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Characterization of archaeological frankincense by gas chromatography–mass spectrometry

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Abstract

A simple gas chromatography–mass spectrometry (GC–MS) method has been developed for the characterization of frankincense in archaeological samples. After trimethylsilylation of the methanolic extract, 15 triterpenoids have been found among the chemical constituents of commercial olibanum (α-boswellic acid, 3-*O*-acetyl-α-boswellic acid, β-boswellic acid, 3-*O*-acetyl-β-boswellic acid, α-amyrin, β-amyrin, lupeol, 3-*epi*-α-amyrin, 3-*epi*-β-amyrin, 3-*epi*-lupeol, α-amyrenone, β-amyrenone, lupenone, 3α-hydroxy-lup-20(29)-en-24-oic acid and 3-*O*-acetyl-hydroxy-lup-20(29)-en-24-oic acid). These compounds have been unequivocally identified by retention time and mass spectral comparison with pure standards previously isolated, for the most part, in our laboratory. Within these triterpenes, acid ones, the corresponding *O*-acetates, and their products of degradation were found to be characteristic of frankincense (*Boswellia* resin). The presence of these unusual triterpenic compounds in an archaeological resinous sample, recovered during excavations from Dahshour site (Egypt, XIIth Dynasty), enabled us to identify unambiguously frankincense resin among several other materials. Additional chromatographic peaks of this sample were assigned to broad chemical classes using retention time and mass spectra features.

Keywords: Olibanum; Frankincense; *Boswellia*; Triterpenoids; Boswellic acids; Lupane triterpenes; Oleanane triterpenes; Ursane triterpenes

1. Introduction

The aromatic oleo-gum-resin known as frankincense or olibanum has been obtained since ancient times from trees belonging to the genus *Boswellia* (family Burseraceae, tribe Bursereae, subtribe Boswellinae [1]).

Generally, the frankincense tree is a small, 3–6 m high, and scrubby tree which grows in rough, wild and inhospitable arid mountainous regions. The resin is harvested by scraping and/or making shallow incisions in the bark. The white emulsion produced solidifies, when exposed to the air and sun, into globular, pear or club shaped tears [2].

Actually, commercial frankincense comes from three distinct regions: East Africa (Eritrea, Ethiopia, Somalia and Sudan), Southern part of the Arabian peninsula (Yemen and Oman) and North-Western India. However, even if the botanical origin of commercial resins and the name ascribed to their source are always a subject of uncertainty [3], four main producing species are recognized. The species in question are *Boswellia sacra* in Arabia, *B. serrata* in India, *B. frereana* in Somalia and *B. carteri* (syn. *B. sacra* [4]) which is commonly found at the Horn of Africa [4–6]. Furthermore, inferior forms of frankincense come from a fifth species, *B. papyrifera*, present in East Africa. This last species is claimed to be the source of olibanum during Antiquity [2].

Olibanum is the best known of the ancient plant resins: it has been used as an incense, in embalming and in preparation of medicines, cosmetics and perfumes since the Egyptians, and nowadays it is still used therapeutically.

This chemically complex material has been reported to contain a rich array of terpenes. The major constituents described from the non-volatile fraction are cembrane and verticillane diterpenes [7–12], tetracyclic triterpenes with

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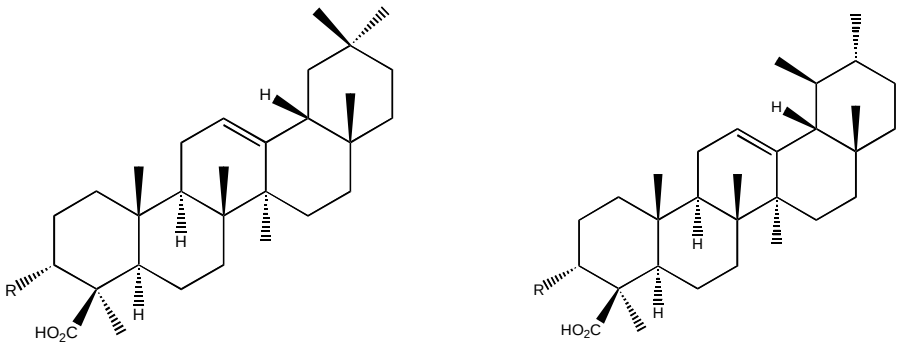


R = -OH : α-boswellic acid (**1**)
R = -OAc : 3-*O*-acetyl-α-boswellic acid (**3**)

R = -OH : β-boswellic acid (**2**)
R = -OAc : 3-*O*-acetyl-β-boswellic acid (**4**)

Fig. 1. Main chemical constituents of frankincense.

dammarane or tirucallane skeletons [13,14], and pentacyclic triterpenoids belonging to the oleanane, ursane or lupane groups [15–21]. Such a chemical composition is nonetheless surprising because, usually, di- and triterpenes are not found together in a same resinic material [22]. In fact, this could be explained by the absence of informations concerning the botanical origin of the material. In literature dealing with diterpenic structures, the described compounds have always been isolated from commercial resins without botanical precisions [7–12], whereas, in all the analyses concerning triterpenes isolated from resins of well-defined species of *Boswellia*, there is no mention of the presence of diterpenes [13–21]. However, the majority of the papers referring to the chemical composition of olibanum, without geographical or botanical distinctions, are agreed with the presence of the α- and β-boswellic acids (compounds **1** and **2**, Fig. 1), and their *O*-acetates (**3** and **4**, Fig. 1), as main constituents of the methanol-soluble fraction of the natural resin.

So, these unusual pentacyclic acids, which have been only isolated from olibanum (commercial and/or certified resins), are specific chemical markers of this resin and could be very useful in order to characterize the presence of frankincense in archaeological resinous material.

The aim of this study was: (i) to isolate and characterize individual components present in the most widely trade olibanum, the "Eritrean-type" resin (produced by Ethiopian and Sudanese *Boswellia* [6]), (ii) to find, among these molecules, specific markers of olibanum, and (iii) to develop a method for the detection of such markers in an archaeological context.

In a first step, we have isolated and structurally characterized 15 triterpenes (α-boswellic acid, 3-*O*-acetyl-α-boswellic acid, β-boswellic acid, 3-*O*-acetyl-β-boswellic acid, α-amyrin, β-amyrin, lupeol, 3-*epi*-α-amyrin, 3-*epi*-β-amyrin, 3-*epi*-lupeol, α-amyrenone, β-amyrenone, lupenone, 3α-hydroxy-lup-20(29)-en-24-oic acid and 3-*O*-acetyl-hydroxy-lup-20(29)-en-24-oic acid) from the most common commercial olibanum: the "Eritrean-type" resin [23]. Thus, a GC–MS analysis was performed in order to detect these compounds in the methanolic extracts of commercial frankincense. The identification of the GC peaks was made by mass spectral and retention time comparison with pure standards previously described. Finally, the suitability of this procedure was proved by the chemical analysis of an archaeological sample suspected to contain frankincense, coming from the funeral endowment of the Egyptian princess Sat-mer-Hout (Dahshour, XIIth Dynasty). For such an analysis, a solvent extraction followed by derivatization and GC–MS is the most often used technique for the characterization and identification of a wide variety of organic compounds with a very small amount of sample. By using silylation instead of methylation, we are able to differentiate between natural methyl ester and free acids.

According to earlier studies [24,25], the analysis of an archaeological sample revealed the presence of characteristic components of frankincense (α- and β-boswellic acids, and their *O*-acetates) together with their products of degradation (24-noroleana-3,12-diene and 24-norursa-3,12-diene). In addition to these markers, we also characterize the presence of olibanum in this sample by the detection in small amount of 3α-hydroxy-lup-20(29)-en-24-oic acid, its *O*-acetyl derivative and their analogue (24-norlupa-3,20(29)-diene) stemming from chemical degradation of the sample. Mass spectra and mass spectral fragmentation of trimethylsilylated triterpenes of olibanum are discussed.

2. Experimental

2.1. Sample description

The analyzed sample (reference n°: L41, Victor Loret Egyptologic Institute, Lyon, France) originated from excavations conducted by J. de Morgan in 1894–1895 at Dahshour (Egypt). This sample, a black and amorphous piece of resin-like material, has been taken from an ointment

vase coming from the tomb of the princess Sat-mer-Hout (~1897–1844 B.C., XIIth Dynasty), sister of the pharaoh Amenemhat I. This vase has been found in a scent casket which had belonged to the funeral endowment of the princess. This sample was ground prior to chemical analysis.

2.2. Materials

Solvents and reagents were all of analytical grade from Merck (Darmstadt, Germany). Crystalline reference samples of all triterpenic standards from frankincense were isolated and characterized in our laboratory [23]. The methanolic extracts of a commercial "Eritrean-type" resin (Les Encens du Monde-Asie Concept, Castelnau-le-Lez, France) were filtered and fractionated by liquid chromatography using silica gel (Merck). Fractions eluted respectively with EtOAc/cyclohexane (10/90), (30/70) and (50/50) were further purified by HPLC (refractometric detection) on a C-18 reverse-phase column (Merck, Superspher 100 RP-18e, 250 × 4 mm). From these separations, we obtained α-boswellic acid, 3-*O*-acetyl-α-boswellic acid, β-boswellic acid, 3-*O*-acetyl-β-boswellic acid, 3-*epi*-α-amyrin, 3-*epi*-β-amyrin, 3-*epi*-lupeol, α-amyrenone, 3α-hydroxy-lup-20(29)-en-24-oic acid and 3-*O*-acetyl-hydroxy-lup-20(29)-en-24-oic acid. All structures were characterized on the basis of chemical and spectral evidence including two dimensional NMR experiments (COSY and NOESY ^{1}H–^{1}H, HMQC and HMBC) and mass spectrometric techniques (EI, HR-MS). For this chromatographic study, we have also used the following commercial triterpenic standards: α-amyrin, β-amyrin and lupeol from Extrasynthese (Genay, France). From β-amyrin and lupeol, we have obtained β-amyrenone and lupenone by a classical oxidation with PCC in dichloromethane.

Abietic, dehydroabietic and 7-oxo-dehydroabietic acids standards were purchased from Helix Biotech (Vancouver, Canada), retene from Extrasynthese and azelaic acid from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Sample preparation

A 5 mg amount of the sample analyzed (commercial resin, triterpenic standards or archaeological sample) was trimethylsilylated with a solution consisting of pyridine (0.5 ml), HMDS (0.45 ml) and TMSCl (0.3 ml). The reaction was conducted at room temperature for 30 min and then the solution was dried with a stream of nitrogen while heating (<40° C). Thereafter, the residue was immediately dissolved in 0.6 ml of diethyl ether. Each sample was treated in triplicate.

2.4. Gas chromatography–mass spectrometry

GC–MS analysis was carried out in a Varian Saturn 3900 gas chromatography, with a Varian 1177 injector, coupled with a Varian 2100 T ion trap mass spectrometer (Varian, Walnut Creek, CA, USA). The gas chromatograph was equipped with a 30 m × 0.25 mm i.d. fused-silica capillary column coated with a 0.25 μm film of poly(5% phenyl, 95% dimethylsiloxane): CP-Sil 8 CB low bleed/MS (Varian). The MS electron multiplier voltage was set at 1400 V and an ionization time of 25000 μs was used, running in the electron impact (EI) mode, with transfer line, ion trap and manifold temperatures of 300 °C, 200 °C and 50 °C, respectively. The mass spectrometer was set to scan 40–650 *m/z* with an ionizing voltage at 70 eV. Samples were injected (1 μl) with a splitting ratio of 1:20 and the injector temperature was set to 250 °C. A continuous flow-rate of 1 ml/min of chromatographic grade helium was used. The column oven was initially at 50 °C and was held for 2 min after injection, followed by temperature ramping at 8 °C/min up to 250 °C, and 250–350 °C at 3 °C/min. No hold time was performed at the upper limit. The total run time was approximately 61 min.

Identification of common fatty acids was performed using the NIST'98 mass spectral database.

3. Results and discussion

Chemical components of the resin part of frankincense are di- or triterpenes which can be unfunctionalized but many of them contain one or several oxygenated functions. For our part, only triterpenes have been studied: they belong in majority to ursane, oleanane or lupane families but we have also isolated in very small amount compounds with tirucallane skeleton. Methanolic extracts, in which only the triterpenoid (and/or the diterpenoid) part(s) are dissolved, were analyzed in order to remove the polymeric fractions present in fresh olibanum resin.

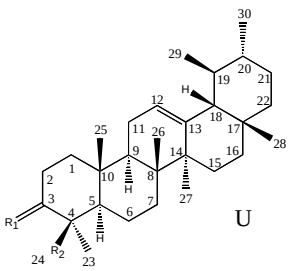
Since the amount of archaeological material available was more often very low, a method of chemical analysis using GC–MS was chosen. As triterpenes contain oxygenated function(s) and because of their relatively high molecular weight, these compounds should be derivatized to increase their volatility when using such a technique. In this work, trimethylsilylation performed at room temperature was preferred to methylation (already applied on resins from *Boswellia* sp. by Hairfield et al. [26]) since the former method should allow determination of naturally occuring methyl esters in commercial frankincense or archaeological samples. Injector parameters and GC operating conditions were adjusted to be able to obtain the best separation for triterpenes as well as for other types of chemical compounds (sesqui- and diterpenes, hydrocarbons, fatty acids, ...) which could be of any interest in an archaeological context.

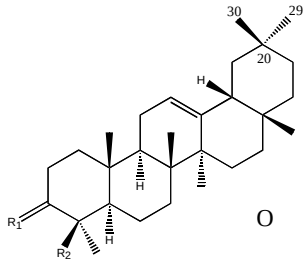
3.1. GC–MS analysis of triterpenic standards

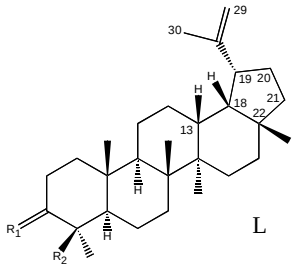
The triterpenic standards used for this study (Table 1) were either purified and structurally characterized (compounds **I**,

Table 1
Chemical structure of triterpenic standards

Structure type







Standard number	Triterpenes		Structure type	R_1	R_2
	Common name	Systematic name			
I	α-Amyrenone	Urs-12-en-3-one	U	O	CH_3
II	α-Amyrin, β-OTMS ether	3β-Hydroxy-urs-12-en-3-ol, β-OTMS ether	U	α-H, β-OTMS	CH_3
III	3-*epi*-α-Amyrin, α-OTMS ether	3α-Hydroxy-urs-12-en-3-ol, α-OTMS ether	U	α-OTMS, β-H	CH_3
IV	β-Boswellic acid, α-OTMS ether, TMS ester	3α-Hydroxy-urs-12-en-24-oic acid, α-OTMS ether, TMS ester	U	α-OTMS, β-H	CO_2TMS
V	3-*O*-Acetyl-β-boswellic acid, TMS ester	3α-*O*-Acetyl-urs-12-en-24-oic acid, TMS ester	U	α-OAc, β-H	CO_2TMS
VI	β-Amyrenone	Olean-12-en-3-one	O	O	CH_3
VII	β-Amyrin, β-OTMS ether	3β-Hydroxy-olean-12-en-3-ol, β-OTMS ether	O	α-H, β-OTMS	CH_3
VIII	3-*epi*-β-Amyrin, α-OTMS ether	3α-Hydroxy-olean-12-en-3-ol, α-OTMS ether	O	α-OTMS, β-H	CH_3
IX	α-Boswellic acid, α-OTMS ether, TMS ester	3α-Hydroxy-olean-12-en-24-oic acid, α-OTMS ether, TMS ester	O	α-OTMS, β-H	CO_2TMS
X	3-*O*-Acetyl-α-boswellic acid, TMS ester	3α-*O*-Acetyl-olean-12-en-24-oic acid, TMS ester	O	α-OAc, β-H	CO_2TMS
XI	Lupenone	Lup-20(29)-en-3-one	L	O	CH_3
XII	Lupeol, β-OTMS ether	3β-Lup-20(29)-en-3-ol, β-OTMS ether	L	α-H, β-OTMS	CH_3
XIII	3-*epi*-Lupeol, α-OTMS ether	3α-Lup-20(29)-en-3-ol, α-OTMS ether	L	α-OTMS, β-H	CH_3
XIV	–	3α-Hydroxy-lup-20(29)-en-24-oic acid, α-OTMS ether, TMS ester	L	α-OTMS, β-H	CO_2TMS
XV	–	3α-*O*-Acetyl-lup-20(29)-en-24-oic acid, TMS ester	L	α-OAc, β-H	CO_2TMS

III–**V**, **VIII**–**X** and **XIII**–**XV**), or from commercial origin (**II**, **VII** and **XII**) or hemi-synthetized from commercial material (**VI** and **XI**).

Their retention time was influenced by the number and the type of functional groups present, and generally increased with increasing molecular weight of derivatized triterpenes. Concerning the role played by their chemical skeleton in the retention mechanism, lupane standards were always retained longer than their ursane isomers and even longer than oleanane ones. Moreover, the absolute configuration of C-3 proved an important criterion for retention: β-configuration always gave a longer retention time than α-configuration. This fact allows to differentiate unambiguously lupeol (**XII**), α- (**II**) and β-amyrin (**VII**) from their respective 3-epimer (**XIII**, **III** and **VIII**) in the chromatogram. Under chromatographic conditions described in experimental section, compounds of a same family are always eluted according to the following order: 3α-alcohol, 3-ketone, 3β-alcohol, 3α-alcohol with a carboxylic function at C-24 and finally the corresponding *O*-acetate.

About the mass spectral study, the occurence of a strong peak at *m*/*z* 218, arising from a classical retro Diels Alder fission of ring C, in the mass spectra of ingredients **I**–**X** is characteristic of olean-12-ene or urs-12-ene derivatives without functionalization on rings C, D and E [27–29]. Among these compounds, the comparison in their mass spectra between peaks at *m*/*z* 203 and *m*/*z* 189 (and not *m*/*z* 191 [28]) allows to make the distinction between oleanane and ursane standards. In fact, for a Δ^{12}-oleanene derivative the fragment ion at *m*/*z* 203 is more intense than the peak at *m*/*z* 189, while usually the reverse occurs (or at least, peaks in question have similar intensities) in the mass spectra of an identical Δ^{12}-ursene derivative (Table 2). For standards belonging to the lupane group (**XI**–**XV**), mass spectra are much less characteristic than those of oleanane or ursane derivatives. In fact, it was found that usually lupane triterpenoids with an isopropenyl group in ring E could be characterized by an intense peak at *m*/*z* 189 [28,29]. This diagnostic information is correct in the case of lupeol (**XII**), 3-*epi*-lupeol (**XIII**) and lupenone (**XI**), but not for the acid **XIV** and its acetoxy derivative (**XV**) where the intensity of this peak is not remarkable. Effect of TMS-derivatization on mass spectra of these triterpenic standards is visible by the presence of: (i) peaks at *m*/*z* 73 ($(CH_3)_3Si^+$), *m*/*z* 147 ($TMSOSi^+(CH_3)_2$) and for [M-15] (loss of CH_3 from TMS) and [M-90] (loss of TMSOH) for all standards, (ii) a peak at *m*/*z* 292, for standards with a carboxylic acid group, due to the rDA fragment containing rings A and B with loss of TMSOH for compounds **IV**, **IX** and **XIV** or AcOH for their corresponding *O*-acetates (**V**, **X** and **XV**). Furthermore, the

Table 2
Gas chromatographic and mass spectrometric data of triterpenic standards

Standard number	t_R (min)	M^+ (relative intensity) (%)	*m/z* values of characteristic fragment of TMS standards (relative intensity) (%)
I	44.22	424 (14)	55 (16), 189 (6), 203 (5), 218 (100), 409 (8) [M–CH_3]
II	44.52	498 (4)	189 (64), 203 (40), 218 (100), 408 (1) [M–TMSOH], 483 (1) [M–CH_3]
III	41.77	498 (7)	189 (64), 203 (38), 218 (100), 408 (2) [M–TMSOH], 483 (1) [M–CH_3]
IV	45.75	600 (4)	189 (15), 203 (22), 218 (48), 292 (100), 510 (4) [M–TMSOH], 585 (9) [M–CH_3]
V	49.17	570 (4)	189 (37), 203 (47), 218 (100), 292 (94), 510 (8) [M–AcOH], 555 (2) [M–CH_3]
VI	43.83	424 (5)	55 (12), 189 (38), 203 (100), 218 (67), 409 (2) [M–CH_3]
VII	44.06	498 (2)	189 (55), 203 (100), 218 (75), 408 (1) [M–TMSOH], 483 (1) [M–CH_3]
VIII	41.40	498 (3)	189 (54), 203 (100), 218 (73), 408 (3) [M–TMSOH], 483 (1) [M–CH_3]
IX	45.15	600 (2)	189 (15), 203 (21), 218 (48), 292 (100), 510 (4) [M–TMSOH], 585 (9) [M–CH_3]
X	48.55	570 (2)	189 (31), 203 (100), 218 (70), 292 (56), 510 (4) [M–AcOH], 555 (1) [M–CH_3]
XI	44.66	424 (32)	55 (35), 189 (58), 203 (55), 205 (100), 218 (29), 409 (40) [M–CH_3]
XII	44.95	498 (16)	175 (28), 189 (100), 203 (59), 218 (30), 408 (12) [M–TMSOH], 483 (10) [M–CH_3]
XIII	41.87	498 (12)	175 (30), 189 (100), 203 (36), 218 (14), 408 (16) [M–TMSOH], 483 (4) [M–CH_3]
XIV	45.63	600 (12)	73 (49), 121 (37), 147 (36), 175 (40), 292 (32), 472 (100), 510 (39) [M–TMSOH], 585 (32) [M–CH_3]
XV	49.28	570 (11)	73 (100), 173 (63), 292 (53), 510 (31) [M–AcOH], 555 (4) [M–CH_3]

presence of a prominent peak at [M-15] in the mass spectra of triterpenic acids (at C-24) with a hydroxyl group at C-3, due to the TMS-derivatization of the two functions, could be used as a diagnostic tool in order to recognize these type of compounds between the other trimethylsilylated standards.

Figs. 2 and 3 describe the main plausible cleavages in TMS-triterpenoid standards of the ursane and lupane families in accord with literature [27–29]: high resolution or other mass spectrometry techniques experiments would be necessary in order to prove the correctness of these mass spectral fragmentation pathways. Fragmentation pattern of oleanane triterpenes is not described because of its similarity (except intensity of peak at *m/z* 203) with the one of ursane standards.

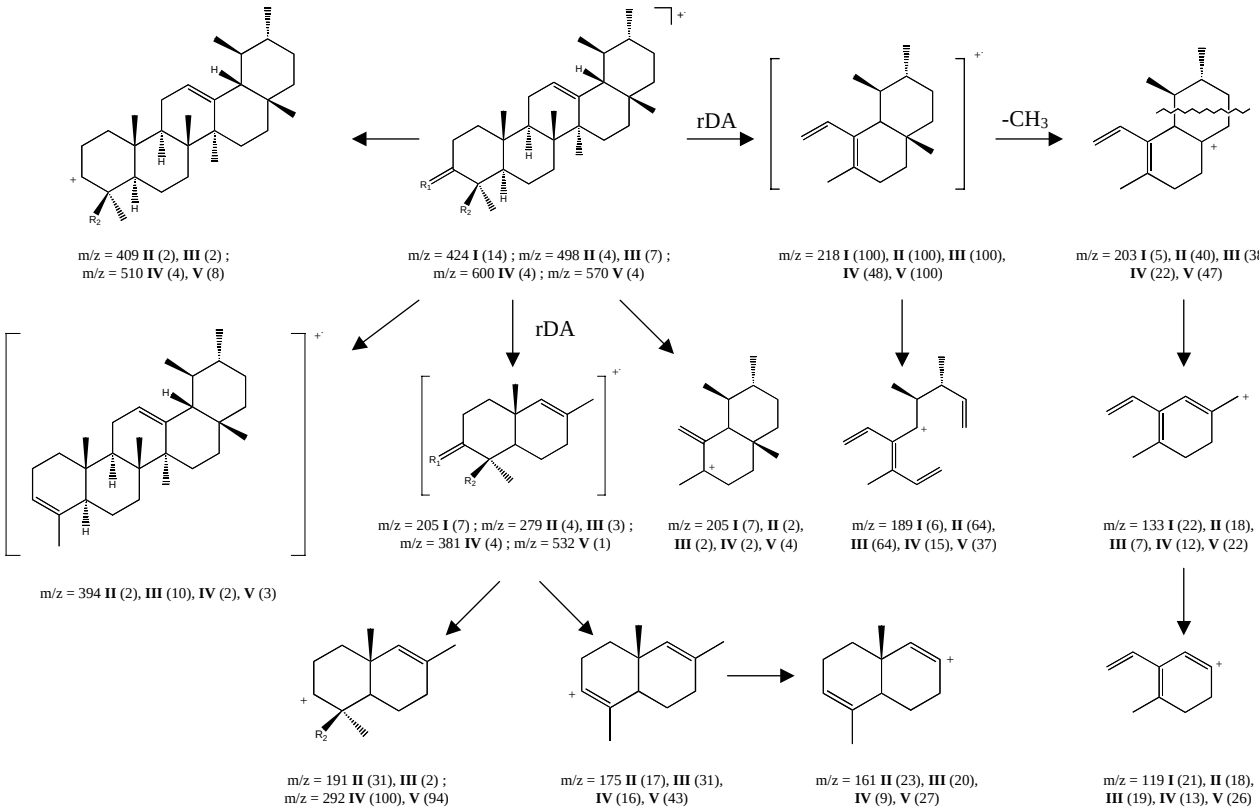


Fig. 2. Main cleavages of trimethylsilylated ursane triterpenoids under EI conditions.

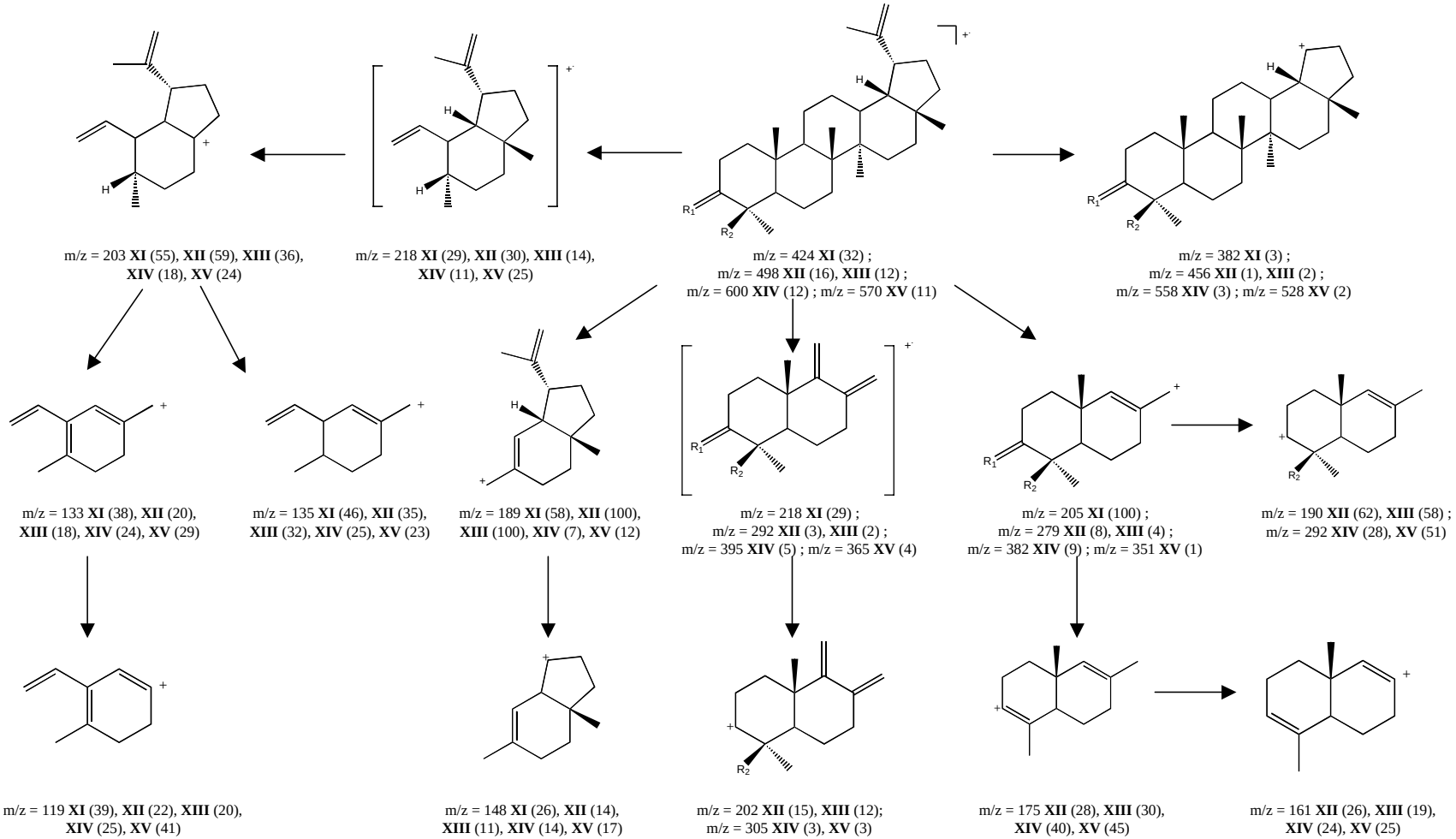


Fig. 3. Main cleavages of trimethylsilylated lupane triterpenoids under EI conditions.

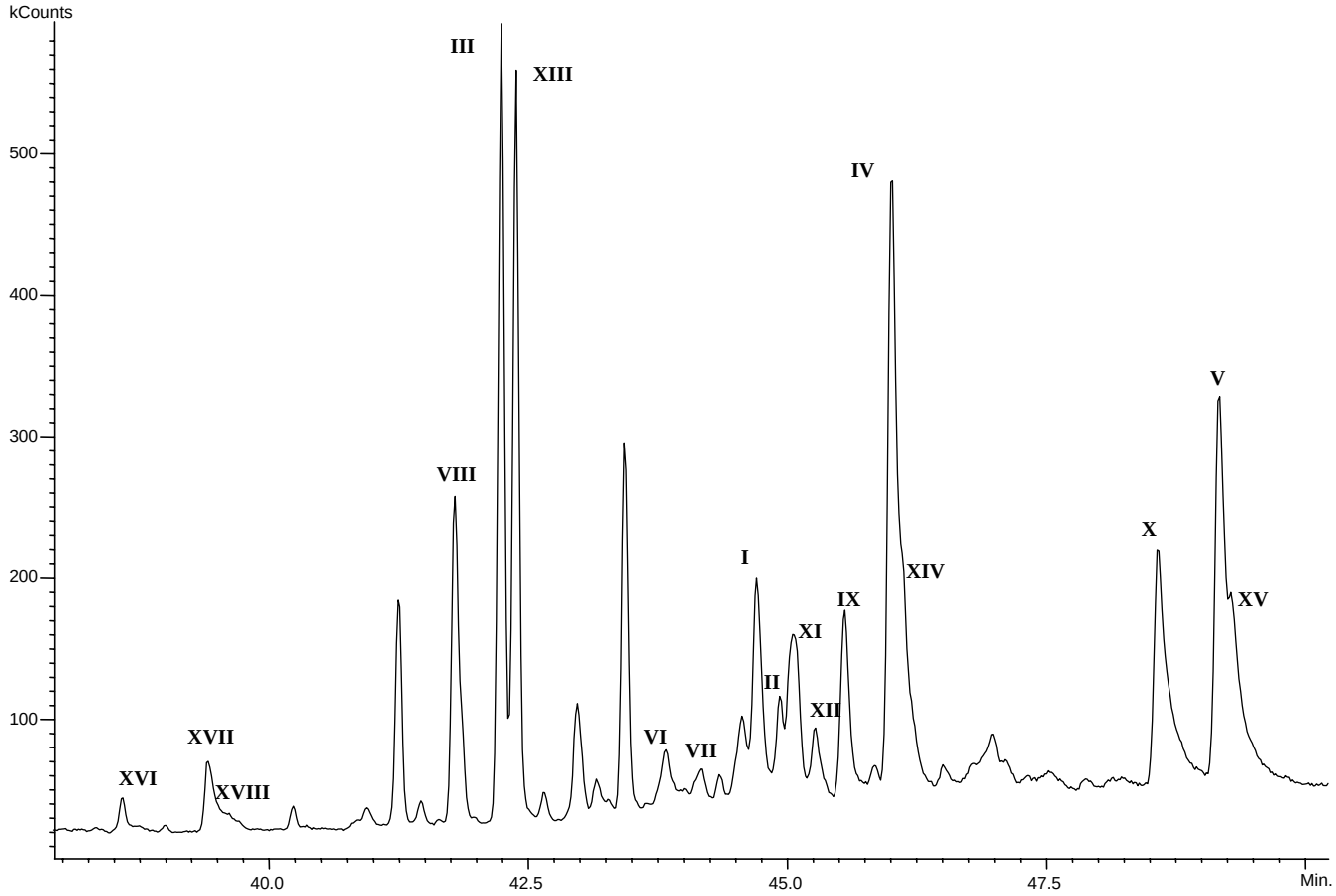


Fig. 4. Total ion current (TIC) chromatogram of the triterpenic zone of commercial frankincense ("Eritrean-type" resin). See Section 2 for GC–MS conditions. Peak numbers refer to compounds in Tables 1 and 3.

3.2. GC–MS analysis of commercial frankincense ("Eritrean-type" resin)

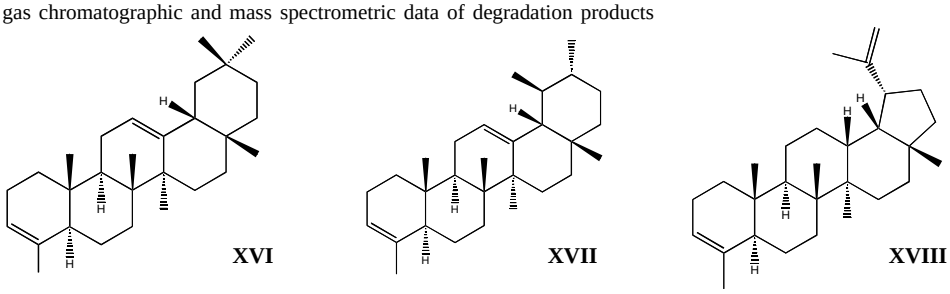
Analysis of a trimethylsilylated sample of a commercial olibanum coming from Eritrea was performed in scan mode and allowed detection of numerous triterpenes. All standards isolated from this resin (compounds **I**, **III**–**V**, **VIII**–**X** and **XIII**–**XV**) were obviously present in the chromatogram together with five other common triterpenes (compounds **II**, **VI**, **VII**, **XI** and **XII**) (Fig. 4).

It can be emphasized that among triterpenes with an alcohol function at C-3, those with an α position of the hydroxyl group are in majority. This fact, in accord with literature on the chemistry of *Boswellia* species [13–21,23], indicates a preferential biosynthetic pathway for metabolites exudated from trees of this genus. The presence of α-amyrenone (**I**), β-amyrenone (**VI**) and lupenone (**XI**) is not surprising because these compounds are frequent constituents of higher plants or could have been formed by trivial oxidation of the corresponding 3-alcohols. Furthermore, α-amyrin (**II**), β-amyrin (**VII**) and lupeol (**XII**), present in small amounts, are probably products of enzymatic reduction of these ketones.

In addition to these standards, 24-noroleana-3,12-diene (**XVI**) and 24-norursa-3,12-diene (**XVII**) have been identified by their mass spectra and their position on the GC profile (a shorter retention time than those of triterpenic structures) (Table 3). These components are known degradation products of α- and β-boswellic acids (**IX**, **IV**) and their *O*-acetates (**X**, **V**) [25]: their formation is favoured by the anti-periplanar conformation of the carboxyl and hydroxyl (or acetate) groups leading to the Δ^3 double bond. In the same manner, 24-norlupa-3,20(29)-diene (**XVIII**), a degradation product of compounds **XIV** and **XV**, has been unambiguously recognized. For confirming the structure of these compounds, triterpenic acid standards (**IV**, **IX** and **XIV**) and their *O*-acetates (**V**, **X** and **XV**) have been subjected to a GC–MS analysis without TMS-derivatization in order to carry out their thermal degradation in the injector of the GC: in each case, a single peak corresponding to the appropriate degradation product was observed in the chromatogram.

Table 3
Chemical structure, gas chromatographic and mass spectrometric data of degradation products

Structure type



Number	Systematic name	t_R (min)	M^+ (relative intensity) (%)	*m/z* values of characteristic fragment (relative intensity) (%)
XVI	24-noroleana-3,12-diene	38.58	394 (3)	175 (40), 189 (31), 203 (100), 218 (62), 379 (1) [M–CH_3]
XVII	24-norursa-3,12-diene	39.41	394 (8)	175 (45), 189 (36), 203 (46), 218 (100), 379 (4) [M–CH_3]
XVIII	24-norlupa-3,20(29)-diene	39.49	394 (34)	175 (100), 189 (44), 203 (41), 218 (24), 379 (32) [M–CH_3]

3.3. GC–MS analysis of a resinous archaeological sample ("L41", Dashour, Egypt)

Analysis of a trimethylsilylated piece of this archaeological sample was performed using the proposed procedure. In the triterpenic area of the chromatogram (Fig. 5a), several triterpenes were detected and these peaks were assigned to 3-*epi*-β-amyrin (**VIII**), 3-*epi*-α-amyrin (**III**), 3-*epi*-lupeol (**XIII**), and more especially 3α-hydroxy-lup-20(29)-en-24-oic acid (**XIV**), α- and β-boswellic acids (**IX** and **IV**), their *O*-acetates (**XV**, **X** and **V**) and their products of degradation (**XVIII**, **XVI** and **XVII**) by comparison of spectral and retention time data. The presence of such metabolites, characteristic of resin obtained from *Boswellia* species, firmly proves that frankincense goes into these archaeological resinous mixture. Concerning the nor-triterpenes **XVI**,

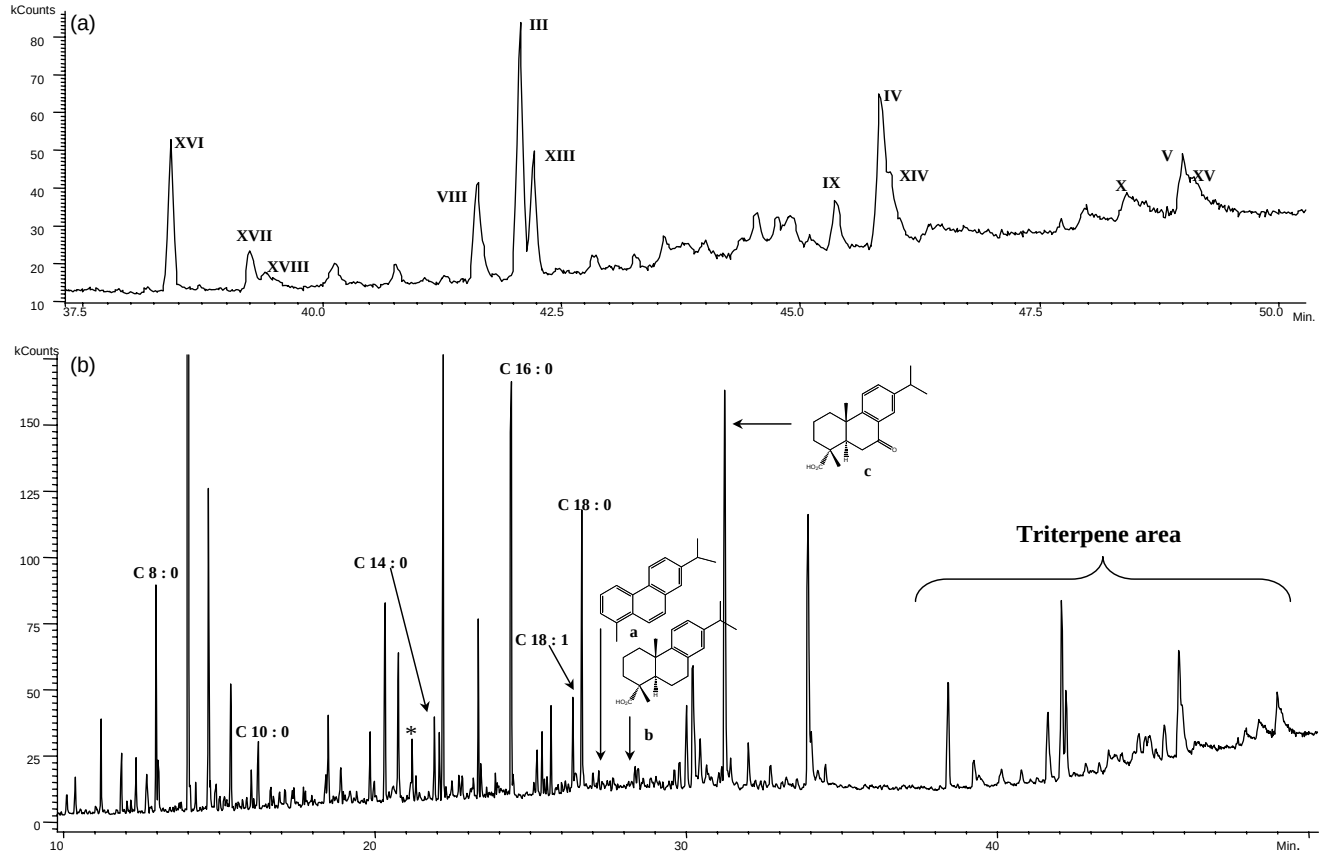


Fig. 5. Total ion current (TIC) chromatogram of sample L41. See Section 2 for GC–MS conditions. (a) Chromatogram of the triterpenic zone. Peak numbers refer to compounds in Tables 1 and 3. (b) Complete chromatogram. Standards: (∗): azelaic acid; a: retene; b: dehydroabietic acid; and c: 7-oxo-dehydroabietic acid.

XVII and **XVIII**, their presence in important amounts is in agreement with the nature of the studied sample: these compounds are probably due in majority to the ageing degradation or the original thermal treatment of the sample but also, to a lesser extent, to the thermal degradation of some triterpenes occuring in the injector of the GC.

In addition, further investigations on the rest of the chromatogram obtained (Fig. 5b) reveal the presence of diterpenoid acids, belonging to the abietane and pimarane groups, characteristic of a conifer resin [30]. The occurence among them of oxidized abietic acid derivatives (retene (**a**), dehydroabietic acid (**b**) and 7-oxo-dehydroabietic acid (**c**)) and the absence of abietic acid are in agreement with the great age of the sample and/or could confirm that the resin was heated during the preparation of this archaeological mixture. On the other hand, fatty acids have also been detected: the presence of azelaic acid (a product of degradation of oleic acid) and the predominance of palmitic acid in comparison to stearic acid seems to favor a vegetable origin for the oil probably used by the maker of this ancient preparation [31]. Common fatty acids were identified by comparison of their mass spectra with those from the NIST'98 database, while for retene (**a**) and azelaic, 7-oxo-dehydroabietic (**c**) and dehydroabietic acids (**b**), the identification was also confirmed by the injection of the corresponding standards.

4. Conclusion

The study of archaeological sample extractives is often a challenge for a chemist because of the wide range of compound classes present, the frequent occurence of degradation products and the small quantities of ancient matter available. In this context, in order to characterize frankincense without losing informations on other hypothetical original ingredients, a simple procedure using trimethylsilylation followed by GC–MS analysis, has been developed. For this, chemical markers of olibanum—3α-hydroxy-lup-20(29)-en-24-oic acid (**XIV**), α- and β-boswellic acids (**IX** and **IV**) and their *O*-acetates (**XV**, **X** and **V**)—have been isolated from an actual commercial resin, structurally characterized and then detected in the archaeological resinous matter studied. The presence of olibanum was also confirmed by the occurence of the typical products of degradation (**XVI**, **XVII** and **XVIII**) of these triterpenic acids and their *O*-acetyl derivatives.

In addition, the presence on one hand of characteristic fatty acids and on the other hand of abietane and pimarane diterpenic acids seems to indicate that an oil of vegetable origin and a conifer oleoresin have been used originally, as well as frankincense, for the preparation of this ancient mixture.

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