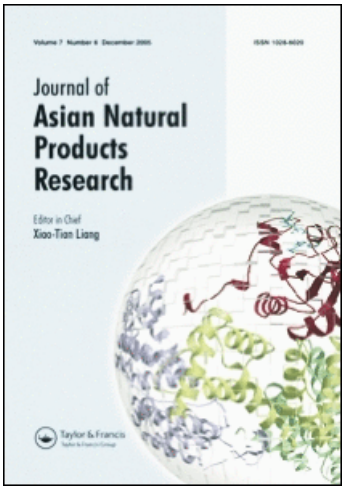

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Note: A potential antiviral flavone glycoside from the seeds of Butea monosperma O. Kuntze

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Note

A potential antiviral flavone glycoside from the seeds of *Butea monosperma O. Kuntze*

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A potential antiviral flavone glycoside has been isolated from the seeds of *Butea monosperma O. Kuntze* and its structure determined as 5,2′-dihydroxy-3,6,7-trimethoxyflavone-5-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-glucopyranoside (**1**) by various spectral analysis and chemical degradations.

Keywords: *Butea monosperma O. Kuntze*; *Leguminosae*: A potential antiviral flavone glycoside

1. Introduction

Butea monosperma, *O. Kuntze* (Leguminosae) is commonly known as "Palash", in Hindi, and is distributed throughout the greater part of India [1,2]. Its bark is used to cure night blindness and elephantiasis, and its seeds are used as an anthelmintic. The flowers are astringent, depurative, diuretic and aphrodisiac. The present paper deals with the isolation and characterization of a potential antiviral flavone glycoside from the seeds of this plant.

2. Results and discussion

The acetone-soluble fraction of the seeds of the plant afforded compound **1**, which was crystallized from methanol as light brownish needles (mp 268–269°C). A molecular formula of $C_{29}H_{34}O_{16}$ was established from elemental analysis and MS data $[M]^+$ 638 (EIMS). It gave a positive response to both the Molisch and Shinoda tests [3]. The IR spectrum of compound **1** shows absorption bands at 3244 (—OH), 2907 (CH), 1652 (C═O) and 1599 (aromatic ring system).

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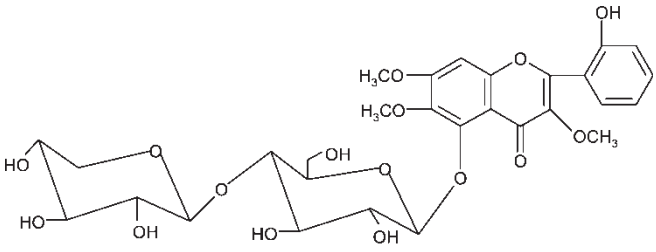
On acid hydrolysis with 10% H_2SO_4 compound **1** gave an aglycone (**2**) with $C_{18}H_{16}O_7$, mp 206–208°C, $[M]^+$ 344(EIMS) and sugars that were identified as D-xylose, and D-glucose by (Co-PC and Co-TLC). The aglycone was identified as 5,2′-dihydroxy-3,6,7-trimethoxyflavone by comparison of its spectral data with that reported in the literature [4].



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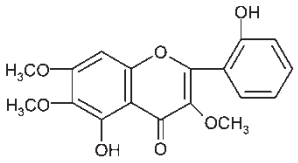

2

The ^{1}H NMR spectrum of **1** shows four aromatic proton signals at δ 7.12 (br, d, $J = 8.2$ Hz), 7.46 (br, t, $J = 8.2$ Hz), 7.08 (br, t, $J = 8.2$ Hz) and 7.67 (dd, $J = 8.3$, 2.2 Hz), assigned to H-3′, H-4′, H-5′and H-6′, respectively, and three proton singlets at δ 3.87, 3.95 and 3.93 due to OMe-3, OMe-6 and OMe-7, respectively. A singlet at δ 6.52 is due to H-8.

Signals for anomeric protons observed at δ 4.42 (1H, d, $J = 7.9$ Hz, H-1″), and 5.44 (1H, d, $J = 8.5$ Hz, H-1‴) are assigned to D-glucose and D-xylose respectively.

The position of sugar moiety in **1** was established by permethylation of **1** [5] followed by acid hydrolysis, affording a methylated aglycone identified as 5-hydroxy-3,6,7,2′-tetramethoxyflavone, which confirmed that the hydroxyl group at C-5 position was involved in the glycosidation; the methylated sugars were identified as 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-xylose, showing that C-1‴ of D-xylose was linked with C-4″ of D-glucose and C-1″ of D-glucose was linked with C-5 of aglycone **2**. The interlinkage (1 → 4) between the sugars was further confirmed by the ^{13}C NMR spectrum (see Experimental).

Periodate oxidation [6] of **1** consumed 3.02 moles of periodate with the liberation of 1.14 moles of formic acid, showing that both sugars are in the pyranose form.

Enzymatic hydrolysis of glycoside **1** by almond emulsin yielded D-xylose, D-glucose and aglycone, confirming a β-linkage between D-xylose and D-glucose as well as between D-glucose and aglycone.

On the basis of the above evidence, compound **1** was identified as 5,2′-dihydroxy-3,6,7-trimethoxyflavone-5-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-glucopyranoside.

3. Experimental

3.1 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 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.

3.2 Plant material

Seeds of *Butea monosperma O. Kuntze* were collected from the Sagar region, and taxonomically identified by Professor T.R. Sahu, Botany Department, Dr H.S. Gour University, Sagar (M.P.), India; a voucher specimen has been deposited in the Herbarium of the Chemistry Department of this University.

3.3 Extraction and isolation

Air-dried and powdered seeds (3 kg) of *Butea monosperma O. Kuntze* were extracted with 95% ethanol in a Soxhlet extractor. The total ethanolic extract was concentrated under reduced pressure to yield a light brown viscous mass (2.53 g), which was successively extracted with light petroleum (60–80°C), benzene, chloroform, ethyl acetate, acetone and methanol. The concentrated acetone-soluble part was then subjected to TLC examination, showing a single spot. It was further purified by column chromatography over silica-gel G column and eluted with $CHCl_3$—CH_3OH in various proportions. The fraction collected from $CHCl_3$—MeOH (5:3) gave compound **1**, which was crystallized from MeOH as light brown needles. It has an mp of 268–269°C and $[M]^+$ 638 (EIMS). (Elemental analysis found (%): C 54.57, H 5.30; calcd for $C_{29}H_{34}O_{16}$, C 54.55, H 5.33.) ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.52 (1 H, s, H-8), 7.12 (1 H, br, d, $J = 8.2$ Hz, H-3′), 7.46 (1H, br, t, $J = 8.2$ Hz, H-4′), 7.08 (1H, br, t, $J = 8.2$ Hz, H-5′), 7.67 (1H, dd, $J = 8.3, 2.2$ Hz, H-6′), 3.88 (3 H, s, OMe-3), 3.95 (3H, s, OMe-6), 3.92 (3H, s, OMe-7), 4.42 (1H, d, $J = 7.9$ Hz, H-1″), 2.94–3.10 (4H, m, H-2″, H-3″, H-4″, H-5″), 3.94 (2H, dd, $J = 2.1, 4.5$ Hz, H-6″), 5.44 (1H, d, $J = 8.5$ Hz, H-1‴), 3.78–3.95 (5H, m, H-2‴, H-3‴, H-4‴, 2H-5‴) ^{13}C NMR (90 MHz, DMSO-d_6) δ (ppm): 155.3 (C-2), 137.3 (C-3), 175.3 (C-4), 152.7 (C-5), 132.6 (C-6), 159.2 (C-7), 90.4 (C-8), 153.1 (C-9), 106.5 (C-10), 118.1 (C-1′), 155.4 (C-2′), 120.7 (C-3′), 133.6 (C-4′), 119.7 (C-5′), 129.6 (C-6′), 104.7 (C-1″), 74.9 (C-2″), 77.4 (C-3″), 71.2 (C-4″), 78.1 (C-5″), 62.5 (C-6″), 106.2 (C-1‴), 72.6 (C-2‴), 76.2 (C-3‴), 71.1 (C-4‴), 68.7 (C-5‴).

3.3.1 Acid hydrolysis of compound 1. Compound **1** was hydrolysed with 10% H_2SO_4 for 2 h, yielding aglycone **2** which was then recrystallised from methanol to yield brownish needles, $C_{18}H_{16}O_7$, mp 206–208°C, $[M]^+$ 344 (EIMS) (elemental analysis, found (%): C 62.81, H, 4.68; calcd for $C_{18}H_{16}O_7$, C, 62.79, H 4.65) and identified as 5,2′-dihydroxy-3,6,7-trimethoxyflavone.

The aqueous hydrolysate was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The concentrated filtrate was then subjected to PC examination using n-BuOH—AcOH—H_2O (4:1:5) as solvent and aniline hydrogen phthalate as detecting agent. The sugars were identified as D-glucose (R_f 0.19) and D-xylose (R_f 0.26) (by Co-PC and Co-TLC).

3.3.2 Permethylation of 1 followed by acid hydrolysis. Compound **1** was treated with MeI and Ag_2O in DMF at room temperature 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 it yielded aglycone **2** and methylated sugars that were identified as 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-xylose (by Co-PC and Co-TLC).

3.3.3 Periodate oxidation of compound 1. Compound **1** was dissolved in MeOH and treated with sodium metaperiodate for 36 h. The liberation of formic acid and consumed periodate were estimated by the Jones' method [6], which suggested that both sugars are in the pyranose form.

3.3.4 Enzymatic hydrolysis of compound 1. Compound **1** was treated with an aqueous solution of almond emulsin (5 ml) and left at room temperature for 24 h to yield D-xylose, D-glucose and aglycone, confirming the presence of a β-linkage between D-xylose (R_f 0.26) and D-glucose (R_f 0.19) as well as between D-glucose and aglycone.

3.3.5 Quantitative estimation of sugars. A quantitative estimation of the sugars in the glycoside was carried out using the procedure of Mishra and Rao, [7] which revealed that two sugars are present in equimolar ratio (1:1).

Acknowledgements

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