Phytochemistry, Vol. 26, No. 9, pp. 2537–2541, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

STEROLS AND FATTY ACIDS OF THE RED TIDE FLAGELLATES *HETEROSIGMA AKASHIWO* AND *CHATTONELLA ANTIQUA* (RAPHIDOPHYCEAE)

PETER D. NICHOLS, JOHN K. VOLKMAN, GUSTAAF M. HALLEGRAEFF* and SUSAN I. BLACKBURN*

CSIRO Division of Oceanography, and *CSIRO Division of Fisheries Research, Marine Laboratories, GPO Box 1538, Hobart, Tasmania, 7001, Australia

(*Revised received* 18 *February* 1987)

Key Word Index—*Heterosigma akashiwo*; *Chattonella antiqua*; algae; Raphidophyceae; lipids; fatty acids; sterols; chemotaxonomy; red tides.

Abstract—The fatty acids and sterols of the raphidophyte flagellates, *Heterosigma akashiwo* (Australian and Plymouth strains) and *Chattonella antiqua* (Japanese strain) are reported. The major sterol of both species is 24-ethylcholesterol, which is more commonly associated with higher plants and has rarely been reported in unicellular algae. *C. antiqua* also contained 24-dihydrozymosterol [cholest-8(9)-en-3β-ol], which is also uncommon in marine algae. The major fatty acids in both raphidophytes are 16:0, 18:4ω3, 20:5ω3, 16:1ω7 and 14:0. Polyunsaturated fatty acids accounted for 46–50% of the total fatty acids in both species. The fatty acid 18:5ω3 was detected in *H. akashiwo*, but not in *C. antiqua*. This acid is found in some dinoflagellates and Prymnesiophycean algae, but this is the first report of its presence in the Raphidophyceae. The lipid distributions obtained for *H. akashiwo* and *C. antiqua* provide unique signature profiles for use in taxonomic, food-web and organic geochemical studies. The fatty acid and sterol distributions of these two raphidophytes justify their assignment to a separate class within the 'brown algal' line.

INTRODUCTION

The Raphidophyceae (=Chloromonadophyceae) are a small group of little studied golden-brown flagellates. They were originally described from fresh waters, but are now also widely recognised in coastal waters [1], where they can cause 'red-tide' phenomena and associated fish kills [2].

Basic features of the cell structure of raphidophytes include two unequal flagella arising from an apical depression: the forward flagellum bears two rows of fine hairs, while the trailing flagellum is smooth and lies close to the surface of the cell. The flagellar bases have a unique system of cross-banded, fibrous roots [3]. The cells are naked, dorsiventrally flattened and contain numerous trichocysts and mucocysts that readily discharge, thereby rendering these species difficult subjects for microscopic studies (M. Vesk and O. Moestrup, personal communication).

The taxonomy of this algal class is the subject of controversy [4]. Raphidophyte taxa have often been mistakenly assigned to other algal classes such as cryptomonads, chrysophytes, dinoflagellates and euglenoids.

Characterization of carotenoid, sterol and fatty acid patterns in unicellular algae can clarify disputed taxonomic affinities based on cell morphology alone. A recent study of carotenoids of the Raphidophyceae identified two species groups [5]. The freshwater taxa *Gonyostonum semen* and *Vacuolaria verscens* lack fucoxanthin and their pigment composition resembles that of the Xanthophyceae with diadinoxanthin, dinoxanthin and heteroxanthin as major xanthophylls. Two marine strains of *Fibrocapsa japonica* (=*Chattonella japonica*) examined contained fucoxanthin and violaxanthin [5], and have a pigment composition similar to that of the Chrysophyceae and Phaeophyceae. The same pigments were also reported for *Heterosigma akashiwo* (as *Olisthodiscus luteus*) [6], which at that time was assigned to the Chrysophyceae.

In a previous study [7], we examined the sterols and fatty acids of the raphidophyte FCRG 51 (=*Fibrocapsa japonica*). An unexpected affinity with the Dinophyceae, rather than the Chrysophyceae, was noted.

We report here the fatty acid and sterol composition of the controversial taxon *Heterosigma akashiwo* (two strains) [8], as well as the typical raphidophyte *Chattonella antiqua* [9]. Our results show that lipid profiles assist the assignment of algal taxonomic position, and also provide signature lipid profiles (biological markers) for use in organic geochemistry and food web studies. Such biochemical analyses may also prove useful in studying the abundance and ecology of these species in marine environments.

RESULTS AND DISCUSSION

Lipid abundances

Thin-layer chromatography–FID analyses of the total lipids of *H. akashiwo* and *C. antiqua* indicated that more than 90% of the lipid fraction consisted of polar material. The proportion of triacylglycerols was low. This observation is probably due to the harvesting of cells during late-log phase, since it has been observed that concentrations of triacylglycerols increase in stationary phase or with nitrogen limitation [10].

The ratios of fatty acids:sterols:phytol are similar in the two species (Table 1). The ratio of fatty acids:sterols (14–20) is lower than that generally found in diatoms (average of 54, $n = 11$) [11], but higher than that found in some dinoflagellates (1.2–9.2) [12, 13]. We believe that such ratios will prove useful in chemotaxonomic studies, but presently there are still too few data available for them to be used to assign an alga to a particular class. Such information is also of use in organic geochemistry studies where the flux of planktonic matter through the water column, or rates of degradation of individual compounds, are calculated [14].

Sterols

Five sterols were identified in *H. akashiwo* (two strains) and *C. antiqua*, using GC-MS techniques (Table 2). The major sterol in both algae was 24-ethylcholesterol (73–94% of total sterols). *H. akashiwo* also contained 24-methylcholesterol (3–6%), which was not detected in *C. antiqua*, and trace amounts of cholesterol. *C. antiqua* contained higher relative amounts of cholesterol and two sterols not found in *H. akashiwo*: cholest-8(9)-en-3β-ol (24-dihydrozymosterol, 11%) and 24-ethylcholesta-5,24(28)*Z*-dien-3β-ol (28-isofucosterol, 4%). Other minor sterols were detected in both species but the amounts were too small to enable identification of these components.

The distributions of 4-desmethyl sterols in unicellular algae have been reviewed by Volkman [15]. As very few species have been studied with modern analytical techniques, and the taxonomy of some species is problematical, reliable data are still too sparse to identify specific sterol markers for each algal class. Nonetheless, characteristic sterol distributions can be defined for some algal classes. In the following discussion, we attempt to interpret the sterol data obtained for *H. akashiwo* and *C. antiqua* in relationship to their taxonomic affinities within the 'brown algal' line (Bacillariophyceae, Chrysophyceae, Phaeophyceae, Prymnesiophyceae and Xanthophyceae).

The dominant sterol in *H. akashiwo* and *C. antiqua*, 24-ethylcholesterol, has been found in very few algae, and seldom as a major component. The diatom *Asterionella glacialis* (Bacillariophyceae) [15, 16], some strains of *Pavlova lutheri* (Prymnesiophyceae) [15, 17], and three species from the Xanthophyceae [18] contain 24-ethylcholesterol at concentrations greater than 65% of the total sterols. The major sterols of the Bacillariophyceae and Prymnesiophyceae are usually 24-methylcholesta-5,22*E*-dien-3β-ol, 24-methylenecholesterol and cholesterol [15]. Few species contain 24-ethylcholesterol, so the occurrences noted above are not typical of these two algal classes.

Carotenoid studies on *Fibrocapsa japonica* and *H. akashiwo* (as *Olisthodiscus luteus*) have previously suggested a link between the Raphidophyceae and the Chrysophyceae [5, 6]. *H. akashiwo* and *C. antiqua* had similar carotenoid profiles to other members of the Raphidophyceae: fucoxanthin, violaxanthin and ββ-carotene were present as the major carotenoids (S. Wright,

Table 1. Relative abundances of lipid classes in *Heterosigma akashiwo* and *Chattonella antiqua**

Lipid class	*Heterosigma akashiwo* CS-39	*Heterosigma akashiwo* CS-169	*Chattonella antiqua* CS-171
Fatty acids	1.0	1.0	1.0
Sterols	0.05	0.07	0.05
Phytol	0.06	0.07	0.07
Fatty acid/sterol	20	14	14

*Concentration of fatty acids set equal to 1.0. Values calculated from calibrated GC response of lipid classes relative to internal injection standard.

Table 2. Sterol compositions of *Heterosigma akashiwo* and *Chattonella antiqua*

Sterol	Common name	M_r*	RR_t†	Percentage composition: *Heterosigma akashiwo* CS-39	*Heterosigma akashiwo* CS-169	*Chattonella antiqua* CS-171
Cholest-5-en-3β-ol	cholesterol	458	1.00	0.5	0.4	7.2
Cholest-8(9)-en-3β-ol	24-dihydrozymosterol	458	1.07	—‡	—	11.1
24-Methylcholest-5-en-3β-ol	24-methylcholesterol	472	1.32	6.0	2.9	—
24-Ethylcholest-5-en-3β-ol	24-ethylcholesterol	486	1.63	90.8	94.1	73.1
24-Ethylcholesta-5,24(28)*Z*-dien-3β-ol	28-isofucosterol	484	1.65	—	—	4.1
Other				2.6	2.6	4.5

*Molecular weight, as TMSi ether.

†RR_t, relative retention time on a methyl silicone (BP1) column: cholesterol = 1.00, 24-ethylcholesterol = 1.63.

‡— not detected.

unpublished data). The sterol distributions of the two algal classes are, however, quite different. The major sterol in three species of the Chrysophycean genus *Ochromonas* is 24-ethylcholesta-5,22*E*-dien-3β-ol [15, 19, 20], while two unidentified species contain significant proportions of C_{30} sterols [15] not found in *H. akashiwo* or *C. antiqua*. Whether these sterol distributions are representative for the Chrysophyceae will not be determined until more species are studied.

The taxonomy of the unicellular alga FCRG 51 (*Fibrocapsa japonica*) has been the subject of much controversy. It is now assigned to the Raphidophyceae [1, 21], but initially it was associated with the dinoflagellate genus *Exuviaella*, and then transferred to the Chloromonadophyceae (=Raphidophyceae), and also the Chrysophyceae [4]. The sterol distribution of FCRG 51 differs markedly from that of *H. akashiwo* and *C. antiqua* and suggests a biochemical link with the Dinophyceae [7]. FCRG 51 contains dinosterol (specific to dinoflagellates), other 4-methyl sterols and 24-methylenecholesterol, while 24-ethylcholesterol was not detected.

C. antiqua is one of the few unicellular algae known to contain cholest-8(9)-en-3β-ol (24-dihydrozymosterol). The occurrence of this sterol is one biochemical feature by which the two Raphidophycean taxa studied here can be distinguished from each other. The mass spectra of C_{27} sterols with $\Delta^{8(14)}$, $\Delta^{8(9)}$ and Δ^{7} double bonds are similar, with prominent ions at *m/z* 458 (M^+ TMS-ether), 443, 368, 353, 255, 229 and 213. In this study the mass spectra of the $\Delta^{8(9)}$ and $\Delta^{8(14)}$ isomers were nearly identical; these two isomers could not, therefore, be distinguished by mass spectral data alone. The mass spectrum of the Δ^{7} isomer, compared with the other isomers, showed a higher abundance of *m/z* 255 relative to *m/z* 213 and 229. The three isomers can be identified by their very different retention times. RR_t values of 1.01, 1.07 and 1.17 were determined for the $\Delta^{8(14)}$, $\Delta^{8(9)}$ and Δ^{7} isomers relative to cholesterol TMS-ether, and the C_{27} sterol present in *C. antiqua* co-chromatographed with authentic cholest-8(9)-en-3β-ol.

Cholest-8(9)-en-3β-ol has been found in the diatom *Biddulphia aurita*, together with approximately equal amounts of fucosterol, 24-methylcholesta-5,22*E*-dien-3β-ol and cholesterol [11]. The reasons why this sterol accumulates in so few species and its significance in terms of sterol biosynthetic pathways require more studies.

The occurrence of 28-isofucosterol in *C. antiqua* suggests that, in this alga at least, this sterol is the biosynthetic precursor of 24-ethylcholesterol. The absence of the 24(28)*E* isomer, fucosterol, is noteworthy and suggests that the enzyme producing the 24(8)*Z* double-bond is specific for this isomer.

Unicellular algae use several mechanisms to elaborate sterol side-chain structures, and many details of the mechanisms involved are still not known. *Ochromonas malhamensis* (Chrysophyceae) has been shown to convert 28-isofucosterol to 24β-ethylcholesta-5,22*E*-dien-3β-ol (poriferasterol) [22], and in *O. danica* the side-chain double-bond is completely reduced to produce a small amount of 24β-ethylcholesterol [19]. The pathway operating in *C. antiqua* produces no 24-ethylcholesta-5,22*E*-dien-3β-ol, and 28-isofucosterol is almost entirely converted to 24-ethylcholesterol. It remains to be established whether the C_{29} sterols of the Raphidophyceae have the 24β stereochemistry found in *Ochromonas* or the 24α stereochemistry found in diatoms.

The occurrence of 24-ethylcholesterol in both species of Raphidophyceae has implications for the study of the origins of organic matter in seawater and sediments. This sterol is often used as a marker for higher plant input to marine sediments [15, 23, 24]. However, recent work suggests that marine sources of this sterol must be significant to account for the high concentrations of 24-ethylcholesterol in seawater particulate matter and some marine sediments [15]. This study has identified two bloom-forming coastal algal species that produce high concentrations of 24-ethylcholesterol. It is likely that other little-studied algal classes will be found to contain this sterol.

These results are also relevant to studies of ancient sediments and crude oils. Sterols are converted to steranes in ancient sediments, and the ratio of C_{27} to C_{29} steranes is often used as an index of the relative amounts of marine and terrigenous organic matter. The present finding of C_{29} sterols in marine algae further supports the view [15, 16] that other higher-plant derived lipids must be present, before it can be assumed that the C_{29} sterols or steranes are derived from terrigenous sources.

Table 3. Fatty acid composition of *Heterosigma akashiwo* and *Chattonella antiqua*

	Percentage composition		
	Heterosigma akashiwo		*Chattonella antiqua*†
Fatty acid*	CS-39	CS-169	CS-171
14:0	5.9	6.0	7.9
15:0	0.6	0.4	0.7
16:1ω7*c*	8.6	9.3	8.0
16:ω5*c*	1.2	1.7	0.2
16:1ω13*t*	4.8	6.8	3.0
16:0	25.8	19.8	24.7
18:5ω3	4.6	5.2	—
18:3ω6	0.7	0.4	0.4
18:4ω3	18.1	16.5	20.1
18:2ω6	3.7	2.3	4.2
18:3ω3	3.5	3.0	4.3
18:1ω9*c*	0.7	0.5	2.2
18:1ω7*c*	0.8	1.0	0.9
18:0	0.3	0.4	0.9
20:4ω6	1.5	1.2	2.7
20:5ω3	11.7	16.3	14.1
22:6ω3	2.1	3.0	2.1
22:5ω3	0.1	0.1	1.9
Other	5.3	6.2	1.6
Total PUFA‡	46.0	48.0	49.8

*Tabulation indicates elution order on non-polar BP1. Coeluting components quantified using a polar BP20 column.

†Also contained traces of 12:0, 13:0.

‡Sum of polyunsaturated fatty acids ($\geq$ 2 double bonds).

Fatty acids

The fatty acid compositions of *H. akashiwo* (two strains) and *C. antiqua* are shown in Table 3. Each of the

three samples contained similar distributions with major acids being 16:0, 18:4ω3, 20:5ω3, 16:1ω7 and 14:0, in decreasing order of abundance. Both algae showed a low 16:1ω7c/16:0 ratio (< 0.3). C_{18}, C_{20} and C_{22} polyunsaturated fatty acids (PUFA) accounted for 46–50% of the total fatty acids. The fatty acids in the Australian (CS-169) and Plymouth (CS-39) strains of *H. akashiwo* were closely similar.

H. akashiwo and *C. antiqua* contained the same profile of fatty acids, except that *C. antiqua* contained no detectable 18:5ω3 (i.e. < 0.05% of total fatty acids). The small differences in the relative abundances of minor fatty acids could be due to differences in physiological state of the algae. *C. antiqua* contained lower relative amounts of 16:1ω5 and 16:1ω13t (the latter associated with chloroplast phospholipids [25]), and higher proportions of 18:1ω9, 20:4ω6 and 22:5ω3.

A comparison with published data on other members of the 'brown algal' group provides insights into possible biochemical relationships. The relative proportions of major fatty acids and the presence or absence of specific compounds can be used to distinguish between different groups, with the proviso that culture conditions may also affect fatty acid compositions. This problem can be avoided if culture conditions and analytical procedures are standardized.

Diatoms (Bacillariophyceae) contain high concentrations of 16:0, 16:1 and 20:5ω3 (often with 16:1ω7 predominating) and low amounts of C_{18} PUFA. They may also contain C_{16} PUFA [e.g. 11, 26]. Members of the Xanthophyceae do not contain 18:4ω3, and characteristically show a high 16:1/16:0 ratio. In many species, C_{16} PUFA are present [18, 27]. Chrysophyceae usually have high 16:1/16:0 ratios (> 2) and contain appreciable amounts of C_{16} PUFA, which are not found in the Raphidophyceae [27]. The fatty acid distributions of *H. akashiwo* and *C. antiqua* are therefore distinct from those of the diatoms, the Chrysophyceae and the Xanthophyceae. Also, none of these algal classes has been reported to contain the uncommon acid 18:5ω3 found in *H. akashiwo*.

The fatty acids found in members of the Dinophyceae and the Prymnesiophyceae show a closer correspondence to those in the two Raphidophycean algae. However, there are significant differences in the proportions of some major acids. Dinoflagellates usually contain higher abundances of 18:5 and 22:6 fatty acids. The fatty acid 18:5ω3 is often more than twice as abundant as 18:4ω3, whereas in *H. akashiwo* it is only one-third as abundant. The Prymnesiophyceae contain 14:0, 16:0, 18:3ω3, 22:6ω3 and 18:4ω3 as major acids [27, 28, 29], often with 18:4ω3 predominating. This is broadly similar to the fatty acid distributions found in *H. akashiwo* and *C. antiqua*, although 14:0, 18:1ω9 and 22:6ω3 seem to be more abundant in the Prymnesiophyceae.

Phylogenetic implications and conclusions

Sterol and fatty acid profiles of the two raphidophyte algae *Heterosigma akashiwo* and *Chattonella antiqua* support assignment of these algae to a separate class within the 'brown algal' line. Although ultrastructure studies and pigment analyses indicate a close link between the Raphidophyceae and Chrysophyceae, this linkage is not supported by the sterol and fatty acid data obtained in this study.

Both red-tide flagellates contain 24-ethylcholesterol as the most abundant sterol. This sterol has previously been found as a major component in only a few unicellular algae, and has been used by organic geochemists as a marker for organic matter from terrestrial sources. The presence of 24-ethylcholesterol in *H. akashiwo* and *C. antiqua* indicates that this sterol is more common in marine algae than previously believed; it would be unwise, therefore, to use it as a terrigenous marker in all environments.

The fatty acid distributions of the two raphidophytes resemble those of the Dinophyceae and Prymnesiophyceae, although there are significant differences in the proportions of some acids. The occurrence of the uncommon acid 18:5ω3 in *H. akashiwo* is the first report of this acid in the Raphidophyceae. Therefore, this compound can no longer be considered to be restricted to members of the Dinophyceae and Prymnesiophyceae.

Data on the lipids and carotenoid pigments of *H. akashiwo* and *C. antiqua* have provided a basis for assigning biochemical affinities of the Raphidophycean algae. The occurrence of specific sterols and fatty acids and selected ratios of compounds should also be useful in assessing the input of such organisms to marine sediments and food-webs. The latter is of ecological importance as dense blooms of *H. akashiwo*, and especially *C. antiqua*, have caused extensive mortalities in cultured fish and other marine organisms [30] in Japanese waters.

EXPERIMENTAL

Culture conditions. The two strains of *Heterosigma akashiwo* (CS-39 and CS-169) and *Chattonella antiqua* (CS-171) were obtained from the CSIRO Algal Culture Collection. The CS-39 isolate came from F. T. Haxo's culture collection at the Scripps Institution of Oceanography and was isolated by M. Parke from Plymouth, England as *Olisthodiscus luteus*. CS-169 was isolated by J. L. Stauber from West Lakes, South Australia, while CS-171 is strain Ho-1 from the NIES-collection, Japan, isolated by M. Watanabe from the Seto Inland Sea. Cultures (1 l) were grown in Erlenmeyer flasks at 19° in half-strength G medium (enriched seawater soil extract) for marine dinoflagellates [31] at 100 $\mu E/m^2$ sec (Philips fluorescent tube-near daylight special white light: TL20W/47 deluxe) on a 12:12 hr light:dark cycle. Cells in late log-phase (8 days) were harvested for lipid analysis by filtration onto precleaned (muffle furnace, 450°) glass-fibre filters (Whatman GF/C) and transferred immediately to the extracting solvent mixture. Cultures were maintained under the same conditions as for the experiments, with the exception that they were grown in 75 ml of medium in 125 ml Erlenmeyer flasks.

Lipid extraction and fractionation. Lipids were quantitatively extracted by the one-phase Bligh and Dyer procedure [32]. After phase separation, lipids were recovered in the $CHCl_3$ phase, the solvents removed *in vacuo*, and the lipids stored under nitrogen at −20°. A portion of the total lipid extract was analysed with an Iatroscan Mk III TH-10 TLC-FID analyser (Iatroscan Laboratories, Japan) [33]. Total neutral lipid and fatty acid fractions were obtained following alkaline saponification as described previously [7]. Fatty acid methyl esters (FAME) were formed by acid methanolysis [34] and stored at −20°. Carotenoid analyses were performed using HPLC methods described in detail elsewhere [35].

GC and GCMS analyses. The neutral lipid fraction, containing predominantly sterol and alcohol components, was treated with BSTFA (30 min, 60°) to produce the corresponding TMS-ethers.

All samples were analysed with a Shimadzu 9A gas chromatograph equipped with an FID and OCI-3 on-column injector (SGE, Australia). Samples were injected at 40° onto a nonpolar BP1 fused-silica capillary column (25 × 0.25 mm i.d., SGE). After 1 min, the oven temperature was raised to 120° at 30° per min, then to 310° at 4° per min. Hydrogen was used as carrier gas and the detector temperature was 340°. FAME were further analysed using a polar BP-20 (carbowax) fused-silica column (25 × 0.25 mm i.d., SGE). Similar GC conditions to those described above were employed, except that 240° was the upper limit of the second temperature ramp. Peak areas were quantified with a Shimadzu C-R3A integrator. Lipid analyses were performed in duplicate. Components were quantitated by calibrated GC response and are subject to errors of up to 5% for major peaks and up to 10% for minor peaks. Peak identifications, prior to GC-MS confirmation, were based on comparison of retention times with data obtained for donated, commercial, and previously identified laboratory standards. Identifications were confirmed by comparison of mass spectra (sterols and alcohols as TMS-ethers) with those of previously reported spectra [e.g. 36–39]. GC-MS analyses were performed with an HP 5890 GC and 5790 MSD fitted with an open-split interface. The nonpolar column, injector and chromatography conditions were similar to those described above. Mass spectra were acquired and processed with a 59970A Workstation operated in scan acquisition mode. Typical MSD operating conditions were: electron multiplier 2600 volts; transfer line 310°; autotune file DFTPP normalized; electron impact energy = 70 eV; scan threshold = 30; 0.8 scans per sec.

Fatty acid nomenclature. Fatty acids are designated as total number of carbon atoms: number of double bonds followed by the position of the double-bond from the ω (aliphatic) end of the molecule. The suffixes *c* and *t* indicate *cis* and *trans* geometry. PUFA indicates polyunsaturated fatty acids. Double-bonds of PUFA are methylene interrupted.

Acknowledgements—We thank Dr S. W. Jeffrey of CSIRO Division of Fisheries Research for the algal cultures; Dr S. Wright, of the Australian Antarctic Division and David Everitt of CSIRO Division of Oceanography for performing carotenoid analyses; Mark Rayner of CSIRO Division of Oceanography for assistance during lipid work-up. Gifts of sterol standards from the Steroid Reference Collection of the Medical Research Council (U.K.) are gratefully acknowledged.

REFERENCES

1. Loeblich, A. R. and Fine, K. E. (1977) *Proc. Biol. Soc. Wash.* **90**, 388.
2. Shimada, M., Murakami, T. H., Imahayashi, T., Ozaki, H. S., Toyoshima, T. and Okaichi, T. (1983) *Acta Histochem. Cytochem.* **16**, 232.
3. Moestrup, O. (1982) *Phycologia* **21**, 427.
4. Gibbs, S. P., Chu, L. L. and Magnussen, C. (1980) *Phycologia* **19**, 173.
5. Fiksdahl, A., Withers, N., Guillard, R. R. L. and Liaaen-Jensen, S. (1984) *Comp. Biochem. Physiol.* **78B**, 265.
6. Withers, N. W., Fiksdahl, A., Tuttle, R. C. and Liaaen-Jensen, S. (1981) *Comp. Biochem. Physiol.* **68B**, 345.
7. Nichols, P. D., Volkman, J. K. and Johns, R. B. (1983) *Phytochemistry* **22**, 1447.
8. Leadbeater, B. S. C. (1969) *Br. Phycol. J.* **4**, 3.
9. Hara, Y. and Chihara, M. (1982) *Jap. J. Phycol.* **30**, 47.
10. Lewin, R. A. (1962) *Physiology and Biochemistry of Algae.* Academic Press, New York.
11. Orcutt, D. M. and Patterson, G. W. (1975) *Comp. Biochem. Physiol.* **50B**, 579.
12. Jones, G. J., Nichols, P. D. and Johns, R. B. (1983) *J. Phycol.* **19**, 416.
13. Nichols, P. D., Jones, G. J., de Leeuw, J. W. and Johns, R. B. (1984) *Phytochemistry.* **23**, 1043.
14. Gagosian, R. B., Nigrelli, G. E. and Volkman, J. K. (1983) in *Coastal Upwelling Part A* (Suess, E. and Thiede, J. eds) pp. 241–272. Plenum Press, New York.
15. Volkman, J. K. (1986) *Org. Geochem.* **9**, 83.
16. Jones, G. J., Nichols, P. D., Johns, R. B. and Smith, J. D. (1987) *Phytochemistry* 1343.
17. Ballantine, J. A., Lavis, A. and Morris, R. J. (1979) *Phytochemistry* **18**, 1459.
18. Mercer, E. I., London, R. A., Kent, I. S. A. and Taylor, A. J. (1974) *Phytochemistry* **13**, 845.
19. Gershengorn, M. C., Smith, A. R. H., Goulston, G., Goad, L. J., Goodwin, T. W. and Haines, T. H. (1968) *Biochemistry* **7**, 1698.
20. Avivi, L., Iaron, O. and Halevy, S. (1967) *Comp. Biochem. Physiol.* **21**, 321.
21. Loeblich, A. R. (1977) *J. Phycol.* **13**, Suppl. 42.
22. Knapp, F. F., Greig, J. B., Goad, L. J. and Goodwin, T. W. (1971) *Chem. Commun.* 707.
23. Huang, W.-Y. and Meinschein, W. G. (1976) *Geochim. Cosmochim. Acta* **40**, 323.
24. Huang, W.-Y. and Meinschein, W. G. (1979) *Geochim. Cosmochim. Acta* **43**, 739.
25. Harwood, J. L. and Russell, N. J. (1984) *Lipids in Plants and Microbes.* George Allen and Unwin, London.
26. Volkman, J. K., Eglinton, G. and Corner, E. D. S. (1980) *Phytochemistry* **19**, 1809.
27. Chuecas, L. and Riley, J. P. (1969) *J. Mar. Biol. Assoc. U.K.* **49**, 97.
28. Sargent, J. R., Eilertsen, H. C., Falk-Petersen, S. and Taasen, J. P. (1985) *Mar. Biol.* **85**, 109.
29. Volkman, J. K., Smith, D. J., Eglinton, G., Forsberg, T. E. V. and Corner, E. D. S. (1981) *J. Mar. Biol. Assoc. U. K.* **61**, 509.
30. Okaichi, T. (1985) *Bull. Mar. Science* **37**, Abs. 772.
31. Loeblich III, A. R. and Smith, E. V. (1968) *Lipids* **3**, 5.
32. White, D. C., Davis, W. M., Nickels, J. S., King, J. D. and Bobbie, R. J. (1979) *Oecologia* **40**, 51.
33. Volkman, J. K., Everitt, D. A. and Allen, D. I. (1986) *J. Chromatogr.* **356**, 147.
34. Edlund, A., Nichols, P. D., Roffey, R. and White, D. C. (1985) *J. Lipid Res.* **26**, 982.
35. Wright, S. W. and Shearer, J. D. (1984) *J. Chromatogr.* **294**, 281.
36. Brooks, C. J. W., Horning, E. C. and Young, J. S. (1968) *Lipids* **3**, 391.
37. de Leeuw, J. W., Rijpstra, W. I. C., Schenck, P. A. and Volkman, J. K. (1983) *Geochim. Cosmochim. Acta* **47**, 455.
38. Knight, B. A. (1969) *J. Gas Chromatogr.* **5**, 273.
39. Ryhage, R. and Stenhagen, E. (1958) *Arkiv. Kemi.* **13**, 523.