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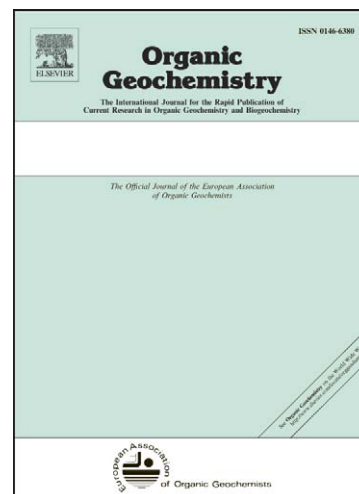
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1 Thermochemolysis of organic matter preserved in stalagmites: a preliminary study

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9
10 **ABSTRACT**

11
12 The analysis of organic matter (OM) preserved in stalagmites is a growing field,
13 but there have been few studies of biomarker compounds such as lignin phenols that are
14 widely used in other palaeoenvironmental contexts. Here we present a preliminary
15 qualitative study of the organic matter in six stalagmite samples from contrasting
16 environments, using thermochemolysis in the presence of tetramethylammonium
17 hydroxide (TMAH). The results indicate that a wide variety of products is preserved,
18 including several potential lignin-derived compounds, but also that further research is
19 needed to maximise compound recovery and allow the analysis of dissolved OM
20 preserved in stalagmites to reach its full potential.

21
22 **Keywords**

23 Stalagmite; organic matter; lignin; tetramethylammonium hydroxide;

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24 **1. Introduction**

25

26 Lignin phenols are established as one of the most effective chemical tracers of
27 terrestrial organic matter (OM) as they are unambiguously linked to vascular plants (e.g.
28 Hedges and Mann, 1979) and may be used to distinguish between different plant
29 sources (angiosperm vs. gymnosperm; woody vs. non-woody (e.g. Hedges and Mann,
30 1979; Chefetz et al., 2000; Wysocki et al., 2008)). They have been used in marine and
31 freshwater contexts as tracers for OM sources (e.g. Goñi and Hedges, 1995; Frazier et
32 al., 2003), as well as in soils for studies of OM decomposition and preservation (e.g.
33 Chefetz et al., 2000; Nierop and Filley, 2007).

34 Stalagmites are precipitated mounds of calcium carbonate that grow
35 incrementally beneath drip points in caves. They are relatively easy to date using
36 radiometric techniques and have a strong connectivity with the overlying soil via the
37 drip water. This enables them to preserve records of external environmental change.
38 OM forms a small but significant part of the stalagmite matrix (Ramseyer et al., 1997)
39 and is currently an area of considerable interest in the development of new
40 environmental proxies (for a review see Blyth et al., 2008). A few recent studies (Xie et
41 al., 2003; Blyth et al., 2007; Huang et al., 2008) have investigated lipid biomarkers such
42 as *n*-alkanes, alkanols, fatty acids and sterols within stalagmites, and have suggested
43 that they show a noticeable correspondence with both climate and vegetation change.
44 However, the potential use of lignin-derived products in the field has not been explored.

45 Here we present thermochemolysis results from a set of modern stalagmite
46 samples from a diverse range of environmental contexts, in order to investigate whether
47 (i) lignin products can be recovered from stalagmites at workable sample sizes, (ii) they

48 provide a potentially coherent signal and (iii) initial results might indicate that they will
49 be of use in the development of new palaeoenvironmental records. There is debate over
50 the most appropriate method for the preparation of lignin phenols for analysis. A recent
51 paper (Wysocki et al., 2008) indicated that CuO oxidation methods may give more
52 reliable results for samples of low organic content than tetramethylammonium
53 hydroxide (TMAH) thermochemolysis, which produces more complex results,
54 especially in the absence of ^{13}C labelling (Filley et al., 1999). However, TMAH
55 techniques have the advantage of being simple and rapid. As the present experiment
56 was primarily intended as a qualitative screening exercise, on-line thermochemolysis
57 with unlabelled TMAH was considered an appropriate approach.

58

59 **2. Methods and materials**

60

61 *2.1. Samples*

62

63 Six modern stalagmite samples were taken from caves beneath a range of
64 contrasting environments with different vegetation regimes: Tar-a (Scotland, peat); SX-
65 10 (Northern England, upland grass); Merc1i (Ethiopia, arable); Merc 1ii (Ethiopia,
66 scrub); Tur-1 (Turkey, grass); BR-36 (SE Australia, scrub). The stalagmites were all
67 actively forming at collection. The samples used were sectioned from the outer layers of
68 the stalagmite (so as to recover the youngest material) using a drill-mounted 2 cm
69 diamond tipped saw. The pieces of calcite were washed in 1 M HCl to remove the outer
70 1 mm and sonicated in high purity MeOH and dichloromethane (DCM) to remove any
71 remaining surface contamination.

72

73 *2.2. Extraction and analysis*

74

75 The samples were dissolved in excess DCM-extracted 1 M HCl. After complete
76 dissolution, an aliquot of each solution equivalent to 1 g of the original calcite was
77 loaded onto a conditioned non-encapped C₁₈ solid phase extraction cartridge (IST
78 Isolute). SPE cartridges were pre-conditioned with hexane, DCM, MeOH and 0.01 M
79 HCl. Once the dissolved OM had adsorbed onto the SPE cartridge any salts were
80 removed by rinsing with 4 ml 0.01 M HCl, the cartridge was dried by flushing with He.
81 The organics were eluted from the column into clean vials with 4 ml MeOH, dried
82 under N₂ and rediluted in 50 µl MeOH.

83 Ca. 10 µl of sample in MeOH was absorbed onto quartz wool in a quartz
84 pyrolysis tube. The samples were left to dry overnight, before 10 µl of 25%
85 tetramethylammonium hydroxide (TMAH) in MeOH were added and allowed to dry
86 overnight. Addition of an excess of TMAH ensures a basic environment for the
87 reaction, with a pH of 14. Thermochemolysis was carried out under the following
88 conditions: heated to 300 °C (held 15 s) at 20 °C ms⁻¹ in a flow of He using a CDS
89 Pyroprobe 5000 fitted with a 1500 valve interface (CDS Analytical, Oxford, PA) and
90 coupled to a gas chromatography-mass spectrometry (GC-MS) instrument. GC-MS was
91 carried out using an Agilent Technologies 6890 gas chromatograph coupled to a 5973
92 mass spectrometer. Separation was performed on a S.G.E. (U.K.) BPX-5 column (30x
93 m x 0.25 mm i.d., 0.25 µm film thickness). He at a flow rate of 1.1 ml min⁻¹ was used
94 as carrier gas. Injection was at a 5:1 split and injector temperature was 270 °C. The GC

95 oven temperature was held for 1 min at 50 °C and then programmed at 5 °C min⁻¹ to
96 310 °C (held 9 min).

97

98 3. Results and discussion

99

100 Fig. 1 shows a typical total ion chromatogram of the TMAH pyrolysis products
101 of a stalagmite sample. All six samples produce a number of products, including
102 methyl esters of butanoic and pentanoic acids (previously identified as general TMAH
103 products (Frazier et al., 2003); 1,4-dimethoxy benzene; 3-methyl- and 4-methylbenzoic
104 acid methyl esters; 2,5- and 2,6-dimethoxy toluene; a series of dimethyl benzoic acid
105 methyl esters (2,6-; 2,5-; 2,4-; 2,3-; 3,5-, and 3,4-); 3-methoxy benzoic acid methyl
106 ester; 3,5-benzoic acid methyl ester; cinnamic acid methyl ester; methyl-3-methoxy-4-
107 methyl benzoic acid methyl ester; and a number of compounds from the *p*-courmaryl,
108 guaiacyl and syringyl groups (Fig. 1). Of these, 4-methoxybenzaldehyde (P4), 4-
109 methoxybenzoic acid methyl ester (P6), 1,2-dimethoxybenzene (G1), 3,4-
110 dimethoxytoluene (G2) and 3,4-dimethoxybenzoic acid methyl ester (G6) were present
111 in the products from all samples, and 4-methoxybenzenepropanoic acid methyl ester
112 (P12), 1-(4-methoxyphenyl)-2-methoxypropane (P23), 3,4-dimethoxybenzaldehyde
113 (G4), 3,4-dimethoxyacetophenone (G5), 1,2,3-trimethoxybenzene (S1), 3,4,5-
114 trimethoxytoluene (S2), and 3,4,5-trimethoxybenzoic acid methyl ester (S6) were
115 identifiable in the products from some of them. Also present were a range of *n*-alkanoic
116 acid methyl esters. Methylated carbohydrate and protein derivatives were also present,
117 but were not identified because of the degree of GC coelution.

118 The presence of abundant products from a relatively small sample size and a
119 wide range of environments is encouraging, although the absence of compounds such as
120 S4, S5, P18 and G18, proposed as being useful in the interpretation of TMAH lignin
121 data (Challinor, 1995; Mason et al., 2009) suggests that identification of the overlying
122 vegetation regime from this technique alone will be difficult without refinement of the
123 analytical method and sample size. However, given the known different responses of
124 particular compounds to degradation and alteration in the soil column (e.g. Chefetz et
125 al., 2000), it may be that the approach has promise in assessing the degree of
126 degradation and speed of transport of the OM to the stalagmite, an understanding of
127 which is important in the interpretation of both wider OM signals and carbon isotope
128 records preserved in the stalagmite calcite (Genty et al., 2003; Blyth et al., 2008).

129

130 **4. Conclusions**

131

132 The analysis of organic matter preserved in stalagmites by thermochemolysis is
133 feasible and potentially has much to offer in the study of terrestrial palaeoenvironments
134 by providing insights into both the overlying vegetation and the degradation processes
135 in the overlying soil. However, further techniques need to be tested in this context
136 before the potential of the approach can be fully realised. Given the findings of
137 Wysocki et al. (2008) suggesting the CuO oxidation may be more effective in
138 recovering lignin data from environments low in organic matter, clearly there is scope
139 for the approach to be applied to stalagmites, especially where identification of the
140 overlying vegetation regime is of primary interest. However, given the wide range of
141 compounds present, many of which, such as hydrolysable tannins, would not be

142 detected using CuO extraction, the application of ^{13}C -labelled TMAH
143 thermochemolysis also seems appropriate, as does the application of comprehensive two
144 dimensional GC-MS to provide increased chromatographic resolution and increased
145 sensitivity.

146

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148

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151

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153

154 *Associate Editor – G. D. Abbott*

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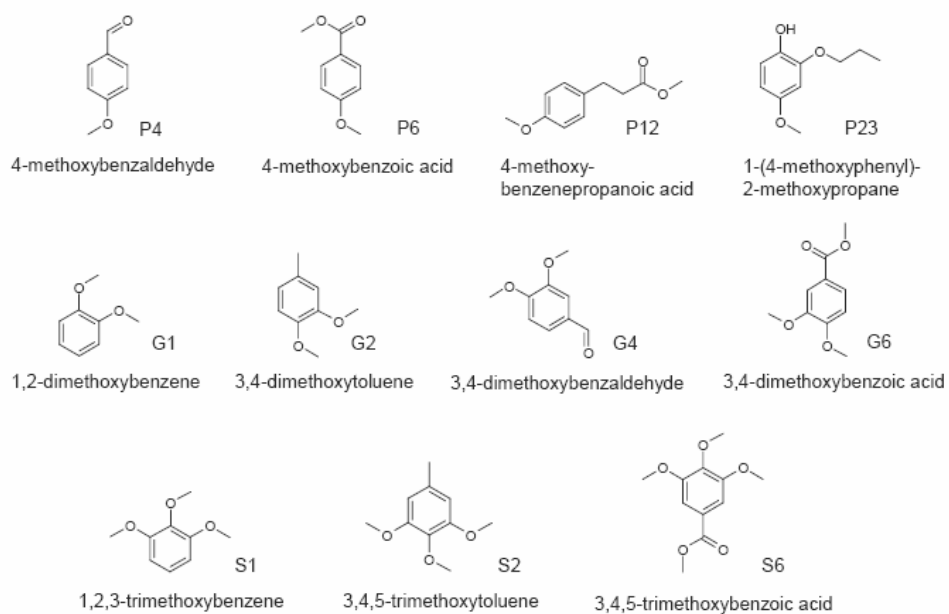
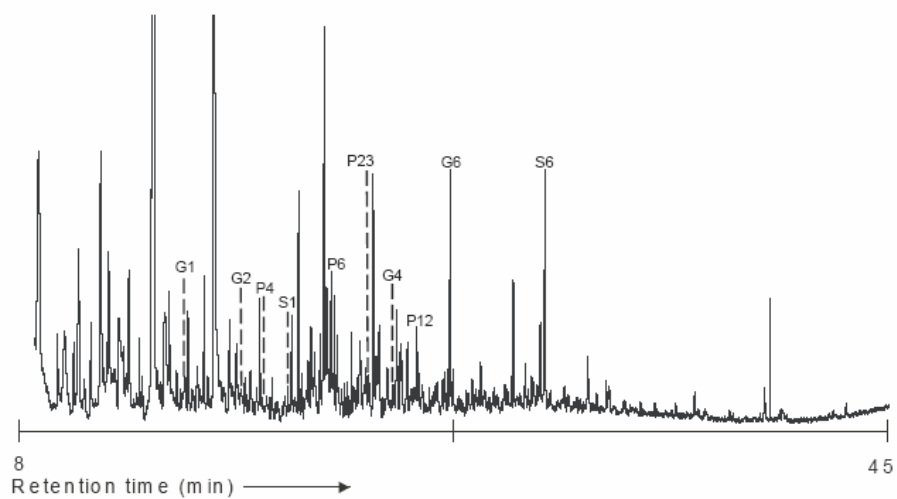
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207 **Figure captions**

208

209 **Fig. 1.** Total ion chromatogram from sample Tar-a (deposited under peatland in NW

210 Scotland), showing *p*-coumaryl, guaiacyl and syringyl peaks and structures.



211

212

213 FIGURE 1