A Novel Flavone Glycoside-6,4′-Dihydroxy-3′-Prenyl-3,5,7,5′-Tetramethoxy Flavone-6-O-α-L-rhamnopyranoside from the Seeds of *Bauhinia purpurea*

R.N. YADAVA* and SHEETAL SODHI
Natural Products Laboratory, Department of Chemistry
Dr. H. S. Gour University, Sagar-470 003, India

Bauhinia purpurea is commonly known as 'Kaniar' in Hindi. It is found in sub-Himalayan tracts up to 4000 ft above sea level. A novel bio-active flavone glycoside, m.f. $C_{30}H_{36}O_{12}$, m.p. 272–273°C, M^+ 588 [EIMS] was isolated from the acetone souble fraction of ethanolic extract from the seeds of *B. purpurea* and its structure was established as 6,4′-dihydroxy-3′-prenyl-3,5,7,5′-tetramethoxy flavone-6-O-α-L-rhamnopyranoside by various chemical degradations, chromatographic and spectroscopic methods.

INTRODUCTION

Bauhinia purpurea[1, 2] Linn. (Leguminosae) is commonly known as 'Kaniar' in Hindi. It is found in sub-Himalayan tracts up to 4000 ft. above sea level. The roots are carminative and flowers are laxative. The bark is used as an astringent in diarrhoea and the ethanolic extract of its leaves shows anti-diarrhoeal property[3]. Earlier workers[4–6] have reported the presence of chalcone glycoside from the seeds and various amino acids from the seeds and leaves of this plant.

EXPERIMENTAL

Plant Material: The seeds of *Bauhinia purpurea* were collected from the Dhamoni forest of Sagar (M.P.), India in the month of Feb-March 1999 and

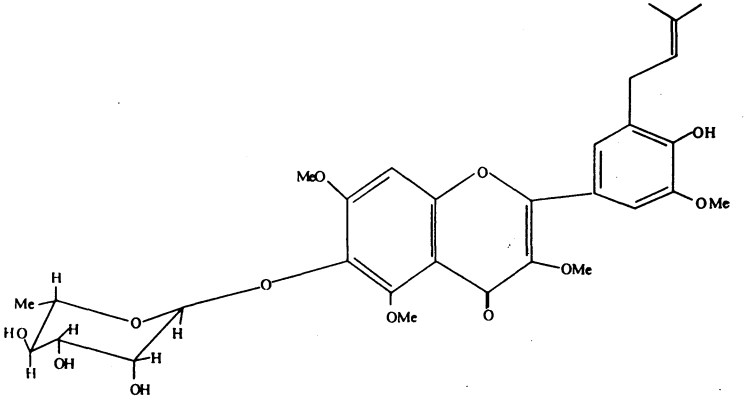

Fig. 1. A novel flavone glycoside: 6,4′-dihydroxy-3′-prenyl-3,5,7,5′-tetramethoxy flavone-6-O-α-L-rhamnopyranoside

identified by the Taxonomist, Department of Botany of this University. A voucher specimen (No. XV) has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr H. S. Gour University, Sagar (M.P.), India.

General Experimental Procedure: UV spectra were obtained on Hitachi 320 spectrometer (run in MeOH); IR spectra were recorded on KBr pellets using a Perkin-Elmer-577 spectrophotometer; mass spectra were recorded on a Jeol-D-300 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-300 using $CDCl_3$ and DMSO-d_6 as solvents and TMS as an internal standard. Melting points were determined in capillaries and are uncorrected.

Extraction and Isolation: Air dried and powdered seeds (2.5 kg) of *B. purpurea* were extracted with 75% aqueous EtOH and concentrated under reduced pressure. The concentrated ethanolic extract was partitioned successively with petroleum ether (60–80°C), Ac_2O, $CHCl_3$, EtOAc and MeOH. The acetone-soluble part was chromatographed on Si-gel G column using $CHCl_3$-MeOH mixture in different proportions. Fractions 12–32, on evaporation of solvent, gave an amorphous compound **1** (280 mg), which was found to be homogeneous on TLC examination.

Study of Compound 1: Crystallized from MeOH as light yellow needles, $C_{30}H_{36}O_{12}$, m.p. 272–273°C, M^+ at m/z 588 (found: C, 61.39; H, 61.36; calcd.: C, 61.22; H, 61.22%). IR (KBr): ν_{max} 3540 ν(OH), 2865 ν(OMe), 2882 (C—H stret.), 1658 ν(α-β unsaturated C=O), 1535 ν(Ar-ring), I510–1025ν (O-gly), 1205 (C—O—C bending), 1130 (C—O—C stret.), 1320 (*gem*-dimethyl groups), 870 (two adjacent H), 822 cm^{-1}. UV (MeOH) λ_{max} 262, 354; (+$AlCl_3$) 264, 354; ($AlCl_3$/HCl) 264, 352; (+NaOMe) 262, 402; (+NaOAc) 263, 352; (+NaOAc-H_3BO_3) 265, 352 nm. ^{13}C NMR (300 MHz, DMSO-d_6): δ 163.50 (C-2), 134.66 (C-3), 162.20 (C-4), 152.64 (C-5), 131.50 (C-6), 160.56 (C-7), 94.63 (C-8), 157.62 (C-9), 106.92 (C-10), 120.7 (C-1′), 127.64 (C-2′), 116.62 (C-3′) 115.56 (C-5′), 120.41 (C-6′), 101.7 (C-1″), 70.3 (C-2″), 70.4 (C-3″), 71.5 (C-4″), 70.1 (C-5″), 70.6 (C-6″), 23.4 (C-1‴), 123.67 (C-2‴), 131.42 (C-3‴), 26.8 (C-4‴), 19.88 (C-5‴), 101.94 (C-6‴), 59.64 (OMe). EIMS m/z (rel int.): [M^+] absent, 442 [M^+-sugar moiety] (15), 427 [M^+-Me] (100), 414 [M^+-CO] (65), 399 [M^+-COMe] (12), 387 [M^+-C_4H_7] (50), 386 [M^+-C_4H_6] (12), 216 (10), 196 (8), 168 (6).

Acetylation of Compound 1: Compound **1** with Ac_2O/pyridine gave a tetra-acetate derivative as light brown amorphous powder **1a**, m.p. 285–268°C, m.f. $C_{38}H_{44}O_{16}$ (found: C, 60.41; H, 5.87; calcd.: C, 60.32; H, 5.82%). ^{1}H-NMR (300 MHz,$CDCl_3$): δ 6.88 (1H, s, H-8), 7.76 (1H, d, J = 2.6 Hz, H-2′), 7.52 (1H, d, J = 2.6 Hz, H-6′), 1.35 (6H, br s, —C $(Me)_2$), 5.18 (1H, br t, J= 7.1 Hz, =C_2''H), 3.68 (1H, m, —C_1''' H_a), 3.71 (1H, m, —C_1''' H_b), 3.933 (3H, s, OMe-3), 3.99 (1H, s, OMe-5), 3.88 (lH, s, OMe-7), 4.02 (1H, s, OMe-5′), 2.33 (3H, s, 4′-OAc), 1.89–2.10 (9H, m, sugar acetoxyl), 4.39–5.25 (5H, m, remaining sugar H′s), 1.27 (3H, d, Me-rhamnose), 4.64 (1H, br s, H″ anomeric proton).

Acid hydrolysis of Compound 1: Compound **1** (30 mg) was refluxed with 10% HCl solution (5 mL) for 3 h at 100°C. The reaction mixture was cooled and extracted with ether to yield a brown amorphous compound **2**, identified as

6,4′-dihydroxy-3′-prenyl-3,5,7,5′-tetramethoxy flavone by various spectral data. The aqueous hydrolysate after neutralization with $BaCO_3$ was subjected to Co-PC using n-BuOH-OHAc-H_2O (4 : 1 : 5) and the sugar was identified as L-rhamnose (R_f 0.36).

Study of Compound 2: Crystallized from ether as light brown amorphous powder, m.p. 252–253°C, m.f. $C_{24}H_{26}O_8$ (found: C, 65.26; H, 5.89; calcd.: C, 65.16; H, 5.88%), M^+ at m/z 442. IR (KBr) ν_{max}: 3450, 2880, 2860, 1650, 1530, 1320, 871, 820 cm^{-1}. UV (MeOH) λ_{max}: 256, 348; (+$AlCl_3$) 264, 355; (+$AlCl_3$/HCl) 284, 372; (+NaOMe) 286, 346; (+NaOAc) 352, 346; (+NaOAc – H_3BO_3) 262, 350 nm.

Cyclization of the aglycone-2 followed by alkaline oxidation: Aglycone-2 (40 mg) was heated with 95% formic acid (1 mL) in presence of H_2SO_4 at 110°C for 1 h in a 100 mL round-bottomed flask. The reaction mixture was then diluted with ice-cold water, filtered and crystallized with acetone to give a crystalline chroman derivative **2a** (60 mg). The chroman derivative **2a** (30 mg) was dissolved in MeOH (8 mL) and 7% KOH (5 mL) in a 150 mL conical flask and 12% H_2O_2 was added to it with constant stirring. The contents were left for 5 h, extracted with ether and acidified with dil. H_2SO_4. The ethereal solution was dried to yield colourless compound **2b**, m.f. $C_{13}H_{16}O_4$, m.p. 179–180°C, M^+ at m/z 236 and was identified as 8-methoxy-2,2-dimethyl-chroman-6-carboxylic acid by mmp and superimposable spectral analysis.

Permethylation of Compound 1 followed by acid hydrolysis: Compound **1** (20 mg) was dissolved in DMF (5 mL) in a 100 mL conical flask and then treated with CH_3I (2 mL) and Ag_2O (25 mg). The total mixture was left at room temperature for two days. The contents were filtered and the residue was treated with ethanol (3 mL). The syrupy mass was hydrolysed with 10% HCl (6 mL) and heated on a steam bath for 2 h. The reaction mixture was extracted with $CHCl_3$, after cooling, to yield permethylated aglycone, 6-hydroxy-3,5,7,4′,5′-pentamethoxy-3′-prenyl flavone **3**, m.p. 233–234°C, m.f. $C_{25}H_{28}O_8$, M^+ at m/z 456. UV (MeOH) λ_{max}: 264, 360; (+NaOMe) 266, 403; (+NaOAc) 268, 354; (+$AlCl_3$) 268, 321; (+$AlCl_3$/HCl) 280, 375 nm. IR (KBr) ν_{max}: 3440, 2872 (OMe), I645, 1600, 1105, 872, 823. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.84 (1H, s, H-8), 1.77 (1H, d, J = 2.7 Hz, H-2′), 7.54 (1H, d, J = 2.7 Hz, H-3′), 3.94 (3H, s, OMe-3), 3.98 (3H, s, OMe-5), 3.89 (3H, s, OMe-7), 4.01 (3H, s, OMe-4′), 4.03 (3H, s, OMe-5′), 9.16 (2H, s, OH, exchangeable with D_2O), 3.59 (2H, d, J = 7.2 Hz, H-I″), 5.67 (1H, t, J = 7.2 Hz, H-2″), 1.70 (3H, s, Me-5″), 1.77 (3H, s, Me-4″); ^{13}C-NMR (DMSO-d_6) 163.52 (C-2), 134.67 (C-3), 162.22 (C-4), 152.65 (C-5), 131.52 (C-6), 160.58 (C-7), 94.64 (C-8), 157.63 (C-9), 106.94 (C-10), 120.78 (C-1′), 127.66 (C-2′), 116.66 (C-3′), 115.58 (C-5′), 120.43 (C-6′), 23.5 (C-1‴), 123.68 (C-2‴), 131.44 (C-3‴), 26.9 (C-4‴), 19.89 (C-5‴), 101.95 (C-6‴).

Enzymatic hydrolysis of Compound 1: Compound **1** (8 mg) was treated with enzyme takadiastase and kept in a 50 mL round-bottomed flask for 30 h at room temperature. It was extracted with n-butanol, after addition of water and then chromatographed on silica-gel G colurnn to give L-rhamnose (R_f 0.36).

RESULTS AND DISCUSSION

The acetone soluble fraction of the ethanolic extract from the seeds of *B. purpurea* yielded a new compound **1**, $C_{30}H_{36}O_{12}$, m.p. 272–273°C, M^+ 588. It gave positive response to Molisch[7] and Shinoda[8] tests, confirming **1** to be a flavonoidal glycoside. A bathochromic shift of 48 nm in band I with NaOMe (relative to MeOH) sugesested the presence of a free hydroxyl group at C-4′ position. No remarkable shift in band II on addition of NaOAc (relative to MeOH) and in band I on addition of $AlCl_3$ (relative to MeOH) indicated the presence of methoxyl groups at C-7 and C-5 positions, respectively. Absence of characteristic shift in band I on addition of $AlCl_3$/HCl and in band II on addition of $AlCl_3$ indicated the presence of a blocked hydroxyl group at C-6 position and presence of a methoxyl group at C-3 position, respectively. The IR absorption bands were at 3450 ν(OH), 2882 (C—H stretching), 2865 ν(OCH_3), 1658 ν(α,β-unsaturated C=O), 1535 ν(Ar-ring), 1510–1025 ν(O-gly), 1205ν (C—O—C bending), 1130 ν(C—O—C stretching), 870 (two adjacent H's), 822 cm^{-1}. Acid hydrolysis of compound **1** yielded an aglycone **2**, m.f. $C_{24}H_{26}O_8$, m.p. 252–253°C, M^+ at m/z 442 and L-rhamnose as sugar moiety. The aglycone **2** was identified as 6,4′-dihydroxy-3′-prenyl-3,5,7,5′-tetramethoxy flavone, hy its m.p., UV, IR, ^{1}H-NMR and MS data studies (see Experimental).

The compound **1** with Ac_2O/pyridine gave a tetraacetate derivative **1a**, m.f. $C_{38}H_{44}O_{16}$, m.p. 285–286°C. ^{1}H-NMR of **1a** showed four singlets at δ 3.93, δ 3.99, δ 3.88 and δ 4.02, each of three proton intensity, indicating the presence of four methoxy groups. A sharp singlet at δ 6.88, of one proton intensity, was assigned to H-8 proton. Two doublets at δ 7.78 (J = 2.8 Hz) and δ 7.53 (J = 2.8 Hz), each of one proton intensity, were assigned for H-2′ and H-6′ protons, respectively. The chemical shifts at δ 1.35 (6H, br s, —C $(Me)_2$), δ 3.68 (1H, m, —$C_1'''H_a$), 3.71 (1H, m, —$C_1'''H_b$), 5.18 (1H, br, t, —$C_2'''H$) were diagnostic for a prenyl unit[9]. A signal at δ 4.64 was obtained for an anomeric proton of L-rhamnose and a doublet at δ 1.27 was due to rhamnosyl methyl. A sharp singlet at δ 2.33, of three proton intensity, was assigned to the phenolic acetoxyl at C-4′ position and a multiplet in the range of δ 1.89–δ 2.10, of nine proton intensity, was assigned to the remaining sugar acetoxyls.

The MS data of **1a** was in full agreement with the proposed structure **1**. Mass fragmentation pattern showed the base peak at m/z 427 [M^+-Me] which is characteristic of 3-methoxy flavone. The fragmentation peaks at 387 and 386 by the loss of M-55 and M-56, indicated the presence of a prenyl unit and also suggested that the prenylation is adjacent to the —OH group[10, 11]. RDA fragment at m/z 196 suggested the presence ot two methoxy and one hydroxy group in ring A, while a fragment at 216 indicated the presence of a methoxy, a prenyl and a hydroxy group in ring B of the aglycone.

The structure of aglycone **2** and position of attachment of prenyl unit was also established by its cyclization with formic acid followed by alkaline oxidation (H_2O_2), which yielded a compound **2b**, m.f. $C_{13}H_{16}O_4$ (found: C, 66.23 ; H, 6.79; calcd.: C, 66.10; H, 6.78%); m.p. 178–179°C, M^+ at m/z 236, which was identified

as 8-methoxy-2,2-dimethyl-chroman-6-carboxylic acid by mmp and superimposable spectral analysis.

Permethylation of compound **1** (MeI/Ag_2O/DMF) followed by acid hydrolysis with 10% HCl afforded compound **3**, m.f. $C_{25}H_{28}O_8$ (found: C, 65.88; H, 6.19; calcd.: C, 65.79; H, 6.14%), m.p. 233–234°C, [M^+] at m/z 456.The permethylated aglycone **3** showed a bathochromic shift of 26 nm in band I with $AlCl_3$/HCl (relative to MeOH), suggesting that a free —OH at C-6 position was originally involved in the glycosidation. The permethylated aglycone **3** was identified as 6-hydroxy-3,5,7,4′,5′-pentamethoxy-3′-prenyl flavone by its ^{1}H-NMR, UV and IR spectral data studies (see Experimental). The methylated sugar was identified as 2,3,4-tri-O-methyl rhamnose, according to Petek[12].

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

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

ACKNOWLEDGEMENTS

The authors are thankful to Prof S.P. Banerjee, Head, Department of Chemistry, Dr. H.S. Gour University, Sagar, India for providing laboratory facilities and Head RSIC, Central Drug Research Institute, Lucknow for various spectral analyses.

REFERENCES

1. R.N. Chopra, S.L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants, CSIR Publication, Hillside Road, New Delhi, p. 35 (1996).
2. The Wealth of India: A Dictionary of Raw Materials and Plant Products, CSIR Publication, Vol. 1, p. 160 (1948).
3. P.K. Mukerjee, T.K. Gopal, T. Subburaju, S.P. Dhanbal, S.P. Duraiswami, K. Elango and B. Suresh, *Natural Product Science*, **4**, 234 (1998).
4. H.P. Bhartiya and P.C. Gupta, *Phytochemistry*, **20**, 2051 (1981).
5. N.F. Hall, N.S. Berry and E.Robert, Proc. Fla. State Horti. Soc., **88**, 486 (1976).
6. R. Pant and P.L. Bishoi, *Curr. Sci. (India)*, **43**, 213 (1974).
7. C.D. Douglass, G.L. Morris and S.H. Wendev, *J. Am. Chem. Soc.*, **703**, 4023 (1951).
8. J. Shinoda, *Pharm. Soc. Japan*, **48**, 214 (1928).
9. I. Jayaraman, A. Ghanimi and H.A. Khan, *Phytochemistry*, **19**, 1267 (1988).
10. E. Ritchie, W.D. Taylor and D.H. Williams, *Tetrahedron Lett.*, 1437 (1964).
11. A.V. Subba Rao, S.S. Rathi and K. Venkataraman, *Indian J. Chem.*, **10**, 989 (1972).
12. F. Petek, *Bull. Soc. Chim. France*, 263 (1965).
13. M. Somogyis, *Bio. Chem.*, **195**, 19 (1952).

(*Received*: 28 September 2000; *Accepted*: 27 December 2000) AJC-2216