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LIPIDS OF THE ANTARCTIC SEA ICE DIATOM *NITZSCHIA CYLINDRUS**

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Abstract—The sterol and neutral, glyco- and phospholipid fatty acid profiles of the sea ice diatom *Nitzschia cylindrus*, isolated from McMurdo Sound, Antarctica, are reported. Two sterols were detected, *trans*-22-dehydrocholesterol (66% of total sterols) and cholesterol (34%); no sterols containing alkyl groups at the C_{24} position were present. The major fatty acids in *N. cylindrus*, 16:1Δ9c, 14:0, 16:0, 20:5Δ5,8,11,14,17 and 20:4Δ5,8,11,14, were typical of previous reports of diatom fatty acids. A number of long-chain monounsaturated fatty acids were also detected, with higher relative proportions present in the phospholipid fraction. GC-MS analysis of the dimethyldisulphide adducts of these monounsaturated components showed that 24:1Δ13c, 24:1Δ15c, 26:1Δ15c and 26:1Δ17c were the major components. The distribution of these fatty acids suggests that chain elongation of monounsaturated fatty acids was occurring in *N. cylindrus*. The proposed chain lengthening occurring for *N. cylindrus* represents, to our knowledge, the first report of possible chain lengthening of monounsaturated fatty acids in microscopic algae. These features, the presence of long-chain monounsaturated fatty acids and the sterol profile, may allow the input of this alga into benthic marine sediments or food webs to be monitored.

INTRODUCTION

Primary production occurring in sea ice has been described for the Antarctic [1–4] and Arctic [5–7] regions. The contribution of ice microalgae to overall primary productivity in polar oceans has recently prompted a resurgence in the investigation of sea ice microbial communities [8–10]. Ice algae, predominantly diatoms, are commonly found in the diffuse lower layers of congelation ice in the Antarctic [11–13] as well as in the lower platelet ice layer in some areas of Antarctica [1, 2].

Our laboratories are examining the microbial biomass and community structure of Antarctic sea ice and marine benthic sediments [10, 14]. Techniques being utilized include analysis of various lipid classes present and measurement of ^{14}C-acetate and ^{3}H-thymidine incorporation into lipids and DNA, respectively [14]. The quantitative recovery and measurement of cellular lipid components, in particular the phospholipid ester-linked fatty acids, has been applied to a wide range of environmental samples [15–18]. Microbial biomass and community structure can be determined by measuring properties common to all cells and identifying specific signature or biological marker lipids. The advantages of chemical procedures, compared to classical enumeration procedures, have been described [15].

Analysis of axenic monoalgal or unibacterial cultures is necessary in order that the signature lipid approach can be applied to Antarctic benthic microbial ecology. In this report a detailed lipid analysis of the common sea ice diatom *Nitzschia cylindrus*, isolated from a site adjacent to Cape Armitage, McMurdo Sound, Antarctica, is presented. Lipid classes analysed include phospholipid, glycolipid and triglyceryl-linked fatty acids and sterols. These data, to our knowledge, represent only the third report of lipid constituents for an Antarctic sea ice diatom. The double bond configuration of monounsaturated fatty acids has been determined, since such data are essential for biochemical and geochemical interpretation of the components present [16–20].

RESULTS AND DISCUSSION

The absolute abundances of total fatty acids in *N. cylindrus* (15.6 μ moles/g or 0.5%, dry wt basis, Table 1) is lower than values reported previously for 11 other diatoms [21]. The value obtained for *N. cylindrus* is closer to that reported for the marine diatom *Biddulphia sinensis* [22]. The concentration of sterols present in *N. cylindrus* is at the low end of the range reported by Orcutt and Patterson [21], and is similar to that noted for *B. sinensis* [22]. The low absolute abundances of sterols and fatty acids may be indicative of the highly silicified nature of the cell wall in *N. cylindrus* as proposed for *B. sinensis*. Analysis of the fractionated lipid classes showed that the glycolipids contained the highest proportion of fatty acids, followed by the neutral lipids and phospholipids. Bacteria, in particular Gram-negative organisms which predominate in marine sediments [23], contain low amounts of neutral and glycolipids [24]. Analysis of these

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Table 1. Absolute abundances of lipid classes in *Nitzschia cylindrus*

Lipid fraction	μmoles/g* tissue
Neutral lipid fatty acids†	5.3
Glycolipid fatty acids	6.8
Phospholipid fatty acids	3.5
Total fatty acids‡	15.6
Phospholipid phosphate	2.1
Total sterols§	1.1

*Dry wt basis.
†TLC revealed predominantly triacylglycerol.
‡Sum of neutral, glyco- and phospholipids.
§Obtained following alkaline saponification of an aliquot of the neutral lipid fraction.

two lipid fractions may thus offer the potential to gain a greater insight into the eukaryotic, including diatom, population in Antarctic sediments. TLC analysis of the neutral lipid fraction revealed that triacylglycerols were the predominant lipid class present. Comparison of our lipid concentration data with those obtained for other polar diatoms is difficult because of the scarcity of such data.

Microbial, including microalgal, biomass and community structure estimations for marine and estuarine sediments have been based on the use of an average bacterial phospholipid ester-linked fatty acid composition of 100 μ moles/g (dry wt basis) [25, 26]. The data reported here for *N. cylindrus* suggests that the use of this factor for Antarctic sediments rich in a diatomaceous input may underestimate the microbial biomass if diatoms low in absolute lipid content represent the dominant species present. Further work is necessary, such as (1) the analysis of a larger number of both sea ice and benthic diatoms and (ii) variation of culture age and growth conditions, before a value similar to the one used by White *et al.* [25, 26] can be confidently used for eukaryotic biomass calculations in Antarctic sediments. Lipid compositional data, as discussed here, have been shown previously to be important for biomass calculations [25, 26] and toxonomic considerations [27, 28]. These data can also be useful for organic geochemical studies, where the flux of planktonic organic matter and degradation rates of individual compounds are calculated [29].

Sterols

The taxonomic significance of sterols in diatoms has not been fully explored. Colonial diatoms, however, generally have a more complex profile than algae existing as solitary cells [21]. The simple sterol profile observed for *N. cylindrus*, which is non-colonial, is in accord with this feature. Two sterols were detected in *N. cylindrus*: *trans*-22-dehydrocholesterol (66% of total sterols) and cholesterol (34%) (Table 2). The sterol profile in *N. cylindrus* differs from that reported for four other *Nitzschia* species [21]. Three of the four, *N. ovalis*, *N. frustulum* and *N. closterium*, contained (24*S*)-24-methylcholesta-5,22*E*-dien-3β-ol the 24α-epimer of brassicasterol, as the major component. The fourth alga, *N. longissima* contained cholesterol as the major component with small proportions of ergost-5-enol and fucosterol also present. *N. ovalis* contained *trans*-22-dehydrocholesterol as a major component in addition to the 24-methylcholesta-5,22*E*-dien-3β-ol noted above and cholesterol.

The absence of sterols alkylated at the C_{24} position distinguishes *N. cylindrus* from not only the other *Nitzschia* species discussed above, but also many other diatoms including the sea ice diatom *Stauroneis amphioxys* [19, 21, 22]. Previous work has indicated that light intensity can alter the proportions of sterols in *N. closterium* occurring as free, esterified and conjugate forms [30]. No study has determined the effect of environmental influences on the proportions of individual sterols in diatoms. Volkman *et al.* [22], in their study of the sterols and fatty acids of *B. sinensis*, suggested that culture conditions could influence the sterol composition and postulated that culture age may be an important factor in determining the level of C_{24} alkylation. At the present time no explanation can be proposed for the absence of C_{24} alkylated sterols in *N. cylindrus*. Mechanisms similar to those discussed above may, however, be responsible for this taxonomically distinguishing feature.

Fatty acids

The fatty acid profiles obtained for the neutral, glyco- and phospholipid fractions of *N. cylindrus* are shown in Table 3. The major acids detected; 16:1Δ9c, 14:0, 16:0, 20:5Δ5, 8, 11, 14, 17 and 20:4Δ5, 8, 11, 14, are similar to those present in most diatoms [19, 21, 22, 24]. C_{18} fatty acids were only minor components as previously reported. A number of interesting features are observed when

Table 2. Sterol composition of *Nitzschia cylindrus*

RR_s*	Sterol†	Percentage composition‡	Mass spectral data	
			M_r§	Major ions detected
0.90	Cholest-5,22*E*-dien-3β-ol (*trans*-22-dehydrocholesterol)	66	456	69(100), 111(75), 129(70) 255(71), 327, 327(86), 351(39) 366(64), 441(11), 456(18)
1.00	Cholest-5-en-3β-ol (cholesterol)	34	458	129(54), 145(29), 225(26), 329(100), 353(44), 368(76), 443(11), 458(34)

*RR_s, cholesterol = 1.00, 24-ethylcholesterol = 1.63.
†Trivial name in parentheses.
‡Sterol composition is expressed in terms of the total sterols.
§M_r of TMSi ether derivatives.

Table 3. Fatty acid composition of *Nitzschia cylindrus*

Fatty acid	Percentage composition*		
	Neutral lipid	Glycolipid	Phospholipid
14:1	0.1	0.6	0.1
14:0	24.3	22.6	25.5
16:4Δ6,9,12,15	0.3	2.5	0.5
16:3Δ6,9,12	1.7	2.5	0.5
16:1Δ9*c*	44.9	32.8	26.5
16:1Δ9*t*	TR†	0.2	0.2
16:1Δ11*c*	0.4	0.5	0.4
16:1Δ3*t*	1.8	0.3	0.8
16:0	14.6	10.9	11.9
18:3Δ6,9,12	0.1	0.4	0.4
18:4Δ6,9,12,15	TR	0.4	0.3
18:2Δ9,12	0.6	0.2	0.7
18:3Δ9,12,15	TR	0.3	1.0
18:1Δ9*c*	1.0	0.3	0.3
18:1Δ11*c*	0.8	0.4	0.5
18:0	1.1	1.0	1.3
20:4Δ5,8,11,14	1.0	7.0	5.0
20:5Δ5,8,11,14,17	2.2		
20:4Δ8,11,14,17	TR	0.6	0.2
20:3Δ8,11,14			
20:*u*‡	TR	TR	TR
20:u	0.2	TR	TR
20:u	0.2	—§	—
20:0	0.1	TR	0.1
21:0	TR	TR	0.1
22:6Δ4,7,10,13,16,19	0.1	TR	0.2
22:1	TR	0.1	0.3
22:1	TR	0.1	TR
22:0	0.1	0.2	0.5
23:0	TR	TR	0.1
24:1Δ13*c*	0.2	1.5	3.2
24:1Δ15*c*	0.2	0.7	1.7
24:1	TR	0.1	0.3
24:0	TR	1.5	4.9
25:0	TR	TR	TR
26:1	0.1	0.5	1.1
26:1Δ15*c*	0.1	0.2	0.6
26:1Δ17*c*	0.3	1.5	4.3
26:1	TR	0.1	0.4
26:0	0.1	0.7	2.0
Others¶	3.4	5.2	4.9
Saturated	40.3	36.9	46.3
Monounsaturated	49.9	39.9	39.9
PUFA¶	6.4	13.9	8.8
C24-C26	1.0	6.8	18.5

*Fatty acid composition is expressed in terms of the total fatty acids.

†Trace, <0.1%.

‡u = unidentified unsaturated fatty acid.

§—Not detected.

‖Includes small amounts of *i*15:0, *a*15:0, 15:0, *i*15:1 and *a*15:1.

¶PUFA, polyunsaturated fatty acids, identified by GC and GC-MS data.

comparing the *N. cylindrus* fatty acid profile to other diatom profiles. The relative levels of polyunsaturated fatty acids (PUFA, 6–14% of total acids) were significantly lower than values reported for the sea ice diatoms *S. amphioxys* [19] and *Naviculi glaciei* [31]. These two diatoms contained between 40 and 50% of the total fatty acids as PUFA.

The inverse relationship between temperature and degree of unsaturation has been well documented [e.g. 32, 33]. Although in *N. cylindrus* the relative level of PUFA is lower than might be expected for this alga, we can offer an alternate suggestion as to how *N. cylindrus* maintains its membrane integrity in the polar environment. Approximately 18% of the phospholipid fatty acids are long chain components, in the range C_{24}–C_{26}, with the majority present as monounsaturated fatty acids (Table 3). Lower proportions of these longer chain fatty acids were present in the neutral and glycolipid fractions. GC-MS analysis of the dimethyldisulphide adducts of the monounsaturated components was performed, and a list of the ion fragments and GC retention data is shown in Table 4. Four of the major components were identified as: 24:1Δ13c, 24:1Δ15c, 26:1Δ15c and 26:1Δ17c. Insufficient material was available for positive identification of the other components. GC-MS analysis of a replicate sample of *N. cylindrus* grown using ^{13}C-labelled Na_2CO_3 showed the presence of the labelled substrate in these relatively novel components. The predominance of 16:1Δ9c in *N. cylindrus*, and the small quantity of 18:1Δ9c indicates that the Δ9 desaturase acts almost exclusively on 16:0 and that a small proportion of the 16:1Δ9c is chain elongated to 18:1Δ11c as observed for *B. sinensis* [22].

The presence and distribution of the long chain monounsaturated fatty acids suggests that chain elongation of the monounsaturated fatty acids is occurring in *N. cylindrus*. The possible occurrence of chain lengthening of long chain monounsaturated fatty acids in *N. cylindrus* represents to our knowledge the first report of chain lengthening of monounsaturated fatty acids in microscopic algae.

The information presented here provides base-line data on the sterol and fatty acid composition of the sea ice diatom *N. cylindrus*. Two novel features were observed. Sterols containing C_{24} alkyl groups were not detected, and a suite of long chain monounsaturated fatty acids was noted. These features may allow the input of this alga to benthic marine sediments or food webs to be monitored.

EXPERIMENTAL

Nitzschia cylindrus (Grunow) Hasle cell material was isolated, grown at 4° and harvested as described previously [34]. A second algal culture was grown under identical conditions, with the exception that $Na_2{}^{13}CO_3$ was used as carbon source.

Lipids were extracted from lyophilized cells with the modified one-phase $CHCl_3$–MeOH Bligh and Dyer [35] extraction [25]. Lipids were recovered in the lower $CHCl_3$ phase, solvents removed *in vacuo* and the lipids were stored under N_2 at −20°. Duplicate sample extractions were performed. Total lipid was separated into three general classes: neutral, glyco- and phospholipid, by silicic acid CC [36, 37]. Neutral lipids were further fractionated into hydrocarbons, triacylglycerols and free sterols using a second silicic acid column [38]. Fractions were collected in test tubes, dried under a stream of N_2 and stored at −20° until further analysis. Lipid phosphate was measured as described in ref. [39]. The mild alkaline methanolysis procedure [25] was applied to the triacylglycerol, glycolipid and phospholipid fractions. The technique was modified slightly in that

Table 4. Long chain monounsaturated fatty acids from *Nitzschia cylindrus*, GC retention data and characteristic ion fragments of derivatised products formed by reaction of the fatty acids with dimethyldisulphide (DMDS)

Fatty acid	Retention time	Ion fragments (*m/z*) of DMDS adducts ω-fragment*	Δ-fragment†
24:1Δ13*c*	36.07	201	273
24:1Δ15*c*	36.16	173	301
24:1	36.31	—‡	—
26:1	39.63	—	—
26:1Δ15*c*	39.69	201	301
26:1Δ17*c*	39.79	173	329
26:1	39.92	—	—

*ω-fragment indicates fragment including aliphatic end of the molecule.
†Δ-fragment indicates fragment including carboxylic end of the molecule.
‡—, Not detected in GC-MS analysis due to insufficient sample material.

hexane–$CHCl_3$ (4:1) rather than $CHCl_3$ was used to extract the resulting fatty acid Me esters (FAME).

FAME samples were taken up in hexane with Me nonadecanoate as int. inj. standard. Initial identification of individual normal fatty acid components was performed by HRGC using a Hewlett–Packard 5880A gas chromatograph equipped with an FID. Samples were injected at 50° in the splitless mode using an autosampler onto a non-polar, cross-linked Me silicone capillary column (50 m × 0.2 mm i.d.). The oven was temp. programmed from 50° to 160° at 5° per min, then at 2° per min to 300°. H_2 was used as carrier (1 ml/min). The injector and FID were maintained at 300°. Tentative peak identification, prior to GC-MS analysis, was based on comparison of R_ts with data for commercial standards and previously identified laboratory standards. Sterol identifications were confirmed by comparison of MS (as TMSi ethers) and R_ts with those of authentic standards, laboratory standards and previously reported spectra [e.g. 40, 41]. Peak areas were quantified using a programmable laboratory data system operated in an int. standard prog. Fatty acid and sterol compositional data reported for these samples were the mean of two analyses. Standard deviations of replicate analyses for individual fatty acids were in the range 0–30%, typically < 5%.

GC-MS analyses were performed on a Hewlett–Packard 5996A system fitted with a direct capillary inlet. The same column type as the one described above was used for analyses. Samples were injected in the splitless mode at 100° and the oven was programmed from 100 to 300° at 4° per min. He was used as carrier. MS operating parameters were: electron multiplier 1300–1400 V, transfer line 300°, source and analyser 250°, autotune file DFTPP normalized, optics tuned at *m/z* 502, MS peak detect threshold = 300 triggered on total ion abundance, electron impact energy = 70 eV. MS data were acquired and processed using a Hewlett–Packard RTE 6 data system.

The dimethyldisulphide (DMDS) adducts of monounsaturated FAME were formed, using the method described in ref. [42] to locate the double bond positions. Samples in hexane (50 μl) were treated with 100 μl DMDS and 1–2 drops of I_2 soln (6.0% in Et_2O). Reactions were performed in a standard GC vial fitted with a Teflon lined screw cap. After reaction at 50° for 48 hr, the mixture was cooled and diluted with hexane (200 μl). I_2 was removed by shaking with 5% aq. $Na_2S_2O_3$ soln (100 μl). The organic layer was removed and the aq. layer re-extracted with hexane–$CHCl_3$ (4:1). The combined organic layers were concd under a stream of N_2 prior to GC analysis. GC-MS analysis of the DMDS adducts showed major ions attributable to fragmentation between the SMe groups at the original site of unsaturation. Discrimination between *cis* and *trans* geometry of the double bond in the original monoenoic FAME is possible. The *erythro* isomer (originally the *trans* acid) elutes after the *threo* isomer (originally the *cis* acid). The different positional isomers of the same geometry were separated under the GC conditions used in this study.

Fatty acids were designated as total number of carbon atoms: number of double bonds followed by the position of the double bond from the Δ (carboxylic) end of the molecule. The prefixes *i* and *a* refer to *iso* and *anteiso* branching, respectively. The suffixes *c* and *t* indicate *cis* and *trans*.

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