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Composition and early diagenesis of fatty acids in lacustrine sediments, lake Aydat (France)

Maya Stefanova, Jean Robert Disnar*

UMR 6531 & FR 09 du CNRS, Université d'Orléans, Bâtiment Géosciences, BP 6759, 45067 Orléans Cedex 02, France

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Abstract

The organic acids from recent sediments from lake Aydat, a small eutrophic lake located in the French Massif Central, have been analysed to determine their possible source(s) and their fate during early diagenesis. The analytical procedure yields three groups of acids — free, H^+-labile and OH^--labile — , successively obtained by solvent extraction, acid hydrolysis and saponification in toluene, in the presence of a phase transfer catalyst (PTC). The free components are relatively minor except for the presence of amounts of ββ-hopanoic acids ($C_{32} > > C_{30}$). The H^+-labile components, assumed to be mostly of bacterial origin, disappear very rapidly at depth. In contrast, notable proportions of OH^--labile compounds, mostly derived from algae and terrestrial plants, survive in the deepest samples analysed, i.e. >40 cm. The various fatty acids (FA) identified comprise normal saturated FA (with a strong C_{20}- dominance), monounsaturated FA (*cis* and *trans* $C_{16:1}$, $C_{18:1}$, $C_{24:1}$ with ω5, ω7 and ω9 double bond position), branched (*iso*, *anteiso*) FA, 3-hydroxy FA and 3-methoxy FA, α,ω- dicarboxylic acids, as well as hopanoic acids and a few lignin-derived aromatic acids. A notable part of the most unusual of these various compound types are classically attributed to methanotrophs, which implies that markers from other micro-organisms are already destroyed in the water column. A distinctive acid composition at 20 cm depth might be explained by intensive microbial activity induced by unusual climatic conditions (i.e. an exceptional drought).

Keywords: Eutrophic lake; Lacustrine sediment; Fatty acid; Early diagenesis

1. Introduction

Molecular studies on recent and ancient sediments provide information on the origins of organic matter and the depositional conditions at the time of incorporation into the sediment (Meyers and Ishiwatari, 1993a,b, 1995). Among the various organic constituents that can be analysed, fatty acids (FA) have frequently been considered because they have been proven to be biomolecules that frequently survive the transformations that occur during diagenesis.

Isolation of FA can be carried out by different treatments to yield various compound classes that have distinctive compositions (Barnes and Barnes, 1978; Nishimura and Baker, 1987). Usually, most attention is paid to the 'bound' lipids classically released by saponication. However, various authors have pointed out that more detailed information can be obtained by the study of the various pools of FA that can be released successively by different chemical treatments. According to Barnes and Barnes (1978) and Albaigés et al. (1984), hydrolysis releases acids sorbed to clays, trapped in occlusions and bound to humates and fulvates by covalent linkages. In addition, it is well-known that FA exist under different types of chemical combinations in living organisms. For example, the cell walls of gram-negative bacteria contain both ester- and amide-linked hydroxyacids that are most effectively released by either saponification or acid hydrolysis, respectively (Rietschel et al., 1972). Mendoza et al. (1987a,b) applied a multi-step extraction with acid and alkaline solutions of increasing strength for

* Corresponding author.

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'bound' (i.e. unsubstituted and β-hydroxy-) FA isolation from recent lacustrine sediments. Goossens et al. (1989a,b) applied a three step extraction procedure to obtain 'free' extractable, OH^-- labile and H^+- labile FA, first from a gram-negative bacterium and second, from a lake sediment. Fukushima et al. (1992a,b) combined acid digestion and saponification to extract hydroxy- and other FA from recent sediments. More recently Zegouagh et al. (1996) obtained five distinct fractions from Arctic surface sediments. The most original points of the approach developed by these authors are most probably (i) the adoption of the saponification with KOH/phase transfer catalyst (PTC) previously applied to kerogen analysis (Amblès et al., 1987; Baudet et al., 1993; Kribii, 1994) and (ii) the application of a pyrolysis technique to complete the whole extraction procedure.

Aydat, a small lake located in the French Massif Central, has previously given rise to various studies mostly dealing with the biology (Amblard, 1988; Aleya and Devaux, 1989) and chemistry (Sarazin and Devaux, 1991; Sarazin et al., 1992,1995) of the water column, in connection to eutrophication. The present work was done as part of an extensive study of the reactions and element transfer processes that occur at or near the water-sediment interface (DBT-INSU project "Sédiments continentaux actuels"). We report here the detailed analysis of the free and bound organic acids in the upper sediment levels of the lake that was done to determine the molecular compositions of micro-organisms involved in early diagenetic transformations and to evaluate the extent of organic matter diagenesis.

2. Materials and analytical methods

2.1. Study site

Aydat lake is located at an altitude of 850 m, about 25 km NW from Clermont-Ferrand, in the French Massif Central. It has a surface area of 0.6 km^2, an average depth of 7 m and a maximum depth of 15 m (Sarazin and Devaux, 1991; Albéric et al., 1996). The lake originates from the damming of a small river, La Veyre, by a basaltic flow 7000 years ago.

The lake, which is eutrophic, has an anoxic hypolimnion from May until October during the period of water stratification and seasonal algal production. Maximum water stratification occurs in September with an oxycline at 4–5 m below the surface. In contrast to the water column, the sediment is permanently anoxic.

The algal production is dominated by diatoms (*Fragillaria crotonensis* and *Melosira italica*) and cyanobacteria (*Anabaena* and *Microcystis*) (Aleya and Devaux, 1989; Philippe, 1989). The occurrence of a given species at a certain period of the anoxia may change from year to year but those cited remain the main ones (Aleya and Devaux, 1989). Organic matter mineralization occurs mainly through methane fermentation (ca. 97%; Sarazin et al., 1995). The sedimentation rate, which was estimated at about 5 mm per year from ^{210}Pb determinations (Sarazin et al., 1992), is presently thought to be higher, perhaps as great as 1 cm per year (see Section 4).

2.2. Core extraction

A sediment core (60 mm diameter and about 40 cm in length) was obtained in autumn 1996 from the deeper part of the lake by a diver using a plastic corer. This operation was done very carefully in order to prevent any disturbance of the upper part of the sedimentary column where the porosity is very high (98% at the sediment surface; 92% at 40 cm depth). The core was extruded immediately from the sleeve and split in 2 cm thick slices. Bulk samples were weighed, frozen for transportation and freeze-dried when back in the laboratory (Sarazin et al., 1995).

Most of the inorganic material is amorphous silica of diatom origin (i.e. 70–80% SiO_2) (Sarazin and Devaux, 1991).

2.3. Sequential lipid extraction

Isolation of 'free' extractable lipids — The lyophilised sediment samples (1–2 g) were soaked overnight in acetone-pentane (1:1), with a 1:50 w/v sample:solvent ratio. The mixture was sonicated 2×5 min at ambient temperature. The extracts were separated by filtration and the residues were carefully washed with acetone:pentane. The extract and the washes were combined and the solvent was eliminated under vacuum.

Isolation of H^+-labile lipids — The solid residues from the previous treatment step were transferred to 25 ml Pyrex® tubes. Methanol (10 ml) and 3N HCl (10 ml) were added. The tubes were tightly closed under vacuum and left overnight in an oven at $80 \pm 2°C$.

The insoluble residues were isolated by filtration and washed with distilled water. The filtrates were combined, concentrated under reduced pressure to about 10 ml and extracted with diethyl ether (2×20 ml). The soluble portions were dried with anhydrous Na_2SO_4, filtered and finally concentrated for subsequent chromatographic separation.

Isolation of OH^--labile lipids — The solid residues of the previous treatment were transferred to Pyrex tubes in which they were saponified with KOH in the presence of 'crown' ether 18-C-6. The complex 18-C-6/KOH was prepared according to Weber and Gokel (1977). The complex 0.02 M KOH/18-C-6 was dissolved and stored in toluene. The residues of sediment samples were suspended in 10 ml of the complex in Pyrex tubes. The

mixtures were shaken vigorously, closed and left for 48 h at 110°C in an oven. After cooling, the reaction products were neutralised with dilute HCl solution. The insoluble residue was separated by filtration and washed with water and diethyl ether. The organic phase was separated from the aqueous solution. The latter was extracted with diethyl ether until colourless. Then, the organic phases were combined, dried and concentrated under reduced pressure.

Fatty acid analysis — All the lipid extracts were separated into neutral and acidic portions by column chromatography on KOH- impregnated silica gel columns (McCarthy and Duthie, 1962). The acidic fractions, eluted by diethyl ether-HCOOH (9:1, v/v) were concentrated and esterified by diazomethane (Lombardi, 1990) prior to GC and GC–MS.

The methylated fractions were analysed by means of a DI 700 Delsi® gas chromatograph equipped with a CP sil 8 CB column (0.22 mm×30 m; 0.25 μm film thickness), a flame ionisation detector (300°C), and a split/splitless capillary injector maintained at 300°C and used in the splitless mode (valve reopened 1 min after injection). After 1 min hold at 50°C the oven temperature was increased from 50 to 120°C at 30°C/min, and then from 120 to 300° at 5°/min, maintaining this final temperature for a period of 1 hour. The carrier gas was helium.

Gas chromatography–mass spectrometry (GC–MS) analyses were performed with a Varian 300 chromatograph connected to an Ion Trap ITD 800 (Finnigan Mat) by a 2m capillary interface heated to 300°C. Other GC conditions were identical to those described above. Operating conditions for the Ion Trap were — temperature: 220°C; ionization energy: 80 eV; 1 scan/ 2s, from 50 to 500 amu.

Determination of double bond position by DMDS derivatisation, was realized according to Scribe et al. (1988). Briefly, aliquots of FA methyl esters were dissolved in heptane and introduced in Pyrex® tubes. Then, 100 μl of dimethyldisulphide (DMDS) and 20 μl of iodine solution in diethyl ether (60 mg I_2 for 1 ml of ether) were added. The tubes were tightly closed and heated for 48 h at 50°C. Thereafter, 200 μl heptane and 200 μl of a 5% $Na_2S_2O_3$ aqueous solution of were added. After a vigorous shaking, the organic phases were collected and concentrated under nitrogen flow. The reaction products were analysed by GC–MS using SIM at m/z 362, 390 and 474.

3. Results

The three distinguished lipid fractions are denoted 'free', 'OH^--(PTC)-labile' (or simply 'OH^--labile') and 'H^+-labile', respectively. The main quantitative data, including TOC contents, are presented in Table 1. TOC values first decrease sharply from more than 10% at the top of the core to less than 8% in the immediate underlying level. This downward decrease goes on but more smoothly to reach values of ca. 4% TOC at about 40 cm depth. In Fig. 1 are presented the yields of the 'free' extractable, H^+- and OH^--labile gross extracts. The amounts of free components decrease regularly with increasing depth, while the other two fractions mostly show a very sharp drop at about 7 cm depth.

An example of a GC trace showing the peaks of most of the various compounds analysed is presented in Fig. 2. The main compounds identified by mass-spectrometry are listed in Table 2; selected structures are shown in the

Table 1
Sample bulk characteristics, amounts of extractable fractions and contents in saturated and unsaturated fatty acids

No	Depth (cm)	TOC (%)	Extractable fractions (mg/g)[a]			Fatty acid contents (μg/g)[b]					
			free	H^+-labile	OH^--labile	free		H^+-labile		OH^--labile	
						Sat.	Unsat.	Sat.	Unsat.	Sat.	Unsat.
1	0–2	11.54	3.30	8.62	8.60	96.44	136.60	366.68	32.40	484.00	72.0
2	−4	7.99	2.45	8.39	7.60	54.92	96.92	286.56	27.60	360.00	64.0
3	−6	6.50	1.92	7.77	8.00	44.68	68.84	129.20	20.80	348.00	20.8
4	−8	6.65	2.15	4.30	3.80	29.36	38.32	21.80	4.80	200.00	33.2
5	−10	6.62	2.15	4.75	4.20	18.68	39.04	21.68	–	196.00	52.8
6	−16	6.30	1.60	4.70	3.80	15.48	9.76	14.64	–	161.20	19.2
7	−20	n.d.[c]	1.50	4.87	4.50	18.24	13.76	50.80	–	154.40	24.0
8	−24	6.25	1.70	4.59	3.70	16.52	19.00	24.32	–	80.80	16.8
9	−28	6.13	1.12	4.52	3.40	15.32	3.04	13.36	–	92.40	16.4
10	−32	n.d.	1.00	5.00	3.40	10.52	–	16.92	–	107.20	12.8
11	−36	6.12	0.77	4.36	3.30	4.56	–	11.60	–	116.00	16.0
12	−40	4.63	20.26	3.88	n.d	2.96	–	5.32	–	n.d.	n.d.
13	−42	4.58	0.18	3.83	3.50	3.76	–	10.20	–	90.40	8.4

[a] Isolated after KOH/SiO_2 separation.
[b] After GC analysis.
[c] n.d. = Not determined.

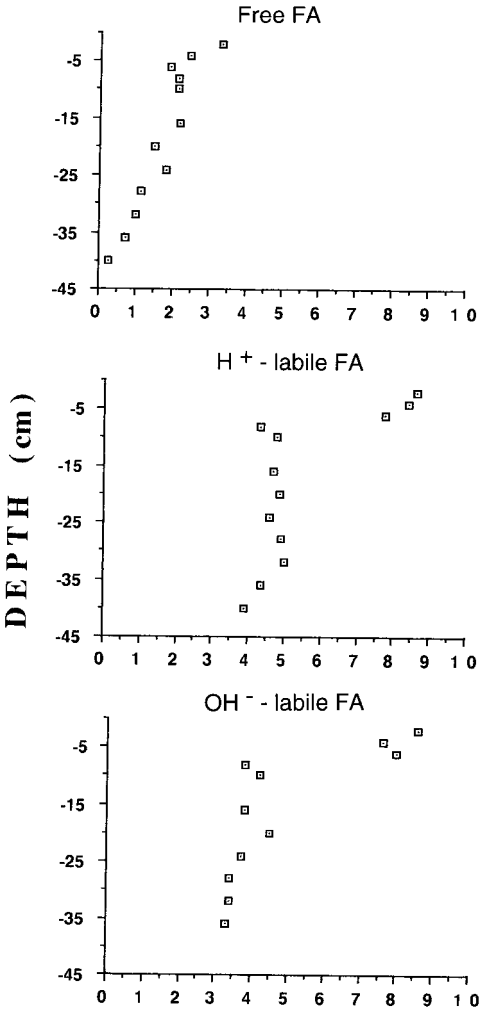


Fig. 1. Yields of lipid extracts in relation to depth: (a) 'free' extractable lipids; (b) H^+-labile lipids; (c) OH^--labile lipids.

Appendix. The main quantitative information of the nature and distribution of the various compound types that are found in the three different fractions is summarized in Table 3. The amounts of normal saturated and monounsaturated FA that dominate other compound classes (branched, hydroxy-, methoxy-FA; Fig. 1) are presented vs depth in Fig. 3. Only OH^--(PTC)-labile saturated components remain at non negligible levels below 10 cm depth; both the free and H^+-labile saturated and unsaturated components disappear rapidly at shallow depth. Saturated FA distributions, normalized to *n*-C_{16} contents, are presented as histograms in Fig. 4. Classically, these saturated FA distributions are strongly dominated by even-carbon numbered compounds. In addition, except at 20 cm depth, the lighter *n*-$C_{16:0}$ and *n*-$C_{18:0}$ always strongly dominate their higher C_{20+} homologues. *n*-C_{16} and *n*-$C_{16:1}$, which constitute the dominant compound pair in the 'free' extractable lipids, both decrease rapidly with depth (Fig. 5), but the *n*-$C_{16:1}$ more rapidly than the *n*-C_{16}. *n*-$C_{16:1}$ also disappears more rapidly than its higher homologue *n*-$C_{18:1}$ (result not shown).

Results on stereoisomer and double bond determination for compounds under study are presented in Table 4. The DMDS derivatization procedure was initially claimed to be suitable for homologues up to C_{20+}; higher members being assumed to be too thermally labile or of insufficient volatility (Scribe et al., 1988). However, we were pleasantly surprised to detect *cis* and *trans* $C_{24:1}\omega 9$ isomers (Fig. 6).

The dependence of FA yields on extraction treatment and depth of burial is illustrated for three samples taken from the top, the middle and the base of the core, respectively (i.e., 2, 20 and 42 cm depth; Fig. 7).

One of the most obvious changes, depicted by the C_{20+}/C_{20-} ratio profiles presented in Fig. 8, deals with the much higher abundance of the saturated C_{20+} FA in the free and H^+-labile fraction of the 20 cm sample compared to the OH^--labile fraction of the same sample as well as to all the other fractions from the other samples.

The absolute and relative amounts of the less common FA compound types found in Lake Aydat sediments can be appreciated through the data on the OH^--labile midcore C_{20-} FA presented in Fig. 9. According to that example, branched (*iso* and *anteiso*) and 3-OH^-FA occur in significant amounts (ca. 8 $\mu g\, g^{-1}$ individual compounds basis) while the 3-MeOH^-FA and the dicarboxylic acids are only subordinate. A further insight into the variation with depth of the composition of the OH^--labile fraction (the only one which remains significant at depth), shows that *n*-FA are always strongly dominant and represent 60–80% of the total. The relative proportions of the other compound types mostly vary between 5 and 15% for unsaturated fatty acids, between 5–10% for *iso*-FA and for 3-hydroxyfatty and between ca. 2–6% for *anteiso*-FA. The proportions of unsaturated fatty acids tend to decrease with increasing depth while the highest proportions of *iso* - acids occur in midcore extracts. There is no clear trend for other compound types.

4. Discussion

The sequential extracts represent three different pools of sedimentary lipids: (i) easily extractable 'free' compounds plus (ii) amide and (iii) ester combined forms for the acid and alkaline treatments, respectively. While the absolute and relative proportions of the different FA pools vary considerably with depth (Table 1; Fig. 7), many of the various compound identified occur in the three fractions (Table 3).

4.1. Unsaturated fatty acids

No polyunsaturated FA (PUFAs) have been detected in the samples studied. Linear monounsaturated FA

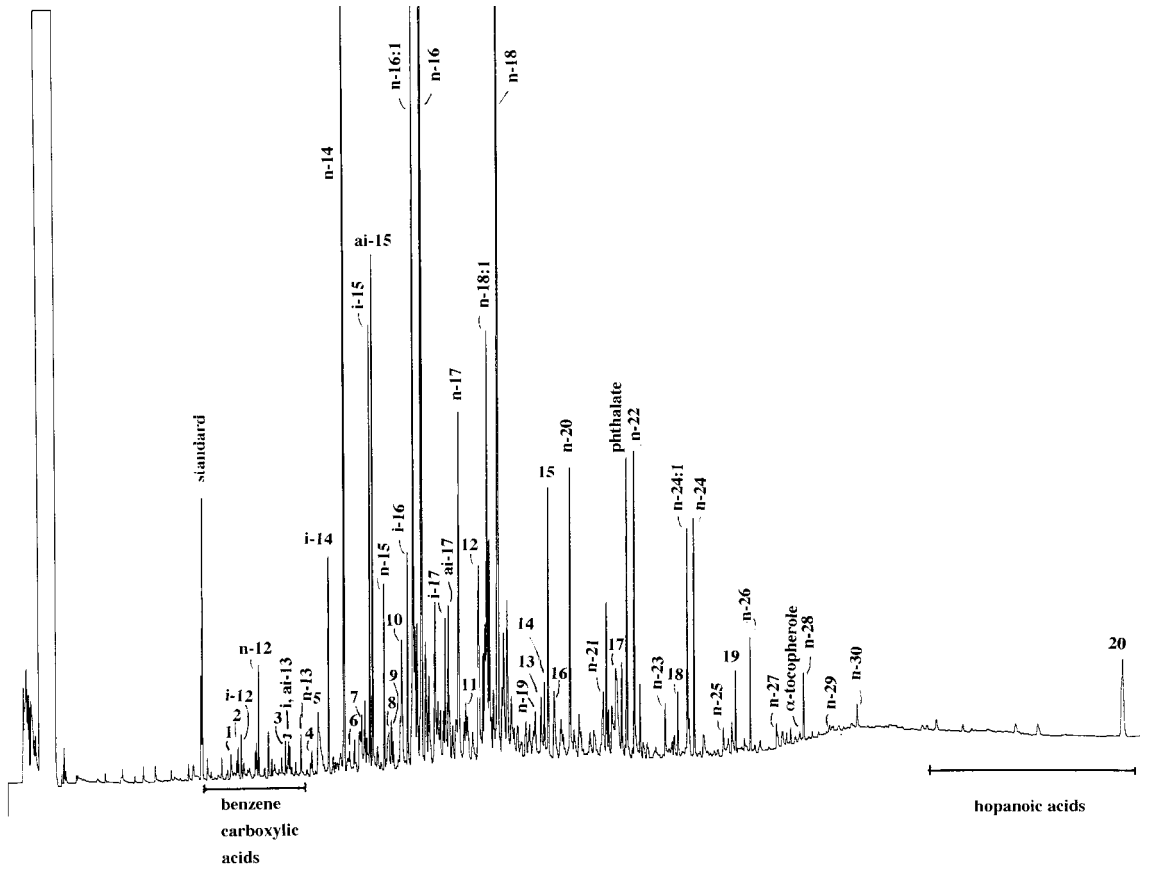


Fig. 2. GC trace of fatty acid methyl esters (FAME) in the OH^--labile acid fraction of a midcore sample (peak identifications in Table 2).

dominate the 'free' lipid distributions from the upper levels of the sedimentary record (Fig. 5). In these levels, the *n*-$C_{16:1}$ and *n*-$C_{18:1}$ on the one hand and the *n*-$C_{24:1}$ on the other hand dominate the lighter (C_{20-}) and heavier (C_{20+}) ends, respectively. These compounds also dominate unsaturated acid distributions from the free fractions from the lower sedimentary levels as well as all the extracts obtained from the H^+ and OH^- fractions (Table 3). As illustrated in Fig. 5 for *n*-$C_{16:1}$ and its saturated counterpart *n*-C_{16}, the unsaturated compounds disappear more rapidly than the saturates. However, neither the *n*-C_{16} nor the *n*-$C_{16:1}$ remain at very significant levels below ca. 10 cm depth. In addition, the lighter monounsaturated FA also disappear more rapidly than their heavier homologues. The same observations hold for the "bound" acids as well (data not shown).

Because of both their ubiquity in the biological realm and their labile character, unsaturated FA are of limited value as source indicators. As with saturated FA and *n*-alkanes, the longer chain members (C_{20+}) are contributed by higher plants (e.g. Cranwell, 1974) whereas the short chain homologues (C_{20-}) usually indicate autochthonous algal and bacterial sources (Holton and Blecker, 1970; Brooks et al., 1976). Double bond position and stereochemistry determination allow some further differentiation of the potential sources. As a matter of fact, the high predominance of *cis* $C_{16:1}\omega7$ and *cis* $C_{18:1}\omega9$ observed in all the studied samples is consistent with the abundance of these two compounds in many organisms: phytoplankton, zooplankton, fungi, mycobacteria and higher plants (Matsuda and Koyama, 1977; Volkman et al., 1980; Volkman, 1986). However, contrary to oleic acid, *cis* $C_{18:1}\omega9$ which is the most abundant unsaturated acid in our downcore sample and which is also dominant in many common organisms (e.g. diatoms; Volkman, 1986), vaccenic acid, *cis* $C_{18:1}\omega7$ is an indicator for bacterial input (Matsuda and Koyama, 1977; Sicre et al., 1988). In our samples, vaccenic acid *cis* $C_{18:1}\omega7$ is not present in 'free' fractions, only traces of the *trans* stereoisomer being observed. In contrast, it is released in notable amounts by the acidic and alkaline treatment as expressed by $C_{18:1}\omega9$/*cis* $C_{18:1}\omega7$ ratio values of 1.3 and 2.2 for H^+-labile and OH^--labile FA, respectively. *Trans* stereoisomers of most of the unsaturated FA have also been observed in all fractions (Table 4). Even in the free fraction, *trans*- $C_{18:1}\omega7$ and *trans*- $C_{24:1}\omega9$ were determined (e.g., in the free fraction of the upper sample,

Table 2
Compounds identified by GC–MS

No	Compound	MW	Formula	Number in Appendix
1	Benzoic acid, 4-hydroxy, methyl ester	152	$C_8H_8O_3$	**I**
2	Benzoic acid, 4-hydroxy-3-methoxy, methyl ester	182	$C_9H_{10}O_4$	**II**
3	Benzoic acid, 3,4-dimethoxy, methyl ester	196	$C_{10}H_{12}O_4$	**III**
4	Benxoic acid, 2,4-dihydroxy-3,5,6-trimethyl, methyl ester	210	$C_{11}H_{14}O_4$	**IV**
5	3-OH-C_{12}, methyl ester	230	$C_{13}H_{26}O_3$	
6	2-propenoic acid, 3-(4-hydroxy-3-methoxyphenyl), methyl ester	208	$C_{11}H_{12}O_4$	**V**
7	Benzoic acid, 3,4,5-trimethoxy, methyl ester	226	$C_{11}H_{14}O_5$	**VI**
8	2-propenoic acid 3-(3,4-dimethoxy phenyl), methyl ester	222	$C_{12}H_{14}O_4$	**VII**
9	3-OCH_3-C_{14}, methyl ester	272	$C_{16}H_{32}O_3$	
10	3-OH-C_{14}, methyl ester	258	$C_{15}H_{30}O_3$	
11	3-OCH_3-C_{16}, methyl ester	300	$C_{18}H_{36}O_3$	
12	3-OH-C_{16}, methyl ester	286	$C_{17}H_{34}O_3$	
13	3-OCH_3-C_{18} methyl ester	328	$C_{20}H_{40}O_3$	
14	α,ω-C_{16}, dimethyl ester	314	$C_{18}H_{34}O_4$	
15	Dehydroabietic acid, methyl ester	314	$C_{21}H_{30}O_2$	**VIII**
16	3-OH-C_{18}, methyl ester	314	$C_{19}H_{38}O_3$	
17	α,ω-C_{18}, dimethyl ester	342	$C_{20}H_{38}O_4$	
18	α,ω-C_{20} dimethyl ester	370	$C_{22}H_{42}O_4$	
19	α,ω-C_{22}, dimethyl ester	398	$C_{24}H_{46}O_4$	
20	17β(H),21β(H)-bishomohopanoic acid, methyl ester	484	$C_{33}H_{56}O_2$	**IX**

Table 3
Acid series distributions: carbon range (and dominant compounds)

Acids	Free	H+ labile	OH − labile	Content μg g^{-1}
n-Saturated	*n*C_{14}-*n*C_{28} (*n*C_{14}, *n*C_{16})	*n*C_{12}–*n*C_{30} (*n*C_{16})	*n*C_{12}-*n*C_{30} (*n*C_{16})	see Fig. 4
n-Monounsaturated (even numbered)	*n*$C_{16:1}$, *n*$C_{18:1}$, *n*$C_{24;1}$	*n*$C_{16:1}$, *n*$C_{18:1}$ *n*$C_{24:1}$	*n*$C_{16:1}$, *n*$C_{18:1}$, *n*$C_{24:1}$	see Fig. 5
Branched	Trace	*i*-C_{15}, *ai*-C_{15} *i*-C_{17}, *ai*-C_{17} (*i*-C_{15})	*i*-C_{13}, *ai*-C_{13} *i*-C_{15}, *ai*-C_{15} *i*-C_{17}, *ai*-C_{17} *i*-C_{16},*i*-C_{18} (*ai*-C_{15})	see Fig. 9
3-OH Saturated	–	3-OH-C_{12} 3-OH-C_{18} (3-OH-C_{16})	3-OH-c_{12} 3-OH-C_{18} (3-OH-C_{16})	see Fig. 9
3-OCH_3 Saturated		Trace	3-OCH_3-C_{12}-C_{18} (3-OCH_3-C_{16})	see Fig. 9
α,ω- Dicarboxylic, (even numbered)	Trace	α,ω-C_{16}-α,ω-C_{22} (α,ω)-C_{16})	α,ω-C_{16}-α,ω-C_{22} (α,ω-C_{16})	see Fig. 9
17β(H),21β(H) Hopanoic	ββC_{30} ββC_{32} (ββC_{32})	ββC_{30}-ββC_{32} (ββC_{32})	ββC_{30}-ββC_{32} (ββC_{32})	0.0–5.0
Dehydroabietic	Trace	Trace	+	2.0–5.0
Benzene carboxylic, (hydroxy-/methoxy-)	–	C_7–C_{10} (C_8)	Trace	0.8–2.0
2-propenoic acid, 3-phenyl		C_{10}, C_{11}	Trace	0.4–0.8

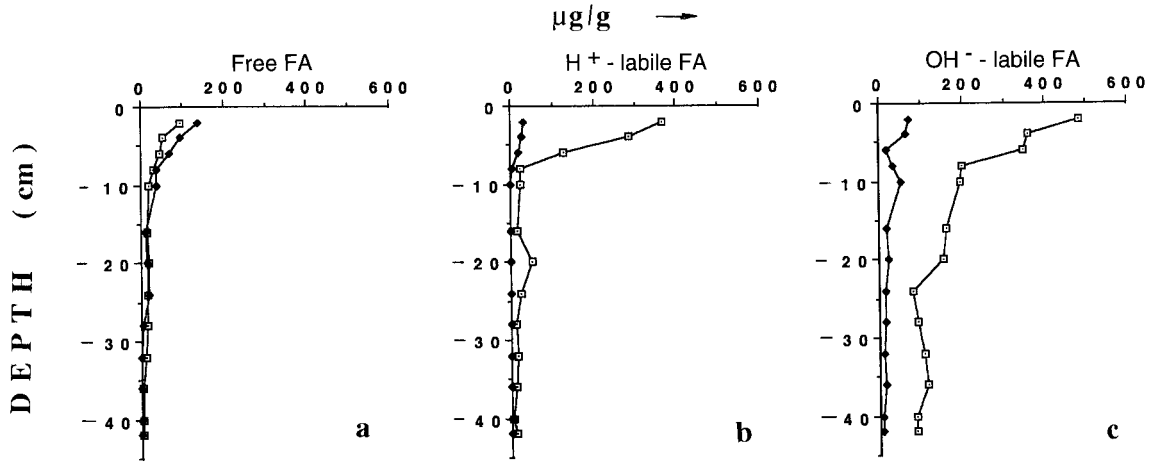


Fig. 3. Yields of saturated (□) and monounsaturated (◆) fatty acids in lipid extracts downcore correlated.

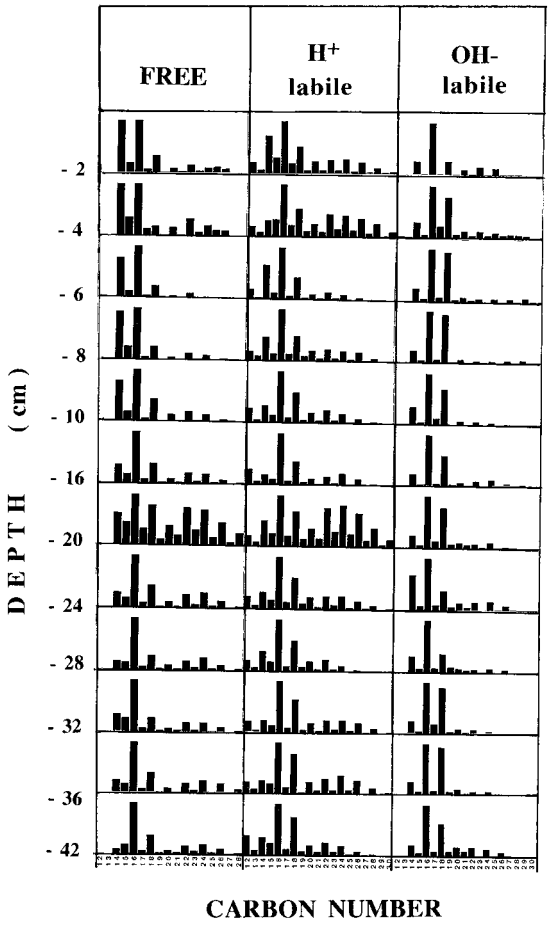


Fig. 4. Histograms of *n*-fatty acids distributions normalized to $C_{16:0}$.

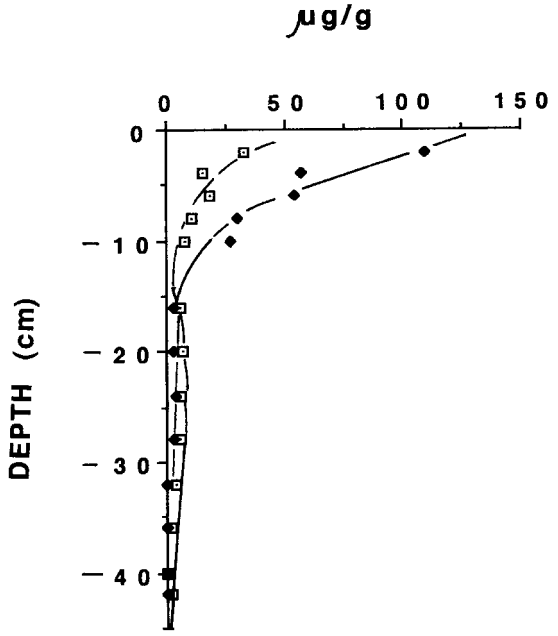


Fig. 5. Variation with depth of the amounts of $C_{16:1}$ (◆) and $C_{16:0}$ (□) in the 'free' fractions.

the $C_{24:1}\omega9$ *cis*/*trans* ratio is ca. 1.5; no *cis* $C_{18:1}\omega7$ was found). *Trans* unsaturated acids could originate from direct microbial input or from microbial modification of pre-existing sedimentary FA or even, from clay catalysed isomerisation (Perry et al., 1979). In this respect, the possibility that a high quantity of *trans* monounsaturated FA could be typical for methane-utilising bacteria (Gillan and Johns, 1986), might very well apply to Aydat lake which is a site with considerable methane production and consumption (Sarazin et al., 1995).

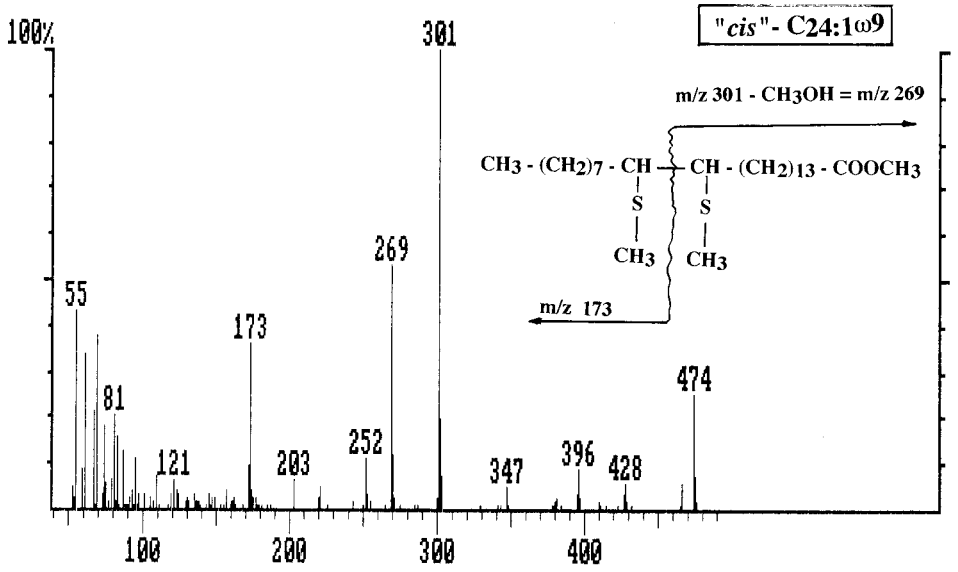


Fig. 6. MS spectrum of DMDS adduct of $C_{24:1}\omega9$ FAME.

For $C_{24:1}\omega9$, only the *cis* stereoisomer was detected in 'bound' lipids. Heavier (C_{20+}) monoenoic acids with a double bond at Δ^{13} or Δ^{15} position are thought to be derived from yeast and, in addition, Δ^{9} $C_{18:1}$ presence might explain Δ^{15} $C_{24:1}$ occurrence by possible chain elongation (Boon et al., 1978).

4.2. Saturated fatty acids

The FA distributions, dominated by shorter-chain members (C_{20-}), are typical for eutrophic and mesotrophic lakes because of the important contribution of plankton-derived short chain lipids (Cranwell, 1978; Robinson et al., 1984; Cranwell et al., 1987; Kawamura et al., 1987). The 'free' lipids of our uppermost samples (Fig. 4), the least affected by diagenetic tranformations, display (i) a preponderance of the *n*-$C_{14:0}$ and *n*-$C_{16:0}$, and (ii) *n*-$C_{16:1}/C_{16:0}$ ratio values of 1.5–3.5, two features that are characteristic for diatoms (Boon et al., 1975), organisms which play a pre-eminent role in organic production in Lake Aydat.

High proportions of longer chain FA classically assumed to represent terrestrial input, are recognised in the 'free' extractable and H^+-labile lipid fractions at 16–20 cm depth. This preponderance results in a steep enhancement in the calculated C_{20+}/C_{20-} ratio, which thus become higher than unity (Fig. 8). No such terrestrial contribution was detected for OH^--labile lipids at the same level. This discrepancy fully illustrates the fact that the various treatment steps effectively release different pools of FA.

Table 4
Distributions of monounsaturated acids in the three separated fractions

Fraction	*n*-$C_{16:1}$	*n*-$C_{18:1}$	*n*-$C_{24:1}$
"free"	*cis*-$C_{16:1}\omega7$	*cis*-$C_{18:1}\omega9$ *trans*-$C_{18:1}\omega7$	*cis*-$C_{24:1}\omega9$ *trans*-$C_{24:1}\omega9$
H^+-labile	*cis*-$C_{16:1}\omega7$ *trans*-$C_{16:1}\omega7$ *cis*-$C_{16:1}\omega5$ *trans*-$C_{16:1}\omega5$	*cis*-$C_{18:1}\omega9$ *cis*-$C_{18:1}\omega7$	*cis*-$C_{24:1}\omega9$
OH^--labile	*cis*-$C_{16:1}\omega7$ *trans*-$C_{16:1}\omega7$ *cis*-$C_{16:1}\omega5$ *trans*-$C_{16:1}\omega5$	*cis*-$C_{18:1}\omega9$ *cis*-$C_{18:1}\omega7$ *trans*-$C_{18:1}\omega7$	*cis*-$C_{24:1}\omega9$

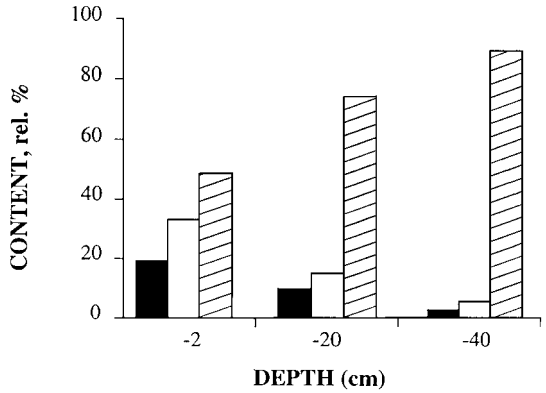


Fig. 7. Relative amounts (%wt) of 'free' and 'bound' lipid extracts in up- mid- and downcore samples; Black: 'free' extractable lipids; white: H^+-labile lipids; hatched: OH^--labile lipids.

4.3. Branched chain fatty acids

Because of microbially mediated diagenesis of labile organic matter in sediments, the proportion of bound lipids increases with depth. Moreover, 'bound' acids (H^+ and OH^--labile) are progressively enriched in bacterially altered components. According to Cranwell (1973, 1984) and Huang et al. (1996) a mark for intensive bacterial activity is the increase in (*iso*+*anteiso*)-C_{15}/*n*-C_{15} ratio values. Ratios lower than 1 in 'free' extractable lipids correspond to low relative amounts of branched *iso*- and *anteiso*-C_{15} acids. In contrast, the enrichment of bacterial constituents leads to ratio values in the 2–3 range for H^+-labile acids and up to 4–5 for OH^-- labile lipids.

In productive lakes *iso*- and *anteiso*-C_{15} acids are dominant over their linear isomer, with the *anteiso*- homologue more abundant (Cranwell, 1973). Such features are effectively observed in Lake Aydat sediments (Figs. 2 and 9). An increase in *iso* - acids occurs in midcore extracts of OH^--labile components and the highest values (>5) are reached at this depth. The diagenetic transformation of biolipids into 'geo' lipids is normally accompanied by an increase in the relative abundance of branched chain FA, an indication that microbial activity has participated. However, such a diagenetic trend is not always observed, variations in depositional or environmental lacustrine conditions evidently provoking fluctuations in the rate of early diagenesis (Ho and Meyers, 1994). No clear trend is observed in our extracts.

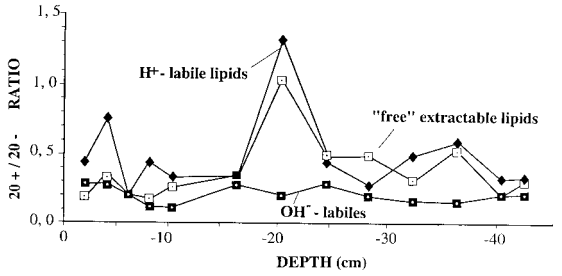


Fig. 8. Variation with depth of the $20^+/20^-$ *n*-fatty acid ratio.

4.4. Hydroxy and methoxy fatty acids

Hydroxy fatty acids are ubiquitous in sediments, and they are generally considered as meaningful source indicators. 3-OH^-FA have been found in various microorganisms such as bacteria, yeasts and fungi (Matsumoto and Nagashima, 1984). However, such compounds especially occur in gram-negative bacteria (Boon et al. 1977; Cranwell, 1981; Goossens et al. 1989a; Fukushima et al., 1992a,b). In gram-negative bacteria, 3-OH^-FA more specifically occur in cell-wall lipopolysaccharides (LPS) where they are linked to the NH_2 and OH groups of D-glucosamine disaccharide units by amide and ester bonds, respectively (Rietschel et al., 1972). In as much as amides are much more difficult to hydrolyse than esters, sequential hydrolysis is commonly used to differentiate acids linked by either of these two functional groups (Mendoza et al., 1987a,b; Fukushima et al., 1992a,b). In our study 3-OH^-FA were not detected in the 'free' fractions but were present in notable amounts among the "bound" acids (Table 3; Figs. 2 and 9). The 3-OH^-FA distributions are dominated by even homologues and maximise at 3-OH-C_{16}. No heavy compounds, i.e. C_{18+}, were found. In the GC–MS chromatograms of 3-OH-FA (SIM *m*/*z* 103) a small quantity of unsaturated homologues were detected, i.e. 3-OH-$_{16:1}$ and 3-OH-$_{18:1}$. *Iso*- and *anteiso*-3-OH-FA C_{15} and C_{17} were identified as well.

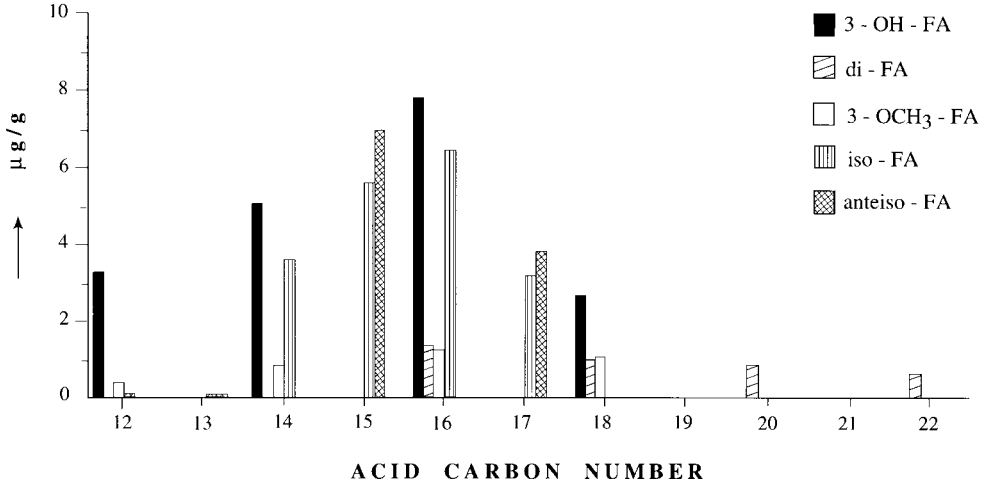


Fig. 9. Relative amounts of *iso*- FA, *anteiso*- FA, 3-OH-FA, 3-OCH_3-FA and α,ω- dicarboxylic acids in midcore extract of OH^--labile acids.

Fukushima et al. (1992b) have determined a high quantity of 3-OH-FA in estuarine and coastal marine sediments. 2- and 3-OH-FA have been reported in diverse sediment types such as diatomaceous oozes (Boon et al., 1977), lacustrine sediments (Cardoso and Eglinton, 1983) and cyanobacterial mats (Cardoso et al., 1975). These investigators have related them to microbial input, a conclusion that is supported by GC separation of R diastereoisomers attributed to microbial cell wall lipids (Cranwell, 1981).

Methanotrophic bacteria are a common source of 3-OH-FA (Skerratt et al., 1992; Huang et al., 1996). As mentioned above, methanotrophs effectively play a considerable role in Lake Aydat organic matter mineralisation and thus constitute a highly probable source of 3-OH-FA. Another confirmation of methanotrophic bacteria contribution is the presence of hopanoid acids, recently shown to have typically low $\delta^{13}C$ ratio values (Huang et al., 1996). These compounds, which are present at rather low levels (i.e., ca. 3–5 $\mu g\ g^{-1}$), display an increase in our midcore extracts and especially at 20 cm depth where the maximum amounts are reached thus denoting that intensive microbial activity has occurred (see Section 5).

A peculiar series of FA detected in OH^--labile extracts exists as methoxylated FA, homologues (3-OCH_3-FA)(Fig. 9; Table 3). Their GC–MS distribution parallels that of the 3-OH-FA, but they occur in ten-fold lower quantities. Mendoza et al., (1987b) have detected 3-OCH_3-FA in carboxylic acid fractions released from recent sediments by methanolic saponification. However, they demonstrated that these compounds were simple artefacts resulting from the reaction of MeOH with naturally occurring β-acyloxy FA esters. We similarly found 3-OCH_3-FA in fractions obtained by saponification, but this treatment was here carried out in a methanol-free medium. There remains the possibility that the reaction with methanol occurred during the previous acid hydrolysis realised in water/methanol (50:50 v/v). However, if this explanation is right, the methoxylated compounds, while not natural, might nevertheless have some value as molecular tracers derived from very specific precursors such as the β-acyloxyesters mentioned above. Further work is needed to fully determine the origin and significance of these compounds.

4.5. Dicarboxylic acids

Even carbon numbered α,ω,w-saturated dicarboxylic acids in the range C_{16} to C_{22}, are detected in notable amounts in "bound" extracts and in traces in 'free' extractable lipids (Table 3). Three sources are proposed to explain the existence of these compounds: (i) terrestrial higher plant input, (ii) water grasses, (iii) in situ formation from ω-OH-FA or even FA by oxidation (Volkman et al. 1980; Kawamura et al., 1987). The lack of ω-OH- and (ω-1)-OH-FA, the typical constituents of cutin and suberin, makes a terrestrial origin for the dicarboxylic acids improbable. The presence in diatoms of small amounts of diacids with only slight even-carbon atom predominance suggests a probable contribution from this source.

4.6. Benzene carboxylic acids

A suite of benzene, hydroxy/methoxy acids and 2-propenoic acid, 3-(4-hydroxy/methoxy phenyl) exists in H^+-labile lipid extracts. In conjunction with dehydroabietic acid (Table 3), these compounds are unequivocal indications of terrestrial contribution since they obviously derive from lignin. As a matter of fact the compounds listed in Table 2 can be grouped in vanillyl (V) and cinnamyl (C) structures, typical for lignin (Maman et al., 1996). The lack of syringyl acids' usually interpreted as an evidence that lignin derives from non-woody gymnosperm/angiosperm tissues (Hedges and Mann, 1979), is here to be considered very carefully because the studied compounds were not obtained by classical CuO oxidation. Despite this limitation, our data are in a good agreement with those obtained by Maman et al., (1996) and Maman (1997), who showed that CuO oxidation products from Lake Aydat sediments derived from non-woody tissues of monocotyledons.

5. General considerations

Unlike previous work (Goossens et al., 1989b; Zegouah et al., 1996; other refs in Mendoza et al., 1987a), we applied the acid treatment step first for two main reasons: (i) we assumed acid hydrolysis with dilute acid solution to be less drastic than saponification, especially when carried out in presence of a phase transfer catalyst; (ii) the acidic treatment applied to release bacterial components was supposed to be harmless to silica diatomaceous tests and thus, might avoid extensive alteration of their algal organic matter content. The first of these two statements is consistent with the observation by Mendoza et al. (1987b) that large proportions of hydroxyacid which can be released by acid hydrolysis are destroyed by alkali. One point in favour of the acidic treatment before saponification is that it obviously releases — and thus also reveals — a pool of compounds mostly or even totally of microbial origin that are very labile in as much as they disappear very rapidly during early diagenesis (Fig. 3).

From the data obtained from the upper sediment samples, it appears that the sum of the three overall acid fractions isolated from the sequential treatment accounts for less than 20% of the TOC. Despite the three-step procedure and the use of the PTC catalyst, the amounts of well-identified organic acids account for less than 1% of the TOC, or more exactly 0.87% of TOC

in the upper sediment level and ca. 0.2% of TOC at depth. These very low amounts of compounds that are usually considered to exist in combinations that make them resistant to biodegradation, illustrate the efficiency of the organic carbon recycling in Lake Aydat. Moreover, comparison of TOC values up to ca. 25% found in sediments trapped at the base of the photic zone during the period of biological production (Albéric, unpublished data) with the 10% TOC found in the surface bottom sediments (Table 1) provides clear evidence that a large part of the mineralisation of the primary production occurs during settling, even in shallow and anoxic waters.

Polyunsaturated FA, which have been found in settling particles (Bourdier and Debroas, pers. comm.), completely disappear before reaching the floor of the lake. Other diagenetic changes that occur in the sediment are fully consistent with now classical observations (e.g., Haddad et al., 1992; Canuel and Martens, 1996), mainly: (i) monounsaturated compounds disappear more rapidly than their saturated counterparts, and (ii) lighter ends more rapidly than their heavier homologues.

The considerable diagenetic changes that occur within the upper few centimetres of the sediment are well documented by the almost complete disappearance of FA released by acid hydrolysis (Fig. 3), assumed to have a predominant or even exclusive bacterial origin. A different evolution trend is depicted by the OH^--labile components assumed to be mostly derived from ester-bound fatty acids from algae and/or terrestrial higher plants. The amounts of these compounds display an exponential-like decrease with increasing depth much like the TOC profile. The preservation of notable proportions of OH^--(PTC)-labile FA in the lower sediment levels, is consistent with the now well-known refractory character of the biological polymer-like constituents from which these FA are released (Largeau et al., 1986; Derenne et al., 1989). The general correspondence of the trend of the OH^--(PTC)-labile FA and of the TOC results in a more or less constant contribution of organic acids to the sedimentary organic matter in the downcore sequence. This apparent constancy, added to that of the C_{20-}/C_{20+} alkanoic acid ratio in the chemical fraction considered, suggests that the autochthonous (algal) and allochthonous (terrestrial) contributions to the sediment have not undergone significant proportional change during the deposition of these sediments.

The almost complete disappearance of the FA released by acid hydrolysis from 6–8 cm depth can be interpreted in two non-mutually exclusive ways: (i) the rapid consumption of remains of bacteria that lived in the water column (and thus contributed to the authochtonous input) and/or (ii) the rapid decrease of in situ bacterial activity. We favor the first hypothesis or, more explicitly, we believe that the observed decrease corresponds to the bacterial consumption of sedimented bacterial remains. Whatsoever, the organic acid fraction released by acid hydrolysis represents a pool of very labile components, especially when compared to those liberated by saponification.

The sample taken at 20 cm depth differs from the upper and lower levels by the presence of relatively high proportions of C_{20+} even alkanoic acids, especially in the H^+-labile fraction and also in the corresponding free fraction. These features are most intriguing, since C_{20+} fatty acids dominated by n-$C_{24:0}$ or n-$C_{26:0}$ are classically assumed to be derived from cuticular waxes of higher plants and should be released by saponification rather than by acid hydrolysis. This observation leads us to hypothesize that these heavy compounds might have an unusual source, e.g. from algae. This latter possibility is strengthed by the presence, at 20 cm depth, of remains of alga Staurastrum (F. Laggoun-Défarge, pers. comm.), which were not found in any other level examined. The unusual presence of this alga in Lake Aydat was noted in 1976 (Bourdier, pers. comm.), a year memorably hot and dry. If this assumption is right, it indicates that the sedimentation rate would be of the order of 1 cm yr^{-1}. Previous estimates from ^{210}Pb radioactive disequilibrium measurements led to a value of 0.46 or 0.9 cm yr^{-1} depending on the interpretative model used (linear or non-linear fitting of ages vs depth; Sarazin et al., 1992). The authors of this earlier work favor the lower estimate, but recent sediment flux calculations are in much better agreement with a value of ca. 1 cm yr^{-1} (Michard, pers. comm.). This hypothesis is presently under examination.

Finally, it can also be supposed that exceptional climatic or other environmental conditions might have triggered the settling of an unsual microbial community. In a recent work, Gong and Hollander (1997) assume that microbes can produce long chain FA identical to those usually attributed to higher plants. Actually, the fact that the maximum amounts (ca. 5 μg g^{-1}) of hopanoic acids also occur at 20 cm depth strengthen the possibility that high microbial activity occurred at that level.

In our discussion, many of the less common organic acids identified i.e. branched, hydroxylated, methoxylated FA and hopanoids, have been attributed to methanotrophic bacteria by reference to previous work. Even if the eutrophic Lake Aydat is the site for important methane production and consumption, methanotrophs constitute only one of the various microbial communities that contribute to the lacustrine food chain. Methanotrophs utilise methane produced by methanogens, which themselves require other substrates (acetate, molecular hydrogen, carbon dioxide and/or other compounds such as trimethylamine), produced by different genera of fermentative bacteria (Marty et al., 1989; Botz et al., 1996). The apparent absence of molecular diversity consistent with the large spectrum of microorganisms which might have contributed to biological production and consumption, might be explained by the following reasons: (i) some bacteria might not produce distinctive FA (e.g.

methanogen lipids are mainly synthesized from long chain alcohols connected by ether bonds and were not sought in the present study, Ourisson et al., 1984); (ii) distinctive markers of given species might have been replaced by subsequent biomarkers. The rapid disappearance of the bacterial acids released by acid hydrolysis as well as independant indications of extensive biodegradation in the water column strongly support the second hypothesis without totally excluding the first one. This interpretation is also supported by the possibility of inhibition of the growth and respiration of gram-positive bacteria by antibiotic substances released by algae during blooming (Barnes and Barnes, 1978). Methanotrophic bacteria, which are gram-negative, might have been extremely abundant under such conditions and could have produced hopanoic acids, 3-OH-FA/3-OCH_3-FA by active reworking of plankton biomass, and especially saturated and unsaturated fatty acids.

6. Conclusions

In the present study a 'reverse' sequential extraction scheme of lipids was applied; acid attack preceding saponification in organic medium in the presence of a phase transfer catalyst. In addition to free acids, this stepwise treatment releases acids bound in various types of chemical combinations, amides and esters principally. Despite this rather aggressive treatment, the total amounts of FA extracted still only represent relatively small portions of TOC, e.g. 0.87% of TOC in the upper core samples and ca. 0.2% of TOC at depth.

The free components comprise only simple normal saturated and monosaturated FA, which disappear rapidly at depth, plus hopanoic acids strongly dominated by the C_{32} component. The two bound acid fractions additionally comprise branched and 3-OH FA, diacids, plus lignin-derived aromatic acids for the H^+-labile fraction and 3-methoxy-unsaturated FA for the OH^--labile one. Further work is needed to determine if the latter compounds are natural or simple artifacts. In contrast to the ester-derived OH^--labile compounds, which survive in notable proportions at depth, the H^+-labile acids, thought to derive predominantly from bacterial amides, disappear very rapidly in the sediment.

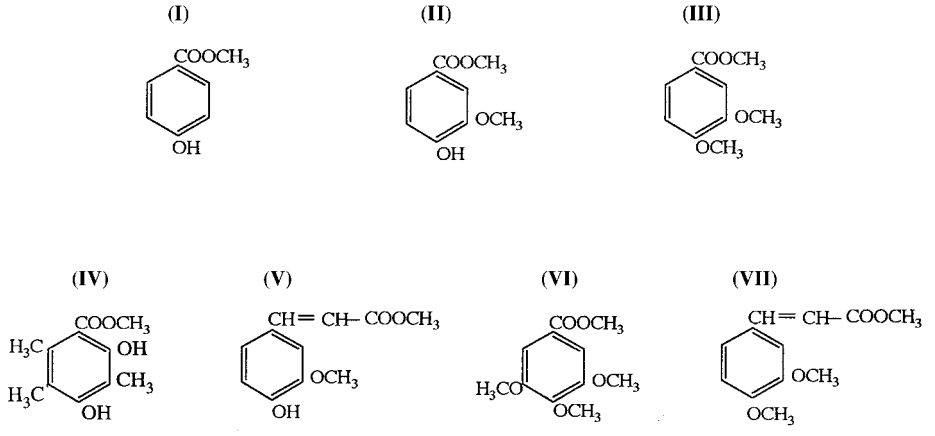
Most of the most unusual fatty acids, i.e. branched, 3-OH-, 3-MeOH-, plus the hopanoids might derive from methane-utilizing bacteria. Like algal polyunsaturated fatty acids (PUFAs), which disappear in the water column, some bacterial markers, especially from methanogens, might have also been destroyed soon after being produced during settling.

Even in conditions of high autochthonous biological production, anoxic waters and shallow depth, only limited amounts of planktonic lipids reach the bottom of the lake. Notable proportions of those latter ones disappear in the upper centimetres of the sediment.

A minor contribution from the watershed is revealed by low relative amounts of C_{20+} normal saturated FA classically attributed to higher plant wax esters and by the presence of few lignin-derived aromatic acids.

Associate Editor—**J.O. Grimalt**

Appendix



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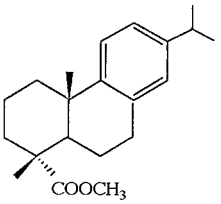

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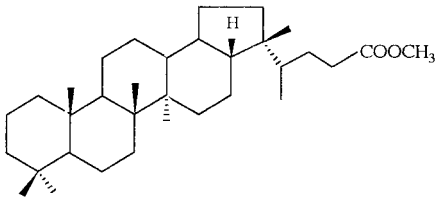

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