

Isolation of New Chalcone from the Leaves of *Bauhinia variegata*

M. MAHESWARA, Y. KOTESWARA RAO, V. SIDDAIAH and C. VENKATA RAO*

Natural Products Division, Department of Chemistry

Sri Venkateswara University, Tirupati-517 502, India

Fax: (91)(877)2248499; Tel: (91)(877)2248457; E-mail: cvr_svu@yahoo.com

A new chalcone, 2'-hydroxy-4',6'-dimethoxy-3,4-methylene-dioxy chalcone (1) together with two known flavonol glycosides, kaempferol-3-O-D-glucopyranoside (2) and kaempferol-3-O- α -L-rhamnoside (3) were isolated from the leaves *Bauhinia variegata*. The structure of 1 was elucidated by ESIMS, UV, IR and 2D NMR spectroscopy including COSY, HSQC and HMBC experiments. These compounds were found to display interesting antifungal activity against *Aspergillus niger* and *Candida albicans*.

Key Words: *Bauhinia variegata*, Flavonol glycosides, A new chalcone, Antifungal activity.

INTRODUCTION

Bauhinia variegata (Caesalpeniaceae) a small size tree, is widely distributed throughout the greater part of India and is used in traditional medicine in the treatment of snake-bite, tumours, antipyretic, skin diseases, leprosy, asthma^{1,2}. Earlier phytochemical investigations³⁻⁷ were limited to the isolation and characterization of few flavonoids only. The present paper reports the isolation and characterization of a new chalcone, 2'-hydroxy-4',6'-dimethoxy-3,4-methylene-dioxy chalcone (1) together with two known flavonol glycosides, kaempferol-3-O- β -D-glucopyranoside (2) and kaempferol-3-O- α -L-rhamnoside (3). These compounds exhibit antifungal activity.

EXPERIMENTAL

The ¹H and ¹³C NMR spectra were recorded on a Bruker AC-400 MHz spectrometer using TMS as internal standard. UV and IR spectra recorded on Shimadzu UV-240 and Perkin-Elmer 240C instrument, respectively. The mass spectra were recorded on API Q-STAR PULSA of applied biosystems. All the compounds were tested for their antifungal activity against *Aspergillus niger* and *Candida albicans*.

The leaves of *Bauhinia variegata* were collected in December 2000 from the Tirumala Hills, Tirupati, India and a voucher specimen (CVR-006) was deposited

in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati. The air-dried and powdered leaves (1.9 kg) were successively extracted with acetone and MeOH. The acetone and MeOH extracts on purification over a silica gel column individually using *n*-hexane/EtOAc and EtOAc/MeOH step gradient yielded **1** (30 mg), **2** (27 mg) and **3** (15 mg), respectively.

2'-Hydroxy-4',6'-dimethoxy-3,4-methylenedioxy chalcone (1): Pale yellow solid; m.p. 164–166°C; UV (MeOH): λ_{\max} (log ϵ) 250 sh (3.28), 304 (3.46), 375 (3.54) nm; (MeOH + NaOMe) 284 sh, 324, 324, 433 nm; (MeOH + NaOAc) 250 sh, 304, 375 nm; (MeOH + AlCl₃) 264, 310, 420 nm; (MeOH + AlCl₃ + HCl) 264, 310, 420 nm; IR (KBr): ν_{\max} 3250 (—OH), 1620 (>C=O), 1580, 1555, 1506, 1460, 1430 and 1296 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) (Table-1); ¹³C NMR (75 MHz, CDCl₃) (Table-1); EIMS: m/z 328 (M⁺) (100), 301 (20), 207 (45), 181 (55), 180 (30), 175 (5), 152 (25), 148 (75)*; 147 (15), 146 (10). ESIMS (m/z): 329.106 (M⁺ + H for C₁₈H₁₇O₆, for 329.3263).

TABLE-1
¹H* AND ¹³C† NMR SPECTRAL DATA OF COMPOUND 1

Position	δ	δ_{H} mult (J = Hz)	HMBC	COSY	NOESY
1	130.0				
2	106.6	7.04, 1H, d (1.6)	C-3, C-4, C-6, C-7	H-6	H-7, H-8
3	148.3				
4	149.5				
5	108.6	6.80, 1H, d (8)	C-1, C-3, C-4, C-6	H-6	H-6
6	125.0	7.07, 1H, dd (1.6, 8)	C-2, C-4, C-5, C-7	H-2, H-5	H-5, H-7
7	142.4	7.73, 1H, d (15.5)	C-1, C-2, C-6, C-8, C-9	H-8	H-2, H-6, H-11
8	125.5	7.68, 1H, d (15.5)	C-1, C-1', C-7, C-9	H-7	H-11, H-2, H-6
9	192.4				
10	101.5	5.99, 2H, s	C-3, C-4		
11	55.5	3.80, 3H, s	C-6'		
12	55.8	3.88, 3H, s	C-4'		
1'	106.3				
2'	168.4	14.34, 1H, s	C-1', C-2', C-3'		
3'	93.8	6.07, 1H, d (2.4)	C-1', C-2', C-4', C-5'	H-5'	H-12
4'	166.0				
5'	91.2	5.93, 1H, d (2.4)	C-1', C-3', C-4', C-6'	H-3'	H-11, H-12
6'	162.4				

*CDCl₃, 400 MHz; †CDCl₃, 75 MHz

RESULTS AND DISCUSSION

Compound 1 was obtained as pale yellow needles, m.p. 164–166°C. It was analyzed for $C_{18}H_{16}O_6$ which is consistent with the presence of a $[M + H]^+$ ion as m/z 329.1016 in its ESIMS spectrum, which was further corroborated by the appearance of all the 18 carbon signals in its proton decoupled ^{13}C NMR spectrum. The UV absorption at 250, 309 and 375 nm and colour reactions suggested that 1 was a chalcone derivative^{8,9}. Addition of sodium acetate did not cause any shift of the UV absorption maxima indicating the absence of a free hydroxyl at C-4'. A bathochromic shift of 45 nm in band I UV absorption maximum with $AlCl_3/HCl$ and a downfield signal at δ 14.34 in its 1H NMR spectrum revealed the presence of a chelated hydroxyl. The IR spectrum showed two strong absorption bands at 3250 and 1620 cm^{-1} due to hydroxyl and conjugated carbonyl functions, respectively.

The proton NMR spectrum of 1 showed a pair of AB doublets ($J = 15.5$ Hz) at δ 7.73 and 7.68 consistent with *trans*-olefinic protons of a chalcone moiety¹⁰. It also exhibited two methoxyl signals at δ 3.88 and 3.80 and they were placed at C-4' and C-6', as they showed NOE cross peaks with H-3', H-5' and H-5' protons, respectively in its NOESY spectrum, further supported by 3J correlation of these methoxyl protons with C-4' and C-6' in its HMBC spectrum (Fig. 1). The signals at δ 6.07 (1H, d, $J = 2.4$ Hz) and 5.93 (1H, d, $J = 2.4$ Hz) correspond to 3',5' protons, respectively of a 2',4',6'-trisubstituted ring-A of a chalcone moiety¹¹. The presence of three aromatic proton signals at δ 7.07 (1H, d, $J = 1.6$ Hz), 7.04 (1H, dd, $J = 8.0, 1.6$ Hz) and 6.80 (1H, d, $J = 8.0$ Hz) in the 1H NMR spectrum of 1 were assigned to protons at 2, 6 and 5 positions of ring B. A strong two proton singlet at δ 5.99 was attributed to a methylenedioxy group at 3 and 4 positions, further evidenced by the presence of two strong 3J correlations with C-3 and C-4 in its HMBC spectrum. The 1H and ^{13}C NMR spectral assignments

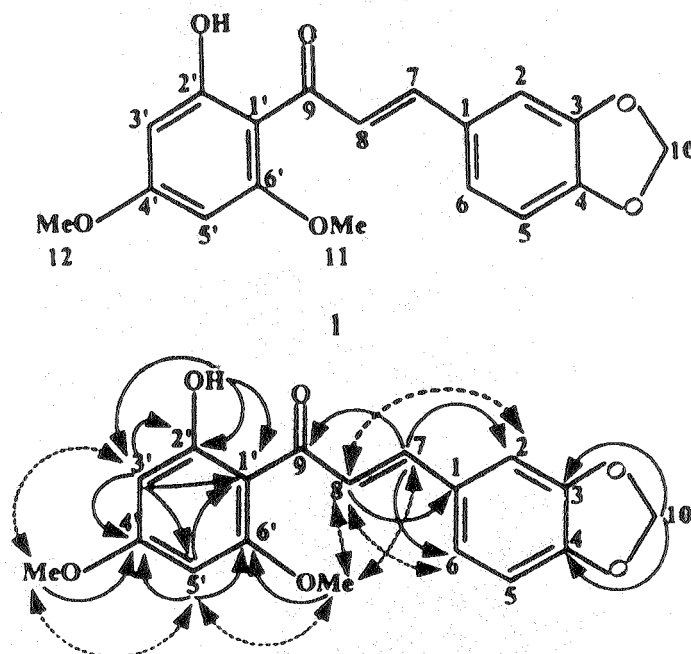


Fig. 1. Significant HMBC (\rightarrow) and NOESY (\leftrightarrow) correlation of 1

were confirmed by ^1H - ^1H COSY, HSQC and HMBC studies. The structure assigned for **1** was further supported by its EIMS which showed the molecular ion peak at m/z 328 and retro-Diels-Alder fragments at m/z 181, 180, 148 and 146 besides other significant fragments at 301, 207, 175, 152 and 147. Thus from the foregoing spectral studies compound **1** was characterized as 2'-hydroxy-4'-6'-dimethoxy-3,4-methylenedioxy chalcone (**1**).

The structures of the known compounds **2** and **3** were established by comparison with the literature data^{12, 13}.

Antifungal activity

These compounds were found to display interesting antifungal activity by filter paper disc method¹⁴. The tests were carried out by taking 5 mm diameter filter paper disc against *Aspergillus niger* and *Candida albicans* (Table-2). Griseofulvin was also tested under similar conditions for comparison.

TABLE-2
ANTIFUNGAL ACTIVITY OF THE COMPOUNDS

S. No.	Microorganism	% (ppm)	1	2	3	GF
1.	<i>Aspergillus niger</i>	25	+++	+++	+++	+++
		50	++++	+++	++++	++++
2.	<i>Candida albicans</i>	22	+++	+++	+++	+++
		50	+++	++++	+++	++++

Inhibition zone diameter in mm: +++: 20–25; ++++: 26–32; GF: griseofulvin.

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