12. Buschi, C. A., Pomilio, A. B. and Izquierdo, J. A. (to be published).
13. Chadwick, D. J. and Hodgson, S. T. (1983) *J. Chem. Soc. Perkin Trans. I* 93.
14. van Leusen, A. M., Siderius, H., Hoogenboom, B. E. and van Leusen, D. (1972) *Tetrahedron Letters* 5337.

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N$_b$-DEMETHYLALSTOPHYLLINE OXINDOLE, AN OXINDOLE ALKALOID FROM THE LEAVES OF *ALSTONIA MACROPHYLLA*

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Key Word Index *Alstonia macrophylla*; Apocynaceae; *N*$_b$-demethylalstophylline oxindole; NMR.

Abstract -An oxindole alkaloid, *N*$_b$-demethylalstophylline oxindole has been isolated from the leaves of *Alstonia macrophylla*. Its structure has been elucidated on the basis of spectroscopic studies.

INTRODUCTION

Alstonia macrophylla has been introduced to Sri Lanka as a forest tree and has rapidly become naturalized in the moist region up to an elevation of 1200–1500 m [1]. An alkaloid, *N*$_b$-demethylalstophylline oxindole (1), has been isolated from its leaves and its structure elucidated on the basis of mass spectrometry and ^{1}H NMR including two-dimensional NMR (2D *J*-resolved, COSY 45), ^{13}C NMR (BB and DEPT) and NOE difference studies.

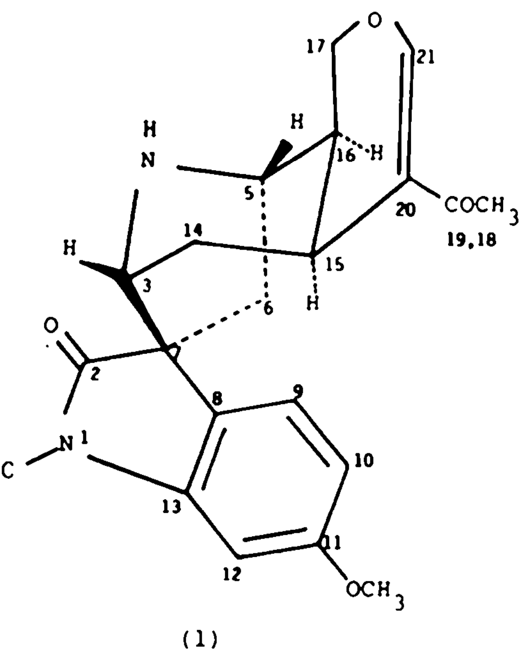
RESULTS AND DISCUSSION

The methanolic extract of the dried leaves was concentrated under reduced pressure and the crude alkaloids were isolated by acid–base extraction. A combination of CC and prep. TLC afforded the new alkaloid named *N*-demethylalstophylline oxindole. It was obtained as a white amorphous solid, $[\alpha]_D^{27}$ +76° (CHCl$_3$, *c* 0.25).

The UV spectrum displayed characteristic absorptions for the oxindole system, with λ_{max}^{MeOH} nm (log ε) 223 (4.29), 256 (3.98), 286 sh (3.44), 294 sh (3.31) and λ_{min}^{MeOH} nm (log ε) 243 (3.91). The IR spectrum showed typical bands at $\nu_{max}^{CHCl_3}$ cm^{-1} 1650 (conj C=O) and 1705 (lactam C=O).

The mass spectrum of 1 showed a $[M]^+$ at *m/z* 368 and the high resolution spectrum showed the exact mass of this peak to be 368.1738 in agreement with the molecular formula $C_{21}H_{24}N_2O_4$ (calcd. 368.1736). Similar measurements on some of the prominent ions served to establish the mass fragmentation pattern of 1. *N*$_b$-Demethylalstophylline oxindole displayed a base peak at *m/z* 179.0949. As reported for other oxindole alkaloids [2, 3] this ion is formed due to cleavage of the spiran ring. The accompanying peaks at *m/z* 161.0837 and 136.0765 are associated with the loss of water and an acyl radical (CH$_3$CO˙) from the base peak. Further decomposition of the base peak results in the formation of the second intense fragment at *m/z* 56.0503. The retro Diels–Alder type fragmentation of ring D gives rise to the indole-containing fragment at *m/z* 244.1211. Another fragment with *m/z* 190.0868 bears the indole skeleton.

The 300 MHz (PFT) ^{1}H NMR spectrum of 1 in CDCl$_3$ showed three methyl singlets at δ2.24, 3.17 and 3.85. These signals were assigned to the acetyl methyl, *N*$_a$-methyl and methoxy groups, respectively. The rather low field value of



(1)

the N_a-methyl group suggested that the nitrogen on which the methyl group was attached was adjacent to the lactam carbonyl group. A one-proton double doublet at δ6.80 was assigned to the H-10 proton, showing *ortho* coupling to H-9 ($J_{10,9}$ = 8.4 Hz) and *meta* coupling to H-12 ($J_{10,12}$ = 2.4 Hz). One proton doublets at δ8.16 ($J_{9,10}$ = 8.4 Hz) and δ6.45 ($J_{12,10}$ = 2.4 Hz) were attributed to H-9 and H-12, respectively. Irradiation of the H-10 proton resulted in the collapse of the doublets at δ8.16 and 6.45 into singlets. Correspondingly the double doublet at δ6.80 for the H-10 proton collapsed into a doublet ($J_{10,9}$ = 8.4 Hz) when the H-12 proton at δ6.45 was irradiated. COSY 45 [4] spectra (Fig. 1) of **1** showed spin–spin coupling of signals of H-10 with H-9. The typical pattern of signals at δ6.45, 6.80 and 8.16 confirmed the presence of a substituent at C-11 of the aromatic nucleus (methoxy group). A low field singlet at δ7.62 was assigned to the olefinic proton, H-21, its shift to low field being on account of its β-position to the carbonyl group and the presence of an adjacent oxygen. Two multiplets centred at δ1.99 and 3.37 were assigned to H-16α and H-15α, respectively. The H-5β proton vicinal to the N_b atom appeared as an unresolved broad doublet at δ3.74 whereas the H-3β proton appeared as a multiplet at δ3.21. The coupling between H-15α and H-14β ($J_{15\alpha,14\beta}$ = 11.9 Hz), as well as the coupling between H-15α and H-14α ($J_{15\alpha,14\alpha}$ = 6.3 Hz) could be observed more clearly when the coupling with the H-16α proton was eliminated by irradiation at δ1.99. The H-14β proton resonated as a broad triplet at δ1.66 (J = 11.2 Hz) while the H-14α proton appeared as a multiplet at δ2.29. Irradiation of the H-14β proton (δ1.66) resulted in the collapse of the multiplets at δ3.21 into a doublet ($J_{3\beta,14\beta}$ = 6.5 Hz). From the magnitude of the coupling constants of proton H-3β, H-14 and H-15α, a chair conformation of ring D can be postulated. A one-proton double doublet at δ2.53 was assigned to the H-6β proton showing coupling with the geminal H-6α proton ($J_{6\alpha,6\beta}$ = 13.5 Hz) and vicinal coupling with the H-5β proton ($J_{6\beta,5\beta}$ = 7.6 Hz). The H-17α proton was found to resonate as a double doublet at 4.28 ($J_{17\alpha,16\alpha}$ = 7, $J_{17\alpha,17\beta}$ = 11 Hz) whereas the H-17β proton resonated as a broad triplet at δ4.58 due to geminal coupling with H-17α ($J_{17\alpha,17\beta}$ = 11 Hz) and vicinal *trans* diaxial coupling with H-16α ($J_{17\beta,16\alpha}$ = 10.5 Hz).

Irradiation of the H-17α proton at δ4.28 led to the collapse of the broad triplet at δ4.58 to a doublet ($J_{17\beta,16\alpha}$ = 10.5 Hz). On the other hand irradiation of the H-17β

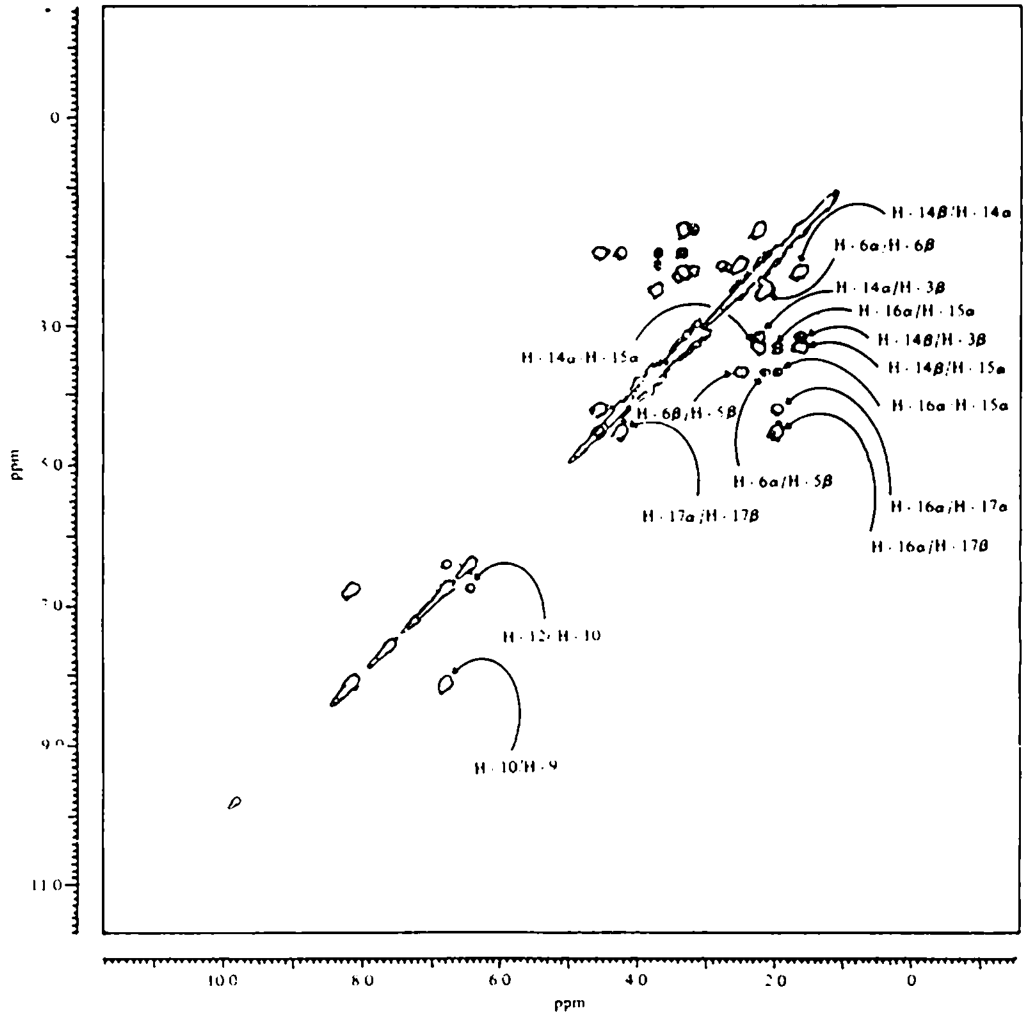


Fig. 1.

proton resulted in the collapse of the double doublet at δ4.28 into a doublet ($J_{17\alpha,16\alpha} = 7$ Hz). Most of the coupling constants were calculated after homo decoupling experiments which led to much simpler spin decoupled patterns of complex multiplets. The spin–spin coupling interactions were determined through the COSY-45 [4] spectra while the multiplicities of the proton signals were unambiguously determined from the 2D *J*-resolved [5] spectrum of **1**. The assignments were made by comparison of the NMR spectra with known related heteroyohimbine and oxindole alkaloids [6–8].

In order to confirm the relative stereochemistry at the various centres NOE difference [9, 10] experiments were carried out. Irradiation of the H-15 multiplet at δ3.37 gave a 13% increase of the double doublet at δ8.16 assigned to the H-9 proton. This shows the proximity of the H-15 and H-9 protons. On the other hand irradiating the H-9 proton at δ8.16 resulted in an 11% NOE at the H-15 proton. These results established that the C-15 proton is transdiaxially disposed to the C-16/C-17 bond. These NOE effects could only be obtained with a *cis* C/D ring junction with the C-7/C-3 bond having a β, and the C-7/C-6 bond an α-configuration. Irradiation of the H-15 proton at δ3.37 led to a 5.5% enhancement of H-16 (δ1.99). This confirmed the configuration of the H-16 proton and *allo* stereochemistry of D and E rings (D/E *cis* ring junction). Irradiation of the methoxy singlet at δ3.85 resulted in an overall 17% increase in the area of the H-10 proton at δ6.80 as well as a 7% increase of the H-12 proton at δ6.45. Corresponding 9% and 3.5% NOE of the aromatic methoxy group were observed by irradiation of the H-10 and H-12 protons, respectively. A NOE enhancement of 6.5% for H-6 was observed upon irradiation of the proton at 3.37 (H-5β proton). The NOE between the geminal protons at C-14, C-6 and C-17 confirmed their ^{1}H NMR assignments (see Fig. 1). The observed NOE values are given in Fig. 2.

75 MHz (PFT) ^{13}C NMR (BB) spectra of N_b-demethylalstophylline oxindole in CDCl$_3$ showed 21 carbon resonances. The multiplicities of each carbon atom were determined using DEPT experiments [11] with polarization pulses of 45°, 90° and 135°. These experiments revealed the presence of three methyl carbons, three methylene carbons and eight methine carbons in agreement with the structure **1**. The C-10 and C-12 aromatic carbons resonated at 106.59 and 96.73, respectively, due to the shielding effect of the C-11 methoxy group, whereas C-11 resonated at δ160.30 due to the deshielding caused by the same group. Two quarternary carbons at δ128.0 and 145.30 in the aromatic region were assigned to the C-8 and C-13 carbons. The N_a methyl carbon appeared at δ26.29 indicating that the nitrogen on which the methyl carbon was attached was a part of a lactam ring. An upfield carbon resonance at δ196.50 was assigned to the C-19 carbon and suggested the presence of an α,β unsaturated ketone moiety. This is supported by the presence of an olefinic methine carbon resonance at δ157.70. The assignments were made by analogy to related alkaloids [12, 13]. On the basis of the results presented here and correlation of the data with Drieding molecular models, structure **1** is assigned to N_b-demethylalstophylline oxindole.

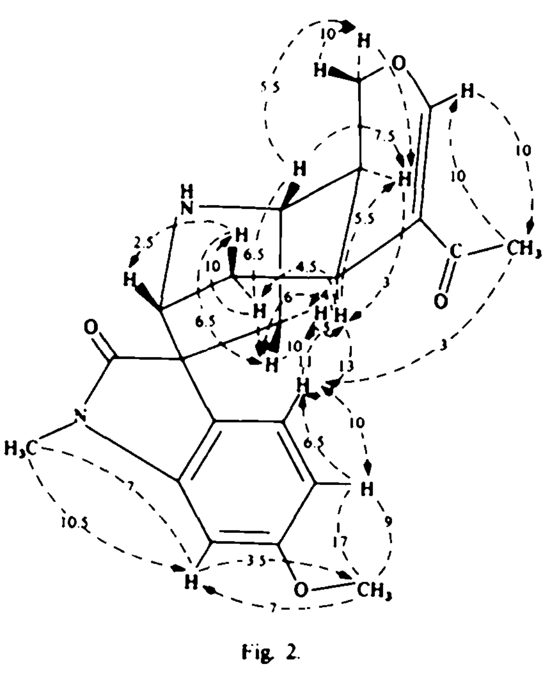


Fig. 2.

EXPERIMENTAL

General. Optical rotation was measured in CHCl$_3$. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR in CDCl$_3$ at 75 MHz with TMS as int. std. DEPT expts were carried out with the polarization pulse $\theta = 45°$ and 90° and 135°. For NOE difference studies the sample soln was degassed by freeze–thaw cycles on a high vacuum line and the normal spectrum was subtracted from the enhanced spectrum so that only differences were recorded.

Plant material was collected from Colombo district and identified by Prof. S. Balasubramaniam from the University of Peradeniya, Sri Lanka.

Extraction and fractionation. Powdered dried leaves (10 kg) of *A. macrophylla* Wall were extracted with MeOH (30 l.) by cold percolation. The solvent was evapd *in vacuo* and the residue macerated with 5% HCl (5 l.). The aq. acidic layer was extracted with CHCl$_3$ (5 l.) for acidic, neutral and weakly basic substances. The aq. acid layer was rendered alkaline to pH 9 with NH$_3$ and extracted with CHCl$_3$ (5 × 5 l.) and dried (Na$_2$SO$_4$). The CHCl$_3$ extract was evapd to dryness to yield crude alkaloids (20 g).

The crude alkaloidal fraction (20 g) was fractionated by flash chromatography over silica gel (75–230 mesh, 1 kg). Elution was carried out with increasing polarities of petrol (1 l.), petrol–CHCl$_3$ (1 l.), CHCl$_3$ (1 l.), CHCl$_3$–MeOH (1 l.) and MeOH (1 l.).

The fraction obtained on elution with petrol–CHCl$_3$ (1:1) was chromatographed on prep. TLC plates (2 mm). Multiple development with petrol–Me$_2$CO (4:1) was carried out to afford the alkaloid N_b-demethylalstophylline oxindole. Detection of alkaloidal bands was carried out using Dragendroff's reagent or UV light (254 and 336 nm).

N$_b$-*Demethyalstophylline oxindole* (**1**). $[\alpha]_D^{22}$ +76; IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1} 1090 (cyclic etc.) 1620 (C=O) 1650 (α,β-unstd C = 20) 1705 (lactam C=O) 2950 (CH); MS: *m/z* (rel. int.) 368 ([M]$^+$, $C_{21}H_{24}N_2O_4$, 85) 244 (5) 190 (68) 179 (100) 161 (40) 136 (54) 118 (13) 56 (82); ^{13}C NMR (75 MHz, CDCl$_3$): 189.50 (C-2) 63–49 (C-3) 56.12 (C-5) 41.72 (C-6) 56.60 (C-7) 128.00 (C-8) 126.33 (C-9) 106.59 (C-10) 160.30 (C-11) 96.73 (C-12) 145.30 (C-13) 30.67 (C-14) 24.15 (C-15) 37.09 (C-16) 68.10 (C-17) 24.90 (C-18) 196.50 (C-19) 121.50 (C-20) 157.70 (C-21) 26.29 (N$_a$–CH$_3$) 55.58 (Ar–OCH$_3$).

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REFERENCES

1. Abeywickrama, B. A. *Flora of Ceylon* (Revised hand book), Vol. 1, p. 12.
2. Gilbert, B., Brissolese, J. A., Finch, N., Taylor, W. I., Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 1523.
3. Shamma, M. and Foley, K. F. (1967) *J. Am. Chem. Soc.* **32**, 4141.
4. Bax, A. and Freeman, R. (1981) *J. Magn. Reson.* **42**, 64.
5. Aue, W. P., Karhan, J. and Ernst, R. R. (1976) *J. Chem. Phys.* **64**, 4226.
6. Hofle, G., Heinstein, P., Stockigt, J. and Zenk, M. H. (1980) *Planta Med.* **40**, 120.
7. Phillipson, J. D. and Hemingway, S. R. (1973) *Phytochemistry* **12**, 1481.
8. Beecham, A. F., Hart, N. K., Johns, S. R. and Lamberton, J. A. (1968) *Aust. J. Chem.* **21**, 491.
9. Noggle, J. H. and Schirmer, R. F. (1971) *The Nuclear Overhauser Effect*. Academic Press, New York.
10. Sanders, J. K. M. and Mersh, J. D. (1982) *Prog. NMR Spectrosc.* **13**, 353.
11. Doddrell, D. M., Pegg, D. T. and Bendall, M. R. (1982) *J. Magn. Reson.* **48**, 323.
12. Wenkert, E., Bindra, J. S., Chang, C. J., Cohran, D. W. and Schell, F. M. (1974) *Acc. Chem. Res.* **7**, 46.
13. Wenkert, E., Chang, C. J., Chawla, H. P. S., Cochran, D. W., Hagaman, E. W., King, J. C. and Orito, K. (1976) *J. Am. Chem. Soc.* **98**, 3045.

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ALKALOIDS OF *ALSTONIA MACROPHYLLA*

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Key Word Index—*Alstonia macrophylla*; Apocynaceae; stem bark; leaves; indole alkaloids; ^{13}C NMR; DEPT.

Abstract—A new alkaloid, 19-hydroxyvincamajine, has been isolated from the leaves of *Alstonia macrophylla*. In addition to this, eight other indole alkaloids, alstonerine, alstophylline, macralstonine, anhydromacralstonine, talcarpine, vincamajine, vincorine and cabucraline, were also isolated and identified from the bark and leaves of *A. macrophylla* of Sri Lanka. The last five alkaloids have been isolated for the first time from this species and the ^{13} NMR of alstonerine and alstophylline are reported.

INTRODUCTION

Alstonia macrophylla is a common plant in Sri Lanka. Several studies on this species growing in other countries have been reported [1-10] and the plant is used in medicinal preparations in the Philippines [2]. However, little previous work has been done on *A. macrophylla* growing in Sri Lanka. Therefore, an investigation of its alkaloidal constituents was initiated.

RESULTS AND DISCUSSION

Four alkaloids, namely talcarpine, alstonerine, macralstonine and anhydromacralstonine, have been isolated and identified from the bark of *A. macrophylla* [12], and vincorine, vincamajine, cabucraline, alstophylline from the leaves, by comparing their spectra with those of known compounds. A new vincamajine derivative was also isolated from the leaves of the plant. Its IR spectrum contained an acetyl band at 1730 cm^{-1}, a band at 740 cm^{-1} characteristic of an *ortho* disubstituted benzene and another band at 1608 cm^{-1}. The ^{1}H NMR spectrum confirmed the presence of an acetyl group at δ3.7 (3H, *s*), a *N*-methyl group at δ2.6 and showed a doublet at δ1.0 (J = 7 Hz). The NMR spectrum of the new compound therefore shows a close resemblence to that of vincamajine [13-15] except for the doublet at δ1.0. The doublet at δ1.0 (J = 7 Hz) can be assigned to a C-19 methyl group. The mass spectrum showed the $[M]^+$ at m/z 384 and a peak at m/z 360 which can be formed by the loss of water from the $[M]^+$. Peaks in the spectrum of lower m/z values can be explained by comparison with the fragmentation patterns established for vincamajine. The fragment ion at m/z 222 is characteristic of alkaloids belonging to the quebrachidine group and the ion at m/z 190 is formed by the loss of MeOH from the former ion. These spectral data indicate that the compound is 19-hydroxyvincamajine (**1**) which is a new alkaloid. Recently *O*-