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.

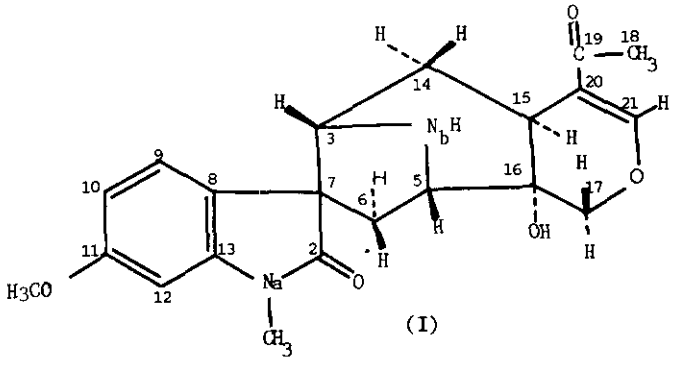
Alstonia macrophylla has been introduced to Sri Lanka as a forest tree and rapidly become naturalized up to an elevation of 1200-1500[1]m. It is a rich source of monomeric and dimeric indole alkaloids. 16-Hydroxy-N_b-demethylalstophylline oxindole (I), a new oxindole alkaloid, has been isolated from the leaves and its structure elucidated on the basis of mass spectrometry and ^{1}H-nmr including two dimensional nmr (2D J-resolved, COSY 45°), ^{13}C-nmr (BB and DEPT) and NOE difference studies. Another alkaloid, N_a-methyl-1,2-dihydrostrictamine[2] (II), has also been isolated from the leaves of Alstonia macrophylla, and its ^{1}H-nmr and ^{13}C-nmr spectral data recorded.

16-Hydroxy-N_b-demethylalstophylline oxindole (I) was obtained as a white crystalline material, mp 215°C. 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 $C_{21}H_{24}N_2O_5$ indicating the presence of eleven double bond equivalents in the molecule. The base peak appeared at m/z 176.0711, corresponding to the formula $C_{10}H_{10}NO_2$. The loss of CH_2O from M^+ gave a daughter peak at m/z 354.1603 ($C_{20}H_{22}N_2O_4$). The peak at m/z 190.0867 represented the ion corresponding to the formula $(C_{11}H_{12}NO_2)^+$. An important peak appearing at m/z 195.0895 for $(C_{10}H_{13}NO_3)^+$ ion, after removal of a water molecule, afforded a peak at m/z 177.0765 having the formula $C_{10}H_{11}NO_2$. This suggested that there is a hydroxyl group at C-16 in the molecule.

The ^{1}H-nmr spectrum (300 MHz) of (I) in $CDCl_3$ showed three methyl singlets at δ2.23, 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 group[6]. 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 δ6.42 was irradiated. The COSY 45° [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-15α and C-5β protons respectively. The COSY 45° spectrum showed prominent cross peaks suggesting that C-15α proton is coupled to *C-14 protons while* C-5 β *proton* is coupled to C-6 methylene protons. When the ^{1}H-nmr spectrum of the compound was compared with that of closely related compounds[6], it was found that the multiplet for C-16 proton (which resonates at δ1.99) is absent, indicating a substitution at this position. This was substantiated by *the presence of a clean one-proton* doublet for the C-17β proton at δ 4.70 ($J_{17\beta,17\alpha}$ = 11.5 Hz), showing only geminal coupling with the C-17α proton which in turn showed a split doublet at δ3.98 showing geminal coupling with C-17β 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 δ2.29 ($J_{14\alpha,14\beta}$ = 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-14α6β and -6α protons, respectively. The C-14β proton appeared as a double triplet at δ1.45 ($J_{14\beta,14\alpha}$ = 13.0 Hz, $H_{14\beta,15\alpha}$ = 4.0 Hz). A broad singlet appearing at δ3.17 was assigned to the C-3β 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° [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* δ3.05 *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-5β proton when irradiated caused 5.4% enhancement of the C-17α proton resonating at δ 3.98 whereas 8% NOE effect was observed in the case of the C-6β proton at δ2.38. The observed NOE values are given in fig. 1.



(I)

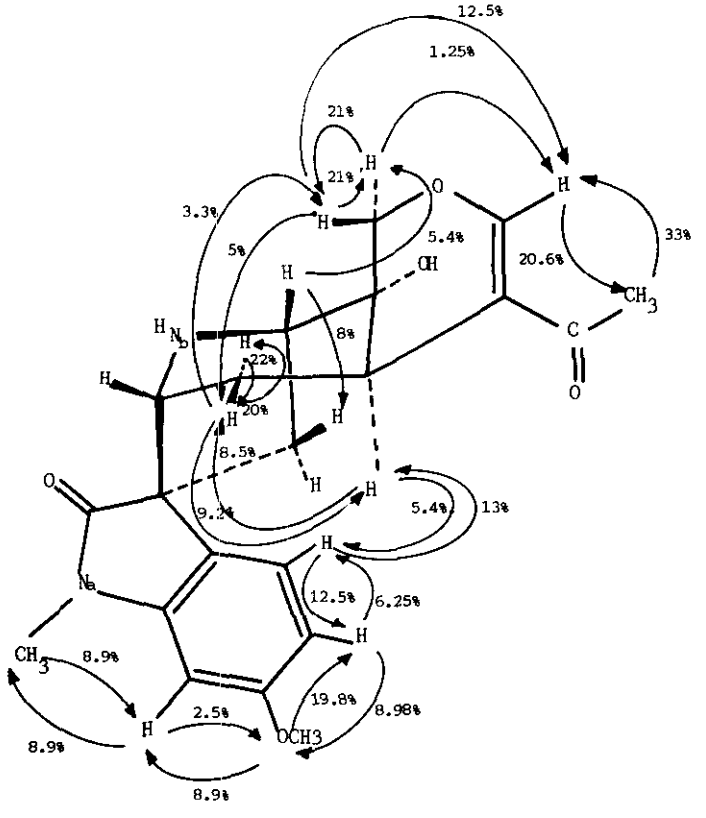


Fig. 1

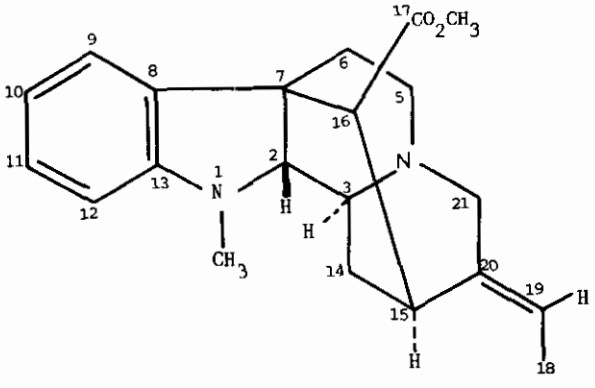
The ^{13}C-nmr spectrum of 16-hydroxy-N_b-demethylalstophylline oxindole (Table I) showed 21 carbon resonances. The multiplicities of each carbon atom were determined using DEPT experiments[10,14] with polarization pulses of 45^o, 90^o and 135^o. These experiments revealed the presence of three methyl, three methylene and seven methine carbons in agreement with structure (I). The three methyl signals appeared at δ25.09, 26.32 and 55.59. The upfield signal at δ25.09 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 ring[6], 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 δ160.20. The signal at δ196.73 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 alkaloids[3-8]. 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-
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
16	68.23	-C-
17	71.60	$-CH_2$
18	25.09	$-CH_3$
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-dihydrostrictamine[2] (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 $C_{21}H_{26}N_2O_2$. The ^{1}H-nmr spectrum exhibited the presence of 4 aromatic protons. The signal at δ1.47 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 ^{1}H-nmr values were in complete agreement with the data reported earlier[2].

The ^{13}C-nmr (BB, 75 MHz) spectra of N_a-methyl-1,2-dihydrostrictamine (II) (Table-II) in $CDCl_3$ 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 carbons 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 δ34.42, 47.24, 52.87 and 79.38. These signals were assigned to the C-15, C-3, C-16 and C-2 carbon atoms respectively. An upfield signal at δ43.06 was assigned to the C-7 quaternary carbon atom.



(II)

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	$-CH_2$
7	43.06	-C-
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	$-CH_2$
16	52.87	-CH
17	173.04	-C=O
18	12.96	$-CH_3$
19	127.22	-CH
20	139.35	-C-
21	54.85	$-CH_2$
N_a-CH_3	33.99	$-CH_3$
$CO.O\underline{CH}_3$	51.47	$-CH_3$

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 ^{1}H-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 $\theta = 45^o$, 90^o and 135^o. For NOE difference studies the sample was degassed by freeze-thaw cycles on a high vacuum line and the normal spectrum was substracted 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

I) 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, $[\alpha]_D$ $CHCl_3$ = + 104.47° (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 $C_{21}H_{24}N_2O_5$: 384.1685) (87.8), 354.1603 ($C_{20}H_{22}N_2O_4$) (18.5), 244.1164 ($C_{14}H_{16}N_2O_2$) (3.4), 195.0895 ($C_{10}H_{13}NO_3$) (6.8), 190.0867 ($C_{11}H_{12}NO_2$) (85.5), 177.0765 ($C_{10}H_{11}NO_2$) (42.6), 176.0711 ($C_{10}H_{10}NO_2$) (100), 160.0767 ($C_{10}H_{10}NO$) (25.9), 152.0672 ($C_8H_{10}NO_2$) (20.9), 134.0607 (C_8H_8NO) (39.9), 122.0610 (C_7H_8NO) (20.1), 96.0458 (C_5H_6NO) (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**). $[\alpha]_D$ $CHCl_3$ = - 58.13° (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 $C_{21}H_{26}N_2O_2$: 338.1994) (18.3), 323.1792 ($C_{20}H_{23}N_2O_2$) (1.5), 307.1816 ($C_{20}H_{23}N_2O$) (2.5), 265.1701 ($C_{18}H_{21}N_2$) (12.7), 194.1173 ($C_{11}H_{16}NO_2$) (75.9). ^{1}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_{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_{gem} = 16.5 Hz, H-21b), 2.68 (3H, s, N_a-CH_3), 2.60 (1H, dd, J_{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_{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_{gem} = 15.0 Hz, $J_{6b,5a}$ = 6.0 Hz, H-6b).

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