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New antifungal flavone glycoside from *Butea monosperma* O. Kuntze

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Abstract

A new bioactive flavone glycoside was isolated from the methanol soluble fraction of the flowers of *Butea monosperma* O. Kuntze, which was identified as 5,7-dihydroxy-3,6,4′-trimethoxyflavone-7-O-α-L-xylopyranosyl-(1 → 3)-O-α-L-arabinopyranosyl-(1 → 4)-O-β-D-galactopyranoside (**1**) by several colour reactions, chemical degradations and spectral analysis. The compound **1** shows antimicrobial activity against various fungal species.

Keywords: Butea monosperma *O. Kuntze, new flavone glycoside, antifungal activity*

Abbreviations: *TMS, Tetramethylsilane; DMSO, Dimethyl sulphoxide; DMF, Dimethyl formamide; Co-PC, Paper chromatography with authentic sample*

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Introduction

Butea monosperma O. Kuntze belongs to the family Leguminosae sub-family mimosae and is commonly known as ‘Palash’ in Hindi being distributed throughout the greater part of India [1–2]. Its bark is used to cure night blindness and elephantiasis, its seeds are used as an anthelmintic whereas the flowers are astringent, depurative, diuretic and aphrodisic. Various compounds have been reported by earlier workers [3] from this plant. The present paper deals with the isolation and structural elucidation of a new flavone glycoside 1 from the flowers of *B. monosperma* which shows antifungal activity.

Materials and methods

Plant material

The flowers of *Butea monosperma* O. Kuntze, were collected around the Sagar region and taxonomically authenticated by the Department of Botany, Dr H.S. Gour University, Sagar, India. The voucher specimen (No. XII) is deposited in the Natural Products Laboratory, Department of Chemistry, Dr H.S. Gour University, Sagar (M.P.) India.

General

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

Extraction and isolation

The powdered air-dried flowers (3 kg) of this plant were extracted with 95% MeOH by a Soxhlet apparatus for 6-7 days. The total methanolic extract was concentrated under reduced pressure to give a light brown amorphous mass, 4.75 g, which was successively partitioned with various solvents of increasing polarity such as pet-ether (60–80°C), C_6H_6, $CHCl_3$, EtOAc, Me_2CO and MeOH. The petroleum ether, benzene, chloroform, ethyl acetate and acetone soluble parts after concentration gave very small amounts of residue and therefore these

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Table I. ^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of compound **1**.

No.	δ Value	Pattern	J value (Hz)	No. of protons	Assignment
1.	6.71	d	8.3	1	H-8
2.	7.78	d	8.6	2	H-2′, H-6′
3.	7.21	d	8.5	2	H-3′, H-5′
4.	3.85	s		3	OMe-3
5.	3.93	s		3	OMe-6
6.	3.75	s		3	OMe-4′
7.	5.48	d	7.8	1	H-1″
8.	4.60	dd	3.6, 9.5	1	H-2″
9.	4.53	dd	9.6, 3.21	1	H-3″
10.	4.62	d	3.0	3	H-4″
11.	4.58	m		3	H-5″
12.	4.38	d	6.4	2	H-6″
13.	4.98	d	6.8	1	H-1″
14.	4.40	m		1	H-2″
15.	3.98	m		1	H-3″
16.	4.16	m		1	H-4″
17.	3.50	m		2	H-5″
18.	5.63	d	6.2	1	H-1″
19.	4.19	m		1	H-2″
20.	4.17	m		1	H-3″
21.	4.15	m		1	H-4″
22.	4.50	dd	10.6, 3.4	1	H-5″

fractions were rejected. The methanol fraction was concentrated under reduced pressure to a light brownish syrupy mass, 2.85 g, at room temperature. Thin layer chromatography showed three spots, indicating it to be a mixture of three compounds **1, 1a** and **1b** which were separated by TLC and purified by colomn chromatography over a SiO_2-gel column using chloroform and methanol as solvents in various proportions (8:2, 8:4, 8:6). The eluents (8:4) and (8:2) obtained from the various fractions on the removal of the solvent gave compounds **1a** and **1b** which were found in very small quantity so that it was not possible to further examine these two compounds. The eluents (8:6) obtained from the various fractions on evaporation of the solvent gave Compound **1** which was further purified by colomn chromatography and found to be homogeneous on thin layer chromatorgraphy.

Compound **1** was crystallized from acetone solution to yield 1.75 g. It had $C_{34}H_{42}O_{20}$, $[M]^{+}$770 (FABMS), m.p. 260–262°C, (found: C, 54.53; H, 5.46%. calcd.: C, 54.55; H, 5.45%). IR ν_{max}^{KBr} cm^{-1} 3452, 2990, 2918 (—CH-stretching), 2870 (—OMe), 1630 (C=O), 1625 (aromatic ring system) and 1530, 1495, 1245, 1136, 1062, 874, 830 cm^{-1}. Compound **1** was also characterized by ^{1}H-NMR and ^{13}C-NMR spectrum (See Tables I and II).

Acid hydrolysis of compound 1

Compound **1** (900 mg) was refluxed with 15 ml of 10% H_2SO_4 on a water bath for 7–8 hr. On cooling a crystalline compound **2** deposited which was separated by filtration. Compound **2** (615 mg) was crystallized from methanol to give light yellow needles m.p. 214–216°C, $C_{18}H_{16}O_7$, $[M]^{+}$ 344 (FABMS) (found: C, 62.81; H, 4.62. calcd. C, 62.79; H,

Table II. ^{13}C-NMR (90 MHz, DMSO-d_6) spectrum of compound **1**.

No.	δ Value	Assignment
1.	158.4	C-2
2.	135.8	C-3
3.	178.4	C-4
4.	162.5	C-5
5.	98.4	C-6
6.	167.2	C-7
7.	95.2	C-8
8.	157.8	C-9
9.	105.3	C-10
10	122.9	C-1′
11.	118.5	C-2′, C-6′
12.	115.8	C-3′, C-5′
13.	146.5	C-4′
14.	101.5	C-1″
15.	71.3	C-2″
16.	72.5	C-3″
17.	70.8	C-4″
18.	73.4	C-5″
19.	63.2	C-6″
20.	103.8	C-1″
21.	73.5	C-2″
22.	74.4	C-3″
23.	69.8	C-4″
24.	67.5	C-5″
25.	104.5	C-1″
26.	75.5	C-2″
27.	78.4	C-3″
28.	70.3	C-4″
29.	67.4	C-5″

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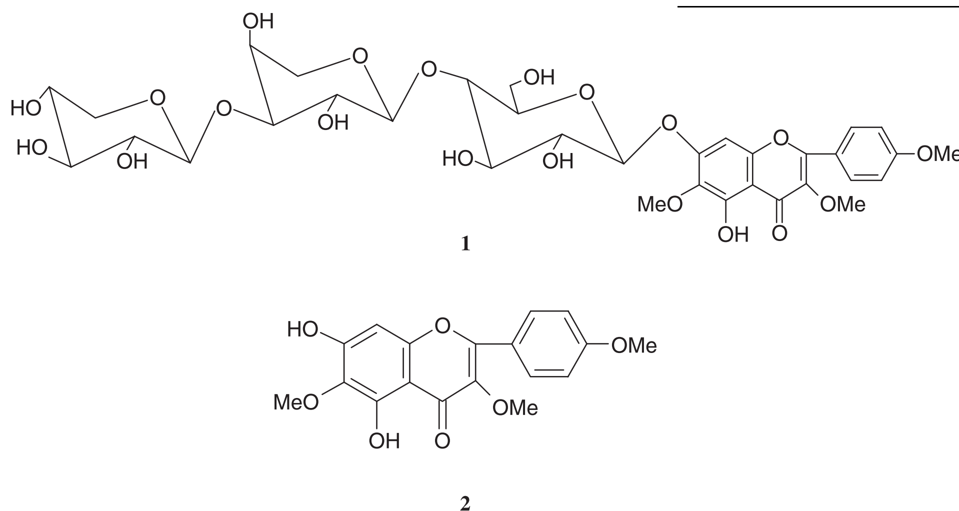
4.65%). It was identified as 5,7 dihydroxy-3,6,4′-trimethoxyflavone as aglycone by comparison of its spectral data with reported literature values.

under reduced pressure and subjected to paper chromatography using n-Butanol: Acetic acid: Water (4:1:5) as solvent and aniline hydrogen phthalate as



spraying reagent. The sugars were identified as 2, 3, 6-tri-O-methyl-D-galactose, 2, 4, di-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-L-xylose (Co-PC).

The aqueous hydrolysate was neutralized with $BaCO_3$, and $BaSO_4$ was filtered off. The filtrate was concentrated under reduced pressure and examined by paper chromatography examination on Whatman filter paper No.1 (30 × 6 cm) using n-Butanol:Acetic acid: Water (B:A:W, 4:1:5) as solvent mixture and aniline hydrogen phathalate as spraying agent. After one hour, the sugars were identified as D-galactose (R_f 0.16), L-arabinose (R_f 0.21) and L-xylose (R_f 0.27) (Co-PC).

Alkaline degradation of compound 2

Compound **2** (50 mg) was treated with 50 ml of 35% KOH in 15 mL of MeOH. The contents were cooled, acidified with HCl and extracted with Et_2O. The ethereal layer was washed with water and dried over Na_2CO_3, to yield a compound with $C_7H_8O_4$, m.p. 217–220°C, $[M]^+$156 and identified as monomethoxy phloroglucinol. The aqueous phase was acidified with HCl, extracted with Et_2O and washed with water to give a compound with $C_8H_8O_3$, mp 182–185°C, $[M]^+$152 and identified as ρ-methoxy benzoic acid.

Permethylation of 1 followed by acid hydrolysis

Compound **1** (35 mg) was treated with 10 ml of MeI and 25 mg of Ag_2O in 12 mL of DMF for two days and then filtered. The filtrate was concentrated under reduced pressure to yield a syrupy mass which was hydrolysed with 12% methanolic H_2SO_4 for 8 h to give a methylated aglycone and methylated sugars and filtered. The methylated aglycone was identified as 7-hydroxy-5,3,6,4′, tetramethoxyflavone. The aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ filtered off. The filtrate was concentrated

Enzymatic hydrolysis of compound 1

Compound **1** (25 mg) was treated with 5 ml of an aqueous solution of takadiastase at 40° for 38 hr to yield L-xylose (R_f 0.27) first, followed by L-arabinose (R_f 0.21) and 5, 7 dihydroxy-3, 6, 4′-trimethoxy flavone–7-O-β-D-galactopyranoside as proaglycone. The proaglycone was hydrolysed with almond emulsion, which liberated D-galactose (R_f 0.16) and the aglycone.

Antifungal activity of compound 1

The antifungal activity of the compound **1** was examined by the Filter Paper Disc Method [9]. The compound (400 mg) was tested at different concentrations using methanol as a solvent. The compound was prepared in methanol and water in different concentrations. The sterile filter paper discs (5 mm diam.) were soaked with various test samples dried at 50°C. Saboraud's broth[10] media with 4% agar was used for the preparation of plates and inoculated with the spore and mycelium suspension (2×10^6 spore/ml) of fungi obtained from a 10 days old culture. The diameters of the zone of inhibition were recorded at 27 ± 1°C after 48 hr, and the results are reported in Table III (See in Results and discussion).

Results and discussion

Compound **1** $C_{34}H_{42}O_{20}$, $[M]^+$**m/z** 770 (FABMS) gave a positive response to Shinoda [4] and Molisch test indicating it to be a flavonoid glycoside. Acid

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Table III. Antifungal activity of compound **1**.

No.	Fungal Species	Inhibition (mm)*					
		Compound 1, at concentration (%)					Std**
		100	80	60	40	20	100
1	*Aspergillus niger*	11.2	7.5	5.6	3.4	2.2	16.9
2.	*Fusarium oxysporum*	18.4	16.3	13.2	11.7	9.1	21.9
3.	*Tricoderma viride*	12.3	8.5	5.2	2.5	1.6	23.1
4.	*Penicillium digitatum*	17.5	15.4	13.4	9.5	6.4	23.5

*The zone of inhibition (mm) taken in different directions; **Griseofulvin used as standard antifungal agent.

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hydrolysis of **1** with 12% methanolic sulphuric acid gave the aglycone **2** identified as 5, 7 dihydroxy-3, 6, 4′ trimethoxy flavone by comparison of its spectral data with reported literature values [5]. The structure of the aglycone was further confirmed by alkaline degradation of **2** which afforded two products; one with $C_7H_8O_4$, mp 217-220°C, $[M]^+156$ identified as monomethoxy phloroglucinol and *ρ*-methoxy benzoic acid mp 182–185°C, $C_8H_8O_3$, $[M]^+152$, which further confirmed the position of the hydroxyl and methoxyl groups in compound **1**.

The ^{1}H-NMR spectrum of compound **1** (see Table I) showed a doublet at δ 6.71 for H-8, δ 7.21 for H-3′, H-5′, doublet at δ 7.78 for H-2′, H-6′, three singlets at δ 3.85, δ 3.93, δ 3.75 for 3-OMe, 6-OMe and 4′-OCH_3, respectively. The anomeric protons of the sugars showed doublets at δ 5.48, δ 4.98, δ 5.63 which were assigned to H-1″, H-1‴ and H-1⁗ of D-galactose, L-arabinose and L-xylose, respectively.

The position of the sugar moiety in compound **1** was established by permethylation [6] of **1** followed by acid hydrolysis. The permethylated sugars, which were identified as 2, 3, 6-tri-**O**-methyl-D-galactose, 2, 4-di-**O**-methyl-**L**-arabinose and 2, 3, 4-tri-**O**-methyl-**L**-xylose [7–8], showed that the C-1⁗ of **L**-xylose was linked with C-3‴ of **L**-arabinose, C-1‴ of **L**-arabinose was linked to C-4″ of **D**-galactose and C-1″ of **D**-galactose was linked to C-7 of the aglycone. The linkage (1 → 3) and (1 → 4) between both the sugars were further confirmed by ^{13}C-NMR spectrum [see Table II].

Enzymatic hydrolysis of compound **1** with taka-diastase enzyme liberated L-xylose first followed by L-arabinose and 5, 7-dihydroxy-3, 6, 4′-trimethoxyflavone 7-**O**-β-D galactopyranoside as proaglycone, which confirmed the presence of the α-linkage between L-xylose and L-arabinose as well as between L-arabinose and the pro-aglycone. The proaglycone on further hydrolysis with almond emulsin enzyme confirmed the presence of the β-linkage between the aglycone and D-galactose.

On the basis of the above evidence, the structure of compound **1** was identified as 5, 7-dihydroxy-3, 6, 4′-trimethoxyflavone-7-O-α-L-xylopyranosyl-(1 → 3)-O-α-L-arabinopyranosyl-(1 → 4)-O-β-D-galacto-pyranoside (**1**).

The results given in Table III show that the antifungal activity of the methanol soluble fraction of compound **1**, was strong (high) against *Fusarium oxysporum* and *Penicillium digitatum* and less active against *Aspergillus niger* and *Tricoderma viride* even at very dilute concentrations. The investigation thus reveals that compound **1** may be potentially useful for diseases caused by these microorganisms.

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