

NOTE

5-Hydroxy 6,2'-Dimethoxy isoflavone 7-O- β -D-galactopyranoside from the Stem Bark of Antirheumatic Plant *Liriodendron tulipifera* Linn

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The glycoside 5-hydroxy 6,2'-dimethoxy isoflavone 7-O- β -D-galactopyranoside has been isolated from the stem bark of *Liriodendron tulipifera* Linn. This glycoside has been identified by chemical degradations and spectral analysis.

Key Words : *Liriodendron tulipifera* Linn., Isoflavone glycoside, Stem bark.

Liriodendron tulipifera Linn.¹ belongs to the natural order Magnoliaceae and is commonly known as 'Tulip tree'. It is a native tree of North America and introduced into some hill stations in India, where it is grown for its handsome foliage and large flowers. Different parts of this plant are used medicinally. The bark of the root, trunk and branches is a bitter tonic, febrifuge, restorative and also used in the treatment of rheumatism and dyspepsia.

The present paper deals with the isolation and study of the isoflavone glycoside, 5-hydroxy-6,2'-dimethoxy isoflavone 7-O- β -D-galactopyranoside from the stem bark of this plant.

Liriodendron tulipifera Linn. (stem bark) was procured from a standard herb supplier and identified by a reputed taxonomist.

The air dried and powdered bark of *L. tulipifera* Linn. was extracted with 95% ethanol in a Soxhlet apparatus. The concentrated extract was successively extracted with solvents: petroleum ether, benzene, chloroform, acetone, ethyl acetate and methanol. The acetone-soluble fraction was concentrated and subjected to silica gel column chromatography using acetone : methanol in varying proportions. The fractions eluted with acetone : methanol (6 : 2) were found to have same R_f values and so were mixed and crystallized from methanol as yellow needles (700 mg); m.f. $C_{23}H_{24}O_{11}$; m.p. 210–212°C and $[M^+]$ 476 (FABMS). UV λ_{max} (MeOH) nm: 262, 320 (sh); (+NaOMe) 266, 318 (sh); $(AlCl_3)$ 268, 330; $(AlCl_3-HCl)$ 270, 330; (+NaOAc) 260, 332; $(NaOAc + H_3BO_3)$ 260, 325. IR ν_{max} (KBr, cm^{-1}): 3375, 2856, 1660, 1650, 1600, 1520, 1120, 1070–1040, 860.

¹H NMR (300 MHz, DMSO) of acetyl derivative: δ 8.22 (1H, s, H-2), δ 6.47 (1H, s, H-8), δ 7.0 (1H, d, J = 8.0 Hz, H-3'), δ 7.42 (1H, d, J = 8.5 Hz, H-4'), δ 7.12 (1H, d, J = 8.3 Hz, H-5'), δ 7.27 (1H, d, J = 7.0 Hz, H-6'), δ 2.52 (3H, s, OAc-5), δ 3.80 (3H, s, -OMe-6), δ 3.71 (1H, s, OMe-2'), δ 4.05–5.56 (m, sugar protons), δ 5.12 (1H, d, J = 1.5 Hz, H-1'', galactose anomeric proton). FABMS m/z 476 [M⁺], 314, 300, 299, 296, 283, 182 and 132.

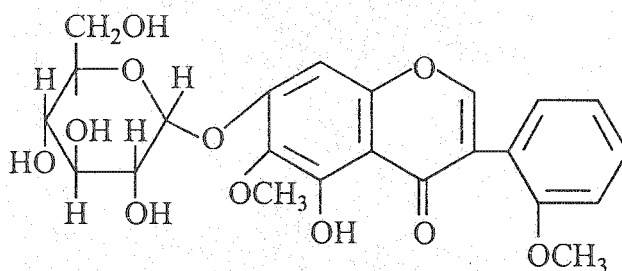
Acid hydrolysis of glycoside: The glycoside was refluxed with 7% aqueous H₂SO₄ for 8 h and subsequently extracted with ethyl acetate. The ethyl acetate layer when worked up yielded aglycone which was identified as 5,7-dihydroxy-6,2'-dimethoxy isoflavone [m.p. 230–232°C and [M⁺] 314 (FABMS)]. The aqueous layer on paper chromatographic examination was found to be D-galactose [PC, *n*-BuOH : AcOH : H₂O (4 : 1 : 5)]

Enzymatic hydrolysis of glycoside: Glycoside (30 mg) was dissolved in aqueous ethanol (30 mL) 1 : 1 (v/v) and then treated with almond emulsion (10 mL) for 3 h at 45°C followed by 48 h at room temperature. This enzymatic hydrolysis liberated D-galactose which was detected by PC and suggested the presence of β-linkage between D-galactose and the aglycone.

Based on the above experimental facts, the glycoside was finally confirmed as 5-hydroxy-6,2'-dimethoxy isoflavone, 7-O-β-D-galactopyranoside.

The glycoside (m.f. C₂₃H₂₄O₁₁, m.p. 210–212°C) responded positive to all characteristic colour reactions of isoflavone and also gave positive Mokich's test for glycoside thereby confirming it to be an isoflavone glycoside. In UV spectrum, it showed an absorption maximum at 262 nm in MeOH which was typical of isoflavone nature. IR bands observed at 3375 cm⁻¹ (chelated —OH), 2856 cm⁻¹ (—OMe), 1660 cm⁻¹ (α, β unsaturated >C=O); 1070–1040 cm⁻¹ glycosidic O—C—O *str.*). The presence of a free hydroxyl group at C-5 was indicated by a bathochromic shift⁴ of 10 nm in the band I (relative to MeOH) on addition of AlCl₃ + HCl.

The glycoside on acid hydrolysis with 7% H₂SO₄ yielded the aglycone and D-galactose (CoPC and CoTLC) which was identified as 5,7-dihydroxy-6,2'-dimethoxy isoflavone [m.f. C₃₁H₃₂O₁₄, m.p. 230–232°C and [M⁺] 314 (FABMS)] by direct comparison of its m.p., UV, IR and ¹H NMR spectral data with those reported in literature^{5–7}. The glycoside on acetylation (Ac₂O/pyridine) gave a pentaacetyl derivative [m.f. C₃₁H₃₂O₁₄, m.p. 155–156°C and [M⁺] 628 (FABMS)] which confirmed the presence of two free hydroxyl groups in the aglycone. The positions of different substituents were indicated by ¹H NMR spectrum. The H-3' proton appeared at δ 7.0 as a doublet which was coupled with protons at C-4' and C-5'. The doublet at 7.42 for C-4' proton was due to its coupling with C-3', C-5' and C-6' protons, whereas the H-5' proton which appeared at δ 7.12 as doublet indicated its coupling with C-4' and C-6' protons. On the other hand, a doublet at δ 7.27 was due to C-6' proton coupled with C-5' and C-4' protons. Also strong peak in FABMS of m/z 299 corresponded to loss of methyl group and thereby confirmed the presence of methoxy group in ring A at C-6 position⁸.



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