Organic chemistry of balms used in the preparation of pharaonic meat mummies

Katherine A. Clark[a], Salima Ikram[b], and Richard P. Evershed[a,1]

[a]Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom; and [b]Department of Egyptology, American University in Cairo, New Cairo 11835, Egypt

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The funeral preparations for ancient Egyptian dead were extensive. Tomb walls were often elaborately painted and inscribed with scenes and objects deemed desirable for the afterlife. Votive objects, furniture, clothing, jewelry, and importantly, food including bread, cereals, fruit, jars of wine, beer, oil, meat, and poultry were included in the burial goods. An intriguing feature of the meat and poultry produced for the deceased from the highest levels of Egyptian society was that they were mummified to ensure their preservation. However, little is known about the way they were prepared, such as whether balms were used, and if they were used, how they compared with those applied to human and animal mummies? We present herein the results of lipid biomarker and stable carbon isotope investigations of tissues, bandaging, and organic balms associated with a variety of meat mummies that reveal that treatments ranged from simple desiccation and wrapping in bandages to, in the case of the tomb of Yuya and Tjuia (18th Dynasty, 1386–1349 BC), a balm associated with a beef rib mummy containing a high abundance of *Pistacia* resin and, thus, more sophisticated than the balms found on many contemporaneous human mummies.

food mummies | pharaohs | Egypt | triterpenoids | fatty acyl lipids

Food to sustain the deceased in the afterlife was perhaps the most important item in a burial and has been found in interments from the earliest periods (3300 BC) to the latest (fourth century AD). The burial of King Tutankhamun (died c. 1323 BC) comprised 48 carved wooden cases containing a variety of joints from cattle and poultry (1, 2). Virtually all of the food found in these tombs was preserved through dehydration, save for the meat, as untreated meat would not last more than a few hours in the Egyptian heat. A solution to preserving meat in the burials would have been to preserve it in the same manner as human mummies. Indeed, "meat" or "victual" mummies have been found in many high status tombs (3). Meat mummies are but one type of animal mummy produced by the Egyptians, the others including votives, pets to be left in the tomb with their owner, and sacred animals, such as the Apis bull (1, 4, 5).

Hundreds of examples of meat mummies are known from ancient Egypt (see appendix II in ref. 1). Until recently, these had been neglected as objects of study and as a consequence are poorly understood. Recent investigations have established that for the most part victual mummies are joints of meat or poultry prepared as if for eating, which are wrapped, encoffined, and placed in the tomb (1, 4, 5). SEM has shown that salt and natron were used for desiccation (1, 5). The question that remains is whether organic balms were applied? The presence of dark residues is consistent with the appearance of balms applied to human and animal mummies (6–12). The Cairo Museum and British Museum graciously provided us with samples of tissues and balms from several meat mummies (Table 1), allowing us to assess their chemical compositions and make comparisons with the compositions of balms from ongoing investigations of human and animal mummies.

Materials and Methods

Chemical analyses were performed using the same methods for human and animal mummies (6, 7) to ensure detection of the range of balm components detected in earlier studies, namely beeswax, animal fats and plant oils, plant resins, petroleum bitumen, and essential oils (6–19).

Ground samples of balm were extracted with $CHCl_3$/MeOH [2:1 (vol/vol), 3×] using ultrasonication. The total lipid extracts (TLEs) were combined, and the solvent was removed under a gentle stream of N_2 at 40 °C. Extract yields were determined gravimetrically. Aliquots of the TLE were trimethylsilylated [*N,O*-bis(trimethylsilyl)trifluoroacetamide, 40 μL, 70 °C, 1 h] and submitted to analysis by GC and GC/MS to identify the major compounds present.

All TLEs were initially screened in a Hewlett-Packard 5890 Series II GC equipped with a fused-silica capillary column (15 m × 0.32 mm) coated with dimethyl polysiloxane stationary phase (DB-1; film thickness, 0.1 μm). Derivatized extracts and fractions (1.0 μL) were injected on-column. The temperature was held isothermally for 2 min at 50 °C and then increased at a rate of 10 °C·min^{-1} and held at 350 °C for 10 min. The flame ionization detector (FID) was set at a temperature of 350 °C. Hydrogen was used as a carrier gas and maintained at a head pressure of 10 psi.

GC/MS analyses of TLEs were performed using a TSQ GC/MS with an on-column injector. The MS was set to scan in the range of *m/z* 50–850 in a total time of 1.5 s. The MS was operated with an electron ionization potential of 70 eV. The GC column was a fused silica capillary coated with DB-1 (15 m × 0.32 mm × 0.1 μm), and the operating conditions were a start temperature of 50 °C, held isothermally for 2 min, followed by an increase to 350 °C at a rate of 10 °C·min^{-1}, and held isothermally for 20 min. Helium was used as a carrier gas, the electron energy was maintained at 300 μA, the ion source temperature was 200 °C, and the GC/MS interface was maintained at a temperature of 350 °C. Data were acquired and processed using an Xcalibur data system, and peak identifications were aided by a National Institute of Standards and Technology database.

For sterane and hopane petroleum biomarker analyses, the extracts were separated into acid and neutral fractions using bonded aminopropyl solid-phase extraction cartridges (100 mg; Varian), with the neutral fraction collected by elution with CH_2Cl_2/propan-2-ol [2:1 (vol/vol); 3 mL]. The neutral fraction was further separated using a column of activated silica gel following elution with hexane to give a saturated hydrocarbon fraction. All of the saturated hydrocarbon factions were submitted to GC/MS using a Finnigan Trace instrument (Finnigan MAT GmbH) equipped with an on-column injector. For selected ion monitoring (SIM), the MS was set to monitor *m/z* 191 and 217. The GC column was a fused silica capillary coated with CPSIL-5

Significance

This unique research on the chemical composition of organic balms of food mummies completes the trilogy of mummy types known from Ancient Egypt, complementing previous investigations of human and animal mummies. Our findings show that the Ancient Egyptians prepared the food offerings they made to their dead using preservation techniques at least as exotic as those used in embalming human and animal mummies. The discovery of the precious *Pistacia* resin on a beef rib mummy is especially noteworthy because the use of this substance is rare even in human mummies.

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[1]To whom correspondence should be addressed. E-mail: r.p.evershed@bristol.ac.uk.

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Table 1. Meat mummies investigated and $\delta^{13}C$ values recorded for fatty acid components of balms

Mummy	Museum number	Date	Provenance	Sample description	$\delta^{13}C_{16:0}$	$\delta^{13}C_{18:0}$
Beef ribs from the tomb of Yuya and Tjuiu	CG5109 (Cairo Museum)	1386–1349 BC	Thebes	Stained bandaging	−23.3	−25.0
Calf victual mummy of Isetemkheb D	CG29852 (Cairo Museum)	1064–948 BC	Thebes	Bandaging	ND	ND
Duck from food box from tomb of Henutmehyt	EA51812 (British Museum)	c.1290 BC	Thebes	Tissue (skin)	ND	ND
Goat(?) from food box from tomb of Henutmehyt	EA51812 (British Museum)	c.1290 BC	Thebes	Tissue (skin)	−16.0	−16.8

ND, not determined.

(60 m × 0.32 mm × 0.1 μm; dimethyl polysiloxane equivalent) and the operating conditions were 50–130 °C at 20 °C·min^{-1}, to 300 °C (held 30 min) at 4 °C·min^{-1}. He was used as carrier gas, the electron emission current was 300 μA, the ion source temperature was 170 °C, and the GC/MS interface was maintained at 350 °C. The electron ionization potential was 70 eV. The sterane and hopane fractions of the balms and tissues were determined using coinjected standards to provide a basis for assessing the presence or absence of bitumen in the mummy balms and semiquantitative estimates of biomarker concentrations.

The compound-specific $\delta^{13}C$ values were determined by GC combustion isotope ratio MS (GC-C-IRMS) (20–22) by taking further aliquots of the TLE treated with NaOH/H_2O [9:1 (wt/vol)] in methanol [5% (vol/vol), 70 °C, 1 h]. Following neutralization, lipids were extracted into chloroform (3 × 3 mL), and excess solvent was evaporated under a gentle stream of nitrogen. Fatty acid methyl esters (FAMEs) were prepared by reaction with BF_3-methanol [14% (wt/vol), 70 °C, 1 h]. FAMEs were extracted with chloroform (3 × 2 mL), and the solvent was removed with a gentle stream of nitrogen. FAMEs were then redissolved into hexane for analysis by GC-C-IRMS using a Varian 3400 GC coupled to a Finnigan MAT Delta-S IRMS via a modified Finnigan MAT combustion interface: Cu and Pt wires (0.1-mm outer diameter) in an alumina reactor (0.5-mm inner diameter). The reactor temperature was maintained at 860 °C, the MS ion source pressure was 6×10^{-6} mbar, and faraday cups were used for the detection of ions of mass 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$), and 46 ($^{12}C^{18}O^{16}O$). Sample injections were performed using a septum-equipped programmable (SPI) injector. The GC column was a fused silica capillary column (50 m × 0.32 inner diameter) coated with a dimethyl polysiloxane stationary phase (CP Sil-5-CB, 0.12-μm film thickness). The temperature program consisted of a 1-min isothermal period at 50 °C followed by an increase to 300 °C at 10 °C·min^{-1}, and finally an isothermal period of 10 min. By convention, stable carbon isotope ratios are reported as $\delta^{13}C$ values and are expressed relative to the VPDB (Vienna Pee Dee Belemnite), $\delta^{13}C$ (‰) = 1,000 $[(R_{sample} - R_{standard})/R_{standard}]$, where R is the $^{13}C/^{12}C$ ratio. Analytical error was ±0.3‰.

In addition to running each of the samples in duplicate, the integrity of the data was assured through the regular analysis of a laboratory standard mix ($C_{11:0}$, $C_{13:0}$, $C_{16:0}$, $C_{21:0}$, and $C_{23:0}$ FAMEs) of known isotopic composition. Results were calibrated against a reference CO_2 standard, which was injected directly into the ion source eight times (four times at the beginning and four at the end of the run). $\delta^{13}C$ values for the individual fatty acids were obtained from their corresponding FAMEs by correcting for the derivatizing carbon using a mass balance calculation.

Results and Discussions

Bandaging taken from a victual calf mummy dating to the 21st Dynasty from the funerary goods of Isetemkheb D CG 29857 (c. 1070–945 BC) was found to contain a mixture of long-chain fatty acids, diacids, and dihydroxyacids, of which the latter oxygenated derivatives are known to form through oxidation of unsaturated fatty acids (23, 24). The ratio of the abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids of 0.92 suggests an animal fat origin. These compounds were present in the external bandages that were not in contact with the meat itself, making it likely that they derive through deliberate application of a balm rather than originating from the meat. No other components were detected, indicating that waxes or resins were not applied to this meat mummy. GC/MS SIM of the hydrocarbon fraction failed to provide any evidence of the sterane and terpane biomarkers characteristic of petroleum bitumen (10). A similar range of diacids and fatty acids was found on the mummified tissue of the goat leg from the food box of Henutmehyt (c. 1290 BC) (EA 51812); however, due to the nature of this sample, it was impossible to know whether these components derived through deliberate application of a balm or from the meat itself. The $\Delta^{13}C$ value ($\delta^{13}C_{16:0}$–$\delta^{13}C_{18:0}$) of −1.1‰ points to a ruminant carcass fat origin (22). The $\delta^{13}C$ value of the $C_{16:0}$ fatty acid of –15.9‰ indicates a strong C_4 plant contribution to the diet of the animal from which this fat derived. A sample of duck tissue (EA 51812) from the same food box contained no detectable lipid.

Analysis of the balm taken from a victual mummy consisting of beef ribs from the tomb of Yuya and Tjuiu (CG 51098) (Fig. 1) revealed an entirely different mixture of components indicating the application of an elaborate balm. The balm was found to contain a mixture of fat/oil and *Pistacia* resin (Fig. 2). The fat/oil is characterized by the presence of abundant long chain fatty acids $C_{16:0}$ and $C_{18:0}$, which indicates the excellent preservation typical of many Egyptian mummies. The high abundance of $C_{18:0}$ suggests the origin of the fatty acids is most likely to be an animal fat rather than a plant oil. Again the $\Delta^{13}C$ value (–1.5‰) lies in the range for ruminant carcass fat, although the $\delta^{13}C$ value for

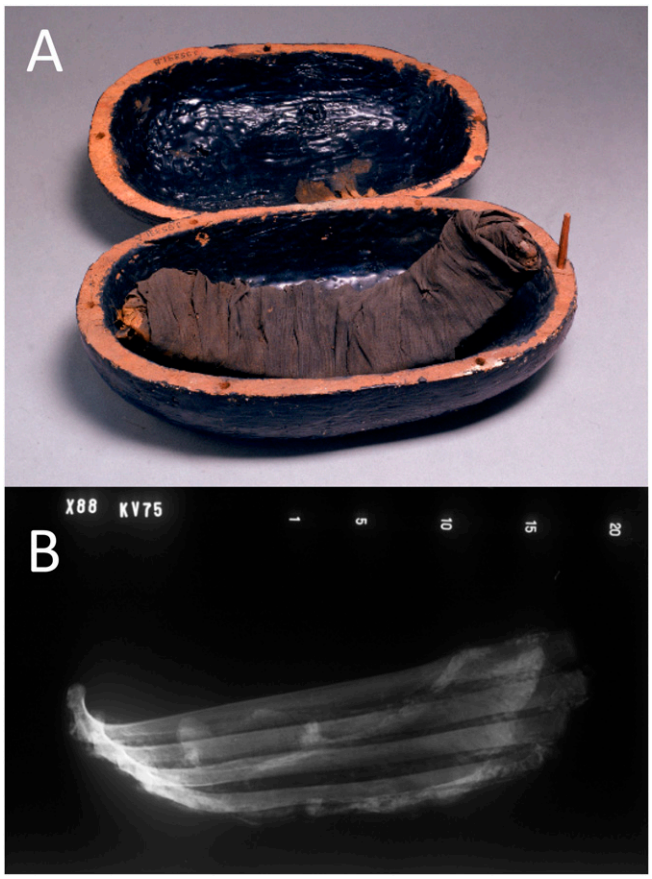

Fig. 1. Beef rib meat mummy from the tomb of Yuya and Tjuiu (1386–1349 BC). Photographic (*A*) and X-ray (*B*) images. Photo by Anna-Marie Kellen, courtesy Egyptian Museum, Cairo.

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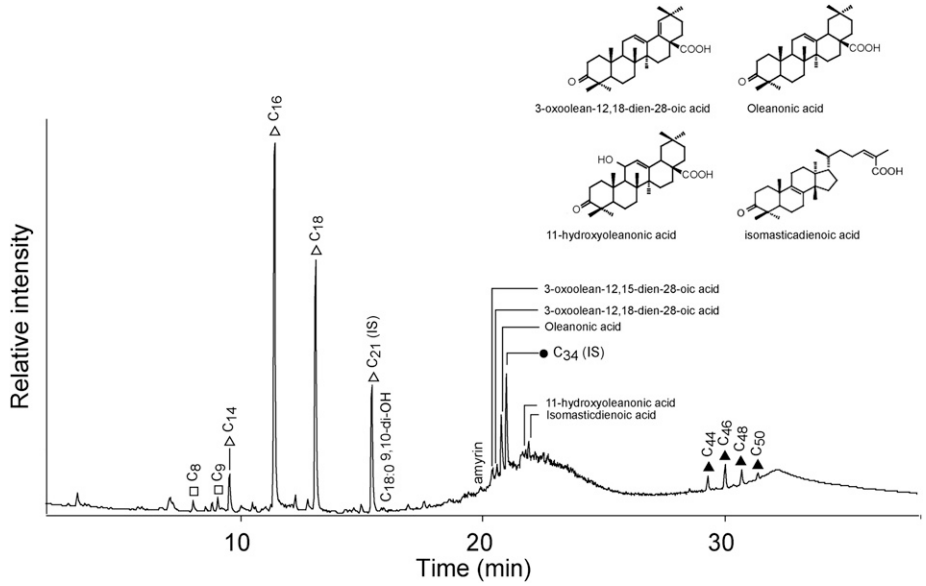

Fig. 2. Partial high temperature gas chromatogram of the trimethylsilylated extract of blackened bandaging of the beef ribs meat mummy from the tomb of Yuya and Tjuiu (1386–1349 BC). Peak identities (x indicates carbon chain length): open squares, C_x indicates α,ω-dicarboxylic acids; open triangles, C_x indicates saturated fatty acids; filled circles, C_x indicates *n*-alkane; filled triangles, C_x indicates wax esters of palmitic acid containing 44–50 carbon atoms. $C_{18:0}$ 9,10-di-OH is 9,10-dihydroxyoctadecanoic acid. Labelled peaks eluting between 20 and 22 min and inset structures: 3-oxoolean-12,18-dien-28-oic acid, 3-oxoolean-12,15-dien-28-oic acid, oleanoic acid, 11-hydroxyoleanonic acid, isomasticadienoic acid, are triterpenoid biomarkers of *Pistacia* resin. I.S., internal standards (*n*-heneicosanoic acid and *n*-tetratriacontane).

$C_{16:0}$ (−23.4‰) indicates a markedly different origin for this fat to that derived from the bandaging associated with the goat leg mummy. As above, the balm analyzed derives from the bandaging not in direct contact with the meat; hence, these fatty acids are most likely the result of the deliberate addition of a fat/oil to the balm treated wrappings. The presence of low concentrations of C_{48} to C_{50} palmitate wax esters point to the possible presence of beeswax in the balm, although hydroxy palmitate wax esters were not detectable probably due to the low overall abundance of wax in the balm.

A high abundance of *Pistacia* resin in the balm was indicated by the presence of the triterpenoids oleanonic and isomasticadienoic acid and a range of oxidation products, including 11-hydroxyoleanonic acid, 3-oxoolean-12,18-dien-28-oic acid and 3-oxoolean-12,15-dien-28-oic acid. The presence of an unresolved complex mixture (a 'hump') around the retention time of the triterpenoids suggested there may have been a bitumen component of this balm; however, SIM analyses of the saturated hydrocarbon fraction failed to detect any of the characteristic bitumen biomarkers, i.e., steranes and triterpanes (10). Interestingly, such humps have also been observed in GC analyses of *Pistacia* resins from Canaanite jars from Amarna (25). Such humps can also arise through heating. However, this *Pistacia* resin does not appear to have been subjected to strong heating, given the absence of the biomarker 28-norolean-17-en-3-one (8) and significant components in the *m/z* 453 mass chromatogram (25).

Assigning the exact *Pistacia* species yielding archaeological resins is problematic. Four Mediterranean species of *Pistacia* (*P. atlantica, P. khinjuk, P. lenticus,* and *P. terebinthus*) have been regarded as possible sources (26); however, only two (*P. lenticus* and *P. terebinthus*) produce significant quantities of resin to make collection in appreciable quantities feasible. The resins from these trees are commonly referred to as Mastic Gum (*P. lenticus*) or Chios Turpentine (*P. terebinthus*). Living representatives of the trees are now restricted in their distribution to the island of Chios. Chemical analysis of the resins from *P. lenticus* and *P. terebinthus* has shown that their triperpenoid compositions are rather similar, differing only in the relative proportions of their various components (27, 28). Although there are subtle differences in their triterpenoid compositions that can be used to distinguish between the two species, such as the presence of 3,4-seco-28-norolean-12 (18)-en-3-oic acid, 18-αH-oleanonic acid, and dammaradienol in *P. terebinthus*, the use of these as criteria for the identification of aged (degraded) resins should be approached with much caution.

Despite being unable to assign the exact species origin for the *Pistacia* resin, its association with the beef rib meat mummy is highly significant. *Pistacia* resin has a long association with ancient Egypt, with a number of lines of evidence indicating that *Pistacia* resin was referred to as *senetjer* by the Egyptians (29). Resin was a valued commodity in ancient Egypt and regarded as a luxury item because it was imported and difficult to obtain (30). *Pistacia* resin was used in other Egyptian contexts, all related to royal or elite consumption. It was used as incense (25) in temple and funerary rituals, the idea being that gods were pleased when they inhaled sweet smoke, and it was also used as varnish on elite coffins (19, 31). Its use in mummification has often been suggested, although has rarely been observed. Thus, the presence of these *Pistacia* resin triterpenoid biomarkers in a mummy is very unusual, making the identification in a meat mummy especially noteworthy. Perhaps this material was used specifically for victual mummies as the *Pistacia* resin might have imparted a special and desirable flavor to the meat—certainly *P. lentiscus* is used in flavoring foods, particularly sea food, ice cream, and desserts, even now in the Mediterranean and Levant (32). The date of the occurrence of *Pistacia* resin associated with this meat mummy predates any known association with human mummies by some 600 y (6–9, 13, 18, 33), although this might change with further investigations of human mummies. The finding of *Pistacia* resin on this meat mummy likely relates to the status of the burial; this meat mummy was part of the funerary assemblage of the parents of Queen Tiye, wife of Amenhotep III (c. 1386–1349 BC) (34), making it among the highest status mummy balm thus far chemically analyzed in modern times.

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Conclusions

The results of this investigation indicate that meat mummies, as with human (7–12) and animal mummies (6), were subjected to a diverse range of treatments to ensure their preservation in ancient Egyptian tombs. The degree of complexity of preparing and provisioning a tomb depended on the tomb owner's wealth and access to luxury goods, which would clearly have related to the status of the burial (35). Our findings show that the sophistication of the burial extended not only to the organic embalming treatments applied to the bodies themselves but also to the foods, particularly the meats, interred with them.

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