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THE ROYAL SOCIETY

Lipids as carriers of anthropogenic signals from prehistory

Richard P. Evershed, Stephanie N. Dudd, Stephanie Charters†, Hazel Mottram, Andrew W. Stott, Anthony Raven, Pim F. van Bergen‡ and Helen A. Bland

Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

Studies performed during the last two decades have shown that lipids are preserved in association with a wide range of artefacts and ecofacts recovered from archaeological sites, e.g. pottery vessels and skeletal remains. The majority of work in this area has focused on the use of molecular structures ('biomarkers') and distributions ('fingerprints') to assess the nature and origin of commodities associated with past cultural, economic and agricultural practices. However, since lipids, like all other classes of biomolecule, are affected by degradation (both pre- and post-burial), emphasis is now being placed on the complementary use of diagenetically robust, compound-specific stable isotope measurements to enhance the scope and reliability of archaeological interpretations. A feature of the $\delta^{13}C$ values of individual lipids, rather than bulk measurements of biochemically more heterogeneous materials, lies in their capacity to reflect differences in both the isotopic composition of the carbon sources used in their biosynthesis and the routing of dietary lipids and their metabolites in consumer organisms. This isotopic information, accessible by gas chromatography–combustion-isotope ratio mass spectrometry, has opened up new avenues of investigation concerning human activity in prehistory.

Keywords: archaeology; organic residues; lipids; biomarkers; stable isotopes; pottery

1. INTRODUCTION

(a) *Biomarkers and organic geochemistry*

For the last 30 years organic geochemists have used the concept of chemical or geochemical fossils, biological markers or biomarkers to mean those compounds that occur in ancient sedimentary materials that can be linked with biochemical precursor compounds as a result of their basic carbon skeletons surviving in a recognizable form through deposition and diagenesis over geological time-scales (Eglinton & Calvin 1967). Lipids, or more correctly their diagenetic (and catagenetic) hydrocarbon products, have been the most widely studied group of compounds by organic geochemists (Johns 1986; Peters & Moldowan 1993). The focus on lipids stems from their survival in recent and geological age sediments owing to their inherent resistance to decay relative to other compound classes, such as proteins and carbohydrates (Eglinton & Logan 1991). Equally important has been the availability and development, throughout the last 30 years, of the techniques of gas chromatography (GC) and combined GC/mass spectrometry (GC/MS) which allow diagnostic lipid distributions and structures to be recognized among the highly complex mixtures of components present in extracts of ancient sedimentary materials.

†Present address: The Biochemical Society, 59 Portland Place, London WIN 3AJ, UK.

‡Present address: Organic Geochemistry Group, Geochemistry, Faculty of Earth Sciences, Utrecht University, PO Box 80021, 3508 TA Utrecht, The Netherlands.

(b) *Archaeological biomarkers*

In the late 1970s and 1980s it began to be realized that lipids are preserved under favourable conditions in association with various classes of archaeological artefact and ecofact. Thus, it was recognized that the concept of chemical fossils or biomarkers might be equally well applied to deriving information relating to the activities of ancient peoples (Evershed 1993*a*). It has now been shown that lipids occur very widely at archaeological sites. For example, steroidal compounds, e.g. stanols, sterols and bile acids, preserved in soils, sediments and coprolites have been used to provide information concerning waste disposal, manuring, other agricultural practices and palaeodiet (Bethell *et al.* 1994; Bull *et al.* 1998*a*,*b*; Evershed & Bethell 1996; Evershed *et al.* 1997*a*; Knights *et al.* 1983; Pepe *et al.* 1989; Pepe & Dizabo 1990; Simpson *et al.* 1997, 1998*a*,*b*). Lipids have also been shown to occur in skeletal and other human remains, e.g. bog bodies (Evershed & Connolly 1988, 1994) and mummies (Gulaçar *et al.* 1990), with the cholesterol component being investigated as a carrier of a robust stable isotope signal for use in palaeodietary studies (Evershed *et al.* 1995*a*; Jim *et al.* 1998; Stott & Evershed 1996; Stott *et al.* 1997, 1998). Furthermore, the results of analyses of lipids in desiccated plant remains (O'Donoghue *et al.* 1996*a*; van Bergen *et al.* 1997*a*), are complementing those obtained from studies of other classes of biomolecule (Bland *et al.* 1998; Evershed *et al.* 1997*b*; O'Donoghue *et al.* 1994, 1996*a*,*b*) and are helping to improve our understanding of the preservational and degradative processes, which is essential in underpinning archaeological interpretations

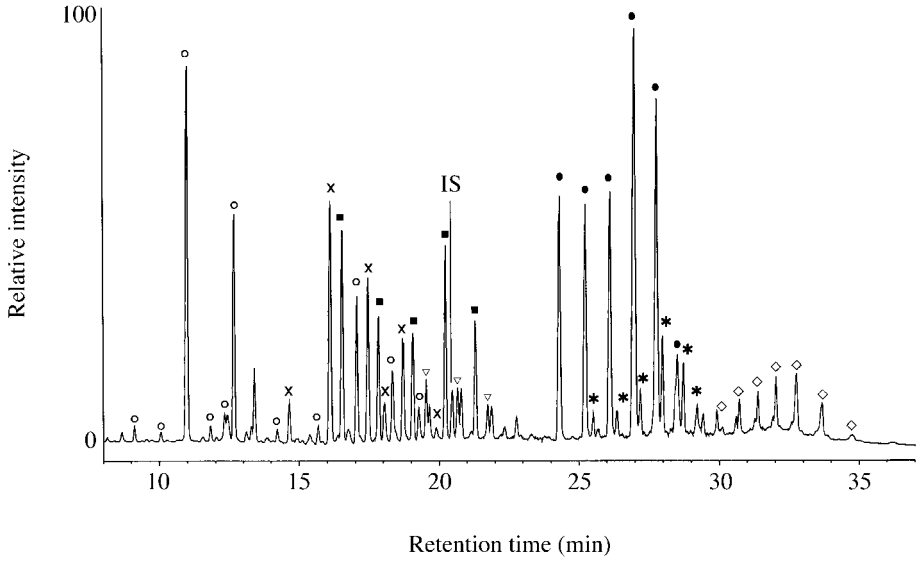

Figure 1. Partial high temperature gas chromatogram of the trimethylsilylated total lipid extract of a potsherd from a domestic, medium-sized Saxon jar form vessel recovered from excavations at Brandon, Norfolk, UK. The analysis serves to illustrate the wide range of compound types that can be revealed in a single analytical run by use of short capillary columns coated with thin films of high temperature-stable cross-linked dimethyl polysiloxane-type stationary phases. See § 2c for further experimental details. This residue comprises a mixture of degraded animal fat (based on high content of saturated C_{16} and C_{18} fatty acids) and beeswax (characterized by the presence of a mixture of long-chain alcohols, high carbon number fatty acids ($>C_{22}$), *n*-alkanes and wax esters; GC profiles for fresh and ethnographic beeswax are given in Charters *et al*. (1995) and Evershed *et al*. (1997*e*), respectively). The mid-chain secondary ketones are formed by condensation of fatty acids at $>300\,^{\circ}C$ and their presence indicates that the vessel has been subjected to intense heating. Peak identities: ○, free fatty acids in the $C_{14:0}$ to $C_{28:0}$ carbon number ranges; ×, *n*-alkanes in the C_{25} to C_{33} range; ■, long-chain *n*-alkanols in the range C_{24} to C_{32}; ▽, C_{31} to C_{35} 2° ketones; ●, palmitic acid wax esters in the C_{40} to C_{52} carbon number range; *, hydroxypalmitic acid wax esters in the C_{42} to C_{50} range; ◇, high molecular weight acyl lipids, possibly diesters or triacylglycerols. IS, *n*-tetratricontane (*n*-C_{34}) added as internal standard.

based on all types of organic residues of archaeological interest.

Perhaps the most well-developed area of the application of biomarker analyses to archaeology is in the investigation of natural bitumens (or asphalts), plant resins and plant pyrolysis products, i.e. tars, pitches, etc. (Mills & White 1994; Pollard & Heron 1996). Such materials arise through Man's need to exploit his environment to provide glues, sealants and coating materials (Aveling & Heron 1998; Beck *et al*. 1989, 1998; Beck & Borromeo 1990; Charters *et al*. 1993*a*; Evershed *et al*. 1985; Robinson *et al*. 1987; Regert *et al*. 1998*a*) and more precious medicinal and ritual/funerary substances (van Bergen *et al*. 1997*b*; Evershed *et al*. 1997*d*). The use of biomarker techniques, such as GC and GC/MS, offer the only reliable means of identifying the origins of such materials. The work of Connan and co-workers has provided the most obvious conjoining of archaeology and organic geochemistry. Sterane and triterpane biomarker distributions derived by GC/MS have been employed to confirm the use of petroleum bitumens in the prehistoric Near East (Connan *et al*. 1992; Boeda *et al*. 1996; see also Connan, this issue).

(c) *Archaeological pottery and organic residues*

Potsherds are one of the most common classes of artefacts recovered from archaeological excavations (Gibson & Woods 1990; Rice 1987), and recent work has shown that unglazed ceramic vessels possess the notable property of absorbing substantial quantities of organic matter from the commodities processed or stored in them during their lifetime of use. Equally important for the purposes of biomarker analyses is the property of fired clay to function as a molecular sieve or trap which can preserve organic biomolecules during burial over many millennia. Often more obvious, but occurring with much lower frequency at most sites, are charred organic deposits visible on the inner or outer surfaces of vessels (Oudemans & Boon 1991; Regert *et al*. 1998*b*). Recent work has shown that the chemical analysis of such residues can provide information concerning the original uses of ancient pottery vessels. Traditional methods of pottery analysis by archaeologists rely on typological or stylistic criteria and/or features to provide information relating to the means and place of manufacture of individual vessels, their relative dates and possible functions (Rice 1987). However, only chemical investigations of organic residues have the capacity to identify commodities that can be directly linked with vessel use in antiquity. Although it has recently been shown that proteinaceous matter survives in archaeological ceramics (Evershed & Tuross 1996), by far the best preserved and most extensively studied class of compounds are the solvent-extractable lipids, i.e. the fats, oils and waxes, that are ubiquitous components of plants and animals. The earliest studies of lipids from archaeological ceramics employed solvent extraction followed by methylation and gas chromatographic analysis to identify the fatty acid components of extracts (e.g. Condamin *et al*. 1976; Patrick *et al*. 1985). Attempts to define the origin of the lipids were then based on drawing comparisons between the relative proportions

of the fatty acids present in the extracts of the ancient vessels and those of modern reference fats. The saturated fatty acid components were generally preferred in these comparisons since they are more resistant to the inevitable degradation that occurs during vessel use and burial than their unsaturated counterparts.

In 1990, we introduced a new approach to the analysis of lipid residues from archaeological ceramics, preferring to use high temperature-gas chromatography (HT-GC) and HT-GC/mass spectrometry (HT-GC/MS) to derive detailed compositional information directly from extracts without chemically degrading them to release their simpler fatty acid moieties (Evershed *et al*. 1990). An important advantage of this technique was the ability to reveal a very broad range of lipid classes within a single analytical run (figure 1). We have now used this approach routinely for over eight years to screen solvent extracts of >1000 potsherds for the presence of lipid biomarkers in organic residues. The effectiveness of this approach has been demonstrated through our identifications of residues of plant leaf waxes (Charters *et al*. 1997; Evershed *et al*. 1991, 1992, 1994), beeswax (Charters *et al*. 1995; Evershed *et al*. 1997*e*) and birch bark tar (Charters *et al*. 1993*a*; Dudd & Evershed 1998*a*).

As this work has progressed, it has become increasingly apparent that for the vast majority of lipid extracts of archaeological pottery, identifications of commodities based only on simple comparisons of compound structures or distributions are rather limited in scope. Shortcomings in this approach arise from the fact that similar compounds or mixtures of compounds are produced by different plants and animals, and perhaps more importantly, that lipid biomarker distributions may be altered either during vessel use or during burial in such way as to limit, or completely confound, subsequent interpretations of the analytical data. Such factors are particularly manifest in the case of degraded animal fats and plant oils, which are the most common class of lipid encountered in archaeological pottery. In this contribution we review our current analytical approaches to the study of organic residues in archaeological ceramics, focusing particularly on investigations that have benefited from the use of compound-specific stable isotope measurements ($\delta^{13}C$ values) derived through the GC/combustion-isotope ratio monitoring MS (GC/C-IRMS) technique developed by Hayes and co-workers (Matthews & Hayes 1978).

2. EXPERIMENTAL METHODS AND APPROACHES

(a) *Samples*

We have studied more than 1000 archaeological potsherds from locations throughout the UK, Europe and the Near East, varying in age from the early Neolithic to the medieval periods. All pottery is unglazed and for the most part lacks surface residues; hence, investigations have focused primarily on lipid absorbed within the vessel walls. Where possible, samples are taken from more than one position on the vessel profile (Charters *et al*. 1993*b*). Studies of archaeological pottery are complemented by analyses of modern pottery that have been employed in laboratory experiments aimed at simulating vessel use and/or burial (Charters *et al*. 1997; Evershed *et al*. 1995*b*; Dudd *et al*. 1998). Investigations have used pottery from archaeological archives (stored for up to 20 years) although freshly excavated pottery is preferred; the latter is by far the most common class of material analysed.

(b) *Extractions and derivatizations*

Subsamples of archaeological potsherds (1–10 g) are cleaned with a modelling drill fitted with an abrasive bit to remove soil contaminants, then crushed using a pestle and mortar. The powdered fabric is then extracted (ultrasonication or Soxhlet) with organic solvent (e.g. dichloromethane or chloroform–methanol, 2:1 v/v). Aliquots of the resulting extract are either trimethylsilylated directly and analysed by GC and GC/MS (see below) or separated into different lipid classes using either solvent partitioning, solid phase extraction or 'flash' chromatography. The resulting fractions are derivatized as required [hydroxy compounds (mono- and diacylglycerols, sterols, triterpenols, long-chain alcohols, hydroxy carboxylic acids) are trimethysilylated (Evershed 1993*b*), carboxylic acids (fatty acids, dicarboxylic acids, hydroxy fatty acids) are methylated (Hamilton *et al*. 1992), and monounsaturated fatty acids converted to methyl ester-dimethyl disulphide derivatives (Evershed 1992; Mottram *et al*. 1998)] and analysed by GC, GC/MS and GC/C-IRMS as described below. Where appropriate intact acyl lipids (i.e. mono-, di- and triacylglycerols and wax esters) are hydrolysed to their component neutral and acidic moieties prior to derivatization as described above. Quantitative estimates of the amounts of lipid obtained from potsherds are obtained from GC analyses by comparing GC peak areas with those of appropriate internal standard(s) added at the extraction stage (Charters *et al*. 1993*b*).

(c) *Instrumental methods*

Screening of the components of trimethylsilylated total lipid extracts containing high molecular weight lipids (e.g. wax esters and triacylglycerols) is achieved by HT-GC and HT-GC/MS (Evershed *et al*. 1990) using capillary columns coated with a thin film of high temperature-stable stationary phase (e.g. DB-1 (cross-linked dimethyl polysiloxane) 15 m × 0.32 m; 0.12 μm film thickness). High resolution capillary GC and GC/MS analyses of lower molecular weight lipids (including free sterols and long-chain alcohols as TMS derivatives) are typically performed on a 50 m fused silica capillary column coated with a CP-Sil 5 stationary phase. Methyl esters of fatty acids are generally analysed on a 25 m × 0.25 mm i.d. fused silica capillary coated with polar stationary phase (e.g. SGE BPX70 (70% cyanopropyl equivalent) or CP-WAX 52CB (Carbowax equivalent); typically 0.25 μm film thickness). All standalone GC analyses employ flame ionization detection to monitor the column effluent. Either hydrogen or helium are used as carrier gas.

Compound structures are determined by GC/MS employing the same GC columns as described above. The vast majority of mass spectra are recorded employing electron ionization (70 eV). Mass spectral interpretations are based on the known fragmentations of organic compounds and searching of NIST or NBS mass spectral libraries.

GC/C-IRMS analyses were performed with a Finnigan MAT Delta S mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) coupled to a Varian GC (Varian Associates, Inc., Walnut, Creek, CA) via a Pt/Ni/CuO combustion interface, which was maintained at 850 °C, with a constant purge of O_2. Removal of water was facilitated by Nafion tubing (Perma Pure Products Inc., Toms River, NJ), and standardization of runs was achieved with six portions of CO_2 gas of known $\delta^{13}C$ value ($\delta^{13}C_{(CO_2)} = -31.8‰$) injected directly into the ion source of the

mass spectrometer. The longer high-efficiency capillary columns described above were used for compound-specific stable isotope analyses since baseline resolution is required. Data were collected and processed with Finnigan MAT isobase software. The $\delta^{13}C$ values of derivatized (methylated or trimethylsilylated) compounds were corrected for the added carbon by using the mass balance equation of Jones *et al*. (1991).

3. RESULTS AND DISCUSSIONS

While the application of lipid biomarker techniques has increased the scope of investigations in several areas of archaeology, their impact has been especially significant in studies of lipids in potsherds. Recent work has shown that the insights into the origins, fate and processes of alteration of lipids associated with archaeological pottery come by using the more conventional structural and distributional information provided by GC and GC/MS in conjunction with $\delta^{13}C$ values of individual compounds. Even at this relatively early stage in the application of these techniques we have found that compound-specific $\delta^{13}C$ values are often indispensable, providing the only means of accessing new sources of information or resolving ambiguities concerning the origins of certain key classes of lipid. The following sections review the current status of our research in this area.

(a) *Plant epicuticular waxes*

One of the most striking examples of the use of lipid biomarkers to identify a commodity processed in archaeological vessels came from our earliest investigations of a large number of domestic cooking vessels recovered from the site of the late Saxon/medieval settlement of West Cotton (Evershed *et al*. 1991). The lipid extracts of a significant number of vessels yielded a distribution of lipids that comprised three major components, including *n*-nonacosane, nonacosan-15-one and nonacosan-15-ol, in proportions exactly analogous to those of the epicuticular leaf wax of *Brassica oleracea* (cabbage; Evershed *et al*. 1991, 1992; Charters *et al*. 1997). The obvious conclusion was that the vessels had been used for boiling cabbage, an inference that is supported by the results of laboratory experiments using replica cooking jars (Evershed *et al*. 1995*b*; Charters *et al*. 1997).

In view of this identification and the availability of appropriate reference plant material (wild-type cabbage collected from the UK), compound-specific stable isotope measurements were performed using GC/C-IRMS (Evershed *et al*. 1994). The analyses that were carried out on the *Brassica* *n*-alkanes produced $\delta^{13}C$ values that fell within the accepted limits of precision (± 0.3‰). The most significant finding was that the $\delta^{13}C$ values for the potsherd alkanes and ketone are consistent with their being of a C_3 plant origin (Rieley *et al*. 1991; Rieley 1993; Lockheart *et al*. 1997). The $\delta^{13}C$ values for the C_{29} *n*-alkane from two different vessels, i.e. -34.8‰ and -33.1‰, are typical for those of higher plant leaf waxes as shown by the similarity to the $\delta^{13}C$ values obtained for the contemporary wild-type *Brassica* (-35.8‰ $\pm$ 0.1). While these values are in keeping with those for contemporary C_3 plants, the results serve to highlight a number of factors relevant to future investigations. For example, an inevitable source of variation in the $\delta^{13}C$ values for the contemporary plant extracts and potsherd lipids concerns the primary carbon source. Carbon dioxide in ancient times (prior to the Industrial Revolution) was enriched in ^{13}C by *ca*. 1‰ compared to present-day atmospheric carbon dioxide (Friedli *et al*. 1986). Hence, the $\delta^{13}C$ values determined for the potsherd lipids will represent the effects of the ^{13}C content of the atmospheric CO_2 at the time of cultivation and of isotopic fractionation. Interestingly, as would be predicted, the isotope value of the modern *Brassica* leaf wax C_{29} *n*-alkane is lighter than that of the ancient leaf wax *n*-alkane (by *ca*. 1.8‰; mean of extracts of two vessels). Although this difference might not be significant, as it is known that within *n*-alkanes of this carbon number range variability of up to 6‰ can occur even within a single leaf type throughout a growing season (Lockheart *et al*. 1997), we do routinely correct the $\delta^{13}C$ values for our modern reference materials for the fossil fuel effect, i.e. in accordance with Friedli *et al*. (1986), modern values are increased by 1.2‰ to account for the lighter carbon introduced through fossil fuel burning depleting the $\delta^{13}C$ value of the CO_2 of the present-day atmosphere. The $\delta^{13}C$ value for the mid-chain ketone (-34.6‰) was congruent with the values obtained for the *n*-alkane (see above); indicating that the stable isotope content of these biochemically closely related compounds were subject to analogous influences (see discussions given above). This investigation served to confirm that the lipid extracts of ancient potsherds contained sufficient lipid to allow high precision $\delta^{13}C$ values to be obtained on individual lipids.

(b) *Resolving questions of pre- and post-burial alterations*

(i) *Pre-burial alterations*

The oxidizing conditions and high temperatures that pottery vessels may be subjected to during their use can result in chemical changes being induced in lipids associated with surface or absorbed residues. The chemical changes occurring in lipids during the modern processing of foods have been extensively studied (for a review, see Davídek *et al*. 1990), allowing some of the major degradative reactions likely to impact on the processing of lipid-containing foodstuffs in pottery vessels to be at least partly anticipated. We have discussed some of these previously and presented data to show that some of the expected changes, e.g. hydrolysis, oxidation and polymerization, do indeed occur in pottery vessels during the cooking of foods (Evershed *et al*. 1992; Regert *et al*. 1998*b*).

A novel degradative reaction has been revealed through analyses of lipid extracts of potsherds recovered from excavations at a number of sites in the UK and mainland Europe (dating from the Early Bronze Age to the medieval period; Evershed *et al*. 1995*c*; Raven *et al*. 1997). A typical gas chromatogram is shown in figure 2 which reveals, in addition to the saturated fatty acids that occur very commonly in potsherds of cooking jars, a series of long-chain ketones (K) containing 29, 30, 31, 32, 33, 34 and 35 carbon atoms. These ketones correspond to nonacosan-14-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

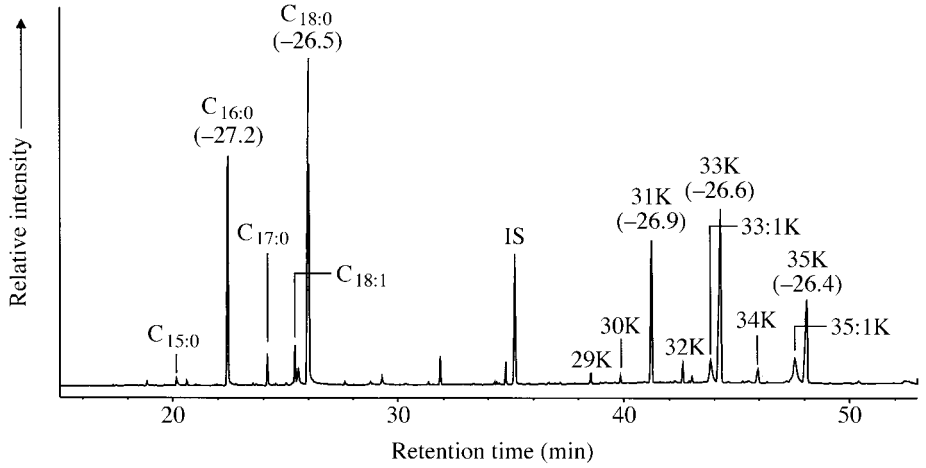

Figure 2. Partial gas chromatogram of the total lipid extract (trimethylsilylated) of an Early Bronze Age cooking vessel from St Veit-Klinglberg, Austria, showing the presence of long-chain 2° ketones formed by thermal free radical condensation of the fatty acids present in the same extract (see figure 3). Peak identities are: $C_{16:0}$, $C_{18:0}$, etc. indicate saturated fatty acids and $C_{18:1}$ indicates a monounsaturated fatty acid; K, long mid-chain ketones with the preceding number corresponding to the number of carbon atoms in each component; 33:1 indicates monounsaturated ketone; IS, internal standard (5α-cholestane). Values in parentheses refer to the $\delta^{13}C$ values of the individual compounds determined by GC/C-IRMS (modified from Raven *et al.* (1997)).

containing 33 and 35 carbon atoms are also detectable, eluting immediately prior to the fully saturated components of the same carbon number. It should be emphasized that these series of ketones are always accompanied by mixtures of fatty acid indicative of degraded animal fats.

Long-chain ketones have been widely reported as components of the epicuticular waxes of higher plants (see above and Kolattukudy (1976); Walton 1990). Significantly, and as already discussed above, nonacosan-15-one and biochemically related compounds (*n*-nonacosane and nonacosan-15-ol) have previously been detected in the total lipid extracts of pottery vessels and are used to demonstrate the use of ancient cooking jars in the cooking of leafy vegetables (Evershed *et al.* 1990, 1991, 1992, 1994; Charters *et al.* 1997). Thus, although the most obvious source of the series of long-chain ketones shown in figure 2 was higher plant leaf waxes, further study of such extracts by means of 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 of −26.8, −26.5 and −25.9‰, respectively. Hence, these compounds are too enriched in ^{13}C for them to derive from the epicuticular leaf waxes of C_3 plants (Rieley *et al.* 1991; Lockheart *et al.* 1997), e.g. they are *ca.* 10‰ more enriched than the nonacosan-15-one of *Brassica oleracea* (cabbage; −35.4‰) and hentricontan-16-one of *Allium porrum* (leek; −35.2‰; Evershed *et al.* 1995*c*). These anomalously enriched $\delta^{13}C$ values forced us to consider alternative origins for these mixtures of long-chain ketones.

Significantly, the two major fatty acids ($C_{16:0}$ and $C_{18:0}$) present in the extracts of the Early Bronze Age vessels have $\delta^{13}C$ values of −25.5 and −26.6‰, respectively, as these are similar to those of the three major ketones present in the same extract (figure 2). The close similarity between the $\delta^{13}C$ values of the fatty acids and ketones suggested a precursor–product relationship between these two classes of compounds. Since the 'cooking' vessels from which the potsherds originated will have been subjected to extensive heating during the processing of their contents, the possibility exists that the series of ketones has formed by condensation of fatty acid moieties derived from fats absorbed into the vessel wall during use. This is a known organic reaction, involving a free radical-induced dehydration and decarboxylation, which has been reported to occur for a variety of carboxylic acid salts at temperatures, generally, in excess of 400 °C (March (1977) and references therein). Compelling 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: (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; and (iii) relative abundance of the ketones compared with the relative abundances of the fatty acids present. The structures of the ketones, derived through GC/MS analysis of the extract shown in figure 2 are summarized in figure 3. Formation of the C_{33} and C_{35} ketones bearing one unsaturated alkyl moiety presumably 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 a low fired (800 °C) marl clay (Raven *et al.* 1997) showed that such mixtures of long-chain ketones form readily under conditions that might be encountered during the heating of fats at high temperatures in ancient pottery vessels. These findings show 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. Most notably, the $\delta^{13}C$ values of the individual compounds (figure 2) were pivotal in establishing the product–precursor relationship between the ketones and the fatty acids.

(ii) *Post-burial alterations*

Changes in the distributions of labile lipids, e.g. polyunsaturated compounds, are inevitable during either vessel use and/or long-term burial. The mode and extent

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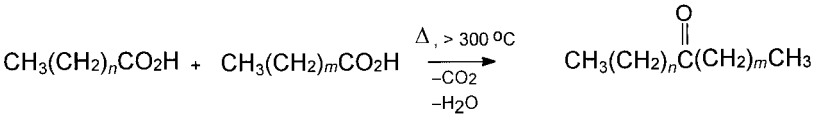

Figure 3. Ketonic decarboxylation leading to the formation of 2° ketones by self- and cross-head-to-head condensation of fatty acids. The reaction is catalysed by metal oxides and proceeds at temperatures in excess of 300 °C. The subcripts *n* and *m* correspond to alkyl chain lengths in the range 13–16 (modified from Evershed *et al.* (1995*c*)).

of alteration of either specific lipid components or their distributions will depend on both the properties of the compounds themselves, e.g. nature and number of functional groups and molecular weights, and the burial environment, e.g. sealed deposits versus exposed surface finds. There are no substantive field data available as yet to allow predictions to be made concerning the likelihood for survival of different lipid types under varying burial conditions. However, we have obtained evidence from laboratory degradation experiments which documents the more rapid decay of lipids under oxic as compared to anoxic conditions (Evershed *et al.* 1995*b*; Evershed & Charters 1995; Charters 1996). Moreover, we have also shown that the inherently more refractory plant epicuticular leaf wax lipids degrade more rapidly than the triacylglycerols with which they frequently co-occur in archaeological pottery (Charters 1996). The post-burial degradation of animal fats is discussed in more detail below.

Changes in lipid distributions occurring as a result of post-burial alteration may be sufficiently extreme as to confound efforts to identify commodities associated with ancient vessels. For example, we have been involved in examining a number of different vessels from Classical and ancient Greek sites for the presence of beeswax. One study (Evershed *et al.* 1997*a*) involved ceramic vessels used as lamps during the Neopalatial period at the settlement of Mochlos on the north coast of east Crete. Lipid analyses were performed with a view to determining the nature of the fuel used. GC analyses showed that 9 out of the 14 lamps studied contained only trace amounts of lipid. However, the remaining five sherds contained variable amounts of lipid (up to *ca.* 700 $\mu g\,g^{-1}$) comprising mixtures of wax esters, long-chain alcohols and *n*-alkanes. Compounds of this type occur widely as components of plant epicuticular waxes and insect waxes, notably beeswax. The mixture of compounds observed in one lamp bore a strong resemblance to beeswax, especially with respect to the distribution of long-chain wax esters (figure 4); the *n*-alkane components characteristic of beeswax were present but their distribution was somewhat different to that normally seen for fresh beeswax (analogous to 100-year-old beeswax shown in figure 4*a*). Free long-chain fatty alcohols were present, with a carbon number distribution that indicated they originated from the long-chain wax esters co-occurring in the extract. Although a similar range of compounds was seen in the remaining four lamps, the variations in their carbon number distributions indicated that either degradation had occurred during use or burial or that they originated from some other natural source, possibly plants.

Verification of the origin of these altered beeswax residues was achieved by use of GC/C-IRMS to determine the $\delta^{13}C$ values of individual components of the lipid extracts. Close similarities were found to exist in the $\delta^{13}C$ values of the long-chain alcohols and palmitic acid moieties derived from the wax esters of the lamp and conical cup extracts and of the reference beeswax, confirming that the lamp lipids derive from beeswax (figure 4). The identification of beeswax as an illuminant burned in prehistoric Aegean lamps is surprising and significant in view of the persistent conventional wisdom that olive oil was the fuel used in lamps. However, the analytical data is compelling, and the desirable burning properties of beeswax are well recognized (Crane 1983) and were being exploited by the inhabitants of Late Minoan Crete. Compound-specific stable isotope values for the individual lipids, derived through GC/C-IRMS, has provided a secure means of distinguishing between multiple putative sources.

(c) *Identification of animal fats*

GC/MS analyses have shown the incidence of degraded animal fats in ancient domestic pottery vessels is very high, with >40% of all sherds studied yielding appreciable lipid residues (Charters *et al.* 1993*b*; Evershed *et al.* 1992, 1995, 1997*b*). While both ourselves and others have had no problem in detecting degraded animal fats, identifying the origin of the fats or specifying whether they are mixtures of fats is much more difficult. Indications that the origins of fats may be classified using subtle differences in the properties of preserved fatty acids, including their $\delta^{13}C$ values, came during an investigation of two types of English medieval vessels, classified as lamps and 'dripping dishes' (Evershed *et al.* 1997*b*). Previous analyses of examples of such vessels from other excavations had consistently shown them to contain appreciable quantities (10^2 to 10^3 µg of lipid g^{-1} 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 putative use as receptacles for fat collection from carcasses during spit-roasting (McCarthy & Brooks 1988). The recovery of sherds from a number of vessels of each type from a single site, and from similar burial contexts, provided the opportunity for direct comparison of their lipid content.

Our initial aim was to determine whether or not compound-specific stable isotope analyses of individual compounds by GC/C-IRMS could be used as a basis for classifying the origins of preserved fats. Indeed, the results obtained from the GC/C-IRMS analysis of the fatty acid (as their methyl ester derivatives) components of the vessel showed that the $\delta^{13}C$ values of the major *n*-alkanoic acids correlated directly 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 5; see also fig. 2*c* in Evershed *et al.* (1997*c*)). Significantly, the $\delta^{13}C$ values correlated with those of the fats of contemporary animals considered to be the major domesticated species in

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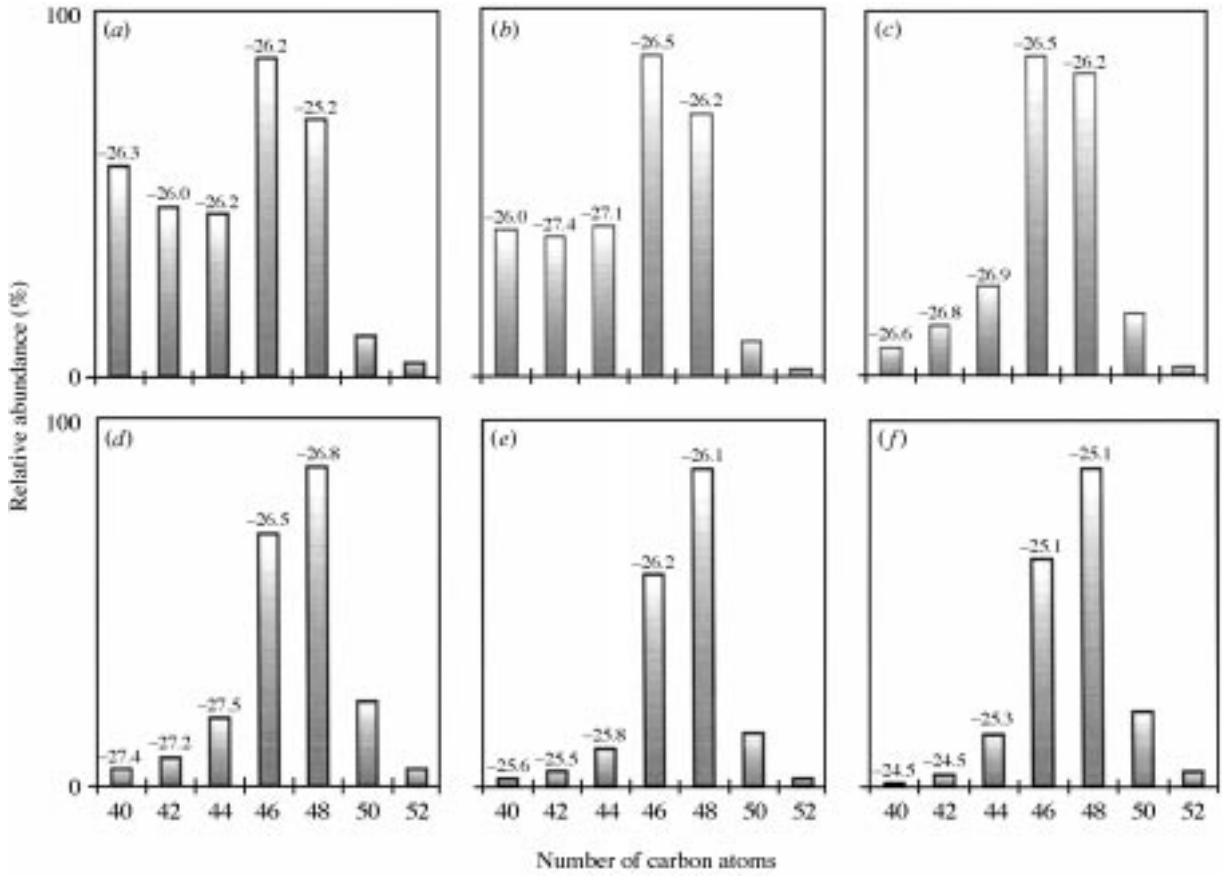

Figure 4. Histograms showing the carbon number distributions of the palmitic acid ($C_{16:0}$) wax esters of 100-year-old beeswax from Crete (*a*), and those of the total lipid extracts of lamps (*b–f*) from the Minoan site of Mochlos, eastern Crete. While the distribution in lamp (*b*) is clearly recognizable as that of beeswax, the identification is less secure for (*c–f*) without reference to the $\delta^{13}C$ values of the long-chain alcohol moieties (negative value given above the peaks). The departures from the distribution of the reference beeswax (*a*) is presumed to be the result of preferential diagenetic loss of the shorter-chain components. The $\delta^{13}C$ values of the long-chain alcohol moieties remove any possibility that these components derived from the leaf waxes of C_3 plants (modified from Evershed *et al.* (1997*a*)).

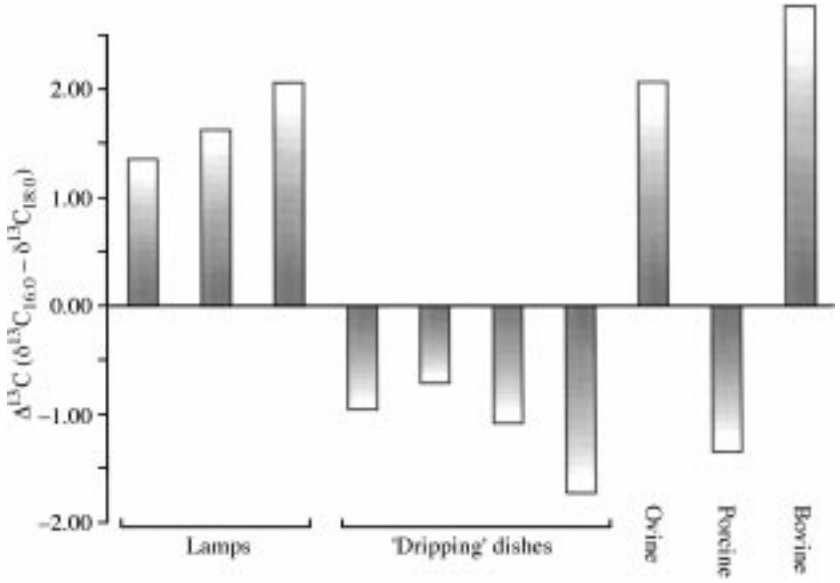

Figure 5. The differences ($\Delta^{13}C$ ‰) between the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids isolated from three medieval lamps and four 'dripping dishes', compared with those of reference ovine, bovine and porcine fats (each value is the mean of three determinations; modified from Evershed *et al.* (1997*c*) and Mottram *et al.* (1998)).

the medieval period in the UK. These preliminary findings suggested that the lipids preserved in the 'dripping dishes' are derived from monogastric animals, such as pigs, while those from the lamps are derived from ruminant animals such as sheep and cattle (Evershed *et al.* 1997*c*).

The ruminant (ovine and bovine) and non-ruminant (porcine) animals which provided the reference fats were reared on C_3 diets of grasses or cereals with mean bulk $\delta^{13}C$ values of −29.3 and −24.4‰, respectively. Hence, the differences in the isotopic composition of the $C_{16:0}$ and $C_{18:0}$ *n*-alkanoic acid components of the body fats and total lipid extracts of the archaeological pottery reflect the fundamental differences between the diets, metabolisms and physiologies of the different classes of modern and ancient

domesticated animals (DeNiro & Epstein 1978; Koch *et al.* 1994). None of the other modern reference fats (chicken, horse and deer) displayed δ^{13}C values consistent with those obtained from the 'dripping dishes' (S. N. Dudd and R. P. Evershed, unpublished data).

Consideration was also given to the overall structures and distributions of the fatty acids in the two vessel types to find data to support the distinction drawn on the basis of the stable isotope measurements. For example, GC 'fingerprints' of the methyl esters of the alkanoic and alkenoic acids showed the two vessel types also to be clearly separable on the basis of these distributions, 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. While the high saturated alkanoic acid content of both vessel types confirmed an animal source (Enser 1991), 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 $C_{19:0}$. In addition, GC/MS analysis of the dimethyl disulphide 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 (Enser 1991). In marked contrast, the fats of monogastric animals, such as pigs, contain only a single isomer, $\mathcal{Z}$-9-octadecenoic acid, a finding which is entirely consistent with the isotopic evidence for the origin of the lipid in the 'dripping dishes'. On the basis of these studies it was therefore possible for the first time to suggest species-specific functional interpretations for vessel use. The pronounced differences in the δ^{13}C values of the individual fatty acids obtained by GC/C-IRMS are entirely consistent with the differences detected in the structures and distributions of the alkanoic and alkenoic acids revealed by GC and GC/MS analysis of the lipid extracts of the archaeological vessels and modern reference fats. The high content of saturated *n*-alkanoic acids, the presence of a mixture of positional isomers of monounsaturated alkenoic acids and branched-chain components clearly excludes vegetable oils as the potential source(s) of lipid in the ancient lamps. Earlier work showed the promise of bulk isotope analyses for the study of carbonized surface residues in archaeological pottery (Hastorf & DeNiro 1985). However, the evidence presented above clearly demonstrates the value of applying compound-specific stable isotope measurements to absorbed lipids, thereby further enhancing the value of ancient pottery as a source of archaeological information. We have confirmed the robustness of the δ^{13}C values of the individual fatty acids by investigating the lipids remaining at the end of laboratory degradation experiments involving fats dosed into modern potsherds which were then incubated in damp mushroom compost (dark at 45 °C, either sealed under N_2 (anoxic) or with air diffusion or purging (oxic); Evershed *et al.* 1995*b*; Charters 1996). Table 1 shows the δ^{13}C values of the major saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) of ovine fat before and after laboratory degradation under oxic and anoxic conditions for 100 days. The results clearly show that the δ^{13}C values are highly robust, being essentially unchanged within the precision of the measurements. The results obtained for the degradation performed under oxic conditions are especially noteworthy, since >90% of the original fat dosed into the experimental sherd had been hydrolysed to free fatty acids and then consumed by micro-organisms during the course of the experiment.

Table 1. *δ^{13}C values obtained for the major fatty acids present in ovine adipose fat before and after degradation under oxic and anoxic conditions for* 100 *days at* 45 °C

incubation time T_{days} (incubation conditions)	δ^{13}C values (‰)			
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$
T_0 (pre-incubation)	−28.4	−29.8	−31.8	−30.4
T_{100} (oxic)	−28.1	−29.5	−31.9	−30.1
T_{100} (anoxic)	−28.8	−29.5	−32.3	−31.0

This approach has recently been applied to the classification of fats in larger assemblages of domestic pottery from archaeological sites. Figure 6 is a plot of the stable isotope values of the major saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) in 30 vessels from the Late Saxon/early medieval site of West Cotton, Northamptonshire, UK, together with the values for the corresponding reference fats from present-day farm animals reared on known diets, i.e. similar pastures from the same farm for all the ruminant animals and cereals for the pigs. The data show that both ruminant and non-ruminant fats are present in the archaeological vessels with the mixing of fats being indicated by the points following the mixing line drawn between the mean 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 (Evershed *et al.* 1997*b*; Dudd & Evershed 1998*b*; Dudd *et al.* 1999).

(d) *Dairying in prehistory*

One of the major categories of fat that we should encounter in pottery vessels is that derived from milk, i.e. butter fat. Just as with the adipose fats, the processing of milk, e.g. pasteurizing, or cooking involving milk or butter, would result in the absorption of appreciable quantities of fat into the walls of unglazed pottery vessels. Significantly, milk fats differ from adipose fats in their fatty acid composition through the presence of short-chain saturated fatty acids in the C_4 to C_{14} carbon number range (McDonald *et al.* 1988). However, while it is known that dairying was widely practiced in the Roman and later periods we have consistently failed to detect fatty residues containing these characteristic shorter-chain fatty acids. There are two possible explanations for this: (i) that dairy products were not processed

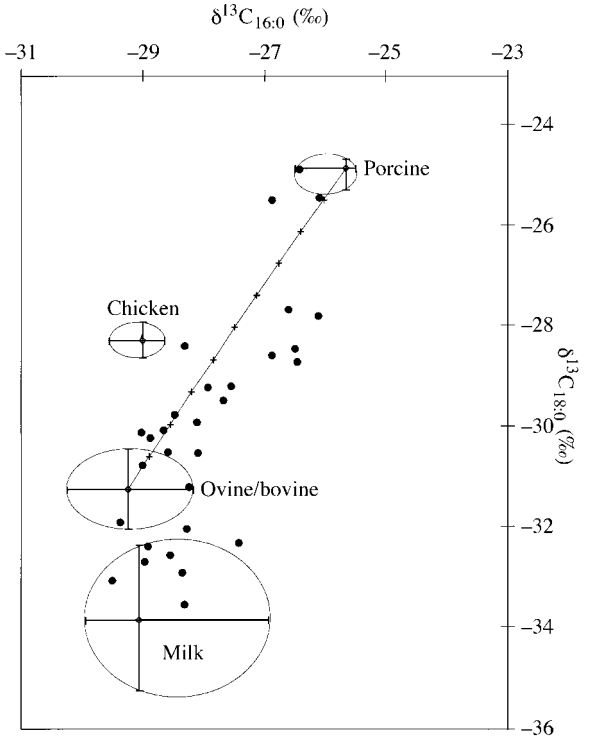

Figure 6. Plot of the $\delta^{13}C$ values of the major *n*-alkanoic acid components ($C_{16:0}$ and $C_{18:0}$) from the lipid extracts of potsherds from the Late Saxon/early medieval site of West Cotton, Northamptonshire, UK. The ringed fields encompass the ranges for present-day reference animal fats with the range bars crossing at the arithmetic mean. A significant number of the archaeological fats cluster near to the reference ruminant adipose and milk fats (bovine and ovine) with fewer plotting in the region of the porcine and chicken adipose fats. The mixing curves have been calculated (Woodbury *et al*. 1995) to illustrate the $\delta^{13}C$ values which would result from the mixing of a theoretical average ruminant adipose fat (based on the mean fatty acid composition of ovine and bovine adipose fats) with average porcine fat. The points adjacent to the mixing line are presumed to be those of vessels used to process mixtures of ruminant and non-ruminant animal products. The more depleted $\delta^{13}C$ values for the $C_{18:0}$ fatty acid lying within the field for the milk fats arises through routing of depleted dietary fatty acids (following biohydrogenation) in milk production (see main text for further explanation, modified from Dudd & Evershed 1998*b*).

to any significant extent in pottery vessels, and (ii) that while dairy fats were present in vessels at the time of discard and burial they become altered, through decay, in such a way as to make them indistinguishable from adipose fats.

Indeed, it can be argued that the short-chain, fatty acyl moieties are more susceptible to hydrolysis, due to reduced steric effects at ester linkages in triacylglycerols compared with their long-chain counterparts (Laakso (1996) and references therein). Furthermore, once released from triacylglycerols by hydrolysis the short-chain fatty acids are appreciably more water soluble than their long-chain counterparts. Significantly, there is an *ca.* tenfold decrease in solubility for each added methylene group. These two factors alone are probably sufficient to explain our failure to observe milk fats in ancient pottery vessels. We have tested this hypothesis in laboratory degradation experiments and shown that the milk fats absorbed in pottery vessels rapidly hydrolyse with preferential decay ('loss') of their short-chain fatty acid moieties to produce a distribution of fatty acids, dominated by $C_{16:0}$ and $C_{18:0}$, reminiscent of adipose fat (Dudd *et al*. 1998; Dudd & Evershed 1998*b*). Thus, it is becoming apparent that the failure by ourselves and others (e.g. Rottländer 1990) to detect appreciable numbers of dairy fats in pottery vessels could be the direct result of diagenetic alteration of milk lipids leading to a distribution of fatty acids resembling that of degraded adipose fats. Hence, while the fats preserved in archaeological pottery in principle offer an excellent source of information concerning the exploitation of dairy products by early farmers, our ability to recognize them through chemical analysis has up to now been thwarted on account of diagenetic alteration.

Figure 6 shows that a significant number of the vessels studied contain 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 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 acid components was milk (Christie 1981; Byers & Schelling 1988; Dudd & Evershed 1998*b*). The distinctive trends seen in figure 6 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). There is enhanced routing of dietary fatty acids during lactation (Christie 1981; Byers & Schelling 1988) resulting 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.

The ability to distinguish between milk and adipose fats using the $\delta^{13}C$ values of their $C_{16:0}$ and $C_{18:0}$ fatty acids represents an elegant application of GC/C-IRMS. Previously, the routing of different carbon pools from the diet to the body tissues could only have been undertaken in living animals by use of radiolabelling techniques. However, the use of stable isotope measurements of individual compounds at natural abundance levels directly reveals this routing phenomenon. Thus, we have established a new means of recognizing milk fats in archaeological pottery which provides for the first time, to our knowledge, a potentially reliable means of recognizing dairying as a component of prehistoric economies (Dudd & Evershed 1998*b*).

4. CONCLUSIONS

The compound-specific stable isotope approach represents a significant development from the earlier studies that used bulk stable isotope measurements to determine the sources of organic residues (Hastorf & DeNiro 1985; Sherriff *et al*. 1995). Bulk stable isotope studies of carbon (e.g. Macko, this issue) allow broad distinctions to be drawn between isotopically distinct marine C_3 and C_4 resources. Compound-specific stable isotope measurements

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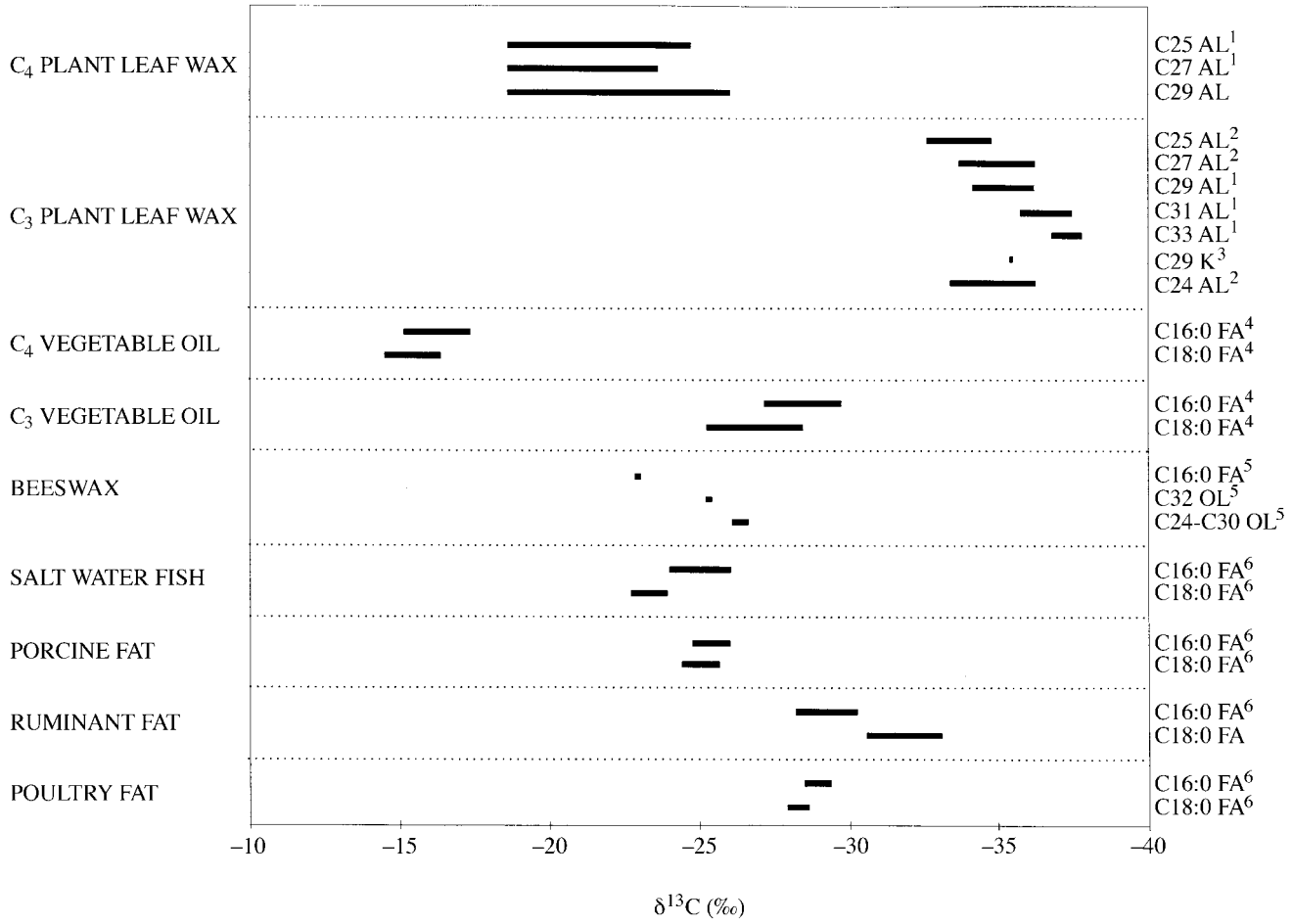

Figure 7. Summary of $\delta^{13}C$ values of individual lipids determined by GC/C-IRMS in various plant and animal products of archaeological importance. AL, *n*-alkane; FA, fatty acid; OL, long-chain alcohol; K, 2° long-chain ketone. The numbers refer to the carbon numbers of the various compounds and $C_{16:0}$ and $C_{18:0}$ are saturated C_{16} and C_{18} fatty acids. [1]Rieley *et al*. 1993; Collister *et al*. 1994; [2]Lockheart *et al*. 1997; [3]Evershed *et al*. 1994; [4]Woodbury *et al*. 1998*a*,*b*; [5]Evershed *et al*. 1997; Dudd & Evershed 1998*b*; [6]S. N. Dudd, unpublished data.

of archaeological materials provide a powerful complement to the traditional compositional information based on structures and distributions. Figure 7 summarizes the compound-specific stable isotope values of various lipid components of archaeological interest. Significantly, structurally similar lipids can vary quite substantially in their stable isotope composition depending on the source organism. For example, long-chain ketones from heated animal fats and plant leaf waxes differ by 10‰, and even with changing physiological status of the same organism, the stearic acid component of adipose and milk fats of ruminant animals differ by up to 6‰. Such variations are readily revealed in individual compounds by use of the GC/C-IRMS technique, for which precisions of ± 0.3‰ are routinely achieved. Since nitrogen-containing lipids are rather uncommon, compound-specific measurements of nitrogen will inevitably be largely restricted to proteinaceous materials of archaeological interest (Simpson *et al*. 1997). However, the possibility now exists for performing $\delta^{18}O$ measurements on individual oxygen-containing lipids, e.g. fatty acids, thus opening up a number of new avenues of application directly relevant to archaeology, e.g. palaeoenvironmental reconstruction.

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Discussion

M. Jones (*University of Cambridge, UK*). Would you expect the use of dung or other types of organic matter as temper in pottery to affect (i) diagenesis, dung being acid, or (ii) the signal obtained from pottery through the introduction of other organic compounds.

R. Evershed. The effects of organic tempers on the lipid signals we obtain from archaeological pottery have not been studied in any systematic way. Overall, I would expect little effect in terms of residual contamination since the temperatures attained during the firing of even low-temperature fired prehistoric pottery will very extensively degrade any lipid, or indeed other organic compounds, present in an organic temper. It is generally accepted that a temperature of at least 600 °C would have been required to produce archaeological pottery that will survive burial, and at such temperatures all compounds of the classes we are using in our work will be degraded beyond meaningful recognition. The question of the effects of organic temper on diagenesis is also an interesting one. I am not sure whether the acidity of the dung will be so much of a factor as the residual carbon that is seen extensively in thin section analyses of prehistoric pottery. This particulate carbon is believed to derive from degraded organic temper or organic matter endogenous to many clays and may provide active sites for the adsorption of organic matter introduced during vessel use, thereby enhancing the possibilities for preservation of organic components during burial.

J. Bada (*University of California, USA*). In the sheep, cow and pig fat reference samples, pigs have a δ^{13}C value which is heavier than the other two. Is this because the pigs are being fed some C_4 plant material?

R. Evershed. No, all the pig reference fats came from animals that had never eaten any C_4 plant material. In fact, one of the most time-consuming aspects of this work is obtaining archaeologically meaningful reference materials for stable isotope analysis. We essentially live in a stable isotopically 'polluted' environment with many wild and domesticated animals having isotopically anomalous diets due to them consuming plants, e.g. maize, or supplements, e.g. fish oils or meals, or cane sugar, which would not have been available in prehistoric Britain or other parts of Europe. Two options exist for obtaining reliable reference materials: (i) by raising animals on strictly controlled diets; or (ii) obtaining materials from animals raised on farms where unnatural (in archaeological terms) plants, supplements and concentrates are never used; we use both approaches. A further refinement required in this stable carbon isotope work results from the lighter values obtained from modern animals and plants due to the effect on the isotope composition of atmospheric carbon dioxide of burning fossil fuels; in view of this we apply a correction factor to all our modern reference materials in order to bring them as close as possible to anticipated pre-industrial revolution values.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES