Pergamon

Tetrahedron 56 (2000) 6143–6150

TETRAHEDRON

Novel Macroline Oxindoles from a Malayan *Alstonia*

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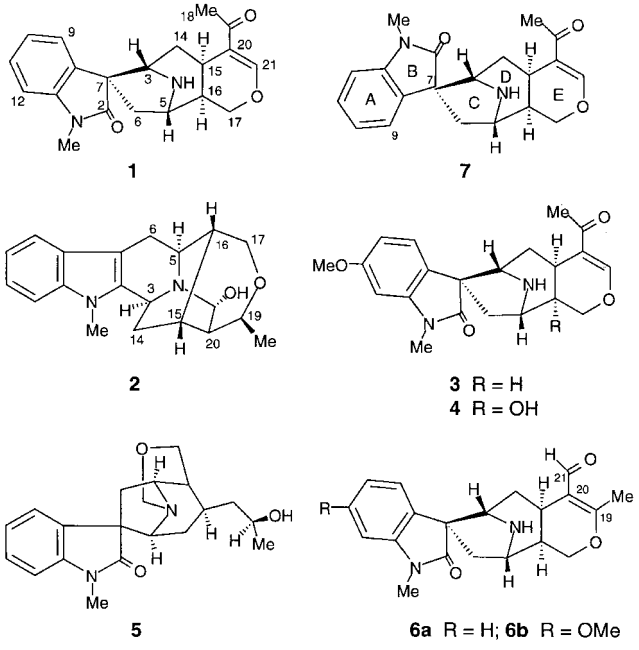
Received 5 April 2000; revised 25 May 2000; accepted 8 June 2000

Abstract—Seven new macroline-type oxindole alkaloids, including several with novel structural features, were obtained from the Malayan *Alstonia macrophylla* and their structures established by spectral analysis.

The genus *Alstonia* is characterised by the preponderance of macroline-type indole and oxindole alkaloids.[1,2] The first macroline oxindole, alstonisine **1**, was reported by Gilman from *Alstonia muelleriana*.[3] Single crystal X-ray analysis[4] provided the relative configuration while a subsequent biomimetic transformation of alstonisine **1** to talpinine **2** allowed the absolute configuration of **1** to be inferred.[5] In the event, interest in the enantiospecific synthesis of alstonisine continues unabated.[6–8] Alstonisine has since then been reported from other *Alstonia* species, including *A. angustifolia*[9] and *A. macrophylla*.[10] Two additional oxindoles, N_b-demethylalstophylline oxindole **3** and 16-hydroxy-N_b-demethylalstophylline oxindole **4** were obtained from the Sri Langkan *A. macrophylla*.[11,12] NOE experiments were carried out which provided confirmation of the configuration of the spirocyclic centre in these two compounds, which were the same as that for alstonisine. Three other macroline oxindoles, macroxine **5**, alstonal **6a**, and N_b-demethylalstophyllal oxindole **6b** were also subsequently obtained from *Alstonia*, for which the configuration at C(7) were not addressed, but were presumed to be similar to that of the known oxindole compounds.[13,10] We have now obtained from the Malayan *A. macrophylla*, in addition to alstonisine **1** and alstonal **6a**, seven new macroline oxindoles, **7**–**13**. Two of these, viz. isoalstonisine **7** and macrogentine **8**, represent the first macroline oxindoles from *Alstonia* which possess opposite stereochemistry at the spirocyclic carbon (*S*) compared to that of the hitherto known macroline oxindoles.



To provide direct confirmation of the C(7) configuration, NOE experiments were first carried out on alstonisine **1**. Irradiation of H(9) causes enhancement of H(15) in **1** and vice versa, thus confirming the configuration of the spirocentre (carbon-7) in alstonisine **1** as *R*. The other NOE's are shown in Fig. 1 and allow the complete assignment of the stereo-chemistry of **1**.

Isoalstonisine **7** showed a molecular ion at *m/z* 338 which analysed for $C_{20}H_{22}N_2O_3$, indicating that it is isomeric with alstonisine **1**. The IR (1704 cm^{-1}) and UV spectrum (λ_{max} 210, 255 nm) indicated an oxindole derivative. The ^{13}C NMR spectral data were very similar to that of alstonisine showing marked departure only for C(2) and C(8). Thus the

Keywords: alkaloids; oxindoles; NMR; plants.
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PII: S0040-4020(00)00564-0

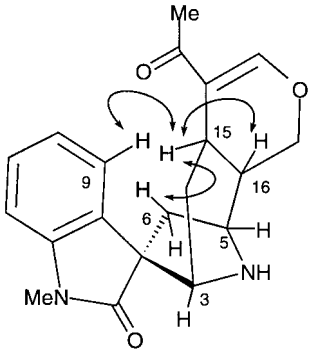


Figure 1. Selected NOE's of **1**.

C(2) resonance in alstonisine **1** is found at δ 181.9 while that in isoalstonisine **7** is shifted upfield to δ 177.5. Likewise, the C(8) resonance in isoalstonisine **7** is shifted downfield to δ 137.6 from 128.7 in alstonisine **1**. The same behaviour was also shown by the second new oxindole macrogentine **8** which also possesses the opposite configuration at the spirocyclic carbon (*S*), with the C(2) and C(8) shifts observed at δ 177.4 and 138.1, respectively. This pattern appears to be quite general for the unsubstituted oxindole compounds. The known oxindole alkaloids alstonisine **1**, alstonal **6a**, and the new macroline oxindoles, alstonoxine A **9**, alstonoxine B **10**, alstofoline **11**, *N*(1)-demethylalstonisine **12**, and *N*(1)-demethylalstonal **13** all showed the C(2) resonance at ca. δ 182 and the C(8) resonance at ca. δ 129. (The 11-methoxysubstituted derivatives **3**, **4**, and **6b** showed a similar pattern for C(2), but the C(8) resonances are shifted slightly upfield to ca. δ 121), while for the C(7) diastereomeric oxindoles **7** and **8**, the resonances for C(2) and C(8) are shifted to δ 177 and 138, respectively. A distinct pattern also emerges on examination of the ^{1}H NMR spectral data. Comparison of isoalstonisine **7** on the one hand, with the C(7) diastereomeric pentacyclic oxindoles exemplified by alstonisine **1**, indicates that H(9) in isoalstonisine **7** (δ 7.15) is shifted upfield when compared with H(9) in alstonisine **1** (δ 8.25). The lower field resonance of H(9) in alstonisine **1** (and in the related 7*R* pentacyclic oxindoles **3**, **4**, **6**, **11**, **12**, **13**) is caused by the anisotropic effect of the proximate C(20)–C(21) double bond[14] (Fig. 1). In the pentacyclic 7*S* oxindole, isoalstonisine **7**, the change in configuration at the spirocentre results in the same double bond being too far removed from the aromatic ring to exert any anisotropy on H(9) (Fig. 2). This conclusion is also supported by NOE

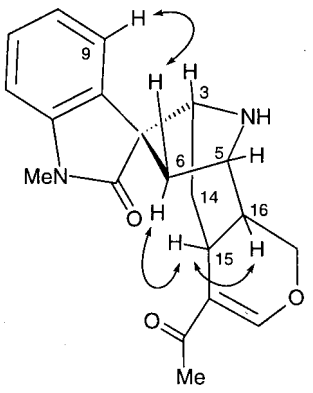


Figure 2. Selected NOE's of **7**.

experiments. It has been mentioned earlier that irradiation of H(9) in alstonisine **1** causes enhancement of H(15) and vice versa. Similar irradiation of H(9) in the case of isoalstonisine **7** results instead in enhancement of H(6β, pseudoaxial) but not H(15). The other NOE's are shown in Fig. 2 which are in agreement with the proposed structure. The major and diagnostic differences in the observed NOE's of alstonisine **1** and isoalstonisine **7** are also consistent with the predicted distances in the energy minimised structures (MM2, CS Chem3D Pro). Thus the calculated H(9)/H(15) distance is 2.11 Å in alstonisine **1**, whereas it is 5.52 Å in isoalstonisine **7**, and the H(9)/H(6) distances in **1** is 4.14 Å whereas it is 2.71 Å in **7**. In addition the H(6β)/H(15) distances in **1** and **7** are 2.24 and 2.33 Å, respectively, which are in agreement with the observed NOE's.

The NOE interaction between H(6α, pseudoequatorial) and H(15) fixes the stereochemistry of H(15) as α. The NOE interaction between H(15) and H(16) coupled with the observed J_{15-16} value of 6 Hz establish the D/E ring junction as *cis*. Finally the observed NOE interaction between H(6α) and H(15) also establishes the *cis* C/D ring junction and the β stereochemistry of H(3) and H(5). Isoalstonisine **7** is obtained in low yield from alstonisine **1** by prolonged reflux in pyridine, which resulted in an equilibrium mixture of **7** and **1**, with the 7*R* diastereomer predominating.[15]

Macrogentine **8** is another novel macroline oxindole with similar C(7) configuration as isoalstonisine **7**. In common with **7**, the C(2) and C(8) resonances are observed at δ 177 and 138, respectively. The EI mass-spectrum showed an M^+ at *m/z* 354 which analysed for $C_{21}H_{26}N_2O_3$ and the UV spectrum is characteristic of an oxindole. The IR spectrum showed a broad band at 1704 cm^{-1} due to various carbonyl groups, which can be attributed to lactam and ketone functions from the observed carbon resonances at δ 177.4 and 208.8, respectively. Analysis of the ^{1}H and ^{13}C NMR spectral data suggested that **8** possesses a carbon skeleton in which, while the A, B, C, and D rings of a macroline oxindole system are essentially intact, cleavage and rearrangement have occurred involving ring E.

Thus in comparison to alstonisine for example, the characteristic low field signal of the vinylic H(21) is missing while the oxymethylene H(17), and the H(16) methine signals have been shifted upfield. Furthermore, a virtually coincident pair of doublet of doublets due to the diastereotopic hydrogens of a methylene group (δ 2.51, 2.56, AA′BB′ system), and a methyl doublet (δ 1.29) are now observed in the spectrum of **8**, while the methyl signals of the *N*(1)-Me and acetyl functions appear to be intact. The COSY and HETCOR spectrum revealed in addition to the C(5)–C(6) fragment, two other partial structures, viz. *N*CH–CH_2–$CH(CH_2)$–CH–CH_2O and CH_3CH–O corresponding to the C3–C14–C15(C20)–C16–C17 and C22–C21 fragments, respectively. The chemical shift of the C(20)-methylene group (δ_H 2.51, 2.56; δ_C 48.7) suggested that it could be α to a carbonyl function.

This was confirmed by the observed three-bond and two-bond correlations from C(20) to H(18) and from C(19) to H(18), respectively. This coupled with the observed ^2J and ^3J correlations from C(20) to H(15) and H(14) in the HMBC

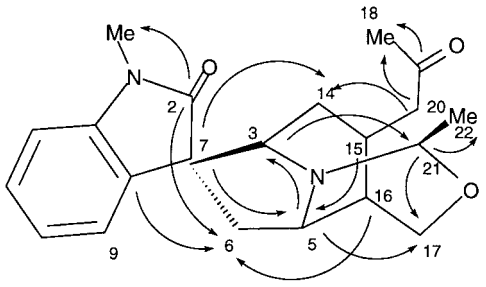
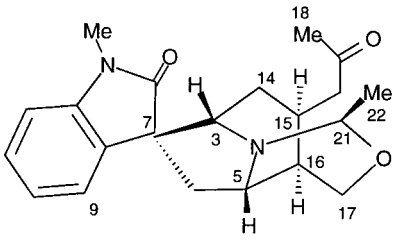


Figure 3. Selected HMBC correlations for macrogentine **8**.

spectrum, provided firm evidence for a 2-oxopropyl side chain attached to ring D at C(15). The molecular formula of **8** (DBE 10) indicates a pentacyclic structure which requires formation of a fifth ring. Furthermore, **8** has an additional carbon atom which corresponds to the high-field methyl doublet observed in the NMR spectrum. The carbon resonance of C(21) at δ 85.1 indicated that it is adjacent to both an oxygen and a nitrogen atom. Insertion of the CH_3CH fragment to link up *N*(4) and the ether oxygen attached to C(17) would provide a new six-membered ring. This proposal is supported by the HMBC data which showed two-bond correlation from C(21) to H(22) and three-bond correlation to H(17). Other HMBC correlations (Fig. 3) are in agreement with the proposed structure. The stereochemistry at carbon-21 is established by the observed NOE interactions (Fig. 4) between H(5) and H(21), and H(3) and the C(21)-methyl, indicating that the configuration of C(21) is *S*. Macrogentine **8** is the second macroline oxindole after isoalstonisine **7** to possess the 7*S* configuration at the spirocentre, as shown by the characteristic C(2) and C(8) shifts as well as by the observed NOE interaction between H(9) and H(6β), H(3β) (calculated H(9)/H(6β) distance 2.77 Å versus H(9)/H(15) distance of 5.49 Å). The structure of macrogentine resembles that of the recently reported oxindole alkaloid macroxine **5**.[13] Macroxine should also possess the *S* configuration at the spirocarbon, from the reported carbon shifts of C(2) and C(8) (δ 177.7 and 138.1, respectively), which are characteristic of the 7*S* series as discussed above, although direct confirmation of this would require additional results from NOE experiments.

Two other new ring-opened macroline oxindoles were also obtained in this study, viz. alstonoxine A **9** and alstonoxine B **10**, in which cleavage has occurred in ring E. Compound **9** showed an M^+ at *m/z* 328 which analysed for $C_{19}H_{24}N_2O_3$, i.e. possessing one carbon less than alstonisine **1**. The IR spectrum indicated the presence of carbonyl (1694 cm^{-1}), NH (3288 cm^{-1}) and hydroxyl (3390 cm^{-1}) functions while the carbon resonances at δ 182.3, 65.8 and 208.4 confirm the presence of lactam, oxymethylene and ketone groups, respectively. The observed C(2) and C(8) resonances, and the H(9)/H(15) NOE interaction, indicated an *R* configuration at the spirocarbon C(7). As in compound **8**, the vinylic H(21) signal of a normal pentacyclic oxindole is missing, the oxymethylene H(17) are observed as a pair of doublet of doublets at δ 3.80 and 4.02, and additional signals due to the C(20) methylene hydrogens are observed at δ 2.72 and 2.79. These diastereotopic methylene hydrogens are clearly distinguishable in **9**, whereas in the previous compound **8**, the signals of these hydrogens were almost coincident. The COSY and HETCOR spectrum revealed the same fragments as in compound **8**, with the exception of the CH_3CH–O fragment. These similarities, and the C(20) to H(18) and C(19) to H(18) correlations shown in the HMBC spectrum confirm the presence of the same 2-oxopropyl side chain linked to ring D at carbon-15 and reveal the *seco*-oxindole **9**. Compound **9** is the sole product formed on acidic hydrolysis (dilute HCl) of alstonisine **1** (Scheme 1).[16]

Alstonoxine B **10**, shows an M^+ at *m/z* 330 ($C_{19}H_{26}N_2O_3$) which is two mass-units more when compared to alstonoxine A **9**. The UV and IR spectrum of these two compounds are very similar. The NMR spectra for both compounds are also generally similar but for a few differences. The most notable difference centres on the signals due to the side chain attached to C(15). The signals due to the acetyl group of compound **9** (δ_H 2.21, s, *Me*C=O; δ_C 208.4, Me*C*=O) have been replaced in **10** by a hydroxyethyl group (δ_H 1.30, d, *Me*CH–OH; δ_C 65.1, Me*C*H–OH). These observations are consistent with structure **10** although the configuration at C(19) remains to be established. It was mentioned previously (vide supra) that in the case of the pentacyclic macroline oxindoles, the H(9) shift is also of diagnostic significance for the assignment of the spirocentre configuration. However, the same criterion cannot be applied to the ring-opened tetracyclic oxindoles such as **9** and **10**. This is because for the ring-opened compounds such



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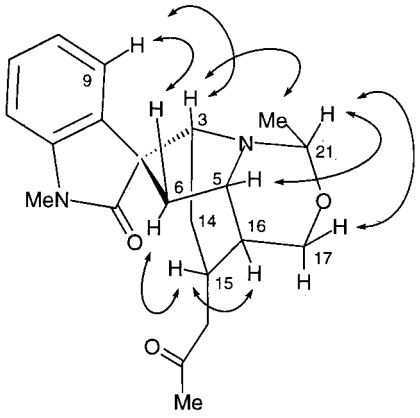
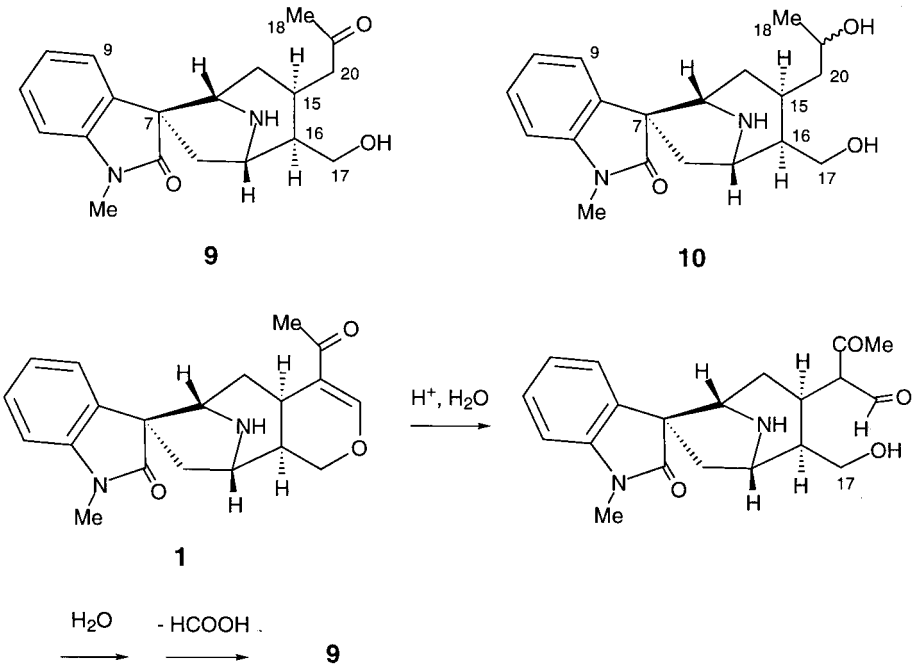


Figure 4. Selected NOE's of **8**.



Scheme 1.

as **9** and **10**, the aromatic H(9) in the 7*R* compounds are no longer placed within the anisotropic influence of the C(20)–C(21) double bond.

Alstofoline **11** showed IR and UV spectra indicating an oxindole. The mass-spectrum showed an M^+ at *m/z* 366, analysing for $C_{21}H_{22}N_2O_4$, differing from alstonisine **1** by replacement of H with CHO. The IR spectrum showed in addition to the characteristic broad carbonyl band at 1706 cm^{-1}, due to lactam and conjugated ketone functions, another carbonyl band at 1662 cm^{-1} which can be ascribed to a formamide function. This assignment is further supported by the ^{13}C NMR spectral data which showed in addition to the usual lactam (δ 179) and ketone (δ 196) resonances, another resonance at δ 160 due to a formamide carbonyl. The ^1H NMR spectrum showed the formamide-H as a singlet at δ 8.1. (The signals at δ_H 8.1/δ_C 160 and that at δ_H 7.6/δ_C 157 are indistinguishable. Assignment of the former pair to the formamide is based on the observed NOE interaction between the formamide-H and H(3)).

The NMR spectral data are in fact very similar to that of alstonisine **1** except for the presence of the signals due to the formyl group and the more pronounced changes in the chemical shifts of H(3) and H(5), indicating that **11** is *N*(4)-formylalstonisine. In addition irradiation of the *N*(1)-methyl signal causes NOE enhancement of H(12) (Fig. 5), furnishing additional proof that the formyl group is on *N*(4). Another clear indication of the presence of a formamide group is shown by the observation that although two separate bands are resolved by centrifugal TLC, equilibration is rapid in solution at room temperature, yielding an equilibrium mixture of two rotamers, with one form predominating. This is clearly seen in both the ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum showed two sets of signals corresponding to the two conformers with a four-fold predominance of the major rotamer. Only for seven of the hydrogens are the signals of the two forms overlapped; for the remaining hydrogens, the signals due to both forms are distinguishable. The same is true of the ^{13}C NMR spectrum, where except for coincidence involving four carbon signals, the

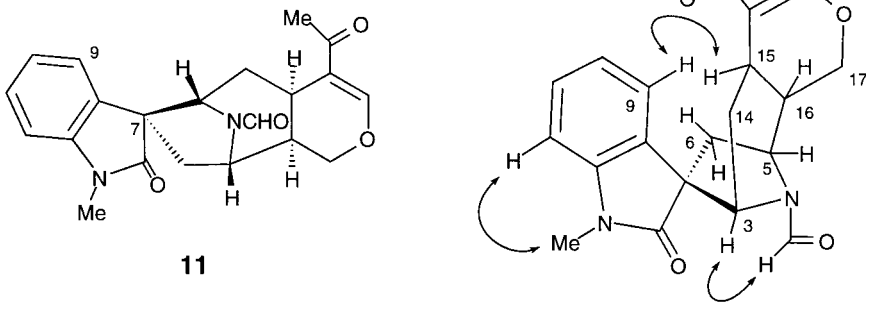
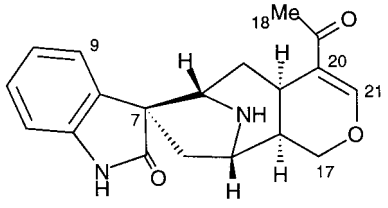


Figure 5. Selected NOE's of **11**.

rest of the carbon signals occur in pairs with one set clearly predominant. The configuration of the spirocyclic C(7) is deduced to be *R* from the diagnostic C(2) and C(8) shifts (δ 180 and 129, respectively) and the observed NOE interaction between H(9) and H(15). Examination of the ^{1}H NMR spectrum showed that among the pairs of signals, the signals corresponding to H(3) and H(5) of the two rotamers are notably distinct. This allows the assignment of the major conformer **11a** as the one in which the carbonyl C=O and H(5) are approximately *syn*-coplanar resulting in the deshielding of H(5), whereas the minor conformer **11b** is the one in which the carbonyl C=O and H(3) are approximately *syn*-coplanar, resulting in the deshielding of H(3). Irradiation of the formamide-H (coincident for both conformers) resulted in NOE enhancement of only H(3) of the major rotamer, and H(5) of the minor rotamer, without any effect on H(14) or H(17), indicating that in compound **11**, the *N*(4)-lone pair is directed away from the lactam carbonyl, while the formyl group is directed towards the lactam carbonyl (Fig. 5). Examination of models indicates that such an arrangement would result in considerable hindrance to free rotation about the formamide C–N bond; free rotation about the C–N bond would result in the lone pair electrons of both the lactam carbonyl oxygen and the formyl oxygen being brought into undue proximity causing severe repulsive interactions.[17] In agreement with this, the major conformer is also the one which has the formyl oxygen further removed from the lactam carbonyl oxygen, compared to the case in the minor conformer.



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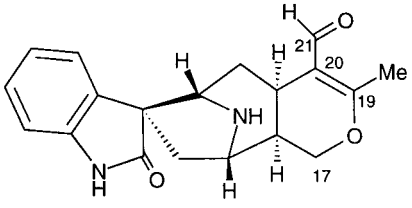

13

Compounds **12** and **13** were obtained as a mixture which proved intractable to further resolution by chromatography as well as fractional crystallisation. This behaviour has been previously documented in the case of the type A and type B macrolines, alstonerine and alstonerinal.[18] The same behaviour is also shown by the oxindoles, alstonal (type A) and alstonisine (type B) which also coeluted in chromatography, but which fortuitously could be readily separated by repeated fractional crystallisation (see Experimental section). The spectral data (MS and NMR) clearly showed that **12** and **13** correspond to the *N*(1)-demethyl derivatives of alstonisine **1** and alstonal **6a**, respectively. As in the case of the previously documented alstonerine and alstonerinal, the type B isomer is the major compound, predominating by a six-fold excess compared to the type A isomer. In common with the above compounds, most of the signals in the ^{1}H and ^{13}C NMR spectra of **12** and **13** are overlapped or nearly so, with the exception of a few, such as the H(21) signal (vinylic-H for **12**, aldehyde-H for **13**) in ^{1}H NMR, and the C(18), C(19), C(20), and C(21) signals in ^{13}C NMR. The tendency of the type A compound to coelute as a minor component with the type B isomer, coupled with the similarity of the NMR spectral data, reinforce the need for caution, since such compounds may easily escape detection.[18]

Experimental

General

UV spectra were recorded on a Shimadzu UV-3101PC spectrophotometer. IR spectra were recorded on a Perkin–Elmer 1600 Series FT-IR spectrophotometer. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. LCMS was obtained on a Perkin–Elmer API 100 instrument. EIMS and HREIMS were determined on a JEOL JMS-AX505H mass spectrometer, courtesy of Dr K. Komiyama, The Kitasato Institute, Japan. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-LA400 spectrometer at 400 and 100 MHz, respectively. Molecular modelling were carried out using the MM2 facility of CS Chem3D Pro (1999 Cambridge Soft Corporation) with the default parameters set.

Collection, extraction and isolation

Plant material was collected in the Genting Highlands area, Selangor, Malaysia and was identified by Dr K. M. Wong, Institute of Biological Sciences, University of Malaya. Herbarium voucher specimens are deposited at the Herbarium of the Department of Chemistry, University of Malaya. Extraction of the ground leaves was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid as has been described in detail elsewhere.[19] The alkaloids were isolated by initial column chromatography on Si gel using $CHCl_3$ with increasing proportions of MeOH followed by rechromatography of the appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal TLC were Et_2O–hexane (2:1), Et_2O, Et_2O–EtOAc (1:1), EtOAc–cyclohexane (3:1), EtOAc and 2% MeOH–EtOAc. The yields (g kg^{-1}) of the alkaloids are as follows: **1** (0.0725), **6a** (0.0312), **7** (0.0014), **8** (0.0015), **9** (0.0039), **10** (0.0040), **11** (0.0039), **12** (0.0083) and **13** (0.0014). Alstonal **6a** (orange prisms) was separated from partially resolved mixtures containing **6a** and **1** by fractional crystallisation from petroleum ether (60–80°C)–acetone.

Isoalstonisine 7, $[\alpha]_D$=+207 ($CHCl_3$, *c* 0.07); IR (dry film) ν_{max} 1704 cm^{-1} (C=O, lactam, ketone); UV (EtOH), λ_{max} nm (log ϵ): 210 (3.93), and 255 (3.80). EIMS, *m/z* (rel. int.): 338 [M$^+$] (100), 295 (7), 256 (27), 231 (24), 219 (49), 193 (27), 179 (71), 160 (44), and 111 (58). HREIMS found, *m/z* 338.1635, calcd for $C_{20}H_{22}N_2O_3$, 338.1630. ^{13}C and ^{1}H

Table 1. ^{13}C NMR spectral data of compounds **1**, **6a**–**13** ($CDCl_3$, 100 MHz)

C	1	6a	7	8	9	10	11a[a]	11b[b]	12	13
2	181.9	181.9	177.5	177.4	182.3	182.4	179.7	179.5	184.2	184.2
3	63.4	63.4	66.9	62.6	63.1	63.5	60.9	56.3	64.0	64.0
5	55.9	55.9	57.0	60.0	61.7	61.7	50.3	55.1	56.4	56.4
6	41.5	41.5	42.3	39.1	40.7	40.5	39.4	38.6	41.9	41.9
7	56.5	56.5	57.3	55.0	57.2	57.4	53.2	53.0	57.3	57.3
8	128.7	128.7	137.6	138.1	129.0	129.2	129.0	129.0	129.6	129.6
9	125.1	125.1	120.9	123.6	125.0	124.3	125.8	125.8	126.1	126.0
10	122.8	122.8	122.3	122.6	123.1	122.8	123.5	123.2	123.4	123.3
11	127.5	127.5	127.8	127.5	128.1	128.1	127.0	126.8	128.0	128.0
12	107.5	107.5	107.7	107.1	108.1	108.2	108.1	108.1	109.4	109.4
13	143.6	143.6	142.4	141.9	144.1	144.2	144.1	144.3	140.8	140.8
14	30.6	30.3	32.7	29.8	33.0	42.3	32.3	31.9	31.0	30.7
15	23.8	23.5	23.9	27.7	26.1	26.8	24.5	24.4	24.2	23.9
16	36.5	36.5	37.8	34.7	41.4	40.7	37.1	37.0	37.1	37.1
17	68.2	67.9	67.8	68.4	65.8	65.2	66.5	66.1	68.6	68.4
18	24.5	16.2	25.8	31.0	30.8	24.7	24.9	25.0	24.9	16.6
19	196.0	170.5	197.1	208.8	208.4	65.1	196.3	196.3	196.6	171.1
20	121.3	117.7	121.9	48.7	47.0	34.0	120.6	120.9	121.8	118.1
21	157.2	189.0	155.9	85.1	–	–	157.5	157.1	157.6	189.5
22	–	–	–	19.8	–	–	–	–	–	–
N(1)Me	25.8	25.8	26.5	26.3	26.2	26.5	26.2	26.4	–	–
N(4)CHO	–	–	–	–	–	–	160.1	158.8	–	–

[a] Major conformer.
[b] Minor conformer.

NMR: see Tables 1 and 2. HMBC: ^2J C(3) to H(14); C(5) to H(6); C(7) to H(6); C(14) to H(15); C(15) to H(14); C(16) to H(17), H(15); C(19) to H(18); C(20) to H(15), H(21). ^3J C(2) to H(6), *N*-Me; C(3) to H(5), H(6); C(5) to H(3); C(7) to H(5), H(9); C(8) to H(6), H(10), H(12); C(9) to H(11); C(10) to H(12); C(11) to H(9); C(12) to H(10); C(13) to H(9), H(11), *N*-Me; C(15) to H(3), H(5), H(17), H(21); C(16) to H(6); C(17) to H(21); C(19) to H(15), H(21); C(20) to H(18); C(21) to H(15), H(17).

Isomerisation of alstonisine 1 to isoalstonisine 7, Alstonisine **1** (24 mg) was refluxed in pyridine for 20 h. The excess pyridine was then removed under reduced pressure and the mixture extracted with $CHCl_3$. The extract was dried (Na_2SO_4) and then chromatographed (SiO_2, centrifugal TLC, Et_2O) to give isoalstonisine **7** (2 mg, 8%) and unreacted alstonisine **1** (20 mg). Extending the reaction time (65 h) resulted in the same ratio of **1**:**7**.

Macrogentine 8, $[\alpha]_D=-21$ ($CHCl_3$, *c* 0.15); IR (dry film) ν_{max} 1704 cm^{-1} (C=O, lactam, ketone); UV (EtOH), λ_{max} nm (log ϵ): 208 (4.44), 258 (3.74), and 284 (3.15). EIMS, *m*/*z* (rel. int.): 354 [M^+] (100), 324 (69), 311 (18), 267 (65), 253 (51), 213 (60), 195 (73), 172 (66), 160 (47), 108 (68), 93 (37), and 43 (59). HREIMS found, *m*/*z* 354.1947, calcd for $C_{21}H_{26}N_2O_3$, 354.1943. ^{13}C and ^1H NMR: see Tables 1 and 2. HMBC: ^2J C(3) to H(14); C(5) to H(6); C(7) to H(6); C(15) to H(14), H(20); C(16) to H(15); C(19) to H(18), H(20); C(20) to H(15); C(21) to H(22); C(22) to H(21). ^3J C(2) to H(6), *N*-Me, C(3) to H(5), H(21); C(5) to H(3), H(17); C(6) to H(3); C(7) to H(5), H(9), H(14); C(8) to H(6), H(10), H(12); C(9) to H(11); C(10) to H(12); C(11)

Table 2. ^1H NMR spectral data of compounds **1**, **7**–**10** ($CDCl_3$, 400 MHz)

Position	1	7	8	9	10
3	3.18 brs	3.15 t (3)	3.52 d (4)	3.25 brs	3.25 brs
5	3.68 brd (7)	3.76 brd (7)	3.74 dd (7, 2)	3.90 brd (8)	3.91 m
6	2.19 brd (13)	2.61 dd (14, 2)	2.40 dd (13, 2)	2.15 dd (13, 2)	2.13 dd (14, 1)
	2.52 dd (13, 7)	2.35 dd (14, 8)	2.26 dd (13, 7)	2.43 dd (13, 8)	2.41 dd (14, 8)
9	8.25 brd (8)	7.15 dd (8, 1)	7.57 dd (8, 1)	7.84 brd (8)	7.52 brd (8)
10	7.30 td (8, 1)	7.00 td (8, 1)	7.00 td (8, 1)	7.20 td (8, 1)	7.10 td (8, 1)
11	7.33 td (8, 1)	7.27 td (8, 1)	7.25 td (8, 1)	7.32 td (8, 1)	7.31 td (8, 1)
12	6.88 brd (8)	6.82 dd (8, 1)	6.79 dd (8, 1)	6.87 brd (8)	6.88 brd (8)
14	1.55 ddd (14, 12, 3)	1.49 ddd (14, 12, 3)	1.88 ddd (13, 11, 4)	1.71 m	1.53 ddd (14, 9, 5)
	2.25 ddd (14, 6, 3)	2.33 ddd (14, 6, 3)	1.74 dd (13, 6)	1.87 ddd (14, 6, 2)	1.85 ddd (14, 9, 3)
15	3.40 dt (12, 6)	4.03 dt (12, 6)	3.44 m	3.05 m	2.72 m
16	1.96 m	2.01 m	1.49 dd (5, 2)	1.71 m	1.77 m
17	4.26 ddd (11, 4, 2)	4.17 ddd (11, 4, 2)	3.84 d (12)	3.80 dd (12, 2)	3.91 m
	4.45 t (11)	4.26 t (11)	4.19 dd (12, 2)	4.02 dd (12, 1)	4.01 dd (11, 1)
18	2.24 s	2.25 s	2.25 s	2.21 s	1.30 d (6)
19	–	–	–	–	3.91 m
20	–	–	2.51 dd (15, 7)	2.72 dd (18, 6)	1.77 m
			2.56 dd (15, 8)	2.79 dd (18, 7)	1.77 m
21	7.62 s	7.54 s	4.90 q (6)	–	–
22	–	–	1.29 d (6)	–	–
N(1)Me	3.20 s	3.26 s	3.22 s	3.20 s	3.20 s

Table 3. ^{1}H NMR spectral data of compounds **11–13** ($CDCl_3$, 400 MHz)

Position	**11a**[a]	**11b**[b]	**12**	**13**
3	3.82 brs	4.49 brs	3.27 brs	3.27 brs
5	4.91 brd (7)	4.31 brd (7)	3.69 brd (7)	3.69 brd (7)
6	2.22 m	2.22 m	2.20 brd (13)	2.19 brd (13)
	2.68 dd (13, 7)	2.77 (13, 7)	2.57 dd (13, 7)	2.56 dd (13, 7)
9	8.28 dd (8, 1)	8.25 dd (8, 1)	8.22 brd (8)	8.22 brd (8)
10	7.39 td (8, 1)	7.39 td (8, 1)	7.25 m	7.25 m
11	7.32 td (8, 1)	7.32 td (8, 1)	7.20 m	7.20 m
12	6.88 dd (8,1)	6.87 dd (8, 1)	6.91 brd (8)	6.91 brd (8)
14	1.57 ddd (14, 12, 2)	1.56 m	1.57 ddd (14, 12, 2)	1.55 ddd (14, 12, 2)
	2.53 ddd (14, 6, 2)	2.42 ddd (14, 6, 2)	2.26 ddd (14, 6, 2)	2.30 ddd (14, 6, 2)
15	3.59 dt (12, 6)	3.59 dt (12, 6)	3.39 dt (12, 6)	3.35 dt (12, 6)
16	2.22 m	2.22 m	1.98 m	1.98 m
17	4.42 ddd (11, 4, 2)	4.27 ddd (11, 4, 2)	4.26 ddd (11, 4, 2)	4.28 ddd (11, 4, 2)
	3.90 t (11)	3.96 t (11)	4.46 t (11)	4.52 t (11)
18	2.26 s	2.26 s	2.24 s	2.24 s
21	7.63 s	7.61 s	7.63 s	9.86 s
N(1)Me	3.18 s	3.17 s	–	–
N(4)CHO	8.10 s	8.10 s	–	–
*N*H	–	–	8.54 s	8.57 s

[a] Major conformer.
[b] Minor conformer.

to H(9); C(12) to H(10); C(13) to H(9), H(11), *N*-Me; C(15) to H(3), H(5), H(17); C(16) to H(6), H(14); C(17) to H(15); C(18) to H(20); C(20) to H(14), H(18); C(21) to H(17).

Alstonoxine A 9, $[\alpha]_D=-34$ ($CHCl_3$, *c* 0.19); IR (dry film) ν_{max} 3390 cm^{-1} (OH), 3288 cm^{-1} (NH), and 1694 cm^{-1} (C=O, lactam, ketone); UV (EtOH), λ_{max} nm (log ϵ): 216 (3.89), 255 (3.70), and 285 (3.18). EIMS, *m/z* (rel. int.): 328 [M^+] (93), 310 (30), 298 (14), 267 (7), 253 (6), 241 (5), 169 (58), 160 (37), 126 (34), 112 (59), 108 (25), 80 (100), and 43 (20). HREIMS found, *m/z* 328.1778, calcd for $C_{19}H_{24}N_2O_3$, 328.1787. ^{13}C and ^{1}H NMR: see Tables 1 and 2. HMBC: ^2J C(3) to H(14); C(5) to H(6); C(7) to H(6); C(9) to H(10); C(14) to H(15); C(15) to H(14), H(16), H(20); C(16) to H(5), H(15); C(19) to H(18), H(20); C(20) to H(15). ^3J C(2) to H(6), *N*-Me; C(3) to H(5), H(6); C(5) to H(3), H(17); C(6) to H(3); C(7) to H(5), H(9), H(14); C(8) to H(6), H(10), H(12); C(9) to H(11); C(10) to H(12); C(11) to H(9); C(12) to H(10); C(13) to H(9), H(11), *N*-Me; C(14) to H(20); C(15) to H(3), H(5), H(17); C(16) to H(6), H(14), H(20); C(17) to H(15); C(19) to H(15); C(20) to H(16), H(14), H(18). NOE H(9)/H(15), H(10); H(5)/H(6β), H(16); H(15)/H(6α), H(9), H(14), H(16).

Acidic hydrolysis of alstonisine 1 to alstonoxine A 9, Alstonisine **1** (24 mg) was refluxed in dilute HCl (5 ml, 2 N) for 17 h. The mixture was then basified with NaOH and extracted with $CHCl_3$. The extract was dried (Na_2SO_4) and chromatographed (SiO_2, centrifugal TLC, EtOAc) to give alstonoxine A **9** (14 mg, 60%).

Alstonoxine B 10, $[\alpha]_D=-12$ ($CHCl_3$, *c* 0.41); IR (dry film) ν_{max} 3370 cm^{-1} (OH), 3288 cm^{-1} (NH), and 1693 cm^{-1} (lactam); UV (EtOH), λ_{max} nm (log ϵ): 213 (4.15), 255 (3.65), and 286 (3.04). EIMS, *m/z* (rel. int.): 330 [M^+] (100), 300 (24), 171 (33), 160 (31), 140 (28), 112 (8), 96 (71), 82 (47), 57 (35), 44 (82), and 43 (27). HREIMS found, *m/z* 330.1934, calcd for $C_{19}H_{26}N_2O_3$, 330.1943. ^{13}C and ^{1}H NMR: see Tables 1 and 2. HMBC: ^2J C(3) to H(14); C(5) to H(6); C(7) to H(6); C(14) to H(15); C(15) to H(14), H(16), H(20); C(19) to H(18). ^3J C(2) to H(6), *N*-Me; C(3) to H(5), H(6); C(5) to H(17); C(6) to H(16); C(7) to H(5), H(9), H(14); C(8) to H(6), H(10), H(12), C(9) to H(11); C(10) to H(12); C(11) to H(9); C(12) to H(10); C(13) to H(9), H(11), *N*-Me; C(15) to H(5), H(17), H(19); C(16) to H(6), H(14), H(20); C(17) to H(15); C(19) to H(15); C(20) to H(14). NOE H(9)/H(15), H(10); H(6β)/H(5); H(6β)/H(15), H(15)/H(6α), H(9), H(14), H(16).

Alstofoline 11, $[\alpha]_D=+39$ ($CHCl_3$, *c* 0.21); IR (dry film) ν_{max} 1706 cm^{-1} (C=O, lactam, ketone), and 1662 cm^{-1} (amide); UV (EtOH), λ_{max} nm (log ϵ): 214 (4.02), 246 (4.01), 254 (4.05), and 290 (3.13). EIMS, *m/z* (rel. int.): 366 [M^+] (53), 338 (32), 317 (17), 286 (12), 267 (22), 236 (22), 212 (17), 179 (42), 160 (88), 151 (38), 117 (39), and 100 (100). HREIMS found, *m/z* 366.1573, calcd for $C_{21}H_{22}N_2O_4$, 366.1580. ^{13}C and ^{1}H NMR: see Tables 1 and 3.

***N*(1)-demethylalsonisine 12** and ***N*(1)-demethylalstonal 13** were obtained as a mixture. API-LCMS, MH^+, *m/z* 325. EIMS, *m/z* (rel. int.): 324 [M^+] (100), 179 (67), 178 (31), 151 (23), 136 (27), 111 (23), 85 (33), 83 (51), 57 (45), and 43 (63). HREIMS found, *m/z* 324.1477, calcd for $C_{19}H_{20}N_2O_3$, 324.1474. ^{13}C and ^{1}H NMR: see Tables 1 and 3.

Acknowledgements

We would like to thank the University of Malaya and IRPA for financial support, Dr K. M. Wong, University of Malaya for identification of plant material, and Dr K. Komiyama, The Kitasato Institute, Tokyo, Japan for mass spectra.

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