Phytochemistry 98 (2014) 204–215

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Macroline, akuammiline, sarpagine, and ajmaline alkaloids from *Alstonia macrophylla*

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ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form 4 November 2013
Available online 13 December 2013

Keywords:
Alstonia macrophylla
Apocynaceae
Indole alkaloids
NMR
X-ray crystallography
Reversal of multidrug-resistance

ABSTRACT

A total of seventeen alkaloids, comprising six macroline (including alstofolinine A, a macroline indole incorporating a butyrolactone ring-*E*), two ajmaline, one sarpagine, and eight akuammiline alkaloids, were isolated from the stem-bark and leaf extracts of the Malayan *Alstonia macrophylla*. The structure and relative configurations of these alkaloids were established using NMR, MS and in several instances, confirmed by X-ray diffraction analysis. Six of these alkaloids were effective in reversing multidrug-resistance (MDR) in vincristine-resistant KB cells.

Introduction

Plants of the genus *Alstonia* (Apocynaceae), which are shrubs or trees, are distributed over tropical parts of Central America, Africa, and Asia (Whitmore, 1973; Markgraf, 1974; Sidiyasa, 1998) and are usually rich in alkaloids. A prominent feature of the *Alstonia* alkaloids is the preponderance of the macroline unit, which abounds in the alkaloids found in plants of the genus (Kam, 1999; Kam and Choo, 2006). About six species occur in Peninsular Malaysia and several of these (local name *Pulai*) are used in traditional medicine, for example, in the treatment of malaria and dysentery (Burkill, 1966; Perry and Metzger, 1980). In Peninsular Malaysia, these plants are mainly found in secondary and primary forest from sea level to about 3000 m altitude, as well as in swampy areas (Whitmore, 1973; Sidiyasa, 1998; Middleton, 2011). Recently the structure and absolute configurations of a number of new bisindoles isolated from a sample of *A. macrophylla* Wall collected from the western coast of Peninsular Malaya (Perak) were disclosed (Lim et al., 2011, 2012, 2013). In addition, a potentially useful method for the determination of the configuration at C-20 in *E-seco* macroline–macroline bisindoles, such as perhentinine, *seco*-macralstonine, and perhentidines A–C, was also reported. This was based on comparison of the NMR chemical shifts of the bisindoles and their acetate derivatives, in addition to X-ray determination of the absolute configuration of perhentinine and macralstonine (Lim et al., 2012). Reported herein are the isolation and structure determination of 17 new indole alkaloids (Fig. 1) from the leaf and stem-bark extracts of this plant.

Results and discussion

Compound **1** was a minor alkaloid isolated from the leaf extract of *A. macrophylla*. It was obtained as a light yellowish oil, with $[\alpha]_D$ −104 (*c* 0.36, $CHCl_3$). The UV spectrum showed two absorption bands (227 and 285 nm) characteristic of an indole chromophore. The IR spectrum showed a sharp band at 1769 cm^{-1} due to a lactone function. The EIMS of compound **1** had a molecular ion at *m*/*z* 296, and high resolution measurements yielded the molecular formula $C_{18}H_{20}N_2O_2$. Other notable fragment peaks observed at *m*/*z* 197, 182, 181, 170, and 144, are typical of macroline derivatives (Mayerl and Hesse, 1978), while the mass fragment at *m*/*z* 281 can be attributed to loss of a CH_3. The ^{13}C NMR spectrum (Table 1) displayed a total of 18 carbon resonances, corresponding to two methyl, three methylene, eight methine and five quaternary carbons. The presence of the lactone functionality, and an oxymethylene carbon, was supported by the observed carbon signals at δ 181.0 and δ 70.7, respectively. The ^1H NMR spectrum (Table 2) showed the presence of an unsubstituted indole moiety (δ 7.11–7.49), two methyl groups corresponding to N1-Me at δ 3.64 and N4-Me at δ 2.42, and two downfield resonances at δ 4.42 (H-17β, t, *J* = 8 Hz) and 4.52 (H-17α, dd, *J* = 11, 8 Hz) due to the geminal

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http://dx.doi.org/10.1016/j.phytochem.2013.11.014

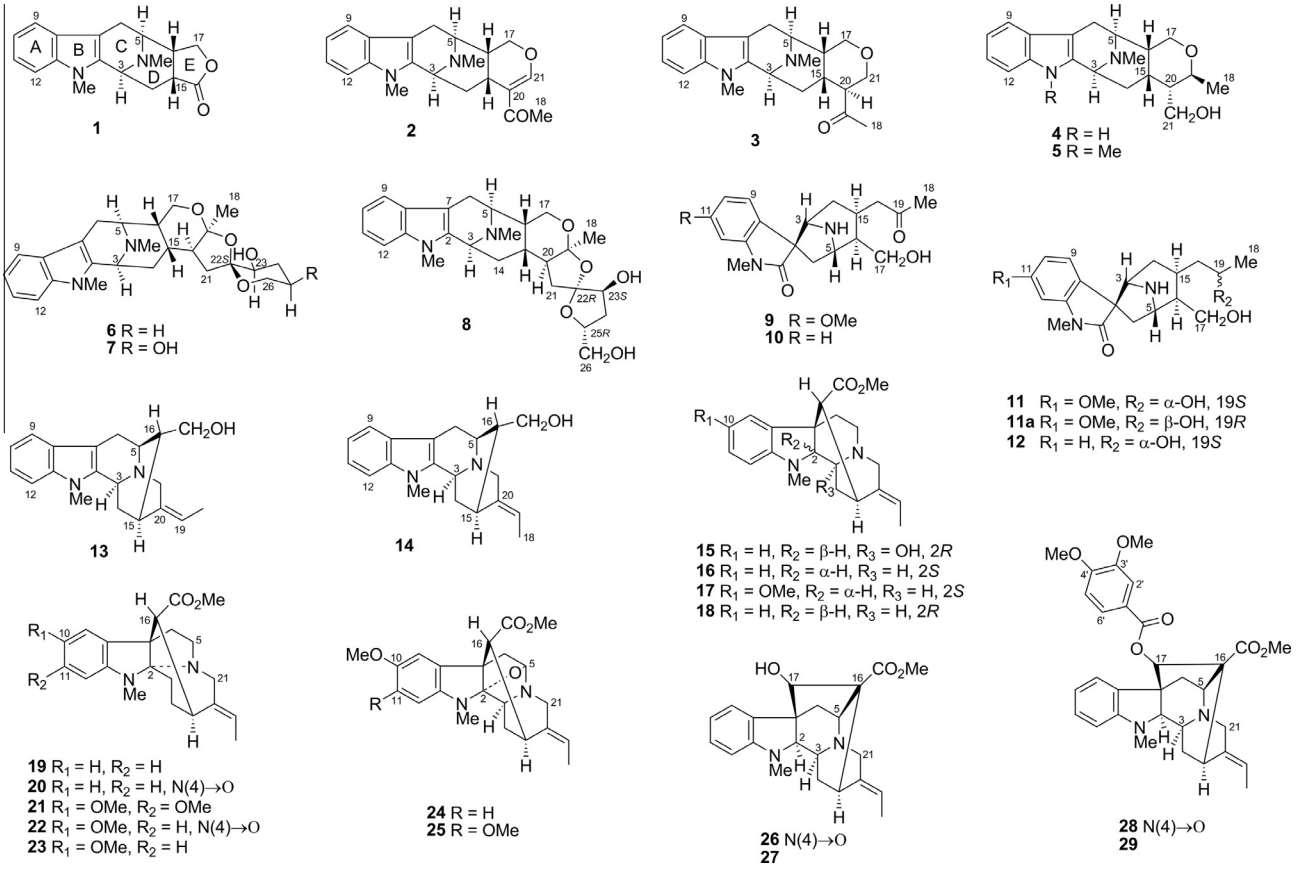

Fig. 1. Structures of compounds **1**–**29**.

hydrogens of an oxymethylene corresponding to C-17. The COSY and HSQC data disclosed partial structures that are characteristic of a macroline-type skeleton, such as $NCHCH_2$ and $NCHCH_2$-$CHCHCH_2O$, corresponding to the N-4–C-5–C-6 and N-4–C-3–C-14–C-15–C-16–C-17–O fragments, respectively. The NMR spectroscopic data were suggestive of a macroline derivative, such as alstonerine (**2**). (Ratnayake et al., 1987; Ghedira et al., 1988; Kam et al., 1999), except for the absence of the typical α,β unsaturated ketone group and the associated vinyl-H, in the ring E of compound **1**. In addition, both H-17 signals in **1** were shifted downfield to δ_H 4.42 and 4.52, when compared to those in alstonerine (**2**) (δ_H 4.16 and 4.40), as well as other typical macroline alkaloids (Kam et al., 2004a; Kam and Choo, 2004a). Furthermore, the observed coupling constants for the H-17 resonances in the case of **1** differed significantly when compared to those in **2** (**1**: H-17β, t, J = 8 Hz; H-17α, dd, J = 11 and 8 Hz; **2**: H-17β, ddd, J = 11, 4, 2 Hz; H-17α, t, J = 11 Hz), indicating changes in ring E of **1** ($J_{17\text{-}17}$ = 8 Hz) compared to normal macrolines with a six-membered ring E ($J_{17\text{-}17}$ = 11 Hz) as exemplified by alstonerine (**2**). The presence of a lactone functionality as a part of ring E (a butyrolactone moiety) was deduced from the observed three-bond correlations from H-14α, H-14β, and H-17α, to the lactone carbonyl C-18 in the HMBC spectrum (Fig. 2). The relative configurations at the various stereogenic centers of **1** were established by NOESY, and were similar to those in other macroline alkaloids. Alstofolinine A (**1**) is the first example of a macroline indole alkaloid incorporating a γ-butyrolactone moiety in ring E.

Compound **3** was isolated in small amounts as a light yellowish oil, with $[\alpha]_D$ −31 (*c* 0.11, $CHCl_3$). The IR spectrum showed a band at 1710 cm^{-1} due to a ketone function. The presence of a ketone function was confirmed by the observed resonance at δ 208.6 in the ^{13}C NMR spectrum. The ESIMS of **3** showed an $[M+H]^+$ ion at *m*/*z* 339, which analyzed for $C_{21}H_{26}N_2O_2$. The UV spectrum showed absorption maxima at 228 and 286 nm, which are characteristic of an indole chromophore.

The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) showed the presence of an unsubstituted indole moiety (δH 7.09–7.50, δ_C 108.7–120.9), two N-methyl signals (N4-Me, δ_C 41.8, δH 2.29; N1-Me, δ_C 29.1, δ_H 3.61), a methyl ketone function (δ_C 208.6; δ_C 28.4, δH 2.12), an oxymethylene, characteristic of C-17 in macroline alkaloids (δ_C 68.6, δ_H 3.72, and 3.95), and another oxymethylene signal at δ_C 64.3 (δ_H 3.86 and 4.18). The NMR signals, assigned with the aid of COSY and HSQC, indicated that **3** is a macroline-type alkaloid. The NMR spectroscopic data resembled those of alstonerine (**2**), which was also isolated from the extract of this plant, except for the absence of signals associated with the trisubstituted C-20−C-21 double bond, such as the olefinic carbon resonances at C-20 (δ 126.5) and C-21 (δ 157.4), and the signal due to the vinylic H-21 in the ^1H NMR spectrum (δ 7.52). These resonances have in **3** been replaced by a methine at C-20 (δ_C 51.9, δ_H 1.97, m) and a methylene at C-21 (δ_C 64.3; δ_H 3.86, dd, J = 12.5, 3 Hz, δ_H 4.18, d, J = 12.5 Hz), consistent with saturation of the C-20−C-21 double bond in **3**. Less substantial changes were observed for the signals of carbons β to both carbons (C-20, C-21) in the ^{13}C NMR spectrum. The configuration at C-20 can be deduced from the observed NOEs, viz., H-20/H-14β, H-18, H-21α; H-21α/H-14α, H-20, H-21β, which indicated the orientation of H-20 is α. Compound **3** is, therefore, the 20,21-dihydro derivative of alstonerine (**2**), which, while previously encountered as an intermediate compound in synthesis (Zhang and Cook, 1990), is here encountered as an optically active natural product for the first time.

Macrocarpine D (**4**) was obtained as a light yellowish oil, with $[\alpha]_D$ −43 (*c* 0.89, $CHCl_3$). It was isolated from the stem-bark extract of *A. macrophylla*, as well as *A. angustifolia* (Tan, 2011). The IR spectrum indicated the presence of hydroxyl and secondary amine

Table 1
^{13}C (100 MHz) NMR spectroscopic data for compounds **1**, **3**, **4**, **6**, **9**, **11**, **13**, **15**–**17**, **19**–**22**, **24**, **26**, and **28**.[a]

C	1	3	4	6	9	11	13	15	16	17	19	20	21	22	24	26	28
2	132.4	133.0	132.2	132.7	183.0	183.1	139.8	80.7	70.1	70.7	97.4	102.5	98.2	102.7	109.3	69.8	70.0
3	52.0	53.6	55.0	53.5	62.9	63.4	49.2	85.3	48.7	48.9	20.8	17.5	20.8	17.1	49.7	69.8	69.7
5	52.4	54.8	55.1	54.9	61.7	61.8	54.9	51.5	46.5	46.6	55.0	66.3	55.2	66.1	87.2	76.0	75.9
6	23.6	22.4	22.5	22.6	41.0	40.8	27.3	30.8	28.7	28.7	41.0	35.1	41.4	34.9	40.5	30.3	31.6
7	106.8	107.0	107.7	106.7	56.8	57.0	103.4	46.3	42.9	43.2	57.0	54.1	57.4	54.3	50.8	56.1	55.5
8	127.3	126.6	127.1	126.4	120.8	121.1	127.4	140.0	137.8	139.4	137.0	135.5	128.0	136.6	136.5	128.9	127.1
9	119.0	118.4	118.0	118.2	125.8	125.1	118.2	120.9	123.3	110.6	123.5	122.6	111.9	110.6	112.1	128.8	123.6
10	120.0	118.9	119.4	119.2	106.4	106.6	118.9	119.4	119.5	153.8	116.9	119.4	140.9	153.6	154.3	109.7	119.7
11	122.3	120.9	121.3	121.1	160.2	160.2	120.9	127.3	127.5	112.3	127.8	129.0	150.1	112.8	112.6	124.6	129.0
12	109.8	108.7	110.9	108.9	96.6	96.5	108.8	109.8	109.1	109.7	105.3	107.8	92.3	107.9	108.8	119.6	109.8
13	138.1	137.1	135.5	137.0	145.5	145.6	137.4	153.8	152.6	146.8	149.0	149.7	144.5	143.9	144.2	154.3	153.9
14	29.5	30.3	26.1	32.0	33.1	34.2	34.5	42.3	25.2	25.2	26.2	25.3	26.6	25.2	25.9	22.2	22.1
15	34.1	24.6	27.1	26.7	26.2	26.9	34.9	36.5	32.5	32.6	34.8	33.8	34.9	33.8	31.3	29.5	29.7
16	43.2	39.3	43.6	37.0	41.6	40.7	44.1	52.0	49.6	49.6	50.6	49.3	51.5	49.3	51.6	60.6	59.8
17	70.7	68.6	67.7	64.6	65.9	65.3	65.4	–	–	–	–	–	–	–	–	74.0	74.9
18	181.0	28.4	20.3	23.8	31.0	24.9	12.6	13.6	12.9	13.0	13.5	13.7	13.9	13.6	12.8	12.6	12.6
19	–	208.6	70.5	105.1	208.7	65.5	117.0	119.1	119.5	119.9	122.4	128.3	123.1	128.4	120.6	119.6	120.4
20	–	51.9	46.9	44.1	47.1	42.5	136.9	137.5	138.1	138.0	138.9	132.0	139.0	131.9	136.0	130.1	129.5
21	–	64.3	61.7	32.2	–	–	53.9	55.0	56.2	56.3	58.3	74.0	58.6	73.7	46.4	70.8	71.0
22	–	–	–	108.6	–	–	–	–	–	–	–	–	–	–	–	–	–
23	–	–	–	70.1	–	–	–	–	–	–	–	–	–	–	–	–	–
24	–	–	–	27.9	–	–	–	–	–	–	–	–	–	–	–	–	–
25	–	–	–	23.0	–	–	–	–	–	–	–	–	–	–	–	–	–
26	–	–	–	64.0	–	–	–	–	–	–	–	–	–	–	–	–	–
N_1-*Me*	30.1	29.1	–	30.1	26.4	26.4	29.4	37.6	34.0	34.9	27.3	32.9	28.4	33.3	30.0	34.8	34.6
N_4-*Me*	42.2	41.8	41.6	42.2	–	–	–	–	–	–	–	–	–	–	–	–	–
CO_2*Me*	–	–	–	–	–	–	–	51.7	51.0	51.1	51.5	52.1	51.9	52.1	51.7	52.3	52.5
CO_2Me	–	–	–	–	–	–	–	173.0	172.8	172.9	173.8	172.6	174.0	172.5	172.5	170.8	169.9
10-OMe	–	–	–	–	–	–	–	–	–	56.0	–	–	58.2	56.1	56.0	–	–
11-OMe	–	–	–	–	55.7	55.6	–	–	–	–	–	–	56.4	–	–	–	–
1′	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	121.5
2′	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	111.9
3′	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	148.8
4′	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	153.4
5′	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	110.4
6′	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	123.4
3′-OMe	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	56.1
4′-OMe	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	56.1
C=O	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	163.7

[a] Measured in $CDCl_3$.

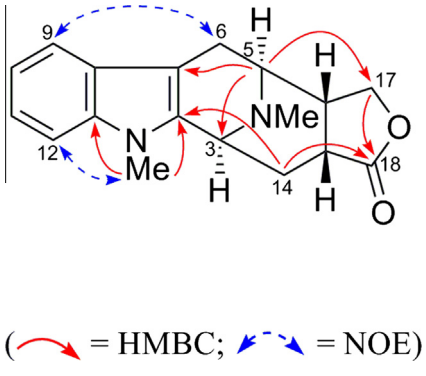

Fig. 2. Selected HMBCs and NOEs of **1**.

functions at 3395 and 3292 cm^{-1}, respectively, while the UV spectrum indicated an indole chromophore (λ_{max} 231 and 286 nm). The ESIMS of **4** showed an $[M+H]^+$ peak at *m*/*z* 327, and HRESIMS measurements established the molecular formula as $C_{20}H_{26}N_2O_2$, 15 mass units less than that of macrocarpine B (**5**) (Kam et al., 2004a). Comparison of the NMR spectroscopic data of **4** with those of **5** (Tables 1 and 2) indicated replacement of the N1-Me group by an NH function in **4**. The NMR data also indicated that the relative configurations at the various stereogenic centers of **4** follow those in the macroline-type alkaloids. In common with compound **5**, the orientation of H-19 and H-20 in **4**, were deduced to be α and β, respectively, from the observed NOEs for H-19/H-14α and H-20/H-15, H-16.

Macrodasine H (**6**) was obtained as a light yellowish oil, with $[\alpha]_D$ −11 (*c* 0.14, $CHCl_3$). The UV spectrum had two absorption maxima at 233 and 288 nm, indicating the presence of an indole chromophore. The IR spectrum displayed a broad band at 3423 cm^{-1} due to a hydroxyl function. The ESIMS of **6** showed an $[M+H]^+$ peak at *m*/*z* 439, and high-resolution measurements yielded the molecular formula $C_{26}H_{34}N_2O_2$. The ^{13}C NMR spectrum (Table 1) had a total of 26 carbon signals comprising three methyl, seven methylene, ten methine and six quaternary carbons, in agreement with the molecular formula. The observed carbon resonance at δ 70.1 was due to an oxymethine carbon, while the carbon resonances at δ 64.6 (C-17) and 64.0 (C-26) were due to two oxymethylene carbons. In addition, two low-field quaternary carbon signals were observed at δ 108.6 and 105.1, each of which was attached to two oxygen atoms. The ^1H NMR spectrum (Table 2) showed the presence of an unsubstituted indole moiety (δ 7.11–7.50), three methyl groups corresponding to N1-Me, N4-Me, and 18-Me, at δ 3.62, 2.33, and 1.62, respectively, and two sets of resonances due to two oxymethylenes at δ 3.75, 4.05; and at δ 3.39, 3.84.

The COSY spectrum disclosed some partial structures, which are characteristic of a macroline-type skeleton, such as $NCHCH_2$, $CHCH_2$, and $NCHCH_2CHCHCH_2O$, corresponding to the N-4–C-5–C-6, C-20–C-21, and N-4–C-3–C-14–C-15–C-16–C-17-O fragments,

Table 2
^{1}H (400 MHz) NMR spectroscopic data for compounds **1**, **3**, **4**, **6**, **9**, **11**, **13**, **15**, and **16**.[a]

H	**1**	**3**	**4**	**6**	**9**	**11**	**13**	**15**	**16**
2	–	–	–	–	–	–	–	2.89 s	3.25 d (4)
3	3.91 br s	3.97 m	3.95 m	3.93 m	3.19 m	3.20 m	4.14 dd (10, 2)	–	3.98 m
5a	3.06 d (6)	2.83 d (7)	2.94 d (7)	2.96 d (7)	3.86 m	3.90 m	2.73 t (6)	2.77 dd (13, 6.5)	2.58 dd (14, 7)
5b	–	–	–	–	–	–	–	4.02 td (13, 5)	3.45 td (14, 6)
6b	2.47 br d (16)	2.52 dd (16)	2.46 d (16)	2.41 d (16)	2.08 d (14)	2.06 d (14)	2.60 br d (15) (b)	1.32 dd (15, 5)	2.07 dd (14, 6)
6a	3.27 dd (16, 6)	3.24 dd (16, 7)	3.27 dd (16, 7)	3.26 dd (16, 7)	2.39 dd (14, 8)	2.38 dd (14, 8)	3.03 dd (15, 5) (a)	2.87 m	3.35 m
9	7.49 d (8)	7.50 d (8)	7.49 d (7.5)	7.50 d (8)	7.76 d (9)	7.39 d (8)	7.41 d (8)	6.91 dd (8, 1)	6.98 d (7)
10	7.11 td (8, 1)	7.09 td (8, 1)	7.11 t (7.5)	7.11 t (8)	6.70 dd (9, 2)	6.60 dd (8, 2)	7.08 td (8, 1)	6.68 td (8, 1)	6.64 t (7)
11	7.22 td (8, 1)	7.18 td (8, 1)	7.15 t (7.5)	7.20 t (8)	–	–	7.19 td (8, 1)	7.09 td (8, 1)	7.01 t (7)
12	7.31 d (8)	7.27 d (8)	7.32 d (7.5)	7.30 d (8)	6.45 d (2)	6.45 d (2)	7.29 d (8)	6.61 br d (8)	6.51 d (7)
14b	2.10 m	1.42 dt (12, 3)	1.62 dt (13, 4)	1.50 m	1.69 m	1.72 m	1.53 m (b)	1.90 dd (14, 3) (b)	1.99 dt (15, 4) (a)
14a	2.13 m	2.44 dd (12, 4)	2.28 td (13, 4)	2.41 m	1.86 dd (13, 5)	1.82 m	2.04 td (12, 2) (a)	2.20 dd (14, 3) (a)	2.13 dd (15, 3) (b)
15	2.17 m	2.35 m	2.01 m	1.84 m	2.97 m	2.69 m	2.23 br s	3.61 m	3.39 m
16	2.54 m	2.12 m	1.89 dt (12, 4)	2.09 m	1.69 m	1.72 m	1.62 m	2.90 d (4)	2.72 d (4)
17b	4.42 t (8)	3.72 dd (11.5, 5)	3.74 dd (12, 5)	3.75 dd (12, 5)	3.81 m (a)	3.90 m	3.47 m	–	–
17a	4.52 dd (11, 8)	3.95 t (11.5)	4.08 t (12)	4.05 t (12)	4.01 d (12) (b)	4.00 dd (11, 1)	3.47 m	–	–
18	–	2.12 s	1.16 d (6)	1.62 s	2.20 s	1.29 d (6)	1.57 d (7)	1.49 dd (7, 2)	1.46 dd (7, 2)
19	–	–	3.50 m	–	–	3.90 m	5.28 q (7)	5.41 br q (7)	5.41 q (7)
20a	–	1.97 m	1.50 m	1.96 dd (12.5, 8)	2.69 dd (18, 5)	1.53 m	–	–	–
20b	–	–	–	–	2.80 dd (18, 8)	1.86 m	–	–	–
21a	–	3.86 dd (12.5, 3) (a)	3.34 dd (11, 8)	1.81 dd (12.5, 8) (a)	–	–	3.63 m	2.98 m	2.98 d (16)
21b	–	4.18 d (12.5) (b)	3.50 dd (11, 5)	2.16 t (12.5) (b)	–	–	3.63 m	4.10 br d (17)	3.95 d (16)
23	–	–	–	3.51 dd (10, 5) (ax)	–	–	–	–	–
24a	–	–	–	1.37 m (ax)	–	–	–	–	–
24b	–	–	–	2.05 m (eq)	–	–	–	–	–
25	–	–	–	1.56 m	–	–	–	–	–
	–	–	–	1.56 m	–	–	–	–	–
26a	–	–	–	3.39 td (10, 4.5) (ax)	–	–	–	–	–
26b	–	–	–	3.84 m (eq)	–	–	–	–	–
NH	–	–	7.89 br s	–	–	–	–	–	–
N_1-*Me*	3.64 s	3.61 s	–	3.62 s	3.16 s	3.17 s	3.61 s	2.95 s	2.68 s
N_4-*Me*	2.42 s	2.29 s	2.34 s	2.33 s	–	–	–	–	–
CO_2*Me*	–	–	–	–	–	–	–	3.78 s	3.53 s
11-OMe	–	–	–	–	3.84 s	3.83 s	–	–	–

[a] Measured in $CDCl_3$.

respectively. An additional fragment, viz., $OCHCH_2CH_2CH_2O$, was also indicated from the COSY spectrum which, taken with the other NMR spectroscopic data, indicated affinity to the macrodasine group of alkaloids reported recently (Tan et al., 2011). These macroline alkaloids incorporate additional fused spirocyclic tetrahydrofuran–tetrahydrofuran (macrodasines A (**8**), B, C, G) and tetrahydrofuran–tetrahydropyran (macrodasines D, E, F (**7**)) rings. The $OCHCH_2CH_2CH_2O$ partial structure noted from the COSY spectrum suggested that compound **6** belonged to the latter group of macrodasine alkaloids (macrodasines D, E, and F (**7**)) characterized by incorporation of fused 5/6 (F/G) spirocyclic rings (Tan et al., 2011). This was further supported by the following correlations observed in the HMBC spectrum, viz., H-26b, H-24 to C-22, H-24 to C-26, H-25 to C-23, and, H-21 to C-15, C-23. The proposed structure is consistent with the full HMBC data (Fig. 3). Compound **6** differs from macrodasine F (**7**), by the absence of the OH substituent at C-25.

The ring junction stereochemistries between rings C, D, E, and F were deduced to be similar to those of macroline alkaloids, as well as macrodasines (A–G) from the NOE data. The reciprocal NOEs observed for H-23/H-21β, H-25a and H-24a/H-26a suggested a chair conformation adopted by the tetrahydropyran ring G, as shown in Fig. 3, in which H-24a, H-25a, and H-26a are all axially oriented. As in the case of macrodasine F (**7**), a NOE was not observed between the C-26 hydrogens and 18-Me, suggesting that the configuration of the spirocyclic C-22 is *S*, i.e., the C-26 hydrogens and 18-Me are directed away from each other (Tan et al., 2011).

Alstonoxine C (**9**) was isolated as a light yellowish oil, with $[\alpha]_D$ −30 (*c* 0.39, $CHCl_3$), and subsequently crystallized from CH_2Cl_2–hexane as colorless block crystals. The UV spectrum showed absorption maxima at 216, 266, and 291 nm, indicative of an oxin-

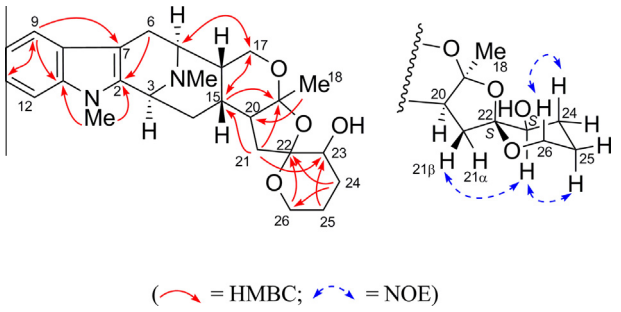

Fig. 3. Selected HMBCs and NOEs of **6**.

dole chromophore. The IR spectrum showed the presence of lactam and ketone carbonyl (1694 cm^{-1}), NH (3295 cm^{-1}), and hydroxyl (3390 cm^{-1}) functions, while the carbon resonances at δ 183.0, 208.7, 65.9 confirmed the presence of lactam (oxindole), ketone, and oxymethylene groups, respectively. The ESIMS showed an $[M+H]^+$ peak at m/z 359, analyzing for $C_{20}H_{26}N_2O_4$. The ^1H NMR spectroscopic data (Table 2) showed features typical of macroline oxindole alkaloids. Notable features include presence of an N1-Me (δ_H 3.16 s), a methyl ketone (δ_H 2.20 s; δ_C 31.0, C-18), and an oxymethylene (δ_H 3.80 m, and 4.01 br d, J = 12 Hz; δ_C 65.9; C-17). In addition, signals due to the presence of a methylene group α to a carbonyl group were observed at δ 2.69 (dd, J = 18, 5 Hz) and 2.80 (dd, J = 18, 8 Hz). These features are reminiscent of the C-18–C-19–C-20 side-chain in the *E*-seco macroline oxindole alstonoxine A (**10**). The NMR spectroscopic data (Tables 1 and 2) of **9** did in fact show a close resemblance to those of alstonoxine A (**10**) (Kam and Choo, 2000), except for the absence of an aromatic hydrogen resonance at C-11, which was replaced in **9** by a 3H singlet at δ 3.84 due to an aromatic methoxy substituent. The placement of the latter at C-11 was further confirmed by the observed NOEs between OMe and H-10, as well as H-12. The configuration of the spirocyclic C-7 was assigned as *S* from the observed reciprocal NOEs between H-9 and H-15. In view of the availability of suitable crystals (crystals were obtained from hexane–CH_2Cl_2), an X-ray diffraction analysis was carried out which provided confirmation of the structure and relative configuration of alstonoxine C (**9**) deduced from the spectroscopic data (Fig. 4). The X-ray structure of alstonoxine C (**9**) also provided additional support for the original assignment of the structure and relative configuration of alstonoxine A (**10**).

Alstonoxine D (**11**) was isolated as a light yellowish oil, with $[\alpha]_D$ −16 (*c* 0.23, $CHCl_3$). The UV and IR spectra of **11** were similar to those of alstonoxine B (**12**) (Kam and Choo, 2000), while the ESIMS showed an $[M+H]^+$ peak at m/z 361 ($C_{20}H_{28}N_2O_4$ + H). The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **11** were generally similar to those of alstonoxine B (**12**), except for the presence of an additional aromatic methoxy singlet at δ 3.83, and the absence of one aromatic-H signal. Placement of the methoxy substituent at C-11 was supported by the observed coupling behaviour of the aromatic hydrogens, as well as from the observed three-bond correlations from H-9 to C-11 and C-13, in the HMBC spectrum. This assignment was further supported by the observed NOEs between 11-OMe and H-10, H-12. The configuration of the spirocenter C-7 was assigned as *S* from the observed NOEs between H-9 and H-15. The difference between alstonoxine D (**11**) and the previous compound **9** resides in the C-18–C-19–C-20 side-chain. Whereas C-19 in **9** is a ketone carbonyl, the C-19 in **11** is an oxymethine associated with a secondary alcohol functionality. This difference was also reflected in the respective NMR spectroscopic data of **9** and **11**. The NMR data of **11** were, however, insufficient to establish the stereochemistry of C-19. Towards this end, alstonoxine C (**9**) was treated with $NaBH_4$/MeOH, which gave a

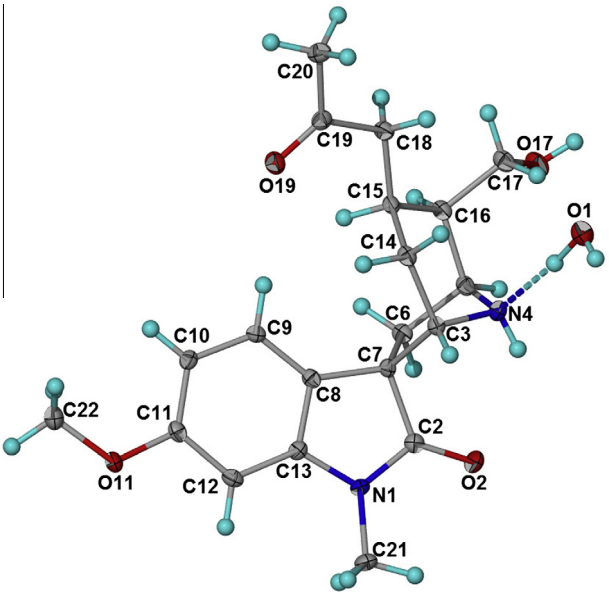

Fig. 4. X-ray crystal structure of **9**.

mixture of the epimeric alcohol products in approximately equal amounts, which were separated by preparative centrifugal TLC (SiO_2, 1% MeOH/$CHCl_3$, NH_3-saturated). The NMR data indicated that the slower eluting compound corresponded to compound **11**. With sufficient amounts obtained in this manner, the configuration at C-19 could be determined by Horeau's procedure (see Experimental) (Horeau and Kagan, 1964; Barnekow and Cardellina, 1989), which showed that the C-19 configuration in **11** is *S* (the faster eluting compound **11a** therefore corresponded to the 19*R* epimer). The structure and relative configuration of alstonoxine B (**12**) was previously reported from *Alstonia angustifolia* var. *latifolia* (Kam and Choo, 2000), based on analysis of the NMR and MS data which at the time were insufficient to assign the configuration at C-19. Alstonoxine B (**12**) was also isolated in the present study, and since suitable crystals were obtained from CH_2Cl_2–hexane

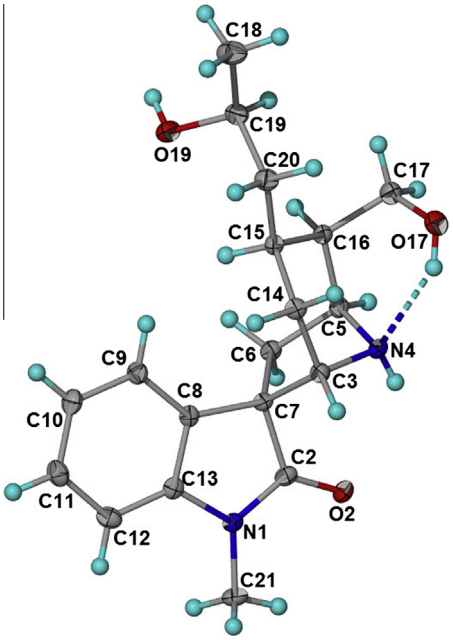

Fig. 5. X-ray crystal structure of **12**.

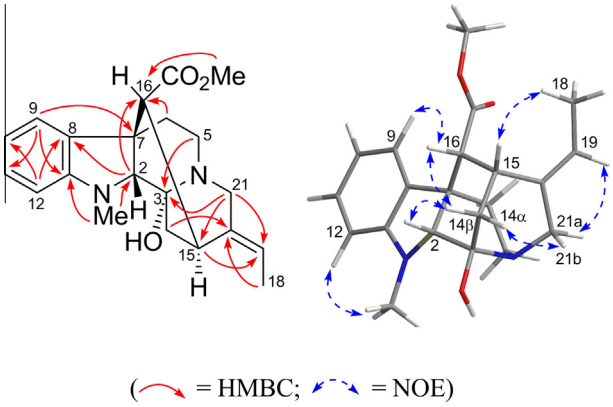

Fig. 6. Selected HMBCs and NOEs of compound **15**.

solution in this instance, X-ray diffraction analysis was carried which established the relative configuration of C-19 in alstonoxine B (**12**) as *S* (Fig. 5). The X-ray structure of **12** also provided additional support for the determination of the C-19 configuration of **11** as 19*S*, using Horeau's procedure (*vide supra*), since the NMR spectroscopic data of **11** and **12** indicated that the non-aromatic portion of **11** was virtually identical to the non-aromatic portion of **12**. Therefore the configuration of C-19 in **11** can be assumed to be similar to that of alstonoxine B (**12**).

Compound **13** was obtained as a light yellowish oil, $[\alpha]_D$ +8 (*c* 0.45, $CHCl_3$). The UV spectrum showed absorption maxima at 229, 254, and 284 nm, indicative of an indole chromophore. The IR spectrum had a broad band at 3372 cm^{-1} due to an OH function. The ESIMS of **13** showed an $[M+H]^+$ peak at *m*/*z* 309, which analyzed for $C_{20}H_{24}N_2O$. The ^{13}C NMR spectrum (Table 1) displayed a total of 20 resonances, comprising two methyl, four methylene, nine methine, and five quaternary carbon atoms, in agreement with the molecular formula. Analysis of the ^1H NMR data (Table 2) established the presence of an unsubstituted indole moiety (δ 7.08–7.41), an N1-Me (δ 3.61), an aminomethylene (δ 3.63), an oxymethylene associated with a hydroxymethyl group (δ 3.47), and an ethylidene side-chain (δ 1.57, 5.28). The COSY spectrum yielded a fragment consistent with a sarpagine-type compound, viz. $NCHCH_2CHCH(CH_2O)CHCH_2$. Analysis of the 2-D NMR spectroscopic data indicated that **13** possessed the same molecular connectivity as affinisine (**14**) (Clivio et al., 1991), which was also isolated. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **13** were generally similar to those of **14** except for notable differences in the chemical shifts of C-15 in the ^{13}C NMR spectrum, and H-15 in the ^1H NMR spectrum. The configurations at the various stereogenic centers were deduced from the NOESY data which indicated similarity with those in affinisine. The observed H-16/H-6β NOE indicated that the configuration of C-16 is *R*. This left the geometry of the C-19–C-20 double bond as a possible point of departure between the two compounds. This was confirmed by the observed reciprocal NOEs for H-19/H-15 and H-21/H-18, which established the geometry of the 19,20-double bond as *Z*. Compound **13** is therefore the 19,20-*Z* isomer of affinisine (19,20-*E*).

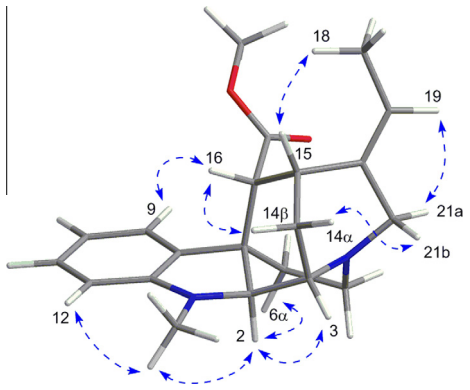

Fig. 7. Selected NOEs of compound **16**.

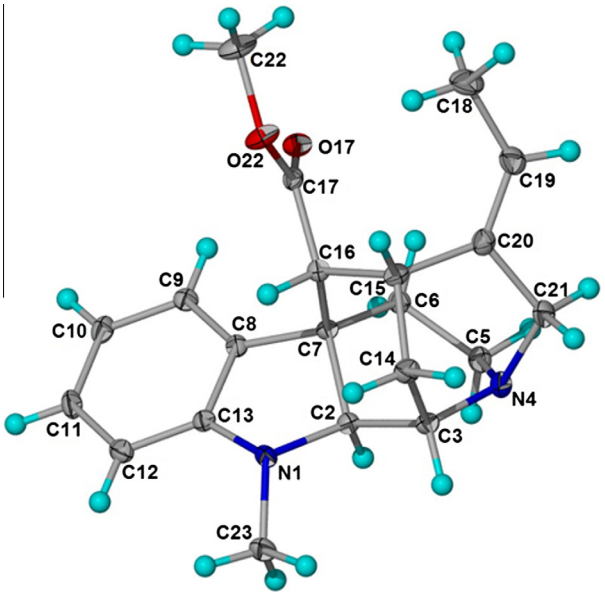

Fig. 8. X-ray crystal structure of compound **16**.

Compound **15** (2(*R*)-3-hydroxycathafoline) was isolated as a light yellowish oil, with $[\alpha]_D$ –48 (c 0.24, $CHCl_3$). The IR spectrum showed a band at 1739 cm^{-1} due to an ester group. The UV spectrum showed typical dihydroindole absorptions at 202, 252, and 295 nm. The ESIMS of **15** showed an $[M+H]^+$ peak at *m*/*z* 355, which analyzed for $C_{21}H_{26}N_2O_3$. The ^{13}C NMR spectrum (Table 1) displayed a total of 21 resonances, comprising three methyl, four methylene, eight methine, and six quaternary carbon atoms. The ^1H NMR spectrum (Table 2) showed the presence of an unsubstituted indole moiety (δ 6.61–7.09), an N1-Me (δ 2.95), a methyl ester (δ 3.78), an aminomethylene (δ_H 2.98, 4.10; δ_C 55.0), and an ethylidene side-chain (δ 1.49, 5.41). The COSY spectrum yielded the following fragments, *viz*., NCH_2CH_2 and CH_2CHCH. The ^{13}C NMR spectrum (Table 1) showed the presence of two downfield resonances, a methine at δ 80.7, and an oxygenated quaternary carbon at δ 85.3. The former resonance was characteristic of C-2 of cathafoline alkaloids with a H-2β orientation (Das et al., 1977), while the latter resonance at δ 85.3 was characteristic of a carbinolamine moiety, suggesting the presence of hydroxy-substitution at C-3. This was further supported by the observed three bond correlations from H-5 and H-21 to the oxygenated C-3 in the HMBC spectrum (Fig. 6). The ^1H and ^{13}C NMR spectroscopic data of **15** showed a close correspondence with those of cathafoline (**18**) (Atta-ur-Rahman et al., 1988), except for the downfield shift of the C-3 signal in the ^{13}C NMR spectrum (from δ 47.2 in **18** to δ 85.3 in **15**) due to substitution by the OH group. The configurations at the various stereogenic centers of **15** were similar to those of cathafoline (**18**) as indicated by the observed NOEs from the NOESY spectrum (Fig. 6). The observed NOE between H-16 and H-9 indicated that the configuration of C-16 is *R* (H-16 directed towards the indole moiety). The observed NOE between H-16 (δ 2.90) and the H-14 signal at δ 1.90 allowed the attribution of this signal to H-14β. The NOE between this H-14β and H-2 indicated that the ori-

Table 3
^{1}H (400 MHz) NMR spectroscopic data for compounds **17**, **19**–**22**, **24**, **26**, and **28**.[a]

H	17	19	20	21	22	24	26	28
2	3.21 d (4)	–	–	–	–	–	3.86 d (5)	3.93 m
3	3.92 m	1.68 m (b)	1.73 m	1.68 m (b)	1.71 m	3.72 m	3.76 m	3.93 m
	–	2.32 m (a)	2.90 m	2.32 m (a)	2.85 m	–	3.96 m	–
5a	2.55 dd (14, 7)	2.73 br t (11) (b)	3.42 dd (11, 9)	2.75 br t (10.5) (b)	3.35 br t (10)	4.70 d (3)	2.53 m	4.13 d (4)
5b	3.43 td (14, 6)	3.33 td (11, 9) (a)	3.91 m	3.38 m (a)	3.94 m	–	2.78 br d (13)	–
6a	2.02 dd (14, 6)	1.97 ddd (14, 9, 1) (a)	2.06 dd (15, 9)	1.97 dd (14, 8) (a)	2.06 dd (15, 9)	2.17 dd (14, 3)	–	2.62 m
6b	3.28 m	2.50 ddd (14, 11, 9) (b)	2.81 m	2.41 m (b)	2.74 m	3.37 br d (14)	–	3.00 br d (13)
9	6.72 d (2)	7.22 dd (7.5, 1)	7.11 d (8)	7.02 s	6.76 d (2.5)	6.76 d (2.5)	7.16 m	6.82 m
10	–	6.58 td (7.5, 1)	6.69 td (8, 1)	–	–	–	6.80 t (8)	6.51 m
11	6.66 dd (9, 2)	7.06 td (7.5, 1)	7.13 td (8, 1)	–	6.67 dd (8.5, 2.5)	6.69 dd (9, 2.5)	7.18 m	7.07 m
12	6.52 br d (9)	6.27 br d (7.5)	6.47 d (8)	5.96 s	6.37 br d (8.5)	6.54 br d (9)	6.66 d (8)	6.63 br d (8)
14a	1.97 m (a)	1.76 m	1.85 m	1.77 m	1.83 m	1.79 br d (14) (b)	1.82 td (10, 3)	1.96 m
14b	2.13 dd (14, 3) (b)	1.76 m	1.92 m	1.77 m	1.91 m	2.11 m (a)	2.55 m	2.81 dd (14, 5)
15	3.36 m	3.61 m	3.65 m	3.62 m	3.64 m	3.27 br s	3.56 m	3.61 m
16	2.71 d (4)	2.83 br s	2.80 br s	2.79 br s	2.77 br s	2.41 d (4)	–	–
17	–	–	–	–	–	–	4.13 s	5.76 br s
18	1.43 dd (7, 2)	1.59 dd (7, 2)	1.60 dd (7, 2)	1.60 dd (7, 2)	1.58 d (7)	1.48 dd (7, 2)	1.60 dd (7, 1)	1.53 d (7)
19	5.38 br q (7)	5.40 q (7)	5.73 q (7)	5.41 q (7)	5.69 q (7)	5.39 q (7)	5.29 br q (7)	5.35 q (7)
21a	2.96 br d (16)	3.00 br d (15)	3.91 br d (15)	3.02 br d (15)	3.85 br d (15)	3.06 br d (18)	3.72 m	3.87 m
21b	3.94 m	3.81 m	4.20 m	3.84 m	4.18 br d (15)	3.75 m	3.96 m	4.25 br d (16)
N_1-*Me*	2.61 s	2.63 s	3.07 s	2.63 s	3.02 s	2.90 s	2.61 s	2.62 s
10-OMe	3.70 s	–	–	3.78 s	3.69 s	3.64 s	–	–
11-OMe	–	–	–	3.86 s	–	–	–	–
CO_2*Me*	3.52 s	3.79 s	3.79 s	3.80 s	3.77 s	3.70 s	3.70 s	3.38 s
2′	–	–	–	–	–	–	–	7.30 d (2)
5′	–	–	–	–	–	–	–	6.84 m
6′	–	–	–	–	–	–	–	7.49 dd (9, 2)
3′-OMe	–	–	–	–	–	–	–	3.84[b] s
4′-OMe	–	–	–	–	–	–	–	3.89[b] s

[a] Measured in $CDCl_3$.
[b] Assignments may be reversed.

entation of H-2 is β as in cathafoline (2*R*), which was also consistent with the observed C-2 resonance at δ 79.1. Furthermore, in the cathafoline alkaloids, the rigid architecture of the molecule restricts the orientation of any substituent at C-3 (including H) to be α only and as such, the orientation of the C-3–OH is therefore by necessity, α. This is the first isolation of a naturally occurring 3-hydroxycathafoline, although a compound previously assigned as 3-hydroxycathafoline has been encountered in transformations of the echitamine alkaloids (Massiot et al., 1983; the ^{13}C NMR data of the reported compound at C-2, C-3, C-14, and C-21, however, were different from those of **15**).

Compound **16** (2(*S*)-cathafoline) was isolated as a light yellowish oil, with $[\alpha]_D$ −175 (*c* 0.22, $CHCl_3$), and was subsequently crystallized from CH_2Cl_2–hexane as colorless block crystals. The UV spectrum showed absorption maxima at 208, 246, and 308 nm, consistent with a dihydroindole chromophore. The IR spectrum showed a band at 1737 cm^{-1} due to an ester function, which was confirmed by the observed carbon shift of the carbonyl function at δ 172.8 in the ^{13}C NMR spectrum. The ESIMS showed an $[M+H]^+$ peak at *m*/*z* 339, which analyzed for $C_{21}H_{26}N_2O_2$.

The ^{13}C NMR spectrum (Table 1) showed a total of 21 resonances, comprising three methyl, four methylene, nine methine, and five quaternary carbon atoms. The ^{1}H NMR spectrum (Table 2) showed many features which were also present in **15** indicating the presence of similar groups, such as an unsubstituted indole moiety, an N1-Me, a methyl ester, an aminomethylene, and an ethylidene side-chain. The COSY spectrum, however, showed the presence of the following partial structures, *viz*., NCH_2CH_2 and $CHCHCH_2CHCH$, which differed from the previous compound by the replacement of CH_2CHCH with a $CHCHCH_2CHCH$ fragment. Furthermore, the downfield resonance at ca. δ 85 in the ^{13}C NMR spectrum, associated with the carbinolamine moiety in **15**, was not observed in the spectrum of **16**. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) indicated a general similarity with those of cathafoline (**18**), except for the more notable differences in the chemical shifts of C-2 and C-14 in the ^{13}C NMR spectrum, and H-2 in the ^{1}H NMR spectrum. This observation, as well as the fact that **16** is isomeric with cathafoline (**18**) as shown by the MS data (*vide supra*), suggested that **16** is a stereoisomer of **18**. The relative configurations at the various stereogenic centers of **16** were similar to those of cathafoline (**18**) as deduced from the NOESY data, except for the configuration at C-2. In the case of **16**, a NOE was not observed between H-2 and H-14β (which was the case in **15**), but was instead observed between H-2 and H-6α, N1-Me, and H-3. These NOEs were different from those observed in the 2*R* cathafoline alkaloids, and indicated that the relative configuration of C-2 in **16** is *S* (H-2α) (Fig. 7), i.e. an inference which was also consistent with the observed resonance of C-2 at δ 70.1 (Das et al., 1977; Massiot et al., 1983). Since suitable crystals of **16** were obtained from CH_2Cl_2–hexane, an X-ray diffraction analysis was carried out which confirmed the assignment of configuration of C-2 as *S* based on the NMR spectroscopic data (Fig. 8). Compound **16** is therefore the C-2 epimer of cathafoline (**18**).

Compound **17** (2(*S*)-10-methoxycathafoline) was obtained as a light yellowish oil, with $[\alpha]_D$ −138 (*c* 0.47, $CHCl_3$). The UV and IR spectra were similar to those of compound **16**. The ESIMS showed

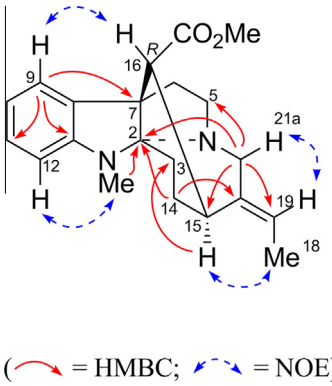

Fig. 9. Selected HMBCs and NOEs of compound **19**.

an $[M+H]^+$ peak at *m/z* 369, which is 30 mass-units more than **16**. The NMR spectroscopic data of **16** and **17** (Tables 1 and 3) were generally similar, except for the presence of a methoxy group in **17**. The substitution of the methoxy group at C-10 was deduced from the coupling behaviour of the aromatic hydrogens, the observed three-bond correlations from H-12 and 10-OMe to C-10 in the HMBC spectrum, and the following observed NOEs: H-9/10-OMe; H-11/10-OMe, H-12; and, H-12/H-11, N1-Me. In view of the diagnostic NOEs observed for H-2/H-6α, N1-Me, H-3, and the observed C-2 resonance at δ 70.7, the configuration at C-2 in **17** is also *S* (H-2α). Compound **17** is therefore, 2(*S*)-10-methoxycathafoline.

Compound **19** (10-demethoxyvincorine) was isolated as a light yellowish oil, with $[\alpha]_D$ −127 (*c* 0.48, $CHCl_3$). The UV spectrum showed absorption bands at 205, 256, and 308 nm, characteristic of a dihydroindole chromophore. The IR spectrum showed a band at 1736 cm^{-1} due to an ester carbonyl function (δ_C 173.8). The ESIMS of **19** gave an $[M+H]^+$ ion at *m/z* 339 and high-resolution measurements gave the formula $C_{21}H_{26}N_2O_2$. The ^1H NMR spectroscopic data (Table 3) displayed a number of features, which were characteristic of akuammiline type alkaloids and which were also common with those present in the previous compounds **15**–**17**, such as the presence of an unsubstituted indole moiety, an N1-Me, a methyl ester, an aminomethylene, and an ethylidene side-chain. A downfield quaternary carbon resonance was observed at δ 97.4 in the ^{13}C NMR spectrum (Table 1), which was assigned to C-2, from the observed three-bond correlation from N1-Me, H-14, and H-21 to C-2 in the HMBC spectrum (Fig. 9). The downfield shift of this carbon was consistent with it being linked to two nitrogen atoms, which is a characteristic of the vincorine type alkaloids. The NMR spectroscopic data (Tables 1 and 3) indicated a close resemblance to those of vincorine (**23**) (Das et al., 1974; Morfaux et al., 1992), except for the absence of the methoxy group at C-10. The relative configuration of C-16 and the geometry of the 19,20-double bond, were similar to those of vincorine (**23**) as shown by the NOE data (Fig. 9).

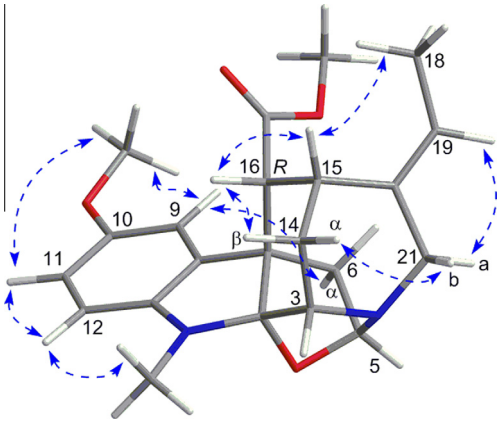

Fig. 10. Selected NOEs of compound **24**.

Compound **20** (10-demethoxyvincorine N(4)-oxide) was obtained as a yellowish oil, with $[\alpha]_D$ −62 (c 0.5, $CHCl_3$). The UV spectrum (208, 247, and 299 nm) showed absorption maxima characteristic of a dihydroindole chromophore, while the IR spectrum showed the presence of an ester carbonyl (1734 cm^{-1}) function. The ESIMS of **20** showed an $[M+H]^+$ peak at *m/z* 355, which analyzed for $C_{21}H_{26}N_2O_3$, 16 mass units higher than that of **19**. Compound **20** was readily identified as the N4-oxide of 10-demethoxyvincorine from its NMR spectroscopic data (Tables 1 and 3), in particular, the characteristic downfield shifts of the carbon resonances for C-2, C-5, and C-21, when compared with those of 10-demethoxyvincorine (**19**).

Compound **21** (11-methoxyvincorine) was obtained as a light yellowish oil, $[\alpha]_D$ −86 (*c* 0.27, $CHCl_3$). The UV spectrum showed dihydroindole absorption maxima at 208, 256 and 317 nm, while the IR spectrum indicated the presence of an ester carbonyl (1733 cm^{-1}) function. The ESIMS of **21** had an $[M+H]^+$ peak at *m/z* 399, corresponding to the molecular formula $C_{23}H_{30}N_2O_4$, differing from vincorine (**23**) by addition of 30 mass units. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 3) were similar in all respects to those of **23**, except for the aromatic resonances and the presence of an additional aromatic methoxy substituent at δ_H 3.86 (δ_C 56.4). In the ^1H NMR spectrum of **21**, two aromatic singlets were observed at δ 7.02 and 5.96, which were assigned to H-9 and H-12, respectively based on the NOE data (NOEs observed for H-9/10-OMe, H-16; H-12/NMe, 11-OMe). These features are consistent with 10,11-dimethoxy-substitution on the indole moiety. Compound **21** is therefore assigned as 11-methoxyvincorine.

Compound **22** (vincorine N(4)-oxide) was obtained as a yellowish oil, with [α]D −84 (*c* 0.4, $CHCl_3$). The UV spectrum (211, 250 and 323 nm) was characteristic of a dihydroindole chromophore, while the IR spectrum showed the presence of an ester (1734 cm^{-1}) function. The ESIMS of **22** had an $[M+H]^+$ at *m/z* 385, which analyzed for $C_{22}H_{28}N_2O_4$, 16 mass units higher than that of vincorine (**23**). Compound **22** was readily identified as the N4-oxide of vincorine

Table 4
Cytotoxic effects of compounds **1**, **3**, **4**, **6**, **9**, **11**, **13**, **15**–**17**, **19**, **21**, and **24**.

Compound	IC_{50}, μg/mL		
	KB/S[a]	KB/VJ300[a]	KB/VJ300(+)[b]
Alstofolinine A (**1**)	>25	>25	>25
20,21-Dihydroalstonerine (**3**)	>25	>25	20.77
Macrocarpine D (**4**)	>25	>25	>25
Macrodasine H (**6**)	>25	>25	13.2
Alstonoxine C (**9**)	>25	>25	>25
Alstonoxine D (**11**)	>25	>25	>25
19,20-*Z*-Affinisine (**13**)	>25	>25	7.47
2(*R*)-3-Hydroxycathafoline (**15**)	>25	>25	>25
2(*S*)-Cathafoline (**16**)	>25	>25	23.58
2(*S*)-10-Methoxycathafoline (**17**)	>25	>25	8.14
10-Demethoxyvincorine (**19**)	>25	>25	11.23
11-Methoxyvincorine (**21**)	>25	>25	4.60
11-demethoxyquaternine (**24**)	>25	>25	6.60
Vincristine	0.015	5.88	–
Kopsamine	>25	>25	6.38 (5.6)[c]

[a] KB/S and KB/VJ300 are vincristine-sensitive and vincristine-resistant human oral epidermoid carcinoma cell lines, respectively.
[b] With added vincristine, 0.1 μg/mL, which did not affect the growth of the KB/VJ300 cells.
[c] With added vincristine, 0.25 μg/mL, which did not affect the growth of the KB/VJ300 cells (Kam et al., 1998b).

from its NMR spectroscopic data (Tables 1 and 3), in particular the characteristic downfield shifts of the carbon resonances for C-2, C-5, and C-21, when compared with those of vincorine (**23**).

Compound **24** (11-demethoxyquaternine) was obtained as a light yellowish oil, $[\alpha]_D$ −10 (*c* 0.21, $CHCl_3$). The UV spectrum had absorption maxima at 208, 241, 307 nm, suggesting the presence of a dihydroindole chromophore, and the IR spectrum displayed an ester carbonyl band at 1736 cm^{-1} (δ_C 172.5). The ESIMS showed an $[M+H]^+$ peak at *m*/*z* 383, which analyzed for $C_{22}H_{26}N_2O_4$. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 3) established the presence of a substituted indole moiety, an ethylidene side-chain, and three 3H singlets at δ_H 3.70, 3.64, and 2.90, due to a methyl ester (δ_C 172.5, 51.7), an aromatic methoxy (δ_C 56.0), and an NMe (δ_C 30.0) group, respectively. In addition, a deshielded methine was observed as a doublet at δ_H 4.70 (*J* = 3 Hz), with the corresponding carbon resonance observed at δ_C 87.2. The observed downfield ^1H and ^{13}C shifts shown by this methine, is characteristic of a carbon, which is adjacent to a nitrogen and an oxygen atom. The NMR spectroscopic data indicated a similarity to those of quaternine (**25**) (Abe et al., 1994a), which was also present in the same plant. The main difference noted from the NMR spectroscopic data was the absence of one of the aromatic methoxy groups (two aromatic methoxy groups were present in quaternine). The aromatic doublet at δ 6.76 was assigned to H-9 from its NOE with H-6α (Fig. 10), while the other aromatic doublet at δ 6.54 was assigned to H-12 from the observed NOE between this hydrogen and N1-Me. The observed NOEs for OMe/H-9, H-11 confirmed the placement of the aromatic methoxy group at C-10. The relative configuration of C-16 was assigned as *R* (H-directed towards the indole moiety), from the observed NOEs between H-16 and H-14β, H-15 (Fig. 10).

Compound **26** (vincamajine N(4)-oxide) was obtained as a yellowish oil, with [α]D −29 (*c* 0.04, $CHCl_3$). The UV spectrum (210, 231 and 282 nm) was characteristic of a dihydroindole chromophore, while the IR spectrum showed the presence of OH and ester groups (3400 and 1737 cm^{-1}). The presence of an ester function was further confirmed by the observed signals at δ 52.3 and 170.8 in the ^{13}C NMR spectrum. The ESIMS of **26** showed an $[M+H]^+$ at *m*/*z* 383 ($C_{22}H_{26}N_2O_4$), which was 16 mass units higher than that of vincamajine (**27**). The NMR spectroscopic data (Table 1 and 3) indicated an alkaloid of the ajmaline type and were in fact similar to those of vincamajine (**27**) (Cherif et al., 1989), which was also isolated, except that the resonances of C-3, C-5, and C-21, were shifted downfield in **26**. Based on these observations, compound **26** was readily identified as the N4-oxide of vincamajine.

Compound **28** (vincamajine 17-*O*-veratrate N(4)-oxide) was obtained as a yellowish oil, with [α]D −75 (*c* 0.94, $CHCl_3$). The UV spectrum (209, 254 and 292 nm) was characteristic of a dihydroindole chromophore, while the IR spectrum displayed the presence of an ester (1737 cm^{-1}) function. The ESIMS of **28** had an $[M+H]^+$ at *m*/*z* 547, which analyzed for $C_{31}H_{34}N_2O_7$. The ^1H NMR (Table 3) spectrum showed similar features as those shown by vincamajine (**27**), except for the presence of additional signals due to the acid residue (3′,4′-dimethoxybenzoic acid or veratric acid) associated with an ester group at C-17 (vincamajine 17-*O*-veratrate (**29**)) (Abe et al., 1994b), and the downfield shifts of the carbon resonances for C-3, C-5, and C-21, when compared with those of vincamajine 17-*O*-veratrate (**29**). Compound **28** (measured mass was 16 mass units higher than that of **29**) is therefore readily identified as the N4-oxide of vincamajine 17-*O*-veratrate.

All the new compounds tested showed no appreciable cytotoxicity against drug-sensitive and vincristine-resistant KB cells (IC_{50} >25 μg/ml in all cases). However, 11-methoxyvincorine (**21**), 11-demethoxyquaternine (**24**), 19,20-*Z*-affinisine (**13**), 2(*S*)-10-methoxycathafoline (**17**), 10-demethoxyvincorine (**19**), and macrodasine H (**6**), were found to reverse multidrug resistance in vincristine-resistant KB (VJ300) cells (Table 4).

The structures of several new macroline oxindole alkaloids from various *Alstonia* species were previously reported (Kam and Choo, 2000). In view of a systematic error involving the labeling of the configuration (*R* or *S*) at the spirocyclic carbon (C-7) in these alkaloids, a list of these alkaloids is now included with the correct *R*/*S* labeling for C-7: isoalstonisine (reported 7*S*, corrected 7*R*) (Kam and Choo, 2000), macrogentine (reported 7*S*, corrected 7*R*) (Kam and Choo, 2000), alstonoxine A (reported 7*R*, corrected 7*S*) (Kam and Choo, 2000), alstonoxine B (reported 7*R*, corrected 7*S*) (Kam and Choo, 2000), alstofoline (reported 7*R*, corrected 7*S*) (Kam and Choo, 2000), N1-demethylalstonisine (reported 7*R*, corrected 7*S*) (Kam and Choo, 2000), affinisine oxindole (reported 7*R*, corrected 7*S*) (Kam and Choo, 2004b).

Concluding remarks

The present investigation reports the presence of 17 new indole alkaloids and completes the study of the alkaloids of this particular sample of *A. macrophylla*. The new indole alkaloids found were mainly of the macroline, sarpagine, akuammiline/vincorine and ajmaline types. It is noted that there is some variation in the alkaloid composition between the indole alkaloids found in the present sample collected on the western coast of Peninsular Malaysia (Perak), compared to a sample collected from the eastern coast of Peninsular Malaysia (Terengganu) (Kam and Choo, 2004a). Although a number of alkaloids from the various subtypes were common in both samples, nevertheless, alkaloids which were found in one sample, were absent in the other, and vice versa. For instance, sarpagine and ajmaline alkaloids found in the present sample were not found in the previous sample. There is also a greater predominance of the akuammiline alkaloids, as well as bisindole alkaloids (Lim et al., 2011, 2012, 2013) in the present sample. Among the alkaloids tested, the akuammiline alkaloids, 11-methoxyvincorine (**21**) and 11-demethoxyquaternine (**24**), were the most active in reversing multi-drug resistance in vincristine-resistant KB cells.

Experimental

General

Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. Optical rotations were determined on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Perkin-Elmer Spectrum 400 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. NOESY experiments were carried out on a Bruker Avance III 600 spectrometer at 600 MHz. ESIMS and HRESIMS were obtained on an Agilent 6530 Q-TOF or on a JEOL AccuTOF-DART mass spectrometer.

Plant material

A. macrophylla was collected in Perak, Malaysia and identification was confirmed by Dr. Richard C. K. Chung, Forest Research Institute, Malaysia, and Dr. K. T. Yong, Institute of Biological Sciences, University of Malaya. Herbarium voucher specimens (K671) are deposited at the Herbarium, University of Malaya.

Extraction and isolation

The leaf (7 kg) and stem-bark (9 kg) were exhaustively extracted with EtOH at room temperature and the concentrated EtOH extract was then partitioned with dilute acid (3% tartaric acid) 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 silica gel column chromatography (CC) using $CHCl_3$ with increasing proportions of MeOH, followed by further chromatography of the appropriate partially resolved fractions using centrifugal preparative TLC. The solvent systems used for centrifugal preparative TLC were Et_2O–hexane (4:1; NH_3-saturated), Et_2O (NH_3-saturated), Et_2O–MeOH (100:1; NH_3-saturated), Et_2O–MeOH (20:1; NH_3-saturated), Et_2O–MeOH (10:1; NH_3-saturated), EtOAc–hexane (1:5; NH_3-saturated), EtOAc–hexane (1:4; NH_3-saturated), EtOAc–hexane (1:1; NH_3-saturated), EtOAc–hexane (2:1; NH_3-saturated), EtOAc–hexane (3:1; NH_3-saturated), EtOAc (NH_3-saturated), EtOAc–MeOH (10:1; NH_3-saturated), $CHCl_3$–hexane (1:1; NH_3-saturated), $CHCl_3$–hexane (1:2; NH_3-saturated), $CHCl_3$ (NH_3-saturated), $CHCl_3$–MeOH (100:1; NH_3-saturated), and $CHCl_3$–MeOH (50:1; NH_3-saturated). The yields (mg kg^{-1}) of the alkaloids from the leaf extract were as follows: **1** (0.9), **9** (7.9), **13** (4.0), **15** (5.0), **16** (4.7), **17** (6.9), **19** (1.0), **20** (1.8), **21** (0.7), **22** (20.6), **24** (13.2), **26** (0.2), and **28** (1.9). The yields (mg kg^{-1}) of the alkaloids from the stem-bark extract were as follows: **3** (0.2), **4** (3.5), **6** (0.3), **11** (2.2), **13** (4.3), and **16** (0.1).

Characterization data

*Alstofolinine A (**1**)*

Light yellowish oil; $[\alpha]^{25}_D$ −104 (*c* 0.36, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 227 (4.10), 285 (3.43) nm; IR (dry film) ν_{max} 1769 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 1, respectively; EIMS *m*/*z* 296 $[M]^+$ (91), 281 (7), 265 (4), 240 (5), 227 (40), 212 (5), 197 (78), 182 (23), 181 (98), 170 (24), 167 (36), 154 (12), 144 (7), 119 (11); HREIMS *m*/*z* 296.1528 (calcd for $C_{18}H_{20}N_2O_2$, 296.1525).

*20,21-Dihydroalstonerine (**3**)*

Light yellowish oil; $[\alpha]^{25}_D$ −31 (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 228 (4.50), 286 (3.79) nm; IR (dry film) ν_{max} 1710 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 1, respectively; EIMS *m*/*z* 338 $[M]^+$ (100), 307 (8), 295 (11), 264 (10), 251 (6), 238 (8), 223 (5), 210 (14), 197 (78), 182 (19), 170 (13), 158 (10), 119 (4), 84 (12), 70 (25), 57 (6), 49 (10); HREIMS *m*/*z* 338.1993 (calcd for $C_{21}H_{26}N_2O_2$, 338.1994); ESIMS *m*/*z* 339 $[M+H]^+$; HRESIMS *m*/*z* 339.2080 (calcd for $C_{21}H_{27}N_2O_2$, 339.2073).

*Macrocarpine D (**4**)*

Light yellowish oil; $[\alpha]^{25}_D$ −43 (*c* 0.89, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 231 (5.16), 286 (4.48) nm; IR (dry film) ν_{max} 3395, 3292 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 1, respectively; EIMS *m*/*z* 324 $[M]^+$ (100), 306 (9), 295 (18), 277 (28), 240 (55), 212 (29), 184 (53), 145 (60), 117 (54), 95 (27); ESIMS *m*/*z* 327 $[M+H]^+$; HRESIMS *m*/*z* 327.2079 (calcd for $C_{20}H_{27}N_2O_2$, 327.2073).

*Macrodasine H (**6**)*

Light yellowish oil; $[\alpha]^{25}_D$ −11 (*c* 0.14, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 233 (4.22), 288 (3.55) nm; IR (dry film) ν_{max} 3423 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 1, respectively; ESIMS *m*/*z* 439 $[M+H]^+$; HRESIMS *m*/*z* 439.2598 (calcd for $C_{26}H_{35}N_2O_4$, 439.2591).

*Alstonoxine C (**9**)*

Light yellowish oil and subsequently colorless block crystals from CH2Cl2−hexane; mp 130–132 °C; $[\alpha]^{25}_D$ −30 (*c* 0.39, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 219 (5.17), 266 (4.33) and 291 (4.24) nm; IR (dry film) ν_{max} 3390, 3295, 1694 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 1, respectively; ESIMS *m*/*z* 359 $[M+H]^+$; HRESIMS *m*/*z* 359.1979 (calcd for $C_{20}H_{27}N_2O_4$, 359.1971).

*Alstonoxine D (**11**)*

Light yellowish oil; $[\alpha]^{25}_D$ −16 (*c* 0.23, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 224 (4.62), 274 (3.80) and 286 (3.80) nm; IR (dry film) ν_{max} 3391, 3298, 1695 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 1, respectively; ESIMS *m*/*z* 361 $[M+H]^+$; HRESIMS *m*/*z* 361.2125 (calcd for $C_{20}H_{29}N_2O_4$, 361.2127).

*19,20-Z-Affinisine (**13**)*

Light yellowish oil; $[\alpha]^{25}_D$ +8 (*c* 0.45, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 229 (5.37), 254 (4.92), 284 (4.73) nm; IR (dry film) ν_{max} 3372 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 1, respectively; ESIMS *m*/*z*: 309 $[M+H]^+$; HRESIMS *m*/*z*: 309.1973 $[M+H]^+$ (calcd for $C_{20}H_{25}N_2O$, 309.1967).

*2(R)-3-Hydroxycathafoline (**15**)*

Light yellowish oil; $[\alpha]^{25}_D$ −48 (*c* 0.24, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 202 (4.64), 252 (4.11), 295 (3.69) nm; IR (dry film) ν_{max} 1739 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 1, respectively; ESIMS *m*/*z*: 355 $[M+H]^+$; HRESIMS *m*/*z*: 355.2028 $[M+H]^+$ (calcd for $C_{21}H_{27}N_2O_3$, 355.2022).

*2(S)-Cathafoline (**16**)*

Light yellowish oil and subsequently colorless needles from CH_2Cl_2-hexanes; mp 121–123 °C; $[\alpha]^{25}_D$ −175 (*c* 0.22, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 208 (4.68), 246 (4.38), 308 (4.06) nm; IR (dry film) ν_{max} 1737 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 2 and 1, respectively; ESIMS *m*/*z*: 339 $[M+H]^+$; HRESIMS *m*/*z*: 339.2071 $[M+H]^+$ (calcd for $C_{21}H_{27}N_2O_2$, 339.2073).

*2(S)-10-Methoxycathafoline (**17**)*

Light yellowish oil; $[\alpha]^{25}_D$ −138 (*c* 0.47, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 208 (4.85), 245 (4.60), 307 (4.17) nm; IR (dry film) ν_{max} 1737 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 3 and 1, respectively; ESIMS *m*/*z*: 369 $[M+H]^+$; HRESIMS *m*/*z*: 369.2172 $[M+H]^+$ (calcd for $C_{22}H_{29}N_2O_2$, 369.2178).

*10-Demethoxyvincorine (**19**)*

Light yellowish oil; $[\alpha]^{25}_D$ −127 (*c* 0.48, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 205 (5.21), 256 (4.78), 308 (4.29) nm; IR (dry film) ν_{max} 1736 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 3 and 1, respectively; ESIMS *m*/*z*: 339 $[M+H]^+$; HRESIMS *m*/*z*: 339.2072 $[M+H]^+$ (calcd for $C_{21}H_{27}N_2O_2$, 339.2073).

*10-Demethoxyvincorine N(4)-oxide (**20**)*

Light yellowish oil; $[\alpha]^{25}_D$ −62 (*c* 0.5, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 208 (5.43), 247 (5.19), 299 (4.70) nm; IR (dry film) ν_{max} 1734 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 3 and 1, respectively; ESIMS *m*/*z*: 355 $[M+H]^+$; HRESIMS *m*/*z*: 355.2019 $[M+H]^+$ (calcd for $C_{21}H_{27}N_2O_3$, 355.2022).

*11-Methoxyvincorine (**21**)*

Light yellowish oil; $[\alpha]^{25}_D$ −86 (*c* 0.27, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 208 (4.16), 256 (3.75), 317 (3.54) nm; IR (dry film) ν_{max} 1733 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 3 and 1, respectively; ESIMS *m*/*z*: 399 $[M+H]^+$; HRESIMS *m*/*z*: 399.2078 $[M+H]^+$ (calcd for $C_{23}H_{31}N_2O_4$, 399.2084).

*Vincorine N(4)-oxide (**22**)*

Light yellowish oil; $[\alpha]^{25}_{D}$ −84 (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 211 (4.88), 250 (4.51), 323 (4.05) nm; IR (dry film) ν_{max} 1734 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 3 and 1, respectively; ESIMS *m*/*z*: 385 $[M+H]^+$; HRESIMS *m*/*z*: 385.2130 $[M+H]^+$ (calcd for $C_{22}H_{29}N_2O_4$, 385.2127).

*11-Demethoxyquaternine (**24**)*

Light yellowish oil; $[\alpha]^{25}_{D}$ −10 (*c* 0.21, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 208 (4.64), 241 (4.35), 307 (3.96) nm; IR (dry film) ν_{max} 1736 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 3 and 1, respectively; ESIMS *m*/*z*: 383 $[M+H]^+$; HRESIMS *m*/*z*: 383.1978 $[M+H]^+$ (calcd for $C_{22}H_{27}N_2O_4$, 383.1971).

*Vincamajine N(4)-oxide (**26**)*

Light yellowish oil; $[\alpha]^{25}_{D}$ −29 (*c* 0.04, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 210 (4.94), 231 (4.70) and 282 (4.30) nm; IR (dry film) ν_{max} 3400 and 1737 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 3 and 1, respectively; ESIMS *m*/*z*: 383 $[M+H]^+$; HRESIMS *m*/*z*: 383.1980 $[M+H]^+$ (calcd for $C_{22}H_{27}N_2O_4$, 383.1971).

*Vincamajine 17-O-veratrate N(4)-oxide (**28**)*

Light yellowish oil; $[\alpha]^{25}_{D}$ −75 (*c* 0.94, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 209 (5.75), 254 (5.40) and 292 (5.07) nm; IR (dry film) ν_{max} 1737 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 3 and 1, respectively; ESIMS *m*/*z*: 547 $[M+H]^+$; HRESIMS *m*/*z*: 547.2443 $[M+H]^+$ (calcd for $C_{31}H_{35}N_2O_7$, 547.2444).

*$NaBH_4$ reduction of alstonoxine C (**9**)*

To a mixture of compound **9** (12.4 mg, 0.035 mmol) in MeOH (5 ml) at 0 °C was added $NaBH_4$ (6.5 mg, 0.17 mmol). The solution was stirred at 0 °C for 1 h. Saturated Na_2CO_3 (5 ml) solution was added, and the product was extracted with CH_2Cl_2 (3 × 10 ml). The combined organic extract was dried (Na_2SO_4), filtered, and concentrated *in vacuo*, and the residue was purified by centrifugal preparative TLC (SiO_2, 1% MeOH:CHCl3, NH_3-saturated) to afford **11** (5.1 mg, 41%) and **11a** (4.8 mg, 39%). Compound **11a**: colorless oil; $[\alpha]^{25}_{D}$ −33 (*c* 0.24, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.69 (1H, d, *J* = 8.3 Hz), 6.58 (1H, dd, *J* = 8.3, 2.2 Hz), 6.45 (1H, d, *J* = 2.2 Hz), 4.02 (1H, m), 3.99 (1H, m), 3.96 (1H, m), 3.92 (1H, m), 3.83 (3H, s), 3.24 (1H, m), 3.17 (3H, s), 2.76 (1H, m), 2.38 (1H, dd, *J* = 8.4, 2.3 Hz), 2.06 (1H, d, *J* = 8.4 Hz), 1.94 (1H, m), 1.77 (1H, m), 1.72 (1H, m), 1.58 (1H, m), 1.53 (1H, m), 1.29 (1H, d, *J* = 6 Hz); HRESIMS *m*/*z* 327.1718 (calcd for $C_{19}H_{23}N_2O_3$, 327.1703).

Determination of the C-19 configuration of compound 6 by Horeau's method

To a solution of compound **11** (5 mg, 0.038 mmol) and anhydrous pyridine (1 ml), was added, racemic 2-phenylbutyric anhydride (0.1 ml). The resulting mixture was stirred for 24 h at rt. H_2O (3 ml) was then added and the mixture was allowed to stand for 30 min. The pH of the solution was adjusted to pH 9 by dropwise addition of NaOH (0.1 M), after which the solution was extracted with CH_2Cl_2 (3 × 20 ml). The aqueous layer was acidified to pH 3 using 1.0 M HCl and extracted with CH_2Cl_2 (3 × 10 ml). Evaporation of the solvent from the organic phase gave the unreacted 2-phenylbutyric acid. The optical rotation of the unreacted 2-phenylbutyric acid was found to be negative (*R*), indicating the *S* configuration at C-19 in compound **11**.

*X-ray crystallographic analysis of alstonoxine C (**9**), alstonoxine B (**12**), and 2(S)-cathafoline (**16**)*

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 = 0.71073 Å), at 100 K. The structure was 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 the relative isotropic parameters. Crystallographic data for compounds 9, 12, and 16 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 alstonoxine C (**9**): Colorless block crystals, $C_{20}H_{26}N_2O_4.H_2O$, M_r = 376.44, orthorhombic, space group $P2_12_12_1$, *a* = 7.3753(2) Å, *b* = 12.6375(3) Å, *c* = 19.5260(4) Å, α = β = γ = 90°, *V* = 1819.93(8) $Å^3$, *T* = 100 K, *Z* = 4, D_{calcd} = 1.374 g cm^{-3}, crystal size 0.16 × 0.18 × 0.24 mm^3, *F*(000) = 704. The final R_1 value is 0.0383 (wR_2 = 0.0898) for 3750 reflections [$I > 2\sigma(I)$]. CCDC number: 935820.

Crystallographic data of alstonoxine B (**12**): colorless block crystals, $C_{19}H_{26}N_2O_3$, M_r = 330.42, monoclinic, space group $P2_1$, *a* = 10.7388(4) Å, *b* = 10.5321(3) Å, *c* = 15.2354(5) Å, α = γ = 90°, β = 92.851(2), *V* = 1721.02(10) $Å^3$, *T* = 100 K, *Z* = 4, D_{calcd} = 1.275 g cm^{-3}, crystal size 0.21 × 0.41 × 0.49 mm^3, *F*(000) = 712. The final R_1 value is 0.0382 (wR_2 = 0.1076) for 7729 reflections [$I > 2\sigma(I)$]. CCDC number: 935819.

Crystallographic data of 2(*S*)-cathafoline (**16**): Colorless block crystals, $C_{21}H_{26}N_2O_2$, M_r = 338.44, monoclinic, space group $P2_1$, *a* = 7.0991(3) Å, *b* = 8.6382(4) Å, *c* = 14.4902(6) Å, α = γ = 90°, β = 101.237(4), *V* = 871.55(7) $Å^3$, *T* = 100 K, *Z* = 2, D_{calcd} = 1.290 g cm^{-3}, crystal size 0.08 × 0.26 × 0.37 mm^3, *F*(000) = 364. The final R_1 value is 0.0367 (wR_2 = 0.0928) for 2138 reflections [$I > 2\sigma(I)$]. CCDC number: 936523.

Cytotoxicity assays

Cytotoxicity assays (Mosmann, 1983) were carried out following essentially the same procedure as described previously (Kam et al., 1998a, 2004b). Human oral epidermoid carcinoma cells (KB) and vincristine-resistant KB cells (VJ-300) were maintained in Eagle's MEM, supplemented with 10% fetal bovine serum and 2% penicillin/streptomycin. The cells were cultured at 37 °C under a humidified atmosphere in a CO_2 incubator. The cells were then seeded in a 96-well microtiter plate (Nunc, Germany) at a concentration of 70,000 cells/mL, and incubated in a CO_2 incubator at 37 °C. After 24 h, the cells were treated with samples at six different concentrations (0.1, 0.3, 1, 3, 10 and 30 μg/ml) and incubated for 72 h. Wells containing untreated cells (without addition of sample) were regarded as negative controls. DMSO was used to dilute the samples and the final concentration of DMSO in each well was not in excess of 0.5% (v/v). No adverse effect due to presence of DMSO was observed. At the end of the incubation period, 20 μl of MTT working solution (5 mg MTT in 1 ml phosphate-buffered saline) was added into each well and the 96-well microtiter plate was incubated for another three hours at 37 °C. The medium was then gently aspirated from each well and 200 μl of DMSO were added to effect formazan solubilization. After agitation for 15 min, the absorbance of each well was measured with a micro plate reader (Emax, Molecular Devices, USA) at 540 nm with 650 nm. The cytotoxic activity of each sample was expressed as the IC_{50} value, which is the concentration of the test sample that causes 50% inhibition of cell growth. All the samples were assayed in three independent experiments.

Acknowledgments

We thank the University of Malaya and MOHE Malaysia (HIR-F005) for financial support, and Dr. K. Komiyama (Center for Basic

Research, Kitasato University, Tokyo, Japan), for a gift of the KB cells.

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