Phytochemical Investigation of Roots of *Pterocarpus marsupium*. Isolation and Structural Studies of Two New Flavanone Glycosides

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Pterocarpus marsupium, Leguminosae, Flavanone Glycosides

From the roots of *Pterocarpus marsupium* 7-Hydroxy-6, 8-dimethyl flavanone-7-O-α-L-arabinopyranoside and 7,8,4′-trihydroxy-3′, 5′-dimethoxy flavanone-4′-O-β-D-glucopyranoside have been isolated and their structure elucidated.

Introduction

Pterocarpus marsupium is well known for its medicinal properties. Various parts of plant are known to possess antidiabetic properties. It is also used in diarrhoea, pyrosis, sores, leucoderma, leprosy and skin diseases.

In India it is found in Madras, Western Peninsula, extending from north to Raj Mahal hills in Bihar and Central India.

Several isoflavonoids and terpenoids have been reported in this species [1, 2] but flavanone glycosides are for the first time being reported in *Pterocarpus* species.

From the ethanolic extract of roots of *Pterocarpus marsupium* two new flavanone glycosides were identified.

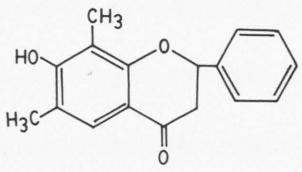
Compound (**I**) $C_{22}H_{24}O_7$, m.p. 129 °C, was found to be a glycoside and on acid hydrolysis with 7% aqueous sulphuric acid gave L-arabinose (co-chromatography with authentic sample and osazone derivative) and an aglycone **Ia** $C_{17}H_{16}O_3$, m. p. 179 °C. The aglycone gave positive test with 2,4-dinitrophenyl hydrazine [3] and sodium borohydride [4] reagents indicating it to be a flavanone which was further confirmed, by its UV and ^{1}H NMR spectra (λ_{max}^{MeOH}: 279, 311 (sh), ^{1}H NMR 5.2) [5].

The aglycone was analyzed for one hydroxyl (monoacetate, IR 3450 cm^{-1}) and two C-methyl groups (^{1}H NMR δ 2.21 and 2.42) [6].

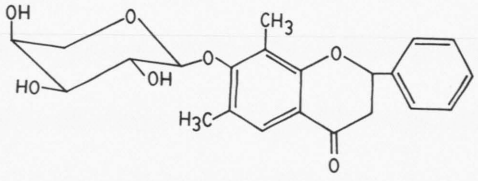
UV spectral studies of aglycone confirmed the presence of one hydroxyl group at position 7 (bathochromic shifts with NaOMe, NaOAc and 1N–NaOH) [7]. Alkaline degradation of aglycone gave 2,4-dimethyl resorcinol, m.p. 149 °C (lit. 149 °C–150 °C) and benzoic acid, m.p. 121 °C (lit. 122 °C). Formation of these products confirms the location of one hydroxyl at position 7 and methyl groups at both the 6 and 8 position of ring A. The aglycone and its methyl ether gave benzoic acid on oxidation with neutral potassium permangnate, which confirmed no substitution in ring B.

^{1}H NMR spectrum of the aglycone showed singlets at δ 7.5 (five protons of ring B) and δ 7.9 (C-5 proton of ring B).

The above results showed that the aglycone is 7-hydroxy-6,8-dimethyl flavanone. The mass fragments at *m/z* 164 ($A_1^{+\cdot}$), 165 ($A_1 + H$)$^+$ and 104 ($B_3^{+\cdot}$) also confirmed the proposed structure of aglycone [8].



Ia AGLYCONE



I FLAVANONE GLYCOSIDE

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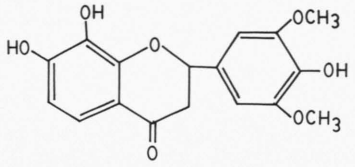
The sugar linkage was found to be of C–O–C type (acid hydrolysis, peak at 810 cm^{-1} in IR). The only position for attachment of arabinose to the aglycone is at C-7 hydroxyl. It was further confirmed by comparison of UV spectral data of glycoside and aglycone [7]. Arabinose is in the pyranose form since periodate oxidation consumed 2 mol of periodate with the liberation of 1 mol of formic acid per mol of the glycoside. The glycoside could be hydrolyzed with takadiastase showing the presence of an α-linkage.

The flavanone glycoside is thus 7-hydroxy 6,8-dimethyl 7-O-α-L-arabino pyranoside.

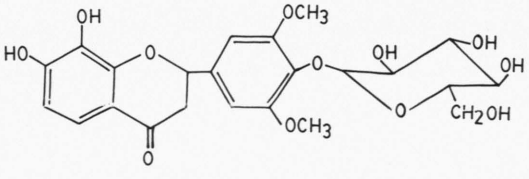
Compound (**II**) $C_{23}H_{26}O_{12}$, m.p. 190 °C, was also a flavanone glycoside, and on acid hydrolysis it gave glucose (co-chromatography with authentic sample and osazone derivative) and yellowish aglycone **IIa,** $C_{17}H_{16}O_7$, m.p. 226 °C.

The aglycone analyzed for three hydroxyl groups (triacetate and IR 3340 cm^{-1}) and two methoxyl groups (Zeisel, IR 2878 and 1176 cm^{-1}; ^1H NMR δ 3.8, 6H, $-OCH_3$).

UV spectral studies of aglycone confirmed the presence of a hydroxyl group at position 7 (bathochromic shift with NaOAc) and ortho-dihydroxyl groups in the aglycone (bathochromic shift with $AlCl_3$ and hypsochromic shift with $AlCl_3$/HCl reagent) [7].



IIa AGLYCONE



II FLAVANONE GLYCOSIDE

^1H NMR signals at δ 7.9, 5.9, 6.7 and 6.8 confirmed that positions 5,6 of ring A and 2′, 6′ of ring B are not substituted.

In order to assign the position of methoxyl and hydroxyl groups the aglycone was subjected to alkaline degradation which gave pyrogallol and 3,5 dimethyl ether of gallic acid.

The above results showed that the aglycone is 7,8,4′-trihydroxy-3′,5′-dimethoxy flavanone.

Comparative study of spectral shifts of the glycoside and aglycone showed that the sugar is attached to the hydroxyl at position 4′ of ring B [7].

Thus, the aglycone has been identified to be 7,8,4′-trihydroxy-3′,5′-dimethoxy flavanone-4′-O-β-D glucopyranoside.

Experimental

Isolation

The root (2.5 kg collected from forest of M.P., Central Province of India) of P. marsupium was identified* by Botanical Survey of India (Allahabad). The root was extracted with boiling ethanol (4 litres) and the concentrated extract (150 ml) was subjected to continuous liquid-liquid extraction employing hexane, benzene, ethylacetate and acetone as solvents. The acetone fraction was concentrated and chromatographed on silica gel column with different solvents and their mixtures. The benzene: ethyl acetate (3:7, v/v) eluate contained a single entity on silica gel TLC, a flavanone glycoside I, m.p. 129 °C whereas benzene:ethylacetate (6:4, v/v) eluate also contained a single entity on TLC, a flavanone glycoside m.p. 190 °C (**II**). Purity of the compounds was checked by PC and TLC.

*Flavanone glycoside (**I**):* $C_{22}H_{26}O_7$, m.p. 129 °C (Found C, 65.92%, H, 5.63%; requires C, 66.00% and H, 5.63%).

UV λ_{max}^{MeOH} *nm:* 272; +NaOMe 272; +$AlCl_3$ 273; +$AlCl_3$/HCl 272; +NaOAc 272; +NaOAc/H_3BO_3 273.

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* The plant was identified with the help of flowers, leaves and fruits (Reference of Family Characters – Flora of British India by J. D. Hooker, Reprinted by B. S. Pal Singh Dehradun and Periodical Experts 42-D Vivek Vihar Delhi-32). We are doing research on the flowers too. A herbarium sheet of the plant material is deposited in Botanical Survey of India (*P. mursupium* No. 43220 Accession No. 50789, collected by R. C. Srivastava, Raigarh, M.P.).

ACID hydrolysis

300 mg (**I**) was heated with 7% aqueous sulphuric acid for 4 h at 80 °C. The solution was extracted with ether. The ether extract was evaporated to yield an aglycone **Ia** crystallized with ethylacetate–light petrol (40–60 °C, 3.5:0.5, m.p. 179 °C).

The aqueous layer was chromatographed on Whatman No. 1 paper in (a) ethyl acetate : pyridine : H_2O (12:5:4) and (b) butanol : acetic acid : water, using glucose, arabinose, xylose, rhamnose and mixtures of these as standards. 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 (at 120–130 °C for 10–15 min).

*Aglycone (**Ia**):* $C_{17}H_{16}O_3$, m.p. 179 °C (Found C, 75.98% H, 5.63% requires C, 76.11%; H, 5.97%).

UV λ_{max}^{MeOH} *nm:* 279; +NaOMe 284; +$AlCl_3$ 279; +$AlCl_3$/HCl 278; +NaOAc 283; NaOAc/H_3BO_3 279; +1N–NaOH 291.

^1H NMR (90 MHz, $CDCl_3$ Values in δ): 2.5 (S, 3H, 8C-Me), 2.2 (S, 3H, 6C-Me), 7.9 (S, 1H, C-5), 2.8 (dd, J = 17 Hz, 2H, C-3), 5.2 (dd, J_{cis} = 5 Hz and J_{trans} = 11 Hz, 1H, C-2), 7.5 (S, 5H, 2′, 3′, 4′, 5′ and 6′).

Mass spectrum – Aglycone: MS (70 eV): *m/z* 268, 191, 165, 164, 104 and 78.

Acetate: Aglycone was suspended in acetic anhydride (3 ml) and pyridine (0.5 ml) and kept at room temp. for 24 h, crystallized with ethyl acetate petroleum ether m.p. 154 °C.

Methyl ether: Aglycone was refluxed with acetone (40 ml) methyl sulphate (1.5 ml) and anhydrous potassium carbonate (2 g) for 4 h. Crystallized with ethyl acetate petroleum ether m.p. 130 °C.

*Flavanone glycoside (**II**):* $C_{22}H_{26}O_{12}$, m.p. 190 °C (Found: C, 55.62%; H, 4.88%; requires C, 55.87%; H, 5.26%).

UV λ_{max}^{MeOH} *nm:* 270; +NaOMe 320; +$AlCl_3$ 281; +$AlCl_3$/HCl 270; +NaOAc 321; +NaOAc/H_3BO_3 282.

^1H NMR (90 MHz, $CDCl_3$ Values in δ): 7.9 (S, 1H, J = 9 Hz C-5), 5.9 (S, 1H, J = 9 Hz, C-6) 5.2 (dd, J_{cis} = 5.1 Hz and J_{trans} = 11 Hz, C-2), 2.8 (Quartets, J = 17 Hz, C-3), 6.8 (S, 1H, C-2′), 6.82 (S, 1H, C-6′), 3.8–3.9 (m, 6H of 2-OCH_3, C-3′ and C-5′) 4.9 (d, J = 7Hz, 1H, C-1 glucosyl) 3.7–3.8 (br. sugar protons).

IR ν_{max}^{KBr} *cm^{-1}:* 3340, 2964, 2876, 1679, 1540, 1510, 1260, 1175, 884, 870, 800 and 750.

*Aglycone (**IIa**):* $C_{17}H_{16}O_7$, m.p. 226 °C (Found C, 60.97%; H, 4.63%; requires C, 61.44%; H, 4.81%).

UV λ_{max}^{MeOH} *nm:* 278; +NaOMe 329; +$AlCl_3$ 290; $AlCl_3$/HCl 278, $AlCl_3$/HCl 278; +NaOAc 331; +NaOAc/H_3BO_3 291.

^1H NMR (90 MHz, $CDCl_3$, Values in δ): 7.9 (d, 7H, J = 9 Hz, C-5), 5.9 (d, 1H, J = 9 Hz, C-6), 5.2 (dd, J_{cis} = 5.1 Hz and J_{trans} = 11 Hz, C-2), 2.8 (quartets overlapping, J = 17 Hz, C-3), 6.7 (S, 1H, C-2′), 6.8 (S, 1H, C-6′), 3.8 (m, 6H of 2-OCH_3, C-3′ and C-5′).

Acetate: AC_2O/Py (24 h at room temp.) crystallized with ethyl acetate, m.p. 202 °C.

Methyl ether: $(CH_3)_2SO_4/K_2CO_3$; crystallized with ethyl acetate m.p. 207 °C.

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