Molecular analysis of black coatings and anointing fluids from ancient Egyptian coffins, mummy cases, and funerary objects

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Black organic coatings and ritual deposits on ancient Egyptian coffins and cartonnage cases are important and understudied sources of evidence about the rituals of funerary practice. Sometimes, the coatings were applied extensively over the surface of the coffin, resembling paint; in other cases, they were poured over the mummy case or wrapped body, presumably as part of a funerary ritual. For this study, multiple samples of black coatings and ritual liquids were taken from 20 Egyptian funerary items dating to a specific time period (c. 943 to 716 BC). Multiple sampling from each object enabled several comparisons to be made: the variability of the black coating within one application, the variability between two applications on one object, and the variability from object to object. All samples were analyzed for lipids using gas chromatography–mass spectrometry (GC-MS), and 51 samples from across the 20 items were further analyzed for the presence of bitumen using solid phase separation followed by selected ion monitoring GC-MS. The majority of the black substances were found to comprise a complex mixture of organic materials, including bitumen from the Dead Sea, conifer resin, and *Pistacia* resin, providing evidence for a continuation in international trade between Egypt and the eastern Mediterranean after the Late Bronze Age. Both the coating and the anointing liquid are very similar to mummification balms, pointing to parallels with Egyptian embalming rituals and raising questions about the practical aspects of Egyptian funerary practice.

archaeology | ancient Egypt | coffins | chromatography | mass spectrometry

To the ancient Egyptians, black symbolized the underworld and Osiris (often shown with black skin) and also night, especially when used in contrast with yellow and gold (for the sun) and the rich black soil of fertility and regeneration (1–3). The practice of using black coatings on Egyptian coffins is first attested in the Middle Kingdom (e.g., the cartonnage cases of Nepthys and Hapiankhtifi, Metropolitan Museum of Art 11.150.15 and 12.183.11c.1-0.2) and occurs in the New Kingdom, when black coatings are also used on funerary objects such as divine figures, shabtis, and shabti boxes (3–5). A secondary application of a black anointing liquid in a funerary context is known from at least as early as the burial of Tjuyu (18th Dynasty, c. 1375 BC), whose gilded funerary mask was anointed with black fluid (6).

The application of black substances as coatings and anointing liquids on coffins and mummy cases also occurs in the Third Intermediate Period c. 1086 to 664 BC. This period in Egypt was a time of decentralized rule. In its first phase (21st Dynasty c. 1086 to 943 BC), kings based in the eastern Delta ruled the northern part of Egypt, sharing power with a line of generals, who also acted as high priests of Amun at Thebes, and controlled the southern stretch of the Nile valley. A return to centralized administration in the early 22nd Dynasty (c. 943 to 716 BC) was followed by a gradual fragmentation of the country into smaller political units, some of which were ruled by individuals claiming kingly status, while others were controlled by rulers of Libyan chiefdoms (7), thus Dynasties 22 to 24 and the early 25th Dynasty overlap. The end of the Bronze Age had seen catastrophic events across the eastern Mediterranean, with the Mycenaean and Hittite states collapsing (8, 9). Egypt itself saw incursions and lost control of land in Syria Palestine. Evidence from Egypt and the Levant during this time suggests fluctuating levels of contact and influence (10).

During this period, burial practices were changing; instead of a decorated tomb and a wide array of tomb goods, the focus turned toward the body and the coffin (11, 12). The elite continued to lavish expenditure on their burials, but due to high levels of tomb raiding and tomb and coffin appropriation, emphasis was no longer placed on the funerary goods placed in the tomb, as was common in the New Kingdom (13). Instead, the expenditure was concentrated on the body and the coffin, which could be moved to a more secure location if necessary. The 21st Dynasty saw developments in mummification that indicate an increase in the resources allocated for those procedures (14). In the 22nd Dynasty, further changes in the treatment of the body were introduced, most notably the introduction of the cartonnage case, into which the mummy was tightly laced (3). Cartonnage is made with layers of linen, plaster, and glue, similar to papier maché, which can be molded to shape and which dries to make a hard case. These cases were valuable in

Significance

Previous studies of ritual black coatings on coffins from ancient Egypt have taken single small samples from objects of wide-ranging time periods and have conflated different types of application. This study takes 100 samples of black ritual liquids identified by type of application and precisely located on the objects, to allow the results to be fully contextualized within the wider discipline. It shows that black coatings on coffins were made using a remarkably consistent selection of natural products. The molecules identified in these black coatings overlap with those used in Egyptian mummification balms, which may suggest a link among separate applications: preparation of the body for burial, decoration of the coffin, and rites performed during the funeral.

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terms of a vehicle for display as they could be highly decorated and gilded (14), and cartonnage is much more difficult to repurpose than other funerary goods because the body of the deceased is sewn inside. Concentrating resources into the preparation of the body, mummy case, and coffin allowed the elite to display their wealth and power while at the same time securing their investment.

Black substances painted on the inside and/or outside of coffins and other funerary items or poured over cartonnage as part of the funerary ritual are a material source of evidence for examining the resourcing of funerary activities during this time of political upheaval. A research project on the 22nd-Dynasty coffins, cartonnage mummy cases, shabti boxes, and Osiris figures in the collection of the British Museum offered the opportunity to address this subject. Using analytical chemistry to examine the complex molecular composition of the black ritual substances, the study aimed to identify the materials used and their likely sources. Determination of chemical composition also offered a means to investigate how the materials were processed and applied as well as patterns of use within the funerary context.

Previous research on similar materials has been limited and varied in terms of analytical techniques, covering multiple time periods and considering ritual applications alongside mummification balms (15, 16) or considering a small subset of samples (5, 17–19).* By focusing on burial goods of the 22nd Dynasty, this research is a more targeted examination of funerary ritual residues than has been previously undertaken.

Objects Studied

Color photographs of all of the items sampled and sampling locations are supplied in the *SI Appendix*. The samples were taken from five empty coffins, one empty cartonnage case, two cartonnage cases still containing human remains, one coffin containing a cartonnage case which itself contained human remains, one coffin containing a wrapped mummy, one wrapped mummy with no coffin or case, two shabti boxes, and seven Osiris figures. Samples were taken from the exterior and interior of coffins, from the exterior of cartonnage cases, and from the exterior wrappings of mummified human remains.

The majority of the 20 items studied can be dated stylistically to the 22nd Dynasty; this term is used here in a broad sense. Some of the cartonnage cases and coffins (EA6684, C12 and EA24906, C15) belong at the end of the period and might even be classed as early 25th Dynasty in chronological terms, although stylistically they are firmly in the 22nd-Dynasty tradition. The mummy EA6697 (C20) is Third Intermediate Period (a broad term for Dynasties 21 to 25) but cannot be dated more specifically. Of the Osiris figures, EA9862 (B4) is New Kingdom, possibly reused in the 21st Dynasty; EA9864 (B8) and EA 33885 (B9) are probably 21st Dynasty. Since the precise dates of these dynastic lines are still somewhat uncertain (20), only approximate dating is possible at present. We suggest a date range of c. 950 to 700 BC for the coffins, cartonnage cases, mummies, and other objects discussed here, thus embracing the period covered by the 22nd to early 25th Dynasties.

All of these objects were purchased through intermediaries in Egypt in the 19th century, and so their precise provenances are unknown. The majority probably came from Thebes, but EA20744 (C1) is not definitely from Thebes and has several stylistic features characteristic of northern Upper Egypt (21). In many instances, due to collection practices in the 19th century, the full set of body containers is not preserved with the body, so there is either a cartonnage case with no coffin (e.g., EA20744, C1), or a coffin with no body (e.g., EA24906, C15), or a body with no container (e.g., EA6697, C20).

Fig. 1. EA6662. Mummified male called Djedkhonsuiufankh in cartonnage case in wooden coffin. The coffin has been coated with type 1 black substance, and a large quantity of type 2 black anointing fluid has been poured over the top of the mummy case, cementing it into the coffin.

The black liquid (now solidified) appears to have been applied in two different ways: a black coating on coffins, which has been painted on as part of the decoration (type 1), and a secondary application of a black liquid that was poured (or occasionally brushed) over the cartonnage case, coffin, or mummy, called here "anointing fluid" (type 2).† A few examples have small amounts of black material preserved only in incidental drips or smears on the surface (type 3), probably accidentally introduced. Also classified as type 3 are samples from black encrusted bandages in the base of EA29578 (C3), which may originate from the mummification treatment (there is no body for this coffin). One, two, or all three types of black substance may occur on one group (Fig. 1). Two shabti boxes and seven Osiris figures were also studied. Each was originally coated with a type 1 black substance, which survives to varying degrees (*SI Appendix*). The shabti boxes are made of wood and were made to contain the shabti figures that performed work for the deceased in the afterlife. The Osiris figures are wooden figures of the mummified deity between 38 and 58 cm tall. They stand on long wooden bases (where preserved) with a niche in the side or in the body of the figure, into which funerary papyri were sealed before being placed in the tomb.

Results

The majority of the samples were composed of organic substances. Seven different predominant materials were identified in the samples based on characteristic molecular components detected by gas chromatography–mass spectrometry (GC-MS) analysis; their occurrence in the different objects and sample types is summarized in Table 1. The basis for these identifications is outlined below. More detailed results are provided in *SI Appendix*, Table S3, which lists the molecules that were identified in each case. Two methods

*M. Serpico, B. Stern, Second Vatican Coffin Conference, June 6–9, 2017, Vatican City, Italy.

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Table 1. Summary of results

Object	British Museum no.	Description	Sample type	No. of samples	Oil*	Fat*	Conifer resin	Conifer pitch	Beeswax	Pistacia	Bitumen
C1	EA20744	Mummified female Tjayasetimu in cartonnage case	2	4	+				+		+
C2	EA6666	Coffin of Horaawesheb	1	4	+				+		+
C2	EA6666	As above	2	4	+						+
C3	EA29578	Coffin of Padihorpakhered	2	4	+		+	tr	+		+
C3	EA29578	As above	4	2	+		+		+		nt
C5	EA6662	Mummified male Djedkhonsuiufankh in cartonnage case in coffin	1	13	+		+		+		+
C5	EA6662	As above	2	6	+						+
C12	EA6684	Cartonnage case of Djedhoriufankh	3	4							tr
C13	EA6660	Mummified male Denytenamun in coffin	1	13		+					+
C13	EA6660	As above	2	9	+		+	tr		tr	+
C13	EA6660	As above	3	1	+				+		nt
C14	EA6659	Mummified male Hor in coffin	1	2		+					
C15	EA24906	Coffin of Pasenhor	2	3							tr
C18	EA30720	Coffin of Nesperennub	3	1	+	+			+		+
C19	EA22939	Mummified female Tamut in cartonnage case	3	3	+	+			+		tr
C20	EA6697	Mummified female	2	4	+	+	+		+		+
C20	EA6697	As above	3	1							tr
B1	EA8530	Shabti box	1	5	+				+		+
B2	EA33882	Osiris statue	1	3	+				+		+
B3	EA8540	Shabti box of Djedamuniuesankh	1	2							
B4	EA9862	Osiris statue	1	3						+	
B5	EA35814	Osiris statue	1	2		+					+
B6	EA9880	Osiris statue	1	1							
B7	EA9870	Osiris statue	1	2							
B8	EA9864	Osiris statue	1	2	+					+	+
B9	EA33885	Osiris statue	1	2	+				+	+	+

Results have been given by object and type of sample. So, for example, C5 appears twice in this table, once for type 1 material and once for type 2 material. Type 1 = black coating painted onto the coffins; type 2 = black anointing fluid poured over the coffin/case; and type 3 = drips or smears of black; tr = trace; nt = not tested.

*Suggested based on distribution of fatty acids, see text.

were applied; method A to analyze samples for lipids and method B to target biomarkers for bitumen (22).

Results were consistent across samples taken from the same coffin/case of the same type of substance (e.g., all the samples of type 1 from coffin C5 had the same components in their chromatograms, although relative abundance might vary, sometimes significantly, see *SI Appendix*, Table S4). For coffins where both type 1 coating and type 2 anointing fluid were present, the components differed. For example, on coffin C2 the type 1 coating contained beeswax, which the type 2 anointing fluid did not, and on coffin C5, the type 1 coating contained conifer resin and beeswax, neither of which were present in the type 2 anointing fluid (Fig. 2). All three types of deposit were present for C13, and each deposit contained a different set of components.

Most of the samples contained a range of linear saturated fatty acids with carbon chain lengths in the range of 8 to 18, indicating the presence of a degraded oil or fat (23). Plant oils are composed of triacylglycerols (TAGs), esters of glycerol with fatty acids, with a small amount of phytosterols (24). Animal fats are also largely composed of TAGs, with steroids present as minor components, cholesterol and its esters being the most significant (25). The TAGs in animal fats contain more saturated fatty acids, which makes them solid at room temperature, whereas liquid plant oils contain more unsaturated fatty acids (24). In archaeological contexts, fats and oils degrade to a mixture of TAGs, diacylglycerols and monoacylglycerol, and free fatty acids. Degradation is influenced by many factors, combining millennia of aging in largely unknown conditions with the consequences of anthropogenic activities such as heating and mixing of materials (25), and under some conditions, the acylglycerols may be lost completely, leaving only the free fatty acids. It is seldom possible to be specific about the origin of the lipid material unless diagnostic biomarkers are present. For example, ricinoleic acid (12-hydroxy-9-octadecenoic acid), a fatty acid highly abundant in castor oil, was identified in the type 2 anointing fluid from C2, indicating that castor oil is present, although not necessarily as the only lipid ingredient (26). Nevertheless, the presence of TAGs and higher amounts of stearic than palmitic acid is considered to be typical for degraded animal fat (25, 27), whereas a predominance of palmitic acid over stearic acid is considered to be more characteristic of plant oil (25), although caution is necessary in mixtures with beeswax where beeswax-derived palmitic acid will skew the ratio (28). Diacids, as degradation products of unsaturated fatty acids, may point to plant oil rather than fat when present in abundance, as in C1. The designation of "oil" and "fat" in Table 1 is based on this distinction, which is well illustrated by the results from C13 (Fig. 3). No sterols were detected in any of the samples, which is not surprising given the low rate of detection of sterols in archaeological contexts, especially in the case of residues that have been heated (29).

The presence of conifer resin was determined by the identification of dehydroabietic acid (DHA) or its oxidation products (7-oxo-DHA and 15-hydroxy-7-oxo-DHA), which are formed from the degradation of abietadienic acids found in the diterpenic resin of conifer trees (30). Retene is a stable end product of the degradation of all these diterpenic materials and is an indicator that the resin has been strongly heated to produce wood tar or pitch (30–32). Retene could be identified in the type 2 anointing fluid on C3 and C13 but only when specifically targeted by selected

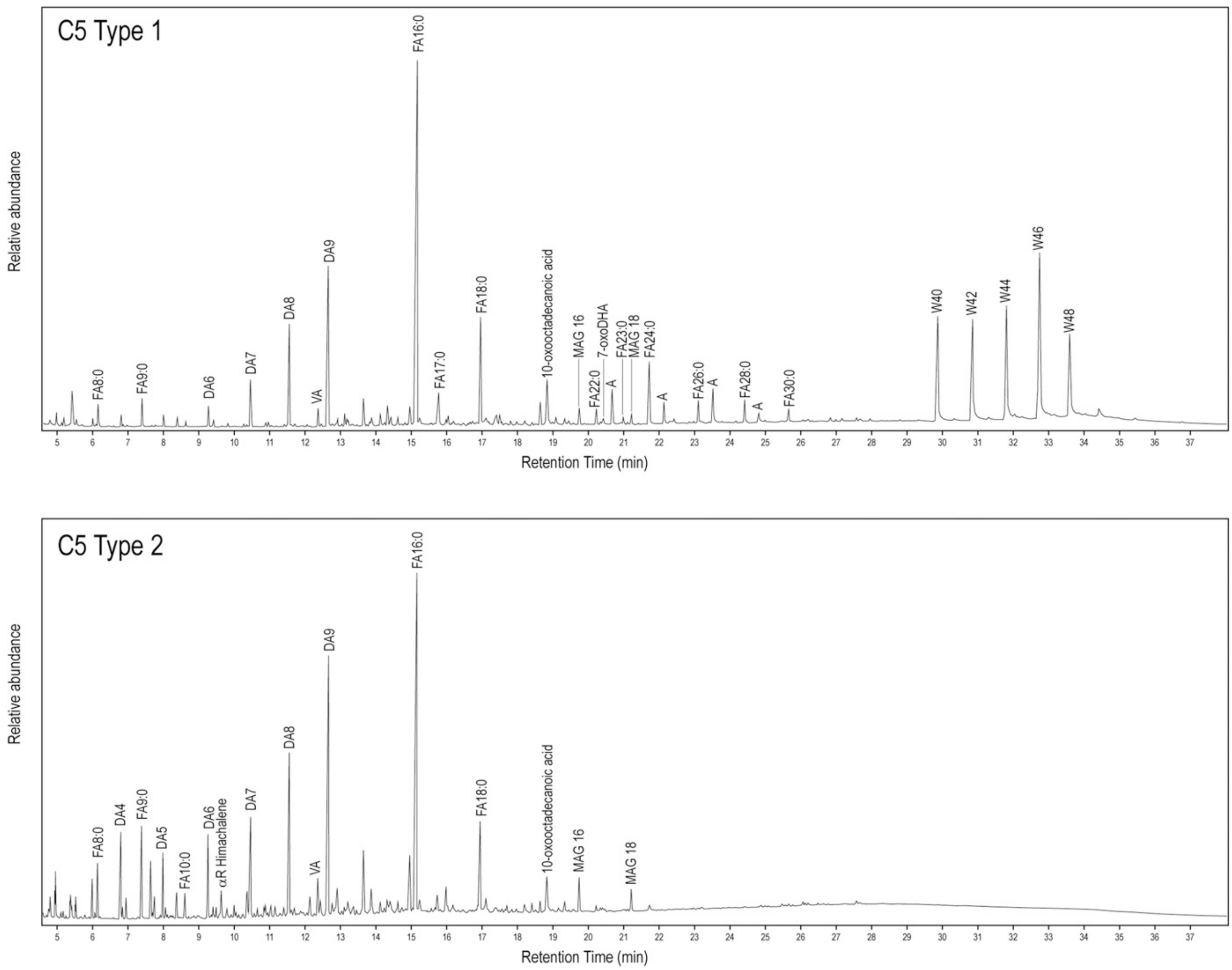

Fig. 2. Partial total ion chromatogram (5 to 38 min) for method A scan of C5 type 1 (sample R7, black coating from inside wall of coffin) and C5 type 2 (sample R1, anointing fluid pooled inside coffin behind head). FAn = fatty acid with n carbons; DAn = diacid with n carbons; MAG n = monacylglycerol with n carbons; VA = vanillic acid; Wn = wax ester with n carbons; DHA = dehydroabietic acid; and A = alkane.

ion monitoring (SIM) analysis (*m*/*z* 219). Although retene is usually interpreted as indicator of high temperature processing for pitch production, trace amounts can form at lower temperatures, perhaps after repetitive heating (33) or from anaerobic degradation by microorganisms (34). Therefore, this trace of retene may represent a minor pitch component or repeated heating of the liquid and/or degradation of the conifer resin over time. DHA or its oxidation products was found in high abundance in the type 2 anointing fluid on coffin C3. In the type 1 coating on coffin C5, the relative abundance of DHA and 7-oxo-DHA varied hugely, 7-oxo-DHA varying from 42% of the abundance of palmitic acid to 2% in different samples (*SI Appendix*, Table S4). This suggests either that there was more than one application of type 1 coating, with the amount of conifer resin mixed in varying from one application to the next, or that the type 1 coating had not been well mixed. The type 2 fluid on coffins C13 and C20 contained 7-oxo-DHA in small amounts, and the abundance was consistent across all samples.

Evidence of beeswax is present in some type 1 and some type 2 residues. The presence of beeswax was evidenced by palmitic wax esters of carbon chain length 40, 42, 44, 46, and 48 accompanied by saturated long chain fatty acids in the range 22 to 30 carbons, peaking at 24, and odd-numbered n-alkanes from 25 to 33 carbons (28, 35). The alcohols usually associated with archaeological beeswax were not observed in any of the samples with wax esters, indicating that the beeswax was not significantly degraded.

Pistacia resin is often found as a "varnish" (possibly a simplification of its intended use) applied over polychrome decoration on coffins and cartonnage cases (5), but in the black deposits studied here, it was rarely present. The resin acids oleanonic acid, moronic acid, and the isomers masticadienonic and isomasticadienonic acid are markers for resin from *Pistacia sp.*, although oleanonic acid is also found in other plant resins so is not itself diagnostic (36–38). Peaks for moronic acid were very low in scan mode and usually only detectable in SIM mode at a retention time determined by running reference samples. *Pistacia* resin was identified in in C13 type 2 anointing fluid and the type 1 coating on Osiris statues B4, B8, and B9. None of these were accompanied by 28-norolean-17-en-3-one, which has been suggested by some as a marker for heated *Pistacia* (5 28, cf. 29).

Both hopanes (*m*/*z* 191) and steranes (*m*/*z* 217) were identified in 37 of the 46 samples run using method B, confirming the presence of bitumen in these samples (39, 40). All of the type 1 coatings and type 2 anointing fluids from coffins and cartonnage cases contained

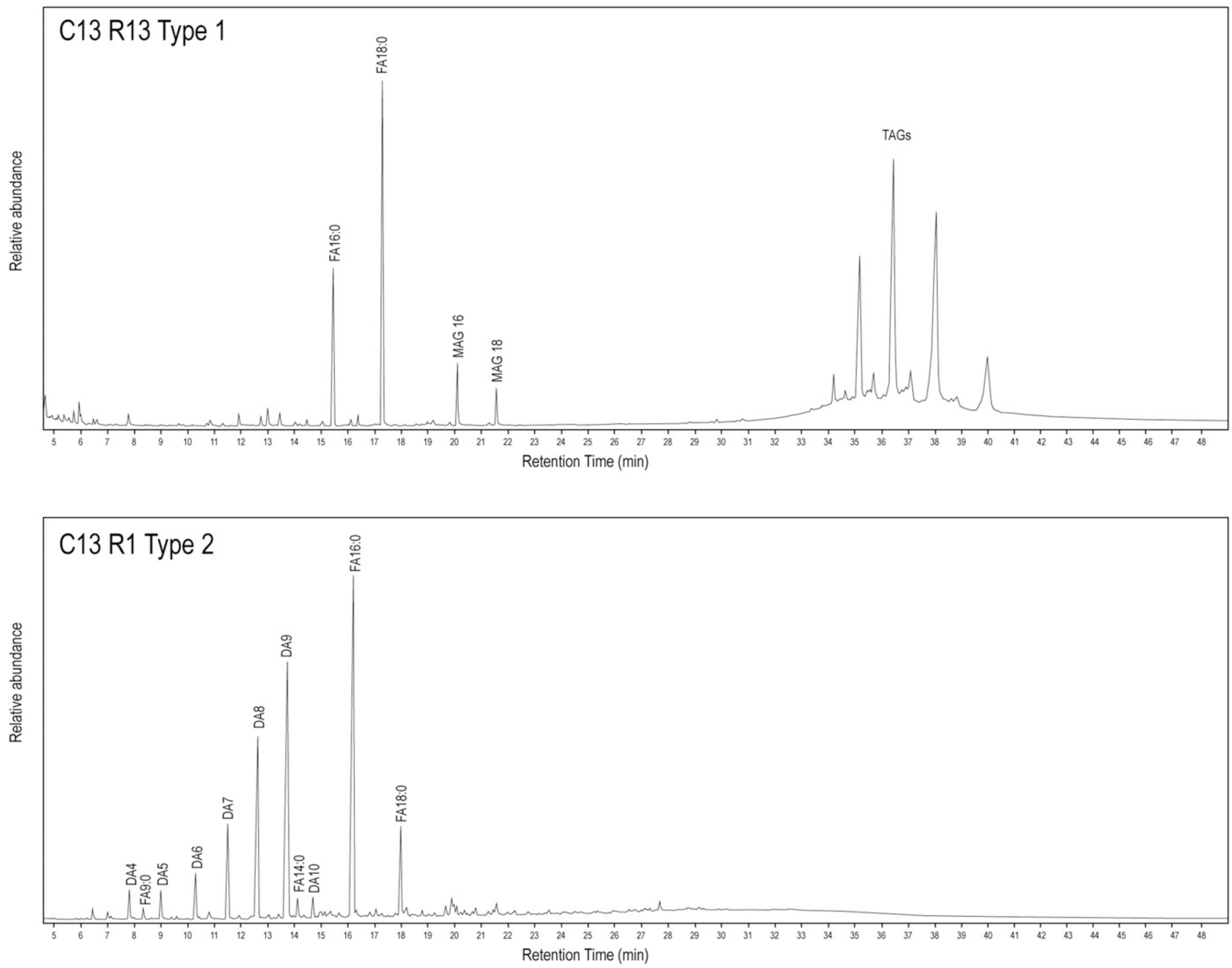

Fig. 3. Total ion chromatograms for method A scan for C13 type 1 (sample R13, black coating from the underside of the coffin base) and C13 type 2 (sample R1, anointing fluid from mummy wrappings). The top chromatogram shows TAGs and higher stearic than palmitic acid, in contrast to the bottom chromatogram, which shows the diacids peaking at 9 carbons and higher palmitic than stearic acid. For key, see Fig. 2.

bitumen, with the exception of the black coating from the underside of coffin C14. Black coatings from the shabti boxes and Osiris statues (B numbers) contained bitumen with the exception of B3, B4, B6, and B7.

The pattern of hopanes and steranes can be used in combination with reference materials and published data to identify the source of the bitumen (40). Sources of bitumen that have been identified in studies of mummification balms include the Dead Sea (22, 41–43), Gebel Zeit, which is a land seep at the southern end of the Gulf of Suez (44, 45), and Hit in Iraq (42, 46); other sources may have been available but have not been identified (46). A trade route for bitumen from the Dead Sea into Egypt appears to have been established from 3900 to 3500 BC (47).

The pattern of bitumen biomarkers was remarkably consistent across all of the tested samples. The high gammacerane, absent oleanane, complete set of triterpenes peaking at C23, slightly higher peaks of C35 homohopanes, and low diasteranes indicate that the bitumen used was most likely from the Dead Sea (Fig. 4) (45, 47, 48). Data taken from clear chromatograms with flat baselines (*SI Appendix*, Table S2) is very similar for the Dead Sea archaeological samples and C2, C5, and C13 (all types of deposit). In contrast, the Ts/Tm ratio for C1 type 2 is well above the values quoted in the literature and other samples run in this study, and the oleanane peak is visible, although very small, whereas it is absent in other samples in this study. In addition, the pattern of steranes is slightly different; in particular, the 28αββR+S peaks are lower than for the Dead Sea reference and other samples. This indicates that this bitumen came from a source other than the Dead Sea, which has not been identified. Biomarker indices for bitumen from Gebel Zeit were taken from the literature and compared to these results (44), but it is not a match for any of the data presented here.

A range of lower-molecular–weight compounds were observed in some samples, and other volatile components may have been lost over time. Vanillic acid and 4-hydroxybenzoic acid were seen in the majority of samples (*SI Appendix*, Table S3); however, since they appear in a range of plant oils and resins (49) and have been identified as degradation products of beeswax (28), which is also sometimes present, they were not considered to be diagnostic. It has previously been suggested that the concurrent occurrence of phenolic compounds such as vanillic acid and polyaromatic hydrocarbons (PAHs) may indicate the use of wood tar (22), but PAHs can also come from bitumen (50), which was present in nearly all samples analyzed here. Some samples were taken from areas where the material was in contact with wood or textile, which is another

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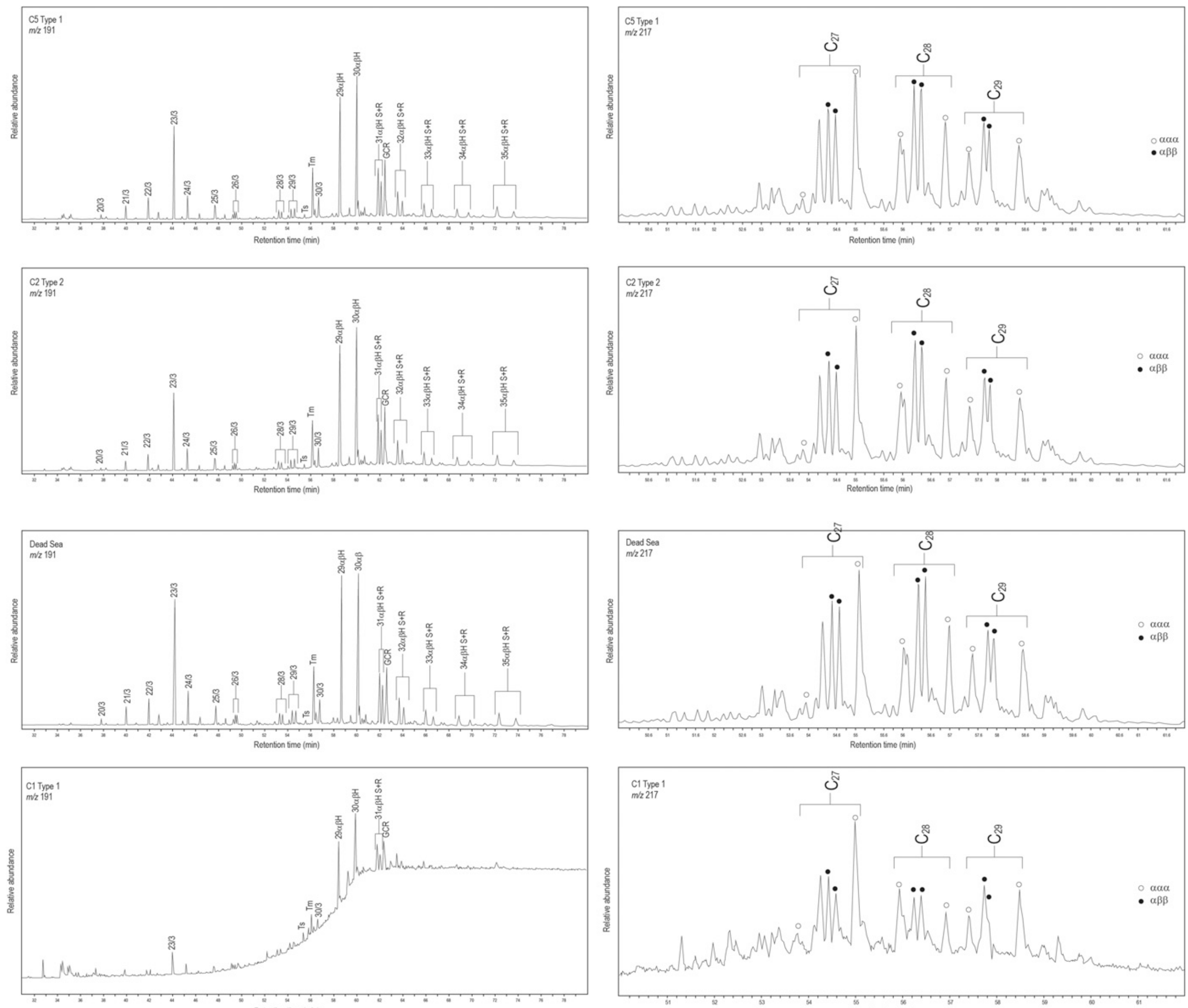

Fig. 4. Partial chromatograms of SIM *m/z* 191 (*Left*, 30 to 80 min) and SIM *m/z* 217 (*Right*, 40 to 72 min) for C2 type 2 (sample R7), C5 type 1 (sample R8), reference material from the Dead Sea, and C1 type 2 (sample R1), prepared using method B (first elute). *m/z* 191 chromatograms show positions of terpanes (20/3 to 30/3), hopanes (29αβH to 35αβH; hopanes 31 to 34 are split into S and R), 18α-22,29,30-trisnorneohopane (Ts), 17α-22,29,30-trisnorhopane (Tm), and gammacerane (GCR). *m/z* 217 chromatograms show Cn long chain ααα- and αββ-steranes with n carbon atoms in R and S configurations.

potential source of phenolic compounds (51). αR-Himachalene and cuparene were both observed in scan mode in some of the samples prepared by method B (C2 type 2; C5 type 1 and type 2; C13 type 2). Sarret and colleagues analyzed black residue from an Egyptian jar dating to the first or second Dynasty c. 3100 to 2700 BC and also identified both himachalene compounds and cuparene (52). They suggested that the himachalene compounds were evidence for the use of a product from *Cedrus libani*, and cuparene was evidence for the use of a product from the Cupressaceae family of junipers and cypresses. However, their appearance together in several samples from this study of 22nd-Dynasty material 2,000 y later, despite variances in the ingredients used, may suggest that the two compounds occur in the same genus and requires further investigation.

Of the six samples taken from EA29578 (C3), four were from the black anointing residue applied to the face of the coffin, and two samples were taken from black residue associated with textile inside the coffin base. The latter two samples contained elevated levels of phenolic compounds and lower levels of DHA compared to the others. It is possible that this residue came from the body that was originally in the coffin; the location of the body is not known.

A few of the samples were found to contain no organic material that could be identified using these methods. One of these samples was from the head end of the exterior of the coffin C2. Analysis of this sample using polarized light microscopy confirms the presence of black particles that are opaque in plane-polarized light and anisotropic in crossed polars, which suggests that this is a carbon pigment (53). This painted black area formed part of the decorative scheme of the coffin rather than a coating and therefore paint may have been the more usual material to use. None of the samples from shabti box B3 or Osiris statues B4, B6, and B7 contained detectable organic material and thus may also have been paints. Polarized light microscopy conducted on these samples showed carbon pigment in the case of B3 and a mixture of carbon pigment and a yellow and translucent material, probably organic, for B4; samples from B6 and B7 showed only brown and translucent particles and thus appeared to be wholly organic. If organic, they were not identifiable using the methods we employed; it is possible that the organic material had polymerized to the extent that it could not be sufficiently solubilized.

The samples of the type 2 anointing fluid in coffin C15 and the type 3 drips on cartonnage case C12 returned no data at all via method A; the method B analysis found traces of hopanes and steranes. The material from C15 has been analyzed three times previously (16, 41, 44). The 2002 and 1988 studies were looking only for the bitumen component of the residue, using the same analysis technique as used here (method B). They identified high oleanane and low gammacerane and high diasteranes (41, 44). The 1988 study lacked comparisons for the data, but the 2002 study found it to be similar to bitumen from Gebel Zeit (44). McCreesh also looked at a sample from C15 and concluded that it "only showed traces of terpenoids, n-alkanes, and benzene" (16). Rullkotter and Nissenbaum (41) reported an extraction yield of only 0.4% compared with c. 50% for samples from other sources, and the published data are noisy compared to other samples. It seems likely that the sample used in this study was too small to yield a useable result, despite being comparable in weight to other samples analyzed here.

Discussion

Information about the rites performed during Egyptian funerals is limited. The Book of the Dead (properly the Book of Going Forth by Day) is a series of texts and vignettes used from the New Kingdom (c. 1550 BC) onward (although with very ancient origins), preserved on papyri in tombs and drawn on tomb walls (54, 55). Some of the chapters provide hints of what was happening during the funeral, but these texts are mainly spells to be read aloud and deal with the equipping of the deceased with knowledge and power for the journey through the underworld, so provide little evidence for real physical activities. The main ritual that was performed at the tomb, the Opening of the Mouth, is alluded to in Book of the Dead chapter 23 and, in much greater detail, in the separate composition known as the Opening of the Mouth (56). Tools and implements which have been found in tombs show that this was a ritual which was actually carried out (57), but although anointings may have formed part of these procedures, the textual and iconographic sources are too standardized to provide useful insights into the practical realities of the ritual. Thus, the analysis of material remains provides us with a great deal of information that we can use to learn more about the rituals surrounding the funeral in ancient Egypt.

The black coating on coffins (type 1) and black anointing fluid (type 2) are very similar substances. Although the components vary from one example to another, there appears to be considerable consistency in the ingredients which were used, with some room for variation.[‡] The ingredients that have been identified are as follows: plant oil, animal fat, *Pistacia* resin, conifer resin, beeswax, and bitumen. The variations in the components are not specific to application type; any can occur in type 1 or type 2. Previous studies of black coatings and anointing fluids have also identified some combination of oils or fats, *Pistacia* resin, bitumen, conifer resin or pitch, and beeswax (5, 16, 19).

Type 1 black coating must have been applied during the decoration of the coffin, as drawings in yellow are applied over the top of the coating. This implies that the person decorating the coffin had access to these materials during the course of their work. Texts on ostraca from Deir el Medina (from a slightly earlier period c. 1300 BC) indicate the existence of what Cooney refers to as an "informal workshop," by which she means a collective of men who were otherwise employed by the state to decorate the tombs in the Valley of the Kings but who in their spare time produced craft items for the private funerary market, including painted coffins (58). The term "workshop" refers to an association of craftsmen, not to a location; the space in which they would have produced these goods is not known but may have been in or on top of their own houses, or their huts in the hills where they lived while working on the royal tombs (58). These texts also indicate that the craftsmen were ordering supplies of pigments and varnish for the private work and that similar arrangements existed in other areas, and that sometimes the various "workshops" traded with each other (5, 58). Since the type 1 black coating forms part of the decorative scheme on some of the coffins, it may have been applied by people operating in a similar way, ordering in their own ingredients (5). The components of the black coating are remarkably consistent across coffins that have several areas of black coating. For example, the coating on C5 is the same on the underside of the lid, the inside of the trough, and outside of the trough (although the amount of conifer resin varies), suggesting either that the person decorating the coffin may have had a vaguely consistent recipe, or they were painted with the same batch of black liquid.

The type 2 anointing fluid appears to have often been poured over, sometimes brushed onto, the finished and occupied coffin or cartonnage case. In some cases, the surface of the dried anointing fluid is smooth, and it has run in rivulets down the sides (*SI Appendix*, Fig. S2). Thus, it seems that this practice must have taken place when the coffin and/or cartonnage case was lying flat. The location of the event is unknown, although the body does not seem to have been substantially moved afterward, at least before the liquid dried, because the surface is usually smooth and unmarked. It seems possible that this could have been a funerary ritual which was sometimes enacted within the tomb. If so, this has implications for thinking about the practicalities of funerary rituals. If the anointing fluid was applied at the tomb, it would have to be transported to that location from where it had been mixed, in some cases in quite large quantities. The area of the base of C5 has been calculated as ~0.53 m^2; if the type 2 fluid in the base is assumed to be on average 2.5 cm deep (it is difficult to be precise), then this would be about 13 L of fluid. The cartonnage case would have displaced some of the fluid, so this is an upper estimate. However, the volume of fluid to transport would have been fairly large in this case. If it was applied while warm, this raises further questions about the provision of fire at the tomb or the transport of a warm liquid to the tombs.

Type 3 drips and smears may be accidental additions of a type 1 coating or type 2 anointing fluid. Although the full range of ingredients have not been observed in type 3 samples, this is probably due to the lower number of samples and possibly the smaller sample size. The type 3 smear on C13 is different to the type 1 and type 2 applications on the same coffin and associated mummy, therefore it must come from a third source, not otherwise used on this coffin, that has transferred somehow. A scenario can be imagined wherein a craftsman applying a black coating was working on more than one coffin at once and making up the black liquid as required. One day, a black liquid was painted onto Coffin A, in this scenario, and on a separate day, another black liquid was mixed for painting onto Coffin B, and during this, some of the Coffin B liquid transferred onto Coffin A from a dirty hand or the coffins bumping together from being in close proximity. Type 3 smears are also present on coffins that otherwise have no black substance applied to them (e.g., C18, EA30720), which is further support for this accidental transfer.

Although applications of type 1 coatings and type 2 anointing fluid are known from earlier periods (3–5, 59), instances of the type 2 anointing fluid seem to increase in the 22nd Dynasty. The focus on the body and the coffin during the Third Intermediate Period may have caused a rise in the popularity of the ritual pouring of

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black liquid during the funeral (type 2). Alongside developments in funerary belief and practice, one function of this may have been to prevent the reuse of the mummy case and/or coffin, which was a common fate of coffins of the New Kingdom and 21st Dynasty (11). It also concentrated the expense of the funeral onto the body of the deceased and perhaps publicly demonstrated the expenditure of the family at the point of interment (14). It has been suggested that the lack of gilding on coffins in the 21st Dynasty was partly due to the desire to reduce the looting and usurping of elite coffin elements because "ostentatious displays of wealth could be profoundly dangerous" (14); black anointing fluid could be used to demonstrate wealth and simultaneously render the coffin and cartonnage case safe forever. Extreme examples, such as C5 (EA6662), where the cartonnage case was cemented into the coffin by the anointing fluid, meant that even prising the mummy out to search for amulets and jewelry would have been a very difficult task.

The origin of several of the components of the black material lie outside of Egypt. The bitumen, conifer resin, and *Pistacia* resin were probably all imported. The bitumen is from the Dead Sea, an origin which has been identified for bitumen in many mummification balms, mostly dating from the Greek and Roman Period (22, 39, 41, 43, 44, 46, 60, 61), despite there being available sources within Egypt (44, 45). Conifers do not grow in Egypt but are widely available to the north, around the eastern Mediterranean (62). *Pistacia* resin was found in large quantities in the Uluburun shipwreck in amphorae and at Amarna in Egypt in the same type of vessels; the ceramic fabrics used for these amphorae have been sourced to near Haifa on the Levant coast and further south to the mid coast of modern Israel, which is also a likely geographic origin for the resin (38, 63–65). *Pistacia* resin was also used for "varnishing" coffins and other funerary equipment and as incense, which was burned in bowls (5, 38, 64), all of which can be considered to be ritual, funerary, and/or religious uses. Similarly, bitumen seems to have been so far identified in Egypt mainly in funerary contexts. This study provides evidence that large amounts of organic materials continued to be imported into Egypt specifically for funerary ritual uses in the Third Intermediate Period, despite the breakdown of the empire and subsequent loss of influence in the Levant.

Published analyses of mummification balms identify the same range of components as have been found in this study (15, 22, 39, 66–69). Therefore, the same range of materials were being applied on three separate occasions: 1) during mummification, 2) painting the coffin (coating, type 1), and 3) pouring on anointing fluid (type 2), probably during the funeral, possibly inside the tomb. The similarity of components of the black applications suggests that they may have stemmed from a common set of ritual requirements and served similar purposes. Those purposes probably included the successful preservation of the body and the transitioning of the deceased to the afterlife as Osiris (70).

Conclusions

The components of the black coating material and the black anointing material are similar but variable; they both draw on a consistent group of ingredient materials, of which seven have here been identified using molecular analysis: fat, oil, conifer resin, pitch, *Pistacia* resin, beeswax, and bitumen. In addition, there are aromatic compounds that could indicate that other ingredients may have been present that cannot currently be identified. Many other volatiles will have been lost over time, which may have been evidence for further components. The material properties of organic components have particular significance for the interpretation of practical activities associated with funerary rituals and the organization of the process, including movement of materials and provision of other resources, such as fire, during ritual events. Many of the identifiable organics in the black liquids would have been imported into Egypt, which is direct evidence for continuing trade with the eastern Mediterranean through the Third Intermediate Period and indicates the importance of this practice among the elite during this time.

Methods

Samples were taken using a clean scalpel and stored in glass vials. Where possible, samples were taken from several places across one coffin, cartonnage case, or mummy in order to assess the variability of the black substance within one context. Sample sizes ranged from less than 0.1 mg to 10 mg. For the exact location of the samples, see *SI Appendix*.

All samples were analyzed by high temperature gas chromatography mass spectrometry after solvent extraction (method A) to look for lipids and waxes. A total of 51 samples (*SI Appendix*, Table S1) were additionally fractionated to isolate saturated hydrocarbons followed by targeted GC-MS analysis (method B) to look for hopanes and steranes, which are biomarkers for bitumen (40). Reference samples of *Pistacia* resin (mastic), conifer tar, pine resin, and bitumen were also analyzed.

Method A. Samples were solvent extracted three times using 1 mL dichloromethane (DCM); extracts were combined and dried by off-gassing in a fume cupboard at 35 °C. Dried extracts were derivatized with 100 μL silylating reagent *N*,*O*-bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane, heated at 70 °C for 1 h. Derivatized samples were autoinjected into a 12 m SGE HT5 column, internal diameter 0.22 mm, film thickness 0.1 μm, and fitted with a 1 m × 0.53 mm precolumn. The oven was set at 50 °C for 2 min, ramped 10 °C/min to 370 °C, and held for 15 min. The detector was an Agilent 5975 mass spectrometer using EI at 70 eV. Each sample was analyzed twice, first in scan mode over the range 50 to 750 amu, then in SIM mode targeting different ions over three time periods: 0 to 15 min (aromatics) *m/z* 105, 205, 267, and 297 (49); 15 to 25 min for conifer resin, pitch, and bitumen *m/z* 219, 239, 253, 459, 191, and 217 (30–32, 40); and 25 to 35 mins for *Pistacia* resin and bitumen *m/z* 189, 409, 421, 526, 191, and 217 (36, 40).

Method B. Samples were solvent extracted three times using 1 mL DCM each time; extracts were combined and dried by off-gassing in a fume cupboard at 35 °C. Subsequently, each extract was deasphalted by adding 1 mL hexane, leaving the asphalt to settle out, and extracting the solute to a clean vial where it was dried under a gentle stream of nitrogen. The de-asphalted samples were then fractionated using column chromatography. The columns were created using glass pipettes held upright and plugged with glass wool and half filled with dried silica (chromatography grade 60–120 μm, pre-extracted with DCM/methanol 97:3, followed by hexane, then oven dried) to which hexane was added to exclude moisture. Each de-asphalted sample was redissolved in 100 μL hexane and decanted into a glass pipette. The first fraction was extracted using 3 mL hexane washed through the pipette; the second using 3 mL DCM:hexane 1:3; and the third using 3 mL DCM:methanol 2:1. Each fraction was collected at the base of the pipette and dried under nitrogen. Only the first elute (saturated hydrocarbons) was analyzed. The first elutes were redissolved in hexane and autoinjected into either a 30 m Agilent HP5-MS column (C1 to C5) or a 30 m Frontier Lab Ultra Alloy 5 gas column (C13 to C15), both with internal diameter 0.25 mm and film 0.25 μm. The oven was set at 60 °C for 2 min, ramped 4 °C/min to 290 °C, and held for 30.5 min. The gas column was coupled to an Agilent 5973 mass spectrometer using EI at 70 eV. A third set (C18 to C20) were run through an Agilent 30 m HP5-MS column, internal diameter 0.25 mm and film thickness 0.25 μm connected to an Agilent 5977 mass spectrometer on the same method. Each sample was scanned over the range 50 to 550 amu then analyzed using SIM mode searching for ions *m/z* 191 and 217 (71).

Molecules were identified by comparison with reference samples, NIST database version 2.3, and published information (5, 25, 28, 30, 36–38, 41, 44, 45, 47, 49, 62, 67, 72–75).

Data Availability. All study data are included in the article and/or *SI Appendix*.

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