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MACROXINE-A NOVEL OXINDOLE ALKALOID FROM *ALSTONIA MACROPHYLLA*

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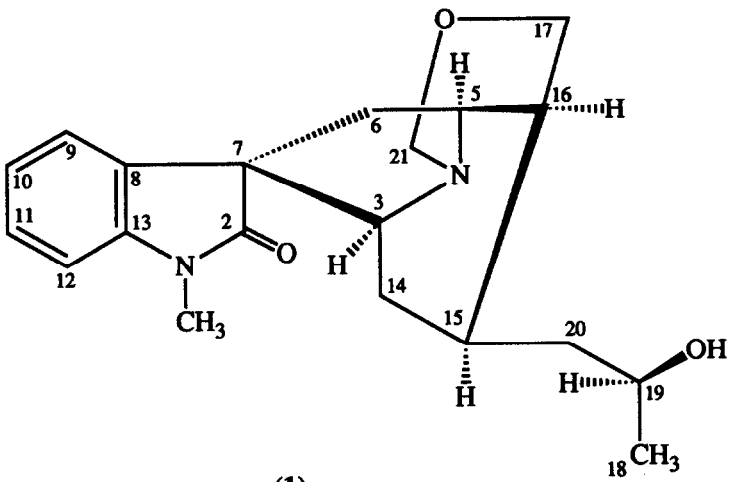
Abstract- A novel oxindole alkaloid macroxine (1) has been isolated from the leaves of *Alstonia macrophylla* and its structure was established on the basis of 1D and 2D NMR experiments including HOHAHA, HMQC and HMBC measurements. It is the first member of a new class of oxindole alkaloids.

Alstonia macrophylla Wall. (Apocynaceae) is native to Sri Lanka. Several oxindole alkaloids have been reported from *Alstonia* species.[1,2] Our investigations on the leaves of *A. macrophylla* have led to the isolation of a novel oxindole alkaloid named macroxine (**1**) from the ethanolic extracts by repeated column chromatography.

The high resolution mass spectroscopy of (**1**) confirmed its mass as 342. 1947 thus establishing its molecular formula as $C_{20}H_{26}N_2O_3$. An important fragment at m/z152.1066 (40%) in its mass spectrum corresponded to the formula $C_9H_{14}NO$, not usually found in oxindole alkaloids. An important peak at m/z 284.1563 corresponding to the formula $C_{17}H_{20}N_2O_2$ appeared to be due to the loss of a hydroxylated propyl unit. The UV spectrum of (**1**) exhibited absorption at 256 nm characteristic of the oxindole chromophore. In its IR spectrum it showed an absorption at 1700 cm^{-1} (oxindole carbonyl) and 3300 cm^{-1} (hydroxyl group).

The ^{1}HNMR spectrum (400 MHz, $CDCl_3$, Table 1) afforded AB doublets for two protons at δ 4.71 and 4.88 (J = 11.4Hz). The downfield chemical shifts and the presence of geminal coupling suggested that the two protons were those of a methylene group which was located between two hetero atoms. The four aromatic protons were found to resonate at δ 7.55, 6.92, 7.20 and 6.73, their number and splitting pattern, indicating the lack of any other substituent on the benzene ring. Of the two methyl groups one resonated as a singlet at δ 3.20 assigned to the N_a-CH_3 protons, while the other appeared as a doublet at δ 1.31 showing vicinal coupling in the COSY spectrum with a split quartet for the methine proton at δ 3.95. The downfield chemical shift of this methine proton and the coupling pattern established the presence of a CH_3 CHOH grouping. The methine proton of the CHOH group was found to be coupled in the COSY spectrum with two double doublets at δ 1.50 and 1.66 indicating the presence of a hydroxylated propyl side chain. The



(1)

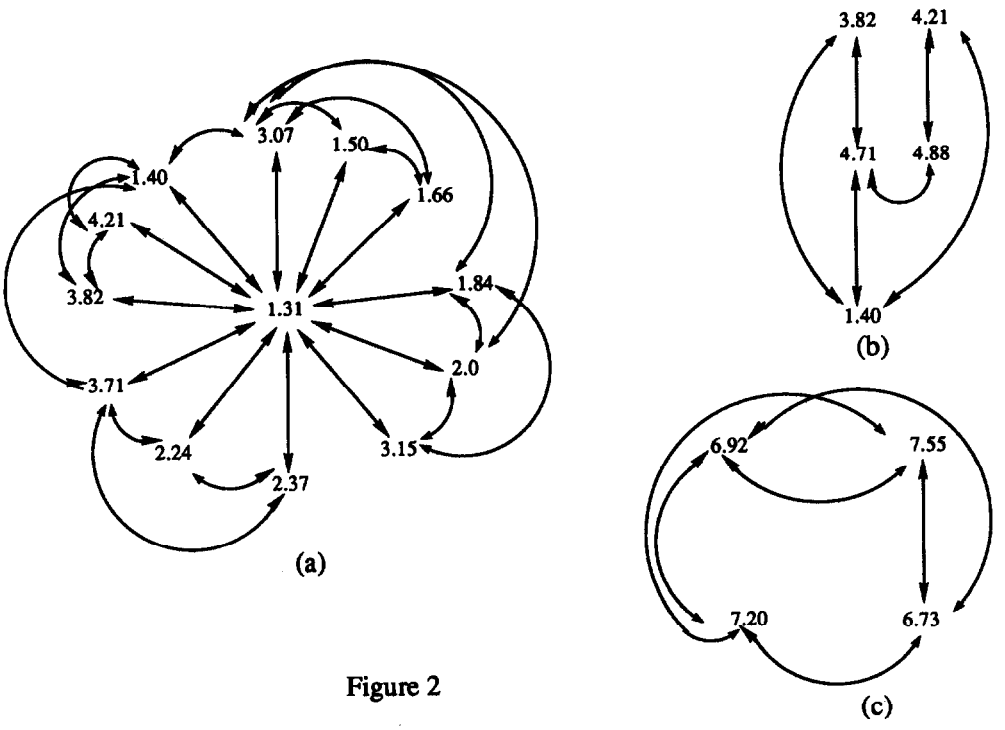


Figure 2

Figure 2 (a), (b), (c): HOHAHA (straight arrows) and COSY (curved arrows) interactions for macroxine (1)

Table 1. ^{1}H (400 MHz) and ^{13}C(100 MHz) NMR data for macroxine (1) in $CDCl_3$

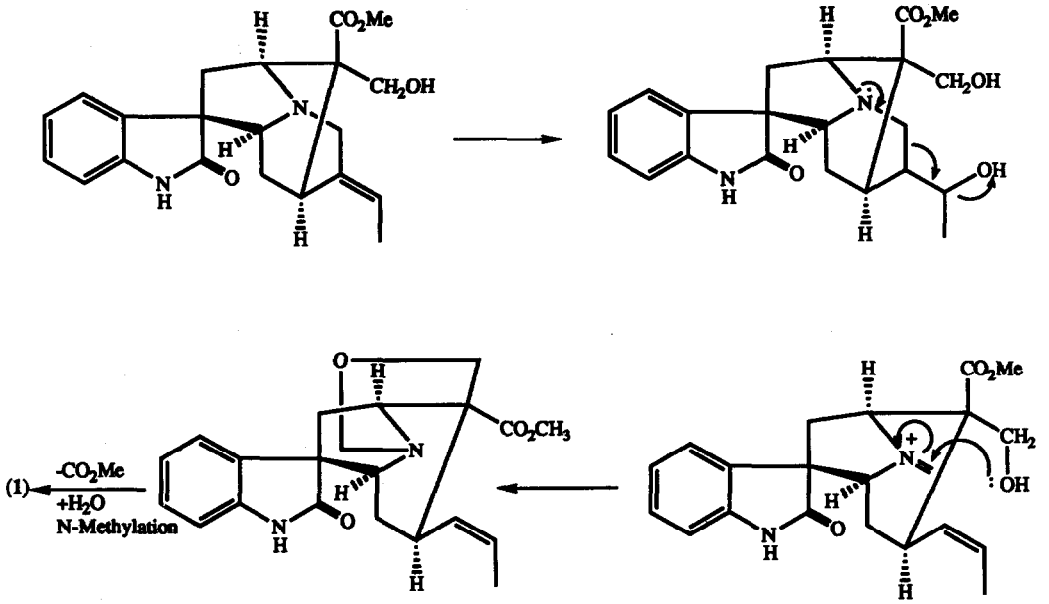
Carbon No.	δ_C	multiplicity(DEPT)	δ_H
2	177.71	s	-
3α	68.48	d	3.15(d,J = 6)
5α	59.05	d	3.71(dd, J = 7.7,2.1)
6α	39.80	t	2.24(dd,J = 13.3,7.7)
6β			2.37(d,J = 13.3)
7	55.82	s	-
8	138.13	s	-
9	123.88	d	7.55(dd,J = 7.5,1)
10	122.61	d	6.92(dt,J = 7.5,1)
11	127.59	d	7.20(dt, J = 7.5,1)
12	107.22	d	6.73(d,J = 7.5)
13	141.94	s	-
14α	33.09	t	1.84(dd,J = 13.3,6)
14β			2.0(m)
15α	28.02	d	3.07(m)
16α	36.98	d	1.40(d,deformed)
17α	68.50	t	3.82(d,J = 11.5)
17β			4.21(dd, J = 11.5, 2.1)
18	23.64	q	1.31(d, J = 6.1)
19	66.42	d	3.95(split q, J = 6.1)
20a	44.39	t	1.66(dd, J = 12,5.9)
20b			1.50(ddd, J = 12,7.6,2.1)
21α	79.79	t	4.71(d, J = 11.4)
21β			4.88 (d, J = 11.4)
N-CH_3	26.30	q	3.20(s)

presence of a secondary hydroxyl group was also confirmed by Horeau's procedure[3] by which its 'S' configuration was established.

The spin systems present in (1) were investigated by HOHAHA and COSY-45 experiments. The HOHAHA was recorded with mixing times of 40ms, 80ms and 120ms.[4-6] At larger mixing intervals (120ms) total correlation between all protons in each spin system (irrespective of whether they were directly coupled or not with each other) was observed, whereas at shorter mixing times the magnetization spread to closer protons within the spin system.[5-8] The HOHAHA and COSY-45 spectra indicated the presence of three spin systems [Fig. 2 (a-c)].

The ^{13}C NMR spectrum (DEPT, Table 1) showed the presence of two methyl, five methylene, nine methine and four quaternary carbons in the molecule. A low field methylene signal at δ 79.79 was assigned to C-21 located between the oxygen and nitrogen atom. The lactam carbonyl carbon resonated at δ 177.71. One bond correlations between ^{1}H and ^{13}C-nuclei were established by the inverse ^{1}H-detected heteronuclear multiple quantum coherence (HMQC)[8-12] experiment and the results are presented in Table 1.

The $^{1}H/^{13}C$ long range connectivity information obtained from the inverse heteronuclear multiple bond connectivity HMBC[8] experiment allowed the various fragments to be connected together. The protons at δ 2.24 (C-6αH) and δ 2.37 (C-6βH) exhibited $^{2}J_{CH}$ cross-peak with C-7 (δ55.82) and with C-5 (δ59.05) and $^{3}J_{CH}$ cross-peaks with C-8 (δ138.13) and C-2 (δ 177.71), while C-3 αH (δ 3.15) showed $^{3}J_{CH}$ interaction with C-8 (δ 138.13) indicating that C-6 and C-3 were both directly attached to the C-7 quaternary carbon. The hydroxypropyl unit must be attached to C-15 (δ28.02) since C-20Ha (δ 1.66) and C-20Hb (δ 1.50) showed $^{2}J_{CH}$ cross-peak with C-15 (δ 28.02) and $^{3}J_{CH}$ connectivities with C-16 (δ36.98) and C-14 (δ33.09). The proton at δ 3.71 (C-5αH) showed $^{3}J_{CH}$ connectivities with C-15 (δ 28.02), C-3 (δ 68.48), C-7 (δ55.82) and C-17 (δ 68.50) carbons. The proton at δ 1.40 (C-16αH) must be attached to a carbon located between C-15 and C-17 since it showed $^{2}J_{CH}$ interaction with C-17 (δ 68.50) and $^{3}J_{CH}$ interaction with C-20 (δ44.39). The protons at δ1.84 (C-14αH) and δ 2.0 (C-14βH) showed $^{2}J_{CH}$ coupling with C-3 (δ 68.48) and C-15 (δ 28.02) indicating the attachment of C-14 with C-3 and C-15. The C-14 protons also showed $^{3}J_{CH}$ interaction with C-7 (δ55.82) and C-20 (δ 44.39) as expected for structure (1). The protons at δ4.21 (C-17βH) and 3.82 (C-17αH) showed $^{3}J_{CH}$ interaction with C-21 (δ79.79) while the protons at δ4.71 (C-21αH) and 4.88 (C-21βH) showed $^{3}J_{CH}$ interaction with C-17 (δ68.50). The above data resulted in the assignment of the structure (1) to macroxine. Lowest energy conformation (Fig. 3) was determined by using MM2 calculation[13]. The calculated coupling constants for low energy conformation (Fig.3) were found identical with the observed Js.



Scheme 1

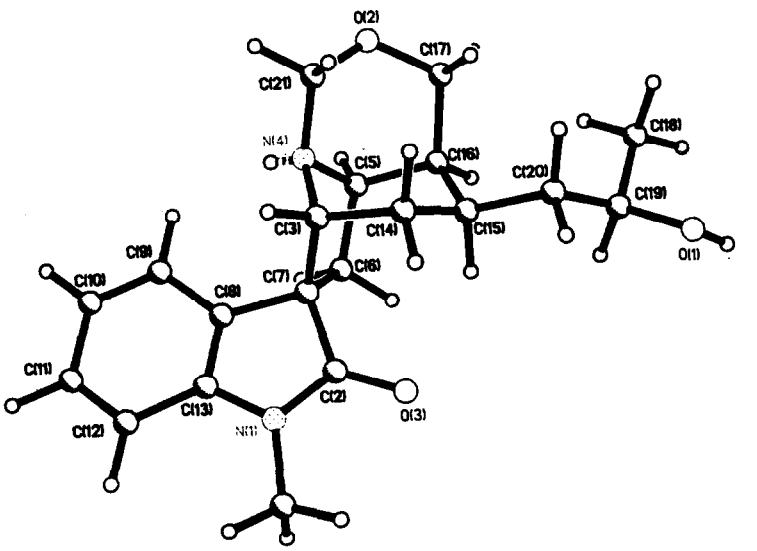


Figure 3: Lowest energy conformation of macroxine (1) determined by MM2 calculations.

Biogenetically, macroxine (1) may arise from voachalotine oxindole[14] (3) or a closely related compound, by fragmentation of the intermediate (4), trapping of the immonium species by ether formation and hydration of the olefin (Scheme 1). Macroxine (1) is the first oxindole alkaloid having a linkage between C-17 and C-21 through oxygen in which the C-20 and C-21 bond has been broken giving rise to the propyl side chain.

EXPERIMENTAL

General Methods: The UV spectrum was recorded on a Shimadzu UV-240 spectrophotometer; the IR spectrum was recorded on JASCO A-302 spectrophotometer; HRMS were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 (DEC) computer system. 1D and 2D ^{1}HNMR spectra were recorded at 400 MHz in $CDCl_3$ on a Bruker AM-400 NMR spectrophotometer.

Plant Material: The leaves of *Alstonia macrophylla* were collected from colombo and Peradeniya, Sri Lanka and identified by Prof. S. Balasubramonium. Department of Botany, Peradeniya University, Srilanka.

Isolation of Alkaloids: The ethanolic extract of the dried leaves (30Kg) of *A. macrophylla* was concentrated and acidified with 10% HCl and extracted with hexane. The aqueous acidic extract was then basified with NH_3 and extracted with $CHCl_3$ at pH9. The crude alkaloidal content at pH 9 (30g) was subjected to column chromatography (silica gel). A fraction obtained in $CHCl_3$: MeOH (9:1) was again subjected to column chromatography (silica gel). A fraction obtained in neat $CHCl_3$ deposited crystals at room temperature. The crystals were soluble in ether wshing with ether left behind an amorphous solid (6 mg) showing a single spot on tlc in the solvent system hexane: acetone (8.5:1.5) which gave an orange colour with Dragendorffs's reagent and no colour with ceric sulfate, identified as macroxine (1)

Macroxine (1): $[\alpha]$ + 72.2° (C = 0.002, $CHCl_3$); IR ($CHCl_3$) 3300, 1700 cm^{-1}; UV (CH_3OH) 256nm (log = 4.2)), ^{1}HNMR and ^{13}CNMR-Table 1; HRMS found m/z 342.1947, $C_{20}H_{26}N_2O_3$ (100%), 325.1942, ($C_{20}H_{25}N_2O_2$) (18%), 284.1563, $C_{17}H_{20}N_2O_2$ (10%), 152.1066,$C_9H_{14}NO$ (90%).

Acid Hydrolysis of Macroxine (1): To 4mg of macroxine (1) 1ml of MeOH and 1ml of 10% aqueous HCl were added and the solution kept overnight at room temperature. No reaction took place indicating that the compound (1) was stable to aqueous acid at room temperature. The solution was then refluxed for six hours. Then the reaction mixture was cooled down by adding ice and NaOH solution was added to basify it. At pH 9 the reaction mixture was extracted with ethylacetate. The ethyl acetate extract was evaporated and tlc was checked showing one alkaloidal spot on tlc (hexane: acetone, 7:3) with some non-alkaloidal impurities. The compound was purified on tlc. The molecular ion of the compound was observed at m/z 324, indicating that dehydration occurred. The ^{1}HNMR spectrum could not be recorded due to paucity of sample.

Table 2. Long range ^{1}H-^{13}C connectivity (HMBC) for macroxine (1) in $CDCl_3$.

^{1}H δ	$^{2}J_{CH}$ δ	$^{3}J_{CH}$ δ	$^{4}J_{CH}$ δ	Minor correlations δ
7.55(C-9H)		127.59 (C-11)		
		141.94 (C-13)		
7.20(C-11H)	122.61(C-10)	123.88(C-9)		
		141.94(C-13)		
6.92(C-10H)		138.13(C-8)		
		107.22(C-12)		
6.73(C-12H)		138.13(C-8)	123.88(C-9)	
		122.61(C-10)		
4.88(C-21βH)		68.48(C-3)	55.82 (C-7)	
		68.50(C-17)		
4.71(C-21αH)		68.48(C-3)	55.82(C-7)	
		68.50(C-17)		
		59.05(C-5)		
4.21 (C-17βH)		79.79(C-21)	39.80(C-6)	
		59.05(C-5)		
3.82 (C-17αH)		79.79 (C-21)	39.80(C-6)	68.48(C-3)
		59.05(C-5)		
		28.02(C-15)		
3.71(C-5αH)		28.02(C-15)		
		68.48(C-3)		
		68.50(C-17)		
		55.82(C-7)		
3.15(C-3αH)		28.02(C-15)		
		59.05(C-5)		
		39.80(C-6)		
		138.13(C-8)		
2.24(C-6αH)	55.82(C-7)	138.13(C-8)	68.50(C-17)	
	59.05(C-5)	177.71(C-2)	79.79(C-21)	
2.37(C-6βH)	55.82(C-7)	138.13(C-8)	68.50(C-17)	
	59.05(C-5)	177.71(C-2)	79.79(C-21)	
2.0(C-14βH)	68.48(C-3)	55.82(C-7)	68.50(C-17)	
	28.02(C-15)	44.39(C-20)		
1.84(C-14αH)	68.48(C-3)	55.82(C-7)	68.50(C-17)	
	28.02(C-15)	44.39(C-20)		
1.66(C-20Ha)	66.42(C-19)	36.98(C-16)		
	28.02(C-15)	33.09(C-14)		
		23.64(C-18)		
1.50(C-20Hb)	66.42(C-19)	36.98(C-16)		
	28.02(C-15)	33.09(C-14)		
		23.64(C-18)		
1.40(C-16α H)	68.50(C-17)	44.39(C-20)		
1.31(C-18H)	66.42(C-19)	44.39(C-20)		

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