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TWO TRITERPENOID LACTONES FROM THE RESIN OF *BURSERA DELPECHIANA**

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Key Word Index—*Bursera delpechiana*; Burseraceae; resin; triterpenoid lactones; ursane type.

Abstract—Two new triterpenoid lactones have been isolated from the resin of *Bursera delpechiana* and their structures assigned as 3β-acetoxy-11α,12α-epoxyurs-28,13-olide and 3β-acetoxy-12β-hydroxyurs-28,13-olide respectively on the basis of their spectroscopic properties and chemical evidence.

INTRODUCTION

We have recently reported on the isolation and characterization of 11-oxoursolic acid and its acetate together with α-amyrin, 3β-acetoxyurs-11-en-28, 13-olide, acetyl ursolic acid, ursolic acid and ursonic acid from the resin of *Bursera delpechiana* [1]. In a continuation of our studies on the minor constituents of the resin of this plant, we have now isolated two new triterpenoid lactones (**1** and **2**) together with the known compounds α-amyrin acetate and 11-dehydroursolic acid lactone. The present paper deals with the structure elucidation of these new compounds.

RESULTS AND DISCUSSION

Compound **1**, $C_{32}H_{48}O_5$ ($[M]^+$ at *m/z* 512 was characterized as a triterpenoid lactone on the basis of a positive LB reaction and its IR spectrum. The ^1H NMR spectrum showed the presence of seven C-methyl, one acetate methyl, one acetoxymethine and two epoxide protons. From the ^1H NMR, ^{13}C NMR and mass spectral data (see experimental), **1** was assigned the structure 3β-acetoxy-11α,12α-epoxyurs-28,13-olide. The structure was confirmed by direct comparison of the compound obtained by epoxidation with H_2O_2–HOAc of 3β-acetoxyurs-11-en-28,13-olide which is also a constituent of the resin. This is the first report of the occurrence of compound **1** in nature although it was reported earlier as a product of oxidation of acetyl ursolic acid and acetyl methyl ursolate [2–4]. The isolation of the deacetyl derivative of **1** from the leaves of *Thevetia nerifolia* has been reported recently [5].

Compound **2**, $C_{32}H_{50}O_5$ ($[M]^+$ at *m/z* 514) was shown to be a triterpenoid lactone by a positive LB test and its IR spectrum. It contained two secondary and five tertiary methyl groups, one acetate methyl, one acetoxymethine, one secondary hydroxyl, one hydroxymethine and a γ-lactone group (^1H NMR and IR spectra). The presence of the secondary hydroxyl group was supported by the formation of the acetate **3** on acetylation and the ketone **4** on Jones oxidation. The MS of compound **2** showed the formation of fragment ions with *m/z* 249 and 189 which were diagnostic of rings A and B with an acetoxyl group at C-3 [6]. The hydroxyl was therefore not present in rings A and B. The spectral data of **2** agreed well with those of 3β-acetoxy-12α-hydroxyurs-28,13-olide (**5**) [2, 3] except for the coupling constant for H-12 in the ^1H NMR spectrum. The 270 MHz spectrum of **2** showed the signal for H-12 as a double doublet at δ3.98 (J = 11.5, 6 Hz). The large coupling constant indicated diaxial coupling with H-11 while the small coupling constant was due to axial-equatorial coupling with the H-11 eq. H-12 in **2** was therefore axial and consequently the hydroxyl was equatorial. The structure of **2** appeared therefore to be 3β-acetoxy-12β-hydroxyurs-28,13-olide. The signal for H-18 appeared as a doublet at δ2.26 (J = 11.2 Hz) supporting its ursane skeleton [7]. The ^{13}C NMR spectrum (see experimental) is in agreement with the structure assigned. A comparison of the ^{13}C NMR spectra and that of acetyl ursolic lactone [3] showed the deshielding effects on C-11 and C-13 and shielding effects on C-18, C-9 and C-14 [8]. Further support for the β-configuration of the hydroxyl at C-12 was obtained by comparing the ^1H NMR spectra of compounds **2** and 3β-acetoxy-12α-hydroxyurs-28,13-olide **5**. Compound **5** was prepared by reduction of the ketone **4** with $NaBH_4$. The ^1H NMR spectrum of **5** showed the signal for H-12 as an unresolved triplet at δ3.71 (Wh/2 7 Hz). On acetylation, compound **5** formed the acetate **6** the IR spectrum of which showed the absence of hydroxyl absorption. The ^1H NMR spectrum of **6** showed the signal for H-12 at δ4.80 (*m*, Wh/2 7 Hz) whereas the signal for H-12 in **3** appeared at δ5.16 (*m*,

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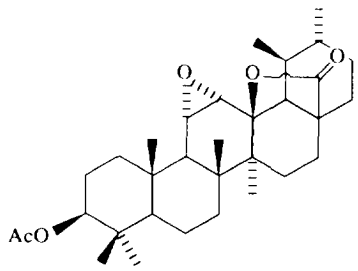

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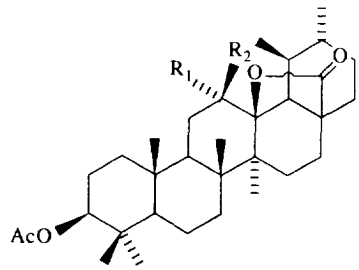

	R_1	R_2
2	H	OH
3	H	OAc
4	O	O
5	OH	H
6	OAc	H

Wh/2 18 Hz). Further the signal for H-29 in compound **5** appeared at δ1.24 (*d*, J = 6.3 Hz) whereas in the ^{1}H NMR spectrum of **2** it appeared at δ1.15 (*d*, J = 7 Hz). The downfield shift may be due to the proximity of the axial hydroxyl group in **5** to the methyl group at C-19. Thus compound **5** was identified as 3β-acetoxy-12α-hydroxyurs-28,13-olide and compound **2** as 3β-acetoxy-12β-hydroxyurs-28,13-olide. It is surprising to note that $NaBH_4$ reduction of the 12-oxo-lactone **4** yielded mostly the 12α-hydroxy-lactone **5** and a small amount of the 12β-epimer **2**. This may be due to the steric hindrance at C-12 of **4** and the attack of hydrogen during reduction from the β-side of the molecule resulting in the formation of the compound **5**. This is the first report of the occurrence of compound **2** in nature.

After the manuscript of this paper was prepared, we came across a paper on the stereochemistry of H_2O_2–HOAc oxidation of ursolic acid and related compounds [9] in which compounds **2** and **5** are described. The physical and spectral characteristics of the natural compound **2** agrees well with the product of H_2O_2–HOAc oxidation of acetyl ursolic acid reported in the paper.

EXPERIMENTAL

Details of the extraction of the plant material and the fractionation of the extract are described in the previous paper [1]. ^{1}H NMR: 90 and 270 MHz, $CDCl_3$ using TMS as int. stand.; ^{13}C NMR: 22.5 MHz, $CDCl_3$ with TMS as int. stand.; MS: 70 eV, direct inlet. The known compounds were identified by their physical and spectroscopic properties.

The nonpolar fractions from Fraction A of the previous work-up [1] were pooled and rechromatographed over silica gel using *n*-hexane-EtOAc mixtures of increasing polarity. α-Amyrin acetate (10 mg), 3β-acetoxyurs-11-en-28,13-olide (25 mg), 3β-hydroxyurs-11-en-28,13-olide (10 mg) [10], 11-oxo acetyl ursolic acid (15 mg) and **1** (25 mg) were obtained by subjecting the various fractions to repeated CC and prep. TLC on silica gel plates. Similarly Fraction B from the earlier work was subjected to repeated CC and prep. TLC to obtain **2** (32 mg) and ursolic acid (5 mg).

3β-*acetoxy*-11α,12α-*epoxyurs*-28,13-*olide* **1**. Needles from MeOH, mp 285–288° (lit. 288° [2]); $C_{32}H_{48}O_5$; MS *m/z* (rel. int.); 512 (9.4) $[M]^+$, 497 (4.8), 452 (5.2), 424 (2.8), 300 (3.0), 277 (18.9), 263 (26.5), 249 (11.9), 234 (7.5), 232 (7.1), 231 (7.1), 217 (18.9), 205 (15.4), 203 (21.9), 189 (32.6), 175 (16.2), 173 (7.2), 163 (10.8), 161 (10.9), 147 (21.7), 135 (19.3), 119 (26.9), 107 (29.8), 95 (32.2), 81 (34.0), 69 (36.7), 55 (32.8) and 43 (100); IR ν_{max}^{KBr} cm^{-1}: 1770 (γ-lactone), 1735, 1245 (acetate), 875 (epoxide); ^{1}H NMR: δ0.88, 0.96, 0.98, 1.08, 1.12, (7 × CH_3), 2.08 (*s*, 3H, OAc), 2.95 (*d*, 1H, J = 4 Hz; H-12), 3.12 (*m*, 1H, Wh/2 = 5 Hz; H-11), 4.52 (*dd*, 1H, J = 7.0, 9.5 Hz; H-3); ^{13}C NMR: δ37.5 (C-1), 23.2 (C-2), 80.4 (C-3), 37.7 (C-4), 54.5 (C-5), 17.3 (C-6), 31.3 (C-7), 41.2 (C-8), 51.2 (C-9), 36.3 (C-10), 54.5 (C-11), 56.1 (C-12), 88.8 (C-13), 41.2 (C-14), 26.8 (C-15), 22.6 (C-16), 45.0 (C-17), 60.5 (C-18), 37.2 (C-19), 40.2 (C-20), 30.4 (C-21), 31.3 (C-22), 27.7 (C-23), 16.2 (C-24), 16.2 (C-25), 17.3 (C-26), 20.2 (C-27), 179 (C-28), 17.3 (C-29), 19.5 (C-30), 170.6 ($\underline{C}OCH_3$) and 21.2 ($CO\underline{C}H_3$).

Action of H_2O_2-HOAc *on* 3β-*acetoxyurs*-11-*en*-28,13-*olide*. 3β-Acetoxyurs-11-en-28,13-olide (20 mg) was dissolved in glacial HOAc (1.5 ml) and a soln of 30% H_2O_2 (0.5 ml) in HOAc (1 ml) was added dropwise over 15 min. The mixture was stirred for about 2 hr at room temp. and then diluted with H_2O (10 ml). The solid which separated was found by TLC to contain a mixture of three compounds. It was taken up into Et_2O, washed thoroughly with H_2O and dried (Na_2SO_4). Prepn TLC of the mixture gave the unreacted starting compound (2 mg), **1** (10 mg), mp 280–282° (MeOH) (mmp, TLC, IR) and an unidentified compound (2 mg).

3β-*acetoxy*-12β-*hydroxyurs*-28,13-*olide* (**2**). Needles from MeOH, mp 283–284°; $C_{32}H_{50}O_5$ ($[M]^+$ at *m/z* 514); MS (rel. int.): *m/z* 514 (7.9), 499 (18.2), 496 (6.5), 453 (24.6), 333 (22.1), 264 (24.9), 249 (20.0), 246 (14.9), 218 (35.8), 207 (16.8), 203 (13.7), 189 (31.2), 177 (38.0), 175 (29.1), 154 (34.4), 135 (13.6), 121 (30.7), 119 (17.0), 111 (29.2), 93 (26.8), 81 (32.0), 69 (18.7), 55 (16.9) and 43 (100);

IR ν_{max}^{KBr} cm^{-1}: 3500 (hydroxyl), 1770 (γ-lactone), 1720, 1250 (acetate); ^1H NMR (270 MHz, $CDCl_3$): δ0.85, 0.86, 0.91, 1.16, 1.19 (*s*, 5 × *ter* Me), 0.93 (*d*, 3H, J = 6.5 Hz), 1.15 (*d*, 3H, J = 7.0 Hz) (2 × sec. Me), 2.05 (*s*, 3H, OAc), 2.26 (*d*, 1H, J = 11.2 Hz, H-18), 3.97 (*dd*, 1H, J = 11.2, 5.3 Hz, H-12) and 4.45 (*dd*, 1H, J = 11.5, 5.5 Hz, H-3); ^{13}C NMR: δ38.2 (C-1), 23.4 (C-2), 80.0 (C-3), 37.5 (C-4), 55.1 (C-5), 17.2 (C-6), 33.9 (C-1), 42.2 (C-8), 49.0 (C-9), 36.9 (C-10), 29.0 (C-11), 69.1 (C-12), 94.4 (C-13), 43 (C-14), 27.6 (C-15), 22.3 (C-16), 45.2 (C-17), 52.1 (C-18), 38.2 (C-19), 38.2 (C-20), 30.5 (C-21), 31.2 (C-22), 27.2 (C-23), 16.5 (C-24), 16.5 (C-25), 18.3 (C-26), 19.5 (C-27), 178.9 (C-28), 17.0 (C-29), 19.5 (C-30), 170.8 ($\underline{C}OCH_3$) and 21.4 ($CO\underline{C}H_3$).

Acetylation of **2**. A mixture of **2** (10 mg), pyridine (0.2 ml) and Ac_2O (1 ml) was heated on a water bath for about 2 hr and then left overnight. The reaction mixture was worked up in the usual manner to obtain the diacetate **3**, needles from MeOH, mp 298–300°; IR ν_{max}^{KBr} cm^{-1}: 1775, 1725, 1240; ^1H NMR: δ0.86, 0.91, 1.24, 1.26, 1.28 (*s*, 3H each, 5 × *ter*. Me), 0.93, 0.97 (*d*, 3H each, 2 × *sec*. Me), 2.06, 2.08 (*s*, 3H each, 2 × OAc), 2.20 (*d*, 1H, J = 10 Hz, H-18), 4.50 (*m*, 1H, Wh/2 = 18 Hz, H-3) and 5.15 (*m*, 1H, Wh/2 = 18 Hz, H-12).

Oxidation of **2** *to give ketone* **4**. To a soln of **2** (15 mg) in Me_2CO (2 ml) was added dropwise a soln of CrO_3 (0.5 ml) (CrO_3 (0.13 g + con. H_2SO_4 2 drops + H_2O 0.5 ml) and the mixture constantly shaken for about 30 min. at room temp. It was then diluted with water and the precipitate obtained was extracted with Et_2O. The Et_2O extract after several washings with water and drying (Na_2SO_4) was evaporated to dryness to obtain the ketone **4**, crystallized from MeOH–$CHCl_3$ as needles, mp 268–271°. IR ν_{max}^{KBr} cm^{-1}: 1760, 1730, 1700, 1245; ^1H NMR: δ0.88 (*s*, 9H), 0.94 (*s*, 6H), 0.96 (*d*, 3H, J = 4 Hz), 1.10 (*d*, 3H, J = 4 Hz), 2.05 (*s*, 3H, OAc), 2.30–2.60 (*m*, 2H, H_2-11).

Reduction of ketone **4** *with* $NaBH_4$. A soln of ketone **4** (10 mg) in EtOH (5 ml) was treated with $NaBH_4$ (10 mg) at room temp. After keeping for 3 hr, the mixture was diluted with water and the precipitated solid was taken up into Et_2O. The Et_2O extract after the usual work-up gave a solid which was shown by TLC to contain two close-moving compounds. Separation of the mixture by prepn TLC gave **2** (3 mg) and 3β-acetoxy-12α-hydroxyurs-28,13-olide (**5**), feathery needles (MeOH) (5 mg), mp 250–54°, $C_{32}H_{50}O_5$ ($[M]^+$ at m/z 514). MS (rel. int.): m/z 514 (30), 499 (32), 496 (30), 454 (15), 436 (10), 300 (35), 251 (20), 250 (22), 249 (25), 218 (42), 205 (100), 203 (22), 189 (80), 175 (15), 161 (26), 135 (32), 121 (28), 109 (25), 107 (22) and 105 (12); IR ν_{max}^{KBr} cm^{-1}: 3445, 1761, 1725, 1238; ^1H NMR: δ0.82 (*s*, 6H), 0.88 (*s*, 3H) 0.96 (*d*, 3H, J = 5.5 Hz), 1.18 (*s*, 3H), 1.22 (*s*, 3H), and 1.24 (*d*, 3H, J = 6.3 Hz). 2.02 (*s*, 3H, OAc), 3.72 (*m*, 1H, H-12), 4.48 (*dd*, J = 9.5, 5.5 Hz, H-3).

Acetylation of compound **5**. Compound **5** was treated with Ac_2O/pyridine at 100° for 2 hr. After the usual work-up, the product crystallized from MeOH to give the acetate **6**, mp 241–45°, IR: ν_{max}^{KBr} cm^{-1}: 1765, 1745, 1238; ^1H NMR: δ0.86 (*s*, 6H), 0.91 (*s*, 3H), 0.97 (*d*, 3H, J = 5.4 Hz), 1.25 (*d*, 3H, J = 5.4 Hz), 1.26 (*s*, 6H), 2.06, 2.08 (*s*, 3H each, 2 × OAc), 4.50 (*m*, 1H, Wh/2 = 18 Hz, H-3) and 5.15 (*m*, 1H, Wh/2 7 Hz, H-12).

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