Org. Geochem. Vol. 15, No. 5, pp. 503–508, 1990
Printed in Great Britain.

0146-6380/90 $3.00 + 0.00

Occurrence of novel C_{30} sterols in Antarctic sea-ice diatom communities during a spring bloom

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(*Received* 21 *June* 1989; *accepted* 14 *June* 1990)

Abstract—The sterol compositions of natural populations of diatom communities in the sea-ice at McMurdo Sound, Antarctica were determined during the austral spring bloom of 1985, using capillary GC and GC–MS. A range of sterols (C_{26}–C_{30}) were detected in the sea-ice diatom communities; 24-methylenecholesterol, brassicasterol and 24-ethylcholesterol were the major sterols at the Cape Armitage, Erebus, Cape Evans and Wohlschlag Bay sites. The similarity of the sterol profiles to those observed in previous studies of Antarctic freshwater algal communities strongly indicates that diatoms, rather than cyanobacteria or other algal groups previously proposed, are a more probable source of C_{29} sterols in these extreme environments. Two novel 4-methyl-C_{30} sterols were also detected: a C_{30} sterol showing a similar mass spectrum to 4-methyl-24-ethyl-5α-cholest-22-en-3β-ol derived from the prymnesiophyte microalga *Pavlova lutheri*, but which was chromatographically resolved from this compound; and a stanol presumed to be derived from the C_{30} sterol. 4-Methyl C_{30} sterols have not been reported previously in diatoms; their presence, and the occurrence of 4-methyl C_{28} and C_{29} sterols, may be due to temperature-induced adaptations in sterol biosynthetic pathways.

Key words—sea-ice diatoms, spring bloom, sterols, 4-methylsterols

INTRODUCTION

During the austral spring and summer at McMurdo Sound, Antarctica, microalgal communities composed almost exclusively of diatoms are found near the bottom of the hard congelation ice and in the underlying platelet ice when it is present (Bunt, 1963; Palmisano and Sullivan, 1983, 1985). As sea-ice diatoms are important components of the carbon and energy flux in the polar oceans, their development, physiology and ecology are being studied intensively.

Our groups have recently examined assimilation of ^{14}C into specific lipid fractions (neutral, glycolipid and phospholipid) over the course of the Antarctic sea-ice algal bloom and its decline (Palmisano *et al.*, 1985a). The most dramatic changes in ^{14}C incorporation were found in the neutral lipid fraction (Palmisano *et al.*, 1988). In a related study of the lipid distribution in the sea-ice algal communities, high neutral lipid content was observed in communities at two study sites shortly after the chlorophyll maximum (Nichols *et al.*, 1988).

In this study, the sterol composition of natural populations of sea-ice diatom communities during a spring bloom of sea-ice microalgae was analysed. The distribution and significance of sterol profiles obtained for the sea-ice diatom communities are examined. Few reports of the sterol and hydrocarbon distributions of Antarctic freshwater algal communities, including stromatolites, are available (Matsumoto *et al.*, 1982, 1983; Orcutt *et al.*, 1986; Volkman *et al.*, 1986). The data presented here provide new information on the possible origins of 4-desmethyl and 4-methyl sterols in Antarctic sediments and food-webs.

MATERIALS AND METHODS

Sea-ice communities were sampled during December 1985 at three sites: Cape Armitage, the Erebus Ice Tongue and Wohlschlag Bay in McMurdo Sound, Antarctica (Table 1, Fig. 1). The Cape Armitage site has been described in detail elsewhere (e.g. Grossi *et al.*, 1984; Palmisano *et al.*, 1985b).

Congelation ice samples (2–3 cores) at each site were collected with a SIPRE ice auger (7 cm core diameter) from annual sea-ice with thicknesses between 0.9 and 2.5 m (Table 1). The lower 20 cm sections, in which 99% of the chlorophyll *a* is found (Palmisano and Sullivan, 1983), were cut from each core with a stainless-steel handsaw, then wrapped in an opaque black plastic and placed in Freezesafe styrofoam containers for transport to the laboratory. Each 20 cm section was melted at less than 5°C in 1.2 liters of filtered seawater.

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Table 1. Ice thickness, water depth and chlorophyll *a* data for sea-ice diatom sampling sites in McMurdo Sound, Antarctica

Parameter	Site						
	Erebus			Cape Armitage		Cape Evans	Wohlschlag Bay
	Dec 13	Dec 18	Dec 28	Dec 18	Dec 28		
Ice thickness (m)	—	—	—	2.5	2.42	1.53	0.92
Water depth (m)	500	500	500	20	20	20	300
Chlorophyll a (mg/m^2)	92	54	15	110	—	—	—

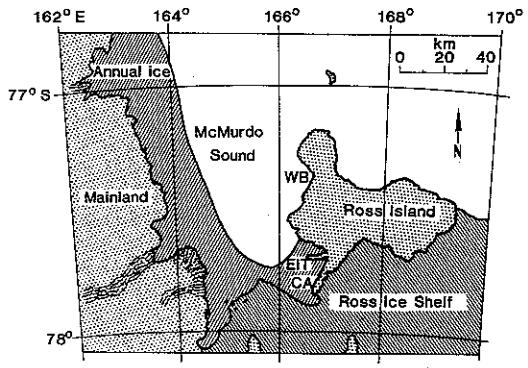

Fig. 1. McMurdo Sound, Antarctica. Sampling sites: WB = Wohlschlag Bay; EIT = Erebus Ice Tongue; CA-Cape Armitage. Cape Evans (CE) site is located approximately 5 km northeast of EIT on Ross Island.

Freshly collected, unpreserved, sea-ice microalgae samples were examined by phase-contrast microscopy (Zeiss) to determine species composition. Sea-ice algal cells were prepared for lipid analysis by filtration onto glass fiber filters (Whatman GF/C) prewashed with $CHCl_3$–MeOH.

Samples were quantitatively extracted at the Eklund Biological Laboratory at McMurdo Station with the modified one-phase $CHCl_3$-MeOH Bligh and Dyer extraction (Bligh and Dyer, 1959; White *et al.*, 1979). After phase separation, the lipids were recovered in the lower $CHCl_3$ layer (solvents were removed *in vacuo*) and were stored sealed under nitrogen at −20°C.

A portion of the total lipid extract was separated into individual lipid classes by column chromatography on silicic acid (3 g) deactivated with 5% distilled water. Eight lipid fractions were obtained. The sterol fraction was eluted with 15 ml hexane–ethylacetate (85/15; v/v).

Gas chromatographic (GC) analyses were performed with a Hewlett–Packard 5890 GC equipped with a 50 m × 0.20 mm i.d. cross-linked methyl silicone fused-silica capillary column and a flame ionization detector. Details are provided in Nichols *et al.* (1988). Sterol compositional data reported for these samples are the means of 2–3 replicate cores.

Gas chromatography–mass spectrometric (GC–MS) analyses of sterols (as OTMSi ethers) were performed on a Hewlett–Packard (HP) 5890 GC and 5970 Mass Selective Detector (MSD) fitted with a direct capillary inlet. Operating conditions have been described in detail in Nichols *et al.* (1988). Identifications of individual sterols were confirmed by comparing mass spectra with those of previously reported spectra (e.g. Brooks *et al.*, 1968; de Leeuw *et al.*, 1983), and by comparing retention time data with data for commercial, donated and previously identified laboratory standards.

RESULTS AND DISCUSSION

Species composition

The composition of sea-ice algal communities of Cape Armitage the Erebus Ice Tongue and Wohlschlag Bay has been previously reported (Palmisano *et al.*, 1988). The sea-ice diatom communities in mid-December were dominated by *Amphiprora* sp., *Nitzschia stellata* Manguin and *Berkeleya* sp. at Cape Armitage; *N. stellata*, *Amphiphora*, *Pleurosigma*, *N. kerguelensis* (O'Meara) Hasle and some small centrics at the Erebus Ice Tongue site; and *Porosira pseudodenticulata* (Hustedt) Jouse at Wohlschlag Bay.

4-Desmethyl sterols

Sterol distributions in the sea-ice diatom communities are shown in Table 2. A representative reconstructed ion chromatogram, showing the sterol distribution of a sample collected at the Erebus Ice Tongue site, is illustrated in Fig. 2. A wide range of C_{26}–C_{30} sterols was detected in the congelation sea-ice diatom communities (Table 2, Fig. 2). At the Cape Armitage and Erebus Ice Tongue sites, C_{28} (mainly 24-methylenecholesterol and brassicasterol) and C_{29} (mainly 24-ethylcholesterol) sterols were the major components. Brassicasterol and 24-methylenecholesterol were the dominant components at Cape Evans and Wohlschlag Bay, respectively. These components are typically the major sterols found in diatoms (Volkman 1986; and references cited therein). The

Table 2. Sterol composition of Antarctic sea-ice diatom communities from McMurdo Sound

Sterol	Trivial name	Peak no.	Percentage composition of total sterols: SITE — Erebus Dec 13	Erebus Dec 18	Erebus Dec 24	Cape Armitage Dec 18	Cape Armitage Dec 28	Cape Evans	Wohlschlag Bay
24-norcholesta-5,22-dien-3ß-ol		1	0.8	0.2	0.4	1.8	2.3	3.1	—
27-nor-24-methylcholesta-5,22E-dien-3ß-ol		2	0.2	0.5	0.9	0.8	1.2	0.9	—
cholesta-5,22E-dien-3ß-ol	22–dehydrocholesterol	3	7.0	3.8	4.2	5.4	6.1	3.7	1.2
cholest-5-en-3ß-ol	cholesterol	4	8.2	5.3	9.3	4.9	4.3	6.6	4.2
24-methylcholesta-5,22E-dien-3ß-ol	brassicasterol	5	18.3	15.6	25.9	42.0	48.8	57.5	1.4
24-methylcholesta-5,24(28)-dien-3ß-ol	24–methylenecholesterol	6	20.8	19.5	24.9	3.4	4.7	8.2	89.5
24-methylcholest-5-en-3ß-ol / 4-methyl-5 α-cholestan-3ß-ol	24 methylcholesterol	7	7.4	4.2	9.1	5.3	5.3	6.5	0.2
24-ethylcholesta-5,22E-dien-3ß-ol		8	2.1	2.9	4.9	3.9	4.4	0.5	0.2
24-ethylcholest-5-en-3ß-ol	24–ethylcholesterol	9	16.0	28.8	13.7	16.0	16.1	12.9	0.2
4,23,24-trimethyl-5 α-cholest-22E-en-3ß-ol	dinosterol	10	0.4	3.2	0.4	0.1	—	—	—
C_{30} sterol*		11	6.4	3.8	1.3	5.8	3.6	—	—
C_{30} stanol		12	7.7	6.5	2.6	3.6	1.3	—	0.8
Others			4.7	5.8	2.3	6.8	1.9	—	2.1
Sum C_{26}			0.8	0.2	0.4	1.8	2.3	3.1	—
Sum C_{27}			15.3	9.6	14.3	11.2	11.6	11.2	5.4
Sum C_{28}			46.5	39.2	59.9	50.7	58.8	72.3	91.2
Sum C_{29}			18.1	31.7	18.6	19.9	20.5	13.4	0.5
Sum C_{30}			14.5	13.5	4.4	9.6	4.9	—	0.8

Peak numbers refer to Fig. 2.
*Sterol 11 showed a similar mass spectrum to 4-methyl-24-ethyl-5 α-cholest-22-en-3ß-ol
Others includes: 4,24-dimethyl-5 α-cholest-24(28)-en-3ß-ol, 4,24-dimethyl-5 α-cholestan-3ß-ol

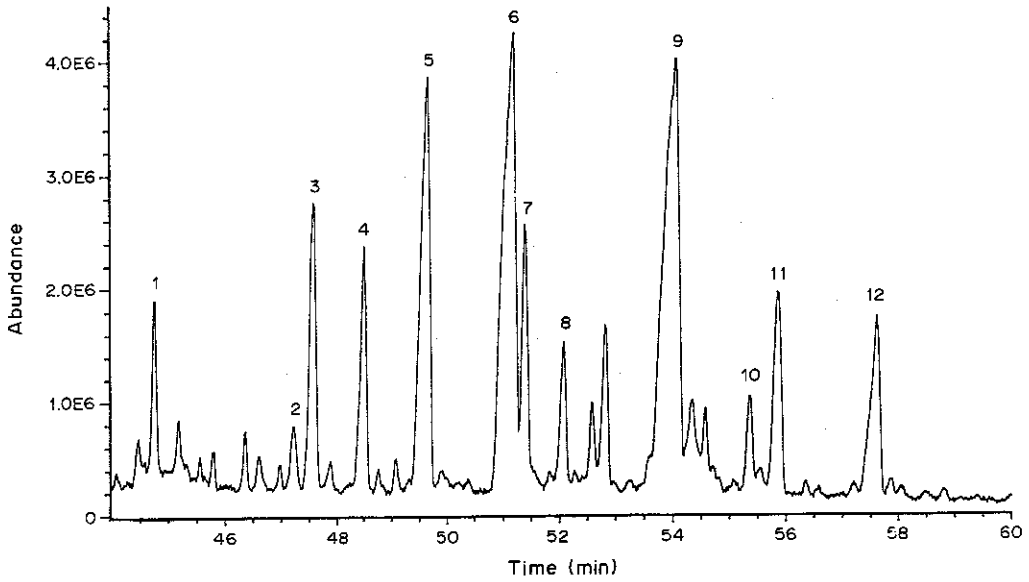

Fig. 2. Partial reconstructed ion chromatogram (44–60 min) illustrating a representative sterol profile obtained for Erebus Ice Tongue sea-ice diatom community, December 18, 1985. Peak numbers refer to Table 2.

similar profiles at the Cape Armitage, Erebus Ice Tongue and Cape Evans sites reflect the similarity of the species composition at these three sites. Differences in the relative proportions of individual sterols at these three sites reflect the different proportions of the species. Likewise, the vastly different species composition of the Wohlschlag Bay sea-ice diatom community, compared with the other three sites, is reflected in the sterol profiles (Table 2).

The sterol profiles showed several interesting features. 24-Ethylcholesterol was the main sterol in the Erebus Ice Tongue sea-ice diatom community on one occasion (18 December), and in two other collections its abundance was between 50 and 80% of the C_{28} sterols. 24-Ethylcholesterol was also the second or third most abundant sterol detected in the Cape Armitage and Cape Evans samples. This sterol has been commonly used as a marker for higher plants (Huang and Meinschein, 1976, 1979). In presence in Antarctic marine sediments in McMurdo Sound (Smith *et al.*, 1989; Venkatesan, 1988), in soils associated with the dry valley lakes of Victoria Land (Matsumoto *et al.*, 1982) and in cyanobacterial mats (modern stromatolites) from Antarctic oasis lakes

(Orcutt *et al.*, 1986), where terrestrial input is negligible, has been attributed to cyanobacterial or green algal input. The similarity of the sterol profiles of sea-ice diatom communities (Table 2) to those observed in previous Antarctic studies strongly indicates that diatoms, rather than cyanobacteria or other algal groups previously proposed, are a more probable source of C_{29} sterols, including 24-ethylcholesterol, in these extreme environments.

The occurrence of 24-ethylcholesterol at high relative abundance in the sea-ice diatom communities extends the number of marine algal classes in which this sterol is found as a major component. Sterols are converted to steranes in ancient sediments, and the ratio of C_{27} to C_{29} steranes is often used as an index of the relative amounts of marine and terrigenous organic matter. The finding of C_{29} sterols at high abundance in marine algae, and in this instance sea-ice diatoms, supports the view that it cannot be assumed that C_{29} sterols or steranes are necessarily derived from terrigenous sources (Jones *et al.*, 1987; Volkman, 1986). In Antarctic studies, variations in the ratio of C_{27} to C_{29} sterols or steranes are more likely to reflect changes in species composition than variations in the proportions of marine and terrestrial input (Smith *et al.*, 1989).

Analysis of sea-ice diatom communities provides an opportunity to follow a natural algal bloom through development and senesence. Interfering processes encountered in oceanographic studies, such as vertical mixing and horizontal advection, are absent. Similarly, losses from grazing and sinking are minor. Studies of sea-ice diatom communities are thus more likely to reflect physiological changes in the algal community than are open-water studies. Changes in the proportion of C_{27}, C_{28} and C_{29} sterols and individual sterol components were observed at the Erebus and Cape Armitage sites over the 1985 study period (Table 2). These changes are most likely the result of changing environmental conditions (e.g. nitrate or silicate concentration or light intensity), and provide a field example of algal sterol profiles changing with environmental conditions as observed with sterol profiles from pure cultures of algae by Ballantine *et al.* (1979).

A C_{26} sterol was detected as a minor component in the sea-ice diatom communities. A C_{26} sterol was previously noted as a minor constituent of the diatom *Thalassionema nitzschioides* (Ballantine *et al.*, 1979). A number of studies have used 24-norcholesta-5,22-dien-3β-ol as a marker for animals or invertebrates (Gillan, 1981; Volkman *et al.*, 1981). The discovery of C_{26} sterolds in diatoms suggests their use as invertebrate markers should be qualified with additional data.

4-Methyl sterols

Three C_{30} sterols were detected in the sea-ice diatom communities. Dinosterol, identified from mass spectral data and coinjection with the authentic sterol, was present as a minor component (0.1–3.2% of total sterols, Table 2) in the Erebus Ice Tongue and Cape Armitage samples. Dinosterol was below detection at the Cape Evans and Wohlschlag sites. Dinosterol has to date only been reported in dinoflagellates and has therefore been used as a biomarker for this algal group (de Leeuw *et al.*, 1983). However, in a study of the sterol distribution of microbial mats in Solar Lake adjacent to the Gulf of Aqaba in north-east Sinai, Edmunds and Eglinton (1984) suggested that dinosterol and related sterols are not exclusive markers for dinoflagellates, but may be produced by other organisms. In our study, dinoflagellates were not observed during microscopy

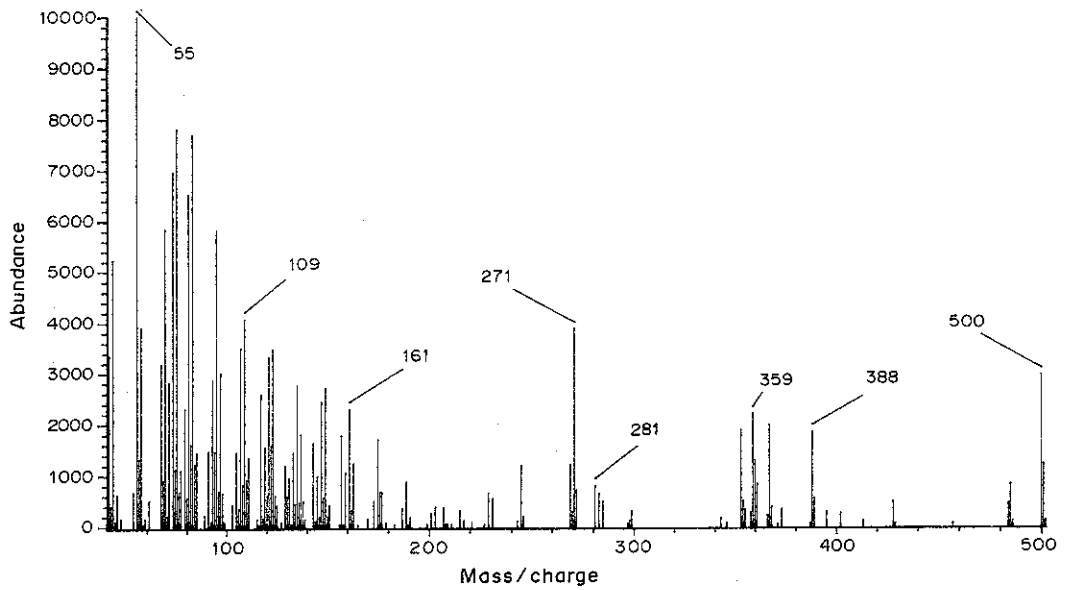

Fig. 3. Mass spectrum of sterol #11. The spectrum is similar to that obtained for 4-methyl-24-ethyl-5α-cholest-22-3β-ol (isolated from *Pavlova lutheri*), which elutes just before sterol #11.

of freshly prepared samples, which suggests that sources other than dinoflagellates may be responsible for the presence of dinosterol in the sea-ice diatom communities.

Two other C_{30} sterols (sterols #11 and 12; Table 2, Figs 2 and 3) were detected in both the Cape Armitage and Erebus Ice Tongue samples. Although standards are not presently available for these two compounds, the mass spectrum of sterol #11 was similar to that of a C_{30} sterol identified as 4-methyl-24-ethyl-5α-cholest-22-en-3β-ol found in the prymnesiophyte *Pavlova lutheri* (Ballantine *et al.*, 1979; Volkman *et al.*, unpublished data). Sterol #11 eluted, however, just after the sterol isolated from *Pavlova*. From the data presently available, sterol #11 is structurally very similar to 4-methyl-24-ethyl-5α-cholest-22-en-3β-ol, but may differ in the position, or more probably the stereochemistry, of a side-chain substituent group. The mass spectrum of sterol #11 (and of 4-methyl-24-ethyl-5α-cholest-22-en-3β-ol) are distinguished from that of dinosterol by their higher m/z 83 to m/z 69 ratio. Sterol #12 was identified as a C_{30} stanol from GC–MS data, and eluted just after authentic dinostanol. It is presumed to be the saturated analogue of sterol #11.

The relative proportion of C_{30} sterols present at the Cape Armitage and Erebus sites decreased during the study period (Table 2). These differences may again be due to either differences in species composition or growth-phase induced changes. We believe the latter to be the more important mechanism in this instance; microscopy of the samples did not show a marked decrease in the cell numbers of any species that paralleled the observed decrease in 4-methyl sterols. However, further studies with pure cultures of these algae are required before this can be confirmed. To data, these sea-ice diatom species have not been isolated and cultured.

Whilst the precise structures of the C_{30} sterols (#11 and 12) in these Antarctic sea-ice diatom communities have not been determined, their occurrence raises a number of interesting points. C_{30} sterols have not been previously reported in diatoms. Their occurrence in natural sea-ice diatoms suggests that other flagellates not observed by microscopy may be present or, more probably, that sea-ice diatoms may biosynthesize C_{30} sterols containing a methyl group at the C_4 position. Small amounts of C_{28} and C_{29} sterols containing a methyl group at the C_4 position were also detected in the Cape Armitage and Erebus Ice Tongue sea-ice diatom communities; these sterols are most likely precursors to the C_{30} sterols. These components have also not been reported previously in diatoms; their presence, as well as that of the 4-methyl C_{30} sterols, may be due to temperature-induced adaptations in sterol biosynthetic pathways.

There has been increased interest recently in the occurrence of dinosterane and other 4-methyl steranes in ancient sediments and petroleum (Wolffe *et al.*, 1986a,b; Summons *et al.*, 1987). In this study, we have identified a third 4-methyl C_{30} sterol (in addition to dinosterol and 4-methyl-24-ethyl-5α-cholest-22-en-3β-ol) in sea-ice diatoms that may be a potential precursor to 4-methyl cyclic hydrocarbons found in ancient sediments. Further studies on the occurrence of these biomarkers will enhance our knowledge of the biology of fossil algae as well as providing an additional tool for investigating the relationships between petroleums and their source rocks.

Sea-ice diatoms are important constituents of the carbon and energy flux in polar regions. The data presented in this report give an insight into changes in the sterol compositions of sea-ice diatom communities during a spring bloom. Analysis of sterols and other lipid classes, such as hydrocarbons, at the molecular level has also yielded information important to studies of chemotaxonomy, dietary transfer of lipids in polar marine food-webs, and aspects of the microbial ecology, oceanography and organic geochemistry of diatom communities and associated sediments.

Acknowledgements—This research was supported in part by National Science Foundation Grants DPP-8415215 to ACP and C. W. Sullivan, and DPP-82-1379601 to DCW. We thank C. W. Sullivan and M. P. Lizotte, of the University of Southern California, for field assistance, unpublished microscopic data and valuable discussions; J. K. Volkman of the CSIRO Division of Oceanography, and Professor E. Holdsworth and C. A. Mancuso of the University of Tasmania for their comments and discussion during manuscript preparation; V. Mawson for editing of the text. Gifts of sterol standards from the Steroid Reference Collection of the National Research Council (U.K.) are gratefully acknowledged.

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