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A Novel Flavone Glycoside: 5,7,3′-trihydroxy-6,4′,5′-Trimethoxyflavone-3′O-α-L-rhamnopyranoside from the Leaves of *Mimosa rubicaulis*

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A novel flavone glycoside was isolated from the leaves of *Mimosa rubicaulis*. Its structure was determined as 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone-3′-O-α-L-rhamnopyranoside by various chemical degradations and spectral analysis.

INTRODUCTION

The *Mimosa rubicaulis*[1–3] (N.O. Leguminosae) commonly known as shiahkanta in Hindi is distributed throughout India. The plant was locally collected from Sagar hills. The Ayurevedic system of medicine describes that the leaves of this plant are used in the treatment of piles. The powdered form of its roots is useful in vomiting. The bruised leaves are applied to burns.

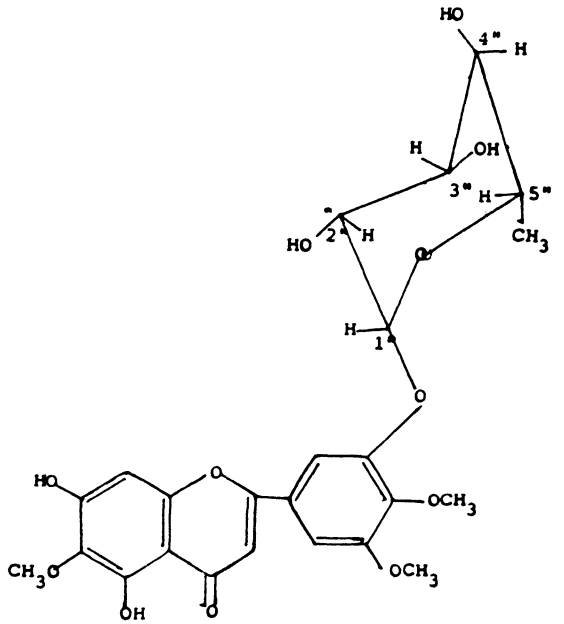
In the present paper, we report the isolation and characterization of a novel flavoneglycoside: 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone-3′-O-α-L-rhamnopyranoside from this plant.

RESULTS AND DISCUSSION

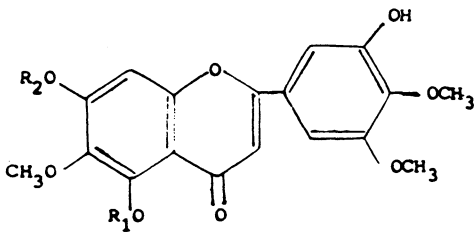
The EtOAc solubles of EtOH extract from the leaves of *Mimosa rubicaulis* yielded a novel compound (I,) m.f. $C_{24}H_{26}O_{12}$, m.p. 265°C, [M^+] m/z 506 which gave a positive response to Molisch test and Shinoda test[4] confirming it to be a flavonoid glycoside. The UV spectrum of compound I showed strong absorption band at 348 nm and 270 nm with diagnostics shift[5,6] reagent suggesting the presence of free hydroxyl group at C-7 (1 NaOAc band II + 15 nm) and C-5 (1 $AlCl_3$ band I + 22 nm) position and blocked hydroxyls at C-4′ and C-6 position. Its IR spectrum showed strong absorption peak at 3450 ν(OH), 2980 ν(C—H), 2870 ν(OMe), 1650 ν(α-β-unsaturated C=O) and 1125–1025 cm^{-1} (O-gly). Acid hydrolysis of compound I yielded an aglycone II, $C_{18}H_{16}O_8$ which was identified as 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone, by comparison of its m.p., UV, IR, ^1H NMR and mass data with literature values.[7,8]

The compound I with Ac_2O/pyridine gave a penta-acetate derivative III, [M^+] m/z 716, m.p. 194°C. ^1H NMR of III indicated the presence of three methoxy groups which appeared as three singlets, each of three proton intensity at δ 3.84, δ 3.72, δ 3.90. A singlet of two proton intensity at δ 6.90 was assigned to H-2′

and H-6′ protons of ring B. Signals of protons at H-3 and H-8 appeared as two singlets each of one proton intensity at δ 6.24 and δ 6.51 respectively. A signal for an anomeric proton was observed at δ 4.63 (1H, br, S, H-1″) and a complex signal at δ 1.28 was due to the rhamnosyl methyl. Two singlets each of three proton intensity at δ 2.42 and δ 2.34 were assigned to phenolic acetoxyls at C-5 and C-7 respectively. Sugar acetoxyls appeared as a multiplet of nine hydrogen intensity in the range of δ 1.85–δ 2.05. A multiplet of five proton intensity in the range of δ 4.40–δ 5.55 were assigned to the remaining sugar protons.



Flavone Glycoside, 3′,5,7-trihydroxy-6,4′,5′-trimethoxy flavone-3′-O-α-L-rhamno pyranoside.



II. $R_1 = R_2 = H$
IV $R_1 = R_2 = CH_3$

The mass spectral analysis of III was in full agreement with the proposed structure. RDA fragment at m/z 332(A_1^+) showed the presence of two OH groups and one OMe group on ring A, while a fragment at m/z 178 (B_1^+) indicated the

presence of two OMe and one OH groups on ring B of aglycone. ^{13}C NMR spectra were in accord with the proposed structure revealing the presence of 24 C atoms which were correlated with a compound having similar oxygenation. The structure of aglycone was confirmed by alkaline degradation which yielded two products, (IIa) and (IIb). These were identified as methoxyphloroglucinol, m.f. $C_7H_8O_4$ [M^+] 156, m.p. 186°C.[9] Found: C: 53.84; H, 5.12; calculated: C, 53.89, H, 5.16 and 3-hydroxy-4,5-dimethoxybenzoic acid, m.f. $C_9H_{10}O_5$ [M^+] 198, m.p. 197–198°C.[10] Found: C, 54.54; H, 5.05; calculated: C, 54.57; H, 5.08%.

Permethylation of glycoside I (Me_2CO/K_2CrO_3) followed by acid hydrolysis with 10% HCl afforded compound IV, 3′-hydroxy-5,6,7,4′,5′-pentamethoxy-flavone, m.f. $C_{20}H_{20}O_8$, m.p. 158°C. The aglycone IV showed a bathochromic shift of 45 nm in band I with decreasing intensity on addition of NaOMe which showed a free hydroxyl group attached at C-3′ position which was involved in glycosidation. The methylated sugar 2,3,4-tri-O-methylrhamnose was identified according to Petek.[11]

Quantitative estimation of sugar with Somogyis's[12] showed the presence of one sugar unit per mole aglycone. Enzymatic hydrolysis of I by Takadiastase liberated L-rhamnose confirming α-linkage between aglycone and L-rhamnose.

EXPERIMENTAL

Plant Material: The plant material was collected locally around Sagar region and was identified by Department of Botany, Dr. H.S. Gour University, Sagar, M.P., India.

General: UV were run in MeOH and IR spectra were measured in KBr disc, ^1H NMR spectra at 300 MHz using TMS as int. standard and $CDCl_3$ as solvent and ^{13}C NMR spectra (300 MHz) using DMSO-d_6 as solvent. M.p.s. were determined in capillaries and are uncorrected.

Extraction and Identification: Air dried and powdered leaves of *Mimosa rubicaulis* were extracted with 95% aqueous MeOH and concentrated under reduced pressure. The concentrated MeOH extract was successively partitioned with n-hexane, chloroform and ethyl acetate. The concentrated EtOAc soluble part was chromatographed over silica-gel column using solvent with increasing polarity. The fraction collected from chloroform : methanol (9:1) gave compound I, crystallised from ether as light yellow needles, $C_{24}H_{26}O_{12}$, [M^+] 506, m.p. 265°C which gave a single spot on TLC (C_6H_6 : AcOH : H_2O, 40:20:1), over silica gel G, [M^+] 506, found: C, 56.90; H, 5.17%; IR ν_{max}^{KBr} (cm^{-1}) 3450 ν(OH), 2980 ν(C—H), 2870 ν(OMe), 1650 ν(α-β unsaturated C=O), 1600, 1125–1025 (-O-gly), 1200, 870, UV λ_{max}^{nm} (MeOH) 264, (+NaOMe) 374, 303, 273; ($AlCl_3$) 352, 295 sh, 284, ($AlCl_3$-HCl) 354, 287, (NaOAc) 366, 279 sh, 270, (NaOAc-H_3BO_3) 337, 271. ^1H NMR of 1H, (300 MHz; $CDCl_3$), δ 6.24 (1H, S, H-3), δ 6.51 (1H, S, H-8), δ 6.92 (2H, S, H-2′,6′), δ 3.84 (3H, S, OMe) δ 3.72 (3H, S, OMe), δ 3.90 (3H, S, OMe), δ 2.42 (3H, S, O-Ac-5), δ 2.34 (3H, S, -O-Ac-7), δ 1.85–δ 2.05 (9H, m, sugar acetoxyl), δ 4.40–δ 5.55 (5H, m, remaining sugar H's), δ 1.28 (complex signal rhamnosyl methyl), δ 4.63 (1H, br, S, H-1″). ^{13}C NMR (300 MHz DMSO-d_6), 163.62 (C-2), 102.48 (C-3), 181.80

(C-4), 152.56 (C-5), 131.34 (C-6), 152.48 (C-7), 94.20 (C-8), 156.94 (C-9), 104.14 (C-10), 120.30 (C-1′), 105.15 (C-2′), 148.24 (C-3′), 139.54 (C-4′), 148.18 (C-5′), 104.14 (C-6′), 101.8 (C-1″), 70.3 (C-2″), 70.8 (C-3″), 71.4 (C-4″), 70.2 (C-5″), 17.4 (Rhm-Me). EIMS m/z 716 [M^+](absent), 360[M^+-acetylated sugar-2Ac] (100%), 359(10), 345(68), 342(57), 331(7), 317(39), 167(12), 139(16), 111(4), 178(3), 181(3).

Acid-Hydrolysis of Compound I: The glycoside I was refluxed with 10% HCl (5 mL) for 2 h at 100°C. The mixture was extracted with EtOAc. The EtOAc fraction containing aglycone was crystallised from $CHCl_3$:MeOH (8:2) as yellow needles II m.p. 243–244°C, [M^+] 360, m.f. $C_{18}H_{16}O_8$. Found: C, 60.01; H, 4.4; calculated: C, 60.06; H, 4.43%. The aglycone was identified as 5,7,3′-trihydroxy-6,4′,5-trimethoxyflavone by comparison of its spectral data with known reported sample.

The aqueous hydrolysate after neutralisation with Na_2CO_3 was subjected to Co-Pc using butanol : acetic acid : water (4:1:5) and authentic sugar was used as checks. The R_f value of unknown sugar was (R_f 0.36) which was same to the R_f value of known sample rhamnose.

Enzymatic hydrolysis of compound I: A 10 mg sample of compound I was treated with Takadiastase and kept in a round-bottom flask (100 mL) at 25°C for 30 h. After addition of water it was extracted with *n*-butanol and was chromatographed over silica-gel column to give L-rhamnose.

Alkaline degradation of the aglycone II: 150 mg of the aglycone was refluxed with 50% KOH (20 mL) in EtOH (10 mL) for 24 h in a 250 mL round- bottom flask. The reaction mixture was cooled and acidified with HCl (7%) and extracted with EtOAc. The EtOAc fraction was washed with H_2O, dried (Na_2SO_4), distilled in *vaccuo* to give IIa, which was identified as methoxyphloroglucinol (IIa), m.f. $C_7H_8O_4$, m.p. 186°C, [M^+] 156; found: C, 53.84; H, 5.12%. The aqueous phase was acidified with HCl, extracted with EtOAc, washed with H_2O, dried (Na_2SO_4), and cooled in *vacuo* to give 3-hydroxy-4,5-dimethoxybenzoic acid (IIb), m.f. $C_9H_{10}O_5$, m.p. 197–198°C, [M^+] 198, (by Co-Pc, Co-TLC); found: C, 54.54; H, 5.05%.

Permethylation of Compound I: CH_3I (1 mL) and Ag_2O (30 mg) were added to a solution of I (25 mg) in DMF (5 mL). The mixture was stirred in dark at room temperature for 48 h. The contents were filtered and the residue was treated with ethanol (2.5 mL). The syrupy residue was heated with 10% HCl on steam bath for 2 h. After cooling, the reaction mixture was extracted with $CHCl_3$ to give the aglycone IV. The aglycone IV showed a bathochromic shift of 45 nm in band I with decreasing intensity on addition of NaOMe which showed a free hydroxyl group attached at C-3′ position which was involved in glycosidation. The methylated sugar was identified according to Petek.

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