

Two New Flavonoids from *Andrographis rothii*

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Two new flavonoids, 5,7,2',5'-tetramethoxyflavanone (1) and 5-hydroxy-7,2'-dimethoxyflavone (2), together with two known flavones, skullcapflavone I (3) and echiodin (4) were isolated from the whole plant of *Andrographis rothii*. The structures of the new compounds were established by extensive one- and two-dimensional (1D- and 2D-) NMR spectral studies.

Key words *Andrographis rothii*; Acanthaceae; flavonoid

Andrographis rothii C. B. CLARKE (Acanthaceae) is a straggling undershrub, occurs widely in the South Carnatic, plains of Tinnevely and dry districts of tropical South India.¹⁾ *Andrographis* species find extensive application in traditional medicine in the treatment of dyspepsia, influenza, malaria and respiratory infections.²⁾ In continuation of our investigations on the flavonoid constituents of *Andrographis* species,^{3–8)} we examined the whole plant of *A. rothii* and report here the isolation and characterization of two new flavonoids (**1**, **2**), in addition to two known flavones (**3**, **4**).

Results and Discussion

Compound **1**, isolated as a colourless solid, showed $[M+H]^+$ peak at m/z 345.1153 in its high resolution electrospray ionisation mass spectrometry (ESI-TOF-MS) corresponding to the molecular formula $C_{19}H_{20}O_6$. This was supported by the appearance of 19 carbon signals in its ^{13}C -NMR spectrum. The UV spectrum of **1** in MeOH at 283 and 324 (sh) nm suggested a flavanone structure.⁹⁾ Its UV absorption maxima was unaffected by the addition of NaOAc and $AlCl_3/HCl$ indicating the absence of free hydroxyls at C-7 and C-5 positions, respectively.

The 1H -NMR spectrum of **1** showed the presence of four methoxyl groups at δ 3.76, 3.79, 3.81 and 3.87. It also exhibited three sets of double doublets at δ 5.72 (1H, dd, $J=12.3$, 4.1 Hz), 2.85 (1H, dd, $J=16.6$, 12.3 Hz) and 2.79 (1H, dd, $J=16.6$, 4.1 Hz) characteristic of H-2, H-3_{ax} and H-3_{eq}, respectively of a flavanone moiety.¹⁰⁾ Two *meta* coupled doublets at δ 6.07 and 6.15, each integrating for one proton, were assigned to H-6 and H-8, respectively. The electrospray ionisation tandem mass spectrometry (ESI-MS/MS) fragmentation of $[M+H]^+$ ion (m/z 345.1) yielded a *retro* Diels–Alder fragment at m/z 181.1 ($^{1,3}A^+$)¹¹⁾ which is consistent with the presence of two methoxyl groups in ring-A, and hence the remaining two methoxyl groups in **1** should be present in ring-B. Of the four methoxyl groups in compound **1**, the one at δ 3.81 was placed at C-7 based on 3J correlations of these protons with C-7 at 165.8 ppm in its heteronuclear multiple bond connectivity (HMBC) spectrum and two strong nuclear Overhauser effect (NOE) correlations with H-6 (δ 6.07) and H-8 (δ 6.15) in its nuclear Overhauser enhancement spectroscopy (NOESY) spectrum (Fig. 1). The second methoxyl group at δ 3.87 was placed at C-5 as these protons showed HMBC correlation with this carbon at 162.3 ppm and

NOE correlation with H-6 in its NOESY spectrum (Fig. 1). The appearance of C-2 signal at an unusually upfield at 74.2 ppm in the ^{13}C -NMR spectrum of **1** indicated C-2' oxygenation in ring-B.¹²⁾ The presence of two aromatic signals at δ 6.82 (2H, m) and 7.14 (1H, d, $J=1.4$ Hz) in the 1H -NMR spectrum of **1** were assigned to H-3' and H-4', and H-6', respectively of ring-B. The chemical shift values of ring-B carbons of **1** were very similar to those observed for ring-B carbons of 2',5'-dioxxygenated flavanones.¹³⁾ The methoxyl groups at δ 3.76 and 3.79 were placed at C-2' and C-5' as they showed NOE correlations with H-3 (δ 2.85) and H-3' (δ 6.82), and H-4' (δ 6.82) and H-6' (δ 7.14), respectively in its NOESY spectrum (Fig. 1). The relative stereochemistry at C-2 was shown to be *S*,¹⁴⁾ as it showed positive and negative Cotton effects at 324 and 283 nm, respectively in its circular dichroism (CD) spectrum. Thus, from the foregoing spectral studies the structure of compound **1** was elucidated as (2*S*)-5,7,2',5'-tetramethoxyflavanone.

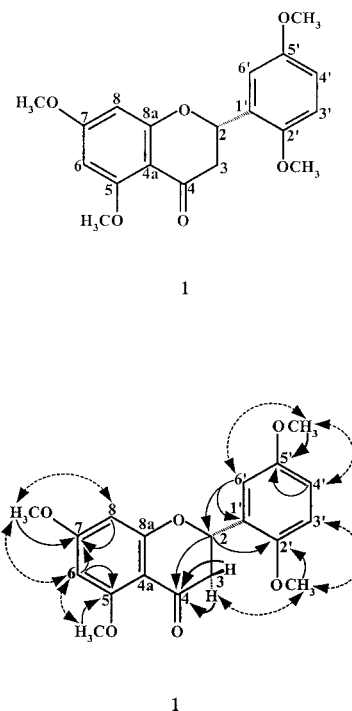


Fig. 1. Significant HMBC (—→) and NOESY (---→) Correlations for **1**

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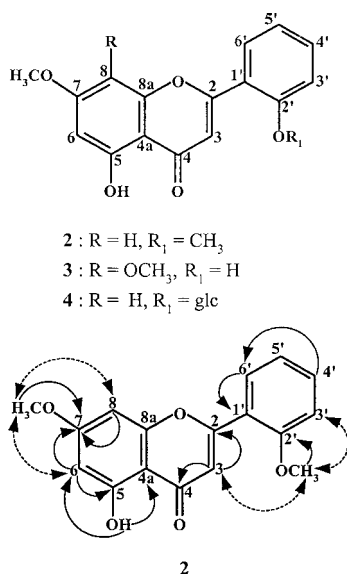


Fig. 2. Significant HMBC (→) and NOESY (↔) Correlations for **2**

Compound **2**, isolated as pale yellow amorphous solid, showed $[M+H]^+$ peak at m/z 299.0859 in its ESI-TOF-MS corresponding to the molecular formula C₁₇H₁₄O₅. The UV absorption maxima of **2** at 266 and 329 nm is typical of a flavone derivative.¹⁵ Its UV spectrum was unaffected by the addition of NaOAc indicating the absence of a free hydroxyl at C-7. A bathochromic shift of 13 nm of band I absorption maximum with AlCl₃/HCl indicated the presence of a chelated hydroxyl group at C-5 position.

The ¹H-NMR spectrum of **2**, showed a D₂O exchangeable downfield signal at δ 12.81 corresponding to a chelated hydroxyl proton at C-5 position. It also exhibited signals for two methoxyl groups at δ 3.92 and 3.85. A set of *meta* coupled doublets ($J=2.2$ Hz) at δ 6.35 and 6.44 were assigned to H-6 and H-8, respectively. A sharp one-proton singlet at δ 7.0 correlated with C-3 in its heteronuclear single quantum coherence (HSQC) spectrum was characteristic of C-3 proton of a 2'-oxygenated flavone.¹⁶ It also displayed the characteristic ABCD signal pattern of a 2'-oxygenated B-ring³) at δ 7.04 (1H, dd, $J=8.3, 1.7$ Hz), 7.09 (1H, ddd, $J=8.3, 7.5, 1.7$ Hz), 7.46 (1H, ddd, $J=8.3, 8.3, 1.7$ Hz) and 7.85 (1H, dd, $J=7.5, 1.7$ Hz) assigned to 3',5',4' and 6' protons, respectively. The methoxyl groups at δ 3.85 and 3.92 were placed at C-7 and C-2' positions, as strong NOEs were observed between the methoxyl protons at δ 3.85 with H-6 (δ 6.35) and H-8 (δ 6.44), and between the methoxyl protons at δ 3.92 with H-3 (δ 7.0) and H-3' (δ 7.04) in its NOESY spectrum (Fig. 2). Thus, from the foregoing spectral studies the structure of compound **2** was elucidated as 5-hydroxy-7,2'-dimethoxyflavone.

The structures of known compounds, **3** and **4** were established by comparison of their spectral data with literature values.^{6,8,17}

Experimental

General Procedures Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The CD spectrum was recorded in MeOH at 25 °C on a JASCO J 715 spectropolarimeter. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. Optical rotations were measured in MeOH at 25 °C on a Perkin-Elmer 241 polarimeter. IR spectra

were determined in KBr discs on a Perkin-Elmer 283 double beam spectrophotometer. ¹H-NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400.13 MHz and ¹³C-NMR spectra on a Bruker AC 300 spectrometer operating at 75.43 MHz in DMSO-*d*₆ and CDCl₃ using tetramethylsilane (TMS) as an internal standard. ¹H-¹H COSY, HSQC, HMBC, NOESY (with 500 ms mixing time) spectra were recorded using the standard pulse sequences. ESI-TOF-MS and ESI-MS/MS were recorded on a API Q-STAR PULSA of Applied Biosystem. EI-MS were obtained on a Nermag R10-10 mass spectrometer at 70 eV by direct inlet probe. FAB-MS was obtained on a 700 JEOL mass spectrometer in glycerol matrix. Column chromatography (CC) separations were carried out by using Acme silica gel (100–200 mesh).

Plant Material The whole plant of *Andrographis rothii* C. B. CLARKE was collected in October 1999 at Tinnevely, Tamil Nadu, South India. A voucher specimen (DG-993) was deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, India.

Extraction and Isolation The shade dried and powdered whole plant (4 kg) of *A. rothii* was exhaustively extracted with MeOH (121×3). The MeOH extract was solvent fractionated with *n*-hexane and Me₂CO. The *n*-hexane soluble portion on purification over a silica gel column with *n*-hexane-EtOAc (7:3) afforded **1** (25 mg). The Me₂CO soluble portion was column chromatographed over silica gel using *n*-hexane-EtOAc step gradients (1:1, 4:6, 1:9) to yield **2** (20 mg), **3** (18 mg) and **4** (15 mg), respectively.

5,7,2',5'-Tetramethoxyflavone (1): Colourless solid (MeOH), mp 198–200 °C [α]_D²⁵ –18.6° ($c=0.14$, MeOH). UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 283 (4.13), 324 (sh) (3.86). IR (KBr) $\nu_{\max}$ cm⁻¹: 2923 (OMe), 1685 ($>C=O$), 1601, 1560, 1492, 1446, 1369, 1276, 1215, 1159. CD ($c=0.14$, MeOH): [θ]₃₂₄+0.21, [θ]₂₈₃–0.68. ¹H-NMR (CDCl₃) δ : 7.14 (1H, d, $J=1.4$ Hz, H-6'), 6.82 (2H, m, H-3', 4'), 6.15 (1H, d, $J=2.2$ Hz, H-8), 6.07 (1H, d, $J=2.2$ Hz, H-6), 5.72 (1H, dd, $J=12.3, 4.1$ Hz, H-2), 3.87 (3H, s, OMe-5), 3.81 (3H, s, OMe-7), 3.79 (3H, s, OMe-5'), 3.76 (3H, s, OMe-2'). 2.85 (1H, dd, $J=16.6, 12.3$ Hz, H-3_{ax}), 2.79 (1H, dd, $J=16.6, 4.1$ Hz, H-3_{eq}). ¹³C-NMR (CDCl₃) δ : 189.8 (C-4), 165.9 (C-8a), 165.8 (C-7), 162.3 (C-5), 153.8 (C-5'), 149.5 (C-2'), 128.5 (C-1'), 113.5 (C-3'), 112.0 (C-6'), 111.5 (C-4'), 106.1 (C-4a), 93.5 (C-8), 93.1 (C-6), 74.2 (C-2), 56.1 (OMe-5), 55.8 (OMe-2',5'), 55.6 (OMe-7), 44.7 (C-3). ESI-MS/MS (positive mode) m/z (%): 345.1 $[M+H]^+$ (3), 191.1 (⁰B⁺) (44), 181.1 (¹A⁺) (100), 176.1 (⁰B⁺–CH₃) (18), 166.1 (¹A⁺–CH₃) (3), 163.1 (⁰B⁺–CO) (3). ESI-TOF-MS m/z : 345.1153 $[M+H]^+$ (Calcd for C₁₉H₂₁O₆: 345.1332).

5-Hydroxy 7,2'-dimethoxyflavone (2): Pale yellow amorphous solid (MeOH), mp 222–224 °C. UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 266 (4.17), 329 (3.95); (AlCl₃) 277, 342; (AlCl₃+HCl) 277, 342. IR (KBr) $\nu_{\max}$ cm⁻¹: 3413 (OH), 2934 (OMe), 1656 ($>C=O$), 1609, 1497, 1454, 1329, 1238, 1159. ¹H-NMR (CDCl₃) δ : 12.81 (1H, s, OH-5), 7.85 (1H, dd, $J=7.5, 1.7$ Hz, H-6'), 7.46 (1H, ddd, $J=8.3, 8.3, 1.7$ Hz, H-4'), 7.09 (1H, ddd, $J=8.3, 7.5, 1.7$ Hz, H-5'), 7.04 (1H, dd, $J=8.3, 1.7$ Hz, H-3'), 7.00 (1H, s, H-3), 6.44 (1H, d, $J=2.2$ Hz, H-8), 6.35 (1H, d, $J=2.2$ Hz, H-6), 3.92 (3H, s, OMe-2'), 3.85 (3H, s, OMe-7). ¹³C-NMR (CDCl₃) δ : 182.8 (C-4), 165.4 (C-7), 162.1 (C-5), 161.3 (C-2'), 158.0 (C-2'), 156.9 (C-8a), 132.6 (C-6'), 129.2 (C-6'), 120.7 (C-1'), 120.3 (C-5'), 111.7 (C-3'), 110.9 (C-3), 105.6 (C-4a), 97.9 (C-6), 92.4 (C-8), 55.8 (OMe-7), 55.7 (OMe-2'). ESI-MS/MS (positive mode) m/z (%): 299.1 $[M+H]^+$ (14), 284.1 $[M+H-CH_3]^+$ (50), 269.1 $[M+H-2CH_3]^+$ (5), 256.0 $[M+H-CO]^+$ (85), 166.0 (¹A⁺) (45), 138.0 (¹A⁺–CO) (100), 133.0 (¹B⁺) (1), 118.0 (¹B⁺–CH₃) (51). ESI-TOF-MS m/z : 299.0859 $[M+H]^+$ (Calcd for C₁₇H₁₅O₅: 299.0915).

Skullcapflavone I (3): Pale yellow needles (MeOH), mp 254–256 °C UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 270 (4.03), 340 (sh) (3.76); (NaOMe) 275, 398; (NaOAc) 270, 340; (AlCl₃) 280, 290, 350, 362 (sh); (AlCl₃+HCl) 275, 293, 315, 355. IR (KBr) $\nu_{\max}$ cm⁻¹: 3434 (OH), 2937, 1651 ($>C=O$), 1611, 1575, 1508, 1453. ¹H-NMR (DMSO-*d*₆) δ : 12.76 (1H, s, OH-5), 9.71 (1H, s, OH-2'), 8.01 (1H, dd, $J=8.0, 1.7$ Hz, H-6'), 7.43 (1H, ddd, $J=8.0, 7.3, 1.7$ Hz, H-4'), 7.14 (1H, s, H-3), 7.11 (2H, m, H-3',5'), 6.49 (1H, s, H-6), 3.97 (3H, s, OMe-7), 3.88 (3H, s, OMe-8). ¹³C-NMR (DMSO-*d*₆) δ : 182.1 (C-4), 161.7 (C-2), 158.4 (C-7), 157.5 (C-2'), 156.6 (C-5), 149.5 (C-8a), 133.0 (C-4'), 128.3 (C-8), 128.2 (C-6'), 119.2 (C-5'), 117.3 (C-3'), 117.2 (C-1'), 108.6 (C-3), 103.5 (C-4a), 95.8 (C-6), 61.1 (OMe-8), 56.7 (OMe-7). EI-MS m/z (%): 314 (M⁺, 44), 299 (100), 284 (1), 271 (8), 257 (2), 196 (2), 181 (19), 168 (3), 153 (45), 121 (4), 118 (9).

Echioidin (4): Yellow needles (MeOH), mp 276–278 °C (dec.). UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 268 (4.38), 320 (4.25); (NaOMe) 275, 375; (NaOAc) 268, 320; (NaOAc/H₃BO₃) 268, 320; (AlCl₃) 280, 291, 342, 378 (sh); (AlCl₃+HCl) 280, 291, 330, 375. IR (KBr) $\nu_{\max}$ cm⁻¹: 3415 (OH), 2942 (OMe), 1660 ($>C=O$), 1554, 1492. ¹H-NMR (DMSO-*d*₆) δ : 12.87 (OH-5),

7.92 (1H, dd, $J=7.5, 2.0$ Hz, H-6'), 7.55 (1H, ddd, $J=7.5, 7.5, 2.0$ Hz, H-4'), 7.35 (1H, dd, $J=7.5, 2.0$ Hz, H-3'), 7.18 (1H, ddd, $J=7.5, 7.5, 2.0$ Hz, H-5'), 7.08 (1H, s, H-3), 6.75 (1H, d, $J=2.0$ Hz, H-8), 6.39 (1H, d, $J=2.0$ Hz, H-6), 5.31 (1H, d, $J=7.0$ Hz, H-1'), 3.86 (3H, s, OMe-7), 3.18–3.80 (6H, m, sugar protons). ^{13}C -NMR (DMSO- d_6) δ : 182.1 (C-4), 165.2 (C-7), 161.1 (C-5), 161.0 (C-2), 157.5 (C-8a), 155.5 (C-2'), 133.0 (C-4'), 129.9 (C-6'), 121.9 (C-5'), 120.0 (C-1'), 115.5 (C-3'), 110.4 (C-3), 104.7 (C-4a), 100.2 (C-1'), 97.9 (C-6), 92.6 (C-8), 77.1 (C-5'), 76.7 (C-3'), 73.3 (C-2'), 69.5 (C-4'), 60.5 (C-6'), 56.0 (OMe-7). FAB-MS (positive mode) m/z (rel. int.): 447 $[\text{M}+\text{H}]^+$ (50), 285 $[\text{M}+\text{H}-\text{glucosyl}]^+$ (100). EI-MS m/z (rel. int.): 284 $[\text{M}-\text{glucosyl}]^+$ (100), 267 (7), 255 (11), 166 (19), 122 (9), 118 (9).

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