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TWO TRITERPENOIDS FROM *BOSWELLIA SERRATA* GUM RESIN

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Key Word Index—*Boswellia serrata*; Burseraceae; boswellic acids; new triterpenoids.

Abstract—Two new triterpenoids, 2α,3α-dihydroxy-urs-12-ene-24-oic acid and urs-12-ene-3α,24-diol, have been isolated from the gum resin of *Boswellia serrata*.

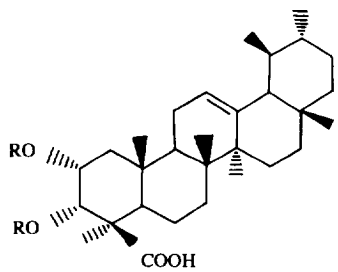
INTRODUCTION

Gum resin of *Boswellia serrata* is well known for its medicinal properties [1, 2]. The resin is known to contain monoterpenes [3], diterpenes [4] and triterpenes [5, 6]. In the Ayurvedic system of medicine it is used as a cure for rheumatoid arthritis and gout. Various extracts of the gum have been subjected to pharmacological tests [7, 8]. In our laboratory, a detailed pharmacological study by Singh and co-workers [9, 10] established that an alcoholic extract of the gum displayed marked anti-inflammatory activity in mice and rats. The extract also inhibited the formation of leukotrienes in rat peritoneal neurophiles [11]. Our attempts to identify the active principles of the gum of *B. serrata* resulted in the isolation and purification of 12 compounds, two of which are now being reported for the first time. The remainder were made up of five known urasanoic acids commonly known as boswellic acids, α- and β-amyrin, and three unidentified compounds.

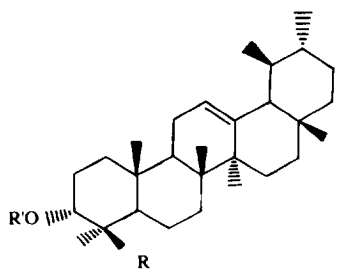
RESULTS AND DISCUSSION

Repeated chromatography of the acidic fraction from an ethanolic extract on silica gel resulted in the separation of nine compounds; of which five are known triterpenoid acids, i.e. β-boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic acid, acetyl-11-keto-β-boswellic acid and 3α-hydroxy-urs-9,12-diene-24-oic acid; three are unidentified triterpenoids and **1**. Compound **2** was obtained by chromatographic fractionation of the neutral fraction.

Compound **1** analysed for $C_{30}H_{48}O_4$. In its IR spectrum strong peaks at 3460 and 1690 cm^{-1} indicated the presence of hydroxyl and carbonyl functions, respectively. The MS showed the $[M]^+$ at *m/z* 472 and a base peak at *m/z* 218, alongwith a strong peak at *m/z* 203 due to a classical retro-Diels fission, diagnostic of olenanes or ursanes. The ^1H NMR spectrum of **1** is typical of an ursane skeleton related to boswellic acids. Two methine proton signals at δ4.08 (*brm*) and 4.26 (*dd*, *J* = 12.6 and 2.70 Hz) are assigned to an equatorial proton at C-3 and an axial proton at C-2, respectively. The downfield location of H-2a is caused by the presence of the axial carboxylic function at C-4. Rao *et al.* [12] have recorded a similar downfield shift of the H-2a signal in bartogenic acid isolated from *Barringtonia speciosa* Forst. In the ^{13}C NMR spectrum the signal (C-24) for the carboxylic function was observed at δ181.6. The olefinic proton H-12 is located at δ5.10. Compound **1** formed the diacetate **1a** on acetylation with the expected shifts of H-2a and H-3e to



1, R = H
1a, R = -COMe



2, R' = H, R = $-CH_2OH$
2a, R' = $-COOH_3$, R = $-CH_2O$ COMe
3, R' = H, R = - COMe

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δ5.30 and 5.50, respectively. It also formed an acetonide, thus confirming the 2α,3α positions for the two hydroxyl functions. Finally, the mass fragmentation pattern and ^{13}C NMR supported the proposed structure of **1** as 2α,3α-dihydroxy-urs-12-ene-24-oic acid. Another triterpenoid acid i.e. "commic acid D" with the same molecular formula and the same ring A substitution pattern, has been isolated from *Commiphloria pyracanthoides* oil. However, it differs from **1** in its physical data (mp 373°) as well as in having the opposite configuration of its ring A substituents [13].

Compound **2** analysed for $C_{30}H_{50}O_2$ (M^+ at *m/z* 442). In its IR spectrum a strong band at 3460 cm^{-1} indicated the presence of a hydroxyl function. The mass fragments displayed a base peak at *m/z* 218 of a retro-Diels fragment, along with a peak at *m/z* 203. In its ^{1}H NMR spectrum **2** displayed two doublets at δ3.36 and 3.68 due to a primary alcoholic group at C-4 and a signal at δ3.78 for the methine proton H-3e. The signal at δ5.12 was assigned to the olefinic proton H-12. The methyl signals of an ursane skeleton were observed at their normal positions. In its diacetate, **2a**, the methylene and methine proton signals were shifted to δ3.86 (*d*), 4.16 (*d*) and 4.92 (*br s*), respectively.

The positions and stereochemistry of two hydroxyl functions in **2** were confirmed by comparing **2** with the $LiAlH_4$ reduced product of the methyl ester of β-boswellic acid (**3**). The reduced product and **2** were found to be identical in all respects. On the basis of the above studies **2** was assigned the structure urs-12-ene-3α,24-diol. Compound **2** is also known synthetically [14] (lit. mp 184°, $[\alpha]_D$ +69°), but ours is the first report of its natural occurrence.

EXPERIMENTAL

MPs: uncorr.; IR: KBr; NMR: ^{1}H at 90 MHz, ^{13}C at 22.5 MHz, with TMS as int. strandard; MS: 70 eV.

Isolation. The gum resin of *Boswellia serrata* was collected from the forests of Gujarat and Madhya Pradesh and identified by the botany division of our laboratory. The lumps or the granules (1 kg) of dried gum resin were extracted with (95%) EtOH (5 l) and the concd extract was fractioned into acidic and neutral frs by alkali treatment (3% KOH). The following compounds were obtained on CC with gradient elution with petrol–EtOAc: acetyl-β-boswellic acid, acetyl-11-keto-β-boswellic acid, β-boswellic acid, 11-keto-β-boswellic acid, 3α-hydroxy-urs-9,12-diene-24-oic acid, 3 unidentified compounds and **1**. Similarly, the neutral part on CC on silica gel and elution with petrol–EtOAc yielded: α-amyrin, β-amyrin and **2**.

Compound **1**. Crystals (petrol–EtOAc) mp 174°, analysed for $C_{30}H_{48}O_4$; requires: C, 76.27; H, 10.0, observed; C, 75.18; H, 9.8%. IR ν_{max}^{KBr} cm^{-1}: 3460, 1690, 1480, 1200, 1050, 750; MS *m/z*: 472 $[M]^+$, 218, 203, 194; ^{1}H NMR ($CDCl_3$): δ0.80 (3H, *s*, Me), 0.94 (6H, *m*, 2 × Me), 1.10 (6H, *m*, 2 × Me), 1.18 (3H, *s*, Me), 1.38 (3H, *s*, Me), 4.08 (1H, *br m*, H-2), 4.26 (1H, *dd*, J = 12.6 and 2.7 Hz, H-3) and 5.1 (1H, *br s*, H-12); ^{13}C NMR ($CDCl_3$): δ14.0 (*q*, C-23), 14.5 (*q*, C-26), 17.0 (*q*, C-30), 18.0 (*q*, C-28), 21.5 (*q*, C-25), 23.0 (*q*, C-27), 23.5 (*q*, C-29), 24.2 (*t*, C-11), 26.5 (*t*, C-16), 28.0 (*t*, C-15), 29.0 (*t*, C-21), 31.0 (*t*, C-7), 34.0 (*s*, C-17), 37.0 (*d*, C-19), 38.8 (*s*, C-10), 39.5 (*t*, C-22), 41.2 (*t*, C-1), 42.4 (*s*, C-8), 43.4 (*s*, C-14), 47.5 (*s*, C-4), 49.0 (*s*, C-9), 54.9 (*d*, C-5), 58.5 (*d*, C-18), 69.0 (*d*, C-2), 70.5 (*d*, C-3), 129.5 (*d*, C-12), 142.5 (*s*, C-5), 181.6 (*s*, C-24).

Acetylation of **1**. Compound **1** (20 mg) was dissolved in Ac_2O (3 ml) and pyridine (0.5 ml) by warming and kept at room temp. overnight. Usual work-up and purification on silica gel gave diacetate **1a**, mp 120°, analysed for $C_{34}H_{52}O_6$ (requires: C, 73.3; H, 9.3, observed: C, 72.9; H, 9.2%). $[M]^+$ at *m/z* 556; ^{1}H NMR ($CDCl_3$): δ0.80 (3H, *s*, Me), 1.22 (15H, *m*, 5 × Me), 1.96 (*s*, 3H, -OCOMe), 2.18 (*s*, 3H, OCOMe), 5.12 (1H, *br s*, H-12), 5.30 (1H, *br s*, H-2) and 5.50 (1H, *dd*, J = 12.60 and 2.70 Hz, H-3).

Preparation of acetonide. This was prepd by stirring 10 mg **1** in 5 ml Me_2CO and concd H_2SO_4 (0.10 ml) overnight at room temp. Neutralization with carbonate sol., extraction in $CHCl_3$ and crystallization furnished the acetonide, mp 140°.

Compound **2**. Crystals (petrol–EtOAc), mp 180°, analysed for $C_{30}H_{50}O_2$ (requires; C, 81.4; H, 11.3, observed; C, 80.9; H-11.1%). IR ν_{max}^{KBr} cm^{-1} 3460, 2980, 1470, 1400, 1040, 770; MS *m/z*: 442 $[M]^+$ 218, 203, 175, 109, 95, 81, 69, 55; ^{1}H NMR ($CDCl_3$): δ0.80 (3H, *s*, Me), 0.98 (18H, *m*, 6 × Me), 3.36 (1H, *d*, J = 10.8 Hz, H-24), 3.68 (1H, *d*, J = 10.8 Hz, H-24), 3.78 (2H, *br s*, H-3) and 5.12 (1H, *br s*, H-12).

Acetylation of **2**. The conditions used were the same as those used for the acetylation of **1**. Compound **2a**: crystals (MeOH) mp 160°. Analysed for $C_{34}H_{54}O_4$ (requires; C, 77.5; H, 11.3, observed: C, 77.1; H, 10.8%). ^{1}H NMR ($CDCl_3$: δ0.76 (18H, *m*, 6 × Me), 1.2 (3H, *s*, Me), 2.06 (6H, *s*, 2 × OCOMe), 3.86 (1H, *d*, J = 10.8 Hz, C-24), 4.16 (1H, *d*, J = 10.8 Hz, C-24), 4.92 (1H, *br s*, H-3), 5.12 (1H, *br s*, H-12).

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REFERENCES

1. Kirtikar, K. R. and Basu, B. D. (1933) Vol. 1, p. 521. Lalit Mohan Basu 49 Leader Road, Allahabad, India.
2. Chatterjee, G. K. and Pal, S. D. (1984) *Ind. Drugs* **21**, 431.
3. Dennis, T. J. (1980) *Bull. Med. Eth. Bot. Res.* **1**, 353.
4. Pardhy, R. S. and Bhattacharya, S. C. (1978) *Ind. J. Chem.* **16B**, 171.
5. Pardhy, R. S. and Bhattacharya, S. C. (1978) *Ind. J. Chem.* **16B**, 174.
6. Pardhy, R. S. and Bhattacharya, S. C. (1978) *Ind. J. Chem.* **16B**, 176.
7. Atal, C. K., Gupta, O. P. and Singh, G. B. (1981) *Br. Pharm.* **74**, 203.

8. Atal, C. K., Singh, G. B., Batra, S., Sharma, S. and Gupta, O. P. (1980) *Ind. J. Pharm.* **12**, 59.
9. Singh, G. B. and Atal, C. K. (1986) *Agents and Action* **1B**, 407.
10. Sharma, M. L., Bani, S. and Singh, G. B. (1989) *Ind. J. Immunopharmac.* **11**, 647.
11. Ammon, H. P., Mack, J., Singh, G. B. and Safayhi, H. (1991) *Planta Med.* **57**, 203.
12. Subba Rao, G. R. S., Prasana, S., Kumar, V. P. S. and Yadgiri, B. (1986) *Ind. J. Chem.* **25B**, 113.
13. Thomas, A. F. (1961) *Tetrahedron* **15**, 212.
14. Allan Graham, G. (1968) *Phytochemistry* **7**, 963.