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Thermochemolysis of organic matter preserved in stalagmites: a preliminary study

Alison J. Blyth \textsuperscript{a,b,*}, Jonathan S. Watson \textsuperscript{b,a}

\textsuperscript{a} Department of Earth and Environmental Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK

\textsuperscript{b} Planetary and Space Sciences Research Institute, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK

ABSTRACT

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

Keywords

Stalagmite; organic matter; lignin; tetramethylammonium hydroxide;

\* Corresponding author. Tel: +44 (0)1908 659883 ; fax: +44(0)1908 655151.
E- mail address: a.blyth@open.ac.uk. (Alison Blyth).
1. Introduction

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

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

Here we present thermochemolysis results from a set of modern stalagmite samples from a diverse range of environmental contexts, in order to investigate whether (i) lignin products can be recovered from stalagmites at workable sample sizes, (ii) they
provide a potentially coherent signal and (iii) initial results might indicate that they will be of use in the development of new palaeoenvironmental records. There is debate over the most appropriate method for the preparation of lignin phenols for analysis. A recent paper (Wysocki et al., 2008) indicated that CuO oxidation methods may give more reliable results for samples of low organic content than tetramethylammonium hydroxide (TMAH) thermochemolysis, which produces more complex results, especially in the absence of $^{13}$C labelling (Filley et al., 1999). However, TMAH techniques have the advantage of being simple and rapid. As the present experiment was primarily intended as a qualitative screening exercise, on-line thermochemolysis with unlabelled TMAH was considered an appropriate approach.

2. Methods and materials

2.1. Samples

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

The samples were dissolved in excess DCM-extracted 1 M HCl. After complete dissolution, an aliquot of each solution equivalent to 1 g of the original calcite was loaded onto a conditioned non-endcapped C$_{18}$ solid phase extraction cartridge (IST Isolute). SPE cartridges were pre-conditioned with hexane, DCM, MeOH and 0.01 M HCl. Once the dissolved OM had adsorbed onto the SPE cartridge any salts were removed by rinsing with 4 ml 0.01 M HCl, the cartridge was dried by flushing with He. The organics were eluted from the column into clean vials with 4 ml MeOH, dried under N2 and rediluted in 50 µl MeOH.

Ca. 10 µl of sample in MeOH was absorbed onto quartz wool in a quartz pyrolysis tube. The samples were left to dry overnight, before 10 µl of 25% tetramethylammonium hydroxide (TMAH) in MeOH were added and allowed to dry overnight. Addition of an excess of TMAH ensures a basic environment for the reaction, with a pH of 14. Thermochemolysis was carried out under the following conditions: heated to 300 °C (held 15 s) at 20 °C ms$^{-1}$ in a flow of He using a CDS Pyroprobe 5000 fitted with a 1500 valve interface (CDS Analytical, Oxford, PA) and coupled to a gas chromatography-mass spectrometry (GC-MS) instrument. GC-MS was carried out using an Agilent Technologies 6890 gas chromatograph coupled to a 5973 mass spectrometer. Separation was performed on a S.G.E. (U.K.) BPX-5 column (30x 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 as carrier gas. Injection was at a 5:1 split and injector temperature was 270 °C. The GC
oven temperature was held for 1 min at 50 °C and then programmed at 5 °C min⁻¹ to 310 °C (held 9 min).

3. Results and discussion

Fig. 1 shows a typical total ion chromatogram of the TMAH pyrolysis products of a stalagmite sample. All six samples produce a number of products, including methyl esters of butanoic and pentanoic acids (previously identified as general TMAH products (Frazier et al., 2003); 1,4-dimethoxy benzene; 3-methyl- and 4-methylbenzoic acid methyl esters; 2,5- and 2,6-dimethoxy toluene; a series of dimethyl benzoic acid methyl esters (2,6-; 2,5-; 2,4-; 2,3-; 3,5-, and 3,4-); 3-methoxy benzoic acid methyl ester; 3,5-benzoic acid methyl ester; cinnamic acid methyl ester; methyl-3-methoxy-4-methyl benzoic acid methyl ester; and a number of compounds from the p-coumaryl, guaiacyl and syringyl groups (Fig. 1). Of these, 4-methoxybenzaldehyde (P4), 4-methoxybenzoic acid methyl ester (P6), 1,2-dimethoxybenzene (G1), 3,4-dimethoxytoluene (G2) and 3,4-dimethoxybenzoic acid methyl ester (G6) were present in the products from all samples, and 4-methoxybenzenepropanoic acid methyl ester (P12), 1-(4-methoxyphenyl)-2-methoxypropane (P23), 3,4-dimethoxybenzaldehyde (G4), 3,4-dimethoxyacetophenone (G5), 1,2,3-trimethoxybenzene (S1), 3,4,5-trimethoxytoluene (S2), and 3,4,5-trimethoxybenzoic acid methyl ester (S6) were identifiable in the products from some of them. Also present were a range of n-alkanoic acid methyl esters. Methylated carbohydrate and protein derivatives were also present, but were not identified because of the degree of GC coelution.
The presence of abundant products from a relatively small sample size and a wide range of environments is encouraging, although the absence of compounds such as S4, S5, P18 and G18, proposed as being useful in the interpretation of TMAH lignin data (Challinor, 1995; Mason et al., 2009) suggests that identification of the overlying vegetation regime from this technique alone will be difficult without refinement of the analytical method and sample size. However, given the known different responses of particular compounds to degradation and alteration in the soil column (e.g. Chefetz et al., 2000), it may be that the approach has promise in assessing the degree of degradation and speed of transport of the OM to the stalagmite, an understanding of which is important in the interpretation of both wider OM signals and carbon isotope records preserved in the stalagmite calcite (Genty et al., 2003; Blyth et al., 2008).

4. Conclusions

The analysis of organic matter preserved in stalagmites by thermochemolysis is feasible and potentially has much to offer in the study of terrestrial palaeoenvironments by providing insights into both the overlying vegetation and the degradation processes in the overlying soil. However, further techniques need to be tested in this context before the potential of the approach can be fully realised. Given the findings of Wysocki et al. (2008) suggesting the CuO oxidation may be more effective in recovering lignin data from environments low in organic matter, clearly there is scope for the approach to be applied to stalagmites, especially where identification of the overlying vegetation regime is of primary interest. However, given the wide range of compounds present, many of which, such as hydrolysable tannins, would not be
detected using CuO extraction, the application of $^{13}$C-labelled TMAH
thermochemolysis also seems appropriate, as does the application of comprehensive two
dimensional GC-MS to provide increased chromatographic resolution and increased
sensitivity.

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**Figure captions**

**Fig. 1.** Total ion chromatogram from sample Tar-a (deposited under peatland in NW Scotland), showing $p$-crownaryl, guaiacyl and syringyl peaks and structures.
FIGURE 1