

1 Microwave-assisted extraction and hydrolysis: An alternative tool to pyrolysis for the analysis
2 of recalcitrant organic matter? Application to a forest soil (Landes de Gascogne, France)

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7

8 **Abstract**

9

10 A comparison was made between the composition of the recalcitrant organic matter (ROM)
11 isolated from a sandy forest soil as revealed by microwave assisted extractions and/or
12 hydrolyses and by usual pyrolysis techniques. Successive microwave irradiation treatments
13 were performed in H₂O, 0.1 and 1 M HCl and 0.1 and 1 M KOH. At each step of the
14 treatment the insoluble residue was examined via Curie point pyrolysis (CuPy) and Curie
15 point thermally assisted hydrolysis and methylation (CuTHM). Sequential irradiation
16 treatments resulted in ca 35% degradation of the ROM. Compounds released on microwave
17 irradiation in H₂O and in HCl were dominated by glucose, suggesting the occurrence of
18 carbohydrate-containing molecular associations in the soil organic matter (SOM) which were
19 not disrupted during acid hydrolyses and extractions as applied for the isolation of the ROM.
20 The product distribution from the microwave irradiation in KOH showed an important
21 contribution to the ROM from the higher plant polyesters cutin and suberin, and to a lesser
22 extent from lignin. Different lignin-derived compounds were specifically released upon
23 microwave acid or base hydrolyses. This suggested that two types of lignin monomers, ether
24 or ester linked, occurred in the ROM. The changes observed in the composition of the CuPy
25 pyrolysates of the residues from the different microwave hydrolyses are consistent with the
26 near complete removal of carbohydrates by microwave HCl hydrolysis. The changes observed
27 in the composition of the CuTHM pyrolysates of the residues from the different microwave
28 acid and base hydrolyses are in agreement with a major release of cutin- and suberin-derived
29 compounds upon microwave KOH hydrolysis. The CuPy and CuTHM pyrolysates of the final
30 residue consists predominantly in lignin-derived compounds. This study emphasizes the
31 potential of microwave-assisted hydrolyses to give a better estimate of the actual contribution

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32 of cutin to the ROM than pyrolysis. However, this technique appears to be unable to
33 completely release the lignin-based constituent of the ROM. Microwave irradiation appears to
34 provide great potential as a tool for extraction and chemical characterization of complex OM
35 and could be an attractive additional technique to pyrolyses.

36

37 **1. Introduction**

38

39 Soil organic matter (SOM) is one of the major carbon pools playing an important role in the
40 global carbon cycle. It contains a chemically recalcitrant (i.e. inert towards drastic laboratory
41 acid hydrolyses) organic matter fraction (ROM). This recalcitrant, non-hydrolysable SOM
42 fraction may account for a substantial part of the total organic carbon of the soil (Poirier et al.,
43 2002, 2003; Quénéa et al., 2005a, 2006a; Mikutta et al., 2006) and can contribute to the stable
44 carbon pool in the soil (Paul et al., 1997, 2006; Mikutta et al., 2006). The inherent or acquired
45 recalcitrance of the ROM fraction and consequent long mean residence time in the soil might
46 contribute to the potential role of SOM as a sink for atmospheric CO₂. The chemical
47 composition and origin of ROM have been chiefly studied using spectroscopic and pyrolytic
48 methods (Poirier et al., 2002, 2003; Naafs, 2004; Quénéa et al., 2005a, 2006a, b). Few
49 chemical degradation studies have been applied to characterise its composition (Quénéa et al.,
50 2005b; Naafs, 2004; Winkler et al., 2005). These studies have pointed out the diversity of
51 aliphatic structures making up the ROM network and the important role of ester functions to
52 link these constituents. Aliphatic constituents are usually considered to derive predominantly
53 from the biopolyesters cutin and/or suberin (Quénéa et al., 2005a,b) or from cutan and/or
54 suberan (Augris et al., 1998; Naafs, 2004), the recalcitrant biopolymers originating from plant
55 cuticles and suberized plant cell walls respectively (Nip et al., 1986; Tegelaar et al., 1995).
56 Some of the pyrolytic studies have also pointed out the significant preservation of
57 polysaccharide-type materials in the ROM (Quénéa et al., 2005a, 2006b). However, one of the
58 main drawbacks of the usually on-line pyrolytic techniques performed is the lack of
59 quantitative data about abundances of compounds released upon pyrolysis.

60 Microwave assisted extraction of organic compounds from matrices such as soils, seeds or
61 food was introduced by Ganzler et al. (1986). This extraction technique has been extended to
62 environmental analysis of contaminants in soils, sediments or water and to the extraction of
63 natural products (e.g. Letellier and Budzinski, 1999; Camel, 2000). Microwaves are high
64 frequency electromagnetic waves which are strongly absorbed by polar molecules.
65 Absorption results in rapid and intensive dielectric heating. Microwave systems using closed

66 vessels can operate at elevated temperature and pressure and temperature of solvents
67 submitted to microwave irradiation can be raised above their boiling point (e.g. Letellier and
68 Budzinski, 1999). The complex macromolecular recalcitrant fraction of SOM contains polar
69 constituents and strong localised heating can be expected to occur at these polar targets under
70 microwave irradiation. This could result in extraction and/or release of some of the
71 constituents of the matrix, opening up a new analytical possibility for obtaining information
72 on the chemical composition of such macromolecular material.

73 In this paper a comparison was made between microwave assisted extraction and/or
74 hydrolysis and pyrolytic methods for the chemical characterisation of recalcitrant organic
75 matter. Capabilities of the two methods were illustrated by analysis of the ROM isolated from
76 a sandy forest soil. The compositions of products released after neutral, acid and base
77 microwave irradiation treatments were analysed. We compared these compositions to those of
78 products released upon standard Curie point pyrolysis (CuPy/GC-MS) and Curie point
79 thermally assisted hydrolysis and methylation (CuTHM) with tetramethylammonium
80 hydroxyde (TMAH). The comparison was used to evaluate the potential of the two methods
81 for chemical characterization of complex organic matter.

82 **2. Materials and methods**

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84 All chemicals used were analytical grade.

85 86 *2.1. Sample*

87
88 The ROM sample was isolated from a soil collected from a maritime pine (*Pinus pinaster*)
89 forest. The dominant forest undergrowth was composed of ferns (*Pteridium aquilinum*) and
90 perennial grasses (*Molinia coerulea*; Jolivet, 2000). The isolation protocol and bulk features
91 of the ROM have been previously described (Quénéa et al., 2005a). Briefly, lipid- and humic
92 substance-free soil was submitted to stepwise acid hydrolyses using trifluoroacetic acid and
93 hydrochloric acid. The hydrolysed material was demineralised using HCl/HF treatment. The
94 ROM was recovered as the insoluble residue remaining after neutralisation and extraction
95 with CHCl₃/MeOH (2/1, v/v). The ROM accounted for 1.6% of the whole soil, i.e. about 34%
96 of the total initial carbon. (Quénéa et al., 2005a). Its elemental composition was 58.7% C;
97 4.2% H; 1.0% N; 4.5% ash (Quénéa et al., 2005a).

98 99 *2.2. Microwave assisted extraction and hydrolysis*

100
101 An outline of the sequential microwave assisted extractions and hydrolyses is shown in Fig. 1.
102 The treatments were performed with a single-mode CEM Discover[®] microwave reactor at a
103 frequency of 2450 MHz (0-300 W) in closed reaction vessels. The temperature was measured
104 with an infrared sensor outside the reaction vessel. The samples were subjected to 20
105 irradiation cycles. An irradiation cycle consisted in an irradiation period of 40 s followed by a
106 phase of cooling, without transfer of microwave energy, for 1 min.

107 The ROM (ca. 100 mg) in 2 ml H₂O was subjected to the irradiation cycles using 100 W
108 microwave energy. The maximum temperature and pressure were 140 °C and 2.5 10⁵ Pa
109 respectively. After the irradiation cycles, the reaction vessel was cooled to room temperature
110 with compressed air and the reaction mixture extracted at room temperature during 4 h with
111 CH₂Cl₂/MeOH (1:1 v/v; 30 ml). The reaction mixture was centrifuged at 1,400 g for 15 min.
112 The extract **1** was dried under reduced pressure, treated with 4 M HCl in MeOH [prepared by
113 mixing CH₃COCl with MeOH (1:2.5 v/v)] for 1 h at 60 °C to esterify carboxyl groups and
114 then with a mixture of pyridine/*N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; 10:1 v/v)
115 for 10 min at 60 °C to convert hydroxyl groups to trimethylsilyl ethers.

116 The insoluble residue **1** from the CH₂Cl₂/MeOH extraction was dried, transferred to a reaction
117 vessel and 2 ml 0.1 M HCl were added. The reaction mixture was subjected to 20 irradiation
118 cycles using 100 W microwave energy. The maximum temperature and pressure were 150 °C
119 and 10⁶ Pa respectively. After irradiation, H₂O (10 ml) was added and the reaction mixture
120 was centrifuged at 1,400 g for 15 min. An aliquot of the aqueous extract was dried under
121 reduced pressure and derivatized with BSTFA before GC-MS analysis. Another aliquot was
122 reduced with NaBH₄ (50 mg) for 1 h at room temperature (Albersheim et al., 1967). The
123 resulting products (alditols) were silylated with BSTFA. These alditols were identified using
124 GC comparison with trimethylsilyl ethers of standard alditols. The pellet was extracted at
125 room temperature during 4 h with CH₂Cl₂/MeOH (1:1 v/v; 30 ml) and centrifuged at 1,400 g
126 for 15 min. The organic phase (extract **2**) was dried under reduced pressure and the products
127 derivatised with HCl/MeOH and BSTFA. Aliquots of the dried residue **2** (ca. 3 mg) were
128 analysed using CuPy/GC-MS and CuTHM/GC-MS.

129 The remaining residue **2** was subjected to 20 irradiation cycles with 100 W microwave energy
130 in 2 ml 1 M HCl. The maximum temperature and pressure were 150 °C and 9 10⁵ Pa
131 respectively. After irradiation, H₂O (10 ml) was added and the reaction mixture was
132 centrifuged (1,400 g, 15 min). The aqueous extract was dried under reduced pressure,
133 derivatised with BSTFA and analysed using GC-MS. The pellet was extracted at room

134 temperature during 4 h with CH₂Cl₂/MeOH (1:1 v/v; 30 ml) and centrifuged (1,400 g, 15
135 min). The extract **3** was dried under reduced pressure and the products derivatised with
136 HCl/MeOH and BSTFA. Aliquots of the residue **3** were analysed using CuPy/GC-MS and
137 CuTHM/GC-MS. The residue **3** was subjected to 20 irradiation cycles with 100 W microwave
138 energy in 2 ml 0.1 M KOH. The maximum temperature and pressure were 150 °C and 6 10⁵
139 Pa respectively. After irradiation, the reaction mixture was acidified with 1 M HCl and
140 centrifuged at 1400 g for 15 min. The pellet was extracted at room temperature during 4 h
141 with CH₂Cl₂/MeOH (1:1 v/v; 30 ml) and centrifuged at 1,400 g for 15 min. The supernatant
142 (extract **4**) was dried under reduced pressure and the products derivatised with HCl/MeOH
143 and BSTFA. The residue **4** was subjected to 20 irradiation cycles with 100 W microwave
144 energy in 1 M KOH. The maximum temperature and pressure were 150 °C and 5 10⁵ Pa
145 respectively. The reaction mixture was acidified with 1 M HCl and centrifuged at 1,400 g for
146 15 min. The pellet was extracted at room temperature during 4 h with CH₂Cl₂/MeOH (1:1 v/v;
147 30 ml) and centrifuged (1,400 g, 15 min). The supernatant (extract **5**) was dried under reduced
148 pressure and the products derivatised with HCl/MeOH and BSTFA. The residue **5** was
149 analysed using CuPy/GC-MS and CuTHM/GC-MS. Yields were determined by weighting the
150 extracts and residues.

151

152 2.3. Analytical techniques

153

154 Gas chromatography-mass spectrometry (GC-MS) analysis was performed with an Agilent
155 6890 gas chromatograph coupled to an Agilent 5973N mass spectrometer with electron
156 ionization at 70 eV. Separation was achieved using a fused silica column coated with DB-
157 5MS (30 m, i.d. 0.25 mm, film thickness 0.5 µm) with He as carrier gas. The GC oven was
158 programmed from 100 °C to 320 °C at 4 °C min⁻¹. Compound identification was based on the
159 NIST mass spectrum library or interpretation of the spectra and comparison of GC retention
160 times with those of standards. When determined, the relative abundances of the positional
161 isomers of mid-chain hydroxyl alkanolic acids and mid-chain alkanedioic acids (compounds
162 **23**, **24**, **26**, **27** and **29** in Table 1) were estimated using the mass spectral α -fragmentation at
163 the secondary hydroxy group yielding the most intense fragment ion m/z
164 [MeOOC(CH₂)_nCHOTMSi]⁺ and assuming that the sensitivity was the same for each isomer.

165

166 2.4. Pyrolytic studies

167

168 Curie point pyrolysis gas chromatography-mass spectrometry (CuPy/GC-MS) was performed
169 using a Pilodist Curie point pyrolyzer. Samples (ca. 1 mg) were pyrolysed for 10 s using
170 ferromagnetic wires with a Curie temperature of 610 °C under a He flow of 5 ml min⁻¹. The
171 pyrolysis unit was directly coupled to the GC-MS system. The pyrolysis products were
172 separated using a Thermo Trace GC Ultra gas chromatograph equipped with a 30 m Rtx-5Sil
173 MS column (0.25 mm i.d., 0.5 µm film thickness). The oven temperature was held at 50 °C
174 for 10 min and raised to 300 °C at 2°C min⁻¹. The gas chromatograph was coupled to a thermo
175 DSQ mass spectrometer operating at 70 eV. For Curie point thermally assisted hydrolysis and
176 methylation (CuTHM) with tetramethylammonium hydroxide (TMAH), the same GC-MS
177 conditions as above were used. Samples (ca. 1 mg) were mixed with 100µL TMAH (25%
178 w/w in H₂O), partially dried under reduced pressure and loaded on the ferromagnetic wire
179 (Curie temperature 610 °C).

180

181 **3. Results**

182

183 *3.1. Microwave irradiation conditions*

184

185 The microwave reactor operated up to 2×10^6 Pa. In the case of higher pressures the system
186 was automatically vented. In the closed vessels used in this study, the maximum allowed
187 pressure corresponded to maximum temperatures of ca. 220 °C and 200 °C in H₂O and 0.1 M
188 HCl respectively. Preliminary experiments on humic acids (unpublished results) showed that,
189 upon continuous microwave irradiation in H₂O at 220 °C for 30 min, the temperature
190 (corresponding to a pressure of ca. $1.7 \cdot 10^6$ Pa) was rapidly reached (< 1 min). When the
191 temperature was reached the microwave energy strongly decreased (ca. 5-20 W). A two fold
192 increase in the irradiation time did not improve the yield of extraction. Conversely, the use of
193 several periods of irradiation followed by a cooling phase allowed to keep a high microwave
194 energy during a longer time and increased extraction yield (ca. 10% increase in the case of the
195 studied humic acids). Although we were aware that optimum irradiation energy, temperature
196 and numbers of cycles depended on the sample matrix, optimisation study was not carried out
197 for the ROM sample studied owing to the small available amounts of this sample. In this
198 study we used cycles consisting of irradiation at 100 W for 40 s followed by a cooling phase
199 of 1 min.

200

201 *3.2. Microwave irradiation in H₂O*

202
203 The microwave assisted H₂O extraction yielded very low amounts of products (1-2% of the
204 initial ROM). These products (Fig. 2a and Table 1 for peak annotations) were largely
205 dominated by monosaccharides. Small amounts of disaccharides were also detected at longer
206 retention times. Apart from predominant sugars, the extract afforded two sets of compounds.
207 The first one contained aliphatic compounds. It included (i) *n*-alkanoic acids ranging from C₁₄
208 to C₂₆ with C₁₆ and C₁₈ as the major components. A C₁₈ monounsaturated acid was also
209 present in the extract. (ii) *n*-alkan-1-ols ranging from C₁₆ to C₂₆ and dominated by C₁₈ and
210 C₂₄, (iii) ω-hydroxy acids ranging from C₁₆ to C₂₆ with C₁₆ and C₂₂ as major components and
211 mid-chain hydroxy acids consisting of 8/9/10-hydroxy octadecanoic acid (**23**), 8/9/10,16-
212 dihydroxy hexadecanoic acid (**27**) and 9,10-dihydroxy octadecanoic acid (**28**). 8-, 9- and 10-
213 Hydroxy octadecanoic acids accounted respectively for ca. 10, 45 and 45% of the total
214 isomeric mixture **23** (see 2.4 of Materials and Methods). 10,16- Dihydroxy hexadecanoic acid
215 was the major isomer (ca. 60% of the total isomeric mixture **27**) followed by 9,16- and 8,16-
216 dihydroxy hexadecanoic acids (ca. 30 and 10% of the isomeric mixture respectively). The
217 second set contained aromatic compounds consisting of substituted phenols (**4**, **9**, **12**; Table
218 1), substituted 2-methoxyphenols (**7**, **14**, **15**, **16**) and substituted 2,6-dimethoxyphenol (**13**).
219 Based on their mass spectra (Fig. 3), compounds **14** and **15** were considered to belong to the
220 the guaicyl units. The relative abundances of *p*-hydroxyphenyl, guaiacyl and syringyl units
221 were estimated using the peak areas in the mass chromatogram, assuming a similar sensitivity
222 for each compound. Guaiacyl units dominated the aromatics (ca. 45% of the total aromatics
223 including ca. 4% ferulic acid) followed by *p*-hydroxyphenyl (ca. 20% of the total aromatics
224 including 11% *p*-coumaric acid) and syringyl units (ca. 11% of the total aromatics).

225

226 3.3. Microwave irradiation in HCl

227

228 We could not undoubtedly state that products released using microwave irradiation in aqueous
229 HCl originated from hydrolysis rather than from an extraction process. However, comparison
230 of the product distributions between microwave irradiation in HCl and in H₂O (see
231 Discussion) suggested that a proportion of the products released from microwave irradiation
232 in HCl likely originated from acid hydrolysis of some constituents of the ROM. Therefore, in
233 this paper, we termed microwave irradiation in HCl as microwave HCl hydrolysis, keeping in
234 mind that this term likely grouped hydrolysis and extraction together.

235

236 3.3.1. Microwave irradiation in 0.1 M HCl

237

238 The products released upon microwave irradiation in HCl 0.1 M accounted for ca. 7% of the
239 residue **1** remaining after microwave irradiation in H₂O and consisted mainly of
240 monosaccharides. The aqueous extract (Fig. 1) accounted for ca. 70% of the products released
241 upon microwave irradiation in 0.1 M HCl and consisted predominantly of monosaccharides.
242 Low abundances of disaccharides and trace amounts of amino acids (mainly aspartic acid and
243 5-oxoproline) were also detected through GC-MS. Analysis of the trimethylsilyl ethers of the
244 alditols of the aqueous extract revealed that glucose was by far the predominant
245 monosaccharide present in the aqueous extract from microwave 0.1 M HCl hydrolysis.
246 Xylose and mannose were observed at extremely low levels. Arabinose and galactose were
247 not found.

248 The organic extract (extract **2**) was also largely dominated by monosaccharides (Fig 2b),
249 glucose being the major one based on retention times. Apart from the largely dominant
250 glucose, the organic extract from microwave 0.1 M HCl hydrolysis gave two additional
251 compound classes (Fig. 2b and Table 1 for peak annotations). The first one consisted of
252 aliphatic compounds and included (i) *n*-alkanoic acids ranging from C₁₄ to C₃₂ with C₁₆ and
253 C₁₈ as major components. A C₁₈ monounsaturated acid was also detected, (ii) *n*-alkan-1-ols
254 from C₁₂ to C₃₂ with C₂₂ and C₂₄ as major components, (iii) ω -hydroxy alkanolic acids ranging
255 from C₁₆ to C₂₈ and dominated by C₂₂, (iv) mid-chain hydroxy alkanolic acids comprising
256 8/9/10-hydroxy octadecanoic acid (each isomer accounted respectively for ca. 10, 45 and 45%
257 of the isomeric mixture **23**), 8/9/10,16-dihydroxy hexadecanoic acid (each isomer accounted
258 respectively for ca. 10, 30 and 60% of the isomeric mixture **27**) and 7/8-hydroxy
259 hexadecanedioic (**26**). The relative abundances of these isomers were respectively ca. 40 and
260 60% of the isomeric mixture **26**. α,ω -Alkanedioic acids were also detected in trace amounts.
261 The second class contained aromatic compounds comprising substituted phenols (**1**, **2**, **3**, **4**, **9**,
262 **12**), substituted 2-methoxyphenols (**6**, **7**, **16**) and substituted 2,6-dimethoxy phenols (**13**). On
263 the basis of their mass spectra tentatively identified and unidentified compounds (**8**, **14**, **15**,
264 **17**, **18** and **19**, Fig. 3) were considered to belong to this aromatic class. This class was largely
265 dominated by guaiacyl units (ca. 57% of the total aromatics including 1% ferulic acid). *p*-
266 Hydroxy phenols (including 2% *p*-coumaric acid) and syringyl units accounted for ca. 8 and
267 16% of the total aromatics, respectively. Isomeric cyclodimers of *p*-coumaric acid (**20**) were
268 also detected in small amounts. The mass spectra of isomers **20** (as methyl ester,
269 trimethylsilyl ether derivatives) were similar and exhibited the same major ions (*m/z* 250,

270 235) as in the mass spectrum of the methyl ester, trimethylsilyl ether of *p*-coumaric acid and
271 their fragmentation patterns (Fig.3g) were consistent with cyclodimers of *p*-coumaric acid
272 (Ford and Hartley, 1989).

273

274 3.3.2. Microwave irradiation in 1 M HCl

275

276 The products released upon microwave irradiation in 1 M HCl accounted for ca. 6% of the
277 residue **2** remaining after microwave irradiation in 0.1 M HCl. The aqueous extract (ca. 60%
278 of the products released upon microwave irradiation) and the organic extract (extract **3**) were
279 qualitatively the same as those resulting from microwave irradiation in 0.1 M HCl. Glucose
280 largely dominated both aqueous and organic extracts. In the aqueous extract, disaccharides
281 were hardly detected and trace amounts of the same amino acids as in the aqueous extract
282 from microwave irradiation in 0.1 M HCl were present (data not shown). Similar aromatic
283 and aliphatic compounds were identified in the organic extract **3** (data not shown). However,
284 the relative abundances of the aromatic and aliphatic compounds were much lower than in the
285 organic extract from the microwave irradiation in 0.1 M HCl.

286

287 3.4. Microwave irradiation in KOH

288

289 As in the case of microwave irradiation in HCl, it could be assumed that hydrolysis
290 (particularly of esters) occurred upon microwave irradiation in KOH. Microwave irradiation
291 of the residue **3** in 0.1 M KOH yielded very low amounts of products. The organic extract
292 (extract **4**) accounted for ca. 1% of the residue. By contrast, the organic extract from
293 microwave irradiation in 1 M KOH (extract **5**) accounted for 22% of the residue **4**. Similar
294 products were identified in both extracts, so only the products released upon microwave
295 irradiation in 1 M KOH were presented here. Fig. 2c depicted the distribution of the products
296 extracted from the microwave 1 M KOH hydrolysate (extract **5**). The microwave KOH
297 hydrolysis yielded two main compound classes. The first contained aliphatic compounds
298 which largely dominated the extract. It consisted in (i) *n*-alkanoic acids ranging from C₁₄ to
299 C₃₂ with C₁₆, C₁₈ and C₂₈ as the major constituents. A C₁₈ monounsaturated acid was also
300 present, (ii) *n*-alkan-1-ols from C₁₆ to C₃₂ with maximum at C₂₂ and C₂₄, (iii) ω-hydroxy
301 alkanolic acids ranging from C₁₄ to C₂₈ and maximising at C₂₂. A monounsaturated C₁₈ ω-
302 hydroxy acid was also detected in low amounts, (iv) mid-chain hydroxy alkanolic acids (**21**,
303 **22**, **23**, **25**, **27**, **28**, **29**, **30**, Table 1 for peak annotations) dominated by 8/9/10,16 dihydroxy

304 hexadecanoic acids (each isomer accounted respectively for ca 10, 30 and 60% of the
305 isomeric mixture **27**) and hydroxy alkanedioic acids (**24**, **26**) comprising C₁₅ and C₁₆
306 homologues with 7/8-hydroxy hexadecanedioic (each isomer accounted for ca. 40 and 60% of
307 the isomeric mixture **26**) as the major component. α,ω -Alkanedioic acids ranging from C₁₆ to
308 C₂₈ with C₂₂ and C₂₄ as the major constituents were also detected in trace amounts. The
309 second class comprised aromatic compounds consisting of substituted phenols (**1**, **2**, **3**, **4**, **9**,
310 **10**, **12**, Table 1 for peak annotations), substituted 2-methoxyphenols (**5**, **6**, **7**, **14**, **16**) and
311 substituted 2,6-dimethoxyphenols (**13**). Isomeric cyclodimers of *p*-coumaric acid (**20**) were
312 also present in substantial amount. This compound class was dominated by guaiacyl units (ca.
313 46% of the total aromatics, including 9% ferulic acid) and *p*-hydroxy phenols (ca. 38% of the
314 total aromatics, including 29% *p*-coumaric acid). Syringic units accounted for ca. 6% of the
315 total aromatics.

316

317 *3.5. Pyrolytic study of the residues*

318

319 In order to substantiate the efficiency and specificity of the microwave irradiation under
320 acidic and basic conditions, the insoluble residues obtained after 0.1, 1 M HCl and 1 M KOH
321 microwave hydrolyses (residues **2**, **3** and **5**) were subjected to Curie point pyrolysis
322 (CuPy/GC-MS) and thermally assisted hydrolysis and methylation (CuTHM/GC-MS).
323 Microwave irradiation in H₂O and in 0.1 M KOH releasing very low amounts of products,
324 therefore, the CuPy/GC-MS and CuTHM/GC-MS of the corresponding residues were not
325 performed.

326

327 *3.5.1. CuPy/GC-MS*

328

329 CuPy/GC-MS traces for the ROM and for the residues **2**, **3** and **5** were shown in Fig. 4. The
330 identified products were listed in Table 2. The ROM pyrolysate (Fig. 4a) was characterised by
331 the presence of four main compound classes. The first was constituted of furans (**1**, **2b**, **8**),
332 levoglucosenone (**6**) and levoglucosan (**15**) which were generally considered as
333 polysaccharide pyrolysis products. Aromatic compounds (**2a**, **3**, **4**, **5**, **7**, **9-14**, **16**), likely
334 related to lignin, constituted the second class. The third class was composed of *n*-alkane/*n*-
335 alkene doublets. The series, ranging from C₉ to C₃₂ did not show any clear carbon number
336 predominance, except the relatively high abundance of the C₂₀, C₂₂ and C₂₄ homologues. The

337 last class was constituted by *n*-alkan-2-one/*n*-alken-2-one doublets. The series, with a strong
338 odd/even predominance, ranged from C₁₉ to C₃₅ with C₂₉ and C₂₇ as the major components.

339 The pyrochromatogram of the residue obtained after microwave 0.1 M HCl hydrolysis (Fig.
340 4b; Table 2 for peak annotations) shown a strong decrease in the relative abundance of
341 levoglucosan (**15**). The decrease in the relative abundances of the other compounds related to
342 polysaccharides (i.e. **1**, **2b**, **6**, **8**) was more pronounced after microwave 1 M HCl hydrolysis
343 (Fig. 4c; Table 2 for peak annotations). Within the other classes (aromatic, *n*-alkane/*n*-alkene
344 and *n*-alkan-2-one/*n*-alken-2-one), the relative abundances of the constituents appeared to
345 remain rather constant after these irradiations.

346 The pyrochromatogram of the residue obtained after microwave 1 M KOH hydrolysis (Fig.
347 4d) shown a strong decrease of the relative abundances of the *n*-alkane/*n*-alkene and *n*-alkan-
348 2-one/*n*-alken-2-one doublets. It was dominated by 2-methoxy phenol (**5**) and 4-methyl-2-
349 methoxyphenol (**7**).

350

351 3.5.2. CuTHM/GC-MS

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353 Fig. 5 shown the total ion current (TIC) chromatograms of the thermochemolysates of the
354 ROM and of the residues **2**, **3** and **5**. The compounds identified as methyl esters and methyl
355 ethers were listed in table 3.

356 The CuTHM pyrolysate of the ROM (Fig. 5a) was quite similar to that obtained by Quénéa et
357 al. (2005a). Two main product classes constituted this pyrolysate. The first was constituted by
358 aromatic compounds (**4-19**, **27**) which were dominated by the methylated counterparts of
359 vanillic acid (**14**) and *p*-coumaric acid (**17**). Isomeric cyclodimers of *p*-coumaric acid (**27**)
360 were also present at longer retention time. The second class contained aliphatic compounds
361 including as the major constituents: (i) *n*-alkanoic acids (a C₁₈ monounsaturated acid was
362 identified), ranging from C₁₆ to C₃₂ with a strong even carbon number predominance. The
363 distribution was dominated by long chain components (>C₂₀); (ii) *n*-alkan-1-ols ranging from
364 C₂₀ to C₃₂ with a strong even/odd predominance and a maximum at C₂₂. (iii) ω -hydroxy acids
365 ranging from C₁₆ to C₃₀ with an even/odd predominance and C₂₂ and C₂₄ as the major
366 components; (iv) long chain α,ω -alkanedioic acids, present in lower relative abundances,
367 ranging from C₂₀ to C₂₈. The distribution exhibited an even/odd predominance and was
368 dominated by C₂₄; (v) mid-chain hydroxy alkanolic and hydroxy alkanedioic acids (**20-26**,
369 Table 3 for peak annotation). The tentative identification of these compounds was based on
370 the interpretation of their mass spectra (Fig. 6). When several structures were possible, the

371 carbon chain lengths found for the mid-chain hydroxy compounds identified in the extracts
372 were selected (i.e. C₁₅, C₁₆ and C₁₈). The resulting identification suggested an incomplete
373 methylation of some hydroxyl groups (compounds **22**, **24** and **26**). A minor series of *n*-
374 alkane/*n*-alkene doublets ranging from C₁₁ to C₃₃ with no obvious carbon number
375 predominance was also identified.

376 The pyrochromatograms of the residues obtained after microwave HCl hydrolyses shown a
377 decrease in the relative abundances of the aliphatic compounds (Fig. 4b, c) with the exception
378 of *n*-alkane/*n*-alkene doublets whose relative abundances appeared to increase. The decrease
379 in the relative abundances of aliphatic compounds was clearly more pronounced after
380 microwave KOH hydrolysis (Fig. 4d).

381 The TIC trace for the residue remaining after microwave irradiation in 1 M KOH was largely
382 dominated by aromatic compounds with vanillic acid (**14**) and *p*-coumaric acid (**17**) as the
383 major constituents. Within the aliphatic compound class, the decrease in the relative
384 abundances of ω -hydroxy acids resulting from microwave KOH hydrolysis, appeared to be
385 stronger than that of the other constituents (e.g. alkanolic acids or alkanols).

386

387 **4. Discussion**

388

389 *4.1. Comparison between microwave irradiation in H₂O and HCl. Extraction or hydrolysis?*

390

391 Although we cannot completely rule out the possibility of bond cleavage under the influence
392 of temperature and pressure, we can assume that products released on microwave irradiation
393 in H₂O arose mainly from a desorption process. It is then likely that monosaccharides, lignin-
394 derived and aliphatic compounds found in the H₂O extract are present as such in the ROM.
395 These compounds, likely adsorbed on (or entrapped in) the macromolecular structure of the
396 ROM, were not released during the isolation of the ROM despite the drastic acid hydrolyses
397 applied (Quénéa et al. 2005a). This could indicate that microwave energy interacts efficiently
398 with either the macromolecular structure or the adsorbed (or entrapped) compounds and is
399 able to disrupt such interactions. Contrary to the extract from microwave irradiation in H₂O
400 and 0.1 M HCl, disaccharides were hardly detected in the extract from microwave irradiation
401 in 1 M HCl, suggesting that at least part of the monosaccharides in 1 M HCl extract arises
402 from an acid hydrolysis of oligo- and/or polysaccharides. The occurrence of acid hydrolysis
403 during microwave irradiation in HCl is also supported by the presence of minute amounts of
404 amino acids which were not observed in the extract from microwave irradiation in H₂O. The

405 predominance of monosaccharides in the extracts from microwave irradiation in H₂O and HCl
406 indicates that intact monosaccharides, and probably oligo- and polysaccharides, are still
407 present in the ROM despite the intensive trifluoroacetic acid and HCl hydrolyses performed
408 during isolation (Quénéa et al., 2005a).

409 The GC-MS traces revealed a very similar distribution of aliphatic compounds in the extracts
410 from microwave irradiation in both 0.1 M HCl and H₂O (Figs. 2a, b). By contrast, some
411 differences are observed for the aromatic compound distributions between these extracts. The
412 greatest difference is a greater range of aromatic compounds in the extract from microwave
413 HCl hydrolysis. Seventeen aromatic compounds and cyclodimers of *p*-coumaric acid were
414 identified in the extract from microwave irradiation in 0.1 M HCl while only eight aromatic
415 compounds were present in the extract from microwave irradiation in H₂O. This difference,
416 together with the strong decrease in the relative abundances of disaccharides and the presence
417 of amino acids in the extracts from microwave HCl hydrolyses, suggest that hydrolysis of
418 some constituents of the ROM, in this case, carbohydrate, protein and lignin moieties,
419 occurred upon microwave irradiation in HCl.

420

421 4.2. Origin of aliphatic compounds

422

423 Aliphatic compounds in all the extracts from microwave irradiation in H₂O, HCl and KOH,
424 are well known constituents of cutin and suberin. The polyester cutin, present in the majority
425 of the aerial parts of vascular plants, and suberin, present in the bark and roots of vascular
426 plants, differ in the chain length and the substitution patterns of their monomers (Walton,
427 1990; Kolattukudy, 2001). Long chain ($\geq C_{20}$) *n*-alkanedioic acids, ω -hydroxy acids, *n*-
428 alkanolic acids and, to a lesser extent, *n*-alkan-1-ols are frequently dominant monomers of
429 suberin, while mid-chain substituted monomers are usually minor constituents. On the
430 contrary, cutin is characterised by substantial abundances of C₁₆ and C₁₈ mid-chain
431 substituted monomers, while long chain monomers ($\geq C_{20}$) are rarely present. In the extracts
432 from microwave irradiation in H₂O and in HCl (Figs. 2a, b), the distribution patterns of
433 aliphatic compounds are quite similar. Apart from ubiquitous C₁₆ and C₁₈ *n*-alkanoic acids,
434 the aliphatic compounds are dominated by long chain ($\geq C_{20}$) components which can be
435 considered as predominantly from a suberin source. However, the high relative abundances of
436 C₂₂ and C₂₄ *n*-alkan-1-ols suggests they could also originate from the plant wax esters in

437 which they are widespread (Bianchi, 1995). The mid-chain hydroxy constituents, probably
438 originating from cutin, are present in lower amounts.

439 Microwave irradiation in KOH is more efficient for promoting hydrolysis of ester linkages of
440 cutin and suberin than microwave irradiation in HCl. Therefore, the distribution pattern of
441 aliphatic compounds in the extract from 1 M KOH hydrolysis (Fig. 2c) strongly differs from
442 that of aliphatic compounds in the extracts from microwave irradiation in HCl (Fig. 2b). ω -
443 Hydroxy alkanolic acids with chain length $\geq C_{20}$, likely originating from suberin, largely
444 dominate. In addition, this extract contains C_{15} , C_{16} and C_{18} mid-chain hydroxy alkanolic and
445 C_{15} and C_{16} mid-chain alkanedioic acids in high amounts. This indicates a significant
446 contribution of cutin to the ROM. The contribution of cutin from pine, the predominant
447 vegetation, is indicated by the presence of trace amounts, of C_{12} and C_{14} ω -hydroxy alkanolic
448 acids, which have been reported as typical constituents of cutin of the needles of numerous
449 species of gymnosperms (Matzke and Riederer, 1991; Nierop, 2001; Nierop and Verstraten,
450 2004; Otto and Simpson, 2006). The contribution of cutin from pine needles could be also
451 suggested by the presence of mid-chain hydroxy pentadecanoic acids (**21**, **22**, **25**; Table 1)
452 and pentadecanedioic acid (**24**; Table 1), which have been reported as monomers of cutin of
453 some gymnosperms (Hunneman and Eglinton, 1972), pine needles and grasses (Otto and
454 Simpson, 2006). The dominant contribution of 8,16- and 10,16-dihydroxy hexadecanoic acid
455 to the dihydroxy hexadecanoic isomeric mixture (respectively ca. 30 and 60% of the isomeric
456 mixture **27** in all the extracts) suggests a significant contribution of cutin from the
457 undergrowth (predominantly grasses and ferns). Indeed according to Goñi and Hedges (1990),
458 in contrast to most of the gymnosperm species, Poaceae species produced high yield of cutin-
459 derived 8,16- and 10,16-dihydroxy hexadecanoic acids. Moreover, 10,16-dihydroxy
460 hexadecanoic acid has been reported as the most abundant component in the solvent-extracted
461 soil under grasses and ferns (Naafs and van Bergen, 2002) The relative contributions of
462 suberin and cutin to the ROM may be estimated using monomers typical of cutin or suberin
463 and monomers common to both polyesters as suggested by Otto and Simpson (2006). The
464 suberin/ cutin ratio of 1.8 calculated in this way from the microwave 1 M KOH hydrolysis for
465 the ROM corroborates the predominance of suberin input previously reported (Quénéa et al.,
466 2005a). According to Otto and Simpson (2006), this value resembles that obtained for
467 grassland soils rather for pine forest ones for which values < 1 were found. This could
468 indicate an important contribution of suberin from the undergrowth in addition to that of the
469 cutin previously suggested.

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4.3. Origin of aromatic compounds

Apart from the tentatively identified (**14**, **15**) and unidentified (**17-19**) aromatic compounds which were mainly found in the extract from microwave irradiation in 0.1 M HCl (Fig. 2b), similar aromatic compounds were present in all the extracts (Fig. 2; Table 1). These compounds were predominantly guaiacyl, *p*-hydroxy and syringyl phenols and were generally considered to be derived from lignin (eg. Brunow, 2001). However, substituted dihydroxyphenols (e.g. **9**, detected in significant amounts in the extract from microwave irradiation in H₂O and 0.1 M HCl, and **10**, present in the extract from microwave irradiation in 1 M KOH) could also originate from (condensed) tannins (e.g. Galletti et al., 1995; Nierop et al., 2005). *p*-Coumaric (**12**), ferulic (**16**) acids and 4-hydroxybenzaldehyde (**1**) can also arise from the aromatic domain of suberin (Walton, 1990; Bernards, 2002) to which they could be linked via ester bonds (Bernards and Lewis, 1998). As in the case of monosaccharides, the presence of these aromatic compounds in the extract from microwave irradiation in H₂O suggests that lignin monomers were present as such in the ROM. They could be adsorbed on (or entrapped in) the macromolecular structure of the ROM or they could form molecular associations with carbohydrates and could not be released upon extractions and hydrolyses of the isolation process. The relative abundances of guaiacyl and syringyl units increase upon microwave irradiation in 0.1 M HCl. The units released upon these conditions are likely present as arylglycerol- β -aryl ether structures, such structures being acid-hydrolysable (Adler et al., 1957; Lundquist and Lundgren, 1972). The tentative structures attributed to compounds **8**, **14**, **15**, **17**, **19** present in the extract from microwave irradiation in 0.1 M HCl (Fig. 3), could support this suggestion. Indeed similar types of ketones were found upon acid degradation of lignin (Adler et al., 1957; Lundquist and Lundgren, 1972). On the other hand, microwave irradiation in KOH leads to an increase in the relative abundances of *p*-coumaric, and ferulic acids. This suggests that these acids were predominantly in an ester-linked form in the ROM. They could be present esterified to lignin-polysaccharide complex or in the aromatic domain of suberin (e.g. Kolattukudy, 2001). The predominance of the guaiacyl over the syringyl units in all the extracts is consistent with the dominant gymnosperm vegetation. However, the significant amounts of *p*-hydroxyphenyl units, including high amount of *p*-coumaric acid, and ferulic acid, particularly in the extract from microwave irradiation in KOH, suggests a significant non-woody angiosperm undergrowth (likely gramineous plants) influence.

504 Interestingly is the presence of cyclodimers of *p*-coumaric acid (**20**) in the extract from
505 microwave HCl and KOH hydrolyses. To our knowledge, these aromatic compounds have
506 never been reported in soil lipids or in degradation products of soils or non-hydrolysable OM
507 from soils. Cyclodimers of *p*-coumaric (or ferulic) acid have been reported as constituents of
508 gramineous plant cell walls (Krauze-Baranowska, 2002 and references therein). They are
509 thought to be synthesized by photodimerisation of *p*-coumaric acid and to reduce the
510 biodegradability of the cell walls (Ford and Hartley, 1989). Their presence supports a
511 contribution of gramineous-derived compounds to the ROM although cyclodimerisation of *p*-
512 coumaric acid under microwave irradiation cannot be completely disregarded.

513

514 *4.4. Pyrolysis of the residues. Comparison of microwave extractions/hydrolyses with pyrolysis*

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516 In good agreement with earlier pyrolytic studies of the same ROM sample (Quénéa et al.,
517 2005a), the pyrochromatogram obtained by CuPy/GC-MS of the ROM (Fig. 4a) is dominated
518 by products commonly observed for the pyrolysis of cellulose and related carbohydrates
519 (Pouwels et al., 1989; Pastorova et al., 1994, Gauthier et al., 2003) i.e. levoglucosan,
520 levoglucosenone and 2,3-dihydrobenzofuran. A series of *n*-alkane/*n*-alkene doublets is
521 present in high relative abundance. Two main origins are generally considered for these
522 doublets, i.e. non-hydrolysable macromolecules of higher plants such as cutan or suberan
523 (Tegelaar et al., 1989; 1995; Augris et al., 1998; Nierop, 1998) or lipids incorporated through
524 covalent bonds into the recalcitrant macromolecular structures (Almendros and Sanz, 1992;
525 Almendros et al., 1996). The distribution pattern of this series appears to result from a
526 combination of a smooth distribution centered between C₂₄ and C₂₇ and separate C₂₀, C₂₂ and
527 C₂₄ doublets. This suggests that the *n*-alkane/*n*-alkene doublets likely derive from the two
528 aforementioned origins, the dominant C₂₀, C₂₂ and C₂₄ doublets likely originating from
529 incorporated lipids. The series of *n*-alkan-2-ones observed here, was not detected by Quénéa
530 et al. (2005a) in the pyrolysate of the same ROM sample, while it was observed, with a
531 similar distribution, in the pyrochromatogram of the double-shot pyrolysis of the same ROM
532 sample (Quénéa et al., 2006b). The differences in the techniques (e.g. magnetic wire vs
533 magnetic tubes) and/or temperature (610 °C in the present study, 650 °C for Curie point
534 pyrolysis and 600 °C for the double-shot pyrolysis) could explain these discrepancies. The
535 origin of *n*-alkan-2-ones is still a subject of speculation. β -Oxidation of *n*-alkanes (e.g.
536 Cranwell et al., 1987; Jaffé et al., 1996), β-oxidation and subsequent decarboxylation of *n*-
537 alkanolic acids (Amblès et al., 1989) and direct inputs of *n*-alkan-2-ones naturally occurring in

538 living organisms (e.g. Volkman et al., 1983; Quéneá et al. 2006b) have been postulated as
539 possible origins for *n*-alkan-2-ones present in sediments, soils or recalcitrant organic matter.
540 Moreover, as far as we are aware, such ketone doublets have not been previously reported in
541 pyrolysates. Their presence would indicate that the keto group is not formed upon pyrolysis,
542 the doublets being formed in a similar way as the *n*-alkane/*n*-alkene doublets.

543 Several phenolic compounds generally considered to originate from pyrolysis of lignin (e.g.
544 Gauthier et al., 2003) were also detected in high relative abundances (Fig. 4a; Table 2). It
545 should be noted that most of the phenolic compounds detected may be related to guaiacyl
546 units. Only phenol (**3**, Table 2), which displays a rather low relative abundance, may be
547 related to *p*-coumaric acid. However the composition of the extracts, especially that from
548 microwave 1 M KOH hydrolysis (Fig 2c) indicates a substantial contribution of *p*-coumaric
549 acid to the ROM. In addition, the methylated form of *p*-coumaric acid is present as one of the
550 major aromatic compounds in the CuTHM pyrolysate of the ROM (Fig. 5; Table 3). This
551 indicates that CuPy technique likely underestimates the contribution of *p*-coumaric acid to the
552 ROM.

553 The strong decrease in the relative abundances of the compounds derived from
554 polysaccharides (**1**, **2b**, **6**, **8**, **15**; Table 2) in residues from the microwave HCl hydrolyses
555 (Figs. 4b, c) is consistent with the predominance of monosaccharides in the extracts from
556 microwave HCl hydrolyses. In spite of drastic acid hydrolyses and extractions applied for the
557 ROM isolation, mono- and oligosaccharides are still present in significant amounts in the
558 ROM. The presence of such compounds in the ROM could be explained by the fact that
559 mono- and oligosaccharides belong to molecular associations (e.g. lignin-carbohydrate
560 complex) which could not be disrupted by the conventional heating used during the isolation
561 process. However, these molecular associations containing highly polar carbohydrates could
562 be disrupted upon microwave irradiation. Therefore it appears that carbohydrates could be
563 released from covalent or physicochemical entrapment. However, although greatly reduced,
564 the polysaccharidic contribution to the ROM was not completely removed, even after
565 microwave irradiation in 1 M HCl (Fig. 4c). The microwave HCl hydrolyses do not result in a
566 noticeable decrease in the relative abundance of compounds considered to derive from lignin
567 (i.e. **2a**, **3**, **5**, **7**, **9-14**, **16**; Table 2). Similar trend is observed for the *n*-alkane/*n*-alkene and *n*-
568 alkanon-2-one/*n*-alken-2-one doublets. This is consistent with the low relative abundances of
569 aromatic and aliphatic compounds present in the extract from microwave irradiation in HCl
570 (Fig. 2b).

571 The pyrochromatogram of the residue obtained after microwave 1 M KOH hydrolyses reveals
572 a strong decrease in the relative abundances of *n*-alkane/*n*-alkene and *n*-alkan-2-one/*n*-alken-
573 2-one doublets (Fig. 4d). Furthermore, among the series of *n*-alkane/*n*-alkene doublets, the
574 relative abundances of C₂₂ and C₂₄ homologues (assumed to derive from covalent linked
575 lipids) tend to decrease and fall in the same range as the whole series. This could be correlated
576 to the high relative abundances of C₂₂ and C₂₄ *n*-alkan-1-ols and/or ω-hydroxy alkanolic acids
577 observed in the extract from microwave 1 M KOH hydrolysis (Fig 2c) and in the CuTHM
578 pyrolysates of the ROM and the residues obtained after microwave HCl hydrolyses (Figs. 5a,
579 b, c). This suggests that at least part of the predominant C₂₂ and C₂₄ *n*-alkane/*n*-alkene
580 doublets originate from such C₂₂ and C₂₄ alcohols and/or ω-hydroxy alkanolic acids (or from
581 the moieties (eg. esters) from which they derive).

582 The microwave irradiation in 1 M KOH results in a substantial decrease in the relative
583 abundances of phenolic compounds with the exception of 2-methoxyphenol (**5**) and 4-methyl-
584 2-methoxyphenol (**7**) which largely dominate the pyrochromatogram (Fig. 4d). These two
585 compounds can be related to guaiacyl units of lignin

586 The distribution pattern of the compounds released upon CuTHM of the ROM (Fig. 5a) is in
587 good agreement with that obtained by Quéneá et al. (2005a) for the same sample. The
588 pyrochromatogram is dominated by aliphatic and aromatic compounds considered to derive
589 respectively from cutin and/or suberin and lignin. However, notable differences between the
590 two distributions are observed. *n*-Alkan-1-ols, mid-chain hydroxy alkanolic acids and
591 cyclodimers of *p*-coumaric acid observed in the present CuTHM pyrolysate have not been
592 identified by Quéneá et al. (2005a). Mid-chain hydroxy alkanolic acids (**20-26**; Table 3) were
593 tentatively identified on the basis of their mass fragmentation and by selecting the carbon
594 chain lengths found for this type of compounds in the extracts from the microwave
595 irradiations (ie C₁₅, C₁₆ and C₁₈). The proposed structures (Fig.6) involve the presence of non-
596 methylated hydroxyl groups in some of these compounds. Providing that these structures are
597 correct, this indicates that hydroxyl groups are only partially methylated upon CuTHM. The
598 partial methylation of hydroxyl groups have been reported in earlier studies (de Leeuw and
599 Baas, 1993, Kralert et al., 1995). It has been suggested that partial methylation of alkanols
600 upon CuTHM is due to competition between methylation and thermovaporization of these
601 compounds (de Leeuw and Baas, 1993, Kralert et al., 1995). The aromatic compounds are
602 dominated by the methylated counterparts of vanillic (**14**) and *p*-coumaric (**17**) acids (Fig. 5a;
603 Table 3).

604 Contrary to CuPy, typical products related to carbohydrates are not detected in the CuTHM
605 pyrolysate of the ROM. This is consistent with previous observations (Quénéa et al., 2005a).
606 It must also be noted that the major aromatic products from the CuPy (**5** and **7**; Fig. 4) appear
607 in CuTHM pyrolysate as rather minor products (**4** and **6**; Fig 5) whereas vanillic (**14**) and *p*-
608 coumaric (**17**) acids largely dominate. This highlights the much higher efficiency of CuTHM
609 when compared to CuPy for the detection of lignin-derived compounds.

610 The CuTHM pyrolysates of the residues obtained after microwave HCl hydrolyses (Figs. 5b,
611 c) reveal a decrease in the relative abundances of aliphatic compounds. By contrast, and as
612 already noticed for the CuPy pyrolysates of these residues, no notable change in the relative
613 abundances of aromatic compound is observed. The decrease in the relative abundances of ω -
614 hydroxy acids and, to a lesser extent that of *n*-alkan-1-ols, appears to be more pronounced
615 than that of *n*-alkanoic acids. For example, when the CuTHM pyrolysate of the ROM and that
616 of the residue from microwave 1 M HCl hydrolysis are compared (Fig. 5a, c), the C₂₂ ω -
617 hydroxy acid to C₂₂ *n*-alkanoic acid and C₂₂ *n*-alkan-1-ol to C₂₂ *n*-alkanoic acid ratios are
618 respectively ca. 3 times and 2 times lower in the latter pyrolysate, while the C₂₂ ω -hydroxy
619 acid to C₂₂ *n*-alkan-1-ol ratio is only 1.3 times lower. The same trend is observed for C₂₄
620 homologues (Table 4). It appears that *n*-alkanoic acids are more difficult to release by
621 microwave HCl hydrolyses. This is consistent with the distribution of the aliphatic
622 compounds in the extract from microwave HCl hydrolyses. Indeed, in these extracts, the
623 aliphatic compounds were dominated by ω -hydroxy acids and *n*-alkan-1-ols. This result
624 suggests the existence of different pools for *n*-alkanoic acids on the one hand, and for ω -
625 hydroxy acids and *n*-alkan-1-ols on the other hand.

626 In good agreement with the composition of the extract from microwave 1M KOH hydrolysis,
627 the decrease in the relative abundances of aliphatic compounds is especially marked in the
628 CuTHM pyrolysate of the final residue (Fig. 5d). However, in contrast to the CuTHM of the
629 residues from microwave HCl hydrolyses, the decrease in the relative abundances of the
630 different aliphatic compounds appears to be uniform (Table 4). Regarding the aromatic
631 compound class (**1-18**; Figs 5a-d) no noticeable change in the relative abundance of its
632 constituents were observed between the CuTHM pyrolysates of the ROM and the residues **2**,
633 **3** and **5**. The final residue obtained after microwave extractions and hydrolyses appears to be
634 predominantly composed of aromatic compounds with vanillic and *p*-coumaric acids as the
635 major components. This indicates that at least part of lignin-derived constituents are resistant
636 to microwave acid and base hydrolyses. By contrast, these constituents can be released by

637 thermal cracking. However, the microwave irradiation of commercial lignin (Lignin,
638 hydrolytic, Aldrich) in 0.1 M NaOH resulted in almost 100% degradation (unpublished
639 results). This suggests that lignin in the ROM is efficiently protected.

640 At this stage, work is preliminary and the mechanistic aspects of microwave hydrolyses of
641 such recalcitrant macromolecular material must be studied and the range of samples
642 expanded.

643

644 **5. Conclusions**

645

646 The recalcitrant organic matter (ROM) isolated from a forest soil was submitted to sequential
647 microwave assisted extractions and/or hydrolyses in H₂O, HCl and KOH, resulting in ca. 35%
648 degradation of the initial ROM. The overall extracts consisted in ca. 10% carbohydrates and
649 ca. 25% aliphatic and aromatic compounds.

650 The differences between the distributions in the extracts from microwave irradiation in H₂O
651 and in HCl suggest that acid hydrolysis of oligo(poly)saccharides and oligo(poly)peptides
652 occurred upon microwave irradiation in HCl.

653 Products resulting from microwave irradiation in H₂O and in HCl are strongly dominated by
654 glucose. The resistance of carbohydrates to the acid hydrolyses and extractions performed
655 during the isolation of the ROM is thought to originate from the presence of carbohydrate-
656 containing molecular associations. These molecular associations contain polar functions
657 suitable for localized superheating effects under microwave irradiations and subsequent
658 disruption, whereas they are resistant to conventional heating.

659 The distribution of compounds released from microwave irradiation in KOH indicates an
660 important suberin- and cutin-derived contribution to the ROM. Moreover, this distribution,
661 together with the value of the suberin/cutin ratio suggests a significant input of both cutin and
662 suberin from the undergrowth.

663 Lignin-derived compounds, present in all the extracts from microwave hydrolyses are
664 dominated by guaiacyl moieties, in agreement with the predominance of pines at the study
665 site. A significant contribution from the non-woody angiosperm undergrowth to the ROM is
666 also suggested by the presence of *p*-hydroxyphenyl units (mainly *p*-coumaric acid) together
667 with that of cyclodimers of *p*-coumaric acid. Two types of lignin monomers are shown to be
668 engaged in different linkages (ether vs ester) as they are specifically released through
669 microwave HCl or KOH hydrolyses.

670 The composition of the CuPy and CuTHM pyrolysates of the residues obtained after
671 microwave irradiations were consistent with the distribution patterns of the corresponding
672 extracts.

673 Finally, the present study illustrates how complementary the microwave irradiation and
674 pyrolysis methods are. Each method presents its own advantages and drawbacks. Microwave
675 assisted extractions and hydrolyses afford quantitative (e.g. yield of degradation) as well as
676 qualitative data (e.g. nature of constituent monosaccharides) which cannot be obtained
677 through on-line pyrolysis. In addition, this technique appears to provide a more accurate
678 measure of the contribution of cutin-derived constituent (i.e. mid-chain hydroxy alkanolic
679 acids) due to the incomplete methylation of the hydroxyl groups occurring during CuTHM
680 pyrolysis. On the other hand, contrary to pyrolysis, microwave assisted hydrolyses appears to
681 be unable to release the major part of the lignin-derived constituent of the ROM. On-line
682 pyrolysis is much less time consuming. However, it is necessary to perform both CuPy and
683 CuTHM to have a holistic view of the composition of the ROM (e.g. carbohydrate only
684 detected in CuPy pyrolysate and polar constituents only detected in CuTHM pyrolysate).
685 Microwave assisted extractions and/or hydrolyses appear as particularly suitable for the
686 analysis of complex matrices and could be an attractive and complementary technique to
687 pyrolysis methods.

688

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698

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- 836

837 Table 1
 838
 839 Compounds identified in the extracts from the sequential microwave irradiation treatments
 840 (black numbers in Figure 2).

841
 842

1	4-hydroxybenzaldehyde
2	3-hydroxybenzoic acid
3	3(4)-hydroxyacetophenone
4	4-hydroxybenzoic acid
5	4-hydroxy-3-methoxybenzaldehyde
6	4-hydroxy-3-methoxyacetophenone
7	4-hydroxy-3-methoxybenzoic acid (vanillic acid)
8	3-(4-hydroxy-3-methoxyphenyl)-propan-2,3-dione (see Fig. 3a)
9	3,4-dihydroxybenzoic acid
10	3,5-dihydroxybenzoic acid
11	4-hydroxy-2,6-dimethoxyacetophenone
12	3-(4-hydroxyphenyl)-2-propenoic acid (<i>p</i> -coumaric acid)
13	4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid)
14	3-(4-hydroxy-3-methoxyphenyl)-3-oxopropanol (see Fig. 3b)
15	3-(4-hydroxy-3-methoxyphenyl)-3-oxo-1-propen-1(2)-ol (see Fig. 3c)
16	3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid (ferulic acid)
17	2-(4-hydroxy-3,5-dimethoxyphenyl)-2-oxo-ethanoic acid (see Fig. 3d)
18	unidentified (see Fig. 3e)
19	2-(4-hydroxy-3,5-dimethoxyphenyl)-2-oxo-ethanoic acid (see Fig. 3f)
20	Cyclodimers of <i>p</i> -coumaric acid (see Fig. 3g)
21	9-hydroxy pentadecanoic acid
22	10-hydroxy pentadecanoic acid
23	8/9/10-hydroxy octadecanoic acid
24	6/7 hydroxy pentadecanedioic acid
25	9,15-dihydroxy pentadecanoic acid
26	7/8-hydroxy hexadecanedioic acid
27	8/9/10,16-dihydroxy hexadecanoic acid
28	9,10-dihydroxy octadecanoic acid
29	9/10/11,18-dihydroxy octadecanoic acid
30	9,10,18-trihydroxy octadecanoic acid

843
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845
 846 Table 2
 847 Compounds identified in the 610 °C Curie point pyrolysates of the ROM and residues
 848 remaining after microwave irradiation in 0.1 M HCl, 1 M HCl and 1 M KOH (black numbers
 849 in Figure 4)

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1	2-furancarboxaldehyde
2a + 2b	benzaldehyde + methylfurancarboxaldehyde ^a
3	phenol
4	acetophenone
5	2-methoxyphenol
6	levoglucosenone
7	4-methyl-2-methoxyphenol
8	dihydrobenzofuran
9	4-ethenyl-2-methoxyphenol
10	3-methoxy-4-hydroxybenzaldehyde
11	4-(propen-2-yl)-2-methoxyphenol
12	3-methoxy-4-hydroxyacetophenone
13	3-methoxy-4-hydroxybenzoic acid methyl ester
14	3-methoxy-4-hydroxyphenylacetone
15	levoglucosan
16	3,4-dimethoxyphenylacetone ^b

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 854 ^a The ratio of **2b/2a** estimated through the intensity of peaks at m/z 110 and 106 decreases
 855 from ca. 1.8 for the ROM to ca. 0.9 and 0.2 for the residues from microwave hydrolyses in 0.1
 856 M and 1 M HCl respectively. ^b tentatively identified (m/z 123, 151 (base peak), 194)
 857

858

859 Table 3

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861 Compounds identified in the 610 °C Curie point thermally assisted hydrolysis and
 862 methylation pyrolysates of the ROM and residues remaining after microwave irradiation in
 863 0.1 M HCl, 1 M HCl and 1 M KOH (black numbers in Figure 5)

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1	methoxy toluene		
2	2-methoxyphenol		
3	benzoic acid		
4	1,2-dimethoxybenzene		
5	4-methoxy ethenylbenzene		
6	3,4-dimethoxytoluene		
7	4-methoxybenzaldehyde		
8	1,2,3-trimethoxybenzene		
9	3-methoxybenzoic acid		
10	{ 3,4-dimethoxy ethenylbenzene 1,2,4-trimethoxybenzene 4-methoxybenzoic acid		
		11	3,4,5-trimethoxy ethenylbenzene
		12	3,4-dimethoxybenzaldehyde (vanillic aldehyde)
13	3,4-dimethoxyacetophenone (acetovanillone)		
14	3,4-dimethoxybenzoic acid (vanillic acid)		
15	3,4,5-trimethoxybenzaldehyde (syringaldehyde)		
16	3,4-dimethoxybenzeneacetic acid		
17	3-(4-methoxyphenyl)-2-propenoic acid (<i>p</i> -coumaric acid)		
18	3,4,5-trimethoxybenzoic acid (syringic acid)		
19	3-(3,4-dimethoxyphenyl)-2-propenoic acid (ferulic acid)		
20	9/10,16-dimethoxy hexadecenoic acid (Fig. 6a)		
21	8/9/10,16-dimethoxy hexadecanoic acid (Fig. 6b)		
22	7/8/9-methoxy-15-hydroxy pentadecanoic acid (Fig. 6c)		
23	7/8-dimethoxy hexadecanedioic acid (Fig. 6d)		
24	8/9-methoxy-16-hydroxy hexadecanoic acid (Fig. 6e)		
25	9,18-dimethoxy octadecanoic acid (Fig. 6f)		
26	9/10-hydroxy-18-methoxy octadecanoic acid (Fig. 6g)		
27	cyclodimers of <i>p</i> -coumaric acid		

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870 Table 4

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872 ω -Hydroxy acid/*n*-alkanoic acid, ω -hydroxy acid/*n*-alkan-1-ol and *n*-alkan-1-ol I/*n*-alkanoic
 873 acid ratios calculated from the peak areas of GC-MS traces of CuTHM pyrolysates.

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	ROM	Residue after microwave irradiation in 1 M HCl	Residue after microwave irradiation in 1 M KOH
C ₂₂ ω -hydroxy acid/C ₂₂ <i>n</i> -alkanoic acid	7.4	2.4	2.4
C ₂₂ ω -hydroxy acid/C ₂₂ <i>n</i> -alkan-1-ol	2.3	1.7	2.1
C ₂₂ <i>n</i> -alkan-1-ol I/ C ₂₂ <i>n</i> -alkanoic acid	3.2	1.4	1.1
C ₂₄ ω -hydroxy acid/C ₂₄ <i>n</i> -alkanoic acid	4.6	1.5	1.6
C ₂₄ ω -hydroxy acid/C ₂₄ <i>n</i> -alkan-1-ol	2.6	1.7	1.9
C ₂₄ <i>n</i> -alkan-1-ol I/ C ₂₄ <i>n</i> -alkanoic acid	1.8	0.9	0.8

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Figure Captions

Fig. 1. Sequential microwave irradiation treatments of the ROM from the forest soil sample.

Fig. 2. Total ion current (TIC) traces of the extracts from microwave irradiation in (a) H₂O; (b) 0.1 M HCl; (c) 1 M KOH. The compounds were identified as methyl ester and trimethylsilyl ether derivatives. Black numbers correspond to compounds listed in Table 1. White numbers correspond to carbon chain length. ▼: *n*-alkanoic acids; ○: *n*-alkan-1-ols; ●: ω-hydroxy acids; ▽: *n*-alkanedioic acids; **S**: mono- or disaccharides; * : pollutant. When two different peaks are labelled with the same number (e.g. **7** or **9**), that with the highest retention time corresponds to the trimethylsilyl ester and ether derivative.

Fig. 3. Mass spectra of tentatively identified and unidentified aromatic compounds found in the organic extracts from the microwave extractions and hydrolyses (Table 1 for peak annotations). The analysis of compounds as trimethylsilyl ester and ether derivatives reveals (i) two compounds with the same retention times and mass spectra as compounds **8** and **15**, indicating the absence of carboxyl groups in these compounds. (ii) a compound, at longer retention time than compound **14**, with a mass spectrum exhibiting peaks at *m/z* 193, 223 (base peak), 325, 340 and 383. This suggests the presence of one carboxyl group for compound **14**. Compound **17** has the molecular weight of a trimethylsilyl ether, trimethylsilyl ester of methoxyhydroxybenzoic acid or trimethylsilyl ether, methyl ester of dihydroxybenzoic acid. However the mass spectra of these latter compounds do not match with that of compound **17**. Compounds **18** and **19** have the molecular weight of a trimethylsilyl ether, trimethylsilyl ester of dihydroxybenzoic acid, but no match was found between the mass spectra of these different compounds.

Fig. 4. TIC traces of 610 °C Curie point (CuPy) pyrolysate of (a) ROM and residues remaining after microwave irradiation in (b) 0.1 M HCl; (c) 1 M HCl; (d) 1 M KOH. Black numbers correspond to compounds listed in Table 2. White numbers correspond to carbon chain length. ▼: *n*-alkanoic acids; ◇: *n*-alkane/*n*-alkene doublets; ◆: *n*-alkan-2-one/*n*-alken-2-one doublets.

913 Fig. 5. TIC traces of 610 °C Curie point thermally assisted hydrolysis and methylation
914 (CuTHM) pyrolysate of (a) ROM and residues remaining after microwave irradiation in (b)
915 0.1 M HCl; (c) 1 M HCl; (d) 1 M KOH. Black numbers correspond to compounds listed in
916 Table 3. White numbers correspond to carbon chain length. ▼: *n*-alkanoic acids; ○: *n*-alkan-
917 1-ols; ●: ω-hydroxy acids; ▽: *n*-alkanedioic acids; ◇: *n*-alkane/*n*-alkene doublets; *:
918 hexahydro-1,3,5-trimethyl-1,3,5-triazine, a by product from TMAH.

919
920 Fig. 6. Mass spectra of the tentatively identified mid-chain substituted ω-hydroxy alkanolic
921 and alkanedioic acids found in the CuTHM pyrolysates of the ROM and residues from
922 microwave irradiation in 0.1 M HCl, 1 M HCl and 1 M KOH. (Table 3 for peak annotation).

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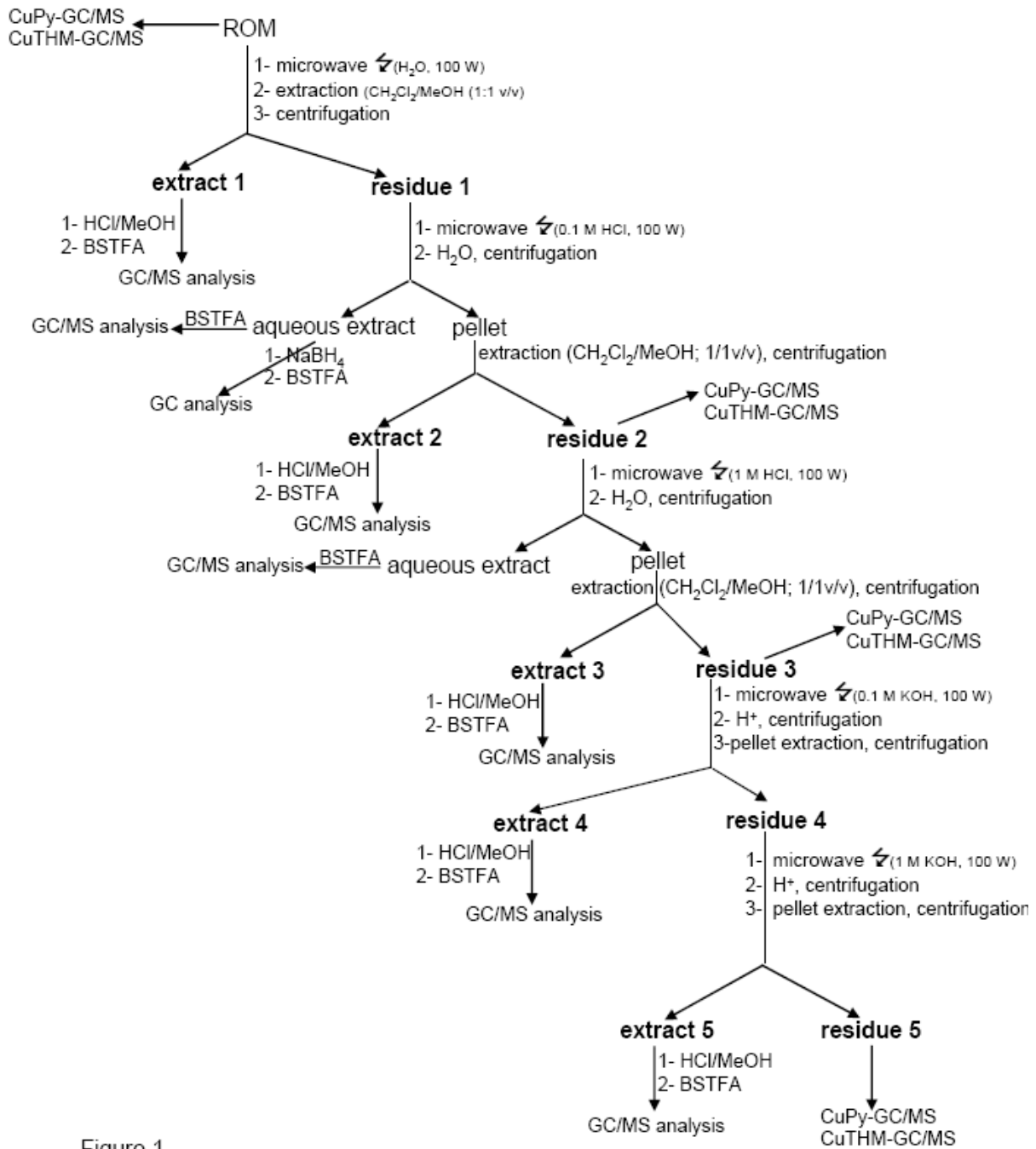


Figure 1

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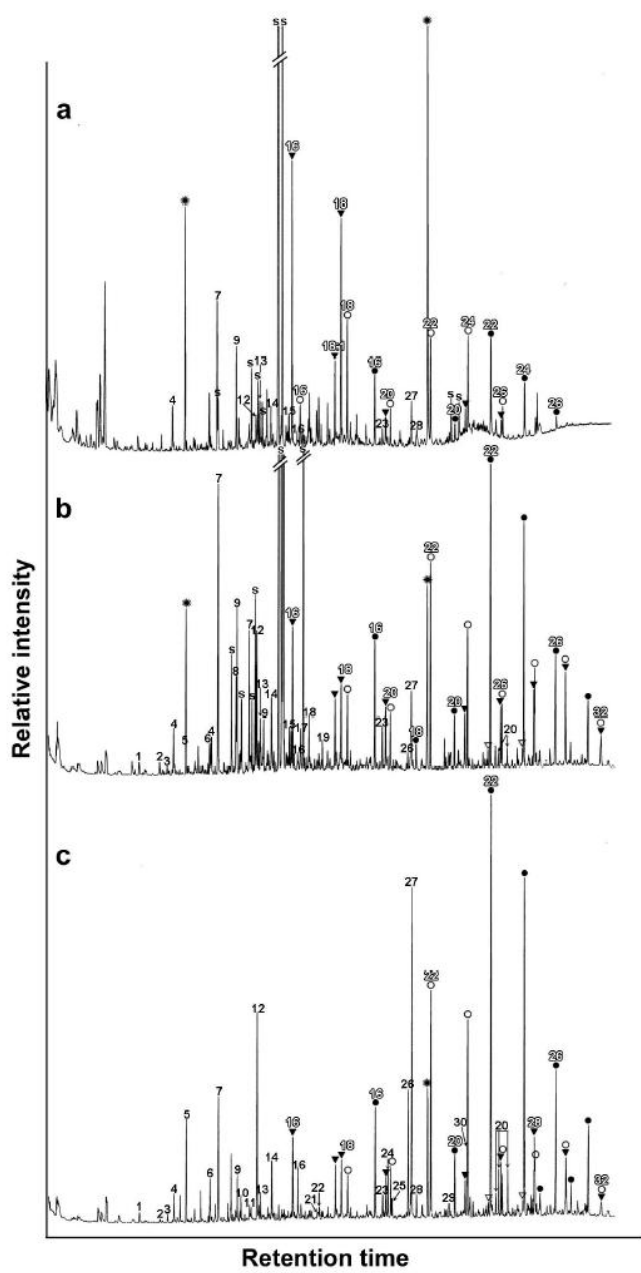


Figure 2

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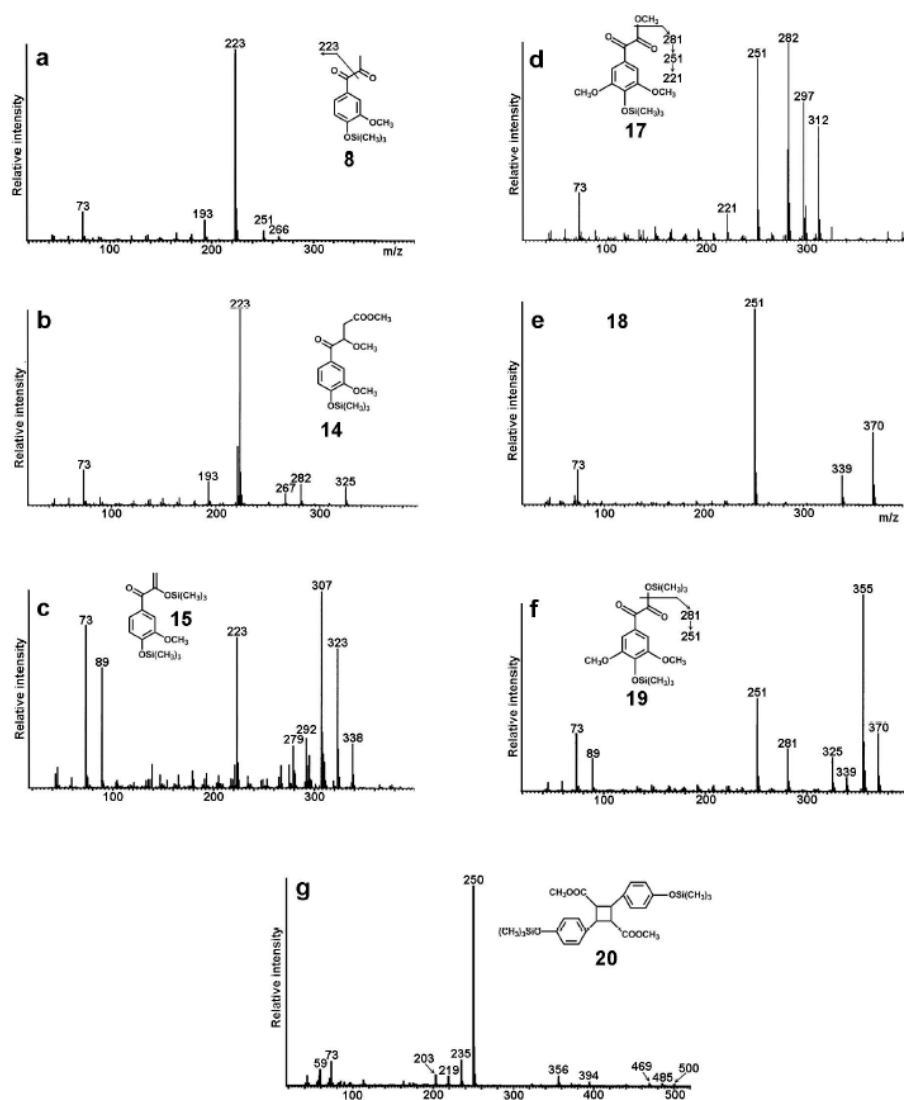


Figure 3

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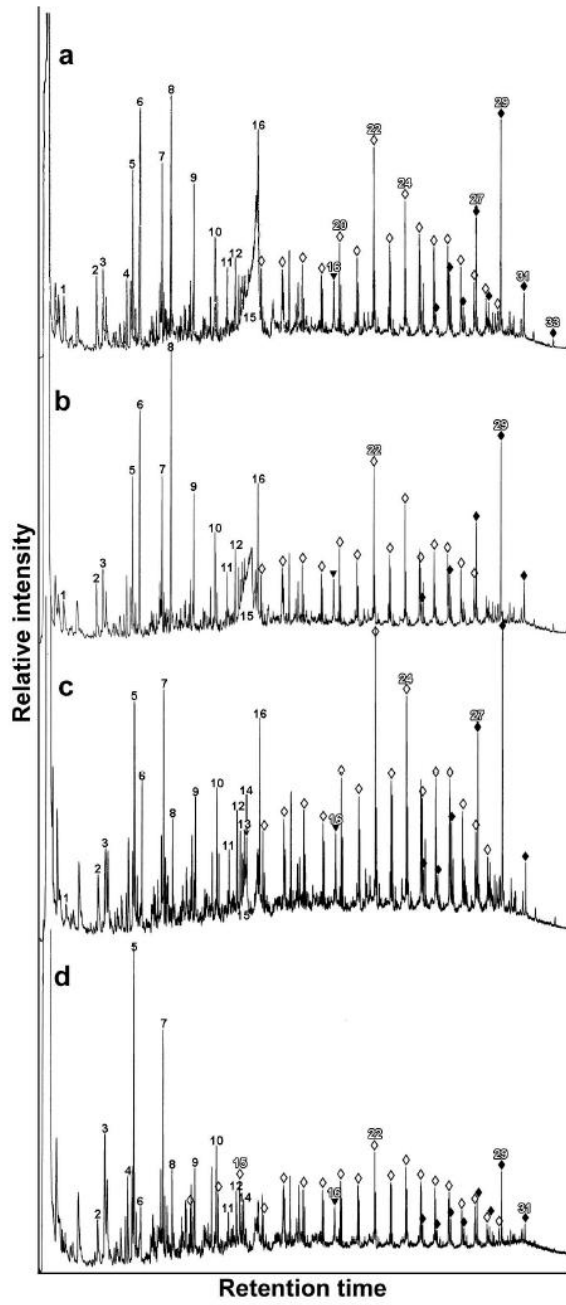


Figure 4

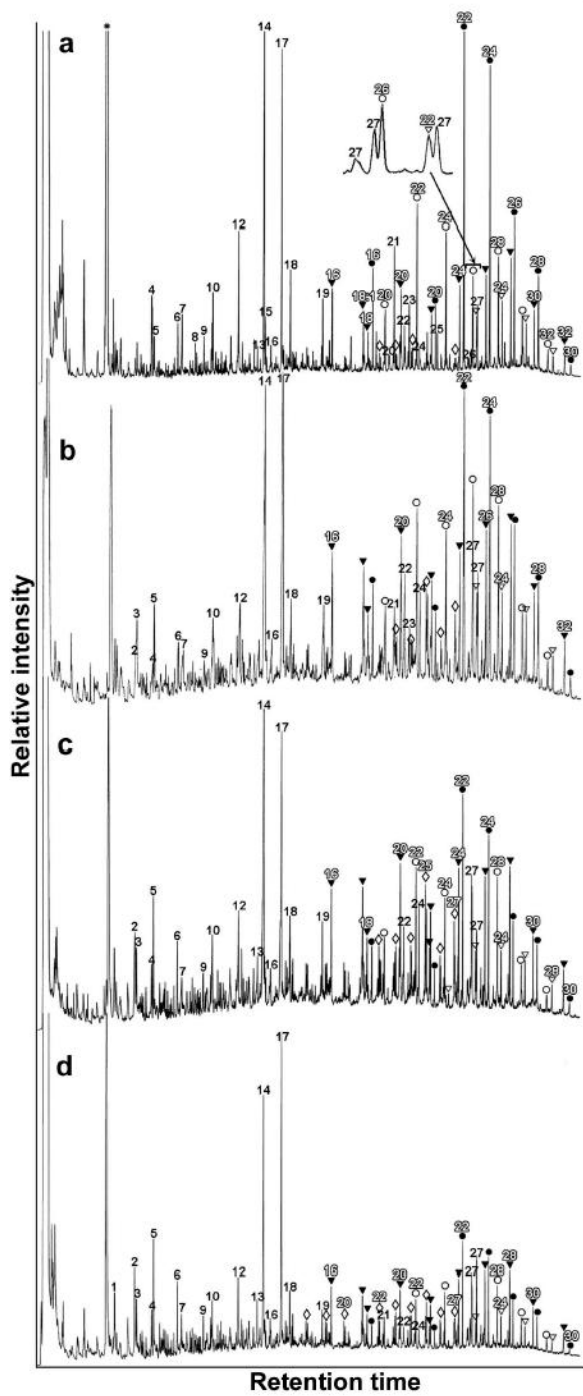


Figure 5

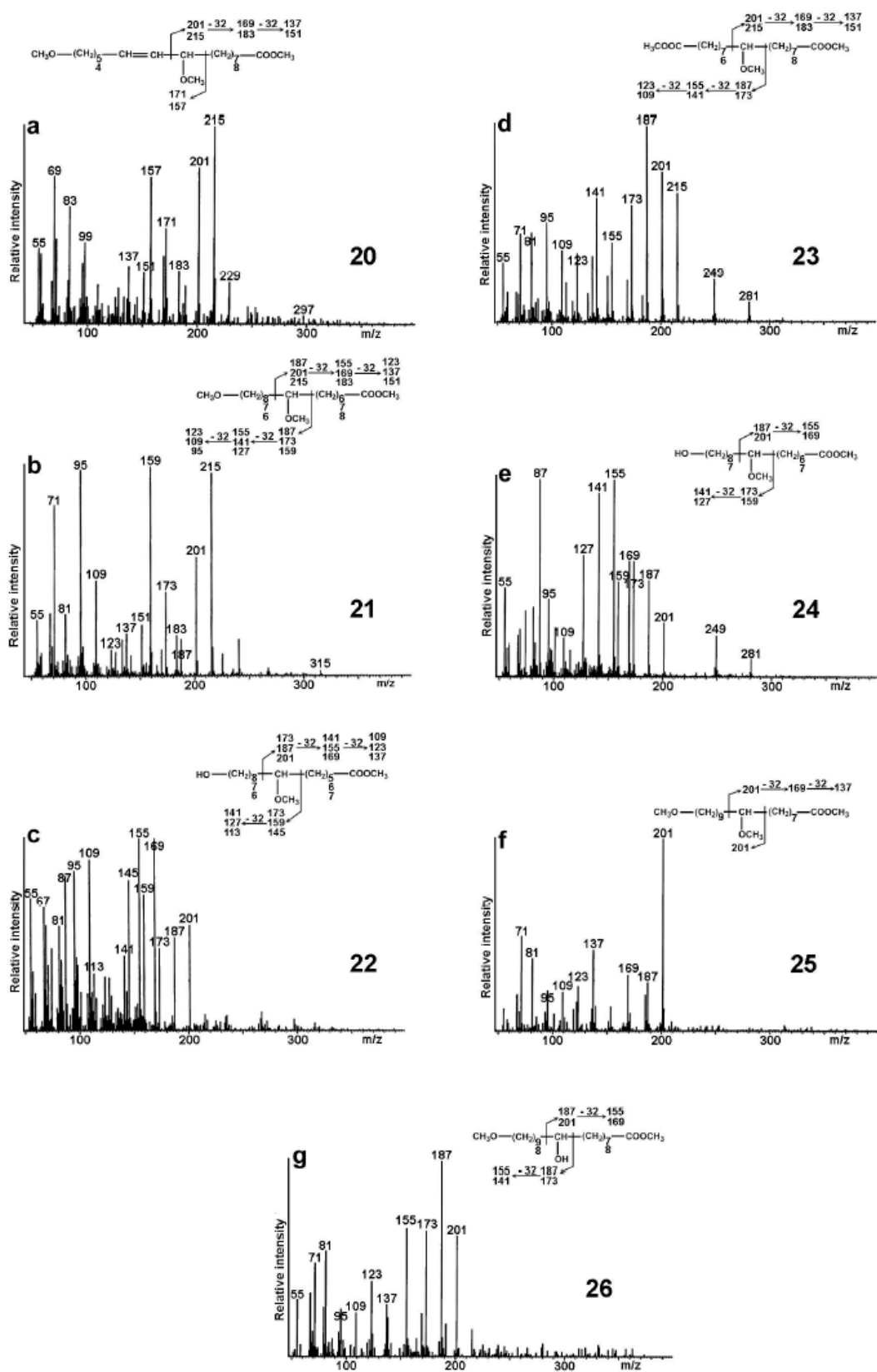


Figure 6