

A new isoflavone glycoside from *Heliotropium indicum*

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Abstract : A new isoflavone glycoside helioside, together with 7-hydroxyflavanone and naringenin-5-methylether has been isolated from the whole plant of *Heliotropium indicum*. The structure of helioside has been established as genistein-7-*O*-(α -L-rhamnosyl-(1 $\rightarrow$ 2)-[β -D-glucosyl-(1 $\rightarrow$ 3)]- β -D-glucoside) by chemical and spectral data.

Keywords : *Heliotropium indicum*, Boraginaceae, isoflavone glycoside.

The plant *Heliotropium indicum* Linn. (Family : Boraginaceae) is distributed throughout India. It is used in the treatment of boils, ulcers, wounds and also used as diuretics in Indian System of Medicine^{1,2}. A number of pyrrolizidine alkaloids³⁻⁶ and non-alkaloids^{7,8} have earlier been reported from this plant. Here we report the isolation of a new isoflavone glycoside, helioside together with 7-hydroxyflavanone and naringenin-5-methyl ether.

Results and discussion

The non-alkaloidal fraction of the methanolic extract of *Heliotropium indicum* furnished a new isoflavone glycoside, designated helioside together with 7-hydroxyflavanone⁹, m.p. 189–190 °C and naringenin-5-methyl ether¹⁰, m.p. 257–259 °C.

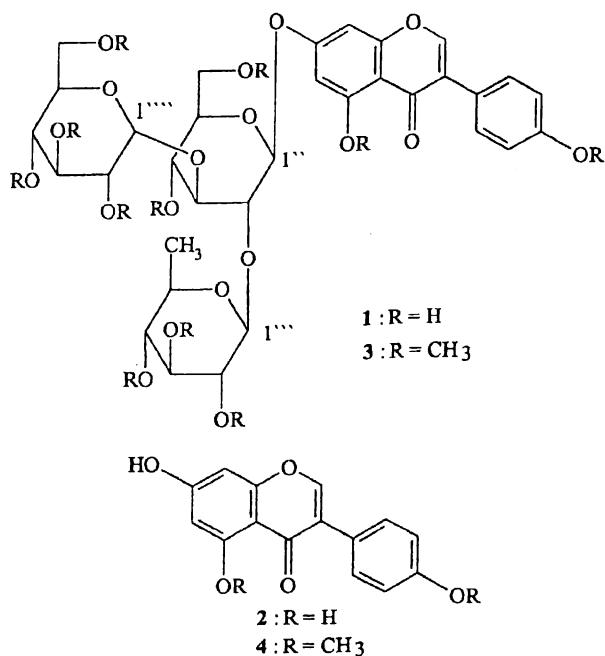
Helioside (1), m.p. 233–235 °C, C₃₃H₄₀O₁₉ (*m/z* 741, [M + H]⁺, FAB-MS) was recognised as isoflavonoid by UV absorption maxima at 261 and 330 (sh) as well as characteristic proton singlet at δ 8.22 (H-2) in ¹H NMR spectrum. IR spectrum showed bands for hydroxyl and conjugated carbonyl groups. Acid hydrolysis of 1 gave genistein¹¹ (2) characterised by spectral data and the sugars D-glucose and L-rhamnose were identified in the hydrolysate by co-PC with authentic sugars. The UV spectrum with NaOMe and AlCl₃ exhibited bathochromic shift of 26 nm and 46 nm, respectively but showed no shift with NaOAc and NaOAc/H₃BO₃. This indicated free OH at C-4' and C-5 and substitution at C-7 positions.

Methylation of 1 by Hakomori method¹² gave permethylated product (3) (IR : absence of OH group). Compound 3 on acid hydrolysis furnished 7-hydroxy-5,4'-dimethoxyisoflavone (4) characterised by spectral analysis and the sugar residues were identified in the hydrolysate as 2,3,4,6-tetra-*O*-methylglucose, 2,3,4-tri-*O*-methyl rhamnose and 4,6-di-*O*-methyl glucose by co-PC with authentic methylated sugars. The structure of 1 is thus settled as genistein-7-*O*-(α -L-rhamnosyl-(1 $\rightarrow$ 2)-[β -D-glucosyl-(1 $\rightarrow$ 3)]- β -D-glucoside). ¹H and ¹³C NMR spectra exhibited three anomeric hydrogens at δ 4.80, 4.91 and 5.00 and three anomeric carbon signals at δ 97.2, 97.5 and 101.2 together with chemical shifts for one mole of genistein and L-rhamnose and two moles of D-glucose units, which was in conformity of structure 1.

Experimental

Plant material : The whole plant of *Heliotropium indicum* was collected from Varanasi District and authenticated by the Department of Botany, Banaras Hindu University, Varanasi and specimen sample is kept in the Department.

Extraction and isolation : Dried and powdered whole plant was extracted with MeOH by cold percolation. The MeOH extract (4 kg) was stirred with 7% aqueous citric acid to remove alkaloids. The acid insoluble fraction (65 g) was chromatographed over silica gel column eluting with solvents of increasing polarity.



The eluates from C₆H₆-CHCl₃ (2 : 1), (4 : 1) and CHCl₃-MeOH (1 : 4) on crystallisation from MeOH furnished respectively 7-hydroxyflavanone (22 mg), naringenin-5-methyl ether (26 mg) and helioside (20 mg) (1).

Helioside (1) : Compound (1) yellow granules, m.p. 233–235 °C; C₃₃H₄₀O₁₉ ([M + H]⁺, *m/z* 741); UV, λ_{max} (MeOH) nm : 261, 330 (sh), λ_{max} (MeOH + NaOMe) nm : 271, 356 (sh), λ_{max} (MeOH + AlCl₃) nm : 272, 308 (sh), 375, λ_{max} (MeOH + AlCl₃ + HCl) nm : 272, 307 (sh), 374, λ_{max} (MeOH + NaOAc) nm : 261, 331 (sh); IR ν_{max} (KBr) cm⁻¹ : 3100–3550, 1640, 1610, 1570, 1500, 1240; ¹H NMR (300 MHz, DMSO-*d*₆) δ : 1.10 (3H, s, rhamnosyl CH₃), 3.00–3.70 (16H, m, two glucosyl and one rhamnosyl protons), 4.80 (1H, br s, rhamnosyl anomeric H), 4.91 (1H, br s, glucosyl anomeric H), 5.00 (1H, br s, glucosyl anomeric H), 6.19 (1H, br s, H-6), 6.32 (1H, br s, H-8), 6.80 (2H, d, *J* 8 Hz, H-3', H-5'), 7.35 (2H, d, *J* 8 Hz, H-2', H-6'), 8.22 (1H, s, H-2), 9.65 (1H, s, exchangeable with D₂O, HO-4'), 12.90 (1H, s, exchangeable with D₂O, HO-5); ¹³C NMR (100 MHz, DMSO-*d*₆) δ : 154.0 (C-2), 122.4 (C-3), 180.3 (C-4), 162.1 (C-5), 99.1 (C-6), 164.4 (C-7), 95.5 (C-8), 157.5 (C-9), 104.6 (C-10), 121.4 (C-1'), 130.3 (C-2'), 115.1 (C-3'), 157.7 (C-4'), 115.1 (C-5'), 130.3 (C-6'), 97.2 (C-1''), 75.3 (C-2''), 73.4 (C-3''), 71.7 (C-4''), 76.2 (C-5''), 61.4 (C-6''), 101.2 (C-1'''), 69.9 (C-2'''), 70.6 (C-3'''), 71.6 (C-4'''), 70.7 (C-5'''), 18.2 (C-6'''), 97.5 (C-1'''), 74.2 (C-2'''), 73.0 (C-3'''), 72.7 (C-4'''), 75.8 (C-5'''), 60.5 (C-6''').

Acid hydrolysis : Compound 1 (30 mg) was dissolved in MeOH (10 ml) and H₂O (2 ml) and refluxed with H₂SO₄ (1.5 ml) for 5 h. The reaction mixture was poured into H₂O (20

ml) and the MeOH was removed by evaporation on water bath and the remained aqueous solution was extracted with CHCl₃. The chloroform extract yielded genistein (2), C₁₅H₁₀O₅ ([M]⁺, 270), yellow granules, m.p. 290–292 °C; UV λ_{max} (MeOH) nm : 261, 328 (sh); IR ν_{max} (KBr) cm⁻¹ : 3400, 1640, 1610, 1570, 1500, 1300; ¹H NMR (300 MHz, DMSO-*d*₆) δ : 6.20 (1H, br s, H-6), 6.35 (1H, br s, H-8), 6.80 (2H, br d, *J* 8 Hz, H-3', H-5'), 7.35 (2H, br d, H-2', H-6'), 8.25 (1H, s, H-2), 9.60 (1H, br s, HO-4'), 10.70 (1H, br s, HO-7), 12.90 (1H, br s, HO-5); MS : *m/z* 270 ([M]⁺), 242, 152, 124, 118. The hydrolysate showed two spots on PC which corresponded to glucose and rhamnose (co-PC with authentic samples).

Methylation by Hakomori method : Compound 1 (41 mg) was treated with NaH (300 mg) and MeI (4 ml) in DMSO (25 ml) for 4 h. The reaction mixture was diluted with H₂O and extracted with CHCl₃ in the usual way. The methylated product (3) on purification by preparative TLC gave a semi solid mass; IR ν_{max} (KBr) cm⁻¹ : 1635, 1615, 1490.

Hydrolysis of permethylated product (3) : Compound 3 was refluxed with 6% HCl in MeOH for 2 h. The MeOH was removed from the reaction mixture which was then extracted with CHCl₃. The CHCl₃ extract on crystallisation gave dirty yellow amorphous solid of 7-hydroxy, 5,4'-dimethoxyisoflavone (4), ¹H NMR (300 MHz, CDCl₃) δ : 3.95 (6H, s, 2 × OCH₃), 6.22 (1H, br s, H-6), 6.30 (1H, br s, H-8), 6.82 (2H, br d, *J* 8 Hz, H-3', H-5'), 6.34 (2H, br d, *J* 8 Hz, H-2', H-6'), 8.30 (1H, s, H-2), 10.72 (1H, br s, HO-7). The hydrolysate gave three spots on PC which corresponded to 2,3,4,6-tetra-*O*-methylglucose; 2,3,4-tri-*O*-methylrhamnose and 4,6-di-*O*-methylglucose (co-PC with authentic samples).

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