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New oleanene and fernane-type triterpenes from the stem bark of *Betula pendula* Roth

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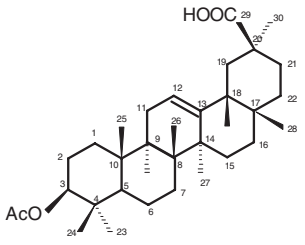
A new oleanene-type triterpene, named betuloleanolic acid acetate and fernane-type triterpenes, namely betufernanediol A and betufernanediol B (isomers) have been isolated from the stem bark of *Betula pendula*. Their structures have been established as olean-12-en-3β-ylacetate-29-oic acid, fern-22(29)-en-3β,12β-diol and fern-22(29)-en-3α,12β-diol, respectively, on the basis of spectral data analysis and chemical reactions.

1. Introduction

The genus *Betula* (Betulaceae) is represented by a handsome slender deciduous tree growing to a height of 30 m. It is commonly called as "silver birch". It has pale grey papery bark, toothed leaves in spring [1]. The bark is smooth, shining, reddish white or white with horizontal lenticles; the outer bark possesses numerous thin papery layers. It is found throughout the main Himalayan range from Kashmir to Bhutan, ascending to an altitude of 4,200 m [2], in Japan and Afghanistan [3]. The bark is pungent, healing, tonic, alexiteric, useful in convulsions, bronchitis, leprosy and diseases of blood and the ear. The decoction of the bark is used as a wash for otorrhoea and poisoned wounds [3]. The bark contains betulin, betulinic acid, lupeol, oleanolic acid [4, 5], acetyl oleanolic acid [5], lupenone, sitosterol, methyl betulonate, methyl betulate [4], karachic acid [6], leucocyaniden and polymeric leucoanthocyanidins [5]. This paper describes the isolation and characterization of oleanene-type and fernane-type triterpenes from the ethanolic extract of the stem bark of *B. pendula.*



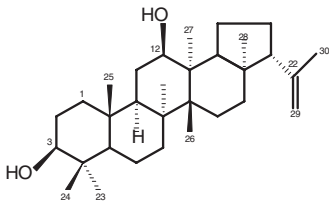
(**1**)

2. Investigations, results and discussion

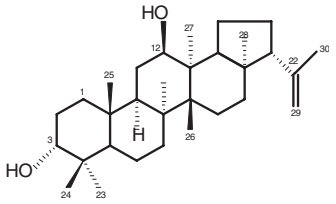
Betuloleanolic acid acetate (**1**), was obtained as colourless shining needles from benzene eluents. It gave a positive Liebermann-Burchard reaction and showed IR absorption bands, for ester (1730 cm^{-1}) and carboxylic (34000–3250, 1690 cm^{-1}) groups and unsaturation (1640 cm^{-1}). It had a molecular ion peak at m/z 498, consistent of a pentacyclic triterpenic formula, $C_{32}H_{55}O_4$. It indicated eight degrees of unsaturation, five of them were adjusted to the pentacyclic carbon skeleton and one each in olefinic linkage, acetyl group and carboxylic group. The MS displayed characteristic ion fragments at m/z 250 and 248 generated due to Retro-Diels-Alder fragmentation suggesting Δ^{12} olefinic linkage in ring C. The important ion peaks at m/z 438 $[M\text{-}AcOH]^+$, 190 $[250\text{-}AcOH]^+$, 207 $[250\text{-}Ac]^+$ and 236 $[250\text{-}Me]^+$ indicated the acetyl function in the ring A/B which was placed at C-3 on biogenetic grounds. The ion peaks at m/z 204 $[248\text{-}CO_2]^+$, 202 $[248\text{-}HCOOH]^+$ and 134 $[248\text{-}C_{18,19}\text{-}C_{17,22}$ fission$]^+$ supported the carboxylic groups in ring E at C-17/20 [8]. The ^1H NMR spectrum of **1** showed one-proton downfield broad signal at δ 5.26 assigned to H-12. A one-proton double-doublet at δ 4.49 was ascribed to the C-3 carbinol proton deshielded by an acetyl function and its coupling interactions of 5.23 and 8.18 Hz supported α-orientation of the proton coupling with $H_{2a\alpha}$ and H_{2eq} protons. A three-proton broad signal at δ 2.04 was attributed to acetyl protons. The seventertiary methyl signals appeared at δ 1.12 (Me-23), 0.94 (Me-25), 0.92 (Me-26), 0.90 (Me-30), 0.86 (Me-24), 0.85 (Me-28) and 0.73 (Me-27). The methyl resonances between δ 1.12–0.73 indicated the location of these functionalities of saturated carbons. The remaining methine and methylene protons appeared between δ 2.83–1.30. The ^{13}C NMR spectrum of **1** showed 32 carbon signals. The presence of important signals at δ 184.55 (C-29), 170.99, 21.22 ($COCH_3$) and 122.46 (C-12), 143.54 (C-13) confirmed the existence of carboxylic group, acetyl group and one olefinic linkage in the molecule. The comparison of carbon signals with pentacyclic triterpenes possessing carboxylic group at C-14, C-17 and C-29 supported its location at the latter carbon C-29 position [7]. Consequently, the structure of betuloleanolic acid ester **1** was proved to be olean-12-en-3βyl acetate 29-oic acid. This is the first report for the isolation of **1** from the *Betula* species which preferably yields triterpenes of dammarane series. The presence of oleanene-type triterpenes indicates that these

compounds play a role as an intermediate for the formation of dammaranes with an effective and specific oxidase enzyme in the plant.

Betufernanediol A (**2**), showed positive Buchard-Liebermann test and IR absorption bands for hydroxy group (3430 cm^{-1}) and unsaturation (1640 cm^{-1}). Its ^{13}C NMR and MS established its molecular weight at m/z 442 ($C_{30}H_{50}O_2$). The MS of **2** displayed important ion peaks at m/z 427 $[M\text{-}Me]^+$, 424 $[M\text{-}H_2O]^+$, 412 $[427\text{-}Me]^+$, 58 $[C_{1,10}\text{-}C_{3,4}$ fission$]^+$, 83 $[C_{3,4}\text{-}C_{5,10}\text{-}C_{7,8}$ fission$]^+$, 69 $[83\text{-}CH_2]^+$, 55 $[69\text{-}CH_2]^+$, 168 $[C_{7,8}\text{-}C_{9,10}$ fission$]^+$, 154 $[168\text{-}CH_2]^+$, 140 $[154\text{-}CH_2]^+$, 150 $[168\text{-}H_2O]^+$ and 136 $[154\text{-}H_2O]^+$ supporting the saturated nature of rings A and B and the presence of hydroxy group in ring A placed at C-3 on biogenetic grounds. The ion peaks at m/z 194, 248 $[C_{8,14}\text{-}C_{9,11}$ fission$]^+$, 176 $[194\text{-}H_2O]^+$, 207 $[248\text{-}C_3H_5]^+$, 189 $[207\text{-}H_2O]^+$, 208, 233 $[C_{11,12}\text{-}C_{8,14}$ fission$]^+$, 190 $[208\text{-}H_2O]^+$ and 203 $[C_{8,14}\text{-}C_{12,13}$ fission$]^+$ indicated saturated nature of the ring C and the location of another hydroxyl group at C-12. The existence of isopropenyl side chain in ring E was inferred from the ion peaks which arose at m/z 122 $[C_{16,17}\text{-}C_{13,18}$ fission$]^+$ and 81 $[122\text{-}C_3H_5]^+$ [8]. The ^1H NMR spectrum of **2** displayed two one-proton downfield signals at δ 4.68 and 4.56 assigned to H-29a and H-29b, respectively. Two one-proton carbinol protons appearing as double doublets at m/z 3.59 (J = 5.5, 10.73 Hz) and 3.02 (J = 5.83, 9.82 Hz) were ascribed to H-3α and H-12α, respectively. A three-proton signal at δ 1.67 was attributed to Me-30 attached to unsaturated C-22 carbon. The six tertiary methyl signals resonated as three-proton singlets at δ 1.39 (Me-23), 1.03 (Me-28), 0.97(Me-27), 0.92 (Me-24), 0.82 (Me-26) and 0.65 (Me-25), all linked to saturated carbons. The ^{13}C NMR spectrum of **2** exhibited 30 carbon signals. The δ_c values of **2** were compared with fernane type molecule [7]. The olefinic carbons resonated at δ 149.90 (C-22) and 108.70 (C-29). On the foregoing discussion, the structure of **2** was established as fern-22(29)-en-3β,12β-diol. This is, the first report of occurrence of fernane-type triterpene in *Betula* species.



(**2**)



(**3**)

Betufernanediol B (**3**), (M^+m/z 44.2, $C_{30}H_{50}O_2$), exhibited identical spectral data resemblance with that of betufernanediol A (**2**). However, its melting point, chromatographic behaviour and physical appearance were different from those of **2**, suggesting it to be an isomer of **2**. The C-3 carbinol proton signal of **3** appeared at δ 3.88 as a double doublet with coupling constants of 4.92 and 5.00 Hz, indicating 3β-orientation of the methine proton. The C-12 methine proton resonates at δ 3.03 with coupling interactions of 4.81 and 9.10 Hz (H-12α). From these evidences, the structure of **3** was elucidated as fern-22(29)-en-3α,12β-diol.

3. Experimental

3.1. General procedure

M.p.'s were determined on a Perfit apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker DRX-300–300 MHz instrument in DMSO-d_6 using TMS as internal standard. ^{13}C NMR spectra were screened on a Bruker DRX-300 75.50 MHz instrument in DMSO-d_6. MS were scanned on a Jeol D-300 instrument. CC was carried out using silica gel (60–120 mesh). Homogenity of the compounds was checked on silica gel G coated TLC plates in solvent systems; (a) C_6H_6-petroleum ether (1 : 1), C_6H_6–$CHCl_3$ (9 : 1) and petroleum ether-EtOAc (9 : 1). Iodine vapours, perchloric acid, ceric sulphate and UV lamp were used for visualization of TLC spots.

3.2. Plant material

Stem bark of *B. pendula* was purchased from the local market of Khari Baoli, Delhi and identified by Dr. M. P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard. A voucher specimen is deposited in the herbarium of the Department.

3.3. Extraction

The dried and pulverised stem bark of *B. pendula* (2 kg) was extracted with EtOH (95%) in a Soxhlet apparatus. The extract was concentrated under reduced pressure to get a dark brown viscous semi-solid mass (195 g). The dry extract was further dissolved in $CHCl_3$, acetone and MeOH successively to get $CHCl_3$, acetone and MeOH soluble fractions, respectively. The dried $CHCl_3$ soluble fraction was dissolved in minimum amount of MeOH and adsorbed on silica gel to form a slurry. The slurry was air-dried and chromatographed over a silica gel column prepared in petroleum ether. The column was eluted with petroleum ether, petroleum ether-$CHCl_3$ (9 : 1, 3 : 1, 1 : 1, 1 : 3 v/v), $CHCl_3$, $CHCl_3$–MeOH (99 : 1, 99 : 2, 95 : 5, 3 : 1, 1 : 1, 1 : 3 v/v) and MeOH to isolate the following compounds:

3.4 Characterisation of the compounds

3.4.1. Betuloleanolic acid acetate (**1**)

Elution of the column with C_6H_6, (fractions 1–7), yielded colourless, lustrous needles of **1**, recrystalized from ethanol, 70 mg (0.0023% yield), m.p. 280–281 °C, $[\alpha]_D^{25}$ + 18, R_f 0.507 (C_6H_6–MeOH, 9 : 1), UV λ_{max} (MeOH) 209 nm (log ε 6.2), IR νmax (KBr) 3400–3250, 950, 2890, 1730, 1690, 1640, 1460, 1365, 1255, 1020, 805 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.26 (1 H, brs, H-12), 4.49 (2 H, dd, J = 5.23, 8.18 Hz, H-3α), 2.83 (1 H, d, J = 10.28 Hz, H-11a), 2.80 (1 H, d, J = 1028 Hz, H-11b), 2.04 (3 H, brs, $COCH_3$), 1.97 (1 H, m, H-1a), 1.87 (1 H, m, H-2a), 1.80 (1 H, m, H-9), 1.76 (1 H, m, H-5α), 1.71 (1 H, m, H-1b), 1.62 (4Hm, 2x, CH_2), 1.52 (2 H, m, CH_2), 1.54 (1 H, m, H-18), 1.45 (2 H, m, CH_2), 1.41 (2 H, m, CH_2), 1.38 (2 H, m, CH_2), 1.30 (2 H, m, CH_2), 1.12 (3 H, brs, Me-23), 0.94 (3 H, brs, Me-25), 0.92 (3 H, brs, Me-26), 0.90 (3 H, brs, Me-30), 0.86 (3 H, brs, Me-24), 0.85 (3 H, brs, Me-28), 0.73 (3 H, brs, Me-27). ^{13}C NMR (DMSO-d_6): δ 37.98 (C-1), 27.59 (C-2), 80.86 (C-3), 37.61 (C-4), 55.21 (C-5), 18.09 (C-6), 33.71 (C-7), 39.20 (C-8), 47.48 (C-9), 36.92 (C-10), 22.76 (C-11), 122.46 (C-12), 143.54 (C-13), 41.43 (C-14), 27.97 (C-15), 23.50 (C-16), 32.41 (C-17), 46.47 (C-18), 45.76 (C-19), 40.79 (C-20), 30.59 (C-21), 33.00 (C-22), 27.97 (C-23), 16.59 (C-24), 15.31 (C-25), 17.13 (C-26), 25.85 (C-27), 23.50 (C-28), 184.55 (C-29), 23.33 (C-30), 70.99, 21.22 ($COCH_3$). EIMS *m/z* (rel.int.) 498$[M]^+$ ($C_{32}H_{50}O_4$) (1.1), 438(6.1), 423(5.2), 393(3.1), 250(27.1), 248(80.6), 236(100), 207(61.2), 204(18.6), 202(67.1), 190(46.5), 181(23.2), 165(28.7), 147(23.2), 134(21.6), 119(8.8), 69(15.2), 43(54.2).

3.4.2. Betuferananediol A (**2**)

Elution of the column with C_6H_6, (fractions 34–36), yielded colourless, amorphous powder of **2**, recrystalized from MeOH, m.p. 260–261^0, $[\alpha]_D^{25}$ 22, R_f, 0.352 (C_6H_6–MeOH, 9 : 1); UV λmax 217 nm (log ε 6.1), IR ν_{max} (KBr) 3430, 2950, 2885, 1640, 1465, 1385, 1355, 1310, 1195, 1115, 1030, 885 cm^{-1}. ^1H NMR (DMSO-d_6) δ 4.68 (1 H, brs, H-29a), 4.56 (1 H, brs, H-29b), 3.59 (1 H, dd, J = 5.5, 10.73 Hz, H-3α), 3.02 (1 H, dd, J = 5.83, 9.82 Hz, H-12α), 2.40 (1 H, m, H-18), 2.39 (1 H, m, H-21), 2.01 (1 H, m, H-5), 1.92 (2 H, brs, CH_2), 1.90 (2 H, brs, CH_2), 1.76 (1 H, m, H-18), 1.71 (2 H, m, CH_2), 1.67 (3 H, brs, Me-30), 1.62 (1 H, m, H-9α), 1.57 (2 H, brs, CH_2), 1.49 (2 H, brs, CH_2), 1.39 (3 H, brs, Me-23), 1.30 (2 H, brs, CH_2), 1.28 (1 H, m, H-8), 1.26 (2 H, m, CH_2), 1.20 (2 H, m, CH_2), 1.15 (2 H, m, CH_2), 1.03 (3 H, brs, Me-28), 0.97 (3 H, brs, Me-27), 0.92 (3 H, brs, Me-24), 0.82 (3 H, brs, Me-26), o.65 (3 H, brs, Me-25) ^{13}C NMR

(DMSO-d_6): δ 40.29 (C-1), 24.72 (C-2), 76.65 (C-3), 33.48 (C-4), 54.75 (C-5), 18.48 (C-6), 17.66 (C-7), 40.05 (C-8), 49.74 (C-9), 36.55 (C-10), 20.17 (C-11), 76.61 (C-12), 38.12 (C-13), 41.95 (C-14), 29.33 (C-15), 26.82 (C-16), 48.15 (C-17), 46.96 (C-18), 39.77 (C-19), 26.82 (C-20), 58.11 (C-21), 149.90 (C-22), 33.71 (C-23), 27.67 (C-24), 14.15 (C-25), 15.18 (C-26), 15.38 (C-27), 15.38 (C-28), 108.70 (C-29), 28.94 (C-30). EIMS m/z (rel. int. 442$[M]^+$ ($C_{30}H_{50}O_2$) (10.1), 427 (6.1), 424 (9.4), 412 (31.2), 394 (9.1), 385 (10.7), 325 (5.2), 288 (12.3), 248 (7.1), 233 (25.6), 208 (19.5), 207 (63.5), 203 (63.8), 194 (18.2), 190 (43.6), 189 (100), 176 (21.5), 175 (38.6), 168 (19.2), 161 (29.6), 154 (11.1), 150 (34.5), 140 (26.2),136 (3.6), 122 (62.8), 107 (81.0), 97 (74.2), 83 (18.2), 81 (78.5), 69 (71.2), 58 (65.2), 55 (71.5), 43 (69.8).

3.4.3. Betufernanediol B (**3**)

Elution of the column with C_6H_6, (fractions 43–52), yielded colourless, amorphous powder of **3**, recrystallised from MeOH, m.p. 245–246 °C, $[\alpha]_D^{25}$ + 22, Rf, 0.466 (C_6H_6–MeOH, 9 : 1) (MeOH, 0.1) UV λ_{max} 216 nm (log ε 6.2), IR ν_{max} (KBr) 3500, 2945, 2865, 1655, 1455, 1400, 1375, 1315, 1235, 1205, 1185, 1035, 955, 880 cm^{-1}. ^1H NMR (DMSO-d_6) δ 4.68 (1 H, brs, H-29a), 4.56 (1 H, brs, H-29b), 3.88 (1 H, dd, J = 4.92, 5.00 Hz, H-3β), 3.03 (1 H, dd, J = 4.81, 9.10 Hz, H-12α), 2.51 (1 H, m, H-21), 2.40 (1 H, m, H-18), 1.95 (1 H, m, H-5), 1.90 (2 H, m, CH_2), 1.76 (1 H, m, H-8), 1.71 (2 H, m, CH_2), 1.66 (3 H, brs, Me-30), 1.62 (1 H, m, H-9), 1.57 (2 H, m, CH_2), 1.48 (2 H, m, CH_2), 1.38 (3 H, brs, Me-23), 1.33 (2 H, m, CH_2), 1.28 (2 H, m, CH_2), 1.15 (2 H, m, CH_2), 1.04 (3 H, brs, Me-28), 0.98 (3 H, brs, Me-27), 0.96 (3 H, brs, Me-24), 0.86 (3 H, brs, Me-26) and 0.70 (3 H, brs, Me-25) ^{13}C NMR (DMSO-d_6): δ 41.94 (C-1), 24.72 (C-2), 76.62 (C-3), 33.66 (C-4), 54.67 (C-5), 18.46 (C-6), 17.63 (C-7), 40.05 (C-8), 49.69 (C-9), 36.53 (C-10), 20.17 (C-11), 76.62 (C-12), 38.06 (C-13), 40.94 (C-14), 29.28 (C-15), 26.48 (C-16), 48.12 (C-17), 46.94 (C-18), 40.92 (C-19), 26.81 (C-20), 58.08 (C-21), 150.00 (C-22), 33.45 (C-23), 27.68 (C-24), 14.15 (C-25), 15.18 (C-26), 15.39 (C-27), 15.39 (C-28), 108.74 (C-29), 28.92 (C-30). EIMS m/z (rel.int.): 442 $[M]^+$ ($C_{30}H_{50}O_2$) (20.1), 427 (5.2), 424(5.7), 412 (40.7), 394 (9.8), 385 (14.3), 288 (9.3), 248 (9.6), 233 (23.5), 208 (13.1), 207 (67.8), 203 (75.1), 189 (97.8), 175 (43.6), 168 (10.2), 161(27.8), 154 (38.9), 150 (23.6), 136 (82.1), 122 (58.3), 107 (67.8), 97 (15.3), 95 (100), 83 (22.6), 81 (78.3), 69 (73.5).

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