A New Chalcone and a Flavone from *Andrographis neesiana*

Muntha Kesava Reddy,[a] Mopuru Vijaya Bhaskar Reddy,[a] Bandi Anil Kumar Reddy,[a] Duvvuru Gunasekar,*[,a] Cristelle Caux,[b] and Bernard Bodo[b]

[a] *Natural Products Division, Department of Chemistry, Sri Venkateswara University; Tirupati 517 502, India: and* [b] *Laboratoire de Chimie des Substances Naturelles, ESA 8041 CNRS, Museum National d'Histoire Naturelle; 63 rue Buffon, 75005 Paris, France.* Received January 14, 2003; accepted March 31, 2003

Two new flavonoids, 2′,4′,6′,2,3,4-hexamethoxychalcone (1) and 5-hydroxy-7,2′,5′-trimethoxyflavone (2) together with a known flavone glycoside, echioidinin 5-*O*-β-D-glucopyranoside (3) were isolated from the whole plant of *Andrographis neesiana*, and the structures were elucidated by electrospray ionization tandem mass spectrometry (ESI-MS/MS) and one- and two-dimensional (1D- and 2D)-NMR spectral studies including ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC) and nuclear Overhauser enhancement spectroscopy (NOESY) experiments.

Key words *Andrographis neesiana*; Acanthaceae; flavonoid

Andrographis neesiana Wt.Ic.t. (Acanthaceae) is an erect handsome shrub with brownish purple flowers found in Western Ghats, Anamalais, Pulneys and hills of Tinnevelly, South India.[1)] As part of our continuing investigations on the flavonoid constituents of *Andrographis* species,[2—7)] we have examined the whole plant of *A. neesiana*, a plant hitherto not investigated for its chemical constituents and report here the isolation and structure elucidation of two new flavonoids (**1** and **2**), together with a known flavone glycoside (**3**).

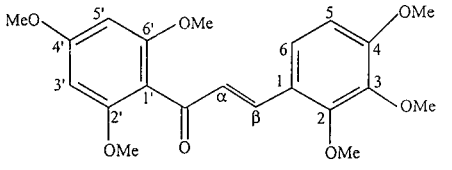
Results and Discussion

Compound **1**, obtained as pale orange-yellow solid, showed $[M+H]^+$ peak at *m*/*z* 389.1340 in the positive electrospray ionization time of flight mass spectrum (ESI-TOF-MS) corresponding to the molecular formula $C_{21}H_{24}O_7$. This was corroborated by the decoupled ^{13}C-NMR spectrum which showed signals for all the twenty one carbons of the molecule. Negative ferric chloride test and the UV absorption maxima of **1** in MeOH at 252 (sh), 308 and 330 nm suggested that compound **1** was a non phenolic flavonoid.[8)]

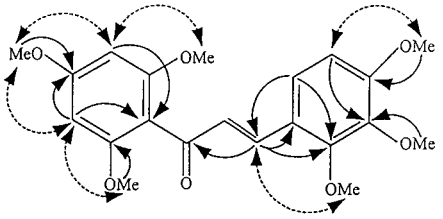
The ^{1}H-NMR spectrum of **1** showed a pair of AB doublets (J=16.2 Hz) at δ 7.55 and 6.90 consistent with *trans* olefinic-protons of a chalcone moiety.[9)] It also showed signals for four aromatic protons at δ 6.13 (2H, s), 6.66 (1H, d, J=8.8 Hz) and 7.29 (1H, d, J=8.8 Hz). It also exhibited six methoxyl groups at δ 3.74 (6H), 3.80 (3H), 3.82 (3H), 3.83 (3H) and 3.86 (3H). A two-proton singlet at δ 6.13 (2H, s) was assigned to 3′ and 5′ protons, respectively of a 2′,4′,6′-trisubstituted ring-A of chalcone moiety.[10)] The electrospray ionization tandem mass spectrometry (ESI-MS/MS) fragmentation of $[M+H]^+$ ion (*m*/*z* 389.1) of **1** yielded two diagnostic fragments at *m*/*z* 195.0 and 194.0 indicating the presence of three methoxyl groups in ring-A and three methoxyl groups in ring-B, respectively. The methoxyl group at δ 3.83 was placed at the C-4′ as it showed ^{3}J correlation with this carbon at 162.2 ppm in the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 1), and two strong nuclear Overhauser effect (NOE) correlations with H-3′ and H-5′ in the nuclear Overhauser enhancement spectroscopy (NOESY) spectrum (Fig. 1). The signal at δ 3.74 (6H, s) correspond to two methoxyl groups at C-2′ and C-6′ as they showed HMBC correlations with these two carbons at 158.7 ppm, and NOE correlations with H-3′ and H-5′ (δ 6.13) in its NOESY spectrum. The β-carbon in C-2 unsubstituted chalcones usually resonates around 144 ($\pm$2) ppm. However, in compound **1** the β-carbon appeared at 139.6 ppm, which is unusually upfield, indicating the presence of C-2 oxygenation.[12)]

The methoxyl group at δ 3.80 was placed at the C-2 position as these protons showed HMBC correlation with this carbon at 152.9 ppm, which in turn showed cross correlation with H-β (δ 7.55). One of the *ortho*-coupled aromatic proton signals at δ 7.29 was assigned to H-6 based on HMBC correlation of this carbon with β-carbon (139.6 ppm). This fixes the other *ortho*-coupled aromatic proton at δ 6.66 to C-5 position. The methoxyl group at δ 3.86 was placed at the C-4 position as they showed a strong NOE correlation with H-5 (δ 6.66). Therefore, the third methoxyl group at δ 3.82 in ring-B should be present at the C-3 position. Thus, from the foregoing spectral studies compound **1** was established as 2′,4′,6′,2,3,4-hexamethoxychalcone. Incidentally, the occurrence of **1** constitutes the first report of a new hexamethoxychalcone with 2,3,4-trioxygenation from an *Andrographis* species.

Compound **2**, isolated as pale yellow solid, showed



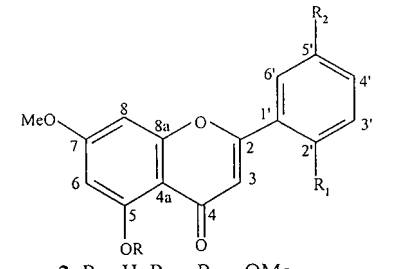
1



1

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

* To whom correspondence should be addressed. e-mail: duvvurusekarg@rediffmail.com



2: R = H, R_1 = R_2 = OMe
3: R = glc, R_1 = OH, R_2 = H

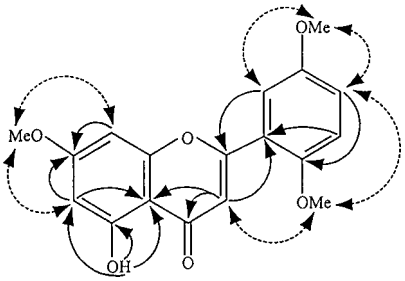


Fig. 2. Significant HMBC (⟶) and NOESY (⇢) Correlations for **2**

$[M+H]^+$ peak at *m/z* 329.0790 in the positive ESI-TOF-MS corresponding to the molecular formula $C_{18}H_{16}O_6$. This was supported by the appearance of 18 carbon signals in the ^{13}C-NMR spectrum. The UV absorption maxima of **2** in MeOH at 277 and 322 nm is typical of a flavone derivative.[13] Its UV spectral maxima was unaffected by the addition of NaOAc indicating the absence of a free 7-hydroxyl group. A bathochromic shift of 31 nm of band I absorption maxima on addition of $AlCl_3$ and $AlCl_3$/HCl, and a downfield signal at δ 12.78 in the ^1H-NMR spectrum of **2** revealed the presence of a chelated hydroxyl group at C-5 position.

A pair of *meta*-coupled doublets at δ 6.37 and 6.45 (1H, J=2.2 Hz), correlated with the carbons at 97.9 and 92.2 ppm, respectively in the heteronuclear single quantum coherence (HSQC) spectrum, were assigned to H-6 and H-8. A sharp one-proton singlet at δ 7.02 which correlated to the carbon at 112.8 ppm in the HSQC spectrum was ascribed to H-3 of a 2′-oxygenated flavone.[14] It also showed three methoxyl groups at δ 3.86, 3.84 and 3.81. The ESI-MS/MS fragmentation[11] of $[M+H]^+$ ion (*m/z* 329.1) of **2** yielded a *retro* Diels–Alder fragment at *m/z* 167.0 ($^{1,3}A^+$) which is consistent with the presence of a methoxyl and a hydroxyl group in ring-A. Therefore, the remaining two methoxyl groups in **2** should be present in ring-B. The methoxyl group at δ 3.84 was placed at C-7 based on HMBC correlation with this carbon at 165.3 ppm and two strong NOE correlations with H-6 (δ 6.37) and H-8 (δ 6.45) in the NOESY spectrum (Fig. 2). The methoxyl group at δ 3.86 was placed at C-2′ based on NOE correlations with H-3 (δ 7.02) and H-2′ (δ 6.94) in the NOESY spectrum. One of the *meta* coupled aromatic proton signals at δ 7.38 was assigned to H-6′ as it showed HMBC correlation with C-2 (160.7 ppm). The methoxyl group at δ 3.81 was placed at C-5′ based on NOE correlations with H-6′ (δ 7.38) and H-4′ (δ 6.99) in the NOESY spectrum. Thus, from the foregoing spectral studies compound **2** was established as 5-hydroxy-7,2′,5′-trimethoxyflavone.

The structure of the known compound **3** was established by comparison of its spectral data with the literature values.[2]

Experimental

General Procedures Melting points were determined on a Kofler hot stage apparatus and are uncorrected. 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 ^{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. ^1H–^1H correlation spectroscopy (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. 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 neesiana* Wt.Ic.t. was collected in December 2000 from Anamalai hills, Tamil Nadu, South India. A voucher specimen (DG-994) has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, India.

Extraction and Isolation The shade dried and powdered whole plant (3.5 kg) of *A. neesiana* was extracted with *n*-hexane, Me_2CO and MeOH. The hexane extract (24 g) on purification over a silica gel column using *n*-hexane–EtOAc (8 : 2) as eluent yielded **1** (20 mg). The Me_2CO (20 g) and MeOH (15 g) extracts were found to be similar on paper and thin layer chromatograms and hence they were combined. The combined extracts (35 g) on purification over a silica gel column employing *n*-hexane–EtOAc (6 : 4) and EtOAc as eluents yielded **2** (42 mg) and **3** (25 mg), respectively.

2′,4′,6′,2,3,4-Hexamethoxychalcone (**1**): Pale orange-yellow solid ($CHCl_3$), mp 174—176 °C. UV λ_{max} (MeOH) nm (log ε): 252 (sh) (3.89), 308 (4.09), 330 (4.16); (MeOH+NaOAc): 252 (sh), 308, 331; (MeOH+$AlCl_3$): 253 (sh), 309, 330. IR (KBr) ν_{max} cm^{-1}: 2904 (–OMe), 1653 (>C=O), 1612, 1504, 1348, 1296, 1219, 851, 772. ^1H-NMR ($CDCl_3$) δ: 7.55 (1H, d, J=16.2 Hz, H-β), 7.29 (1H, d, J=8.8 Hz, H-6), 6.90 (1H, d, J=16.2 Hz, H-α), 6.66 (1H, d, J=8.8 Hz, H-5), 6.13 (2H, s, H-3′, 5′), 3.86 (3H, s, OMe-4), 3.83 (3H, s, OMe-4′), 3.82 (3H, s, OMe-3), 3.80 (3H, s, OMe-2), 3.74 (6H, s, OMe-2′, 6′). ^{13}C-NMR ($CDCl_3$) δ: 194.8 (>C=O), 162.2 (C-4′), 158.7 (C-2′, 6′), 155.4 (C-4), 152.9 (C-2), 142.5 (C-3), 139.6 (C-β), 128.2 (C-α), 123.2 (C-6), 122.1 (C-1′), 111.9 (C-1), 102.6 (C-5), 90.7 (C-3′, 5′), 61.4 (OMe-2), 60.9 (OMe-3), 56.0 (OMe-4), 55.9 (OMe-2′, 6′), 55.4 (OMe-4′). ESI-MS/MS (positive mode) *m/z* (%): 389.1 $[M+H]^+$ (2), 195.0 (59), 194.0 (3), 180.0 (53), 165.0 (8), 163.0 (27), 152.0 (100), 151.0 (38), 137.0 (70). ESI-TOF-MS *m/z*: 411.1234 $[M+Na]^+$ (22) 389.1340 $[M+H]^+$ (100) (Calcd for $C_{21}H_{24}O_7$+H: 389.1600).

5-Hydroxy-7,2′,5′-trimethoxyflavone (**2**): Pale yellow solid (MeOH), mp 196—198 °C. UV λ_{max} (MeOH) nm (log ε): 277 (3.99), 322 (3.54); (MeOH+NaOAc): 277, 322; (MeOH+$AlCl_3$): 277, 353; (MeOH+$AlCl_3$/HCl): 277, 353. IR (KBr) ν_{max} cm^{-1}: 3414 (–OH); 2944 (–OMe), 2842, 1647 (>C=O), 1589, 1492, 1464, 1414, 1339, 1298, 1262, 1229, 1208, 1160, 1127, 1029, 943, 804. ^1H-NMR ($CDCl_3$) δ: 12.78 (1H, br s, OH-5), 7.38 (1H, d, J=3.0 Hz, H-6′), 7.02 (1H, s, H-3), 6.99 (1H, dd, J=9.0, 3.0 Hz, H-4′), 6.94 (1H, d, J=9.0 Hz, H-3′), 6.45 (1H, d, J=2.2 Hz, H-8), 6.37 (1H, d, J=2.2 Hz, H-6), 3.86 (3H, s, OMe-5′), 3.84 (3H, s, OMe-7), 3.81 (3H, s, OMe-2′). ^{13}C-NMR ($CDCl_3$) δ: 182.7 (C-4), 165.3 (C-7), 160.7 (C-2), 157.8 (C-8a), 152.3 (C-2′), 161.9 (C-5), 153.3 (C-5′), 120.5 (C-1′), 117.4 (C-4′), 114.4 (C-6′), 112.8 (C-3), 105.5 (C-4a), 110.9 (C-3′), 97.9 (C-6), 92.2 (C-8), 56.0 (OMe-7), 55.8 (OMe-2′), 55.7 (OMe-5′). ESI-MS/MS (positive mode) *m/z* (%): 329.1 $[M+H]^+$ (24), 314.0 $[M+H-CH_3]^+$ (25), 299.0 $[M+H-2CH_3]^+$ (100), 271.0 $[M+H-2CH_3-CO]^+$ (89), 256.0 $[M+H-3CH_3-CO]^+$ (33), 167.0 ($^{1,3}A^+$) (34), 163.0 ($^{1,3}B^+$) (4), 148.0 ($^{1,3}B^+-CH_3$) (71), 139.0 ($^{1,3}A^+-CO$) (6), 133.0 ($^{1,3}B^+-2CH_3$) (34). ESI-TOF-MS *m/z*: 329.0790 $[M+H]^+$ (100) (Calcd for $C_{18}H_{16}O_6$+H: 329.1025).

Echioidinin 5-*O*-β-D-glucopyranoside (**3**): Pale yellow solid (MeOH), mp 245—246 °C $[\alpha]_D^{28}$ −70.1° (c=0.2, C_5H_5N). UV λ_{max} (MeOH) nm (log ε) 257 (4.38), 305 (4.33), 330 (4.25); (MeOH+NaOMe): 257 (sh), 300 (sh), 400; (MeOH+NaOAc): 257, 305, 342 (sh); (MeOH+NaOAc/H_3BO_3): 257, 305, 342 (sh); (MeOH+$AlCl_3$): 257, 315, 348 (sh); (MeOH+$AlCl_3$/HCl): 257, 288 (sh) 335. IR (KBr) ν_{max} cm^{-1}: 3500 (–OH), 2950 (–OMe), 1652 (>C=O), 1618, 1560, 1450, 1340, 1260, 1204, 1160, 1100, 1088, 1040, 820, 736. ^1H-NMR (DMSO-d_6) δ: 10.70 (1H, br s, OH-2′, exchangeable with D_2O), 7.94 (1H, dd, J=2.0, 8.0 Hz, H-6′), 7.38 (1H, dt, J=2.0, 8.0 Hz,

H-4′), 7.06 (1H, s, H-3), 7.04 (1H, m, H-5′), 7.02 (1H, dd, J=2.0, 8.0 Hz, H-3′), 6.98 (1H, d, J=2.5 Hz, H-8), 6.92 (1H, d, J=2.5 Hz, H-6), 4.78 (1H, d, J=7.0 Hz, H-1″), 3.90 (3H, s, OMe-7), 3.79 (1H, dd, J=12.0, 2.0 Hz, H-6″a), 3.40 (1H, dd, J=12.0, 5.0 Hz, H-6″b), 3.42 (1H, dd, J=7.0, 9.0 Hz, H-2″) 3.40 (1H, dd, J=9.0, 9.0 Hz, H-3″), 3.35 (1H, ddd, J=9.0, 5.0, 2.0 Hz, H-5″), 3.21 (1H, dd, J=9.0, 9.0 Hz, H-4″). ^{13}C-NMR (DMSO-d_6) δ: 177.1 (C-4), 163.5 (C-7), 158.7 (C-8a), 158.5 (C-2), 158.0 (C-5), 156.6 (C-2′), 132.4 (C-4′), 128.2 (C-6′), 119.2 (C-5′), 117.0 (C-1′), 116.9 (C-3′), 112.0 (C-3), 109.1 (C-4a), 104.1 (C-1″), 103.3 (C-6), 96.4 (C-8), 77.5 (C-3″), 75.6 (C-5″), 73.5 (C-2″), 69.8 (C-4″), 60.8 (C-6″), 55.9 (OMe-7). FAB-MS (Positive mode) m/z (%): 447 $[M+H]^+$ (7), 285 $[M+H\text{-glucosyl}]^+$ (24), 284 (5), 166 (5), 149 (6), 138 (16), 107 (10).

Acknowledgements The authors thank Dr. J. P. Brouard, Laboratoire de Chimie des Substances Naturelles, MNHN, Paris, France for providing mass spectral data.

References

1) Gamble J. S., "Flora of the Presidency of Madras," Vol. 2, Botanical Survey of India, Calcutta, 1956, pp. 1048—1051.
2) Damu A. G., Jayaprakasam B., Rao K. V., Gunasekar D., *Phytochemistry*, **49**, 1811—1813 (1998).
3) Damu A. G., Jayaprakasam B., Gunasekar D., *J. Asian Nat. Products Res.*, **1**, 133—138 (1998).
4) Damu A. G., Jayaprakasam B., Gunasekar D., Blond A., Bodo B., *Phytochemistry*, **52**, 147—151 (1999).
5) Jayaprakasam B., Damu A. G., Gunasekar D., Blond A., Bodo B., *Phytochemistry*, **52**, 935—937 (1999).
6) Jayaprakasam B., Gunasekar D., Rao K. V., Blond A., Bodo B., *J. Asian Nat. Products Res.*, **3**, 43—48 (2001).
7) Jayakrishna G., Harikishore P., Venkata Rao C., Gunasekar D., Blond A., Bodo B., *Chem. Pharm. Bull.*, **49**, 1555—1557 (2001).
8) Markham K. R., "Techniques of Flavonoid Identification," Academic Press, New York, 1982, pp. 49—50.
9) Mabry T. J., Markam K. R., Thomas M. B., "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1970, p. 267.
10) Chibber S. S., Sharma R. P., Dutt S. K., *Phytochemistry*, **18**, 2056 (1979).
11) Ma Y. L., Li Q. M., Van den Heuvel H., Claeys M., *Rapid Commun. Mass Spectrom.*, **11**, 1357—1364 (1997).
12) Agrawal P. K., Bansal M. C., "The Carbon-13 NMR of Flavonoids," ed. by Agrawal P. K., Elsevier, Amsterdam, 1989, pp. 368—369.
13) Mabry T. J., Markham K. R., Thomas M. B., "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1970, pp. 41—44.
14) Tanaka T., Inuma M., Mizuno M., *Chem. Pharm. Bull.*, **34**, 1667—1671 (1986).