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INDOLE ALKALOIDS FROM *ALSTONIA MACROPHYLLA*

NIWAT KEAWPRADUB and PETER J. HOUGHTON*

Pharmacognosy Research Laboratories, Department of Pharmacy, King's College London, Manresa Road, London SW3 6LX, U.K.

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Key Word Index—*Alstonia macrophylla*; Apocynaceae; root bark; indole alkaloids; alstomacrophylline; alstomacroline.

Abstract—Two new bisindole alkaloids, alstomacrophylline and alstomacroline, have been isolated from the root bark of *Alstonia macrophylla*, along with six known alkaloids, alstonerine, alstophylline, macrocarpamine, alstoumerine, 20-*epi*-antirhine and villastonine *N*-oxide. The monomeric indole alkaloid, 20-*epi*-antirhine, has been isolated for the first time from this species. Structures of the alkaloids were determined by spectroscopic methods, especially 2D NMR. © 1997 Elsevier Science Ltd

INTRODUCTION

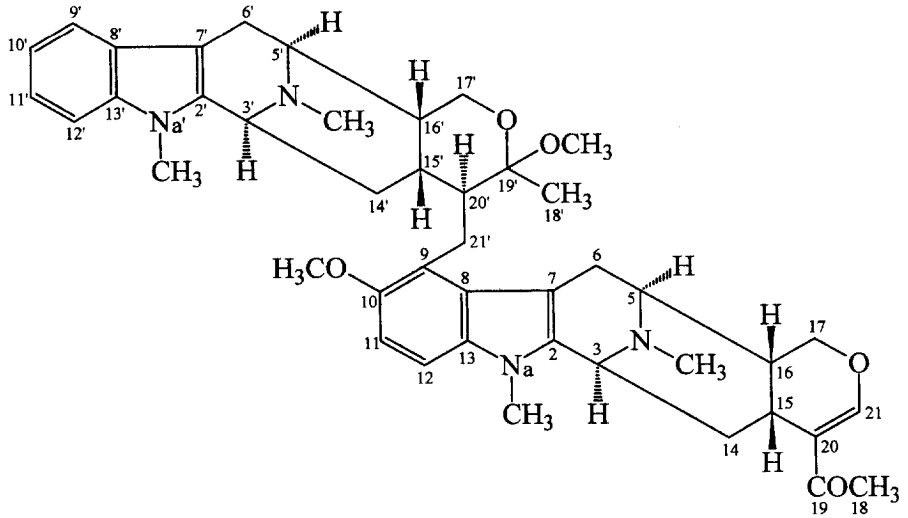
Alstonia macrophylla belongs to the section Monuraspermum of the Apocynaceae [1]. This medium-sized tree grows widely throughout the forests of southern Thailand and has been used for medicinal purposes in traditional medicine [2, 3]. Investigations on this species, mainly on leaves and stem bark, have revealed that there are wide diversities in alkaloid composition among plant material from various countries; altogether, 40 alkaloids have been isolated. In a preceding communication, we describe the isolation of a novel bisindole alkaloid, *O*-methylmacralstonine, and four known alkaloids, talcarpine, peliocarpamine, villalstonine and macralstonine, from the most active fraction (against human lung cancer cell lines) of the root bark of *A. macrophylla* collected from Thailand [4]. The purpose of the present paper is to give a complete account of the alkaloid composition of the root bark of this particular species, since it has not previously been investigated. Herein, we report the isolation and structural elucidation of two new bisindole alkaloids, alstomacrophylline (**1**) and alstomacroline (**2**), together with six known alkaloids, from the less active fractions of this material.

RESULTS AND DISCUSSION

A crude alkaloid mixture was extracted by acid-base workup from the root bark of *A. macrophylla*. Extensive TLC and VLS resulted in the isolation of 13 alkaloids, five of which have been described previously [4]. The UV spectra of the remaining eight alkaloids suggested the presence of the indole chromophore with maxima in the range 226–232 nm. By comparison of their spectral properties (UV, mass, ^1H and ^{13}C NMR spectra) with literature data, six of these alkaloids were identified as alstonerine [5], alstophylline [5–7], macrocarpamine [8], alstoumerine [9], 20-*epi*-antirhine [10] and villalstonine *N*-oxide [11]. 20-*epi*-antirhine has never been reported previously from any material of *A. macrophylla*. The data for the remaining two alkaloids **1** and **2** did not agree with any previously published.

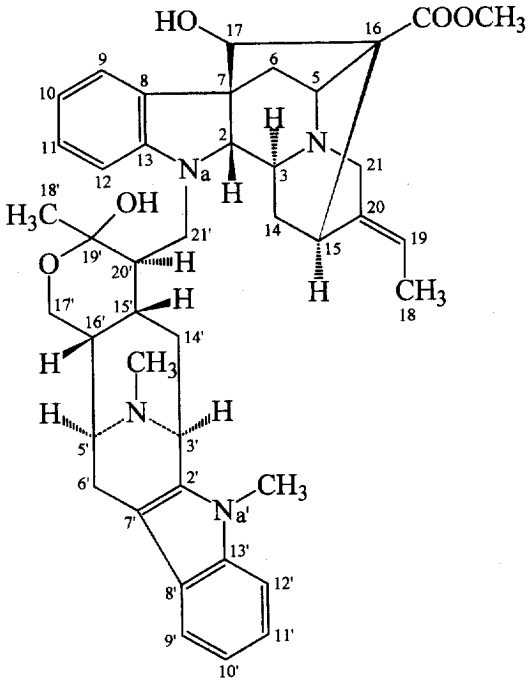
The high-resolution FAB mass spectrum of **1** showed a $[M+H]^+$ peak at *m/z* 719.4180 consistent with the molecular formula $C_{44}H_{54}N_4O_5$ (D.B.E. = 20), which is identical to that of *O*-methylmacralstonine [4]. The EI and FAB mass spectra of **1** also exhibited the characteristic fragments of the macroline-derived moiety at *m/z* 379, 197 and 170 [12, 13]. The vinyl proton (H-21) of the lower macroline-derived unit was observed as a one-proton singlet at δ 7.56. The coupling patterns of the six aromatic protons obtained from the ^1H NMR spectrum of **1** indicated the existence of a bisindole skeleton, four of which at δ 7.32, 7.16, 712 and 7.05 were attributed to H-9′, H-11′, H-10′ and H-12′ of the upper macroline-derived unit, respectively, on the basis of ^1H–^1H COSY and NOESY spectra (Scheme 1). A distinction between the structures of **1** and *O*-methylmacralstonine was clearly observed in their ^1H–^1H COSY spectra. In *O*-methylmacralstonine, the two aromatic protons (H-9 and H-12) of the lower part appeared as two one-proton singlets (*para*-position) indicating C-10 as the point of linkage to the upper macroline-derived unit (confirmed by NOESY),

* Author to whom correspondence should be addressed.

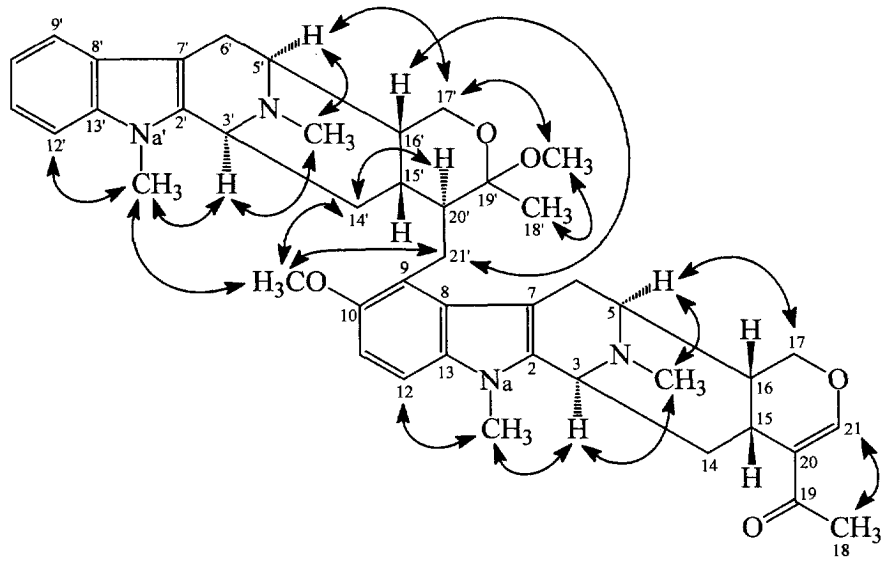


Alstomacrophylline (1)

Alstomacroline (2)



whereas the multiplicities of the two aromatic protons of the lower part of **1** exhibited *ortho*-coupling (*d*, $J = 8.5$ Hz). The linkage between the two parts and the methoxyl group must therefore be at the 9, 10 or 11, 12 positions. The correlation peak between signals at δ 5.83 and 3.64 (N_a–CH_3) in the NOESY spectrum of **1** showed that these protons must be at H-11 and H-12. The assignment was also fully supported by the one-bond ^{13}C-^1H correlated spectrum, in which the corresponding carbon signals of H-11 (δ 6.21) and H-12 (δ 5.83) were observed at δ 115.5 and δ 104.5, respectively. Consequently, only two positions, i.e. C-9 and C.10, were available for the linkage of the two moieties and the methoxyl substitution. The substantial upfield-shift of the signals due to H-3′ (−0.26 ppm), H-14′ (−0.72 ppm), H-15′ (−0.75 ppm) and N_a-CH_3 (−0.36 ppm) in the ^1H NMR or **1** compared with those of *O*-methylmacralstonine, revealing the shielding effect from the electron-donating function in the indole nucleus of the lower part of **1**, strongly suggested the presence of a methoxyl group at C-10 in **1**. The presence of cross-peaks between signals at δ 3.66 (10-OCH_3)/3.13 (N_a-CH_3) and 3.66/1.15 (H-14′) observed from the NOESY spectrum of **1** provided additional support for this assignment. Furthermore, the possibility of C-10/C-21′ linkage was also excluded by the lack of NOEs between the signals of OCH_3 (δ 3.66)/18′-CH_3 (δ 1.37) and OCH_3 (δ 3.66)/H-6 (δ 3.19 and 2.27). This provided evidence of a new type of bisindole linkage in the structure of **1**. It was deduced, therefore, that the point of attachment of the two parts of **1** be at C-9. The two signals of protons at δ 3.35 and 3.02, and their corresponding methylenic carbon resonating at δ 24.2 were attributed to 21′-CH_2 on the basis of ^1H–^1H COSY, NOESY and one-bond ^{13}C–^1H correlated spectra. The relative stereochemistry at C-20′ of **1** was also determined by the NOESY experiment, by comparison with *O*-methylmacralstonine. The presence of cross-peaks in the NOESY spectrum of **1** at δ 1.85 (H-20′)/1.15 (H-14′) and 3.35 (H-21′)/2.00 (H-16′) on one hand, and the absence of the correlation peak between H-20′ and H-16′ on the other hand, strongly suggested the α-orientation at H-20′. The configuration at C-20′ was thus assigned to be *S*. All hydrogen-bonded carbons of **1** were conclusively allocated by the one-bond ^{13}C–^1H correlations and DEPT spectra (Table 1). For quaternary carbons, the assignments were achieved by analogy with *O*-methylmacralstonine. On the basis of the above spectra argument, the structure of alkaloid **1** was proposed and named, alstomacrophylline.



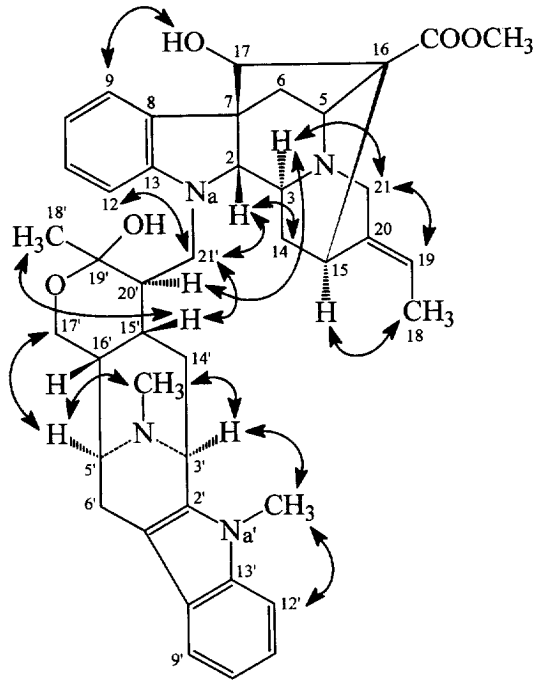
Scheme 1.

The high-resolution FAB mass spectrum of **2** displayed a protonated $[M]^+$ peak at *m/z* 691.3850 consistent with the molecular formula $C_{42}H_{50}N_4O_5$ (M_r690), revealing twenty degrees of unsaturation. The chemical shifts and coupling patterns of the eight aromatic protons from the ^1H NMR of **2** indicated the existence of a bisindole skeleton lacking any substitution on the benzene portions of the two indole moieties. The characteristic chemical shifts and multiplicities of the vinyl proton and the corresponding methyl group of an ethylidene side-chain were observed at δ 5.21 (H-19) and 1.63 (18-CH_3), respectively. the EI mass spectral fragmentation pattern of **2** at *m/z* 197 (base peak), 181 and 170, and two 3H-singlets at δ 3.67 (N_a-CH_3) and 2.41 (N_b-CH_3), together with their corresponding carbons resonating at δ 28.7 (N_a-CH_3) and 41.9 (N_b-CH_3), indicated the existence of a macroline-derived moiety as one half of the molecule. The correlation peaks of the signals at δ 7.34 (H-12′)/3.67 (N_a-CH_3), 3.96 (H-3′)/3.67, 3.96/2.41 (N_b-CH_3), and 3.06 (H-5′)/2.41 observed from the NOESY spectrum of **2** were used to distinguish the two parts of the molecule from each other. The chemical shifts and coupling information of carbons and protons obtained from the ^1H–^1H COSY, NOESY and ^{13}C–^1H correlations spectra of the other half (non-macroline portion) of **2** was reminiscent of those belonging to vincamajine and quebrachindine [14–17], however, with slight differences. The signals at δ 3.12 (*d*, J = 4.4 Hz) and 3.22 (*m*) were attributed to H-2 and H-3, respectively, according the ^1H–^1H COSY and NOESY spectra. The interlocking cross-peaks of the proton signals at δ 6.58 (H-12)/3.25 (H-21′) and 3.12 (H-2)/2.71(H-21′) from the NOESY of **2** provided evidence of an unusual C–N linkage between the two units (Scheme 2). This was strongly supported by the lack of the N–H peak in the IR spectrum and the downfield-shift of C-21′, resonating at δ 49.3 in the ^{13}C–^1H correlated spectrum. The very upfield signal of the one-proton doublet at δ 0.66 (J = 6.1 Hz), which coupled with the proton at δ 2.11 (*d*, J = 6.1 Hz, H-17) but did not correlate to any carbon in the one-bond ^{13}C–^1H correlations spectrum was, therefore, assigned to the 17-OH function. This was also confirmed by the absence of this signal in the ^1H NMR spectrum of the monoacetate product of **2** (17-*O*-acetylalstomacroline). Studies with models showed that the 17-OH was shielded by the benzene ring of the quebrachidine-derived moiety and this would account for the upfield shift. Interesting to note, was the failure to obtain the diacetate product of **2** under normal conditions of acetylation. This may partly be due to steric hindrance of the tertiary 19′-OH function. However, the chemical shift of C-19′ at δ 97.0, which was almost the same as that of macralstonine [4, 13], suggested the existence of an OH function at C-19′. Moreover, it has been reported that no loss of water or an OH group could be observed in the EI mass spectra of quebrachindine and alstonisidne which contained only one OH group at C-17 [18]. Obviously, the intense peak at *m/z* 672 $[M\text{-}18]^+$ in the EI mass spectrum of **2** represented the loss of H_2O form the OH function at C-19′. The methine, methylene and methyl carbons of **2** were straightforwardly assigned by the one-bond ^{13}C–^1H correlations and the DEPT spectra (Table 1). The chemical shifts of quaternary carbons were allocated by an ^{13}C–^1H COLOC experiment as depicted in Table 2.The relative stereochemistry at C-2 and C-20′ of **2** was also proposed by the NOSEY experiment. Since the macroline-derived moiety of **2** was similar to those of villalstonine and alstonisidine, of which the configurations of H-15′ and H-16′ have been confirmed by X-ray analysis as β-orientation [19, 20], this could

Table 1. ^{13}C NMR assignments of compounds **1** and **2** [100 MHz, $CDCl_3$]

Carbons	1	2
C-2	132.9	76.1
C-3	53.9	54.2
C-5	54.6	61.3
C-6	22.8	22.4
C-7	105.9	59.4
C-8	122.3	128.9
C-9	111.3	125.2
C-10	154.1	118.4
C-11	115.5	127.7
C-12	104.5	108.0
C-13	136.3	153.5
C-14	32.3	36.1
C-15	22.9	30.0
C-16	38.7	56.5
C-17	67.8	74.9
C-18 (CH_3)	25.1	12.8
C-19	195.1	116.0
C-20	121.1	136.7
C-21	157.4	55.3
$\underline{C}OOCH_3$	—	172.6
$COO\underline{C}H_3$	—	51.3
N_a-CH_3	32.0	—
N_b-CH_3	41.8	—
10-OCH_3	56.5	—
C-2′	133.1	133.0
C-3′	54.4	54.5
C-5′	55.1	55.0
C-6′	22.2	22.3
C-7′	106.3	107.6
C-8′	126.7	126.2
C-9′	118.2	118.0
C-10′	118.1	117.8
C-11′	120.0	120.0
C-12′	108.2	109.1
C-13′	136.9	136.5
C-14′	32.4	31.7
C-15′	25.6	24.2
C-16′	37.2	38.0
C-17′	61.6	61.2
C-18′ (CH_3)	20.9	28.8
C-19′	101.3	97.0
C-20′	43.8	43.2
C-21′	24.2	49.4
$N_{a'}$-CH_3	28.4	28.7
$N_{b'}$-CH_3	41.7	41.9
19′-OCH_3	47.4	—

be applied to those of **2** on the basis of NOSEY and ^1H–^1H COSY spectra. The presence of cross-peaks at δ 3.22 (H-3)/1.23 (H-20′) and 2.30 (H-15′)/1.29 (18′-CH_3), and the lack of NOEs between H-16′/H-20′ and 18′-CH_3/H-20′ in the NOESY spectrum of **2** indicated the α-orientation of H-20′; thus, the configuration at C-20′ was concluded to be *S*. The correlation peak of signals at δ 3.12 (H-2)/1.48 (H-14) also clearly indicated the β-orientation of H-2, therefrom, the configuration at C-2 of **2** was deduced to be *S*, the same



Scheme 2.

as that of alstonisidine [20, 21]. According to these spectral data, the structure of alkaloid **2** was proposed and named alstomacroline.

The biogenesis of **2** could be speculated at arising by an electrophilic substitution on the indoline aromatic nucleus of a quebrachidine-derived moiety by the enone function of macroline unit as in alstonisidine [18, 21] but without ring closure. This may be an explanation of the absence of alstonisidine in *A. macrophylla* since, so far, it has been isolated only from *A. muelleriana* [21, 22]. The isolation and structures of **1** and **2** is of considerable biogenetic interest, as additional examples of macroline-derived alkaloids which are considered as real chemotaxonomic mar-

Table 2. Long-range ^{13}C–^1H correlations of the quaternary carbons observed in the COLOC spectrum of compound **2**

$\delta^{13}C$	δ^2J_{CH}	δ^3J_{CH}
172.6 ($\underline{C}OOCH_3$)	—	3.79 (OCH_3)
153.5 (C-13)	—	6.94 (H-9)
136.7 (C-20)	—	1.63 (18-CH_3)
136.5 (C-13′)	—	7.03 (H-11′)
		6.87 (H-9′)
		3.67 ($N_{a'}$-CH_3)
133.0 (C-2′)	3.96 (H-3′)	3.67 ($N_{a'}$-CH_3)
123.9 (C-8)	—	6.72 (H-10)
126.2 (C-8′)	—	—
107.6 (C-7′)	3.10 (H-6′)	—
	2.28 (H-6′)	
97.0 (C-19′)	1.29 (18′-CH_3)	—
59.4 (C-7)	1.26 (H-6)	—
56.5 (C-16)	3.28 (H-5)	—

kers of the section Monuraspermum of the genus *Alstonia* [11].

EXPERIMENTAL

General. UV: in MeOH. IR: KBr discs. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, using TMS as int. standard. DEPT expts were carried out with the polarization pulse $\theta = 45$, 90 and 135°. Standard programme for the library $^nJ_{CH} = 10$ Hz was used for the COCLOC expt. FABMS and EIMS data were recorded on a high-resolution spectrometer. Anal. TLC: precoated plates of silica gel 60 F_{254} (Merck) and precoated plates of $Al_2O_360F_{254}$ (Merck, neutral, type E). Prep. TLC: silica gel PF_{254} (Merck), 1 mm thick. The zone was eluted with $CHCl_3$–MeOH (1:1) and evapd to dryness under red. pres. The following developing solvent systems were used: S1, EtOAc–MeOH (7:3); S2, petrol–EtOAc–*iso*-PrOH (8:6:1); S3, EtOAc–$CHCl_3$–MeOH (40:40:1); S4, $CHCl_3$–MeOH (14:1); S5, EtOAc–MeOH–35%NH_3 (14:2:1); S6, EtOAc–$CHCl_3$–MeOH (5:4:2); S7, EtOAc–*iso*-PrOH–35%NH_3 (28:3:1); S8, $CHCl_3$–MeOH (9:1). Detection for TLC: UV at 254 nm and Dragendorff's spray reagent.

Plant material. Root bark of *Alstonia macrophylla* Wall. ex G.Don was collected at Songkla, Thailand, in 1994. Authentication of plant materials was carried out at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand, where the herbarium vouchers (numbers APOPS 014–015) have been kept. Dried powdered root bark (250 g) was precolated with MeOH (5 × 500 ml). The comb. MeOH percolate was concd to dryness under red. pres. The procedure of alkaloid extraction has been described elsewhere [4]. Fractionation of the alkaloid extract (2.0 g) was achieved by means of repeated prep. TLC (silica gel, S1) to afford 4 frs (from lower to higher polarity): FA2a–FA2d. Fr. FA2a was subjected to prep. TLC (silica gel, S2) to yield alstonerine (116 mg) and alstophylline (8 mg). FA2b: see ref. [4]. FA2c was separated by VLC (silica gel, TLC grade) using $CHCl_3$–MeOH (18:1, 9:1 and 1:1) as eluents to give 3 pooled frs (I–III). Pool I was purified by prep. TLC (Al_2O_3, 0.2 mm, S3) to give macrocarpamine (19 mg), and the remaining mixt. was further purified by prep. TLC (silica gel, S4) to afford **1** (alstomacrophylline, 5 mg). Pool II was submitted to prep. TLC (silica gel, S5 and S6) to yield alstoumerine (7 mg) and **2** (alstomacroline, 14 mg), respectively. Pool III was purified by prep. TLC (silica gel, S7 and S8) to afford 20-*epi*-antirhine (6 mg). FA2d was subjected to RP-VLC (Davisil 60A, C_{18}, 30–40 μm) eluted with MeOH. The main frs which showed one spot on TLC were pooled and identified as villalstonine *N*-oxide (11 mg).

Alstomacrophylline (**1**). Amorphous powder. $[\alpha]_D^{20}$ +42.8° (MeOH; *c* 0.093). UV λ_{max} nm ($\log\varepsilon$): 232 (4.27), 260 (3.67), 292 (3.51). IR ν_{max} cm^{-1}: 2895, 1616, 1384. HR-FABMS: found 719.4180 $[M+H]^+$, calcd for $C_{44}H_{55}N_4O_5$: 719.4172. EIMS (probe) 70 eV, *m/z* (rel. int.): 718 $[M]^+$ (26), 686 $[M\text{-}H\text{-}OCH_3]^+$ (100), 656 (7), 486 (12), 379 (6), 197 (15), 170 (2). FABMS (thioglycerol), *m/z* (rel. int.): 719 $[M+H]^+$ (96), 687 (100), 379 (56), 239 (44), 237 (34), 197 (5), 170 (10), 131 (53). ^1H NMR (400 MHz, $CDCl_3$): δ 7.56 (1H, *s*, H-21), 7.32 (1H, *br d*, $J = 7.4$ Hz, H-9′), 7.16 (1H, *td*, $j = 7.1$, 1.3 Hz, H-11′), 7.12 (1H, *td*, $J = 7.1$, 1.0 Hz, H-10′), 7.05 (1H, *br d*, $J = 7.6$ Hz H-12′), 6.21 (1H, *d*, $J = 8.5$ Hz, H-11), 5.83 (1H, *d*, $J = 8.5$ Hz, H-12), 4.41 (1H, *t*, $J = 11.4$ Hz, H-17), 4.19 (1H, *dd*, $J = 11.4$, 2.5 Hz, H-17), 4.06 (1H, *t*, $J = 11.7$ Hz, H-17′), 3.74 (1H, *br s*, H-3), 3.67 (1H, overlapped with δ 3.66, H-3′), 3.66 (3H, *s*, 10-OCH_3), 3.64 (3H, *s*, N_a-CH_3), 3.47 (1H, *dd*, $J = 11.7$, 4.8 Hz, H-17′), 3.35 (1H, *dd*, $J = 14.2$, 11.7 Hz, H-21′), 3.30 (3H, *s*, 19′-OCH_3), 3.19 (1H, *dd*, $J = 16.4$, 6.9 Hz, H-6), 3.13 (3H, *s*, N_a-CH_3), 3.06 (1H, *d*, $J = 7.1$ Hz, H-5), 3.02 (1H, *dd*, $J = 14.2$, 5.8 Hz, H-21′), 3.00 (1H, *dd*, $J = 15.7$, 6.1 Hz, H-6′), 2.82 (1H, *td*, $J = 12.9$, 3.6 Hz, H-14′), 2.80 (1H, *d*, $J = 6.5$ Hz, H-5′), 2.51 (1H, *m*, H-15), 2.30 (3H, *s*, N_b-CH_3), 2.27 (1H, *d*, $J = 16.4$ Hz, H-6), 2.24 (3H, *s*, N_b-CH_3), 2.12 (3H, *s*, 18-CH_3), 2.05 (1H, *br*, *d*, $J = 12.8$ Hz, H-14), 2.00 (1H, *m*, H-16′), 1.91 (1H, *d*, $J = 15.7$ Hz, H-6′), 1.88 (1H, *m*, H-16), 1.85 (1H, *m*, H-20′), 1.74 (1H, *td*, $J = 12.8$, 3.8 Hz, H-14), 1.37 (3H, *s*, 18′-CH_3), 1.15 (1H, *dt*, $J = 12.9$, 3.8 Hz, H-14′), 0.95 (1H, *m*, H-15′). ^{13}C NMR Table 1.

Alstomacroline (**2**). Amorphous powder. $[\alpha]_D^{20}$ +54.6° ($CHCl_3$; *c* 0.366) UV λ_{max} nm ($\log\varepsilon$): 232 (4.44), 251 (3.88), 293 (3.78). IR λ_{max} cm^{-1}: 3416, 2909, 1734, 1616, 741. HR-FABMS: found 691.3850 $[M+H]^+$, calcd for $C_{42}H_{51}N_4O_5$: 691.3859. EIMS (probe) 70 eV, *m/z* (rel. int.): 690 $[M]^+$ (7), 672 $[M\text{-}H_2O]^+$ (18), 365 (24), 324 (32), 292 (33), 197 (100), 182 (62), 181 (49), 170 (58), 130 (36). FABMS (thioglycerol), *m/z* (rel. int.): 691 $[M+H]^+$ (100), 365 (67), 237 (18), 170 (4). ^1H NMR (400 MHz, $CDCl_3$): δ 7.34 (1H, *d*, $J = 7.8$ Hz, H-12′), 7.20 (1H, *t*, $J = 7.9$ Hz, H-11), 7.03 (1H, *t*, $J = 7.8$ Hz, H-11′), 6.94 (1H, *d*, $J = 7.1$ Hz, H-9), 6.87 (1H, *d*, $J = 7.7$ Hz, H-9′), 6.77 (1H, *t*, $J = 7.4$ Hz, H-10′), 6.72 (1H, *t*, $J = 7.3$ Hz, H-10), 6.58 (1H, *d*, $J = 7.9$ Hz, H-12), 5.21 (1H, *q*, $J = 6.5$ Hz, H-19), 4.50 (1H, *t*, $J = 11.6$ Hz, H-17′), 3.96 (1H, *br s*, H-3′), 3.79 (3H, *s*, OCH_3) 3.67 (3H, *s*, N_a-CH_3), 3.57 (1H, *dd*, $J = 11.6$, 4.1 Hz, H-17′), 3.32 (2H, *m*, 2 × H-21), 3.28 (1H, *m*, H-5), 3.25 (1H, *m*, H-21′), 3.22 (1H, *m*, H-3), 3.18 (1H, *m*, H-15), 3.12 (1H, *d*, $J = 4.4$ Hz, H-2), 3.10 (1H, *m*, H-6′), 3.08 (1H, *m*, H-14′), 3.06 (1H, *d*, $J = 5.6$ Hz, H-5′), 2.71 (1H, *dd*, $J = 14.4$, 5.2 Hz, H-21′), 2.41 (3H, *s*, N_b-CH_3), 2.34 (1H, *m*, H-14), 2.30 (1H, *m*, H-15′), 2.28 (1H, *d*, $J = 16.0$ Hz, H-6′), 2.19 (1H, *m*, H-16′), 2.11 (1H, *d*, $J = 6.1$ Hz, H-17), 1.63 (3H, *d*, $J = 6.5$ Hz, 18-CH_3) 1.48 (1H, *d*, $J = 11.6$ Hz, H-14), 1.35 (1H, *br d*, $J = 12.8$ Hz, H-14′), 1.29 (3H, *s*, 18′-CH_3), 1.26 (2H, *m*, 2 × H-6), 1.23 (1H, *m*, H-20′) 0.66 (1H, *d*, $J = 6.1$ Hz, 17-OH). ^{13}C NMR: Table 1.

Acetylation of **2**. A soln of **2** (4 mg) in pyridine (0.3

ml) and Ac_2O (0.3 ml) was stirred at room temp. for 16 hr. The reaction mixt. was basified with 17.5% NH_3 and extracted with $CHCl_3$. The organic layer was washed with brine, dried and evapd to dryness. The residue was purified by prep. TLC [silica gel, $CHCl_3$–MeOH (14:1)] to afford 2.3 mg of 17-*O*-acetylalstomacroline. Amorphous powder. UV λ_{max} nm: 231, 251, 286. EIMS (probe) 70 eV, *m/z* (rel. int.): 732 $[M]^+$ (3), 714 $[M\text{-}H_2O]^+$ (4), 197 (14), 170 (18), 141 (90), 113 (100). FAMBS (thioglycerol), *m/z* (rel. int.): 733 $[M+H]^+$ (100). ^1H NMR (400 MHz, $CDCl_3$): new 3H-singlet at δ 2.23 (OAc).

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