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OCCURRENCE OF AN ISOPRENOID C_{25} DIUNSATURATED ALKENE AND HIGH NEUTRAL LIPID CONTENT IN ANTARCTIC SEA-ICE DIATOM COMMUNITIES[1]

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ABSTRACT

The lipid and hydrocarbon composition of natural populations of diatom communities collected during the austral spring bloom of 1985 in the sea-ice at McMurdo Sound, Antarctica was analyzed by TLC-FID, GC and GC-MS. Sea-ice diatom communities were dominated by Amphiprora *sp.,* Nitzschia stellata *Manguin and* Berkeleya *sp. at Cape Armitage;* N. stellata, Amphiprora, Pleurosigma, N. kerguelensis *(O'Meara) Hasle and some small centric diatoms adjacent to the Erebus Ice Tongue; and* Porosira pseudodenticulata *(Hustedt) Jouse at Wohlschlag Bay. Lipid distributions of the sea-ice diatom communities from the Cape Armitage and Erebus sites were characterized by high concentrations of triacylglycerol (triacylglycerol/polar lipid = 1.0 to 1.5). The hydrocarbon n-$C_{21:6}$, common in temperate diatoms, and an isoprenoid C_{25} diunsaturated alkene were the dominant hydrocarbons detected at these two sites. Hydrogenation of the C_{25} diene produced the known alkane 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane. The C_{25} diene is one of several structurally related hydrocarbons reported in many estuarine, coastal and oceanic sediments. We propose that certain species of diatoms are a likely source of these alkenes in sediments. The first reported biological occurrence of the C_{25} diene in the green seaweed* Enteromorpha prolifera *may have been due to the presence of epiphytic microalgae in the field sample analysed.*

Key index words: Antarctic sea-ice diatoms; lipids; triacylglycerols; hydrocarbons; isoprenoid C_{25} diene; 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane; GC-MS

During the austral spring and summer, land-fast sea ice in the Antarctic and Arctic regions supports the growth of rich and diverse sea-ice microbial communities (10,15). The recognition that psychrophilic microalgae significantly contribute to productivity in polar oceans has resulted in a range of studies of the development, growth and ecology of sea-ice microbial communities (3,4,8,9,14,25).

As part of a continuing research program our group has been examining changes in the photosynthetic metabolism of sea-ice microalgae during the spring bloom period at McMurdo Sound, Antarctica. Our principle techniques are measurement of ^{14}C assimilation into crude fractions of protein, lipid (including subfractions), carbohydrate and small molecular weight metabolites (SMWM) (23,26), and analysis of microbial (algal and bacterial) biomass and community structure by measuring properties common to all cells and identifying specific signature pigments (Palmisano, unpubl. data) and phospholipids (20). Recent ^{14}C-productivity experiments showed that the proportion of label assimilated into lipid, protein, carbohydrate and SMWM did not change greatly during the spring bloom period (26). However, assimilation of ^{14}C into specific lipid fractions (neutral, glyco- and phospholipids) changed dramatically over the course of the bloom and its decline (23).

These observations prompted an analysis of the neutral lipid material, including hydrocarbons, of sea-ice diatom communities from McMurdo Sound, Antarctica. Limited data are available concerning the lipid composition of Antarctic sea-ice diatoms (12,20,42) and Antarctic freshwater cyanobacterial mats (21). However, no information has been reported as yet on the hydrocarbon distributions of these algae. Characterization of these and other lipid components of members of the sea-ice diatom community may provide signature lipids that can be used to determine the contribution of organic matter from sea-ice algae to benthic sediments or foodwebs. Aspects of the biosynthesis and the oceanographic and organic geochemical significance of the hydrocarbon profiles observed are discussed.

MATERIALS AND METHODS

Sea-ice communities were sampled during December 1985 at three sites: Cape Armitage, the Erebus Ice Tongue and Wohlshlag Bay in McMurdo Sound, Antarctica (Fig. 1). The Cape Armitage site is described in detail elsewhere (e.g. 14,23,24). 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 1.6 to 2.5 m. The lower 20 cm sections of each core were obtained with a stainless steel handsaw, and samples were maintained in darkness by wrapping them in opaque black plastic and placing them in Freezesafe styrofoam containers. It was previously demonstrated that 99% of the chlorophyll *a* is found in

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the bottom 20 cm of the congelation ice in McMurdo Sound, Antarctica (24,25). Each core was melted in 1.2 L of filtered seawater with care taken to ensure sample temperature did not rise above 5° C. The freshly collected unpreserved sea-ice microalgae samples were examined under a Zeiss phase-contrast microscope to determine species composition. Sea-ice algal cells were prepared for lipid analysis by filtration onto glass fiber filters (Whatman GF/C) that had been 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 (6,43,44). 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.

Subsequent lipid fractionation and analysis were performed at the CSIRO Marine Laboratories in Hobart. A portion of the total lipid extract was analyzed with an Iatroscan MK III TH10 TLC-FID analyzer (Iatron Laboratories, Japan) as described in detail elsewhere (39). Samples were applied to silica gel SII chromarods (5 μm particle size) using 1-μL disposable micropipettes (Drummond). Chromarods were developed in a glass tank lined with preextracted filter paper. The solvent system used for the lipid separation was hexane-diethyl ether-acetic acid (60/17/0.5; v/v/v). This solvent system was designed to clearly resolve triacylglycerols and free fatty acids as well as other common neutral lipid classes (39). After development, the chromarods were oven dried for 10 min at 100° C and analyzed immediately in order to minimize adsorption of atmospheric contaminants. The FID was calibrated for each compound class (0.1–20 μg range). Total lipid content represents the sum of the individual lipid classes determined using the Iatroscan TLC-FID (Tables 1, 2). A further 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. The hydrocarbon fraction was eluted using 4 mL hexane and 7 mL hexane-toluene (1/1; v/v).

Gas chromatographic (GC) analyses were performed using 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. Samples were injected at 40° C in the splitless mode with a 0.5-min venting time. The injector was fitted with an insulation cover to maximize transfer of high-boiling components from the heated injector to the column. Quantitative recovery was checked using an n-alkane mixture (n-C_{12} to n-C_{30}). After 1 min, the oven was temperature-programmed from 40 to 100° C at 30° C/min, then at 4° C/min to 280° C. Hydrogen was used as carrier gas, and the injector and detector were maintained at 280 and 300° C respectively. Peak areas were quantified using chromatography software (DAPA Scientific Software, Kalamunda, Western Australia) operated using an IBM-XT personal computer. Lipid and hydrocarbon compositional data reported for these samples are the means of 2–3 replicate analyses. Hydrogenations were carried out by adding the hydrocarbons to a suspension of PtO_2 (Adams catalyst) in purified iso-octane and bubbling H_2 through the suspension for 3 h.

Gas chromatography-mass spectrometric (GC-MS) analyses of hydrocarbon samples were performed on a Hewlett Packard (HP) 5890 GC and 5970 Mass Selective Detector (MSD) fitted with a direct capillary inlet. The column, injector and chromatography conditions were similar to those described above, with the exception that helium was used as the carrier gas. Mass spectra were acquired and processed using an HP 59970C Workstation operated in scan acquisition mode. Typical MSD operating conditions were: electron multiplier 1600–1800 volts; transfer line 300° C; autotune file DFTPP normalized; electron impact energy 70 eV; scan threshold = 1000; 0.8 scans/sec; mass range 40–600 amu; solvent delay 5 min. Identifications were confirmed by comparison of mass spectra with those of previously reported spectra (e.g. 1,2,5,33,34) and by comparison of retention data (both before and after hydrogenation) with data obtained for commercial and previously identified laboratory standards (e.g. hydrocarbons isolated from Peruvian coastal sediments; 40).

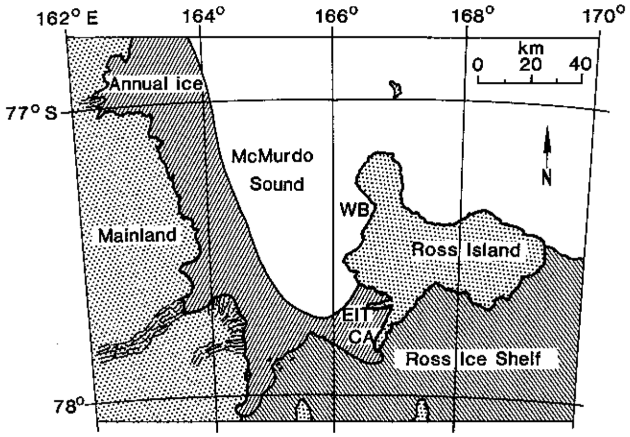

FIG. 1. Sampling sites at McMurdo Sound, Antarctica: CA = Cape Armitage, EIT = Erebus ice tongue, WB = Wohlschlag Bay.

RESULTS AND DISCUSSION

Species Composition

Microscopic examination of freshly collected unpreserved sea-ice algal communities revealed that diatoms were the only microalgae present in the samples from all three sites. The sea-ice diatom communities were dominated by *Amphiprora* sp., *Nitzschia stellata* Manguin and *Berkeleya* sp. at Cape Armitage; *N. stellata, Amphiprora, Pleurosigma, N. kerguelensis* (O'Meara) Hasle and some small centric diatoms adjacent to the Erebus Ice Tongue; and *Porosira pseudodenticulata* (Hustedt) Jouse at Wohlschlag Bay. Representative species composition data are shown in Table 1. Grossi et al. (14) estimated a maximum bacterial carbon contribution of 0.002 g C·m^{-2}. This value is insignificant (0.05%) compared to the average carbon contributed by algae, which has been estimated at up to 4.1 g C·m^{-2} (22). Similarly, carbon/Chl *a* values of less than 40 were previously reported for Antarctic sea-ice algae communities (9,25,41), further supporting the view that algae, in this case diatoms, are the primary source of carbon and therefore lipids in sea-ice microbial communities.

Lipid Composition

TLC-FID chromatograms of lipids obtained from the sea-ice diatom communities are shown in Figure 2. Six peaks were present in all samples analyzed: hydrocarbon (including carotenes), triacylglycerol, free fatty acid, sterol, an unidentified polar lipid (probably diacylglycerol) and polar lipid (including phospholipid, glycolipid and chlorophylls). The Wohlschlag Bay community, dominanted by *Porosira pseudodenticulata,* contained predominantly polar lipid, with small amounts of neutral lipid components present (Fig. 2a, Tables 1, 2). This profile is

TABLE 1. *Abundances of lipid, hydrocarbon and chlorophyll in Antarctic sea-ice communities.*

	Lipid abundances (mg·m^{-2})		
Lipid class	Cape Armitage 18 Dec 1985[a]	Erebus Ice Tongue 18 Dec 1985	Wohlschlag Bay 12 Dec 1985
Total lipid content	2577 (885)[b]	1830 (140)	1200 (30)
Total hydrocarbon content	63 (21)	29 (14)	24 (15)
Total chlorophyll content	100 (24)	50 (17)	—[c]

[a] Collection dates.

[b] Mean of 2 or 3 cores (±SD). Data for total lipid and hydrocarbon content were obtained using an Iatroscan TLC-FID analyzer (39). The total lipid data represents the sum of the individual lipid classes determined using the TLC-FID analyzer. Chlorophyll content courtesy of Palmisano et al. (23). For the Cape Armitage site the relative proportion of ice-diatom species (not including *Berkeleya* sp.) was *Nitzschia stellata,* 58% of total; *Amphiprora* sp., 29%; *N. kerguelensis,* 3%; *Pleurosigma* sp., 2%; small centrics, 2%; naviculoid species, 6%. Total cell concentration was 5.3 × 10^4 cells·mL^{-1}. At the Wohlschlag Bay site *Porosira pseudodenticulata* accounted for around 90% of the total population.

[c] No data.

similar to that reported for a natural population of the ice diatom *Navicula glaciei* (42).

Lipids from the Cape Armitage and Erebus Ice Tongue sites, in contrast to the Wohlschlag Bay sample, contained high proportions of triacylglycerol (Fig. 2b, c). The high triacylglycerol content of the Cape Armitage and Erebus Ice Tongue communities was confirmed by GC analysis of the fatty acid methyl esters formed by direct transesterification of the triacylglycerols using BF_3/MeOH, and additionally by fluorometric determination of the neutral lipid content of the cells using Nile Red (13). The fatty acid profiles were similar to those typically reported for diatoms (12,20,42) with 14:0, 16:1*w*7c, 16:0, and 20:5*w*3 present as major components. The differences in neutral lipid content, particularly triacylglycerol, between the Wohlschlag Bay sea-ice diatom communities and those at Cape Armitage and the Erebus Ice Tongue may possibly reflect differences in the physiological state between these communities. We suggest, however, that the markedly differing species composition at these sites is a more likely explanation.

The high triacylglycerol content of the two Antarctic sea-ice diatom communities at the Cape Armitage and Erebus Ice Tongue sites occurred at a time close to the observed chlorophyll maximum of the austral 1985–1986 spring bloom (23). Samples collected later in the season contained relatively lower proportions of triacylglycerol (unpubl. data). However, the triacylglycerol concentration for the late-season samples was still significantly greater than that observed in the Wohlschlag Bay sea-ice diatom community. The lipid compositional data obtained for the Cape Armitage and Erebus sites follow the same trends established by Morris (18) who examined changes in lipids in a temperate, planktonic, diatom-dominated experimental water column. In his study, during the initial stages of a bloom, the diatom lipids were dominated by triacylglycerols. As the bloom progressed, triacylglycerols decreased whereas polar lipids increased.

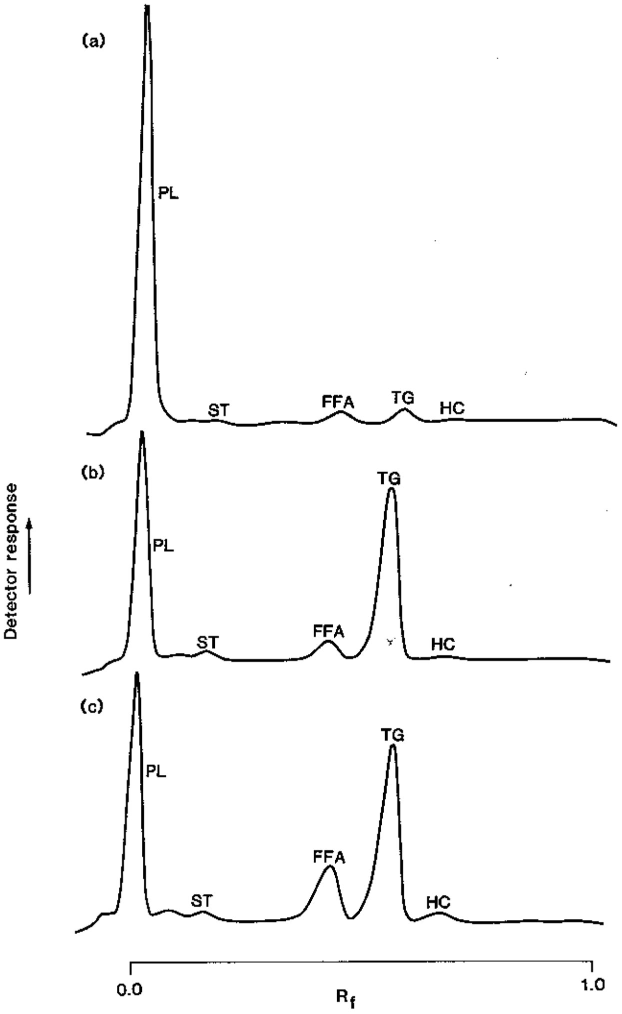

FIG. 2a–c. Iatroscan TLC-FID chromatograms of total lipids from sea-ice diatom communities. (a) Wohlschlag Bay, (b) Cape Armitage, (c) Erebus Ice Tongue. HC = hydrocarbons, TG = triacylglycerols, FFA = free fatty acids, ST = sterols, PL = polar lipid.

Hydrocarbons

The hydrocarbon profiles obtained for the sea-ice diatom communities at the Cape Armitage and Erebus Ice Tongue sites were dominated by two unsaturated components (Fig. 3, Table 3). Compound I, n-heneicosahexaene (n-$C_{21:6}$), is often the major hydrocarbon in photosynthetic diatoms (7,16), but it is not found in nonphotosynthetic species. The very low relative abundance of n-$C_{21:6}$ in lipids of diatoms from Wohlschlag Bay (Table 3) again reflects the different species composition found in this environment as suggested above. *Porosira pseudodenticulata,* based on these data, contains only trace amounts of n-$C_{21:6}$.

TABLE 2. *Percentage composition of lipid classes for Antarctic sea-ice diatom communities.*

		Percent composition[b]		
Lipid class	Abbreviation[a]	Cape Armitage	Erebus Ice Tongue	Wohlschlag Bay
Hydrocarbons	HC	2.7 (1.5)[c]	1.5 (0.9)	1.7 (1.6)
Triacylglycerols	TG	49.4 (7.0)	43.4 (8.8)	7.0 (1.1)
Free fatty acids	FFA	9.3 (6.5)	6.0 (1.3)	6.4 (3.0)
Sterols	ST	1.6 (0.1)	1.9 (0.4)	1.8 (0.3)
Polar lipid	PL	34.9 (2.9)	45.3 (8.7)	81.0 (1.8)
Other	—	1.8 (1.1)	2.0 (2.0)	2.4 (0.6)

[a] Abbreviations refer to Figure 2.

[b] Percent composition of total lipids. Data obtained using an Iatroscan TLC-FID analyzer (39). See Table 1 footnote.

[c] Mean of 2 or 3 cores (±SD).

The second most abundant hydrocarbon in all samples was identified as a diunsaturated isoprenoid C_{25} alkene containing an unusual linkage of C_5 isoprenoid units (II). The mass spectrum of alkene II and GC retention data are identical to those reported for the same compound isolated from marine sediments by Albaiges et al. (1,2) and other workers (5,27,28,30–38,40). Attempts to establish double bond positions using derivatization procedures have not been successful to date. Other isomers of the diene were previously reported (32) but were not present in the sea-ice diatom hydrocarbons.

The electron impact mass spectrum and GC retention data (Table 4) of the parent alkane III, formed by catalytic hydrogenation of compound II, were identical to those observed in previous studies (1,2,33); hence, structure III was identified as 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane (Fig. 4). Alkane III also coeluted with the isoprenoid C_{25} alkane formed by hydrogenation of the C_{25} alkene fraction characterized for Peruvian upwelling sediments (40). This alkane was not detected in the sea-ice diatom communities.

Although hydrocarbon biosynthesis in unicellular algae has not been extensively examined, the formation of straight-chain alkanes and alkenes by decarboxylation of the corresponding fatty acid is well established (17). Thus, heneicosahexaene (n-$C_{21:6}$) is formed from 22:6ω3 fatty acid (16), which was present as a minor component in the fatty acids of the sea-ice diatom communities. The isoprenoid alkene is apparently not formed by the same mechanism since no long-chain branched fatty acids were detected in these samples. It is likely that biosynthesis proceeds by condensation of 5 isoprene units, with concurrent or subsequent loss of the double bonds. The sea-ice diatom communities contained no C_{20} or C_{30} homologues, although these chain-lengths have been found in some sediments (1,2).

In a recent study, Rowland et al. detected the C_{25} diene (II) in field samples of the macroscopic green seaweed *Enteromorpha prolifera* (34). This was the first report of the biological occurrence of these unusual isoprenoid hydrocarbons, although they are commonly found in marine sediments (e.g. 1,2,30–38,40).

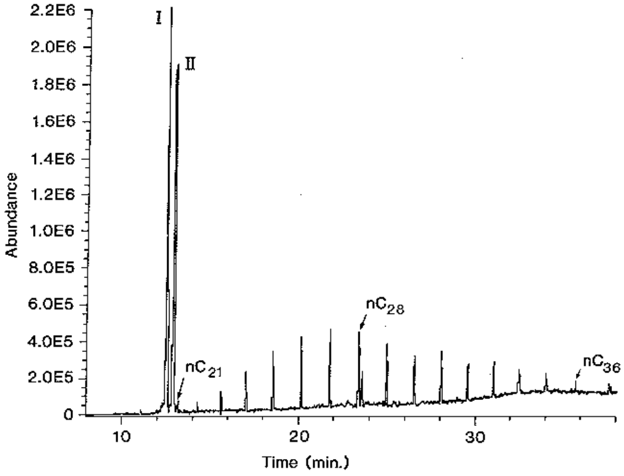

FIG. 3. GC-MS total ion chromatogram illustrating a representative hydrocarbon profile obtained for the sea-ice diatom community from Erebus Ice Tongue site. Peak numbers: I = n-$C_{21:6}$, II = isoprenoid C_{25} diene. Chain lengths of n-alkanes are as shown on the chromatogram.

The isolation of the isoprenoid C_{25} diene as a major hydrocarbon in sea-ice diatom communities is, to our knowledge, the first report of such a compound in unicellular algae. It is noteworthy that Rowland et al. also reported n-$C_{21:6}$ in *Enteromorpha* (34). As this compound had not been previously detected in *Enteromorpha,* but is common in diatoms, they proposed that epiphytic microalgae were present. They also pointed out that as their *Enteromorpha* samples were from the field, it remained to be proven that the isoprenoid C_{25} diene and related hydrocarbons were synthesized by the macroalga itself. In our opinion, the co-occurrence of the C_{25} diene and n-$C_{21:6}$ in sea-ice diatom communities supports the hypothesis that the detection of the C_{25} diene in *Enteromorpha* was due to the presence of epiphytic microalgae. The absence of this alkene in the related species *E. intestinalis* (unpubl. data) adds further support to this suggestion.

TABLE 3. *Composition of hydrocarbons for Antarctic sea-ice diatom communities.*

		Percent composition[b]		
Hydrocarbon	Peak no.[a]	Cape Armitage	Erebus Ice Tongue	Wohlschlag Bay
n-$C_{21:6}$	I	59.0	41.9	TR[c]
$C_{25:2}$	II	32.0	24.0	11.9
n-C_{14} to n-C_{37} Alkanes	—	8.8	34.1	62.0
Squalene	—	0.21	ND[d]	26.1

[a] Peak numbers refer to Figure 3 and Table 4.

[b] Percent composition of total hydrocarbons. Other minor components present but not quantified.

[c] Trace.

[d] Not detected.

III

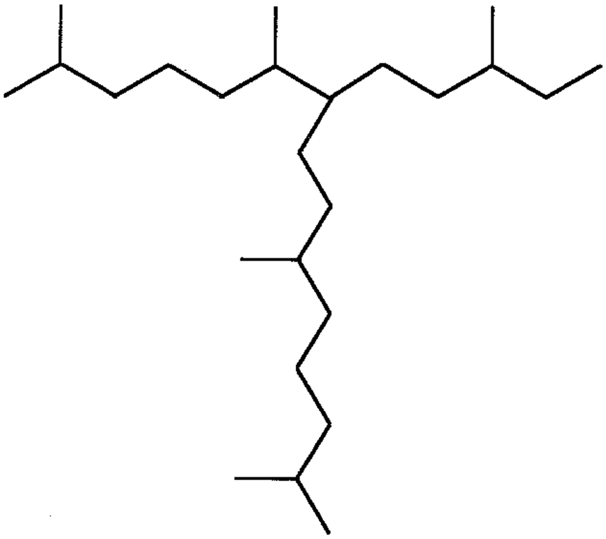

FIG. 4. Carbon skeleton of compound III: 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane (isoprenoid C_{25} alkane) obtained from hydrogenation of compound II (isoprenoid $C_{25:2}$).

Previous studies reported the presence of C_{25} isoprenoid alkenes in particulate matter obtained from sediment traps deployed in coastal waters off Peru (40) and Washington State (27). Since the material in these traps is derived from many different sources, including zooplankton, phytoplankton, detritus from these sources, and associated bacteria, the exact source of the alkenes cannot be determined. Our observation of a C_{25} diene in sea-ice algae indicates that unicellular algae can synthesize these hydrocarbons, and it seems likely that diatoms are a major source of these alkenes in particulate matter and sediments. The low abundance of the alkene in the Wohlschlag Bay diatom community indicates that it probably occurs in only a few species. We have yet to identify any temperate diatom species in culture that contain these alkenes, and to date axenic cultures of sea-ice diatoms have not been available. The alkenes are not present in the temperate diatoms *Biddulphia sinensis, Thalassiosira eccentrica, T. pseudonana, T. oceanica, Asterionella glacialis, Chaetoceros debilis* or *Skeletonema costatum* (unpubl. data).

Bacteria have been proposed as a source of C_{25} alkenes in marine sediments (32). Requejo and Quinn also reported that C_{25} alkenes were formed during laboratory decomposition of samples of *Cladophora* algal mat inoculated with homogenized sediment (30). As the alkenes were not detected in *Cladophora* itself, Requejo and Quinn hypothesized that they were formed during early diagenetic processes or perhaps from de novo synthesis by organisms such as purple photosynthetic bacteria, which are active in anaerobic decomposition of organic matter. We suggest that a bacterial source is unlikely and that diatoms were the more probable source of alkenes in the sediments they analyzed. This explanation is supported by the knowledge that epiphytic diatoms are commonly abundant in such algal mats (21).

A series of n-alkanes, ranging from n-C_{14} to n-C_{37}, was present in the hydrocarbon fractions from all environments (Table 3, Fig. 3). Some n-alkanes were present in control analyses ($<10\%$ of that found in samples), but this could not account for those found in the samples. Moreover, petroleum-derived components such as pristane, phytane and unresolved complex material were absent in the sea-ice diatom community lipids (Fig. 3). These data support the view that the n-alkane series detected in the sea-ice samples is biogenic in origin. Alkanes showing a high predominance of even-carbon chains have been found in Antarctic marine animals (19,29). It was proposed that an unusual biosynthetic pathway, in

TABLE 4. *Mass spectral characteristics and structural assignments for major hydrocarbons present in sea-ice diatom communities.*

Peak no.[a]	ECL[b]	Identification	M^+ (%)	Diagnostic ions (%)
I	20.48	n-$C_{21:6}$	ND[c]	41 (70), 67 (53), 79 (100), 91 (65) 105 (28), 108 (29), 119 (21), 133 (10) 145 (7), 159 (5), 173 (4), 187 (2) 199 (1), 201 (1), 215 (2), 241 (1) 255 (1)
II	20.88	ip$C_{25:2}$	348 (1.3)	43 (100), 55 (94), 69 (80), 81 (59) 95 (68), 109 (47), 123 (33), 137 (17) 151 (9.1), 165 (11), 180 (12), 193 (5.2) 207 (8.7), 221 (1.6), 235 (5.5) 263 (3.5), 266 (4.7), 320 (3)
III[d]	21.11	ipC_{25}	ND	43 (32), 57 (100), 71 (75), 85 (49) 99 (24), 113 (21), 127 (18), 141 (14) 153 (8), 155 (13), 169 (10), 183 (6) 197 (2), 210 (3), 238 (21), 266 (1) 295 (0.2)

[a] Refers to Figure 3 and Table 3.

[b] ECL: equivalent chain length on fused silica cross-linked methyl silicone column.

[c] Not detected.

[d] Structure III, 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane (ipC_{25}, see Fig. 4), formed from II (ip$C_{25:2}$) by hydrogenation.

either the predatory animals analyzed or in the lower phytoplanktonic members of the Antarctic food chain, may be responsible for the observed even-chain predominance (19). The n-alkane distribution in all three sea-ice diatom communities showed little or no even- or odd-carbon chain predominance (Fig. 3). Based on these data, we suggest that the animals themselves, rather than the lower phytoplanktonic members of the food-web, may be the source of alkane distributions showing a predominance of even-carbon chains. These data also add to the growing collection of reports of long-chain alkanes in microscopic algae (11).

CONCLUSIONS

Knowledge of the lipid composition of Antarctic sea-ice diatoms is important to studies of chemotaxonomy, dietary transfer and conversion of lipids in polar marine food-webs, and aspects of the microbial ecology, oceanography and organic geochemistry of these communities and associated sediments. This report provides base-line data on the lipid and hydrocarbon composition of sea-ice diatom communities collected from McMurdo Sound, Antarctica. A source for the unusual biological marker isoprenoid C_{25} alkenes, commonly detected in recent sediments, has been determined.

Future studies are planned to examine the distribution of components within other lipid classes and to follow changes occurring during the spring bloom period. Such data will permit a better understanding of the development, growth, ecology, biochemistry and other aspects of sea-ice microbial communities.

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