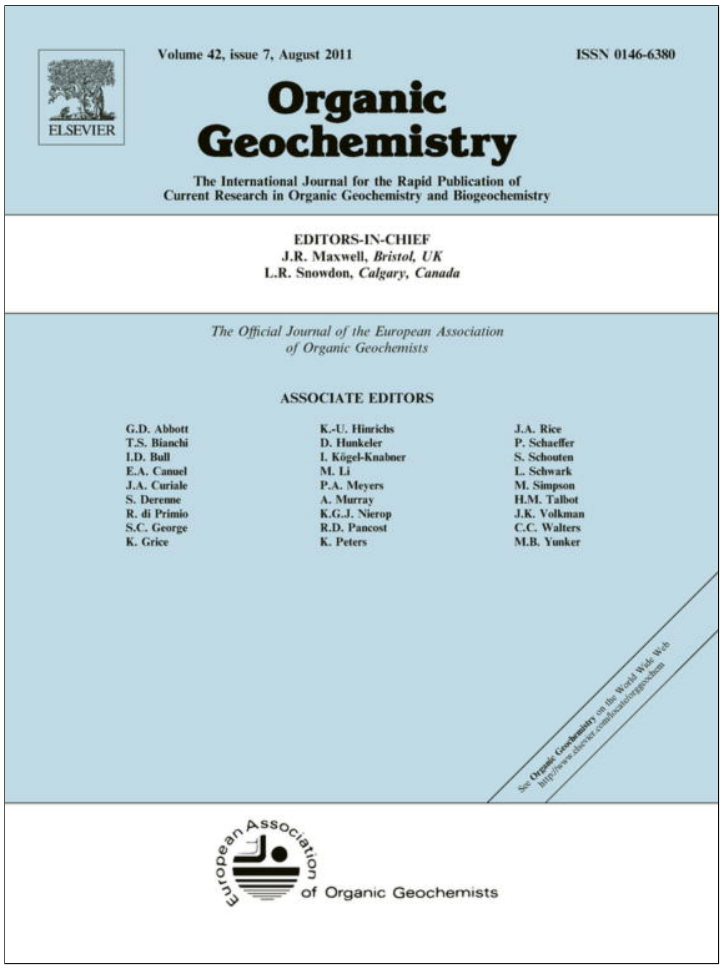

Organic Geochemistry 42 (2011) 823–837

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Co-occurrence of long chain diols, keto-ols, hydroxy acids and keto acids in recent sediments of Lake El Junco, Galápagos Islands

Zhaohui Zhang [a,b,*], Pierre Metzger [c], Julian P. Sachs [b,1]

[a] State Key Laboratory For Mineral Deposits Research (Nanjing University), School of Earth Sciences and Engineering, Nanjing University, 22 Hankou Road, Nanjing 210093, China
[b] Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA
[c] Université Pierre et Marie Curie, BioEMco, CNRS UMR 7618, 4 Place Jussieu, Paris F-75252, France

ARTICLE INFO

Article history:
Received 28 November 2010
Received in revised form 5 April 2011
Accepted 19 April 2011
Available online 5 May 2011

ABSTRACT

Lipid biomarkers in lacustrine sediments provide valuable information about lake history and environmental change. Here we report the occurrence of a unique set of lipid biomarkers from a freshwater crater lake, El Junco, in the Galápagos. In addition to previously reported alkadienes, botryococcenes and lycopadienes indicative of *Botryococcus braunii* A, B and L races, we find highly branched C_{25} isoprenoids (HBIs) from diatoms, monomethyl alkanes likely from insect epicuticular waxes, long chain alkenols, diols and a triol, keto-ols, hydroxy acids and keto acids. Saturated and mono-unsaturated long chain diols from C_{30}–C_{36} had terminal hydroxyl groups and hydroxyl groups between the ω16 and ω20 positions. Vicinal diols with hydroxyl groups at ω9 and ω10 were likely from the floating fern *Azolla*. C_{30}–C_{36} keto-ols, mid-chain hydroxy and keto acids had mid-chain functional groups at similar positions to the diols, suggesting common origins. The predominance of ω20-hydroxy acids and diols, together with 20,21-dihydroxy-nonacosanoic acid is indicative of an *Azolla* source, while ω16 and ω18 hydroxy acids and diols imply a microalgal source.

1. Introduction

Lacustrine sediments provide excellent archives of historical environmental variations due to high sedimentation rates and quick responses to environmental changes. Biomarkers preserved in lake sediments play a unique role in deciphering the water column in which microorganisms live and the watershed environment (e.g. Cranwell et al., 1987; Volkman et al., 1998). A wealth of knowledge on the lipids produced by microalgae, bacteria, zooplankton and other organisms has been accumulated over the last 40 years. Less is known about biomarkers in lacustrine ecosystems than marine environments, limiting both our ability to understand the relationships between different classes of biomarkers and means to apply them in paleoenvironmental reconstructions.

Located at 760 m above sea level in the highlands of San Cristobal, the easternmost island in the Galápagos archipelago, Laguna El Junco is the only permanent freshwater lake in the Galápagos. Far from any continents and without any river influence, this closed basin crater lake provides a unique setting for evaluating biomarker distributions and their responses to environmental variations.

The sediment of El Junco is characterized by a rich variety of biomarkers which can be traced to different origins. In a previous work (Zhang et al., 2007) we reported biomarker evidence for the co-occurrence of three different races of *Botryococcus braunii* (A, B & L) in the water column and organic matter from sediments of El Junco Lake. We also showed for the first time the existence of C_{40} isoprenoids (lycopadienes and lycopatrienes) in suspended particles and sediments. Biomarker indicators of the three races were: (*E*)- and (*Z*)- C_{25}–C_{31} *n*-alkadienes and a C_{29} triene for race A, a series of $C_{34}H_{58}$ botryococcenes for race B, and a $C_{40}H_{78}$ hydrocarbon, *trans*,*trans*-lycopadiene accompanied by minor amounts of isomers, for race L. Concentrations of botryococcene and lycopadiene varied over four orders of magnitude in a 75 cm long surface core and such down-core variations in these lipids likely reflects changes in lake hydrology, alternately favoring the bloom or diminishment of *B. braunii* race B.

In this paper we further explore the biomarkers present in El Junco sediments, speculate on their origin and evaluate the implications for ecological and environmental reconstructions at this site. The lipid classes we identified include hydrocarbons (e.g. monomethyl alkanes), highly branched isoprenoids (HBIs), *n*-alkyl esters, *n*-alkenols, *n*-alkyl diols and keto-ols, hydroxy acids and keto acids. They can variously be attributed to microalgae, bacteria, ferns and insects.

* Corresponding author. Present address: Department of Earth Sciences, Nanjing University, 22 Hankou Road, Nanjing 210093, China. Tel.: +86 25 8359 2024; fax: +86 25 8368 6016.
E-mail address: zhaohui@nju.edu.cn (Z. Zhang).
[1] Present address: University of Washington, School of Oceanography, Box 355351, Seattle, WA 98195, USA.

0146-6380/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.orggeochem.2011.04.012

2. Methods

2.1. Site and samples

Located about 1000 km west of Ecuador and on the equator, the Galápagos Islands have a semi-arid to arid climate. The only permanent freshwater lake, Laguna El Junco (0.30°S, 91.00°W) is located at 760 m above sea level in the highlands of the easternmost island in the Galápagos archipelago. The lake itself is contained within the summit crater of an extinct volcano and is about 300 m wide. Apart from an overflow (3 m deep and 2 m wide) and possible seepage through the rim, the lake basin is closed. The water depth varies with precipitation, being at its deepest in the rainy season (October to May). It was 6 m deep when we sampled the lake in September 2004, and it was well mixed as indicated by temperature and oxygen profiles.

Samples from four different cores were studied. Two parallel surface cores (75 cm long) EJ7-MW1 and EJ5-MW2 were sampled at 3 cm and 10 cm intervals, respectively. In addition, a 3 m long Nesje core (EJ-N) was collected by a group of scientists from the University of Arizona. One sample from a fourth core, EJ-3 200–201 cm, was also analyzed for this study. The sample names were presented as core name and composite depth below the surface, for example, EJ-N 344–345 cm indicates the sample was from EJ-N core with the composite depth of 344–345 cm. The 75 cm long surface core covers approximately the last 500 years and the bottom of the EJ-N core has composite depth of 372 cm and represents about 9100 yr.

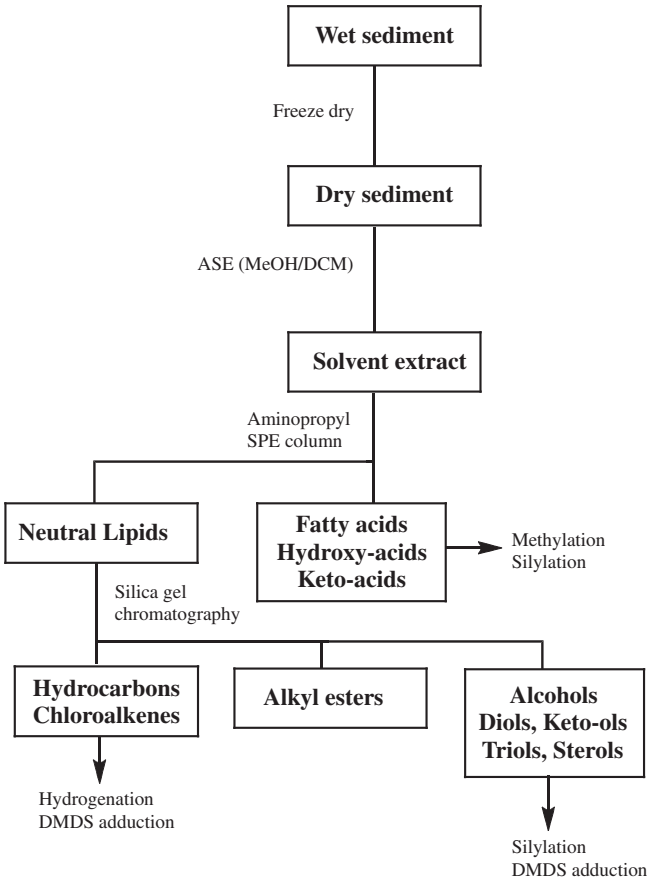

Fig. 1. Scheme for extracting and analyzing lipid biomarkers in El Junco sediments.

2.2. Lipid extraction, separation and identification

The method of lipid extraction was presented in Zhang and Sachs (2007) and Zhang et al. (2007). The separation and analysis scheme is shown in Fig. 1. Briefly, sediment samples were freeze dried and internal standards were added prior to extraction. Extractions were performed on a Dionex ASE-200 pressurized fluid extractor with dichloromethane (DCM) and methanol (MeOH) (9:1) at 10,342 kPa (1500 psi) and 150 °C. Neutral lipids were separated from fatty acids on an aminopropyl cartridge-style SPE column (Burdick & Jackson, size 500 mg/4 ml) by eluting with DCM:isopropyl alcohol (3:1), followed by 4% acetic acid in diethyl ether to recover fatty acids (F5) (Zhang and Sachs, 2007). Neutral lipids were fractionated by column chromatography with 5% water-deactivated silica gel. Hydrocarbons were eluted with hexane (F1), wax esters with 10% ethyl acetate (EtOAc) in hexane (F2), sterols/alcohols with DCM and/or DCM:MeOH (9:1) (F3), MeOH (F4).

All fractions were analyzed using an Agilent 5890 gas chromatograph coupled to an Agilent 5989 mass spectrometer (GC–MS). The GC was equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm) coated with polydimethyl-siloxane (film thickness 0.25 μm). The oven temperature began at 100 °C and was increased to 300 °C at 4 °C/min. The ion source temperature in the mass spectrometer was 220 °C and the ionization energy was 70 eV.

2.3. Separation and identification of alkyl esters

Aliquots of F2 fractions isolated from EJ5-MW2 0–10, 10–20 and 40–50 cm intervals (composite depths 0–10, 10–20 and 40–50 cm, respectively) were purified by silica gel TLC using heptane:diethyl ether (91:9 v:v) as eluent. The bands were visualized under UV light and those exhibiting R_f values between 0.65 and 0.80 were scraped off and extracted with diethyl ether. The extracts were concentrated and the recovered oils were analyzed by GC–MS. Saponification of the oils was performed in 1 N KOH/MeOH for 1 h under reflux. Thereafter the reaction mixture was diluted with water, acidified with 1 N aqueous HCl and extracted with diethyl ether. The concentrated extracts were refluxed for 1 h in MeOH/HCl generated by the addition of a few drops of acetyl chloride in MeOH, in order to esterify the acids released by saponification. The reaction mixture was then dried under reduced pressure, followed by the addition of MeOH and evaporation. The procedure was repeated three times to eliminate HCl traces. Finally, aliquots of the recovered mixtures containing fatty acid methyl esters and fatty alcohols were treated with *N*,*N*-bis trimethylsilyltrifluoroacetamide (BSTFA):pyridine (1:1), 200 μl at 60 °C for 15 min and then directly analyzed by GC–MS.

Standards of stearyl *iso*-palmitate, stearyl palmitate and palmityl stearate were prepared by reaction of the relevant alcohols and acids in the presence of *N*,*N*'-dicyclohexylcarbodiimide and 4-dimethylaminopyridine, according to the procedure of Neises and Steglich (1978).

2.4. Dimethyldisulfide derivatization of unsaturated compounds

Dimethyldisulfide (DMDS; 200 μl) and 20 μl of a solution of iodine (60 mg) in 1 ml diethyl ether were added to a 20 μl solution of heptane containing ~1 mg of lipids. After 48 h at 50 °C, the reaction was quenched by the addition of 200 μl aqueous sodium thiosulfate solution (10% w/v). The heptane solution was decanted and the aqueous phase was re-extracted twice with 200 μl heptane. The combined extracts were evaporated under a stream of nitrogen and analyzed by GC–MS.

2.5. Hydrogenation

Hydrogenation of an aliquot of the hydrocarbon fraction isolated from the EJ-N 344–345 cm sediment interval was performed in heptane in the presence of a rhodium catalyst (Rh/C, 5%) at 70 °C and 20 atm pressure for 1 h. The reaction mixture was then centrifuged; the supernatant was concentrated under vacuum and analyzed by GC–MS.

3. Results and discussions

3.1. Hydrocarbons

In addition to lycopadienes and lycopatrienes unique to *B. braunii* race L, botryococcenes characteristic of *B. braunii* race B, and C_{25}–C_{31} *n*-alkadienes and trienes indicative of race A (Zhang et al., 2007), a suite of hydrocarbons were present in the El Junco sediment, including *n*-alkanes, *n*-alkenes, phytadienes and their derived thiophenes, highly branched isoprenoids (HBIs), hopanoids, monomethyl alkanes and fernenes (Fig. 2).

3.1.1. Alkanes and alkenes

In the surface sediment, the *n*-alkane profile had a unimodal distribution from C_{17}–C_{35}, with a strong odd/even carbon number predominance and C_{max} at C_{25} (Fig. 2a). In some deeper intervals of the Nesje core (EJ-N 289–290, 294–295, 306–307, 344–345 and 351–352 cm) both C_{19}-C_{35} *n*-alkanes and *n*-alkenes exhibit an odd/even carbon number predominance. The 289–290 cm interval is characterized by the dominance of C_{25} and C_{27} *n*-alkenes (Fig. 3a), distinguishing it from the other intervals (e.g. Fig. 3b). The predominance of alkenes over alkanes was previously noticed in some upper sediment intervals from El Junco Lake (Zhang et al., 2007) as well as in sediments from Lake Titicaca (Theissen et al., 2005). In the latter case *B. braunii* race A was proposed as the potential source of $C_{25:1}$ and $C_{27:1}$. In El Junco, $C_{25:1}$ and $C_{27:1}$ predominated in the surface sediments but the characteristic hydrocarbons produced by *B. braunii* race A in the same interval were the $C_{29:2}$ and $C_{31:2}$ dienes (Zhang et al., 2007). Therefore the high $C_{25:1}/C_{25:0}$ and $C_{27:1}/C_{27:0}$ ratios result from the contribution of other algae (Zhang et al., 2004, 2007).

3.1.2. Monomethyl alkanes

Monomethyl alkanes consisting of C_{22} (11-Me–C_{21}), C_{23} (11-Me–C_{22}), C_{24} (11-Me–C_{23}) and C_{26} (11-Me–C_{25} and 13-Me–C_{25}) (Fig. 2b–d) are present in low concentrations in sediments from 0–50 cm in depth. The mid-chain position of branching markedly differs from that observed in monomethyl alkanes present in torbanite extracts (Audino et al., 2001), precluding *B. braunii* as the source. Moreover, their chain length is inconsistent with a cyanobacterial origin (Han and Calvin, 1970; Shiea et al., 1990 and references cited therein). The most likely source for the El Junco methyl alkanes is the epicuticular waxes of insects (Nelson and Blomquist, 1995 and references cited therein; Saïd et al., 2005), corroborated by the observation of large insect population observed on and around the lake during the field trip. The monomethyl alkanes from El Junco sediments are characterized by branching points at odd numbered carbon atoms and aliphatic chains containing predominantly odd numbers of carbon atoms (C_{21}–C_{25}) (Fig. 2b), which is in good agreement with methyl alkanes (C_{15}–C_{55}) from some aquatic beetles isolated from a freshwater pond in Ontario (Alarie et al., 1998). Contributions of insect lipids to sediments, while unusual, have been reported in some Holocene cyanobacterial mats that contained C_{24}–C_{45} methyl alkanes (Kenig et al., 1995) and sediments from a freshwater lake in Tanzania that contained C_{37}–C_{43} *n*-alkenes (de Mesmay et al., 2007).

3.1.3. Highly branched isoprenoids

Minor amounts of two $C_{25:1}$ HBIs (Fig. 2b) characteristic of diatoms were also present. Their structures were determined by GC–MS after catalytic reduction and formation of DMDS adducts and by comparison with published mass spectra. They appear to be two diastereoisomers of 2,10,14-trimethyl-6-methylene-7-(3′-methylpentane)pentadecane (Dunlop and Jefferies, 1985), the isomerism being very likely related to the existence of two configurations at C-7. Commonly found in marine environments (Rowland and Robson, 1990), HBIs have also been identified in some lacustrine sediments (de Mesmay et al., 2007; Balascio et al., 2011). $C_{25:1}$ HBI mono-enes were first reported in epipelic diatoms isolated from the Tarmar Estuary, UK (Hird and Rowland, 1995) and were later found in sediments from Florida Bay (Xu et al., 2006) and in sea ice (Belt et al., 2007). Freshwater diatoms from the *Navicula* genus, some of which occur in El Junco Lake (Steinitz-Kannan et al., 1998), are reported to produce C_{25} HBIs, but only those with more than one double bond (Belt et al., 2001). The source of $C_{25:1}$ HBIs in El Junco is therefore unknown.

3.1.4. Isoprenoid thiophenes

Two isomers of C_{20} isoprenoid thiophenes were found in the 0–10 cm sediment interval based on EI mass spectra (Fig. 2b), eluting between the C_{21} and C_{22} *n*-alkanes. Such thiophenes are most likely formed through the reaction of chlorophyll derived phytol and hydrogen sulfide (H_2S) via a phytadiene intermediate (Fukushima et al., 1992; Rowland et al., 1993). The required reductive environment is corroborated by the existence of elemental sulfur (S_8) in El Junco sediment (Fig. 2b).

3.1.5. Reduced squalenes

The presence of several compounds exhibiting mass spectral ions at *m/z* 420, 418 and 416 suggests the occurrence of partially reduced squalenes (squalane has *m/z* of 422). The most abundant derivative, a $C_{30:2}$ squalene, is likely 2,3,6,7,18,19,22,23–octahydrosqualene based on a comparison of its EI mass spectrum (Fig. 2e) with that of $C_{40:2}$ *trans,trans*-lycopadienes (Fig. 2f). The two mass spectra have similar fragmentation patterns in the mid-chain and homologous fragment ions, differing by 70 amu and corresponding to one isoprenoid unit. Methanogens and halophilic archaea are generally considered as the source of di-, tetra- and hexahydrosqualenes in sediments (e.g. Evans et al., 1980; Ferrante et al., 1986; Volkman et al., 1986; Stiehl et al., 2005). In El Junco sediments, the slightly anoxic conditions revealed by the presence of molecular sulfur (S_8) and phytadiene thiophenes (Fig. 2b) imply that methanogenic archaea may have been present. However, given the high abundance of *Botryococcus* lipids in the sediment, a eukaryotic algal origin is more likely. A recent survey of 28 axenic isolates belonging to the genus *Choricystis*, inhabitants of freshwater lakes and, like *Botryococcus*, members of the Trebouxiophyceae class (Senousy et al., 2004), indicates that all synthesize significant proportions of dihydrosqualene and trace amounts of tetrahydrosqualene in addition to squalene (Fawley and Metzger, unpublished results). In El Junco, we propose that *B. braunii* race L is a likely source of squalene derivatives. In Zhang et al. (2007) we suggested that lycopadiene in El Junco particulate matter and sediments may originate from the enzymatic reduction of an acyclic carotenoid such as lycopene, based on the presence of small concentrations of lycopatriene isomers. The formation of octahydrosqualene, and possibly squalane as well, would therefore reflect a lack of specificity of the enzymatic system involved in the reduction of the lycopadiene precursors.

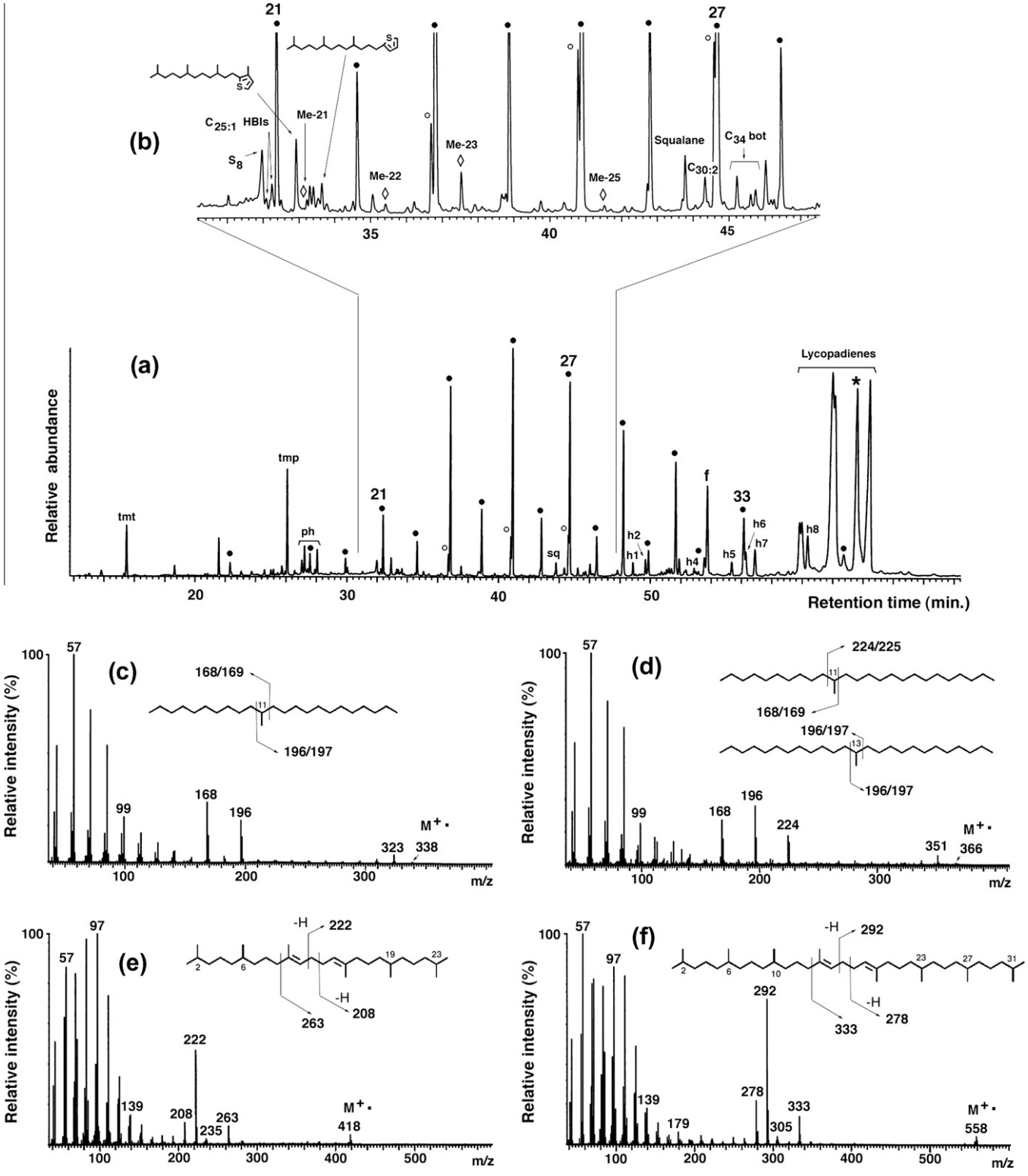

Fig. 2. Total ion chromatogram (TIC) and EI mass spectra of hydrocarbons in El Junco surface (EJ5-MW2 0–10 cm) sediment. (a) Total ion chromatogram showing the predominance of $C_{40}H_{58}$ lycopadienes; (b) expanded portion of the TIC containing several peaks of branched acyclic compounds; (c–d) EI mass spectra of monomethyl alkanes: 11-Me-tricosane (c) and 11-Me-pentacosane coeluting with its 13-Me isomer (d); (e) EI mass spectra of reduced squalene: 2,3,6,7,18,19,22,23–octahydrosqualene; (f) EI mass spectra of *trans*,*trans*-lycopadiene marked by ∗ in the TIC. ●: *n*-alkanes, ○: *n*-alkenes, ◇: mid-chain methyl branched C_{22}–C_{26} alkanes, bot: botryococcenes, $C_{30:2}$: octahydrosqualene, f: fernenes, h1: 22,29,30-trisnorhop-17(21)-ene, h2: 17(β)-22,29,30-trisnorhopane, h3: hop-17(21)-ene is shown in Fig. 3, h4: 17(α),21(β)-hopane, h5: 17(α),21(β)-homohopane, h6: 17(β),21(β)-hopane, h7: hop-22(29)-ene, h8: 17(β),21(β)-homohopane, ph: phytadienes, sq: squalane, tmp: 6,10,14-trimethylpentadecan-2-one, tmt: 2,6,10-trimethyltridecane.

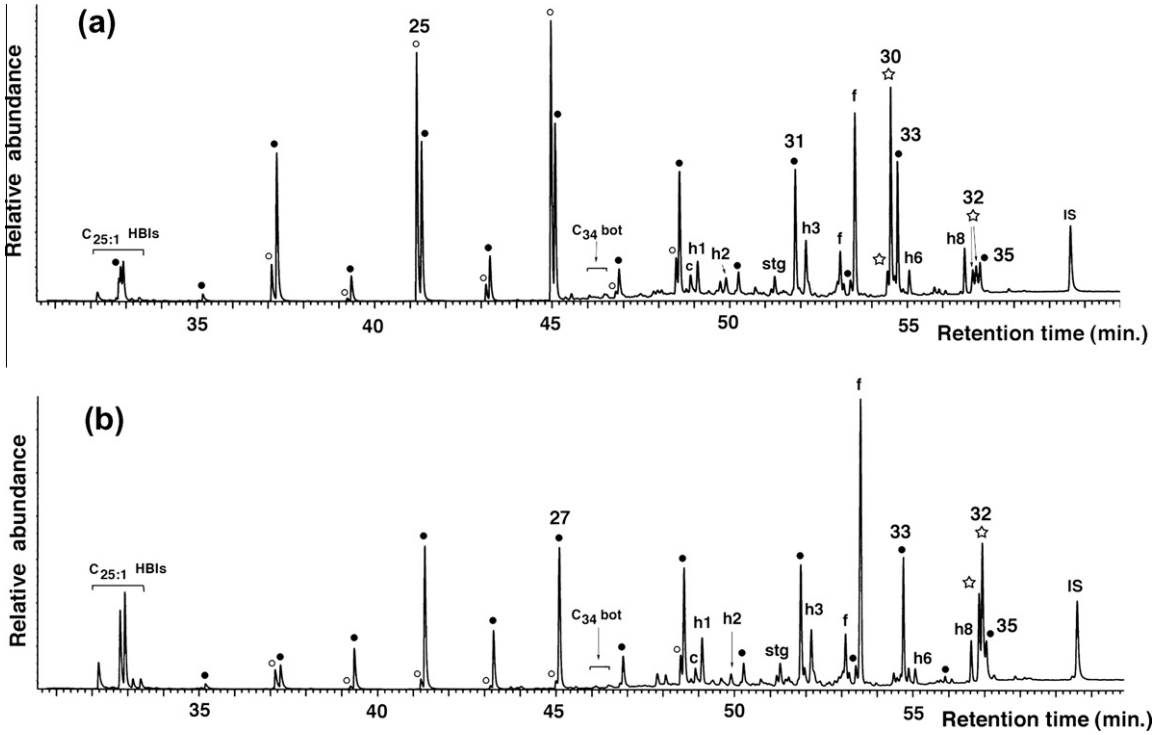

Fig. 3. TICs of hydrocarbon fractions obtained from EJ-N 289–290 cm (a) and EJ-N 294–295 cm (b) sediment intervals. Identities of peaks labelled ●, ○, f and h are given in Fig. 2. ☆: 1-chloroalkenes, stg: 24-ethyl-cholesta-3,5,22-triene, IS: internal standard (*n*-heptatriacontane).

3.1.6. Hopene, fernene and chloroalkenes

Hopanoids and fernenes are important constituents of the hydrocarbon fractions from deeper sediment intervals. Hopanoids, which are commonly attributed to a prokaryote source, may also derive from ferns, such as hop-22(29)-ene and hop-17(21)-ene (Fig. 3a and b) (Ageta and Arai, 1983). Two fernene isomers also are present (f in Fig. 3) that may derive from the water fern *Azolla microphylla* whose remains are abundant in the sediments of El Junco Lake (Schofield and Colinvaux, 1969).

In addition, unusual long chain chloroalkenes and chloroalkanes appear to be present in El Junco sediments (Fig. 3). Long chain chloroalkanes have been reported in halophytes (Grossi and Raphel, 2003), but never before in sediments. Details of their identification and down-core variation in El Junco sediment will be the subject of a subsequent paper.

3.2. Alkyl esters

Alkyl esters (C_{25}–C_{36}) were present in appreciable concentrations in near surface sediments, but their concentration decreased rapidly with depth, disappearing below the 50–60 cm interval (Table 1). Saturated and monounsaturated wax esters usually co-eluted during our analyses of El Junco sediments. Their molecular compositions were derived from both the GC–MS analysis of fatty acids and fatty alcohols released by saponification and the relative intensities of the $[RCO_2H_2]^+$ ions in summation spectra obtained from each GC peak, as previously reported (Cranwell et al., 1988). Both alcohols and acids exhibited ranges between C_{13} and C_{20}, while *iso*-branching was limited to the C_{16} and C_{18} compounds. The proportion of *iso*-branched and $>C_{30}$ components increased as a function of depth (Table 1).

The distribution of C_{25}–C_{36} alkyl esters in El Junco sediment were similar to those reported in three other freshwater lakes (Cranwell et al., 1983; Robinson et al., 1986). The presence of *iso*-branching in some alkyl and acyl chains is consistent with a bacterial origin (Kaneda, 1977). All freshwater microalgae wax esters exhibit straight-chain components although alkyl esters occur in the range C_{26}–C_{38} (Weete, 1976; Cranwell et al., 1988, 1990). In higher plants the carbon chain lengths are typically longer (e.g. de Leeuw, 1986; van Bergen et al., 1997; Jansen et al., 2006). Two other possible sources of long chain alkyl esters in El Junco Lake may be copepods (Volkman et al., 1980; Cavaletto et al., 1989) and ciliated protozoans (Wheeler and Holmlund, 1975). Copepods are abundant in El Junco (Zhang et al., 2007) but the connection between the alkyl esters and copepods remains tentative. Furthermore, some bacteria may produce and accumulate high proportions of wax esters under certain environmental conditions, such as during nitrogen deprivation and/or when consuming hydrocarbons (Makula et al., 1975; Fixter et al., 1986).

3.3. Alcohols

3.3.1. Alkanols

C_{20}–C_{32} *n*-alkan-1-ols exhibited a strong even/odd predominance and had a mean chain length centered at C_{28} throughout the sediment interval (Fig. 4). This distribution suggests a terrigenous plant leaf wax origin, but an algal origin cannot be excluded (Volkman et al., 1998). Alkanol abundance varied down-core: appreciable concentrations in surface sediments (Fig. 4a), low concentrations at 200–201 cm (Fig. 4b), and high concentrations at EJ-N 344–345 cm (Fig. 4c).

3.3.2. Alkenols

C_{30} and C_{32} alken-1-ols were present in the surface sediment (Fig. 4a) and at 344–345 cm interval (Fig. 4c). DMDS adduction followed by GC–MS analyses indicated the presence of three main C_{30} alkenol adducts with molecular ions at *m*/*z* 602: two isomers co-eluting (ca 95% of the whole) and a minor one (ca 5%) exhibiting a slightly greater retention time. Mass fragmentation patterns established that the minor C_{30} alkenol was triacont-21(ω9)-en-1-ol

Table 1
Composition of alkyl esters in a mud-water interface core (EJ5-MW2) from Lake El Junco.

Branch[a]/carbon number	0–10 cm depth		10–20 cm depth		40–50 cm depth	
	% Total esters	Dominant esters[b] (%)	% Total esters	Dominant ester (%)	% Total esters	Dominant ester (%)
n,n-25	0.1	10–15 (0.1)	nd		nd	
n,n-26	2.2	11–15 (1.3)	0.8	11–15 (0.5)	nd	
i,n-27	0.3	12–15 (0.1)	nd		nd	
n,n-27	5.9	11–16 (2.7)	2.1	11–16 (1.0)	0.8	11–16 (0.8)
i,n-28	0.7	13–15 (0.3)	0.5	13–15 (0.2)	nd	
n,n-28	16.5	13–15 (9.4)	5.8	13–15 (3.1)	3.6	13–15 (2.1)
i,n-29	1.4	13–16 (0.7)	0.8	14–15 (0.4)	nd	
n,n-29	22.4	13–16 (14.1)	9.2	13–16 (5.7)	4.4	13–16 (3.1)
i,i-30	Traces		1.6	14–16 (1.1)	3.8	14–16 (3.8)
i,n-30	2.7	14–16 (1.7)	4.8	14–16 (3.4)	4.9	14–16 (4.9)
n,n-30	23.0	14–16 (10.3)	13.0	14–16 (6.6)	12.5	14–16 (5.5)
i,n-31	1.5	15–16 (0.8)	1.5	15–16 (0.6)	2.3	15–16 (1.0)
n,n-31	11.3	15–16 (6.2)	6.4	15–16 (3.3)	6.3	15–16 (3.4)
i,i-32	0.6	16–16 (0.6)	4.0	16–16 (2.7)	6.2	16–16 (4.1)
i,n-32	2.0	16–16 (0.8)	9.6	16–16 (5.2)	12.0	16–16 (6.5)
n,n-32	5.7	15–17 (3.0)	12.2	14–18 (4.9)	10.9	16–16 (3.4)
i,n-33	Traces		1.1	16–17 (0.6)	1.2	16–17 (0.7)
n,n-33	1.5	16–17 (0.8)	2.5	16–17 (1.0)	2.0	16–17 (1.0)
i,i-34	Traces		2.6	16–18 (2.0)	4.4	16–18 (4.4)
i,n-34	0.6	16–18 (0.6)	7.1	16–18 (6.2)	9.4	16–18 (8.5)
n,n-34	1.6	16–18 (1.6)	6.4	16–18 (5.1)	6.3	16–18 (5.0)
n,n-35	Traces		1.3	17–18 (0.7)	1.0	18–17 (0.4)
i,i-36	nd		1.3	18–18 (0.9)	2.2	18–18 (2.2)
i,n-36	nd		2.5	18–18 (2.0)	2.8	18–18 (2.2)
n,n-36	Traces		2.9	18–18 (2.4)	2.3	18–18 (1.9)
$\sum i,i + i,n$	9.2		37.4		49.2	
$\sum > C_{30}$	24.8		61.4		69.3	

nd: not detected.
[a] *i* = iso branching, *n* = straight chain; *i* and *n* can apply to acyl or alkyl moieties.
[b] Carbon numbers of acid-alcohol.

(ion fragments at *m/z* 173 and 429 from vicinal α-cleavage), while the two co-eluting isomers were derived from triacont-14(ω16)-en-1-ol (characteristic ions at *m/z* 271 and 331; Fig. 5a) and triacont-15(ω15)-1-ol (characteristic fragment ions at *m/z* 257 and 345; Fig. 5a), respectively. A small fragment ion at *m/z* 285 (Fig. 5a) suggested the existence of a third co-eluting DMDS adduct of triacont-13(ω17)-en-1-ol. Due to co-elution of the DMDS adducts of C_{32} alkenols with unknown compounds, the position of the double bond could not be determined in these higher homologues. Although alkenols occurrence in numerous eustigmatophytes (Volkman et al., 1992, 1998, 1999a; Gelin et al., 1997; Méjanelle et al., 2003) and in some freshwater chlorophytes (Allard and Templier, 2000) would suggest a microalgal origin, long chain *n*-alkenols are relatively uncommon in sediments (Xu et al., 2007). Short chain *n*-alkenols ($C_{20:1}$ and $C_{22:1}$) were reported in suspended particles and sediments from the East China Sea and were proposed to derive from copepods (Jeng and Huh, 2004). Given the fact that a large amount of copepods throughout the water column of El Junco was observed in 2004, they might be a source for the long chain *n*-alkenols in this lake in addition to microalgal source.

3.3.3. Sterols

4-Desmethylsterols were the main steroidal alcohols in El Junco surface sediments (Fig. 4a). Relatively high abundances of cholesterol, 24-methylcholest-5-en-3β-ol, 24-ethylcholesta-5,22-dien-3β-ol and 24-ethylcholesta-7,22-dien-3β-ol, and low concentrations of 24-ethylcholest-5-en-3β-ol indicates a predominantly microalgal source (Volkman et al., 1998). Moreover, the higher relative abundance of 5(α) H stanols (cholestanol, 24-methylcholestanol and 24-ethylcholestanol; Fig. 4a) is indicative of bacterial reduction at the water/sediment interface rather than a direct input from microalgae (Gaskell and Eglinton, 1975; Smith et al., 1983a; Volkman et al., 1990). In sharp contrast, the sterol fraction from 344–345 cm (Fig. 4c) was dominated by 4α,23,24-trimethyl-5α-cholest-22-en-3β-ol and 4α,23,24-trimethyl-5α-cholestan-3β-ol, commonly referred to as dinosterol and dinostanol, respectively, and indicative of a dinoflagellate source (Boon et al., 1979; Robinson et al., 1984, 1987), though some diatoms also produce these lipids (Volkman et al., 1993). At 200–201 cm steroidal alcohols occurred in low concentration and were dominated by 24-ethylcholesterol (Fig. 4b).

3.4. Long chain diols, triols and keto-ols

3.4.1. Diols and triols

Both vicinal and terminal/mid-chain diols occurred in El Junco sediments, though the majority of them contained one hydroxyl group at the terminal position. In the three sediment intervals examined about 32 distinct C_{30}–C_{36} diols were identified based on the mass spectra of their TMSi ethers (Table 2). C_{30} diols were dominant in the 0–10 and 344–345 cm intervals, while C_{32} diols predominated in the 200–201 cm interval (Fig. 4). In the latter interval minor amounts of C_{36} diols were also detected (TIC peak not shown in Fig. 4). Each mass spectrum exhibited ions at *m/z* [M^+-90] due to the loss of HOTMSi, and fragment ions resulting from the cleavage around the carbon bearing the mid-chain OTMSi group. The presence of homologous fragment ions in almost all the spectra indicated the presence of co-eluting isomers with the mid-chain hydroxyl group occurring at various ω positions (Table 2). Thus the spectrum of the co-eluting $C_{32:0}$ diols in the 344–345 cm sediment interval showed ion pairs at *m/z* 313/415, 327/401, 341/387 and 369/359, indicative of mid-chain hydroxyl groups at ω16 (1,17-dotriacontanediol), ω17 (1,16-dotriacontanediol), ω18 (1,15-dotriacontanediol) and ω20 (1,13-dotriacontanediol), respectively (Figs. 4c and 5b).

The relative proportions of the isomeric diols were calculated based on the relative intensities of the fragment ions in the summation spectra obtained from each GC–MS peak (Table 2). The

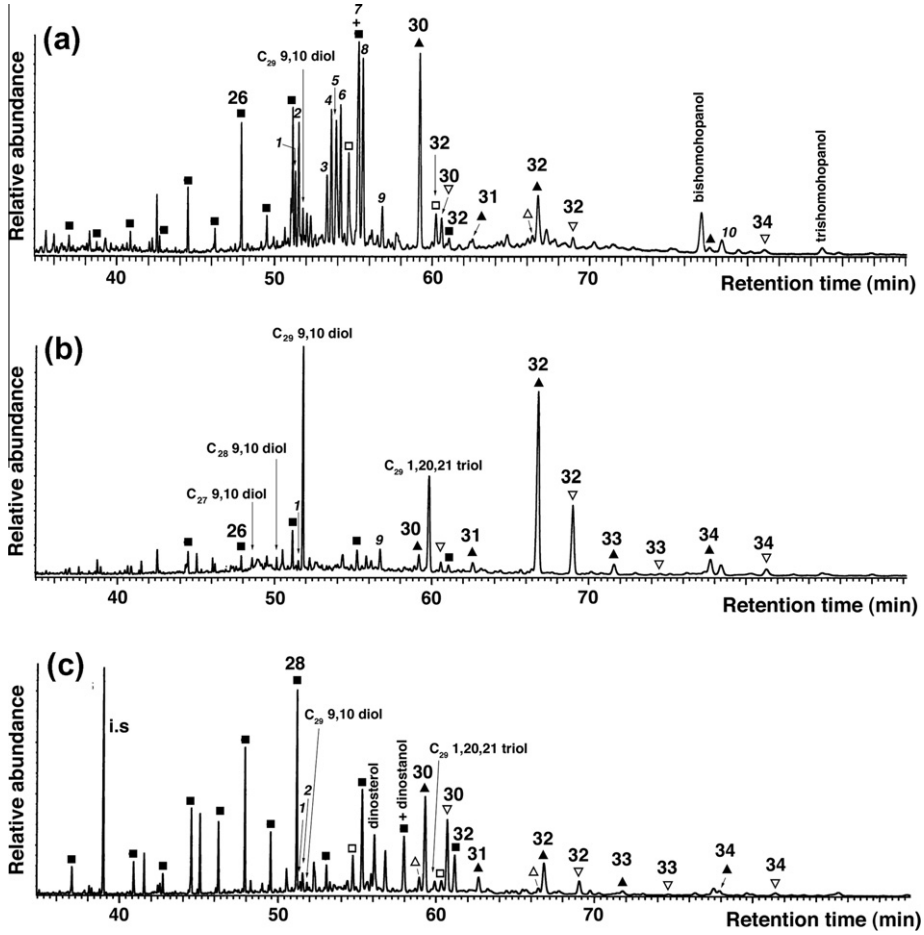

Fig. 4. TICs of trimethylsilyl derivatives of polar fractions (MeOH eluates from silica gel) of lipid extracts from three sediment intervals: (a) EJ5-MW2 0–10 cm, (b) EJ-3 200–201 cm and (c) EJ-N 344–345 cm. ■: *n*- alkan-1-ols, □: *n*- alken-1-ols, ▲: mid-chain alkyl diols, △: mid-chain alkenyl diols, ▽: mid-chain keto-ols, i.s. internal standard (*n*-tricosan-1-ol), *1*: cholest-5-en-3β-ol, *2*: 5αH-cholestan-3β-ol, *3*: 24-methylcholest-5-en-3β-ol, *4*: 24-methylcholestan-3β-ol, *5*: 24-ethylcholesta-5,22-dien-3β-ol, *6*: 24-ethylcholest-22-en-3β-ol (24-ethyl Δ22-coprostenol), *7*: 24-ethylcholesta-7,22-dien-3β-ol, *8*: 24-ethylcholestan-3β-ol, *9*: 24-ethylcholest-7-en-3β-ol.

preferential positions of the hydroxy group in the mid-chain appeared variable among different sediment intervals. While the mid-chain hydroxyl group was mainly located at ω20 in C_{30}–C_{36} diols at 200–201 cm, the ω18 and ω16 positions characterized the C_{32}–C_{34} and C_{30}–C_{31} diols at 0–10 cm and 344–345 cm, respectively (Table 2).

Occurring in a variety of marine and freshwater sediments (Versteegh et al., 1997; Xu et al., 2007; Speelman et al., 2009; Shimokawara et al., 2010), long mid-chain diols have a wide diversity of sources, including the marine eustigmatophyte genus *Nannochloropsis* (Gelin et al., 1997; Méjanelle et al., 2003; Volkman et al., 1992), some freshwater eustigmatophytes of the genus *Vischeria* (Volkman et al., 1998), marine diatoms of the genus *Proboscia* (Sinninghe Damsté et al., 2003), two terrestrial *Osmunda regalis* ferns (Jetter and Riederer, 1999), the aquatic fern *Azolla filliculoides* (Speelman et al., 2009), and the cuticular waxes of some terrestrial plants (Jetter et al., 1996; Jetter, 2000; Wen et al., 2006).

Two C_{32} (1,16-dotriacontenediol and 1,15-dotriacontenediol) and one C_{30} (1,16-triacontenediol) monounsaturated diols were also identified in two sediment intervals (0–10 and 344–345 cm) (Fig. 4a, c; Table 2). The fragment ions formed by cleavage around the carbon bearing the mid-chain OTMSi group suggested that the double bond was located between the terminal methyl group and C-16 in the $C_{30:1}$ diol, and between C-1 and C-15 (or C-16) in the two $C_{32:1}$ isomers. The occurrence of unsaturated long mid-chain alkenediols has been reported in some *Nannochloropsis* species (Volkman et al., 1992) and in some recent sediments (Smith et al., 1983b; Morris and Brassell, 1988). The occurrence in both the surface sediments and deeper zones implies favorable conditions for preservation of these lipids in El Junco.

Three diols and one triol with hydroxyl groups at ω9 and ω10 (vicinal diols) occurred in relatively high concentration at 200–201 cm, and at low concentrations at 0–10 cm and 344–345 cm (Fig. 4). Structures were assigned based on mass spectra of their TMSi ether derivatives which exhibited a common *m*/*z* 215 ion resulting from cleavage of the bond between the carbons bearing the two vicinal $OSiMe_3$ groups (Fig. 6a–c). They were identified as 9,10-heptacosanediol (Fig. 6a), 9,10-octacosanediol and 9,10-nonacosanediol (Fig. 6b) and 1,ω9,ω10-nonacosanetriol (Fig. 6c). A C_{29} diol and triol were recently identified in the leaf wax of the aquatic fern *Azolla filliculoides* and in Eocene sediments from the Arctic containing *Azolla* remains (Speelman et al., 2009). The co-variation of El Junco diols and triols with fernenes (Figs. 3 and 4) suggests they may have a common source in the aquatic fern *Azolla* whose fossil remains are abundant in the sediments of El Junco Lake (Schofield and Colinvaux, 1969). The additional presence of minor amounts of C_{27} and C_{28} 9,10 diols (Fig. 4b) may result from diagenesis or a biosynthetic response *of Azolla* to environmental conditions.

3.4.2. Keto-ols

A variety of C_{30}–C_{34} and C_{36} keto-ols occurred in El Junco sediments (Fig. 4; Table 3). The mass spectra of the TMSi ethers of the 22 keto-ols each exhibited intense ions at *m*/*z* $(M–Me)^+$,

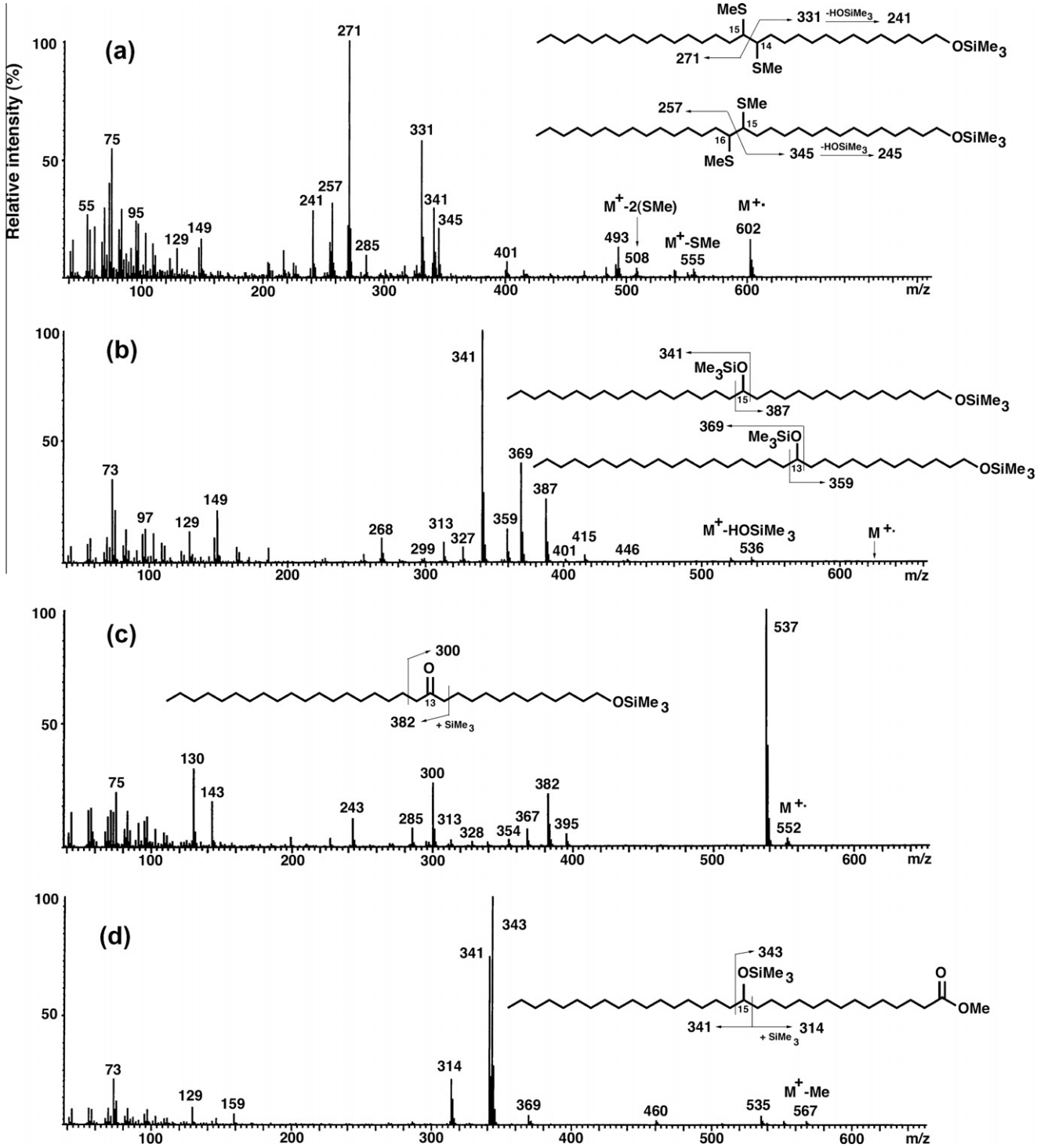

Fig. 5. EI mass spectra of coeluting (a) DMDS adducts of C_{30} alk-14,15-en-1-ol and alk-15,16-en-1-ol, (b) a series of C_{32} mid-chain diols with 1,13 and 1,15 diols predominating, (c) 1,13 (major) and 1,15 C_{32} keto-ols, and (d) C_{32} 15-hydroxy (major) and 13-hydoxy fatty acid methyl esters; spectra shown in (a) and (b) were obtained from the analyses of lipid fractions isolated from the EJ-N 344–345 cm interval, (c) from EJ-3 200–201 cm interval, and (d) from EJ7-MW2 0–10 cm interval.

characteristic ions at 130 and 143, and fragment ions from C–C cleavages β to the carbonyl group (Fig. 5c). Typically 2–5 isomers co-eluted at each carbon chain length (Table 3), with the predominant position for the carbonyl group usually the same as that observed for the mid-chain hydroxyl groups in the same sample (i.e. at ω16 for C_{30} at 0–10 and 344–345 cm; ω18 for C_{32}, C_{33}, C_{34} and C_{36} at 344–345 cm, and ω18 and ω20 for C_{30}-C_{36} at 200–201 cm). Long chain keto-ols generally co-occur with the corresponding diols in marine and lacustrine sediments (de Leeuw et al., 1981; Smith et al., 1983b; Versteegh et al., 1997; Xu et al., 2007; Speelman et al., 2009). The source of keto-ols in sediments remains debated (Xu et al., 2007), particularly whether they represent primary inputs (Jetter and Riederer, 1999) or diagenetic alteration products (Ferreira et al., 2001; Sinninghe Damsté et al., 2003).

Table 2
Distribution of long chain diol isomers[a] in three selected sediment intervals with characteristic [fragment ions].

Chain length	Positions of the mid-chain hydroxyl group					
	ω15	ω16	ω17	ω18	ω19	ω20
EJ5-MW2 0–10 cm						
$C_{30:0}$	1,16 (18) [299, 401]	1,15 (100) [313, 387]				
$C_{30:1}$	1,16 (100) [299, 399]					
$C_{31:0}$	1,17 (4) [299, 415]	1,16 (20) [313, 401]	1,15 (100) [327, 387]	1,14 (10) [341, 373]		
$C_{32:0}$		1,17 (10) [313, 415]	1,16 (4) [327, 401]	1,15 (100) [341, 387]		1,13 (21) [369, 359]
$C_{32:1}$			1,16 (8) [325, 401]	1,15 (100) [339, 387]		
$C_{34:0}$		1,19 (56) [313, 443]	1,18 (17) [327, 429]	1,17 (100) [341, 415]	1,16 (10)	
EJ-3 200–201 cm						
$C_{30:0}$	1,16 (4) [299,401]	1,15 (35) [313, 387]	1,14 (9) [327, 373]	1,13 (57) [341, 359]	1,12 (3) [355, 345]	1,11 (100) [369, 331]
$C_{31:0}$			1,15 (10) [327, 387]	1,14 (4) [341, 373]	1,13 (10) [355, 359]	1,12 (100) [369, 345]
$C_{32:0}$				1,15 (7) [341, 387]		1,13 (100) [369, 359]
$C_{33:0}$		1,18 (6) [313, 429]	1,17 (19) [327, 415]	1,16 (11) [341, 401]	1,15 (4) [355, 387]	1,14 (100) [369, 373]
$C_{34:0}$				1,17 (90) [341, 415]	1,16 (2) [355, 401]	1,15 (100) [369, 387]
$C_{36:0}$				1,19 (6) [341, 443]	1,18 (3) [355, 429]	1,17 (100) [369, 415]
EJ-N 344–345 cm						
$C_{30:0}$	1,16 (14) [299, 401]	1,15 (100) [313, 387]	1,14 (3) [327, 373]			
$C_{30:1}$	1,16 (100) [299, 399]					
$C_{31:0}$		1,16 (52) [313, 401]	1,15 (100) [327, 387]	1,14 (28) [341, 373]		
$C_{32:0}$		1,17 (8) [313, 415]	1,16 (7) [327, 401]	1,15 (100) [341, 387]		1,13 (43) [369, 359]
$C_{32:1}$				1,15 (100) [339, 387]		
$C_{33:0}$		1,18 (49) [313, 429]	1,17 (92) [327, 415]	1,16 (100) [341, 401]	1,15 (16) [355, 387]	1,14 (61) [369, 373]
$C_{34:0}$		1,19 (12) [313, 443]	1,18 (6) [327, 429]	1,17 (100) [341, 415]	1,16 (6) [355, 401]	1,15 (88) [369, 387]

[a] For a given chain length the relative proportions of isomers (in brackets) were determined by comparing in the mass spectra the relative intensities of the fragment ions resulting from the cleavage of the C–C bonds vicinal to the $OSiMe_3$ group as shown in Fig. 5b.

3.5. Long chain hydroxy acids and keto acids

3.5.1. Hydroxy acids

A series of hydroxy acids occurred in conjunction with the ubiquitous C_{16}-C_{34} *n*-fatty acids centred at either C_{26} or C_{28} (Fig. 7a–d). GC-MS spectra of methylated and silylated acids (F5 fraction; Fig. 1) revealed a homologous series of C_{14}–C_{36} ω-hydroxy acids, maximizing at C_{26} or C_{28} and exhibiting an even/odd predominance (Fig. 7). These hydroxy acids are generally considered to originate from cutins, suberins and higher plant epicuticular waxes (Eglinton et al., 1968), though microalgal sources exist for compounds with >28 carbon atoms (Blokker et al., 1998; Volkman et al., 1999b; Allard and Templier, 2001). The long chain (ω-1)-hydroxy acids were also observed (C_{26}, C_{28}, C_{30}, C_{32} and C_{34}; Fig. 7) which may derive from methylotrophic bacteria (Nichols et al., 1985; Urakami and Komagata, 1987; Skerratt et al., 1992), symbiotic *Rhizobium* (Hollingsworth and Carlson, 1989; Gil-Serrano et al., 1994), and/or certain cyanobacteria (Volkman et al., 1998).

In addition, very long chain hydroxy acids ranging from C_{30}–C_{36} (excluding C_{35}), exhibiting one mid-chain hydroxyl group, were present in all sediment intervals examined (e.g. Fig. 7). The mass spectra were dominated by intense fragment ions resulting from cleavages around the carbon bearing the $OSiMe_3$ group (Fig. 5d). As with the diols, the mass spectra of hydroxy acids had homologous fragment ions indicative of co-eluting isomers (Table 4). At 344–345 cm ω16 (15-OH–C_{30}, 19-OH–C_{34} and 21-OH–C_{36}) and ω18 (15-OH–C_{32}) hydroxy acids dominated while the ω20 (11-OH–C_{30}, 13-OH–C_{32}, 15-OH–C_{34} and 17-OH–C_{36}) counterparts were dominant at 200–201 cm. This difference in the hydroxy acid distribution between the two intervals is similar to that observed for long chain diols (Tables 2 and 4). The dominance of ω20-hydroxy acids and diols at 200–201 cm is indicative of substantial input of *Azolla* lipids (Speelman et al., 2009), a premise corroborated by the existence of the C_{29} 20,21-dihydroxy-nonacosanoic acid (Figs. 6d and 7b). By contrast, the predominance of ω16- and ω18-hydroxy acids and diols at 344–345 cm implies a microalgal source (Versteegh et al., 1997).

3.5.2. Keto acids

Two series of keto acids were found in El Junco sediments. The first is a series of C_{28}, C_{30} and C_{32} (ω-1)-keto acids (Fig. 7). Less abundant than the hydroxy acids, these compounds were identified as methyl esters on the basis of mass spectra containing fragment ions at *m/z* 58 (McLafferty rearrangement), [M^+–MeOH], [M^+–CH_2COCH_3] and [M^+–CH_2COCH_3–MeOH–H] (Fig. 8a). In addition, a previously unreported homologous series of long chain keto

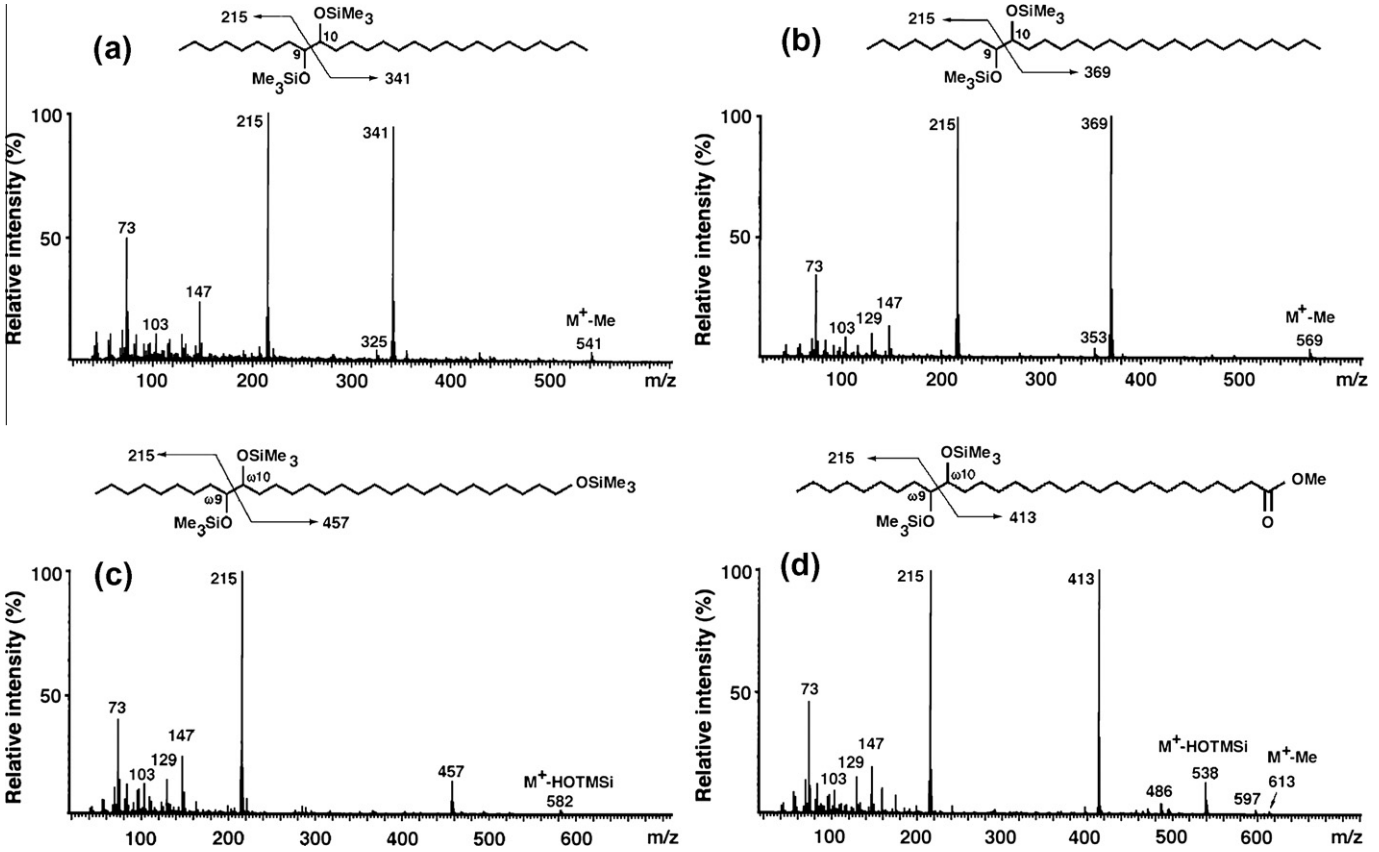

Fig. 6. EI mass spectra and fragmentation pattern of TMSi ether derivatives of (a) C_{27} 9,10 diol, (b) C_{29} 9,10 diol, (c) C_{29} 1,20,21 triol, and (d) C_{29} 20,21 dihydroxy fatty acid as methyl ester identified in the EJ3 200–201 cm interval.

Table 3
Distribution[a] of isomers in long chain keto-1-ols in three selected sediment intervals with characteristic [fragment ions].

Chain length	Position of the mid-chain keto group					
	ω15	ω16	ω17	ω18	ω19	ω20
EJMW2 0–10 cm						
$C_{30:0}$	1,16 (6) [312, 342]	1,15 (100) [326, 328]	1,14 (5) [340,314]			
$C_{32:0}$		1,17 (17) [326, 356]	1,16 (15) [340, 342]	1,15 (100) [354, 328]	1,14 (5) [368, 314]	1,13 (18) [382, 300]
EJ-3 200–201 cm						
$C_{30:0}$		1,15 (34) [326, 328]	1,14 (11) [340,314]	1,13 (44) [354,300]		1,11 (100) [382, 272]
$C_{32:0}$				1,15 (9) [354, 328]		1,13 (100) [382, 300]
$C_{34:0}$				1,17 (100) [354, 356]		1,15 (60) [382, 328]
$C_{36:0}$				1,19 (6) [354, 384]	1,18 (3) [368, 370]	1,17 (100) [382, 356]
EJ-N 344–345 cm						
$C_{30:0}$	1,16 (13) [312, 342]	1,15 (100) [326, 328]	1,14 (15) [340,314]			
$C_{32:0}$		1,17 (14) [326, 356]	1,16 (7) [340, 342]	1,15 (100) [354, 328]		1,13 (18) [382, 300]
$C_{33:0}$		1,18 (17) [326, 370]	1,17 (25) [340, 356]	1,16 (100) [354, 342]		1,14 (40) [382, 314]
$C_{34:0}$		1,19 (12) [326, 384]	1,18 (6) [340, 370]	1,17 (100) [354, 356]	1,16 (9) [368, 342]	1,15 (33) [382, 328]
$C_{36:0}$				1,19 (100) [354, 384]		1,17 (20) [382, 356]

[a] For a given chain length, the relative proportions of isomers (in brackets) were determined by comparing in the mass spectra the relative intensities of the fragment ions resulting from the cleavage of the C–C bonds β to the keto group according to the schema shown in Fig. 5c.

acids with the oxo-group in the mid-chain was observed. Even numbered from C_{30}–C_{36}, these acids were characterized on the basis of the mass spectra of their methyl esters showing pairs of fragment ions resulting from McLafferty rearrangements (cf. Fig. 8b). All the spectra contained several ion pairs suggesting the presence of co-eluting isomers. Thus, the mass spectrum of the

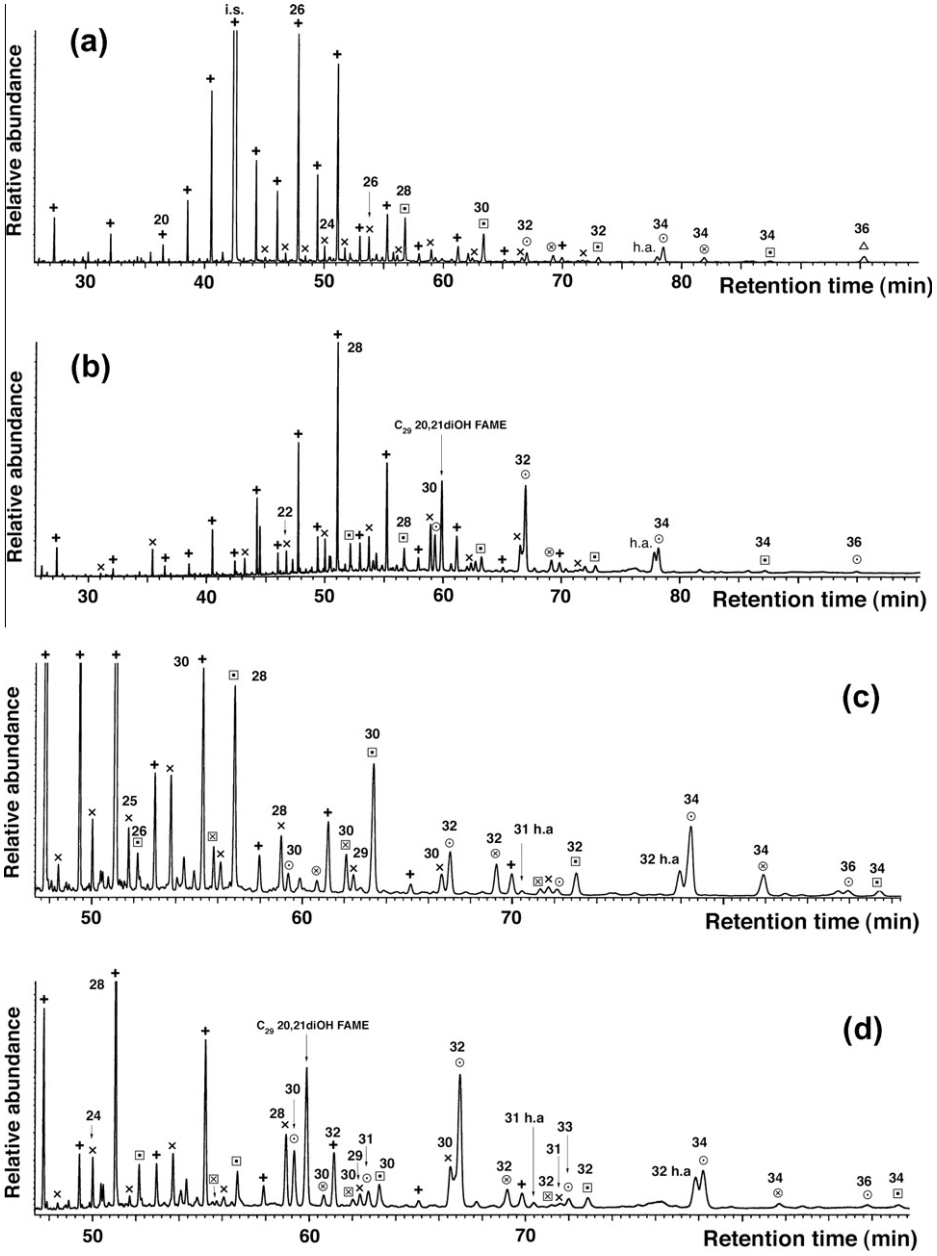

Fig. 7. TICs of methylated and silylated acid fractions from EJ-3 200–201 cm interval and EJ-N 344–345 cm interval in El Junco sediments. TIC traces of 200–201 cm (a) and 344–345 cm (b) intervals; enhanced partial ion chromatograms of EJ-3 200–201 cm (c) and EJ-N 344–345 cm (d) intervals. +: fatty acids, ×: ω-hydroxy acids, ▣: ω-1 hydroxy acids, ⊠: ω-1 keto acids, ⊙: mid-chain hydroxy acids, ⊗: mid-chain keto acids, h.a.: C_{31} and C_{32} hopanoic acids, i.s.: internal standard (tricosanoic acid methyl ester).

C_{34} keto acids from 344–345 cm (Fig. 8b; Table 5) exhibited ions at *m*/*z* 354/240, 340/254, 326/268, 312/282, 298/296 and 284/310 indicative of the presence of 20-, 19-, 18-, 17-, 16- and 15-keto acids, respectively, with 19(ω16)-keto predominating. The relative proportions of the isomeric keto acids were estimated based on the relative intensities of these ions (Table 4) and indicated a preferential position of the keto group at ω16 at 344–345 cm and ω20 at 200–201 cm.

(ω-1)-Keto acids have been rarely reported in sediments (Haugh et al., 1971; Barakat et al., 1994). To our knowledge, in living organisms they have only been reported in *Legionella* (Moll et al., 1992). These Gram-negative bacteria are known to inhabit various anthropogenic and natural freshwater environments at very low concentrations (Declerck et al., 2007). Given the reducing conditions prevailing in El Junco sediments, (ω-1)-keto acids are likely to originate from biotic oxidation of the corresponding (ω-1)-hydroxy acids by anaerobic bacteria.

Similar positions of the mid-chain hydroxy and keto groups suggest these lipids may share a common source. Reports of such long chain keto acids in living organisms are sparse, but they do occur in the rhizomes of some higher plants (Gupta et al., 1983, 1986). In El Junco sediments mid-chain keto acids may derive from biotic oxidation of diols through diol → keto-ol → keto acid and/or diol → hydroxy acid → keto acid pathways.

3.6. Ecological and environmental implications

A wide diversity of lipid biomarkers in El Junco Lake implies that multiple sources of plants, plankton, microbes and invertebrates contribute substantial quantities of lipids to the sediment (Table 6). While some of them can be linked to specific sources, others cannot. By evaluating the entire suite of lipids we can begin to infer certain first and second order characteristics of the environment and ecology of the El Junco Lake catchment over time.

Table 4
Distribution[a] of isomers in long chain mid-chain hydroxy acids in two different sediment intervals with characteristic [fragment ions].

Chain length	Positions of the hydroxyl group						
	ω15	ω16	ω17	ω18	ω19	ω20	ω22
EJ-3 200–201 cm							
$C_{30:0}$		15-OH (12) [313, 343]	14-OH (2) [327, 329]	13-OH (19) [341, 315]		11-OH (100) [369, 287]	9-OH (3) [397, 259]
$C_{32:0}$				15-OH (6) [341, 343]		13-OH (100) [369, 315]	
$C_{34:0}$		19-OH (3) [313, 399]		17-OH (4) [341, 371]		15-OH (100) [369, 343]	
$C_{36:0}$		21-OH (34) [313, 427]	20-OH (3) [327, 413]	19-OH (39) [341, 399]	18-OH (2) [355, 385]	17-OH (100) [369, 371]	
EJ-N 344–345 cm							
$C_{30:0}$	16-OH (23) [299, 357]	15-OH (100) [313, 343]	14-OH (9) [327, 329]	13-OH (17) [341, 315]		11-OH (31) [369, 287]	
$C_{32:0}$	18-OH (47) [299, 385]	17-OH (74) [313, 371]	16-OH (9) [327, 357]	15-OH (100) [341, 343]		13-OH (33) [369, 315]	
$C_{34:0}$	20-OH (10) [299, 413]	19-OH (100) [313, 399]	18-OH (4) [327, 385]	17-OH (7) [341, 371]		15-OH (11) [369, 343]	
$C_{36:0}$		21-OH (100) [313, 427]	20-OH (3) [327, 413]	19-OH (21) [341, 399]		17-OH (4) [369, 371]	

[a] For a given chain length, the relative proportions of isomers (in brackets) were determined by comparing in the mass spectra the relative intensities of the characteristic [fragment ions] resulting from the cleavage of the C–C bonds vicinal to the $OSiMe_3$ group as shown in Fig. 5d.

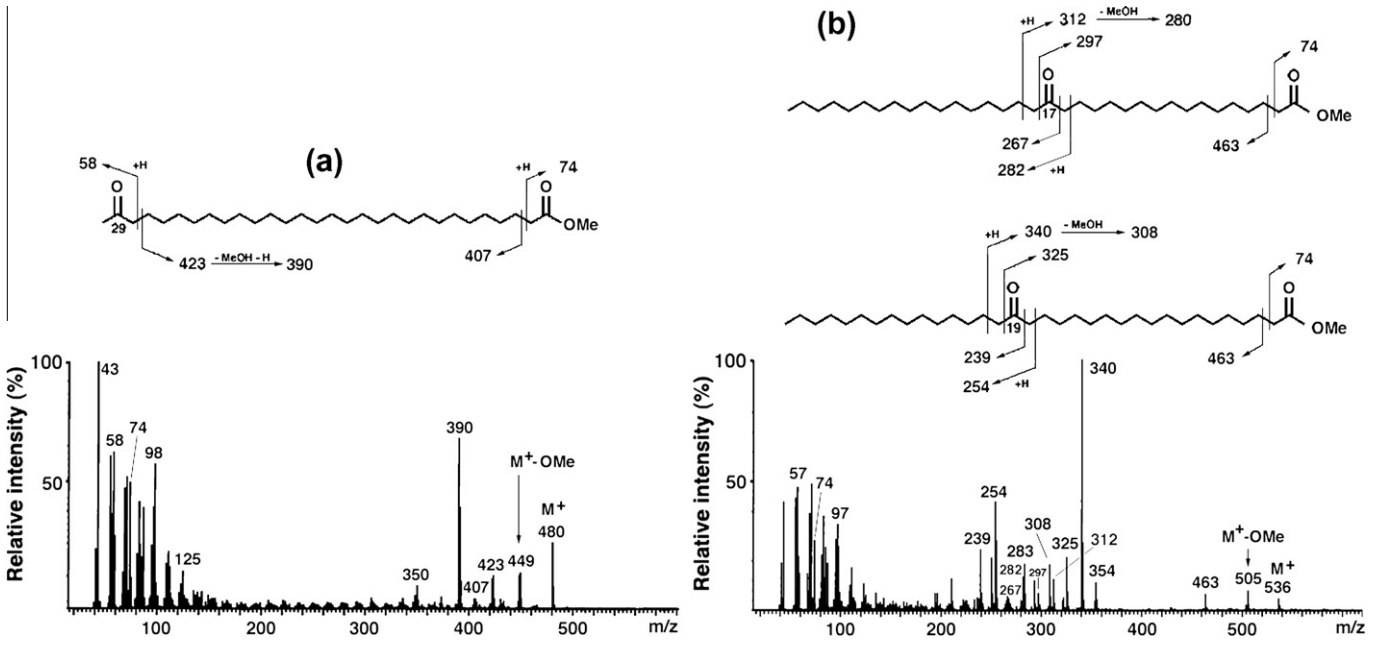

Fig. 8. EI mass spectra and fragmentation patterns of (a) 29-oxo-triacontanoic acid methyl ester and (b) co-eluting isomers of oxo-tetratriacontanoic acid methyl esters (oxo group at positions C-15 to C-20) in the EJ-N 344–345 cm interval, dominated by the 17- and 19-oxo compounds.

Leaf wax from higher plants in the catchment is the source of both long chain *n*-alkanes with an odd/even predominance and long chain *n*-alkanols with an even/odd predominance. Leaf cutin may be a source for ω-hydroxy acids in the sediments.

B. braunii races A, B and L contribute alkadienes/alkatrienes, botryococcenes and lycopadienes/lycopatrienes, respectively, to the water column and sediments of El Junco Lake. The relatively high abundance of alkadienes in the water column and near absence in the surface sediment leads us to hypothesize that they may be metabolized by hydrocarbon degrading bacteria. Hydrogenated squalenes could be synthesized in a similar fashion to lycopadienes, reflecting a possible lack of specificity of the enzymatic system involved in the reduction of the lycopadiene precursors.

Copepods are red zooplankton that graze extensively on *B. braunii* (Zhang et al., 2007). They may contribute to the substantial amount of wax esters in the surface sediment (Volkman et al., 1980). The insects inhabiting the lake catchment are the likely source of mid-chain methyl branched alkanes (C_{21}–C_{25}).

Phytadienes in the sediment was formed by the biodegradation of phytol by anaerobic bacteria (Table 6). Meanwhile the reduced environment evidenced in the existence of elemental sulfur (S_8) enable the formation of thiophenes through abiotic sulfurization. Such environment may also facilitate the formation of (ω-1)-hydroxy acids by methylotrophic bacteria.

Navicula diatoms are the likely source of HBI alkenes in El Junco sediments. They may also contribute long, mid-chain diols as well, though this conclusion is tentative.

Short chain alkenes and alkanes are likely from microalgal sources. Freshwater eustigmatophytes can be potential sources of sterols, C_{22}–C_{28} *n*-alcohols and C_{28}–C_{32} *n*-alkyl diols. Although a

Table 5
Distribution[a] of isomers in long chain keto acids in two different sediment intervals with characteristic [fragment ions].

Chain length	Positions of the oxo group[a]					
	ω15	ω16	ω17	ω18	ω19	ω20
EJ-3 200–201 cm						
$C_{30:0}$		15-CO (9) [284, 254]	14-CO (4) [270, 268]	13-CO (15) [256, 282]	12-CO (3) [242, 296]	11-CO (100) [228, 310]
$C_{32:0}$				15-CO (17) [284, 282]	14-CO (10) [270, 296]	13-CO (100) [256, 310]
$C_{34:0}$		19-CO (16) [340, 254]	18-CO (3) [326, 268]	17-CO (36) [312, 282]	16-CO (3) [298, 296]	15-CO (100) [284, 310]
EJ-N 344–345 cm						
$C_{30:0}$	16-CO (13) [298, 240]	15-CO (100) [284, 254]	14-CO (10) [270, 268]	13-CO (28) [256, 282]	12-CO (18) [242, 296]	11-CO (47) [228, 310]
$C_{32:0}$	18-CO (37) [326, 240]	17-CO (100) [312, 254]	16-CO (7) [298, 268]	15-CO (18) [284, 282]	14-CO (13) [270, 296]	13-CO (12) [256, 310]
$C_{34:0}$	20-CO (10) [354, 240]	19-CO (100) [340, 254]	18-CO (7) [326, 268]	17-CO (14) [312, 282]	16-CO (6) [298, 296]	15-CO (7) [284, 310]
$C_{36:0}$		21-CO (100) [368, 254]	20-CO (5) [354, 268]	19-CO (33) [340, 282]		17-CO (4) [312, 310]

[a] For a given chain length, the relative proportions of isomers (in brackets) were determined by comparing in the mass spectra the relative intensities of the characteristic [fragment ions] resulted from MacLafferty rearrangement as shown in Fig. 5e. Due to the low resolution of their mass spectra, the distribution of the odd carbon numbered keto acids could not be estimated.

Table 6
Possible sources of lipid biomarkers isolated from sediments of El Junco Lake.

Biomarkers	Sources
Hydrocarbons	
n-C_{17}–C_{35} alkanes and alkenes	Microalgae and higher plants
$C_{34:6}$ botryococcenes	*Botryococcus braunii* race B
$C_{40:2}$ lycopadienes	*Botryococcus braunii* race L
$C_{29:2}$ and $C_{31:2}$ alkadienes	*Botryococcus braunii* race A
Phytadienes	Biodegradation of phytol by anaerobic bacteria
Sulfurized phytadienes	Abiotic sulfurization of phytadienes
Hydrogenated squalenes	*Botryococcus braunii* race L (?)
Fernenes	*Azolla microphylla*, ferns, bacteria
$C_{25:1}$ HBIs	Diatoms
Mid-chain Me-*br* alkanes (C_{21}–C_{25})	Insect cuticules
Hopanoids	Bacteria
Alkyl esters	Copepods or bacteria?
Dinosterols/dinostanols	Dinoflagellate
n-Alkanols and *n*-alkenols	Higher plants and microalgae/copepods, respectively
Long chain mid-chain diols and mid-chain hydroxy acids	Microalgae (Eustigmatophytes? Diatoms?), *Azolla microphylla*
Long chain mid-chain keto-ols and keto acids	Biotic oxidation of diols and hydroxy acids, respectively.
ω-Hydroxy acids	Higher plant cutins, microalgae
(ω-1)-Hydroxy acids	Methylotrophic bacteria, cyanobacteria

variety of sources could contribute long mid-chain diols to freshwater sediment, the co-occurrence of both diols and triols as well as mono-unsaturated diols seems to indicate that freshwater eustigmatophytes are likely to be the major source.

Vicinal diols with 27–29 carbon atoms and hydroxyl groups at ω9 and ω10 are most likely sourced from *Azolla*, a floating fern, whose fossil remains are abundant in the sediments of El Junco Lake. *Azolla* is also the main source of 20,21-dihydroxy-nonacosanoic acid and ω20-hydroxy acids. On the other hand, ω16 and ω18 hydroxy acids and diols imply a microalgal source.

The long, mid-chain keto-ols and keto acids likely to derive from biotic oxidation of diols and hydroxy acids, respectively, given their similarities in chain length and the position of functional groups (hydroxyl group and keto group position in the middle of a chain).

4. Conclusions

Sediments from El Junco Lake contain a rich variety of unusual lipids. Long chain *n*-alkan-1-ols, alken-1-ols, mid-chain terminal diols and unsaturated diols, vicinal diols and triols, and keto-ols occurred together in the three Holocene sediment intervals investigated. Long chain C_{30}–C_{36} diols with one terminal hydroxyl group and one hydroxyl group at ω15-ω19 are likely from microalgae, as are the mono-unsaturated C_{30} and C_{32} diols. Vicinal diols with two hydroxyl groups at ω9 and ω10, together with one C_{29} triol indicate that they are probably derived from the aquatic fern *Azolla*. C_{30}–C_{36} keto-ols in surface and deep sediments may be derived from living organisms or biotic oxidation.

In addition to those biomarkers featured by hydroxyl groups, a series of acids, including *n*-fatty acids, hydroxy acids and keto acids also co-occurred in El Junco sediments. Similar to those in diols, hydroxyl groups in C_{30}–C_{36} hydroxy acids occurred at the ω16 and ω18 positions in some sedimentary intervals, and at ω20 in other intervals. The dominance of ω20-hydroxy acids and diols at 200–201 cm is indicative of substantial lipid contribution from *Azolla*, corroborated by the presence of 20,21-dihydroxy-nonacosanoic acid. By contrast, the predominance of ω16 and ω18 in the hydroxyl acids and the diols in the deeper portion of the core supports a microalgal origin. Long chain (ω-1)-hydoxy acids (C_{26}–C_{34}) could be the signature of methylotrophic bacteria,

symbiotic *Rhizobium* and/or cyanobacteria. C_{28}, C_{30} and C_{32} (ω-1)-keto acids most likely originated from the biotic oxidation of the corresponding (ω-1)-hydroxy acids. The discovery of long chain keto acids with the oxo-group in the mid-chain has not previously been reported.

Acknowledgments

This material is based upon work supported by the US National Science Foundation under Grants ESH-0639640 (J.S.) and EAR-0823503 (J.S.); by the US National Oceanic and Atmospheric Administration under Grant NA08OAR4310685 (J.S.); by the Gary Comer Science and Education Foundation (J.S.); and by the Chinese National Science Foundation under Grants NSFC 40873056 and 40973052 (Z.Z.). We are grateful to Drs. Vincent Grossi, John Volkman and an anonymous reviewer for their thoughtful reviews. We acknowledge Rienk Smittenberg, Paul Colinvaux, Jonathan Overpeck, Miriam Steinitz-Kannan, and Jessica Conroy for assistance in the field. We thank the Galápagos National Park and the Charles Darwin Foundation for their assistance with fieldwork and for their permission to work on El Junco Lake.

Associate Editor—**John Volkman**

References

Ageta, H., Arai, Y., 1983. Fern constituents: pentacyclic triterpenoids isolated from *Polypodium niponicum* and *P. formosanum*. Phytochemistry 22, 1801–1808.

Alarie, Y., Joly, H., Dennie, D., 1998. Cuticular hydrocarbon analysis of the aquatic beetle *Agabus anthracinus* Mannerheim (Coleoptera: Dytiscidae). The Canadian Entomologist 130, 615–629.

Allard, B., Templier, J., 2000. Comparison of neutral lipid profile of various trilaminar outer cell wall (TLS)-containing microalgae with emphasis on algaenan occurrence. Phytochemistry 54, 369–380.

Allard, B., Templier, J., 2001. High molecular weight lipids from the trilaminar outer wall (TLS)-containing microalgae *Chlorella emersonii*, *Scenedesmus communis* and *Tetrahedron minimum*. Phytochemistry 57, 459–467.

Audino, M., Grice, K., Alexander, R., Boreham, C.J., Kagi, R.I., 2001. Unusual distribution of monomethylalkanes in *Botryococcus braunii*-rich samples: origin and significance. Geochimica et Cosmochimica Acta 65, 1995–2006.

Balascio, N.L., Zhang, Z.H., Bradley, R.S., Perren, B., Dahl, S.O., Bakke, J., 2011. A multi-proxy approach to assessing isolation basin stratigraphy from the Lofoten Islands, Norway. Quaternary Research 75, 288–300.

Barakat, A.O., Peakman, T.M., Rullkötter, J., 1994. Isolation and structural characterization of 10-oxo-octadecanoic acid in some lacustrine sediments from the Nördlinger Ries (southern Germany). Organic Geochemistry 21, 841–847.

Belt, S.T., Massé, G., Allard, W.G., Robert, J.M., Rowland, S.J., 2001. Identification of a C_{25} highly branched isoprenoid triene in the freshwater diatom *Navicula sclesvicensis*. Organic Geochemistry 32, 1169–1172.

Belt, S.T., Masse, G., Rowland, S.J., Poulin, M., Michel, C., LeBlanc, B., 2007. A novel chemical fossil of palaeo sea ice: IP25. Organic Geochemistry 38, 16–27.

Blokker, P., Schouten, S., van den Ende, H., de Leeuw, J.W., Sinninghe Damsté, J.S., 1998. Cell wall-specific ω-hydroxy fatty acids in some freshwater green microalgae. Phytochemistry 49, 691–695.

Boon, J.J., Rijpstra, W.I.C., de Lange, F., de Leeuw, J.W., Yoshioka, M., Shimizu, Y., 1979. Black Sea sterol – a molecular fossil for dinoflagellate blooms. Nature 277, 125–127.

Cavaletto, J.F., Vanderploeg, H.A., Gardner, W.S., 1989. Wax esters in two species of freshwater zooplankton. Limnology and Oceanography 34, 785–789.

Cranwell, P.A., 1983. Alkyl esters in recent sediments of two productive lakes. In: Bjorøy, M. et al. (Eds.), Advances in Organic Geochemistry 1981. Wiley, Chichester, pp. 299–308.

Cranwell, P.A., Eglinton, G., Robinson, N., 1987. Lipids of aquatic organisms as potential contributors to lacustrine sediments—II. Organic Geochemistry 11, 513–527.

Cranwell, P.A., Creighton, M.E., Jaworski, G.H.M., 1988. Lipids of four species of freshwater chrysophytes. Phytochemistry 27, 1053–1059.

Cranwell, P.A., Jaworski, G.H.M., Bickley, H.M., 1990. Hydrocarbons, sterols, esters and fatty acids in six freshwater chlorophytes. Phytochemistry 29, 145–151.

de Leeuw, J.W., 1986. Higher-molecular-weight markers. In: Johns, R.B. (Ed.), Biological Markers in the Sedimentary Record. Elsevier, Amsterdam, pp. 249–260.

de Leeuw, J.W., Rijpstra, W.I.C., Schenck, P.A., 1981. The occurrence and identification of C_{30}, C_{31}, C_{32} alkan-1,15-diols and alkan-15-one-1-ols in Unit I and Unit II Black Sea sediments. Geochimica et Cosmochimica Acta 45, 2281–2285.

de Mesmay, R., Grossi, V., Williamson, D., Kajula, S., Derenne, S., 2007. Novel mono-, di- and tri-unsaturated very long chain (C_{37}–C_{43}) *n*-alkenes in alkenone-free lacustrine sediments (Lake Masoko, Tanzania). Organic Geochemistry 38, 323–333.

Declerck, P., Behets, J., van Hoef, V., Ollevier, F., 2007. Detection of *Legionella* spp. and some of their amoeba hosts in floating biofilms from anthropogenic and natural aquatic environments. Water Research 41, 3159–3167.

Dunlop, R.W., Jefferies, P.R., 1985. Hydrocarbons of the hypersaline basins of Shark Bay, Western Australia. Organic Geochemistry 8, 313–320.

Eglinton, G., Hunneman, D.H., Douraghi-Zadeh, K., 1968. Gas chromatographic–mass spectrometric studies of long chain hydroxy acids. II. The hydroxy acids and fatty acids of a 5000-year-old lacustrine sediment. Tetrahedron 24, 5929–5941.

Evans, R.W., Kushawa, S.C., Kates, M., 1980. The lipids of *Halobacterium marismortiu*, an extremely halophilic bacterium in the Dead Sea. Biochimica et Biophysica Acta 619, 533–544.

Ferrante, G., Ekiel, I., Sprott, G.D., 1986. Structural characterization of the lipids of *Methanococcus voltae*, including a novel *N*-acetylglucosamine 1-phosphate diether. The Journal of Biological Chemistry 261, 17062–17066.

Ferreira, A.M., Miranda, A., Caetano, M., Baas, M., Vale, C., Sinninghe Damsté, J.S., 2001. Formation of mid-chain alkane keto-ols by post-depositional oxidation of mid-chain diols in Mediterranean sapropels. Organic Geochemistry 32, 271–276.

Fixter, L.M., Nagi, M.N., McCormack, J.G., Fewson, C.A., 1986. Structure, distribution and function of wax esters in *Acinetobacter calcoaceticus*. Journal of General Microbiology 132, 3147–3157.

Fukushima, K., Yasukawa, M., Muto, N., Uemura, H., Ishiwatari, R., 1992. Formation of C_{20} isoprenoid thiophenes in modern sediments. Organic Geochemistry 18, 83–91.

Gaskell, S.J., Eglinton, G., 1975. Rapid hydrogenation of sterols in a contemporary lacustrine sediment. Nature 254, 209–211.

Gelin, F., Volkman, J.K., de Leeuw, J.W., Sinninghe Damsté, J.S., 1997. Mid-chain hydroxy long-chain fatty acids in microalgae from the genus *Nannochloropsis*. Phytochemistry 45, 641–646.

Gil-Serrano, A.M., González-Jiménez, I., Tejero-Mateo, P., Megías, M., Romero-Vázquez, M.J., 1994. Analysis of the lipid moiety of lipopolysaccharide from *Rhizobium tropici* CIAT899: identification of 29-hydroxytriacontanoic acid. Journal of Bacteriology 176, 2454–2457.

Grossi, V., Raphel, D., 2003. Long-chain (C_{19}–C_{29}) 1-chloro- *n*-alkanes in leaf waxes of halophytes of the Chenopodiaceae. Phytochemistry 63, 693–698.

Gupta, M.M., Shukla, Y.N., Lal, R.N., 1983. Oxo fatty acid esters from *Cryptocoryne spiralis* rhizomes. Phytochemistry 22, 1969–1971.

Gupta, M.M., Verma, R.K., Akhila, A., 1986. Oxo acids and branched fatty acid esters from rhizomes of *Costus speciosus*. Phytochemistry 25, 1899–1902.

Han, J., Calvin, M., 1970. Branched alkanes from blue-green algae. Journal of the Chemical Society Chemical Communication, 1490–1491.

Haugh, P., Schnoes, H.K., Burlingame, A.L., 1971. Studies of the acidic components of the Colorado Green River Formation oil shale: mass spectrometric identification of the methyl esters of extractable acids. Chemical Geology 7, 213–236.

Hird, S.J., Rowland, S.J., 1995. An investigation of the sources and seasonal variations of highly branched isoprenoid hydrocarbons in intertidal sediments of the Tamar Estuary, UK. Marine Environmental Research 40, 423–437.

Hollingsworth, R.I., Carlson, R.W., 1989. 27-Hydroxyoctacosanoic acid is a major structural fatty acyl component of the lipopolysaccharide of *Rhizobium trifolii* ANU843. Journal of Biological Chemistry 264, 9300–9303.

Jansen, B., Nierop, K.G.J., Hageman, J.A., Cleef, A.M., Verstraten, J.M., 2006. The straight-chain lipid biomarker composition of plant species responsible for the dominant biomass production along two altitudinal transects in the Ecuadorian Andes. Organic Geochemistry 37, 1514–1536.

Jeng, W.-L., Huh, C.-A., 2004. Lipids in suspended matter and sediments from the East China Sea Shelf. Organic Geochemistry 35, 647–660.

Jetter, R., 2000. Long-chain alkanediols from *Myricaria germanica* leaf cuticular waxes. Phytochemistry 55, 169–176.

Jetter, R., Riederer, M., 1999. Long-chain alkanediols, ketoaldehydes, ketoalcohols and ketoalkyl esters in the cuticular waxes of *Osmunda regalis* fronds. Phytochemistry 52, 907–915.

Jetter, R., Riederer, M., Seyer, A., Mioskowski, C., 1996. Homologous long-chain alkanediols from *Papaver* leaf cuticular waxes. Phytochemistry 42, 1617–1620.

Kaneda, T., 1977. Fatty acids of the genus *Bacillus*: an example of branched-chain preference. Bacteriological Review 41, 391–418.

Kenig, F., Sinninghe Damsté, J.S., Kock-VanDalen, A.C., Rijpstra, W.I.C., Huc, A.Y., De Leeuw, J.W., 1995. Occurrence and origin of mono-, di-, and trimethylalkanes in modern and Holocene cyanobacterial mats from Abu Dhabi, United Arab Emirates. Geochimica et Cosmochimica Acta 59, 2999–3015.

Makula, R., Lockwood, P., Finnerty, W., 1975. Comparative analysis of the lipids of *Acinetobacter* species grown on hexadecane. Canadian Journal of Microbiology 121, 250–258.

Méjanelle, L., Sanchez-Gargallo, A., Bentaleb, I., Grimalt, J.O., 2003. Long chain *n*-alkyl diols, hydroxy ketones and sterols in a marine eustigmatophyte, *Nannochloropsis gaditana*, and in *Brachionus plicatilis* feeding on the algae. Organic Geochemistry 34, 527–538.

Moll, H., Sonesson, A., Jantzen, E., Marre, R., Zähringer, U., 1992. Identification of 27-oxo-octacosanoic acid and heptacosane-1,27-dioic acid in *Legionella pneumophila*. FEMS Microbiology Letters 97, 1–6.

Morris, R.J., Brassell, S.C., 1988. Long-chain alkanediols: biological markers for cyanobacterial contributions to sediments. Lipids 23, 256–285.

Neises, B., Steglich, W., 1978. Simple method for the esterification of carboxylic acids. Angewandte Chemie International Edition 17, 522–524.
Nelson, D.R., Blomquist, G.J., 1995. Insect waxes. In: Hamilton, R.J. (Ed.), Waxes: Chemistry, Molecular Biology and Functions. The Oily Press, Dundee, pp. 1–129.
Nichols, P.D., Smith, G.A., Antworth, L.P., Hanson, R.S., White, D.C., 1985. Phospholipid and lipopolysaccharide normal and hydroxy fatty acids as potential signatures for methane-oxidizing bacteria. FEMS Microbiology Ecology 31, 327–335.
Robinson, N., Cranwell, P.A., Finlay, B.J., Eglinton, G., 1984. Lipids of aquatic organisms as potential contributors to lacustrine sediments. Organic Geochemistry 6, 143–152.
Robinson, N., Cranwell, P.A., Eglinton, G., Brassell, S.C., Sharp, C.L., Gophen, M., Pollingher, U., 1986. Lipid geochemistry of Lake Kinneret. Organic Geochemistry 10, 733–742.
Robinson, N., Cranwell, P.A., Eglinton, G., Jaworski, G.H.M., 1987. Lipids of four species of freshwater dinoflagellates. Phytochemistry 26, 411–421.
Rowland, S.J., Robson, J.N., 1990. The widespread occurrence of highly branched acyclic C_{20}, C_{25} and C_{30} hydrocarbons in recent sediments and biota – a review. Marine Environmental Research 30, 191–216.
Rowland, S., Rockey, C., Allihaibi, S.S., Wolff, G.A., 1993. Incorporation of sulfur into phytol derivatives during simulated early diagenesis. Organic Geochemistry 20, 1–5.
Saïd, I., Costagliola, G., Leoncini, I., Rivault, C., 2005. Cuticular hydrocarbon profiles and aggregation in four *Periplaneta* species (Insecta: Dictyoptera). Journal of Insect Physiology 51, 995–1003.
Schofield, E.K., Colinvaux, P.A., 1969. Fossil *Azolla* from the Galapagos Islands. Bulletin of the Torey Botanical Club 96, 623–628.
Senousy, H.H., Beakes, G.W., Hack, E., 2004. Phylogenetic placement of *Botryococcus braunii* (Trebouxiophyceae) and *Botryococcus sudeticus* isolate UTEX 2629 (Chlorophyceae). Journal of Phycology 40, 412–423.
Shiea, J., Brassell, S.C., Ward, D.M., 1990. Mid-chain branched mono- and dimethyl alkanes in hot spring cyanobacterial mats: a direct biogenic source for branched alkanes in ancient sediments? Organic Geochemistry 15, 223–231.
Shimokawara, M., Nishimura, M., Matsuda, T., Akiyama, N., Kawai, T., 2010. Bound forms, compositional features, major sources and diagenesis of long chain alkyl mid-chain diols in Lake Baikal sediments over the past 28,000 years. Organic Geochemistry 41, 753–766.
Sinninghe Damsté, J.S., Rampen, S., Rijpstra, W.I.C., Abbas, B., Muyzer, G., Schouten, S., 2003. A diatomaceous origin for long-chain diols and mid-chain hydroxy methyl alkanoates widely occurring in Quaternary marine sediments: indicators for high-nutrient conditions. Geochimica et Cosmochimica Acta 67, 1339–1348.
Skerratt, J.H., Nichols, P.D., Bowman, J.P., Sly, L.I., 1992. Occurrence and significance of long chain (ω-1)-hydroxyacids in methane-utilizing bacteria. Organic Geochemistry 18, 189–194.
Smith, D.J., Eglinton, G., Morris, R.J., Poutanen, E.L., 1983a. Aspect of the steroid geochemistry of an interfacial sediment from the Peruvian upwelling. Oceanologica Acta 6, 211–219.
Smith, D.J., Eglinton, G., Morris, 1983b. Occurrence of long-chain alkan-diols and alkan-15-one-1-ols in a Quaternary sapropel from the Eastern Mediterranean. Lipids 18, 902–905.
Speelman, E.N., Reichart, G.-J., de Leeuw, J.W., Rijpstra, W.I., Sinninghe Damsté, J.S., 2009. Biomarker lipids of the freshwater fern *Azolla* and its fossil counterpart from the Eocene Arctic Ocean. Organic Geochemistry 40, 628–637.
Steinitz-Kannan, M., Riedinger, M.A., Last, W., Brenner, M., Miller, M.C., 1998. A 6000-year record of intense evidence of El Niño from sediments in a Galápagos Islands lake. Bulletin de l'Institut Français d'Etudes Andines 27, 581–592 (in Spanish with English abstract).
Stiehl, T., Rullkötter, J., Nissenbaum, A., 2005. Molecular and isotopic characterization of lipids in cultured halophilic microorganisms from the Dead Sea and comparison with the sediment record of this hypersaline lake. Organic Geochemistry 36, 1242–1251.
Theissen, K.M., Zinniker, D.A., Moldowan, J.M., Dunbar, R.B., Rowe, H.D., 2005. Pronounced occurrence of long-chain alkenones and dinosterol in a 25,000-year lipid molecular fossil record from Lake Titicaca, South America. Geochimica et Cosmochimica Acta 69, 623–626.
Urakami, T., Komagata, K., 1987. Cellular fatty acid composition with special reference to the existence of hydroxy fatty acids in Gram-negative methanol-, methane-, and methylamine-utilizing bacteria. Journal of General and Applied Microbiology 33, 135–165.
van Bergen, P.F., Bull, I.D., Poulton, P.R., Evershed, R.P., 1997. Organic geochemical studies of soils from the Rothamsted Classical Experiments—I. Total lipids extracts, solvent insoluble residues and humic acid from Broadbalk Wilderness. Organic Geochemistry 26, 117–135.
Versteegh, G.J.M., Bosch, H.J., de Leeuw, J.W., 1997. Potential palaeoenvironmental information of C_{24} to C_{36} mid-chain diols, keto-ols and mid-chain hydroxy fatty acids; a critical review. Organic Geochemistry 27, 1–13.
Volkman, J.K., Gatten, R.R., Sargent, J.R., 1980. Composition and origin of milky water in the North Sea. Journal of the Marine Biological Association UK 60, 759–768.
Volkman, J.K., Allen, D.I., Stevenson, P.L., Burton, H.R., 1986. Bacterial and algal hydrocarbons in sediments from a saline Antarctic lake, Ace Lake. Organic Geochemistry 10, 671–681.
Volkman, J.K., Kearney, P., Jeffrey, S.W., 1990. A new source of 4-methyl sterols and 5α(H)-stanols in sediments: prymnesiophyte microalgae of the genus *Pavlova*. Organic Geochemistry 15, 489–497.
Volkman, J.K., Barrett, S.M., Dunstan, G.A., Jeffrey, S.W., 1992. C_{30}–C_{32} alkyl diols and unsaturated alcohols in microalgae of the class Eustigmatophyceae. Organic Geochemistry 18, 131–138.
Volkman, J.K., Barrett, S.M., Dunstan, G.A., Jeffrey, S.W., 1993. Geochemical significance of the occurrence of dinosterol and other 4-methyl sterols in a marine diatom. Organic Geochemistry 20, 7–15.
Volkman, J.K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L., Gelin, F., 1998. Microalgal biomarkers: a review of recent research developments. Organic Geochemistry 29, 1163–1179.
Volkman, J.K., Barrett, S.M., Blackburn, S.I., 1999a. Eustigmatophyte microalgae are potential sources of C_{29} sterols, C_{22}–C_{28} *n*-alcohols and C_{28}–C_{32} *n*-alkyl diols in freshwater environments. Organic Geochemistry 30, 307–318.
Volkman, J.K., Barrett, S.M., Blackburn, S.I., 1999b. Fatty acids and hydroxy fatty acids in three species of freshwater Eustigmatophytes. Journal of Phycology 35, 1005–1012.
Weete, J.D., 1976. Algal and fungal waxes. In: Kolattukudy, P.E. (Ed.), Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam, pp. 349–418.
Wen, M., Buschhaus, C., Jetter, R., 2006. Nanotubules on plant surfaces: chemical composition of epicuticular wax crystals on needles of *Taxus baccata* L. Phytochemistry 67, 1808–1817.
Wheeler, M.A., Holmlund, C.E., 1975. Identification of wax esters in *Tetrahymena pyriformis*. Lipids 10, 260–262.
Xu, Y.P., Jaffé, R., Wachnicka, A., Gaiser, E.E., 2006. Occurrence of C_{25} highly branched isoprenoids (HBIs) in Florida Bay: paleoenvironmental indicators of diatom-derived organic matter inputs. Organic Geochemistry 37, 847–859.
Xu, Y.P., Simoneit, B.R.T., Jaffé, R., 2007. Occurrence of long chain *n*-alkenols, diols, keto-ols and *sec*-alkanols in a sediment core from a hypereutrophic, freshwater lake. Organic Geochemistry 38, 870–883.
Zhang, Z.H., Sachs, J.P., 2007. Hydrogen isotope fractionation in freshwater algae: I. Variations among lipids and species. Organic Geochemistry 38, 582–608.
Zhang, Z.H., Zhao, M., Yang, X., Wang, S., Jiang, X., Oldfield, F., Eglinton, G., 2004. A hydrocarbon biomarker record for the last 40 kyr of plant input to Lake Heqing, southwestern China. Organic Geochemistry 35, 595–613.
Zhang, Z.H., Metzger, P., Sachs, J.P., 2007. Biomarker evidence for the co-occurrence of three races (A, B and L) of *Botryococcus braunii* in El Junco Lake, Galápagos. Organic Geochemistry 38, 1459–1478.