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Free and esterified aliphatic carboxylic acids in humin and humic acids from a peat sample as revealed by pyrolysis with tetramethylammonium hydroxide or tetraethylammonium acetate

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Abstract

The combination of TMAH thermochemolysis and TEAAc treatment makes it possible to discriminate between the different forms of mono- and dicarboxylic acids present in the structure of humin and humic acids, that is, "free" uncombined acids, methyl or ethyl esters present as tightly trapped molecules within the matrix, or acids chemically linked to the matrix by ester groups. The results confirm that ester groups are involved in the structure of humin and humic acids. The cross-linking of moieties originating from microbial metabolism or inherited from higher plants is partly ensured by these chemical groups. On the other hand, significant amounts of fatty monocarboxylic acids and linear dicarboxylic acids are present as free acids in the humin of the studied sample. Humin contains also fatty acid methyl esters. Free, uncombined α,ω-dicarboxylic acids were only found in humin. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Humin and humic acids are known to play a key role in the soil, and environment, for fertility and interactions with pollutants. Their role in the global carbon cycle is also significant. As a consequence, determination of their chemical composition and structure at a molecular level is of importance for soil scientists. In this way, the relationship between "free" (not chemically bound) compounds present in the directly extractable lipid fraction and lipid components physically trapped within the macromolecular network of humin or humic acids must be established for a better understanding of humification processes and capacities of soils to retain or liberate autochthonous or allochthonous (xenobiotic) compounds. In addition, important information concerning the prediagenetic and diagenetic evolution of lipids could arise from the study of free and combined forms of mono- and dicarboxylic acids present in soils or sediments.

Studies devoted to this problem frequently involve the use of different pyrolysis techniques. Pyrolysis with in situ methylation in the presence of tetramethylammonium hydroxide (TMAH) is an efficient method for transesterification of esters and methylation of fatty acids: in both cases, methyl esters are obtained. This method is now commonly used for the structural investigation of complex forms of natural organic matter present in soils (humic substances, macromolecular lipids, clay-organic complexes, total organic matter) and in ancient sediments, or for the investigation of synthetic polymers (Challinor, 1989, 1994, 1996; Saiz-Jimenez et al., 1993; Saiz-Jimenez, 1994a,b; Hatcher and Clifford, 1994; Martin et al., 1994; del Rio et al., 1994, 1996; del Rio and Hatcher, 1996; Schulten and Sorge, 1995; Schulten et al., 1996; Gobé et al., 2000) via flash (or analytical)

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pyrolysis used on a very small quantity of sample. Nevertheless, in order to obtain information about yields of the various pyrolysis products and identification of minor products often hidden in complex flash pyrolysates, it is necessary to obtain degradation products in sufficient amounts for liquid chromatographic separation. In this way, preparative, off-line pyrolysis was applied to kerogens (Largeau et al., 1986; Behar et al., 1989; Baudet et al., 1994). A new preparative thermochemolysis method using TMAH, which allows for the treatment of large quantities (1–2 g) of product (Grasset and Amblès, 1998a) was recently reported for the analysis of humin and humic acids. In this study, the formation of methylated short- and long-chain monocarboxylic acids, dicarboxylic acids and alkanols upon TMAH thermochemolysis was interpreted as reflecting the occurrence of a cross-linked macromolecular matrix, ester groups being involved in the cross-linking (Grasset and Amblès, 1998b). Aliphatic carboxylic acids were considered as molecular entities esterified to the matrix. This emphasizes the importance of esterification processes occurring during early diagenesis in young sediments. Nevertheless, some of the carboxylic acids (e.g. fatty acids) may have been present in a “free” form, strongly trapped (with non-covalent linkages) within the macromolecular network. To test this assumption complementary investigations were needed. The use of tetramethylammonium acetate (TMAAc) for the selective alkylation of free acids (yielding methyl esters) without transesterification of the present esterified fatty acids was recently reported for the study of wood pulp (Hardell and Nilvebrant, 1999). Owing to the efficiency of the method, and taking into account that methyl esters can occur in soil organic matter, a modified thermochemolysis method using TMAH and tetraethylammonium acetate (TEAAc) was used in the present work to discriminate between free and esterified mono- and dicarboxylic acids present in the humin and humic acids of a peat sample. They were compared to mono- and dicarboxylic acids from the corresponding lipid fraction of the sample.

2. Experimental

2.1. Sample description and isolation of organic fractions

A representative sample was collected from a calcic peat (Marais Poitevin, Deux-Sèvres, Western part of France) at 30–50 cm depth (Jambu, 1971). Lipid, fulvic acid, humic acid and humin fractions were obtained following the IHSS procedure (Grasset, 1997; Grasset and Amblès, 1998a,b). The sample contained 80 wt.% of total organic matter (TOM) based on the oven-dried soil (measured by combustion). Free lipids were first extracted (Soxhlet, $CHCl_3$), the residue was then washed with a 1 M HCl solution. Humic acids were extracted with 1 M NaOH (under a nitrogen atmosphere) and separated from fulvic acids by precipitation at pH 1 (with 6 M HCl solution). The alkaline-insoluble residue corresponded to a humin concentrate, associating insoluble organic matter (humin) with the remaining mineral matter, as defined for kerogen in old sediments (Durand, 1980). Prior to characterisation, humic acids and humin were extracted again with chloroform (Soxhlet, 2×24 h) to remove possibly adsorbed residual lipids. The detailed procedure is described in Grasset (1997) and Grasset and Amblès (1998a,b). The prepared humin concentrate contained 83.5 wt.% of organic matter. Humin, humic acids and lipids corresponded to 60, 27 and 2 wt.% of TOM, respectively. The elemental composition of humin and humic acids is given in Table 1.

Acids and neutrals from the free lipid fraction were separated on a SiO_2/KOH column (McCarthy and Duthie, 1962). Esters were separated from the other neutrals by liquid chromatography using diethyl ether/petroleum ether mixtures of increasing polarity for elution. Acids were identified as methyl esters after classical methylation of the acid fraction with trimethylsilyldiazomethane (Hashimoto et al., 1981) and separation on a SiO_2 column (elution with diethyl ether/petroleum ether mixtures of increasing polarity).

2.2. Pyrolyses of humin and humic acids

The samples (humic acids: 1.02 g; humin: 1.28 g containing 1.07 g of pure organic matter) were placed in a ceramic boat after overnight moistening with 2 ml of a 50% (w/w) methanol solution of tetramethylammonium hydroxide (TMAH). For the treatment with tetraethylammonium acetate (TEAAc), the samples (humic acids: 1.00 g; humin: 1.00 g containing 0.83 g of pure organic matter) were moistened overnight with 3 ml of a 25% (w/w) ethanol solution of TEAAc. Each sample was then transferred in a 60×3 cm i.d. Pyrex® tube and heated at 400 °C (1 h isothermal period). Thermochemolysis products were swept by helium (flow rate: 100 ml min^{-1}) to 2 successive traps containing chloroform cooled at −20 °C. After evaporation of the solvent, both trapped pyrolysates were combined and further separated on a SiO_2 column.

Table 1
Elemental composition of the humic fractions

	C	H	O[a]	N	S	Ash
Humin	40.9	4.9	32.8	3.4	1.5	16.5
Humic acids	41.2	4.2	47.7	2.8	1.3	2.8

[a] By difference.

2.3. Gas chromatography–mass spectrometry

The products were analysed by capillary GC and GC–MS. GC separations were carried out with a Shimadzu GC 14A or Hewlett Packard HP 6890 Series gas chromatograph using a CP Sil 5 CB (Chrompack) capillary column (25 m length). The temperature of the column was programed from 60 °C (10 min isothermally) to 300 °C (20 min isothermally) at 3 °C min^{-1}. GC–MS were performed on a Finnigan Incos 500 mass spectrometer. The GC conditions were the same as for GC analysis. The various products were identified on the basis of their GC retention times and their mass spectra (comparison with standards) and literature data.

3. Results and discussion

The yields of pyrolysis products in the presence of TMAH or TEAAc are given in Table 2. The yields of total organic compounds are approximately equivalent for humin with TMAH and TEAAc and surprisingly two times larger with TEAAc vs TMAH for humic acids. The products of interest for the present work are aliphatic monocarboxylic acids and aliphatic dicarboxylic acids, mostly present as α,ω-dicarboxylic acids. The amounts of these different kinds of acids, in comparison with the quantities present in the free lipid fraction of the sample, are given in Table 3. It can be noted that:

1. fatty acid methyl (FAMEs) and ethyl (FAEEs) esters are present in trace amounts in the free lipid fraction, the main acids being aliphatic mono- and dicarboxylic acids. FAMEs were detected in very low amounts in the TEAAc products from humic acid.
2. humin afforded on both treatments higher quantities of products (×8) than humic acids;
3. quantitative differences are noticeable between the two pyrolysis techniques: the yield of acidic products (as acids or acid derivatives) is higher with TMAH than with TEAAc. This result is logical as TMAH is known to react with free acidic groups (yielding methyl esters) as well as with esterified acids after thermally assisted hydrolysis and methylation (THM), (Challinor, 1989, 1994, 1996; del Rio et al., 1994, 1995, 1996; del Rio and Hatcher, 1996) leading to methyl esters, whereas TEAAc is described to react only with free acidic groups leading to ethyl ester derivatives (Hardell and Nilvebrant, 1999).
4. aliphatic ethyl esters are significantly present with TEAAc, compared with the reaction of TMAH.

Table 2
Yields (%) of organic compounds obtained after pyrolysis[a]

	With TMAH	With TEAAc
Humin	13.9	13.7
Humic acids	3.3	6.8

[a] % Of the initial material.

3.1. Alkanoic monocarboxylic acids

The distribution pattern of directly-extractable alkanoic monocarboxylic acids (free lipid fraction), identified in the C_{14}–C_{36} range, presents a long mode maximizing at C_{28} with a strong even-over-odd carbon number predominance as indicated by the value of the carbon preferential index (even over-odd components) or CPI: 7.30. Such a distribution (Fig. 1) indicates a dominant higher plant origin for these acids (Kolattukudy, 1976). The branched iso- and anteiso-C_{15} and C_{17} acids were identified in the short mode as the signature of bacterial contribution (Boon et al., 1977, 1978).

Table 3
Acids obtained on pyrolysis (mg/kg of total organic matter) in comparison with acids from bitumen

Main series of products[a]	Free lipid fraction	Humin		Humic acids	
		TMAH	TEAAc	TMAH	TEAAc
FAs	2135	n.d.	tr	n.d.	n.d.
FAMEs	tr[b]	4020	420	450	tr
FAEEs	tr	n.d.	1900	n.d.	300
DAs	350	n.d.	n.d.	n.d.	n.d.
DAMEs	n.d.[b]	450	n.d.	140	n.d.
DAEEs	n.d.	n.d.	180	n.d.	tr

[a] FAs: fatty acids; FAMEs: fatty acid methyl esters; FAEEs: fatty acid ethyl esters; DAs: α,ω-dicarboxylic acids; DAMEs: α,ω-dicarboxylic acid methyl esters; DAEEs: α,ω- dicarboxylic acid ethyl esters.

[b] n.d.: not detected; tr: trace amounts.

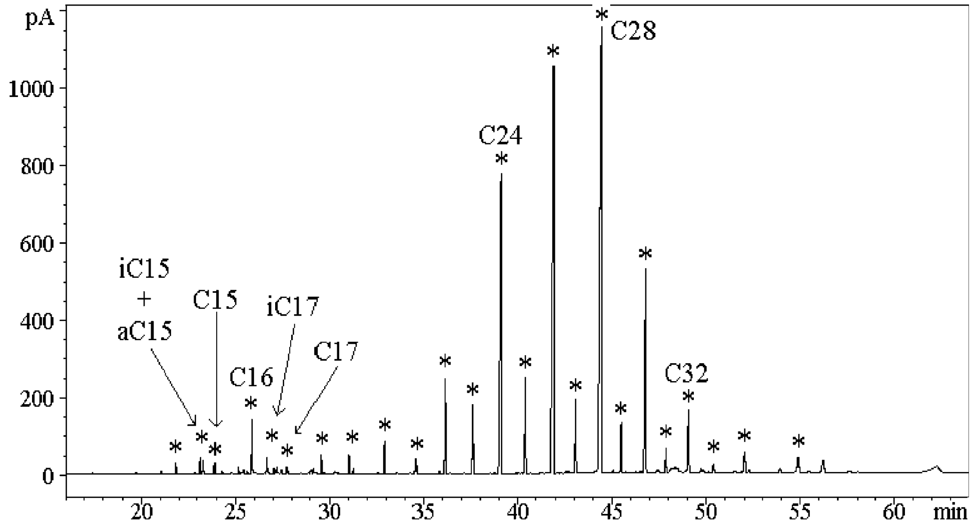

Fig. 1. GC trace of the acid fraction of the bitumen after methylation showing the fatty acids or FAs (*) present in the directly-extractable fraction. (i: iso; a: anteiso).

After TMAH treatment of humin, the monocarboxylic acids released as FAMEs (major acids, Table 3) are C_{14}–C_{32} members and their distribution (Fig. 2) shows also a strong predominance of the even components (CPI: 5.52). The $C_{16:0}$ compound is dominant, while $C_{14:1}$, $C_{16:1}$, $C_{18:1}$, branched iso- and anteiso-C_{15} and C_{17} acid methyl esters are found in minor concentrations. These compounds were released after ester bond cleavage and/or they result from the methylation of "free", uncombined fatty acids which were initially trapped in the humin and freed during the alteration of the matrix. Their distribution reflects a double origin: higher plants (long acid methyl esters) and microbial (short chain linear and branched acid methyl esters) (Boon et al., 1977, 1978; Perry et al., 1979).

Fatty acid ethyl esters (FAEEs) were the main acid derivatives obtained after TEAAc treatment of humin (Table 3). Their distribution pattern (Fig. 3) (CPI value: 5.92) shows a higher relative proportion of the long homologues of higher plant origin, when compared with FAMEs from TMAH (Fig. 2). Moreover, their amount is two times lower. The presence of FAMEs as TEAAc products from humin shows clearly that a part of the FAMEs released after the TMAH treatment was initially present as FAMEs in the studied sample: naturally-occurring fatty acid methyl esters were present as trapped molecules in the humin structure. The high proportion of FAEEs obtained indicates that a large part of the FAMEs released after the TMAH treatment were initially trapped as "free", uncombined fatty acids. This

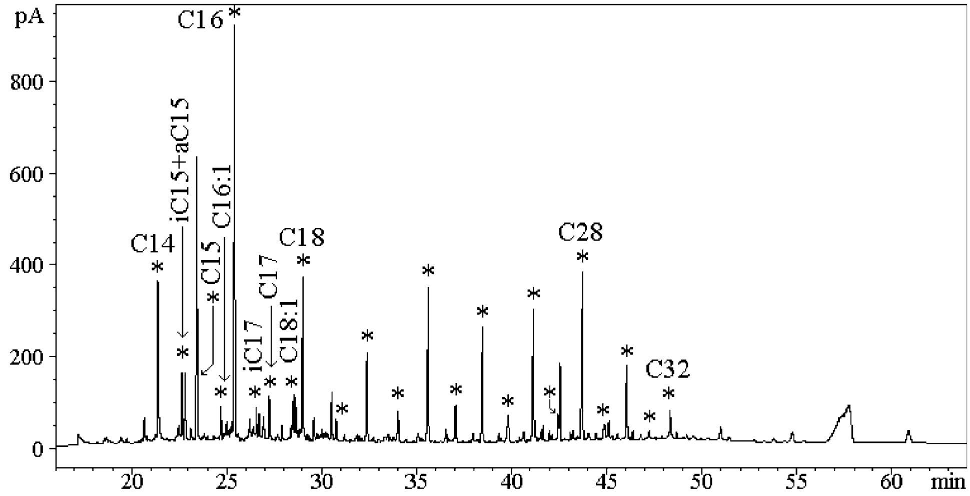

Fig. 2. FAMEs (*) obtained by TMAH treatment of humin (i, iso; a, anteiso; Cx:1, unsaturated acids with x carbon atoms).

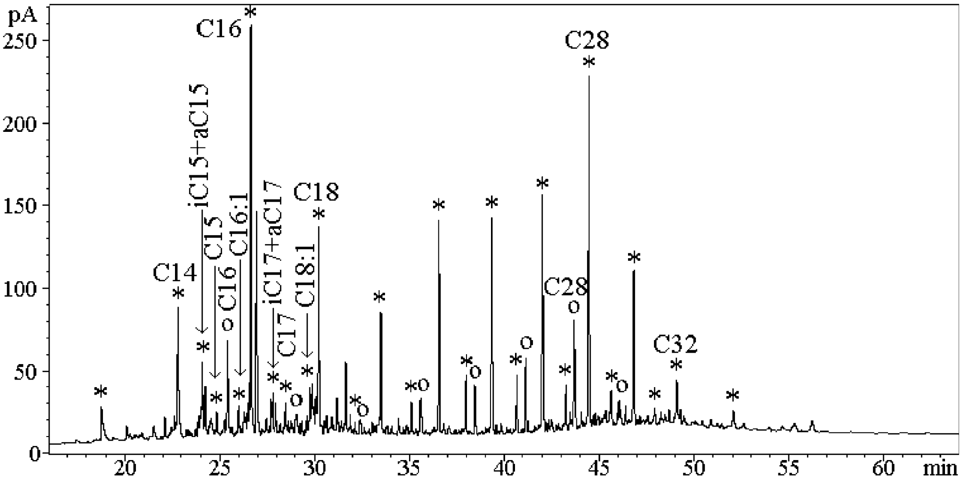

Fig. 3. FAEEs (*) and FAMEs (°) obtained after TEAAc treatment of humin (legend is the same as in Fig. 2).

proportion appears to be higher regarding only fatty acids originating from higher plants.

FAMEs (after TMAH treatment, Fig. 4) (CPI: 3.44) and FAEEs (after TEAAc treatment Fig. 5) (CPI: 4.50) from humic acids display chiefly the same distribution as that of FAMEs (after TMAH treatment) from humin (CPI: 5.52). Quantitative results (Table 3) show that a major part of the monocarboxylic fatty acids in humic acid of this sample was present as “free”, uncombined compounds, leading to FAEEs on TEAAc treatment.

3.2. α,ω-*Dicarboxylic acids*

Dicarboxylic acids (DAs) were present only in the bitumen fraction. Methyl ester derivatives (DAMEs) were identified only in the TMAH products from humin and humic acid and dicarboxylic acid ethyl esters (DAEEs) were only found after TEAAc pyrolysis (in trace amounts in the case of humic acids (Table 3). The distributions of these different forms of dicarboxylic acids are shown in Fig. 6. C_{22}–C_{30} α,ω-dicarboxylic acids are present in the free lipid extract with a maximum at C_{28}. DAs with even carbon numbers (CPI value: 3.77) are prominent (Fig. 6a).

α,ω-Dicarboxylic acids released as methyl esters (DAMEs) after TMAH thermochemolysis of humin were identified in the C_9–C_{30} range (Fig. 6d) The C_9 diacid is dominant. The long mode presents a Gaussian distribution maximising at C_{26} with a very low contribution of members with odd carbon numbers (CPI value: 6.67). TEAAc treatment of humin results in a homologous series of DAEEs, ranging from C_{17} to C_{30} with a maximum at C_{26} (Fig. 6b). The CPI value is 2.30. Sources of long-chain members are cutin and suberin

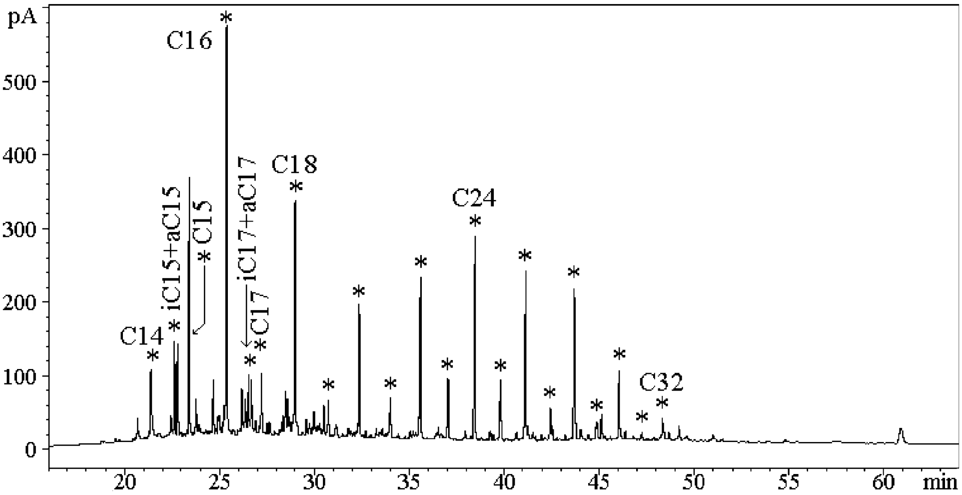

Fig. 4. FAMEs (*) formed by TMAH treatment of humic acids (legend is the same as in Fig. 1).

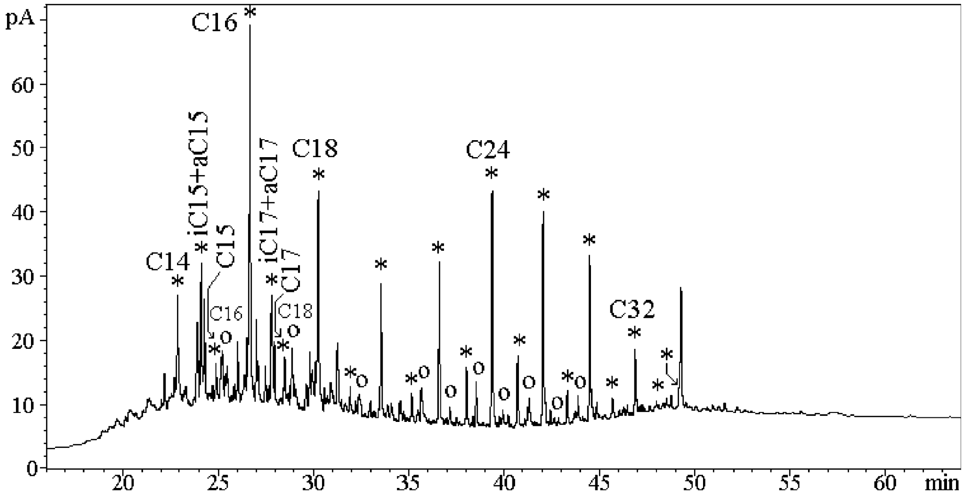

Fig. 5. FAEEs (*) and FAMEs (°) formed after TEAAc treatment of humic acids.

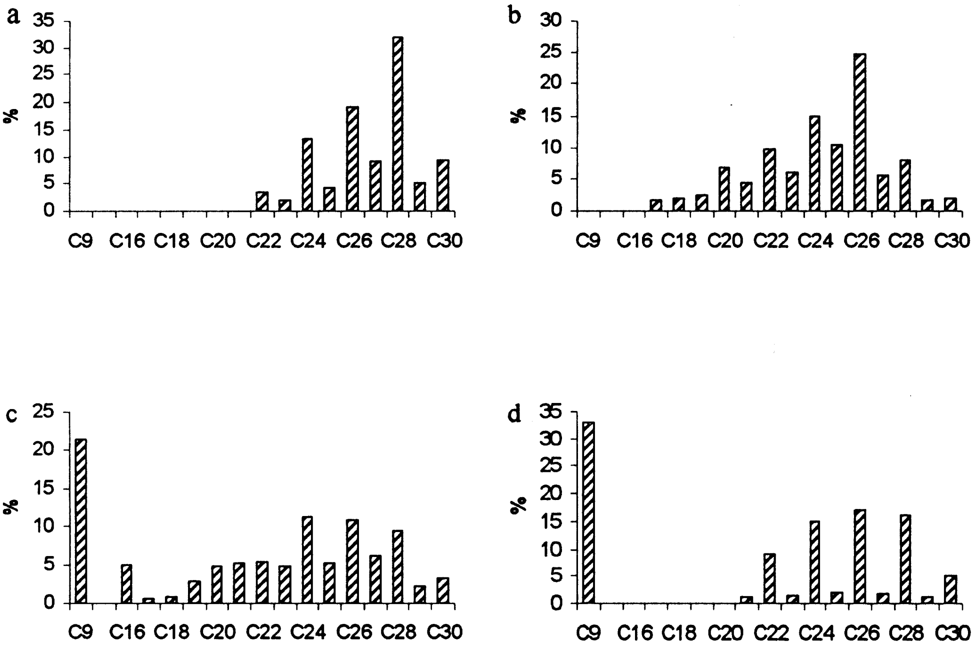

Fig. 6. Distribution of linear dicarboxylic acids. (a) DAs from lipids; (b) DAEEs after TEAAc treatment of humin; (c) DAMEs after TMAH treatment of humic acids; (d) DAMEs after TMAH treatment of humin.

from higher plants (Kolattukudy, 1976) or microbial ω-oxidation of fatty acids. α,ω-Dicarboxylic acids components with odd carbon numbers may also originate from further oxidative degradation of fatty acids via ω-hydroxycarboxylic acids (Amblès et al., 1994). The C_9 diacid probably results from the oxidation of the carbon-carbon double bonds of Δ-9 fatty acids (Amblès et al., 1994).

The significant difference between the distribution of the various α,ω-dicarboxylic acids obtained from humin is the absence of the C_9 component after TEAAc treatment (Fig. 6b). This C_9 compound was, therefore, exclusively linked to the organic matrix in humin and not present under a trapped, "free" form. The presence of substantial amounts of C_9 α,ω-dicarboxylic acid bound to the humin matrix by ester linkages was previously demonstrated for an acidic peaty soil (Grasset and Amblès, 1998b). On the other hand, quantitative results from Table 3 show that the amount of α,ω-dicarboxylic acids is only 2.5 times lower after TEAAc than after TMAH treatment (180 vs 450 mg/kg of total organic matter). No α,ω-dicarboxylic acid methyl ester was detected after the TEAAC treatment. These results indicate clearly that an important part of the DAMEs obtained by TMAH treatment was initially present in humin under a "free", uncombined acidic form, the other part corresponding to dicarboxylic acids bound to the humin matrix by ester groups (they probably correspond to bridges between alkyl chains). Substantial differences are observed between TMAH and TEAAc treatments of humin: in the TEAAc treatment (Fig. 6b), the C_9 diacid is absent and the relative abundance of even-carbon-numbered diacids compared to odd-carbon-numbered ones, is lower than in the TMAH treatment (Fig. 6d) as expressed by the CPI values in the C_{16}–C_{30} range (TEAAc: 2.30; TMAH: 6.67). It thus appears that the ester-bound acids are predominantly even-carbon-numbered compounds, i.e. they show a less altered distribution when compared to biological acids than the trapped ones. This suggests that the ester-bound acid moieties are better preserved towards degradation than the trapped ones, in agreement with the results of Zegouah et al. (2000).

Compared with that of humin (Fig. 6d), the distribution of DAMEs obtained after TMAH treatment of humic acids (Fig. 6c) exhibits a similar range of α,ω-dicarboxylic acids but with different distributions. Only trace amounts of α,ω-dicarboxylic acids as methyl (DAMEs) or ethyl (DAEEs) esters were detected after TEAAc treatment (Table 3). As a consequence, it can be concluded that α,ω-dicarboxylic acids present in humic acids are linked to the humic acid matrix by ester groups. The lower CPI value found in humic acids (1.92 compared to 6.67 for humin) probably indicates that esterified dicarboxylic acids are more accessible to biodegradation in humic acids than in humin.

4. Conclusion

Preparative off-line thermochemolysis provides significant advantages over classical flash pyrolysis for the analysis of humic materials. Relatively large amounts can be treated and the quantity of pyrolysis products thus obtained in one experiment enables chromatographic separation, as well as precise qualitative and quantitative analyses.

The combination of TMAH thermochemolysis and TEAAc treatment makes it possible to discriminate between the different forms of mono- and dicarboxylic acids present in humin and humic acids: "free" uncombined acids, methyl or ethyl esters present as tightly trapped molecules within the organic matrix, acids chemically linked to the matrix by ester groups. The results of the present work confirm that lipid moieties contribute to the structure of humin and humic acids and are bound by ester groups. The cross-linking of moieties originating from microbial metabolism or inherited from higher plants is partly assumed by these chemical groups.

Fatty monocarboxylic acids and linear dicarboxylic acids are present as free acids in the humin of the peat sample. Humin also contains fatty acid methyl esters. Free, uncombined α,ω-dicarboxylic acids were only found in humin. The commonly used TMAH treatment does not allow such a discrimination. The results of the work suggest that acid moieties chemically bound to humin or humic acids are better preserved towards biodegradation than the trapped ones, and that humin constituents are more resistant than humic acid ones, maybe as a consequence of more complex, sterically hindered structure.

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