

ISOLATION AND STRUCTURAL STUDIES ON THE ALKALOIDS OF ALSTONIA MACROPHYLLA

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Abstract - A new oxindole alkaloid, 16-hydroxy-N_b-demethylalstophylline oxindole (I) has been isolated from the leaves of Alstonia macrophylla. Its structure has been determined by modern spectroscopic studies, particularly by NOE difference, COSY 45° and DEPT experiments. N_a-Methyl-1,2-dihydrostrictamine (II) has also been isolated from this new source.

Alstonia macrophylla has been introduced to Sri Lanka as a forest tree and rapidly become naturalized up to an elevation of 1200-1500¹m. It is a rich source of monomeric and dimeric indole alkaloids. 16-Hydroxy-N_b-demethylalstophylline oxindole (I), a new oxindole alkaloid, has been isolated from the leaves and its structure elucidated on the basis of mass spectrometry and ¹H-nmr including two dimensional nmr (2D J-resolved, COSY 45°), ¹³C-nmr (BB and DEPT) and NOE difference studies. Another alkaloid, N_a-methyl-1,2-dihydrostrictamine² (II), has also been isolated from the leaves of Alstonia macrophylla, and its ¹H-nmr and ¹³C-nmr spectral data recorded.

16-Hydroxy-N_b-demethylalstophylline oxindole (I) was obtained as a white crystalline material, mp 215⁰C. It afforded a uv spectrum characteristic of oxindole alkaloids, showing absorption maxima at 218, 255, 285 and 294 nm, while the absorption minimum was observed at 242 nm. The ir spectrum exhibited peaks at 1690 (lactam C=O) and 1620 cm⁻¹ (keto C=O). The mass spectrum of the compound (I) showed the molecular ion at m/z 384.1672 corresponding to the formula C₂₁H₂₄N₂O₅ indicating the presence of eleven double bond equivalents in the molecule. The base peak appeared at m/z 176.0711, corresponding to the formula C₁₀H₁₀NO₂. The loss of CH₂O from M⁺ gave a daughter peak at m/z 354.1603 (C₂₀H₂₂N₂O₄). The peak at m/z 190.0867 represented the ion corresponding to the formula (C₁₁H₁₂NO₂)⁺. An important peak appearing at m/z 195.0895 for (C₁₀H₁₃NO₃)⁺ ion, after removal of a water molecule, afforded a peak at m/z 177.0765 having the formula C₁₀H₁₁NO₂. This suggested that there is a hydroxyl group at C-16 in the molecule.

The ^1H -nmr spectrum (300 MHz) of (I) in CDCl_3 showed three methyl singlets at δ 2.23, 3.14 and 3.82. These signals were assigned to the acetyl methyl, N_a -methyl and methoxy methyl protons, respectively. The rather low field value of N_a -methyl protons suggested that the nitrogen on which the methyl group was attached was adjacent to the lactam carbonyl group⁶. A one-proton double-doublet at δ 6.77 was assigned to C-10 proton, showing *ortho* coupling to C-9 proton ($J_{10,9} = 8.4$ Hz) and *meta* coupling to C-12 proton ($J_{10,12} = 2.3$ Hz). This was confirmed by irradiation of the C-10 proton, which resulted in the collapse of the doublets at δ 8.05 (C-9H) and δ 6.42 (C-12H) into singlets. Correspondingly the double doublet at δ 6.77 for the C-10 proton collapsed into a doublet ($J_{10,9} = 8.4$ Hz) when the C-12 proton at δ 6.42 was irradiated. The COSY 45 $^\circ$ 9,10 spectrum established the spin-spin coupling between signals at δ 6.77 (C-10H) with the signals at δ 8.05 (C-9H). The typical pattern of signals at δ 6.42, 6.77 and 8.05 confirmed the presence of a substituent (OMe) at C-11 of the aromatic nucleus. A downfield singlet at δ 7.62 was assigned to the olefinic proton (C-21H), its downfield position being on account of its β -disposition to the carbonyl group and the presence of an adjacent oxygen atom. Two multiplets centred at δ 3.05 and 3.48 were assigned to C-15 $_\alpha$ and C-5 $_\beta$ protons respectively. The COSY 45 $^\circ$ spectrum showed prominent cross peaks suggesting that C-15 $_\alpha$ proton is coupled to C-14 protons while C-5 $_\beta$ proton is coupled to C-6 methylene protons. When the ^1H -nmr spectrum of the compound was compared with that of closely related compounds⁶, it was found that the multiplet for C-16 proton (which resonates at δ 1.99) is absent, indicating a substitution at this position. This was substantiated by the presence of a clean one-proton doublet for the C-17 $_\beta$ proton at δ 4.70 ($J_{17_\beta, 17_\alpha} = 11.5$ Hz), showing only geminal coupling with the C-17 $_\alpha$ proton which in turn showed a split doublet at δ 3.98 showing geminal coupling with C-17 $_\beta$ proton and a W-coupling with C-21H ($J_{17_\alpha, 17_\beta} = 11.5$, $J_{17_\alpha, 21} = 2.04$ Hz respectively). Three doublets showing geminal coupling were observed at δ 2.29 ($J_{14_\alpha, 14_\beta} = 13.0$ Hz), 2.38 ($J_{6_\beta, 6_\alpha} = 13.0$ Hz) and 2.48 ($J_{6_\alpha, 6_\beta} = 13.0$ Hz) and assigned to C-14 $_\alpha$, 6 $_\beta$ and 6 $_\alpha$ protons, respectively. The C-14 $_\beta$ proton appeared as a double triplet at δ 1.45 ($J_{14_\beta, 14_\alpha} = 13.0$ Hz, $H_{14_\beta, 15_\alpha} = 4.0$ Hz). A broad singlet appearing at δ 3.17 was assigned to the C-3 $_\beta$ proton which is vicinal to N_b -atom. Most of the coupling constants were calculated from the decoupled spectra, which led to much simpler spin decoupled patterns of the previously complex multiplets. The spin-spin coupling interactions were determined through the COSY 45 $^\circ$ 9,10 spectrum and the multiplicities of the protons were confirmed from the 2D J-resolved^{10,11} spectrum of (I).

In order to confirm the relative stereochemistry at the various centres, NOE difference^{10,12,13} experiments were carried out. Irradiation of the multiplet for C-15H resonating at δ 3.05 gave a 5.4% increase in the intensity of the doublet at δ 8.05 due to the C-9 proton which in turn gave a 13% NOE

of the C-15 proton. These results led to the conclusion that the C-15 proton is trans diaxially disposed to the C-16/C-17 bond. These NOE effects could only be achieved from a cis C/D ring junction with the C-7/C-3 bond having a β and C-7/C-6 bond an α -configuration. The C-5 β proton when irradiated caused 5.4% enhancement of the C-17 α proton resonating at δ 3.98 whereas 8% NOE effect was observed in the case of the C-6 β proton at δ 2.38. The observed NOE values are given in fig. 1.

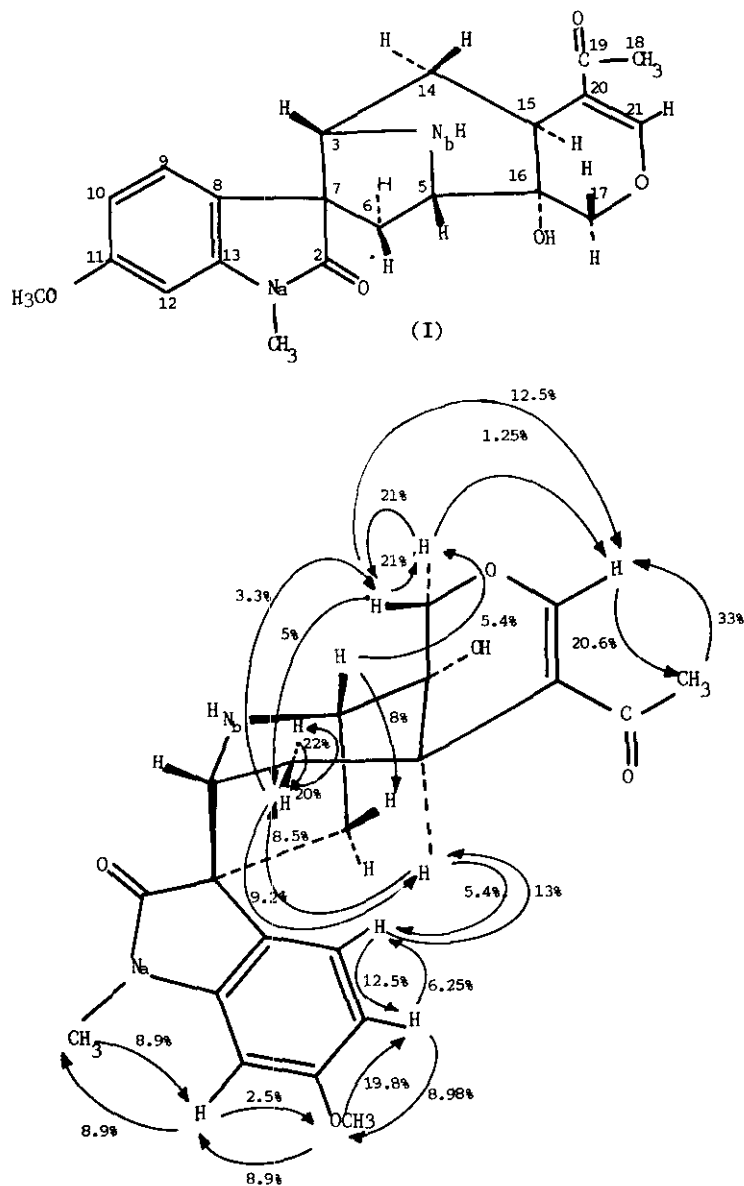


Fig. 1

The ^{13}C -nmr spectrum of 16-hydroxy- N_b -demethylalstophylline oxindole (Table I) showed 21 carbon resonances. The multiplicities of each carbon atom were determined using DEPT experiments^{10,14} with polarization pulses of 45° , 90° and 135° . These experiments revealed the presence of three methyl, three methylene and seven methine carbons in agreement with structure (I). The three methyl signals appeared at δ 25.09, 26.32 and 55.59. The upfield signal at δ 25.09 was assigned to the methyl carbon of the acetyl group present at C-20. The N_a -methyl carbon appeared slightly downfield at δ 26.32 indicating that the nitrogen on which the methyl carbon was attached was a part of a lactam ring⁶, whereas the downfield methyl carbon resonated at δ 55.59 being assigned to the aromatic methoxy carbon present at C-11. The C-10 and C-12 aromatic carbons resonated at δ 106.42 and δ 96.66 respectively, due to the shielding effect of the methoxy group whereas the deshielding effect of the group was responsible for the downfield shift of the C-11 carbon atom appearing at δ 160.20. The signal at δ 196.73 was assigned to the 19-carbonyl carbon. Its upfield shift suggested the presence of an α , β -unsaturated ketone moiety. An olefinic methine carbon resonance was observed at δ 156.63. The downfield resonance of the C-16 quaternary carbon atom (δ 68.23) suggested the presence of an OH group on it. These assignments were made by analogy to related alkaloids³⁻⁸. On the basis of results presented here and correlation of the data with Drieding molecular models, structure (I) (16-hydroxy- N_b -demethylalstophylline oxindole) is assigned to the new alkaloid.

Table-I: ^{13}C -nmr (75 MHz, CDCl_3) of (I).

Carbon	Chemical shift (ppm)	Multiplicity (DEPT)
2	182.95	C=O
3	63.45	-CH
5	61.19	-CH
6	37.99	-CH ₂
7	56.89	-C- ²
8	120.82	-C-
9	126.06	-CH
10	106.42	-CH
11	160.20	-C-
12	96.66	-CH
13	145.31	-C-
14	33.49	-CH ₂
15	33.18	-CH ₂
16	68.23	-C-
17	71.60	-CH ₂
18	25.09	-CH ₂
19	196.73	-C=O
20	119.75	-C-
21	156.63	-CH
N_a -CH ₃	26.32	-CH ₃
OCH ₃	55.59	-CH ₃

^{N^a}-Methyl-1,2-dihydrodrositricamine² (II) was isolated by a combination of column chromatography over silica gel and thin layer chromatography (silica). It afforded uv absorptions at 246 and 292 nm, indicating the presence of a dihydroindole chromophore. The ir spectrum showed an ester carbonyl peak at 1730 cm⁻¹. The mass spectrum showed the molecular ion at m/z 338.1989 corresponding to the molecular formula C₂₁H₂₆N₂O₂. The ¹H-nmr spectrum exhibited the presence of 4 aromatic protons. The signal at δ 1.47 was assigned to C-18 methyl protons. The ester methyl protons appeared at δ 3.77 as a singlet. Another three-proton singlet resonating at δ 2.68 was due to the presence of the methyl group at N^a. The C-2 methine proton resonated as a sharp singlet and did not show any coupling with the C-3 proton, suggesting that these protons have a trans orientation. The mass and ¹H-nmr values were in complete agreement with the data reported earlier.²

The ¹³C-nmr (BB, 75 MHz) spectra of N^a-methyl-1,2-dihydrodrositricamine (II) (Table-II) in CDCl₃ showed 21 carbon resonances. The multiplicities of each carbon atom were determined using DEPT experiments. These experiments revealed the presence of three methyl, four methylene and nine methine carbon atoms in agreement with the proposed structure (II). The three methyl carbon atoms appearing at δ 12.96, 33.99 and 51.47 were assigned to C-18, N^a-methyl and ester methyl carbon atoms respectively. The C-5 and C-21 methylene carbons resonated at δ 50.72 and δ 54.85 respectively, whereas the vinylic methine carbon appeared at δ 127.22. The other methine carbon atoms showed their resonances at δ 34.42, 47.24, 52.87 and 79.38. These signals were assigned to the C-15, C-3, C-3, C-16 and C-2 carbon atoms respectively. An upfield signal at δ 43.06 was assigned to the C-7 quaternary carbon atom.

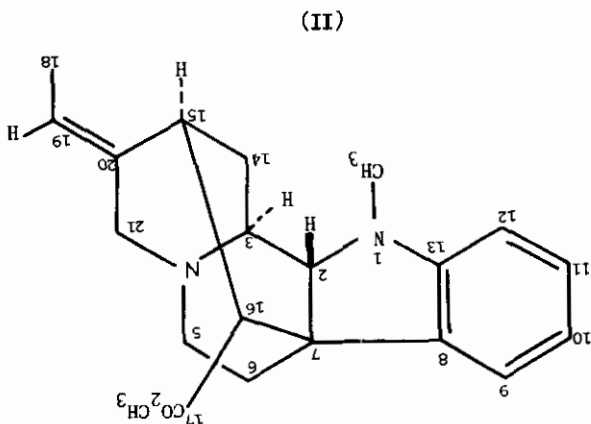


Table-II: ^{13}C -nmr (75 MHz, CDCl_3) of (II)

Carbon	Chemical shift (ppm)	Multiplicity (DEPT)
2	79.38	-CH
3	47.24	-CH
5	50.72	-CH ₂
6	31.25	-CH ₂ ²
7	43.06	-C- ²
8	140.53	-C-
9	109.44	-CH
10	120.96	-CH
11	119.38	-CH
12	119.00	-CH
13	153.28	-C-
14	33.94	-CH ₂
15	34.42	-CH ₂ ²
16	52.87	-CH
17	173.04	-C=O
18	12.96	-CH ₃
19	127.22	-CH ₃
20	139.35	-C-
21	54.85	-CH ₂
N-CH ₃	33.99	-CH ₃ ²
CO ₂ OCH ₃	51.47	-CH ₃ ³

EXPERIMENTAL

Optical rotation was measured in CHCl_3 . Ir and uv spectra were measured on JASCO - IRA-1 and Pye-Unicam SP-800 spectrometers respectively. Mass spectra were recorded on Finnigan MAT 112 and 312 double focussing spectrometers. The ^1H -nmr spectra were recorded on Bruker AM 300 NMR spectrometer. The ^{13}C -nmr spectra were recorded in CDCl_3 at 75 MHz with TMS as int.std. DEPT experiments were carried out with the polarization pulse $\theta = 45^\circ, 90^\circ$ and 135° . For NOE difference studies the sample 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.

EXTRACTION AND FRACTIONATION

Powdered dried leaves (10 kg) of A. macrophylla Wall were extracted with MeOH (30 l) by cold percolation. The solvent was evaporated in vacuo and the residue macerated with 5% HCl (5 l). The aqueous acidic layer was extracted with CHCl_3 (5 l) for acidic, neutral and weakly basic substances. The aqueous acidic layer was rendered alkaline to pH 9 with NH_3 and extracted with CHCl_3 (5 x 5

l) and dried (Na_2SO_4). The CHCl_3 extract was filtered and evaporated to dryness to yield the crude alkaloids (20 g). These were fractionated by column chromatography over silica gel. Elution was carried out with increasing polarities of pet. ether, pet. ether : acetone, acetone, acetone : ethylacetate, ethylacetate, ethylacetate : MeOH, and MeOH.

Isolation of 16-Hydroxy- N_b -demethylalstophylline Oxindole (I)

The fraction obtained on elution with pet. ether : acetone (50:50) was kept in MeOH in the refrigerator to afford white crystals. mp 215°C , $[\alpha]_D \text{CHCl}_3 = +104.47^\circ$ ($c = 0.067$), uv: λ_{max} (MeOH) nm: 218, 255, 285, 294, λ_{min} (MeOH) nm: 242. Ir ν_{max} (CHCl_3) cm^{-1} : 1690 (lactam C=O), 1620 (keto C=O). HRMS m/z (%): 384.1672 (M^+ , calcd. for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_5$: 384.1685) (87.8), 354.1603 ($\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_4$) (18.5), 244.1164 ($\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_2$) (3.4), 195.0895 ($\text{C}_{10}\text{H}_{13}\text{NO}_3$) (6.8), 190.0867 ($\text{C}_{11}\text{H}_{12}\text{NO}_2$) (85.5), 177.0765 ($\text{C}_{10}\text{H}_{11}\text{NO}_2$) (42.6), 176.0711 ($\text{C}_{10}\text{H}_{10}\text{NO}_2$) (100), 160.0767 ($\text{C}_{10}\text{H}_{10}\text{NO}$) (25.9), 152.0672 ($\text{C}_8\text{H}_{10}\text{NO}_2$) (20.9), 134.0607 ($\text{C}_8\text{H}_8\text{NO}$) (39.9), 122.0610 ($\text{C}_7\text{H}_8\text{NO}$) (20.1), 96.0458 ($\text{C}_5\text{H}_6\text{NO}$) (29.8).

Isolation of N_a -Methyl-1,2-dehydrostrictamine (II)

The fraction obtained on elution with 20% MeOH/ethyl acetate was chromatographed on precoated silica gel plates to afford compound (II). $[\alpha]_D \text{CHCl}_3 = -58.13^\circ$ ($c = 0.172$), uv: λ_{max} (MeOH) nm: 246 and 292. Ir ν_{max} (CHCl_3) cm^{-1} : 1730 (ester C=O). HRMS m/z (%): 338.1990 (M^+ , calcd. for $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_2$: 338.1994) (18.3), 323.1792 ($\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}_2$) (1.5), 307.1816 ($\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}$) (2.5), 265.1701 ($\text{C}_{18}\text{H}_{21}\text{N}_2$) (12.7), 194.1173 ($\text{C}_{11}\text{H}_{16}\text{NO}_2$) (75.9). $^1\text{H-nmr}$ (300 MHz, CDCl_3) δ : 7.07 (1H, dt, $J_{11,12} = 7.7$ Hz, $J_{11,9} = 1.0$ Hz, H-11), 6.93 (1H, dd, $J_{9,10} = 7.4$ Hz, $J_{9,11} = 1.0$ Hz, H-9), 6.68 (1H, dt, $J_{10,9} = 7.4$ Hz, $J_{10,12} = 1.0$ Hz, H-10), 6.59 (1H, d, $J_{12,11} = 7.7$ Hz, H-12), 5.37 (1H, q, $J_{19,18} = 6.9$ Hz, H-19), 4.09 (1H, d, $J_{3,14a} = 5.0$ Hz, H-3), 3.90 (1H, d, $J_{\text{gem}} = 16.5$ Hz, H-21a), 3.77 (3H, s, OCH_3), 3.76 (1H, m, H-6a), 3.58 (1H, d, $J_{15,16} = 3.0$ Hz, H-15), 3.05 (1H, m, H-5b), 2.93 (1H, d, $J_{16,15} = 3.0$ Hz, H-16), 2.88 (1H, d, $J_{\text{gem}} = 16.5$ Hz, H-21b), 2.68 (3H, s, $\text{N}_a\text{-CH}_3$), 2.60 (1H, dd, $J_{\text{gem}} = 13.0$ Hz, $J_{5a,6b} = 6.0$ Hz, H-5a), 2.49 (1H, s, H-2 β), 2.36 (1H, m, H-14a), 1.57 (1H, dd, $J_{\text{gem}} = 13.5$ Hz, H-14b), 1.47 (3H, dd, $J_{18,19} = 6.9$ Hz, $J_{18,21} = 2.4$ Hz, H-18), 1.42 (1H, dd, $J_{\text{gem}} = 15.0$ Hz, $J_{6b,5a} = 6.0$ Hz, H-6b).

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