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# Terpenoid Indole Alkaloids from *Winchia calophylla*

Li-She Gan, Sheng-Ping Yang, Yan Wu, Jian Ding, and Jian-Min Yue\*

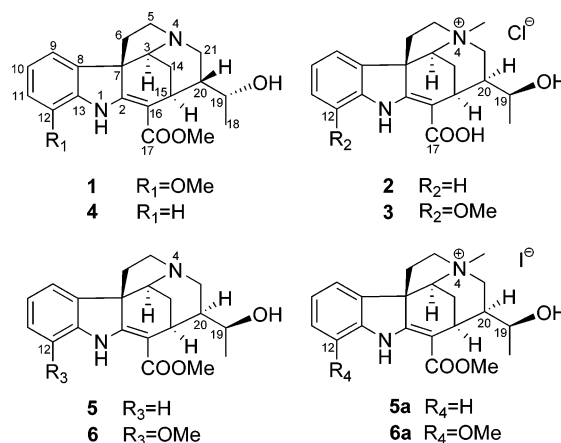
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Three new indole alkaloids, *N*(4)-demethyl-12-methoxyalstogustine (**1**), 17-carboxyl-*N*(4)-methylechitamidinium chloride (**2**), and 17-carboxyl-12-methoxy-*N*(4)-methylechitamidinium chloride (**3**), along with 15 known alkaloids, were isolated from the ethanolic extract of the stem bark of *Winchia calophylla*. The structures of **1**–**3** were elucidated on the basis of spectroscopic means and chemical methods. The determination of relative configurations at C-19 and C-20 for **1**–**3** was aided by  $^{13}\text{C}$  NMR spectroscopic data. The absolute configurations of alkaloids **1**–**3** were determined by direct comparison of their CD spectra with those of known alkaloids. All the alkaloids were tested in cytotoxic assays against P-388 and A-549 tumor cell lines, and only two of them showed weak activity against the A-549 cell line.

The medical applications of terpenoid indole alkaloids, such as anticancer drugs vinblastine and vincristine, have been of great interest to the fields of natural products, medicinal chemistry, and pharmacology.<sup>1</sup> The plant families Apocynaceae, Loganiaceae, and Rubiaceae are three important sources of structurally diverse terpenoid indole alkaloids.<sup>2</sup> *Winchia calophylla* A. DC. (Apocynaceae) is mainly distributed in southern China, India, Myanmar, Vietnam, and Indonesia.<sup>3</sup> The stem bark of *W. calophylla* has been traditionally applied as a folk medicine in China to treat cough and asthma.<sup>4</sup> Previous studies on the stem bark and root of *W. calophylla*, collected from Yunnan Province of China, have reported a number of indole alkaloids<sup>5</sup> and some nonalkaloid compounds.<sup>6</sup> In the present research, three new indole alkaloids, *N*(4)-demethyl-12-methoxyalstogustine (**1**), 17-carboxyl-*N*(4)-methylechitamidinium chloride (**2**), and 17-carboxyl-12-methoxy-*N*(4)-methylechitamidinium chloride (**3**), along with 15 known compounds, *N*(4)-demethylalstogustine, echitamidinium, 12-methoxyechitamidinium, *N*(4)-methylechitamidinium, echitamine, *N*(4)-demethylechitamine, akuammicine, tubotaiwine, 12-methoxytubotaiwine, 1,2-dehydroaspidoaspermidine, 6,7-*seco*-angustilobine B, undulifoline, panarine, 19,20-*E*-vallesamine, and 19,20-*Z*-vallesamine, were isolated from the ethanolic extract. The structures of these new alkaloids were elucidated on the basis of spectroscopic evidence and chemical methods. The assignment of relative configurations at C-19 and C-20 of the *N*(4)-demethyl alkaloids (**1**, **4**–**6**) by  $^{13}\text{C}$  NMR data is briefly discussed. The absolute configurations of alkaloids **1**–**3** were determined by direct comparison of their CD spectra with those of known alkaloids. We report herein the isolation and structural elucidation of these alkaloids, two of which, 12-methoxyechitamidinium (**6**) and akuammicine, showed weak cytotoxic activities against the A-549 human lung carcinoma cell line.

## Results and Discussion

*N*(4)-Demethyl-12-methoxyalstogustine (**1**) was obtained as a white amorphous solid. Its molecular formula was determined as  $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_4$  by positive mode HRESIMS at  $m/z$  371.1978 [ $\text{M} + \text{H}$ ]<sup>+</sup>, which was 30 amu more than that of the known alkaloid *N*(4)-demethylalstogustine (**4**).<sup>7</sup> The IR spectrum indicated absorptions for an ester carbonyl ( $1734\text{ cm}^{-1}$ ), double bond ( $1674\text{ cm}^{-1}$ ), and aromatic ring ( $1614$ ,  $1492$ , and  $1458\text{ cm}^{-1}$ ). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **1** exhibited similarity with those of **4**, except for the loss of one aromatic proton and the presence of an additional methoxyl. These data indicated that compound **1** was an analogue of *N*(4)-demethylalstogustine (**4**). By comparing the  $^{13}\text{C}$  NMR data of **1**



with that of **4** (Table 2), the C-12 carbon signal was significantly deshielded, and the C-11 and C-13 carbon signals were relatively shielded, suggesting that the methoxy was attached to C-12. The structure of **1** and the linkage of the methoxy at C-12 were confirmed by an HMBC experiment in which the methoxy protons at  $\delta$  3.87 correlated to C-12 at  $\delta$  144.4 (Figure 1a). The remaining NMR assignments of **1** matched those of alkaloid **4**, suggesting that both compounds have the same relative configuration. This was confirmed by a ROESY spectrum that revealed correlations of H-3 with H-5 $\alpha$  and H<sub>2</sub>-14, H-15 with H<sub>2</sub>-14 and H-20, and H-19 with H-21 $\alpha$  (Figure 1b). The specific rotation of **1** was found to be  $-442$  (in  $\text{CHCl}_3$ ), similar to that of **4** ( $-424$ , in  $\text{CHCl}_3$ ), indicating that they might have the same absolute configuration. Thus, the structure of **1** was elucidated as *N*(4)-demethyl-12-methoxyalstogustine.

17-Carboxyl-*N*(4)-methylechitamidinium chloride (**2**), a colorless amorphous solid, showed a molecular formula of  $\text{C}_{20}\text{H}_{25}\text{N}_2\text{O}_3\text{Cl}$  as determined by the positive mode HRESIMS peak at  $m/z$  341.1859 [ $\text{M} - \text{Cl}$ ]<sup>+</sup> and by negative mode ESIMS peaks at  $m/z$  375 [ $\text{M} - \text{H}$ ]<sup>−</sup> (100%) and  $m/z$  377 [ $\text{M} + 2 - \text{H}$ ]<sup>−</sup> (35%, isotope peak). The IR spectrum showed a typical absorption band between 3000 and  $2500\text{ cm}^{-1}$  for carboxylic acid. An *ortho*-disubstituted benzene ring was implied by the presence of four coupled aromatic proton signals in the  $^1\text{H}$  NMR spectrum (Table 1). A proton signal at  $\delta$  3.41 (3H, s) was assigned to a methyl bound to a heteroatom (O or N). The spectroscopic data, including NMR of **2**, showed high similarity with those of the known alkaloid echitamidinium (**5**).<sup>8</sup> The structure of **2** was finally established by an HMBC spectrum (see Figure 2a), in which the key linkage of the *N*-methyl was verified by its HMBC correlations with C-3, C-5, and C-21. The relative configuration of alkaloid **2** was established by ROESY correlations (see

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**Table 1.**  $^1\text{H}$  NMR Data of Alkaloids **1–3** (400 MHz)

H	<b>1</b> ( $\text{CDCl}_3$ )	<b>2</b> ( $d_6$ -DMSO)	<b>3</b> ( $\text{D}_2\text{O}$ )
NH	8.38 (1H, br s)	10.1 (1H, br s)	
3	4.08 (1H, s)	4.57 (1H, s)	4.44 (1H, s)
5	5 $\alpha$ 3.24 (1H, m)	5 $\alpha$ 3.80 (1H, m)	5 $\alpha$ 3.77 (1H, m)
	5 $\beta$ 3.02 (1H, m)	5 $\beta$ 3.57 (1H, m)	5 $\beta$ 3.68 (1H, m)
6	6 $\alpha$ 2.02 (1H, m)	6 $\alpha$ 1.91 (1H, m)	6 $\alpha$ 2.17 (1H, m)
	6 $\beta$ 2.30 (1H, m)	6 $\beta$ 2.83 (1H, m)	6 $\beta$ 2.90 (1H, m)
9	6.86 (1H, d, 7.4)	7.56 (1H, d, 7.3)	7.03 (1H, d, 6.9)
10	6.90 (1H, m)	6.80 (1H, t, 7.4)	6.98 (1H, t, 8.2)
11	6.77 (1H, d, 7.6)	7.09 (1H, t, 7.6)	6.95 (1H, d, 8.2)
12		6.88 (1H, d, 7.6)	
14	14 $\alpha$ 1.19 (1H, br d, 14.1)	14 $\alpha$ 1.30 (1H, br d, 13.9)	14 $\alpha$ 1.53 (1H, br d, 15.6)
	14 $\beta$ 2.27 (1H, br d, 14.1)	14 $\beta$ 2.19 (1H, br d, 14.0)	14 $\beta$ 2.25 (1H, br d, 15.0)
15	2.96 (1H, br s)	3.27 (1H, br s)	3.35 (1H, m)
18	1.12 (3H, d, 6.1)	1.02 (3H, d, 5.7)	1.17 (3H, d, 6.4)
19	3.58 (1H, m)	3.10 (1H, m)	3.37 (1H, m)
20	1.81 (1H, m)	1.89 (1H, m)	2.09 (1H, m)
21	21 $\alpha$ 2.90 (1H, br t, 13.3)	21 $\alpha$ 3.31 (1H, m)	21 $\alpha$ 3.38 (1H, m)
	21 $\beta$ 2.65 (1H, dd, 14.5, 6.1)	21 $\beta$ 2.90 (1H, dd, 13.4, 10.9)	21 $\beta$ 3.08 (1H, dd, 13.2, 13.5)
12-OMe	3.87 (3H, s)		3.89 (3H, s)
17-OMe	3.85 (3H, s)		
N(4)-Me		3.41 (3H, s)	3.40 (3H, s)

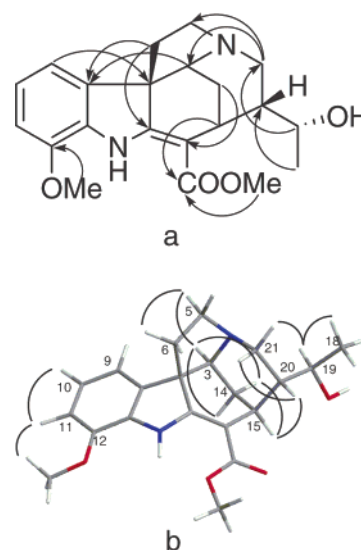
**Table 2.**  $^{13}\text{C}$  NMR Data of Alkaloids **1–6** (100 MHz)

C	<b>1</b> <sup>a</sup>	<b>2</b> <sup>b</sup>	<b>3</b> <sup>c</sup>	<b>4</b> <sup>a,d</sup>	<b>5</b> <sup>a</sup>	<b>6</b> <sup>a</sup>
2	167.1	160.0	166.5	167.5	168.9	168.9
3	58.9	69.9	73.9	59.0	60.9	61.0
5	53.5	62.6	66.5	53.8	54.2	54.2
6	46.0	38.2	40.4	46.6	43.6	43.4
7	59.2	52.5	57.0	58.5	57.3	57.9
8	136.2	133.2	136.2	135.6	135.7	136.7
9	113.3	120.8	115.6	121.1	121.4	112.2
10	121.9	119.6	125.2	120.8	119.8	122.1
11	110.3	128.2	114.9	127.9	127.6	110.0
12	144.4	109.8	147.3	109.6	109.6	144.2
13	132.4	145.8	136.1	143.7	143.7	132.3
14	27.0	26.6	29.1	27.3	31.1	31.0
15	29.2	27.6	30.5	29.2	28.9	28.9
16	103.1	107.5	108.4	102.8	96.9	97.2
17	167.8	172.5	178.1	167.8	172.5	172.3
18	20.2	20.2	21.3	20.2	19.8	19.8
19	71.0	66.1	70.0	71.0	68.4	68.4
20	45.2	41.5	44.1	45.4	45.9	46.0
21	48.1	56.4	59.3	48.3	48.2	48.2
12-OMe	55.5		58.7			55.4
17-OMe	51.5			51.4	51.9	51.8
N(4)-Me		47.6	51.4			

<sup>a</sup> In  $\text{CDCl}_3$ . <sup>b</sup> In  $d_6$ -DMSO. <sup>c</sup> In  $\text{D}_2\text{O}$ . <sup>d</sup> Data cited from ref 7 and our data were obtained in  $d_6$ -acetone.

Figure 2b). The structure of **2** was confirmed by a chemical conversion from echitamine (**5**). Methylation of **5** with  $\text{CH}_3\text{I}$  in acetone gave *N*(4)-methylechitamine iodide (**5a**), which, upon LiOH hydrolysis in aqueous  $\text{CH}_3\text{OH}$ , and by an anion exchange process gave 17-carboxyl-*N*(4)-methylechitamine chloride (**2**) (see Experimental Section). The negative specific rotation of **2** ( $-207$ , in  $\text{H}_2\text{O}$ ), compared to that of **5** ( $-505$ , EtOH),<sup>9</sup> suggested that they might have the same absolute configuration.

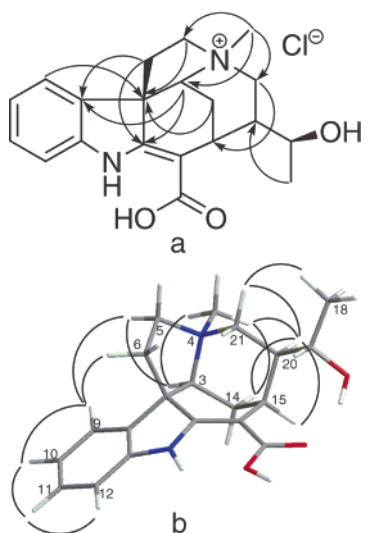
17-Carboxyl-12-methoxy-*N*(4)-methylechitamine chloride (**3**) was isolated as a colorless amorphous solid. Its molecular formula was determined to be  $\text{C}_{21}\text{H}_{27}\text{N}_2\text{O}_4\text{Cl}$  by positive mode HRESIMS ( $m/z$  371.1977 [ $\text{M} - \text{Cl}]^+$ ) and was supported by negative mode ESIMS ( $m/z$  405 [ $\text{M} - \text{H}]^-$  and  $m/z$  407 [ $\text{M} + 2 - \text{H}]^-$ ,  $\sim 3:1$  ratio). Overall spectroscopic analyses indicated that the structure of **3** is closely related to that of **2**; the only difference was the presence of a methoxy group at C-12. The structure of **3** and the linkage of the methoxy at C-12 were confirmed by the HMBC spectrum, in which the methoxy correlated with C-12 (Figure 3a). The relative configuration of **3** was deduced by ROESY correlations (Figure 3b). The structure of **3** was further confirmed by chemical conversion from the known alkaloid 12-methoxyechitamine (**6**)<sup>9</sup>

**Figure 1.** (a) Selected HMBC ( $\text{H} \rightarrow \text{C}$ ) correlations of **1**; (b) Key ROESY ( $\text{H} \rightarrow \text{H}$ ) correlations and conformation (generated by computer modeling) of **1**.

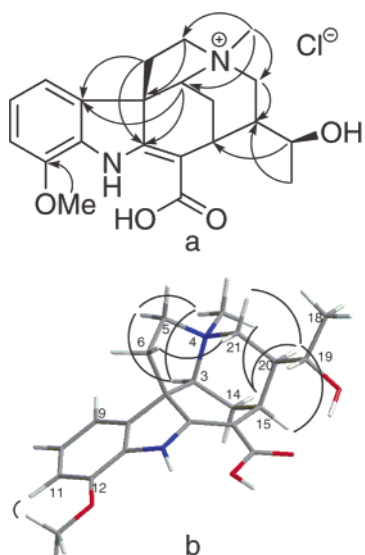
via the two-step transformation (method used for **2** above). Compounds **3** ( $-133$ , in  $\text{H}_2\text{O}$ ), **2** ( $-207$ , in  $\text{H}_2\text{O}$ ), and **6** ( $-445$ , in  $\text{CHCl}_3$ ) all showed negative specific rotations, indicating that they may take the same absolute configuration.

The chemical shift changes of C-14 and C-16 in alkaloids **1** (or **4**) and **5** (or **6**) could be explained in terms of the  $\gamma$ -gauche effects (Figure 4a,b).<sup>10</sup> Compared with the known compound **5** (or **6**) (Table 2), the chemical shift of C-14 in **1** (or **4**) was shielded by about 4 ppm due to  $\gamma$ -gauche effects with both C-21 and C-19, while C-16 in **1** (or **4**) was deshielded about 6 ppm due to the  $\gamma$ -gauche effect with C-21. The chemical shift of C-19 in alkaloid **1** (or **4**) was deshielded ( $\Delta$  2.6 ppm) relative to **5** (or **6**), due to intramolecular H-bonding between the nitrogen atom and the C-19-OH (Figure 4c,d).<sup>11</sup> On the basis of these analyses, the relative configurations at C-19 and C-20 of these *N*(4)-demethyl alkaloids were deduced.

The similar patterns of Cotton effects in the CD spectra corresponding to the UV absorption maxima of alkaloids **1–6** (see Figure 5) indicated that the chiral centers have absolute configuration identical to that of *N*(4)-demethylalstogustine (**4**) or other known analogues.<sup>7,12</sup> These observations further supported the relative configuration assigned for the new alkaloids **1–3**.



**Figure 2.** (a) Selected HMBC (H→C) correlations of **2**; (b) Key ROESY (H→H) correlations and conformation (generated by computer modeling) of **2**.

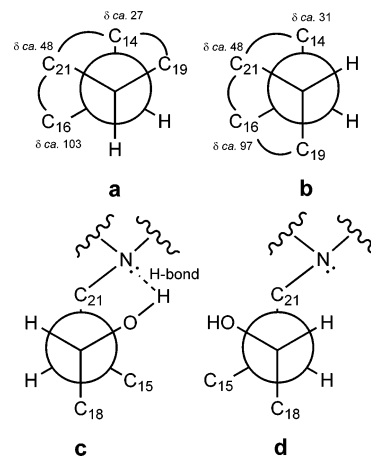


**Figure 3.** (a) Selected HMBC (H→C) correlations of **3**; (b) key ROESY (H→H) correlations and conformation (generated by computer modeling) of **3**.

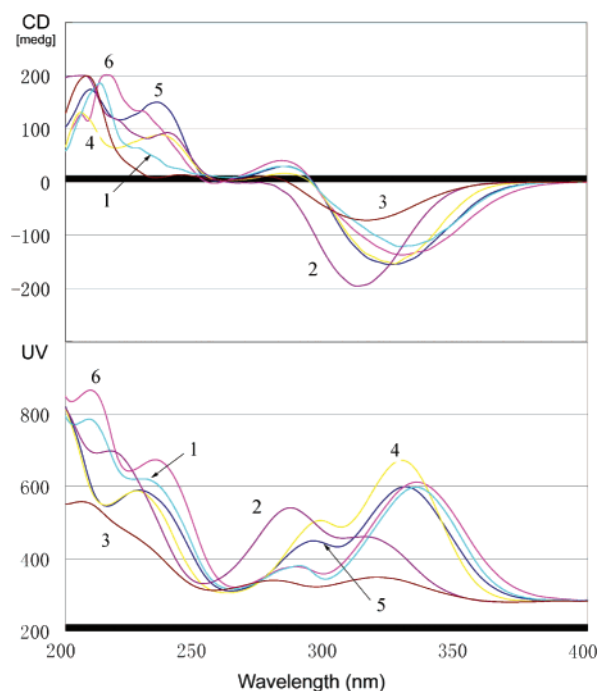
*N*(4)-Demethylstogustine (**4**) was identified by  $^1\text{H}$  and  $^{13}\text{C}$  NMR data and also by comparison with its synthetic epimer 20-epi-echitamidine.<sup>7,13</sup> Echitamidine (**5**),<sup>8</sup> 12-methoxyechitamidine (**6**),<sup>9</sup> *N*(4)-methylakuammidine,<sup>7</sup> echitamine,<sup>8</sup> *N*(4)-demethylechitamine,<sup>8</sup> akuammicine,<sup>14</sup> tubotaiwine,<sup>15</sup> 12-methoxytubotaiwine,<sup>16</sup> 1,2-dehydroaspidospermidine,<sup>17</sup> 6,7-*seco*-angustilobine B,<sup>18</sup> undulifoline,<sup>19</sup> and panarine<sup>20</sup> were determined by EIMS and  $^1\text{H}$  and  $^{13}\text{C}$  NMR data. The structures of 19,20-*E*-vallesamine<sup>21</sup> and 19,20-*Z*-vallesamine<sup>21</sup> were established by EIMS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, and HMBC spectra.

The *in vitro* activity of all the alkaloids against two tumor cell lines, P-388 murine leukemia and A-549 human lung carcinoma, was evaluated by using the MTT<sup>22</sup> and SRB<sup>23</sup> methods, respectively, with pseudolaric acid B<sup>24</sup> as a positive control. In this test,  $\text{IC}_{50}$  values of more than 50  $\mu\text{M}$  were defined as inactive. Among the tested compounds, 12-methoxyechitamidine (**6**) and akuammicine showed weak cytotoxic activities against the A-549 cell line, with  $\text{IC}_{50} = 11.5$  and 15.0  $\mu\text{M}$ , respectively.

In conclusion, the present study adds three more new structurally interesting terpenoid indole alkaloids and provides the results of *in vitro* cytotoxic evaluation on 18 terpenoid indole alkaloids. It is



**Figure 4.** Views along the C-20–C-15 bond of alkaloids **1/4** (a) and **5/6** (b). Views along the C-19–C-20 bond of alkaloids **1/4** (c) and **5/6** (d).



**Figure 5.** CD and UV spectra of **1–6**.

also helpful that the determination of relative configurations at C-19 and C-20 for this kind of terpenoid indole alkaloids, such as compounds **1–3**, was aided by  $^{13}\text{C}$  NMR spectroscopic data.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were measured on a CARY 300 Bio UV–visible spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer with KBr disks. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as internal standard. EIMS (70 eV) and ESIMS were carried out on a Finnigan MAT95 mass spectrometer and a Finnigan LC Q<sup>DECA</sup> instrument, respectively. All solvents were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China). Silica gel (200–300 mesh) was used for column chromatography, and precoated silica gel GF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for TLC. C<sub>18</sub> reversed-phase silica gel (150–200 mesh, Merck), MCI gel (CHP20P, 75–150  $\mu\text{m}$ , Mitsubishi Chemical Industries Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) were also used for column chromatography.

**Plant Material.** *W. calophylla* was collected from Hainan Province of People's Republic of China and authenticated by Prof. Shi-Man



Huang, Department of Biology, Hainan University of People's Republic of China. A voucher specimen has been deposited in Institute of Materia Medica, SIBS, Chinese Academy of Sciences (accession number: WCA-2003-2Y).

**Extraction and Isolation.** The powder of air-dried stem bark of *W. calophylla* (4.5 kg) was extracted with 95% EtOH at room temperature to give a crude extract (612 g), which was dissolved in 1.5 L of H<sub>2</sub>O to form a suspension and adjusted with 2 N H<sub>2</sub>SO<sub>4</sub> to pH  $\approx$  4. The acidic mixture was partitioned with EtOAc (1000 mL  $\times$  4), and the aqueous layer was basified with 5% Na<sub>2</sub>CO<sub>3</sub> to pH  $\approx$  10 and extracted with *n*-BuOH (800 mL  $\times$  3) to obtain 254 g after evaporation. This combined *n*-BuOH extract was subjected to a silica gel column, eluted with CHCl<sub>3</sub>/MeOH/Et<sub>2</sub>NH (30:1:0.1 to 0:1:0.1), to give two fractions of crude alkaloids Alk-1 (151 g) and Alk-2 (31 g), as well as pure echitamine (15 g). Alk-1 was subjected to an MCI gel column, eluted with H<sub>2</sub>O/MeOH (1: 1 to 0: 1), to afford two fractions, Alk-1a and Alk-1b. Fraction Alk-1a was subjected to a silica gel column eluted with petroleum ether/acetone/Et<sub>2</sub>NH (4:1:0.1) to obtain an enriched alkaloid fraction, which was then separated on a C<sub>18</sub> reversed-phase silica gel column eluted with MeOH/H<sub>2</sub>O (1:1 to 1:0) to give *N*(4)-demethylechitamine (35 mg), along with five major fractions, Alk-1a-1 to Alk-1a-5. The fractions Alk-1a-1 to Alk-1a-5 were successively purified by the following: preparative TLC with cyclohexane/EtOAc/Et<sub>2</sub>NH (1:1:0.1) to obtain 19,20-*Z*-vallesamine (5 mg); Sephadex LH-20 chromatography eluted with 100% MeOH to give undulifoline (10 mg); silica gel column eluted with cyclohexane/EtOAc (2:1) to afford 6,7-*seco*-angustilobine B (5 mg); silica gel column eluted with CHCl<sub>3</sub>/MeOH/Et<sub>2</sub>NH (30:1:0.1) to obtain **4** (30 mg); silica gel column eluted with petroleum ether/EtOAc/Et<sub>2</sub>NH (1:1:0.1) to give **1** (8 mg). Fraction Alk-1b was separated on a silica gel column eluted with a mixture of petroleum ether/EtOAc/Et<sub>2</sub>NH (4:1:0.1) to give five subfractions, Alk-1b-1 to Alk-1b-5, which were successively purified as follows: for Alk-1b-1, a C<sub>18</sub> reversed-phase silica gel column eluted with MeOH/H<sub>2</sub>O (9:1) to give 1,2-dehydroaspidospermidine (15 mg); for Alk-1b-2, a silica gel column eluted with CHCl<sub>3</sub>/MeOH/Et<sub>2</sub>NH (40:1:0.1) to obtain akuammicine (6 mg); for Alk-1b-3, a C<sub>18</sub> reversed-phase silica gel column eluted with MeOH/H<sub>2</sub>O (8:2) to give tubotaiwine (25 mg); for Alk-1b-4, a preparative TLC developed with petroleum ether/EtOAc/Et<sub>2</sub>NH (1:1:0.1) to afford 12-methoxytubotaiwine (2 mg) and 19,20-*E*-vallesamine (3 mg); for Alk-1b-5, a C<sub>18</sub> reversed-phase silica gel column eluted with MeOH/H<sub>2</sub>O (6:4) to give **5** (30 mg) and **6** (20 mg). The Alk-2 part was subjected to an MCI gel column eluted with H<sub>2</sub>O/MeOH (1: 0 to 3: 2) to give two fractions, Alk-2a and Alk-2b. Fraction Alk-2a was purified by a C<sub>18</sub> reversed-phase silica gel column eluted with MeOH/H<sub>2</sub>O (1:9) to collect three major fractions, each of which was then purified by preparative TLC developing with *n*-BuOH/MeOH (2:1) to obtain **2** (20 mg), **3** (8 mg), and panarine (10 mg). Fraction Alk-2b was purified by a silica gel column with EtOAc/MeOH (10:1) as solvent to obtain *N*(4)-methylakuammidine (15 mg).

***N*(4)-Demethyl-12-methoxyalstogustine (1):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> −442 (c 0.48, CHCl<sub>3</sub>); UV (H<sub>2</sub>O)  $\lambda_{\max}$  (log  $\epsilon$ ) 211 (4.27), 290 (3.75), 333 (4.18) nm; CD (MeOH) 213 ( $\Delta\epsilon$  +26.6), 283 ( $\Delta\epsilon$  +4.22), 328 ( $\Delta\epsilon$  −17.3) nm; IR (KBr)  $\nu_{\max}$  3390, 2928, 1734, 1674, 1614, 1492, 1458 cm<sup>−1</sup>; <sup>1</sup>H NMR, Table 1; <sup>13</sup>C NMR, Table 2; ESIMS (positive) *m/z* 371 [M + H]<sup>+</sup> (100), 339 [M − OMe]<sup>+</sup> (20); HRESIMS *m/z* 371.1978 (calcd for C<sub>21</sub>H<sub>27</sub>N<sub>2</sub>O<sub>4</sub><sup>+</sup>, 371.1965).

**17-Carboxyl-*N*(4)-methylechitamine chloride (2):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> −207 (c 0.52, H<sub>2</sub>O); UV (H<sub>2</sub>O)  $\lambda_{\max}$  (log  $\epsilon$ ) 219 (3.92), 287 (3.81), 314 (3.71) nm; CD (H<sub>2</sub>O) 206 ( $\Delta\epsilon$  +35.7), 239 ( $\Delta\epsilon$  +16.6), 312 ( $\Delta\epsilon$  −34.9) nm; IR (KBr)  $\nu_{\max}$  3423, 2973, 1637, 1606, 1548, 1480, 1461 cm<sup>−1</sup>; <sup>1</sup>H NMR, Table 1; <sup>13</sup>C NMR, Table 2; ESIMS (positive) *m/z* 341 [M − Cl]<sup>+</sup> (100), 323 [M − Cl − H<sub>2</sub>O]<sup>+</sup> (15); ESIMS (negative) *m/z* 375 [M − H]<sup>−</sup> (100), 325 [M − HCl − CH<sub>3</sub>]<sup>−</sup> (65); HRESIMS *m/z* 341.1859 (calcd for C<sub>20</sub>H<sub>25</sub>N<sub>2</sub>O<sub>3</sub><sup>+</sup>, 341.1860).

**17-Carboxyl-12-methoxy-*N*(4)-methylechitamine chloride (3):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> −133 (c 0.46, H<sub>2</sub>O); UV (H<sub>2</sub>O)  $\lambda_{\max}$  (log  $\epsilon$ ) 208 (4.04), 280 (3.63), 318 (3.67) nm; CD (H<sub>2</sub>O) 208 ( $\Delta\epsilon$  +28.8), 245 ( $\Delta\epsilon$  +1.99), 277 ( $\Delta\epsilon$  +1.53), 316 ( $\Delta\epsilon$  −10.4) nm; IR (KBr)  $\nu_{\max}$  3419, 2972, 1639, 1616, 1549, 1491, 1460 cm<sup>−1</sup>; <sup>1</sup>H NMR, Table 1; <sup>13</sup>C NMR, Table 2; ESIMS (positive) *m/z* 393 [M − HCl + Na]<sup>+</sup> (42), 371 [M − Cl]<sup>+</sup> (100), 353 [M − Cl − H<sub>2</sub>O]<sup>+</sup> (29); ESIMS (negative) *m/z* 405 [M − H]<sup>−</sup> (100), 375 [M − OMe]<sup>−</sup> (25), 355 [M − HCl − CH<sub>3</sub>]<sup>−</sup> (70), 339 [M − HCl − OCH<sub>3</sub>]<sup>−</sup> (30), 325 [M − HCl − COOH]<sup>−</sup>; HRESIMS *m/z* 371.1977 (calcd for C<sub>21</sub>H<sub>27</sub>N<sub>2</sub>O<sub>4</sub><sup>+</sup>, 371.1965).

**Chemical Transformation of Alkaloid 5 (6) into Alkaloid 2 (3).** Echitamine (**5**) (8 mg) was dissolved in 5 mL of acetone, and then 0.5 mL of CH<sub>3</sub>I was added at room temperature. The reaction mixture was stirred for 2 h to produce alkaloid **5a**, which was then treated with LiOH in an aqueous MeOH (1:1), and the reaction mixture was stirred for 8 h at room temperature. The resulting mixture was then adjusted to pH = 6 with 2 N HCl. The crude alkaloid was purified by column chromatography (C<sub>18</sub> silica gel eluted with H<sub>2</sub>O/MeOH from 1:0 to 2:1) to give 17-carboxyl-*N*(4)-methylechitamine iodide, which was then treated with an anion-exchange resin (717 type) to obtain 17-carboxyl-*N*(4)-methylechitamine chloride (2 mg), whose ESIMS, <sup>1</sup>H NMR, and TLC were identical to those of **2**. In the same way, 12-methoxyechitamine (**6**) (9 mg) was transformed into 17-carboxyl-12-methoxy-*N*(4)-methylechitamine chloride (**3**) (3 mg).

***N*(4)-Methylechitamine iodide (5a):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> −357 (c 0.53, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  7.58 (1H, d, *J* = 7.1 Hz), 7.22 (1H, ddd, *J* = 7.8, 7.6, 1.0 Hz), 7.04 (1H, d, *J* = 7.6 Hz), 6.97 (1H, dd, *J* = 7.8, 7.1 Hz), 4.62 (1H, br s), 3.88 (3H, s), 3.87 (1H, m), 3.74 (1H, m), 3.54 (1H, m), 3.52 (3H, s), 3.42 (2H, m), 3.24 (1H, dd, *J* = 3.9, 3.3 Hz), 3.02 (1H, m), 2.36 (1H, dt, *J* = 14.8, 3.0 Hz), 2.13 (2H, m), 1.57 (1H, br d, *J* = 14.5 Hz), 1.20 (3H, d, *J* = 6.2 Hz); EIMS 70 eV *m/z* (rel int) 354 (5), 336 (40), 322 (50), 282 (55), 180 (90), 94 (100).

**12-Methoxy-*N*(4)-methylechitamine iodide (6a):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> −415 (c 0.14, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  7.20 (1H, dd, *J* = 7.2, 1.2 Hz), 6.98 (1H, dd, *J* = 7.2, 5.3 Hz), 6.97 (1H, dd, *J* = 5.3, 1.1 Hz), 4.59 (1H, d, *J* = 3.1 Hz), 3.90 (3H, s), 3.87 (3H, s), 3.86 (1H, m), 3.74 (1H, dd, *J* = 11.5, 7.4 Hz), 3.55 (1H, br d, *J* = 2.6 Hz), 3.50 (3H, s), 3.49 (1H, m), 3.41 (1H, dd, *J* = 14.0, 4.3 Hz), 3.26 (1H, dd, *J* = 4.3, 3.4 Hz), 3.05 (1H, m), 2.36 (1H, dt, *J* = 14.9, 3.0 Hz), 2.15 (2H, m), 1.58 (1H, br d, *J* = 14.9 Hz), 1.21 (3H, d, *J* = 6.2 Hz); EIMS 70 eV *m/z* (rel int) 384 (1), 326 (20), 312 (61), 212 (52), 188 (59), 151 (79), 94 (100).

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**Supporting Information Available:** IR, ESIMS, <sup>1</sup>H NMR, <sup>13</sup>C NMR, and 2D NMR of *N*(4)-demethyl-12-methoxyalstogustine (**1**), 17-carboxyl-*N*(4)-methylechitamine chloride (**2**), and 17-carboxyl-12-methoxy-*N*(4)-methylechitamine chloride (**3**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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