Phytochemical Investigation of *Cassia spectabilis* Seeds

Isolation and Structural Studies of Two New Flavone Glycosides

M. Singh and J. Singh*

Department of Chemistry, University of Allahabad, Allahabad, India

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From the seeds of *Cassia spectabilis* 6-hydroxy-4'-methoxy flavone-6-O-arabinopyranoside and 3,5-dihydroxy-7,3',4'-trimethoxy flavone-3-O-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 $C_{23}H_{24}O_{11}$ (**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, -OCH₃). UV spectral studies of the aglycone (λ_{max}^{EtOH} 240, 345 nm) indicated the absence of hydroxyl group at positions 5,7 and 4' (No shift with AlCl₃, 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 δ 7.28 ppm (2H, H-2', H-6') and 7.00 ppm (2H, H-3', H-5') representing A₂B₂ 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-O-arabinopyranoside (**1**). This glycoside is new and was not reported earlier.

* Reprint requests to Dr. J. Singh.

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$C_{23}H_{24}O_{11}$ (**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'-trimethoxyflavone 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'-trimethoxyflavone-3-O-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_2O to give coloured ppt and an aqueous solution.

The aqueous fraction was concentrated and successively extracted with ether and ethyl acetate. Extraction with Et_2O 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**).

$C_{21}H_{20}O_8$ (**1**), m.p. 185 °C (d)

Calcd C 63.00 H 5.00,

Found C 62.48 H 4.91.

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

UV λ_{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_2O . The Et_2O extract was evaporated to yield an aglycone crystallised from ethyl acetate – petroleum ether (3:1) m.p. 245 °C.

The aq layer was chromatographed on Whatman No. 1 paper in (a) EtOAc–Py– H_2O (12:5:4) and (b) EtOAc–*iso*PrOH– H_2O (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 ν_{max}^{KBr} 3380, 2915, 2865, 1700, 1625, 1530, 1185, and 900 cm^{-1} .

UV λ_{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_2O/Py) crystallised from ethyl acetate m.p. 207 °C. Methyl ether ($(CH_3)_2SO_4/K_2CO_3$) 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.

$IR \nu_{\max}^{KBr}$: 3350, 2900, 2850, 1640, 1500, 1450, 1185, 1150, 1130, 835, 810, 760 cm^{-1} .

$UV \lambda_{\max}^{EtOH}$ 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: $UV \lambda_{\max}^{EtOH}$ nm: 255, 367; $AlCl_3$ 429; $AlCl_3 + HCl$ 412; NaOEt 417; NaOAc 255; $H_3BO_3 + NaOAc$ 410.

$IR \nu_{\max}^{KBr}$: 3350, 2900, 2850, 1640, 1500, 1450, 1185, 1150, 1130, 835, 810 cm^{-1} .

NMR [$CDCl_3$, 100 MHz] δ : 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.

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- [1] J. Singh and M. Singh, Z. Naturforsch. **39b**, 1425 (1984).
 [2] L. H. Briggs, L. D. Calebook, and W. Wildman, Anal. Chem. **29**, 904 (1954).
 [3] J. B. Harborne, T. J. Mabry, and H. Mabry, Chapman and Hall (London) first edition p. 64 (1975).

- [4] Ibid, p. 82.
 [5] K. Subrahmanyam, M. J. Rao, and J. K. V. Rao, Ind. J. Chem. **15B**, 105 (1977).
 [6] E. Wollenweber and K. Egger, Tetrahedron Lett. **1970**, 1601.