Chemical Constituents and Biological Activities of the Fruit of *Zanthoxylum integrifoliolum*

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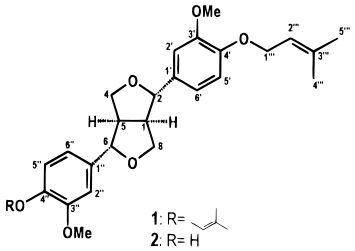
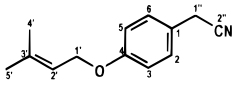
Through continuing studies on the chemical constituents and antiplatelet aggregation principles of the fruit of the Formosan *Zanthoxylum integrifoliolum*, four new compounds—including two new lignans, (+)-pinoresinol-di-3,3-dimethylallyl ether (**1**), and (+)-pinoresinol-3,3-dimethylallyl ether (**2**); zanthonitrile (**3**), and one new flavonoid, 3,5-diacetyltambulin (**4**)—and 18 known compounds were isolated from the $CHCl_3$-soluble fraction. Their structures were elucidated on the basis of spectral data and chemical evidence. Among the isolates, including the previously reported isobutylamides, 13 compounds showed strong in vitro antiplatelet aggregation activity, with only (−)-tetrahydroberberine showing weak vasorelaxing effect in high potassium- or norepinephrine-induced contraction of rat aorta.

Zanthoxylum integrifoliolum (Merr.) Merr. (Rutaceae) is an evergreen tree distributed in northern Philippine and Lanyu Island in Taiwan.[1] Its bark was utilized as remedy for snake-bite by Ya-Mei aborigines in Lanyu Island and was also a good source for antiplatelet agents such as chelerythrine and avicine pseudocyanide.[2] The alkaloids of benzo[*c*]phenanthridines, quinolines, and triterpenoids, were the major constituents of this plant from the past reports.[3−5] Recently, we have isolated three indolopyridoquinazoline alkaloids with strong antiplatelet aggregation activity[6] from a small amount of the fruit of this plant. Examination of the chemical constituents and antiplatelet principles from the recollected fruit has led to the isolation of nine isobutylamides[7] from the $CHCl_3$-soluble fraction. Further investigation of the same fraction has now resulted in the characterization of four new compounds—(+)-pinoresinol-di-3,3-dimethylallyl ether (**1**), (+)-pinoresinol-3,3-dimethylallyl ether (**2**), zanthonitrile (**3**), and 3,5-diacetyltambulin (**4**)—along with 18 known compounds—(−)-tetrahydroberberine,[8] skimmianine,[4] canthin-6-one,[4] 11-methoxycanthin-6-one,[9] rutaecarpine,[6] 1-hydroxyrutaecarpine,[6] 14-formylrutaecarpine,[10] norchelerythrine,[11] nornitidine,[12] decarine,[13] atanine,[14] tambulin,[15,16] prudomestin,[17,18] scopoletin,[19] (+)-piperitol-3,3-dimethylallyl ether,[20] (+)-sesamin,[4] pregnenolone,[21] and 2-tridecanone.[22] These known compounds were identified by comparisons of their IR, UV, ^{1}H NMR, TLC, and/or mixed melting points with corresponding authentic samples or literature data. In this paper, we report the structure elucidation of the four new compounds and the antiplatelet aggregation activity of the isolates. Compounds with vasorelaxing activity are also mentioned.





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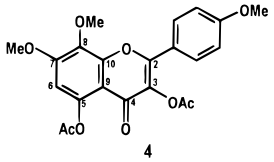

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Results and Discussion

(+)-Pinoresinol-di-3,3-dimethylallyl ether (**1**) was isolated as a colorless oil. Its molecular formula of $C_{30}H_{38}O_6$ was established by EIMS ([M]$^+$, *m*/*z* 494) and HREIMS (found 494.2677, calcd 494.2668). The ^{1}H NMR spectrum for **1** was very similar to that of the lignan, (+)-sesamin,[4] in the 3,3-*O*-bicyclooctane moiety with eight aliphatic protons [δ 3.12 (2H, m, H-1a and H-5a), 3.89 (2H, dd, J = 9.2, 3.6 Hz, H-4β and H-8β), 4.25 (2H, dd, J = 9.2, 7.2 Hz, H-4a and H-8a), and 4.75 (2H, d, J = 4.4 Hz, H-2β and H-6β)] and in the two trisubstituted benzene moieties with six aromatic protons [δ 6.83 (2H, d, J = 8.2 Hz, H-5′ and H-5″), 6.85 (2H, br d, J = 8.2 Hz, H-6′ and H-6″) and 6.90 (2H, br s, H-2′ and H-2″)], except that a methoxyl group at δ 3.88 (3H, s) and one 3, 3-dimethylallyloxyl group [δ 4.57 (2H, d, J = 6.8 Hz, H-1‴), 5.50 (1H, br t, J = 8.0 Hz, H-2‴), 1.72 (3H, d, J = 0.4 Hz, H-4‴), and 1.77 (3H, d, J = 0.8 Hz, H-5‴)] in **1** was in place of a methylenedioxyl group in each of two aryl groups in (+)-sesamin. The coupling constants of H-2 and H-6 were 4.4 Hz and showed the relative configuration of H-1 and H-2, H-5 and H-6 to be *trans*-form.[24,25] The simplicity of the ^{1}H NMR spectrum revealed **1** to be a symmetrical lignan. According to the above data and the similarity of the 3,3-dimethylallyloxyl and methoxyl groups in the ^{1}H NMR spectrum between piperitol-3,3-dimethylallyl ether[23] and **1**, the 3,3-dimethylallyloxyl group was probably at C-4′, and the methoxyl group thus was at C-3′. The correlations of H-5′ and H-1‴, H-2′ and 3′-OCH_3 were observed in the NOESY experiment (Figure 1) and further supported the 3,3-dimethylallyloxyl group and the methoxyl group at C-4′ and C-3′, respec-

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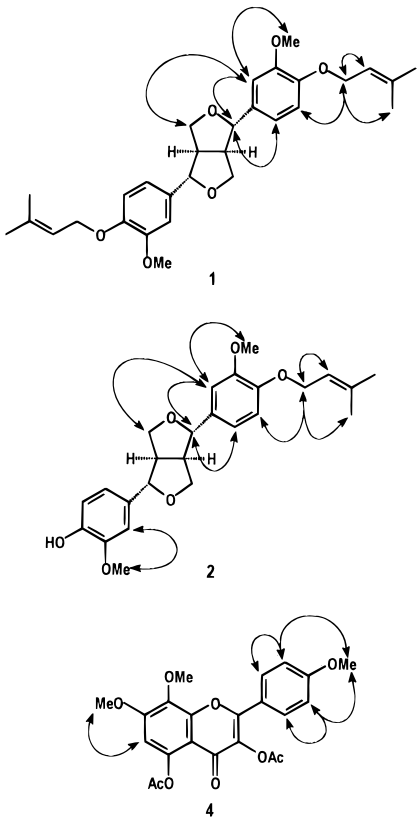

Figure 1. NOESY correlations for compounds **1**, **2**, and **4**.

tively. With the dextrorotatory optical rotation, $[\alpha]^{25}_D$ +41.6°(*c* 0.13, $CHCl_3$), the structure of **1** was elucidated as (+)-pinoresinol-di-3,3-dimethylallyl ether, which was further confirmed by ^{13}C NMR, DEPT, and HETCOR spectra.

(+)-Pinoresinol-3,3-dimethylallyl ether (**2**) was isolated as colorless oil with $[\alpha]^{25}_D$ +35.8°(*c* 0.065, $CHCl_3$). The EIMS spectrum afforded the molecular ion $[M]^+$ at *m/z* 426, implying a molecular formula of $C_{25}H_{30}O_6$, which was confirmed by the HREIMS (found 426.2051, calcd 426.2043). The ^1H NMR spectrum of **2** was also similar to that of (+)-sesamin, except that a 3,3-dimethylallyloxyl group [δ 4.57 (2H, d, J = 6.8 Hz, H-1‴), 5.51 (1H, br t, J = 8.0 Hz, H-2‴), 1.73, 1.77 (each 3H, s, H-4‴ and H-5‴)], two methoxyl groups [(δ 3.88 (3H, s, OCH_3), 3.91(3H, s, OCH_3)], and a hydroxyl group [δ 5.58 (1H, s, OH)] in **2** were in place of two methylenedioxyl groups in (+)-sesamin. The presence of a hydroxyl group and a methoxyl group on an aryl group was supported by a base peak of *m/z* 151 belonging to a hydroxymethoxybenzoyl ion. The Gibbs test was negative. The NOESY experiment (Figure 1) showed that both methoxyl signals at δ 3.88 and 3.91 can correlate with H-2′ and H-2″ (2H, d, J = 2.0 Hz). According to the above data, the 3,3-dimethylallyloxyl group and the hydroxyl group were reasonably assigned at C-4′ and C-4″, respectively, and the two methoxyl groups were assigned to C-3′ and C-3″, respectively. Thus, the structure of **2** was elucidated as (+)-pinoresinol-3,3-dimethylallyl ether.

Zanthonitrile (**3**) was obtained as a yellowish oil with a slightly pungent odor. The EIMS afforded the molecular ion $[M]^+$ at *m/z* 201, implying a molecular formula of $C_{13}H_{15}NO$. The IR spectrum revealed a nitrile absorption at 2250 cm^{-1}. The ^1H NMR spectrum of **3** showed a AA′XX′ system [δ 6.91 (2H, d, J = 8.6 Hz, H-3 and H-5), 7.22 (2H, d, J = 8.6 Hz, H-2 and H-6)] suggesting a 1,4-disubstituted benzene in **3**. Furthermore, one substituent was proved to be a 3,3-dimethylallyloxyl group [δ 4.50 (2H, d, J = 6.8 Hz, H-1′), 5.48 (1H, t, J = 6.8 Hz, H-2′), 1.78 and 1.80 (each 3H, s, H-4′ and H-5′)], and an acetonitrile substituent was suggested by a singlet at δ 3.68 (2H, H-1″). The acetonitrile substituent was further confirmed with a C-1″ at δ 22.8 and a nitrile at δ 118.2 (C-2″) by ^{13}C NMR spectrum. According to the above data and the prominent fragments at *m/z* 69 $[-CH_2CHC(CH_3)_2]^+$ and *m/z* 133 $[HOC_6H_4CH_2CN]^+$ in the MS, the structure of **3** was elucidated as 4-(3,3-dimethylallyloxy)phenylacetonitrile and named zanthonitrile.

3,5-Diacetyltambulin (**4**) was isolated as yellowish needles. The molecular formula was determined as $C_{22}H_{20}O_9$ by EIMS (M^+, *m/z* 428) and HREIMS (found 428.1099, calcd 428.1107). The UV absorption at 259, 326, 403 was similar to that of tambulin[16] and indicated the presence of a flavonoid moiety. No bathochromic shift after adding $AlCl_3$ or NaOAc revealed that **4** is a nonphenolic flavonoid skeleton. The ^1H NMR of **4** showed three methoxyl groups [δ 3.89 (3H, s), 3.97 (6H, s)], four aromatic protons [δ 7.02 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 7.87 (2H, d, J = 9.0 Hz, H-2′ and H-6′)] in AA′XX′ system, and one aromatic proton at δ 6.68 (1H, s, H-6), similar to those of tambulin, except for two additional acetoxyl groups [δ 2.34, 2.43 (each 3H, s)] in **4** in place of two hydroxyl groups [δ 6.58, 11.61 (each 1H, s)] in tambulin. Thus, the structure of **4** might be *O*,*O*-3,5-diacetyltambulin, in accord with the change of chemical shift of H-6 at δ 6.68, H-2′ and H-6′ at δ 7.87 in **4** in comparison to H-6 at δ 6.41, H-2′ and H-6′ at δ 8.25 in tambulin. Acetylation of tambulin with acetic anhydride in pyridine afforded a diacetyl product that was completely identical to **4** by comparison of their IR, UV, ^1H NMR, and TLC. Accordingly, the structure of **4** was undoubtedly elucidated as 3,5-diacetyltambulin, which was further confirmed by COLOC, DEPT, ^{13}C NMR, and NOESY (Figure 1) spectra.

The chloroform-soluble fraction of the fruit of *Z. integrifoliolum* showed strong antiplatelet aggregation activity in vitro using the turbidimetric method.[26] The absorbance of platelet suspension was taken as 0% aggregation and that of platelet-free Tyrode's solution as 100% aggregation. Each fraction or compound was preincubated with platelet suspension for 3 min at 37 °C before the aggregation inducer was added. The percent aggregation was measured at 6 min. Bioassay-guided fractionation led to the isolation of 3,5-diacetyltambulin (**4**), prudomestin, skimmianine,[27] canthin-6-one, decarine,[27] atanine, lanyuamide I, lanyuamide II, tetrahydrobungeanool, (2*E*,4*E*,8*Z*,11*Z*)- and (2*E*,4*E*,8*Z*,-11*E*)-2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide, γ-sanshoöl, hydroxy γ-sanshoöl as the active principles with antiplatelet aggregation activity (Table 1). The flavonoid prudomestin and the pungent isobutylamides, lanyuamides I and II, tetrahydrobungeanool, (2*E*,4*E*,8*Z*,-11*Z*)- and (2*E*,4*E*,8*Z*,11*E*)-2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide, γ-sanshoöl, and hydroxy γ-sanshoöl were the major active principles. It is interesting to observe that the greater the number of double bonds connected to the *trans*-2-*trans*-4-dienamide of isobutylamides, the lower the inhibitory activity of platelet aggregation induced by thrombin or PAF, as in the cases of (2*E*,4*E*,8*Z*,11*Z*)- and (2*E*,4*E*,8*Z*,11*E*)-2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide, γ-sanshoöl, and hydroxy γ-sanshoöl. Among the isolates, 11 compounds were also evaluated for their vasorelaxing effect (Table 2) using a published method of bioassay.[28] Rat aorta was preincubated with each fraction or compound at 37 °C for 15 min, then high potassium (80 nm) or norepinephrine (3 μM) was added. In this experi-

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

		aggregation (%)			
compound	(μg/mL)	Thr (0.1 U/mL)	AA (100 μM)	Col (10 μg/mL)	PAF (2 nM)
control		87.8 ± 0.9(3)	82.2 ± 0.5(3)	83.5 ± 0.7(3)	86.3 ± 0.9(5)
3,5-diacetyltambulin (**4**)	100	88.5 ± 1.2(3)[b]	0.0 ± 0.0(4)[d]	5.1 ± 2.6(4)[d]	0.0 ± 0.0(3)[d]
	50				46.7 ± 6.0(3)[d]
	20		0.0 ± 0.0(4)[d]		71.2 ± 2.3(3)[d]
	10		29.0 ± 11.2(4)[d]		78.0 ± 3.2(3)[d]
	5		51.1 ± 13.6(4)[c]		
(−)-tetrahydroberberine	100	91.2 ± 1.4(3)	45.8 ± 10.9(4)[d]	76.4 ± 4.8(4)	54.7 ± 8.5(4)[d]
canthin-6-one	100	85.3 ± 1.6(3)[c]	4.2 ± 1.5(4)[d]	7.9 ± 3.6(4)[d]	36.4 ± 10.0(4)[c]
	50		57.3 ± 4.0(4)[d]		
11-methoxycanthin-6-one	25	90.3 ± 0.7(3)	80.4 ± 2.2(3)[c]	76.3 ± 4.9(3)	84.8 ± 3.3(3)
atanine	100	84.3 ± 2.8(3)[c]	0.0 ± 0.0(4)[d]	19.5 ± 12.1(4)[c]	48.2 ± 13.0(4)[c]
	50		0.0 ± 0.0(4)[d]		
	20		73.7 ± 4.2(4)[c]		
tambulin	100	81.1 ± 2.2(3)[c]	73.3 ± 2.4(3)[c]	64.9 ± 10.3(3)[b]	78.6 ± 1.3(3)[b]
prudomestin	100	87.5 ± 2.7(3)	0.0 ± 0.0(3)[d]	58.1 ± 17.7(3)	73.3 ± 5.9(3)[c]
	20		0.0 ± 0.0(3)[d]		
	10		64.5 ± 8.5(3)[c]		
scopoletin	100	89.8 ± 0.9(3)	80.5 ± 0.8(3)[d]	80.9 ± 2.4(3)	78.2 ± 2.6(3)[d]
(+)-sesamin	100		cause platelet aggregation		
	50		cause platelet aggregation		
	20	87.8 ± 1.7(3)	82.3 ± 3.9(3)	83.2 ± 3.1(3)	83.7 ± 3.2(4)
pregnenolone	100	91.8 ± 1.3(3)	86.5 ± 1.7(3)	84.7 ± 0.9(3)	77.0 ± 4.3(3)[c]
2-tridecanone	50		cause platelet aggregation		
	20		63.5 ± 5.6(3)		
lanyuamide I	100	8.9 ± 7.2(3)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]
	50	75.7 ± 2.2(3)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]	20.0 ± 12.8(3)[d]
	20	80.4 ± 1.3(3)[d]	15.6 ± 12.7(3)[d]	2.7 ± 2.2(3)[d]	78.6 ± 1.1(3)[d]
lanyuamide II	100	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(4)[d]
	50	35.9 ± 15.4(3)[c]			0.0 ± 0.0(4)[d]
	20	75.2 ± 1.0(3)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]	70.5 ± 2.8(4)[d]
	10	77.6 ± 0.3(3)[d]	44.2 ± 6.6(3)[d]	10.5 ± 8.5(3)[d]	77.8 ± 1.6(4)[d]
tetrahydrobungeanool	100	74.0 ± 4.6(3)[c]	0.0 ± 0.0(4)[d]	0.0 ± 0.0(3)[d]	6.7 ± 5.8(4)[d]
	50		58.3 ± 10.9(4)		82.7 ± 3.4(4)[d]
(2*E*,4*E*,8*Z*,11*Z*) and (2*E*,4*E*,8*Z*,11*E*)-2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide	100	89.2 ± 0.8(3)[d]	0.0 ± 0.0(3)[d]	0.0 ± 0.0(3)[d]	38.4 ± 6.0(4)[d]
	50		0.0 ± 0.0(3)[d]		
	20		4.0 ± 3.0(3)[d]		
	10		27.8 ± 14.8(3)		
	5		46.2 ± 19.7(3)[b]		
γ-sanshoöl	100	88.7 ± 1.2(3)	0.0 ± 0.0(3)[d]	1.4 ± 1.2(3)[d]	72.7 ± 4.0(3)[c]
	50		69.7 ± 4.5(3)[c]		
hydroxy γ-sanshoöl	100	87.7 ± 0.5(3)[d]	0.0 ± 0.0(5)[d]	0.0 ± 0.0(3)[c]	78.6 ± 3.0(3)[d]
	50		19.9 ± 6.6(5)[d]		
aspirin	50	92.1 ± 1.3(3)	0.0 ± 0.0(5)[d]	87.1 ± 2.5(3)	90.1 ± 1.4(3)
	20		42.7 ± 15.8(3)		
	10		90.2 ± 0.9 (5)		

[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 ± S. E. M. (*n*). [b] $p < 0.05$. [c] $p < 0.01$. [d] $p < 0.001$, as compared with respective control.

ment, only (−)-tetrahydroberberine showed a weak vasorelaxing effect in high potassium- or norepinephrine-induced contraction of rat aorta.

Experimental Section

General Experimental Procedures. Melting points were determined with a YANACO micro-melting point apparatus and were uncorrected. Optical rotations were measured using a JASCO DIP-370 polarimeter in $CHCl_3$. IR spectra were taken on a Hitachi 260−30 (KBr and neat) spectrophotometer. UV spectra were obtained on a JASCO UV-240 spectrophotometer. EIMS spectra were recorded on a VG Biotech Quattro 5022 spectrometer. HREIMS were recorded on a JEOL JMX-HX 110 mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were measured on either a Varian Gemini 200 or a Varian Unity Plus 400 spectrometer and are given in parts per million (δ) downfield from internal TMS. Si gel 60 (Merck 70−230 mesh, 230−400 mesh, ASTM) was used for column chromatography, and Si gel 60 F_{254} (Merck) for TLC.

Plant Material. *Z. integrifoliolum* fruits were collected at Lanyu Island, Taitung County, Taiwan, in August 1995. A voucher sample (no. Chen 5528) was deposited in the herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. Dried fruits (16.5 kg) were crushed, extracted with MeOH, and concentrated in vacuo to leave a brownish fluid. The MeOH extract was partitioned between $CHCl_3$ and H_2O (1:1). The H_2O-soluble fraction was further partitioned between H_2O and *n*-BuOH (1:1) to afford a H_2O fraction (Fraction D, 620 g) and *n*-BuOH fraction (Fraction B, 130 g). The $CHCl_3$-soluble fraction was also partitioned between 90% aqueous MeOH and *n*-hexane (1:1), and then an *n*-hexane fraction was obtained (Fraction A, 420 g). The 90% aqueous MeOH extract was first treated with $CHCl_3$ to produce yellowish crystal I (2.54 g), then a second batch of yellowish crystal II (0.58 g) was obtained from the filtrate. The filtrate was concentrated in vacuo to obtain a $CHCl_3$ fraction (220 g). A part of the $CHCl_3$-soluble fraction (99 g) was chromatographed over Si gel, eluting with CH_2Cl_2 and gradually enriched with EtOAc to give 12 fractions (C1−C12). Fraction C1 (3.75 g) was washed by Et_2O to yield tambulin (187 mg) after recrystallization from MeOH−Et_2O. Fraction C3 (2.92 g) was washed with Et_2O to give prudomes-

Table 2. Vasorelaxing Effect[a] of Compounds on the Contraction Induced by K^+ and Norepinephrine in Rat Aorta

compound	(μg/mL)	contraction (% of control)		
		K^+	NE (phasic)	NE (tonic)
control		100.0 ± 4.7	100.0 ± 3.9	100.0 ± 5.1
3,5-diacetyltambulin (**4**)	50	25.3 ± 0.9	74.6 ± 1.3	58.7 ± 0.6
	15	57.1 ± 2.0	94.6 ± 8.0	86.3 ± 7.6
	5	84.8 ± 8.4		
(−)-tetrahydroberberine	50	13.6 ± 2.5	11.2 ± 0.9	25.6 ± 2.1
	15	49.8 ± 2.9	79.1 ± 2.9	70.0 ± 4.5
	5	82.8 ± 2.0		
11-methoxycanthin-6-one	12.5	57.9 ± 3.9	88.4 ± 3.1	78.9 ± 5.3
	3.8	74.5 ± 1.9		
atanine	50	33.8 ± 1.1	87.8 ± 0.2	56.8 ± 0.5
	15	67.1 ± 1.5		
tambulin	100	104.7 ± 17.5	100.2 ± 4.6	98.6 ± 4.7
prudomestin	100	36.7 ± 2.4	20.0 ± 0.0	46.7 ± 9.0
	30	83.4 ± 1.1	100.6 ± 4.3	97.8 ± 7.3
scopoletin	50	88.4 ± 1.1	91.9 ± 14.5	81.6 ± 10.9
pregnenolone	50	93.6 ± 1.6	114.1 ± 4.1	102.6 ± 1.9
lanyuamide I	50	28.6 ± 5.1	77.1 ± 3.8	45.5 ± 1.2
	15	47.6 ± 4.9	100.0 ± 14.1	77.1 ± 6.8
	5	82.0 ± 5.6		
lanyuamide II	50	36.1 ± 4.8	83.8 ± 2.7	78.5 ± 1.1
	15	72.5 ± 1.8		
γ-sanshoöl	50	92.3 ± 2.6	120.0 ± 0.0	107.0 ± 2.2
nifedipine	1	0.0 ± 0.0		
prazosin	1		0.0 ± 0.0	0.0 ± 0.0

[a] Rat aorta were preincubated with various compounds, DMSO (0.1%, control), nifedipine, or prazosin at 37 °C for 15 min, then high potassium (K^+, 80 mM) or norepinephrine (NE, 3 μM) was added. Percentages of the control contraction were calculated and presented as means ± S. E. M. (n = 3).

tin (179 mg) after recrystallization from $CHCl_3$−MeOH. The Et_2O washings (2.546 g) were chromatographed over Si gel eluting with $CHCl_3$−EtOAc (40:1), gradually increasing the polarity with EtOAc, and seven fractions (C3-1−C3-7) were collected. Fraction C3-1 (340.8 mg) was washed by Et_2O to yield (+)-sesamin (239 mg), and the washings were purified by preparative TLC (C_6H_6−EtOAc, 10:1) to obtain norchelerythrine (8 mg), 1-hydroxyrutaecarpine (1.5 mg), and zanthonitrile (13 mg). Fraction C4 (11.4 g) was washed with Et_2O to yield prudomestin (2.58 g). A mixture (4.41 g) of prudomestin and tambulin was removed from the Et_2O washings, then the filtrate (982 mg) was chromatographed on Si gel using n-hexane−Me_2O (3:1) to yield seven fractions (C4-1−C4-7). Fraction C4-2 was purified with preparative TLC (C_6H_6−EtOAc, 20:1) to obtain (+)-sesamin (20.4 mg) again, and fraction C4-4 (12 mg) was purified with preparative TLC (n-hexane−EtOAc, 2:1) to yield rutaecarpine (1.6 mg). Fraction C5 (1.63 g) was chromatographed on Si gel using n-hexane−Me_2O (2:1) and enriched gradually with Me_2O to obtain 12 fractions (C5-1−C5-12). Fraction C5-4 (45 mg) was washed with $CHCl_3$−MeOH to yield decarine (1.7 mg), and the washings were purified with preparative TLC ($CHCl_3$−Me_2O, 25:1) to yield nornitidine (0.4 mg). Fraction C7 (1.63 g) was washed with Me_2O to get pregnenolone (54 mg). The washings (1.49 g) were rechromatographed on Si gel eluting with $CHCl_3$−EtOAc, gradually increased the polarity with EtOAc, and 11 fractions (C7-1−C7-11) were collected. Fraction C7-2 (46 mg) was purified with preparative TLC (C_6H_6−EtOAc, 10:1) to get (+)-piperitol-3,3-dimethylallyl ether (13 mg) and (+)-pinoresinol-di-3,3-dimethylallyl ether (11 mg). Fraction C7−3 (25 mg) was purified by preparative TLC (CH_2Cl_2−EtOAc, 20:1) to afford (+)-pinoresinol-3,3-dimethylallyl ether (1.4 mg). Fraction C7-7 (380 mg) was rechromatographed on Si gel eluting with n-hexane−EtOAc (2:1), then enriched with EtOAc to obtain eight fractions (C7-7-1−C7-7-8). Fraction C7-7-6 (62 mg) was recrystallized from MeOH to afford 14-formylrutaecarpine (26 mg), and scopoletin (1.4 mg) was obtained after purification using preparative TLC (C_6H_6−MeOH, 30:1) from fraction C7-7-8 (23 mg). Fraction C8 (19.1 g) was rechromatographed on Si gel eluting with $CHCl_3$−MeOH mixtures to yield 13 fractions (C8-1−C8-13). Fraction C8-5 (4.1 g) was separated by Si gel eluting with $CHCl_3$−Me_2O to obtain 10 fractions (C8-5-1−C8-5-10). Fraction C8-5-2 (340 mg) was rechromatographed on Si gel and eluting with $CHCl_3$−Me_2O to afford six fractions (C8-5-2-1−C8-5-2-6). Fraction C8-5-2-2 (129 mg) was chromatographed on Si gel and eluted with n-hexane−EtOAc (1:1) and enriched with EtOAc to obtain (−)-tetrahydroberberine (8.7 mg) and skimmianine (6.9 mg). Fraction C8-5-3 (500 mg) was chromatographed on Si gel and eluting with n-hexane−EtOAc (1:1), gradually increased polarity with EtOAc to obtain 13 fractions (C8-5-3-1−C8-5-3-13). Fraction C8-5-3-2 (70 mg) was recrystallized from Et_2O to yield atanine (17 mg). Fraction C8-5-3-8 (24 mg) was purified with preparative TLC (CH_2Cl_2−Me_2O, 4:1) to afford 11-methoxycanthin-6-one (7 mg) and canthin-6-one (4.5 mg). A part of n-hexane fraction (Fraction A, 6.33 g) was rechromatographed on Si gel using $CHCl_3$−Me_2O to get 10 fractions (A1−A10). Fraction A-2 (63 mg) was purified by preparative TLC (n-hexane−EtOAc, 25:1) to get 2-tridecanone (41 mg). The ppt I (Fraction E, 2.54 g) was chromatographed on Si gel using C_6H_6−EtOAc to get seven fractions (E1−E7). Fraction E-5 (135 mg) was rechromatographed with CH_2Cl_2−EtOAc, and three fractions (E5-1−E5-3) were collected. Fraction E-5-3 (44 mg) was further purified by preparative TLC (CH_2Cl_2−EtOAc, 20:1) to afford 3,5-diacetyltambulin (2.3 mg).

(+)-Pinoresinol-di-3,3-dimethylallyl ether (1): colorless oil; $[\alpha]^{25}_D$ +41.6° (c 0.13, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 280.5 (5.93), 233.5 (5.40) nm; IR (neat) ν_{max} 1580, 1500 (aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.72 (6H, d, J = 0.4 Hz, H-4‴), 1.77 (6H, d, J = 0.8 Hz, H-5‴), 3.12 (2H, m, H-1α, 5α), 3.88 (6H, s, OCH_3), 3.89 (2H, dd, J = 9.2, 3.6 Hz, H-4β, 8β), 4.25 (2H, dd, J = 9.2, 7.2 Hz, H-4α, 8α), 4.57 (4H, d, J = 6.8 Hz, H-1‴), 4.75 (2H, d, J = 4.4 Hz, H-2β, 6β), 5.50 (2H, br t, J = 8.0 Hz, H-2‴), 6.83 (2H, d, J = 8.2 Hz, H-5′, 5″), 6.85 (2H, br d, J = 8.2 Hz, H-6′, 6″), 6.90 (2H, br s, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.2 (q, C-4‴), 25.8 (q, C-5‴), 54.1 (d, C-1, 5), 55.9 (q, 3′-OCH_3, 3″-OCH_3), 65.8 (t, C-1‴), 71.7 (t, C-4, 8), 85.8 (d, C-2, 6), 109.5 (d, C2′, -2″), 113.0 (d, C-5′, -5″), 118.1 (d, C-6′, -6″), 119.9 (d, C-2‴), 133.6 (s, C-1′, -1″), 137.5 (s, C-3‴), 147.8 (s, C-3′, -3″), 149.7 (s, C-4′, -4″); EIMS m/z 494 $[M]^+$ (0.7), 426 (2), 358 (33), 163 (31), 152 (19), 151 (72), 150 (27), 137 (59), 131 (21), 69 (100); HREIMS m/z 494.2677 (calcd for $C_{30}H_{38}O_6$, 494.2668).

(+)-Pinoresinol-3,3-dimethylallyl ether (2): colorless oil; $[\alpha]^{25}_D$ +35.8° (c 0.065, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.73, 1.77 (each 3H, s, CH_3), 3.11 (2H, m, H-1α, 5α), 3.89 (2H, dd, J = 9.0, 3.2 Hz, H-4β, 8β), 3.91 (6H, s, OCH_3), 4.25 (2H, dd, J = 9.0, 6.8 Hz, H-4α, 8α), 4.57 (2H, d, J = 6.8 Hz, H-1‴),

4.75 (2H, dd, $J = 5.6$, 4.8 Hz, H-2β, 6β), 5.51 (1H, br t, $J = 8.0$ Hz, H-2‴), 5.58 (1H, br s, OH, exchangeable with D_2O), 6.82 (2H, dd, $J = 8.2$, 2.0 Hz, H-6′, 6″), 6.85, 6.88 (each 1H, d, $J = 8.2$ Hz, H-5′, 5″), 6.89 (2H, d, $J = 2.0$ Hz, H-2′, 2″); EIMS *m*/*z* 426 $[M]^+$ (1.3), 359 (6), 358 (26), 327 (4), 205 (14), 163 (29), 151 (100), 150 (25), 137 (59), 131 (29), 124 (13), 69 (31); HREIMS *m*/*z* 426.2051(calcd for $C_{25}H_{30}O_6$, 426.2043).

Zanthonitrile (3): colorless oil; IR (neat) ν_{max} 2250 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.74 (3H, s, H-4′), 1.80 (3H, s, H-5′), 3.68 (2H, s, H-1″), 4.50 (2H, d, $J = 6.8$ Hz, H-1′), 5.48 (1H, t, $J = 6.8$ Hz, H-2′), 6.91 (2H, d, $J = 8.6$ Hz, H-3, 5), 7.22 (2H, d, $J = 8.6$ Hz, H-2, 6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.1 (q, C-4′), 22.8 (t, C-1″), 25.8 (q, C-5′), 64.9 (t, C-1′), 115.3 (d, C-3, 5), 118.2 (q, C-2″), 119.4 (d, C-2′), 121.7 (s, C-1), 129.0 (d, C-2, 6), 138.4 (s, C-3′), 158.6 (s, C-4); EIMS *m*/*z* 201 $[M]^+$ (6), 200 (13), 133 (100), 107 (19), 77 (63), 69 (98).

3,5-Diacetyltambulin (4): yellowish prisms ($CHCl_3$−MeOH); mp 156−158 °C; UV (MeOH) λ_{max} (log ϵ) 259 (4.02), 326 (3.89), 403 (3.12) nm; IR (KBr) ν_{max} 1765 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.34 (3H, s, 3-OCOCH_3), 2.43 (3H, s, 5-OCOCH_3), 3.89 (3H, s, 4′-OCH_3), 3.97 (6H, s, 7, 8-OCH_3), 6.68 (1H, s, H-6), 7.02 (2H, d, $J = 9.0$ Hz, H-3′, -5′), 7.87 (2H, d, $J = 9.0$ Hz, H-2′, -6′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.7 (q, 3-OCO*C*H_3), 21.1 (q, 5-OCO*C*H_3), 55.4 (q, 4′-OCH_3), 56.7 (q, 7-OCH_3), 61.6 (q, 8-OCH_3), 104.7 (d, C-6), 111.2 (s, C-10), 114.3 (d, C-3′, -5′), 122.1 (s, C-1′), 129.9 (d, C-2′, -6′), 132.6 (s, C-3), 134.9 (s, C-7), 145.3 (s, C-5), 150.5 (s, C-2), 154.8 (s, C-9), 156.2 (s, C-8), 161.9 (s, C-4′), 168.0 (s, 3-O*C*OCH$_3$), 169.8 (s, 5-O*C*OCH$_3$), 170.5 (s, C-4); EIMS *m*/*z* 428 $[M]^+$ (2), 386 (34), 344 (100), 329 (98), 135 (14); HREIMS *m*/*z* 428.1099 $[M]^+$ (calcd for $C_{22}H_{20}O_9$, 428.1107).

Acetylation of Tambulin. A mixture of tambulin (22 mg), Ac_2O (1 mL), and pyridine (1 mL) was allowed to stand at room temperature overnight. After workup, it yielded **4**, as yellowish prisms ($CHCl_3$−MeOH) (10.2 mg), mp 162−163 °C.

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