

Isolation, Synthesis, and Bioactivity of Homoisoflavonoids from *Caesalpinia pulcherrima*¹⁾

Biswanath DAS,^{*,a} Ponnaboina THIRUPATHI,^a Bommena RAVIKANTH,^a Rathod ARAVIND KUMAR,^a Akella Venkata Subramanya SARMA,^b and Shaik Jilani BASHA^b

^a Organic Chemistry Division-I, Indian Institute of Chemical Technology; and ^b NMR Division, Indian Institute of Chemical Technology; Hyderabad–500 007, India.

Received May 29, 2009; accepted July 29, 2009; published online July 30, 2009

One new homoisoflavonoid, (3*E*)-2,3-dihydro-6,7-dimethoxy-3[(3-hydroxy-4-methoxyphenyl)methylene]-4*H*-1-benzopyran-4-one and four naturally new analogues, (3*E*)-3-(1,3-benzodioxol-5-ylmethylene)-2,3-dihydro-7-hydroxy-4*H*-1-benzopyran-4-one, (3*E*)-3-(1,3-benzodioxol-5-ylmethylene)-2,3-dihydro-7-methoxy-4*H*-1-benzopyran-4-one, (3*E*)-2,3-dihydro-7-hydroxy-3-[(3-hydroxy-4-methoxyphenyl)methylene]-4*H*-1-benzopyran-4-one and (3*E*)-2,3-dihydro-3-[(3,4-dimethoxyphenyl)methylene]-7-methoxy-4*H*-1-benzopyran-4-one, along with four known homoisoflavonoids, bonducellin, sappanone A, 2'-methoxybonducellin and 7-*O*-methylbonducellin were isolated from aerial parts of *Caesalpinia pulcherrima*. The structures of the new compounds were elucidated by interpretation of their 1D and 2D NMR spectra. Syntheses of the naturally new compounds and the known compounds have also been accomplished. The antibacterial and antifungal activities of the isolated homoisoflavonoids were studied.

Key words *Caesalpinia pulcherrima*; homoisoflavonoid; synthesis; antibacterial activity; antifungal activity

Caesalpinia pulcherrima SWARTZ is a Leguminous, perennial large shrub or small tree found throughout India. It has several medicinal properties, used in the treatment of ulcer, asthma, fever, tumors, and skin diseases.²⁾ Previous studies on this plant led to the isolation of several diterpenoids,^{3–6)} peltogynoids,⁷⁾ flavonoids,^{7,8)} chalcones,⁸⁾ and homoisoflavonoids.^{7–13)}

In continuation of our search for new plant metabolites^{14–17)} we carried out the chemical investigation on the chloroform–methanol (1 : 1) extract of the fresh collection of the aerial parts of *C. pulcherrima*. The crude extract was subjected to column chromatography over silica gel using hexane/ethyl acetate mixtures to obtain nine homoisoflavonoids 1–9 (Fig. 1). Compounds 1 [(3*E*)-3-(1,3-benzodioxol-5-ylmethylene)-2,3-dihydro-7-hydroxy-4*H*-1-benzopyran-4-one], 2 [(3*E*)-3-(1,3-benzodioxol-5-ylmethylene)-2,3-dihydro-7-methoxy-4*H*-1-benzopyran-4-one], 4 [(3*E*)-2,3-dihydro-7-hydroxy-3-[(3-hydroxy-4-methoxyphenyl)methylene]-4*H*-1-benzopyran-4-one], and 5 [(3*E*)-2,3-dihydro-3-[(3,4-dimethoxyphenyl)methylene]-7-methoxy-4*H*-1-benzopyran-4-one] are reported here for the first time from a natural source but they were synthesized before.^{8,18–24)} On the other hand, compounds 6 [(3*E*)-2,3-dihydro-7-hydroxy-3-[(4-methoxyphenyl)methylene]-4*H*-1-benzopyran-4-one] (bonducellin),^{3–6)} 7 [(3*E*)-2,3-dihydro-3-[(3,4-dihydroxyphenyl)methylene]-7-hydroxy-4*H*-1-benzopyran-4-one] (sappanone A),^{8,9)} 8 [(3*E*)-2,3-dihydro-7-methoxy-3-[(4-methoxyphenyl)methylene]-4*H*-1-benzopyran-4-one] (7-*O*-methyl bonducellin)¹²⁾ and 9 [(3*E*)-2,3-dihydro-3-[(2,4-dimethoxyphenyl)methylene]-7-hydroxy-4*H*-1-benzopyran-4-one] (2'-methoxybonducellin)¹¹⁾ were isolated previously from the title plant. The structures of these naturally new and known homoisoflavonoids were settled by comparison of their spectral (IR, ¹H- and ¹³C-NMR and MS) data with those reported earlier.

Compound 3 is a new constituent. It was isolated as an yellow amorphous powder, mp 168–170 °C. Its molecular

formula was assigned as C₁₉H₁₈O₆ from its mass spectrum ([M+H]⁺ at *m/z* 343), elemental analysis and ¹³C-NMR spectrum. A comparison of its molecular formula with that of the known constituent, 4 (molecular formula: C₁₇H₁₂O₅) indicated that 3 contained a methoxy group instead of a hydroxy group present in 4 along with an additional methoxy group. The appearance of two fragment peaks at *m/z* 180 and 162 in the mass spectrum due to *retro* Diels–Alder cleavage suggested the presence of two methoxy group in ring A while a hydroxy and a methoxy groups in ring B. The ¹H- and ¹³C-NMR spectral data of the compounds also revealed that it

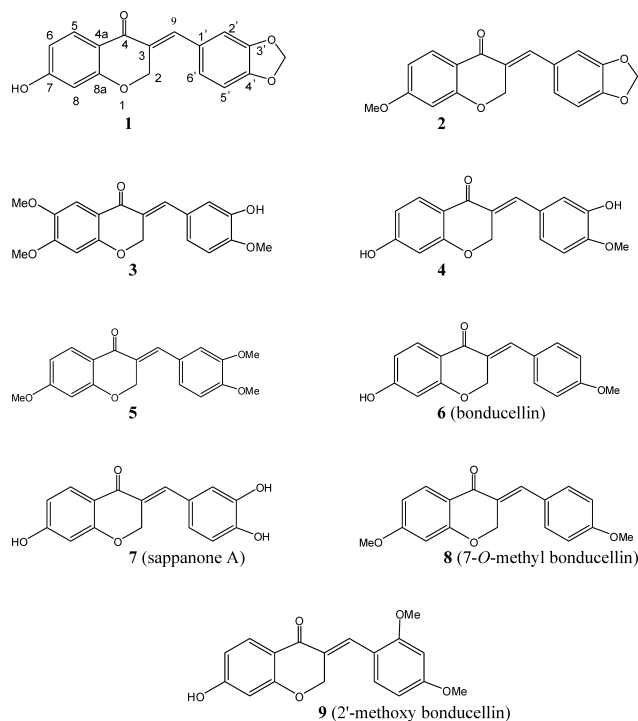


Fig. 1. Structures of Homoisoflavonoids 1–9

* To whom correspondence should be addressed. e-mail: biswanathdas@yahoo.com

contained three methoxy groups (δ 3.95, 3H, s and 3.92, 6H, s in the ^1H -NMR spectrum and δ 56.3, 56.2, and 56.0 in the ^{13}C -NMR spectrum) and a hydroxy group (δ 5.72, br s in the

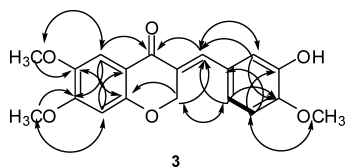
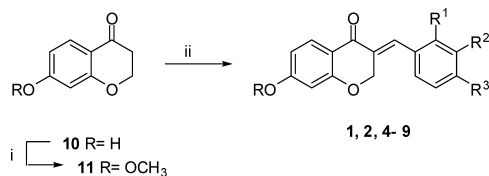


Fig. 2. Significant DQF-COSY (—) and NOESY (↷) Correlations and HMBC (↶) for **3**



Reagent and conditions (i) CH_3I , K_2CO_3 , acetone, 2 h, reflux, (91%); (ii) substituted benzaldehyde, piperidine, 2 h (58–69%).

Chart 1

Table 1. Antibacterial Activity of the Homoisoflavonoids **1–9**^{a–c)}

Compound	Gram-positive organisms						Gram-negative organisms					
	<i>Bacillus subtilis</i>		<i>Bacillus sphaericus</i>		<i>Staphylococcus aureus</i>		<i>Pseudomonas aeruginosa</i>		<i>Klebsiella aerogenes</i>		<i>Chromobacterium violaceum</i>	
	A	B	A	B	A	B	A	B	A	B	A	B
1	+	+	+	+	+	++	–	–	+	++	+	++
2	+	+	+	+	+	+	–	–	+	+	+	+
3	+	+	+	+	+	+	–	–	+	+	+	+
4	+	+	+	+	+	+	–	–	+	+	+	+
5	+	+	+	+	+	+	–	–	+	++	+	+
6	+	+	+	+	+	+	–	–	+	+	+	+
7	+	+	+	+	+	++	–	–	+	+	+	+
8	+	+	+	+	+	+	–	–	+	+	+	+
9	+	+	+	+	+	+	–	–	+	+	+	+
(Positive controls)												
Penicillin G	++		++		++							
Streptomycin							++++		++++		++++	

a) Results after 24 h. b) Inhibitory zone measured in mm, (–) no activity; (+) inhibitory zone 5–10 cm; (++) inhibitory zone 11–15 cm; (+++) inhibitory zone 16–20 cm; (+++++) inhibitory zone 21–25 cm. c) Compounds were tested with a concentration of 30 $\mu\text{g/ml}$ (A), or 100 $\mu\text{g/ml}$ (B).

Table 2. Antifungal Activity of the Homoisoflavonoids **1–9**^{a–c)}

Compound	Microorganisms					
	<i>Aspergillus niger</i>		<i>Candida albicans</i>		<i>Rhizopus oryzae</i>	
	B	C	B	C	B	C
1	+	+	+	+	–	–
2	+	+	+	++	–	–
3	+	++	+	++	–	–
4	+	++	+	++	–	–
5	+	+	+	++	–	–
6	+	+	+	+	–	–
7	+	++	+	+	–	–
8	+	+	+	+	–	–
9	+	+	+	++	–	–
(Positive controls)						
Clotrimazole	++++		++++		++++	

a) Results after 48 h. b) Inhibitory zone measured in mm, (–) no activity, (+) inhibitory zone 5–10 cm; (++) inhibitory zone 11–15 cm; (+++) inhibitory zone 16–20 cm; (+++++) inhibitory zone 21–25 cm. c) Compounds were tested with a concentration of 100 $\mu\text{g/ml}$ (B), or 150 $\mu\text{g/ml}$ (C).

^1H -NMR spectrum). The 2D-NMR (double quantum filtered (DQF)-COSY, NOESY and HMBC) experiments confirmed the positions of the methoxys at C-6, C-7 and C-4' while that of the hydroxy group at C-3' (Fig. 2). The *E*-geometry of the double bond at C-3 and C-9 in **3** was clearly indicated by the characteristic of the methylene proton (δ 5.35, d, $J=1.5$ Hz) at C-2 and the vinylic proton (δ 7.75, 1H, br s) at C-9.⁸⁾ Thus the structure of **3** was confirmed as (3*E*)-2,3-dihydro-6,7-dimethoxy-3[(3-hydroxy-4-methoxyphenyl)methylene]-4*H*-1-benzopyran-4-one.

We extended our work to syntheses of isolated homoisoflavonoids **1**, **2**, and **4–9** according to the literatures.^{25–27)} Thus the piperidine catalyzed condensation of 7-hydroxy-4-chromanone **10** with 4-methoxy, 3,4-methylenedioxy, 3-hydroxy-4-methoxy, 2,4-dimethoxy, and 3,4-dihydroxybenzaldehydes afforded **6**, **1**, **4**, **9** and **7** in 68, 63, 58, 60, and 59% yields, respectively (Chart 1). The condensation of 7-methoxy-4-chromanone (**11**) (obtained by methylation of 7-hydroxy-4-chromanone (**10**) using iodomethane and K_2CO_3) with 3,4-methylenedioxy, 3,4-dimethoxy, and 4-methoxy benzaldehydes using piperidine gave compounds **2**, **5** and **8** in 65, 69 and 61% yields, respectively (Chart 1).

In all the cases single geometrical isomer (*E*) was obtained. The stereochemistry at the double bond was confirmed by the characteristic $^1\text{H-NMR}$ spectral value of H-9 which appeared around δ 7.5.¹⁸ The spectral (NMR and MS) data of **1**, **2**, and **4**—**9** were identical with those of the natural products.

Activity of Homoisoflavonoids The homoisoflavonoids possess antibacterial and antifungal activities. The antibacterial activity of the homoisoflavonoids, **1**—**9** was evaluated (Table 1) following the reported.⁴ Agar cup bioassay method. All the compounds showed moderate activity against the Gram-positive organisms, *Bacillus subtilis*, *Bacillus sphaericus* and *Staphylococcus aureus*. However, they were inactive against the Gram-negative organism, *Pseudomonas aeruginosa*, and weakly active against *Klebsiella aerogenes* and *Chromobacterium violaceum*.

The antifungal activity of all the compounds **1**—**9** (Table 2) was also moderate against the organisms, *Apergillus niger* and *Candida albicans* but they were inactive against *Rhizopus oryzae*.

Experimental

General Experimental Procedures Melting points were measured in a Buchi-510 instrument and are uncorrected. Spectra were recorded with the following instruments: IR: Perkin-Elmer spectrophotometer and ^1H - and ^{13}C -NMR; Gemini 200 spectrometer using CDCl_3 and $\text{DMSO}-d_6$ with TMS as an internal standards. HSQC, DQF-COSY, HMBC, and phase sensitive NOESY (with 150 ms mixing time) experiments were carried out using the standard pulse sequences. EI-MS were recorded on VG-micromass 7070H (70 eV) and ESI-MS on Thermo Finnigan LCQ ion trap mass spectrometer. Column chromatography was performed on silica gel (BDH 100—200 mesh) and TLC with silica gel GF 254.

Plant Material The aerial parts of *C. pulcherrima* were collected from Osmania University campus in May, 2007 and identified botanically. A voucher specimen (No CP-AP-1) was preserved in our laboratory and another voucher specimen (IICP-150908) in IICT herbarium.

Extraction and Isolation The air-dried and powdered whole plant material (5.5 kg) of *C. pulcherrima* was successively extracted thrice with CHCl_3 and MeOH (1 : 1) (12 l) at room temperature. The extract was filtered and concentrated by rotary evaporator. The thick brown residue was chromatographed over silica gel, the column being eluted with solvents of increasing polarity using *n*-hexane and EtOAc. The fraction eluted with 15% EtOAc in hexane afforded compound **5** (13 mg). A mixture of three compounds was obtained when the column was eluted with 20% EtOAc in hexane. These were separated by preparative TLC using 10% EtOAc in hexane to obtain pure **2** (12 mg), **3** (6 mg) and **8** (8 mg). Subsequent elution of the main column with 30% EtOAc in hexane yielded **1** (7 mg) and **6** (10 mg). The column was next eluted with 35% EtOAc in hexane to produce the compound **9** (7 mg). Further elution of the column with 40% EtOAc in hexane afforded **4** (12 mg) and **7** (5 mg).

Compound 3 (3*E*)-2,3-Dihydro-6,7-dimethoxy-3[(3-hydroxy-4-methoxyphenyl)methylene]-4*H*-1-benzopyran-4-one Yellow amorphous powder, mp 168—170 °C; IR (KBr) ν_{max} : 3382, 2928, 1610, 1507, 1265, 1129, 756 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3) δ : 7.75 (1H, br s, H-9), 7.42 (1H, s, H-5), 6.91 (1H, d, J =8.5 Hz, H-5'), 6.90 (1H, d, J =2.0 Hz, H-2'), 6.86 (1H, dd, J =8.5, 2.0 Hz, H-6'), 6.43 (1H, s, H-8), 5.72 (1H, br s, -OH), 5.35 (2H, d, J =1.5 Hz, H-2), 3.95 (3H, s, OMe-4'), 3.92 (6H, s, OMe-6, OMe-7). $^{13}\text{C-NMR}$ (CDCl_3) δ : 180.9 (C-4), 157.5 (C-8a), 156.1 (C-7), 147.5 (C-4'), 154.5 (C-3'), 144.1 (C-6), 136.5 (C-9), 129.3 (C-1'), 128.0 (C-3), 123.3 (C-6'), 115.8 (C-2'), 114.2 (C-4a), 110.5 (C-5'), 107.4 (C-5), 100.0 (C-8), 68.1 (C-2), 56.3 (MeO-4'), 56.2 (MeO-6), 56.0 (MeO-7); ESI-MS $[\text{M}+\text{H}]^+$ at m/z 343; Anal. Calcd for $\text{C}_{19}\text{H}_{18}\text{O}_6$: C, 66.66; H, 5.26 Found: C, 66.87; H, 5.34.

Synthesis of Homoisoflavonoids. Preparation of 7-Methoxy-4-chromanone 11 A mixture of **10** (1 g) and iodomethane (1.5 g) was taken into acetone (10 ml). K_2CO_3 (1.2 g) was added and the mixture was stirred for 6 h at room temperature. The reaction was monitored by TLC. After completion of the reaction, the mixture was extracted with EtOAc (2×50 ml). The combined organic layers were washed with brine (2×25 ml), dried over Na_2SO_4 and filtered. Concentration of the filtrate in vacuo gave crude compound

which was subjected to column chromatography to yield the pure 7-methoxy-4-chromanone (**11**, 91%) as a colorless amorphous powder, mp 51—53 °C.

Syntheses of Homoisoflavonoids To a mixture of substituted 4-chromanone (**10** or **11**) (2 mmol) and appropriate substituted benzaldehyde (2.2 mmol) piperidine (6 drops) was added and the total mass was heated at 70—80 °C for 2 h. The mixture was cooled and diluted with water (100 ml), acidified with dil. HCl and extracted with EtOAc (3×50 ml). The combined EtOAc layer was washed with water (50 ml) and dried over Na_2SO_4 . The residue obtained after evaporation of the solvent was chromatographed over silica gel using mixtures of *n*-hexane and EtOAc as eluent to afford pure homoisoflavonoid (**1**, **2**, **4**—**9** in 58—69%). The physical (R_f and mp) and spectral (IR, ^1H - and ^{13}C -NMR and MS) properties of the compounds were identical to those of the natural products.

Studies on Antibacterial and Antifungal Activities The methods have exactly been followed from our earlier work.⁸

Acknowledgements The authors thank CSIR and UGC, New Delhi, for financial assistance.

References and Notes

- Part 63 in the series, "Studies on Phytochemicals."
- Kirtikar K. R., Basu B. D., "Indian Medicinal Plants," Vol. 2, Periodical Experts, New Delhi, 1935, pp. 848—849.
- Che C. T., McPherson D. D., Cordell G. A., Fong H. H. S., *J. Nat. Prod.*, **49**, 561—569 (1986).
- McPherson D. D., Che C. T., Cordell G. A., Soejarto D. D., Pizzuto J. M., Fong H. H. S., *Phytochemistry*, **25**, 167—170 (1986).
- Patel A. D., Freyer A. J., Webb R. L., Zuber G., Rechwein R., Bean M. F., Fautt L., Johnson R. K., *Tetrahedron*, **53**, 1583—1589 (1997).
- Ragasa C. Y., Hofilena J. G., Rideout J. A., *J. Nat. Prod.*, **65**, 1107—1110 (2002).
- McPherson D. D., Cordell G. A., Soejarto D. D., Pizzuto J. M., Fong H. H. S., *Phytochemistry*, **22**, 2835—2838 (1983).
- Srinivas K. V. N. S., Rao Y. K., Mahender I., Das B., Rama Krishna K. V. S., Harakishore K., Murty U. S. N., *Phytochemistry*, **63**, 789—793 (2003).
- Namikoshi M., Nakata H., Yamada H., Nagai M., Saitoh T., *Chem. Pharm. Bull.*, **35**, 2761—2773 (1987).
- Namikoshi M., Nakata H., Saitoh T., *Phytochemistry*, **26**, 1831—1833 (1987).
- Wall M. E., Wani M. C., Manikumar G., Taylor H., McGivney R., *J. Nat. Prod.*, **52**, 774—778 (1989).
- Zhao P., Iwamoto Y., Kouno I., Egami Y., Yamamoto H., *Phytochemistry*, **65**, 2455—2461 (2004).
- Maheswara M., Siddaiah V., Venkata Rao C., *Chem. Pharm. Bull.*, **54**, 1193—1195 (2006).
- Ramesh C., Ravindranath N., Das B., Prabhakar A., Bharatam J., Ravikumar K., Kashinatham A., *Phytochemistry*, **64**, 841—844 (2003).
- Das B., Ramu R., Rao Y. K., Reddy M. R., Harish H., Reddy V. S., Rama Krishna K. V. S., *Phytochemistry*, **67**, 978—983 (2006).
- Das B., Ravindranath N., Reddy M. R., Mahender G., Ramu R., Ravi Kumar K., *Phytochemistry*, **65**, 2387—2390 (2004).
- Das B., Reddy M. R., Ramu R., Ravindranath N., Harish H., Rama Krishna K. V. S., Rao Y. K., Hara Kihore K., Murty U. S. N., *Phytochemistry*, **66**, 633—638 (2005).
- Bohler P., Tamm Ch., *Tetrahedron Lett.*, **8**, 3479—3483 (1967).
- Blasko G., Cordell G. A., *Heterocycles*, **27**, 445—452 (1988).
- Davis F. A., Chen B. C., *J. Org. Chem.*, **58**, 1751—1753 (1993).
- Jain A. C., Sharma A., Srivastava R., *Indian J. Chem. B*, **22**, 1119—1121 (1983).
- Yoshihama M., Nakakoshi M., Nakamura J., Nakayama S., *PCT Int. Appl.*, **28**, (1998).
- Pfeiffer P., Breith E., Hoyer H., *J. Prakt. Chem.*, **129**, 31—54 (1931).
- Nakib T., Bezjak V., Meegan M. J., Chandy R., *Eur. J. Med. Chem.*, **25**, 455—462 (1990).
- Kock K., Biggers M. S., *J. Org. Chem.*, **59**, 1216—1218 (1994).
- Siddaiah V., Rao C. V., Venkateshwerlu S., Krishnaraju A. V., Subbaraju G. V., *Bioorg. Med. Chem.*, **14**, 2545—2551 (2006).
- Foroumadi A., Samzadeh-Kermani A., Emami S., Dehghan G., Sorkhi M., Arabsorkhi F., Heidari M. R., Abdollahi M., Shafiee A., *Bioorg. Med. Chem. Lett.*, **17**, 6764—6769 (2007).