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A chemical investigation by headspace SPME and GC–MS of volatile and semi-volatile terpenes in various olibanum samples

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Abstract

Six different olibanum samples with certified botanical origin were analyzed by headspace SPME–GC/MS in order to define their mono-, sesqui- and diterpenic composition, as pertinent criteria of identification. *Boswellia carteri* and *Boswellia sacra* olibanum have quite similar chemical composition, with isoincensole acetate as the main diterpenic biomarker. Although *Boswellia serrata* olibanum also exhibits this biomarker, the presence of methylchavicol, methyleugenol and an unidentified oxygenated sesquiterpene distinguishes *B. serrata* olibanum from the two other species. The characteristic chemical compounds of *Boswellia papyrifera* are the diterpenic biomarkers incensole and its oxide and acetate derivatives, *n*-octanol and *n*-octyl acetate. *Boswellia frereana* olibanum is devoid of diterpenes of the incensole family but contains a high amount of many dimers of α-phellandrene. The chemical composition of olibanum, which is demonstrated to be different for each *Boswellia* species allowed the determination of the taxonomic origin of frankincense samples purchased on various markets in East Africa, in the Near East and in Yemen. Moreover, terpenic fingerprints allowed the botanical origin of olibanum used in traditional incense mixtures to be identified. Furthermore, this study gave us the opportunity to assign a botanical origin to an archaeological frankincense sample.

Keywords: *Boswellia*; Olibanum; Frankincense; Gum resin; Terpenes; SPME; Archaeological sample; GC–MS

1. Introduction

One of the research axes of our laboratory is the study of mummification balms and unguents of the Ancient Egypt. According to ancient papyrus and hieroglyphs on the walls inside temples, the recipe of these balms is elaborate and needs many constituents with their own ritual significance: vegetable oils, animal fats, pitches, waxes, honey, gums, resins, gum resins (Tchapla et al., 1999). Among the gum resins, olibanum (also called frankincense), used alone or in mixture, represents the universe of the Punt country from where the Gods of Egypt originate. Egyptologists think that olibanum as well as myrrh, another precious gum resin, was essentially used either in perfumes, or in fumigation during embalming ceremonies or possibly as a component of mummification balms for Pharaoh, persons of rank, or priests (at least from the 18th Dynasty). Incense burners have been found in graves since the Fifth Dynasty (Lucas and Harris, 1989). As incense was a generic term in Ancient Egypt, and frankincense could be obtained from different *Boswellia* trees (Baum, 1999), it is of fundamental importance to develop an analytical method in order to discriminate olibanum from other substances used as incense and to identify its botanical origin.

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Our aim was to determine the possible occurrence of olibanum in mummification balms and unguents by headspace SPME. Among the methods of sample pre-treatment for GC–MS analysis, headspace SPME offers certain advantages when dealing with archaeological samples. Firstly, it is a sample non-destructive method. Secondly, it concentrates volatile compounds allowing their detection even at trace level and so requires only small amount of sample. Thirdly, non-volatile compounds such as fats, waxes and polysaccharides, often dominant in mummification balms, are not extracted by such a method, thus avoiding the fastidious sample pre-treatment required for classical GC–MS analysis. Previously, we have successfully applied headspace SPME to a wide range of resins and gum resins and to archaeological samples (Hamm et al., 2004).

Olibanum, is a natural oleo-gum-resin that exudes from tappings in the bark of *Boswellia* trees. The genus *Boswellia* of the Burseraceae family includes approximately 23 species of small trees that grow mainly in Arabia, on the eastern coast of Africa and in India. Frankincense is a complex mixture composed of about 5–9% highly aromatic essential oil (mono- and sesquiterpenes), 65–85% alcohol-soluble resins (diterpenes, triterpenes), and the remaining water-soluble gums (polysaccharides) (Tucker, 1986; Khan and Farooqi, 1991). Mono- and sesquiterpenes are highly volatile compounds, diterpenes exhibit low volatility, triterpenes very low volatility and polysaccharides are not volatile. Mono- and sesquiterpenes can be easily trapped by headspace SPME, but they are rarely characteristic of olibanum, because of their frequent occurrence in the vegetable kingdom. However, considering compounds with low volatility, conclusions could be very different. Diterpenes like incensole or isoincensole, their oxide or acetate derivatives and triterpenes like the boswellic acids can be considered as biomarkers of olibanum. Because headspace SPME is not relevant to the analysis of compounds of very low volatility, such as triterpenes, the present work focuses on the semi-volatile diterpene biomarkers. A previous paper presents the optimization of experimental parameters for headspace SPME of the diterpenes of a commercial olibanum sample (Hamm et al., 2003). Several publications deal with the GC or GC–MS analysis of volatile components of olibanum extracts or essential oils (Baser et al., 2003; Basar et al., 2001; Baratta et al., 1998; Dekebo et al., 1999; Hayashi et al., 1998; Ammar et al., 1994; Lawrence, 1992; Chiavari et al., 1991; Ma et al., 1991; Vernin et al., 1990; etc.), but samples were often purchased from markets and so lacked certified botanical origin. Thus, their conclusions cannot be used for positive identification.

From an archaeological point of view, knowing the botanical origin of olibanum used in mummification balms would be key information, documenting traditional craftsmanship and possible trade routes from ancient Egypt. To fill this gap in knowledge, the present contribution focuses on possible ways of determining the botanical origin of olibanum samples, of both ancient and modern origin.

The first step of this work was to build a database of the volatile terpenes of each olibanum with certified botanical origin and to derive characteristic signatures associated with the botanical origin. A second step was to apply these signatures to define the species of samples purchased in markets from various countries, alone or mixed with other plant products. Finally, we tested our method on an archaeological frankincense sample, not only to find its botanical origin, but also to determine which compounds it could still contain after approximately 2000 years.

2. Results and discussion

2.1. Olibanum of certified botanical origin

The main compounds detected by headspace SPME–GC/MS in the six olibanum samples of certified origin are collected in Table 1. We have verified that qualitative results obtained with headspace SPME were identical with those obtained when simply dissolving olibanum samples in dichloromethane, except for triterpenes.

The first observation is the similarity in chemical composition between the two *Boswellia carteri* samples and the sample of *Boswellia sacra*. For these three olibanum, we found α-pinene (**2**), β-myrcene (**8**) and limonene (**14**) as the predominant monoterpenes. β-Caryophyllene (**73**) is the major sesquiterpene as well as α-copaene (**65**), α-humulene (**78**) and caryophyllene oxide (**95**). The characteristic olibanum compounds: isoincensole and isoincensole acetate (**128**) together with cembrene A (**120**) are the main diterpenes. These three samples (*B. carteri* olibanum and *B. sacra* olibanum) differ by only small compositional variations: the occurrence of ledol (**96**), octyl acetate (**40**) and dimer 2 (**111**) being the single dimer of α-phellandrene in *B. sacra*. The main difference between the two *B. carteri* olibanum samples is the absence of dimers of α-phellandrene (**108**, **111**, **114**, **115**, **117**, **118**) in detectable amount in *B. carteri* from Aden. Moreover, β-cubebene (**68**) occurs in a relatively high amount (1.1%) in *B. carteri* olibanum from Aden whilst it is absent or less than 0.1% in *B. carteri* olibanum from Somalia.

Boswellia serrata olibanum has a chemical composition close to that of the two *B. carteri* and of *B. sacra*, however some components distinguish it: Firstly, α-thujene (**1**) predominates over α-pinene (**2**). Secondly, *B. serrata* olibanum contains compounds that are absent in those from other *Boswellia* and consequently could be used as markers: methylchavicol (**38**), *p*-anisaldehyde

Table 1
Main components detected by headspace SPME–GC/MS in the six reference olibanum samples with certified botanical origin and in eight olibanum samples without botanical origin

Peak no.	Compound	RI[a]	*B. carteri* Somalia	*B. carteri* Aden	*B. frereana* Somalia	*B. papyrifera* Ethiopia	*B. sacra* Oman	*B. serrata* India	Somalia (Wolf)	Somalia (Asie Concept)	Eritrea (Wolf)	Israel (Jerusalem)	Lebanon (Beirut)	Yemen (Sa'da)	Yemen (Ta'izz)	Yemen (San'a)
1	α-Thujene	916	–	0.1	**9.8**	–	2.0	**11.7**	0.1	0.2	–	–	–	**6.5**	1.4	**3.5**
2	α-Pinene	942	**23.2**	**6.3**	**12.4**	0.5	**13.9**	3.4	**3.9**	**4.7**	2.2	–	–	**4.3**	**5.5**	**4.0**
3	4-Methylene-1-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene	946	0.1	0.1	0.4	–	0.2	0.3	–	–	–	–	–	6.6	1.7	3.0
4	Camphene	949	0.6	0.2	0.6	–	0.8	0.1	0.9	0.4	–	–	–	–	–	1.1
5	Isomer of 4-methylene-1-(1-methyl-ethyl)-bicyclo-[3.1.0]hex-2-ene	952	0.2	0.2	0.6	–	0.7	tr	–	1.2	–	–	–	–	–	1.8
6	Sabinene	963	2.2	0.3	3.2	–	2.1	3.0	0.3	0.6	–	–	–	4.8	0.7	1.5
7	β-Pinene	966	1.5	0.4	1.4	–	0.9	0.5	0.3	0.6	–	–	–	–	0.7	1.5
8	β-Myrcene	994	**4.5**	**4.4**	0.3	–	**5.2**	**7.0**	**4.0**	**0.4**	–	–	–	1.7	**1.7**	0.2
9	α-Phellandrene	1003	2.1	0.3	1.1	–	0.8	0.9	0.5	–	–	–	–	1.3	0.2	1.5
10	*n*-Hexanoic acid	1003	–	–	–	**0.2**	–	–	–	–	**1.8**	**8.1**	**4.2**	–	–	–
11	*o*-Methylanisole	1005	–	0.2	–	–	–	–	1.2	0.5	–	–	–	–	0.5	–
12	δ-3-Carene	1006	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–
13	*p*-Cymene	1014	2.5	1.5	**7.8**	0.2	**5.5**	**4.7**	1.2	1.7	–	–	–	**9.3**	1.2	**7.8**
14	Limonene	1020	**22.4**	**10.2**	1.7	0.1	**6.9**	2.6	**7.1**	**3.3**	**5.2**	–	–	–	0.9	–
15	Eucalyptole	1022	–	–	1.7	0.1	–	–	0.9	–	–	1.4	–	3.7	1.2	4.1
16	Thujol	1049	0.1	tr	0.6	–	0.2	0.4	–	–	–	–	–	3.1	–	2.4
17	γ-Terpinene	1054	tr	–	0.1	–	0.1	0.2	tr	–	–	–	–	0.8	–	0.6
18	*n*-Octanol	1063	–	–	–	**13.9**	–	–	–	–	**11.7**	**7.7**	**12.7**	–	–	–
19	*cis*-Linalool oxide	1073	–	tr	tr	0.2	–	–	–	–	–	–	–	–	–	–
20	*n*-Heptanoic acid	1076	–	–	–	**tr**	–	–	–	–	**1.8**	**5.9**	**2.1**	–	–	–
21	Linalool	1077	0.2	0.9	1.5	0.2	1.2	1.6	1.0	–	0.5	–	–	–	0.9	–
22	Oxygenated monoterpene[b]	1108	0.3	0.8	2.7	–	2.1	tr	–	0.9	–	–	–	–	1.7	–
23	Thujone	1111	0.1	–	0.8	–	0.6	1.0	–	–	–	–	–	3.5	–	1.8
24	*trans p*-Mentha-2,8-dienol	1113	0.3	0.3	–	–	0.3	–	0.1	0.2	–	–	–	2.0	–	1.6
25	*n*-Octyl formiate	1117	–	–	–	**tr**	–	–	–	–	**1.5**	**0.9**	**2.8**	–	–	–
26	α-Campholenal	1119	0.4	0.2	0.7	tr	0.8	0.1	–	0.2	–	–	–	–	0.7	0.9
27	*trans p*-2,8-Menthadien-1-ol	1123	0.1	0.3	–	–	–	–	0.2	0.1	–	–	–	–	–	–
28	*trans*-Pinocarveol	1129	0.9	0.5	1.7	0.1	1.9	0.2	0.4	0.6	–	–	–	1.9	0.9	2.2
29	*cis*-Verbenol	1131	1.5	1.1	3.5	–	3.5	0.7	0.1	1.0	–	–	–	–	1.5	2.3
30	*cis*-Sabinol	1170	0.5	–	1.5	–	0.3	0.9	–	–	–	–	–	5.1	–	5.9
31	4-Terpineol	1182	0.1	tr	2.8	–	0.6	0.1	0.1	–	–	–	–	6.5	0.2	4.2
32	Methyl-acetophenone	1186	tr	tr	0.2	–	0.2	–	–	0.2	–	–	–	–	–	–
33	*p*-Cymen-8-ol	1187	1.1	0.9	0.3	–	0.7	0.4	0.3	1.1	–	–	–	4.0	1.0	6.0
34	α-Terpineol	1193	0.3	0.4	0.9	–	0.2	–	0.4	0.2	–	–	–	3.0	0.4	3.3
35	Myrtenal	1197	–	0.6	0.7	–	0.8	–	–	0.3	–	–	–	–	1.4	0.6
36	*n*-Octanoic acid	1197	–	–	–	**0.8**	–	–	–	–	**2.3**	**6.9**	**3.7**	–	–	–
37	Myrtenol	1198	0.7	–	0.3	–	0.4	–	–	0.4	–	–	–	–	–	–
38	Methylchavicol	1200	–	–	–	–	–	**8.9**	–	–	–	–	–	–	–	–
39	2-Methyl-5-(1-Methylethyl)-1,3-cyclohexadiene monoepoxide	1202	0.9	–	1.0	–	–	0.2	0.6	–	–	–	–	–	–	–
40	*n*-Octyl acetate	1220	–	–	–	**64.6**	0.3	–	–	0.4	**36.8**	**35.6**	**50.1**	–	–	–
41	Verbenone	1221	1.4	0.9	3.1	–	3.0	0.2	0.1	1.2	–	–	–	0.8	2.2	3.6
42	*trans*-Carveol	1225	1.3	1.4	0.9	0.3	1.4	0.6	0.4	1.3	2.5	–	–	0.6	1.6	2.1
43	*cis*-Carveol	1230	0.4	0.4	–	–	0.2	–	0.1	0.3	0.6	–	–	–	0.1	–
44	Cumaldehyde	1235	0.3	0.1	0.5	–	0.3	0.1	0.1	0.1	–	–	–	1.6	0.1	1.9

(*continued on next page*)

Table 1 (*continued*)

Peak no.	Compound	RI[a]	*B. carteri* Somalia	*B. carteri* Aden	*B. frereana* Somalia	*B. papyrifera* Ethiopia	*B. sacra* Oman	*B. serrata* India	Somalia (Wolf)	Somalia (Asie Concept)	Eritrea (Wolf)	Israel (Jerusalem)	Lebanon (Beirut)	Yemen (Sa'da)	Yemen (Ta'izz)	Yemen (San'a)
45	Carvone	1237	1.0	0.8	0.1	0.3	0.7	0.1	0.3	0.6	1.6	–	–	0.3	0.4	0.5
46	α-Cyclogeranyl acetate	1241	–	–	2.2	–	–	–	–	–	–	–	–	–	–	–
47	*p*-Anisaldehyde	1242	–	–	–	–	–	**0.5**	–	–	–	–	–	–	–	–
48	3,5-Dimethoxy-toluene	1264	0.7	1.1	–	–	0.5	–	1.5	0.3	–	0.6	1.5	1.3	0.8	1.2
49	*n*-Nonanoic acid	1265	–	–	–	**0.5**	–	–	–	–	**1.2**	**3.4**	**2.9**	–	–	–
50	Bornyl acetate	1275	0.9	0.3	3.8	0.2	1.3	–	0.3	0.5	0.4	3.8	–	0.6	0.7	2.4
51	*m*-Thymol	1276	0.5	0.1	tr	–	tr	0.2	–	–	–	–	–	3.5	–	1.8
52	Cuminol	1276	tr	0.1	0.3	–	0.2	tr	0.1	0.3	–	–	–	0.5	0.1	1.2
53	Carvacrol	1281	tr	–	1.9	–	0.3	0.2	0.2	0.2	–	–	–	2.4	0.2	4.0
54	Perillol	1282	tr	0.3	–	–	tr	0.2	0.1	0.1	–	–	–	–	0.2	–
55	Unidentified compound[b]	1312	–	–	**1.9**	–	–	–	–	–	–	–	–	**2.3**	–	**1.0**
56	Unidentified compound[b]	1314	–	–	**2.0**	–	–	–	–	–	–	–	–	**1.7**	–	**2.6**
57	1-Methyl-4-(1-methyl-ethenyl)-1,2-cyclohexanediol	1321	0.6	0.3	–	tr	0.4	–	–	–	0.8	–	–	–	–	–
58	Terpinyl acetate	1344	0.3	–	–	–	tr	0.1	0.4	–	–	–	–	–	–	–
59	α-Cubebene	1347	0.2	0.8	–	–	0.6	0.1	1.8	3.0	–	–	–	0.5	1.0	–
60	3,4-Dimethoxystyrene	1354	0.1	0.6	0.5	–	0.1	–	tr	–	–	–	–	–	–	–
61	Cyclosativene	1360	–	–	–	–	tr	0.1	–	–	–	–	–	–	0.8	–
62	α-Ylangene	1362	–	–	–	–	–	0.1	tr	–	–	–	–	–	–	
63	*n*-Decanoic acid	1367	–	–	–	**0.1**	–	–	–	–	**1.4**	**1.1**	**2.5**	–	–	–
64	Neryl acetate	1375	–	–	–	0.1	–	–	–	–	1.0	–	–	–	–	–
65	α-Copaene	1383	**1.6**	**5.5**	0.9	–	**4.0**	1.1	**2.7**	**2.6**	–	–	–	0.5	**2.8**	tr
66	*n*-Hexyl hexanoate	1387	–	–	–	**0.9**	–	–	–	–	**0.2**	**2.5**	**0.7**	–	–	–
67	β-Bourbonene	1388	0.1	–	0.2	–	0.3	4.2	0.9	–	–	–	–	2.1	7.2	1.1
68	β-Cubebene	1390	–	1.1	–	–	0.4	–	–	–	–	–	–	–	–	–
69	β-Elemene	1393	0.9	–	2.7	–	1.6	–	5.5	5.6	–	–	–	–	1.1	–
70	Methyl-eugenol	1394	–	–	–	–	–	**3.7**	–	–	–	–	–	–	–	–
71	*n*-Decyl acetate	1406	–	–	–	**0.9**	–	–	–	–	**1.9**	**2.0**	**4.8**	–	–	–
72	(*Z*)-Caryophyllene	1418	–	–	–	–	–	–	–	–	–	–	–		3.1	–
73	β-Caryophyllene	1426	**6.9**	**16.9**	–	–	**4.7**	–	**12.0**	**5.5**	–	–	–	–	**6.0**	–
74	β-Gurjunene	1427	–	–	–	–	tr	–	1.0	–	–	–	–	–	1.7	–
75	(*Z*)-α-*trans*-Bergamotene	1428	–	–	–	–	tr	0.2	–	1.1	–	–	–	–	–	–
76	α-Guaiene	1441	–	tr	–	–	tr	–	0.2	0.1	–	–	–	–	–	–
77	Aromadendrene	1444	–	–	–	–	–	0.2	0.3	–	–	–	–	–	–	–
78	α-Humulene	1454	**1.1**	**5.2**	tr	–	**3.1**	–	**5.1**	**3.3**	–	–	–	–	**5.3**	–
79	allo-Aromadendrene	1456	0.2	0.6	–	–	0.6	0.1	1.2	1.1	–	–	–	–	2.1	–
80	γ-Muurolene	1475	0.4	1.0	–	–	1.1	0.4	3.0	3.2	–	–	–	–	1.6	–
81	Germacrene D	1479	0.2	0.2	–	–	0.8	1.5	1.4	–	–	–	–	0.2	0.4	–
82	Isocaryophyllene	1480	–	–	–	–	–	**1.7**	–	–	–	–	–	–	–	–
83	β-Eudesmene	1483	0.6	0.1	0.1	–	0.5	–	3.2	2.9	–	–	–	–	0.7	–
84	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphtalene	1485	0.5	1.6	–	–	1.4	–	2.0	–	–	–	–	–	1.0	–
85	α-Muurolene	1501	1.0	0.6	–	–	0.6	0.1	2.6	1.1	–	–	–	0.1	1.6	–
86	δ-Guaiene	1503	–	tr	–	–	–	–	0.3	0.3	–	–	–	–	–	–
87	γ-Cadinene	1509	0.1	0.2	–	–	1.9	0.2	2.9	1.3	–	–	–	–	2.0	–

88	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	1511	1.9	5.7	–	–	2.4	–	0.9	9.6	–	–	–	0.4	3.6	–
89	δ-Cadinene	1524	1.0	2.0	0.1	–	2.2	0.3	1.9	1.6	–	–	–	0.1	2.6	–
90	1,2,3,4,6,8a-Hexahydro-1-isopropyl-4,7-dimethylnaphtalene	1528	tr	0.1	–	–	tr	–	0.6	1.2	–	–	–	–	–	–
91	Oxygenated sesquiterpene[b]	1529	–	–	–	–	–	**8.0**	–	–	–	–	–	–	–	–
92	Elemicin	1533	–	–	–	–	–	**0.9**	–	–	–	–	–	–	–	–
93	Lauric acid	1572	–	–	–	**0.3**	–	–	–	–	**1.5**	**0.7**	**2.8**	–	–	–
94	Hexyl caprylate + another ester	1579	–	–	–	**1.2**	–	–	–	–	**0.7**	**5.3**	**0.5**	–	–	–
95	Caryophyllene oxide	1582	**2.0**	**13.1**	0.1	–	**4.9**	–	**4.6**	**11.0**	–	–	–	0.2	**3.8**	0.1
96	Ledol	1587	–	–	–	–	2.0	–	0.3	0.4	–	–	–	0.7	0.7	–
97	Ethyl laurate	1597	–	–	–	–	–	–	–	–	0.4	–	–	–	–	–
98	Oxygenated sesquiterpene[b]	1605	–	–	–	–	0.2	–	0.8	–	–	–	–	–	4.0	–
99	1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1607	0.2	2.4	0.2	–	1.8	–	0.6	5.4	–	–	–	–	1.8	–
100	γ-Eudesmol	1609	0.5	–	–	–	0.2	0.6	–	–	–	–	–	–	–	–
101	τ-Cadinol	1634	1.2	0.1	–	–	2.7	–	1.6	1.8	–	–	–	tr	2.7	0.2
102	δ-Cadinol	1636	–	tr	–	–	–	–	0.3	0.2	–	–	–	0.1	0.6	–
103	α-Eudesmol or β-Eudesmol	1649	0.2	–	tr	–	tr	–	0.1	0.9	–	–	–	–	0.4	0.1
104	α-Cadinol	1651	–	tr	–	–	0.2	–	0.3	0.6	–	–	–	tr	0.3	–
105	Oxygenated sesquiterpene[b]	1653	–	0.3	–	–	0.1	–	0.5	1.5	–	–	–	–	0.5	–
106	Oxygenated sesquiterpene[b]	1661	–	1.0	–	–	0.4	–	0.6	2.2	–	–	–	–	0.6	–
107	(*E*)-9-Dodecen-1-ol acetate	1700	–	–	–	–	–	–	–	–	0.4	–	0.5	–	–	–
108	Dimer of α-phellandrene 1[c]	1732	tr	–	0.6	–	–	–	–	–	–	–	–	0.4	–	0.4
109	Benzyl benzoate	1761	–	–	–	–	–	–	–		0.3	0.4	0.5	–	–	–
110	Octyl caprylate	1779	–	–	–	–	–	–	–		0.3	1.6	0.2	–	–	–
111	Dimer of α-phellandrene 2[b]	1795	1.5	–	–	–	0.3	1.0	0.8	–	–	–	–	–	0.6	0.2
112	Ethyl myristate	1797	–	–	–	–	–	–	–		0.3	–	–	–	–	–
113	Dimer of α-phellandrene 3[c]	1810	–	–	**15.6**	–	–	–	–	–	–	–	–	**6.0**	–	**7.3**
114	Dimer of α-phellandrene 4[c]	1815	0.2	–	1.6	–	–	0.1	–	–	–	–	–	0.3	–	1.6
115	Dimer of α-phellandrene 5[c]	1837	0.2	–	2.4	–	–	–	0.1	–	–	–	–	1.5	0.1	–
116	Unidentified diterpene[b]	1865	–	–	tr	–	–	–	tr	–	–	–	–	0.6	0.1	0.6
117	Dimer of α-phellandrene 6[b]	1904	0.4	–	tr	–	–	–	0.4	–	–	–	–	0.2	0.4	0.5
118	Dimer of α-phellandrene 7[b]	1918	0.2	–	tr	–	tr	–	0.3	–	–	–	–	0.1	0.2	0.3
119	Unidentified diterpene[b]	1945	0.1	0.1	–	–	0.1	1.9	0.3	tr	0.3	–	–	–	0.1	–
120	Cembrene A	1959	0.3	0.7	–	1.7	0.3	2.5	2.0	0.5	0.7	0.7	0.2	–	1.2	–
121	Cembrene?	1964	–	–	–	–	–	–	–	–	0.7	–	–	–	–	–
122	Unidentified diterpene[b]	1981	tr	–	–	–	tr	0.7	0.1	–	–	–	–	–	0.1	–
123	Cembrene C	2002	tr	0.1	–	tr	tr	tr	0.4	0.1	0.5	–	–	–	0.1	–
124	Unidentified diterpene[b]	2003	–	–	–	–	–	**0.1**	–	–	–	–	–	–	–	–
125	Verticilla-4(20),7,11-triene	2004	0.1	0.1	–	–	0.1	0.3	–	–	1.6	–	3.1	–	–	–
126	Unidentified diterpene[c]	2141	**0.1**	**0.1**	–	–	**0.1**	**1.1**	**0.7**	**0.2**	–	–	–	–	**0.3**	–
127	Incensole	2150	–	–	–	**1.0**	–	–	–	–	**5.0**	**0.6**	**0.5**	–	–	–
128	Isoincensole + isoincensole acetate	2152	**1.4**	**2.1**	–	–	**1.4**	**6.9**	**8.0**	**2.5**	–	–	–	–	**3.3**	–

(*continued on next page*)

Table 1 (*continued*)

Peak no.	Compound	RI[a]	*B. carteri* Somalia	*B. carteri* Aden	*B. frereana* Somalia	*B. papyrifera* Ethiopia	*B. sacra* Oman	*B. serrata* India	Somalia (Wolf)	Somalia (Asie Concept)	Eritrea (Wolf)	Israel (Jerusalem)	Lebanon (Beirut)	Yemen (Sa'da)	Yemen (Ta'izz)	Yemen (San'a)
129	Incensole acetate	2189	–	–	–	**10.8**	–	–	–	–	**9.4**	**6.0**	**1.3**	–	–	–
130	Incensole oxide[d]	–	–	–	–	**0.4**	–	–	–	–	**0.4**	**1.6**	**0.4**	–	–	–
131	Incensole oxide acetate[d]	–	–	–	–	**0.1**	–	–	–	–	**0.2**	**1.0**	**0.2**	–	–	–

Components are presented by increasing retention indices, with their corresponding relative peak area (%). Values in bold correspond to compounds present at high levels or characteristic of an olibanum.

[a] Retention indices relative to C_9–C_{22} alkanes obtained on a DB5 column. tr: traces (<0.1%).

[b] Spectral data of the unidentified terpenes, *m*/*z* (relative intensity):

Oxygenated monoterpene (**22**): 43 (100), 109 (46), 41 (42), 69 (33), 95 (32), 39 (30), 67 (29), 82 (29), 55 (21), 91 (21), 84 (20), 29 (11), 137 (10), 119 (7), 152 (5, M^+).

Unidentified compound (**55**): 43 (100), 71 (36), 87 (36), 41 (28), 126 (24), 111 (24), 109 (23), 55 (22), 58 (22), 74 (18), 81 (14), 39 (14), 95 (13), 29 (11), no M^+.

Unidentified compound (**56**): 43 (100), 126 (50), 111 (50), 71 (34), 41 (32), 109 (22), 55 (21), 39 (18), 95 (15), 29 (14), no M^+.

Oxygenated sesquiterpene (**91**): 43 (100), 126 (86), 108 (85), 41 (48), 81 (43), 82 (34), 55 (34), 55 (34), 93 (28), 67 (28), 83 (27), 69 (23), 71 (23), 222 (2, M^+).

Oxygenated sesquiterpene (**98**): 109 (100), 95 (92), 161 (81), 43 (80), 41 (77), 81 (60), 67 (54), 55 (51), 105 (44), 119 (41), 204 (27), 179 (19), 191 (14), 189 (14), 207 (12), 222 (5, M^+).

Oxygenated sesquiterpene (**105**): 41 (100), 43 (86), 55 (62), 93 (53), 79 (50), 91 (49), 81 (45), 107 (44), 109 (43), 69 (42), 39 (40), 105 (37), 29 (33), 121 (27), 131 (25), 157 (20), 187 (14), 203 (7), 220 (2, M^+).

Oxygenated sesquiterpene (**106**): 41 (100), 43 (70), 93 (57), 55 (56), 91 (56), 79 (53), 81 (43), 69 (41), 107 (40), 92 (39), 95 (38), 105 (35), 109 (30), 131 (29), 121 (26), 135 (18), 149 (17), 159 (13), 187 (11), 164 (10), 205 (6), 202 (3), 220 (2, M^+).

Dimer of α-phellandrene 2 (**111**): 93 (100), 92 (58), 136 (27), 77 (22), 43 (13), 41 (12), 65 (3), 55 (2), 105 (2), no M^+.

Unidentified diterpene (**116**): 81 (100), 93 (60), 91 (59), 92 (53), 41 (34), 43 (32), 69 (26), 95 (26), 136 (24), 105 (24), 229 (49), 55 (13), 272 (4, M^+).

Dimer of α-phellandrene 6 (**117**): 93 (100), 92 (55), 41 (39), 136 (20), 77 (15), 69 (15), 43 (13), 105 (10), 55 (6), 229 (5), 272 (2, M^+), 257 (1).

Dimer of α-phellandrene 7 (**118**): 93 (100), 92 (61), 136 (36), 91 (14), 41 (12), 94 (9), 77 (8), 105 (7), 69 (6), 43 (4), 137 (4), 119 (3), 79 (3), 55 (2), 95 (2), 229 (2), 272 (1, M^+).

Unidentified diterpene (**119**): 41 (100), 69 (91), 91 (25), 105 (16), 119 (15), 79 (15), 55 (14), 133 (8), 147 (8), 161 (7), 229 (7), 203 (5), 187 (4), 272 (3, M^+), 257 (2).

Unidentified diterpene (**122**): 69 (100), 41 (96), 93 (72), 91 (42), 109 (30), 105 (29), 107 (26), 119 (25), 79 (21), 133 (17), 67 (17), 161(15), 229 (14), 147 (13), 55 (11), 203 (9), 187 (8), 272 (4, M^+), 257 (2).

Unidentified diterpene (**124**): 69 (100), 41 (60), 93 (40), 119 (27), 203 (19), 133 (17), 79 (15), 107 (15), 109 (13), 105 (13), 147 (10), 187 (9), 159 (4), 215 (4), 229 (4), 257 (1), 272 (1, M^+).

[c] See Fig. 2.

[d] Retention indices of these compounds were not calculated because their retention times were higher than the one of the last alkanes used (C_{22}).

(**47**), methyleugenol (**70**), isocaryophyllene (**82**), sesquiterpene **91**, elemicin (**92**) and an unidentified diterpene (**124**) eluting between cembrene C (**123**) and verticilla-4(20),7,11-triene (**125**). Thirdly, it is devoid of β-caryophyllene (**73**), α-humulene also called α-caryophyllene (**78**), caryophyllene oxide (**95**) and bornyl acetate (**50**).

The four aforementioned olibanum (*B. carteri* from Somalia and Aden, *B. sacra* and *B. serrata*) contain the same main diterpenes: an unidentified diterpene (**119**) eluted before cembrene A, cembrene A (**120**), cembrene C (**123**), verticilla-4(20),7,11-triene (**125**), an unidentified diterpene (**126**) and isoincensole co-eluting with isoincensole acetate (**128**). The mass spectra of these two latter compounds are presented in Fig. 1. The spectrum of **126** resembles that of cembrene but its different retention index indicates another structure. Since the presence of diterpene **126** seems to be related to that of isoincensole acetate (**128**), it can also be considered as a chemical biomarker of these four olibanum.

Boswellia frereana and *Boswellia papyrifera* olibanum have very different terpenic composition from the others. Although *B. frereana* olibanum contains very similar monoterpenes to olibanum from *B. carteri*, *B. sacra* or *B. serrata*, it is very poor in sesquiterpenes and it contains none of the biomarker diterpenes cited previously. Moreover, two unidentified compounds (**55** and **56**) characterize the composition of *B. frereana* olibanum. Its main diterpenes, present in high level, are four dimers of α-phellandrene (see Fig. 2 for their mass spectra). Three of them (dimer 1 (**108**), dimer 4 (**114**) and dimer 5 (**115**)) are present in very small amounts or at trace level in *B. carteri* frankincense from Somalia and in *B. serrata* frankincense. Dimer 3 (**113**) is the major component of *B. frereana* olibanum. Because of its

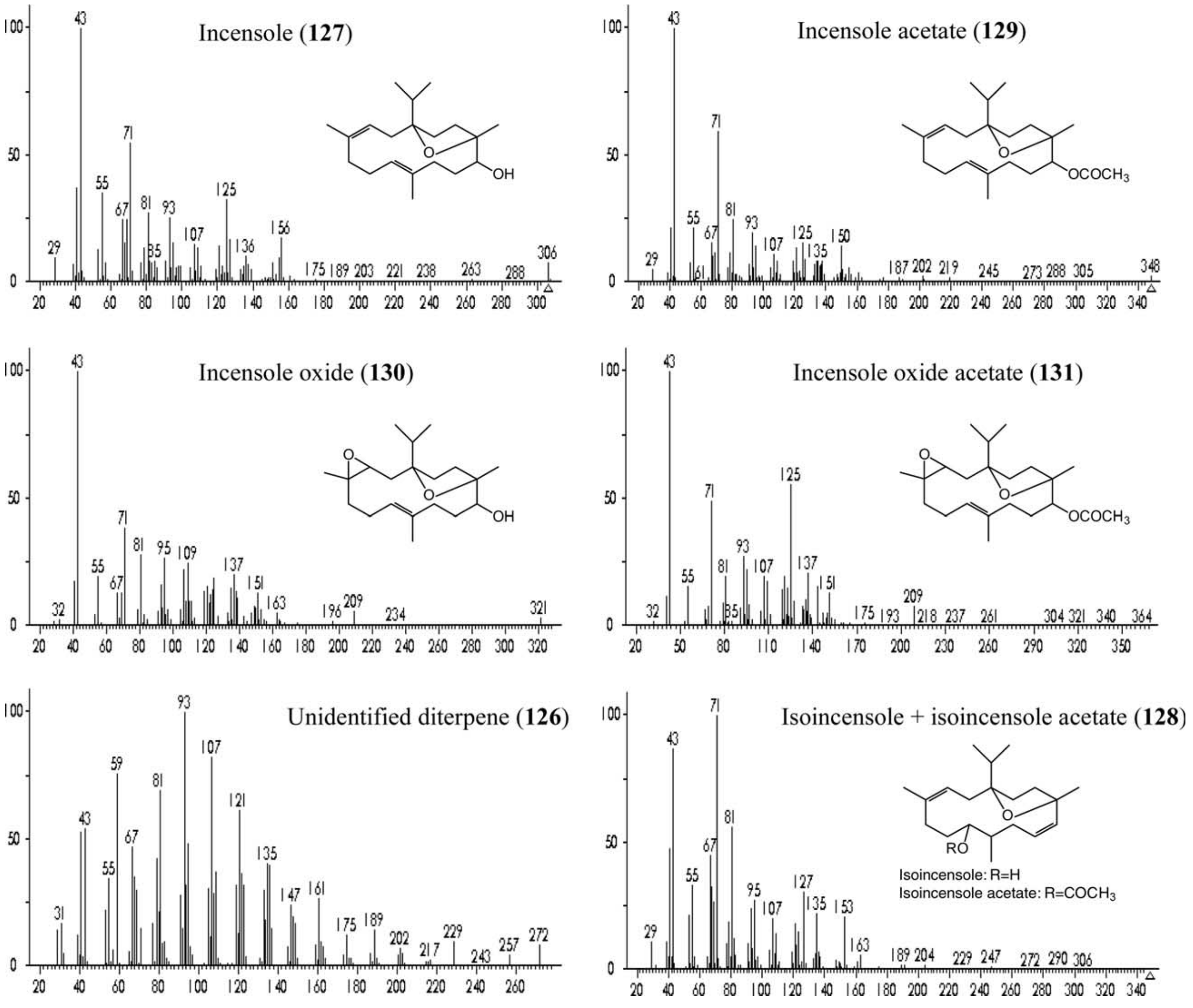

Fig. 1. Mass spectra of the six diterpenes biomarkers of olibanum. Incensole (**127**), incensole acetate (**129**), incensole oxide (**130**) and incensole oxide acetate (**131**) are characteristic of *B. papyrifera* olibanum. In contrast, diterpene **126** and coeluting isoincensole and isoincensole acetate (**128**) occured in olibanum from *B. carteri*, *B. sacra* and *B. serrata*. The latter structures were deduced from isoincensole oxide (Basar et al., 2001).

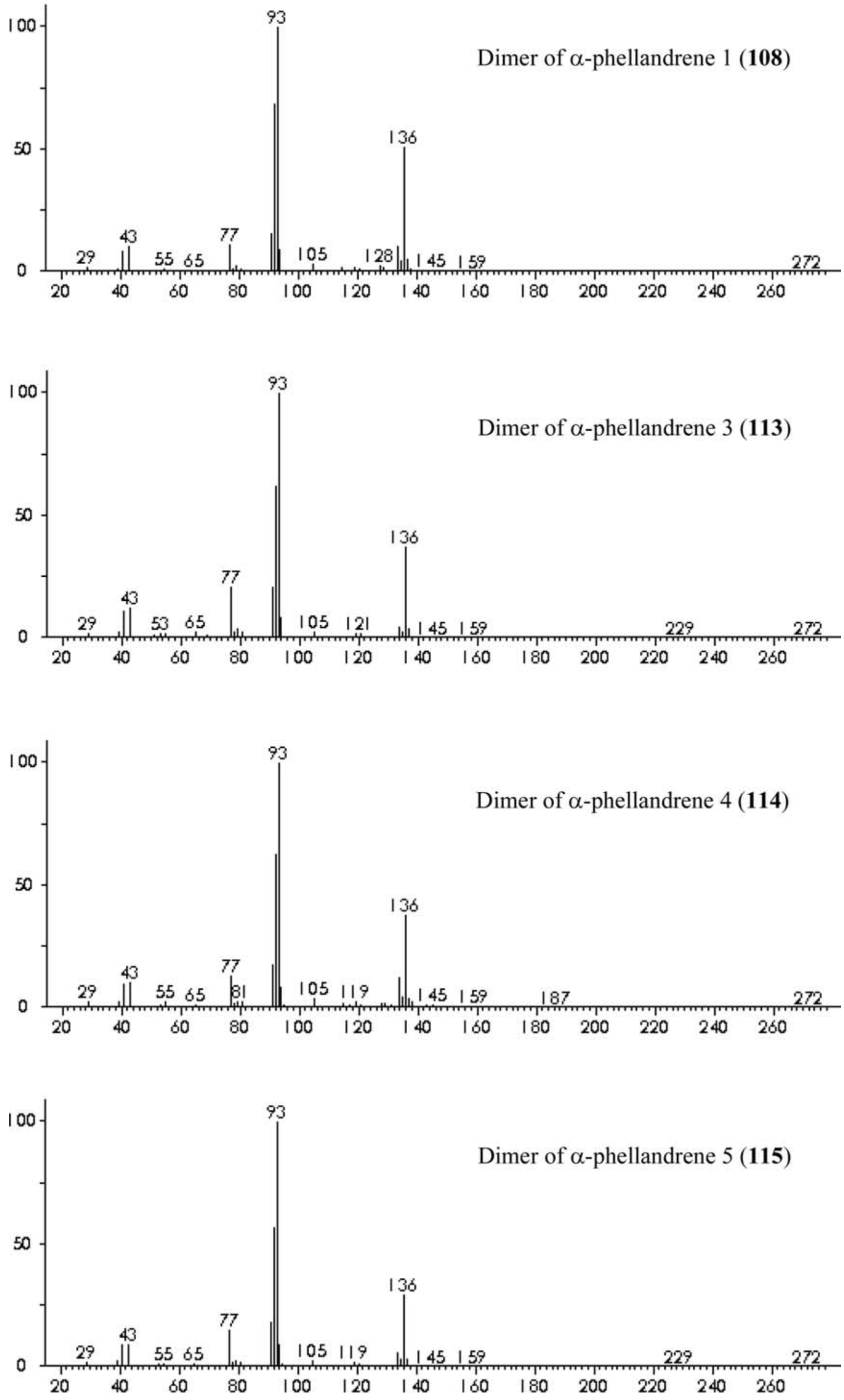

Fig. 2. Mass spectra of the four main diterpenes in *B. frereana* olibanum.

absence in the other olibanum, it can be considered as characteristic of *B. frereana* olibanum. Conversely, dimer 2 (**111**) occurring in *B. carteri*, *B. sacra* and *B. serrata* olibanum, is lacking in *B. frereana* olibanum. The hypothetical structures of these dimers of α-phellandrene can be related to the photosensitized dimerization of α-phellandrene (Baldwin and Nelson, 1966) and were also evidenced in an oleoresin of *Canarium luzonicum*, a tree that also belongs to the Burseraceae family (Brieskorn and Krauss, 1986).

The chemical composition of *B. papyrifera* olibanum is markedly different from that of other *Boswellia*. *B. papyrifera* contains small amounts of mono- and sesquiterpenes, and large amounts of *n*-octanol (**18**) and *n*-octylacetate (**40**), the major compound. *B. papyrifera* also contains particular diterpenes: incensole (**127**), incensole acetate (**129**), incensole oxide (**130**) and incensole oxide acetate (**131**) although isoincensole and isoincensole acetate (**128**) are absent. Linear carboxylic acids from pentanoic acid to lauric acid (**93**) were also identified in

B. papyrifera olibanum exclusively. Pentanoic acid and undecanoic acid (present at trace level) are not reported in Table 1. Shorter-chain acids were not included in the analytical window defined by the chromatographic conditions used here, and longer-chain acids were not efficiently trapped by the SPME fiber. Their presence in this olibanum cannot be excluded.

2.2. Comparison with the literature

We compared our results for each olibanum sample with certified botanical origin with those of the literature. Whilst abundant literature documenting olibanum triterpene composition exists (because of their pharmaceutical properties) in contrast, the descriptions of the volatile terpenes of olibanum are scarce. Quasi all-chemical analyses were carried out on the essential oil of frankincense. This is an ingredient (obtained by hydrodistillation) which is widely used in perfumery, particularly as a fixative. However, the botanical origin of the olibanum sample was often not specified.

The present work on olibanum of certified botanical origins has demonstrated the similar chemical composition of olibanum from *B. carteri* and *B. sacra*. This is in agreement with the species synonymy described by Pernet, evidenced by an ethnobotanical and chemical survey of the Burseraceae family (Pernet, 1972) and with the conclusion of Thulin and Warfa who considered that the two should not be considered as separate species since they differ only in the geographical location in which they are encountered: *B. sacra* grows in Arabia whereas *B. carteri* grows in Somalia (Thulin and Warfa, 1987). In contrast, Dupéron presented anatomical arguments for maintaining *B. sacra* and *B. carteri* as two distinct species (Dupéron, 1993). More recently, in a paper on the analysis of triterpenes of various olibanum samples by HPLC, Mathe et al. found the same components for *B. sacra* and *B. carteri* olibanum (Mathe et al., 2004). Surprisingly, our description of the chemical composition of *B. sacra* olibanum agrees with that described in the literature, but this is not the case for *B. carteri* olibanum.

2.2.1. *Boswellia sacra*

B. sacra is a tree indigenous to the Dhofar region of the Sultanate of Oman (Champault, 1996). Its gum resin was possibly imported to Egypt for embalming or fumigation purposes. It was reported that Queen Hatchepsout tried unsuccessfully to cultivate this tree in Egypt (Ammar et al., 1994). Our sample also comes from Oman. The chemical composition of the volatile oil of *B. sacra* studied by Ammar is very similar to that which we obtained by headspace SPME, with α-pinene (**2**) as the major compound (30%) and several identical monoterpenes: camphene (**4**), sabinene (**6**), β-pinene (**7**), *p*-cymene (**13**), limonene (**14**), α-campholenal (**26**), pinocarveol (**28**), 4-terpineol (**31**), myrtenal (**35**), myrtenol (**37**), verbenone (**41**), carveol (**42** and **43**), carvone (**45**), bornyl acetate (**50**) and cuminol (**52**).

2.2.2. *Boswellia carteri*

A number of publications report octylacetate (**40**), octanol (**18**), α-pinene (**2**) and incensole (**127**) as the main components of essential oil of *B. carteri* olibanum (Abdel Wahab et al., 1987; Vernin et al., 1990; Basar et al., 2001). However, these reported chemical compositions correspond to the pattern which we observed in *B. papyrifera*.

The literature on the chemical composition of olibanum is often confused as to the true origin of the analyzed olibanum. Two denominations exist for olibanum gum resins: Aden and Eritrea. According to Peyron, both are gathered by artisans, then conveyed to Aden where they are washed, separated and exported, but the relationship between their botanical origin and their denomination is unclear (Peyron et al., 1981). Three independent studies showed that the chemical composition of "Aden" denominated olibanum was similar to that of our *B. carteri* olibanum from Aden, with α-pinene (**2**), limonene (**14**) and *p*-cymene (**13**) as the main components (Obermann, 1977; Peyron et al., 1981; Maupetit, 1985). An olibanum marketed under the "Eritrea" denomination showed the predominance of octyl acetate (Peyron et al., 1981), characteristic of *B. papyrifera* olibanum (Table 1). Bearing in mind the limited data on "Eritrea olibanum", it can be speculated that this denomination is allocated to olibanum from *B. papyrifera*.

2.2.3. *Boswellia papyrifera*

The gum resin from *B. papyrifera* is claimed to be the principal source of frankincense in antiquity; coming from Ethiopia, Sudan or East Africa, and was considered an inferior form of frankincense (Tucker, 1986). *B. papyrifera* occurs on rocky slopes between altitudes of 950 and 1800 m in Eritrea, Nigeria, Cameroon, Tchad, Sudan, and Uganda and its resin is the raw material of the Ethiopian frankincense (Demissew, 1993). Hairfield has identified by TLC an olibanum from Eritrea as *B. papyrifera* (Hairfield et al., 1984). Dekebo et al. confirm that *B. papyrifera* was known to occur in Ethiopia, Sudan and in some parts of West Africa (Dekebo et al., 1999). The unique analysis of an olibanum of an identified plant of *B. papyrifera* is in agreement with ours, with the predominance (56%) of octyl acetate (**40**), a high content (8%) of octanol (**18**), some monoterpenes, namely α-pinene (**2**), limonene (**14**), linalool (**21**), and only traces of sesquiterpenes. As aforementioned, the chemical composition of the *B. papyrifera* olibanum sample examined in this study is very similar to that often described for *B. carteri*. Indeed, a recent study of the essential oil of an authentic and certified *B. carteri* resinoid from Ethiopia, by GC–MS, reported octylacetate (**40**) as the major compound, a high content of octanol (**18**) and the five diterpenes: cembrene A (**120**), cembrene C (**123**), incensole (**127**), incensole acetate (**129**) and verticilla-4(20),7,11-triene (**125**), as well as some monoterpenes and (*S*)-*trans*-nerolidol as the only sesquiterpene (Basar et al., 2001). Apart from (*S*)-*trans*-nerolidol (which we did not detect), this description of the chemical composition of *B. carteri* olibanum is identical to that of the *B. papyrifera* gum resin from our sample set. Of particular note is the occurrence of the same diterpenes. As both samples are of certified origin, such discrepancies remain unexplained until further analysis of independently certified *B. carteri* olibanum.

2.2.4. *Boswellia frereana*

The habitat of the *B. frereana* tree is restricted to North Somalia, from near sea-level up to 1000 m (Thulin and Warfa, 1987). The terpenic composition of *B. frereana* olibanum from our data set confirms previous reports on this olibanum, in particular a report showing the predominance of α-pinene (**2**) and other compounds in similar relative abundances (Vernin et al., 1990). The similarity extends to the four diterpenes (**108**, **113**, **114** and **115**) which we attributed to dimers of α-phellandrene, as shown by the identical mass spectra (this paper, Fig. 2; Vernin et al., 1990, Fig. 7). Thus, the simultaneous presence in a sample of these dimers could serve as diagnostic biomarkers for *B. frereana* olibanum. A sample of *B. frereana* olibanum collected in Somalia exhibited some minor differences in composition though its predominant volatile was *p*-cymene and the occurrence of α-terpinene, α-cubebene, cembrene and isocembrene was restricted to it (Strappaghetti et al., 1982).

2.2.5. *Boswellia serrata*

B. serrata is commonly found on dry hills throughout greater parts of India. Several authors reported α-thujene (**1**) as the major compound in olibanum from *B. serrata*, which distinguishes it from African olibanum (Girgune and Garg, 1979; Verghese et al., 1987). We found most of the components cited by Girgune and Verghese, notably methylchavicol (**38**) in high proportion, elemicin (**92**), *p*-cymene (**13**) and β-bourbonene (**67**). The occurrence of methylchavicol and elemicin constitutes a specific fingerprint of this olibanum. The only diterpene identified in the literature from this olibanum is serratol, an isomer of cembrenol (Pardhy and Bhattacharyya, 1978). This compound was not identified in our sample.

2.3. Olibanum of unknown botanical origin

Eight olibanum samples were purchased from various locations where it is traditionally crafted and sold. Their chemical compositions are summarized in Table 1. The two olibanum from Somalia and that from a market in Ta'izz (Yemen) can be attributed to *B. carteri* or *B. sacra* on the basis of the occurrence of the characteristic diterpenes isoincensole and isoincensole acetate (**128**) together with diterpene **126**. The absence of methylchavicol (**38**), oxygenated sesquiterpene **91** and diterpene **124** and the presence in relatively large amount of β-caryophyllene (**73**), α-humulene (**78**) and caryophyllene oxide (**95**) exclude the hypothesis of a *B. serrata* origin.

The samples from Eritrea, Jerusalem and Lebanon are undoubtedly *B. papyrifera*, because of their characteristic constituents: octanol (**18**), octyl acetate (**40**), linear caboxylic acids (**10**, **20**, **36**, **49**, **63**), incensole (**127**) and its acetate and oxide derivatives (**129**, **130**, **131**). As aforementioned, an olibanum from Eritrea has already been attributed to *B. papyrifera* on the basis of TLC chemical characterization (Hairfield et al., 1984). Moreover, oils obtained by simultaneous distillation extraction from olibanum produced in Turkey and Israel were rich in octyl acetate and octanol (Hayashi et al., 1998). All these results point to the crafting of *B. papyrifera* olibanum in these regions.

Samples purchased on markets in Sa'da and San'a in Yemen exhibit no diterpenes from the incensole family but large amounts of dimers of α-phellandrene, in particular dimer 3 (**113**), and the two compounds **55** and **56** are present. This pattern identifies them as *B. frereana* olibanum. The taxonomic origin of an olibanum oil from Oman, a country neighbouring Yemen, was also determined to be *B. frereana* (Hayashi et al., 1998). As *B. frereana* grows exclusively in Somalia, these olibanum were certainly imported to Yemen and Oman.

2.4. Present day traditional incenses and archaeological samples

In traditional incenses, olibanum is mixed with other ingredients. The question arises whether mono-, sesqui- and diterpene specific fingerprints could still be recognized when such complex mixtures were submitted to SPME–GC/MS analysis. Two traditional mixtures of the present epoch were selected to explore this question: incense from the monastery at Mount Athos and Kyphi, a re-creation of an ancient Egyptian recipe. Finally, an attempt to characterize terpene fingerprints was carried out on a sample of incense from antique Yemen.

2.4.1. Mount Athos traditional incense

The chromatogram of a traditional incense from the Mount Athos monastery, in Greece is shown in Fig. 3. The occurrence of octanol (**18**), octyl acetate (**40**), linear carboxylic acids (**10, 20, 36, 49**, **63**, **93**), incensole (**127**), incensole acetate (**129**), incensole oxide (**130**) and incensole oxide acetate (**131**), demonstrates that it contains

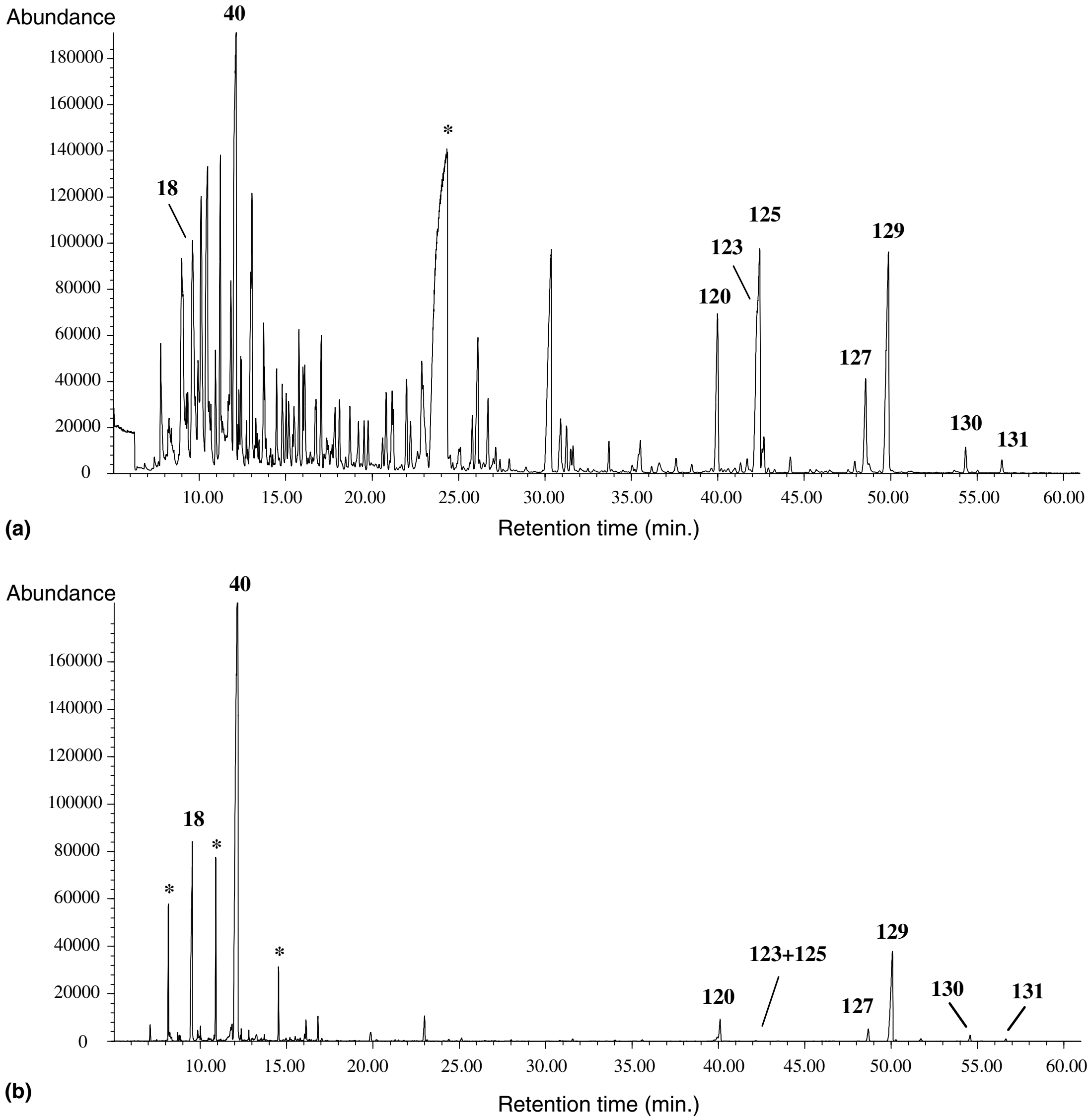

Fig. 3. Total ion current chromatograms of the incense from Mount Athos (a) and of *B. papyrifera* olibanum (b). Peak labels correspond to compound identification given in Table 1. The occurrence of the following biomarkers of *B. papyrifera* olibanum in the incense from Mount Athos are a clear signature of its botanical origin: *n*-octanol (**18**), *n*-octylacetate (**40**), incensole (**127**), incensole acetate (**129**), incensole oxide (**130**) and incensole oxide acetate (**131**); artefacts (∗).

B. papyrifera olibanum. This olibanum is mixed with other substances, probably Damask rose and jasmine.

2.4.2. Reconstituted Kyphi

Kyphi was an ancient Egyptian mixture burned in temples. It includes saffron, frankincense, mastic, benzoin, cardamon seeds, galangal root, lemongrass, sandalwood, aloeswood, rose petals, raisins, red wine, honey and herbs. The chromatogram of its terpenes shows isoincensole and isoincensole acetate (**128**) (Fig. 4), thus indicating that the frankincense used in this mixture originated from a *B. carteri*, a *B. sacra* or a *B. serrata* tree. The absence of methylchavicol excludes *B. serrata* olibanum in this sample. Moreover, all the monoterpenes, sesquiterpenes and diterpenes of *B. carteri* (or *B. sacra*) olibanum were also clearly detected in the Kyphi. Our *B. sacra* and *B. carteri* olibanum and the Kyphi were purchased from the same manufacturer. It was interesting for us to determine whether our analytical method allowed us to recognize which olibanum was used in the Kyphi. The person who had crafted this Kyphi certified that he used *B. sacra* olibanum from Oman and the results agree. Indeed, the occurrence of the oxygenated sesquiterpene **98** and of dimer 2 (**111**) only, support that the Kyphi contains *B. sacra* olibanum. Of the other substances used in the manufacture of this sample of Kyphi, some characteristic compounds of myrrh could be recognized: curezerene, furanoeudesma-1,3-diene, lindestrene, 2-methoxyfuranodiene, 2-acetoxyfuranodiene (Hamm et al., 2004).

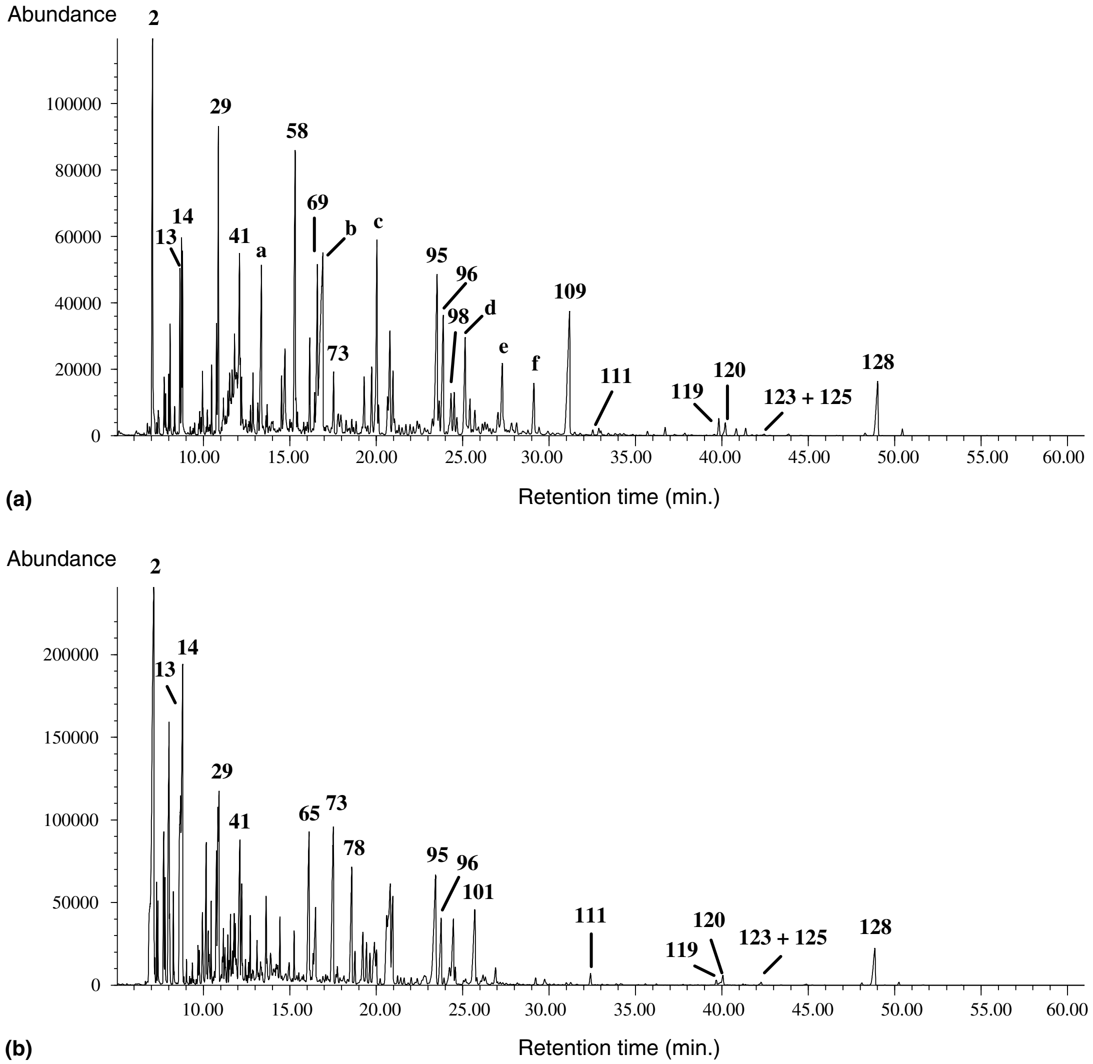

Fig. 4. Total ion current chromatograms of Kyphi (a) and of *B. sacra* olibanum (b). Peaks are labelled with numbers according to compounds identification given in Table 1. The occurrence of isoincensole acetate (**128**) as well as the occurrence of the oxygenated sesquiterpene **98** and only of the dimer 2 (**111**) in the Kyphi are clear fingerprints of the botanical origin of the olibanum used. Peaks labelled by letters correspond to the following compounds: **a** – cinnamaldehyde, **b** – vanilline, **c** – curzerene, **d** – furanoeudesma-1,3-diene, **e** – α-santalol, **f** – 2-methoxyfuranodiene.

2.4.3. Archaeological incense

An archaeological incense from a warehouse in Qana (Yemen, I–IV century AD), stored in a sealed amphora, provided a unique opportunity to test the potential of our method on an ancient object. Our first objective was to confirm whether it really was frankincense. Some of its irregular and extremely friable grains were sufficiently large to suggest that volatile terpenes were still trapped in their core. While triterpenes of archaeological incense have already been documented (Mathe et al., 2004; Evershed et al., 1997; Van Bergen et al., 1997), this, to the best of our knowledge, is the first report on the volatile and semi-volatile compounds of an archaeological incense. This archaeological sample provides an opportunity to assess which volatile compounds still remain after 2000 years and, in turn, could be used to determine the potential presence of frankincense in archaeological mummification balms. On the chromatogram obtained from the archaeological incense, terpenes yield a smaller signal than that of a modern olibanum sample (Fig. 5). The major component is verbenone (**41**), that was present in small amounts in reference olibanum samples and that derives from the degradation of limonene. The main monoterpenes and sesquiterpenes that were detected in the archaeological incense as well as in modern olibanum samples are α-pinene (**2**), camphene (**4**), isomer of 4-methylene-1-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene (**5**), *p*-cymene (**13**), limonene (**14**), *trans*-pinocarveol (**28**), methylacetophenone (**32**), myrtenal (**35**), cyclosativene (**61**), α-copaene (**65**), β-bourbonene (**67**), β-elemene (**69**), γ-muurolene (**80**) and β-eudesmene (**83**). Although occurring at low levels,

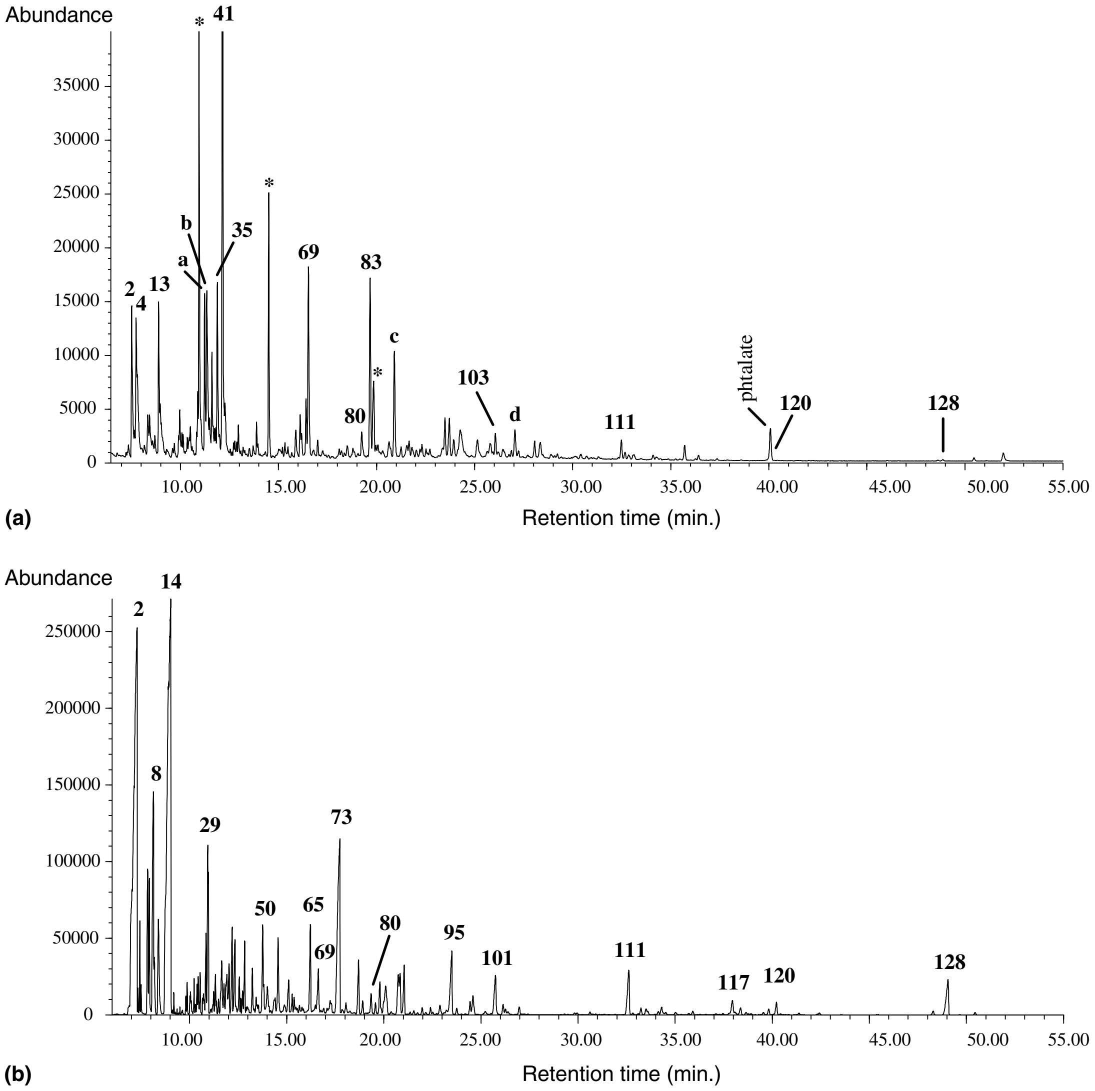

Fig. 5. Total ion current chromatograms of the archaeological incense (a) and of *B. carteri* olibanum (b). Peaks are labelled with numbers according to compounds identification given in Table 1. Peaks labelled by letters correspond to the following compounds: **a** – pinocamphone, **b** – borneol, **c** – calamenene, **d** – cadalene. Among other fingerprints, the occurrence of dimer 2 (**111**), cembrene A (**120**) and isoincensole acetate (**128**) certify that this sample indeed is olibanum and attributes its botanical source to *B. carteri* or to *B. sacra*.

the following series of compounds adds to the olibanum components evidenced in the archaeological incense: α-phellandrene (**9**), eucalyptole (**15**), α-campholenal (**26**), 4-terpineol (**31**), α-terpineol (**34**), *trans*-carveol (**42**), cumaldehyde (**44**), carvone (**45**), 3,5-dimethoxytoluene (**48**), bornyl acetate (**50**), carvacrol (**53**) and α-muurolene (**85**). Most important is that three characteristic diterpenes of olibanum: dimer 2 (**111**), cembrene A (**120**) and isoincensole acetate (**128**) give a clear signal on the chromatogram of this 2000-year-old sample (Fig. 5). These three compounds confirm that the sample is olibanum. Their occurrence yields an important piece of information, as they demonstrate that this old incense is an olibanum from a *B. carteri*, a *B. sacra* or a *B. serrata* tree (Table 1). This latter species is less likely, because of the occurrence of myrtenal (**35**), β-elemene (**69**) and β-eudesmene (**83**) in high levels and of 3,5-dimethoxytoluene (**48**) and bornyl acetate (**50**) in low levels in the archaeological sample, whereas they are absent in our modern *B. serrata* olibanum. To the best of our knowledge, a botanical origin is assigned to an archaeological frankincense for the first time. Some compounds were characterized in the archaeological sample whereas they are lacking in modern olibanum: pinocamphone (**a**), borneol (**b**), calamenene (**c**) and cadalene (**d**), and in smaller quantity, dehydro-*p*-cymene, camphor, naphthalene and some of its derivatives and some ketones (norinone, carvotanacetone). All these compounds are probably degradation products of terpenes and are commonly characterized in archaeological samples (Hamm et al., 2004). This study proves that headspace SPME–GC/MS is well adapted to the analysis of archaeological samples especially if they are not too finely powdered. As well as the feasibility of the analytical method, the information provided by the volatile and semi-volatile terpene composition demonstrates the high potential of this approach in the field of archaeology. This line of research is still little explored and, in our opinion, deserves further scientific attention.

3. Conclusion

The terpenic composition (particularly the diterpenic biomarkers) characteristic to five species of *Boswellia* (*B. carteri*, *B. sacra*, *B. frereana*, *B. papyrifera* and *B. serrata*) was successfully established by headspace SPME and GC–MS. The database developed during this study has allowed us to determine the botanical origin of olibanum samples purchased in souks in various countries as well as the botanical origin of frankincense used in traditional incense mixtures, and validates the use of volatile and semi-volatile terpenes to derive the specific origin of olibanum. When combining olibanum analyzed in the present study: those of certified botanical origin and those supplied by manufacturers whose origin was attributed on the basis of their terpenic composition, botanical origins of olibanum cluster in geographic regions. The olibanum samples from Somalia were only from *B. carteri* (or *B. sacra*) and *B. frereana*. Those from Eritrea and Ethiopia were from *B. papyrifera*. However, in the case of samples bought from markets, the botanical origin of olibanum is not necessarily linked to a unique geographical origin. For instance, among the three olibanum purchased from markets in Yemen, two were from *B. frereana* and one from *B. carteri* (or *B. sacra*). *B. papyrifera* frankincense was found from markets in the Near East as well as being a component used in the traditional incense from Mount Athos in Greece. *B. papyrifera* frankincense is probably the main one used by the Church, however this needs to be confirmed by the analysis of further samples. The occurrence of olibanum from *B. serrata*, a tree growing mainly in India, seems to be limited to the Asian continent and thus was not encountered in the markets of the regions under study. The present results of volatile and semi-volatile terpenes of olibanum of certified botanical origin build a preliminary database and would strongly benefit further documentation, provided that the botanical origin of olibanum samples are accurately determined, a point which we strongly recommend.

The analytical method presented here has also permitted us to assert that a likely archaeological incense sample was effectively frankincense and that it originated from a *B. carteri* (or *B. sacra*) tree. Understanding the chemical composition of a 2000-year-old frankincense sample is pre-requisite to exploring the potential presence of olibanum in complex mixtures such as Egyptian mummification balms or unguents. The information obtained from this archaeological sample demonstrates that using volatile mono- and sesquiterpenes and semi-volatile diterpenes to elucidate the botanical origin of natural craft components is a very promising approach and encourages the development of this innovative line of research in the field of archaeology.

4. Experimental

4.1. Samples

The six olibanum samples with certified botanical origin, i.e., *B. carteri* (Somalia), *B. carteri* (Aden), *B. sacra* (Oman), *B. papyrifera* (Ethiopia), *B. serrata* (India), *B. frereana* (Somalia), and Kyphi were purchased from Scents of Earth (Sun City, USA). The Kyphi is a recreation by Scents of Earth of an ancient Egyptian recipe. The Greek incense was brought by A. Tchapla from a monastery at Mount Athos. It is a traditional incense, burned during religious ceremonies. Wolf society (15 rue de l'Arsenal, 75004, Paris, France) kindly donated one sample of olibanum from Somalia (Somalie no. 1, type 8767, no. 1336) and one sample of olibanum from Eritrea (no. 1070, March 1989). The second sample of olibanum from Somalia (no. 1118) was bought from Asie Concept by Dr. J. Connan from a market at Crozon (Brittany, France). The olibanum from the souk of San'a in Yemen (no. 961) and the archaeological sample (I–IV century AD, no. 1286) from a warehouse (sector 6) at the Bir Ali site were kindly donated by M. Mouton. Bir Ali, known as Qana in the classical literature, was the main harbour of the Hadramawt kingdom in antiquity. Other olibanum samples were purchased from markets in Jerusalem (Israel), Beirut (Lebanon), Sa'da and Ta'izz (Yemen).

4.2. SPME procedure

Polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm (Stable Flex), was purchased from Supelco (Bellefonte, PA, USA). Before each extraction, the fiber was conditioned at 250 °C for 10 min.

All extractions were performed in 2 ml glass vials equipped with screw caps and PTFE/silicone septa, using 5 mg of powdered frankincense. Because of its heterogeneous and gummy nature, the Kyphi could not be powdered and a higher weight was used (22 mg) in order to provide a more representative sample. The vial was immersed to a depth of 5 mm in a thermostatically controlled bath at 80 °C for 60 min. The SPME fiber was maintained 1 cm above the solid sample. After extraction, the analytes were thermally desorbed for 5 min at 250 °C in the injector of the gas chromatograph.

4.3. GC–MS conditions

GC–MS analyses were performed on a 3400 gas chromatograph (Varian, Walnut Creek, CA, USA) coupled to an INCOS 50 quadrupole mass spectrometer (Finnigan, San Jose, CA, USA). Analytes were separated on a DB5 (J&W Scientific, Folsom, USA) or a CP Sil 8 CB LB/MS (Varian/Interchim, Montluçon, France) capillary column of 30 m × 0.25 mm with a phase thickness of 0.25 μm. The injector temperature was set to 250 °C and the temperature program was 40 °C for 1 min, 9 °C/min increase rate up to 130 °C, followed by a 2 °C/min increase rate to 230 °C. The carrier gas was helium with a column head pressure of 10 psi. Splitless injection (1 min) was used. The temperatures of the transfer line and the source were 250 and 150 °C, respectively. The mass spectrometer was operated in electron-impact mode (EI) at 70 eV, in the scan range *m/z* 29–400. Compounds were identified by use of the NIST98 spectral library, as well as literature MS data and Kovatz indices (Adams, 1989; Vernin et al., 1990; Basar et al., 2001; Barakat and Rullkötter, 1993). Identification of incensole, incensole acetate and isoincensole acetate was carried out on the basis of the mass spectra published by Vernin et al. Each analysis was made at least twice.

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