Dihydrobenzo[*c*]phenanthridine Alkaloids from Stem Bark of *Zanthoxylum nitidum*

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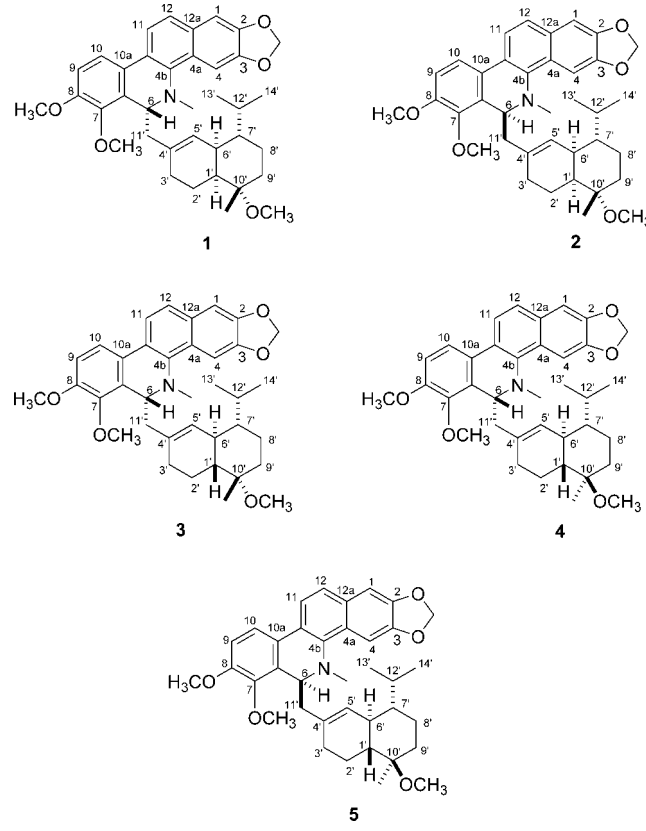
Five novel alkaloids, zanthomuurolanine (**1**), *epi*-zanthomuurolanine (**2**), zanthocadinanines A (**3**) and B (**4**), and *epi*-zanthocadinanine B (**5**), composed of dihydrochelerythrine and a cadinane-type sesquiterpene linked by a methylene bridge, have been isolated from stem bark of *Zanthoxylum nitidum*. These structures were elucidated by spectroscopic techniques (UV, IR, MS, CD, ^{1}H NMR, ^{13}C NMR, DEPT, COSY, NOESY, HSQC, and HMBC analyses). Single-crystal X-ray diffraction studies confirmed the relative configurations of **1** and **4** and provided additional support for the structures of **2**, **3**, and **5**.

Zanthoxylum nitidum (Roxb.) DC. (*Xanthoxylum nitidum* (Roxb.) DC., *Fagara nitida* Roxb.) (Rutaceae) is a scandent prickly shrub. It is distributed in the Molucca Islands, New Guinea, South China, and Ryukyus and also grows at low altitudes throughout Taiwan.[1,2] Its stems and leaves have been used as folk medicines to relieve toothache and sore throat.[3,4] The root has been used to promote blood circulation, to dissipate blood stasis to treat traumatic injury, and to cure snake bite.[4] Pharmacological investigations of *Z. nitidum* have reported antitumor,[5] anti-inflammatory,[6] and analgesic activities.[6] Benzo[*c*]phenanthridine alkaloids, quinolines, phenylpropenoids, lignans, and coumarins have been isolated from this plant.[6–13] Earlier chemical studies of *Z. nitidum* frequently focused on its polar alkaloidal constituents. Examination of the nonpolar fraction of the stem bark of *Z. nitidum* in this report led to the isolation of five novel dihydrobenzo[*c*]phenanthridine alkaloids, zanthomuurolanine (**1**), *epi*-zanthomuurolanine (**2**), zanthocadinanine A (**3**), zanthocadinanine B (**4**), and *epi*-zanthocadinanine B (**5**). The structural elucidations of **1**–**5** were based on spectroscopic analyses and X-ray diffraction studies.



Results and Discussion

Zanthomuurolanine (**1**) was obtained as colorless needles. The molecular formula was established as $C_{37}H_{45}NO_5$ by HRESIMS analysis (584.3375 $[M + H]^+$), indicating the existence of 16 degrees of unsaturation. UV absorptions at 229 (3.90), 284 (4.53), and 322 sh (4.05) nm suggested the presence of a dihydrochelerythrine moiety,[14] and IR absorptions indicated the existence of a methylenedioxy group (1041, 947 cm^{-1}). The ^{1}H NMR spectrum (C_6D_6, 500 MHz) (Table 1) showed six aromatic protons, including two sets of *ortho*-coupled spin systems at δ 6.65 (d, $J = 8.5$ Hz, H-9) and 7.50 (d, $J = 8.5$ Hz, H-10) and 7.78 (d, $J = 8.5$ Hz, H-11) and 7.38 (d, $J = 8.5$ Hz, H-12) and proton singlets at δ 7.06 (H-1) and 7.97 (H-4), a benzylic proton at δ 4.81 (dd, $J = 9.3$, 4.2 Hz, H-6), a methylenedioxy group at δ 5.35 and 5.41 (each 1H, d, $J = 0.1$ Hz), two methoxy groups at δ 3.39 (3H, s, OCH_3-8) and 3.86 (3H, s, OCH_3-7), and an *N*-methyl group at δ 2.61 (s). The data suggested the partial structure of **1** to be a 6-substituted dihydrochelerythrine moiety,[14] with 13 degrees of unsaturation, a structure supported by an ESI mass fragmentation pattern characteristic for a parent peak at *m*/*z* 348.[14]

The remaining signals in the ^{1}H NMR spectrum (Table 1) revealed signals of an isopropyl group, three methines [δ 1.27 (m, H-7′), 1.94 (br d, $J = 12.5$ Hz, H-1′), and 2.45 (m, H-6′)], an olefinic proton at δ 5.63 (d, $J = 6.0$ Hz, H-5′), five methylenes, one methyl group (δ 1.04, s, CH_3-10′), and a methoxy group (δ 3.05, s, OCH_3-10′). The above-mentioned proton signals and the residual molecular formula of $C_{21}H_{27}O$ suggested that the residual moiety was a cadinane-type sesquiterpene with a methoxy group rather than a

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Table 1. ^{1}H NMR (C_6D_6, 500 MHz) Data (δ) for **1–5**

	δ_H (J in Hz)				
position	**1**	**2**	**3**	**4**	**5**
1	7.06, s	7.05, s	7.09, s	7.08, s	7.07, s
4	7.97, s	7.95, s	7.93, s	7.87, s	7.95, s
6	4.81, dd (9.3, 4.2)	4.85, dd (10.5, 5.5)	4.92, dd (11.0, 5.0)	4.86, dd (11.0, 4.5)	4.85, dd (9.5, 5.5)
9	6.65, d (8.5)	6.65, d (8.5)	6.64, d (8.5)	6.64, d (8.5)	6.64, d (8.5)
10	7.50, d (8.5)	7.51, d (8.5)	7.50, d (8.5)	7.50, d (8.5)	7.51, d (8.5)
11	7.78, d (8.5)	7.78, d (8.5)	7.77, d (8.5)	7.76, d (8.5)	7.78, d (8.5)
12	7.38, d (8.5)	7.38, d (8.5)	7.39, d (8.5)	7.38, d (8.5)	7.38, d (8.5)
1′	1.94, br d (12.5)	2.05, d (13.0)	1.39, m	1.79, br t (12.5)	1.56, td (14.0, 7.8)
2′α	1.57, m	1.65, m	2.09, dd (12.9, 6.0)	2.43, dd (12.5, 5.4)	1.44, m
2′β	1.49, m	1.50, m	1.89, dddd (12.9, 12.9, 12.9, 6.0)	1.29, dddd (12.5, 12.5, 12.5, 5.8)	2.38, m
3′α	2.23, m	2.21, dd (15.0, 5.5)	2.50, m	2.52, m	2.42, dd (17.0, 5.0)
3′β	2.33, dd (17.0, 5.8)	2.29, m	2.23, dd (17.0, 6.0)	2.17, dd (16.8, 5.4)	2.18, m
5′	5.63, d (6.0)	5.50, br d (5.5)	5.23, s	5.08, s	5.21, br s
6′	2.45, m	2.50, m	2.39, m	1.69, m	1.86, m
7′	1.27, m	1.36, m	1.03, m	0.96, m	0.84, tt (11.3, 3.0)
8′α	1.35, m	1.28, m	1.40, m	1.54, m	1.00, qd (15.5, 4.9)
8′β	1.52, m	1.56, m	1.31, dd (13.8, 2.5)	0.97, m	1.48, m
9′α	1.71, br d (13.5)	1.68, m	1.20, ddd (14.0, 10.5, 3.5)	1.64, m	1.41, q (4.0)
9′β	1.24, td (13.5, 4.0)	1.24, m	2.00 (dt, 14.0, 3.5)	1.81, m	1.70, m
11′	2.26, dd (14.0, 9.3)	2.27, dd (13.5, 10.5)	2.30, dd (13.5, 11.0)	2.29, dd (13.8, 11.0)	2.32, dd (14.5, 9.5)
	2.43, dd (14.0, 4.2)	2.35, dd (13.5, 5.5)	2.34, dd (13.5, 5.0)	2.38, dd (13.8, 4.5)	2.32, dd (14.5, 5.5)
12′	2.01, br sept (7.0)	1.81, br sept (7.0)	1.60, br sept (7.0)	1.48, br sept (7.0)	1.72, br sept (7.0)
13′	0.85, d (7.0)	0.75, d (7.0)	0.62, d (7.0)	0.50, d (7.0)	0.67, d (7.0)
14′	1.01, d (7.0)	0.88, d (7.0)	0.83, d (7.0)	0.77, d (7.0)	0.74, d (7.0)
CH_3-10′	1.04, s	1.07, s	1.13, s	1.06, s	1.17, s
OCH_3-10′	3.05, s	3.16, s	3.00, s	3.21, s	3.13, s
OCH_3-7	3.86, s	3.84, s	3.85, s	3.83, s	3.83, s
OCH_3-8	3.39, s	3.40, s	3.39, s	3.39, s	3.40, s
OCH_2O	5.35, 5.41, d (0.1)	5.35, 5.36, s	5.38, 5.52, d (1.0)	5.39, 5.52, d (0.5)	5.38, 5.45, s
N-CH_3	2.61, s	2.59, s	2.67, s	2.50, s	2.56, s

hydroxy group at C-10′. Furthermore, the cadinane moiety was connected via a methylene group [δ 2.26 (dd, J = 14.0, 9.3 Hz, H-11′a), 2.43 (dd, J = 14.0, 4.2 Hz, H-11′b)] to C-6 of the dihydrochelerythrine moiety. The COSY spectrum (Figure 1) showed contiguous correlations from H-5′ to H-3′ and H-9′ and the HMBC spectrum (Figure 1) revealed ^2J and ^3J correlations between H-11′ (δ 2.26, 2.43) and C-4′ (δ 135.7) and C-3′ (δ 30.8), respectively. Furthermore, H-5′ (δ 5.63) gave a ^2J correlation with C-4′ (δ 135.7), which further supported the argument that **1** was a cadinane-type derivative of dihydrochelerythrine.

Cadinane-type sesquiterpenes have been divided into three subclasses, muurolane,[15,16] cadinane,[15] and amorphane,[17] on the basis of the nature of the ring fusion and the orientation of the isopropyl group. The relative configuration of the sesquiterpene moiety of **1** was deduced from its NOESY spectrum (Figure 2). The observation of cross-peaks between H-1′ (δ 1.94) and H-6′ (δ 2.45) indicated a *cis*-fused ring system, which was supported by the olefinic proton (H-5′) appearing as a doublet (J = 6.0 Hz),[15] resulting from the dihedral angle between H-5′ and H-6′ being close to 30°. On the other hand, H-6′ displayed correlations with OCH_3-10′, in agreement with a 1,3-diaxial interaction. To reduce the steric effect, the isopropyl group at C-7′ should be equatorial in orientation, which was confirmed by NOESY correlations between H-6′ and H-13′. By measuring **1** in $CDCl_3$ and comparing the sesquiterpenoid portion of the NMR data with that of the known sesquiterpene T-muurolol,[16] the only observed differences were that the OH group at C-10 in T-muurolol was replaced by a methoxy group (δ 3.05, 3H, s) at C-10′ in **1**, and the methyl group (C-11) in T-muurolol was converted to a methylene linkage (C-11′) of **1**. Thus, the planar structure of **1** was elucidated as shown, and it was named zanthomuurolanine.

The absolute configuration of dihydrochelerythrine at C-6 has never been determined, and NOESY experiments failed to determine the relative configuration of **1** at C-6. Thus, single-crystal X-ray diffraction of **1** was carried out and the relative configuration of **1** was determined to be *rel*-6*R*,1′*S*,6′*S*,7′*S*,10′*S* (Figure 3).

The molecular formula of **2** was $C_{37}H_{45}NO_5$ by HRESIMS (*m*/*z* found 584.3378 $[M + H]^+$, calcd 584.3376), which was the same as that of **1**. Structural determination of **2** was conducted using 1D and 2D NMR and revealed features similar to those of **1** (Tables 1 and 2). NOEs were observed in which the cross-peaks appearing between H-1′ (δ 2.05), H-6′ (δ 2.50), and OCH_3-10′ (δ 3.16) indicated a *cis*-fused ring system and an axial-orientated methoxy group, which indicated the presence of a T-muurolol *O*-methyl ether, the same as in **1**. Compound **2** ($[\theta]_{287}$ +6626, $[\theta]_{248}$ +28 931,

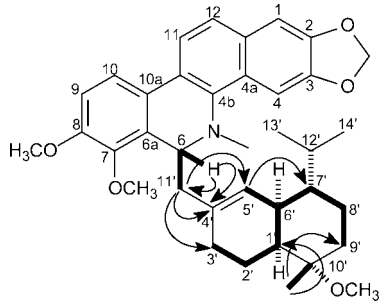

Figure 1. Key ^{1}H−^{1}H COSY (—) and HMBC (H→C) correlations of **1**.

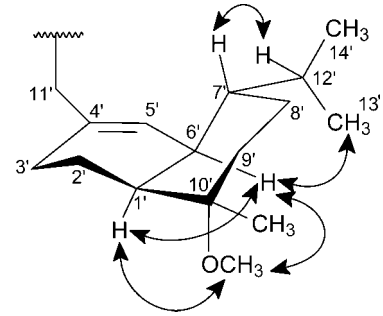

Figure 2. Key NOESY (H↔H) correlations of **1**.

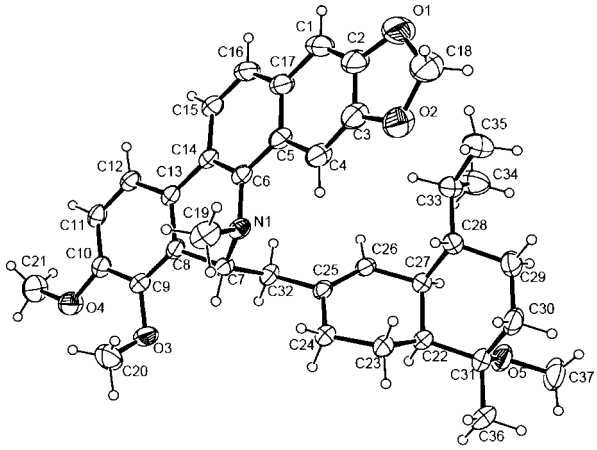

Figure 3. ORTEP drawing of **1** as determined by X-ray analysis.

Table 2. ^{13}C NMR (C_6D_6, 125 MHz) Data for **1**–**5**[a]

position	1	2	3	4	5
1	105.2	105.1	105.0	104.9	105.0
2	148.4	148.4	148.3	148.3	148.4
3	148.9	148.8	148.5	148.5	148.8
4	101.9	102.0	102.6	102.7	102.4
4a	132.1	132.1	132.0	132.0	132.1
4b	141.3	141.1	141.1	141.1	141.1
NCH_3	43.3	43.2	43.2	43.1	43.2
6	58.5	57.4	56.6	56.3	57.4
6a	131.1	131.2	131.5	131.4	131.3
7	147.0	147.0	147.0	147.0	147.1
7-OCH_3	60.9	60.9	61.0	61.0	61.0
8	153.0	153.0	153.0	153.0	153.0
8-OCH_3	55.8	55.6	55.7	55.7	55.7
9	112.1	111.8	112.0	112.0	111.9
10	119.3	119.3	119.3	119.3	119.2
10a	125.8	125.8	125.9	125.9	125.8
10b	124.8	124.8	124.8	124.8	124.9
11	120.7	120.7	120.8	120.7	120.7
12	124.4	124.5	124.4	124.3	124.4
12a	128.7	128.7	128.7	128.7	128.6
OCH_2O	101.2	101.3	101.3	101.3	101.3
1′	48.2	42.6	50.7	47.9	47.3
2′	21.7	21.6	23.6	23.4	23.6
3′	30.8	30.8	29.9	29.5	29.4
4′	135.7	134.6	135.5	136.4	136.3
5′	128.2	128.2	126.0	125.2	125.9
6′	35.2	35.0	38.0	40.3	40.3
7′	44.6	44.6	46.7	46.5	47.1
8′	20.0	20.0	20.4	17.7	22.3
9′	32.4	32.8	34.5	36.3	36.2
10′	76.1	76.1	74.3	76.3	76.3
11′	43.1	43.0	44.1	44.2	43.4
12′	27.4	27.3	26.6	26.2	26.4
13′	15.9	15.8	15.6	15.4	15.4
14′	22.4	22.2	21.8	21.8	22.0
CH_3-10′	23.0	23.1	23.2	22.3	18.2
OCH_3-10′	48.1	48.3	48.7	48.4	48.3

[a] Chemical shifts are in ppm (δ).

$[\theta]_{218}$ –13 367) gave a CD spectrum opposite of that of **1** ($[\theta]_{284}$ –2609, $[\theta]_{249}$ –9031, $[\theta]_{221}$ +3721). Thus, the evidence indicated that **2** (*rel*-6*S*,1′*S*,6′*S*,7′*S*,10′*S*) was the C-6 epimer of **1**, and **2** consequently was named *epi*-zanthomuurolanine.

HRESIMS revealed the molecular formula of **3** to be $C_{37}H_{45}NO_5$ (*m*/*z* found 584.3375 $[M + H]^+$, calcd 584.3376), which implied that **3** was another isomer of **1** and **2**. The ^1H NMR spectrum of **3** was similar to those of **1** and **2**, but the olefinic proton H-5′ was a broad singlet indicating a *trans*-fused ring system[15] rather than a doublet as in **1** (J = 6.0 Hz) and **2** (J = 5.5 Hz; *cis*-fused rings). NOESY correlations were observable between H-6′ and OCH_3-10′ and between H-1′ and CH_3-10′. Comparing the NMR data of **3** to that of α-cadinol,[15] which was also isolated in this study, showed that **3** had an OCH_3 group rather than an OH group as in α-cadinol. The CD spectrum had a Cotton effect (CE) identical to that of **1** and a negative specific rotation ($[\alpha]^{20}_D$ –71.5 (*c* 0.13, $CHCl_3$)), indicating that **3** and **1** are C-1′ epimers. Thus, the relative configuration of **3** is proposed as *rel*-6*R*,1′*R*,6′*S*,7′*S*,10′*S*.

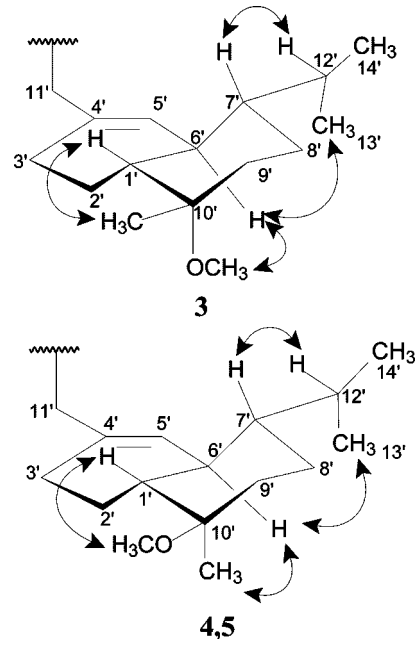

Figure 4. Key NOESY correlations of compounds **3**–**5**.

The molecular formula of **4** was $C_{37}H_{45}NO_5$ (*m*/*z* found 584.3375 $[M + H]^+$, calcd 584.3376) by HRESIMS, the same as that of **1**. Structure determinations were conducted by 1D and 2D NMR, which indicated that **4** also was a sesquiterpenoid dihydrochelerythrine (Tables 1 and 2). The sesquiterpenoid moiety of **4** was deduced to have a *trans*-fused ring system, as the trisubstituted olefinic proton (H-5′) appeared as a broad singlet.[15] NOESY correlations of **4** (Figure 4) were observed between H-6′ and CH_3-10′ and between H-1′ and OCH_3-10′, indicating that **3** and **4** are C-10′ epimers. The CD spectrum of **4** showed negative CE at 283, 249 nm ($[\theta]_{283}$ –10 197, $[\theta]_{249}$ –24 315) and a positive CE at 223 nm ($[\theta]_{223}$ +15 446), which was similar to **1** and **3**. Thus, the relative configuration of **4** was proposed as *rel*-6*R*,1′*R*,6′*S*,7′*S*,10′*R*, which was supported by single-crystal X-ray diffraction (Figure 6).

Compound **5** also had the molecular formula $C_{37}H_{45}NO_5$ (*m*/*z* found 584.3374 $[M + H]^+$, calcd 584.3376). NOESY analysis (Figure 4) of *epi*-zanthocadinanine B (**5**) showed a sesquiterpenoid moiety with a decalin system similar to that of **4**. The CD spectrum of **5** indicated that **4** and **5** are C-6 epimers. The relative configuration of **5** was proposed to be *rel*-6*S*,1′*R*,6′*S*,7′*S*,10′*R*.

Compounds **1**–**5** were unstable in solvents such as chloroform. To ensure stability of compounds, all NMR spectra were measured in C_6D_6. Due to instability of the compounds, we were not able to add heavy atoms by chlorination or bromination for the determination of absolute configuration using X-ray measurements. Thus, the relative configurations of **1**–**5**, which differ in configuration at C-6, C-1′, C-6′, and C-10′, respectively, were determined by 1D and 2D NMR spectra or supported by X-ray diffraction studies of **1** and **4**. The biological activity of benzo[*c*]phenanthridine alkaloids has been studied extensively. These alkaloids are known to have antitumor activity through DNA intercalation or interference with apoptotic protein.[18] Compounds **1**–**5**, which contain the benzo[*c*]phenanthridine moiety, were nontoxic against the non-small cell lung (NCI-H460), human breast (MCF-7), and CNS (SF-460) cancer

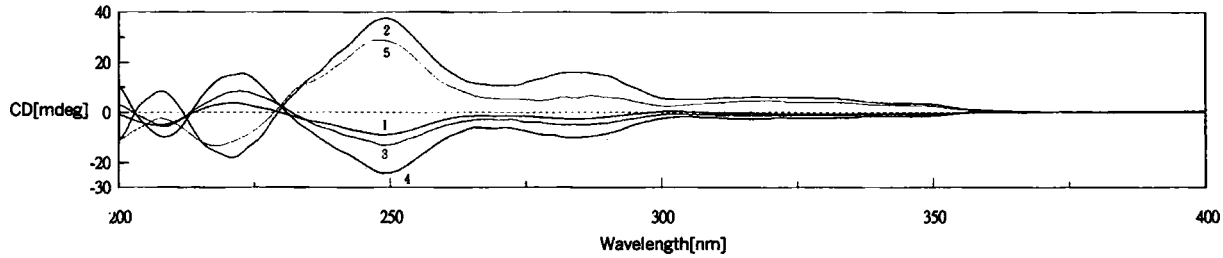

Figure 5. CD spectra of **1**−**5**.

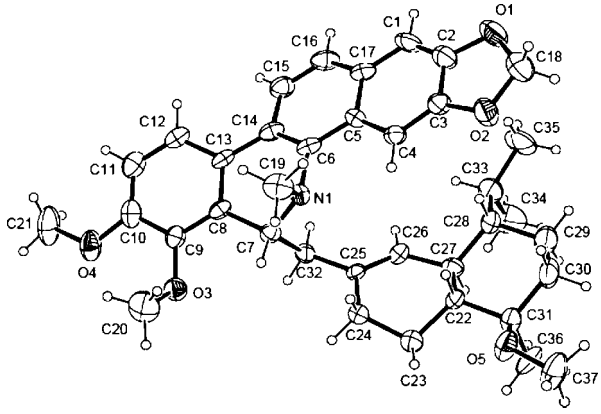

Figure 6. ORTEP drawing of **4** as determined by X-ray analysis.

cell lines. The lack of toxicity could be due to the absence of an iminium ion (–C=N$^+$) in ring B of **1**–**5**, since the carbon of the iminium function represents an effective alkylation site that has been invoked to account for the cytotoxicity of related alkaloids.[19]

Experimental Section

General Experimental Procedures. All melting points were determined on a Yanaco micromelting point apparatus and are uncorrected, optical rotations were measured on a Jasco P-1020 digital polarimeter, UV spectra were obtained on a Jasco UV-240 spectrophotometer in MeOH, and IR spectra (KBr or neat) were taken on a Perkin-Elmer System 2000 FT-IR spectrometer. 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, NOESY, TOCSY, HSQC, HMBC) NMR spectra using $CDCl_3$ or C_6D_6 as solvent were recorded on Varian INOVA-500 (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) spectrometers. Chemical shifts were internally referenced to the solvent signals in $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.0) or C_6D_6 (^{1}H, δ 7.16; ^{13}C, δ 128.5), with TMS as the internal standard. Low-resolution ESIMS spectra were obtained on an API 3000 (Applied Biosystems); high-resolution ESIMS spectra, on a Bruker Daltonics APEX II 30e spectrometer. Low-resolution EIMS spectra were recorded on a Quattro GC/MS spectrometer having a direct inlet system. Silica gel (70–230, 230–400 mesh) (Merck) was used for column chromatography, and silica gel 60 F-254 (Merck) was used for TLC and PTLC.

Plant Material. The stem bark of *Z. nitidum* was collected in Laiyi, Pingtung County, Taiwan, in June 2006, and a voucher specimen (Chen 5645) was deposited in the Herbarium of the Faculty of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. Dried stem bark (10.6 kg) was sliced into chips, extracted with cold MeOH, and concentrated under reduced pressure. The MeOH extract (500 g) was partitioned between $CHCl_3$−H_2O (1:1) to provide $CHCl_3$- (220 g) and H_2O-soluble fractions. The $CHCl_3$-soluble fraction was partitioned between *n*-hexane and 90% MeOH to give *n*-hexane- (fraction A, 90 g) and 90% MeOH-soluble fractions (fraction B, 48 g). The H_2O-soluble fraction was partitioned with *n*-BuOH to give *n*-BuOH- (fraction C, 227 g) and H_2O-soluble (fraction D, 126 g) fractions. The *n*-hexane-soluble fraction (90 g) was chromatographed over silica gel (2000 g) eluting with *n*-hexane, enriched with EtOAc and acetone, to furnish 13 fractions: A1 (500 mL, *n*-hexane), A2 (10 L, *n*-hexane–EtOAc, 30:1), A3 (4 L, *n*-hexane–EtOAc, 7:1), A4 (1 L, *n*-hexane–EtOAc, 5:1), A5−A9 (each 1 L, *n*-hexane–EtOAc, 5:1), A10–12 (each 3 L, *n*-hexane–EtOAc, 1:1), and A13 (2 L, MeOH). Fraction A5 (2.6 g) was rechromatographed over silica gel (500 g) eluting with *n*-hexane−EtOAc (40:1–1:1) to obtain 30 fractions (each 150 mL, fractions A5-1–A5-30). Fraction A5-8 (50 mg) was chromatographed over Sephadex LH-20 (50 g) eluting with MeOH to obtain seven fractions (each 10 mL, fractions A5-8-1–A5-8-7). Fraction A5-8-5 was separated by PTLC (*n*-hexane−acetone, 100:1) to afford **3** (3.7 mg). Fraction A5-9 (114 mg) was chromatographed over silica gel eluting with *n*-hexane–EtOAc (10:1) to obtain **5** (10.0 mg). Fraction A6 (17.7 g) was chromatographed over silica gel (500 g) eluting with *n*-hexane−EtOAc (20:1–1:1) to obtain 27 fractions (each 150 mL). Fraction A6-13 (1.4 g) was chromatographed over silica gel (50 g) eluting with *n*-hexane−EtOAc, 10:1, to obtain 10 fractions (each 50 mL). Fraction A6-13-8 was crystallized from MeOH to afford **1** (4.7 mg). Fraction A6-14 (1.5 g) was chromatographed over silica gel (50 g) eluting with *n*-hexane−EtOAc, 8:1, to obtain 10 fractions (each 50 mL, fractions A6-14-1–A6-14-10). Fraction A6-14-2 was filtered, then the crude crystals were recrystallized from MeOH to afford **4** (5.6 mg). Fraction A6-16 (200 mg) was chromatographed over Sephadex LH-20 (50 g) eluting with MeOH to obtain **2** (5.6 mg).

Zanthomuurolanine (1): colorless needles; mp 198–199 °C; $[\alpha]^{20}_D$ –51.3 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 229 (3.90), 284 (4.53), 322 (sh) (4.05) nm; IR (KBr) ν_{max} 1041, 947 cm^{-1} (OCH_2O); CD (*c* 0.23, MeOH) $[\theta]_{284}$ –2609, $[\theta]_{249}$ –9031, $[\theta]_{221}$ +3721; ^{1}H NMR (C_6D_6, 500 MHz) see Table 1; ^{13}C NMR (C_6D_6, 125 MHz) see Table 2; ESIMS *m*/*z* 584 [M + H]$^+$; HRESIMS *m*/*z* 584.3375 (calcd for $C_{37}H_{46}NO_5$, 584.3376).

***epi*-Zanthomuurolanine (2):** colorless oil; $[\alpha]^{20}_D$ +86.9 (*c* 0.22, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 227 (4.37), 283 (4.39), 324 (sh) (3.96); IR (neat) ν_{max} 1037, 942 cm^{-1} (OCH_2O); CD (*c* 0.36, MeOH) $[\theta]_{287}$ +6626, $[\theta]_{248}$ +28 931, $[\theta]_{218}$ –13 367; ^{1}H NMR (C_6D_6, 500 MHz) see Table 1; ^{13}C NMR (C_6D_6, 125 MHz) see Table 2; ESIMS *m*/*z* 584 [M + H]$^+$; HRESIMS *m*/*z* 584.3378 (calcd for $C_{37}H_{46}NO_5$, 584.3376).

Zanthocadinanine A (3): colorless needles; mp 190–191 °C; $[\alpha]^{20}_D$ –71.5 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ) 229 (3.90), 284 (4.20), 320 (sh) (3.10); IR (KBr) ν_{max} 1040, 946 cm^{-1} (OCH_2O); CD (*c* 0.42, MeOH) $[\theta]_{286}$ –5063, $[\theta]_{249}$ –13 149, $[\theta]_{222}$ +8377; ^{1}H NMR (C_6D_6, 500 MHz) see Table 1; ^{13}C NMR (C_6D_6, 125 MHz) see Table 2; FABMS *m*/*z* 584 [M + H]$^+$; HRFABMS *m*/*z* 584.3375 (calcd for $C_{37}H_{46}NO_5$, 584.3376).

Zanthocadinanine B (4): colorless needles; mp 160–161 °C; $[\alpha]^{20}_D$ –52.5 (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ) 229 (4.67), 284 (4.81), 322 (sh) (4.33); IR (KBr) ν_{max} 1041, 947 cm^{-1} (OCH_2O); CD (*c* 0.23, MeOH) $[\theta]_{283}$ –10 197, $[\theta]_{249}$ –24 315, $[\theta]_{223}$ +15 446; ^{1}H NMR (C_6D_6, 500 MHz) see Table 1; ^{13}C NMR (C_6D_6, 125 MHz) see Table 2; ESIMS *m*/*z* 584 [M + H]$^+$; HRESIMS *m*/*z* 584.3375 (calcd for $C_{37}H_{46}NO_5$, 584.3376).

***epi*-Zanthocadinanine B (5):** colorless needles; mp 162–163 °C; $[\alpha]^{20}_D$ +108.1 (*c* 0.87, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ) 228 (4.73), 283 (4.75), 322 (sh) (4.32); IR (KBr) ν_{max} 1041, 947 cm^{-1} (OCH_2O); CD (*c* 0.84, MeOH) $[\theta]_{283}$ +16 032, $[\theta]_{249}$ +37 587, $[\theta]_{221}$ –18 129; ^{1}H NMR (C_6D_6, 500 MHz) see Table 1; ^{13}C NMR (C_6D_6, 125 MHz) see Table 2; ESIMS *m*/*z* 584 [M + H]$^+$; HRESIMS *m*/*z* 584.3374 (calcd for $C_{37}H_{46}NO_5$, 584.3376).

Biological Assay. MCF-7 (human breast adenocarcinoma), NCI-H460 (non-small-cell lung cancer), and SF-268 (glioblastoma) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and nonessential amino acid (Life Technolo-

gies, Inc.) and maintained at 37 °C in a humidified incubator with an atmosphere of 5% CO_2. Human cancer cells were seeded in 96-well microtiter plates in 100 μL of culture medium at cell number/well of 6500, 2500, and 7500 for MCF-7, NCI-H460, and SF-268, respectively. After an overnight adaptation period, the cells were treated with at least eight different concentrations of test compounds in a CO_2 incubator for 72 h. The number of viable cells was estimated using the 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazoyl)-3-(4-sulfophenyl) tetrazolium salt (MTS) reduction assay,[20] and the experiment was performed with the manufacturer's recommendations (Promega, Madison, WI). DMSO 0.1% (final concentration) was used as vehicle control. Results were expressed as a percentage of DMSO control. The results of these assays were used to obtain the dose–response curves from which IC_{50} values were determined. The values represent averages of three independent experiments, each with duplicate samples. The clinically applied anticancer agent actinomycin D was used as the reference compound. A value of $IC_{50} \leq 4$ μgmL^{-1} is considered to be indicative of significant cytotoxicity. Compounds **1**–**5** were all inactive at concentrations up to 29.2 μg mL^{-1}.

X-ray Crystallographic Study of Zanthomuurolanine (1): colorless crystals of **1** were obtained by recrystallization from acetone. The crystal (0.8 × 0.6 × 0.5 mm) belonged to the orthorhombic system, with the formula $C_{37}H_{45}NO_5$ ($M_r = 583.74$), space group $P2_12_12_1$ with $a = 6.415(3)$ Å, $b = 19.316(7)$ Å, $c = 26.196(8)$ Å; $\alpha = \beta = \gamma = 90°$; $V = 3246(2)$ Å^3; $Z = 4$; and $\rho_{calcd} = 1.194$ mgm^{-3}. A total of 4150 reflections were collected to a maximum 2θ value of 52° by using the ω scan technique at 298(2) K. The data were solved using the direct method, and the structure was refined by full-matrix least-squares procedure. All non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. The refinement converged to the final $R = 0.0426$, $wR = 0.1003$ for 2789 observed reflections ($I > 2\sigma(I)$, $2\theta = 52°$) and 395 variables.[21]

X-ray Crystallographic Study of Zanthocadinanine B (4). Colorless crystals of **4** were obtained by crystallization from acetone. The crystal (0.6 × 0.3 × 0.1 mm) belonged to the monoclinic system, with formula $C_{37}H_{45}NO_5$ ($M_r = 583.74$), space group $P2_1$ with $a = 6.959(2)$ Å, $b = 12.409(3)$ Å, $c = 18.863(4)$ Å; $\alpha = \gamma = 90°$, $\beta = 90.00(2)°$; $V = 1628.8(7)$ Å^3; $Z = 2$; and $\rho_{calcd} = 1.190$ mg m^{-3}. A total of 4161 reflections were collected to a maximum 2θ value of 52° by using the ω scan technique at 298(2) K. The data were solved using the direct method, and the structure was refined by full-matrix least-squares procedure. All non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. The refinement converged to the final $R = 0.0466$, $wR = 0.1113$ for 1565 observed reflections ($I > 2\sigma(I)$, $2\theta = 52°$) and 395 variables.[21]

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Supporting Information Available: CIF data of zanthomuurolanine (**1**) and zanthocadinanine B (**4**). This material is available free of charge on the Internet at http://pubs.acs.org.

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(21) CCDC 659324 contains the supplementary crystallographic data for compound **1** and CCDC 664140 for compound **4**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.ac.uk/data_request/cif.

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