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Chemical analyses of Egyptian mummification balms and organic residues from storage jars dated from the Old Kingdom to the Copto-Byzantine period

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ABSTRACT

Twenty three samples of Egyptian organic materials, spanning from the Old Kingdom to the Copto-Byzantine Period, were investigated by gas chromatography-mass spectrometry. The sample set was comprised of ten balm samples from human mummies, three balms from shrews, and ten samples of residues scraped from jars and amphora from storehouses.

This research program was undertaken with two main goals:

Firstly to provide complementary data on the mummification balms from both humans and animals with an emphasis on the occurrence of bitumen in mummification mixtures.

Secondly to explore whether the jar residues were mixtures that were used for mummification purposes or whether they were pure ingredients stored for various uses including ritual practices.

The analysis highlighted that the most abundant constituents of the mummification balms were: fats or oils, waxes, conifer resin, pitch, mastic resin, castor oil, and bitumen. Balms from animal mummies were not found to be significantly different from the balms from human mummies. Residues from potsherds appeared to belong to two categories: pure products (fats and castor oil) and mixtures containing fats, Pinaceae resin and pitch, mastic resin, and castor oil, i.e. the constituents also identified in mummification balms. The mixtures were thus residues of preparations for ritual practices and embalming.

This study demonstrates that bitumen is underestimated by the chemical approach currently applied in most archaeometric studies of Egyptian organic residues, which are better suited for the identification of lipids and resinous materials. We thus applied a specific analytical design, targeted at bitumen. Bitumen from the Dead Sea was conclusively identified using as reference materials for comparison, i.e. the present day bitumen from the Dead Sea floating blocks, as well as several bitumens from mummification balms and bitumen lumps unearthed from the archaeological site of Tell Yarmouth near Jerusalem in Israel.

1. Introduction

Recent advances in analytical techniques have enabled the chemical composition of various archaeological residues of organic materials from ancient Egypt to be investigated, in order to identify the ingredients used to prepare human and animal mummies (Brettell et al., 2017; Buckley et al., 2004; Buckley and Evershed, 2001; Colombini et al., 2000; Macke et al., 2002; Ménager et al., 2014, 2013, Perraud, 2012) and to investigate funerary artefacts such as boxes for canopic jars, ushabtis, Osiris statuettes, and coffins (Charrié-Duhaut et al., 2007; Serpico and Raymond, 2001). Even burial food, such as meat and poultry from Pharaonic tombs has been investigated (Clark et al., 2013).

The various recipes used in mummification can be revealed by

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collecting data on the residues of organic materials stored in jars and amphoras. Such residues may be either ingredients stored for the subsequent preparation of mummification balms, or mixtures which were prepared and used for ritual or funerary purposes.

The aim of the study was to provide new data on the chemical composition of various mummification balms ranging from the New Kingdom to the 30th dynasty, i.e. between 1550 and 342 BCE, and on several organic residues, scraped from the inside of a stone vase from the Old Kingdom, as well as from potsherds of jars and amphora covering the time range from the New Kingdom (1552-1069 BCE) to the Copto-Byzantine period (395-645 AD).

The molecular profile of the balms was determined by gas chromatography-mass spectrometry (GC-MS). We adopted two different sample pre-treatments. The first is commonly used for characterising lipids and resinous materials in mummification balms, and is based on saponification, the extraction of neutral and acidic fractions, and derivatisation before injection in the GC-MS (Lucejko et al., 2012). For some samples, we also adopted a specific analytical procedure designed for the analysis of petroleum and rock extracts and for the identification of bitumen (Connan, 2012; Connan and Dessort, 1991).

The aim of the work was also to attempt to answer the question raised by the organic residues stuck on various potsherds from the various containers was: were they examples of the storage of pure organic materials (foodstuff? ingredients for special uses?) or mixtures of several natural products prepared for a specific purpose, for instance for embalming practices?

2. Experimental

2.1. Samples

The samples are described in Table 1 and are subdivided into three classes:

1- Samples from the embalming of human mummies. Ten samples were collected from various parts of the mummies (skull, chest, shroud) with an emphasis on samples inside the skull (Fig. 1). One sample came from a Duamoutef canopic jar and was associated with embalmed viscera. The dates of the mummies spanned from the New Kingdom (1550-1070 BCE)

Table 1
Information on the analyzed samples.

Sample number	Sample	Dynasty	Date range	Area	Context
1896	Balm of human mummy collected along the thoracic vertebra, on the right side of a man wrapped in bandages	New Kingdom	1550-1070 BCE	Thebes	Burial vault
2504	Red balm at the level of the neck, probably originally in the mouth. It was also located on the palate	XXIe or XXIIe	1069-945 or 945-715 BCE	Thebes	Tomb excavation
2759	Balm from human mummy	XXI-XXIIe	1069-715 BCE	Thebes	Tomb excavation
2678	Balm from a canopic jar with a jackal head (Duamoutef) containing remains of viscera wrapped in linen soaked with “black resin”	XXIIe	945-715 BCE	Thebes	Burial vault
2679	Balm stuck to the linen of the shroud. Black balm smeared over the shroud to ensure adhesion	XXIIe	945-715 BCE	Thebes	Tomb excavation near surface
2680	Balm in the mouth. Red balm filling the mouth, pressed agianst the tongue, retracted into the back of the mouth	XXIIe	945-715 BCE	Thebes	Tomb excavation near surface
2681	Balm from the thoracic cavity. The bottom of the left chest cavity is lined with a black balm, poured hot when embalming and forming a horizontal surface	XXIIe	945-715 BCE	Thebes	tomb excavation near surface
2770	Balm from human mummy originating from the excerebration hole in the skull	XXIIe	945-715 BCE	Thebes	Tomb excavation
2771	Balm in the skull (sag in the skull that runs from front to back)	XXIIe	945-715 BCE	Thebes	Tomb excavation
2774	Balm collected deep inside the pharynx, which is completely obstructed by the black material	XXIIe	945-715 BCE	Thebes	Tomb excavation
2702	Balm from a mummified shrew	XXXe	380-342 BCE	Giza	Necropolis excavation
2703	Balm from a mummified shrew	XXXe	380-342 BCE	Giza	Necropolis excavation
2704	Balm on linens from a shrew	XXXe	380-342 BCE	Giza	Necropolis excavation
1811	Deposit in a stone vase discovered in a pit	Old Kingdom	2686-2181 BCE	Saqqarah	Pit excavation
2785	Yellow powder with mineral grains	New Kingdom	1552-1069 BCE	Thebes	Temple storehouse
2795	Deposit scraped from a potsherd	New Kingdom	1552-1069 BCE	Thebes	Temple storehouse
2775	Reddish amorphous material scraped from a potsherd	XIX-XXe (Rammesid period)	1296-1069 BCE	Thebes	Temple storehouse
2776	Amorphous material scraped from a potsherd	XIX-XXe (Rammesid period)	1296-1069 BCE	Thebes	Temple storehouse
2779	Amorphous material on a jar handle	XIX-XXe (Rammesid period)	1296-1069 BCE	Thebes	Temple storehouse
2783	Gray flakes scraped from a potsherd	XIX-XXe (Rammesid period)	1296-1069 BCE	Thebes	Temple storehouse
2786	Yellow/Orange amorphous material scraped from a potsherd	Copto-Byzantine period	395-645 AD	Thebes	Temple storehouse
2790	Deposit scraped from the bottom of an amphora	Copto-Byzantine period	395-645 AD	Thebes	Temple storehouse
2794	Deposit scraped from the bottom of an amphora	Copto-Byzantine period	395-645 AD	Thebes	Temple storehouse

Fig. 1. The location of sample # 2774.

to the Third Intermediate Period (22nd dynasty, 945-715 BCE). The mummies all came from the Thebes area.

2- Samples from the embalming of animal mummies. Three samples were collected from the remains of shrews in the area of Guizeh, and dated from the 30th dynasty (380-342 BCE),

3- Resin-like samples scraped from containers: one sample was extracted from a stone vase from the Old Kingdom, which was discovered in a pit near Saqqara. The other nine samples were scraped from potsherds of jars and amphoras dated between the New Kingdom and the Copto-Byzantine period.

As references for the Dead Sea bitumen, present-day specimens of pure bitumen floating on the Dead Sea surface, and archaeological bitumen, unearthed from the Tel Yarmouth site in Israel were used. To complete the references for a comparison, data were also added on bitumen identified as Dead Sea bitumen in balms of Egyptian mummies (Connan, 2005; Macke et al., 2002).

2.2. Analytical procedures and equipment

Procedure A. All the archaeological samples were subjected to an analytical procedure based on gas chromatography-mass spectrometry (Colombini et al., 2003) for the identification of acyllipids, waxes, resinous materials in the same micro-sample. Samples (1–5 mg) were subjected to saponification with 10% hydroalcoholic KOH. Neutral organic components were extracted with *n*-hexane and, after acidification, the acidic organic components were then extracted from the residual solution with diethyl ether. Aliquots of both extracts were derivatised with N,O-Bis(trimethyl)-silyl-trifluoroacetamide (BSTFA) containing 1%

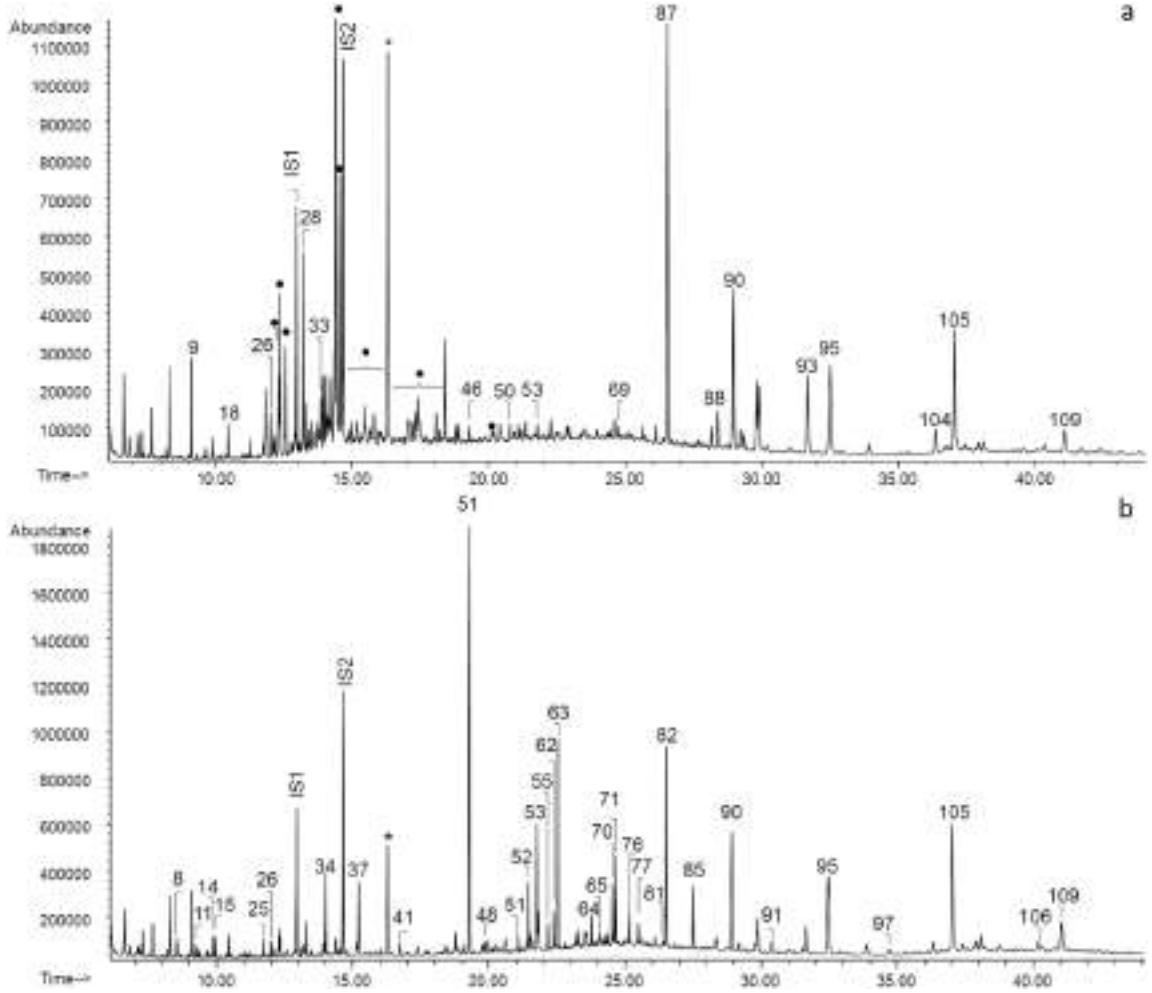

Fig. 2. Total ion current chromatogram of a) neutral and b) acidic fraction of sample #2678. (IS1 = hexadecane, IS2 = tridecanoic acid). The acidic and alcoholic species are present as TMS-derivatives, "•": sesquiterpenes, "*": phthalate contamination. The numbers refer to Table 2.

trimethylchlorosilane (Sigma) using isooctane as a solvent. 2 μl were analyzed by GC-MS using hexadecane and tridecanoic acid as internal standards.

Milestone ETHOS microwave oven was used for the saponification. The program applied consisted of a first step of 2 min during which the temperature was increased gradually to 60 °C with a power of 550 W, followed by a second step of 3 min, which maintained a temperature of 60 °C with a power of 500W.

The GC-MS instrumentation consisted of a 6890 N Network GC System (Agilent Technologies, Palo Alto, CA, USA) equipped with a PTV injector and coupled to a 5973 MS detector with a quadrupole analyzer.

MS parameters: electron impact ionisation (EI, 70 eV) in positive mode; ion source temperature 230 °C; scan range 50–700 *m/z*; interface temperature 280 °C.

GC separation was performed on an HP-5MS column (J&W Scientific, Agilent Technologies, stationary phase 5% phenyl–95% methylpolysiloxane 30 m length, 0.25 mm i.d., 0.25 μm film thickness) connected to a deactivated fused silica precolumn (J&W Scientific, Agilent Technologies, 2 m length, 0.32 mm i.d.).

GC conditions: the PTV injector was used in splitless mode at 300 °C and the chromatographic oven was programmed as follows: 80 °C, for 2 min isothermal, 10 °C/min up to 200 °C, 4 min isothermal, 6 °C/min up to 280 °C, 40 min isothermal; constant He flow 1.2 ml/min, injector temperature 280 °C.

Peak assignation was based on the interpretation of mass spectra and a comparison with reference compounds and materials, with library spectra (NIST 1.7), and with spectra reported in the literature.

Procedure B. The samples were subjected to a second sample pre-treatment to quantify free alkanes, aromatic and polar compounds of a bituminous material in order to recognise the geographical origin. A brief description of Procedure B is reported in the literature (Connan, 1999; Connan et al., 2006b, 2013). In brief, the dichloromethane extract was deasphalted using hexane. The deasphalted fraction was separated into saturated hydrocarbons, aromatic hydrocarbons and resins by gravity flow column chromatography using a 100–200 mesh silica gel support activated at 400 °C prior to use. Hexane, dichloromethane and dichloromethane/ethanol (50:50) were used to elute saturate, aromatic and NSO fractions, respectively. Following solvent evaporation, the recovered fractions were quantified gravimetrically. The C_{15+} saturated hydrocarbon fraction was subjected to molecular sieve filtration (Union Carbide S-115 powder) after the technique described by (West et al., 1990). An aliquot of the total alkane fraction was kept to give access to the n-alkane distribution.

Gas chromatography-Mass spectrometry (GC-MS) analysis of hydrocarbon fractions was performed using an HP5890 chromatograph (split injection) interfaced to an HP5971 mass spectrometer. An HP-2 column (50 m length, 0.2 mm i.d., 0.11 μm film

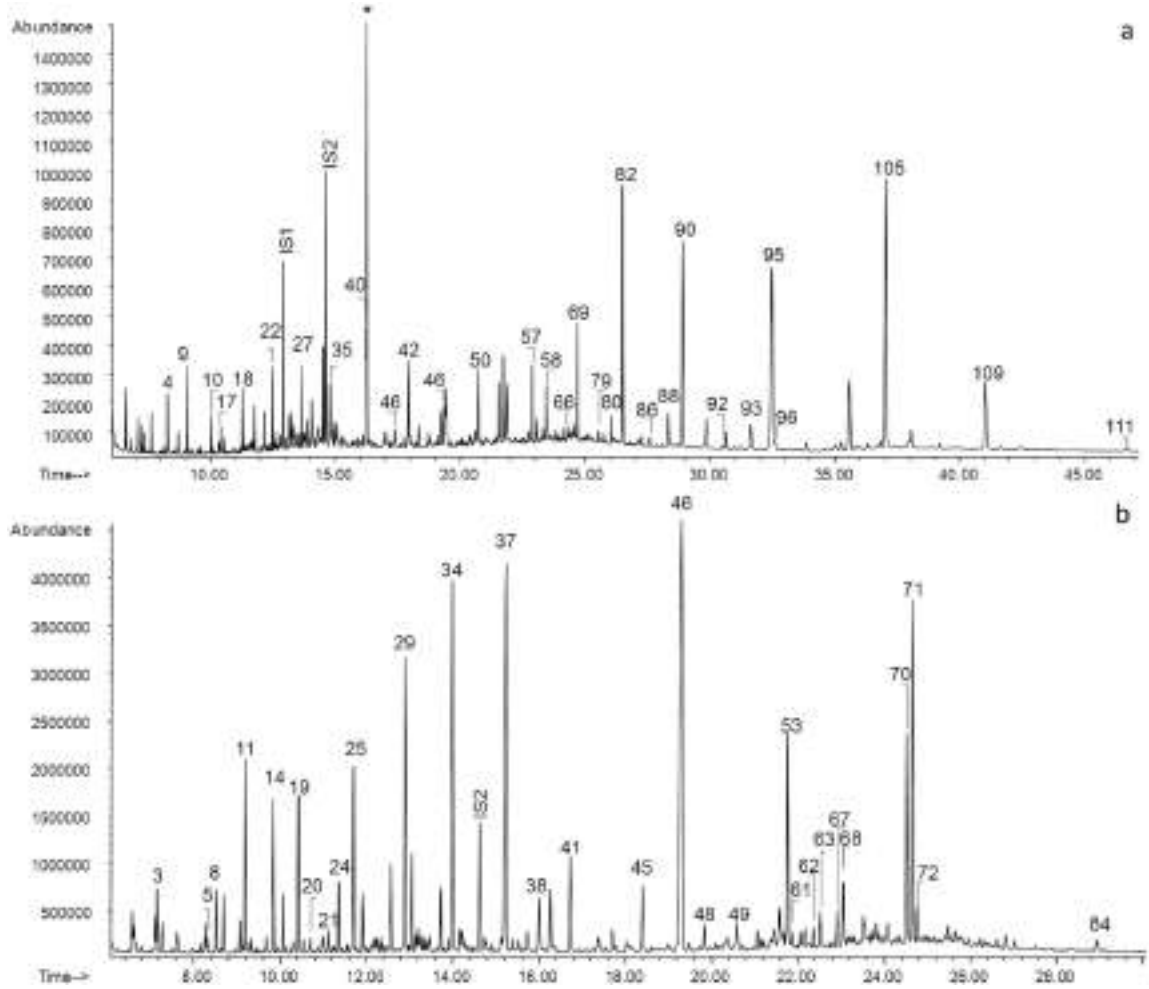

Fig. 3. Total ion current chromatogram of a) neutral and b) acidic fraction of sample #2679. (IS1 = hexadecane, IS2 = tridecanoic acid). The acidic and alcoholic species are present as TMS-derivatives. "*": phthalate contamination. The numbers refer to Table 2.

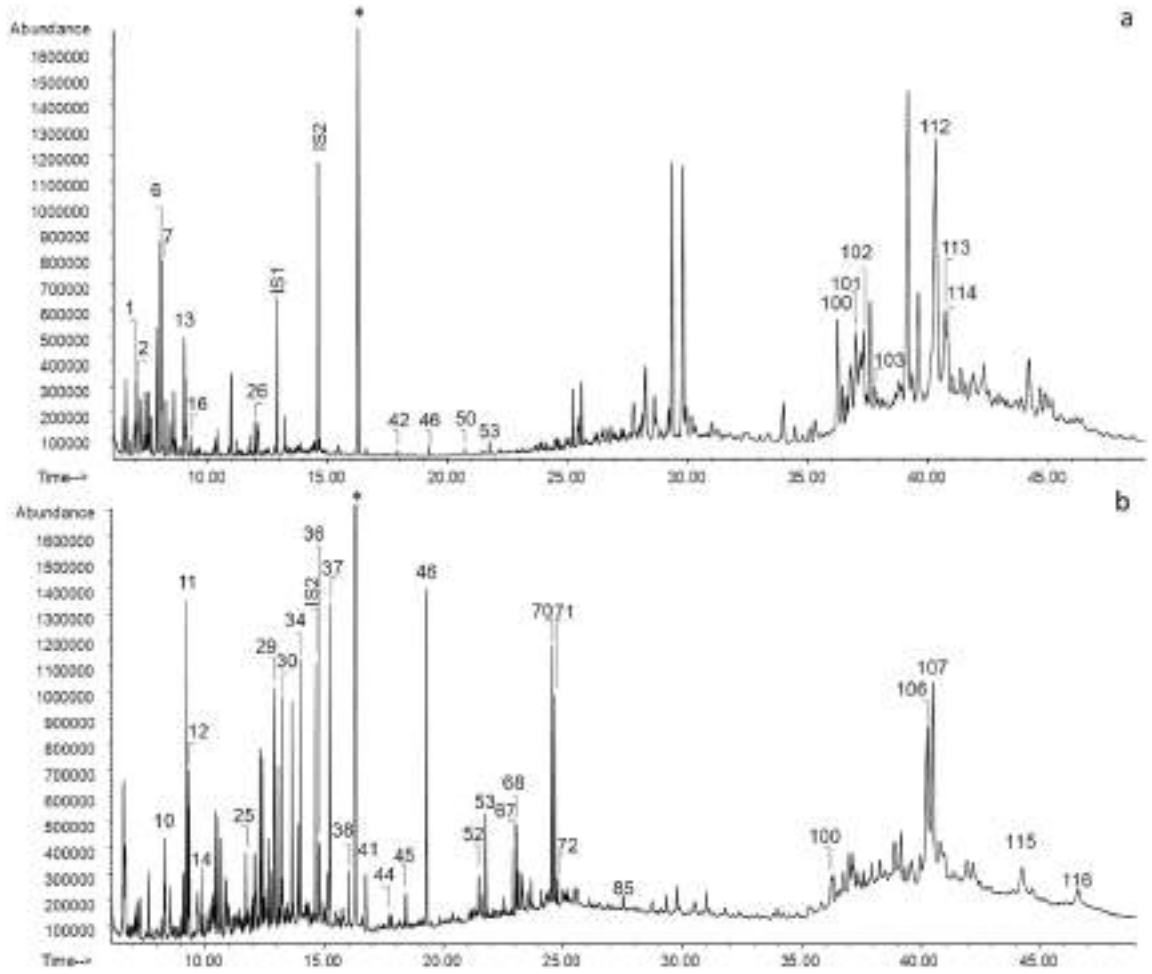

Fig. 4. Total ion current chromatogram of a) neutral and b) acidic fraction of sample #2680. (IS1 = hexadecane, IS2 = tridecanoic acid). The acidic and alcoholic species are present as TMS-derivatives. "*": phthalate contamination. The numbers refer to Table 2.

thickness) was used for chromatographic separation. GC conditions: the chromatographic oven was programmed from 150 °C to 325 °C at 2 °C/min, and then held for 10 min. The spectrometer was run in the selected ion mode (SIM), monitoring ions at *m/z* 177, 191, 205, 217, 218, 221, 231 and 253. In order to determine the concentrations of biomarkers, a deuterated internal standard (d4-C29ααα20 R sterane, Chiron lab, Norway) was added to the C_{15+}branched/cyclic hydrocarbon fraction. Response factors (RFs) at *m/z* 221 for the deuterated standard to hopane (*m/z* 191) and sterane (*m/z* 217) authentic standards were found to be approximately 1.4 and 1.0, respectively. The concentration of individual biomarkers was determined using the following equation: Conc. [ppm] = (peak height biomarker) [ng standard] (RF) [mg B/C fraction]. Stable carbon isotope ratios ($\delta^{13}C$ in ‰ vs. VPDB) of the C_{15+}saturates, C_{15+}aromatics, NSO and asphaltenes were determined using Sofer's combustion technique (Sofer, 1980) along with a Finnigan Delta E isotope mass spectrometer. Uncertainty is ±0.05‰.

3. Results and discussion

3.1. Identification of resinous and lipid materials via procedure A

The samples submitted to saponification and extraction (procedure A) showed some differences in the molecular patterns of both the acidic and neutral fractions. For example, Figs. 2–5 show the chromatograms of both the acidic and neutral fractions for balm samples #2678, #2679, #2680 and #2681, respectively. Table 2 lists the components identified in the various samples.

The main acidic components in all the samples were linear monocarboxylic acids from short to long chain (9–32 carbon atoms). The most abundant were palmitic acid (hexadecanoic acid, C16:0) and stearic acid (octadecanoic acid, C18:0). The length of the acyl chains suggested that they originated from the saponification of both glycerolipids (contained in drying oils) and cerides (long chain esters contained in natural waxes).

The second most abundant compounds in the samples after monocarboxylic acids were α,ω-dicarboxylic acids ranging from 5 to 24 carbon atoms, with azelaic acid (nonandioic, diC9) as the most abundant, along with suberic (octandioic, diC8) and sebacic (decandioic, diC10) acids. Dicarboxylic acids are not by nature present in waxes, oils and fats, but can be formed during curing and ageing as a result of preferential oxidation and bond cleavage at the double bonds in the acylic chain. The prevalence of azelaic acid is thus an indication of the predominance of oleic acid (9-octadecenoic acid, C18:1) in the original material, which had undergone degradative oxidation, and is also highlighted by the presence of 9,10-hydroxyoctadecanoic acid. (Copley et al., 2005).

The lipid profile thus seems to indicate that a plant oil or a mixture of plant oils was present in the mixtures. We were unable to identify the type of plant oil due to the un-specificity of the fatty acids, except for samples #2504, #2759, #2771, #2775 and #2795

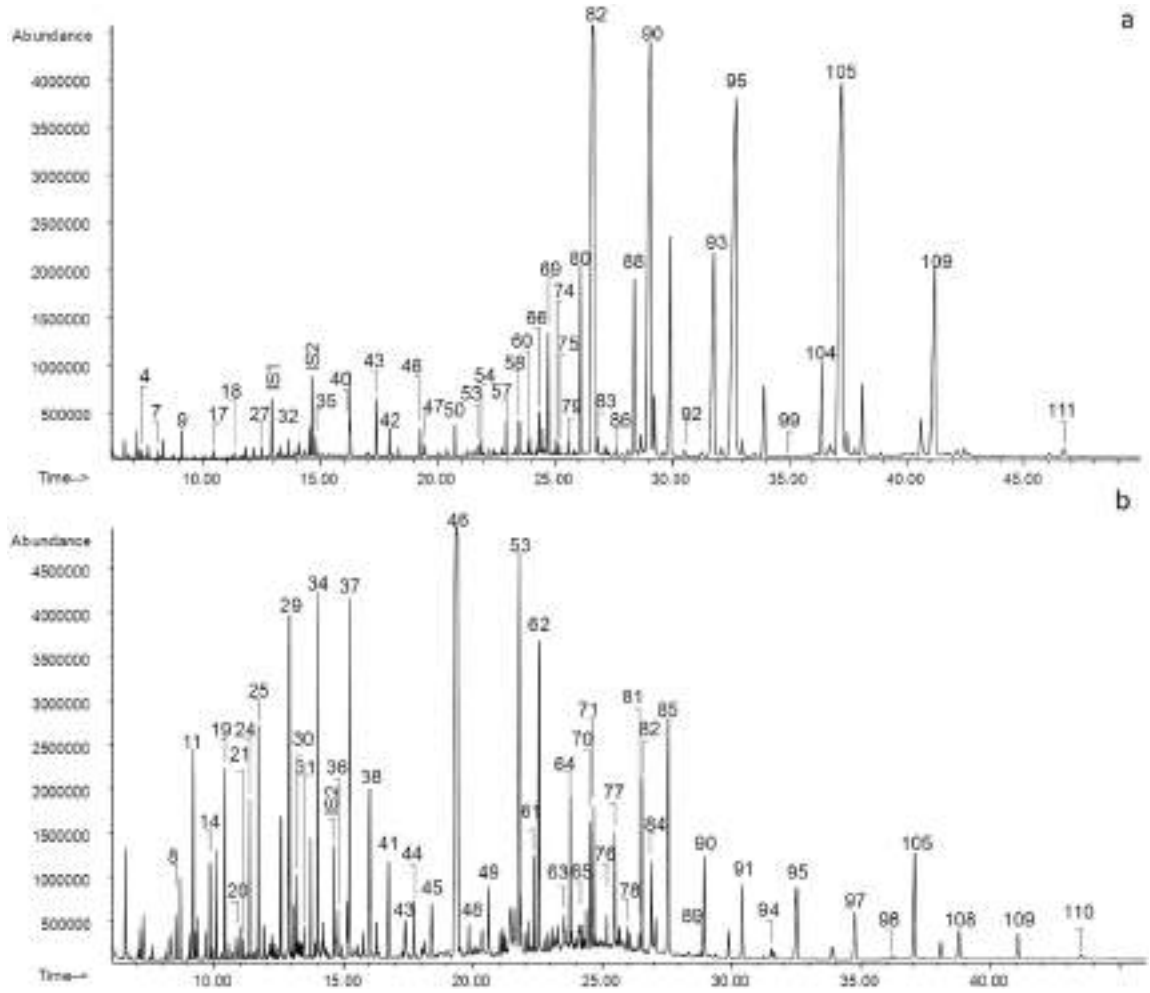

Fig. 5. Total ion current chromatogram of a) neutral and b) acidic fraction of sample #2681. (IS1 = hexadecane, IS2 = tridecanoic acid). The acidic and alcoholic species are present as TMS-derivatives. "*": phthalate contamination. The numbers refer to Table 2.

which showed a peak of ricinoleic acid (12-hydroxy-9-cis-octadecenoic), which is a marker of castor oil obtained from the seeds of *Ricinus communis* L. (Euphorbiaceae). This acid has been previously identified in Egyptian mummy samples (Copley et al., 2005; Tchapla et al., 2004).

The presence of non-negligible amounts of odd chain length fatty acids and cholesterol indicates that animal fats were present in the samples. They could result from either lipid tissues from the body or from an ingredient of animal origin specifically added in the mummification recipe (Evershed et al., 1999). Odd-carbon-numbered straight-chain fatty acids (in particular pentadecanoic acid, C15:0 and heptadecanoic acid, C17:0) and the corresponding branched chain fatty acids suggest the presence of animal fats (in particular ruminant fats such as sheep, cattle, goats, etc.) or that the lipids had undergone bacterial degradation.

In all the samples, except for #2785, #2786, #2790 and #2794, the presence of long-chain monocarboxylic fatty acids along with the co-occurrence in the neutral fraction of long chain alcohols and alkanes, indicated the presence of natural waxes (see Fig. 5). In fact, natural waxes are complex lipid mixtures mainly consisting of long chain esters (cerides) of fatty acids with long chain alcohols, free fatty acids, hydroxy acids, alcohols, diols, and alkanes.

The molecular profile varies according to the type of wax and to the degree of ageing (Evershed et al., 1997; Heron et al., 1994; Regert et al., 2001). Here the hydroxy acids were mainly (ω-1)−OH-acids, with (ω-1)−OH-hexadecanoic acid as the most abundant. This is in line with the identification of the wax as beeswax together with the peaks corresponding to lignoceric acid (tetracosanoic acid, C24:0) in the fatty acid profile of almost all the samples. However although beeswax is likely to be the main constituent added, other waxes originating from plant epicuticular waxes could be also present, as shown in other archaeological cases (Ribechini et al., 2008). In addition, mummy lipids themselves could have undergone post-mortem transformations leading to the formation of oxydised fatty acids (Gulacara et al., 1990).

Another significant component of many of the analyzed samples (except for samples #2679, #2770, #2774, #2783 and #2795) was the terpenic fraction: monoterpenes, sesquiterpenes, diterpenes and triterpenes. Monoterpenes and sesquiterpenes, which can derive from resin, wood or wood extracts, were very evident in the chromatograms of the neutral fractions. The identified species included borneol, longiborneol, camphor, myrtenol, verbenone, cuparene and others, which can derive from many plant extracts or essential oils, but due to their volatility their profile is deeply altered with ageing, so they can't be considered markers of a specific botanical specie.

Diterpenes, above all dehydroabietic acid and 7-oxo-dehydroabietic acid, highlight that an ingredient in the material used for embalming came from a resin exuded from plants of the *Pinaceae* family (Colombini et al., 2005; Mills and White, 1994; Regert and

Table 2
Peak identification for the chromatograms in Figs. 2–5.

N°	Identified compound	2678	2679	2680	2681	N°	Identified compound	2678	2679	2680	2681
1	Pinocarveol			✓		59	Pentadecanedioic Acid				✓
2	Camphor			✓		60	Eneicosanol				✓
3	Heptanoic Acid		✓			61	14-Hydroxy-Hexadecanoic Acid	✓	✓		✓
4	Octanol		✓		✓	62	15-Hydroxy-Hexadecanoic Acid				✓
5	Benzoic Acid		✓			63	16-Hydroxy-Hexadecanoic Acid	✓	✓		✓
6	Berbenone			✓		64	Eicosanoic Acid	✓			✓
7	Borneol			✓	✓	65	Hexadecanedioic Acid	✓			
8	Octanoic Acid	✓		✓	✓	66	Pentacosane		✓		✓
9	Tridecane	✓	✓		✓	67	11,12-Dihydroxy-Hexadecanoic Acid		✓	✓	
10	Nonanol		✓		✓	68	11,12-Dihydroxy-Hexadecanoic Acid		✓	✓	
11	Butanedioic Acid	✓	✓	✓	✓	69	Docosanol C22OH	✓	✓		✓
12	Dimethyl Butanedioic Acid			✓		70	9,10-Dihydroxy-Octadecanoic Acid	✓	✓	✓	✓
13	Myrtenol			✓		71	9,10-Dihydroxy-Octadecanoic Acid	✓	✓	✓	✓
14	Nonanoic Acid	✓	✓		✓	72	11,12-Dihydroxy-Octadecanoic Acid		✓	✓	
15	2-Methyl Benzoic Acid	✓				73	11,12-Dihydroxy-Octadecanoic Acid		✓	✓	
16	Verbenone			✓		74	7-Oxo-Dehydroabietic Acid Methylester				✓
17	Tetradecane	✓			✓	75	Hexacosane				✓
18	Decanol		✓		✓	76	7-Oxo-Dehydroabietic Acid	✓			✓
19	Pentanedioc Acid		✓			77	Docosanoic Acid	✓			✓
20	6-Hydroxyhexanoic Acid		✓		✓	78	7-Oxo-Didehydroabietic Acid				✓
21	Decanoic Acid		✓		✓	79	Tricosanol		✓		✓
22	Undecanol				✓	80	Heptacosane		✓		
23	Pentadecane				✓	81	Tricosanoic Acid	✓			
24	Heptanedioic Acid Methylester		✓		✓	82	Tetracosanol	✓			✓
25	Hexanedioic Acid	✓	✓	✓	✓	83	Dehydroabietic Acid Methyl Ester				✓
26	Cuparene	✓		✓		84	15-Hydroxy-Dehydroabietic Acid		✓		✓
27	Dodecanol		✓		✓	85	Tetracosanoic Acid	✓		✓	✓
28	Longiborneol	✓				86	Pentacosanol		✓		✓
29	Heptanedioic Acid		✓	✓	✓	87	Eicosanedioic Acid				✓
30	4-Hydroxybenzoic Acid			✓	✓	88	Nonacosane	✓	✓		✓
31	Dodecanoic Acid				✓	89	Pentacosanoic Acid				✓
32	Tridecanol				✓	90	Hexacosanol	✓	✓		✓
33	Calamenene	✓				91	Hexacosanoic Acid	✓			✓
34	Octanedioic Acid	✓	✓	✓	✓	92	Heptacosanol		✓		✓
35	Tetradecanol		✓		✓	93	Hentriacontane	✓	✓		✓
36	Vanillic Acid			✓	✓	94	24-Hydroxytetracosanoic Acid				✓
37	Nonanedioic Acid	✓	✓	✓	✓	95	Octacosanol	✓	✓		✓
38	Tertadecanoic Acid		✓	✓		96	3-Hydroxycholestestane		✓		
39	Hexadecenoic Acid		✓	✓	✓	97	Octacosanoic Acid	✓			✓
40	Pentadecanol		✓		✓	98	26-Hydroxyhexacosanoic Acid				✓
41	Decanedioic Acid	✓	✓	✓	✓	99	Nonacosanol				✓
42	Hexadecanoic Acid		✓	✓	✓	100	Nor-B-Amyrone			✓	
43	Hexadecanoic Acid Methylester		✓		✓	101	A-Amyrine			✓	
44	Pentadecanoic Acid			✓	✓	102	Lupeon			✓	
45	Undecanedioic Acid		✓	✓	✓	103	Lupeol			✓	
46	Hexadecanoic Acid	✓	✓	✓	✓	104	Tritriacontane	✓			✓
47	Heptadecanol				✓	105	Triacontanol	✓	✓		✓
48	Dodecanedioic Acid	✓	✓		✓	106	Moronic Acid	✓		✓	
49	Heptadecanoic Acid		✓		✓	107	Oleanonic Acid			✓	
50	Octadecanol	✓	✓	✓	✓	108	Triacontanoic Acid				✓
51	Tridecanedioic Acid	✓				109	Dotriacontanol	✓	✓		✓
52	Octadecenoic Acid	✓		✓	✓	110	Dotriacontanoic Acid				✓
53	Octadecanoic Acid	✓	✓	✓	✓	111	Tetratriacontanol		✓		✓
54	Nonadecanol				✓	112	20,24-Epoxy-25-Hydroxydammaren-3-One			✓	
55	Tetradecanedioic Acid	✓				113	Hydroxydammarenone			✓	
56	Nonadecanoic Acid				✓	114	Oleanonic Aldehyde			✓	
57	Eicosanol		✓		✓	115	Masticadienoic Acid			✓	
58	Retene		✓		✓	116	Isomasticadienoic Acid			✓	

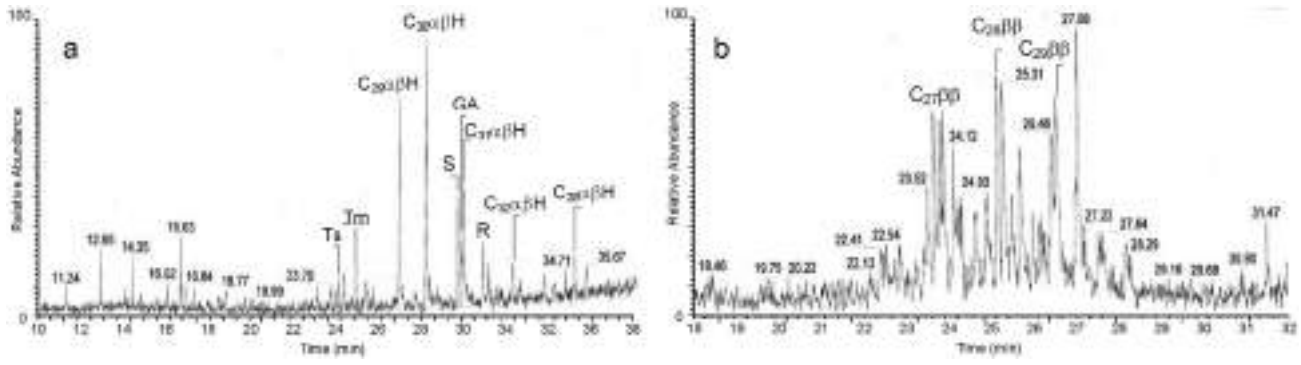

Fig. 6. Mass fragmentograms *m/z* 191 (terpanes) (a) and *m/z* 217 + 218 (steranes) (b) obtained for sample #2680 with procedure B.

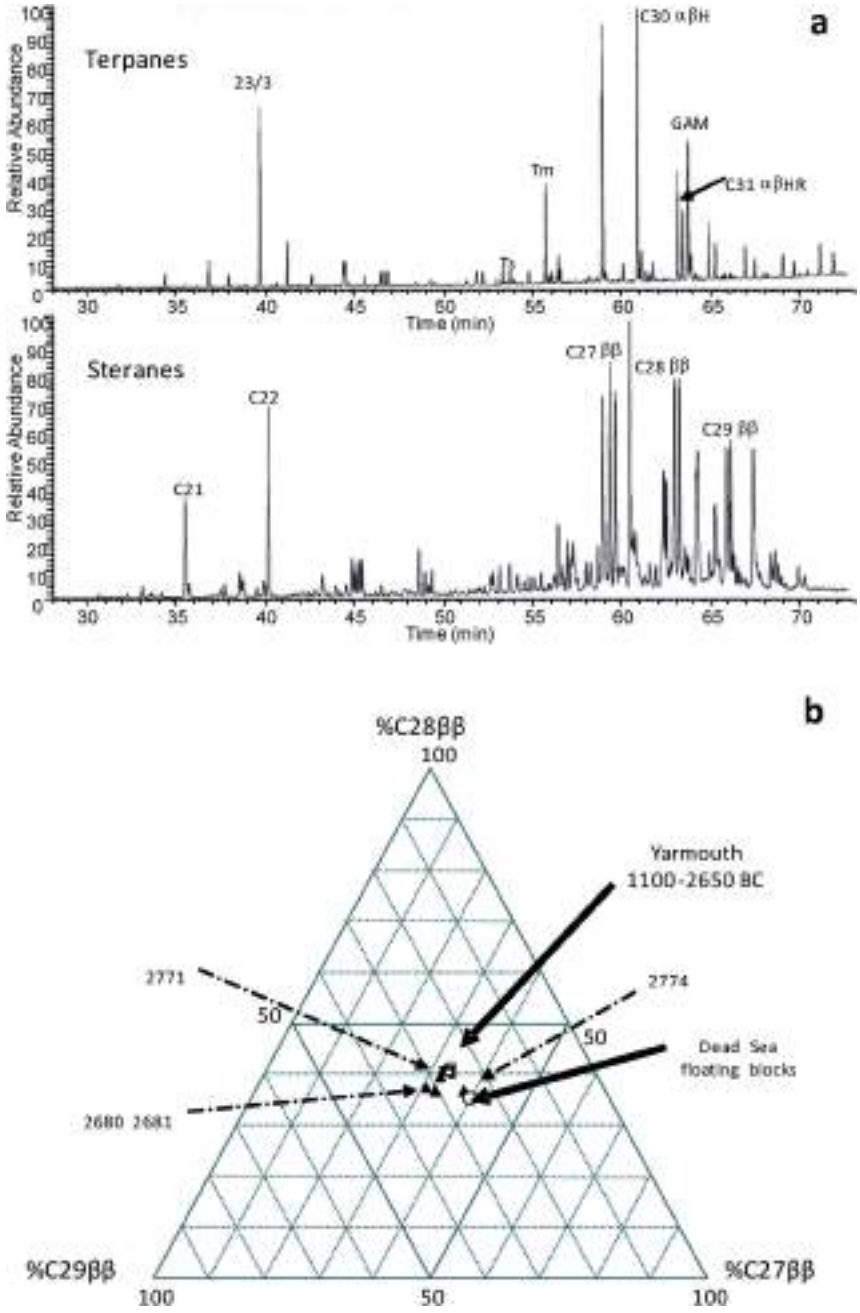

Fig. 7. a) Mass fragmentograms *m/z* 191 (terpanes) and *m/z* 217 (steranes) obtained for sample #2774, b) Composition of ββ steranes in a ternary diagram.

Table 3
Gross composition, isotope and molecular data of samples #2680, #2681, #2771, #2774. Sat.:saturated hydrocarbons; Aro.: aromatic hydrocarbons.

Sample number	Type of sample	Extractable organic matter (% by w./sample)	Sat. (%)	Aro (%)	Resins (%)	Asphaltenes (%)	$\delta^{13}C$ sat.	$\delta^{13}C$ aro	$\delta^{13}C$ res	$\delta^{13}C$ asp	GA/ C31R	GA/ C30αβH	Ts/ Tm	C29 20 S/ R	ppm C30 hopane	% C27	% C28	% C29
2680	Mummy										1.75	0.45	0.24	0.27		32.7	36.8	30.5
2681	Mummy										2	0.61	0.09	0.35		31	37.2	31.8
2771	Mummy	2.2	0.4	1	11.7	86.9	−28	−25	−24.6	−25.5	1.59	0.44	0.09	0.26	481	32.4	39.6	28
2774	Mummy	3	0.3	0.6	11.2	87.9	−26	−26	−25.3	−25.1	1.96	0.51	0.13	0.56	192	37.7	36.8	25.5

Rolando, 2002; Stacey et al., 2006).

Large amounts of phenolic compounds (such as acetovanillone, hydroxybenzoic and vanillic acids) (Lucejko et al., 2009) and of condensed aromatic hydrocarbons (PAHs), mainly phenanthrene, were revealed in many of the samples. The concomitance of these two classes of compounds suggests that one component of the mummification balm was obtained from combustion or partial dry-distillation of wood tar, tar, oil or wood. Phenanthrenes include

Table 4
Gross composition, isotope and molecular data of samples used as references for bitumen. Sat.:saturated hydrocarbons; Aro.: aromatic hydrocarbons.

Sample number	Type of sample	Date	Extractable organic matter (% by w./sample)	Sat. (%)	Aro (%)	Resins (%)	Asphaltenes (%)	$\delta^{13}C$ aro	$\delta^{13}C$ asp	GA/C31R	GA/C30αβH	Ts/Tm	C29 20 S/R	ppm C30 hopane	% C27	% C28	% C29	Origin of bitumen
69B	Geological sample	Dead Sea	98.7	1.8	10.2	21.9	66.1		−29.9		0.44	0.04	0.71		40	35	25	
68B	Geological sample	Dead Sea							−29.4		0.44	0.06						
69A	Geological sample	Dead Sea							−29.9		0.44	0.05						
Yarmouth-2233	Archaeological bitumen	1100-1200 BCE		0.1	1.2	7.6	91.2	−29.7	−29.2	1.56	0.46	0.07	0.64	1682	32.7	40.6	26.8	Dead Sea[a]
Yarmouth-2234	Archaeological bitumen	2650-2200 BCE		0.4	0.5	4	95.2	−30.4	−29.3	1.32	0.47	0.08	0.7	387	33	41.1	26	Dead Sea[a]
Yarmouth-2235	Archaeological bitumen	2650-2200 BCE		0.2	0.5	4.2	95.1	−30.1	−29.3	1.56	0.43	0.07	0.65	824	33.4	41.2	25.4	Dead Sea[a]
Yarmouth-2236	Archaeological bitumen	2650-2200 BCE		0.1	0.6	4.9	94.4	−29.9	−29.3	1.63	0.46	0.07	0.66	1194	33.4	40.3	26.4	Dead Sea[a]
Yarmouth-2239	Archaeological bitumen	2650-2200 BCE		0.3	0.5	4.1	95.2	−29.7	−29.4	1.63	0.44	0.07	0.67	568	34.2	39.7	26.1	Dead Sea[a]
Yarmouth-2249	Archaeological bitumen	2650-2200 BCE		0.8	1.1	4.8	93.3	−29.7	−29.4	1.53	0.45	0.08	0.75	325	33.8	40.5	25.7	Dead Sea[a]
Yarmouth-2250	Archaeological bitumen	2650-2200 BCE		0.3	0.7	5.4	93.6	−29.6	−29.3	1.58	0.44	0.07	0.67	750	33.7	40.3	26	Dead Sea[a]
Yarmouth-2252	archaeological bitumen	1300-1400 BCE		0.4	1.3	9.5	88.8	−29.7	−29.4	1.62	0.45	0.07	0.68	509	32.8	40.8	26.5	Dead Sea[a]
237	Mummy	40-405 AD							−26.7		0.49	0.04						Dead Sea[b]
255	Mummy	1105-800 BCE							−24.5		0.51	0.07						Dead Sea[b]
254	Mummy	50-405 AD							−25		0.42	0.05						Dead Sea[b]
323	Mummy	753-404 BCE							−25.2		0.39	0.08						Dead Sea[b]
238	Mummy	50-405 AD							−25.8		0.16	0.06						Dead Sea[b]
322	Mummy	753-404 BCE							−23.3		0.38	0.1						Dead Sea?[b]
252	Mummy	50-405 AD							−24		0.29	0.11						Dead Sea?[b]

[a] Connan (*paper in preparation*).
[b] (Macke et al., 2002).

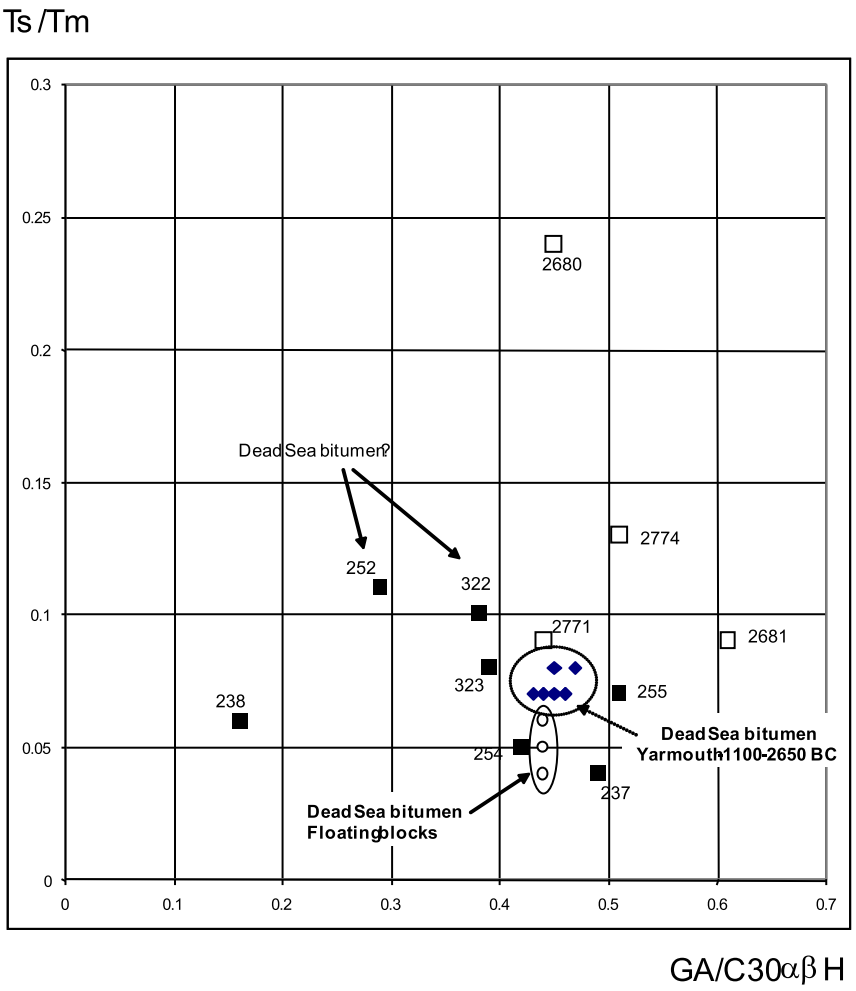

Fig. 8. Plot of Ts/Tm vs. GA/C29αβH: comparison of the four selected samples (#2680, #2681, #2771, #2774 – Table 3) analyzed with references from Dead Sea floating blocks, mummies and bitumen from Tell Yarmouth (Table 4).

retene (1-methyl-7-isopropyl phenanthrene) and abietatrienone, indicating that the pitch was obtained from Pinaceae wood. This evidence is also supported by the presence of methyl-dehydroabietate, which derives from the reaction of dehydroabietic acid with methanol produced during the dry distillation of resinous wood (Colombini et al., 2005).

In six samples (#2504, #2678, #2680, #2770, #2775, #2704 and #2779), the high retention time region was characterized by the presence of triterpenoids in the neutral as well as in the acidic fractions (Fig. 4). Moronic, oleanonic, iso-masticadienonic and masticadienonic acids were observed in the acidic fraction, while nor-β-amyrone, hydroxydammarenone and 17-nor-oleanone, were found in the neutral fraction (Fig. 4a). All these compounds are characteristic biomarkers of mastic resin. Mastic resin, obtained from the *Pistacia genus*, was widespread in the Mediterranean in ancient times, and has been found to be an ingredient in chemical balms of mummification in ancient Egypt (Buckley et al., 2004; Colombini et al., 2000; Lucejko et al., 2012).

3.2. Bitumen analysis via procedure B

Bitumen was detected thanks to the occurrence of specific biomarkers in seven samples by procedure A. However, procedure A does not allow us to obtain a comprehensive compositional profile of the bitumen and to quantify the biomarkers in order to achieve information on bitumen geographical origin. To overcome these problems, the dedicated protocol of procedure B (Connan et al., 2006a) was used. The focus was thus on the isolation of saturated hydrocarbons. To investigate their profile the diagnostic mass fragmentograms corresponding to the *m/z* 217 and 218, and *m/z* 177 and 191 of steranes and terpanes were extracted and are shown in (Fig. 6).

In the four samples examined by this specialized protocol, namely #2680, #2681, #2771, #2774, the fossil biomarkers, steranes and terpanes were clearly identified. Their terpane distribution is characterized by the occurrence of the complete family of C19-C28 tricyclopolyprenanes, a low Ts/Tm ratio and a high amount of gammacerane (Fig. 7a). In addition the sterane distribution (Fig. 7a, Table 3) looks similar to that of Dead Sea bitumen exemplified by samples of the floating blocks from the Dead Sea and by bitumen excavated from Tell Yarmouth and covering different periods from 1100 BCE to 2650 BCE (Table 4). At Tell Yarmouth, located at 45 km from the Dead Sea, a complete set of massive lumps of pure bitumen, some of them weighting over 400 g, was unearthed. All of them were identified as originating from the Dead Sea (Connan, *paper in preparation*). This set gave a reference suite of archaeological bitumen from the Dead Sea which completes the data on the Dead Sea floating blocks, which are present day reference. These archaeological bitumen are likely more appropriate references for mummy bitumen for they are ancient materials

Table 5
List of materials identified in analyzed samples.

Samples	Glycerolipids	Waxes	Pinaceae resin	Castor oil	Pinacea pitch	Triterpenoids	Bitumen	
	Fatty acids	Long chain alcohols; acids and hydroxyacids	Didehydroabietic dehydroabietic; 7-oxo-dehydroabietic; 15-hydroxy-7-oxo-dehydroabietic; 15-hydroxy-dehydroabietic acids	Ricinoleic acid	Retene; PAH-polycyclic aromatic hydrocarbon; 18-norabietatriene; 19-norabietatriene; abietatrienone; methyl-dehydroabietate	Nor-β-amyrone; α-amyrine; lupeon, lupeol; 20,24-epoxy-25-hydroxydammarenone; hydroxydammarenone; oleanonic aldehyde; moronic, oleanonic, masticadienoic, isomasticadienoic acids	Hopanes; steranes; gamma cerane	
Procedure	**A**							**B**
1896	x	x					x	n.a.
2504	x	x		x		x		
2759	x	x	x	x	x		x	n.a.
2678	x	x	x			x	x	n.a.
2679	x	x			x			
2680	x		x			x		x
2681	x	x	x		x			x
2770	x	x				x	x	n.a.
2771	x	x	x	x	x		traces	x
2774	x	x					traces	x
2702	x	x	x		x			
2703	x	x	x		x		x	n.a.
2704	x	x	x			x		
1811	x	x			x			
2785	x		x		x			
2795	x	x		x				
2775	x	x		x		x		
2776	x	x	x		x			
2779	x	x				x		
2783	x	x						
2786	x		x		x			
2790	x		x		x			
2794	x		x		x			

n.a.: samples not analyzed by procedure B.

which may have been submitted to aging. Apart from its use for mummification purposes, Dead Sea bitumen was obviously considered as a precious material for it was discovered at Toukh el-Qaramous, Egypt (Edgar, 1906) in a monetary treasure from the Ptolemaic period associated with gold Egyptian figurines and statues, silver dishes, etc. and more recently in a grave of the 7th Century-Mound Ship-Burial at Sutton Hoo (Suffolk, UK) (Burger et al., 2016). The composition of ββ steranes (Tables 3 and 4) reproduced in a ternary diagram (Fig. 7b) confirms that our four mummy samples are in good agreement with the references, both the present day bitumen from the Dead Sea and the archaeological bitumen from Tell Yarmouth. The plot of the diagnostic terpane ratios GA/C30αβH vs. Ts/Tm (Fig. 8) matches well with the reference Dead Sea bitumen. However the distribution of balms from mummies are slightly more scattered than the reference data. Balms from mummies from this study showed higher values in Ts/Tm and GA/C30αβH, which suggests that the Dead Sea bitumen may have been affected by an incipient biodegradation (Frontera-Suau et al., 2002). However the biodegradation effects here are limited and do not hamper the recognition of diagnostic Dead Sea molecular characteristics.

4. Conclusions

Our investigations show that the examined balms from animal mummies were not significantly different from the balms from human mummies. The constituents identified in both types of balms were: fats or oils of uncertain origin, beeswax or other waxes, castor oil, conifer resins but also pitch derived from the pyrolysis of conifer wood, mastic resin and castor oil (Table 5). Our results are consistent with recently published findings by other authors.

The residues isolated from potsherds of jars and amphorae were found to be both pure products and mixtures. As pure natural products, the following were identified: fat in a stone vase from the Old Kingdom discovered in a pit from the Sakkara area, castor oil on a potsherd dated from the New Kingdom, Thebes area, and fat from the Ramesside period on a potsherd from the area of Thebes. In the seven remaining samples, the organic residue stuck on potsherds was composed of a mixture which was likely to have been prepared for ritual purposes, including mummification. These mixtures were very similar to those identified in balms from mummies and contained: fat, pinaceae resin, pinaceae pitch, mastic resin, castor oil. The mixtures dated from the Copto-Byzantine period were based on fat, Pinaceae resin and pitch: above all conifer products mixed with oil and fat. Other samples, dated from the New Kingdom and Ramesside period were more diversified, including not only Pinaceae products but also *Pistacia* resin, castor oil, beeswax and fat.

This study suggests that the methods used by most researchers tend to underestimate the presence of bitumen in mummy balms. In fact procedure A, optimised to analyse the majority of organic constituents present in mummification balms, such as acyl-lipids, waxes and resinous materials, led to the qualitative detection of bitumen only in few cases. Therefore a specifically optimised procedure (procedure B) was necessary to quantitatively study the molecular pattern of bitumen constituents, in order to identify the geographical origin of the bituminous materials. In particular, the

bitumen in the balms was identified as Dead Sea bitumen (Connan et al., 2006a; Macke et al., 2002).

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