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NOTE

Chemical Constituents from the Pods of *Cassia glauca*, Lam.

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The present paper deals with the isolation and structural elucidation of a new flavonol glycoside: 5,7-dihydroxy-4′-methoxy flavonol-3-O-β-D-galactopyranoside along with chrysophenol, physcion, kaempferide and quercetin from the pods of *Cassia glauca*.

In course of our studies on plant pigments of Cassia species[1-3], we undertook a systematic chemical investigation of the pods of *Cassia glauca*, a medicinally important plant.[4] In this short communication the isolation and structure elucidation of a new flavonol glycoside besides the known compounds like chrysophanol, physcion, kaempferide and quercetin are being reported from the pods of this plant.

The air-dried and crushed pods (5 kg) were extracted with boiling methanol. The alcoholic extract was concentrated under reduced pressure to a dark brown viscous mass which was extracted continuously with n-hexane by liquid-liquid extractor to remove chlorophyll. It was then extracted with acetone and the extract was concentrated under reduced pressure, mixed with cold water and allowed to stand for 48 h in an ice-bath. The resulting water-soluble part and water-insoluble (coloured residue) were worked up separately.

The ethyl acetate extract of the water-soluble part after concentration under reduced pressure was subjected to column chromatography on silica gel and eluted with ETOAc→MeOH in varying proportion. Elusion of the column with ETOAc—MeOH (7 : 3 v/v) afforded compound-A which was crystallised from methanol as a light yellow coloured plates (0.6 g), m.p. 140–41°C, $R_f = 0.78$ (PC, BuOH : AcOH : H_2O : : 4 : 1 : 5 v/v) and 0.60 (20% AcOH). It gave a green colour with alcoholic ferric chloride and a bright yellow colour with conc. H_2SO_4. It gave red colour with Mg/HCl and positive Molisch's test. The compound-A analysed for $C_{22}H_{22}O_{11}$ (Found: C, 57.94; H, 4.98. Calc. for $C_{22}H_{22}O_{11}$: C, 57.14; H, 4.76%); UV (EtOH) λ_{max}: 268,355 nm; +$AlCl_3$: 271, 401; +$AlCl_3$/HCl: 272, 401; +NaOAc: 280, 358; +NaOAc/H_3BO_3: 267, 355 nm;

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IR (KBr) ν_{max} (cm^{-1}): 3420 ν(OH), 2880 $\nu(OCH_3)$ 1640 (chelated >C=O), 1610 (aromatic), 1520, 1425, 1360, 1300, 1260, 1220, 1170, 1120, 1040, 970, 880 and 730 cm^{-1}. The chromatographic spot, conclusive colour reactions and UV spectrum with different chemical shifts indicated[5] it to be a flavonol glycoside having hydroxyls probably at C-5 and C-7 positions and methoxyl at C-4′ position in the molecule.

The glycoside on hydrolysis with 7% ethanolic H_2SO_4 yielded a yellow aglycone and sugar in the hydrolysate identified as D-galactose by R_f value 0.16 (PC, solvent—BAW, 4:1:5 v/v, spray-A HP) and co-chromatography with an authentic sample.

The aglycone was obtained as yellow crystalline solid on crystallisation from methanol, m.p. 228–29°C, $R_f = 0.90$ (PC, BAW, 4:1:5 v/v). The aglycone analysed for $C_{16}H_{12}O_6$ (Found: C, 64.30; H, 4.15; Calc. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.00%); λ_{max}: 267, 367 nm; ν_{max} (cm^{-1}): 3400, 2875, 1640, 1600, 1520,1420, 1350, 1300, 1260, 1220, 1180, 1160, 1120, 1025, 980, 940, 900, 730 cm^{-1}. It formed triacetate, m.p. 194°C and analysed for one methoxyl group.[6] On alkali fusion with 50% KOH, it produced phloroglucinol, m.p. 217°C and anisic acid, m.p. 182°C which clearly indicated that hydroxyl groups are present at C-5 and C-7 positions and methoxyl at C–4′ position. Thus the aglycone was characterised and identified as 5,7-dihydroxy-4′-methoxy flavonol (kaempferide) by direct comparison of m.m.p., Co-TLC and superimposable IR spectra with an authentic sample of kaempferide.

The point of attachment of sugar moiety to the aglycone was deduced from UV spectral shift studies with diagnostic reagents. On comparing UV data of the aglycone λ_{max}^{EtOH} 267, 367 nm with that of the glycoside λ_{max}^{EtOH} 268, 355 nm, there was found hypsochromic shift of 12 nm in band I (from 367 to 355 nm) in EtOH indicating the glycosidation at C-3[7]. The glycoside was methylated with $(CH_3)_2SO_4/K_2CO_3$ in dry acetone and the methylated product on hydrolysis with $4N\text{-}H_2SO_4$ yielded a compound $C_{18}H_{16}O_6$, m.p. 152°C, identified as 5,7,4′-trimethoxy flavonol (Kaempferol-5,7,4′-tri-O-methyl ether) by m.m.p. and co-chromatography with an authentic sample. This also confirmed the glycosidic linkage through C-3 of the aglycone.

The glycoside consumed two moles of periodate per mole of the glycoside and liberated one mole of formic acid showing that the sugar is mono saccharide in pyranose form. Hydrolysis of the glycoside with emulsin enzyme solution indicated the β-linkage between the sugar and the aglycone. The usual procedures for establishing the structure of flavonol glycoside were made as in our previous papers[1, 8]. Thus, the above reactions and spectral data led to the characterisation of compound-A as 5,7-dihydroxy-4′-methoxy flavonol-3-O-β-D-galactopyranoside. Although the aglycone, kaempferide, is known but its glycoside with galactose is being reported for the first time in genus Cassia.

The water-insoluble part (coloured residue) was dissolved in minimum quantity of MeOH, chromatographed over silica gel and eluted with petrol → C_6H_6 → EtOAC → MeOH gradient. Elusion with solvents of increasing polarity furnished B [0.5 g, C_6H_6-EtOAc (9 : 1 v/v)]. C [0.75 g, C_6H_6–EtOAc (8 : 2 v/v)], D [0.4 g, EtOAc-MeOH (9 : 1 v/v)]. E [0.35 g, EtOAC-MeOH (8 : 2

v/v)] and F [0.05 g, EtOAc—MeOH (1 : 1 v/v)]. These compounds were characterised as chrysophanol (B), physcion (C), kaempferide (D), quercetin (E). The identities were confirmed by direct comparison with the corresponding known samples. Compound-F was crystallised from MeOH as light yellow amorphous solid, m.p. 185°C (d) which could not be characterised due to the material being available in very small amount.

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