Quinoline Alkaloids and Other Constituents of *Melicope semecarpifolia* with Antiplatelet Aggregation Activity

Ih-Sheng Chen,*,† Hui-Fang Chen,† Ming-Jen Cheng,† Ya-Ling Chang,‡ Che-Ming Teng,‡ Ishikawa Tsutomu,§ Jih-Jung Chen,⊥ and Ian-Lih Tsai†

Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China, Pharmacological Institute, College of Medicine, National Taiwan University, Taipei 100, Taiwan, Republic of China, Faculty of Pharmaceutical Sciences, Chiba University, 1-33, Yayoi, Inage Chiba 263-8522, Japan, and Department of Pharmacy, Tajen Institute of Technology, Pingtung, Taiwan, Republic of China

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Three new quinoline alkaloids, 2-acetylevolitrine (**1**), 2-acetylpteleine (**2**), and semecarpifoline (**3**), along with 26 known compounds were isolated from the root bark of *Melicope semecarpifolia*. The structures of **1**−**3** were elucidated by means of spectral analysis. In addition, (2*S*)-(−)-7,8-dimethoxyplatydesmine (**4**), *cis*-(+)-7,8-dimethoxymyrtopsine (**5**), and (3*R*)-(−)-8,9-dimethoxygeibalansine (**6**) were isolated as new natural products. Several of these isolates were determined as exhibiting significant antiplatelet aggregation activities in vitro.

Melicope semecarpifolia (Merr.) T. Hartley [*Evodia merrillii* Kanehira & Sasaki ex Kanehira; *Melicope confusa* (Merr.) Liu] (Rutaceae) is a mid-sized evergreen trifoliated tree occurring in Taiwan and the Philippines.[1] This plant has undergone some phytochemical studies, and its characteristic constituents are furoquinoline alkaloids, acetophenones, coumarins, and flavonoids.[2−7] The roots of this plant have been used as a carminative in folk medicine.[8] Since the methanolic extract of the root bark of *M. semecarpifolia* shows antiplatelet aggregation activity, this was used as the rationale for the current study to identify the substances responsible for this effect. Consequently, three new quinoline alkaloids (**1**−**3**), together with 26 known compounds, were isolated from the methanolic extract of this species. This paper describes the structural elucidation of **1**−**3** and the antiplatelet aggregation activity of the isolates obtained.

Results and Discussion

2-Acetylevolitrine (**1**) was obtained as yellowish needles. Its molecular formula of $C_{15}H_{13}NO_4$ was determined by EIMS ([M]$^+$, *m*/*z* 271) and HREIMS. The IR spectrum indicated the presence of a carbonyl group at 1728 cm^{-1}. UV absorptions at 235, 260, and 351 nm showed the presence of a furoquinoline moiety.[2] The ^{1}H NMR spectrum of **1** showed three protons of an ABX system at δ 8.17 (1H, d, J = 9.4 Hz), 7.11 (1H, dd, J = 9.4, 2.6 Hz), and 7.32 (1H, d, J = 2.6 Hz), which were assigned to H-5, H-6, and H-8, respectively. Two singlets at δ 4.49 and 3.96 (each 3H, s) suggested the presence of C-4 and C-7 methoxyl groups. Three protons at δ 2.67 (3H, s) and a singlet proton at δ 7.82 (1H, s) were assignable to a C-2 acetyl group and H-3, respectively, in the furan ring. The NOE-DIF experiment (Figure 1) showed that the characteristic C-4 methoxyl group at δ 4.49 correlated with H-5 at δ 8.17 and H-3 at δ 7.82. The acetyl group (δ 2.67) at C-2 was thus confirmed. On the basis of the above data, the structure of **1** was elucidated as 2-acetylevolitrine, which was supported by carrying out ^{1}H−^{1}H COSY, ^{13}C NMR, HMQC, and HMBC experiments (Figure 2).

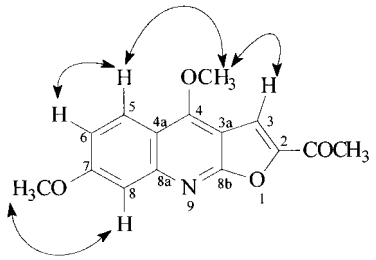

Figure 1. NOE-DIF correlations of **1**.

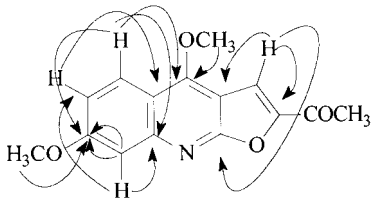

Figure 2. HMBC correlations of **1**.

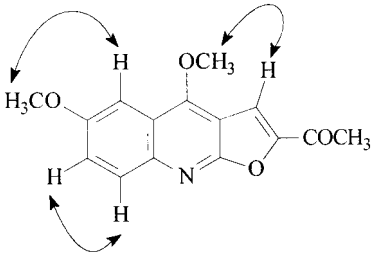

Figure 3. NOE-DIF correlations of **2**.

2-Acetylpteleine (**2**) was isolated as yellowish needles. Its molecular formula of $C_{15}H_{13}NO_4$ was determined by EIMS ([M]$^+$, *m*/*z* 271) and HREIMS. The IR spectrum showed the presence of a carbonyl group at 1728 cm^{-1}. UV absorptions at 236, 303, and 330 nm showed the presence of a furoquinoline moiety.[2] The ^{1}H NMR spectrum of **2** revealed the presence of an acetyl group at δ 2.68 (3H, s), an olefinic proton at δ 7.83 (1H, s), and a methoxyl group at δ 4.52 (3H, s), indicating that **2** has the same 2-acetyl-4-methoxyfuroquinoline moiety as **1**. The chemical shifts

* To whom correspondence should be addressed. Tel: (886) 73121101, ext. 2191. Fax: (886) 73210683. E-mail: m635013@cc. kmu.edu.tw.
† Kaohsiung Medical University.
‡ National Taiwan University.
§ Chiba University.
⊥ Tajen Institute of Technology.

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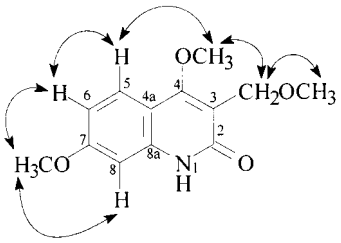


Figure 4. NOE-DIF correlations of **3**.

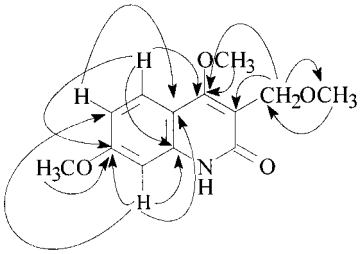


Figure 5. HMBC correlations of **3**.

of the three ABX protons at δ 7.42 (1H, dd, J = 9.5, 3.0 Hz), 7.53 (1H, d, J = 3.0 Hz), and 7.93 (1H, d, J = 9.5 Hz) and a methoxyl group [δ 3.95 (3H, s)] were similar to those present [δ 7.36 (1H, dd, J = 9.6, 2.8 Hz, H-7), 7.52 (1H, d, J = 2.8 Hz, H-5), 7.91 (1H, d, J = 9.6 Hz, H-8), 3.95 (3H, s, OCH_3-6)] in pteleine (**8**), a compound also isolated in this study. Thus, the structure of **2** was proposed as 2-acetylpteleine, which was further supported by a NOE-DIF experiment (Figure 3).

Semecarpifoline (**3**) was obtained as yellowish needles. Its molecular formula of $C_{13}H_{15}NO_4$ was determined by

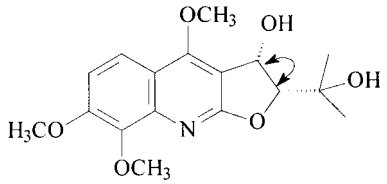
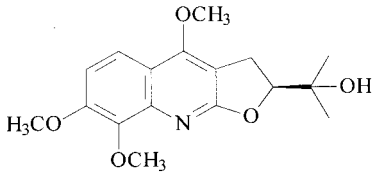


Figure 6. NOESY correlations of **5**.

EIMS ([M]$^+$, *m*/*z* 249) and HREIMS. The IR spectrum indicated the presence of an amidoamino group at 3440 cm^{-1} and an amidocarbonyl group at 1644 cm^{-1}. UV absorptions at 218, 284, 325, and 337 nm showed the presence of a 2-quinolone moiety.[9] The ^{1}H NMR spectrum showed three ABX protons [δ 7.73 (1H, d, J = 8.8 Hz), 6.81 (1H, dd, J = 8.8, 2.4 Hz), 6.61 (1H, d, J = 2.4 Hz)], two methoxyl groups [δ 4.10, 3.89 (each 3H, s)], and an NH group [δ 10.25 (1H, s)]. In addition, a methoxymethyl group [δ 3.47 (3H, s), 4.54 (2H, s)] was present in **3** instead of a H-3 singlet, which is usually observed at ca. δ 6.00 in typical 2-quinolone alkaloids. From the above data, the structure of **3** could be either 4,7-dimethoxy-3-methoxymethyl-2-quinolone or 4,6-dimethoxy-3-methoxymethyl-2-quinolone. NOE-DIF (Figure 4), and HMBC (Figure 5) experiments confirmed that the structure of semecarpifoline (**3**) was the former of these two possibilities.

Compound **4** was identified by comparison with literature data of 7,8-dimethoxyplatydesmine,[10,11] but showed a levorotatory optical activity with $[\alpha]^{23}_D$ −10.3° (*c* 0.16, $CHCl_3$). Without an enantiomeric antipode for comparison, but with the reference to (*R*)-(+)-8-methoxyplatydesmine,[12] the stereochemistry at C-2 of **4** would

Chart 1



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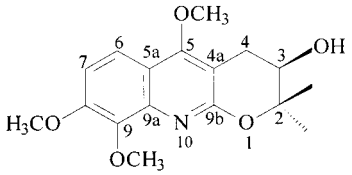

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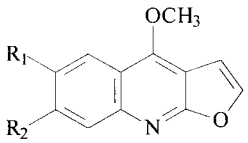
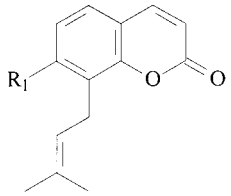
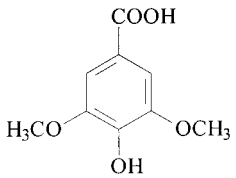


7. R_1=H, R_2=OH
8. R_1=R_2=OCH_3



9. R_1=OH
10. R_1=OCH_3



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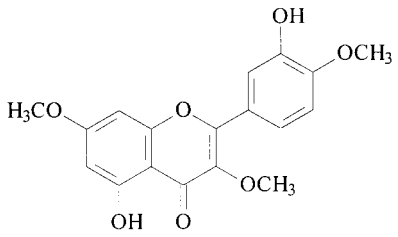

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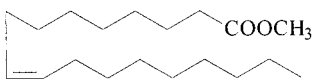

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Table 1. Inhibitory Effects of Compounds on the Aggregation of Washed Rabbit Platelets Induced by Thrombin (Thr), Arachidonic Acid (AA), Collagen (Col), and Platelet-Activating Factor (PAF)[a]

compound	(μg/mL)	aggregation (%) Thr (0.1 U/mL)	AA (100 μM)	Col (10 μg/mL)	PAF (2 ng/mL)
semecarpifoline (**3**)	100	85.8 ± 0.9(3)	81.0 ± 0.7(3)***	82.7 ± 1.7(3)	88.8 ± 0.1(3)***
(−)-7,8-dimethyoxyplatydesmine (**4**)	100	86.5 ± 0.6(3)	81.7 ± 1.0(3)**	84.4 ± 0.9(3)	89.8 ± 0.5(3)
(+)-7,8-dimethoxymyrtopsine (**5**)	100	85.4 ± 0.5(3)	78.3 ± 1.2(3)***	79.0 ± 3.5(3)	89.4 ± 0.1(3)***
confusameline (**7**)	100		0.0 ± 0.0(3)***	11.4 ± 3.4(3)***	78.6 ± 2.6(3)*
kokusaginine (**8**)	100		0.0 ± 0.0(3)***	15.2 ± 1.6(3)***	70.7 ± 1.4(3)**
	50		0.0 ± 0.0(3)***	80.6 ± 2.1(3)*	88.9 ± 2.7(3)
osthenol (**9**)	100	90.4 ± 0.5(3)	23.2 ± 18.9(3)**	13.7 ± 4.1(3)***	89.8 ± 0.3(3)*
	50		84.8 ± 1.8(3)*	79.2 ± 4.1(3)*	
	20			87.0 ± 1.6(3)	
osthole (**10**)	100	86.6 ± 1.9(3)*	0.0 ± 0.0(3)***	7.4 ± 5.1(3)***	0.0 ± 0.0(3)***
	50		52.6 ± 11.8(3)**	7.0 ± 5.4(3)**	88.6 ± 1.0(3)
	20		84.5 ± 1.3(3)*	88.1 ± 0.5(3)	90.0 ± 0.6(3)
syringic acid (**11**)	100	88.3 ± 0.1(3)	83.2 ± 0.8(3)	85.1 ± 1.3(3)	89.6 ± 0.4(3)***
ayanin (**12**)	100	85.4 ± 0.5(3)	13.3 ± 5.4(3)***	43.1 ± 9.6(4)**	88.3 ± 0.6(3)***
	50		38.0 ± 12.1(3)**	66.1 ± 6.0(4)*	
	20		66.3 ± 4.9(3)**	80.3 ± 0.8(4)***	
	10		77.2 ± 2.9(3)*		
	5		81.9 ± 1.7(3)		
methyl oleate (**13**)	50		cause platelet aggregation		
	20	86.7 ± 0.3(3)	81.7 ± 1.1(3)*	84.4 ± 0.9(3)	89.4 ± 0.7(3)*
aspirin[b]	50	94.2 ± 1.9(3)	7.8 ± 6.8(4)***	88.4 ± 1.2(4)	91.2 ± 1.5(3)
	20		11.7 ±10.1(4)***		
	10		84.3 ± 0.6(4)***		
control		93.8 ± 1.5(5)	90.3 ± 1.0(5)	89.9 ± 0.8(5)	90.5 ± 1.3(4)

[a] Platelets were preincubated with DMSO (0.5%, control) or each compound at 37 °C for 3 min, and then the inducer was added. Aspirin was used as a reference control. Values are presented as means ± SEM (*n*). [b] Positive control. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$, as compared with respective control.

appear to be of the *S*-configuration. Accordingly, (*S*)-(−)-7,8-dimethoxyplatydesmine (**4**) was found in nature for the first time.

Compound **5** was identical to 7,8-dimethoxymyrtopsine[10] and showed a dextrorotatory optical activity with $[\alpha]^{23}_D$ +16.2° (*c* 0.165, $CHCl_3$). The relative configuration at H-2 and H-3 was in the *cis*-form, as confirmed by analysis of the NOESY spectrum (Figure 6).

Compound **6** was in accordance with the literature data of 8,9-dimethoxygeibalansine,[10,11] for which there has been no reported $[\alpha]_D$ value. Isolate **6** showed levorotatory optical activity with $[\alpha]^{23}_D$ −18.6° (*c* 0.065, $CHCl_3$), and by reference to (*S*)-(+)-9-methoxygeibalansine, the configuration of **6** at C-3 was proposed as being in the *R*-form. Thus, the isolation of (3*R*)-(−)-8,9-dimethoxygeibalansine (**6**) as a natural product is also reported for the first time.

The methanolic extract of the root bark of *M. semecarpifolia* showed strong antiplatelet activity in vitro using the turbidimetric method.[13] In washed rabbit platelets, thrombin (0.1 U/mL), arachidonic acid (AA 100 μM), collagen (100 μg/mL), and PAF (2 ng/mL) all caused about 90−94% aggregation. The fractionation led to the isolation of 11 compounds, confusameline (**7**),[7] kokusaginine (**8**),[7] osthenol (**9**), osthole (**10**),[14] ayanin (**12**), evolitrine,[7] dictamnine,[15] skimmianine,[16] haplopine,[17] platydesmine,[18] and anisocoumarin H,[18] as the active principles with antiplatelet aggregation activity (Table 1). The furoquinolines confusameline, kokusaginine, evolitrine, dictamnine, skimmianine, and haplopine all showed inhibitory activity on platelet aggregation at 100 μg/mL induced by AA and collagen, and kokusaginine (**8**) at 50 μg/mL showed selective inhibitory activity of platelet aggregation induced by AA. The dihydrofuroquinoline platydesmine,[18] with no substituent in ring A, and the coumarin anisocoumarin H[18] both showed a selective inhibitory activity of platelet aggregation at 100 μg/mL induced by collagen. Semecarpifoline (**3**), (−)-7,8-dimethoxyplatydesmine (**4**), (+)-7,8-dimethoxymyrtopsine (**5**), and syringic acid (**11**) all showed no obvious activity at 100 μg/mL.

The remaining compounds were all of previously known structure and comprised confusameline (**7**),[2] kokusaginine (**8**),[2] osthenol (**9**),[19] osthole (**10**),[20] syringic acid (**11**),[21] ayanin (**12**),[6] methyl oleate (**13**),[22] evolitrine,[2] pteleine,[23] dictamnine,[15] skimmianine,[2] haplopine,[17] platydesmine,[6] 7-*O*-prenylumbelliferone,[24] umbelliferone,[15] anisocoumarin H,[25] xanthyletin,[15] and a mixture of β-sitosterol,[26] stigmasterol,[26] campesterol,[26] methyl vanillate,[27] tetracosylferulate,[16] and 2-hydroxymethylfurfural.[28] These compounds were identified by comparison of their spectral (UV, IR, ^{1}H NMR, and MS) and/or mp data with those of corresponding authentic samples or data from the literature. Osthole, syringic acid, methyl vanillate, evolitrine, dictamnine, anisocoumarin H, and 2-hydroxymethylfurfural were isolated for the first time from this plant.

Experimental Section

General Experimental Procedures. All melting points were determined on a Yanaco micro-melting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-370 polarimeter in $CHCl_3$. UV spectra were obtained on a Hitachi U-2000 spectrophotometer. IR spectra (KBr or neat) were taken on a Perkin-Elmer system 2000 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on Varian Unity 400, Varian Inova 500, and Varian Gemini 200, Bruker AMX-400, and JEOL-600 spectrometers, and chemical shifts are given in ppm (δ) with TMS as internal standard. EIMS were recorded on a VG Biotech Quattro 5022 spectrometer. HREIMS were recorded on a JEOL JMX-HX 110 spectrometer. Si gel 60 (Merck 70−230, 230−400 mesh) was used for column chromatography, Si gel 60 F_{254} (Merck) for TLC, and Si gel 5715 and 5744 (Merck) for preparative TLC.

Plant Material. The root bark of *M. semecarpifolia* was collected from Lai-I, Pingtung County, Taiwan, in April 1998. A voucher specimen (Chen 5685) is deposited in the herbarium of the School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. The root bark (5.5 kg) was chipped and extracted with MeOH, and the extract was concentrated under reduced pressure. The MeOH extract was

partitioned between $CHCl_3$ and H_2O (1:1). The $CHCl_3$-soluble fraction was then extracted with 2% aqueous H_2SO_4 to afford a neutral $CHCl_3$-soluble fraction (fraction C, 160 g) and acid-soluble fraction. The acid-soluble part was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ solution was treated with 2% aqueous NaOH, then dried with K_2CO_3 to give a mixture of tertiary nonphenolic bases (fraction A, 1.51 g). The NaOH solution was treated with NH_4Cl and extracted with $CHCl_3$. The $CHCl_3$ solution was dried with $MgSO_4$ and evaporated in vacuo to afford a mixture of tertiary phenolic bases (fraction B, 370 mg). The H_2O solution was partitioned between EtOAc, then *n*-BuOH to give the EtOAc fraction (fraction D, 3.8 g), the *n*-BuOH fraction (fraction E, 3.5 g), and a H_2O fraction (fraction F, 50 g).

Fraction A (1.51 g) was chromatographed over Si gel, eluting with CH_2Cl_2, and then gradually increasing the polarity with MeOH, to obtain seven fractions (A1−A7). Fraction A3 (24 mg) was purified by preparative TLC (C_6H_6−EtOAc, 10:1) to yield evolitrine (1.5 mg) and pteleine (0.7 mg). Fraction A-6 (831 mg) was chromatographed on Si gel and eluted with CH_2Cl_2 and CH_2Cl_2−MeOH mixtures to obtain three fractions (A6-1−A6-3). Fraction A6-2 (507 mg) was chromatographed over Si gel, eluted with *n*-hexane, and enriched with acetone to obtain seven fractions (A6-2-1−A6-2-7). Of these, fraction A6-2-3 (70 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 2:1) to yield (*R*)-(+)-platydesmine (2.8 mg). Fraction A6-2-4 (65.4 mg) was purified by preparative TLC (*n*-hexane−acetone, 10:1) to yield (*S*)-(−)-7,8-dimethoxyplatydesmine (**4**) (2.7 mg) and (3*R*)-(−)-8,9-dimethoxygeibalansine (**6**) (1 mg). In turn, fraction A6-2-6 (22 mg) was purified by preparative TLC (*n*-hexane−acetone, 2:1) to yield semecarpifoline (**3**) (2.5 mg) and *cis*-(+)-7,8-dimethoxymyrtopsine (**5**) (1.3 mg).

Fraction B (370 mg) was chromatographed over Si gel eluting with CH_2Cl_2, gradually increasing the polarity with MeOH, to obtain five fractions (B1−B5). Fraction B2 (36.2 mg) was then chromatographed on Si gel and eluted with CH_2Cl_2 and CH_2Cl_2−EtOAc mixtures to obtain six fractions (B2-1−B2-6). Fraction B2-2 (5.3 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 2:1) to yield ayanin (**12**) (1.7 mg). Fraction B2-3 (6.4 mg) was purified by preparative TLC (C_6H_6−EtOAc, 10:1) to yield 2-acetylevolitrine (**1**) (1.2 mg) and 2-acetylpteleine (**2**) (0.5 mg). Fraction B2-4 (2.6 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 2:1) to obtain confusameline (**7**) (1.0 mg). Fraction B4 (28.3 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 1:5) to yield *cis*-(+)-7,8-dimethoxymyrtopsine (**5**) (2.2 mg).

Fraction C (160 g) was chromatographed over Si gel eluting with CH_2Cl_2 and CH_2Cl_2−MeOH mixtures to obtain six fractions (C-1−C-6). Fraction C2 (740 mg) was chromatographed on Si gel and eluted with *n*-hexane and mixtures of *n*-hexane and EtOAc gradually increasing in polarity, to obtain 12 fractions (C 2-1−C2-12). Fraction C2-8 (60.3 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 10:3) to yield tetracosyl ferulate (18.6 mg). Fraction C3 (9.84 g) was chromatographed over Si gel eluting with *n*-hexane, and the polarity was gradually increased with EtOAc−acetone to obtain five fractions (C3-1−C3-5). Fraction C3-3 (220 mg) was purified by preparative TLC (CH_2Cl_2−EtOAc, 15:1) to yield osthenol (**9**) (7.2 mg) and osthole (**10**) (5.2 mg). Fraction C3-4 (1.3 g) was chromatographed on Si gel eluting with *n*-hexane−acetone (10:1), gradually increasing the polarity with acetone to obtain four fractions (C3-4-1−C3-4-4). Fraction C3-4-3 (30 mg) was purified by preparative TLC ($CHCl_3$−EtOAc, 5:1) to yield skimmianine (14.2 mg) and haplopine (27.4 mg). Part (30 g) of fraction C4 (100 g) was chromatographed on Si gel, eluting with *n*-hexane, gradually increasing the polarity with acetone, to obtain nine fractions (C4-1−C4-9). Part (50 mg) of fraction C4-1 (289 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 10:1) to yield methyl oleate (12 mg) and 7-*O*-prenylumbelliferone (2.3 mg). A portion (50 mg) of fraction C4-4 (109 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 2:1) to yield methyl vanillate (1.7 mg) and dictamnine (3.5 mg). Fraction C4-6 (95 mg) was washed with MeOH to yield a mixture of *β*-sitosterol, stigmasterol, and campesterol in a ratio of 2:1:2 (21 mg). Fraction C5 (45 g) was chromatographed on Si gel, eluting with *n*-hexane−acetone (20:1), and gradually increasing the polarity with acetone, to obtain five fractions (C5-1−C5-5). Fraction 5-2 (24 mg) was purified by preparative TLC (C_6H_6−EtOAc, 10:1) to yield evolitrine (3.2 mg) and pteleine (1.1 mg). Fraction C5-3 (32 mg) was purified by preparative TLC ($CHCl_3$−acetone, 5:1) to yield kokusaginine (**8**) (5.2 mg).

Fraction D (3.8 g) was chromatographed on Si gel eluting with CH_2Cl_2−MeOH (20:1), by gradually increasing the polarity with MeOH to obtain four fractions (D1−D4). Fraction D2 (93 mg) was purified by preparative TLC ($CHCl_3$−acetone, 10:1) to yield 2-hydroxymethylfurfural (3.2 mg). Fraction D3 (200 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 5:1) to produce xanthyletin (5.9 mg).

Fraction E (5.0 g) was chromatographed on Si gel, eluting with $CHCl_3$, gradually increasing the polarity with MeOH and H_2O, to afford four fractions (E1−E4). Part (25 mg) of fraction E2 (95 mg) was purified by preparative TLC ($CHCl_3$−acetone, 10:1) to yield anisocoumarin H (8.4 mg).

Fraction F (50 g) was chromatographed on Diaion HP-20, eluting with H_2O, gradually decreasing the polarity with MeOH, to yield 12 fractions (F1−F12). Fraction F6 (32 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 2:1) to yield umbelliferone (3.2 mg).

2-Acetylevolitrine (1): yellow needles (MeOH); mp 121−124 °C; UV (MeOH) λ_{max} (log ϵ) 235 (4.41), 260 (4.40), 351 (4.43) nm; IR (KBr) ν_{max} 1728 (CO), 1614, 1558 (aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.67 (3H, s, Ac-2), 3.96 (3H, s, OCH_3-7), 4.49 (3H, s, OCH_3-4), 7.11 (1H, dd, J = 9.4, 2.6 Hz, H-6), 7.32 (1H, d, J = 2.6 Hz, H-8), 7.82 (1H, s, H-3), 8.17 (1H, d, J = 9.4 Hz, H-5); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 26.9 ($COCH_3$-2), 55.6 (OCH_3-7), 59.5 (OCH_3-4), 102.6 (C-3a), 106.2 (C-8), 109.9 (C-3), 113.5 (C-4a), 117.5 (C-6), 124.1 (C-5), 149.9 (C-8a), 150.0 (C-8b), 159.7 (C-4), 162.2 (C-7), 163.6 (C-2), 189.4 (CO); EIMS *m/z* 271 $[M]^+$ (100), 256 (35), 228 (16), 186 (23), 170 (17), 134 (11), 43 (44); HREIMS *m/z* 271.0845 (calcd for $C_{15}H_{13}NO_4$, 271.0845).

2-Acetylpteleine (2): yellow needles (MeOH); mp 123−126 °C; UV (MeOH) λ_{max} (log ϵ) 236 (4.01), 303 (3.70), 330 (3.76) nm; IR (KBr) ν_{max} 1728 (CO), 1614, 1558 (aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.68 (3H, s, Ac-2), 3.95 (3H, s, OCH_3-6), 4.52 (3H, s, OCH_3-4), 7.42 (1H, dd, J = 9.5, 3.0 Hz, H-7), 7.53 (1H, d, J = 3.0 Hz, H-5), 7.83 (1H, s, H-3), 7.93 (1H, d, J = 9.5 Hz, H-8); EIMS *m/z* 271 $[M]^+$ (100), 257 (12), 256 (78), 228 (17), 222 (12), 221 (51), 207 (16), 198 (18), 186 (35), 148 (12), 147 (70), 106 (22), 85 (10), 75 (13), 74 (16), 73 (40), 72 (20), 63 (23), 59 (23), 53 (12), 43 (75); HREIMS *m/z* 271.0845 (calcd for $C_{15}H_{13}NO_4$, 271.0845).

Semecarpifoline (3): yellow needles (MeOH); mp 123−127 °C; UV (MeOH) λ_{max} (log ϵ) 218 (4.57), 284 (3.83), 325 (4.06), 337 (3.97) nm; IR (KBr) ν_{max} 3440 (NH), 1644 (CO), 1580, 1500 (aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 3.47 (3H, s, CH_2OCH_3-3), 3.89 (3H, s, OCH_3-7) 4.10 (3H, s, OCH_3-4), 4.54 (2H, s, CH_2OCH_3-3), 6.61 (1H, d, J = 2.4 Hz, H-8), 6.81 (1H, dd, J = 8.8, 2.4 Hz, H-6), 7.73 (1H, d, J = 8.8 Hz, H-5), 10.25 (1H, br s, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 55.6 (OCH_3-7), 58.5 (CH_2OCH_3-3), 63.1 (OCH_3-4), 63.6 (CH_2OCH_3-3), 98.1 (C-8), 111.0 (C-4a), 112.0 (C-6), 114.5 (C-3), 126.4 (C-5), 140.9 (C-8a), 162.4 (C-7), 165.5 (C-4), 166.0 (C=O); EIMS *m/z* 249 $[M]^+$ (16), 235 (11), 234 (100), 232 (24), 219 (44), 218 (33), 204 (38), 202 (13), 191 (13), 190 (11), 160 (34), 59 (19); HREIMS *m/z* 249.0845 (calcd for $C_{13}H_{15}NO_4$, 249.0845).

(*S*)-(−)-7,8-Dimethoxyplatydesmine (4): $[\alpha]^{23}_D$ −10.3°(*c* 0.16, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.0 (CH_3), 26.3 (CH_3), 29.0 (C-3), 56.5 (OCH_3-7), 58.2 (OCH_3-4), 61.5 (OCH_3-8), 71.5 (C-1′), 86.3 (C-2), 99.8 (C-4a), 110.5 (C-6), 115.8 (C-3a), 117.7 (C-5), 142.8 (C-8), 143.1 (C-8a), 152.8 (C-7), 163.5 (C-8b), 169.1 (C-4).

***cis*-(+)-7,8-Dimethoxymyrtopsine (5):** $[\alpha]^{23}_D$ +16.2° (*c* 0.165, $CHCl_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.8 (CH_3), 25.4 (CH_3), 56.6 (OCH_3-7), 58.3 (OCH_3-4), 61.4 (OCH_3-8), 70.9 (C-3), 71.3 (C-1′), 94.8 (C-2), 101.9 (C-4a), 110.5 (C-6), 115.4 (C-3a), 118.5 (C-5), 141.5 (C-8), 143.1 (C-8a), 153.3 (C-7), 161.6 (C-8b), 168.5 (C-4).

(3*R*)-(−)-8,9-Dimethoxygeibalansine (6): $[\alpha]^{23}_{D}$ −18.6° (*c* 0.065, $CHCl_3$).

Antiplatelet Aggregation Test. Washed rabbit platelets were obtained from EDTA-anticoagulated platelet-rich plasma according to the washing procedures described previously.[29] The platelet pellets were suspended in Tyrode's solution of the following composition (nM): NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2PO_4 (0.33), $CaCl_2$ (1.0), and glucose (11.2), containing bovine serum albumin (0.35%). Platelet aggregation was measured by the turbidimetric method as described by O'Brien.[13] Percentages of aggregation were calculated using the absorbance of platelet suspension to represent 0% aggregation and the absorbance of Tyrode's solution as 100% aggregation.

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