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A flavanone and a dihydrodibenzoxepin from *Bauhinia variegata*

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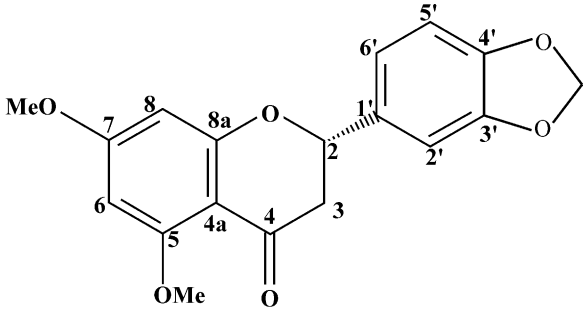
Abstract

Phytochemical analysis of the root bark of *Bauhinia variegata* Linn yielded a new flavanone, (2*S*)-5,7-dimethoxy-3′,4′-methylenedioxyflavanone (**1**) and a new dihydrodibenzoxepin, 5,6-dihydro-1,7-dihydroxy-3,4-dimethoxy-2-methyldibenz [b,f]oxepin (**2**) together with three known flavonoids (**3–5**). The structures of the new compounds were determined on the basis of spectral studies.

Keywords: *Bauhinia variegata*; Leguminosae; Flavonoids; Dihydrodibenzoxepin

1. Introduction

Bauhinia variegata Linn (Leguminosae) is a medium-sized deciduous tree found on the rocky hills of Circars, Deccan, and Carnatic regions of South India (Gamble, 1956). An infusion from its bark is used as an astringent, tonic and useful in scrofula, skin diseases, and ulcers. The decoction of the roots is used in dyspepsia and as an antidote to snake poison (Thammanna et al., 1990). Previous phytochemical studies on the stems (Gupta et al., 1979, 1980, 1984), flowers (Rahman and Begum, 1966; Wahab et al., 1987), and seeds (Yadava and Reddy, 2001) of this species have led to the isolation of several flavonoids. To the best of our knowledge, no phytochemical investigation on the root bark of this species has been reported. The present work on the root bark has resulted in the isolation of a new flavonoid, (2*S*)-5,7-dimethoxy-3′,4′-methylenedioxyflavanone (**1**), and a new dihydrodibenzoxepin, 5,6-dihydro-1,7-dihydroxy-3,4-dimethoxy-2-methyldibenz [b,f]oxepin (**2**) together with three known flavonoids, quercetin 7-methyl ether (**3**) (Barbera et al., 1986), kaempferol 7,4′-dimethyl ether 3-*O*-*β*-D -glucopyranoside (**4**) (Kumar et al., 1985), and kaempferol 3-*O*-*β*-D-glucopyranoside (**5**) (Hari Kishore et al., 2003).



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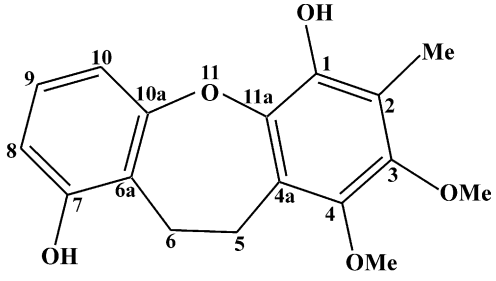

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2. Results and discussion

Compound **1**, obtained as colourless needles, showed an $[M+H]^+$ peak at m/z 329.1097 and an $[M+Na]^+$ peak at m/z 351.0958 in its positive ESITOFMS corre-

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sponding to the molecular formula $C_{18}H_{16}O_6$. The ^{13}C NMR spectrum of 1 showed resonances for all the 18 carbons present in the molecule. The UV absorption maxima of 1 in MeOH at 283 and 320 (sh) nm and negative ferric chloride test suggested compound **1** to be a non-phenolic flavanone (Mabry et al., 1970). The IR spectrum of **1** exhibited two strong absorption bands at 1658 and 940 cm^{-1} attributable to carbonyl function and a methylenedioxy group.

The ^1H NMR spectrum of **1**, showed three sets of double doublets at δ 5.27 (1H, *dd*, $J = 13.0$, 3.0 Hz), 2.95 (1H, *dd*, $J = 16.5$, 13.0 Hz), and 2.71 (1H, *dd*, $J = 16.5$, 3.0 Hz) typical of H-2, H-3_{ax}, and H-3_{eq} of ring-C of a flavanone moiety (Mabry et al., 1970). It also showed signals for two aromatic methoxyl groups at δ 3.78 and 3.85. A sharp two-proton singlet at δ 5.95, correlating with the carbon at δ 101.1 in the HSQC spectrum of 1 indicated a methylenedioxy group. The ESI-MS/MS fragmentation of $[M+H]^+$ ion at m/z 329.1 yielded diagnostic RDA fragments (Ma et al., 1997; Stevens et al., 2000) at m/z 181.0 ($^{1,3}A^+$) and 148.0 ($^{1,3}B^+$) consistent with the presence of two methoxyl groups in ring-A and a methylenedioxy group in ring-B, respectively. The *meta*-coupled doublets at δ 6.05 and 6.10, which correlated to carbons at δ 93.0 and 93.5, respectively, in the HSQC spectrum, were assigned to H-6 and H-8 of ring-A. The methoxyl groups at δ 3.78 and 3.85 were placed at C-5 and C-7 positions, respectively, as they showed ^3J correlation with these carbons at δ 165.8 and 162.1 in the HMBC spectrum. These assignments were also supported by NOE correlations between C-5 methoxy protons and H-6 (δ 6.05), and C-7 methoxy protons with H-6 (δ 6.05) and H-8 (δ 6.10) in the NOESY spectrum. The ^1H NMR spectrum also exhibited a typical ABX spectrum at δ 6.93 (1H, d, $J = 1.7$ Hz), 6.85 (1H, *dd*, $J = 8.0$, 1.7 Hz), and 6.79 (1H, d, $J = 8.0$ Hz) corresponding to three aromatic protons of ring-B. The chemical shift position of C-2 at δ 78.9 in compound **1** indicated that both the C-2′ and C-6′ positions are unsubstituted (Agrawal, 1989). Two strong NOE correlations of H-2 (δ 5.27) with the aromatic protons at δ 6.93 and 6.85 showed their attachment to C-2′ and C-6′, respectively. This fixes the *ortho*-coupled aromatic proton at δ 6.79 to C-5′. On the basis of these observations the methylenedioxy group at δ 5.95 was found to be located between 3′ and 4′ positions. HMBC experiments also confirmed the ring-B to have a methylenedioxy group between C-3′ and C-4′ positions, as these protons (δ 5.95) showed ^3J correlations with C-3′ (δ 147.9) and C-4′ (δ 147.7), which in turn showed cross correlations with H-5′ (δ 6.79) and H-2′ (δ 6.93), respectively. The absolute configuration at C-2 was found to be *S* as it showed positive and negative Cotton effects at 318 and 283 nm, respectively in the CD spectrum (Gaffield, 1970). Thus, the structure of compound 1 was established as (2*S*)-5,7-dimethoxy-3′,4′-methylenedioxyflavanone.

Compound **2**, isolated as colourless needles, showed an $[M+H]^+$ peak at m/z 303.1317 and an $[M+Na]^+$ peak at m/z 325.1138 in its positive ESITOFMS consistent with the molecular formula $C_{17}H_{18}O_5$, supported by the presence of 17 carbon signals in its ^{13}C NMR spectrum. A positive ferric chloride test and a strong IR absorption band at 3420 cm^{-1} indicated the presence of a phenolic hydroxyl in **2**.

The ^1H NMR spectrum of **2** (Table 1) was strikingly similar to pacharin, previously isolated from *Bauhinia racemosa* (Anjaneyulu et al., 1984) except for the presence of signals corresponding to an additional methoxyl group (δ 3.75) and two methylene groups (δ 3.04 and 3.11) in **2** instead of an aromatic proton signal (δ 6.29, s) and two olefinic proton signals (δ 6.65 and 6.89) in pacharin. The additional methoxyl group (δ 3.75) in **2** was placed at C-4 as these protons showed a ^3J correlation with this carbon at δ 143.2 which in turn showed a ^3J correlation with the methylene protons at δ 3.11 in the HMBC spectrum. A strong NOE correlation of the methylene protons at δ 3.11 with C-4 methoxyl protons at δ 3.75 in the NOESY spectrum showed their attachment to C-5. The HMBC correlations of the methylene protons at δ 3.04 with C-5 (δ 22.9), C-7 (δ 154.3), and C-10a (δ 157.8) showed their linkage to C-6 (δ 23.5). The ^1H-^1H COSY correlations observed between the methylene protons at δ 3.11 and the methylene protons at δ 3.04 also supported their attachment to C-5 and C-6, respectively. Thus, the structure of compound **2** was elucidated as 5,6-dihydro-1,7-dihydroxy-3,4-dimethoxy-2-methyldibenz[b,f]oxepin.

Table 1
^1H and ^{13}C NMR spectral data for compound **2**[a] and pacharin[b] (δ in ppm)

H	^1H NMR			^{13}C NMR	
	2	Pacharin	C	**2**	Pacharin
4	–	6.29 (s)	1	142.0	146.8
5	–	6.89 (d, 11.0)	2	116.1	113.4
6	–	6.65 (d, 11.0)	3	148.1	154.1
8	6.54 (dd, 8.10, 1.80)	6.64 (dd, 8.0, 2.0)	4	143.2	111.5
9	6.98 (t, 8.10)	7.12 (t, 8.0)	4a	123.6	127.9
10	6.72 (dd, 8.10, 1.80)	7.00 (dd, 8.0, 2.0)	5	22.9	129.5
5-CH_2	3.11 (m)	–	6	23.5	128.7
6-CH_2	3.04 (m)	–	6a	119.3	118.0
2-Me	2.14 (s)	2.02 (s)	7	154.3	155.1
3-OMe	3.76 (s)	3.71 (s)	8	111.3	100.5
4-OMe	3.75 (s)	-	9	126.9	124.3
1-OH	5.82 (s)	9.45 (s)	10	113.0	112.2
7-OH	4.98 (s)	9.45 (s)	10a	157.8	158.8
			11a	140.0	138.3
			2-Me	8.8	8.5
			3-OMe	60.5	55.4
			4-OMe	61.0	–

[a] Measured in $CDCl_3$.
[b] Measured in DMSO-d_6; multiplicities and coupling constants (J in Hz) in parentheses.

The structures of the known compounds **3** (Barbera et al., 1986), **4** (Kumar et al., 1985), and **5** (Hari Kishore et al., 2003) were established by comparison of their spectral data with literature values.

3. Experimental

3.1. General

Melting points were measured on a Kofler hot-stage apparatus and are uncorr. Optical rotations were measured in MeOH at 25 °C on a Perkin-Elmer 241 polarimeter. UV absorptions were measured in MeOH on a Shimadzu UV-240 spectrophotometer and IR spectra were recorded in KBr discs on a Perkin-Elmer 283 double beam spectrophotometer. The CD spectrum was recorded in MeOH at 25 °C on a JASCO J 715 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were determined on Bruker Avance 400 and Bruker AC 300 spectrometers using DMSO-d_6 and $CDCl_3$ with TMS as internal standard. ^{1}H-^{1}H COSY, HSQC, HMBC, and phase sensitive NOESY (with 500 ms mixing time) spectra were recorded using the standard pulse sequences. ESITOFMS and ESI-MS/MS were recorded in positive ion mode on a API Q-STAR PULSA of Applied Biosystems. CC was performed on Acme silica gel finer than 200 mesh (0.08 mm).

3.2. Plant material

The root bark of *B. variegata* was collected in December 2000 from Tirumala Hills, Andhra Pradesh, South India. A voucher specimen (DG 003) has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, India.

3.3. Extraction and isolation

Air-dried and powdered root bark of *B. variegata* (2.5 kg) was successively extracted with *n*-hexane (3×7 l), Me_2CO (3×7 l), and MeOH (3×7 l). The Me_2CO extract was triturated with *n*-hexane and the hexane insoluble residue was purified over a silica gel column using a *n*-hexane–EtOAc step gradient. The *n*-hexane–EtOAc, 7:3, 1:1, and 1:9 eluates yielded **1** (17 mg), **2** (11 mg), and **3** (9 mg), respectively. The MeOH extract was purified over a silica gel column using a *n*-hexane–EtOAc step gradient. The *n*-hexane–EtOAc (2:8) and EtOAc eluates yielded **4** (21 mg) and **5** (30 mg), respectively.

3.4. (2S)-5,7-Dimethoxy-3′,4′-methylenedioxyflavanone (1)

Colourless needles ($CHCl_3$), mp 192–194 °C; $[\alpha]_D^{25}$ −18.5° (MeOH, *c* 0.1); UV_{max}^{MeOH} nm (log ε): 283 (4.32), 320 (sh) (3.69); (MeOH + NaOAc): 282, 320 (sh); (MeOH + $AlCl_3$): 283, 320 (sh); CD: $\Delta\varepsilon_{283}$ −0.14, $\Delta\varepsilon_{318}$ + 0.04 (MeOH, *c* 0.15); IR ν_{max}^{KBr} cm^{-1}: 2953 (–OMe), 1658 (>C=O), 1600, 1597, 1480, 1450, 1340, 1300, 1280, 1180, 1150, 940 (OCH_2O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.93 (1H, *d*, *J* = 1.7 Hz, H-2′), 6.85 (1H, *dd*, *J* = 8.0, 1.7 Hz, H-6′), 6.79 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.10 (1H, *d*, *J* = 2.3 Hz, H-8), 6.05 (1H, *d*, *J* = 2.3 Hz, H-6), 5.95 (2H, *s*, OCH_2O), 5.27 (1H, *dd*, *J* = 13.0, 3.0 Hz, H-2), 3.85 (3H, *s*, 5-OMe), 3.78 (3H, *s*, 7-OMe), 2.95 (1H, *dd*, *J* = 16.5, 13.0 Hz, H-3$_{ax}$), 2.71 (1H, *dd*, *J* = 16.5, 3.0 Hz, H-3$_{eq}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.0 (C-4), 165.8 (C-7), 164.7 (C-8a), 162.1 (C-5), 147.9 (C-3′), 147.7 (C-4′), 132.4 (C-1′), 119.8 (C-6′), 108.2 (C-5′), 106.6 (C-2′), 105.7 (C-4a), 101.1 (OCH_2O), 93.4 (C-8), 93.0 (C-6), 78.9 (C-2), 56.0 (7-OMe), 55.4 (5-OMe), 45.4 (C-3); ESI-MS/MS (positive mode) *m*/*z* (rel. int.): 329.1 $[M+H]^+$ (1), 181.0 ($^{1,3}A^+$) (60), 166.0 ($^{1,3}A^+$−Me) (41), 153.1 ($^{1,3}A^+$−CO) (3), 151.0 ($^{1,3}A^+$−2Me) (8), 149.0 ($^{0,2}B^+$) (2), 148.0 ($^{1,3}B^+$) (6), 138.0 ($^{1,3}A^+$−Me−CO) (100), 123.0 ($^{1,3}A^+$−2Me−CO) (21), 121.0 ($^{0,2}B^+$−CO) (6); ESITOFMS (positive mode) *m*/*z* 351.0958 $[M+Na]^+$, 329.1097 $[M+H]^+$ ($C_{18}H_{17}O_6$ requires 329.1025).

3.5. 5,6-Dihydro-1,7-dihydroxy-3,4-dimethoxy-2-methyldibenz[b,f]oxepin (2)

Colourless crystalline solid ($CHCl_3$), mp 215–217 °C; UV_{max}^{MeOH} nm (log ε): 225 (sh) (4.32), 279 (3.74); (MeOH + NaOMe): 285, 304; (MeOH + NaOAc): 225, 280; (MeOH + $AlCl_3$): 226, 281; IR ν_{max}^{KBr} cm^{-1}: 3420 (−OH) 2850 (−OMe), 1614, 1480, 1100, 1015, 1005, 960, 850, 800; ^{1}H and ^{13}C NMR data, see Table 1; ESI-MS/MS (positive mode) *m*/*z* (rel. int.): 303.1 $[M+H]^+$ (3), 288.1 $[M+H-Me]^+$ (18), 285.1 $[M+H-H_2O]^+$ (16), 273.1 $[M+H-2Me]^+$ (19), 257.1 $[M+H-Me-OMe]^+$ (15), 243.1 $[M+H-2OMe+2H]^+$ (46), 181.1 (100), 166.0 (70); ESITOFMS (Positive mode) *m*/*z* 325.1138 $[M+Na]^+$, 303.1317 $[M+H]^+$ ($C_{17}H_{19}O_5$ requires 303.1232).

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