α(H),14β(H), 17β(H)-steranes in an immature sediment. *Geochimica et Cosmochimica Acta*, **33**, 2001–2009.

PETERS, K. E. & MOLDOWAN, J. M. 1993. *The Biomarker Guide. Interpreting Molecular Fossils in Petroleum and Ancient Sediments*. Prentice Hall, Englewood Cliffs, New Jersey.

PRAHL, F. G., MUEHLAUSEN, L. A. & ZAHNLE, D. L. 1988. Further evaluation of long-chain alkenones as indicators of paleooceanographic conditions. *Geochimica et Cosmochimica Acta*, **52**, 2303–2310.

QUANDT, I., GOTTSCHALK, G., ZIEGLER, H. &

STICHLER, W. 1977. Isotope discrimination by photosynthetic bacteria. *FEMS Microbiological Newsletters*, 125–128.

RALPH, J. & HATFIELD, R. D. 1991. Pyrolysis–GC–MS characterization of forage materials. *Journal of Agricultural Food Chemistry*, **39**, 1426–1437.

REPETA, D. J. 1993. A high resolution historical record of Holocene anoxygenic primary production in the Black Sea. *Geochimica et Cosmochimica Acta*, **57**, 4337–4342.

REQUEJO, A. G., ALLAN, J., CREANEY, S., GRAY, N. R. & COLE, K. S. 1992. Arylisoprenoids and diaromatic carotenoids in Paleozoic source rocks and oils from the Western Canada and Williston Basins. *Organic Geochemistry*, **19**, 245–264.

ROBSON, J. N. & ROWLAND, S. J. 1986. Identification of novel widely distributed sedimentary acyclic sesterpenoids. *Nature*, **324**, 561–563.

ROHMER, M., BOIVIER-NAVE, P. & OURISSON, G. 1989. Distribution of hopanoid triterpenes in prokaryotes. *Journal of Genetic Microbiology*, **130**, 1137–1150.

SAIZ-JIMENEZ, C. & DE LEEUW, J. W. 1986. Lignin pyrolysis products: Their structures and their significance as biomarkers. *Organic Geochemistry*, **10**, 869–876.

SARKANEN, K. V. & LUDWIG, C. H. 1971. *Lignins. Occurrence, Formation, Structure and Reactions.* Wiley-Interscience, New York.

SCHAEFLE, J., LUDWIG, B., ALBRECHT, P. & OURISSON, G. 1977. Hydrocarbures aromatiques d'origine geologique. *Tetrahedron Letters*, **41**, 3673–3676.

SCHOELL, M., SCHOUTEN, S., SINNINGHE DAMSTÉ, J. S. & DE LEEUW, J. W. 1994. A molecular isotope record of Miocene climate changes. *Science*, **263**, 1122–1125.

SCHOLL, D. W. 1964. Recent sedimentary record in mangroves swamps and the rise in sea level over the southwestern coast of Florida: Part 1. *Marine Geology*, **1**, 344–366.

SCHOUTEN, S., DRIEL, G., SINNINGHE DAMSTÉ, J. S. & DE LEEUW, J. W. 1993*b*. Natural sulphurization of ketones and aldehydes: A key reaction in the formation of organic sulphur compounds. *Geochimica et Cosmochimica Acta*, **57**, 5111–5116.

———, PAVLOVIC, D. SINNINGHE DAMSTÉ, J. S. & DE LEEUW, J. W. 1993*a*. Nickel boride: an improved desulphurizing agent for S-rich geomacromaolecules in polar and apolar asphaltene fractions. *Organic Geochemistry*, **20**, 901–909.

———, SCHOELL, M., SINNINGHE DAMSTÉ, J. S., SUMMONS, R. E. & DE LEEUW, J. W. 1994. Molecular biogeochemistry of Monterey sediments (Naples Beach, USA). II Carbon isotopic compositions of free and sulphur-bound carbon skeletons. *In:* ISAACS, C. M. & RULLKÖTTER, J. (eds) *Studies in Geology*. American Association of Petroleum Geologists.

SHEIA, J., BRASSELL, S. C. & WARD, D. M. 1990. Mid chain branched mono- and dimethyl alkanes in hot spring cyanobacterial mats: a direct biogenic source for branched alkanes in ancient sediments. *Organic Geochemistry*, **15**, 223–231.

SINNINGHE DAMSTÉ, J. S. & DE LEEUW, J. W. 1990. Analysis, structure and geochemical significance of organically bound sulphur in the geosphere: State of the art and future research. *Organic Geochemistry*, **16**, 1077–1101.

———, EGLINTON, T. I., RIJPSTRA, W. I. C. & DE LEEUW, J. W. 1990*a*. Molecular characterisation of organically-bound sulphur in sedimentary high-molecular-weight organic matter using flash pyrolysis and Raney Ni desulphurisation. *In:* ORR, W. L. & WHITE, C. M. (eds) *Geochemistry of Sulfur in Fossil Fuels*. American Chemical Society, Washington, 486–528.

———, KEELY, B. J., BETTS, S. E., BAAS, M., MAXWELL, J. R. & DE LEEUW, J. W. 1993*a*. Variations in abundances and distributions of isoprenoid chromans and long chain alkylbenzenes in sediments of the Mulhouse Basin: a molecular sedimentary record. *Organic Geochemistry*, **20**, 497–503.

———, KOCK-VAN DALEN, A. C., DE LEEUW, J. W. SCHENCK, P. A., GUOYING, S. & BRASSELL, S. C. 1987. The identification of mono- di- and trimethyl 2-methyl-2-(4,8,12-trimethyltridecyl) chromans and their occurrence in the geosphere. *Geochimica et Cosmochimica Acta*, **51**, 2393–2677.

———, KOHNEN, M. E. L. & DE LEEUW, J. W. 1990*b*. Thiophenic biomarkers for palaeoenvironmental assessment and molecular stratigraphy. *Nature*, **345**, 609–611.

———, RIJPSTRA, W. I. C., DE LEEUW, J. W. & SCHENCK, P. A. 1988. Origin of organic sulphur compounds and sulphur containing high molecular weight substances in sediments and immature crude oils. *In:* MATAVELLI, L. & NOVELLI, L. (eds) *Advances in Organic Geochemistry 1987*. Organic Geochemistry, **13**, 593–606.

———, ———, KOCK-VAN DALEN, A. C., DE LEEUW, J. W. & SCHENCK, P. A. 1989*b*. Quenching of labile functionalised lipids by inorganic sulphur species: Evidence for the formation of sedimentary organic sulphur compounds at the early stages of diagenesis. *Geochimica et Cosmochimica Acta*, **53**, 1443–1455.

———, VAN KOERT, E. R., KOCK-VAN DALEN, A. C., DE LEEUW, J. W. & SCHENCK, P. A. 1989*a*. Characterisation of highly branched isoprenoid thiophenes occurring in sediments and immature crude oils. *Organic Geochemistry*, **14**, 555–567.

———, WAKEHAM, S. G., KOHNEN, M. E. L., HAYES, J. M. & DE LEEUW, J. W. 1993*b*. A 6,000 year sedimentary molecular record of chemocline excursions in the Black Sea. *Nature*, **362**, 827–829.

SIREVAG, R., BUCHANAN, B. B., BERRY, J. A. & TROUGHTON, J. H. 1977. Mechanisms of CO_2 fixation in bacterial photosynthesis studied by the carbon isotope technique. *Archaeological Microbiology*, **112**, 35–38.

SUMMONS, R. E., BARROW, R. A., CAPON, R. J., HOPE, J. M. & STRANGER, C. 1993. The structure of a new C_{25} isoprenoid alkane biomarker from diatomaceous microbial communities. *Australian Journal of Chemistry*, **46**, 907–915.

——— & POWELL, T. G. 1987. Identification of aryl isoprenoids in source rocks and crude oils: Bio-

logical markers for the green sulphur bacteria. *Geochimica et Cosmochimica Acta*, **51**, 557–566.

———, VOLKMAN, J. K. & BOREHAM, C. J. 1987. Dinosterane and other steroidal hydrocarbons of dinoflagellate origin in sediments and petroleum. *Geochimica et Cosmochimica Acta*, **51**, 3075–3082.

TAPPAN, H. 1980. *The Paleobiology of Plant Protists*. W. H. Freeman and Co. San Francisco.

TEGELAAR, E. W., DE LEEUW, J. W. & SAIZ-JIMENEZ, C. 1989*a*. Possible origin of aliphatic moieties in humic substances. *Science of the Total Environment*, **81/82**, 1–17.

———, HOLLMAN, G., VAN DE VEGT, P., DE LEEUW, J. W. & HOLLOWAY, P. J. 1994. Chemical characterisation of the outer bark tissue of some angiosperm species: recognition of an insoluble, non-hydrolysable, highly aliphatic biopolymer (suberan). *Organic Geochemistry*, in press.

———, KERP, J. H. F., VISSCHER, H., SCHENCK, P. A. & DE LEEUW, J. W. 1991. Bias of the paleobotanical record as a consequence of variations in the chemical composition of higher vascular plant cuticles. *Paleobiology*, **17**, 133–144.

———, MATTEZING, R. M., JANSEN, B. H., HORSFIELD, B. & DE LEEUW, J. W. 1989*b*. Possible origin of *n*-alkanes in high wax crude oils. *Nature*, **342**, 529–531.

———, WATTENDORFF, J. & DE LEEUW, J. W. 1993. Possible effects of chemical heterogeneity in higher land plant cuticles on the preservation of its ultrastructure upon fossilization. *Review of Palaeobotany and Palynology*, **77**, 149–170.

TEN HAVEN, H. L., PEAKMAN, T. M. & RULLKÖTTER, J. 1992. Δ^2-Triterpenes – Early intermediates in the diagenesis of terrigenous triterpenoids. *Geochimica et Cosmochimica Acta*, **56**, 1993–2000.

——— & RULLKÖTTER, J. 1988. The diagenetic fate of taraxer-14-ene and oleanene isomers. *Geochimica et Cosmochimica Acta*, **52**, 2543–2548.

THOMAS, J. B., MARSHALL, J., MANN, A. L., SUMMONS, R. E. & MAXWELL, J. R. 1993. Dinosteranes (4,23,24-trimethylsteranes) and other biological markers in dinoflagellate rich marine sediments of Rhaetian age. *Organic Geochemistry*, **20**, 91–105.

TRAVERSE, A. 1988. *Paleopalynology*. Unwin Hyman, Boston.

VALISOLALAO, J., PERAKIS, N., CHAPPE, B. & ALBRECHT, P. 1984. A novel sulphur containing C_{35} hopanoid in sediments. *Tetrahedron Letters*, **25**, 1183–1186.

VAN AARSSEN, B. G. K., COX, H. C., HOOGENDOORN, P. & DE LEEUW, J. W. 1990. A cadinene biopolymer present in fossil and extant dammar resins as a source for cadinanes and bicadinanes in crude oils from South East Asia. *Geochimica et Cosmochimica Acta*, **54**, 3021–3031.

———, DE LEEUW, J. W., COLLINSON, M. E. & BOON, J. J. 1994. Occurrence of polycadinane in fossil and recent resisns. *Geochimica et Cosmochimica Acta*, **58**, 223–230.

VAN BERGEN, P. F., COLLINSON, M. E. & DE LEEUW, J. W. 1993. Chemical composition and ultrastructure of salvinialean microspore massulae and megaspores. *Grana 1993*, supplement 1, 18–30.

———, ———, HATCHER, P. G. & DE LEEUW, J. W. 1994*c*. Lithological control on the state of preservation of fossil seed coats of water plants. *In:* VAN GRAASS, G., TELNAES, N. & ØYGARD, K. (eds) *Advances in Organic Geochemistry 1993*. Organic Geochemistry, in press.

———, ———, SINNINGHE DAMSTÉ, J. S. & DE LEEUW, J. W. 1991. A newly discovered polyphenolic substance in seed walls as an alternative source for phenol moieties in brown coals. *In:* MANNING, D. C. (ed.) *Advances and Applications in Energy and the Natural Environment*. Manchester University Press, Manchester, 458–460.

———, ———, ——— & ——— 1994*a*. Chemical and microscopical characterization of inner seed coats of fossil water plants. *Geochimica et Cosmochimica Acta*, **58**, 231–239.

———, GONI, M., COLLINSON, M. E., BARRIE, P. J., SINNINGHE DAMSTÉ, J. S. & DE LEEUW, J. W. 1994*b*. Chemical and microscopic characterization of outer seed coats of fossil and extant water plants. *Geochimica et Cosmochimica Acta*, **58**, 3823–3844.

VOLKMAN, J. K., BARRATT, S. M., DUNSTAN, A., BLACKBURN, S. I. & JEFFREY, S. W. 1994. Biomarkers for marine microlagae. *In:* VAN GRAASS, G., TELNAES, N. & ØYGARD, K. (eds) *Advances in Organic Geochemistry* 1993. Organic Geochemistry, in press.

———, ———, ——— & ——— 1992. Advances in the biomarker chemistry of marine algae. Abstracts, Third Conference on Petroleum Geochemistry and Exploration in the Afro-Asian Region, Melbourne, February 1992.

———, ———, ——— & ——— 1993. Geochemical significance of the occurrence of dinosterol and other 4-methyl sterols in diatoms. *Organic Geochemistry*, **20**, 7–15.

———, EGLINTON, G., CORNER, E. D. S. & SARGENT, J. R. 1980. Novel unsaturated straight-chain C_{37}–C_{39} methyl and ethyl ketones in marine sediments and a coccolithophore *Emiliania huxleyi*. *In:* DOUGLAS, A. G. & MAXWELL, J. R. (eds) *Advances in Organic Geochemistry 1979*. Pergamon Press, Oxford, 219–227.

——— & MAXWELL, J. R. 1986. Acrylic isoprenoids as biological markers. *In:* JOHNS, R. B. (ed.) *Biological Markers in the Sedimentary Record*. Elsevier, Amsterdam, 1–42.

WHITEHEAD, E. V. 1974. The structure of petroleum pentacyclanes. *In:* TISSOT, B. & BIENNER, F. (eds) *Advances in Organic Geochemistry*, 225–243.