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Unusual spirocyclic macroline alkaloids, nitrogenous derivatives, and a cytotoxic bisindole from *Alstonia*

Toh-Seok Kam,[a,*] Yeun-Mun Choo[a] and Kanki Komiyama[b]

[a]*Department of Chemistry, University of Malaya, Pantai Valley, 50603 Kuala Lumpur, Malaysia*
[b]*The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan*

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Abstract—The bark extract of the Malayan *A. macrophylla* provided several novel indoles with unprecedented carbon skeletons, an unusual nitrogenous compound, a cytotoxic bisindole, several new macroline alkaloids, in addition to other known alkaloids. The structures of the new compounds were established by spectroscopic analysis.

1. Introduction

The genus *Alstonia* is characterised by a preponderance of the macroline-type indole and oxindole alkaloids.[1–9] We have previously reported the presence of new macroline indoles as well as oxindoles from the Malayan species, *A. angustifolia* var. *latifolia*, including isoalstonisine and macrogentine, which represent the first macroline oxindoles possessing the *S* configuration at the spirocyclic carbon.[3,4] In continuation of our studies of Malaysian *Alstonia*, we would like to report the structures of new alkaloids from *A. macrophylla*, including novel macroline alkaloids incorporating an unprecedented spiroketal unit,[9] unusual nitrogenous derivatives, and a cytotoxic bisindole.

2. Results and discussion

Macrodasine A **1** was obtained from the bark extract of *A. macrophylla* as a colourless oil, with $[\alpha]_D$=+36 (*c* 0.36, $CHCl_3$). The UV spectrum was characteristic of an indole chromophore with absorption maxima at 230 and 287 nm, while the IR spectrum (3411 cm^{-1}, broad) indicated the presence of hydroxyl functions. The EIMS of **1** showed a molecular ion at *m/z* 454, which analyzed for $C_{26}H_{34}N_2O_5$, requiring 11 degrees of unsaturation while the mass fragments which were observed at *m/z* 197, 182, 181, 170, and 144 are typical of macroline derivatives[10] and provided early indication that **1** contained a macroline core. The ^{13}C NMR spectrum (Table 2) gave a total of 26 separate carbon resonances (three methyls, six methylenes, 11 methines, and six quaternary carbons) in agreement with the molecular formula. In addition to the eight signals associated with the indole moiety, the ^{13}C NMR spectrum is notable for the presence of two oxymethylenes (δ 63.9, 64.3), two oxymethines (δ 77.7, 79.2), and two quaternary carbons each of which are flanked by two oxygen atoms (δ 105.5, 114.8), consistent with a highly oxygenated molecule as indicated by the molecular formula. The ^1H NMR spectrum of **1** (Table 1) showed the presence of an unsubstituted indole chromophore, from the signals due to four aromatic hydrogens, the presence of three methyl groups corresponding to the *N*(1)–Me (δ 3.63), *N*(4)–Me (δ 2.33), and Me(18) (δ 1.59), and a hydroxymethyl group from the presence of a pair of doublet of doublets at δ 3.43 and 3.77 (corresponding to the carbon resonance at δ 63.9).

The COSY spectrum disclosed some partial structures which are characteristic 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.[3,4] This is further supported by the observed hydrogen chemical shifts and coupling behaviour for H(3), H(5), H(16), H(17), as well as the three characteristic methyl groups which are typical of a macroline compound (e.g., alstonerine).[3] At this stage, further analysis of the COSY spectrum was complicated by overlap of some key signals. Thus, two sets of partial structures can be proposed for the remaining fragments, viz., $CHCH_2$ and $OCHCH_2CHCH_2O$, versus $CHCH_2CHO$ and CH_2CHCH_2O, which with the aid of the HMBC data led to two possible structures, **1** and **2**, respectively. Structure **1** is distinguished by the incorporation of a 1,6-dioxaspiro[4.4]nonane substructure fused onto a macroline residue, while structure **2** on the other hand, is distinguished by the incorporation of contiguously fused tetrahydropyran and tetrahydrofuran rings onto the

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* Corresponding author. Tel.: +60379674266; fax: +60379674193; e-mail address: tskam@um.edu.my

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Table 1. ^{1}H NMR spectral data of **1**, **3**, **4**, **5**, **11**, **12**, **13**, **14**, **15**, and **18**[a]

Position	**1**	**3**	**4**	**5**	**11**	**12**	**13**	**14**	**15**	**18**	Position	**18**
3	3.95 t (3)	4.79 brs	3.94 t (3)	4.78 br s	3.96 m	3.98 t (3)	3.97 t (4)	3.82 br s	3.82 br s	4.09 dd (4, 2)	3′	3.79 t (3)
5	2.98 d (7)	3.56 d (7)	2.99 d (7)	3.57 d (7)	2.87 d (7)	2.91 d (7)	2.91 d (7)	3.10 d (6)	3.10 d (6)	3.46 d (7)	5′	2.99 d (7)
6	2.39 m	3.04 d (18)	2.41 d (17)	3.02 d (17)	2.47 d (17)	2.43 d (17)	2.45 d (17)	2.48 d (16)	2.48 d (16)	2.54 m	6′	2.28 m
	3.27 dd (17, 7)	3.45 dd (18, 7)	3.28 dd (17, 7)	3.46 dd (17, 7)	3.25 dd (17, 7)	3.26 dd (17, 7)	3.27 dd (17, 7)	3.30 dd (16, 6)	3.30 dd (16, 6)	3.08 m		3.32 m
9	7.50 br d (8)	7.56 br d (8)	7.50 br d (8)	7.55 br d (8)	7.49 br d (8)	7.49 br d (8)	7.49 dd (8, 1)	7.33 d (8)	7.33 d (8)	7.52 br d (8)	9′	6.90 s
10	7.12 br t (8)	7.23 td (8, 1)	7.12 td (8, 1)	7.23 td (8, 1)	7.10 td (8, 1)	7.10 td (8, 1)	7.09 td (8, 1)	6.76 dd (8, 2)	6.76 dd (8, 2)	7.13 td (8, 1)	10′	—
11	7.21 td (8, 1)	7.35 td (8, 1)	7.21 td (8, 1)	7.35 td (8, 1)	7.19 td (8, 1)	7.18 td (8, 1)	7.17 td (8, 1)	—	—	7.22 td (8, 1)	11′	—
12	7.31 br d (8)	7.40 br d (8)	7.31 br d (8)	7.40 br d (8)	7.29 br d (8)	7.29 br d (8)	7.27 dd (8, 1)	6.84 d (2)	6.84 d (2)	7.32 br d (8)	12′	6.69 s
14	1.55 ddd (13, 5, 3)	1.78 br d (14)	1.56 m	1.79 dt (14, 5)	1.42 ddd (13, 5, 2)	1.54 ddd (12, 4, 3)	1.39 dt (13, 4)	1.81 m	1.81 m	1.98 m	14′	1.75 td (12, 3)
												2.04 m
	2.39 m	3.33 td (14, 4)	2.42 m	3.35 td (14, 3)	2.50 td (13, 4)	2.29 m	2.26 td (13, 4)	2.13 m	2.13 m	2.41 m	15′	2.54 m
15	1.85 m	1.92 dt (14, 5)	1.84 dt (12, 5)	1.96 dt (14, 5)	2.06 dt (13, 5)	1.97 dt (13, 4)	1.86 m	2.64 dt (11, 5)	2.64 dt (11, 5)	2.14 m	16′	1.84 dt (11, 4)
16	2.03 dt (12, 5)	2.33 dt (12, 5)	2.14 m	2.49 dt (13, 5)	2.15 dt (11, 5)	1.86 dt (11, 4)	1.86 m	1.92 m	1.92 m	1.57 m	17′	4.13 ddd
17	3.70 dd (12, 5)	3.75 dd (12, 5)	3.85 dd (12, 5)	3.88 dd (13, 5)	3.79 dd (11, 5)	3.73 dd (11, 4)	3.74 dd (11, 4)	4.17 ddd (11, 4, 2)	4.19 ddd (11, 4, 2)	3.95 dd (11, 3)		(11, 4, 1)
												4.37 t (11)
	4.04 t (12)	4.82 t (12)	4.08 t (12)	4.92 t (13)	4.07 t (11)	4.06 t (11)	4.07 t (11)	4.45 t (11)	4.50 t (11)	4.01 dd (11, 2)	18′	2.05 s
18	1.59 s	1.72 s	1.54 s	1.71 s	1.24 d (7)	1.15 d (6)	1.13 d (6)	2.09 s	2.17 s	1.72 s	21′	7.51 s
19	—	—	—	—	3.96 m	3.49 m	3.51 dq (10, 6)	—	—	—	*N*(1)-Me′	3.65 s
20	2.01 dd (12, 8)	2.07 m	2.02 m	2.10 m	1.07 m	1.46 m	1.69 m	—	—	3.32 m	*N*(4)-Me′	2.25 s
21	1.85 m	1.85 dd (13, 8)	2.02 m	2.10 m	3.69 dd (11, 4)	3.31 dd (11, 8)	3.83 d (7)	7.54 s	9.66 s	2.41 m	11′-OMe	3.87 s
	2.39 m	2.17 t (13)	2.15 dd (13, 11)	2.10 m	3.81 dd (11, 6)	3.49 m	3.83 d (7)	—	—	3.08 m		
23	4.13 d (5)	5.21 d (4)	—	—	—	—	1.68 s	—	—	—		
24	1.85 m	2.03 m	2.50 dd (17, 7)	2.28 dd (19, 7)	—	—	—	—	—	—		
	2.39 m	2.14 td (9, 4)	2.52 dd (17, 8)	2.69 dd (19, 7)	—	—	—	—	—	—		
25	4.42 m	4.37 dtd (9, 7, 4)	4.56 m	4.69 tt (7, 4)	—	—	—	—	—	—		
26	3.43 dd (12, 3)	3.98 dd (12, 7)	3.61 dd (12, 4)	4.15 dd (12, 4)	—	—	—	—	—	—		
	3.77 dd (12, 2)	4.21 dd (12, 4)	3.96 dd (12, 3)	4.30 dd (12, 3)	—	—	—	—	—	—		
N(1)–Me	3.63 s	3.69 s	3.63 s	3.69 s	3.62 s	3.62 s	3.60 s	—	—	3.55 s		
N(4)–Me	2.33 s	2.88 s	2.34 s	2.88 s	2.31 s	2.30 s	2.34 s	2.36 s	2.36 s	2.34 s		
23-OAc	—	2.02 s	—	—	—	—	—	—	—	—		
26-OAc	—	2.06 s	—	2.02 s	—	—	—	—	—	—		
11-OMe	—	—	—	—		—	—	3.84 s	3.84 s	—		

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

Table 2. ^{13}C NMR spectral data of **1**, **3**, **4**, **5**, **7**, **9**, **10**, **11**, **12**, **13**, **14**, **15** and **18**[a]

Position	**1**	**3**	**4**	**5**	**7**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**18**	Position	**18**
2	132.8	[b]	131.5	125.9	60.2	132.8	132.8	133.2	133.2	133.0	129.2	129.2	131.3	2′	132.9
3	53.3	55.1	53.3	55.7	36.9	53.5	53.1	53.7	53.6	53.5	54.8	54.8	53.1	3′	53.7
4	—	—	—	—	220.0	—	—	—	—	—	—	—	—	5′	54.7
5	54.8	56.2	54.7	56.4	69.1	54.5	54.9	54.6	55.1	54.9	55.1	55.1	59.2	6′	22.0
6	22.5	23.4	22.6	23.8	30.7	22.5	22.4	22.6	22.5	22.4	22.9	22.5	22.6	7′	105.4
7	106.4	105.4	106.5	105.9	26.7	106.6	106.6	106.6	106.7	106.7	106.4	106.4	105.9	8′	120.1
8	126.4	[b]	126.2	124.9	36.5	126.2	126.2	126.4	126.4	126.3	121.6	121.6	126.3	9′	118.7
9	118.0	118.4	118.0	118.6	68.4	118.1	117.9	118.1	117.9	117.9	118.2	118.2	118.2	10′	119.1
10	118.9	120.0	118.9	120.4	18.9	118.9	118.9	118.8	118.8	118.8	108.8	108.8	119.0	11′	153.6
11	121.0	[b]	121.0	123.2	69.4	121.0	121.0	120.8	120.7	120.8	156.2	156.2	120.9	12′	91.3
12	108.0	109.5	108.8	109.7	54.2	108.7	108.9	108.7	108.8	108.5	95.4	95.4	108.7	13′	136.5
13	136.9	137.5	137.1	137.6	203.4	137.2	137.0	136.9	137.0	136.9	136.8	136.8	137.0	14′	32.4
14	31.9	[b]	31.9	[b]	—	30.1	26.7	30.7	25.3	25.0	32.9	32.9	32.3	15′	22.8
15	26.5	25.9	27.1	25.9	—	27.0	26.1	28.6	26.7	27.1	23.0	23.0	31.5	16′	38.3
16	36.9	36.8	36.6	36.8	—	39.4	42.5	39.3	43.5	43.4	38.6	38.6	43.1	17′	67.7
17	64.3	[b]	68.8	62.9	—	68.8	67.1	68.9	67.6	67.5	67.7	68.1	66.5	18′	24.9
18	24.2	[b]	23.5	22.7	—	19.2	20.2	18.8	20.2	20.1	25.0	16.5	31.1	19′	195.4
19	105.5	105.4	106.1	104.9	—	69.4	67.8	71.2	70.5	70.3	195.6	170.0	213.2	20′	120.8
20	44.3	43.6	45.8	45.3	—	54.6	57.7	43.6	46.8	43.2	121.6	121.6	54.5	21′	157.4
21	34.7	35.2	35.9	35.6	—	204.7	203.0	63.1	61.6	62.9	157.6	188.8	32.0	*N*(1)-Me′	29.0
22	114.8	113.7	106.4	105.6	—	—	—	—	—	170.8	—	—	—	*N*(4)-Me′	41.2
23	77.7	78.8	209.4	208.6	—	—	—	—	—	20.3	—	—	—	11′-OMe	55.5
24	33.0	33.0	34.7	35.3	—	—	—	—	—	—	—	—	—		
25	79.2	76.4	75.0	72.4	—	—	—	—	—	—	—	—	—		
26	63.9	67.2	63.1	64.9	—	—	—	—	—	—	—	—	—		
N(1)–Me	29.0	29.4	29.0	29.5	42.2	29.1	29.0	29.0	29.0	28.9	—	—	28.9		
N(4)–Me	41.6	40.8	41.7	40.4	—	41.8	41.6	41.7	41.7	41.6	41.5	41.5	41.7		
23-OAc	—	21.1 170.0	—	—	—	—	—	—	—	—	—	—	—		
26-OAc	—	20.9 170.8	—	20.7 170.6	—	—	—	—	—	—	—	—	—		
11-OMe	—	—	—	—	—	—	—	—	—	—	55.8	55.8	—		

[a] $CDCl_3$, 100 MHz; assignments based on HMQC and HMBC.
[b] Not detected.

same macroline unit. Both structures accommodate the observed NMR chemical shifts as well as the HMBC correlation data. To resolve the difficulty in distinguishing the two structures, acetylation (Ac_2O, pyridine) was carried out which yielded a single diacetylated derivative, providing cogent support for structure **1**. Furthermore, conversion to the acetylated derivative resulted in a better resolved ^1H NMR spectrum (Table 1), which removed the earlier ambiguity associated with some of the key signals. Specifically, the signals for H(21), H(23), H(24) and H(25) were now sufficiently clear and well resolved in the acetate derivative **3** {whereas H(24) and H(21) were overlapping multiplets in **1**}, and indicated the presence of the key $OCHCH_2CHCH_2O$ fragment, corresponding to the C(23)–C(24)–C(25)–C(26) partial structure in **1**. In addition, the observed carbon resonance of δ 114.8 for the spirocyclic centre was in good agreement with that previously noted for the spirocarbon in compounds containing a 1,6-dioxaspiro[4.4]nonane unit.[11–14]

The ring junction stereochemistry between rings C, D, and E, is assumed to follow that in the known macroline compounds (e.g., alstonerine)[3] from the similarity of the chemical shifts and coupling patterns observed for the ring junction hydrogens, a supposition which is also in agreement with the NOE and NOESY data. The observed NOE between 18-methyl and H(17α) as well as H(20), fixes the E/F ring junction stereochemistry as *cis* {18-Me and H(20) both α}. The resonance for H(20) was a doublet of doublets with J=12, 8 Hz. Decoupling experiments indicated that the splittings were due to coupling with the two H(21). Since the stereochemistry of H(20) has been fixed as α, the 12 Hz coupling must be due to coupling to H(21β). Irradiation of H(23) causes NOE enhancement of H(25) and vice versa, indicating that they are *syn* to each other. Aside from these, further assignment of the remaining stereochemistry, such as that of the spirocyclic centre at C(22), was precluded by the unresolved signals of H(21) in **1**, which were fortuitously well resolved in the diacetate derivative **3**. Thus observation of the key NOE interaction between H(23) and H(21β) in **3**, not only allowed assignment of the configuration at the spirocarbon as *R*, but also fixes the stereochemistry of C(23) and C(25), respectively, as *R*, *R*.

Macrodasine B **4** was also obtained from the bark extract of *A. macrophylla*, as a colourless oil, with $[\alpha]_D$=+149 (*c* 0.067, $CHCl_3$). The UV spectrum was very similar to that of **1** with absorption maxima at 230 and 287 nm (log ε 3.95 and 3.23, respectively), characteristic of an unsubstituted indole chromophore, while the IR spectrum showed in addition to a broad OH band at 3435 cm^{-1}, another band at 1765 cm^{-1}, indicative of a five-membered cyclic ketone. The EIMS of **4** showed a molecular ion at *m*/*z* 452, which analyzed for $C_{26}H_{32}N_2O_5$, two mass units less than that of **1**, and requiring 12 degrees of unsaturation. In common with **1**, the mass fragments which were observed at *m*/*z* 197, 182, 181, 170, and 144 are characteristic of macroline derivatives,[10] and indicated that **4** also contained a macroline-like residue. The ^{13}C NMR spectrum (Table 2) gave a total of 26 separate carbon resonances (three methyls, six methylenes,

10 methines, and seven quaternary carbons) in agreement with the molecular formula, but differing from that of **1** by the addition of a quaternary carbon at the expense of a methine.

The ^{1}H and ^{13}C NMR spectral data share a number of common features with that of a typical macroline (as well as with **1**), indicating that rings A–E are essentially unchanged, but that substantial changes have occurred affecting rings F and G. Thus the ^{1}H NMR spectrum (Table 1) showed the presence of three methyl groups corresponding to the *N*(1)–Me (δ 3.63), *N*(4)–Me (δ 2.34), and Me(18) (δ 1.54), and a hydroxymethyl group, from the presence of a pair of doublet of doublets at δ 3.61 and 3.96 (corresponding to the carbon resonance at δ 63.1), which are similar to **1**. The COSY spectrum of **4** showed in addition to the $NCHCH_2$, $CHCH_2$, and $NCHCH_2CHCHCH_2O$ fragments, which are common to **1**, a CH_2CHCH_2O fragment, in place of the $OCHCH_2CHCH_2O$ fragment observed in **1**. Comparison of the ^{13}C NMR spectra of 1 and 4, showed that the two oxymethylenes at δ 68.8 and 63.1, corresponding to C(17) and C(26), respectively, are intact, as is the oxymethine corresponding to C(25) {δ 75.0 c.f. 79.2 in **1**}, and the quaternary carbon resonance due to the spiroacetal C(22), which was observed at δ 106.4. However, the other oxymethine at δ 77.7 corresponding to C(23) in **1**, is absent in the spectrum of **4**. Instead a ketone carbonyl resonance at δ 209.4 was observed in its place.

At this stage the structure of macrodasine B can be assembled as shown in structure **4**, which reveals it to be the 23-oxo derivative of **1**. The structure is consistent with the HMBC data (Fig. 1), as well as the observed cyclic ketone absorption at 1765 cm^{-1} in the IR spectrum, which is in excellent agreement with that of 3-oxacyclopentanones (1764 cm^{-1}) versus that for 3-oxacyclohexanones (1725 cm^{-1}).[15] In addition, the chemical shift and geminal coupling constant for H(24) {δ 2.50 dd, J=17, 7 Hz; 2.52, dd, J=17, 8 Hz} are highly diagnostic of geminal hydrogens adjacent to a carbonyl carbon.[4,16] Reaction of **4** with Ac_2O/pyridine yielded the monoacetate derivative **5**, in agreement with the proposed structure. The NOESY and NOE data are similar to those observed for **1** and confirmed the stereochemistry of the E/F ring junction {18-Me and H(20) both α},[9] in addition to the characteristic ring junction stereochemistries for the C/D/E rings, which correspond to that of a typical macroline.[3,4] In the case of macrodasine A **1**, assignment of the configuration at the spirocyclic C(22) was facilitated by the well-resolved H(21) and H(23) signals in the diacetate derivative **3**, which permitted NOE experiments to be carried out {NOE between H(23) and H(21β)},[9] which is precluded in the case of macrodasine B **4**, where C(23) is now a ketone carbonyl. The configuration at the spirocyclic C(22) and at C(25) in **4** are therefore tentatively assigned as *R* and *R*, respectively, on the grounds of a presumed close biogenetic relationship with **1**.

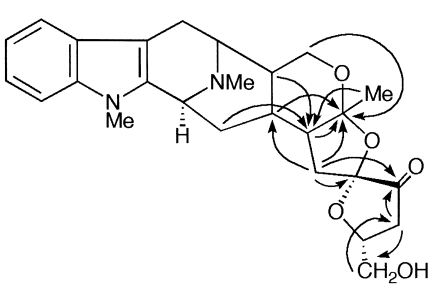


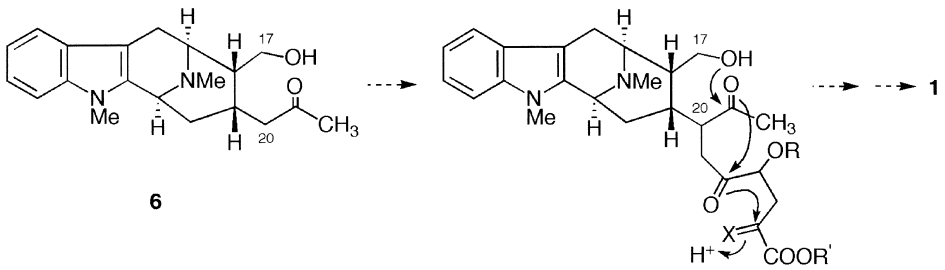
Figure 1. Selected HMBC (H to C) of **4**.

Macrodasines A **1** and B **4**, represent the first members of an unusual class of macroline compounds which have incorporated additional novel structural features, in the form of fused spirocyclic tetrahydrofuran rings, incorporating an unprecedented spiroacetal moiety. The spiroketal unit has been previously encountered in insect pheromones,[17–19] marine natural products,[11–14,20,21] microbial compounds,[17,22–26] plant steroidal derivatives[17] and various other plant secondary metabolites.[17] It has however not been found as a substructure in alkaloids. The macrodasines **1** and **4**, thus represent the first instances of the incorporation of a spiroketal unit in an indole alkaloid.

A tentative proposal for a possible pathway to these unusual compounds is from the ring-opened form of alstonerine **6**,[4,30] which on alkylation by a six-carbon fragment at C(20), followed by tandem intramolecular hemiketal formation (Scheme 1), yields the ring system of the macrodasines.[9]

An unusual nitrogenous compound, angustimalal **7** was also obtained from this study. It was isolated as a colourless oil, with $[\alpha]_D$=+78 (*c* 0.064, $CHCl_3$). The IR spectrum showed two carbonyl bands, one at 1717 cm^{-1}, corresponding to an aldehyde carbonyl, and another at 1741 cm^{-1}, indicative of a five-membered ring ketone. The characterstic Fermi doublets at 2767 and 2867 cm^{-1} were clear in this instance, and taken with the ^{1}H NMR signal at δ 10.0, confirmed the presence of the aldehyde function. The EIMS of **7** showed a molecular ion at *m/z* 237, the odd mass indicating the presence of a single nitrogen. HREIMS measurements gave the formula $C_{13}H_{19}NO_3$. The ^{13}C NMR spectrum (Table 2) showed a total of 13 peaks in agreement with the molecular formula (two methyls, three methylenes, seven methines and one quaternary carbon). Two methyl groups were indicated, a CH_3CH (δ 1.42) and an NCH_3 (δ 2.30). The quaternary carbon resonance at δ 220.0 is due to a ketone function while the methine at δ 203.4 corresponds to the aldehyde group. The COSY spectrum revealed the following partial structures, $NCHCH_2$, $NCHCH_2CHCHCH_2O$, and $CH_3CHCHCH{=}O$. The former two fragments are characteristic of macroline compounds and correspond to the C(5)–C(6) and C(3)–C(14)–C(15)–C(16)–C(17) fragments, respectively, of a macroline alkaloid, while the latter fragment, together with the two methine resonances at δ 26.7 and 36.5, corresponds to the ring E portion of a type-A macroline, such as talcarpine **9**. The molecule can therefore be assembled accordingly and requires only insertion of a ketone function to complete the structure of angustimalal as shown in **7**, which is in perfect agreement with the HMBC data (Fig. 2).

The ring junction stereochemistry was established from the NOESY spectrum and was in agreement with that in a typical macroline alkaloid (e.g., talcarpine **9**). The stereochemistries of the tetrahydropyran ring substituents were



Scheme 1. (X=O; or OH, H).

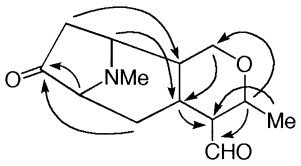


Figure 2. Selected HMBC (H to C) of **7**.

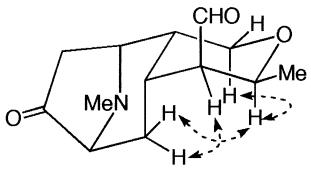


Figure 3. Selected NOE's of **7**.

also established on the basis of the NOESY spectrum (Fig. 3).

The stereochemistry of the two H(9) can be determined on the basis of their respective coupling constants (see Section 3). The α-oriented H(9) which is *trans*-diaxial with H(8) is seen as a triplet, with J=11 Hz. The observed NOE interaction of H(9α) with H(11) indicated that the methyl substituent is β. Similarly H(6α) can be distinguished from H(6β) on the basis of their coupling interactions. The NOE observed between H(6α)/H(11α), and between H(6β)/H(12) confirmed the β-stereochemistry of the C(12) aldehyde substituent (Fig. 3). This assignment is further vindicated by comparison of the chemical shifts of the aldehyde-H in angustimalal **7** (δ 10.0) with the shifts observed for the corresponding macroline alkaloids, talcarpine **9** (β-CHO, δ 9.95) and *N*(4)-methyl-*N*(4), 21-*seco*talpinine 10 (α-CHO, δ 9.41) (see Section 3).

The structure of angustimalal **7** shows that it retains all the features of the non-indole portion of a type-A macroline compound, except for the presence of an additional oxygenated carbon. A similar compound, angustimaline **8** (corresponding to the non-indole portion of a type-B macroline alkaloid in this case) has been encountered once recently, from the bark extract of another *Alstonia* species.[27] The origin of such compounds remains enigmatic, although a simple assumption (in the case of **7**) is that it is probably derived from fragmentation of a macroline-type precursor, possibly talcarpine **9** (which also occurs in the same plant), or its as yet unknown oxindole.

Three other new macroline indole derivatives were also obtained from the bark extract, macrocarpines A **11**, B **12**, and C **13**. A common feature of these three alkaloids is that they contain a saturated ring E, as exemplified by talcarpine **9**. Macrocarpine A **11** was obtained as a light yellowish oil, $[\alpha]_D$=+117 (c 0.11, $CHCl_3$). The IR spectrum showed the presence of a hydroxyl function (3400 cm^{-1}), while the UV spectrum indicated an indole chromophore. The EIMS of **11** showed a molecular ion at m/z 340, which analysed for $C_{21}H_{28}N_2O_2$. Examination of the ^1H and ^{13}C NMR spectral data (Tables 1 and 2, respectively) revealed a macroline compound resembling talcarpine **9** in all respects except for changes involving the substituents in the saturated E-ring, viz., the replacement of the 20β-CHO substituent by a 20β-hydroxymethyl substituent in **11**. This is clearly indicated by the presence of the hydroxymethyl signals (δ_H 3.69, 3.81; δ_C 63.1) in place of the aldehyde signals of talcarpine. The stereochemistry of the hydroxymethyl substituent at C(20) is readily confirmed from the observed NOE interaction between H(14β) and H(20) which is only possible if H(20) is α. The assignment is also confirmed by chemical correlation, by conversion of talcarpine **9** to **11** by $NaBH_4$ reduction, and oxidation of **11** to talcarpine **9** by PCC. Macrocarpine B **12** was obtained as a light yellowish oil, $[\alpha]_D$=−51 (c 0.34, $CHCl_3$). The IR (OH, 3400 cm^{-1}), UV (indole), and EIMS (M^+ m/z 340) spectral data were similar to that of **11**, as were the NMR spectral data (Tables 1 and 2), which were generally similar except for the noticeable difference in the shifts of C(14) and C(16). These similarities indicated that **12** is the C(20) epimer of **11** which is confirmed from the NOESY spectrum which showed NOE interaction between H(20)/H(16). The assignment was also confirmed by chemical correlation with *N*(4)-methyl-*N*(4), 21-*seco*talpinine **10**, via $NaBH_4$ reduction. Macrocarpine C **13** is readily shown to be the acetate derivative of macrocarpine B **12** from the spectral data. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) were similar to that of **12** except for the presence of a methyl resonance at δ 1.68 and the carbon signals at δ 20.3 and 170.8. The assignment was again supported by correlation with **12** via acetylation (Ac_2O/pyridine).

Two other new macroline derivatives **14** and **15** were obtained as an inseparable mixture of type-B and type-A forms (ratio 3:1, respectively), which co-eluted 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 **15**, vinylic-H for **14**} signals are clearly distinguishable in the ^1H NMR spectrum (Table 1), while the signals of H(17) are

partially overlapped. The rest of the hydrogen resonances of the two isomers are coincident. In the ^{13}C NMR spectrum (Table 2), the majority of the signals are coincident with the exception of C(6), C(17), C(18), C(19), and C(21). This behaviour has been observed previously in the case of the macroline indoles, alstonerine (type-B) and alstonerinal (type-A),[3] and in the case of the macroline oxindoles, *N*(1)-demethylalstonisine (type-B) and *N*(1)-demethylalstonal (type-A).[4] In the event, the spectral data (Tables 1 and 2) indicated that **14** and **15** are the *N*(1)-demethyl derivatives of alstophylline **16** and alstophyllal **17**, respectively. The latter two compounds also occur as a pair of unresolvable type-A and type-B isomers in both the stem and leaf extracts.

Two bisindole alkaloids were obtained, of which one was a new natural product. Perhentinine **18** was obtained as a light yellowish oil, $[\alpha]_D=-61$ (*c* 1.19, $CHCl_3$). The IR spectrum showed the presence of hydroxyl (3400 cm^{-1}), ketone (1701 cm^{-1}), and α,β-unsaturated ketone (1651, 1616 cm^{-1}) functions, while the UV spectrum indicated an indole chromophore, with charcteristic absorption maxima at 231 and 298 nm. The LSIMS spectrum of **18** showed the MH^+ ion at *m/z* 705, which analysed for $C_{43}H_{52}N_4O_5$. The ^1H NMR spectrum (Table 1) showed several clear features, *inter alia*, four aromatic hydrogen signals associated with an unsubstituted indole moiety, two aromatic singlets associated with another indole substituted at positions 10′ and 11′, a total of seven methyl singlets corresponding to two *N*(1)-methyls, two *N*(4)-methyls, two acetyls, and an aromatic methoxy group. Since only six aromatic hydrogens are observed and both indolic nitrogens are substituted, it is reasonable to conclude that the bisindole is branched from one of the aromatic carbon atoms of one monomer, with the adjacent position occupied by the methoxy substituent. The low field region also showed the presence of a vinylic singlet (δ 7.51), which with the associated 18′-methyl singlet at δ 2.05, indicated one monomer to be a type-B macroline. This is supported by the observation of the characteristic C(17′) hydrogen signals as a ddd and a triplet at δ 4.13 and 4.37, respectively.[3,4] The ^{13}C NMR spectrum (Table 2) showed a total of 43 carbon signals, comprising seven methyls, seven methylenes, 16 methines, and 13 quaternary carbon atoms.

Examination of the carbon spectrum revealed that one set of signals showed a correspondence to a 10′, 11′-disubstituted alstonerine.[3] Furthermore, the observed low-field resonances of both H(12′) and C(12′) at δ 6.69 and 91.3, respectively, are characteristic of oxygenation at the adjacent C(11′),[28,29] thus indicating position 11′ as the site of methoxy substitution and position 10′ as the site of branching of the bisindole from this monomeric unit.

The second moiety constituting the bisindole was deduced to be another macroline derivative from initial inspection of the NMR spectral data. The C(17) hydogens are observed as doublets of doublets at δ 3.95 and 4.01 and the acetyl hydrogens of C(18) are seen as a singlet at δ 1.72, features which are characteristic of ring E-opened, *seco*-macrolines.[4] Further examination of the NMR spectral data indicated that C(20), which is expected to be a methylene, is now substituted, appearing as a methine at δ 3.32 (δ_C 54.5). The NMR spectral data also revealed that this monomeric unit corresponds to the new *seco*-macroline, alstomicine, isolated from the leaf-extract of the same plant.[30] The point of branching in this second macroline unit must therefore be from C(20). This leaves the methylene C(21) unaccounted for, which is observed at δ 32.0 in the ^{13}C NMR spectrum (δ_H 2.41, 3.08). This methylene is directly attached to C(20) of the *seco*-macroline unit, from the COSY spectrum, as well as the HMBC spectrum {^3J from H(21) to C(19)}. In addition, the observation of another key three-bond correlation from H(9′) to this C(21) provided cogent support for the proposed structure, in which the two macroline units are connected by a methylene bridge, as shown in **18**, although the configuration at C(20) could not be established based on the present data. Perhentinine and the other known bisindole obtained, villalstonine, both showed moderate in vitro cytotoxicity towards the P388 murine leukemia cell line (IC_{50} 12.3 and 4.4 μg/ml, respectively) (the biological activity of *Alstonia* alkaloids will be reported separately).

In addition to the above new alkaloids, 12 other known alkaloids were also obtained from the bark extract of this plant, as detailed in Section 3. A notable feature of the alkaloidal composition, in addition to the novel structures discussed above, is the predominance of the macroline skeleton, which is a characteristic of *Alstonia*.

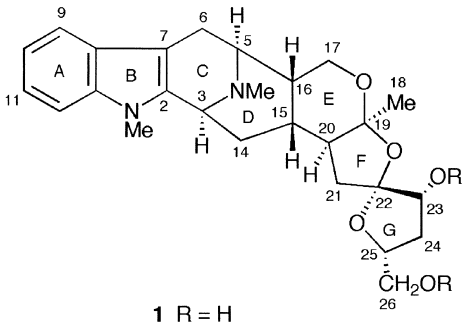
3. Experimental

3.1. General

UV spectra were recorded on a Shimadzu UV-3101PC spectrophotometer. IR spectra were recorded on a Perkin–Elmer RX1 FT-IR spectrophotometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter or an Atago Polax-D polarimeter. ESIMS was obtained on a Perkin–Elmer API 100 instrument. HREIMS and HRLSIMS measurements were carried out at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JMN-LA400 spectrometer at 400 and 100 MHz, respectively. Assignments are confirmed by COSY, HMQC, HMBC, NOESY and NOE experiments. All solvents were of analytical grade and were distilled before use.

3.2. Collection, extraction and isolation

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 of the ground bark material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid as has been described in detail elsewhere.[31] 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



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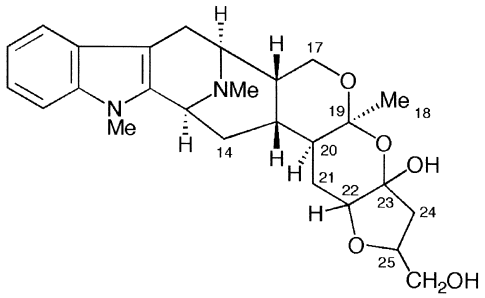

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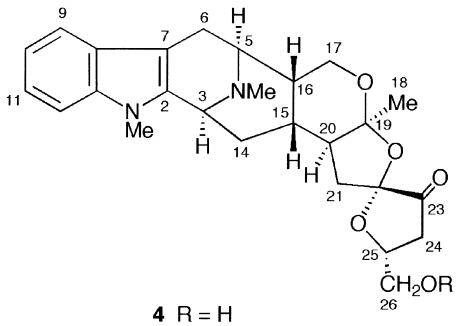

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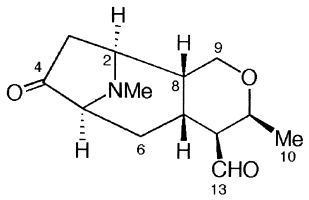

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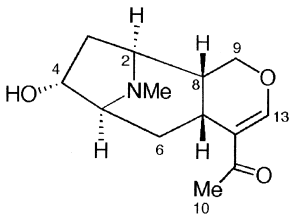

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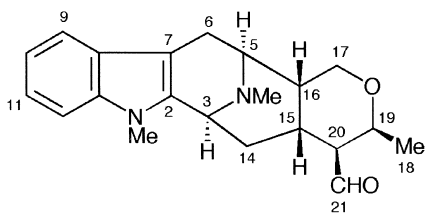
TLC were Et_2O–petroleum ether (1:1; 2:1), Et_2O, $CHCl_3$–MeOH (100:1), $CHCl_3$ (NH_3-saturated), and EtOAc (NH_3-saturated). The yields (g kg^{-1}) of the alkaloids were as follows: **1** (0.004), **4** (0.0012), **7** (0.0012), **9** (0.0005), **10** (0.010), **11** (0.002), **12** (0.031), **13** (0.028), **14** (0.0004), **15** (0.0002), **16** (0.027), **17** (0.016), **18** (0.0249), alstonisine (0.028), alstonal (0.0085), *N*(4)-demethylalstophylline oxindole (0.054), *N*(4)-demethylalstophyllal oxindole (0.035), villalstonine (0.393), pleiocarpamine (0.071), fluorocarpamine (0.029), 16*R*,19*E*-isositsirikine (0.004), and 11-methoxyakuammicine (0.0012).

3.2.1. Macrodasine A 1. $[\alpha]_D$=+36 ($CHCl_3$, *c* 0.36); IR (dry film) ν_{max} 3411 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 230 (3.88) and 287 (3.17). EIMS, *m/z* (rel. int.): 454 [M^+] (78), 439 (4), 424 (44), 367 (7), 197 (100), 182 (27), 181 (16), 170 (34), 144 (13), 70 (26), 57 (16) and 43 (36). HREIMS found, *m/z* 454.2462, Calcd for $C_{26}H_{34}N_2O_5$, 454.2468. ^1H and ^{13}C NMR: see Tables 1 and 2, respectively.

3.2.2. Macrodasine B 4. $[\alpha]_D$=+149 ($CHCl_3$, *c* 0.07); IR (film) ν_{max} 3435 and 1765 cm^{-1}; (EtOH), λ_{max} nm (log ε): 230 (3.95) and 287 (3.23). EIMS, *m/z* (rel. int.): 452 [M^+] (64), 437 (3), 421 (12), 366 (11), 322 (17), 293 (4), 237 (6), 197 (100), 182 (26), 181 (19), 170 (29), 144 (9), 85 (13), 70 (19), 57 (16) and 40 (35). HREIMS found, *m/z* 452.2326, Calcd for $C_{26}H_{32}N_2O_5$, 452.2311. ^1H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.3. Acetylation of macrodasine A 1. Macrodasine A **1** (11 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 5 mg (38%) of the diacetate derivative **3** as a colourless oil; $[\alpha]_D$=+119 ($CHCl_3$, *c* 0.06); IR (film) ν_{max} 1739 and 1234 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 221 (4.17), 229 (4.22), 286 (3.56) and 293 (3.52). EIMS, *m/z* (rel. int.): 538 [M^+] (29), 465 (4), 281 (14), 253 (4), 207 (100), 197 (99), 182 (23), 167 (20), 144 (8), 96 (9), 70 (23) and 55 (18). ^1H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.4. Acetylation of macrodasine B 4. Acetylation of macrodasine B **4** (4 mg) with Ac_2O/pyridine as described above gave the monoacetate derivative 5 as a colourless oil (2 mg, 46%); $[\alpha]_D$=+147 ($CHCl_3$, *c* 0.02); IR (film) ν_{max} 1767, 1739, and 1237 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 222 (4.41), 228 (4.48), 285 (3.79) and 293 (3.75). EIMS, *m/z* (rel. int.): 494 [M^+] (30), 366 (8), 322 (14), 197 (100), 182 (23), 170 (30), 158 (10), 144 (10) and 70 (33). ^1H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.



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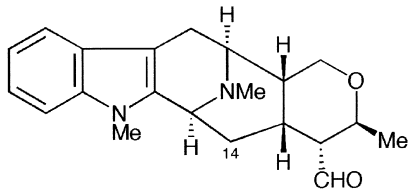

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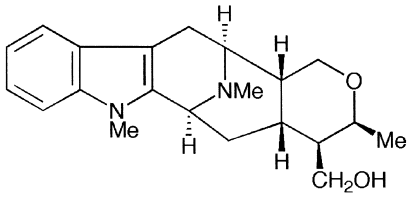

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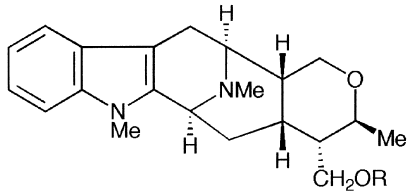

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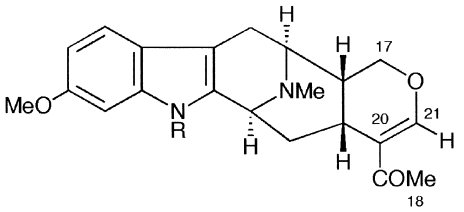
13 R = Ac



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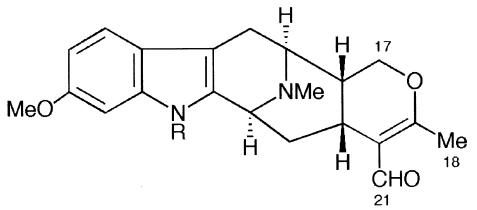
16 R = Me



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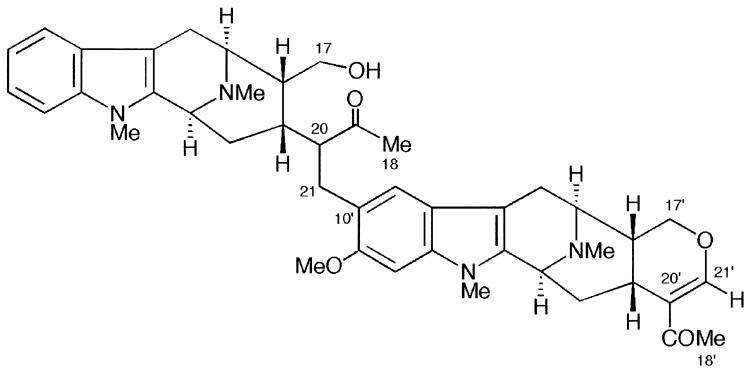
17 R = Me



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3.2.5. Angustimalal 7. $[\alpha]_D$=+78 ($CHCl_3$, *c* 0.06); IR (film) ν_{max} 1741 and 1711 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 211 (3.09) and 256 (2.42). EIMS, *m/z* (rel. int.): 237 [M^+] (2), 209 (74), 194 (39), 180 (100), 166 (24), 150 (25), 138 (13), 124 (28), 110 (32), 94 (65), 84 (97), 70 (26), 57 (50) and 42 (74). HREIMS found, *m/z* 237.1375, Calcd for $C_{13}H_{19}NO_3$, 237.1365. ^1H (400 Hz; $CDCl_3$; Me_4Si) δ 1.42 (3H, d, *J*=7 Hz, 10-Me), 1.67 (1H, m, H-6), 1.95 (1H, s, H-12), 1.98 (1H, m, H-8), 2.06 (1H, d, *J*=19 Hz, H-3), 2.11 (1H, td, *J*=13, 3 Hz, H-6), 2.30 (3H, s, *N*-Me), 2.44 (1H, dt, *J*=13, 6 Hz, H-7), 2.68 (1H, dd, *J*=19, 7 Hz, H-3), 2.90 (1H, br s, H-5), 3.23 (1H, d, *J*=7 Hz, H-2), 3.90 (1H, dd, *J*=11, 5 Hz, H-9), 3.94 (1H, qd, *J*=7, 2 Hz, H-11), 4.15 (1H, t, *J*=11 Hz, H-9), 10.0 (1H, d, *J*=2 Hz, H-13); ^{13}C NMR spectral data, see Table 2.

3.2.6. Talcarpine 9. $[\alpha]_D$=−26 ($CHCl_3$, *c* 0.12); UV (EtOH), λ_{max} nm (log ε): 209 (3.86), 226 (4.01), 277 (2.65), 285 (2.91) and 294 (2.65). ESIMS, *m/z* (rel. int.): 339 [MH^+]. ^1H (400 Hz; $CDCl_3$; Me_4Si) δ 1.30 (3H, d, *J*=7 Hz, 18-Me), 1.45 (1H, ddd, *J*=12, 4, 3 Hz, H-14), 1.79 (1H, br s, H-20), 2.06 (1H, dt, *J*=11, 5 Hz, H-16), 2.20 (1H, m, H-15),

2.32 (3H, s, *N*(4)-Me), 2.45 (1H, d, *J*=16 Hz, H-6), 2.50 (1H, td, *J*=12, 4 Hz, H-14), 2.90 (1H, d, *J*=7 Hz, H-5), 3.27 (1H, dd, *J*=16, 7 Hz, H-6), 3.62 (3H, s, *N*(1)–Me), 3.89 (1H, dd, *J*=12, 5 Hz, H-17), 3.98 (2H, m, H-3 and H-19), 4.14 (1H, t, *J*=12 Hz, H-17), 7.10 (1H, td, *J*=8, 1 Hz, H-10), 7.19 (1H, td, *J*=8, 1 Hz, H-11), 7.29 (1H, br d, *J*=8 Hz, H-12), 7.49 (1H, br d, *J*=8 Hz, H-9), 9.95 (1H, d, *J*=3 Hz, H-21); ^{13}C NMR spectral data, see Table 2.

3.2.7. N(4)-Methyl-N(4), 21-*seco*talpinine 10. $[\alpha]_D$=+19 ($CHCl_3$, *c* 0.45); UV (EtOH), λ_{max} nm (log ε): 205 (3.95), 228 (4.21), 280 (3.00), 285 (3.42) and 300 (3.12). ESIMS, *m/z* (rel. int.): 339 [MH^+]. ^{1}H (400 Hz; $CDCl_3$; Me_4Si) δ 1.20 (3H, d, *J*=7 Hz, 18-Me), 1.28 (1H, m, H-14), 1.93 (1H, m, H-16), 2.31 (3H, s, *N*(4)-Me), 2.37 (3H, m, H-14, H-15, H-20), 2.49 (1H, d, *J*=16 Hz, H-6), 2.96 (1H, d, *J*=7 Hz, H-5), 3.30 (1H, dd, *J*=16, 7 Hz, H-6), 3.58 (3H, s, *N*(1)-Me), 3.75 (1H, dd, *J*=12, 5 Hz, H-17), 3.93 (2H, m, H-3, H-19), 4.06 (1H, t, *J*=12 Hz, H-17), 7.13 (1H, td, *J*=8, 1 Hz, H-10), 7.21 (1H, td, *J*=8, 1 Hz, H-11), 7.31 (1H, br d, *J*=8 Hz, H-12), 7.52 (1H, br d, *J*=8 Hz, H-9), 9.41 (1H, br s, H-21); ^{13}C NMR spectral data, see Table 2.

3.2.8. Macrocarpine A 11. $[\alpha]_D$=+117 ($CHCl_3$, *c* 0.11); IR (film) ν_{max} 3400 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 230 (4.15) and 286 (3.46). EIMS, *m/z* (rel. int.): 340 [M^+] (100), 309 (14), 226 (19), 197 (75), 182 (23), 170 (13) and 70 (18). HREIMS found, *m/z* 340.2142, Calcd for $C_{21}H_{28}N_2O_2$, 340.2151. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.9. Reduction of 9 to 11. $NaBH_4$ (27 mg) was added to a solution of **9** (24 mg) in MeOH (2 ml) at room temperature and the mixture stirred for 5 h. Excess solvent was removed under reduced pressure and water (5 ml) was then added. The mixture was extracted with $CHCl_3$, dried (Na_2SO_4), and then chromatographed (SiO_2, centrifugal TLC, 1% MeOH–$CHCl_3$) to give macrocarpine A **11** (12 mg, 50%).

3.2.10. Oxidation of 11 to 9. PCC (9 mg) was added to solution of **11** (12 mg) in CH_2Cl_2 (2 ml) at room temperature and the mixture stirred for 4 h. Water (5 ml) was then added and the mixture was extracted with $CHCl_3$, dried (Na_2SO_4), and then chromatographed (SiO_2, centrifugal TLC, $CHCl_3$) to give **9** (3 mg, 25%).

3.2.11. Macrocarpine B 12. $[\alpha]_D$=−51 ($CHCl_3$, *c* 0.34); IR (film) ν_{max} 3400 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 230 (4.34) and 288 (3.64). EIMS, *m/z* (rel. int.): 340 [M^+] (75), 325 (10), 309 (13), 226 (15), 197 (100), 182 (43), 170 (19), 158 (16), 144 (13), 83 (11), 70 (31) and 40 (48). HREIMS found, *m/z* 340.2149, Calcd for $C_{21}H_{28}N_2O_2$, 340.2151. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.12. Reduction of 10 to 12. Reduction of **10** (47 mg) with $NaBH_4$ (52 mg) as described above gave macrocarpine B **12** (27 mg, 57%).

3.2.13. Macrocarpine C 13. $[\alpha]_D$=−35 ($CHCl_3$, *c* 1.55); IR (film) ν_{max} 1737 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 230 (4.30) and 287 (3.62). EIMS, *m/z* (rel. int.): 382 [M^+] (85), 307 (11), 197 (100), 182 (33), 170 (26), 70 (32) and 43 (12). HREIMS found, *m/z* 382.2252, Calcd for $C_{23}H_{30}N_2O_3$, 382.2256. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.14. Acetylation of 12 to 13. Acetylation of **12** (27 mg) with Ac_2O/pyridine as described above gave **13** (16 mg, 53%).

3.2.15. N(1)-Demethylalstophylline 14 and N(1)-demethylalstophyllal 15. EIMS, *m/z* (rel. int.): 352 [M^+] (74), 337 (6), 283 (12), 265 (4), 228 (12), 213 (79), 197 (28), 186 (100), 170 (19), 143 (12), 118 (5), 91 (6), 70 (40) and 40 (41). HREIMS found, *m/z* 352.1773, Calcd for $C_{21}H_{24}N_2O_3$, 352.1787. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.16. Perhentinine 18. $[\alpha]_D$=−61 ($CHCl_3$, *c* 1.19); IR (film) ν_{max} 3400, 1701, 1651 and 1616 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 231 (4.25) and 298 (3.45). LSIMS, *m/z* (rel. int.): 705 [MH^+] (51), 661 (14), 379 (58), 239 (19) and 197 (100). HRLSIMS found, *m/z* 705.4019, Calcd for [$C_{43}H_{42}N_4O_5$+H], 705.4016. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

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