CHAPTER 3
RESULTS AND DISCUSSION

3.1 MALAYSIAN *Alstonia spatulata* AND *Alstonia macrophylla*

Alstonia is a genus of trees or sometimes shrubs of the family Apocynaceae and is found in many locations stretching from Africa to the Pacific. The Malaysian *Alstonia* species are known locally as "pulai" and have a characteristic appearance with leaves in whorls of three to eight, small white flowers, slender follicles, and the trunk often buttressed (Keng, 1969 and Ridley, 1923).

The Malaysian *Alstonia spatulata* Bl. is a tree 80 to 90 feet tall, with leaves 3 to 5 in a whorl, obovate or spatulate, tip rounded and the fruits are glabrous. (Ridley, 1923; Markgraf, 1974 and Whitmore, 1973). It is found mostly in swamps in the Malay Peninsula and also in Borneo (Burkill, 1966). The Malaysian *A. spatulata* Bl. used in this study is collected from Sabah. The bark was collected, dried and then the alkaloid extracted for the present study.

The Malaysian *Alstonia macrophylla* Wall. is found on hill forests from Penang to Malacca in West Malaysia as well as in Borneo (Burkill, 1966). It is a medium sized tree, with leaves that are chartaceous, four in a whorl, obovate and the fruits are long and glabrous follicles (Ridley, 1923; Markgraf, 1974 and Whitmore, 1973). The Malaysian *Alstonia macrophylla* Wall. used in this study was collected from Sabah. The bark was collected, dried and then extracted for alkaloids.

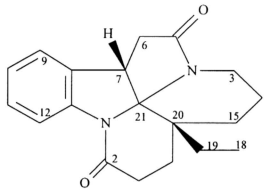
3.2 ALKALOIDS FROM *Alstonia spatulata*

The bark of *Alstonia spatulata* Blume gave a very low yield of alkaloids approximately 0.0005g/g of bark. Only two alkaloids were isolated and they are: **AS1** (8mg) identified as Leuconoxine [**76**] and **AS2** identified as an alkaloid with similar skeleton to the methoxy epi-echitamidine type. Other alkaloids were found only in trace amounts.

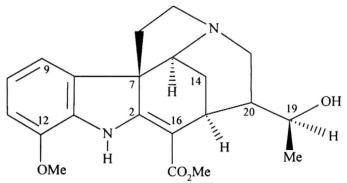
3.2.1 Leuconoxine [76] (AS1)

HREIMS of **AS1** gave an exact mass of 310.1675 corresponding to the molecular formula $C_{19}H_{22}N_2O_2$ (calcd. 310.1681).

^{1}H NMR (Fig. 3.1) and ^{13}C NMR (Fig. 3.2 and Table 3.1), for **AS1** are in accord with that reported in the literature for Leuconoxine [**76**] (Abe & Yamauchi, 1994). The ^{1}H NMR displayed four protons in the aromatic region, which indicated an unsubstituted aromatic ring. The ^{13}C NMR and DEPT experiments afforded four methine proton in the low field region which corresponds to the four aromatic carbons (C-9, C-10, C-11 and C-12) signals. Other lower field signals observed were for the quaternary carbons of C-8 (δ135.1), C-13 (δ142.0), and the two lactam carbonyls C-2 (δ172.9) and C-5 (δ170.8). The other characteristic feature of Leuconoxine [**76**] is the absence of N-H signal and the presence of quaternary C-21 (δ92.5) signal which indicates C-21 to be a diazaspirocarbon (Abe & Yamauchi, 1994 and Goh et al., 1989).



Leuconoxine [**76**]



Scholarine [**77**]

Table 3.1 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for Leuconoxine [76] (AS1) in $CDCl_3$

Position	δH	δC	Multiplicity (DEPT)
2	-	172.9	s
3	3.95 brd (13) 2.72 - 2.79 m	36.8	t
5	-	170.8	s
6	2.86 dd (16.5, 7) 2.67 d (16.5)	37.6	t
7	3.82 d (7)	41.9	d
8	-	135.1	s
9	7.12 - 7.19 m	123.8	d
10	7.12 - 7.19 m	125.5	d
11	7.23 - 7.28 m	127.9	d
12	7.77 d (7.5)	120.1	d
13	-	142.0	s
14	1.58 - 1.64 m 1.58 - 1.64 m	20.1	t
15	1.96 - 2.02 m 1.72 - 1.78 m	26.2	t
16	2.72 - 2.79 m 2.49 ddd (19, 6, 1)	29.4	t
17	1.82 - 1.88 m 1. 62 - 1.67 m	26.6	t
18	0.92 t (6)	7.3	q
19	1.38 qui (6)	26.9	t
20	-	38.1	s
21	-	92.5	s

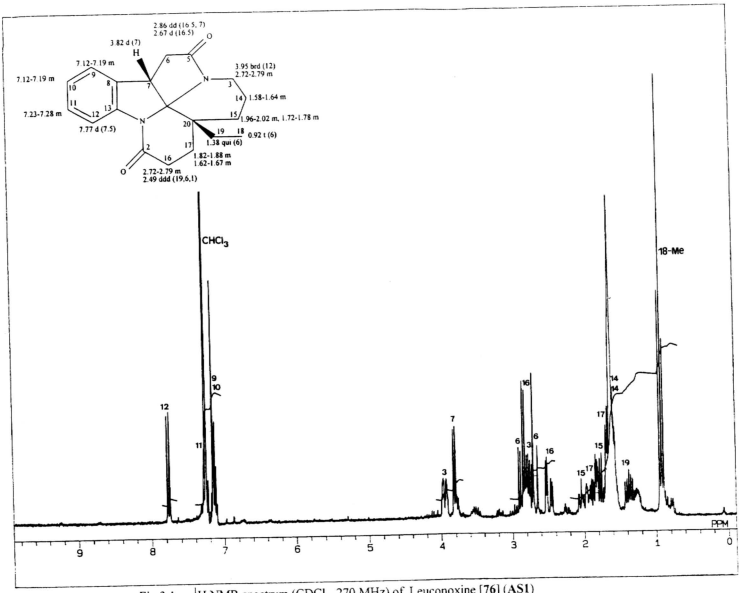


Fig 3.1 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of Leuconoxine **[76] (AS1)**

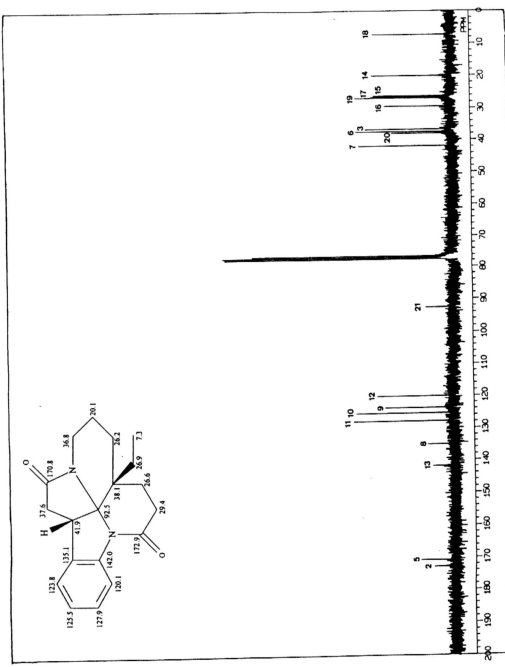


Fig 3.2 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of Leuconoxine [76] (**AS1**)

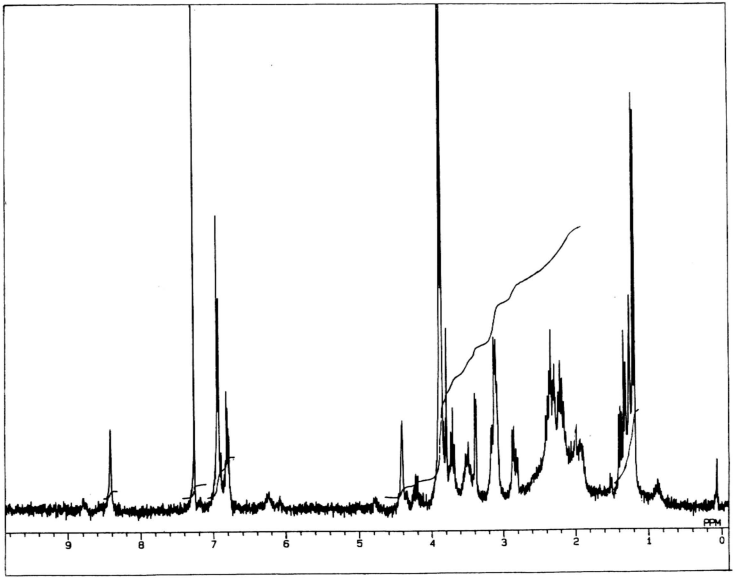


Fig 3.3 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of **AS2**

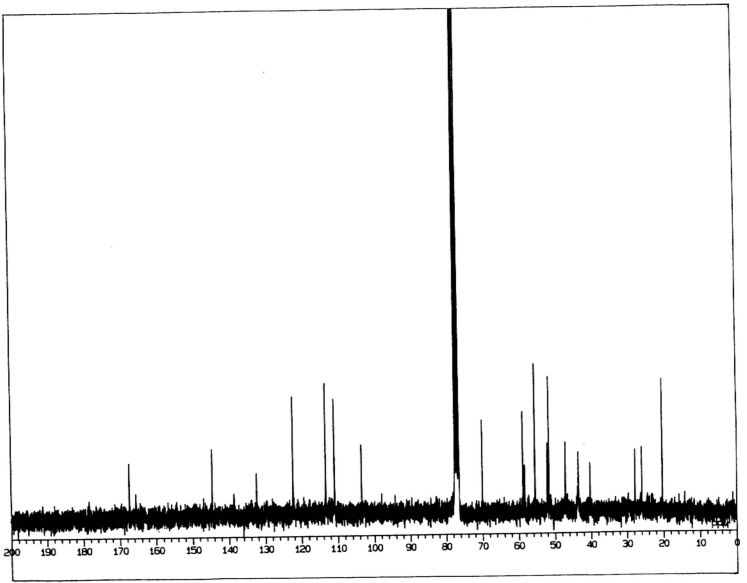


Fig 3.4 ^{13}C NMR spectrum ($CDCl_3$, 67.8 Mhz) of **AS2**

3.3 ALKALOIDS FROM *Alstonia macrophylla*

The bark of *Alstonia macrophylla* Wall. upon exhaustive extraction afforded an alkaloid mixture of 0.0083g/g bark. Following are the alkaloids isolated and documented : (-)-Talcarpine **[54]** (**AM1**), (+)-N_b-Methyl-N_b,21-secotalpinine**[78]** (**AM2**), Alstophylline **[6]** (**AM3**), 19,20-Dehydro-10-methoxy talcarpine **[80]** (**AM4**), N_b-Demethylalstophylline oxindole **[59]** (**AM5**), N_b-Demethylalstophyllal oxindole **[81]** (**AM6**), Alstonisine **[58]** (**AM7**), Alstonal **[82]** (**AM8**), Pleocarparmine **[3]** (**AM9**), Villalstonine **[10]** (**AM10**) and Macralstonine hydroxyketone **[83]** (**AM11**).

Of the eleven alkaloids isolated, the N_b-demethylalstophyllal oxindole **[81]** (**AM6**) and alstonal **[82]** (**AM8**) are new alkaloids (Wong et al., 1996). (+)-N_b-Methyl-N_b,21-secotalpinine **[78]** (**AM2**) was isolated for the first time as a natural product. (-)-Talcarpine **[54]** (**AM1**) was first isolated from *Pleiocarpa talbotii* Benth. (Naranjo et al., 1972) and later from *A. macrophylla* Wall. of Sri Lanka (Ratnayake et al., 1987). Alstophylline **[6]** (**AM3**) has been isolated from *A. macrophylla* Wall. found in the Philippines (Kishi et al., 1965 and Arambewela et al., 1990), Sri. Lanka (Ratnayake et al., 1987) and Thailand (Abe et al., 1994a). In the New Guinean *A. glabriflora* Mgf., alstophylline **[6]** (**AM3**) has also been documented to be present (Hart et al., 1972). Alstophylline **[6]** (**AM3**) and 19,20-dehydro-10-methoxytalcarpine **[80]** (**AM4**) have both been reported present in the Malaysian *A. angustifolia* Wall. (Ghedira et al., 1988). **AM1**, **AM2**, **AM3** and **AM4** are all the macroline (C4e)-type alkaloid.

AM5, **AM6**, **AM7** and **AM8** all fall under the macroline related oxindole (C6e)-type alkaloid. Among these oxindole alkaloids, N_b-demethylalstophylline oxindole **[59]** (**AM6**) and alstonisine **[58]** (**AM7**) have both been isolated from the Sri Lanka *A. macrophylla* Wall. (Atta-ur-Rahman et al., 1987 and

Atta-ur-Rahman et al., 1990b). More recently, **AM5** has also been documented to be present in the *A. macrophylla* Wall. from Thailand (Abe et al., 1994a). Alstonisine [**58**] (**AM7**) has also been found to be present in *A. muelleriana* Domin. from Australia (Elderfield & Gilman, 1972).

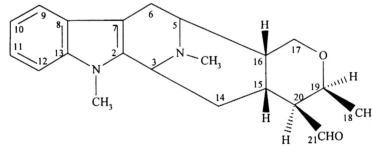
Pleiocarpamine [**3**] (**AM9**) is a well documented alkaloid found in the family of *Apocynaceae* (Gilbert, 1968). This tertiary alkaloid was first isolated from *Pleiocarpa mutica* Benth. (Kump & Schmid, 1961). It was also found to be present in the Sri Lanka and the Philippines *A. macrophylla* Wall. (Arambewela et al., 1990, Manalo, 1968 and Waldner et al., 1967).

Villalstonine [**10**] (**AM10**) and macralstonine hydroxyketone [**83**] (**AM11**) are both dimeric alkaloids which have been isolated from *A. macrophylla* Wall. (Hesse et al., 1966; Kishi et al., 1966 and Ratnayake et al., 1987). Both **AM10** and **AM11** were also found in *A. angustifolia* Wall from Malaysia (Ghedira et al., 1988), *A. glabriflora* Mgf. from New Guinea (Hart et al., 1972) and *A. muelleriana* Domin. from Australia (Cook & Le Quesne, 1971 and Elderfield & Gilman, 1972). Villalstonine [**10**] (**AM10**) was also reported present in *A. spectabilis* R.Br from New Guinea (Hart et al., 1972).

3.3.1 (-)-Talcarpine [54] (AM1)

The EI mass spectrum of **AM1** gave a M^+ at *m/z* 338 ($C_{21}H_{26}N_2O_2$ and the fragmentation pattern is similar to that reported for (-)-talcarpine **[54]** (Naranjo et al., 1972). The ^1H NMR (Fig. 3.5) of **AM1** is also consistent with the reported structure (Table 3.2). The ^{13}C NMR (Fig. 3.6), 2D ^1H-^1H COSY and 2D ^{13}C-^1H COSY experiments were carried out and assignments are given in Table 3.2. The assignments of the quaternary carbons were done by comparison with the reported data on (-)-talcarpine **[54]** (Takayama et al., 1991).

The NOE difference (Fig. 3.7a & 3.7b) experiment confirmed the configuration of H-19 and 18-Me to be consistent with the stereochemistry previously reported for talcarpine **[54]** (Takayama et al., 1991). Irradiation of the 18-Me doublet at δ1.30 resulted in a 4% increase in the H-21 doublet at δ9.94, whilst irradiation of H-21 resulted in an enhancement of 4% at 18-Me. Irradiation at the δ3.92-4.05 multiplet resonance (H-19) gave an increase of 4% to the H-20 broad singlet at δ1.78 and conversely irradiation at δ1.78 broad singlet (H-20) also gave an enhancement of 6% at δ3.92-4.05 multiplet (H-19). This observation confirms the correlation in space where the H-19 has an α configuration similar to H-20α and the 18-Me as having the same β configuration as H-21.



(-)-Talcarpine **[54]**

Table 3.2 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for (-)-Talcarpine [54] (AM1) in $CDCl_3$

Position	δH	δC
2		132.6
3	3.92 - 4.05 m	53.5
5	2.89 d (7)	54.5
6	2.45 d (16)	22.4
	3.24 dd (16, 7)	
7		106.6
8		126.3
9	7.48 brd (7)	118.1
10	7.09 brt (7)	118.9
11	7.19 brt (7)	120.9
12	7.28 brd (7)	108.7
13		136.9
14	2.41 - 2.55 m	30.0
	1.40 - 1.48 m	
15	2.15 - 2.24 m	27.0
16	2.00 - 2.09 m	39.4
17	4.13 t (11)	68.8
	3.89 dd (11,5)	
18	1.30 d (6.5)	19.2
19	3.92 - 4.05 m	69.4
20	1.78 brs	54.5
21	9.94 d (3)	204.7
N_a-Me	3.62 s	28.9
N_b-Me	2.32 s	41.7

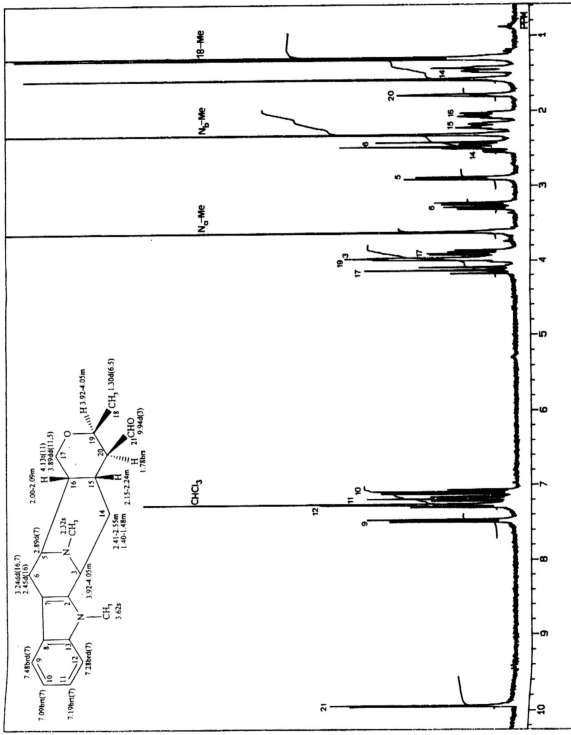


Fig 3.5 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of (-)-Talcarpine [54] (AM1)

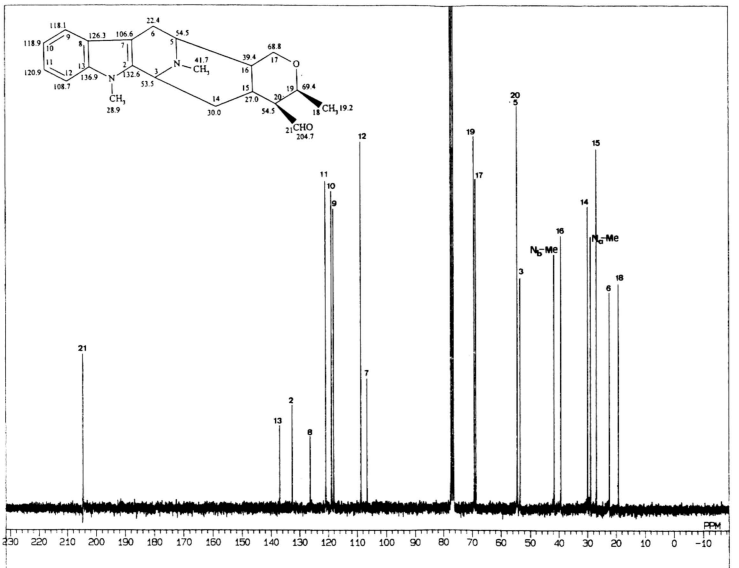


Fig 3.6 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of (-)-Talcarpine **[54]** **(AM1)**

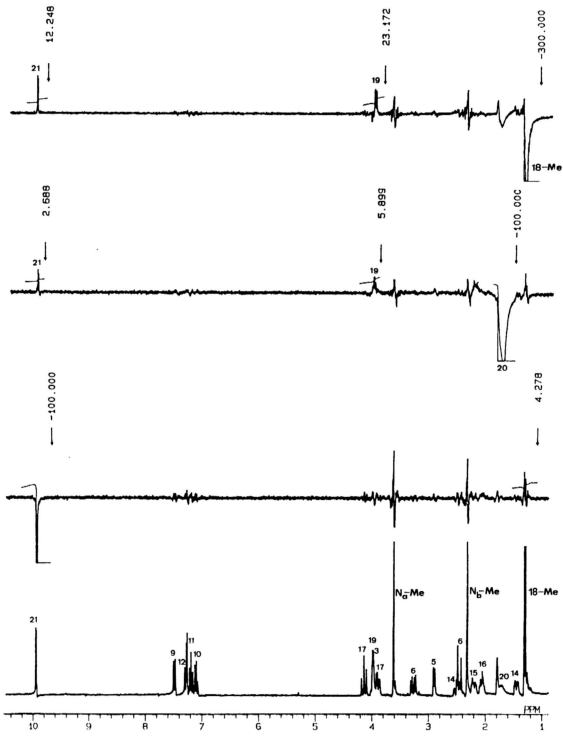


Fig 3.7a NOE spectrum of (-)-Talcarpine [**54**] (**AM1**)

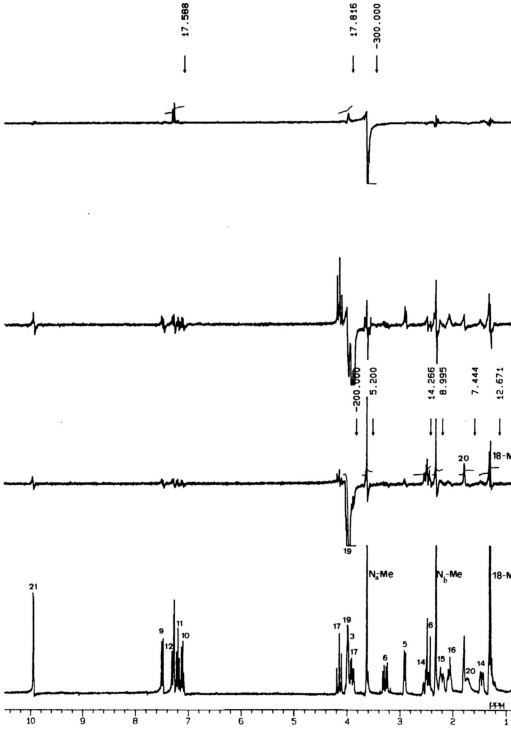
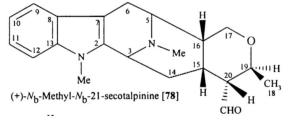


Fig 3.7b NOE spectrum of (-)-Talcarpine [**54**] (**AM1**)

3.3.2 (+)-N_b-Methyl-N_b, 21-secotalpinine [78] (AM2)

In this present study, alkaloid **AM2** is identified as (+)-N_b-methyl-N_b, 21-secotalpinine [**78**]. (+)-N_b-Methyl-N_b, 21-secotalpinine [**78**] was isolated for the first time as a natural product. **78** was first reported as a synthetic product from treatment of talpinine [**79**] with methyl iodide in benzene (Naranjo et al., 1972). Garnick and Le Quesne (1978) reported **78** as a synthetic product prepared from alstonerine [**51**]. The proposed structure for **78** as epimeric with (-)-Talcarpine [**54**] at the carbon bearing the formyl group, was based on analyses of IR, UV, Mass and ^{1}H NMR spectra (Naranjo et al., 1972). UV spectrum of **78** is characteristic of an unsubstituted dihydroindole chromophore and the absorption at 1717cm^{-1} and 2713cm^{-1} in the IR spectrum indicated presence of an aldehyde function. EIMS of **78** showed a M^+ of 338 and the fragmentation pattern is reminiscent to that of **54**. However, characteristic difference of relative intensity in the peak at *m/z* 310 was also noted and is attributed to decarbonylation at the epimeric centers (Naranjo et al., 1972 and Garnick et al., 1978). The optical rotation of **78**, $[\alpha]_D$ = -49° also supports that it is an epimer of **54**, $[\alpha]_D$ = +41°. The ^{1}H NMR (Fig. 3.8) and ^{13}C NMR (Fig. 3.9) of **78** is in accord with those reported in the literature (Naranjo et al., 1972 and Takayama et al., 1991). By comparison with **54**, and analysis of the 2D NMR spectra, the ^{1}H NMR and ^{13}C NMR assignments for **78** are given in Table 3.3.



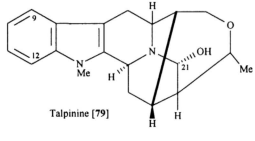


Table 3.3 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for (+)-N_b-Methyl-N_b-21-secotalpinine [78] (AM2) in $CDCl_3$

Position	δH	δC
2		132.7
3	3.91 - 3.98 m	53.0
5	2.97 d (7)	54.8
6	2.5 d (17)	22.4
	3.31 dd (17, 7)	
7		106.5
8		126.2
9	7.53 brd (7)	117.8
10	7.14 brt (7)	118.9
11	7.22 brt (7)	120.9
12	7.32 brd (7)	108.8
13	-	136.9
14	2.36 - 2.40 m	26.6
	1.28 - 1.32 m	
15	2.36 - 2.40 m	26.0
16	1.95 brd (11.5)	42.4
17	4.08 t (11.5)	67.0
	3.76 dd (11.5, 5)	
Me-18	1.21 d (6)	20.2
19	3.91 - 3.98 m	67.7
20	2.36 - 2.40 m	57.6
21	9.41 brs	203.0
N_a-CH_3	3.59 s	28.9
N_b-CH_3	2.32 s	41.6

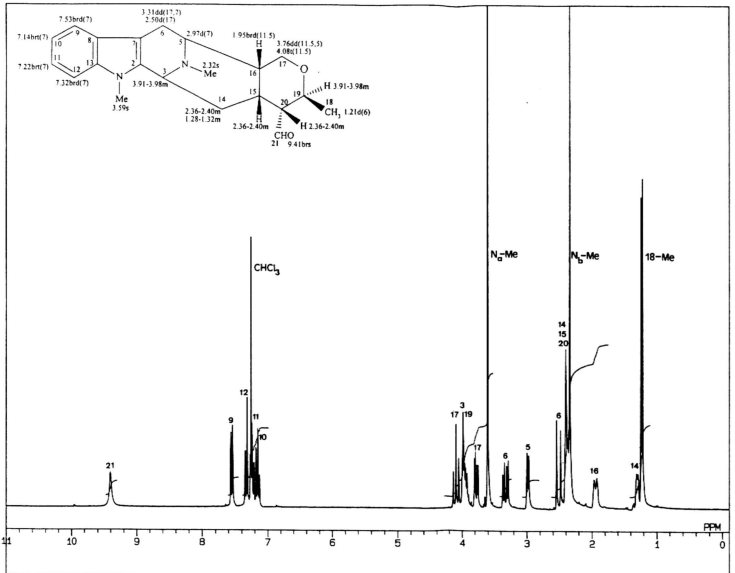


Fig 3.8 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of (+)-N_a-Methyl-N_b 21-secotalpinine **[78]** (**AM2**)

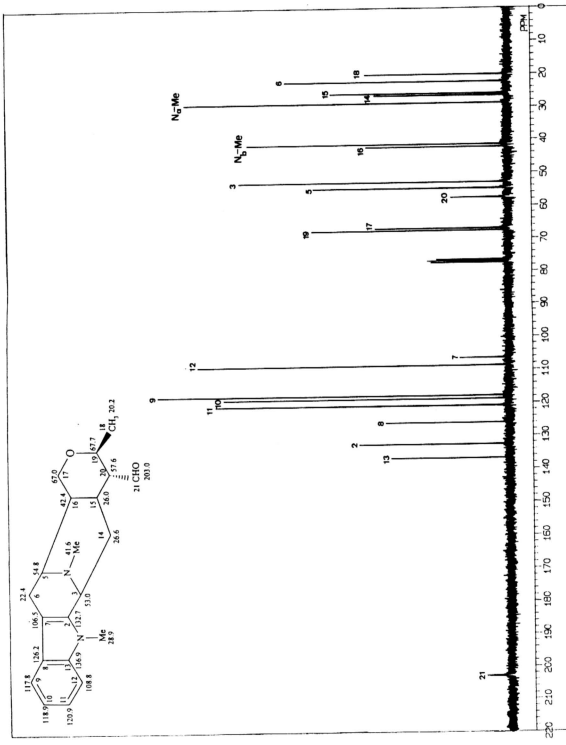
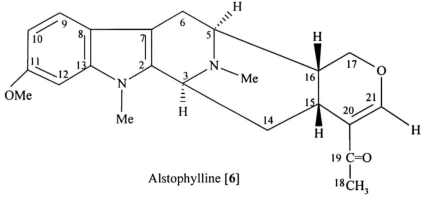


Fig 3.9 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of (+)-N_b-Methyl-N_a 21-secotalpinine [78] (**AM2**)

3.3.3 Alstophylline [6] (AM3)

EIMS of **AM3** shows the M^+ at 366 and the fragmentation pattern concurs with that reported for alstophylline **[6]** in the literature(Kishi et al., 1965) . The ^1H NMR (Fig. 3. 10) and ^{13}C NMR (Fig. 3.11) (Table 3.4) assignments are also in good agreement with recent data on alstophylline **[6]** (Abe et al., 1994a). ^1H-^1H COSY experiment also show the substructure sequences which fitted well with the proposed structure.

Analysis of the **AM3** ^1H NMR spectra revealed similarities in structure to (-)-talcarpine **[54]** (**AM1**) and N_b-methyl-N_b, 21-secotalpinine **[78]** (**AM2**), where characteristic macroline related signals of three-proton singlets for N_a and N_b methyls, a one-proton triplet between 4 and 4.5 ppm (H-17), one doublet of doublets between 3 and 4 ppm (H-17 and H-6) and two doublets between 2 and 3 ppm (H-5 and H-6) were observed (Ghedira et al., 1988). **AM3** upon closer comparison with (-)-talcarpine **[54]** (**AM1**) and N_b- methyl-N_b, 21-secotalpinine **[78]** (**AM2**), exhibits a slight downfield shift for the one-proton triplet (H-17) to δ 4.40 and the other H-17 proton to δ 4.16 and also the spin-spin coupling pattern at δ4.16 (H-17) is no longer observed as a dd but as a ddd system. The slight downfield shift for the C-17 protons in **AM3** is attributed to the presence of the olefinic C-20/C-21 bond.



Alstophylline **[6]**

Table 3.4 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for Alstophylline [6] (AM3) in $CDCl_3$

Position	δH	δC
2	-	132.0
3	3.84 brs	53.8
5	3.06 d (7)	54.8
6	3.29 dd (16,7)	22.9
	2.45 d (16)	
7	-	105.8
8	-	121.1
9	7.33 d (8.5)	118.3
10	6.75 dd (8.5, 2)	108.2
11	-	156.0
12	6.81 d (2)	93.3
13	-	138.0
14	2.11 - 2.14 m	32.4
	1.74 - 1.84 m	
15	2.57 - 2.65 m	23.0
16	1.86 - 1.92 m	38.5
17	4.40 t (11)	67.8
	4.16 ddd (11, 4, 2)	
18	2.09 s	25.0
19	-	195.3
20	-	121.1
21	7.53 s	157.4
O-Me	3.89 s	56.0
N_a-Me	3.59 s	29.1
N_b-Me	2.32 s	41.8

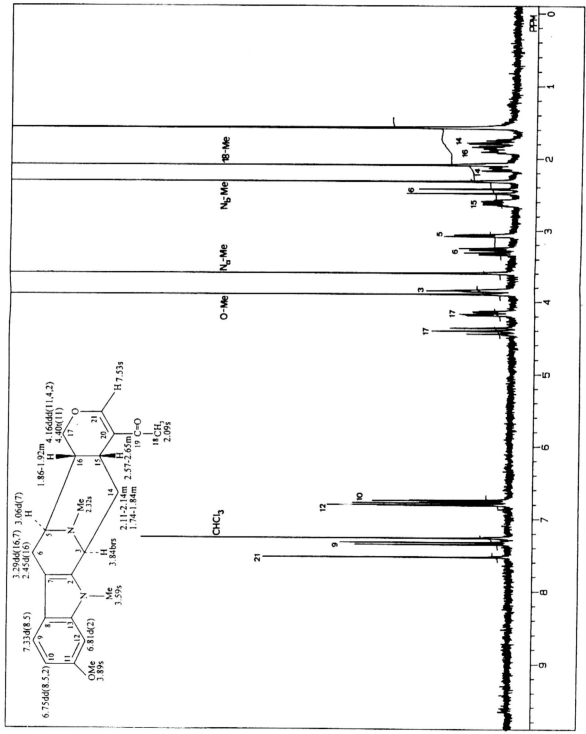


Fig 3.10 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of Alstophylline [6] (**AM3**)

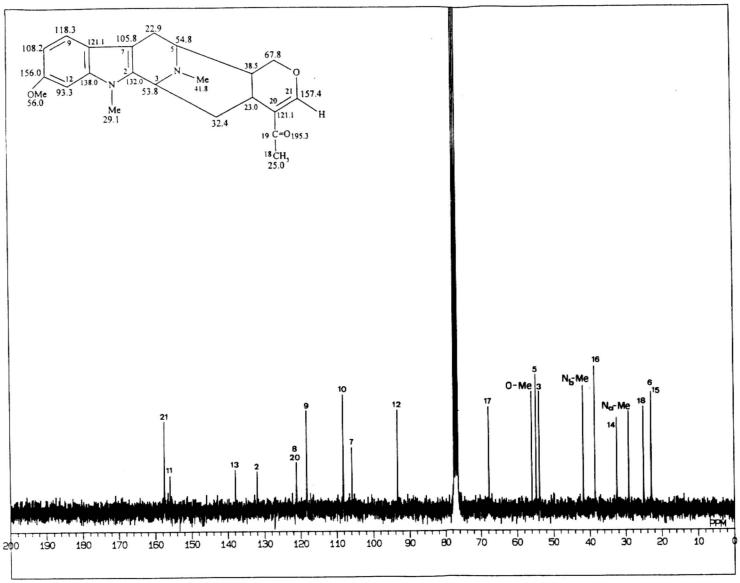
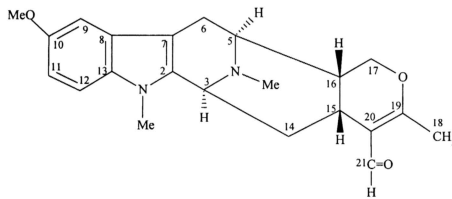


Fig 3.11 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of Alstophylline [**6**] (**AM3**)

3.3.4 19,20-Dehydro-10-methoxytalcarpine [80] (AM4)

EIMS of **AM4** gave a M^+ of 366 and the fragmentation pattern is similar to that of 19,20-dehydro-10-methoxytalcarpine [**80**] (Ghedira et al., 1988). Characteristic signals in the aromatic region of the **AM4** ^{1}H NMR (Fig. 3.12) indicated the presence of a substituent in the benzene ring. The presence of a three proton singlet at δ 3.86 is characteristic of *O*-methoxy substitution in the aromatic ring and this is further supported by ^{13}C NMR spectra (Fig. 3.13) indicating that the methoxy substituent is at C-10. The ^{1}H NMR also shows typical macroline unit [**21**] related coupling patterns and chemical shifts for H-5, H-6, H-17, N_a-Me and N_b-Me which are present in the spectra of macroline related compounds (Ghedira et al., 1988).

The ^{1}H NMR and ^{13}C NMR assignments of **AM4** (Table 3.5) are in good agreement with those reported in literature (Ghedira et al., 1988) with the exception of C-19 assignment. By comparing the similarities in ring E of structures N_b-demethylalstophyllal oxindole [**81**] and alstonal [**82**] the low field peak at δ 170.9 in ^{13}C NMR spectrum of **AM4** is assigned to the C-19 carbon in **AM4**.



19,20-Dehydro-10-methoxytalcarpine [**80**]

Table 3.5 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for 19,20-Dehydro-10-Methoxytalcarpine [80] (AM4) in $CDCl_3$

Position	δH	δC
2	-	132.6
3	3.82 - 3.85 m	53.8
5	3.08 d (6.5)	54.7
6	3.29 dd (16.5,6.5)	22.9
	2.46 d (16.5)	
7	-	105.4
8	-	126.7
9	6.93 d (2.5)	100.4
10	-	153.8
11	6.85 dd (8.5,2.5)	110.5
12	7.20 d (8.5)	109.6
13	-	nd
14	2.08 - 2.13 m	nd
	1.78 - 1.85 m	
15	2.57 - 2.63 m	31.9
16	1.86 - 1.97 m	38.5
17	4.43 t (11)	68.1
	4.19 ddd (11, 4, 1.5)	
18	2.17 s	22.4
19	-	170.9
20	-	117.4
21	9.66 s	188.6
O-Me	3.86 s	56.1
N_a-CH_3	3.63 s	29.2
N_b-CH_3	2.33 s	41.7

nd : Not detected

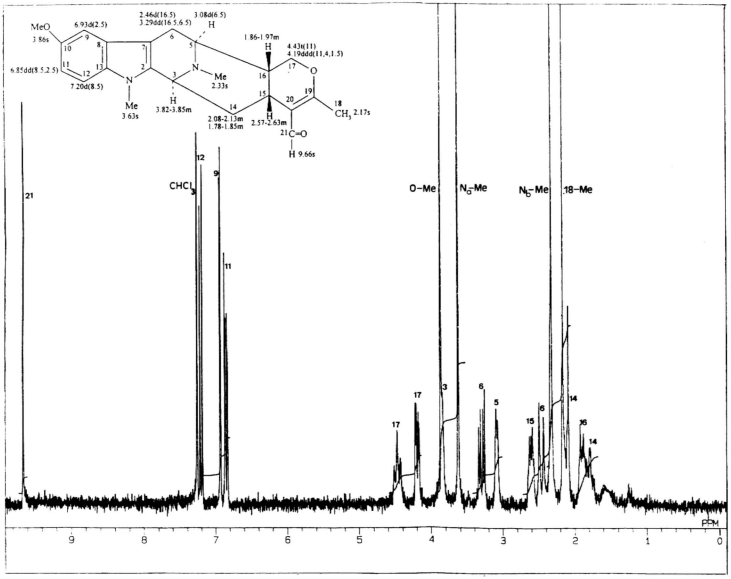


Fig 3.12 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of 19,20-Dehydro-10-methoxytalcarpine **[80]** (**AM4**)

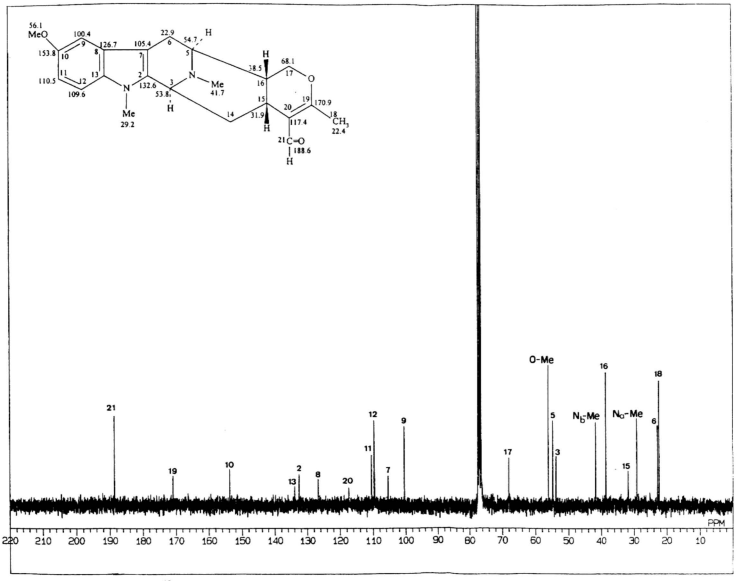
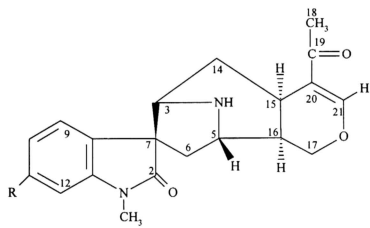


Fig 3.13 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of 19,20-Dehydro-10- methoxytalcarpine [**80**] (**AM4**)

3.3.5 N_b-Demethylalstophylline oxindole [59] (AM5)

The HREIMS of **AM5** gave the exact mass peak at 368.1745 consistent with the molecular formula $C_{21}H_{24}N_2O_4$ (calcd. 368.1736). This bears great resemblance to N_b-demethylalstophylline oxindole [**59**] (Atta-ur-Rahman et al., 1987). ^{1}H NMR (Fig. 3.14) and ^{13}C NMR (Fig. 3.15) data (Table 3.6) were also found to be in agreement with those in the literature (Abe et al., 1994 and Atta-ur-Rahman et al., 1987).

The 2D ^{1}H-^{1}H COSY afforded substructure connectivities that fitted well with the assignments. NOE difference (Fig. 3.16) experiment of **AM5** confirmed the stereochemistry as similar to that of the reported structure. Irradiation of H-15 multiplet at δ3.31-3.42 brought about 29% enhancement of the H-9 doublet at δ8.17 indicating proximity of H-15α to H-9. This is possible when the C-7/C-3 bond is in the β-configuration and the C-7/C-6 bond has the α-configuration (Atta-ur-Rahman et al., 1987 and Peterson & Cook, 1994).



N_b-Demethylalstophylline oxindole [**59**] R:OCH_3
Alstonisine [**58**] R:H

Table 3.6 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for N_b-Demethylalstophylline oxindole [59] (AM5) in $CDCl_3$

Position	δH	δC
2	-	183.1
3	3.10 - 3.22 m	63.7
5	3.66 brd (7)	56.3
6	2.13 d (14)	42.1
	2.50 dd (14,7)	
7	-	56.4
8	-	120.9
9	8.17 d (8)	126.2
10	6.80 dd (8,2)	106.3
11	-	160.1
12	6.45 d (2)	96.6
13	-	145.3
14	1.52 ddd (14,12,3.5)	31.1
	2.17 - 2.30 m	
15	3.31 - 3.42 m	24.2
16	1.90 - 2.07 m	37.0
17	4.25 ddd (11,4,1.5)	68.4
	4.45 t (11)	
18	2.24 s	24.9
19	-	196.7
20	-	121.8
21	7.62 s	157.7
N-Me	3.16 s	26.2
O-Me	3.85 s	55.6

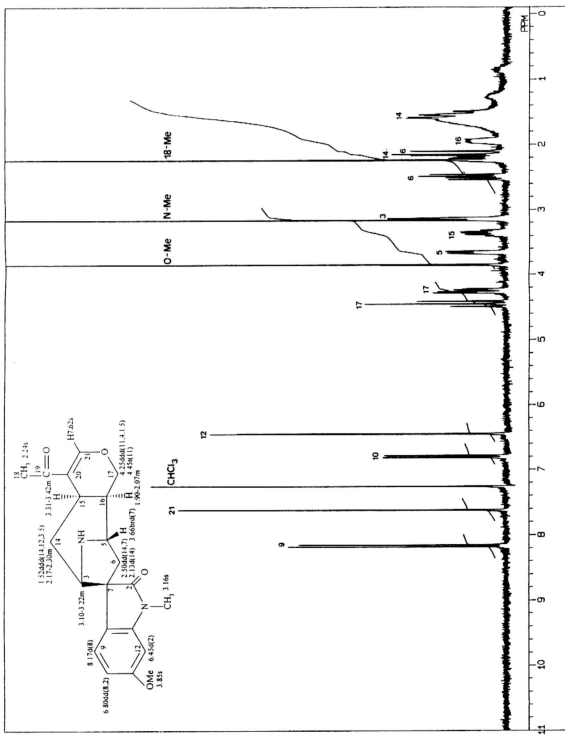


Fig 3.14 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of N_b-Demethylalstophylline oxindole **[59]** (AM5)

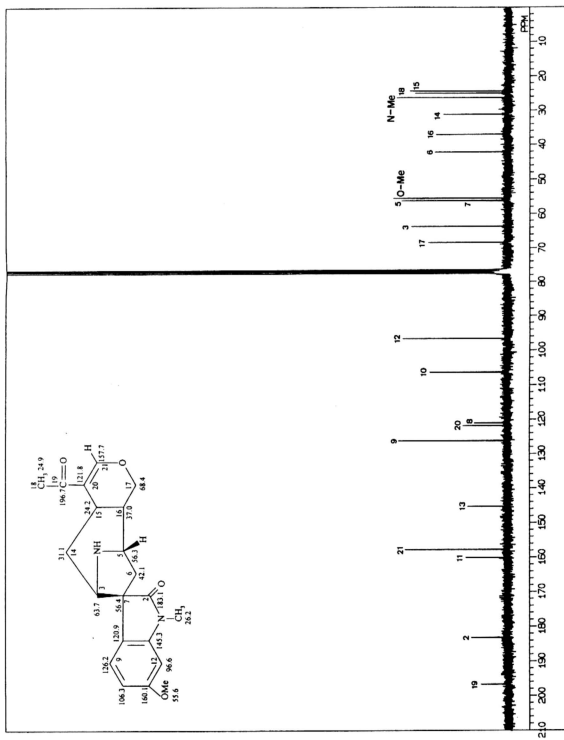


Fig 3.15 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of N_b-Demethylalstophylline oxindole [59] (AM5)

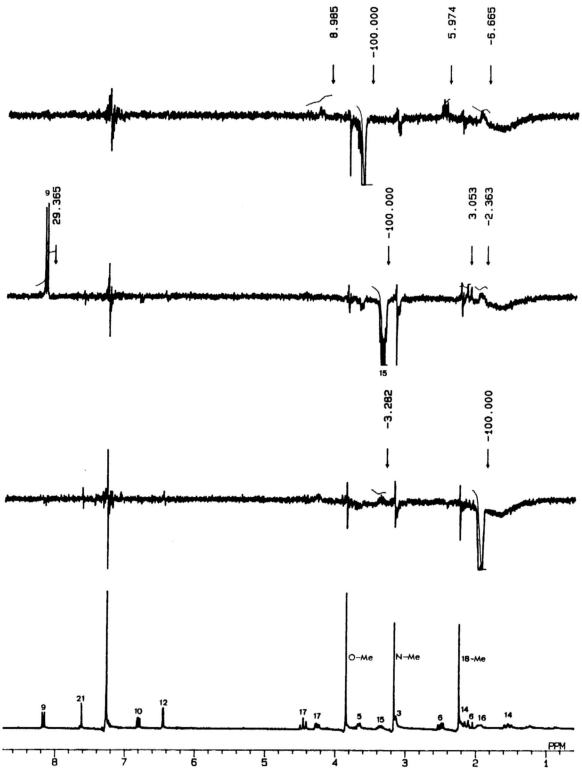
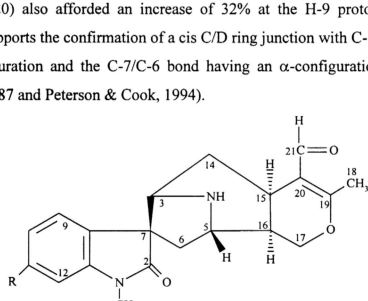


Fig 3.16 NOE spectrum of N_b-Demethylalstophylline oxindole **[59]** (**AM5**)

3.3.6 N_b -Demethylalstophyllal oxindole [81] (AM6)

AM6 is a new alkaloid isolated as colourless crystalline needles and identified as N_b-demethylalstophyllal oxindole [**81**] (Wong et al., 1996). The UV spectrum of **AM6** displayed characteristic absorptions for an oxindole system and the typical oxindole absorptions were also noted in the IR spectrum (Atta-ur-Rahman et al., 1987). HREIMS of **AM6** disclosed a molecular ion at *m/z* 368.1742 (calcd. 368.1736) consistent with the molecular formula $C_{21}H_{24}N_2O_4$ which is identical with the molecular formula of N_b-demethylalstophylline oxindole [**59**]. Furthermore, the EIMS fragmentation pattern in of N_b-demethylalstophyllal oxindole [**81**] was identical to that of N_b-demethylalstophylline oxindole [**59**] suggesting that these two molecules are closely related in structure. Analysis of the ^{1}H NMR (Fig. 3.17) and ^{13}C NMR (Fig. 3.18) spectra of N_b-demethylalstophyllal oxindole [**81**] revealed congruence with N_b-demethylalstophylline oxindole [**59**] in rings A, B, C and D, however substantial differences in ring E were observed (Table 3.7) (Wong et al., 1996). The 2D ^{1}H-^{1}H COSY (Fig. 3.19) of N_b-demethylalstophyllal oxindole [**81**] also fitted well with the proposed structure. Irradiation of H-15α proton in the NOE difference experiment (Fig. 3.20) also afforded an increase of 32% at the H-9 proton resonance. This again supports the confirmation of a cis C/D ring junction with C-7/C-3 bond having a β-configuration and the C-7/C-6 bond having an α-configuration (Atta-ur-Rahman et al., 1987 and Peterson & Cook, 1994).



N_b-Demethylalstophyllal oxindole [**81**] : R = OCH_3
Alstonal [**82**] : R = H

Table 3.7 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for N_b-Demethylalstophyllal oxindole [81] (AM6) in $CDCl_3$

Position	δH	δC
2	-	183.1
3	3.09 - 3.21 m	63.7
5	3.66 brd (7)	56.3
6	2.12 d (14)	42.1
	2.49 dd (14,7)	
7	-	56.3
8	-	120.9
9	8.16 d (8)	126.3
10	6.80 dd (8,2)	106.4
11	-	160.0
12	6.40 d (2)	96.5
13	-	145.3
14	1.52 ddd (14,12,3.5)	30.8
	2.20 - 2.34 m	
15	3.30 - 3.42 m	24.0
16	1.90 - 2.07 m	37.0
17	4.26 ddd (11,4,1.5)	68.7
	4.50 t (11)	
18	2.24 s	16.7
19	-	171.0
20	-	118.2
21	9.85 s	189.5
N-Me	3.16 s	26.2
O-Me	3.86 s	55.6

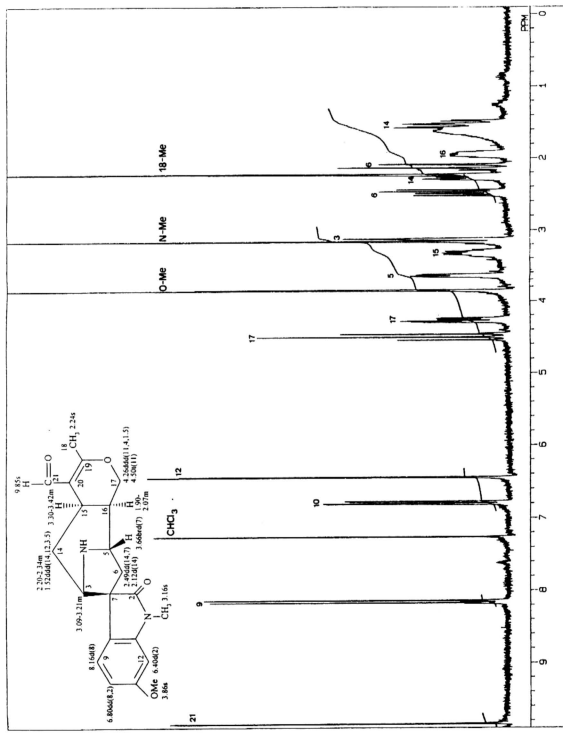


Fig 3.17 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of N_b-Demethylalstophyllal oxindole [81] (AM6)

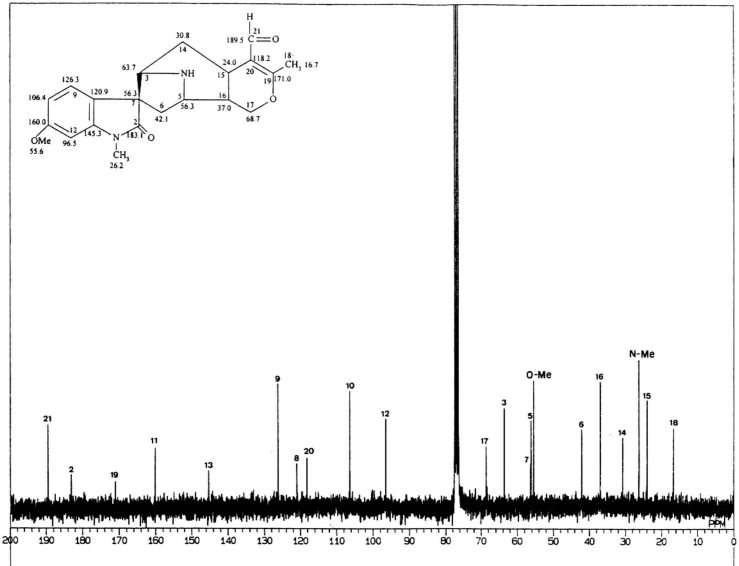


Fig.3.18 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of N_b-Demethylalstophyllal oxindole **[81]** (**AM6**)

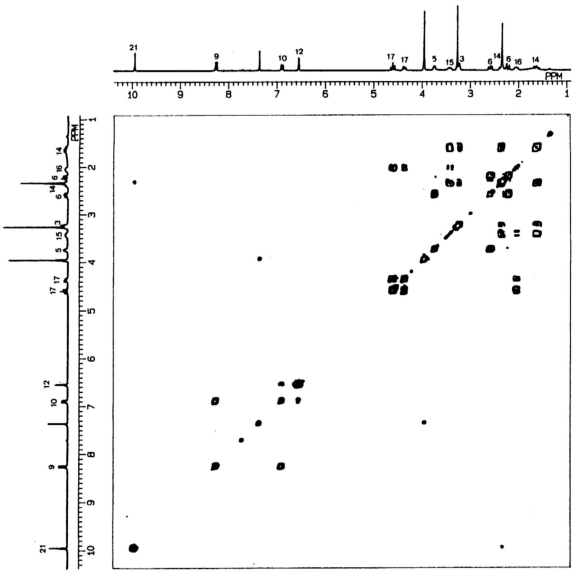


Fig.3.19 ^{1}H-^{1}H COSY spectrum of N_b-Demethylalstophyllal oxindole **[81]** (**AM6**)

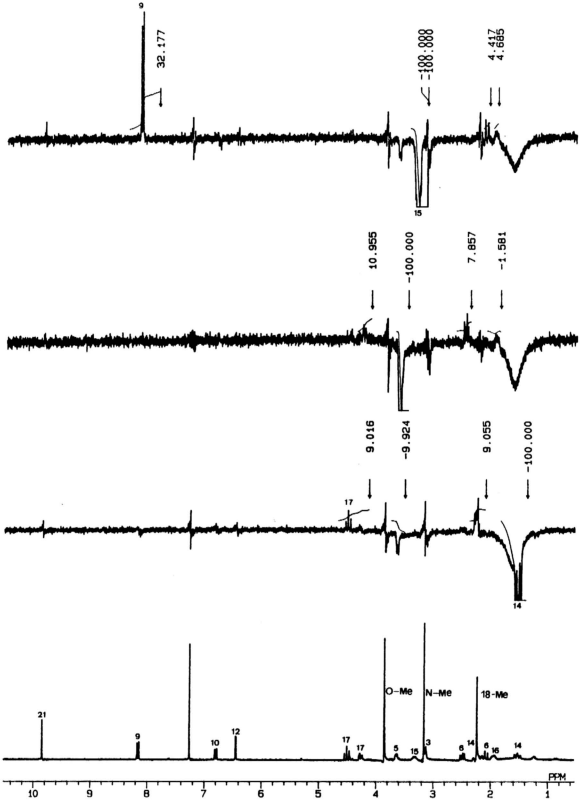


Fig.3.20 NOE spectrum of N_b-Demethylalstophyllal oxindole **[81]** (**AM6**)

The doublet at δ3.66 (H-5β) correlated to the doublet of doublets at δ2.49 (H-6β) with the coupling constant of 7 Hz. Whilst no correlation between H-5β and the doublet at δ2.12 (H-6α) was observed, this indicated a torsional angle of approximately 90° between H-5β (equatorial) and H-6α (axial). Subsequently the correlation observed between the δ2.49 doublet of doublets (H-6β) and the doublet at δ2.12 (H-6α) accounts for the geminal coupling constant of 14Hz. The geminal coupling constant of 14Hz is lower compared to the C-6 protons geminal coupling constant (16-17Hz) in the other macroline system compounds: (-)-Talcarpine [**54**], N_b-Methyl-N_b,21-secotalpinine [**78**], Alstophylline [**6**] and 19,20-Dehydro 10-methoxytalcarpine [**80**] (**AM1**, **AM2**, **AM3** and **AM4**, respectively). The lower value of geminal coupling constant (14Hz) between the C-6 protons in **AM6** is mainly due to C-6 of **AM6** being no longer adjacent to π orbitals, which are present in the C-2/C-7 double bond.

The other downfield one proton multiplet signal at δ3.09-3.21 was assigned to H-3β which is adjacent to the amine function. Correlation was observed in the 2D ^{1}H-^{1}H COSY (Fig. 3.19) between δ3.09-3.21m (H-3β) and δ1.52 ddd(14Hz, 12Hz, 3.5Hz) and between the H-3β signal and δ2.20-2.34 multiplet . Thus the δ1.52 ddd(14Hz, 12 Hz, 3.5 Hz) signal is assigned to H-14β and the δ2.20-2.34 multiplet to H-14α. This assignment was further supported by NOE difference (Fig. 3.20) experiment and the Drieding molecular model. Irradiation of H-14β caused an enhancement of 9% at the δ 4.50 triplet signal (H-17β). The Drieding molecular model of N_b-demethylalstophyllal oxindole [**81**] shows H-14α to be deshielded in comparison to H-14β because of proximity to the deshielding region of the benzene ring. Strong correlation H-14β resonance with the H-14α resonance accounts for the geminal coupling (14Hz) value. Cross peak of the H-14β resonance to multiplet at δ 3.30-3.42 (H-15α) was evident, whilst a coupling constant of 12Hz was observed. This indicated H-15α is in the axial position with a dihedral angle of about 180° with

respect to the H-14β. The third cross peak in relation H-14β is that of H-14β to H-3β, whilst the coupling constant is 3.5Hz, which shows that H-3β is in the equatorial position with respect to H-14β.

Similar to H-14α, the H-15α resonance is also shifted downfield due to deshielding effect of the benzene ring. Besides correlation to the C-14 protons the 2D ^{1}H-^{1}H COSY also shows the correlation with the multiplet at δ1.90-2.07. Therefore the multiplet at δ 1.90-2.07 was assigned to H-16α. Correlation of this multiplet to the resonance at 4.26 ddd (11Hz, 4Hz, 1.5Hz) and 4.50 t (11Hz) consequently attributed these signals to H-17α and H-17β respectively. The spin-spin coupling pattern of H-17α was attributed to geminal coupling (11Hz) with H-17β, the vicinal (equatorial-axial) coupling (4Hz) with H-16α and a long range W coupling of 1.5Hz. The H-17β manifestation as a triplet (11Hz) shows that H-16α is in the trans diaxial position to H-17β.

^{13}C NMR gave characteristic low field carbon resonance at δ183.1, δ145.3, δ120.6 and δ56.3 which are characteristic of ring B of oxindole systems, where the amide carbonyl function at C-2 was assigned to the singlet at δ183.1. The HMQC (Fig. 3.21) experiment performed on **AM6** disclosed correlation of the characteristic unsaturated carbonyl signal at δ189.5 to the singlet at δ9.85. This confirms the presence of the vinyl aldehyde function and subsequently the signal at δ9.85 was assigned to H-21 and the signal at δ189.5 to C-21. The resonance at δ16.7 correspond to the three proton singlet at δ2.24, characteristic of a vinyl methyl group and thus was assigned as 18-Me and C-18. Whilst from the HMBC (Fig. 3.22) long range coupling (J^2) was observed between singlet at δ9.85 to signal at δ118.2, which indicates δ118.2 as C-20. Long range coupling (J^2) between the 18-Me resonance and C-19 signal in the HMBC spectrum further supports the proposed assignment.

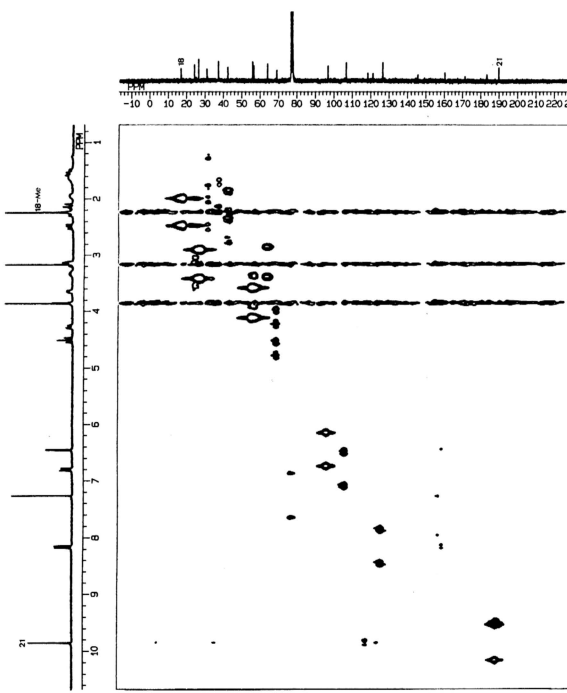


Fig.3.21 HMQC spectrum of N_b-Demethylalstophyllal oxindole [81] (AM6)

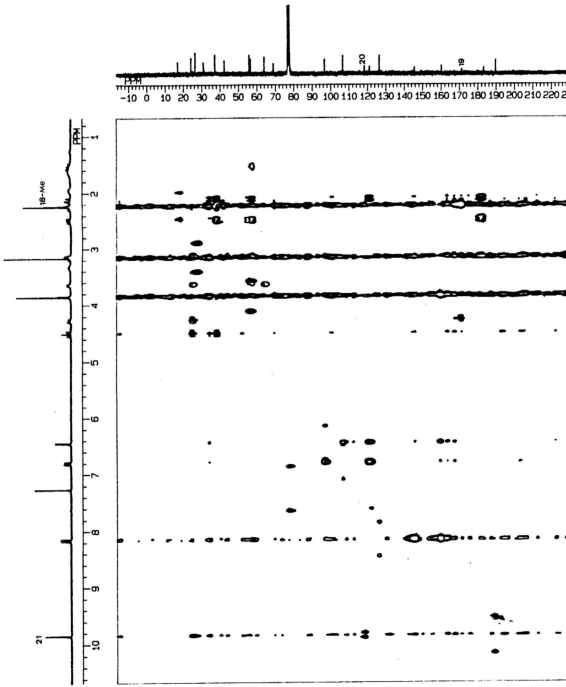


Fig.3.22 HMBC spectrum of N_b-Demethylalstophyllal oxindole [**81**] (**AM6**)

3.3.7 Alstonisine [58] (AM7)

HREIMS of **AM7** showed a molecular ion at 338.1625 (calcd. 338.1630) suggesting a molecular formula of $C_{20}H_{22}N_2O_3$. The EIMS fragmentation pattern in **AM7** shows similarity to **AM5**, where both indicated presence of 179 alicyclic moiety due to the cleavage of the spiran ring (Atta-ur-Rahman et al., 1987). The *m/z* 160 peak in EIMS of **AM7** was observed to be shifted by 30mu (*m/z* 190) in the EIMS of **AM5**. The difference (30mu) is attributed to the methoxy substitution in **AM5** and not in **AM7**. The proposed cleavage of the spiran ring is shown in Fig. 3.23 and is based on reported mass spectral studies of oxindole compounds (Gilbert et al., 1963 and Atta-ur-Rahman et al., 1987).

^{1}H NMR (Fig. 3.24) for alstonisine **[58]** relates well to the reported data (Ghedira et al., 1988), but there has been no ^{13}C NMR data reported. The assignment for the 13 carbon resonances of **[58]** (Fig. 3.25) were based on comparison to data from compounds **AM5**, **AM6** and **AM8** (Table 3.8). Characteristic vinyl ketone group signals similar to NMR signals of **AM5** were observed at δ196.6 (C-19), δ24.9 (C-18) and the three proton singlet at δ2.24 (18-Me), and also the vinyl methine group resonances at δ157.6 (C-21) and one proton singlet at δ7.62 (H-21) were noted. The absence of methoxy peak at δ3.85 in the ^{1}H NMR of **AM7** further supports **AM7** as the demethoxy of **AM5**. The spin-spin coupling pattern also characterizes **AM7** as an unsubstituted oxindole moiety.

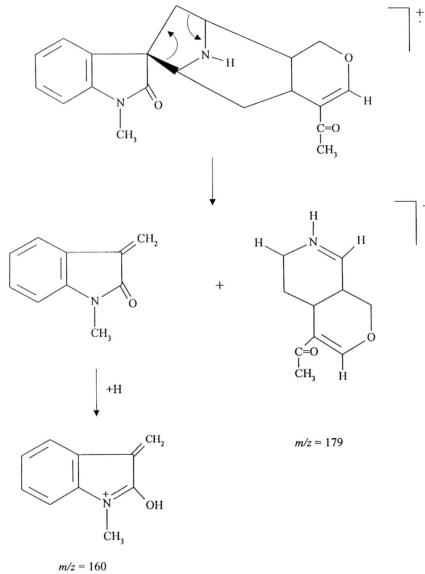


Figure 3.23 : Spiran Cleavage of Alstonisine [**58**]

Table 3.8 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for Alstonisine [58] (AM7) in $CDCl_3$

Position	δH	δC
2	-	182.5
3	3.15 - 3.20 m	63.8
5	3.68 d (7)	56.4
6	2.19 d (14)	41.9
	2.52 dd (14,7)	
7	-	56.9
8	-	129.1
9	6.86 dd (7,1.5)	107.9
10	7.30 dd (7,1.5)	123.4
11	7.32 dd (7,1.5)	127.9
12	8.25 dd (7,1.5)	125.6
13	-	144.0
14	1.47 - 1.58 m	31.0
	2.20 - 2.33 m	
15	3.35 - 3.41 m	24.2
16	1.90 - 2.00 m	37.0
17	4.26 ddd (11,4,1.5)	68.4
	4.45 t (11)	
18	2.24 s	24.9
19	-	196.6
20	-	121.8
21	7.62 s	157.6
N-Me	3.19 s	26.2

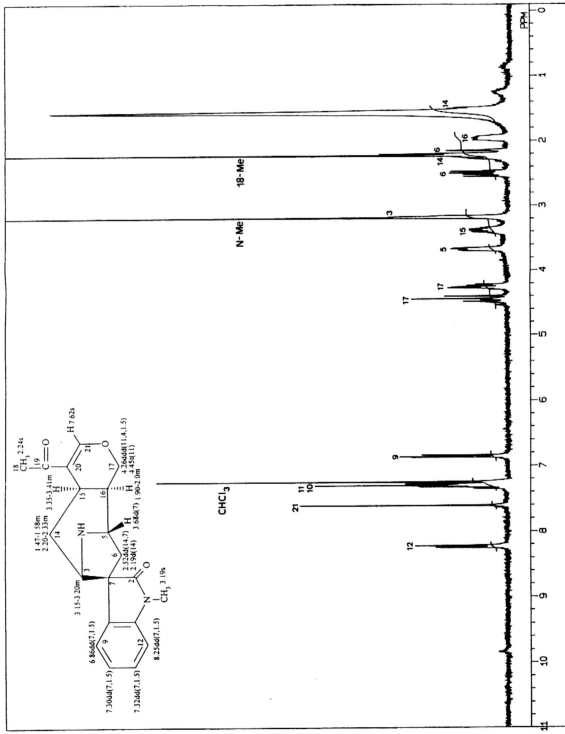


Fig.3.24 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of Alstonisine [58] (AM7)

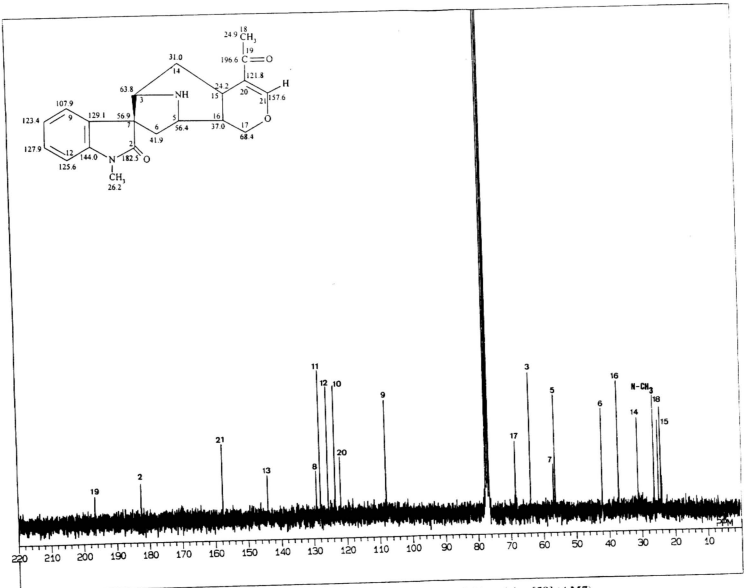


Fig.3.25 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of Alstonisine [**58**] (**AM7**)

3.3.8 Alstonal [82] (AM8)

AM8 is a new compound obtained as colourless crystalline needles m.p 198-199° and identified as Alstonal **[82]** (Wong et al., 1996). HREIMS of **AM8** showed a molecular ion at *m/z* 338.1638 (calcd. 338.1630 for $C_{20}H_{22}N_2O_3$). EIMS provided fragmentation pattern similar to Alstonisine**[58]**. Fragments at *m/z* 179 and 160 indicated cleavage at the spiran ring to produce an alicyclic fragment (*m/z* 179) and the oxindole fragment (*m/z* 160).

The ^{1}H NMR of **AM8** (Fig. 3.26) exhibits similarities to the ^{1}H NMR of Alstonisine **[58]**, particularly at the oxindole moiety. However, upon closer examination of the ^{1}H NMR and ^{13}C NMR spectra of **AM8** (Fig. 3.27) a better resemblance to N_b-demethylalstophyllal oxindole **[81]** in ring E was noted. The features similar to ring E of N_b-demethylalstophyllal oxindole **[81]** were the vinyl aldehyde function : H-21 singlet at δ9.85 and C-21 at δ189.5; and the vinyl methyl group : three proton singlet at δ2.23 and C-18 at δ16.7 (Table 3.9). Therefore the structure for **AM8,** alstonal **[82]** was put forward as the 11-demethoxy derivative of N_b-demethylalstophyllal oxindole **[81]**. The ^{1}H-^{1}H COSY spectrum of **AM8** (Fig. 3.28) showed correlation peaks which establishes substructure sequences for rings C and D, indicating connectivities of the C-5 protons to the C-6 protons, the C-3 proton to the C-14 protons; the C-14 protons to the C-15 proton; the C-15 proton to the C-16 proton and the C-16 proton to the C-17 protons.

Similarly the HMQC and HMBC spectrums (Fig. 3.29 & Fig. 3.30) of **AM8** supports the ring E features of **AM8** as identical to that observed in the HMQC and HMBC spectrums of **AM6** (discussed in Section 3.3.6).

Table 3.9 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for Alstonal [82] (AM8) in $CDCl_3$

Position	δH	δC
2	-	182.5
3	3.10 - 3.20 m	63.8
5	3.66 d (7)	56.4
6	2.17 d (14)	41.9
	2.51 dd (14,7)	
7	-	56.9
8	-	129.1
9	6.86 dd (7,1.5)	107.9
10	7.30 dd (7,1.5)	123.4
11	7.32 dd (7,1.5)	127.9
12	8.24 dd (7,1.5)	125.6
13	-	143.9
14	1.54 ddd (15,11.5,3)	30.8
	2.22 - 2.34 m	
15	3.33 - 3.39 m	23.9
16	1.90 - 2.00 m	37.0
17	4.27 ddd (11,4,1.5)	68.6
	4.50 t (11)	
18	2.23 s	16.7
19	-	170.9
20	-	118.1
21	9.85 s	189.5
N-Me	3.19 s	26.2

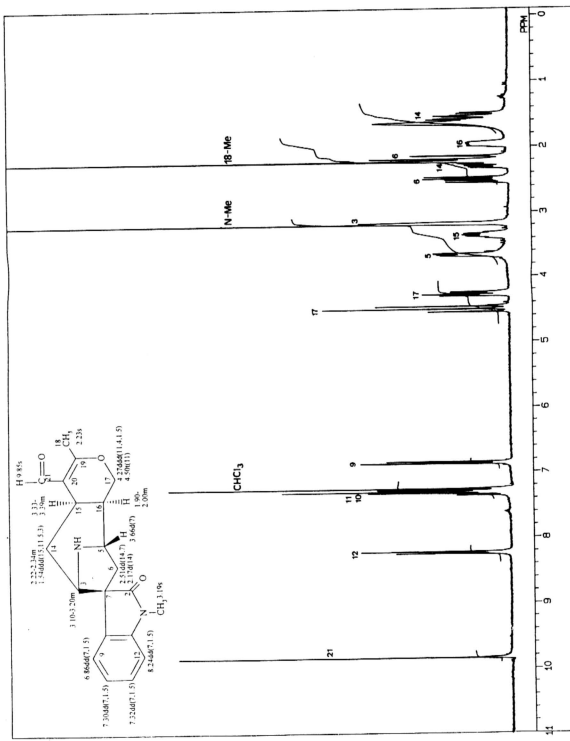


Fig.3.26 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of Alstonal [82] (**AM8**)

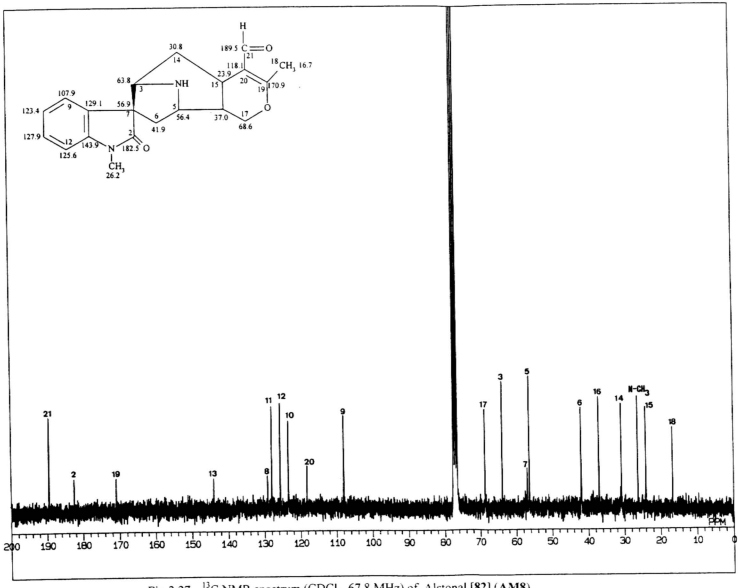


Fig.3.27 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of Alstonal **[82]** (**AM8**)

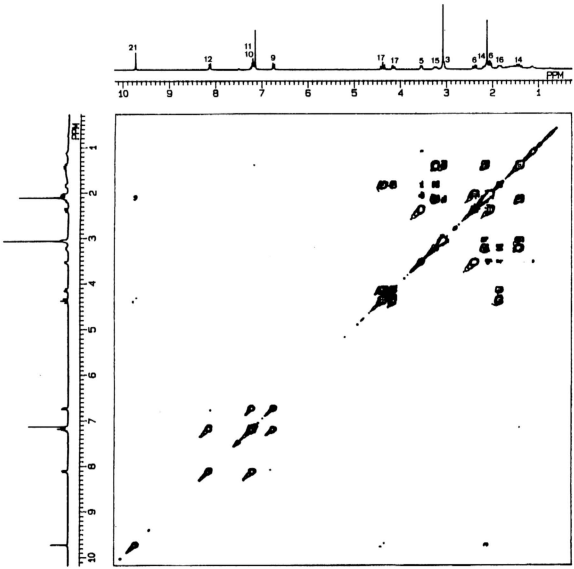


Fig.3.28 ^{1}H-^{1}H COSY spectrum of Alstonal **[82] (AM8)**

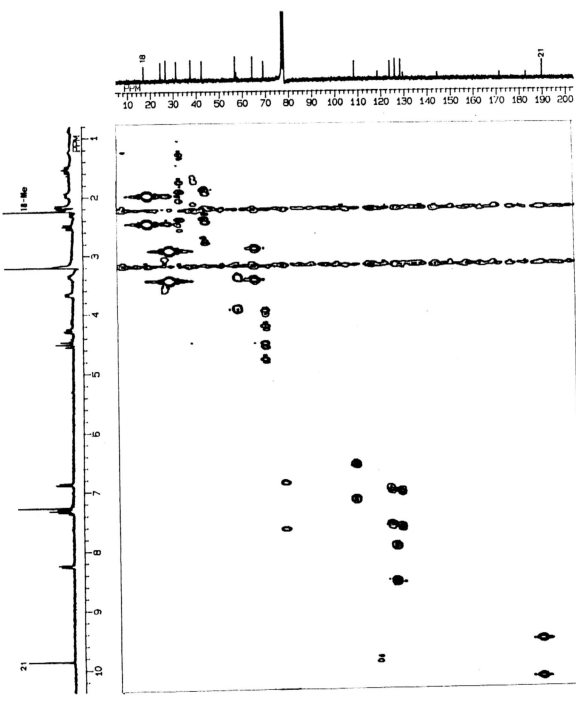


Fig.3.29 HMQC spectrum of Alstonal [82] (AM8)

3.3.9 Pleoicarpamine [3] (AM9)

EIMS of **AM9** shows M^+ at 322 and the fragmentation pattern is similar to those reported for pleiocarpamine **[3]** (Hesse et al., 1964 and Kump & Schmid, 1961). The peak at *m/z* 263 is characteristic for the loss of the C-16 methyl ester substituent. Subsequent loss of the ethylidene side chain gave rise to a peak at *m/z* 234 . Further decomposition probably involving initial retro Diels-Alder cleavage of ring C giving a fragment at *m/z* 180 (Hesse et al., 1964 and Kump et al., 1961).

The ^{1}H NMR recorded (Fig. 3.31) is also in accord with that reported (Hesse et al., 1964 and Kump et al., 1961). In particular, the characteristic high field signal was observed for one of the C-21 proton at δ1.75 (brd, J=12.5Hz), which is unusual for a proton between nitrogen and a double bond. Drieding molecular model shows that this shielding effect is due to the position of the C-21 proton being directly over the C-2/C-7 double bond of the indole ring. Similarly, from the Drieding molecular model H-15 is located in the plane of the 19,20-double bond, and is observed at δ3.52-3.54 as a multiplet (Gilbert, 1968).

The ^{1}H-^{1}H COSY (Fig. 3.33) spectrum of **AM9** afforded substructure connectivities that fitted well with the pleiocarpamine **[3]** structure. Homoallylic coupling (2Hz) is observed between the doublet of doublets at δ1.49 (18-Me) and the broad doublet at δ1.75 (H-21). In addition, the allylic coupling of H-19 to H-21 is also noted.

^{13}C NMR (Fig. 3.32) and HMQC (Fig. 3.34) spectra further supported the pleiocarpamine **[3]** structure. The assignments of ^{13}C NMR signals correlates well to the structure where the typical ester function was depicted by signals at δ169.1 (C-17) and δ51.9 (methoxy carbon) which were observed to correlate to a three proton singlet at δ3.58 (characteristic of a methyl ester group). C-21, C-5, C-3 have been assigned

Table 3.10 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for Pleiocarpamine [3] (AM9) in $CDCl_3$

Position	δH	δC
2	-	136.8
3	3.86 brs	50.6
5	2.36 ddd (13.5,8.5,6)	49.9
	3.38 ddd (13.5,10,2.5)	
6	2.69 ddd (16,10,6)	20.6
	3.16 ddd (16,8.5,2.5)	
7	-	108.0
8	-	128.5
9	7.54 - 7.57 m	118.3
10	7.08 - 7.18 m	119.9
11	7.08 - 7.18 m	120.6
12	6.95 - 6.98 m	112.3
13	-	137.6
14	2.22 ddd (13,4,2)	28.4
	2.51 ddd (13,4,2)	
15	3.52 - 3.54 m	33.7
16	5.22 d (4)	61.2
18	1.49 dd (7,2)	12.5
19	5.32 qd (7,1.5)	122.9
20	-	133.0
21	1.75 brd (12.5)	56.4
	2.62 d (12)	
$\underline{C}OOCH_3$	-	169.1
$COO\underline{C}H_3$	3.58 s	51.9

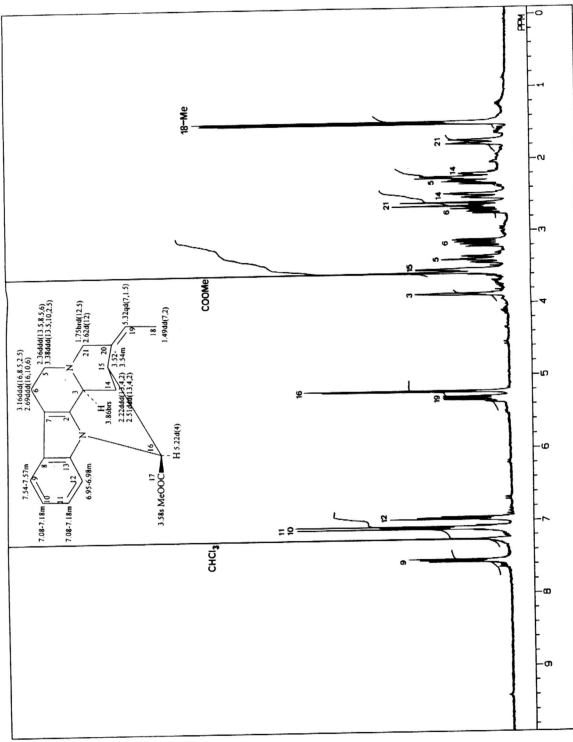


Fig.3.31 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of Pleiocarpamine [3] (AM9)

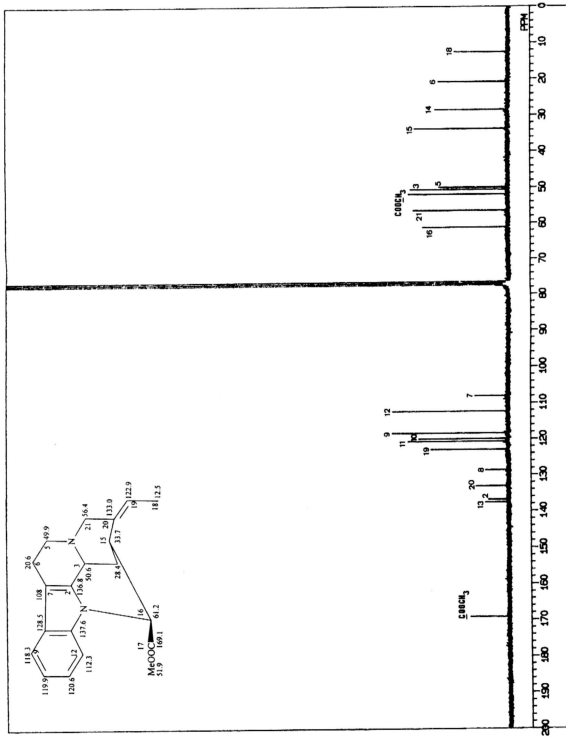


Fig.3.32 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of Pleiocarpamine [3] (AM9)

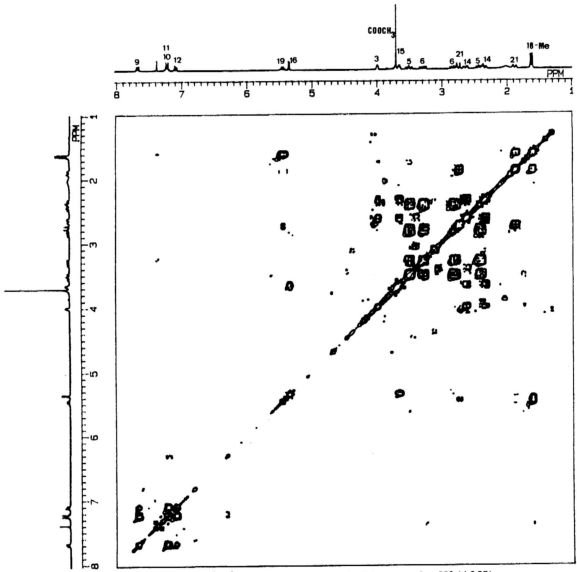


Fig.3.33 ^{1}H-^{1}H COSY spectrum of Pleiocarpamine [3] (AM9)

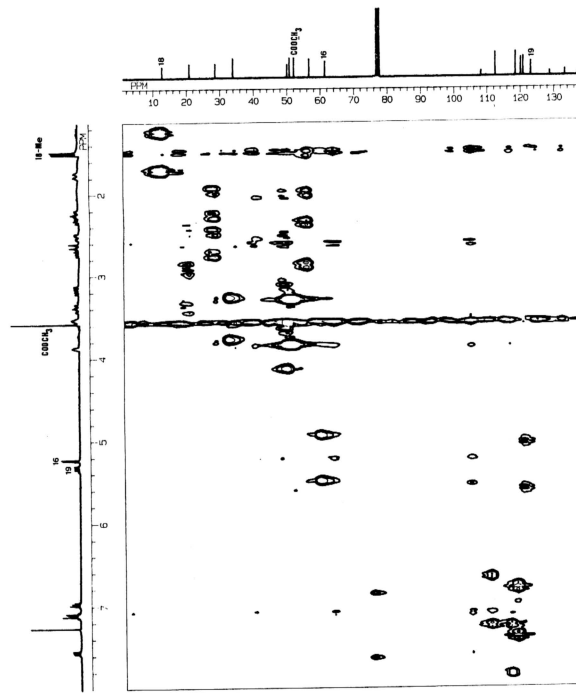
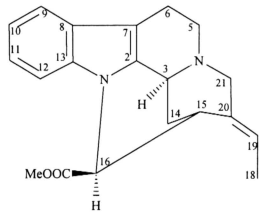


Fig.3.34 HMQC spectrum of Pleiocarpamine [3] (AM9)

δ56.4, δ49.9 and δ50.6 respectively, all showing typical values for carbon adjacent to N atom. Another characteristic feature observed is the ethylidene side chain as indicated by signals at δ122.9 (C-19), quartet of doublets at δ5.32 (H-19) and δ12.5 (C-18) along with the methyl signal at δ1.49 (18-Me). The C-16 resonance at δ61.2 (typical of carbon adjacent to N atom and a carbonyl function) correlated to the doublet at δ5.22 (H-16). Assignments of the remaining carbon signals were done by comparison to the literature information and by HMBC experiment.



Pleiocarpamine [3]

3.3.10 Villalstonine [10] (AM10)

EIMS of **AM10** shows a M^+ at 660 similar to that reported for Villalstonine [**10**]. The presence of a macroline unit was indicated by peaks at *m/z* 338, 197, 182, 181 and 170 (Hesse et al., 1965). Ions diagnostic of pleiocarpamine [**3**] unit were observed at *m/z* 322 and 263 (Hesse et al., 1964 and Kump & Schmid, 1961). Fragment at *m/z* 352 indicated a double junction between the two monomers (Ghedira et al., 1988). It has been postulated that the fragmentation involves the fission of the tetrahydropyran ring in villalstonine [**10**] to give *m/z* 352 which further degrades to give radical ions at *m/z* 135, 121 and 107 (Hesse et al., 1965). This double junction was also established by chemical analysis and X-ray crystallography (Saxton, 1970; Hesse et al., 1965 and Nordman & Kurma, 1965).

^{1}H NMR (Fig. 3.35) and ^{13}C NMR (Fig. 3.36) assignments (Table 3.11) are based on that reported values in the literature (Ghedira et al., 1988). The 2D COSY supports the proposed structure. Characteristic peaks for macroline [**21**] unit and pleiocarpamine [**3**] units were also observed. Typical signals for macroline unit [**21**] observed were : two 3-proton singlets for N_a and N_b methyls; the 1-proton triplet at δ3.99 (H-17); two 1-proton doublets at δ2.91 (H-5)and at δ2.47 (H-6); and the doublet of doublets at δ3.29 (H-6) (Ghedira et al., 1988). The characteristic resonance arising from the pleiocarpamine unit [**3**] observed were : a 3-proton doublet of doublets at δ1.55 (7Hz, 1.5Hz) and a 1-proton quartet at δ5.36 (6.5Hz) from the ethylidene side chain; a 3-proton singlet at δ3.67 for the methyl ester group.

However, the characteristic shielded C-21' proton in the pleiocarpamine unit [**3**] no longer resonate at shielded values indicating presence of reduced C-2'/C-7' double bond. Furthermore low field C-2' and C-7' carbon resonance was no longer

Table 3.11 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for Villalstonine [10] (AM10) in $CDCl_3$

Position	δH	δC	Position	δH	δC
2		135.9	2'		92.1
3	3.86 brs	53.4	3'	3.70 - 3.75 m	51.8
5	2.91 d (6.5)	54.4	5'	2.63 - 2.73 m	47.4
6	2.47 d (16.5)	22.9		3.13 brdd (14, 2.5)	-
	3.29 dd (16.5, 6.5)	-	6'	1.11 brd (13)	31.3
7	-	106.6		2.01 - 2.12 m	-
8	-	126.4	7'	-	44.1
9	7.54 d (7.5)	118.1	8'	-	132.9
10	7.15 t (7.5)	120.9	9'	6.88 d (7.5)	120.8
11	7.22 t (7.5)	118.9	10'	6.98 t (7.5)	118.1
12	7.33 d (7.5)	108.8	11'	6.69 t (7.5)	126.4
13	-	137.0	12'	6.14 d (7.5)	109.3
14	1.44 brd (12.5)	32.5	13'	-	146.9
	2.36 - 2.46 m	-	14'	1.67 - 1.72 m	27.5
15	1.56 - 1.66 m	32.4		2.63 - 2.73 m	-
16	2.01 - 2.12 m	37.9	15'	3.21 brd (3.5)	31.8
17	3.70 - 3.75 m	65.6	16'	4.44 d (3.5)	57.8
	3.99 t (12)	-		-	-
18	1.25 s	26.5	18'	1.55 dd (7, 1.5)	12.3
19		98.6	19'	5.36 q(6.5)	118.5
20	1.16 - 1.19 m	36.8	20'	-	136.1
21	1.56 - 1.66 m	28.5	21'	2.96 d (12.5)	52.9
	2.36 - 2.46 m	-		4.19 brd (12.5)	-
N_a-Me	3.62 s	29.0	$\underline{C}OOCH_3$	-	171.0
N_b-Me	2.31 s	41.8	$COO\underline{C}H_3$	3.67 s	51.7

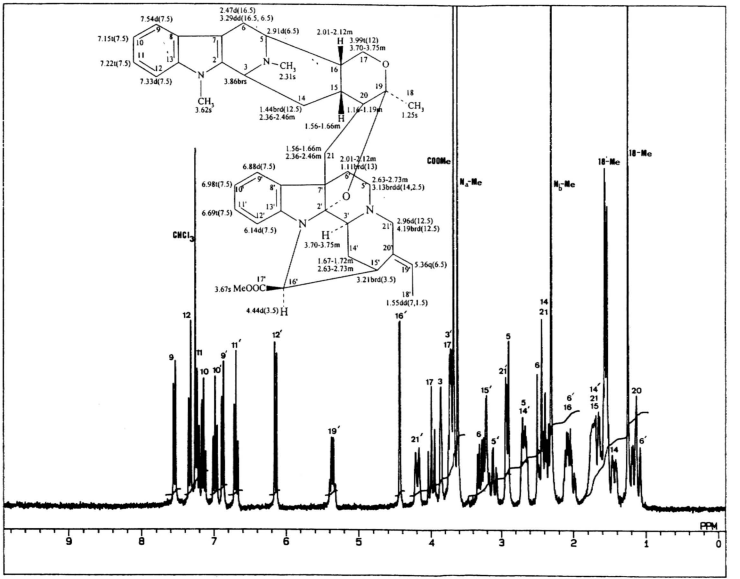


Fig 3.35 ^{1}H NMR spectrum (CDCl$_3$, 270 MHz) of Villalstonine [101 (AM10)

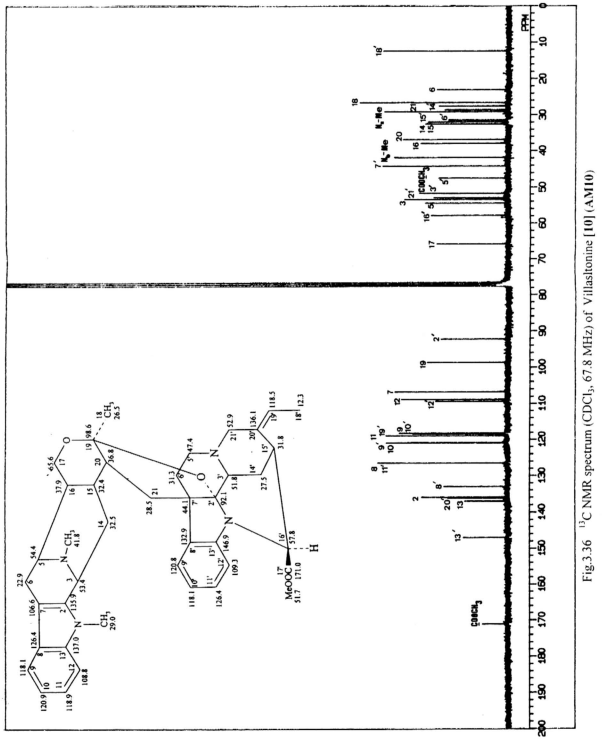
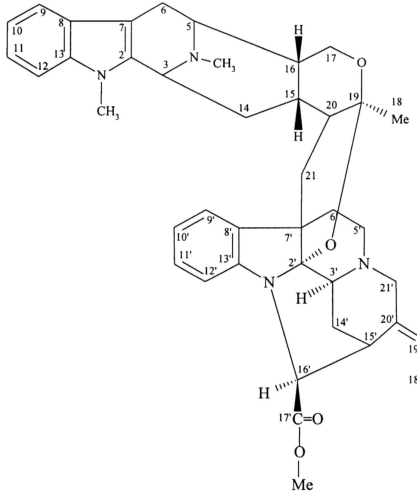


Fig.3.36 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of Villalstonine **[10]** (**AM10**)

observed. This is indicative that the double junction between the two monomers involves both C-2' and C-7' to the unsaturated ketone function of the macroline unit, which no longer exhibits low field resonance due to carbonyl and enolic influences. The absence of carbonyl and enolic influences in the macroline unit [**21**] are characterized in the ^{13}C NMR mainly by the absence of low field C-19, C-20 and C-21 resonance, which were shifted to δ98.6 (C-19), δ36.8 (C-20) and δ28.5 (C-21) respectively.



Villalstonine [**10**]

3.3.11 Macralstonine hydroxyketone [83] (AM11)

EIMS of **AM11** showed a M^+ at 704 and fragmentation pattern reminiscent of that reported for macralstonine [**22**] (Kishi, et al., 1966) . The ^1H NMR and ^{13}C NMR spectra (Fig. 3.37 & 3.38) indicated that **AM11** is not a mixture of two forms (the hemiketal form [**22**] and the hydroxy ketone form [**83**]) but the hydroxyketone form of macralstonine [**83**]. Complete assignments of the ^1H NMR and ^{13}C NMR (Table 3.12) were based on ^1H-^1H COSY (Fig. 3.39), DEPT (Fig. 3.40), HMQC (Fig. 3.41) and HMBC (Fig. 3.42) experiments.

Thus far all the NMR documentation of macralstonine [**22**] involved were for mixtures of isomers (Gheidra et al., 1988 and Kishi et al., 1966) . ^1H NMR (Fig. 3.37) spectrum for **AM11** showed no splitting between the C-18 methyl group and the C-9' and C-12' protons which were observed in the isomeric mixture (Kishi et al., 1966). The C-9' and C-12' protons were observed as singlets at δ6.9 and δ6.7 respectively. Integration values of all the other signals also indicated presence of a single form of macralstonine (the hydroxyketone form) [**83**].

The ^{13}C NMR (Fig. 3.38) and DEPT (Fig. 3.40) experiment further supported the hydroxy ketone form of macralstonine [**83**]. Two carbonyl peaks were observed at δ213.2 and δ195.5 whilst no peaks for the quaternary C-19 of the hemiketal form was observed. The ^1H-^1H COSY (Fig. 3.39) experiment afforded identification of the substructure sequences of the 2 monomers. Hence signals for the ring C and D of the macroline half and ring C', D' and E' for alstophylline half was established and the assignments were made accordingly.

Table 3.12 : ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) Spectral Data for Macralstonine hydroxyketone [83] (AM11) in $CDCl_3$

Position	δH	δC	Position	δH	δC
2		132.9	2'		131.3
3	4.08 - 4.18 m	53.2	3'	3.80 brs	53.8
5	3.48 d (7.5)	59.3	5'	3.02 - 3.05 m	54.8
6	2.53 d (16.5)	22.7	6'	2.35 - 2.38 m	22.1
	3.28 dd (16.5, 7.5)			3.02 - 3.05 m	
7	-	105.5	7'	-	106.0
8	-	126.4	8'	-	120.9
9	7.51 - 7.53 m	118.3	9'	6.90 s	118.9
10	7.13 t (7.5)	119.2	10'	-	119.1
11	7.20 - 7.26 m	121.0	11'	-	153.7
12	7.33 d (7.5)	108.8	12'	6.70 s	91.3
13	-	137.1	13'	-	136.7
14	1.96 - 1.98 m	32.4	14'	1.75 - 1.77 m	32.5
	2.38 - 2.41 m			2.02 - 2.04 m	
15	2.10 - 2.15 m	31.6	15'	2.49 - 2.54 m	22.9
16	1.58 - 1.61 m	43.2	16'	1.83 - 1.87 m	38.4
17	3.92 - 4.03 m	66.5	17'	4.08 - 4.18 m	67.8
	3.92 - 4.03 m			4.39 t (11.5)	
18	1.71 s	31.2	18'	2.07 s	25.0
19	-	213.2	19'	-	195.5
20	3.27-3.30 m	54.7	20'	-	120.1
21	2.42 dd (13, 4)	32.0	21'	7.51 s	157.5
	3.08 dd (13, 4)			-	
N_a-Me	3.55 s	29.0	N'_a-Me	3.66 s	29.1
N_b-Me	2.36 s	41.7	N'_b-Me	2.27 s	41.2
			O-Me	3.87 s	55.6

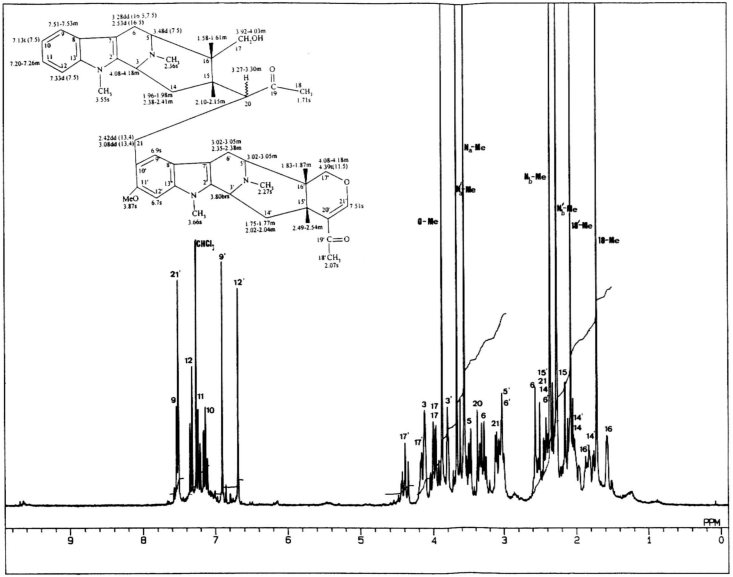


Fig.3.37 ^{1}H NMR spectrum ($CDCl_3$, 270 MHz) of Macralstonine hydroxyketone [**83**] (**AM11**)

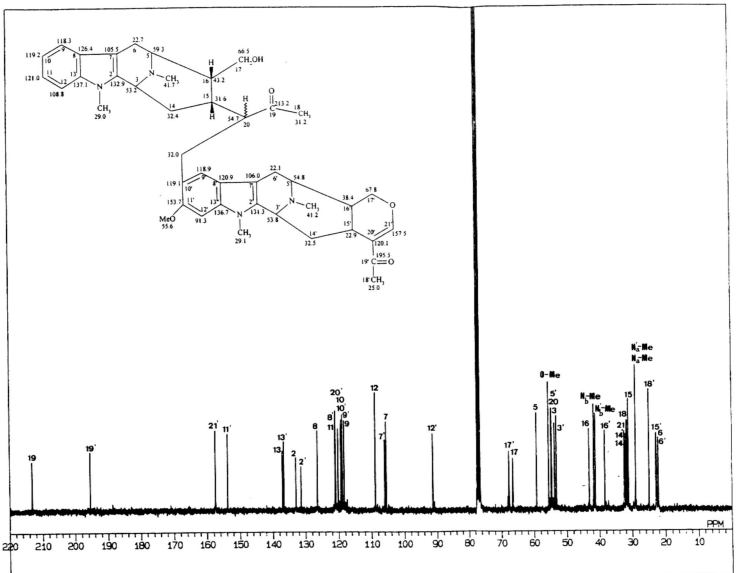


Fig.3.38 ^{13}C NMR spectrum ($CDCl_3$, 67.8 MHz) of Macralstonine hydroxyketone **[83] (AM11)**

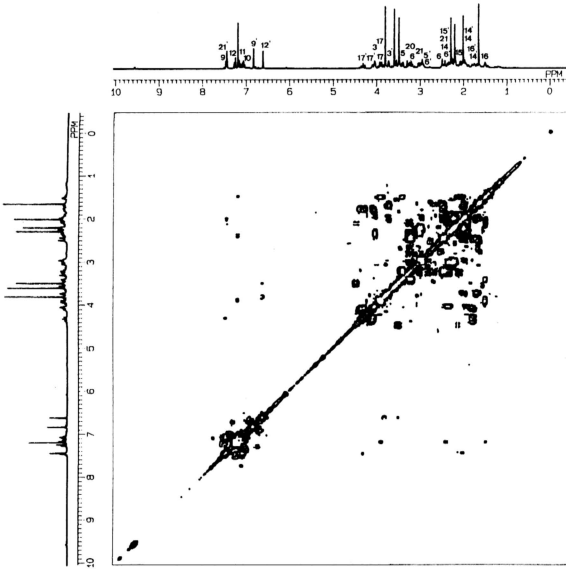


Fig.3.39 ^{1}H-^{1}H COSY spectrum of Macralstonine hydroxyketone [**83**](**AM11**)

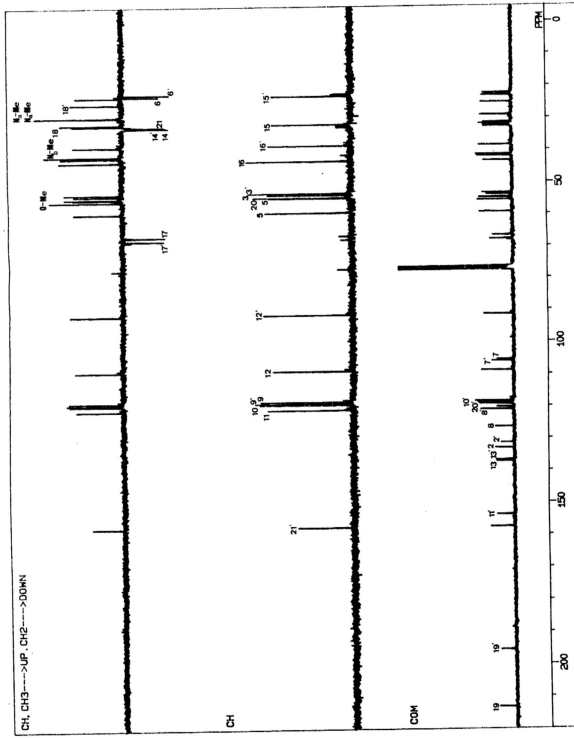


Fig 3.40 DEPT spectrum of Macralstonine hydroxyketone [83] (AM11)

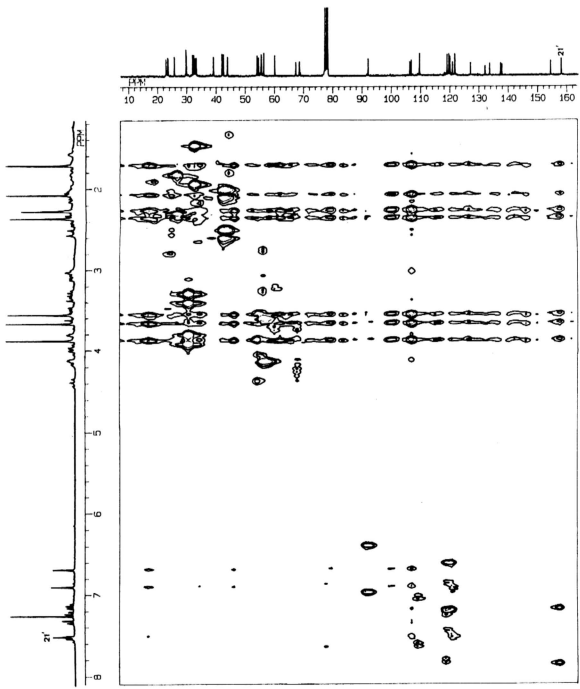


Fig.3.41 HMQC spectrum of Macralstonine hydroxyketone [83] (AM11)

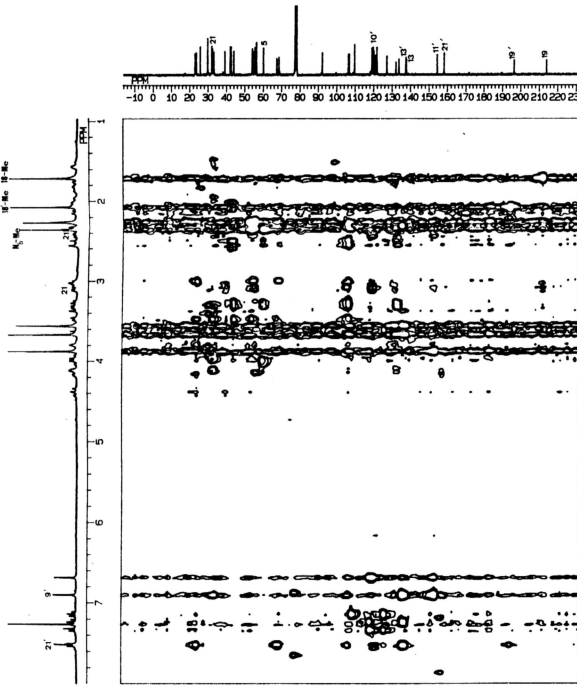
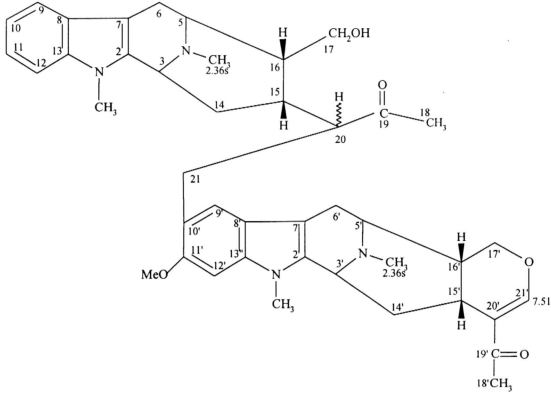


Fig.3.42 HMBC spectrum of Macralstonine hydroxyketone [83] (AH11)



Macralstonine hydroxyketone [**83**]

Long range connectivity (J^3) in the HMBC spectrum (Fig. 3.42) was observed between C-5 resonance at δ59.3 and N_b-methyl at δ2.36, and hence it is expected that the other aliphatic N methyl signal at δ2.27 follows as N'_b-methyl of the alstophylline unit. The carbonyl peak at δ195.5 and δ213.2 showed long range (J^2) connectivity to the 18'-Me (δ2.07) and (J^2) connectivity to the 18-Me (δ1.71) respectively, thus establishing the resonance at δ195.5 and δ213.2 for C-19' and C-19 respectively.

The C-19' signal also showed long range connectivity (J^3) to H-21' one proton singlet signal at δ7.51.

Both the aromatic protons of the alstophylline unit were observed as one proton singlet at δ6.9 (H-9') and δ6.7 (H-12') indicating both to be situated para to each other. This suggests that the attachment is between the C-21 of macroline unit and the C-10' of ring A'(Kishi et al., 1966). This proposed attachment is further supported by long range coupling observed in the HMBC, between H-9' and C-21 (J^3); C-21 protons and C-10' (J^2). Long range coupling (J^3) between C-13' resonance (δ136.7) and one proton singlet at δ6.9 (H-9'); C-11' resonance (δ153.7) and the one proton singlet at δ6.9 (H-9') confirms the H-9' assignment.