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# Structural characterization of humic substances from an acidic peat using thermochemolysis techniques

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**Abstract** – The general aim of the present work is a study of the insoluble organic matter (humic acids, humin) present in an acidic wetland, and more particularly lipidic parts involved in their chemical structure. Humic acids and humin from an acidic peat bog located at Brennilis (Sphagnum, pH 4.7) in Brittany (France) were characterised by spectroscopic properties (FT-IR and solid state  $^{13}\text{C}$  NMR) of their functional groups. Analytical and preparative thermochemolysis (pyrolysis using tetraalkylammonium hydroxide) were also used to investigate humic substances. This technique yielded various ranges of hydrocarbons, fatty acid methyl esters and  $\alpha,\omega$ -dicarboxylic acid methyl esters. In both cases, pyrolysates contained high amounts of polar macromolecular components and aromatic products, mainly derived from lignin moieties, as syringic and p-coumaric units. Our results indicate that structural similarities exist between humic acids and humin in the Brennilis sample, but important structural differences can be pointed out by thermochemolysis.

**peat / thermochemolysis / humin / humic acids**

**Résumé** – **Étude structurale de substances humiques d'une tourbe acide par thermochimiole.** L'objectif général de ce travail est l'étude des formes insolubles de matière organique (humine, acides humiques) présentes dans une tourbe acide, et plus particulièrement de leur composante lipidique. Les principaux groupes fonctionnels des acides humiques et de l'humine de la tourbe acide de Brennilis (Sphaignes, pH 4,7) en Bretagne (France) ont été caractérisés par spectroscopie (FT-IR et RMN  $^{13}\text{C}$  du solide). Plusieurs techniques de thermochimiole (pyrolyse en présence d'hydroxyde de tétraalkyl ammonium) analytique et préparative ont également été utilisées pour étudier les substances humiques. Ces techniques permettent d'isoler différentes séries d'hydrocarbures, d'esters méthyliques d'acides gras et d'esters  $\alpha, \omega$ -diméthyliques. Dans tous les cas, les pyrolysats contiennent une grande quantité de composés macromoléculaires et de produits aromatiques dérivés principalement de motifs ligneux, tels que les acides syringique et p-coumarique. Nos résultats indiquent que des similitudes existent entre les acides humiques et l'humine de l'échantillon de Brennilis, mais que des différences structurales importantes apparaissent par thermochimiole.

**tourbe / thermochimiole / humine / acides humiques**

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## 1. Introduction

Humins and humic acids correspond often to the main part of soil organic matter, and are well-known for playing an important role in soil processes [32]. Despite their geochemical importance, the structure of humic substances remains largely unknown, as a consequence of their heterogeneity and their great complexity. In this work, the structure of humins and humic acids from an acidic peat was investigated by Fourier transform Infrared and solid state  $^{13}\text{C}$  NMR spectroscopy, flash pyrolysis in the presence of tetramethylammonium hydroxide (TMAH) or tetrapropylammonium hydroxide (TPAH), and with a new preparative TMAH pyrolysis technique.

Pyrolysis with in situ methylation is a new, powerful technique for the structural analysis of macromolecules. This technique was firstly introduced by Challinor [5–7], and has been applied to several biopolymers [11, 33] like lignin [8, 24], humic substances [10, 13, 18, 22, 23, 26, 27, 29, 30, 34] or whole soil organic matter [31]. The presence of TMAH avoids decarboxylation of aromatic acids and dehydration of alcohol moieties and, as a consequence, prevents the loss of important structural information. Most of the pyrolysis products also become more volatile and more easily analysable by Gas Chromatography-Mass Spectrometry (GC/MS). Moreover, this procedure of TMAH pyrolysis has been described by several authors [9, 18, 22] as a thermally assisted chemolysis or thermochemolysis, rather than a classical pyrolysis. Recently, McKinney et al. [21] introduced an innovating thermochemolysis technique in sealed Pyrex<sup>®</sup> tubes, at a subpyrolysis temperature of 300 °C.

The preparative off-line thermochemolysis procedure used in this study has been developed in our laboratory [15,16] to treat large quantities of humic material (up to 2 g), in order to produce a high amount of pyrolysate that can therefore be separated by liquid chromatography prior to GC/MS analyses.

## 2. Materials and methods

### 2.1. Samples and preparation of humic material

The peat samples were collected in the Brennilis wetland (tourbière du Venec, Brittany, France), then freeze dried and sieved to 2 mm before analysis. After Soxhlet extraction of the free lipids with chloroform (3 × 24 h), a part of fulvic acids and minerals were removed from the residue by treatment with 1 M HCl (10 mL per g). Humic and fulvic acids were then extracted by 0.1 M NaOH (10 mL per g) under a nitrogen atmosphere. The alkaline treatment was reiterated 3 times to ensure total extraction. Humic acids were separated from fulvic acids by acidification to pH 1 (6 M HCl solution) and centrifugation (30 min, 7000 × g). The alkaline-insoluble residue was Soxhlet extracted with dichloromethane (2 × 24 h) to extract possibly adsorbed lipids. The final residue corresponded to a humin concentrate containing insoluble organic matter (humins) and the remaining mineral matter.

### 2.2. Analytical thermochemolysis

Humic samples (about 30 mg in 5 elemental pyrolysis) were moistened with a TMAH (Aldrich) solution (50% w/w in methanol) or a TPAH (Aldrich) solution (1 M in water) then introduced in a quartz tube before pyrolysis. The used pyrolysis device in a CDS Pyroprobe 1000. The temperature was raised from 350 °C to 650 °C (10 s isothermally) at a rate of 5 °C·ms<sup>-1</sup> under a helium flow. The products were directly analysed by GC/MS.

### 2.3. Preparative thermochemolysis

About 1 g of humic sample was placed in a ceramic boat and moistened with a TMAH/MeOH solution (50% w/w). The sample was dried under reduced pressure and introduced to a 60 × 3 cm i.d. Pyrex<sup>®</sup> tube. The tube was then placed in an oven heated at 400 °C for 1 hour. Thermochemolysis products were flushed out by a helium flow

(100 mL·min<sup>-1</sup>) to two successive traps containing chloroform cooled at -15 °C. Degradation products were obtained between 5 and 30 min after the tube was introduced in the oven. After evaporation of the solvent, pyrolysis products were separated by liquid chromatography on a SiO<sub>2</sub> column. Mixtures of diethyl ether/petroleum ether of increasing polarity were used for elution.

## 2.4. Analyses

Elemental analyses were provided by combustion on a CE Instruments NA 2100 analyser.

Fourier transform-Infrared spectroscopy (FT-IR) was performed on a Nicolet 750 spectrometer, after sample incorporation in KBr pellets.

The solid state <sup>13</sup>C NMR spectrum was obtained with a Bruker MSL 400 spectrometer at 4 kHz with magic angle spinning and cross-polarisation with a contact time of 1 ms. The chemical shifts were measured downfield of tetramethylsilane (TMS).

Thermochemolysis products were analysed by capillary GC and GC/MS. The GC was a Hewlett-Packard model 6890 equipped with a split/splitless injector, a FID detector, a 30 m × 320 μm × 0.25 μm fused silica capillary column containing a 5% methyl phenyl siloxane phase (Hewlett Packard HP-5). The GC oven temperature was raised from 60 °C to 300 °C (20 min isothermally) at a rate of 5 °C·min<sup>-1</sup>.

The GC/MS analyses were carried out on a Varian model 3400 interfaced with a Finnigan IncoS quadrupole mass spectrometer. The oven conditions were the same as for GC analyses. The structures of the various products were determined on the basis of their comparison with standards, of their mass spectra and by comparison with literature data.

## 3. Results and discussion

### 3.1. General properties

The studied soil sample was acidic (pH 4.7), it contained a high amount of organic matter (85 weight %, determined by combustion).

**Table I.** Amounts of the different forms of O.M.

	Humin*	Humic Acids	Fulvic Acids**	Lipids
g·kg <sup>-1</sup> o.d. soil	276	424	95	58
% of the T.O.M.	32.3	49.8	11.1	6.8

\* As pure humin.

\*\* By difference.

determined by combustion). The different forms of organic matter were separated using chemical selective extractions. The final residue or humin concentrate contained 83.74 wt % of organic matter (humin). As shown in Table I, humin and humic acids contributed respectively to 32.3 wt % and 49.8 wt % of the original organic matter, whereas lipids and fulvic acids represented a lower amount, 6.8 wt % and 11.1 wt %, respectively.

The elemental composition of humin and humic acids is given in Table II. The value of the atomic H/C ratio in humin and humic acids (1.63 and 1.38, respectively) indicates a highly aliphatic nature of these two types of organic matter.

FT-IR spectra of both humin concentrate and humic acids, presented in Figure 1, reveal a wide hydroxyl band from 2500 to 3500 cm<sup>-1</sup>, as well as polymethylenic bands between 2850 and 2925 cm<sup>-1</sup>. The band centred at 1652 cm<sup>-1</sup> is due to C=C overlapping the aromatic C=C band (~1600 cm<sup>-1</sup>), arising partly from lignin moieties. In the case of humic acids, the band near 1715 cm<sup>-1</sup> corresponds to C=O vibration from carbonyl or carboxyl (esters and acids) groups. The absorbance in the 1000–1100 cm<sup>-1</sup> range may partly arise from C–O vibration, but it can also originate from residual mineral matter, due to Si–O vibration [20], especially in the case of the humin concentrate.

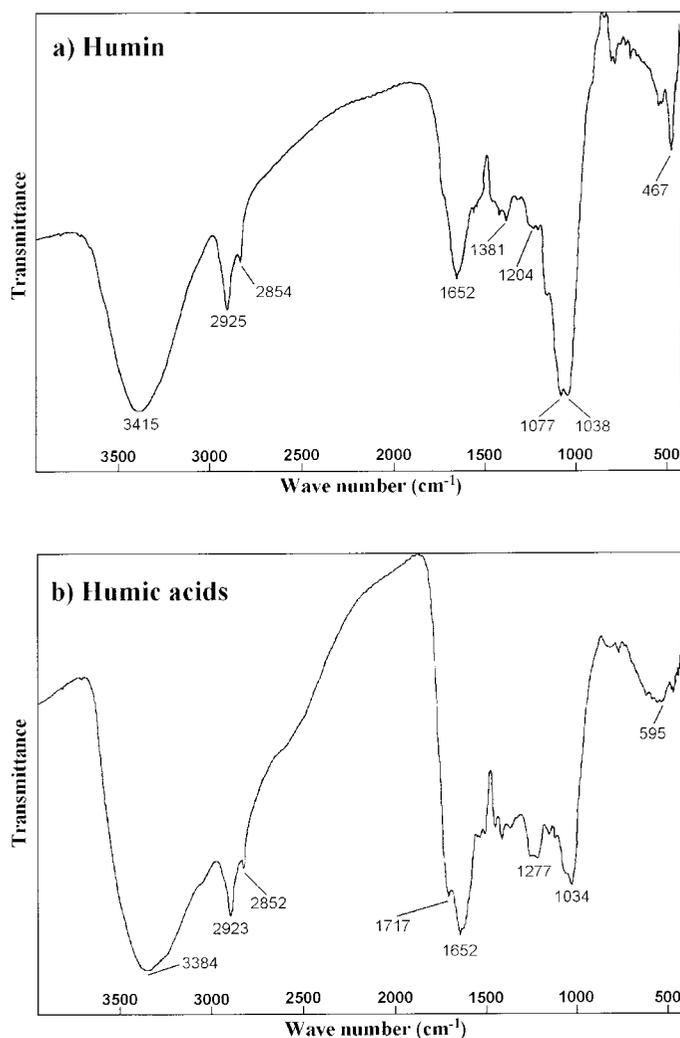
<sup>13</sup>C NMR spectroscopy (Fig. 2) confirms the high aliphaticity of humin concentrate and humic acids:

**Table II.** Elemental composition (%) of humin and humic acids.

Fraction	C	H	O*	N	S	Atomic H/C ratio
Humin (83.74)	42.33	5.75	49.56	2.36	0.00	1.63
Humic acids (94.40)	47.05	5.40	43.97	3.53	0.05	1.38

Numbers in brackets: percentage of organic matter, determined by combustion.

\* By difference.

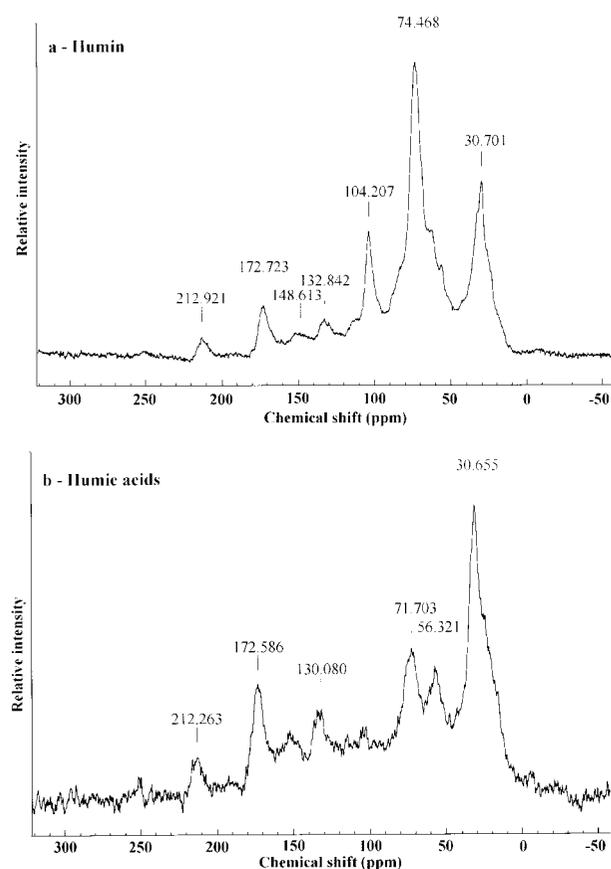


**Figure 1.** FT-IR spectra of humin and humic acids (wave numbers in  $\text{cm}^{-1}$ ).

the important (humin; Fig. 2a) or prominent (humic acids; Fig. 2b) signal at 30 ppm corresponds to paraffinic structures. Aromatic carbon signals (centred around 130 ppm) are present in both spectra, as well as phenolic carbons (150 ppm). The carboxyl signal at 172 ppm suggests the occurrence of ester and acid groups in the structure. The signal centred at 74.5 ppm; (humin; Fig. 2a) or 71.7 ppm (humic acids; Fig. 2b), which is particularly intense in humin, is attributable to O-alkyl carbons from carbohydrate units of cellulose and hemicellulose [1] and probably to some alcohol groups. The peak at 104 ppm in the humin spectrum corresponds to ketals and acetals from carbohydrates. Methoxyl

carbons from lignin are detected at 56 ppm [13] in the humic acids spectrum, and only as a shoulder in the case of humin. The observed signal centred at 212–213 ppm corresponds to carbonyl groups from ketones and possibly aldehydes. Nevertheless, a part of the signal at 212 ppm and at 130 ppm in humic acids (Fig. 2a) could be due to rotation side-bands of the signal at 172 ppm.

Finally, even if no precise quantitative determination is available,  $^{13}\text{C}$  CP/MAS NMR spectroscopy indicates a wide aliphatic contribution in the studied humic substances, and points out the occurrence of carbohydrate and/or cellulose and hemicellulose moieties, particularly intense in



**Figure 2.** Solid state CP/MAS  $^{13}\text{C}$  NMR spectra of humin concentrate and humic acids.

humin. Methoxyl carbons arising from lignin are more discernible in the case of humic acids.

### 3.2. Flash thermochemistry

#### 3.2.1. TMAH pyrolysis

Humin and humic acids were characterised by flash pyrolysis using tetramethylammonium hydroxide (TMAH). As a fact, the chromatogram of the pyrolysate appeared much more complex in humin than in humic acids (Fig. 3). In both cases, fatty acid methyl esters (FAMEs) arising from the transesterification of esters [14] were produced in the  $\text{C}_{14}$ - $\text{C}_{30}$  range (maxima at  $\text{C}_{16}$  and  $\text{C}_{24}$ ) with a strong even/odd predominance, the long  $\text{C}_{20}$ - $\text{C}_{30}$  members being more abundant in the case of

humin. The unsaturated  $\text{C}_{18:1}$  FAME is observed in both pyrolysates. Linear  $\text{C}_{15}$ - $\text{C}_{31}$  hydrocarbons (as alkene/alkane doublets) were released by humin treatment, whereas *n*-alkanes ( $\text{C}_{21}$ - $\text{C}_{27}$ ) are detected as traces in the case of humic acids. Prist-1-ene and the isoprenoid  $\text{C}_{18}$  methylketone were also present in the products from humin.

$\alpha$ ,  $\omega$ -dicarboxylic acid methyl esters were identified in the  $\text{C}_{16}$ - $\text{C}_{26}$  range (maxima at  $\text{C}_{16}$  and  $\text{C}_{22}$ ) with a strong even/odd predominance in the humin pyrolysate. The short  $\text{C}_8$  and  $\text{C}_9$  members were also strongly present. In the case of humic acids, only the  $\text{C}_{16}$  (maximum) and  $\text{C}_{18}$  members were detected.

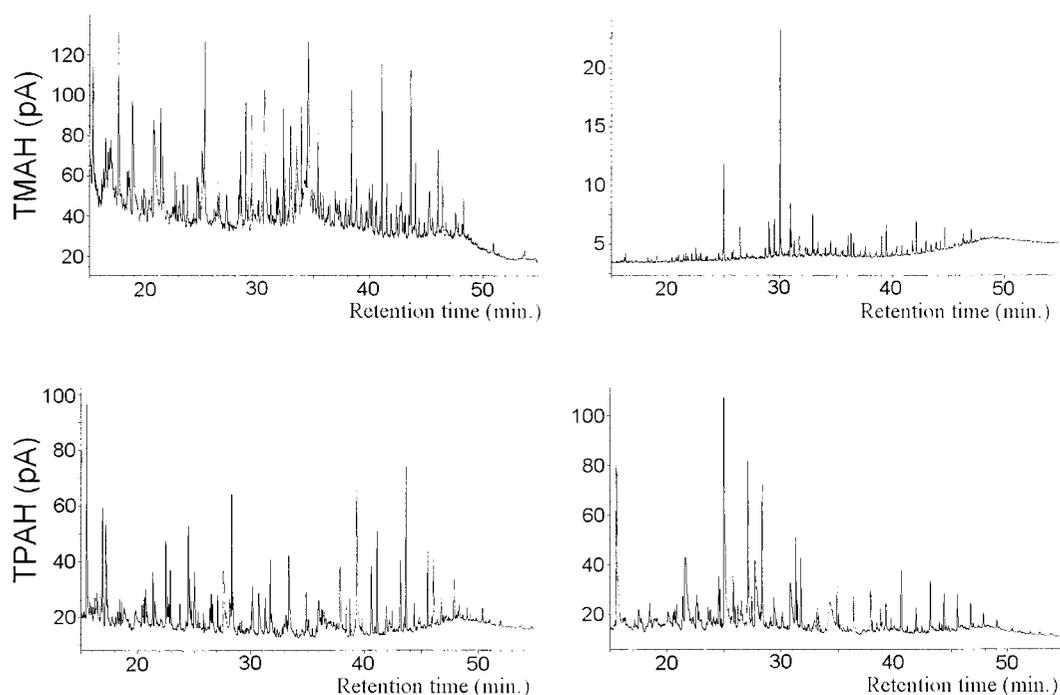
$\omega$ -methoxy methyl esters represented major components in both pyrolysates. They were found in the  $\text{C}_{16}$ - $\text{C}_{28}$  range (maxima at  $\text{C}_{16}$  and  $\text{C}_{24}$ ), they were exclusively even carbon-numbered. The  $\text{C}_{16}$  member is largely prominent in the case of humic acids, whereas the long  $\text{C}_{20}$ - $\text{C}_{28}$  mode is higher in humin.

Pyrolysates contained also a wide range of aromatic compounds bearing one or more oxygenated group, like methoxyl or methylester groups, presenting consequently high similarities with lignin monomers. Factually, the observed methoxyl groups could arise either from lignin monomers, and/or hydroxyl groups methylated by TMAH. In order to differentiate these two origins, flash-pyrolysis using an alternative alkylating agent, tetrapropylammonium hydroxide (TPAH) has been applied.

#### 3.2.2. TPAH pyrolysis

The aromatic compounds obtained by TPAH-thermochemistry of humin and humic acids had the same structure as those obtained with TMAH, one or more methyl groups being replaced by propyl groups. The main aromatic compounds observed with TMAH and the corresponding TPAH products are reported in Table III.

As a fact, no methylester derivative was observed in the TPAH thermochemistry products. Thus, the methylester (with TMAH) and propylester (with TPAH) groups originated exclusively from free or esterified carboxylic acids derivatised or transesterified by the alkylating agent.



**Figure 3.** Pyrograms obtained by flash pyrolysis of humin (left) and humic acids (right).

As seen in Table III, some methoxyl groups are also present in the TPAH products. It is an indication that they were initially present in the matrix as methoxyl groups, and not as free or bounded hydroxyl groups. Conversely, methoxyl groups observed with TMAH and replaced by propyloxyl groups with TPAH correspond either to free hydroxyl groups or to hydroxyl groups bound to the matrix by ester groups.

**Table III.** Main aromatic compounds obtained by flash TMAH and TPAH thermochemolysis of humin and humic acids (Me =  $-\text{CH}_3$ ; Pr =  $-(\text{CH}_2)_2-\text{CH}_3$ ).

<b>TMAH</b>								
<b>TPAH</b>								

In addition to these aromatic compounds, *n*-alkene/alkane doublets are observed in the TPAH products, as well as propyl esters outcoming from fatty acids. The propyl esters present the same distribution as the FAMES obtained with TMAH, confirming they were initially present as esterified acids in the macromolecular matrix.

### 3.3. Preparative thermochemolysis

#### 3.3.1. Quantitative results

A quantity of about 1 g of humic acids and humin was submitted to preparative thermochemolysis. The yields given in Table IV indicate that humin is slightly more reactive than humic acids. The products were separated by liquid chromatography on a silica column which allows the quantification of each individual class of components. The amounts of the various kinds of products are reported in Table IV. At this level, some differences between humin and humic acids are observable, humin releasing higher amounts of hydrocarbons and  $\alpha,\omega$ -dicarboxylic acid methyl esters.

**Table IV.** Quantitative results of thermochemolysis (in mg·kg<sup>-1</sup> of humic O.M.).

Fraction	Humins	Humic acids
Yield*	21%	16%
Hydrocarbons	11466	857
Fatty acid methyl esters	6454	5790
Methoxyacid methyl esters	767	437
$\alpha,\omega$ -dicarboxylic acid methyl esters	3430	809
Aldehydes	794	–
Ketones	1549	95
Aromatic compounds	18510	14901
Polar products	86330	60686

\* Based on the total obtained products.

We can notice that the major part of the thermochemolysis products is constituted by polar compounds, which are not compliant with GC/MS analyses. These products are obviously highly macromolecular units from humic material.

### 3.3.2. Detailed study of the thermochemolysis products

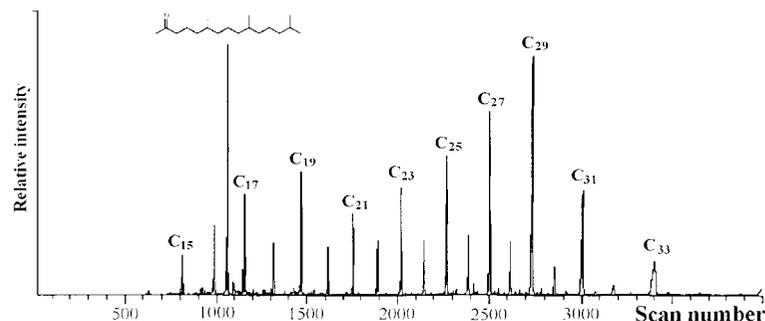
Both humin and humic acids released linear hydrocarbons, as classical *n*-alkene/*n*-alkane doublets from C<sub>16</sub> to C<sub>35</sub>, without any even/odd predominance. Unsaturated and saturated hydrocarbons can originate from the  $\beta$ -scission of radicals, but alk-1-enes can also derive from the decarboxylation of acids or the dehydration of alcohols. Actually, even if such secondary reactions are minimized in the presence of TMAH, it cannot entirely be prevented. The thermal degradation of esters is also known to generate alkanes (from the acid moiety) and alkenes (from the alcohol part). Prist-1-ene and prist-2-ene were identified in substantial

amounts, manifestly deriving from ether-bound phytol or tocopherol [16].

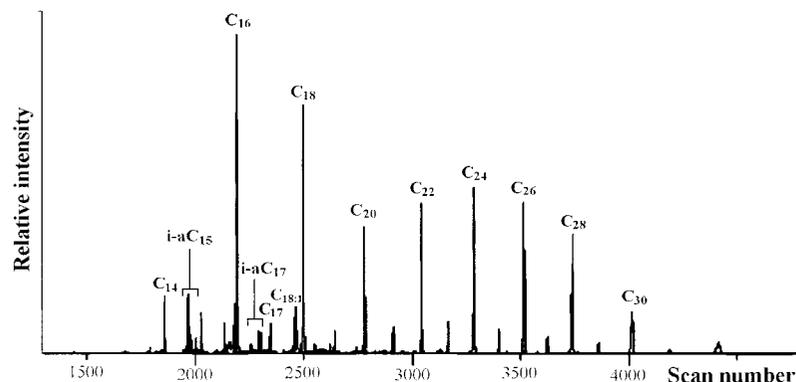
The presence of various hopanoid hydrocarbons with 17 $\beta$  (H), 21 $\beta$  (H) configuration (derived from bacteriohopanetetrol) was observed, ranging from C<sub>27</sub> to C<sub>31</sub>. The main compounds are the C<sub>27</sub>, C<sub>29</sub> hopenes and the C<sub>27</sub> hopane. 24-ethyl-cholesta-3,5-diene was also detected as traces. These polycyclic compounds were certainly bound to the macromolecular matrix via ether or ester linkage.

Thermochemolysis of humin freed a wide range (C<sub>15</sub>-C<sub>33</sub>) of linear methylketones (Fig. 4), with a marked odd/even carbon number predominance, in addition to an abundant C<sub>18</sub> isoprenoid methylketone (6,10,14-trimethylpentadecan-2-one). The origin of these compounds has not been clearly established. The presence of the C<sub>18</sub> isoprenoid methylketone was reported in the pyrolysis products from chlorophylls and cyanobacteria, the later containing also a series of linear methylketones [28]. As a consequence, the observed methylketones could be pyrolysis products from plant and microbial organic matter. However, no phytene nor phytadiene were found in the pyrolysates to confirm this assumption. Several mechanisms [12, 17], involving ether or C-C bond cleavage, can explain the formation of methylketones from alkyl chains bound to the matrix by ether group or from chains acting as substituent of the matrix and bearing an alcohol or a keto group.

Likewise, C<sub>17</sub> and C<sub>19</sub> aldehydes are produced by thermochemolysis of humin, as several branched and/or unsaturated isomers. Their origin could be explained by the same mechanisms as ketones but



**Figure 4.** Distribution of methylketones released by thermochemolysis of humin (m/z 58 selected ion fragmentogram).



**Figure 5.** Distribution of fatty acids methyl esters released from humin by thermochemolysis ( $m/z$  74 selected ion monitoring; Cn: linear; iCn: iso; aCn: anteiso).

we have no direct evidence. Moreover, methylketones and aldehydes could be trapped in the macromolecular network, and then freed after breakage of the complex matrix.

Fatty acids released as methyl esters follow a quite similar distribution in samples of humin and humic acids (Fig. 5), with a strong even/odd carbon number predominance. The long  $C_{20}$ - $C_{32}$  mode, centred on  $C_{24}$ , is representative of a plant input. The ubiquitous stearic and palmitic acids dominate the short  $C_{14}$ - $C_{18}$  mode. Unsaturated and/or branched isomers are also observed, specially  $C_{16:1}$  and  $C_{18:1}$ . Iso- and anteiso  $C_{15}$  and  $C_{17}$  isomers, characteristic of a bacterial contribution [3, 4, 25] are detected. The distribution of FAMEs released after preparative thermochemolysis of humic acids is almost the same as that from humin, except for the occurrence of higher  $C_{16:1}$  and  $C_{18:1}$  compounds.

The presence of  $\alpha$ - and  $\omega$ -methoxy fatty acid methyl esters, derived from hydroxyacids, are observed in humic acids and humin pyrolysates.  $\omega$ -methoxy methyl esters, detected in the  $C_{16}$ - $C_{30}$  range, are exclusively even carbon-numbered, with maxima at  $C_{16}$  and  $C_{28}$ .  $\alpha$ -methoxy methyl esters, from  $C_{22}$  to  $C_{26}$ , were produced in minor amounts by humin treatment. Only the  $C_{24}$  and  $C_{26}$  members are detected as traces in the case of humic acids.

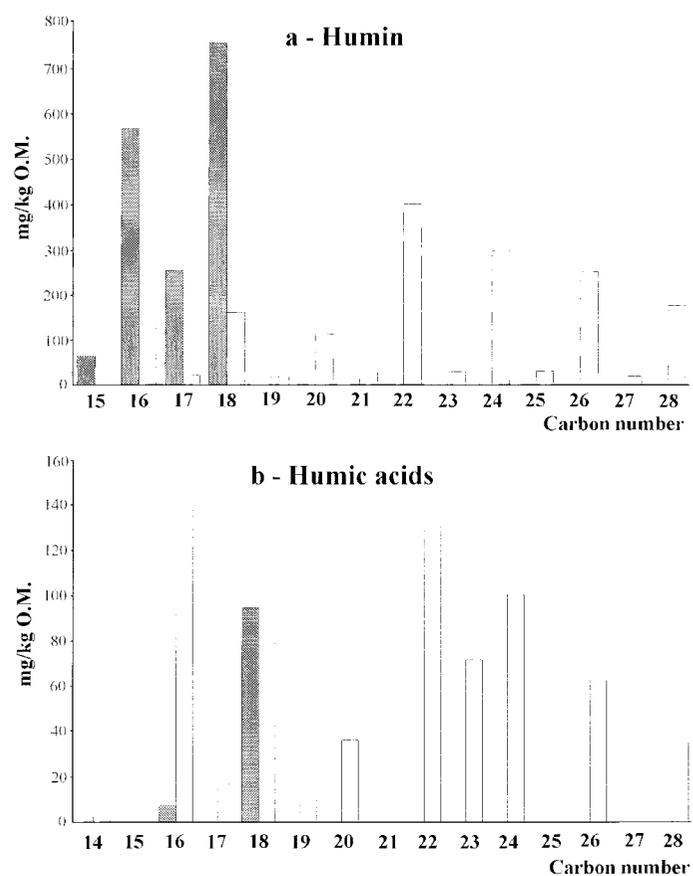
The obtained methoxyl group can derive from free hydroxyl, but it can also arise from the rupture of an ester (by transalkylation) or ether linkage.

$\alpha,\omega$ -dicarboxylic acid methyl esters were observed in the  $C_{14}$ - $C_{28}$  range, with a strong even/odd predominance. The  $C_{16}$ ,  $C_{18}$  and  $C_{22}$  members are prominent, as well as the unsaturated  $C_{16:1}$  and  $C_{18:1}$ , especially in the case of humin (Fig. 6).  $\alpha,\omega$ -dicarboxylic acids can directly be inherited from higher plants [19]. They can also result from the bacterial terminal oxidation of fatty acids [2], followed by the incorporation to the humic matrix by esterification of both acid chemical groups. The observed double bond is maybe the consequence of carbon-heteroatom bond breakage, or more probably indicates the incorporation of unsaturated fatty acids, as oleic acid.

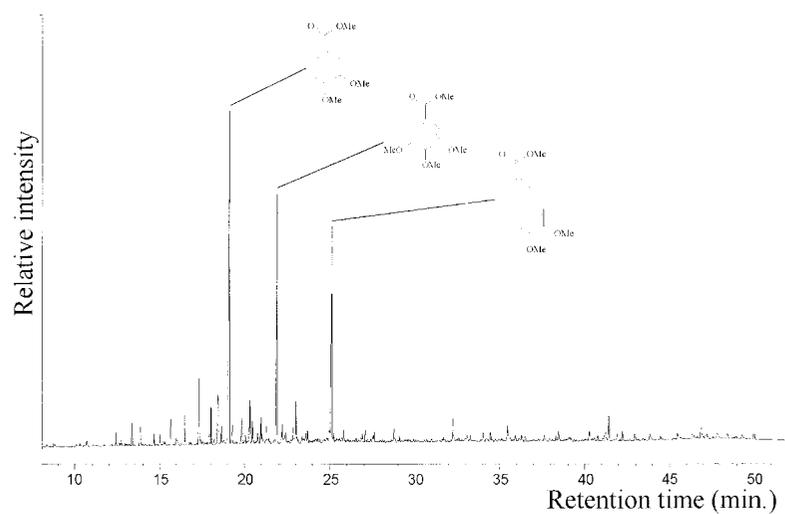
Aromatic compounds represent the main proportion of identifiable compounds in the thermochemolysis products of both humin and humic acids. They were the same as those observed by flash TMAH-pyrolysis (Fig. 7), vanillic, syringic and ferulic acids (as esters) being the most abundant in both humin and humic acids. As an evidence, these compounds indicate a high contribution of lignin moieties in the studied humic substances.

## 4. Conclusions

Infrared and NMR spectroscopy indicates that structural and functional similarities exist between



**Figure 6.** Distribution of dicarboxylic acids released as dimethyl esters by thermochemolysis of humin and humic acids (□ saturated; ■ unsaturated).



**Figure 7.** Chromatogram of the aromatic fraction obtained by thermochemolysis of the humic acids.

humins and humic acids, but important differences can also be found in their lipidic constituents when using analytical TMAH and TPAH thermochemolysis, then preparative TMAH pyrolysis; humins releasing a wider range of products, with a higher chemical diversity. Moreover, lignin derivatives play an important role in the retention of more aliphatic products in both humins and humic acids. Furthermore, ester (and certainly ether) groups have proved to be essential in the reticulation of those macromolecules.

The preparative thermochemolysis is an efficient method in complement to spectroscopic analyses, and permits confirmation of the results obtained with flash pyrolysis techniques. It allows the treatment of greater quantities, and so permits the estimation of quantitative results and the detection of trace compounds. However, these results have to be confirmed by selective chemical degradations, the mechanisms occurring during thermochemolysis being insufficiently known at the moment.

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