Staffordshire Hoard
Research Report 5

FTIR and GC-MS Analysis of Pastes and Soils from the Staffordshire Hoard

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2013

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Information about this report

This report was produced in 2013 as part of Stage 1 of the project, i.e. before fragments were joined and catalogued. The concordance of the K numbers given in the report to the catalogue numbers as they appear in the final publication is as given below. The list also includes the names of the objects as used in the final publication.

The owners, Historic England and Barbican Research Associates are grateful to National Geographic for the funding which enabled the work to be carried out.

K number	Catalogue number	Name in publication
131	559	Strip-mount in gold with garnet cloisonné decoration and filigree serpent mounts (part).
270	542	Eye-shaped mount in gold with garnet cloisonné decoration.
273	550	Strip-mount in gold with garnet cloisonné decoration.
291	76	Pommel in cast silver, of cocked-hat form with double sword-rings, with cast interlace and niello inlay, and mounts with cloisonné and filigree decoration (part).
356	562	Edge-mount in gold of L-shaped form with garnet cloisonné.
447	548	Strip-mount in gold with garnet cloisonné decoration.
451		Pyramid
546	589	Helmet crest (part).
662	557	Strip-mount in gold with garnet cloisonné decoration and serpent filigree mounts (part).
673	553	Strip-mount in gold with garnet cloisonné decoration and one pointed end.
716	557	Strip-mount in gold with garnet cloisonné decoration and serpent filigree mounts (part).
1003	174	Hilt-collar in gold, of narrow form, with garnet cloisonné decoration.
1062	556	Strip-mount in gold with garnet cloisonné decoration and serpent filigree mounts.
1388	639	Gold boss with filigree collar.

The British Museum

DEPARTMENT OF CONSERVATION AND SCIENTIFIC RESEARCH

FTIR and GC-MS analysis of pastes and soil from Staffordshire Hoard artefacts

Science Report
Envelope No. PR07444-7

Valerie Steele and Marei Hacke

Abstract:
The aim of this study was to examine pastes and other organic materials from a selection of objects from the Staffordshire Hoard. Eleven samples of pastes from nine objects and five soil samples were analysed by Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry. In addition five other samples of materials that were not pastes from within cloisonné cells but appeared to contain organic material were analysed by FTIR. Seven of the paste samples (K131_6, K270_13, K273_7, K356_2, K451_5 K673 and K1003_4) were confirmed to contain organic material. In all cases this was identified as beeswax with another material which appears to be proteinaceous, possibly animal glue. Two samples (K270_13 and K673) also contained the sugars characteristic of a plant gum. All samples also contained an inorganic component consisting either of copper corrosion products or material derived from clay and/or sandy material. The four samples that contained no organic compounds (K279_19, K356_10, K447_8 and K716_1) were either copper corrosion products or (alumino)silicate minerals such as are present in iron oxide-containing earth pigments and quartz, in one case also with the clay mineral kaolinite. The soil samples all yielded organic compounds. However these were present at much lower concentrations in the soils with a different range and distribution compared with compounds present in the paste samples. In addition the soils contained large amounts of compounds, some unidentified, which are mostly attributable to modern contamination. Four (K291, K546_1, K1062_1 and K1388) of the five samples which were not from the interior of cloisonné cells also yielded organic material. All of these contained beeswax, while K1388 also contained what is believed to be proteinaceous components. K546_1 and K1062 also contained calcium carbonate and K1388 also contained (alumino)silicate minerals such as are present in iron oxide-containing earth pigments. The remaining sample, K360_5, only contained copper corrosion products.

CSR Project no. PR07444
Staffordshire Hoard numbers: K131, K270, K273, K291, K356, K447, K451, K546, K662 (soil only), K673, K716, K1003, K1062, K1388

March 2013

Introduction

Eleven samples, from nine objects, thought to be pastes used to support the inlaid garnets in cloisonné cells (K131_6, K270_13, K270_19, K273_7, K356_2, K447_8, K451_5, K662_4, K673, K716_1 and K1003_4) and five soil samples (K270_1, K356_1, K447_1, K451_1 and K1003_1) retrieved from objects in the Staffordshire Hoard during conservation were examined using Fourier transform-infrared microscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS). The aim of the analysis was to determine whether these pastes contained any organic material and to characterise any organic components present. Further, it was of interest to determine whether all samples of paste were the same.

In addition four samples of material that appeared to be organic (from objects K291, K360_5, K546_1, K1062_1 and K1388) were sampled and examined by FTIR to determine the nature of these materials. In general FTIR was used as a screening method as it provides general information about the nature of the material and whether organic compounds are present. It can also provide information about any inorganic compounds present in the material. GC-MS analysis was carried out to provide more detailed information about the organic components of the pastes, if any, and several different sample preparations and methods of analysis were used to target different materials. The results of the FTIR analyses were used to inform the choice of methods used for GC-MS analysis. In the last five cases (K291, K360_5, K546_1, K1062_1 and K1388) the FTIR results were specific enough to identify the materials.

Methods

Most of the pastes and soil samples analysed arrived in small plastic or glass vials. Of those that were sampled at the British Museum, the sample from K673 was removed from behind the gold foil inside one of the cells containing garnets. During conservation work one of the garnets was found to be loose and was removed prior to consolidation and the opportunity was taken to sample the paste in the cell. As this material is known to be undisturbed material which has not been exposed to soil, this was an important addition to the samples available. It was sampled with a clean needle and transferred to a small glass sample vial. For samples from objects K291, K360_5, K546_1, K1062_1 and K1388 small amounts of the materials to be analysed were removed from the objects by scraping with a fine needle (0.25 mm) using stereoscopic magnification to ensure that the sample size was minimal and, in most cases, the sampled area was invisible to the naked eye.

For FTIR of the pastes and soils a small sub-sample was removed from each vial using a fine needle or scalpel, under a stereoscopic microscope. As most were complex mixtures, use of the microscope enabled the different components of the mixtures to be analysed separately. In all cases the subsamples were transferred onto the window of a diamond micro-compression cell for FTIR analysis. In the five cases where objects were sampled these samples were transferred directly onto a diamond cell window.

Samples for GC-MS were removed from the vials in a similar way, transferred to glass vials and accurately weighed prior to sample preparation. All the samples analysed by GC-MS were initially solvent-extracted to analyse for lipids (e.g. fats, waxes, oils, bituminous materials) using a mixture of dichloromethane and methanol (2:1 v/v). Following extraction they were derivatised and an internal standard was added to allow quantification before analysis by GC-MS. In two cases (K270_13 and K673) the FTIR analysis indicated the presence of a plant gum. Sub-samples of these two pastes were removed from the samples as above and extracted using methanolic hydrochloric acid to release the neutral sugars and

uronic acids present in plant gums. In four cases where FTIR indicated the presence of proteinaceous material, sub-samples of these pastes were extracted by acidic hydrolysis to release individual amino acids present in the proteins. However it was not possible to analyse these extracts by GC-MS as the extraction method also appears to have formed or released a component/components which was incompatible with the stationary phase on the GC-MS column resulting in damage to the column. It has not been possible to identify this component/components which are incompatible with the GC-MS column as FTIR of the extracts has not provided a definite identification.

Full details of the samples analysed are given in Table 1 and a full description of the methods is given in the Appendix.

Results and discussion

Of the pastes and residues analysed, five of the total of 16 contained no organic material with the remaining 11 samples all yielding evidence of at least one organic component. The soils analysed did contain some organic material but this was of a different nature and at far lower concentrations than in the samples from objects.

FTIR results

The FTIR analysis produced results which divided the samples into two main groups – those which did not contain any organic material and those which gave spectra indicative of organic materials.

Samples showing no evidence of organic materials

The samples which did not contain any organic material could be divided into two groups. The first of these groups comprised K270_19, K447_8 and K716_1 and these gave FTIR spectra typical of soil-derived materials. These showed the presence of (alumino)silicate materials such as are present in iron oxide-containing earth pigments, with quartz and/or kaolinite (a clay mineral). A typical spectrum is shown in Figure 1.

The second group (K356_10 and K360_5) were predominantly brochantite ($Cu_4SO_4(OH)_6$) and malachite ($Cu_2CO_3(OH)_3$) which are corrosion products of copper or copper alloys. Some areas of K356_10 also contained (alumino)silicate minerals such as are present in iron oxide-containing earth pigments The spectrum for K360_5 is shown in Figure 2.

Samples showing evidence of organic materials

Of the remaining 11 samples, nine gave spectra which indicated the presence of beeswax with the spectra obtained from some samples yielding very close matches to reference spectra. This suggests that these samples were composed almost entirely of beeswax (see Figure 3). The two samples where evidence for the presence of beeswax was not apparent on the basis of FTIR analysis (K131_6 and K270_13) did produce evidence of beeswax upon GC-MS analysis (see below).

The spectra of eight samples (K131_6, K270_13, K273_7, K356_2, K451_5, K673, K1003_4 and K1388) also suggested the presence of proteinaceous material (see Figure 4). Proteinaceous binders might include egg, animal glue and milk proteins: the spectra of K131_6, K270_13, K271_7, K356_2, K451_5 and K1003_4 matched most closely with reference spectra of animal glue, although the spectrum of K673 was a closer match to a reference spectrum of egg.

Table 1: Details of samples and analyses carried out. Key: Y – analysis carried out; N – analysis not attempted; A – analysis attempted but failed due to technical difficulties.

Sample	Description	Analytical methods			
		FTIR	GC-MS		
			Solvent extract	Sugars extract	Amino acid extract
	Pastes	Y	Y	N	A
K131_6	Black material found with soil in damaged cloisonné cell of gold strip inlaid with garnets	Y	Y	Y	A
K270_13	Loose greenish deposit found in cloisonné cell of gold and garnet lentoid object	Y	Y	N	N
K270_19	Dark material from the bottom of the wide depressed area around centre of K270	Y	Y	N	A
K273_7	Clump of material from inside cloisonné cell of gold strip inlaid with garnets	Y	Y	N	A
K356_2	Material from empty cell on side of gold and garnet edging strip	Y	Y	N	A
K356_10	Green material from empty cell on side of K356	Y	N	N	N
K447_8	Material from empty cloisonné cell at the end of gold and garnet strip	Y	Y	N	N
K451_5	Material from cloisonné cell along bottom edge of gold, garnet and glass sword pyramid	Y	Y	N	A
K673	Material which fell out of cloisonné cell when garnet also fell out during conservation work. From behind gold foil under garnet of gold and garnet fitting – from undisturbed cell	Y	Y	Y	A
K716_1	Gold strip inlaid with garnets. Sample from black material from central empty panel area thought to have held filigree panel in place	Y	Y	N	N
K1003_4	Material from empty cloisonné cell of gold and garnet hilt collar	Y	Y	N	A
	Other organics				
K291	Sample from white, waxy smear on surface of filler in fragmentary, silver pommel cap	Y	N	N	N
K360_5	Sample of green material on exterior of silver pommel cap	Y	N	N	N
K546_1	Silver gilt channelled strip – sample from crumbly, white and shiny material from a section of box strip	Y	N	N	N

Sample	Description	Analytical methods			
		FTIR	GC-MS		
			Solvent extract	Sugars extract	Amino acid extract
K1062_1	White/green/black material at the back of the central panel of K1062 – white part of material analysed	Y	N	N	N
K1388	Material from interior surface of silver boss	Y	N	N	N
	Soils				
K270_1	Soil from top of object K270	Y	Y	N	N
K356_1	Soil from exterior of K356	Y	Y	N	N
K447_1	Soil from front of K447	Y	Y	N	N
K451_2	Soil from object K451	Y	Y	N	N
K662_4	Soil from edges of K662	Y	Y	N	N
K1003_1	Soil from the inner side of the collar K1003	Y	Y	N	N

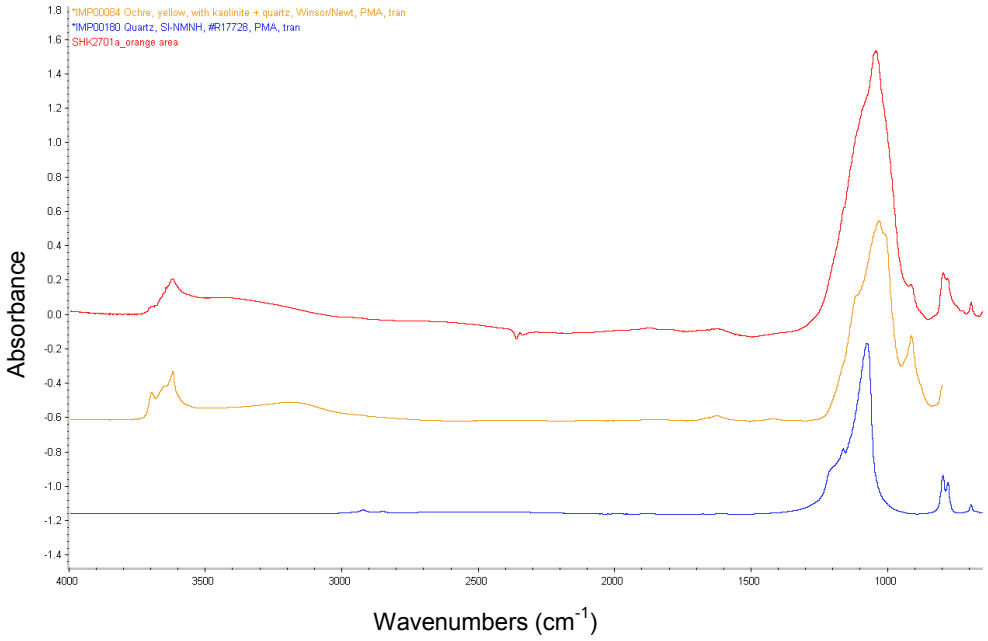

Figure 1: The FTIR spectrum of paste K270_19 (top, red), with the reference spectra for yellow ochre (an earth pigment containing (alumino)silicate minerals and iron oxide, with this particular sample containing kaolinite and quartz) (centre, yellow/brown) and quartz (bottom, blue) for comparison

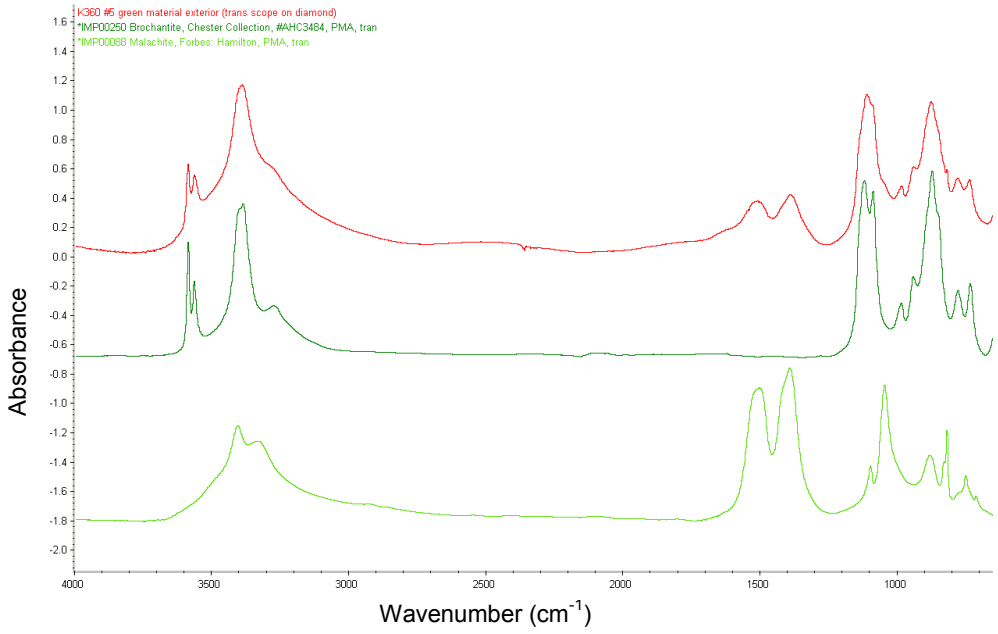

Figure 2: FTIR spectrum of sample K360_5 (top, red) with reference spectra for brochantite (middle, dark green) and malachite (bottom, pale green) for comparison

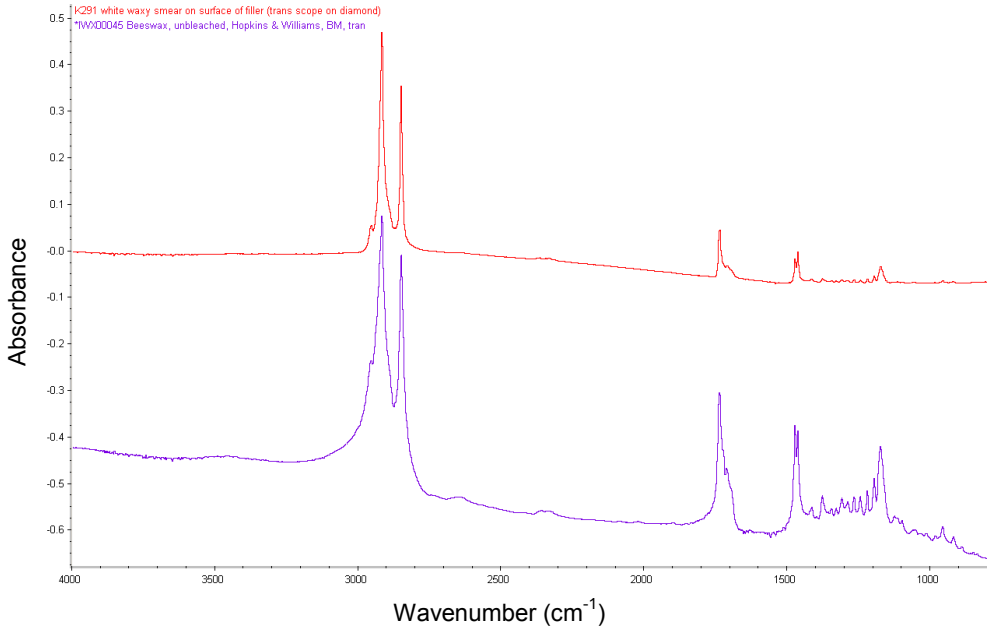

Figure 3: FTIR spectrum of the sample from K291 (top, red) with a reference spectrum for beeswax (bottom, purple) for comparison.

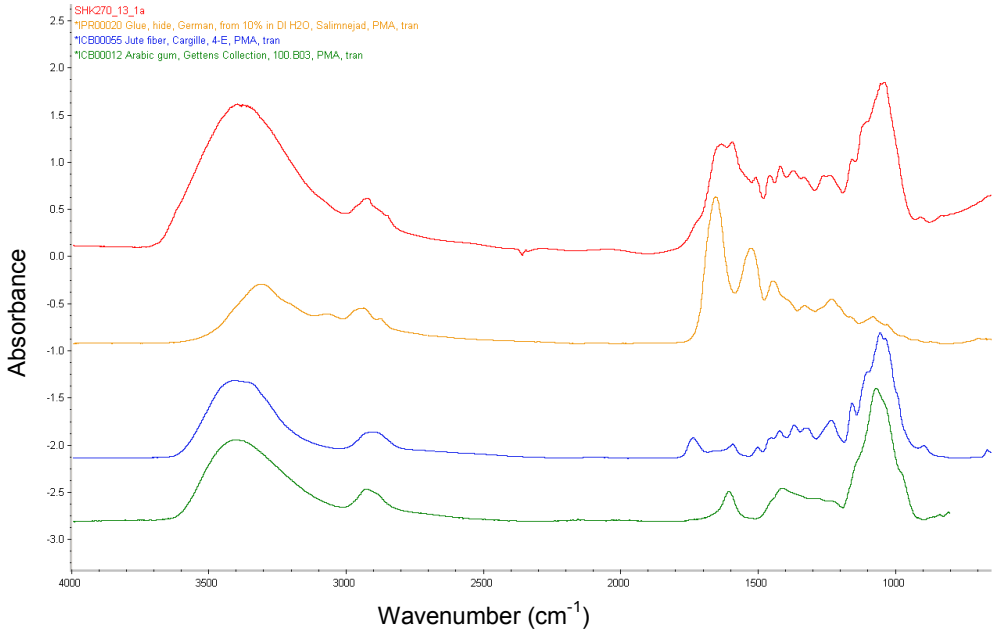

Figure 4: FTIR spectrum of paste K270_13 (top, red). Also shown are the reference spectra for protein- and carbohydrate-based materials for comparison: German hide glue (next to top, yellowish/brown), jute fibre (next to bottom, blue) and gum arabic (bottom, green).

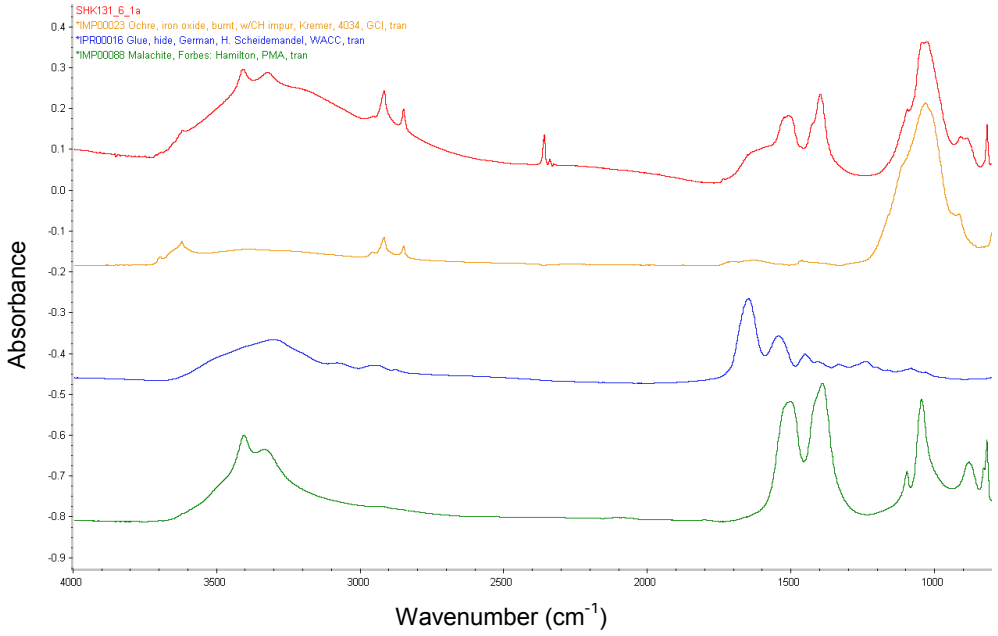

Figure 5: FTIR spectrum of K131_6 (top, red) with reference spectra of burnt ochre (an earth pigment containing (alumino)silicate minerals and iron oxide) (second, yellow/brown), German hide glue (third, blue) and malachite (bottom, green) for comparison.

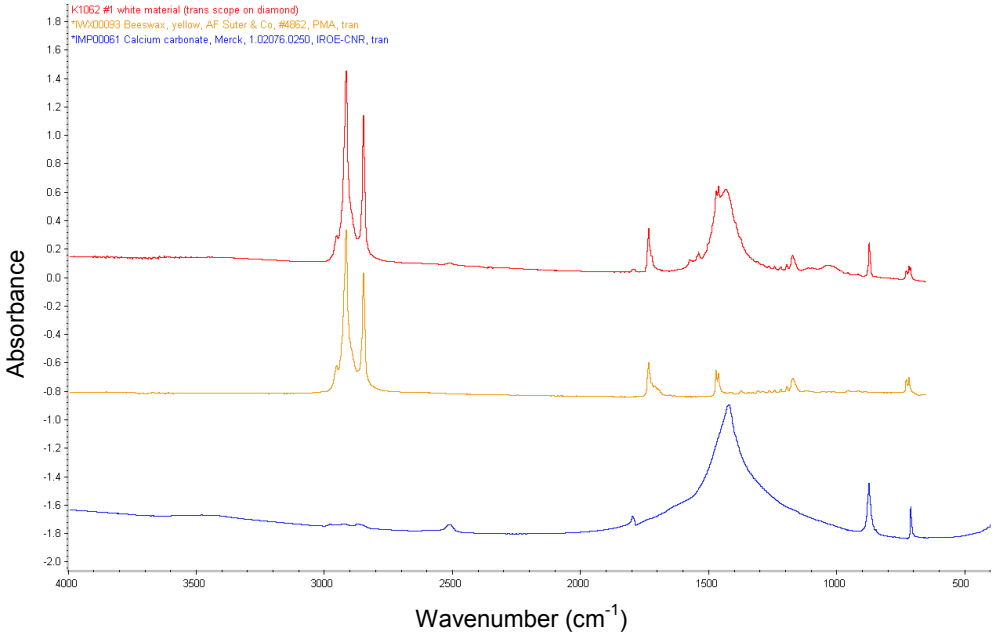

Figure 6: FTIR spectrum of sample K1062_1 (top, red). Reference spectra of beeswax (yellow/brown, middle) and calcium carbonate (bottom, blue) are shown for comparison.

Sample K270_13 also showed features of a plant gum and possibly plant fibre or wood (Figure 4). K673 did not show any features of plant gum in the FTIR spectrum but as it was largest of all the samples and was also taken from an area previously completely enclosed within a cloisonné cell in its original position, it was therefore felt useful to examine this sample for all possible types of organic material. This sample was therefore extracted for sugars as discussed below.

In addition to beeswax, proteinaceous glue and plant gum, all of these samples also contained inorganic material. Six samples (K131_6, K356_2, K451_5, K673, K1003_4 and K1388) showed evidence of (alumino)silicate minerals such as are present in iron oxide-containing earth pigments; two samples (K131_6 and K273_7) contained copper corrosion products, probably azurite ($Cu_3(CO_3)_2(OH)_2$) or malachite; and two samples contained calcium carbonate (K546_1 and K1062_1) (see Figures 5 and 6).

On the basis of these results all but one of the pastes were analysed by GC-MS following solvent extraction to identify lipid components. K356_10 was not analysed as it showed no evidence of organic material in the FTIR spectrum and a further paste from this object (K356_2) was analysed by GC-MS. The samples of residues which were not pastes were not analysed by GC-MS due to the very clear nature of the FTIR results obtained and the limited sampling area available without causing visual disturbance.

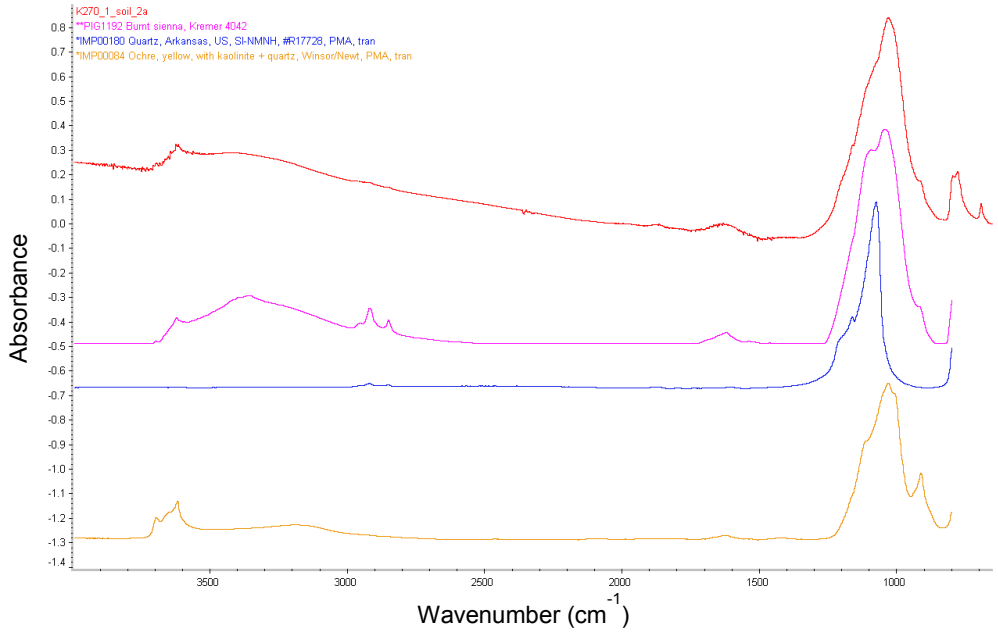

Figure 7: A typical example of an FTIR spectrum of soil (K270_1) (top, red) with a reference spectra for a sample of burnt sienna (a calcined earth pigment containing (alumino)silicate minerals and iron oxide (second, magenta)), quartz (third, blue) and a sample of yellow ochre, an earth pigment containing (alumino)silicate minerals and iron oxide, with this particular sample containing kaolinite and quartz (bottom, yellow/brown) for comparison.

Analysis of the soils

The six soil samples (K270_1, K356_1, K447_1, K451_2, K662_4 and K1003_1) all yielded spectra dominated by (alumino)silicate minerals such as are found in iron oxide-containing earth pigments. In addition the spectra contained contributions from the clay mineral

kaolinite (K270_1 and K662_4), quartz (K270_1, K356_1, K447_1 and K1003_1), additional (alumino)silicates (K447_1), a carbonate mineral (K451_2), copper corrosion products (K451_2) and possibly some plant material (K356_1 and K447_1). A typical spectrum is shown in Figure 7. Only K356_1 and K447_1 showed any evidence in the FTIR spectra of organic material. However, as soil usually contains some organic material, all these samples were subjected to solvent extraction and analysis by GC-MS.

GC-MS results

Solvent-extracted samples – lipid analysis

Of the samples subjected to solvent extraction and GC-MS analysis, only K270_19, K447_8 and K716_1 yielded no evidence of any significant organic compounds (Tables 1 and 2). These samples all yielded trace levels of *n*-alkanes, which are ubiquitous in the environment, and phthalate plasticizers which probably come from the storage of the samples in plastic vials or storage of the objects in plastic bags/boxes and/or in close proximity to expanded plastic foam. This confirmed the results of the FTIR analysis of these samples which found no evidence of organic materials.

All of the other samples analysed by GC-MS yielded a suite of compounds which are indicative of the presence of degraded beeswax (Figure 8). These included a range of long-chain alcohols with even numbers of carbon atoms in the range 22 to 34; high levels of the saturated fatty acids with 16 ($C_{16:0}$) and 24 ($C_{24:0}$) carbon atoms plus lower levels of a range of even carbon-numbered fatty acids between $C_{22:0}$ and $C_{34:0}$; hydroxy fatty acids, predominantly 14-hydroxyhexadecanoic and 15-hydroxyhexadecanoic acids; even carbon numbered wax esters with 42–50 carbon atoms; and a similar range of hydroxy wax esters (Heron *et al.*, 1994; Evershed *et al.*, 1997; Regert *et al.*, 2001; Jiménez *et al.*, 2003; Jiménez *et al.*, 2004; Jiménez *et al.*, 2007; Ribechini *et al.*, 2009). Fresh beeswax also contains a series of odd, or predominantly odd, carbon numbered *n*-alkanes with 23 to 33 carbons (Regert *et al.*, 2001; Jiménez *et al.*, 2004; Jiménez *et al.*, 2007). Alkanes are present in all of these samples and in K451_5 and K1003_4 odd alkanes within this range are dominant. However in the other samples, although alkanes are present, they are only present in trace amounts and show no odd over even preference. This depletion of alkanes has been observed in degraded beeswax and is attributed to loss by sublimation of the alkanes during heating (Heron *et al.*, 1994; Evershed *et al.*, 1997; Regert *et al.*, 2001). Experimental work by Regert *et al*. (2001) has shown that mild heating (60° C to 100° C) is enough to cause this loss. The melting point of raw beeswax is approximately 60 - 65° C (Tulloch and Hoffman, 1972; Fabra *et al.*, 2009) so melting the wax to mix it with other materials or to pour would be sufficient to cause loss of at least some alkanes.

In three samples (K270_13, K273_7 and K356_2) no saturated or unsaturated fatty acid with 18 carbons ($C_{18:0}$ and $C_{18:1}$) was detected and in the other samples it was present at much lower abundances than the $C_{16:0}$. This suggests that the presence of any animal or plant fats is unlikely as $C_{16:0}$ and $C_{18:0}$ and/or $C_{18:1}$ fatty acids are compounds characteristic of (degraded) fats and oils (Evershed, 1993; Heron and Evershed, 1993; Evershed *et al.*, 2002; Evershed, 2008)

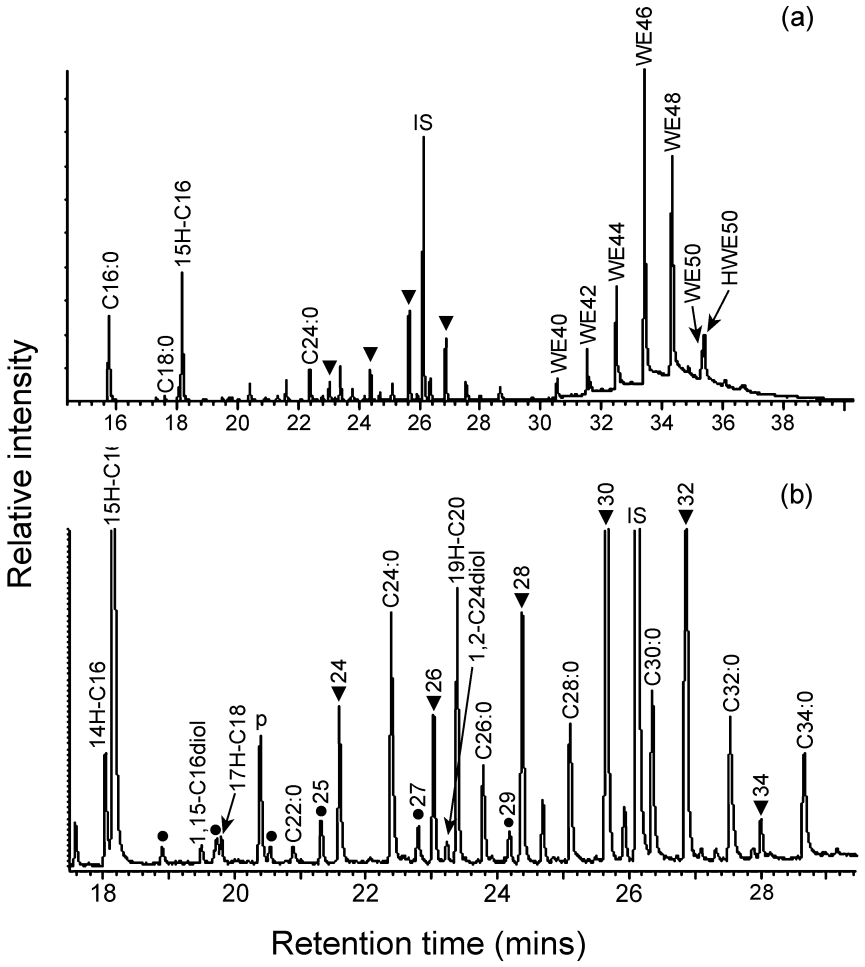

Figure 8: (a) Total ion chromatogram of the solvent extract from K1003_4; (b) an enlargement of the chromatogram between 18 and 29 minutes retention time. Key: x – number of carbon atoms; Cx:y – fatty acid with x carbon atoms and y double bonds; zH-Cx – hydroxy fatty acid, hydroxyl group on carbon z; ▼ – alcohols; m,n-Cxdiol – diol with alcohol groups on carbon atoms m and n;● – *n*-alkanes; WE – wax ester; HWE – hydroxy wax ester; IS – internal standard

Samples extracted for sugars and uronic acids – carbohydrate analysis

The two samples extracted for sugars and uronic acids (K270_13 and K673) both yielded a range of sugars characteristic of plant gums (Figure 9). A selection of modern reference gums (gum arabic, gum tragacanth, plum gum and locust bean gum) was prepared and run at the same time as the samples for comparison. Neither of the Staffordshire Hoard samples exactly matched any of the reference gums either in the literature or analysed with the samples. However polysaccharide gums are generally very hydrophilic and are also vulnerable to microbial degradation and chemical attack (Bleton *et al.*, 1996; Bonaduce *et al.*, 2007; Colombini and Modugno, 2009). Reactions with any substrate or, in this case, material mixed with the gum may also alter the composition of gums (Bleton *et al.*, 1996; Bonaduce *et al.*, 2007). It seems that arabinose is particularly susceptible to loss although the processes of ageing and alteration of plant gums are poorly understood (Bleton *et al.*, 1996; Bonaduce *et al.*, 2007; Colombini and Modugno, 2009). It is also important to note that plant gums are natural materials produced by plants growing in a wide range of different

habitats under different conditions and this will produce a natural variation even between gum samples from the same tree in different years (Colombini and Modugno, 2009).

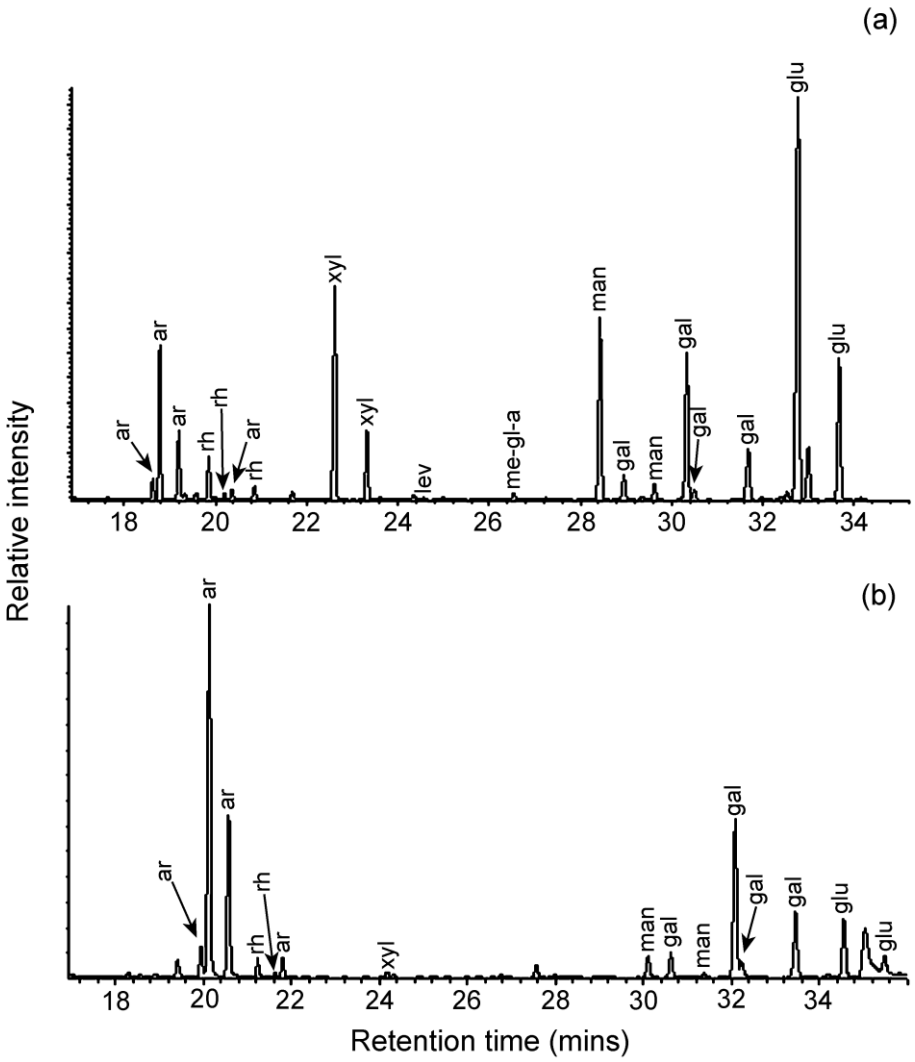

Figure 9: Chromatograms of sugar extractions of (a) K270_13 and (b) K673. Key: ar – arabinose; rh – rhamnose; xyl – xylose; lev – levoglucosan; me-gl-a – methyl glucuronic acid; man – mannose; gal – galactose; glu - glucose

Allowing for the processes of degradation and alteration, the nearest match to K270_13 in terms of sugar composition was a plum or cherry tree gum, or possibly an aged gum arabic (Figure 10). The high proportion of glucose in the sample may indicate the presence of another sugary material, such as honey, starch or cellulosic plant material (hemicellulose) (Colombini *et al.*, 2002b; Bonaduce *et al.*, 2007). Glucose is present in gum tragacanth, but not at the high concentration seen in K270_13. In addition the composition of the sample is unlike that of gum tragacanth which contains fucose and galacturonic acid both of which are absent from the sample (Figure 10).

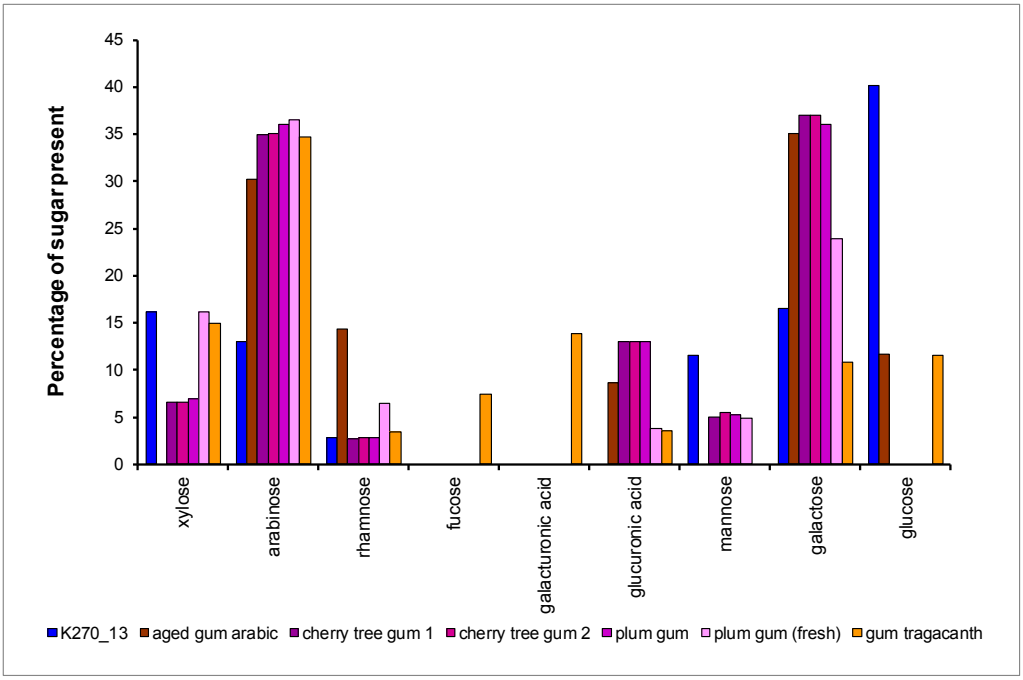

Figure 10: Comparison of the sugar composition of paste K270_3 with aged gum arabic, four examples of tree gums (two cherry tree and two plum tree), and gum tragacanth. Data for cherry tree gum and first example of plum gum from Bonaduce *et al.* (2007), the other data from analysis of reference gums at the British Museum

The pattern of sugars released from sample K673 was a reasonable, although not exact, match to the composition of gum arabic or aged gum arabic (see Figure 11). The sample contains very little xylose which reduces the possibility of a plum or cherry tree gum and gum tragacanth. Gum tragacanth is excluded by the absence of galacturonic acid and the very low levels of fucose in K673, despite the presence of a moderate amount of glucose which might suggest this gum. It is also possible that this is a product such as galactan from the European larch (*Larix decidua*) which consists mainly of arabinose and galactose in a ratio of 6:1 (Aspinall *et al.*, 1958). This ratio is much higher than that in the sample (only 1.8:1) but the amount of arabinose is unusually high in this sample compared with all the other reference gums, especially when taking into consideration the propensity for arabinose to disappear as gums age. Lack of detailed compositional data and the lack of any reference sample of this gum make it difficult to come to any firm conclusions regarding the presence of larch galactan.

In both of these cases it is possible that the sample is a mixture of different gums but this is very hard to determine without a series of very precise, quantified analyses of reference gums and samples followed by principal component analysis (Bonaduce *et al.*, 2007), which is beyond the scope of this project.

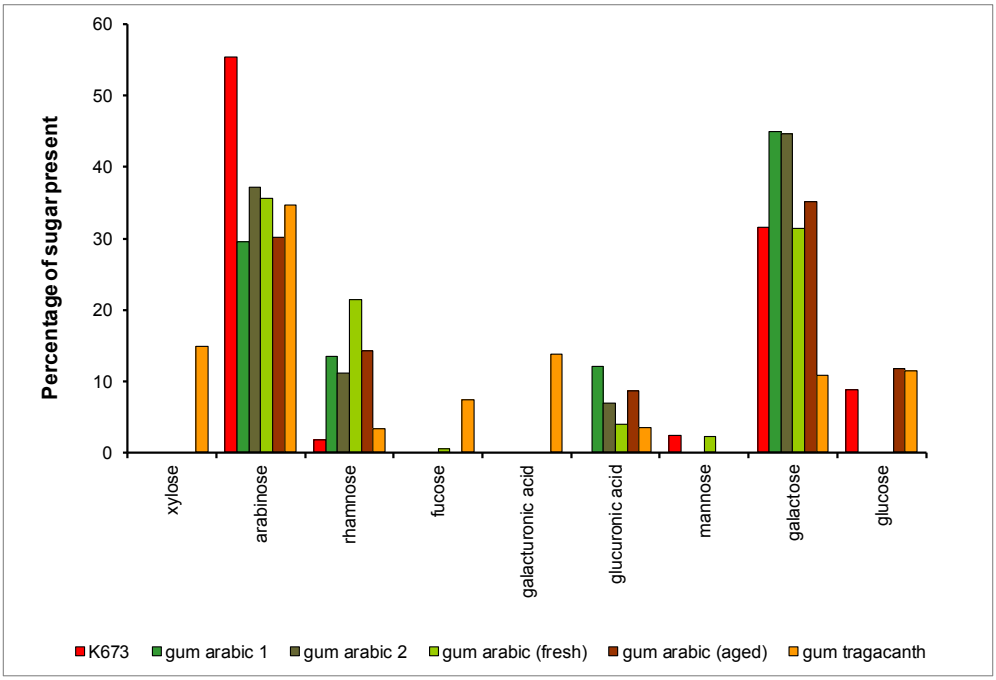

Figure 11: Comparison of the sugar composition of paste K673 with four examples of gum arabic, one of aged gum arabic and one of gum tragacanth. Data on gum arabic 1 from Colombini *et al*. (2002a), gum arabic 2 from Bonaduce *et al*. (2007), the rest of the samples are reference gums analysed at the British Museum.

Amino acid extractions – protein analysis

Unfortunately, as noted above, although samples K131_6, K270_13, K273_7, K356_2, K451_5, K673 and K1003_4 were prepared by acid hydrolysis for amino acid analysis, it proved impossible to analyse these samples using GC-MS. However in most cases, including sample K1388 where GC-MS analysis was not attempted, the FTIR results clearly show the presence of proteinaceous material. From this it can be concluded that these samples probably contained a proteinaceious material which may have been in the form of animal glue.

Analysis of the soils

The soils were all very similar and yielded a range of compounds which superficially appeared the same as those in the pastes containing beeswax (Figure 12 – *cf* Figure 8). These included $C_{16:0}$; even carbon-numbered fatty acids in the range $C_{22:0}$ to $C_{28:0}$ with a maximum at $C_{24:0}$; even carbon-numbered alcohols (C22 – C32); alkanes in the range C21 to C33 which were usually present in amounts too small to quantify; and traces of wax esters. However they also yielded compounds not present in the pastes including $C_{18:0}$ at levels of the same order of magnitude as $C_{16:0}$; shorter chain saturated fatty acids in the range $C_{7:0}$ to $C_{14:0}$; unsaturated fatty acids $C_{16:1}$ and $C_{18:1}$; mono- and di-acylglycerols; the animal sterol cholesterol; sugars; a range of compounds ubiquitous in the environment and soils in particular including vanillin, iso-vanillin and polyaromatic hydrocarbons; and a range of contaminants, many unidentified, which were present at very high levels compared to other components of the organic material in the soils. All of these compounds, including many of the identified contaminants, have been reported in soils and sediments (Heron *et al.*, 1991; Rushdi *et al.*, 2005; Otto and Simpson, 2007; Rogge *et al.*, 2007).

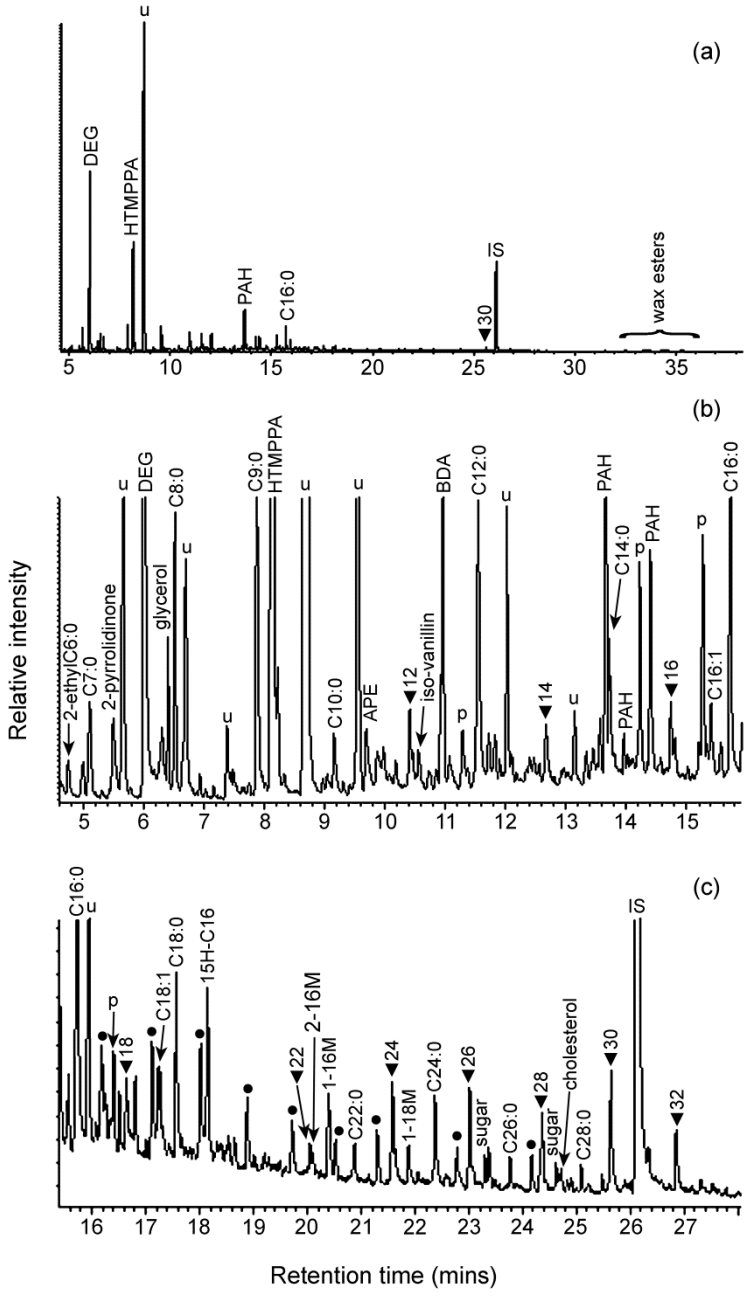

Figure 12: Soil from the inner side of collar K1003 (a) total ion chromatogram of solvent extract; (b) enlargement of the area between 5 and 16 minutes retention time; (c) enlargement of the area between 16 and 28 minutes. Key: DEG – diethylene glycol; HTMPPA – 2-methylpropanoic acid, 3-hydroxy-2,4,4-trimethylpentyl ester; BDA – 1,3-butanediol diacrylate; APE – acetophenyl ethanone; PAH – polyaromatic hydrocarbon; Cx:y – fatty acid with x carbon atoms and y double bond; zH-Cx – hydroxy fatty acid with x carbons, hydroxyl group attached at carbon z; 1- or 2-xM – monoacylglycerol with x carbons in the fatty acid chain; ▼x – alcohol with x carbons; ● – *n*-alkane; u – unidentified; IS internal standard

Despite the superficial similarity between the organic compounds found in the pastes and those in the soil, a closer inspection reveals that it is not possible for the material in the pastes to have come from the soil. Firstly, the organic compounds in the soil are present at much lower concentrations that those in the pastes with the total residues in soil ranging from 1.9 µg/g to 370 µg/g while those in the pastes range from 720 µg/g to 40.2 mg/g. The range and distribution of the compounds in the soils are also different. For example, in degraded beeswax the alcohol distribution usually has its maximum at C30 while in the soils it is consistently at C26. The soils also show a much wider range of different compounds many of which, such as the lower carbon number fatty acids, mono- and diacylglycerols, unsaturated fatty acids, cholesterol and compounds such as vanillin, are not present in the pastes. In addition the contaminants which form a large proportion of all the soil samples are not present in the pastes at all which would be expected if the paste residues had been composed mainly of material from the soil.

The complete results for the pastes and other organic materials (but excluding the soil samples) are summarized in Table 2.

Conclusions

Of the 11 potential paste samples and five other samples of possible organic materials, 11 (seven of the pastes and four of the other samples) yielded evidence of organic materials. These included over half the paste samples taken from inside cloisonné cells in gold and garnet objects. All of these samples (K131_6, K270_13, K273_7, K356_2, K451_5, K673 and K1003_4 of the pastes and K291, K546_1, K1032_1 and K1388 of the other samples) contained beeswax. It appears that beeswax was the main organic ingredient in the pastes and in the other fillers/packing materials/glues used in this group of objects. FTIR analysis also identified proteinaceous material, possibly animal glue, in all the paste samples containing organic material (K131_6, K270_13, K273_7, K356_2, K451_5, K673 and K1003_4) and in the material from the silver boss K1388. Unfortunately it was not possible to investigate the proteinaceous component of the materials further by GC-MS as the extraction method appears also to have formed or released a component/components which was totally incompatible with the stationary phase on the GC-MS column. However in most cases the FTIR results clearly indicate the presence of protein and it can safely be assumed that a proteinaceous material was used as part of the mixture of organic materials in these samples.

In addition to beeswax and proteinaceous material, two pastes, K270_13 and K673, contained the sugars indicative of a plant gum. In the case of K270_13 this is probably a fruit tree gum, such as plum or cherry, or possibly aged gum arabic. The gum in K673 is not a complete match for any one reference gum. It is closest in composition to gum arabic but could also be a degraded larch galactan gum or a mixture of the two.

It appears that, in general, beeswax and proteinaceous material mixed with an inorganic component derived from clay soil and/or sandy material was the most usual ‘recipe’ for the pastes and in a few cases plant gum or similar carbohydrate-based material was also added to the mixture. Other organic materials used on/in the objects are also based on beeswax with an inorganic material.

The samples which showed no evidence of organic materials were composed mainly of copper degradation products (K356_10 and K360_5) or soil-derived material (K279_19, K447_8 and K716_1).

Table 2: A summary of the results from the analysis of the pastes and other organic materials

Sample	FTIR	GC-MS solvent extract	GC-MS sugar extract
Pastes			
K131_6	Degraded proteinaceous material, copper corrosion products, (alumino)silicate minerals as in iron oxide-containing earth pigments	Beeswax	Not analysed
K270_13	Proteinaceious material, plant fibre/wood, plant gum – possibly degraded gum arabic	Beeswax	Plant gum, possibly fruit tree gum, with cellulosic plant material
K270_19	(Alumino)silicate minerals as in iron oxide-containing earth pigments, kaolinite, quartz	No organics	Not analysed
K273_7	Degraded beeswax, proteinaceous material, copper corrosion products	Beeswax	Not analysed
K356_2	Beeswax, proteinaceous material, (alumino)silicate minerals as in iron oxide-containing earth pigments	Beeswax	Not analysed
K356_10	Copper corrosion products	No analysis	Not analysed
K447_8	(Alumino)silicate minerals as in iron oxide-containing earth pigments, quartz	Not analysed	Not analysed
K451_5	Beeswax, proteinaceous material, (alumino)silicate minerals as in iron oxide-containing earth pigments	Beeswax	Not analysed
K673	Beeswax, (alumino)silicate minerals as in iron oxide-containing earth pigments, gypsum, proteinaceous material	Highly degraded beeswax	Plant gum, possibly gum arabic, perhaps with larch galactan
K716_1	(Alumino)silicate minerals as in iron oxide-containing earth pigments, quartz	No organics	Not analysed
K1003_4	Beeswax, proteinaceous material, (alumino)silicate minerals as in iron oxide-containing earth pigments	Beeswax	Not analysed
Other organics			
K291	Beeswax	Not analysed	Not analysed
K360_5	Copper corrosion products	Not analysed	Not analysed

Sample	FTIR	GC-MS solvent extract	GC-MS sugar extract
K546_1	Beeswax, calcium carbonate	Not analysed	Not analysed
K1062_1	Beeswax, calcium carbonate	Not analysed	Not analysed
K1388	Beeswax, proteinaceous material, (alumino)silicate minerals as in iron oxide-containing earth pigments	Not analysed	Not analysed

The presence or absence of organic material within the samples appears to bear no relation to the visual appearance of the pastes, e.g. the greenish sample K270_13 was rich in organic material while the blackish sample K716_1 yielded no organic components. It is however worth noting that all the samples are inhomogeneous, containing particles of brown, dark, light and greenish material, and with FTIR care was taken to ensure analysis of different areas within samples. For GC-MS analysis a larger sample size is required so will include material from different areas of the samples giving a 'bulk' analysis but, even here, the sample may not be totally representative of the materials present on the objects analysed.

It is hard to detect any pattern in terms of the type of object or position of the sampled material on the individual objects. It appears that, where organic material is present at all, a beeswax mixture is generally used within the cells behind garnets. Its use in other areas of objects appears to be variable. In K270_13 it was present within the cell sampled but not in the larger depressed area around the centre of the object (K270_19). It was also absent from the empty central panel in K716.

In addition the samples from the surface of the bulk filler in the fragmentary, silver pommel cap K291, the white material from the section of box strip K546, the material from the back of the central panel on the gold and garnet strip K1063 and the white residue inside the gold boss K1388 all contained beeswax, although only K1388 also contained proteinaceous material. Of these latter samples, which are from contexts other than the cloisonné cells, three (K546, K1062_1 and K1388) contained inorganic material, calcium carbonate in the first two cases and (alumino)silicate minerals as in iron oxide-containing earth pigments in K1388. This does seem to indicate that a slightly different material, although still based on beeswax, was being used for glues, fillers etc outside the context of the cells.

Potential for further work

The work presented here has established that, in general, pastes within the cloisonné cells of gold and garnet objects are a mixture of organic and inorganic materials. In general the organic components appear to be beeswax and what is probably a proteinaceous glue, sometimes with the addition of plant gum or other carbohydrate-based material. There does not seem to be any discernable variation between objects but investigation of this would need a larger number of samples to provide a clear answer. It would be possible to target particular questions, for example intra-object variation in the types of paste used. By removing a few of the garnets from each of several objects and analysing the pastes it would be possible to determine whether all the cells in one object are similar. Variation between objects of different styles (for example later or earlier examples) could potentially be investigated in a similar way. However it is quite probable that the results will all be similar with the organic component of pastes being basically beeswax and proteinaceous material.

Further analysis of the proteinaceous material in the pastes will be complex and may not produce any useful results. Separating the amino acids of interest from the portion of the sample that is damaging to the GC-MS cannot be carried out until the nature of the samples has been more firmly established. The effort and time required to make any progress with this may not be worthwhile when the FTIR results clearly show the presence of proteinaceous material and in most cases appear to give a reasonable match to animal glue.

The analysis of potential organic material from other objects does seem to be worthwhile, although it seems that the range of materials used is still primarily limited to beeswax and proteinaceous material.

The results of any further analyses will be limited by the size of samples available. FTIR can be carried out on very small samples but does not always detect minor components of a mixture – such as the plant gum in sample K673. GC-MS can detect materials down to fractions of µg but in the samples analysed here only a proportion of each sample is organic, sometimes a small proportion. This means that, although quite small samples can be analysed successfully, in this case down to 0.1 mg, very small samples will not provide enough organic material to give useful results. The results reported here have come from the largest of the samples supplied and in some cases have proved hard to interpret due to the low levels of individual organic compounds in the sample. A sample may contain 40 mg/g of organic compounds but if only 0.1 mg of sample is available (as in the smallest of these samples) the total organic material will only amount to 400 µg. As each sample of organic material may contain tens of compounds (typically 20 to 40 in these samples) only 1 µg of a minor compound may be present. In this case it can be seen that samples much smaller than 0.1 mg will be too small to produce good results when analysed by GC-MS. It is therefore recommended that GC-MS analysis should only be attempted where a suitably sized sample is available.

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References

Aspinall, G.O., Hirst, E.L. and Ramstad, E. 1958. The constitution of larch ε-galactan. *Journal of the Chemical Society (Resumed),* 1958: 593-601.

Bleton, J., Mejanelle, P., Sansoulet, J., Goursaud, S. and Tchapla, A. 1996. Characterization of neutral sugars and uronic acids after methanolysis and trimethylsilylation for recongnition of plant gums. *Journal of Chromatography A,* 720: 27-49.

Bonaduce, I., Brecoulaki, H., Colombini, M.P., Lluveras, A., Restivo, V. and Ribechini, E. 2007. Gas chromatographic-mass spectrometric characterisation of plant gums in samples from painted works of art. *Journal of Chromatography A,* 1175: 275-282.

Colombini, M.P., Ceccarini, A. and Carmignani, A. 2002a. Ion chromatography characterization of polysaccharides in ancient wall paintings. *Journal of Chromatography A,* 968: 79-88.

Colombini, M.P. and Modugno, F. 2009. Organic materials in art and archaeology. *In:* Colombini, M. P. & Modugno, F. (eds.) *Organic Mass Spectrometry in Art and Archaeology.* Chichester, West Sussex, UK: John Wiley & Sons Ltd: 3-36.

Colombini, M.P., Modugno, F., Fuoco, R. and Tognazzi, A. 2002b. A GC-MS study on the deterioration of lipidic paint binders. *Microchemical Journal,* 73: 175-185.

Evershed, R.P. 1993. Biomolecular archaeology and lipids. *World Archaeology,* 25(1): 74-93.

Evershed, R.P. 2008. Organic residue analysis in archaeology: the archaeological biomarker revolution. *Archaeometry,* 50(6): 895-924.

Evershed, R.P., Dudd, S.N., Copley, M.S., Berstan, R., Stott, A.W., Mottram, H.R., Buckley, S.A. and Crossman, Z. 2002. Chemistry of archaeological animal fats. *Accounts of Chemical Research,* 35(8): 660-668.

Evershed, R.P., Vaughan, S.J., Dudd, S.N. and Soles, J.S. 1997. Fuel for thought? Beeswax in lamps and conical cups from Late Minoan Crete. *Antiquity,* 71: 979-985.

Fabra, M.J., Talens, P. and Chiralt, A. 2009. Microstructure and optical properties of sodium caseinate films containing oleic acid-beeswax mixtures. *Food Hydrocolloids,* 23(3): 676-683.

Heron, C. and Evershed, R.P. 1993. The analysis of organic residues and the study of pottery use. *In:* Schiffer, M. (ed.) *Archaeological Method and Theory.* Arizona: University of Arizona Press: 247-284.

Heron, C., Evershed, R.P. and Goad, L.J. 1991. Effects of migration of soil lipids on organic residues associated with buried potsherds. *Journal of Archaeological Science,* 18: 641-659.

Heron, C., Nemcek, N., Bonfield, K.M., Dixon, D. and Ottaway, B.S. 1994. The chemistry of Neolothic beeswax. *Naturwissenschaften,* 81: 266-269.

Jiménez, J.J., Bernal, J.L., Aumente, S., Del Nozal, M.J., Martin, M.T. and Bernal Jr., J. 2004. Quality assurance of commercial beeswax. Part I. Gas chromatography-electron impact ionization mass spectrometry of hydrocarbons and monoesters. *Journal of Chromatography A,* 1024: 147-154.

Jiménez, J.J., Bernal, J.L., Aumente, S., Toribio, L. and Bernal Jr., J. 2003. Quality assurance of commercial beeswax II. Gas chromatography-electron impact ionization mass spectrometry of alcohols and acids. *Journal of Chromatography A,* 1007: 101-116.

Jiménez, J.J., Bernal, J.L., Jesús Del Nozal, M., Toribio, L. and Bernal, J. 2007. Detection of beeswax adulterations using cencentration guide-values. *European Jornal of Lipid Science and Technology,* 109(7): 682-690.

Otto, A. and Simpson, M.J. 2007. Analysis of soil organic biomarkers by sequential chemical degradation and gas chromatography - mass spectrometry. *Journal of Separation Science,* 30: 272-282.

Regert, M., Colinart, S., Degrand, L. and Decavallas, O. 2001. Chemical alteration and use of beeswax through time: accelerated ageing tests and analysis of archaeological samples from various environmental contexts. *Archaeometry,* 43(4): 549-569.

Ribechini, E., Orsini, S., Silvano, F. and Colombini, M.P. 2009. Py-GC/MS, GC/MS and FTIR investigations on Late Roman-Egyptian adhesives from *opus sectile*: new insights into ancient recipes and technologies. *Analytica Chimica Acta,* 638: 79-87.

Rogge, W.F., Medeiros, P.M. and Simoneit, B.R.T. 2007. Organic marker compounds in surface soils of crop fields from the San Joaquin Valley fugitive dust characterization study. *Atmospheric Environment,* 41: 8183-8204.

Rushdi, A.I., Al-Mutlaq, K. and Simoneit, B.R.T. 2005. Sources of organic compounds in fine soil and sand particles during winter in the metropolitan area of Riyadh, Saudi Arabia. *Archives of Environmental Contamination and Toxicology,* 49: 457-470.

Tulloch, A.P. and Hoffman, L.L. 1972. Canadian beeswax: analytical values and composition of hydrocarbons, free acids and long chain esters. *Journal of the American Oil Chemists Society,* 49: 696-699.

Appendix – Methods

FTIR

FTIR Spectroscopy was performed on a Nicolet 6700 with Continuum IR microscope equipped with MCT/A detectors. The samples were analysed in transmission mode, flattened in a diamond micro-compression cell. The cell was opened and the flattened sample supported on one diamond window, a clean area of which was used for background spectra. The field of view was controlled by the sliding aperture which, when fully open, gives a maximum area of analysis of 150x150 µm. The spectra were acquired over a range of 4000-650 cm^{-1} using 32 scans at a resolution of 4 cm^{-1} and automatic gain.

GC-MS

To prevent contamination during sample preparation nitrile gloves were worn at all times, all glassware and tools were washed in solvent and all reagents were Analar or HPLC grade (99.5% pure or better). Blank samples were prepared and analysed with each batch of samples to check for contamination introduced during sample preparation and analysis.

Sample preparation – solvent extraction

Each sample was crushed in an agate pestle and mortar and accurately weighed into in a clean glass vial. Samples were then placed in an ultrasonic bath for 15 minutes with a mixture of dichloromethane (DCM) and methanol (2:1 v/v). After centrifuging for 10 minutes at 2000 rpm the supernatants were pipetted off into clean vials. This was repeated twice more, combining the three extracts of each sample. The excess solvent was evaporated off the samples under a gentle stream of dry nitrogen (N_2) while heating at 40° C. A measured amount of an internal standard, C34 *n*-alkane, was added to each sample to allow quantification. Samples were derivatised for GC-MS by heating at 70° C in closed vials with 30 – 50 µl N,O-bis(trimethylsilyl)fluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS). After cooling the excess BSTFA was evaporated off under dry N_2 as above. The samples were re-dissolved in approximately 25 µl DCM prior to analysis by GC-MS.

Sample preparation for the extraction of neutral sugars and uronic acids

To extract sugars and uronic acids a 0.5M solution of methanolic hydrochloric acid was prepared by adding 400 µl acetyl chloride to 15 ml anhydrous methanol. Samples were weighed and placed in clean vials as above and heated with 100 µl of the methanolic hydrochloric acid at 80° C for 24 hours. Samples were evaporated to dryness as above and derivatised by heating at 80° C for 1.5 hours with 100 µl Sigma-Sil-A (1:3:9 mixture of trimethylchlorosilane:hexamethyldisilazane:pyridine). After derivatisation the samples were again evaporated to dryness and re-dissolved in hexane prior to GC-MS analysis.

Four reference gums (gum arabic, plum gum, gum tragacanth and locust bean gum) were prepared at the same time for comparison purposes. The same method was used but 200 µl methanolic hydrochloric acid and 300 µl Sigma-Sil-A were used.

Sample preparation for extraction of amino acids

Samples were weighed and placed in clean glass vials as above. A measured amount (30 µl) of a standard norleucine solution (1 mg norleucine in 6 ml 0.1N hydrochloric acid (HCl)) was added to each sample as an internal standard. Excess HCl was evaporated off and 100 µl 6.0N HCl added to each sample. Samples were then heated in closed vials for 24 hours

at 105° C, allowed to cool, centrifuged and evaporated to dryness under dry N_2 at 60° C. Samples were then agitated with 40 µl high purity water, centrifuged and evaporated to dryness. This was then repeated with 40 µl denatured alcohol. A silylating agent was made up by first dissolving 40 mg pyridine hydrochloride per ml in silylation grade pyridine to create a saturated solution. For the silylation reagent 300 µl N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) with 1% *tert*-butyldimethylchlorosilane (BDMCS) was added to 700 µl of the saturated pyridine hydrochloride solution. Samples were agitated with 50 µl derivatising reagent, warmed at 60° C for 30 minutes and then heated in an oven at 105° C for 5 hours. After cooling these were centrifuged before injecting into the GC-MS. Some samples were prepared twice by this method but both times they could not be run as something in the samples from both preparation attempts was very destructive to the stationary phase on the GC-MS column.

GC-MS analysis

Solvent extracted samples

These were run on an Agilent 6890 gas chromatograph attached to an Agilent 5973 mass selective detector. The gas chromatograph was fitted with an SGE HT-5 column, 12 m x 0.22 mm x 0.1 µm. The oven was programmed for an isothermal hold at 50° C for 2 minutes, rise at 10° per minute to 370° C and a final isothermal hold for 15 minutes. Helium was used as the carrier gas at a constant flow of 1.5 ml per minute. The Programmable Variable Temperature (PTV) injector was operated in on-column mode with the temperature programmed to match the oven temperature. The column was inserted directly into the mass spectrometer with the interface at 350° C. Mass spectral data was acquired in scan mode over a mass range of 50 to 750.

Neutral sugars and uronic acids

Samples were analysed on an Agilent 6890 gas chromatograph linked to an Agilent 5973 mass selective detector. The gas chromatograph was fitted with a 30 m x 0.25 mm x 0.25 µm Agilent HP-5ms column. The split/splitless injector was operated in splitless mode. Helium was used as the carrier gas at a constant flow of 1.3 ml per minute. The oven was programmed to start at 40° C, rise to 130° C at 9° C per minute, then to 290° C at 2° per minute with a final hold of 10 minutes. The column was inserted directly into the mass spectrometer with the interface at 280° C. Data was acquired in scan mode over a mass range of 29 to 650.

Amino acids

An attempt was made to analyse these samples on the same GC-MS and column as that used for sugars and uronic acids. The oven was programmed to start at 80° C for 1 minute, rise to 300° C at 20° C per minute with a final isothermal hold of 6 minutes. The column interface with the mass spectrometer was at 300° C. Data was collected in scan mode over a mass range of 45 to 700. Unfortunately at approximately 8.5 minutes into the first run the baseline rose to a level higher than any peaks in the sample and remained at this level despite baking out the column, running solvent blanks and injecting derivatising agent. This indicates a breakdown of the stationary phase in the column and cannot be reversed. It occurred with both sets of samples prepared by this method and appears to result from an unidentified component in the samples.

All data were analysed using the Agilent MSD Chemstation data analysis software. Mass spectra were identified by comparison with the NIST mass spectral database and published mass spectra.

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