A Novel Flavanol Glycoside from *Crotalaria prostrata* Rottl.

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THE medicinal value of the plant *Crotalaria prostrata* Rottal.[1] (N.O. Leguminosae) is said to be useful in derangements of the stomach and infantile diarrhoea.

Experimental

The plant material was supplied by United Chemicals and Allied Products (Calcutta) and authenticated by the Department of Botany, University of Sagar. A herbarium specimen (No. XXII) has been deposited in Natural Products Laboratory. Air-dried and powdered seeds were extracted with hot 95% EtOH. The extract was concentrated under reduced pressure to a viscous mass and separated into water-soluble and -insoluble part. The water-soluble part was concentrated to a viscous mass (0.80%) and extracted successively with C_6H_6, $CHCl_3$, EtOAc, Me_2CO and MeOH.

The water-soluble part of the methanol-soluble fraction gave a single spot on tlc (Si-gel G ; $CHCl_3$ – MeOH – H_2O, 13 : 7 : 2) on I_2 vapour as visualisation. It gave all the colour reactions of flavanoidal glycosides. It was purified by column chromatography (Si-gel G) to give a cream coloured crystalline solid (0 036%), $C_{27}H_{32}O_{17}$, m.p. 200–01° (Found : C, 59.53 ; H, 5.02. Calcd. for : C, 51.59 ; H, 5.09%), $[\alpha]_D^{27}$ +30.7, $[M]^+$ 628. It formed an acetyl derivative, $C_{42}H_{54}O_{28}$, m.p. 184–85° (Found : C, 53.90 ; H, 4.90. Calcd. for : C, 53.94 ; H, 4.95%) ; λ_{max} (MeOH) 280, 320sh, λ_{max} (NaOMe) 240, 330, 377sh, λ_{max} ($AlCl_3$) 292, 312, 340, 390sh, λ_{max} (NaOAc) 287, 362sh, λ_{max} (NaOAc+H_3BO_3) 265, 296sh, λ_{max} ($AlCl_3$+HCl) 290, 310, 340, 354sh ; ν_{max} (KBr) 3 332 (OH), 2 900, 1 680, 1 670, 1 452, 1 260, 1 125, 820 cm^{-1} ; ^1H nmr (90 MHz) δ ($CDCl_3$) 5.20 (1H, d, *J* 9.9 Hz, C2), 6.72 (2H, d, *J* 7.0 Hz, C2′, C6′), 6.88 (1H, d, *J* 6.5 Hz, C5′), 2.16 (3H, s, C3′-OAc), 2.18 (3H, s, C4′-OAc), 2.19 (3H, s, C5-OAc), 5.80 (1H, s, C6), 2.12 (3H, s, C7-OAc), 5.90 (1H, d, *J* 2.5 Hz, C8), 4.32 (1H, d, *J* 7.0 Hz, C_1'' anomeric proton), 2.05 (3H, s, C2″-OAc), 2.14 (3H. s, C3″-OAc), 2.08 (3H, s, C4″-OAc), 4.10–4.60 (6H, m, protons of glucose), 4.42 (1H, d, *J* 7.0 Hz, Cl‴ anomeric proton), 2.04 (3H, s, C2‴-OAc), 2.12 (3H, s, C3‴-OAc), 2.10 (3H, s, C4‴-OAc), 2.07 (3H, s, C6‴-OAc), 4.20–4.30 (6H, m, protons of galactose) ; $[M]^+$ 628, *m/z* 465, 449, 304, 303, 276, 153, 152, 124.

The compound was hydrolysed with (7% ethanolic H_2SO_4) bv refluxing for about 10 h and after removal of EtOH it yielded on aglycone, m.p. 238–39° (Found : C, 59.20 ; H, 3.90. Calcd. for : C, 59.25 ; H, 3.91%), $[\alpha]_D^{27}$ +30.2, $[M]^+$ 304. The hydrolysate neutralised with $BaCO_3$, $BaSO_4$ was filtered off. The filtrate was concentrated under reduced pressure. Pc examination (n-BuOH – HOAc – H_2O, 4 : 1 : 5) showed the presence of D-glucose and D-galactose (1 mol) (co-pc, co-tlc). The quantitative estimation of sugar in the hydrolysate confirmed the presence of one molecule of glucose and galactose.

The white crystalline microneedles (0.027%), $C_{15}H_{12}O_7$, m.p. 238–39° (Found : C, 59.20 ; H, 3.90. Calcd for : C, 59.25 ; H, 3.9%) , $[\alpha]_D^{28}$ +30.2, $[M]^+$ 304, on tlc examination gave a single spot (R_f=0.69, $CHCl_3$ – MeOH – H_2O, 13 : 7 : 2) and was purified by column chromatography. It formed a penta-acetyl derivative, $C_{25}H_{22}O_{12}$, m.p. 225° (Found : C, 53 90 ; H, 4.90. Calcd for : C, 53 94 ; H, 4.95%), $[M]^+$ 514, λ_{max} (MeOH) 287, 330sh, λ_{max} (NaOAc) 304, 324sh, λ_{max} ($AlCl_3$) 274, 325sh, λ_{max} (NaOMe) 260, 328, 345sh, λ_{max} (NaOAc+H_3BO_3) 263, 359sh ; ν_{max} (KBr) 3 315, 2 904, 1 672, 1 670, 1 650, 1 284, 825 cm^{-1} ; ^1H nmr (90 MHz) δ ($CDCl_3$) 5.20 (1H, d, *J* 9.9 Hz, C2), 6.72 (2H, d, *J* 7.0 Mz, C2′, C6′), 2.16 (3H, s, C3′-OAc), 2.18 (3H, s, C4′-OAc), 6.88 (1H, d, *J* 6.5 Hz, C5′) 2.20 (3H, s, C3-OAc), 2.19 (3H, s, C5-OAc), 5.80 (1H, s, C6), 2.17 (3H, s, C7-OAc), 5.92 (1H, d, *J* 2.5 Hz, C8), $[M]^+$ 304, *m/z* 303, 276, 153, 152, 150, 125.

The glycoside was dissolved in MeOH and treated with sodium metaperiodate[2] for 5 days and the liberated HCOOH and the consumed periodate were estimated[3].

The glycoside in EtOH was mixed with an equal volume of almond emulsin solution and left at 30° for 24 h. The examination of the hydrolysate on pc examination (n-BuOH – HOAc – H_2O, 4 : 1 : 5) showed the presence of β-linkage between the sugars as well as to the aglycone.

Results and Discussion

The water-soluble part of the methanol-soluble fraction gave a compound which showed all the colour reactions of the flavanoidal glycosides (1) and gave positive molisch test for glycoside and all the colour tests for flavanone[4]. Its acetyl derivative suggested the presence of eleven acetylysable OH

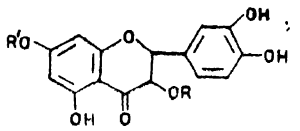
groups. Compound **1** on acid hydrolysis gave an aglycone (**2**).

Compound **2** formed a penta-acetate, $C_{25}H_{22}O_{12}$. On KOH degradation, **2** gave phloroglucinol and protocatechuic acid (m.m.p., co-tlc), which confirmed the hydroxyl group at C5, C7, C3′ and C4′ positions[5]. A characteristic colour reaction with Zn/HCl[6] and zirconium oxychloride in citric acid[7] further confirmed the presence of OH group at C_3. Yellow fluorescence of the aglycone in uv light and the spectral shift with $AlCl_3$ of 60 nm relative to band-I in MeOH suggested a free OH group at C3 in the aglycone.

The signals for C2 appeared at δ 5.20 while the aromatic protons appeared at δ 6.72 (C2′, C6′) and 6.88 (C5′). The singlet at δ 5.80 was indicative of a proton at C6. ^{1}H nmr spectra of the acetyl derivative (**2**) showed a singlet at δ 2.16, 2.18, 2.19 and 2.11, indicative of the 3′-OAc, 4′-OAc, 5′-OAc and 7-OAc.

On permethylation[8] followed by acid hydrolysis, gave 2,3,4-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-galactose and confirmed that Cl of the glucose was attached at C3 of the aglycone which showed (1→6) linkage between D-galactose and D-glucose. Compound **1** on periodate oxidation also indicated the presence of dissaccharide with both the sugars in pyranose form.

The emulsin enzymatic hydrolysis confirmed the presence of a β-linkage between the sugars and aglycone. Thus compound **1** was identified as taxifolin 3-*O*-β-D-galactopyranosyl (1→6)-*O*-β-D-glucopyranoside.



1; R=Glc(1→6)Glu, R′=H
(Glc=β-D-galactopyranosyl, Glu=β-D-glucopyranoside)
2; R=H, R′=H

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