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Lipid Markers for Marine Organic Matter

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Abstract An astonishing variety of different lipids have been found in marine sediments and the water column attesting to the diversity of biosynthetic pathways employed by aquatic organisms. Many of these compounds have distinctive structures allowing them to be used as biomarkers for particular sources of organic matter in marine ecosystems. Microalgae synthesize many unusual compounds, such as long-chain alkenones, alkenoates and alkenes, long-chain alkyl diols, highly branched isoprenoid alkenes as well as distinctive sterols and unsaturated fatty acids, thus enabling inputs of microalgal organic matter to be easily recognized. The input of terrestrial organic matter to marine environments can be recognised from lipids of higher plant origin, such as long-chain alcohols, alkanes and fatty acids, and C_{29} sterols, although marine sources for some of these compounds are now recognised. Bacteria synthesize a diverse range of compounds, such as branched fatty acids, hopanoids and isoprenoids, many of which are particularly stable, for instance those that contain an ether bond. Qualitative assignments of organic matter sources are thus reasonably straightforward, although even now lipids can be found for which no source is known. However, quantitative assessments are more difficult since lipid contents vary greatly between different organisms and lipids display a wide range of reactivities. The combination of lipid biomarker data with information from stable isotopes can provide good estimates of organic matter sources, provided that the isotope signatures of the contributing sources (end-members) are known. This chapter provides a review of biomarkers commonly found in sediments together with notes on their identification and source specificity.

Keywords Biomarkers · Lipids · GC-MS · Phytoplankton · Higher plants

1 Introduction

The lipid constituents of seawater and marine sediments have been the subject of numerous investigations over several decades. Some of the first work described the use of branched alkanes to identify algal contributions to sediments [1]. The lipids in phytoplankton and zooplankton communities were studied by Jeffries [2], who showed how specific fatty acids might be useful in food-web studies. Later work examined the occurrence of lipids in the surface microlayer of the oceans [3]. From these early beginnings a large body of research has developed – variously called environmental organic geochemistry or marine organic chemistry or marine lipid chemistry – that has demonstrated the value of studying individual compounds (biomarkers) as well as the bulk organic matter in order to understand carbon sources and cycling.

Organic geochemists have discovered many new and unusual lipid components during their studies of Recent and ancient sediments. Information ob-

tained from the distribution of components within a variety of lipid classes in such samples has led to searches for the same compounds in possible source organisms, in particular microalgae and bacteria. Indeed, the identification of many compounds in sediments occurred some years before a biological source was recognized [4]. This chapter provides an overview of the types of lipids found in marine ecosystems, and information on how to identify those lipids that are commonly found, together with some notes on their significance. Reference is made to review articles and in particular some early papers to illustrate how the field has developed.

2 Lipid Extraction

2.1 Extractable Lipids

Most studies of lipids in sediments and marine organisms use the Bligh and Dyer method of extraction based on mixtures of chloroform, methanol and water [5], or some modification of it such as the additional use of sonication to liberate more tightly bound lipids. Also used is the earlier Folch extraction method [6]. Recently, these two methods have been compared. In animal tissues containing < 2% lipids both methods give similar yields, but at higher lipid contents the Bligh and Dyer method appears to be less effective than the Folch method [7]. Some geochemical studies just use a hydrocarbon solvent such as hexane, but it should be noted that this is only suitable for extraction of hydrocarbons and even here the solubility of long-chain alkanes in hexane is quite low and this could lead to a biased distribution. Chlorinated solvents such as methylene chloride (DCM) and chloroform provide good extraction of neutral lipids, but are not suitable for the extraction of complex lipids such as phospholipids where recoveries can be as low as 60–65% [8].

The extracts obtained by solvent extraction need to be further fractionated in order to provide fractions with simpler distributions that are more amenable to analysis by gas chromatography-mass spectrometry (GC-MS) or high performance liquid chromatography. Saponification is often used to separate fatty acids from "neutral" components such as hydrocarbons, sterols, alcohols and the like. This process breaks down polar lipids such as triacylgycerols and phospholipids into their constituent fatty acids, plus glycerol, phosphate and sugar groups. Intact polar lipids can be analyzed by HPLC (see later) or by Iatroscan TLC-FID [9, 10].

Fatty acids are converted into methyl esters for GC-MS analysis: diazomethane is often used although the need to prepare the reagent each time is seen as a disadvantage. Dry methanol containing HCl gives good results, but the use of BF_3 in methanol can lead to artifacts [11]. Extracts are often further

fractionated into compound classes either by column chromatography, thin-layer chromatography or in a few cases by HPLC. The book by Christie [12] provides a good overview of methodologies available for lipid analysis.

2.2 Free and Bound Lipids

A few researchers have examined both the lipids that are readily extracted into organic solvents (usually designated as free or unbound lipids) and those that are bound more tightly to the sediment matrix (bound lipids). Several approaches have been employed, but most treat the extracted sediments with base and acid (Fig. 1) to liberate esterified (OH^--labile) compounds and amide-linked (H^+-labile) compounds [13–15], while a few studies have further examined the remaining residue by pyrolysis or chemolysis with tetramethylammonium hydroxide (TMAH) to liberate ether- or polymer-bound lipids [15]. It is commonly observed that the distributions of lipids in each fraction show some significant differences, and that the bound lipids usu-

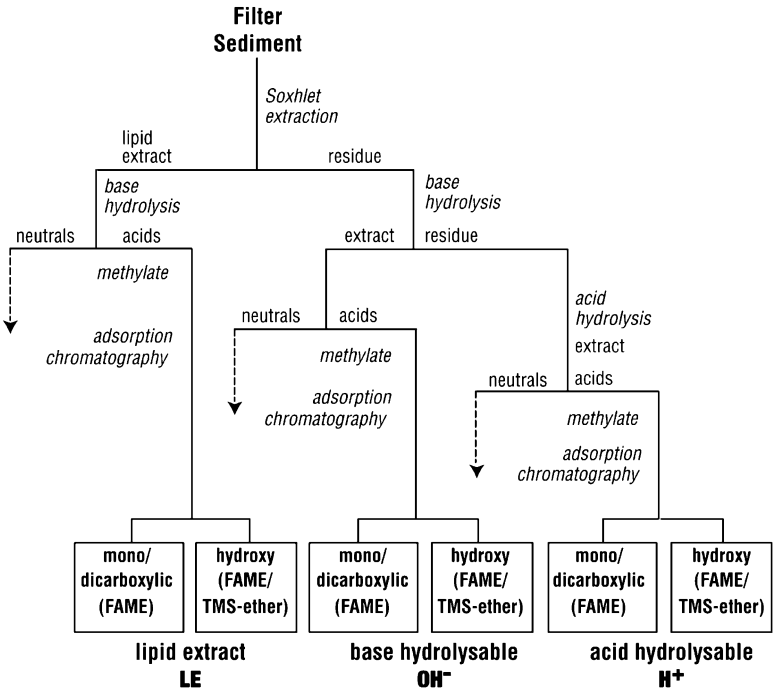

Fig. 1 Lipid analysis scheme from Wakeham [14] which provides fractions contain OH^- labile (i.e. ester-linked) and H^+-labile (i.e. amide-linked) compounds. Many variants on this scheme exist (e.g. sonication is used instead of soxhlet extraction) and so direct comparisons of data between studies can be difficult

ally contain a higher proportion of labile lipids presumably indicating that adsorption to sediments protects the lipids to some extent from microbial attack. Such studies are much more time consuming than those that involve analyses of only the extractable lipids, but they do reveal much more about the chemical forms in which lipids exist in the sample.

3
Identification of Biomarkers in Sediments and Seawater

3.1
Hydrocarbons

Hydrocarbons are those compounds that contain only carbon and hydrogen. This simple description conceals the great variety of chemical structures that can be found in marine organisms and the multitude of structures formed by degradation of functionalized lipids. For further information, the reader is referred to recent books [16–20].

3.1.1
Straight-Chain Alkanes

Alkanes isolated from marine environments typically fall into two categories. Those with odd-chains such as *n*-C_{15}, *n*-C_{17} and *n*-C_{19} are indicative of algal inputs [1] and are often accompanied in higher abundance by the corresponding alkenes. Long-chain (*n*-C_{20}–C_{35+}) alkanes that display a strong predominance of odd-chain lengths indicates a contribution from terrestrial plants. Alkanes from petroleum show little or no predominance of either odd- or even-chain lengths [21]. Erosion of ancient sediments can also be a source of similar *n*-alkane distributions in some aquatic sedimentary environments [22]. Hydrocarbons from eroded sediments often display distinctive sterane and hopane distributions [23].

n-Alkanes give characteristic mass spectra showing a monotonic series of C_nH_{2n+1} ions which decrease in abundance with increasing m/z value. Molecular ions are usually obvious, as is the M^+-15 ion. These distributions are usually visualized using m/z 57, 71, or 85 mass fragmentograms, but these cannot be used directly for quantification since the proportion of these ions in the total ion current decreases with chain-length.

3.1.2
Branched Acyclic Alkanes (Including Isoprenoids)

Simple branched alkanes such as 7- and 8-methylheptadecane are found in many species of cyanobacteria [24], and in algal mats and lagoonal sedi-

ments. Series of longer mid-chain monomethyl alkanes have been identified in Precambrian oils and kerogens [25], testifying to the early evolution of these compounds. Some cyanobacteria contain more complex distributions of mono-, di- and trisubstituted methyl alkanes [26–28], but these are only infrequently encountered in sediments. Long-chain *iso*- and *anteiso*-branched alkanes found in some higher plants [29], are rarely seen in sediments. An exception is the report of abundant $C_{20} - C_{30}$ *anteiso*-alkanes in some Antarctic rocks, but these are derived from cryptoendolithic microbial communities composed of microalgae, cyanobacteria, black and colorless fungi and heterotrophic bacteria [30].

The C_{19} isoprenoid alkane pristane is common in marine samples, reflecting its abundance in some zooplankton species [31]. The C_{20} isoprenoid phytane can also be found, either reflecting petroleum inputs or a contribution from Archaebacteria [32]. Two isomeric C_{25} isoprenoid alkanes are commonly encountered: 2,6,10,14,18- and 2,6,10,15,19-pentamethylicosane in sediments (Fig. 2). The former is known from halophilic bacteria [33], while the latter is usually considered to be a biomarker for methanogenic bacteria [34]. However, the finding of exceptionally light $\delta^{13}C$ values for this C_{25} isoprenoid and the C_{20} isoprenoid crocetane (2,5,10,14-tetramethylhexadecane) in reducing sediments of the Hydrate Ridge of the Cascadia continental margin implies a methanotrophic source, at least in this environment [35], while other work suggests the possibility of algal sources [36].

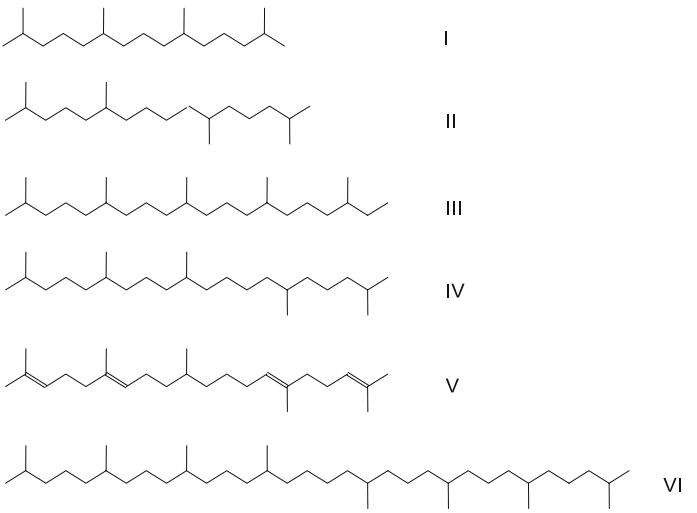

Fig. 2 Structures of some of the isoprenoid hydrocarbons found in sediments and seawater. I: pristane; II: crocetane; III: 2,6,10,14,18-pentamethylicosane; IV: 2,6,10,15,19-pentamethylicosane (PME: mainly methanogenic bacteria); V: 2,6,10,15,19-pentamethylicos-2,6,14,18-tetraene (methanogens); VI: lycopane

Polyunsaturated alkenes based on the 2,6,10,15,19-pentamethylicosane skeleton have been identified in methanogenic archaea [37]. For a review of the lipids in archaebacteria, see the comprehensive compilation of De Rosa and Gambacorta [38].

Another isoprenoid that is ubiquitous in sediments is lycopane [39]. It seems to be particularly abundant in sediments that were deposited under anoxic conditions, suggesting that the lycopane/C_{31} *n*-alkane ratio could be used as a proxy for oxic conditions [39]. The origin of lycopane is still unknown, but it has been suggested that a marine photoautotroph may be the source [39, 40].

Branched alkanes produce similar spectra to those of *n*-alkanes, but the ion due to α-cleavage is more intense so the position of branching is usually easy to identify [30]. More highly branched alkanes can also be identified from their mass spectra [26–28] (and references therein), but possible problems with co-elution must be taken into account. For example, Han et al. [24] showed that the "7,9-dimethylhexadecane" previously identified in some cyanobacteria was in fact a mixture of 7- and 8-methylheptadecane. In such cases, retention indices can be very helpful to distinguish between possible isomers [26–28, 41]. Isoprenoid alkanes are generally identified using a m/z 183 or 113 mass fragmentogram, since these ions are enhanced in abundance compared with *n*-alkanes. The C_{40} isoprenoid lycopane co-elutes with the *n*-C_{35} alkane on most non-polar capillary columns [39], but can be recognized by enhanced ions due to cleavage next to the methyl groups to give characteristic ions at m/z 477, 406/7, 336/7, 308/9 and 183.

3.1.3 *n*-Alkenes

A variety of unsaturated hydrocarbons (alkenes) are found in marine samples. The most common is the hexa-unsaturated alkene *n*-$C_{21:6}$, which is produced by many species of microalgae by decarboxylation of the 22:6(*n*–3) fatty acid [42]. It is particularly abundant in diatoms, which explains why these species rarely have significant amounts of the 22:6 PUFA. Conversely it is a relatively minor component in dinoflagellates which have abundant 22:6 fatty acid. It is not found in cyanobacteria or chlorophytes that lack longer-chain PUFA. In diatoms and dinoflagellates this alkene can be accompanied by the 21:5 and 21:4 alkenes [43]. The presence of such highly labile alkenes in a sediment usually points to the presence of intact (perhaps living) algal cells.

Microalgae and some prokaryotes are likely sources of long-chain alkenes in marine environments [44]. These include, *inter alia*, diunsaturated C_{31}, tri- and tetraunsaturated C_{33} alkenes (with *cis*-double bonds), in some haptophytes (e.g. [45–47]) and di- and tri-unsaturated C_{37} and C_{38} alkenes (with *trans*-double bonds) which occur in the haptophyte *Emiliania huxleyi* [45]. These alkenes are found in marine sediments [48] and fauna such as filter-

feeding mussels [49]. Saturated and polyunsaturated $C_{14} - C_{31}$ hydrocarbons with a strong odd-over-even carbon predominance have been isolated from two marine *Nannochloropsis* species [50]. A North Atlantic strain of *Rhizosolenia setigera* biosynthesizes a C_{25} HBI alkene (see below) as well as C_{25} and C_{27} *n*-polyenes with six or seven double bonds [51]. These alkenes may be the precursors for C_{25} and C_{27} 2-*n*-alkylthiophenes found in sediments from palaeo upwelling regions [51]. Prokaryotes are also potential sources, although their contributions are more obvious in specialized environments such as algal mats. For example, $C_{19} - C_{29}$ alkenes occur in the cyanobacterium *Anacystis montana* [52], and green photosynthetic bacteria of the genus *Chloroflexus* contain a $C_{31:3}$ alkene all-*cis* hentriaconta-9,15,22-triene [53].

Under electron impact conditions, alkenes give mass spectra with prominent molecular ions (provided that the number of double bonds is 4 or less) and major ions at C_nH_{2n-1}. It is usually not possible to assign the position or geometry of the double bond, but derivatization techniques such as formation of DMDS adducts [53] can help identify double bond positions, although mass spectra can be difficult to interpret where the number of double bonds is greater than 3.

3.1.4 Highly Branched Isoprenoid (HBI) Alkenes

An unusual class of highly branched polyunsaturated alkenes (now termed HBI alkenes) have been recognized in many studies of marine sediments; Gearing et al. [54] seems to be the first report. HBIs have an unusual coupling of C_5 isoprene units producing a "T" shaped molecule and typically have 2–4 double bonds (for C_{25} alkenes; Fig. 3) or 4–6 double bonds (for C_{30} alkenes). A C_{35} HBI with 7 double bonds has also been observed in sediments from the Arabian Sea [55]. The parent C_{20} alkane was first noted in the Rozel Point crude oil [56], but in modern marine environments it is much more common to find C_{25} and C_{30} chain-lengths, with saturated and monounsaturated C_{20} hydrocarbons much less frequent [57]. A diatom origin was suggested by the high abundance of C_{25} HBIs in sediments and seawater from the Peru upwelling [58] and the isolation of a diunsaturated C_{25} HBI from sea-ice communities [59]. This was confirmed when high contents of C_{25} highly branched isoprenoid alkenes were identified in laboratory cultures of the diatom *Haslea ostrearia* and C_{30} HBI alkenes were found in *Rhizosolenia setigera* [43]. Since then the C_{25} and C_{30} HBI alkenes have been found in several species of *Haslea*, *Navicula*, *Pleurosigma* and *Rhizosolenia* (e.g. [51, 60–63]).

Genetic differences between strains, environmental factors and growth stage appear to be important determinants of relative abundances of the various homologues identified [64]. Recently, HBI-related monocyclic compounds containing a single cyclohexane ring (Fig. 3) have also been identified

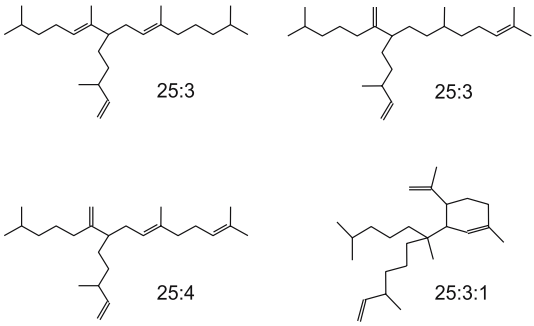

Fig. 3 Structures of a few of the many highly branched isoprenoid alkenes identified in sediments. Note that the 25:3:1 nomenclature here refers to 3 double bonds and 1 ring. The latter compound is difficult to hydrogenate and thus has been confused as a diene with 2 rings. Massé et al. [66] give full details of structures and retention times

in *Rhizosolenia setigera* [65]. Even though these are relatively minor constituents they can be readily recognized in the hydrocarbon fraction after hydrogenation. Massé et al. [66] have shown that these monocyclic alkenes also occur in a variety of sedimentary environments along with the corresponding HBI alkenes.

An extensive literature is now available to identify HBI alkenes in marine samples. Retention data are a good guide to which particular isomer is present [43, 60]. Mass spectra have been reported for the parent alkanes [60] and for most of the alkene isomers [51, 67, 68]. The paper by Massé et al. [66] provides a very good summary of RRT data and structures for HBI alkenes and their cyclohexyl analogs. NMR data for some the hydrocarbons isolated in nearly pure form are also available through the work of Rowland and coworkers [61, 62, 69, 70].

3.1.5 Other Branched Alkenes

Another class of unusual isoprenoid alkenes are the botryococcenes formed by green microalgae of the genus *Botryococcus* [71–73]. Some strains of this alga also synthesize unusual lycopadienes [74]. *Botryococcus* is found in terrestrial freshwater aquatic ecosystems, and so will not be discussed further here. However, it should be noted that compounds thought to be derived from *Botryococcus* have been detected in ancient hypersaline environments [75], so a marine source for this compound type cannot be entirely discounted.

$C_{32} - C_{36}$ polymethylene isoprenoid-like branched alkenes have been found in Black Sea sediments and attributed to an unknown photoautotroph source [76]. Their structures suggest that they were biosynthesized by methylation of an unsaturated n-C_{31} precursor at specific positions. The mass

spectra of the corresponding alkanes show characteristic ion pairs due to cleavage α to the methyl group.

3.1.6
Polycyclic Alkenes and Petroleum Biomarkers

In seawater and Recent sediments one usually finds a mixture of steroidal alkenes (ster-2-enes, ster-4-enes and ster-5-enes) as degradation products of sterol and/or steroidal ketone precursors [77–79]. The presence of fully saturated steranes is indicative of thermally altered organic matter and/or petroleum. Petroleum hydrocarbons are ubiquitous in the marine environment: they originate from many sources such as shipping accidents, ships' discharge, urban and industrial effluents and natural oil seeps [21]. Sterane distributions are characterized from m/z 217 and 218 mass fragmentograms, hopanes from m/z 177, 191 and 205 mass fragmentograms and diasteranes from m/z 259 mass fragmentograms. Aromatic steroidal hydrocarbons with 1 or 3 aromatic rings are also useful compounds for fingerprinting petroleum samples.

3.2
Fatty Acids

3.2.1
Monocarboxylic Fatty Acids

Straight-chain fatty acids are often the most abundant lipids found in seawater particulate matter and in Recent marine sediments. Sources include bacteria, microalgae, higher plants and marine fauna; each of which has a distinctive fatty acid profile so that sources can usually be assigned. However, some fatty acids such as palmitic and stearic acids (16:0 and 18:0 respectively) are ubiquitous. Terrestrial plants are usually considered the main source of long-chain ($C_{20} - C_{30}$) fatty acids, showing a strong even over odd predominance, but some microalgae and/or bacteria are also suspected to be a minor source of these acids [80].

Bacteria are the major source of *iso*-, *anteiso*-, cyclopropyl and mid-chain branched fatty acids in marine ecosystems [81], but it is often overlooked that bacteria can also be a significant source of palmitoleic (16:1(n–7)) and palmitic (16:0) acids in addition to the more commonly used biomarker *cis*-vaccenic acid (18:1(n–7)) [82–84]. A typical example of a fatty acid distribution found in a modern marine sediment is shown in Fig. 4.

A fascinating recent finding is the identification in planctomycete-like bacteria capable of anaerobic ammonium oxidation of highly unusual fatty acids and related compounds containing concatenated cyclobutane rings [85]. These lipids can contain up to 5 linearly fused cyclobutane rings joined by

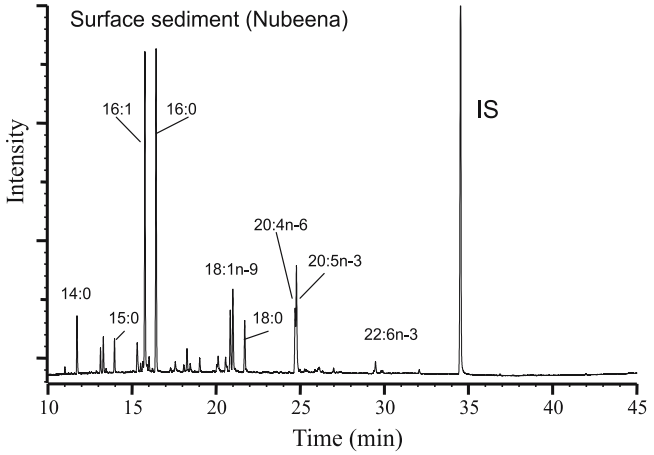

Fig. 4 Capillary gas chromatogram of total fatty acids (as methyl esters) in a surface sediment from coastal Tasmania (Nubeena), Australia. The predominance of 16:0, 16:1 and 20:5 is typical of sediments where diatoms are a major source of organic matter

cis-junctions, hence giving rise to the common name of ladderanes for these compounds [85]. These anammox bacteria contain a unique organelle called the anammoxasone in their cytoplasm [86], and it is postulated that the close packing of the ladderane lipids in the cell membrane reduces its permeability to toxic intermediates (hydrazine and hydroxylamine) produced in the anammox reaction [85]. Examples of this lipid type include fatty acid methyl esters, *sn*-2-alkyl glycerol monoethers, alcohols, *sn*-1,2-dialkyl glycerol diethers and *sn*-2-O-alkyl, *sn*-1-acyl glycerol [86]. Illustrative structures are shown in Fig. 5. Recent field work has established that coupled nitrite reduction and ammonia oxidation can be an important source of dinitrogen gas production in many aquatic systems including the Black Sea [87].

$C_{18} - C_{22}$ polyunsaturated fatty acids (PUFA) are found in almost all marine organisms, often in amounts comparable to the saturated fatty acids. Their primary source is from the phytoplankton. Marine animals obtain most of their PUFA from the food-web and further modify the distributions by chain-elongation and desaturation of the ingested fatty acids. Many studies have used fatty acid distributions to identify food items [88]; one interesting example used fatty acids in benthic foraminifera to show that these animals selectively fed on settled phytodetritus to secure their needs for PUFA [89].

The PUFA composition of microalgae has been well studied [80] and a comprehensive review of fatty acids in marine organisms has recently been published [90]. Diatoms and eustigmatophytes are rich in 20:5(n–3) (EPA) and produce small amounts of arachidonic acid, 20:4(n–6) (AA) with negligible amounts of 22:6(n–3) (DHA). Diatoms synthesize unusual C_{16} PUFA such as 16:4(n–1) and 16:3(n–4). In contrast, dinoflagellates have high con-

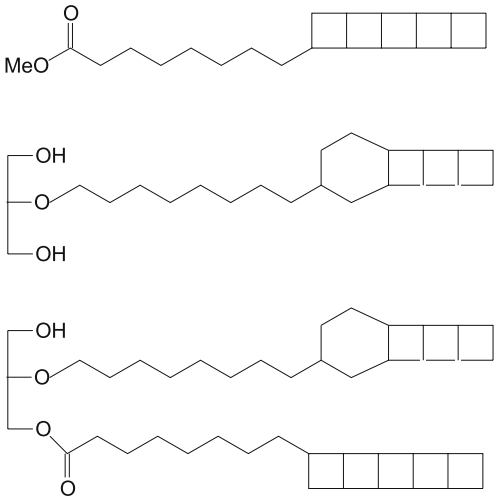

Fig. 5 Structures of ladderane lipids found in planctomycete-like anammox bacterium *Candidatus B. anammoxidans*. Modified from [86]

centrations of DHA and moderate to high proportions of EPA and precursor C_{18} PUFA [18:5(*n*–3) and 18:4(*n*–3)]. Haptophytes also contain EPA and DHA with EPA the dominant PUFA. Cryptomonads are a rich source of the C_{18} PUFA 18:3(*n*–3) (ALA α-linolenic acid) and 18:4(*n*–3) (stearidonic acid), as well as EPA and DHA. Chlorophytes (green microalgae) typically contain low concentrations of C_{20} and C_{22} PUFA, but have abundant 18:3(*n*–3) and 18:2(*n*–6), and are also able to make 16:4(*n*–3). Two very-long-chain highly unsaturated (C_{28}) VLC-HUFA: octacosaheptaenoic acid [28:7(*n*–6) (4,7,10,13,16,19,22)] and octacosaoctaenoic acid [28:8(*n*–3) (4,7,10,13,16,19,22,25)] were discovered in seven marine dinoflagellates [91]. More recent work has shown that the glycolipid fraction of the dinoflagellate *Karenia brevis* contains 18:5(*n*–3), while the phospholipid fraction contained small amounts of both 28:8(*n*–3) and 28:7(*n*–6) [92, 93].

Saturated fatty acid methyl esters (FAME) give characteristic mass spectra showing a base peak at m/z 74 and important ions at m/z 87, 143, M^+, M^+-29, M^+-31 and M^+-43. *Iso*- and *anteiso*-branched acids also show weak ions due to α-cleavage at the branching point. Thus, *anteiso*-fatty acids show an enhanced M^+-29 ion [30]. Monounsaturated FAME show a reduced abundance for the m/z 74 and 87 ions and an enhanced m/z 55 ion. The molecular ion is readily discerned as is the M^+-32 ion. The geometry of the double bond has minimal effect on the mass spectrum, but E (*trans*) and Z (*cis*) isomers can be identified from their relative retention times (E-isomers elute just after Z-isomers on non-polar columns) [94]. Much information is available from the human nutrition and clinical literature [94, 95] due to the health concerns about *trans*-fatty acids in margarines. Double bond positions can be

confirmed by mass spectrometry of dimethyl disulphide (DMDS) adducts as described by [95–99]. Mass spectra of the adducts show ions due to cleavage between the two SCH_3 groups. E and Z isomers can be distinguished, since the erythro isomer (originally E) elutes after the threo isomer (originally Z).

Polyunsaturated fatty acids may be more difficult to identify since they can exist as different positional isomers. It is often helpful to analyse these on two capillary columns differing in polarity. Extensive sets of relative retention times (RRTs) are available [98]. A useful rule-of-thumb is that on non-polar columns, PUFA elute as pairs if they differ by one double bond and the position of the last double bond differs by three carbon atoms. Thus 20:4(n–6) elutes just before 20:5(n–3), 16:2(n–4) elutes with 16:1(n–7), 18:2(n–6) elutes just before 18:3(n–3) etc. Mass spectra are less useful for positive identification, since the intensity of the molecular ion decreases with increasing degree of unsaturation and may not observed in FAME having 4 or more double bonds (although this is somewhat instrument dependent). The base peak is usually m/z 79.

Other useful techniques are argentation TLC, column chromatography or HPLC [99, 100]. A typical TLC procedure involves double development in hexane-Et_2O-HOAc (94 : 4 : 2) on silica gel (7 g) plates loaded with 3% $AgNO_3$ (w/w). Bands can be visualized under 366 nm UV light after spraying with 2′,7′-dichlorofluorescein, and extracted into hexane-$CHCl_3$ (4:1, ×3). The FAME extract needs to be washed with saturated NaCl and 2 M NH_3 to remove Ag^+ and 2′,7′-dichlorofluorescein, respectively, before further analysis.

A range of derivatization methods are available for PUFA analysis, although their utilization by marine chemists and organic geochemists is still infrequent. A detailed account of the methods available is given by Christie [101]. Double bonds can be located by EI GC-MS of picolinyl esters or 4,4-dimethyloxazoline (DMOX) derivatives of the PUFA. Both techniques can be useful and should be considered complementary [101], although the picolinyl esters have long retention times and thus DMOX derivatives are often preferred. A general rule has been formulated to interpret the mass spectra of DMOX derivatives. It states that: if an interval of 12 Dalton, instead of the regular 14 Dalton, is observed between the most intense peaks of clusters of fragments containing n and n – 1 carbon atoms in the mass spectrum, then the double bond occurs between carbons n and n + 1 in the molecule (reviewed in [102]). Examples of DMOX mass spectra are given by Christie [101] and Spitzer [102].

A recent example of the application of these techniques is provided by Méjanelle et al. [103]. These authors used a combination of DMOX and DMDS derivatizations to identify the FAME in marine flagellates (smaller than 10 μm) that colonize marine particles in deep Atlantic waters. Among the compounds identified was a novel non-methylene-interrupted fatty acid $20:3\Delta^{7,13,17}$, which occurred in quite high amounts.

Nichols and Davies [104] report a highly sensitive method based on analysis of 2-oxo-phenylethyl esters by high-performance liquid chromatography-

mass spectrometry (LC-MS) combined with ultra violet (UV) detection. The technique was applied to identify PUFA in *Shewanella* bacteria. It is now recognized that some bacteria, including deep-sea and coastal species, can synthesize PUFA such as 20:5(n–3) and thus can be a source of PUFA in particular sedimentary environments (e.g. [105]).

3.2.2
α,ω-Dicarboxylic Fatty Acids

C_{16} and C_{18} α,ω-dicarboxylic fatty acids are major constituents of the higher plant polyester cutin and $C_{16} - C_{22}$ even-chain dicarboxylic fatty acids are often abundant in the plant biopolymer suberin [106–108]. Seagrasses also contain α,ω-dicarboxylic fatty acids, so most sediments containing higher-plant organic matter can be expected to contain these long-chain dicarboxylic acids (e.g. [109]). It is usually necessary to hydrolyze the sediments to release these esterified fatty acids [14].

Shorter-chain dicarboxylic fatty acids such as azelaic acid (C_9) occur as natural constituents of some plants and as degradation products from oxidative scission of the double bonds in unsaturated fatty acids. Azelaic and other short-chain dicarboxylic acids have been found in marine sediments and in aerosols [110]. The presence of long-chain α,ω-dicarboxylic fatty acids in Black Sea sediments in which shorter-chain α,ω-dicarboxylic fatty acids were absent suggested to Wakeham [14] that they were derived from plant matter rather than oxidation of hydroxyl fatty acids in the sediments.

3.2.3
Monohydroxy Monocarboxylic Fatty Acids

Hydroxylated fatty acids have been found in many sediments (see [44] for a review), but most reports are from lacustrine settings. Aliphatic α- and β-monohydroxy fatty acids (i.e. 2- and 3-hydroxy monocarboxylic acids) occur in a wide range of organisms [111] and are typically produced as intermediates in the α- and β-oxidation of monocarboxylic fatty acids. β-Oxidation occurs more widely than α-oxidation, although the latter is known in plants, animals and bacteria. In yeasts, α-hydroxy fatty acids are intermediates in fatty acid biosynthesis [112]. Long-chain hydroxy fatty acids are rarely reported in microalgae, although C_{22} to C_{26} saturated and monounsaturated α-hydroxy fatty acids have also been found as major lipid components of the cell wall of three marine chlorophytes [50] and a series of saturated α- and β-hydroxy acids ranging from C_{24} to C_{30} with C_{28} predominating was detected in some freshwater eustigmatophytes [113].

Shorter-chain ($C_{12} - C_{18}$) β-hydroxy fatty acids are commonly found in Gram-negative bacteria as amide-bound constituents of the lipid A component of the cell wall polysaccharide [114, 115]. The C_{14} β-hydroxy fatty

acid usually predominates, but C_{15} and C_{17} *iso*- and *anteiso*-branched β-hydroxy fatty acids predominate in the common marine bacterium *Desulfovibrio desulfuricans* [116]. These biomarkers can be used to characterize microbial communities [116], and they provide a useful complement to phospholipid fatty acid analysis [117].

$C_{30} - C_{34}$ mid-chain hydroxy fatty acids were identified in several marine eustigmatophytes of the genus *Nannochloropsis* [118]. The predominant positional isomer contained an hydroxy group at the ω18 position, suggesting that the series was produced by chain-shortening or elongation of a single major precursor.

C_{16} and C_{18} ω-hydroxy fatty acids are common constituents in plant cutin while $C_{16} - C_{22}$ ω-hydroxy fatty acids are found in suberin [119]. Long-chain ω-hydroxy fatty acids and α-hydroxy fatty acids also occur in the seagrass *Zostera* [109]. (ω – 1)-Hydroxy long-chain fatty acids have been identified in some marine and lacustrine environments, but their origin is still debated [116, 120]. Methane-utilizing bacteria are a possible source of C_{26}, C_{28} and C_{30} (ω – 1)-hydroxy fatty acids [121], and a C_{26} (ω – 1) hydroxy acid has also been found in the two cyanobacteria, *Anabaena cylindrica* [122] and *Aphanizomenon flos-aquae* [123].

Hydroxy fatty acids (as methyl esters) can be separated by silica gel TLC into different categories according to the number and position of the hydroxyl groups. These fractions are then derivatized with BSTFA (N,O-*bis*-(trimethylsilyl)-trifluroacetamide) to convert the free OH group(s) into the TMSi-ether. α- and β-hydroxy acids coelute even on the high resolution columns [106, 113], but they can be identified from their characteristic mass spectra. Mass spectra of β-hydroxy acids (as methyl esters, TMSi-ethers) show major ions at m/z 175 [$(CH_3)_3SiO = CHCH_2CO_2CH_3$] and M^+-15 (base peak) with minor ions at m/z 103, 89, 133, 159 and M^+-31 [106]. The mass spectra of α-hydroxy fatty acids (as methyl esters, TMSi-ethers) show major ions at m/z 73 (base peak), M^+-15 and M^+-59 with minor ions at m/z 89, 103, 129, 159 and M^+-43 [106]. α- and β-hydroxy fatty acids in soils, sediments and biofilms have also been identified as the methyl ester with the OH group underivatized [117].

Quantification of α- and β-hydroxy fatty acids can be problematic since they co-elute. Volkman et al. [113] estimated the contribution of α-hydroxy acids to each peak in the hydroxy fatty acids of some freshwater eustigmatophytes from the proportion of the M^+-59 ion in the background-subtracted mass spectrum using the fact that this was about 11.5% of the total ions in the mass spectra of peaks containing only α-hydroxy acids. This value was determined from the mass spectrum of the C_{26} saturated α-hydroxy fatty acid. Note that this value may vary under different GC-MS conditions and there will be a small change in the abundance of this ion with increasing chain length.

3.2.4
Polyhydroxy Monocarboxylic Fatty Acids

$C_{16} - C_{22}$ polyhydroxy fatty acids are common constituents of higher plants where they occur inter-esterified in the cutin and suberin biopolymers. C_{16} and C_{18} polyhydroxy fatty acids are common constituents in plant cutin while $C_{16} - C_{22}$ fatty acids are found in suberin. In cutin, the most abundant ω-hydroxy fatty acid is usually 16-hydroxyhexadecanoic acid, which is formed from palmitic acid by ω-oxidation [124]. This in turn is hydroxylated at the 10- or 9-position to form 10,16- and 9,16-dihydroxy hexadecanoic acids. Oxidation of the ω-hydroxy fatty acid forms the corresponding α,ω-dicarboxylic acid. The C_{18} fatty acids are usually less abundant than the C_{16} and here the main precursor is oleic acid [18:1(n–9)] giving rise to 18-hydroxyoctadec-9-enoic acid which is further epoxidized to 18-hydroxy-9,10-epoxy octadecanoic acid which in turn is converted to 9,10,18-trihydroxyoctadecanoic acid [107, 125]. These hydroxy fatty acids, $C_{16} - C_{22}$ dicarboxylic acids and ω-hydroxy fatty acids can be excellent markers for terrestrial organic matter in sediments [106], especially when combined with compound-specific isotope data [126]. Note, however, that two dihydroxy fatty acids (identified as 15,16-dihydroxy-dotriacontanoic acid and 16,17-dihydroxytritriacontanoic acid) have been found in a marine eustigmatophyte [118], so this general type of biosynthesis is not unique to plants.

Polyhydroxy fatty acids are usually identified as their methyl ester, TMSi-ether. Strong ions occur due to α-cleavage to the hydroxy group. Thus 10,16-dihydroxyhexadecanoic acid (FAME, TMSi-ether) gives a weak molecular ion at m/z 446 and major ions at m/z M-15, M-31, M-47, m/z 373 and 275. The presence of any co-eluting 9,16-dihydroxy isomer is shown by ions at m/z 259 and 289.

3.3
Intact Esterified Lipid Classes

The fatty acids in phytoplankton occur in a variety of more complex polar lipids. Neutral lipids such as triacylglycerols (TAG; also referred to as triglycerides in the older literature), and glycolipids such as monogalactosyldiacylglycerols (MGDG), and digalactosyldiacylglycerols (DGDG) are often predominant with lesser amounts of phospholipids (PL) and so-called acetone mobile polar lipids [127]. Wax esters are very uncommon in phytoplankton, although abundant in many zooplankton [128]. Information about the polar lipids in seawater and natural phytoplankton is surprisingly sparse so we are heavily reliant on data published for microalgal species cultured in the laboratory. Often the conditions used are very different from those occurring in the field, and it is well documented that the proportions of lipid classes is very dependent on environmental conditions (e.g. [129] and references therein).

3.3.1 Triacylglycerols

Lipids of microalgae are often rich in polar lipids during logarithmic phase growth, but many species accumulate triacylglycerol during stationary phase when nitrogen is limiting [130]. The fatty acid amount and composition is dependent on both the growth conditions and the physiological state (e.g. [131]). In contrast, there are very few reports of TAG in bacteria or other prokaryotes so TAG in marine samples is almost always due to eukaryotic organisms. Triacylglycerols are also used by many marine animals as an energy reserve [132], but the proportion of PUFA is often quite low compared with either the triacylglycerols in microalgae or the phospholipids of zooplankton [133, 134]. Wax esters (WE; see later) are another lipid store, especially used by some species of zooplankton. The composition of fatty acids in these TAG or WE can provide useful clues about prey species of the animal in question (e.g. [135]).

Triacylglycerols are readily isolated by column chromatography on silica [135]. Their fatty acid composition can be determined after saponification or transesterification. If information on the composition of intact triacylglycerols is required then high temperature GC and GC-MS methods are available [134–137], although loss of the more unsaturated triacylglycerols through adsorption on the GC column can be a problem. An alternative technique is probe MS analysis of the entire TAG fraction isolated by TLC or HPLC. This was used by Boon et al. [138] to demonstrate the presence of intact TAG in a diatomaceous ooze from off-shore Namibia.

3.3.2 Phospholipids

Phospholipids are key components of biological membranes. They contain glycerol (glycerophosphatides) with a least one O-acyl, O-alkyl or O-alky-1′-enyl attached to the glycerol plus a polar head group (nitrogenous base, glycerol or inositol). Common examples containing one nitrogenous base include PC (phosphatidylcholine), PE (phosphatidylethanolamine) and PS (phosphatidyl serine), or inositol (PI: phosphatidyl inositol) or two glycerol molecules (PG: phosphatidylglycerol; and DPG: diphosphatidylglycerol or cadiolipin). Related compounds include the sphingosyl phosphatides.

The research group of Professor D.C. White pioneered the use of total phospholipid fatty acids (PLFA) as a measure of microbial biomass in sediments and other environments (e.g. [139–141]). Dobbs and Findlay [141, 142] report a set of values for converting lipid-P concentrations to carbon biomass for bacteria, cyanobacteria, yeasts and some microalgae. Viable microbes have an intact membrane which contains phospholipids and their constituent fatty acids. When these cells die, enzymes hydrolyze the phosphate group within minutes to hours leaving a diacylglycerol (diglyceride: DG) [143]. The

resulting DG has the same signature fatty acids as the phospholipids (until it degrades), so a comparison of the ratio of PLFA to DG provides an indication of the proportions of viable and non-viable microbes.

A development of this approach is to analyze the intact phospholipids directly as a marker for intact viable cells in marine sediments. Rütters et al. [144, 145] have shown that intact phospholipids isolated from a sediment extract can be identified using liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS). The combined analysis of phospholipid types and their fatty acid substituents allows various groups of microorganisms living in the sediment to be differentiated. Note that phospholipids and their degradation products (free fatty acids, mono- and diacylglycerols) are rapidly recycled in sediments and so their abundances show a sharp decline with sediment depth [145, 146].

3.3.3 Glycolipids

Glycolipids are important constituents of cell membranes. Common glycolipids include monogalactosyldiacylglycerol MGDG, digalactosyldiacylglycerol DGDG, and sulfoquinovosyldiacylglycerol SQDG (e.g. [128, 147, 148]), but many other more complex glycolipids (i.e. polar molecules containing sugars) exist. New galactolipids based on a sugar galactopyranoside and 16:1 and 20:5 fatty acids have been identified in the diatom *Nitzschia* sp. [149]. Glycolipids can be purified by chromatography on DEAE-cellulose and silica-gel columns [150], since most glycolipids are less polar than most phospholipids [151]. Very few data have been published on glycolipids in marine waters or sediments, reflecting their low concentration due rapid

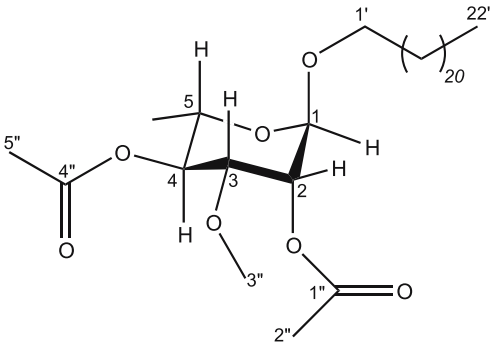

Fig. 6 Structure of the unusual glycolipid found in sediments from Ace Lake Antarctica [55]. The presence of C_{22} and C_{24} carbon chains ether-linked at position 1 of the sugar shows that some common biomarkers (in this case C_{22} and C_{24} alcohols) may be derived from quite unexpected molecules. The source organism of this compound is still unknown

hydrolysis following their liberation from the microbial cell (e.g. [151]). An interesting exception was the isolation of intact novel glycolipids from anoxic sediments in an Antarctic lake [152]. These unusual compounds were identified as docosanyl 3-O-methyl-α-rhamnopyranoside and docosanyl 3-O-methylxylopyranoside (Fig. 6). These compounds were a major and unexpected source of C_{22} and C_{24} alcohols in the sediment.

Analysis of the polar lipids in the Dead Sea biomass during a mass bloom of halophilic archaea in 1992 showed one major glycolipid to be present in the extracts, corresponding with the sulfated diglycosyl diether lipid (S-DGD-1) characteristic of the genus *Haloferax* [153]. Other glycolipids indicative of *Halobacterium sodomense* or *Haloarcula marismortui*, or other *Halobacterium* species were not found. These data indicated that the major archaea in the lake was not one of those that had been cultured.

3.3.4 Betaine Lipids

DGCC (1,2-diacylglyceryl-3-(O-carboxyhydroxymethymethylcholine)) is a betaine lipid which is a common constituent of the Haptophyceae [154]. It also occurs in dinoflagellates and in at least one diatom [154]. DGTA (1,2-diacylglyceryl-O-2′-(hydroxymethyl)-(*N*,*N*,*N*-trimethyl)-β-alanine) was detected in five out of 16 species of Haptophyceae, whereas DGTS (1,2-diacylglyceryl-O-4′-(*N*,*N*,*N*-trimethyl)homoserine) was not detected. There are no reports of betaine lipids in seawater, particulate matter or sediments, but it is doubtful that analysts would have been looking for these compounds.

3.3.5 Wax (Alkyl) Esters

Commonly used chromatographic lipid separation schemes typically provide a fraction containing long-chain wax esters, steryl ethers and steryl esters (e.g. [135, 137]), Although these quite different lipid classes have quite different origins and geochemical significance they are often analyzed together.

Wax esters (or more correctly alkyl esters) are particularly common in the marine environment as they are used as an energy store by many species of zooplankton [133, 155]. Wax esters of zooplankton origin have been identified in sediments deposited under the upwelling cells of Walvis Bay [137], the Peru margin [155], and the Arabian Sea [55]. The fatty acid composition of these wax esters generally resembles that of the phytoplankton diet and contains PUFA such as 20:5(n–3) and 22:6(n–3) [133]. The alcohols consist mainly of saturated and monounsaturated $C_{16} - C_{22}$ moieties. Indeed, elevated levels of 20:1 and 22:1 alcohols in sediments is usually indicative of hydrolysis of marine wax esters. Note that the distributions of wax esters found in lacustrine and terrestrial sediments usually show a much higher proportion of saturated

fatty acids and alcohols extending to much longer chain-lengths (e.g. [156]). Some bacteria also synthesize wax esters [157], but it seems that bacteria are rarely a significant source of these lipids in aquatic environments.

Wax esters can be analyzed on low bleed non-polar capillary columns able to operate up to 350 °C. They give readily interpretable mass spectra [137, 159]. A wax ester of general formula R_FCOOR_A gives a strong molecular ion and cleavage ions that include the fatty acid moiety $[R_FCOO + 2H]^+$ and the alcohol moiety $[R_A + H]^+$. Thus within each set of wax esters having the same overall carbon number, the various combinations of alcohols and fatty acids present can be readily identified and quantified from the abundance of the cleavage ions.

3.4 Alicyclic Alcohols

$C_{22} - C_{32}$ even-chain *n*-alkanols are found in most marine sediments and are usually attributed to an input from plant waxes, especially when they are accompanied by *n*-alkanes showing a strong predominance of odd chain-lengths. Marine sources of *n*-alkanols include hydrolysis of the wax esters of zooplankton which gives rise to saturated and unsaturated alcohols from C_{14} to C_{22}. These distributions often show a predominance of 16:0, 20:1, and 22:1 alcohols [128]. A specific, but unidentified, marine source of the 22:0 alcohol has also been suggested (Fig. 6; [44, 152]). An interesting finding is the presence of straight-chain monounsaturated C_{32} and C_{30} alcohols and a diunsaturated C_{32} alcohol in acid hydrolysates of the total extract from marine eustigmatophytes [160]. The relative proportions of the $C_{30} - C_{32}$ alcohols were very similar to those of the corresponding alkyl diols, having the same carbon number but one less double bond. Free alcohols were present only in trace amounts, suggesting that the alcohols are bound to extractable polar lipids by bonds that are resistant to base hydrolysis (perhaps by amide bonds).

Alcohols are usually examined as their TMSi-ethers although some researchers prefer to use acetate derivatives because of their greater stability. The TMSi-ethers give a strong M-15 ion, weak M^+ and typically show a base peak at m/z 75 and a major ion at m/z 103.

3.5 Long-Chain Alkyl Diols

$C_{30} - C_{32}$ alkyl 1,15-diols were first discovered in Black Sea sediments [161], which was soon followed by numerous reports on their occurrence in other sediments (e.g. [162–164]). After initial suggestions that the alkyl diols were derived from cyanobacteria, it was shown that saturated and monounsaturated $C_{30} - C_{34}$ alkyl diols occur in marine eustigmatophytes from the

genus *Nannochloropsis* [160] together with alcohols of the same chain-length (Fig. 7). These compounds are probably building blocks for biopolymers called algaenans found in these microalgae [50]. Algaenans such as this are quite resistant to bacterial degradation and thus they can survive into the sediment record ultimately to become an important source of hydrocarbons in some crude oils [165, 166]. The biosynthesis of these alkyl diols remain unknown although some clues are provided by the occurrence of long-chain alcohols and hydroxy fatty acids in the same species (e.g. [167]).

The distribution of alkyl diols in eustigmatophytes is not an exact match with those found in some sediments, indicating that other organisms might synthesize these compounds [160]. An additional source has now been found with the identification of C_{28}, $C_{28:1}$, C_{30}, and $C_{30:1}$ alkyl 1,14-diols, together with C_{27} and C_{29} 12-hydroxy methyl alkanoates, as major neutral lipids in rhizosolenid diatoms belonging to the widespread diatom genus *Proboscia* [168]. These components were abundant in sediment traps and sediments, especially in areas with elevated primary production such as upwelling regions of the Arabian Sea.

Alkyl diols are often accompanied by the corresponding long-chain keto-ols where a carbonyl group replaces the mid-chain hydroxy group (Fig. 7). These have no known biological source, but they occur ubiquitously in marine sediments. It seems highly likely that they are formed by oxidation of long-chain diols, both in the water column and in the sediment [169].

Alkyl diols are readily identified from their mass spectra as TMSi-ethers which show intense ions due to cleavage α to the mid-chain hydroxyl group. Illustrative mass spectra are provided by de Leeuw et al. [161] and Volkman et al. [160]. The mass spectrum of the TMSi-ether derivative of the C_{32} saturated 1,15-diol shows a molecular ion at m/z 626, and major ions at m/z 341 and 387 due to cleavage either side of the C-15 hydroxy group. The mass spectrum of the of the $C_{32:1}$ 1,15-diol (as the TMSi-ether) shows cleavage ions at m/z 339 and 387, and an M^+-90 ion at m/z 534 due to loss of the OTMSi group. Alkyl

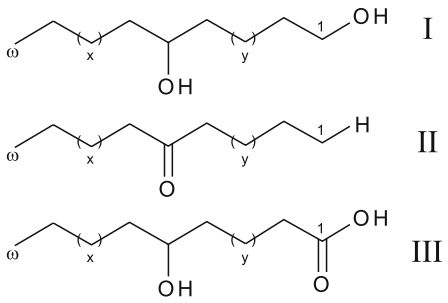

Fig. 7 Structures of alkyl keto-ols (I), alkyl diols (II) and corresponding hydroxyl fatty acids (III). In eustigmatophytes the chain-lengths are usually C_{28}, C_{30} or C_{32} and the mid-chain hydroxyl group is on C-15. In diatoms, the hydroxyl group is at C-14

keto-ols are easily identifiable from their mass spectra which show a base peak at m/z 130 and major ions at m/z 143 and M^+-15 [161].

3.6 Isoprenoid Ether Lipids

The lipids of organisms from the archaea contain ether-linked lipids [38] rather than the more common ester-linked lipids of eukaryotic organisms. A common constituent is archaeol (Fig. 8) in which C_{20} isoprenoid chains are ether-linked to the glycerol backbone. Related compounds with a hydroxyl group on the isoprenoid chain (*sn*2- or *sn*3-hydroxyarchaeols; Fig. 8) are typical constituents of the methanogenic order Methanosarcinales [170, 171]).

Glyceryl dialkyl glyceryl tetraethers (GDGTs) are specific to the archaea. A variety of chemical forms are known having different numbers of cyclopentyl rings usually designated by a numeral after GDGT. Thus GDGT-0 is the base skeleton having no cyclopentyl rings (Fig. 8). Wakeham et al. [173] showed that bicyclic and tricyclic GDGTs and their constituent ^{13}C-depleted

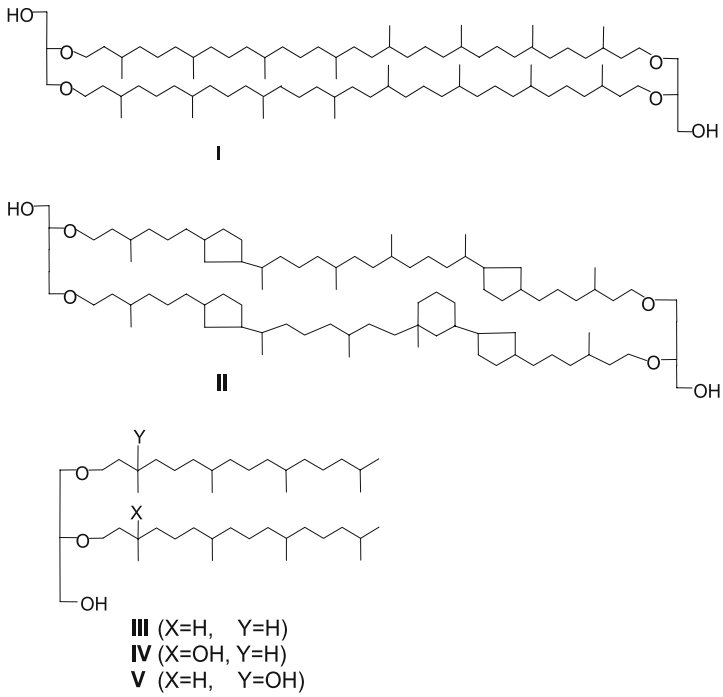

Fig. 8 Structures of some isoprenoid ether lipids of prokaryotic origin. I: GDGT-0 (from methanogenic archaea); II: Crenarchaeol; III: archaeol (X = H, Y = H) (from methanogenic archaebacteria); IV: *sn*-2-hydroxyarchaeol (X = OH, Y = H); V: *sn*-3-hydroxyarchaeol (X = H, Y = OH) (from methanogens)

monocyclic and bicyclic biphytanes (^{13}C down to – 67 ‰) in the Black Sea were indicative of archaea involved in anaerobic oxidation of methane (AOM).

An exciting discovery was an unusual glycerol dibiphytanyl glycerol tetraether (GDGT) termed crenarchaeol in both oxic and anoxic marine waters and sediments (e.g. [174]). Its structure (Fig. 8) was identified using high field two-dimensional NMR techniques which demonstrated the presence of an unusual cyclohexane ring and four cyclopentane rings formed by internal cyclization of the biphytanyl chains [175]. Its structure is thus similar to that of GDGTs biosynthesized by (hyper)thermophilic crenarchaeota apart from the cyclohexane ring. This compound is now recognized as derived from the membrane lipids of cosmopolitan pelagic crenarchaeota. In the Black Sea, GDGT-0 and crenarchaeol dominated the distributions of ether lipids in the oxic surface and shallow anoxic waters reflecting their origin from planktonic crenarchaeota.

Compound-specific isotope analyses of the carbon skeletons suggest that planktonic archaea utilize an isotopically heavy carbon source such as algal carbohydrates and proteins or dissolved bicarbonate. Due to their high preservation potential, these lipids provide a fossil record of planktonic archaea and suggest that they have thrived in marine environments for more than 50 million years [174]. Sinninghe Damsté et al. [176] suggest that planktonic Crenarchaeota are probably facultative anaerobes and that the world's oceans contain ca. 10^{28} cells of planktonic Crenarchaeota.

Intact tetraether lipids in archaeal cell material and sediments can be analyzed by high performance liquid chromatography in combination with atmospheric pressure chemical ionization mass spectrometry [177, 178]. Non-isoprenoid dialkyl diglycerol tetraethers containing 13,16-di- or 5,13,16-trimethyloctacosanyl moieties have been identified in peats and coastal marine and lake sediments using similar techniques of HPLC-MS and high-field NMR spectroscopy [178, 179]. The ratio of these branched isoprenoids to themselves plus crenarchaeol (BIT index) has been used to estimate the relative amounts of terrestrial and marine organic inputs to marine sediments [178].

3.7 Aliphatic Ketones

A surprising diversity of aliphatic compounds containing carbonyl groups has been found in sediments and seawater. Some of these are natural products, while others are formed by diagenetic reactions.

3.7.1 *n*-Alkan-2-ones

Distributions of long-chain ($C_{19} - C_{35}$) ketones having a carbonyl at the 2-position (i.e. methyl ketones) have been found in soils [180], peats [181],

lacustrine sediments [182] and some coastal marine sediments (e.g. [58]). These compounds can be derived from oxidation of *n*-alkanes *via* the intermediate alkan-2-ols [182], but the mismatch of chain-lengths between alkanes and ketones in coastal sediments suggests that they are not produced *in-situ*, but rather they are transported with terrestrial organic matter to the marine environment [58]. Hernandez et al. [183] reported distributions of alkan-2-ones maximizing at C_{25} in a subtropical estuary for which they proposed a seagrass source. It is not yet clear whether this could be a source of alkan-2-ones in other coastal environments. *n*-Alkan-2-ones are readily identified by GC-MS. Their mass spectra are similar to those of *n*-alkanes, but show a base peak at m/z 58 which shifts to m/z 59 with increasing chain-length [58].

3.7.2 Mid-Chain Ketones

Long-chain ketones having a carbonyl group near the centre of the chain (mid-chain ketones) are infrequently found in marine samples. Boon and de Leeuw [137] reported a series of these compounds, dominated by C_{38} and C_{40} ketones with the carbonyl at C-19, in Walvis Bay diatomaceous ooze. The origin of these compounds remains unknown, but the predominance of even carbon atoms rules out higher plants which typically contain odd-chain mid-chain ketones such as those that have been found in lacustrine settings [158].

Mid-chain ketones occur in the same chromatographic fraction as wax esters and have similar GC retention times and thus may be overlooked. A simple procedure is to re-examine the "wax ester" fraction after saponification or transesterification to see whether long-chain components are still present. Mid-chain ketones R_1COR_2 give prominent ions from cleavage α to the carbonyl group together with H and CH_3 rearrangement ions $[R_1CO]^+$, $[R_2CO]^+$, $[R_1CO + H]^+$, $[R_2CO + H]^+$, $[R_1COCH_3]^+$, $[R_2COCH_3]^+$, $[R_1COCH_3 + H]^+$, $[R_2COCH_3 + H]^+$ [137].

3.7.3 Alkenones

Very long-chain ($C_{35} - C_{40}$) unsaturated methyl and ethyl ketones termed alkenones, having a carbonyl at the 2 or 3 position respectively, are found in haptophytes including the coccolithophores *Emiliania huxleyi* and *Gephyrocapsa oceanica* [45, 48, 184]. Alkenones are straight-chain alkyl lipids with *trans* double bonds. The most abundant are C_{37} and C_{38} chain-lengths with 2 or 3 double bonds with the carbonyl group at C-2 or C-3. Recent work has demonstrated the presence of several new alkenones in these microalgae, including monounsaturated homologs, as well as the corresponding long-chain

alkenols [185]. Alkenones are ubiquitous in marine sediments (e.g. [162, 186–189]), and the ratio of components has been found to vary systematically with the seawater temperature in which the microalgae grow [186, 189]. This has prompted many paleoceanographic studies that have used the ratio of concentrations of tri- to di-unsaturated C_{37} ketones in sediments (some over 100 million years old) to estimate the paleotemperature when the sediments were deposited (e.g. [187, 189, 190] and references therein). Environmental factors other than temperature such as salinity may also influence alkenone distributions (e.g. [191]), but these effects are not well understood or quantified [188].

3.8 Steroidal Compounds

Sterols and compounds derived from them by diagenetic reactions are ubiquitous in sediments. Their structures contain a number of unique features such as positions of double bonds, alkylation in the ring system and side-chain, and stereochemistry (Fig. 9) which makes them ideal for assigning sources of organic matter and for studying its short-term fate (e.g. [44, 192–194]). A diversity of steroidal compounds are found in marine waters and sediments, reflecting the variety of sterol distributions found in microalgae, plants and animals.

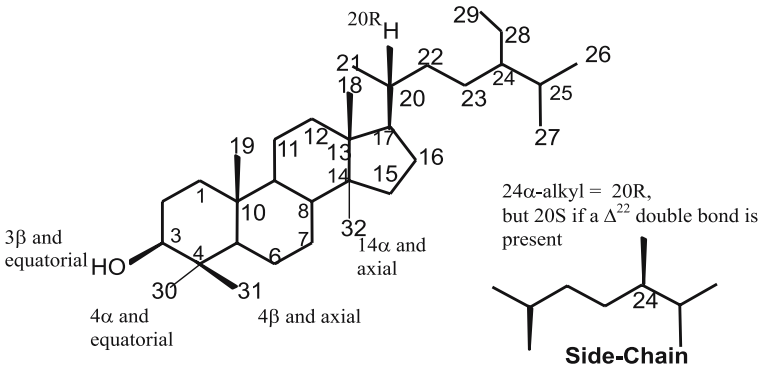

Fig. 9 Generalized structure of a 4,4-dimethyl, 24-ethyl sterol showing numbering system and stereochemistry at important centre; adapted from [194]

3.8.1 Sterols (Stenols, Stanols)

Microalgae are the primary source of sterols in the sea. Some species show a predominance of a single sterol, such as cholesterol in marine eustigmatophytes and 24-methylcholesta-5,22E-dien-3β-ol in some diatoms and haptophytes, to complex mixtures of 4-desmethyl and 4-methylsterols found in

some species of dinoflagellates [192]. Some sterols are widely distributed, but others are useful chemotaxonomic markers. For example, the C_{28} sterol 24-methylcholesta-5,22E-dien-3β-ol (*epi*-brassicasterol or brassicasterol depending on the stereochemistry of the methyl group at C-24) is sometimes incorrectly thought of as a unique marker for diatoms, but it only occurs in some diatoms and it is abundant in haptophytes and cryptophytes [192, 195]. A more specific marker is 24-methylcholesta-5,24(28)-dien-3β-ol, which is abundant in some centric diatoms such as *Thalassiosira* and *Skeletonema* [196]. Some diatoms have a high content of the cholesta-5,22E-dien-3β-ol, while others have abundant 24-ethylcholesta-5,22E-dien-3β-ol, or cholesterol or cholesta-5,24-dien-3β-ol [196]. Given the diversity of sterols now known to be present in diatoms and the importance of diatoms as a source of organic matter in marine systems, it is not surprising that the sterol distributions found in many marine sediments show a variety of structures.

Other algal groups display characteristic sterol distributions. For example, 24-*n*-propylidenecholesterol seems to be a marker for some chrysophytes [197] and eustigmatophytes are a source of cholesterol [160, 167]. Green microalgae can have a wide variety of sterols, many of which have double bonds at Δ^7 [198].

Dinoflagellates are the major source of 4-methyl sterols in marine systems and the C_{30} sterol 4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol (dinosterol) is often used as a biomarker for dinoflagellates (although it does occur as a minor constituent of a few diatoms [199]). However species from the genus *Pavlova* (Prymesiophyceae) also contain 4-methyl sterols and 5α(H)-stanols as well as 3,4-dihydroxy-4α-methylsterols termed pavlovols, but these rarely occur in marine sediments [200, 201]. An important consideration is that not all dinoflagellates have high contents of 4-methyl sterols [202] and some species lack dinosterol (Fig. 10). For example, *Amphidinium* spp. synthesize amphisterol (4α,24-dimethyl-5α-cholesta-8(14),24(28)-dien-3β-ol) as their major sterol [203] whereas several *Gymnodinium* species have 4α,24-dimethylcholestanol as a major sterol and contain little dinosterol [204]. An illustration of the diversity in proportions of 4-desmethyl sterols and 4-methyl sterols, even in closely related species, is shown in Fig. 10.

An uncommon example where a 4-methylsterol may not be derived from dinoflagellates comes from work by Santos et al. [205], who found high contents of 4-methylcholestanol in abyssal sediments from the Porcupine Abyssal Plain. This was also the most abundant sterol in the hindgut of holothurians (sea cucumbers), *Oneirophanta mutabilis*, suggesting that this sterol might be a marker for holothurian fecal matter in the sediments. Indeed, the significant role of benthic animals in reprocessing deposited organic matter is often overlooked in organic geochemical studies of surface sediments.

Fig. 10 Although many dinoflagellates contain a high proportion of 4-methylsterols, often dominated by dinosterol, this example shows the great range in the proportion of 4-methyl and 4-desmethylsterols in these microalgae. The dinoflagellates analysed were: A: *Gymnodinium sanguineum*; B: *Symbiodinium microadriaticum*; C: *Gymnodinium* sp.; D: *Scrippsiella* sp.; E: *Fragilidium* sp.; F: *Prorocentrum mexicanum*; G: *Prorocentrum micans*. Selected data were taken from [113] and [202]

Many microalgae synthesize C_{29} sterols and a number of examples are now known where the major sterol is 24-ethylcholesterol or 24-ethylcholesta-5,22E-dien-3β-ol [196], both of which are more commonly associated with higher plants [192]. In many near-shore sediments it is likely that much of the 24-ethylchoelsterol present is derived from plants. Figure 11 shows an example where the content of the C_{29} sterol in sediments from the Huon estuary in southern Tasmania [206] is plotted against the proportion of terrestrial organic matter determined independently from stable isotope data. The good correlation shows that little of the 24-ethylchoelsterol present in this case is derived from microalgae. However, in off-shore sediments where much of the organic matter is from phytoplankton, then algal sources can also be important contributors [58].

Sterols with a fully saturated ring system (5α(H)-stanols) occur in all marine sediments where they are thought to be formed by bacterial reduction of stenols. A typical distribution of stenols and stanols in a modern coastal sediment is shown in Fig. 12. Direct inputs from dinoflagellates are also possible [207], as well as minor inputs from diatoms (e.g. [196, 208]) and some haptophytes [200, 201]. The presence of 5β(H)-stanols in sediments is often taken as evidence for the presence of fecal-derived organic matter [209], since the 5β(H)-stanol coprostanol constitutes approximately 60% of the total sterols found in human feces. More detailed analyses of the carbon number distributions of 5β(H)-stanols indicate that human-derived fecal matter can be distinguished from other sources [210]. Note, however, that 5β(H)-stanols can also be formed in sediments under highly reducing conditions [192] and they can be derived from marine mammals [211]. Stanols with 3α(OH) stereochemistry can also be found; epicoprostanol (5β-cholestan-3α-ol) is particularly abundant in Antarctic sediments and in the feces of baleen whales [210].

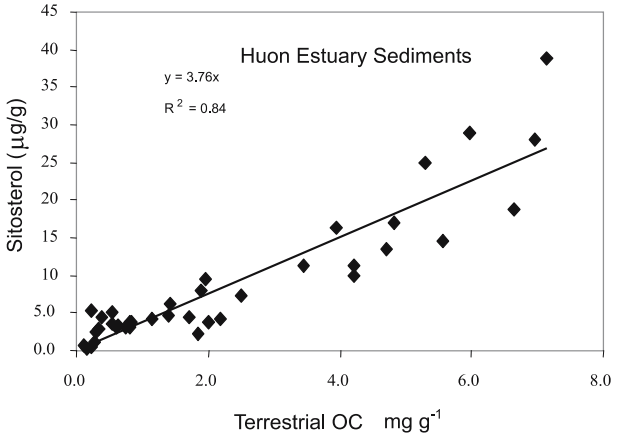

Fig. 11 Plot of sitosterol (24-ethylcholesterol) contents in sediments from the Huon estuary, Tasmania against an independent measure of terrestrial organic carbon derived from $\delta^{13}C$ values. In this instance, sitosterol provides a quantitative index of plant organic matter in the sediments, whereas in most marine sediments a contribution from microalgae may also be important. Data from Butler et al. [206]

Sterols are readily identified from their mass spectra and are usually studied as their acetate or trimethylsilyl-ether derivative, although free sterols can also produce useful mass spectra [212]. TMSi-ethers are generally preferred because they produce more diagnostic mass spectra, but are less stable than acetates [213, 214]. Acetate derivatives, however, may show a weak molecular ion, or even no molecular ion in which case the M^+-60 ion is abundant [214].

In analyses of TMSi-ethers, a few simple rules can help identify particular series. For example sterols with Δ^5-unsaturation show a base peak at m/z 129 and fragmentation ions at M^+-90 and M^+-129. Fully saturated sterols elute slightly later (Fig. 12) and show mass spectra with m/z 215 as the base peak (m/z 229 if a 4-methyl group is present). 5β(H)-stanols elute much earlier than 5α(H)-stanols. Sterols with Δ^7, Δ^8 or $\Delta^{9(11)}$ lack the ion at m/z 129, and show major ions at m/z 213, 229 and 255. These mass spectra are very similar [214], but retention indices can be used to identify which isomer is present [215]. The presence of a stenol/stanol pair of peaks is good indication for a Δ^5 double bond in the first eluting compound, and conversely the absence of a second peak indicates that the ring double bond must be at a position other than Δ^5. Sterols with $\Delta^{5,24(28)}$ unsaturation produce a major ion at m/z 386 (c.f. m/z 388 if the double bond at Δ^5 is absent). Sterols with $\Delta^{5,22}$ unsaturation show a base peak at m/z 255 (c.f. m/z 257 if the Δ^5 double bond is absent). Comprehensive compilations of retention time data on various GC phases are available for many of the commonly encountered structures [215, 216].

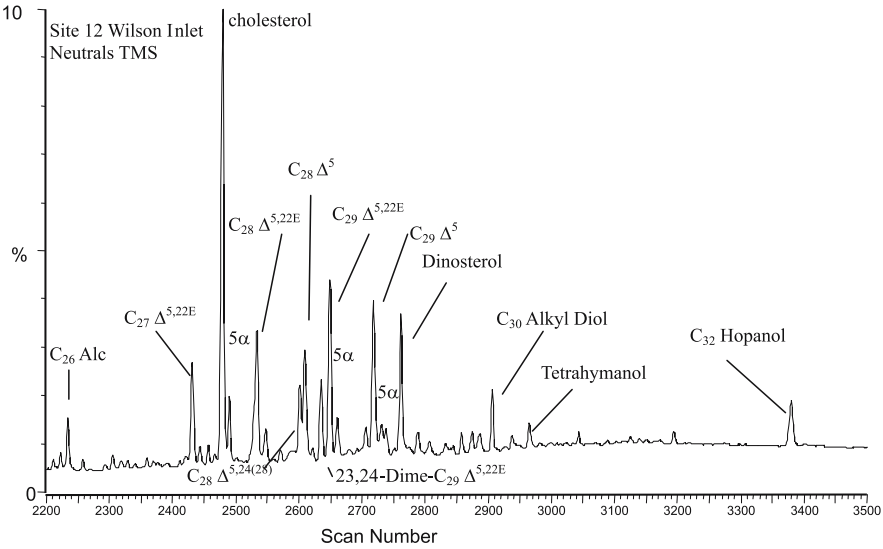

Fig. 12 Section of the chromatogram from GC-MS analysis of total neutrals in a surface sediment from Wilson Inlet, Western Australia. The sterols show a diversity of structures indicative of mixed inputs from algae, fauna, and plants. Note that each Δ^5 sterol is accompanied by smaller amounts of the corresponding 5α(H)-stanol which elutes immediately afterwards. Also shown are C_{30} alkyl diols (microalgae), tetrahymanol (protozoans) and the C_{32} triterpenoid alcohol (bacteria and/or cyanobacteria). The 4-methyl sterols are dominated by dinosterol from dinoflagellates

3.8.2 Steroid Ketones

Steroidal ketones are often found in marine environments (e.g. [217]). These distributions reflect early stage oxidation of the sterols present [217], plus direct inputs from a small subset of microalgae, such as the dinoflagellates. For example, the dinoflagellate *Scrippsiella* contains at least 21 steroidal ketones including dinosterone (4α,23,24-trimethyl-5α-cholest-22E-en-3-one), dinostanone and 4α,23,24-trimethyl-5α-cholest-8(14)-en-3-one [202, 218]. Mass spectra for these same steroid ketones isolated from a toxic dinoflagellate *Pfiesteria piscida* are provided by Leblond and Chapman [219].

Δ^5-unsaturated sterols can be converted to Δ^4-3-one stenones in sediments by microbial processes similar to those operating in the rumen [220]. These compounds are readily recognized by a base peak at m/z 124. Reduction of these unsaturated steroidal ketones can then give rise to both 5α(H)- and 5β(H)-stanols.

Steroid ketones with the carbonyl group at C-3 from oxidation of the sterol 3-hydroxy group are most common. Saturated ketones (stanones) have a base peak at m/z 231 while the 4-methyl equivalents (4-methylstanones) show

a base peak at m/z 245 (see examples in [217] and [219]). The mass spectrum of Δ^{22}-unsaturated steroid ketones show strong ions due to cleavage about the side-chain. The most common example of the latter is dinosterone which has major ions at m/z 69(b), 285, 287, 314, 383 and 436 [217, 219].

Benfenati et al. [221] reported that stanones such as coprostanone (5β-cholestan-3-one) and cholestanone can be readily converted to the silylated enol ether with the silylating reagent MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide). The more commonly used reagent BSTFA is less effective and often leads to a mixture of silylated and non-silylated forms. These enol derivatives give a strong molecular ion, M-15 and M-29 ions and a major ion at m/z 143. This side-reaction needs to be considered when analyzing a total neutral extract containing sterols and steroidal ketones after silylation. Coprostanone is found in animal feces. On non-polar GC columns it elutes just before the *n*-C_{28} alcohol and is readily distinguished from the 5α(H)-isomer by the presence of an M-70 ion in its mass spectrum [210].

3.8.3
Steryl Chlorin Esters (SCEs)

Steryl esters of the chlorophyll a transformation product pyropheophorbide a (SCEs; also known as phorbin steryl esters – PSEs) occur in most Recent sediments and to a much lesser degree in the particulate matter in seawater [222, 223]. It is has demonstrated that they are formed from zooplankton grazing on microalgae. For example, the copepod *Calanus helgolandicus* fed the marine diatom *Thalassiosira weissflogii* produced fecal pellets containing SCEs in which all of the animal and algal sterol components were found, although in different proportions [223]. When similar experiments were carried out with the dinoflagellates *Prorocentrum micans* and *Alexandrium tamarense*, there was a clear discrimination against the uptake of 4-methyl sterols into the SCEs [224]. SCEs degrade more slowly than free sterols thus providing a means for preserving phytoplankton-derived sterols in sediments [224].

Intact SCEs are analyzed by HPLC and identified by HPLC-MS using APCI. Chlorins can be identified by electronic (UV-visible) spectroscopy and the sterols by GC-MS [223, 224].

3.8.4
Steryl Esters and Steryl Ethers

There are relatively few reports of steryl esters in seawater and marine sediments, and in some cases their presence has been inferred by isolation of sterols from the "wax ester" fraction after saponification (e.g. [161]). Intact steryl esters have been found in sediment trap samples from the North At-

lantic [134]. The most abundant constituents were C_{16} and C_{18} fatty acids esterified to cholesterol, which suggests a primary source from zooplankton. Many microalgae also synthesize steryl esters (although amounts vary greatly relative to free sterols depending on the species analyzed), so this should also be considered as a possible source in surface waters and some sediments (e.g. [137]).

Steryl ethers found in marine sediments tend to be dominated by cholesteryl ethers with a $C_8 - C_{10}$ alkyl chain ether-linked at C-3 of the cholesterol molecule. Reports of steryl ethers are very limited [55, 137] and, indeed, there are few reports of their occurrence in the biota. Trace amounts have been found in bovine cardiac muscle [225], although quite a bit is known of their chemical properties from compounds synthesized to study sterol absorption in animals (e.g. [172]). The noticeable occurrence of these compounds in sediments underlying waters with high abundances of diatoms and the disparity between the sterol moiety of the ethers compared to the free sterols studied by Schouten et al. [55] led these authors to suggest that diatoms may be a direct source of sterol ethers, but this still remains unproven despite the numerous lipid studies of diatoms.

Steryl ethers isolated from marine sediments elute in the same general region of the chromatogram as wax esters [137]. The major ions are the molecular ion and two ions at m/z 368 and 370 due to loss of the cholesteryl group. A prominent ion at m/z 329 occurs due to cleavage of the A-ring. Boon and de Leeuw [137] provide a spectrum of 3-nonoxycholest-5-ene, while Schouten et al. [55] show a spectrum for the C_{10} analog.

Steryl esters elute much later than wax esters on non-polar GC columns and thus high temperature columns able to operate to 370 °C or higher are required for direct analysis [134, 136]. GC-MS analysis is not straightforward due to this temperature limitation, but has nonetheless been used to identify steryl esters in sediment trap material [134]. Steryl esters rarely give molecular ions in EI or CI mode, but useful fragment ions can be obtained using CI with methane as the reagent gas [134].

3.9 Triterpenoid Alcohols

This is a very broad collection of compounds and so the discussion here will be brief. The term "triterpenoid" alcohols is usually restricted to those compounds with a 6-membered E-ring (e.g. compounds based on the oleanane or ursane skeletons), but the term can be taken to include those with a 5-membered ring such as hopanoids (Figs. 13 and 14 [194]). Triterpenoids have proven to be particularly useful to assign sources of organic matter in sediments and petroleum.

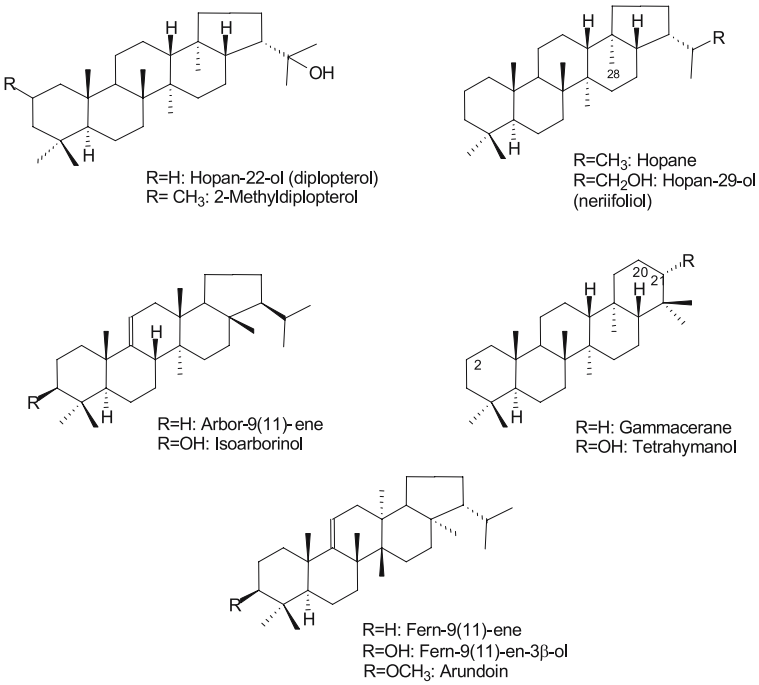

Fig. 13 Structures of some triterpenoids derived primarily from prokaryotes found in sediments

3.9.1 Hopanoids

The most common triterpenoid class in sediments is the hopanoids. Indeed, they may even be the most abundant natural product on earth [226]. Hopanoids are synthesized in bacteria from squalene which is cyclized by squalene-hopene cyclase (e.g. [227]). This reaction shares considerable similarities with that of the conversion of oxidosqualene to sterols (e.g. [194]). Hopanoids can occur in a variety of functionalized forms including alkenes, ketones, acids and alcohols (Fig. 13). Most are derived either directly from bacterial sources and have an extended alkyl chain at C-22 (e.g. [228]), or are diagenetic products. These diagenetic reactions occur very early in the diagenetic sequence in sediments (e.g. [229, 230]). For example, sediments of the highly productive lake (Priest Pot, UK) contain not only the parent bacteriohopanetetrol (BHT), but a series of hopanediols and triols such as trishomohopane-32,33-diol and bishomohopane-30,31,32-triol which represent intermediate stages in the conversion of biologically produced hopanoids to the diagenetic products, hopanes [231]. This process can be mimicked in the laboratory by oxidizing the biohopanoid in the presence of cupric chloride in pyridine [232].

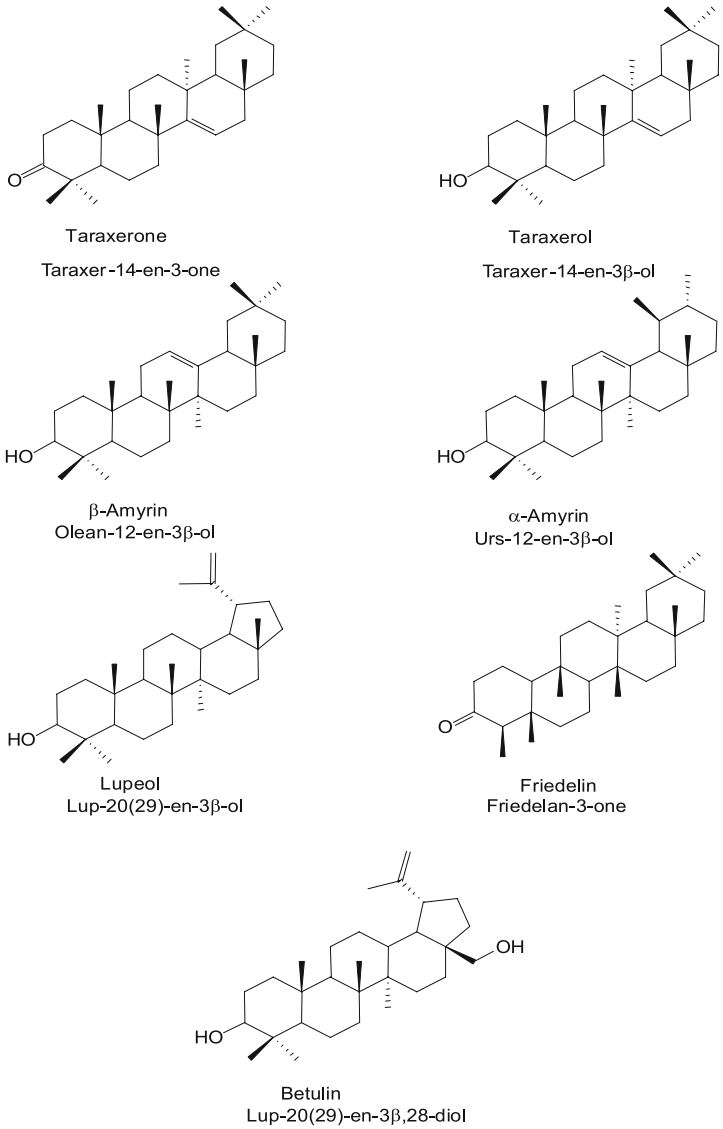

Fig. 14 Structures of some triterpenoids commonly found in higher plants

Unsaturated hydrocarbons (hopenes) such as hop-22(29)-ene, hop-17(21)-ene and 17β(H)-hop-21-ene [233] and C_{31} and C_{32} $\beta\beta$-isomers of diagenetically formed hopanes derived from polyhydroxyhopanoids of bacterial origin [234] are often present in immature sediments. Some unusual hopenes with the "moretane" stereochemistry such as 17β(H)-moret-22(29)-ene have also been reported in sediments [235]. Fully saturated hopanes are rare in

Recent sediments and seawater and in most cases these are attributable to ancient organic matter or petroleum contamination [21], rather than biological sources. This is most clearly shown when the carbon number range extends from C_{27} to C_{35} and several 17,21 α- and β-isomers are present. A full discussion of these compounds would take a chapter in their own right, so the reader is referred to leading reviews [18–20].

Most hopanoids can be identified directly by straightforward GC-MS techniques (e.g. [216]), and are usually characterized with a m/z 191 mass fragmentogram (or m/z 205 for hopanoids with a methyl group in the A ring or m/z 177 for demethylated hopanes). The direct detection of most intact biohopanoids is not possible due to their highly functionalized and amphiphilic nature. Talbot et al. [236] have developed a new reversed-phase high-performance liquid chromatography method for the direct analysis of acetylated, intact bacteriohopanepolyols and applied it to solvent extracts of methanotrophic bacteria. This method was suitable for identifying the four biohopanoids: bacteriohopanetetrol and aminobacteriohopanetriol, -tetrol and -pentol.

3.9.2
"Higher Plant" Triterpenoids

A major source of triterpenoid alcohols in modern sediments is from the angiosperms, which are the most abundant plant group on the earth today. Angiosperms contain triterpenoids of the β-amyrin (oleananoid) type, which on diagenesis ultimately produces the C_{30} triterpenoid hydrocarbon oleanane. The occurrence of oleanane in the geological record broadly correlates with the emergence of angiosperms in the Early Cretaceous and radiation in the Late Cretaceous and Tertiary (e.g. [237]).

Triterpenoids with a single hydroxyl group such as α- and β-amyrin, germanicol and taraxerol (Fig. 14), are widely used as terrestrial plant markers. For example, Killops and Frewin [238] used β-amyrin and taraxerol and their degradation products to trace organic matter from mangroves (*Rhizophora mangle*) in sediments from Florida Bay. Koch et al. [239] have had similar success in using such compounds as tracers. Volkman et al. [240] used a dihydroxylated triterpenoid betulin (Fig. 14) and other triterpenoid alcohols to show that sediments from the Wadden Sea contained organic matter from eroded peats. Note, however, that these compounds are not source-specific and can be found in a variety of plants [241], so care must be taken when using them to trace organic matter from specific plants.

3.9.3
Other Triterpenoids

Unfortunately, higher plants are not the only source of triterpenoid skeletons. For example, Smetanina et al. [242] identified the 3β-methoxyolean-18-ene

(miliacin – the methoxy ether derivative of germanicol) in a marine fungus *Chaetomium olivaceum* (Fig. 13). α-Amyrin has been positively identified in the spores from 16 species of arbuscular mycorrihizal fungi (AMF) belonging to the order Glomales [243]. Triterpene methyl ethers have been reported in a few sediments (e.g. [244]), but their origins remain obscure. Methyl ethers of other common triterpene series (e.g. cylindrin – isoarborinol methyl ether; sawamilletin - taraxerol methyl ether; isosawamilletin – β-amyrin methoxy ether) are also known from a variety of plants, but most commonly from grasses [245, 246].

Fernene isomers of bacterial origin have also been observed in some Recent sediments (e.g. [233, 247]). The distribution of fernenes appears to follow progressive isomerization from the Δ^7 to the thermodynamically more stable Δ^8 and $\Delta^{9(11)}$ isomers (structures in Fig. 13) with depth, although the latter can also have natural sources [248]. These C_{30} triterpenes have a prominent m/z 243 ion in their mass spectra. Fernenes and fernenols are abundant in contemporary ferns (e.g. [249, 250]), but their most likely source in most sediments is from bacteria [233, 251]. Fern-7-ene and fern-9(11)-ene have been isolated from a purple, non-sulfur bacterium *Rhodomicrobium vanniellii* [248].

Another interesting class of triterpenoids is based on the isoarborinol skeleton (Fig. 13). Isoarborinol has been found in quite high concentration in sediments from the Permian and Triassic [252], but reports in contemporary environments are rare. It has been suggested that it is derived from a microbial source [253–255].

Gammacerane has been found in sediments from the Late Proterozoic which also contain some of the earliest examples of fossil protozoans [256]. It is presumed to be derived from tetrahymanol (gammaceran-21α-ol) which is found in ciliates, ferns, fungi and bacteria [257, 258]. Tetrahymanol is both common and abundant in recent marine sediments (e.g. [259]).

3.10 Chlorophyll and Carotenoid Pigments

Microalgae contain a diverse array of carotenoid and chlorophyll pigments, including a number of newly discovered components. Some pigments are specific to one or a limited number of algal classes and thus can be used as biomarkers in the same way as lipids. Indeed, a valuable development has been the software program CHEMTAX [260], which uses specific pigment ratios to ascertain the abundance of microalgal groups in seawater samples. A comprehensive review of pigments is provided in the SCOR-UNESCO publication on phytoplankton pigments in oceanography [261]. This information is updated and summarized in the chapter by Wright and Jeffrey. The combination of pigment markers with lipid biomarkers such as fatty acids has proven to be very useful for elucidating sources of organic matter in the sea and for studying their rates of degradation [262].

3.11
Organic Sulfur Compounds

A great variety of organic sulfur compounds (OSCs) have been identified in marine sediments and in anoxic seawater. Most of these compounds result from incorporation of sulfur into functionalized biolipids, especially when the content of reactive iron in the sediments is low. Both intra- and intermolecular incorporation of sulfur has been documented. These OSCs thus contain biomarker information about the originally deposited organic matter. Further details can be found in recent papers [263–265].

4
Summary

A wide range of chemically distinct organic compounds can be found in marine sediments and seawater. The structures of many of these have been elucidated, although it seems that every year brings the discovery of some new class of compound. Some chemical structures can be related to specific groups of organisms, or even to a particular Class, Order or genus and thus they can be used as biomarkers for elucidating the sources of the organic matter. Many biomarkers degrade over time as a result of chemical and biological processes. The distributions of these degradation products can thus also provide a history of the breakdown of organic matter in the sediments or water column. Advances in analytical and chemical identification techniques now make it possible to identify very small amounts (ng to mg) in aquatic environment even when present in complex mixtures. Moreover, it is now possible to determine the ^{13}C and ^{14}C content in many compounds, thus providing additional information about their source and age.

Acknowledgements I wish to pay tribute to my colleagues for their assistance, advice and fruitful conversations over many years which has led to this overview of organic geochemical biomarkers. Funding from CSIRO Marine Research, the Fisheries Research and Development Corporation, Aquafin CRC, Antarctic Science Advisory Committee and the Australian Research Council is gratefully acknowledged.

References

1. Han J, McCarthy ED, Van Hoeven W, Calvin M, Bradley WH (1968) Proc Natl Acad Sci USA 59:29
2. Jeffries HP (1970) Limnol Oceanogr 15:419
3. Larsson K, Odham G, Södergren A (1974) Mar Chem 2:49
4. Albrecht P, Ourisson G (1971) Angew Chem Int Ed Engl 10:209
5. Bligh EG, Dyer WJ (1959) Can J Biochem Physiol 37:912

6. Folch J, Lees M, Sloane Stanley GH (1957) J Biol Chem 226A:497
7. Iverson SJ, Lang SLC, Cooper MH (2001) Lipids 36:283
8. Delmas RP, Parrish CC, Ackman RG (1984) Anal Chem 56:1272
9. Parrish CC, Ackman RG (1983) J Chromatogr 262:103
10. Volkman JK, Everitt DA, Allen DI (1986) J Chromatogr 356:147
11. Lough AK (1964) Nature 202:795
12. Christie WW (2003) Lipid analysis: isolation, separation, identification and structural analysis of lipids, 3rd edn. Oily Press, England
13. de Leeuw JW, Rijpstra WIC, Schenck PA, Volkman JK (1983) Geochim Cosmochim Acta 47:455
14. Wakeham SG (1999) Org Geochem 30:1059
15. Garcette-Lepecq A, Largeau C, Bouloubassi I, Derenne S, Saliot A, Lorre A, Point V (2004) Org Geochem 35:959
16. Johns RB (ed) (1986) Biological markers in the sediment record. Methods in geochemistry and geophysics, vol 24. Elsevier, Amsterdam
17. Engel MH, Macko SA (eds) (1993) Organic geochemistry. Principles and applications. Plenum, NY
18. Philp RP (1985) Fossil fuel biomarkers. Applications and spectra. Methods in geochemistry and geophysics, vol 23. Elsevier, Amsterdam
19. Moldowan JM, Albrecht P, Philp RP (1992) Biological markers in sediments and petroleum. Prentice Hall, Englewood Cliffs, NJ
20. Killops SD, Killops VJ (1993) An introduction to organic geochemistry. Longman Scientific and Technical, NY
21. Volkman JK, Revill AT, Murray AP (1997) Applications of biomarkers for identifying sources of natural and pollutant hydrocarbons in aquatic environments. In: Eganhouse RP (ed) Molecular markers in environmental geochemistry. ACS Symposium Series 671. American Chemical Society, NY p 110
22. Giger W, Schaffner C, Wakeham SG (1980) Geochim Cosmochim Acta 44:119
23. Rowland SJ, Maxwell JR (1984) Geochim Cosmochim Acta 48:617
24. Han J, McCarthy ED, Calvin M, Benn MH (1968) J Chem Soc C 2785
25. Höld IM, Schouten S, Jellema J, Sinninghe Damsté JS (1999) Org Geochem 30:1411
26. Köster J, Volkman JK, Rullkötter J, Scholz-Böttcher BM, Rethmeier J, Fischer U (1999) Org Geochem 30:1367
27. Kenig F, Sinninghe Damsté JS, Dalen ACK, Rijpstra WIC, Huc AY, de Leeuw JW (1995) Geochim Cosmochim Acta 59:2999
28. Kenig F, Simons DJH, Crich D, Cowen JP, Ventura GT, Rehbein-Khalily T, Brown TC, Anderson KB (2003) Proc Natl Acad Sci USA 100:12554
29. Nishimoto S (1974) J Sci Hiroshima Univ A 38:165
30. Matsumoto GI, Friedmann EI, Watanuki K, Ocampo-Friedmann R (1992) J Chromatogr 598:267
31. Blumer M, Mullin MM, Thomas DW (1963) Science 140:974
32. Volkman JK, Maxwell JR (1986) Acyclic isoprenoids as biological markers. In: Johns RB (ed) Biological markers in the sedimentary record. Elsevier, Amsterdam, p 1
33. Waples DW, Haug P, Welte DH (1974) Geochim Cosmochim Acta 38:381
34. Brassell SC, Wardroper AMK, Thomson ID, Maxwell JR, Eglinton G (1981) Nature 290:693
35. Elvert M, Suess E, Whiticar MJ (1999) Naturwiss 86:295
36. Freeman KH, Wakeham SG, Hayes JM (1994) Org Geochem 21:629
37. Sinninghe Damsté JS, Schouten S, van Vliet NH, Huber R, Geenevasen JAJ (1997) Tetrahedron Lett 38:6881

38. De Rosa M, Gambacorta A (1994) Archaeal lipids. In: Goodfellow M, O'Donnell AG. Chemical methods in prokaryotic systematics. John Wiley and Sons, Chichester p 197
39. Sinninghe Damsté JS, Kuypers MMM, Schouten S, Schulte S, Rullkötter R (2003) Earth Planet Sci Letts 209:215
40. Wakeham SG, Freeman KH, Pease TK, Hayes JM (1993) Geochim Cosmochim Acta 57:159
41. Kissin YV, Feulmer GP, Payne WB (1986) J Chrom Sci 24:164
42. Lee RF, Loeblich III AR (1971) Phytochem 10:593
43. Volkman JK, Barrett SM, Dunstan GA (1994) Org Geochem 21:407
44. Volkman JK, Barrett SM, Blackburn SI, Mansour MP, Sikes EL, Gelin F (1998) Org Geochem 29:1163
45. Volkman JK, Eglinton G, Corner EDS, Forsberg TEV (1980) Phytochem 19:2619
46. Volkman JK, Smith DJ, Eglinton G, Forsberg TEV, Corner EDS (1981) J Mar Biol Assoc UK 61:509
47. Rieley G, Teece MA, Peakman TM, Raven AM, Greene KJ, Clarke TP, Murray M, Leftley JW, Campbell CN, Harris RP, Parkes RJ, Maxwell JR (1998) Lipids 33:617
48. Volkman JK, Eglinton G, Corner EDS, Sargent JR (1980) Novel unsaturated straight-chain $C_{37}-C_{39}$ methyl and ethyl ketones in marine sediments and a coccolithophorid *Emiliania huxleyi*. In: Douglas AG, Maxwell JR (eds) (1979) Advances in organic geochemistry. Pergamon, Oxford, p 219
49. Rowland SJ, Volkman JK (1982) Mar Environ Res 7:117
50. Gelin F, Boogers I, Noordeloos AAM, Sinninghe Damsté JS, Riegman R, de Leeuw JW (1997) Org Geochem 26:659
51. Sinninghe Damsté JS, Rijpstra WIC, Schouten S, Peletier H, van der Maarel MJEC, Gieskes WWC (1999) Org Geochem 30:95
52. Gelpi E, Oró J, Schneider HJ, Bennett EO (1968) Science 161:700
53. van der Meer MTJ, Schouten S, Ward DM, Geenevasen JAJ, Sinninghe Damsté JS (1999) Org Geochem 30:1585
54. Gearing P, Gearing JN, Lytle TF, Lytle JS (1976) Geochim Cosmochim Acta 40:1005
55. Schouten S, Hoefs MJL, Sinninghe Damsté JS (2000) Org Geochem 31:509
56. Yon DA, Maxwell JR, Ryback G (1982) Tetrahedron Lett 23:2143
57. Hird SJ, Evens R, Rowland SJ (1992) Mar Chem 37:117
58. Volkman JK, Farrington JW, Gagosian RB, Wakeham SG (1983) Lipid composition of coastal marine sediments from the Peru upwelling region. In: Bjorøy M et al. (eds) Advances in organic geochemistry 1981. John Wiley, Chichester p 228
59. Nichols PD, Volkman JK, Palmisano AC, Smith GA, White DC (1988) J Phycol 24:90
60. Rowland SJ, Robson JN (1990) Mar Environ Res 30:191
61. Belt ST, Massé G, Allard WG, Robert J-M, Rowland SJ (2001) Org Geochem 32:1271
62. Belt ST, Allard WG, Massé G, Robert J-M, Rowland SJ (2001) Tetrahedron Lett 42:5583
63. Schouten S, Klein Breteler WCM, Blokker P, Rijpstra WIC, Grice K, Baas M, Sinninghe Damsté JS (1998) Geochim Cosmochim Acta 62:1397
64. Rowland SJ, Allard WG, Belt ST, Massé G, Robert J-M, Blackburn S, Frampton D, Revill AT, Volkman JK (2001) Phytochem 58:717
65. Belt ST, Massé G, Allard WG, Robert JM, Rowland SJ (2003) Tetrahedron Lett 44:9103
66. Massé G, Belt ST, Allard WG, Lewis CA, Wakeham SG, Rowland SJ (2004) Org Geochem 35:813.
67. Wraige EJ, Belt ST, Lewis CA, Cooke DA, Robert J-M, Massé G, Rowland SJ (1997) Org Geochem 27:497

68. Wraige EJ, Johns L, Belt ST, Massé G, Robert J-M, Rowland S (1999) Phytochem 51:69
69. Belt ST, Cooke DA, Hird SJ, Rowland SJ (1994) J Chem Soc Chem Comm 2077
70. Belt ST, Allard WG, Massé G, Robert J-M, Rowland SJ (2000) Geochim Cosmochim Acta 64:3839
71. Cox RE, Burlingame AL, Wilson DM, Eglinton G, Maxwell JR (1973) J Chem Soc Chem Comm 284
72. Metzger P, Casadevall E, Coute A (1988) Phytochem 27:1383
73. Okada S, Murakami M, Yamaguchi K (1997) Phytochem Anal 8:198
74. Metzger P, Allard B, Casadevall E, Berkaloff C, Cout A (1990) J Phycol 26:258
75. Grice K, Schouten S, Nissenbaum A, Charrach J, Sinninghe Damsté JS (1998) Org Geochem 28:195
76. Sinninghe Damsté JS, Erkes AMWEP, Rijpstra WIC, de Leeuw JW, Wakeham SG (1995) Geochim Cosmochim Acta 59:347
77. Mackenzie AS, Brassell SC, Eglinton G, Maxwell JR (1982) Science 217:491
78. Peakman TM, ten Haven HL, Rechka JR, de Leeuw JW, Maxwell JR (1989) Geochim Cosmochim Acta 53:2001
79. Brassell SC, McEvoy J, Hoffmann CF, Lamb NA, Peakman TM, Maxwell JR (1984) Isomerisation, rearrangement and aromatisation of steroids in distinguishing early stages of diagenesis. In: Schenck PA, de Leeuw JW, Lijmbach GWM (eds) (1983) Advances in organic geochemistry. Pergamon, Oxford, p 11
80. Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) J Exp Mar Biol Ecol 128:219
81. Perry GJ, Volkman JK, Johns RB, Bavor HJ (1979) Geochim Cosmochim Acta 43:1715
82. Fulco AJ (1983) Prog Lipid Res 22:133
83. Volkman JK, Johns RB (1977) Nature 267:693
84. Ivanova EP, Zhukova NV, Svetashev VI, Gorshkova NM, Kurilenko VV, Frolova GM, Mikhailov VV (2000) Curr Microbiol 41:341
85. Sinninghe Damsté JS, Strous M, Rijpstra WIC, Hopmans EC, Geenevasen JAJ, van Duin ACT, van Niftrik LA, Jetten MSM (2002) Nature 419:708
86. Jetten MSM, Sliekers O, Kuypers M, Dalsgaard T, van Niftrik L, Cirpus I, van de Pas-Schoonen K, Lavik G, Thamdrup B, Le Paslier D, Op den Camp HJM, Hulth S, Nielsen LP, Abma W, Third K, Engström P, Kuenen JG, Jørgensen BB, Canfield DE, Sinninghe Damsté JS, Revsbech NP, Fuerst J, Weissenbach J, Wagner M, Schmidt I, Schmid M, Strous M (2003) Appl Microbiol Biotechnol 63:107
87. Kuypers MMM, Sliekers AO, Lavik G, Schmid M, Jørgensen BB, Kuenen JG, Sinninghe Damsté JS, Strous M, Jetten MSM (2003) Nature 422:608
88. Napolitano GE, Pollero RJ, Gayoso AM, MacDonald BA, Thompson RJ (1997) Biochem Syst Ecol 25:739
89. Suhr SB, Pond DW, Gooday AJ, Smith CR (2003) Mar Ecol Prog Ser 262:153
90. Dalsgaard J, St John M, Kattner G, Muller-Navarra D, Hagen W (2003) Adv Mar Biol 46:225
91. Mansour MP, Volkman JK, Holdsworth DG, Jackson AE, Blackburn SI (1999) Phytochem 50:541
92. Leblond JD, Chapman PJ (2000) J Phycol 36:1103
93. Leblond JD, Evans TJ, Chapman PJ (2003) Phycologia 42:324
94. Shantha NC, Napolitano GE (1992) J Chromatogr 624:37
95. Moss CW, Daneshvar MI (1992) J Clin Microbiol 30:2511
96. Dunkelblum E, Tan SH, Silk PJ (1985) J Chem Ecol 11:265
97. Nichols PD, Guckert JB, White DC (1986) J Microbiol Met 5:49
98. Ackman RG, Burgher RD (1964) J Fish Res Bd Canada 21:319

99. Ghosh A, Hogue M, Dutta J (1972) J Chromatogr 69:207
100. Dobson G, Christie WW, Nikolovada-Myanova B (1995) J Chromatogr B Biomed Appl 671:197
101. Christie WW (1998) Lipids 33:343
102. Spitzer V (1996) Prog Lipid Res 35:387
103. Méjanelle L, Laureillard J, Saliot A (2002) J Microbiol Met 48:221
104. Nichols DS, Davies NW (2002) J Microbiol Met 50:103
105. Skerratt JH, Bowman JP, Nichols PD (2002) Int J Syst Evol Microbiol 52:2101
106. Eglinton G, Hunneman DH, Douraghi-Zadeh K (1968) Tetrahedron 24:5929
107. Kolattukudy PE (1980) Cutin, suberin, and waxes. In: Stumpf PK (ed) The biochemistry of plants. A comprehensive treatise, vol 4, Lipids: Structure and Function. Academic, New York, p 571
108. Kolattukudy PE (1984) Can J Bot 62:2918
109. Volkman JK, Johns RB, Gillan FT, Perry GJ, Bavor HJ (1980) Geochim Cosmochim Acta 44:1133
110. Kawamura K, Gagosian RB (1990) Naturwiss 77:25
111. Downing DT (1961) Rev Pure Appl Chem 11:196
112. Fulco AJ (1967) J Biol Chem 242:3608
113. Volkman JK, Barrett SM, Blackburn SI (1999) J Phycol 35:1005
114. Edlund A, Nichols PD, Roffey R, White DC (1985) J Lipid Res 26:982
115. Kaltashov IA, Doroshenko V, Cotter RJ, Takayama K, Qureshi N (1997) Anal Chem 69:2317
116. Boon JJ, de Leeuw JW, van der Hoek GJ, Vosjan JH (1977) J Bacteriol 129:1183
117. Keinanen MM, Korhonen LK, Martikainen PJ, Vartiainen T, Miettinen IT, Lehtola MJ, Nenonen K, Pajunen H, Kontro MH (2003) J Chromatogr B 783:443
118. Gelin F, Volkman JK, de Leeuw JW, Sinninghe Damsté JS (1997) Phytochem 45:641
119. Kolattukudy PE, Croteau R, Walton TJ (1975) Plant Physiol 55:875
120. Fukushima K, Kondo H, Sakata S (1992) Org Geochem 18:913
121. Skerratt JH, Nichols PD, Bowman JP, Sly LI (1992) Org Geochem 18:189
122. Abreu-Grobois FA, Billyard TC, Walton TJ (1977) Phytochem 16:351
123. de Leeuw JW, Rijpstra WIC, Mur LR (1992) Org Geochem 18:575
124. Kolattukudy PE, Walton TJ (1972) Biochemistry 11:1897
125. Walton TJ, Kolattukudy PE (1972) Biochem Biophys Res Comm 46:16
126. Huang YS, Lockheart MJ, Logan GA, Eglinton G (1996) Org Geochem 24:289
127. López Alonso D, Belarbi E-H, Rodríguez-Ruiz J, Segura CI, Giménez A (1998) Phytochem 47:1473
128. Sargent JR, Gatten RR, Henderson RJ (1981) Pure Appl Chem 53:867
129. Pernet F, Tremblay R, Demers E, Roussy M (2003) Aquaculture 221:393
130. Dunstan GA, Volkman JK, Barrett SM, Garland CD (1993) J Appl Phycol 5:71
131. Siron R, Giusti G, Berland B (1989) Mar Ecol Prog Ser 55:95
132. Mayzaud P, Errhif A, Bedo A (1998) J Mar Syst 17:391
133. Lee RF, Nevenzel JC, Paffenhofer G-A (1971) Mar Biol 9:99
134. Wakeham SG (1982) Geochim Cosmochim Acta 46:2239
135. Wakeham SG, Farrington JW, Volkman JK (1983) Fatty acids, wax esters, triacylglycerols, and alkyldiacylglycerols associated with particles collected in sediment traps in the Peru upwelling. In: Bjorøy M et al (eds) (1981) Advances in organic geochemistry. John Wiley, Chichester, p 185
136. Wakeham SG, Frew NM (1982) Lipids 17:831
137. Boon JJ, de Leeuw JW (1979) Mar Chem 7:117

138. Boon JJ, Rijpstra WIC, de Leeuw JW, Burlingame AL (1980) Geochim Cosmochim Acta 44:131
139. Bobbie RJ, White DC (1980) Appl Environ Microbiol 39:1212
140. White DC (1993) Phil Trans Roy Soc Lond A 344:59
141. Dobbs FC, Findlay RH (1993) Quantitative description of microbial communities using lipid analysis. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p 271
142. Findlay RH, Dobbs FC (1993) Analysis of microbial lipids to determine biomass and detect the response of sedimentary microorganisms to disturbance. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p 347
143. White DC, Davis WM, Nickels JS, King JD, Bobbie RJ (1979) Oecologia 40:51
144. Rütters H, Sass H, Cypionka H, Rullkötter J (2002) J Micro Met 48:149
145. Rütters H, Sass H, Cypionka H, Rullkötter J (2002) Org Geochem 33:803
146. Laureillard J, Pinturier L, Fillaux J, Saliot A (1997) Deep Sea Res II 44:1085
147. Okuyama H, Morita N, Kogame K (1992) J Phycol 28:465
148. Budge SM, Parrish CC (1999) Phytochem 52:561
149. Son BW, Cho YJ, Choi JS, Lee WK, Kim DS, Choi HD, Choi JS, Jung JH, Im KS, Choi WC (2001) Nat Prod Letts 15:299
150. Batrakov SG, Nikitin DI, Pitryuk IA (1996) Biochim Biophys Acta-Lipids Lipid Metab 1303:39
151. Gillan FT, Sandstrom MW (1985) Org Geochem 8:321
152. Sinninghe Damsté JS, van Dongen BE, Rijpstra WIC, Schouten S, Volkman JK, Geenevasen JAJ (2001) Org Geochem 32:321
153. Oren A, Gurevich P (1993) FEMS Microbiol Ecol 12:249
154. Kato M, Adachi K, Hajiro-Nakanishi K, Ishigaki E, Sano H, Miyachi S (1994) Phytochem 37:279
155. ten Haven HL, Littke R, Rullkötter J, Stein R, Welte DH (1990) Accumulation rates and composition of organic matter in Late Cenozoic sediments underlying the active upwelling area off Peru. In: Suess E, von Huene R et al. (eds) Proceedings of the Ocean Drilling Program, scientific results, vol 112. Ocean Drilling Program, College Station, p 591
156. Cranwell PA, Volkman JK (1981) Chem Geol 32:29
157. Russell NJ, Volkman JK (1980) J Gen Microbiol 118:131
158. Cranwell PA (1984) Org Geochem 6:115
159. Aasen AJ, Hofstetter HH, Iyengar BTR, Holman RT (1971) Lipids 6:502
160. Volkman JK, Barrett SM, Dunstan GA, Jeffrey SW (1992) Org Geochem 18:131
161. de Leeuw JW, Rijpstra WIC, Schenck PA (1981) Geochim Cosmochim Acta 45:2281
162. Nichols PD, Johns RB (1986) Org Geochem 9:25
163. Versteegh GJM, Bosch HJ, de Leeuw JW (1997) Org Geochem 27:1
164. Versteegh GJM, Jansen JHF, de Leeuw JW, Schneider RR (2000) Geochim Cosmochim Acta 64:1879
165. Tegelaar EW, Matthezing RM, Jansen JBH, Horsfield B, de Leeuw JW (1989) Nature 342:529
166. Largeau C, de Leeuw JW (1995) Insoluble, non-hydrolysable aliphatic macromolecule constituents of microbial cell walls. In: Jones JG (ed) Advances in microbial ecology, vol 14. Plenum, New York, p 77
167. Volkman JK, Barrett SM, Blackburn SI (1999) Org Geochem 30:307
168. Sinninghe Damsté JS, Rampen S, Rijpstra WIC, Abbas B, Muyzer G, Schouten S (2003) Geochim Cosmochim Acta 67:1339

169. Ferreira AM, Miranda A, Caetano M, Baas M, Vale C, Sinninghe Damsté JS (2001) Org Geochem 32:271
170. Schouten S, Rijpstra WIC, Kok M, Hopmans EC, Summons RE, Volkman JK, Sinninghe Damsté JS (2001) Geochim Cosmochim Acta 65:629
171. Sprott GD, Dicaire CJ, Choquet CG, Patel GB, Ekiel I (1993) Appl Environ Microbiol 59:912
172. Paltauf F (1983) Ether lipids containing sterol moieties. In: Mangold HK and Paltauf F, Ether lipids: biochemical and biomedical aspects. Academic, NY, p 177
173. Wakeham SG, Lewis CM, Hopmans EC, Schouten S, Sinninghe Damsté JS (2003) Geochim Cosmochim Acta 67:1359
174. Hoefs MJL, Schouten S, de Leeuw JW, King LL, Wakeham SG, Sinninghe Damsté JS (1997) Appl Environ Microbiol 63:3090
175. Sinninghe Damsté JS, Schouten S, Hopmans EC, van Duin ACT, Geenevasen JAJ (2002) J Lipid Res 43:1641
176. Sinninghe Damsté JS, Rijpstra WIC, Hopmans EC, Prahl FG, Wakeham SG, Schouten S (2002) Appl Environ Microbiol 68:2997
177. Hopmans EC, Schouten S, Pancost RD, van der Meer MTJ, Sinninghe Damsté JS (2000) Rapid Comm Mass Spectrom 14:585
178. Hopmans EC, Weijers JWH, Schefuß E, Herfort L, Sinninghe Damsté JS, Schouten S (2004) Earth Planet Sci Lett 224:107
179. Sinninghe Damsté JS, Hopmans EC, Pancost RD, Schouten S, Geenevasen JAJ (2000) Chem Comm 1683
180. Morrison RI, Bick W (1966) Chem Ind April 2, p 596
181. Lehtonen K, Ketola M (1990) Org Geochem 15:275
182. Cranwell PA, Eglinton G, Robinson N (1987) Org Geochem 11:513
183. Hernandez ME, Mead R, Peralba MC, Jaffé R (2001) Org Geochem 32:21
184. Volkman JK, Barrett SM, Blackburn SI, Sikes EL (1995) Geochim Cosmochim Acta 59:513
185. Rontani J-F, Marchand D, Volkman JK (2001) Org Geochem 32:1329
186. Marlowe IT, Brassell SC, Eglinton G, Green JC (1984) Org Geochem 6:135
187. Sikes EL, Volkman JK, Robertson LG, Pichon JJ (1997) Geochim Cosmochim Acta 61:1495
188. Sikes EL, Sicre MA (2002) Geochem Geophys Geosyst 3: art no. 1063
189. Prahl FG, Wakeham SG (1987) Nature (Lond) 330:367
190. Brassell SC, Eglinton G, Marlowe IT, Pflaumann U, Sarnthein M (1986) Nature (Lond) 320:129
191. Rosell-Melé A (1998) Paleoceanogr 13:694
192. Volkman JK (1986) Org Geochem 9:83
193. Volkman JK (2003) Appl Micro Biotech 60:495
194. Volkman JK (2004) Org Geochem (in press)
195. Goad LJ, Holz GGJ, Beach DH (1983) Phytochem 22:475
196. Barrett SM, Volkman JK, Dunstan GA, LeRoi JM (1995) J Phycol 31:360
197. Moldowan MJ, Fago FJ, Lee CY, Jacobson SR, Watt DS, Slougui N-E, Jeganathan A, Young DC (1990) Science 247:309
198. Patterson GW (1971) Lipids 6:120
199. Volkman JK, Barrett SM, Dunstan GA, Jeffrey SW (1993) Org Geochem 20:7
200. Volkman JK, Kearney P, Jeffrey SW (1990) Org Geochem 15:489
201. Volkman JK, Farmer CL, Barrett SM, Sikes EL (1997) J Phycol 33:1016
202. Mansour MP, Volkman JK, Jackson AE, Blackburn SI (1999) J Phycol 35:710
203. Withers NW, Goad LJ, Goodwin TW (1979) Phytochem 18:899

204. Withers N (1987) Dinoflagellate sterols. In: Taylor FJR (ed) The biology of dinoflagellates. Biological Monographs, vol 21. Blackwell Scientific, Oxford, p 316
205. Santos VLCS, Billett DSM, Wolff GA (2000) Aquat Eco Health Management 3:397
206. Butler E, Parslow J, Volkman J et al. (2000) Huon Estuary Study – Environmental research for integrated catchment management and aquaculture. FRDC Final Report Project No. 96/284. Fisheries Research and Development Corporation, Canberra
207. Robinson N, Eglinton G, Brassell SC, Cranwell PA (1984) Nature (Lond) 308:439
208. Nishimura M, Koyama T (1977) Geochim Cosmochim Acta 41:379
209. Nishimura M (1982) Geochim Cosmochim Acta 46:423
210. Leeming R, Latham V, Rayner M, Nichols P (1997) Detecting and distinguishing sources of sewage pollution in Australian inland and coastal waters and sediments. In: Eganhouse RP (ed) ACS Symposium Series No. 617. Molecular markers in environmental geochemistry. American Chemical Society, NY p 306
211. Venkatesan MI, Santiago CA (1989) Mar Biol 102:431
212. Knights BA (1967) J Gas Chromatog 5:273
213. Brooks CJW, Henderson W, Steel G (1973) Biochim Biophys Acta 296:431
214. Rahier A, Benveniste P (1989) Mass spectral identification of phytosterols. In: Nes WD, Parish EJ (eds) Analysis of sterols and other biologically significant steroids. Academic, San Diego, p 223
215. Itoh T, Tani H, Fukishima K, Tamura T, Matsumoto T (1982) J Chromatogr 234:65
216. Jones GJ, Nichols PD, Shaw PM (1994) Analysis of microbial sterols and hopanoids. In: Goodfellow M, O'Donnell AG (eds) Chemical methods in prokaryotic systematics. John Wiley and Sons, Chichester, p 163
217. Gagosian RB, Smith SO (1979) Nature 277:287
218. Harvey RH, Bradshaw SA, O'Hara SCM, Eglinton G, Corner EDS (1988) Phytochem 27:1723
219. Leblond JD, Chapman PJ (2004) J Phycol 40:104
220. Parmentier G, Eyssen H (1974) Biochim Biophys Acta 348:279
221. Benfenati E, Cools E, Fattore E, Fanelli R (1994) Chemosphere 29:1393
222. King LL, Repeta DJ (1994) Geochim Cosmochim Acta 58:4389
223. Talbot HM, Head RN, Harris RP, Maxwell JR (1999) Org Geochem 30:1163
224. Talbot HM, Head RN, Harris RP, Maxwell JR (2000) Org Geochem 31:871
225. Fumasaki J, Gilbertson JR (1968) J Lipid Res 9:766
226. Ourisson G, Albrecht P (1992) Acc Chem Res 25:398
227. Kannenberg EL, Poralla K (1999) Naturwiss 86:168
228. Rohmer M, Bouvier-Navé P, Ourisson G (1984) J Gen Microbiol 130:1137
229. Rodier C, Llopiz P, Neunlist S (1999) Org Geochem 30:713
230. Tritz JP, Herrmann D, Bisseret P, Connan J, Rohmer M (1999) Org Geochem 30:499
231. Watson DF, Farrimond P (2000) Org Geochem 31:1247
232. Bisseret P, Rohmer M (1995) Tetrahedron Lett 36:7077
233. Volkman JK, Allen DI, Stevenson PL, Burton HR (1986) Org Geochem 10:671
234. Ourisson G, Albrecht P, Rohmer M (1984) Sci Am 251:44
235. Uemura H, Ishiwatari R (1995) Org Geochem 23:675
236. Talbot HM, Watson DF, Murrell JC, Carter JF, Farrimond P (2001) J Chromatogr A 921:175
237. Moldowan JM, Dahl J, Huizinga BJ, Fago FJ, Hickey LJ, Peakman TM, Taylor DW (1994) Science 265:768
238. Killops SD, Frewin NL (1994) Org Geochem 21:1193
239. Koch BP, Rullkötter J, Lara RJ (2003) Wetlands Ecol Management 11:257

240. Volkman JK, Rohjans D, Rullkötter J, Scholz-Böttcher BM, Liebezeit G (2000) Cont Shelf Res 20:1139
241. Abe I, Rohmer M, Prestwich GD (1993) Chem Rev 93:2189
242. Smetanina OF, Kuznetzova TA, Denisenko VA, Pivkin MV, Khudyakova YV, Gerasimenko AV, Popov DY, Il'in SG, Elyakov GB (2001) Russ Chem Bull 50:2463
243. Grandmougin-Ferjani A, Dalpé Y, Hartmann M-A, Laruelle F, Sancholle M (1999) Phytochem 50:1027
244. Grimalt JO, Yruela I, Saiz-Jimenez C, Toja J, de Leeuw JW, Albaigés J (1991) Geochim Cosmochim Acta 55:2555
245. Jacob J, Disnar J-R, Boussafir M, Albuquerque ALS, Sifeddine A, Turcq B (2005) Org Geochem 36:449
246. Ohmoto T, Nikaido T, Nakadai K, Tohyama E (1970) Yakugaku Zasshi 90:390
247. ten Haven HL, Rullkötter J, Stein R (1989) Preliminary analysis of extractable lipids in sediments from the Eastern North Atlantic (Leg 108): comparison of a coastal upwelling area (Site 658) with a nonupwelling area (Site 659). In: Ruddiman W, Sarnthein M et al. (eds) Proceedings of the Ocean Drilling Program, scientific results, vol 108. Ocean Drilling Program, College Station, p 351
248. Howard DL, Simoneit BRT, Chapman DJ (1984) Arch Microbiol 137:200
249. Ageta H, Shiojima K, Arai Y, Masuda K (1987) J Pharm Sci 76:S213
250. Shiojima K, Sasaki Y, Ageta H (1993) Chem Pharm Bull 41:268
251. Brassell SC, Eglinton G (1983) Steroids and triterpenoids in deep sea sediments as environmental and diagenetic indicators. In: Bjorøy M et al. (eds) Advances in organic geochemistry 1981. John Wiley, Chichester, p 684
252. Hauke V, Adam P, Trendel JM, Albrecht P, Schwark L, Vliex M, Hagemann H, Püttmann W (1995) Org Geochem 23:91
253. Hauke V, Graff R, Wehrung P, Trendel JM, Albrecht P, Schwark L, Keely BJ, Peakman TM (1992) Tetrahedron 48:3915
254. Hauke V, Graff R, Wehrung P, Trendel JM, Albrecht P, Riva A, Hopfgartner G, Gülaçar FO, Buchs A, Eakin PA (1992) Geochim Cosmochim Acta 56:3595
255. Jaffé RI, Hausmann KB (1995) Org Geochem 22:231
256. Summons RE, Brassell SC, Eglinton G, Evans E, Horodyski RJ, Robinson N, Ward DM (1988) Geochim Cosmochim Acta 52:2625
257. Kleemann G, Poralla K, Englert G, Kjøsen H, Liaaen-Jensen S, Neunlist S, Rohmer M (1990) J Gen Microbiol 136:2551
258. Bravo J-M, Perzl M, Härtner T, Kannenberg EL, Rohmer M (2001) Eur J Biochem 268:1323
259. Harvey HR, McManus GB (1991) Geochim Cosmochim Acta 55:3387
260. Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996) Mar Ecol Prog Ser 144:265
261. Jeffrey SW, Mantoura RFC, Wright SW (1997) Phytoplankton pigments in oceanography: guidelines to modern methods. UNESCO Paris
262. Tolosa I, Vescovali I, Leblond N, Marty JC, De Mora S, Prieur L (2004) Mar Chem 88:103
263. Werne JP, Lyons TW, Hollander DJ, Formolo MJ, Sinninghe Damsté JS (2003) Chem Geol 195:159
264. Aycard M, Derenne S, Largeau C, Mongenot T, Tribovillard N, Baudin F (2003) Org Geochem 34:701
265. Sinninghe Damsté JS, Schouten S, de Leeuw JW, van Duin ACT, Geenevasen JAJ (1999) Geochim Cosmochim Acta 63:31