Phytochemistry, Vol. 23, No. 5, pp. 1043–1047, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

THE FATTY ACID AND STEROL COMPOSITION OF TWO MARINE DINOFLAGELLATES

PETER D. NICHOLS,* GARY J. JONES,† JAN W. DE LEEUW‡ and R. B. JOHNS*

*Organic Chemistry Department and †Marine Chemistry Laboratory, University of Melbourne, Parkille, Victoria, Australia, 3052; ‡Delft University of Technology, Department of Chemistry and Chemical Engineering, Organic Geochemistry Unit, de Vries van Heystplantsoen 2, 2628 RZ Delft, The Netherlands

(*Received* 8 *June* 1983)

Key Word Index—*Gymnodinium wilczeki*; *Prorocentrum cordatum*; Dinophyceae; unicellular alga; lipids; fatty acids; sterols; pigments.

Abstract—The fatty acid, sterol and chlorophyll pigment compositions of the marine dinoflagellates *Gymnodinium wilczeki* and *Prorocentrum cordatum* are reported. The fatty acids of both algae show a typical dinoflagellate distribution pattern with a predominance of C_{18}, C_{20} and C_{22} unsaturated components. The acid 18:5ω3 is present at high concentration in these two dinoflagellates. *G. wilczeki* contains a high proportion (93.4 %) of 4-methyl-5α-stanols including 4,23,24-trimethyl-5α-cholest-22*E*-en-3β-ol (dinosterol), dinostanol and 4,23,24-trimethyl-5α-cholest-7-en-3β-ol reported for the first time in dinoflagellates. The role of this sterol in the biosynthesis of 5α-stanols in dinoflagellates is discussed. *P. cordatum* contains high concentrations of a number of $\Delta^{24(28)}$-sterols with dinosterol, 24-methylcholesta-5,24(28)-dien-3β-ol, 23,24-dimethylcholesta-5,22*E*-dien-3β-ol, 4,24-dimethyl-5α-cholest-24(28)-en-3β-ol and a sterol identified as either 4,23,24-trimethyl- or 4-methyl-24-ethyl-5α-cholest-24(28)-en-3β-ol present as the five major components. The role of marine dinoflagellates in the input of both 4-methyl- and 4-desmethyl-5α-stanols to marine sediments is discussed.

INTRODUCTION

Dinoflagellates are important components of the phytoplankton, being the foundation of many food chains and are major producers of dietary sterols. The lipid, particularly the sterol, compositions of marine dinoflagellates are currently the subject of detailed investigation [1–5]. The analysis of fatty acid and sterol compositions as a chemotaxonomic guide has provided much information on algal (including members of the Dinophyceae) classification [6–10]. 4α,23,24-Trimethyl-5α-cholest-22*E*-en-3β-ol (dinosterol), first identified in the photosynthetic dinoflagellate *Gonyaulax tamarensis* [11], appears to be unique to dinoflagellates and has been used as a 'biological marker' for dinoflagellate input to marine sediments [12]. The synthesis of a wide range of 4-methyl sterols is a characteristic feature of dinoflagellates [10]. This feature can be useful in the classification of dinoflagellates and also in the identification of the source of organic matter in marine sediments where dinoflagellates are thought to be important input components.

We report here a detailed analysis of the fatty acid and sterol composition of two marine dinoflagellates *Gymnodinium wilczeki* Pouchet (?) and *Prorocentrum cordatum* (Ostenfeld) Dodge. A recent study has shown, based on lipid composition data, that the alga FCRG 51 (Scripps Institute of Oceanography clonal designation number) is a member of the Dinophyceae [10]. FCRG 51 was originally assigned to the genus *Exuviaellae*, but later transferred to the Chloromonadophyceae [13, 14] and Chrysophyceae [15]. The genus *Exuviaella* has been transferred to the genus *Prorocentrum* [16]. The data reported here will not only aid the assignment of algal taxonomic position but also provide essential data for organic geochemical studies.

RESULTS AND DISCUSSION

Lipid and pigment composition

Lipid (free and esterified) concentration data, expressed on a dry weight basis, are presented in Table 1. Fatty acid : sterol ratios of 9.2 : 1 and 1.9 : 1 were obtained for *G. wilczeki* and *P. cordatum* respectively. These values, in particular the value of 1.9 : 1 obtained for *P. cordatum*, are

Table 1. Lipid composition of the marine dinoflagellates *Gymnodinium wilczeki* and *Prorocentrum cordatum*

	Percentage composition (dry wt) of tissue	
Lipid fraction	*G. wilczeki*	*P. cordatum*
Fatty acids	14.0	5.8
Sterols	1.5	3.1
Fatty acids/sterols	9.2	1.9
Phytol	0.42	0.30
Chlorophyll *a*	0.72	0.50
(μg/10^6 cells)	(10.3)	(1.5)
Chlorophyll *c*	0.29	0.23
(μg/10^6 cells)	(4.1)	(0.68)
Chlorophyll *a* : *c*	2.5	2.2

significantly lower (due to the higher sterol concentration) than the average value of 54 : 1 reported for eleven diatom species [17]. These data support the hypothesis that dinoflagellates are important contributors of sterols into marine sediments in environments where they are a major input source, and even in environments where they may be less abundant than other phytoplankton classes. This observation, in conjunction with the identification of individual components, is important when calculating (i) the flux of planktonic organic matter into marine sediments and (ii) the degradation rates of individual organic compounds in the marine environment [18].

The cell chlorophyll concentrations and chlorophyll *a* : *c* ratios for *G. wilczeki* and *P. cordatum* (Table 1) are consistent with values reported previously for marine dinoflagellates [19]. The high absolute levels of chlorophylls *a* and *c* in *G. wilczeki* compared with those reported by Jeffrey *et al.* [19], are indicative of the larger cell size of this alga. Chlorophyll concentrations and chlorophyll *a* : *c* ratios vary widely within and between algal classes and, taken in isolation, cannot be used as definitive chemotaxonomic indicators. The phytol concentration in both algae is significantly higher than that reported for the marine diatom *Biddulphia sinensis* [20]. To our knowledge the phytol content of dinoflagellates has not been reported.

Fatty acids

The fatty acid compositions of *G. wilczeki* and *P. cordatum* are reported in Table 2. The high proportions of C_{18}, C_{20} and C_{22} polyunsaturated fatty acids (PUFAs) in both algae are in agreement with previous analyses for dinoflagellates [21–24]. Clearly the range of fatty acids is similar for both dinoflagellates, yet the distribution patterns are distinctive enough for the species to be readily distinguished one from the other.

The acid 18:5ω3,* which has been proposed as a biological marker for dinoflagellates in marine food chain studies [22], is present in high concentration in both algae. Although 18:5ω3 has recently been reported in several coccolithophorids at relative levels of 3.3% to 10.1% of the total fatty acids [7], this acid has not been detected in other classes of algae. The coccolithophores examined in that study all contained high concentrations of the acid 18:3ω3 [7]. This acid is however, only a minor component in *P. cordatum* and *G. wilczeki* and other dinoflagellates [21–24], hence the high ratio of 18:5ω3 to 18:3ω3 may be a taxonomic feature of dinoflagellates.

The detection of the monounsaturated acid 18:1ω7 at high relative concentrations in both dinoflagellates is significant. This acid has generally been associated with bacterial fatty acid biosynthesis [25]. Diatom cultures grown concurrently with this experiment, under identical conditions, did not contain this acid indicating that it is endemic to these two dinoflagellates, rather than of a bacterial origin. We have previously reported the presence of 18:1ω7 in small quantities in the unicellular alga FCRG 51 [10] and *Thoracosphaera heimii* [24], which are taxonomically related to the Dinophyceae based on their lipid composition. This acid has also been reported in the lipids of *Prorocentrum minimum* [23], which contains a similar range of fatty acid components as found here for *G. wilczeki* and *P. cordatum*. The presence of 18:1ω7 in the two dinoflagellates *G. wilczeki* and *P. cordatum*, which have been cultured aseptically for this study, further strengthens the view that this acid is distributed more widely in marine algae than hitherto realized [6, 20, 26].

Table 2. Fatty acid composition of the marine dinoflagellates *Gymnodinium wilczeki* and *Prorocentrum cordatum*

		Percentage composition	
Fatty acid	ECL*	*G. wilczeki*	*P. cordatum*
12:0	12.00	0.1	TR†
14:1	13.72	TR	0.3
14:0	14.00	3.4	3.9
iso 15:0	14.65	TR	0.6
anteiso 15:0	14.70	TR	0.2
15:0	15.00	1.3	0.4
16:1ω7	15.73	0.3	9.5
trans 16:1ω13	15.93	0.1	0.2
16:0	16.00	35.4	25.7
17:0	17.00	0.2	TR
18:5ω3	17.35	13.3	27.8
18:3ω6	17.46	0.1	0.1
18:4ω3	17.46	0.1	0.1
18:2ω6	17.57	3.0	1.1
18:3ω3	17.57	0.8	0.8
18:1ω9	17.68	3.2	2.2
18:1ω7	17.75	4.5	4.5
18:0	18.00	2.6	1.2
20:4ω6	19.14	TR	0.8
20:5ω3	19.14	12.1	4.4
20:2ω6	19.62	0.3	—
20:1	19.80	TR	0.5
20:0	20.00	0.1	TR
22:6ω3	21.02	18.5	14.5
22:5ω3	21.12	0.2	1.0
22:1	21.79	0.1	0.2
22:0	22.00	0.3	TR
PUFAs‡		48.4	50.6
Saturated fatty acids		43.4	32.0
Monenoic fatty acids		8.2	17.4

*Equivalent chain length (ECL) on SE 30.

†TR: trace, < 0.1%.

‡PUFAs, polyunsaturated fatty acids, ⩾ 2 double bonds.

Sterols

The sterol compositions of the two dinoflagellates *G. wilczeki* and *P. cordatum* are presented in Table 3. The sterol composition of algae from these two genera have to our knowledge, not been reported previously. *G wilczeki* contains eight sterol components and is characterized by a very high abundance of 4-methyl sterols. The five 4-methyl sterols represented 93.4% of the total sterols and are identified in decreasing abundance as: 4,23,24-trimethyl-5α-cholest-22*E*-en-3β-ol (dinosterol), 4,23,24-trimethyl-5α-cholestan-3β-ol (dinostanol), 4,23,24-trimethyl-5α-cholest-7-en-3β-ol, 4,24-dimethyl-5α-cholestan-3β-ol and a sterol, which based on RR_t and mass spectral data can be identified either as 4,22,23- or 4,22,24-trimethyl-5α-cholestan-3β-ol.

---

*Double bonds are numbered from the methyl end of the fatty acid; all subsequent double bonds are methylene interrupted.

Table 3. Sterol composition of the marine dinoflagellates *Gymnodinium wilczeki* and *Prorocentrum cordatum*

Sterol no.	*RR*$_t$*	MW†	Identification	Percentage of total sterols	
				G. wilczeki	*P. cordatum*‡
1	0.89	456	cholesta-5,22*E*-dien-3β-ol	2.3	—
2	1.00	458	cholest-5-en-3β-ol	1.7	4.2
3	1.12	470	24-methylcholesta-5,22*E*-dien-3β-ol	—	2.5
4	1.28	470	24-methylcholesta-5,24(28)-dien-3β-ol	—	35.4
5	1.39	484	23,24-dimethylcholesta-5,22*E*-dien-3β-ol	—	12.5
6	1.42	486	23,24-dimethyl-5α-cholest-22*E*-dien-3β-ol	—	1.5
7	1.47	486	4,24-dimethyl-5α-cholest-22*E*-en-3β-ol	—	1.4
8	1.63	486	24-ethylcholest-5-en-3β-ol	2.6	—
9	1.65	486	4,24-dimethyl-5α-cholest-24(28)-en-3β-ol§	—	5.2
10	1.69	488	4,24-dimethyl-5α-cholestan-3β-ol	3.5	—
11	1.79	500	4,23,24-trimethyl-5α-cholest-22*E*-en-3β-ol	47.9	28.7
12	1.93	500	4,23,24-trimethyl-5α-cholest-24(28)-en-3β-ol or 4-methyl-24-ethyl-5α-cholest-24(28)-en-3β-ol	—	8.6
13	1.96	500	4,23,24-trimethyl-5α-cholest-7-en-3β-ol	15.5	—
14	2.02	502	4,22,23- or 4,22,24-trimethyl-5α-cholestan-3β-ol	2.3	—
15	2.08	502	4,23,24-trimethyl-5α-cholestan-3β-ol	24.2	—
			4-desmethyl sterols	6.6	56.1
			4-methyl sterols	93.4	43.9

**RR*$_t$ (Relative retention time): cholesterol 1.00; 24-ethylcholesterol 1.63.

†Molecular weight of the TMSi-ether derivative.

‡Also contained trace levels of a $C_{28:3}$ sterol (*RR*$_t$ 1.23) and a $C_{29:3}$ sterol (*RR*$_t$ 1.53).

§Also contained some 24-ethylcholesta-5,*Z*-24(28)-dien-3β-ol.

The 4-desmethyl sterols isolated from *G. wilczeki* (6.6% of total sterols) are: 24-ethyl-cholest-5-en-3β-ol, cholest-5,22*E*-dien3β-ol and cholest-5-en-3β-ol. The isolated sterols have been identified by comparison of their *RR*$_t$'s and mass spectra with literature data [10, 20, 27–29]. 4-23,24-Trimethyl-5α-cholest-7-en-3β-ol has been tentatively identified based on the similarity of its mass spectrum with that of 5α-cholest-7-en-3β-ol and its 4-methyl analogue. Δ^7-Sterol TMSi ethers have the molecular ion as one of the most abundant peaks. A peak at *m/z* 269 represents the nuclei fragment and peaks at *m/z* 243 and 227 represent further degradation of ring D in the 4-methyl-Δ^7-sterol [27, 28].

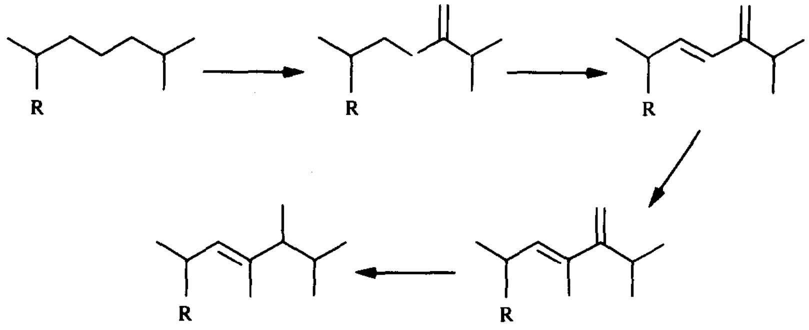
The presence of Δ^7-sterols(cholest-7-en-3β-ol, cholesta-5,7-dien-3β-ol and 24-methylcholesta-5,7-dien-3β-ol) has been reported in symbiotic dinoflagellates (zooxanthellae) associated with the gorgonian coral *Briareum asbestinium* [30, 31] and from the nonphotosynthetic dinoflagellate *Crypthecodinium cohnii* [32]. The finding of 4,23,24-trimethyl-5α-cholest-7-en-3β-ol in high concentration in *G. wilczeki* further increases the number of Δ^7-sterols encountered in dinoflagellates. Based on the co-occurrence of dinosterol, Δ^5-dehydrodinosterol and dinosterone in *C. cohnii*, Withers *et al.* [32] suggested that the biosynthetic route to dinosterol is similar to the one of cholestan-3β-ol in animal tissue [33, 34], in which Δ^7 intermediates play a role in the sequence 3β-hydroxy-Δ^7-sterol → 3β-hydroxy-$\Delta^{5,7}$ → 3β-hydroxy-Δ^5 → 3-oxo-Δ^5 → 3-oxo-Δ^4 → 3-oxo-5α → 3β-hydroxy-5α-stanol. The presence of 4,23,24-trimethyl-5α-cholest-7-en-3β-ol in *G. wilczeki* indicates that this sterol may be an intermediate in the biosynthesis of dinosterol. Moreover, side chain methylation in *G. wilczeki* in fact may occur prior to conversion of the 3β-hydroxy-Δ^7-sterol nucleus to the 3β-hydroxy-5α-stanol.

The high absolute and relative (93.4% of total sterols) abundance of 4-methyl-5α-stanols in *G. wilczeki* is significant. 5α-Stanols are commonly found in marine sediments as a result of direct input from source organisms or from *in situ* microbial hydrogenation of the sterols deposited in the sediment [35–37]. 4-Methyl-5α-stanols present in marine sediments will originate, based on the high level of 4-methyl-5α-stanols found in *G. wilczeki* and in other marine dinoflagellates, almost exclusively from dinoflagellates.

The origin of 4-desmethyl-5α-stanols in marine sediments is to the present time explained by similar processes to those described above. Although these stanols have been found in low concentration in marine dinoflagellates [4, 30, 31], a major biological source has yet to be discovered. We have recently detected high relative levels of 4-desmethyl-5α-stanols (40.3% of total sterols) in the unicellular alga *Thoracosphaera heimii* which, based on its lipid composition [24] and reproductive cycle [38], is a member of the Dinophyceae [10]. These limited data suggest that marine dinoflagellates may also be a source of 4-desmethyl-5α-stanols in marine sediments.

Nine sterols have been detected in *P. cordatum* (Table 3) with 4-methyl sterols and 4-desmethyl sterols representing 43.9% and 56.1% respectively of the total sterols. The distribution of sterols in *P. cordatum* is similar to that found in the unicellular alga FCRG 51 [10]. The significance of the observed sterol distribution pattern in terms of the side chain alkylation pathway has been discussed in that report.

The two 4-methyl-$\Delta^{24(28)}$ unsaturated sterols found in *P. cordatum* (**9** and **12**) are identical to the two unidentified 4-methyl sterols present in FCRG 51. The presence of 4,24-dimethyl-5α-cholest-24(28)-en-3β-ol (**9**) and either 4,23,24-trimethyl- or 4-methyl-24-ethyl-5α-



Scheme 1. Probable pathway of the sterol side-chain biosynthesis in *Prorocentrum cordatum*.

cholest-24(28)-en-3β-ol (**12**), in both *P. cordatum* and FCRG 51 indicates that the biosynthesis of the 23,24-dimethyl-Δ^{22} side chain (as in dinosterol) may involve other intermediate sterols than those suggested for the biosynthesis of the 23,24-dimethyl-Δ^{22} side chain of 4-desmethyl sterols (3β-hydroxy-$\Delta^5 \rightarrow$ 24-methyl-3β-hydroxy-$\Delta^{5,24(28)} \rightarrow$ 24-methyl-3β-hydroxy-$\Delta^{5,22} \rightarrow$ 23,24-dimethyl-3β-hydroxy-$\Delta^{5,22}$ [39]). The data for *P. cordatum* and FCRG 51 suggest that introduction of a methyl group at the C_{23} position may proceed before the 24-methylene group is reduced to a methyl group (Scheme 1). The similar distribution pattern of sterols, including relative levels of individual components, in *P. cordatum* and FCRG 51 is further confirmatory evidence that the alga FCRG 51 is a dinoflagellate [cf. 10] and indeed is related to the genus *Prorocentrum*.

Knowledge of the sterol compositions of members of the Dinophyceae is essential for both the interpretation of dietary conversions of sterols in the marine food web and the understanding of processes involving 4-methyl and 4-desmethyl sterols in sedimentary environments.

EXPERIMENTAL

Prorocentrum cordatum (clonal designation DINO-EX, Marine Science Laboratory, Queenscliff, Victoria) and *Gymnodinium wilczeki* (clonal designation NI-5) were aseptically maintained in f/2 medium [40] at 19° under 14:10 hr illumination:dark cycle provided by cool-white fluorescent lights. Cells in late log-phase were harvested for lipid analysis by filtration onto $CHCl_3$-washed glass fibre filters (Whatman GF/C) and transferred immediately to 75 ml $CHCl_3$–MeOH (2:1 + 0.05% pyridine) [8, 41].

Cell dry-wt was determined by filtering a known volume of cell culture onto a pre-weighed (Perkin-Elmer AM-2 Autobalance) 1.0 μ Nucleopore filter. The harvested cells and filter were rinsed 2 × with 5 ml 35% ammonium formate, dried overnight at 70° and reweighed. Cell density was determined microscopically using an Improved Neubauer haemacytometer.

Total neutral lipid and fatty acid methyl esters were obtained as described previously [25, 41]. The neutral lipid fraction, containing predominantly sterol components, was treated with BSTFA to produce the corresponding sterol-TMSi ethers. Fatty acid methyl esters and sterol-TMSi ethers were analysed using a nonpolar SE-30 wallcoated fused silica (12 m × 0.2 mm i.d.) capillary column, temperature programmed from 100° to 255° at 3° per min. Helium was used as the carrier gas (linear flow 20 cm/sec). Fatty acid methyl esters were also analysed on a polar SP1000 glass SCOT (100 m × 0.3 mm i.d.) capillary column temperature programmed from 100° to 220° at 1.5° per min. Fatty acid methyl esters were identified by co-chromatography with authentic standards (Analabs, Supelco, App. Sci.) and by ECL measurements [42–45]. Each lipid component was quantified from the calibrated FID response. Relative proportions are subject to maximum errors of ± 10%. Replicate GC analyses were performed in each case.

Chlorophyll *a*:*c* ratios and absolute abundances were determined using the equations of Jeffrey and Humphrey [46] for 90% Me_2CO solns of the solvent extracts (after solvent removal) using a Varian Superscan 3 scanning spectrometer. Sterol identifications were based on RR_t measurements, co-injection with authentic standards and by comparison of mass spectra with those of standards and previously reported spectra [10, 20, 27–29, 47, 48]. GS/MS analyses were performed on a HP 5995A GC-MS system fitted with an open split interface between the capillary (SE-30 vitreous silica, WCOT, 50 m × 0.2 mm) column and the mass spectrometer (Electron multiplier 1400 V). Major ions in the MS of the TMSi derivatives of sterols **9**, **12** and **13** were as follows. Sterol **9**; m/z (rel. int.): 486 $[M]^+$ (4), 471 (8), 402 (15), 387 (12), 359 (15), 269 (10), 229 (18), 121 (37), 109 (45), 95 (40), 81 (62), 75 (100). Sterol **12**; m/z (rel. int.): 500 $[M]^+$ (5), 485 (3), 402 (5), 387 (20), 359 (8), 297 (15), 283 (10), 121 (50), 95 (65), 57 (100); sterol **13** m/z (rel. int.): 500 $[M]^+$ (45), 485 (7), 410 (3), 395 (8), 369 (4), 359 (5), 342 (2), 269 (10), 243 (15), 227 (25), 173 (23), 161 (20), 147 (50), 131 (30), 105 (40), 81 (30), 75 (45), 73 (40), 57 (70), 43 (100).

Acknowledgements—P.D.N. and G.J.J. acknowledge University of Melbourne and C.P.R. Awards respectively. The authors thank Dr Bob Western, CSIRO, Division of Materials Science, for access to the HP 5995A GC-MS system, Darryl Perkins for technical assistance, Brett Errey for supplying the cultures, Dr R. Wetherbee for the use of his growth cabinet, Mr K. Blazé for his assistance with algal identification, and Mr D. Sewell for his help in obtaining scanning electron micrographs of *P. cordatum*.

REFERENCES

1. Kokke, W. C. M. C., Fenical, W. and Djerassi, C. (1981) *Phytochemistry* **20**, 127.
2. Alam, M., Schram, K. H. and Ray, S. M. (1978) *Tetrahedron Letters* 3517.
3. Kokke, W. C. M. C., Withers, N. W., Massey, I. J., Fenical, W. and Djerassi, C. (1979) *Tetrahedron Letters* 3601.
4. Wengrovitz, P. S., Sanduja, R. and Alam, M. (1981) *Comp. Biochem. Physiol.* **69B**, 535.
5. Djerassi, C. (1981) *Pure Appl. Chem.* **53**, 873.

6. Johns, R. B., Nichols, P. D. and Perry, G. J. (1979) *Phytochemistry* **18**, 799.
7. Volkman, J. K., Smith, D. J., Eglinton, G., Forsberg, T. E. V. and Corner, E. D. S. (1981) *J. Mar. Biol. Ass. U.K.* **61**, 509.
8. Johns, R. B., Nichols, P. D., Gillan, F. T., Perry, G. J. and Volkman, J. K. (1981) *Comp. Biochem. Physiol.* **69B**, 843.
9. Gillan, F. T., McFadden, G. I., Wetherbee, R. and Johns, R. B. (1981) *Phytochemistry* **20**, 1935.
10. Nichols, P. D., Volkman, J. K. and Johns, R. B. (1983) *Phytochemistry* **22**, 1447.
11. Shimuzu, Y., Alam, M. and Kobayashi, A. (1976) *J. Am. Chem. Soc.* **98**, 1059.
12. Boon, J. J., Rijpstra, W. I. C., de Lange, F., de Leeuw, J. W., Yoshioka, M. and Shimizu, Y. (1979) *Nature* **277**, 125.
13. Loeblich, A. R. (1977) *J. Phycol.* **13**, Suppl. 42.
14. Loeblich, A. R. and Fine, K. E. (1977) *Proc. Biol. Soc. Wash.* **90**, 388.
15. Gibbs, S. P., Chu, L. L. and Magnussen, C. (1980) *Phycologia* **19**, 1973.
16. Dodge, J. D. (1975) *Bot. J. Linn. Soc.* **71**, 103.
17. Orcutt, D. M. and Patterson, G. W. (1975) *Comp. Biochem. Physiol.* **50B**, 579.
18. Gagosian, R. B., Nigrelli, G. E. and Volkman, J. K. (1984) in *Nato Advanced Research Institute on Coastal Upwelling and its Sediment Record* (Suess, E. and Thiede, J., eds). (in press).
19. Jeffrey, S. W., Sielicki, M. and Haxo, F. T. (1975) *J. Phycol.* **11**, 374.
20. Volkman, J. K., Eglinton, G. and Corner, E. D. S. (1980) *Phytochemistry* **19**, 1809.
21. Harrington, G. W., Beach, D. H., Dunham, J. E. and Holz, G. G., Jr. (1970) *Protozool.* **17**, 213.
22. Mayzaud, P., Eaton, C. A., and Ackman, R. G. (1976) *Lipids* **11**, 858.
23. Joseph, J. D. (1975) *Lipids* **10**, 395.
24. Jones, G. J., Nichols, P. D. and Johns, R. B. (1983) *J. Phycol.* **19**, 416.
25. Perry, G. J., Volkman, J. K., Johns, R. B. and Bavor, H. J. (1979) *Geochim. Cosmochim. Acta* **43**, 1715.
26. Ackman, R. G. and McLachlan, J. (1978) *Proc. N. S. Inst. Sci.* **28**, 47.
27. Brooks, C. J. W., Horning, E. C. and Young, J. S. (1968) *Lipids* **3**, 391.
28. Knight, B. A. (1969) *J. Gas Chromatogr.* **5**, 273.
29. de Leeuw, J. W., Rijpstra, W. I. C., Schenck, P. A. and Volkman, J. K. (1983) *Geochim. Cosmochim. Acta* **47**, 455.
30. Bohlin, L., Kokke, W. C. M. C., Fenical, W. and Djerassi, C. (1981) *Phytochemistry* **20**, 2397.
31. Withers, N. W., Kokke, W. C. M. C., Fenical, W. and Djerassi, C. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3764.
32. Withers, N. W., Tuttle, R. C., Holz, G. G., Beach, D. H., Goad, L. J. and Goodwin, T. W. (1978) *Phytochemistry* **17**, 1987.
33. Miller, W. L. and Gaylor, J. L. (1970) *J. Biol. Chem.* **245**, 5375.
34. Mulheirn, L. J. and Ramm, P. J. (1972) *Chem. Soc.Rev.* **1**, 259.
35. Nishimura, M. and Koyama, T. (1977) *Geochim. Cosmochim. Acta* **41**, 379.
36. Nishimura, M. (1978) *Geochim. Cosmochim. Acta* **42**, 349.
37. Lee, C., Gagosian, R. B. and Farrington, J. W. (1977) *Geochim. Cosmochim. Acta* **41**, 985.
38. Tangen, K., Brand, L. E., Blackwelder, P. L. and Guillard, R. R. L. (1982) *Mar. Micropaleontology* **7**, 193.
39. Withers, N. W., Tuttle, R. C., Goad, L. J. and Goodwin, T. W. (1979) *Phytochemistry* **18**, 71.
40. Guillard, R. R. L. and Ryther, J. H. (1962) *Can. J. Microbiol.* **8**, 229.
41. Nichols, P. D., Klumpp, D. W. and Johns, R. B. (1982) *Phytochemistry* **21**, 1613.
42. Ackman, R. G. and Castell, J. D. (1967) *J. Gas Chromatogr.* **5**, 489.
43. Ackman, R. G., Sipos, J. C. and Jangaard, P. M. (1967) *Lipids* **2**, 251.
44. Ackman, R. G. (1972) in *Progress in the Chemistry of Fats and Other Lipids* (Holman, R. T., ed.) Vol. 12, pp. 165–284. Pergamon Press, Oxford.
45. Ackman, R. G., Hooper, S. N. and Hingley, J. (1972) *J. Chromatogr. Sci.* **10**, 430.
46. Jeffrey, S. W. and Humphrey, G. F. (1975) *Biochem. Physiol. Pflanzen* **167**, 191.
47. Alam, M., Sansing, T. B., Busby, E. L., Martinez, D. R. and Ray, S. M. (1979) *Steroids* **33**, 197.
48. Wardroper, A. M. K., Maxwell, J. R. and Morris, R. J. (1978) *Steroids* **32**, 203.