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A NEW NARINGENIN GLYCOSIDE FROM *CLEOME VISCOSA*

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Key Word Index—*Cleome viscosa*; Capparaceae; flavanone glycoside; naringenin 4′-(xylosyl-β-(1 → 4)-glucoside).

Cleome viscosa is a medicinal plant [1, 2] used in the indigenous system of medicine. Extraction of the whole plant gave a glycoside, mp 92–95°, with the characteristic reactions of a flavanone. Acid hydrolysis gave 1 mol each of glucose and xylose and 5,7,4′-trihydroxyflavanone (naringenin). The aglycone, $C_{15}H_{12}O_5$, analysed for three OH groups (acetate) and gave positive bathochromic shifts with $AlCl_3$ [3] and NaOAc [4] for free OH groups at positions 5 and 7. The above positions were further confirmed by specific tests [5]. The aglycone, as well as its methyl ether, on $KMnO_4$ oxidation gave *p*-hydroxybenzoic acid and *p*-methoxy benzoic acid, respectively, which indicates another OH group at position 4′. On 50% KOH degradation [6] the aglycone gave phloroglucinol and *p*-hydroxybenzoic acid. Its identity as naringenin was further confirmed by co-chromatography with an authentic sample [7].

Preparation of the decamethyl ether of the glycoside by the Kuhn method [8], followed by its methanolysis [9] and then hydrolysis with aq. HCl gave 2,3,6-tri-*O*-methyl-D-glucose (1 mol), 2,3,4-tri-*O*-methyl-D-xylose (1 mol) and naringenin. The observed consumption of 3.2 mol of periodate with the liberation of 1.2 mol of formic acid, together with methylation studies, indicates that the glucopyranose unit is joined to the xylopyranose unit by a 1 → 4 linkage. The sugar moiety was shown to be linked at 4′-OH by the study of the colour reactions of the glucoside and complete methylation followed by 50% alkali degradation of the glycoside. The results of partial and enzymatic hydrolysis revealed that xylose is the terminal sugar and is linked to the glucose through a β-linkage which in its turn is linked to the aglycone through a β-linkage. Hence the glycoside is thus naringenin 4′-*O*-β-D-xylopyranosyl-(1 → 4)-β-D-glucopyranoside.

EXPERIMENTAL

Sugars were characterized spectrophotometrically by the PhOH–H_2SO_4 method [10, 11] using a Klett–Summerson photoelectric colorimeter and also by Whatman No. 1 filter paper, descending PC using BAW (4:1:5) as solvent and AHP for revealing the spot.

Isolation and purification. The air-dried and powdered whole plant (2 kg) of *C. viscosa* was extracted with EtOH under reflux for 20 days. The extract (2.5 l.) was concd to 100 ml and poured into distilled water (500 ml) with continuous stirring. The water soluble portion, on extraction with methanol, gave the glycoside (yield 1.3 g). It was purified on a column of Si gel, crystallized as yellow needles from EtOAc–petrol, mp 92–95°. The homogeneity of the glycoside was checked by TLC (R_f 0.43, MeOH–$CHCl_3$, 3:7) and PC (R_f 0.59 in BAW). (Found: C, 55.11; H, 5.30. $C_{26}H_{30}O_{14}$ requires: C, 55.12; H, 5.30%). IR (KBr) cm^{-1}: 3335 (OH), 2900 (—C—H str.), 1680 (—C—O str.), 1604, 1536, 1450 (ring str.), 1282 (—C—O—C— vib.), 1120 (—C—O— str. in —C—OH), 825 (pyranose form of the sugar) and 770 (—C—H in —CH_2).

Acid hydrolysis. The glycoside (500 mg) was hydrolysed with boiling 7% ethanolic H_2SO_4 (50 ml) for 6 hr. The sugars produced were separated by CC on cellulose in *n*-BuOH–*i*-PrOH–H_2O (11:6:3) [12] and characterized; glucose; osazone and *p*-nitrophenylhydrazone, mp and mmp 204–205° and 184–86°; xylose; osazone and *p*-nitrophenylhydrazone, mp and mmp 158° and 156°, respectively. Quantitative analysis [13] revealed the presence of 1 mol of each sugar.

Characterization of the aglycone. The aglycone was purified by PC and crystallized from EtOAc–petrol as yellow needles, mp 248°. The homogeneity was checked by TLC and PC in different organic solvents. (Found: C, 66.15; H, 4.40. $C_{15}H_{12}O_5$ requires: C, 66.17; H, 4.41%). M^+ 272. ^1H NMR spectrum (100 MHz, $(CD_3)_2CO$, −60°): δ 2.79 (*d*, J_{gem} = 17 Hz, 3-H (eq)), 3.30 (*q*, J_{gem} = 2 Hz, 3-H (ax)), 7.09 (*s*, 6- and 8-H), 7.5 (3′- and 5′-H), 7.85 (2′- and 6′-H); −7.60 (*s*, 5-OH). The acetyl derivative was crystallized from MeOH, mp 54°. The percentage of acetyl groups was determined by the method of ref. [14] as described by ref. [15]. (Found: C, 63.30; H, 4.52; 3 × acetyl, 32.40. $C_{21}H_{18}O_8$ requires: C, 63.31; H, 4.53; 3 × acetyl, 32.41%). M^+ 398. ^1H NMR signal of acetate δ 2.09.

Methylation of the glycoside. The glycoside (500 mg) was methylated by Kuhn's procedure with MeI–Ag_2O–$HCONMe_2$. Methanolysis [9] followed by hydrolysis with 7% aq. HCl at 100° for 4 hr gave 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-xylose in 1:1 ratio, indicating a 1 → 4 linkage of glucose and xylose.

Periodate oxidation. The glycoside (30 mg) was oxidized by $NaIO_4$ at room temp. in 20 ml 90% EtOH. A blank was run similarly. Results after 60 hr showed the consumption of 3.2 mol of periodate with the liberation of 1.20 mol of formic acid per 1 mol of the glycoside.

Enzymatic hydrolysis. About (100 mg) of the glycoside was suspended in an almond emulsin solution (20 ml) and kept at 45° for 18 hr. The appearance of glucose and xylose [11] in this hydrolysate indicated it to be β-linked in both the sugars as well as sugar with aglycone.

Partial hydrolysis. The glycoside (15 mg) was dissolved in dioxane (5 ml) containing HCl (1%) and allowed to stand at room temp. Aliquots were taken at different intervals and examined by PC. The results indicated that xylose was liberated first after 80 hr while glucose appeared after 130 hr.

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TAXIFOLIN 3,5-DIRHAMNOSIDE FROM THE SEEDS OF *CORDIA OBLIQUA*

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Key Word Index—*Cordia obliqua*; Boraginaceae; taxifolin 3, 5-dirhamnoside; flavanonol glycoside.

Cordia obliqua is a medicinal plant [1, 2], but little work has been reported on the seeds [3] and roots [4–6]. We now report the identification of taxifolin 3,5-dirhamnoside.

The glycoside gave the characteristic reactions of flavanone, while acid hydrolysis gave 2 mol of rhamnose and taxifolin (dihydroquercetin). This aglycone was identified by analysis, spectral procedures, alkaline degradation and finally by co-chromatography with an authentic specimen [7].

Preparation of the nonamethyl ether of the glycoside by the Kuhn method [8] followed by its methanolysis [9] and then hydrolysis with aq. HCl gave 2,3,4-tri-*O*-methyl-L-rhamnose (2 mol) and taxifolin 7,3′,4′-trimethyl ether, which on demethylation with HBr–HOAc gave taxifolin (mp, mmp, co-IR and co-TLC). The observed consumption of 4.01 mol of periodate with the liberation of 1.01 mol of HCOOH together with methylation studies indicated that the L-rhamnose is present as two monosaccharide units in the pyranose form (if it was present as a disaccharide, the glycoside would have consumed *ca* 3 mol of periodate instead of *ca* 4 mol).

The position of the sugars in the glycoside was determined by the comparison of the spectral shifts of the glycoside with those of the aglycone. The glycoside was methylated, followed by acidic hydrolysis to afford an aglycone which displayed signal in the NMR (τ-2.40 (1H at C-5); 6.00 (9H, OMe at C-7, C-3′ and C-4′), −5.5 (2H at C-3)) which was identified as taxifolin 7,3′,4′-trimethyl ether (confirmed by UV, IR and KOH degradation), further confirming the rhamnose unit at C-3 and C-5. The fact that glycoside could not be hydrolysed with almond emulsin indicated the presence of α-linkages. Hence the glycoside is taxifolin 3,5-*O*-α-L-dirhamnopyranoside.

EXPERIMENTAL

Isolation and purification. The air-dried and powdered seeds of *Cordia obliqua* were extracted with $CHCl_3$ under reflux. The extract was filtered and concd. It deposited a yellow amorphous compound. It was purified over a Si gel column and eluted with MeOH–EtOAc. It was crystallized as yellow needles from EtOH, mp 148°(d). The homogeneity of the glycoside was checked by TLC (R_f 0.54 in MeOH–$CHCl_3$, 3:7) and PC (R_f 0.89 in BAW, 4:1:5). (Found: C, 54.35; H, 5.37. $C_{27}H_{32}O_{15}$ requires: C, 54.36; H, 5.37%.) ν_{max}^{KBr} cm^{-1}: 3400, 2900, 1680, 1604, 1536, 1455, 1285, 1125, 822 and 725. λ_{max} nm: 288 (MeOH); 290 (+$AlCl_3$); 323 (+NaOAc).

Acidic hydrolysis. The glycoside (500 mg) was hydrolysed with 50 ml 7% ethanolic H_2SO_4 for 4 hr under reflux. It was poured into distilled H_2O (500 ml). On cooling a solid precipitated which was filtered off. The aq. hydrolysate, after neutralization with $BaCO_3$, was identified as L-rhamnose (co-PC and osazones). The solid was purified over a Si gel column and crystallized as golden needles from aq. EtOH. It was homogeneous by TLC (R_f 0.44 in $CHCl_3$–MeOH, 8:2) and PC (R_f 0.90 in BAW, 4:1:5), mp 237–38°, M^+ 304, $C_{15}H_{12}O_7$. (Found: C, 59.20; H, 3.94. $C_{15}H_{12}O_7$ requires: C, 59.19; H, 3.95%.) ν_{max}^{KBr} cm^{-1}: 3350, 2910, 1685, 1602, 1535, 1450, 1278, 1130, and 1030. λ_{max} nm: 290 (MeOH); 314 (+$AlCl_3$); 325 (+NaOAc). NMR (100 MHz, $(CD_3)_2CO$, −60°): τ 7.20 (3H, d, J_{gem} = 17 Hz), 6.70 (3H, *q*, J = 2 Hz), 2.90 (*s*, 6- and 8-H), 3.21 (5′-H), 2.2 (2′- and 6′-H), −2.40 (*s*, 5-OH), −4.04 (*s*, C_6- or C_8-H), 4.02 (1H, *d* centred at 4.75, C_2-H). The aglycone formed a penta-acetate (Ac_2O–Py), mp 150–51°, M^+ 514. (Found: C, 58.35; H, 4.25; acetate, 41.80. $C_{25}H_{22}O_{12}$ requires: C, 58.36; H, 4.28; 5 × acetate. 41.82%.) NMR at τ 7.90 (acetate group).

$KMnO_4$ *oxidation of the aglycone.* The aglycone (50 mg) was dissolved in 20 ml Me_2CO and 10% aq. $KMnO_4$ soln was added until its colour persisted. The reaction mixture was refluxed on a water bath for 4 hr. It was cooled, $NaHSO_3$ was added to remove excess MnO_2, it was made acidic (conc HCl), and then extracted with Et_2O. The ethereal layer was washed with H_2O to remove mineral acid. It was dried and concd to 1 ml. The