

Antimicrobial Activity of a Novel Flavonol Glycoside Isolated from the Roots of *Clitoria ternatea* Linn.

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A novel biologically active flavonol glycoside **1**, m.p. 260–261°C, m.f. C₃₃H₄₀O₂₀, [M]⁺ 756 (EIMS) was isolated from the ethyl acetate soluble fraction of the defatted seeds of *Clitoria ternatea* Linn. It was characterized as a novel biologically active flavonol glycoside 3,5,4'-trihydroxy-7-methoxyflavonol-3-O- α -L-xylopyranosyl-(1 \rightarrow 3)-O- β -D-galactopyranosyl (1 \rightarrow 6)-O- β -D-glucopyranoside by several colour reactions, spectral analysis and chemical degradations. The compound **1** showed antimicrobial activity against various bacteria and fungi.

Key Words: Flavonol glycoside, *Clitoria ternatea*, Antimicrobial activity.

INTRODUCTION

Clitoria ternatea Linn.¹⁻³ belongs to family Leguminosae which is commonly known as "Aparajita" in Hindi. It is found all over the tropical region from the Himalayas to Ceylon. The root is also used as tonic to brain, cures leucoderma, burning sensation, ulcers and snakebite. Its seeds are purgative and aperient. The infusion of the leaves is used for eruption⁴.

The present paper deals with the isolation and structure elucidation of a new biologically active flavonol glycoside 3,5,4'-trihydroxy-7-methoxyflavonol-5-O- α -L-xylopyranosyl-(1 \rightarrow 3)-O- β -D-galactopyranosyl-(1 \rightarrow 6)-O- β -D-glucopyranoside (**1**) by various spectral and chemical analyses. The compound (**1**) showed antimicrobial activity against several bacteria and fungi.

EXPERIMENTAL

Melting points are uncorrected. The IR spectra was recorded in KBr disc. ¹H-NMR spectra were run at 300 MHz using TMS as internal standard and CDCl₃ as solvent. ¹³C-NMR spectra were run at 90 MHz using DMSO-d₆ as solvent.

Plant material: The roots of *Clitoria ternatea* Linn. were collected around Sagar region and taxonomically authenticated by the Department of Botany, Dr. H.S. Gour University Sagar, India. The voucher specimen was deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar, India.

Extraction and Isolation: Air-dried and powdered seeds (3 kg) of this plant were extracted with pet-ether (60–80°C) in a Soxhlet apparatus for 75–80 h. The total defatted seeds of the plant were concentrated at room temperature, which was successively extracted with C₆H₆, CHCl₃, CH₃COOC₂H₅, CH₃COCH₃ and MeOH. The ethyl acetate soluble fraction of the defatted seeds of the plant was concentrated at room temperature under reduced pressure, which showed three spots on TLC

examination which were separated by TLC and gave three compounds **1**, **1a** and **1b** which were purified by column chromatography over silica gel G. The compounds **1a** and **1b** were obtained in very small quantity, therefore it was not possible to carry out further examination. Compound **1** was further purified by column chromatography, which was found to be homogeneous on TLC examination. It was crystallized from methanol to yield 1.06 g.

Study of compound 1: It had m.p. 260–262°C, m.f. $C_{33}H_{40}O_{20}$. (Element analysis: found: C 52.37%, H 5.28%; calcd. for $C_{33}H_{40}O_{20}$: C 52.38%, H 5.29%). 1H -NMR (300 MHz, $CDCl_3$): δ 6.38 (1H, d, $J = 2.0$ Hz, C-6), 6.57 (1H, d, $J = 2.0$ Hz, H-8), 8.15 (2H, d, $J = 8.7$ Hz, H-2' and H-6'), 6.92 (2H, d, $J = 8.7$ Hz, H-3' and H-5'), 3.68 (3H, s, 7-OMe), 5.44 (1H, d, $J = 7.7$ Hz, H-1''), 3.30 (1H, m, H-2''), 3.42 (1H, m, H-3''), 3.43 (1H, m, H-4''), 3.54 (1H, m, H-5''), 3.79 (1H, dd, $J = 11.4, 5.2$ Hz, H-6''), 5.47 (1H, d, $J = 3.7$ Hz, H-1'''), 4.63 (1H, dd, $J = 3.7, 9.7$ Hz, H-2'''), 4.56 (1H, dd, $J = 9.7, 3.3$ Hz, H-3'''), 4.58 (1H, d, $J = 3.1$ Hz, H-4'''), 4.61 (1H, m, H-5'''), 4.42 (2H, d, $J = 6.3$ Hz, H-6'''), 5.66 (1H, d, $J = 6.2$ Hz, H-1'''), 4.22 (1H, m, H-2'''), 4.21 (1H, m, H-3'''), 4.20 (1H, m, H-4'''), 4.49 (1H, dd, $J = 11.4, 3.9$, H-5'''). ^{13}C -NMR (90 MHz, $DMSO-d_6$), δ 157.4 (C-2), 134.6 (C-3), 178.7 (C-4), 162.3 (C-5), 98.4 (C-6), 165.6 (C-7), 92.4 (C-8), 157.4 (C-9), 106.4 (C-10), 55.7 (-OCH₃, C-7), 122.2 (C-1'), 132.4 (C-2'), 116.4 (C-3'), 161.8 (C-4'), 116.4 (C-5'), 132.4 (C-6'), 95.4 (C-1''), 73.8 (C-2''), 77.6 (C-3''), 70.6 (C-4''), 77.5 (C-5''), 69.2 (C-6''), 100.5 (C-1'''), 70.7 (C-2'''), 71.5 (C-3'''), 71.2 (C-4'''), 72.4 (C-5'''), 62.6 (C-6'''), 104.9 (C-1'''), 75.1 (C-2'''), 78.7 (C-3'''), 70.8 (C-4'''), 67.3 (C-5''').

Acid hydrolysis of compound 1: Compound **1** (200 mg) was dissolved in MeOH (25 mL) and refluxed with 10 mL of 9% HCl on water bath for 6–7 h. The contents were allowed to cool and residue was separated with Et_2O . The ethereal layer was washed with water and the residue was chromatographed over silica-gel G using methanol-chloroform (8 : 6) as solvent to give compound **2**, m.p. 219–220°C, m.f. $C_{16}H_{12}O_6$, $[M]^+$ 300 (Elemental analysis: found: C 64.1%, H. 4.1%; calcd. for $C_{16}H_{12}O_6$: C 64.00%, H 4.00%).

The aqueous hydrolysate was neutralized with $BaCO_3$ and $BaSO_4$, filtered off. The filtrate was concentrated and subjected to paper chromatography examination using BAW (4 : 1 : 5) as solvent and aniline-hydrogen-phthalate as spraying agent, which showed the presence of D-glucose (R_f 0.19), D-galactose (R_f 0.15) and L-xylose (R_f 0.28) (Co-PC and Co-TLC).

Permethylation followed by acid hydrolysis of compound 1: Compound **1** (30 mg) was treated with MeI (7 mL) and Ag_2O (30 mg) in 8 mL of DMF for 48 h and then filtered. The filtrate was dried in vacuum and hydrolyzed with 9% methanolic HCl for 8–9 h to yield methylated aglycone which was identified as 5-hydroxy-3,7,4'-trimethoxy-flavonol and methylated sugars, which were identified as 2,3,4-tri-O-methyl-D-glucose, 2,4,6-tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-xylose according to Petek⁵.

Enzymatic hydrolysis of the compound 1: 50 mg of the compound **1** was dissolved in MeOH (50 mL) and hydrolyzed with equal volume of Takadiastase at room temperature yielding L-xylose first, confirming the presence of the α -linkage between L-xylose and proaglycone; and on further hydrolysis with almond emulsion liberated D-galactose, D-glucose and aglycone confirming the

presence of β -linkage between aglycone and D-glucose as well as between D-glucose and D-galactose.

RESULTS AND DISCUSSION

A novel biologically active flavonol glycoside **1**, m.p. 260–262°C, m.f. $C_{33}H_{40}O_{20}$, $[M]^+$ 756 (EIMS) was isolated from the ethyl acetate soluble fraction of the defatted seeds of the plant. It gave characteristic colour reactions of flavonoid⁴. Its IR spectrum showed absorption band at 3450, 2870, 2785, 1645, 1560, 1530, 1495, 1025, 820 cm^{-1} . ¹H-NMR spectrum of compound **1** showed doublet at δ 6.38, δ 6.57 for H-6 and H-8; δ 8.15 for H-2' and H-6'; δ 6.92 for H-3' and H-5'; singlet at δ 3.92 for —OCH₃ group at H-7. The anomeric proton double doublet at δ 5.44, δ 5.47 and δ 5.66 were assigned for H-1'', H-1''' and H-1'''' of D-glucose, D-galactose and L-xylose respectively.

Acid hydrolysis of compound **1** with 9% methanolic HCl gave aglycone **2**, m.p. 219–220°C, m.f. $C_{16}H_{12}O_6$, $[M]^+$ 300 (EIMS) which was identified as 3,5,4'-tri-hydroxy-7-methoxyflavonol **2** by comparison of its spectral data with literature values⁶.

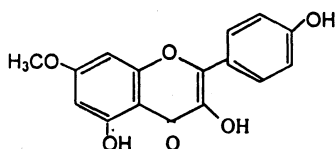
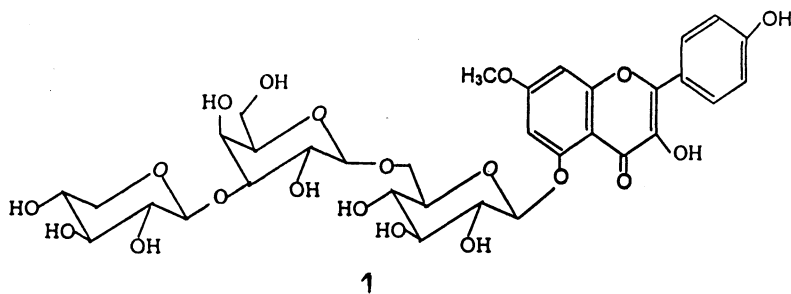
The aqueous hydrolysate after the removal of the aglycone was neutralized with BaCO₃ and BaSO₄ and filtered off. The filtrate was concentrated and subjected to PC using nBAW (4 : 1 : 5) and sugars were identified as D-glucose (R_f 0.19), D-galactose (R_f 0.15) and L-xylose (R_f 0.28) (Co-PC and Co-TLC). Periodate oxidation⁷ of compound **1** further confirmed that all the sugars were present in pyranose form.

The position of sugar moiety in compound **1** was established by permethylation⁸ of **1** followed by acid hydrolysis. The permethylated aglycone was identified as 5-hydroxy-3,7,4'-tri-methoxy-flavonol and methylated sugars, which were identified as 2,3,4-tri-O-methyl-D-glucose, 2,4,6-tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-xylose according to Petek⁵, which showed that the C-1'''' of L-xylose was linked with C-3''' of D-galactose, C-1''' of D-galactose was linked to C-6'' of D-glucose and C-1'' of D-glucose was linked to C-5 of aglycone. The interlinkages (1→3) and (1→6) between sugars were further confirmed by ¹³C-NMR spectrum.

Enzymatic hydrolysis of compound **1** with Takadiastase liberated L-xylose first confirming the presence of the α -linkage between L-xylose and proaglycone; and on further hydrolysis with almond emulsin liberated D-galactose, D-glucose and aglycone confirming the presence of β -linkage between aglycone and D-glucose as well as between D-glucose and D-galactose.

On the basis of the above evidences, the structure of compound **1** was identified as 3,5,4'-trihydroxy-7-methoxyflavonol-5-O- α -L-xylopyranosyl-(1→3)-O- β -D-galactopyranosyl-(1→6)-O- β -D-glucoopyranoside (**1**).

Antimicrobial study of compound 1: The antibacterial and antifungal activity of the ethyl acetate soluble fraction of the compound **1** was tested at its various dilutions using ethyl acetate as solvent at different concentrations. The various bacterial species were first incubated at 40°C for 48 h. The zones of inhibition were recorded at $34 \pm 1^\circ C$ for 48 h for bacteria and $32 \pm 1^\circ C$ after 24 h for fungi.



The antimicrobial activity was determined by Whatman No. 1 filter paper discs (6 mm) method⁹. Paper discs were soaked with various samples tested and were dried at 50°C. The discs were then kept on soft nutrient agar (2%) petri discs previously seeded with suspension of each bacterial species.

For the fungus, petri discs were placed on Sabouraud's broth¹⁰ medium (1%). The zones of inhibition were expressed as an average of maximum diameter in four different directions. The various results are recorded in Table-1.

TABLE-1
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF COMPOUND 1

S.No.	Micro organism	Diameters of zone of inhibition (mm)*					Std.†
		Chloroform fraction	8 : 2	6 : 4	4 : 6	2 : 8	
Bacterial species:							
1.	(-) <i>Escherichia coli</i>	11.0	9.6	6.5	4.2	1.8	16.0
2.	(-) <i>Salmonella typhimurium</i>	15.2	13.5	11.0	9.5	7.0	20.0
3.	(+) <i>Staphylococcus aureus</i>	10.0	8.5	6.2	4.0	2.1	17.0
4.	(+) <i>Bacillus coagulans</i>	14.5	11.3	8.02	5.2	2.5	18.0
5.	(+) <i>Bacillus subtilis</i>	23.2	20.5	16.5	14.3	8.5	25.0
Fungal species:							
6.	<i>Penicillium digitatum</i>	14.0	12.2	9.5	6.2	2.1	21.0
7.	<i>Rhizopus oligosporus</i>	12.8	10.4	8.4	6.3	4.1	18.5
8.	<i>Aspergillus fumigatus</i>	9.5	5.9	3.6	1.8	0.0	15.0
9.	<i>Penicillium notatum</i>	11.3	10.2	8.3	6.4	4.0	19.0
10.	<i>Fusarium oxysporum</i>	13.5	11.0	9.2	7.5	5.0	20.0

*The zone of inhibition (mm) taken as average of four determinations in four different directions and Whatman no. 1 filter paper (6 mm) were soaked with each sample tested for their activity.

†Streptomycin used as standard antibacterial agent.

‡Chloramphenicol used as standard antifungal agent.

The results showed that the antibacterial activity of the compound **1** was found to be fairly good against gram +ve bacteria, *e.g.*, *B. subtilis* and gram -ve bacteria, *e.g.*, *Salmonella typhymurium*. Antifungal activity of the compound **1** was found to be more active against *Fusarium oxysporum* and *Rhizopus oligosporus*.

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