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Flavonoids from *Limnophila indica*

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Abstract

Two flavonoids, (2*S*)-5,7,3′,4′-tetramethoxyflavanone (**1**) and 5,7,2′,5′-tetramethoxyflavone (**2**) together with three known flavonoids, 7-*O*-methylwogonin (**3**), skullcapflavone I (**4**) and 5-hydroxy-7,2′-dimethoxyflavone (**5**) were isolated from the whole plant of *Limnophila indica*. The structures of compounds **1**–**5** were elucidated on the basis of spectral and chemical studies.

Keywords: *Limnophila indica*; Scrophulariaceae; Flavonoids

1. Introduction

Limnophila indica (L.) Druce (Scrophulariaceae) is a small herb, used widely in traditional Indian medicine in the treatment of pestilent fevers, dysentery and elephantiasis (Ambasta, 1986; Thammanna et al., 1994; Satyavati et al., 1987). Previous phytochemical studies on this plant have resulted in the isolation of 5-hydroxy-6,8-dimethoxy-3′,4′-methylenedioxyflavone (Mukherjee et al., 1998) and 5,8-dihydroxy-6,7,4′-trimethoxyflavone (Bramhachari et al., 2004). In our systematic search for polyphenolic constituents from Indian medicinal plants, we have investigated the whole plant of *L. indica* and report herein the isolation and structural elucidation of two new flavonoids, 5,7,3′,4′-tetramethoxyflavanone (**1**) and 5,7,2′,5′-tetramethoxyflavone (**2**) besides three known flavonoids, 7-*O*-methylwogonin (**3**), skullcapflavone I (**4**) and 5-hydroxy-7,2′-dimethoxyflavone (**5**).

2. Results and discussion

Compound **1**, obtained as colourless crystalline solid, showed $[M+H]^+$ and $[M+Na]^+$ peaks at *m*/*z* 345.1284 and 367.1238, respectively in its 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 283 and 324 (sh) nm and negative ferric chloride test suggested that compound **1** was a non-phenolic flavanone (Mabry et al., 1970a). The ^1H NMR spectrum of **1** showed three signals at δ 5.42 (1H, *dd*, $J = 12.8$, 2.9 Hz), 3.09 (1H, *dd*, $J = 16.4$, 12.8 Hz) and 2.58 (1H, *dd*, $J = 16.4$, 2.9 Hz) characteristic of H-2, H-3_{ax} and H-3_{eq}, respectively of a flavanone moiety (Mabry et al., 1970a). Two *meta*-coupled doublets ($J = 2.3$ Hz) at δ 6.19 and 6.21, each integrating for one proton, were assigned to H-6 and H-8, respectively. It also showed signals for four aromatic methoxyl groups at δ 3.79, 3.77, 3.76 and 3.75. The MS–MS fragmentation (Hughes et al., 2001) of $[M+H]^+$ ion (*m*/*z* 345.1) of **1** yielded a diagnostic RDA fragment ion at *m*/*z* 181.0 ($^{1,3}A^+$) indicating the presence two methoxyl groups in ring A, and hence the remaining two methoxyl groups in **1** should be present in ring B. Two of the four methoxyl proton signals present at δ 3.77 and

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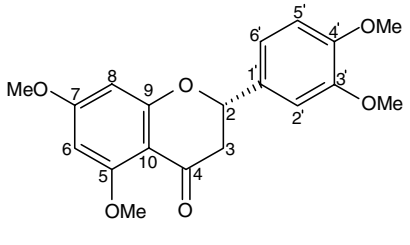
3.79 showed long range correlations with the carbons at 161.7 and 165.4 ppm, respectively and were assigned to C-5 and C-7 as they showed cross correlations with H-6 (δ 6.19), and H-6 (δ 6.19) and H-8 (δ 6.21), respectively in its HMBC spectrum. These assignments were further evidenced by NOE correlations of C-5 methoxyl protons (δ 3.77) with H-6 (δ 6.19), and C-7 methoxyl protons (δ 3.79) with H-6 (δ 6.19) and H-8 (δ 6.21) in the NOESY spectrum (Fig. 1). The ^{1}H NMR spectrum also showed a typical ABX system of three aromatic proton signals at δ 6.95 (1H, *d*, $J = 8.5$ Hz, H-5′), 7.11 (1H, *d*, $J = 2.0$ Hz, H-2′) and 7.01 (1H, *dd*, $J = 8.5$, 2.0 Hz, H-6′). The remaining two methoxyl groups at δ 3.76 and 3.75 in ring B should therefore be placed at C-3′ and C-4′ positions. The methoxyl group at δ 3.75 was placed at C-4′ based on its long range HMBC correlation with this carbon at 149.0 ppm and a strong NOE correlation with H-5′ (δ 6.95). The remaining methoxyl group at δ 3.76 was found to be attached to C-3′ based on its long range HMBC correlation with this carbon at 148.7 ppm and a strong NOE correlation with H-2′ (δ 7.11). The relative stereochemistry at C-2 was shown to be *S* (Gaffield, 1970) as it showed positive and negative Cotton effects at 324 and 283 nm, respectively in its CD spectrum. Thus, from the foregoing spectral studies the structure of compound **1** was elucidated as (2*S*)-5,7,3′,4′-tetramethoxyflavanone. Although compound **1** has been reported synthetically (Babber et al., 1987), this is the first report of its isolation from a natural source.

Compound **2**, isolated as colourless needles, showed $[M+H]^+$ peak at *m*/*z* 343.1224 and $[M+Na]^+$ peak at *m*/*z* 365.1144 in its positive ESITOFMS corresponding to the molecular formula $C_{19}H_{18}O_6$. This was corroborated by the ^{13}C NMR spectrum which showed 19 carbon resonances. The UV absorption maxima of **2** in MeOH at 245, 263, 303 and 345 nm and negative ferric chloride test suggested that compound **2** was a non-phenolic flavone (Geissman, 1962). The ^{1}H NMR spectrum of **2** showed a sharp one-proton singlet at δ 6.97, correlated with C-3 (114.1 ppm) in its HSQC spectrum, characteristic of a 2′-oxygenated flavone (Tanaka et al., 1986). Two *meta*-coupled doublets ($J = 2.3$ Hz) at δ 6.29 and 6.46, each integrating for one proton, were assigned to H-6 and H-8, respectively. It also showed signals for four aromatic methoxyl groups at δ 3.89, 3.83, 3.82 and 3.78. The MS–MS fragmentation (Ma et al., 1997) of $[M+H]^+$ ion (*m*/*z* 343.1) 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 hence the remaining two methoxyl groups in **2** should be present in ring B. Of the four methoxyl groups in **2**, the one at δ 3.83 was placed at C-7 based on ^3J correlation of these protons with C-7 at 163.8 ppm in its HMBC spectrum and two strong NOE correlations with H-6 (δ 6.29) and H-8 (δ 6.46) in its NOESY spectrum. The second methoxyl group at δ 3.89 was placed at C-5 as these protons showed HMBC correlation with this carbon at 160.7 ppm and a strong NOE correlation with H-6 (δ 6.29) in its NOESY spectrum (Fig. 2). The appearance of C-3 resonance at 114.1 ppm is characteristic of 2′-oxygenated flavones (Agrawal, 1989) and the chemical shift values of ring B carbons of **2** were very similar to those observed for the B ring carbons of 2′,5′-dioxygenated flavones (Zhang et al., 1994; Iinuma and Mizuno, 1989). The methoxyl groups at δ 3.78 and 3.82 were placed at C-2′ and C-5′ on the basis of NOE correlations of C-2′ methoxyl protons (δ 3.78) with H-3 (δ 6.97) and H-3′ (δ 6.88), and



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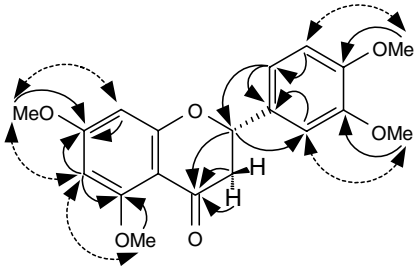
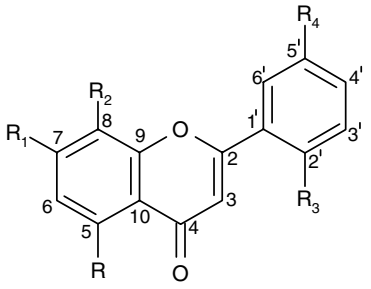


Fig. 1. Significant HMBC (→) and NOESY (↔) correlations for **1**.



2 R = R_1=R_3 = R_4=OMe, R_2=H
3 R = OH, R_1=R_2=OMe, R_3=R_4=H
4 R= R_3=OH, R_1=R_2=OMe, R_4=H
5 R=OH, R_1=R_3=OMe, R_2=R_4=H

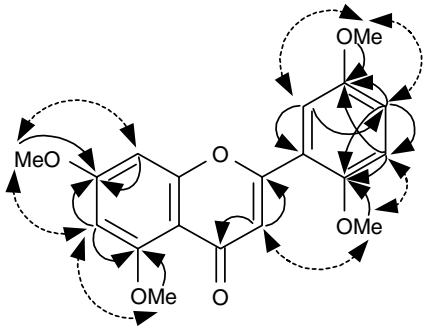


Fig. 2. Significant HMBC (→) and NOESY (↔) correlations for **2**.

C-5′ methoxyl protons (δ 3.82) with H-6′ (δ 7.35) and H-4′ (δ 6.92) in the NOESY spectrum. Thus, from the foregoing spectral studies, compound **2** was characterized as 5,7,2′,5′-tetramethoxyflavone.

Compounds **3**–**5** were identified by comparison of their spectral data with the literature values as 7-*O*-methylwogonin (**3**) (Kuroyanagi et al., 1987), skullcapflavone I (**4**) (Jalal et al., 1979) and 5-hydroxy-7,2′-dimethoxyflavone (**5**) (Kesava Reddy et al., 2003).

3. Experimental

3.1. General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured 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. NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Bruker Avance 400 spectrometer or at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Bruker AC 300 spectrometer using either DMSO-d_6 or $CDCl_3$ with TMS as internal standard. ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY (500 ms mixing time) spectra were obtained using the standard pulse sequences. ESITOFMS and ESI-MS/MS were recorded in positive mode on a API Q-STAR PULSA of Applied Biosystem. CC was carried out on silica gel (Acme) finer than 200 mesh (0.08 mm).

3.2. Plant material

The whole plant of *L. indica* was collected from Tirumala hills, Andhra Pradesh, South India in January 2004. A voucher specimen (DG-041) 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 whole plant of *L. indica* (2.1 kg) was exhaustively extracted with MeOH (3×8 l). The MeOH extract was solvent fractionated with *n*-hexane and Me_2CO. Silica gel column chromatography of the hexane soluble portion on elution with *n*-hexane–ethyl acetate step gradients yielded **1** (15 mg), **2** (10 mg) and **3** (18 mg). The Me_2CO extract on purification over a silica gel column using *n*-hexane–ethylacetate step gradients gave **4** (20 mg) and **5** (16 mg).

3.4. 5,7,3′,4′-Tetramethoxyflavanone (*1*)

Colourless crystalline solid (MeOH), m.p. 119–121 °C; $[\alpha]_D^{25}$ −18.2° (*c* 0.16, MeOH); UV λ_{max}^{MeOH} nm (log ε): 283 (4.22), 324 (sh) (3.67); CD (MeOH, *c* 0.16): $\Delta\varepsilon_{283}$ −0.21, $\Delta\varepsilon_{324}$ + 0.07; IR ν_{max}^{KBr} cm^{-1}: 2823 (−OMe), 1669 (>C=O), 1619, 1515, 1460, 1390; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.11 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.01 (1H, *dd*, *J* = 8.5, 2.0 Hz, H-6′), 6.95 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.21 (1H, *d*, *J* = 2.3 Hz, H-8), 6.19 (1H, *d*, *J* = 2.3 Hz, H-6), 5.42 (1H, *dd*, *J* = 12.8, 2.9 Hz, H-2), 3.79 (3H, *s*, OMe-7), 3.77 (3H, *s*, OMe-5), 3.76 (3H, *s*, OMe-3′), 3.75 (3H, *s*, OMe-4′), 3.09 (1H, *dd*, *J* = 16.4, 12.8 Hz, H-3$_{ax}$), 2.58 (1H, *dd*, *J* = 16.4, 2.9 Hz, H-3$_{eq}$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 188.0 (C-4), 165.4 (C-7), 164.3 (C-9), 161.7 (C-5), 149.0 (C-4′), 148.7 (C-3′), 131.1 (C-1′), 119.0 (C-6′), 111.5 (C-5′), 110.4 (C-2′), 105.4 (C-10), 93.6 (C-8), 92.7 (C-6), 78.5 (C-2), 55.8 (OMe-5), 55.7 (OMe-7), 55.6 (OMe-3′), 55.5 (OMe-4′), 44.8 (C-3); ESI-MS/MS (positive mode) *m*/*z* (rel. int.): 345.1 $[M+H]^+$ (45), 191 ($^{0,4}B^+$–H_2O) (26), 181 ($^{1,3}A^+$) (100), 176 ($^{0,4}B^+$–H_2O–CH_3) (1), 166.0 ($^{1,3}A^+$–CH_3) (9), 163 ($^{0,4}B^+$–H_2O–CO) (4); ESITOFMS (positive mode) *m*/*z* 711.2000 $[2M+Na]^+$, 367.1238 $[M+Na]^+$, 689.2305 $[2M+H]^+$, 345.1284 $[M+H]^+$ (calc. for $C_{19}H_{21}O_6$ 345.1332).

3.5. 5,7,2′,5′-Tetramethoxyflavone (*2*)

Colourless needles ($CHCl_3$), m.p. 190–191 °C; UV λ_{max}^{MeOH} nm (log ε): 245 (4.09), 263 (4.11), 303 (3.87), 345 (3.78); IR ν_{max}^{KBr} cm^{-1} : 2937(−OMe), 1607(>C=O), 1496, 1458, 1347; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (1H, *d*, *J* = 2.9 Hz, H-6′), 6.97 (1H, *s*, H-3), 6.92 (1H, *dd*, *J* = 9.0, 2.9 Hz, H-4′), 6.88 (1H, *d*, *J* = 9.0 Hz, H-3′), 6.46 (1H, *d*, *J* = 2.3 Hz, H-8), 6.29 (1H, *d*, *J* = 2.3 Hz, H-6), 3.89 (3H, *s*, OMe-5), 3.83 (3H, *s*, OMe-7), 3.82 (3H, *s*, OMe-5′), 3.78 (3H, *s*, OMe-2′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9 (C-4), 163.8 (C-7), 160.7 (C-5), 159.8 (C-9), 157.5 (C-2), 153.2 (C-2′), 152.1 (C-5′), 120.9 (C-1′), 116.6 (C-4′), 114.4 (C-6′), 114.1 (C-3), 112.7 (C-3′), 109.0 (C-10), 95.8 (C-6), 92.6 (C-8), 56.3 (OMe-7), 56.0 (OMe-2′), 55.8 (OMe-5′), 55.6 (OMe-5); ESI-MS/MS (positive mode) *m*/*z* (rel. int.): 343.1 $[M+H]^+$ (33), 328.1 $[M+H-CH_3]^+$ (6), 313.1 $[M+H-2CH_3]^+$ (100), 298.0 $[M+H-3CH_3]^+$ (20), 181.1 ($^{1,3}A^+$) (15), 166.1 ($^{1,3}A^+$–CH_3) (10); ESITOFMS (positive mode) *m*/*z* 707.1765 $[2M+Na]^+$, 365.1144 $[M+Na]^+$, 685.1998 $[2M+H]^+$, 343.1224 $[M+H]^+$ (calc. for $C_{19}H_{19}O_6$ 343.1174).

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