Chemistry of Archaeological Animal Fats

RICHARD P. EVERSHED,*
STEPHANIE N. DUDD, MARK S. COPLEY, ROBERT BERSTAN, ANDREW W. STOTT, HAZEL MOTTRAM, STEPHEN A. BUCKLEY, AND ZOE CROSSMAN

Organic Geochemistry Unit, Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, U.K.

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ABSTRACT

Animal fats are preserved at archaeological sites in association with unglazed pottery, human and animal remains, and other deposits or hoards. High-temperature gas chromatography (HT-GC) and combined HT-GC/mass spectrometry (HT-GC/MS) has confirmed the presence of animal fats in lipid extracts of artifacts. Degradation products and pathways have been discerned through the analyses of archaeological finds and the products of laboratory and field-based decay experiments. The origins of preserved fats have been determined through detailed compositional analysis of their component fatty acids by GC, by GC/MS of dimethyl disulfide derivatives of monoenoic components, and by GC−combustion−isotope ratio-MS (GC−C−IRMS), to derive diagenetically robust $\delta^{13}C$ values. Regiospecific analysis of intact triacylglycerols by high-performance liquid chromatography/MS (HPLC/MS), with atmospheric pressure chemical ionization, provides a further criterion for establishing the origin of fats. Preparative GC has been employed to isolate individual fatty acids from archaeological pottery in sufficient amounts for ^{14}C dating.

Introduction

In the late 1970s and 1980s it began to be realized that lipids are preserved under favorable conditions in association with various classes of archaeological artifact and ecofact.[1] The prominence of animal fats among the solvent-soluble components of various organic residues encountered at archaeological sites is consistent with their wide range of potential uses in antiquity, i.e., diet, art materials, lubricants, illuminants, binders, waterproofing agents, bases for cosmetics, ointments, and perfumes, use in religious rituals and burial practices, varnishes, glues, polishes, etc.[2]

Until the 1990s the identification of archaeological fats generally proceeded with base treatment of solvent extracts followed by methylation to yield fatty acid methyl esters and subsequent analysis by GC. Interpretations of the origins of the remnant fats were then based on comparisons between the proportions of the major fatty acids in the ancient fats and the known compositions of the fats of modern animals (reference fats). The early literature in this area contains numerous references to identifications of ancient fats or oils which are unsubstantiated by rigorous analytical chemical data.[3] Major hurdles in the identification of ancient fats are the effects of decay which complicate direct comparison of fatty acid compositions of the aged materials with modern reference data. Although degradation of archaeological fats is retarded due to preservation or burial in favorable environments, it can never be halted. Thus, all archaeological fats are degraded and, without detailed analyses, the most realistic interpretations based on the earlier studies would be of general more easily distinguishable categories of fats or oils, e.g. adipose fats of terrestrial animals, marine mammals, and fish or vegetable oils.[4,5]

During the past 10 years we have undertaken extensive chemical investigations of lipids from a wide range of archaeological finds, including pottery, soils, human and animal remains, and various aged amorphous organic deposits, addressing a range of archaeological questions.[6] We review herein the analytical techniques employed in this work, providing examples of compositional ranges that exist for ancient animal fats and case studies to illustrate degree of specificity that can now be achieved in assigning the origins of archaeological animal fats.

Richard P. Evershed obtained his first degree (B.Sc.) in applied chemistry from Trent Polytechnic, Nottingham, U.K., in 1978 and Ph.D. from the University of Keele in 1981. He held postdoctoral research positions at the Universities of Bristol (1981−1984) and Liverpool (1984−1993) before moving to the School of Chemistry, University of Bristol as Lectuer in 1993. He was awarded his Personal Chair in Biogeochemistry in 2000. In 2002 he received the Royal Society of Chemistry 2002 Theophilus Redwood lectureship for his application of chromatography and mass spectrometry to provide stable isotope and molecular information to enhance our understanding of modern and ancient environments.

Stephanie N. Dudd obtained her first degree (B.A.) in environmental systems at the University of Wales in 1991 and then completed her Ph.D. in 1999 at the University of Bristol in the chemical identification of animal fats preserved in prehistoric pottery. She is now a technical sales specialist with Micromass UK Ltd.

Mark S. Copley obtained a B.Sc. in biomolecular archaeology sciences at the University of Bradford in 1996 and then an M.Sc. in biomolecular archaeology (UMIST/Sheffield University). In 2002 he completed his Ph.D. at Bristol University, where he is currently a postdoctoral research fellow investigating the antiquity of dairying using isotopic and biomolecular methods.

Rob Berstan obtained his first degree (B.Sc.) in chemistry from the University of Bristol in 1997 and is currently completing his Ph.D. in archaeological chemistry investigating aspects of the identification of animal fats in archaeological pottery and radiocarbon dating.

Andrew W. Stott completed his B.Sc. in geology at the University of Newcastle-upon-Tyne in 1990, where he then undertook a Ph.D. in organic geochemistry. From 1994 to 1999 he undertook postdoctoral research at the University of Bristol, investigating compound-specific $\delta^{13}C$ and ^{14}C approaches to the analysis of lipids in archaeological bones and pottery. He is currently manager of the NERC ^{15}N Stable Isotope Facility, CEH, Merlewood, UK.

Hazel Mottram received her B.Sc. in chemistry (1995) and Ph.D. (1999) from the University of Bristol. She is currently undertaking postdoctoral reseach in stable isotopes and microbial ecology.

Stephen A. Buckley completed his B.Sc. degree in chemistry at the University of Sheffield in 1994, then obtained an M.Sc. degree in analytical chemistry and instrumentation in 1995. He is currently completing a Ph.D. in the organic chemistry of embalming agents in Egyptian mummies at the University of Bristol.

Zoe Crossman obtained her first degree (B.Sc.) in chemistry from the University of Bristol and is currently completeing her Ph.D. in stable isotope and microbial ecology.

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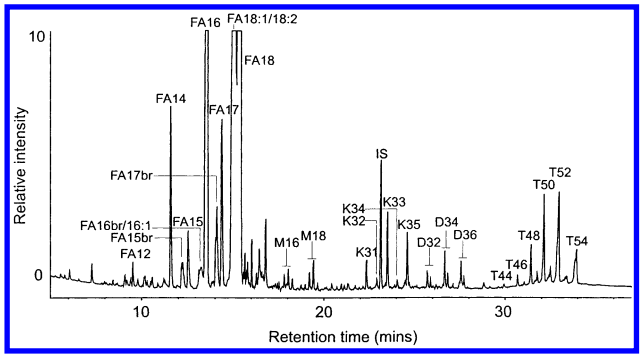


FIGURE 1. Partial HTGC profile of the trimethylsilylated extract from a Romano−British sherd from Stanwick, Northamptonshire, U.K. The analysis was performed on a 15 m × 0.32 mm i.d. fused silica capillary column coated with HP1 stationary phase (immobilized dimethyl polysiloxane; 0.1 μm film thickness; J&W Scientific) using hydrogen as carrier gas (column head pressure 10 psi). Temperature program: 2 min of isothermal at 50 °C and then 50 to 350 °C at 10 °C min^{-1}, followed by a 10 min hold at 350 °C. Sample introduction was by on-column injection. Major peaks in chromatogram expanded off scale to reveal the minor constituents. Peak identities: FA12, FA14, FA15, etc. = *n*-alkanoic acids with 12, 14, and 15 carbon atoms, etc., respectively; FA17br = branched-chain alkanoic acid with 17 carbon atoms; FA16:1 and FA18:1 = monounsaturated *n*-alkanoic acids containing 16 and 18 carbon atoms, respectively; M16 and M18 = monoacylglycerols containing 16 and 18 acyl carbon atoms, respectively (the 1-isomer elutes before the 2-isomer); K31, K32, etc., = midchain ketones, discussed further below; D30, D32, etc., = diacylglycerols containing 30, 32, etc., acyl carbon atoms, respectively (the 1,2-isomer elutes before the 1,3-isomer); T44, T46, T48, etc., = triacylglycerols bearing 44, 46, 48, etc., acyl carbon atoms, respectively; IS = internal standard (*n*-tetratriacontane). All peak assignments were confirmed by GC/MS.

Experimental Approaches

The most extensively studied materials have been unglazed pottery, human and animal remains, soils, and other amorphous deposits. Archaeological pottery or bones (1−10 g) are cleaned with a modeling drill fitted with an abrasive bit to remove soil and postexcavation contaminants and then crushed in a pestle and mortar. Soils (10−50 g) are sieved (2 mm). Dried tissues or amorphous organic remains (1−10 mg) are crushed to a powder. The powdered material is then extracted (ultrasonication or Soxhlet) with organic solvent (e.g. dichloromethane or chloroform/methanol, 2:1 v/v). Aliquots of extract are either trimethylsilylated directly and analyzed by GC and GC/MS or separated into different compound classes using either solvent partitioning, solid-phase extraction, or "flash" column chromatography. The resulting fractions are derivatized as required. Hydroxy compounds (mono- and diacylglycerols, hydroxy carboxylic acids) are trimethysilylated,[7] carboxylic acids (fatty acids, dicarboxylic acids, hydroxy fatty acids) are methylated,[8] and monounsaturated fatty acids are converted to methyl ester−dimethyl disulfide derivatives[9,10] and analyzed as required by GC, GC/MS,[11] and/or GC−C−IRMS.[12] Where appropriate, intact acyl (i.e. mono-, di-, and triacylglycerols) or "bound" lipids are hydrolyzed to their component neutral and acidic moieties prior to derivatization as described above.[13] Quantitative analyses use internal standard(s) added at the extraction stage.[14] Regiospecific analyses of triacylglycerols are performed by high-performance liquid chromatographic−MS analyses of triacylglycerols employing atmospheric pressure chemical ionization (APCI).[15]

High-Temperature-GC and HT-GC/Mass Spectrometry (HT-GC/MS)

In 1990 we reported the use of HT-GC and HT-GC/MS for the analysis of lipid residues from archaeological ceramics to derive detailed compositional information directly from extracts without chemically degrading them to release their simpler fatty acid moieties.[11] An example of a HT-GC/MS analysis of the trimethylsilylated solvent extract of an ancient potsherd using a capillary column coated with a thin film (0.1 μm) of high-temperature stable stationary phases (dimethyl polysiloxane) is shown in Figure 1.[11] The advantage of the approach lies in the simplicity of performing such analyses, i.e., minimal sample manipulation, and the wide range of lipid classes revealed within a single analytical run. By basing our analytical protocols around HT-GC and HT-GC/MS, we have been able screen large numbers of extracts of archaeological finds for the presence of lipid residues. Moreover, the results of such analyses have revealed the presence of hitherto unrecognized components and provide the starting point for more detailed compositional investigations of archaeological animal fats.

Degraded Animal Fats

Hydrolyzed Fats. Figure 1 shows the GC profile typical of many lipid residues detected in archaeological pottery. Such residues are characterized by a readily recognizable distribution of free fatty acids, mono-, di-, and triacylglycerols dominated by $C_{16:0}$ and $C_{18:0}$ acyl moieties.[11,16,17] The distribution is consistent with the degradative pathway shown in Figure 2. Mono- and diacylglycerols produced by the loss of two or one fatty acyl moieties, respectively, are often low in abundance since complete hydrolysis is rapid following the loss of one fatty acid. Animal fat triacylglycerols commonly range in acyl carbon number (C_n) between C_{40} and C_{54}, with the C_{50} and C_{52} components the most abundant. The laboratory decay of animal fat produces a pattern of acyl lipids components exactly analogous to that shown in Figure 1, via a combination of enzymatic or chemical hydrolysis.[17,18]

The advantages of applying HT-GC and HT-GC/MS are immediately apparent from the profile shown in Figure 1. First, the state of preservation is readily assessed; such information is completely lost if lipid extracts are hydrolyzed and only analyzed for their fatty acid content. Although the HT-GC profile shown in Figure 3 reveals that extensive hydrolysis has taken place, it is remarkable that intact triacylglycerols are preserved intact in pottery vessels of this age (ca. 6000 years B.P.); the ceramic fabric appears to be a highly protective environment for such readily degraded compounds. Second, additional diagnostic neutral lipids are readily detected. For example, the

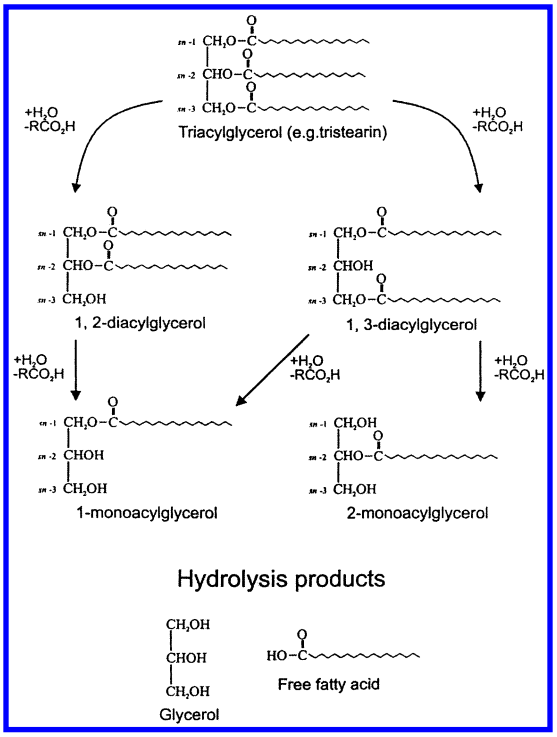


FIGURE 2. Hydrolytic pathway for the transformation of triacylglycerols to free fatty acids.

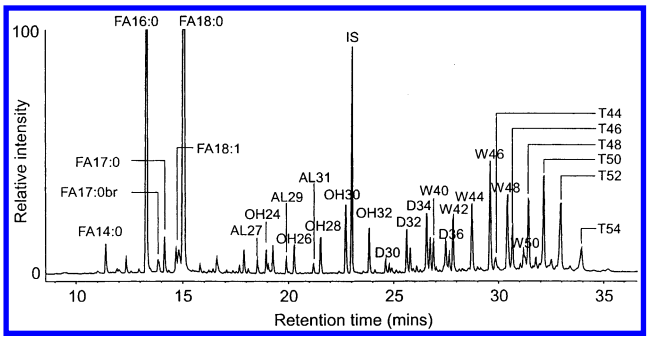


FIGURE 3. Partial HTGC profile of the trimethylsilylated total lipid extract of the rim sherd of an early Neolithic bowl. GC conditions and peak identities are as for Figure 1. OH24−OH32 refer to primary alcohols with 24−32 carbon atoms, respectively.

Neolithic potsherd lipid extract shown Figure 3 also contains a high proportion of *n*-alkanes, long-chain alcohols and wax esters, characteristic of beeswax.

Secondary Long-Chain Ketone Formation. The series of 2° ketones eluting at 20−25 min in the chromatogram shown in Figure 1 occur commonly in lipid extracts of cooking vessels of widely varying age and origin. The series includes components containing 29−35 carbon atoms and correspond to nonacosan-15-one, triacontan-14-one, triacontan-15-one, hentriacontan-16-one, dotriacontan-15-one, dotriacontan-16-one, tritriacontan-16-one, tetratriacontan-17-one, and pentatriacontan-18-one. Monounsaturated ketones containing 33 and 35 carbon atoms are also detectable in some instances, eluting immediately prior to the fully saturated components of the same carbon number. Similar compounds are common constituents of plant leaf waxes,[19] have previously been detected in the total lipid extracts of pottery vessels, and

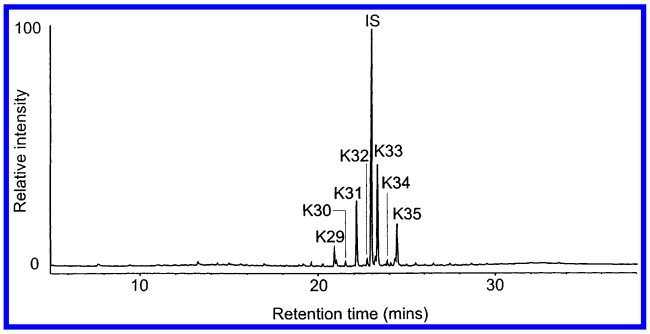
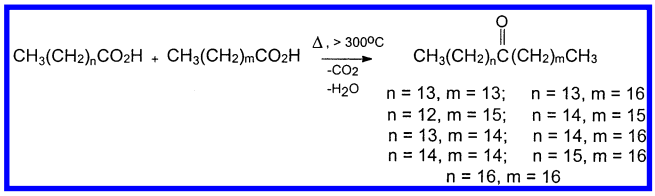


FIGURE 4. Partial HTGC profile of the trimethylsilylated total lipid extract of an early Neolithic Fengate ware rim sherd from Eton Rowing Lake, showing the distribution of midchain ketones. GC conditions and peak identities are as for Figure 1.

Scheme 1. Ketonic Decarboxylation Leading to the Formation of 2° Ketones by Self- and Cross-Head-to-Head Condensation of Fatty Acids (Modified from Refs 24 and 27)



are used to demonstrate the use of ancient cooking jars in the processing of leafy vegetables.[11,12,16,20,21] However, GC−C−IRMS showed the three major long-chain ketone components, i.e., the saturated 31, 33, and 35 carbon number components, to have $\delta^{13}C$ values ca. 10‰ too enriched in ^{13}C for them to derive from the epicuticular leaf waxes of C_3 plants,[22,23] suggesting an alternative origin for these long-chain ketones.[24] Significantly, comparable $\delta^{13}C$ values of the two major fatty acids ($C_{16:0}$ and $C_{18:0}$) and the ketones present in the same extract suggested a precursor−product relationship. Indeed a free radical-induced dehydration and decarboxylation has been reported to occur for a variety of carboxylic acid salts at temperatures, generally, in excess of 400 °C.[25,26] Further evidence for this mechanism of ketone formation comes from closer inspection of the structures and compositions of the components of the mixture of ketones, most notably the following: (i) carbon number range of the ketones and of the putative precursor fatty acids; (ii) position of the carbonyl group in the long-chain ketones; (iii) relative abundance of the ketones compared with the relative abundances of the co-occurring fatty acids. The structures of the ketones typically identified are shown in Scheme 1; formation of the C_{33} and C_{35} ketones bearing unsaturated alkyl moieties occurs by condensation of the monounsaturated $C_{18:1}$ fatty acid with the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively.

Laboratory experiments involving the heating (≥300 °C) of either triacylglycerols or free fatty acids, in the presence of fired marl clay, showed that such mixtures of long-chain ketones to form readily.[27] These findings indicate that caution must be exercised in interpreting the origins of long-chain ketones in archaeological pottery, given the close similarity of the ketones produced by pyrolysis of acyl lipids and those biosynthesized by higher plants. In

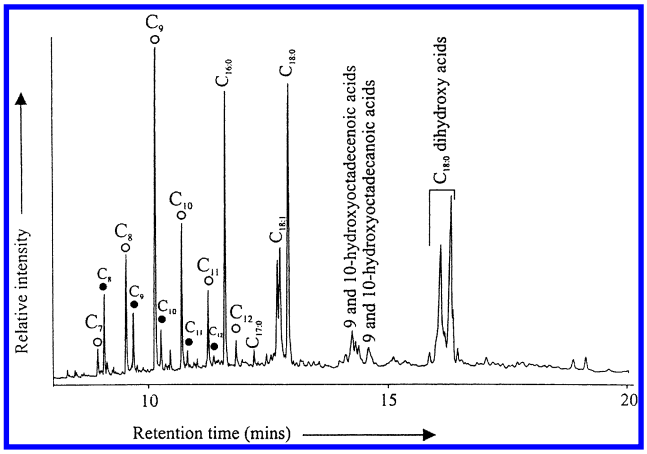


FIGURE 5. Partial gas chromatogram of the base treated residue (as methyl ester−trimethylsilyl ester derivatives) of a potsherd from a Neolithic cooking vessel. Peak identities: $C_{16:0}$, $_{18:0}$, etc., saturated fatty acids; $C_{18:1}$, monounsaturated fatty acid; ○ C_x, α,ω-diacrboxylic acids with carbon chain length *x*; ● C_x, ω-hydroxy acids with carbon chain length *x*.

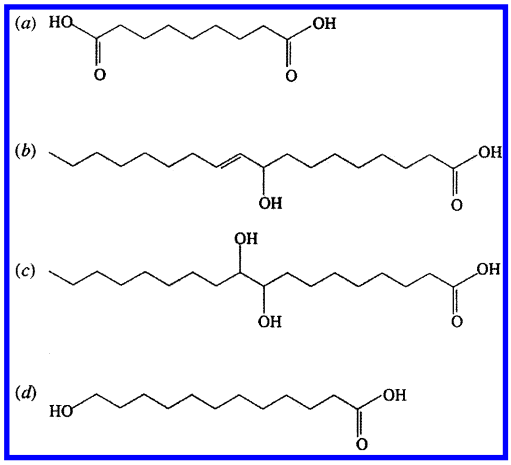


FIGURE 6. Structures of fatty acid oxidation products found in the saponified residue of a Neolithic cooking potsherd shown in Figure 5. Structures: (a) C_9 indicates α,ω-diacrboxylic or nonanedioic acid (azaleic acid); (b) 9-hydroxyoctadecenoic acid; (c) 9,10-dihydroxy-octadecanoic acid; (d) ω-hydroxydodecanoic acid.

some cases the ketones become the dominant components of potsherd extracts (Figure 4) presumably due to free fatty acids being leached from the sherds by percolating groundwater. The significantly more hydrophobic ketones are well-preserved, with the C_{31} and C_{35} components likely to reflect the original distribution of the $C_{16:0}$ and $C_{18:0}$ free fatty acids, confirming the vessel had been used to process animal products.

Oxidized and Polymerized Fats. One of the major processes of transformation of fats and oils is via the "drying" reaction to produce a semisolid polymer. Polymerized and oxidized fats are encountered in many situations in art and archaeology[2] notably in association with mummified human remains from arid locations.[28,29] Recent work has also provided examples of polymerized and oxidized fats in archaeological pottery.[13] Elemental analyses (organic carbon) of the extracted potsherds suggested that "unextractable" or "bound" lipid may still be present. Alkaline treatment of the insoluble residues of previously solvent-extracted potsherds yielded GC chromatograms of the type shown in Figure 5, with GC/MS confirming the presence of oxygenated fatty acid derivatives (Figure 6), including α,ω-dicarboxylic acids ranging from C_7 to C_{12}, with azelaic (C_9) acid the dominant component. C_8−C_{12} α-hydroxy carboxylic acids were also present, together with the saturated carboxylic acids, 9- and 10-hydroxyoctadecanoic acids, unsaturated hydroxy-octadecenoic acids and 9,10-dihydroxyoctadecanoic acids. These data indicated a new mechanism for the preservation of degraded lipids in archaeological pottery involving chemical or physical "bonding", most likely via ester linkages or strong dipole or ionic interactions.

Figure 7 shows the results of the py-GC/MS analysis of a microgram fragment of tissue taken from an Egyptian mummy.[28] The analysis was aimed at determining the presence or otherwise of embalming agents.[30] The data show homologous series of alkanes and alkenes identified from mass spectra and *m*/*z* 55 (alkenes) and 57 (alkanes) mass chromatograms. Since the sample was exhaustively

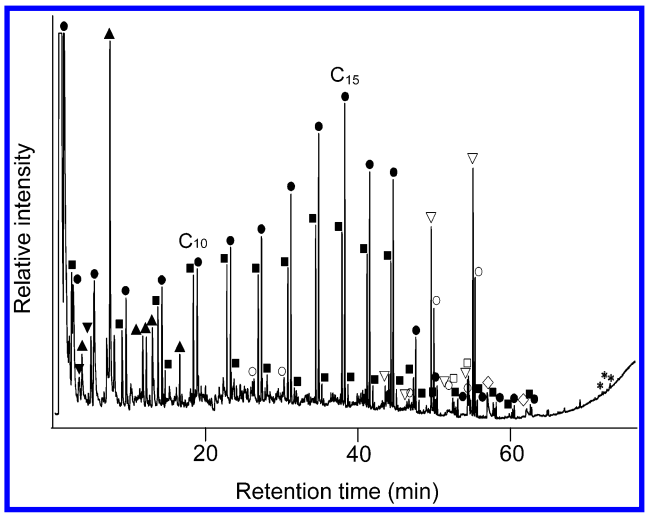


FIGURE 7. GC/MS analysis of the pyrolysis (610 °C) products of bandage/tissue of an Egyptian mummy (Khnum Nakt, ca. 2000 B.C.). Peak identities are the following: ■ = alkenes; ● = alkanes; ▼ = alicyclic hydrocarbons; ▲ = aromatic hydrocarbons; ○ = 2-alkanones; □ = 3-alkanones; ▽ = nitriles; ◇ = amides; ∗ = steroids. C_{10} and C_{15} refer to the carbon numbers of the alkenes and alkanes.

solvent extracted prior to py-GC/MS analysis, it is assumed that the pyrolysis products derive from an insoluble, possibly highly aliphatic polymer probably derived through the polymerization of animal fat either endogenous to the body or added as part of the embalming process. Similar py-GC/MS profiles have been obtained from charred surface residues of potsherds where they are also ascribed to aliphatic polymers formed through the free radical cross-linking of animal fats or plant oils.[31]

Origins of Animal Fats

The question of the origin of animal fats is perhaps most crucial in the case of archaeological pottery due to the importance of archaeological pottery and the high frequency of occurrence of animal fats detected therein; >40% of all sherds studied yield appreciable lipid resi-

Table 1. Ratios of $C_{16:0}$ to $C_{18:0}$ Fatty Acids in Lamps and Dripping Dishes Determined by GC

vessel type	% $C_{16:0}$	% $C_{18:0}$	ratio $C_{16:0}$:$C_{18:0}$
lamp	19	36	0.5
lamp	19	34	0.5
lamp	17	54	0.3
dripping dish	39	17	2.3
dripping dish	49	15	3.3
dripping dish	38	17	2.3
dripping dish	36	15	2.5

dues.[6,14,16,32] While the detection of degraded animal fats is straightforward (see above), identifying the origin of the fats or specifying whether they are mixtures of fats is much more challenging. Evidence that the origins of fats may be established using subtle differences in the chemical compositions of preserved fatty acids came during an investigation of two types of English medieval vessel, classified as lamps and "dripping dishes".[10,32] Previous analyses of such vessels from other excavations had consistently shown them to contain appreciable quantities (10^2-10^3 μg of lipid g^{-1} of dry weight of potsherd) of degraded animal fat. In the case of the lamps this represents the residue of fuel burned, while its presence in "dripping dishes" is consistent with their use as receptacles for fat collection from carcasses during spit-roasting.[33]

Fatty Acid Compositions. GC "fingerprints" of the trimethylsilylated lipid extracts or the methyl esters of the alkanoic and alkenoic acids showed the two vessel types to be clearly separable; i.e., in the lamps $C_{18:0}$ was more abundant than $C_{16:0}$, while the "dripping dishes" showed $C_{16:0}$ in greater abundance (Table 1). While the high $C_{18:0}$ saturated alkanoic acid content of both vessel types confirmed an animal source,[34] distinct differences were also apparent in the distributions of the minor components for the two vessel types. For example, the lamps contained significant amounts of branched-chain alkanoic acids which were undetectable in all but one of the "dripping dishes" and then only in very low abundance. The lamps also displayed a higher abundance of odd carbon numbered, straight-chain components, specifically $C_{15:0}$, $C_{17:0}$, and $C_{19:0}$ (Figure 8). These compositions pointed to ruminant origin for the lamp lipid and monogastric origin for the dripping dish residue.

Positional Isomers of Monounsaturated Fatty Acids. GC-MS analysis of the dimethyl disulfide derivatives of the monounsaturated acids in the extracts of the lamp lipid residues revealed a complex mixture of positional isomers of octadecenoic acid with the double bonds located at the 9-, 11-, 13-, 14-, 15-, and 16-positions. Such mixtures of isomers appear in the fats of ruminant animals, such as sheep and cattle, as a result of biohydrogenation of unsaturated dietary fats in the rumen.[34] In contrast, the fats of monogastric animals, such as pigs, contain only a single isomer, *Z*-9-octadecenoic acid, a finding consistent with the conclusions drawn from the alkanoic acid compositions.

Compound-Specific Stable Isotope Analyses. The application of GC−C−IRMS to the analysis of animal fats for the first time (Figure 9) revealed pronounced differences in the $\delta^{13}C$ values of the individual fatty acids of the ancient fats and reference animal fats, consistent with the differences detected in the structures and distributions of the alkanoic and alkenoic acids. The $\delta^{13}C$ values of the major *n*-alkanoic acids also correlated with vessel type. In the lamps the $C_{16:0}$ was enriched in ^{13}C relative to $C_{18:0}$, whereas in the "dripping dishes" the situation was reversed (Figure 10). Significantly, the $\delta^{13}C$ values correlated with those of the fats of animals considered to be the major domesticated species in the medieval period in the U.K. These preliminary findings suggested that the lipids preserved in the "dripping dishes" derived from monogastric animals, such as pigs, while those from the lamps derived from ruminant animals, such as sheep and/or cattle.[10,32] None of the other modern reference fats (chicken, horse, and deer) displayed $\delta^{13}C$ values consistent with those obtained from the "dripping dishes" (Figure 10). The high content of saturated *n*-alkanoic acids and the pres-

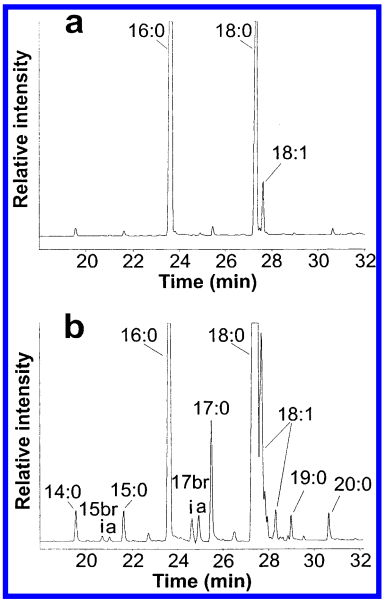


FIGURE 8. Partial GC chromatograms of fatty acid methyl esters recovered from a dripping dish (a) and lamp (b). Peak identities are the following: *x*:*y*, where *x* is the carbon number of the fatty acid and *y* is the degree of unstauration; 15 br and 17 br, i and a are iso- and anteiso isomers of branched C_{15} and C_{17} alkanoic acids.

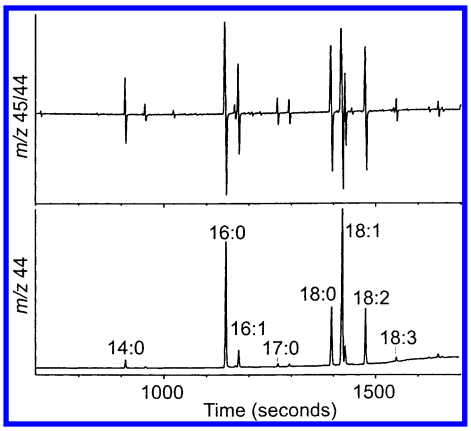


FIGURE 9. Partial *m*/*z* 44 and *m*/*z* 45/44 traces obtained by GC−C−IRMS analysis of fatty acids (as their methyl ester derivatives) in modern reference pig adipose fat.

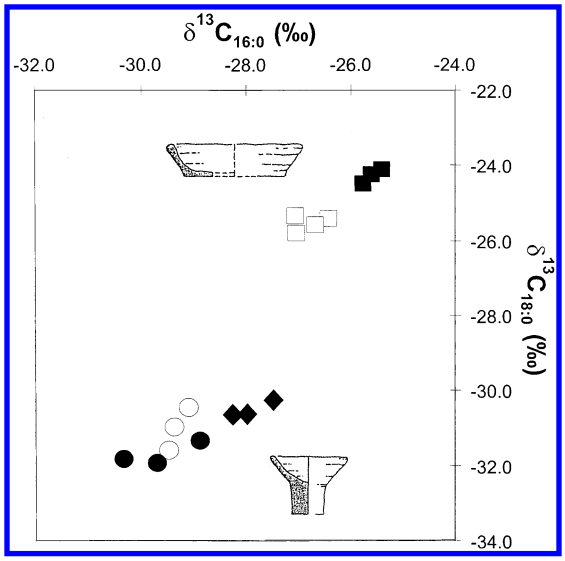


FIGURE 10. Plot showing the $\delta^{13}C$ values of the 16:0 and 18:0 fatty acids form archaeological lamps (○) and dripping dishes (□) and from modern reference animal fats [cattle (◆), sheep (●), and pig (■)].

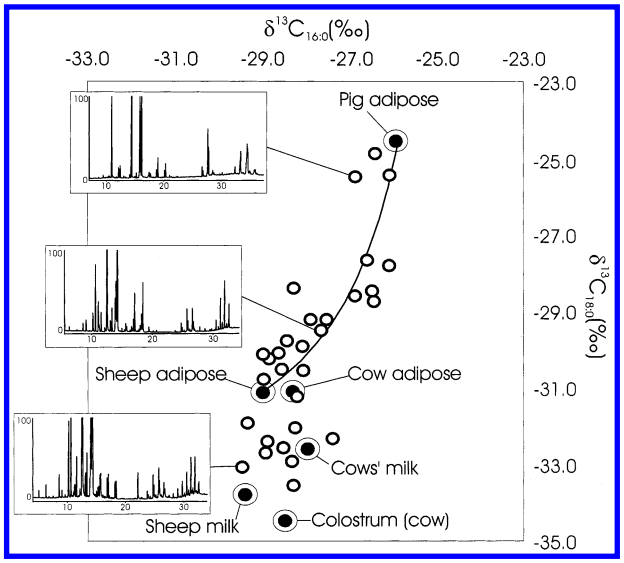


FIGURE 11. Plot of the $\delta^{13}C$ values of the major *n*-alkanoic acid components ($C_{16:0}$ and $C_{18:0}$) of modern reference fats and the lipid extracts of potsherds from the Late Saxon/early medieval site of West Cotton, Northamptonshire, U.K. The filled circles represent the archaeological fats. The mixing curve was determined as in Woodbury et al.[38] to illustrate the $\delta^{13}C$ values which would result from mixing ovine and porcine fats in the vessels. Reference fat fatty acid $\delta^{13}C$ values have been corrected for the post-Industrial Revolution effects of fossil fuel burning.[39] Instrumental error is ±0.3‰, and samples were run in triplicate. The inset HT-GC chromatograms show the total lipid extracts obtained from selected vessels to illustrate characteristic differences in the distributions of triacylglycerols (eluting >30 min).

ence of a mixture of positional isomers of monounsaturated alkenoic acids and branched-chain components excludes vegetable oils as the potential source(s) of lipid in the ancient lamps.

Laboratory degradation experiments of fats dosed into modern potsherds have confirmed the robustness of the $\delta^{13}C$ values of the individual fatty acids.[6,35,36] The results presented in Table 2 shows the $\delta^{13}C$ values of the major saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) of ovine fat are unchanged after laboratory degradation for 1300 days. The results obtained for the oxic degradation are especially noteworthy, since >90% of the original fat dosed into the experimental sherd had been consumed by microorganisms during the experiment.

Table 2. $\delta^{13}C$ Values Obtained by GC−C−IRMS for the Major Fatty Acids Present in Ovine Adipose Fat before and after Accelerated Laboratory Degradation under Oxic and Anoxic Conditions for 1300 days at 45 °C

incubation time T_{days} (incubation conditions)	$\delta^{13}C$ values (‰)	
	$C_{16:0}$	$C_{18:0}$
T_0 (preincubation)	−30.1	−31.9
	−30.1	−32.3
T_{1300} (oxic)	−30.1	−32.2
	−30.0	−32.3
T_{1300} (anoxic)	−30.0	−32.5
	−29.8	−32.3

Figure 11 shows the application of this approach to 30 vessels from the Late Saxon/early medieval site of West Cotton, Northamptonshire, U.K.[37] The data show that both ruminant and nonruminant fats are present in the archaeological vessels with the mixing of fats being indicated by the points following the mixing line drawn between the means values for the ruminant and pig reference fats. Interestingly, the trends in the processing of animal products in the various archaeological vessels revealed by these data follow the statistical trends seen in the animal bone assemblage from the site. Application of this technique to pottery from prehistoric periods is beginning to reveal important cultural biases in the exploitation of animal products.[32,40]

Dairy Fats in Prehistory. Milk fats differ from adipose fats in their fatty acid composition through the presence of short-chain saturated fatty acids in the C_4−C_{14} carbon number range.[41,42] Surprisingly, animal fat residues containing these characteristic shorter chain fatty acids are detected very rarely in archaeological pottery. Possible explanations for this are that either dairy products were not processed to any significant extent in pottery vessels or that diary fats become altered, through decay, in such a way as to make them indistinguishable from adipose fats. Indeed, it can be argued that the short fatty acyl moieties would be more susceptible to hydrolysis, due to reduced steric effects at ester linkages in triacylglycerols compared with their long-chain counterparts. Furthermore, once released from triacylglycerols by hydrolysis the short-chain fatty acids are appreciably more water soluble (and volatile) than their long-chain counterparts. We have tested this hypothesis in laboratory degradation experiments and shown that the milk fats absorbed in pottery vessels rapidly hydrolyze with preferential decay ("loss") of their short-chain fatty acid moieties to produce a distribution of fatty acids and triacylglycerols, dominated by $C_{16:0}$ and $C_{18:0}$, reminiscent of adipose fat,[18,37] thereby explaining why ourselves and others[43] have consistently failed to detect dairy fats in pottery vessels.

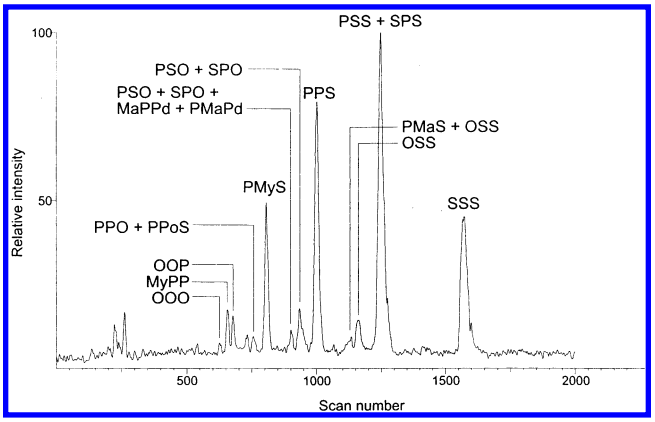


FIGURE 12. HPLC-APCI MS profile of the TAG fraction obtained from potsherd-containing ruminant fat. Fatty acyl moieties indicated by peak annotations: P, palmitic acid (16:0); Po, palmitoleic acid (16:1); Ma, margaric acid (17:0); S, stearic acid (18:0); O, oleic acid (18:1). The regioisomerism of the TAG is reflected in the abbreviation.

Significantly, Figure 11 shows that a number of the vessels studied from West Cotton contained fatty residues in which the $C_{18:0}$ fatty acid is significantly depleted in ^{13}C compared with the reference adipose fats. Consideration of the biochemistry and physiology of milk production in ruminant animals[44,45] and the subsequent analysis of reference milk fats obtained from C_3 pasture-reared sheep and cattle strongly indicates that the origin of these ^{13}C-depleted $C_{18:0}$ fatty acids was milk.[37] The distinctive trends seen in Figure 11 for the $\delta^{13}C$ values of the dairy product $C_{16:0}$ and $C_{18:0}$ fatty acids reflects de novo synthesis of the $C_{16:0}$ fatty acid from acetate, with the $C_{18:0}$ component deriving in part directly from the dietary fatty acids, i.e., mainly $C_{18:2}$ and $C_{18:3}$, by biohydrogenation (bacterial reduction) in the rumen, and in part from acetate (derived largely from dietary carbohydrate). During lactation there is a shift in the energy balance such that a greater proportion of the $C_{18:0}$ fatty acid present in milk is derived directly from the unsaturated C_{18} fatty acids in the diet. Thus, the enhanced routing of dietary fatty acids during lactation results in more negative $\delta^{13}C$ values for the $C_{18:0}$ fatty acid of milk fats compared with the adipose fats of animals feeding on the same diets, thereby establishing a new means of detecting anicent milk fats and investigating the importance of dairying as a component of prehistoric economies.[37]

High-Performance Liquid Chromatography/Mass Spectrometry. Since intact triacylglycerols survive in archaeological pottery and bog butters, the opportunity exists to investigate their compositions and origin as intact triacylglycerol molecular species. HPLC/MS with APCI allows positional distributions of fatty acids in the individual triacylglycerol molecular species to be determined via spectra which show the protonated molecular ion, $[M + H]^+$, diacylglycerol ions, $[M - RCO_2]^+$, and acylium ions, RCO^+.[15,46] The positional distributions can be identified by the abundance of the diacylglycerol ions, since the least abundant diacylglycerol ion is formed by cleavage of the *sn*-2-position fatty acid as this is energetically less favorable than the *sn*-1 or *sn*-3-positions.[47]

Table 3. Relative Abudances of $C_{16:0}$ (P) and $C_{18:0}$ (S) Fatty Acyl Moieties, Overall and in the 2-Position, in Preserved Triacylglycerols Determined by HPLC/MS with APCI

	archaeological samples		ref samples	
P:S ratios	"ruminant"	"nonruminant"	cattle fat	pig fat
overall	67:33	41:58	44:56	60:40
2-position	45:55	81:19	41:59	86:14

The triacylglycerol fractions of potsherds containing appreciable abundances of triacylglycerols have been analyzed by HPLC/MS with APCI. Previous analyses of the lipids present in these potsherds had indicated the presence of degraded animal fats of ruminant and nonruminant origin, respectively. The HPLC/MS profile for the sherd containing the ruminant fat is shown in Figure 12. The triacylglycerol distribution is dominated by saturated components, the loss of unsaturated fatty acyl moieties having occurred through oxidative decay. Regiospecific analysis of the triacylglycerols showed that some components were present as more than one regioisomer (e.g. PSO and SPO). In addition, some triacylglycerols were present as more than one chromatographic peak, indicating that more than one isomer of the 18:1 fatty acid may be present, which is entirely consistent with the fat being of ruminant origin.

Assessment of the ratio of palmitic (16:0) to stearic (18:0) acid present in the 2-positions of TAGs indicates that this may provide information on the origin of archaeological animal fats. Ruminant fats show a P:S ratio in the 2-position of ca. 60:40 whereas, in nonruminants, the ratio is closer to 95:5.[48] The percentage of each fatty acid present in the 2-position of the samples was calculated from their HPLC-APCI MS profiles using a quantification method described previously.[15,46] The results shown in Table 3 (along with P:S ratios calculated for modern reference fats, pig and cattle) indicate a clear difference between the archaeological samples. The extract thought to contain ruminant fat shows a 2-position P:S ratio of 45:55, while that believed to contain nonruminant fat shows a 2-position P:S ratio of 81:19. These correlate well with the ratios obtained for modern pig and cattle adipose fats, which were 41:59 and 86:14, respectively. These preliminary results suggest that postburial transesterification is not significant in this case and that HPLC/MS of intact TAGs provides a further means of determining the origins of ancient animal fats.

Compound-Specific Radiocarbon Dating

The lipids preserved in archaeological pottery constitute a potentially important source of carbonaceous material for radiocarbon dating. We have recently used archaeological pottery to explore the method described by Eglinton and co-workers[49] for the radiocarbon dating of individual lipids. The method uses automated preparative capillary GC employing wide bore capillary columns to isolate individual compounds from lipid extracts of archaeological pottery. Fatty acids (as methyl ester derivatives) are suitable target compounds since they are often present in high concentrations. As shown by the chro-

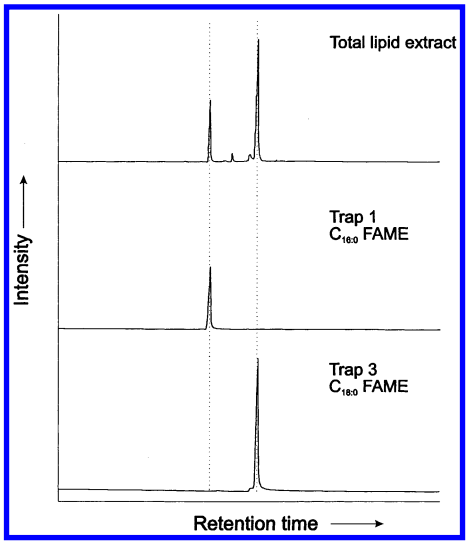


FIGURE 13. Partial gas chromatogram of products collected from 95 preparative GC runs in which $C_{16:0}$ and $C_{18:0}$ fatty acids were trapped from a Neolithic potsherd dated at ca. 4000 years B.C. The upper chromatogram corresponds to the fatty acid methyl ester components of the TLE, while those below are analyses of the contents of the traps after trapping prior to AMS dating.

matograms presented in Figure 13, fatty acids can be isolated in high purity (>95%) and amounts (>250 μg; external standard used for quantification) sufficient for radiocarbon analysis by accelerator mass spectrometry (AMS). The advantage of compound-specific dating is that ^{14}C dates are obtained for individual compounds that can be directly linked to the components of commodities processed in the vessels during their use, e.g. animal fats. So far dates have been obtained on archaeological pottery whose predicted ages spanned a 6000 year date range.[50] Initial results indicate that meaningful correlation can be obtained between the predicted date of the pottery and the ^{14}C date of the preserved lipids. These results constitute an important step forward in the direct dating of archaeological pottery.

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