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Isolation and characterization of Trimethyl Ether Glycoside from *Lawsonia inermis*

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Abstract:
Lawsonia inermis is a very common shrub, cultivated throughout India. Earlier workers have reported compounds of medicinal use in different parts of the plant. A flavone glycoside Tricetin 3′,4′,5′ trimethyl ether 7-O-α-L rhamnopyranosyl (1→6) β-D glucopyranoside was isolated from the whole plant of *Lawsonia inermis*.
Keywords: *Lawsonia inermis,* Lythraceae, Tricetin 3′,4′,5′ trimethyl ether 7-O-α-L rhamnopyranosyl (1→6) β-D glucopyranoside, Spectral analysis.

Introduction

A new flavon glycoside Tricetin 3′,4′,5′ trimethyl ether 7-O-α-L rhamnopyranosyl (1→6) β-D glucopyranoside was obtained from the whole plant of *Lawsonia inermis*.

The glycoside was identified by the joint application of chemical as well as spectral analysis.

Lawsonia inermis (Lytharaceae), commonly known as "Mehndi", is a shrub, cultivated throughout India. All parts of the plant were reported to have significant medicinal properties. Bark was given in jaundice, skin diseases and leprosy, leaves were used externally in headache [1]. Compounds of significant medicinal value were reported by earlier workers [2]. Substituted xanthones were obtained from all parts of plant, stearic palmitic olieic and linoliec acids were obtaines from the seed [3], [4]. β-sitosterol and triterpenes were also obtained from the bark of *Lawsonia inermis*. A new flavon glycoside Tricetin 3′,4′,5′ trimethyl ether (5,7 dihydroxy 3′,4′,5′ trimethoxyflavon)7-O-

α-L rhamnopyranosyl (1→6) β-D pyranoside (**1**) was obtained from *Lawsonia inermis.*

Compound (**1**) was isolated as yellowish crystals, gave positive Molish and Shinoda test. Permethylatin (MeI, DMF/Ag2 O) of (**1**) followed by acid hydrolysis yielded 7 Hydroxy

3′,4′,5′, 5 tetramethoxy flavon, tri-O-methyl D-glucose and tri-O-methyl L-rhamnose (CO PC and CO TLC).

Material and Method

The plant material was collected locally and identified by Dr. Archana Shortriya Deptt. of Botany Govt. P.G. College, Guna, Madhya Pradesh state, India.

The dried and powdered whole plant (excluding root about 3 Kg) of *Lawsonia inermis* were extracted with 90% EtOH in a soxhlet apparatus. After extraction, extract was
concentrated under reduced pressure. The residue (5%) was then subjected to successive extractions with petroleum ether, C6H6, CHCl3 EtoAc and MeOH.

The residue of MeOH extract (50g) was subjected to column chromatography in silica gel column with CHCl3, MeOH mixtures. The CHCl3:MeOH (6:4) affording yellowish crystals of compound (**1**) (100mg).

Acid hydrolysis [5] of compound (**1**) – It was hydrolysed (7% HCl) for 6 hours at 100°C. On cooling crystals of (**2**) obtained, which was identified as 5,7 dihydroxy 3′,4′,5′ trimethoxy flavone (tricetin 3′,4′,5′ trimethyl ether). Molecular wt. detected by MS of protonated aglycone is $[A^+H]^+$ ion at m/z 345. The molecular formula was determined as C18H16O7. The structure of (**2**) was confirmed by the comparision of its spectral data with available spectral data of tricetin 3′,4′,5′ trimethyl ether. The hydrolysate then neutralized with BaCO3 and subjected to CO PC and CO TLC (BAW 4:1:5 v/v). Two

sugars were identified as D-glucose and L-rhamnose by their comparison with Rf values of authentic sugars.

Permethylation [6] of compound (**1**) followed by acid hydrolysis of (**1**) yielded 7 hydroxy

3′,4′,5′ 5 tetramethoxy flavone and methylated sugar, 2, 3, 4 tri- O-methyl-D-glucose and 2,3,4 tri-O-methyl-L-rhamnose, which shows C-6′′ –OH attachment of D-glucose to C-1′′ OH attachment of L-rhamnose.

Enzymatic hydrolysis [7] of compound (**1**) with diastase yielded L-rhamnose (CO PC)

and glycoside (**3)** which was identified as tricetin 3′,4′,5′ trimethyl ether 7-O-β-D

glucopyranoside C24H26O12 M^+ 506 [8]. Glycoside (**3)** was hydrolysed by almond

emulsion yielded (**2)** and sugar, identified as D-glucose. Quantitative estimation of sugars was carried out by the method given by Mishra and Rao [9] indicated, that two sugars

are in equimolar ratio.

Results

Tricetin 3′,4′,5′ trimethyl ether 7-O-α-L rhamnopyranosyl (1→6) β-D glucopyranoside (**1**) C30H27O16 M^+ 653 m.p. 241°C : IR bands (nujol)cm^{-1}: 2876 (-OMe), 1648(>C=O), 3600(-OH). UV λmax (MeOH)nm: 270, 330. ^{1}H NMR (DMSO-d6): δ7.14(1H, S, H-3), 6.51(1H, d, *J*= 2.2, H-6), 6.96(1H, *J*= 2.2, H-8), 7.38(2H, S, H-2′ and H-6′), 3.88(6H, S, 3′ and 5′ OMe), 3.75 (3H, S, 4′ OMe), 11.26(1H, br, S, OH-5), 4.92 (1H, d, *J*=7.3, H-1′′′): ^{13}C NMR (DMSO-d6) 159.8 (C-2), 99.7(C-3), 179.4(C-4), 160.3(C-5), 99.1 (C-6), 161.4(C-7), 95.0 (C-8), 155.8 (C-9), 104.9 (C-10), 125.1 (C-1′), 103.8(C-2′), 150.8 (C-3′), 139.9 (C-4′), 151.2(C-5′), 104.1 (C-6′), 56.2 (3′,5′ OMe), 59.1(4′OMe), 102.1(C-1′′), 72.9 (C-2′′), 75.8(C-3′′), 69.1(C-4′′), 75.9(C-5′′), 65.3(C-6′′), 100.1 (C-1′′′), 71.1(C-2′′′), 70.9(C-3′′′), 72.1(C-4′′′), 68.4(C-5′′′), 17.9(C-6′′′).

Discussion

Long wave length band at 330nm in the UV spectrum of compound (**1)** indicated that it is glycoside of tricetin trimethyl ether with a substituted 4′hydroxyl group. In ^{1}HNMR

spectrum, two metacoupled protons at δ6.51 (1H, d, *J*= 2.2 Hz) and 6.96 (1H, d, *J* =2.2 Hz) were assigned to H-6 and H-8 positions of the ‘A’ ring of flavone. A broad singlet at downfield shift at δ11.26 (1H, br S) is assigned for OH group at 5 position in the same

ring. At δ 3.88, 6H singlet was observed, which was assigned for two OMe groups at 3′

and 5′ positions in ‘B’ ring. Another singlet of 3H at δ 3.75 confirms the presence of one

–OMe group at 4′ in the same rings. ^{13}C NMR spectral data indicated the presence of two hexose residues. All these data confirmed that (**2)** is 5,7 dihydroxy 3′,4′, 5′ trimethoxy flavone (Tricetin 3′,4′, 5′ trimethyl ether).

Acknowledgement:

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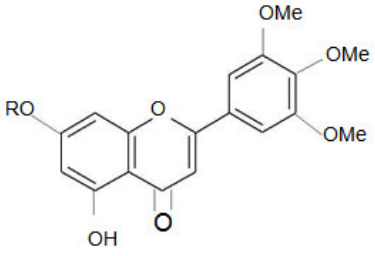
Legends for figure

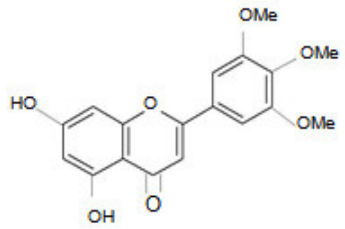
Fig. 1 Chemical structure of Tricetin 3′,4′,5′ trimethyl ether 7-O-α-L rhamnopyranosyl (1→6) β-D glucopyranoside (**1**), 5,7 dihydroxy 3′,4′, 5′ trimethoxy flavones (**2**), 3′,4′,5′ trimethyl ether 7-O-β-D glucopyranoside (**3**)

Fig.**1**



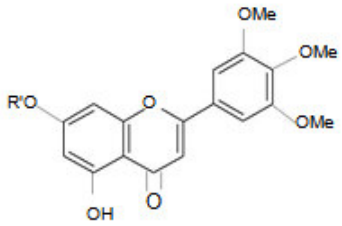
1

(R= α-L rhamnopyranosyl (1→6) β-D glucopyranoside)



2

(5,7 dihydroxy 3′,4′, 5′ trimethoxy flavone)



3
(R′= Glucopyranoside)

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