Two New 5-Deoxyflavonoids from *Calliandra inermis*

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Two new 5-deoxyflavonoids, 7,2′,3′,4′-tetramethoxyflavone (1) and 7,2′,3′,4′-tetramethoxyflavanone (2) together with a known flavone 7,4′-dimethoxy-3′-hydroxyflavone (3) were isolated from the whole plant of *Calliandra inermis*. The structures of these new compounds were elucidated by high resolution electron impact mass spectrometry (HR-EI-MS) and 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).

Key words *Calliandra inermis*; Leguminosae; 5-deoxyflavonoid

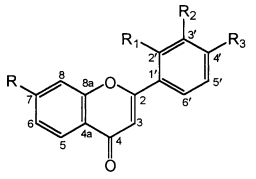
Calliandra inermis (Leguminosae) is an herbaceous plant distributed in the Rayalaseema region of Andhra Pradesh, South India.[1)] In continuation of our search for new flavonoids,[2)] we have examined the whole plant of *C. inermis*, and report here the isolation and structure elucidation of two new 5-deoxyflavonoids, 7,2′,3′,4′-tetramethoxyflavone (**1**) and 7,2′,3′,4′-tetramethoxyflavanone (**2**) in addition to a known flavone, 7,4′-dimethoxy-3′-hydroxyflavone (**3**).

Results and Discussion

Compound **1**, was isolated as an amorphous powder. The high resolution electron impact mass spectrum (HR-EI-MS) of **1** showed the $[M]^+$ peak at *m/z* 342.1061 in agreement with the molecular formula $C_{19}H_{18}O_6$ (Calcd 342.1058). This was corroborated by the decoupled ^{13}C-NMR spectrum which showed signals for all the nineteen carbons of the molecule. The UV absorption in MeOH (254, 330 nm) suggested compound **1** to be a flavone.[3)] The IR absorption bands at 1650, 1625 and 1596 cm^{-1} and negative ferric chloride test indicated compound **1** to be a non-phenolic flavone.

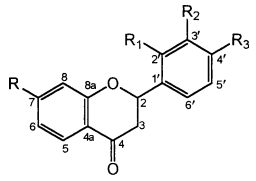
The ^{1}H-NMR spectrum of **1** showed four methoxyl singlets at δ 3.95, 3.93, 3.92 and 3.89 and a sharp one-proton singlet at δ 6.92 ascribed to H-3. The eletron impact mass spectrometry (EI-MS) of **1** showed two *retro*-Diels Alder fragments[4)] at *m/z* 151 $[A_1+H]^+$ and *m/z* 192 $[B_1]^+$ indicating the presence of one methoxyl group in ring-A and three methoxyl groups in ring-B, respectively. The ^{1}H-NMR sepectrum of **1** further showed a ABX spin coupled system at δ 8.11 (1H, d, J=8.8 Hz), 6.94 (1H, dd, J=8.8, 2.3 Hz) and 6.88 (1H, d, J=2.3 Hz) was assigned to H-5, H-6 and H-8, respectively, as the H-6 and H-8 protons showed nuclear Overhauser enhancement spectroscopy (NOESY) correlation with the methoxyl group at C-7 position. Two *ortho* coupled protons at δ 7.50 and 6.77 were assigned to H-6′ and H-5′ respectively. The three methoxyl groups in ring-B at δ 3.93, 3.92 and 3.89 were assigned to C-2′, C-3′ and C-4′ positions as these three methoxyl protons and H-5′ and H-6′ protons showed heteronuclear multiple bond connectivity (HMBC) correlations with these carbons at 152.7 (C-2′), 142.5 (C-3′) and 156.0 (C-4′) ppm,[5)] respectively (Fig. 1). Thus from the foregoing spectral studies the structure of compound **1** was characterised as 7,2′,3′,4′-tetramethoxyflavone.

Compound **2**, was isolated as an amorphous powder. Comparison of the ^{1}H-NMR data of **2** with those of **1**, revealed that compound **2** was the 2,3-dihydro derivative of **1**, based on the appearance of new signals at δ 5.65 (dd, $J_{2ax.\,3ax}$=12.5



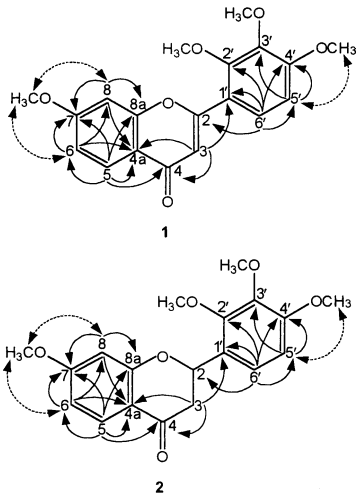
1: R = R_1 = R_2 = R_3 = OCH_3
3: R = R_3 = OCH_3, R_1 = H, R_2 = OH

Fig. 1. Structures of Compounds **1** and **3**



2: R = R_1 = R_2 = R_3 = OCH_3

Fig. 2. Structure of Compound **2**



1

2

Fig. 3. Significant HMBC (◀——▶) and NOESY (◀- - -▶) Correlations for **1** and **2**

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Table 1. ^{13}C-NMR Data (125 MHz, $CDCl_3$) of **1**, **2** and **3**[a)]

Carbon	1	2	3
2	161.0	75.0	162.5
3	110.9	43.5	105.3
4	178.1	191.0	176.3
4a	117.6	114.8	117.1
5	126.9	128.6	126.2
6	114.1	109.0	114.5
7	164.0	165.9	163.8
8	100.3	100.8	100.8
8a	158.1	163.7	157.4
1′	119.0	124.7	123.5
2′	152.7	151.2	112.9
3′	142.5	142.0	146.8
4′	156.0	154.0	150.8
5′	107.3	107.4	112.1
6′	124.0	121.3	118.4
7-OMe	55.8	55.5	56.0
2′-OMe	60.9	60.7	—
3′-OMe	61.1	61.2	—
4′-OMe	56.1	56.0	55.7

a) DMSO-d_6.

Hz, $J_{2ax.\,3eq}$=3.1 Hz), 3.01 (dd, $J_{3ax.\,3eq}$=17.1 Hz, $J_{3ax.\,2ax}$= 12.5 Hz) and 2.75 (dd, $J_{3eq.\,3ax}$=17.1 Hz, $J_{3eq.\,2ax}$=3.1 Hz) for the protons of H-2, H-3$_{ax}$ and H-3$_{eq}$ respectively,[6,7] and the loss of the resonance of the olefinic H-3. The other resonances in the ^{1}H-NMR spectrum were comparable with those of **1**. The ^{13}C-NMR data (Table 1) of **2** supported this finding through the appearance of C-2 and C-3 signals at upfield 75.06, 43.50 ppm respectively, and the absence of olefinic resonance of C-3, and C-4 signal also appeared as downfield 191.0 ppm. The remaining ^{13}C resonances were comparble with those of compound **1**. The comparison of the EI-MS of **1** and **2** further evidences the dihydro derivative nature with compound **2**, as compound **2** M^+ (344) is more by two atomic mass units than compound **1** M^+ (342). The laevorotatory nature of **2** indicated the normal flavone stereochemistry, *S* at C-2. A final proof of the proposed structure for **2** was obtained by dehydrogenation[8] of **2** which resulted compound **1**. Thus the structure of compound **2** was characterised as (2*S*)-7,2′,3′,4′-tetramethoxyflavanone.

The structure of the known compound as 7,4′-dimethoxy-3′-hydroxyflavone (**3**) was established by comparison of its spectral data with literature values.[9]

Experimental

General Procedures Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded in KBr discs on a Bio-Rad win FT-IR spectrophotometer and UV spectra on a Shimadzu UV-240 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were determined on a Varian VRX 500 Spectrometer operating at 500 MHz and 125 MHz, respectively using TMS as an internal standard. ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), HMBC and the phase-sensitive NOESY (with 150 ms mixing time) spectra were recorded using the standard pulse sequences. Liquid chromatography (LC)-MS was recorded on a AGILENT-1100 periods LC/MSD (VL). HR-EI-MS were recorded at 70 eV (direct probe) on a Nermag R 10-10 mass spectrometer. Column chromatography (CC) was perfomred on acme silica gel finer than 200 mesh (0.08 nm).

Plant Material The whole plant of *C. inermis* was collected in September 2001 at Tirumala Hills, Tirupati, Andhra Pradesh, India.

Extraction and Isolation The air-dried and powdered whole plant (2.5 kg) of *C. inermis* was successively extracted with *n*-hexane, Me_2CO and MeOH. The *n*-hexane extract was purified over a silica gel column using *n*-hexane and EtOAc, and their step gradient mixture as eluents. The *n*-hexane and EtOAc (3 : 7, 2 : 8) eluents yielded **1** (15 mg) and **2** (20 mg) respectively. The acetone extract on similar purification using hexane and EtOAc, 6 : 4 yielded **3** (23 mg).

7,2′,3′,4′-Tetramethoxyflavone (**1**): Amorphous powder, mp 192—194 °C (MeOH). UV λ_{max} (MeOH) nm (log ε): 254 (4.23), 330 (4.14). IR (KBr) ν_{max} cm^{-1}: 1648 (>C=O), 1620, 1596, 1490. ^{1}H-NMR ($CDCl_3$) δ: 8.11 (1H, d, *J*=8.8 Hz, H-5), 7.50 (1H, d, *J*=8.8 Hz, H-6′), 6.94 (1H, dd, *J*=8.8, 2.3 Hz, H-6), 6.88 (1H, d, *J*=2.3 Hz, H-8), 6.77 (1H, d, *J*=8.8 Hz, H-5′), 6.92 (1H, s, H-3), 3.95 (3H, s, OMe-7), 3.93 (3H, s, OMe-2′), 3.92 (3H, s, OMe-3′), 3.89 (3H, s, OMe-4′); ^{13}C-NMR ($CDCl_3$): see Table 1. EI-MS *m/z* (%): 342 $[M]^+$ (65), 192 (42), 177 (26), 151 (100), 134 (26), 119 (18), 79 (20). HR-EI-MS *m/z* 342.1061 $[M]^+$ (Calcd for $C_{19}H_{18}O_6$: 342.1058), LC-MS *m/z* 343 $[M+H]^+$ and 365 $[M+Na]^+$.

7,2′,3′,4′-Tetramethoxyflavanone (**2**): Amorphous powder, mp 180—182 °C (MeOH), $[\alpha]_D^{28}$ −16.3° (*c*=0.12, MeOH). UV λ_{max} (MeOH) nm (log ε): 261 (4.32), 339 (4.03). IR (KBr) ν_{max} cm^{-1}: 1670 (>C=O), 1600, 1509, 1440, 1376. ^{1}H-NMR ($CDCl_3$) δ: 7.84 (1H, d, *J*=8.8 Hz, H-5), 6.58 (1H, dd, *J*=8.6, 2.3 Hz, H-6), 6.40 (1H, d, *J*=2.3 Hz, H-8), 5.65 (1H, dd, $J_{2ax.\,3ax}$=12.5 Hz, $J_{2ax.\,3eq}$=3.1 Hz, H-2$_{ax}$), 3.01 (1H, dd, $J_{3ax.\,3eq}$=17.1 Hz, $J_{3ax.\,2ax}$=12.5 Hz), 2.75 (1H, dd, $J_{3eq.\,3ax}$=17.1 Hz, $J_{3eq.\,2ax}$=3.1 Hz), 3.98 (3H, s, OMe-7), 3.90 (3H, s, OMe-2′), 3.83 (3H, s, OMe-3′), 3.74 (3H, s, OMe-4′). ^{13}C-NMR ($CDCl_3$): see Table 1. EI-MS *m/z* (%): 344 $[M]^+$ (25), 313 (100), 194 (33), 179 (41), 151 (55), 121 (19). HR-EI-MS *m/z* 344.1251 $[M]^+$ (Calcd for $C_{19}H_{20}O_6$: 344.1255), LC-MS *m/z* 345 $[M+H]^+$ and 367 $[M+Na]^+$.

Dehydrogenation of **2**: A mix of 12 mg of 2, 0.15 g of KOAc and 25 mg of I_2 in glacial HOAc (2 ml) was heated under reflux for 2 h on oil bath the reaction mix. was cooled and poured into crushed ice and extracted with EtOAc. The solvent was removed *in vacuo* and a satd soln of $NaHSO_3$ was added to the residue to destroy excess of I_2. It was filtered and the residue obtained was purified from MeOH to yield colourless compound which is identical in all respects with compound **1**.

7,4′-Dimethoxy-3′-hydroxyflavone (**3**): Colourless needles, mp 190—192 °C (MeOH). UV λ_{max} (MeOH) nm (log ε): 235 (4.41), 314 sh, 338 (4.20). IR (KBr) ν_{max} cm^{-1}: 3278 (OH), 2972, 2840, 1643 (>C=O), 1602, 1511, 1440, 1379. ^{1}H-NMR (DMSO-d_6) δ: 9.44 (1H, s, OH-3′), 7.89 (1H, d, *J*=8.9 Hz, H-5), 7.51 (1H, dd, *J*=8.9, 1.5 Hz, H-6′), 7.43 (1H, d, *J*=1.5 Hz, H-2′), 7.21 (1H, d, *J*=1.5 Hz, H-8), 7.03 (2H, m, H-5′, 6), 6.70 (1H, s, H-3), 3.88 (3H, s, OMe-7), 3.84 (3H, s, OMe-4′). ^{13}C-NMR (DMSO-d_6) δ: see Table 1. EI-MS *m/z* (%): 298 $[M]^+$ (100), 283 (5), 270 (4), 255 (11), 165 (10), 151 (32).

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