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# Microalgal biomarkers: A review of recent research developments

JOHN K. VOLKMAN[1,2]*, STEPHANIE M. BARRETT[1], SUSAN I. BLACKBURN[1], MAGED P. MANSOUR[1], ELISABETH L. SIKES[2,3] and FRANÇOIS GELIN†[4]

[1]CSIRO Division of Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia, [2]Antarctic CRC, GPO Box 252-80, Hobart, Tasmania 7001, Australia, [3]AGSO, GPO Box 252-80, Hobart, Tasmania 7001, Australia and [4]Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands

**Abstract**—Microalgae are major sources of lipids in lacustrine and marine environments. This paper provides a review of some recent advances in our knowledge of the wide variety of lipid types that have been isolated from microalgae with an emphasis on those likely to be useful biomarkers for identifying sources of organic matter in sediments. Extensive data are now available on the fatty acids in all of the major classes of microalgae and some useful characteristic features have been observed in the abundance of particular polyunsaturated fatty acids. Despite several decades of study, it is now apparent that some of the biosynthetic steps leading to the formation of these unsaturated fatty acids are still not known with certainty as shown by the occurrence of $C_{28}$ polyunsaturated fatty acids in some dinoflagellates and the likely involvement of chain-shortening reactions. Considerable data have also been obtained on the sterols in microalgae, but some classes of organisms are still not well documented (e.g. cryptomonads, eustigmatophytes, xanthophytes and raphidophytes). Diatoms show a great variety of sterol compositions and no sterol appears to be either unique or representative. However, 24-methylenecholesterol in sediments is probably derived in most cases from diatoms. High contents of $C_{25}$ highly branched isoprenoid (HBI) alkenes have been identified in the diatom *Haslea ostrearia* and both $C_{25}$ and $C_{30}$ HBI alkenes have been found in diatom strains thought to be *Rhizosolenia setigera*. Genetic and environmental factors appear to be important controls on the relative abundances of the various homologues identified. Microalgae are also suspected to be a source of long-chain saturated fatty acids having an even carbon number predominance and of long-chain alkanes with no odd over even carbon number predominance, although the available data are not conclusive. An exciting development in recent years is the identification of highly aliphatic biopolymers (algaenans) in some species of marine and freshwater green algae and eustigmatophytes. This material persists in sediments and may be a source of the alkyl chains in ancient kerogens and crude oil constituents. Algaenans do not occur in all algal species and may be absent from some classes, such as diatoms. This implies that the organic matter preserved in sediments is strongly influenced by a subset of the microalgal contributors of organic matter. Although reasonable sources have been identified for many of the lipids in sediments, there are still many gaps in our knowledge and further studies are clearly required. 

*Key words*—biomarkers, fatty acids, sterols, alkenones, alkyl diols, hydrocarbons, alcohols, chemotaxonomy, microalgae, algaenans

## INTRODUCTION

Over recent years, many new compounds have been identified in sediments deposited in marine and lacustrine environments. Despite the fact that our knowledge of algal lipids is still far from comprehensive, microalgal sources have now been identified for many of the lipids that are widely distributed in Recent sediments (e.g. Boon *et al.*, 1979; Albaiges *et al.*, 1984; Volkman, 1986; ten Haven *et al.*, 1987; Volkman *et al.*, 1992, 1994; Conte *et al.*, 1994). Some of these compounds are only slowly degraded or are transformed to more stable chemical structures (e.g. Gagosian *et al.*, 1980; Kohnen *et al.*, 1990), and thus they can be used as biomarkers for assessing the sources of the organic matter in sediments. However, it is also apparent that some compounds are more widely distributed in the biosphere than previously thought and thus their value as specific fossil biomarkers has diminished. Sterols provide a good example of this; some sterols are found only in a few classes while others are now known to be quite widely distributed (Table 1; Volkman, 1986; Patterson, 1991). The identification of several distinctive types of aliphatic biopolymer in some classes of microalgae

*To whom correspondence should be addressed. Tel: +61-3-62325218; Fax: +61-3-62325123; E-mail: john.volkman@marine.csiro.au

†Present address: Shell International Exploration and Production BV, Research and Technical Services, P.O. Box 60, 2280 AB Rijswijk, The Netherlands.

Table 1. Major sterols in the different microalgal classes and cyanobacteria (sterols listed may not occur in all species)

| Algal class | Major sterols |
|---|---|
| Bacillariophyceae | $C_{28}\Delta^{5,22}$, $C_{28}\Delta^{5,24(28)}$, $C_{27}\Delta^{5}$, $C_{29}\Delta^{5}$, $C_{27}\Delta^{5,22}$ |
| Bangiophyceae | $C_{27}\Delta^{5}$, $C_{27}\Delta^{5,22}$, $C_{28}\Delta^{7,22}$ |
| Chlorophyceae | $C_{28}\Delta^{5}$, $C_{28}\Delta^{5,7,22}$, $C_{28}\Delta^{7,22}$ $C_{29}\Delta^{5,22}$, $C_{29}\Delta^{5}$ |
| Chrysophyceae | $C_{29}\Delta^{5,22}$, $C_{29}\Delta^{5}$, $C_{28}\Delta^{5,24(28)}$, $C_{30}\Delta^{5,24(28)}$ |
| Cryptophyceae | $C_{28}\Delta^{5,22}$ |
| Cyanobacteria | $C_{27}\Delta^{5}$, $C_{29}\Delta^{5}$ |
| Dinophyceae | 4Me, dinosterol, $C_{27}\Delta^{5}$, $C_{28}\Delta^{5,24(28)}$ |
| Euglenophyceae | $C_{28}\Delta^{5,7,22}$, $C_{29}\Delta^{5}$, $C_{28}\Delta^{7}$, $C_{29}\Delta^{5,7}$, $C_{28}\Delta^{7,22}$ |
| Eustigmatophyceae | $C_{27}\Delta^{5}$ or $C_{29}\Delta^{5}$ |
| Haptophyceae | $C_{28}\Delta^{5,22}$, $C_{27}\Delta^{5}$, $C_{29}\Delta^{5,22}$, $C_{29}\Delta^{5}$ |
| Prasinophyceae | $C_{28}\Delta^{5}$, $C_{28}\Delta^{5,24(28)}$, $C_{28}\Delta^{5}$ |
| Raphidophyceae | $C_{29}\Delta^{5}$, $C_{28}\Delta^{5,24(28)}$ |
| Rhodophyceae | $C_{27}\Delta^{5}$, $C_{27}\Delta^{5,22}$ |
| Xanthophyceae | $C_{29}\Delta^{5}$, $C_{27}\Delta^{5}$ |

References: Patterson (1991); Patterson and Van Valkenburg (1990); Jones *et al.* (1994); Volkman (1986) and references cited therein. The nomenclature is $C_x\Delta^y$ where $x$ is the total number of carbon atoms and $y$ indicates the positions of the double bonds. $C_{28}$ sterols have a methyl group at C-24, and $C_{29}$ sterols have a 24-ethyl substituent.

(Gelin *et al.*, 1998; de Leeuw and Largeau, 1993) provides a new type of biomarker and highlights the value of studies of the bound lipids and macromolecular organic matter in organisms and sediments. This paper provides a review of some of these new findings on microalgal lipid compositions and their relevance to organic geochemical studies.

## EXPERIMENTAL

### *Microalgal cultures*

Microalgae were obtained from the CSIRO culture collection of living microalgae (Jeffrey, 1980). *Rhizosolenia setigera* (CS-389/1) was isolated from water of the Huon Estuary, Tasmania by Dr A. Jackson. The freshwater eustigmatophyte *Vischeria punctata* CS-142 was originally obtained from the University of Texas culture collection in 1982 as UTEX 153. The dinoflagellate *Scrippsiella* sp. CS-295/c was isolated from ship ballast water.

Cultures were grown under a 12:12 h light–dark cycle, at 18.5°C under cool white fluorescent light at an irradiance of 80 $\mu Em^{-2}\,s^{-1}$ measured with a Biospherical Optics light meter. Cultures were not axenic, but had a very low bacterial content. The eustigmatophyte culture was grown in a 2 L Erlenmeyer flask containing 1.5 L of Bolds Basal (BB) culture medium (Nichols, 1973). *R. setigera* was cultured in fE/2-1, a CSIRO modification of culture medium f (Guillard and Ryther, 1962). *Scrippsiella* sp. was grown in 2 L Erlenmeyer flasks containing 1 L of GSe medium, which is a modification of the GP medium of Loeblich (1975), with selenium added as selenite at $10^{-8}$ M and salinity adjusted to 28 psu. The *V. punctata* culture was harvested after 15 d and the *R. setigera* culture after 6 days, all towards the end of logarithmic growth phase by filtering through 47 mm diameter glass fibre filters (Whatman GFC, nominal pore size 1.2 $\mu$m) and stored in liquid $N_2$ prior to analysis.

### *Lipid extraction and analysis*

Lipids were extracted using a modification of the method of Bligh and Dyer (1959) as described by Volkman *et al.* (1997). Non-saponifiable lipids were obtained by saponifying an aliquot of the total solvent extract in 5% KOH in methanol–water (80:20 v/v) under $N_2$ at 80°C for 2 h. The non-saponifiable lipids were extracted with hexane:chloroform (4:1 v/v) and then treated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) immediately before gas chromatographic (GC) analysis to convert compounds containing free hydroxyl groups to their trimethylsilyl–ether (TMS) derivatives. A further aliquot was treated with methanol/chloroform/HCl (10:1:1, v/v/v; 3 mL) for 2 h at 80°C to convert free and esterified fatty acids to methyl esters (FAME).

Samples were analyzed with a Varian High Temperature Series 5410 gas chromatograph with a septum-equipped programmable injector (SPI) using a non-polar methyl silicone fused-silica capillary column (HP1; 50 m × 0.32 mm i.d., 0.17 $\mu$m film thickness; Hewlett Packard). GC-MS analyses used a Fisons Instruments MD800 equipped with a Carlo Erba on-column injector. Electron impact mass spectra were acquired and processed with Fisons Masslab software on a PC. Typical mass spectrometer operating conditions were: transfer line, 310°C; electron impact energy, 70 eV; 0.8 scans $s^{-1}$; mass range, $m/z$ 40–650.

## RESULTS AND DISCUSSION

All species of microalgae contain sterols and fatty acids, and most contain small amounts of hydrocarbons. However, some species have been shown to contain unusual lipids (Table 2), and some of these may proved to be useful as biomarkers in organic geochemical studies. The following discussion examines many of these lipid classes in more detail, with an emphasis on identifying characteristics that might be restricted to a particular class of microalgae.

### *Fatty acids*

Fatty acids are abundant in most organisms, and thus they are often the most abundant lipid type in Recent sediments. Sources of fatty acids include bacteria, microalgae, higher plants and marine fauna; each of these has a distinctive fatty acid profile. However, some fatty acids such as palmitic and stearic acids (16:0 and 18:0 respectively) are ubiquitous. Bacteria are the major source of *iso*-, *anteiso*- and mid-chain branched fatty acids, but they can also be a significant source of palmitoleic (16:1*n*-7)

Table 2. Occurrence of unusual or distinctive lipids in microalgal classes, cyanobacteria and prochlorophytes (compounds mentioned may only be present in a few species)

| Algal class | Unusual lipids | Reference |
|---|---|---|
| Bacillariophyceae (diatoms) | $C_{25}$ and $C_{30}$ HBI alkenes, 34:1 alcohol (rare), 4-methylsterols (rare), 5α-stanols (minor); $C_{25}$ and $C_{27}$ alkenes; 16:4*n*-1 fatty acid | Belt *et al.* (1996); Volkman *et al.* (1993, 1994); Wraige *et al.* (1997); Schouten *et al.* (1998) |
| Chlorophyceae (green algae) | $\Delta^7$ sterols, chlorosulfolipids, algaenans, $C_{20}$–$C_{26}$ α-hydroxy fatty acids, 28:0 fatty acid, wax esters, $C_{25}$ and $C_{27}$ alkenes; *Botryococcus*: botryococcenes (~50), aldehydes, lycopadiene, $C_{27}$–$C_{31}$ alkadienes | Gelin *et al.* (1998); Gelpi *et al.* (1968); Mercer and Davies (1979); Metzger *et al.* (1991); Patterson (1991); Schouten *et al.* (1998) |
| Cryptophyceae | $C_{30}$ sterols | Rohmer *et al.* (1980) |
| Cyanophyceae (cyanobacteria) | Hopanoids, branched alkanes, $C_{19}$–$C_{29}$ alkenes, (ω-1)-hydroxy-26:0 fatty acid, chlorosulfolipids, various toxins | Abreu-Grobois *et al.* (1977); Gelpi *et al.* (1968); de Leeuw *et al.* (1992); Mercer and Davies (1979) |
| Dinophyceae (dinoflagellates) | 4-methyl sterols, steroid ketones, 5α-stanols, 18:5*n*-3 fatty acid, $C_{28}$ polyunsaturated fatty acids | Joseph (1975); Withers (1983, 1987); Mansour *et al.* (unpublished data) |
| Euglenophyceae | $\Delta^7$ sterols, chlorosulfolipids | Mercer and Davies (1979) |
| Eustigmatophyceae | $C_{28}$–$C_{34}$ alkyl diols and mid-chain hydroxy fatty acids, algaenans 22:0, 26:1 and 28:1 alcohols, $C_{26}$–$C_{30}$ α- and β-hydroxy fatty acids chlorosulfolipids, $C_{22}$ diol | Gelin *et al.* (1997a,b, 1998); Mercer and Davies (1979); Volkman *et al.* (1992) |
| Haptophyceae | $C_{37}$–$C_{39}$ alkenones, alkenes and alkenoates, $C_{31}$–$C_{33}$ alkenes, 18:5*n*-3 fatty acid; *Pavolovales*: pavlovols, 5α-stanols, 4-methyl sterols | Marlowe *et al.* (1984); Volkman *et al.* (1980a, 1990, 1995) |
| Prochlorophyta | Hop-22(29)-ene, no sterols, 16:2*n*-12 fatty acid | Volkman *et al.* (1988a) |
| Raphidophyceae | 18:5*n*-3 fatty acid, 24-ethylcholesterol and 24-ethylcholestanol; 4-methyl-$\Delta^8$-sterols | Beastall *et al.* (1974); Bell *et al.* (1997); Patterson and Van Valkenburg (1990) |
| Xanthophyceae | $C_{22}$ diol, chlorosulfolipids; 24-ethylcholesterol | Mercer and Davies (1979); Mercer *et al.* (1974) |

and *cis*-vaccenic acids (18:1*n*-7) (Volkman *et al.*, 1980b; Fulco, 1983).

Microalgae are a major source of fatty acids in most sedimentary environments. The contribution from different microalgal classes can often be discerned from characteristic differences between the distributions, especially if the positions of double bonds in polyunsaturated fatty acids are considered (Volkman and Johns, 1977). Some microalgae contain high concentrations of certain long-chain essential polyunsaturated fatty acids such as 20:5*n*-3 and 22:6*n*-3 (e.g. Volkman *et al.*, 1989). For example, marine eustigmatophytes such as *Nannochloropsis* spp. contain 20:5*n*-3 but little 22:6*n*-3, whereas haptophytes (prymnesiophytes) such as *Pavlova* spp. contain both 20:5*n*-3 and 22:6*n*-3. Chlorophytes rarely contain significant amounts of these fatty acids, but instead have a predominance of $C_{18}$ polyunsaturated fatty acids such as 18:2*n*-6 and 18:3*n*-3. Dinoflagellates have high levels of 20:5*n*-3 and 22:6*n*-3 and many contain the unusual fatty acid 18:5*n*-3. However, the latter is not unique to dinoflagellates and has been found in algae as diverse as haptophytes, raphidophytes and some prasinophytes (Volkman *et al.*, 1989; Bell *et al.*, 1997).

In most marine organisms, fatty acids occur predominantly as polar lipids, such as glyco- and phospholipids, although levels of triacylglycerols can be high in some zooplankton and in microalgae grown under nitrogen-deficient growth conditions. Free fatty acids are rarely abundant in living organisms (Berge *et al.*, 1995), but in sediments they can be the major form of fatty acids due to rapid chemical or enzymatic hydrolysis of polar lipids. In contemporary sediments, intact esterified lipids are usually associated with the indigenous animals, microalgae and bacteria. Indeed, fatty acid distributions in phospholipids have been used successfully to characterize bacterial populations (Guckert *et al.*, 1985).

The pathways by which unsaturated fatty acids are biosynthesized by microalgae and other organisms are now being reinvestigated and it appears highly likely, based on animal studies, that 22:6*n*-3 is formed by chain-shortening of 24:6*n*-3 (Voss *et al.*, 1991; Buzzi *et al.*, 1997). The high abundance of 18:5*n*-3 in some dinoflagellates, prasinophytes, haptophytes and raphidophytes (see Bell *et al.*, 1997 for references) may also be due to a similar chain-shortening mechanism from 20:5*n*-3 as originally proposed by Joseph (1975). Recently, we identified small amounts of highly unsaturated $C_{28}$ fatty acids containing 7 and 8 methylene-interrupted double bonds in several species of dinoflagellates (Mansour *et al.*, unpublished data, 1997). The 28:7*n*-6 fatty acid and other very long-chain polyunsaturated fatty acids had been found in fish oil (Rezanka, 1990), and these had probably been derived from the diet. The mode of biosynthesis of these very-long-chain fatty acids and unusual methyl and ethyl esters of 36:2 and 36:3 fatty acids in *Emiliania huxleyi* (Volkman *et al.*, 1980a) remains to be elucidated.

A common feature of the fatty acid distributions in sediments is the presence of $C_{20}$–$C_{30}$ saturated straight-chain fatty acids that show a strong predominance of even chain-lengths. In many sediments, particularly those from lacustrine environments, these are probably derived from the surface waxes of higher plants (e.g. Eglinton *et al.*, 1968).

However, an increasing body of analytical data suggests that microalgae (e.g. Volkman *et al.*, 1980b, 1989; Nichols *et al.*, 1986; Rezanka and Podojil, 1986; Dunstan *et al.*, 1992) and perhaps bacteria (e.g. Volkman *et al.*, 1988b) can also produce these fatty acids, albeit in small amounts (typically $<2\%$) relative to $C_{14}$–$C_{20}$ fatty acids. These long-chain fatty acids are minor constituents in the gas chromatogram of fatty acids from *Vischeria punctata* shown in Fig. 1. Recent data for *Scenedesmus communis* suggest that the $C_{28}$ fatty acid in this green alga may play a role in the formation of the aliphatic biopolymer in its cell wall (Schouten *et al.*, 1998), although this does not preclude other roles within the cell.

Long-chain saturated fatty acids appear to be more stable that the shorter-chain fatty acids and thus can persist in sediments. For example, Volkman *et al.* (1980b) estimated that diatoms contributed from 30 to 80% of $C_{24}$ to $C_{28}$ fatty acids in an intertidal sandy sediment based on the occurrence of these fatty acids in a mixed diatom culture. Middelburg *et al.* (1993) suggested that freshwater microalgae might be a source of long-chain fatty acids in sediments from Kau Bay due to the co-occurrence of a 34:1 $\omega$-hydroxy fatty acid that they indicated was characteristic of an algal origin. Proof of an algal origin for such fatty acids in contemporary marine sediments has yet to be obtained.

*Hydroxy fatty acids*

A wide range of hydroxylated fatty acids is found in sediments (e.g. Eglinton *et al.*, 1968; Cranwell, 1981; Kawamura and Ishiwatari, 1982; Cardoso and Eglinton, 1983; Volkman *et al.*, 1980b), but unfortunately these compounds have received little attention from organic geochemists. These compounds can be separated into different categories according to the number and position of the hydroxyl groups. Aliphatic $\alpha$- and $\beta$-monohydroxy fatty acids occur in a wide range of organisms (Downing, 1961) and are typically produced as intermediates in the $\alpha$- and $\beta$-oxidation of monocarboxylic fatty acids. $\beta$-Oxidation occurs more widely than $\alpha$-oxidation, although the latter is known in plants, animals and bacteria. $\alpha$-Hydroxy fatty acids are intermediates in the fatty acid biosynthesis in yeasts (Fulco, 1967). Long-chain $C_{10}$–$C_{24}$ $\alpha$- and $\beta$-monohydroxy acids were observed in a 5000 year-old lacustrine sediment from the English Lake district (Eglinton *et al.*, 1968), and were attributed to microbial oxidation of monocarboxylic fatty acids.

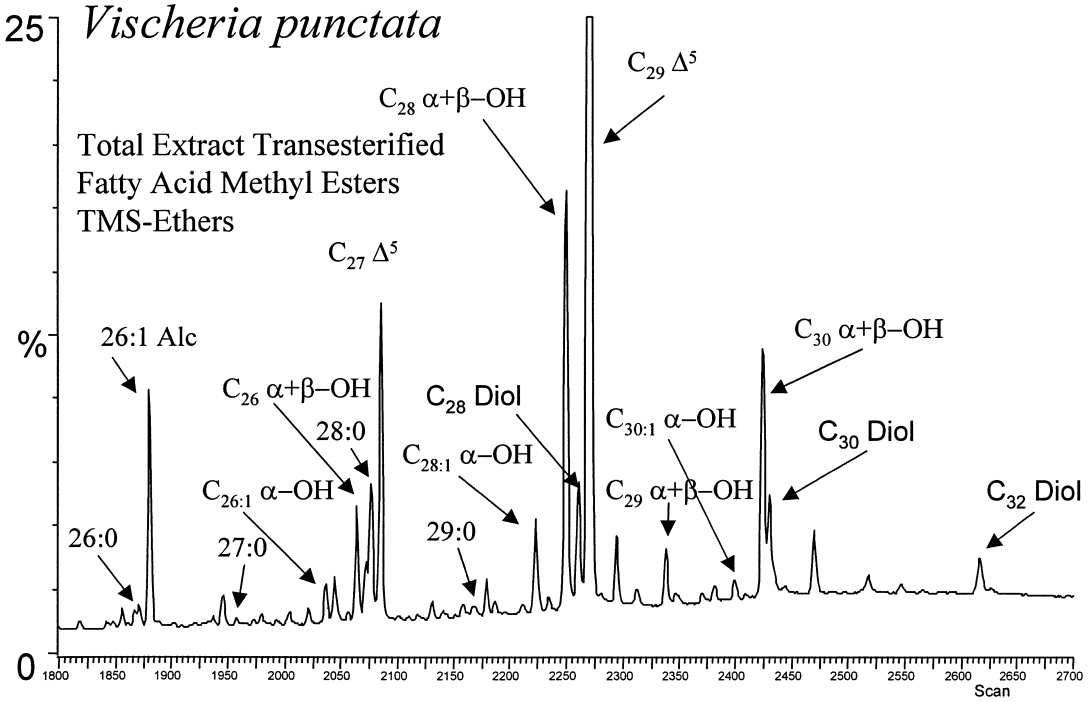


Fig. 1. Partial capillary total ion chromatogram from GC-MS analysis of the transesterified total extract of the freshwater eustigmatophyte *Vischeria punctata*. This procedure converts all fatty acids, whether free or esterified in complex lipids, to fatty acid methyl esters. Alcohols are designated *x*:*y* Alc where *x* is the chain length and *y* is the number of double bonds. The two sterols are cholesterol (labelled $C_{27}\Delta^5$) and 24-ethylcholesterol (labelled $C_{29}\Delta^5$; this peak is off-scale by a factor of 4). Long-chain alkyl diols are denoted by $C_x$ where *x* is the number of carbon atoms (the $C_{28}$ diol co-elutes with the 30:1 alcohol). $C_{26}$–$C_{30}$ long-chain $\alpha$- and $\beta$-hydroxy fatty acids are also indicated (odd-chain $\beta$-hydroxy acids were not detected). Minor amounts of long-chain *n*-alkanoic acids are designated by *x*:*y*.

Cranwell (1981) determined the stereochemistry of α- and β-hydroxy fatty acids in a lacustrine sediment from Loch Clair in Scotland. He deduced that free and bound α-hydroxy fatty acids with chain-lengths greater than $C_{20}$ were characteristic of the by-products formed during fatty acid metabolism by α-oxidation. In contrast, the shorter-chain $C_{14}$–$C_{18}$ bound α- and β-hydroxy fatty acids showed a predominance of the *R* configuration usually associated with microbial cell wall lipids. Indeed, bacterially-derived β-hydroxy fatty acids are found in most, if not all, Recent sediments (Kawamura and Ishiwatari, 1982; Cardoso and Eglinton, 1983). The carbon number range is typically from $C_{10}$ to $C_{20}$, often with 14:0 predominating, which is typical of the lipid distributions in the cell wall lipopolysaccharide of gram-negative bacteria (Klok *et al*., 1988; Skerratt *et al*., 1992). Bacteria also contribute significant amounts of *iso*- and *anteiso*-branched $C_{12}$–$C_{18}$ β-hydroxy fatty acids to sediments.

$C_{16}$–$C_{28}$ saturated α-hydroxy acids, with maxima at 16:0 and 24:0, were reported in intertidal sediments from Victoria, Australia (Volkman *et al*., 1980b). Similar distributions were also observed in the seagrass *Zostera muelleri* growing at the same site, which suggested that the predominant source in this sediment was seagrass detritus rather than bacteria. Higher plant cutin and suberin can also be a significant source of esterified $C_{16}$–$C_{22}$ α-, β-, and ω-monohydroxy and $C_{16}$ and $C_{18}$ polyhydroxylated fatty acids in sediments (Cardoso and Eglinton, 1983).

Our recent work suggests that some microalgae are also a potential source of monohydroxy fatty acids. $C_{30}$–$C_{34}$ mid-chain hydroxy fatty acids were identified in hydrolysed extracts from marine eustigmatophytes of the genus *Nannochloropsis* (Gelin *et al*., 1997a). The predominant positional isomer in each homologue contained a hydroxy group at the ω18 position suggesting that the series is produced by chain-shortening or elongation from a single major precursor. Two dihydroxy fatty acids identified as 15,16-dihydroxydotriacontanoic acid and 16,17-dihydroxytritriacontanoic acid were also found.

In addition, we recently detected a series of α- and β-hydroxy acids ranging from 26:0 to 30:0 (with traces of 18:0, but no intermediate chain-lengths) in the freshwater eustigmatophyte *Vischeria punctata*. Similar distributions occur in the eustigmatophytes *Vischeria helvetica* and *Eustigmatos vischeri* (Volkman *et al*., unpublished data, 1997). Figure 1 shows a partial chromatogram of the total extract of *V. punctata* after transesterification to form methyl esters of any free and esterified fatty acids, and treatment with BSTFA to convert hydroxyl groups to TMSi-ethers. The major peak is due to a mixture of $C_{28}$ α- and β-hydroxy fatty acids, with the latter more abundant. Smaller amounts of 26:0 and 30:0 β-hydroxy fatty acid homologues are also present. In contrast, the saturated α-hydroxy acids range from 24:0 to 30:0 and include odd carbon number components. Small amounts of a series of monounsaturated α-hydroxy acids ranging from 26:1 to 30:1 are also present. $C_{22}$ to $C_{26}$ saturated and monounsaturated α-hydroxy fatty acids have also been found as major lipid components of the cell wall of several marine chlorophytes (Gelin *et al*., 1997b). Since few studies of microalgal lipids have examined bound or esterified lipids, long-chain hydroxy fatty acids are likely to be more widely distributed in microalgae. Further studies of bound and esterified lipids in microalgae would clearly be valuable.

The source of (ω-1)-hydroxy long-chain fatty acids in sediments has long been the subject of conjecture (Boon *et al*., 1977; Fukushima *et al*., 1992). A possible bacterial source was identified by Skerratt *et al*. (1992) who found $C_{26}$, $C_{28}$ and $C_{30}$ (ω-1)-hydroxy fatty acids in five out of the 16 methane-utilizing strains of bacteria that they examined. Similar distributions of (ω-1)-hydroxy fatty acids were found in several lacustrine lake sediments by Fukushima *et al*. (1992), but they were apparently unaware of the Skerratt *et al*. (1992) work and attributed the likely source to higher plants. Although reports of such fatty acids in the biosphere are comparatively rare, a $C_{26}$ (ω-1) hydroxy acid has also been found in the two cyanobacteria, *Anabaena cylindrica* (Abreu-Grobois *et al*., 1977) and *Aphanizomenon flos-aquae* (de Leeuw *et al*., 1992). In *A. cylindrica* it occurs as the glycoside 25-hydroxyhexacosanoic acid (1-α-D-glucopyranose) ester. A $C_{16}$ (ω-1) hydroxy fatty acid has been found in some mosses and liverworts (Caldicott and Eglinton, 1976). However, sediment data suggest additional sources. In the Lake Clarkia deposit it appears from isotope data that these compounds are derived from photosynthetic aquatic organisms, even though ω-hydroxy acids in the same lacustrine sediment were derived from higher plants (Huang *et al*., 1996).

### *Long-chain alcohols*

There are few reports of long-chain alcohols in microalgae and it appears that microalgae are not a major source of these lipids in most sediments. A $C_{34}$ *n*-alkenol with four double bonds was identified in a diatom from the genus *Navicula* (Volkman *et al*., 1993) and an 18:1 fatty alcohol has been found in the diatom *Skeletonema costatum* (Berge *et al*., 1995). A series of $C_{22}$–$C_{28}$ saturated *n*-alcohols, with even carbon numbers predominating, and a maximum at $C_{26}$ and $C_{28}$, has been identified in the heterocyst glycolipids of the cyanobacterium *Anabaena cylindrica* (Abreu-Grobois *et al*., 1977). The green alga *Chlorella kessleri* contains $C_{10}$–$C_{20}$ saturated and mono-unsaturated fatty alcohols,

with 16:0 most abundant, esterified to long-chain fatty acids (Rezanka and Podojil, 1986; Rezanka *et al.*, 1986). $C_{30}$–$C_{32}$ alcohols having one or two double bonds are significant constituents of the lipids of marine eustigmatophytes of the genus *Nannochloropsis* (Volkman *et al.*, 1992). The freshwater eustigmatophytes *V. punctata* contains saturated and monounsaturated *n*-alkanols from $C_{16}$ to $C_{28}$ showing a strong predominance of 22:0 and 26:1, respectively (Fig. 2). Similar distributions were found in *V. helvetica* and *E. vischeri* (Volkman *et al.*, unpublished data, 1997). Note that in both series of alcohols there is not a steady decline in abundances with increasing chain length, but rather a strong predominance of just a few homologues.

Morris and Brassell (1988) reported that the major alcohol in a phytoplankton sample from the Baltic Sea was 22:0, although details of the distribution were not reported. The major species in this sample was the cyanobacterium *Aphanizomenon flos-aquae*, but de Leeuw *et al.* (1992) did not find significant contents of 22:0 in this species when grown in laboratory culture. Several authors have reported high contents of the 22:0 alcohol in sediments where an algal origin is plausible. For example, the major alcohol in a sample of the lacustrine Green River Shale of Eocene age is also 22:0 which comprises over 50% of the alcohols present (Sever and Parker, 1969). We have also found that 22:0 was the major *n*-alkanol in sediments from Ace Lake, Antarctica (unpublished data), and it is also the major alcohol in sediments from the Indus Fan in the northeast Arabian Sea (ten Haven and Rullkötter, 1991).

### *Long-chain alkenones, alkenonates and alkenes*

Long-chain unsaturated ketones (alkenones) have been found in several species of haptophytes (Volkman *et al.*, 1980a; Marlowe *et al.*, 1984; Conte *et al.*, 1994) including the widely distributed coccolithophorids *Emiliania huxleyi* and *Gephyrocapsa oceanica*. The biosynthesis and function of these compounds is still not known, but in both species, the ratio of tri- to di-unsaturated $C_{37}$ alkenones increases with decreasing growth temperature (Brassell *et al.*, 1986; Conte *et al.*, 1994; Volkman *et al.*, 1995). This observation forms the basis for the use of the $U^{K'}_{37}$ parameter to estimate palaeo surface seawater temperatures from the ratio of the di- and tri-unsaturated alkenones in marine sediments. Attempts to use the ratios of alkenones in some lacustrine sediments have been less successful (e.g. Li *et al.*, 1996) and it seems likely from the anomalous compositional data reported from some aquatic environments that environmental factors in

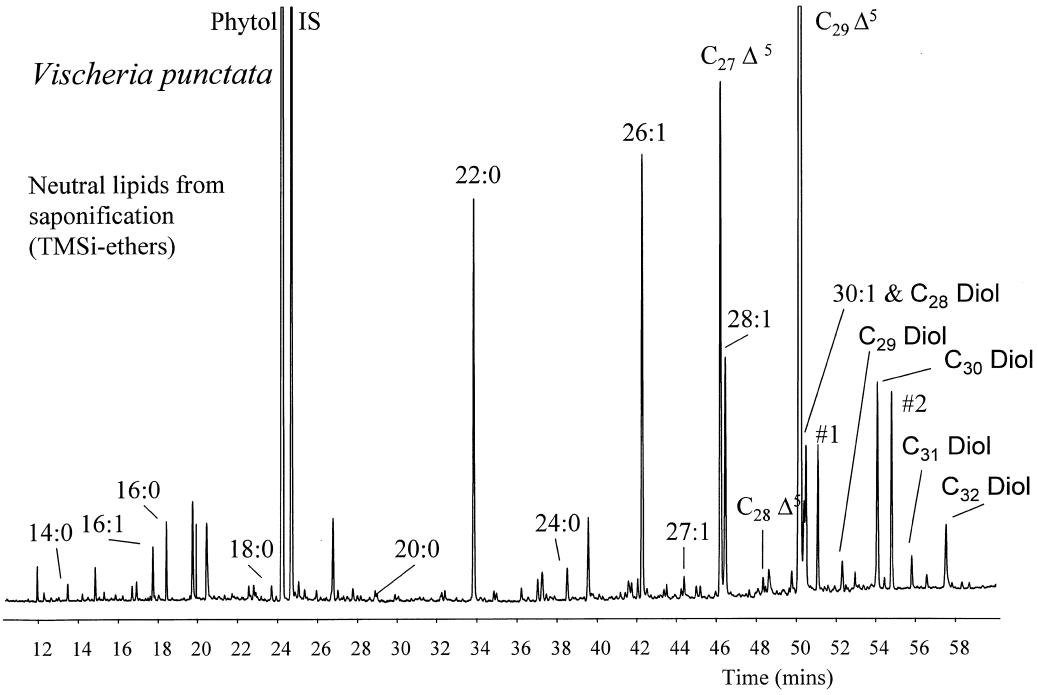


Fig. 2. Capillary gas chromatogram of the neutral lipids from saponification of the lipid extract of the freshwater eustigmatophyte *Vischeria punctata*. Alcohols are designated *x*:*y* where *x* is the chain length and *y* is the number of double bonds. The three sterols are cholesterol (labelled $C_{27}\Delta^5$), 24-methylcholesterol (labelled $C_{28}\Delta^5$) and 24-ethylcholesterol (labelled $C_{29}\Delta^5$; this peak is off-scale by a factor of 4). Long-chain alkyl diols are denoted by $C_x$ where *x* is the number of carbon atoms. The $C_{28}$ diol coelutes with the 30:1 alcohol, but the elution order relative to sterols differs from that shown in Fig. 1.

addition to temperature might also influence the proportions of these compounds.

Our studies of the cold waters of the Southern Ocean have revealed that $U^{K'}_{37}$ remains little changed at temperatures below about 5°C (Sikes and Volkman, 1993). Thus, we have investigated the use of other biomarkers, such as long-chain unsaturated alkenes, in an attempt to derive alternative palaeotemperature parameters for this low temperature regime. These alkenes increase in relative abundance relative to alkenones with decreased water temperature and the proportion of the more unsaturated $C_{37}$ alkene also increases. Unfortunately, these alkenes are rarely abundant in sediments and so their usefulness as a palaeotemperature proxy in ancient sediments remains to be established (Sikes *et al.*, 1997).

*Hydrocarbons including highly branched isoprenoid (HBI) alkenes*

Biogenic alkanes and alkenes are a common feature of the hydrocarbon distributions in sediments. Many microalgae contain the highly unsaturated alkene *n*-$C_{21:6}$ (*n*-heneicosa-3,6,9,12,15,18-hexaene) formed by decarboxylation of the 22:6*n*-3 fatty acid (Lee and Loeblich, 1971). A few species also contain the *n*-$C_{21:5}$ alkene *n*-heneicosa-3,6,9,12,15-pentaene (Volkman *et al.*, 1994). These alkenes are only rarely found in sediments, presumably because they are rapidly degraded. If present in sediments, one would suspect that intact algal cells were the source. There are several reports of shorter-chain *n*-$C_{15}$, *n*-$C_{17}$ and *n*-$C_{19}$ alkanes and monounsaturated alkenes in microalgae (e.g. Weete, 1976), and these too are likely formed by decarboxylation of the saturated fatty acids. The $C_{17}$ *n*-alkane is common in contemporary sediments.

An interesting feature of the hydrocarbons in some Recent sediments is the occurrence of a distribution of long-chain *n*-alkanes that shows little or no odd carbon number predominance. In many instances this is due to contamination with petroleum products based on the presence of an unresolved complex mixture (UCM) and typical sterane and hopane biomarker distributions (e.g. Albaiges and Albrecht, 1979). In a few cases it might be due to laboratory contamination. However, there are now several examples which suggest the possibility of a natural source. For example, Nichols *et al.* (1988) found *n*-$C_{21}$–$C_{36}$ alkanes in a diatom sea-ice sample together with *n*-$C_{21:6}$ and a diunsaturated $C_{25}$ HBI alkene, both of which were attributed to diatoms. Similar distributions of alkanes have been found in diatomaceous sediments (Volkman *et al.*, 1983) and a mixed diatom culture (Volkman *et al.*, 1980b). Recently Bieger *et al.* (1997) found long-chain *n*-alkane distributions having no odd over even carbon number predominance in spring bloom and sediment samples from Conception Bay, Newfoundland. The isotope signature of the alkanes changed over the course of the bloom suggestive of a biogenic source rather than an origin from petroleum products. In contrast, Eglinton *et al.* (1997) attributed *n*-alkane distributions with no odd carbon number predominance in the Black Sea to a petrogenic source based on their $^{14}C$ age determined by carbon-specific radiocarbon analysis. In this instance, no UCM was observed. Although this powerful technique is not yet widely available, it promises to provide a valuable new tool to distinguish between modern and fossil organic matter in aquatic environments.

Data on hydrocarbons in axenic cultures of microalgae are unfortunately rather rare, so it is not clear whether such compounds might also have a bacterial or microalgal origin. Available evidence suggests that microalgae including diatoms are at least one source (Volkman *et al.*, 1980b, 1983). Robinson *et al.* (1987) reported distributions of *n*-alkanes lacking an odd carbon number predominance in some freshwater dinoflagellates. Bacteria may be another source based on the occurrence of *n*-alkanes showing no odd carbon number predominance in the $< 53\ \mu$m fraction of particulate matter from anoxic waters in the Black Sea (Wakeham *et al.*, 1991). Long-chain $C_{24}$–$C_{35}$ *n*-alkanes showing no predominance of odd carbon numbers have been found in the sulfate-reducing bacterium *Desulfovibrio desulfuricans* (Davis, 1968), although the chromatogram shown had a significant UCM and no information on blanks was provided. Similar distributions have been found in aquatic macrophytes (Nishimoto, 1974) and in the internal lipids of some plants (Kaneda, 1969).

Several microalgae contain very-long-chain alkenes (Gelpi *et al.*, 1968, 1970). These include, *inter alia*, diunsaturated $C_{31}$, tri- and tetraunsaturated $C_{33}$ alkenes, and di- and tri-unsaturated $C_{37}$ and $C_{38}$ (probably with *trans*-double bonds) in the haptophyte *Emiliania huxleyi* (Volkman *et al.*, 1980a), a $C_{31}$ diunsaturated alkene in the haptophyte *Isochrysis galbana* (Volkman *et al.*, 1981), $C_{23}$–$C_{33}$ odd-chain diunsaturated alkenes in the chlorophyte *Botryococcus braunii* (Gelpi *et al.*, 1968), $C_{25}$ and $C_{27}$ monounsaturated alkenes in the chlorophyte *Chlorella emersonii* (Afi *et al.*, 1996) and $C_{19}$–$C_{29}$ alkenes in the cyanobacterium *Anacystis montana* (Gelpi *et al.*, 1968).

Saturated and polyunsaturated $C_{14}$–$C_{31}$ hydrocarbons with a strong predominance of odd carbon numbers have recently been isolated from two marine *Nannochloropsis* species (Gelin *et al.*, 1997b). The polyunsaturated compounds are thought to be biosynthetically linked to the long-chain diols and the aliphatic biopolymer also produced by these species. Polyunsaturated $C_{25}$ and $C_{27}$ alkenes also occur in a strain of *Rhizosolenia setigera* analyzed by Schouten *et al.* (1998). These alkenes have not

been found in other species of *Nannochloropsis* or *Rhizosolenia* (Fig. 3), suggesting either that genetic differences between strains or environmental conditions control their abundance.

Despite these occurrences, surprisingly few studies report algal-derived long-chain *n*-alkenes in sediments, although $C_{37}$–$C_{39}$ alkenes derived from *Emiliania huxleyi* are abundant in some Black Sea sediments (Wakeham *et al.*, 1991; Eglinton *et al.*, 1997). In other cases where long-chain alkenes have been found a higher plant origin has been proposed (e.g. Albaiges *et al.*, 1984).

Perhaps the best studied source of branched and straight-chain alkenes is the freshwater green alga *Botryococcus braunii* (Metzger *et al.*, 1991). This alga exists as three or more distinct races, each of which has a distinctive distribution of hydrocarbons. The A race is characterised by $C_{27}$–$C_{31}$ alkadienes and minor amounts of $C_{29}$ and $C_{31}$ alkatrienes. These are now known to be biosynthesized from oleic acid (18:1*n*-9) and together with very long-chain fatty acyl derivatives act as precursors to the resistant biopolymer in these species through metabolism of the alkadienes into epoxides (Templier *et al.*, 1993). The B and L races are characterized by distributions of isoprenoid-derived botryococcenes and lycopadiene, respectively (Metzger *et al.*, 1991).

Highly branched unsaturated $C_{20}$, $C_{25}$ and $C_{30}$ alkenes, however, are observed in most marine sediments (reviewed by Rowland and Robson, 1990). They also occur as sulphurized derivatives due to the early incorporation of sulfur into these highly unsaturated compounds (Kohnen *et al.*, 1990). Recently, we reported the occurrence of $C_{25}$ alkenes with 3, 4, and 5 double bonds in the diatom *Haslea ostrearia* and $C_{30}$ alkenes with 4, 5, and 6 double bonds in the diatom *Rhizosolenia setigera* (Volkman *et al.*, 1994). The full structures of the $C_{25}$ compounds have now been elucidated using a combination of NMR spectroscopy, epoxide derivatization and mass spectrometry (Rowland *et al.*, 1995; Belt *et al.*, 1996; Wraige *et al.*, 1997). These researchers have also raised the possibility that the positions of some double bonds may rearrange when the alkenes are deposited in sediments. They also found that the abundances of individual compounds varied with culturing conditions.

Figure 3 shows a capillary gas chromatogram of the neutral constituents after saponification of an extract from a strain of *Rhizosolenia setigera* isolated from temperate Tasmanian estuarine waters. This strain synthesizes a distribution of $C_{30}$ HBI alkenes similar to that previously published for another strain of this species (Volkman *et al.*, 1994), but with two important differences. The abundance of the last eluting 30:5 alkene (labeled R3 in Volkman *et al.*, 1992; their Fig. 2) is much reduced in the new strain. Also, the HBI alkenes are more abundant, as a group, relative to the *n*-$C_{21:6}$ alkene. The alkenes are also more abundant than phytol derived from saponification of chlorophyll *a* (Fig. 3). These results clearly indicate that

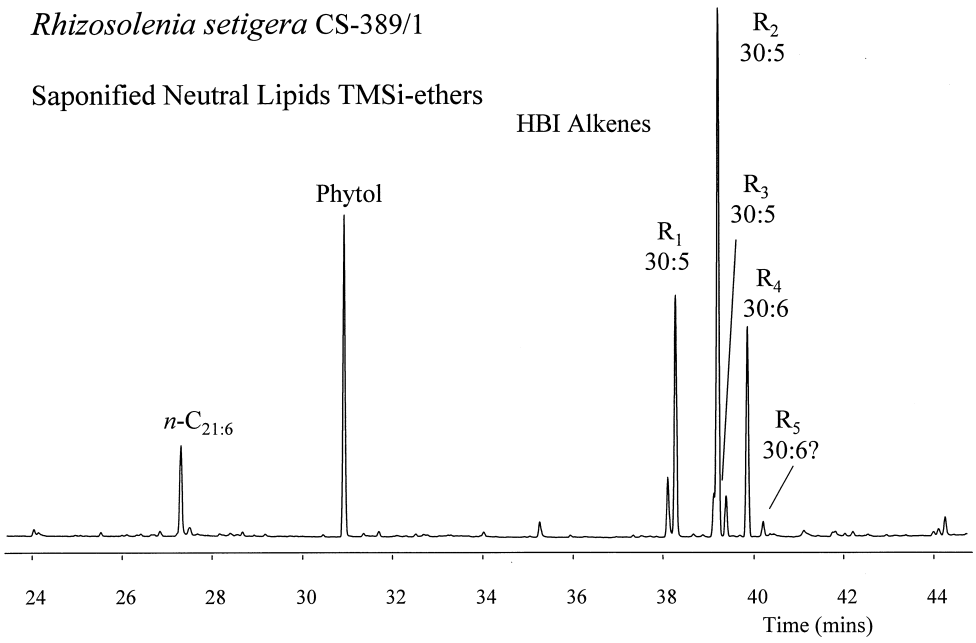


Fig. 3. Partial capillary gas chromatogram of the neutral lipids obtained from saponification of the lipid extract from a culture of *Rhizosolenia setigera* (CSIRO strain CS-389/1) isolated from the Huon Estuary, near Hobart, Tasmania, Australia. Note the high abundance of $C_{30}$ highly branched isoprenoid (HBI) alkenes labelled R1–R5 as in Volkman *et al.* (1994).

distributional variations occur between strains of the same species, in addition to effects due to changes in environmental conditions. A more dramatic difference has been found by the NIOZ group who found $C_{25}$ and not $C_{30}$ HBI alkenes in yet another isolate of a *Rhizosolenia* species (Schouten *et al.*, 1998). This suggests further genetic variability, although it should be pointed out that the taxonomy of this genus is debated and that some species may need to be reassigned (Hallegraeff, personal communication, 1996). Such differences between strains (or species) might account for the occurrence of $C_{25}$ and lack of $C_{30}$ HBI alkenes in waters and sediments from Conception Bay (Bieger *et al.*, 1997). An alga reported to be *Rhizosolenia setigera* was abundant in the plankton at that site (Bieger *et al.*, 1997), and it seems to us that it was the likely source of the $C_{25}$ HBI alkenes.

While it seems clear that diatoms are a major source of HBI alkenes in sediments, much still needs to be known about the function and bioactivity of these unusual hydrocarbons in these algae as well as the effects of environmental conditions on their composition and abundance. No algal source has been identified for the $C_{20}$ HBI alkane (Yon *et al.*, 1982) and its corresponding monounsaturated alkene apart from their occurrence in a field sample of the green macroalga *Enteromorpha prolifera* (Rowland *et al.*, 1985). This occurrence might be due to the presence of epiphytes. Diatoms have been proposed as the source for unusual $C_{26}$ HBI alkenes (Summons *et al.*, 1993), $C_{26}$ thiophenes, and HBI alkane (Rospondek *et al.*, 1997), novel $C_{25}$ HBI-derived thiophenes (Sinninghe Damsté and Rijpstra, 1993) and $C_{35}$ highly branched isoprenoid alkene with 7 double bonds (Hoefs *et al.*, 1995) in some sediments. The origins of these compounds have yet to be confirmed by algal culture studies.

*Sterols including 5α(H)-stanols and 4-methyl sterols*

A great diversity of sterols are found in microalgae (Patterson, 1991). These distributions range from the 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 mixtures of 10 or more 4-desmethyl and 4-methylsterols in some species of dinoflagellates (for an example see Fig. 4). Some sterols are widely distributed, but others are useful chemotaxonomic markers. Barrett *et al.* (1995) carried out a detailed analysis of the sterols in 14 species of marine diatoms and found, contrary to expectations, a range of distributions. The $C_{28}$ sterol 24-methylcholesta-5,22E-dien-3β-ol (diatomsterol) was only found as a minor constituent in three species and yet this sterol is sometimes incorrectly stated to be a unique marker for diatoms. While it is certainly abundant in some species it is also found in

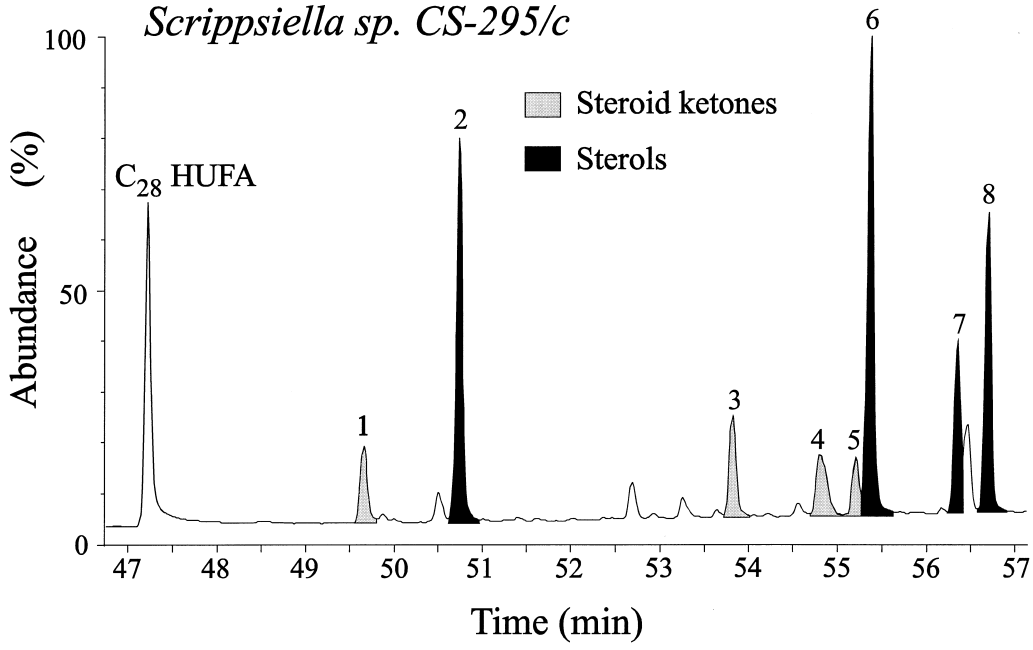


Fig. 4. Partial capillary gas chromatogram showing the distribution of 4-desmethyl sterols, 4-methyl sterols, 5α(H)-stanols (all as TMSi-ethers) and 4-methylstanones from transesterification of the total extract of the dinoflagellate *Scrippsiella* sp. CS-295/c. For each of the major 4-methyl sterols there is a corresponding steroidal ketone. The peak labelled $C_{28}$ HUFA (highly unsaturated fatty acid) is the $C_{28}$ highly unsaturated fatty acid 28:7*n*-6. Other peak identifications are: 1: 5α-cholestan-3-one, 2: 5α-cholestanol, 3: dinosterone, 4: 4α,23,24-trimethyl-5α-cholest-8(14)-en-3-one, 5: dinostanone, 6: dinosterol, 7: 4α,23,24-trimethyl-5α-cholest-8(14)-en-3-ol, 8: dinostanol.

other microalgae, such as the haptophytes and cryptophytes (Goad *et al.*, 1983; Volkman, 1986). A better marker might be 24-methylcholesta-5,24(28)-dien-3$\beta$-ol since this is abundant in some centric diatoms such as *Thalassiosira* and *Skeletonema*. Unfortunately, it is also found in a few dinoflagellates and prasinophytes. Several of the diatoms had a high content of the $C_{27}$ sterol cholesta-5,22E-dien-3$\beta$-ol, two had abundant 24-ethylcholesta-5,22E-dien-3$\beta$-ol, one had cholesterol as the major sterol and in another cholesta-5,24-dien-3$\beta$-ol was the only sterol detected (Barrett *et al.*, 1995). Given the diversity of sterols present in diatoms and the importance of diatoms as a source of organic matter in marine systems, it is not surprising that a diversity of sterol distributions occurs in marine sediments.

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-3$\beta$-ol (e.g. Barrett *et al.*, 1995), both of which are more commonly associated with higher plants (Volkman, 1986). For example, a high predominance of 24-ethylcholesterol is found in the diatoms *Asterionella glacialis* (Volkman, 1986), *Haslea ostrearia* and *Amphiprora hyalina* (Barrett *et al.*, 1995), and the raphidophyte *Olisthodiscus luteus* (Patterson and Van Valkenburg, 1990). Many cyanobacteria also contain high proportions of 24-ethylcholesterol compared with other sterols, although concentrations per cell are low (Paoletti *et al.*, 1976; Volkman, 1986). $C_{29}$ sterols having a C-24 ethyl substituent and $\Delta^7$ double bond are also abundant in many green microalgae (e.g. Rezanka *et al.*, 1986; Patterson, 1991).

We recently found that the distribution of sterols in three species of freshwater eustigmatophytes is remarkably simple and consists predominantly of 24-ethylcholesterol with a small amount of cholesterol, 24-methylcholesterol and isofucosterol (Fig. 2). In addition, the freshwater xanthophytes *Botrydium granulatum* and *Tribonema aequale* and the freshwater eustigmatophyte *Monodus subterraneus* also contain simple distributions of sterols consisting of 24-ethylcholesterol (66–86% of total sterols) and cholesterol (Mercer *et al.*, 1974). This contrasts with the sterol distributions of marine eustigmatophytes of the genus *Nannochloropsis* which consist mainly of cholesterol (Volkman *et al.*, 1992).

Sterols derived from dinoflagellates are often major constituents of the sterol distributions in sediments, although this does not always mean that dinoflagellates are major constituents of the phytoplankton in that environment. For example, dinosterol is the major sterol in the Unit 1 section of the Black Sea (de Leeuw *et al.*, 1983), even though this is a coccolith ooze. The sterol composition of dinoflagellates is dominated by 4$\alpha$-methyl sterols, including dinosterol (4$\alpha$,23,24-trimethyl-5$\alpha$-cholest-22E-en-3$\beta$-ol) which is found in many dinoflagellate species (e.g. Withers, 1983; Pirretti *et al.*, 1997) and hence has been used as an indicator of dinoflagellate contribution to marine sediments (e.g. Boon *et al.*, 1979; Robinson *et al.*, 1984). However, not all dinoflagellates have high contents of 4-methyl sterols and some species lack dinosterol. For example, *Amphidinium* spp. synthesize amphisterol (4$\alpha$,24-dimethyl-5$\alpha$-cholesta-8(14),24(28)-dien-3$\beta$-ol) as their major sterol (Withers *et al.*, 1979), whereas several *Gymnodinium* species have 4$\alpha$,24-dimethylcholestanol as a major sterol and contain little dinosterol (Withers, 1987). A freshwater dinoflagellate, *Ceratium furcoides*, contains no 4-methyl sterols at all (Robinson *et al.*, 1987).

Sterols with a fully saturated ring system (5$\alpha$(H)-stanols) often occur in dinoflagellates (e.g. Figure 4), but are not common in other marine microalgae with a few notable exceptions (Nishimura and Koyama, 1977; Volkman *et al.*, 1990). Hence, dinoflagellates are often the major direct source of 5$\alpha$(H)-stanols in marine sediments (Robinson *et al.*, 1984), in addition to those formed by bacterial reduction of stenols and 5$\beta$(H)-stanols derived from faecal matter (Nishimura, 1982). Diatoms can also be a minor source of 5$\alpha$(H)-stanols (Barrett *et al.*, 1995), and the raphidophyte *Olisthodiscus luteus* contains a high content (30%) of 24-ethylcholestanol (Patterson and Van Valkenburg, 1990). The presence of 5$\beta$(H)-sterols in sediments is often taken as evidence for the presence of faecal-derived organic matter, since the 5$\beta$(H)-stanol coprostanol constitutes approximately 60% of the total sterols in human faeces. Recently, Leeming *et al.* (1996) extended this concept and showed that faecal matter from a variety of animals could be distinguished on the basis of $C_{27}$ and $C_{29}$ stanol ratios. As might be expected the principal faecal biomarker of herbivores was 24-ethylcoprostanol with smaller amounts of coprostanol. Mixtures of 5$\alpha$- and 5$\beta$-stanols can, however, be formed in highly or permanently anoxic sediments (e.g. Nishimura, 1982; Volkman, 1986), and some marine mammals are also a natural source of coprostanol (Venkatesan and Santiago, 1989).

### *Steroidal ketones*

Saturated 4-methyl steroidal ketones and their 4-desmethyl counterparts have been reported in several Recent marine sediments (Gagosian and Smith, 1979). They have been proposed as intermediates in the microbiological or chemically mediated degradation in sediments of stenols to sterenes (Gagosian and Smith, 1979). However a direct origin from the lipids of dinoflagellates (Robinson *et al.*, 1984) must also be considered since these compounds have been reported in a several species (Withers *et al.*, 1978; Kokke *et al.*, 1982; Robinson *et al.*, 1987; Harvey *et al.*, 1988). The presence of 3-keto steroids

in sediment traps and surface sediments (Gagosian *et al*., 1980, Smith *et al*., 1983) supports the idea of a direct origin from phytoplankton (Harvey *et al*., 1988). These compounds are not commonly reported in other microalgae and they may be absent from many dinoflagellates as well (e.g. Robinson *et al*., 1987). For example, Harvey *et al*. (1988) identified 21 steroidal ketones in *Scrippsiella trochoidea*. We found three of the same major steroidal ketones (dinosterone, dinostanone and 4α,23,24-trimethyl-5α-cholest-8(14)-en-3-one) in another *Scrippsiella* sp. (Fig. 4), but steroidal ketones were not present in four other species (Mansour *et al*., unpublished data, 1997). Note that in this example, there is a steroidal ketone corresponding to each of the major 4-methyl sterols although there are differences in the relative abundance of components within each series of compounds.

*Steroidal diols*

In previous work (Volkman *et al*., 1990) we established that species from the genus *Pavlova* (Haptophyta) contain novel 3,4-dihydroxy-4α-methylsterols now termed pavlovols. The co-occurrence of 4α-methyl sterols and 5α-stanols in these microalgae confirmed that such sterols are not unique to dinoflagellates. Our recent identification of dinosterol (or its epimer) and other 4-methyl sterols in a diatom (Volkman *et al*., 1993) suggests a wider distribution for 4α-methyl sterols in microalgae. Recently, we have confirmed the occurrence of pavlovols in several other closely-related species including *Pavlova pinguis* and *Diacronema vlkianum*. This is the first identification of these unusual compounds in a species not in the genus *Pavlova*, which suggests that they might be more general chemotaxonomic markers for species from the order Pavlovales (Volkman *et al*., 1997).

*Long-chain alkyl diols*

Long-chain saturated $C_{30}$–$C_{32}$ alkyl diols occur in most marine sediments (reviewed by Versteegh *et al*., 1998) and in a few instances, such as in Black Sea Unit 1 sediments, they can be the major lipids (de Leeuw *et al*., 1981). The occurrence of these compounds in a natural phytoplankton bloom suggested that cyanobacteria might be the source of these compounds (Morris and Brassell, 1988), but this was challenged by de Leeuw *et al*. (1992) who were unable to find alkyl diols in the cyanobacterium *Aphanizomenon flos-aquae*. A microalgal source for these compounds was discovered when Volkman *et al*. (1992) identified $C_{30}$–$C_{32}$ alcohols and diols in marine eustigmatophytes from the genus *Nannochloropsis* although the distribution of homologues found in *Nannochloropsis* differs significantly from those in most sediments (Volkman *et al*., 1992; Versteegh *et al*., 1998). At the time of their discovery, the function of such compounds was not known, but work by Gelin (1996) and Gelin *et al*. (1996) has suggested that these diols are building blocks for novel highly aliphatic biopolymers produced by these microalgae. More recent work has also indicated the likely involvement of long-chain mid-chain hydroxy acids in the formation of these biopolymers (Gelin *et al*., 1997a).

Most studies of mid-chain-hydroxy alkyl diols in sediments report the presence of $C_{30}$ and $C_{32}$ saturated constituents having a predominance of 1,15-isomers (reviewed by Versteegh *et al*., 1998). Relatively few studies report high contents of $C_{28}$ alkyl diols (e.g. ten Haven and Rullkötter, 1991), and in several cases these show a strong predominance of the 1,14-isomer suggesting that these are derived from another unidentified algal source (Versteegh *et al*., 1998).

Although most reports of alkyl diols in sediments refer to marine environments, only a few describe their occurrence in lacustrine sediments (e.g. Cranwell *et al*., 1987). This may not be a true indication of the occurrence of alkyl diols in lacustrine systems, but rather may reflect the limited number of studies in which these compounds have been investigated. Of interest is our recent finding that three freshwater eustigmatophytes (*Vischeria punctata*, *Vischeria helvetica* and *Eustigmatos vischeri*) also contain $C_{28}$–$C_{32}$ saturated alkyl diols. In these species, the major constituent was $C_{30}$ closely followed in abundance by the $C_{28}$ alkyl diol with smaller amounts of $C_{32}$, $C_{31}$ and $C_{29}$ alkyl diols (Fig. 2). Unlike the case with marine eustigmatophytes, unsaturated alkyl diols were not detected. The positional isomers are dominated by the 1,15-dihydroxy isomer, although other isomers are present. In these species, the alkyl diols are much less abundant than the major sterol, 24-ethylcholesterol (Figs 1 and 2).

Structurally similar mid-chain alkyl diols, with 22 and 24 carbon atoms, have been found in a number of freshwater algal species (e.g. Haines, 1973; Mercer and Davies, 1979). In all but one case, these were chlorinated and non-chlorinated derivatives of a long-chain $C_{22}$ 1,14-diol present as a disulfate. The exception was *Ochromonas malhamensis* where the parent compound was identified as the $C_{24}$ 1,15-diol disulfate (Mercer and Davies, 1979). These compounds have not been reported in lacustrine sediments, but it may be that they have been overlooked because they partition into the aqueous phase when conventional solvent extraction methods based on chloroform-(or dichloromethane)–methanol–water mixtures are used. Although alkyl diols having chain-lengths greater than $C_{24}$ have yet to be reported in species outside of the Eustigmatophyceae, there is a strong possibility that other algal classes might contain such compounds.

*Algaenans*

A major advance over the past decade is the recognition that some species of microalgae contain highly aliphatic, resistant biomacromolecules now termed algaenans (Philp and Calvin, 1976; Derenne *et al.*, 1992; de Leeuw and Largeau, 1993; Afi *et al.*, 1996; Gelin, 1996; Gelin *et al.*, 1998). These are much more resistant to bacterial and chemical degradation than free lipids or macromolecular components derived from proteins and polysaccharides, and hence they can survive in sediments. These studies have lead to the discovery of another pathway for the preservation of organic matter in sediments, which has been called the selective preservation pathway (Tegelaar *et al.*, 1989). According to this theory, sedimentary organic matter becomes enriched in algaenans which ultimately serve as a major source of the hydrocarbon constituents in marine crude oils. Furthermore, algaenans may play an important role in carbon cycling in marine environments ultimately acting as a sink for organic carbon.

The distribution of algaenans in microalgae appears to be quite limited, and several structural types have been identified. Algaenans have been reported in several freshwater and marine green algae (e.g. Derenne *et al.*, 1992; Gelin *et al.*, 1997b) and marine eustigmatophytes (Gelin *et al.*, 1996). The presence of long-chain alkyl diols in some freshwater eustigmatophytes (Fig. 2) suggests that algaenans similar to those found in marine eustigmatophytes (Gelin *et al.*, 1996) are also likely to be present in these freshwater species. A recent survey of marine microalgae, however, failed to find algaenans in several diatoms, dinoflagellates, two haptophytes and a prasinophyte although evidence for its possible occurrence in a dinoflagellate was obtained (Gelin *et al.*, 1998). Since algaenans persist in sediments, their occurrence in microalgae has important consequences for the types and compositions of organic matter preserved in different aquatic environments. It seems likely that the preserved organic matter will represent only a proportion of the diversity of organic matter contributed to the sediment.

*A caveat on comparisons of microalgal lipid compositions*

Environmental conditions can lead to significant changes in the lipid compositions of microalgae (e.g. Shifrin and Chisholm, 1981) and this can complicate comparisons with sedimentary compositions. Sterol distributions are usually fairly robust and distributional variations with changes in environmental conditions are usually small (e.g. Hallegraeff *et al.*, 1991), although significant variations have been documented in a few species. For example, Pirretti *et al.* (1997) investigated the 4-methyl sterol composition of a *Gymnodinium* sp. isolated from the Adriatic Sea and cultured in the laboratory. They found 4$\alpha$,24-dimethylcholestan-3$\beta$-ol as the only 4-methyl sterol detected at exponential phase in a batch culture. However, at stationary phase the proportion of 4$\alpha$,24-dimethylcholestan-3$\beta$-ol was reduced to 16.1% of total 4-methyl sterols and the proportions of dinosterol and dinostanol increased from 0 to 30.3% and 22.2% of total 4-methyl sterols, respectively. Such environmental extremes may be infrequent in aquatic ecosystems, but they can occur when microalgae form large blooms that can strip the water of the major inorganic nutrients. Such blooms are thought to be geochemically important because they can be associated with high fluxes of organic matter to the sediments. Thus, the lipid compositional variations seen with batch cultures may provide a guide to the variations possible in the biosphere and must be considered when comparing algal and sediment lipid data.

Environmental conditions can have an even more dramatic effect on fatty acid distributions (Dunstan *et al.*, 1993). Fatty acids occur in various lipids having different physiological functions. The proportions of these lipids can vary significantly in response to changes in light intensity, salinity and most importantly nutrient levels. Less is known about effects on other lipid classes. Alkenone abundances change with temperature although there has been speculation that changes in salinity might also have an effect (e.g. Li *et al.*, 1996). Attempts to find other temperature proxies have thus far not been particularly successful. ten Haven *et al.* (1987) suggested that a plot of the $C_{27}$ sterol cholesta-5,22E-dien-3$\beta$-ol to the same sterol plus 27-nor-24-methylcholesta-5,22E-dien-3$\beta$-ol when present in esterified form might provide a sterol-based temperature index, but this concept has not been adopted.

Bell and Pond (1996) recently examined the lipids of flagellated and coccolith cell types of the geochemically-important species *Emiliania huxleyi* throughout its growth cycle. The coccolith form had slightly elevated levels of neutral lipids compared with the flagellate, but the distributions were generally similar and methyl and ethyl alkenones were present in both cell types as also found by Volkman *et al.* (1980a). The proportion of phospholipids and glycolipids increased during logarithmic growth phase, while neutral lipids (free fatty acids, triacylglycerols, ketones and hydrocarbons) achieved their highest levels in the late stationary growth phase. Changes in polyunsaturated fatty acid composition over the growth cycle were surprisingly small, although the abundance of 22:6*n*-3 peaked in the late stationary phase.

## CONCLUSIONS

Studies of contemporary marine sediments and of living microalgae continue to demonstrate the pre-

sence of novel lipids. Many of these have quite long alkyl chains, and it is becoming clear that the biosynthetic pathways in microalgae are far more diverse than had been suspected from earlier studies. Some lipid classes such as alkyl diols, alkenones, HBI alkenes, and botryococcenes appear to have a restricted occurrence in microalgae (Table 2) and thus are useful biomarkers for identifying sources of organic matter in sediments. However, many microalgal classes have not been studied extensively and further work may yet identify additional sources for some of these compounds. More widely distributed lipid classes, such as sterols and fatty acids, can still be useful indicators of carbon sources. However, it is becoming increasingly apparent that some sterols are widely distributed and that care must be exercised when using these compounds to infer sources. Hydroxy fatty acids appear to have considerable potential as biomarkers, but information on their occurrence in organisms and sediments is still limited and, as shown here for the freshwater eustigmatophytes, some surprising results are still being obtained. Since many of the simple lipids found in sediments are those that have escaped degradation because they were originally bound to resistant organic matrix, we suggest that hydrolysates of the extractable and non-extractable fractions of algal cultures should be systematically examined for the presence of new biomarkers.

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