Phytochemical Investigation of *Cassia spectabilis* Seeds
Isolation and Structural Studies of Two New Flavone Glycosides

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Z. Naturforsch. 40b, 550–552 (1985); received July 27, 1984

*Cassia Spectabilis*, Leguminosae, Flavone Glycosides

From the seeds of *Cassia spectabilis* 6-hydroxy-4’-methoxy flavone-6-0-arabinopyranoside and 3,5-dihydroxy-7,3',4'-trimethoxy flavone-3-0-arabinopyranoside have been isolated and their structure elucidated.

Introduction

*Cassia spectabilis* is known for its medicinal properties and the plant is grown throughout India.

In previous paper [1] we have reported the occurrence of two esters and a chromone glycoside from *Cassia spectabilis* seeds. In the present investigation of the same plant, we report the isolation and characterisation of two new flavone glycosides.

Results and Discussion

From the ethanolic extract of the seeds of *Cassia spectabilis* two flavone glycosides C_{21}H_{20}O_{8} (1) m.p. 185 °C (d) and C_{23}H_{22}O_{11} (2) m.p. 280 °C (d) have been isolated. Homogeneity and purity of these compounds were established by chromatography. C_{21}H_{20}O_{8} (1) was found to be a glycoside. On acid hydrolysis it gave arabinose and an aglycone C_{16}H_{12}O_{4}, m.p. 245 °C which showed colour reactions characteristic of flavone.

The aglycone analysed for one hydroxyl group (mono acetate) and one methoxyl group (IR 2915 and 1185 cm\(^{-1}\) [2]; NMR δ 3.75, 3H, -OCH\(_3\)). UV spectral studies of the aglycone 240, 345 nm) indicated the absence of hydroxyl group at positions 5,7 and 4' (No shift with AICl\(_3\), NaOAc, NaOMe). The aglycone gave anisic acid on oxidation with neutral potassium permanganate, which confirmed the position of methoxyl group at position-4' in ring B. NMR spectrum of the aglycone showed doublets at 7 7.28 ppm (2H, H-2', H-6') and 7.00 ppm (2H, H-3', H-5') representing A\(_2\)B\(_2\) system in ring C. NMR spectrum of the aglycone also showed signals of three protons for ring A, one at δ 6.60 ppm (J = 2.5 cps, 9 cps, 1H, H-7); second at δ 6.88 ppm (J = 9 cps, 1H, H-8) and third at δ 7.60 ppm (J = 2.5 cps, 1H, H-5). The doublet at δ 7.60 ppm was strongly deshielded by the 4-keto group and was assigned to H-5 proton [3].

The above results showed that the aglycone is 6-hydroxy-4’-methoxy flavone. The mass fragments at m/z 136 (A\(_1^+\)), m/z 132 (B\(_1^+\)) and m/z 135 (B\(_2^+\)) also confirmed the proposed structure of the aglycone [4]. The aglycone is new and has not been isolated earlier from any plant source. Structure of the aglycone is finally confirmed by its synthesis: 2,5-dihydroxy acetophenone and anisaldehyde on condensation in alkaline medium gave 4-methoxy 2',5'-dihydroxy chalcone m.p. 175 °C. The chalcone when treated with DDQ gave 6-hydroxy-4’-methoxy flavone [5]. This synthetic flavone was identified in every respect with the aglycone isolated from *Cassia spectabilis* seeds.

The only position for attachment of arabinose to the aglycone is at C-6 hydroxyl. That arabinose in the glycoside is in the pyranose form has been proved by periodate oxidation when it consumed 2 moles of periodate per mole of the glycoside and produced one mole of formic acid. Thus the glycoside has been identified to be 6-hydroxy-4’-methoxy flavone-6-0-arabinopyranoside (1). This glycoside is new and was not reported earlier.
C_{23}H_{24}O_{11} (2) m.p. 280 °C (d) was also a glycoside and gave characteristic colour reactions of flavonol. On acid hydrolysis it gave arabinose and an aglycone C_{18}H_{16}O_{7}, m.p. 205 °C. The aglycone gave characteristic colour reactions of flavonol and has been identified to be 3,5-dihydroxy-7,3',4'-trimethoxy flavone by standard colour reactions, spectral data (UV, IR, NMR and mass) and co-chromatography with an authentic sample.

Methylation of 2 followed by acid hydrolysis gave quercetin 5,7,3',4'-tetra-0-methyl ether (m.p., m.m.p., co-chromatography) showing that sugar was linked at position-3 of the aglycone. That arabinose is present in the pyranose form has been proved by periodate oxidation. Thus the glycoside has been identified to be 3,5-dihydroxy-7,3',4'-trimethoxy flavone-3-0-arabinopyranoside (2). This glycoside is also new and was not reported earlier.

**Experimental**

The seeds were extracted with petrol ether. Petrol ether extract yielded two esters [1]. The defatted seeds were then extracted with EtOH and the conc. extract diluted with H_{2}O to give coloured ppt and an aqueous solution.

The aqueous fraction was concentrated and successively extracted with ether and ethyl acetate. Extraction with Et_{2}O and chromatography over silica gel with benzene-ethyl acetate (1:1) gave 1 m.p. 185 °C (d). The ethyl acetate extract of the original aqueous solution gave two compounds (Sigel TLC; Benzene-Ethyl acetate, 1:9) which were separated by column chromatography. The benzene-ethylacetate (4:6) eluate contained a single entity on TLC, a chromone glycoside [1] whereas benzene-ethylacetate (1:9) eluate also contained single entity on TLC (Sigel, EtOAc), a yellow glycoside m.p. 280 °C (d) (2).

C_{23}H_{26}O_{8} (1), m.p. 185 °C (d)

Calcd C 63.00 H 5.00,
Found C 62.48 H 4.91.

IR_{max}^{KBr} 3380, 2915, 2860, 1700, 1620, 1530, 1185, 860, 800, 760, and 690 cm^{-1}.

UV_{max}^{EtOH} nm 240, 345; AlCl_{3} 350, NaOAc 240, and NaOMe 365.

Acid hydrolysis. 400 mg 1 were heated with 7% sulphuric acid for 4 h at 80 °C, diluted with water. The solution was extracted with Et_{2}O. The Et_{2}O extract was evaporated to yield an aglycone crystallised from ethyl acetate – petroleum ether (3:1) m.p. 245 °C.

The ag layer was chromatographed on Whatman No. 1 paper in (a) EtOAc–Py–H_{2}O (12:5:4) and (b) EtOAc–isoPrOH–H_{2}O (3:1:1) using glucose, arabinose, xylose and mixtures of these as standard. The chromatogram was developed with (a) p-anisidine hydrochloride (1 g) and NaHSO_{3} (0.1 g) in MeOH (10 ml) diluted to 100 ml with n-BuOH and (b) aniline hydrogen phthalate spray at 120–130 °C for 10–16 min.

Aglycone (C_{16}H_{12}O_{4}) m.p. 245 °C

Calcd C 71.64 H 4.48,
Found C 71.22 H 4.52.

IR_{max}^{KBr} 3380, 2915, 2860, 1700, 1625, 1530, 1185, and 900 cm^{-1}.

UV_{max}^{EtOH} nm 240, 345; AlCl_{3} 245, 350; NaOAc 245, 355; NaOMe 245, 370. NMR (CdCl_{3}, 100 MHz) δ: 3.75 (S, 3H, -OCH_{3}); 6.34 (S, 1H, H-3); 6.60 (dd, J = 2.5 cps, and 9 cps, 1H, H-7); 6.88 (d, J = 9 cps, 1H, H-8); 7.60 (d, J = 2.5 cps, 1H, H-5); 7.28 (d, J = 9 cps, 2H, H-2', H-6'), and 7.00 (d, J = 9 cps, 2H, H-3', H-5').

Acetate (Ac_{2}O/Py) crystallised from ethyl acetate m.p. 207 °C. Methyl ether ((CH_{3})_{2}SO_{4}/K_{2}CO_{3}) crystallised from ethyl acetate: Pt ether m.p. 144 °C.

**Synthesis:** 2,5-dihydroxy acetophenone (2 g) and 4'-methoxy benzaldehyde (1.85 g) were dissolved in ethanol (10 ml) and an aqueous solution of potassium hydroxide (20%, 8 ml) added to the mixture kept at 0 °C. After the usual work up the crude product crystallised from absolute alcohol, m.p. 175 °C. This chalcone (200 mg) was refluxed with DDQ in dry benzene medium for 20 h. After work up the reaction, the residue was purified using column...
chromatography when flavone crystallised as light yellow coloured needles (ethyl acetate – petroleum ether; m.p. 245 °C) yield, 100 mg (C = 71.26%; H = 4.50%).

C_{23}H_{24}O_{11} (2): m.p. 280 °C (d) crystallised from ethyl acetate – Pt ether (3:1)
  Calcd  C 57.98  H 5.04,
  Found   C 57.45  H 5.28.
  IR_{\text{KBr}}^{\text{max}}: 3350, 2900, 2850, 1640, 1500, 1450, 1185, 1150, 1130, 835, 810, 760 cm^{-1}.
  UV_{\text{EtOH}}^{\lambda_{\text{max}}} nm: 255, 370, NaOAc 255; H_3BO_3+NaOAc 360; AlCl_3 425; AlCl_3+HCl 410; EtONa 415.

_Acid hydrolysis:_ On acid hydrolysis (7% H_2SO_4) it gave a yellow coloured aglycone and arabinose. The aglycone was crystallised from ethyl acetate – petroleum ether (3:1) m.p. 205 °C [6].
  Calcd  C 62.79  H 4.65,
  Found   C 62.70  H 4.75.

Aglycone: UV_{\text{EtOH}}^{\lambda_{\text{max}}} nm: 255, 367; AlCl_3 429; AlCl_3+HCl 412; NaOEt 417; NaOAc 255; H_3BO_3+NaOAc 410.
  IR_{\text{KBr}}^{\text{max}}: 3350, 2900, 2850, 1640, 1500, 1185, 1150, 1130, 835, 810 cm^{-1}.
  NMR [CDCl_3, 100 MHz] \delta: 4.00 (S, 9H, -OCH_3); 6.30 (d, J = 2.5 cps, 1H, H-6); 6.66 (d, J = 2.5 cps, 1H, H-8); 7.80 (dd, J = 2.5 cps, 9 cps, 2H, H-2', H-6'), and 6.90 (d, J = 9 cps, 1H, H-5').
  Mass (70 eV, direct inlet) m/e 344, 329, 328, 314, 279, 270, 181, 175, 166, 162, 72.
  Acetate (Ac_2O/Py) m.p. 180 °C.
  Methyl ether ((CH_3)_2SO_4/K_2CO_3) m.p. 278 °C.

One of us (M. Singh) is grateful to C.S.I.R. New Delhi, India for the award of Senior Research Fellowship.

[4] Ibid, p. 82.