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 echioidin (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.

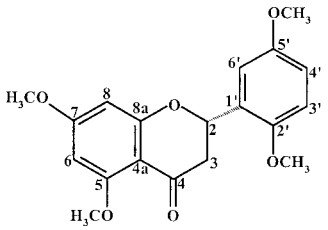
Key words *Andrographis rothii*; Acanthaceae; flavonoid

Andrographis rothii C. B. Clarke (Acanthaceae) is a straggling undershrub, occurs widely in the South Carnatic, plains of Tinnevelly 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**).

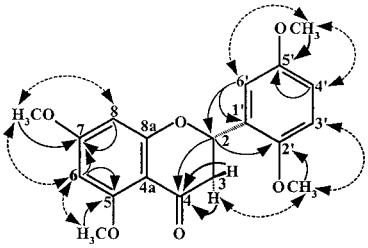
Results and Discussion

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 ^{1}H-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 ^{1}H-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′-dioxygenated 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.



1



1

Fig. 1. Significant HMBC (⟶) and NOESY (⟷) Correlations for **1**

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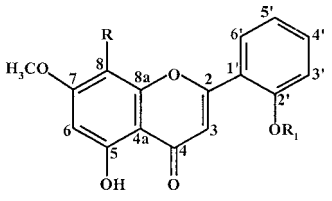
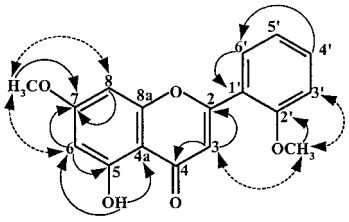


2 : R = H, R_1 = CH_3
3 : R = OCH_3, R_1 = H
4 : R = H, R_1 = glc



2

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 $C_{17}H_{14}O_5$. 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 $AlCl_3$/HCl indicated the presence of a chelated hydroxyl group at C-5 position.

The ^{1}H-NMR spectrum of **2**, showed a D_2O 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-ring[3] 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. ^{1}H-NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400.13 MHz and ^{13}C-NMR spectra on a Bruker AC 300 spectrometer operating at 75.43 MHz in DMSO-d_6 and $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. ^{1}H–^{1}H 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 Tinnevelly, 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 (12 l×3). The MeOH extract was solvent fractionated with *n*-hexane and Me_2CO. The *n*-hexane soluble portion on purification over a silica gel column with *n*-hexane–EtOAc (7 : 3) afforded **1** (25 mg). The Me_2CO 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′-Tetramethoxyflavanone (**1**): Colourless solid (MeOH), mp 198—200 °C $[\alpha]_D^{25}$ −18.6° (c=0.14, MeOH). UV λ_{max} (MeOH) nm (log ε): 283 (4.13), 324 (sh) (3.86). IR (KBr) ν_{max} cm^{-1}: 2923 (OMe), 1685 (>C=O), 1601, 1560, 1492, 1446, 1369, 1276, 1215, 1159. CD (c=0.14, MeOH): $[\theta]_{324}$+0.21, $[\theta]_{283}$−0.68. ^{1}H-NMR ($CDCl_3$) 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}). ^{13}C-NMR ($CDCl_3$) 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 ($^{0,4}B^+$) (44), 181.1 ($^{1,3}A^+$) (100), 176.1 ($^{0,4}B^+-CH_3$) (18), 166.1 ($^{1,3}A^+-CH_3$) (3), 163.1 ($^{0,4}B^+-CO$) (3). ESI-TOF-MS *m/z*: 345.1153 $[M+H]^+$ (Calcd for $C_{19}H_{21}O_6$: 345.1332).

5-Hydroxy 7,2′-dimethoxyflavone (**2**): Pale yellow amorphous solid (MeOH), mp 222—224 °C. UV λ_{max} (MeOH) nm (log ε): 266 (4.17), 329 (3.95); ($AlCl_3$) 277, 342; ($AlCl_3$+HCl) 277, 342. IR (KBr) ν_{max} cm^{-1}: 3413 (OH), 2934 (OMe), 1656 (>C=O), 1609, 1497, 1454, 1329, 1238, 1159. ^{1}H-NMR ($CDCl_3$) 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). ^{13}C-NMR ($CDCl_3$) 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-4′), 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 $[M+H-CH_3-CO]^+$ (85), 166.0 ($^{1,3}A^+$) (45), 138.0 ($^{1,3}A^+-CO$) (100), 133.0 ($^{1,3}B^+$) (1), 118.0 ($^{1,3}B^+-CH_3$) (51). ESI-TOF-MS *m/z*: 299.0859 $[M+H]^+$ (Calcd for $C_{17}H_{15}O_5$: 299.0915).

Skullcapflavone I (**3**): Pale yellow needles (MeOH), mp 254—256 °C UV λ_{max} (MeOH) nm (log ε): 270 (4.03), 340 (sh) (3.76); (NaOMe) 275, 398; (NaOAc) 270, 340; ($AlCl_3$) 280, 290, 350, 362 (sh); ($AlCl_3$+HCl) 275, 293, 315, 355. IR (KBr) ν_{max} cm^{-1}: 3434 (OH), 2937, 1651 (>C=O), 1611, 1575, 1508, 1453. ^{1}H-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). ^{13}C-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 λ_{max} (MeOH) nm (log ε): 268 (4.38), 320 (4.25); (NaOMe) 275, 375; (NaOAc) 268, 320; (NaOAc/H_3BO_3) 268, 320; ($AlCl_3$) 280, 291, 342, 378 (sh); ($AlCl_3$+HCl) 280, 291, 330, 375. IR (KBr) ν_{max} cm^{-1}: 3415 (OH), 2942 (OMe), 1660 (>C=O), 1554, 1492. ^{1}H-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 $[M+H]^+$ (50), 285 $[M+H\text{-glucosyl}]^+$ (100). EI-MS m/z (rel. int.): 284 $[M\text{-glucosyl}]^+$ (100), 267 (7), 255 (11), 166 (19), 122 (9), 118 (9).

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