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Flavonoids from *Andrographis lineata*

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Abstract

Three flavonoids, 5,7,2′,3′,4′-pentamethoxyflavone (**1**), 2′-hydroxy-2,4′,6′-tri methoxychalcone (**2**) and dihydroskullcapflavone I (**3**), together with 17,19,20-trihydroxy-5β, 8α H, 9β H,10α-labd-13-en-16,15-olactone (**4**), a known diterpenoid and six known flavonoids, 5-hydroxy-7,8-dimethoxyflavanone (**5**), 5-hydroxy-7,8,2′,3′,4′-pentamethoxyflavone (**6**), 5,2′-dihydroxy-7-methoxyflavanone (**7**), 5,2′-dihydroxy-7,8-dimethoxyflavone (**8**), 5,2′-dihydroxy-7-methoxyflavone (**9**) and 5,2′-dihydroxy-7-methoxyflavone 2′-*O*-β-D-glucopyranoside (**10**) were isolated from the whole plant of *Andrographis lineata*. The structures of these compounds were elucidated on the basis of spectral and chemical studies.

Keywords: *Andrographis lineata*; Acanthaceae; Whole plant; Flavonoids; Diterpenoid

1. Introduction

Andrographis lineata Nees (Acanthaceae) is an erect herb found widely in Deccan, Carnatic, Yercaud and Nilgiri hills of South India (Gamble, 1956). In continuation of our investigations on *Andrographis* species (Damu et al., 1998a,b, 1999; Jayaprakasam et al., 1999, 2001; Jaya Krishna et al., 2001), we examined the whole plant of *A. lineata* and report here the isolation and structure elucidation of three new flavonoids, 5,7,2′,3′,4′-pentamethoxyflavone (**1**), 2′-hydroxy-2,4′,6′-trimethoxychalcone (**2**) and dihydroskull capflavone I (**3**) together with a known diterpene (**4**) and six known flavonoids (**5**–**10**).

2. Results and discussion

Compound **1**, obtained as colourless needles, showed $[M+H]^+$ and $[M+Na]^+$ peaks at *m*/*z* 373.0953 and 395.0970, respectively in its positive HRESIMS corresponding to the molecular formula $C_{20}H_{20}O_7$. The ^{13}C NMR spectrum of **1** showed resonances for all the 20 carbons present in the molecule. The UV absorption maxima of **1** in MeOH (271 and 329 nm) and negative ferric chloride test suggested compound **1** to be a non-phenolic flavone.

The ^1H NMR spectrum of **1** showed a sharp one-proton singlet at δ 6.80, correlated with C-3 (δ 112.4) in its HSQC spectrum, characteristic of a 2′-oxygenated flavone (Tanaka et al., 1986). Two *meta*-coupled doublets ($J=2.1$ Hz) at δ 6.32 and 6.46, each integrating for one proton, were assigned to H-6 and H-8, respectively. It also showed signals for five aromatic methoxyl groups at δ 3.91, 3.89, 3.88, 3.86 and 3.85. The MS-MS fragmentation (Ma et al., 1997, 1999) of $[M+H]^+$ ion (*m*/*z* 373.1) yielded a diagnostic RDA fragment ion at *m*/*z* 181.1 ($^{1,3}A^+$) indicating the presence of two methoxyl groups in ring A, and therefore the remaining methoxyl groups in 1 should be present in ring B. Two of the five methoxyl groups present at δ 3.91 and 3.85 showed long range correlations with the carbons at 160.8 and 163.7 ppm, respectively and were assigned to C-5 and C-7 as they showed cross correlations with H-6 (δ 6.32), and H-6 (δ 6.32) and H-8 (δ 6.46), respectively in its HMBC spectrum. These assignments were further evidenced by

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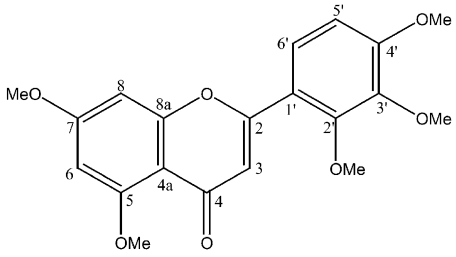

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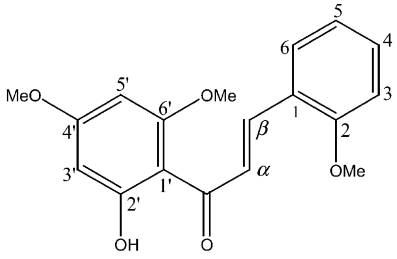

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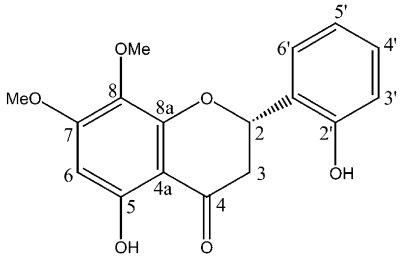

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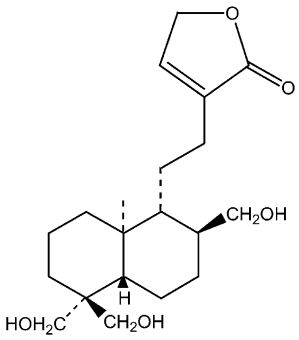

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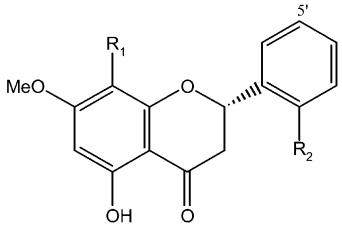
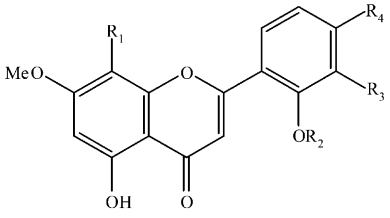


	R_1	R_2
5	OMe	H
7	H	OH



	R_1	R_2	R_3	R_4
6	OMe	Me	OMe	OMe
8	OMe	H	H	H
9	H	H	H	H
10	H	Glc	H	H

NOE correlations of C-5 methoxyl protons (δ 3.91) with H-6 (δ 6.32), and C-7 methoxyl protons (δ 3.85) with H-6 (δ 6.32) and H-8 (δ 6.46) in the NOESY spectrum. The ^{1}H NMR spectrum also showed a typical AB spectrum of two *ortho*-coupled ($J = 8.8$ Hz) aromatic protons of ring B at δ 6.73 and 7.45. Of the three methoxyl groups in ring B, the one at δ 3.89 was placed at C-2′ as it showed long range HMBC correlation with this carbon at 152.8 ppm which in turn showed cross correlation with H-6′ (δ 7.45). A strong NOE correlation of this methoxyl group (δ 3.89) with H-3 (δ 6.80) in its NOESY spectrum also supported its attachment to C-2′. The long range HMBC correlation of one of the *ortho*-coupled aromatic protons at δ 7.45 with C-2 (158.5 ppm) fixes its attachment to C-6′, and this fixes the other *ortho*-coupled aromatic proton at δ 6.73 to C-5′ position. The ^{1}H–^{1}H COSY correlations between the protons at δ 6.73 and 7.45 also supported their placement at C-5′ and C-6′ positions, respectively. The remaining two methoxyl groups at δ 3.86 and 3.88 in ring B should therefore be placed at C-3′ and C-4′ positions. The methoxyl group at δ 3.88 was placed at C-4′ based on its long range HMBC correlation with this carbon at 155.8 ppm and a strong NOE correlation with H-5′ (δ 6.73). The methoxyl group at δ 3.86 was found to be attached to C-3′ and is evidenced by the appearance of a ^{13}C NMR signal at 60.8 ppm, characteristic of a di-*ortho* substituted methoxyl group (Kuroyanagi et al., 1987). Thus, from the foregoing spectral studies compound **1** was characterized as 5,7,2′,3′,4′-pentamethoxyflavone.

Compound **2**, isolated as yellow needles, showed a protonated molecular ion at m/z 315.1052 in its HRESIMS spectrum consistent with the molecular formula $C_{18}H_{18}O_5$, further supported by the presence of 18 carbon signals in its ^{13}C NMR spectrum. The UV absorption maxima (251 sh, 306 sh and 363 nm) and colour reactions suggested that 2 was a chalcone derivative (Mabry et al., 1970). Addition of NaOAc did not cause any shift in its UV absorption maxima indicating the absence of a free hydroxyl group at C-4′. A bathochromic

shift of 43 nm in band I UV absorption maxima with $AlCl_3$/HCl, and a downfield signal δ 14.39 in the ^1H NMR spectrum of **2** revealed the presence of a chelated hydroxyl group at C-2′ position.

The ^1H NMR spectrum of **2** showed a pair of AB doublets ($J = 15.7$ Hz) at δ 7.94 and 8.12 consistent with *trans*-olefinic protons of a chalcone moiety (Mabry et al., 1970). It also showed signals for three aromatic methoxyl groups at δ 3.88 (6H) and 3.80 (3H). A set of *meta*-coupled doublets ($J = 2.3$ Hz) at δ 6.08 and 5.93, each integrating for one proton, were attributed to H-3′ and H-5′. The MS-MS fragmentation of $[M+H]^+$ ion (m/z 315.1) yielded a diagnostic RDA fragment ion at m/z 181.1 ($^{1,3}A^+$) indicating the presence of a hydroxyl and two methoxyl groups in ring A. The methoxyl groups at δ 3.80 and 3.88 showed long range HMBC correlations with the carbons at 166.0 and 162.5 ppm, respectively and were assigned to C-4′ and C-6′ as they showed cross correlations with H-5′ (δ 5.93) and H-3′ (δ 6.08), and H-5′, respectively. These assignments were further evidenced by NOE correlations of C-4′ methoxyl protons with H-3′ and H-5′, and C-6′ methoxyl protons with H-5′ in the NOESY spectrum. The β-carbon in C-2 unsubstituted chalcones usually resonates around 144 (± 2) ppm. However, in compound **2** it appeared at 137.8 ppm, which is unusually upfield, indicating the presence of C-2 oxygenation (Agrawal, 1989). This fixes the attachment of the methoxyl group at δ 3.88 to C-2 position, further evidenced by long range HMBC correlation of these protons with C-2 (158.6 ppm) which in turn showed cross correlation with H-β (δ 8.12). The presence of four aromatic proton signals at δ 6.91, 7.34, 6.96 and 7.57 in the ^1H NMR spectrum of **2** were assigned to protons at 3,4,5 and six positions of ring B, respectively. Thus, the structure of **2** was elucidated as 2′-hydroxy-2,4′,6′-trimethoxychalcone.

Dihydroskullcapflavone I (**3**), obtained as colorless needles, showed $[M+H]^+$ peak at m/z 317.1021 in its HRESIMS, consistent with the molecular formula $C_{17}H_{16}O_6$. This was supported by the appearance of 17 carbon signals in its ^{13}C NMR spectrum. The UV absorption maxima of **3** in MeOH (287 and 329 sh nm) suggested a flavanone structure (Mabry et al., 1970). Its UV spectral maxima was unaffected by the addition of NaOAc indicating the absence of a free 7-hydroxyl. A bathochromic shift of 23 nm in its UV absorption maxima on addition of $AlCl_3$ and $AlCl_3$/HCl suggested the presence of a chelated hydroxyl group at C-5 position (Mabry et al., 1970).

The ^1H NMR spectrum of **3**, showed two D_2O exchangeable signals at δ 11.90 and 6.85 indicating the presence of a chelated hydroxyl at C-5 and a non-chelated hydroxyl group, respectively. The presence of three sets of double doublets at δ 5.73 (1H, *dd*, $J = 13.5$, 3.1 Hz), and 3.12 (1H, *dd*, $J = 17.3$, 13.5 Hz) and 3.07 (1H, *dd*, $J = 17.3$, 3.1 Hz) were typical of H-2 and, H-3_{ax} and H-3_{eq} of ring C of a flavanone moiety (Mabry et al., 1970). A sharp one-proton singlet at δ 6.11 correlated with C-5 (160.0 ppm) in its HMBC spectrum was assigned to H-6. It also showed signals for two methoxyl groups at δ 3.88 and 3.78. The MS-MS fragmentation of $[M+H]^+$ ion (m/z 317.1) yielded a diagnostic RDA fragment ion at m/z 197.0 ($^{1,3}A^+$) indicating the presence of a hydroxyl and two methoxyl groups in ring A. The methoxyl groups at δ 3.88 and 3.78 placed at C-7 and C-8 positions as they showed long range HMBC correlations with these carbons at 161.6 and 129.8 ppm, respectively which in turn showed cross correlation with H-6 (δ 6.11). The presence of a methoxyl at C-8 was also evidenced by the appearance of C-8 methoxy carbon resonance at 61.5 ppm, characteristic of a methoxyl group in a di-*ortho* substituted environment (Kuroyanagi et al., 1987). The non-chelated hydroxyl at δ 6.85 was linked to C-2′ as it showed two strong NOE correlations with H-3_{eq} (δ 3.07) and H-2 (δ 5.73) in its NOESY spectrum. A typical ABCD spectrum at δ 6.86, 7.22, 6.94 and 7.33 established the presence of four adjacent aromatic protons (3′, 4′, 5′ and 6′) in ring B. The absolute configuration at C-2 was found to be *S* (Gaffield, 1970) as it showed positive and negative Cotton effects at 329 and 287 nm, respectively in its CD spectrum. Thus, the structure of dihydroskullcapflavone I was established as (2*S*)-5,2′-dihydroxy-7,8-dimethoxyflavanone (**3**).

Compounds **4**–**10** were identified by comparison of their spectral data with literature values as 17,19,20-trihydroxy-5β, 8αH, 9βH,10α-labd-13-en-16,15-olactone (**4**) (Joshi et al., 1974), 5-hydroxy-7,8-dimethoxyflavanone (5) (Gupta et al., 1983; Kuroyanagi et al., 1987), 5-hydroxy-7,8,2′,3′,4′-pentamethoxyflavone (**6**) (Govindachari et al., 1968), 5,2′-dihydroxy-7-methoxyflavanone (**7**) (Jayaprakasam et al., 1999), 5,2′-dihydroxy-7,8-dimethoxyflavone (**8**) (Jalal et al., 1979; Jayakrishna et al., 2001), 5,2′-dihydroxy-7-methoxyflavone (**9**) (Govindachari et al., 1965a,b; Damu et al., 1998) and 5,2′-dihydroxy-7-methoxyflavone 2′-*O*-β-D-glucopyranoside (**10**) (Govindachari et al., 1965b; Jayaprakasam et al., 1999).

3. Experimental

3.1. General

All mps were determined on a Kofler hot-stage apparatus and are uncorr. Optical rotations were measured in MeOH at 28 °C on a Perkin-Elmer 241 polarimeter. IR spectra were recorded in KBr discs on a Perkin-Elmer 283 double beam spectro-photometer and UV spectra on a Shimadzu UV-240 spectrophotometer. The CD spectrum was recorded in MeOH at 25 °C on a

JASCO J 715 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were determined on Brüker Avance 400 and AC 300 spectrometers using $CDCl_3$ with TMS as internal standard. ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectra were recorded using standard pulse sequences. HRESIMS and ESI-MS/MS were recorded in positive mode on a API Q-STAR PULSA of Applied Bio-system. CC was performed on Si gel (Acme) finer than 200 mesh.

3.2. Plant material

The whole plant of *A. lineata* Nees was collected from Yercaud hills, Tamilnadu, South India in February 2000. A voucher specimen (DG 001) documenting its collection is on deposit at the Department of Botany, Sri Venkateswara University, Tirupati.

3.3. Extraction and isolation

Air dried, powdered whole plant of *A. lineata* (1.5 kg) was successively extracted with *n*-hexane, Me_2CO and MeOH. The *n*-hexane extract on purification over a Si gel column using hexane and hexane/EtOAc step gradient yielded **1** (12 mg); **2** (10 mg); **3** (23 mg); **4** (320 mg) and **5** (12 mg). The Me_2CO extract on purification over a Si gel column employing hexane, hexane/EtOAc mixtures yielded **6** (7 mg); **7** (25 mg); **8** (30 mg); **9** (38 mg) and **10** (40 mg).

3.3.1. 5,7,2′,3′,4′-Pentamethoxyflavone (*1*)

Colourless needles ($CHCl_3$). mp 166–167 °C; UV λ_{max}^{MeOH} nm (log ε): 271 (3.94), 329 (3.61); (MeOH + NaOAc): 270, 330; (MeOH + $AlCl_3$): 272, 330; (MeOH + $AlCl_3$ + HCl): 272, 330; IR ν_{max}^{KBr} cm^{-1}: 2943 (–OMe), 1632 (>C=O), 1590, 1491, 1459, 1414, 1342. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (1H, *d*, J = 8.8 Hz, H-6′), 6.80 (1H, *s*, H-3), 6.73 (1H, *d*, J = 8.8 Hz, H-5′), 6.46 (1H, *d*, J = 2.1 Hz, H-8), 6.32 (1H, *d*, J = 2.1 Hz, H-6), 3.91 (3H, *s*, OMe-5), 3.89 (3H, *s*, OMe-2′), 3.88 (3H, *s*, OMe-4′), 3.86 (3H, *s*, OMe-3′), 3.85 (3H, *s*, OMe-7); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.8 (C-4), 163.7 (C-7), 160.8 (C-5), 159.9 (C-8a), 158.5 (C-2), 155.8 (C-4′), 152.8 (C-2′), 142.6 (C-3′), 123.7 (C-6′), 118.6 (C-1′), 112.4 (C-3), 109.1 (C-4a), 107.2 (C-5′), 95.8 (C-6), 92.6 (C-8), 60.9 (OMe-2′), 60.8 (OMe-3′), 56.3 (OMe-5), 56.0 (OMe-4′), 55.6 (OMe-7); ESI-MS/MS (positive mode) m/z (rel. int.): 373.1 $[M+H]^+$ (1), 358.0 $[M+H–CH_3]^+$ (3), 330.0 $[M+H–CH_3–CO]^+$ (4), 302.0 $[M+H–CH_3–2CO]^+$ (10), 237.0 ($^{0,4}B^+$) (6), 195.0 ($^{0,2}B^+$) (4), 193.0 ($^{1,3}B^+$) (3), 181.1 ($^{1,3}A^+$) (100); HRESIMS (positive mode) m/z: 373.0953 $[M+H]^+$ ($C_{20}H_{21}O_7$ requires 373.1287).

3.3.2. 2′-Hydroxy-2,4′,6′-trimethoxychalcone (*2*)

Yellow needles ($CHCl_3$). mp 171–173 °C; UV λ_{max}^{MeOH} nm (log ε): 251 sh (4.13), 306 sh (3.87), 363 (4.36); (MeOH + NaOAc): 251 sh, 306 sh, 363; (MeOH–H + $AlCl_3$): 270, 406; (MeOH + $AlCl_3$ + HCl): 270, 406; IR ν_{max}^{KBr} cm^{-1}: 3416 (–OH), 2885 (–OMe), 1612 (>C=O), 1511, 1458, 1259, 1155; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.39 (1H, *s*, OH-2′), 8.12 (1H, *d*, J = 15.7 Hz, H-β), 7.94 (1H, *d*, J = 15.7 Hz, H-α), 7.57 (1H, *dd*, J = 8.7, 1.7 Hz, H-6), 7.34 (1H, *dt*, J = 7.8, 1.7 Hz, H-4), 6.96 (1H, *dt*, J = 7.6, 1.8 Hz, H-5), 6.91 (1H, *dd*, J = 8.3, 1.9 Hz, H-3), 6.08 (1H, *d*, J = 2.3 Hz, H-3′), 5.93 (1H, *d*, J = 2.3 Hz, H-5′), 3.88 (6H, *s*, OMe-2, OMe-6′), 3.80 (3H, *s*, OMe-4′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.0 (C=O), 168.3 (C-2′), 166.0 (C-4′), 162.5 (C-6′), 158.6 (C-2), 137.8 (C-β), 131.3 (C-4), 128.7 (C-6), 127.8 (C-α), 124.5 (C-1), 120.6 (C-5), 111.1 (C-3), 106.4 (C-1′), 93.7 (C-3′), 91.1 (C-5′), 55.7 (OMe-2), 55.5 (OMe-4′), 55.4 (OMe-6′); ESI-MS/MS (positive mode) m/z (rel. int.): 315.1 $[M+H]^+$ (1), 181.0 ($^{1,3}A^+$)(71), 166.0 ($^{1,3}A^+$–CH_3)(48), 138.0 ($^{1,3}A^+$–CH_3–CO)(100); HRESIMS (positive mode) m/z: 315.1052 $[M+H]^+$ ($C_{18}H_{19}O_5$ requires 315.1232).

3.3.3. Dihydroskullcapflavone I (*3*)

Colourless needles ($CHCl_3$). mp 151–153 °C; $[\alpha]_D^{28}$ −21.7° (MeOH, *c* 0.15); UV λ_{max}^{MeOH}nm (log ε): 287 (4.13), 329 sh (3.86); (MeOH + NaOAc): 287, 329 sh; (MeOH + $AlCl_3$): 310, 358; (MeOH + $AlCl_3$ + HCl): 310, 358; CD: $\Delta\varepsilon_{287}$ −0.86, $\Delta\varepsilon_{329}$ +0.20 (MeOH, *c* 0.15); IR ν_{max}^{KBr} cm^{-1}: 3200 (–OH), 1637 (>C=O), 1605, 1498, 1458, 1352; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.90 (1H, *s*, OH-5), 7.33 (1H, *dd*, J = 7.7, 1.7 Hz, H-6′), 7.22 (1H, *ddd*, J = 7.7, 7.7, 1.7 Hz, H-4′), 6.94 (1H, *ddd*, J = 7.7, 7.7, 1.1 Hz, H-5′), 6.86 (1H, *dd*, J = 8.1, 1.1 Hz, H-3′), 6.85 (1H, *s*, OH-2′) 6.11 (1H, *s*, H-6), 5.73 (1H, *dd*, J = 13.5, 3.1 Hz, H-2), 3.88 (3H, *s*, OMe-7), 3.78 (3H, *s*, OMe-8), 3.12 (1H, *dd*, J = 17.3, 13.5 Hz, H-3$_{ax}$), 3.07 (1H, *dd*, J = 17.3, 3.1 Hz, H-3$_{eq}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.5 (C-4), 161.6 (C-7), 160.0 (C-5), 153.9 (C-2′), 153.0 (C-8a), 129.9 (C-4′), 129.8 (C-8), 126.4 (C-6′), 124.1 (C-1′), 120.7 (C-5′), 116.7 (C-3′), 102.9 (C-4a), 93.5 (C-6), 76.6 (C-2), 61.5 (OMe-8), 56.3 (OMe-7), 41.3 (C-3); ESI-MS/MS (positive mode) m/z (rel. int.): 317.1 $[M+H]^+$ (9), 197.0 ($^{1,3}A^+$)(100), 182.0 ($^{1,3}A^+$–CH_3)(38), 164.0 ($^{1,3}A^+$–CH_3–H_2O)(18), 154.0 ($^{1,3}A^+$–CH_3–CO)(50); HRESIMS (positive mode) m/z: 317.1021 $[M+H]^+$ ($C_{17}H_{17}O_6$ requires 317.1024).

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