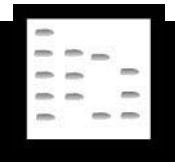
ARCHAEOLOGY: USES OF CHROMATOGRAPHY IN



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History

Although recent advances in analytical methods have accelerated the study of these materials, the analysis and identification of ancient organic residues has a long history. An early example, in the 1920s, was the use of wet chemical techniques by the chemist Alfred Lucas to study organic material from pottery and mummified human remains from the tomb of Tutankhamun. Over the last 20 years or so, the analysis of organic residues has grown into a recognized field in its own right. Examples of organic residues include the debris associated with the remains of food and other natural products as a result of their manipulation in pottery containers (e.g. cooking of food), the balms in the wrappings of mummified bodies and traces of colouring dyes impregnated in ancient textiles. Given the amorphous character of organic residues, the most effective approach to their identification lies in their chemical composition. Characterization of organic residues generally relies upon the principles of chemotaxonomy, where the presence of a specific compound or distribution of compounds in an unknown sample is matched with its presence in a contemporary natural substance. The use of such molecular markers is not without its problems, since many compounds are widely distributed in a range of natural materials, and the composition of an ancient residue may have changed significantly during burial. In general, molecular markers belong to the compound class defined as the lipids, a heterogeneous group of molecules which includes fats and oils and molecules with common solubilities, such as the constituents of resins and waxes.

Early work in this field relied heavily on either thin-layer chromatography (TLC) or gas chromatography (GC) alone to characterize residues. Today, combined GC–mass spectrometry (GC-MS) and, to a lesser extent, high-performance liquid chromatography–MS (LC-MS) are demonstrating considerable value in identifying ancient organic matter. The wider availability of these techniques and, in particular, the introduction of GC–isotope ratio mass spectrometry (GC–IRMS), is contributing to more specific identifications than was possible before. GC–IRMS allows the ratios of abundances of stable isotopes of elements such as carbon and nitrogen to be determined for individual compounds introduced via a gas chromatograph. Stable isotope ratios are of particular importance to studies of foodwebs due to the characteristic isotope signatures of plants utilizing different photosynthetic pathways. These distinctive ratios are passed along the food chain to herbivores and carnivores. The method requires very small samples and is being applied to trace organic residues in pottery vessels to establish their origin with a high degree of precision.

Methods

Analysis of archaeological material presents a number of challenges, including the small amount of sample available, the presence of complex molecular mixtures from more than one source, chemical alteration due to processing or degradation, and contamination. Furthermore, every sample is unique. These factors mitigate against simple interpretations of analytical results.

Recent developments in instrumental chromatographic techniques have enabled trace amounts of organic residues to be detected. Hence it is possible to analyse molecules surviving in an inorganic matrix such as pottery or soil, or surviving in morphological organic remains such as lipids in seeds or bone. Insoluble or polymeric fractions of residues that are not themselves volatile enough for conventional analysis can be broken up by pyrolysis, thereby allowing separation and identification of the fragments. Pyrolysis-GC-MS has been successfully applied to the recognition of biopolymers in fossil and recent higher plant resins, and to macromolecular debris remaining from the burning of food in archaeological pottery vessels.

Preparation of ancient lipids and natural products normally involves solvent washing of samples. Prefractionation of the lipid residue can be undertaken using microscale column chromatography or TLC. Prior to analysis, unhindered acid functionalities are derivatized by treatment with diazomethane. Trimethylsilylation using *N*,*O*-*bis*(trimethylsilyl) triflouroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) is used for the derivatization of hindered carboxyl groups and alcohols. In some cases an

internal standard is added to the sample to quantify yield.

GC remains a useful screening technique prior to GC-MS and can provide fingerprint chromatograms, whereby a complex set of peaks in a mixture can be matched to those in reference samples. Nevertheless, since molecular alteration is likely, this approach must be exercised with caution. Combined GC-MS provides valuable structural information on each of the components separated, and permits identification of molecular modification.

Applications

Residues Associated with Pottery

Fragments of broken and discarded pottery vessels are one of the most common classes of archaeological find. These sherds offer few immediate clues as to their original content and use – a significant point of enquiry in archaeology. During use, however, pottery vessels are known to accumulate residues of foods processed in them. If these residues survive long-term burial then they offer potential for determining artefact use. The residues occur as both charred or burnt deposits, which can be observed on the surface of the pottery, and as absorbed residues whereby organic components migrate into the pores of the vessel fabric. The porous microstructure of the fabric offers some protection to the residue from the effects of biodegradation and leaching during burial. The lipid constituents of these residues preserve rather well, and these can be extracted (by solvent washing of powdered sherd) from excavated sherds and analysed by GC and GC-MS.

A gas chromatogram from a typical degraded fat residue recovered from an archaeological sherd of Iron Age date (*c.* 100 BC) is shown in **Figure 1**B. The residue is rich in acylglycerols and free fatty acids and is typical of a partially hydrolysed lipid. This can be compared with the composition of fresh mammalian depot fat (**Figure 1**A), which is dominated by intact triacylglycerols. The monoacylglycerols, diacylglycerols and free fatty acids in the degraded fat result from the hydrolytic processes which begin as the pot is used (e.g. during boiling of food) and continue during burial. Furthermore, lipid residues are depleted in unsaturated fatty acids (such as oleic acid; $C_{18:1}$). This illustrates the problems in making simple comparisons between ancient lipids and fatty acid compositions of modern fats and oils.

Fractionation of the lipid to obtain minor constituents, such as sterols, can assist in determining a plant or animal source (or indeed whether lipids from both are present). Odd and branched-chain fatty acids may also be present. These are characteristic components of bacteria. They are, however, also introduced into ruminant adipose tissue by bacteria in the rumen and migrate throughout the animal's body, contributing to all the tissues. The presence of appreciable levels of these components, both in the free state and as components of the acylglycerol fraction, supports the view that the predominant source of lipid in the example shown derives from a ruminant animal.

Identification of thermal degradation products may give clues to vessel use. The long chain ketones in Figure 1 are formed by a high temperature reaction of fatty acids that is catalysed by the mineral matrix of the pottery fabric. Thus vessel use may be further understood by linking the molecular composition of the residues with exposure to high temperatures during formation, for example, during cooking. Recent research suggests that animal fats (such as adipose tissue, dairy products and fish/marine mammal oils) and plant tissues (notably the waxy compounds coating the surfaces of leaves) have the ability, under favourable burial conditions, to survive.

Pottery sherds may also exhibit the remains of organic surface treatments or sealants preserved as surface deposit. These are often resins, waxes or tars. GC analysis of one such deposit, a burnt surface residue on a neolithic potsherd, from Ergolding Fischergasse, Germany (mid 4th millennium BC), led to its identification as beeswax (**Figure 2**). The chromatograms shown compare wax ester distributions in fresh beeswax (*Apis mellifera*) with the fraction extracted from the surface deposit removed from the neolithic sherd. The principal wax esters in both samples are even-carbon-numbered aliphatic chains of saturated alcohols and fatty carboxylic acids with total carbon numbers in the range C_{40} to C_{50}, with the C_{46} wax ester the most abundant. The unsaturated wax esters present in the natural beeswax are absent from the neolithic residue. This is due to the deleterious effects of burial, during which the double bond is rendered susceptible to oxidation or reduction reactions. Natural beeswax also contains a considerable alkane component (in the range C_{21}–C_{33}), yet this was severely depleted in the archaeological sample, suggesting its combustion when the beeswax was burned. The sealing and water-repelling properties of beeswax suggest that it may have been used to seal the vessel to enable it to hold liquids. It is possible, however, that the vessel was used to store the beeswax for other uses. The identification of this commodity also implies the availability of honey to

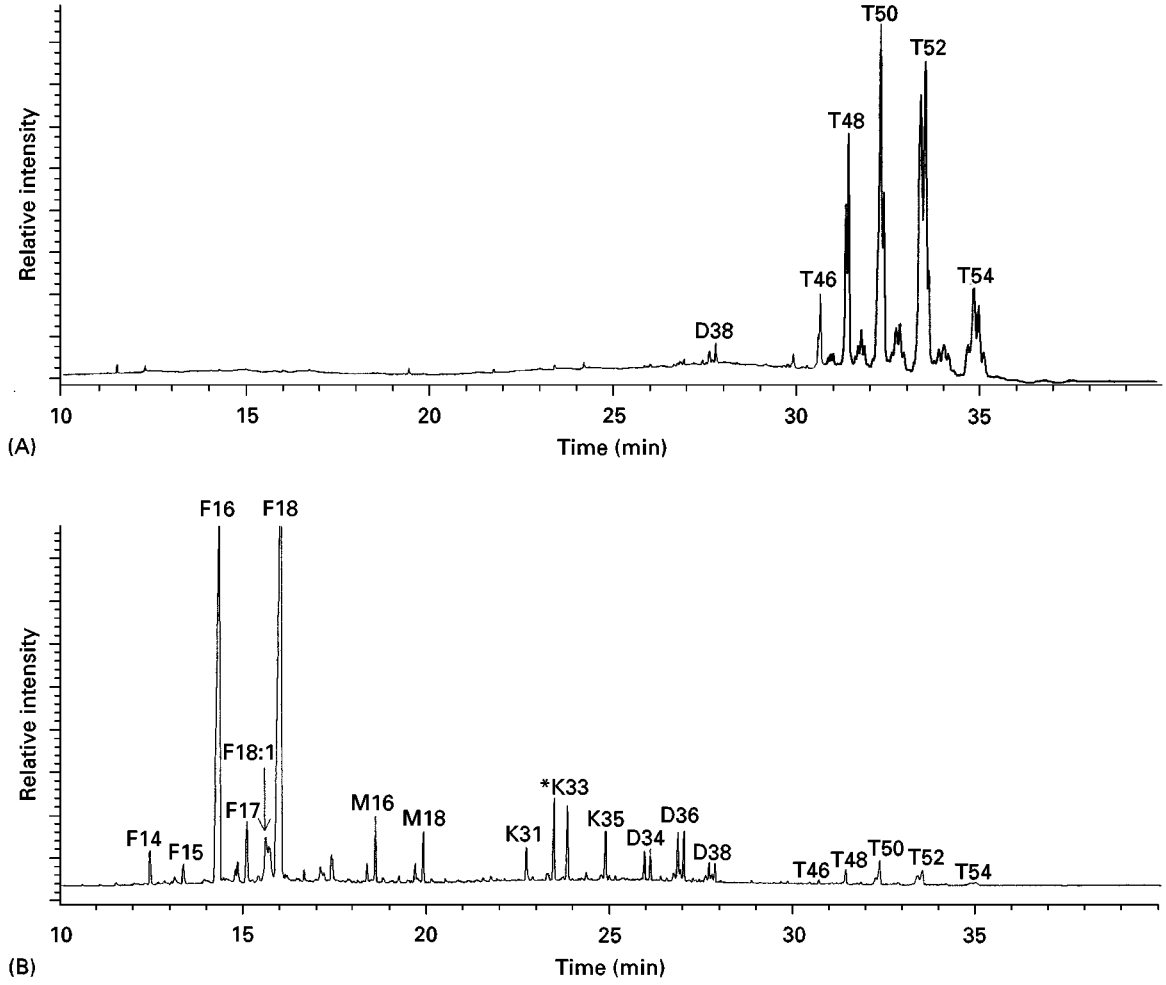


Figure 1 Partial gas chromatograms showing the compositions of (A) fresh beef fat and (B) the lipid residue extracted from an Iron-Age cooking vessel from Easingwold, Yorkshire, UK. The peak identities were established by GC-MS and are as follows: F14–F18 denote saturated fatty carboxylic acids with 14–18 carbon atoms respectively; F18:1 denotes a monounsaturated fatty acid with 18 carbon atoms; M16 and M18 are monoacylglycerols with 16 and 18 fatty acyl carbon atoms respectively; K31, K33 and K35 are mid-chain ketones with 31, 33 and 35 carbon atoms respectively; D34 and D36 represent diacylglycerols with 34 and 36 fatty acyl carbon atoms respectively. T46–T54 are triacylglycerols with 46–54 fatty acyl carbon atoms respectively. * Internal standard.

Analytical conditions: gas chromatography was carried out on a Hewlett Packard 5890 series II gas chromatography, equipped with a flame ionization detector. Samples were introduced by on-column injection into a 60 cm $\times$ 0.32 mm i.d. retention gap of deactivated polyimide-clad fused silica capillary tubing connected to the analytical column via a capillary connector. The carrier gas was helium at a constant flow of 1 mL min^{-1}. The temperature of the oven was programmed from 50 to 340°C at a rate of 10°C min^{-1} following a 2-min isothermal hold at 50°C after injection, with the final temperature held for 8 min.

The combined GC-MS was performed using a Hewlett Packard 5972A quadrupole mass selective detector in conjunction with a Hewlett Packard 5890 series II gas chromatograph. Samples were introduced via a splitless injector at 340°C with a 3-min purge time. Helium carrier gas was at constant pressure of 10 psi. Mass spectra were recorded over a mass range of 50–700 μm. The MSD interface temperature was 340°C, and the temperature was programmed from 50 to 340°C at a rate of 10°C min^{-1} following a 2-min isothermal hold at 50°C after injection, with the final temperature held for 12 min.

In both cases, the analytical column was a polyimide-clad 12 m $\times$ 0.22 mm i.d. fused silica capillary coated with BP1 stationary phase (immobilized poly(dimethylpolysiloxane), OV-1 equivalent, 0.1 μm film thickness, SGE, UK).

neolithic communities in Europe. GC-MS has also been used to identify beeswax residues associated with medieval ceramics, and in lamps from late Minoan Crete where the wax was burned as a fuel.

Analysis of a large number of vessels from an archaeological site enables correlation between residue type and pottery form and fabric, providing general assessments of use within assemblages.

Amorphous Residues and Adhesives

Amorphous organic substances can survive in other contexts, such as on stone tools, or as isolated

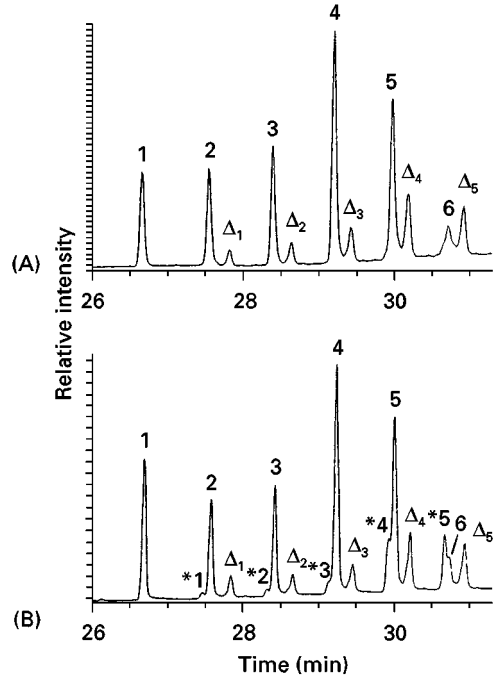


Figure 2 Partial gas chromatograms comparing the wax ester distribution in (A) Neolithic deposit on a potsherd from Ergolding Fischergasse, Germany with (B) authentic beeswax (*Apis mellifera*). Peak identities: 1–6 are wax esters in the range C_{40} (peak 1) to C_{50} (peak 6) comprising mostly hexadecanoic (palmitic) acid esterified with alcohols of increasing chain length (C_{24} to C_{34}). Peaks Δ_1 to Δ_5 represent co-elution of hydroxymonoester isomers and are seen in both samples. In contrast, peaks $*_1$ to $*_5$ are only present in the authentic sample and represent wax esters comprising an unsaturated (octadecanoyl) fatty acid moiety. Their absence in the ancient samples is not unexpected given the susceptibility of the double bond to oxidation or reduction reactions. Reproduced with permission from Heron C, Nemcek N, Bonfield KM *et al.* (1994). The chemistry of Neolithic beeswax. *Naturwissenschaften* 81: 266–269. Courtesy of Springer Verlag.

aggregates. An example is birch bark tar, which has been used as multipurpose natural product for at least 10 000 years, and its use continues to the present day in some parts of eastern and south-eastern Europe. The tar is obtained by heating fresh birch bark (*Betula* sp.) at temperatures of 250–350°C. Spectroscopic and chromatographic investigations of the material began during the 1960s, and a variety of techniques such as TLC, infrared and nuclear magnetic resonance (NMR) spectroscopy were used to identify birch bark tar on flint implements, lithics, ceramic and lumps of tar with human tooth impressions. More recent analysis has revealed that the tar was used to glue flint tips to arrows belonging to Ötzi – the 'ice man' discovered in the Tyrolean Alps in 1991. The tar has also been identified on potsherds, stone implements and worked bone from Ergolding Fischergasse (mid 4th millennium BC). Many of the early analyses have recently been confirmed by GC-MS, including lumps with tooth impressions, interpreted as a very early form of chewing gum.

Figure 3 compares fresh birch bark tar with samples from the Mesolithic site of Star Carr (Yorkshire, UK). The triterpenoids of the outer bark of *Betula* sp. are derivatives of lup-20(29)-ene and, to a lesser extent, olean-2-enes. The triterpenoid composition is modified slightly by heating the bark and by some 9000 years of water-logged burial, but the identity and relative abundance of these biomarkers is sufficient to characterize the archaeological samples. Tars produced from other bark and wood samples have a very different molecular composition. For example, softwoods produce diterpenoid compounds and are easily distinguishable, while the barks and tars of other trees such as hazel, rowan and willow produce triterpenoids but with different carbon skeletons or relative abundance of the lup-20(29)-enes. Analysis by GC-MS enables identification of the molecular markers of the heating of the bark and post-depositional alteration (**Figure 4**).

Bitumen represents the fraction of sedimentary organic matter which is soluble in organic solvents. The liquid or semi-solid varieties of bitumen were widely used in the Near East and Middle East in antiquity, serving as a multipurpose glue and water-proofing material, a building mortar, medicinal agent and as one of the constituents of the organic preparations applied to mummified bodies in Ancient Egypt. Compounds consistent with a bituminous substance include saturated hydrocarbons which have linear (alkylated alkanes) or cyclic (steranes, terpanes) carbon skeletons. These molecules largely derive from microscopic plants deposited in the sediments as well as bacterial inputs. It has proved possible to identify molecular and isotopic characteristics of the bitumen, which enables archaeological finds to be assigned to a particular source of bitumen. At the site of Susa, Iraq (dating from the beginning of the 4th millennium BC), bitumen was deliberately mixed and heated with mineral elements, to produce a substance known as bitumen mastic – a product ideal for fashioning decorative objects by sculpture.

Understanding Archaeological Sites

In addition to extant residues, chromatographic analyses can be used to identify the remains of ancient human activities that are invisible to the archaeological excavator. Identification of β-stanols, which are faecal biomarkers, in soil samples from archaeological sites have enabled identification of specific site features such as cess pits. The approach may also be used on a large scale to look at issues of waste

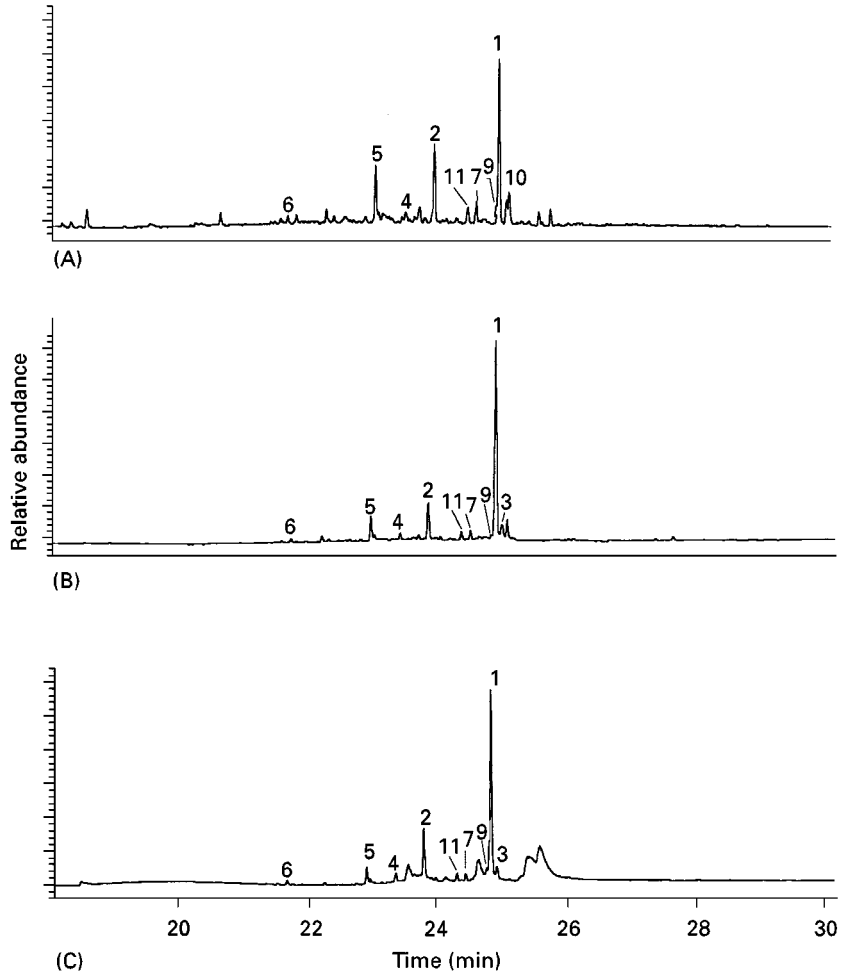


Figure 3 Partial gas chromatograms obtained by analysis of the solvent-soluble portions of samples of birch bark tar (*Betula pendula*) (peak identities were confirmed by GC-MS and are identified in Figure 4). (A) Birch bark tar prepared from fresh bark in the laboratory (350°C); (B) mesolithic sample from Star Carr ('resin cake'); (C) mesolithic sample from Star Carr (hafting glue). Reproduced with permission from Aveling EM and Heron C (1998). Identification of birch bark tar at the mesolithic site of Star Carr. *Ancient Biomolecules* 2(1): 69–80. Reproduced with permission of the copyright owners OPA (Overseas Publishers Association) N.V.

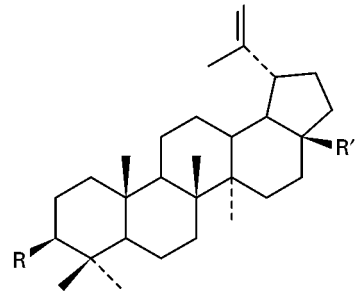
disposal and manuring patterns and early results suggest that the identification of specific sources of faecal matter may be possible.

Other Applications

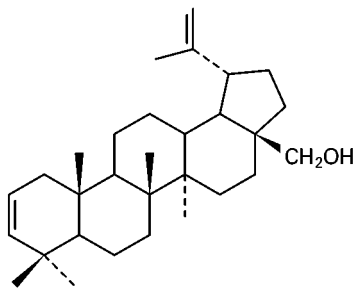
These examples cover only a small part of the spectrum of archaeological approaches making use of chromatographic techniques. It should be emphasized that high performance liquid chromatography has been used not only for the separation of amino acids and peptides (for the purposes of dating, amino acid racemization studies and isotopic investigations), but also in the study of ancient wine residues in pottery containers from the Old World, the analysis of ancient dyes, the identification of alkaloids (such as caffeine and theobromine characteristic of cacao in Mayan archaeological ceramics from Mexico) and in tracing alteration products of purine and pyrimidine bases in nucleic acid extracts of animal and plant remains.

Summary

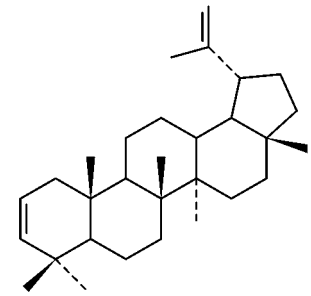
Today, chromatography is embedded in the battery of analytical approaches used to interrogate the surviving materials and residues of past societies. The acceleration of research in bimolecular archaeology in the last decade can largely be attributed to the availability of increasingly sophisticated analytical techniques. GC-MS is becoming the routine approach for the characterization of lipids and natural products, and compound-specific carbon isotope determinations are proving their value in identifying the origin of residue components. Chromatographic



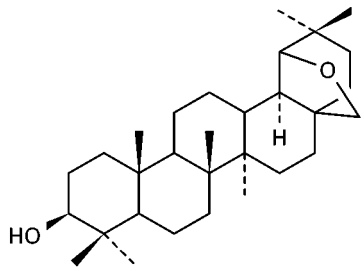
Peak number	*R*	*R′*	*Compund name*
1	OH	CH_2OH	Betulin (lup-20(29)-en-3β,28-diol)
2	OH	CH_3	Lupeol (lup-20(29)-en-3β-ol)
3	OH	COOH	Betulinic acid (3β-hydroxylup-20(29)-en-28-oic acid)
4	O=	CH_3	Lupenone (lup-20(29)-en-3-one)
7	O=	CH_2OH	Betulone (lup-20(29)-en-3-one-28-ol)
9	OH	CHO	Betulinic aldehyde (3β-hydroxylup-20(29)-en-28-al)



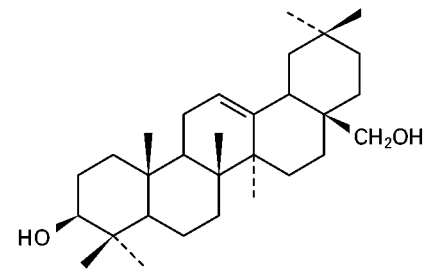
Peak 5: Lupa-2,20(29)-dien-28-ol



Peak 6: *Possibly* Lupa-2,20(29)-diene



Peak 10: Allobetulinol
(19β,28-epoxy-3β-hydroxy-18α(H)-oleanane)



Peak 11: Erythrodiol
(Olean-12-ene-3β,28-diol)

Figure 4 Structures of birch bark triterpenoids identified in Figure 3. (Reproduced with permission from Gundel LA and Lane DA (1999).)

techniques are contributing ever more to our understanding of the relationship between past human populations and their use of plant and animal resources, and of myriad ways in which artefacts were used.

See Colour Plate 55.

See also: **II/Chromatography: Gas:** Derivatization; Detectors: Mass Spectrometry; Pyrolysis Gas Chromatography. **Chromatography: Liquid:** Detectors: Mass Spectrometry. **III/Alkaloids:** Liquid Chromatography; Gas Chromatography; Thin-Layer (Planar) Chromatography. **Amino Acids:** Gas Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Amino Acids and Derivatives: Chiral Separations. Lipids:** Gas Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography.

Further Reading

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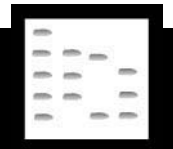
Includes a chapter on the identification of natural products (resins, pitch and waxes) from European prehistoric sites.

AROMAS: GAS CHROMATOGRAPHY

See **III / FRAGRANCES: GAS CHROMATOGRAPHY**

ART CONSERVATION: USE OF CHROMATOGRAPHY IN



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Introduction

Analytical science plays a vital role in the conservation of our artistic heritage and chromatography is one of the most valuable techniques available to the conservation scientist.

In order to design the optimum safe conservation/restoration treatment plan, which takes account of the nature of the original materials used by the artist, conservators require a detailed knowledge of the materials used. The microscopic samples characteristic of work in this area are notoriously problematic to deal with and the sensitivity of the analytical technique is paramount.

The question why are some painted works in better condition than others of a similar age? is an important one for the conservator and specific information regarding the nature of the media used in such works may offer some insight as to why variations in the ageing characteristics of individual paintings occur.

Paint Media

Artists have traditionally used a diverse range of materials as binding media for their pigments: natural oils, gums and proteinaceous materials such as egg, milk and collagen glues have all been incorporated into paint layers.

Oil painting was popular in northern Europe from before the 13th century and analytical evidence suggests that linseed oil was favoured, whilst in Italy, where oil painting was introduced in the 15th century, walnut oil was initially preferred. The oils most widely used in western European art are linseed, walnut and poppy, though the use of other oils, such as tung and safflower, has become more common in recent years.

Plant gums are commonly found as adhesives and binders. Gum arabic is primarily used as a painting medium, but others such as gum tragacanth (a medium for painting on linen) and cherry gum (which results in an enamel-like effect when mixed with egg or casein emulsions) are used less frequently. There is documentary evidence to suggest that gums have been employed as binding media and sizing materials for centuries: gum was used as a replacement for sun-dried oil as early as the 12th century.

Proteinaceous media include gelatine, milk and egg proteins. Animals and fish collagen glues are widely used as strong adhesives for wood, binders in the preparation of grounds, size for canvas, and pigment binders in decorative paints. Casein (a mixture of related phosphoproteins found in milk products), egg albumin (glair) and egg yolk (tempera) have traditionally found uses as pigment binders, temporary