



AN URSANE DIOL FROM THE LEAVES OF *PRISTIMERA GRAHAMII**

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Key Word Index—*Pristimera grahamii*; Hippocrateaceae; triterpenoid; grahamidiol.

Abstract—A new pentacyclic triterpenoid (grahamidiol) of the ursane-series was isolated from the leaves of *Pristimera grahamii* and its structure was elucidated as urs-12-en-3 β ,30-diol based on the spectroscopic data of its diacetyl derivative.

INTRODUCTION

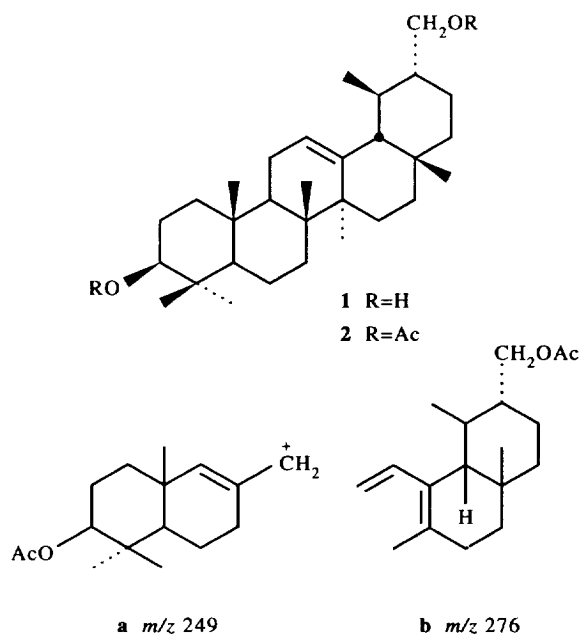
In continuation of our work [1, 2] on *Pristimera grahamii* A. C. Smith [Syn: *Reissantia grahamii* (Wight) Ding Hou and *Hippocratea grahamii* Wight], we report herein the isolation and structural elucidation of a new triterpenoid, grahamidiol (1).

RESULTS AND DISCUSSION

The chloroform extract of the leaves of *Pristimera grahamii* gave, in addition to the compounds reported earlier [1], grahamidiol in a poor yield and in an impure state even after repeated column chromatography over silica gel. The impure compound showed only the presence of hydroxyl in its IR spectrum. As minor impurities could not be removed by column, the compound was acetylated with acetic anhydride and pyridine, and the acetyl derivative (2) was used for spectroscopic studies.

Compound 2, molecular formula $C_{34}H_{54}O_4$, gave a positive Liebermann–Burchard colour test for triterpenoids. It showed IR bands at 1724 and 1213 cm^{-1} (acetyl carbonyl), and 1640 and 840 cm^{-1} (trisubstituted double bond). The 1H NMR spectrum of 2 had signals due to six tertiary methyls and one secondary methyl in the region δ 0.81–1.11 and a triplet at δ 5.14 ($J = 3.5$ Hz) due to H-12. In the ^{13}C NMR spectrum of 2, the olefinic carbons C-12 and C-13 appeared at δ 125.1 and 138.5, respectively. The upfield value of C-13 clearly suggested the compound to be an ursane derivative rather than an oleanane [3]. The 1H and ^{13}C NMR spectra further showed the presence of one primary and one secondary acetoxyl groups in the molecule.

The mass spectrum of 2 revealed characteristic retro Diels–Alder fragments at m/z 249 (fragment a) and 276



(fragment b) due to the cleavage of the ring at C-12. This showed that one of the acetoxyl groups is present in ring A/B and another in ring D/E portions of the molecule [4]. The ready loss of the methyl group at C-17 together with acetic acid from fragment b gave a fragment at m/z 201. Based on biogenetic considerations and spectral data, the secondary acetoxyl is fixed at C-3, the orientation being β -equatorial. The primary acetoxyl may be at either C-29 or C-30, as each of the methylene protons of the acetoxymethylene group gave a double-doublet showing a coupling with an adjacent hydrogen atom. The position is fixed as C-30 by comparison with the methyl shift values of 1H and ^{13}C NMR signals of some ursane derivatives reported earlier [5–9].

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EXPERIMENTAL

Mp: uncorr.; IR spectrum: CHCl_3 ; ^1H and ^{13}C NMR: CDCl_3 , TMS as int. stand; MS: 70 eV.

Pristimera grahamii was collected from the Bhimashankar hill forests (altitude 1200 m), near Pune (Maharashtra, India) and identified by Dr S. Usman Ali. Voucher specimens were deposited at Jawaharlal Nehru Ayurvedic Medicinal Plants Garden and Herbarium (CCRAS, Govt. of India), Pune.

Shade dried and powdered leaves (ca 4.0 kg) were exhaustively extracted with CHCl_3 by the cold percolation method and the extract was subjected to CC over silica gel using benzene.

Isolation of grahamidiol acetate (2). Elution of the column with benzene–EtOAc (3:1) gave a green mass which on repeated CC afforded impure grahamidiol (1, 40 mg) which on acetylation with Ac_2O and pyridine at room temp. yielded **2** (30 mg), crystallized from CHCl_3 –MeOH, $\text{C}_{34}\text{H}_{54}\text{O}_4$ (M_r 526 from DEPT), mp 165–167°. IR $\nu_{\text{max}}^{\text{CHCl}_3}$, cm^{-1} : 3018, 2955, 2875, 1724, 1640, 1460, 1377, 1213, 1113, 1032, 986, 879, 840, 768, 677; ^1H NMR (300 MHz, CDCl_3): δ 0.81 (3H, s, H-28), 0.82 (3H, d, $J=6$ Hz, H-29), 0.86 (3H, s, H-23), 0.87 (3H, s, H-24), 0.98 (3H, s, H-25), 1.05 (3H, s, H-26), 1.11 (3H, s, H-27), 5.14 (1H, t, $J=3.5$ Hz, H-12), 2.04 and 2.05 (3H each, s, $2 \times \text{OAc}$), 3.93 and 4.20 (1H each, dd, $J=11$ and 7.1 Hz; 11 and 3.6 Hz, CH_2OAc), 4.50 (1H, t, $J=8$ Hz, H-3); ^{13}C NMR (25 MHz, CDCl_3): δ 38.5 (C-1), 25.4 (C-2), 80.9 (C-3), 37.7 (C-4), 55.3 (C-5), 18.3 (C-6), 32.8 (C-7), 40.0 (C-8), 47.6 (C-9), 36.0 (C-10), 23.5 (C-11), 125.1 (C-12), 138.5 (C-13), 42.0 (C-14), 26.9 (C-15), 29.3 (C-16), 33.6 (C-17), 58.8 (C-18), 36.8 (C-19), 43.9 (C-20), 28.1 (C-21), 40.8 (C-22), 28.1 (C-23), 15.7 (C-24), 16.1 (C-25), 16.8 (C-26), 23.5 (C-27), 28.5 (C-28), 17.1 (C-29), 68.2 (C-30), 21.0 and 21.3

($2 \times \text{OCOMe}$), 170.9 and 171.3 ($2 \times \text{OCOMe}$); MS m/z : 526 $[\text{M}]^+$, 511 $[\text{M} - \text{Me}]^+$, 466 $[\text{M} - \text{HOAc}]^+$, 453 $[\text{M} - \text{CH}_2\text{OAc}]^+$, 451 $[\text{M} - \text{HOAc} - \text{Me}]^+$, 276 (b), 255, 249 (a), 216 $[\text{b} - \text{HOAc}]^+$, 201 $[\text{b} - \text{HOAc} - \text{Me}]^+$, 189 $[\text{a} - \text{HOAc}]^+$, 133, 42 (100%).

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