# Antiplatelet Arylnaphthalide Lignans from *Justicia procumbens*

Chien-Chih Chen,* Wen-Chi Hsin, Feng-Nien Ko,† Yu-Lin Huang, Jun-Chih Ou, and Che-Ming Teng†

*National Research Institute of Chinese Medicine, No. 155-1, Sec 2, Li Nung St. Peitou, Taipei, Taiwan, Republic of China, and Department of Pharmacology, National Taiwan University, Taipei, Taiwan, Republic of China*

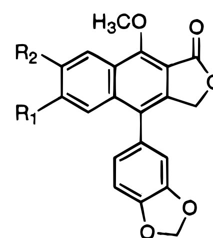
*Received May 9, 1996*⊗

Fractionation of the EtOH extract of *Justicia procumbens*, guided by antiplatelet bioassay, led to the isolation of nine known arylnaphthalide lignans, neojusticin A (**1**), justicidin B (**2**), justicidin A (**3**), taiwanin E methyl ether (**4**), neojusticin B (**5**), chinensinaphthol methyl ether (**6**), taiwanin E (**8**), chinensinaphthol (**9**), and diphyllin (**10**), and a new arylnaphthalide lignan that was characterized by spectral means as 4′-demethylchinensinaphthol methyl ether (**7**). Compounds **1**, **2**, **4**, and **8** significantly inhibited platelet aggregation.

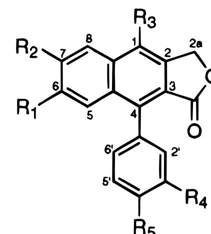
The whole plant of *Justicia procumbens* L. (Acanthaceae) is used as an herbal remedy for the treatment of fever, pain due to pharyngolaryngeal swelling,[1] and cancer.[2] In Taiwan, the juice of this plant has also been used as a fish-killing material. Previous phytochemical studies on this plant have afforded arylnaphthalide lignans.[3−5] Recently, we have been searching for pharmacologically active principles among medicinal plants used in traditional medicine in Taiwan,[6−9] and we found that the EtOH extract of the whole plant of *J. procumbens*, at 20 $\mu$g/mL, exhibited 50% inhibitory activity to the arachidonic acid (AA)-induced aggregation of rabbit platelets. This activity was followed in a bioassay-directed fractionation. The EtOH extract was extracted with $CHCl_3$ to give an active $CHCl_3$-soluble fraction (5 $\mu$g/mL, 88% inhibition to AA). This was chromatographed on a Si gel column and eluted successively with *n*-hexane, $CHCl_3$, $CHCl_3$−$Me_2CO$ (1:1), and $Me_2CO$ to give an active fraction (1 $\mu$g/mL, 82% inhibition to AA) eluted with $CHCl_3$. This active fraction was further separated by preparative TLC, Sephadex LH-20, and HPLC to give 10 arylnaphthalide lignans, neojusticin A (**1**),[3,5,10] justicidin B (**2**),[3,4] justicidin A (**3**),[3−5] taiwanin E methyl ether (**4**),[3] neojusticin B (**5**),[3,5,10] chinensinaphthol methyl ether (**6**),[11] 4′-demethylchinensinaphthol methyl ether (**7**), taiwanin E (**8**),[12] chinensinaphthol (**9**),[11] and diphyllin (**10**).[5] Among these, compound **7** is a novel natural product. Compounds **6**, **8**, and **9** were isolated for the first time from *Justicia* spp. The known lignans were identified by spectral analyses and comparison of their data (MS, NMR, IR) with published reports.[3−5,11,12]

4′-Demethylchinensinaphthol methyl ether (**7**) was isolated as colorless prisms, mp 240−242 °C, and its high-resolution mass spectrum indicated a molecular formula $C_{21}H_{16}O_7$ $[M]^+$ at *m/z* 380.0901. The IR spectrum contained bands associated with an aromatic $\gamma$-lactone (1755 $cm^{-1}$) and a methylenedioxy ether (933 $cm^{-1}$). The $^1H$ NMR spectrum contained the signals of five aromatic protons [$\delta$ 7.57 (s), 7.09 (s), 7.05 (d, $J$ = 8.4 Hz), and 6.82 (2H, m)], a methylenedioxy group [$\delta$ 6.08 (2H, s)], a $\gamma$-lactone methylene group [$\delta$ 5.51 (2H, s)], a hydroxy group [$\delta$ 5.74 (s)], and two methoxyl groups ($\delta$ 4.08 and 3.87). The signal due to the $\gamma$-lactone methylene protons appeared at $\delta$ 5.51, indicating that it was a 1-aryl-2,3-naphthalide lignan.[12,13] These observations implied a substitution pattern similar to that of chinensinaphthol (**9**).[11] The location of the methoxyls in **7** was confirmed by NOE measurements. Irradiation of the 1-$OCH_3$ at $\delta$ 4.08 enhanced the signals at $\delta$ 7.57 (H-8) and 5.51 ($H_2$-2a) by 3% and 5%, respectively. Irradiation of the 3′-$OCH_3$ at $\delta$ 3.87 enhanced the signal at $\delta$ 6.82 (H-2′). On the basis of the above results, the structure of 4′-demethylchinensinaphthol methyl ether was assigned as **7**.

With the exception of **7** and **9**, which were unavailable in sufficient quantity, these lignans were tested for their *in vitro* antiplatelet activity on rabbit platelets against AA-induced aggregation. As shown in Table 1, all of the lignans tested were less active than indomethacin. However, compounds **1**, **2**, **4**, and **8** were significantly more active than aspirin. The rank order of antiplatelet potency was **1** > **4** > **2** = **8**.




**1** $R_1$-$R_2$ = -O-$CH_2$-O-
**5** $R_1$ = $R_2$ = -$OCH_3$




**2** $R_1$ = $R_2$ = -$OCH_3$, $R_3$ = -OH, $R_4$-$R_5$ = -$OCH_2O$-
**3** $R_1$ = $R_2$ = $R_3$ = -$OCH_3$, $R_4$-$R_5$ = -$OCH_2O$-
**4** $R_1$-$R_2$ = $R_4$-$R_5$ = -$OCH_2O$-, $R_3$ = -$OCH_3$
**6** $R_1$-$R_2$ = -O-$CH_2$-O-, $R_3$ = $R_4$ = $R_5$ = -$OCH_3$
**7** $R_1$-$R_2$ = -O-$CH_2$-O-, $R_3$ = $R_4$ = -$OCH_3$, $R_5$ = -OH
**8** $R_1$-$R_2$ = $R_4$-$R_5$ = -$OCH_2O$-, $R_3$ = -OH
**9** $R_1$-$R_2$ = -$OCH_2O$-, $R_3$ = -OH, $R_4$ = $R_5$ = -$OCH_3$
**10** $R_1$ = $R_2$ = -$OCH_3$, $R_3$ = -OH, $R_4$-$R_5$ = -$OCH_2O$-

* To whom correspondence should be addressed. Tel.: 886-2-8201999 ext. 6701. Fax: 886-2-8250743.
† National Taiwan University.
⊗ Abstract published in *Advance ACS Abstracts,* November 1, 1996.

S0163-3864(96)00443-0 CCC: $12.00 © 1996 American Chemical Society and American Society of Pharmacognosy

**Table 1.** Effect of Compounds, Isolated from *J. procumbens*, on AA-Induced Platelet Aggregation to Rabbit Platelet Suspension[a]

| compounds | $IC_{50}$ ($\mu$M) |
|---|---|
| neojusticin A (**1**) | 1.1 ± 0.3 |
| justicidin B (**2**) | 8.0 ± 1.2 |
| justicidin A (**3**) | 31.7 ± 4.5 |
| taiwanin E methyl ether (**4**) | 1.7 ± 0.3 |
| neojusticin B (**5**) | 50.8 ± 8.3 |
| chinensinaphthol methyl ether (**6**) | 18.8 ± 3.0 |
| taiwanin E (**8**) | 8.0 ± 1.1 |
| diphyllin (**10**) | >100 |
| aspirin[b] | 20.3 ± 2.1 |
| indomethacin[b] | 0.21 ± 0.04 |

[a] Platelets were preincubated with various concentrations of each compound or the solvent (0.5% DMSO, control) at 37 °C for 3 min, and then AA (100 $\mu$M) was added to trigger the reaction. The activity of antiplatelet aggregation (%) was calculated by the following equation: antiplatelet aggregation (%) = [1 − (platelet aggregation potency of sample/platelet aggregation potency of control)]100. Then, the $IC_{50}$ value of each compound was calculated and shown as mean ± SD ($n$ = 3−6). [b] Positive control standard.

## Experimental Section

**General Experimental Procedures.** Melting points were measured on a Yanaco micro-melting point apparatus and are uncorrected. The IR spectra were recorded on a JASCO-IR-100 spectrometer. $^1$H NMR spectra were taken on a Varian Gemini 200 FT-NMR. EIMS spectra were recorded on a JEOL JMS-HX100 spectrometer. HPLC was carried out using a UV detector (254 nm) and a Cosmosil 5C18-AR (5 $\mu$m, 10 × 250 mm) column with a solvent ($CH_3CN$-$H_2O$ = 1:1) flow rate of 2 mL/min.

**Plant Material.** The whole plants of *J. procumbens* L. were collected in July 1993 at Yang-Ming Mountain, Taipei, under the direction of Mr. M. T. Kao of the National Research Institute of Chinese Medicine, A voucher specimen is maintained in the herbarium of the Research Institute of Chinese Medicine.

**Extraction and Isolation.** Air-dried whole plant of *J. procumbens* (3.0 kg) was chipped and refluxed with 95% EtOH (10 L × 3). After filtration and evaporation of the EtOH at reduced pressure to afford a brown syrup (232 g), the EtOH extract was mixed thoroughly with $CHCl_3$. Filtration and evaporation of the $CHCl_3$ then gave a $CHCl_3$-soluble fraction (54 g). The $CHCl_3$-soluble fraction (54 g) was chromatographed over a Si gel (70−230 mesh, 1.5 kg) column that was eluted successively with *n*-hexane, $CHCl_3$, $CHCl_3$−$Me_2CO$ (1:1), and $Me_2CO$ to give an active fraction (4.1 g) that eluted with $CHCl_3$. This active fraction was further separated by preparative TLC (Si gel, $CH_2Cl_2$−$Me_2CO$ = 25:1). Six blue fluoresent zones were detected by 365 nm UV light. Material from the first zone ($R_f$ 0.74) was recrystallized with MeOH to give neojusticin A (**1**, 12.4 mg). Material from the second zone ($R_f$ 0.66) was further separated by HPLC to give justicidin B (**2**, 11.3 mg, $t_R$ = 28.01 min), justicidin A (**3**, 8.1 mg, $t_R$ = 34.18 min), taiwanin E methyl ether (**4**, 4.6 mg, $t_R$ = 44.43 min), and neojusticin B (**5**, 10.6 mg, $t_R$ = 50.03 min). Material from the third zone ($R_f$ 0.55) was subjected to Sephadex LH-20 CC (MeOH) to obtain chinensinaphthol methyl ether (**6**, 3.3 mg). Material from the fourth zone ($R_f$ 0.41) was further purified by HPLC to give 4′-demethylchinensinaphthol methyl ether (**7**, 1.4 mg). Material from the fifth zone ($R_f$ 0.30) was purified by Si gel (230−400 mesh, $CH_2Cl_2$−$Me_2CO$ = 30:1) and Sephadex LH-20 CC (MeOH) to give taiwanin E (**8**, 2.0 mg). Material from the sixth zone ($R_f$ 0.20) was further separated by HPLC to give chinensinaphthol (**9**, 2.1 mg, $t_R$ = 18.98 min) and diphyllin (**10**, 2.4 mg, $t_R$ = 20.50 min).

**4′-Demethylchinensinaphthol methyl ether (7):** colorless prisms (MeOH); mp 240−242 °C; EIMS (70 ev) $m/z$ $[M]^+$ 380 (100), 365 (5), 337 (10), 305 (50); HREIMS $m/z$ 380.0901 ($C_{21}H_{16}O_7$ requires 380.0895); IR (KBr) $\nu_{max}$ 3300, 1755, 1610, 933 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 3.87 (3H, s, 3′-$OCH_3$), 4.08 (3H, s, 1-$OCH_3$), 5.51 (2H, s, $H_2$-2a), 5.74 (1H, s, −OH), 6.08 (2H, s, −$OCH_2O$−), 6.82 (2H, m, H-2′,6′), 7.05 (1H, d, $J$ = 8.4 Hz, H-5′), 7.09 (1H, s, H-5), 7.57 (1H, s, H-8).

**Platelet Aggregation.** Washed platelets were obtained from fresh rabbit blood according to the washing procedures described previously.[14] Aggregation was measured by an aggregometer (Chrono-Log Co., USA) using the turbidimetric method[15] and assigned the absorbance of platelet suspension as 0% aggregation and the absorbance of platelet-free Tyrode solution as 100% aggregation. The final concentration of the solvent DMSO was fixed at 0.5%, which did not affect the aggregation. Aspirin and indomethacin were used as positive controls.

**Acknowledgment.** This work was supported by a research grant of the National Science Council of Republic of China (NSC 85-2331-B-077-003).

## References and Notes

(1) Kan, W. S. *Pharmaceutical Botany*; National Research Institute of Chinese Medicine: Taiwan, R.O.C., 1981; p 513.
(2) Hsu, H. Y. *Treating Cancer with Chinese Herbs*; Oriental Healing Arts Institute: Los Angeles, CA, 1982; p 238.
(3) Ohta, K.; Munakata, K. *Tetrahedron Lett.* **1970**, 923−925.
(4) Okigawa, M.; Maeda, T.; Kawano, N. *Chem. Pharm. Bull.* **1970**, *18*, 862−863.
(5) Fukamiya, N.; Lee, K. H. *J. Nat. Prod.* **1986**, *49*, 348−350.
(6) Teng, C. M.; Yu, S. M.; Chen, C. C.; Huang, Y. L.; Huang, T. F. *Thromb. Res.* **1990**, *59*, 121−130.
(7) Chen, C. C.; Huang, Y. L.; Ou, J. C.; Su, M. J.; Yu, S. M.; Teng, C. M. *Planta Med.* **1991**, *57*, 406−408.
(8) Chen, C. C.; Wu, L. G.; Ko, F. N.; Teng, C. M. *J. Nat. Prod.* **1994**, *57*, 1271−1274.
(9) Chen, C. C.; Lin, C. F.; Huang, Y. L.; Ko, F. N.; Teng, C. M. *J. Nat. Prod.* **1995**, *58*, 1423−1425.
(10) Okigawa, M.; Maeda, T.; Kawano, N. *Tetrahedron* **1970**, *26*, 4301−4305.
(11) Ghosal, S.; Chauhan, R. P. S.; Srivastava, R. S. *Phytochemistry* **1974**, *13*, 1933−1936.
(12) Horii, Z.; Tsujiuchi, M.; Momose, T. *Tetrahedron Lett.* **1969**, 1079−1082.
(13) Sheriha, G. M.; Amer, K. M. A. *Phytochemistry* **1984**, *23*, 151−153.
(14) Teng, C. M.; Chen, W. Y.; Ko, W. C.; Ouyang, C. *Biochim. Biophys. Acta* **1987**, *924*, 375−382.
(15) O'Brien, J. R. *J. Clin. Path.* **1962**, *15*, 452−455.

NP960443+