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Flavonoids and andrographolides from *Andrographis paniculata*

Y. Koteswara Rao [a], G. Vimalamma [b], C. Venkata Rao [b], Yew-Min Tzeng [a,*]

[a] *Department of Applied Chemistry, Chaoyang University of Technology, 168 Gifeng E. Road, Wufeng, Taichung 413, Taiwan*
[b] *Natural Products Division, Department of Chemistry, Sri Venkateswara University, Tirupati 517 502, India*

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Abstract

Two flavonoids, identified as 5,7,2′,3′-tetramethoxyflavanone and 5-hydroxy-7,2′,3′-trimethoxyflavone, as well as several other flavonoids, andrographolide diterpenoids, and polyphenols, were obtained from the phytochemical investigation of the whole plant of *Andrographis paniculata*, a well known medicinal plant. The structures of these compounds were established with the aid of spectroscopic methods, including analysis by 2D NMR spectroscopy.

Keywords: *Andrographis paniculata*; Acanthaceae; Flavonoids; Andrographolide diterpenoids; Benzenoids

1. Introduction

Andrographis is a genus of the Acanthaceae family comprising of about 40 species several members of which enjoy a reputation in traditional medicine. Particularly, *Andrographis paniculata* Nees is used for several applications such as an antidote for snake-bite and poisonous stings of some insects, and to treat dyspepsia, influenza, dysentry, malaria and respiratory infections (Kirtikar and Basu, 1975; Chopra et al., 1980). It is also considered to be a latent-heat clearing, antipyretic, detoxicant, anti-inflammatory, detumescent, febrifugal, antiphlogistic and analgesic agent for the treatment of acute infections of the gastrointestinal tract, respiratory organs and urinary system (Nazimudeen et al., 1978; Choudhury and Poddar, 1985). *A. paniculata* is an erect handsome herb well known in Asia. It occurs widely in the plains of India, Sri Lanka, Mainland China and Taiwan (Gamble, 1956). Previous investigations on the chemical composition of this well studied herb showed that it is a rich source of 2′-oxygenated flavonoids (Govindachari et al., 1969; Jalal et al., 1979; Gupta et al., 1983, 1996; Kuroyanagi et al., 1987), and labdane diterpenoids (Kleipool, 1952; Chan et al., 1971; Balmain and Connolly, 1973; Fujita et al., 1984; Puri et al., 1993; Matsuda et al., 1994; Jantan and Waterman, 1994; Munta et al., 2003). In the present study, we report the isolation and characterization of 23 compounds (**1**–**23**), (Fig. 1) including two new flavonoids, (2*S*)-5,7,2′,3′-tetramethoxyflavanone (**6**) and 5-hydroxy-7,2′,3′-trimethoxyflavone (**12**), as well as 21 known compounds (**1**–**5**, **7**–**11** and **13**–**23**) from the extracts of the whole plant of *A. paniculata.*

2. Results and discussion

The MeOH extract of the whole plant of *A. paniculata* was divided into $CHCl_3$, Me_2CO and MeOH soluble fractions. Each fraction was submitted to a series of chromatographic separations individually to yield two new flavonoids (**6**, **12**) and twenty one known compounds (**1**–**5**, **7**–**11** and **13**–**23**).

Compound **6**, obtained as colourless solid, gave a molecular ion peak at m/z 344.1331 in its HREIMS corresponding to the molecular formula $C_{19}H_{20}O_6$. This was further supported by ^{13}C NMR spectral analysis, which displayed 19 signals for all carbon atoms in the molecule including one carbonyl, seven nonprotonated, six methine, one methylene and four methoxyl carbons. The UV spectrum of **6** in MeOH at 263 and 336 nm

* Corresponding author. Tel.: +886-4-23304898/23323000x3003; fax: +886-4-23304896.
E-mail address: ymtzeng@cyut.edu.tw (Y.-M. Tzeng).

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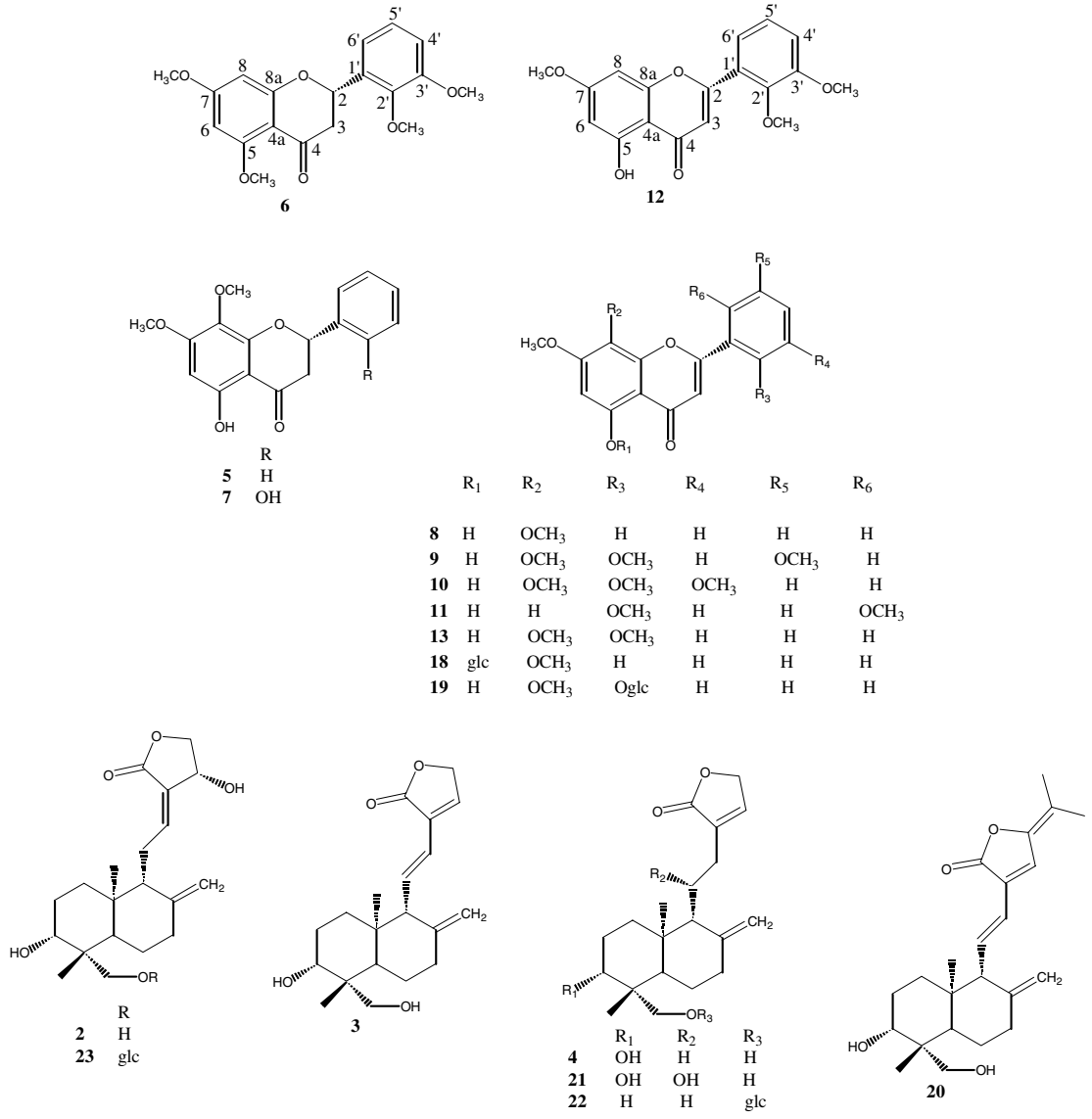

Fig. 1. Structures of compounds isolated from *A. paniculata*.

suggested a flavanone skeleton for the molecule (Mabry et al., 1970); 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 **6** showed the presence of four methoxyl groups at δ 3.81, 3.86, 3.88 and 3.90. It also exhibited three sets of double doublets of an AMX system at δ 5.76 (1H, $J = 13.3$, 3.0 Hz), 2.98 (1H, $J = 16.7$, 13.3 Hz) and 2.77 (1H, $J = 16.7$, 3.0 Hz) which were characteristic of H-2, H-3_{ax} and H-3_{eq}, respectively, of the ring C of a flavanone moiety (Mabry et al., 1970). Two *meta*-coupled doublets ($J = 2.3$) at δ 6.09 and 6.14, each integrating for one proton, were assigned to H-6 and H-8, respectively. The EIMS of compound **6** displayed diagnostic peaks of retro-Diels–Alder (RDA) cleavage of ring C at m/z 180 and 164 suggesting the presence of two methoxyl groups in ring A, and hence the remaining two methoxyl groups should be in ring B. Two of the four methoxyl groups at δ 3.88 and 3.81 were situated on C-5 and C-7, as they showed HMBC correlations with the carbons at 162.2 and 165.8 ppm, assignable to C-5 and C-7, respectively. These assignments were further supported by NOE correlations of the methoxyl protons (δ 3.88) with H-6 (δ 6.09), and the methoxyl protons (δ 3.81) with H-6 (δ 6.09) and H-8 (δ 6.14) in the NOESY spectrum. The C-2 signal in 2′-unsubstituted flavanones usually appears at δ 79.0. However, in compound **6**, the C-2 signal appeared at an upfield position (δ 74.2), indicating the presence of C-2′ oxygenation in ring B (Agrawal, 1989). Thus a methoxyl group at δ 3.90 was attached to C-2′ on the basis of its HMBC correlation with C-2′(δ 146.8), and was further confirmed by its NOE correlation with H-3_{eq}. The final

methoxyl group at δ 3.86 was placed at C-3′, as evidenced by its HMBC correlation with C-3′ at δ 152.5, and two strong NOEs with 2′-OMe and a proton at δ 6.93 (H-4′) (Fig. 2). The remaining aromatic proton signals at δ 6.93 (1H, *dd*, $J = 7.0$, 2.7 Hz) and 7.15 (2H, *m*) were attributed to H-4′ and H-5′ and H-6′, respectively, of ring B. The chemical shift values of ring B carbons of **6** were similar to those observed for ring B carbons of 2′,3′-dioxygenated flavanones (Kojima et al., 1997). The absolute configuration at C-2 was shown to be *S*-configuration (Iinuma et al., 1994), as the CD spectrum of **6** exhibited a negative Cotton effect at 292 nm ($\Delta\varepsilon - 1.05$) and positive Cotton effects at 262 nm ($\Delta\varepsilon + 0.53$) and 338 nm ($\Delta\varepsilon + 0.46$). Thus, from the foregoing spectral studies the structure of compound **6** was elucidated as (2*S*)-5,7,2′,3′-tetramethoxyflavanone.

Compound **12,** isolated as a yellow amorphous solid, gave a positive ferric chloride test. Its HREIMS displayed a $[M]^+$ peak at m/z 328.1052 consistent with the molecular formula $C_{18}H_{16}O_6$. This was further corroborated by the 18 carbon signals in its ^{13}C NMR spectrum, which include a conjugated carbonyl, eight nonprotonated, six methine and three methoxyl carbons. The UV absorption maxima of **12** at 241 and 327 nm were typical of a flavone derivative (Mabry et al., 1970). The UV spectrum was unaffected by the addition of NaOAc again suggesting the absence of a free hydroxyl at C-7. A bathochromic shift of 42 nm of the band I absorption maximum with $AlCl_3$/HCl indicated the presence of a chelated hydroxyl group at the C-5 position. The IR spectrum exhibited bands at 3158 and 1656 cm^{-1} corresponding to hydroxyl and α,β-unsaturated carbonyl functionalities, respectively.

The ^1H NMR spectrum of **12** showed a D_2O exchangeable downfield signal at δ 12.82 assignable to a hydrogen-bonded hydroxyl group at C-5. A pair of *meta*-coupled doublets ($J = 2.2$ Hz) at δ 6.36 and 6.44 were attributed to H-6 and H-8, respectively. It also exhibited signals due to three methoxyl groups at δ 3.92, 3.89 and 3.86. The EIMS fragmentation of the molecular ion at m/z 328 of **12** in its RDA cleavage at ring C yielded diagnostic peaks at m/z 166 and 162 thereby inferring that a hydroxyl and a methoxyl group were in ring A and two methoxyl groups were in ring B of the molecule. The methoxyl group at δ 3.89 was placed at C-7, on the basis of its ^3J correlation with a carbon at δ165.5 (C-7) in the HMBC spectrum and NOE crosspeaks with H-6 (δ 6.36) and H-8 (δ 6.44) in NOESY spectrum. A sharp one-proton singlet at δ 6.98 correlating with C-3 (110.5 ppm) in the HSQC spectrum was characteristic of H-3 of a 2′-oxygenated flavone (Tanaka et al., 1986). A typical ABC spectrum at δ 7.07 (1H, *dd*, $J = 8.1$, 1.5 Hz), 7.21 (1H, *dd*, $J = 8.1$, 7.9 Hz) and 7.33 (1H, *dd*, $J = 7.9$, 1.5 Hz) for three adjacent protons, 4′, 5′ and 6′ protons, respectively, established a 2′,3′-dioxygenated B ring in the molecule (Kuroyanagi et al., 1987). Thus, the methoxyl groups at δ 3.92 and 3.86 were placed at C-2′ and C-3′ positions, as they have HMBC connectivities with C-2′ (δ 148.0) and C-3′ (δ 153.3), respectively. This was also inferred by the NOEs, OCH_3-2′/OCH_3-3′, OCH_3-3′/H-4′, and H-5′/H-4′, H-6′ in the NOESY experiment (Fig. 2). From these findings, compound **12** was established as 5-hydroxy-7,2′,3′-trimethoxyflavone.

The structures of known isolates (Fig. 1) from the whole plant of *A. paniculata* were identified by comparison of their spectral data with literature values as β-sitosterol (**1**) (Ali et al., 2002); andrographolide (**2**), 14-deoxy-ll, 12-dedihydroandrographolide (**3**) and 14-deoxyandrographolide (**4**) (Matsuda et al., 1994); 7-*O*-methyldihydrowogonin (**5**) (Gupta et al., 1983); dihydroskullcapflavone I (**7**) (Hari Kishore et al., 2003); 7-*O*-methylwogonin (**8**) (Kuroyanagi et al., 1987); 5-hydroxy-7,8,2′,5′-tetramethoxy-flavone (**9**) (Mopuru et al., 2003); 5-hydroxy-7,8,2′,3′-tetramethoxyflavone (**10**) (Kuroyanagi et al., 1987); 5-hydroxy-7,2′,6′-trimethoxyflavone (**11**) (Munta et al., 2003); skullcapflavone 12′-methylether (**13**) (Jalal et al., 1979); cinnamic acid (**14**), caffeic acid (**15**), ferulic acid (**16**) and chlorogenic acid (**17**) (Satyanarayana et al., 1978); 7-*O*-methylwogonin 5-glucoside (**18**) (Kuroyanagi et al., 1987); skullcapflavone I 2′-glucoside (**19**) (Gupta et al., 1996); 14-deoxy- 15-isopropylidene-11,12-didehydro-andrographolide (**20**) (Munta et al., 2003); 14-deoxy-11-hydroxyandrographolide (**21**), neoandrographolide (**22**) and andrographoside (**23**) (Matsuda et al., 1994).

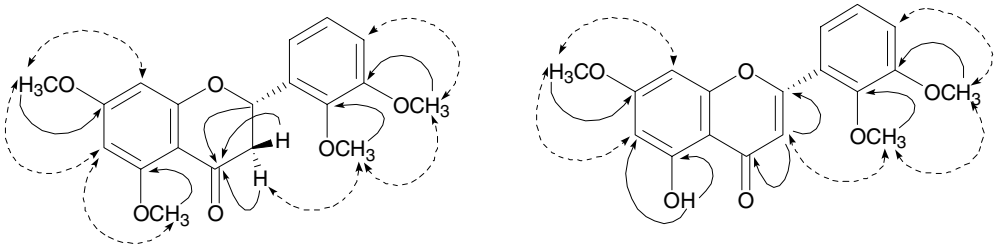

Fig. 2. Significant HMBC (→) and NOESY (⇢) correlations of **6** and **12**.

Isolation of two new and nine known 2′-oxygenated flavonoids, which occur rarely in nature, in addition to seven andrographolide diterpenoids, from *A. paniculata* provided strong evidence for the statement, "*Andrographis* species are noted for profuse production of 2′-oxygenated flavonoids and andrographolide diterpenoids" (Iinuma and Mizuno, 1989). Accordingly, the above class of compounds isolated from Acanthaceae so far were confined to *Andrographis* species only; this shows promise of being a useful chemotaxonomic marker for *Andrographis* in the Acanthaceae.

3. Experimental

3.1. General

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. CD spectra were recorded in MeOH at 25 °C on a JASCO J 715 spectropolarimeter, where UV spectra were obtained on a Shimadzu UV-240 spectrophotometer. Optical rotations were measured in MeOH at 25°C on a Perkin–Elmer 241 Polarimeter, whereas IR spectra were determined KBr discs using 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 $CDC1_3$ using TMS as internal standard. ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY (with 150 ms mixing time) spectra were recorded using the standard pulse sequences. EIMS were obtained on a Nermag RI0-10 mass spectrometer at 70 eV by direct inlet probe, whereas HREIMS were recorded on a Jeol JMS HX 110 mass spectrometer. CC was performed on Acme Si gel finer than 200 mesh (0.08 mm).

3.2. Plant material

The whole plant of *A. paniculata* Nees was collected in September 2002 at Tirumala Hills, Andhra Pradesh, S. India. A voucher specimen (No. 0024/AP) has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, India.

3.3. Extraction and isolation

The air-dried and powdered whole plant (10.5 kg) of *A. paniculata* was extracted with MeOH (5×151, reflux for 8 h) and the combined extracts were evaporated in vacuo to yield a dark brown residue (1.2 kg). This was fractionated by its solubility in $CHCl_3$, Me_2CO, and MeOH, respectively. The residue (650 g) obtained from evaporation in vacuo of the $CHCl_3$ solubles was defatted with *n*-hexane and then separated by Si gel CC using *n*-hexane–EtOAc step gradients as eluents to afford five fractions (I–V). Fraction I yielded **1** (64.5 mg) and **3** (1.3 g) on purification with Si gel column by eluting with the mixtures of *n*-hexane and EtOAc. Fraction II gave **2** (2.2 g), **4** (1.2 g), and **5** (25.6 mg) after a series of chromatographic separations with mixtures of *n*-hexane and EtOAc. Fraction III was subjected to Si gel CC with *n*-hexane–EtOAc step gradients followed by prep. TLC, developed with benzene:acetone (9:1) to give **6** (17.1 mg) and **7** (5.2 mg). Workup of fraction IV by Si gel column afforded **7** (25.2 mg) and **8** (32.3 mg). The Me_2CO solubles were defatted with *n*-hexane and the residue (150 g) obtained was purified over a Si gel column using *n*-hexane and EtOAc step gradient mixtures as eluents to give three fractions. Workup of these three fractions, individually by repeated Si gel CC with $CHCl_3$–EtOAc mixtures followed by prep. TLC developed with benzene:acetone (7:3) yielded **9** (18 mg) and **10** (25 mg); **11** (20 mg) and **12** (15 mg); and **13** (22 mg), respectively. The MeOH solubles were extracted with *n*-hexane using a soxhlet apparatus. The *n*-hexane insoluble portion was concentrated to dryness and the residue obtained (250 g) was subjected to Si gel CC using $CHCl_3$–MeOH step gradients to give four fractions A–D. Fraction A was purified with Si gel column using CHC_3–MeOH step gradients followed by prep.TLC with $CHCl_3$–MeOH (9:1) to obtain **14** (18 mg) **15** (10 mg), **16** (5 mg), and **17** (12 mg). Fraction B on repeated CC over Si gel using $CHCl_3$–MeOH step gradients afforded **18** (22 mg), **19** (33 mg), and 20 (14 mg). Fraction C on purification with Si gel CC by eluting with $CHCl_3$–MeOH step gradients gave **21** (15 mg), **22** (20 mg), and **23** (28 mg).

3.4. 5,7,2′,3′-Tetramethoxyflavanone (*6*)

Colorless solid ($CHCl_3$); m.p. 164–166 °C; $[\alpha]_D^{25}$ −34.8° (MeOH; *c*, 0.01), CD (MeOH; *c*, 0.01): $\Delta\varepsilon_{338}$ +0.46, $\Delta\varepsilon_{292}$ −1.05, $\Delta\varepsilon_{262}$ +0.53; UV (MeOH) nm $\lambda_{max}(\log\varepsilon)$: 263 (4.16), 290 (sh) (3.85), 336 (3.76); IR ν_{max}^{KBr} cm^{-1}: 2904, 2843 (OMe), 1662 (>C=O), 1610, 1594, 1450, 1100; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.77 (1H, *dd*, $J = 16.7$, 3.0 Hz, H-3_{eq}), 2.98 (1H, *dd*, $J = 16.7$, 13.3 Hz, H-3_{ax}), 3.81 (3H, *s*, OMe-7), 3.86 (3H, *s*, OMe-3′), 3.88 (3H, *s*, OMe-5), 3.90 (3H, *s*, OMe-2′), 5.76 (1H, *dd*, $J = 13.3$, 3.0 Hz, H-2), 6.09 (1H, *d*, $J = 2.3$ Hz, H-6), 6.14 (1H, *d*, $J = 2.3$ Hz, H-8), 6.93 (1H, *dd*, $J = 7.0$, 2.7 Hz, H-4′), 7.15 (2H, *m*, H-5′, 6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 45.0 (C-3), 55.4 (OMe-7), 56.2 (OMe-5), 56.5 (OMe-3′), 60.1 (OMe-2′), 74.2 (C-2), 93.1 (C-8), 93.9 (C-6), 106.1 (C-4a), 112.0 (C-4′), 117.3 (C-6′), 123.2 (C-5′), 131.6 (C-1′) 146.8 (C-2′), 152.5 (C-3′), 162.2 (C-5), 165.8 (C-7), 166.5 (C-8a), 189.8 (C-4); EIMS *m*/*z* (rel. int): 344 $[M]^+$ (100), 343 (7), 207 (16), 181 (10), 180 (65), 164 (17), 152 (6); HREIMS: found 344.1331, $C_{19}H_{20}O_6$ requires 344.1338.

3.5. 5-Hydroxy-7,2′,3′-trimethoxyflavone (**12**)

Yellow amorphous solid ($CHCl_3$); m.p. 191–192 °C; UV (MeOH) nm $\lambda_{max}(\log \varepsilon)$: 241 (3.62), 327 (3.02); (MeOH + $AlCl_3$): 241, 369; (MeOH + $AlCl_3$ + HCl): 241, 369; IR ν_{max}^{KBr} cm^{-1}: 3158 (OH), 2900, 2889 (OMe), 1656 (>C=O), 1607, 1508, 1450, 1135; ^1H NMR (400 MHz, $CDC1_3$): δ 3.86 (3H, *s*, OMe-3′), 3.89 (3H, *s*, OMe-7), 3.92 (3H, *s*, OMe-2′), 6.36 (1H, *d*, $J = 2.2$ Hz, H-6), 6.44 (1H, *d*, $J = 2.2$ Hz, H-8), 6.98 (1H, *s*, H-3), 7.07 (1H, *dd*, $J = 8.1$, 1.5 Hz, H-4′), 7.21 (1H, *dd*, $J = 8.1$, 7.9 Hz, H-5′), 7.33 (1H, *dd*, $J = 7.9$, 1.5 Hz, H-6′), 12.82 (1H, *s*, OH-5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.7 (OMe-7), 56.0 (OMe-3′), 60.9 (OMe-2′), 92.4 (C-8), 97.9 (C-6), 105.6 (C-4a), 110.5 (C-3), 115.2 (C-1′), 120.6 (C-6′), 124.2 (C-4′), 126.0 (C-5′), 148.0 (C-2′), 153.3 (C-3′), 158.0 (C-8a), 162.0 (C-5), 162.1 (C-2), 165.5 (C-7), 182.7 (C-4); EMS *m/z* (rel. int): 328 $[M]^+$ (100), 300 (2), 166 (70), 165 (8), 162 (15), 161 (10), 149 (1), 138 (2), 107(1); HREIMS: found 328.1052, $C_{18}H_{16}O_6$ requires 328.1025.

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References

Agrawal, P.K., 1989. The Carbon-13 NMR of Flavonoids. Elsevier, Amsterdam.

Ali, M.S., Saleem, M., Ahmad, W., Parvez, M., Yamdagni, R., 2002. A chlorinated monoterpene ketone, acylated *p*-sitosterol glycosides and a flavanone glycoside from *Mentha longifolia* (Lamiaceae). Phytochemistry 59, 889–895.

Balmain, A., Connolly, J.D., 1973. Minor diterpenoid constituents of *Andrographis paniculata* Nees. Journal of Chemical Society Perkin Transactions I, 1247–1251.

Chan, W.R., Taylor, D.R., Willis, C.R, Bodden, R.L, Fehlhaber, H.W., 1971. The structure and stereochemistry of neoandrographolide, a diterpene glycoside from *Andrographis paniculata* Nees. Tetrahedron 27, 5081–5091.

Chopra, R.N., Nayer, S.L., Chopra, I.C., 1980. Glossary of Indian Medicinal Plants. CSIR, New Delhi. p. 18.

Choudhury, B.R., Poddar, M.K., 1985. Andrographolide and kalmegh *(Andrographis paniculata)* extract: effect on intestinal brush-border membrane-bound hydrolases. Methods and Findings in Experimental and Clinical Pharmacology 7, 617–621.

Fujita, T., Fujitani, R., Takeda, Y., Yakaishi, Y., Yamada, T., Kido, M., Miura, I., 1984. On the diterpenoids of *Andrographis paniculata*: X-ray crystallographic análysis of andrographolide and structure determination of new minor diterpenoids. Chemical and Pharmaceutical Bulletin 32, 2117–2125.

Gamble, J.S., 1956. In: Flora of the Presidency of Madras, vol. 2. Botanical Survey of India, Calcutta. pp. 1048–1052.

Govindachari, T.R., Pai, B.R., Srinivasan, M., Kalyanaraman, P.S., 1969. Chemical investigation *of Andrographis paniculata*. Indian Journal of Chemistry 7, 306.

Gupta, K.K., Taneja, S.C., Dhar, K.L., Atal, C.K., 1983. Flavonoids *of Andrographis paniculata*. Phytochemistry 22, 314–315.

Gupta, K.K., Taneja, S.C., Dhar, K.L., 1996. Flavonoid glycoside of *Andrographis paniculata*. Indian Journal of Chemistry 35B, 512–513.

Hari Kishore, P., Reddy, M.V.B., Reddy, M.K., Gunasekar, D., Caux, C., Bodo, B., 2003. Flavonoids from *Andrographis lineata*. Phytochemistry 63, 457–461.

Iinuma, M., Mizuno, M., 1989. Natural occurrence and synthesis of 2′-oxygenated flavones, flavonols, flavanones and chalcones. Phytochemistry 28, 681–694.

Iinuma, M., Yokoyama, J., Ohyama, M., Tanaka, T., Ruangrungsi, X., 1994. Eight phenolic compounds in roots *of Sophara exigua*. Phytochemistry 35, 785–789.

Jalal, M.A.F., Overton, K.H., Rycroft, D.S., 1979. Formation of three new flavones by differentiating callus cultures of *Andrographis paniculata*. Phytochemistry 18, 149–151.

Jantan, I., Waterman, P.G., 1994. E*nt*-14-β-hydroxy-8(17), 12-labdadien-16,15-olide-3β,19-oxide: a diterpene from the aerial parts of *Andrographis paniculata*. Phytochemistry 37, 1477–1479.

Kirtikar, K.R., Basu, B.D., 1975. In: Indian Medicinal Plants, vol. 3. Periodical Experts, New Delhi. pp. 1884–1886.

Kleipool, R.J.C., 1952. Constituents of *Andrographis paniculata* Nees. Nature 169, 33–34.

Kojima, K., Gombosurengyin, P., Ondognyi, P., Begzsurengyin, D., Zevgeegyin, O., Hatano, K., Ogihara, Y., 1997. Flavanones from *Iris tenuifolia*. Phytochemistry 44, 711–714.

Kuroyanagi, M., Sato, M., Ueno, A., Nishi, K., 1987. Flavonoids from *Andrographis paniculata*. Chemical and Pharmaceutical Bulletin 35, 4429–4435.

Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer Verlag, New York.

Matsuda, T., Kuroyanagi, M., Sugiyama, S., Umehara, K., Ueno, A., Nishi, K., 1994. Cell differentiation inducing diterpenes from *Andrographis paniculata* Nees. Chemical and Pharmaceutical Bulletin 42, 1216–1225.

Mopuru, V.B.R., Hari Kishore, P., Venkata Rao, C., Gunasekar, D., Caux, C., Bodo, B., 2003. New 2′-oxygenated flavonoids from *Andrographis qffinis*. Journal of Natural Products 66, 295–297.

Munta, K.R., Reddy, M.V.B., Gunasekar, D., Murthy, M.M., Caux, C., Bodo, B., 2003. Aflavone and an unusual 23-carbon terpenoid from *Andrographis paniculata*. Phytochemistry 62, 1271–1275.

Nazimudeen, S.K., Ramaswamy, S., Kameswaram, L., 1978. Effect of *Andrographis paniculata* on snake venom induced death and its mechanism. Indian Journal of Pharmaceutical Sciences 40, 132–133.

Puri, A., Saxena, R., Saxena, R.P., Saxena, K.C., Srivastava, V., Tandon, J.S., 1993. Immunostimulant agents from *Andrographis paniculata*. Journal of Natural Products 56, 995–999.

Satyanarayana, D., Mythirayee, C., Krishnamurthy, V., 1978. Polyphenols of *Andrographis paniculata* Nees. Leather Science 25, 250–251.

Tanaka, T., Inuma, M., Mizuno, M., 1986. Spectral properties of 2′-oxygenated flavones. Chemical and Pharmaceutical Bulletin 34, 1667–1671.