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New Polyphenolic Aromatic Glycoside from *Bauhinia variegata* L. Stem Bark

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A new polyphenolic aromatic glycoside characterized as 2,4,8,9,10-pentahydroxy-3,7-dimethoxyanthracene-6-O-α-L-rhamnopyranoside has been isolated from stem bark of *Bauhinia variegata* L. The structure was established primarily on the basis of NMR spectra and chemical transformation.

Key Words: *Bauhinia variegata*, *Leguminosae*, Aromatic glycoside, Favanone.

INTRODUCTION

Bauhinia variegata L. (*Leguminosae*) is a medium sized deciduous tree. The bark is astringent to the bowel and tonic to the liver[1]. Various flavone glycosides have been isolated from the seeds and roots of *B. variegata*[2,3]. The flavonoids isolated from various parts have been reported as effective hypoglycemic agent[4]. In present studies, the isolation and characterization of a new polyphenolic aromatic glycoside along with three known compounds namely 4-O-β-D-glucosylbenzoic acid, kaempferol-7-O-methylether and 5,3'-dihydroxy-6,7,4'-trimethoxyflavanone from stem bark of *B. variegata* are reported.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument at working frequencies 500 and 125 MHz in C_5D_5N at 30 ºC with TMS as standard. Two-dimensional spectra were measured using standard methods of Bruker. IR spectra were recorded on a Shimadzu FTIR 8400S in KBr pellets. UV spectra recorded in Beckman DU 700 UV spectrophotometer. Column chromatography was carried out on silica gel (Kieselgel 60-120 and 70-230 mesh, Merck). Sugars were chromatographed on plates impregnated with 0.3 M solution of NaH_2PO_4. The phenolic hydroxy groups and glycosides were detected by alcoholic ferric chloride and Molisch test.

Stem bark of *Bauhinia variegata* Linn was collected from G.G. University campus in October, 2005 and was authenticated by plant taxonomist Rajasekharan. A voucher specimen (SLT-Med. Plant.-721) was deposited in the S.L.T. Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur (Chhattisgarh, India).

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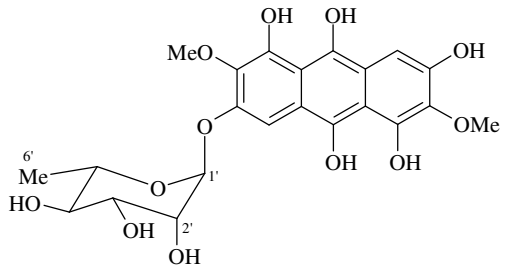
Stem bark were separated from wood and used for extraction. The collected materials were washed thoroughly in water, chopped, air dried for a week at 35-40 ºC and pulverized in electric grinder.

Extraction and isolation: The air-dried and powdered stem bark (2.5 kg) was exhaustively defatted with light petroleum ether (60-80 ºC). The petroleum free mass was then extracted with 80 % aqueous ethanol. The ethanol extract (15 g) was concentrated under reduced pressure in vacuum and dried. A suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with $CHCl_3$:H_2O:MeOH (6:4:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract. The aqueous layer was partitioned with butanol (BuOH:H_2O; 6:4). The butanol layer was concentrated under reduced pressure and digested with MeOH:H_2O (8:2) and filtered. The filtrate was evaporated to dryness under reduced pressure to give methanol extract. The methanol extract (7.0 g) was subjected to column chromatography over Si-gel using gradient elution with $CHCl_3$:MeOH (10:0 → 17:3) to isolate compounds from various fractions. The $CHCl_3$:MeOH (90:10) fraction (3.5 g) was subjected to column chromatography over Si-gel using gradient elution with $CHCl_3$:MeOH (98:2 → 90:10) afforded compound **2** (33 mg) and compound **3** (54 mg). The fractions obtained with $CHCl_3$:MeOH (95:5) was subjected to column chromatography over Sephadex LH-20 eluted with MeOH:$CHCl_3$ (2:1) afforded compound **4** (105 mg) and compound **1** (100 mg).



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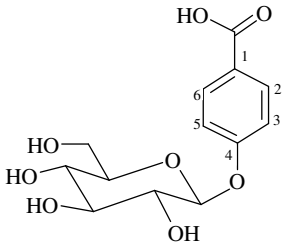

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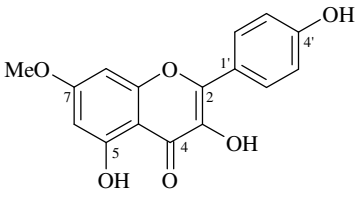

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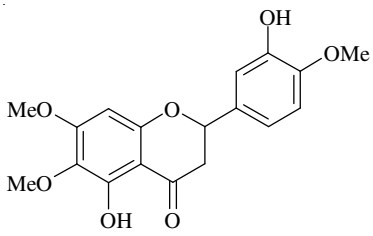

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Structure of compounds **1-4**

2,4,8,9,10-Pentahydroxy-3,7-dimethoxyanthracene-6-O-α-L-rhamnopyranoside (1): White amorphous powder, m.p. 296-298 ºC (MeOH); $[\alpha]_D^{25}$ -36 (c = 0.02, MeOH); IR (KBr, ν_{max}, cm^{-1}): 3430, 2921, 1605, 1582, 1285, 1259, 1210, 1053, 981, 972, 895; UV (MeOH, λ, nm): 228 (3.83), 237 (4.71); HR-FAB MS m/z: 503.11663 $[M + Na]^+$ (calcd. for $C_{22}H_{24}O_{12}Na$: 503.11652); ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 HMz, C_5D_5N) spectra are given in Table-1.

TABLE-1
NMR SPECTRAL DATA OF COMPOUND **1**

C/H	δ C	δ H (*J* in Hz)	HMBC Correlation
1	113.07	8.10, 1H, s	C-2, C-3, C-4a*, C-9, C-9a
2	154.45	–	–
3	141.34	–	–
4	142.42	–	–
4a	113.96	–	–
5	113.18	8.40, 1H, s	C-6, C-7, C-8a*, C-10, C-10a
6	151.37	–	–
7	143.02	–	–
8	141.93	–	–
8a	114.97	–	–
9	159.02	–	–
9a	111.73	–	–
10	159.07	–	–
10a	112.99	–	–
3-OCH_3	61.33	4.20, 3H, s	C-3
7-OCH_3	61.74	4.10, 3H, s	C-7
1'	101.59	6.30, 1H, s	C-6, C-2'
2'	72.62	4.71, 1H, dd (*J* = 2.5, 8.9 Hz)	C-3'
3'	71.77	4.37, 1H, m	C-2'
4'	71.73	4.82, 1H, m	C-5'
5'	73.46	4.38, 1H, m	C-4'
6'	18.59	1.65, 3H, d (*J* = 2.5 Hz)	C-4', C-5'

*: Weak interactions.

Acid hydrolysis of 1: 5 mg compound was dissolved in 5 mL of 7 % H_2SO_4 and refluxed for 3 h on water bath. The reaction mixture was cooled and neutralized with saturated solution of $BaCO_3$ and filtered. The filtrate was extracted with diethyl ether to remove the aglycone. The aqueous layer was concentrated to dryness and the residue was dissolved in 50 % methanol. The sugar was identified as rhamnose by paper chromatography (*n*-BuOH-CH_3COOH-H_2O; 4:1:5) and spraying with aniline hydrogen phthalate.

RESULTS AND DISCUSSION

The methanol extract of the stem bark of *B. variegata* L. on repeated column chromatography led to the isolation of a new compound **1**. Compound **1** was obtained

from methanol as white amorphous solid. It responded positive to alcoholic ferric chloride and Molisch test indicating the presence of phenolic and hydroxyl group(s) and glycosidic nature. The UV maxima at 227 and 238 nm indicated the presence of aromatic ring(s) in the molecule. The IR spectrum showed characteristic absorption bands at 3430 cm^{-1} (for hydroxyl), 2921 cm^{-1} (C-H stretching), 1605 and 1582 (aromatic ring), 1053 cm^{-1} (glycosidic linkage), indicating it to be an aromatic glycoside. The molecular formula $C_{22}H_{24}O_{12}$, was determined on the basis of positive HR-FAB MS data, m/z: 503.11663 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_{12}Na$: 503.11652); ^1H and ^{13}C NMR (C_5D_5N) spectra are given in Table-1.

The ^1H NMR spectrum (Table-1) showed two isolated singlets, each for one proton, at δ 8.10 and 8.40 in the aromatic region indicating the presence of 2-*penta*-substituted aromatic rings in the molecule. In aliphatic region, the ^1H NMR spectrum displayed two singlets each integrated for three protons at δ 4.20 and 4.10 indicating the presence of two methoxy groups in the molecule. In addition to this a singlet at δ 6.30 for one proton attributed for anomeric proton of rhamnose together with other sugar protons. The ^{13}C NMR spectrum displayed presence of 22 carbon atoms, of which 16 were assigned for aglycone moiety and 6 for sugar part. The DEPT spectrum displayed absence of methylene carbons, presence of 3 methyl, 7 methine and 12 quaternary carbons in the molecule. The assignments of protonated carbons were made by COSY and HMQC experiment. The downfield chemical shifts in the region between δ 159.07-141.34 (Table-1) indicated the presence of eight oxygenated carbons atoms in the molecule, which led to the conclusion that the molecule contained anthracene nucleus. The presence of 2-methoxy groups was confirmed by the ^{13}C-chemical shifts at δ 61.3 and 61.7. An α-rhamnopyranosyl moiety was recognized from signals at δ 101.59, 73.46, 72.62, 71.77, 71.73 and 18.39 in the ^{13}C NMR spectrum and signals at 6.30 (1H, s, H-1'), 4.71, (1H, ddI, J = 2.5, 8.9 Hz, H-2'), 4.37 (1H, m, H-3'), 4.82 (1H, m, H-4'), 4.38 (1H, m, H-5'), 1.65 (3H, d, J = 2.5 Hz, H-6') in the ^1H NMR-spectrum. The identification of rhamnose in aqueous solution after hydrolysis of **1** in 7 % H_2SO_4 supported this confirmation, which was coincident with authentic α-L-rhamnopyranose on paper chromatography (PC). The ^{13}C NMR spectrum of **1** contained signals at δ 151.37 attributed to C-6 showing that the hydroxyl group at this carbon is glycosylated. The HMBC spectrum displayed $^3J_{CH}$ long-range correlation between anomeric proton of rhamnose and C-6 (δ 151.37) of anthracene moiety, indicating that the sugar is linked with the C-6 of anthracene. The methoxy protons at δ 4.10 and 4.20 showed long-range correlation with C-7 (143.02) and C-3 (141.34) carbons of anthracene, respectively, which confirmed their location at C-7 and C-3. The other long-range correlation identified from HMBC experiment is shown in Fig. 1, which mapped out the entire molecule. Therefore, compound **1** was determined to be 2,4,8,9,10-*penta*-hydroxy-3,7-dimethoxy-anthracene-6-O-α-L-rhamnopyranoside.

Compounds **2-4** were identified as 4-O-β-D-glucosylbenzoic acid[5], kaempferol-7-O-methylether[6], 5,3'-dihydroxy-6,7,4'-trimethoxyflavanone[7], respectively by comparison of NMR and mass spectral data with the literature values.

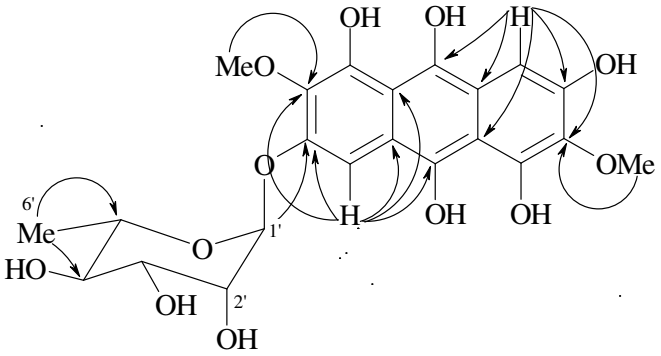

Fig. 1. Key HMBC correlations of **1**

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