



Flavones from *Andrographis viscosula*

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Abstract

Two new 2'-oxygenated flavones, 5,7,2'-trimethoxyflavone (**1**) and 5,7,2',4',6'-pentamethoxyflavone (**2**) were isolated from the whole plant of *Andrographis viscosula* along with three known flavones, echiodinin (**3**), 5,2',6'-trihydroxy-7-methoxyflavone (**4**), and echiodin (**5**). The structures of these compounds were elucidated on the basis of 1D and 2D NMR spectral studies.

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1. Introduction

Andrographis viscosula Nees (Acanthaceae) is an erect herb found widely in the Hills of Tinnevely, South India (Gamble, 1956). In traditional Indian medicine some of the *Andrographis* species are used in the treatment of dyspepsia, influenza, dysentery, malaria and respiratory infections (Kirtikar and Basu, 1975). Continuing our investigations on *Andrographis* species (Damu et al., 1998a,b, 1999; Jayaprakasam et al., 1999, 2001; Jayakrishna et al., 2001), we investigated the whole plant of *A. viscosula* and report here the isolation and structure elucidation of two new flavones 5,7,2'-trimethoxyflavone (**1**) and 5,7,2',4',6'-pentamethoxyflavone (**2**), together with three known flavones, 5,2'-dihydroxy-7-methoxyflavone (**3**), 5,2',6'-trihydroxy-7-methoxyflavone (**4**) and 5,2'-dihydroxy-7-methoxyflavone 2'-O- β -D-glucopyranoside (**5**) (Fig. 1).

2. Results and discussion

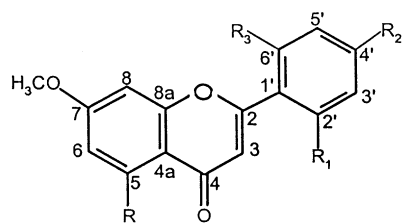
Compound **1** isolated as colourless needles, showed $[M]^+$ peak at m/z 312 and $[M+H]^+$ peak at m/z

313.1077 in its EIMS and HRCIMS, respectively consistent with the molecular formula $C_{18}H_{16}O_5$ which was further supported by the presence of 18 carbon signals in its ^{13}C NMR spectrum. The UV spectrum of **1** in MeOH (261 and 322 nm) suggested a flavone structure and further its UV spectral maxima were unaffected by the addition of $AlCl_3/HCl$ and $NaOAc$ indicating the absence of free hydroxyls at 5 and 7 positions, respectively (Mabry et al., 1970). The IR absorption bands at 1630, 1602 and 1491 cm^{-1} , and negative ferric chloride test indicated that compound **1** had no free hydroxyl groups.

The 1H NMR spectrum of **1** showed signals for three aromatic methoxyl groups at δ 3.90, 3.87 and 3.81, and a sharp one-proton singlet at δ 6.69 characteristic of 2'-oxygenated flavone (Tanaka et al., 1986). Two *meta*-coupled doublets at δ 6.48 and 6.78, each integrating for one proton, were assigned to H-6 and H-8, respectively. It also showed a typical ABCD spectrum for four aromatic protons of ring-B at δ 7.12, 7.21, 7.53 and 7.89 for H-5', H-3', H-4' and H-6', respectively. The EIMS of **1** showed two *retro*-Diels–Alder fragments at m/z 180 and 132 consistent with the presence of two methoxyls groups in ring-A and one methoxyl group in ring-B, respectively. The methoxyl groups at δ 3.81 and 3.87 were placed at C-5 and C-7, respectively as they showed 3J correlation with these carbons at 160.2 and 163.7 ppm in its HMBC spectrum and strong NOE correlations of

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- 1 R, R₁ = OCH₃; R₂, R₃ = H
- 2 R, R₁, R₂, R₃ = OCH₃
- 3 R, R₁ = OH; R₂, R₃ = H
- 4 R, R₁, R₃ = OH; R₂ = H
- 5 R = OH; R₁ = OGlc; R₂, R₃ = H

Fig. 1. Structures of compounds 1–5.

these methoxyl protons with H-6, and H-6 and H-8 in its NOESY spectrum (Fig. 2). The lone methoxyl group at δ 3.90 in ring-B was placed at C-2' as it showed 3J correlation with this carbon at 157.5 ppm in its HMBC spectrum, and a strong NOE correlation with H-3' in its NOESY spectrum (Fig. 2). The ^{13}C NMR spectrum of **1** further supported the presence of a 2'-methoxyl group in **1** as the chemical shifts of ring-B carbons of **1** were very similar to the literature values of 2'-methoxyflavones (Agrawal, 1989). Thus from the foregoing spectral studies the structure of compound **1** was established as 5,7,2'-trimethoxyflavone.

Compound **2** obtained as a pale yellow solid, showed positive ESIMS at m/z 395.0994 $[\text{M} + \text{Na}]^+$ and

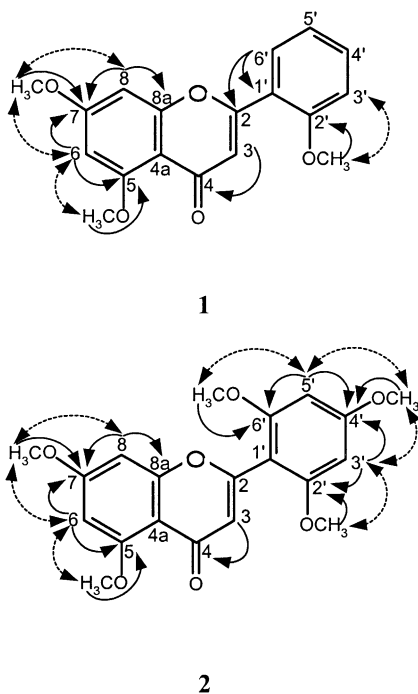


Fig. 2. Significant HMBC ($^2-^3J_{\text{CH}}$) (\longrightarrow) and NOESY (\longleftrightarrow) correlations for **1** and **2**.

373.0698 $[\text{M} + \text{H}]^+$, consistent with the molecular formula $\text{C}_{20}\text{H}_{20}\text{O}_7$, further supported by the presence of 20 carbon signals in its ^{13}C NMR spectrum. The UV absorption maxima of **2** in MeOH at 252 and 300 nm were typical of a flavone derivative (Mabry et al., 1970). Addition of AlCl_3/HCl , NaOAc and NaOMe didn't cause any shift in its UV absorption maxima indicating the absence of free hydroxyl groups at 5 and 7 and 4 positions, respectively. The IR absorption bands of **2** at 1660, 1610 and 1590 cm^{-1} , and negative ferric chloride test indicated compound **2** also had no free hydroxyl groups.

The ^1H NMR spectrum of **2** showed signals for five aromatic methoxyl groups at δ 3.91 (3H), 3.83 (3H), 3.82 (3H) and 3.74 (6H). A sharp one-proton singlet at δ 6.23 and two meta-coupled doublets at δ 6.32 and 6.43 were assigned to H-3, and H-6 and H-8, respectively on the basis of HSQC and HMBC studies (Fig. 2). The ESIMS of **2** showed two *retro*-Diels–Alder fragments, at m/z 181.0568 and m/z 192.0876 indicating the presence of two methoxyl groups in ring-A and three methoxyl groups in ring-B, respectively. A two-proton singlet at δ 6.13 was assigned to H-3' and H-5' as the NOE experiments (Fig. 2) indicated that both the *ortho* positions of these aromatic protons were substituted with methoxyl groups. This clearly showed that ring-B is symmetrically substituted with three methoxyl groups at C-2 (δ 3.74), C-4' (δ 3.83) and C-6' (δ 3.74) positions. This fixes the placement of the remaining two methoxyl groups at C-5 (δ 3.91) and C-7 (δ 3.82) positions, further supported by NOE correlations of these methoxyl protons with H-6, and H-6 and H-8 (Fig. 2) respectively. The ^1H and ^{13}C NMR spectral signals for **2** were unambiguously assigned on the basis of HSQC, HMBC and NOESY studies. Thus from the foregoing spectral studies the structure of **2** was established as 5,7,2',4',6'-pentamethoxyflavone. This is the first report of a 2',4',6'-trioxygenated flavone in Nature.

The structures of the known compounds **3**, **4** and **5** were established by comparison with the literature data (Damu et al., 1998a,b).

3. Experimental

3.1. General

Mps are uncorr. UV spectra were recorded in MeOH and IR spectra were recorded in a KBr disk. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 300, 400 spectrometer using $\text{DMSO}-d_6$, and CDCl_3 with TMS as int. standard. HSQC, HMBC and phase-sensitive NOESY (with 150 ms mixing time) spectra were recorded using the standard pulse sequences. EIMS were recorded at 70 eV (direct probe) on a Nermag R 10–10 mass spectrometer. HRCIMS was obtained on a 700 JEOL mass spectrometer (direct probe) using CH_4 as the ionizing gas at 500°C . ESIMS was registered in

positive ion mode on a Autospec mass spectrometer using NaCl matrix. CC was performed on Acme silica gel finer than 200 mesh (0.08 mm).

3.2. Plant material

The whole plant of *A. viscosula* Nees was collected in December, 1998 from the Hills of Tinnevely, South India. A voucher specimen (CVR-989) was deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

3.3. Extraction and isolation

Dried and powdered whole plant (2.5 kg) was successively extracted with *n*-hexane, Me₂CO and MeOH. The *n*-hexane and Me₂CO extracts on purification over a silica gel column individually using *n*-hexane/EtOAc step gradient yielded **1** (20 mg) and **2** (25 mg); **3** (20 mg) and **4** (20 mg), respectively. The MeOH extract on purification over a silica gel column employing EtOAc/MeOH step gradient afforded **5** (12 mg).

3.4. 5,7,2'-Trimethoxyflavone (**1**)

Colourless needles (CHCl₃), mp 177–178 °C, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 261 (4.32), 322 (4.14); +NaOAc: 261, 322; +AlCl₃: 261, 322; +AlCl₃/HCl: 261, 290 sh, 323. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1699 (>C=O), 1602 (C=C), 1491, 1458, 1427, 1341. ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.89 (1H, *dd*, *J*=8.1, 1.7 Hz, H-6'), 7.53 (1H, *dt*, *J*=8.1, 1.7 Hz, H-4'), 7.22 (1H, *dd*, *J*=8.1, 0.9 Hz, H-3'), 7.13 (1H, *dt*, *J*=7.8, 0.9 Hz, H-5'), 6.69 (1H, *s*, H-3), 6.78 (1H, *d*, *J*=2.3 Hz, H-8), 6.48 (1H, *d*, *J*=2.3 Hz, H-6), 3.90 (3H, *s*, OMe-2'), 3.87 (3H, *s*, OMe-7), 3.81 (3H, *s*, OMe-5); ¹³C NMR (75 MHz, DMSO-*d*₆): see Table 1. HRCIMS (Positive ion mode) *m/z*: 313.1077 [M+H]⁺ (Calc. for C₁₈H₁₇O₅, 313.1075); EIMS *m/z* (rel. int.): 312 [M]⁺ (100), 311 (55), 283 (35), 266 (40), 180 (10), 151 (44), 131 (40).

3.5. 5,7,2',4',6'-Pentamethoxyflavone (**2**)

Pale yellow solid, mp 192–194 °C, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 252 (4.30), 300 (4.09); +NaOAc: 252, 300; +NaOMe: 252, 300; +AlCl₃: 252, 301; +AlCl₃/HCl: 252, 301. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1660 (>C=O), 1610 (C=C), 1590, 1440, 1428, 1340, 1240. ¹H NMR (400 MHz, CDCl₃): δ 6.43 (1H, *d*, *J*=2.2 Hz, H-8), 6.32 (1H, *d*, *J*=2.2 Hz, H-6), 6.23 (1H, *s*, H-3), 6.13 (2H, *s*, H-3', 5'), 3.91 (3H, *s*, OMe-5), 3.83 (3H, *s*, OMe-4'), 3.82 (3H, *s*, OMe-7), 3.74 (6H, *s*, OMe-2',6'); ¹³C NMR (75 MHz, CDCl₃): see Table 1. ESIMS (Positive ion mode) *m/z* (rel. int.): 395.0994 [M+Na]⁺ (10), 373.0698 [M+H]⁺ (100); 329.1014 (5), 192.0876 (15), 181.0568 (100), 177.0625 (65), 167.0400 (65), 166.0323 (50).

Table 1
¹³C NMR data (ppm) of **1** and **2** (75 MHz, CDCl₃)

C	1	2
2	157.4	157.8
3	112.4	116.4
4	175.8	177.8
4a	108.0	109.3
5	160.2	160.8
6	96.1	95.8
7	163.7	163.6
8	93.2	92.8
8a	159.3	160.8
1'	119.4	104.3
2'	157.5	159.5
3'	113.0	90.6
4'	132.5	163.1
5'	120.7	90.6
6'	128.7	159.5
5-OMe	56.1	56.3
7-OMe	55.9	55.6
2'-OMe	55.9	55.9
4'-OMe	—	55.4
6'-OMe	—	55.9

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