Cytotoxic Bisbenzylisoquinoline Alkaloids from the Roots of *Cyclea racemosa*

Jian-Zhong Wang, Qiao-Hong Chen, and Feng-Peng Wang*

Department of Chemistry of Medicinal Natural Products, West China College of Pharmacy, Sichuan University, No. 17, Duan 3, Renmin Nan Road, Chengdu 610041, People's Republic of China

Received March 17, 2010

Six new bisbenzylisoquinoline alkaloids, racemosidines A−C (**1**−**3**) and racemosinines A−C (**4**−**6**), and four known compounds were isolated from the roots of *Cyclea racemosa*. Compound **1** is the first bisbenzylisoquinoline alkaloid reported that has diphenyl ether bridges at C-11/C-7′ and C-8/C-12′ and a benzyl−phenyl ether bridge at C-7/C-11′. Structures and absolute configurations of **1**−**6** were established by interpretation of spectroscopic data and confirmed by X-ray crystallographic analysis of representative compounds. Compounds **1**−**3** exhibited significant cytotoxicity against HCT-8 and BEL-7402 tumor cells, and compound **1** was also cytotoxic against A2780 tumor cells.

Bisbenzylisoquinoline alkaloids are very important components of plants of the Menispermaceae family.[1−5] The bisbenzylisoquinoline alkaloids have various pharmacological activities, including cytotoxic activity toward some cancer cell lines and antitumor effects.[6−10] In our search for new natural products with cytotoxic activity, we examined the constituents of roots of *Cyclea racemosa* Oliv. f. *emeiensis* Lo et. S. Y. Zhao (Menispermaceae). This species grows on Emmei Mountain, People's Republic of China, and the dried roots are used traditionally in China for treatment of gastric ulcers and tooth pain.[11] The present paper deals with the chemical and biological investigation of the roots of *C. racemosa* Oliv. f. *emeiensis*. The chemical investigation led to the isolation of six new bisbenzylisoquinoline alkaloids, named racemosidines A−C (**1**−**3**) and racemosinines A−C (**4**−**6**), and four known compounds, (−)-curine,[12] α-cyclanoline,[13] 7-*O*-methylhayatidine,[14] and stepononine.[13] Herein, we report the structure determination of these new alkaloids and their in vitro cytotoxic activity against a small panel of tumor cell lines.

Results and Discussion

Roots of *C. racemosa* were extracted with 95% ethanol, and the dried extract was partitioned between ethyl acetate and water containing HCl (pH 3). The aqueous layer was then basified and extracted with chloroform. The dried chloroform extract was subjected repeatedly to column chromatography (CC) to afford the new bisbenzylisoquinoline alkaloids **1**−**6** and four known compounds.

Racemosidine A (**1**), colorless needles, gave a molecular formula of $C_{37}H_{38}N_2O_6$ with 20 degrees of unsaturation, as determined by HRESIMS at *m/z* 607.2786 $[M + H]^+$ (calcd 607.2803). IR absorptions at 3441, and 1611 and 1503 cm^{-1} indicated the presence of OH groups and aromatic rings. The ^{13}C NMR spectrum, along with the HMQC and DEPT data (see Table 1), displayed 37 carbon resonances, assignable to 15 aromatic quaternary carbons, nine aromatic methines, two aliphatic methines, seven aliphatic methylenes [one oxymethylene (δ_H 4.93, 1H, d, J = 14.2 Hz; δ_H 5.18, 1H, d, J = 14.2 Hz; δ_C 73.1)], two *N*-methyl groups, and two aromatic OCH_3 groups. These characteristic data, in combination with biogenetic considerations, suggested that **1** was a bisbenzylisoquinoline alkaloid.[1−5] The ESIMS fragmentation peak at *m/z* 309 (Figure 1), due to the fragment after the facile cleavage of the two benzylic bonds, is characteristic of head-to-tail BBI alkaloids.[15,16] The NMR data featured two trisubstituted benzyl moieties (δ_H 5.76, 1H, d, J = 1.6 Hz; δ_H 6.68, 1H, d, J = 8.0 Hz; δ_H 6.96, 1H, dd, J = 8.0, 1.6 Hz and δ_H 6.29, 1H, br s; δ_H 7.29, 1H, d, J = 8.4 Hz; δ_H 7.19, 1H, dd, J = 8.4, 1.2 Hz) (Table 1) and three aromatic proton singlets at δ_H 6.31, 6.58, and 5.36. The correlations between H-10 (δ_H 5.76, d, J = 1.6 Hz) and C-α and between H-10′ (δ_H 6.29, br s) and C-α′ in the HMBC spectrum suggested that the substituents of these two benzyl moieties could be at C-9, C-11, and C-12, and C-9′, C-11′, and C-12′, respectively. These assignments were supported by the NOESY correlations between H-α and H-1 and NCH_3-2 and between H-α′ and H-1′. Three aromatic singlet protons were located at C-5, C-5′, and C-8′ on the basis of the HMBC correlations between the signal at δ_H 6.31 s and C-8a (δ_C 120.0) and C-7 (δ_C 139.6), between the signal at δ_H 6.58 s and C-8a′ (δ_C 127.9), C-7′ (δ_C 142.8), and C-4′ (δ_C 25.5), and between the signal at δ_H 5.36 s and C-6′ (δ_C 147.5), C-4a′ (δ_C 128.4), and C-1′ (δ_C 64.9). The two OCH_3 groups were assigned at C-6 and C-6′, which was corroborated by long-range correlations between the signal at δ_H 3.80 s and C-6 (δ_C 150.1) and between the signal at 3.84 s and C-6′ (δ_C 147.5). Therefore, the two moieties of compound **1** were determined to be a 6,7,8,11,12-pentasubstituted tetrahydrobenzylisoquinoline and a 6′,7′,11′,12′-tetrasubstituted tetrahydrobenzylisoquinoline.

The two moieties accounted for 18 degrees of unsaturation, and the remaining two degrees of unsaturation indicated the existence of three bridges between these two moieties. The presence of a methyleneoxy bridge was indicated by the diagnostic AB system at δ 4.93 and 5.18 (J = 14.2 Hz). This linkage was assigned to C-7 and C-11′ due to the HMBC correlations between the methylene protons and C-7, C-10′, C-11′, and C-12′. NOESY correlations between the methylene protons and C-11 also supported the existence of a 7/11′ bridge. According to the molecular formula and the established two benzylisoquinoline moieties, the other two bridges should be diphenyl ether bridges. It was proposed that compound **1** had a diphenyl ether bridge at C-8/C-12′ according to the existence of the methyleneoxy bridge at C-7/C-11′ and the substituted pattern of two tetrahydrobenzylisoquinoline moieties. This proposal was supported by the NOESY correlation between H-14 and H-1. The NOESY correlation between H-8′ and H-10 suggested the existence of a C-11/C-7′ or C-12/C-7′ bridge. However, the HMBC data did not provide direct evidence for a C-11/C-7′ or C-12/C-7′ ether bridge. The negative optical rotation and circular dichroism information might indicate the absolute configuration of **1** to be 1-*R*, 1′-*R* by comparison with those of known head-to-tail bisbenzylisoquinoline alkaloids.[1−5] Luckily, we obtained **1** as colorless needles from MeOH−H_2O, which were analyzed on a X-ray diffractometer with a mirror Cu Kα (λ = 1.54184 Å) radiation (ω scans, $2\theta_{max}$ = 144.94°). By anomalous dispersion methods with Flack x = 0.10(13), the absolute configuration of **1** was confirmed as 1-*R*, 1′-*R* (Figure 2).[17] The existence of a bridge between C-11 and C-7′ was confirmed as well.

* To whom correspondence should be addressed. Tel/Fax: +86-28-85501368. E-mail: wfp@scu.edu.cn.

10.1021/np1001863
Published on Web 07/01/2010

Table 1. NMR Spectroscopic Data of **1**−**3**[a]

	1		**2**		**3**	
position	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.
1	4.25 m	59.5, CH	4.01 br d (9.6)	59.3, CH	3.89 br d (8.0)	58.3, CH
3	3.05 m, 2.58 m	48.0, CH_2	3.38 m, 2.85 m	44.2, CH_2	3.25 m, 2.77 m	44.2, CH_2
4	2.61 m, 2.33 m	26.8, CH_2	2.94 m, 2.55 m	23.3, CH_2	2.92 m, 2.50 m	23.3, CH_2
4a		131.8, C		129.1, C		130.4, C
5	6.31 s	107.9, CH	6.56 s	108.8, CH	6.59 s	112.2, CH
6		150.1, C		151.7, C		148.2, C
7		139.6, C		140.2, C		138.6, C
8		149.7, C		143.1, C		143.4, C
8a		120.0, C		125.5, C		124.1, C
a	3.13 dd (14.4,2.4)	37.3, CH_2	2.75 m	40.2, CH_2	2.75 m	39.3, CH_2
	3.40 dd (14.4, 5.2)		2.58 m			
9		130.6, C		132.8, C		133.4, C
10	5.76 d (1.6)	124.4, CH	6.38 d (1.6)	121.3, CH	6.26 br s	119.8, CH
11		141.4, C		144.1, C		144.8, C
12		146.2, C		146.0, C		148.7, C
13	6.68 d (8.0)	115.1, CH	6.78 d (8.0)	115.2, CH	6.74 d (8.4)	114.2, CH
14	6.96 dd (8.0, 1.6)	128.3, CH	6.85 dd (8.0, 1.6)	125.8, CH	6.81 br d (8.4)	124.7, CH
2-*N*CH_3	2.63 s	43.0, CH_3	2.27 s	42.5, CH_3	2.15 s	42.5, CH_3
6-OCH_3	3.80 s	55.9, CH_3	3.87 s	55.9, CH_3		
7-OCH_3			3.70 s	60.6, CH_3	3.74 s	60.9, CH_3
12-OCH_3					3.72 s	56.0, CH_3
1′	3.44 dd (8.0, 3.2)	64.9, CH	3.50 dd (10.4, 3.2)	64.6, CH	3.52 dd (8.4, 4.8)	64.2, CH
3′	3.19 m, 2.75 m	46.1, CH_2	3.25 m, 2.75 m	46.8, CH_2	3.30 m, 2.77 m	47.1, CH_2
4′	2.85 m, 2.65 m	25.5, CH_2	2.92 m, 2.85 m	25.8, CH_2	2.90 m, 2.78 m	25.5, CH_2
4a′		128.4, C		129.7, C		128.0, C
5′	6.58 s	111.5, CH	6.63 s	111.8, CH	6.60 s	112.3, CH
6′		147.5, C		148.0, C		148.2, C
7′		142.8, C		143.1, C		143.1, C
8′	5.36 s	116.2, CH	5.88 s	117.9, CH	5.75 s	117.5, CH
8a′		127.9, CH		128.6, CH		127.6, CH
a’	3.29 dd(14.4, 3.2)	39.6, CH_2	3.20 dd (13.2, 4.0)	39.0, CH_2	3.10 dd (13.2, 3.6)	38.2, CH_2
	2.58 m		2.75 m		2.85 m	
9′		133.5, C		131.9, C		132.0, C
10′	6.29 br s	129.6, CH	6.60 dd (8.8, 2.0)	132.3, CH	6.58 dd (8.0, 2.4)	132.1, CH
11′		126.3, C	6.88 dd (8.8, 2.0)	115.0, CH	6.78 dd (8.0, 2.4)	112.3, CH
12′		152.6, C		155.7, C		155.1, C
13′	7.29 d (8.4)	121.3, CH	6.74 dd (8.8, 2.0)	113.3, CH	6.64 dd (8.0, 2.4)	113.7, CH
14′	7.19 dd (8.4, 1.2)	128.6, CH	7.12 dd (8.8, 2.0)	129.9, CH	6.96 br d (8.0, 2.4)	130.5, CH
15′	4.93 d (14.2)	73.1, CH_2				
	5.18 d (14.2)					
2′-*N*CH_3	2.50 s	42.3, CH_3	2.50 s	42.5, CH_3	2.49 s	42.5, CH_3
6′-OCH_3	3.84 s	55.8, CH_3	3.84 s	55.9, CH_3	3.80 s	55.9, CH_3

[a] Data were measured in $CDCl_3$ at 400 MHz for ^1H, 100 MHz for ^{13}C; chemical shifts are expressed in ppm; the spin coupling (*J*) is given in parentheses (Hz).

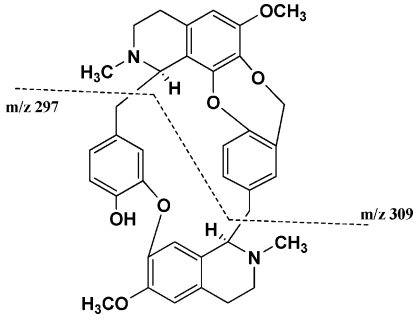


Figure 1. Key MS fragmentation of **1**.

It was reported[12] that the bisbenzylisoquinoline alkaloids like compound **1** possess a strained structure adopting extended and folded conformations for rings ABC and A′B′C′, respectively. In addition, the substituent effects observed on the ^{13}C and ^1H NMR signals could be produced by a gradual rotation of ring C leading C-12 to the external part of the molecule, which adopts a less hindered conformation. The X-ray crystallographic analysis of compound **1** also supported this conformation. Subsequently, H-10, H-8′, and H-1′ of compound **1** were directed to the internal part of the molecule, and anisotropic shielding from the aromatic rings explained their chemical shifts upfield. H-1 and H-10′ were directed toward the external part of the molecule, away from anisotropic shielding of the aromatic rings, resulting in their downfield chemical shifts.

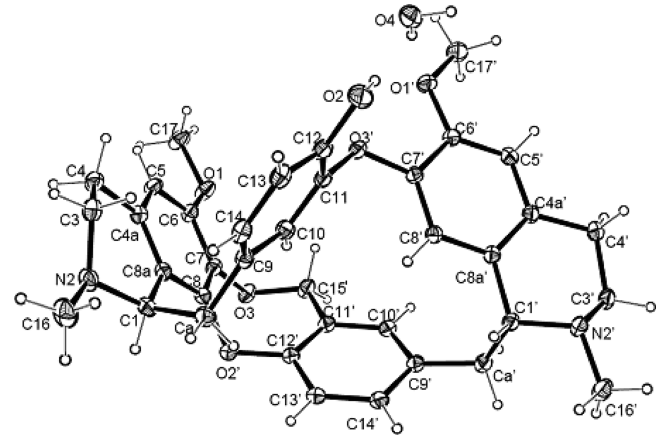
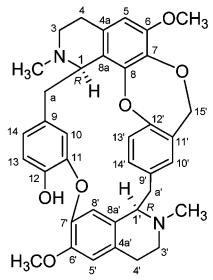


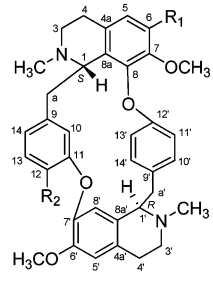
Figure 2. ORTEP diagram for **1**.

To date, four head-to-tail bisbenzylisoquinoline alkaloids (insularine, insulanoline, insularine-2*β*-*N*-oxide, and insularine-2′*β*-*N*-oxide) with two diphenyl ether bridges and a benzyl−phenyl ether

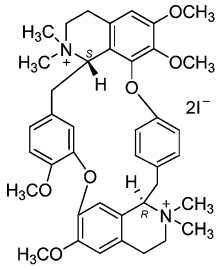
bridge have been isolated from plants.[18] However, racemosidine A (**1**) is the first example that possesses two diphenyl ether bridges at C-11/C-7′ and C-8/C-12′, and a benzyl−phenyl ether bridge at C-7/C-11′.



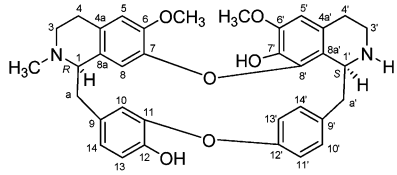
1 racemosidine A



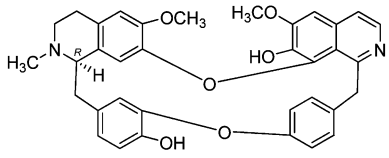
2 racemosidine B $R_1 = OCH_3$ $R_2 = OH$
3 racemosidine C $R_1 = OH$ $R_2 = OCH_3$



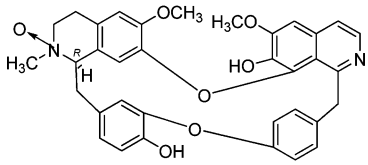
2a



4 racemosinine A



5 racemosinine B



6 racemosinine C

The HRESIMS of racemosidine B (**2**) showed the $[M + H]^+$ at *m/z* 609.2968, corresponding to $C_{37}H_{40}N_2O_6$, indicating 19 degrees of unsaturation. The IR spectrum exhibited absorptions at 3425 cm^{-1} (OH) and 1608 and 1508 cm^{-1} (aromatic rings). All of the available NMR data indicated that **2** was a bisbenzylisoquinoline alkaloid.[1−5] The ^1H NMR spectrum of **2** displayed a *para*-disubstituted phenyl moiety [δ_H 6.60, 1H, dd, $J = 8.8$, 2.0 Hz; 6.88, 1H, dd, $J = 8.8$, 2.0 Hz; 6.74, 1H, dd, $J = 8.8$, 2.0 Hz; 7.12 (1H, dd, $J = 8.8$, 2.0 Hz)], an ABX coupling system of a 1,2,4-trisubstituted phenyl moiety (δ_H 6.38, 1H, d, $J = 1.6$ Hz; 6.78, 1H, d, $J = 8.0$ Hz; 6.85, 1H, dd, $J = 8.0$, 1.6 Hz), three aromatic proton singlets (δ_H 6.56, 6.63, and 5.88), three OCH_3 groups (3.70, 3.84, and 3.87), and two methyl groups each connected to a nitrogen atom (δ_H 2.27, 2.50; which was supported by HMBC correlations between these methyl groups and C-1 or C-1′). These NMR features indicated that the structure of **2** was very similar to that of **1** except that (i) a trisubstituted benzyl moiety of **1** was replaced by a *para*-disubstituted benzyl moiety in **2** and (ii) a methyleneoxy bridge at 7/11′ in **1** was replaced with an OCH_3 group at C-7 in **2**. The difference of 2 mass units in their molecular weights also supported these differences. The additional OCH_3 at C-7 was also supported by the related HMBC correlations. The planar structure of **2** was assigned as shown, which was corroborated by the HMBC correlations. The CD spectrum of **2** was significantly different from that of **1**, but was inconclusive as to its absolute configuration. Introduction of heavy atoms to compound **2** was completed by preparing its methyl iodide derivative **2a**. An X-ray study demonstrated that the absolute configuration of **2a** was 1-*S*, 1′-*R*, with Flack $x = 0.007(9)$ (Figure 3).[17]

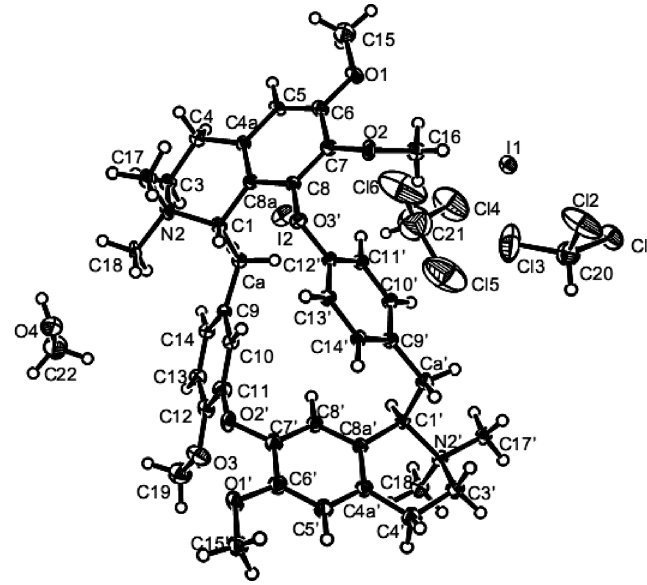


Figure 3. ORTEP diagram for **2a**.

Racemosidine C (**3**) was also a new bisbenzylisoquinoline alkaloid, as shown by the UV and IR absorptions and the NMR data. The molecular formula was $C_{37}H_{40}N_2O_6$ (HRESIMS), suggesting that compounds **3** and **2** were a pair of isomers. Comparisons of the NMR data of **3** (Table 1) with those of **2** indicated that the OCH_3 group (δ_H 3.87) at C-6 in **2** was switched to C-12 in **3**. Further evidence was provided by NOE experiments, which showed enhancement of the signal at δ_H 6.74 (H-13) by irradiation of the resonance at δ_H 3.72 (OCH_3-12). COSY, HMQC, and HMBC correlations confirmed the similarity of **2** and **3** and led to the complete assignment of all the remaining protons of **3** (Table 1). The CD spectrum of **3** was very similar to that of **2**, implying that the absolute configuration of **3** was 1-*S*, 1′-*R*. In addition, methylation of **3** and **2** yielded the same derivative (**2a**).

Racemosinine A (**4**) exhibited NMR features characteristic of the head-to-head and tail-to-tail bisbenzylisoquinoline alkaloids.[1−5] The NMR spectra of **4** were very similar to those of known bisbenzylisoquinoline alkaloid hamoaromoline.[7] The main differences were the absence of two methyl signals at δ_H 3.76 s, 2.43 s and δ_C 55.7, 41.5 due to the OCH_3 group at C-12 and the methyl group at N′ in the NMR spectra of homoaromaline. The mass spectrum of **4** presented a molecular ion at *m/z* 581 $[M + H]^+$ ($C_{35}H_{36}N_2O_6$), smaller than the molecular ion of homoaromoline by 28 mass units. The remaining two OCH_3 groups in **4** were assigned to C-6 and C-6′ according to the HMBC correlations between OCH_3-6 (δ_H 3.60, s) and C-6 (δ_C 148.4) and between OCH_3-6′ (δ_H 3.82, s) and C-6′ (δ_C 146.0). The difference between **4** and homoaromoline should therefore be the presence of an OH group at C-12 and a proton at N′ in **4** instead of the OCH_3 group at C-12 and the methyl group at N′ in homoaromoline. The hypothesis was supported by 2D NMR experiments, and the unambiguous assignments of the ^1H and ^{13}C NMR data of **4** were completed by 2D NMR techniques ($^1H-^1H$ COSY, HMQC, HMBC, and NOESY). Both alkaloids presented very similar CD curves,[19] indicating that the absolute configuration of **4** is 1-*R*, 1′-*S*.

Racemosinine B (**5**) gave a molecular formula of $C_{35}H_{32}N_2O_6$, as determined by HRESIMS and NMR data. The fact that the upper

Table 2. NMR Data of **4**−**6**[a]

	4[b]		**5**[b]		**6**[b]	
position	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.
1	3.57 br s	64.0, CH	3.58 br s	62.0, CH	4.23 br s	76.4, CH
3	2.78 m, 2.46 m	50.1, CH_2	2.66 m, 2.40 m	47.0, CH_2	2.99 m, 2.90 m	58.3, CH_2
4	2.51 m, 2.51 m	27.4, CH_2	2.66 m, 2.20 m	26.0, CH_2	3.22 m, 2.32 m	23.7, CH_2
4a		130.2, C		128.4, C		124.7, C
5	6.40 s	111.4, CH	6.55 s	111.2, CH	6.61 s	110.8, CH
6		148.4, C		147.0, C		148.1, C
7		143.9, C		143.5, C		144.1, C
8	6.64 s	117.2, CH	5.95 s	111.4, CH	5.92 s	111.2, CH
8a		128.5, C		126.5, C		121.6, C
a	3.18 m	38.0, CH_2	2.94 dd (14.4, 3.2), 2.40 m	36.8, CH_2	3.07 dd (16.0, 4.4)	37.1, CH_2
	3.00 (14.4, 3.6)				2.77 (16.0, 4.4)	
9		130.4, C		129.4, C		124.2, C
10	5.69 br s	117.4, CH	4.74 d (2.0)	116.2, CH	4.74 d (1.6)	116.4, CH
11		143.8, C		142.7, C		144.5, C
12		147.0, C		148.1, C		149.0, C
13	6.78 d (8.0)	114.8, CH	6.66 d (8.0)	114.5, CH	6.70 d (8.0)	115.2, CH
13	6.74 dd (8.0, 1.6)	124.1, CH	6.60 dd (8.0, 2.0)	122.3, CH	6.49 dd (8.0, 1.6)	121.9, CH
2-*N*CH_3	2.51 s	43.1, CH_3	2.28 s	42.2, CH_3	3.32 s	57.3, CH_3
6-*O*CH_3	3.60 s	55.3, CH_3	4.05 s	55.4, CH_3	4.06 s	55.5, CH_3
1′	4.54 dd (6.4, 2.0)	53.8, CH		156.1, C		155.7, C
3′	3.33 m, 2.90 m	38.0, CH_2	8.30 d (5.6)	138.7, CH	8.30 d (5.6)	138.7, CH
4′	2.92 m, 2.77 m	27.5, CH_2	7.54 d (5.6)	119.3, CH	7.54 d (5.6)	119.3, CH
4a′		123.7, C		133.3, C		133.2, C
5′	6.37 s	105.1, CH	7.03 s	101.5, CH	7.03 s	101.7, CH
6′		146.0, C		152.2, C		153.0, C
7′		133.7, C		135.4, C		135.2, C
8′		141.0, CH		138.0, CH		138.7, CH
8a′		123.4, C		118.7, C		118.7, C
a′	3.22 m	43.9, CH_2	5.43 d (14.4)	44.0, CH_2	5.46 d (14.4)	43.9, CH_2
	3.04 dd (14.0, 6.8)		4.43 d (14.4)		4.44 d (14.4)	
9′		138.1, C		132.2, C		137.4, C
10′	6.82 dd (8.4, 1.6)	130.9, CH	6.88 dd (8.4, 1.6)	128.2, CH	6.75 br d (8.0)	128.2, CH
11′	6.45 dd (8.4, 1.6)	120.4, CH	6.60 dd (8.4, 2.4)	122.8, CH	6.51 dd (8.0, 2.0)	122.0, CH
12′		153.1, C		152.1, C		151.8, C
13′	6.87 dd (8.4, 1.6)	124.1, CH	6.49 dd (8.0, 2.4)	121.8, CH	6.56 dd (8.0, 2.0)	121.5, CH
14′	7.43 dd (8.4, 1.6)	128.7, CH	7.43 dd (8.0, 1.6)	130.9, CH	7.47 br d (8.0)	130.8, CH
6′-OCH_3	3.82 s	56.1, CH_3	4.05 s	55.8, CH_3	4.04 s	55.8, CH_3

[a] Recorded at 400 MHz for ^{1}H, 100 MHz for ^{13}C, δ in ppm, J in Hz. [b] Recorded in $CDCl_3$−CD_3OD.

part of the dimer was not observed in the mass spectrum suggested that an imine was present in aromatic ring B (or B′). The ^{1}H NMR spectrum displayed a two-proton AB system (δ 8.30 and 7.54, J = 5.6 Hz), typical of the pyridine moiety within a true isoquinoline nucleus. Two doublets at δ 5.43 and 4.43, with a large coupling constant (14.4 Hz), are due to the two geminal protons of the C-α benzylic methylene adjacent to the pyridine ring. The ^{1}H NMR spectrum of **5** was similar to that of stephasubisine.[19] A noticeable difference was the absence of a three-proton singlet at δ 3.88 attributable to the OCH_3 group at C-12 in stephasubimine, implying that the OCH_3 at C-12 in stephasubisine was replaced by an OH in **5**. The CD spectrum of **5** was similar to that of stephasubisine, indicating the absolute configuration of **5** is 1-*R*. The ^{1}H NMR and ^{13}C NMR assignments (Table 2) were completed by interpretation of the 2D NMR spectra.

Racemosinine C (**6**) had the molecular formula $C_{35}H_{32}N_2O_7$, as established by HRESIMS. The ^{1}H NMR spectrum was very similar to that of **5** as far as the aromatic protons and the aromatic substituents were concerned. However, differences were observed with signals for the 2-*N*-methyl group and the adjoining H-1, which were both shifted downfield. This indicated that **6** was the 2-*N*-oxide of **5**, which was further evidenced by the 16 additional mass units in the mass spectrum of **6**. The 2-*N*-methyl singlet at δ 3.32 and the H-1 broad singlet at δ 4.23 are characteristic of a *trans*-relationship between the *N*-oxide oxygen and H-1.[16] This *trans*-relationship was confirmed by a NOESY correlation between the δ 3.32 *N*-methyl singlet and the H-1 signal at δ 4.23.

Five new alkaloids, **1**, **2**, **3**, **5**, and **6**, were selected for in vitro evaluation of their cytotoxicity against HCT-8, Bel-7402, and A2780 cancer cell lines by the MTT assay using paclitaxel as a positive control. As shown in Table 3, racemosidine A (**1**) exhibited significant cytotoxic activity toward the three cell lines, with IC_{50} values of 2.80−6.77 μM. Racemosidines B and C also exhibited cytotoxic activities against HCT-8 and Bel-7402 cancer cell lines.

Table 3. Cytotoxic Activity of Compounds **1**, **2**, **3**, **5**, and **6** against Cultured HCT-8, Bel-7402, and A2780 Cancer Cells

	IC_{50} (μM)		
compound	HCT-8	Bel-7402	A2780
1	2.80	6.09	6.70
2	9.72	4.62	>10
3	6.04	7.53	>10
5	>10	>10	>10
6	>10	>10	>10
paclitaxel	0.67	1.80	0.65

Experimental Section

General Experimental Procedures. Melting points were taken on a micro melting point apparatus (Kexing-X4) without correction. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were measured on a PuXi Tu 1800 PC spectropolarimeter. IR spectra were obtained on a Nicolet FT-IR 200 SXV spectrophotometer. CD spectra were obtained with a JASCO J-810 spectropolarimeter. NMR spectra were recorded on a Varian Unity INOVA 400/45 NMR spectrometer. Mass spectra were carried out on Waters Q-TOF-Premier spectrometers. X-ray crystallographic analysis was carried out on an Oxford Diffraction Gemini S Ultra CCD diffractometer with Cu Kα radiation. Silica gel H (Qindao Marine Chemical Factory, P. R. China) was used for column chromatography. Zones on TLC plates (silica gel G, Qindao Marine Chemical Factory) were detected with the modified Dragendorff's reagent.

Plant Material. Roots of *Cyclea racemosa* Oliv. f. *emeiensis* were collected from Emmei Mountain, Sichuan Province, People's Republic of China, in June 2008. The plant was identified by Professor Guang-Hua Lu, Chengdu University of Traditional Chinese Medicine. A voucher specimen (No. 20071116) was deposited in the West China College of Pharmacy at Sichuan University.

Extraction and Isolation. The air-dried and powdered roots (14.5 kg) of *C. racemosa* were extracted three times with 95% EtOH at room temperature. After removal of the solvent, the crude extract (870 g) was suspended in 3.0 L of H_2O and acidified with 2 N hydrochloric acid to pH 3. The acidic mixture was defatted with ethyl acetate (2000 mL × 2) and then basified with 10% aqueous NH_4OH to pH 10. Extraction of the subsequent mixture with $CHCl_3$ (2000 mL × 3) afforded 63.0 g of crude alkaloids, which were subjected to silica gel CC, eluting with $CHCl_3$−MeOH−diethylamine (99:1:0.5 to 20:10:0.5), to give five major fractions (F1−F5). Fraction F2 (31.0 g) was further chromatographed on a silica gel column employing $CHCl_3$−MeOH (95:5 to 70:30) as eluent to afford six subfractions (A−F). Purification of subfraction A (4.5 g) by silica gel CC, eluting with $CHCl_3$−MeOH (95:5−8:2), yielded 7-*O*-methylhayatidine (350 mg). CC of subfraction B (16.0 g) over silica gel using $CHCl_3$−MeOH (95:5−7:3) as eluent afforded racemosidine A (**1**) (380 mg), racemosidine B (**2**) (1.2 g), racemosidine C (**3**) (200 mg), and (−)-curine (1.3 g). Separation of subfraction D (5.1 g) by silica gel CC using $CHCl_3$−MeOH (9:1−6:4) provided racemosinine A (**4**) (1.0 g), racemosinine B (**5**) (120 mg), and racemosinine C (**6**) (85 mg). CC of subfraction E over silica gel, using MeOH−H_2O (99:1 to 8:2), yielded steponine (80 mg) and α-cyclanoline (56 mg).

Racemosidine A (1): colorless needles (MeOH); mp 273−275 °C; $[\alpha]_D^{20}$ −29.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 279 (3.55) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 213 (−52), 237 (+2.04), 243 (+0.69), 259 (+4.57), 268 (+3.91), 282 (+4.55), 301 (−0.86) nm; IR (KBr) ν_{max} 3441, 2935, 1611, 1503, 1274, 1256, 1217, 1122, 812 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 607, 309, 307, 192; HRESIMS *m/z* 607.2786 $[M + H]^+$ (calcd for $C_{37}H_{39}N_2O_6$, 607.2803).

Racemosidine B (2): amorphous powder; mp 224−228 °C; $[\alpha]_D^{20}$ −252 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.28), 280 (3.91) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 211 (−147), 230 (−21.4), 259 (−2.10), 289 (−6.94), 308 (−0.859) nm; IR (KBr) ν_{max} 3425, 2934, 1608, 1508, 1449, 1275, 1216, 1114, 1008, 838 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 609, 312, 297 192; HRESIMS *m/z* 609.2968 $[M + H]^+$ (calcd for $C_{37}H_{41}N_2O_6$, 609.2965).

Compound 2a: colorless needles ($CHCl_3$−MeOH); mp 242−246 °C; $[\alpha]_D^{20}$ −173 (*c* 0.17, MeOH); ESIMS *m/z* 652 $[M - 2I]^+$, 637, 594, 549, 326.

Racemosidine C (3): white, amorphous powder; mp 173−177 °C. $[\alpha]_D^{20}$ −202 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.55), 278 (4.15) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 212 (−142), 231 (−22.6), 261 (−1.71), 289 (−7.31), 308 (−0.297) nm; IR (KBr) ν_{max} 3424, 2934, 1609, 1508, 1441, 1264, 1219, 1127, 1013, 835 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 609, 314, 312, 309 178; HRESIMS *m/z* 609.2977 $[M + H]^+$ (calcd for $C_{37}H_{41}N_2O_6$, 609.2965).

Racemosinine A (4): white, amorphous powder; mp 216−219 °C; $[\alpha]_D^{20}$ +95 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 283 (3.64) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 197 (−49.7), 222 (+52.7), 238 (+30.1), 268 (+2.81), 289 (+5.60), 307 (−0.37) nm; IR (KBr) ν_{max} 3420, 2936, 1615, 1509, 1450, 1272, 1222, 1117, 1020, 829 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 2; ESIMS *m/z* 581 $[M + H]^+$, 367, 353, 336, 291; HRESIMS *m/z* 581.2644 $[M + H]^+$ (calcd for $C_{35}H_{37}N_2O_6$, 581.2652).

Racemosinine B (5): white, amorphous powder; mp 214−216 °C; $[\alpha]_D^{20}$ +297 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.45), 283 (4.04), 330 (3.94) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 197(−79.9), 211 (−18.1), 232 (+5.30), 249 (+80.7), 261 (+27.5), 294 (+7.35), 327 (−2.86) nm; IR (KBr) ν_{max} 3425, 2928, 1607, 1511, 1432, 1270, 1118, 852 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; ESIMS *m/z* 577 $[M + H]^+$, 471, 413, 342, 291; HRESIMS *m/z* 577.2333 $[M + H]^+$ (calcd for $C_{35}H_{33}N_2O_6$, 577.2339).

Racemosinine C (6): white, amorphous powder; mp 237−240 °C; $[\alpha]_D^{20}$ −137 (*c* 0.17, EtOH); UV (MeOH) λ_{max} (log ε) 239 (4.41), 284 (3.88), 335 (3.77) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 197 (−79.5), 211 (−29.4), 226 (+9.82), 249 (+92.3), 287 (+2.29), 296 (+5.88), 334 (−2.73) nm; IR (KBr) ν_{max} 3418, 2930, 1613, 1514, 1433, 1252, 1121, 853 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; ESIMS *m/z* 593, 437, 301, 192, 151; HRESIMS *m/z* 593.2287 $[M + H]^+$ (calcd for $C_{35}H_{33}N_2O_7$, 593.2288).

Crystallographic data for 1: $C_{37}H_{38}N_2O_6 \cdot H_2O$, monoclinic, space group *P*21, a = 10.9877(2) Å, b = 9.8470(3) Å, c = 15.1430(3) Å, V = 1600.10(7) $Å^3$, Z = 2, d = 1.297 g/cm^3. A crystal of dimensions 0.36 × 0.30 × 0.24 mm was used for measurement on an Oxford Diffraction Gemini S Ultra CCD diffractometer with a mirror Cu Kα (λ = 1.54184 Å) radiation at room temperature (ω scans, $2\theta_{max}$ = 144.94°). The total number of independent reflections measured was 5849, of which 5280 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices (($|F|^2 \geq 2\sigma|F|^2$): R_1 = 0.0308, wR_2 = 0.0810, S = 1.001, $(\Delta/\sigma)_{max}$ = 0.031, $(\Delta\rho)_{min}$ = −0.159 e/$Å^3$, $(\Delta\rho)_{max}$ = 0.350 e/$Å^3$. Flack x = 0.10(13).

Crystallographic data for 2a: $C_{40}H_{48}N_2O_6I_2 \cdot CH_3OH \cdot 2CHCl_3$, orthorhombic, space group $P2_12_12_1$, a = 15.2225(2) Å, b = 15.8415(2) Å, c = 19.7187(3) Å, V = 4755.11(11) $Å^3$, Z = 4, d = 1.645 g/cm^3, crystal dimensions 0.30 × 0.30 × 0.24 mm was used for measurements on an Oxford Diffraction Gemini S Ultra CCD diffractometer with a mirror Cu Ka (λ = 1.54184 Å) radiation at 100 K (ω scans, $2\theta_{max}$ = 139.40°). The total number of independent reflections measured was 8756, of which 8044 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices ($|F|^2 \geq 2\sigma|F|^2$): R_1 = 0.0685, wR_2 = 0.1798, S = 1.067, $(\Delta/\sigma)_{max}$ = 0.181, $(\Delta\rho)_{min}$ = −1.727 e/$Å^3$, $(\Delta\rho)_{max}$ = 2.375 e/$Å^3$. Flack x = 0.007(9).

Cytotoxic Assays. The following human tumor cell lines were used in the assay: HCT-8, Bel-7402, and A2780. All cells were cultured in RPMI-1640 medium supplemented with 5% fetal bovine serum. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 5000 cells per well with compounds added from DMSO-diluted stock. After three days in culture, attached cells were stained with MTT (3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium] bromide). The absorbance at 540 nm was measured using a microplate reader after solubilizing the bound dye. The IC_{50} is the concentration of agent that inhibited cell growth by 50% under the experimental conditions and is the average from triplicate determinations that were reproducible and statistically significant.

Acknowledgment. We thank Prof. Xiao-Guang Chen (Chinese Academy of Medical Sciences and Peking Union Medical College) for the cytotoxic assays and Dr. Shi-Ming Zhou (University of Science and Technology of China) for the X-ray crystallographic analyses.

Supporting Information Available: 1D and 2D NMR spectra of compounds **1**−**6**. These materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Guha, K. P.; Mukherjee, B.; Mukherjee, R. *J. Nat. Prod.* **1979**, *42*, 1–84.
(2) Schiff, P. L., Jr. *J. Nat. Prod.* **1983**, *46*, 1–43.
(3) Schiff, P. L., Jr. *J. Nat. Prod.* **1987**, *50*, 529–599.
(4) Schiff, P. L., Jr. *J. Nat. Prod.* **1991**, *54*, 645–749.
(5) Schiff, P. L., Jr. *J. Nat. Prod.* **1997**, *60*, 934–953.
(6) Chen, Y.; Chen, J. C.; Tseng, S. H. *Int. J. Cancer* **2009**, *124*, 2260–2269.
(7) Lin, L. Z.; Shieh, H. L.; Angerhofer, C. K.; Pezzuto, J. M.; Cordell, G. A.; Xue, L.; Johnson, M. E.; Ruangrungsi, N. *J. Nat. Prod.* **1993**, *56*, 22–29.
(8) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M.; Ruangrungsi, N. *J. Nat. Prod.* **1993**, *56*, 30–38.
(9) Chang, F. R.; Wu, Y. C. *J. Nat. Prod.* **2005**, *68*, 1056–1060.
(10) Angerhofer, C. K.; Guinaudeau, H.; Wongpanich, V.; Pezzuto, J. M.; Cordell, G. A. *J. Nat. Prod.* **1999**, *62*, 59–66.
(11) Zhu, Z. Y.; Feng, Y. X.; He, L. Y.; Wang, Y. C. *Acta Pharm. Sci.* **1983**, *18*, 535–540.
(12) Koike, L.; Marsaioli, A. J.; Reis, F. de A. M. *J. Org. Chem.* **1981**, *46*, 2385–2389.
(13) Tanahashi, T.; Su, Y.; Nagakura, N.; Nayeshiro, H. *Chem. Pharm. Bull.* **2000**, *48*, 370–373.
(14) Baldas, J.; Bick, I. R. C.; Porter, Q. N.; Vernengo, M. J. *J. Chem. Soc., Chem. Commun.* **1971**, 132–133.
(15) Tomita, M.; Kikuchi, T.; Fujitani, K.; Kato, A.; Furukawa, H.; Aoyagi, Y.; Kitano, M.; Ibuka, T. *Tetrahedron Lett.* **1966**, *8*, 857–864.
(16) Guinaudeau, H.; Freyer, A. J.; Shamma, M. *Nat. Prod. Rep.* **1986**, 476–488.

(17) Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers CCDC 778705 and CCDC 778706). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

(18) Lai, S.; Zhao, T. F.; Wang, X. K. *Acta Chim. Sin.* **1993**, *51*, 1133–1138.

(19) Patra, A.; Eyerh, U.; Inaudeau, B.; Shamma, M. *J. Nat. Prod.* **1986**, *49*, 424–427.

NP1001863