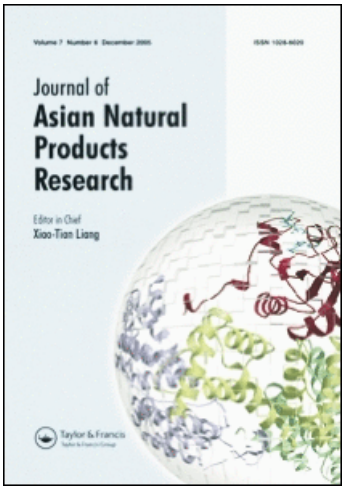

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A new biologically active flavone glycoside from the seeds of *Cassia Fistula* (Linn.)

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A NEW BIOLOGICALLY ACTIVE FLAVONE GLYCOSIDE FROM THE SEEDS OF *CASSIA FISTULA* (LINN.)

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A new bioactive flavone glycoside **1** [mp 252–254°C, $C_{28}H_{32}O_{16}$, $[M]^+$ 624 (EIMS)] was isolated from the acetone soluble fraction of the defatted seeds of *Cassia fistula* (Linn.). It was characterized as a new bioactive flavone glycoside 5,3′,4′-tri-hydroxy-6-methoxy-7-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-galactopyranoside by several colour reactions, spectral analysis and chemical degradations. Compound **1** showed anti-microbial activity.

Keywords: *Cassia fistula* (Linn.); Leguminosae; Flavonoid; Antimicrobial activity

INTRODUCTION

Cassia fistula (Linn.) [1–3] belongs to the family Leguminosae, which is commonly known as "Amaltas" in Hindi. It is found throughout India, Ceylon, Malaya, China, South Africa and the West Indies. The Ayurvedic system of medicine describes that its seeds are used in the treatment of biliousness and to improve the appetite. Its root is useful in the treatment of skin diseases, leprosy, tuberculous glands and syphilis. It also cures burning sensation. Its fruits are useful in inflammation, throat troubles, liver complaints, chest complaints, rheumatism and asthma. The present paper deals with isolation and characterisation of a new biologically active flavone glycoside from the seeds of this plant.

RESULTS AND DISCUSSION

A new bioactive flavone glycoside [mp 252–254°C, $C_{28}H_{32}O_{16}$, $[M]^+$ 624 (EIMS)] was isolated from the acetone soluble fraction of the defatted seeds of the plant. It gave characteristic colour reactions of a flavonoid [4]. Its IR spectrum showed absorption bands at 3421 cm^{-1} (–OH), 1622 cm^{-1} (α,β-unsaturated C=O), 2920 cm^{-1} (C–H), and 1075 cm^{-1} (–C–O). ^{1}H-NMR spectrum of compound **1** showed signals at δ 6.87, δ 7.61 and δ 7.72 for H-5′, H-6′ and H-2′, and a singlet at δ 6.72 for H-8 and another singlet at δ 3.85 for $-OCH_3$ group at the C-6 position. The anomeric proton signals at δ 5.50 and δ 5.20 were assigned to H-1″ and H-1‴ of D-galactose and L-rhamnose.

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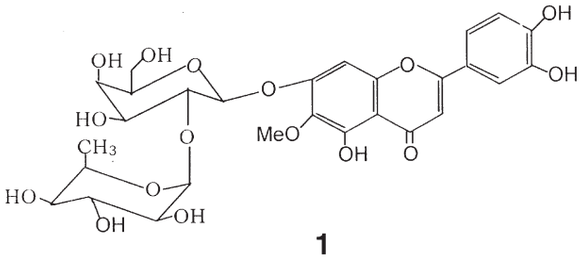
Acid hydrolysis of compound **1** with 9% methanolic HCl gave aglycone **2** [mp 218–220°C, $C_{16}H_{12}O_7$, $[M]^+$ 316], which was identified as 5,7,3′,4′-tetrahydroxy-6-methoxyflavone **2** by comparison of its spectral data with literature values [5].



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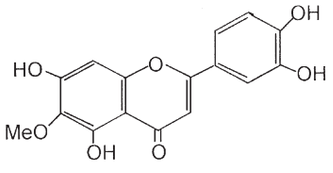

2

The aqueous hydrolysate obtained after the removal of the aglycone was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to PC using nBAW(4:1:5) and the sugars were identified as D-galactose (R_f 0.16), and L-rhamnose (R_f 0.37) (Co-PC and Co-TLC). Periodiate oxidation [6] of compound **1** further confirmed that both the sugars were present in the pyranose form.

The position of the sugar moiety in compound **1** was established by permethylation [7] of **1** followed by acid hydrolysis. The aglycone **2** was identified as 5,6,7,3′,4′-pentamethoxyflavone and the methylated sugars were identified as 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-L-rhamnose (Co-PC and Co-TLC) according to Petek [8] which showed that the C-1‴ of rhamnose was linked with C-2″ of galactose and C-1″ of galactose was linked to C-7 of aglycone. The interlinkages between both sugars were further confirmed by the ^{13}C-NMR spectrum (see Experimental section).

Enzymatic hydrolysis of compound **1** with takadiastase liberated L-rhamnose first confirming the presence of the α-linkage between L-rhamnose and D-galactose and on hydrolysis with almond emulsin liberated D-galactose confirming the presence of the β-linkage between D-galactose and aglycone.

On the basis of above evidence, the structure of compound **1** was identified as 5,3′,4′-trihydroxy-6-methoxy-7-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-galactopyranoside **1**.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points are uncorrected. The IR spectra were recorded in KBr discs. ^{1}H-NMR spectra were run at 300 MHz using TMS as internal standard and $CDCl_3$ as solvent. ^{13}C-NMR spectra were run at 90 MHz using DMSO-d_6 as solvent.

Plant Material

The seeds of *C. fistula* (Linn.) were collected around the Sagar region and were 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 the *C. fistula* (Linn.) were extracted with petroleum ether (40–60°C) in a Soxhlet apparatus for 6–7 days. The totally defatted seeds of the plant were concentrated at room temperature, and were successively extracted with C_6H_6, $CHCl_3$, $CH_3COOC_2H_5$, CH_3COCH_3 and MeOH. The acetone 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**. Compounds **1a** and **1b** were found in very small quantity, therefore, it was not possible for further examination of these two compounds. Compound **1** was purified by column chromatography, which was found to be homogeneous on TLC examination. It was crystallised from methanol to yield 1.04 g. It had mp 252–254°C, $C_{28}H_{32}O_{16}$ (elemental analysis: found C 53.83%, H 5.12% calcd for $C_{28}H_{32}O_{16}$, C 53.84%, H 5.13%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.75 (1H, s, H-8), 7.73 (1H, s, H-2′), 7.20 (1H, d, $J = 8.4$ Hz, H-3′), 7.65 (1H, d, $J = 8.4$ Hz, H-6′), 3.85 (3H, s, OCH_3, C-6), 5.50 (1H, d, $J = 8.3$ Hz, H-1″), 5.46 (1H, dd, $J = 8.3$, 9.7 Hz, H-2″), 3.86 (1H, dd, $J = 3.5$, 9.7 Hz, H-3″), 3.95 (1H, t, $J = 3.5$ Hz, H-4″), 3.87 (1H, t, $J = 7.2$ Hz, H-5″), 4.26 (2H, dd, $J = 5.7$, 11.2 Hz, H-6″), 5.27 (1H, br, s, H-1‴), 4.14 (1H, br, d, $J = 3.8$ Hz, H-2‴), 3.82 (1H, dd, $J = 8.6$, 3.2 Hz, H-3‴), 3.42 (1H, dd, $J = 9.1$, 9.1 Hz, H-4‴), 3.36 (1H, dq, $J = 9.0$, 6.0 Hz, H-5‴), 1.15 (3H, d, $J = 5.9$, Rham-Me). ^{13}C-NMR (90 MHz, DMSO-d_6) δ 149.1 (C-2), 108.2 (C-3), 178.5 (C-4), 152.5 (C-5), 135.0 (C-6), 164.5 (C-7), 95.8 (C-8), 154.2 (C-9), 107.2 (C-10), 125.0 (C-1′), 116.8 (C-2′), 146.9 (C-3′), 150.0 (C-4′), 116.9 (C-5′), 123.2 (C-6′), 58.8 ($-OCH_3$), 97.9 (C-1″), 76.8 (C-2″), 77.5 (C-3″), 72.0 (C-4″), 77.2 (C-5″), 61.2 (C-6″), 111.2 (C-1‴), 71.2 (C-2‴), 69.8 (C-3‴), 73.0 (C-4‴), 67.9 (C-5‴), 18.8 (C-6‴).

Acid Hydrolysis of Compound 1

Compound **1** (200 mg) was dissolved in MeOH (25 ml) and refluxed with 10 ml of 9% HCl on a water bath for 6–7 h. The contents were allowed to cool and the 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**: mp 218–220°C, $C_{16}H_{12}O_7$, $[M]^+$ 316 (EIMS) (elemental analysis: found C 60.07%, H 3.78%; calcd for $C_{16}H_{12}O_7$, C 60.08%, H 3.79%).

The aqueous hydrolysate was neutralized with $BaCO_3$, and $BaSO_4$, and then 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-galactose (R_f 0.16) and L-rhamnose (R_f 0.37) (Co-PC and Co-TLC).

Permethylation Followed by Acid Hydrolysis of Compound 1

Compound **1** (25 mg) was treated with MeI (5 ml) and Ag_2O (25 mg) in 5 ml of DMF for two days and then filtered. The filtrate was dried *in vacuo* and hydrolysed with 9% methanolic

HCl for 6–7 h, and gave methylated aglycone which was identified as 5,6,7,3′,4′-pentamethoxy-flavone and methylated sugars, which were identified as 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-L-rhamnose according to Petek [6].

Enzymatic Hydrolysis of Compound 1

Compound **1** (40 mg) was dissolved in MeOH (15 ml) and hydrolysed with an equal volume of takadiastase at room temperature to yield L-rhamnose confirming the presence of the α-linkage between L-rhamnose and D-galactose and on hydrolysis with almond emulsin liberated D-galactose confirming the presence of the β-linkage between D-galactose and aglycone.

Antimicrobial Study of Compound 1

The antibacterial and antifungal activity of the acetone soluble of fraction of the compound was tested at various dilutions using methylene glycol as solvent, at a concentration of 6 mg/ml of phosphate-buffered saline (w/v). The various bacterial species were first incubated at 40°C for 48 h. The zones of inhibition were recorded at 34 ± 1°C for 48 h for bacteria and 33 ± 1°C after 24 h for fungi.

The antimicrobial activity was determined by the Whatman No.1 filter paper discs (6 mm) method [9]. 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 dishes previously seeded with a suspension of each bacterial species.

For the fungus, petri dishes were placed on Sabouraud's broth [10] 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 I and II.

The results from the tables showed that the antibacterial activity of compound **1** was found to be fairly good against Gram +ve bacteria, e.g. *Staphylococcus aureus* and *B. subtilis* and Gram −ve bacteria, e.g. *Klebsiella pneumoniae* and *E. coli*. The antifungal activity of compound **1** was found to be greater against *A. niger* and *Fusarium oxysorum*.

TABLE I Antibacterial activity of compound **1**

		*Diameters of zone of inhibition (mm)**				
S. No.	*Bacterial species*	*Acetone fraction*	*1:4*	*1:8*	*1:12*	*1:16*
1	(−) *Proteus vulgaris*	12.5	10.2	8.0	7.5	0
2	(−) *Pseudomonas aeruginosa*	11.5	9.5	7.0	6.1	0
3	(−) *Klebsiella pneumoniae*	20.5	11.5	9.5	8.5	7.5
4	(−) *Escherichia coli*	19.5	9.5	7.5	6.1	5.5
5	(+) *Bacillus anthracis*	11.2	9.3	7.02	4	0
6	(+) *Staphylococcus aureus*	22.7	18.0	16.5	15.5	10.5
7	(+) *Bacillus subtilis*	20.8	17.5	15.4	12.5	9.5
8	(+) *Streptococcus pyogenes*	18.5	12.7	10.5	8.4	6.5

* 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 at a concentration of mg/ml of PBS (w/v).

TABLE II Antifungal activity of compound **1**

		*Diameters of zone of inhibition (mm)**				
S. No.	*Fungal species*	*Acetone fraction*	*1:4*	*1:8*	*1:12*	*1:16*
1	*Penicillium digitatum*	8.0	6.2	4.0	1.0	0
2	*Penicillium notatum*	6.8	2.2	1.4	0	0
3	*Fusarium oxysorum*	13.5	11.5	9.5	7.5	5.5
4	*Aspergillus niger*	16.5	9.8	9.0	8.5	6.5
5	*Aspergillus fumigatus*	7.8	4.8	2.6	1.2	0
6	*Aspergillus terreus*	8.7	7.2	5.0	3.0	1.5
7	*Trich viride*	6.8	2.2	1.4	0	0
8	*Rhizopus oligosporus*	11.5	7.5	5.5	3.5	2.5
9	*Fusarium moniliforme*	9.5	6.5	4.5	2.5	1.3

* 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 at a concentration of mg/ml of PBS (w/v).

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