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TWO FLAVONOID GLYCOSIDES FROM THE BARK OF *PROSOPIS JULIFLORA*

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Key Word Index—*Prosopis juliflora*; Leguminoseae; flavonol glycoside; kaempferol 4′-methyl ether 3-*O*-β-D-galactopyranoside; isoflavone glycoside; retusin 7-*O*-neohesperidoside.

Abstract—Two new glycosides, kaempferol 4′-methyl ether 3-*O*-β-D-galactopyranoside and retusin 7-*O*-neohesperidoside, have been characterized from the stem bark of *Prosopis juliflora*.

Plants of the genus *Prosopis* (Leguminoseae) are well known for their medicinal value [1]. The leaf extract of *P. juliflora* has been shown to have antibiotic properties [2] and tannins have been reported from the bark [3].

The stem bark was extracted successively with boiling acetone and ethanol. The ether soluble fraction from the acetone concentrate yielded a flavonol glycoside (**1**), while an isoflavone glycoside was obtained from the ethanol extract (**2**).

1, $C_{22}H_{22}O_{11}$, mp 168° (*d*), gave a positive Molisch test. Acid hydrolysis yielded an aglycone and galactose. UV spectral data and the characteristic colour tests of the aglycone (**1a**) indicated its flavonol nature [4]. The formation of a triacetate and a tetramethyl ether suggested the presence of three free hydroxyls and one methoxyl group in the molecule. Positive colour tests for C-5 and C-7 hydroxyls were observed [5]. Bathochromic shifts in the absorption maxima with $AlCl_3$–HCl and fused NaOAc confirmed the presence of free C-5 and C-7 hydroxyls. $KMnO_4$ oxidation of the aglycone yielded anisic acid, fixing the methoxyl group at the 4′-position and alkaline fission yielded phloroglucinol and anisic acid thus establishing the structure of **1a** as kaempferol 4′-methyl ether (kaempferide).

Spectral shifts and colour reactions indicated that **1** also contained free hydroxyls at C-5 and C-7. The aglycone gave a positive colour test for a free C-3 hydroxyl but the glycoside did not [6]. Methylated **1**, on acid hydrolysis, yielded kaempferol 5,7,4′-trimethyl ether, mp 150° (lit. 151°) [7], confirming the sugar attachment at C-3. Quantitative sugar estimation and periodate oxidation of the methylated glycoside confirmed the presence of one mol of galactose per mol of the glycoside, and that the sugar was in the pyranose form. Hydrolysis of **1** with almond emulsin gave galactose confirming the sugar linkage as β. Thus **1** is characterized as kaempferide 3-*O*-β-D-galactopyranoside. Although kaempferide occurs commonly in nature, its galactoside has not been reported previously.

2, $C_{28}H_{32}O_{14}$, mp 177° (*d*), gave a green ferric reaction and a positive Molisch test. Acid hydrolysis yielded an aglycone (**2a**), glucose and rhamnose. The colourless aglycone, mp 249°, analysed for $C_{16}H_{12}O_5$. Characteristic colour reactions and λ_{max}^{EtOH} nm at 261 and 308 (*sh*) indicated its isoflavonoid nature [4]. Formation of a diacetate and a trimethyl ether indicated the presence of two free hydroxyls and one methoxyl group in the molecule. Bathochromic shifts in the UV spectrum with NaOAc, NaOAc–H_3BO_3 and $AlCl_3$ indicated a free C-7 hydroxyl and another free hydroxyl *ortho* to the C-7 hydroxyl. ^{1}H NMR of the aglycone diacetate (90 MHz, $CDCl_3$) δ: 7.94 (1H, *s*, C-2), 6.96 (2H, *d*, $J = 9$ Hz, C-3′ and C-5′), 7.47 (2H, *d*, C-2′ and C-6′), 7.25 (1H, *d*, C-6) fixed the other hydroxyl at C-8. The proton at C-5, *ortho* to the carbonyl group, occurred downfield as a doublet ($J = 9.0$ Hz at δ 8.20). Anisic acid was obtained as one of the products of $KMnO_4$ oxidation, fixing the methoxyl group at position 4′. Alkaline fission of the aglycone dimethyl ether gave formic acid and a deoxybenzoin, identified as 4-methoxybenzyl-(2-hydroxy-3,4-dimethoxy)phenyl ketone. The aglycone was thus characterized as 7,8 dihydroxy-4′-methoxyisoflavone (retusin).

Methylation of **2** followed by acid hydrolysis yielded an aglycone, mp 221° (lit. mp 221°) [8], characterized as 8-*O*-methylretusin. It could thus be concluded that both sugars are linked at position 7. The λ max of **2** did not give bathochromic shifts with NaOAc, NaOAc–H_3BO_3 or $AlCl_3$.

Quantitative sugar estimation and periodate oxidation of the glycoside methyl ether confirmed the sugar moiety as a disaccharide, and that both sugars are in the pyranose form. The disaccharide moiety was confirmed as rhamnosylglucose by mild acid hydrolysis, rhamnose being released first, followed by glucose. Permethylation of the glycoside followed by hydrolysis yielded 3,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose, confirming a 1 → 2 linkage between the two sugars. A doublet at δ 1.32 in the ^{1}H NMR spectrum of **2** due to the rhamnose methyl protons, gave further evidence for a 1 → 2 linkage [9, 10]. Hydrolysis of the glycoside with diastase liberated only rhamnose indicating the α-nature

of the intersugar linkage. The liberation of glucose on hydrolysis with almond emulsin showed the β-nature of the glycosidic linkage. The compound is thus characterized as retusin 7-*O*-neohesperidoside (**2**), the first report of its occurrence in nature.

EXPERIMENTAL

Plant material. The stem bark of *Prosopis juliflora* (Hindi: Kabuli kikar) was collected locally and identified by the Allahabad branch of the Botanical Survey of India.

Isolation and purification. The air-dried and powdered stem bark (2.75 kg) was exhaustively extracted with Me_2CO (5 × 4 l.) and EtOH (5 × 4 l.), respectively. Both extracts were concd under red. pres. to 150 ml and 250 ml, respectively, and separately extracted with petrol, Et_2O and EtOAc. The concd Et_2O fraction from EtOAc–petrol (bp 40–60) as yellow needles. The concd column. The C_6H_6–$CHCl_3$ (1:4) eluate yielded **1**. TLC on Si gel G, R_f 0.38 (C_6H_6–EtOAc, 3:2; spray: 2 N H_2SO_4), which cryst. from EtOAc–petrol (bp 40–60°) as yellow needles. The concd Et_2O fraction from the EtOH extract was also chromatographed on a Si gel column. The C_6H_6–EtOAc (1:1) eluate yielded **2**. TLC R_f 0.47 (C_6H_6–MeOH, 4:1; spray: 2 N H_2SO_4) and cryst. from EtOAc–petrol (bp 40–60°) as cream crystals, mp 177° (*d*).

1. Found: C, 57.09; H, 4.73. $C_{22}H_{22}O_{11}$ requires: C, 57.14; H, 4.76%. λ_{max}^{MeOH} (nm): 269, 345; +$AlCl_3$ 272, 399; +$AlCl_3$–HCl 271, 397; +NaOAc 276, 353. *Hydrolysis.* **1** (0.5 g) was refluxed with H_2SO_4 (7% aq. 5 ml) for 3 hr. The aglycone (**1a**) crystallized from EtOAc–petrol (bp 40–60°) as cream crystals, mp 227°. Found: C, 63.97; H, 3.96. Calculated for $C_{16}H_{12}O_6$: C, 64.00; H, 4.00%. λ_{max}^{MeOH} (nm): 267, 367; +$AlCl_3$ 271, 423; +$AlCl_3$ HCl 270, 422; +NaOAc 274, 384.

1a *Methyl ether.* **1a** was (0.1 g) refluxed with Me_2SO_4–K_2CO_3–Me_2CO for 4 hr giving a solid which cryst. from EtOAc–petrol (bp 40–60°) as colourless prisms, mp 165°. The percentage of methoxyls in (i) the aglycone and (ii) its methyl ether, was estimated [11]. (i) Found: –OMe, 10.31. Calculated for $C_{15}H_9O_5$.OMe; OMe, 10.33%. (ii) Found: OMe, 36.19. Calculated for $C_{15}H_9O_2(OMe)_4$: OMe, 36.25%.

1a *Acetate.* **1a** (0.05 g) was kept with Ac_2O (5 ml) and pyridine (2 ml) at room temp. for 48 hr. The product cryst. from EtOAc–petrol (bp 40–60°), mp 193°. Found: Ac, 30.21. Calculated for $C_{16}H_9O_6(Ac)_3$: Ac, 30.28% [12].

$KMnO_4$ *oxidation of* **1a**. **1a** (0.1 g) was refluxed with 10% aq. $KMnO_4$ for 4 hr. Anisic acid was obtained, mp 183° (lit. 184°), Co–PC with an authentic sample gave a single spot, R_f 0.37 (*n*-BuOH satd. with NH_3; spray: bromophenol blue).

Alkaline hydrolysis of **1a**. **1a** (0.1 g) dissolved in EtOH (25 ml) was refluxed with 25% aq. KOH (10 ml) for 4 hr.

Periodate oxidation. Methylated **1** (0.05 g) was treated with 0.1 M $NaIO_4$ (25 ml) in aq. EtOH at room temp. (25°C) for 48 hr. For each mol of glycoside, 2.1 mol of periodate were consumed and 1.0 mol of HCOOH liberated.

2. Found: C, 56.69; H, 5.44. $C_{28}H_{32}O_{14}$ requires: C, 56.75; H, 5.40%. λ_{max}^{EtOH} (nm): 257, 313; +$AlCl_3$ 258, 315; +$AlCl_3$–HCl 257, 313; +NaOAc 257, 313; +NaOAc–H_3BO_3 256, 313. ^{1}H NMR (90 MHz, $CDCl_3$) δ: 1.32 (3H, *d*, *J* = 12 Hz, rhamnose Me), 4.00 (3H, *s*, –OMe), 4.90 (1H, *s*, H-1″ rhamnosyl), 5.20 (1H, *br*, H1″ glucosyl), 3.82 (10H, *br*, sugar protons), 7.94 (1H, *s*, C-2), 6.95 (2H, *d*, *J* = 8.5 Hz, C-3′,5′), 7.46 (2H, *d*, *J* = 8.5 Hz, C-2′,6′), 7.28 (1H, *d*, *J* = 9.0 Hz, C-6), 8.23 (1H, *d*, *J* = 9.0 Hz, C-5).

Aglycone (**2a**). Found: C, 67.8; H, 4.29; –OMe, 10.86. Calculated for $C_{16}H_{12}O_5$: C, 67.6; H, 4.22; –OMe, 10.91%. λ_{max}^{EtOH} (nm): 261, 308; +$AlCl_3$ 281, 322; +$AlCl_3$–HCl 261, 308; +NaOAc 277, 314; +NaOAc–H_3BO_3 269, 310.

2a *Diacetate.* Cryst. from EtOAc–petrol (bp 40–60°) as colourless needles, mp 166°. Found: C, 65.17; H, 4.34; –Ac, 23.29. Calculated for $C_{16}H_{10}O_5(Ac)_2$: C, 65.2; H, 4.38; –Ac, 23.36%. ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.94 (1H, *s*, C-2), 4.00 (3H, *s*, OMe), 6.96 (2H, *d*, *J* = 9.0 Hz, C-3′, 5′), 7.47 (2H, *d*, *J* = 9.0 Hz, C-2′, 6′), 7.25 (1H, *d*, *J* = 9.0 Hz, C-6), 8.20 (1H, *d*, *J* = 9.0 Hz, C-5).

2a *Methyl ether.* Methylation of **2a** with Me_2SO_4–K_2CO_3–Me_2CO for 2 hr gave a solid which cryst. from EtOAc–petrol (bp 40–60°) as colourless needles, mp 151°. Found: C, 69.31; H, 5.08; OMe, 26.69. Calculated for $C_{15}H_7O_2(OMe)_3$: C, 69.2; H, 5.16; OMe, 29.8%.

Alkaline hydrolysis of **2a** *dimethyl ether.* **2a** Dimethyl ether (0.25 g) was heated under reflux with EtOH (25 ml) and 10% aq. KOH (4.0 ml) for 1 hr. The product recryst. from Me_2CO–MeOH to yield 4-methoxybenzyl-(2-hydroxy-3,4-dimethoxy)phenyl ketone as long colourless needles, mp 122°, which gave an intense wine-red colour with alcoholic $FeCl_3$. Found: C, 67.4; H, 6.02; –OMe, 30.7. Calculated for $C_{17}H_{18}O_5$: C, 67.5; H, 6.00; $(-OMe)_3$, 30.8%. Methylation of this compound gave 4-methoxybenzyl-(2,3,4-trimethoxy)phenyl ketone, as glistening needles, mp 54°, which did not give positive colour with alcoholic $FeCl_3$.

Permethylation of **2** *and hydrolysis of the permethylated product.* **2** (0.20 g) was permethylated by repeated treatment with Me_2SO_4 (10 ml) and 10% aq. NaOH (10 ml) and the product hydrolysed with 2 N H_2SO_4. PC, R_G: 0.79 and 1.01, of hydrolysate (*n*-BuOH–EtOH–H_2O, 5:1:4; spray, AHP) corresponded to 3,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose, respectively.

Periodate oxidation of methylated **2**. For each mol of **2** 3.01 mol of periodate were consumed and 1.1 mol of HCOOH liberated.

Enzymic hydrolysis of **2**. The glycoside (0.03 g) dissolved in EtOH and diastase solution (0.1 g in 25 ml) was left at 40° for 48 hr. The mixture was extracted with EtOAc and the concd aq. layer gave rhamnose, R_f 0.34 (*n*-BuOH–HOAc–H_2O, 4:1:5, spray: AHP). The EtOAc extract was taken up in EtOH, 10 ml of emulsin soln added and the mixture kept at 40° for 48 hr. The hydrolysate gave glucose (PC, R_f 0.18).

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