CHAPTER IV

RESULTS AND DISCUSSION

A combination of silica gel column chromatography, gel filtration on Sephadex LH-20 and preparative TLC were applied to the ethanolic extract of *A. salviifolium* root to yield 4 alkaloids (AL-1 to AL-4) and applied to the methanolic fraction of the leaves to yield a mixture of 2 alkaloids (AL-1L).

1. Structure Elucidation of AL-1

Compound AL-1 was obtained as amorphous powder from fraction F-061 (Table 3). It gave orange color to Dragendroff's test. and thus was likely an alkaloid. The EIMS spectrum (Figure 2) exhibited a molecular ion peak at *m/z* 464, suggesting a molecular formula of $C_{28}H_{36}N_2O_4$. The IR spectrum (Figure 3) displayed the absorption bands of O-H stretch of hydroxyl group at 3430 cm^{-1}, methyl C-H stretch of methoxyl group at 2955 cm^{-1}, and C=C stretch of aromatic ring at 1512 cm^{-1}. The UV spectrum showed the absorption maxima at 207 nm (log ε 3.59), 277 (log ε 3.01), 406 (log ε 3.20) (Figure 4).

For mass fragment analyses from the EIMS spectrum (Figure 2), there were major fragments observed at *m/z* 191, 216, 244 and 273 which are the characteristics of benzoquinolizidine and dihydroisoquinoline moieties of tetrahydroisoquinoline monoterpene alkaloid. The γ-H rearrangement (McLafferty rearrangement) and allylic cleavage gave the peak at *m/z* 273 and 244 representing the presence of benzoquinolizidine moiety bearing two methoxyl groups and the peak at *m/z* 191 representing the dihydroisoquinoline moiety containing a methoxyl and a hydroxyl group (Scheme 9). The cleavage of ring C by retro-Diels Alder gave the isoquinoline moiety with 2 methoxyl groups at *m/z* 191 and 190 and dihydroisoquinoline moiety with a methoxyl and a hydroxyl

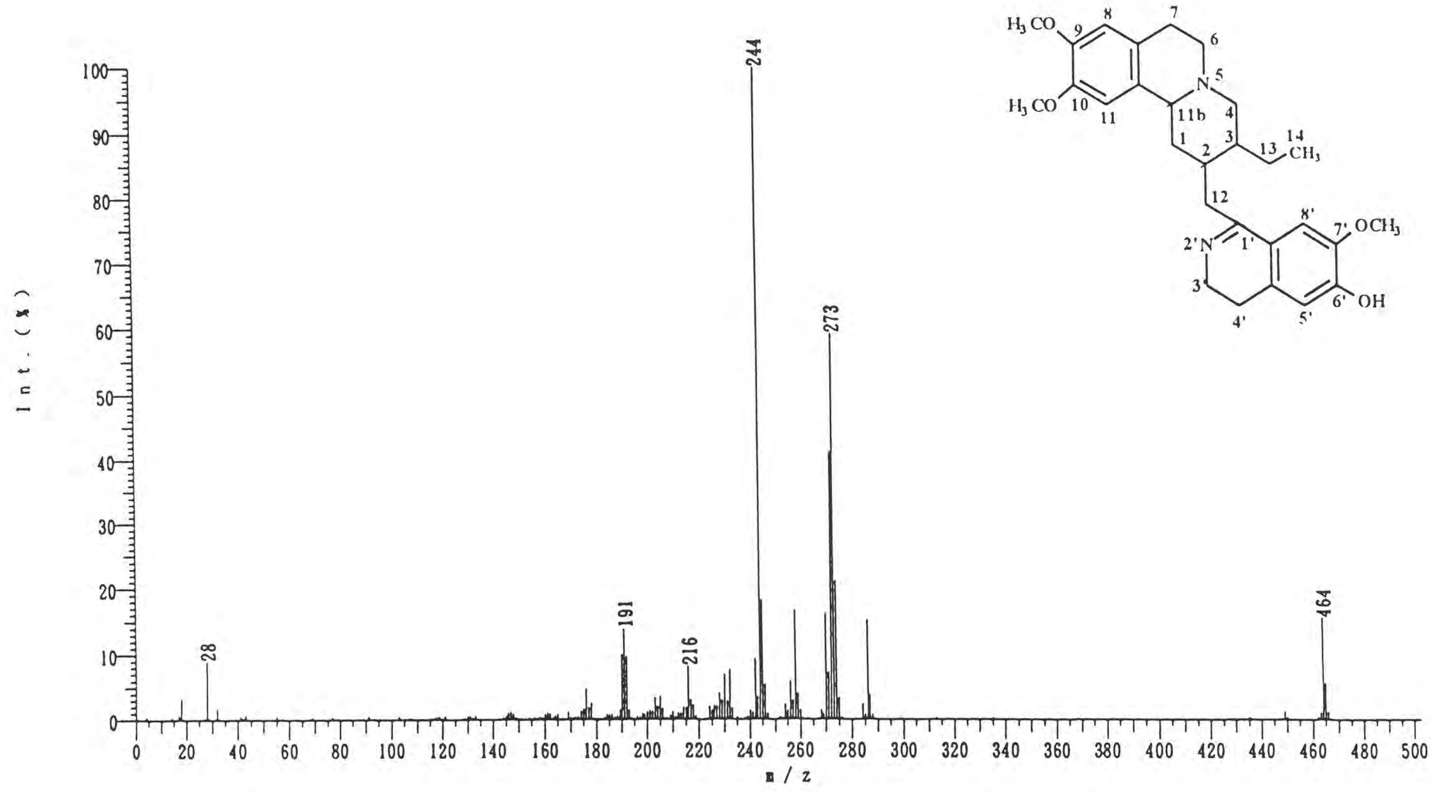


Figure 2 EIMS spectrum of AL-1

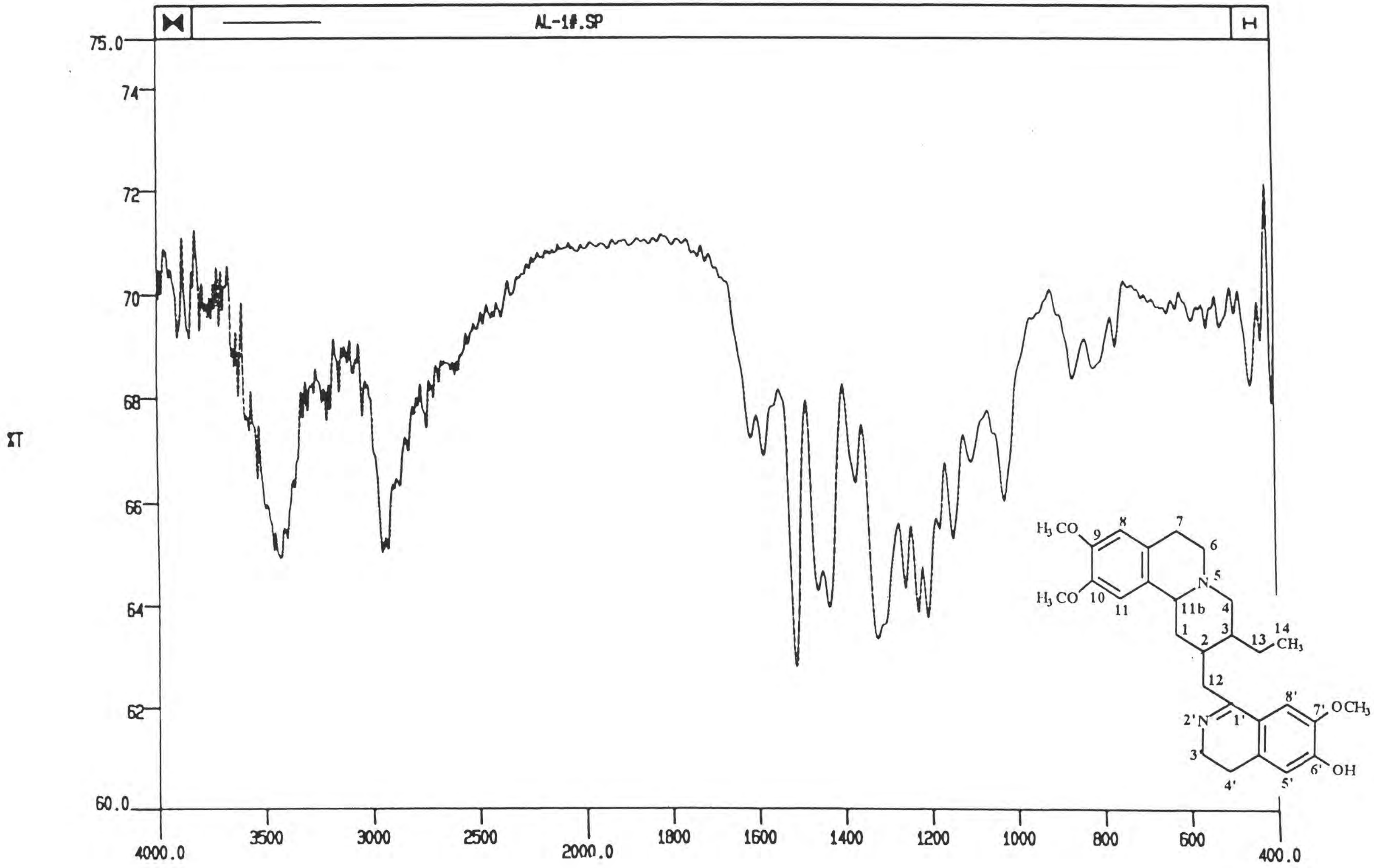


Figure 3 IR spectrum of AL-1 (KBr disc)

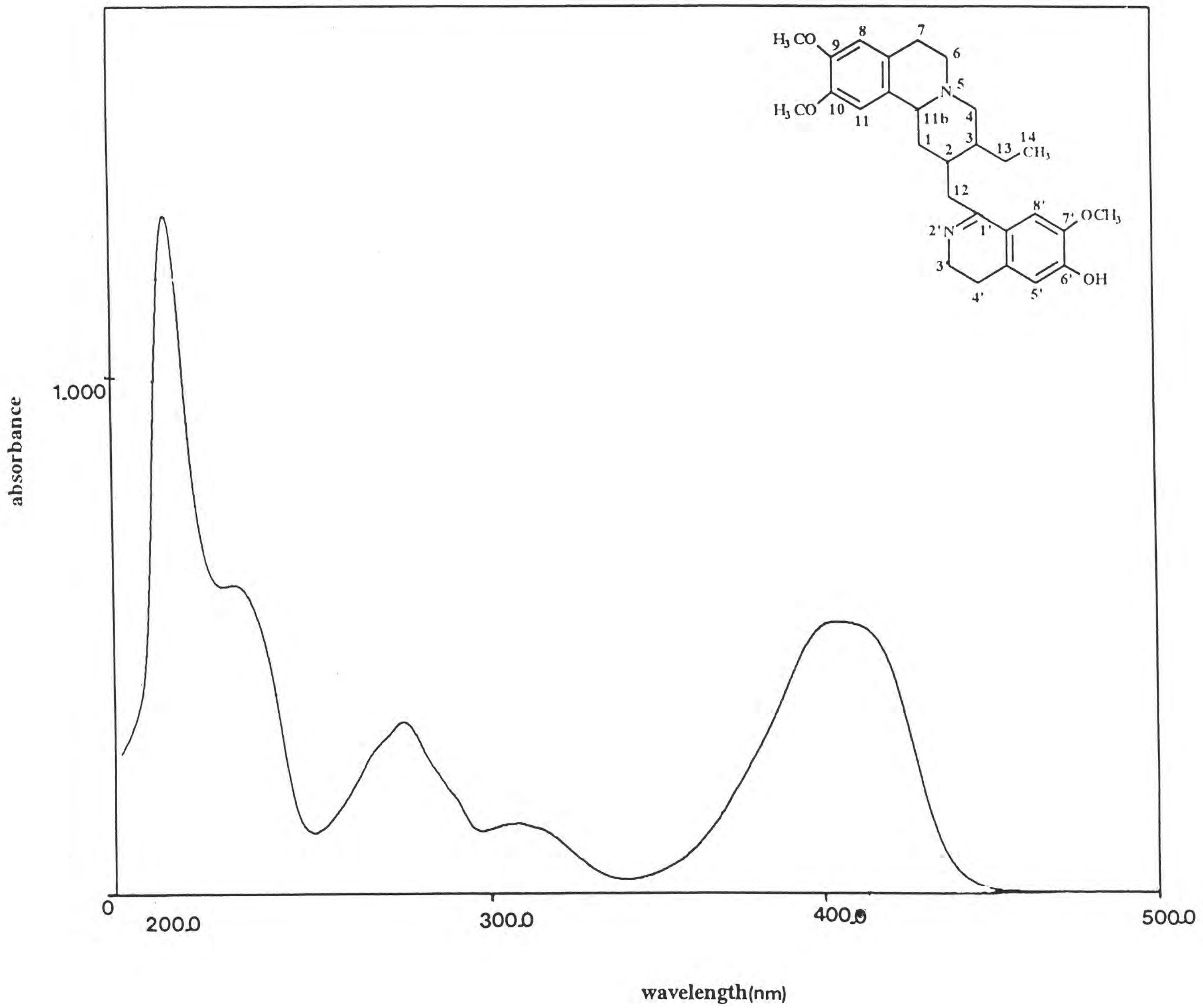
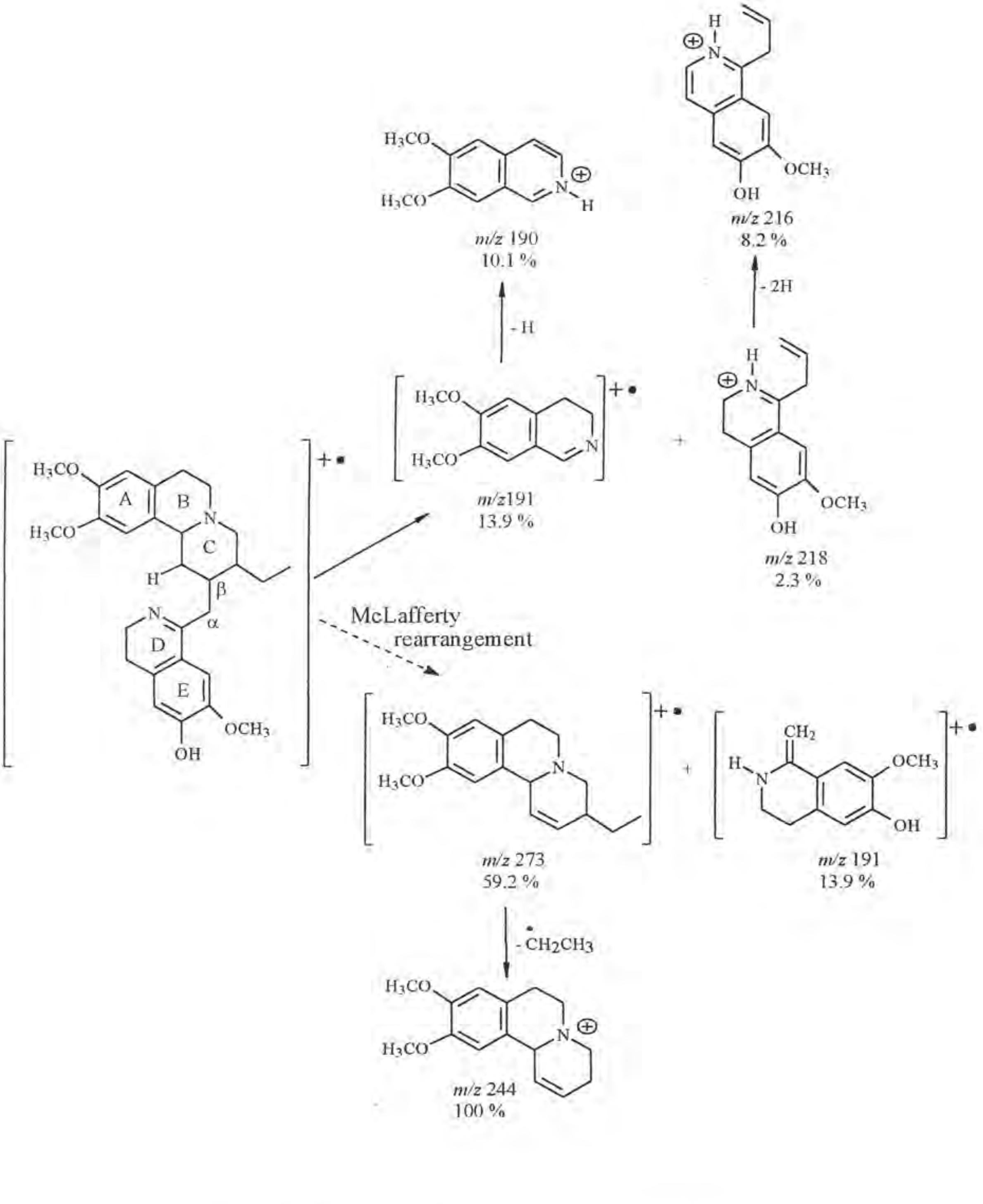


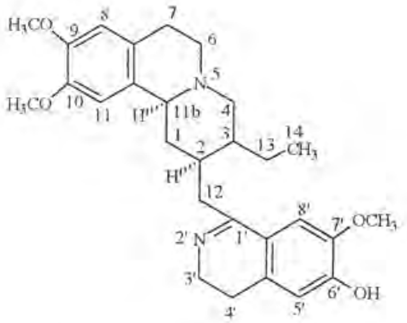
Figure 4 UV spectrum of AL-1 (in methanol)



Scheme 9 Mass fragmentation pattern of AL-1

group at m/z 216.

Hence, it was proposed that the alkaloid AL-1 was a tetrahydroisoquinoline monoterpene alkaloid possesing 2 methoxyl groups on the benzoquinolizidine moiety and a methoxyl and a hydroxyl group on the isoquinoline moiety. Comparison of the published data (Wiegrebe, Kramer, and Shamma, 1984), with the mass fragmentation pattern of the alkaloid Al-1, it could be identified as the known alkaloid psychotrine. Its structure is shown below.



psychotrine

The structure of psychotrine was confirmed by its ^1H NMR (Figures 5, 6, 7) and ^{13}C NMR (Figures 8, 9) spectral data. The presence of three methoxyl groups in the structure was confirmed by the appearance of three methoxyl protons as singlet at δ 3.72, 3.82, and 3.89 ppm in the ^1H NMR spectrum and the presence of downfield aromatic carbons at δ 146.0, 146.5 and 146.8 ppm together with the presence of methoxyl carbons at δ 55.0 , 55.1 and 55.3 ppm in ^{13}C NMR spectrum. The hydroxyl group was confirmed by the presence of downfield aromatic carbon at δ153.8 ppm in ^{13}C NMR spectrum. It should be noted that the hydroxyl proton could not be seen in ^1H NMR spectrum .

The ^1H NMR spectrum exhibited signals for aromatic protons as four singlets at δ 6.45, 6.54, 6.76 and 6.99 ppm. The singlet signals of all aromatic proton suggested that each proton is on the *para* position. Therefore, the two aromatic protons of benzoquinolizidine moiety were assigned to position 8 and 11 and the two methoxyl groups were substituted at position 9 and 10. The aromatic protons of isoquinoline moiety were assigned to the position $5'$ and $8'$.

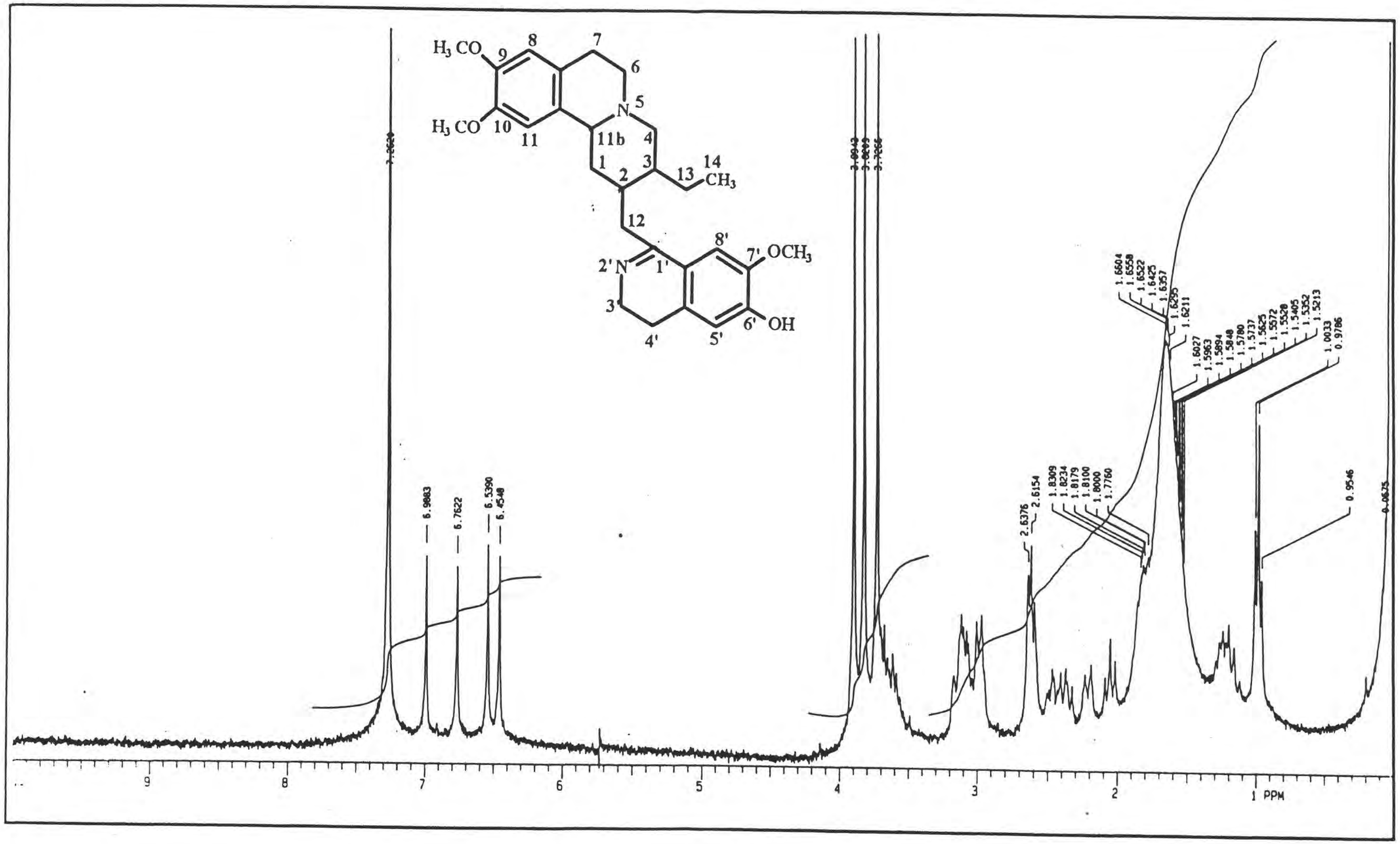


Figure 5 The 300 MHz ^{1}H NMR spectrum of AL-1 (in $CDCl_3$)

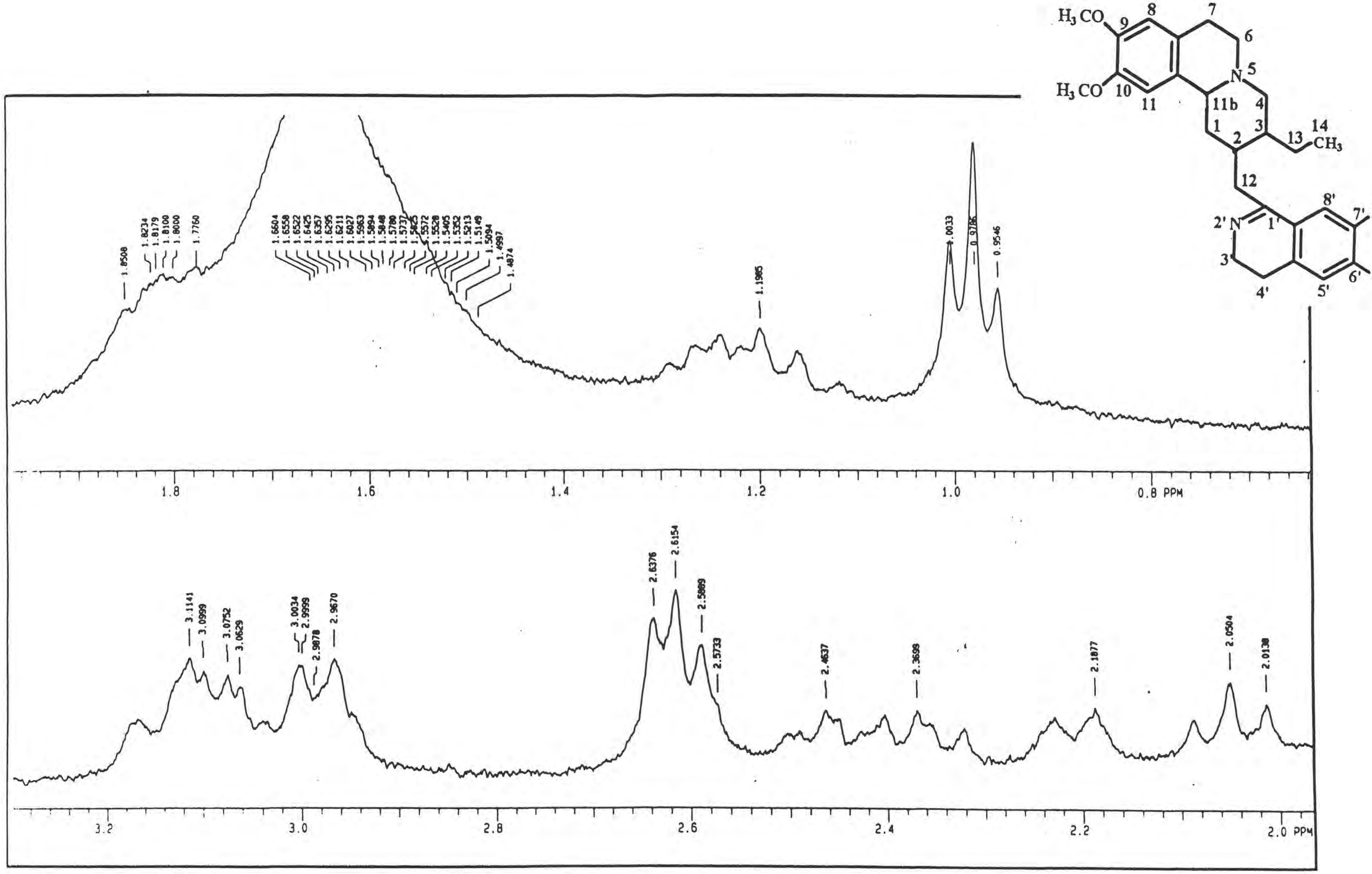


Figure 6 The 300 MHz ^{1}H NMR spectrum of AL-1 (in $CDCl_3$)

(expanded from 0.8-1.8 ppm and 2.0-3.2 ppm)

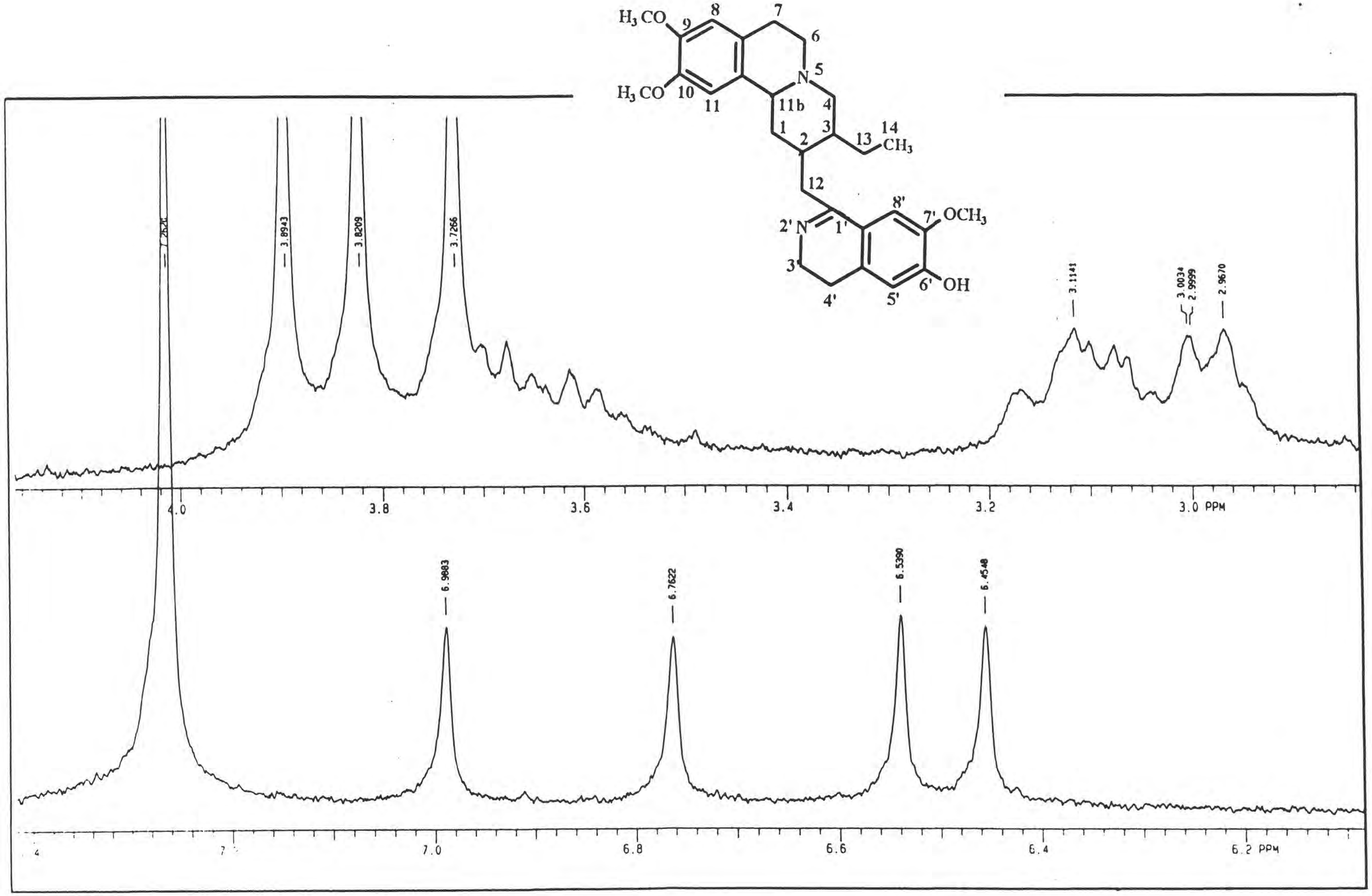


Figure 7 The 300 MHz ^{1}H NMR spectrum of AL-1 (in CDCl$_3$)
(expanded from 3.0-4.0 ppm and 6.2-7.4 ppm)

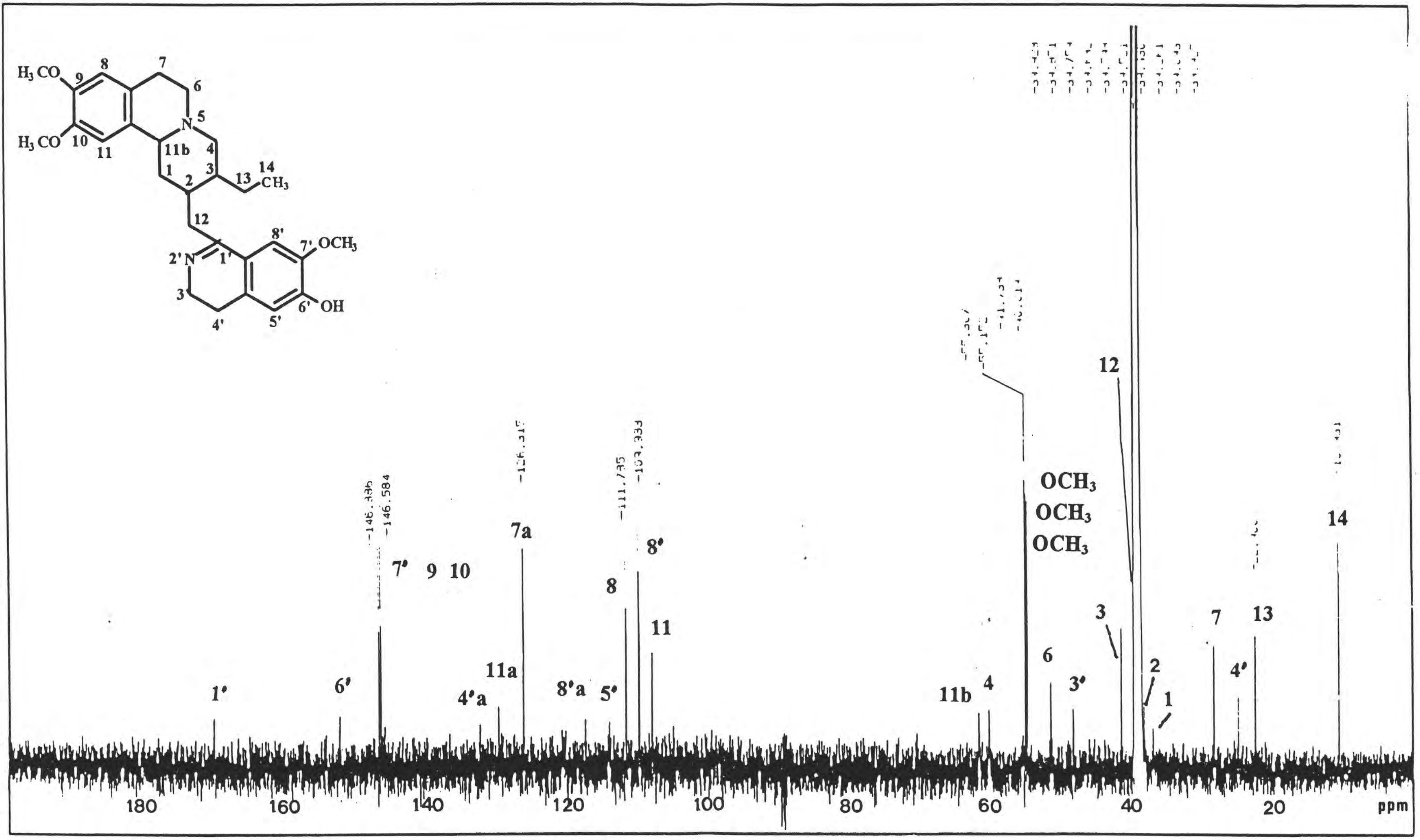


Figure 8 The 125 MHz ^{13}C NMR spectrum of AL-1 (in DMSO)

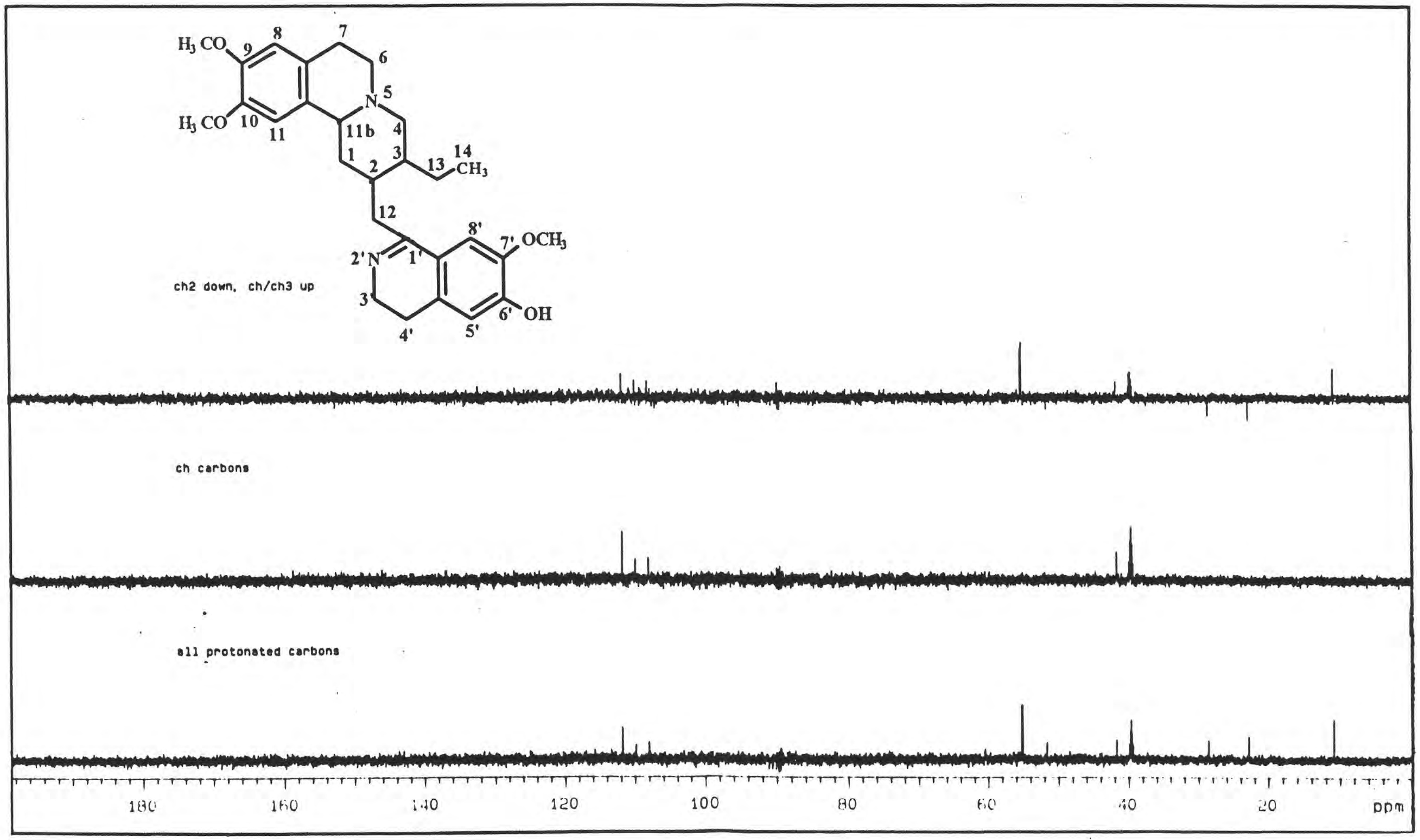


Figure 9 The 125 MHz DEPT spectrum of AL-1 (in DMSO)

Establishment of the hydroxyl group to the position 6′ in psychotrine was based on Brindley and Pyman's assumption that the 6′-methoxyl in *O*-methylpsychotrine was the most labile of the four methoxyls in the molecule (Teitel and Brossi, 1966). Hence, the substitution of the hydroxyl and the methoxyl was at position 6′ and 7′ respectively.

The assignment of AL-1 as psychotrine was also confirmed by comparison of the carbon signals to the reported data of psychotrine (Fujii *et al.*, 1983) as summarized in Table 4.

Table 4 Comparative ^{13}C NMR data for AL-1 and reported psychotrine

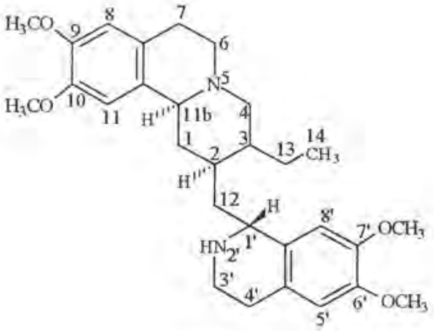
Carbon	Chemical shift		Carbon	Chemical shift	
	AL-1	psychotrine		AL-1	psychotrine
1	37.2	37.2	13	22.9	23.7
2	40.0	40.2	14	10.9	11.3
3	41.7	42.4	1′	165.4	167.5
4	60.5	61.1	3′	48.4	45.0
6	51.6	52.4	4′	25.7	26.3
7	28.6	29.1	4′a	132.5	133.4
7a	126.3	126.6	5′	114.1	115.4
8	111.7	111.4	6′	153.8	155.9
9	146.8^b	147.0^a	7′	146.0^b	147.4^a
10	146.5^b	147.1^a	8′	109.9	109.1
11	108.1	108.2	8′a	117.4	117.5
11a	129.8	129.2	9-OMe	55.3^c	55.8
11b	61.9	62.3	10-OMe	55.1^c	55.8
12	38.4	38.6	7′-OMe	55.0^c	55.8

a,b,c) = Assignment indicated by a given superscript may be reversed

2. Structure Elucidation of AL-2

Compound AL-2 was obtained as amorphous powder from fraction F-081 (Table 3). It gave positive test with Dragendorff's reagent, suggesting that it was an alkaloid. The EIMS spectrum (Figure 10) of AL-2 showed a molecular ion peak at *m/z* 480, suggesting a molecular formula of $C_{29}H_{40}N_2O_4$. The IR spectrum (Figure 11) displayed the absorption bands of N-H stretch at 3330 cm^{-1} , methyl C-H stretch of methoxyl group at 2900 cm^{-1}, and C=C stretch of aromatic ring at 1517 cm^{-1}.

The mass spectrum of AL-2 (Figure 10) displayed the characteristic fragmentation pattern of benzoquinolizidine and dihydroisoquinoline moieties (Scheme 10). The peak at *m/z* 192 represented the benzoquinolizidine moiety bearing two methoxyl groups, while the peaks at *m/z* 289 and 260 represented the dihydroisoquinoline moiety bearing two methoxyl groups (Scheme 10). Based on comparison of the molecular weight and mass fragmentation pattern to the published data (Wiegrebe, Kramer, and Shamma, 1984 ; Cordell *et al.*, 1989), AL-2 could be assigned as the known alkaloid emetine. The structure of emetine is shown below.



emetine

The structure was confirmed by ^1H NMR (Figure 12, 13) and ^{13}C NMR (Figure 14, 15, 16) spectral data. The ^1H NMR exhibited signals for 4 aromatic protons at δ 6.56 ppm. The signals at δ 3.80, 3.81, 3.83, 3.86 ppm were the signals of 4 methoxyl protons and the triplet signal at δ 0.90 ppm could be assigned for the methyl group of ethyl residue which was coupling to neighbouring methylene protons.

EIMS AL2

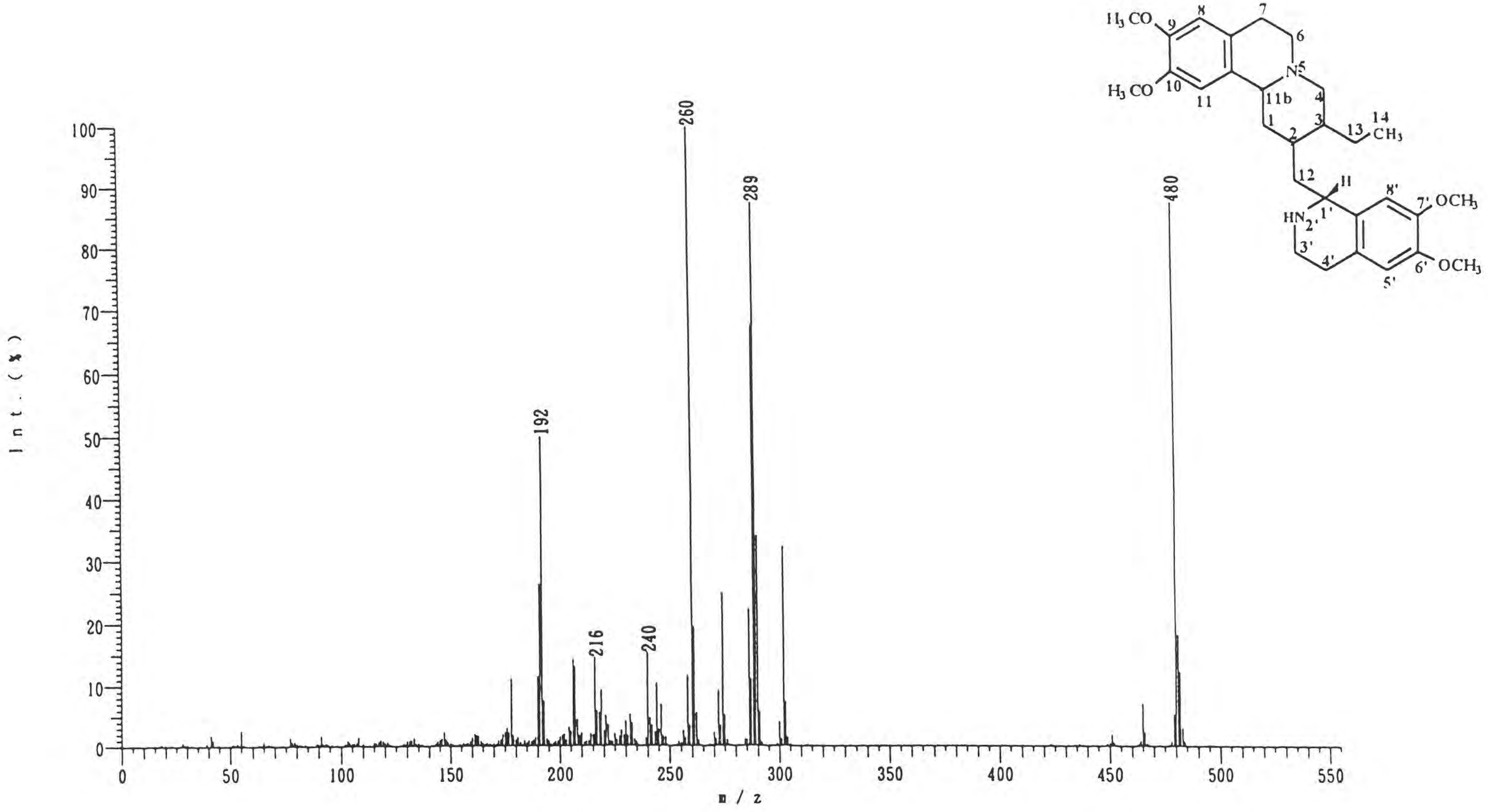


Figure 10 EIMS spectrum of AL-2

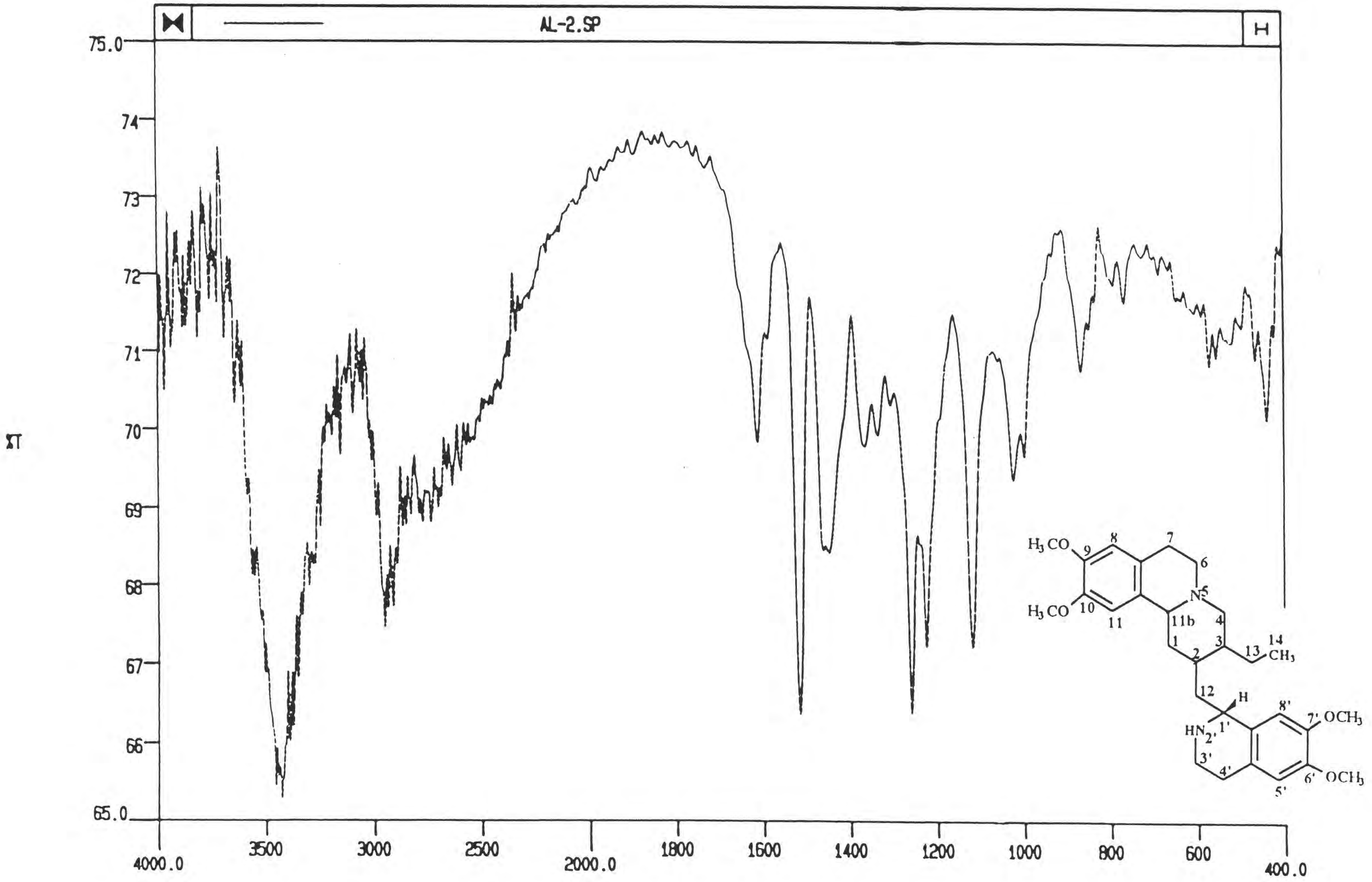
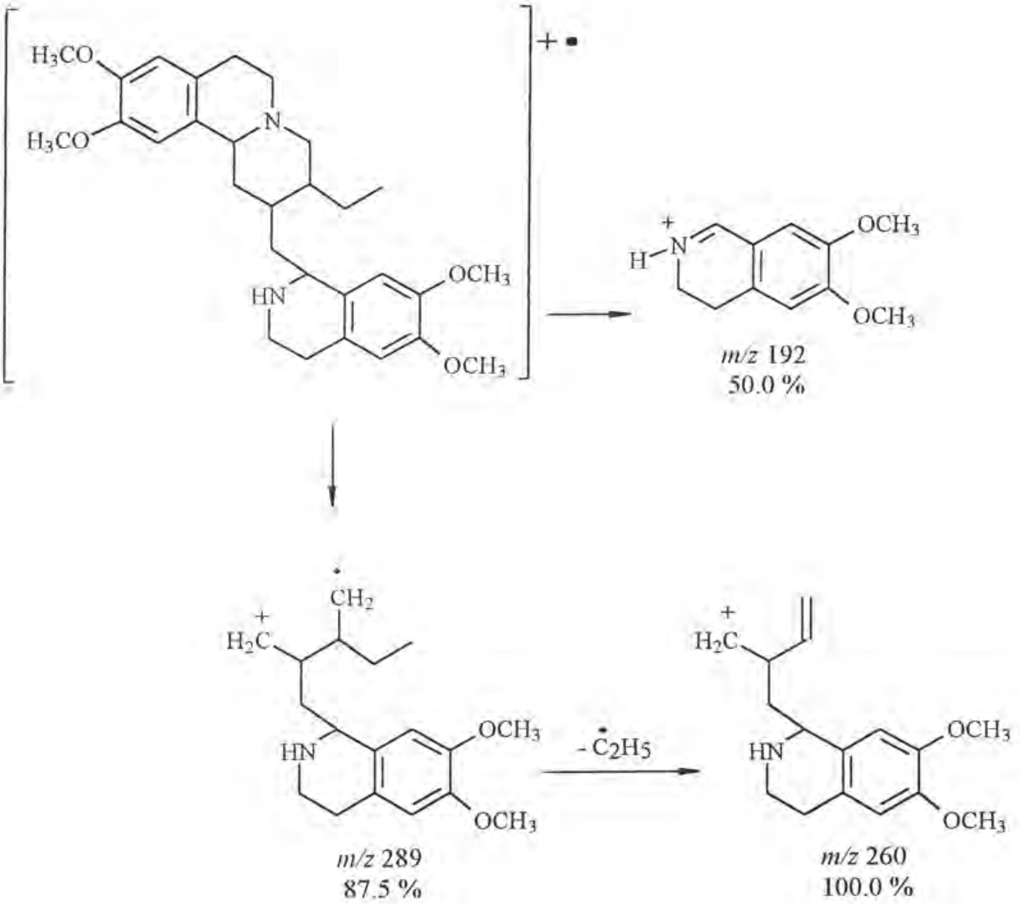


Figure 11 IR spectrum of AL-2 (KBr disc)



Scheme 10 Mass fragmentation pattern of AL-2

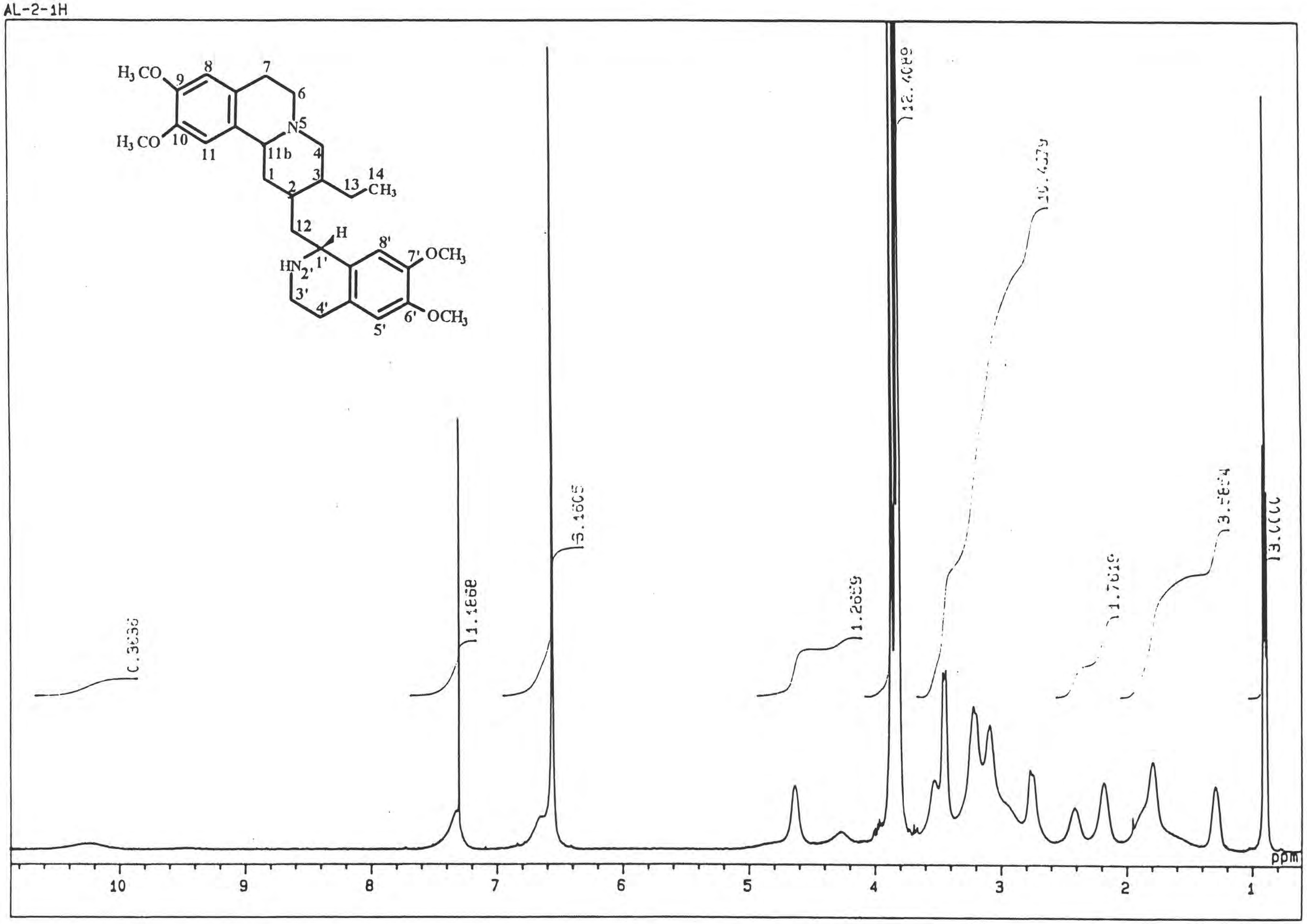


Figure 12 The 500 MHz ^{1}H NMR spectrum of AL-2 (in $CDCl_3$)

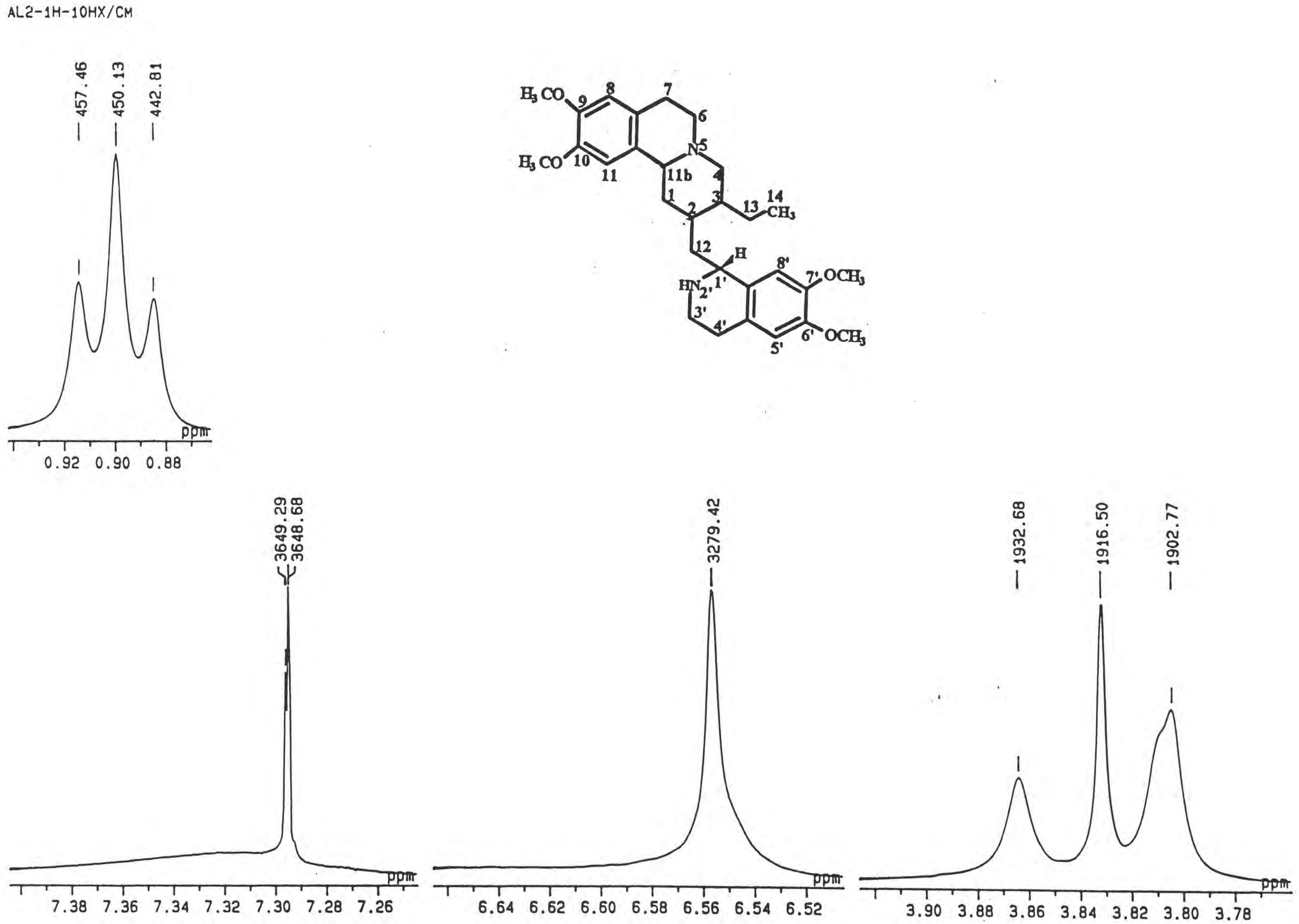


Figure 13 The 500 MHz ^{1}H NMR spectrum of AL-2 (in $CDCl_3$)

(expanded from 0.88-0.92, 3.78-3.90, 6.52-6.64 and 7.26-7.38 ppm)

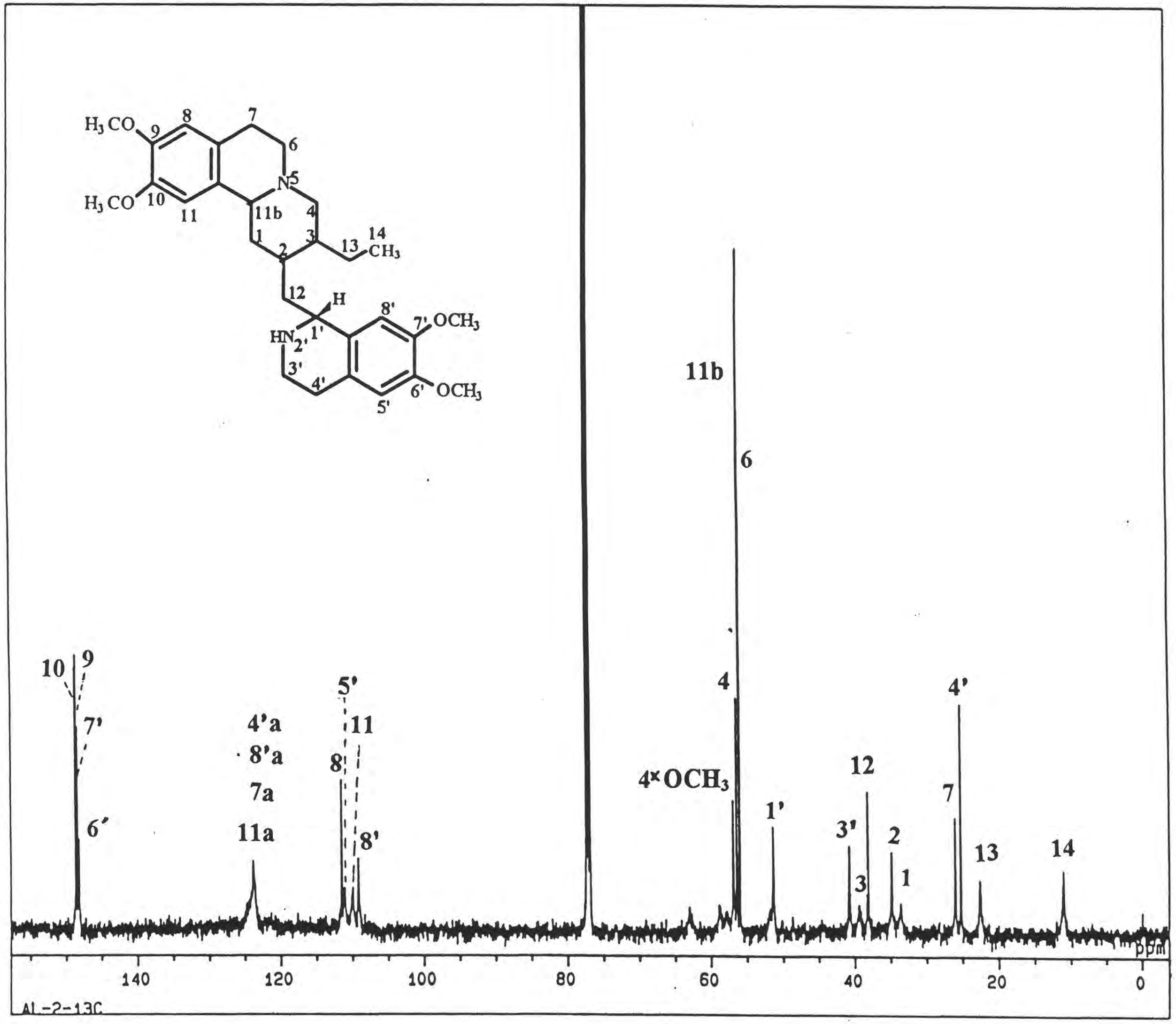


Figure 14 The 125 MHz ^{13}C NMR spectrum of AL-2 (in $CDCl_3$)

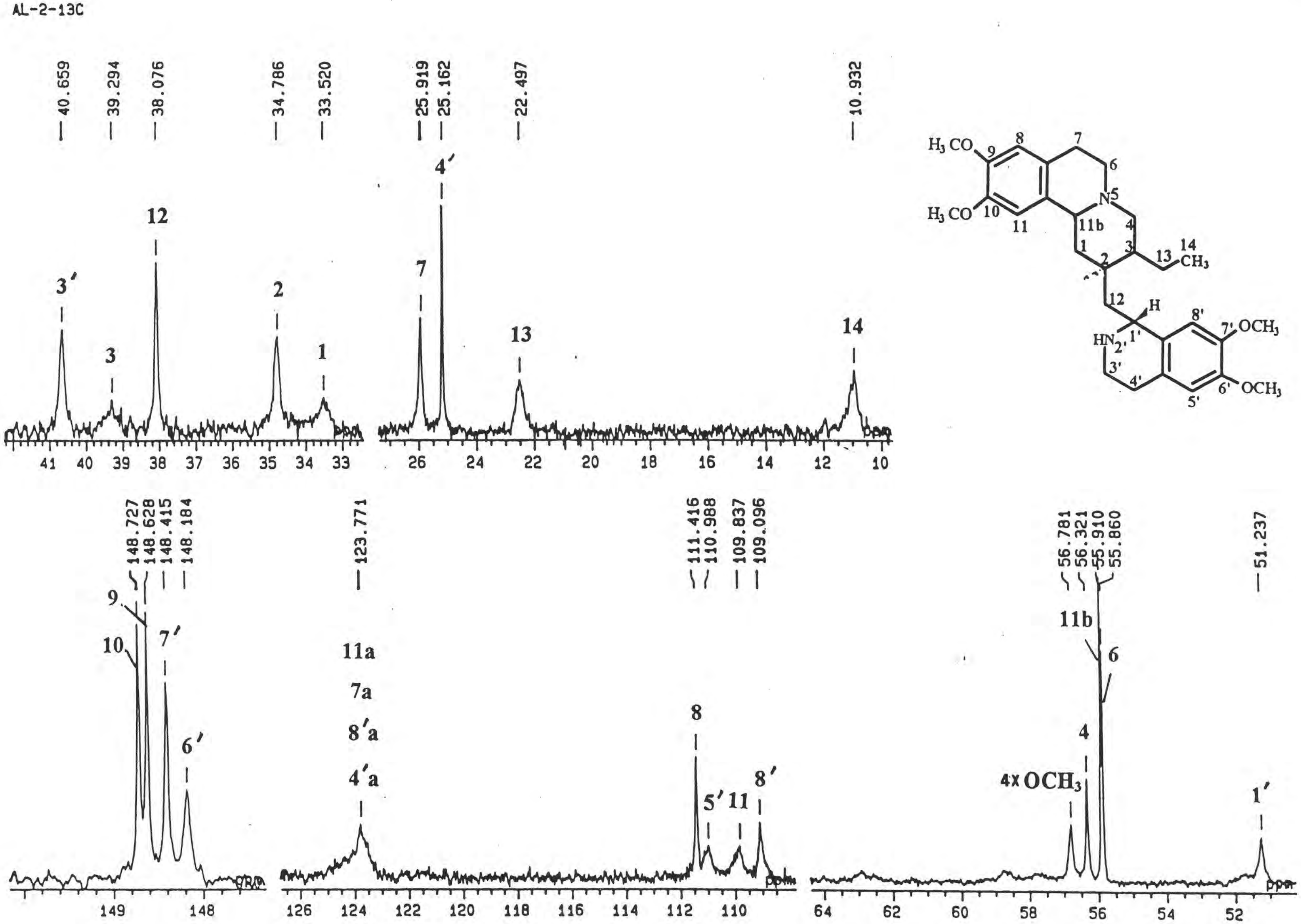


Figure 15 Expansions of the 125 MHz ^{13}C NMR spectrum of AL-2 (in CDCL$_3$)

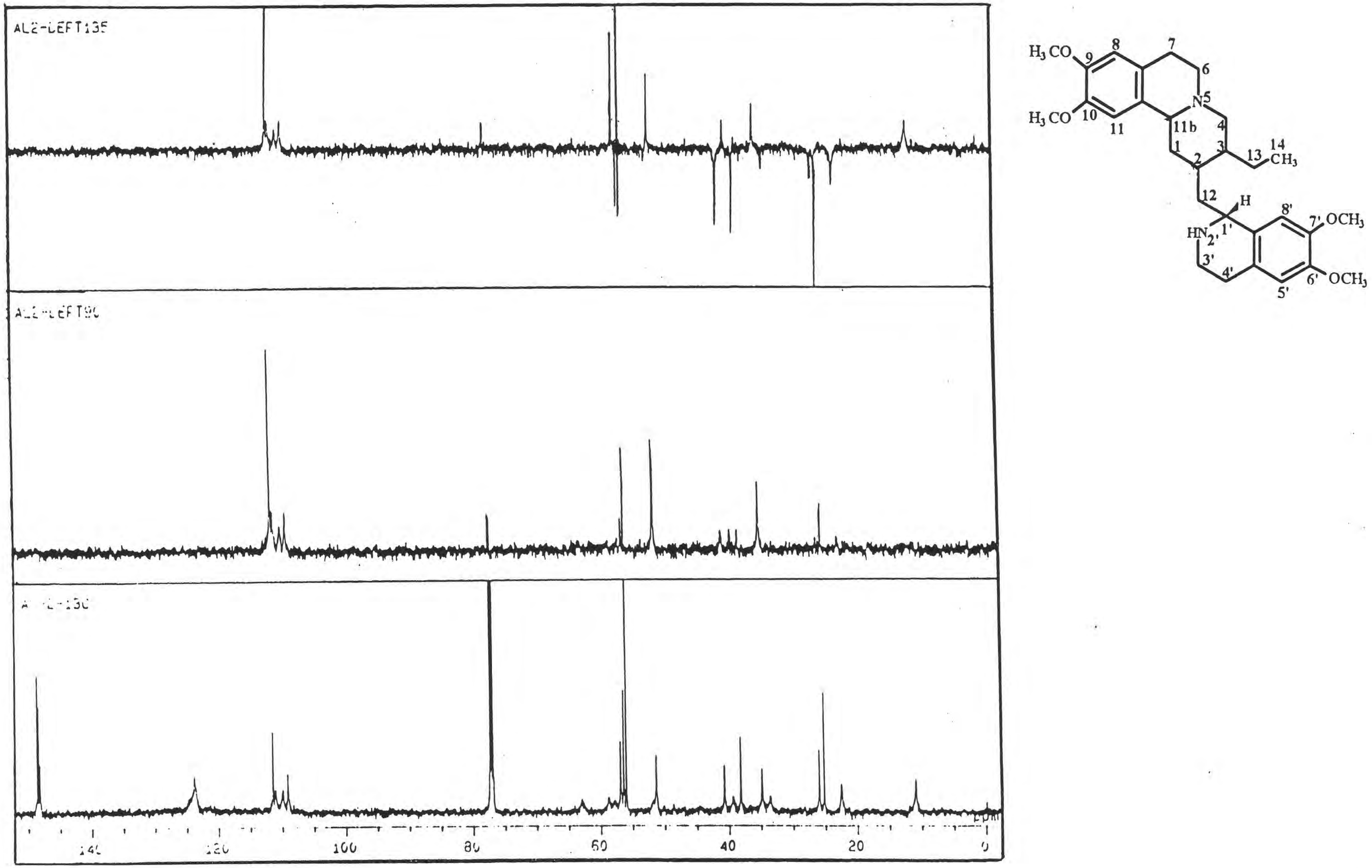


Figure 16 The 125 MHz DEPT spectrum of AL-2 (in CDCl$_3$)

The ^{13}C NMR spectrum (Figure 14, 15) showed the signals of 29 carbons. The DEPT experiment (Figure 16), indicated that AL-2 consists of 8 quarternary, 8 methine, 8 methylene and 5 methyl carbons. Almost all of the quarternary carbons were sp^2 (δ 148.1, 148.4, 148.6, 148.7 ppm and 123.7 ppm for 4 carbons), thus they were assigned to the two aromatic rings. Four of the methine carbons were sp^2 (δ 109.1, 109.8, 110.9 and 111.4 ppm), they were also assigned to the aromatic rings. Other methine carbons were sp^3 and were assigned as shown in Table 5. The four methoxyl groups were confirmed by the presence of four downfield quarternary carbon signals at δ 148.1, 148.4, 148.6 and 148.7 ppm and the presence of methyl signals at δ 56.7 ppm. The carbon assignments of AL-2, based on the reported chemical shifts of emetine (Wiegrebe, Kramer and Shamma, 1984), are shown in Table 5

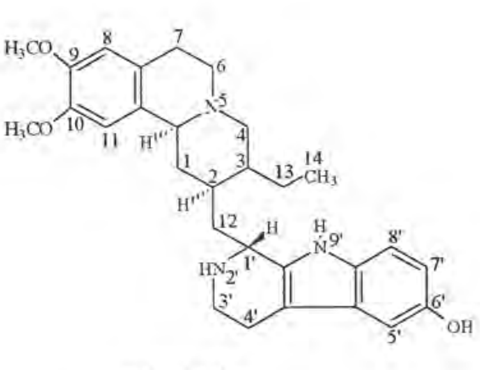
Table 5 ^{13}C NMR assignments for AL-2

Carbon	Chemical shift (ppm)	Carbon	Chemical shift (ppm)
1	33.5	14	10.9
2	34.8	1′	51.2
3	39.2	3′	40.7
4	56.3	4′	25.2
6	55.9	4′a	123.8
7	25.9	5′	111.0
7a	123.8	6′	148.2
8	111.4	7′	148.4
9	148.6	8′	109.1
10	148.7	8′a	123.8
11	109.8	9-OMe	56.8
11a	123.8	10-OMe	56.8
11b	55.9	6′-OMe	56.8
12	38.1	7′-OMe	56.8
13	22.5		-

3. Structure Elucidation of AL-3

Compound AL-3 was obtained as amorphous powder from fraction F-06 Table 3). It was suggested to be an alkaloidal compound since it gave orange color to Dragendorff's test. The EIMS (Figure 17) exhibited a molecular ion peak at m/z 475, suggesting a molecular formula of $C_{29}H_{37}N_3O_3$. The IR spectrum (Figure 18) exhibited a sharp peak at 3381 cm^{-1}, corresponding to the N-H stretch of indole molecule. A band near 2800 cm^{-1} indicated the C-H stretch of methyl and methylene groups and a band at 1,512 indicated C=C stretch of aromatic ring. The UV spectrum (Figure 19) showed the absorption maxima at 205 nm (log ε 3.35), 281 nm (log ε 2.71) and shoulder at 225 nm.

The mass spectrum (Figure 17) displayed a series of characteristic peaks representing benzoquinolizidine and β-carboline moieties. The peak at m/z 205, 246 and 272 represented the benzoquinolizidine moiety with two methoxyl groups and the peak at m/z 187 represented the β-carboline moiety bearing a hydroxyl group (Scheme 11). Comparison to the reported data (Brauchli *et al.*,1964; Wiegrebe, Kramer and Shamma, 1984; Cordell *et al.*, 1989), suggested that the alkaloid AL-3 could be assigned as the known alkaloid tubulosine. Its structure is shown below.



tubulosine

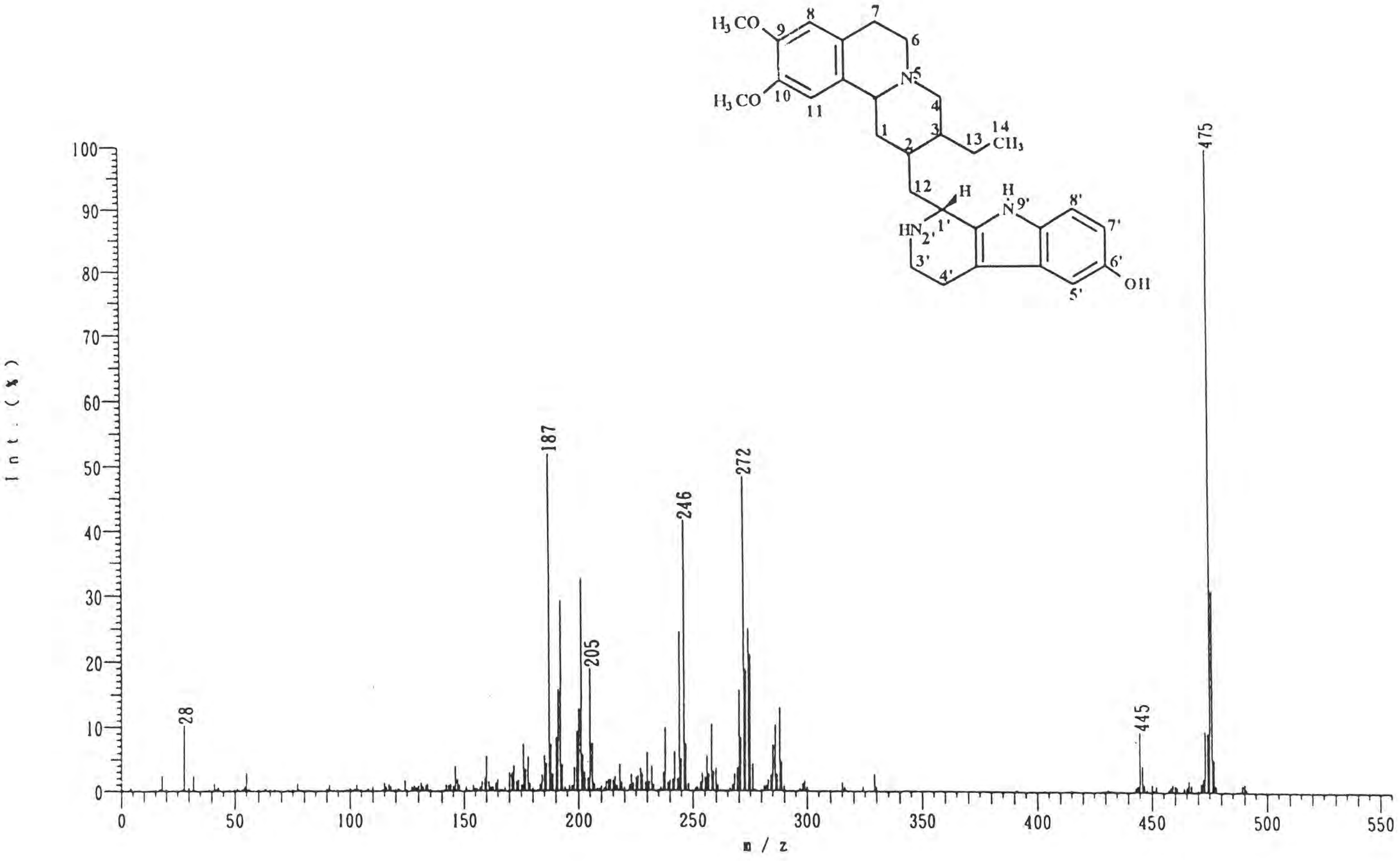


Figure 17 EIMS spectrum of AL-3

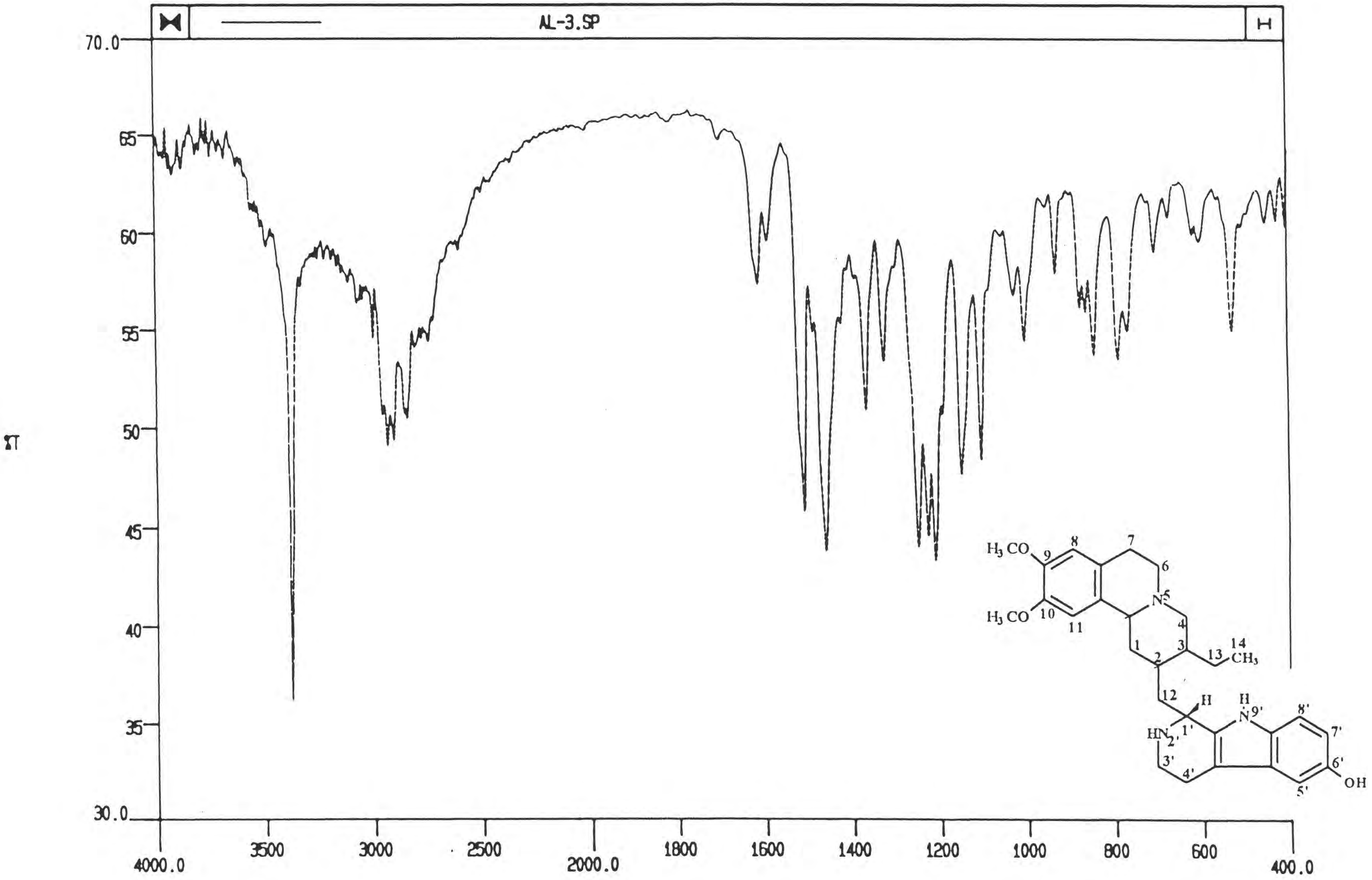


Figure 18 IR spectrum of AL-3 (KBr disc)

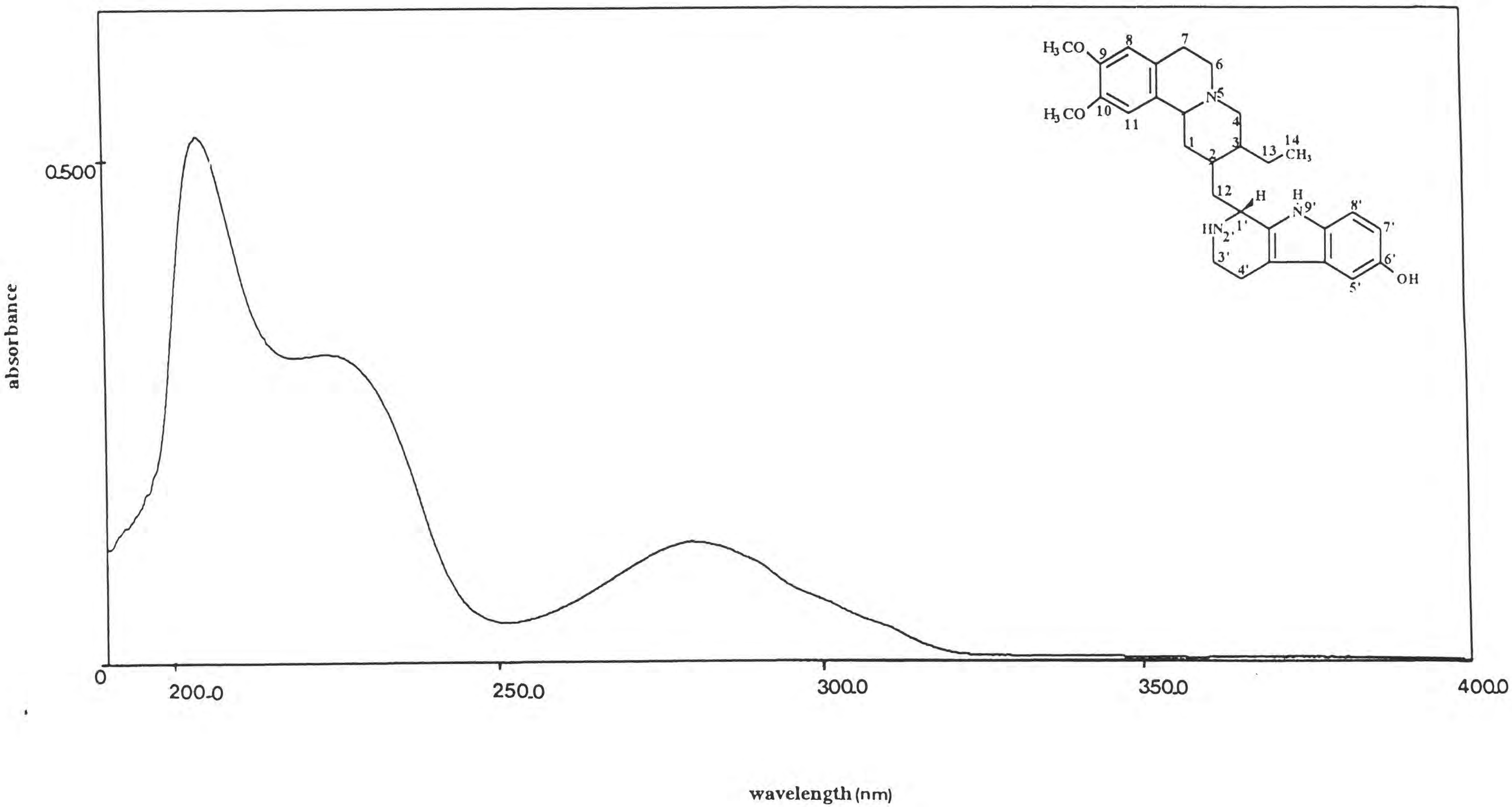
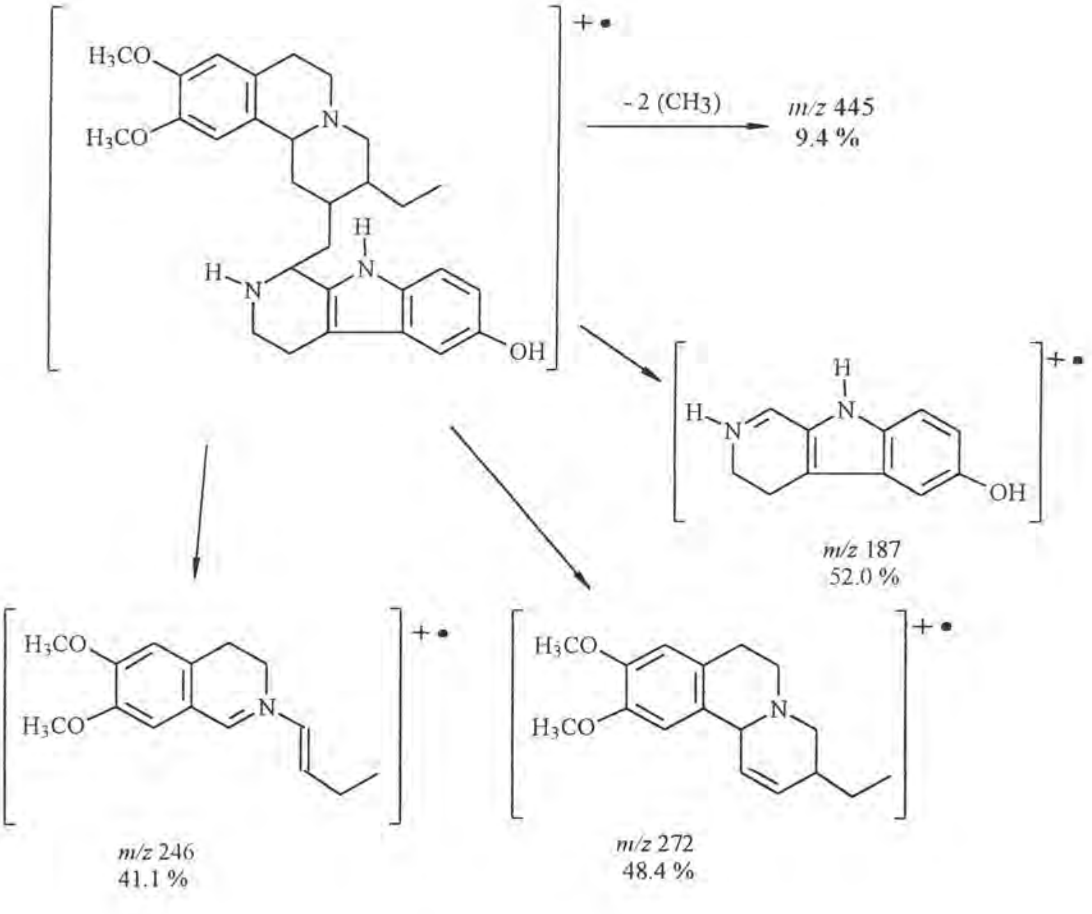


Figure 19 UV spectrum of AL-3 (in methanol)



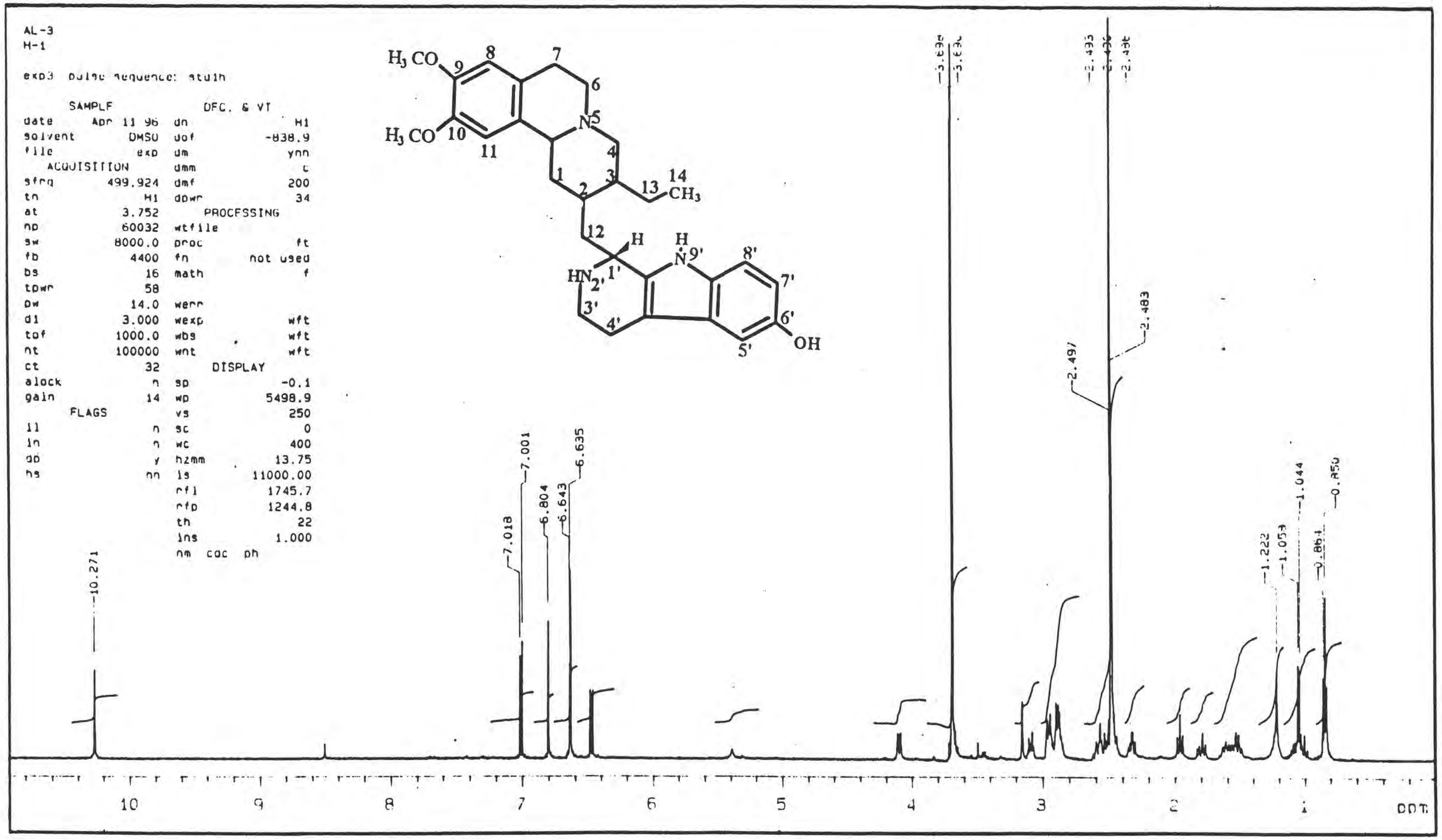
Scheme 11 Mass fragmentation pattern of AL-3

The structure of AL-3 was confirmed by ^{1}H NMR (Figures 20, 21, 22, 23), ^{13}C NMR (Figure 24) and DEPT (Figure 25) spectra. The presence of a hydroxyl group was confirmed by the downfield shift of an aromatic carbon signal to δ 150.0 ppm. The presence of two methoxyl groups was confirmed by the presence of methoxy-substituted aromatic carbon signals at δ 147.1 and 146.8 ppm, methoxyl carbon signals at δ 55.3 and 55.8 ppm and methoxyl proton singlets at δ 3.69 and 3.70 ppm.

The ^{1}H NMR spectrum also showed the signal of indolic proton at δ 10.27 ppm and hydroxyl proton at δ 8.45 ppm. The aromatic proton signals could also be integrated for 5 protons at δ 6.46 ppm (dd, J= 6.8, 1.6 Hz), 6.63 ppm (s), 6.64 ppm (d, J=1.6 Hz), 6.80 ppm (s) and 7.01 ppm (d, J = 6.8 Hz).

The singlet signals at δ 6.63 and 6.80 ppm were assigned to the aromatic protons attached to benzoquinolizidine moiety. The multiplicity of the signals suggested that the protons were *para* to each other and thus the two methoxyl groups were at C-9 and C-10.

The other three were assigned to aromatic protons attached to β-carboline moiety. The proton signal at δ 6.46 ppm showed *ortho* coupling with a coupling constant of 6.8 Hz to the doublet at δ 7.01 ppm and *meta* coupling with a coupling constant of 1.6 Hz to the signal at 6.64 ppm (Figures 26, 27). To locate the position of these protons, the substitution of hydroxyl group at C-6$'$ must be confirmed. The chemical shifts of each aromatic protons in β-carboline moiety was calculated and compared to the chemical shift of closely related structures in the literature (Fresenius *et al.*, 1989). The structures and their chemical shift are shown below.



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AL-3
H-1

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date        Apr 11 96  dn              H1
solvent          DMSO  dof         -838.9
file              exp  dm             ynn
   ACQUISITION         dmm              c
sfrq          499.924  dmf            200
tn                 H1  dpwr            34
at              3.752      PROCESSING
np              60032  wtfile
sw             8000.0  proc            ft
fb               4400  fn        not used
bs                 16  math             f
tpwr               58
pw               14.0  werr
d1              3.000  wexp           wft
tof            1000.0  wbs            wft
nt             100000  wnt            wft
ct                 32        DISPLAY
alock               n  sp            -0.1
gain               14  wp          5498.9
        FLAGS          vs             250
il                  n  sc               0
in                  n  wc             400
dp                  y  hzmm         13.75
hs                 nn  is        11000.00
                       rfl         1745.7
                       rfp         1244.8
                       th              22
                       ins          1.000
                       nm  cdc  ph
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Figure 20 The 500 MHz ^{1}H NMR spectrum of AL-3 (in DMSO)

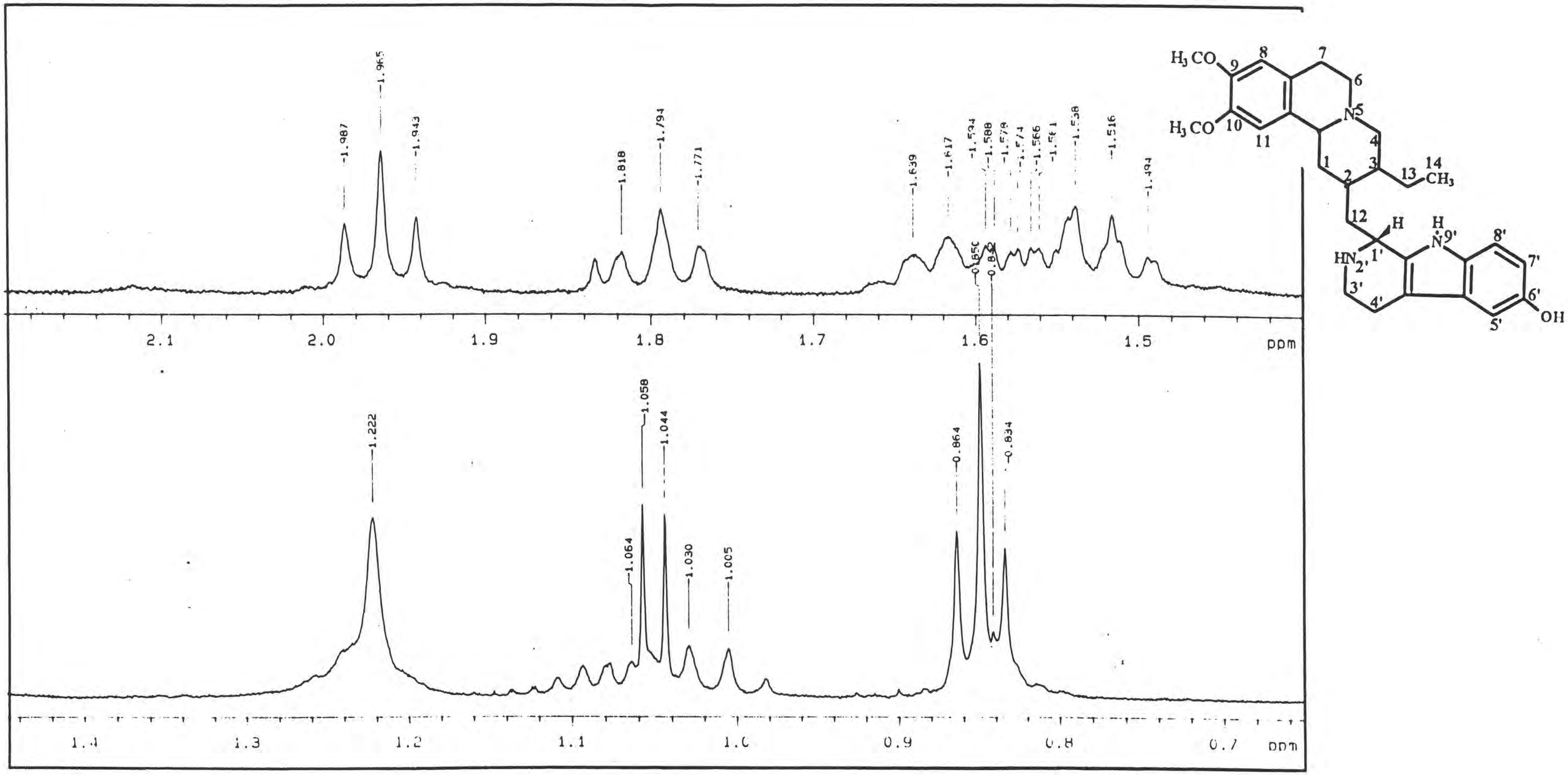


Figure 21 The 500 MHz ^{1}H NMR spectrum of AL-3 (in DMSO)
(expanded from 0.70-2.10 ppm)

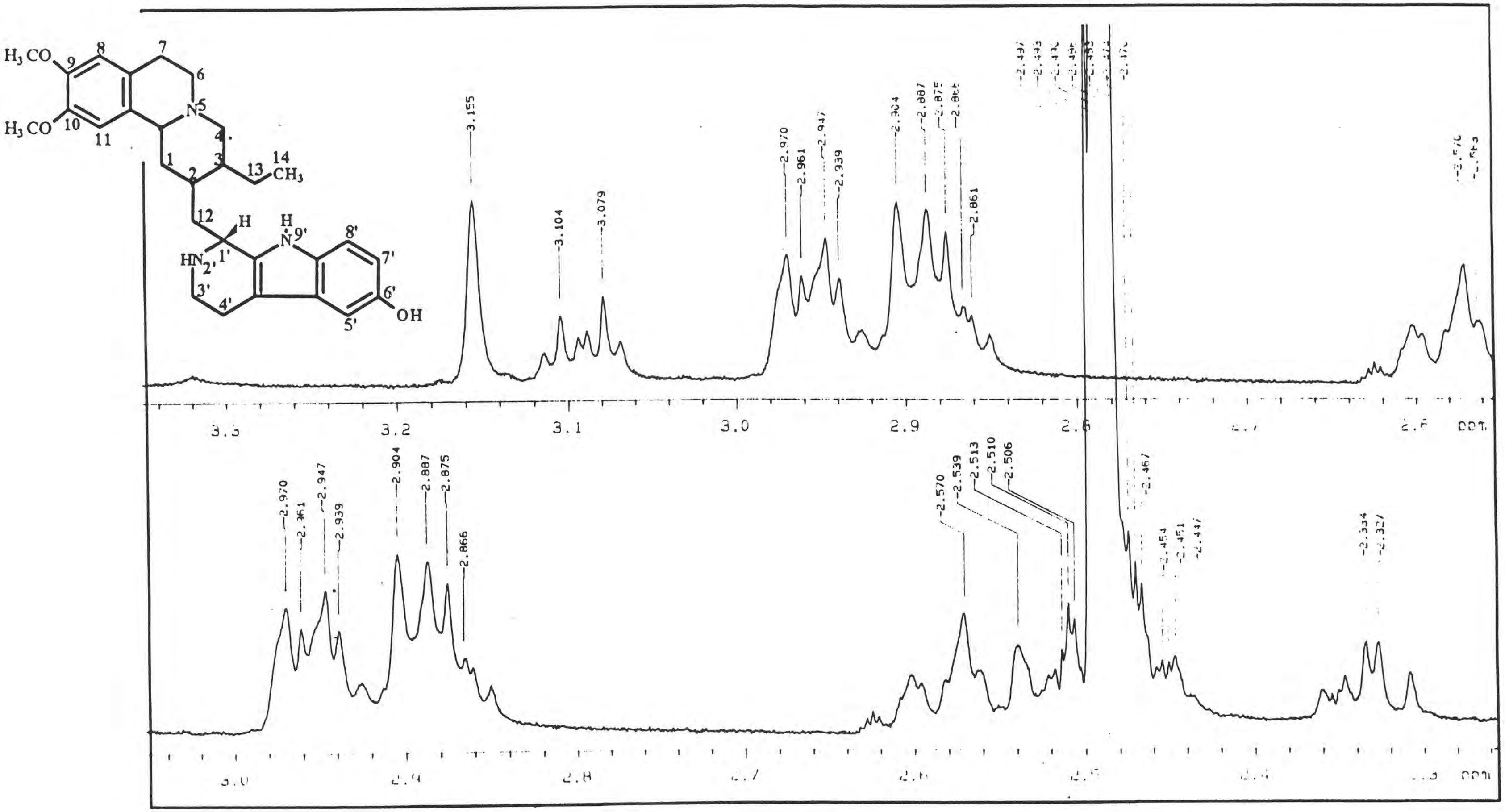


Figure 22 The 500 MHz ^{1}H NMR spectrum of AL-3 (in DMSO)
(expanded from 2.30-3.30 ppm)

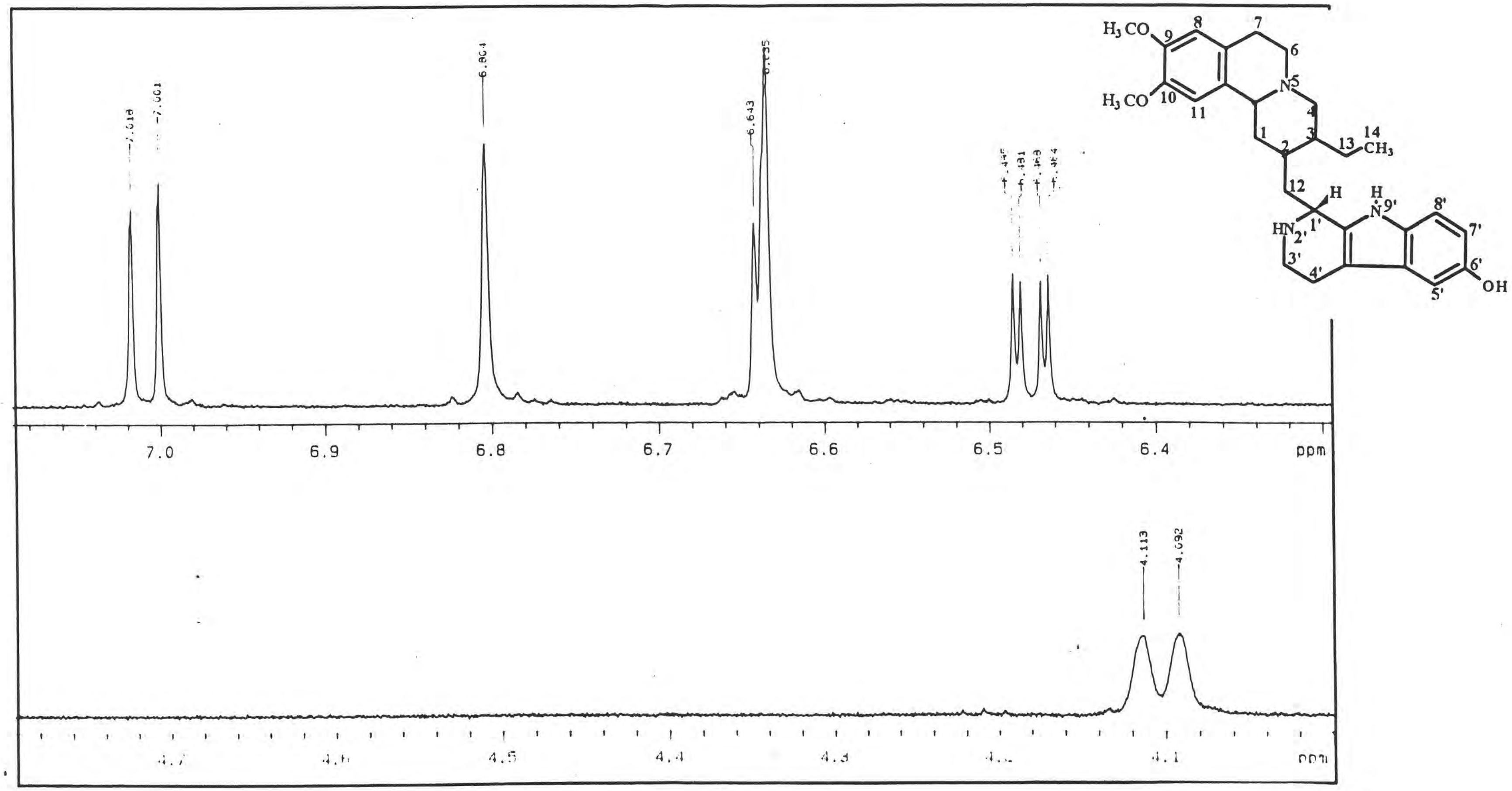


Figure 23 The 500 MHz ^{1}H NMR spectrum of AL-3 (in DMSO) (expanded from 4.10-7.00 ppm)

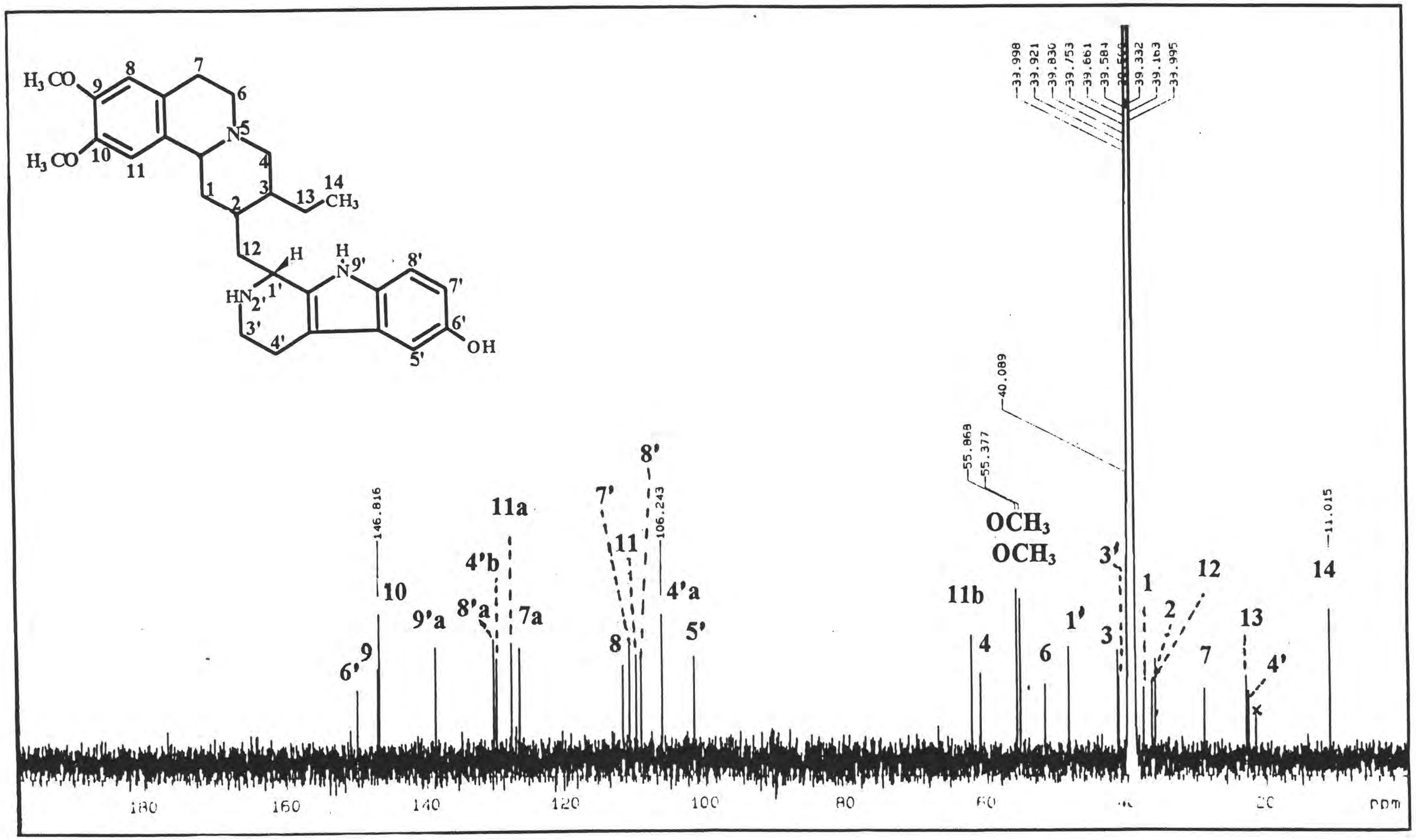


Figure 24 The 125 MHz ^{13}C NMR spectrum of AL-3 (in DMSO)

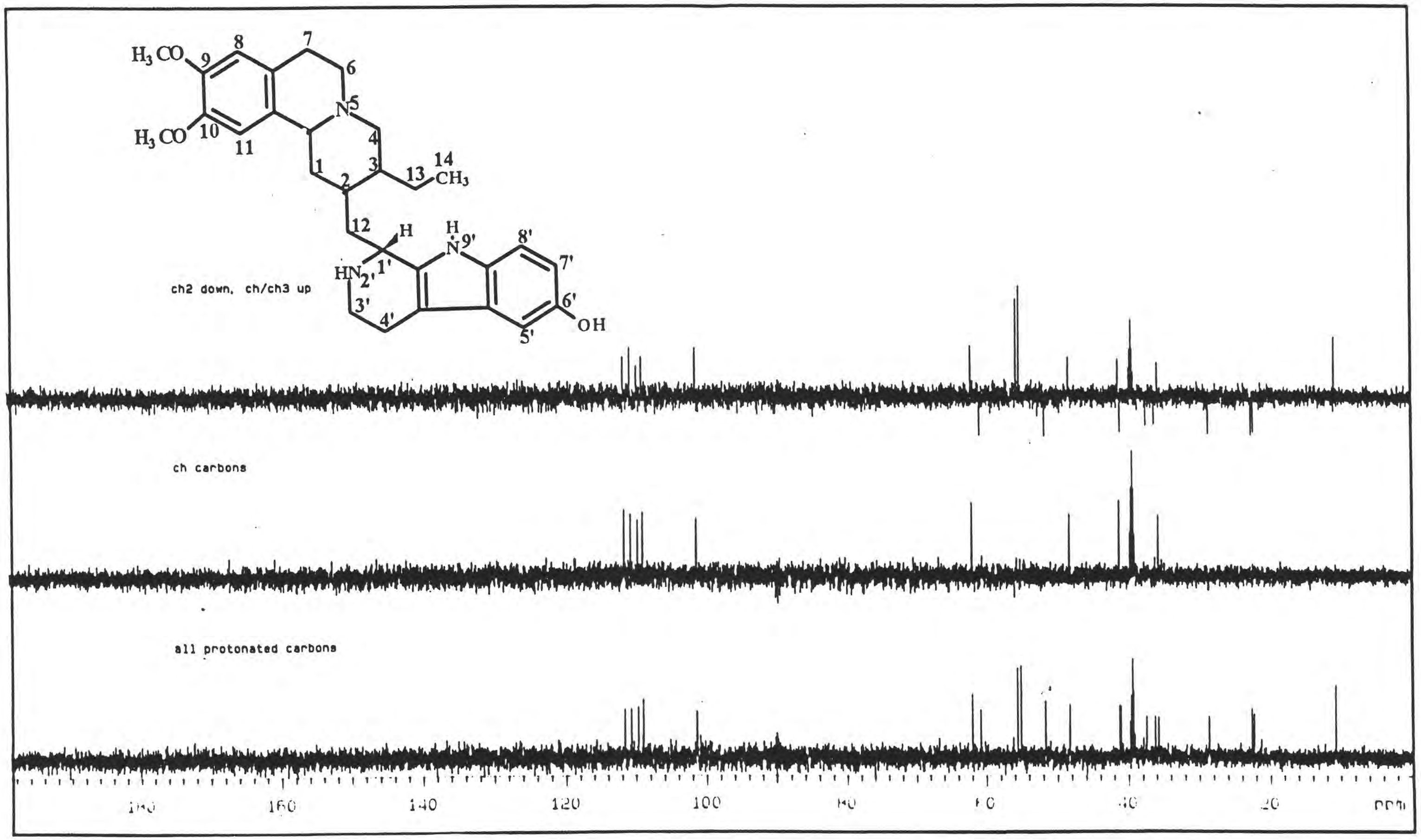


Figure 25 The 125 MHz DEPT spectrum of AL-3 (in DMSO)

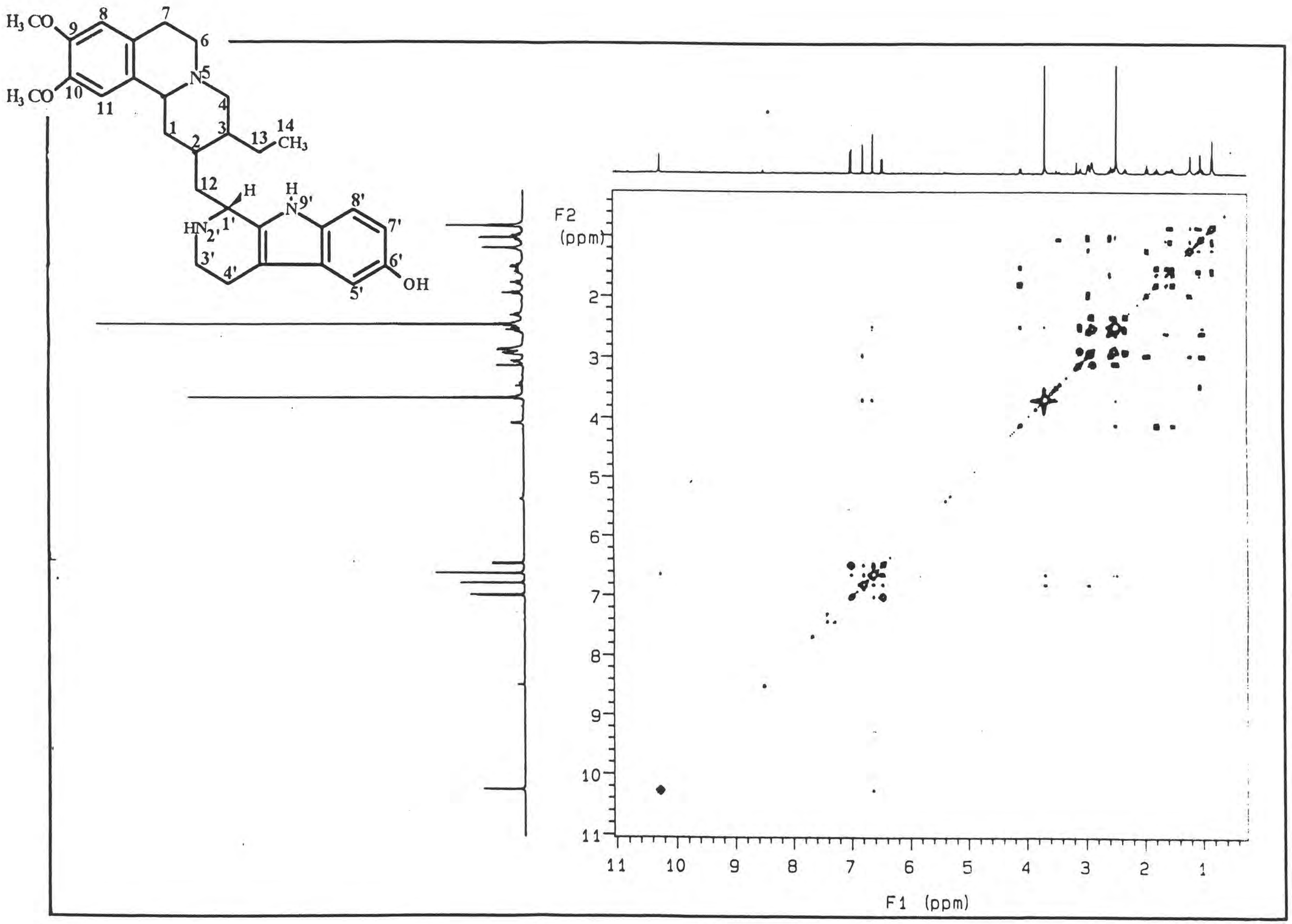


Figure 26 The 500 MHz ^1H-^1H COSY spectrum of AL-3 (in DMSO)

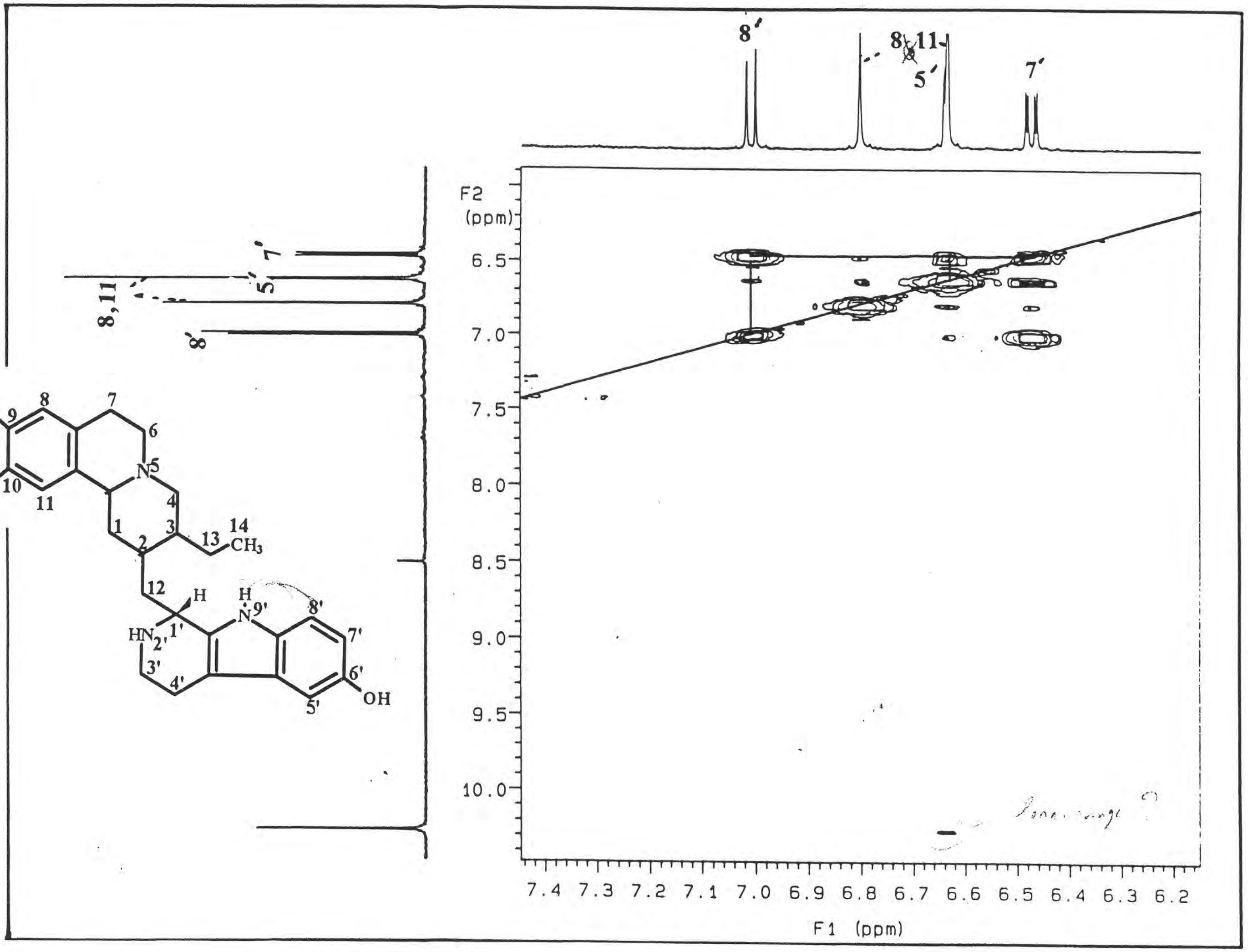
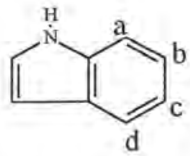
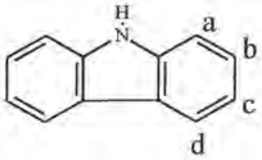


Figure 27 The 500 MHz ^{1}H-^{1}H COSY spectrum of AL-3 (in DMSO) (expanded from 6.20-10.00 ppm)

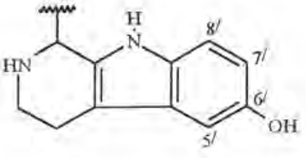


a) 7.40 ppm b) 7.09 ppm c) 6.99 ppm d) 7.55 ppm



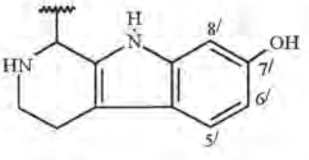
a) 7.47ppm b) 7.36 ppm c) 7.16 ppm d) 8.08 ppm

The proton coupling pattern revealed that there were 2 possibilities for the substitution of hydroxyl group in β-carboline moiety. The first possibility was that the hydroxyl group was substituted at C-6$'$. The chemical shift of each aromatic proton should be assigned as shown below.



$5'$) 6.64 ppm	$7'$) 6.46 ppm	$8'$) 7.01 ppm
(d J=1.6 Hz)	(dd, J=6.8,1.6 Hz)	(d, J=6.8 Hz)

The second possibility was the substitution of the hydroxyl group at C-7$'$ and the chemical shift should be assigned as shown below.



$5'$) 7.01 ppm	$6'$) 6.46 ppm	$8'$) 6.64 ppm
(d, J= 6.8 Hz)	(dd, J=6.8,1.6 Hz)	(d, J= 1.6 Hz)

In the first possibility the chemical shifts were in accordance with the fact that the mesomeric effect of hydroxyl group causes upfield shift of protons *ortho* and *para* to the hydroxyl group in benzene ring and does not cause any effect to *meta* proton. The protons at C-5$'$ and C-7$'$ were upfield compared to the chemical shift of d and b respectively in the above data and proton at C-8$'$ (compared to a) did not change much in its chemical shift. Whereas, in the second possibility, the chemical shift of proton *meta* to hydroxyl group (C-5$'$) was rather upfield when compared to position d in the above reference data.

Based on these results, it was concluded that the substitution of hydroxyl group was at C-6′ and the chemical shift of each aromatic proton in β-carboline moiety was assigned as in the first possibility.

The structure could also be confirmed by the comparison of carbon chemical shifts to the reported data (Fujii *et al.*, 1983; Fujii and Ohba, 1985; Itoh *et al.*, 1995). The carbon assignments were summarized as in Table 6.

Table 6 The ^{13}C NMR assignments of AL-3

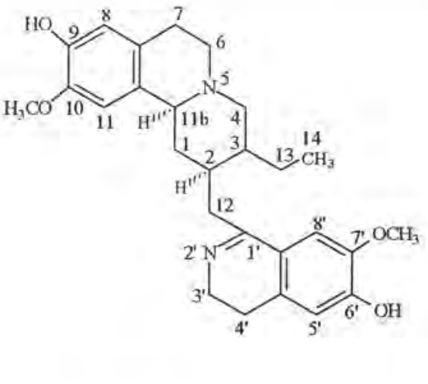
Carbon	Chemical shift (ppm)	Carbon	Chemical shift (ppm)
1	37.7	14	11.0
2	36.0	1′	48.5
3	41.5	3′	41.3
4	61.0	4′	22.5
6	51.9	4′a	106.5
7	28.8	4′b	129.9
7a	126.6	5′	101.6
8	111.2	6′	150.0
9	147.1[a]	7′	110.9
10	146.8[a]	8′	109.2
11	109.9	8′a	130.3
11a	127.8	9′a	138.6
11b	62.2	9-OMe	55.8[b]
12	36.5	10-OMe	55.3[b]
13	22.9		

a,b) = Assignment indicated by a given superscript may be reversed.

4. Structure Elucidation of AL-4

Compound AL-4 was obtained as amorphous powder from fraction F-09 (Table 3). It gave positive result to Dragendorff's reagent, thus it appeared to be an alkaloid. Its mass spectrum (Figure 28) gave molecular ion peak at m/z 450, suggesting the molecular formula of $C_{27}H_{34}N_2O_4$. In the IR spectrum (Figure 29), absorption bands at 3400 - 3200 cm^{-1} (O-H stretch of hydroxy group) and 2900 -2800 cm^{-1} (C-H stretch of methyl and methylene groups) were observed. The UV spectrum (Figure 30) showed the absorption maxima at 205 nm (log ε 3.52) and 362 nm (log ε 2.73).

The mass spectrum (Figure 28) displayed a peak at m/z 191 due to the isoquinoline moiety bearing a hydroxyl group and a methoxyl group and fragment peaks at m/z 230 and 259 due to the benzoquinolizidine moiety bearing a hydroxyl group and a methoxyl group (Scheme 12). The mass fragmentation pattern suggested that the structure of AL-4 was closely related to psychotrine (AL-1). The difference was the decrease in 14 mass unit of benzoquinolizidine moiety in AL-4. Hence, AL-4 could be proposed as 9 or 10-demethyl derivative of psychotrine.



9-demethylpsychotrine (R = OH, R$'$ = OCH_3)
10-demethylpsychotrine (R = OCH_3, R$'$ = OH)

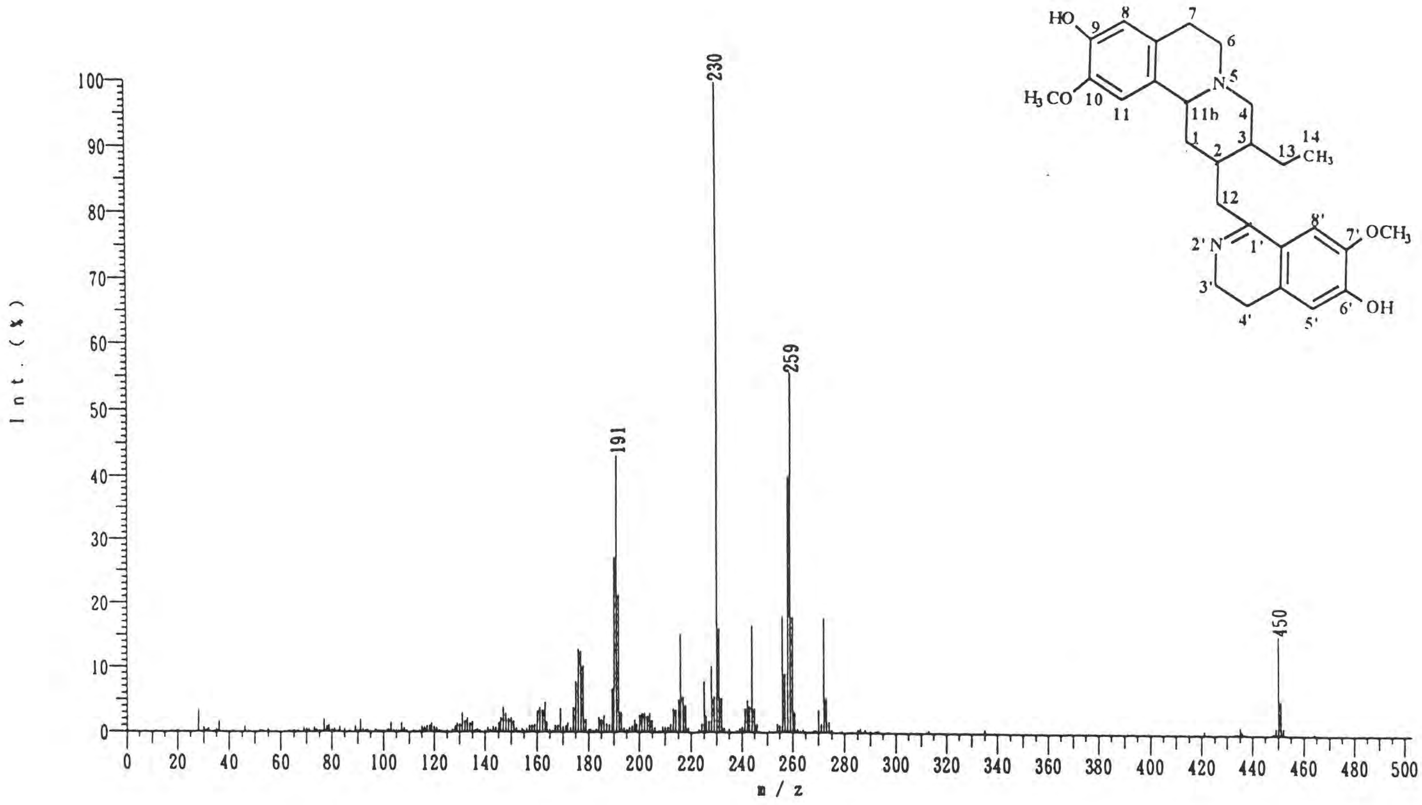


Figure 28 EIMS spectrum of AL-4

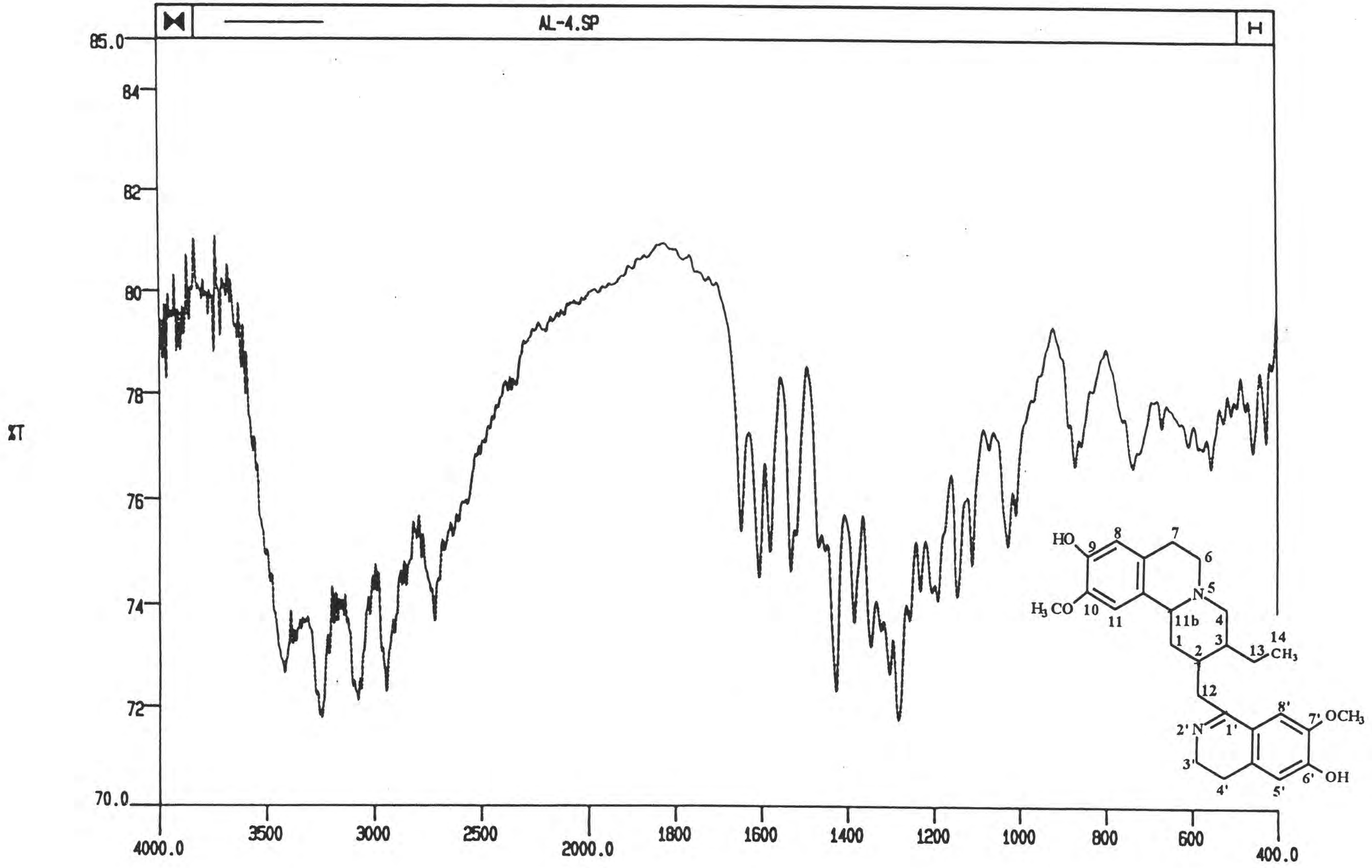


Figure 29 IR spectrum of AL-4 (KBr disc)

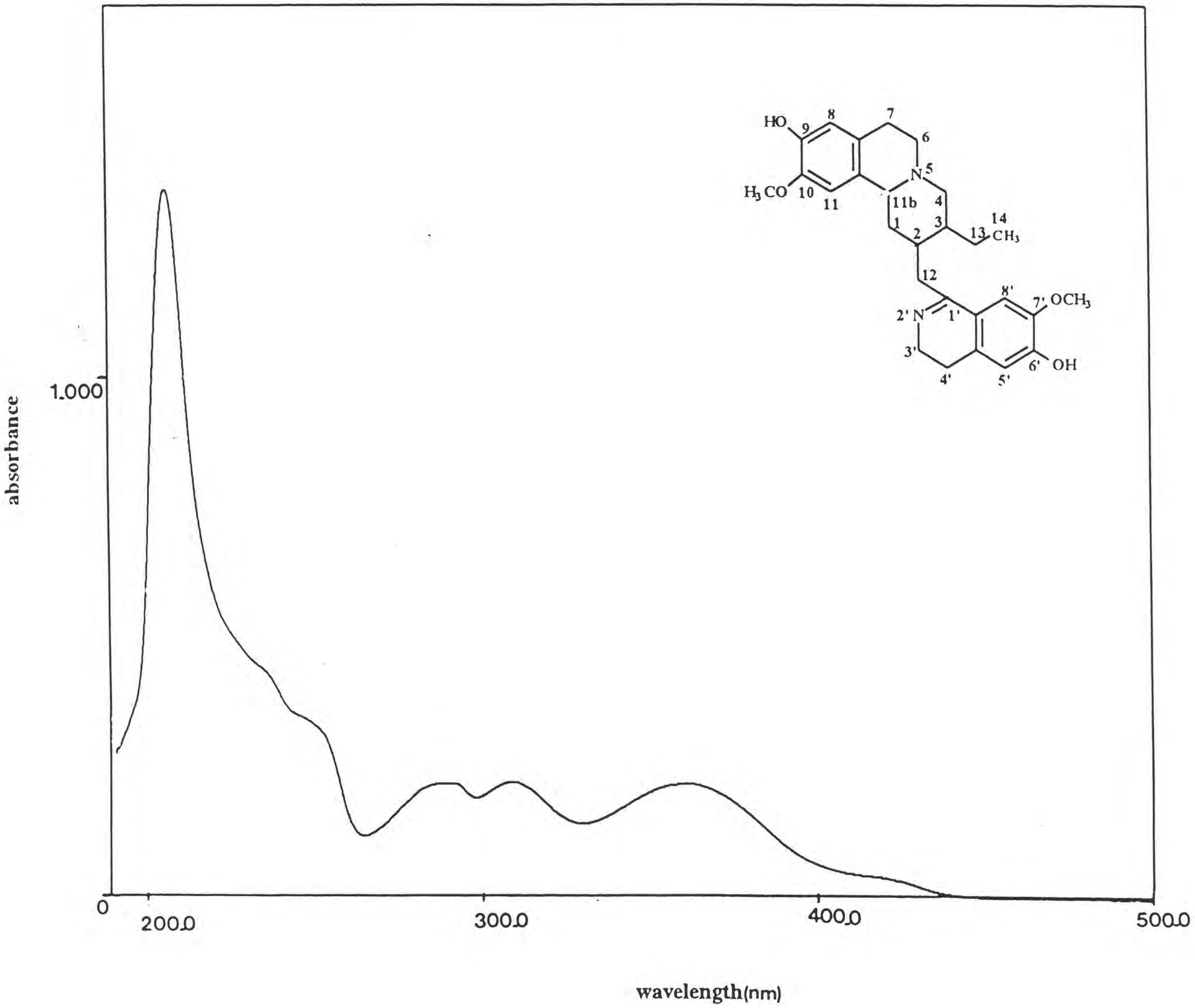
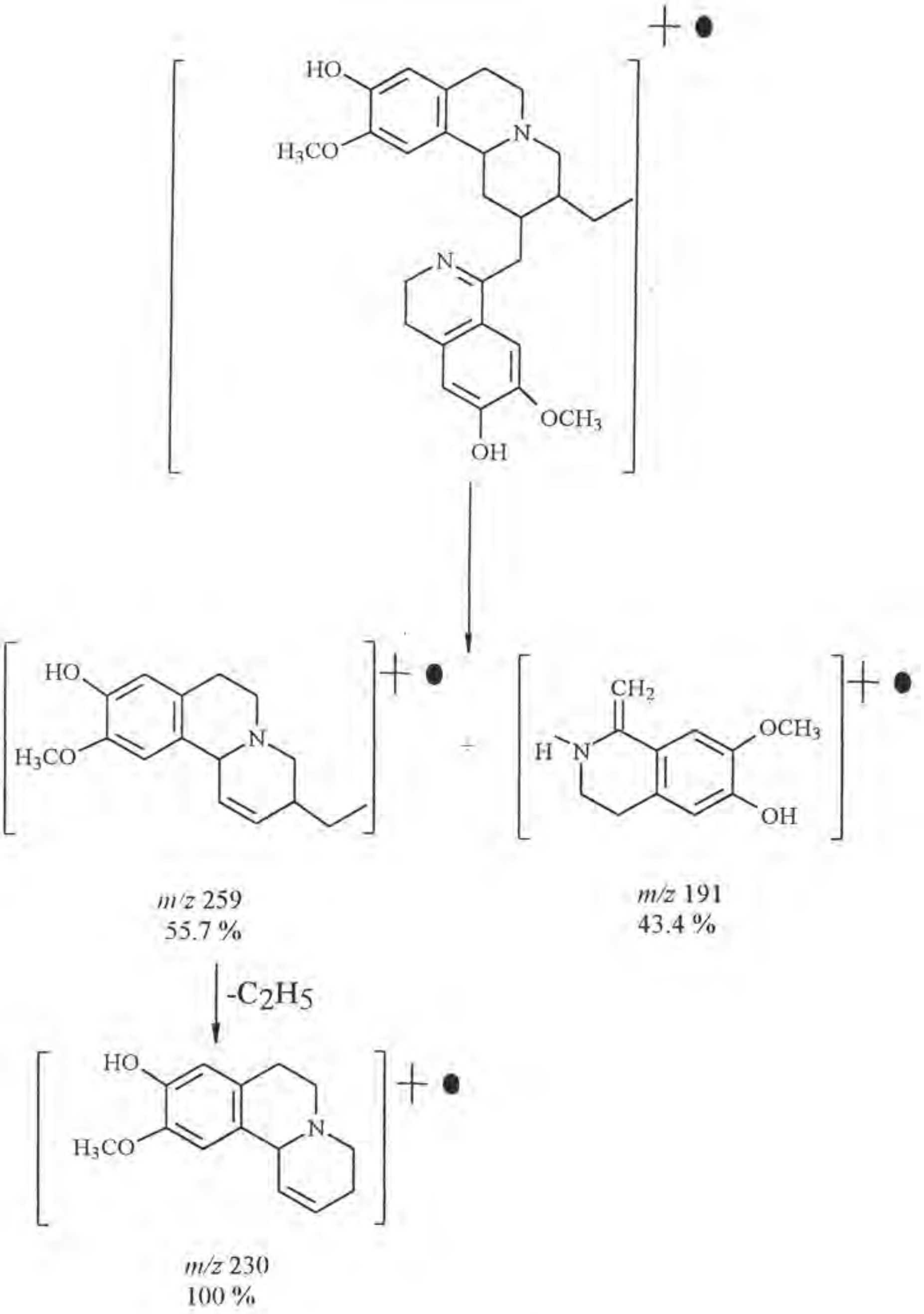


Figure 30 UV spectrum of AL-4 (in methanol)



Scheme 12 Mass fragmentation pattern of AL-4

^{1}H NMR spectrum (Figure 31, 32, 33, 34) revealed the presence of signals of 4 aromatic protons as singlets at δ 6.57, 6.59, 6.92, and 7.41 ppm, protons of methoxyl groups as two singlets at δ 3.66 and 3.85 ppm, protons of hydroxyl group as broad singlet at δ 9.14 ppm and methyl protons of ethyl residue as triplet at δ 0.93 ppm. The presence of two group of methoxyl protons instead of three also confirmed that the structure was demethyl derivative of psychotrine. The singlet signals of all aromatic protons suggested that each proton is *para* to one another. Therefore, the two aromatic protons of benzoquinolizidine moiety were assigned to position 8 and 11 and the aromatic protons of isoquinoline moiety were assigned to the position $5'$ and $8'$. As the same manner as psychotrine, establishment of the hydroxyl group to the position $6'$, was based on Brindley and Pyman's assumption that the $6'$-methoxyl in O-methylpsychotrine was the most labile of the four methoxyls in the molecule (Teitel and Brossi, 1966), Hence the substitution of the hydroxyl and the methoxyl was at position $6'$ and $7'$ respestively.

The assignments of methoxyl and hydroxyl groups at position 9 and 10 could not be indicated since more experiments were needed.

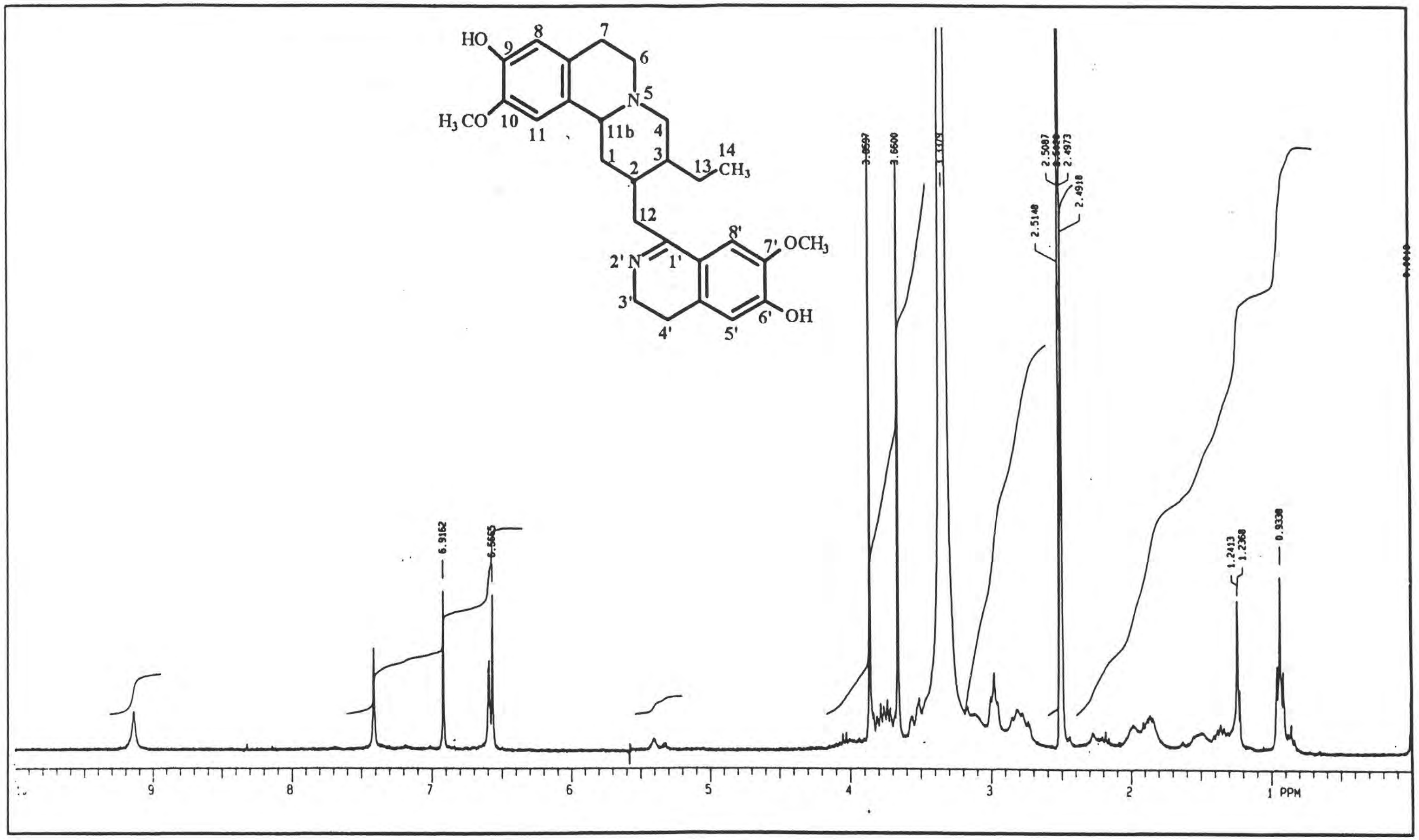


Figure 31 The 300 MHz ^{1}H NMR spectrum of AL-4 (in DMSO)

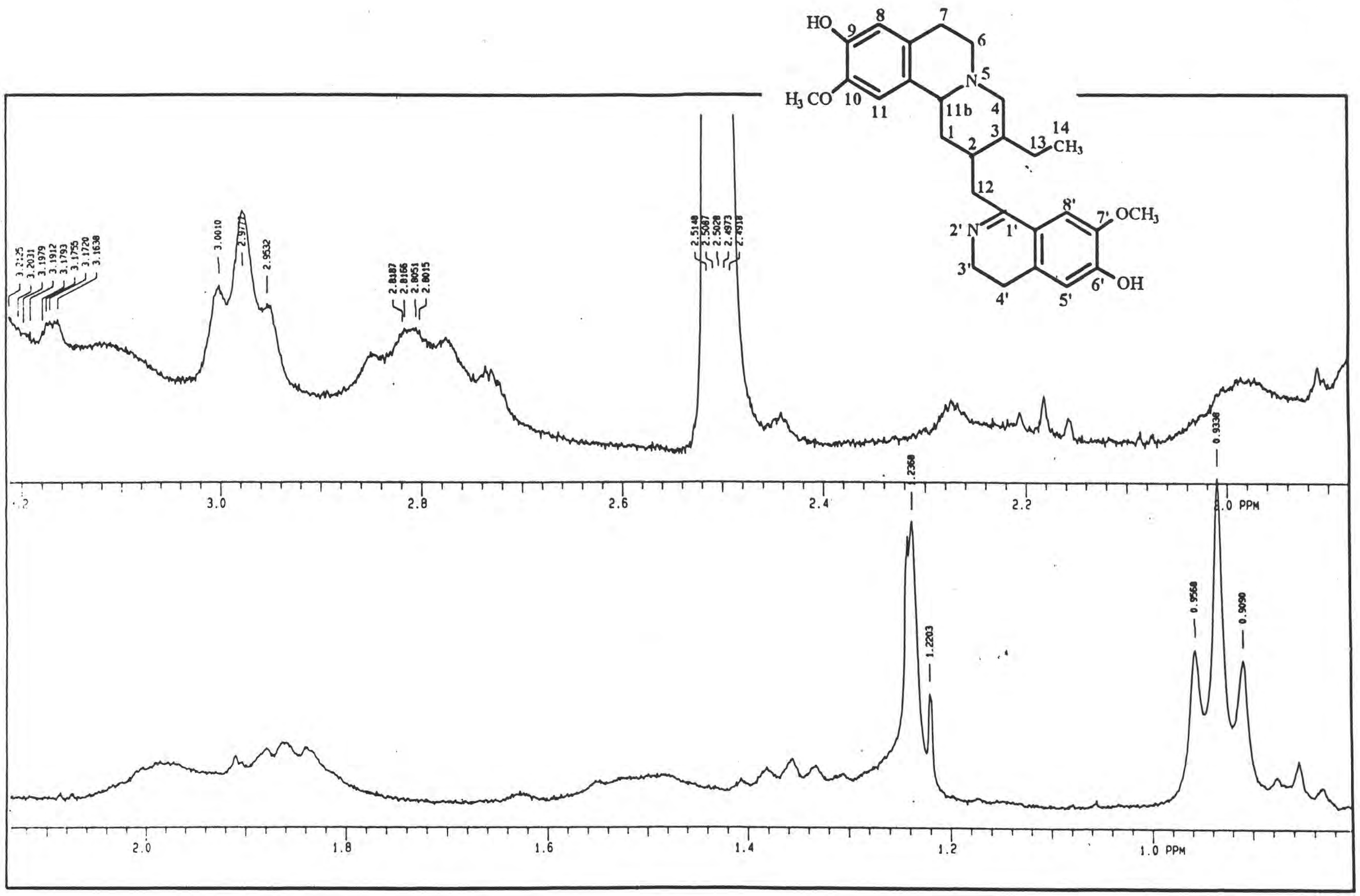


Figure 32 The 300 MHz ^{1}H NMR spectrum of AL-4 (in DMSO)

(expanded from 1.0-2.0 ppm and 2.0-3.2 ppm)

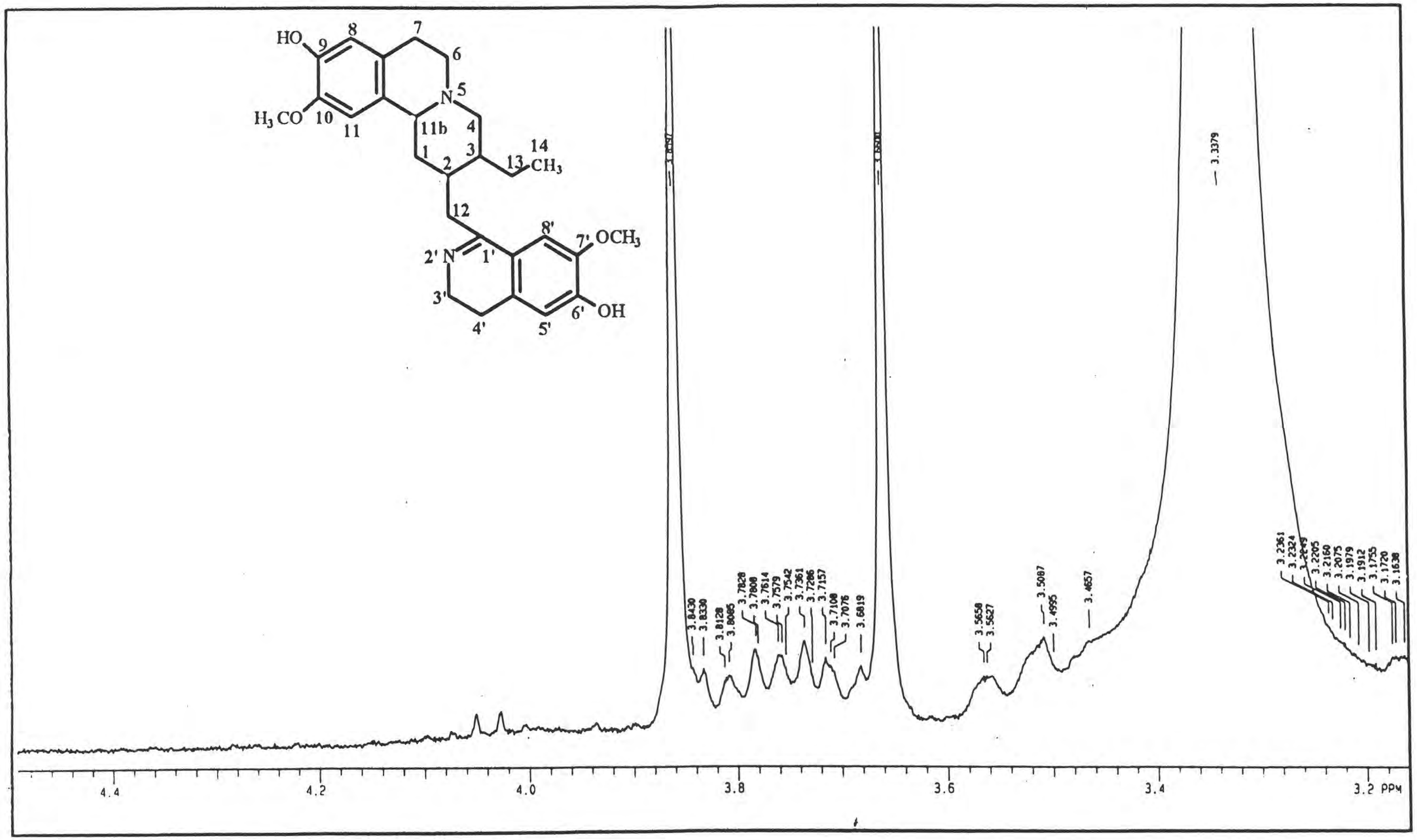


Figure 33 The 300 MHz ^{1}H NMR spectrum of AL-4 (in DMSO)

(expanded from 3.2-4.4 ppm)

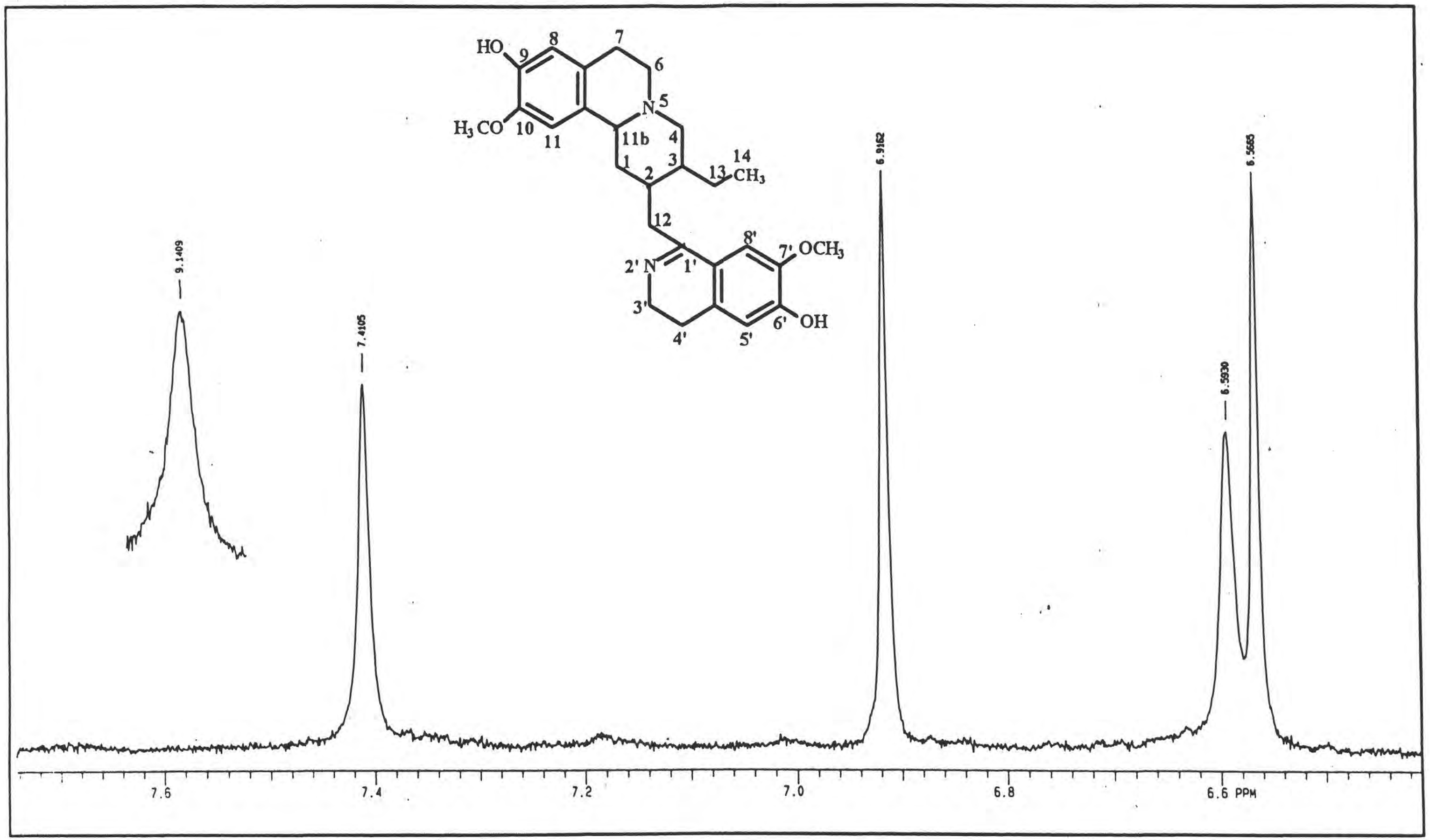


Figure 34 The 300 MHz ^{1}H NMR spectrum of AL-4 (in DMSO)

(expanded from 6.6-7.6 ppm)

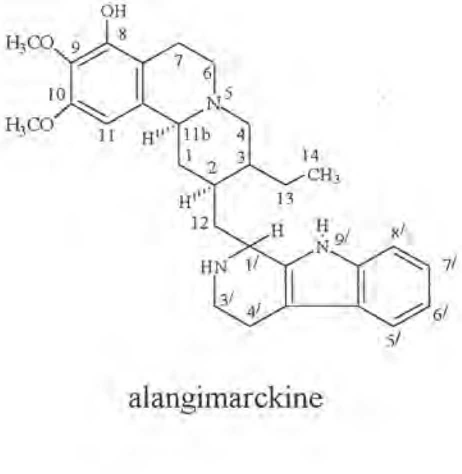
5. Structure Elucidation of AL-1L

Compound AL-1L was obtained as yellow amorphous powder from the leaves. It gave orange color to Dragendorff's test and thus was likely alkaloidal in nature.

The IR spectrum (Figure 35) exhibited the absorption bands of N-H stretch of indole molecule at 3300 - 3400 cm^{-1}, phenolic O-H stretch near 3300 cm^{-1} and C-H stretch of methyl and methylene groups near 2,900 cm^{-1}.

The EIMS spectrum (Figure 36) showed the molecular ion peak at *m/z* 475. The mass fragmentation showed characteristic pattern of benzoquinolizidine and β-carboline moieties. The peaks at *m/z* 208, 262, and 288 indicated the presence of benzoquinolizidine bearing two methoxyl and a hydroxyl group. On the other hand, the peak at *m/z* 185 indicated the presence of non-substituted β-carboline (Scheme 13).

By comparision of the mass fragmentation and the molecular weight to the published data (Wiegrebe, Kramer, and Shamma, 1984; Cordell *et al.*, 1989), the molecular formula of the alkaloid AL-1L was determined as $C_{29}H_{37}N_3O_3$ and it could be assigned the structure of the known compound, alangimarckine. Its structure is shown below.



alangimarckine

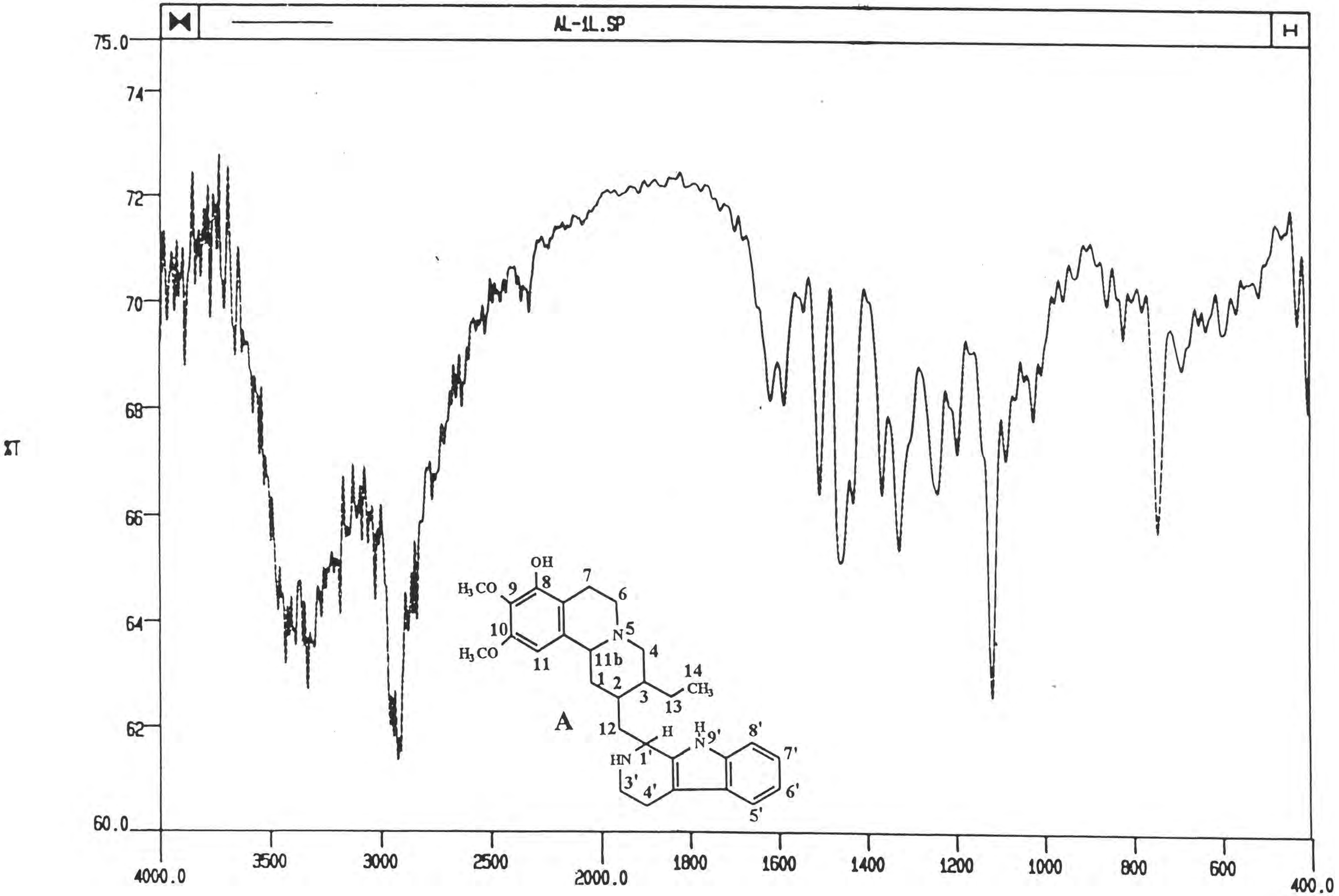


Figure 35 IR spectrum of AL-1L (KBr disc)

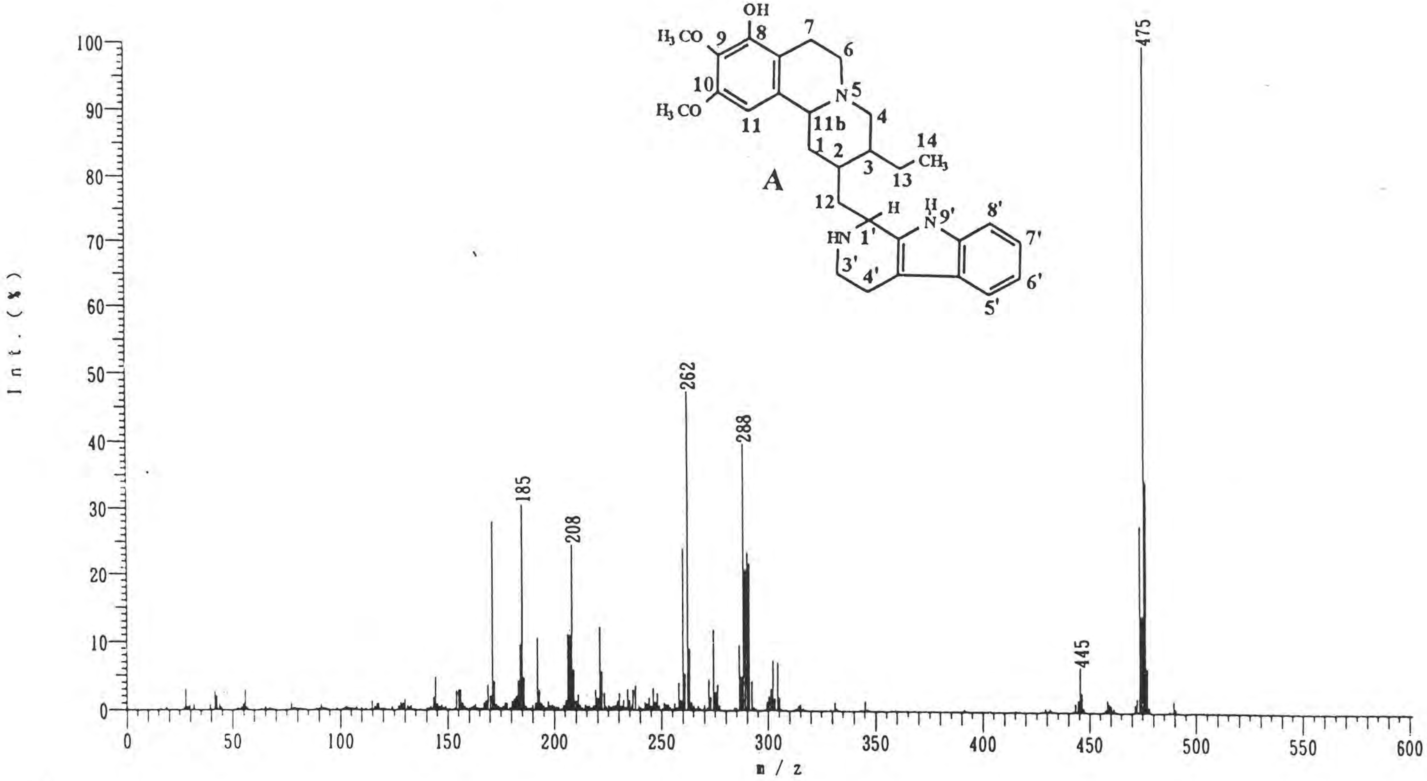
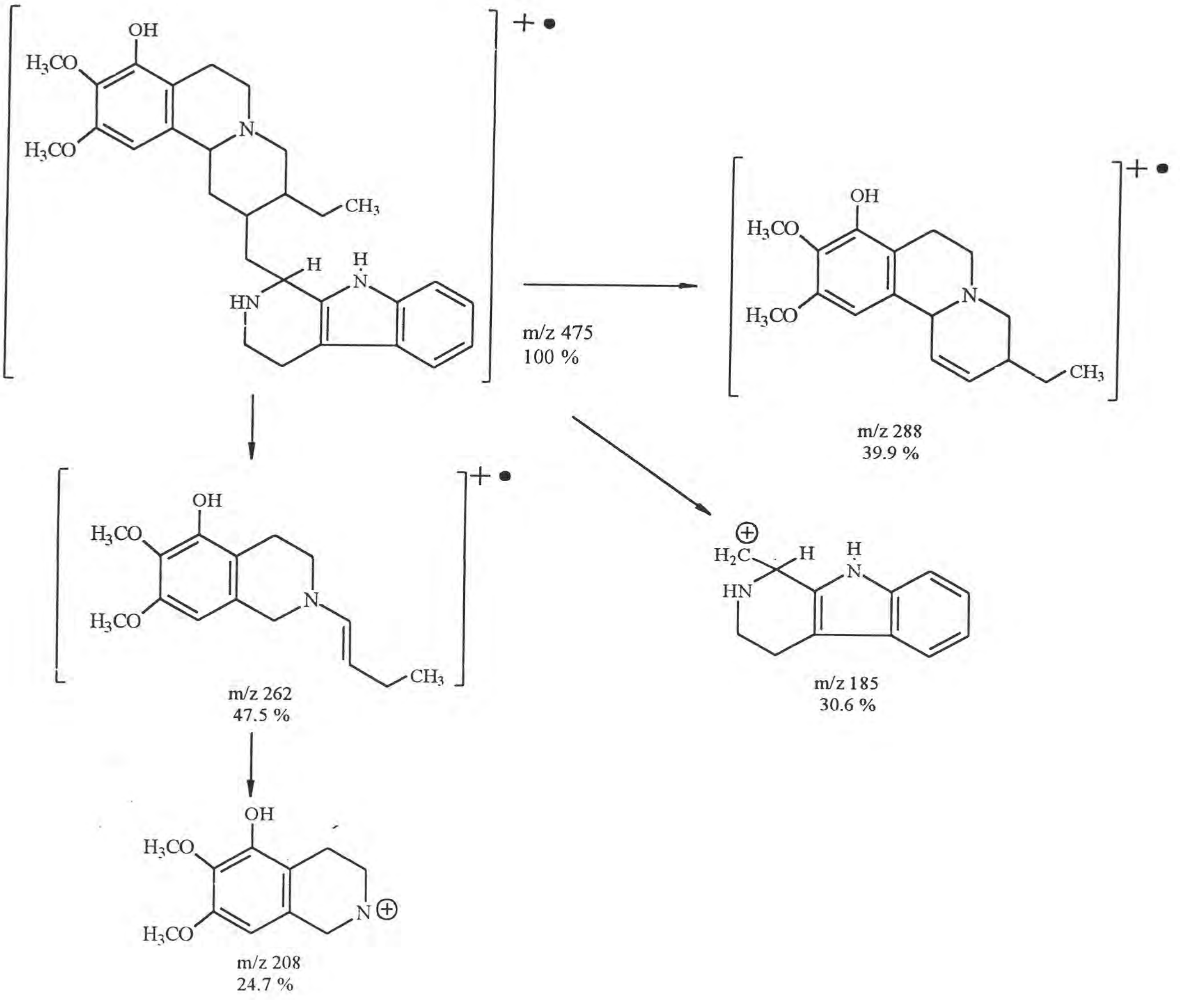


Figure 36 EIMS spectrum of AL-1L



Scheme 13 Mass fragmentation pattern of AL-1L

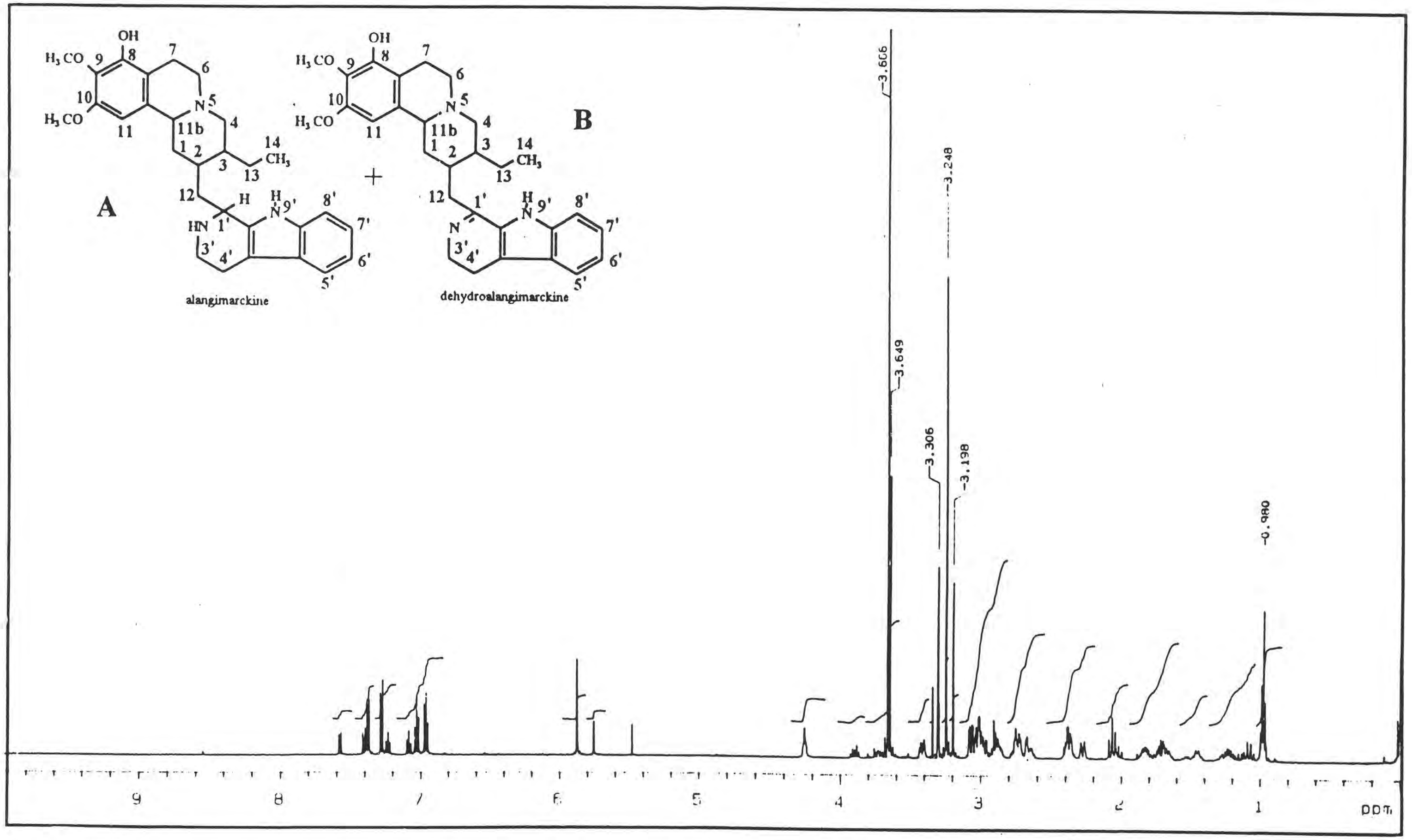


Figure 37 The 500 MHz ^{1}H NMR spectrum of AL-1L (in DMSO)

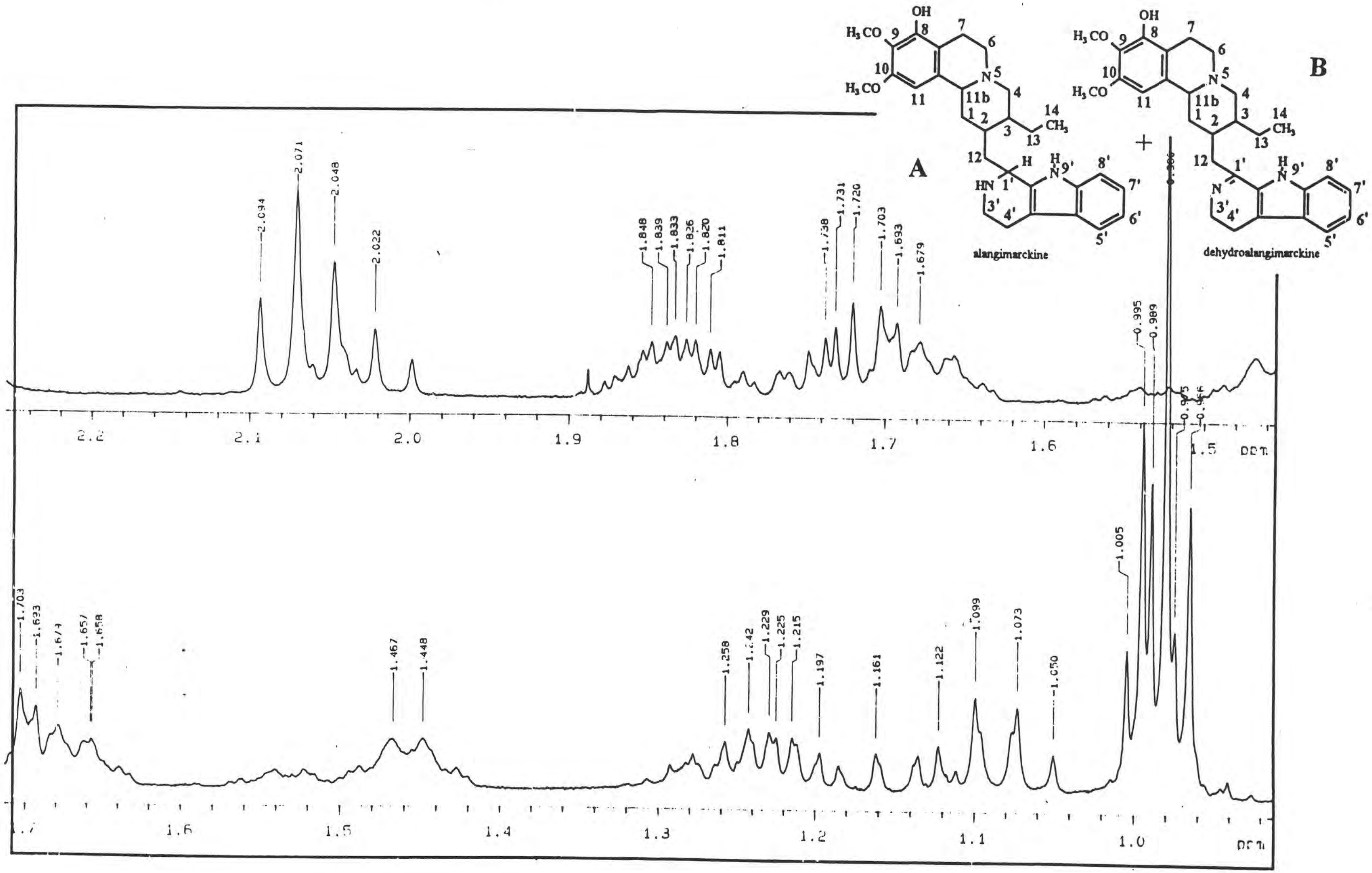


Figure 38 The 500 MHz ^{1}H NMR spectrum of AL-1L (in DMSO) (expanded from 1.00-2.20 ppm)

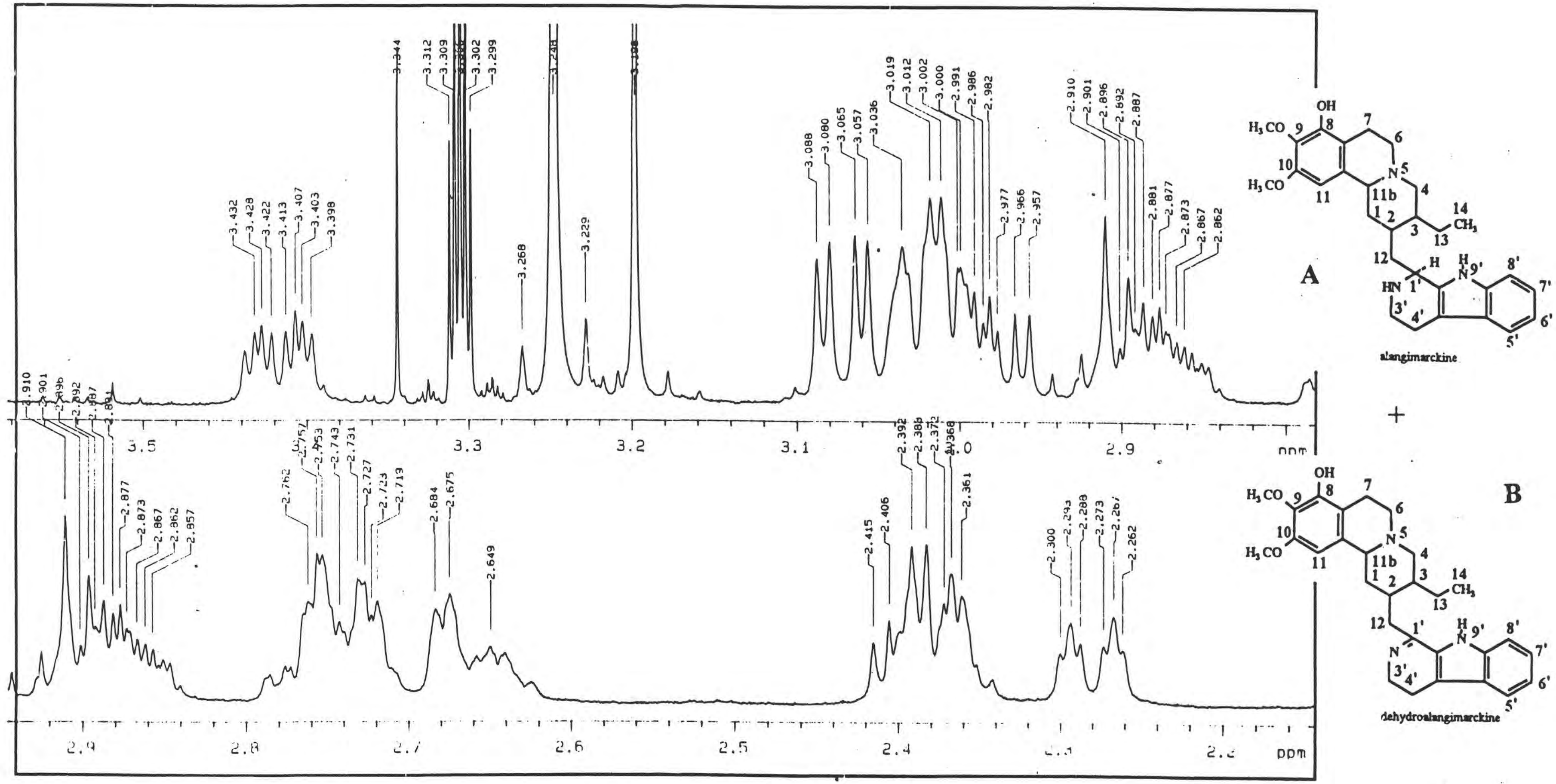


Figure 39 The 500 MHz ^{1}H NMR spectrum of AL-1L (in DMSO)
(expanded from 2.20-3.50 ppm)

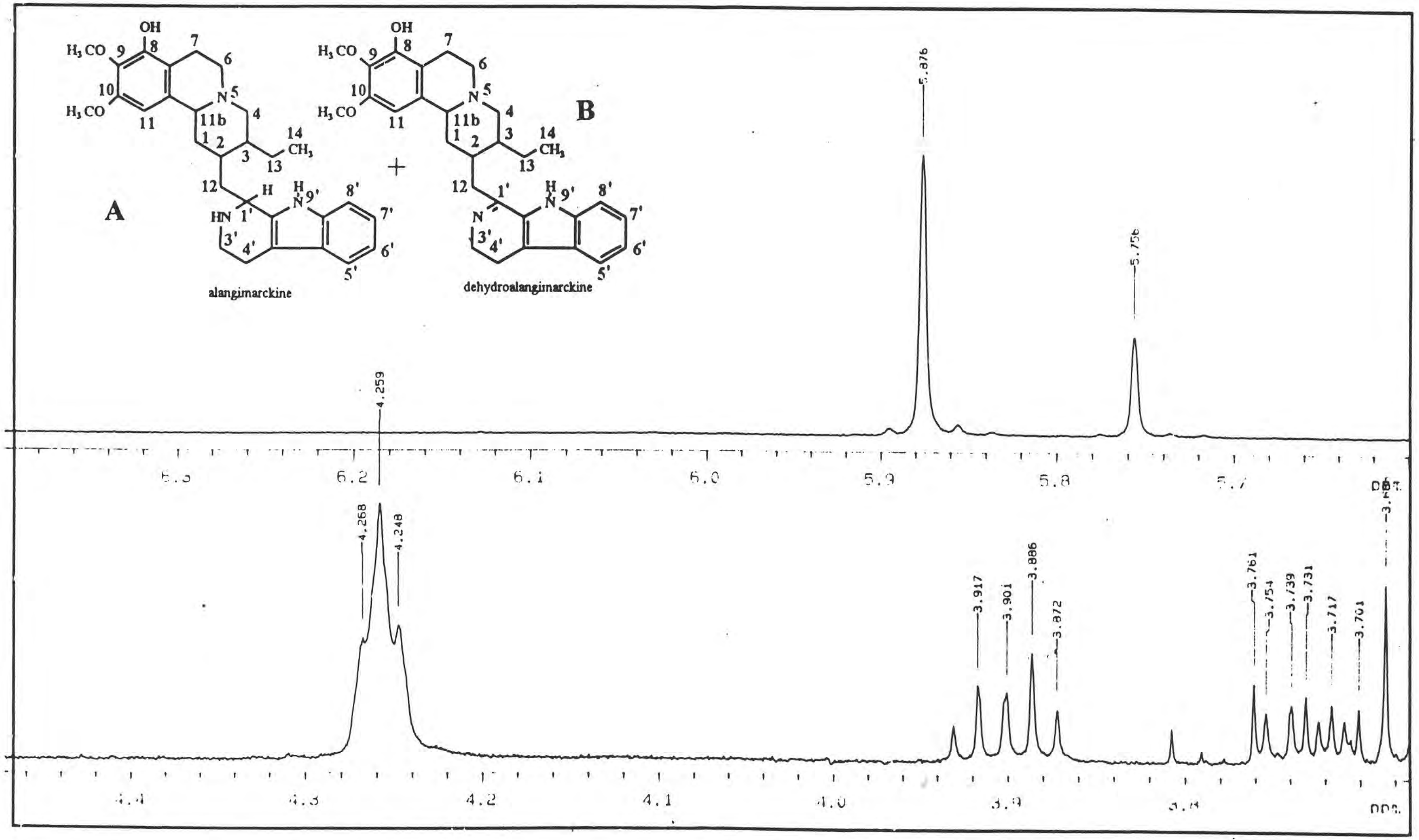


Figure 40 The 500 MHz ^{1}H NMR spectrum of AL-1L (in DMSO)

(expanded from 3.70-6.30 ppm)

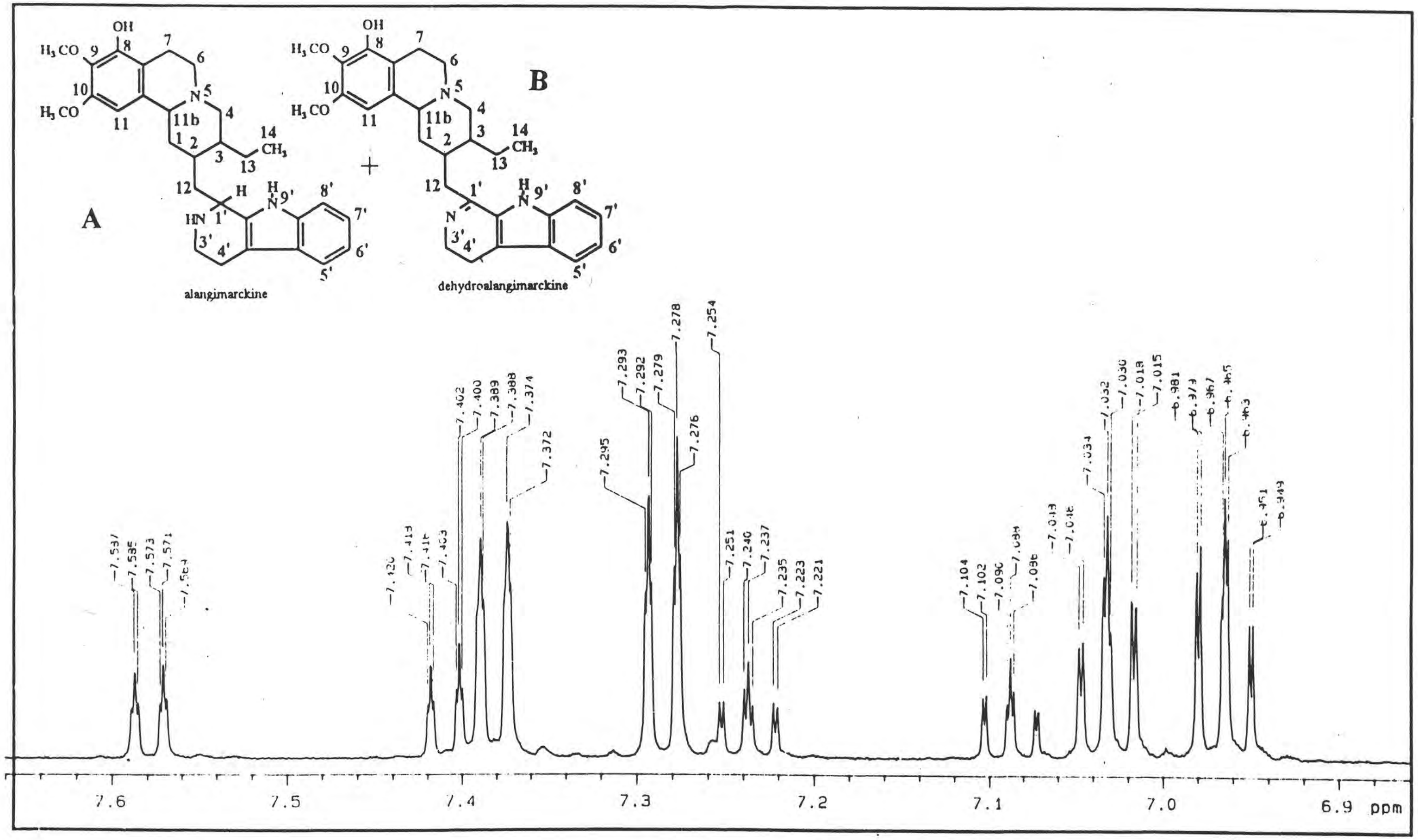


Figure 41 The 500 MHz ^{1}H NMR spectrum of AL-1L (in DMSO)
(expanded from 6.90-7.60 ppm)

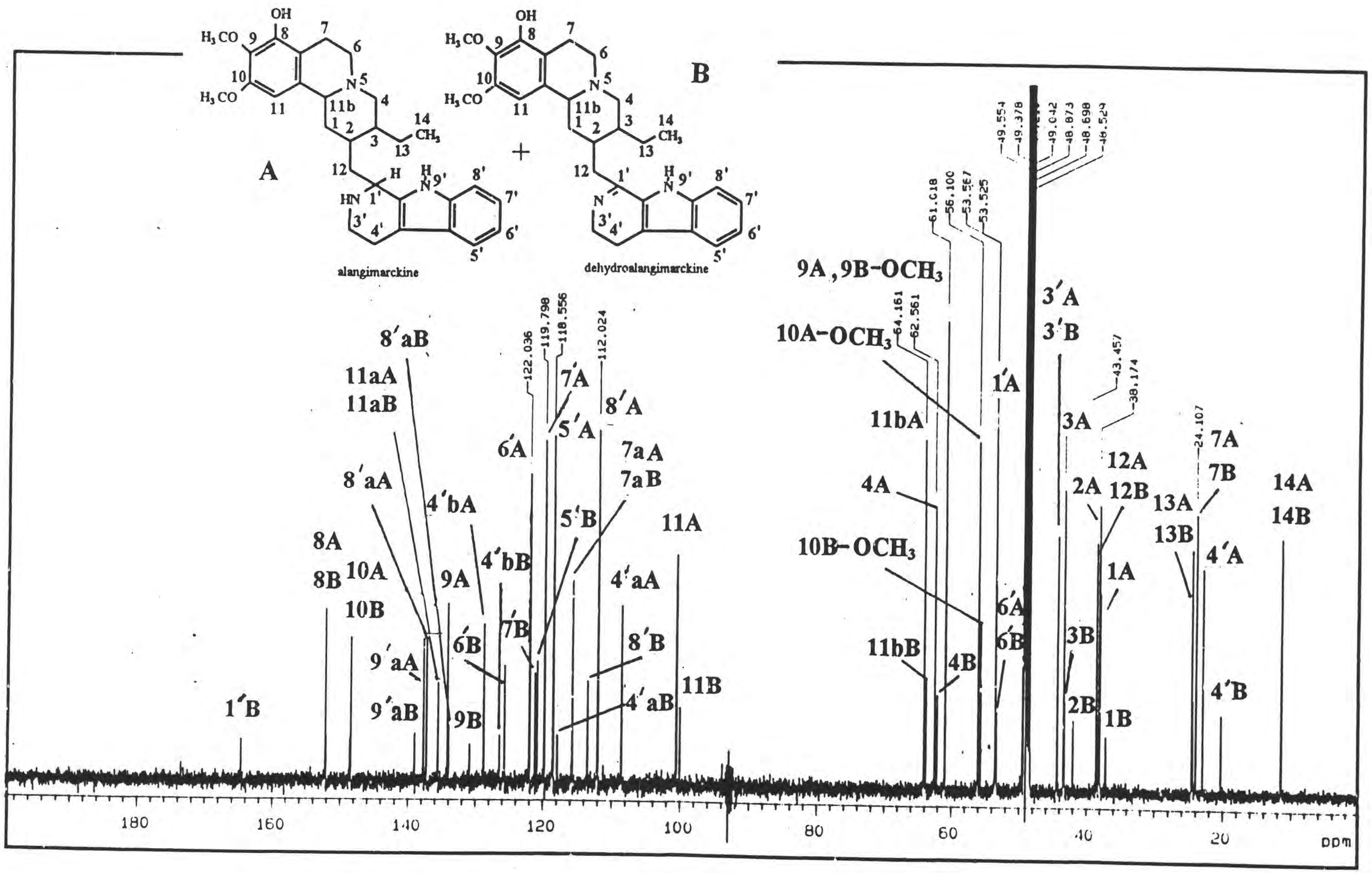


Figure 42 The 125 MHz ^{13}C NMR spectrum of AL-1L (in DMSO)

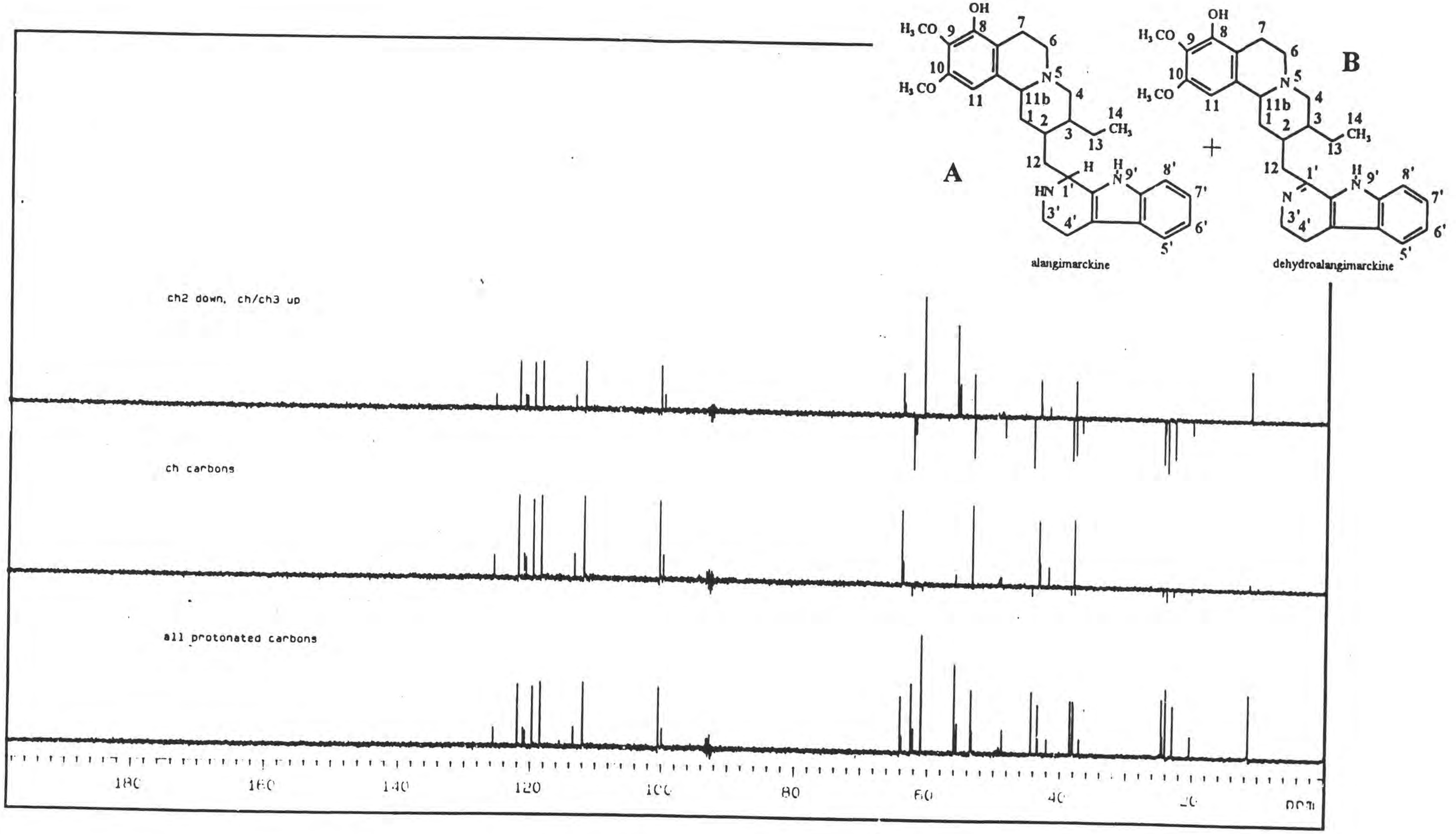


Figure 43 The 125 MHz DEPT spectrum of AL-1L (in DMSO)

However the ^{1}H NMR (Figures 37,38, 39, 40, 41) and ^{13}C NMR (Figures 42, 43) spectra showed that there were two sets of carbon signals with difference intensity (approximately 3:1). It was, therefore, likely that AL-1L was the mixture of two alkaloids in which alangimarckine was the main compound. For convenience in the elucidation, the compound with higher intensity was given the code, AL-1L(A) and the lower one AL-1L(B).

To confirm the presence of alangimarckine in the AL-1L mixture, and also to identify the structure of AL-1L(B), the ^{13}C NMR of the mixture was analysed and compared with reported data (Fujii *et al.*,1985). The results are summarized in Table 7.

Table 7 Comparative ^{13}C NMR data for alangimarckine and AL-1L mixture.

carbon	chemical shift (ppm)		
	alangimarckine[a]	AL-1L(A)[b]	AL-1L(B)[b]
1	36.8	38.0	37.1
2	36.5	38.1	42.0
3	41.6	43.4	43.3
4	61.4	62.5	52.1
6	51.9	53.5	53.4
7	23.5	24.1	23.0[f]
7a	115.1	115.7	115.8
8	150.2	152.2	152.1
9	134.2[c]	134.2	130.9
10	145.6	148.6	148.4
11	100.5	100.6	100.0
11a	133.9[c]	135.5	135.5
11b	62.5	64.1	63.8
12	38.9	38.5	38.5
13	23.5	24.7	24.5

Table 7 (Continued)

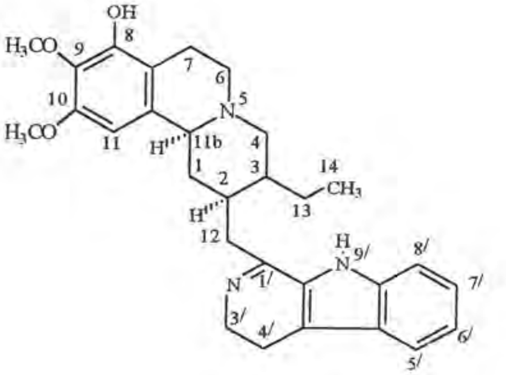
carbon	chemical shift (ppm)		
	alangimarckine[a]	AL-1L(A)[b]	AL-1L(B)[b]
14	11.2	11.5	11.5
1′	49.3	53.567	164.714
3′	42.1	44.8	44.8
4′	22.9	23.1	20.4[f]
4′a	108.9[c]	108.5	117.9
4′b	127.9	128.7	126.4
5′	117.9	118.5	120.7
6′	121.5	119.7[d]	125.7[e]
7′	119.4	122.0[d]	121.1[e]
8′	110.7	112.0	113.4
8′a	136.5	137.7	134.0[g]
9′a	135.5	137.3	139.0[e]
9-OMe	60.9	61.0	61.0
10-OMe	56.0	56.1	55.7

a) Chemical shift in $CDCl_3$

b) Chemical shift in CD_3OD

c,d,e,f,g) Assignment may be interchanged

Based on Table 7, it appeared that the chemical shift of each carbon of AL-1L(A) closely resembled that of alangimarckine. For AL-1L(B), the data was different from alangimarckine in the chemical shift at 1′-position which was extremely down field (δ 164.7 ppm). This down field shift represented the presence of unsaturation between position 1′ and 2′ similar to that of psychotrine, in which the C-1 signal shifted downfield to 167.5 ppm (Fujii *et al.*, 1983). Thus, AL-1L(B) could be assigned as a new alkaloid dehydroalangimarckine and the structure is shown below.



dehydroalangimarckine

The proton assignments of AL-1L were based on H-H COSY (Figures 44, 45), HMQC (Figures 46, 47, 48, 49, 50), and HMBC (Figures 51, 52, 53, 54, 55, 56, 57, 58, 59) spectra. The upfield proton signals were rather complicated, the differentiation of each proton signal for AL-1L(A) and AL-1L(B) could not be completely done. However, the ^{1}H-NMR spectrum clearly showed 2 different sets of aromatic proton signals with 2 separated set of H-H COSY correlation.

For AL-1L(A); the H-NMR spectrum (Figure 41) showed an aromatic proton appeared as a singlet at δ 5.87 ppm and from HMQC spectrum (Figure 50), this signal was asssigned to position 11. Another set of aromatic protons was at δ 6.97, 7.03, 7.27 and 7.38 ppm. Following, ganalyses of HMQC and H-H COSY spectra, the chemical shift of each proton could be assigned as summarized in Table 8.

Table 8 Chemical shifts of aromatic protons of AL-1L(A).

position	chemical shift (ppm) (multiplicity, *J* Hz)	H-H correlation in H-H COSY spectrum
11	5.87 (s)	-
5′	7.38 (dd, *J*=6.0, 0.8)	6′-H , 7′-H
6′	6.97 (td, *J*=6.0, 0.8)	5′-H , 7′-H , 8′-H
7′	7.03 (td, *J*=6.0, 0.8)	6′-H , 7′-H , 8′-H
8′	7.27 (dd , *J*=6.0, 0.8)	6′-H , 7′-H

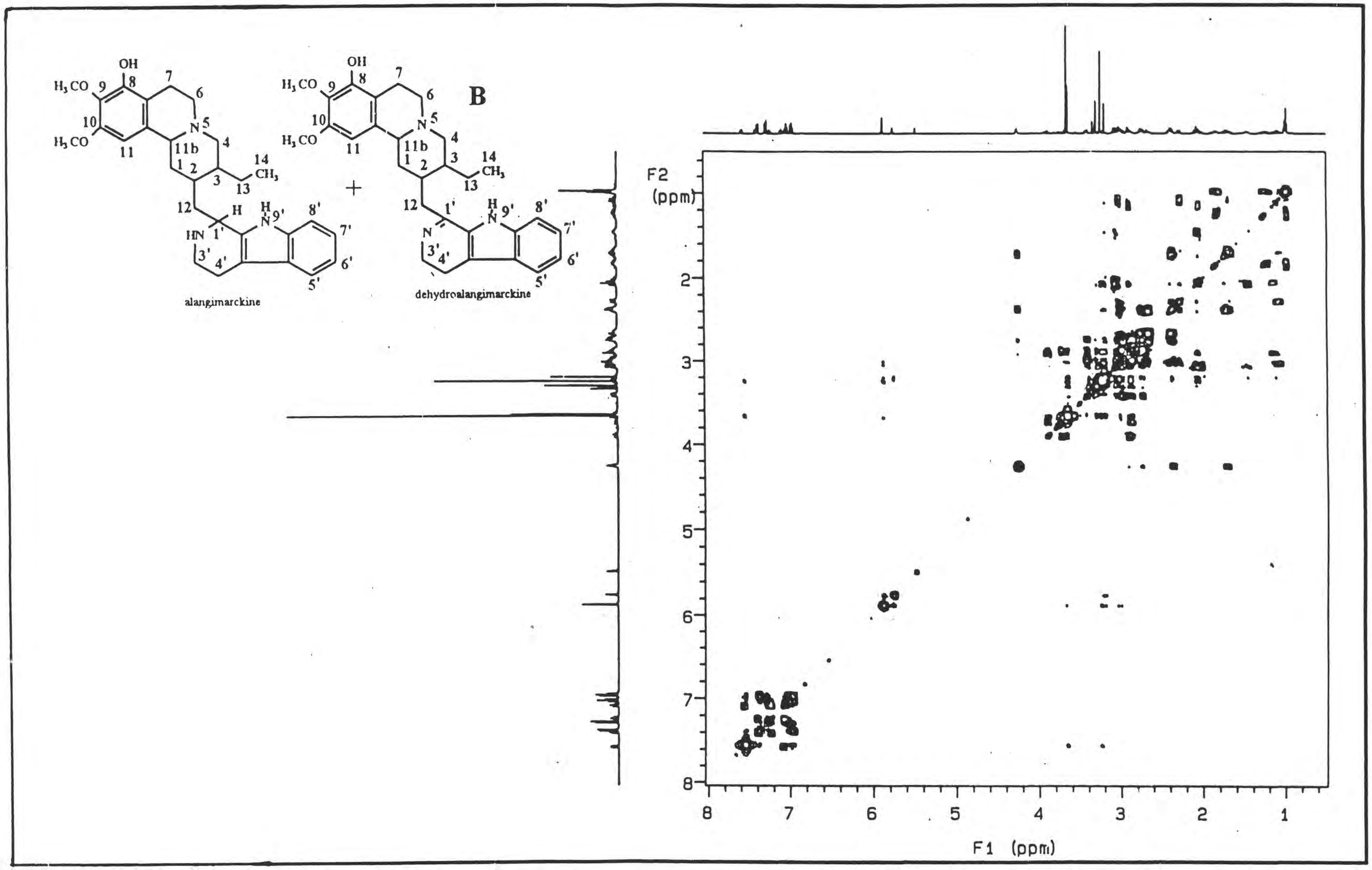


Figure 44 The 500 MHz ^1H-^1H COSY spectrum of AL-1L (in DMSO)

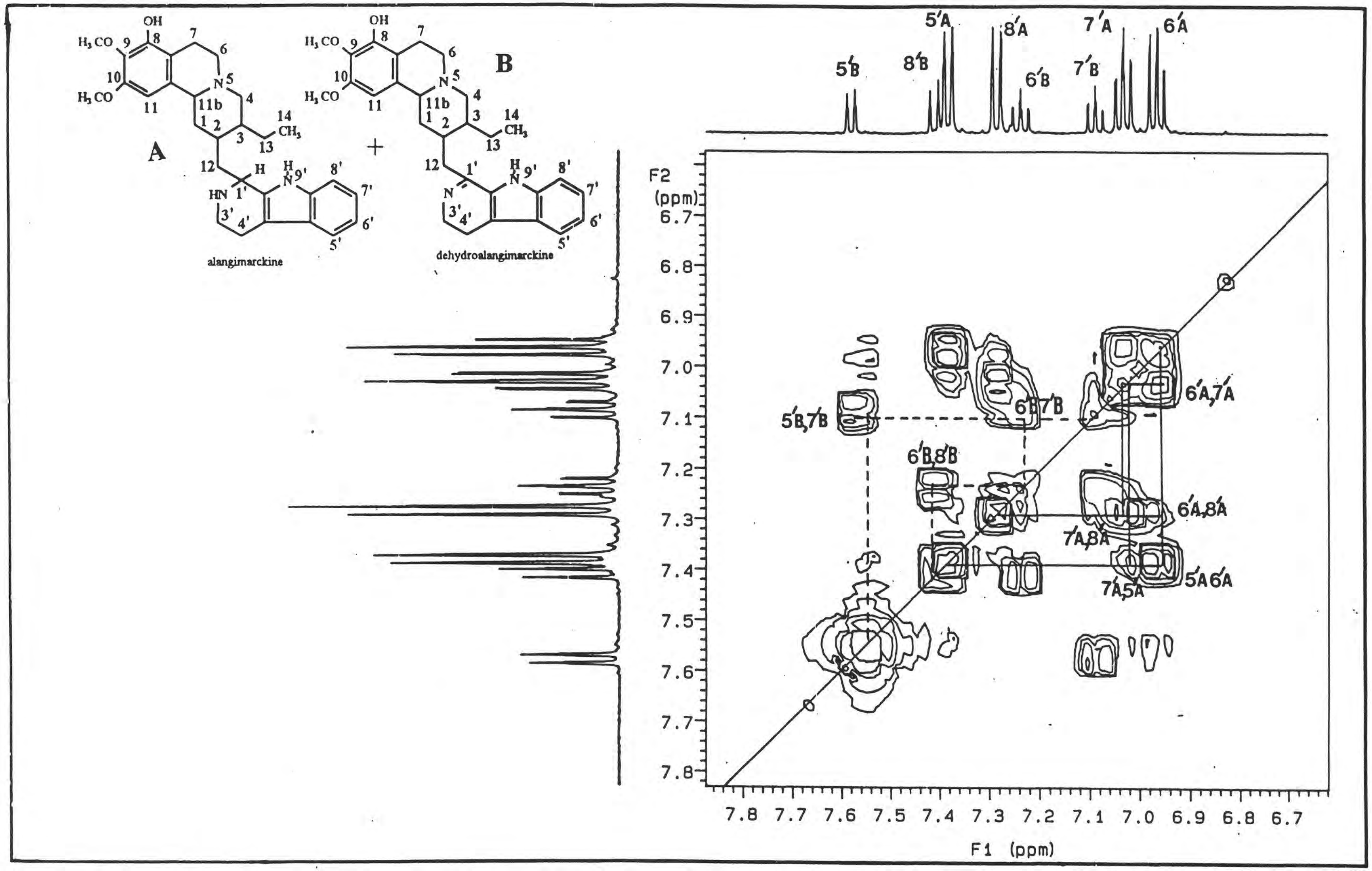


Figure 45 The 500 MHz ^{1}H-^{1}H COSY spectrum of AL-1L (in DMSO)

(expansion of the aromatic region)

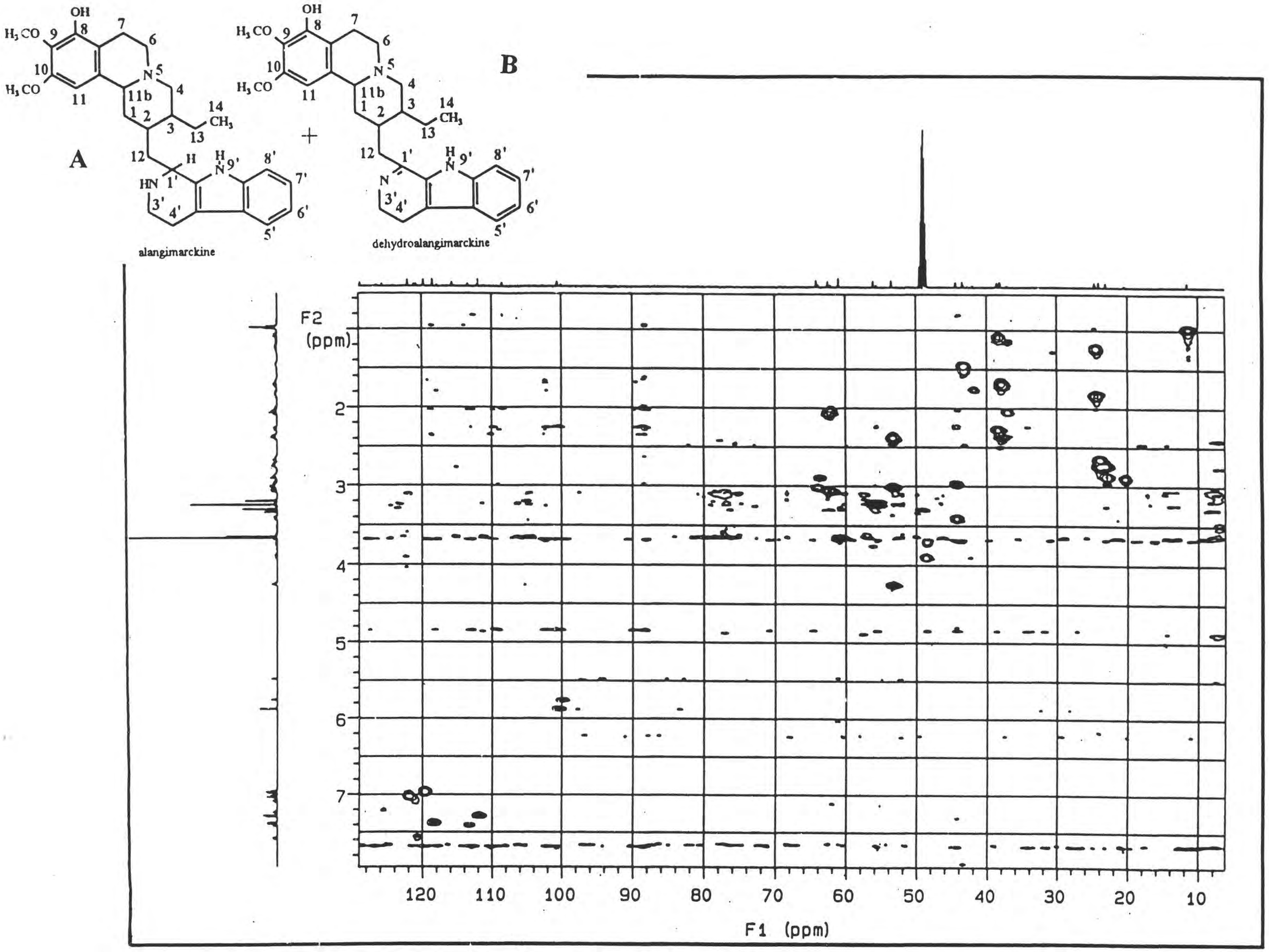


Figure 46 The 500 MHz HMQC spectrum of AL-1L (in DMSO)

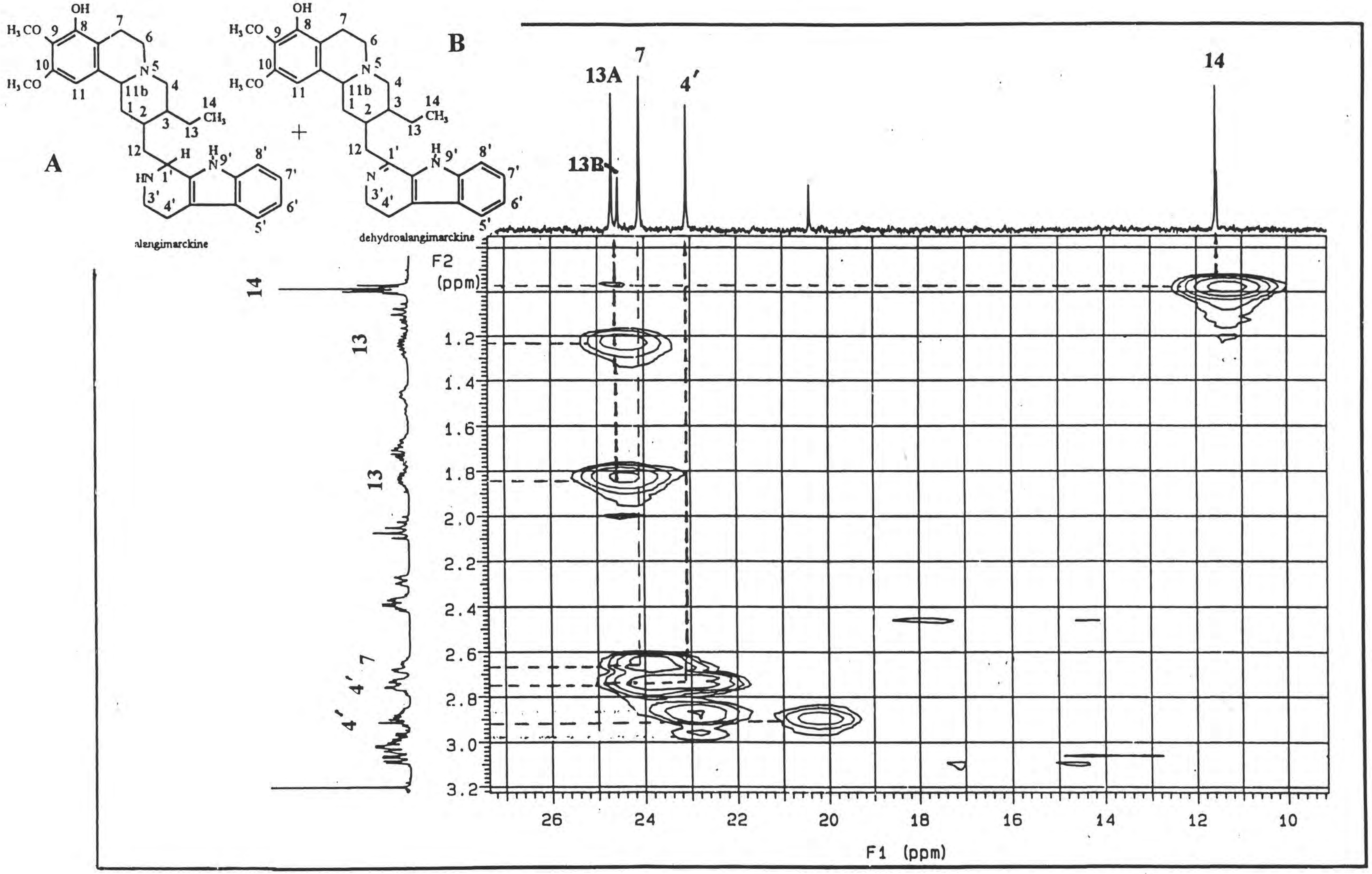


Figure 47 The expansion of 500 MHz HMQC spectrum of AL-1L (in DMSO)

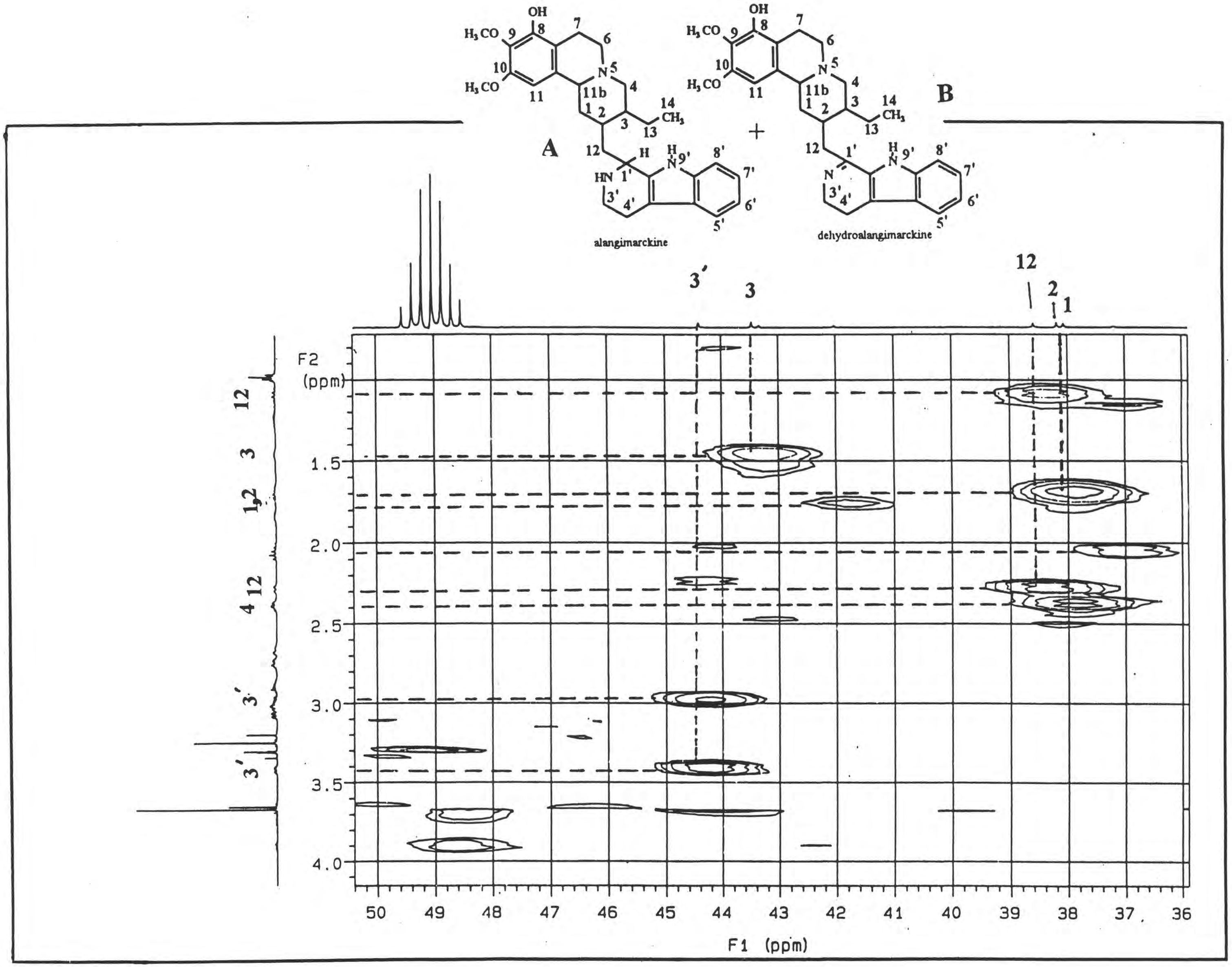


Figure 48 The expansion of 500 MHz HMQC spectrum of AL-1L (in DMSO)

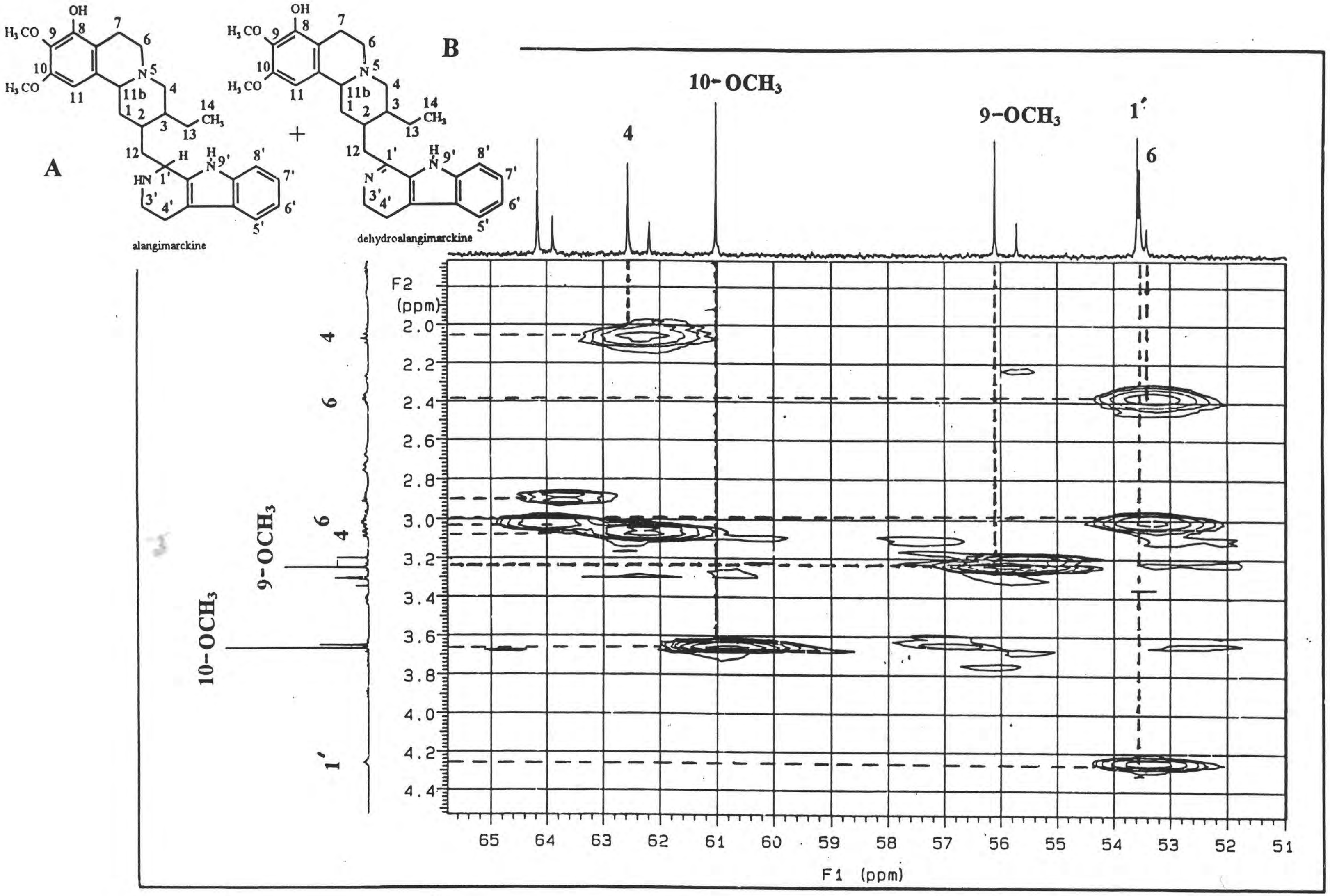


Figure 49 The expansion of 500 MHz HMQC spectrum of AL-1L (in DMSO)

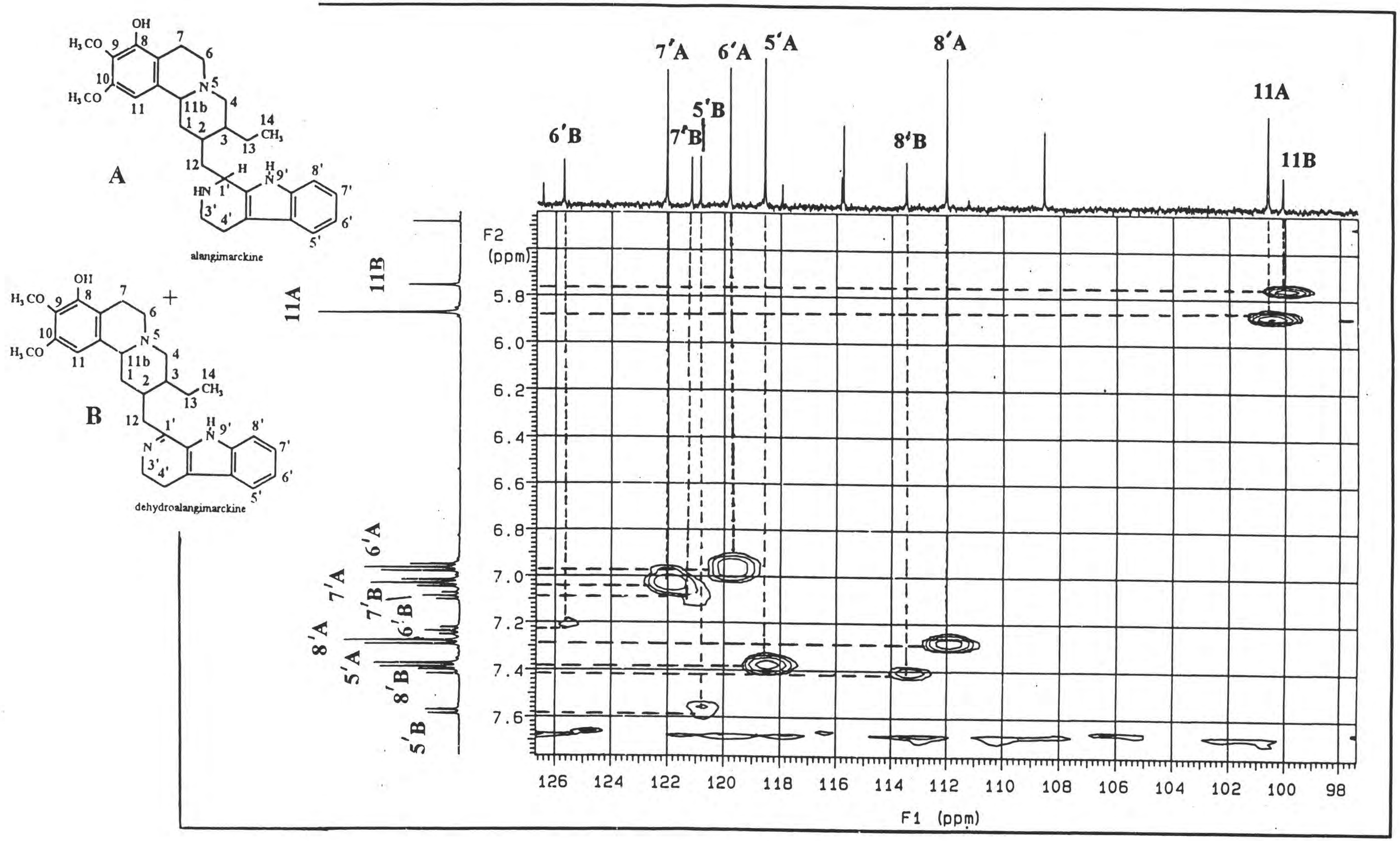


Figure 50 The expansion of 500 MHz HMQC spectrum of AL-1L (in DMSO)

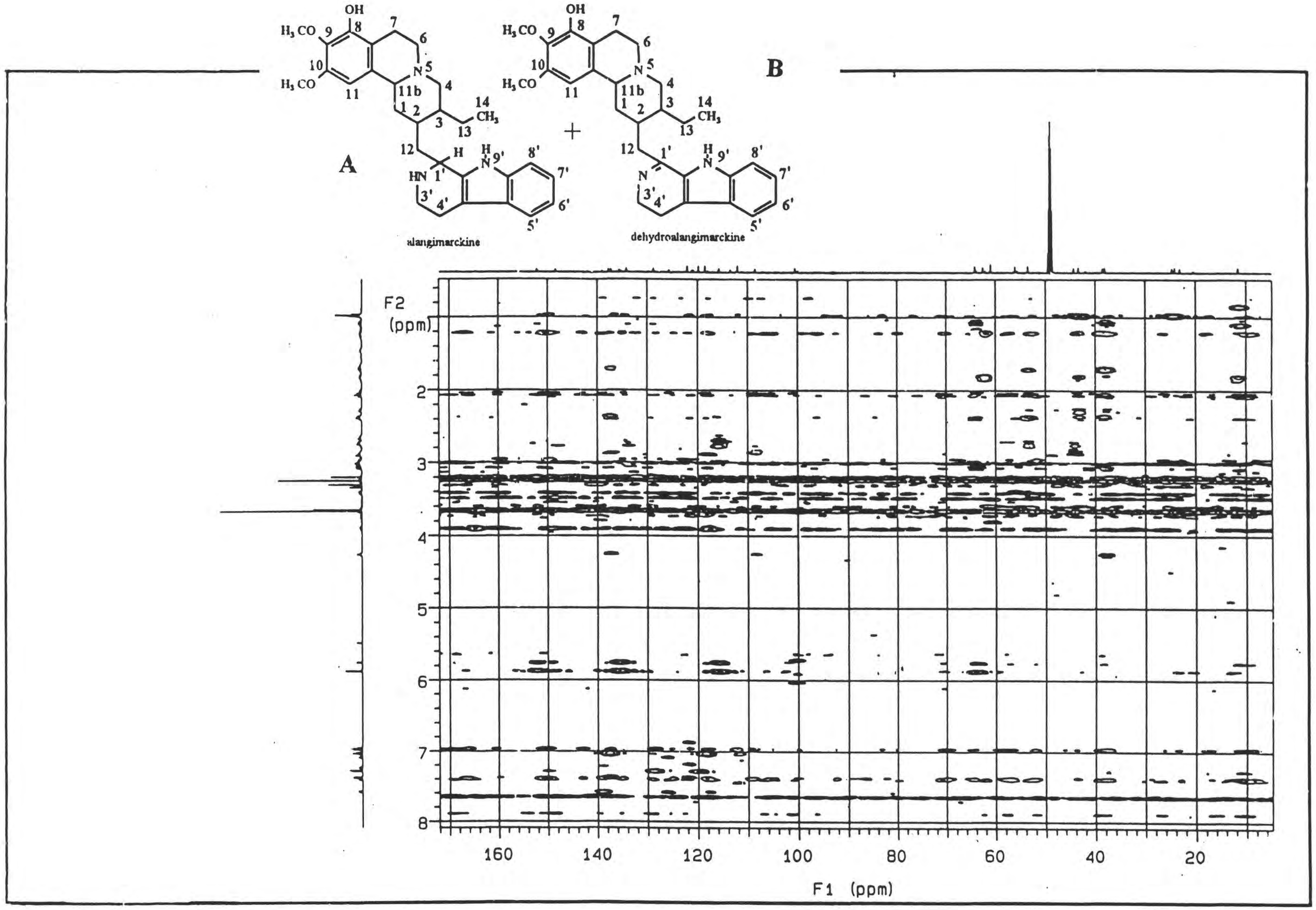


Figure 51 The 500 MHz HMBC spectrum of AL-1L (in DMSO)

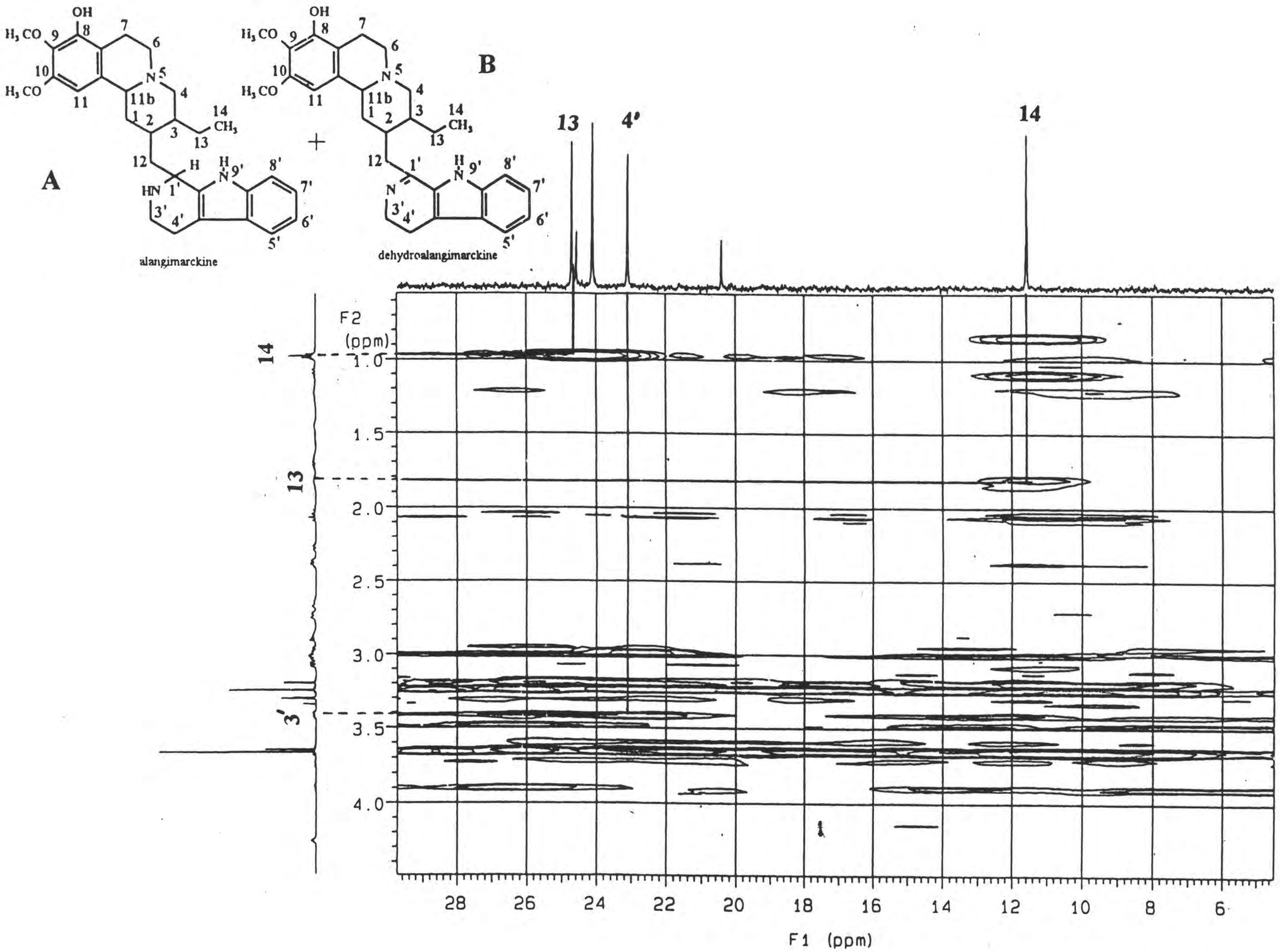


Figure 52 The expansion of 500 MHz HMBC spectrum of AL-1L (in DMSO)

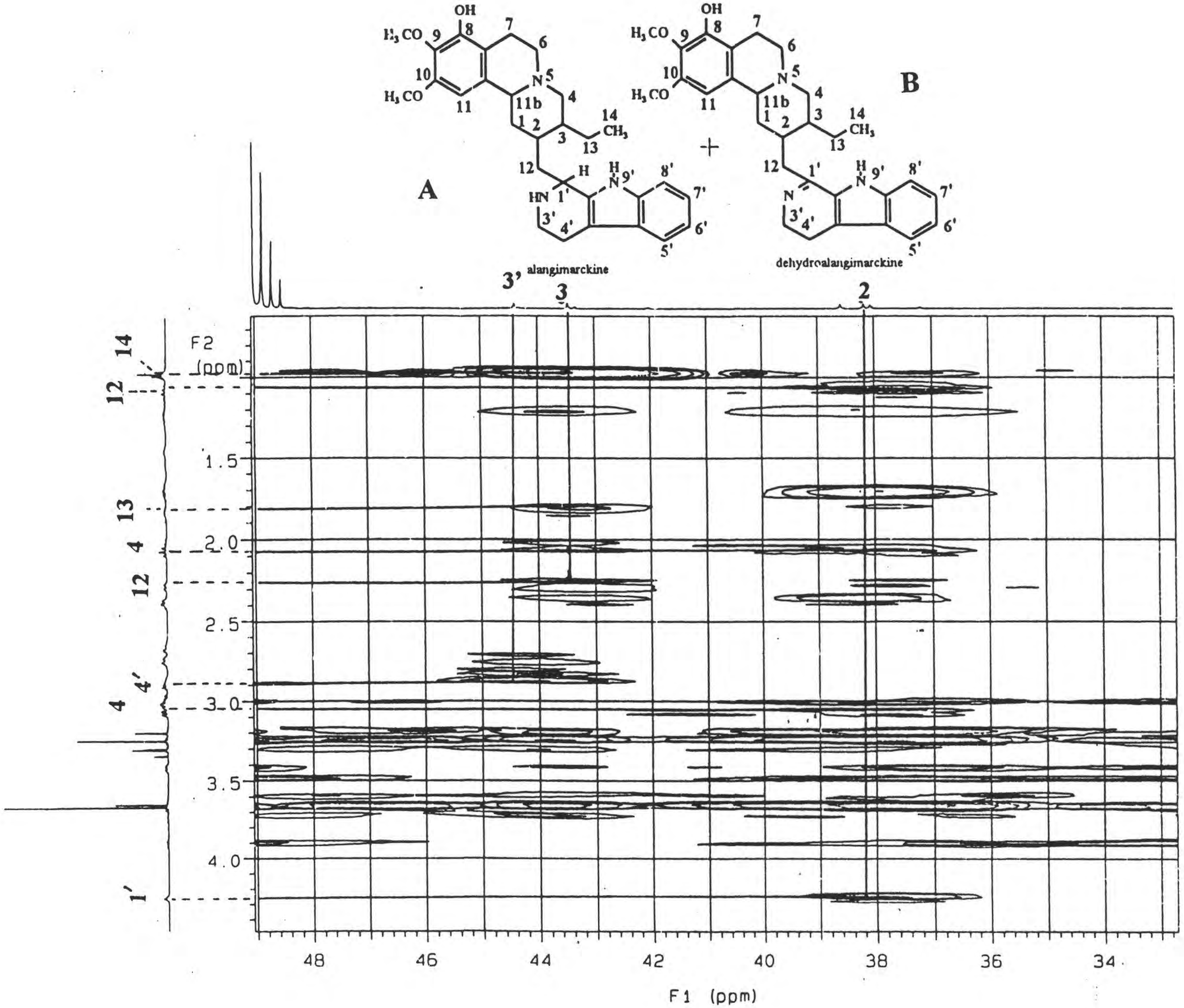


Figure 53 The expansion of 500 MHz HMBC spectrum of AL-1L (in DMSO)

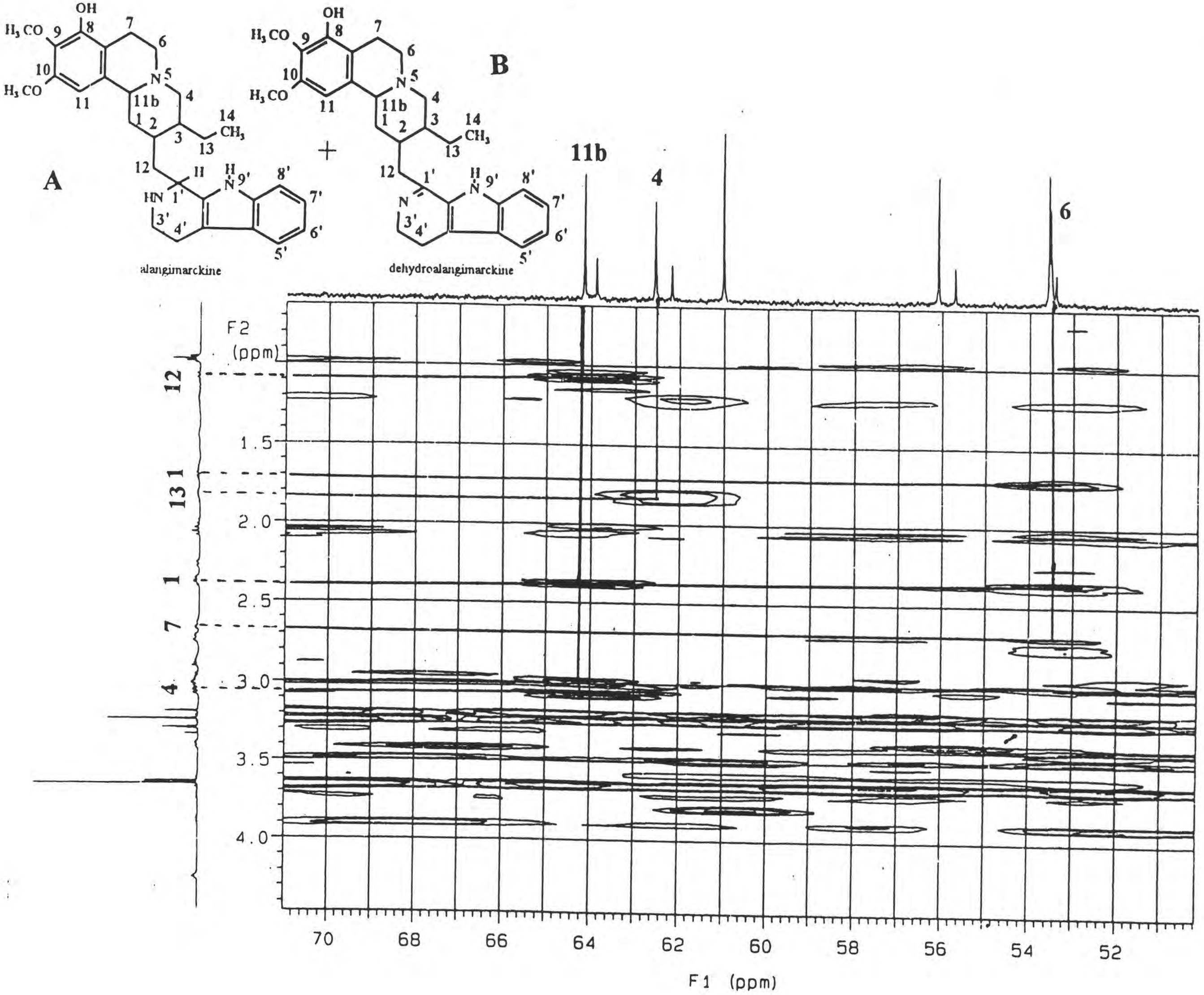


Figure 54 The expansion of 500 MHz HMBC spectrum of AL-1L (in DMSO)

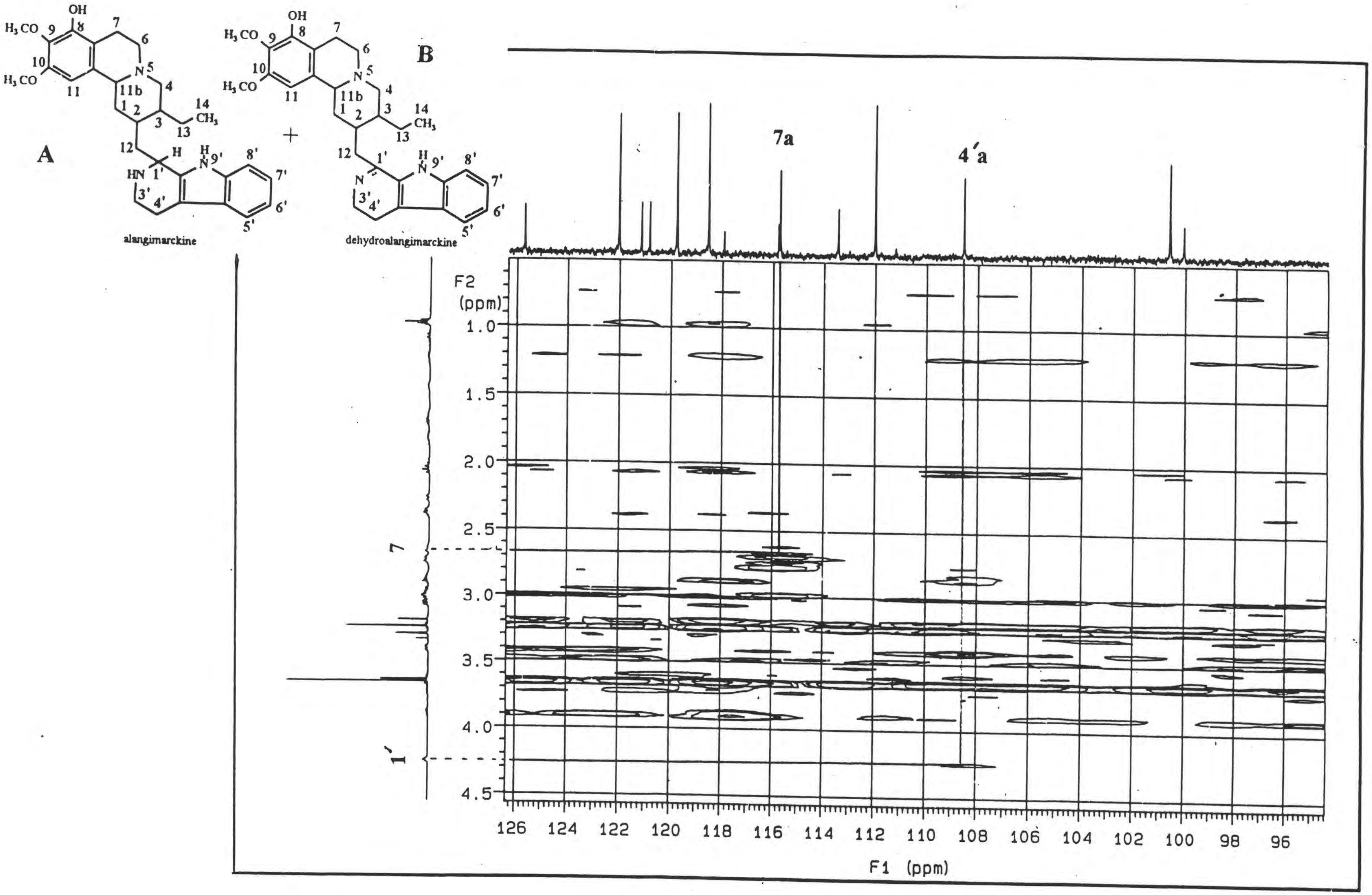


Figure 55 The expansion of 500 MHz HMBC spectrum of AL-1L (in DMSO)

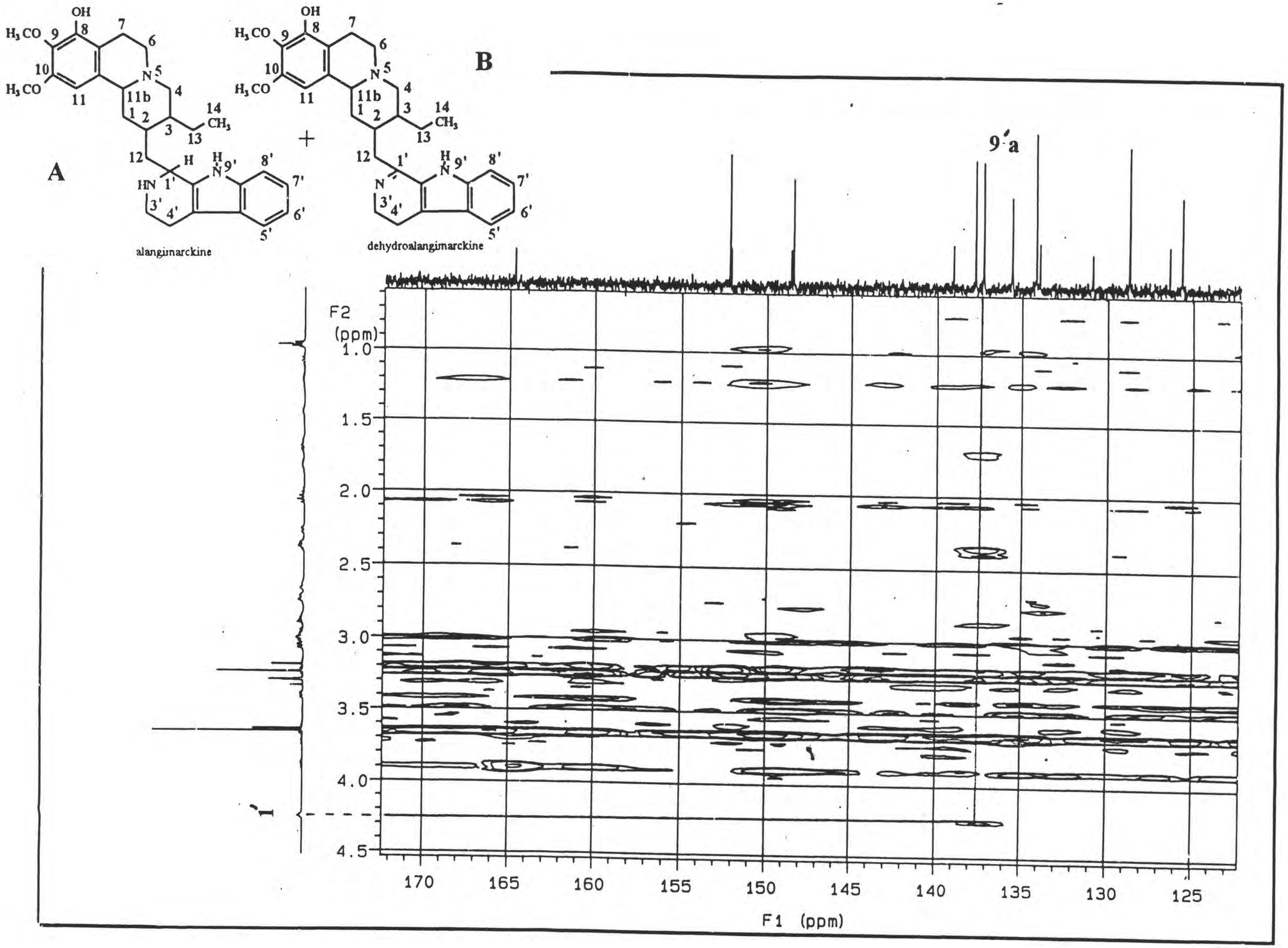


Figure 56 The expansion of 500 MHz HMBC spectrum of AL-1L (in DMSO)

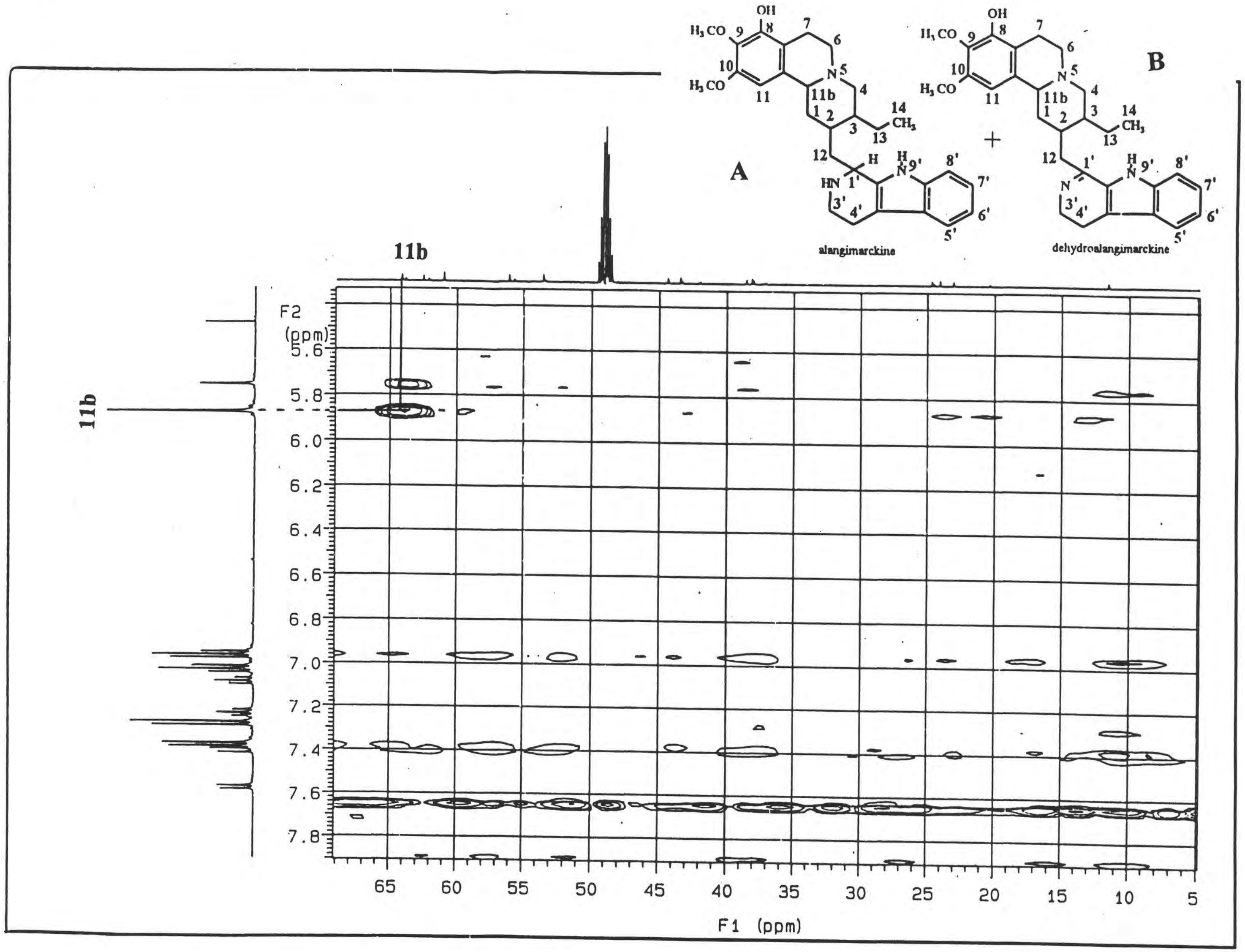


Figure 57 The expansion of 500 MHz HMBC spectrum of AL-1L (in DMSO)

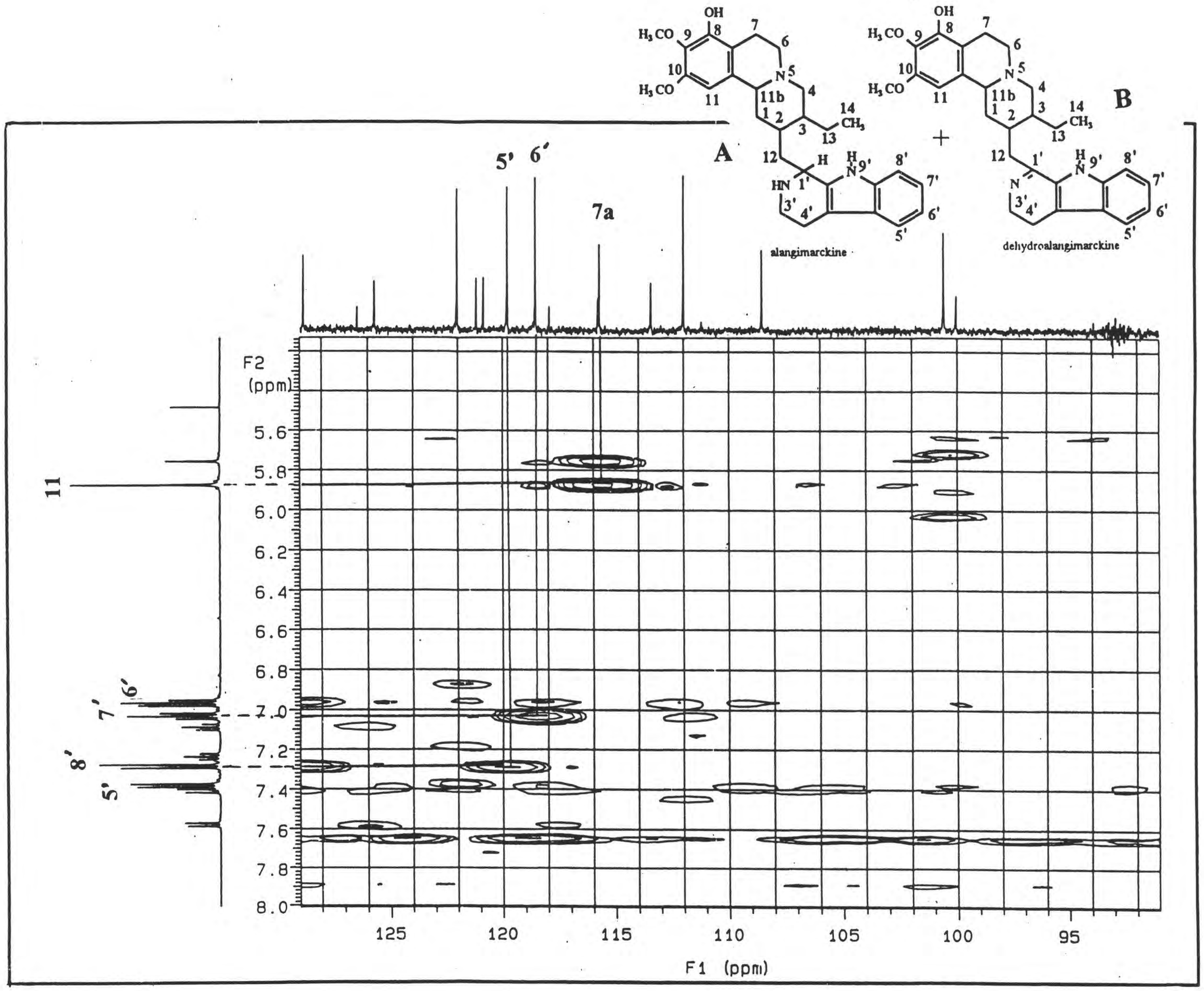


Figure 58 The expasion of 500 MHz HMBC spectrum of AL-1L (in DMSO)

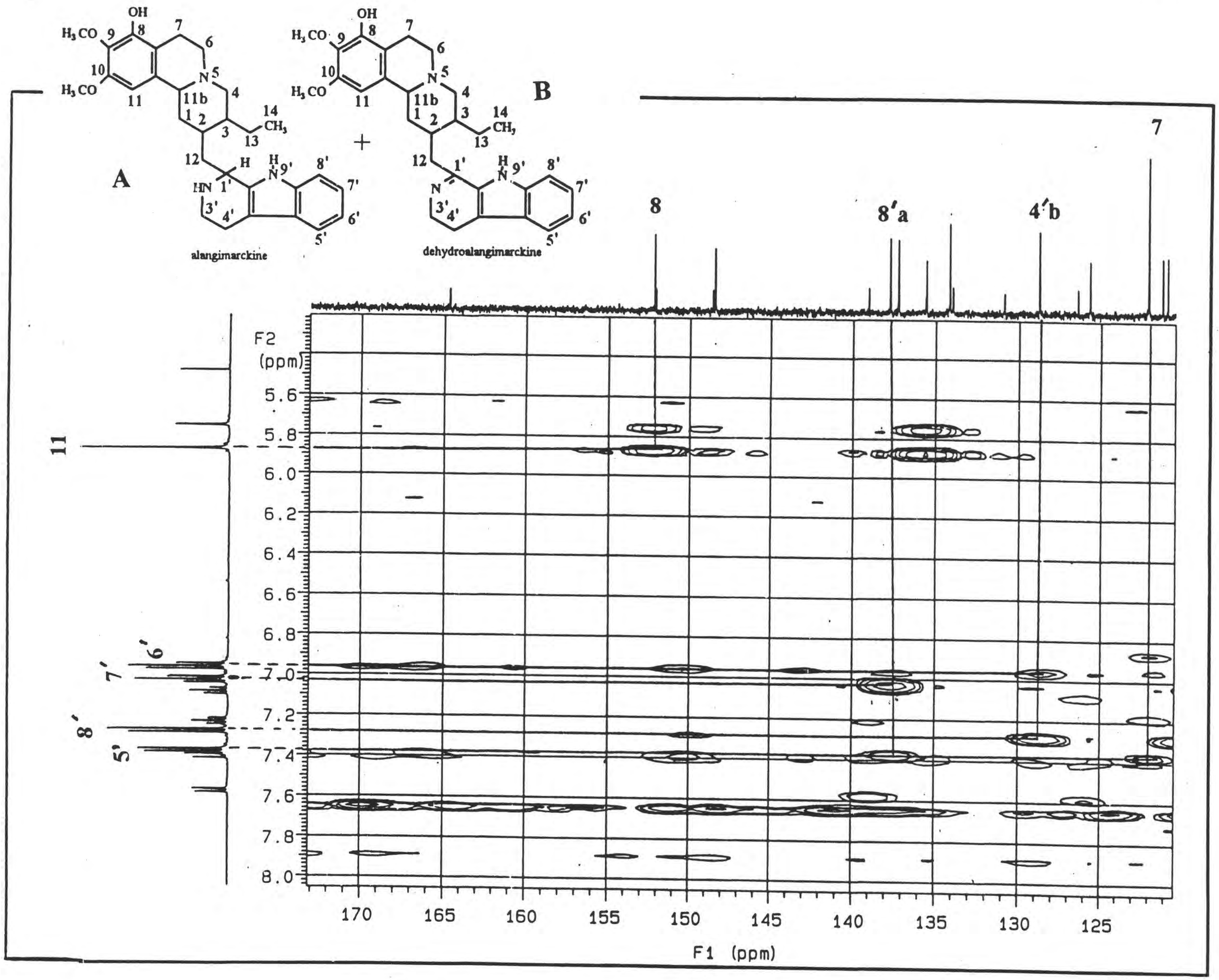


Figure 59 The expasion of 500 MHz HMBC spectrum of AL-1L (in DMSO)

The chemical shift of aromatic protons in AL-1L(B) could also be similarly assigned and the result was summarized in Table 9.

Table 9 Chemical shifts of aromatic protons of AL-1L(B).

position	chemical shift (ppm) (multiplicity, *J* Hz)	H-H correlation in H-H cosy spectra
11	5.75 (s)	-
5′	7.57 (dd, *J* =6.0, 0.8)	7′-H
6′	7.23 (td, *J* =6.0, 0.8)	8′-H
7′	7.08 (td, *J* =6.0, 0.8)	6′-H
8′	7.41 (dd, *J* =6.0, 0.8)	6′-H

Summary of proton assignments of AL-1L including correlations between protons and carbons by HMBC and HMQC spectra are shown in Table 10.

Table 10 Carbon and proton assignments of AL-1L and correlations between protons and carbons.

position	δC (ppm)	δH (ppm) (multiplicity, *J* Hz) (from C-H correlations in HMQC spectrum)	long-range correlation from H to C in HMBC spectrum
1	38.0	1.69 (m), 2.38 (m, *J* 9.2, 3.6)	C-11b, C-6
2	38.1	1.72 (m)	n.d.
3	43.4	1.45 (broad d, *J* 7.6 Hz)	n.d.
4	62.5	2.07 (q, *J* 9.2 Hz), 3.08 (dd, *J* 9.2, 3.2)	C-2, C-3, C-11b
6	53.5	2.38 (m, *J* 9.2, 3.6), 2.91 (m)	n.d.
7	24.1	2.67 (m), 2.74 (m)	C-6, C-7a
7a	115.7	-	-
8	152.2	-	-
9	135.5	-	-
10	148.6	-	-

Table 10 (Continued)

position	δC (ppm)	δH (ppm) (multiplicity, *J* Hz) (from C-H correlations in HMQC spectrum)	long-range correlation from H to C in HMBC spectrum
11	100.6	5.87 (s)	C-7a, C-11a, C-11b
11a	135.5	-	-
11b	64.1	3.00 (m)	n.d.
12	38.5	1.09 (m, *J* 10.4, 9.2), 2.28 (m, *J*10.4, 2.4)	C-2, C-3, C-11b
13	24.7	1.22 (m), 1.83 (m)	C-3, C-14, C-4
14	11.2	0.98 (t, *J* 5.6)	C-3, C-13
1′	53.5	4.25 (t, *J* 3.6, 4.4)	C-2, C-4′a, C-9′a
3′	44.8	2.96 (dd, *J* 0, 3.6) , 3.42 (ddd, *J* 10, 2.4)	C-4′
4′	23.0	2.74 (m), 2.87 (ddd, *J* 3.6)	C-3′
4′a	108.5	-	-
4′b	128.7	-	-
5′	118.5	7.38	C-7′, C8′a
6′	119.7	6.97	C-4′b
7′	122.0	7.03	C-6′, C8′a
8′	112.0	7.27	C-4′b
8′a	137.2	-	-
9′	-	-	-
9′a	137.7	-	-
9-OCH$_3$	61.0	3.66 (s)	-
10-OCH$_3$	56.1	3.24 (s)	-

n.d. = not determined