Two new flavonoids from *Andrographis macrobotrys*

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Two new flavonoids, 5,7,8,2′-tetramethoxyflavanone 1 and 2′-hydroxy-2,3,4′-trimethoxychalcone 3, together with three known flavones, 5-hydroxy-7-methoxyflavone 2, 5,2′,6′-trihydroxy-7-methoxyflavone 4 and 5,7,2′,6′-tetrahydroxyflavone 5 have been isolated from the whole plant of *Andrographis macrobotrys*. The structures of the new compounds 1 and 3 have been established using extensive 2D NMR and ESI-MS/MS studies.

**Keywords:** tetramethoxyflavanone, trimethoxychalcone, methoxyflavone, *Andrographis macrobotrys*

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*Andrographis macrobotrys* Nees (Acanthaceae) is a pubescent herb found widely in Western Ghats, Anamalais, Pulneys and hills of Travancore, South India. In continuation of our investigations on *Andrographis* species, we examined the whole plant of *A. macrobotrys* and report herein the isolation and structure elucidation of two new flavonoids 1 and 3, besides three known flavones 2, 4 and 5.

**Results and Discussion**

Compound 1, isolated as a colourless solid, showed [M + H]^+ peak at m/z 345.1373 in the positive ESITOFMS corresponding to the molecular formula C_{19}H_{20}O_{6}. This was corroborated by the $^{13}$C NMR spectrum which showed 19 carbon resonances. The UV absorption maxima of 1 in MeOH at 262 and 316 (sh) nm and negative ferric chloride test suggested that compound 1 was a non-phenolic flavanone.

The $^1$H NMR spectrum of 1 showed three characteristic signals for H-2, H-3ax and H-3eq at δ 5.76 (1H, dd, J = 12.5, 3.2 Hz), 2.92 (1H, dd, J = 16.7, 12.5 Hz) and 2.83 (1H, dd, J = 16.7, 3.2 Hz) respectively, indicating that 1 had a flavanone skeleton. It also showed signals for four aromatic methoxyl groups at δ 3.79, 3.80, 3.90 and 3.92. The ESI-MS/MS fragmentation of [M + H]^+ ion (m/z 345.1) yield a diagnostic RDA fragment ion at m/z 211.0 ($^{13}$A^+ indicating the presence of three methoxyl groups in ring-A. A sharp one-proton singlet at δ 6.11 correlating with the carbon at δ 89.2 in the HSQC spectrum was assigned to H-6 as it showed HMBC correlations with C-5 (δ 157.8), C-7
Thus, the structure of compound

derivative. A bathochromic shift of 45 nm in band

hydroxyl in doublets (\(\delta\) 6.95, 7.03, 7.30 and 7.59) showed HMBC correlation with H-6 (\(\delta\) 6.11) in the NOESY spectrum (Figure 1). The third methoxyl group at \(\delta\) 3.79 was placed at C-8 as these protons showed HMBC correlation with this carbon at \(\delta\) 130.7 and a strong NOE correlation with H-6' (\(\delta\) 7.59). The methoxyl group at \(\delta\) 3.80 was placed at C-2' as it showed two strong NOE correlations with H-2 (\(\delta\) 5.76) and H-3' (\(\delta\) 6.95) in the NOESY spectrum. The relative stereochemistry at C-2 was shown to be \(S\) as it showed positive and negative cotton effects at 317 and 262 nm, respectively in its CD spectrum. Thus, the structure of compound 1 was elucidated as (2S)-5,7,8,2'-tetramethoxyflavanone. The isolation of compound 1 constitutes a rare case of occurrence of a flavanone with 2'-oxygenation.

Compound 3, obtained as yellow needles, showed a protonated molecular ion peak at m/z 315.1206 in the positive ESI-TOFMS corresponding to the molecular formula C18H18O5. This was corroborated by the decoupled \(^1^3\)C NMR spectrum, which showed 18 carbon resonances. The UV absorption maxima of 3 in MeOH at 252 (sh), 310 and 358 nm and the color reactions suggested that 3 was a chalcone derivative. A bathochromic shift of 45 nm in band I absorption maximum with AlCl\(_3\) and AlCl\(_3\)/HCl and a downfield signal at \(\delta\) 13.40 in the \(^1\)H NMR spectrum of 3 revealed the presence of a chelated hydroxyl in 3.

The \(^1\)H NMR spectrum of 3 showed a pair of AB doublets (\(J = 15.7\) Hz) at \(\delta\) 7.93 and 8.07 consistent with trans olefinic protons of a chalcone moiety. It also showed signals for three methoxyl groups at \(\delta\) 3.82 (6H) and 3.79 (3H). The ESI-MS/MS fragmentation\(^{20}\) of [M + H]\(^+\) ion (m/z 315.1) yields a diagnostic fragment ion at m/z 151.0 (\(^{13}\)A\(^-\)) indicating the presence of a hydroxyl and a methoxyl group in ring-A. Therefore the remaining two methoxyl groups in 3 should be present in ring-B. The signals at \(\delta\) 6.49 (1H, d, \(J = 2.4\) Hz), 6.54 (1H, dd, \(J = 9.0, 2.4\) Hz) and 8.21 (1H, d, \(J = 9.0\) Hz) correspond to 3', 5' and 6' protons, respectively of a 2', 4'-disubstituted chalcone moiety. The methoxyl group at \(\delta\) 3.82 was placed at C-4' as it showed \(^3\)J correlation with C-4' at \(\delta\) 166.0 in the HMBC spectrum and two strong NOE correlations with H-3' (\(\delta\) 6.49) and H-5' (\(\delta\) 6.54) in the NOESY spectrum (Figure 2). The \(\beta\)-carbon in C-2 unsubstituted chalcones usually resonates around 144 (+2) ppm. However, in compound 3 it appeared at \(\delta\) 137.9, which is unusually upfield, indicating C-2 oxygenation in ring-B. The methoxyl group at \(\delta\) 3.82 was placed at C-3, as it showed \(^3\)J correlation with this carbon at \(\delta\) 152.7 and a strong NOE correlation with H-4 (\(\delta\) 7.08) in the NOESY spectrum. The three aromatic proton signals at \(\delta\) 7.08, 7.12 and 7.65 in the \(^1\)H NMR spectrum of 3 were assigned to H-4, H-5 and H-6, respectively. The methoxyl group at \(\delta\) 3.79 was placed at C-2 based on two strong NOE correlations observed for the methoxyl protons with H-\(\beta\) (\(\delta\) 8.07) and H-8 with H-6 (\(\delta\) 7.65) in the NOESY spectrum. This assignment was further evidenced by the appearance of C-2 methoxyl carbon at \(\delta\) 61.0, which is characteristic of a di-ortho-substituted methoxyl group. Thus, the structure of compound 3 was elucidated as 2'-hydroxy-2,3,4'-trimethoxychalcone.
Incidentally, the isolation of compound 3 constitutes the first report of the natural occurrence of a chalcone with 2,3-dioxygenation in the B-ring.

The structures of known compounds, 2, 4 and 5 were established by comparison of their spectral data with literature values7,23,24.

**Experimental Section**

**General.** 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 obtained on a Shimadzu UV-240 spectrophotometer. IR spectra were recorded in KBr discs on a Perkin-Elmer 241 polarimeter. Optical rotations were measured in MeOH at 25 °C with a Perkin-Elmer 283 double beam spectrophotometer. Column chromatography (CC) was performed on Acme silica gel (100-200 mesh). Chromatographic cracking (CC) was performed on Acme silica gel (100-200 mesh).

**Plant material.** The whole plant of *A. macrobotrys* Nees was collected from Anamalai hills of W.Ghats, South India in December 2001. A voucher specimen (DG-007) was deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, India.

**Extraction and isolation**

The air-dried and powdered whole plant of *A. macrobotrys* (2 kg) was successively extracted with *n*-hexane, Me2CO and MeOH. The air-dried and powdered whole plant of *A. macrobotrys* (6H, s, OMe-3, 4′), 3.92 (3H, s, OMe-7), 3.90 (3H, s, OMe-5), 3.80 (3H, s, OMe-2′), 3.79 (3H, s, OMe-8), 2.92 (1H, dd, J = 16.7, 12.5 Hz, H-3eq), 2.83 (1H, dd, J = 16.7, 3.2 Hz, H-2), 3.92 (3H, s, OMe-7), 3.90 (3H, s, OMe-5), 3.80 (3H, s, OMe-2′), 3.79 (3H, s, OMe-8), 2.92 (1H, dd, J = 16.7, 12.5 Hz, H-3eq), 2.83 (1H, dd, J = 16.7, 3.2 Hz, H-2), 3.92 (3H, s, OMe-7), 3.90 (3H, s, OMe-5), 3.80 (3H, s, OMe-2′), 3.79 (3H, s, OMe-8), 2.92 (1H, dd, J = 16.7, 12.5 Hz, H-3eq), 2.83 (1H, dd, J = 16.7, 3.2 Hz, H-2).

**5,7,8,2′-Tetramethoxyflavanone 1:** Colourless solid (CHCl3), m.p. 155-57°C; [α]25D = 19.2° (c 0.18, MeOH); UV (MeOH) (log ε): 262 (4.49), 316 (sh) (4.33) nm; IR (KBr): 2859 (-OMe), 1640 (>C=O), 1580, 1219, 772 cm⁻¹, CD (c 0.4, MeOH): [θ]315 + 0.07, [θ]262 - 0.26; 1H NMR (CDCl3): δ 7.59 (1H, dd, J = 7.52, 1.5 Hz, H-6′), 7.30 (1H, ddd, J = 7.5, 7.5, 1.5 Hz, H-4′), 7.03 (1H, ddd, J = 7.5, 7.5, 1.5 Hz, H-5′), 6.95 (1H, dd, J = 7.5, 1.5 Hz, H-3′), 6.11 (1H, s, H-6), 5.76 (1H, dd, J = 12.5, 3.2 Hz, H-2), 3.92 (3H, s, OMe-7), 3.90 (3H, s, OMe-5), 3.80 (3H, s, OMe-2′), 3.79 (3H, s, OMe-8), 2.92 (1H, dd, J = 16.7, 12.5 Hz, H-3eq), 2.83 (1H, dd, J = 16.7, 3.2 Hz, H-2), 3.92 (3H, s, OMe-7), 3.90 (3H, s, OMe-5), 3.80 (3H, s, OMe-2′), 3.79 (3H, s, OMe-8), 2.92 (1H, dd, J = 16.7, 12.5 Hz, H-3eq), 2.83 (1H, dd, J = 16.7, 3.2 Hz, H-2).

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References
