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TRITERPENOIDS FROM *SWERTIA PETIOLATA*

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Abstract—From the petrol extract of *Swertia petiolata* a new natural triterpene, 3β-hydroxylup-13(18)-ene has been isolated in addition to 3β-hydroxylup-12en-28-oic acid and ursolic acid.

Swertia petiolata Royle is a high altitude perennial medicinal herb growing on grassy and moist meadows of Kashmir (2800–3700 m). The plant has not been chemically investigated previously, although other members of the genus have been studied extensively for xanthone pigments. However, in addition to ursolic acid and oleanolic acid, only two other triterpenes, namely C-12 unsaturated hydroxy triterpenes of ursane/oleanane type [1] have been reported from the genus *Swertia*. The present communication deals with the isolation and characterization of 3β-hydroxylup-13(18)-ene (**1**) and 3β-hydroxylup-12-en-28-oic acid (**2**). These triterpenoids are being reported for the first time from the genus *Swertia* and to our knowledge compound **1** has not been reported previously from any natural source. Besides these triterpenoids, ursolic acid was also isolated from the plant.

The petrol extract of the whole plant on repeated column chromatography over silica gel furnished compounds **1** and **2**. Compound **1**, mp 268°, $C_{30}H_{50}O$, gave a positive Liebermann test for triterpenes and did not show any absorption above 220 nm in the UV spectrum. A broad band at 3400–3280 cm^{-1}, and the absence of any absorption due to a carbonyl group in the IR spectrum, was ascribed to an alcoholic function, which was proved to be equatorial and present at the C-3 position of an A/B *trans*-triterpene [2, 3] by the –COH stretching vibrations at 1042 and 1018 cm^{-1}. In addition absorptions at 1390, 1382 and 1368 cm^{-1} were suggestive of bending vibrations due to *gem* dimethyl and/or an isopropyl group in the molecule. Furthermore the C-3 axial proton appeared in the ^1H NMR spectrum as a double doublet at δ3.24 with coupling constants of 10.3 Hz (J_{aa}) and 6.1 Hz (J_{ae}), supporting the equatorial orientation of the C-3 hydroxy group. The ^1H NMR spectrum also showed the presence of six tertiary methyl groups and a doublet for two secondary methyl groups at δ0.93 (6H, *d*, $J = 7.2$ Hz). The mass fragmentation pattern of 1, with M^+ at *m/z* 426 and other prominent fragments at *m/z* 411 $[M-Me]^+$, 383 $[M-isopropyl]^+$ and a base peak at 189 supported a lupane or hopane type of carbon skeleton [4–7], with unsaturation in ring D or E, as the ^1H NMR spectrum did not support this linkage in the isopropyl side chain. Examination of the IR, ^1H NMR and mass spectral data so far suggests **1** to be a lupane or hopane type of triterpene with a C-3 OH group and unsaturation in ring D or E. The compound was converted into a monoacetate (**3**) with acetic anhydride–pyridine at room temperature. The acetyl carbonyl absorbed at 1741 cm^{-1} in the IR spectrum and the acetyl protons exhibited a sharp singlet at δ2.06 in the ^1H NMR spectrum. The C-3 axial proton appeared as a double doublet at δ4.52 (1H, *dd*, $J_{aa} = 9$ Hz, $J_{ae} = 5.9$ Hz), again proving the equatorial position of the hydroxylic function. The secondary nature of the hydroxy group was reconfirmed by its oxidation. The compound appeared to be unstable towards Jone's oxidation. Consequently, a better reagent for acid sensitive compounds, pyridinium chlorochromate (PCC) [8, 9] adsorbed on alumina in *n*-hexane at room temperature was employed for the oxidation of the C-3 hydroxy group. The oxidation product **4** exhibited a band for a six-membered cyclic ketone at 1718 cm^{-1} in the IR spectrum and the C-2 protons appeared as a multiplet at δ2.50 in the ^1H NMR spectrum. The unsaturation in ring D/E was proved to be tetrasubstituted, as compound **1** resisted prolonged catalytic hydrogenation in EtOAc–HOAc. This observation was supported by the absence of any olefinic proton signal in the ^1H NMR spectrum and by the appearance of two singlets at δ133 and 134.5 in the ^{13}C NMR spectrum. This linkage could, therefore, be placed at $\Delta^{17(21)}$ in hopane and $\Delta^{13(18)}$ or Δ^{18} in a lupane skeleton. However, it was settled to be $\Delta^{13(18)}$ in the lupane skeleton on the basis of the mass fragmentation pattern and the following observations. A high intensity fragment at *m/z* 218, characteristic of a Δ^{12} olefinic linkage [10], was observed in the mass spectrum of **1**. However, the ^1H NMR spectrum did not support such an assumption. Although this fragment can be accounted for in the mass fragmentation of compound **1** (Scheme 1), its high abundance cannot be

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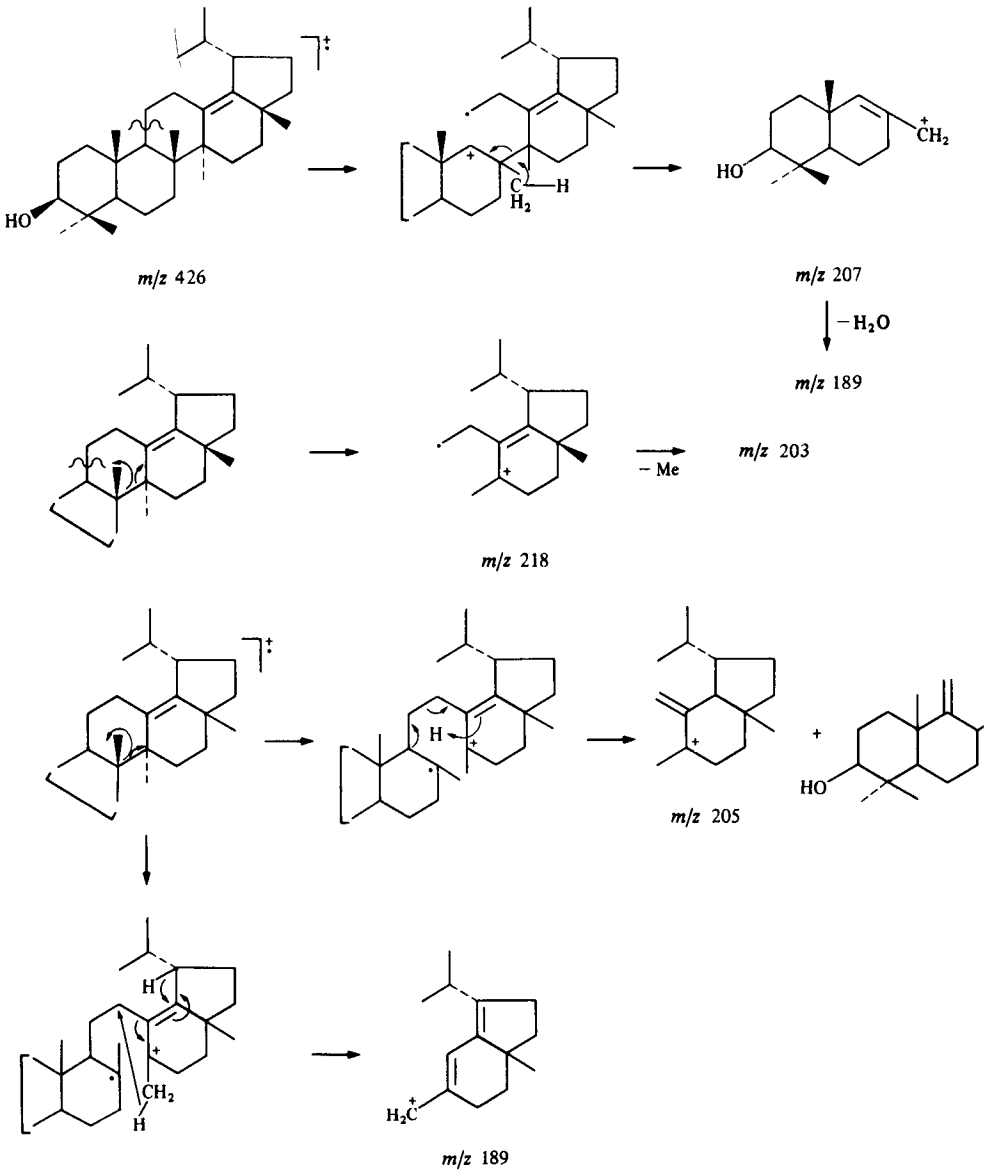
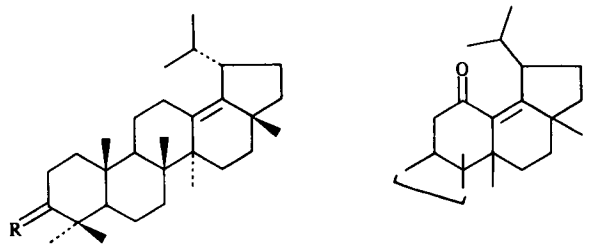


Scheme 1



1 R = β-OH, α-H
3 R = β-OAc, α-H
4 R = O

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justified on the basis of this fragmentation scheme, because comparison with mass fragmentation data of known hopenes and lupenes shows that this fragment was either totally absent or was present in an insignificant percentage. However, in the mass fragmentation pattern reported for hopanes/lupanes [11] with a double bond at $\Delta^{13(18)}$, a comparable high abundance of *m/z* 218 was observed. It is assumed here that besides the normal cleavage of ring C, also the $\Delta^{13(18)}$-bond migrates under electron impact [12] to Δ^{12}, which then undergoes retro-Diels–Alder fragmentation, leading to a high intensity of the *m/z* 218 ion. This type of migration is not possible in the $\Delta^{17(21)}$-hopane or Δ^{18}-lupane skeleton. The ^{1}H NMR spectrum of **1** also showed the C-12 methylene protons, adjacent to the $\Delta^{13(18)}$-double bond, as broad singlets at δ2.32 and 2.19. The above position of the double bond was confirmed by subjecting the monoacetate **3** to oxidation with ruthenium tetroxide in carbon tetrachloride at room temperature. The main oxidation product (**5**) displayed a band in the IR spectrum at 1685 cm^{-1} due to a six-membered ring α,β-unsaturated carbonyl, which resulted from allylic oxidation at C-12 [13–16]. Two poorly defined quartets in the ^{1}H NMR spectrum of **1**, integrating for only one proton at δ2.72 and 2.58, were attributed to the C-19 tertiary proton because of its coupling to the C-20 and C-21 protons. Study of molecular models showed that this splitting was only possible when the C-19 proton was given a β-orientation. In the case of an α-orientation this proton bisects the H-C(21)-H bond and should, therefore, appear as a multiplet. On this basis the isopropyl group was given an α-orientation. The ^{13}C NMR data also supports the proposed structure for **1** and is based on comparison with the data reported for lupane/hopane triterpenoids [17, 18]. The above data thus proved compound **1** to be 3β-hydroxylup-13(18)-ene.

Compound **2**, mp 290°, $C_{30}H_{48}O$, was shown by comparison of melting points of **2**, its acetate and methyl ester acetate and their spectral data to be 3β-hydroxylup-12-en-28-oic acid [19]. Ursolic acid was identified by co-TLC and mixed melting point with an authentic sample.

EXPERIMENTAL

Mps: uncorr. The air-dried whole plant material (5.5 kg) of *S. petiolata* Royle (voucher No. 4536 U.D., deposited with the Herbarium of Botany Department, University of Kashmir) collected in July–August 1986 from Gulmarg (Kashmir), was extracted with petrol and the extract allowed to stand overnight when a pale green dust settled down, which was filtered and, after chromatographic purification, identified as ursolic acid by mixed mp and co-TLC with an authentic sample. The filtrate was concd to a gum-like residue (120 g) which was chromatographed over silica gel (650 g, 60–120 mesh) and eluted with petrol and petrol–EtOAc. The middle collections of petrol–EtOAc elute (96:4) were re-chromatographed over silica gel, eluted with petrol–EtOAc (98:2) and recrystallized from MeOH to give **1** (1.5 g). Similarly initial fractions of the petrol–EtOAc (8:2) elute of the main extract on repeated chromatography over silica gel with $CHCl_3$–MeOH (99:1) gave **2** (167 mg).

Compound **1**, mp 268°, $C_{30}H_{50}O$, IR ν_{max}^{KBr} cm^{-1}: 3400–3280, 2880, 1460, 1390, 1382, 1368, 1042 and 1018. ^{1}H NMR (100 MHz, $CDCl_3$): δ3.24 (1H, *dd*, J_{aa}=10.3, J_{ae}=6.1 Hz, axial H-3), 2.72 (1/2 H, *q*) and 2.58 (1/2 H, *q*) [H-19], 2.32 (1H, *br s*) and 2.19 (1H, *br s*) [H_2-12], 0.93 (6H, *d*, *J*=7.2 Hz, 29, 30 Me), 1.16 (3H, *s*), 1.02 (3H, *s*), 1.0 (3H, *s*), 0.87 (3H, *s*), 0.77 (3H, *s*), 0.70 (3H, *s*) [23–28 Me]. EIMS (probe) 70 eV, *m/z* (rel. int.): 426 [M]$^+$ (65), 411 (25), 408 [M−H_2O]$^+$ (2), 393 [M−Me−H_2O]$^+$ (10), 383 (16), 257 (10), 218 (62), 207 (28), 206 (28), 205 (64), 204 (28), 203 (51), 189 (base peak), 175 (10), 151 (10), 135 (30), 109 (73), 95 (68), 69 (40), 55 (50), 43 (47). (Found: C, 83.98; H, 11.67; calcd. for $C_{30}H_{50}O$ C, 84.51; H, 11.74%). ^{13}C NMR (25.2 MHz, $CDCl_3$): δ38.8 (C-1), 27.1 (C-2), 79.3 (C-3), 39.0 (C-4), 55.5 (C-5), 18.4 (C-6), 34.5 (C-7), 41.0 (C-8), 50.8 (C-9), 37.0 (C-10), 21.0 (C-11), 25.0 (C-12), 134.5 (C-13), 45.2 (C-14), 28.0 (C-15), 36.5 (C-16), 47.0 (C-17), 133.0 (C-18), 44.8 (C-19), 26.4 (C-20), 32.2 (C-21), 39.2 (C-22), 33.0 (C-23), 15.0 (C-24), 16.0 (C-25), 17.2 (C-26), 21.7 (C-27), 24.0 (C-28), 21.7 (C-29), 23.5 (C-30).

Acetylation of compound **1**. Compound **1** (150 mg) was acetylated with pyridine (4 ml) and Ac_2O (8 ml) at room temp. (24 hr). Usual work-up and recrystallization from $CHCl_3$–MeOH (1:1) afforded white needles of **3** (135 mg) mp 253–254.5°. IR ν_{max}^{KBr} cm^{-1}: 2930, 1741 (OAc), 1380, 1362, 1268, 1255, 1032, 1015, 985, 978, and 905. ^{1}H NMR (100 MHz, $CDCl_3$): δ4.52 (1H, *dd*, J_{aa}=9, J_{ae}=5.9 Hz, axial H-3), 2.73 (1/2 H, *m*) and 2.58 (1/2 H, *m*) [H-19], 2.35 (1H, *br s*) and 2.21 (1H, *br s*) [H_2-12], 2.06 (3H, *s*, -OAc), 1.28 (3H, *s*), 1.17 (3H, *s*), 1.02 (3H, *s*), 0.95 (3H, *s*), 0.89 (3H, *s*), 0.88 (3H, *s*), 0.86 (3H, *s*), 0.71 (3H, *s*) [23–30 Me].

PCC oxidation of compound **1**. Compound **1** (200 mg) in *n*-hexane (150 ml) was stirred with PCC adsorbed on alumina (16 g) under dry conditions at room temp. (16 hr) and the mixture after filtration was CC over silica gel to give **4** (80 mg) mp 260–261°. Compound **4** gave a yellow spot on TLC when sprayed with 2:4 DNP. IR ν_{max}^{KBr} cm^{-1}: 2857, 1718 (six-membered cyclic C=O), 1468, 1395, 1377, 1258, 1156, 1122, 1053, 1025, 980, 925 and 838. ^{1}H NMR (100 MHz, $CDCl_3$): δ2.75 (1/2H, *m*) and 2.60 (1/2H, *m*) [H-19], 2.50 (2H, *m*, H_2-2), 2.35 (1H, *br s*) and 2.20 (1H, *br s*) [H_2-12], 0.99 (6 H, *d*, *J*=7 Hz, 29, 30 Me), 1.17 (3 H, *s*), 1.10 (3H, *s*), 1.03 (3H, *s*), 0.95 (3H, *s*), 0.90 (3H, *s*), 0.71 (3H, *s*) [23–28 Me].

RuO_4 *oxidation of compound* **3**. Compound **3** (50 mg) in CCl_4 was oxidized with $RuO_2 \cdot H_2O$ (50 mg/CCl_4) and $NaIO_4$ (600 mg in H_2O) by stirring at room temp. (2 hr). The organic layer upon chromatography over silica gel with petrol–EtOAc (60:1) provided **5** (23 mg) mp 281–284°. IR ν_{max}^{KBr} cm^{-1}: 2915, 1740 (-OAc), 1685 (six-membered ring α,β unsaturated C=O), 1465, 1378, 1365, 1248, 1030. Compound **2**, mp 290°, $C_{30}H_{48}O_3$, IR ν_{max}^{KBr} cm^{-1}: 3470–3392 (OH), 2920, 1697 (C=O), 1642 (>C=C<), 1450, 1407, 1391, 1385, 1360, 1278, 1032 and 1020. ^{1}H NMR (80 MHz, DMSO-d_6 + acetone-d_6): δ5.17 (1 H, *br s*, H-12), 4.40 (1H, *m*, $W_{1/2}$=16.4 Hz, axial H-3), 1.18 (3H, *s*), 1.12 (3H, *s*), 1.02 (3H, *s*), 1.0 (3H, *s*), 0.92 (3H, *s*), 0.77 (3H, *s*), 0.69 (3H, *s*) [23–27, 29, 30 Me]. EIMS (probe) 70 eV *m/z* (rel. int.): 456 [M]$^+$ (48), 439 (6), 438 [M−H_2O]$^+$ (8), 413 [M−43]$^+$ (11), 411 [M−COOH]$^+$ (32), 395 [M−43−H_2O]$^+$ (7), 248 (base peak), 208 (17), 207 (21), 205 (14), 203 (37), 191 (42), 189 (68), 175 (18) and 43 (21). (Found: C, 78.51; H, 10.43; calcd. for $C_{30}H_{48}O_3$ C, 78.94; H, 10.52%). Methylation of compound **2** (65 mg) (CH_2N_2–Et_2O) gave the monomethyl ester, mp 208°, IR ν_{max}^{KBr} cm^{-1}: 3400–3380 (OH), 1742(ester C=O), 1640 and 820 (trisubstituted olefinic linkage), 1382, 1360, 1218. Acetylation of **2** (Ac_2O–pyridine) afforded a monoacetate, mp 294.5°. IR ν_{max}^{KBr} cm^{-1}: 3410–3372, 1738 (OAc), 1694 (carboxyl), 1641, 1395, 1387, 1359, 1245, 821. The monoacetate of **2** underwent methylation (CH_2N_2–Et_2O) within 12 min to give methyl ester acetate, mp 216°. IR ν_{max}^{KBr} cm^{-1}: 1744, 1638, 1380, 1367, 1233, 1165, 1021, 872.

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