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STEROLS AND FATTY ACIDS OF THE MARINE DIATOM *BIDDULPHIA SINENSIS*

JOHN K. VOLKMAN, GEOFFREY EGLINTON and ERIC D. S. CORNER*

Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol, BS8 1TS, U.K.; * Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, U.K.

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Abstract—Nine sterols, most showing Δ^5- or $\Delta^{5,22}$-unsaturation, were identified in the marine diatom *Biddulphia sinensis*. One sterol, cholesta-5,22*E*-dien-3β-ol, comprised 70–80% of the total sterols which is the first such predominance noted in a diatom. The only Δ^7-sterol detected was cholest-7-en-3β-ol and this was a very minor component. A sterol showing unusual side-chain alkylation, 23,24-dimethylcholesta-5,22*E*-dien-3β-ol, was identified for the first time in a diatom. Total fatty acids exhibited a predominance of Δ^9-16:1, 14:0, 20:5 and 16:0, typical of diatoms, although the proportions of these acids were found to vary with culture maturity. *n*-Heneicosahexaene was the major hydrocarbon together with a small amount of squalene.

INTRODUCTION

Despite the abundance and species diversity of diatoms in the marine environment and their fundamental importance in the marine food web, there are still relatively few studies of their lipid content. Available data suggest widely differing sterol distributions although comparisons are hampered by many erroneous reports occurring in the early literature prior to the more widespread use of techniques such as capillary GLC and GC–MS. As part of a study of the lipid metabolism of zooplankton [1, 2] and a wider study of lipids, including those from diatoms, in sediments [3, 4] we have identified the total sterols, fatty acids and hydrocarbons of the marine diatom *Biddulphia sinensis* Grev. (Bacillariophyceae, Bacillariales).

RESULTS AND DISCUSSION

Concentrations of total fatty acids, sterols, phytol and hydrocarbons for two laboratory cultures of the large marine diatom *Biddulphia sinensis* are shown in Table 1. Comparisons with other species are hampered by the lack of appropriate data, as in only a few studies has more than one lipid class been examined. An exception is the useful work by Orcutt and Patterson [5] who studied 11 different species. They determined average fatty acid and sterol concentrations of 12.5 and 0.23% of dry wt respectively, which are considerably higher than the corresponding values for *B. sinensis*. This is a reflection of the unusually low lipid content of this diatom (7% of dry wt) which was also noted by Klenk and Eberhagen [6] in a study of a natural phytoplankton sample reported to be mainly *B. sinensis*. The only other *Biddulphia* species thus far examined for lipid content is *B. aurita* and this too showed a low lipid content [5] despite a very different cell size and habitat compared with *B. sinensis*. This probably reflects, some extent, the highly silicified nature of the *Biddulphia* cell wall.

Sterols

Analysis of the total sterol fraction by computerized capillary GC–MS indicated the presence of nine major sterols and some components present at relative

Table 1. Lipid concentrations in *B. sinensis*

	Sample 2		Sample 3	
	(pg/cell)§	%Dry wt*	(pg/cell)§	%Dry wt†
Total sterols	7.2	0.02	16	0.073
Total fatty acids	460	1.4	470	2.1
Total phytol	8.6	0.025	13	0.059
Cholesta-5,22*E*-dien-3β-ol	5.9	0.017	12	0.054
n-Heneicosahexaene	0.33	0.001	0.40	0.002
Chlorophyll *a*‡	26	0.076	39	0.17

* Based on an average cell mass of 3.4×10^{-8} g.
† Based on an average cell mass of 2.2×10^{-8} g.
‡ Based on the total phytol measurement.
§ Values are believed accurate to ± 10%
Dry wt averaged 10.5% of wet wt.

concentrations less than 0.2% (Table 2). Virtually all of this sterol fraction was solvent-extractable from the diatom and no indication of steryl esters or conjugates was obtained. One sterol, cholesta-5,22*E*-dien-3β-ol (dehydrocholesterol), predominated and represented 70–80% of the total sterols. This C_{27} sterol was tentatively identified in *Nitzschia ovalis* (33% of total sterols) on the basis of GLC retention time [5], in a natural sample of *Thalassionema nitzschiodes* (22.7%) [7] and as a trace constituent of *Nitzschia closterium* [5]. It has apparently not been detected in other diatoms. It is found in small amounts throughout the marine environment [4, 5] but significant amounts have only been reported in red algae [8], some jellyfish [9] and scallops [10]. Analysis on the capillary GLC columns confirmed the absence of the 22*Z*(*cis*)-isomer as this could be completely separated from the 22*E*-isomer on the columns used. The biosynthesis of this sterol has not yet been elucidated, but if it involves direct insertion of the double bond into the saturated side-chain of cholesterol the process is obviously highly specific.

The second most abundant sterol, 24-methylcholesta-5,22*E*-dien-3β-ol, is found in many diatoms, sometimes as the major component [11, 12]. Studies of this sterol isolated from *Phaeodactylum tricornutum* [11] and *Nitzschia closterium* [5] indicate that the 24-methyl group has the *S*(α) configuration which is opposite to that of 24-methylsterols synthesized by green algae. Minor sterols in *B. sinensis* included 24-methylcholest-5-en-3β-ol and its probable precursor 24-methylenecholest-5-en-3β-ol. Both sterols are common in diatoms and the latter is the major sterol in *Nitzschia alba* [13], a non-photosynthetic diatom, and in *Chaetoceros simplex calcitrans* [14]. The low abundance of cholesterol in *B. sinensis* is fairly typical of diatoms although a few exceptions have been noted [5, 14, 15]. 24-Ethylidenecholest-5-en-3β-ol is also common but it is of interest that no reduction of the double bond has occurred to produce 24-ethylcholest-5-en-3β-ol.

The unusual sterol, 23,24-dimethylcholesta-5,22-dien-3β-ol, has not to our knowledge been reported previously in diatoms. This sterol has been detected in some molluscs [16], a soft coral [17] and in a diatomaceous ooze [4], and in each instance an algal origin is possible. It is thought to be the biosynthetic precursor of gorgosterol (22,23-methylene-23,24-dimethylcholest-5-en-3β-ol) although no trace of this C_{30} sterol could be detected in *B. sinensis* presumably due to the lack of the appropriate enzyme. In the dinoflagellate *Crypthecodinium cohnii* [18] the 4α-methyl analogue, dinosterol (4α,23,24-trimethylcholesta-5,22-dien-3β-ol), has been shown to arise via a direct transmethylation at the C_{23} position by a methyl group from methionine. A similar transmethylation probably produces the 23,24-dimethylsterol in *B. sinensis*. We have also identified 23,24-dimethylcholesta-5,22-dien-3β-ol as one of the three sterols synthesized by the unicellular flagellate *Hymenomonas carterae* (Haptophycea) [19] and it seems likely that further work will indicate its presence in other unicellular algae.

The predominance of Δ^5- and $\Delta^{5,22}$-sterols in *B. sinensis* (90% of total sterols, Table 2) has been noted in other diatoms although in one study [5] several diatoms were reported to contain significant amounts of sterols containing Δ^7-unsaturation. The only Δ^7-sterol identified in *B. sinensis* was cholest-7-en-3β-ol and this was only a very minor constituent (0.4% of total sterols). No sterols lacking a nuclear double bond (i.e. stanols) were detected in common with most other diatom analyses, [7] the only exception being the freshwater diatom *Melosira granulata* which contains small amounts (1.2% of total sterols) of 5α-stanols [15].

The proportions of the sterols in each of the three samples were very similar, the only significant changes being in the amounts of cholesta-5,22*E*-dien-3β-ol and 24-methylcholesta-5,22*E*-dien-3β-ol. This is not attributable to differences in the extraction procedures used since analyses of a single sample indicated that the amount of sterol obtained by solvent extraction and by saponification were virtually identical. Rather they must reflect some variation in the culture conditions used although we have no data to suggest which environmental factor is important. Previous work with *Nitzschia closterium* [20] has shown that light intensity can alter the proportions of total sterols occurring in free, esterified and conjugate forms but no study has been made of environmental influences on the proportions of individual sterols. Our data suggest that culture age may be an important factor and that this may be manifested at the level of C_{24} alkylation. Culture maturity may also account for the different sterol concentrations in the two samples (Table 1). Interestingly the ratio of phytol to total sterols showed less variation between the samples, the average value for the ratio of chlorophyll *a* to sterols being *ca* 3:1 for this diatom.

Table 2. Percentage composition of the total sterol fraction of *B. sinensis*

	Co-injected*	RR_t†	Sample 1	Sample 2	Sample 3
Cholesta-5,22*E*-dien-3β-ol (**1**)	+	2.22	81.7	70.1	75.9
Cholest-5-en-3β-ol (**2**)	+	2.45	2.4	0.9	3.0
Unknown $\Delta^{5,22}$-C_{28} (**3**)	–	2.63	2.4	2.2	4.2
24-Methylcholesta-5,22*E*-dien-3β-ol (**4**)	+	2.76	6.8	17.3	11.2
Cholest-7-en-3β-ol (**5**)	–	2.84	0.4	0.4	0.3
24-Methylenecholest-5-en-3β-ol (**6**)	+	3.17	2.7	4.0	3.1
24-Methylcholest-5-en-3β-ol (**7**)	+	3.25	1.0	1.5	0.3
23,24-Dimethylcholesta-5,22*E*-dien-3β-ol (**8**)	+	3.42	0.9	2.9	1.2
24*E*-Ethylidenecholest-5-en-3β-ol (**9**)	–	4.15	1.5	0.4	0.8
Others	–	–	0.2	0.3	0.3

* Co-injected on OV-1, SE-52 and OV-17 capillary columns.

† Relative retention times calculated from 5α-cholestane on a 20 m × 0.3 mm i.d. OV-1 glass WCOT capillary column, isothermal at 230°.

Fatty acids

The total fatty acid distribution (Table 3) was typical of most diatoms [5, 21–23] showing a strong predominance of Δ^9-16:1, 14:0, 16:0 and 20:5. These data are broadly similar to those of a previous analysis by Klenk and Eberhagen [6] but differ in the proportions of the major acids 14:0, 16:1 and 20:5. These differences were not due to degradative loss of the more polyunsaturated acids during work-up as shown by parallel analyses of cod liver oil fatty acids as a reference standard [24]. The proportion of 20:5 was particularly variable being 7.2% in sample 2, compared with 24.2% in sample 3. Klenk and Eberhagen [6] report an intermediate value of 15.1% for a natural population of *B. sinensis*. The proportion of 20:5 seemed to be inversely related to the proportion of 14:0. A decrease in 20:5 with concomitant increase in 14:0 was observed by Ackman *et al.* [25] in the diatom *Skeletonema costatum* as the culture aged. This may also be true for *B. sinensis* since sample 2 was grown for 17 days compared with only 12 days in the case of sample 3. Neither sample contained as much 16:4 fatty acid as that reported by Klenk and Eberhagen [6] and both samples contained significantly more 16:1 than the previous analysis. The relative amounts of individual fatty acids may be dependent on the culture conditions employed and since in our study the only variable factor was the period of culture it seems likely that the nutritional status of the culture could be important.

Data on the double-bond positional isomers of the mono-unsaturated fatty acids, not given by Klenk and Eberhagen [6], are shown in Table 3. The significant proportion of vaccenic acid (Δ^{11}-18:1) compared with oleic acid (Δ^9-18:1), especially in sample 2, is particularly noteworthy as most previous diatom analyses have suggested that the common plant isomer predominates. Recent work [26,27] has shown that this is certainly not the case in many macroscopic algae. Significant amounts of Δ^{11}-18:1 have been noted in the unicellular alga *Isochrysis galbana* [28] and smaller quantities occur in other species [29]. In view of the predominance of Δ^9-16:1 and small quantity of Δ^9-18:1, it seems probable that the Δ^9-desaturase in *B. sinensis* acts almost exclusively on 16:0 and that a small proportion of the resultant Δ^9-16:1 is chain-elongated to Δ^{11}-18:1. The small amounts of Δ^{11}-16:1 are probably formed in the same way by chain-elongation of Δ^9-14:1. The latter was only detected in trace amounts despite the abundance of the 14:0 fatty acid. No evidence has been presented to date for the existence in microscopic algae of chain lengthening of mono-unsaturated fatty acids.

C_{18}-Fatty acids were very minor components which is a common feature of diatom lipids. Only trace quantities of 18:3 could be detected, in agreement with Klenk and Eberhagen [6], although a small amount of 18:4 occurred. Two isomers of 16:2, viz. $\Delta^{6,9}$-16:2 and $\Delta^{9,12}$-16:2, were identified and these are probably formed by further desaturation of Δ^9-16:1 on either side of the Δ^9-double bond [25]. The predominance of $\Delta^{9,12}$-16:2 over $\Delta^{6,9}$-16:2 seems to be a common feature in those diatoms where the 16:2 isomers have been characterized [22, 25], although the factors determining this have not been elucidated. Chlorophyceae apparently synthesize mainly $\Delta^{7,10}$-16:2, $\Delta^{7,10,13}$-16:3 and $\Delta^{4,7,10}$-16:3 [21, 25, 27] which thus differ from the isomers synthesized by diatoms (Table 3). The identification of these isomers can be useful for assigning the origin of algal fatty acids in sediments [3].

Hydrocarbons

Hydrocarbons were minor components of the diatom lipids and these consisted almost exclusively of *n*-heneicosahexaene and squalene (ratio 8:1), together with trace amounts of *n*-alkanes and other components which were not characterized. Only one isomer of *n*-heneicosahexaene was detected and this had an identical MS to 3,6,9,12,15,18-heneicosahexaene [30] which is often the major hydrocarbon (80–90%) in photosynthetic diatoms [31]. Although this alkene is thought to arise from decarboxylation of the 22:6 fatty acid, only traces of this acid were detected, in common with the findings of other workers [31,32]. Either this conversion is highly efficient, substrate specific and goes to completion, or else some other biosynthetic pathway is involved. The concentration of *n*-heneicosahexaene, expressed on a per cell basis (Table 1), is higher by over two orders of magnitude than any of the algal species analysed by Blumer *et al.* [32], no doubt reflecting the large cell volume of *B. sinensis* (*ca* 2 $\times 10^5$ μm^3). However when expressed on a dry wt basis, it is slightly less than in the six diatom species analysed by Lee *et al.* [30] for which comparable data are available. The

Table 3. Percentage composition of total fatty acids of *B. sinensis*

	Sample			Sample	
Fatty acid*	2 (%)	3 (%)	Fatty acid	2 (%)	3 (%)
14:0	19.9	11.3	$\Delta^{6,9,12,15}$-16:4	1.2	2.5
15:0	0.7	0.2	18:0	0.6	1.1
16:0	15.3	13.4	Δ^9-18:1	0.3	0.5
Δ^9-16:1	38.4	37.7	Δ^{11}-18:1	1.5	0.4
Δ^{11}-16:1	0.2	0.4	$\Delta^{9,12}$-18:2	0.7	0.4
trans Δ^3-16:1	2.4	1.1	$\Delta^{6,9,12,15}$-18:4	0.2	0.2
$\Delta^{6,9}$-16:2	2.5	0.6	$\Delta^{5,8,11,14,17}$-20:5	7.2	24.2
$\Delta^{9,12}$-16:2	4.3	2.5	22:5	0.6	0.9
$\Delta^{6,9,12}$-16:3	3.0	1.8	Others	1.0	0.8

* Fatty acids are designated by No. of carbon atoms: No. of double bonds. Double bond positions are numbered from the carboxyl end.

presence of squalene was not unexpected in a sterol-producing organism, although its presence in other diatoms has not apparently been noted previously.

The general conclusions from this study are that the hydrocarbon and fatty acid distributions of *B. sinensis* are fairly typical of diatoms, while the sterol distribution is quite unusual and highlights the diversity of sterols biosynthesized by diatoms. This diversity appears to extend even to species level, as previously shown for some *Nitzschia* species [5], and by comparison of our results for *B. sinensis* with those for *B. aurita* which has very different Δ^7- and Δ^8-sterols and no cholesta-5,22-dien-3β-ol.

EXPERIMENTAL

Biddulphia sinensis Grev. was obtained from the Marine Biological Association (Plymouth, U.K.) culture collection and grown at 15° in Erd Schreiber culture medium [33] until dense cultures were obtained, according to our previously described methods [1]. Final cell densities differed in the three cultures used for lipid analysis; sample 1:1.46 × 10^6 cells/l.; sample 2:2.82 × 10^6 cell/1.; sample 3:1.05 × 10^6 cells/1. The higher cell density in sample 2 was achieved by growing the culture for 17 days compared with a 12 day culture period used in sample 3. The solvent-extractable lipids from samples 1 and 3 were extracted into CH_2Cl_2–MeOH with sonication, reduced to dryness and saponified in aq. KOH–MeOH (pH 12) under reflux for 3 hr. Non-saponifiable lipid was extracted into hexane–Et_2O (9:1) and after acidification with HCl (pH 2) the fatty acids were similarly extracted. Total sterols were the only lipid class examined in sample 1. Total lipids from sample 2 were obtained by direct saponification of the algal cells in KOH–MeOH (pH 12) under reflux for 3 hr followed by the same work-up procedure as for sample 1. A portion of the extract from sample 3 was examined by TLC for the presence of steryl esters and sterol sulphates but they were not detected. Fatty acids were converted to methyl esters using BF_3–MeOH, and sterols were converted to TMSi-ethers using BSTFA, prior to GLC analyses on 20 m × 0.3 mm i.d. glass capillary columns coated with OV-1, SE-52 or OV-17. The Grob [34] method of splitless injection was used with injection at 40° followed after 1 min by a rapid programme to 120° and a subsequent programme from 120 to 280° at 4°/min. Helium was used as the carrier gas at 1.5 ml/min and FID and injector temps were 300°. Sterol–TMSi ethers were also analysed isothermally at 230° on these GLC columns (Table 2). Double-bond positions of the fatty acid methyl esters were determined using a 100 m × 0.3 mm i.d. WCOT DEGS column by comparison with the fatty acids of cod liver oil as a secondary ref. standard [24].

Combined GC–MS used a quadrupole filter instrument operating at 350 μA, 40 eV electron energy and on ion-source temp. of 250°. An OV-1 capillary column, coupled directly to the ion-source via glass-lined stainless steel tubing, was used with similar GLC conditions to those above. MS were taken every 2.5 sec and processed using an on-line 32K computer data system. Compounds were identified by co-injection, retention time and by comparison of MS with standards. Major ions in the MS of the TMSi derivatives of each of the sterols were as follows. Sterol **1** *m/e* (rel. int): 456 (M^+; 11), 366 (22), 351 (10), 327 (22), 255 (31), 129 (66), 111 (100), 69 (80); sterol **2**: 458 (M^+, 2.4), 368 (15), 353 (7.4), 329 (24), 255 (7.4), 247 (12), 129 (100); sterol **3**: 470 ($M^{+\cdot}$, 2.6), 380 (5.5), 372 (5.8), 365 (2.4), 282 (11), 255 (46), 213 (12), 129 (19), 125 (52), 69 (100); sterol **4**: 470 (M^+, 17), 380 (32), 372 (4.0), 365 (12), 341 (17), 282 (5.5), 255 (46), 129 (63), 125 (52), 69 (100); sterol **5**: 458 (M^+, 13), 255 (61), 229 (26), 213 (38), 125 (64), 69 (100); sterol **6**: 470 ($M^{+\cdot}$, 5.6), 455 (3.5), 386 (20), 380 (19), 371 (5.3), 365 (12), 341 (23), 296 (26), 129 (100); sterol **7**: 472 (M^+, 3.0), 382 (11), 343 (17), 261 (7.5), 255 (13), 129 (100); sterol **8**: 484 ($M^{+\cdot}$, 2.6), 394 (6.5), 372 (13), 351 (10), 343 (22), 323 (10), 282 (15), 255 (54), 129 (26), 69 (100); sterol **9**: 484 ($M^{+\cdot}$, 1.0), 386 (12), 355 (5.3), 296 (23), 281 (12), 257 (13), 255 (8.4), 129 (100).

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REFERENCES

1. Corner, E. D. S., Head, R. N. and Kilvington, C. C. (1972) *J. Mar. Biol. Assoc. U.K.* **52**, 847.
2. Sargent, J. R., Gatten, R. R., Corner, E. D. S. and Kilvington, C. C. (1977) *J. Mar. Biol. Assoc. U.K.* **57**, 525.
3. Volkman, J. K. and Johns, R. B. (1977) *Nature* **267**, 693.
4. Wardroper, A. M. K., Maxwell, J. R. and Morris, R. J. (1978) *Steroids* **32**, 203.
5. Orcutt, D. M. and Patterson, G. W. (1975) *Comp. Biochem. Physiol.* **50B**, 579.
6. Klenk, E. and Eberhagen, D. (1962). *Hoppe Seyler's Z. Physiol. Chem.* **328**, 189.
7. Ballantine, J. A., Lavis, A. and Morris, R. J. (1979) *Phytochemistry* **18**, 1459.
8. Tsuda, K., Sakai, K., Tanabe, K. and Kishida, Y. (1960) *J. Am. Chem. Soc.* **82**, 1442.
9. Ballantine, J. A., Roberts, J. C. and Morris, R. J. (1976) *Biomed. Mass Spectrom.* **3**, 14.
10. Tamura, T., Wainai, T., Truscott, B. and Idler, D. R. (1964) *Can. J. Biochem.* **42**, 1331.
11. Rubinstein, I. and Goad, L. J. (1974) *Phytochemistry* **13**, 485.
12. Kanazawa, A., Yoshioka, M. and Teshima, S. (1971) *Bull. Jpn. Soc. Sci. Fish.* **37**, 899.
13. Kates, M., Tremblay, P., Anderson, R. and Volcani, B. E. (1978) *Lipids* **13**, 34.
14. Boutry, J. L. and Barbier, M. (1974) *Mar. Chem.* **2**, 217.
15. Nishimura, M. and Koyama, T. (1977) *Geochim. Cosmochim. Acta* **41**, 379.
16. Ando, T., Kanazawa, A., Teshima, S. and Miyawaki, H. (1979) *Mar. Biol.* **50**, 169.
17. Kanazawa, A., Teshima, S., Ando, T. and Tomita, S. (1974) *Bull. Jpn. Soc. Sci. Fish.* **40**, 729.
18. Withers, N. W., Tuttle, R. C., Goad, L. J. and Goodwin, T. W. (1979) *Phytochemistry* **18**, 71.
19. Volkman, J. K., Corner, E. D. S. and Eglinton, G. (1980) in *Colloq. Int. C.N.R.S.* (in press).
20. Orcutt, D. M. and Patterson, G. W. (1974) *Lipids* **9**, 1000.
21. De Mort, C. I., Lowry, R., Tinsley, I. and Phinney, H. K. (1972) *J. Phycol.* **8**, 211.
22. Chuecas, L. and Riley, J. P. (1969) *J. Mar. Biol. Assoc. U.K.* **49**, 97.
23. Ackman, R. G., Tocher, C. S. and McLachlan, J. (1968) *J. Fish. Res. Board Can.* **25**, 1603.
24. Ackman R. G. and Burgher, R. D. (1964) *J. Am. Oil Chem. Soc.* **42**, 38.
25. Ackman, R. G., Jangaard, P. M., Hoyle, R. J. and Brockerhoff, H. (1964) *J. Fish. Res. Board Can.* **21**, 747.
26. Ackman, R. G. and McLachlan, J. (1978) *Proc. N. S. Inst. Sci.* **28**, 47.

27. Johns, R. B., Nichols, P. D. and Perry, G. J. (1979) *Phytochemistry* **18**, 799.
28. Watanbe, T. and Ackman, R. G. (1974) *J. Fish. Res. Board. Can.* **31**, 403.
29. Jamieson, G. R. and Reid, E. H. (1972) *Phytochemistry* **11**, 1423.
30. Lee, R. F., Nevenzel, J. C., Paffenhofer, G.-A., Benson, A. A., Patton, S. and Kavanagh, T. E. (1970) *Biochim. Biophys. Acta* **202**, 386.
31. Lee, R. F. and Loeblich A. R., III (1971) *Phytochemistry* **10**, 593.
32. Blumer, M., Mullin, M. M. and Guillard, R. R. L. (1970) *Marine Biol.* **6**, 226.
33. Fǿyn, B. (1934) *Arch. Protistenkd.* **83**, 1.
34. Grob, K. and Grob, G. (1979) *J. High Resol. Chromatogr. Chromatogr. Commun.* **2**, 109.