Biomolecular archaeology and lipids

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Introduction

Amongst the first demonstrations of the existence of DNA in archaeological remains was a report by Pääbo (1985) in which DNA was recovered from a mummified Egyptian child (radiocarbon dated to nearly 2,500 years). Since that report considerable interest has developed in the recovery of ancient DNA from archaeological materials and palaeontological specimens. Demonstrating that DNA survives in the archaeological record, albeit in a degraded state, raises many exciting possibilities because of the uniqueness of the information contained in the genetic code (Brown and Brown 1992). Significantly though, in terms of actual mass, DNA is not one of the major organic components of living organisms. Proteins, carbohydrates and lipids are the most abundant constituents of animal tissues, while in plants lignins, other polymeric components and pigments also occur in high proportions. None of these latter classes of biomolecule compares to DNA in terms of the level of genetic or environmental information they carry. However, as biomolecular archaeology embraces any biological molecule that survives from antiquity and that can be used in the retrieval of archaeological information, the potential of all classes of biological molecules must be carefully considered. Although the first demonstrations of the preservation of medium-sized molecules, such as lipids, in archaeological materials were made well before the discovery of ancient DNA, the full potential of the use of lipids in archaeological studies has yet to be fully realized. It is the intention of this article to describe some of the general principles surrounding the use of lipids in archaeological investigations, including their properties, distribution, means of analysis, modes of preservation and decay, and application. Some of the areas in which scope exists for future developments are also discussed.

Orgins of biomolecular archaeology

There has been a long-standing interest in the study of amorphous organic materials found at archaeological sites. Chemical analysis of these materials can provide insights into the natural products such as resins, fats and oils, exploited by people in the past, and the processes used in their collection, refining and manufacture from plants, animals and other

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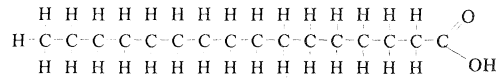
materials, such as natural bitumens. Early investigations of organic residues were inevitably limited by the lack of suitable analytical techniques and relied on determinations of the physical properties of organic residues using simple tests such as melting points and solubility measurements (Gangl 1936; Lucas and Harris 1962; von Stokar 1938). Progress towards precisely identifying materials was inevitably limited by the inherent complexity of the compositions of the substances under investigation, complicated by effects of decay over archaeological time. The advent of modern instrumental chemical and biochemical techniques has provided us with the resources necessary for the effective recovery, detection and characterization of biomolecules and their decay products in archaeological materials.

Biomolecular archaeology is closely related to, if not a generic sub-division of, the developing discipline of biomolecular palaeontology. Biomolecular palaeontology is concerned with understanding the processes which result in the preservation or decay of biomolecules, and their use in the assessment of palaeoenvironments and even the study of evolution itself (Eglinton and Logan 1991). On this basis, the relationship of biomolecular archaeology to biomolecular palaeontology is clear. However, an obvious distinction between the two fields of study derives from biomolecular palaeontology being concerned with the fate of biomolecules *per se*, while biomolecular archaeology focuses on the study of those ancient biomolecules that can yield information relating to human activity in the past.

Structures, properties and occurrence of lipids

Lipids are the most commonly occurring class of medium-sized molecules produced by living organisms and can frequently be recovered from archaeological materials. Lipids are defined, rather imprecisely, as being those components of biological materials that are more soluble in organic solvents than in aqueous media. A mixture of chloroform and methanol (in the volume ratio 2 : 1) is commonly used in the extraction of lipids from biological materials (Folch et al. 1957). An important feature of lipids is the wide range of structures that arise due to the ability of carbon to bond to carbon in a wide variety of arrangements. Lipids are composed largely from *carbon*, *hydrogen and oxygen*, and to a lesser degree phosphorus nitrogen and sulphur. Their structures consist of linear, branched and cyclic (mono- or polycyclic) carbon skeletons (see structures 1–7) which are commonly fully substituted with hydrogen atoms (termed *saturated*). As can be seen from structure 1, the chain of carbon atoms in palmitic acid is effectively covered by a skin of hydrogen atoms. Structure 2 shows a shorthand notation that is used routinely to depict the structures of lipids, and other organic compounds. Oxygen-containing functional groups (e.g. ester moieties in triacylglycerols and 3β-hydroxyl group in sterols) and double bonds (e.g. in unsaturated fatty acids and sterols (3–5) occur with reasonable frequency in lipids. The high proportion of saturated hydrocarbon moieties present in lipids confers on them hydrophobic properties which tend to reduce their solubility in water, hence limiting their loss from artefacts by water leaching. However, many possibilities exist for *in situ* chemical or microbiological alterations occurring to the structures of lipids that can complicate interpretations of their origin.



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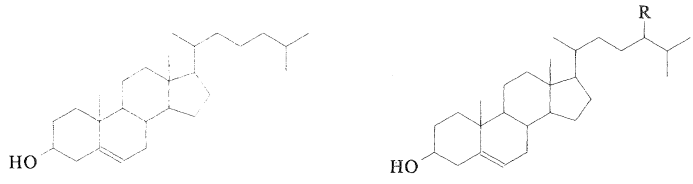
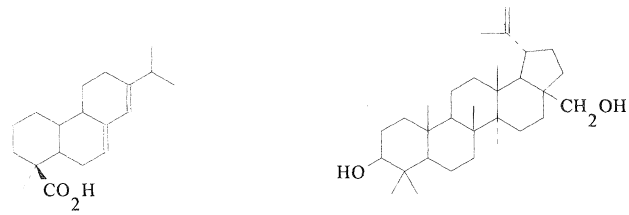


3 4 R = CH_3 5 R = C_2H_5



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Structures 1–7 (see text for explanation)

In living organisms lipids fulfil a variety of structural, metabolic and physiological roles. For example, in animals cholesterol (3) functions as a structural component of cell membranes, and as the precursor of steroid hormones (which are involved in sexual differentiation) and of bile acids (which serve to solubilize fats in digestion). Fatty carboxylic acids are constituents of triglycerides which are the major components of fats and represent important energy stores (Mead et al. 1986). Fatty acids are also constituents of phospholipids, which serve as major components of cell membranes (Mead et al. 1986). Other long chain alkyl compounds, e.g. alcohols, ketones, wax esters, etc., occur widely as components of natural waxes, such as beeswax and plant waxes (Kollattukudy 1976). Numerous other polycyclic diterpenoid (6) and triterpenoid (7) compounds occur in natural resins produced by higher plants. Mills and White (1987) have produced a useful account of the lipid compositions of plant and animal materials found in association with museum objects, thus further coverage of their structure and occurrence will not be given here. However, examples of the occurrence of lipids in archaeological materials will be given in the sections below, where appropriate.

Lipid biomarkers in archaeology

The biomolecules that comprise the plant and animal products associated with artefacts

will be susceptible to the impact of any of the normal mechanisms of decay that operate in a given burial environment, unless protected in some way by the physical state of the artefact or the context of deposition. Few investigations have been conducted on the mechanisms of decay or preservation of biomolecules in environments of archaeological importance. The susceptibility of the major classes of biomolecules to structural modification and degradation follows the order:

Lignin and other plant polymers < Lipids
(including lipopolysaccharides, glycolipids and resins)

< Carbohydrates < Proteins < Nucleotides (RNA and DNA)

This order of preservation potential is related to the susceptibility to chemical and microbiological attack of the various types of linkage which the molecules possess. Although the above stability order was established in assigning the preservation potential of the various types of biomolecule during geological fossilization (Tegelaar 1990), it can be applied equally well to the preservation of biomolecules in archaeological contexts. The degree of preservation (or extent of decay) of a particular class of compound will also be highly dependent on the physico-chemical conditions of the burial environment, e.g. pH, redox potential, temperature, wetness (arid vs waterlogged) and biomass. As can be seen from the above, lipids can be expected to be relatively well preserved compared to carbohydrates, proteins and nucleotides. The resistance of lipids to decay, combined with their likely persistence at the original site of deposition, because of their inherent hydrophobicity, makes them excellent candidates for use as biomarkers in archaeological investigations.

The entrapment of lipids in certain archaeological contexts will lead to an enhancement of their preservation potential. For instance, entrapment either in organic or mineral matrices will generally lead to a reduction in the loss of biomolecules by diffusion and to diminution of microbial activity, because their access to potential lipid substrates would be impeded. In highly dense or vitrified materials microbial activity will be limited because of the reduced porosity and permeability to essential nutrients. Notable examples of entrapment serving to enhance the preservation of lipids in archaeological contexts are seen in the absorbed and carbonized surface residues found in archaeological ceramics (Evershed et al. 1992a; Heron and Evershed 1993). Lipids are well preserved in charred surface residues (Oudemans et al. in press) presumably due to microencapsulation inhibiting microbial activity. Likewise the absorption of lipids into the pores of unglazed ceramic fabrics of pottery will inhibit access by micro-organisms. Adsorption of biomolecules on clay surfaces may also limit their availability as substrates for micro-organisms. However, the presence of molecules adsorbed to the clay surface along with water and other reactive species may encourage certain reactions, e.g. chemical hydrolysis (non-biological). The phenomenon of 'sacrificial' decomposition, which relies upon the preferential decay of co-deposited biological organic matter, may also serve to preserve lipids in some archaeological contexts (Eglinton and Logan 1991).

Finally, and of special importance archaeologically, are those instances where organic matter is found in large quantities due to the requirement of people in the past to

accumulate, store, preserve or transport substantial quantities of natural, or manufactured, products such resins, tars, fats, oils, etc. Examples of this include: the finding of oils in sealed bottles (Beck et al. 1974), bog butters deposited in peat bogs (Morgan et al. 1973) and finds of wood tar or pitch (Evershed et al. 1985). Waste disposal, either of faecal material or household refuse, to produce cess or midden deposits, also falls into this category; such activities will often lead to substantial accumulations of organic matter at specific site locations.

Use of lipids as biomarkers

Definition of archaeological biomarkers

In essence, the biomolecular archaeology of lipids is concerned with recognizing the source of amorphous organic matter of archaeological interest (often termed organic residues) by comparing the properties of the individual compounds or mixtures of compounds (i.e. the biomarkers) present to those occurring in contemporary plants and animals (reference materials). This principle is largely analogous to that which has been applied in the fields of molecular organic geochemistry and biomolecular palaeontology to determine the nature of biological inputs into sedimentary materials, and in the assessment of palaeoenvironments. The above definition might appear somewhat simplistic at first reading, but implicit in it is the need to recognize the range of factors involved in the compositional changes that occur due to decay (taking into account different burial environments) and most importantly, the effects of human intervention, on the composition of lipids, such as due to refining or mixing of natural products during manufacturing processes or culinary activities.

Analytical techniques

Numerous reference texts are available describing analytical methodologies used in the study of lipids (e.g. Kates 1986; Christie 1989; Zweig and Sherma 1984; Hamilton and Hamilton 1992). Discussions of analytical methodologies applied specifically to the investigation of lipid biomarkers in archaeological materials include those of Mills and White (1987) Robinson et al. (1987), Evershed et al. (1990; 1992b) and Heron and Evershed (1993).

Gas chromatography (GC) has a role to play in the analysis of all the major classes of lipids that exist in biological materials. The development of modern capillary columns has greatly extended the scope of GC, offering the necessary resolving power to separate the individual compounds present in the complex mixtures of lipids that are often recovered from archaeological materials. When used in combination with mass spectrometry, termed GC/MS, the facility is provided for both separation and the on-line recording of mass spectra that are required to make the structure assignments necessary for the study of biomarkers. The high sensitivities that are routinely attained by GC and GC/MS are of advantage in the analysis of archaeological materials, particularly where the amounts of sample are limited. Other chromatographic techniques, e.g. thin layer chromatography,

are of value for the purification of lipid extracts prior to GC and GC/MS. The ability to employ GC or GC/MS for the quantification of specific lipid biomarkers in total lipid extracts can also be of use. For example, enhancements above a background concentration of 5β-cholestan-3β-ol (8; coprostanol) in specific soil horizons of pit infills at archaeological sites, provides a means of identifying these features as ancient cesspits (Knights et al. 1983; Pepe et al. 1989; Bethell et al. in press; Evershed et al. in press).

Classifications and identifications based on lipid biomarkers

The most widely used method for identifying the origin of lipids in archaeological materials relies on matching the structures of individual compounds, or the relative proportions of the components of a mixture of compounds, to those found in contemporary plant and animal natural products likely to have been exploited in antiquity. The most detailed information that currently exists concerning lipid composition relates to agriculturally important crops and animals, however, the range of species of plants and animals exploited in the past will generally have been far greater than now. As investigations in this area progress it is inevitable that instances will arise where the identification of the origins of organic residues will be thwarted due to the lack of chemical data on the corresponding contemporary reference material. The only way to overcome this problem will be to expand our investigations of the chemical composition of contemporary plant and animal reference materials to include those likely to have been of importance in the past. An examination of the macroscopic plant and animal remains recovered from excavations may assist in targeting materials for chemical analysis required for constructing the necessary databases.

Table 1 Constant and variant characteristics of the major classes of lipid among species of the same genus in animals and higher plants.

Lipid class[+]	*Essentially constant qualities*	*Main variations*
Sterols	Configurations, extent of alkylation at C-24 position, extent of nuclear unsaturation, and relative quantities	Relative proportions of Δ^{22} and to a slight extent nuclear double bonds
Triterpenoids	Configuration, types of cyclisation, and extent of oxidation	Relative proportions of compounds derived by a family of cylisations and oxidations
Acyclic compounds including fatty acids, fatty alcohols and *n*-alkanes	Range of chain lengths, proportion of even to odd carbon numbers and type of unsaturation	Detailed distribution of chain lengths, which can be substantial

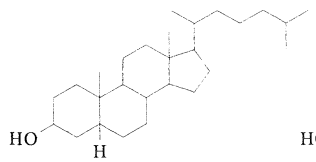
[+] Modified from Nes and Nes (1980)

Some scope exists for drawing deductions based on the known chemosystematics of the substances produced by plants and animals without the need to expand our knowledge base. The use of biomarkers is essentially based on chemotaxonomic and phylogenetic principles. Table 1 lists some of the constant and variant characteristics of the structures of lipids among species of the same genus in animals and higher plants. Essentially what this table is telling us is the characteristics of the main classes of lipids that may be of value in identifying the origins of organic residues based on their lipid compositions. In addition, this table provides a guide to the degree of confidence that can be held in interpretations based on specific classes of compound, together with variations in particular structural features. In general those organisms which, on morphological and other grounds of taxonomy, have sufficiently close affinities to be called species within a genus do usually have close relationships with respect to their lipid compositions (Nes and Nes 1980). Of course there is always the possibility that lipid distributions in archaeological materials have been affected by decay, and great care must be exercised in drawing conclusions on the basis of subtle variations in the abundance of components bearing reactive functional groups, e.g. polyunsaturated fatty acids and ester linkages in triacylglycerols and phospholipids. Some of the modes of degradation of lipids of archaeological interest have been discussed by Evershed et al. (1992a). The following sections provide some examples of the applications of lipids as biomarkers in archaeological investigations.

Sterols and sterol derivatives

The simplest distinction that can be drawn between plants and animals based on their lipid composition relies on the characteristic sterols they biosynthesize. Cholesterol (3) is the most abundant animal sterol, while campesterol (4) and sitosterol (5) are the two major plant sterols. The significant difference in the structures of these latter two molecules, compared to that of cholesterol, concerns the presence of an alkyl group (methyl (CH_3-) in the case of campesterol and ethyl (C_2H_5-) in the case of sitosterol) at the C-24 position in the sterol side-chain. Potential therefore exists to classify plant or animal products of archaeological interest on the basis of their sterol composition. However, this has been attempted on surprisingly few occasions because of the problems that exist in the detection of sterols due to their low abundance in lipid extracts. Uncertainty also surrounds the use of cholesterol because of the potential for its accidental introduction by the handling of artefacts (further discussions of cholesterol as a contaminant are presented below).

Although sterols are relatively more resistant to degradation than fatty acids (see below), structure alteration may still occur, such that oxidized and reduced sterol



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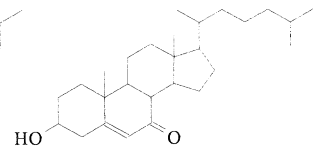

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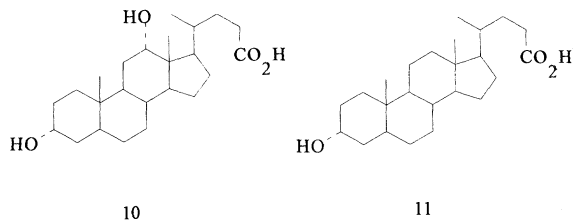
Structures 8 and 9 (see text for explanation)

derivatives, e.g. 5β-cholestanol (coprostanol, 8) and cholest-5-en-7-one-3β-ol (9), can be observed in lipid extracts of archaeological materials. The latter compound, and a range of other oxygenated derivatives, is readily derived from cholesterol by reaction with singlet oxygen (Smith 1981). Although these oxidized cholesterol derivatives may form naturally, due to autoxidation at normal temperatures, their abundance may be enhanced by the application of heat, e.g. fat frying or oil burning. Coprostanol is the product of microbial reduction of cholesterol during its passage through the mammalian gastrointestinal tract (Rosenfeld et al. 1954). As already mentioned above the occurrence of 5β-cholestanol in high abundance in faeces raises the possibility of using this compound as a biomarker of faecal deposition in archaeological soils and sediments (Knights et al. 1983; Pepe et al. 1989; Bethell et al. in press; Evershed et al. in press). Although both compounds are degradation products of cholesterol they retain the arrangement of the original carbon atoms in the parent molecule and permit clear conclusions concerning their animal origin.

The bile acids found in higher animals are biosynthesized directly from cholesterol by saturation of the double bond, epimerization of the 3β-hydroxyl group, introduction of hydroxyls into the 7α and 12α positions, and oxidation of the C_{27} side chain to a C_{24}-carboxylic acid. Bile acids are involved in the emulsification of dietary fats through their detergent action. Although the primary bile acids in mammals are cholic and chenodeoxycholic, they are transformed in the intestine by micro-organisms into the secondary bile acids, deoxycholic (10) and lithicholic (11). Of significance archaeologically



10 11

Structures 10 and 11 (see text for explanation)

is the fact that the formation of bile acids is the most important pathway for the metabolism and excretion of cholesterol in mammals. Hence, as these compounds are excreted in appreciable quantities in the faeces (c. 0.5 g/day) and survive in archaeological soils they can be used as additional biomarkers of faecal inputs, complementary to the coprostanol discussed above (Knights et al. 1983; Bethell et al. in press). Measurement of the relative proportions of different sterol and bile acid structures in individual coprolites has been tentatively proposed as a means of determining the relative contribution of plant and animal foodstuffs to the diet (Lin et al. 1978). The composition of the faecal bile acids also vary with the health or disease state of individuals. However, as little is known of the relative rates of decomposition of different sterols and sterol derivatives (i.e. stanols and bile acids) in archaeological contexts, substantial research is required before such principles can be applied with confidence.

Steroid and triterpenoid hydrocarbons occur widely in petroleum and bituminous materials with distributions that reflect the original biological inputs into the geological

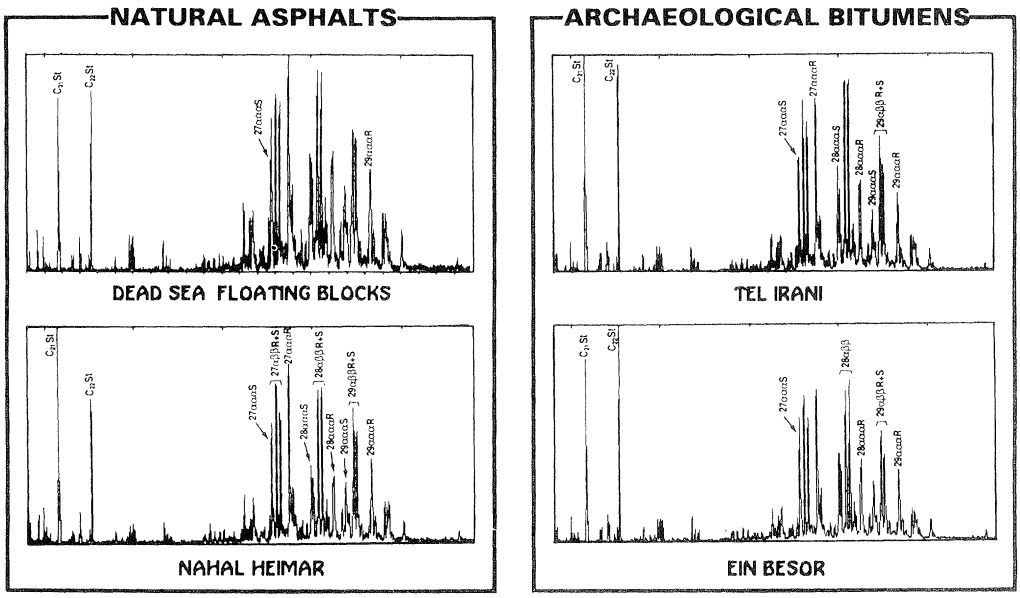


Figure 1 Examples of sterane *m/z* 217 mass chromatograms derived by GC/MS analysis of two natural asphalts and two archaeological bitumens. The mass chromatograms provide highly specific 'fingerprints' that can be used to establish generic differences or similarities (reproduced from Connan et al. 1992 with permission).

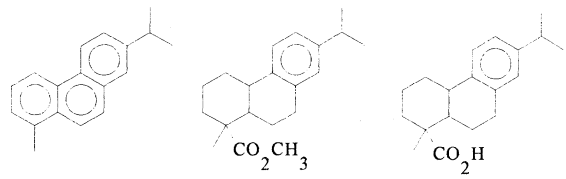
sediments, depositional palaeoenvironments prevailing at the time of deposition, and their subsequent thermal histories. Connan and co-workers (1992) have taken advantage of this to derive biomarker evidence for the export of Dead Sea asphalt to Canaan and Egypt in the Chalcolithic–early bronze age (fourth to third millennium BC). A number of geochemical parameters were measured. Analyses of sterane and triterpane distributions determined by computerized GC/MS, supported by $\delta^{13}C$ measurements, were used to establish genetic relationships between the archaeological and natural bitumens. For example, Figure 1 shows the sterane distribution patterns for two archaeological and two natural bitumens. The close similarity of the sterane distributions for the Tel Irani bitumen and floating block asphalts from the Dead Sea confirmed that these two materials were of the same generic origin. The chemical analyses that were performed also produced evidence for biodegradation and weathering having affected the compositions of the alkane and aromatic components of some of the archaeological bitumens; this would be an entirely expected alteration process for bitumens that have experienced long-term exposure at archaeological sites.

Diterpenoids and triterpenoids

Although terpenoid compounds occur very widely in the plant and animal kingdoms, this section will consider only those compounds that occur in plants. Most archaeological applications in this area have involved the characterization of diterpenoid compounds in

order to determine the nature, origin and possible means of production of resins or resin derivatives, such as wood tars and pitches. Examples of a diterpenoid (abietic acid, 6) and triterpenoid (betulin, 7) that are of archaeological interest have already been presented above. As can be clearly seen from these two structures, diterpenoids contain twenty carbon atoms, while triterpenoids contain thirty carbon atoms; the differences in composition being due to the numbers of isoprene units ($CH_2=C(CH_3)CH=CH_2$) that are combined in their respective biosynthetic pathways. It is particularly convenient for the purposes of archaeological interpretation that di- and triterpenoids are never found together in the same resin. Mills and White (1977; 1987) have provided very useful accounts of the structures and occurrence of di- and triterpenoids in resins likely to be encountered in museum objects.

Chemically altered plant resins and other wood products are often present in finds of tars (distilled) and pitches (undistilled) that are the products of the pyrolysis of resins and wood. In the absence of other direct evidence, the structure of the individual di- or triterpenoid components may provide evidence for the means of production. For example, the high abundance of diterpenoid hydrocarbons (e.g. 12), methyl dehydroabietate (13) and dehydroabietic acid (14) found in the tars and pitches recovered from King Henry

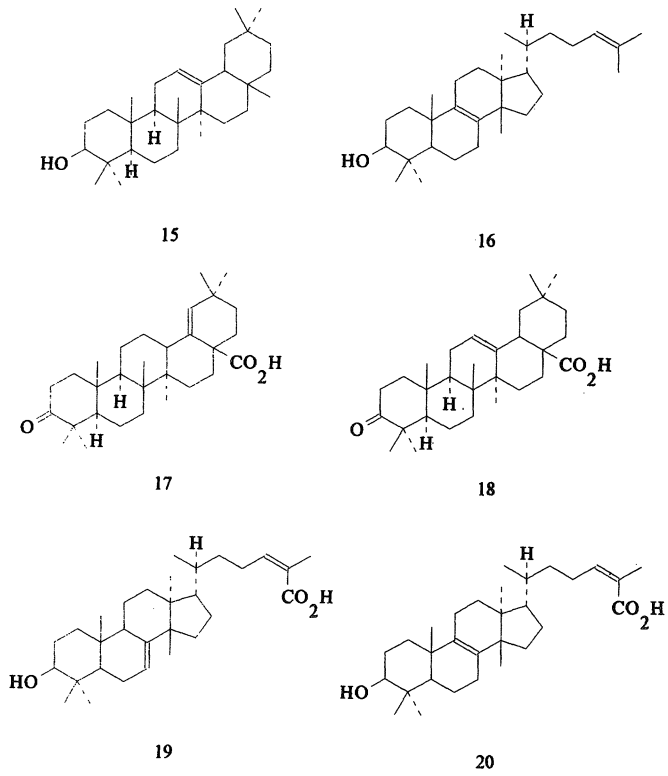


12 13 14

Structures 12–14 (see text for explanation)

VIII's flagship the *Mary Rose* indicated that the pine wood from which they had been generated must have experienced substantial heating, i.e. sufficient to induce dehydrogenation and decarboxylation reactions. Thus, the chemical data was consistent with a manufacturing process involving destructive distillation, comparable to the production of 'Stockholm tar' (Evershed et al. 1985). This can be contrasted with the very low abundance of diterpenoid hydrocarbons detected in 'pitch' balls recovered from a submerged Roman shipwreck off the coast of Guernsey; the high abundance of dehydroabietic acid (14) and the low abundance of defunctionalized diterpenoids indicated that the 'pitch' is more closely related to a resin (colophany) than to 'Stockholm tar' (Evershed and Monaghan, unpublished data).

Amongst the finds of ancient tar, at a number of sites throughout Europe, are those that have been shown to contain triterpenoids related to compounds produced in the bark of *Betula* sp. The detection of betulin (7) and lupeol in the tars, together with various pyrolysis products, provides strong evidence for the use of birch bark in the manufacture of these tars (Hayek et al. 1990; Heron et al. 1991a; Charters et al. 1993). Mills and White (1989) provided compelling evidence for the identity of resins recovered from the Late Bronze Age shipwreck at Ulu Burun in southern Turkey. The resin contained in



Structures 15–20 (see text for explanation)

'Syro-Palestinian' amphorae in a highly preserved state, comprised β-amyrin (15), tirucallol (16), moronic acid (17), oleanoic acid (18), masticadienonic acid (19) and isomasticadienonoic acids (20) which indicated the resins were from a species of *Pistacia*, probably *P. atlantica*. (The use of triterpene hydrocarbon biomarkers present in bitumens to establish generic relationships between archaeological samples and natural asphalts (Connan et al. 1992) has already been alluded to above).

Fatty acids and acyl lipids

Fatty acids and other acyl lipids survive from antiquity in a range of archaeological contexts. Although numerous attempts have been made to determine the origins of lipids in archaeological materials by analysis of their constituent fatty acids, these have generally met with only modest success. Where fatty acids, or other related acyl lipids such as triacylglycerols, diacylglycerols or monoacylglycerols, are detected in appreciable quantities, say in organic residues found in association with an archaeological ceramics, then this may confirm an earlier use in connection with a plant oil or animal fat. However, attempts to make further progress in assigning the origins of archaeological lipids based on their specific fatty acid content is generally limited by the narrow range of fatty acids that comprise most fats and oils. Interpretations are complicated still further by the

susceptibility of the unsaturated fatty acids to polymerization, oxidation, and microbial degradation (Gunstone and Norris 1983; Mills and White 1987; den Dooren de Jong 1961; Morgan et al. 1973; Evershed et al. 1992a; Evershed 1992). Without exception, the more labile unsaturated fatty acids are depleted in archaeological materials. Broad classifications, e.g. 'animal fats', 'dairy products', 'vegetable oils' and 'fish/marine oils', are probably the best that can be hoped to be achieved at present on the basis of the fatty acid content of archaeological fats and oil residues. For example, evidence for remnant dairy fats in archaeological ceramics derives from the detection of short chain acyl moieties present in their tri- and diacylglycerols, and the presence of free fatty acids in the $C_8 - C_{18}$ carbon number range (Evershed et al. 1992b). Difficulties are anticipated in positively identifying dairy fats in some situations in archaeological materials because of preferential hydrolysis and leaching of short chain fatty acids. Phospholipids do not appear to survive intact in archaeological materials.

Long chain alkyl (acyclic) compounds as biomarkers

Long chain alkyl compounds, such as those found in the protective waxes produced by plants and animals, are widely distributed in nature and hold considerable potential for use as biomarkers where they occur in association with archaeological finds. The types of compounds of interest here include long-chain alcohols, hydrocarbons, ketones, aldehydes, wax esters, etc. As such compounds are fully saturated and contain few, if any (in the case of alkanes), reactive functional groups, they are relatively resistant to decay over archaeological time. Although many natural waxes contain relatively similar classes of compound, the relative proportions of the individual components can be used as 'fingerprints' to identify the specific origin of an unknown ancient wax (see Table 1). Beeswax is one of the best known examples of a natural wax that was an important commodity in antiquity. The composition is so constant (see Fig. 2) that it can be used very reliably for the purposes of archaeological interpretation. Figure 2 shows the GC profile for the total lipid extract of a potsherd from an inturned rim bowl of Late Saxon/early medieval date recovered from the multi-period settlement at Raunds in Northamptonshire, UK. The identities of all the major peaks were assigned through their mass spectra and agree with published data on beeswax (Tulloch and Hoffman 1972). This identification provides evidence for the use of the vessel in connection with the collection or processing of the beeswax, and hence indirect evidence for beekeeping at the site. Beeswax was also detected in an unglazed jar (Fig. 2) from the same site (Charters and Evershed, unpublished data).

Despite their ubiquitous occurrence in plants, few examples exist for the identification of plant waxes in archaeological materials. A recent study has however revealed the presence of a mixture of long-chain alkyl compounds, *n*-nonacosane (21), nonacosan-15-ol (22) and nonacosan-15-one (23), in the total lipid extracts of several Late Saxon/early medieval potsherds from the site, Raunds, referred to above (Evershed et al. 1991; 1992a). The composition of the mixture was consistent with a derivation from *Brassica* vegetables that had been processed in the vessels in antiquity. This result provides a novel means of deducing the past exploitation of leafy vegetables that would otherwise remain undetected by conventional palaeobotanical techniques. There would appear to be considerable scope

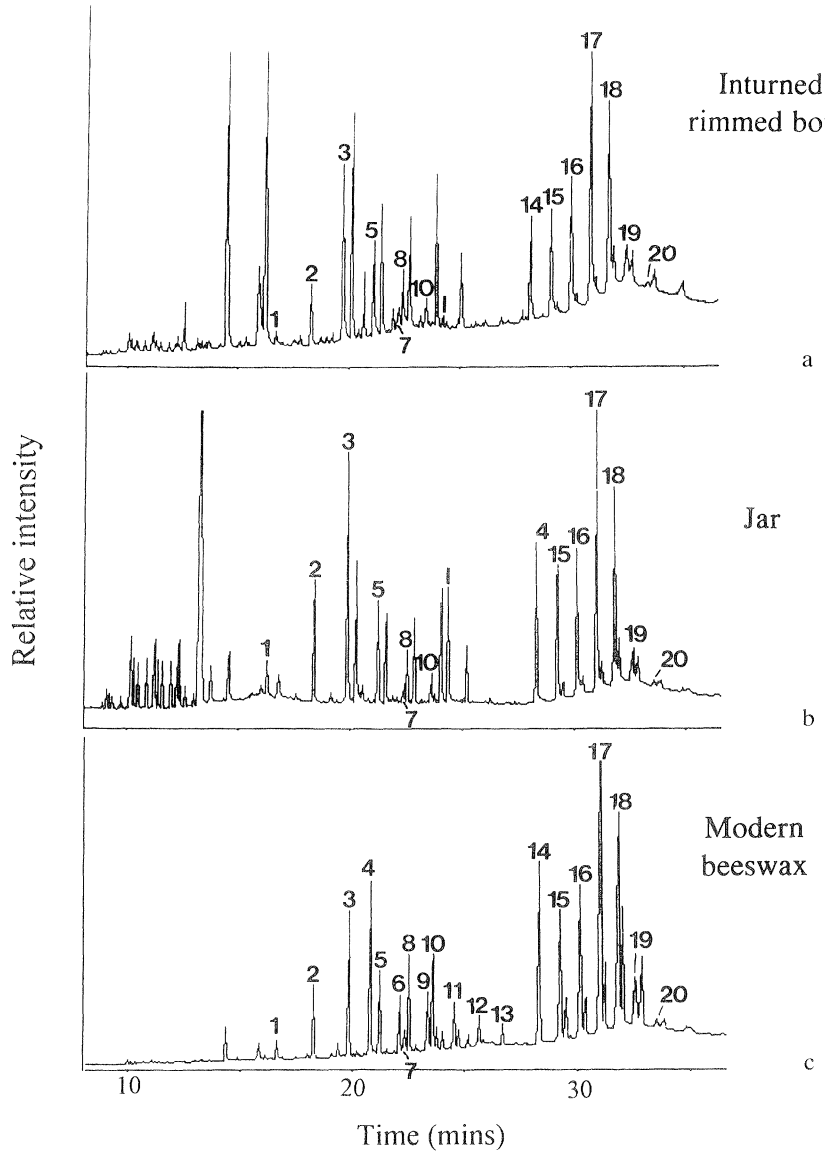
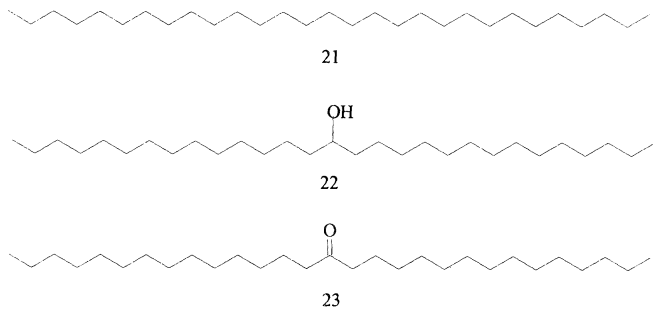


Figure 2 Gas chromatograms of beeswax compared to that obtained from the total lipid extracts obtained by solvent extraction of the based sherds of an inturned rimmed bowl and a jar recovered from the Late Saxon/early medieval hamlet of West Cotton, Raunds, Northamptonshire, U.K. Peak identities: 1 = tricosane; 2 = pentacosane; 3 = heptacosane; 4 = tetracosanoic acid; 5 = nonacosane; 6 = hexacosanoic acid; 7 = hentriacontene; 8 = hentriacontane; 9 = heptacosanoic acid; 10 = tritriacontene; I. = tetratriacontane ($C_{34}H_{70}$; internal standard); 11 = tricontanoic acid; 12 = dotricontanoic acid; 13 = tetratricontanoic acid; 14–20 correspond to palmitic acid wax esters containing 40, 42, 44, 46, 48, 50 and 52 carbon atoms, respectively. The unlabelled peaks eluting with the wax esters correspond esters of fatty alcohols and hydroxy fatty acids. The remaining unlabelled peaks in the chromatograms for the inturned rimmed bowl and jar correspond to free fatty acids and alcohols and are presumed to derive from degradation of the beeswax and mixing with another fatty material during vessel use and burial (see text for further details). GC analyses were performed according to the conditions given in Evershed et al. 1990. All peak identities were determined by GC/MS.



Structures 21–23 (see text for explanation)

for the development of this approach for the identification of other leafy vegetables prepared in unglazed ceramic vessels. However, as noted above, the wider application of this technique will require the development of databases of chemical compositions of waxes of plants, including those likely to have been exploited in antiquity, but which have little economic significance in the modern-day.

A further advance in this area has the application of isotope ratio-monitoring GC/MS to make $\delta^{13}C$ measurements on the individual biomarkers derived from epicuticular waxes preserved in the potsherds (Arnot, Evershed and Eglinton unpublished data). The results obtained to date have confirmed that the methodology is reliable, with the $\delta^{13}C$ values for the individual lipid biomarkers of the remnant *Brassica* epicuticular wax components being consistent with a C_3 plant origin. This new analytical approach has potentially wide application to palaeodietary studies, providing the possibility of making very precise assessments of the contributions of C_3, C_4 and CAM plants to the human diet, on the basis of measurements of $\delta^{13}C$ values for individual lipid biomarkers.

Detection of contamination

Biomarkers can assist in identifying both post-burial and post-excavational contamination. The former may correspond to contamination due to compounds that have migrated into an artefact from the burial environment, or from the remains of micro-organisms. The potential exists for addressing the contributions of both these factors through the use of biomarkers and lipid 'fingerprints'. For example, the possibility of contamination of potsherds by soil lipids has been addressed by comparison of the compositions of the lipid extracts of sherds and their adhering burial soil (Heron et al. 1991b). The soil lipids that were examined were found to be of relatively constant composition, consisting of a complex mixture of lipids originating from plant, animal and microbial detritus; the sherd extracts were generally of simpler and more variable composition. The contamination of lipids in potsherds by migration of soil lipids is not a serious problem and probably relates to the hydrophobic nature of lipids. This method of screening for soil lipid contamination can be equally well applied to other archaeological materials, such as lithics.

The presence of bacterial lipid contamination is rather more difficult to detect. Unfortunately many of the lipids produced by micro-organisms are also produced by plants and animals. Many bacteria produce pentacylic triterpenoids to mimic the

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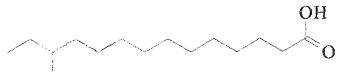

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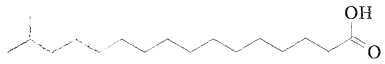

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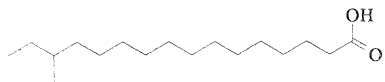

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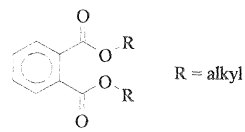
Structures 24–27 (see text for explanation)

functional role of sterols in their membranes, but, as such compounds also occur widely in plants, potential biomarkers of bacterial contamination must be chosen with care. Branched chain fatty acids, e.g. *iso*- and *anteiso*-C_{15} and C_{17} (see structures 24 to 27) are often cited as being characteristic of bacteria. However, assignments of bacterial contamination based on the presence of such compounds should again be made with extreme caution since these compounds occur widely in nature, particularly in the tissues of ruminant animals (Christie 1981). Moreover, as bacteria produce a wide diversity of fatty acids, with straight-chain fatty acids dominating in some species, the lack of branched chain fatty acids must not be taken to constitute a lack of microbial contamination.

Post-excavational lipid contamination may also arise by the growth of bacteria or fungi on archaeological samples stored under inappropriate conditions (e.g. warm and humid). Obviously similar arguments apply to the detection of this type of contamination, using lipid biomarkers. Although not of biological origin, phthalate plasticizers (e.g. 28) are commonly observed as contaminants in lipid extracts, arising by storage of archaeological samples in plastics, e.g. bags, vial caps and 'cling film'. Plasticizers are readily recognized in GC and GC/MS analyses from their characteristic retention times, and mass spectra which are dominated by an abundant *m/z* 149 ion. Other commonly occurring contaminants include the lipids from the skin which can be introduced through the handling of artefacts. Squalene (29, see Fig. 3a) is one of the major lipid components of human



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Structure 28 (see text for explanation)

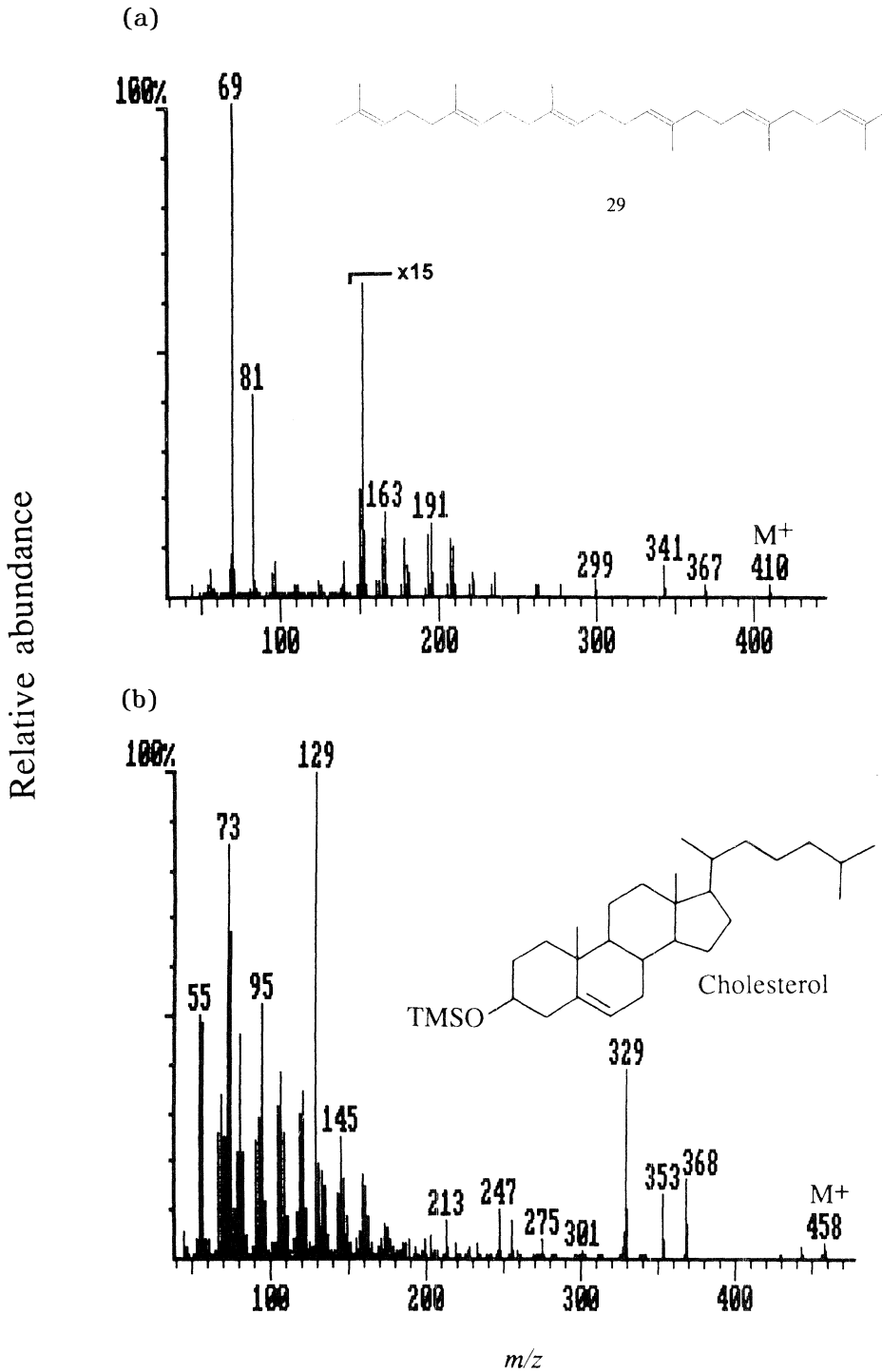

Figure 3 Structures and mass spectra of (a) squalene and (b) cholesterol (as its trimethylsilyl ether derivative) common post-excavational contaminants of lipid extracts of archaeological materials.

'fingerprints'. It is readily recognized from its mass spectrum analyses (Fig. 3a), however, the large number of double bonds in squalene makes it so susceptible to degradation that it would be unlikely to survive from antiquity and its presence in lipid extracts may be a strong indication of post-excavational contamination. Cholesterol (mass spectrum shown in Fig. 3b) also occurs in the surface lipids of human skin, although at a somewhat lower abundance than squalene, hence their co-occurrence in lipid extracts of archaeological materials would point strongly to contamination.

Concluding remarks

It is clear from the above overview that substantial scope exists for the use of lipids in archaeological investigations. However, few if any applications of lipid biomarkers have been developed to a stage that they can be considered to be of routine use. Notable successes include elucidations of the generic origins of ancient resins, tars, pitches, and bitumens based on analyses of their biomarker components. Substantial progress is also being made in the identification of foodstuffs and other natural products, particularly from lipids preserved in unglazed ceramic vessels. In almost every area more systematic work is required to develop general principles of interpretation that will be of wide application. Studies of lipids found in association with inhumations, e.g. mummies (Gulaçar et al. 1990) and bog bodies (Evershed and Connolly 1988; Evershed 1990; 1992), are providing valuable insights into the mechanisms of decay in archaeological burials. The study of decay is of fundamental importance in the investigation of all classes of compounds, and has serious implications for the rigor of archaeological interpretation which can be achieved. Scope exists here for the use of field and laboratory-based simulation studies to examine the relative rates of decay of different compound types in archaeological contexts. As alluded to above, expansion of our knowledge of the chemical compositions of plant and animal natural products likely to have been exploited by man in the past is essential for achieving reliable archaeological interpretations. To be of use these specialist databases will need to include information on animal and plant species that might have been significant in the past but may have little or no economic importance at the present day. While progress can be made in drawing interpretations on the basis of the analysis of lipid biomarker information alone, greater potential exists for its use in conjunction with other data, for example, as an adjunct to analyses of other classes of biomolecule (DNA, proteins, carbohydrates, etc.), radiocarbon dating, stable isotope ratio ($\delta^{13}C$) measurements, trace element analyses, palaeobotanical data and traditional archaeological information.

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References

Beck, C. W., Fellows, C. A. and MacKennan, E. 1974. Nuclear magnetic resonance spectrometry in archaeology. In *Archaeological Chemistry*, vol. 1 (ed. C. W. Beck). Advances in Chemistry Series 138. Washington, DC: American Chemical Society, pp. 226–35.

Bethell, P. H. Evershed, R. P. and Goad, L. J. in press. The investigation of lipids in organic residues by gas chromatography/mass spectrometry: application to palaeodietary studies. In *Molecular Archaeology of Prehistoric Human Bone* (eds J. B. Lambert and G. Grupe). Berlin: Springer Verlag.

Brown, T. A. and Brown, K. A. 1992. Ancient DNA and the archaeologist. *Antiquity*, 66: 10–23.

Charters, S., Evershed, R. P., Goad, L. J., Heron, C. and Blinkhorn, P. 1993. Identification of an adhesive used to repair a Roman jar. *Archaeometry*, 35: 91–101.

Christie, W. W. (ed.) 1981. *Lipid Metabolism in Ruminant Animals*. Oxford: Pergamon Press.

Christie, W. W. 1989. *Gas Chromatography and Lipids*. Ayr: Oily Press.

Connan, J., Nissenbaum, A. and Dessort, D. 1992. Molecular archaeology: export of Dead Sea asphalts to Canaan and Egypt in the Chalcolithic-early bronze age (4th-3rd millennium BC). *Geochimica et Cosmochimica Acta*, 56: 2743–59.

den Dooren De Jong, L. E. 1961. On the formation of adipocere from fats: contribution to the microbiology of systems containing to liquid fats. *Antonie von Leeuwenhoek Journal of Microbiology and Serology*, 27: 337–61.

Eglinton, G. and Logan, G. A. 1991. Molecular preservation. *Philosophical Transactions of the Royal Society of London, Series B*, 333: 315–28.

Evershed, R. P. 1990. Lipids from samples of skin from seven Dutch bog bodies: preliminary report. *Archaeometry*, 32: 139–53.

Evershed, R. P. 1992. Chemical composition of a bog adipocere. *Archaeometry*, 34: 253–65.

Evershed, R. P. and Connolly, R. C. 1988. Lipid preservation in Lindow Man. *Naturwissenschaften*, 75: 143–5.

Evershed, R. P., Bethell, P. H. and Goad, L. J. in press. The use of biological markers to detect faecal remains in archaeological soils. In the proceedings of a meeting on Archaeological Sciences held at the University of York, UK in September 1991.

Evershed, R. P. Heron, C. Charters, S. and Goad, L. J. 1992a. The survival of food residues: new methods of analysis, interpretation and application. *Proceedings of the British Academy*, 77: 187–208.

Evershed, R. P. Heron, C., Charters, S. and Goad, L. J. 1992b. Chemical analysis of organic residues in ancient pottery: methodological guidelines and applications. In *Organic Residues in Archaeology: Their Identification and Analysis* (eds R. White and H. Page). London: UKIC Archaeology Section, pp. 11–25.

Evershed, R. P., Heron, C. and Goad, L. J. 1990. Analysis of organic residues of archaeological origin by high temperature gas chromatography and gas chromatography/mass spectrometry. *The Analyst*, 115: 1339–42.

Evershed, R. P., Heron, C. and Goad, L. J. 1991. Epicuticular wax components preserved in ancient potsherds as chemical indicators of soft tissue vegetables in ancient diets. *Antiquity*, 65: 540–4.

Evershed, R. P., Jerman, K. and Eglinton, G. 1985. Pine wood origin for pitch from the Mary Rose. *Nature*, 314: 528–30.

Folch, J., Lees, M. and Stanley, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226: 497–509.

Gangl, J. 1936. Report giving the results of examination of various materials from Maadi. In *The Excavations of the Egyptian University in the Neolithic Site at Maadi, Second Preliminary Report (Season 1932)* (eds O. Menghin and M. Amer). Cairo: Gov. Press.

Gülaçar, F. O., Susini, A. and Koln, M. 1990. Preservation and post-mortem transformations of lipids in samples from a 4000-year old Nubian mummy. *Journal of Archaeological Science*, 17: 691–705.

Gunstone, F. D. and Norris, F. A. 1983. *Lipids in Foods: Chemistry, Biochemistry and Technology*. Oxford: Pergamon Press.

Hamilton, R. J. and Hamilton, S. (eds) 1992. *Lipid Analysis: A Practical Approach*. Oxford: Oxford University Press.

Hayek, E. W., Krenmayr, P., Lohninger, H., Jordis, U., Moche, W. and Sauter, F. 1990. Identification of archaeological and recent wood tar pitches using gas chromatography/mass spectrometry and pattern recognition. *Analytical Chemistry*, 62: 2038–43.

Heron, C. and Evershed, R. P. 1993. Analysis of organic residues and the study of pottery use. In *Archaeological Method and Theory*, vol. 5 (ed. M. B. Schiffer). Arizona: University of Arizona Press, pp. 247–84.

Heron, C., Evershed, R. P., Chapman, B. and Pollard, A. M. 1991a. Glue, disinfectant and 'chewing gum' in prehistory. In *Archaeological Sciences 1989, Proceedings of a Conference on Archaeological Sciences, University of Bradford* (eds P. Budd, B. Chapman, C. Jackson, R. Janaway and B. Ottaway). Oxford: Oxbow Monograph 9. Oxford: Oxbow Publications, pp. 332–9.

Heron, C., Evershed, R. P. and Goad, L. J. 1991b. Effects of migration of soil lipids on organic residues associated with buried potsherds. *Journal of Archaeological Science*, 18: 641–59.

Kates, M. 1986. *Techniques of Lipidology*. Amsterdam: Elsevier.

Knights, B. A., Dickson, C. A., Dickson, J. H. and Breeze, D. J. 1983. Evidence concerning the Roman military diet at Bearsden Scotland in 2nd century AD. *Journal of Archaeological Science*, 10: 139–52.

Kolattukudy, P. E. (ed.) 1976. *Chemistry and Biochemistry of Natural Waxes*. Amsterdam: Elsevier.

Lin, D. S., Conner, W. E., Napton, L. K. and Heizer, R. F. 1978. The steroids of 2000-year-old human coprolites. *Journal of Lipid Research*, 19: 215–21.

Lucas, A. and Harris, J. R. 1962. *Ancient Egyptian Materials and Industries*, 4th edn. London: Edward Arnold.

Mead, J. F., Alfin-Slater, R. B., Howton, D. R. and Popjak, G. 1986. *Lipids: Chemistry, Biochemistry and Nutrition*. New York: Plenum.

Mills, J. S. and White, R. 1977. Natural resins of art and archaeology. Their sources, chemistry, and identification. *Studies in Conservation*, 22: 3–18.

Mills, J. S. and White, R. 1987. *The Organic Chemistry of Museum Objects*. London: Butterworths.

Mills, J. S. and White, R. 1989. The identity of the resins from the late bronze age shipwreck at Ulu Burun (Kas). *Archaeometry*, 31: 37–44.

Morgan, E. D., Cornford, C., Pollack, D. R. J. and Isaacson, P. 1973. The transformation of fatty material in the soil. *Science and Archaeology*, 10: 9–10.

Nes, W. R. and Nes, W. D. 1980. *Lipids in Evolution*. New York: Plenum Press.

Oudemans, T. F. M., Boon, J. J. and Evershed, R. P. in press. Tracing vessel use: quantitative comparison between absorbed lipids and lipids in surface residues on prehistoric pottery. In the proceedings of a meeting on Archaeological Sciences held at the University of York, UK in September 1991.

Pääbo, S. 1985. Molecular cloning of Ancient Egyptian mummy DNA. *Nature*, 314: 644–5.

Pepe, C., Dizabo, P., Scribe, P., Dagaux, J., Fillaux, J. and Saliot, A. 1989. Les marqueurs biogeochimiques. Application a l'archaeologie. *Revue d'Archaeometrie*, 13: 1-11.

Robinson, N., Evershed, R. P., Higgs, W. J., Jerman, K. and Eglinton, G. 1987. Proof of a pine wood origin for pitch from Tudor (Mary Rose) and Etruscan shipwrecks: application of analytical organic chemistry in archaeology. *The Analyst*, 112: 637–44.

Rosenfeld, R. S., Fukushima, D. K., Hellman, L. and Gallagher, T. F. 1954. The transformation of cholesterol to coprostanol. *Journal of Biological Chemistry*, 211: 301–11.

Smith, L. L. 1981. *Cholesterol Autoxidation*. New York: Plenum Press.

Stokar, W., von 1938. Prehistoric organic remains. *Antiquity*, 12: 82–6.

Tegelaar, E. W. 1990. Resistant biomolecules in morphologically characterised constituents of kerogen: a key to the relationship between biomass and fossil fuels. Ph.D. thesis. Technical University of Delft, The Netherlands.

Tulloch, A. P. and Hoffman, L. L. 1972. Analysis of beeswax by gas liquid chromatography. *Journal of the American Oil Chemists Society*, 49: 696.

Zweig, G. and Sherma, J. (eds) 1984. *Handbook of Chromatography: Lipids*, vols I and II. Boca Raton: CRC Press.

Abstract

Evershed, R. P.

Biomolecular archaeology and lipids

Medium-sized biomolecules, particularly lipids, can frequently be detected in ancient materials. The structures and compositions of mixtures of lipids can provide direct evidence for their origin, and hence, evidence for human activity in the past. An important concept in the field of biomolecular archaeology of lipids is that of 'biomarkers'. Archaeological biomarkers are characteristic compounds (or mixtures of compounds) found in archaeological materials that can be matched to those present in contemporary materials likely to have been exploited in antiquity. Some of the general principles surrounding the use of lipid biomarkers, including their properties, origins, means of detection, characterization, modes of preservation and decay, and application to archaeological investigation, are discussed in this article. Examples are presented to illustrate the scope of the biomolecular archaeology of lipids and some remarks are made concerning possible areas of future development.