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CHEMICAL CONSTITUENTS OF *ALSTONIA MACROPHYLLA*

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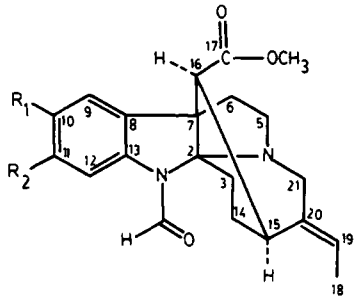
ABSTRACT.—Three new indole alkaloids, alstonamide [**1**], demethoxyalstonamide [**2**], and alstoumerine [**3**], have been isolated from the leaves of *Alstonia macrophylla*. The first two alkaloids are of the vincorine type, and the third is of the sarpagine type.

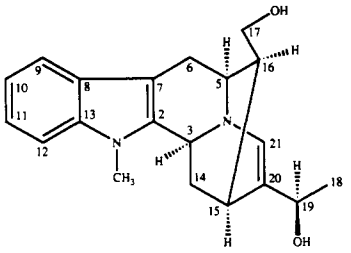
Alstonia macrophylla Wall. (Apocynaceae) is a plant commonly found in Sri Lanka. Several studies on this species growing in other countries have been reported (1–5), and the plant is used in medicinal preparations in the Philippines (1). However, little previous work has been done on *A. macrophylla* of Sri Lankan origin.

Alstonamide [**1**], demethoxyalstonamide [**2**], and alstoumerine [**3**] were isolated by separation of crude alkaloids on the basis of their differential basicities. The fraction obtained at pH 9 was subjected to column and preparative layer chromatography (Si gel).

The uv spectrum of alstonamide [**1**] showed λ max (MeOH) 208, 264, and 303 nm, typical of a vincorine-type system. The ir spectrum ($CHCl_3$) showed intense absorptions at 1600 cm^{-1} (C=C), 1657 cm^{-1} (*N*-formyl C=O), 1723 cm^{-1} (ester C=O), and 2920 cm^{-1} (C-H). The hrms afforded the molecular ion peak at *m/z* 412.1987 (calcd 412.1998), consistent with the molecular formula $C_{23}H_{28}N_2O_5$ and indicating eleven degrees of unsaturation in the molecule. The molecular ion was confirmed by fdms. The peak at *m/z* 397.1768 (calcd 397.1763) suggested the loss of a methyl group. The peak at *m/z* 384.2051 (calcd 384.2048) corresponded to the loss of carbon monoxide, while other fragments appeared at *m/z* 369 $[M-CO-Me]^+$, 356 $[M-CO-C_2H_4]^+$, and 325 $[M-CO-CO_2Me]^+$. This fragmentation pattern is characteristic of the vincorine-type alkaloids (6,7). The ^{1}H-nmr spectrum ($CDCl_3$, 300 MHz), 2D COSY-45, and *J*-resolved and nOe difference measurements helped in assigning each proton and their relative stereochemistries. The ethylidene methyl protons (H-18) appeared as a double doublet at δ 1.60 showing vicinal coupling ($J_{18,19}$ = 7.0 Hz) with the adjacent C-19 olefinic proton and homoallylic coupling ($J_{18,21\beta}$ = 1.8



1 $R_1=R_2=OMe$
2 $R_1=OMe$, $R_2=H$



3

Hz) with H-21β. The C-19 olefinic proton, on the other hand, resonated at δ 5.43 as a split quartet showing vicinal coupling ($J_{19,18}$ = 7.0 Hz) with the ethylidene methyl protons (H-18). The ester methyl protons resonated at δ 3.88. Two three-proton singlets at δ 3.81 and 3.82 were assigned to the 10-OMe and 11-OMe protons, respectively. The presence of two one-proton signals in the aromatic region indicated the existence of a disubstituted indole nucleus. A one-proton singlet at δ 8.47 was assigned to the *N*-formyl proton (8,9).

Structure **1** was further substantiated from the ^{13}C-nmr spectrum (Table 1), which

TABLE 1. ^{13}C-nmr Data of Compounds **1**, **2**, and **3** in $CDCl_3$.[a]

Carbon	Compound		
	1	**2**	**3**
C-2	94.8	94.2	138.8
C-3	26.0	26.4	48.6
C-5	54.0	54.3	56.5
C-6	41.6	41.3	38.4
C-7	58.4	58.3	102.4
C-8	132.6	139.7	127.1
C-9	108.1	112.0	118.1
C-10	148.6	157.0	119.0
C-11	146.1	111.1	121.0
C-12	100.7	117.0	108.7
C-13	129.3	133.0	149.4
C-14	27.7	27.4	25.2
C-15	35.2	35.4	29.6
C-16	52.2	49.9	44.3
C-17	173.3	173.3	64.4
C-18	13.5	13.5	22.3
C-19	123.3	123.3	67.2
C-20	138.6	138.0	137.4
C-21	58.0	58.0	135.4
N-CH_3	—	—	29.2
N-CHO	160.0	160.1	—
OCH_3	56.1	56.2	—
OCH_3	56.3	—	—
$COOCH_3$	51.8	51.9	—

[a] ^{13}C-nmr spectra of **1** and **2** were recorded at 75 MHz, while that of **3** was recorded at 100 MHz.

indicated the presence of six methine, five methylene, four methyl, and eight quaternary carbons. The ^{13}C-nmr assignments of **1** were made by analogy to related compounds (7). The signal due to the C-2 quaternary carbon was found to resonate at δ 94.8. In the aromatic region two methine carbons were found to resonate at δ 108.1 and δ 100.7 and were assigned to C-9 and C-12, respectively. The *N*-formyl carbon resonated at δ 160.0. The three OMe carbons resonated at δ 56.3, 56.1, and 51.8. All of the above spectral studies led us to assign the structure **1** to alstonamide.

The uv spectrum of the second alkaloid, demethoxyalstonamide [**2**], showed λ max 263, 292, and 306 (sh) nm. The ir spectrum ($CHCl_3$) showed intense absorption at 1600 cm^{-1} (C=C), 1657 cm^{-1} (*N*-formyl C=O), and 1723 cm^{-1} (ester C=O). The uv and ir spectra were similar to those of alstonamide. The ei mass spectrum afforded the molecular ion peak at *m/z* 382, while other major fragments appeared at *m/z* 354, 339, 295, 281, 199, 186, and 174. The fragmentation pattern of **2** was found to be very similar to that of alstonamide [**1**]. A comparison of the ei mass spectrum of **2** with that of (−)-norvincorine (7) exhibited a distinct similarity. The molecular ion peak was shifted to a higher mol wt by 28 mu in **2**, which indicated that the substance in hand was probably a formyl derivative of (−)-norvincorine. The ^{1}H-nmr spectrum ($CDCl_3$, 300 MHz) was very similar to that of alstonamide except in the aromatic region where the three aromatic protons were found to resonate, indicating the presence of one substituent on the benzene ring. The ^{13}C-nmr spectrum ($CDCl_3$, 75 MHz, Table 1) closely resembled that of alstonamide, though some chemical shift differences were apparent in the aromatic region due to the absence of the OMe group at the C-11 position. These spectral studies established that the compound possessed structure **2**.

The uv spectrum of alstoumerine [**3**] showed λ max (MeOH) at 225, 285, 290 nm, characteristic of the indolic chromophore. The ir spectrum ($CHCl_3$) showed an intense absorption at 3300 cm^{-1} (OH). The ei mass spectrum of **3** showed the molecular ion peak at *m/z* 324 as the base peak, while hrms established its exact mass to be 324.1836 in agreement with the molecular formula $C_{20}H_{24}N_2O_2$ (calcd 324.1837) revealing ten degrees of unsaturation. A peak at *m/z* 307.1823 ($C_{20}H_{23}N_2O$, calcd 307.1810), indicated the presence of a hydroxyl group in the molecule. Other important peaks were at *m/z* 293.1653 ($C_{19}H_{21}N_2O$, calcd 293.1653), 281.1649 ($C_{18}H_{21}N_2O$, calcd 281.1653), and 182.0878 ($C_{12}H_{10}N_2$, calcd 182.0843). The overall fragmentation pattern was distinctly similar to those of other sarpagine-type alkaloids (10). The ^{1}H-nmr spectrum ($CDCl_3$, 400 MHz) of **3** bore a distinct similarity to those of other sarpagine alkaloids (11–13). It showed a singlet at δ 3.55 assigned to the N_a-methyl protons, its downfield chemical shift being consistent with its attachment to the indole nitrogen atom (14). A triplet at δ 3.08 was assigned to H-5α, as it showed cross peaks with H-6α (δ 3.14) and H-16α (δ 1.63). The coupling constant of H-5α and H-16α confirmed the *S* configuration at C-5. Another doublet at δ 6.58 was due to the H-21 olefinic proton ($J_{21,15\alpha}$ = 1.4 Hz), its downfield chemical shift indicating that the olefinic carbon was directly attached to the nitrogen (15). H-19 resonated at δ 4.55 as a quartet ($J_{19,18}$ = 6.5 Hz), its downfield chemical shift corresponding to the presence of a hydroxyl group at C-19. The doublet at δ 1.37 was assigned to the Me-18 protons ($J_{18,19}$ = 6.5 Hz). The C-17 methylene protons resonated at δ 3.46 ($J_{17a,17b}$ = 11.4 Hz, $J_{17a,16\alpha}$ = 5.0 Hz) and at δ 3.64 ($J_{17b,17a}$ = 11.4 Hz, $J_{17b,16\alpha}$ = 5.0 Hz) as double doublets. The presence of four protons in the aromatic region indicated the presence of an unsubstituted indole nucleus. The ^{1}H-nmr assignments and their relative stereochemistry were made with the help of spin decoupling, COSY-45, and nOe difference measurements (16,17).

The ^{13}C-nmr spectrum ($CDCl_3$, 100 MHz) of **3** showed 20 carbon resonances. The multiplicities of each carbon atom were determined using DEPT experiments, which

revealed the presence of two methyl, three methylene, ten methine, and five quaternary carbons. The two OH-bearing carbon atoms, C-17 and C-19, resonated at δ 64.4 and δ 67.2, respectively. The chemical shift of C-21 at δ 135.4 revealed the location of the double bond, its downfield value being due to the attachment of nitrogen to this carbon. The ^{13}C-nmr assignments were made by analogy to related alkaloids (Table 1) (18).

To establish the absolute configuration of the secondary hydroxyl group present at C-19, Horeau's procedure (19) was employed, which established its *R* configuration. On the basis of the above spectral and chemical studies, structure **3** was assigned to alstoumerine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectrum was recorded on a Shimadzu UV-240 spectrophotometer; the ir spectrum was recorded on JASCO A-302 spectrophotometer. Eims and fdms were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 (DEC) computer system. Hrms was recorded on JEOL MS-HX 110 mass spectrometer connected to DEC PDP 11/73 computer system. 1D and 2D ^{1}H-nmr spectra were recorded at 300 MHz and 400 MHz in $CDCl_3$ on Bruker AM-300 and 400 nmr spectrometers, respectively. The analytical experiments were performed on Si gel plates (GF-254, 0.2 mm, E. Merck).

PLANT MATERIAL.—The leaves of *A. macrophylla* were collected from Colombo and Peradeniya, Sri Lanka and identified by Prof. S. Balasubramonium, Department of Botany, Peradeniya University, Sri Lanka. The herbarium number is Wall number 1648 (K-W).

EXTRACTION AND ISOLATION.—The EtOH extract of the dried leaves (30 kg) of *A. macrophylla* was concentrated, acidified with 10% HCl, and extracted with petroleum ether. The defatted aqueous acidic extract was basified with 20% NH_4OH and extracted with $CHCl_3$ at different pH values. The fraction obtained at pH 9.0 (30 g) was subjected to cc (Si gel), eluted with $CHCl_3$, and marked as A, while the fraction obtained in $CHCl_3$-MeOH (9:1) was marked as B. Fraction A was again subjected to cc with $CHCl_3$-MeOH (9.9:0.1) and was further purified by preparative tlc on Si gel plates (GF-254, 0.2 mm) with hexane-Et_2O (1:1) and a few drops of NH_3. This afforded the pure alkaloids alstonamide [**1**] (10 mg, 3×10^{-4}%) and demethoxyalstonamide [**2**] (8.5 mg, 2.8×10^{-5}%). Fraction B was again subjected to cc. The fraction obtained in $CHCl_3$-MeOH (9.5:0.5) was further purified by preparative tlc on Si gel plates (GF-254, 0.2 mm) with hexane-Me_2CO (1:1) as the solvent system to afford alstoumerine [**3**] (8 mg, 2.6×10^{-5}%).

ALSTONAMIDE [**1**].—[α]D +82° ($CHCl_3$, $c=0.0062$); uv λ max (MeOH) nm 208, 264, 303: ir ($CHCl_3$) ν max (cm^{-1}) 1600 (C=C), 1657 (*N*-formyl C=O), 1723 (ester C=O), 2920 (C-H); eims *m/z* (rel. int. %) 412 (43), 384 (100), 369 (57), 356 (9), 325 (13), 311 (14), 297 (10), 267 (12), 173 (15); fdms 412; hrms 412.1987, 384.2051, 369.1820, 356.1740, 325.1917; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 1.60 (3H, dd, $J_{18,19}=7.0$ Hz, $J_{18,21\beta}=1.8$ Hz, H-18), 3.2 (1H, m, H-6α), 2.8 (1H, m, H-6β), 3.00 (1H, d, $J_{21\alpha,21\beta}=15.4$ Hz, H-21α), 3.92 (1H, d, $J_{21\beta,21\alpha}=15.4$ Hz, H-21β), 2.4 (1H, m, H-15α), 1.98 (1H, d, $J_{16\alpha,15\alpha}=8.4$ Hz, H-16α), 5.43 (1H, split q, $J_{19,18}=7.0$ Hz), 3.88 (3H, s, COOCH_3), 3.81 (3H, s, 10-OCH_3), 3.82 (3H, s, 11-OCH_3), 7.79 (1H, s, H-9), 7.05 (1H, s, H-12), 8.47 (1H, s, *C*HO); ^{13}C-nmr see Table 1.

DEMETHOXYALSTONAMIDE [**2**].—[α]D +74° ($CHCl_3$, $c=0.0049$); uv λ max (MeOH) nm 206, 264, 306; ir ($CHCl_3$) ν max (cm^{-1}) 1600 (C=C), 1657 (*N*-formyl C=O), 1723 (ester C=O), 2920 (C-H); eims *m/z* (rel. int. %) 382 (40), 354 (100), 339 (25), 295 (15), 199 (40), 186 (45), 174 (42); fdms 382; hrms 382.1888, 354.1585, 339.1710, 295.1811; ^{1}H-nmr ($CDCl_3$, 300 MHz) δ 1.60 (3H, dd, $J_{18,19}=7.0$ Hz, $J_{18,21\beta}=1.8$ Hz, H-18), 3.2 (1H, m, H-6α), 2.8 (1H, m, H-6β), 3.00 (1H, d, $J_{21\alpha,21\beta}=15.4$ Hz, H-21α), 3.92 (1H, d, $J_{21\beta,21\alpha}=15.4$ Hz, H-21β), 2.4 (1H, m, H-15α), 1.98 (1H, d, $J_{16\alpha,15\alpha}=8.4$ Hz, H-16α), 5.43 (1H, split q, $J_{19,18}=7.0$ Hz), 3.88 (3H, s, COOCH_3), 3.81 (3H, s, 10-OCH_3), 7.0 (1H, d, $J_{9,11}=2.6$ Hz, H-9), 6.72 (1H, dd, $J_{11,9}=2.6$ Hz, $J_{11,12}=8.7$ Hz, H-11), 8.0 (1H, d, $J_{12,11}=8.7$ Hz, H-12), 8.47 (1H, s, CHO); ^{13}C nmr see Table 1.

ALSTOUMERINE [**3**].—Pale yellowish crystals: mp 170; [α]D −5.5° ($CHCl_3$, 0.0034); uv λ max (MeOH) nm (log ε) 225 (4.7), 285 (4.5), 290 (4.2); ir ν max (cm^{-1}) 3300 (OH); hrms *m/z* (rel. int. %) 324.1836 (100), 307.1823 (20), 293.1653 (50), 281.1649 (25), 182.0878 (70); ^{1}H-nmr ($CDCl_3$, 400 MHz) δ 1.37 (3H, d, $J_{18,19}=6.5$ Hz, H-18), 1.63 (2H, m, H-14β, H-16α), 1.89 (1H, ddd,

$J_{14\alpha,14\beta}$ = 10.8 Hz, $J_{14\alpha,3\alpha}$ = 2.6 Hz, $J_{14\alpha,15\alpha}$ = 1.2 Hz, H-14α), 2.68 (1H, d, $J_{6\beta,6\alpha}$ = 15.4 Hz, H-6β), 2.8 (1H, br s, H-15α), 3.08 (1H, t, $J_{5\alpha,6\alpha}$ = 5.6 Hz, $J_{5\alpha,16\alpha}$ = 12.8 Hz, H-5α), 3.14 (1H, dd, $J_{6\alpha,6\beta}$ = 15.4 Hz, $J_{6\alpha,5\alpha}$ = 5.6 Hz, H-6α), 3.55 (3H, s, N-*Me*), 3.46 (1H, dd, $J_{17a,17b}$ = 11.4 Hz, $J_{17a,16\alpha}$ = 5.0 Hz, H-17a), 3.64 (1H, dd, $J_{17b,17a}$ = 11.4 Hz, $J_{17b,16\alpha}$ = 5.0 Hz, H-17b), 3.93 (1H, dd, $J_{3\alpha,14\alpha}$ = 2.6 Hz, $J_{3\alpha,14\beta}$ = 10.2 Hz, H-3α), 4.55 (1H, q, $J_{19,18}$ = 6.5 Hz, H-19), 6.58 (1H, d, $J_{21,15\alpha}$ = 1.4 Hz, H-21), 7.08 (1H, m, H-10), 7.18 (1H, m, H-11), 7.30 (1H, dd, $J_{12,11}$ = 8.1 Hz, H-12), 7.50 (1H, dd, $J_{9,10}$ = 7.7 Hz, $J_{9,11}$ = 1.8 Hz, H-9); ^{13}C nmr see Table 1.

DETERMINATION OF C-19 CONFIGURATION OF **3**.—Compound **3** (5 mg, ca. 0.000015 mol) was added to a solution of racemic 2-phenylbutanoic anhydride (0.2 ml) in dry C_5H_5N (ca. 0.1 ml). The resulting mixture was allowed to stand for 10 h at 18°, H_2O (0.3 ml) was added, and the mixture was allowed to stand for 30 min. NaOH (0.1 M) was then added dropwise until the pH became 9 and the solution was then extracted with $CHCl_3$. The aqueous layer was acidified to pH 3 using 1 M HCl, and the acidic layer was extracted with C_6H_6 (10 ml). The C_6H_6 extract was evaporated to adjust the volume to 1 ml. The optical rotation of 2-phenylbutanoic acid in solution was found to be positive, establishing the *R* configuration of the hydroxyl group at C-19.

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