New Indole Alkaloids from *Alstonia macrophylla*

Toh-Seok Kam* and Yeun-Mun Choo

Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

Received November 3, 2003

Ten new indole alkaloids, alstomaline (**1**), 10,11-dimethoxynareline (**2**), alstohentine (**3**), alstomicine (**4**), 16-hydroxyalstonisine (**5**), 16-hydroxyalstonal (**6**), 16-hydroxy-*N*(4)-demethylalstophyllal oxindole (**7**), alstophyllal (**8**), 6-oxoalstophylline (**9**), and 6-oxoalstophyllal (**10**), in addition to 21 other known ones, were obtained from the leaf extract of the Malayan *Alstonia macrophylla*. The structures were determined using NMR and MS analysis.

We previously reported the alkaloidal composition of the stem-bark and leaf extract, including the structures of a series of novel macroline indoles and oxindoles, of a Malayan *Alstonia* species, which at the time we erroneously identified as *A. macrophylla* Wall.[1,2] Since then we have obtained and investigated the alkaloidal content of an authentic sample of *A. macrophylla*, which forms the basis of the present paper. The later acquisition of *A. macrophylla* prompted a careful reexamination of the earlier sample, which we concluded to be that of *A. angustifolia* var. *latifolia* K. and G. The common tendency to confuse the latter with *A. macrophylla* Wall has been previously noted.[3−5] We have subsequently reported the full alkaloidal composition of the leaf extract of *A. angustifolia* var. *latifolia*, including the isolation of additional new compounds,[6] and now wish to report the isolation of new alkaloids from *A. macrophylla*. While there has been a previous report on the alkaloids from a North Borneo sample,[7] there has been no previous study of the Malayan species.[8]

Results and Discussion

A total of 31 alkaloids were obtained from the leaf extract of the Malayan *Alstonia macrophylla*, of which 10 are new alkaloids (**1**−**10**). Of these, eight are macroline alkaloids, while the other two represent new akuammiline and nareline compounds.

Alstomaline (**1**) was obtained as a yellow oil, $[\alpha]_D$ −244° (*c* 0.04, $CHCl_3$). The IR spectrum showed bands due to ester (1732 cm^{-1}) and conjugated ketone (1646, 1634 cm^{-1}) functions. The UV spectrum showed absorption maxima at 202, 273, and 317 nm, suggesting the presence of an iminoquinone chromophore.[9,10] The EIMS of **1** showed a molecular ion at *m*/*z* 338, with a peak due to loss of a methyl ester moiety observed at *m*/*z* 279. HRMS measurements gave the molecular formula $C_{20}H_{22}N_2O_3$. The ^{13}C NMR spectrum (Table 1) showed a total of 20 separate resonances, comprising two methyls, five methylenes, six methines, and seven quaternary carbon atoms, in agreement with the molecular formula. The observed quaternary carbon resonances at δ 188.9 and 164.1 (corresponding to C-10 and C-13, respectively) provided strong support for the presence of an iminoquinone chromophore,[9,10] while the signals at δ 172.5 and 51.9 confirmed the presence of the methyl ester function. The ^1H NMR spectrum (Table 2) showed the presence of three aromatic hydrogens and an ethylidene side chain. The upfield shifts of the α-hydrogens H-9 and H-11 and the downfield shift of H-12 are consistent with the iminoquinone structure. Since no other functionalities are revealed from the spectral data, a pentacyclic ring system is indicated from the molecular formula. The COSY spectrum showed the presence of the fragments NCH_2CH_2, CH_2CH_2CHCH, and the ethylidene side chain, the former two corresponding to the C-5, C-6 and C-3, C-14, C-15, C-16 units, respectively. These partial structures are somewhat reminiscent of akuammiline-type alkaloids, and coupled with the unusually low-field signal observed for the saturated quaternary C-2 at δ 104.3, suggesting that it is adjacent to two nitrogen atoms, indicated an alkaloid of the akuammiline subtype, in which bond formation is from C-2 to *N*-4, as exemplified by vincorine, which was also obtained. The signal of the methyl ester singlet at δ 3.79 is consistent with the configuration at C-16, which has the ester function directed away from the anisotropic influence of the aromatic moiety. Alstomaline (**1**) is a new alkaloid, although it constitutes the common monomeric unit present in the bisindoles rausutrine, flexicorine, and rausutranine previously obtained from *Rauwolfia sumatrana*.[10]

Compound **2** was obtained as a light yellow oil, $[\alpha]_D$ −56° (*c* 1.57, $CHCl_3$). The IR spectrum showed bands at 3201 and 1737 cm^{-1} due to OH and ester functions, respectively. The UV spectrum showed absorption maxima at 232 and 294 nm, indicating the presence of an indolenine chromophore,[11] which was confirmed by the observed carbon resonance at δ 183.1 due to the imine carbon. The EIMS of **2** showed a molecular ion at *m*/*z* 412, and HRMS measurements gave the molecular formula $C_{22}H_{24}N_2O_6$. The ^1H NMR spectrum (Table 2) showed the presence of two aromatic methoxy groups, substituted at positions 10 and 11, from the observation of two aromatic singlets at δ 7.27 and 7.33. In addition, the spectrum also showed the presence of an ethylidene side chain and a methyl ester function. The ^{13}C NMR spectrum (Table 1) accounted for all 22 carbon atoms, and in addition to the imine carbon resonance at δ 183.1, another signal due to a methine adjacent to two oxygen atoms was observed at δ 100.2. The NMR spectral data can be assigned by the use of standard 2-D techniques and revealed **2** to possess the nareline ring system.[12−14] Comparison of the NMR spectra (Tables 1 and 2) with that of nareline and its methyl and ethyl ether derivatives[12,13] showed that compound **2** is 10,11-dimethoxynareline.

Compound **3**, alstohentine, was obtained as a light yellow oil, $[\alpha]_D$ −58° (*c* 0.22, $CHCl_3$). The IR spectrum showed a broad band at 3368 cm^{-1} due to an OH function, while the UV spectrum is characteristic of an indole chromophore

* To whom correspondence should be addressed. Tel: 603-79674266. Fax: 603-79674193. E-mail: tskam@um.edu.my.

10.1021/np034041v CCC: $27.50
Published on Web 03/03/2004

Table 1. ^{13}C NMR Spectral Data for **1**−**12** (100 MHz, $CDCl_3$)[a]

C	1	2	3	4	5	6	7	8	9	10	11	12
2	104.3	183.1	135.9	132.5	182.2	182.2	182.9	131.8	147.7	147.7	133.2	134.5
3	27.6	62.4	49.4	53.2	63.5	63.5	63.4	53.6	54.6	54.6	53.7	49.5
5	53.6	100.2	54.7	59.1	61.2	61.2	61.1	54.6	67.3	67.4	54.6	55.0
6	40.8	55.9	20.8	22.2	37.8	37.8	38.0	22.3	192.2	192.2	22.6	21.1
7	56.9	55.5	103.3	105.9	57.3	57.3	56.8	105.8	108.3	108.3	106.6	103.1
8	159.0	131.2	127.0	126.3	128.9	128.9	120.7	121.0	117.8	117.8	126.4	126.8
9	123.0	108.7	118.0	118.1	125.4	125.4	126.1	118.2	122.3	122.3	118.1	118.1
10	188.9	147.4	119.2	119.0	123.4	123.4	106.4	108.1	111.7	111.7	118.8	119.4
11	135.6	149.7	121.4	121.0	128.1	128.0	160.1	155.9	157.3	157.3	120.8	121.7
12	133.8	104.3	108.9	108.9	108.0	108.0	96.6	93.2	94.3	94.3	108.7	109.0
13	164.1	150.7	137.3	137.2	143.9	143.9	145.2	137.9	138.9	138.9	136.9	137.4
14	26.5	35.1	28.6	34.3	33.4	33.1	33.2	31.8	30.1	30.1	30.7	28.1
15	35.6	31.3	34.9	25.1	33.1	32.7	32.8	24.9	22.4	21.9	28.6	33.0
16	50.2	53.9	38.5	43.6	68.2	68.1	68.1	38.4	33.0	32.9	39.3	38.4
17			70.8	66.0	71.5	71.8	71.9	67.7	67.1	67.1	68.9	69.2
18	13.8	12.6	15.0	30.5	25.0	16.5	16.5	16.5	24.9	16.6	18.8	14.7
19	123.7	122.7	79.1	207.5	196.7	170.2	170.4	170.0	195.5	171.4	71.2	77.2
20	138.7	130.6	68.9	46.2	119.6	115.8	115.8	121.0	120.2	116.5	43.6	69.2
21	59.0	65.5	64.7		156.5	189.8	189.8	188.8	157.8	188.6	63.1	64.4
CO_2Me	51.9	51.7										
CO_2Me	172.5	170.9										
10-OMe		56.1										
11-OMe		56.2					55.5	55.8	55.8	55.8		
N(1)-Me			29.5	28.9	26.2	26.2	26.3	29.0	30.3	30.3	29.0	29.6
N(4)-Me			38.7	41.4				41.6	42.9	42.9	41.7	38.7
21-OAc												20.9
												170.5

[a] Assignments based on HMQC and HMBC.

Table 2. ^1H NMR Spectral Data for **1**−**4**, **11**, and **12** (400 MHz, $CDCl_3$)[a]

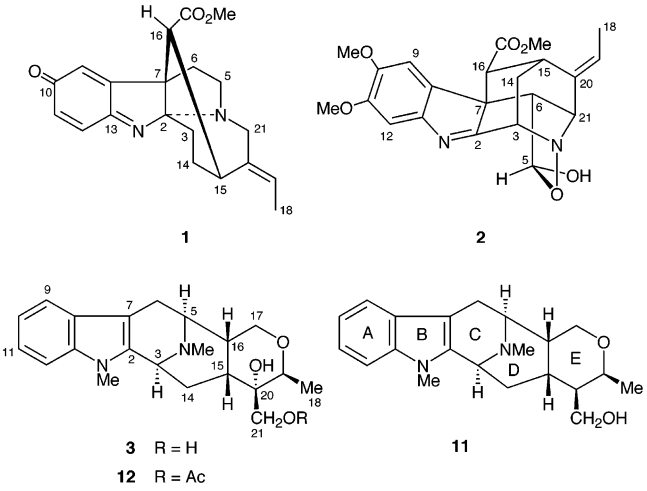
H	1	2	3	4	11	12
3	1.28 ddd (16, 10, 8) 2.75 m	4.59 t (3)	3.83 d (10)	4.01 br s	3.96 m	4.12 d (10)
5	2.75m 2.75 m	4.28 br s	3.31 m	3.49 d (7)	2.87 d (7)	3.46 t (5)
6	1.87 m 2.52 ddd (14, 11, 8)	3.72 m	2.37 d (16) 2.95 dd (16, 4)	2.56 d (17) 3.31 dd (17, 7)	2.47 d (17) 3.25 dd (17, 7)	2.52 d (16) 3.06 dd (16, 5)
9	6.54 d (2)	7.33 s	7.41 br d (8)	7.50 d (8)	7.49 br d (8)	7.48 br d (8)
10			7.04 td (8, 1)	7.11 t (8)	7.10 td (8, 1)	7.12 td (8, 1)
11	6.60 dd (10, 2)		7.14 td (8, 1)	7.20 t (8)	7.19 td (8, 1)	7.23 td (8, 1)
12	7.38 d (10)	7.27 s	7.24 br d (8)	7.31 d (8)	7.29 br d (8)	7.32 br d (8)
14	1.87 m 1.87 m	2.07 dt (14, 3) 2.33 dt (14, 3)	1.77 dd (14, 10) 2.73 dd (14, 10)	1.61 m 2.27 m	1.42 ddd (13, 5, 2) 2.50 td (13, 4)	1.87 dd (14, 1) 2.87 dd (14, 10)
15	3.79 m	3.35 q (3)	1.90 dd (10, 6)	2.27 m	2.06 dt (13, 5)	2.06 m
16	2.75 m	2.26 d (3)	1.61 td (6, 3)	1.69 br s	2.15 dt (11, 5)	1.77 br t (5)
17			3.44 dd (12, 3) 3.70 br d (12)	3.90 br d (11) 3.98 br d (11)	3.79 dd (11, 5) 4.07 t (11)	3.51 m 3.82 br d (12)
18	1.64 dd (7, 2)	1.70 d (7)	1.21 d (6)	2.04 s	1.24 d (7)	1.29 d (6)
19	5.44 q (7)	5.80 q (7)	3.32 q (6)		3.96 m	3.51 m
20				2.43 dd (17, 7) 2.64 dd (17, 7)	1.07 m	
21	3.02 d (16) 4.02 d (16)	4.10 d (3)	3.34 s 3.34 s		3.69 dd (11, 4) 3.81 dd (11, 6)	3.86 d (12) 4.25 d (12)
CO_2Me	3.79 s	3.72 s				
10-OMe		3.90 s				
11-OMe		3.94 s				
N(1)-Me			3.57 s	3.63 s	3.62 s	3.66 s
N(4)-Me			2.37 s	2.37 s	2.31 s	2.54 s
20-OH			9.01 br s			9.40 br s
21-OAc						2.02 s

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

(228 and 289 nm). The EIMS of **3** showed a molecular ion at *m*/*z* 356, and HRMS measurements gave the molecular formula $C_{21}H_{28}N_2O_3$. In addition to the M − H_2O (*m*/*z* 338) and M − CH_2OH (*m*/*z* 325) peaks, the mass fragments that were observed at *m*/*z* 197, 182, 170, and 144 are typical of macroline derivatives.[15] The ^1H NMR spectrum of **3** (Table 2) showed the presence of an unsubstituted indole chromophore, from the signals due to four aromatic hydrogens, the presence of three methyl groups corresponding to *N*(1)-Me (δ 3.57), *N*(4)-Me (δ 2.37), and Me-18 (δ 1.21), and a hydroxymethyl group from the presence of a 2H singlet at δ 3.34 (corresponding to the carbon resonance at δ 64.7). In addition, another OH signal was observed as a broad singlet at δ 9.01. The ^{13}C NMR spectrum (Table 1) accounted for all 21 carbon atoms (3 methyls, 4 methylenes, 9 methines, and 5 quaternary carbon atoms).

The COSY spectrum disclosed partial structures that are characteristic of a macroline skeleton, such as *N*$CHCH_2$ and *N*$CHCH_2CHCHCH_2O$, corresponding to the C-5, C-6 and C-3, C-14, C-15, C-16, C-17 fragments, respectively.[1,2] This is further supported by the presence of the signals due to the three characteristic methyl groups that are

typical of a macroline compound (e.g., alstonerine/alstonerinal). In addition to these however, two other fragments were indicated, viz., CH_3CH and an isolated oxymethylene, which correspond to C-18, C-19, and the hydroxymethyl C-21, respectively, suggesting affinity to a ring E-saturated macroline, exemplified by talcarpine. This leaves one oxygenated quaternary carbon (δ 68.9) unaccounted for, which must correspond to C-20, to which a hydroxyl function is attached. These conclusions are supported by the HMBC data (three-bond correlations from H-14, H-16, H-18, to C-20), which revealed structure **3** for alstohentine.



It transpires that the corresponding 20-deoxy derivative of **3** [macrocarpine A (**11**)], a new alkaloid reported from the stem-bark extract,[16] was also obtained from the leaves. Comparison of the ^{13}C NMR spectrum of **3** (Table 1) with that of the 20-deoxy derivative **11** provided additional support for the structure of **3**. The ^{13}C NMR spectral data of the two compounds are generally similar, except for departure in the shifts for the α-carbons, C-15 and C-19, and a pronounced difference in the shift for C-20 (δ 43.6 in **11** versus 68.9 in **3**), consistent with hydroxy substitution at C-20 in the case of **3**. Additional support for these conclusions was provided by acetylation (Ac_2O, pyridine) of **3**, which yielded the monoacetate derivative **12**, which had NMR spectral data that were similar to that of **3**. Examination of the ^1H NMR spectrum of **3** (Table 2) revealed additional differences when compared to that of other macroline compounds. Specifically, the coupling patterns for the ring D and E hydrogens are distinctly different when compared with those of other macroline derivatives (e.g., **11**), suggesting a change in the conformations of rings D and E in **3**, compared to those in the other macrolines. In most macroline compounds, rings D and E are *cis*-fused and both rings generally adopt the more stable chair conformation, which is accordingly reflected in the coupling behavior of the hydrogens involved. In alstohentine (**3**), these hydrogens show distinctly different coupling behavior. For example, H-17α in a typical macroline (e.g., **11**) is usually a triplet with $J = 12$ Hz due to its being *trans*-diaxially disposed with respect to H-16. Likewise, H-14α is usually seen as a td ($J = 13$, 4 Hz), due to its *trans*-diaxial relation to H-15 $\{J_{14\alpha-15} = 13$ Hz$\}$. In alstohentine, H-17α is instead observed as a broad doublet with $J = 12$ Hz, while H-14α is observed as a dd with $J = 14$ and 10 Hz. In addition to the C-17 and C-14 hydrogens, other hydrogens that showed departure in their coupling behavior are H-16, H-15, and H-3.

A possible explanation for these observations is that hydrogen bonding occurs between the suitably disposed,

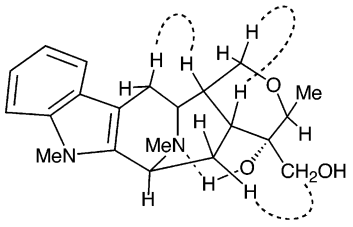


Figure 1. Selected NOEs of **3**.

α-oriented, OH at C-20 and the proximate *N*-4, which also accounts for the observation of the OH signal at δ 9.01. (The OH signal is not usually detected in the ^1H NMR of most macroline compounds. The OH at C-20 is detected in **3**, as well as **12**, presumably due to reduction in the rate of exchange as a result of intramolecular hydrogen bonding.) This hydrogen bonding also results in a new conformational arrangement, with a twist-boat and chair conformation being adopted by the D and E rings, respectively. Such an arrangement would be in agreement with the observed coupling behavior of the D and E ring hydrogens in **3**. For instance, the H-14α and H-14β coupling behavior in **3** ($J_{14\alpha-15} = J_{14\beta-3} = 0$ Hz) indicates an orthogonal relationship between the C−H(15) and C−H(14α) bonds and between the C−H(3) and C−H(14β) bonds, which is entirely consistent with the conformation adopted in **3**. The same is true for H-17α, which has $J_{17\alpha-16} = 0$ Hz, as a result of the conformation adopted.

Further support for this proposal is provided by the results from NOE experiments. Alstohentine (**3**) showed two clear NOEs that are not observed in other macroline compounds (e.g., **11**), viz., that between H-17β/H-15 and H-14α/H-21, which become intelligible on the basis of the new conformation adopted (Figure 1). Additionally, these observations provide further support for the assignment of the relative configuration of C-20 as *R* (β-CH_2OH). The stereochemistry of the methyl substituent at C-19 could not be confirmed by NOE, as H-19 is overlapped with other signals, but is assumed to be α in common with all other known macroline indoles.[8]

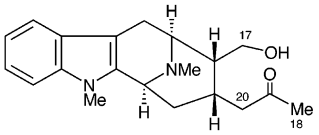
Compound **4**, alstomicine, was obtained as a pale yellowish oil, $[\alpha]_D$ +74° (*c* 0.14, $CHCl_3$). The IR spectrum showed bands at 3400 and 1711 cm^{-1} due to OH and ketone functions, respectively, while the UV spectrum is characteristic of an indole chromophore (230 and 289 nm). The EIMS of **4** showed a molecular ion at *m*/*z* 326, and HRMS measurements gave the molecular formula $C_{20}H_{26}N_2O_2$ (DBE 9), which considering the presence of only a ketone carbonyl as the only other source of unsaturation, indicated a tetracyclic molecule. The ^{13}C NMR spectrum (Table 1) showed a total of 20 carbon signals (three methyls, four methylenes, eight methines, five quaternary carbons). The signals at δ 207.5 and 66.0 are readily attributed to ketone and oxymethylene functions, respectively. The ^1H NMR spectrum (Table 2) showed the presence of an unsubstituted aromatic moiety, three methyl groups at δ 3.63, 2.37, and 2.04, corresponding to *N*(1)-Me, *N*(4)-Me, and COMe, respectively, and two pairs of AB doublets, one at δ 3.90 and 3.98, corresponding to the oxymethylene hydrogens, and another at δ 2.43 and 2.64, corresponding to the methylene (C-20) adjacent to the carbonyl function, the large geminal coupling constant of 17 Hz providing additional confirmation for the assignment. These features are reminiscent of the corresponding ring-opened, *seco*-macroline oxindoles, alstonoxines A and B,[2] and indicated that alstomicine is the ring-opened derivative of the macroline compound alstonerine, which has a hydroxymethyl and a 2-oxopropyl side chain attached to ring D at

Table 3. ^{1}H NMR Spectral Data for **5**−**10** (400 MHz, $CDCl_3$)[a]

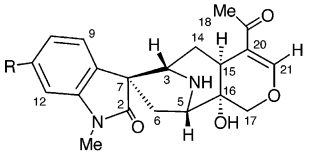
H	5	6	7	8	9	10
3	3.16 t (3)	3.16 br s	3.11 t (3)	3.84 t (3)	4.06 br s	4.06 br s
5	3.53 d (7)	3.52 d (7)	3.49 br d (7)	3.06 d (7)	3.20 br s	3.20 br s
6	2.35 m	2.35 dd (14, 7)	2.37 m	2.45 d (16)		
	2.51 dd (14, 1)	2.49 dd (14, 1)	2.44 br d (14)	3.29 dd (16, 7)		
9	8.17 dd (8, 1)	8.16 dd (8, 1)	8.07 d (8)	7.34 d (8)	8.04 d (8)	8.04 d (8)
10	7.29 td (8, 1)	7.28 td (8, 1)	6.78 dd (8, 2)	6.76 dd (8, 2)	6.96 dd (8, 2)	6.96 dd (8, 2)
11	7.33 td (8, 1)	7.33 td (8, 1)				
12	6.87 dd (8, 1)	6.87 dd (8, 1)	6.45 d (2)	6.81 d (2)	6.82 d (2)	6.82 d (2)
14	1.39 ddd (15, 12, 3)	1.38 ddd (15, 11, 3)	1.36 ddd (14, 11, 3)	1.77 td (12, 4)	2.00 m	2.00 m
	2.35 m	2.40 ddd (15, 7, 3)	2.37 m	2.14 m	2.18 m	2.18 m
15	3.10 ddd (12, 7, 2)	3.06 m	3.03 m	2.61 m	2.69 dt (11, 6)	2.69 dt (11, 6)
16				1.89 m	2.18 m	2.18 m
17	4.01 dd (12, 2)	4.03 dd (12, 2)	4.03 dd (11, 2)	4.19 ddd (11, 4, 2)	4.25 ddd (11, 4, 2)	4.27 ddd (11, 4, 2)
	4.70 d (12)	4.76 d (12)	4.76 d (11)	4.46 t (11)	4.46 t (11)	4.51 t (11)
18	2.26 s	2.30 s	2.30 s	2.17 s	2.11 s	2.19 s
21	7.66 s	9.89 s	9.89 s	9.66 s	7.56 s	9.67 s
11-OMe			3.85 s	3.89 s	3.91 s	3.91 s
N(1)-Me	3.20s	3.20 s	3.17 s	3.60 s	3.69 s	3.70 s
N(4)-Me				2.32 s	2.44 s	2.44 s

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

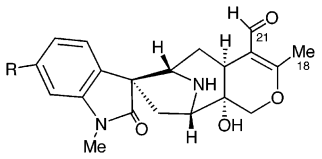
C-16 and C-15, respectively, as shown in structure **4**. The structure is in agreement with the HMBC data (^3J H-5 to C-17, H-20 to C-16, H-18 to C-20), while the ring junction stereochemistry follows that of a normal macroline indole, as confirmed by the NOESY data. Alstomicine (**4**), which is found only in the leaves, contains one of the monomeric units of the novel bisindole, perhentinine, which was obtained from the stem-bark extract of this plant.[16]



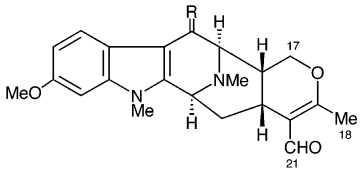
4



5 R = H
13 R = OMe



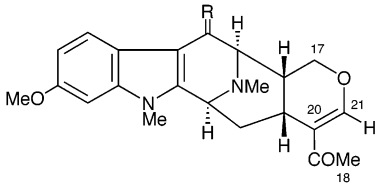
6 R = H
7 R = OMe



8 R = H_2
10 R = O

Two new macroline oxindoles, **5** and **6**, corresponding to the type-B and type-A isomers, respectively, were obtained. Both compounds were separated by chromatography. The EIMS of both showed M^+ at *m*/*z* 354 ($C_{20}H_{22}N_2O_4$). The UV spectra showed absorption maxima (209 and 256 nm for **5**; 207 and 263 nm for **6**) characteristic of an oxindole chromophore, while the IR spectrum showed bands due to hydroxyl (3400 cm^{-1}) and various carbonyl functions (1685 for **5**, 1684 cm^{-1} for **6**). The ^{13}C NMR spectra (Table 1) for both **5** and **6** showed the oxindole lactam carbonyl resonance at δ 182.2 and the spirocyclic C-7 resonance at δ 57.3, which are characteristic of macroline oxindoles possessing the more common *R* configuration at the spirocenter C-7.[2] The ^{1}H NMR spectra (Table 3) of both compounds showed many features in common with other macroline oxindoles, such as the *N*(1)-Me, the characteristic ring junction hydrogens, H-3 and H-5, COMe and vinyl-H for **5**, and aldehyde-H and vinylic methyl for **6**. However, in contrast to other macroline indoles, the signal due to H-16 is absent, and the signal due to H-15 and both the H-17's have undergone a change in their coupling pattern. This is also reflected in the ^{13}C NMR spectral data of both compounds, which showed C-16 to be an oxygenated quaternary carbon (ca. δ 68). This value agrees well with that of 16-hydroxy-*N*(4)-demethylalstophylline oxindole (**13**), which also possesses 16-hydroxy substitution (δ 68.2, C-16).[17] The stereochemistry at C-16 is deduced to be similar to **13** from the similarity in the ^{1}H and ^{13}C shifts, as well as from the observation that the shifts and coupling behavior of the other hydrogens, such as H-3, H-5, H-6, and H-14, have remained essentially unchanged compared to the other macroline oxindoles, indicating that the C/D and D/E ring junction stereochemistries remain intact. Compounds **5** and **6** are therefore 16-hydroxyalstonisine and 16-hydroxyalstonal, respectively.

Compound **7** was obtained pure and in amorphous form, $[\alpha]_D$ +203° (*c* 0.11, $CHCl_3$). The IR spectrum showed bands at 3400 and 1693 cm^{-1} due to OH and various carbonyl functions, respectively, while the UV spectrum is characteristic of an oxindole chromophore (220 and 267 nm). The EIMS of **7** showed a molecular ion at *m*/*z* 384, and HRMS measurements gave the molecular formula $C_{21}H_{24}N_2O_5$. The ^{1}H and ^{13}C NMR spectral data (Tables 3 and 1, respectively) were essentially similar to those of **6**, except for the presence of a 11-methoxy aromatic substituent. Compound **7** is therefore 11-methoxy-16-hydroxyalstonal, which is the type-A isomer of the known type-B compound,



9 R = O
14 R = H_2

16-hydroxy-*N*(4)-demethylalstophylline oxindole (**13**), which was also present.

Unlike the previous compounds **5** and **6**, and **13** and **7**, which were resolvable pairs of type-A and type-B macroline isomers, compounds **8** and **14** were obtained as an inseparable mixture of type-A and type-B forms (ratio 3.5:1, respectively), which coeluted in column chromatography and proved resistant to further attempts at resolution by chromatography or fractional crystallization. The H-18 (methyl) and H-21 (aldehyde-H for **8**, vinylic-H for **14**) signals are clearly distinguishable in the ^{1}H NMR spectrum (Table 3), while the remaining hydrogen resonances are partially overlapped or coincident. In the ^{13}C NMR spectrum (Table 1), the majority of the signals appear in pairs with very similar chemical shifts or are coincident as in the case of the signals due to C-8, C-13, and C-17. This behavior has been observed previously in the case of the macroline indoles, alstonerine (type-B) and alstonerinal (type-A),[1] and in the case of the macroline oxindoles, *N*(1)-demethylalstonisine (type-B) and *N*(1)-demethylalstonal (type-A).[2] The NMR spectral data (Tables 1 and 3) indicated that **8** (alstophyllal) is the type-A isomer of the known type-B compound, alstophylline (**14**). These two compounds are also found in the stem-bark extract of this plant.

Compounds **9** and **10** were also obtained as an inseparable mixture of type-B and type-A macroline isomers, with the former predominating by a 2-fold excess. The EI-mass spectrum showed a M^+ at *m*/*z* 380, which analyzed for $C_{22}H_{24}N_2O_4$. The IR spectrum showed bands at 1642 and 1619 cm^{-1} due to various carbonyl functions. The UV spectrum showed absorption maxima at 211, 248, 273, and 319 nm, which do not correspond to that of a normal macroline indole. The ^{1}H NMR spectrum (Table 3) indicated a macroline indole derivative with aromatic methoxy substitution at C(11). This is revealed by the chemical shift data as well as by HMBC. Other clear signals include those due to the characteristic *N*(1)-Me, *N*(4)Me, and Me-18 groups. In the ^{13}C NMR spectrum (Table 1), the majority of the signals are overlapped or coincident, except for the signals due to C-5, C-15, C-16, C-18, C-19, C-20, and C-21. In the case of the ^{1}H NMR spectrum, the H-18 (methyl) and H-21 (aldehyde-H for **10**, vinylic-H for **9**) signals are clearly distinguishable as before (Table 3), while the remaining hydrogen resonances are partially overlapped or coincident. In a departure from the other macroline compounds, the COSY spectrum showed only one major partial structure, viz., *N*CHCH$_2$CHCHCH$_2$O, corresponding to the C-3, C-14, C-15, C-16, C-17 fragment of a macroline compound. The ^{1}H NMR spectrum is also notable for the absence of peaks normally due to both the H-6's, while the H-5 signal is simplified to a broad singlet. The notable difference in the case of the ^{13}C NMR spectrum is the replacement of the usual methylene C-6 resonance by a conjugated ketone carbonyl signal at δ 192.2 (coincident in both isomers). The placement of this ketone function at position 6 is also in agreement with the HMBC data (see Experimental Section), as well as the downfield shift of H-9 (δ 8.04, cf. ca. 7.3 in corresponding 11-methoxy-substituted macrolines) caused by the anisotropy of the proximate carbonyl function. Compounds **9** and **10** represent the first examples of oxygenation at carbon-6 of the macroline skeleton.

In addition to the new alkaloids discussed above, 21 other known alkaloids were also obtained from the leaf extract of this plant, as detailed in the Experimental Section. A notable feature of the alkaloidal composition, in addition to the new structures discussed above, is the predominance of the macroline skeleton, which is a characteristic of *Alstonia*.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-370 digital polarimeter or an Atago Polax-D polarimeter. IR spectra were recorded on a Perkin-Elmer 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 were obtained on a Perkin-Elmer API 100 instrument. EIMS and HREIMS were obtained at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia.

Plant Material. Plant material was collected in Terengganu, Malaysia (June 2000) and was identified by Dr. K. M. Wong, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia. Herbarium voucher specimens (K 659) are deposited at the Herbarium of the University of Malaya.

Extraction and Isolation. Extraction of the ground leaf material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid, as has been described in detail elsewhere.[18] The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH followed by rechromatography of appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal TLC were Et_2O, Et_2O/petroleum ether (1:1), Et_2O/petroleum ether (1:2), Et_2O/petroleum ether (1:4), Et_2O/petroleum ether (3:1), $CHCl_3$, MeOH/$CHCl_3$ (1:100), MeOH/$CHCl_3$ (1:50), MeOH/$CHCl_3$ (3:100), and MeOH/$CHCl_3$ (1:20). The yields (g kg^{-1}) of the alkaloids were as follows: **1** (0.0006), **2** (0.112), **3** (0.003), **4** (0.002), **5** (0.016), **6** (0.036), **7** (0.016), **8** (0.096), **9** (0.0005), **10** (0.003), **11** (0.002), **13** (0.019), **14** (0.027), macrocarpine B[16] (0.005), alstonerine[1,2] (0.070), alstonerinal[1] (0.022), talcarpine[7,19] (0.026), *N*(4)-methyl-*N*(4),21-*seco*talpinine[19] (0.033), alstonisine[2,7] (0.001), alstonal[7] (0.0003), *N*(4)-demethylalstophylline oxindole[20] (0.0003), *N*(4)-demethylalstophyllal oxindole[7] (0.0001), alstonoxine B[2] (0.040), vincorine[21] (0.004), demethylalstonamide[22] (0.002), vincoridine[23] (0.002), quaternine[24] (0.003), volkensine[24] (0.002), 10,11-dimethoxy-1-methyldeacetylpicraline-3′,4′,5′-trimethoxy-benzoate[25] (0.017), 6-methoxy-4-methylquinoline[26] (0.001), and 6-methoxy-α-methyl-4-quinolinemethanol[26] (0.002).

Alstomaline (1): yellowish oil; $[\alpha]_D$ −244° (*c* 0.04, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 202 (3.77), 273 (3.85), 317 (3.12) nm; IR (dry film) ν_{max} 1732, 1646, 1634 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 1, respectively; EIMS *m*/*z* 338 $[M]^+$ (100), 279 (27), 149 (21), 51 (21); HREIMS *m*/*z* 338.1620 (calcd for $C_{20}H_{22}N_2O_3$, 338.1631).

10,11-Dimethoxynareline (2): light yellowish oil; $[\alpha]_D$ −56° (*c* 1.57, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 232 (4.38), 294 (3.81) nm; IR (dry film) ν_{max} 3201, 1737 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 1, respectively; EIMS *m*/*z* 412 $[M]^+$ (75), 383 (100), 367 (28), 353 (19), 325 (64), 307 (33), 197 (26), 183 (16), 154 (15), 77 (14), 59 (31), 41 (18); HREIMS *m*/*z* 412.1620 (calcd for $C_{22}H_{24}N_2O_6$, 412.1634).

Alstohentine (3): light yellowish oil; $[\alpha]_D$ −58° (*c* 0.22, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 228 (3.91), 289 (3.26) nm; IR (dry film) ν_{max} 3368 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 1, respectively; EIMS *m*/*z* 356 $[M]^+$ (54), 338 (16), 325 (51), 308 (14), 239 (18), 197 (100), 182 (57), 170 (34), 144 (13), 70 (32), 40 (41); HREIMS *m*/*z* 356.2094 (calcd for $C_{21}H_{28}N_2O_3$, 356.2100).

Acetylation of Alstohentine (3). Alstohentine (**3**) (9 mg) was added to a mixture of acetic anhydride/pyridine (1:1; 2 mL) and the mixture stirred at room temperature for 2 h. The mixture was then poured into saturated Na_2CO_3 and extracted with CH_2Cl_2. Removal of the solvent followed by purification by centrifugal chromatography over SiO_2 (2% MeOH−$CHCl_3$) afforded 3 mg (30%) of the monoacetate derivative **12** as a

colorless oil; $[\alpha]_D$ +431° (*c* 0.03, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (4.16), 285 (3.72), 293 (3.67) nm; IR (dry film) ν_{max} 3400, 1739, 1229 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 1, respectively; EIMS *m/z* 398 ($[M]^+$, 66%, $C_{23}H_{30}N_2O_4$), 325 (11), 284 (9), 239 (27), 225 (32), 197 (100), 182 (74), 170 (22), 158 (15) 144 (19), 70 (49).

Alstomicine (4): light yellowish oil; $[\alpha]_D$ +74° (*c* 0.14, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 230 (3.80), 289 (3.11) nm; IR (dry film) ν_{max} 3400, 1711 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 1, respectively; EIMS *m/z* 326 $[M]^+$ (5), 308 (82), 293 (5), 265 (4), 239 (21), 212 (7), 197 (100), 181 (31), 170 (98), 154 (14), 128 (8), 70 (26), 40 (46); HREIMS *m/z* 326.1988 (calcd for $C_{20}H_{26}N_2O_2$, 326.1994).

16-Hydroxyalstonisine (5): white amorphous powder; $[\alpha]_D$ +170° (*c* 0.15, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 209 (4.17), 256 (3.95) nm; IR (dry film) ν_{max} 3400, 1685, 1611 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; EIMS *m/z* 354 $[M]^+$ (58), 176 (100), 160 (64), 134 (41), 40 (17); HREIMS *m/z* 354.1592 (calcd for $C_{20}H_{22}N_2O_4$, 354.1579).

16-Hydroxyalstonal (6): white amorphous powder; $[\alpha]_D$ +153° (*c* 0.26, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 207 (4.61), 263 (4.35) nm; IR (dry film) ν_{max} 3400, 1684, 1610 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; EIMS *m/z* 354 $[M]^+$ (70), 176 (100), 160 (61), 134 (27), 96 (9), 43 (13); HREIMS *m/z* 354.1577 (calcd for $C_{20}H_{22}N_2O_4$, 354.1579).

16-Hydroxy-*N*(4)-demethylalstophyllal oxindole (7): white amorphous powder; $[\alpha]_D$ +203° (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 220 (4.54), 267 (4.20) nm; IR (dry film) ν_{max} 3400, 1693, 1622 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; EIMS *m/z* 384 $[M]^+$ (61), 202 (14), 190 (66), 176 (100), 149 (22), 134 (29), 122 (14), 96 (13), 40 (18); HREIMS *m/z* 384.1692 (calcd for $C_{21}H_{24}N_2O_5$, 384.1685).

Alstophyllal (8) and alstophylline (14): light yellowish oil; ^1H NMR and ^{13}C NMR data of **8**, see Tables 3 and 1, respectively; EIMS *m/z* 366 $[M]^+$ (100), 227 (94), 200 (70), 170 (14), 146 (12), 70 (17); HREIMS *m/z* 366.1952 (calcd for $C_{22}H_{26}N_2O_3$, 366.1943).

6-Oxoalstophylline (9) and 6-oxoalstophyllal (10): light yellowish oil; $[\alpha]_D$ +31° (*c* 0.08, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 211 (4.76), 248 (4.70), 273 (4.65), 319 (4.11) nm; (IR (dry film) ν_{max} 1642, 1619 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 3 and 1, respectively; HMBC of **9**, H-5/C-3, C-6, C-15, C-16, C-17; H-9/C-11, C-13; H-10/C-8, C-12; H-12/C-8, C-10, C-11; H-15/C-20; H-16/C-20; H-17/C-15, C-16, C-21; 18-Me/C-19; H-21/C-15, C-17, C-19, C-20; *N*(1)-Me/C-2, C-13; *N*(4)-Me/C-3, C-5; 11-OMe/C-11; HMBC of **10**, H-5/C-3, C-6, C-15, C-16, C-17; H-9/C-11, C-13; H-10/C-8, C-12; H-12/C-8, C-10, C-11; H-15/C-20; H-16/C-20; H-17/C-15, C-16; 18-Me/C-19; H-21/C-15; *N*(1)-Me/C-2, C-13; *N*(4)-Me/C-3, C-5; 11-OMe/C-11; EIMS *m/z* 380 $[M]^+$ (100), 350 (9), 293 (7), 255 (25), 214 (76), 175 (7), 111 (6), 70 (15); HREIMS *m/z* 380.1742 (calcd for $C_{22}H_{24}N_2O_4$, 380.1736).

Acknowledgment. We thank the University of Malaya and IRPA for financial support.

References and Notes

(1) Kam, T. S.; Iek, I. H.; Choo, Y. M. *Phytochemistry* **1999**, *51*, 839−844.
(2) Kam, T. S.; Choo, Y. M. *Tetrahedron* **2000**, *56*, 6141−6150.
(3) Whitmore, T. C. *Tree Flora of Malaya*; Longman: Kuala Lumpur, 1973; Vol. 2, pp 7−12.
(4) Kochummen, K. M.; Wong, K. M. *Blumea* **1984**, *29*, 513−522.
(5) Sidiyasa, K. *Taxonomy, Phylogeny, and Wood Anatomy of Alstonia (Apocynaceae)*; Blumea Supplement II, 1998; p 230.
(6) Kam, T. S.; Choo, Y. M. *Phytochemistry* **2004**, in press.
(7) Wong, H. H.; Lim, P. B.; Chuah, C. H. *Phytochemistry* **1996**, *41*, 313−315.
(8) Kam, T. S. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon: Amsterdam, 1999; Vol. 14, pp 285−435.
(9) Chatterjee, A.; Ghosh, A. K.; Hagaman, E. W. *J. Org. Chem.* **1982**, *47*, 1732−1734.
(10) Subhadhirasakul, S.; Takayama, H.; Aimi, N.; Ponglux, D.; Sakai, S. I. *Chem. Pharm. Bull.* **1994**, *42*, 1427−1431.
(11) Sangster, A. W.; Stuart, K. L. *Chem. Rev.* **1965**, *65*, 69−130.
(12) Kam. T. S.; Nyeoh, K. T.; Sim, K. M.; Yoganathan, K. *Phytochemistry* **1997**, *45*, 1303−1305.
(13) Achenbach, H.; Waibel, R.; Zwanzger, M. *Phytochemistry* **1994**, *37*, 1737−1743.
(14) Morita, Y.; Hesse, M.; Schmid, H.; Banerji, A.; Banerji, J.; Chatterjee, A.; Oberhansli, W. E. *Helv. Chim. Acta* **1977**, *60*, 1419−1434.
(15) Mayerl, F.; Hesse, M. *Helv. Chim. Acta* **1978**, *61*, 337−351.
(16) Kam, T. S.; Choo, Y. M.; Komiyama, K. To be published.
(17) Atta-ur-Rahman; Qureshi, M. M.; Muzaffar, A.; De Silva, K. T. D. *Heterocycles* **1988**, *27*, 725−732.
(18) Kam, T. S.; Tan, P. S. *Phytochemistry* **1990** *29*, 2321−2322.
(19) Naranjo, J.; Pinar, M.; Hesse, M.; Schimd, H. *Helv. Chim. Acta* **1972**, *55*, 752−771.
(20) Atta-ur-Rahman; Silva, W. S. D.; Alvi, K. A.; De Silva, K. T. D. *Phytochemistry* **1987**, *26*, 865−868.
(21) Das, B. C.; Cosson, J. P.; Lukacs, G.; potier, P. *Tetrahedron Lett.* **1974**, 4299−4302.
(22) Atta-ur-Rahman; Abbas, S. A.; Nighat, F.; Ahmed, G.; Choudhary, M. I.; Alvi, K. A.; Habib-ur-Rehman; De Silva, K. T. D.; Arambewela, L. S. R. *J. Nat Prod.* **1991**, *54*, 750−754.
(23) Caron, C.; Yachaoui, Y.; Massiot, G.; Le Men-Olivier, L.; Pusset, J.; Sevenet, T. *Phytochemistry* **1984**, *23*, 2355−2357.
(24) Abe, F.; Yamauchi, T.; Padolina, W. J. *Phytochemistry* **1994**, *35*, 253−257.
(25) Petitfrere-Auvray, N.; Vercauteren, J.; Massiot, G.; Lukacs, G.; Sevenet, T.; Le Men-Olivier, L.; Richard, B.; Jacquier, M. J. *Phytochemistry* **1981**, *20*, 1987−1990.
(26) Cornforth, J. W.; Cornforth, R. H. *J. Chem. Soc.* **1948**, 93−97.

NP034041V