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Two New and Four Known Triterpenoids from *Boswellia sacra* Fluckiger

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Abstract: Two new *O*-acetyl derivatives of pentacyclic triterpenic acids, 3*α*-acetoxyurs-5:12-dien-24-oic acid (**1**) and 3*α*-acetoxylup-12:20 (29)-dien-24-oic acid (**2**) were isolated from Omani frankincense of *Boswellia sacra* Flueckiger along with four known compounds: commic acid-D (**3**), 9,11-dehydro-*β*-boswellic acid (**4**), 3*β*-Hydroxy-11-oxours-12-ene (**5**), and 11*β*-Hydroxy-3-oxours-12-ene (**6**). The structures of new compounds **1** and **2** were elucidated on the basis of detailed mass and NMR spectroscopic analysis using ^{1}H NMR, ^{13}C NMR, HMQC, HMBC, COSY, and HREIMS techniques. The relative configurations of **1** and **2** were assigned by comparative analysis of the NMR spectral data with the related known triterpenoids together with NOESY experiments. The structures of the known compounds **3**-**6** were confirmed by the comparative analysis of the reported mass and NMR data.

Keywords: Omani Frankincense; HPLC; Pentacyclic Triterpenoids; *Boswellia sacra*.

1. Plant Source

Frankincense is an oleo-gum-resin obtained from the incisions of plants of the genus *Boswellia*. *Boswellia sacra* Flueck., belonging to the family Burseraceae, is a woody plant found in the southern part of the Sultanate of Oman [1]. The frankincense samples of *B. sacra* were collected from the southern part of Oman and were also supplied by a trustful partner (Mr. Saleh Al-Amri, Ministry of Agriculture and Marine Wealth). All these samples were authenticated by Dr. Mustafa Mansi (botanist), Department of Biological Sciences and Chemistry, University of Nizwa, the Sultanate of Oman. A voucher specimen (No: BSHR-01/2012) was deposited in the herbarium of the Department of Biological Sciences and Chemistry.

2. Previous Studies

Boswellic acids (BAs) are a group of pentacyclic triterpenoids isolated from gum resin of *Boswellia* spp. Their bioactivities against inflammation [2], arthritis [3], ulcerative colitis [4], chronic colitis [5], asthma [6] and hepatitis [7] are well documented. The Omani frankincense obtained from *B. sacra* is used for dental infections, as a tonic for the digestive system, to treat stomach aches, to relief joint pain from arthritis, muscle pain, and for the treatment of colds, cough, fever and asthma [8]. However, a very few reports are available on the phytochemical and pharmacological investigations of the title plant itself and also the frankincense from this plant [9].

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3. Present Study

In continuation of our phytochemical investigations on *B. sacra,* we isolated the minor constituents in the HR grade resin by using repeated column chromatography, followed by recycling preparative HPLC separations.

The methanol extract (50 g) was subjected to column chromatography over a silica gel column (1000 g, 70-230 mesh ASTM, Merck 1.07734.9025) using *n*-hexane/CH_2Cl_2 up to 100% CH_2Cl_2, and then by the gradient of MeOH (1%, to 100%) which yielded various sub-fractions. The sub-fraction obtained at 2% methanol/dichloromethane was further subjected to repeated injections on recycling HPLC by using 1:1 solvent system of ethyl acetate/*n*-hexane with a 4 ml/min flow rate in a silica column. After repeated injections and recycles, 3*α*-acetoxyurs-5:12-dien-24-oic acid (**1**, 5.6 mg) and 3*α*-acetoxylup-12:20(29)-dien-24-oic acids (**2**, 6.3 mg) were purified as UV inactive compounds at a retention time of 42 min. and 59 min., respectively. The remaining sub-fractions were loaded on silica gel column to purify the known compounds **3**-**6** (Figure 1). The sub-fraction (F_3) obtained at 1% MeOH/CH_2Cl_2 was loaded on silica gel column (flash silica 230-400 mesh) using 40% dichloromethane/*n*-hexane to afford commic acid-D (**3**, 4.6 mg). The sub-fraction (F_6) obtained at 2% methanol/dichloromethane was also loaded on column chromatography and 9,11-dehydro-*β*-boswellic acid (**4**, 7.5 mg) was eluted at 100% dichloromethane. Similarly 3*β*-Hydroxy-11-oxours-12-ene (**5**, 4.2 mg) and 11*β*-Hydroxy-3-oxours-12-ene (**6**, 6.7 mg) were obtained from fraction F_4 after column chromatography using dichloromethane/*n*-hexane (1:1) and (3:7), respectively. The known compounds were identified by comparison of their physical and spectroscopic data (^{1}H and ^{13}C NMR) with those reported in the literature [10–14].

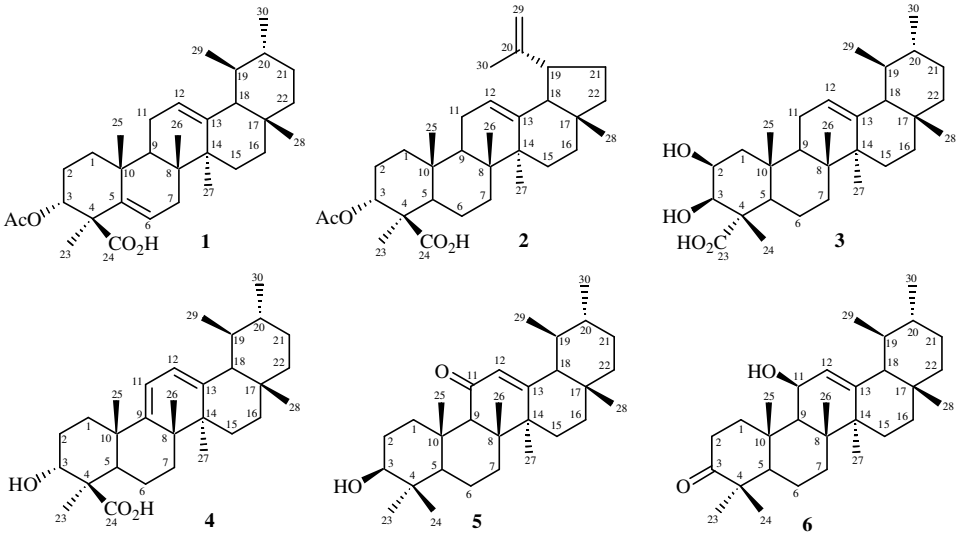

Figure 1. Structures of compounds **1**-**6**

2.4. 3α-acetoxyurs-5:12-dien-24-oic acid (**1**): Colorless amorphous powder (5.6 mg): $[\alpha]_D^{30}$ -17.4 (*c* 0.07, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3435, 3025, 1725, 1695, 1635, and 819 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz): see Table 1; HR-ESI-QTOF-MS: *m/z* 497.3634 $[M + H]^+$, calcd. 497.3632

2.5. 3α-acetoxylup-12:20(29)-dien-24-oic acids (**2**): Colorless amorphous powder (6.3 mg): $[\alpha]_D^{30}$ -37.5 (*c* 0.8, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3415, 3055, 1735, 1695, 1630, and 820 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz): see Table 1; HR-ESI-QTOF-MS: *m/z* 497.3636 $[M + H]^+$, calcd. 497.3632

Compound **1** was isolated as a colorless amorphous powder from the repeated recycles of HPLC separations carried out at 2 % MeOH/dichloromethane fraction. The IR spectrum indicated the presence of hydroxyl (3435 cm^{-1}), carboxyl (1695 cm^{-1}) and ester (1725 cm^{-1}) groups, and double bond (1635 cm^{-1}) functionalities. The molecular mass was obtained through EI-MS, showing molecular ion peak at *m/z* 496. The molecular formula was thus assigned as $C_{32}H_{48}O_4$ on the basis of HR-ESI-QTOF-MS which displayed a protonated molecular ion peak $[M + H]^+$ at *m/z* 497.3634 (calculated for

$C_{32}H_{49}O_4$, 497.3632). The other fragment ions in the EI-MS of **1** at *m/z* 436.3 (18 %), 392.3 (58.5 %), 255.2 (100 %), 237 (12 %), 218.2 (17 %), and 203.2 (17 %) were indicative of the pentacyclic triterpenoid skeleton in the molecule. The fragment ions at *m/z* 238 and 218 could be assigned to the retro-Diels-Alder (RDA) cleavages of ring-B and ring-C respectively [15].

The ^{1}H NMR spectrum of **1** showed two trisubstituted double bonds at δ 5.15 (1H, br. *s*, H-6) and 5.24 (1H, br. *s*, H-12), and one oxymethine proton at δ 4.21 (1H, m, H-3). Five tertiary methyls were observed as singlets at δ 1.22 (3H, s, H-25), 1.19 (3H, s, H-23), 1.13 (3H, s, H-27), 1.09 (3H, s, H-28), and 0.82 (3H, s, H-26), while two additional secondary methyls were observed at δ 0.93 (6H, d, *J* = 6.4 Hz, H-29 and H-30). A sharp signal at δ 2.07 (3H, s, H-32) was assigned to the methyl group of acetyl moiety. The above data was consistent with an ursane-type triterpene carrying one double bond at Δ^5 and the other at Δ^{12} [16–17]. In addition, the ^{13}C NMR spectrum (BB and DEPT) showed signals for eight methyls, eight methylenes, seven methines (including one oxymethine at δ 72.9 for C-3), and nine quaternary carbons, together with one carboxyl group (δ 181.0), one acetyl group (δ 170.3 and 21.3), and four olefinic carbons of two double bonds at Δ^5 and Δ^{12} [δ 141.7 (C-13), 123.0 (C-12), 116.6 (C-6), and 152.5 (C-5)]. The aforementioned data when compared with those of the known structurally related compounds suggested that the ^{1}H and ^{13}C NMR spectra of **1** showed close resemblance with those of boswellic acid [18], except for the signal due to the acetyl substituted group at C-3 and an extra olefinic double bond at C-5 position.

Unambiguous assignments of the olefinic double bonds at Δ^5 and Δ^{12}, the carboxylic group at C-24 and the acetyl group as substituted at OH-3 position was established on the basis of connectivities observed from the HMBC experiments. Thus the structure of **1** was finally confirmed on the basis of combined COSY, HMQC, and HMBC correlations which are mentioned in Figure 2. The relative stereochemistry of the asymmetric centers was determined by the NOESY interactions and also on the basis of biogenetic considerations.

Thus on the basis of the above spectral discussions, the structure of compound **1** was established as 3*α*-acetoxyurs-5:12-dien-24-oic acid as a new secondary metabolite of the boswellic acid series from the resin of *Boswellia sacra*.

The molecular formula of compound **2** was established as $C_{32}H_{48}O_4$ (calculated for $C_{32}H_{49}O_4$, 497.3632) on the basis of HR-ESI-QTOF-MS, which showed the quasi-molecular ion $[M + H]^+$ at *m/z* 497.3636, as well as from its NMR spectroscopic data. In EIMS, the RDA fragmentation of ring C was confirmed by the presence of fragment ions at *m/z* 216 and 220, which were assigned to the fragments of the D/E rings and the deacetylated A/B rings [15]. The other major fragment ions in the EI-MS were observed at *m/z* 482.3 (8.4 %), 421.3 (7 %), 283 (7 %), 255 (23 %), 238 (21 %), 237 (25 %), 220 (25 %), 218.2 (100 %), 216 (5 %), and 203.2 (53 %). These observations indicated the presence of pentacyclic triterpenoid skeleton in the molecule [19]. Unambiguous assignments of the various groups in the skeleton were established on the basis of correlations observed in the HMBC experiments (Figure 4). Accordingly, cross-peak correlations of H-3 to C-4, C-24, C-23, C-31 and H-12 to C-11, C-13, and C-14 confirmed the position of carboxylic group, acetyl group and double bond.

The IR spectrum showed absorption bands for hydroxyl group at 3405 cm^{-1}, an ester group at 1735 cm^{-1}, a carboxylic acid at 1695 cm^{-1} and a double bond at 1640 cm^{-1}. The ^{1}H NMR spectrum of compound **2** showed seven tertiary methyl signals at δ 2.05, 1.69, 1.33, 1.07, 1.02, 0.89 and 0.78, also supported by ^{13}C NMR spectrum. ^{13}C NMR and DEPT experiments revealed the presence of 32 carbons including seven singlet methyls, ten methylenes, six methines, and nine quaternary carbons.

The NMR spectra also revealed the presence of an isopropenyl group [δ_H 1.69 (3H, s), 5.12 (1H, d, *J* = 1.6 Hz), and 5.07 (1H, d, *J* = 1.6 Hz); δ_C 19.7, 110.7, and 150.5], an acetyl group [δ_H 2.05 (3H, s); δ_C 21.4 and 171.7], one trisubstituted double bond [δ_H 5.14 (1H, t, *J* = 3.7 Hz); δ_C 139.5 and 124.4], and one carboxylic group [δ_C 182.3]. The remaining five degrees of unsaturation in **2** suggested it to be a pentacyclic triterpene of the lupane family [20]. The ^{1}H and ^{13}C NMR spectra for compound **2** showed similar pattern to those for the known compound, lupeol [21] with additional trisubstituted double bond at C-12, a carboxylic group at C-24 and OH-3 is substituted as acetyl group.

Finally the structure of **2** was confirmed from COSY, HMQC, and especially important HMBC correlations which are mentioned in Figure 2. The relative stereochemistry of the asymmetric centers was determined by the NOESY interactions and also on the basis of biogenetic considerations, and thus the structure of **2** was elucidated as 3*α*-acetoxylup-12:20(29)-dien-24-oic acid.

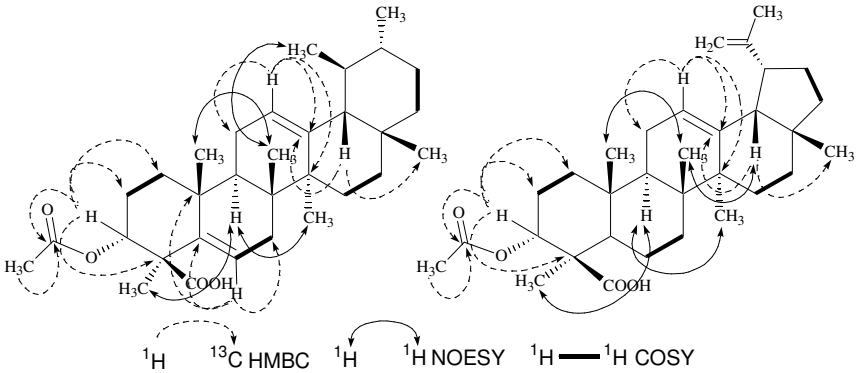

Figure 2. Key COSY, NOESY, and HMBC interactions in compound **1** and **2**

Table 1. NMR data of compound **1** and **2** ($CDCl_3$)

C. No.	**1**			**2**		
	^{13}C (δ)	**^{1}H (δ)**	**Multiplicity**	**^{13}C (δ)**	**^{1}H (δ)**	**Multiplicity**
1	41.3	0.92, m	CH_2	37.5	0.84, m	CH_2
2	19.5	1.89, m	CH_2	23.4	1.92, m	CH_2
3	72.9	4.21, m	CH	70.8	4.04, m	CH
4	46.8		C	47.2		C
5	152.5		C	56.8	0.76, br s	CH
6	116.6	5.15, br s	CH	19.6	1.45, 1.59, each m	CH_2
7	31.2	1.23, m	CH_2	31.2	1.25, m	CH_2
8	43.4		C	40.0		C
9	47.4	1.57, m	CH	48.8	1.57, m	CH
10	39.0		C	37.5		C
11	24.3	1.27, m	CH_2	23.2	1.23, m	CH_2
12	123.0	5.24, br s	CH	124.4	5.14, t, *J* = 3.7	CH
13	141.7		C	139.5		C
14	40.6		C	41.5		C
15	26.2	1.52, m	CH_2	26.4	1.58, m	CH_2
16	28.3	1.69, m	CH_2	28.8	1.98, m	CH_2
17	33.7		C	39.7		C
18	57.3	2.18, d, *J* = 8.2	CH	59.1	2.04, d, *J* = 7.8	CH
19	39.4	1.52, m	CH	48.9	1.59, m	CH
20	39.1	1.67, m	CH	150.5		C
21	31.8	1.40, m	CH_2	26.2	1.47, m	CH_2
22	33.2	1.55, m	CH_2	39.6	1.59, m	CH_2
23	21.8	1.19, s	CH_3	23.4	1.02, s	CH_3
24	181.0		C	182.3		C
25	23.7	1.22, s	CH_3	16.9	0.89, s	CH_3
26	17.4	0.82, s	CH_3	17.5	0.78, s	CH_3
27	23.3	1.13, s	CH_3	23.2	1.33, s	CH_3
28	28.7	1.09, s	CH_3	24.1	1.07, s	CH_3
29	17.4	0.93, d, *J* = 6.4	CH_3	110.7	5.07, 5.12, d, *J* = 1.6	CH_2
30	21.5	0.93, d, *J* = 6.4	CH_3	19.7	1.69, s	CH_3
31	170.3		C	171.7		C
32	21.3	2.07, s	CH_3	21.4	2.05, s	CH_3

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References

[1] A. Coppi, L. Cecch, F. Selvi and M. Raffaell (2010). The frankincense tree (*Boswellia sacra*, Burseraceae) from Oman: ITS and ISSR analyses of genetic diversity and implications for conservation, *Gen. Res. Crop Evol.* **57**, 1041-1052.

[2] S. Singh, A. Khajuria, S. C. Taneja, R. K. Khajuria, J. Singh and G. N. Qazi (2007). Boswellic acids and glucosamine show synergistic effect in preclinical anti-inflammatory study in rats, *Bioorg. Med. Chem. Lett*. **17,** 3706-3711.

[3] D. Khanna, G. Sethi, K. S. Ahn, M. K. Pandey, A. B. Kunnumakkara, B. Sung, A. Aggarwal and B. B. Aggarwal (2007). Natural products as a gold mine for arthritis treatment, *Curr. Opin. Pharmacol*. **7**, 344-351.

[4] I. Gupta, A. Parihar, P. Malhotra, G. B. Singh, R. Ludtke, H. Safayhi and H. P. Ammon (1997). Effects of *Boswellia serrata* gum resin in patients with ulcerative colitis, *Eur. J. Med. Res*. **2**, 37-43.

[5] I. Gupta, A. Parihar, P. Malhotra, S. Gupta, R. Ludtke, H. Safayhi and H. P. Ammon (2001). Effects of gum resin of *Boswellia serrata* in patients with chronic colitis, *Planta Med*. **67**, 391-395.

[6] A. Gupta, V. Gupta, A. Parihar, S. Gupta, R. Ludtke, H. Safayhi and H. P. Ammon (1998). Effects of *Boswellia serrata* gum resin in patients with bronchial asthma: results of a double-blind, placebo-controlled, 6-week clinical study, *Eur. J. Med. Res*. **3**, 511-514.

[7] H. Safayhi, T. Mack and H. P. Ammon (1991). Protection by boswellic acid against galactosamine/endotoxin-induced hepatitis in mice, *Biochem. Pharmacol*. **41**, 1536-1537.

[8] Al-Ghassany (2008). *Dhofar, the Land of Frankincense,* 2nd ed. Al-Maktaba Al-Alamya, Ruwi, Sultanate of Oman.

[9] A. Al-Harrasi and S. Al-Saidi (2008). Phytochemical analysis of the essential oil from botanically certified oleogum resin of *Boswellia sacra* (Omani luban), *Molecules* **13**, 2181-2189.

[10] A. F. Thomas and J. M. Mueller (1960). Triterpene acids from *Commiphora glandulosa* Schinz, *Experientia* **16**, 62-64.

[11] A. F. Thomas (1961). The triterpenes of *Commiphora*-II: The structures of commic acid C and commic acid D, *Tetrahedron* **15**, 212-216.

[12] A. F. Thomas and B. Willhalm (1964). The triterpenes of Commiphora IV mass spectra and organic analysis V mass spectroscopic studies and the structure of commic acids A and B. *Tet. Lett.* **5**, 3177-3183.

[13] S. Matsunaga, R. Tanaka and M. Akagi (1988). Triterpenoids from *Euphorbia maculata* L., *Phytochemistry* **27**, 535-537.

[14] J. G. Luis and L. S. Andres (1999). New ursane type triterpenes from *Salvia mellifera* Greene, *Nat. Prod. Lett.* **13**, 187-194.

[15] A. M. Ayatollahi, M. Ghanadian, S. Afsharypour, O. M. Abdella, M. Mirzai and G. Askari (2011). Pentacyclic triterpenes in *Euphorbia microsciadia* with their T-cell Proliferation activity, *Iranian J. Pharm. Res.* **10**, 287-294

[16] Z. Ahmad, S. Mehmod, R. Ifazal, A. Malik, N. Afza, M. Ashraf and E. Jahan (2007). A new ursane-type triterpenoid from *Salvia santolinifolia, Turk. J. Chem.* **31**, 495-501.

[17] R. W. Kirwacki and T. P. Pitner (1989). Current aspects of two dimentional Nucelar Magnetic Reonance Spectroscopy: Application to structure elucidation, *Pharm. Res.* **6**, 531-554.

[18] E. Fattorusso, C. Santacroce and C. F. Xaasan (1983). 4(23)-Dihydroroburic acid from the resin (incense) of *Boswellia carterii*, *Phytochemistry* **22**, 2868-2869.

[19] L. Ali and F. Shaheen (2013). Isolation and structure elucidation of a new triterpenoid from *Prunus cerasoides* D. Don. *Rec. Nat. Prod.* **7**, 80-85.

[20] I. H. Chen, Y. C. Du, M. C. Lu, A. S. Lin, P. W. Hsieh, C. C. Wu, S. L. Chen, H. F. Yen, F. R. Chang and Y. C. Wu (2008). Lupane-type triterpenoids from *Microtropis fokienensis* and *Perrottetia arisanensis* and the apoptotic effect of 28-hydroxy-3-oxo-lup-20(29)-en-30-al. *J. Nat. Prod.* **71**, 1352-1357.

[21] M. Saleem (2009). Lupeol, a novel anti-inflammatory and anticancer dietary triterpene, *Cancer Lett.* **285**, 109-115.