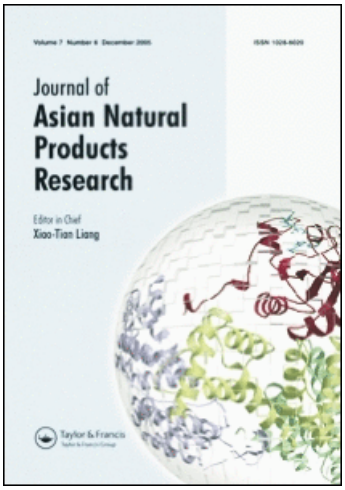

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A New Flavone Glycoside: 5,7,4'-Trihydroxy-6,3'-Dimethoxy Flavone 5-O-α-L-Rhamnopyranoside from the Leaves of *Tridax procumbens* Linn

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A NEW FLAVONE GLYCOSIDE: 5,7,4′-TRIHYDROXY-6,3′-DIMETHOXY FLAVONE 5-*O*-α-L-RHAMNOPYRANOSIDE FROM THE LEAVES OF *TRIDAX PROCUMBENS* LINN.

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Tridax procumbens Linn. (N.O. Compositae) is commonly known as Tikki Kasa in Hindi. It is distributed throughout in India up to 2400 m above sea level and in all hot countries. The present paper deals with the isolation and identification of a new flavone glycoside, 5,7,4′-trihydroxy-6,3′-dimethoxy-flavone 5-*O*-α-L-rhamnopyranoside 1, from the leaves of this plant.

Keywords: *Tridax procumbens*; Compositae; Flavone glycoside; 5,7,4′-trihydroxy-6,3′-dimethoxy-flavone 5-*O*-α-L-rhamnopyranoside

INTRODUCTION

Tridax procumbens [1,2] Linn. (*Compositae*) is commonly known as "Tikki Kasa" in Hindi. It is distributed throughout India up to 2400 m above sea level and in all hot countries. The leaves are cooked as a vegetable and they are also eaten by cattle. The leaves are reported to be employed in catarrh, dysentery and diarrhoea. The leaf juice possesses antiseptic activity and it is also used to treat cuts, burns and wounds.

Earlier workers [3,4] have reported the presence of dexamedthasone and benzyladenine.

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RESULTS AND DISCUSSION

The $CHCl_3$ soluble fraction of the ethanolic extract from the leaves of *T. procumbens* yielded a new compound **1** (Fig. 1), $C_{23}H_{24}O_{11}$, mp 274°C, M^+ 476. It gave a positive response to Molish test and Shinoda test [5] confirming **1** to be a flavonoid glycoside. A bathochromic shift of 68 nm in band **I** with NaOAc and a bathochromic shift of 22 nm in band **I** with NaOMe suggested a free hydroxyl at C-7 and C-4′ positions. Absence of any characteristic shift with $AlCl_3$ indicated a blocked hydroxyl at C-5. The IR absorption bands were at 3450 (OH), 2980 (C–H), 2870 (OMe), 1650 (α,β-unsaturated C=O) and 1125–1025 (O-gly) cm^{-1}. Acid hydrolysis of compound **1** yielded an aglycone $C_{17}H_{14}O_7$, mp 218–219°C, $[M^+]m/z$ 330 and rhamnose as sugar moiety. The aglycone 2 was identified as 6,3′-dimethoxy-5,7,4′,-trimethoxyflavone, by comparison of its mp, UV, IR, and ^1H NMR and MS data with literature values [6].

The compound **1** with Ac_2O/Pyridine gave a penta acetate derivative 3, $C_{33}H_{34}O_{16}$ mp 196–197°C. ^1H NMR of 3 indicated the presence of two methoxy groups which appeared as two singlets each of three proton intensity at δ 3.84, and δ 3.92. Ring B protons showed ABX coupling pattern, fixing 3′,4′ dioxygenation which consisted of a meta coupled doublet at δ 7.32 (1H, $J = 2.5$ Hz) for H-2′ proton, an orthocoupled doublet at δ 6.95 (1H, $J = 8.5$ Hz), for H-5′ proton and a double doublet at δ 7.46 which consisted of both of the ortho ($J = 8.5$ Hz) and meta ($J = 2.5$ Hz) coupling. Signals of proton at H-3 and H-8 appeared as two singlets each of one proton intensity at δ 6.62 and δ 7.32, respectively. Signal for an anomeric proton of

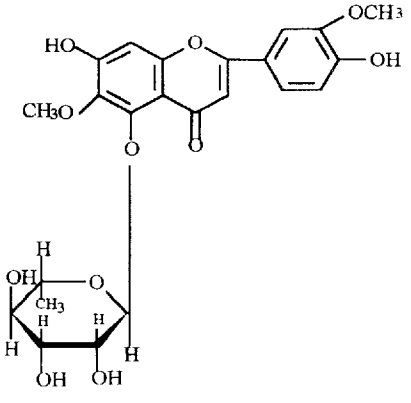

FIGURE 1 5,7,4′-trihydroxy-6,3′-dimethoxy flavone 5-O-α-L-rhamnopyranoside (**1**)

rhamnose was observed at δ 4.65 (1H, br, s, H-1′) and a doublet signal at δ 1.26 was due to the rhamnosyl methyl. Two sharp singlets each of three proton intensity at δ 2.32 and δ 2.44 were assigned to phenolic acetoxyls at C-7 and C-4′, respectively. The remaining sugar protons appeared as a multiplet of five hydrogen intensity in the range of δ 4.38–5.24. A multiplet of nine proton intensity in the range of δ 1.88–2.00 were assigned to remaining sugar acetoxyls.

The MS data of **3** was in full agreement with the proposed structure, molecular ion peak as expected was not observed. MS showed the base peak at m/z 315 (M^+-Me), characteristic of 6-methoxy flavone. The relative intensities of M^+, M^+-15 and M^+-18 were characteristics of 5,7-dihydroxy-6-methoxyflavone [7]. RDA fragment at m/z 167 showed the presence of two hydroxy and one methoxy group on ring A, while a fragment at m/z 148 indicated the presence of a methoxy and a hydroxy group on ring **B** of aglycone. ^{13}C NMR spectrum was in accord with the proposed structure revealing the presence of 23 carbon atoms which were correlated with compound having similar oxygenation [8,9]. The structure of aglycone was confirmed by alkaline degradation which yielded two products **2a** and **b**. These were identified as methoxyphloroglucinol [10], $C_7H_8O_4$ [M^+] 156, mp 186°C. Anal.: C, 53.73; H, 5.09; cald.: C, 53.84; H, 5.16 and 3-methoxy 4 hydroxy benzoic acid [11], $C_8H_8O_7$, mp 210–212°C, M^+ 216 (Anal.: C, 44.31; H, 3.68; calcd.: C, 44.45; H, 3.73).

Permethylation of glycoside **1**, (MeI/Ag_2O/DMF) followed by acid hydrolysis with 10% HCl afforded compound **4**, $C_{19}H_{18}O_7$, mp 252°C. The permethylated aglycone **4** showed a bathochromic shift of 62 nm in band **1** with $AlCl_3$ (relative to MeOH) suggesting a free-OH at C-5 originally involved in glycosidation. The aglycone was identified as 6,7,3′,4′ tetramethoxy-5-hydroxy flavone by study of its ^1H NMR, UV and IR spectral data (see Experimental). The methylated sugar 2,3,4-tri-*O*-methylrhamnose was identified according to Petek [12].

Quantitative estimation of sugar according to Somogyis [13] showed the presence of one sugar unit per mole aglycone.

Enzymatic hydrolysis of **1** by Takadiastase liberated L-rhamnose confirming α-linkage between aglycone and L-rhamnose.

EXPERIMENTAL SECTION

General experimental procedure UV spectra were taken on Hitachi 320, IR spectra were run on a Perkin Elmer 1800 (FTIR) spectrometer, mass spectra

were recorded on a Jeol-D-300 spectrometer. ^{1}H NMR ($CDCl_3$) and ^{13}C NMR (DMSO-d_6) spectra were taken on a Bruker DRX-300 using TMS as international standard. Mps were determined in capillaries and are uncorrected.

Plant material The leaves of *T. procumbens* were collected locally from Sagar region in June–July 1997 and was identified by Taxonomist, Department of Botany of this University. A voucher specimen (No. X-III) has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar, M.P., India.

Extraction and identification Air dried and powdered leaves of *T. procumbens* were extracted with 95% aqueous ethanol and concentrated under reduced pressure. The concentrated ethanol extract was successively extracted with petroleum ether, benzene, acetone, chloroform and ethyl acetate. The concentrated $CHCl_3$ soluble part was chromatographed on silica-gel column using solvents with increasing polarity. The fraction collected from $CHCl_3$: MeOH (9 : 1) gave compound **1**, crystallised from Et_2O as light yellow needles, $C_{23}H_{24}O_{11}$, mp 274°C, M^+ 476 (Anal.: C, 58.03; H, 5.09; calcd.: C, 57.98; H, 5.07), R_f 0.97 (n-BuOH–HOAc–H_2O, 4 : 1 : 5) which gave a single spot on TLC (C_6H_6 : AcOH : H_2O, 40 : 20 : 1) on silica gel G, IR (KBr) ν_{max} 3450 (OH), 2980 (C–H), 2870 (OMe), 1650 (α-β unsaturated C=O), 1605, 1508–1025 (O-gly), 1200, 870 cm^{-1}. UV (MeOH) λ_{max} 272, 330 nm; (+NaOMe) 274, 352 nm; ($AlCl_3$) 272, 332 nm; ($AlCl_3$–HCl) 271, 332 nm; (NaOAc) 270, 398 nm; (NaOAc–H_3BO_3) 271, 335 nm. ^{1}H NMR of **3** (300 MHz; $CDCl_3$), δ 6.62 (1H, s, H-3), 7.32 (1H, s, H-8), 7.32 (1H, d, J = 2.5 Hz, H-2′), 6.95 (1H, d, J = 8.5 Hz, H-5′), 7.46 (1H, dd, J = 2.5, 8.5 Hz, H-6′), 3.84 (3H, s, OMe), 3.92 (3H, s, OMe), 2.32 (3H, s, OAc-7), 2.44 (3H, s, OAc-4′), 1.88–2.00 (9H, m, sugar acetoxyl), 4.38–5.24 (5H, m, remaining sugar H's), 1.26 (doublet rhamnosyl methyl) and 4.65(1H, br, s, H-1″). ^{13}C NMR (75 MHz DMSO-d_6): δ 163.54 (C-2), 102.60 (C-3), 182.06 (C-4), 152.82 (C-5), 131.34 (C-6), 152.34 (C-7), 94.60 (C-8), 157.32 (C-9), 103.88 (C-10), 120.1 (C-1′), 145.8 (C-3′), 149.4 (C-4′), 116.2 (C-5′), 118.6 (C-6″), 101.6 (C-1″), 70.4 (C-2″), 70.5 (C-3″), 71.6 (C-4″), 70.2 (C-5″), 70.5 (C-6″), 56.22 (OMe) and 59.42 (OMe). EIMS m/z 476 [M^+] (absent), 330[M^+-acetylated mono saccharide sugar-2Ac](30.2), 329(14), 315(100), 312(60), 287(40), 167(8.5), 151(6.1) and 148(7.5).

Acid hydrolysis of compound **1**. The glycoside **1** was refluxed with 10% HCl (5 ml) for 2 h at 100°. The mixture was extracted with EtOAc. The EtOAc fraction yielded an aglycone which was crystallised from $CHCl_3$: MeOH (7 : 3) as light yellow needles **2**. $C_{17}H_{14}O_7$, mp 218–219°C, [M^+] 330, (Anal. C, 61.79; H, 4.24 calcd. C, 61.81; H, 4.27). The aglycone was identified as

5,7,4′-trihydroxy-6,3′-dimethoxyflavone by comparison of its spectral data with known reported sample. The aqueous hydrolysate after neutralisation with Na_2CO_3 was subjected to Co-PC. The sugar identified was rhamnose, R_f 0.36 (n-BuOH–HOAc–H_2O, 4 : 1 : 5).

Enzymatic hydrolysis of compound **1** A 10 mg of sample of compound **1** was treated with Takadiastase and kept in a round bottomed flask (100 ml) at 25°C for 30 h. After addition of water it was extracted with n-butanol and was chromatographed on silica-gel column to gave L-Rhamnose.

Alkaline degradation of the aglycone **2** Alkaline degradation was done by refluxing the aglycone (120 mg) for 24 h with 50% KOH (20 ml) in EtOH (10 ml). The reaction mixture was cooled and neutralised with HCl (7%). The EtOAc fraction yielded methoxyphloroglucinol (**2a**) $C_7H_8O_4$, mp 186°C, [M^+] 156 (Anal.: C, 53.73; H, 5.09; Calcd.: C, 53.84; H, 5.16). The aqueous phase yielded 3-methoxy, 4-hydroxy benzoic acid, $C_8H_8O_4$, mp 211–212°C, M^+216, (Anal.: C, 44.31; H, 3.68; Calcd.: C, 44.45; H, 3.73).

Permethylation of compound **1** CH_3I (1 ml) and Ag_2O (30 mg) were added to a solution of **1** (25 mg) in DMF (5 ml). The mixture was stirred in dark at room temperature for 48h. The contents were filtered and the residue was treated with ethanol (2.5 ml). The syrupy residue was heated with 10% HCl on steambath for 2h. After cooling, the reaction mixture was extracted with $CHCl_3$ to give aglycone **4**, $C_{19}H_{18}O_7$ (15 mg), mp 252°C, M^+ m/z 358. UV (MeOH) λ_{max} 274, 328 nm; (+NaOMe) 273, 330, 338; (+NaOAc) 274, 326, 330; (+$AlCl_3$) 275, 390; (+$AlCl_3$+HCl) 226, 332. IR (KBr) ν_{max} 3442, 2872 (OMe), 1645, 1600, 1105, 1040, 924 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$). 6.60 (1H, s, H-3), 7.30 (1H, s, H-8), 7.36 (d, $J = 2$ Hz, H-2′), 7.18 (d, $J = 9$ Hz, H-5′), 7.45 (d, $J = 9.5$ Hz, H-6′), 3.88 (3H, s, OMe), 3.92 (3H, s, OMe), 3.81 (3H, s, OMe), 3.78 (3H, s, OMe).

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