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Oxidized Derivatives of Macroline, Sarpagine, and Pleiocarpamine Alkaloids from *Alstonia angustifolia*

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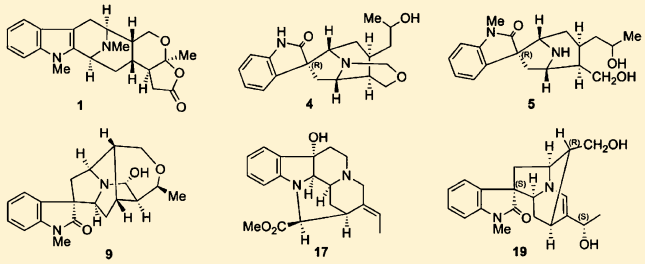
Supporting Information

ABSTRACT: A total of 20 new indole alkaloids comprising mainly oxidized derivatives of macroline- (including alstofonidine, a macroline indole incorporating a butyrolactone ring-F), pleiocarpamine-, and sarpagine-type alkaloids were isolated from the bark and leaf extracts of *Alstonia angustifolia*. The structures and relative configurations of these alkaloids were determined using NMR and MS analyses and in some instances confirmed by X-ray diffraction analyses. Alkaloids **3**, **7**, **35**, and **41** showed moderate to weak activity, while **21** showed strong activity in reversing multidrug resistance in vincristine-resistant KB cells.



The genus *Alstonia* is characterized by the preponderance of macroline-type indole and oxindole alkaloids.[1,2] Plants belonging to this genus are usually rich in alkaloids and are mainly distributed over tropical regions of Central America, Africa, and Asia.[3] In Peninsular Malaysia, these plants are mainly found in secondary and primary forests from sea level to about 3000 m altitude, as well as in swampy areas.[4−6] There are about six Malayan species, and several of these (local name Pulai) are used in traditional medicine, for example, in the treatment of malaria and dysentery.[7,8] Recent investigation of the alkaloids of *A. angustifolia* Wall has resulted in the isolation of a number of unusual alkaloids, e.g., the unusual alkaloid-pyrrole, -pyrone, and -carbamic acid adducts,[9] a group of new macroline indole alkaloids incorporating fused spirocyclic tetrahydrofuran−tetrahydrofuran and tetrahydrofuran−tetrahydropyran rings,[10] and various bisindole alkaloids.[11−13] Reported herein are the isolation and structure determination of 20 new indole alkaloids (**1**−**20**) from the leaf and stem-bark extracts of this plant.

RESULTS AND DISCUSSION

Compound **1** (alstofonidine) was obtained as a light yellowish oil, $[\alpha]_D$ −75 (*c* 0.1, $CHCl_3$). The UV spectrum was characteristic of an indole chromophore with absorption maxima at 229 and 285 nm (log ε 4.27 and 3.60, respectively), while the IR spectrum showed a band at 1780 cm^{-1}, indicative of a five-membered lactone moiety. The presence of a lactone function was confirmed by the resonance at δ 174.8 in the ^{13}C NMR spectrum. The EIMS of **1** showed a molecular ion at *m*/*z* 366, which analyzed for $C_{22}H_{26}N_2O_3$. In addition to the mass fragments characteristic of macroline alkaloids at *m*/*z* 197, 182, 181, 170, and 144, notable fragments were also observed at *m*/*z* 351, 335, and 322, due to loss of Me, CH_2OH, and CO_2, respectively. The ^{13}C NMR data (Table 1) indicated 22 carbon resonances. In addition to the eight resonances associated with the indole moiety, the ^{13}C NMR spectrum is notable for the presence of an ester carbonyl (δ 174.8), an oxymethylene (δ 63.7), and a dioxygenated secondary carbon (δ 106.5).

The ^1H NMR data of **1** (Table 3) showed the presence of an indole moiety with an unsubstituted A-ring from the signals due to four aromatic hydrogens (δ 7.13−7.51), as well as the presence of three methyl groups, corresponding to N1-Me (δ 3.63), N4-Me (δ 2.36), and Me-18 (δ 1.67). The COSY spectrum disclosed partial structures reminiscent of a macroline skeleton, such as $NCHCH_2$ and $NCHCH_2CHCHCH_2O$, corresponding to the C-5−C-6 and C-3−C-14−C-15−C-16−C-17 fragments, respectively. This was further supported by the chemical shifts and coupling patterns of H-3, H-5, H-16, and H-17, as well as the three characteristic methyl groups that are typical of macroline compounds (e.g., alstonerine **21**).[14−16] Furthermore, a $CHCH_2$ fragment was evident from the COSY spectrum. The three-bond correlations from H-17 to C-19, from Me-18 to C-20, and from H_2-21 to C-19 in the HMBC spectrum established the C-18−C-19−C-20−C-21 partial structure and indicated that the $CHCH_2$ fragment corresponds to C-20−C-21. This left the lactone function to be accounted for, which must be linked to C-21 via C-22 and to C-19 via the ester oxygen, forming the γ-lactone F-ring as required by the molecular formula. This is consistent with the deshielding of C-19 at δ 106.5 and the three-bond correlation from H-20 to the C-22 lactone carbonyl in the HMBC spectrum (Figure 1).

The ring junction stereochemistry between rings C, D, and E is assumed to be the same as in the known macroline compounds (e.g., alstonerine **21**) from the similarity of the chemical shifts and coupling patterns for the ring junction hydrogens, which are also in agreement with the NOE and NOESY data. The NOEs (Figure 1) between Me-18 and H-20 as well as

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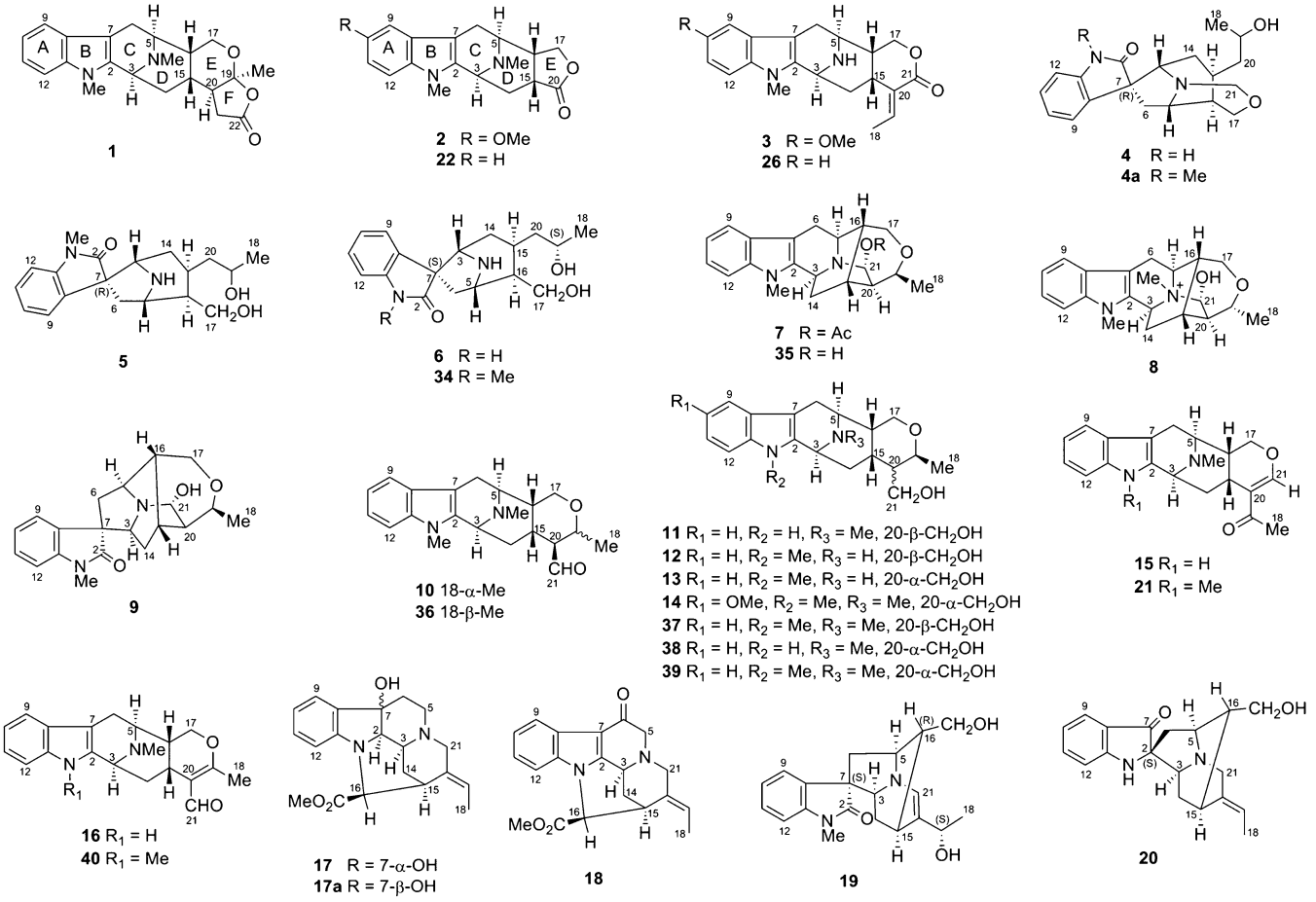
Chart 1



H-17α (δ 4.13, t, J = 12 Hz) fix the E/F ring junction stereochemistry as *cis* (Me-18 and H-20 both α). This is the first example of a macroline indole alkaloid containing an additional (sixth) ring, in this instance a γ-lactone moiety.

A putative pathway to **1** from the *D*-seco macroline alstomicine **23**[17,18] is shown in Scheme 1. Alkylation of the enolate of **23** at C-20 by a two-carbon fragment gives the intermediate keto acid **24**, which then undergoes a hemiketalization–lactonization cascade to furnish alstofonidine (**1**).

Alstofolinine B (**2**) was obtained as a light yellowish oil, with $[\alpha]_D$ −63 (c 0.3, $CHCl_3$). As with compound **1**, the IR spectrum showed the γ-lactone absorption band at 1774 cm^{-1}, which was confirmed by the resonance at δ 180.2 in the ^{13}C NMR spectrum. The EIMS of **2** showed a molecular ion at m/z 326, with the fragments due to loss of Me and OMe at m/z 311 and 295, respectively. HREIMS measurements established the molecular formula as $C_{19}H_{22}N_2O_3$ (DBE 10). The ^1H NMR data of **2** (Table 3) showed the presence of a substituted indole A-ring, an aromatic methoxy group (δ 3.85), and two methyl groups corresponding to N1-Me (δ 3.60) and N4-Me (δ 2.41). The ^{13}C NMR data (Table 1) showed 19 carbon resonances, including three methyl, three methylene, and seven methine carbons. The location of the methoxy group at C-10 was confirmed by NOE experiments (Figure 2). Irradiation of the doublet at δ_H 6.93 resulted in enhancement of the H-6β resonance (δ 2.42), which allowed the assignment of the doublet to H-9, which in turn facilitated assignment of H-11 (δ 6.86) and H-12 (δ 7.18). The COSY spectrum indicated the presence of $NCHCH_2$ and $NCHCH_2CHCHCH_2O$ partial structures, which are characteristic of a macroline structure and corresponded to the C-5–C-6 and C-3–C-14–C-15–C-16–C-17 fragments, respectively. As with the previous compound, C-17 corresponds to an oxymethylene from its resonance at δ 69.9. These partial structures led to the assembly of the macroline A/B/C/D skeleton, and as only the lactone unit remains, this moiety must constitute part of the fifth ring. This was confirmed by the observed correlations from H-14, H-15, and H-17 to the C-20 carbonyl in the HMBC spectrum (Figure 2). This is the second example of a tris-nor macroline indole alkaloid that has incorporated a γ-lactone E-ring. The first member, alstofolinine A (**22**), was recently reported from another *Alstonia*.[19]

A putative origin of the alstofolinines is shown in Scheme 2 from an alstonerine precursor, which on oxidative cleavage gives the formate-dione **25**.[20] An enzyme-mediated hydrolysis of the formate-dione[21] yields the hydroxy acid, en route to the lactone **2** or **22**.

Compound **3** (alstolactone A) was isolated in minute amounts as a colorless oil, $[\alpha]_D$ +110 ($CHCl_3$, c 0.1). The IR spectrum showed bands at 3444 and 1706 cm^{-1} due to NH and lactone carbonyl functions, respectively. The presence of the lactone function was confirmed by the resonance at δ 165.7 in the ^{13}C NMR spectrum (Table 1). The UV spectrum (226 and 285 nm) was characteristic of an indole chromophore. The EIMS of **3** showed a molecular ion at m/z 352, in addition to a significant fragment ion at m/z 321, which can be attributed to loss of OMe. HREIMS measurements (m/z 352.1786) gave the formula $C_{21}H_{24}N_2O_3$ (calcd 352.1787).

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Table 1. ^{13}C NMR Data (δ) for 1−10[a]

C	1	2	3	4	5	6	7	8	9	10
2	132.6	132.4[b]	136.6	180.0	177.6	185.0	138.9	134.5	181.3	134.6
3	53.0	51.3	46.1	68.5	66.5	63.3	41.7	50.8	56.6	52.0
5	54.8	51.6	48.0	58.9	62.4	61.6	50.3	59.0	58.1	54.3
6	22.2	22.9	28.4	39.6	41.5	40.2	26.6	24.5	43.2	21.7
7	106.5	105.5	107.3	56.2 (*R*)	58.1 (*R*)	57.8 (*S*)	103.1	100.0	54.7	105.6
8	126.2	126.8	126.9	138.5	137.2	129.6	127.2	125.9	129.0	126.7
9	118.1	100.5	100.3	124.2	120.7	124.6	118.1	118.4	126.5	117.9
10	119.1	154.0	154.1	122.5	122.5	122.6	120.9	120.2	122.1	118.7
11	121.2	111.3	111.2	127.5	127.9	128.0	118.8	122.8	128.8	120.8
12	108.8	109.7	109.8	108.7	107.9	110.1	108.7	109.7	108.4	108.7
13	137.0	132.6[b]	133.1	138.9	141.9	141.7	137.4	138.0	144.5	136.9
14	32.6	28.8	29.8	32.9	34.3	33.8	32.3	31.4	26.5	32.7
15	27.7	33.4	27.2	27.9	26.1	26.7	23.2	28.6	21.5	26.2
16	35.6	42.4	38.7	36.8	42.3	40.5	35.2	37.0	38.9	37.8
17	63.7	69.9	68.6	68.5	65.2	64.9	63.8	69.8	63.3	66.5
18	25.1		14.0	23.4	23.5	24.5	15.6	17.8	14.7	20.6
19	106.5		142.6	66.1	66.0	65.1	71.6	74.2	72.1	69.0
20	42.1	180.2	128.8	44.2	42.6	42.3	43.6	47.4	42.3	57.6
21	34.0		165.7	79.7			88.9	104.1	84.4	203.3
22	174.8									
N(1)-Me	29.0	29.5	29.2		26.4		29.3	29.9	26.6	29.0
N(4)-Me	41.8	41.5								40.9
N(4)$^+$-Me								42.5		
10-OMe		56.1	56.0							
21-OAc							21.2			
21-OAc							169.6			

[a]$CDCl_3$, 100 MHz; assignments based on HMQC and HMBC. [b]Interchangeable

Table 2. ^{13}C NMR Data (δ) for 11−20[a]

C	11	12	13	14	15	16	17	18	19	20
2	132.1	136.4	136.5[b]	134.2	129.8	129.8	70.7	152.1	181.7	72.6
3	54.9	46.5	46.2	53.8	55.8	55.8	47.8	51.1	64.1	61.2
5	54.5	48.2	48.5	55.2	55.2	55.2	47.6	63.9	60.5	59.1
6	22.5	28.5	28.3	22.7	23.2	23.2	25.9	193.0	42.2	45.8
7	107.4	107.7	107.6	106.4	105.6	105.6	76.6	110.1	55.5	206.1
8	126.9	126.6	126.5	126.7	126.4	126.8	137.2	124.9	131.0	119.1
9	118.0	118.1	117.7	100.4	117.8	117.8	120.9	121.7	126.3	124.7
10	119.3	119.1	118.8	153.9	119.4	119.4	119.7	123.5	121.8	118.7
11	121.2	121.2	120.9	110.5	121.8	121.8	127.9	122.9	128.2	137.1
12	110.8	108.9	108.8	109.6	111.5	111.5	108.6	112.0	107.8	111.9
13	135.7	136.8	136.6[b]	132.4	136.2	136.2	147.6	138.1	144.2	160.5
14	31.3	29.8	24.3	25.5	31.9	31.9	32.4	27.7	32.5	29.5
15	28.6	29.0	27.5	27.1	22.7	22.7	32.4	33.3	27.2	26.5
16	39.2	38.1	42.2	43.6	38.6	38.6	60.9	61.6	51.0	47.6
17	68.9	68.6	67.1	67.7	67.0	67.0	170.4	168.2	64.5	65.1
18	18.7	18.9	20.1	20.4	25.0	16.6	12.4	12.8	22.5	12.2
19	71.1	71.3	70.6	70.7	195.8	171.8	119.7	124.9	67.3	114.9
20	43.5	44.1	46.8	46.9	119.9	116.3	134.9	131.7	150.1	135.8
21	62.8	62.5	60.9	61.7	158.0	188.8	53.7	54.1	132.0	48.8
N(1)-Me		29.0	28.8	29.3					26.5	
N(4)-Me	41.5			41.8	41.1	41.1				
10-OMe				56.1						
CO_2Me							51.9	52.4		
CO_2Me							170.4	168.2		

[a]$CDCl_3$, 100 MHz; assignments based on HMQC and HMBC. [b]Interchangeable

The ^{1}H NMR data (Table 3) showed the presence of four aromatic hydrogens, an N1-Me, and an ethylidene side chain. The COSY spectrum showed the presence of similar macroline fragments to those in the spectra of **1** and **2**. The downfield shifts of the β-olefinic carbon and the corresponding β-olefinic H ($\delta_{C\text{-}19}$ 142.6, $\delta_{H\text{-}19}$ 7.08), compared to the α-olefinic

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Table 3. ^{1}H NMR Data (δ) for 1−10[a]

H	1	2	3	4	5	6	7	8	9	10
3	3.96 m	3.85 m	4.29 br t (3)	3.24 br d (3.6)	3.24 m	3.28 m	4.48 br dd (10, 2)	5.07 d (12)	3.73 d (9)	3.89 br d (6)
5	2.97 d (7)	3.05 d (6)	3.45 br d (7)	3.74 br d (7)	4.04 br d (8)	3.89 m	3.52 br t (5.5)	3.56 m	3.69 dd (7, 1.7)	3.00 m
6β	2.42 d (17)	2.42 d (16)	2.67 d (17)	2.28 dd (13.7, 7.8)	2.24 dd (13.7, 8)	2.10 d (13.5) (a)	2.63 d (15.6)	2.91 d (16.7)	1.83 d (13)	2.34 d (16)
6′α	3.28 dd (17, 7)	3.23 dd (16, 6)	3.24 dd (17, 7)	2.41 d (13.7)	2.52 d (13.7)	2.39 dd (13.5, 8) (b)	3.20 dd (15.6, 5.5)	3.29 dd (16.7, 5)	2.83 dd (13, 7)	3.15 dd (16, 6)
9	7.51 br d (8)	6.93 d (2.3)	6.94 d (2.5)	7.52 d (7.8)	7.10 d (8)	7.47 d (7.5)	7.47 br d (7.5)	7.49 d (8)	7.23 d (7.5)	7.47 d (7.5)
10	7.13 br t (8)			6.97 br t (7.8)	7.03 t (8)	7.01 t (7.5)	7.09 br td (7.5)	7.18 td (8, 1)	7.10 t (7.5, 1)	7.17 t (7.5)
11	7.22 br t (8)	6.86 dd (9, 2.3)	6.87 dd (9, 2.5)	7.17 td (7.8, 1)	7.29 t (8)	7.20 t (7.5)	7.19 td (7.5, 1)	7.30 td (8, 1)	7.33 t (7.5, 1)	7.08 t (7.5)
12	7.31 br d (8)	7.18 d (9)	7.20 d (9)	6.83 d (7.8)	6.84 d (8)	6.92 d (7.5)	7.29 br d (7.5)	7.37 d (8)	6.86 d (7.5)	7.27 d (7.5)
14β	1.54 m	2.10 m	1.66 ddd (13, 5, 3)	1.91 dd (13.7, 6) (α)	1.69 m (a)	1.75 m (a)	1.52 ddd (12, 4, 2.8)	1.74 ddd (12, 5, 2)	1.63 m (α)	1.64 ddd (12.7, 6, 1)
14′α	2.38 m	2.20 m	2.15 td (13, 4)	2.02 ddd (13.7, 11, 5) (β)	1.85 dd (13, 4) (b)	1.75 m (b)	1.89 ddd (12, 10, 1.6)	2.77 br t (12)	2.04 ddd (14, 5, 3) (β)	2.41 m
15	1.80 dt (13, 4)	2.15 m	2.93 m	2.99 m	3.11 m	2.68 m	2.00 m	1.93 m	2.16 br d (3)	2.16 m
16	2.02 m	2.55 dt (11, 8.5)	2.21 m	1.42 m	1.71 m	1.75 m	1.30 m	1.69 m	1.62 br s	1.71 m
17β	3.90 dd (12, 6)	4.42 t (8.5)	4.36 ddd (11, 4.6, 2)	3.83 d (11.9)	3.96 m (a)	3.98 m (a)	3.47 dd (11, 2)	3.50 br d (11.6)	3.54 dd (12, 1.4) (α)	3.57 dd (11, 4.6)
17′α	4.13 t (12)	4.50 dd (11, 8.5)	4.99 t (12)	4.23 dd (11.9, 1.8)	3.96 m (b)	3.98 m (b)	3.71 dd (11, 1)	3.79 dd (11.6, 2)	3.85 dd (12, 1.4) (β)	4.02 dd (11, 7)
18	1.67 s		1.45 d (7)	1.33 d (6)	1.31 d (6)	1.28 d (6)	1.30 d (7)	1.35 d (6.3)	1.33 d (7)	1.31 d (6.5) (α)
19			7.08 qd (7, 1)	3.95 q (6.4)	3.90 m	3.89 m	4.34 q (7)	3.57 m	4.07 q (7)	3.84 m
20	2.05 m			1.50 dt (13.3, 6.4)	1.65 m	1.56 ddd (14, 9, 5)	1.34 m	2.24 m	1.48 m	2.41 m
20′				1.69 dt (13.3, 7.8)	1.65 m	1.82 m				
21	2.48 m			4.73 d (11) (α)			5.63 br d (2)	5.55 m	5.06 d (2)	9.51 br d (2.3)
21′	2.48 m			4.89 d (11) (β)						
N(1)-Me	3.63 s	3.60 s	3.61 s		3.22 s		3.66 s	3.72 s	3.20 s	3.60 s
N(4)-Me	2.36 s	2.41 s								2.32 s
N(4)$^+$-Me								3.01 s		
N(1)-H				8.35 br s		9.42 br s				
N(4)-H			1.76 br s							
10-OMe		3.85 s	3.86 s							
21-OAc							2.16 s			

[a]$CDCl_3$, 400 MHz; assignments based on COSY, HSQC, HMQC, and NOESY/DNOE.

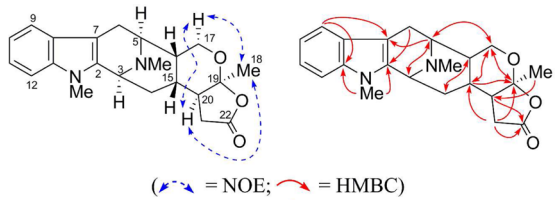
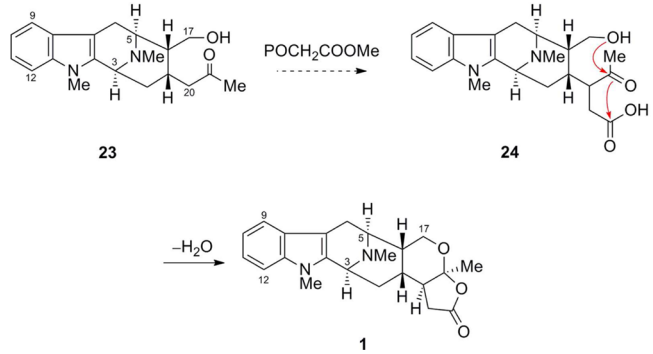


Figure 1. Selected NOEs and HMBCs of 1.

(quaternary) carbon ($\delta_{C\text{-}20}$ 128.8), indicated conjugation of the C-19−C-20 double bond with the ester/lactone function, a feature reminiscent of that in alstolactone (**26**).[22] The three-bond correlations from the oxymethylene H_2-17 and the olefinic H-19 to the C-21 carbonyl provided further confirmation that **3** is an alstolactone derivative. Furthermore, the mass of **3** differed from that of alstolactone (**26**) by 30 mass units, suggesting replacement of H with OMe. The ^{1}H NMR spectrum of **3** showed similarity to that of **26**, except for the presence of the aromatic methoxy resonance at δ 3.86.

Scheme 1. Putative Biosynthetic Pathway to 1



The presence of the aromatic methoxy group was evident from the aromatic proton spin patterns, i.e., δ 6.94, d, J = 2.5 Hz; 6.87, dd, J = 9, 2.5 Hz, and 7.20, d, J = 9 Hz. The placement of the methoxy substituent at C-10 was confirmed by the NOE interactions for H-9/H-6β, H-9/10-OMe, and H-12/N1-Me.

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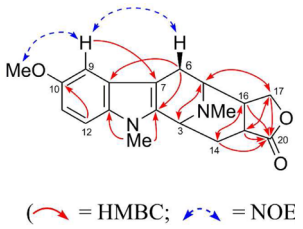

Figure 2. Selected HMBCs and NOEs of **2**.

The relative configurations at C-3, C-5, C-15, and C-16 in **3** were established from NOEs, which showed that these were similar to those in **26**. Similarly, the geometry of the exocyclic double bond is determined to be *E*, from the H_3-18/H-15 NOEs. Alstolactone A (**3**) is therefore the 10-methoxy derivative of alstolactone (**26**), which was also recently isolated from another *Alstonia*.[22]

Macrogentine A (**4**) was obtained as a colorless oil, with $[\alpha]_D$ −7 (*c* 0.1, $CHCl_3$). The UV spectrum showed characteristic oxindole chromophore absorption maxima at 210, 250, and 284 nm, while the IR spectrum showed bands due to OH (3378 cm^{-1}), NH (3288 cm^{-1}), and lactam carbonyl (1702 cm^{-1}) functions. The ESIMS of **4** showed an $[M + H]^+$ ion at *m*/*z* 329, and HRESIMS measurements yielded the molecular formula $C_{19}H_{24}N_2O_3$.

The ^{13}C NMR data (Table 1) showed 19 carbon resonances, including one methyl, five methylene, nine methine, and two quaternary carbon atoms. In addition to the resonance of a lactam function at δ 180.0, the ^{13}C NMR spectrum also indicated the presence of an oxymethylene (δ 68.5) and an oxymethine (δ 66.1). A deshielded methylene resonance at δ 79.7 was assigned to C-21, located between a nitrogen atom (N-4) and an oxygen atom. This is consistent with the deshielded hydrogen resonances of this methylene at δ 4.73 and 4.89 (each d, each *J* = 11 Hz) in the ^1H NMR data (Table 3). The ^1H NMR spectrum also showed the presence of four aromatic hydrogens (δ 6.83−7.52), an indolic NH (δ 8.35), an oxymethine (δ 3.95), and an oxymethylene (δ 3.83 and 4.23). The COSY spectrum indicated that the highest field methyl resonance at δ 1.33 is associated with the oxymethine at δ 3.95, which is in turn coupled with two doublets of triplets at δ 1.50 and 1.69. These observations are consistent the presence of a 2-hydroxypropyl side chain.[23,24]

The COSY spectrum disclosed, in addition to the 2-hydroxypropyl and C-21 methylene fragments, two other partial structures, viz., $NCHCH_2$ and $NCHCH_2CHCHCH_2O$, corresponding to the C-5−C-6 and C-3−C-14−C-15−C-16−C-17 fragments, respectively. Examination of the NMR data (Tables 1 and 3) as well as linking of these fragments based on the HMBC data (Figure 3) revealed the similarity of this compound to macroxine (**27**),[24] except for the presence of an indolic NH (δ 8.35) in place of a N1-Me group in macroxine. The configuration of the spirocarbon C-7 was determined to be *R* from the NOEs between H-9 and H-3, H-6β (pseudoaxial) (Figure 3). The configuration at the spirocyclic center in macroxine was not defined, but can be assigned as 7*R* by analogy with isoalstonisine and macrogentine, based on the diagnostic C-2 and C-8 chemical shifts (vide infra).[19,23] The reciprocal NOEs between H-6α (pseudoequatorial) and H-15 indicated that the orientation of H-15 is α. In addition, the H-15α/H-16 NOE indicated that H-16 is α-oriented. Finally, the H-6α/H-15 NOE established the *cis* C/D ring junction as well as the β-orientations of H-3 and H-5. These NOEs therefore allowed the configurations at C-3, C-5, C-7, C-15, and C-16 to be assigned as *S*, *S*, *R*, *S*, and *R*, respectively (Figure 3), which are consistent with the configurations assigned for the related oxindole alkaloid macrogentine (**28**).[19,23] However, the configuration at C-19 could not be established based on the present data, as the small amount obtained prevented further transformations or crystallization.

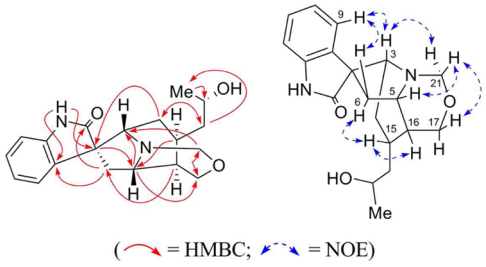

Figure 3. Selected NOEs and HMBCs of **4**.

A putative biosynthetic pathway to the macrogentine A- and macroxine-type alkaloids (**4** and **4a**)[19,23,24] is shown in Scheme 3 from isoalstonisine (**29**, R = Me) or *N*(1)-demethylisoalstonisine (**29a**, R = H), which on hydrolysis leads to the keto aldehyde **30**.[23] Intramolecular nucleophilic addition by N-4 gives the carbinol amine **31**, which in turn furnishes the formamide **32** via a retro-aldol-like reaction. Subsequent hemiacetal formation, followed by generation of the N-4 iminium ion **33** and reduction, leads to the macrogentine A/macroxine alkaloids.

Scheme 2. Putative Biosynthetic Pathway to the Alstofolinines (2 or 22)

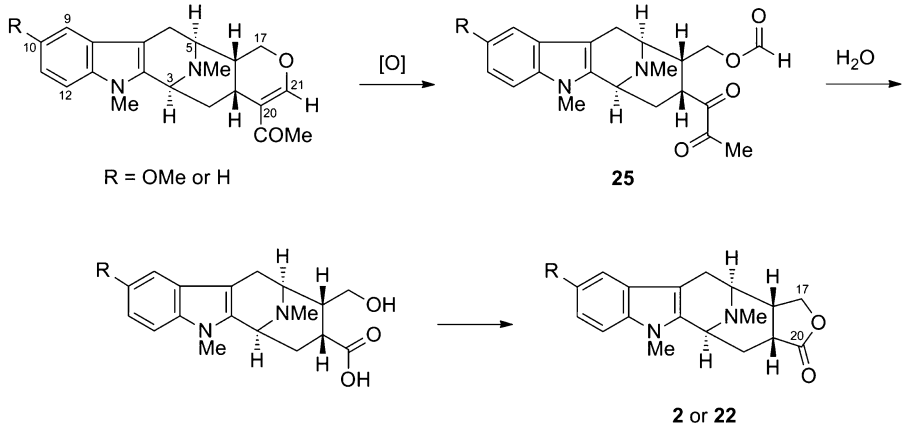

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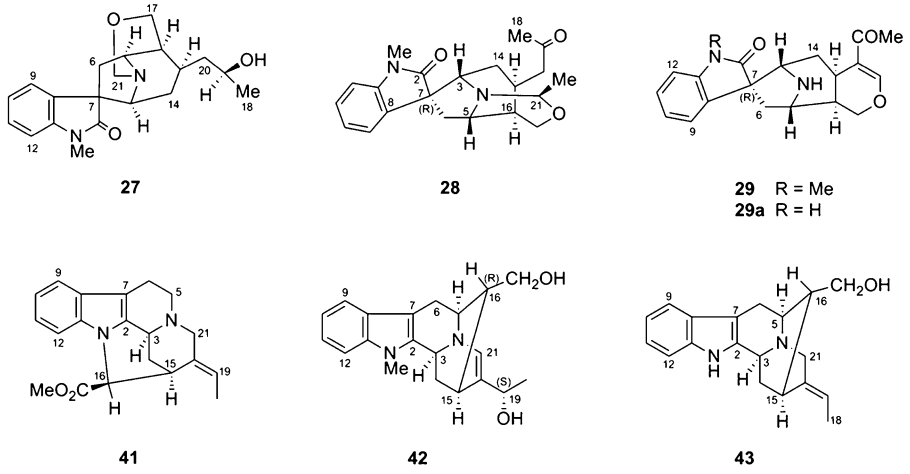
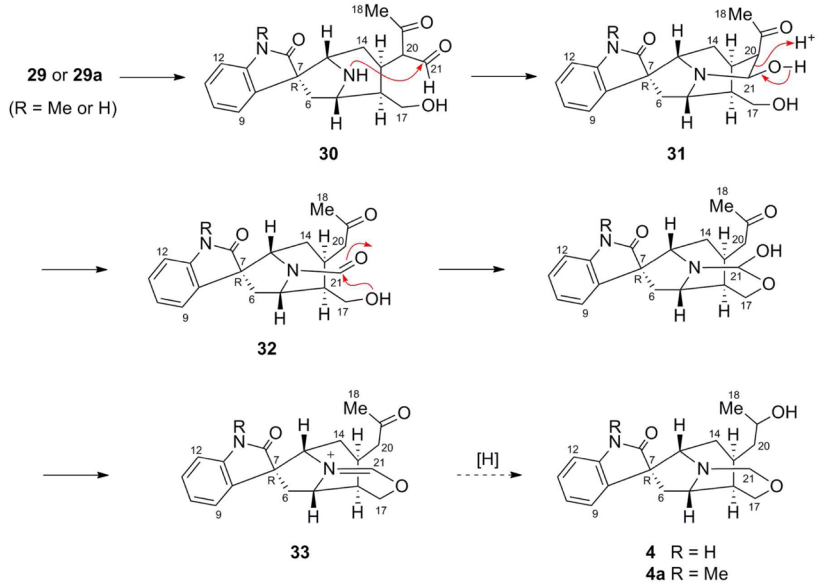
Chart 2



Scheme 3. Possible Biosynthetic Pathway to 4 and 4a



Compound **5** (isoalstonoxine B) was obtained as a colorless oil, with $[\alpha]_D$ +55 (*c* 0.6, $CHCl_3$). The ESIMS of **5** showed an $[M + H]^+$ ion at *m*/*z* 331, and HREIMS measurements yielded the molecular formula $C_{19}H_{26}N_2O_3$ (isomeric with alstonoxine B, **34**).[22] The IR spectrum showed bands at 3394 and 1702 cm^{-1} due to OH/NH and a five-membered lactam carbonyl function, respectively. The UV spectrum showed absorption maxima at 211 and 257 nm, indicative of an oxindole chromophore.

The ^{13}C NMR data of **5** (Table 1) were similar to those of alstonoxine B (**34**), showing differences only for C-2 and C-8.[19,23] It has been demonstrated that the C-2 and C-8 resonances are diagnostic for the assignment of the configuration of the spirocyclic C-7 in the macroline oxindoles.[19,23] The C-2 resonance in **34** was found at δ 182.4, while that in isoalstonoxine B (**5**) was shielded to δ 177.6. Likewise, the C-8 resonance in **5** was deshielded to δ 137.2 from δ 129.2 in **34**. These observations suggested a change in the configuration at the spirocenter C-7 in **5** when compared to that of **34**. The same behavior was also shown by the oxindole alkaloids macrogentine (**28**) and isoalstonisine (**29**), which possess an *R* configuration at the spirocyclic carbon, with the C-2 resonance at ca. δ 177 and the C-8 resonance at ca. δ 138.[19,23]

To provide confirmation of the C-7 configuration, NOE experiments were first carried out on **34**. Irradiation of H-9 resulted in the enhancement of H-15 in **34** and *vice versa*, thus confirming the configuration of the spirocenter C-7 in **34** as *S*. Similar irradiation of H-9 in the case of isoalstonoxine B (**5**) resulted instead in enhancement of H-3, H-5, and H-6β (pseudoaxial), but not H-15 (Figure 4). The other NOEs are shown in Figure 4, which are in agreement with the proposed *R* configuration for C-7 in **5**. These observations are consistent with structure **5**, although the configuration at C-19 remains undefined.

Alstonoxine E (**6**) is also an E-ring-opened macroline oxindole. Compound **6** showed an $[M + H]^+$ ion at *m*/*z* 317 ($C_{18}H_{23}N_2O_3$ + H), which was 14 mass units (CH_2) less when compared to alstonoxine B (**34**). The UV and IR spectra of these compounds were similar. The NMR data (Tables 1 and 3) for both compounds were also generally similar except for the absence of a resonance due to the N1-methyl group in **6**. Instead, an indolic NH was observed as a broad singlet at δ 9.42 in the ^1H NMR spectrum, indicating that **6** is the N1-demethyl derivative of **34**. As in **34**, the configuration at the spirocyclic C-7 was assigned as *S* from the reciprocal NOEs

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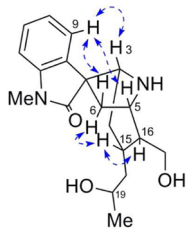

Figure 4. Selected NOEs of **5**.

between H-9/H-15. The NMR data, however, were insufficient to establish the C-19 configuration, and since suitable crystals were obtained in this case, X-ray diffraction analysis was carried out (Figure 5). Compound **6** crystallized from $CHCl_3$ as

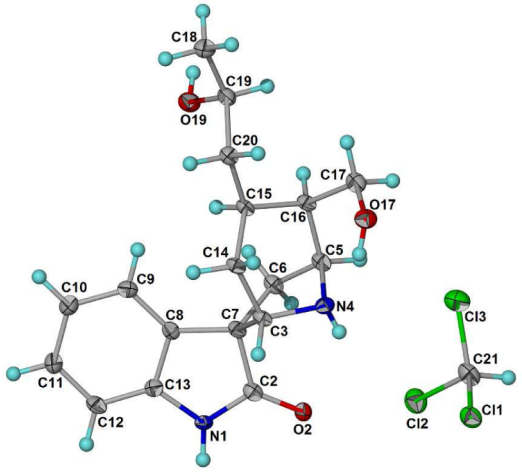

Figure 5. X-ray crystal structure of **6**.

colorless, block-shaped crystals, with mp 110−112 °C, and with incorporation of $CHCl_3$ in the crystal lattice (one molecule of $CHCl_3$ per molecule of **6**). X-ray analysis, therefore, gave the absolute configuration of compound **6**, in addition to establishing the configuration of C-19 as *S*.

O-Acetyltalpinine (**7**) was obtained as a light yellowish oil with $[\alpha]_D$ −8 (*c* 0.2, $CHCl_3$). The IR spectrum showed a carbonyl band at 1734 cm^{-1} due to an ester carbonyl function. The UV spectrum showed typical indole absorption maxima at 226 and 284 nm. The ESIMS of **7** showed an $[M + H]^+$ ion at *m*/*z* 367, and HRESIMS measurements yielded the molecular formula $C_{22}H_{26}N_2O_3$. The ^1H and ^{13}C NMR data (Tables 3 and 1) were essentially similar to those of talpinine (**35**),[25] except for the presence of resonances indicative of an acetyl group (δ_H 2.16; δ_C 21.2 and 169.6), showing that **7** is the *O*-acetyl derivative of **35**. The deshielding of the H-21 resonance from δ 4.72 in **35** to δ 5.63 in **7** indicated that the C-21-OH in **35** has been acetylated. Additional support for these conclusions was provided by chemical correlation, via acetylation (Ac_2O−pyridine) of talpinine (**35**), which yielded **7**. *O*-Acetyltalpinine (**7**) was obtained previously from acetylation of talpinine (**35**), but was isolated for the first time from a natural source in the present study.

N(4)-Methyl-19-epitalpinine (**8**), a quaternary alkaloid, was obtained as a light yellowish oil, with $[\alpha]_D$ −15 (*c* 0.1, $CHCl_3$). The IR spectrum showed a broad band at 3387 cm^{-1} due to an OH function, while the UV spectrum was characteristic of an indole chromophore (223 and 282 nm). The ESIMS data of **8** showed an $[M]^+$ ion at *m*/*z* 339, and HRESIMS measurements yielded the molecular formula $C_{21}H_{27}N_2O_2$, differing from talpinine (**35**) by addition of 15 mass units. Examination of the ^1H and ^{13}C NMR data (Tables 1 and 3) with the aid of COSY, HMQC, and HMBC suggested the presence of a talpinine-type carbon skeleton. However, an additional resonance due to a methyl singlet (δ 3.01) was present, which was absent in the ^1H NMR spectrum of talpinine (**35**). The three-bond correlations from these methyl hydrogens to C-3, C-5, and C-21 in the HMBC spectrum (Figure 6), coupled with the chemical shift

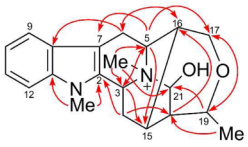

Figure 6. Selected HMBCs of **8**.

of the methyl carbon at δ 42.5, suggested the attachment of a methyl group to N-4. This was in agreement with the deshielding of H-3, H-5, and H-21 in the ^1H NMR spectrum of **8**, as well as the corresponding carbon shifts in the ^{13}C NMR data (Table 1), when compared to those of **35**, on account of N-4 being a quaternary center in **8**. As with **35**, the relative configurations at C-3, C-5, C-15, C-16, and C-21 in **8** were established from the NOEs (Figure 7), which showed that these were similar to those

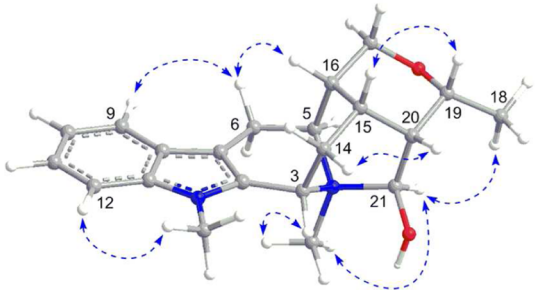

Figure 7. Selected NOEs of **8**.

in **35**. In contrast to **35** however, the orientation of H-19 is β, as deduced from the NOEs for H-19/H-15 and 18-Me/H-21, in addition to the absence of NOEs for H-19/H-21. Compound **8** is therefore *N*(4)-methyl-19-epitalpinine.

7(*S*)-Talpinine oxindole (**9**) was obtained as a light yellowish oil, with $[\alpha]_D$ −4 (*c* 0.2, $CHCl_3$). The IR spectrum indicated the presence of OH (3403 cm^{-1}) and γ-lactam carbonyl (1705 cm^{-1}) functions. The UV spectrum showed oxindole absorption maxima at 204 and 254 nm. The EIMS of **9** showed a molecular ion at *m*/*z* 340 with other major fragments due to M − H_2O (*m*/*z* 322) and M − CO (*m*/*z* 312), while HREIMS measurements gave the formula $C_{20}H_{24}N_2O_3$.

The ^1H NMR data (Table 3) showed the presence of four aromatic hydrogens, an N1-Me (δ 3.20), a methyl doublet (δ 1.33), and two distinct oxymethine resonances at δ 5.06 and 4.07. The ^{13}C NMR data (Table 1) gave a total of 20 carbon resonances including two methyl, three methylene, 11 methine, and two quaternary carbons. The ^{13}C NMR data were generally similar to those of talpinine (**35**), except that the C-2 resonance was deshielded from δ 138.9 to 181.3 (oxindole lactam carbonyl), while the C-7 resonance was shielded from δ 103.3 to 54.7, confirming the presence of an oxindole chromophore.

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Analysis of the NMR data indicated that compound **9** is talpinine oxindole. The configuration of the spirocabon C-7 was determined to be *S* from the NOEs for H-9/H-6β, H-14β, H-16 (Figure 8). In addition, the reciprocal NOEs between H-21 and

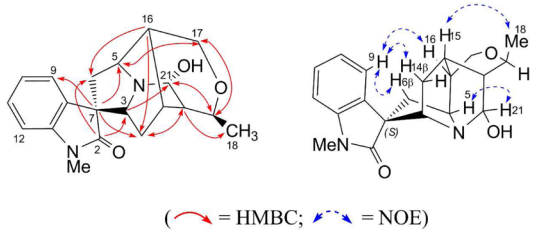

Figure 8. Selected HMBCs and NOEs of **9**.

H-5 established the orientation of the 21-OH group as α, while the orientation of H-19 is assigned as α from the NOEs for 18-Me/H-15.

Compound **10** (19-epitalcarpine) was obtained as a light yellowish oil, $[\alpha]_D$ −59 (*c* 0.2, MeOH). The IR spectrum showed the presence of a formyl function (1720 cm^{-1}), while the UV spectrum was characteristic of an indole chromophore (225 and 283 nm). The ESIMS data of **10** showed an $[M + H]^+$ ion at *m*/*z* 339, and HRESIMS measurements yielded the molecular formula $C_{21}H_{26}N_2O_2$. The ^{13}C NMR data (Table 1) indicated 21 carbon resonances (three methyl, three methylene, 11 methine, and two quaternary carbon atoms), while the resonances at δ 203.3, 69.0 and 66.5 were readily attributed to formyl, oxymethine, and oxymethylene functions, respectively.

The ^{1}H NMR data (Table 3) showed the presence of an unsubstituted indole A-ring (δ 7.08−7.47), a formyl hydrogen (δ 9.51), and three methyl groups corresponding to N1-Me (δ 3.60), N4-Me (δ 2.32), and Me-18 (δ 1.31). The ^{1}H and ^{13}C NMR data (Tables 3 and 1) revealed a macroline compound resembling talcarpine (**36**)[25] except for changes involving the C-19 configuration. The relative configuration of C-19 was deduced from the reciprocal NOEs for Me-18/H-14′α, H-17′α, which indicated that the C-19 methyl substituent has α-orientation (H-19β) (Figure 9). As in **36**, the stereochemistry

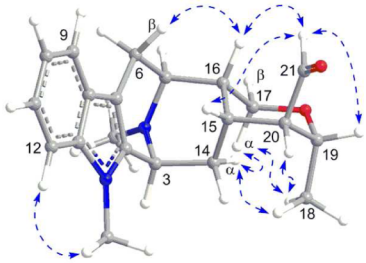

Figure 9. Selected NOEs of **10**.

of the C-20 formyl substituent was readily confirmed from the H-21/H-15, H-16 NOEs, which was only possible if H-20 is α-oriented. These observations suggested that **10** is the C-19 epimer of talcarpine (**36**).

Compound **11** (macrocarpine E) was obtained as a light yellowish oil, $[\alpha]_D$ −12 (*c* 0.8, $CHCl_3$). The UV spectrum was typical of an indole chromophore (227 and 282 nm), and the IR spectrum indicated the presence of NH (3276 cm^{-1}) and OH (3398 cm^{-1}) functionalities. The ^{1}H NMR data (Table 4) showed the presence of an unsubstituted indole A-ring (δ 7.10−7.48), two oxymethylenes (δ 3.76, 4.04; 3.64, 3.71), an indolic NH (δ 8.14), and two methyl groups at δ 2.30 and 1.21, corresponding to N4-Me and Me-18, respectively. The NMR data (Tables 2 and 4) showed that **11** belongs to the macrocarpine group of macroline alkaloids and displayed similarity with those of macrocarpine A (**37**),[17] except for the absence of resonances due to the N1-methyl group in **11**. Compound **11** is therefore the N1-demethyl derivative of **37**. This was further supported by the HRESIMS data, which showed that the measured mass of **11** was 14 mass units less that of **37**. The relative configuration at C-20 was determined from the NOE interaction between H-14β and H-20, which is only possible if H-20 is α-oriented.

Compound **12** (macrocarpine F) was obtained as a colorless oil, $[\alpha]_D$ +38 (*c* 0.6, $CHCl_3$). The UV and IR spectra were similar to those of **11**, while the EIMS data showed a molecular ion at *m*/*z* 326 ($C_{20}H_{26}N_2O_2$), indicating that **12** is isomeric with **11**. Comparison of the ^{1}H and ^{13}C NMR data of **12** with those of **11** (Tables 4 and 2) indicated that they belong to the same macrocarpine group, where the main differences between **12** and **11** were the presence of the N1-Me resonance at δ_H 3.52 (δ_C 29.0), as well as the absence of the N4-Me resonance in **12**. The presence of the methyl group at N-1 was supported by the three-bond HMBC correlations from N1-Me to C-2 and C-13. In addition, the absence of the N4-Me group resulted in the shielding of C-3 and C-5 from δ 54.9 and 54.5 in **11** to δ 46.5 and 48.2 in **12**, respectively. NOE data showed that the configurations at the stereogenic centers in **12** were similar to those in **11**.

Compound **13** (macrocarpine G) is another new macrocarpine type alkaloid. It was obtained as a light yellowish oil, $[\alpha]_D$ +7 (*c* 1.1, $CHCl_3$). The IR and UV spectra were similar to those of macrocarpine D (**38**).[19] The ESIMS data showed an $[M + H]^+$ ion at *m*/*z* 327, and HREIMS measurements gave the molecular formula $C_{20}H_{26}N_2O_2$, indicating that **13** is isomeric with **38**. The ^{1}H and ^{13}C NMR data (Tables 4 and 2) were essentially similar to those of macrocarpine B (**39**),[18] except for the absence of resonances due to the N4-methyl group in **13**, indicating that **13** is the *N*(4)-demethyl derivative of **39**.

Macrocarpine H (**14**) was obtained as a light yellowish oil, $[\alpha]_D$ −17 (*c* 0.3, $CHCl_3$). The UV spectrum showed indole absorption maxima at 230 and 289 nm, while the IR spectrum indicated the presence of a hydroxy (3413 cm^{-1}) function. The ESIMS data of **14** showed an $[M + H]^+$ ion at *m*/*z* 371, corresponding to the molecular formula $C_{22}H_{30}N_2O_3$, differing from macrocarpine B (**39**) by addition of 30 mass units. The ^{1}H and ^{13}C NMR data (Tables 4 and 2) were similar to those of **39**, except for the aromatic region, which indicated the presence of a methoxy group at C-10. The position of methoxy substitution at C-10 was confirmed by the NOE between H-9 and H-6β and between H-12 and N1-Me. The C-20 configuration was confirmed by the NOEs between H-20 and H-15, H-16.

N(1)-Demethylalstonerine (**15**) and *N*(1)-demethylalstonerinal (**16**) were isolated in minute amounts as an inseparable mixture of type-B and type-A macroline isomers (ratio ca. 5:1). The ESIMS data showed an $[M + H]^+$ ion at *m*/*z* 323 corresponding to the formula $C_{20}H_{22}N_2O_2$. The H-18 (methyl) and H-21 (vinylic-H for **15**, formyl-H for **16**) resonances were distinguishable in the ^{1}H NMR spectrum (Table 4), while the remaining hydrogen resonances are partially overlapped or coincident. In the ^{13}C NMR data (Table 2), the majority of the

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Table 4. ^{1}H NMR Data (δ) for 11−20[a]

H	11	12	13	14	15	16	17	18	19	20
2							2.92 m (α)			
3	3.85 br t (3)	4.27 m	4.27 br t (3)	3.94 m	4.14 m	4.14 m	3.30 q (3.5)	4.46 m	3.02 dd (10, 1.5)	3.24 dd (10, 2)
5	2.82 d (7)	3.21 m	3.25 m	2.90 d (7)	3.32 m	3.32 m	2.87 ddd (14, 5, 1.5) (β)	3.09 d (18) (β)	2.84 dd (6.3, 2)	2.97 m
5′							3.19 td (14, 3) (α)	3.69 d (18) (α)		
6β	2.43 d (17)	2.63 d (15)	2.58 d (16)	2.39 d (16.5)	2.70 br d (16) (a)	2.70 br d (16) (a)	1.50 m (α)		1.87 d (13)	1.62 m
6′α	3.23 dd (17, 7)	3.17 m	3.18 dd (16, 7)	3.22 dd (16.5, 7)	3.37 dd (16, 6) (b)	3.37 dd (16, 6) (b)	2.21 td (14, 5) (β)		2.75 dd (13, 6.3)	2.63 dd (12.7, 6)
9	7.48 dd (7, 1)	7.46 br d (8)	7.46 br d (7.5)	6.94 d (2.3)	7.67 d (7)	7.67 d (7)	7.25 dd (7.8, 1)	8.04 br d (8)	7.35 d (7.6)	7.59 br d (8)
10	7.10 td (7, 1)	7.07 br t (8)	7.08 td (7.5, 1)		7.09 t (7)	7.09 t (7)	6.79 td (7.8, 1)	7.28 m	7.08 t (7.6)	6.80 br t (8)
11	7.14 td (7,1)	7.18 br t (8)	7.17 td (7.5, 1)	6.83 dd (8.7, 2.3)	7.15 t (7)	7.15 t (7)	7.06 td (7.8, 1)	7.21 td (8, 1)	7.30 t (7.6)	7.42 td (8, 1)
12	7.31 dd (7, 1)	7.75 br d (8)	7.24 br d (7.5)	7.17 d (8.7)	7.87 d (7)	7.87 d (7)	6.18 br d (7.8)	7.02 br d (8)	6.81 d (7.6)	6.85 br d (8)
14β	1.44 ddd (13, 5, 3)	1.35 m	1.49 dt (12, 2)	1.52 dt (13, 4)	2.04 m (a)	2.04 m (a)	1.87 dt (13, 3.5)	2.27 ddd (13, 4, 2) (α)	1.29 m (α)	1.73 m (α)
14′α	2.43 td (13, 4)	2.45 td (12, 4)	2.18 td (12, 4)	2.25 td (13, 4)	2.22 dt (13, 3) (b)	2.22 dt (13, 3) (b)	2.08 dt (13, 3.5)	2.47 m (β)	2.14 dt (13.4, 2.7) (β)	2.07 ddd (14, 4, 2) (β)
15	1.98 dt (13, 5)	2.10 m	2.02 m	1.97 dq (13, 4)	2.65 dd (12, 5)	2.65 dd (12, 5)	3.27 m	3.67 m	2.98 m	2.80 m
16	2.08 dd (12, 5)	2.08 m	1.81 dt (12, 5)	1.85 dt (11, 5)	2.04 m	2.04 m	4.03 d (3.7) (α)	5.21 d (4) (α)	2.05 m	1.78 m
17β	3.76 dd (12, 5)	3.78 dd (11, 5)	3.72 dd (12, 4)	3.71 dd (12, 5)	4.22 dd (11, 4) (a)	4.22 dd (11, 4) (a)			3.38 t (11) (a)	3.57 m (a)
17′α	4.04 t (12)	4.07 t (11)	4.03 t (12)	4.05 t (12)	4.70 m (b)	4.70 m (b)			3.54 dd (11, 5.6) (b)	3.57 m (b)
18	1.21 d (6.7)	1.21 d (6.8)	1.11 d (6)	1.14 d (6)	2.09 s	2.19 s	1.57 dd (6.8, 2.4)	1.54 dd (6.8, 2)	1.31 d (6.4)	1.59 d (6.8)
19	3.93 (6.7, 2.6)	3.94 qd (6.8, 2)	3.46 m	3.46 m			5.43 qd (6.8, 2.4)	5.45 qd (6.8, 2)	4.55 q (6.4)	5.28 br q (6.8)
20	1.06 m (α)	1.04 m (α)	1.37 m (β)	1.45 m (β)						
21	3.64 dd (11, 4)	3.65 dd (11, 4)	3.22 m	3.31 dd (10.5, 8)	7.56 s	9.63 s	3.01 d (12.4) (α)	2.45 m (β)	6.35 s	3.57 m
21′	3.71 dd (11, 6)	3.72 dd (11, 6)	3.40 dd (11, 5)	3.46 m			4.32 dt (12.4, 2.4) (β)	3.04 d (13.7) (α)		3.57 m
N(1)-Me		3.52 s	3.52 s	3.58 s					3.20 s	
N(4)-Me	2.30 s			2.30 s	2.48 br s	2.48 br s				
N(1)-H	8.14 br s				9.01 br s	9.01 br s				5.21 br s
10-OMe				3.85 s						
CO_2Me							3.71 s	3.55 s		

[a]$CDCl_3$, 400 MHz; assignments based on COSY, HSQC, HMQC, and NOESY/DNOE.

resonances appeared in pairs with similar chemical shifts or were coincident, except for the C-18, C-19, C-20, and C-21 resonances. This behavior has been observed previously in the case of the macroline indoles alstonerine (**21**) (type B) and alstonerinal (**40**) (type A),[16] as well as *N*(4)-demethylalstonerine (type B) and *N*(4)-demethylalstonerinal (type A).[22] Analysis of the NMR spectroscopic data (Tables 2 and 4) and comparison with the NMR data of **21** and **40** indicated that **15** and **16** are the *N*(1)-demethyl derivatives of **21** and **40**, respectively.

Compound **17** (7-hydroxypleiocarpamine) was obtained as a colorless oil, $[\alpha]_D$ +50 (*c* 0.2, $CHCl_3$). The UV spectrum showed absorption maxima at 210, 252, and 292 nm, characteristic of a dihydroindole chromophore. The IR spectrum showed bands at 3382 and 1753 cm^{-1}, suggesting the presence of OH and ester functionalities, respectively. The EIMS data showed a molecular ion at *m*/*z* 340, corresponding to the molecular formula $C_{20}H_{24}N_2O_3$. Other major fragments were observed at *m*/*z* 323 and 281, due to loss of OH and CO_2Me, respectively. The ^{1}H NMR data (Table 4) showed resonances due to four aromatic hydrogens (δ 6.18−7.25), a methyl ester group (δ_H 3.71, δ_C 170.4, 51.9), and an ethylidene side chain (δ 1.57, dd, 3H; 5.43, qd, 1H). A methine doublet at δ 4.03 with *J* = 3.7 Hz (δ_C 60.9) is characteristic of H-16 in pleiocarpamine-type alkaloids.[26] Examination of the ^{1}H and ^{13}C NMR data (Tables 4 and 2) and comparison with pleiocarpamine (**41**) confirmed the presence of a pleiocarpamine moiety. The main difference between **17** and **41** is that the aromatic C-2 and C-7, which are associated with an indole ring in **41**, have been replaced by a methine and an oxygenated tertiary carbon, respectively, in **17**. This conclusion was supported by the shielding of C-2 and C-7 resonances to δ 70.7 and 76.6 in **17**, from δ 136.8 and 107.9 in **41**, respectively, in the ^{13}C NMR spectrum.

The orientation of H-2 was deduced to be α from the reciprocal NOEs for H-2/H-14β and H-2/H-16 (Figure 10).

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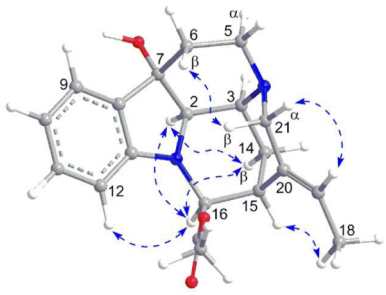

Figure 10. Selected NOEs of **17**.

This leaves only two possible structures (**17** and **17a**) to be considered, which differ only in the configuration at C-7. Structure **17**, which incorporates a *cis*-fused B/C-ring (chair conformation in ring C), should be more stable compared to **17a**, with a *trans*-fused B/C-ring (boat conformation in ring C), an inference supported by NOE experiments, as well as by analysis of the vicinal coupling constants. The H-6β resonance at δ 2.21 was seen as a triplet of doublets, with J = 14 and 5 Hz ($J_{6\beta\text{-}6\alpha}$ = $J_{6\beta\text{-}5\alpha}$ = 14, $J_{6\beta\text{-}5\beta}$ = 5 Hz), indicating that it is *trans*-diaxial with H-5α. This observation coupled with the NOE between H-6β and H-21β provided support for the chair conformation adopted by ring C. On the basis of these observations as well as from energetic considerations, the orientation of the 7-OH group was assigned as α.

Compound **18** (6-oxopleiocarpamine) was obtained as a colorless oil, $[\alpha]_D$ +129 (c 0.2, $CHCl_3$). The UV spectrum (214, 246, and 305 nm) was somewhat similar to that of pleiocarpamine (**41**) with an additional band, possibly due to the presence of additional conjugation to a carbonyl function. The mass spectrum showed a molecular ion at m/z 336, and HREIMS measurements established the molecular formula as $C_{20}H_{20}N_2O_3$. The molecular formula of **18** indicated that it differs from pleiocarpamine (**41**) by replacement of two hydrogen atoms with an oxygen atom. The resonance at δ 193.0 in the ^{13}C NMR data (Table 2) confirmed the presence of a conjugated carbonyl group. The ^1H NMR data of **18** (Table 4) were generally similar to those of pleiocarpamine (**41**), except for the presence of a pair of AB doublets (δ 3.09 and 3.69, J = 18 Hz) in place of the resonance due to the C-5−C-6 ethylene fragment present in **41**, thus indicating that the carbonyl function is located at C-6. This was confirmed by the HMBC data, which showed three-bond correlations from H-5 to C-3, C-7, and C-21.

Alstoumerine oxindole (**19**) crystallized from CH_2Cl_2−hexane as colorless, block-shaped crystals, mp >160 °C (dec), with $[\alpha]_D$ −48 (c 0.5, $CHCl_3$). The IR spectrum indicated the presence of OH (3363 cm^{-1}) and γ-lactam carbonyl (1707 cm^{-1}) functions. The UV spectrum showed absorption maxima at 208, 256, and 288 nm indicative of an oxindole chromophore. This latter observation, coupled with the IR band at 1707 cm^{-1}, and the observed carbon resonances at δ 181.7 and 55.5, due to the oxindole lactam carbonyl C-2 and the spirocyclic C-7, respectively, confirmed the presence of an oxindole alkaloid. The EIMS data showed a molecular ion at m/z 340, with a notable fragment ion peak at m/z 323 due to loss of OH. HREIMS data established the molecular formula of **19** as $C_{20}H_{24}N_2O_3$. The ^{13}C NMR data (Table 2) showed 20 carbon signals including two methyl, three methylene, 10 methine, and three quaternary carbon atoms. The eight carbon resonances of the oxindole moiety could be readily assigned from comparison of their chemical shifts with those of the related oxindoles[27] and were confirmed by the HMBC data. The ^1H NMR data (Table 4) showed the presence of an unsubstituted aromatic moiety (δ 6.81−7.35), an N1-Me group (δ 3.20), a hydroxyethyl group (δ 1.31, 4.55), a hydroxymethyl group (δ 3.38, 3.54), and an isolated olefinic methine resonance at δ 6.35, corresponding to H-21.

The COSY and HMQC data disclosed the following partial structures, viz., $CH_2CHCHCH_2OH$, $NCHCH_2CH$, and $OCHCH_3$, corresponding to the C-6−C-5−C-16−C-17, C-3−C-14−C-15, and C-19−C-18 fragments, respectively. Assembly of the molecule based on the HMBC data revealed **19** to be the oxindole of the sarpagine-type alkaloid, alstoumerine (**42**),[28,29] which was also present in the stem-bark extract. The relative configuration at the spirocyclic C-7 was assigned as *S* from the reciprocal NOEs between H-9 and H-6β, H-14′β, H-16 (Figure 11).

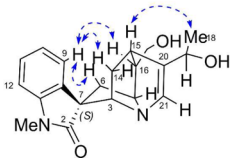

Figure 11. Selected NOEs of **19**.

In addition, the H-9/H-16 NOE also allowed the assignment of the configuration of C-16 as *R*. As the NMR data were insufficient to establish the C-19 configuration, X-ray diffraction analysis was undertaken, which established the relative configuration of the hydroxy-substituted C-19 as *S* (Figure 12) in addition to confirming the various stereochemical assignments of **19** based on NOE experiments (Figure 11).

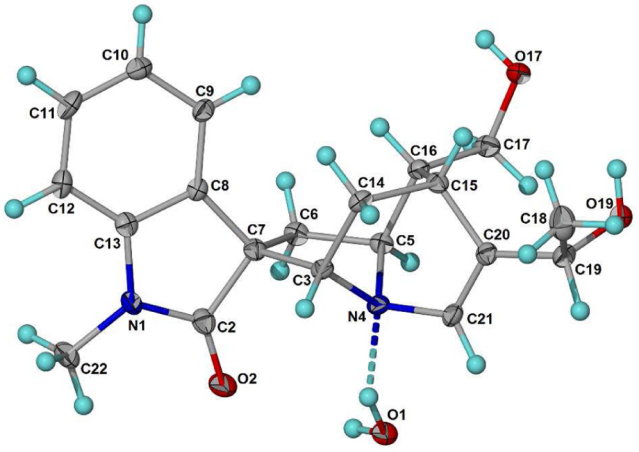

Figure 12. X-ray crystal structure of **19**.

Compound **20** [normacusine B-2(*S*)-pseudoindoxyl] was obtained as a light yellowish oil, $[\alpha]_D$ −107 (c 0.5, $CHCl_3$). The IR spectrum showed bands due to carbonyl (1687 cm^{-1}) and NH/OH (3350 cm^{-1}) functions. The UV spectrum showed absorption maxima at 232, 255 (shoulder), and 284 nm, indicative of a pseudoindoxyl chromophore. The IR band observed at 1687 cm^{-1} coupled with the ^{13}C NMR resonances at δ 206.1 and 72.6, due to the pseudoindoxyl carbonyl C-7 and spirocyclic C-2, respectively, provided further confirmation for the presence of a pseudoindoxyl alkaloid. The EIMS data showed a molecular ion at m/z 310, with two other major fragment ions

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at m/z 293 and 279 due to loss of OH and CH_2OH. HREIMS measurements established the molecular formula of **20** as $C_{19}H_{22}N_2O_2$.

The ^{13}C NMR data (Table 2) showed 19 carbon resonances (one methyl, four methylene, nine methine, and two quaternary carbon atoms). The oxymethylene carbon resonance (δ 65.1) in the ^{13}C NMR data is consistent with the presence of a hydroxymethyl group. The ^1H NMR data (Table 4) showed the presence of an unsubstituted indole A-ring, an indolic NH (δ 5.21), an ethylidene side chain (δ 5.28, 1.59), a hydroxymethyl group (δ 3.57, 2H), and an isolated aminomethylene (δ 3.57, 2H). The COSY spectrum yielded fragments consistent with a sarpagine-type carbon skeleton, such as $NCHCH_2$, $NCHCH_2CHCHCH_2OH$, an isolated aminomethylene, and the ethylidene side chain. Moreover, the molecular formula indicated 10 degrees of unsaturation, which suggested a pentacyclic alkaloid. Assembly of the molecule based on the HMBC data revealed **20** to be the pseudoindoxyl of the sarpagine alkaloid normacusine B (**43**),[30,31] which was also isolated in the present study.

The NOE data (Figure 13) showed that the relative configurations of the stereogenic centers in **20** are similar to those of

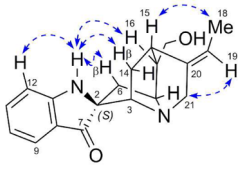

Figure 13. Selected NOEs of **20**.

43. The configuration of the spirocyclic center, C-2, was assigned as *S* from the NOEs between NH and H-6β, H-14′β, and H-16. The NH/H-16 NOE also allowed the assignment of the C-16 configuration as *R*. In addition, the NOEs observed for 18-Me/H-15 and H-19/H-21 confirmed the *E* geometry of the C-19−C-20 double bond.

The new alkaloids as well as selected known alkaloids were screened for their cytotoxic effects on KB cells. All the alkaloids tested did not show any cytotoxic effects against both vincristine-sensitive and vincristine-resistant (KB/VJ300) cells (IC_{50} > 25 μg/mL). Alkaloids **3**, **7**, **35**, and **41** showed moderate to weak activity (IC_{50} 14−22 μg/mL (40−60 μM) in the presence of 0.1 μg/mL (0.12 μM) vincristine), while alstonerine (**21**) showed strong activity in reversing multidrug resistance in drug-resistant KB/VJ300 cells (IC_{50} 3.43 μg/mL (10 μM) in the presence of 0.1 μg/mL (0.12 μM) vincristine).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. Optical rotations were measured 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. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on JEOL JNM-LA 400 and JNM-ECA 400 spectrometers at 400 and 100 MHz, respectively. EIMS and HREIMS data were obtained at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia. ESIMS and HRESIMS were obtained on an Agilent 6530 Q-TOF mass spectrometer.

Plant Material. Plant material was collected in Pahang, Malaysia, and identification was confirmed by Dr. Richard C. K. Chung, Forest Research Institute, Malaysia. Herbarium voucher specimens (K665) are deposited at the Herbarium, University of Malaya.

Extraction and Isolation. The plant material (bark 8 kg, leaf 25 kg) was exhaustively extracted with EtOH (10 L, room temperature), and the concentrated EtOH extract was partitioned with 5% HCl, followed by basification of the aqueous fraction with concentrated NH_4OH solution, and extraction of the liberated alkaloids with $CHCl_3$. The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH, followed by further purification of the appropriate partially resolved fractions using centrifugal preparative TLC. Solvent systems used for centrifugal preparative TLC were Et_2O−hexanes (1:2, NH_3 saturated), Et_2O−hexanes (5:1, NH_3 saturated), Et_2O−methanol (100:1, NH_3 saturated), Et_2O−methanol (50:1, NH_3 saturated), Et_2O−methanol (25:1, NH_3 saturated), EtOAc−hexanes (1:1, NH_3 saturated), EtOAc−hexanes (4:1, NH_3 saturated), EtOAc (NH_3 saturated), EtOAc−methanol (100:1, NH_3 saturated), $CHCl_3$−hexanes (1:1), $CHCl_3$−hexanes (1:1, NH_3 saturated), $CHCl_3$−hexanes (2:1, NH_3 saturated), $CHCl_3$−hexanes (4:1, NH_3 saturated), $CHCl_3$, $CHCl_3$ (NH_3 saturated). The yields (mg kg^{-1}) of the alkaloids from the stem-bark extract were as follows: **1** (5.0), **7** (3.0), **8** (0.34), **9** (1.1), **11** (29.8), **10** (0.44), **12** (1.4), **13** (46.3), **15** (0.4), **16** (0.08), **17** (0.5), **19** (3.5), **20** (1.3), **21** (36.0), **23** (5.0), **34** (19.8), **35** (42.8), **36** (3.8), **38** (56.0), **39** (1.4), **40** (10.2), **41** (112.5), **42** (119.6), **43** (3.8), macrodasine A (3.4), macrodasine B (6.3), macrodasine C (2.1), macrodasine D (1.3), macrodasine E (0.75), macrodasine F (1.5), macrodasine G (2.1), alstopirocine (1.9), 20,21-dihydroalstonerine (2.4), angustimaline (0.64), angustimaline A (0.16), angustimaline B (0.25), angustimaline C (1.25), angustimaline D (3.0), angustimaline E (0.63), alstohentine (2.0), alstomicine (5.0), talcarpine (3.8), *N*(4)-methyl-*N*(4),21-secotalpinine (3.4), pleiomaltinine (6.0), pleiomalicine (0.13), 16-hydroxymethylpleiocarpamine (0.88), fluorocarpamine (4.0), picramicine (0.63), alstonisine (20.7), alstonal (5.6), alstofoline (10.0), affinisine (13.5), affinisine oxindole (18.0), lochnerine (11.3), 16(*R*),19(*Z*)-isositsirikine (1.3), 16(*R*),19(*E*)-isositsirikine (0.63), dihydrocorynantheol (1.8), antirhine (15.3), bipleiophylline (2.4), perhentidine A (0.88), perhentidine C (1.1), perhentinine (5.0), anhydromacralstonidine (0.5), lumutinine E (3.4), perhentisine A (2.5), perhentisine B (1.9), perhentisine C (1.8), villalstonidine A (2.1), villalstonidine B (1.4), villalstonidine C (0.75), villalstonidine D (70.0), villalstonidine E (625.0), villalstonine (404.4), villalstonine *N*-oxide (27.5), and macrocarpamine (2.0). The yields (mg kg^{-1}) of the alkaloids from the leaf extract were as follows: **2** (0.2), **3** (0.05), **4** (0.2), **5** (2.9), **6** (1.3), **14** (0.35), **17** (1.2), **18** (0.19), **34** (89.6), **41** (2.0), **43** (2.6), 10-methoxyalstonerine (1.6), 19,20-dehydro-10-methoxytalcarpine (1.6), alstophylline (12.5), fluorocarpamine (1.5), alstonoxine A (3.2), alstonisine (4.1), alstonal (4.1), affinisine (33.7), *N*1-methylsarpagine (0.4), 10-methoxyaffinisine (2.6), affinisine oxindole (0.97), normacusine B (2.6), 16(*R*),19(*Z*)-isositsirikine (0.55), 18,19-dihydroisositsirikine (0.16), strictamine (0.44), 11-methoxystrictamine (0.65), 11-hydroxystrictamine (9.9), 10,11-dimethoxynareline (0.54), 17-epiyohimbine (0.48), and yohimbine (1.4).

*Alstofonidine (**1**):* light yellowish oil; $[\alpha]_D$ −75 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 229 (4.27), 285 (3.60) nm; IR (dry film) ν_{max} 1780 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; EIMS m/z 366 $[M]^+$ (70), 351 $[M - Me]^+$ (5), 335 $[M - CH_2OH]^+$ (10), 322 $[M - CO_2]^+$ (3), 277 (3), 254 (27), 236 (5), 197 (100), 182 (25), 181 (19), 170 (30), 144 (7), 119 (5), 70 (21), 42 (7); HREIMS m/z 366.1934 $[M]^+$ (calcd for $C_{22}H_{26}N_2O_3$, 366.1943).

*Alstofolinine B (**2**):* light yellowish oil; $[\alpha]_D$ −63 (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 228 (4.11), 284 (3.59) nm; IR (dry film) ν_{max} 1774 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; EIMS m/z 326 $[M]^+$ (78), 311 $[M - CH_3]^+$ (6), 295 $[M - OCH_3]^+$ (2), 257 (23), 227 (44), 212 (100), 197 (18), 181 (8), 157 (5), 43 (6); HREIMS m/z 326.1631 $[M]^+$ (calcd for $C_{19}H_{22}N_2O_3$, 326.1630).

*Alstolactone A (**3**):* colorless oil; $[\alpha]_D$ +110 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 226 (4.48), 285 (3.89) nm; IR (dry film) ν_{max} 3444, 1706 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1,

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respectively; EIMS m/z 352 $[M]^+$ (26), 321 $[M - OMe]^+$ (10), 275 (37), 213 (100), 200 (85), 157 (24), 121 (24), 107 (7), 69 (8), 43 (10); HREIMS m/z 352.1786 $[M]^+$ (calcd for $C_{21}H_{24}N_2O_3$, 352.1787).

Macrogentine A (**4**): colorless oil; $[\alpha]_D$ −7.1 (c 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 210 (3.84), 250 (3.35), 284 (2.86) nm; IR (dry film) ν_{max} 3378, 3288, 1702 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; HRESIMS m/z 329.1861 $[M + H]^+$ (calcd for $C_{19}H_{24}N_2O_3$ + H, 329.1860).

Isoalstonoxine B (**5**): colorless oil; $[\alpha]_D$ +55 (c 0.6, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 211 (4.34), 257 (3.84) nm; IR (dry film) ν_{max} 3394, 1702 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; HRESIMS m/z 331.2021 $[M + H]^+$ (calcd for $C_{19}H_{26}N_2O_3$ + H, 331.2016).

Alstonoxine E (**6**): colorless, block-shaped crystals, from $CHCl_3$; mp 110−112 °C; $[\alpha]_D$ −25 (c 1.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 212 (4.26), 250 (3.74), 284 (3.12) nm; IR (dry film) ν_{max} 3293, 1693 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; HRESIMS m/z 317.1857 $[M + H]^+$ (calcd for $C_{18}H_{24}N_2O_3$ + H, 317.1860).

O-Acetyltalpinine (**7**): light yellowish oil; $[\alpha]_D$ −8 (c 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 208 (4.24), 226 (4.36), 284 (3.74) nm; IR (dry film) ν_{max} 1734 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; HRESIMS m/z 367.2022 $[M + H]^+$ (calcd for $C_{22}H_{26}N_2O_3$ + H, 367.2016).

N(4)-Methyl-19-epitalpinine (**8**): light yellowish oil; $[\alpha]_D$ −15 (c 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 223 (3.74), 282 (3.12) nm; IR (dry film) ν_{max} 3387 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; HRESIMS m/z 339.2067 $[M]^+$ (calcd for $C_{21}H_{27}N_2O_2$, 339.2067).

7(S)-Talpinine oxindole (**9**): light yellowish oil; $[\alpha]_D$ −4 (c 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 204 (4.44), 254 (3.52) nm; IR (dry film) ν_{max} 3403, 1705 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; EIMS m/z 340 $[M]^+$ (62), 322 (15), 312 (9), 279 (8), 267 (12), 251 (58), 241 (8), 215 (18), 197 (13), 172 (36), 160 (100), 130 (27), 110 (30), 96 (93), 80 (81), 69 (30), 56 (45), 43 (16); HREIMS m/z 340.1787 $[M]^+$ (calcd for $C_{20}H_{24}N_2O_3$, 340.1787).

19-Epitalcarpine (**10**): light yellowish oil; $[\alpha]_D$ −59 (c 0.2, MeOH); UV (EtOH) λ_{max} (log ε) 225 (4.94), 283 (4.25) nm; IR (dry film) ν_{max} 1720 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; HRESIMS m/z 339.2067 $[M + H]^+$ (calcd for $C_{21}H_{26}N_2O_2$ + H, 339.2067).

Macrocarpine E (**11**): light yellowish oil; $[\alpha]_D$ −12 (c 0.8, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 227 (4.09), 282 (3.34) nm; IR (dry film) ν_{max} 3398, 3276 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 4 and 2, respectively; HRESIMS m/z 327.2076 $[M + H]^+$ (calcd for $C_{20}H_{26}N_2O_2$ + H, 327.2067).

Macrocarpine F (**12**): colorless oil; $[\alpha]_D$ +38 (c 0.6, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 230 (4.00), 288 (3.12) nm; IR (dry film) ν_{max} 3407 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 4 and 2, respectively; EIMS m/z 326 $[M]^+$ (31), 295 $[M - CH_2OH]^+$ (2), 226 (12), 196 (7), 183 (100), 168 (15), 144 (11), 128 (5), 91 (5), 69 (3), and 44 (7); HREIMS m/z 326.1991 [M]+ (calcd for $C_{20}H_{26}N_2O_2$, 326.1994).

Macrocarpine G (**13**): light yellowish oil; $[\alpha]_D$ +7 (c 1.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 230 (3.55), 289 (2.73) nm; IR (dry film) ν_{max} 3394, 3299 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 4 and 2, respectively; HRESIMS m/z 327.2077 $[M + H]^+$ (calcd for $C_{20}H_{26}N_2O_2$ + H, 327.2067).

Macrocarpine H (**14**): light yellowish oil; $[\alpha]_D$ −17 (c 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 230 (4.47), 289 (2.95) nm; IR (dry film) ν_{max} 3413 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 4 and 2, respectively; HRESIMS m/z 371.2333 $[M + H]^+$ (calcd for $C_{22}H_{30}N_2O_3$ + H, m/z 371.2329).

N(1)-Demethylalstonerine (**15**) *and N(1)-Demethylalstonerinal* (**16**): light yellowish oil; ^1H NMR and ^{13}C NMR data, see Tables 4 and 2, respectively; ESIMS m/z 323 $[M + H]^+$; EIMS m/z 322 $[M]^+$ (86), 291 (8), 253 (10), 210 (11), 198 (19), 183 (88), 167 (53), 156 (100), 129 (33), 115 (10), 91 (10), 70 (58), 57 (19), 43 (24); HREIMS m/z 322.1675 (calcd for $C_{20}H_{22}N_2O_2$, 322.1681).

7-Hydroxypleiocarpamine (**17**): colorless oil; $[\alpha]_D$ +50 (c 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 210 (4.39), 252 (4.27), 293 (3.72) nm; IR (dry film) ν_{max} 3382, 1753 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 4 and 2, respectively; EIMS m/z 340 $[M]^+$ (19), 323 $[M - OH]^+$ (46), 281$[M - CO_2Me]^+$ (35), 263 (27), 247 (7), 232 (16), 216 (100), 204 (9), 180 (41), 168 (9), 156 (29), 144 (18), 107 (27), 93 (12), 82 (18), 69 (12), 43 (23); HREIMS m/z 340.1779 $[M]^+$ (calcd for $C_{20}H_{24}N_2O_3$, 340.1787).

6-Oxopleiocarpamine (**18**): colorless oil; $[\alpha]_D$ +129 (c 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 214 (4.46), 246 (4.21), 305 (3.94) nm; IR (dry film) ν_{max} 1758, 1652 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 4 and 2, respectively; EIMS m/z 336 $[M]^+$ (71), 308 $[M - CO]^+$ (29), 277 $[M - CO_2Me]^+$ (100), 263 (12), 249 (52), 234 (11), 220 (17), 194 (40), 180 (10), 169 (16), 151 (4), 140 (7), 108 (8), 80 (6), 57 (3); HREIMS m/z 336.1468 $[M]^+$ (calcd for $C_{20}H_{20}N_2O_3$, 336.1474).

Alstoumerine oxindole (**19**): colorless, block-shape crystals from CH_2Cl_2/hexanes; mp >160 °C (dec); $[\alpha]_D$ −48 (c 0.5, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 208 (3.84), 256 (3.26), 288 (2.85) nm; IR (dry film) ν_{max} 3363, 1707 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 4 and 2, respectively; EIMS m/z 340 $[M]^+$ (50), 323 $[M - OH]^+$ (55), 296 (96), 251 (13), 199 (23), 172 (100), 144 (58), 130 (22), 94 (12), 55 (8), 43 (15); HREIMS m/z 340.1787 $[M]^+$ (calcd for $C_{20}H_{24}N_2O_3$, 340.1787).

Normacusine B-2(S)-psedoxindoxyl (**20**): light yellowish oil; $[\alpha]_D$ −107 (c 0.5, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 232 (3.72), 255 (3.12) (shoulder), 284 (2.52) nm; IR (dry film) ν_{max} 3350, 1687 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 4 and 2, respectively; EIMS m/z 310 $[M]^+$ (100), 293 $[M - OH]^+$ (18), 279 $[M - CH_2OH]^+$ (20), 237 (10), 213 (7), 197 (22), 185 (14), 170 (16), 158 (26), 146 (16), 108 (8), 70 (5), 43 (5); HREIMS m/z 310.1676 $[M]^+$ (calcd for $C_{19}H_{22}N_2O_2$, 310.1681).

X-ray Crystallographic Analysis of 6 and 19. X-ray diffraction analysis was carried out on a Bruker SMART APEX II CCD area detector system equipped with a graphite monochromator and a Mo Kα fine-focus sealed tube (λ = 0.710 73 Å), at 100 K. The structures were solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on F^2 (SHELXL-97). 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. The absolute structures were determined by refinement of the Flack parameter.[32−34] Crystallographic data for compounds **6** and **19** 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 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).

Crystallographic data of **6**: colorless block crystals, $C_{18}H_{24}N_2O_3 \cdot CHCl_3$, M_r = 435.76, orthorhombic, space group $P2_12_12_1$, a = 9.7811(7) Å, b = 12.2584(8) Å, c = 17.0772(13) Å, Z = 4, D_{calcd} = 1.414 gcm^{-3}, crystal size 0.36 × 0.26 × 0.16 mm^3, $F(000)$ = 912, T = 100 K. The final R_1 value is 0.0487 (wR_2 = 0.1253) for 3357 reflections [$I > 2\sigma(I)$]. Flack parameter, x = −0.04(0.16). CCDC number: 1003755.

Crystallographic data of **19**: colorless block crystals, $C_{20}H_{24}N_2O_3 \cdot H_2O$, M_r = 358.43, orthorhombic, space group $P2_12_12$, a = 11.5360(2) Å, b = 19.2745(3) Å, c = 8.11190(10) Å, Z = 4, D_{calcd} = 1.320 gcm^{-3}, crystal size 0.22 × 0.16 × 0.05 mm^3, $F(000)$ = 768, T = 100 K. The final R_1 value is 0.0444 (wR_2 = 0.0912) for 3699 reflections [$I > 2\sigma(I)$]. CCDC number: 1003756.

Acetylation of Talpinine (35). Talpinine (**35**) (4.4 mg, 0.014 mmol) was added to a mixture of Ac_2O−pyridine (1:1; 1 mL), and the mixture stirred under N_2 at room temperature for 1 h. The mixture was poured into a saturated Na_2CO_3 solution (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). Removal of the solvent, followed by purification by centrifugal preparative TLC over SiO_2 ($CHCl_3$, NH_3-saturated), afforded 4.1 mg (82%) of the *O*-acetyl derivative of **35**.

Cytotoxicity Assays. Cytotoxicity assays were carried out following the procedure that has been described previously.[19]

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■ ASSOCIATED CONTENT

Supporting Information

The authors declare no competing financial interest. This material is available free of charge via the Internet at http://pubs.acs.org.

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