Marine particles
Fatty acids
Monounsaturates
Derivatization
16:1w10c

Particules marines
Acides gras
Monoinsaturés
Derivatisation
16 : 1w10c

Occurrence of *cis*-6-hexadecenoic acid and other unusual monounsaturated fatty acids in the lipids of oceanic particulate matter

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ABSTRACT

Distributions of monounsaturated fatty acids in oceanic particulate matter were determined after capillary GC-MS of their dimethyldisulphide (DMDS) adducts. The technique has permitted the wide range (typically 20-30 monounsaturated fatty acids per sample) of monounsaturated fatty acids present in seawater to be characterised. The unusual fatty acid 16:1*w*10c was detected in several samples as the major C_{16} monounsaturated fatty acid, and was present in samples from the six water masses analysed. The general occurrence of 16:1*w*10c in a range of seawater samples suggests the presence of this component has been previously overlooked. Differences in the relative and absolute abundance of 16:1*w*10c were observed between and within water masses. The limited data currently available suggest that marine invertebrates and bacteria may be sources of 16:1*w*10c . Other Δ6 monounsaturated fatty acids were also detected in marine particulate matter. *Trans* monounsaturated fatty acids were minor components in all samples analysed. *Trans/cis* ratios of less than 0.1 were observed for most of the common monounsaturated fatty acids, whilst ratios greater than 1 were found for several minor components of possible bacterial origin. Long-chain C_{22} to C_{28} monounsaturated (*w*9 and *w*7) fatty acids found in particulate matter from several environments are probably from invertebrate sources; these components may be used as markers for these organisms.

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RÉSUMÉ

Acide cis-6-hexadécénoïque et autres acides gras mono-insaturés peu communs dans les lipides de la matière organique océanique

La répartition des acides gras mono-insaturés dans la matière particulaire océanique a été déterminée par chromatographie en phase gazeuse capillaire couplée à la spectrométrie de masse de leurs adduits thiométhylés. Cette technique a permis de caractériser une gamme étendue d'acides gras mono-insaturés, de 20 à 30 par échantillon. L'acide gras mono-insaturé 16:1*w*10c est le composant majeur des acides gras mono-insaturés en C_{16} dans plusieurs échantillons. Sa présence dans tous les échantillons explique qu'il ait jusqu'alors échappé aux techniques d'analyse. Des différences dans l'abondance relative et absolue de 16:1*w*10c sont observées entre les masses d'eau et au sein de celles-ci. Les quelques données disponibles suggèrent que les invertébrés marins et les bactéries pourraient être des sources de 16:1*w*10c. Les autres acides gras mono-insaturés Δ6 sont détectés également dans la matière particulaire marine. Les acides gras mono-insaturés *trans* sont des composants mineurs des échantillons analysés. Les rapports *trans/cis* sont inférieurs à 0,1 dans la plupart des acides gras mono-insaturés communs, ils sont supérieurs à 1 pour plusieurs composants mineurs d'origine probablement bactérienne. Les acides gras à longue chaîne (C_{22} à C_{28}), mono-insaturés en

position *w*9 et *w*7, trouvés dans la matière particulaire de plusieurs milieux, semblent provenir d'invertébrés et peuvent être utilisés comme marqueurs d'origine de ces organismes.

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INTRODUCTION

Organic geochemists, marine chemists and oceanographers have used the analysis of specific lipid components, such as hydrocarbons, fatty alcohols, sterols and steroid ketones, to determine the chemical composition and flux of particulate organic matter in marine ecosystems. Analysis of specific biological marker compounds has also aided the understanding of the transport, transformation, remineralization and repackaging processes that affect particulate matter (*e.g.* Wakeham *et al.*, 1984).

Fatty acids (free and ester-linked) are the most abundant lipids present in oceanic particulate matter, and have therefore been the subject of many investigations. Whilst sediments and oceanic particulate matter samples from a range of environments have been analysed for fatty acids, few of these studies have provided details on double-bond positions and geometry. Gas chromatography alone cannot provide this information; other procedures such as derivatization combined with GC-MS must be used for precise identification and quantification. It has recently become evident that precise determination of monounsaturated fatty acid double-bond position and geometry is essential for the correct interpretation of increasingly complex data sets. In this way membrane-derived fatty acids have been successfully used as biomarkers in the fields of taxonomy, ecology, organic geochemistry and clinical microbiology (Gillan and Johns, 1986; Guckert *et al.*, 1985). The utility of fatty acids in oceanographic studies may be similarly enhanced through such derivatization procedures.

In our laboratory, analyses of the fatty acids of marine particulate matter using high-resolution capillary GC columns have revealed the presence of other 16:1 isomers apart from the commonly identified 16:1*w*7c. In this study, the dimethyldisulphide (DMDS) derivatization procedure (Dunkelblum *et al.*, 1985; Nichols *et al.*, 1986*a*) was used to perform detailed analyses of the monounsaturated fatty acids of marine particulate matter collected from different oceanic water masses and related samples. Our goal was to positively identify the other major C_{16} monounsaturated fatty acid, along with other monounsaturated fatty acids, present in seawater and, to determine how prevalent this component is in seawater. Characterization of these and other lipid components of oceanic particulate matter may provide signature lipids or chemical tracers to determine sources of organic matter in the marine environment. Aspects of the biosynthesis, and the organic geochemical and oceanographic significance of the monounsaturated fatty acid profiles are discussed.

MATERIALS AND METHODS

Study sites and sampling

The organic geochemical studies of suspended particulate matter were performed on samples from several oceanic areas (Fig. 1).

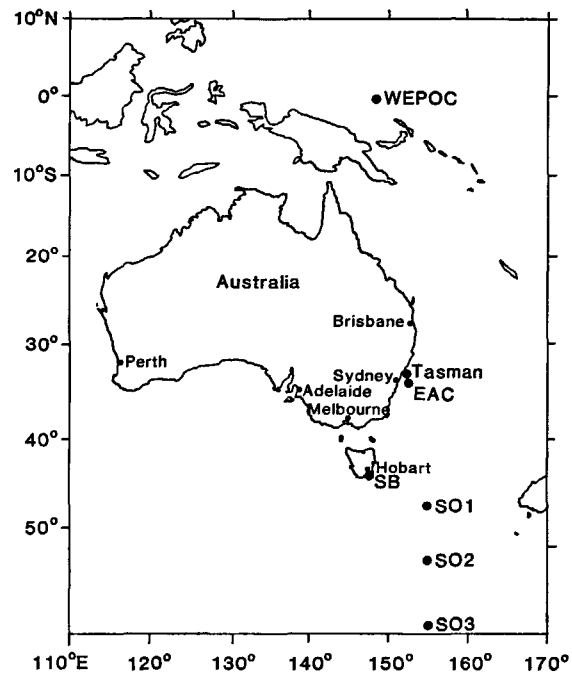

Figure 1

Location of sampling sites. Site abbreviations are as shown in Table 1.

Oceanographic data on these sites are presented in Table 1. The Storm Bay, WEPOCS, East Australian Current and Tasman Sea particulate matter samples were filtered with Whatman GF/F glass fibre filters (nominally 0.7 µm). Samples from the Southern Ocean were collected using Seastar *in situ* water samplers (Seastar Instruments, British Columbia) fitted with particulate filter units (*e.g.* Green *et al.*, 1986) and Schleicher and Schuell No 8 glass fibre filters (nominally 0.5 µm, 150 mm diameter). Previous studies have shown that adsorption of particles of sizes smaller than the filter size occurs in filtration of seawater. For example, 0.176 µm latex beads were retained by GF/C filters (1.2 µm nominal pore size; Johnson and Wangersky, 1985). It has also been recently shown that picoplankton (0.2 to 2.0 µm) are retained on both GF/F and GF/C filters (Prepas *et al.*, 1988). In this study of oceanic particles it has therefore been assumed that picoplankton, flagellates and bacteria were retained on the glass fibre filters, in addition to larger plankton and zooplankton-derived material.

Penaeus esculentus lipid samples were obtained from A. Chandumpai of the CSIRO Division of Fisheries, Cleveland, Queensland, Australia. The mixed zooplankton sample was collected in Storm Bay, Tasmania, with a 100 µm mesh ring net. Samples of tuna larvae *Thunnus alalunga* were provided by T. Davis and J. May of the CSIRO Division of Fisheries, Hobart, Tasmania, Australia.

Lipid extraction and fractionation

Lipids were quantitatively extracted from filters by the modified one-phase Bligh and Dyer procedure (Bligh and Dyer, 1959; White *et al.*, 1979). After phase separation, lipids were recovered in the chloroform phase, the solvents removed under vacuum, and the lipids stored under nitrogen at −20°C. Total neutral lipid and fatty acid fractions were obtained for Storm Bay and WEPOCS samples after alkaline saponification as described in Nichols *et al.* (1983). Lipid material from the Newcastle transect and Southern Ocean samples, which were collected and analysed subsequent to the analysis of the earlier sample material, was fractionated into individual lipid classes on silicic acid (3 g) deactivated with 5% distilled water (Volkman *et al.*, 1983; Nichols *et al.*, 1987) to obtain further information on the distribution of monounsaturated fatty acids between the lipid fractions. Polar lipid (Southern Ocean) and triacylglycerol (Newcastle) were obtained with acetone and methanol and hexane/ethylacetate (95/5; v/v) respectively. Fatty acid methyl esters (FAME) were formed with BF_3-methanol and stored at −20°C.

GC and GC-MS analyses

Gas chromatography (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 (0.11 µm film thickness, Hewlett-Packard, Australia) and a flame ionization detector (FID). Samples were dissolved in $CHCl_3$ with methyl-nonadecanoate (19 : 0) as the internal injection standard and injected at 40°C in the splitless mode, with a 0.5 minute 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 with an *n*-alkane mixture (n-C_{12} to n-C_{30}). After 1 minute, the oven was heated from 40 to 100°C at 30°C/mn, then at 4°C/mn to 300°C. Hydrogen was used as carrier gas, and the injector and detector were maintained at 290 and 310°C respectively. FAME were further analysed using a polar BP20 (carbowax) fused-silica column (25 × 0.25 mm i.d., SGE, Australia). Similar GC conditions to those described above were employed, except that 240°C was the upper limit of the second temperature ramp. Peak areas were quantified using chromatography software (DAPA Scientific Software, Kalamunda, Western Australia) and an IBM-XT personal computer. Components were quantitated by calibrated GC response; they are subject to errors of up to 5% for major peaks and up to 10% for minor peaks.

Gas chromatography-mass spectrometric (GC-MS) analyses of FAME and derivatized FAME 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 59970A Workstation operated in scan acquisition mode. Typical MSD operating conditions were: electron multiplier 1 800-2 200 V; transfer line 310°C; autotune file PFTBA normalized; electron impact energy 70 eV; threshold = 2 500-4 000; 0.8 scans/s; mass range 40-600 amu; solvent delay 7 minutes. Identifications were confirmed by comparison of mass spectra with those of previously reported spectra, and by comparison of retention data (both before and after derivatization) with data obtained for commercial and previously identified laboratory standards where available.

Table 1
Location, water depth, collection date and oceanographic data for marine particulate matter samples.

Sample Code [a]	Location		Sample depth (m)	Date collected	Temperature (°C)	Salinity
WEPOC	Western Equatorial Pacific 01°00′N 148°42′E		86	August 1985	27.0	35.3
EAC	East Australian Current 33°30′S 152°52′E		5	November 1983	21.3	35.7
TASMAN	Tasman Sea 33°13′S 152°19′E		5	November 1983	20.7	35.6
SB	Storm Bay	(i)	10	December 23, 1985	15.6	34.6
	43°10′S 147°32′E	(ii)	10	January 7, 1986	15.1	34.6
		(iii)	10	September 3, 1986	11.2	34.6
SO1	Southern Ocean		10	November 17, 1986	11.8	34.8
	45°59.6′S 155°00′E		50		9.6	34.6
			250		–	–
SO2	Southern Ocean		10	November 14, 1986	9.1	34.6
	51°10.9′S 155°00′E		50		9.6	34.6
			250		8.5	34.5
SO3	Southern Ocean		10	November 9, 1986	2.0	34.2
	57°14.6′S 155°00′E		50		2.0	34.5

[a] Sample codes refer to Figure 1.

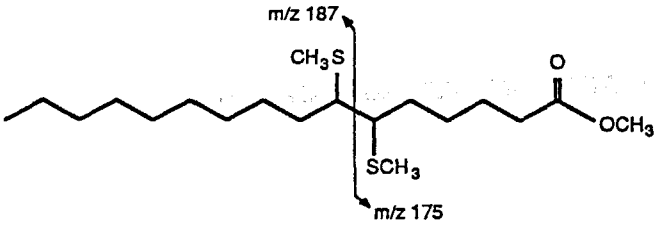

Figure 2
Structure of dimethyldisulphide adduct of 16:1w10c *illustrating the cleavage that gives rise to* Δ6 *fragment* (*m/z* 175) *and* w10 *fragment* (*m/z* 187) *ions observed by GC-MS analysis* (*Tab.* 2)

Determination of monounsaturated fatty acid double-bond position and geometry

The dimethyldisulphide (DMDS) adducts of monounsaturated FAME were formed to determine double-bond position and geometry, using methods previously described (Dunkelblum *et al.*, 1985; Nichols *et al.*, 1986*a*). Samples were analysed by GC and GC-MS as described above. The elution order of methyl thio adducts on the non polar column was the same as that of monounsaturated fatty acids prior to derivatization. GC-MS analysis of the DMDS adducts showed major ions attributable to fragmentation between the two CH_3S groups located at the original site of unsaturation (Fig. 2, Tab. 2). Detection of both the *w* and Δ-fragments was used to verify the original double-bond position of all monounsaturated fatty acids. Discrimination between *cis* and *trans* geometry in the original monounsaturated fatty acid was possible. The erythro isomer (originally the *trans* fatty acid) eluted after the threo isomer (originally the *cis* fatty acid) under the GC conditions employed. Adducts derived from the different positional isomers were generally at least partially separated by GC-MS as evidenced in Figure 3. The relative proportion of the respective *w* and Δ-fragments were used to quantitate individual components when coelution occurred. Further confirmation of double-bond geometry was obtained for selected samples using argentation thin-layer chromatography (TLC; Perry *et al.*, 1979) and GC-FT/IR analysis using a HP5880 GC coupled to a Digilab FT-IR spectrometer. PUFA also reacted completely under the conditions employed; products formed eluted after the region in which the monounsaturated fatty acid methyl thio adducts eluted, and therefore did not interfere with monounsaturated fatty acid identification.

Fatty acid nomenclature

Fatty acids are designated as total number of carbon atoms : number of double-bonds followed by the position of the double-bond from the *w* (aliphatic) or Δ (carboxylic) end of the molecule. The prefixes *i*, *ai* and *cy* indicate iso, anteiso and cyclopropyl branching respectively. The suffixes *c* and *t* indicate *cis* and *trans* geometry respectively. PUFA indicates polyunsaturated fatty acids. Double-bonds of PUFA are methylene interrupted.

RESULTS AND DISCUSSION

Sources of fatty acids

Capillary GC analyses of the fatty acids in 14 samples of oceanic particulate matter from tropical to subantarctic waters are reported in this paper. A representative GC-FID chromatogram of fatty acids in a sample from the Southern Ocean is shown in Figure 4. This chromatogram shows the good separation of most commonly occurring fatty acids that can be achieved with high resolution non polar 50 m capillary columns. The abundance of polyunsaturated fatty acids typical of marine phytoplankton and animals and the presence

Table 2
Characteristic ion fragments of adducts formed by reaction of oceanic particulate matter monounsaturated fatty acids with dimethyldisulphide (*DMDS*).

Fatty acid	Peak Number[a]	Ion fragments (m/z) of DMDS Adducts	
		ω-fragment[b]	Δ-fragment[c]
14 : 1ω9c	1	173	161
14 : 1ω8c	2	159	175
14 : 1ω7c	3	145	189
14 : 1ω5c	4	117	217
br 15 : 1[d]	5	173	175
15 : 1ω9c	6	173	175
15 : 1ω8c	7	159	189
15 : 1ω7c	8	145	203
16 : 1ω10c	9	187	175
16 : 1ω10t	10	187	175
16 : 1ω9c	11	173	189
16 : 1ω8c	12	159	203
16 : 1ω7c	13	145	217
16 : 1ω7t	14	145	217
16 : 1ω6c	15	131	231
16 : 1ω5c	16	117	245
br 17 : 1[d]	17	201	175
br 17 : 1[d]	18	201	175
17 : 1ω11c	19	201	175
17 : 1ω10c	20	187	189
17 : 1ω9c	21	173	203
17 : 1ω8c	22	159	217
17 : 1ω7c	23	145	231
18 : 1ω10c	24	187	203
18 : 1ω10t	25	187	203
18 : 1ω9c	26	173	217
18 : 1ω9t	27	173	217
18 : 1ω8c	28	159	231
18 : 1ω8t	29	159	231
18 : 1ω7c	30	145	245
18 : 1ω7t	31	145	245
18 : 1ω6c	32	131	259
18 : 1ω6t	33	131	259
18 : 1ω5c	34	117	273
18 : 1ω5t	35	117	273
20 : 1ω10	–	187	231
20 : 1ω9c	36	173	245
20 : 1ω7c	37	145	273
22 : 1ω9c	38	173	273
22 : 1ω7c	39	145	301
24 : 1ω9c	40	173	301
24 : 1ω7c	41	145	329
26 : 1ω9c	42	173	329
26 : 1ω7c	–	145	357

[a] Peak numbers refer to Figures 3, 5 and 6.
[b] ω-fragment indicates fragment including aliphatic end of the molecule.
[c] Δ-fragment indicates fragment including carboxylic end of the molecule.
[d] br, branched chain component; position of branching not determined.

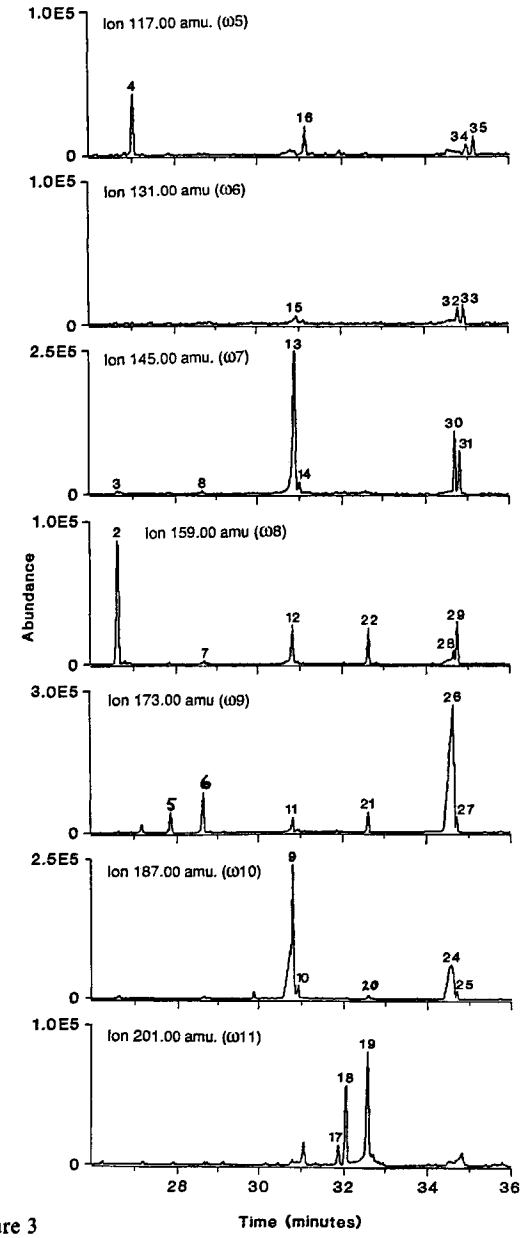

Figure 3

Partial reconstructed ion chromatograms of w-fragmentation ions obtained for monounsaturated fatty acid DMDS adducts in seawater particulate matter (WEPOC). Peak numbers refer to Table 2. Column, fused silica capillary, 50 m × 0.2 mm i. d. cross linked methyl silicone. Oven temperature, 50° for 1 minute, 30°/mn to 150°, then 4°/mn to 300°. Carrier gas was helium.

of two 16:1 isomers should be noted (Tab. 3). When shorter (25 m) columns were used, or when 16:1*w*7c was the dominant C_{16} monounsaturated fatty acid present, the two isomers were not separated by GC-FID analysis.

Compositional features such as the relative abundance of saturated and unsaturated fatty acids and positional isomers of polyunsaturated fatty acids can often provide information about the sources of fatty acids in seawater and sediments and hence of the organic matter in general. Similarly, use of TLC-FID can provide data on the relative proportions of lipid classes and therefore the origin of fatty acids (Volkman *et al.*, 1986). Additional information can be obtained from microscopic studies and from an analysis of chlorophyll and carotenoid pigments and other lipid classes such as sterols.

The samples analysed in this study are all from nutrient-poor waters where nanoplankton dominate the phytoplankton biomass. Diatoms are not abundant, and this is reflected in the low relative abundance of the major diatom-derived fatty acid, 16:1*w*7c, in all samples. In the Southern Ocean samples, the marine prymnesiophyte *Phaeocystis pouchetti* was a major constituent; this is reflected in the high proportion of 18:5*w*3 and 22:6*w*3 fatty acids found there (Tab. 3). Samples from the East Australian current and Western Equatorial Pacific show a high proportion of C_{18}, C_{20} and C_{22} polyunsaturated fatty acids and low amounts of C_{16} unsaturated fatty acids. Such distributions are similar to those found in marine prymnesiophytes, dinoflagellates and crustacea. Pigment analysis of the WEPOCS sample (Everitt *et al.*, in press) showed that the major constituents of the phytoplankton are prymnesiophyte algae, with significant numbers of green algae and cyanobacteria. Cholesterol is the major sterol in all of the Storm Bay, EAC and Tasman Sea samples (unpublished data), which indicates that animals are also important contributors of lipids on particles from these water masses. Fatty acids commonly used as bacterial markers (e. g. il5:0, ai15:0, i17:0, ai17:0, cy17:0, cy19:0; Perry *et al.*, 1979) were usually only minor components (Fig. 4; Tab. 3), indicating that bacteria, whilst present, were generally not major sources of fatty acids in the particulate samples analysed.

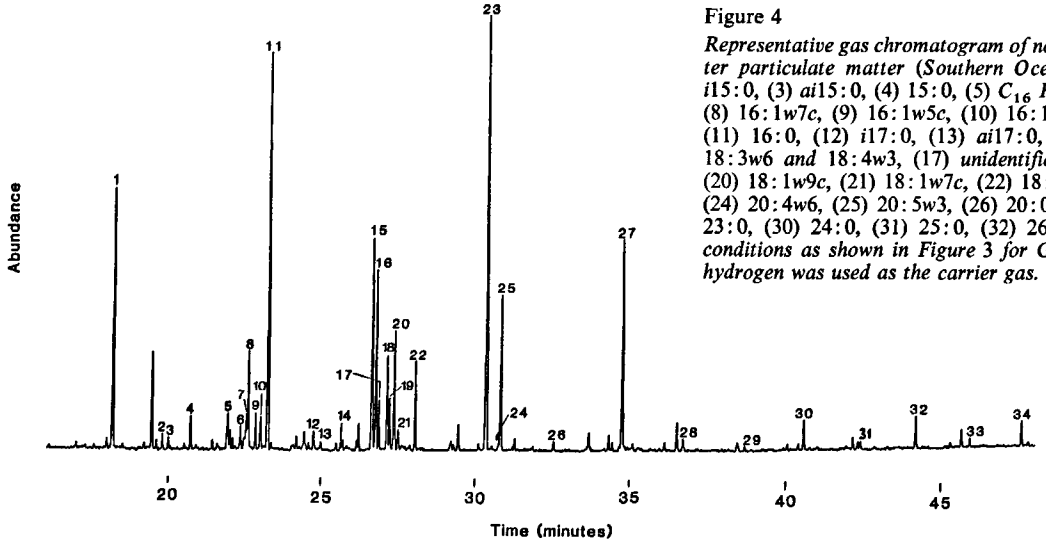

Figure 4

Representative gas chromatogram of non-adducted fatty acids in seawater particulate matter (Southern Ocean; SO1 50 m). (1) 14:0, (2) *i*15:0, (3) *ai*15:0, (4) 15:0, (5) C_{16} *PUFA*, (6) *i*16:0, (7) 16:1*w*10*c*, (8) 16:1*w*7*c*, (9) 16:1*w*5*c*, (10) 16:1*w*13*t and coeluting component*, (11) 16:0, (12) *i*17:0, (13) *ai*17:0, (14) 17:0, (15) 18:5*w*3, (16) 18:3*w*6 *and* 18:4*w*3, (17) *unidentified*, (18) 18:2*w*6, (19) 18:3*w*3, (20) 18:1*w*9*c*, (21) 18:1*w*7*c*, (22) 18:0, (23) 19:0 *internal standard*, (24) 20:4*w*6, (25) 20:5*w*3, (26) 20:0, (27) 22:6*w*3, (28) 22:0, (29) 23:0, (30) 24:0, (31) 25:0, (32) 26:0, (33) 27:0, (34) 28:0. *GC conditions as shown in Figure 3 for GC-MS, with the exception that hydrogen was used as the carrier gas.*

Table 3
Fatty acid composition of selected marine particulate matter samples.

Fatty acid	Percentage composition of total fatty acids — Sample Location [a]						
	WEPOC	EAC	TASMAN	SO1 50 m	SO1 250 m	SO2 50 m	SO3 50 m
14 : 0	14.9	5.2	10.2	15.9	9.5	12	13
br 15 : 0	0.8	0.4	1.6	2.1	2.5	1	1.5
15 : 0	2.9	3.4	3.2	1.1	2.8	0.7	1.6
C_{16}PUFA	0.4	0.3	0.5	1.2	0.4	2.4	1.9
i 16 : 0	–	–	–	0.1	1.3	0.6	0.7
16 : 1ω10c	5.1	5.8	6.6	TR	4.9	0.6	1.8
16 : 1ω7c	4.1	4.2	8.8	11	2.4	9.7	5.1
16 : 1ω5c	–	–	–	0.8	TR	0.8	1.1
16 : 0	29.4	29.4	25.6	22	29	12.6	22.1
br 17 : 0	0.3	0.6	0.6	0.5	2.1	0.4	0.9
17 : 0	0.8	1.4	0.9	0.7	1.2	0.2	0.9
18 : 5ω3	–	–	–	6.5	0.3	18.2	6.6
18 : 4ω3+18 : 3ω6	1.8	–	0.8	3.6	0.1	5.3	5.5
18 : 2ω6	2.1	4.5	5.5	2.5	3.5	1.9	3.7
18 : 3ω3	1	0.4	0.6	1.7	0.7	1.7	1.7
18 : 1ω9c	12.4	23	13.4	5	15.8	3.4	5.7
18 : 1ω7c	1.1	1.9	3.4	3.4	2.9	1.5	1.4
18 : 0	11.3	10.9	4.2	4.2	11	1.6	4.9
20 : 5ω3	0.6	0.4	2.6	4.1	0.5	6.8	5.1
20 : 0	1.1	0.5	0.3	TR	0.6	0.3	0.5
22 : 6ω3	1.7	0.8	4.7	9.2	1.2	13.3	7.1
C_{22}-C_{26}	4	1.6	2.2	0.1	1.8	0.5	2.4
Other	3.9	5.3	4.3	3.6	1.6	3.1	4.2

[a] For sample location and lipid class details, *see* Tables 1 and 4 and Figure 1.

Table 4
Selected lipid compositional data (μg/l) for marine particulate matter samples.

Parameter (μg/l)	Sample location [a]													
				SB			SO1 (m)			SO2 (m)			SO3 (m)	
	WEPOC	EAC	TASMAN	(i)	(ii)	(iii)	10	50	250	10	50	250	10	50
Chlorophyll *a*	0.34	0.17	0.40	0.58	0.72	1.45	–	–	–	–	–	–	–	–
Fatty acid abundance [b]	18.8	0.49	0.89	0.49	2.3	3.2	2.4	2.5	0.27	0.89	1.05	0.08	0.36	0.50
16 : 0	5.5	0.29	0.49	0.19	0.73	0.91	0.39	0.54	0.08	0.11	0.13	0.023	0.12	0.11
16 : 1ω10c	0.89	0.06	0.13	0.28	0.11	0.11	0.001	–	0.013	0.008	0.006	0.003	<0.001	0.009
16 : 1ω7c	0.75	0.04	0.17	0.26	0.21	0.34	0.27	0.27	0.007	0.05	0.10	0.002	0.002	0.026
16 : 1ω10c/16 : 1ω7c [c]	1.2	1.4	0.75	1.1	0.5	0.3	0.004	–	2.0	0.16	0.06	1.6	–	0.36

[a] For sample location details, *see* Table 1 and Figure 1.
[b] Data for WEPOC and SB samples are for total fatty acids. The SO fatty acid data was obtained for fatty acids derived from the acetone and methanol containing fractions of the total lipid extract. Polar lipids typically accounted for 50-90% of the total extractable lipids in the SO samples (TLC-FID unpublished data). EAC and TASMAN data was obtained for triacylglycerol fractions (10-20%) of total lipid.
[c] Ratio for *Penaeus esculentus* cuticle and muscle neutral lipid = 1.1 and 0.54 respectively, ratio for Storm Bay mixed zooplankton sample = 0.05.

Identification of 16:1*w*10c in seawater particles

Several 16:1 isomers were present in all samples, with two closely eluting peaks observed in many of the samples. The commonly identified 16:1*w*7c isomer was generally the principal C_{16} monounsaturated fatty acid. The second major isomer eluted just before 16:1*w*7c on the high-resolution 50 m nonpolar and polar columns used in this study. In several samples this fatty acid was more abundant than 16:1*w*7c (Tab. 4), and in others, the amounts were similar.

To determine double bond position and geometry, the DMDS derivatization method, which has been previously used in taxonomic, microbial ecology and insect lipid studies, was applied to the monounsaturated fatty acids isolated from oceanic particulate matter samples. A representative series of reconstructed ion chromatograms showing *w*-fragment ions is illustrated in Figure 3. The fragmentation pathway for the formation of *w* and Δ-fragments ions is indicated in Figure 2.

Up to 20 to 30 monounsaturated fatty acids in the C_{14} to C_{20} range were found in particulate matter samples, as well as several longer chain homologues (Tab. 2). The unusual 16:1*w*10c isomer was the other major C_{16} monounsaturated fatty acid detected in seawater particles. Prior to the first submission of our paper there were no other reports of 16:1*w*10c in seawater, but recently Sicre *et al.* (1988) have also noted the presence of 16:1*w*10 in marine particles. Based on the similar statistical behaviour of 16:1*w*10 with other bacterial fatty acids, they proposed a microbial origin of this compound. The authors did not unfortunately specify double bond geometry for 16:1*w*10 or other

monounsaturated fatty acids detected. The 16:1*w*10c isomer has been reported in a cultured marine bacterium, and as a minor component in the phospholipid fraction isolated from a deep-sea sediment (Baird and White, 1985; Gillan *et al.*, 1981). Scribe *et al.* (1988), in a very recent report documenting the use of the DMDS procedure applied to the total fatty acids isolated from a recent marine sediment, presented mass fragmentograms and chromatograms that documented the presence of 16:1*w*10c. Unfortunately no quantitative data or discussion of this observation was presented. Boon *et al.* (1978), in a detailed study of Walvis Bay diatomaceous ooze, reported the presence of 16:1*w*10c and related C_{14} and C_{15} monounsaturated fatty acids in sediment core samples.

Previous studies by Ackman and coworkers during the early 1970's (Ackman *et al.*, 1971; 1972; 1973; Hooper *et al.*, 1973; Pascal and Ackman, 1975) and others (Pearce and Stillway, 1976) reported 16:1*w*10t in jellyfish, the Atlantic leatherback turtle, spadefish, sperm whale oil, a gorgonian and sunfish. Filter-feeding jellyfish and similar organisms may have been the source of 16:1*w*10t for animals higher up the food chain. The occurrence of both the *cis* and *trans* isomers of 16:1*w*10 in the marine environment indicates that care needs to be taken with the identification of these components, and that it is important to confirm both double bond position and geometry.

Assignment of the *trans* geometry in the previous studies of marine organisms was largely based upon separation of the *cis* and *trans* monounsaturated fatty acids by thin-layer chromatography on plates impregnated with silver nitrate. Based on the earlier work of Ackman and coworkers which showed that 16:1*w*10t was distributed in a wide range of marine animals, it was expected that the 16:1*w*10 isomer detected in our study would show *trans* geometry. This was not the case, as shown by several lines of evidence : 1) Derivatives formed from the original *trans* and *cis* monounsaturated fatty acids were chromatographically separated (*trans* derivative elutes after *cis* derivative), as were adducts derived from the different positional isomers (Fig. 3). The retention time observed for the major 16:1*w*10 adduct in seawater particles was consistent with the original monounsaturated fatty acid having *cis* geometry; 2) Argentation TLC performed using conditions which separate *cis* and *trans* monounsaturates did not separate the 16:1*w*10c isomer present in seawater and a mixed zooplankton sample (Tab. 4) from the authentic *cis* component 16:1*w*7c; 3) Coinjection experiments showed that 16:1*w*10c from seawater particles was chromatographically resolved from 16:1*w*10t present as the major 16:1*w*10 isomer in a turtle oil sample (sample kindly provided by Prof. R. G. Ackman). The 16:1*w*10t isomer, in contrast to the *cis* isomer, elutes after 16:1*w*7c on the non polar column and between 16:1*w*10c and 16:1*w*7c on the polar carbowax column; 4) GC-FT/IR analysis of the two C_{16} isomers (*w*7c, *w*10c) did not show any appreciable absorbance at 966 cm^{-1}, which is characteristic of *trans* double bonds.

Distribution of 16:1*w*10c in seawater

16:1*w*10c was found in samples from a range of oceanic regions; it is not therefore, confined to specific areas. The general occurrence of this fatty acid is further supported if it is assumed that the 16:1*w*10 identified by Sicre *et al.* (1988) is also the *cis* isomer. Differences in the absolute and relative abundance of this monounsatured fatty acid between and within water masses were apparent (Tab. 3 and 4), suggesting that this fatty acid may be used as a specific marker for measuring differences between water bodies as reflected in the proportion of organisms containing 16:1*w*10c (see below). The WEPOCS, Storm Bay (January 1986), EAC, Tasman Sea and Southern Ocean (250 m) samples all contained 16:1*w*10c at similar concentrations to 16:1*w*7c. In contrast, samples collected at 10 and 50 m in the Southern Ocean (polar lipid data) and other samples from Storm Bay (December 1985, September 1986) contained significantly less 16:1*w*10c than 16:1*w*7c (ratio 16:1*w*10c/16:1*w*7c=0.5 or less). Samples from the surface waters (10 and 50 m) of the Southern Ocean contained the lowest relative proportions of 16:1*w*10c. Microscopy and the observed PUFA profiles showed that the prymnesiophyte flagellate *Phaeocystis pouchetti* was the dominant alga in these samples. This alga is not a likely source of 16:1*w*10c, as it was not noted in detailed fatty acid profiles of *Phaeocystis* isolated from North Sea (Sargent *et al.*, 1985) and Australian (unpublished data) waters.

The two 250 m samples collected from the Southern Ocean both contained high proportions of 16:1*w*10c relative to 16:1*w*7c. Analysis of the acetone-mobile polar lipids demonstrated that this lipid class contained higher portions of 16:1*w*10c relative to 16:1*w*7c than the methanol (phospholipid containing) fraction (unpublished data). Samples from the EAC also generally contained higher portions of 16:1*w*10c relative to 16:1*w*7c than did particles from the Tasman Sea.

The general occurrence of 16:1*w*10c as a major C_{16} monounsaturated fatty acid in a range of seawater samples suggests that the presence of this component in seawater particles has been previously overlooked. In many previous studies of fatty acids in seawater, either the double bond positions have not been indicated, or in a few cases only the major monounsaturated fatty acids have been identified (*e. g.* Ehrhardt *et al.*, 1980; Larsson *et al.*, 1974; Saliot *et al.*, 1982; Schultz and Quinn, 1972; 1977; Tanoue and Handa, 1980; Wakeham *et al.*, 1984; Wakeham, 1985). The use of lower resolution columns or lack of appropriate derivatisation procedures may have prevented those workers from assigning double bond configuration for many or all of the monounsaturated fatty acids. In other studies, workers have simply not attempted to assign double bond position and/or geometry.

Origins of 16:1*w*10c in seawater particulate matter

Marine biological samples were analysed for information on the possible origins of 16:1*w*10c in seawater.

Ten species of axenic unicellular algae from the classes Chlorophyceae, Prasinophyceae, Bacillariophyceae, Prymnesiophyceae and Cryptophyceae were analysed using the DMDS procedure, for their fatty acid content (Volkman *et al.*, 1989). The novel 16:1*w*10c was found in only two species: *Nannochloris atomus* (Chlorophyceae, 1.2% of total fatty acids) and *Chroomonas salina* (Cryptophyceae, trace constituent). Cyanobacteria were an important constituent of the phytoplankton in several WEPOCS samples (Everitt *et al.*, in press), but the monounsaturated fatty acids of members of this algal class have not as yet been studied using the DMDS or similar procedures.

A number of fatty acid samples derived from marine animals were also examined. The monounsaturated fatty acids derived from the cuticle and muscle lipids of the brown tiger prawn *Penaeus esculentus* and a mixed zooplankton sample (predominantly *Calanus australis* and *Nyctophanes australis*) all contained 16:1*w*10c. Larvae of the albacore tuna *Thunnus alalunga* and material from the prawn's digestive gland contained 16:1*w*10t at approximately one third the abundance of 16:1*w*7c; 16:1*w*10c was either not detected or was present as a very minor component. The *trans* monounsaturated fatty acid 16:1*w*10t eluted just after 16:1*w*7c on the non polar capillary column used in this study, and was also identified using SIM analysis of the DMDS adducts. The prawn cuticle contained 16:1*w*10c at similar relative levels to 16:1*w*7c (Tab. 4), whilst in the zooplankton sample and the prawn muscle the novel monounsaturated fatty acid was present at considerably lower abundance than 16:1*w*7c.

The fatty acid 16:1*w*10c has been reported in bacteria, whilst 16:1*w*10t has been found in a variety of marine organisms (Ackman *et al.*, 1971; 1972). Gillan *et al.*, (1981) reported 16:1*w*10c as a major monounsaturated fatty acid (>10%) of the fatty acids in at least one isolate) in a survey of the fatty acids derived from eight marine bacteria. The same authors also noted 16:1*w*10t as a minor component in several cultures. Boon *et al.* (1978) detected 16:1*w*10c in sediment cores from Walvis Bay diatomaceous ooze; however, no quantitative data were reported. As Δ6 desaturases are known to occur in plants (Stearns, 1970), they speculated that such desaturases are present in algae and that algae were the source of these compounds in the sediments studied. The Δ6 desaturases also exists widely in marine animals (*e. g.* 18:2*w*6→18:3*w*6 and 18:3*w*3→18:4*w*3; Bell *et al.*, 1986), confirming that marine animals, as noted above, may have been a source of these compounds.

From the limited data available, it appears that both bacteria and crustaceans may be sources of 16:1*w*10c in marine particles. The low abundance of fatty acids commonly used as bacterial markers (*e.g.* i15:0, ai15:0, i17:0, ai17:0, cy17:0, cy19:0; Perry *et al.*, 1979; White, 1983) in the sea water samples analysed in this study (Tab. 3) indicates that bacteria are generally minor components of the total biomass. Similarly, the detection in several samples (Tab. 3) of 16:1*w*10c in the triacylglycerols and acetone fraction, containing glycolipid, which are generally not abundant in bacteria, suggests that sources other than bacteria must be considered. It is therefore possible that marine invertebrates, or detritus derived from these organisms (including faecal material), may be in some cases the major source of 16:1*w*10c in seawater particles. Differences between water masses in the abundance of 16:1*w*10c will therefore reflect differences in the proportions of these organisms.

Other monounsaturated fatty acids

In addition to 16:1*w*10c, other Δ6 monounsaturated fatty acids were also detected in several of the seawater particulate samples, as evidenced by reconstructed ion chromatograms for m/z 175 (Δ6 DMDS ion fragment; Fig. 5, Tab. 2). Up to eight Δ6 monounsaturated fatty acids were identified in the particulate matter samples: 14:1*w*8c, br15:1, 15:1*w*9c, br16:1, 16:1*w*10c and 16:1*w*10t (the last present in several samples as a trace component), br17:1 (2 isomers) and 17:1*w*11c. It seems reasonable to assume that a common source exists for these novel fatty acids. Apart from the previously reported presence of 16:1*w*10t and 2 analogous branched chain C_{17} isomers in various marine animals (Ackman *et al.*, 1972; Hooper *et al.*, 1973), no data are available on the biological occurrence and distribution of the range of Δ6 monounsaturated fatty acids detected in this study.

Long-chain C_{22} to C_{28} monounsatured fatty acids were also detected in several particulate matter samples and in the lipids of *Penaeus esculentus* and the mixed zooplankton sample. It has been reported that the DMDS adducts of monounsaturated fatty acids containing more than 20 carbon atoms could not be detected by GC-MS analysis (Scribe *et al.*, 1988). Our own data (Nichols *et al.*, 1986*a*, *b*; and the present study) indicate, however, that adducts formed from longer-chain (>C_{20}, $C_{22}-C_{28}$) monounsaturated fatty acids can be analysed using GC-MS. It seems highly likely, with the recent advent of high-temperature aluminium-clad columns, that the carbon-chain range that can be analysed using the DMDS procedure could be extended beyond C_{28}. The DMDS procedure confirmed that the *w*9 and *w*7 isomers were the two major isomers detected for all chain lengths (Fig. 6). These components are presumably formed by chain-elongation of shorter-chain 16:1*w*7 and 18:1*w*9 homologues. Long-chain monounsaturated fatty acids were also present in one Storm Bay particulate matter sample, and

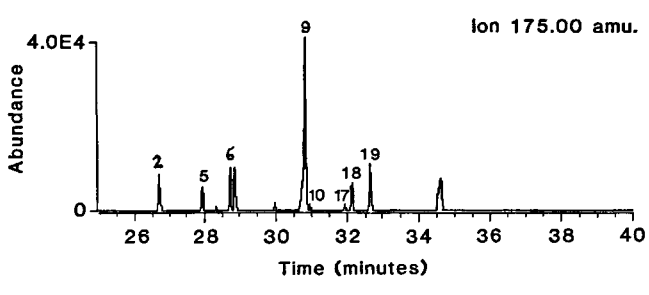

Figure 5

Partial reconstructed ion chromatogram of m/z 175 ion (Δ6 fragmentation) obtained for Storm Bay particulate matter. Peak numbers refer to Table 2. GC-MS conditions as for Figure 3.

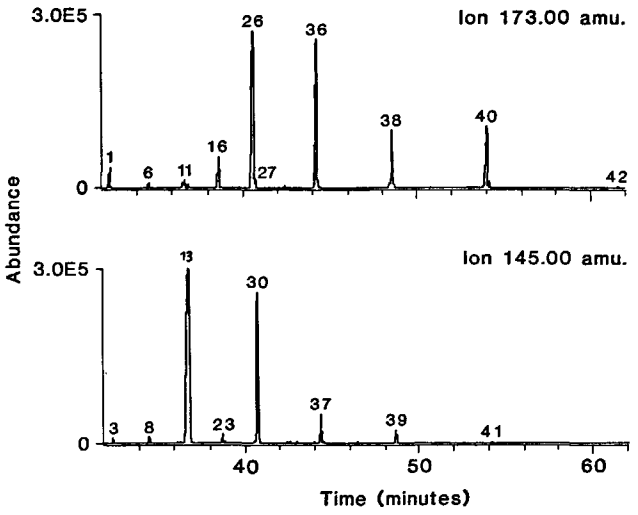

Figure 6
Partial reconstructed ion chromatogram of m/z 173 and 145 ions (w9 and w7 respectively) obtained for mixed zooplankton sample collected in Storm Bay. Peak numbers refer to Table 2. GC-MS conditions as for Figure 3.

these data are consistent with an invertebrate contribution to the particulate matter. Long-chain saturated fatty acids were also detected in many of the oceanic particulate samples. n-C_{24} and n-C_{26} fatty acids have been previously used as terrigenous markers (Brooks *et al.*, 1976; Cranwell, 1974; Shaw and Johns, 1986). More recent reports have documented the presence of these compounds in marine algae (Nichols *et al.*, 1986*b*; Volkman *et al.*, 1989), and an algal source is more likely for the samples analysed in this study. These data support the view that care should therefore be taken with the use of these components as terrigenous markers. Additional information should be sought before it is assumed that only terrestrial sources are involved.

The analysis of membrane-derived fatty acids not only provides insight into microbial community structure or possible carbon sources but also provides information on nutritional status. *Trans* monounsaturated fatty acids accumulated in the bacteria *Vibrio cholera*, *Pseudomonas atlantica* and other organisms when starved (Guckert *et al.*, 1986; Nichols *et al.*, 1986*a*; White *et al.*, pers. comm.). For the common bacterial monounsaturated fatty acids, 16:1*w*7 and 18:1*w*7, *trans/cis* ratios of less than 0.1 have been observed in most bacterial cultures and sediments. During starvation the ratio increases dramatically to values greater than 1. It was proposed that this ratio might be useful in determining the nutritional status of bacteria and other cells in natural aquatic environments and that these organisms may represent a direct source of *trans* monounsaturated fatty acids into marine and estuarine sediments (Guckert *et al.*, 1986).

Trans monounsaturated fatty acids were detected as minor components in all the particulate matter samples (Fig. 3 and 5), and low *trans/cis* ratios were generally observed for 16:1*w*7 and 18:1*w*7. Phytoplankton and marine animals are not known to biosynthesize 16:1*w*7t and 18:1*w*7t, and the low ratios typically observed for these compounds can be attributed to these organisms, rather than bacteria, being the major contributors to the fatty acids in these samples. High *trans/cis* ratios were observed for several of the minor monounsaturated fatty acids that are likely to be of bacterial origin (*e.g.* 18:1*w*8, Fig. 3). There is increasing evidence that bacteria in the open ocean are in a state of near-starvation (*e.g.* Joint and Morris, 1982). Our data suggest that the potential exists to utilize detailed analysis of component monounsaturated fatty acids to provide further information on the trophic status of bacteria in oceanic studies. If this potential is to be fully exploited, then further effort will need to be given to sampling the particulate matter pool; bacteria should be isolated from other planktonic species to obtain an unambiguous signal. Current sampling techniques commonly used in oceanographic studies do not meet this requirement

Small quantities of *trans*-monounsaturated fatty acids have been reported in many marine sediments (Van Vleet and Quinn, 1976; Volkman and Johns, 1977; Boon *et al.*, 1978). From the data presented in this paper and other studies (unpublished), it is apparent that particles from the water column are not major sources of these acids in sediments. High abundances noted in particular sediments (*e.g.* Boon *et al.*, 1978) most probably arise from preferential degradation of *cis*-unsaturated fatty acids and direct inputs from sedimentary bacteria.

CONCLUSION

A wide range of monounsaturated fatty acids have been identified in oceanic particulate matter samples. In particular, the novel fatty acid 16:1*w*10c, not previously characterized in marine particulate matter, was detected in particulate matter collected from 6 distinct water masses. If other sources of 16:1*w*10c are discovered when more marine organisms are analysed, the potential of 16:1*w*10c as a biological marker could be more fully evaluated. Similarly, analysis of individual lipid classes (*e.g.*, wax esters, triacylglycerols, free fatty acids, glycolipids and phospholipids) in a wide range of particulate samples and marine organisms from all trophic levels will provide further information on the origin of this and other unusual fatty acids.

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