Steroids and terpenoid from *Mimosa pudica* roots

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Abstract : One new sterolglucoside **1** along with stigmasterol, β -sitosterol and betulinic acid isolated from the roots of *Mimosa pudica* has been characterized as 4- α ,24-dimethylcholest-7-en-3 β -ol-3 β -D-glucoside. This report of isolation of stigmasterol and betulinic acid is for the first time from this plant and β -sitosterol was reported earlier from its aerial parts.

Keywords : *Mimosa pudica*, Mimosaceae, steroids, terpenoid.

Mimosa pudica L. (Mimosaceae) (local name : Lajak, Lajjabati), a tropical deciduous undershrub of American species¹ is naturalized in tropical and sub-tropical regions of India. In traditional medicine the decoction of the root is used in gravel and other urinary complaints and in the treatment of piles and fistula². Earlier investigations on the aerial parts of the plant reported the isolation of 4-*O*-(3,5-dihydroxybenzoic acid)- β -D-glucuronide, D-pinitol, nor-epinephrine, β -sitosterol, C-glycosyl flavones and phenolic ketone³. We investigated the roots of this plant for the first time to isolate the active principles present in it and isolated one new sterolglucoside **1** along with known compounds, stigmasterol, β -sitosterol and betulinic acid.

Results and discussion

Sterolglucoside **1**, amorphous powder, responded the Liebermann Burchard test for steroids. The positive ion-FAB-mass spectrum showed a quasi-molecular ion peak at m/z 599 $[M+Na]^+$ as well as elemental analysis corresponded to its molecular formula $C_{35}H_{60}O_6$. The IR spectrum in KBr indicated the presence of hydroxyl (3446 cm^{-1}) and trisubstituted olefinic (1650, 824 and 812 cm^{-1}) functions. The 400 MHz 1H NMR spectrum (see Experimental) comprised of a signal for one olefinic proton at δ 5.22 (brd, H-7), two methyl singlets at δ 0.54 (H_3 -18) and 0.86 (H_3 -19), five methyl doublets at δ 0.88 (3H, d, J 6.0 Hz, H_3 -30), 0.78 (3H, d, J 6.8 Hz, H_3 -28), 0.79 (3H, d, J 6.8 Hz, H_3 -27), 0.86 (3H, d, J 6.8 Hz, H_3 -26) and 0.95 (3H, d, J 6.5 Hz, H_3 -21), a secondary

carbinol methine at δ 3.45 (1H, ddd, J 11.5, 4.5, 2.5 Hz, H-3) and an anomeric sugar proton at δ 4.27 (d, J 8.0 Hz) suggesting its 4 α -methyl Δ^7 -sterolglucoside structure^{4,5}. The upfield C-18 and C-19 methyl protons and C-7 olefinic proton resonances indicated that it was a Δ^7 sterol⁶. The chemical shift values of two olefinic carbons in the ^{13}C NMR spectrum (see Experimental) at δ 117.82 (d, C-7) and 140.05 (s, C-8) also corroborated the position of olefinic double bond at C-7. The downfield C-3 and C-5 carbon resonances also supported the alpha (α)-configuration of C-4 methyl group⁷. The positive ion-FAB-mass spectrum recorded a quasi-molecular ion peak at m/z 599 $(M+Na)^+$ and significant mass peaks at m/z 447 (M^+ -side chain (C_9H_{19}) and -2H), 414 (M^+ -glucosyl + H), 398 (M^+ -glucosyl and CH_3), 396 (M^+ -glucose), 381 (M^+ - CH_3 and glucose), 287 (M + H-glucosyl and side chain [SC]), 269 (M -SC and glucose), 245 (287- C_3H_6 [part of ring D]) and 227 (M^+ -glucose, SC and C_3H_6 [part of ring D]) corroborating 4 α ,24-dimethyl sterol structure with a double bond in the nucleus⁸. The compound on hydrolysis with aqueous-methanolic HCl gave a free sterol (**1a**), m.p. 154 °C, $C_{29}H_{50}O$ (M^+ 414), whose spectral data (see Experimental) were similar to those of 24-methylphenol⁵. The sterol **1a** on acetylation with Ac_2O and pyridine afforded monoacetate, **1b** $C_{31}H_{52}O_2$ (M^+ 456), m.p. 139 °C (lit.⁵ 138–139 °C). On the basis of these data, the structure of **1** was deduced as 24-methylphenol glucoside or 4 α ,24-dimethylcholest-7-en-3 β -ol-3 β -D-glucoside. Known compounds, β -sitosterol, stigmasterol and betulinic acid were identified by

comparing their physical and spectral data (see Experimental) with literature values.

Antibacterial screening of the isolated compounds indicated that 24-methylphenol glucoside was active against *Bacillus subtilis* (100 µg/disc) and 24-methylphenol against *Pseudomonas aeruginosa* (100 µg/disc). The details of screening study of several phytochemicals has been communicated to a biomedical Journal.

Experimental

The melting points were recorded on a sulphuric acid bath in open capillary tube and are uncorrected. The UV spectra were recorded on a Perkin UV spectrophotometer and IR spectra on a Perkin FTIR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on a Varian XL 400 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C NMR. EI-MS and FAB-MS were recorded on a JEOL JMS AX 505 HA and a JEOL JMS 700 Mstation spectrometers. For FAB-MS, *m*-NBA, PZG 200 and NaI were used as matrix and chloroform-methanol (1 : 1) as solvent. Column chromatography (CC) was carried out with silica gel (60–120 mesh, Qualigens, India) and thin layer chromatography (TLC) on silica gel G (Merck, India), respectively.

Plant material : The fresh plant material (roots of *M. pudica*) was collected from the campus area of Tripura University (TU) during October, 2003 and was identified by Dr. B. K. Dutta, Taxonomist, Department of Life Sciences, Tripura University.

Extraction and isolation : Air-dried powdered roots (2.5 kg) of *M. pudica* was extracted with MeOH in a percolator. The MeOH extract was concentrated to a resinous residue (50 g). The residue was suspended in water (ca. 50 mL) and extracted with benzene, chloroform and *n*-butanol, respectively. The benzene fraction on CC gave a residue of a sterol mixture from benzene-chloroform (9 : 1) eluate, which on repeated CC afforded β -sitosterol (15 mg), $\text{C}_{29}\text{H}_{50}\text{O}$, m.p. 138 °C and stigmasterol (25 mg), $\text{C}_{29}\text{H}_{48}\text{O}$, m.p. 170 °C. Both these sterols were identified by comparison of spectral data with literature^{4,9} as well as direct comparison (m.m.p. and co-TLC) with the authentic samples. The chloroform fraction on CC afforded stigmasterol (8 mg) from benzene-chloroform (7 : 3) eluate and betulinic acid (30 mg), $\text{C}_{30}\text{H}_{48}\text{O}_3$ (M^+ 456), m.p. 316 °C from chloroform eluate. Betulinic acid was identified by comparison of its spectral (^1H , ^{13}C NMR and EI-MS) data with literature¹⁰ as well as by direct comparison with an authentic sample. The *n*-butanol fraction on CC afforded betulinic acid (15 mg) from

chloroform-methanol (9 : 1) eluate and sterolglucoside 1 (120 mg) from ethyl acetate eluate.

Compound 1 : Amorphous powder, m.p. 245 °C (Found : C, 72.76; H, 10.41. $\text{C}_{35}\text{H}_{60}\text{O}_6$ requires : C, 72.88; H, 10.48%); UV (MeOH) λ_{max} 248 nm (log ϵ , 3.6); IR (KBr) 3446, 1650, 1523, 1460, 1378, 1060, 824, 812 cm^{-1} ; ^1H NMR (400 MHz, $\text{CDCl}_3\text{-CD}_3\text{OD}$, 9 : 1) δ 3.45 (1H, ddd, J 11.5, 4.5 and 2.5 Hz, H-3), 1.76 (1H, m, H-4), 1.40 (1H, m, H-5), 1.82 (2H, m, H₂-6), 5.22 (1H, brd, J 5.5 Hz, H-7), 0.54 (3H, s, H₃-18), 0.86 (3H, s, H₃-19), 0.95 (3H, d, J 6.5 Hz, H₃-21), 0.86 (3H, d, J 6.8 Hz, H₃-26), 0.79 (3H, d, J 6.8 Hz, H₃-27), 0.78 (3H, d, J 6.8 Hz, H₃-28), 0.88 (3H, d, J 6.0 Hz, H₃-30), 4.27 (1H, d, J 8.0 Hz, H-1'), 3.12 (1H, dd, J 9.0 and 8.0 Hz, H-2'), 3.29 (1H, dd, J 9.0 and 9.0 Hz, H-3'), 3.27 (1H, dd, J 9.0 and 8.0 Hz, H-4'), 3.15 (1H, ddd, J 9.0, 4.5 and 2.5 Hz, H-5'), 3.62 (1H, dd, J 12.5 and 4.5 Hz, H_a-6'), 3.71 (1H, dd, J 12.5 and 2.5 Hz, H_b-6'); ^{13}C NMR (100 MHz, $\text{CDCl}_3\text{-CD}_3\text{OD}$, 9 : 1) δ 36.78 (t, C-1), 31.61 (t, C-2), 78.88 (d, C-3), 40.23 (d, C-4), 51.01 (d, C-5), 29.42 (t, C-6), 117.82 (d, C-7), 140.05 (s, C-8), 49.93 (d, C-9), 36.44 (s, C-10), 20.78 (t, C-11), 39.50 (t, C-12), 42.05 (s, C-13), 55.70 (d, C-14), 25.11 (t, C-15), 27.95 (t, C-16), 56.49 (d, C-17), 11.86 (q, C-18), 15.01 (q, C-19), 35.87 (d, C-20), 18.97 (q, C-21), 33.67 (t, C-22), 29.99 (t, C-23), 38.58 (d, C-24), 32.14 (d, C-25), 20.86 (q, C-26), 18.35 (q, C-27), 15.01 (q, C-28), 15.07 (q, C-30), 100.84 (d, C-1'), 73.30 (d, C-2'), 76.19 (d, C-3'), 69.95 (d, C-4'), 75.53 (d, C-5'), 61.59 (t, C-6'); HR-FAB-MS m/z 599.4278 (rel. int. 100%). Calcd. for $\text{C}_{35}\text{H}_{60}\text{O}_6\cdot\text{Na}$ 599.4288; positive ion-LR-FAB-MS m/z (rel. int. %) 599 ($\text{M}+\text{Na}$, 5), 447 (6), 414 (10), 398 (58), 396 (48), 381 (11), 287 (11), 269 (7), 245 (21), 227 (11), 127 (31), 71 (49), 57 (85), 43(100).

Acid hydrolysis of compound 1 : A solution of compound 1 (30 mg) in aqueous methanolic 1 *M* HCl (10 mL) was refluxed for 2 h and monitored by TLC. The TLC of the reaction mixture showed disappearance of 1. The reaction mixture was evaporated on boiling water bath to remove methanol, diluted with water and extracted with chloroform. The chloroform extract was washed with water, concentrated and subjected to CC to get aglycone, 24-methylphenol 1a (12 mg), m.p. 154 °C. The aqueous layer left after chloroform extraction of the concentrated reaction mixture was neutralized with Ag_2CO_3 , filtered and the filtrate was concentrated. The concentrated solution on TLC and optical rotation study (+ve rotation) showed the presence of D-glucose (R_f 0.48, *n*-butanol-pyridine- H_2O , 6 : 4 : 3).

Compound 1a : Colourless, crystals, m.p. 154 °C; IR (KBr) 3616. 1652. 1523, 1461, 1048, 824, 813 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 3.52 (1H, H-3), 5.20 (1H, brd, H-7), 0.54 (3H, s, H_3 -18), 0.85 (3H, s, H_3 -19), 0.94 (3H, d, J 6.5 Hz, H_3 -21), 0.86 (3H, d, J 6.8 Hz, H_3 -26), 0.80 (3H, d, J 6.8 Hz, H_3 -27), 0.78 (3H, d, J 6.8 Hz, H_3 -28), 0.85 (3H, d, J 6.0 Hz, H_3 -30); ^{13}C NMR (100 MHz, CDCl_3) δ 37.00 (t, C-1), 28.33 (t, C-2), 77.82 (d, C-3), 38.20 (d, C-4), 50.13 (d, C-5), 27.95 (t, C-6), 117.22 (d, C-7), 139.40 (s, C-8), 50.15 (d, C-9), 36.50 (s, C-10), 21.18 (t, C-11), 39.51 (t, C-12), 42.21 (s, C-13), 54.39 (d, C-14), 23.06 (t, C-15), 28.18 (t, C-16), 56.11 (d, C-17), 11.97 (q, C-18), 14.10 (q, C-19), 36.14 (d, C-20), 18.77 (q, C-21), 35.67 (t, C-22), 24.29 (t, C-23), 39.07 (d, C-24), 29.12 (d, C-25), 21.02 (q, C-26), 18.97 (q, C-27), 15.36 (q, C-28), 15.43 (q, C-30); EI-MS m/z (rel. int.) 414 (M^+ , 40), 399 (M-Me, 42), 396 (M- H_2O , 22), 381 (M- H_2O and Me, 25), 287 (28), 269 (14), 245 (21), 227 (35), 127 (63), 71 (73), 57 (100), 43 (87).

1a R = H

1b R = Ac

Acetylation of compound 1a : Compound **1a** (6 mg) was acetylated with Ac_2O (1 mL) and pyridine (4 drops) at room temperature for 24 h. The acetylated mixture on usual work-up followed by column purification afforded

monoacetate **1b** in colourless crystals (2 mg), m.p. 139 °C. $\text{C}_{31}\text{H}_{52}\text{O}_2$ (M^+ 456); IR (KBr) 1735, 1260 (OAc), 830, 815 cm^{-1} ($>\text{C}=\text{CH}$).

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