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## Full Papers

### Chemical Constituents from *Cassytha filiformis* II

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Using a bioassay-directed fractionation method, three new compounds, including an aporphine alkaloid, cassyformine (**4**); an oxoaporphine alkaloid, filiformine (**8**), and a lignan, (+)-diasyringaresinol (**10**), along with 14 known compounds, were further isolated and characterized from the MeOH extract of the fresh herbs of *Cassytha filiformis*. Among the isolates of this plant, cathafiline (**1**), cathaformine (**2**), actinodaphnine (**3**), *N*-methylactinodaphnine (**5**), predicentrine (**6**), and ocoteine (**7**) exhibited significant antiplatelet aggregation activity.

In the course of our studies on bioactive natural substances, the MeOH extract of the fresh herbs of *Cassytha filiformis* Linn. (Lauraceae) was found to exhibit significant vasorelaxant activity as well as inhibitory effects on the platelet aggregation induced by several aggregating agents. In a previous communication, we reported two new aporphine alkaloids, cathafiline (**1**) and cathaformine (**2**), along with six known compounds, actinodaphnine (**3**), cassythine, isoboldine, cassameridine, cassamedine, and lysicamine, from the fresh herbs of Formosan grown *Cassytha filiformis*.<sup>1</sup> Further studies on the active fractions of the MeOH extract of this plant led to the isolation of four aporphines, (+)-cassyformine (**4**), (+)-*N*-methylactinodaphnine (**5**),<sup>2</sup> predicentrine (**6**),<sup>3</sup> and ocoteine (**7**);<sup>4</sup> two oxoaporphines, filiformine (**8**) and thalicminine;<sup>5</sup> two proaporphines, stepharine<sup>6</sup> and pronuciferine;<sup>7</sup> one morphinandienone, *O*-methylflavinative;<sup>8</sup> three lignans, (+)-yangambin,<sup>9</sup> (+)-syringaresinol (**9**),<sup>10</sup> and (+)-diasyringaresinol (**10**); two aromatic aldehydes, vanillin and isovanillin; one glycerol ester; and two phytosterols;

as well as a mixture of  $\beta$ -sitosterol and stigmasterol and the mixture of  $\beta$ -sitosterol-D-glucoside and stigmaterol-D-glucoside. Among them, **4**, **8**, and **10** were identified as new compounds. Moreover, compounds **1–3** and **5–7** showed significant selective antiplatelet aggregation activity induced by either ADP or arachidonic acid (AA), respectively.

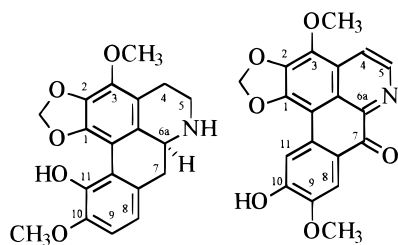
### Results and Discussion

After a series of partition procedures, the alkaloidal extract of this plant displayed significant antiplatelet aggregation activities. The alkaloidal extract was repeatedly subjected to column chromatography on Si gel or preparative TLC to afford nine alkaloids, including two new ones, **4** and **8**. Cassyformine (**4**) was isolated as a yellow amorphous powder from CHCl<sub>3</sub>, mp 135–137 °C. The molecular formula was established as C<sub>19</sub>H<sub>19</sub>O<sub>5</sub>N by HREIMS M<sup>+</sup> ion at *m/z* 341.1256 (calcd 341.1263). The presence of a 1,2,3,10,11-oxygenated aporphine skeleton in the molecule was deduced from the UV spectrum (absorption maxima at 215, 284, and 302 nm).<sup>11</sup> An anticipated bathochromic shift of UV spectrum in alkaline solution and the IR absorption at 3400 cm<sup>-1</sup> suggested the presence of a phenolic function. The IR spectrum also showed a methylenedioxy group at 1040 and 920 cm<sup>-1</sup>. Further inspection of the <sup>1</sup>H

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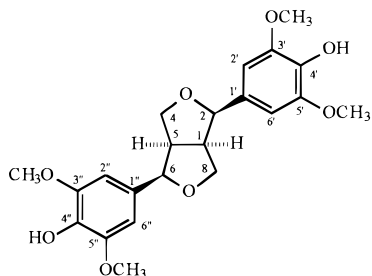
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4

8



10

NMR of **4** revealed the presence of seven protons at  $\delta$  4.14 (1H, m, H-6a), 3.78 (1H, m, H-5a), and 3.25–2.87 (5H, m), which were assigned to aliphatic protons of the aporphine skeleton. A pair of AB-doublets at  $\delta$  6.82 and 6.84 (each 1H, d,  $J = 8.0$  Hz) was assigned to H-9 and H-8 in the aromatic region. The  $^1\text{H}$  NMR of **4** also included signals at  $\delta$  3.91 (3H, s) and 4.09 (3H, s) and two one-proton doublets at  $\delta$  5.97 and 6.15 (each 1H, d,  $J = 1.2$  Hz), which were assigned to OMe-10, OMe-3, and methylenedioxy (C-1, C-2), respectively. A remaining hydroxy group was substituted at C-11. The complete assignment of the relative configuration of aliphatic and aromatic protons of **4** was established by COSY and NOESY (Figure 1) experiments. Thus, the structure of cassyformine was elucidated as **4**.

Filiformine (**8**) was obtained as a red amorphous powder from  $\text{CHCl}_3$ , mp 318–320 °C. The HREIMS gave an  $\text{M}^+$  ion peak at  $m/z$  351.0762 (calcd 351.0743), consistent with a molecular formula of  $\text{C}_{19}\text{H}_{13}\text{O}_6\text{N}$ . The UV absorption bands at 250, 279, 320, and 406 nm and the exhibition of a bathochromic shift on addition of aqueous KOH suggested the presence of a phenolic 1,2,3,9,10-oxygenated oxoaporphine.<sup>12</sup> The IR spectrum showed a broad band of OH absorption at  $3430\text{ cm}^{-1}$ , a conjugated carbonyl group at  $1650\text{ cm}^{-1}$ , and a methylenedioxy group at  $1050$  and  $950\text{ cm}^{-1}$ . Analysis of the  $^1\text{H}$  NMR spectrum of **8** revealed the presence of two methoxy signals at  $\delta$  4.06 (3H, s) and 4.30 (3H, s) and a methylenedioxy signal at  $\delta$  6.35 (2H, s). The aromatic region of the spectrum integrated for four protons: a pair of AB-doublets at  $\delta$  8.19 (1H, d,  $J = 5.4$  Hz) and 8.82 (1H, d,  $J = 5.4$  Hz) due to H-4 and H-5 of typical chemical shifts of oxoaporphine, and two downfield-shifted singlet protons at  $\delta$  7.85 and 8.01 attributed to H-8 and H-11, respectively. The presence of a phenolic function was substantially supported after acetylation of **8** with  $\text{Ac}_2\text{O}$  in pyridine. The acetic derivative **8a** was produced, and a new acetyl signal at  $\delta$  2.36 (3H, s) was observed in  $^1\text{H}$  NMR of **8a**. The phenolic hydroxy group will be located at C-10 because the signal of H-11 in **8**

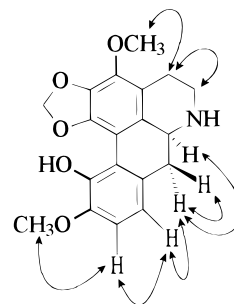


Figure 1. The NOESY interactions observed for **4**.

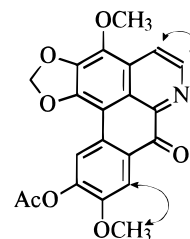


Figure 2. The NOESY interactions observed for **8a**.

was shifted 0.17 ppm downfield in the  $^1\text{H}$  NMR of **8a**. The structural assignment for **8** was further supported by the 1D NOE-DIF and 2D NOESY NMR spectra of **8a** (Figure 2). This compound, an oxoaporphine alkaloid, which we named filiformine.

The nonalkaloidal extract was subjected to column chromatography and HPLC, which led to the purification of three lignans and five other compounds. Compounds **9** and **10** behaved similarly in several chromatographic systems. Two spots, forming brown upon spraying with sulfuric acid, were distinguished by TLC. Compound **10** was isolated as a white amorphous powder from  $\text{CHCl}_3$ , mp 170–172 °C,  $[\alpha]^{25}_{\text{D}} +110^\circ$  ( $c$  0.1,  $\text{CHCl}_3$ ). The molecular formula of **10** was determined to be  $\text{C}_{22}\text{H}_{26}\text{O}_8$  by HREIMS ( $m/z$  found 418.1634, calcd 418.1627). The UV spectrum of **10** showed absorption maxima at 212, 240, and 278 nm, which suggested the exhibition of a furofuran-type lignan.<sup>9,10</sup> The IR spectrum revealed the presence of a hydroxy group absorption at  $3400\text{ cm}^{-1}$ , which was substantiated to be phenolic from a bathochromic shift in the UV spectrum. The  $^1\text{H}$  NMR spectrum of **10** revealed the presence of symmetrical C6–C3 moieties in the molecule, which was strikingly similar to that of (+)-syringaresinol (**9**). The  $^1\text{H}$  NMR spectrum clearly indicated the presence of four methoxy groups, two hydroxy groups, and two pairs of equivalent aromatic protons. Furthermore, signals between  $\delta$  3.0 and 5.0 indicated the presence of a bis-tetrahydrofuran ring of 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane-type lignan. The C-1,C-5 bond of naturally occurring furofuranic-type lignans is characteristically in the *cis* configuration, and the  $^1\text{H}$  NMR spectrum obtained at ca.  $\delta$  3.18 (2H, m) for H-1 and H-5 and ca.  $\delta$  4.90 (2H, d,  $J = 5.2$  Hz) for H-2 and H-6 indicated that is also true for **10**.<sup>9</sup> The aromatic substituents of C-2 and C-6 were assigned to axial/axial orientations (dialignan) allowing for two types of stereoisomers (the *R,R,R,R* or *S,S,S,S* configuration at C-1, C-2, C-5, and C-6).<sup>9</sup> According to literature reports, all (+)-sesamin-type lignans belong to the same series with the absolute configuration *R* at the bridge carbons C-1 and C-5.<sup>9,13</sup> Compound **10**

**Table 1.** Effects of Test Alkaloids on the Platelet Aggregation Induced by ADP, AA, Collagen, and PAF in Washed Rabbit Platelets<sup>a</sup>

compound <sup>b</sup>	Aggregation (%)			
	ADP (20 $\mu$ M)	AA (100 $\mu$ M)	Collagen (10 $\mu$ g/mL)	PAF (3.6 nM)
<b>1</b>		0.0 $\pm$ 0.0*** <sup>c</sup>		
<b>2</b>		0.0 $\pm$ 0.0***	7.6 $\pm$ 4.6***	61.5 $\pm$ 4.8**
<b>3</b>	0.0 $\pm$ 0.0***	0.0 $\pm$ 0.0***	0.0 $\pm$ 0.0***	0.0 $\pm$ 0.0***
<b>5</b>	0.0 $\pm$ 0.0***	0.0 $\pm$ 0.0***	0.0 $\pm$ 0.0***	68.1 $\pm$ 6.2*
<b>6</b>		0.0 $\pm$ 0.0***	0.0 $\pm$ 0.0***	74.0 $\pm$ 1.8*
<b>7</b>		0.0 $\pm$ 0.0***	0.0 $\pm$ 0.0***	87.7 $\pm$ 1.8*
aspirin	77.9 $\pm$ 1.9	0.0 $\pm$ 0.0	87.8 $\pm$ 1.5	90.4 $\pm$ 1.1
control	79.8 $\pm$ 1.8	88.4 $\pm$ 1.1	88.5 $\pm$ 0.4	90.5 $\pm$ 1.1

<sup>a</sup> Platelets were preincubated with DMSO (0.5%, control), aspirin, or test compounds at 37 °C for 3 min, then ADP (20  $\mu$ M), AA (100  $\mu$ M), collagen (10  $\mu$ g/mL), or PAF (3.6 nM) was added. Percentage of aggregation are presented as means S.E. ( $n = 3-5$ ).

<sup>b</sup> The concentration of each test compound was 100  $\mu$ g/mL; aspirin was 25  $\mu$ g/mL. <sup>c</sup> \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  as compared with the respective control.

showed a positive optical rotation and axial/axial aromatic substituents, which suggested the absolute configuration at C-1, C-2, C-5, and C-6 also be assigned to *R*. The COSY spectrum confirmed the relationship of H-2 $\leftrightarrow$ H-1, H-1 $\leftrightarrow$ H-8, H-1 $\leftrightarrow$ H-5, H-5 $\leftrightarrow$ H-4, and H-5 $\leftrightarrow$ H-6. On the basis of these data, compound **10** is suggested as a new lignan, (+)-diasyringaresinol. Mass spectral fragmentation ions of **10** at  $m/z$  193, 183, 182, 181, and 167 supported this conclusion. The known compounds were identified by comparison of their spectral data (UV, IR, <sup>1</sup>H NMR, and MS) and/or the physical properties of authentic samples.

The antiplatelet effects of the alkaloids **1-3**, and **5-7** were studied on the aggregation of washed rabbit platelets induced by either ADP (20  $\mu$ M), arachidonic acid (AA) (100  $\mu$ M), collagen (10  $\mu$ g/mL), or PAF (3.6 nM). The results are shown in Table 1. As indicated in Table 1, at a concentration of 100  $\mu$ g/mL, compound **1** completely inhibited platelet aggregation induced by AA, while compounds **2**, **6**, and **7** completely inhibited platelet aggregation induced by AA and collagen. Compound **5** showed complete inhibition of ADP-, AA-, and collagen-induced platelet aggregation, compound **3** completely inhibited platelet aggregation induced by all four agents (ADP, AA, collagen, and PAF), while compounds **1**, **2**, and **3** exhibited the same effects at 50  $\mu$ M. Because the positive control (aspirin) completely inhibited the AA-, but not the collagen- or the PAF-induced platelet aggregation, the mechanism of antiplatelet effects of these aporphine and oxoaporphine alkaloids appears to be different from that of aspirin, a cyclooxygenase inhibitor.<sup>14</sup>

From a concentration-response experiment, compound **5** gave complete inhibitory effects against AA-induced platelet aggregation even at 20  $\mu$ M.

Of the alkaloids isolated, actinodaphnine (**3**) is the most potent on platelet aggregation induced by ADP, AA, collagen, and PAF in washed rabbit platelets. It is interesting that the six molecules of the aporphines (**1**, **2**, **3**, **5**, **6**, and **7**) are of similar molecular size, and the potency of the alkaloids is sensitive to small structural changes in the molecules, suggesting the action at a specific receptor site. Compounds **3** and **5** showed significant and strong inhibition of the high K<sup>+</sup>- and

norepinephrine-induced contractions in rat thoracic aorta, which had been reported in our previous paper.<sup>15</sup>

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Melting points were determined using a Yanagimoto micro-melting point apparatus and were uncorrected. The UV spectra were obtained on a Hitachi 200-20 spectrophotometer, and IR spectra were measured on a Hitachi 260-30 spectrophotometer. <sup>1</sup>H NMR spectra were recorded with Varian NMR spectrometers (Unity Plus 400 and Gemini 200) at 400 and 200 MHz, and <sup>13</sup>C NMR spectra were recorded with Varian NMR spectrometers at 100 and 50 MHz, in CDCl<sub>3</sub> using TMS as internal standard. LREIMS and LRFABMS spectra were obtained with a JOEL JMS-SX/SX 102A mass spectrometer or a Quattro GC/MS spectrometer having a direct inlet system. HREIMS were measured on a JOEL JMS-HX 110 mass spectrometer. Si gel 60 (Macherey-Nagel, 230-400 mesh) was used for chromatographic column, precoated Si gel plates (Macherey-Nagel, SIL G-25 UV<sub>254</sub>, 0.25 mm) were used for analytical TLC, and precoated Si gel plates (Macherey-Nagel, SIL G/UV<sub>254</sub>, 0.25 mm) were used for preparative TLC. The spots were detected by spraying with either Dragendroff's reagent or 50% H<sub>2</sub>SO<sub>4</sub> and then heated on a hot plate.

**Plant Material.** *C. filiformis* L. were collected from Pingtung-Hsien, Taiwan, in September 1994. A voucher specimen is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

**Extraction and Isolation.** Air-dried, whole plant of *C. filiformis* L. (10 kg) were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated in vacuo. The residue was treated with 3% HCl aqueous and then partitioned with CHCl<sub>3</sub> to yield the CHCl<sub>3</sub> layer (non-alkaloidal portion, Part A, 500 g), the acid layer, and the marc (300 g). The acid layer was adjusted to pH 8 with aqueous NH<sub>3</sub> and extracted with CHCl<sub>3</sub> to yield the free alkaloidal portion (Part B, 60 g). Part A was repeatedly subjected to Si gel column chromatography, using *n*-hexane; CHCl<sub>3</sub>; 2%, 4%, and 8% MeOH-CHCl<sub>3</sub>; and MeOH as eluents. The eluted fractions (100 mL each) were monitored by TLC and combined into 18 fractions. The fractions were rechromatographed, followed by preparative TLC and/or HPLC to afford (+)-yangambin [6 mg, TLC CHCl<sub>3</sub>:MeOH (10:1),  $R_f$  0.75], **9** [12 mg, TLC CHCl<sub>3</sub>:MeOH (10:1),  $R_f$  0.71], **10** [7 mg, TLC CHCl<sub>3</sub>:MeOH (10:1),  $R_f$  0.69], vanillin [50 mg, TLC CHCl<sub>3</sub>:MeOH (10:0.5),  $R_f$  0.62], isovanillin [23 mg, TLC CHCl<sub>3</sub>:MeOH (9:1),  $R_f$  0.55], glycerol ester [47 mg, TLC CHCl<sub>3</sub>:MeOH (10:1),  $R_f$  0.64], the mixture of  $\beta$ -sitosterol and stigmaterol [125 mg, TLC CHCl<sub>3</sub>:MeOH (9:1),  $R_f$  0.55], and a mixture of  $\beta$ -sitosterol-D-glucoside and stigmaterol-D-glucoside [150 mg, TLC CHCl<sub>3</sub>:MeOH (6:1),  $R_f$  0.57]. Part B was repeatedly subjected to Si gel column chromatography, using *n*-hexane, CHCl<sub>3</sub>; 2%, 4%, and 8% MeOH-CHCl<sub>3</sub>; and MeOH as eluents. The eluted fractions (100 mL each) were monitored by TLC and combined into 28 fractions. The fractions were rechromatographed over Si gel, preparative TLC, and/or HPLC to afford **4** [7 mg,



TLC CHCl<sub>3</sub>:MeOH (9:1), *R<sub>f</sub>* 0.43], **5** [15 mg, TLC CHCl<sub>3</sub>:MeOH (15:1), *R<sub>f</sub>* 0.54], **6** [10 mg, TLC CHCl<sub>3</sub>:MeOH (10:1), *R<sub>f</sub>* 0.62], **7** [25 mg, TLC CHCl<sub>3</sub>:MeOH (10:1), *R<sub>f</sub>* 0.73], **8** [50 mg, TLC CHCl<sub>3</sub>:MeOH (10:1), *R<sub>f</sub>* 0.54], thalicminine [34 mg, TLC CHCl<sub>3</sub>:MeOH (9:1), *R<sub>f</sub>* 0.57], stepharine [8 mg, TLC CHCl<sub>3</sub>:MeOH (15:1), *R<sub>f</sub>* 0.47], pronuciferine [48 mg, TLC CHCl<sub>3</sub>:MeOH (15:1), *R<sub>f</sub>* 0.45] and *O*-methylflavinantive [58 mg, TLC CHCl<sub>3</sub>:MeOH (15:1), *R<sub>f</sub>* 0.42].

**Cassyformine (4)**: was isolated as a yellow amorphous powder (CHCl<sub>3</sub>); mp 135–137 °C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +30° (*c* 0.2, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 215 (4.03), 284 (4.10), and 302 (3.77) nm; IR (KBr)  $\nu_{\max}$  3400 (OH) and 1040, 920 (–OCH<sub>2</sub>O–) cm<sup>–1</sup>; EIMS (70 eV) *m/z* (rel int) 341 ([M]<sup>+</sup>, 73), 340 (100), 326 (33), and 310 (47); HREIMS 341.1250 (calcd 341.1263 for C<sub>19</sub>H<sub>19</sub>O<sub>5</sub>N); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) 2.87 (1H, dd, *J* = 14.4, 13.2 Hz, H-7axia), 3.04 (2H, m, H-4a and 4b), 3.10 (1H, m, H-5b), 3.25 (1H, dd, *J* = 14.4, 3.6 Hz, H-7equ), 3.78 (1H, m, H-5a), 4.14 (1H, dd, *J* = 13.2, 3.6 Hz, H-6a), 3.91 (3H, s, C10–OCH<sub>3</sub>), 4.09 (3H, s, C3–OCH<sub>3</sub>), 5.97 and 6.15 (each 1H, d, *J* = 1.2 Hz), 6.82 (1H, d, *J* = 8 Hz, H-9), and 6.84 (1H, d, *J* = 8 Hz, H-8).

**Filiformine (8)**: obtained as a red amorphous powder (CHCl<sub>3</sub>); mp 318–320 °C; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 250 (4.11), 279 (4.20), 320 (4.03), and 406 (3.27) nm; IR (KBr)  $\nu_{\max}$  3300 (OH), 1620 (CO) and 1050, 950 (–OCH<sub>2</sub>O–) cm<sup>–1</sup>; FABMS *m/z* (rel int) 352 ([M + 1]<sup>+</sup>, 24), 351 ([M]<sup>+</sup>, 100), 336 (39), 321 (10), and 308 (15); HREIMS 351.0762 (calcd 351.0743 for C<sub>19</sub>H<sub>13</sub>O<sub>6</sub>N); <sup>1</sup>H NMR [CDCl<sub>3</sub>:CD<sub>3</sub>OD (25:1), 200 MHz] 4.06 (3H, s, C9–OCH<sub>3</sub>), 4.30 (3H, s, C3–OCH<sub>3</sub>), 6.35 (2H, s, –OCH<sub>2</sub>O–), 7.85 (1H, s, H-8), 8.01 (1H, s, H-11), 8.19 (1H, d, *J* = 5.4 Hz, H-4), and 8.82 (1H, d, *J* = 5.4 Hz, H-5).

**Filiformine acetate (8a)**: obtained as a yellow powder (CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  1750 (CO), 1635 (CO), and 1010, 920 (–OCH<sub>2</sub>O–) cm<sup>–1</sup>; EIMS (70 eV) *m/z* (rel int) 393 ([M]<sup>+</sup>, 24), 352 (22), 351 (100), and 336 (43); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz) 2.36 (3H, s, C10–OCOCH<sub>3</sub>), 3.95 (3H, s, C9–OCH<sub>3</sub>), 4.28 (3H, s, C3–OCH<sub>3</sub>), 6.28 (2H, s, –OCH<sub>2</sub>O–), 7.95 (1H, s, H-8), 8.18 (1H, s, H-11), 8.06 (1H, d, *J* = 5.4 Hz, H-4), and 8.85 (1H, d, *J* = 5.4 Hz, H-5).

**(+)-Diasyringaresinol (10)**: isolated as a white amorphous powder (CHCl<sub>3</sub>); mp 170–172 °C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +110° (*c* 0.1, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 212 (4.11), 240 (4.16), and 278 (4.09) nm; IR (KBr)  $\nu_{\max}$  3400, 1600, and 1500 cm<sup>–1</sup>; EIMS (70 eV) *m/z* (rel int) 418 ([M]<sup>+</sup>, 59), 182 (56), 181 (97), and 167 (100); HREIMS 418.1634 (calcd 418.1627 for C<sub>22</sub>H<sub>26</sub>O<sub>8</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) 3.18 (2H, m, H-1 and 5), 3.57 (2H, dd, *J* = 9.6, 3.6 Hz, H-4b and -8b) and 3.73 (2H, dd, *J* = 9.6, 6.8 Hz, H-4a and -8a), 4.90 (2H, d, *J* = 4.3 Hz, H-2 and -6), 3.91

(12H, s, 4-OCH<sub>3</sub>), 5.49 (2H, s, OH) and 6.61 (4H, s, H-2', -6', -2'' and -6''); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) 49.53 (d, C-1 and 5), 56.33 (q, 4-OCH<sub>3</sub>), 68.78 (t, C-4 and -8), 84.17 (d, C-2 and -6), 103.05 (d, C-2', -6', -2'', and -6''), 130.03 (s, C-1' and -1''), 133.87 (s, C-4' and -4''), and 147.03 (s, C-3', -5', -3'', and -5'').

**Assay Method for Platelet Aggregation.** Rabbit washed platelets were obtained from EDTA-anticoagulated blood according to our previously described method.<sup>16</sup> Platelets were suspended in Tyrode's solution, and the numbers were adjusted to 4.5 × 10<sup>8</sup> platelets/mL. Aggregation was measured by turbidity method using a Lumi-aggregometer (Chrono-Log Co.). The absorbance of platelet suspension was taken as 0% aggregation and the absorbance of platelet-free Tyrode's solution as 100% aggregation.

**Data Analysis.** The experimental results are expressed as means ± S. E. and accompanied by the number of observations. A one-way analysis of variance (ANOVA) was used for multiple comparison, and if there was significant variation between treatment groups, then the mean values for inhibitors were compared with those for control by Student's *t* test, and *p* values of <0.05 were considered to be statistically significant.

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