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A lupane triterpene from frankincense (*Boswellia* sp., Burseraceae)

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Abstract

A new lupane-type triterpene, 3α-hydroxy-lup-20(29)-en-24-oic acid, was isolated from the methanolic extract of "Erytrean-type" resin of commercial frankincense together with the known 3α-hydroxy-olean-12-en-24-oic acid (α-boswellic acid) and 3α-hydroxy-urs-12-en-24-oic acid (β-boswellic acid). Their structures were characterized on the basis of chemical and spectral evidence including two dimensional NMR experiments and mass spectrometric techniques.

Keywords: Frankincense; Olibanum; *Boswellia* sp.; Burseraceae; Lupane, oleanane and ursane triterpenes; 3α-Hydroxy-lup-20(29)-en-24-oic acid; Boswellic acids

1. Introduction

In the course of our studies on natural resins, and their possible applications in archaeology and restoration of works of art (Vieillescazes, 1992; Archier and Vieillescazes, 2000; Martin et al., 1999; Pistre et al., 1999), the chemical content of commercial frankincense was investigated. Frankincense, or olibanum, is a natural oleo-gum-resin obtained from trees of the genus *Boswellia* (family Burseraceae). It is one of the oldest fragrant and medicinal resins known throughout the world. The botanical origin of olibanum resins and the names ascribed to their sources have been a subject of much uncertainty and discussion over many years (Coppens, 1995). Actually, the four main producing species are *Boswellia carteri* (East Africa), *B. frereana* (Somalia), *B. sacra* (Southern Arabia) and *B. serrata* (North-Western India) (Thulin and Warfa, 1987; Dupéron, 1993; Coppens, 1995).

Frankincense has been reported to be a rich source of terpenes, the major non-volatile constituents being cembrane and verticillane diterpenoids on one hand, and oleanane and ursane triterpenoids on the other hand (Tücker, 1986; Maupetit, 1984; Basar et al., 2001).

In the present study, we have examined the chemical composition of the most widely traded olibanum: the "Erytrean-type" resin produced by Ethiopian and Sudanese *Boswellia* (Coppens, 1995). In addition to α- and β-boswellic acids (**1** and **2**), previously reported as constituents of incense (Winterstein and Stein, 1932; Savoir et al., 1967; Fattorusso et al., 1983; Mahajan et al., 1995), we now report on the isolation and characterization of a new pentacyclic triterpene (**3**) of the lupane series.

2. Results and discussion

The methanolic extracts of the commercial resin were filtered and fractionated by liquid chromatography using silica gel. The fraction eluted with EtOAc–hexane (1:1) was further purified by HPLC on a C_{18} reverse-phase column. From this separation, we obtained the previously reported triterpenes **1** and **2**, as well as the new compound **3**. The ^{1}H and ^{13}C NMR data for compounds **1** and **2** revealed the presence of structurally similar triterpenes. On the basis of the comparison of

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their physical properties with those reported in the literature (Winterstein and Stein, 1932; Beton et al., 1956) but more especially by the analysis of their mass and NMR spectra, these compounds have been identified as 3α-hydroxy-olean-12-en-24-oic acid (α-boswellic acid) and 3α-hydroxy-urs-12-en-24-oic acid (β-boswellic acid). Their ^{1}H and ^{13}C NMR data have been included since they have been unambiguously assigned (Tables 1 and 2).

Compound **3** was an optically active white amorphous powder. It was assigned the molecular formula $C_{30}H_{48}O_3$ by HRMS ([M]$^+$ at 456.3624; calc. 456.3603). It gave a positive Liebermann-Burchard test for triterpenes. The IR spectrum showed the absorption for a hydroxyl group (3640 cm^{-1}), a carboxylic group (1690 cm^{-1}) and a terminal double bond (3070, 1740 and 885 cm^{-1}).

The formation of a methyl ester (**3a**) and a monoacetyl derivative (**3b**) corroborated the presence of one carboxylic and one hydroxyl function.

The ^{13}C NMR spectra of compound **3** revealed 30 carbon signals which were sorted by DEPT ^{13}C NMR as six methyls, ten methylenes, five methines, five quaternary carbons, one alcoholic methine, one carboxylic acid and two olefinic carbons (one $=CH_2$ and one quaternary). The $\Delta^{20,29}$-functionality of a lupene skeleton was inferred for this compound from the resonances of the sp^2 carbons at C-29 (secondary carbon signal deduced by DEPT pulse sequence) at δ 109.3 and C-20 (quaternary carbon) at δ 151.0.

A detailed analysis of the ^{1}HNMR spectrum of **3** confirmed the characteristic features for a triterpenic lup-20(29)-ene parent structure. It was characterized by signals for five tertiary methyls at δ 0.77, 0.79, 0.96, 1.06 and 1.32 (3H each, *s*, Me-25, Me-28, Me-27, Me-26 and Me-23, respectively), one vinylic methyl at δ 1.68 (3H, *s*, Me-30), two protons of an isoprenyl moiety at δ 4.69 and 4.57 (1H each, *bs*, H$_a$-29 and H$_b$-29),

Table 1
^{1}H NMR data of compounds **1**, **2** and **3** (TMS as int. standard)[a]

H	1	2	3
	$CDCl_3$ (400 MHz)	$CDCl_3$ (500 MHz)	$CDCl_3$ (500 MHz)
1	1.45 *m* 1.29 *m*	1.49 *m* 1.33 *m*	1.48 *m* 1.20 *m*
2	2.22 *m* 1.59 *m*	2.24 *m* 1.60 *m*	2.23 *m* 1.59 *m*
3	4.08 *t* (2.5)	4.08 *t* (2.5)	4.05 *t* (3.0)
5	1.49 *m*	1.50 *m*	1.45 *m*
6	1.85 *m* 1.70 *m*	1.83 *m* 1.70 *m*	1.84 *m* 1.60 *m*
7	1.52 *m* 1.37 *m*	1.58 *m* 1.41 *m*	1.41 *m* 1.37 *m*
9	1.66 *m*	1.63 *m*	1.36 *m*
11	1.88 *m*	1.92 *m* 1.18 *m*	1.47 *m* 1.18 *m*
12	5.19 *t* (3.5)	5.14 *t* (3.5)	1.74 *m* 1.62 *m*
13	–	–	1.66 *m*
15	1.77 *m* 1.00 *m*	1.86 *m* 1.02 *m*	1.65 *m* 1.01 *m*
16	2.00 *m* 0.81 *m*	2.02 *m* 0.88 *m*	1.48 *m* 1.37 *m*
18	1.96 *m*	1.34 *m*	1.37 *m*
19	1.70 *m* 1.02 *m*	1.34 *m*	2.38 *m*
20	–	0.94 *m*	–
21	1.33 *m* 1.10 *m*	1.41 *m* 1.29 *m*	1.90 *m* 1.29 *m*
22	1.44 *m* 1.22 *m*	1.45 *m* 1.27 *m*	1.38 *m* 1.18 *m*
23	1.35 *s*	1.34 *s*	1.32 *s*
25	0.89 *s*	0.91 *s*	0.77 *s*
26	1.00 *s*	1.05 *s*	1.06 *s*
27	1.15 *s*	1.11 *s*	0.96 *s*
28	0.84 *s*	0.83 *s*	0.79 *s*
29	0.87 *s*	0.80 *d* (5.5)	4.69 *bs* (H$_a$) 4.57 *bs* (H$_b$)
30	0.87 *s*	0.94 *d* (6.0)	1.68 *s*

[a] Chemical shifts are δ values, coupling constants (*J* in parentheses) are given in Hz; assignments were confirmed by decoupling and 2D NMR experiments (COSY ^{1}H–^{1}H, HMQC and HMBC).

Table 2
^{13}C NMR data of compounds **1**, **2** and **3** (TMS as int. standard)[a]

C	1	2	3
	$CDCl_3$ (400 MHz)	$CDCl_3$ (500 MHz)	$CDCl_3$ (500 MHz)
1	33.6	33.9	33.8
2	26.2	26.2	26.3
3	70.8	70.8	71.0
4	47.4	47.4	47.5
5	49.1	49.1	49.0
6	19.7	19.7	19.6
7	32.7	33.1	34.1
8	39.8	40.0	40.8
9	46.7	46.8	49.7
10	37.6	37.5	37.8
11	23.5	23.4	21.1
12	121.7	124.5	25.2
13	145.1	139.6	38.0
14	41.9	42.3	42.9
15	26.0	26.5	27.4
16	26.9	28.1	35.5
17	32.5	33.8	43.0
18	47.3	59.2	48.2
19	46.7	39.7	48.0
20	31.1	39.6	151.0
21	34.7	31.3	29.8
22	37.1	41.5	40.0
23	24.2	24.2	24.1
24	183.2	183.1	183.0
25	13.1	13.3	13.4
26	16.7	16.9	15.9
27	25.9	23.2	14.5
28	28.4	28.8	18.0
29	33.3	17.4	109.3
30	23.7	21.4	19.3

[a] Multiplicities were obtained with DEPT sequences.

one carbinolic proton at *δ* 4.05 (1H, *t*, $J=3.0$ Hz, H-3) and the typical lupenyl H_β-19 proton at δ 2.38 (1H, *m*). In addition, the α-position of the isoprenyl group was confirmed by the spatial correlation between H_β-19 and the proton signal at *δ* 0.79 (C-28) in the NOESY experiment.

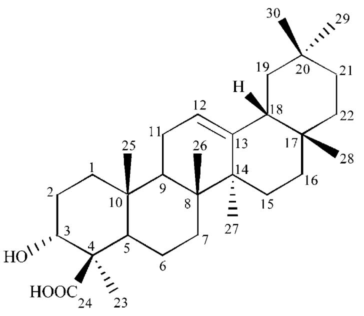
The position of the hydroxyl group at C-3 was determined through an HMBC experiment in which the oxymethine proton signal at *δ* 4.05 (H-3) showed $^2J_{\text{C-H}}$ correlations with C-2 (*δ* 26.3) and C-4 (*δ* 47.5), and $^3J_{\text{C-H}}$ correlations with C-1 (*δ* 33.8), C-5 (*δ* 49.0) and C-23 (*δ* 24.1); while the alcoholic methine carbon signal at *δ* 71.0 (C-3) showed $^3J_{\text{C–H}}$ interactions with H-1 (*δ* 1.48 and 1.20), H-5 (*δ* 1.45) and H-23 (*δ* 1.32). The configuration of the hydroxyl group at C-3 was assigned to be α on the basis of: (i) the absence of interactions between H-3 and H-5 in the NOESY experiment; (ii) the absence of diaxial spin–spin coupling shown by the small *J* values (which indicated an equatorial position for H-3 on the β-face and an axially orientation for the hydroxyl group on the α-face).

Localization of the carboxylic acid function at C-24 was realized through the observation on the HMBC spectrum of $^3J_{\text{C–H}}$ interactions between the carboxylic carbon signal at δ 183.0 (C-24) and H-3 (*δ* 4.05), H-5 (*δ* 1.45) and H-23 (*δ* 1.32).

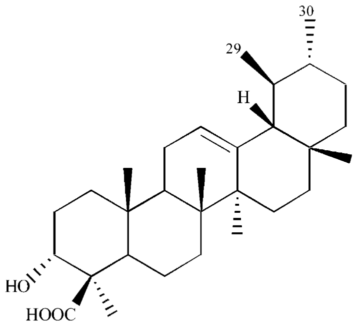
Thus on the basis of its spectroscopic data and comparison of the NMR chemical shifts of all the carbons and protons of rings A and B with those of compounds structurally similar such as **1** and **2** (Tables 1 and 2), compound **3** was assigned to be 3α-hydroxy-lup-20(29)-en-24-oic acid.

For these three compounds, assignment of carbon and proton signals (Tables 1 and 2 respectively) was confirmed by means of homonuclear (COSY) and heteronuclear (HMQC and HMBC) 2D NMR experiments, in particular regarding the location of methyl and quaternary carbons.

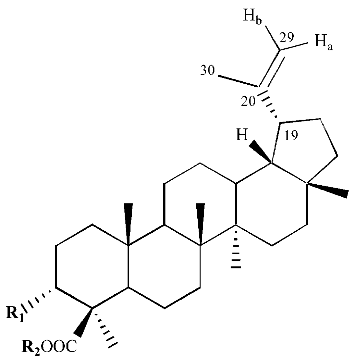
Numerous ursane- and oleanane-type triterpenes have been already described from frankincense (Tücker, 1986), but also from well-defined resins [*Boswellia carteri* (Fattorusso et al., 1983) and *B. serrata* (Mahajan et



1 α-boswellic acid



2 β-boswellic acid



	R_1	R_2
3	-OH	-H
3a	-OH	$-CH_3$
3b	-Ac	-H

al., 1995)]. From *B. frereana* and *B. serrata*, triterpenes with, respectively, dammarane (Fattorusso et al., 1985) and tirucallane (Pardhy and Bhattacharyya, 1978) skeletons have been found. Lupane-type triterpenes are less common because only lupeol and *epi*-lupeol have been isolated from *B. frereana* (Proietti et al., 1981), thus the isolation of compound **3** is the first report of a triterpene with this type of skeleton from commercial olibanum. In addition with α- and β-boswellic acids (compounds **1** and **2**), which have been only isolated from frankincense (i.e. resins harvested on trees of the genus *Boswellia*), the identification of such a specific chemical marker will be very useful in order to characterize the presence of frankincense in archaeological samples.

3. Experimental

3.1. General

MS: direct inlet, 70 eV; IR:CCl_4; ^1H NMR: 400 and 500 MHz; ^{13}C NMR: 100 and 125 MHz. Chemical shifts are quoted in ppm (δ) relative to TMS and coupling constants are in Hz. Optical rotations were determined using a Perkin Elmer 241 polarimeter. Final purification of all metabolites was achieved by HPLC on a C18 reverse-phase column (250×4 mm, Superspher 100 RP-18e) with RI monitoring.

3.2. Material

The "Erytrean-type" resin (olibanum in granis from Erytrea, ref.: OLSOU) was furnished by the society "Les Encens du Monde - Asie Concept", 460, Chemin du mas de Rochet, F-34170 Castelnau-le-Lez, France.

3.3. Extraction and purification

The raw material (30 g) was extracted with MeOH at room temperature. After filtration, the filtrate was concentrated to yield 20.5 g of a methanolic extract which was subjected to CC over silica gel (350 g) eluted with a solvent gradient from hexane to EtOAc and finally MeOH. The new compound (3), α- and β-boswellic acid (1 and 2) were eluted with EtOAc–hexane (1:1) and subsequently purified by reverse-phase HPLC with 100% MeCN to give, in polarity order, 25 mg of **3**, 110 mg of **1** and 200 mg of **2**.

*3.4. α-Boswellic acid (**1**)*

Colourless crystals from MeOH, $[\alpha]_D^{25}+80$ ($CHCl_3$, *c* 0.25); UV λ_{max}^{EtOH} nm: 205; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3637, 3525, 2972, 2950, 2928, 2858, 1736, 1692, 1463, 1382, 1362, 1261, 1022; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS (70 eV) *m*/*z* (rel. Int.): 456 (0.3), 394 (7), 281 (4), 218 (100), 203 (80), 189 (22), 175 (15), 161 (14).

*3.5. β-Boswellic acid (**2**)*

Colourless crystals from MeOH, $[\alpha]_D^{25}$ +105 ($CHCl_3$, *c* 0.85); UV λ_{max}^{EtOH} nm: 204; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3640, 3528, 2975, 2923, 2860, 1735, 1692, 1455, 1379, 1263, 1054, 1024; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS (70 eV) *m*/*z* (rel. Int.): 456 (0.6), 394 (11), 218 (100), 203 (25), 189 (17), 175 (15), 161 (16).

*3.6. 3α-Hydroxy-lup-20(29)-en-24-oic acid (**3**)*

Colourless crystals from MeOH, $[\alpha]_D^{25}$ +16 ($CHCl_3$, *c* 1.15); UV λ_{max}^{EtOH} nm: 208; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3636, 3400, 2930, 2859, 1737, 1693, 1455, 1380, 1282, 1261, 1205, 1054, 1033, 958, 885; ^1H and ^{13}C NMR: Tables 1 and 2; HREIMS:*m*/*z* 456.3624 ($C_{30}H_{48}O_3$ requires m/z 456.3603); EIMS (70 eV) *m*/*z* (rel. Int.): 456 (32), 408 (10), 218 (40), 205 (10), 203 (14), 191 (10), 189 (14), 187 (11), 175 (10), 161 (16), 149 (21), 135 (27), 133 (16), 123 (20), 121 (27), 109 (100).

*3.7. Methylation of compound **3** (compound **3a**)*

Compound **3** (6 mg) was dissolved in 5 ml of MeOH, and treated with an ether solution of CH_2N_2 at room temp. for 2 h. After evaporation of the solvent, 6 mg of methyl ester **3a** were obtained. Colourless crystals from MeOH, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3637, 2930, 2856, 1722, 1696, 1458, 1382, 1231, 1193, 1057, 865; ^1H NMR($CDCl_3$) δ (ppm): 4.69 (1H, *s*, H_a-29), 4.57 (1H, *s*, H_b-29), 4.08 (1H, *bs*, H-3), 3.65 (1H, *s*, $COOC\underline{H}_3$), 2.39 (1H, *m*, H-19), 1.69 (3H, *s*, H-30), 1.26 (3H, *s*, H-23), 1.05 (3H, *s*, H-26), 0.96 (3H, *s*, H-27), 0.80 (3H, *s*, H-28), 0.67 (3H, *s*, H-25) ;^{13}C NMR ($CDCl_3$) δ (ppm): 13.3 (C-25), 14.5 (C-27), 15.9 (C-26), 18.0 (C-28), 19.3 (C-30), 19.8 (C-6), 21.1 (C-11), 24.0 (C-23), 25.2 (C-12), 26.4 (C-2), 27.4 (C-15), 29.9 (C-21), 33.9 (C-1), 34.1 (C-7), 35.6 (C-16), 37.6 (C-10), 38.1 (C-13), 40.0 (C-22), 40.8 (C-8), 42.9 (C-14), 43.0 (C-17), 47.6 (C-4), 48.0 (C-19), 48.2 (C-18), 48.9 (C-5), 49.7 (C-9), 51.1 ($COO\underline{C}H_3$), 71.1 (C-3), 109.3 (C-29), 151.0 (C-20), 177.7 (C-24).

*3.8. Acetylation of compound **3** (compound **3b**)*

Compound **3** (10 mg) was dissolved in pyridine (1 ml) with Ac_2O (3 ml), and the mixture was left overnight at room temperature. Ice was added to the reaction mixture, and it was extracted with EtOAc. The EtOAc layer was evaporated to yield the monoacetate **3b** (7 mg). Colourless crystals from MeOH, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2930, 2857, 1742, 1696, 1454, 1380, 1245, 1119, 1029, 887; ^1H NMR ($CDCl_3$) δ (ppm): 5.29 (1H, *bs*, H-3), 4.69 (1H, *s*, H_a-29), 4.57 (1H, *s*, H_b-29), 2.39 (1H, *m*, H-19), 2.10 (3H, *s*, $C\underline{H}_3COO$), 1.68 (3H, *s*, H-30), 1.22 (3H, *s*, H-23), 1.06 (3H, *s*, H-26), 1.00 (3H, *s*, H-27), 0.80 (3H, *s*, H-28), 0.78 (3H, *s*, H-25);^{13}C NMR ($CDCl_3$) δ (ppm):

12.4 (C-25), 14.1 (C-27), 15.9 (C-26), 18.0 (C-28), 19.3 (C-30), 19.6 (C-6), 21.0 (C-11), 21.4 ($\underline{C}H_3COO$), 23.8 (C-23, C-2), 25.2 (C-12), 27.4 (C-15), 30.0 (C-21), 34.1 (C-7), 34.5 (C-1), 35.6 (C-16), 37.7 (C-10), 38.8 (C-13), 40.0 (C-22), 40.8 (C-8), 42.9 (C-14), 43.0 (C-17), 46. (C-4), 47.9 (C-19), 48.2 (C-18), 49.6 (C-5), 50.5 (C-9), 77.2 (C-3), 109.3 (C-29), 150.9 (C-20), 170.5 ($CH_3\underline{C}OO$), 182.9 (C-24).

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