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Cytotoxic Indole Alkaloids from *Tabernaemontana divaricata*

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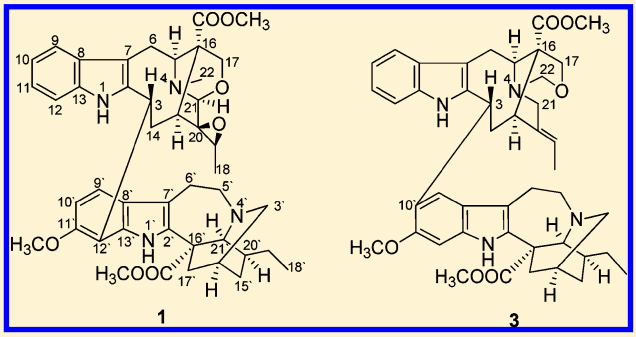
Supporting Information

ABSTRACT: Five new vobasinyl−ibogan-type bisindole alkaloids, tabernaricatines A−E (**1**−**5**), two new monomers, tabernaricatines F and G (**6** and **7**), and 24 known indole alkaloids were isolated from the aerial parts of *Tabernaemontana divaricata*. Alkaloids **1** and **2** are the first vobasinyl−ibogan-type alkaloids possessing a six-membered ring via an ether linkage between C-17 and C-21. All compounds except for **3** were evaluated for their cytotoxicity against five human cancer cell lines; conophylline showed significant bioactivity against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cells with IC_{50} values of 0.17, 0.35, 0.21, 1.02, and 1.49 μM, respectively.



Monoterpenoid indole alkaloids (MIAs) play an important role in natural products due to their complicated structures and biological activities.[1] Additionally, bisindole alkaloids are well known for their antitumor bioactivity, e.g., vincristine derivatives.[2] Plants of the genus *Tabernaemontana* are rich in MIAs, especial their dimeric forms.[3,4] The genus includes about 120 species and are distributed mainly in the tropical and subtropical areas of Asia and Australia. Many of these species are used in folk medicine for the treatment of abdominal pain, hypertension, and sore throat.[5] In our previous study of this genus, a series of cytotoxic monoterpenoid indole and bisindole alkaloids were reported.[6] As a continuation of our studies on bioactive MIAs from Apocynaceae, we investigated the constituents of *Tabernaemontana divaricata*. As a result, seven new (**1**−**7**) and 24 known alkaloids were identified. The known alkaloids were identified as ervachinine C,[7] ervachinine A,[7] ervachinine B,[7] tabernaecorymbosine A,[6] tabernaecorymbosine B,[6] cononitarine B,[8] conofoline,[9] conophylline,[9] hydroxyindolenine,[10] voacangine hydroxyindolenine,[11] voacristina hydroxyindolenina,[12] 3-(2-oxopropyl)voacangine,[13] voacristina,[12] ibogaine,[14] voacristine,[15] tabernanthine,[16] isovoacangine,[17] 19-epi-isovoacristine,[18] 19*S*-heyneanine,[19] 1-methylvoaphylline,[20] voaphyllinediol,[21] 19,20-*E*-vallesamine,[22] and picrinine.[23] The cytotoxicity of these compounds against five human cancer cell lines was evaluated.

RESULTS AND DISCUSSION

Alkaloid **1** was isolated as a colorless powder. The UV absorption bands at 292, 285, and 222 nm suggested an indole chromophore,[24] while the IR absorption bands at 3388 and 1728 cm^{-1} resulted from the −NH and ester carbonyl groups. The molecular formula of **1** was established as $C_{44}H_{52}N_4O_7$ by HREIMS ($[M]^+$ at m/z 748.3854), indicating 21 indices of hydrogen deficiency. Its ^1H NMR spectrum (Table 1) displayed two indole NH signals at δ_H 9.75 (1H, br s) and 7.37 (1H, br s); an unsubstituted indole moiety with signals at δ_H 7.72 (1H, d, J = 7.2 Hz), 7.04 (1H, t, J = 7.2 Hz), 7.06 (1H, t, J = 7.2 Hz), and 7.12 (1H, d, J = 7.2 Hz); a disubstituted indole moiety with signals at δ_H 7.22 (1H, d, J = 8.6 Hz) and 6.85 (1H, d, J = 8.6 Hz); one aromatic methoxy group at δ_H 3.94 (3H, s) together with a nitrogen methyl (δ_H 2.66, 3H, s); and two methyl ester groups (δ_H 2.28 and 3.71, each 3H, s). The former ester methyl, associated with the vobasine unit (unit A, Figure 2), was unusually shielded (δ_H 2.28) by the aromatic ring.[8] The ^{13}C NMR and DEPT spectra of **1** (Table 2), in association with the MS spectrum, suggested that **1** possessed 44 carbons, including six methyl, nine methylene, 14 methine, and 15 quaternary carbons. Compound **1** was thus readily identified as a bisindole alkaloid, comprising a vobasinyl-type unit (A) and an ibogan-type unit (B) (Figure 2), similar to conodiparine B (Figure 1).[8] A significant difference was the presence of a six-membered ring formed via an ether linkage between C-17 and C-21 in unit A, as supported by the HMBC correlations of H-21 (δ_H 3.62, s) with C-5 (δ_C 62.9), C-15 (δ_C 43.5), C-17 (δ_C 73.1), and C-19 (δ_C 59.1) and of H-17 (δ_H 3.69, m) with C-5 (δ_C 62.9), C-21 (δ_C 90.1), and C-15 (δ_C 43.5). An oxirane involving C-19 (δ_C 59.1) and C-20 (δ_C 65.2) was confirmed by their typical chemical shifts in unit A, which was also supported by correlations of H-19 (δ_H 3.03 q, J = 5.6 Hz) with C-18 and C-21 in its HMBC spectrum. In unit B, a hydroxy group was absent at C-19′ in **1** compared with conodiparine B,[8] as confirmed by the upfield shifts of the carbon resonances at δ_C

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Table 1. ^{1}H NMR Spectroscopic Data for 1−5 (δ in ppm and J in Hz)[a]

postion	δ_H (1)	δ_H (2)	δ_H (3)	δ_H (4)	δ_H (5)
NH	9.75, br s	9.66, br s	7.57, br s	9.71, br s	9.18, br s
3	4.93, br d (12.2)	5.15, br d (12.6)	5.14, br d (13.8)	5.30, br d (13.6)	5.15, br d (11.7)
5	3.48, m	3.49, m	4.13, t (9.2)	4.12, t (9.4)	3.78, m
6	3.22, m	3.27, m	3.41, m	3.57, m	3.26, m
	4.13, dd (4.0, 8.0)	4.01, m	3.75, m	3.73, m	3.60, m
9	7.72, d (7.2)	7.71, d (7.5)	7.09, d (7.5)	7.74, d (7.4)	7.53, d (6.8)
10	7.04, t (7.2)	7.03, t (7.5)	7.62, t (7.5)	7.07, t (7.4)	6.95, t (6.8)
11	7.06, t (7.2)	7.01, t (7.5)	7.09, t (7.5)	7.04, t (7.4)	6.96, t (6.8)
12	7.12, d (7.2)	7.08, d (7.5)	7.04, d (7.5)	7.11, d (7.4)	7.10, d (6.8)
14	2.24, m	2.02, m	2.07, m	1.95, m	1.96, m
	3.08, m	2.73, m	2.50, m	2.60, m	2.62, m
15	2.79, m	2.38, m	3.79, m	3.80, m	3.63, overlap
17	3.69, overlap	3.61, overlap	3.63, d (11.0)	3.57, overlap	3.61, overlap
		3.49, overlap	3.69, d (11.0)		3.78, overlap
18	1.36, d (5.6)	0.95, t (7.3)	1.63, d (6.6)	1.60, d (6.7)	1.62, d (6.3)
19	3.03, q (5.6)	1.45, m	5.18, q (6.6)	5.08, q (6.7)	5.31, q (6.3)
		1.49, m			
20		1.39, m			
21	3.62, s	4.05, d (1.8)	3.25, d (16.0)	3.19, d (15.8)	2.86, m
			4.30, d (16.0)	4.24, d (15.8)	3.88, m
22	2.66, s	2.71, s	4.62, d (9.6)	4.55, d (9.7)	2.51, s
			4.74, d (9.6)	4.64, d (9.7)	
$COOCH_3$	2.28, s	2.31, s	2.43, s	2.45, s	2.26, s
NH′	7.37, br s	7.52, br s	7.65, br s	7.70, br s	9.20, br s
3′	2.38, m	2.40, m	2.72, m	2.38, m	3.24, m
	2.66, m	2.65, m	2.83, m	2.65, m	
5′	2.92, m	2.95, m	3.08, m	2.93, m	3.02, m
	3.24, m	3.23, m	3.19, m	3.23, m	3.05, m
6′	2.84, m	2.83, m	2.71, m	2.82, m	2.82, m
	2.93, m	2.95, m	2.94, m	2.93, m	2.58, m
9′	7.22, d (8.6)	7.22, d (8.6)	6.83, s	7.20, d (8.6)	6.77, s
10′	6.85, d (8.6)	6.86, d (8.6)		6.84, d (8.6)	
12′			6.80, s		6.90, s
14′	1.34, m	1.37, m	1.83, m	1.34, m	1.64, m
15′	0.87, m	0.88, m	1.07, m	0.88, m	1.22, m
	1.45, m	1.48, m	1.69, m	1.46, m	1.49, m
17′	0.54, br d (13.2)	0.62, br d (13.5)	1.80, m	0.59, br d (13.9)	1.85, m
	1.66, br d (13.2)	1.77, br d (13.5)	2.50, m	1.72, br d (13.9)	2.68, m
18′	0.81, t (7.4)	0.81, t (7.4)	0.84, t (7.0)	0.81, t (7.4)	0.82, t (7.3)
19′	1.30, m	1.30, m	1.38, m	1.31, m	1.37, m
	1.43, m	1.44, m	1.50, m	1.44, m	1.37, m
20′	1.13, m	1.15, m	1.25, m	1.15, m	1.23, m
21′	3.36, s	3.35, s	3.42, br s	3.35, br s	3.46, s
11′-OCH_3	3.94, s	3.95, s	3.97, s	3.95, s	3.91, s
C′$OOCH_3$′	3.71, s	3.72, s	3.66, s	3.68, s	3.62, s
CH_2COCH_3					2.67, m
					2.48, m
CH_2COCH_3					2.04, s

[a]Compound **3** in $CDCl_3$; **1**, **2**, **4**, and **5** in acetone-d_6.

11.8 (q, C-18′) and 27.4 (t, C-19′) in the ^{13}C NMR and DEPT spectra and corresponding protons H-18′ (δ_H 0.81, 3H, t, J = 7.4 Hz) and H-19′ (δ_H 1.30, 1.43, each 1H, m) in the ^{1}H NMR spectrum. The assumption was further supported by correlations of H-18′ with C-19′ and C-20′ (δ_C 39.4) in the HMBC spectrum. Finally, the linkage of units A and B by C-3 and C-12′ was established by the HMBC correlations of H-3 (δ_H 4.93, br d, J = 12.2 Hz) with C-12′ (δ_C 115.8), C-13′ (δ_C 135.9), C-2 (δ_C 138.7), and C-15 (δ_C 43.5) (Figure 2); a noticeable upfield shift of both the C-17′ methylene protons at δ_H 0.54 and 1.66 confirmed the mode of branching of the monomeric units.[8] The NOE correlations of H-19/H-21, H-21/H-22 (NCH_3), and H-22/H-5 in the ROESY spectrum indicated that the relative configuration of **1** was the same as that of conodiparine B (Figure 1).[8] A detailed analysis of the 2D NMR data (HSQC, HMBC, ROESY) established the structure of **1** to be as shown, and it was named tabernaricatine A.

Compound **2** had the molecular formula $C_{44}H_{54}N_4O_6$, established by HREIMS ([M]$^+$ at m/z 734.3969), with 20

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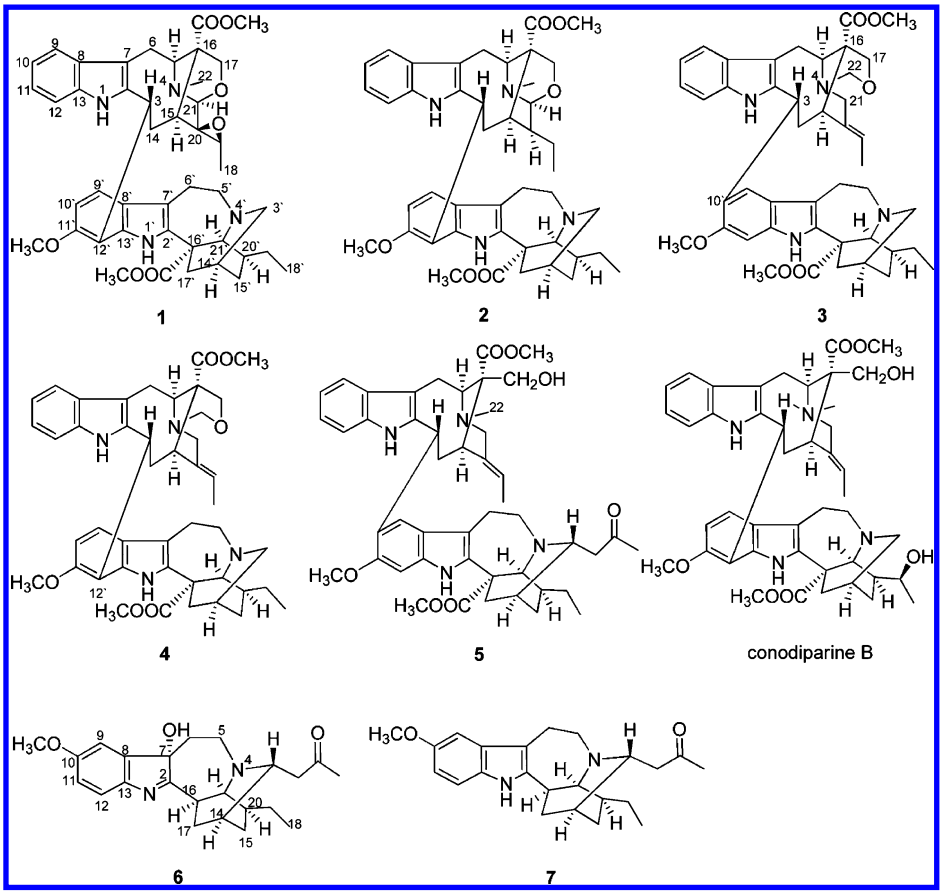

Figure 1. Alkaloids (**1**–**7**) isolated from *T. divaricata*.

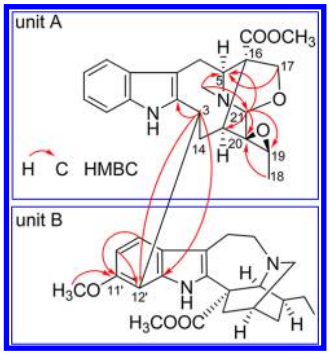

Figure 2. Key HMBC correlations of **1**.

indices of hydrogen deficiency. The UV spectrum displayed absorption maxima characteristic of indole chromophores at 285 and 222 nm, and the IR spectrum showed absorption bands due to −NH (3405 cm^{-1}) and ester carbonyl (1727 cm^{-1}) functions. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) were similar to those of **1**, except that the oxirane between C-19 and C-20 was reduced, as supported by the molecular formula and the HMBC correlations of H-19 (δ_H 1.45 and 1.49, each 1H, m) with C-21 (δ_C 87.0), C-20 (δ_C 51.4), C-15 (δ_C 41.2), and C-18 (δ_C 12.3). Taking the degrees of unsaturation into consideration confirmed the absence of the oxirane ring. Analysis of the 2D NMR data confirmed that the other parts were the same as those of **1**. Hence, the structure of **2** was elucidated as shown, and it was named tabernaricatine B.

The molecular formula $C_{44}H_{52}N_4O_6$ of **3** was established by the molecular ion peak at m/z 732.3726 in the HREIMS. The UV absorption bands at 295 and 225 nm and IR absorption bands at 3424 and 1723 cm^{-1} were characteristic of indole alkaloids. In the ^{1}H NMR spectrum, signals typical of an unsubstituted indole moiety (vobasine unit) were observed at δ_H 7.09 (1H, d, J = 7.5 Hz), 7.62 (1H, t, J = 7.5 Hz), 7.09 (1H, t, J = 7.5 Hz), and 7.04 (1H, d, J = 7.5 Hz). Two aromatic singlets appeared at δ_H 6.83 and 6.80, indicating a 10′,11′-disubstituted indole moiety (iboga unit; Table 1). The ^{13}C NMR and DEPT spectra showed 44 signals arising from two methyl (δ_C 11.8 and 11.8), three methoxy (δ_C 50.5, 52.7, and 56.0), two ester carbonyl (δ_C 173.6 and 176.0), 11 sp^2 quaternary carbon (δ_C 153.4, 141.2, 137.4, 136.3, 135.4, 134.8, 129.9, 127.3, 122.5, 110.2, and 110.1), two sp^3 quaternary carbon (δ_C 55.1 and 47.0), seven sp^2 methine (δ_C 121.9, 119.1, 118.0, 117.7, 114.0, 110.0, and 92.7), six sp^3 methine (δ_C 61.0, 57.7, 39.6, 39.3, 37.0, and 27.4), and 11 sp^2 methylene (δ_C 88.4, 76.7, 53.1, 51.5, 50.0, 38.3, 36.6, 32.1, 26.8, 25.5, and 22.2) carbons. Besides these, two low-field signals were observed at δ_C 76.7 (t, C-17) and δ_C 88.4 (t, C-22). The nitrogen methyl signals were conspicuously absent, which suggested an ether bond between C-22 (nitrogen methyl) and C-17 in unit A. This was also confirmed by the observed HMBC correlations of H-22 (δ_H 4.62, 4.74) to C-21 (δ_C 50.0), C-17 (δ_C 76.7), and C-5 (δ_C 61.0). The NMR data (Tables 1 and 2) were closely related to those of conodirinine A,[25] except for a signal at δ_C 26.8 (t, C-19′) in the ^{13}C NMR spectrum of **3** instead of a signal for an oxymethine in conodirinine A. Therefore, **3** was elucidated as shown by analysis of 2D NMR data (HSQC, HMBC, ROESY), and it was named tabernaricatine C.

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Table 2. ^{13}C NMR Spectroscopic Data for 1−5[a]

position	δ_C (1)	δ_C (2)	δ_C (3)	δ_C (4)	δ_C (5)
2	138.7, C	138.7, C	137.4, C	137.9, C	139.6, C
3	36.2, CH	35.9, CH	37.0, CH	35.9, CH	38.1, CH
5	62.9, CH	64.2, CH	61.0, CH	61.7, CH	61.2, CH
6	31.2, CH_2	32.1, CH_2	25.5, CH_2	26.3, CH_2	18.0, CH_2
7	109.2, C	109.4, C	110.2, C	109.5, C	110.0, C
8	130.0, C	130.1, C	129.9, C	130.2, C	131.1, C
9	119.1, CH	119.0, CH	119.1, CH	118.8, CH	118.1, CH
10	119.7, CH	119.5, CH	117.7, CH	119.5, CH	118.7, CH
11	122.5, CH	122.4, CH	121.9, CH	122.5, CH	121.5, CH
12	110.8, CH	110.7, CH	110.0, CH	110.7, CH	110.6, CH
13	137.4, C	137.5, C	136.3, C	137.7, C	137.5, C
14	33.5, CH_2	41.2, CH_2	38.3, CH_2	36.4, CH_2	38.0, CH_2
15	43.5, CH	41.2, CH	39.6, CH	40.2, CH	36.2, CH
16	50.7, C	48.6, C	47.0, C	47.5, C	53.6, C
17	73.1, CH_2	72.9, CH_2	76.7, CH_2	76.9, CH_2	70.3, CH_2
18	17.6, CH_3	12.3, CH_3	11.8, CH_3	11.6, CH_3	12.2, CH_3
19	59.1, CH	27.6, CH_2	114.0, CH	113.5, CH	118.9, CH
20	65.2, C	51.4, CH	141.2, C	142.6, C	139.1, C
21	90.1, CH	87.0, CH	50.0, CH_2	50.3, CH_2	52.6, CH_2
22	40.8, CH_3	42.4 CH_3	88.4, CH_2	88.7, CH_2	42.4, CH_3
$COO\underline{C}H_3$	50.5, CH_3	50.4, CH_3	50.5, CH_3	50.5, CH_3	49.7, CH_3
$\underline{C}OOCH_3$	172.1, C	172.9, C	173.6, C	173.9, C	173.3, C
2′	136.6, C	136.7, C	135.4, C	136.5, C	136.8, C
3′	52.8, CH_2	52.5, CH_2	51.5, CH_2	52.5, CH_2	56.4, CH_2
5′	53.8, CH_2	53.8, CH_2	53.1, CH_2	53.7, CH_2	52.1, CH_2
6′	22.5, CH_2	22.5, CH_2	22.2, CH_2	22.4, CH_2	22.5, CH_2
7′	109.5, C	109.7, C	110.1, C	109.6, C	109.8, C
8′	125.4, C	125.4, C	122.5, C	125.4, C	123.2, C
9′	117.5, CH	117.5, CH	118.0, CH	117.4, CH	118.2, CH
10′	105.6, CH	106.2, CH	127.3, C	106.2, CH	128.4, C
11′	152.9, C	152.8, C	153.4, C	152.8, C	154.1, C
12′	115.8, C	116.3, C	92.7, CH	116.2, C	93.8, CH
13′	135.9, C	135.9, C	134.8, C	135.9, C	136.2, C
14′	28.1, CH	28.1, CH	27.4, CH	28.0, CH	31.5, CH
15′	32.8, CH_2	32.8, CH_2	32.1, CH_2	32.7, CH_2	27.6, CH_2
16′	55.1, C	55.2, C	55.1, C	55.0, C	55.1, C
17′	35.3, CH_2	35.5, CH_2	36.6, CH_2	35.5, CH_2	38.0, CH_2
18′	11.8, CH_3	11.9, CH_3	11.8, CH_3	11.7, CH_3	11.9, CH_3
19′	27.4, CH_2	27.4, CH_2	26.8, CH_2	27.4, CH_2	27.4, CH_2
20′	39.4, CH	39.4, CH	39.3, CH	39.4, CH	38.8, CH
21′	57.3, CH	57.6, CH	57.7, CH	57.2, CH	58.8, CH
11′-OCH_3	57.0, CH_3	57.4, CH_3	56.0, CH_3	57.4, CH_3	56.1, CH_3
$\underline{C}'OOCH_3'$	174.7, C	174.7, C	176.0, C	174.7, C	175.3, C
$C'OO\underline{C}H_3'$	52.7, CH_3	52.6, CH_3	52.7, CH_3	52.5, CH_3	52.6, CH_3
$\underline{C}H_2COCH_3$					46.6, CH_2
$CH_2\underline{C}OCH_3$					208.2, C
$CH_2CO\underline{C}H_3$					30.7, CH_3

[a]Compound **3** in $CDCl_3$; **1**, **2**, **4**, and **5** in acetone-d_6.

Compound **4** possessed the molecular formula $C_{44}H_{52}N_4O_6$ as determined by HREIMS. The UV (285 and 222 nm) and IR data (3433 and 1725 cm^{-1}) showed the characteristic absorptions of indole chromophores. Comparison of the NMR data of **4** with those of **3** suggested the linkage of the vobasine and iboga units via C-3/12′ in **4**, rather than via C-3/10′ as in **3**, which was supported by the coupling constants of the aromatic protons at δ_H 7.20 (1H, d, J = 8.6 Hz, H-9′) and 6.84 (1H, d, J = 8.6 Hz, H-10′) in **4**. The assumption was further supported by the HMBC correlations of H-3 (δ_H 5.30, br d, J = 13.6 Hz) with C-11′ (δ_C 152.8), C-12′ (δ_C 116.2), and C-13′ (δ_C 135.9). Analysis of 2D NMR data confirmed that the other parts were identical to those of **3**. Hence, the structure of **4** was elucidated as shown, and it was named tabernaricatine D.

Compound **5** was isolated as a light yellow powder. Its UV spectrum showed absorption maxima at 295 and 227 nm, suggesting indole chromophores, and the IR spectrum showed −NH (3440 cm^{-1}), ester carbonyl (1725 cm^{-1}), and carbonyl (1711 cm^{-1}) bands. The molecular formula $C_{47}H_{58}N_4O_7$ was established by HREIMS (m/z 790.4297 $[M]^+$), which is 56 Da higher than that of ervachinine C.[7] Analysis of the NMR data (Tables 1 and 2) indicated that **5** was also a vobasinyl−ibogan

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bisindole alkaloid, and it was readily identified as a 2-oxopropyl derivative of ervachinine C[7] by the presence of signals at δ_C 208.2 (s), 46.6 (t), and 30.7 (q) in its ^{13}C NMR spectrum. The 2-oxopropyl group was substituted at C-3′, as supported by the HMBC correlations of H-3′ (δ_H 3.24) with the carbon at δ_C 208.2. In the ROESY spectrum of **5**, H-17′α (δ_H 1.85) was correlated with H-15′α; thus, the NOE correlation of H-3′ with H-17′β (δ_H 2.68) suggested that H-3′ was β-oriented. Thus, the structure of **5** was elucidated and named tabernaricatine E.

The UV and IR spectra of **6** were similar to those of the above alkaloids. Its molecular formula, $C_{23}H_{30}N_2O_3$, from HREIMS ([M]$^+$ m/z 382.2265) indicatied a monomeric alkaloid with 10 indices of hydrogen deficiency. The ^{13}C NMR and DEPT spectra displayed 23 carbon resonances ascribed to three methyl, six methylene, eight methine, and six quaternary carbons (Table 3). In the ^1H NMR spectrum, three

Table 3. ^1H and ^{13}C NMR Spectroscopic Data for 6 and 7 (δ in ppm and J in Hz)[a]

	6		7	
position	δ_H	δ_C	δ_H	δ_C
NH			9.53, br s	
2		191.5, C		143.7, C
3	3.26, m	52.6, CH	3.60, m	54.0, CH
5	3.00, m	47.6, CH_2	3.01, m	52.9, CH_2
	3.27, m		3.39, m	
6	1.79, m	34.0, CH_2	2.54, m	21.5, CH_2
	1.82, m		3.33, m	
7		88.1, C		108.5, C
8		145.6, C		131.1, C
9	6.88, d (2.5)	108.9, CH	6.89, d (1.9)	100.7, CH
10		159.3, C		154.5, C
11	6.80, dd (8.3, 2.5)	113.8, CH	7.10, dd (8.6, 1.9)	111.5, CH
12	7.21, d (8.3)	120.8, CH	6.64, d (8.6)	110.8, CH
13		147.1, C		131.1, C
14	1.55, m	32.0, CH	1.62, m	30.7, CH
15	1.23, m	28.2, CH_2	1.61, m	36.9, CH_2
	1.52, m		2.12, m	
16	2.96, m	43.8, CH	1.35, m	41.6, CH
17	3.25, m	35.0, CH_2	1.59, m	27.4, CH_2
	2.10, m		1.59, m	
18	0.90, t (7.2)	12.1, CH_3	0.88, t (7.0)	12.1, CH_3
19	1.45, m	27.9, CH_2	1.48, m	28.1, CH_2
	1.52, m		1.55, m	
20	1.44, m	40.6, CH	3.06, m	42.2, CH
21	3.66, d (2.6)	55.3, CH	2.81, s	59.6, CH
7-OH	4.95, br s			
10-OCH_3	3.79, s	55.9, CH_3	3.77, s	55.7, CH_3
$\underline{CH_2}COCH_3$	2.52, m	47.2, CH_2	2.58, m	47.6, CH_2
	2.66, m		2.73, m	
$CH_2\underline{CO}CH_3$		208.2, C		208.2, C
$CH_2CO\underline{CH_3}$	2.07, s	30.6, CH_3	2.04, s	30.7, CH_3

[a]Compounds **6** and **7** in acetone-d_6.

signals [δ_H 6.88 (d, J = 2.5 Hz, H-9), 6.80 (dd, J = 8.3, 2.5 Hz, H-11), 7.21 (d, J = 8.3 Hz, H-12)] revealed the presence of a monosubsitituted A ring found in MIAs. A low-field signal at δ_H 4.95 (br s) was assigned to OH-7, as supported by the HMBC correlations with C-6 (δ_C 34.0), C-7 (δ_C 88.1), and C-2 (δ_C 191.5). These data were similar to those of hydroxyindolenine,[10] with the exception of three additional carbon signals at δ_C 30.6 (q), 208.3 (s), and 47.2 (t) in the ^{13}C NMR spectrum, which suggested that **6** was a 2-oxopropyl derivative of hydroxyindolenine. Moreover, the HMBC correlation of H-3 (δ_H 3.26) with the carbon at δ_C 208.2 placed the 2-oxopropyl moiety at C-3. The NOE correlation of 7-OH/H-16 indicated that the 7-OH group was α-oriented. Analysis of the 2D NMR data (HSQC, HMBC, ROESY) indicated that the rest of **6** was the same as that of hydroxyindolenine. The molecular formula of **7** was established as $C_{23}H_{30}N_2O_2$ by HREIMS ([M]$^+$ m/z 366.2306). The ^1H NMR, ^{13}C NMR, and DEPT data (Table 3) displayed signals for a monosubstituted indole ring [δ_C 143.7 (s, C-2), 108.5 (s, C-7), 131.1 (s, C-8), 100.7 (d, C-9), 154.5 (s, C-10), 111.5 (d, C-11), 110.8 (d, C-12), 131.1 (s, C-13); δ_H 6.89 (d, J = 1.9 Hz, H-9), 7.10 (dd, J = 8.6, 1.9 Hz, H-11), 6.64 (d, J = 8.6 Hz, H-12)]. The NMR data (Table 3) of **7** were similar to those of 3-(2-oxopropyl)voacangine[13] except for the absence of an ester signal at C-16 in **7**. This assignment was supported by the HMBC correlations of H-16 (δ_H 1.35, m) with C-7 (δ_C 108.5), C-14 (δ_C 30.7), and C-20 (δ_C 42.2). Similar to **5**, the ROESY correlation of H-17β with H-3 suggested that H-3 was β-oriented. Thus, the structures of alkaloids **6** and **7** were elucidated and named tabernaricatines F and G, respectively.

Alkaloids **1** and **2** are the first vobasinyl−ibogan-type alkaloids possessing a six-membered ring via an ether linkage between C-17 and C-21. The cytotoxicities of all alkaloids except **3** against five human cancer cell lines were evaluated using the MTT method reported previously.[26] Cononitarine B and conophylline exhibited significant inhibitory effects against these five human cancer cell lines, and alkaloids **1**, **7**, ervachinine A, ervachinine B, ervachinine C, and conofoline displayed moderate cytotoxicity against some of the cell lines (Table 4).

Table 4. Cytotoxicity Data of the Alkaloids (IC_{50}, μM)

compd	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	0.79	3.41	3.52	3.10	2.59
7	3.88	14.75	28.53	13.23	13.01
ervachinine A	2.35	9.86	16.51	13.26	16.54
ervachinine B	3.88	14.75	28.53	13.23	13.01
ervachinine C	2.68	12.35	14.75	12.61	13.43
cononitarine B	2.90	3.75	9.40	3.02	2.72
conofoline	0.80	10.35	10.35	14.00	13.67
conophylline	0.17	0.35	0.21	1.02	1.49
cisplatin	1.14	14.51	12.76	17.18	16.84

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrometer. IR spectra were obtained by a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D NMR spectroscopic data were run on Bruker AVANCE III-600, DRX-500, and AM-400 MHz spectrometers with TMS as an internal standard. HREIMS was recorded on a Waters Auto Premier P776 spectrometer. Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China), RP-18 gel (20−45 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd., Qingdao), and spots were visualized by Dragendorff's reagent.

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Plant Material. *T. divaricata* was collected from Xishuangbanna, Yunnan Province, P. R. China, and identified by Mr. Jing-Yun Cui, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (No. Cui20091027) has been deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. An air-dried and powdered sample (5 kg) was extracted with 90% aqueous MeOH (2 days × 3) at room temperature with the solvent removed *in vacuo*. The extract was partitioned between EtOAc and a 0.5% HCl solution. The water layer was adjusted to pH 9−10 with 10% ammonia and partitioned with EtOAc to give an alkaloid extract layer. The total alkaloid mixture (42 g) was subjected to CC over silica gel and eluted with $CHCl_3$/acetone (from 1:0 to 1:1, v/v) to afford four fractions (I−IV). Fraction II (1.1 g) was further applied to Si gel chromatography using a petroleum ether/EtOAc eluent (from 9:1 to 6:1, v/v) to yield isovoacangine (10 mg), voacristine (6 mg), **6** (3 mg), 1-methylvoaphylline (15 mg), ibogaine (50 mg), voacristine hydroxyindolenine (20 mg), and **7** (12 mg). Fraction III (18.0 g) was subjected to RP-18 (MeOH/H_2O, from 1:9 to 1:0, v/v), affording five subfractions (IIIa−IIIe). Fraction IIIa (850 mg) was subjected to silica gel CC and eluted with petroleum ether/acetone (5:1, v/v) to afford 19-epi-isovoacristine (100 mg), voacristine (14 mg), and (19*S*)-heyneanine (25 mg). Fraction IIIb (6.2 g) was subjected to silica gel CC and eluted with petroleum ether/acetone (from 6:1 to 4:1, v/v) to give mixtures A and B. A and B were further purified by C_{18} CC (MeOH/H_2O, from 6:4 to 8:2, v/v) to yield **3** (5 mg), **5** (7 mg), tabernaecorymbosine A (180 mg), tabernaecorymbosine B (12 mg), cononitarine B (5 mg), ervachinine C (35 mg), and voaphyllinediol (15 mg). Fraction IIIc (2.1 g) was separated by a C_{18} column (MeOH/H_2O, 6:4, v/v) to afford conofoline (21 mg) and conophylline (30 mg). Fraction IIId (4 g) was subjected to a C_{18} silica gel column and eluted with MeOH/H_2O (7:3, v/v) to afford **4** (18 mg) and fraction C (3 g) as a mixture of compounds. C was further purified by Si gel CC (petroleum ether/Me_2CO, 5:1, v/v) to afford ervachinine A (6 mg) and ervachinine B (15 mg). Fraction IIIe (2.5 g) was subjected to C_{18} Si gel CC (MeOH/H_2O, 6:4, v/v) to yield **1** (21 mg) and mixed fraction D (19 mg). D was purified by Sephadex LH-20 (MeOH) to yield **2** (10 mg). Fraction IV (11 g) was separated by silica gel (petroleum ether/acetone, from 5:1 to 1:1, v/v) to afford five subfractions (IVa−IVe). Fraction IVb (250 mg) was further purified by Sephadex LH-20 (MeOH) to yield conofoline (15 mg) and 3-(2-oxopropyl)voacangine (8 mg). Hydroxyindolenine (5 mg) and picrinine (7 mg) were crystallized from fraction IVd (46 mg). Fraction IVe (3.2 g) was subjected to C_{18} CC (MeOH/H_2O, from 4:6 to 9:1, v/v) and then purified by Sephadex LH-20 (MeOH) to yield tabernanthine (14 mg) and mixed fraction E (2.1 g). E was further purified by a C_{18} column (MeOH/H_2O, 5:5, v/v) to afford (19*S*)-heyneanine (26 mg) and 19,20-(*E*)-vallesamine (14 mg).

*Tabernaricatine A (**1**):* colorless powder; $[\alpha]_D^{25}$ −6.9 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 292 (3.49), 285 (3.50), 222 (4.07) nm; IR (KBr) ν_{max} 3388, 2947, 2927, 2857, 1728, 1619, 1461, 1450, 1281, 1240, 1129, 1056, 878, 740 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (150 MHz) data (acetone-d_6), see Tables 1 and 2; positive ion HREIMS *m*/*z* 748.3854 (calcd for $C_{44}H_{52}N_4O_7$ $[M]^{+\bullet}$, 748.3831).

*Tabernaricatine B (**2**):* colorless powder; $[\alpha]_D^{25}$ +26.4 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 285 (3.42), 222 (3.97) nm; IR (KBr) ν_{max} 3405, 2956, 2929, 2870, 2857, 1727, 1619, 1461, 1277, 1243, 1117, 1072, 740 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (acetone-d_6), see Tables 1 and 2; positive ion HREIMS *m*/*z* 734.3969 (calcd for $C_{44}H_{54}N_4O_6$ $[M]^{+\bullet}$, 734.4038).

*Tabernaricatine C (**3**):* light yellowish oil; $[\alpha]_D^{25}$ −86.0 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 295 (3.53), 225 (4.08) nm; IR (KBr) ν_{max} 3424, 2951, 2928, 2857, 1723, 1629, 1462, 1250, 1039, 742 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (150 MHz) data ($CDCl_3$), see Tables 1 and 2; positive ion HREIMS *m*/*z* 732.3726 (calcd for $C_{44}H_{52}N_4O_6$ $[M]^{+\bullet}$, 732.3881).

*Tabernaricatine D (**4**):* light yellowish oil; $[\alpha]_D^{25}$ −28.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 285 (2.83), 222 (3.42) nm; IR (KBr) ν_{max} 3433, 2952, 2925, 2855, 1725, 1628, 1461, 1244, 741 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (acetone-d_6), see Tables 1 and 2; positive ion HREIMS *m*/*z* 732.3881 (calcd for $C_{44}H_{52}N_4O_6$ $[M]^{+\bullet}$, 732.3881).

*Tabernaricatine E (**5**):* light yellow powder; $[\alpha]_D^{25}$ −11.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 295 (3.43), 227 (4.03) nm; IR (KBr) ν_{max} 3440, 2955, 2924, 2855, 1725, 1711, 1630, 1461, 1248, 744 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (acetone-d_6), see Tables 1 and 2; positive ion HREIMS *m*/*z* 790.4297 (calcd for $C_{47}H_{58}N_4O_7$ $[M]^{+\bullet}$, 790.4300).

*Tabernaricatine F (**6**):* colorless powder; $[\alpha]_D^{25}$ +78.2 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 284 (3.13), 223 (3.48) nm; IR (KBr) ν_{max} 3430, 2956, 2930, 2870, 1714, 1626, 1474, 1280, 821 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (acetone-d_6), see Table 3; positive ion HREIMS *m*/*z* 382.2265 (calcd for $C_{23}H_{30}N_2O_3$ $[M]^{+\cdot}$, 382.2251).

*Tabernaricatine G (**7**):* colorless powder; $[\alpha]_D^{25}$ −9.4 (*c* 0.9, MeOH); UV (MeOH) λ_{max} (log ε) 293 (3.17), 226 (3.62) nm, 202 (3.65) nm; IR (KBr) ν_{max} 3387, 3295, 2920, 2872, 1702, 1490, 1453, 1280, 825 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (acetone-d_6), see Table 3; positive ion HREIMS *m*/*z* 366.2306 (calcd for $C_{23}H_{30}N_2O_2$ $[M]^{+\bullet}$, 366.2302).

Cytotoxicity Assay. Five human cancer cell lines, human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW480), were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA), in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates.[26] Briefly, 100 μL of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0624, 0.32, 1.6, 8, and 40 μM in triplicate for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method.[27]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR and MS spectra of tabernaricatines **1**−**7** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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