JOURNAL OF NATURAL PRODUCTS

Article

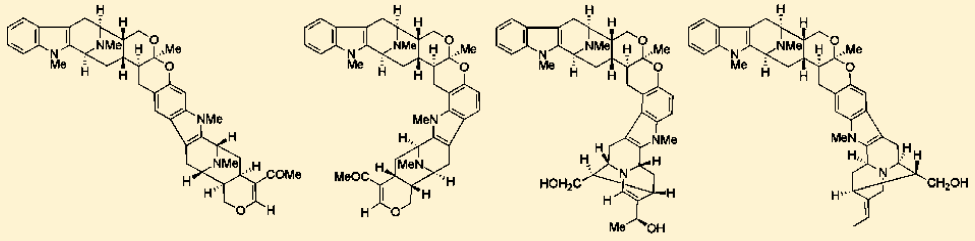
pubs.acs.org/jnp

Lumutinines A–D, Linearly Fused Macroline–Macroline and Macroline–Sarpagine Bisindoles from *Alstonia macrophylla*

Siew-Huah Lim, Shin-Jowl Tan, Yun-Yee Low, and Toh-Seok Kam*

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

Supporting Information



1 2 3 4

ABSTRACT: Four new linearly fused bisindole alkaloids, lumutinines A–D (**1**–**4**), were isolated from the stem-bark extract of *Alstonia macrophylla*. Lumutinines A (**1**) and B (**2**) represent the first examples of linear, ring A/F-fused macroline–macroline-type bisindoles, while lumutinines C (**3**) and D (**4**) were constituted from the union of macroline and sarpagine moieties. A reinvestigation of the stereochemical assignment of alstoumerine (**8**) by NMR and X-ray diffraction analyses indicated that the configuration at C-16 and C-19 required revision.

The genus *Alstonia* (Apocynaceae), which is widely distributed in Southeast Asia,[1−3] is rich in alkaloids,[4−13] and the Malaysian representatives in particular have proven to be fertile sources of novel alkaloids with unusual or intriguing carbon skeletons and interesting biological activity.[14−30] In continuation of our studies on the Malaysian members of this genus, we report the structures of four new bisindole alkaloids, viz., lumutinines A–D (**1**–**4**), from *A. macrophylla* Wall.

Lumutinine A (**1**) was obtained as a light yellowish oil with $[\alpha]^{25}_{D}$ +160 (*c* 0.4, $CHCl_3$). The IR spectrum showed bands at 1617 and 1651 cm^{-1} due to the presence of an α,β-unsaturated carbonyl group, while the UV spectrum showed absorption maxima at 210, 233, 254 (sh), and 283 nm, indicating the presence of indole chromophores and an α,β-unsaturated carbonyl moiety. The ESIMS of **1** showed a pseudomolecular ion $[M + H]^+$ at *m*/*z* 673, which analyzed for $C_{42}H_{48}N_4O_4$ + H. The ^{13}C NMR spectrum (Table 1) showed a total of 42 resonances, comprising six methyl, seven methylene, 16 methine, and 13 quaternary carbon atoms, in agreement with the molecular formula. The observed quaternary carbon resonance at δ 196.4 and the olefinic carbon signals at δ 121.4 and 158.1 are consistent with the presence of the α,β-unsaturated carbonyl group, while the unusually low-field resonance of the β-carbon at δ 158.1 indicated oxygen substitution.

The ^1H NMR spectrum (Table 2) showed the presence of four aromatic hydrogens (δ 7.11–7.50) associated with an unsubstituted indole moiety, two aromatic singlets (δ 6.76, 6.98) associated with another indole moiety substituted at C-10′ and C-11′, a vinylic singlet at δ 7.58 associated with a trisubstituted double bond, and a total of six methyl singlets, corresponding to two N1-Me (δ 3.54, 3.55), two N4-Me (δ 2.27, 2.29), one acetyl methyl (δ 2.15, 18′-Me), and a methyl attached to a quaternary carbon (δ 1.38, 18-Me). Since only six aromatic hydrogens were observed and both indolic nitrogens are substituted, it can be inferred from the observation of the two H-9′ and H-12′ aromatic singlets that the bisindole is branched from C-10′ and C-11′ of one monomeric unit. Furthermore, the observed NOE between the aromatic singlet at δ 6.76 and the N1-Me′ singlet at δ 3.54 allowed this aromatic singlet and the one at δ 6.98 to be assigned to H-12′ and H-9′, respectively. The observation of the low-field vinylic singlet at δ 7.58 (H-21′) with the associated acetyl methyl singlet at δ 2.15 indicated this 10′,11′-branched monomer to be a type-B macroline.[14] Examination of the ^1H and ^{13}C NMR data with the help of 2-D COSY and HSQC experiments indicated that this type-B macroline corresponded to a 10,11-substituted alstonerine,[9,19,31] with C-11′ carrying some form of oxygenation as deduced from the observed ^{13}C NMR shift of this carbon at δ 150.3 and the observed correlation from H-9′ to this carbon (C-11′) in the HMBC spectrum.

The other unit of the bisindole, after discounting the signals due to the substituted alstonerine half, corresponded to that of another macroline derivative with an unsubstituted indole moiety. The C-17 oxymethylene hydrogens were observed as a doublet of doublets at δ 3.69 and a triplet at δ 4.67, while the 18-methyl singlet was observed at δ 1.38. The C-19 resonance at δ 99.5 indicated attachment to two oxygen atoms, while the methine H-20 was seen as a multiplet at δ 1.93. These features corresponded to a pentacyclic macroline alkaloid with a saturated ring E (for example, macrocarpines A–C and talcarpine[21]), which was also supported by the observed three-bond correlation

Received: September 12, 2011
Published: December 7, 2011

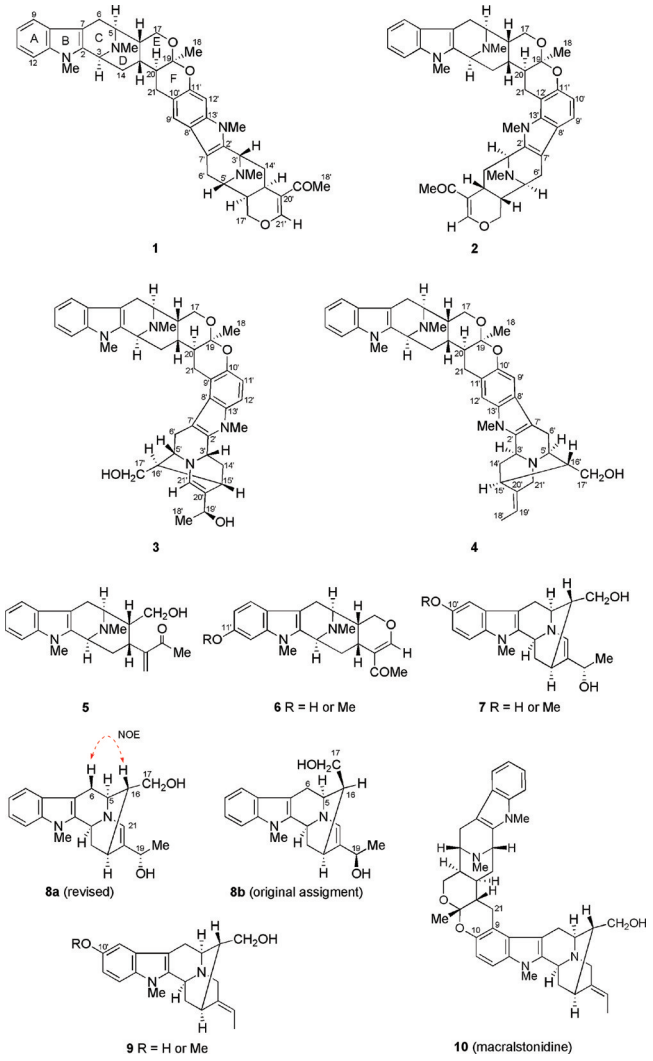
Table 1. ^{13}C NMR Data (δ) for 1−4 and 10 (100 MHz, $CDCl_3$)[a]

C	1	2	3	4	10
2	133.7	136.8[d]	133.4	133.5	133.3
3	53.8	51.3	54.0	53.8	53.9
5	55.2	54.1	55.2	55.2	55.0
6	22.8	21.6	22.8	22.7	22.6
7	106.8	104.7	107.0	106.8	106.8
8	126.5	127.5	126.4	126.5	126.3
9	117.9	118.1	118.1	117.9	117.8
10	118.8	118.9	119.0	118.9	118.7
11	120.7	120.8	120.9	120.8	120.6
12	108.9	108.9	108.9	108.9	108.8
13	137.0	137.1[d]	136.9	137.0	136.8
14	27.1	31.0	26.7	26.9	26.7
15	30.3	28.3	30.4	30.2	30.3
16	43.5	37.3	43.5	43.5	43.3
17	62.5	65.0	62.3	62.4	62.2
18	25.7	25.6	25.4	25.6	25.4
19	99.5	99.4	99.0	99.3	98.8
20	37.5	38.0	37.2	37.4	36.9
21	28.9	24.2	26.8	29.5	26.5
N_1Me	29.2[b]	29.6	29.3	29.3	29.3[e]
N_4Me	41.78[c]	40.2	41.8	41.8	41.6
2′	132.3	132.1	139.3	140.0	139.4
3′	53.9	53.9	48.7	49.7	49.4
5′	54.8	54.7	56.4	54.4	54.2
6′	23.1	22.8	27.9	27.1	29.3
7′	104.9	106.3	102.1	102.8	103.1
8′	112.6	121.8	125.0	126.8	124.9
9′	116.5	116.7	111.4	105.1	111.2
10′	121.9	110.6	147.7	148.3	147.4
11′	150.3	149.6	112.6	114.9	112.3
12′	96.9	103.2	107.7	107.5	107.5
13′	137.2	135.7	132.4	133.5	132.3
14′	32.5	32.5	38.6	32.9	32.8
15′	23.0	23.0	29.2	27.5	27.4
16′	38.7	38.6	45.0	44.4	44.1
17′	68.0	68.0	64.5	64.8	64.7
18′	25.2	25.3	22.5	12.9	12.8
19′	196.4	195.9	67.3	117.0	116.8
20′	121.4	121.3	149.3	135.7	135.5
21′	158.1	157.9	136.1	56.3	55.9
N_1Me'	29.6[b]	32.5	29.4	29.6	29.0[e]
N_4Me'	41.82[c]	41.9			

[a]Assignments are based on COSY, HSQC, and HMBC. [b−e]Assignments are interchangeable.

from the 18-methyl hydrogens to C-20. The observed NOE between 18-Me and H-20 requires a *cis*-orientation (Figure 1). In addition, an NOE was also observed between 18-Me and H-21α, which in turn facilitated the assignment of the orientation of both 18-Me and H-20 as α. Examination of models showed that, had the orientation of 18-Me and H-20 been β, NOE would have been impossible between 18-Me and both the C-21 hydrogens. This mode of ring E/F fusion is also seen in the spirocyclic macrodasines[29] and macralstonidine (**10**, *vide infra*).

Of the two oxygens linked to C-19, one was also attached to C-17 (C-19−O−C-17 connection) from the observed three-bond correlation from H-17 to C-19 in the HMBC spectrum, while the other was attached to the aromatic C-11′, from its observed downfield shift at δ 150.3, which had been previously



noted (*vide supra*). Connection from the ring E C-20 of the upper macroline unit to C-10′ of the lower half was mediated via a methylene bridge (C-21), as shown by the observed H-21 to C-9′ and C-11′ three-bond correlations in the HMBC spectrum, while that from C-19 to C-11′ was via an oxygen atom. These observations revealed the mode of union of the two macroline halves, which from a biogenetic viewpoint (*vide infra*) can be considered as comprising a macroline (**5**) and an 11′-hydroxy- or 11′-methoxyalstonerine (alstophylline) (**6**). The structure is entirely consistent with the full HMBC as well as the NOE data, in particular the observed NOE between H-9′ and H-21 (Figure 1).

The second bisindole, lumutinine B (**2**), was obtained as a light yellowish oil with $[\alpha]^{25}_D$ −11 (*c* 0.5, $CHCl_3$). As in the case of **1** the IR spectrum showed similar bands at 1616 and 1651 cm^{-1}, while the UV spectrum (210, 232, 255, and 285 nm) was also similar to that of **1**. The ESIMS of **2** showed a pseudomolecular ion $[M + H]^+$ at *m/z* 673, which analyzed for $C_{42}H_{48}N_4O_4$ + H, indicating that **1** and **2** are isomers. Examination of the ^{1}H and ^{13}C NMR data (Tables 2 and 1) showed the presence of similar functionalities to those in **1**, such as an unsubstituted indole moiety (δ 7.09−7.49), another indole moiety substituted at C-11′ and C-12′ from the presence of a pair of AB doublets at δ 6.66 and 7.16, a vinylic singlet at δ 7.55 associated with a trisubstituted double bond, an α,β-unsaturated carbonyl moiety (δ_C 195.9, 121.3, and 157.9; δ_H 2.12), and six methyl singlets, corresponding to two

dx.doi.org/10.1021/np200730j | J. Nat. Prod. 2011, 74, 2556−2562

Table 2. ^{1}H NMR Data (δ) for 1−4 and 10 (400 MHz, $CDCl_3$)[a]

H	1	2	3	4	10
3	3.90 br s	3.93 m	3.74 m	3.70 m	3.75 m
5	3.00 d (7)	3.37 m	2.99 m	2.97 m	3.00 d (7)
6β	2.48 m	2.35 m	2.45 d (16)	2.43 d (17)	2.45 br d (16) (*b*)
6α	3.28 m	3.12 dd (16, 5)	3.28 m	3.25 m	3.27 dd (16, 7) (*a*)
9	7.50 d (7.5)	7.49 d (7.5)	7.50 d (7.5)	7.49 d (7.5)	7.51 d (7.5)
10	7.11 t (7.5)	7.09 td (7.5, 1)	7.10 t (7.5)	7.10 t (7.5)	7.11 td (7.5, 1)
11	7.18 t (7.5)	7.17 td (7.5, 1)	7.17 t (7.5)	7.18 t (7.5)	7.18 td (7.5, 1)
12	7.28 d (7.5)	7.28 d (7.5)	7.25 d (7.5)	7.27 d (7.5)	7.27 d (7.5)
14β	1.25 m	1.78 m	1.18 m	1.10 d (13)	1.21 m (*b*)
14α	2.34 m	2.52 m	2.35 td (13, 3)	2.28 m	2.33 td (13, 4) (*a*)
15	1.87 m	1.78 m	1.87 m	1.84 m	1.86 m
16	2.00 m	1.75 m	2.00 m	1.99 m	2.01 m
17β	3.69 dd (12, 4)	3.75 m	3.67 dd (11.5, 4)	3.66 m	3.68 dd (11.5, 4) (*b*)
17α	4.67 t (12)	4.22 dd (11, 3)	4.62 t (11.5)	4.63 t (11.5)	4.62 t (11.5) (*a*)
18	1.38 s	1.54 s	1.35 s	1.39 s	1.37 s
20	1.93 m	2.68 m	1.97 m	1.93 m	1.96 m
21β	2.43 m	3.33 m (α)	2.75 m	2.48 m	2.77 br d (17.5) (*b*)
21α	3.24 m	3.54 m (β)	3.25 m	3.25 m	3.23 m (*a*)
N_1Me	3.55 s	3.65 s	3.40 s	3.47 s	3.45 s
N_4Me	2.29 s	2.39 s	2.26 s	2.24 s	2.28 s
3′	3.83 br s	3.81 m	3.82 m	4.16 m	4.16 br d (10)
5′	3.07 d (6)	3.06 d (7)	2.87 m	2.76 m	2.64 d (5)
6′α	2.33 m	2.41 m (β)	2.71 m	2.57 br d (15) (β)	2.67 br d (15) (*b*)
6′β	3.20 m	3.22 dd (16.5, 7) (α)	3.22 m	3.01 m (α)	3.17 dd (15, 5) (*a*)
9′	6.98 s	7.16 d (8)		6.91 s	
10′		6.66 d (8)			
11′			6.71 d (9)		6.72 d (9)
12′	6.76 s		7.01 d (9)	6.85 s	7.04 d (9)
14′α	1.84 m	1.79 m (β)	1.66 m	1.65 m (*b*)	1.74 m (*b*)
14′β	2.15 m	2.15 m (α)	1.89 m	2.05 m (*a*)	2.10 m (*a*)
15′	2.76 m	2.66 m	2.82 m	2.82 m	2.87 m
16′	1.93 m	1.88 m	1.55 m	1.82 m	1.79 m
17′α	4.19 dd (11.5, 3)	4.16 dd (11, 3) (β)	3.42 m (*b*)	3.48 m (*b*)	3.53 m
17′β	4.44 t (11.5)	4.43 t (11) (α)	3.61 dd (12, 3) (*a*)	3.53 m (*a*)	3.53 m
18′	2.15 s	2.12 s	1.34 d (6)	1.63 d (6.5)	1.65 d (6.8)
19′			4.46 q (6)	5.40 q (6.5)	5.41 q (6.8)
21′	7.58 s	7.55 s	6.44 s	3.59 m	3.53 m
				3.59 m	3.60 m
N_1Me'	3.54 s	3.86 s	3.48 s	3.56 s	3.57 s
N_4Me'	2.27 s	2.29 s			

[a]Assignments are based on COSY, HSQC, and HMBC.

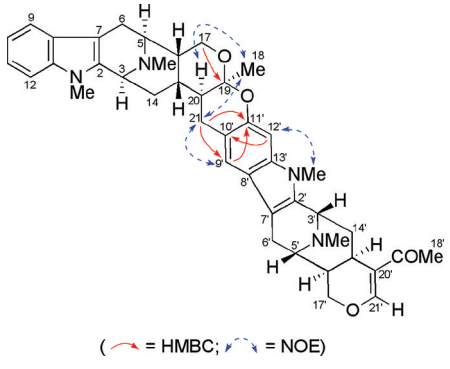


Figure 1. Selected HMBCs and NOEs of **1**.

N1-Me, two N4-Me, one acetyl methyl, and a methyl attached to a quaternary carbon (18-Me). Thus, except for the aromatic AB doublets in place of the two aromatic singlets and, overall, small differences in the chemical shifts of the other signals, the NMR data bear a close resemblance to those of **1**, indicating the presence of the same two constituent units.

The HMBC and NOE data, however, showed significant differences, indicating a different mode of union of the two halves (Figure 2). In the case of **2**, a three-bond correlation from H-10′ to the quaternary C-12′ was observed in the HMBC spectrum. The observed NOE from the aromatic doublet at δ 7.16 to both the H-6′ signals allowed this doublet to be assigned to H-9′ and the other doublet at δ 6.66 to H-10′. This was in contrast to compound **1**, where a three-bond correlation was observed from H-12′ to the quaternary C-10′. In addition, in **2**, three-bond correlations were observed from H-21 and H-9′ to C-11′ (δ 149.6, oxygenated) and C-13′, indicating C-12′ to be a branching point and C-11′ to be the site of oxygen substitution, whereas in **1**, three-bond correlations were observed from H-21 to C-11′ (oxygenated) and C-9′, indicating C-10′ to be the branch-

dx.doi.org/10.1021/np200730j | *J. Nat. Prod.* 2011, 74, 2556−2562

ing point. These observations, therefore, indicated that in **2** the bisindole is branched at C-11′ and C-12′. The branching from C-11′ is mediated by a ketalic oxygen atom, while that from C-12′ is mediated by a methylene (C-21). This difference in the mode of attachment of the two monomeric units was further supported by the NOEs observed for N1-Me′/H-21 and N1-Me′/H-14 (Figure 2). Bisindoles **1** and **2** are, therefore,

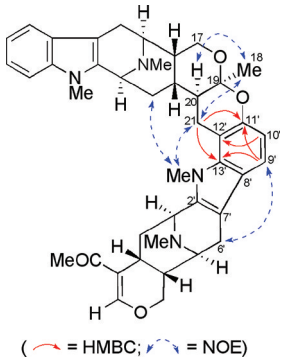


Figure 2. Selected HMBCs and NOEs of **2**.

regioisomers constituted from the union of similar macroline moieties, but differing in the mode of union of these moieties. They also represent the first examples of linear A/F-fused macroline−macroline-type bisindoles.[4,7]

Lumutinine C (**3**) was obtained as a light yellowish oil with $[\alpha]^{25}_{D}$ +84 (*c* 0.32, $CHCl_3$). The IR spectrum showed an absorption band at 3360 cm^{-1} due to the presence of an OH group, while the UV spectrum showed absorption maxima at 208, 228, and 284 nm, consistent with an indole chromophore. The ESIMS of **1** showed a pseudomolecular ion $[M + H]^+$ at *m*/*z* 661, which analyzed for $C_{41}H_{48}N_4O_4$ + H. The ^{13}C NMR spectrum (Table 1) showed a total of 41 resonances, comprising five methyl, seven methylene, 17 methine, and 12 quaternary carbon atoms, in agreement with the molecular formula. Of these, two were oxymethylenes (*δ* 62.3, C-17; 64.5, C-17′), one an oxymethine (*δ* 67.3, C-19′), and another, a quaternary carbon linked to two oxygen atoms (*δ* 99.0, C-19). The olefinic carbon signals at *δ* 136.1 and 149.3 are consistent with the presence of trisubstituted double bond, and the resonance at *δ* 136.1 is consistent with the presence of an N-substituted sp^2 methine (C-21′) with the corresponding hydrogen shift observed as a downfield singlet at *δ* 6.44.

The ^1H NMR spectrum (Table 2) showed the presence of four aromatic hydrogens (*δ* 7.10−7.50) associated with an unsubstituted indole moiety, a pair of aromatic doublets (*δ* 6.71, 7.01) associated with another indole moiety substituted at C-9′ and C-10′, a vinylic singlet at *δ* 6.44 associated with a trisubstituted double bond, and four methyl singlets, corresponding to two N1-Me, one N4-Me, and a methyl attached to a quaternary carbon (18-Me). In addition, a hydroxyethyl side chain was present from the characteristic signals at *δ* 1.34 (d, 3H) and 4.46 (q, 1H). The observed NOE (Figure 3) between the aromatic doublet at *δ* 7.01 and the N1-Me′ signal at *δ* 3.48 permitted the assignment of this signal to H-12′ and the other aromatic doublet at *δ* 6.71 to H-11′. This pattern of aromatic substitution, although similar to that in compound **2**, in that the aromatic ring is vicinally substituted, differs from **2** from the viewpoint of the actual site of aromatic substitution. In **2**, the bisindole is branched at C-11′ and C-12′, with C-11′ (*δ* 149.6) being the site of oxygenation, whereas in **3**, the bisindole is branched at C-9′ and C-10′, with C-10′ (*δ* 147.7) being the site of oxygenation. Examination of the NMR data showed that the monomeric unit corresponding to the upper half and incorporating the unsubstituted indole moiety corresponded to the same macroline-derived moiety present in the previous two compounds. In the case of **3**, therefore, the bisindole is branched at C-9′ and C-10′ of the lower monomeric unit, with C-9′ connected to the upper ring E via a methylene bridge (C-21), and C-10′ connected via an oxygen atom to C-19. This conclusion was supported by the HMBC data (Figure 3), in

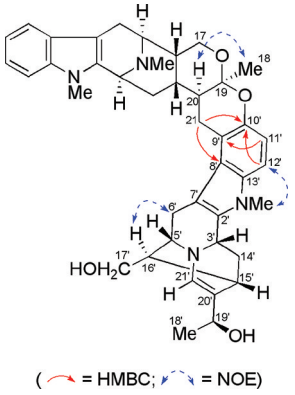


Figure 3. Selected HMBCs and NOEs of **3**.

particular, the observed three-bond correlations from H-21 to C-8′ and to the oxygenated C-10′, from H-11′ to C-9′, and from H-12′ to C-10′.

After discounting the upper macroline-derived half, the monomeric unit corresponding to the lower half was deduced from the NMR data to comprise an alkaloid of the sarpagine type, specifically, a 10-hydroxy- or 10-methoxyalstoumerine (**7**). Comparison of the NMR data with that reported for alstoumerine (**8b**)[32] showed a general agreement for the nonindole portion of the molecule, providing support for such a conclusion. Despite this, some inconsistencies were noted regarding the earlier structure elucidation of alstoumerine (**8b**).

Alstoumerine was first reported from *A. macrophylla* collected in Sri Lanka.[32] The structure was deduced on the basis of NMR data and assigned the structure shown in **8b**. The configuration of the hydroxy-substituted C-19 was determined using Horeau's procedure[33] and was assigned as 19*R*, while the configuration of C-16 was assigned as 16*S* with the hydroxymethyl group pointing toward the indole moiety and H-16 pointing away from the indole moiety. This was despite the observation of the resonance due to H-16 at *δ* 1.63 and those for the C-17 oxymethylene hydrogens at *δ* 3.46 and 3.64. The resonances of H-16 and H-17 are of diagnostic significance for the determination of C-16 configuration in the sarpagine-type alkaloids.[19,23,34,35] The observed resonance for H-16 upfield at *δ* 1.63 is indicative of shielding due to it being located within the shielding zone of the aromatic moiety, which in turn requires H-16 to be oriented toward the indole moiety with the hydroxymethyl group directed away from the indole unit. The original assignment of the C-16 configuration of alstoumerine (**8b**), therefore, requires amendment to 16*R* (**8a**). In the case of lumutinine C (**3**), the resonance due to H-16′ was observed at *δ* 1.55, while the resonances due to the C-17′ oxymethylene hydrogens were seen at *δ* 3.42 and 3.61. These values were similar to those in alstoumerine and require H-16′ to be directed toward the indole moiety (16*R*).

dx.doi.org/10.1021/np200730j | *J. Nat. Prod.* 2011, 74, 2556−2562

Since we were in possession of authentic alstoumerine from our past and ongoing work in alkaloid chemistry, we carried out a rigorous configurational assignment. In addition to the chemical shift considerations mentioned above, the 16*R* configuration of alstoumerine (**8a**) was further confirmed by NOE experiments, which showed a strong NOE between H-16 and H-6*β*, requiring H-16 to be directed toward the indole moiety and hence proximate to H-6*β*. Reinvestigation of the C-19 configuration was also carried out, by repeating the determination using Horeau's procedure,[33,36] which in our hands (see Experimental Section) gave the configuration of C-19 in alstoumerine as 19*S* (**8a**), and not 19*R* (**8b**) as originally reported.[32] In view of the two major discrepancies noted, we carried out an X-ray diffraction analysis (Figure 4), which confirmed the structure and

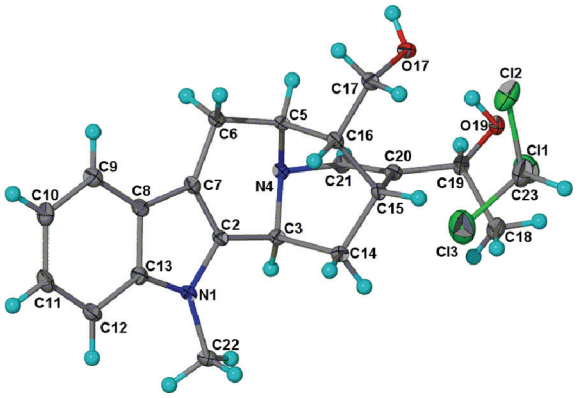


Figure 4. X-ray structure of **8a**.

absolute configuration of alstoumerine (**8a**). We also include the ^{13}C NMR data for alstoumerine (see Experimental Section) in view of revisions required for several of the original assignments.

With the structure of alstoumerine (**8a**) unequivocally established, we can, therefore, conclude that the lower sarpagine half in the bisindole corresponded to that of a 10-hydroxy(or -methoxy)alstoumerine (**7**), and the structure of lumutinine C is as shown in **3**.

Lumutinine D (**4**) was isolated as a light yellowish oil with $[\alpha]^{25}_{D}$ +209 (*c* 0.4, $CHCl_3$). The IR spectrum showed an OH absorption band at 3370 cm^{-1}, and the UV spectrum indicated the presence of an indole chromophore (209, 231, 290 nm). The ESIMS of **4** showed a pseudomolecular ion $[M + H]^+$ at *m*/*z* 645, which analyzed for $C_{41}H_{48}N_4O_3$ + H. The ^{13}C NMR spectrum (Table 1) showed a total of 41 resonances (five methyl, eight methylene, 16 methine, and 12 quaternary carbon atoms). The ^{1}H NMR spectrum (Table 2) showed signals associated with the same macroline-derived upper monomeric unit common to bisindoles **1**–**3**, such as those due to the four aromatic hydrogens of an unsubstituted indole moiety (*δ* 7.10–7.49), three methyl singlets corresponding to N1-Me, N4-Me, and 18-Me (attached to a quaternary carbon), an oxymethylene due to the C-17 hydrogens, and another methylene due to the hydrogens of the C-21 methylene bridge. The remaining discernible signals included the two aromatic singlets (*δ* 6.85, 6.91) associated with another indole moiety substituted at C-10′ and C-11′, a methyl singlet corresponding to N1-Me′, a methyl doublet (*δ* 1.63) and the associated vinylic quartet (*δ* 5.40) due to an ethylidene side chain (H-19′/H-21′ and H-18′/H-15′ NOEs (Figure 5) indicated that the geometry of the 19′,20′-double bond is *E*), and an oxymethylene (δ_C 64.8, δ_H 3.48, 3.53) associated with a hydroxymethyl group. These features suggested

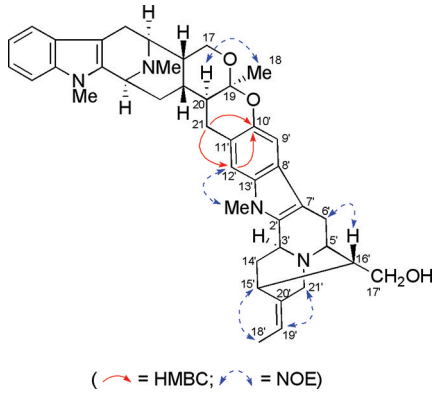


Figure 5. Selected HMBCs and NOEs of **4**.

the presence of another sarpagine unit. Examination of the NMR data and comparison with the literature revealed this lower unit to be a 10-hydroxy(or 10-methoxy)affinisine (**9**).[19,23] The observed shift of H-16′ at *δ* 1.82 and the observed H-16′/H-6′ NOE are consistent with the assigned 16*R* configuration.

The mode of union of the two units can be inferred from the NOE and HMBC data (Figure 5). As in the previous compounds, the observed NOE between the aromatic singlet at *δ* 6.85 and the N1-Me′ singlet at *δ* 3.56 allowed this aromatic singlet to be assigned to H-12′ and the other at *δ* 6.91 to be assigned to H-9′. The three-bond correlation from H-12′ to the oxygenated carbon at *δ* 148.3 in the HMBC spectrum (Figure 5) facilitated the assignment of this quaternary aromatic signal to C-10′. The three-bond correlation from the C-21 methylene bridge hydrogens to C-10′ and C-12′ indicated direct attachment of C-21 to C-11′. These observations revealed the bridging of the affinisine moiety at C-11′ to C-21 and at C-10′, via an oxygen atom, to C-19. It transpires that the structure of lumutinine D (**4**) deduced from the NMR data corresponded to a regioisomer of the known bisindole macralstonidine (**10**),[37,38] differing from **6** in the mode of union of the same constituent monomeric moieties, i.e., C-11′ to C-21, C-10′ to C-19–O in lumutinine D (**4**), cf., C-9′ to C-21, C-10′ to C-19–O in macralstonidine (**10**). Since only partial low-field ^{1}H NMR data of macralstonidine (**10**) were previously available,[37] and the ^{13}C NMR data[38] require revision for some of the original assignments, we include the full ^{13}C and ^{1}H NMR data for **10** in Tables 1 and 2.

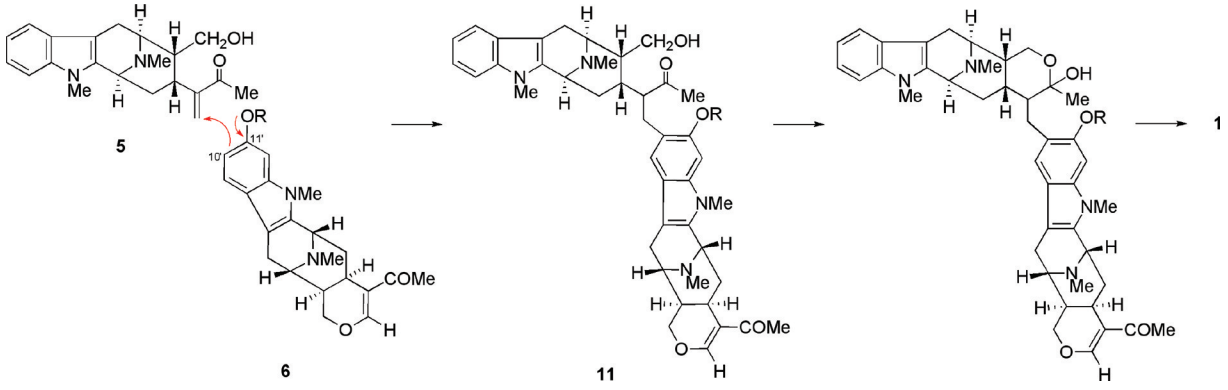
A plausible origin of these bisindoles (illustrated for lumutinine A (**1**), Scheme 1)[14] derives from Michael addition of the electron-rich C-10′ of 11′-hydroxyalstonerine or alstophylline (**6**) onto macroline (**5**) to give the hydroxyketone **11**, which on subsequent ring closure via hemiketal formation, followed by ketalization, yields lumutinine A (**1**). The formation of lumutinine B from attack of C-12′ of **6**, lumutinine C from attack of C-9′ of **7**, and lumutinine D from attack of C-11′ of **9** follows a similar pathway. While the linear, ring A/F-fused macroline–sarpagine-type bisindoles similar to **3** and **4** are known, this is the first report of linear, ring A/F-fused macroline–macroline-type bisindoles (**1** and **2**).[4,7]

EXPERIMENTAL SECTION

General Experimental Procedures. 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 RX1 FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on JEOL JNM-LA 400 and JNM-ECA 400 spectrometers at 400 and 100 MHz, respectively. ESIMS and

dx.doi.org/10.1021/np200730j | J. Nat. Prod. 2011, 74, 2556–2562

Scheme 1. Possible Biogenetic Pathway to 1



HRESIMS were obtained on an Agilent 6530 Q-TOF mass spectrometer. X-ray diffraction analyses were 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 structures were solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on F^2 (SHELXL-97).

Plant Material. Plant material was collected in Perak, Malaysia, and identification was confirmed by Dr. Richard C. K. Chung, Forest Research Institute, Malaysia. Herbarium voucher specimens (K671) are deposited at the Herbarium, University of Malaya.

Extraction and Isolation. Extraction of the bark material and partitioning of the concentrated EtOH extracts with dilute acid were carried out as described in detail elsewhere.[39] The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH, followed by rechromatography of the appropriate partially resolved fractions using centrifugal preparative TLC. Solvent systems used for centrifugal preparative TLC were Et_2O (NH_3-saturated), Et_2O/MeOH (50:1; NH_3-saturated), Et_2O/MeOH (20:1; NH_3-saturated), EtOAc (NH_3-saturated), EtOAc/hexanes (3:1; NH_3-saturated), EtOAc/MeOH (50:1; NH_3-saturated), and $CHCl_3$/hexanes (NH_3-saturated). The yields (g kg^{-1}) of the alkaloids from the bark extract were as follows: **1** (0.0041), **2** (0.0029), **3** (0.0001), and **4** (0.0017).

Lumutinine A (1): light yellowish oil; $[\alpha]^{25}_D$ +160 (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 210 (5.61), 233 (5.61), 254 sh (5.26), 283 (4.94) nm; IR (dry film) ν_{max} 1617, 1651 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 1, respectively; ESIMS *m*/*z* 673 [M + H]$^+$; HRESIMS *m*/*z* 673.3755 (calcd for $C_{42}H_{48}N_4O_4$ + H, 673.3748).

Lumutinine B (2): light yellowish oil; $[\alpha]^{25}_D$ −11 (*c* 0.5, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 210 (5.71), 232 (5.64), 255 (5.35), 285 (4.95) nm; IR (dry film) ν_{max} 1616, 1651 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 1, respectively; ESIMS *m*/*z* 673 [M + H]$^+$; HRESIMS *m*/*z* 673.3751 (calcd for $C_{42}H_{48}N_4O_4$ + H, 673.3748).

Lumutinine C (3): light yellowish oil; $[\alpha]^{25}_D$ +84 (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 208 (5.31), 228 (5.33), 284 (4.78) nm; IR (dry film) ν_{max} 3360 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 1, respectively; ESIMS *m*/*z* 661 [M + H]$^+$; HRESIMS *m*/*z* 661.3749 (calcd for $C_{41}H_{48}N_4O_4$ + H, 661.3748).

Lumutinine D (4): light yellowish oil; $[\alpha]^{25}_D$ +209 (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 209 (5.40), 231 (5.49), 290 (4.89) nm; IR (dry film) ν_{max} 3370 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 1, respectively; ESIMS *m*/*z* 645 [M + H]$^+$; HRESIMS *m*/*z* 645.3809 (calcd for $C_{41}H_{48}N_4O_3$ + H, 645.3799).

Alstoumerine (8a): colorless needles ($CHCl_3$), mp 174−176 °C (lit.[32] mp 170 °C); $[\alpha]^{25}_D$ −13 (*c* 0.94, MeOH) (lit.[32] $[\alpha]_D$ −5.5 (*c* 0.0034, $CHCl_3$)); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.1 (C, C-20), 139.5 (C, C-2), 137.4 (C, C-13), 136.4 (CH, C-21), 127.3 (C, C-8), 121.0 (CH, C-11), 118.9 (CH, C-10), 118.1 (CH, C-9), 108.7 (CH, C-12), 102.5 (C, C-7), 67.4 (CH, C-19), 64.7 (CH_2, C-17), 56.2 (CH, C-5), 48.6 (CH, C-3), 44.4 (CH, C-16), 38.8 (CH_2, C-14), 29.6 (CH, C-15), 29.3 (NMe), 25.4 (CH_2, C-6), 22.5 (CH_3, C-18).

Crystallographic Data of Alstoumerine (8a). A single crystal of **8a** was obtained from $CHCl_3$, $C_{20}H_{24}N_2O_2$, M_r = 563.15, orthorhombic, space group $P2_12_12_1$, *a* = 10.3890(2) Å, *b* = 10.4473(2) Å, *c* = 23.0709(4) Å, *V* = 2504.05(8) Å^3, *Z* = 4, D_{calcd} = 1.494 g cm^{-3}, crystal size 0.69 × 0.18 × 0.16 mm^3, *F*(000) = 1160. The final R_1 value is 0.0397 (wR_2 = 0.0867) for 6345 reflections [$I > 2\sigma(I)$]. The absolute configuration was determined on the basis of a Flack parameter of 0.01(0.04),[40,41] refined using 3167 Friedel pairs.

Crystallographic data for the structure of alstoumerine (**8a**) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition number: CCDC 841573). A copy 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).

Determination of C-19 Configuration of Alstoumerine (8a) by Horeau's Method.[33,36] Alstoumerine (**8a**) (45 mg, 0.145 mmol) was added to a solution of racemic 2-phenylbutyric anhydride (168 μL, 0.58 mmol) in 1 mL of dry pyridine. The resulting mixture was stirred for 20 h at rt. Water (3.0 mL) was 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 (166 mg): $[\alpha]^{25}_D$ −3.1 (*c* 1.66, C_6H_6); $[\alpha]^{25}_D$ −3 (*c* 1.66, $CHCl_3$). The optical rotation of the unreacted 2-phenylbutyric acid was found to be negative (*R*), indicating the *S* configuration at C-19 in alstoumerine (**8a**).

■ ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 603-79674266. Fax: 603-79674193. E-mail: tskam@um.edu.my.

■ ACKNOWLEDGMENTS

We thank the University of Malaya and MOHE Malaysia (UMRG and HIR Grants) for financial support.

■ REFERENCES

(1) Sidiyasa, K. *Taxonomy, Phylogeny, and Wood Anatomy of Alstonia (Apocynaceae)*; Rijksherbarium/Hortus Botanicus: The Netherlands, 1998; Blumea Supplement 11, pp 1−230.

(2) Markgraf, F. *Blumea* **1974**, *22*, 20−29.

(3) Whitmore, T. C. In *Tree Flora of Malaysia*; Longman: London, 1972; Vol. *2*, pp 7−12.
(4) Cordell, G. A.; Saxton, J. E. In *The Alkaloids*; Manske, R. H. F.; Rodrigo, R. G. A., Eds.; Academic Press: New York, 1981; Vol. *20*, pp 1−295.
(5) Hamaker, L. K.; Cook, J. M. In *Alkaloids: Chemical and Biological Perspective*; Pelletier, S. W., Ed.; Pergamon: London, 1994: Vol. *9*, pp 23−84.
(6) Kam, T. S. In *Alkaloids: Chemical and Biological Perspective*; Pelletier, S. W., Ed.; Pergamon: Amsterdam, 1999; Vol. *14*, pp 285−435.
(7) Kam, T. S.; Choo, Y. M. In *The Alkaloids*; Cordel, G. A., Ed.; Academic Press: Amsterdam, 2006; Vol. *63*, pp 181−337.
(8) Abe, F.; Yamauchi, T.; Padolina, W. G. *Phytochemistry* **1994**, *35*, 253−257.
(9) Abe, F.; Yamauchi, T.; Santisuk, T. *Phytochemistry* **1994**, *35*, 249−252.
(10) Keawpradub, N.; Houghton, P. J. *Phytochemistry* **1997**, *46*, 757−762.
(11) Keawpradub, N.; Eno-Amooquaye, E.; Burke, P. J.; Houghton, P. J. *Planta Med.* **1999**, *65*, 311−315.
(12) Keawpradub, N.; Kirby, G. C.; Steele, J. C. P.; Houghton, P. J. *Planta Med.* **1999**, *65*, 690−694.
(13) Carroll, A. R.; Hyde, E.; Smith, J.; Quinn, R. J.; Guymer, G.; Forster, P. I. *J. Org. Chem.* **2005**, *70*, 1096−1099.
(14) Ghedira, K.; Zeches-Hanrot, M.; Richard, B.; Massiot, G.; Le Men-Olivier, L.; Sevenet, T.; Goh, S. H. *Phytochemistry* **1988**, *27*, 3955−3962.
(15) Massiot, G.; Boumendjel, A.; Nuzillard, J. M.; Richard, B.; Le Men-Olivier, L.; David, B.; Hadi, A. H. A. *Phytochemistry* **1992**, *31*, 1078−1079.
(16) Wong, W. H.; Lim, P. B.; Chuah, C. H. *Phytochemistry* **1996**, *41*, 313−315.
(17) Kam, T. S.; Nyeoh, K. T.; Sim, K. M.; Yoganathan, K. *Phytochemistry* **1997**, *45*, 1303−1305.
(18) Kam, T. S.; Jayashankar, R.; Sim, K. M.; Yoganathan, K. *Tetrahedron Lett.* **1997**, *38*, 477−478.
(19) Kam, T. S.; Iek, I. H.; Choo, Y. M. *Phytochemistry* **1999**, *51*, 839−844.
(20) Kam, T. S.; Choo, Y. M. *Tetrahedron* **2000**, *56*, 6143−6150.
(21) Kam, T. S.; Choo, Y. M.; Komiyama, K. *Tetrahedron* **2004**, *60*, 3957−3966.
(22) Kam, T. S.; Choo, Y. M. *Helv. Chim. Acta* **2004**, *87*, 366−369.
(23) Kam, T. S.; Choo, Y. M. *Phytochemistry* **2004**, *65*, 603−608.
(24) Kam, T. S.; Choo, Y. M. *J. Nat. Prod.* **2004**, *67*, 547−552.
(25) Kam, T. S.; Choo, Y. M. *Tetrahedron Lett.* **2003**, *44*, 8787−8789.
(26) Kam, T. S.; Tan, S. J.; Ng, S. W.; Komiyama, K. *Org. Lett.* **2008**, *10*, 3749−3752.
(27) Tan, S. J.; Choo, Y. M.; Thomas, N. F.; Robinson, W. T.; Komiyama, K.; Kam, T. S. *Tetrahedron* **2010**, *66*, 7799−7806.
(28) Koyama, K.; Hirasawa, Y.; Hosoya, T.; Teh, C. H.; Chan, K. L.; Morita, H. *Bioorg. Med. Chem.* **2010**, *18*, 4415−4421.
(29) Tan, S. J.; Robinson, W. T.; Komiyama, K.; Kam, T. S. *Tetrahedron* **2011**, *67*, 3830−3838.
(30) Tan, S. J.; Low, Y. Y.; Choo, Y. M.; Abdullah, Z.; Etoh, T.; Hayashi, M.; Komiyama, K.; Kam, T. S. *J. Nat. Prod.* **2010**, *73*, 1891−1897.
(31) Ratnayake, C. K.; Arambewela, L. S. R.; De Silva, K. T. D.; Atta-ur-Rahman.; Alvi, K. A. *Phytochemistry* **1987**, *26*, 868−870.
(32) Atta-ur-Rahman.; Abbas, S. A.; Nighat, F.; Ahmed, G.; Choudhary, M. I.; Alvi, K. A.; Habib-ur-Rehman. *J. Nat. Prod.* **1991**, *54*, 750−754.
(33) Horeau, A.; Kagan, H. B. *Tetrahedron* **1964**, *20*, 2431−2441.
(34) Pinchon, T. M.; Nuzillard, J. M.; Richard, B.; Massiot, G.; Le Men-Olivier, L.; Sevenet, T. *Phytochemistry* **1990**, *29*, 3341−3344.
(35) Clivio, P.; Richard, B.; Deverre, J. R.; Sevenet, T.; Zeches, M.; Le Men-Olivier, L. *Phytochemistry* **1991**, *30*, 3785−3792.
(36) Barnekow, D. E.; Cardellina, J. H. II. *Tetrahedron Lett.* **1989**, *30*, 3629−3632.
(37) Waldner, E. E.; Hesse, M.; Taylor, W. I.; Schmid, H. *Helv. Chim. Acta* **1967**, *50*, 1926−1939.
(38) Das, B. C.; Cosson, J. P.; Lukacs, G.; Potier, P. *Tetrahedron Lett.* **1974**, 4299−4302.
(39) Kam, T. S.; Tan, P. S. *Phytochemistry* **1990**, *29*, 2321−2322.
(40) Flack, H. D. *Acta Crystallogr. A* **1983**, *39*, 876−881.
(41) Flack, H. D.; Bernardinelli, G. *J. Appl. Crystallogr.* **2000**, *33*, 1143−1148.