Indole Alkaloids from Three Species of the *Ervatamia* Genus: *E. officinalis*, *E. divaricata*, and *E. divaricata* Gouyahua

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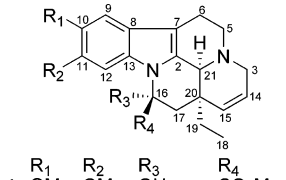
Received July 16, 2006

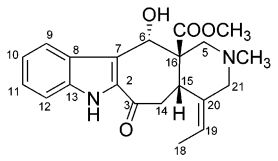
Six new indole alkaloids, 14,15-didehydro-10,11-dimethoxy-16-epivincamine (**1**), 14,15-didehydro-10-hydroxy-11-methoxy-16-epivincamine (**2**), 14,15-didehydro-10,11-dimethoxyvincamine (**3**), 14,15-didehydro-10-hydroxy-11-methoxyvincamine (**4**), 19,20-didehydro-6α-hydroxyervatamine (**5**), and dehydroxyervataminol (**6**), along with 36 known indole alkaloids, were isolated from three species, *E. officinalis*, *E. divaricata*, and *E. divaricata* Gouyahua, of the *Ervatamia* genus. The structures of these alkaloids were characterized on the basis of spectroscopic methods and chemical correlation. The in vitro cytotoxic activities of all the alkaloids except **7**, **18**, **27**, **38**, **40**, and **44** against the tumor cell lines P-388 murine leukemia and A-549 human lung carcinoma were evaluated. Only the dimeric indole alkaloids showed cytotoxic activities.

Plants of the Apocynaceae family are rich sources of structurally diversified indole alkaloids.[1] Some of these alkaloids, such as reserpine, serpentine, and catharanthine, have important pharmaceutical applications.[2] There are about 120 plant species in the genus *Ervatamia* (Apocynaceae family) distributed in the tropical and subtropical areas of Asia and Australia.[2] Fifteen plant species and five varieties of this genus grow in the south of China,[2] and many of them have been administered in traditional Chinese medicine or folklore medicine.[3]

The whole plants of *E. officinalis*, *E. divaricata*, and *E. divaricata* Gouyahua have been applied in China as folklore herbs for the treatment of hypertension and sore throat.[3] Previous studies on *E. officinalis* and *E. divaricata* reported the isolation of more than 30 indole alkaloids and several nonalkaloids,[4] as well as the antitumor activities of some dimeric indole alkaloids.[4b,c,e] Our current studies on *E. officinalis*, *E. divaricata*, and *E. divaricata* Gouyahua have led to the isolation of six new indole alkaloids, 14,15-didehydro-10,11-dimethoxy-16-epivincamine (**1**), 14,15-didehydro-10-hydroxy-11-methoxy-16-epivincamine (**2**), 14,15-didehydro-10,11-dimethoxyvincamine (**3**), 14,15-didehydro-10-hydroxy-11-methoxyvincamine (**4**), 19,20-didehydro-6α-hydroxyervatamine (**5**), and dehydroxyervataminol (**6**), together with 36 known ones. This paper deals with the isolation, structural elucidation, and cytotoxic activities of these alkaloids.



	R_1	R_2	R_3	R_4
1	OMe	OMe	OH	CO_2Me
2	OH	OMe	OH	CO_2Me
3	OMe	OMe	CO_2Me	OH
4	OH	OMe	CO_2Me	OH



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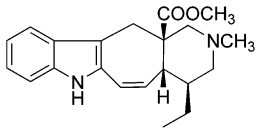

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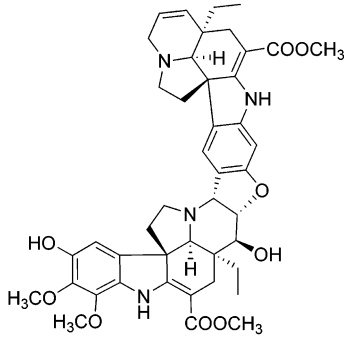

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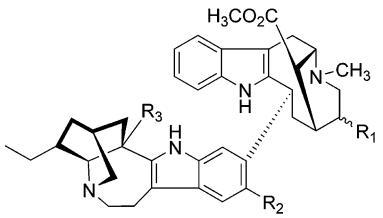

	R_1	R_2	R_3
22	β-Et	OMe	H
23	(*E*)-ethylidene	OMe	H
41	β-Et	OMe	CO_2Me
49	β-Et	H	H

Results and Discussion

14,15-Didehydro-10,11-dimethoxy-16-epivincamine (**1**) was obtained as white, amorphous powders with a specific rotation of $[\alpha]_D^{20}$ +19.0 (*c* 0.30, $CHCl_3$). A molecular formula of $C_{23}H_{28}N_2O_5$ was assigned for **1** on the basis of HREIMS showing an $[M]^+$ at *m*/*z* 412.1971 (calcd 412.1998). Its UV spectrum displayed four maxima at λ_{max} (log ϵ) 303 (3.92), 299 (3.91), 275 (3.86), and 228 (4.42) nm. The IR absorptions at 3427 and 1736 cm^{-1} showed the presence of hydroxy and ester carbonyl groups, respectively. Direct comparison of its NMR data (Tables 1 and 2) with those of 14,-15-didehydro-16-epivincamine[5] indicated that **1** was also an eburna-type indole alkaloid bearing two additional aromatic *O*-methyl groups at δ_H 3.91 (3H, s, 10-OMe) and 3.85 (3H, s, 11-OMe). Two sharp aromatic singlets at δ_H 6.88 (1H, s) and 7.06 (1H, s) in the ^1H NMR spectrum were assignable to H-9 and H-12, respectively, suggesting that the two additional *O*-methyl groups were located at C-10 and C-11, which was further confirmed by the strong NOESY correlations of H-9/10-OMe, 10-OMe/11-OMe, and 11-OMe/H-12. The relative configuration of **1** was established from the NOESY spectrum (Figure 1), in which the correlations between H_2-19 and H-21 indicated their α-cofacial orientation. The CO_2-Me at C-16 was β-configured, as deduced from the NOESY correlation of CO_2*Me*/H-15. The relative configuration of **1** established from the NOESY spectrum was consistent with that of

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10.1021/np060344o CCC: $37.00
Published on Web 01/05/2007

Table 1. ^{1}H NMR Spectroscopic Data for Alkaloids **1**−**6**

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]	**6**[a]
3	3.01 (br s, 2H)	3.01 (m, 2H)	3.11 (ddd, 17.3, 3.8, 2.1) 3.02 (dt, 17.3, 2.4)	3.10 (ddd, 17.4, 3.8, 2.0) 3.00 (dt, 17.4, 2.4)		6.26 (d, 12.1)
5	3.38 (dd, 13.8, 6.9) 3.24 (ddd, 13.8, 11.0, 6.3)	3.35 (dd, 13.9, 6.9) 3.22 (ddd, 13.9, 11.0, 6.3)	3.43 (dd, 13.9, 7.0) 3.33 (ddd, 13.9, 10.6, 6.1)	3.41 (dd, 13.9, 7.2) 3.31 (ddd, 13.9, 10.8, 6.4)	3.34 (d, 12.0) 3.03 (d, 12.0)	3.08[c] 2.23 (d, 10.9)
6	3.07 (m) 2.49 (ddd, 16.0, 6.3, 1.8)	3.04 (m) 2.45 (ddd, 16.1, 6.3, 1.9)	3.05 (m) 2.53 (ddd, 16.1, 6.1, 1.7)	3.03 (m) 2.50 (ddd, 16.1, 6.4, 1.8)	5.72 (s)	3.69 (d, 17.2) 3.48 (dd, 17.2, 1.8)
9	6.88 (s)	6.92 (s)	6.91 (s)	6.96 (s)	7.62 (d, 8.4)	7.56 (dd, 7.8, 0.6)
10					7.15 (m)	7.08 (m)
11					7.34 (m)	7.13 (m)
12	7.06 (s)	7.03 (s)	6.61 (s)	6.57 (s)	7.44 (m)	7.23 (dd, 7.9, 0.6)
14	5.48 (dt, 10.3, 3.1)	5.48 (dt, 10.2, 3.2)	5.60 (ddd, 10.3, 3.8, 2.4)	5.60 (ddd, 10.3, 3.8, 2.4)	4.06 (dd, 16.1, 11.6) 2.32 (dd, 16.1, 0.8)	5.93 (dd, 12.1, 7.4)
15	5.24 (br d, 10.3)	5.24 (br d, 10.2)	5.75 (br d, 10.3)	5.75 (br d, 10.3)	3.77 (d, 11.6)	2.67 (m)
17	2.57 (d, 14.0) 1.98 (d, 14.0)	2.58 (d, 13.9) 2.00 (d, 13.9)	2.38 (d, 14.1) 2.31 (d, 14.1)	2.37 (d, 14.3) 2.31 (d, 14.3)		
18	0.91 (t, 7.6)	0.92 (t, 7.6)	1.00 (t, 7.6)	0.99 (t, 7.6)	1.58 (dd, 6.8, 2.0)	0.91 (t, 7.4)
19	1.76 (dq, 15.0, 7.6) 1.41 (dq, 15.0, 7.6)	1.78 (dq, 15.0, 7.6) 1.42 (dq, 15.0, 7.6)	1.95 (dq, 15.1, 7.6) 1.62 (dq, 15.1, 7.6)	1.95 (dq, 15.1, 7.6) 1.62 (dq, 15.1, 7.6)	5.40 (br q, 6.8)	1.76 (m) 1.27 (m)
20						1.87 (m)
21	3.80 (br s)	3.81 (br s)	4.06 (br s)	4.06 (br s)	3.03 (d, 12.1) 2.90 (br d, 12.1)	3.05[c] 1.76 (m)
10-OMe	3.91 (s)		3.91 (s)			
11-OMe	3.85 (s)	3.85 (s)	3.84 (s)	3.85 (s)		
CO_2Me	3.48 (s)	3.47 (s)	3.87 (s)	3.87 (s)	3.55 (s)	3.40 (s)
NMe					2.36 (s)	2.33 (s)
16-OH	4.24 (br s)		3.86 (s)	3.84 (s)		
NH						7.72 (br s)

[a] Measured in $CDCl_3$. [b]Measured in $CDCl_3/CD_3OD$ (10:1). [c] Overlapping signals.

Table 2. ^{13}C NMR Spectroscopic Data for Alkaloids **1**−**6**

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]	**6**[a]
2	131.2	131.4	130.1	130.1	131.6	131.2
3	43.7	43.6	43.8	43.7	195.2	119.1
5	49.8	49.8	49.6	49.5	57.7	66.0
6	16.7	16.6	16.8	16.6	69.1	28.3
7	106.3	106.2	106.0	105.8	123.2	111.6
8	121.6	122.4	122.0	122.6	126.3	129.2
9	100.1	102.3	100.8	102.8	119.5	118.6
10	145.2	141.1	145.4	141.3	120.9	119.3
11	146.1	143.6	146.5	143.9	126.3	122.0
12	96.9	96.0	94.8	93.7	112.5	110.4
13	131.0	130.8	128.2	128.0	136.3	135.4
14	125.8	125.8	125.7	125.8	43.4	130.9
15	126.6	126.6	128.1	127.9	33.6	47.5
16	84.1	84.1	82.1	82.1	54.0	48.7
17	45.9	45.9	43.6	43.5		
18	8.4	8.3	8.4	8.4	12.3	11.0
19	35.2	35.2	34.8	34.7	121.6	23.8
20	38.5	38.4	36.8	36.7	135.8	40.2
21	57.1	57.1	57.6	57.4	60.6	61.8
10-OMe	56.2		56.4			
11-OMe	56.4	56.4	56.4	56.3		
CO_2Me	172.0	172.0	173.1	173.1	174.3	175.3
CO_2Me	52.6	52.5	54.0	54.0	52.5	51.9
NMe					45.6	46.6

[a] Measured in $CDCl_3$. [b]Measured in $CDCl_3/CD_3OD$ (10:1).

14,15-didehydro-16-epivincamine.[5] The HMQC and HMBC experiments of **1** were also performed to complete the full assignments of ^{1}H and ^{13}C NMR signals. Thus, the structure of alkaloid **1** was elucidated as 14,15-didehydro-10,11-dimethoxy-16-epivincamine.

14,15-Didehydro-10-hydroxy-11-methoxy-16-epivincamine (**2**) showed a molecular ion peak at *m*/*z* 398.1833 in the HREIMS corresponding to the molecular formula $C_{22}H_{26}N_2O_5$ (calcd 398.1842), which was one $-CH_2-$ group less than that of **1**. Its UV spectrum showed the characteristic absorptions of a 10,11-disubstituted indole alkaloid at λ_{max} (log ϵ) 306 (3.81), 301 (3.80), and 228 (4.25) nm with shoulders at 311 (3.77) and 277 (3.71) nm. The ^{1}H and ^{13}C NMR data of **1** and **2** showed high similarity except for the absence of the 10-OMe resonance (δ_H 3.91, δ_C 56.2 for **1**) in **2** (Tables 1 and 2), indicating that **2** was an *O*-demethyl derivative of **1**, which was in good agreement with its molecular formula. The NOESY correlation between H-12 (δ_H 7.03, 1H, s) and 11-OMe (δ_H 3.85, 3H, s) verified that the hydroxyl was located at C-10 (δ_C 141.1); that is, compound **2** was the 10-*O*-demethyl derivative of **1**. The relative configuration of **2** was in accordance with that of **1** on the basis of its NOESY spectrum (Figure S12, Supporting Information). The structure of alkaloid **2** was thereby characterized as 14,15-didehydro-10-hydroxy-11-methoxy-16-epivincamine.

14,15-Didehydro-10,11-dimethoxyvincamine (**3**) had a molecular formula of $C_{23}H_{28}N_2O_5$ as determined by HREIMS ($[M]^+$ at *m*/*z* 412.1998, calcd 412.1998). The IR spectrum displayed absorption bands at 3421 and 1743 cm^{-1} corresponding to hydroxy and ester carbonyl groups, respectively. Comparison of its NMR data (Tables 1 and 2) with those of 14,15-didehydrovincamine[5] revealed that they were analogues, with the two additional aromatic *O*-methyls in **3** resonating at δ_H 3.91 (3H, s) and 3.84 (3H, s). Two sharp aromatic singlets at δ_H 6.91 (1H, s, H-9) and 6.61 (1H, s, H-12) indicated that the two aromatic *O*-methyl groups were located at C-10 and C-11, respectively. The relative configuration of **3** was determined by a combination of the ROESY spectrum and analogous correlation of its NMR data with those of 14,15-didehydrovincamine.[5] The NMR data for the eastern hemispheres of both compounds were identical, suggesting that their relative configurations at C-16, C-20, and C-21 were the same. This was supported by the ROESY spectrum of **3** (Figure 1), in which the ROESY correlation of CO_2*Me*/H-15 was not observed. Therefore, the structure of **3** was assigned as the C-16 epimer of **1**.

14,15-Didehydro-10-hydroxy-11-methoxyvincamine (**4**) exhibited a molecular formula of $C_{22}H_{26}N_2O_5$ as determined by HREIMS ($[M]^+$ at *m*/*z* 398.1839, calcd 398.1842). The ^{1}H and ^{13}C NMR

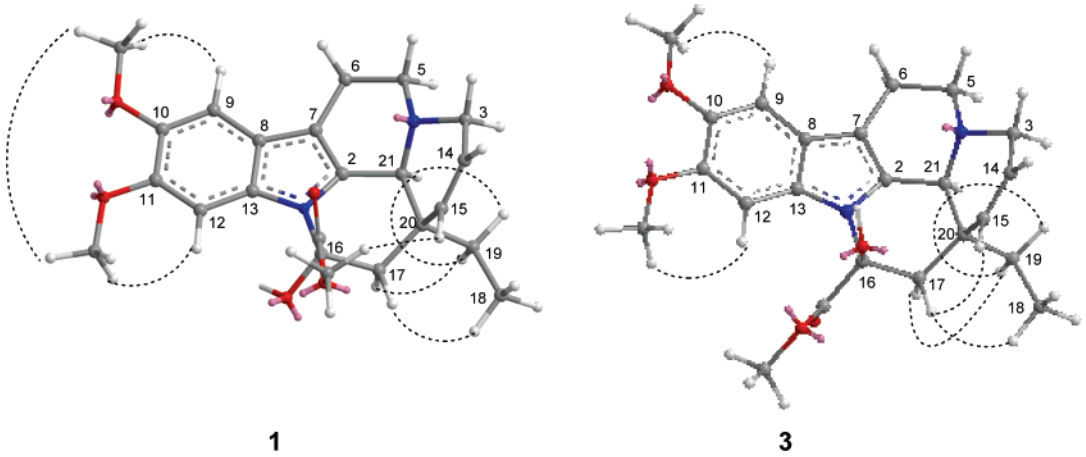


Figure 1. NOESY correlations (dashed lines) of alkaloids **1** and **3**.

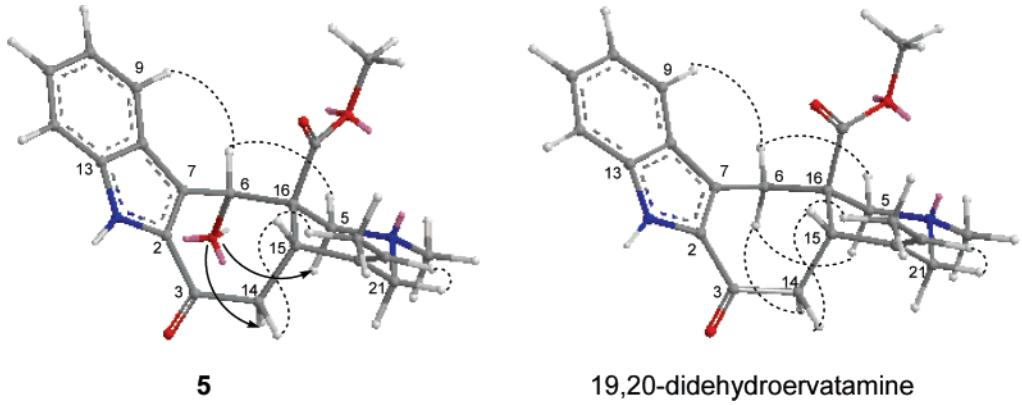


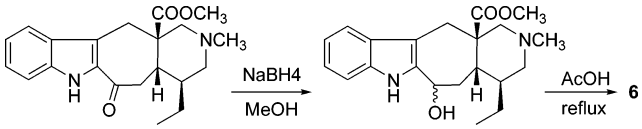
Figure 2. NOESY correlations (dashed lines) of alkaloid **5** and 19,20-didehydroervatamine, and pyridine-induced chemical shift effect (arrow) of alkaloid **5**.

data (Tables 1 and 2) of **4** showed that it also contained the eburna-type indole skeleton, and its structure was closely related to alkaloid **3** and 14,15-didehydrovincamine.[5] Two sharp aromatic singlets at δ_H 6.96 (1H, s, H-9) and 6.57 (1H, s, H-12) were assigned to H-9 and H-12, respectively, indicating that alkaloid **4** was a 10,11-disubstituted eburna-type indole alkaloid. Comparison of its NMR data with those of **3** showed that alkaloid **4** was the 10-*O*-demethyl derivative of **3**. The NOESY correlation between H-12 (δ_H 6.57, 1H, s) and 11-OMe (δ_H 3.85, 3H, s) confirmed that the hydroxyl was located at C-10 (δ_C 141.3). The relative configurations of C-16, C-20, and C-21 were the same as those of **3**, which was supported by a ROESY spectrum (Figure S24, Supporting Information).

19,20-Didehydro-6α-hydroxyervatamine (**5**) was determined by HREIMS to have a molecular formula of $C_{21}H_{24}N_2O_4$ ($[M]^+$ *m*/*z* 368.1737, calcd 368.1736). Its UV spectrum showed the typical absorption maxima at λ_{max} (log ϵ) 312 (4.18) and 235 (4.04) nm of an α-acylindole alkloid. The IR absorptions at 3385−3304, 1720, and 1645 cm^{-1} indicated the presence of a hydroxy (or NH), ester carbonyl, and conjugated ketone carbonyl, respectively. The NMR data of **5** were closely related to the coexisting alkaloid 19,20-didehydroervatamine (**34**),[6] and the only difference was the presence of an additional 6-OH in **5** as judged from the proton resonance at δ_H 5.72 (1H, s, H-6) and carbon at δ_C 69.1 (C-6) (Tables 1 and 2). The C-7 (δ_C 123.2) and C-16 (δ_C 54.0) carbon resonances of **5** were also obviously deshielded as compared with those of 19,20-didehydroervatamine [δ_C 119.7 (C-7) and 49.1 (C-16)]. The molecular formula of **5** showed one oxygen atom more than that of **34**. The 6-OH was α-oriented by comparison of its NOESY spectrum with that of 19,20-didehydroervatamine (**34**) (Figure 2). In the NOESY spectrum of **34**, $H_α$-6 (δ_H 2.89, 1H, d, J = 15.4 Hz) correlated with $H_α$-14 (δ_H 3.10, 1H, dd, J = 15.9, 11.1 Hz), while $H_β$-6 (δ_H 3.63, 1H, d, J = 15.4 Hz) correlated with H-9 (δ_H 7.60, 1H, br d, J = 7.9 Hz). As shown in Figure 2, the conformations of both analogues showed high similarity; the correlation between H-6 (δ_H 5.72) and H-9 (δ_H 7.62, d, J = 8.4 Hz) of **5** therefore suggested a 6α-OH. The pyridine-induced chemical shifts ($\Delta\delta = \delta_{CDCl_3} - \delta_{C_5D_5N}$) of $H_α$-5 (−0.55) and $H_α$-14 (−0.56) were stronger than those of $H_β$-5 (−0.37) and $H_β$-14 (−0.32), respectively, indicating that $H_α$-5 and $H_α$-14 were more deshielded than $H_β$-5 and $H_β$-14 by the anisotropic pyridine molecule coordinating to the hydroxy group. This supported the presence of a 6α-OH in **5** (Figure 2).[7] The NOESY correlation (Figure 2) between H-18 (δ_H 1.58, 3H, dd, J = 6.8, 2.0 Hz) and H-15 (δ_H 3.77, 1H, d, J = 11.6 Hz) revealed an *E*-geometry for the Δ^{19} double bond, which is the same as that of 19,20-didehydroervatamine.[6]

Dehydroxyervataminol (**6**) displayed a molecular ion peak at *m*/*z* 338.1987 (calcd 338.1994) in the HREIMS corresponding to a molecular formula of $C_{21}H_{26}N_2O_2$. Its IR spectrum displayed the presence of NH (3396 cm^{-1}) and ester carbonyl (1730 cm^{-1}) moieties. The spectroscopic data (Tables 1 and 2) of **6** showed high similarity with those of ervatamine (**35**),[8] indicating that **6** was also an ervatamia-type indole alkaloid. The most notable difference between the two alkaloids was the existence of one $\Delta^{3(14)}$ double bond in **6** as determined by the proton resonances at δ_H 6.26 (1H, d, J = 12.1 Hz, H-3) and 5.93 (1H, dd, J = 12.1, 7.4 Hz, H-14) and the carbon resonances at δ_C 119.1 (C-3) and 130.9 (C-14), instead of the C-3 ketone group (δ_C 192.6) and C-14 methylene group (δ_C 44.0) of ervatamine (**35**). In the HMBC spectrum, the correlations from H-15 at δ_H 2.67 (1H, m) to C-3 and C-14 also supported the location of the $\Delta^{3(14)}$ double bond. The structure of alkaloid **6** was finally confirmed by chemical correlation with ervatamine. Thus, ervatamine (**35**) was first reduced with $NaBH_4$, followed by dehydration in glacial AcOH to give compound **6** (Scheme 1 and Experimental Section).

Scheme 1. Chemical Transformation of Ervatamine (**35**) to **6**



The other known alkaloids isolated from the three species of the *Ervatamia* genus were identified by spectroscopic analyses. The four new compounds **1**−**4**, as well as 18 known alkaloids, voaphylline (**7**),[9] 12-methoxyvoaphylline (**8**),[10] (−)-velbanamine (**9**),[11] 20*S*-hydroxy-1,2-dehydropseudoaspidospermidine (**10**),[12] 14,-15-didehydro-16-epivincamine (**11**),[5] voacangine (**12**),[13] ibogaine (**13**),[14] ibogaine hydroxyindolenine (**14**),[15] voacristine (**15**),[13] 19*S*-hydroxyconopharyngine (**16**),[16] 10-demethoxynorvincorine (**17**),[17] picrinine (**18**),[18] rhazinaline (**19**),[19] strictamine (**20**),[20] voachalotine (**21**),[21] 16′-demethoxycarbonyl-19,20-dihydro-20-epivoacamine (**22**),[22] 16′-demethoxycarbonylvoacamine (**23**),[23] and conophyllidine (**24**),[24] were isolated from *E. officinalis* in the current study. In addition, 10 alkaloids, tabernaemontanine (**25**),[25] dregamine (**26**),[25] vobasine (**27**),[25] 16-epiaffinine (**28**),[16] 20-episilicine (**29**),[4a] 6-oxo-16,20-episilicine (**30**),[4a] 16,20-episilicine (**31**),[4a] 6,16-didehydro-20-episilicine (**32**),[4a] methuenine (**33**),[6] and 19,20-didehydroervatamine (**34**),[6] were reported previously. A total of 32 indole alkaloids were isolated by us from *E. officinalis*. From *E. divaricata*, 13 alkaloids, 19,20-didehydro-6α-hydroxyervatamine (**5**), **7**,[9] **12**,[13] **25**,[25] **26**,[25] **34**,[6] ervatamine (**35**),[8] 20-epiervatamine (**36**),[6] tubotaiwine (**37**),[26] (−)-mehranine (**38**),[27] (−)-akuammicine (**39**),[28] ervadivaricatine A (**40**),[4e] and ervadivaricatine B (**41**),[4e] were isolated. From *E. divaricata* Gouyahua, 15 alkaloids, dehydroxyervataminol (**6**), **7**,[9] **12**,[13] **25**,[25] **26**,[25] **36**,[6] **37**,[26] (−)-apparicine (pericalline, **42**),[13] voacangine hydroxyindolenine (**43**),[29] coronaridine hydroxyindolenine (**44**),[29] (−)-coronaridine (**45**),[13] 14*β*,15*β*-epoxytabersonine (**46**),[30] tabersonine (**47**),[31] ibogamine (**48**),[32] and 19,20-dihydrotabernamine (**49**),[33] were isolated.

The isolation of these alkaloids from the three species is summarized in Table 3. Alkaloids **1**−**4**, **8**−**11**, **13**−**24**, and **27**−**33** were isolated only from *E. officinalis*; alkaloids **5**, **35**, and **38**−**41** were obtained only from *E. divaricata*; and alkaloids **6** and **42**−**49** were obtained only from *E. divaricata* Gouyahua. Alkaloid **34** was isolated from both *E. officinalis* and *E. divaricata*, and alkaloids **36** and **37** were obtained from both *E. divaricata* and *E. divaricata* Gouyahua. Alkaloids **7**, **12**, **25**, and **26** were ubiquitous in the three species.

Cytotoxicity Evaluation of the Isolates. The in vitro cytotoxic activities of all the alkaloids except **7**, **18**, **27**, **38**, **40**, and **44** against the tumor cell lines P-388 murine leukemia and A-549 human lung carcinoma were evaluated by using the MTT[34] and SRB[35] methods, respectively, with pseudolaric acid B[36] as positive control. Only the dimeric indole alkaloids showed inhibitory activities (Table 4).

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Hitachi U-2010 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. NMR spectra were measured on a Varian Mercury plus 400 and a Bruker AM-400 instrument. EIMS and HREIMS (70 eV) were done on a Finnigan MAT 95 mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Reagents Company, Ltd.). Silica gel (20−40 μm, Qingdao Haiyang Chemical Company, Ltd.) was used for column chromatography (CC), and precoated silica gel GF254 plates (Yantai Huiyou Silica Gel Exploitation Company, Ltd.) were used for TLC. Amino silica gel (20−45 μm, Fuji Silysia Chemical Ltd.), RP-18 silica gel (150−200 mesh, Merck), and MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Industries, Ltd.) were also used for CC.

Plant Material. The whole plants of *E. officinalis* were collected in November 2003 from Hainan Province, People's Republic of China, and were identified by Prof. Shi-Man Huang, Research Center of Biology, Hainan University, People's Republic of China. The stems and leaves of *E. divaricata* and *E. divaricata* Gouyahua were harvested in May 2005 from Xishuangbanna area of Yunnan Province, People's Republic of China, and were identified by Prof. You-Kai Xu, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, People's Republic of China. Voucher specimens of the three plants *E. officinalis*, *E. divaricata*, and *E. divaricata* Gouyahua were deposited in Shanghai Institute of Materia Medica with accession numbers EO-2003-1Y, ED-2005-1Y, and EDG-2005-1Y, respectively.

Extraction and Isolation. The powder of *E. officinalis* (8.0 kg) was percolated (3 × 5 days) with 90% EtOH at rt to give 400 g of crude extract, which was then suspended in 2.0 L of acidified water (adjusted with 2.0 mol/L H_2SO_4 to pH 1−2). After removal of the nonalkaloids by extracting with Et_2O (1.0 L × 4), the acidic aqueous phase was basified with Na_2CO_3 to pH 8−9 and partitioned with $CHCl_3$ (1.0 L × 4) to afford the crude alkaloids (24.2 g).

The crude alkaloids were subjected to silica gel CC eluted with petroleum ether/Et_2O/MeOH (5:1:0.3 to 1:2:0.6) to give four fractions (F1−F4). F1 (6.01 g) was chromatographed on a silica gel column ($CHCl_3$/MeOH, 200:1 to 5:1) to give six subfractions, F1a−F1f. F1a (0.80 g) was subjected to a silica gel CC eluted with petroleum ether/EtOAc/Et_2NH, 10:1:0.3, to give two major components, each of which was then purified by RP-18 silica gel CC eluted with MeOH/H_2O, 4:1, to afford alkaloids **8** (10 mg) and **12** (10 mg), respectively. F1b (32 mg) was subjected to CC ((1) silica gel, petroleum ether/EtOAc/Et_2-NH, 10:1:0.3; (2) amino silica gel, cyclohexane/EtOAc, 5:1) to obtain **7** (5 mg). F1d (0.88 g) was separated on silica gel CC eluted with petroleum ether/EtOAc/Et_2NH, 10:1:0.3, to give two major fractions, which were purified by preparative TLC ($CHCl_3$/MeOH, 30:1) to yield **13** (15 mg) and **14** (7 mg), respectively. F1f (0.39 g) was further purified on preparative TLC ($CHCl_3$/MeOH, 20:1) to obtain **9** (18 mg). F2 (1.79 g) was chromatographed on a silica gel column (petroleum ether/EtOAc/Et_2NH, 12:1:0.3 to 3:1:0.3) to afford five subfractions (F2a−F2e). F2a (250 mg) was purified on an RP-18 silica gel column eluted with MeOH to afford **22** (43 mg). F2d (69 mg) was first subjected to CC (RP-18 silica gel, MeOH/H_2O, 3:1) to collect the major alkaloid, which was then purified on preparative TLC ($CHCl_3$/MeOH, 80:1) to give **15** (11 mg). F2e (90 mg) was separated on a silica gel column ($CHCl_3$/MeOH, 20:1) to yield **11** (7 mg) and **17** (8 mg). F3 (3.10 g) was chromatographed on a silica gel column (petroleum ether/EtOAc/Et_2NH, 10:1:0.3 to 1:1:0.3) to afford eight subfracions (F3a−F3h). F3b (0.26 g), F3c (60 mg), F3d (78 mg), and F3h (0.52 g) were subjected to CC of RP-18 silica gel (MeOH/H_2O, 1:0, 7:3, 7:3, and 3:2, respectively) to obtain **23** (24 mg), **10** (18 mg), **3** (14 mg), and **18** (44 mg), respectively. F3e (50 mg) was separated on a column of RP-18 silica gel eluted with MeOH/H_2O, 7:3, to obtain a major alkaloid, which was further purified on silica gel CC ($CHCl_3$/MeOH, 40:1) to give **20** (7 mg). F3f (0.20 g) was extensively subjected to CC (first using silica gel eluted with $CHCl_3$/MeOH, 100:1, and then RP-18 silica gel eluted with MeOH/H_2O, 3:1) to afford **21** (10 mg) and **16** (32 mg). F3g (0.52 g) was separated on an RP-18 silica gel column (MeOH/H_2O, 3:1, and MeOH) to yield **1** (20 mg) and **24** (22 mg). F4 (8.13 g) was chromatographed on a silica gel column (petroleum ether/EtOAc/Et_2NH, 10:1:0.3 to 1:1:0.3) to give six subfractions (F4a−F4f). F4b (72 mg) was subjected to CC (first silica gel eluted with $CHCl_3$/MeOH, 30:1, and then RP-18 silica gel eluted with MeOH/H_2O, 3:2) to afford **4** (20 mg). F4d (60 mg) was purified by CC of RP-18 silica gel (MeOH/H_2O, 3:2) to obtain **2** (26 mg). F4e (12 mg) was subjected to an RP-18 silica gel column (MeOH/H_2O, 7:3) to yield **19** (4 mg).

The powder of *E. divaricata* (1.5 kg) was treated in a manner similar to that of *E. officinalis* to afford 5.91 g of crude alkaloids, which was chromatographed on a silica gel column eluted with petroleum ether/EtOAc/Et_2NH (30:1:0.3 to 2:1:0.3) to give five fractions (F1−F5). F1 (0.12 g) was subjected to CC (RP-18 silica gel, MeOH/H_2O, 4:1, and MeOH) to provide **12** (30 mg), **38** (3 mg), and **40** (3 mg). F2 (70 mg) was purified on an RP-18 silica gel column (MeOH/H_2O, 4:1) to give **7** (10 mg). F3 (0.32 g) was subjected to an RP-18 silica gel column (MeOH/H_2O, 3:1, 4:1, and MeOH) and repeated silica gel columns ($CHCl_3$/MeOH) to yield **35** (26 mg), **36** (2 mg), and **41** (15 mg). F4 (0.89 g) was subjected to CC (RP-18 silica gel, MeOH/H_2O, 7:3 to 9:1) and then repeated silica gel columns ($CHCl_3$/MeOH) to afford **34** (97 mg), **25** (51 mg), **26** (83 mg), **39** (20 mg), and **37** (10 mg). F5 (0.39 g) was purified on preparative TLC ($CHCl_3$/MeOH, 10:1) to provide **5** (13 mg).

Table 3. Isolation of the Alkaloids from the Three *Ervatamia* Species

	1−**4**, **8**−**11**, **13**−**24**, **27**−**33**	**5**, **35**, **38**−**41**	**6**, **42**−**49**	**34**	**36**, **37**	**7**, **12**, **25**, **26**
E. officinalis	+			+		+
E. divaricata		+		+	+	+
E. divaricata Gouyahua			+		+	+

Table 4. Inhibitory Activities of the Dimeric Indole Alkaloids to P-388 and A-549 Cell Lines

	IC_{50} (μM)	
	P-388	A-549
22	0.84	1.10
23	0.11	0.52
24	0.025	0.044
41	0.36	0.43
49	0.43	0.26
PAB[a]	3.70	0.30

[a] Pseudolaric acid B was used as positive control.

The sample of *E. divaricata* Gouyahua (1.5 kg) was treated via the same procedures as *E. officinalis* to obtain 4.39 g of crude alkaloids, which was subjected to a CC of silica gel eluted with petroleum ether/EtOAc/Et_2NH (60:1:0.3 to 5:1:0.3) to afford five fractions (F1−F5). F1 (0.29 g) was separated on CC of RP-18 silica gel (MeOH/H_2O, 7:3 then 3:1) to provide **46** (10 mg), **44** (13 mg), **45** (86 mg), and **48** (17 mg). F2 (0.24 g) was extensively separated on CC and preparative TLC ($CHCl_3$/MeOH) to give **42** (126 mg), **6** (4 mg), **43** (14 mg), and **7** (4 mg). F3 (90 mg) was chromatographed on an RP-18 silica gel column (MeOH/H_2O, 7:3 and 3:1) to yield **36** (6 mg) and **25** (41 mg). F4 (0.31 g) was extensively chromatographed on CC (RP-18 silica gel eluted with MeOH/H_2O, 3:1 to 9:1; and silica gel eluted with $CHCl_3$/MeOH, 40:1, 50:1, and 20:1, respectively) to give **26** (11 mg), **37** (20 mg), and **49** (4 mg). F5 (0.91 g) was separated on preparative TLC ($CHCl_3$/MeOH, 40:1) to yield **47** (3 mg) and **12** (16 mg).

Chemical Transformation of Ervatamine (35) to Dehydroxyervataminol (6). To a stirred solution of **35** (10.1 mg) in MeOH (1 mL) was slowly added 3.0 mg of $NaBH_4$. The reaction mixture was kept at room temperature for 1 h, and then diluted with 10 mL of H_2O and extracted with CH_2Cl_2 (3.0 mL × 3). The organic phase was washed with H_2O (5.0 mL × 3) and evaporated to give a residue, which was then dissolved in 1 mL of glacial HOAc and refluxed for 2 h.[37] The solution was diluted with 5 mL of H_2O and basified with saturated $NaHCO_3$. After workup, the crude product was purified on preparative TLC ($CHCl_3$/MeOH, 100:1) to yield **6** (2.1 mg), which was identified by spectroscopic data.

14,15-Didehydro-10,11-dimethoxy-16-epivincamine (1): $[\alpha]^{20}_D$ +19.0 (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 303 (3.92), 299 (3.91), 275 (3.86), 228 (4.42) nm; IR (KBr) ν_{max} 3427, 2929, 2852, 1736, 1626, 1481, 1443, 1365, 1265, 1230, 1205, 1163, 1107, 1032, 781, 719 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 412 $[M]^+$ (100), 394 (9), 383 (19), 365 (23), 353 (14), 344 (50), 326 (13), 310 (43), 309 (33), 295 (10); HREIMS *m*/*z* 412.1971 (calcd for $C_{23}H_{28}N_2O_5$, 412.1998).

14,15-Didehydro-10-hydroxy-11-methoxy-16-epivincamine (2): $[\alpha]^{20}_D$ +8.4 (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 311 (3.77), 306 (3.81), 301 (3.80), 277 (3.71), 228 (4.25) nm; IR (KBr) ν_{max} 3427, 2931, 2852, 1736, 1632, 1578, 1479, 1443, 1367, 1263, 1203, 1161, 1107, 1032, 781, 721 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 398 $[M]^+$ (100), 380 (19), 369 (28), 351 (49), 339 (18), 330 (51), 312 (25), 296 (54), 295 (55), 281 (14); HREIMS *m*/*z* 398.1833 (calcd for $C_{22}H_{26}N_2O_5$, 398.1842).

14,15-Didehydro-10,11-dimethoxyvincamine (3): $[\alpha]^{20}_D$ +86.3 (*c* 0.90, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 303 (3.93), 299 (3.92), 276 (3.83), 229 (4.41) nm; IR (KBr) ν_{max} 3421, 2935, 2852, 1743, 1470, 1340, 1259, 1213, 1149, 1070, 941, 548 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 412 $[M]^+$ (100), 394 (9), 383 (20), 379 (23), 365 (25), 353 (21), 344 (50), 326 (13), 310 (25), 309 (27), 295 (10), 230 (46); HREIMS *m*/*z* 412.1998 (calcd for $C_{23}H_{28}N_2O_5$, 412.1998).

14,15-Didehydro-10-hydroxy-11-methoxyvincamine (4): $[\alpha]^{20}_D$ +81.9 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 306 (3.87), 276 (3.72), 228 (4.29) nm; IR (KBr) ν_{max} 3442, 2931, 2852, 1738, 1660, 1628, 1576, 1481, 1444, 1356, 1271, 1215, 1148, 1103, 1068, 1036, 1103, 856, 552 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 398 $[M]^+$ (100), 380 (31), 369 (27), 365 (34), 351 (81), 339 (24), 330 (53), 312 (43), 296 (32), 295 (42), 281 (14), 216 (50); HREIMS *m*/*z* 398.1839 (calcd for $C_{22}H_{26}N_2O_5$, 398.1842).

19,20-Didehydro-6α-hydroxyervatamine (5): $[\alpha]^{16}_D$ +167.3 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 312 (4.18), 235 (4.04) nm; IR (KBr) ν_{max} 3385, 3304, 2951, 1720, 1645, 1535, 1456, 1333, 1256, 1200, 1074, 1038, 750 cm^{-1}; ^1H NMR (C_5D_5N) δ_H 13.45 (1H, s, NH), 7.86 (1H, d, J = 8.0 Hz, H-8), 7.64 (1H, d, J = 8.3 Hz, H-12), 7.37 (1H, m, H-11), 7.19 (1H, m, H-10), 6.28 (1H, s, H-6), 5.33 (1H, br q, J = 6.6 Hz, H-19), 4.62 (1H, dd, J = 15.8, 11.2 Hz, H_α-14), 4.27 (1H, d, J = 11.2 Hz, H-15), 3.89, 3.40 (each 1H, d, J = 12.2 Hz, H_2-5), 3.56 (3H, s, OCH_3), 3.13, 2.70 (each 1H, d, J = 11.8 Hz, H_2-21), 2.64 (1H, d, J = 15.8 Hz, H_β-14), 2.33 (3H, s, NCH_3), 1.60 (3H, dd, J = 6.6, 1.6 Hz, H-18), ^1H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; EIMS *m*/*z* 368 $[M]^+$ (65), 350 (22), 307 (11), 296 (17), 291 (43), 254 (22), 222 (16), 210 (39), 194 (25), 182 (50), 180 (100); HREIMS *m*/*z* 368.1737 (calcd for $C_{21}H_{24}N_2O_4$, 368.1736).

Dehydroxyervataminol (6): $[\alpha]^{16}_D$ −61.5 (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 315 (4.07), 241 (4.26) nm; IR (KBr) ν_{max} 3396, 2937, 2783, 1730, 1647, 1464, 1446, 1340, 1288, 1227, 1203, 1151, 1082, 768, 741 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 338 $[M]^+$ (68), 309 (5), 295 (5), 279 (14), 236 (9), 194 (13), 182 (13), 180 (16), 98 (100); HREIMS *m*/*z* 338.1987 (calcd for $C_{21}H_{26}N_2O_2$, 338.1994).

Acknowledgment. Financial support of the National Natural Science Foundation (Grant No. 30630072), Shanghai Municipal Scientific Foundation (Grant No. 04XD14019), and the foundation from the Ministry of Science and Technology (Grant No. 2002CB512807) of People's Republic of China is gratefully acknowledged. We thank Prof. S.-M. Huang and Prof. Y.-K. Xu for the collection and identification of the plant materials.

Supporting Information Available: EIMS, IR, and 1D and 2D NMR spectra of the new alkaloids **1**−**6** and NMR spectra of the known compounds **7**−**26** and **34**−**49** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP060344O