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A flavone and an unusual 23-carbon terpenoid from *Andrographis paniculata*

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Abstract

Phytochemical investigation of the roots and aerial parts of *Andrographis paniculata* Nees yielded a new flavone, 5-hydroxy-7,2′,6′-trimethoxyflavone and an unusual 23-carbon terpenoid, 14-deoxy-15-isopropylidene-11,12-didehydroandrographolide together with five known flavonoids and four known diterpenoids. The structures of these compounds were determined on the basis of spectral and chemical studies.

Keywords: *Andrographis paniculata*; Acanthaceae; Flavonoids; Terpenoids

1. Introduction

Andrographis paniculata Nees (Acanthaceae) is an erect herb found in the plains throughout India and Sri Lanka (Gamble, 1956). In the traditional Indian medicine the whole plant of *A. paniculata* is extensively used in the treatment of dyspepsia, dysentry, malaria, respiratory infections, and as an antidote for snake-bite (Kirtikar and Basu, 1975; Chopra et al., 1980). Previous phytochemical studies on this plant have resulted in the isolation of a number of 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; Matsuda et al., 1994).

In our systematic search for chemical constituents of *Andrographis* species (Damu et al., 1998 a,b, 1999; Jayaprakasam et al., 1999, 2001; Jayakrishna et al., 2001), we have investigated the roots and aerial parts of *A. paniculata* and report here the isolation and structure determination of a new flavone (**4**) and a novel 23-carbon terpenoid (**7**) besides five known flavonoids, 7-*O*-methyldihydrowogonin (**1**) (Gupta et al., 1983; Kuroyanagi et al., 1987), 7-*O*-methylwogonin (**2**) (Kuroyanagi et al., 1987), skullcapflavone I 2′-methyl ether (**3**) (Jalal et al., 1979), 7-*O*-methylwogonin 5-*O*-glucoside (**5**) (Kuroyanagi et al., 1987), skullcapflavone I 2′-*O*-glucoside (**6**) (Gupta et al., 1996) and four known labdane type diterpenoids, 14-deoxy-11,12-didehydroandrographolide (**8**) (Balmain and Connolly, 1973; Matsuda et al., 1994), andrographolide (**9**) (Kleipool, 1952; Matsuda et al., 1994), isoandrographolide (**10**) (Matsuda et al., 1994) and neoandrographolide (**11**) (Kleipool, 1952; Chan et al., 1971; Matsuda et al., 1994).

2. Results and discussion

Compound **4**, isolated as colourless crystalline solid, showed $[M+H]^+$ peak at *m*/*z* 329.0816 and $[M+Na]^+$ peak at *m*/*z* 351.0851 in its positive ESITOFMS corresponding to the molecular formula $C_{18}H_{16}O_6$, supported by the presence of 18 carbon signals in its ^{13}C NMR spectrum. The UV absorption maxima of **4** in MeOH

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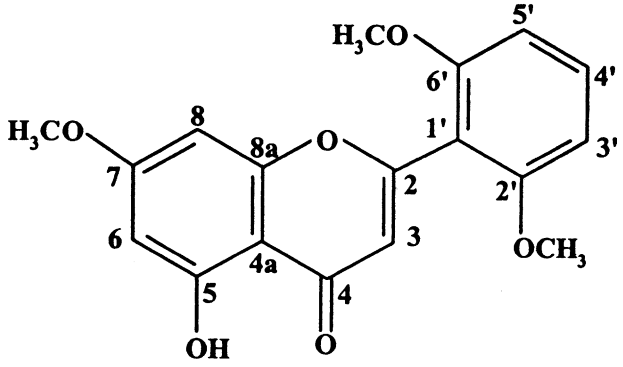
(258 and 297 nm) suggested a flavone structure with 2′,6′-dioxygenation (Zhou et al., 1997; Damu et al., 1998a; Jayakrishna et al., 2001). A bathochromic shift of 72 nm in band I, induced by $AlCl_3$, unchanged on addition of HCl, indicated the presence of a free hydroxyl group at C-5 position. Its IR spectrum showed two strong absorption bands at 3438 and 1684 cm^{-1} corresponding to hydroxyl and carbonyl functions, respectively.

The ^1H NMR spectrum of **4** showed a D_2O exchangeable downfield signal at δ 12.77 corresponding to a chelated hydroxyl group at C-5 position. A pair of *meta*-coupled doublets ($J = 2.2$ Hz) at δ 6.39 and 6.63, each integrating for one proton, were assigned to H-6 and H-8, respectively. A sharp one-proton singlet at δ 6.29 which correlated to the carbon at 112.4 ppm in its HSQC spectrum was ascribed to the C-3 proton. It also showed signals due to three methoxyl groups at δ 3.76 (6H, s) and 3.82 (3H, s). The ESI-MS/MS fragmentation of the $[M+H]^+$ ion (*m*/*z* 329.1) of **4** in its *retro* Diels–Alder fragmentation at ring-C yielded a diagnostic peak at *m*/*z* 167.0 ($^{1,3}A^+$) (Ma et al., 1997) indicating the presence of a methoxyl group in ring-A. Therefore, the other two methoxyl groups in **4** should be present in ring-B. The methoxyl group at δ 3.82 was placed at C-7 based on an HMBC correlation of these protons with C-7 at 165.3 ppm and two strong NOE connectivities with H-6 (δ 6.39) and H-8 (δ 6.63) in its NOESY spectrum. The presence of AB_2 type aromatic proton signals at δ 7.49 (1H, t, $J = 8.4$ Hz) and 6.79 (2H, d, $J = 8.4$ Hz) were typical of 4′, and 3′, 5′ protons of 2′, 6′-dioxygenated flavones (Tomimori et al., 1986; Kikuchi et al., 1991). Therefore, the remaining two methoxyl groups at δ 3.76 in **4** should be present at the 2′ and 6′ positions. This was confirmed by NOE correlation of these methoxyl protons with H-3′ and H-5′ (δ 6.79) in its NOESY spectrum. Thus, compound **4** was characterized as 5-hydroxy-7,2′,6′-trimethoxyflavone.



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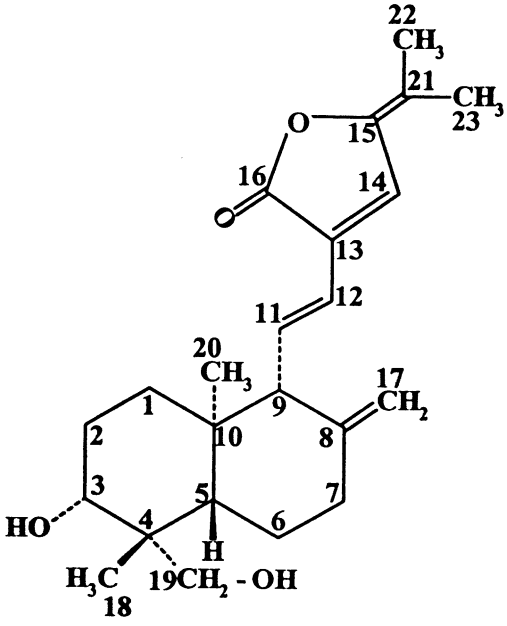
Compound **7**, obtained as colourless needles, showed $[M+H]^+$ peak at *m*/*z* 373.2435 and $[M+Na]^+$ peak at *m*/*z* 395.2117 in its ESITOFMS corresponding to the molecular formula $C_{23}H_{32}O_4$, further corroborated by the presence of 23 carbon signals in its ^{13}C NMR spectrum. The IR spectrum of **7** showed the presence of hydroxyl, α,β-unsaturated-γ-lactone and *exo*-methylene functions at 3376, 1737, and 1645 and 898 cm^{-1}, respectively. Positive Legal colour reaction (Haynes, 1955) also confirmed the presence of an α,β-unsaturated-γ-lactone moiety in **7**. The ^1H and ^{13}C NMR spectral data of **7** were very similar to 14-deoxy-11,12-didehydroandrographolide (**8**) (Table 1) except for the presence of a ^1H NMR signal at δ 1.92 (6H, s) and two ^{13}C NMR signals at 122.7 and 18.4 ppm ($=CMe_2$) corresponding to an isopropylidene moiety in **7** instead of a ^1H NMR signal at δ 4.78 due to methylene protons at C-15 (69.5 ppm) in **8**. The isopropylidene moiety in **7** was found to be present at C-15 as the isopropylidene methyls at δ 1.92 showed ^3J and ^2J correlations with C-15 (144.5 ppm) and C-21 (122.7 ppm) in the HMBC spectrum, and a strong NOE cross peak with H-14 (δ 7.98) in the NOESY spectrum. HMBC correlations of H-14 (δ 7.98) with C-15 and C-21 also confirmed the location of the isopropylidene moiety at C-15. Thus, **7** was characterized as 14-deoxy-15-isopropylidene-11,12-didehydroandrographolide. Incidentally, the isolation of **7** constitutes the first report of an unusual 23-carbon terpenoid from the family Acanthaceae.



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3. Experimental

3.1. General

Melting points were determined on a Kofler hot-stage apparatus and are uncorr. Optical rotations were mea-

Table 1
^{1}H and ^{13}C NMR spectral data for compounds **7** (DMSO-d_6) and **8** ($CDCl_3$) δ (ppm); multiplicity (J in Hz)

	^{1}H NMR			^{13}C NMR	
H	**7**	**8**	C	**7**	**8**
1a	1.48 (*m*)	1.48 (*ddd*, *J* = 13.6, 3.5, 3.5)	1	38.0	38.2
1b	1.12 (*ddd*, *J* = 13.8, 13.8, 4.6)	1.12 (*ddd*, *J* = 13.5, 13.5, 4.2)			
2a	1.74 (*m*)	1.72 (*m*)	2	27.7	28.1
2b	1.74 (*m*)	1.72 (*m*)			
3	3.40 (*dd*, *J* = 11.8, 4.1)	3.44 (*dd*, *J* = 11.4, 4.3)	3	78.6	80.8
4	–	–	4	42.4	43.3
5	1.17 (*dd*, *J* = 12.6, 2.4)	1.17 (*dd*, *J* = 12.8, 2.4)	5	53.7	56.4
6a	1.76 (*m*)	1.76 (*m*)	6	23.2	22.9
6b	1.31 (*dddd*, *J* = 13.6, 12.6, 12.2,4.3)	1.31 (*dddd*, *J* = 12.9, 12.9, 11.9, 4.3)			
7a	2.38 (*ddd*, *J* = 13.6, 4.3, 2.3)	2.42 (*ddd*, *J* = 13.6, 4.2, 2.4)	7	36.3	36.5
7b	2.01 (*ddd*, *J* = 13.6, 13.6, 5.1)	2.03 (*ddd*, *J* = 13.3, 13.3, 5.1)			
8	–	–	8	148.9	148.1
9	2.40 (*d*, *J* = 10.1)	2.28 (*d*, *J* = 10.1)	9	60.7	61.6
10	–	–	10	38.4	38.5
11	6.75 (*dd*, *J* = 15.8, 10.1)	6.83 (*dd*, *J* = 15.8, 10.1)	11	135.6	136.0
12	6.12 (*d*, *J* = 15.8)	6.08 (*d*, *J* = 15.8)	12	121.4	121.0
13	–	–	13	125.8	129.2
14	7.98 (*s*)	7.14 (*dd*, *J* = 2.6, 2.1)	14	132.6	142.9
15	–	4.78 (*br s*)	15	144.5	69.5
16	–	–	16	168.6	172.3
17a	4.74 (*d*, *J* = 1.4)	4.74 (*br d*)	17	108.2	109.1
17b	4.42 (*d*, *J* = 1.4)	4.49 (*br d*)			
18-Me	1.10 (*s*)	1.22 (*s*)	18	23.0	22.6
19a	4.18 (*d*, *J* = 10.6)	4.18 (*d*, *J* = 11.1)	19	62.7	64.2
19b	3.81 (*d*, *J* = 10.6)	3.31 (*d*, *J* = 11.1)			
20-Me	0.78 (*s*)	0.78 (*s*)	20	15.5	15.9
21	–	–	21	122.7	–
22-Me	1.92 (*s*)	–	22	18.4	
23-Me	1.92 (s)	–	23	18.4	–

sured in MeOH at 28 °C on a Perkin-Elmer 241 polarimeter. UV absorptions were measured in MeOH on a Shimadzu UV-240 spectrophotometer and IR spectra were recorded in KBr discs on a Perkin-Elmer 283 double beam spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 and AC 300 spectrometers using DMSO-d_6 and $CDCl_3$ with TMS as internal standard. ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY (500 ms mixing time) were obtained using the standard pulse sequences. ESI-TOFMS and ESI-MS/MS were recorded in positive mode on a API Q-STAR PULSA of Applied Bio-system. CC was carried out on silica gel (Acme) finer than 200 mesh (0.08 mm).

3.2. Plant material

The roots and aerial parts of *A. paniculata* Nees were collected in December 2000 from Talakona forest, Andhra Pradesh, S. India. A voucher specimen has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

3.3. Extraction and isolation

The shade dried and powdered roots of *A. paniculata* (3 kg) were successively extracted with *n*-hexane (3 × 10 l), Me_2CO (3 × 10 l) and MeOH (3 × 10 l). Silica gel column chromatography of the hexane extract on elution with *n*-hexane–EtOAc step gradients yielded **1** (26 mg), **2** (50 mg) and **3** (40 mg). The acetone extract on purification over a silica gel column using *n*-hexane–EtOAc step gradients gave **4** (18 mg), **5** (32 mg) and **6** (35 mg).

Dried and powdered aerial parts of *A. paniculata* (9 kg) were exhaustively extracted with MeOH (5×15 l). The MeOH extract was soxhleted with *n*-butanol. The butanol soluble portion was concentrated to dryness and the residue obtained (152 g) was column chromatographed over silica gel using *n*-hexane–EtOAc step gradients to yield **7** (25 mg), **8** (1.2 g), **9** (1.1 g), **10** (2.2 g) and **11** (1.5 g).

3.4. 5-Hydroxy-7,2′,6′-trimethoxyflavone (4)

Colourless solid (MeOH), mp 196–198 °C; UV_{max}^{MeOH} nm (log ϵ): 258 (4.13), 297 (3.77); (MeOH + $AlCl_3$): 268, 316, 369; (MeOH + $AlCl_3$ + HCl): 268, 316, 369; IR ν_{max}^{KBr} cm^{-1}: 3438 (–OH), 2969 (–OMe), 1684 (>C=O), 1539, 1461, 1262; ^1H NMR (400 MHz, DMSO-d_6): δ 12.77 (1H, s, OH-5), 7.49 (1H, t, J = 8.4 Hz, H-4′), 6.79 (2H, d, J = 8.4 Hz, H-3′,5′), 6.63 (1H, d, J = 2.2 Hz, H-8), 6.39 (1H, d, J = 2.2 Hz, H-6), 6.29 (1H, s, H-3), 3.82 (3H, s, OMe-7), 3.76 (6H, s, OMe-2′,6′); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.9 (C=O), 165.3 (C-7), 161.7 (C-2), 161.2 (C-5), 158.2 (C-8a), 157.9 (C-2′, 6′), 132.9 (C-4′), 112.4 (C-3), 109.0 (C-1′), 104.3 (C-3′,5′), 104.0 (C-4a), 98.2 (C-6), 92.6 (C-8), 56.1 (OMe-2′, 6′), 56.0 (OMe-7); ESI-MS/MS (positive mode) m/z (rel. int): 329.1 $[M+H]^+$ (13), 314.1 $[M+H-CH_3]^+$ (15), 298.0 $[M+H-OCH_3]^+$ (28), 167.0 ($^{1,3}A^+$) (100); ESITOFMS (positive mode) m/z 351.0851 $[M+Na]^+$, 329.0816 $[M+H]^+$ ($C_{18}H_{17}O_6$ requires 329.1025).

3.5. 14-Deoxy-15-isopropylidene-11,12-didehydroandrographolide (7)

Colourless needles (MeOH), mp 207–209 °C; $[\alpha]_D^{28}$ −21.8° (MeOH, c 0.002); UV_{max}^{MeOH} nm (log ϵ): 233 (3.82), 315 (4.46); IR ν_{max}^{KBr} cm^{-1}: 3376 (−OH), 1737 (α,β-unsaturated-γ-lactone), 1645 and 898 (*exo*-methylene); ^1H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) data, see Table 1. ESI-MS/MS (positive mode) m/z (rel. int.): 373.1, $[M+H]^+$ (1), 355 $[M+H-H_2O]^+$ (1), 337 $[M+H-2H_2O]^+$ (5), 235 $[M+H-C_8H_{10}O_2]^+$ (4), 138 $[M+H-C_{15}H_{23}O_2]^+$ (8), 105 $[M+H-C_{15}H_{23}O_2-CH_3-H_2O]^+$ (100); ESI-TOFMS (positive mode) m/z 395.2117 $[M+Na]^+$, 373.2435 $[M+H]^+$ ($C_{23}H_{33}O_4$ requires 373.2379).

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