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GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC STUDIES OF LONG CHAIN HYDROXY ACIDS—II*

THE HYDROXY ACIDS AND FATTY ACIDS OF A 5000-YEAR-OLD LACUSTRINE SEDIMENT

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Abstract—Fatty acids and hydroxy acids have been found in a 5000-year-old sediment from a freshwater lake in the English Lake District. The fatty acids showed the expected distribution for such a sediment. The hydroxy acids, which comprised 0·6% of the dry weight of the sediment, were identified as the even numbered ω-hydroxy acids from C_{16} to C_{24}; 10,16-dihydroxyhexadecanoic acid and the α- and β-hydroxy acids ranging from C_{10} to C_{24}. The ω-hydroxy acids and the 10,16-dihydroxyhexadecanoic acid are almost certainly derived from plant cutin and suberin but the α- and β-hydroxy acids may result from microbial oxidation of fatty acids.

FATTY acids, which occur as esters of simple or complex lipids, are widely distributed in nature.[1] Their presence in a variety of geological sources has been firmly established, for example, petroleum,[2] peats and lignites,[3] Montan wax,[4,5] fossil brine,[6] meteorites,[7] recent sediments[8–12] and ancient sediments.[12–14] Hydroxy acids are also extensively distributed in nature although less so than the fatty acids.[15] β-Hydroxy acids, for example, have been reported in a plant wax[16] and bacteria.[17] A wide variety of hydroxy acids occur in yeasts.[18] ω-Hydroxy, di- and trihydroxy acids occur as constituents of suberin,[19] the cork layer of many plants, and cutin, the varnish-like covering on the aerial parts of plants.[20] The hydroxy acids of cutin are of prime geochemical interest because of their known species variation[20] and their possible use for palaeochemotaxonomy.[21] The chemical resistance of cutin, in which the hydroxy acids exist as a polymerized and interesterified network[22] led us to believe that such hydroxy acids might be recoverable from sediments. Hydroxy acids of a non-cutin nature have been isolated from Recent soils[23] but no work has been done on other sediments nor have cutin acids been isolated from any geological source.

The sediment chosen for study was from a fresh water lake known as Esthwaite Water in the English Lake District. The sediment in this lake varies vertically from 11,000 years old to the present day (radiocarbon dating of immediate post-glacial sediment[24]) and has been studied palaeobotanically and its composition described.[25] The particular part of the sediment core studied here lay 336–382 cm below the lake bottom surface. The age of this layer is approximately 5000 years.[24] At the same depth, pollen analysis[25] of a similar core for the same lake, showed the principal component plants to be *Alnus* (alder), *Quercus* (oak), *Betula* (birch), and members of

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the Graminae (grasses), with smaller amounts of *Pinus* (pine), *Corylus* (hazel), *Ulmus* (elm), *Salix* (willow), and members of the Cyperaceae (sedges). Other workers have studied the diatoms,[26] extractable pigment[27] and inorganic geochemistry of the same sediment.[24]

Correlation of our results with those from other investigations and especially correlation of the hydroxy acid pattern with the known content of plant debris must, of necessity, await a fuller study and examination of all levels; however, the novelty of our results and their obvious biochemical and geochemical importance prompted early publication.

METHODS AND RESULTS

The dried sediment was demineralized with a mixture of hydrochloric and hydrofluoric acids and then hydrolysed with a methanol solution of potassium hydroxide. The sediment was acidified and the products extracted with methanol. The hydroxy and non-hydroxy acids were separated by TLC of their methyl esters and the non-hydroxy esters further resolved (TLC) into monocarboxylic and dicarboxylic esters. Individual components in each fraction were identified by GLC and combined gas chromatography—mass spectrometry (GC-MS).

A GC-MS trace of the monocarboxylic methyl esters is shown in Fig. 1A. The prominent peak was identified as methyl palmitate by its mass spectrum. The identity of each peak as determined by mass spectrometry is marked on the figure and the relative percentages given in Table 1. The mass spectra of the straight chain, iso- (methyl branched on the penultimate carbon) and anteiso- (methyl branched on the antepenultimate carbon) methyl esters were compared with published data.[28,29] The mass spectrum of the compound identified as the methyl ester of the *iso*-C_{14} acid is shown in Fig. 2A.

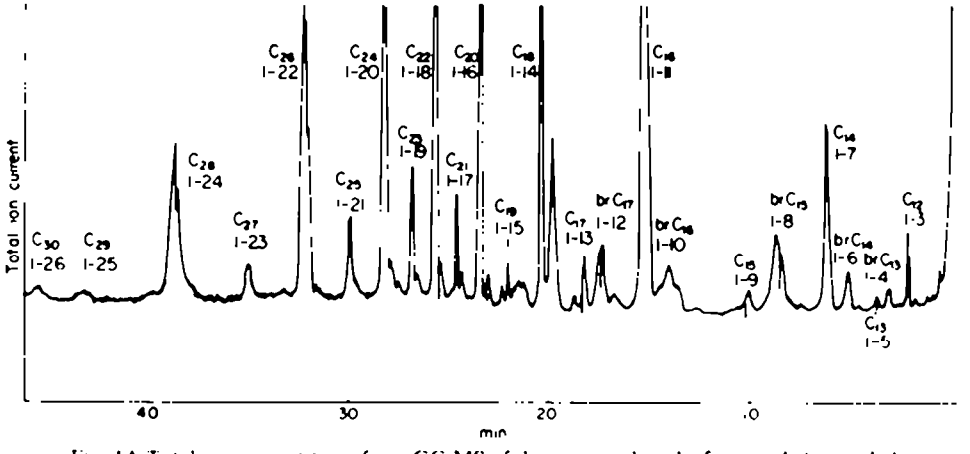

FIG. 1A Total ion current trace from GC-MS of the monocarboxylic fatty acids (as methyl esters) from Esthwaite Water sediment. Conditions: 1% SE30 on 100–120 mesh Gas-Chrom Q, 3 m × 3 mm i.d., He carrier gas at 30 ml/min. Temperature programmed 100–250° at 4°/min. The mass spectra were recorded at 70 eV, the total ion trace at 20 eV. Peak identities as in Table 1, the chain length of each ester is indicated. Mass spectra were taken at the points where there is a vertical line through a peak. Mass spectra of some peaks were determined on other runs.

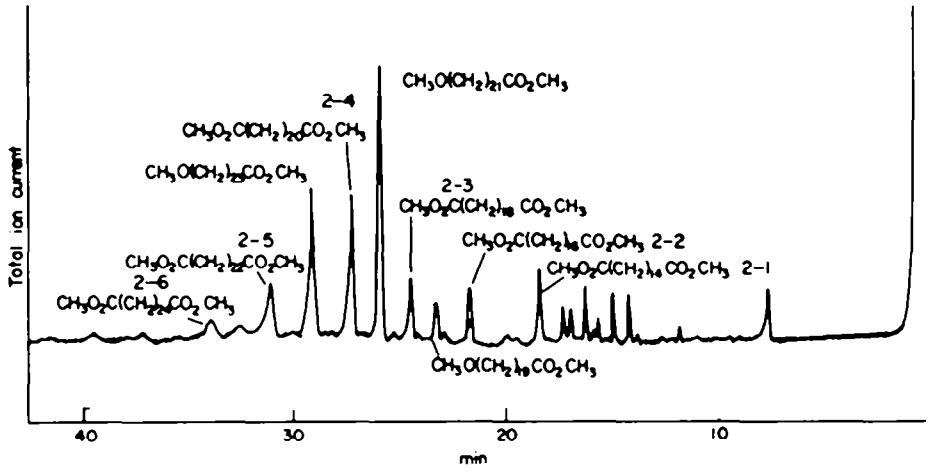

FIG. 1B Total ion current trace from GC-MS of the fraction containing α,ω-dicarboxylic acids (as methyl esters) from Esthwaite Water sediment Conditions as in Fig. 1A. Peak identities as in Table 2

TABLE 1 MONOCARBOXYLIC ACIDS FROM THE ESTHWAITE WATER SEDIMENT, DETERMINED AS METHYL ESTERS, PERCENTAGES CALCULATED FROM RELATIVE AREAS UNDER GLC PEAKS. ALL COMPOUNDS ARE STRAIGHT CHAIN UNLESS INDICATED OTHERWISE. THIS FRACTION COMPOSED 0.1% OF THE DRY WEIGHT OF THE SEDIMENT

Compound (Fig. 1A)	Chain length	Relative percentage	Compound	Chain length	Relative percentage
1-1	10	+	1-14	18	9
1-2	11	+	1-15	19	+
1-3	12	1	1-16	20	8
1-4	13 br	+	1-17	21	1
1-5	13	+	1-18	22	8
1-6	14 br	1	1-19	23	1
1-7	14	5	1-20	24	8
1-8	15 br	5	1-21	25	1
1-9	15	1	1-22	26	6
1-10	16 br	4	1-23	27	1
1-11	16	25	1-24	28	4
1-12	17 br	3	1-25	29	+
1-13	17	2	1-26	30	1

+ = trace amount
br = branched chain (see text)

The TLC fraction containing the dicarboxylic methyl esters also contained the entire series of methoxy esters corresponding to the hydroxy acids also found in the sediment. The GC-MS trace of this fraction is given in Fig. 1B. The dicarboxylic esters were identified on the basis of their mass spectra[30] and the mass spectrum of the compound identified as methyl eicosan-1,20-dioate is given in Fig. 2B. The relative amounts of the diesters are given in Table 2.

The identities of the hydroxy methyl esters were based on the retention indices

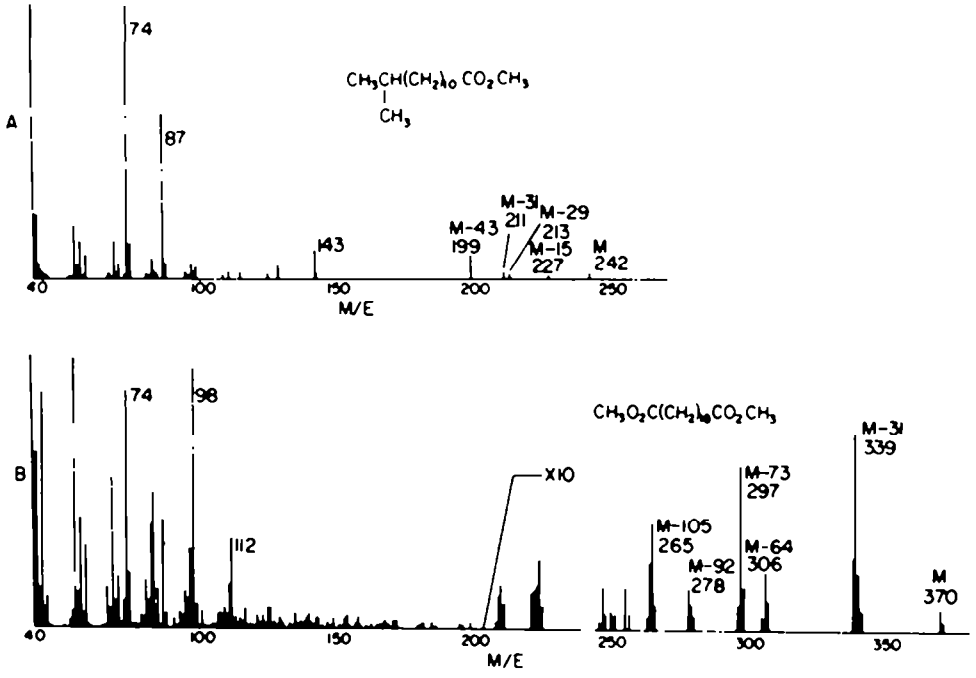

FIG. 2A Mass spectrum of compound identified as methyl 12-methyltridecanoate from Esthwaite Water sediment (Fig. 1A, peak 1-6).

2B Mass spectrum of compound identified as methyl eicosan-1,20-dioate from Esthwaite Water sediment (Fig. 1B, peak 2-3).

TABLE 2. α,ω-DICARBOXYLIC ACIDS FROM THE ESTHWAITE WATER SEDIMENT, DETERMINED AS METHYL ESTERS, PERCENTAGES CALCULATED FROM RELATIVE AREAS UNDER GLC PEAKS. THIS FRACTION COMPOSED 0·05 % OF THE DRY WEIGHT OF THE SEDIMENT

Compound (Fig. 1B)	Chain length	Relative percentage
2-1	16	14
2-2	18	9
2-3	20	14
2-4	22	51
2-5	24	6

and mass spectra of the corresponding trimethylsilyl ethers, which could be compared with those of authentic standards obtained from modern cutin.[31] The GLC trace of the total hydroxy acid fraction (as the methyl esters, trimethylsilyl ethers) is shown in Fig. 3 and the relative percentages given in Table 3. The retention indices were determined by coinjection with n-alkanes on programmed runs. A retention index and mass spectrum were determined for at least one authentic standard from each series of compounds. The hydroxy esters were comprised of three distinct series of compounds: the methyl esters of α- and β-hydroxy acids from C_{12} to C_{24};

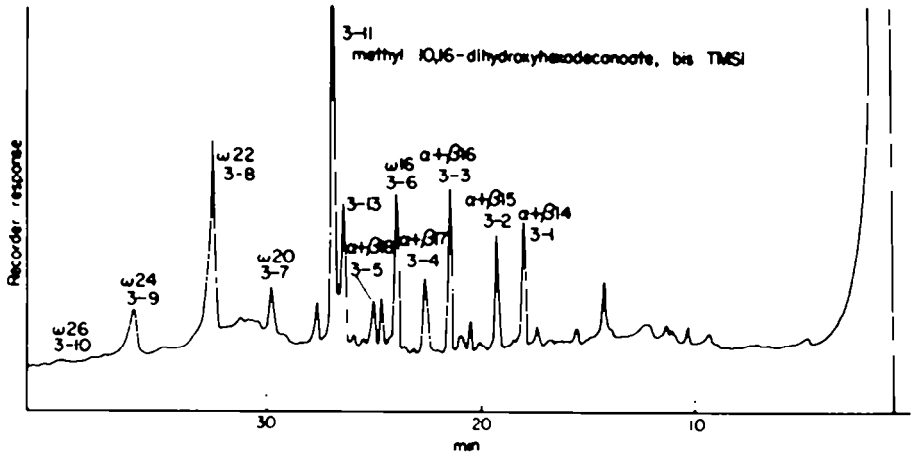

FIG. 3 GLC trace of the hydroxy acids from the Esthwaite Water sediment (as methyl esters, trimethylsilyl ethers). Conditions. 100–260° at 5°/min, N_2 pressure 30 psi, column 10' × $\frac{1}{8}$", 3% SE 30 on Gas-Chrom Q. Peak identities and abbreviations as in Table 3.

the cutin and suberin acids, including ω-hydroxy acids from C_{16} to C_{26} and the 10,16-dihydroxyhexadecanoic acid; and aromatic acids, among which was identified *p*-hydroxybenzoic acid.

TABLE 3 PRINCIPLE HYDROXY ACIDS FROM THE ESTHWAITE SEDIMENT, DETERMINED AS METHYL ESTERS, TMSi ETHERS, PERCENTAGES CALCULATED FROM RELATIVE AREAS UNDER GLC PEAKS. THIS FRACTION COMPOSED 0·6% OF THE DRY WEIGHT OF THE SEDIMENT

Peak No.	Compound (Fig. 3)	Abbreviation	Relative percentage	Retention index
3 1	α- and β-Hydroxytetradecanoic	α + β14	6·4	1930
3 2	α- and β-Hydroxypentadecanoic	α + β15	7·0	2000
3 3	α- and β-Hydroxyhexadecanoic	α + β16	8·1	2124
3 4	α- and β-Hydroxyheptadecanoic	α + β17	5·5	2200
3 5	α- and β-Hydroxyoctadecanoic*	α + β18	2·4	2320
3 6	ω-Hydroxyhexadecanoic*	ω16	8·3	2274
3–7	ω-Hydroxyeicosanoic	ω20	2·9	2680
3 8	ω-Hydroxydocosanoic	ω22	17·3	
3 9	ω-Hydroxytetracosanoic	ω24	6·4	
3–10	ω-Hydroxyhexacosanoic	ω26	trace	
3 11	10,16-Dihydroxyhexadecanoic*		18·8	2470
3 12	Unidentified		2·9	2340
3 13	Unidentified		7·6	2430

* Identity confirmed by retention index of authentic reference standard

The mass spectra of the compounds identified as α- and β-hydroxy methyl esters, TMSi ethers, were compared with that of an authentic sample of methyl β-hydroxy-octadecanoate, TMSi ether. The mass spectrum of this compound, methyl α-hydroxy-eicosanoate, TMSi ether and that of the corresponding derivative of the C_{18} compound from the sediment are given in Fig. 4. The M-59 peak (loss of CO_2CH_3) is

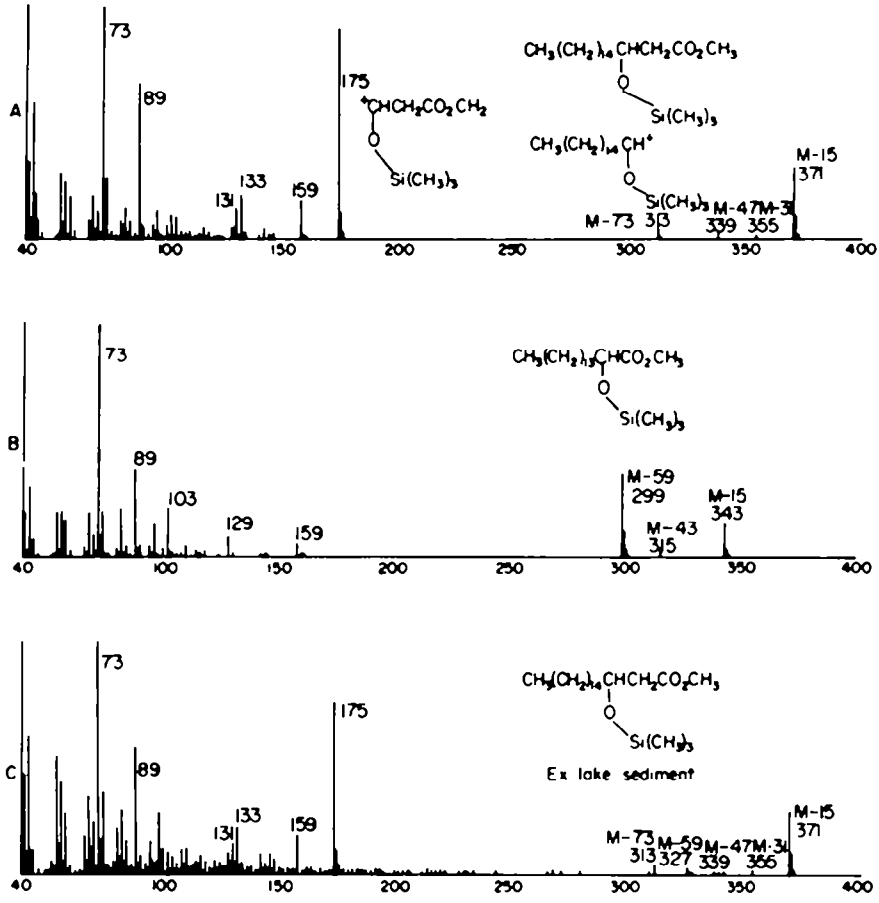

FIG. 4 Mass spectra of hydroxy acid, methyl esters, TMSi ethers. *A*. Methyl β-hydroxyoctadecanoate, TMSi ether (authentic). *B*. Methyl α-hydroxyhexadecanoate, TMSi ether (authentic). *C*. Methyl α- and β-hydroxyoctadecanoate, TMSi ether (ex Esthwaite Water sediment).
All spectra determined on the LKB 9000 at 70 eV.

absent from the mass spectrum of the authentic methyl β-hydroxyoctadecanoate, TMSi ether while it is very strong in the authentic α-compounds. The α- and β-isomers were not separable on this GLC column but from the mass spectra it is obvious that both isomers are present. Indeed, in the spectrum of the TMSi derivative of the C_{24} compound from the sediment the *m/e* 175 peak (indicative of the β-hydroxy compound) is considerably smaller than the M-59 peak (11%/54%) implying that the α-isomer is dominant. To further examine the monohydroxy acid fraction, an aliquot of the sediment extract was chromatographed (TLC) and the two fastest hydroxy ester bands were removed from the plate and examined separately. The GLC traces of these fractions as their TMSi derivatives are given in Fig. 5 and the summation of the relative amounts of the α- and β-hydroxy acids given in Table 4. The parallel distribution of chain length with the fatty acids can be seen by com-

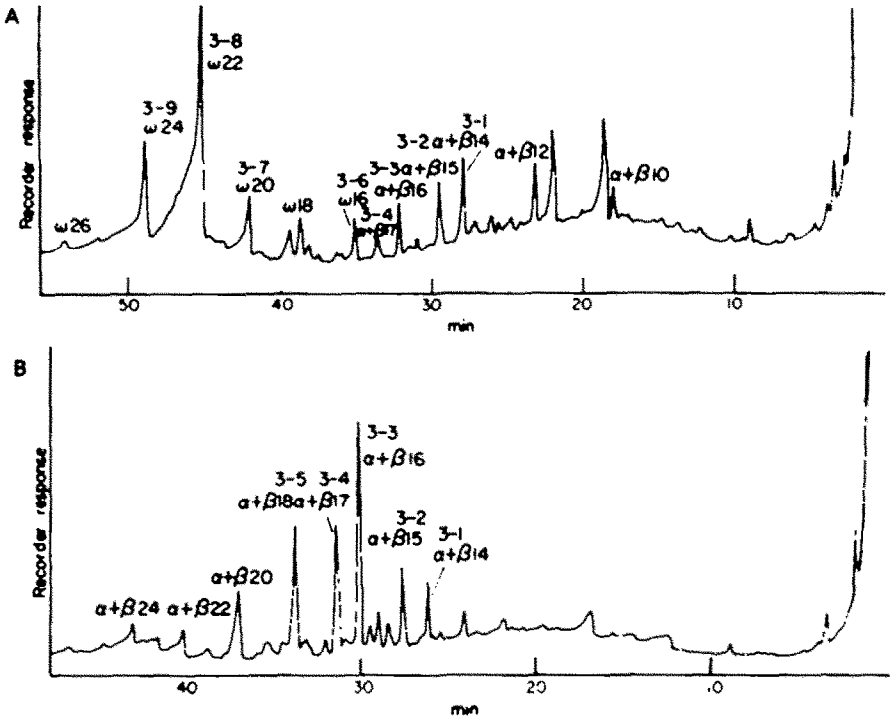

FIG. 5 GLC of the trimethylsilyl ethers, methyl esters of fractions from preparative TLC (ether/hexane/methanol, 40:10:1) of Esthwaite Water sediment hydroxy acids illustrating chain length distribution of the α- and β-hydroxy acids. *A*. TLC band R_f 0·47–0·56. *B*. TLC band R_f 0·56–0·72. GLC conditions as in Fig. 3. Identities as in Tables 3 and 4

TABLE 4. α- AND β-HYDROXY ACIDS OF ESTHWAITE WATER SEDIMENT (DETERMINED AS METHYL ESTERS, TMSi ETHERS); SUMMATION OF AMOUNTS IN FIG. 5A and 5B, TO ILLUSTRATE CHAIN LENGTH DISTRIBUTION OF THESE COMPOUNDS AND PARALLEL DISTRIBUTION WITH FATTY ACIDS (SEE TABLE 1)

Chain length	Retention index	Relative percentage
10	1538	3·2
12	1728	5·5
14	1930	11·2
15 br	2000	12·7
16	2126	23·6
17 br	2200	16·5
18	2320	13·8
20	2515	9·9
22		2·2
24		1·6

parison with Table 1. The retention indices of the C_{15} and C_{17} compounds did not come in the series of the other α- and β-hydroxy compounds, whose retention indices could be compared to authentic methyl β-hydroxyoctadecanoate, TMSi and methyl α-hydroxyhexadecanoate, TMSi. The C_{15} and C_{17} compounds, therefore, are branched, most probably the iso- or anteisostructures.

The ω-hydroxy compounds were also determined on the basis of the mass spectra and retention indices of the methyl ester, TMSi ethers; as was the 10,16-dihydroxyhexadecanoic acid.

The above acids were obtained by basic hydrolysis (KOH/methanol) followed by acid extraction. In an attempt to see if the α- and β-hydroxy acids and the cutin and suberin acids could be differentially extracted, the sediment was first demineralized (HCl/HF) and then extracted with methanol to give a fraction (I). The residues were briefly extracted with methanolic potassium hydroxide to give another fraction (II) and the residues were then subjected to exhaustive alkaline hydrolysis (5% KOH/methanol) under reflux to give a third fraction (III). Examination of the three fractions revealed no clear cut advantages for this method as compared with the previous extraction procedure with respect to the aliphatic hydroxy acids. Indeed, it seemed to give a much more complicated mixture. On the other hand one major difference emerged: fraction III is composed almost exclusively of what appear to be aromatic acids, one of which has been identified (GLC and mass spectrum of its methyl ester, TMSi compared with those of an authentic standard) as *p*-hydroxybenzoic acid. As these acids are outside our present field of interest they were not examined further.

DISCUSSION

The composition of the fatty acid fraction (Table 1) as found in this sediment is very much as expected for Recent sediments, although the bulk of previous work refers to marine environments.[12] The most abundant acid is the saturated C_{16}, while in living organisms the most prominent fatty acids are oleic, linoleic and palmitic. The unsaturated acids are evidently removed rapidly from the sediment by some non-reductive process, i.e. one that does not give stearic acid. The carbon preference index (CPI)[8] is high (11·2) as would be expected for a young sediment, whereas ancient sediments show CPI's approaching unity. One surprising feature in the present results is the absence of observable amounts of isoprenoid acids, which are already known to make up a significant proportion of the acids of an Eocene lacustrine sediment.[32] Branched acids, iso and anteiso are present, however, and, as previously suggested, they probably represent microbial metabolites.[10,11]

We have previously reported the presence of α,ω-dicarboxylic acids in an ancient sediment, Torbanite[14] (Carboniferous) and this has since been extended to the Green River Shale (Eocene).[33,34] Diacids of moderate chain length are found in certain plant waxes[35] and in cutin[31,36] but these sources are probably inadequate to explain their presence in the Esthwaite core (Table 2) and other sediments in such large amounts. Bacterial oxidation of fatty acids is known to produce diacids[37] but it seems more likely that they derive from the oxidation of the ω-hydroxy acids that are prominent constituents of this sediment (Table 3). This is suggested by the parallel distribution of the chain lengths of the diacids and ω-hydroxy acids, with large C_{22}, moderate C_{16} and C_{20}, and lesser amounts of C_{18} and C_{24}.

The presence of α- and β-hydroxy acids in the sediment was unexpected. Such

acids have been found in waxes[16] but not in such large amounts and only in the chain lengths C_{18}–C_{30}. We conclude that these acids are derived from the fatty acids by α- and β-oxidation. This conclusion is based on the wide variation in chain length (C_{10}–C_{24}) and the parallel distribution of chain lengths of the α- and β-hydroxy acids and the fatty acids. The survival of these acids over even a relatively short period of geological time is surprising and so far inexplicable. It is possible, of course, that these compounds are derived from modern bacteria digesting the ancient sediment. The classical method of distinguishing ancient and modern materials, radiocarbon dating, is not applicable in this case since the fatty acids and their oxidation products would show an ancient date even if recently oxidized. To our knowledge this is the first isolation of such a long series of α- and β-hydroxy acids from any material, although shorter series of α-hydroxy acids have been reported.[46] It is certainly the first from a geological sediment. The widespread occurrence of α- and β-oxidation systems[38] when taken with the present finding of the entire range of α- and β-hydroxy acids is strong evidence for α- and β-oxidation as important pathways of degradation for fatty acids in biological and, now, geological systems.

The acids which occur as common components of suberin, i.e. ω-hydroxy C_{20} to C_{26} acids, are present in this sediment in approximately the same relative proportions as in modern plants.[39] Suberin occurs in the cork layer of the woody parts of many plants and is closely related in composition to the cutin which makes up part of the cuticle of leaves and fruits.[40] Both materials are principally composed of interesterified mono-, di-, and trihydroxy fatty acids but differ in that the suberin acids are usually of longer chain length and have a higher proportion of diacids. Cutin acids are characterized by the almost complete dominance of C_{16} and C_{18} chain lengths and the apparently ubiquitous presence of 10,16-dihydroxyhexadecanoic acid.[41] The particular cutin acids which are present in this sediment, i.e. 10,16-dihydroxyhexadecanoic, ω-hydroxyhexadecanoic, and ω-hydroxyoctadecanoic, are significant in the light of the known species variation in cutin composition.[20] However, little can be said specifically until a larger number of plants have been examined. The acids will evidently survive geological conditions, at least for a short time. A study of cutin compositions of those particular plant species, the remains of which are suspected as components of a sediment, should permit geochemical deductions as to the botanical composition. The parameters that may be called upon include the presence (e.g. 10,16-dihydroxyhexadecanoic, ω-hydroxyhexadecanoic, and ω-hydroxyoctadecanoic acids) or absence (e.g. 9,10,18-trihydroxyoctadecanoic acid) of certain acids and their relative proportions. Unsaturated hydroxy acids, such as the ω-hydroxyoctadec-9-enoic acid and ω-hydroxyoctadeca-9,12-dienoic acids are important[31,40] constituents of certain cutins and we have yet to determine whether these compounds might survive over long periods of time. Cutin acid composition might be of more use in examination of specific fossil cuticles, for example, the Indiana Paper Coal,[42] than in generalized sediments which are usually a mixture of a variety of plant species. It could be a useful adjunct to such classical techniques as pollen analysis.

The occurrence of aromatic acids, such as *p*-hydroxybenzoic, is not unexpected as they are common metabolites,[43] especially as constituents of lignin. No attempt in the present study was made to further identify these compounds.

We do not ascribe geological significance to the methoxy fatty acids herein reported. These acids are most likely derived from the corresponding hydroxy compounds during methylation with diazomethane. This possibility is suggested by their chain length distribution which parallels that of the hydroxy acids. Diazomethane is known to methylate alcohols when catalysed with Lewis acids[44] such as $ZnCl_2$, $MgCl_2$, and $FeCl_3$, as well as the more commonly used BF_3. Acidified $FeCl_3$ is soluble in ether[45] and hence its presence is to be expected in the ether soluble acid fraction.

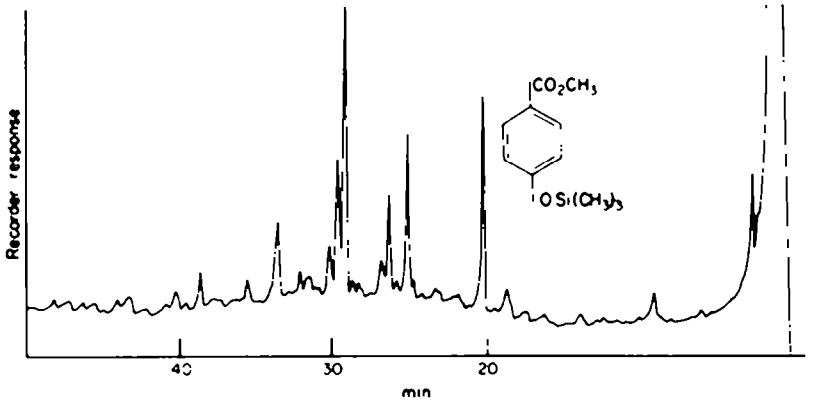

FIG. 6 GLC trace of an acidic fraction (III) containing aromatic hydroxy acids (as methyl esters, TMSi ethers) from Esthwaite Water sediment. Conditions as in Fig. 3.

EXPERIMENTAL

GLC traces were obtained with a Perkin Elmer F 11 gas chromatograph equipped with a coiled stainless steel column (10' × $\frac{1}{8}$") packed with 3% SE 30 on Gas-Chrom Q (coated by Applied Science) which tested for 4700 theoretical plates with n-tetracosane at 200°C and 30 psi N_2 press. Retention indices were determined by coinjecting standard n-alkanes with the sample, the measurements being made on traces from normal temperature programmed operation.

Mass spectra were determined on an LKB 9000 combined gas chromatograph–mass spectrometer operating at 70 eV and using a 3 m × 3 mm (i.d.) coiled glass column packed with 1% SE 30 on acid washed and silanized Gas-Chrom P. This column tested for 3360 theoretical plates with n-pentadecane at 100°. The helium flow rate through the column was 30 ml/min. The GLC trace was given by the total ion current at 20 eV. The scan time was 2 seconds per mass decade. The background mass spectrum for SE 30 was subtracted from each mass spectrum. Only a few background peaks were evident (at *m/e* 147, 207, 221, 281, 341, 355, 429, 503) and they did not amount to more than 1-2% relative intensity. Peaks with a relative intensity greater than 1% were tabulated.

All solvents were of AnalaR grade and were distilled at atmospheric pressure through a column (1 m) packed with glass helices and equipped with a reflux-return head.

Sediment collection. The core was collected in October 1965 by F. J. H. Mackereth of the Freshwater Biological Association.[24] It was cut into different layers and then thoroughly dried at 50° for 48 h. The different layers were stored in tightly closed vessels at room temp and work was started on the one concerned here in May 1967.

Pulverization and demineralization. The dried sediment was broken into smaller lumps and crushed in a Tema mill (17 min). The powdered sediment (100 g) was treated with 6N HCl (90 ml, room temp, for 1½ h) and 40% HF (90 ml, room temp, 4 days) in a Teflon container. The mixture was filtered and washed with water. The acidic aqueous extract was thoroughly extracted with ether and the ether phase added to the wet demineralized sediment.

Hydrolysis and extraction of ether-soluble acid fraction. The combined wet demineralized sediment and the ether extract of the washings were heated under reflux with methanolic KOH (3%, 250 ml, 48 h). The hydrolysed mixture was acidified to pH 2 with 5N HCl and the supernatant removed, concentrated to 100 ml and extracted with ether (5 × 50 ml). The solid residues were ultrasonically extracted (Dawe Ultrasonic Tank, Type 1165) with MeOH (3 × 300 ml) and the MeOH extract then combined with the ether extract and concentrated under vacuum. The combined extracts were rehydrolysed (to liberate any acids converted to methyl esters during HCl/MeOH extraction) with refluxing methanolic KOH (6%, 100 ml, 3 h). The mixture was then diluted with water and washed with hexane (2 × 200 ml) to remove neutral lipids. The mixture was then acidified with 3N HCl and extracted with ether (2 × 100 ml). The ethereal extracts were evaporated and azeotropically dried with benzene to give the ether-soluble acid fraction (1.21 g).

Esterification and separation of ester fractions. The ether-soluble acid fraction was esterified by treatment with excess diazomethane in ether. An aliquot of the crude methyl ester fraction (45 mg) was preparatively thin layer chromatographed on silica gel HF_{254} (E. Merck) (0.25 mm × 20 cm × 20 cm) in ether:hexane:methanol (40:10:1). Two bands were collected, A (R_f = 0·05 – 0·73) and B (R_f – 0·73 – 1·00). In this solvent system the methyl esters of carboxylic acids showed the following R_f values: monobasic acids, 0·94; dibasic acids, 0·85; ω-hydroxy acids, 0·60; di- and trihydroxy acids, < 0·6.[31] Band A weighed 22 mg and band B, 12 mg (48%, and 27% of the ether soluble acid fraction, respectively).

Non-hydroxy methyl esters. Band B was again preparatively thin layer chromatographed on silica gel G (0·25 mm × 20 cm × 20 cm) with hexane:ether (95:5). The plate was sprayed with aqueous Rhodamine 6G (0·0005%) and two bands were removed, band B(i) (R_f 0·12 – 0·24, 2 mg, diesters) and band B(ii) (R_f 0·42 – 0·65, 3·9 mg, monoesters) and an ether soln of each passed down a short column of neutral alumina (to remove Rhodamine 6G).

The two concentrates were then examined by GLC (Tables 1 and 2) and GC-MS. The GC-MS trace of band B(i) is given in Fig. 1B and of band B(ii) in Fig. 1A. Mass spectra for one peak from each are given in Fig. 2.

Hydroxy methyl esters (a) The entire sample from band A was treated with bis(trimethylsilyl)acetamide (BSA) to give the trimethylsilyl (TMSi) ethers.[31] The mass spectra and GLC retention times were then determined on the components of this fraction (Figs. 3 and 4, and Table 3).

(b) An aliquot (30 mg) of the crude methyl ester fraction was thin layer chromatographed on silica gel HF_{254} (0·25 mm × 20 cm × 20 cm) in ether:hexane:methanol (40:10:1). Bands were visualized under UV light and those running at R_f – 0·47 – 0·56 (band A(i) and) R_f – 0·56 – 0·72 (band A(ii)) were collected and eluted with ether. Each fraction was then treated with BSA to make the trimethylsilyl ethers and mass spectra and retention times determined. The two fractions were diluted to the same volume and thus areas under GLC peaks could be added in compiling the data presented in Table 4.

(c) To the powdered sediment (10 g) was added first, 6N HCl (25 ml, 3 h) and then 40% HF (25 ml, 3 days). The mixture was filtered and washed with water. The moist sediment was then extracted in a soxhlet for 24 h with MeOH. The methanol extract (265 mg) upon hydrolysis (3% KOH/MeOH, 6 h reflux), acidification, bicarbonate extraction, and reacidification, followed by extraction with ether gave a crude acid fraction (29 mg). This material was methylated with diazomethane and the hydroxy ester fraction (R_f = 0·05 – 0·80, 14·0 mg) was obtained by preparative TLC. This fraction was made into the TMSi ethers with BSA and was then termed fraction I (methyl esters, TMSi ethers).

The sediment residues from the above extraction were sonicated in cold methanolic potassium hydroxide (5%, 150 ml, ½ hr) and then exhaustively extracted with cold MeOH. The extract was treated as above, yielding the hydroxy ester fraction (14·3 mg) which was treated with BSA to give the methyl esters, TMSi ethers of fraction II.

The sediment residues were then refluxed with methanolic KOH (5%, 24 hr) and exhaustively extracted with cold MeOH. The hydroxy ester fraction (14·5 mg) was obtained in the usual manner and treated with BSA to give the methyl esters, TMSi ethers of fraction III. A GLC trace of this fraction is given in Fig. 6.

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