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ISOLATION AND STUCTURAL STUDY OF 7-HYDROXY, 6,3',4'-TRIMETHOXYISOFLAVONE-5-O-α-L-RHAMNO-PYRANOSYLO [1→6]-O-β-D-GALACTOPYRANOSIDE FROM THE FLOWERS OF *LIMONIA CRENULATA* (ROXB.)

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ABSTRACT

The plant *limonia crenulata* is commonly used as puragative, sudrorific and is also reported to be useful for curing colic and cardiolgia. The dried fruits of their plant work as an antidote to various poisons. They also resist the contagion of small pox along with malignant and pestilent fever.

In the present work, the flowers were phytochemically investigated. The extract (in 95% ethanol) was separated into chloroform, ethyl acetate, acetone and methanol soluble fractions. The acetone soluble fraction on column chromatography gave a compound ($C_{30}H_{36}O_{16}$), which was identified as 7-hydroxy-6,3',4'-trimethoxyisoflaxone-5-0-α-L-rhamnopyranssy (1 → 6) O-β-D-galactopyranoside on the basis of chemical degradation and spectral data.

Key words: *Limonia crenulata*, Isoflavone, Isolation.

INTRODUCTION

The plant *limonia crenulata* (Roxb)[1] is commonly known as Beli in Hindi and belongs to natural-order rutaceae. It occurs in Western and South India, Punjab, N. W. Himalaya, Simla, Kumaon, Bihar, Bengal and Assam.

Its roots are purgative, sudorific and are reported to be useful for curing colic and cardialagia. The dried fruits of this plant work as antidote to various poisons. They function as tonic and diminish intestinal fermentation. They also resist the contagion of small pox

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along with malignant and pestilent fevers. It's leaves are reported to be useful for curing epilepsy.

The flowers of *limonia crenulata* (Roxb) N. O. (Rutaceae) were procured from standard herb supplier from Dehradun and authenticated by a Senior Taxonomist of this region.

In view of it's important medicinal values, it's flowers were investigated phytochemically and the present paper describes the isolation and structural study of an isoflavone glycoside.

EXPERIMENTAL

Extraction and isolation

About 2.5 kg air dried and powdered flowers of *limonia crenulata* (Roxb) were defatted in a Soxhlet apparatus with petroleum ether (40-60^{o}). The defatted flowers were then exttacted with 95% ethanol in a round bottomed flask over a reflux condenser on a bath to give a dark yellow viscous mass. The ethanolic extract was filtered while hot and concentrated. The concentrated extract was separated into chloroform, ethyl acetate, acetone and methanol soluble fractions. The acetone soluble fraction was subjected to column chromatography over silica gel, when the compound 7-hydroxyl-6,3',4'-trimethoxy isoflavone 5-O-α-L-rhamnopyranosy (1 → 6)-O-β-D-galactopyranoside was obtained.

The isoflavone glycoside has molecular formula $C_{30}H_{36}O_{16}$, m. p. 214^{o}-215^{o}C and gave a positive Molisch test along with the characteristic colour reaction[2] of an isoflavonoid[3]. A bathochromic shift of 17 nm in band II with NaOAc in UV spectrum indicated the presence of –OH group at C-7 position[4]. The IR showed strong absorption bands at 3343 cm^{-1} (free OH) 2870 cm^{-1} (OMe), 1615 cm^{-1} (aromatic ring in the glycoside) and 1600 cm^{-1} for (α, β-unsaturated C = O).

On complete acid hydrolysis, the isoflavonoidal glycoside yielded an aglycone, along with galactose and rhamnos. The aglycone was having m.p.193^{o}C. It was analysed for molecular formula $C_{18}H_{16}O_7$ (Found C-62.66%, H-4.48%, Calcd. C-62.76%, H-4.65%). The aglycone was identified as, 7-dihydroxy-6,3',4'-trimethoxy isoflavone by the comparison of its m.p., UV, IR, ^{1}H NMR and MS with those reported in the literature[5]. The aqueous

hydrolysate, after neutralization with Na_2CO_3 was subjected to Co-PC on butanol : acetic acid : water [4 : 1 : 5] using authentic sugars as standard reference. The R_f value of unknown sugars were 0.17 and 0.16, which were same that of known sample of galactose and rhamnose.

The isoflavone glycoside on acetylation yielded a heptacetate derivative $C_{44}H_{50}O_{23}$ m. p. 216°C, (Found : C-56.64%, H 5-42%, Calcd. : C-56.37%. H-5.32%). The ^{1}H NMR showed important resonace signals at δ 7.90 for H-2 proton, which confirmed the isoflavone skeleton in the glycoside. Three sharp singlets, each of three protons intensity were also characteristic of methoxy groups in ^{1}H NMR of the compound at δ 3.91 (3H, s, 6-OMe), d 3.96 (6H, s, 3',4'-OMe), respectively. Another important signal at δ 7.80 of one proton intensity was assigned to H-8 proton[6].

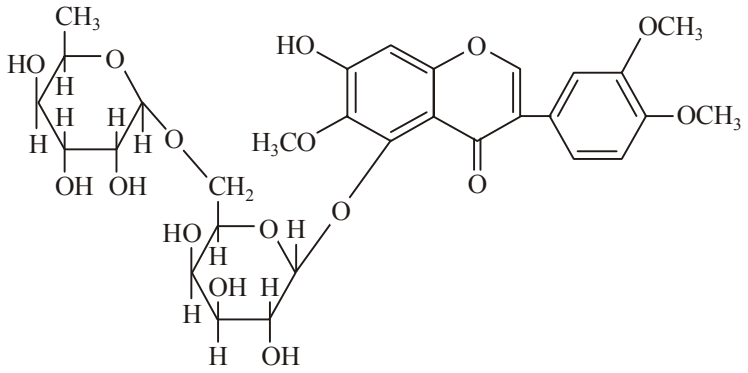
Other important signal in ^{1}H NMR spectrum of isoflavonoidal glycoside at d 2.41 was assigned to phenolic acetoxy, while placement of two methoxy group at C-3' and C-4' positions in B-ring was established by 3H ABX splitting pattern of ring B-protons. On the other hand, separate coupled doublets of two protons intensity at δ 7.31 (J-8.0 Hz) was assigned to H-2',6'. Aso orthocoupled doublet at δ-6.60 of one proton intensity was assigned to H-5'. Two anomeric sugars protons appeared at d 5.65 (1H, J-8 Hz, for 1 H- of galactose) and δ 5.35 (1H, δ, J = 1.4 Hz portion of rhamnose). Also signals for rhamnosyl methyl appeared as doublet at δ 1.08 (J = 7 Hz) for three protons intensity. Characteristic signals in the range of δ 2.04-2.15 (18 H, 6 x OAc) and δ 4.75-5.50 (12 H) were assigned to sugars of acetoxyls and for remaining sugars protons of the glycoside.

The permethylation of isoflavonoidal glycoside, followed by it's acid hydrolysis yielded the aglycone m. p. 175°C; $[M]^+ = 372$ was analysed for molecular formula $C_{20}H_{20}O_5$ (Found C = 64.55%, H = 5.37%, Calcd. C = 64.57%, H = 5.37 %) and methylated sugars, which were identified as 2,3,4-tri-O-methyl-1-rhamnose and 2,3,4-tri-O-methyl galactose. Thereby, confirming a (1 $\rightarrow$ 6) linkage between two sugars, and also estlabishing that the both sugars were present in pyranose form in the glycoside[7].

The mass spectrum of acetylated glycoside was in good agreement of molecular ion peak at m/z = 344, which was attributed to aglycone fragment resulting form the loss of acetylated sugar moiety from acetylated glycoside. RDA fragment at m/z = 170 and 160 further confirmed the presence of two hydroxyl and methoxy groups on ring A and B having two methoxy groups in the isoflavonidal glycoside.

On degradative hydrolysis with Killiani reagent, the isoflavone glycoside gave proaglycone m.f. $C_{24}H_{26}O_{12}$, m. p. 216-217°C $[m]^+ = 506$, which was identified as 7-hydroxy 6,3'4'-trimethoxyisoflavone-5-O-β-D galactopyranoside by studies of superimposable analysis of its UV, IR, ^1H NMR and mas spectra. Hydrolysis of the glycoside with takadiastase liberated L-rhamnose and afforded the same proglycone, which established that L-rhamnose was the terminal sugar and thus, an α-linkage between L-rhamnose and proaglycone. Further enzymatic hydrolysis of the proaglycone liberated the aglycone, which showed β-linkage between galactose and the aglycone.

Thus, on the basis of the above facts, the structure of the isoflavone glycoside has been estlablished as; 7-hydroxy-6,3',4'-trimethoxyisoflavone-5-O-α-L-rhamnopyranosyl (1 → 6)-O-β-D-galactopyranoside.



7-Hydroxy 6,3',4'-trimethoxyisoflavone-5-O-α-L-rhamnopyranosyl (1 → 6)-O-β-D-galactopyranoside

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