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SHORT COMMUNICATION

TERPENOIDS OF *BOSWELLIA NEGLECTA* OLEO-GUM RESIN

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ABSTRACT. Oleo-gum resin exudate from *Boswellia neglecta* afforded a new ursane-type triterpene characterized as 3α-acetoxy-28-hydroxy-11-oxours-12-en-24-ioc acid (**1**) together with twelve known compounds. Their structural elucidation was accomplished using physical, chemical and spectroscopic methods.

KEY WORDS: *Boswellia neglecta*, Burseraceae, Oleo-gum rein, Triterpenes

INTRODUCTION

Boswellia species (Burseraceae) are found in areas from the sea level up to 1000 m, usually in rocky slopes and gullies with growth heights of 3-12 m [1]. In Kenya, the genus *Boswellia* is represented by three species namely; *Boswellia neglecta* S. Moore (syn: *B. hildebrandtii* Engl.), *B. carterii* and *B. rivae* Engl. (syn: *B. boranensis*) and are acclimatized in the arid and semi-arid regions of the country [2]. They are known for frankincense which is an oleo-gum resin exudate obtained from incision or damage to the plants [3]. The resin of *B. neglecta* is burnt to repel insects, used in perfumery, food and beverage flavoring and as antiinflammatory [4, 5]. The resin also finds usage as incense during ceremonies [6]. Previous phytochemical studies on the plant resin resulted in the identification of monoterpenes with α-thujene, α-pinene and terpin-4-ol as major constituents [7], diterpenes [8], triterpenes [9-11]. In this communication, phytochemical analysis of the steam distilled residue of the plant oleo-gum resin resulted in the isolation of one new ursane-type triterpenes characterized as 3α-acetoxy-28-hydroxy-11-oxours-12-en-24-oic acid (**1**) together with known compounds 3α,11α-dihydroxyurs-12-en-24-oic acid (**2**), 11-oxo-β-boswellic acid (**3**), α-Boswellic acid (**4**), β-boswellic (**5**), ursolic acid (**6**), lupeol (**7**) [8], β-amyrin (**8**) and α-amyrin (**9**), α-amyrone (**10**) [9], quercetin (**11**) [12], olean-12-en-3-*O*-β-glucoside (**12**) [13] and catechin-3-*O*-glucoside (**13**) [14]. Compounds **1**, **2**, **11**, **12** and **13** are reported from *B. neglecta* gum resin for the first time.

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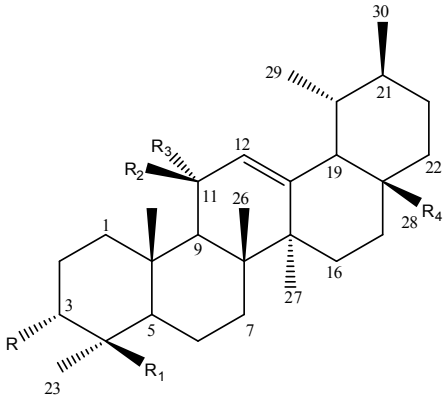

1 R = OAc, R_1 = COOH, R_2 = R_3 = O, R_4 = CH_2OH

EXPERIMENTAL

Instrumentation, solvents and chemicals. Melting points were determined using Gallenkamp melting point apparatus and are uncorrected. The IR spectra were run on Pye-Unicam SP8 spectrophotometer using KBR pellet. The NMR data were measured in $CDCl_3$ and $CDCl_3$-DMSO-d_6 on a Brucker NMR Ultrashield TM operating at 500 and 125 MHz, respectively, with TMS used as internal standard. The mass spectral data were obtained using a Varian MAT 8200 A instrument. Silica gel 60G (0.02-0.7 mm Mesh) Merck was used for medium pressure chromatography. All solvents used were of analytical grade. Glucose, galactose and rhamnose used as reference standards were bought from Kobian Kenya Ltd. On the other hand, authentic standards of quercetin and catechin were stocks at the Natural Products section, Department of Environmental Science, Policy and Management, University of California at Berkeley, California, USA.

Oleo-gum resin source. The resin samples were collected in Wajir County, Kenya and supplied by Ms. Rose Chiteva of Non-Wood Forest products, Kenya Forestry Research Institute (KEFRI). Authentification of the plant was done by Mr. Norman Gachathi (Taxonomist) of the same institution and voucher specimen (No: KEFRI/BN-03/2010) was deposited in the herbarium of Non-Wood Forest Products Programme.

Extraction and isolation of compounds. The residue from hydrodistillated resin (1 kg) was cold extracted with CH_2Cl_2 (3 x 4 L) and MeOH (3 x 4 L), each lasting four days, separately evaporated under reduced pressure yielding yellow-brown (35 g) and dark brown (70 g) materials, respectively.

Fractionation of CH_2Cl_2 extract. A portion of the extract (30 g) was adsorbed onto silica gel and then subjected to column chromatography (3.0 x 60 cm, SiO_2 500 g, pressure ≈ 1 bar) using n-hexane-CH_2Cl_2 gradient (increment 10%) up to 100% CH_2Cl_2 and elution concluded with ethyl acetate, collecting 20 mL each. The process afforded various sub-fractions (I-VI) as determined by TLC profiles [solvent systems: n-hexane-CH_2Cl_2 (1:4, 1:2, 1:1) and n-hexane-EtOAc, 3:2)]. The sub-fraction I (fractions 1-20) showed no spot and solvent recovered. Sub-fraction II (fractions 30-60) produced colorless oil and was kept under freezing conditions a waiting GC-MS analysis.

The sub-fraction III (fractions 65-90, 7 g) was further subjected to medium pressure chromatography using n-hexane-EtOAc (4:1), collecting 10 ml each to afford α-amyrone (**10**), a mixture of α-and β-amyrins (**9** and **8**, 200 mg) and lupeol (**7**, 34 mg). Sub-fraction IV (fraction 93-130, 4 g) showed two spots R_f 0.46 and 0.42 (solvent system: n-hexane-EtOAc, 3:2) and was further purified by medium pressure chromatography to give ursolic acid (**6**, 44 mg) and a mixture of α- and β-boswellic acids (**5** and **4**, 50 mg). Sub-fraction V (fractions 140-170, 6.0 g) afforded three spots on TLC analysis (solvent system: n-haxane-EtOAc, 1:1) with R_f values 0.31, 0.24 and 0.19, respectively. Further purification using 0.2% MeOH in CH_2Cl_2 afforded 11-oxo-β-boswellic acid **3** (R_f 0.31, 25 mg), **2** (R_f 0.24, 30 mg) and **1** (R_f 0.19, 40 mg). Subfraction VI (fractions 174-195, 5 g) was loaded onto silica gel column and further purified by medium pressure chromatography (2.5 x 50 cm, SiO_2 150 g, pressure ≈ 1 bar) to give further compounds **1** and **2** in 70 mg and 40 mg, respectively.

Fractionation of MeOH extract. Medium pressure chromatographic separation of MeOH extract constituents (approx. 65 g, 3.5 x 90 cm, SiO_2 500 g, pressure ≈ 1 bar) using a stepwise gradient mixture of CH_2Cl_2-MeOH (97:3; 95:5; 9:1; 4:1; 2:1) and finally with MeOH afforded 250 fractions each 20 mL. The fractions were grouped into three pools (I-III) depending on TLC profiles. Pool I (fractions 20-40, 4 g) was repeatedly fractionated over silica gel column using CH_2Cl_2-MeOH (97:3) to give quercetin (**11**, 47 mg). Pool II (fractions 64-135, 3.5 g) showed one major spot and was further purified by crystallization (CH_2Cl_2-MeOH, 98:2) to give olean-12-en-3-*O*-β-glucoside (**12**, 76 mg). Fractions 142-242 [constituted pool III (4.5 g)] and on further purification using CH_2Cl_2-MeOH (95:5) followed by same solvent system in the ratio 9:1 afforded further **12** (35 mg) and catechin 3-*O*-β-glucoside (**13**, 45 mg).

Acid hydrolysis of compounds **12** *and* **13**. A solution of **12** and **13** (each 10 mg) in a mixture of 8% HCl (1 mL) and MeOH (20 mL) were separately refluxed for 2 h. The reaction mixtures were reduced *in vacuo* to dryness, dissolved in H_2O (2 mL) and neutralized with NaOH. The neutralized products were then subjected to TLC analysis (eluent: EtOAc-MeOH-H_2O-HOAc, 6:2:1:1). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 100 ^{0}C for 2 min. The presence of glucose was confirmed after comparison with reference samples (glucose, galactose and rhamnose). Similarly, the presence of aglycones quercetin and catechin were confirmed by TLC co-chromatography with authentic samples.

*Compound **1***. An amorphous white powder with m.p. 204-206 ^{0}C; $[\alpha]^{25}_D$ -20.6^0 (MeOH, c 0.6). IR ν_{max} (KBr) cm^{-1}: 3415, 3000-2500, 1740, 1710, 1680, 1640, 1580, 1450, 1340, 1250, 1210, 1110, 1028, 960; ^{1}H NMR ($CDCl_3$+ drop DMSO-d_6) ppm: 5.32 (1H, brs, H-12), 4.75 (1H, dd, *J* = 8.4, 3.6 Hz, H-3), 3.32 (1H, d, *J* = 11.0 Hz, H-28), 3.0 (1H, d, *J* = 11.0 Hz, H-28), 2.25 (1H, s, H-9), 2.10 (3H, s, OAc), 1.98 (1H, m, H-2), 1.75 (1H, m, H-19), 1.65 (1H, m, H-1), 1.45 (2H, m, H-2 and H-16), 1.43 (d, *J* = 12.0 Hz, H-18), 1.42 (1H, dd, *J* = 12.0, 3.1 Hz, H-5), 1.33 (3H, s, CH_3-27), 1.20 (2H, m, H-21), 1.15 (1H, m, H-16), 1.14 (3H, s, CH_3-26), 1.10 (1H, m, H-1), 1.06 (3H, s, CH_3-23), 0.98 (3H, s, CH_3-25), 0.88 (3H, d, *J* = 6.6 Hz, CH_3-30), 0.80 (3H, d, *J* = 6.5 Hz, CH_3-29); ^{13}C NMR ($CDCl_3$+ drop DMSO-d_6) ppm: see Table 1; ESIMS *m/z* (rel. int.) 528 $[M]^+$(4), 487 (5), 368 (1), 448 (3), 289 (7), 280 (21), 257 (14), 248 (21), 221 (8), 220 (6), 218 (100), 217 (12), 208 (2), 203 (50), 135 (60). HRMSHRSMS *m/z:* 528.7357 (calculated for $C_{32}H_{48}O_6$, 528.7366).

*Compound **2***. White crystals from CH_2Cl_2-MeOH (99:1), m.p. 189-193 ^{0}C; $[\alpha]^{25}_D$ -36^0 (MeOH, c 0.5). IR ν_{max} (KBr) cm^{-1}: 3425, 3100-2500, 1705, 1642, 1570, 1454, 1380, 1257, 1208, 1100, 1028, 898; ^{1}H NMR ($CDCl_3$+ drop DMSO-d_6) ppm: 5.45 (1H, d, *J* = 3.6 Hz, H-12), 3.70 (1H, dd, *J* = 8.3. 3.4 Hz, H-11), 3.40 (1H, dd, *J* = 8.3, 2.4 Hz, H-3), 2.18 ((1H, d, *J* = 9.0 Hz, H-9), 1.95 (1H, m, H-2), 1.70 (2H, m, H-1and H-6), 1.55 (1H, dd, *J* = 11.6, 3.4 Hz, H-5), 1.47 (1H,

d, *J* = 12.3 Hz, H-18), 1.40 (2H, m, H-21), 1.30 (3H, s, CH_3-27), 1.25 (1H, m, H-16), 1.20 (3H, s, CH_3-23), 1.18 (1H, m, H-1), 1.10 (3H, s, CH_3-26), 1.02 (3H, s, CH_3-25), 0.95 (3H, s, CH_3-28), 0.93 (3H, d, *J* = 6.5 Hz, CH_3-30), 0.86 (3H, d, *J* = 6.7 Hz, H-29); ^{13}C NMR ($CDCl_3$+ drop DMSO-d_6) ppm: see Tables 1 ESIMS *m/z ;* (rel. int.) 472 $[M]^+$ (2), 456 (4), 454 (8), 436 (2), 238 (15), 234 (22), 220 (15), 218 (100), 216 (10), 203 (50), 189 (30), 160 (13), 55 (70). HRSMS *m/z:* 472.7146 (calcd. for $C_{32}H_{48}O_4$, 472.71457).

Compound **12**. White amorphous powder with m.p. > 250 ^{0}C; IR ν_{max} (KBr) cm^{-1}: 3350, 2980, 2850, 1660, 1380, 1190, 1038, 980; ^{1}H NMR ($CDCl_3$ + drop DMSO-d_6) ppm: 5.31 (1H, t, *J* = 3.5 Hz, H-12), 3.40 (1H, dd, *J* = 12.2, 4.5 Hz, H-3), 1.95 (1H, m, H-15), 1.84 (1H, m, H-18), 1.72 (1H, s, H-1), 1.65 (1H, m, H-19), 1.55 (1H, m, H-2), 1.75 (1H, m, H-9), 1.46 (1H, m, H-5), 1.20 (H, m, H-1), 1.43 (d, *J* = 12.0 Hz, H-18), 1.42 (1H, dd, *J* = 12.0, 3.1 Hz, H-5), 1.33 (3H, s, CH_3-27), 1.32 (1H, m, H-2), 1.24 (3H, s, CH_3-27), 1.10 (3H, s, CH_3-25), 0.99 (3H, s, CH_3-23), 0.96 (3H, s, CH_3-26), 0.90 (3H, s, CH_3-30), 0.86 (3H, s, CH_3-29), 0.82 (3H, s, CH_3-28), 0.78 (3H, s, CH_3-24); glc: 5.01 (1H. d, *J* = 7.2 Hz, H-1'), 3.77 (1H, m, 6'a), 3.64 (1H, m, 6'b), 3.56 (1H, m, H-5'), 3.47 (1H, m, H-2'), 3.36 (1H, m, H-4'), 3.28 (1H, m, H-3'); ^{13}C NMR ($CDCl_3$ + drop DMSO-d_6) ppm: see Table 1; ESIMS *m/z* (rel. int.) 426 $[M]^+$ (10), 208 (15), 218 (100), 203 (56), 189 (45), 135 (32), 55 (86), 41 (75).

Compound **13**. An amorphous white powder with m.p. > 250 ^{0}C; IR ν_{max} (KBr) cm^{-1}: 3450, 2950, 1870, 1660, 1018, 860; ^{1}H NMR ($CDCl_3$ + drop DMSO-d_6) ppm: 7.03 (1H, d, *J* = 1.2 Hz, H-2'), 6.80 (1H, dd, *J* = 8.5, 1.2 Hz, H-6'), 6.67 (1H, d, *J* = 8.5Hz, H-5'), 5.95 (1H, d, *J* = 2.1Hz, H-6), 5.89 (1H, d, *J* = 2.1 Hz, H-8), 5.10 (1H, d, *J* = 7.4 Hz, H-1''), 4.85 (1H, br s, H-2), 4.1 (1H, m, H-3), 3.98 (1H, m, H-6''$_a$), 3.62 (1H, m, H-6''$_b$), 3.46 (1H, m, H-4''), 3.41 (1H, m, H-3''), 3.37 (1H, m, H-2''), 3.20 (1H, m, H-5''); ^{13}C NMR ($CDCl_3$+drop DMSO-d_6) ppm: 79.4 (C-2), 66.7 (C-3), 30.0 (C-4), 157.8 (C-5), 95.8 (C-6), 156.3 (C-7), 96.0 (C-8), 156. 0 (C-8a), 132.6 (C-1'), 116.1 (C-2'), 144.9 (C-3'), 145.0 (C-4'), 116.4 (C-5'), 120.2 (C-6''); Glc: 102.3 (C-1''), 74.0 (C-2''), 76.7 (C-3''), 70. 8 (C-4''), 76.4 (C-5''), 61.6 (C-6''); ESIMS *m/z:* (rel. int.) 290 (57), 373 (24).

RESULTS AND DISCUSSION

Compound **1** was isolated as white amorphous powder with a molecular formula $C_{32}H_{48}O_6$ as evidenced by EI-MS which exhibited a $[M]^+$ ion peak at *m/z* 528 (9 unsaturation equivalents). It showed a positive Liebermann-Burchard test and Molish reaction suggesting a triterpene skeleton [12]. The IR spectrum showed characteristic absorptions attributable to hydroxyl (3415 cm^{-1}), ester carbonyl (1740 cm^{-1}), carboxylic cid (1710 cm^{-1}), conjugated keto (1680 cm^{-1}) and a double bond (1640 cm^{-1}) functional groups. The ^{1}H NMR spectrum of **1** displayed five tertiary methyls (δ 2.10, 1.33, 1.14, 1.06 and 0.98, each singlet including methyl from acetoxy group), two secondary methyls (δ 0.88, d, *J* = 6.6 Hz and 0.80, d, *J* = 6.5 Hz) and a trisubstituted olefinic proton (δ 5.32, s), which are characteristic of acetylated ursane-type triterpenes related to boswellic acids [15, 16]. The ^{13}C NMR spectrum (Table 1) of compound **1** displayed 32 distinct peaks accounted for by 7 methyls, 7 methines, 9 methylenes and 9 quaternary carbons in the 135 DEPT spectrum. Careful analysis of both ^{1}H and ^{13}C NMR data of the compound taking into consideration the fragmentation pattern in the EI-MS (Figure 1) suggested that compound **1** is a 3α-acetoxy-11-keto-β-boswellic acid derivative possibly with acetoxy and carboxylic acid groups in rings A/B [*m/z* 280 ($C_{16}H_{24}O_4$)], while an oxo moiety together with terminal hydroxymethylene are in rings D/E [*m/z* 248 ($C_{16}H_{24}O_2$) [8, 17].

The ^{1}H NMR spectrum confirmed the presence of acetoxy group at C-3 and was in axial orientation as evidenced by the narrow peak half-height width $w_{1/2}$ (3.6 Hz) of the equatorially-positioned geminal proton which appeared relatively downfield at δ 4.75 [18, 19), an interpretation further substantiated by HMBC correlation between H-5 (δ_H 1.42) and C-3 (δ_c

75.6). Similarly, the H-3 showed an HMBC cross-peak with a peak at δ_c182.1 which further supported the presence of carboxylic group in ring A at either C-23 or C-24 positions. The characteristic ^{1}H-^{1}H proximity (NOESY) between CH_3-23 and H-5 allowed the assignment of the functional group at C-24 as previously observed with boswellic acids [8, 16].

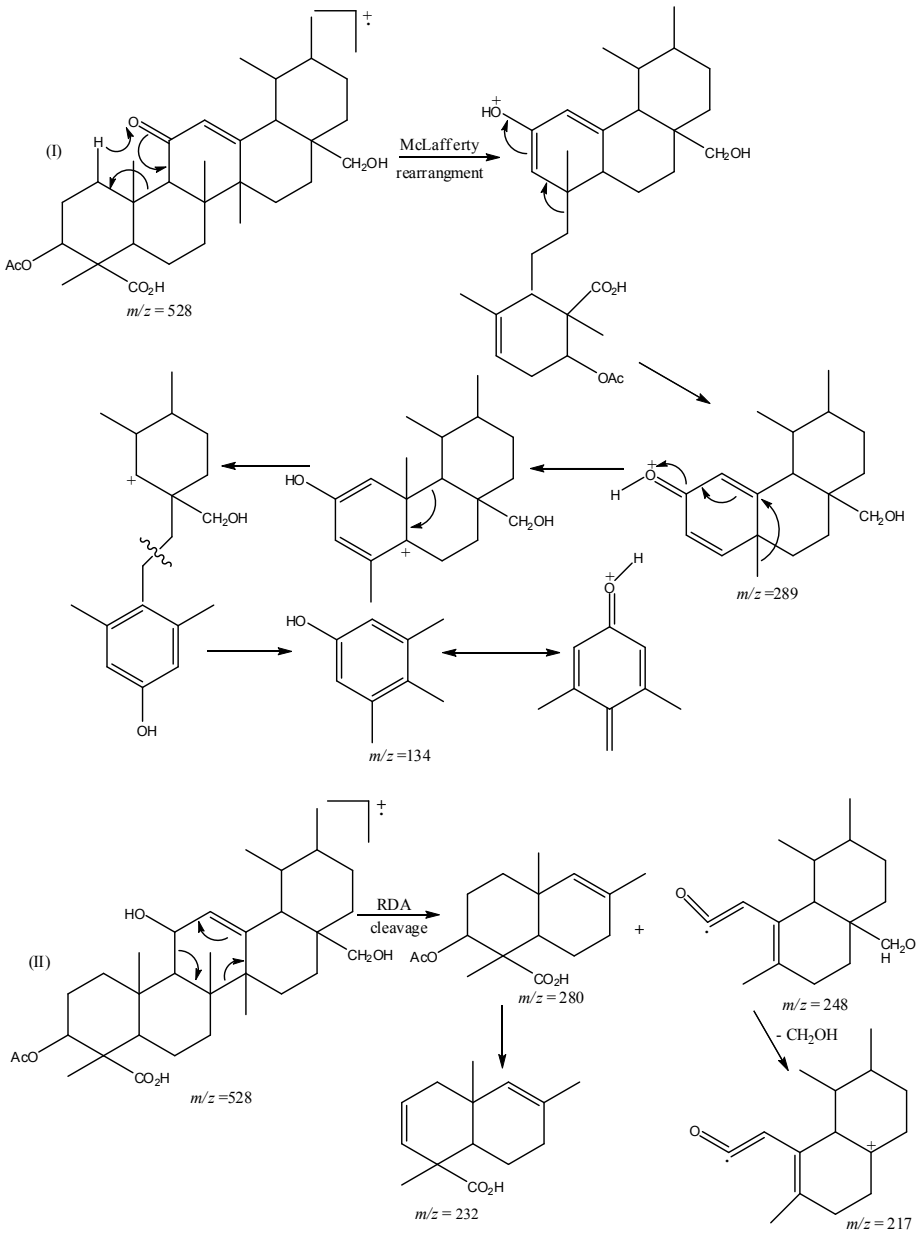

Figure 1. Possible fragmentations of compound **1**.

Table 1. ^{13}C NMR of compounds **1**, **2** and **12**.

H	**1**	**2**	**12**
1	36.2	37.1	37.5
2	24.8	27.7	26.0
3	75.6	70.1	76.2
4	44.0	43.0	41.2
5	50.4	48.0	54.6
6	18.3	20.0	20.8
7	34.0	33.5	33.9
8	41.9	39.4	40.5
9	58.0	47.9	48.0
10	35.0	36.9	36.3
11	205.3	72.1	24.4
12	132.7	122.5	123.7
13	165.5	141.4	143.2
14	44.3	42.1	43.4
15	30.7	29.5	28.5
16	26.6	25.5	26.2
17	34.4	33.8	34.0
18	56.5	55.7	58.1
19	40.0	38.0	39.2
20	43.0	41.4	40.7
21	26.9	28.1	30.8
22	36.9	37.9	41.0
23	24.1	24.2	28.7
24	182.1	179.3	22.0
25	16.0	16.5	16.1
26	17.2	16.8	16.6
27	20.3	21.7	23.6
28	68.8	23.5	27.0
29	16.9	15.9	18.0
30	28.8	25.8	21.8
Me<u>C</u>O	169.8		
CH_3CO	20.5		
Glc			
1’			104.0
2’			74.3
3’			77.6
4’			71.5
5’			75.9
6’			62.6

On the other hand, the singlet peak at δ 5.32 (H-12) correlated with C-11 (δ_c 205.3)/C-8 (δ 58.0) and in turn with C-13 (δ_c165.5)/C-14 (δ_c44.3) and on this basis, the position of the keto group was assigned to C-11; a fact further supported by EI-MS peaks at *m/z* 289 [$C_{19}H_{29}O_2$] originating from McLafferty rearrangement and 248 [$C_{16}H_{24}O_2$] due to *retro*-Diels-Alder cleavage. Similarly, in the HMBC spectrum, the terminal hydroxymethylene protons showed long range correlations with carbons at C-18 (δ_c 56.5)/C-16 (δ_c 26.6)/C-22 (δ_c 36.9) suggesting the location of the group at C-17 [20]. This was further supported by the medium intensity diagnostic EI-MS fragmentation peaks which appeared at *m/z* 289 and 248. The latter fragment lost a terminal hydroxymethylene group to form an *m/z* 218 (100) leading to stable tertiary carbenium, thus confirming the –CH_2OH to be at C-17 (8).

Thus, on the basis of spectroscopic data and also comparison with literature data, structure of compound **1** was deduced as 3α-acetoxy-28-hydroxy-11-oxours-12-en-24-oic acid (3α-acetoxy28-hydroxy-11-keto-β-boswellic acid).

CONCLUSION

Compounds **2-9** isolated from *B. neglecta* oleo-gum resin was consistent with other reports of boswellic acids of ursane-type triterpene [8].

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