Two New Homoisoflavonoids from *Caesalpinia pulcherrima*

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Two new homoisoflavonoids, (*E*)-7-methoxy-3-(4′-methoxybenzylidene)chroman-4-one (1) and (*E*)-7-hydroxy-3-(3′,4′,5′-trimethoxybenzylidene)chroman-4-one (5), along with three known homoisoflavonoids (*Z*)-7-hydroxy-3-(4′-methoxybenzylidene)chroman-4-one (isobon ducellin) (2), (*E*)-7-hydroxy-3-(4′-methoxybenzylidene) chroman-4-one (bonducellin) (3) and (*E*)-7-hydroxy-3-(2′,4′-dimethoxybenzylidene)chroman-4-one (4) were isolated from the whole plant of *Caesalpinia pulcherrima*. The structures of these new compounds were elucidated by electron impact mass spectrometry (EI-MS) and 1D and 2D-NMR spectral studies. Antimicrobial activity of the new compounds was evaluated.

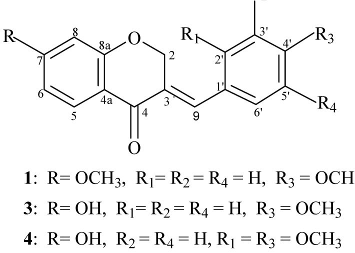
Key words *Caesalpinia pulcherrima*; Leguminosae; homoisoflavonoid; antimicrobial activity

Caesalpinia pulcherrima (Leguminosae) is a small size plant distributed in the hill tracks of Tirumula Hills, Andhra Pradesh, South India.[1] In traditional Indian Medicine *C. pulcherrima* is used in the treatment of tridosha, fever, ulcer, abortifacient, emmenagogue, asthma, tumors, vata and skin diseases.[2] Previous studies on this plant have resulted in the isolation of several diterpenoids,[3—6] flavanoids,[7] peltogynoids[8] and homoisoflavonoids.[7—9] In continuation of our search for new plant secondary metabolites,[10] we have investigated the whole plant of *C. pulcherrima*, and report here the isolation, structure elucidation and antimicrobial activity of two new homoisoflavanoids, (*E*)-7-methoxy-3-(4′-methoxybenzylidene)chroman-4-one (**1**) and (*E*)-7-hydroxy-3-(3′,4′,5′-trimethoxybenzylidene)chroman-4-one (**5**), together with three known homoisoflavonoids, (*Z*)-7-hydroxy-3-(4′-methoxybenzylidene)chroman-4-one (isobonducellin) (**2**), (*E*)-7-hydroxy-3-(4′-methoxybenzylidene)chroman-4-one (bonducellin) (**3**) and (*E*)-7-hydroxy-3-(2′,4′-dimethoxybenzylidene)chroman-4-one (**4**).

Results and Discussion

Compound **1**, was isolated as an amorphous powder. Its molecular formula was assigned as $C_{18}H_{16}O_4$ from its mass spectrum (M^+ at *m*/*z* 296), elemental analysis and ^{13}C-NMR spectrum, with eleven degree of unsaturation. The strong IR absorptions at 1659 (C=O), 1604 (C=C), 829 cm^{-1} (*para* substituted benzene ring), UV absorption maxima at 356 and 320 nm and negative ferric chloride test indicated that **1** was a non-phenolic unsaturated homoisoflavanone.[8]

The ^{1}H-NMR spectrum of **1** revealed the presence of two methoxyl groups as a singlet at δ 3.82 integrating for 6 protons. The EI-MS of **1** showed two *retro*-Diels–Alder fragments at *m*/*z* 151 and *m*/*z* 146 indicating the presence of one methoxyl group in ring-A and another methoxyl group in ring-B, respectively. The correlations observed in the NOESY spectrum of **1** (Fig. 3) indicated that the two methoxyl groups in **1** were placed at C-7 and C-4′ position. The ^{1}H-NMR spectrum of **1** showed an ABX spin coupled system at δ 7.79 (1H, d, J=8.8 Hz), 6.68 (1H, dd, J=8.8, 1.9 Hz) and 6.54 (1H, d, J=1.9 Hz) was assigned to H-5, H-6 and H-8, respectively. Further it also revealed the presence of AA′ XX′ spin coupled system with two protons centered at δ 7.42 (d, J=8.5 Hz) and another two protons at δ 7.04 (d, J=8.5 Hz), which were assigned to the H-2′, 6′ and H-3′, 5′ protons, respectively. The *E*-geometry of the double bond at C-3 and C-9 in **1** was clearly indicated by δ values of the methylene protons at δ 5.40 for C-2 and the vinylic proton at



1: R= OCH_3, R_1= R_2 = R_4 = H, R_3 = OCH_3

3: R= OH, R_1= R_2 = R_4 = H, R_3 = OCH_3

4: R= OH, R_2 = R_4 = H, R_1 = R_3 = OCH_3

5: R= OH, R_1= H, R_2 = R_3 = R_4 = OCH_3

Fig. 1. Structures of Compounds **1**, **3**, **4** and **5**

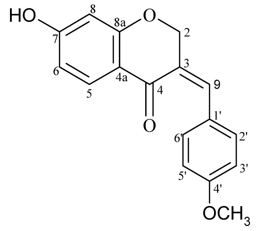


Fig. 2. Structure of Compound **2**

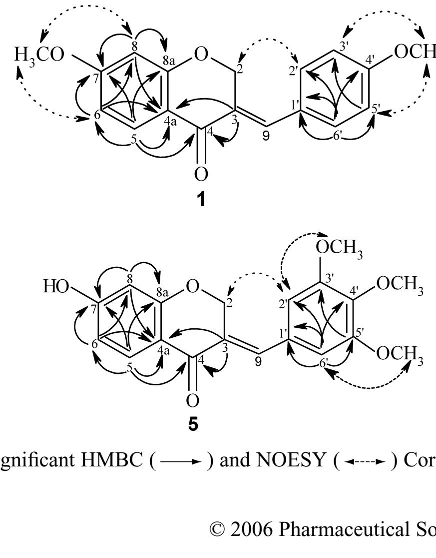


Fig. 3. Significant HMBC (⟶) and NOESY (⇠⇢) Correlations for **1** and **5**

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Table 1. ^{13}C-NMR Data (125, 75 MHz, CDCl$_3$ and DMSO-d_6) of Compounds **1**, **5**, **2**, **3** and **4**

Carbon	1	5	2	3	4
2	67.7	67.5	75.0	67.2	67.4
3	126.4	129.5	126.6	126.6	128.3
4	179.6	179.1	181.6	180.2	179.6
4a	105.4	107.8	115.5	114.3	115.3
5	128.9	129.5	129.1	129.2	128.8
6	110.3	111.2	110.6	110.8	110.5
7	165.5	164.7	164.3	164.3	164.1
8	100.6	102.4	102.1	102.4	102.1
8a	162.5	162.6	162.6	162.5	158.9
9	135.6	135.6	138.6	135.5	131.0
1′	128.5	130.2	127.2	128.7	114.0
2′	132.3	107.8	132.5	131.4	161.7
3′	114.3	152.8	112.9	113.7	99.8
4′	160.3	138.4	160.2	159.9	162.2
5′	114.3	152.8	112.9	113.7	104.4
6′	132.3	107.8	132.5	131.4	130.8
7-OMe	55.8	—	—	—	—
2′-OMe	—	—	—	—	55.6
3′-OMe	—	56.0	—	—	—
4′-OMe	55.8	60.1	54.8	54.9	54.8
5′-OMe	—	56.0	—	—	—

δ 7.65 for C-9, since in the case of the Z-geometry positions the H-9 away from the anisotropic region of the carbonyl group should resonate at a higher field.[11,12] Therefore the structure of **1** was characterized as (E)-7-methoxy-3-(4′-methoxybenzylidene)chroman-4-one.

Compound **5** was isolated as a yellow colour solid. It was analyzed for $C_{19}H_{19}O_6$ from its mass spectrum (M$^+$ at m/z 342), elemental analysis and ^{13}C-NMR spectrum with eleven degree of unsaturation. The UV absorption bands at 355 and 310 nm, the characteristic IR absorption bands at 3233 (OH), 1651 (C=O), 1618 (C=C), 847 cm^{-1} (trisubstituted benzene ring) and positive ferric chloride test indicated that compound **5** was also a unsaturated homoisoflavanone[8] containing phenolic hydroxyl group.

The fragment peaks at m/z 167 and m/z 137 attributable to a *retro*-Diels–Alder reaction in the EI-MS of **5**, suggested that three methoxyl groups were present in the ring-B and a hydroxyl group in ring-A respectively. The ^{1}H-NMR spectrum of **5** was closely related to that of **1**, suggesting that compound **5** also possessed a homoisoflavanone skeleton with three methoxyl and a hydroxyl groups. However, instead of the AA′XX′ coupling system in **1**, compound **5** possessed a symmetric two proton singlet at δ 6.72 (2H, s) assigned to C-2′ and C-6′ positions and two sharp singlets at δ 3.82 (6H, s) and 3.70 (3H, s) assigned for three symmetric methoxyl groups at C-3′, C-4′, and C-5′ positions, respectively. The placements of these three methoxyl groups at C-3′, C-4′, and C-5′ were confirmed by the NOESY and HMBC correlations (Fig. 3). The hydroxyl group was placed at C-7 as this carbon signal was shifted downfield by 29.3 ppm in its ^{13}C-NMR spectrum.[13] Thus the structure of compound **5** was characterized as (E)-7-hydroxy-3-(3′,4′,5′-trimethoxybenzylidene) chroman-4-one.

The known compounds, (Z)-7-hydroxy-3-(4′-methoxybenzylidene)chroman-4-one (isobonducellin) (**2**), (E)-7-hydroxy-3-(4′-methoxybenzylidene)chroman-4-one (bonducellin) (**3**) and (E)-7-hydroxy-3-(2′,4′-dimethoxybenzylidene)chroman-4-one (**4**) were identified by comparison of the spectral data in the literatures.[7—9)]

Experimental

General Procedures Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. UV spectra were recorded in MeOH on a Shimadzu UV-240 spectrophotometer and IR spectra were recorded in KBr discs on a Bio-rad win FT-IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were determined on a Bruker 300, 500 Spectrometer using DMSO-d_6, and CDCl$_3$ with TMS as an internal standard. ^{1}H–^{1}H COSY, HSQC, HMBC and the phase-sensitive NOESY (with 150 ms mixing time) spectra were recorded using the standard pulse sequences. EI-MS were recorded at 70 eV (direct probe) on a Nermag R10-10 mass spectrometer. LC-MS was recorded on a AGILENT-1100 periods LC/MSD (VL). Column chromatography (CC) was performred on acme silica gel finer than 200 mesh (0.08 nm).

Plant Material The whole plant of *C. pulcherrima* was collected in December 2004 at Tirumala Hills, Tirupati, Andhra Pradesh, India.

Extraction and Isolation The air-dried and powdered whole plant (3.5 kg) of *C. pulcherrima* was successively extracted with n-hexane, Me$_2$CO and MeOH. The n-hexane extract was purified over a silica gel column using n-hexane and EtOAc and the fractions eluted with 5% EtOAc in hexane afforded compound **1** (24 mg). Similarly the acetone extract was chromatographed using hexane and EtOAc and their step gradient to obtain a mixture containing **2** and **3** (35 mg). Further elution of the column with 40% EtOAc in hexane afforded **4** (16 mg) and **5** (21 mg). The mixture was rechromatographed using step gradient elution with 25% and 30% EtOAc in hexane to yield **2** (12 mg) and **3** (18 mg) respectively.

(E)-7-Methoxy-3-(4′-methoxybenzylidene)chroman-4-one (**1**): Amorphous powder, mp 130—132 °C (hexane–EtOAc). UV $\lambda_{\max}$ (MeOH) nm (log ε): 356 (4.44), 320 (4.21). IR (KBr) $\nu_{\max}$ cm^{-1}: 2966, 2841, 1659 (C=O), 1604 (C=C), 1587, 1456, 1259, 1157, 1027, 829. ^{1}H-NMR (DMSO-d_6) δ: 7.79 (1H, d, J=8.8 Hz, H-5), 7.65 (1H, s, H-9), 7.42 (2H, d, J=8.5 Hz, H-2′, H-6′), 7.04 (2H, d, J=8.5 Hz, H-3′, H-5′), 6.68 (1H, dd, J=8.8, 1.9 Hz, H-6), 6.54 (1H, d, J=1.9 Hz, H-8), 5.40 (2H, s, H-2), 3.82 (6H, s, OMe-7, OMe-4′). ^{13}C-NMR (DMSO-d_6): see Table 1. EI-MS m/z (%): 296 [M]$^+$ (100), 295 (62), 151 (56), 146 (27), 131 (40), 108 (74), 77 (18). LC-MS m/z 297 [M+H]$^+$ and 319 [M+Na]$^+$. *Anal.* Calcd for $C_{18}H_{16}O_4$: C, 72.97; H, 5.40; Found: C, 72.93; H, 5.45.

(E)-7-Hydroxy-3-(3′,4′,5′-trimethoxybenzylidene)chroman-4-one (**5**): Yellow colour solid, mp 202—204 °C (hexane–EtOAc). UV $\lambda_{\max}$ (MeOH) nm (log ε): 355 (4.60), 310 (3.91). IR (KBr) $\nu_{\max}$ cm^{-1}: 3233 (OH), 2924, 1651 (C=O), 1618 (C=C), 1582, 1468, 1259, 1158, 1008, 847. ^{1}H-NMR (DMSO-d_6) δ: 10.6 (1H, s, OH), 7.73 (1H, d, J=8.7 Hz, H-5), 7.63 (1H, s, H-9), 6.72 (2 H, s, H-2′, H-6′), 6.55 (1 H, dd, J=8.7, 1.9 Hz, H-6), 6.32 (1H, d, J=1.9 Hz, H-8), 5.41 (2H, s, H-2), 3.82 (6H, s, OMe-3′ OMe-4′), 3.70 (3H, s, OMe-5′). ^{13}C-NMR (DMSO-d_6): see Table 1. EI-MS m/z (%): 342 [M]$^+$ (100), 341 (43), 280 (27), 181 (43), 175 (32), 167 (70), 137 (92), 107 (27), 77 (18). LC-MS m/z 341 [M−H]$^-$. *Anal.* Calcd for $C_{19}H_{19}O_6$: C, 66.66; H, 5.55; Found: C, 66.70; H, 5.61.

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