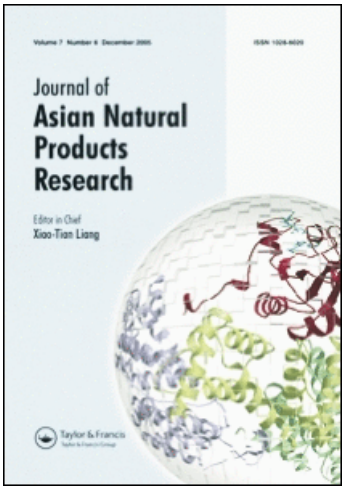

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A NEW BIO-ACTIVE FLAVONOL GLYCOSIDE FROM THE STEMS OF *BUTEA SUPERBA* Roxb

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A new bio-active flavonol glycoside was isolated from the stems of *Butea superba* Roxb, and its structure was determined by spectral analysis and chemical degradations as 3,5,7,3′,4′-pentahydroxy-8-methoxy-flavonol-3-*O*-β-D-xylopyranosyl(1 → 2)-α-L-rhamnopyranoside. The compound **1** showed antimicrobial activity against plant pathogenic fungi *Trich viride*, *Asprgillus fumigatus*, *A. niger*, *A. terreus*, *Penicillium expansum*, *Helmitnhosporium oryzae*, *Botxitis cinerea*, *Rhizopus oligosporus*, *R. chinensis*, *Kelbsiella pneumoniae*, *Fusearium moniliforme* and gram-positive bacteria *Streplococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis* gram-negative bacteria *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. The maximum inhibitory effect was shown by *H. oryzae*, *A. niger*, *B. cinera* and gram-positive bacteria.

Keywords: *Butea superba* Roxb; Leguminosae; A new bio-active flavonol glycoside; Antimicrobial activity

INTRODUCTION

Butea superba Roxb (Leguminosae) is known as 'Palaslata' in Hindi and is distributed in forest area over a large part of the country [1–3]. The root, bark and the flowers are prescribed for the treatment of snake-bite. Earlier workers [4] have reported the preliminary pharmacological examination of the seeds. We have recently reported the chemical examination [5] of the leaves and isolation of a novel flavone glycoside [6] from the EtOAc soluble fraction of the stems of this plant. The present paper deals with the isolation

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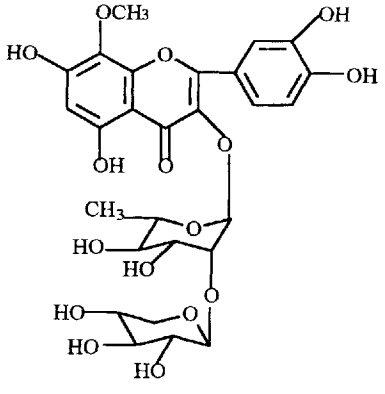
and characterisation of a new bio-active flavonol glycoside from the stems of this plant.

RESULTS AND DISCUSSION

The acetone soluble fraction of the stems of *B. superba* Roxb, afforded a new compound **1** (Fig. 1), $C_{27}H_{30}O_{16}$, mp 223–224°C; $[M]^+$ 610 (EIMS). It gave positive response to Molish test and Shinoda test [7], and also reduced Fehling's solution after acid hydrolysis indicating it to be a flavonol glycoside having no free hydroxyl group at C-3 [8]. The IR spectrum of **1** showed absorption bands at 3352 (–OH), 2865 ($-OCH_3$), 1652 (C=O) 1615 (aromatic ring system) and 1564, 1515, 855 cm^{-1}. The UV spectrum of **1** showed a bathochromic shift of 23 nm in band I with NaOMe and 32 nm in band I



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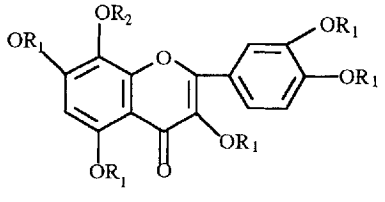

2 R1 = H, R2 = CH_3

FIGURE 1

with NaOAc suggesting the presence of free hydroxyl groups at C-7 and C-4′ [9].

The compound **1** on acid hydrolysis with 7% H_2SO_4 gave an aglycone (2) $C_{16}H_{12}O_8$; mp 286–287°C; $[M]^+$ 332 (EIMS) and sugars which were identified as xylose, and rhamnose (by Co-PC and Co-TLC). The aglycone was identified as 3,5,7,3′,4′-pentahydroxy-8-methoxy flavonol by comparision of its spectral data with known reported literature [10].

The ^{1}H-NMR spectrum of compound **1** showed three aromatic proton signals at δ 7.80 (1H, d, $J = 2.5$ Hz), 6.95 (1H, d, $J = 8.2$ Hz) and 7.65 (1H, dd, $J = 8.2$ and 2 Hz) which were assigned to H-2′, H-5′, H-6′, respectively and a three proton singlet at δ 3.86 due to OMe-8 and singlet at δ 6.93 due to H-6 proton. Signals for anomeric proton were observed at δ 5.34 (1H, br, s, H-1″) and δ 4.24 (1H, d, $J = 7.5$ Hz, H-1‴), assigned to rhamnose and xylose, respectively and a complex signal at δ 1.02 was due to the rhamnosyl methyl group.

The position of sugar moiety in compound **1** was established by permethylation of **1** [11] followed by acid hydrolysis which afforded 3,4,-di-*O*-methyl-L-rhamnose, 2,3,4-tri-*O*-methylxylose and 5,7,8-3′,4′-pentamethoxy-3-hydroxyflavonol showing that the C-1‴ of xylose was linked with C-2″ of rhamnose and the C-3 position of the aglycone(2) originally involved in glycosylation. The inter linkage (1 → 2) between the sugars was further confirmed by its ^{13}C-NMR spectrum (see Experimental).

Periodate oxidation [12] of **1** consumed 3.01 moles of periodate with the liberation of 1.15 moles of formic acid suggesting that the presence of both the sugars were in pyranose form.

Enzymatic hydrolysis of the glycoside **1** by Takadiastase liberated L-rhamnose (by PC) showing its α-linkage with aglycone (2) and also the glycoside hydrolysed by almond emulsin, xylose being observed in the hydrolysate (by PC) showing its β-linkage nature with rhamnose.

On the basis of above evidences the compound **1** is identified as 3,5,7,3′, 4′-pentahydroxy-8-methoxy-flavonol-3-*O*-β-D-xylopyranosyl-(1 → 2)-α-L-rhamnopyranoside.

The compound **1** was tested for antimicrobial activity against plant pathogenic fungi and bacteria.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points are uncorrected. UV spectra were determined in MeOH and IR spectra recorded in KBr discs. ^{1}H-NMR spectra were run at 400 MHz

using TMS as internal standard and $CDCl_3$ as solvent. ^{13}C-NMR spectra were run at 100 MHz using DMSO-d_6 as solvent.

Plant Material

The stems of *B. superba* Roxb were collected from "Pachimarhi" forest area and taxonomically authenticated by staff of Botany Department, Dr. H.S. Gour University, Sagar (M.P.), INDIA, and the herbarium specimen (K196) deposited in room no. 36 of Chemistry Department.

Extraction and Isolation

Air dried and powdered stems of (3 kg) of *B. superba* Roxb were extracted with 90% MeOH in a Soxhlet extractor. The methanolic extract was concentrated under reduced pressure to a viscous mass, which was then dissolved in hot H_2O, and partitioned with petroleum ether, chloroform, ethyl acetate and acetone. The concentrated acetone soluble part was chromatographed on a silica-gel column using solvents with increasing polarity. The fraction collected from $CHCl_3$–MeOH (6 : 2) gave compound **1**, crystallised from MeOH as light yellow crystal which gave a single spot on TLC by using $CHCl_3$–MeOH–H_2O (9 : 2 : 1) as developing solvent system, mp 223–224°C and $[M]^+$ 610 (EIMS). (Anal.: C 53.21%; H 4.90%; calcd for $C_{27}H_{29}O_{16}$: C, 53.20%; H, 4.91%) IR (KBr). ν_{max} 3354, 2865, 1652, 1564, 1515 and 835 cm^{-1}. UV (MeOH) λ_{max}; 266, 371. (+NaOMe): 276, 328, 394 (+NaOAc): 282, 330, 403, (+$AlCl_3$): 280, 309, 346, 439. (+$AlCl_3$|HCl): 278, 305, 366, 414, (+NaOAc|H_3BO_3): 267, 295 nm. ^{1}H-NMR (400 MHz-$CDCl_3$): δ 7.80 (1H, d, J = 2.5 Hz, H-2′), 6.93 (1H, d, J = 8.2 Hz, H-5′), 7.65 (1H, dd, J = 8.2 and 2 Hz, H-6′), 12.26 (1H, s, OH-5), 6.93 (1H, s, H-6), 3.86 (3H, s, OMe-8), 5.34 (1H, br, s, H-1″), 4.24 (1H, d, J = 7.5 Hz, H-1‴), 1.02 (3H, d, J = 6.1 Hz, Rham-Me). ^{13}C-NMR (100 MHz DMSO-d_6) see Table I. EIMS, m/z 610 $[M]^+$, 341, 342, 332(aglycone ion), 317, 289, 167, 139 and 137.

Acid Hydrolysis of Compound 1

Compound **1** was hydrolysed with 7% H_2SO_4 for 2 h. The aglycone (2) which precipated out on cooling was recrystallised from Et_2O as a yellow needles and was identified as 3,5,7,3′,4′-pentahydroxy 8-methoxy flavonol. $C_{16}H_{12}O_8$, mp 286–287°C, $[M]^+$ 332 (EIMS) (Anal.: C, 57.82%; H, 3.60%; calcd for $C_{16}H_{12}O_8$: C, 57.83%; H, 3.61%).

TABLE I ^{13}C-NMR of compound **1**

Atom	*δ-value*
C-2	156.8
C-3	135.2
C-4	178.7
C-5	159.5
C-6	100.4
C-7	161.2
C-8	126.3
C-9	152.6
C-10	105.2
OMe	64.3
C-1′	122.7
C-2′	116.5
C-3′	145.8
C-4′	148.3
C-5′	118.2
C-6′	122.4
C-1″	103.2
C-2″	82.4
C-3″	72.3
C-4″	73.2
C-5″	71.9
C-6″	17.5
C-1‴	107.8
C-2‴	75.2
C-3‴	78.0
C-4‴	70.7
C-5‴	67.1

The aqueous hydrolysate was neutralised with $BaCO_3$ and $BaSO_4$ was filtered off. The concentrated filtrate was developed on PC with upper phase of solvent system n-BuOH–AcOH–H_2O (4 : 1 : 5) and using aniline hydrogen phthalate as detecting agent. The Rf value for rhamnose was 0.36 and for xylose was 0.26.

Permethylation of 1 Followed by Acid Hydrolysis

Compound **1** was treated with MeI and Ag_2O in DMF at room temperatue for 24 h and then filtered. The filtrate was dried *in vacuo* and hydrolysed with 20% ethanolic H_2SO_4 for 6 h, after the usual work up yield aglycone (2) and methylated sugars identified (by Co-PC) as 2,3,4-tri-*O*-methyl-xylose and 3,4-di-*O*-methyl-rhamnose according to Petek.

Periodate Oxidation of Compound 1

Compound **1** was dissolved in MeOH and treated with sodium meta periodate for 48 h. The liberation of formic acid and consumed periodate were

estimated by the Jone's method [12] which suggests the presence of both sugars in pyranose form.

Enzymatic Hydrolysis of Compound 1

Compound **1** was treated with 3 ml of enzyme Takadiastase at 35°C for 24 h to liberate L-rhamnose ($R_f = 0.36$) (by PC) (BAW 4 : 1 : 5) using aniline hydrogen phthalate as detecting reagent. After complete hydrolysis with Takadiastase the glycoside 1 in MeOH was treated with equal volume of Almond emulsion solution and left at room temperature for 24 h. Examination of the hydrolysate on PC (BAW 4 : 1 : 5) showed the presence of xylose (R_f 0.26).

Quantitative Estimation of Sugars

Quantitative estimation of sugars in the glycoside was done by Mishra and Rao procedure [13] which revealed that two sugars were present in equimolar ratio (1 : 1).

Antimicrobial Studies of Compound 1

The antimicrobial activity of the compound **1** was tested by filter paper disc method [14]. The acetone soluble fraction of **1** was added to each medium to give the concentrations of 0.5, 1.0, 1.5 mM. The inhibitory effect of compound **1** against plant pathogenic fungi and bacteria are tabulated in Tables II and III. The maximum inhibitory effect were shown by *H. oryzae* and *A. niger*, *B. cinera* and gram-positive bacteria.

TABLE II Antifungal activity of compound **1**

Fungi	*Incubation time* (h)	*Inhibition* (%) *concentration* (mM)		
		0.5	1.0	1.5
Trich viride	22	25	21	90
Aspergillus fumigatus	14	20	25	86
A. niger	14	52	72	99
A. terreus	24	32	40	100
Penicillium expansum	24		43	85
Helminthosporium oryzae	24	92	97	99
Botrytis cinerea	24	42	45	95
Rhizopus oligosporus	14		25	76
R. chinensis	22			30
Relbsiella pneumoniae	14			82
Fusarium moniliforme	14		73	92

TABLE III Antibacterial activity of compound **1**

Bacteria	*Incubation time* (h)	*Inhibition* (%) *concentration* (mM)		
		0.5	1.0	1.5
Gram-positive				
Streptococcus pyogenes	12	40	92	95
	24	35	52	65
Staphylococcus aureus	12	64	68	68
	24	40	42	50
Bacillus subtilis	12	—	—	72
	24	—	—	68
Gram-negative				
Escherichia coli	12	—	15	22
	24		—	15
Proteus vulgaris	12	—	16	26
	24	—	18	29
Klebsiella pneumoniae	12	—	15	20
	24	—	—	18
Pseudomonas aeruginosa	12	25	32	69
	24	—	25	68

– Not inhibited.

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