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5,6,4′-Trihydroxy-7,8,3′-Trimethoxy Flavone-4′-O-β-D-Xylopyranosyl-(1→2)-O-α-L-rhamnopyranoside: A Novel Bioactive Flavone Glycoside from the *Mucuna prurita* Hook

R.N. YADAVA* and SHIVANI JAIN
Natural Products Laboratory, Department of Chemistry
Dr. H.S. Gour University, Sagar-470 003, India

A novel flavone glycoside isolated from the seeds of *Mucuna prurita* and its structure was identified as 5,6,4′-trihydroxy-7, 8, 3′-trimethoxy flavone-4′-O-β-D-xylopyranosyl-(1→2)-O-α-L-rhamno-pyranoside by various chemical degradations and spectral analysis. This compound showed antimicrobial and antifungal activity against various gram +ve gram –ve bacteria and fungi.

INTRODUCTION

Mucuna prurita[1, 2] belongs to family Leguminosae which is commonly known as 'Kivanchh' in Hindi. It is found in Punjab plains, Himalaya to Ceylon and Burma. The Ayurvedic system of medicine describes that the fruit of this plant is used as aphrodisiac tonic, cures blood diseases, biliousness and ulcers. The seeds are aphrodisiac and cure "Vata". Its roots are useful in the treatment of dysentery and in uterine troubles.

"Yunani" system of medicine also describes that the leaves of this plant are used as tonic and anthelmintic, and it is also useful in inflammations and headache. The seeds are alexipharmic and cure scorpion sting and are also useful in gonorrhoea.

RESULTS AND DISCUSSION

The acetone soluble fraction of the ethanolic extract of the seeds of *M. prurita* afforded a novel compound **(I)**, m.p. 260–262°C., m.f. $C_{29}H_{34}O_{16}$, and $[M]^+$ 638. It gave positive response to Molisch test for glycosidic nature and Shinoda test[3] for its flavonoid nature. A bathochromic shift at 48 nm (MeOH + NaOMe) showed the hydroxy group at C-4′ position and bathochromic shift at 25 nm in band I with MeOH suggested the presence of OH group at C–5 position.[4] Its IR spectrum showed absorption bands at 3445 (—OH), 2971 (—CH), 2873 (—OCH_3), 1626 (x=O), 1495–1020 (—O-gly) and 870 cm^{-1}.

Compound **I**, on acid hydrolysis with 10% HCl yielded anaglycone **(II)**, m.f. $C_{18}H_{16}O_8$, m.p. 270–71°C and $[M]^+$ 360 and sugars were identified as L-rhamnose (R_f 0.35) and D-xylose (R_f 0.29) (Co-PC and Co-TLC). The aglycone (II) was identified as 5,6,4′-trihydroxy-7,8,3′-trimethoxy flavone with reported literature[5].

The ^{1}H-NMR spectrum of 1 showed three singlets at δ 3.85, δ 3.95, δ 3.94

which were assigned to three methoxy groups at C-7, C-8 and C-3′ positions and two aromatic protons as one singlet at δ 7.21 and δ 6.78 assigned to 2′, 6′ and 5′ positions respectively. The anomeric proton signals at δ 5.35 (1H, br, s) and 4.37 (1H, d, J = 7.6 Hz) were assigned to H-1″ and H-1‴ of L-rhamnose and D-xylose respectively and a doublet at δ 1.03 was due to the rhamnosyl methyl group.

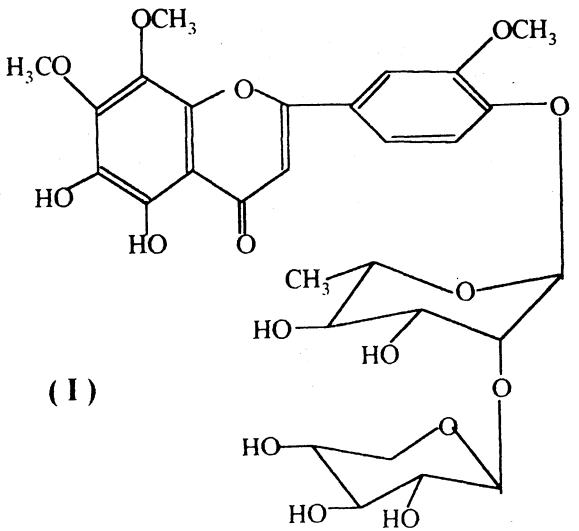
The EIMS at m/z 360 corresponded for aglycone fragment. A fragment at m/z 214 in the Retro-Diels Alder fragmentation suggested the presence of two hydroxyl groups and two methoxy groups in ring A and another fragment at m/z 132 and 149 suggesting the presence of the one methoxy group and one hydroxy group in the B ring.

Permethylation[6] of **I** followed by acid hydrolysis yielded 3,4-di-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-xylose, according to Petek,[7] suggesting that the C-1‴ of xylose was linked with C-2″ of rhamnose and C-1″ of rhamnose was attached to C-4′ of aglycone. The interlinkage (1→2) between both sugars was further confirmed by its ^{13}C-NMR spectrum.

Periodate oxidation[8] of compound **I** consumed 3.01 moles of periodate with the liberation of 1.18 moles of formic acid confirming the presence of both the sugars in pyranose form.

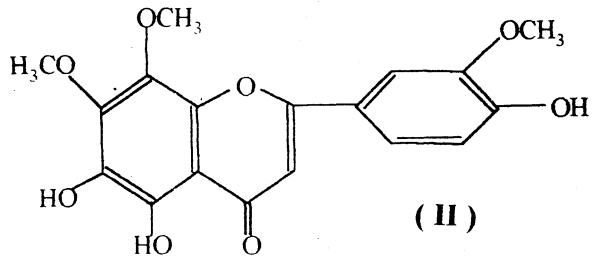
Quantitative estimation of sugars in the glycoside was done by Somogyi's procedure[9] which showed the presence of both the sugars in equimolar ratio (1 : 1).

Enzymatic hydrolysis of compound I with almond emulsion liberated D-xylose first showing the presence of β-linkage between D-xylose and rhamnose, and on hydrolysis with takadiastase liberated L-rhamnose confirming the presence of the α-linkage between the aglycone and L-rhamnose.



(I)

On the basis of above evidences, the structure of compound **I** was assigned as 5,6,4′-trihydroxy-7,8,3′-trimethoxy flavone 4′-O-β-D-xylopyranosyl-(1→2)-O-α-L-rhamnopyranoside.



(II)

EXPERIMENTAL

The seeds of *Mucuna prurita* were collected around sagar region and was taxonomically authenticated by the taxonomist of Botany Department of Dr. H.S. Gour University, Sagar. The voucher specimen was deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar (M.P.).

Extraction and Isolation

The air-dried and powdered seeds of *Mucuna prurita* were extracted with 95% rectified spirit in a Soxhlet extractor. The total extract was concentrated under reduced pressure to yield light brown viscous mass, which was successively extracted with petroleum ether (60–80°C), benzene, chloroform, ethyl acetate, acetone and methanol. The concentrated acetone-soluble fraction on TLC examination using $CHCl_3$: MeOH : H_2O (9 : 6 : 3) and I_2 vapour as visualizing agent gave a single spot. Therefore it was purified by column chromatography over Si-gel-G and eluted with EtOAc : acetone (3 : 1). On evaporation of the solvent it gave an amorphous compound which was found to be homogeneous on TLC examination. It was recrystallised from methanol as light brownish crystals, m.p. 260–262°C, m.f. $C_{29}H_{34}O_{16}$, $[M]^+$ 638 (EIMS); (Found: C 54.55%, H 5.32%; Calcd. for $C_{29}H_{34}O_{16}$: C, 54.56%, H. 5.35%; IR (KBr) ν_{max}: 3445, 2971, 2873, 1626, 1495–1020, 870 cm^{-1}; ^{1}H–NMR (300 MHz, $CDCl_3$) at δ 3.86 (3H, s, C-7 OMe); 3.90 (3H, C-8, OMe); 3.87 (3H, s, C-3′, OMe); δ 6.76 (1H, s, H-3); δ 7.21 (2H, s, H-2′, H-6′); δ 6.78 (1H, s, H-5′); 5.34 (1H, br, s, H-1″); 4.16 (1H, br, d, J = 3.6 Hz, H-2″); 3.81 (1H, dd, H-3″); 3.28 (1H, dd, H-4″); 3.66 (1H, d, H-5″); 1.03 (3H, d, J = 6.0 Hz, Rham-Me); 4.34 (1H, J = 7.8 Hz, H-1‴); 3.24 (1H, dd, H-2‴); 3.36 (1H, dd, H-3‴); 3.38 (1H, H-4‴); 3.12 (2H, dd, H-5‴); ^{13}C-NMR (90 MHz, DMSO-d_6) 161.4 (c-2); 108.5 (C-3); 177.4 (C-4); 158.1 (C-5); 103.5 (C-6); 164.8 (C-7); 96.5 (C-8); 159.1 (C-9); 107.4 (C-10); 126.4 (C-1′); 104.1 (C-2′); 153.3 (C-3′); 141.1 (C-4′); 153.5 (C-5′); 104.5 (C-6); 56.4 (OMe-7); 59.2 (OMe-8); 57.1 (OMe-3′); 103.6 (C-1‴); 82.9 (C-2‴); 71.8 (C-3‴); 74.5 (C-4‴); 72.0 (C-5‴); 18.8 (C-6‴); 108.1 (C-1‴); 74.8 (C-2‴); 77.1 (C-3‴); 70.6 (C-4‴); 67.1 (C-5‴).

Acid Hydrolysis of Compound-I: The compound I was refluxed with 7% ethanolic H_2SO_4 for 8–10 h at 100°C. The contents were allowed to cool and after the removal of the solvent yielded an aglycone II, which was separated by

filtration. The aglycone (II) was recrystallised from EtOH as light brownish crystals, m.f. $C_{18}H_{16}O_8$, m.p. 270–271°C, $[M]^+$ 360 (EIMS), UV, λ_{max} nm, (+MeOH) 250 sh, 292, 343; (+NaOMe) 259, 3921 (+$AlCl_3$) 241 sh, 261, 306, 380; (+$AlCl_3$) 241 sh, 261, 306, 380; (+$AlCl_3$/HCl), 240 sh, 259, 305, 369; (+NaOAc) 296, 310, 341, 392; (NaOAc/H_3BO_3), 296 sh, 333.

The aqueous hydrolysate obtained after the acid hydrolysis of compound (I) was neutralised with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination (n-BAW : 4 : 1 : 5) confirming the presence of L-rhamnose (R_f 0.35) and D-xylose (0.29) (by Co-PC and Co-TLC).

Permethylation of Compound I: Compound I was treated with CH_3I and Ag_2O in DMF at room temperature for 24 h and then filtered. The filtrate was concentrated in vacuum and hydrolysed with ethanolic H_2SO_4 for 8–10 h yielding methylated aglycone identified as 5,6,7,8,3′,4′-hexamethoxy flavone and methylated sugars, which were identified as 3,4-di-O-methyl-L-rhamnose and 2.3.4-tri-O-methyl-D-xylose according to Petek.[7]

Periodate Oxidation of Compound I: The compound I was dissolved in MeOH and treated with sodium metaperiodate for two days. The liberation of formic acid and consumed periodate were estimated by Jone's method,[8] which showed that both the sugars were present in pyranose form.

Microbial Activity of Compound I: The acetone soluble fraction of the ethanolic extract of the plant was tested for antibacterial and antifungal activity at its different dilutions using ethylene glycol as solvent, at a concentration of 6 mg/mL of phosphate buffered saline (w/v). The different bacterial species were first incubated at 40°C for 48 h. The zones of inhibition were recorded at 37 ± 1°C after 48 h for bacteria and at 36 ± 1°C after 24 h for fungi.

The antimicrobial activity was determined by Whatman No. 1 filter paper discs (6 mm) method[10]. Paper discs were soaked with various samples tested and were dried at 50°C. The discs were then placed on soft nutrient agar (2%) petri plates previously seeded with suspension of each bacterial species.

For the fungus, petri plates were placed on Sabouraud's broth[11] medium (1%). The zones of inhibition were expressed as an average of maximum diameter in four different directions. The various results are recorded in Tables 1 and 2.

The results recorded in Tables 1 and 2 showed that the antibacterial activity of the extract of the plant was found to be fairly good against gram +ve bacteria, *e.g.*, *Bacillus anthracis* and gram –ve bacteria, *e.g.*, *Proteus vulgaris, Salmonella newport* and was found to retain its activity even at dilution of 1 : 16. Antifungal activity of the plant extract was found to be more active against *Aspergillus fumigatus* and *Penicillium notatum.*

Thus the above investigations revealed that the acetone-soluble fraction of the ethanolic extract of the plant may potentially be used as therapeutic agent for diseases caused by these microorganisms.

TABLE-1
ANTIBACTERIAL ACTIVITY OF COMPOUND I

S.No.	Bacterial species	Diameters of zone of inhibition (mm)*				
		Acetone fraction	1 : 4	1 : 8	1 : 12	1 : 16
1.	(–) *Klebsiella pneumoniae*	9.5	8.0	7.0	0	0
2.	(+) *Streptococcus agalactiae*	21.5	18.0	15.0	0	0
3.	(–) *Proteus vulgaris*	18.5	12.5	11.5	10.5	10.0
4.	(+) *Bacillus anthracis*	8.5	8.0	7.0	7.0	6.5
5.	(–) *Salmonella newport*	25.0	19.5	18.0	18.0	12.0
6.	(–) *Pseudonomas aeruginosa*	8.5	7.5	7.0	7.5	0

TABLE-2
ANTIFUNGAL ACTIVITY OF THE COMPOUND I

S. No.	Fungal species	Diameters of zone of inhibition (mm)*				
		Acetone fraction	1 : 4	1 : 8	1 : 12	1 : 16
1.	*Aspergillus niger*	9.5	6.5	0	0	0
2.	*A. fumigatus*	8.0	7.5	7.5	7.0	7.0
3.	*Microspermum gypsum*	8.0	7.5	0	0	0
4.	*Penicillium notatum*	9.0	8.5	7.5	7.0	6.5
5.	*Fusarium oxyporum*	3.5	2.0	2.0	0	0

*The zone of inhibition (mm) taken as average of four determinations in four different directions and Whatmann 1 (6 mm) were soaked with each sample tested for their activity at a concentration of 6 mg/mL of PBS (w/v).

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