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Ibogan, Tacaman, and Cytotoxic Bisindole Alkaloids from *Tabernaemontana*. Cononusine, an Iboga Alkaloid with Unusual Incorporation of a Pyrrolidone Moiety

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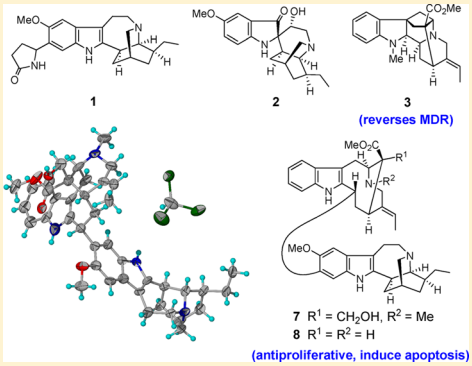
Supporting Information

ABSTRACT: Six new indole alkaloids, viz., cononusine (**1**, a rare example of an iboga−pyrrolidone conjugate), ervaluteine (**2**), vincamajicine (**3**), tacamonidine (**4**), 6-oxoibogaine (**5**), and N^4-chloromethylnorfluorocurarine chloride (**6**), and two new vobasinyl-iboga bisindole alkaloids, ervatensines A (**7**) and B (**8**), in addition to other known alkaloids, were isolated from the stem-bark extract of the Malayan *Tabernaemontana corymbosa*. The structures of these alkaloids were established on the basis of NMR and MS analyses and, in one instance (**7**), confirmed by X-ray diffraction analysis. Vincamajicine (**3**) showed appreciable activity in reversing multidrug resistance in vincristine-resistant KB cells (IC_{50} 2.62 μM), while ervatensines A (**7**) and B (**8**) and two other known bisindoles displayed pronounced in vitro growth inhibitory activity against human KB cells (IC_{50} < 2 μM). Compounds **7** and **8** also showed good growth inhibitory activity against A549, MCF-7, MDA-468, HCT-116, and HT-29 cells (IC_{50} 0.70−4.19 μM). Cell cycle and annexin V-FITC apoptosis assays indicated that compounds **7** and **8** inhibited proliferation of HCT-116 and MDA-468 cells, evoking apoptotic and necrotic cell death.



The genus *Tabernaemontana* (Apocynaceae), comprising a large number of species, is distributed over the tropical and subtropical regions of the world, including parts of the Americas, Africa (including Madagascar), Asia, Oceana, and Australia.[1] The large number of species, in addition to the wide geographical distribution, and the large number of synonyms have in the past given rise to problems and much confusion, associated with the taxonomic classification of the plants belonging to this genus. In a significant advance, the recent comprehensive review of this genus by Leeuwenberg has resulted in a significant reduction in the number of the Old World species.[1] Plants of the genus are well known as rich sources of structurally novel as well as biologically active alkaloids.[2−5] About 13 species are known to occur in Malaysia (Peninsular Malaya and Malaysian Borneo). In our systematic study of the Malaysian representatives of this genus, we have reported many examples of new alkaloids that are distinguished by their structural novelty, as well as useful biological activity.[4,5] For instance, we have previously reported the presence of the cytotoxic *Aspidosperma* alkaloids (jerantinines and jerantiphyllines),[6−8] the unusual alkaloid−lignan conjugates (conoliferine and conomicidines),[9,10] and the hexacyclic indole alkaloid conolutinine,[11] from *T. corymbosa* Roxb. ex Wall. Among the Malayan members of this genus, this species appears to be the most varied from a morphological viewpoint, as well as the most widely distributed.[1,12] We have therefore also investigated the different varieties of this species, occurring in distinctly different geographical locations, in order to ascertain whether there are marked differences in alkaloid composition between the samples. Herein we report the full alkaloidal composition of the stem-bark extract of a sample of *T. corymbosa* collected from the state of Pahang (Tekam Forest) in Peninsular Malaysia, including the isolation, structure determination, and biological activity of additional new alkaloids. We also include a comparison of the alkaloid content between the present sample and that of another, collected near Chenderiang, in the state of Perak, Peninsular Malaysia.

RESULTS AND DISCUSSION

Cononusine (**1**) was obtained as a colorless oil with $[\alpha]^{25}_{D}$ −27 (*c* 0.2, $CHCl_3$). The UV spectrum showed absorption maxima characteristic of an indole chromophore (205, 233, and 299 nm), while the IR spectrum indicated the presence of NH (3378 and 3266 cm^{-1}) and lactam carbonyl (1684 cm^{-1}) functions. The EIMS showed a molecular ion at *m*/*z* 393, the odd mass indicating the presence of a third N atom. HREIMS measure-

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Table 1. ^{1}H NMR Data (δ) for 1−6 (400 MHz)[a]

H	1	2	3	4	5	6
2			3.16 d (4.9)			
3	3.05 m	2.77 dt (11.5, 2.7)	3.48 m	4.36 dt (6.1, 2)	2.92 d (8.5)	4.77 s
	3.05 m	2.99 dt (11.5, 2.0)			3.09 dt (8.5, 3.4)	
5	3.15 br d (13.4)	2.52 dd (13.9, 2.7)	3.54 d (4.6)	3.28 dd (14.2, 6.4)	3.69 d (18.1)	4.20 dd (11.7, 7.1)
	3.38 m	3.05 dd (13.9, 5.4)		3.41 ddd (14.2, 11.7, 5.5)	3.81 d (18.1)	4.11 ddd (13.5, 11.7, 6.1)
6	2.63 m	4.37 dd (11.5, 5.4)	1.80 dd (11.2, 2.7)	2.86 m		2.09 dd (13.5, 6.1)
	3.32 m		2.32 dd (11.4, 4.9)	2.56 ddd (16.9, 5.5, 2.5)		2.65 td (13.5, 7.1)
9	6.90 s	6.97 d (3)	7.03 dd (7.3, 1)	7.45 dd (6.8, 1.5)	8.01 d (2.7)	7.88 d (7.6)
10			6.77 td (7.3, 1)	7.30 td (7.3, 1.5)		6.96 t (7.6)
11		7.08 dd (8.8, 3)	7.14 td (7.8, 1)	7.34 td (7.3, 1.5)	6.84 dd (8.8, 2.7)	7.24 t (7.6)
12	7.14 s	6.89 d (8.8)	6.65 d (7.8)	8.38 dd (7.1, 1.5)	7.21 d (8.8)	7.08 d (7.6)
14	1.84 m	1.67 m	1.46 ddd (13.9, 9.8, 1.1)	2.83 m	1.85 m	1.43 d (14.5)
			2.65 dd (13.9, 5.2)			2.93 d (14.5)
15	1.23 m	1.03 ddt (12.7, 6, 3)	3.29 d (4.6)	0.87 t (13.2)	1.20 m	3.98 m
	1.80 m	1.63 m		1.66 ddd (13.2, 4.6, 2)	1.78 m	
16	2.92 br d (11.7)	1.70 m			3.43 dt (11.7, 2)	
17	1.62 m	1.34 dt (11.9, 2.5)	1.46 d (13.6)	2.20 dd (17.2, 2.2)	1.63 ddt (13, 4, 2)	9.76 s
	2.05 br t (12.5)	2.51 m	2.58 dd (13.7, 2.7)	3.04 dd (17.2, 5.4)	2.12 br t (13)	
18	0.89 td (7.1, 1.7)	0.86 t (6.8)	1.58 ddd (6.8, 2.2, 1.5)	0.86 t (7.6)	0.92 t (7.1)	1.56 d (7)
19	1.48 m	1.40 m	5.26 q (6.8)	1.36 m	1.50 m	5.83 q (7)
	1.55 m	1.49 m		1.36 m	1.57 m	
20	1.55 m	1.45 m			1.54 m	
21	2.84 br s	2.43 br s	3.44 dt (16.1, 2.3)	2.31 d (11.2)	3.00 d (2)	4.37 d (14)
			3.52 m	2.42 dd (11.2, 2)		4.50 d (14)
NH	7.98 br s	4.54 br s				11.3 br s
N-Me			2.67 s			
10-OMe	3.87 s	3.70 s			3.87 s	
CO_2Me			3.67 s			
CH_2Cl						5.88 d (9)
						6.01 d (9)
3′	2.39 m					
	2.39 m					
4′	1.96 m					
	2.58 m					
5′	5.15 dd (7.6, 5.2)					
N1′-H	5.94 br s					

[a]Assignments based on COSY and HMQC. Compounds **1**−**4** were obtained in $CDCl_3$; compound **5** was obtained in $CDCl_3$−CD_3OD; compound **6** was obtained in DMSO-d_6.

ments (m/z 393.2419) and ^{13}C NMR data established the molecular formula as $C_{24}H_{31}N_3O_2$ (calcd for 393.2416), differing from ibogaine by C_4H_5NO. Other significant fragment ion peaks were observed at m/z 378 $[M - CH_3]^+$, 364 $[M - CH_2CH_3]^+$, and 308 $[M - C_4H_6NO]^+$. Comparison of the MS, IR, and ^{13}C NMR data of **1** with those of ibogaine indicated that the C_4H_6NO fragment lost from the molecule of **1** was associated with the intense IR band at 1684 cm^{-1} as well as the carbon shift at δ_C 179.2, indicating that the C_4H_6NO fragment corresponds to a pyrrolidone unit present in **1**. The ^{1}H and ^{13}C NMR data of **1** (Tables 1 and 2) bear a striking similarity to those of ibogaine, except for the presence of signals due to the pyrrolidone unit and the conspicuous absence of the H-11 signal, from the observation of the two remaining aromatic hydrogens as sharp singlets. The two aromatic singlets (δ_H 6.90, H-9; δ_H 7.14, H-12) can be readily distinguished from the NOE interactions observed between H-9/H-6 and H-12/N1-H.

Analysis of the COSY and HMQC data revealed the presence of an NCHCH$_2$CH$_2$ partial structure corresponding to the N-1′−C-5′−C-4′−C-3′ fragment of the lactam unit, in addition to the partial structures corresponding to those in ibogaine. The rather deshielded signal due to H-5′ observed at δ_H 5.15 was consistent with the location of C-5′, α to both N-1′ and the indole aromatic ring. Heteronuclear long-range correlations from H-12 to C-5′; H-5′ to C-10, C-11, C-12, C-2′; H-4′ to C-11, C-2′; and, N1′-H to C-2′, C-4′, C-5′ not only confirmed the attachment of the indole C-11 to C-5′ but also firmly established the presence of the pyrrolidone moiety in **1**.

The NOE interactions observed between H-12/H-5′, N1′-H provided further support for the attachment of a pyrrolidone unit from C-5′ to the ibogaine moiety at C-11. These, as well as other NOEs observed for **1** (Figure 1) were however insufficient to determine the configuration at C-5′. Compound **1** is the second example of a monoterpene indole alkaloid incorporating a pyrrolidone unit, the first being bannucine, in which a vindoline alkaloid is connected from C-10 to the pyrrolidone unit at C-5′.[13]

Compound **2** (ervaluteine) was obtained from the stem-bark extract as a yellowish oil with $[\alpha]^{25}_D$ −37 (c 0.4, $CHCl_3$). The UV spectrum showed absorption maxima at 227, 253 (shoulder), and 414 nm, characteristic of a 10-methoxy pseudoindoxyl chromophore (e.g., iboluteine,[14] voaluteine,[15] and voacristine pseudoindoxyl[16]), while the IR spectrum showed bands due to

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Table 2. ^{13}C NMR Data (δ) for 1−6 (100 MHz)[a]

C	1	2	3	4	5	6
2	143.2	72.7	75.0	130.4	154.4	164.8
3	50.0	53.7	53.5	52.6	52.5	71.8
5	54.3	57.4	63.2	50.3	66.1	61.6
6	20.7	62.0	39.2	16.7	201.1	42.8
7	108.8	203.1	52.2	112.7	113.1	56.0
8	128.9	122.5	132.5	129.7	128.2	133.7
9	98.9	104.3	121.3	118.1	104.4	122.1
10	151.0	153.9	119.2	123.9	155.9	121.3
11	124.9	127.3	127.8	124.5	112.7	129.0
12	106.9	115.0	109.0	116.3	111.1	110.7
13	129.1	156.5	153.9	134.4	130.6	143.1
14	26.4	25.5	21.4	29.9	26.7	27.9
15	31.9	32.7	31.7	35.7	31.0	26.5
16	41.2	41.0	52.0	167.2	41.7	112.0
17	34.2	24.1	42.4	38.8	32.6	185.2
18	11.9	11.9	12.8	6.8	11.4	13.7
19	27.8	28.9	116.1	32.5	26.6	128.7
20	41.9	39.0	137.4	69.7	40.8	131.9
21	57.6	53.2	55.7	53.6	54.9	63.4
10-OMe	55.8	55.6			55.5	
N-Me			34.1			
CO_2*Me*			51.8			
CO_2Me			176.1			
CH_2Cl						70.0
2′	179.2					
3′	29.9					
4′	29.4					
5′	53.1					

[a]Assignments based on HMQC and HMBC. Compounds **1**−**4** were obtained in $CDCl_3$; compound **5** was obtained in $CDCl_3$−CD_3OD; compound **6** was obtained in DMSO-d_6.

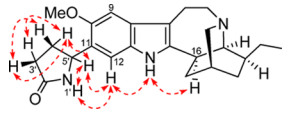

Figure 1. Selected NOEs of **1**.

carbonyl (1670 cm^{-1}) and NH/OH (3336 cm^{-1}) functions. The EIMS showed a molecular ion at m/z 342, with the base peak at m/z 313 due to loss of CH_2CH_3. HREIMS (m/z 342.1943) and ^{13}C NMR data established the molecular formula of **2** as $C_{20}H_{26}N_2O_3$, differing from that of iboluteine [ibogaine-2(R)-pseudoindoxyl] by replacement of H with OH. The ^{13}C NMR data (Table 2) showed a total of 20 carbon resonances, comprising two methyl, five methylene, eight methine, one ketocarbonyl, one methoxy-substituted aromatic carbon (C-10), two tertiary carbons linked to the indolic nitrogen (corresponding to C-2 and C-13), and one quaternary carbon atom (C-8), in agreement with the molecular formula. The oxymethine carbon resonance (δ_C 62.0) observed in the ^{13}C NMR spectrum was consistent with the presence of an OH function. The ^{1}H NMR data (Table 1) showed, in addition to an oxymethine signal at δ_H 4.37, the presence of three aromatic hydrogens, an NH, a methoxy group, and an ethyl side chain. The ^{1}H and ^{13}C NMR data of **2** bear some similarities to those of iboluteine. Analysis of the COSY and HMQC data, however, revealed that the NCH_2CH_2 fragment corresponding to the N−C-5−C-6 unit present in iboluteine was absent in **2** and was replaced instead by an NCH_2CHOH fragment. This observation was further supported by the HMBC data, which showed three-bond correlations from H-6 to C-7 and from H-5 to C-2, C-3, and C-21. Compound **2** is therefore the 6-hydroxy derivative of ibogaine pseudoindoxyl.

The marked departure of the C-2, C-7, C-16, and C-21 resonances in **2** from those of iboluteine suggested that the spirocyclic C-2 in **2** has opposite configuration compared to that in iboluteine [i.e., 2(S) in **2**, 2(R) in iboluteine]. In order to provide direct confirmation for the configuration of C-2 in **2**, NOE experiments were carried out (Figure 2). Irradiation of the

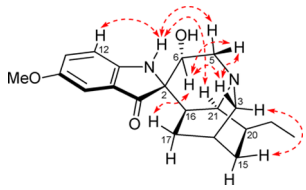

Figure 2. Selected NOEs of **2**.

NH signal resulted in enhancement of the H-5α and H-21 signals and vice versa, thus confirming the configuration of the C-2 spirocenter in **2** as S. In addition, examination of models showed that H-17β in **2** is in close proximity with the C-7 carbonyl function, while in iboluteine, it is H-21 instead, which is in close proximity with the carbonyl function. This is in accord with the observation that while the H-17β signal was relatively deshielded (from δ_H 1.72 in iboluteine to δ_H 2.51 in **2** due to anisotropy from the C-2 carbonyl), the H-21 resonance was relatively shielded (from δ_H 3.52 in iboluteine to δ_H 2.43 in **2** due to absence of anisotropy from the C-2 carbonyl). In addition, the configuration at the hydroxy-substituted C-6 stereocenter was also readily determined to be R from the observed reciprocal NOEs between H-3β/H-5β and H-6/H-3β, H-5β, H-17β (Figure 2). Ervaluteine is therefore 6(R)-hydroxyibogaine-2(S)-pseudoindoxyl.

Compound **3** (vincamajicine), $[\alpha]^{25}_D$ −12 (c 0.3, $CHCl_3$), showed an M^+ at m/z 350 (HRMS m/z 350.1995) in the mass spectrum, which analyzed for $C_{22}H_{26}N_2O_2$, differing from vincamajine by the replacement of OH with H. Other significant fragments were detected at m/z 335 $[M - Me]^+$ and 291 $[M - CO_2Me]^+$. The IR spectrum showed the presence of an ester function (1728 cm^{-1}), while the UV spectrum was typical of a dihydroindole chromophore, with absorption maxima at 207, 250, and 295 nm. The ^{1}H NMR data (Table 1) showed a general similarity to those of vincamajine,[17] except for the replacement of the hydrogen signal due to the isolated C-17 oxymethine present in vincamajine by signals due to a pair of geminal hydrogens observed at δ_H 1.46 and 2.58 in **3**. In addition, the characteristic oxymethine carbon resonance at δ_C 74.5 in vincamajine was also not observed in the ^{13}C NMR spectrum of **3**, while a methylene carbon resonance at δ_C 42.4 was observed instead (Table 2). This suggested that **3** is the C-17-deoxy derivative of vincamajine. This was further supported by the presence of the long-range W-coupling observed between H-17α and H-6α, as well as the NOE interaction observed for H-17β/H-6β.

Compound **4** (tacamonidine) was obtained in minute amounts as a colorless oil with $[\alpha]^{25}_D$ +63 (c 0.2, $CHCl_3$). The UV spectrum showed absorption maxima at 203, 242, 266, and 298 nm typical of an N-acyl indole chromophore (e.g., tacamonine and eburnamonine).[18] The IR band observed at 1702 cm^{-1} as well as the observed carbon resonance at δ_C 167.2 due to the lactam carbonyl associated with the indolic N-1 confirmed the presence of an N-acyl indole chromophore. This

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was also in agreement with the presence of the characteristically deshielded H-12 signal (δ_H 8.38) in the ^{1}H NMR spectrum (Table 1) due to anisotropy from the proximate C-16 lactam carbonyl. The IR spectrum also showed a band at 3387 cm^{-1}, indicating the presence of an OH group. The EIMS of **4** showed a molecular ion at m/z 310, which analyzed for $C_{19}H_{22}N_2O_2$ (HRMS m/z 310.1676), differing from tacamonine by addition of 16 mass units and consistent with replacement of a H by an OH group. This was further supported by the ^{1}H and ^{13}C NMR data (Tables 1 and 2), which showed a close correspondence with those of tacamonine[18] except for some notable differences. For instance, the signal due to H-20 present in tacamonine was conspicuously absent in the ^{1}H NMR spectrum of **4**, while the signal due to C-20 was found to resonate at lower field (δ_C 69.7) in the ^{13}C NMR spectrum, as a result of OH-substitution at C-20. The β-orientation of the OH group at C-20 was indicated by the significant downfield shift observed for H-14 from δ_H 2.45 in tacamonine to δ_H 2.83 in **4**. This observation was due to paramagnetic deshielding of H-14 by the spatially proximate OH group since H-14 is 1,3-*trans*-diaxial to C-20-OH (Figure 3).[19,20]

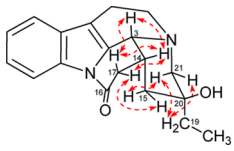
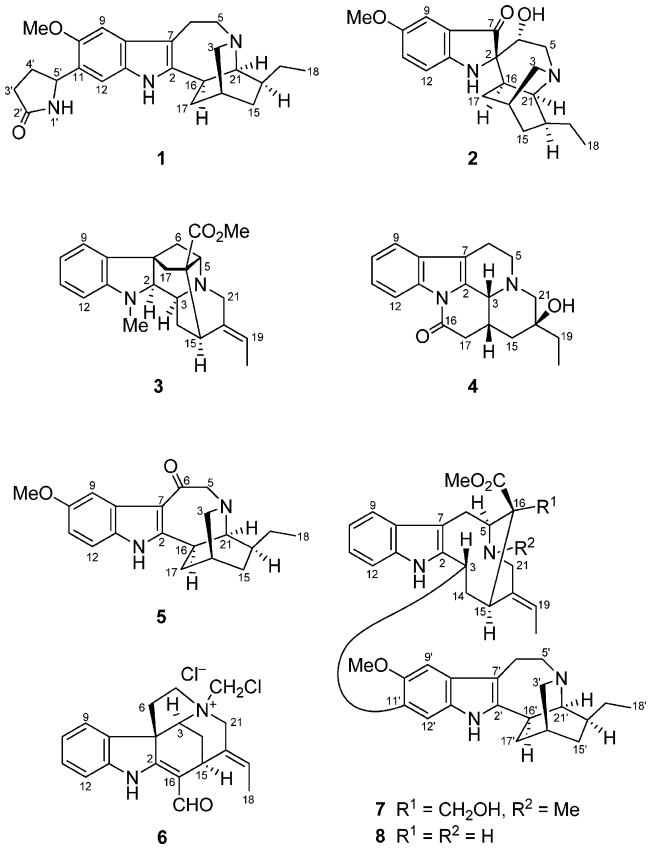


Figure 3. Selected NOEs of **4**.

This in turn required the OH group at C-20 to be β-oriented. Further confirmation of the configuration at C-20 was provided by the reciprocal NOEs observed between H-19 and H-21α, which is possible only if the OH group at C-20 is β-oriented. The relative configurations at other stereogenic centers were firmly established from the examination of coupling constants and the results of extensive NOE experiments (Figure 3).

Compound **5** (6-oxoibogaine) was obtained as a colorless oil with $[\alpha]^{25}_D$ +43 (c 0.1, $CHCl_3$). The UV spectrum (214, 253, 280, and 305 nm) was somewhat similar to that of ibogaine with an additional band, due possibly to the presence of additional conjugation to a ketocarbonyl function. The mass spectrum showed a molecular ion at m/z 310, and HREIMS measurements (m/z 324.1838) and ^{13}C NMR data established the molecular formula as $C_{19}H_{22}N_2O_2$. The molecular formula of **5** indicated that it differed from ibogaine by replacement of two hydrogens with an oxygen atom. The presence of a resonance at δ_C 201.1 in the ^{13}C NMR spectrum (Table 2) confirmed the presence of the conjugated carbonyl moiety. The ^{1}H NMR data of **5** (Table 1) was generally similar to that of ibogaine, except for the presence of a pair of AB doublets (δ_H 3.69 and 3.81, J = 18.1 Hz) in place of the usual signals due to the C-5−C-6 ethylene fragment present in ibogaine, thus indicating that the carbonyl function is located at either C-5 or C-6. Another notable difference between the ^{1}H NMR spectrum of **5** and that of ibogaine is that the H-9 signal of **5** (δ_H 8.01) was significantly deshielded compared to that of ibogaine (δ_H 6.93). The deshielding of H-9 was due to anisotropy from the proximate carbonyl function. Examination of models showed that the carbonyl function has to be located at C-6 in order for H-9 to experience the observed anisotropic deshielding. This was further verified by the HMBC data, which showed three-bond correlations from H-5 to C-3, C-7, and C-21. 6-Oxoibogaine (**5**) was encountered previously in a stereoselective synthesis of the iboga and epi-iboga alkaloids,[21] but was isolated for the first time from a natural source in the present study.



Compound **6** (N^4-chloromethylnorfluorocurarine chloride), $[\alpha]^{25}_D$ −674 (c 0.7, EtOH), was the most polar alkaloid isolated and was obtained as colorless crystals: mp >160 °C (dec), HR-FT-APCIMS m/z 341.1415 (calcd for $C_{20}H_{22}ClN_2O^+$, 341.1415). The ^{1}H NMR data (Table 1) as well as its mass fragmentation pattern strongly suggested a norfluorocurarine structure. However, two additional signals due to a pair of geminal hydrogens (a pair of AB doublets at δ_H 5.88 and 6.01, J = 9 Hz) were seen in the ^{1}H NMR spectrum, which were absent in the ^{1}H NMR spectrum of norfluorocurarine. The HMBC correlations observed from these geminal hydrogens to C-3, C-5, and C-21, coupled with the carbon shift of the methylene at δ_C 70.0, suggested the attachment of a CH_2Cl group at N-4. This was in agreement with the observed downfield shifts of H-3, H-5, and H-21 in the ^{1}H NMR spectrum of **6**, as well as the corresponding carbon shifts in the ^{13}C NMR spectrum (Table 2) when compared to those of norfluorocurarine, on account of N-4 being a quaternary center. These observations are characteristic of alkaloid N-4 oxides or ammonium salts. Compound **6** was therefore deduced to be a norfluorocurarine-CH_2Cl_2 adduct, an artifact formed as a result of the use of CH_2Cl_2 during isolation.[22] The conversion of norfluorocurarine to **6** by refluxing norfluorocurarine in CH_2Cl_2 provided further confirmation for the structure of **6**.

Compound **7** (ervatensine A) was initially obtained as a light yellowish oil and subsequently crystallized from $CHCl_3$/MeOH as colorless block crystals: mp >190 °C dec, with $[\alpha]^{25}_D$ −54 (c 0.2, $CHCl_3$). The IR spectrum showed bands due to OH (3458 cm^{-1}), NH (3393 cm^{-1}), and ester carbonyl (1717 cm^{-1}) functions, while the UV spectrum (209, 232, 288, and 296 nm) is characteristic of an indole chromophore. The ESIMS showed an

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Table 3. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data (δ) for 7 and 8 in $CDCl_3$[a]

	7		8			7		8	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2		138.1		138.0	2′		142.4		142.5
3	5.13 d (11.7)	37.4	5.12 br d (10.5)	37.5	3′	2.94 m	49.8	2.93 m	49.9
5	3.91 t (9)	60.0	4.17 br t (9)	53.4		3.01 d (9)		2.93 m	
6	3.29 m	17.1	3.26 m	24.6	5′	3.09 m	54.1	3.04 m	54.1
	3.55 m		3.60 m			3.35 m		3.32 m	
7		110.4		110.8	6′	2.57 m	20.6	2.59 m	20.7
8		129.7		129.6		3.29 m		3.30 m	
9	7.54 d (6)	117.3	7.54 m	117.4	7′		108.6		108.7
10	7.04 m	118.8	7.02 m	118.8	8′		128.0		128.2
11	6.98 m	121.6	7.02 m	121.4	9′	6.92 s	98.5	6.70 br s	98.6
12	6.94 m	109.9	7.02 m	109.8	10′		150.8		150.7
13		136.0		135.7	11′		128.6		128.6
14	2.00 m	36.4	1.97 m	36.4	12′	6.63 s	110.2	6.91 br s	110.2
	2.49 m		2.56 m		13′		129.1		129.3
15	3.55 m	35.8	3.82 m	34.1	14′	1.78 m	26.3	1.75 m	26.3
16		52.0	2.50 t (2.8)	50.1	15′	1.16 m	31.9	1.15 m	31.9
17	3.71 br s	70.4				1.75 m		1.77 m	
	3.71 br s				16′	2.77 m	41.2	2.76 m	41.2
18	1.65 d (6.3)	12.0	1.63 d (6.5)	12.0	17′	1.48 m	34.0	1.47 m	34.0
19	5.36 q (6.3)	119.6	5.23 q (6.5)	117.1		1.94 t (13)		1.93 m	
20		136.7		140.4	18′	0.86 t (7)	11.8	0.86 t (7.1)	11.8
21	2.94 d (13.4)	51.8	3.08 m	44.1	19′	1.48 m	27.7	1.44 m	27.8
	3.55 m		3.96 m			1.48 m		1.53 m	
CO_2Me	2.39 s	50.0	2.46 s	49.8	20′	1.48 m	41.8	1.50 m	41.8
CO_2Me		173.9		171.3	21′	2.76 br s	57.4	2.80 m	57.5
N1-H	7.64 br s		7.77 br s		10′-OMe	3.98 s	55.8	3.98 br s	55.9
N4-Me	2.55 s	41.8			N1′-H	7.22 br s		7.39 br s	

[a]Assignments based on COSY, HMQC, and HMBC.

$[M + H]^+$ ion at m/z 677 (m/z 677.4061), which analyzed for $C_{42}H_{52}N_4O_4$ + H. The ^{13}C NMR spectrum showed a total of 42 carbon resonances, in agreement with the molecular formula from the HRMS measurements. Examination of the ^{1}H and ^{13}C NMR data (Table 3) with the aid of COSY, HMQC, and HMBC data confirmed the presence of vobasinyl and iboga units. Thus, the ^{1}H NMR spectrum of 7 showed the presence of two indole NH, an unsubstituted indole ring (vobasinyl), another indole ring substituted at C-10′ and C-11′ (iboga), one aromatic methoxy group (iboga), one methyl ester group (vobasinyl), an *N*-methyl (vobasinyl), an ethylidene side chain (vobasinyl), a hydroxymethyl (vobasinyl), and an ethyl side chain (iboga). The ester methyl associated with the vobasinyl unit was unusually shielded (δ_H 2.39), which was in agreement with the configuration of C-16, with the ester function oriented within the shielding zone of the aromatic ring. The H-3 resonance of the vobasinyl unit was observed as a doublet at δ_H 5.13 with J = 11.7 Hz, and since only one H-3 was present, the bisindole must be branched from C-3 of the vobasine half. The attachment to the iboga unit at C-11′ was determined from the observed three-bond correlations from H-3 to C-12′ and from H-12′ to C-3 in the HMBC spectrum. Methoxy substitution at C-10′ of the iboga unit was supported by comparison of the aromatic carbon resonances with those of voacamine and 16′-decarbomethoxyvoacamine, as well as other vobasinyl-iboga bisindoles with similar substitution and branching.[23] The aromatic hydrogens of the iboga unit were observed as two singlets at δ_H 6.63 and 6.92, indicating substitution of the aromatic moiety at positions 10′ and 11′. The signal due to H-12′ (δ_H 6.63, δ_C 110.2) of the iboga moiety was readily recognized from the observed NOE interaction between H-12′ and N1′-H. This was further supported by the observed correlations from H-12′ and 10′-OMe to C-10′ in the HMBC spectrum. The configuration at C-3 can be determined from a combination of two observations. First, the signal for H-3 is a doublet with J = 12 Hz, requiring H-3 and one of the H-14 to be *trans*-diaxial. Furthermore, irradiation of H-3 resulted in NOE enhancement of N1-H and vice versa, requiring these two hydrogens to be in mutual proximity. These two observations are satisfied only in the case where H-3 has a β-orientation, since in the alternative arrangement in which the orientation of H-3 is α, the conformation adopted by the central 10-membered ring in order that one H-14 is *trans* to H-3 would result in H-3 pointing into the concave face of the middle ring and therefore away from N1-H, in which case, NOE between N1-H and H-3 would have been impossible. The ^{1}H and ^{13}C NMR data of 7 (Table 3) showed a general similarity to those of 16′-decarbomethoxyvoacamine,[23] except for the presence of the 16-hydroxymethyl group in place of H-16 present in 16′-decarbomethoxyvoacamine. Ervatensine A (7) is therefore the 16-hydroxymethyl analogue of 16′-decarbomethoxyvoacamine. Although 7 was isolated as a new compound in 2008,[24] an identical bisindole (ervachinine B) was recently reported from *Ervatamia chinensis*.[25] The ^{1}H and ^{13}C NMR data of 7 were generally identical to those reported for ervachinine B, except for the chemical shift of C-2′. The ^{13}C NMR shift obtained for C-2′ in ervatensine A (7, this study) was at δ_C 142.4, while the value reported in the other study was δ_C 136.8. Comparison of several bisindoles that incorporate the 16-decarbomethoxyiboga half present in 7 showed that the ^{13}C NMR shifts for C-2′ in these alkaloids are ca. δ_C 140−142.[26,27] On the other hand, the ^{13}C

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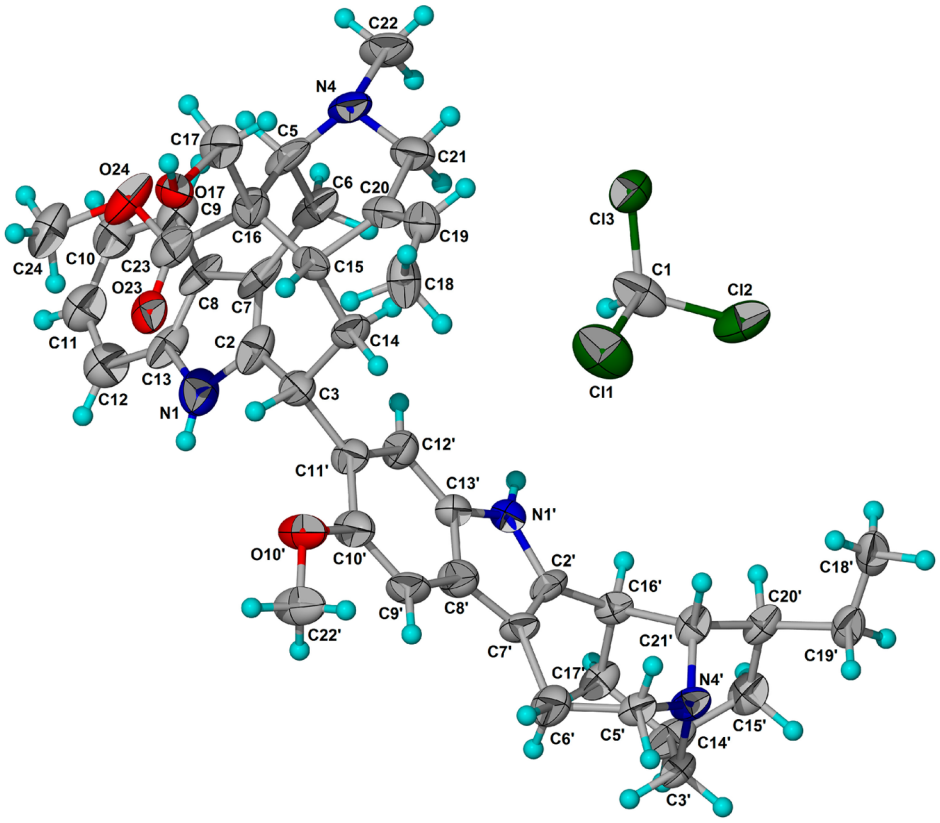

Figure 4. X-ray crystal structure of **7**.

NMR shifts for C-2′ of bisindoles that contain a 16-carbomethoxyiboga half are ca. δ_C 134−137.[26,28−30] These observations are also consistent with the C-2′ shifts reported for ervachinines C (δ_C 136.7) and D (δ_C 141.5),[25] which incorporate 16-carbomethoxy- and 16-decarbomethoxy-iboga halves, respectively. In view of these discrepancies, and since suitable crystals of **7** were eventually obtained, X-ray diffraction analysis was carried out (Figure 4), which furnished verification for the structure proposed above, based on analysis of the spectroscopic data, in addition to providing the absolute configuration for ervatensine A (**7**).

Compound **8** (ervatensine B) was obtained as a light yellowish oil with $[\alpha]^{25}_D$ −74 (*c* 0.4, $CHCl_3$). The UV spectrum was similar to that of **7**, while the IR spectrum showed bands due to NH (3396 cm^{-1}) and ester (1717 cm^{-1}) functions. The ESIMS of **8** showed a $[M + H]^+$ peak at *m*/*z* 633 (*m*/*z* 633.3799), which analyzed for $C_{40}H_{48}N_4O_3$ + H. The ^1H and ^{13}C NMR data of **8** (Table 3) were generally similar to those of 16′-decarbomethoxyvoacamine,[23] except for the signals due to N4-Me, which were absent in **8**. Instead, a broad one-proton singlet due to N4-H of the vobasinyl unit was observed at δ_H 7.77. As in the case of **7** and 16′-decarbomethoxyvoacamine,[23] compound **8** is branched from C-3 of the vobasinyl unit to C-11′ of the iboga unit, as shown by the presence of the two aromatic singlets due to H-9′ and H-12′ and the observed H-3 to C-11′ and C-12′ correlations in the HMBC spectrum. The observed $J_{3,14}$ coupling constant of 11 Hz and the NOE interaction between N1-H and H-3 confirmed the α attachment of the iboga moiety at C-3. Ervatensine B (**8**) is therefore the 16′-decarbomethoxy-N^4-demethyl derivative of voacamine.

Vincamajicine (**3**) and ervaluteine (**2**) showed strong and weak activity, respectively, in reversing multidrug resistance in vincristine-resistant KB cells (Table 4). On the other hand, the four iboga-vobasinyl bisindoles, namely, ervatensine A (**7**), ervatensine B (**8**), 16′-decarbomethoxyvoacamine,[23] and 16′-decarbomethoxyvoacaminepseudoindoxyl,[31] displayed pronounced growth inhibitory activity against drug-sensitive as well as vincristine-resistant KB cells (Table 4). Additionally, compounds **7** and **8** also displayed potent growth inhibitory activity (IC_{50} 0.44−2.83 μg/mL) when tested against A549, MCF-7, MDA-468, HCT-116, and HT-29 cells (Table 4). In contrast, however, ervachinine B from *E. chinensis* was previously reported to possess only weak growth inhibitory activity against a panel of five cancer cell lines including A549 and MCF-7.[25] Cell cycle analysis and annexin V-FITC apoptosis assays were carried out in HCT-116 and MDA-468 cells for compounds **7** and **8** to assess perturbations in the cell cycle and apoptotic events, respectively. Cell cycle analyses revealed that the substantial pre-G1 accumulations evoked by compounds **7** and **8** at 48 h were indicative of cell death (Figure 5), a conclusion further validated by annexin V-FITC apoptosis assays conducted at the same time point (Figure 6). Early and late apoptotic populations emerged following treatment of MDA 468 cells with compounds **7** and **8** and HCT 116 cells with compound **7**; however, the significant PI^+ HCT 116 population observed following exposure of cells to compound **8** at 2 μM (IC_{50}) and 4 μM (2 × IC_{50}) may indicate mixed apoptosis/necrosis.

A comparison of the alkaloids isolated in the present study (sample A)[24] with those of a previous study based on material collected from a different location in Peninsular Malaysia and at a different time (sample B)[32] showed a significant variation in the alkaloid composition. Sample A was collected from the Tekam Forest, Pahang, in June 2003, while sample B was collected near Chenderiang, Perak, in May 1996. While the two samples share a

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Table 4. Cytotoxic Effects of Selected Alkaloids[a]

compound	IC_{50}, μg/mL (μM)							
	KB/S	KB/VJ300	KB/VJ300[b]	A549	MCF-7	MDA-MB-468	HCT-116	HT-29
cononusine (1)	>25	>25	>25					
ervaluteine (2)	>25	>25	18.2 (53.2)					
vincamajicine (3)	>25	>25	0.92 (2.62)					
tacamonidine (4)	>25	>25	>25					
6-oxoibogaine (5)	>25	>25	>25					
N^4-chloromethylnorfluorocurarine chloride (6)	>25	>25	>25					
ervatensine A (7)	0.94 (1.39)	0.82 (1.21)	0.66 (0.98)	2.07 (3.06)	2.83 (4.19)	1.87 (2.77)	1.97 (2.91)	1.74 (2.57)
ervatensine B (8)	0.60 (0.95)	0.70 (1.11)	0.76 (1.20)	1.79 (2.83)	2.04 (3.23)	0.44 (0.70)	1.28 (2.03)	0.50 (1.58)
16′-decarbomethoxyvoacamine	0.99 (1.53)	0.63 (0.98)	0.52 (0.80)					
16′-decarbomethoxyvoacaminepseudoindoxyl	0.70 (1.06)	0.70 (1.06)	0.80 (1.21)					

[a]KB/S: vincristine-sensitive KB carcinoma; KB/VJ300: vincristine-resistant KB carcinoma; A549: human lung carcinoma; MCF-7: estrogen-sensitive human breast adenocarcinoma; MDA-MB-468: estrogen-insensitive human breast adenocarcinoma; HCT-116 and HT-9: human colorectal carcinoma. [b]With added vincristine 0.1 μg/mL, which did not affect the growth of the KB/VJ300 cells.

number of common alkaloid skeletons (with a predominance of the iboga alkaloids), others that were present in sample A were absent in the previous sample (B) and vice versa (Table 5). Notable differences include the following: (a) the presence of *Aspidosperma* (jerantinines A–H and jerantiphyllines A and B),[6,7] ajmaline (vincamajicine), tacaman (tacamonidine), velbanamine (velbanamine and 20(*S*)-hydroxy-1,2-dehydropseudoaspidospermidine), and vallesiachotaman (antirhine) alkaloids in sample A,[24] but not in sample B; (b) the presence of a substantial number of bisindoles (24)[32] in sample B compared to only four in sample A,[24] of which none occurred in sample B; (c) the presence of the dippinines and an akuammiline alkaloid (strictamine) in sample B,[32] but not A; (d) the presence of four unusual iboga alkaloids conjugated to lignan or pyrrolidine moieties (i.e., conoliferine,[9] the conomicidines,[10] and cononusine) in sample A,[24] but not B; (e) the exclusive presence of alkaloids with novel carbon skeletons in both sample, e.g., conolutinine[11] and lirofoline A,[33] in sample A,[24] but not in sample B, and the tronocarpines,[32,34] tronoharine,[35,36] and voastrictine[37] in sample B,[32] but not A.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a PerkinElmer RX1 FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-LA 400 spectrometer at 400 and 100 MHz, respectively. ESIMS and HRESIMS were obtained on an Agilent 6530 Q-TOF mass spectrometer. EIMS, HREIMS, FT-APCIMS, and HR-FT-APCIMS were obtained at OIC Organic Mass Spectrometry, University of Tasmania, Tasmania, Australia.

Plant Material. Plant material was collected in Pahang, Malaysia (June 2003), and identification was confirmed by Dr. K. M. Wong, Institute of Biological Sciences, University of Malaya, Malaysia. Herbarium voucher specimens (K667) are deposited at the Herbarium, University of Malaya.

Extraction and Isolation. Extraction of the stem-bark material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid (5% HCl). The alkaloids were isolated by initial column chromatography on silica gel using CH_2Cl_2 with increasing proportions of MeOH, followed by rechromatography of the appropriate partially resolved fractions using radial chromatography (Chromatotron). Solvent systems used for radial chromatography were Et_2O/hexanes (1:1, NH_3-saturated), Et_2O/hexanes (2:1, NH_3-saturated), Et_2O, Et_2O/MeOH (20:1, NH_3-saturated), CH_2Cl_2 (NH_3-saturated), CH_2Cl_2/hexanes (1:2, NH_3-saturated), CH_2Cl_2/MeOH (100:1, NH_3-saturated), CH_2Cl_2/MeOH (10:1, NH_3-saturated), EtOAc/hexanes (1:1, NH_3-saturated), and EtOAc/hexanes (1:3, NH_3-saturated). The yields (mg kg^{-1}) of the alkaloids from the stem-bark extract of *T. corymbosa* were as follows: **1** (0.51), **2** (1.24), **3** (2.34), **4** (0.44), **5** (1.02), **6** (3.65), **7** (11.8), **8** (6.86), 16′-decarbomethoxyvoacamine (21.2), 16′-decarbomethoxyvoacaminepseudoindoxyl (2.19), conolutinine (0.44),[11] lirofoline A (1.46),[33] conoliferine (4.38),[9] conomicidine A (3.07),[10] conomicidine B (0.51),[10] ibogamine (2.48), ibogaine (50.7), ibogaine-7-hydroxyindolenine (3.28), iboxygaine (0.29), iboluteine (2.19), coronaridine (0.36), heyneanine (0.36), 19-epi-heyneanine (2.77), 7(*R*)-geissoschizol oxindole (2.48),[38] 7(*S*)-geissoschizol oxindole (5.40),[38] 16(*R*),7(*R*)-19,20-*E*-isositsirikine oxindole (1.39),[38] affinisine (0.58), voachalotine (1.09), norfluorocurarine (4.38), antirhine (0.80), velbanamine (23.1), and 20(*S*)-hydroxy-1,2-dehydropseudoaspidospermidine (2.19).

Cononusine (1): colorless oil; $[\alpha]^{25}_D$ −27 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 205 (4.12), 233 (4.12), 299 (3.71) nm; IR (dry film) ν_{max} 3378, 3266, 1684 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m*/*z* 393 $[M]^+$ (67), 378 $[M - CH_3]^+$ (12), 364 $[M - CH_2CH_3]^+$ (4), 308 $[M - C_4H_6NO]^+$ (76), 220 (13),

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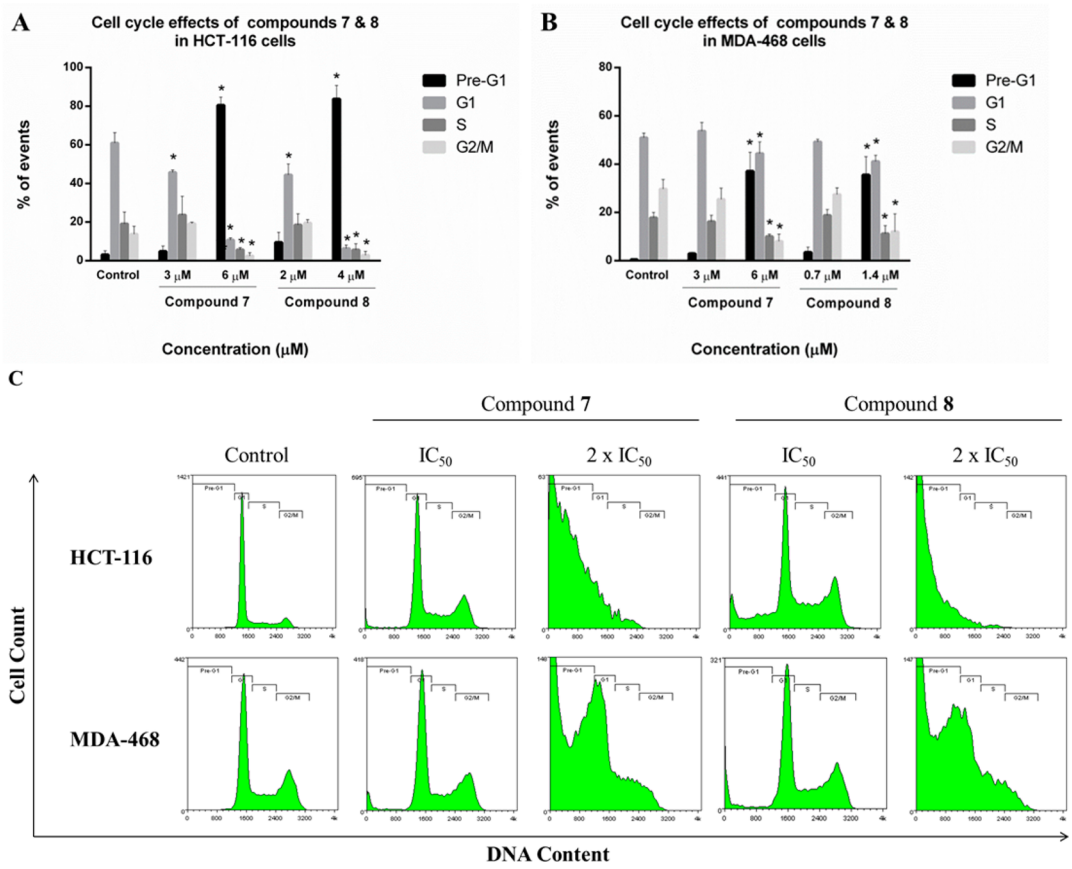

Figure 5. Cell cycle effects of compounds **7** and **8** at respective IC_{50} and $2 \times IC_{50}$ concentrations in HCT-116 (A) and MDA-468 (B) after 48 h exposure. Asterisks indicate significant ($p < 0.05$) increases or decreases in mean events with respect to control (A and B). Representative histograms are presented above (C). Experiments were repeated ≥3 times, where $n = 6$. A minimum of 10 000 events were recorded during acquisition. Both HCT-116 and MDA-468 succumbed to significant accumulations in pre-G1 events after treatment with compounds **7** and **8** (80−83% ± 2.34% and 35−37% ± 1.08%, respectively).

205 (43), 177 (9), 149 (55), 136 (100), 122 (32), 98 (9), 71 (12), 57 (21), 43 (9); HREIMS *m/z* 393.2419 (calcd for $C_{24}H_{31}N_3O_2$, 393.2416).

*Ervaluteine (**2**):* yellowish oil; $[\alpha]^{25}_D$ −37 (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 227 (4.53), 253 (4.10), 414 (3.62) nm; IR (dry film) ν_{max} 3336, 1670 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 342 $[M]^+$ (68), 325 (13), 313 $[M - CH_2CH_3]^+$ (100), 298 (19), 281 (7), 227 (10), 189 (13), 176 (11), 151 (28), 136 (32), 122 (47), 108 (18), 94 (12), 44 (13); HREIMS *m/z* 342.1943 (calcd for $C_{20}H_{26}N_2O_3$, 342.1943).

*Vincamajicine (**3**):* colorless oil; $[\alpha]^{25}_D$ −12 (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 207 (4.32), 250 (3.87), 295 (3.45) nm; IR (dry film) ν_{max} 3387, 1728 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 350 $[M]^+$ (92), 335 $[M - Me]^+$ (8), 307 (7), 291 $[M - CO_2Me]^+$ (27), 263 (7), 248 (17), 233 (8), 219 (14), 206 (55), 194 (15), 182 (30), 157 (100), 144 (98), 131 (24), 109 (19), 91 (23), 83 (18), 71 (23), 57 (29), 43 (35); HREIMS *m/z* 350.1995 (calcd for $C_{22}H_{26}N_2O_2$, 350.1994).

*Tacamonidine (**4**):* colorless oil; $[\alpha]^{25}_D$ +63 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 203 (4.18), 242 (4.20), 266 (3.91), 298 (3.57) nm; IR (dry film) ν_{max} 1702 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 310 $[M]^+$ (100), 291 (26), 281 (10), 266 (23), 238 (11), 224 (7), 210 (29), 196 (96), 180 (19), 168 (47), 149 (19), 115 (10), 105 (36), 77 (14), 69 (10), 57 (21), 43 (15); HREIMS *m/z* 310.1676 (calcd for $C_{19}H_{22}N_2O_2$, 310.1681).

*6-Oxoibogaine (**5**):* colorless oil; $[\alpha]^{25}_D$ +43 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 214 (4.47), 253 (4.25), 280 (4.10), 305 (4.09) nm; IR (dry film) ν_{max} 1573, 1595 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 324 $[M]^+$ (48), 309 $[M - CH_3]^+$ (2), 295 $[M - CH_2CH_3]^+$ (16), 252 (5), 238 (9), 225 (5), 200 (7), 176 (5), 149 (16), 135 (100), 122 (36), 108 (5); HREIMS *m/z* 324.1838 (calcd for $C_{20}H_{24}N_2O_2$, 324.1838).

*N^4-Chloromethylnorfluorocurarine chloride (**6**):* colorless crystals (EtOH); mp >160 °C (dec); $[\alpha]^{25}_D$ −674 (*c* 0.7, EtOH); UV (EtOH) λ_{max} (log ε) 202 (4.09), 242 (3.85), 298 (3.50), 361 (4.05) nm; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; FT-APCIMS *m/z* 341 $[M]^+$; HR-FT-APCIMS *m/z* 341.1415 (calcd for $C_{20}H_{22}ClN_2O^+$, 341.1415).

*Ervatensine A (**7**):* light yellowish oil and subsequently as orange block crystals from $CHCl_3$; mp >190 °C (dec); $[\alpha]^{25}_D$ −54 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 209 (4.71), 232 (4.77), 288 (4.31), 296 (4.33) nm; IR (dry film) ν_{max} 3458, 3393, 1717 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; ESIMS *m/z* 677 $[M + H]^+$; HRESIMS *m/z* 677.4061 (calcd for $C_{42}H_{52}N_4O_4$ + H, 677.4062).

*Ervatensine B (**8**):* light yellowish oil; $[\alpha]^{25}_D$ −74 (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 210 (4.57), 228 (4.61), 287 (4.16), 294 (4.17) nm; IR (dry film) ν_{max} 3396, 1717 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; ESIMS *m/z* 633 $[M + H]^+$; HRESIMS *m/z* 633.3799 (calcd for $C_{40}H_{48}N_4O_3$ + H, 633.3803).

X-ray Crystallographic Analysis of 7. X-ray diffraction analysis was carried out on an Agilent Technologies SuperNova Dual CCD area detector system equipped with mirror monochromator and using Mo Kα radiation (λ = 0.710 73 Å), at 100 K. The structure was solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on F^2 (SHELXL-2014). All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with relative isotropic parameters. Crystallographic data for compound **7** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12

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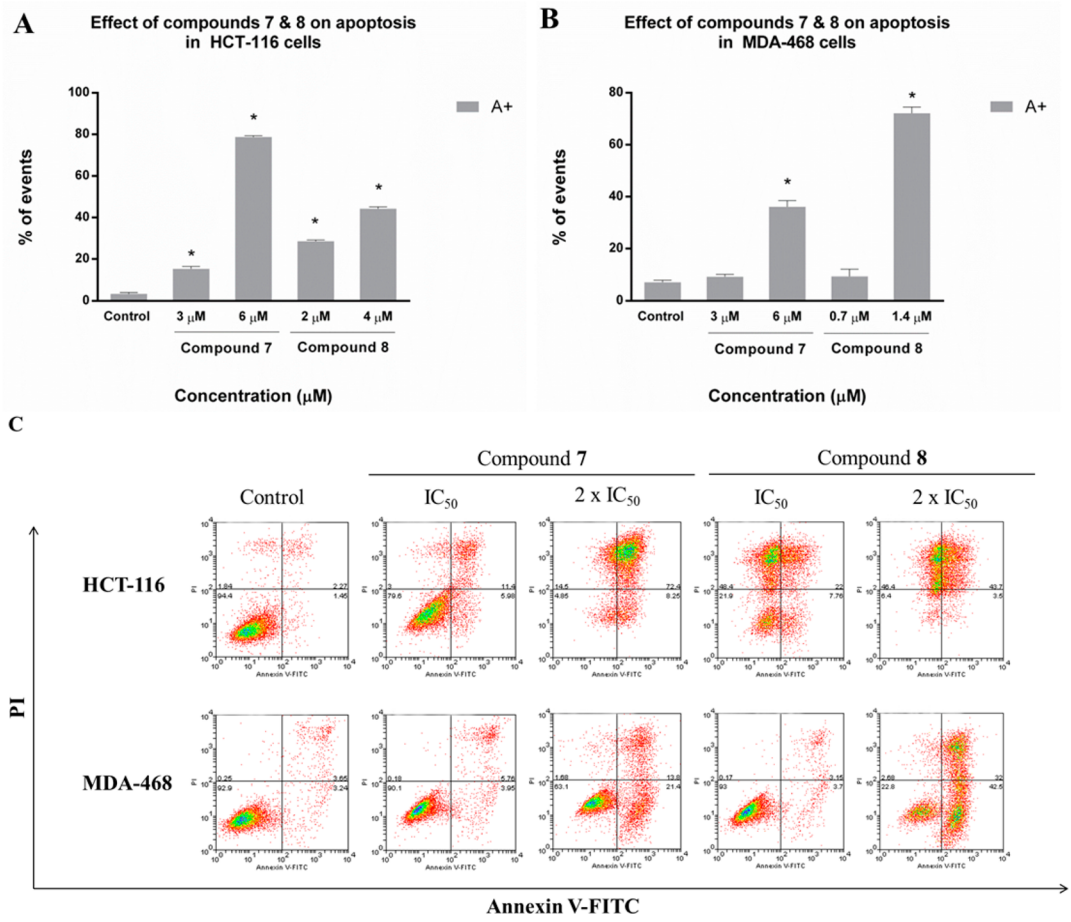

Figure 6. Apoptotic effect of compounds **7** and **8** at respective IC_{50} and $2 \times IC_{50}$ concentrations in HCT-116 (A) and MDA-468 (B) after 48 h exposure. Asterisks indicate significant ($p < 0.05$) increases in mean apoptotic events with respect to control (A and B). Representative dot plots with quadrant gating are presented above (C). Experiments were repeated ≥3 times, where $n = 6$. A minimum of 10 000 events were recorded during acquisition. Dose-dependent increases in apoptotic events corroborated with pre-G1 events seen in the cell cycle. Note that the lower left quadrant signifies a healthy cell population, whereas the lower and upper right signify early and late apoptosis, respectively (cumulatively defined as A^+). The upper left quadrant is representative of necrosis.

Table 5. Comparison of Alkaloid Composition in Two Malayan *T. corymbosa*

alkaloid type	sample A[a]	sample B[b]	number of common alkaloids
bisindole	4	24	
iboga	10	17	4
iboga conjugates	4		
dippinine−chippiine		4	
corynantheine	3	2	2
sapargine/vobasine	2	5	1
Strychnos	2	4	1
Aspidosperma	10		
akuammiline		1	
ajmaline	1		
vincamine	1		
tacaman	1		
vallesiachotaman	1		
velbanamine	2		
new skeletons	2	5	

[a]Collected in Tekam Forest, Pahang, in June 2003.[24] [b]Collected near Chenderiang, Perak, in May 1996.[32]

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Crystallographic data of ***7****:* colorless block crystals, $C_{42}H_{52}N_4O_4 \cdot CHCl_3 \cdot 3H_2O$. $M_r = 850.29$, hexagonal, space group $P6_5$, $a = 25.987(3)$ Å, $b = 25.987(3)$ Å, $c = 12.7789(12)$ Å, $Z = 6$, $D_{calc} = 1.132$ g cm^{-3}, crystal size $0.4 \times 0.2 \times 0.2$ mm^3, $F(000) = 2712$, $T = 100$ K. The final R_1 value is 0.1011 ($wR_2 = 0.2847$) for 3366 reflections [$I > 2\sigma(I)$]. Flack parameter [$x = 0.04(11)$].[39] CCDC number: 1031871.

Growth Inhibitory Assays. MTT assays were carried out following the procedure that has been described in detail previously.[40,41]

Flow Cytometry. Cell cycle and annexin V-FITC apoptosis assays were carried out following procedures that have been detailed previously.[8]

Statistical Analysis. All statistical analyses were performed using GraphPad Prism 6. Cell cycle and apoptosis gating and analyses were carried out using Weasel v3.0 (open source software). Statistical significances ($p < 0.05$) in cell cycle phases and total apoptosis (A^+; i.e., A^+/PI [lower right quadrant], plus A^+/PI^+ [upper right quadrant]) between control and treatment groups were done using two-way analysis of variance (ANOVA) and Student's *t* tests, respectively.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra for compounds **1**−**8**. X-ray crystallographic data in CIF format for compound **7**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00117.

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Notes

The authors declare no competing financial interest.

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DEDICATION

Dedicated to Dr. Kanki Komiyama (Kitasato University and, formerly, The Kitasato Institute, Tokyo, Japan) on the occasion of his 73rd birthday.

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