Phytochemistry, Vol. 22, No. 6, pp. 1447–1452, 1983.
Printed in Great Britain.

0031-9422/83/061447-06$03.00/0

STEROLS AND FATTY ACIDS OF THE MARINE UNICELLULAR ALGA, FCRG 51

PETER D. NICHOLS, JOHN K. VOLKMAN* and R. B. JOHNS

Organic Chemistry Department, University of Melbourne, Parkville, Victoria 3052, Australia

(*Received* 7 *September* 1982)

Key Word Index—Unicellular alga; Dinophyceae; Chloromonadophyceae; Chrysophyceae; fatty acids; sterols; lipids; chemotaxonomy.

Abstract—The fatty acids and sterols of the marine unicellular alga FCRG 51 have been determined. The fatty acids show a predominance of C_{18} and C_{22} polyunsaturated components, typical of the Dinophyceae. Eleven sterols were detected, of which eight have been identified. Both 4-methyl sterols (41%) and 4-desmethyl sterols (59%) are present and these exhibit a wider range of structural diversity than has been reported previously for dinoflagellates. 24-Methylenecholesterol, 23,24-dimethylcholesta-5,22*E*-dien-3β-ol and dinosterol are the three major sterols which together represent 75% of the total sterols. Dinosterol is found only in dinoflagellates and in some organisms which feed on dinoflagellates, but the two 4-desmethyl sterols are rarely reported in these algae. The lipid composition indicates that the unicellular alga FCRG 51 is related to the Dinophyceae and not to the Chloromonadophyceae or Chrysophyceae as previously proposed.

INTRODUCTION

Unicellular algae are important components of the phytoplankton and form the foundation of many marine food chains. The classification of unicellular algae is generally based on pigment composition, food storage products, flagella structure, cell wall structure and cell structure. Chemotaxonomic studies, including the analyses of fatty acid and sterol compositions, have also provided much information on algal classification [1–4]. The unicellular algal strain, FCRG 51, UTEX 2162, was first isolated at a depth of 2 m off the Pt. Loma sewerage outfall at San Diego, California. This organism was originally thought to be a dinoflagellate and was assigned to a species of *Exuviaella*. The pigment composition of FCRG 51 has been documented in a report on the chloroplast pigment patterns in dinoflagellates [5]. Both chlorophylls *a* and *c* (ratio 5.73) were present and fucoxanthin was the major carotenoid detected. In 1977 it was proposed that this alga was in fact conspecific with the marine chloromonad *Fibrocapsa japonica*, which along with *Olisthodiscus luteus* and *Heterosigma inlandica* was transferred to the marine chloromonad genus *Chattonella* [6, 7]. More recently, biochemical and ultrastructural evidence has been reported to show that *Olisthodiscus luteus* is a member of the Chrysophyceae [8]. Clearly, there is a need for further study on these organisms along with other algae of unknown taxonomic standing.

In this paper we report a detailed analysis of the fatty acid and sterol composition of the marine unicellular alga FCRG 51. Such data can be usefully used in assigning the taxonomic position of this alga and other related algae.

*Present address: School of Applied Chemistry, Western Australian Institute of Technology, Hayman Road, South Bentley, Western Australia, Australia.

RESULTS AND DISCUSSION

Lipid concentrations

Concentrations of the lipids in FCRG 51, expressed on a dry wt basis, are presented in Table 1. Few comparable data exist as most previous analyses have been limited to a single compound class, such as fatty acids, sterols or carotenoids [9–16]. Fatty acids are *ca* 17 times more abundant than sterols, which is much lower than the average value of 54 determined for 11 diatom species by Orcutt and Patterson [17]. An even higher value of 67 was determined for the Antarctic Sea ice diatom *Stauroneis amphioxys* [1]. The abundance of sterols in this alga, thus, seems to be slightly higher than that in the diatoms but the relative proportion of phytol is much lower than that in *S. amphioxys* [1]. Such data, when coupled with identification of the individual components, are important for assessments of the flux of planktonic organic matter to marine sediments and calculations of the rates of degradation of individual organic compounds in the marine environment [18].

n-Alkanols were present at low concentration in the

Table 1. Lipid composition of FCRG 51

	% composition (dry wt) of cells	Relative abundance
Fatty acids	9.0	1.0
Sterols	0.5	0.06
Phytol	0.06	0.007
Carotenoids	0.01	0.001
Chlorophylls*	0.18	0.02
Fatty alcohols	0.02	0.002

*Based on phytol measurement.

hydrolysis products suggesting the presence of wax esters in the original lipid extract. The alcohols were identified as 14:0 (5.6%), 16:0 (17.9%), 18:0 (71.4%), 20:0 (3.2%) and 22:0 (1.9%). Significant amounts of phytyl esters have been found in one dinoflagellate, *Peridinium foliaceum* [19], but, in general, high concentrations of wax esters are very uncommon in unicellular algae. Hydrocarbons were only trace components of the total lipid extract and were not examined further.

Pigment composition

The carotenoid composition of FCRG 51 has been reported previously [5], with fucoxanthin present as the major component along with carotene and four other unidentified yellow xanthophyll fractions. HPLC analysis of our sample confirmed the presence of carotene but insufficient material was available to identify the other carotenoids. The low abundance of carotenoids in our sample (0.01%) may be due to the delay between sample preparation and extraction. Loeblich and Fine [7], in reclassifying this alga to the Chloromonadophyceae, asserted that the dinoflagellate plastids did not contain fucoxanthin. The more recent report by Gibbs *et al.* [8] transferring *O. luteus* to the Chrysophyceae emphasized, in contrast, that marine chloromonads do not contain fucoxanthin. The absence of fucoxanthin in chloromonads has been also noted by Chapman and Haxo [20] in two freshwater unrelated members of the Chloromonadophyceae. The chlorophyll *a*:*c* ratio (*ca* 3.0) of *O. luteus* (FCRG 51 chlorophyll *a*:*c* = 5.73) also indicated that this alga was not a xanthophyte, since xanthophytes unlike dinoflagellates and chrysophytes have only traces of chlorophylls c_1 and c_2. The chlorophyll *a*:*c* ratios of xanthophytes were found to range from 55:1 to 116:1 [21]. Thus, based on pigment data alone (in particular the chlorophyll ratios), the unicellular alga FCRG 51 appears linked to either the Dinophyceae or Chrysphyceae.

Fatty acids

The major fatty acids detected in the alga FCRG 51 in decreasing order of abundance were: 16:0; 22:6ω3*, 18:5ω3, 18:4ω3, 18:2ω6, 20:5ω3, 18:1ω9 and 14:0 (Table 2). The high proportion of C_{18} and C_{22} polyunsaturated fatty acids (PUFA's = 48.9%) is in accord with previous analyses of marine dinoflagellates [13–16]. Similar distributions have been noted in several coccolithophores [2]. Members of the Chrysophyta, of which diatoms (Bacillariophyceae) are prominent constituents, usually show a very different distribution with 16:0 and 16:1ω7 predominating [22–24]. Another common feature of diatom lipids is that C_{18} fatty acids are very minor components and high relative concentrations of the C_{20} PUFAs 20:4ω6 and 20:5ω3 are present. Thus, based on its fatty acid composition, the alga FCRG 51 does not relate to the Chrysophyta as proposed by Gibbs [8].

The high abundance of the unusual fatty acid 18:5ω3 is particularly striking. This acid is found in many dinoflagellates [15, 16] and it has been proposed as a biological marker for dinoflagellate lipids in marine food chain studies [15]. Although it has been recently identified in several coccolithophorids [2], this acid is apparently not present in other genera of unicellular algae. Both potential precursors of this acid, viz. 18:4ω3 and 20:5ω3, are present in this alga, so we cannot confirm that 18:5ω3 is solely formed by β-oxidation of 20:5ω3, as suggested previously. Comparison of the fatty acid composition of FCRG 51 with algae of the genus *Exuviaella* or with members of the Chloromonadophyceae is not possible since lipid analyses of these algae have not been previously reported.

Several monounsaturated fatty acids were detected in FCRG 51. In decreasing order of abundance these were: 18:1ω9, 16:1ω7, 20:1ω7, 20:1ω9 and 18:1ω7. Oleic acid (18:1ω9) represented over 60% of the total monoenoic acids (Table 2). The predominance of Δ^9 isomers, such as 18:1ω9 and 16:1ω7, suggests the presence of a Δ^9 desaturase which acts primarily on 18:0 and 16:0. Of the few analyses of fatty acids in dinoflagellates, only one presents details of the double bond isomers [16]. The presence of small quantities of 18:1ω7 in the alga FCRG 51 should be noted. This acid is more commonly associated with bacterial biosynthesis but its isolation here, and from the dinoflagellate *Prorocentrum minimum* [16], is further evidence for its widespread distribution in marine algae [2, 4, 22].

The finding of 18:5ω3 in the alga FCRG 51, along with the similar distribution of fatty acids to that found in

Table 2. Fatty acid composition of FCRG 51

	Acid	ECL*	% composition
	14:0	14.00	3.4
	14:1	14.15	0.05
	15:0	15.00	TR
	16:0	16.00	32.8
	16:1ω7	16.32	2.0
trans	16:1ω13	16.41	TR
	16:1ω5	16.44	TR
	18:0	18.00	1.9
	18:1ω9	18.25	5.7
	18:1ω7	18.33	0.2
	18:2ω6	18.77	6.4
	18:3ω6	19.07	0.3
	18:3ω3	19.42	1.6
	18:4ω3	19.76	6.8
	20:0	20.00	2.4
	18:5ω3	20.22	11.3
	20:1ω9	20.26	0.3
	20:1ω7	20.32	0.6
	20:4ω6	21.25	0.9
	20:5ω3	21.98	3.5
	22:0	22.00	0.7
	22:5ω3	24.04	0.2
	22:6ω3	24.27	17.9
PUFAs†		—	48.9
Saturated fatty acids		—	41.2
Monoenoic fatty acids		—	8.85
Total concentration (% dry wt)		—	9.0

*Equivalent chain length (ECL) on SIL-47-CNP.
†PUFAs, Polyunsaturated fatty acids with ⩾ 2 double bonds.
TR, trace, < 0.05%.

*Double bonds are numbered from the methyl end of the molecule (ω notation) or from the carboxyl end (Δ notation); all subsequent double bonds are methylene interrupted.

other dinoflagellates, suggests that a relationship of this alga to the Dinophyceae exists. An alternative view that the acid 18:5ω3 may be distributed more widely than previously realized is a possibility. It is apparent, however, that further related unicellular algae need to be analysed before classification based on fatty acid data alone can be achieved conclusively.

Sterols

Eleven different sterols were detected in FCRG 51 (Table 3) and these can be conveniently grouped, on biosynthetic grounds, according to whether they have a methyl group at C-4 (4-methyl sterols) or not (4-desmethyl sterols). Six 4-desmethyl sterols were identified and these represented 59% of the total sterols. The major 4-desmethyl components were 24-methylcholest-5,24(28)-dien-3β-ol (24-methylene cholesterol) (36.5%) and 23,24-dimethylcholesta-5,22*E*-dien-3β-ol (19.0%). Five 4-methyl sterols were present, representing 41% of the total sterols, of which only two were identified. These were 4α,23,24-trimethyl-5α-cholest-22-en-3β-ol (dinosterol) (20.0%) and 4α,24-dimethyl-5α-cholest-22*E*-en-3β-ol (3.8%).

The sterol compositions of several species of dinoflagellates have been studied [9–12, 25] following the identification of the unusual sterol dinosterol in the photosynthetic dinoflagellate *Gonyaulax tamarensis* [9]. This sterol appears to be unique to dinoflagellates and, thus, has been used as a 'biological marker' for dinoflagellate input to marine sediments [26].

Djerassi [27] has drawn attention to the presence of trace amounts of 22*S*,23*S*-methylenecholesterol in some marine organisms and suggested that it may be misidentified as 24-methylenecholesterol due to its similar mass spectrum and GC R_t. It is important to discriminate between these two sterols since their presence can be an indication of the biosynthetic pathway of side chain alkylation operating in the alga. To confirm that the major sterol in FCRG 51 was indeed 24-methylenecholesterol, we hydrogenated an aliquot of the total sterol mixture. This produced three major sterols, the most abundant of which had a mass spectrum identical to that of 24-methyl-5α-cholestan-3β-ol and coeluted with it by capillary GC. This is the expected hydrogenation product of 24-methylenecholesterol and, thus, rules out a 22,23-methylene side chain for the major sterol in FCRG 51. The other major sterols in the hydrogenation product were identified, from their mass spectra and GC elution times, as 23,24-dimethyl-5α-cholestan-3β-ol and 4α-23,24-trimethyl-5α-cholestan-3β-ol (dinostanol), thus supporting the sterol identifications in Table 3. The identity of the minor hydrogenation products, which may help to elucidate the structures of sterols 8, 9 and 11 are under investigation.

The structural diversity of sterols found in FCRG 51 is not typical of the dinoflagellate species analysed to date. For example, many species of *Gonyaulax* contain only cholest-5-en-3β-ol (cholesterol) (42–65%) and dinosterol [28]. In the alga, FCRG 51, cholesterol is a very minor sterol and its role as a membrane constituent seems to be taken by sterols alkylated in the side chain. Few dinoflagellates contain high proportions of C_{28} and C_{29} 4-desmethyl sterols; many contain cholesterol [28] and a few contain other C_{27} sterols, such as cholesta-5,7-dien-3β-ol [12] and cholesta-7,22-dien-3β-ol [12]. Exceptions include *Gonyaulax monilata*, which contains gorgostanol and an unusual sterol, 23-methylene-24-methylcholestanol [29], and the freshwater dinoflagellate, *Ceratium hirundinella*, which has been reported to contain 24-methylcholestanol [30]. A number of C_{28} and C_{29} stenols (sterols containing a nuclear double bond) and stanols have been identified in the culture medium of a bioluminescent dinoflagellate *Pyrocystis lunula* [31]. However, many species have yet to be studied and preliminary data (unpublished) suggests that the range of sterols biosynthesized by dinoflagellates may be wider than the present limited number of reports indicate.

The predominance of 24-methylenecholesterol in FCRG 51 is unusual. This sterol has not previously been reported as the major sterol in dinoflagellates but, rather, is more common in diatoms, where it can be the major sterol present [32]. Small amounts of 24-methylenecholesterol have been isolated from the culture medium of

Table 3. Sterol composition of FCRG 51

Sterol	RR_t*	Scan No†	MW‡	Identification	% of total sterols
1	1.00	2458	458	Cholest-5-en-3β-ol	0.4
2	1.12	2503	470	24-Methylcholesta-5,22*E*-dien-3β-ol	1.1
3	1.28	2564	470	24-Methylcholesta-5,24(28)-dien-3β-ol	36.5
4	1.32	2574	472	24-Methyl-5α-cholestan-24(28)-en-3β-ol	0.2
5	1.39	2599	484	23,24-Dimethylcholesta-5,22*E*-dien-3β-ol	19.0
6	1.42	2613	486	23,24-Dimethyl-5α-cholest-22*E*-en-3β-ol	1.5
7	1.47	2626	486	4α,24-Dimethyl-5α-cholest-22*E*-en-3β-ol	3.8
8	1.60	2673	484	4α-24-Dimethylcholesta-5,7-dien-3β-ol	1.8
9	1.66	2695	486	Unidentified; 4-methyl sterol §	6.8
10	1.79	2745	500	4α,23,24-Trimethyl-5α-cholest-22-en-3β-ol	20.0
11	1.95	2798	500	Unidentified; 4-methyl sterol ‖	8.9

*RR_t, cholesterol 1.00; 24-ethylcholesterol 1.63.

†GC/MS scans are 1 sec apart.

‡MW of TMSi ether derivative.

§Identified as 4,24-dimethyl-5α-cholest-24(28)-en-3β-ol.

‖Identified as either 4,23,24-trimethyl- or 4-methyl-24-ethyl-5α-cholest-24(28)-en-3β-ol.

4-Desemethyl sterols, 58.8%.

4-Methyl sterols, 41.3%.

Pyrocystis lunula [31], and it has been identified in some dinoflagellate zooxanthellae [33]. The nuclear saturated analogue, 24-methylenecholestanol, has been reported in a freshwater dinoflagellate but the concentration of this stanol is very low (0.2%) in FCRG 51. Another minor sterol in FCRG 51 is 24-methylcholesta-5,22*E*-dien-3β-ol (1.1%). This sterol is rarely found in dinoflagellates although it is common in diatoms [17] and some coccolithophorids [2].

Another unusual feature of the sterol distribution is the abundance of 23,24-dimethylcholesta-5,22*E*-dien-3β-ol (19% of total sterols). This sterol was first isolated from a soft coral [34] and since then it has been identified in molluscs [35], a diatom [22] and a coccolithophore [2]. There are no reports of its isolation from dinoflagellates despite the abundance of its ring saturated 4-methyl analogue, dinosterol, in these algae [12]. However, this sterol has been detected in a number of marine sediments [36, 37] and it seems likely that it is far more widespread in marine organisms than the present, very limited data, indicate. Since unicellular algae form the base of the marine food web, it is probable that its presence in higher marine organisms, such as corals and molluscs, is due to ingestion of algae in their diet.

Sterol biosynthesis

Withers *et al.* [38] have studied the biosynthesis of the 23,24-dimethyl-Δ^{22} side chain of dinosterol using experiments in which CD_3-labelled methionine was incorporated into the marine dinoflagellate *Crypthecodinium cohnii*. They proposed that the C-24 methyl group is introduced first via a 24-methylene intermediate. The C-22 double bond is then introduced followed by insertion of the C-23 methyl group by transmethylation from the methionine donor. A similar mechanism could account for all the structural features of both the 4-methyl and 4-desmethyl series of sterols in FCRG 51. In the 4-desmethyl sterol series the probable biosynthetic route is: cholesterol → 24-methylenecholesterol → 24-methylcholesta-5,22*E*-dien-3β-ol → 23,24-dimethylcholesta-5,22*E*-dien-3β-ol (Fig. 1). Our inability to detect 24-methylcholesterol in FCRG 51 does not invalidate the proposed pathway but implies only that this sterol is not accumulated by this alga, in common with most unicellular algae.

The alternative possibility that the C-23 methyl group is introduced prior to the C-24 group appears to be ruled out since no 4-desmethyl sterols were detected which contained either a methyl or a methylene group at C-23 and not C-24. Such sterols have been isolated from a few marine organisms, including a dinoflagellate [10]. Djerassi [27] has argued that the direct alkylation of a C-22 double bond at C-23, in the absence of a C-24 substituent, is a feasible process. It may be that several pathways of C-24 alkylation have developed in different unicellular algae.

Small quantities of two 4-desmethyl sterols with saturated ring systems (stanols) were identified in FCRG 51 (Table 3). Stanols do not appear to be common in unicellular algae [22, 38], although a few species have been reported to contain low concentrations [25, 39, 40]. It is noteworthy that the stanol–stenol ratio is very low for the 4-desmethyl sterol fraction, but the reverse is true for the 4-methyl sterols. Small quantities of 4-desmethyl stanols have been isolated from dinoflagellates [12, 25, 30] suggesting that this biochemical feature may be common within this algal group. Dinoflagellates are a likely source of 5α-stanols in marine sediments. In oxic environments, the more facile degradation of unsaturated sterols is likely to lead to much higher stanol–stenol ratios than those typically found in algae.

No data are available concerning the mechanism by which stanols are biosynthesized by dinoflagellates but, perhaps, the mechanism is analogous to that operative in animals and bacteria; that is the Δ^5-sterol is first oxidized to a 3-oxo-Δ^5-sterol, which rearranges to the 3-oxo-Δ^4 system, which in turn is reduced to the 5α-stanol. The only report of 3-oxo steroids in dinoflagellates is the identification of dinosterone in *Crypthecodinium cohnii* [12], but this compound class has not been the subject of detailed study. No steroid ketones were detected in the total neutral extract of FCRG 51 but it is possible that they were present in concentrations below our GC/MS detection limits (*ca* 0.1% of total sterols).

The predominance of dinosterol (4α,23,24-trimethyl-5α-cholest-22*E*-en-3β-ol) in the 4-methyl sterol fraction of FCRG 51 is shared by most, if not all, marine dinoflagellates. Dinosterol has not been isolated from any other class of unicellular algae, which has prompted its use as a 'biological marker' for dinoflagellate lipids in marine sediments [26]. The only other 4-methyl sterol which was fully identified was sterol 7 (4α,24-dimethyl-5α-cholest-22*E*-en-3β-ol). This sterol has a very similar mass spectrum to that of the 4-desmethyl analogue [36], with the exception that ions involving the A ring are shifted up 14 amu due to the 4-methyl group. A similar compound, 4α,23-dimethyl-5α-cholest-22-en-3β-ol (24-desmethyldinosterol), has been found in a dinoflagellate, *Gonyaulax diagenesis* [10], which supports the view of Djerassi [27] that alkylation of a Δ^{22} double bond at C-23 does occur in nature. Our assignment of the side chain methyl group in

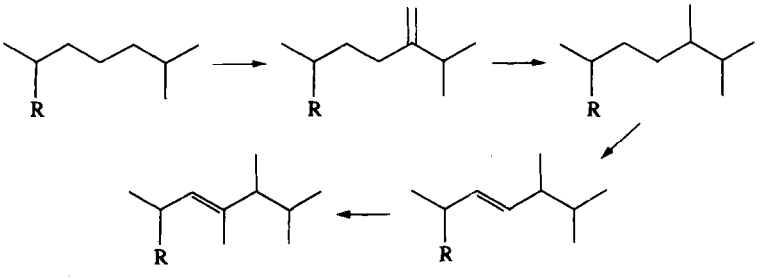

Fig. 1. Probable biosynthetic pathway of the 4-desmethyl sterol series in the alga, FCRG 51.

sterol 7 to C-24 and not C-23 is, thus, of interest. This identification derives from the base peak in the mass spectrum being m/z 69 which we take to be indicative of cleavage of a C_5 group α to the Δ^{22} double bond. This feature is particularly prominent in the spectra of TMSi ethers of 23,24-dimethyl sterols, such as dinosterol, where cleavage α to the Δ^{22} double bond is further enhanced by the 23-methyl group. Side chain cleavages in 24-desmethyldinosterol would not yield the m/z 69 ion since cleavage α to the double bond would yield a C_4 fragment. 4α,24-Dimethylcholest-22-en-3β-ol is probably the biosynthetic precursor of dinosterol and its presence in the alga FCRG 51 provides support for the suggestion by Withers *et al.* [38] that alkylation occurs first at C-24 and then at C-23. This may not be true for all dinoflagellates, but in FCRG 51 it seems very likely that the side chains of both the 4-methyl and 4-desmethyl series of sterols are biosynthesized by the same sequence of alkylations.

Another characteristic feature of dinoflagellates is their ability to biosynthesize a number of other 4-methyl sterols. However, a discussion of the other 4-methyl sterols present in FCRG 51 must await a more detailed study of their structure. In sediments where much of the organic matter is derived from dinoflagellates, as in the Black Sea, a variety of 4-methyl sterols have been identified [41] and it is thought that these components are derived from dinoflagellates. Several of these sterols have yet to be isolated from any aquatic organism and a more detailed search for such lipids in marine plants and animals would be valuable in the interpretation of the organic geochemistry of these Recent sediments.

Chemotaxonomic conclusions

The presence of the 'biological marker' for dinoflagellates, dinosterol, in the unicellular alga, FCRG 51, along with the occurrence of a number of 4-methyl sterols, which are also characteristic of members of the Dinophyceae, would appear to provide strong grounds for assigning this alga to the Dinophyceae. Sterols have proved to be strong biological taxonomic markers to date and, in the case of FCRG 51, there is strong back-up evidence provided by the data from fatty acid and pigment analyses. The strength in the use of these chemical lipid markers as taxonomic indicators increases in the order fatty acids < pigments < sterols in the particular example under study. Taken together, the evidence presented here points strongly to the classification of the unicellular marine alga, FCRG 51, as a member of the Dinophyceae and not to the Chloromonadophyceae or Chrysophyceae as previously proposed [6–8]. Further lipid analyses, of the type described here, of similar algae which have been placed in the Chloromonadophyceae [6, 7] and in the Chrysophyceae [8], based on a variety of cell ultrastructure determinations, would be taxonomically of much importance and clearly needs to be undertaken before classification of these algae can be taken as definitive.

EXPERIMENTAL

A freeze-dried sample of the autotrophic, unialgal, bacteria free unicellular alga, FCRG 51, was obtained from the Scripps Institution of Oceanography culture collection (clonal designation PY-37). Culture conditions have been described previously [5]. Total lipid was obtained by direct saponification of the algal cells in 5.0% KOH in aq. MeOH (pH 12) under reflux for 2 hr. Neutral lipids were extracted into heptane–$CHCl_3$ (4:1) and, after acidification (pH 2), the fatty acids were similarly extracted. Fatty acids were converted to Me esters using BF_3–MeOH, and purified by TLC [4]. The neutral lipid fraction, containing predominantly sterol and alcohol components, was treated with BSTFA to produce the corresponding TMSi ethers. Fatty acid Me esters and sterol-TMSi ethers were analysed by FID GC using a SE30 glass SCOT (45 m × 0.3 mm i.d.) capillary column, temp. programmed from 140° to 280° at 2°/min. He was used as carrier (linear flow: 20 cm/sec). The fatty acid Me esters were also analysed on a polar SIL-47-CNP glass WCOT (43 m × 0.2 mm i.d.) capillary column temp. programmed from 100° to 240° at 3°/min. Injector and manifold temps. of 280° and 300°, respectively, were used. Fatty acid Me esters were identified by co-chromatography with authentic standards and by ECL measurements [42–45]. Each lipid component was quantified from the calibrated FID response. Relative proportions are subject to maximum errors of ±5%.

Sterol identifications were based on co-injection with authentic standards, RR_t measurements and by comparison of MS with standards and previously reported spectra. An aliquot of the total neutral fraction, after the addition of MeOH and Adams catalyst, was hydrogenated for 3 hr with mechanical agitation in a Parr Hydrogenator (270 kPa, 25°). C-GC/MS conditions have been described previously [22]. Major ions in the MS of the TMSi derivatives of each of the sterol components were as follows. Sterol 2 m/z (rel. int.): 470 $[M]^{+\cdot}$ (12), 380 (13), 340 (15), 282 (7), 255 (27), 129 (50), 69 (100); sterol 3: 470 $[M]^{+\cdot}$ (8), 455 (7), 386 (28), 341 (27), 257 (15), 129 (100); sterol 4: 457 (5), 388 (66), 345 (30), 255 (27), 215 (30), 75 (100); sterol 5: 484 $[M]^{+\cdot}$ (4), 394 (3), 372 (5), 351 (4), 343 (10), 323 (4), 255 (16), 139 (16), 129 (13), 97 (16), 83 (23), 69 (100); sterol 6: 374 (6), 345 (16), 257 (18), 139 (11), 97 (24), 83 (25), 69 (100); sterol 7: 486 $[M]^{+\cdot}$ (16), 471 (4), 388 (13), 359 (18), 271 (44), 229 (6), 69 (100); sterol 8: 484 $[M]^{+\cdot}$ (73), 393 (37), 379 (10), 355 (4), 267 (26), 241 (29), 227 (40), 69 (100); sterol 9: 471 (10), 402 (100), 387 (13), 359 (48), 297 (20), 269 (30), 229 (35); sterol 10: 500 $[M]^{+\cdot}$ (3), 388 (7), 359 (19), 271 (21), 139 (10), 97 (24), 83 (25), 69 (100); sterol 11: 500 $[M]^{+\cdot}$ (5), 485 (6), 402 (15), 387 (85), 359 (27), 297 (61), 283 (40), 121 (66), 95 (97), 57 (100).

Acknowledgements—We thank Professor F. Haxo of Scripps Institution of Oceanography for supplying the algal sample, for pertinent comments and generous help during the preparation of the manuscript; Professor G. Eglinton, University of Bristol, for access to the NERC financed C-GC-MS (GR3/2951 and GR3/3758). J.K.V. acknowledges support from NERC grant GR3/3419 while at the University of Bristol. P.D.N. acknowledges a University of Melbourne postgraduate research award. The ARGC is acknowledged for partial financial support.

REFERENCES

1. Gillan, F. T., McFadden, G. I., Wetherbee, R. and Johns, R. B. (1981) *Phytochemistry* **20**, 1935.
2. 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.
3. Johns, R. B., Nichols, P. D., Gillan, F. T., Perry, G. J. and Volkman, J. K. (1981) *Comp. Biochem. Physiol.* **69B**, 843.
4. Johns, R. B., Nichols, P. D. and Perry, G. J. (1979) *Phytochemistry* **18**, 799.
5. Jeffrey, S. W., Sielicki, M. and Haxo, F. T. (1975) *J. Phycol.* **11**, 374.
6. Loeblich, A. R. (1977) *J. Phycol. (Suppl.)* **13**, 42.
7. Loeblich, A. R. and Fine, K. E. (1977) *Proc. Biol. Soc. Wash.* **90**, 388.

8. Gibbs, S. P., Chu, L. L. and Magnussen, C. (1980) *Phycologia* **19**, 173.
9. Shimizu, Y., Alam, M. and Kobayashi, A. (1976) *J. Am. Chem. Soc.* **98**, 1059.
10. Alam, M., Schram, K. H. and Ray, S. M. (1978) *Tetrahedron Letters* 3517.
11. Withers, N. W., Kokke, W. C., Rohmer, M., Fenical, W. H. and Djerassi, C. (1979) *Tetrahedron Letters* 3605.
12. Withers, N. W., Tuttle, R. C., Holz, G. G., Beach, D. H., Goad, L. J. and Goodwin, T. W. (1978) *Phytochemistry* **17**, 1987.
13. Wood, B. J. B. (1974) in *Botanical Monographs* (Stewart, W. D. P., ed.), Vol. 10, pp. 236–265. Blackwell, Oxford.
14. Harrington, G. W., Beach, D. H., Dunham, J. E. and Holz, G. G., Jr. (1970) *J. Protozool.* **17**, 213.
15. Mayzaud, P., Eaton, C. A. and Ackman, R. G. (1976) *Lipids* **11**, 858.
16. Joseph, J. D. (1975) *Lipids* **10**, 395.
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. (1982) in *Nato Advanced Research Institute on Coastal Upwelling and its Sediment Record* (Suess, E. and Thiede, J., eds.) (in press).
19. Withers, N. W. and Nevenzel, J. C. (1977) *Lipids* **12**, 989.
20. Chapman, D. J. and Haxo, F. T. (1966) *J. Phycol.* **2**, 89.
21. Jeffrey, S. W. (1976) *J. Phycol.* **12**, 349.
22. Volkman, J. K., Eglinton, G. and Corner, E. D. S. (1980) *Phytochemistry* **19**, 1809.
23. De Mort, C. L., Lowry, R., Tinsley, I. and Phinney, H. K. (1972) *J. Phycol.* **8**, 211.
24. Volkman, J. K. and Johns, R. B. (1977) *Nature (London)* **267**, 693.
25. Bohlin, L., Kokke, W. C. M. C., Fenical, W. and Djerassi, C. (1981) *Phytochemistry* **20**, 2397.
26. Boon, J. J., Rijpstra, W. I. C., de Lange, F., de Leeuw, J. W., Yoshioka, M. and Shimizu, Y. (1979) *Nature (London)* **277**, 125.
27. Djerassi, C. (1981) *Pure Appl. Chem.* **53**, 873.
28. Alam, M., Sansing, T. B., Busby, E. L., Martinez, D. R. and Ray, S. M. (1979) *Steroids* **33**, 197.
29. Wenogrovitz, P. S., Sanduja, R. and Alam, M. (1981) *Comp. Biochem. Physiol. B* **69**, 535.
30. Cranwell, P. A. (1976) *Freshwater Biol.* **6**, 41.
31. Ando, T. and Barbier, M. (1975) *Biochem. Syst. Ecol.* **3**, 245.
32. Kates, M., Tremblay, P., Anderson, R. and Volcani, B. E. (1978) *Lipids* **13**, 34.
33. Steudler, P. A., Schmitz, F. J. and Ciereszko, L. S. (1977) *Comp. Biochem. Physiol. B* **56**, 385.
34. Kanazawa, A., Teshima, S., Ando, T. and Tomita, S. (1974) *Bull. Jpn. Soc. Sci. Fish.* **40**, 729.
35. Ando, T., Kanazawa, A., Teshima, S. and Miyawaki, H. (1979) *Mar. Biol.* **50**, 169.
36. Wardroper, A. M. K., Maxwell, J. R. and Morris, R. J. (1978) *Steroids* **32**, 203.
37. Volkman, J. K., Gillan, F. T., Johns, R. B. and Eglinton, G. (1981) *Geochim. Cosmochim. Acta.* **45**, 1817.
38. Withers, N., Tuttle, R. C., Goad, L. J. and Goodwin, T. W. (1979) *Phytochemistry* **18**, 71.
39. Ballantine, J. A., Lavis, A. and Morris, R. J. (1979) *Phytochemistry* **18**, 1459.
40. Nishimura, M. and Koyama, T. (1977) *Geochim. Cosmochim. Acta.* **41**, 379.
41. De Leeuw, J. W., Rijpstra, W. I. C., Schenck, P. A. and Volkman, J. K. (1983) *Geochim. Cosmochim. Acta.* (in press).
42. Ackman, R. G. and Castell, J. D. (1967) *J. Gas. Chromatogr.* **5**, 489.
43. Ackman, R. G., Sipos, J. C. 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.