CHAPTER 3

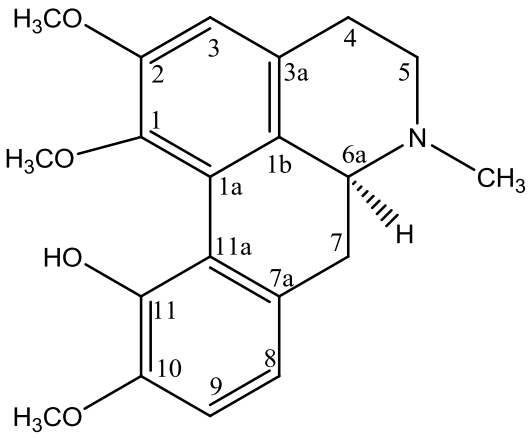
3.0 Introduction

The present work deals with the bioassay-guided isolation and structural elucidation of alkaloids from the leaves and bark of *Alseodaphne corneri* Kosterm. The extraction methods of alkaloids are explained in chapter 5.

3.1. Alkaloids from the Dichloromethane Extract of *Alseodaphne corneri*

Isolation and structural elucidation of alkaloids from the leaves and bark of this species yielded ten known and one new alkaloids. Four aporphine alkaloids isolated from the leaves were known as isocorydine **44**, norisocorydine **45**, *N*-methyl-laurotetanine **46** and *N*-methyl lindcarpine **47**. Purification of the alkaloids from the bark yielded two known apophine alkaloids namely laurotetanine **48** and norboldine **49**; together with five bisbenzylisoquinoline type namely gyrolidine **50**, norstephasubine **51**, 7'-*O*-demethylstebisimine **53,** stephasubimine **54** and also 3′, 4′-dihydronorstephasubine **52** which is a new bisbenzylisoquinoline alkaloid. The structural elucidation was established through several spectroscopic methods; UV, IR, MS, 1D (^{1}H-NMR and ^{13}C-NMR) and 2D-NMR (COSY, DEPT, HMQC, HMBC, NOESY) and also by comparison with literature data. The following sub-chapters discuss briefly the structural elucidation of the isolated compounds.

3.1.1 Isocorydine, 44



44

Alkaloid **44** was isolated as a brownish amorphous powder with $[\alpha]_D^{25}$+196° (c = 0.10,MeOH) indicating the absolute configuration at C-6a as *S* [69]. In the UV spectrum, maxima were observed at 270 and 310 nm which indicated the existence of the conjugated system. Its IR spectrum revealed absorption band due to the conjugated hydroxyl group at about 3202 cm^{-1}.

Its molecular formula was assigned as $C_{20}H_{24}O_4N$ on the basis of HREIMS spectrum, which showed a molecular ion peak at m/z 342.1720, $[M+H]^+$ (calcd. 342.1705.)

^{1}H-NMR spectrum showed the presence of three methoxy groups as a singlet at δ 3.89, 3.88 and 3.68 corresponding to 10-OMe, 2-OMe, 1-OMe respectively. The C-1 methoxy signal was rather shielded compared to the other two aromatic methoxyls since the protons of the former methoxy were forced to place themselves on top of ring A where the electron density is high.

H-3 was observed as a singlet at δ 6.68, indicating that C-1 and C-2 in ring A are substituted with methoxyl groups. In addition, vicinal protons H-8 and H-9 signals

appeared as a doublet at δ 6.81 and 6.84 with a coupling constant of 8.32 Hz. In the aliphatic region, H-5 appeared at deshielding area (δ 2.84, 3.27) compared to H-4 (δ 2.41) due to the withdrawing effect of the neighboring electronegative *N*-atom. A singlet signal was observed at δ 2.50 indicating the presence of *N*-Me group in ring B.

The COSY spectrum showed cross peaks between CH_2-5/ CH_2-4, and CH_2-7/CH-6a, as shown in Figure 3.03.

The ^{13}C- NMR spectrum of Alkaloid **44** showed 20 carbon signals. The DEPT 135 spectrum showed four methyls, three methylenes, four methines carbon signals. Three signals of methoxyl groups were observed at δ 62.8, 56.1 and 55.8. The signals for quaternary carbons appeared at δ 142.8, 151.4, 149.5 and 144.0 which, could be assigned to C-1, C-2, C-10 and C-11, bearing the methoxyl and hydroxyl groups.

The HMBC spectrum Figure 3.07 showed significant cross-peaks of H-9 to C-11 which bearing a hydroxyl group. The correlations of protons 10-OMe and H-8 to C-10 were also seen in the HMBC spectrum, further supported the assignment of protons and carbons as shown in Table 3.1

From the analysis of the spectroscopic data obtained and comparison with literature values, alkaloid **44** was identified as isocorydine.[69-71]

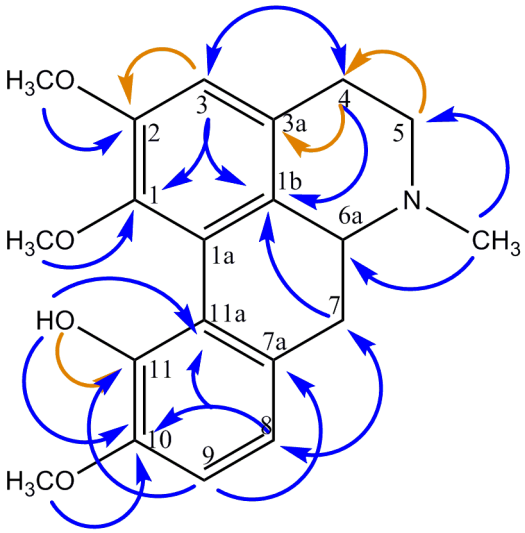
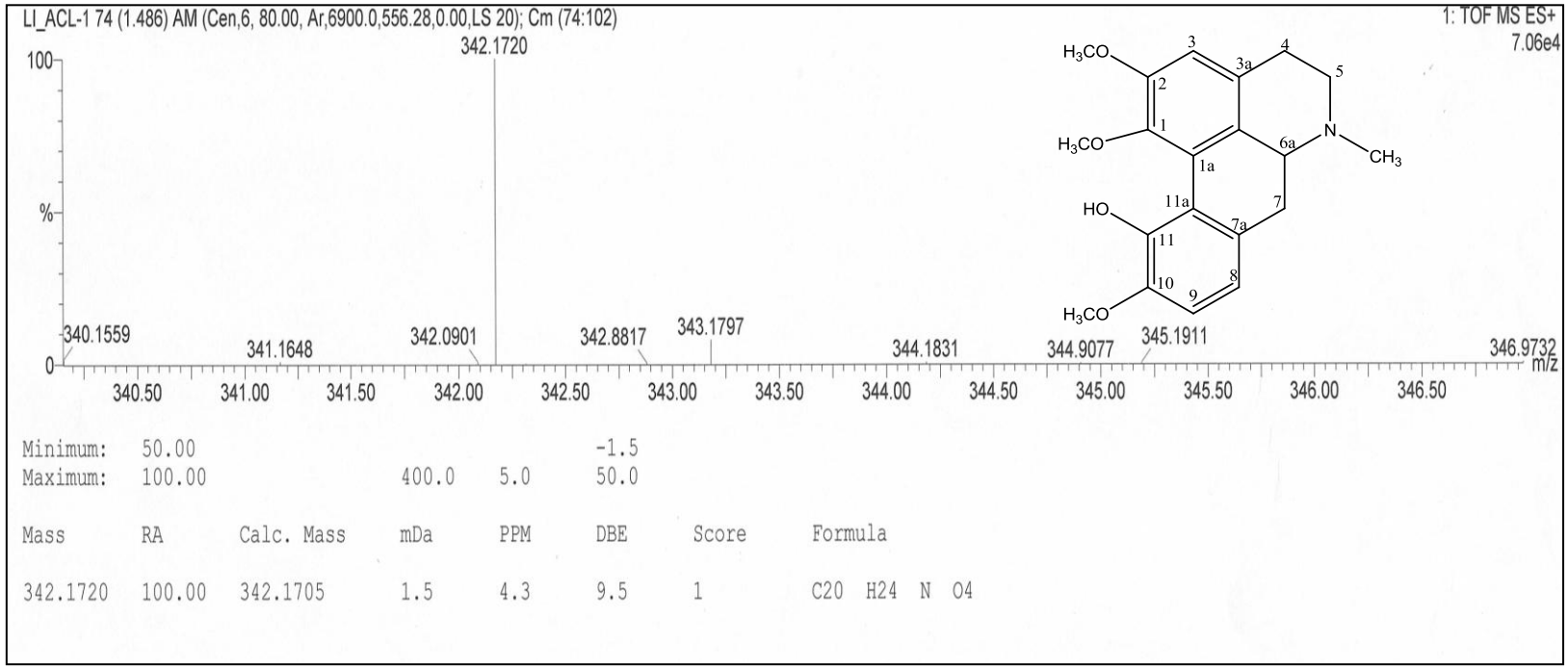


Figure 3.00: ^{1}H-^{13}C correlations observed in HMBC spectrum of Alkaloid **44**

Table 3.1: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **44**

Position	^{1}H- NMR δ (Hz)	^{13}C- NMR (δ)	HMBC
1		142.1	
1a		125.9	
1b		129.1	
2		151.2	
3	6.68 s	110.9	4,2,1,1b
3a		129.9	
4	2.41 m	29.3	1b,3a,3,5
5	α 2.84 dd (1.9,12.9)	52.7	4
	β 3.27 m		
6a	3.23 m	62.8	3a
7	α 2.60 dd (3.1,16.4)	35.8	1b,8
	β 3.05 m		
7a		130.1	
8	6.81 d (8.3)	118.9	7,11a,10
9	6.84 d (8.0)	111.1	7a,11
10		149.4	
11		143.9	
11a		120.1	
1-OMe	3.68 s	62.0	1,2-OMe
2-OMe	3.88 s	55.8	2,1-OMe
10-OMe	3.89 s	56.1	10
N-Me	2.50 s	43.8	5,6a



Minimum:	50.00				-1.5		
Maximum:	100.00		400.0	5.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	Score	Formula
342.1720	100.00	342.1705	1.5	4.3	9.5	1	C20 H24 N O4

Figure 3.01: HREIMS Spectrum of Alkaloid **44**

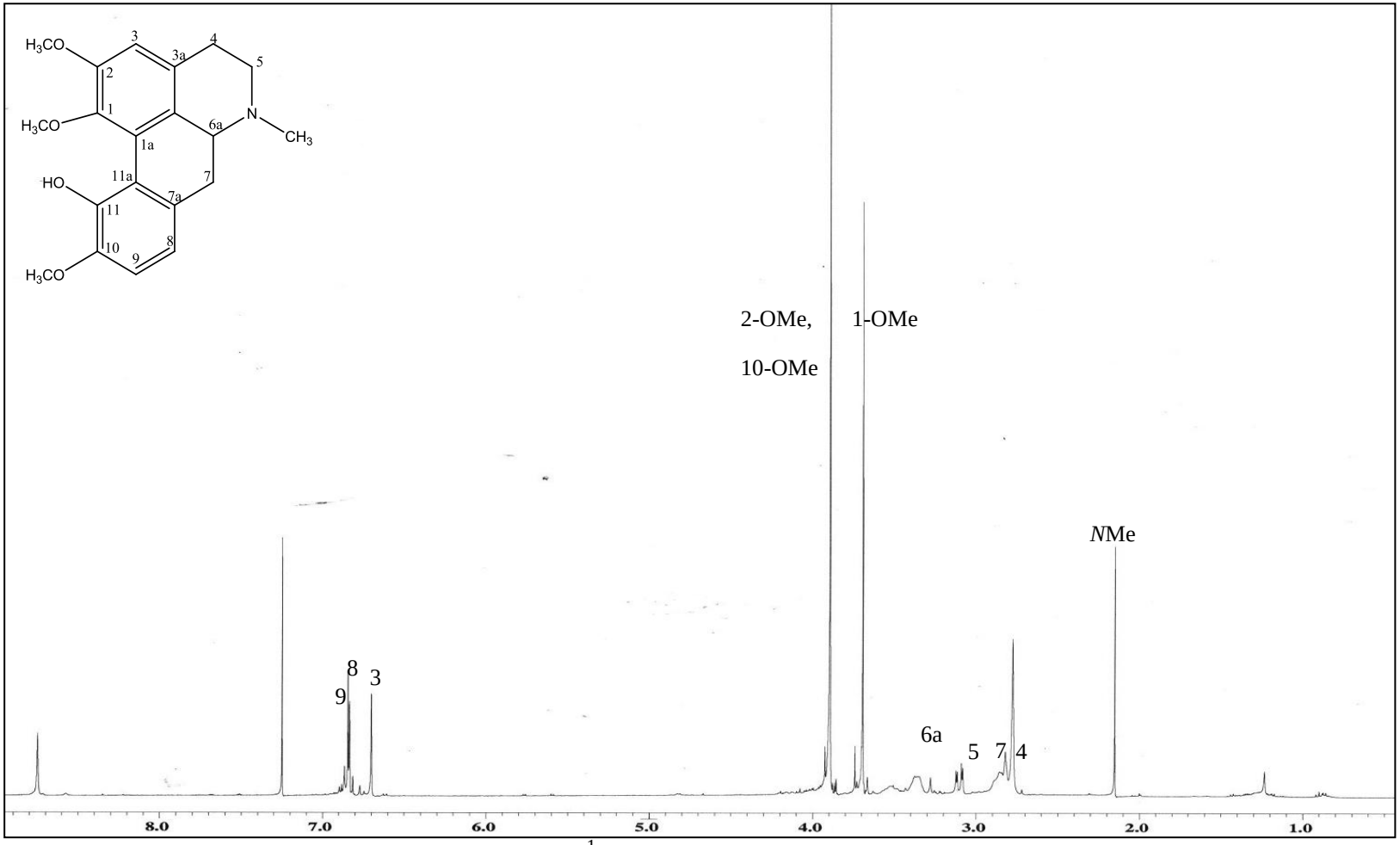


Figure 3.02: ^{1}H NMR Spectrum of Alkaloid **44**

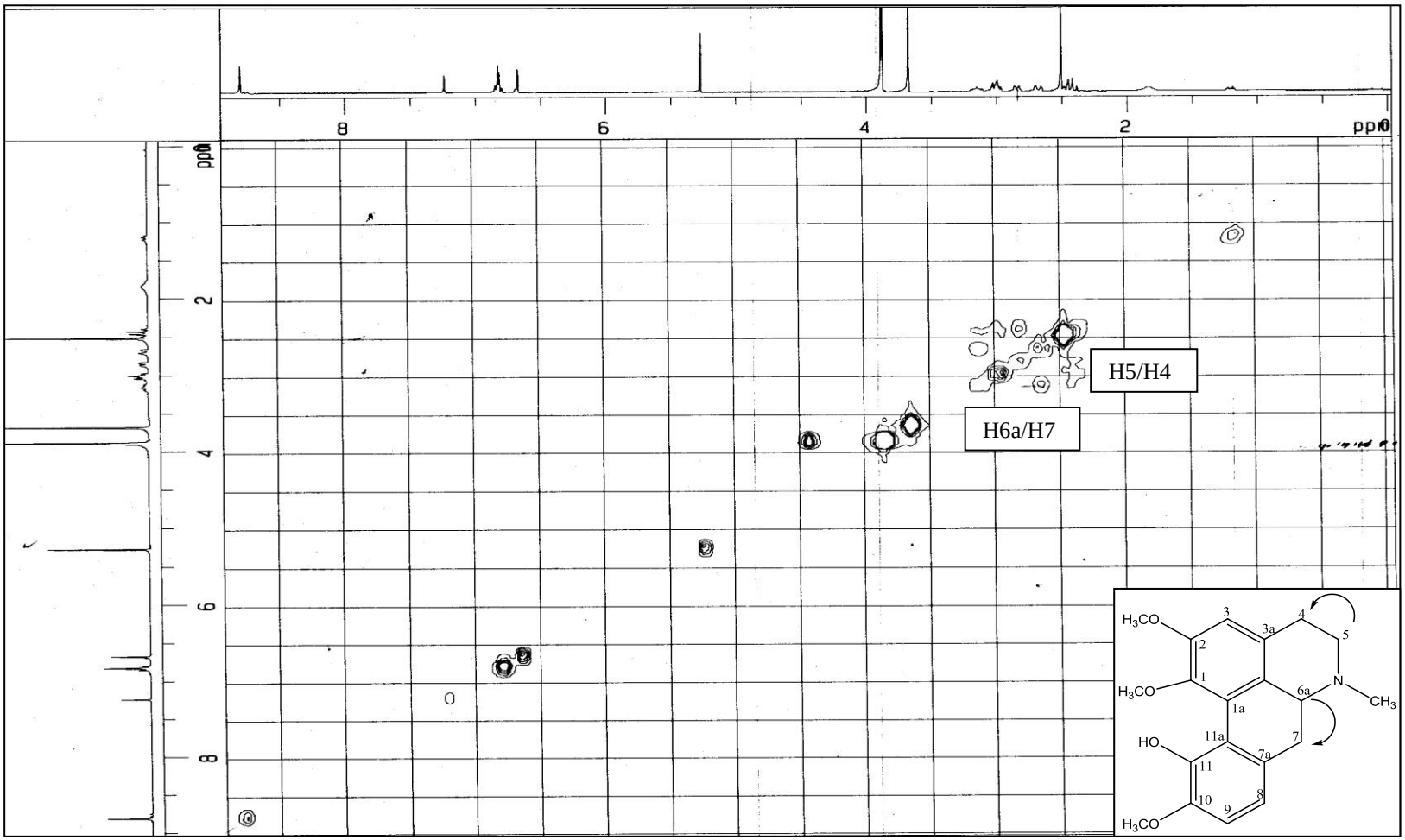


Figure 3.03: COSY Spectrum of Alkaloid **44**

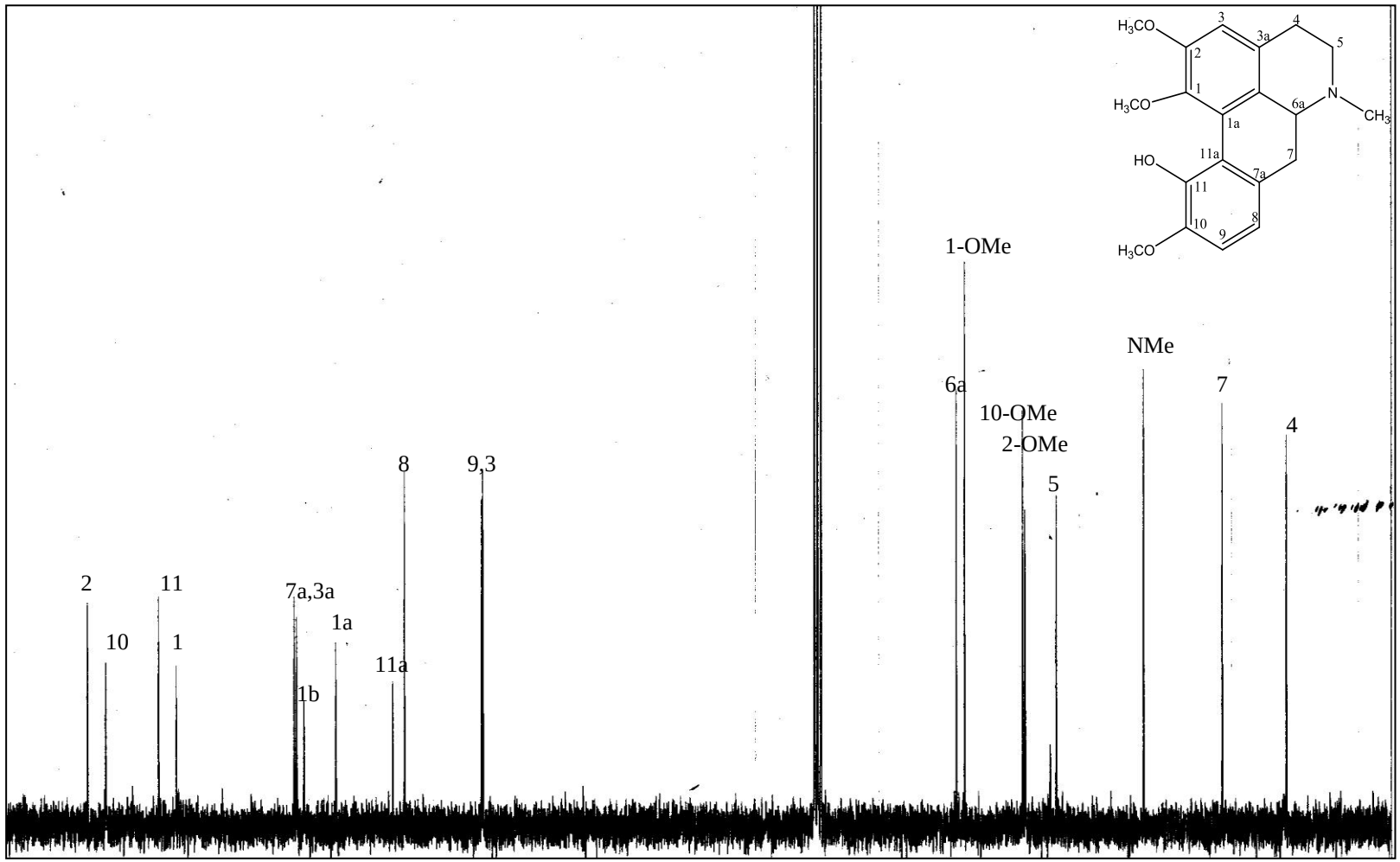


Figure 3.04: ^{13}C NMR Spectrum of Alkaloid **44**

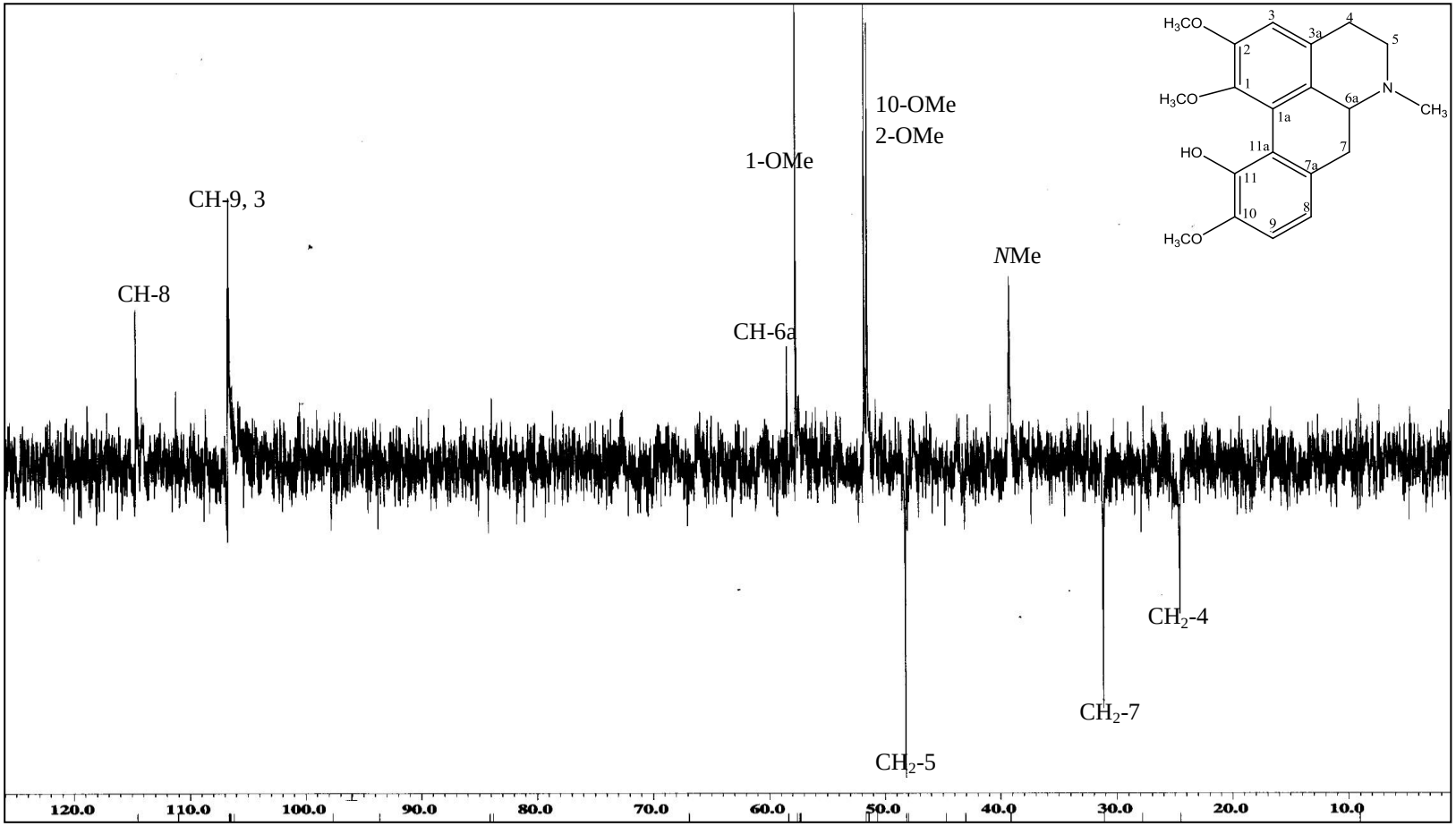


Figure 3.05: DEPT Spectrum of Alkaloid **44**

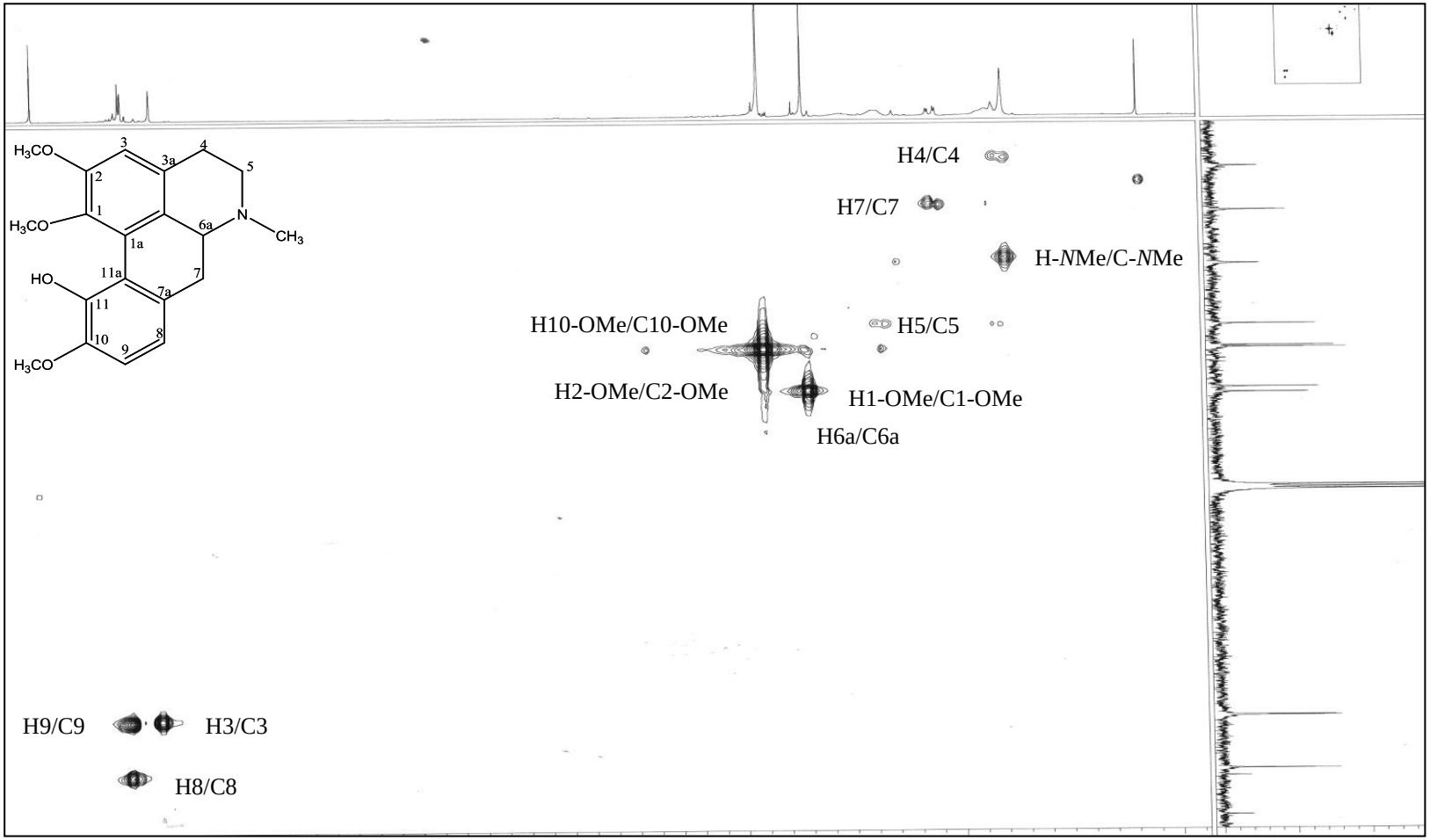


Figure 3.06: HMQC Spectrum of Alkaloid **44**

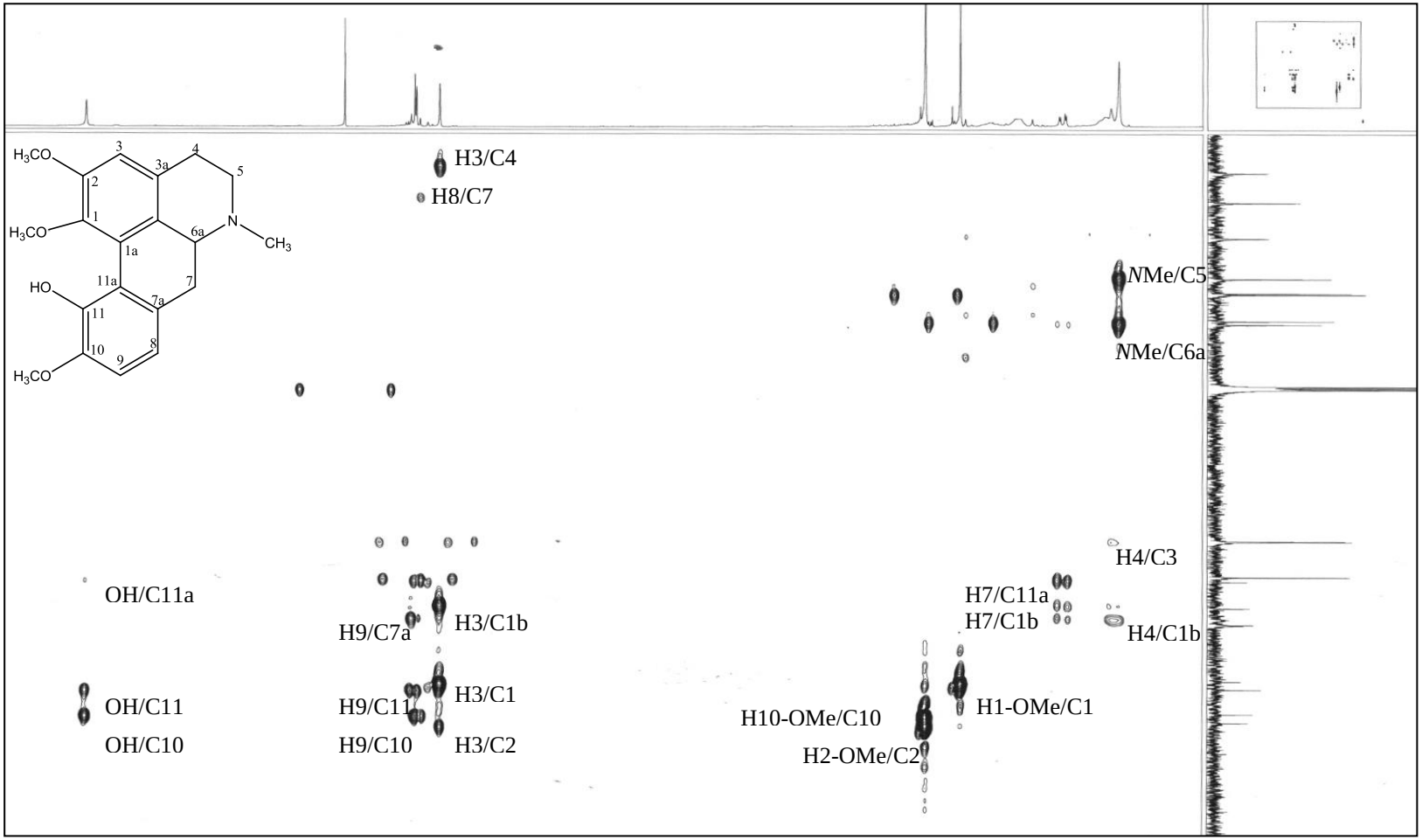
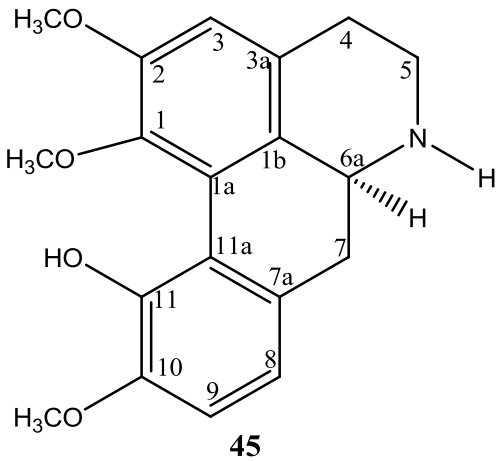


Figure 3.07: HMBC Spectrum of Alkaloid **44**

3.1.2 Norisocorydine, 45



45

Alkaloid **45** was isolated as a brownish amorphous powder with $[\alpha]_D^{25}$+158.5°,(c = 0.10, EtOH) indicated the absolute configuration at C-6a was *S* as in the known alkaloids. [70-72] HRESIMS spectrum revealed a pseudomolecular peak at m/z 328.1538 (calc. 328.1549), corresponding to the molecular formula of $C_{19}H_{22}NO_4$.

The UV spectrum supported a 1,2,10,11-tetrasubstituted aporphine type, with absorptions at λ_{max} 270, 310 nm [26, 73]. In addition, the IR spectrum gave a broad band between 3500 and 2936 cm^{-1} due to the presence of OH and NH groups.

Similar features were observed on ^{1}H NMR and ^{13}C NMR spectra of alkaloid **44** and **45**, which confirmed a close structural relationship between both compounds. However, there was an obvious difference in the ^{1}H NMR spectrum of alkaloid **45**, the *N*-Me signal at δ 2.00-2.50 which was apparent in alkaloid **44** but was absent in alkaloid **45**.

An analysis of COSY spectrum revealed the presence of methylene protons at position C4 (δ 2.70, 3.00) and C5 (δ2.92, 3.42). In the NOESY spectrum, the cross-peaks were observed between H-3/ 2-OMe, H-9/10-OMe thus, confirming the assignment of 2-OMe and 9-OMe position as shown in structure **45**. Other correlations are shown in Figure 3.11.

In the HMBC spectrum, the methylene protons and the methine protons gave cross-peaks with a quaternary carbons at δ 130.4 (C-3a) and δ 129.9 (C-1b). With this observation, the exact position of quaternary carbons could be determined.

The ^{13}C- NMR and DEPT spectrum for Alkaloid **45** showed 19 carbon signals. The DEPT spectrum showed three methyls, three methylenes, four methines and nine quaternary carbon signals. It showed three signals of methoxy groups which were observed at δ 62.2, 56.2 and 56.0. The signals for quaternary carbons appeared at δ 142.1, 151.4, 149.5 and 144.2 which, could be assigned to C-1, C-2, C-10 and C-11.

From the analysis of the spectroscopic data obtained and comparison with literature values, alkaloid **45** is identified as norisocorydine.[70-72]

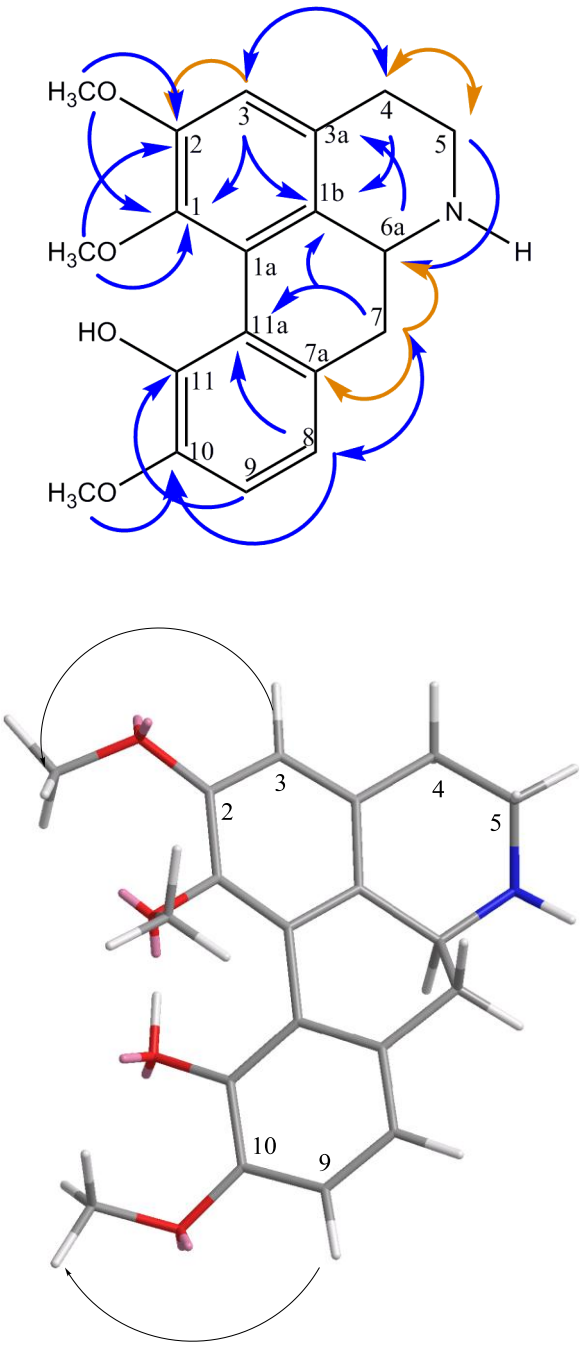


Figure 3.08: HMBC and NOESY Correlation of Alkaloid **45**

Table 3.2: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **45**

Position	^{1}H- NMR δ (Hz)	^{13}C- NMR (δ)	HMBC
1		142.1	
1a		125.8	
1b		129.9	
2		151.4	
3	6.69 s	111.7	1,1b,2,4
3a		130.4	
4	α 2.70 m	29.4	1b,3,5
	β 3.00 m		
5	α 2.92 m	42.8	4, 6a
	β 3.42 m		
6a	3.66 m	54.1	3a
7	α 2.56 t (13.3)	38.4	1b, 6a,7a,8,11a
	β 2.76 dd (4.1,13.3)		
7a		130.5	
8	6.79 d (8.0)	119.0	7,11a
9	6.83 d (8.2)	110.9	11
10		149.5	
11		144.2	
11a		120.2	
1-OMe	3.69 s	62.2	1,2-OMe
10-OMe	3.89 s	56.2	10
2-OMe	3.89 s	56.0	2,1-OMe

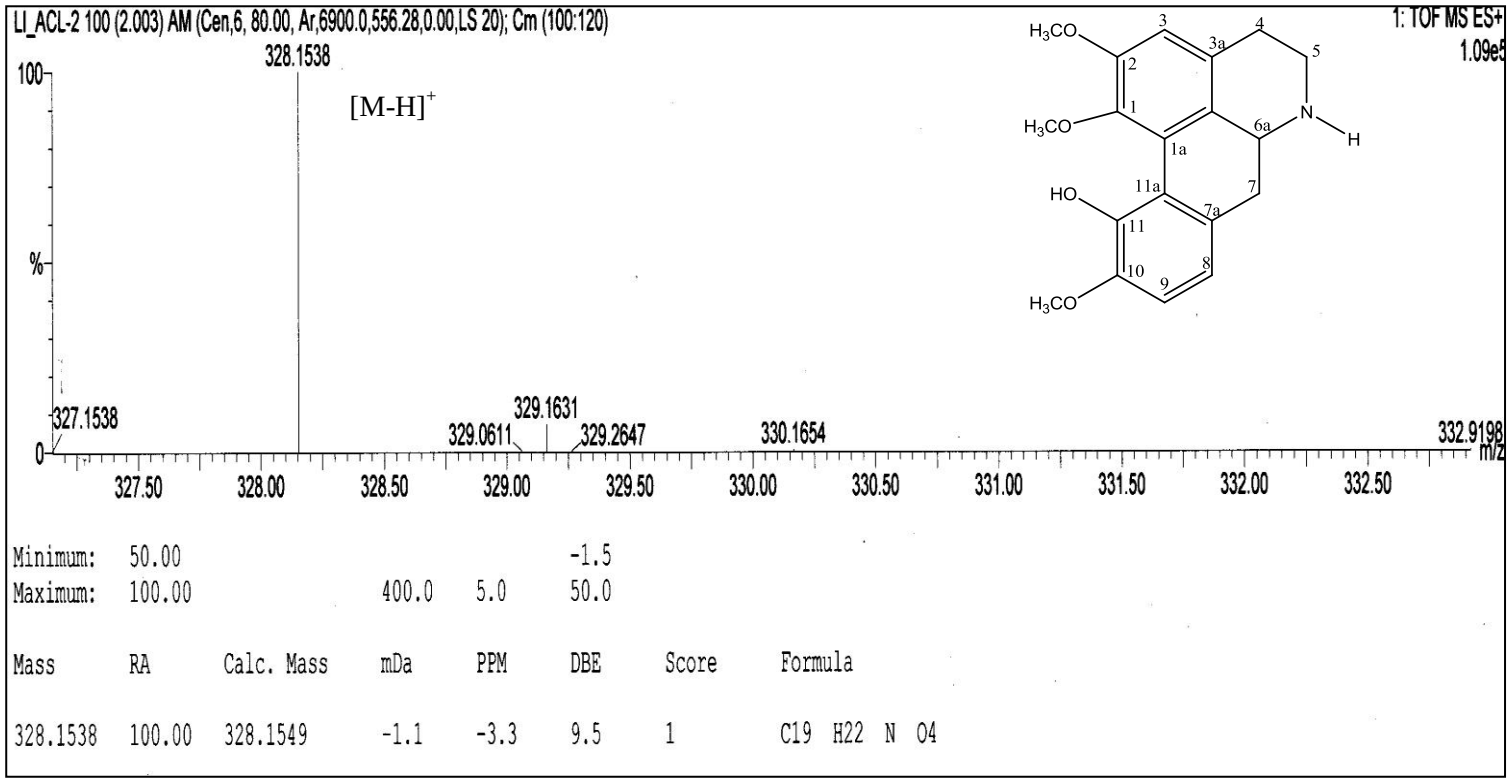


Figure 3.09: HREIMS Spectrum of Alkaloid **45**

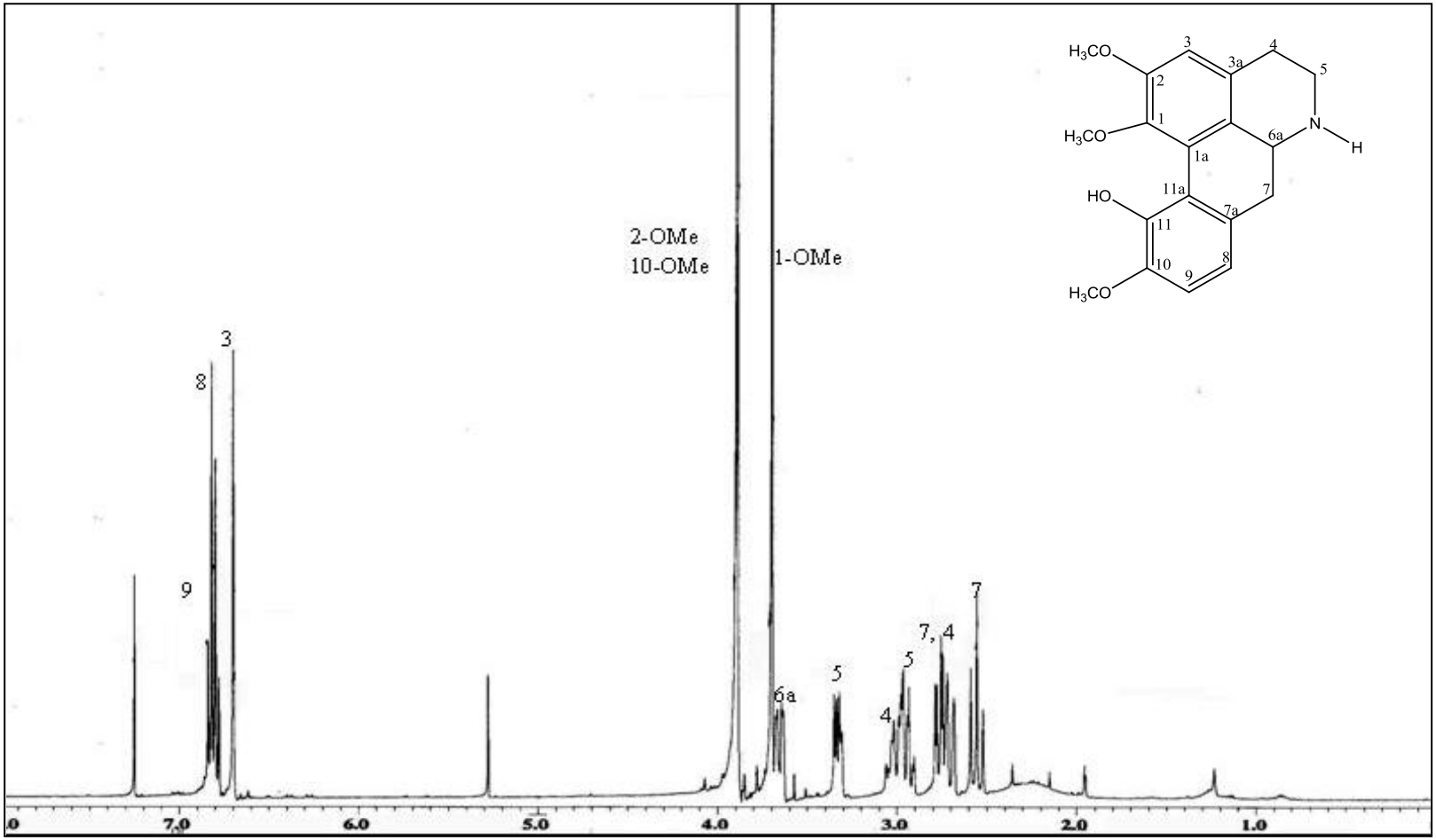


Figure 3.10: ^{1}H-NMR Spectrum of Alkaloid **45**

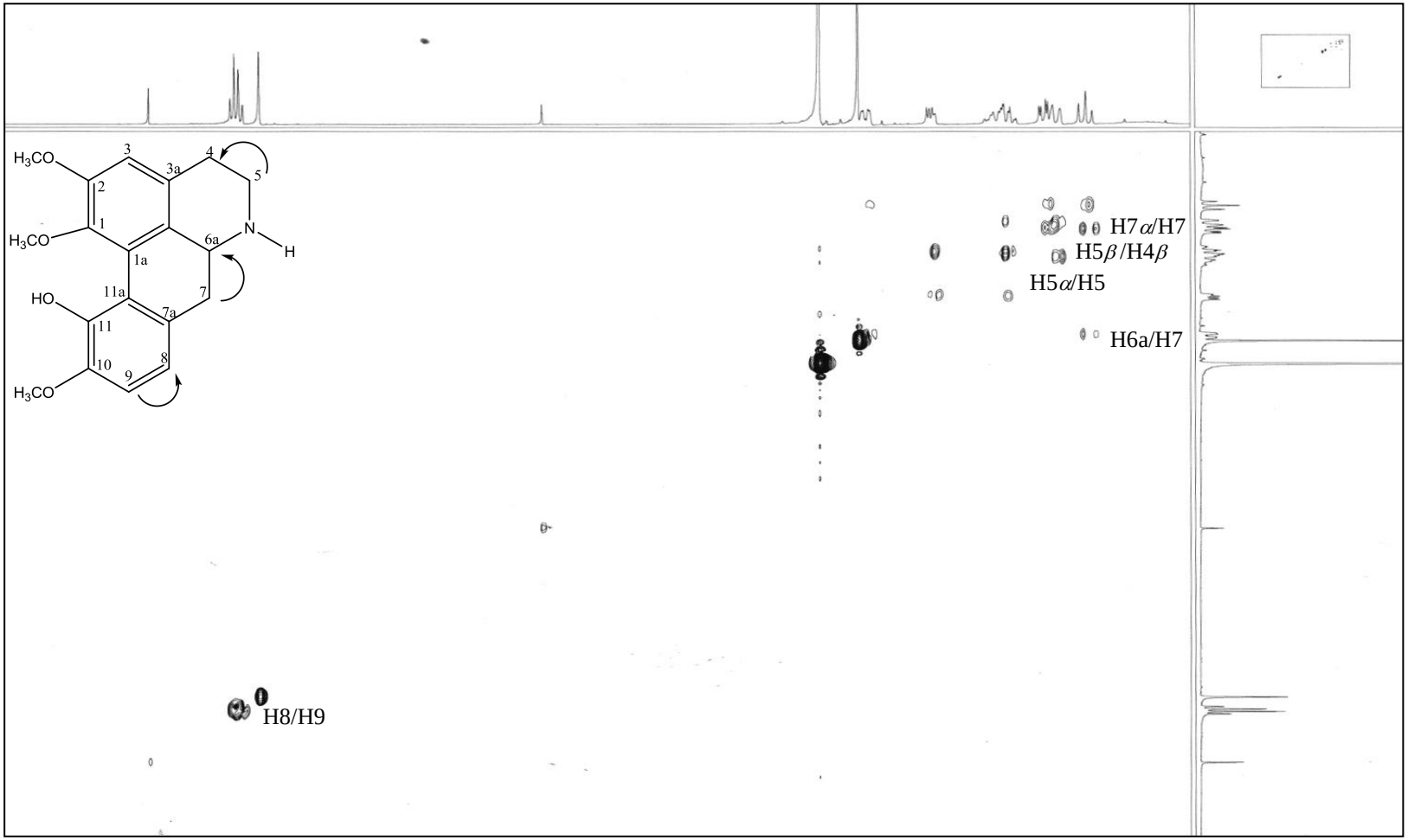


Figure 3.11: COSY Spectrum of Alkaloid **45**

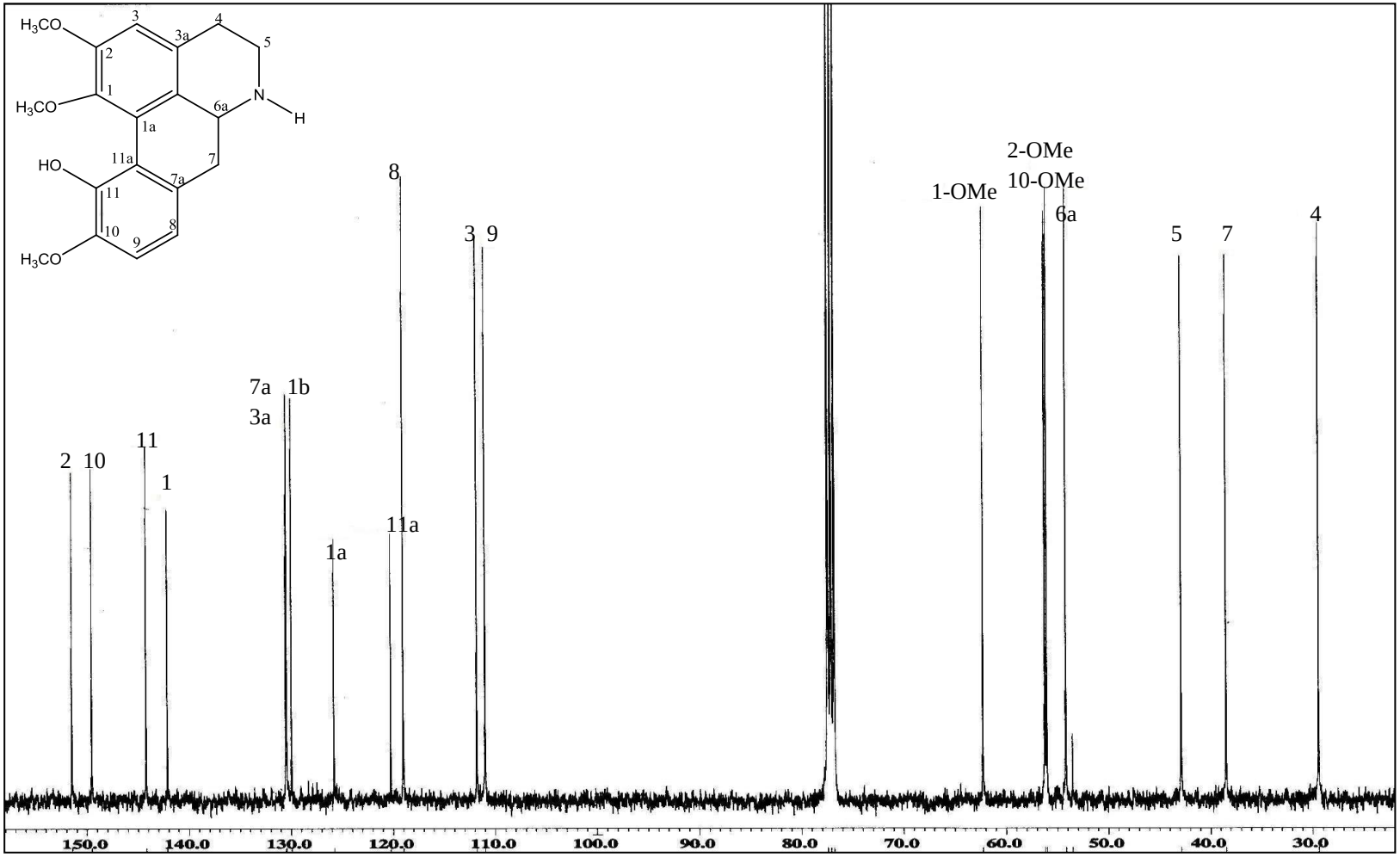


Figure 3.12: ^{13}C NMR Spectrum of Alkaloid **45**

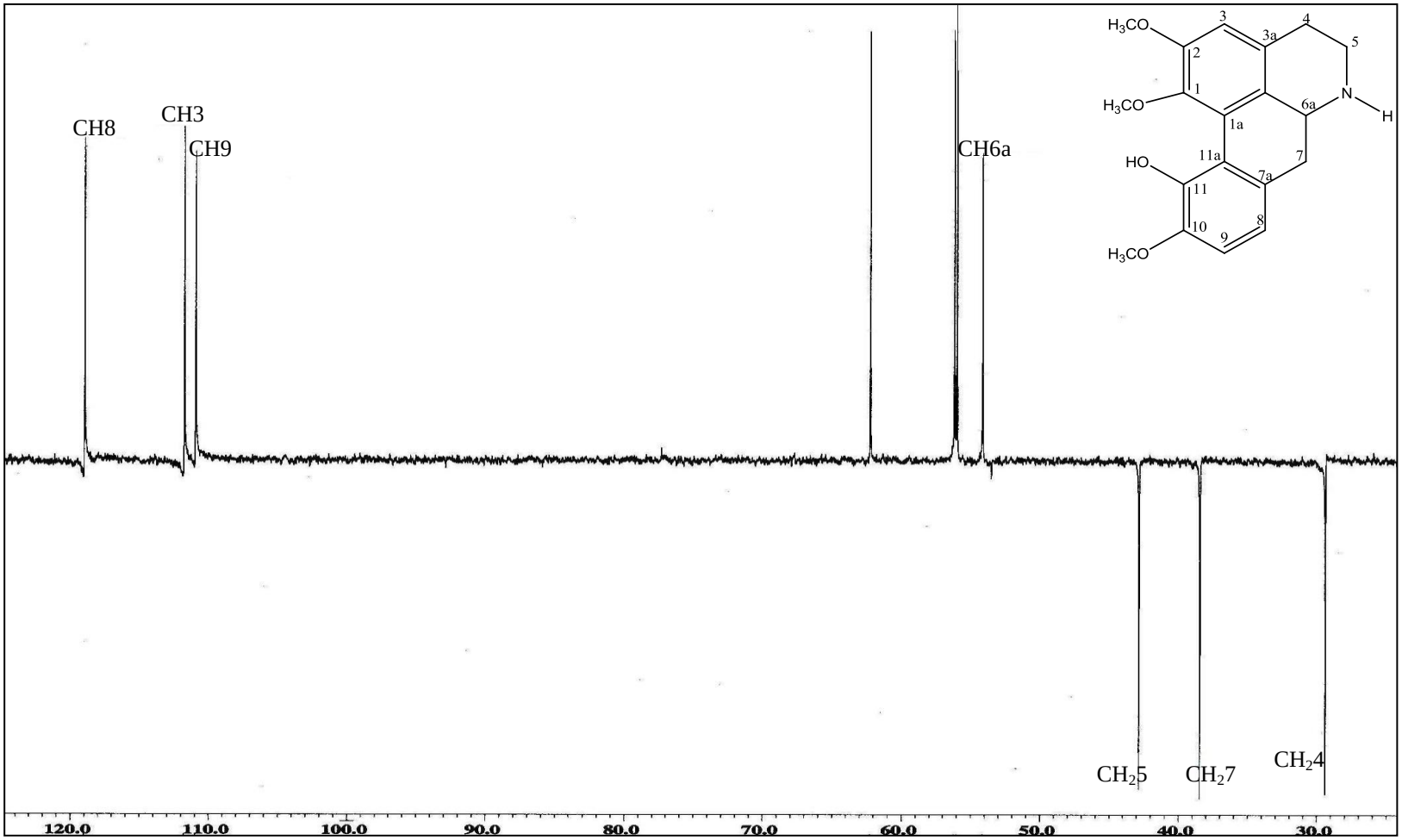


Figure 3.13: DEPT Spectrum of Alkaloid **45**

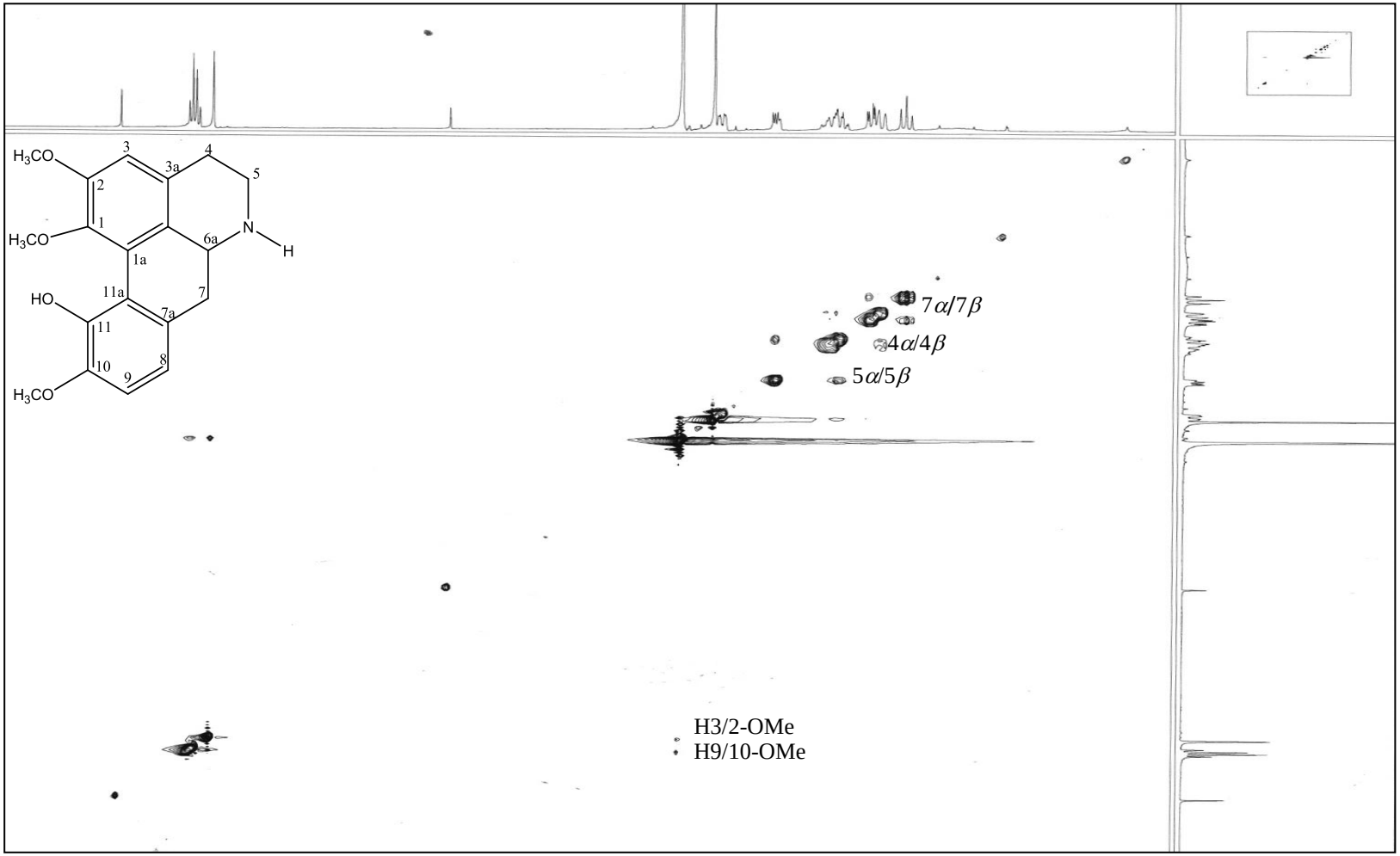


Figure 3.14: NOESY Spectrum of Alkaloid **45**

Figure 3.15: HSQC Spectrum of Alkaloid **45**

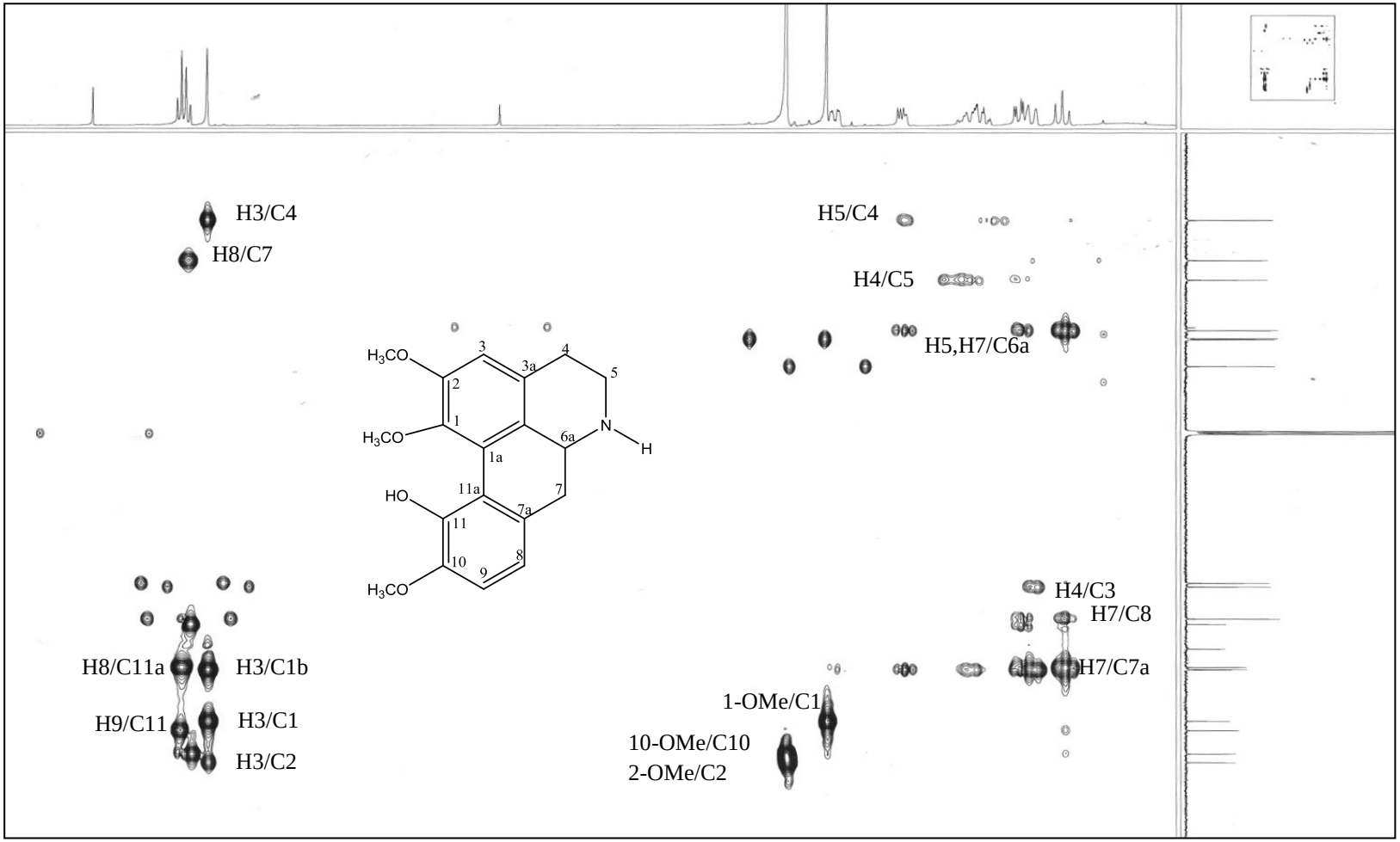
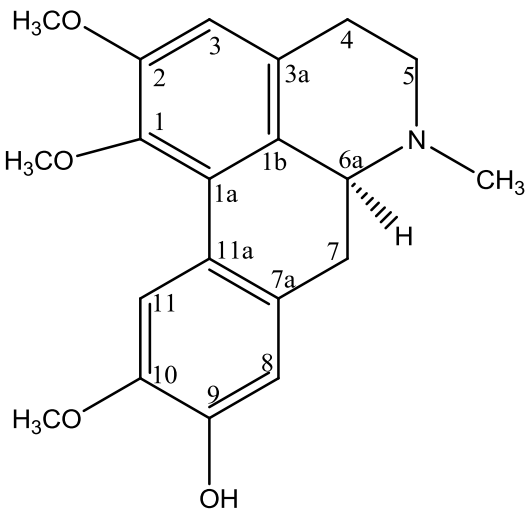


Figure 3.16: HMBC Spectrum of Alkaloid **45**

3.1.3 *N*-Methyl laurotetanine, 46



46

Alkaloid **46** was obtained as a pale brownish amorphous solid with $[\alpha]_D^{25}$+111° (c= 0.86, MeOH) indicating the absolute configuration at C-6a as *S*.[70,74-76] The UV spectrum showed maxima absorptions at 215, 283 and 305 nm, indicating this base as an aporphine substituted at position 1,2,9,10[36, 77]. The IR spectrum showed absorption peak at 3390 cm^{-1} indicating the presence of hydroxyl group in the structure. Its molecular formula was established as $C_{20}H_{24}NO_4$ by HREIMS at m/z 342.17 $[M+H]^+$ (calcd. 342.1705).

The ^1H NMR spectrum of Alkaloid **46** exhibited three methoxyl singlets at δ 3.63, 3.87 and 3.90. The singlet at δ 3.63 is assigned to the methoxyl at C-1 since the protons were shielded by the anistropic effect caused by ring D. Two singlets at δ 6.57 and 6.87 were observed in the spectrum assignable to H-3 and H-8 confirming that the adjacent carbons were substituted. A downfield singlet of H-11 at δ 8.09 suggested that C-10 could be substituted by a methoxy group. The aliphatic protons gave a multiplet between δ 3.20-2.50.

The ^{13}C NMR spectrum established the presence of 20 carbons. The DEPT experiment showed four methyl, three methylenes, four methines and nine quaternary carbon signals in the molecule.

The actual distribution of the OH and OCH_3 substituents was determined by using HMBC and NOESY correlations, thus proving that the oxygenation pattern for the ring A was 1,2-methoxys and for ring D was 9-methoxyl-10-hydoxyl aporphine as shown in figure 3.17.

Comparison of the spectroscopic data from literature, confirmed that the alkaloid **46** was *N*-methyl laurotetanine. [70,74-76]

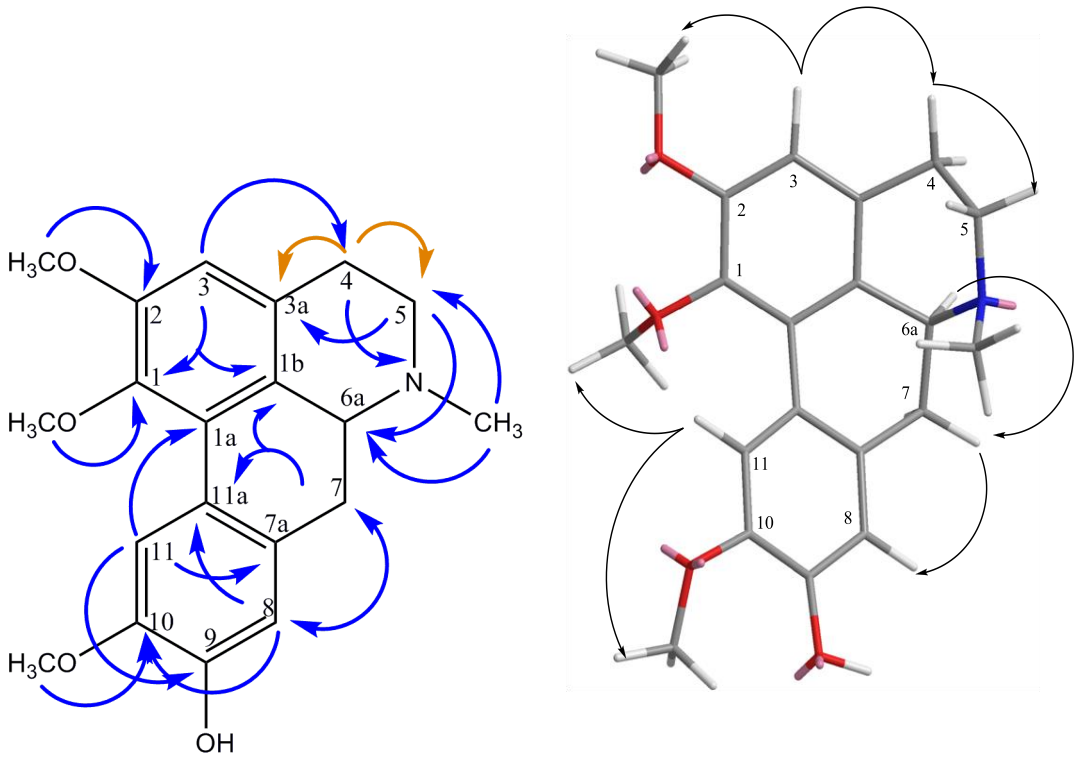


Figure 3.17: HMBC and NOESY Correlations of Alkaloid **46**

Table 3.3: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **46.**

Position	^{1}H- NMR δ (Hz)	^{13}C- NMR (δ)	HMBC
1		144.2	
1a		127.1	
1b		128.9	
2		151.9	
3	6.57s	110.3	1,2,4,1b
3a		127.2	
4	α 2.66 (dd,4.4,16.0)	29.2	1b,5,3a
	β 3.15(m)		
5	α 2.48 (d,3.9)	53.3	3a,6a,1b
	β 3.06 (dd,6.4,12.4)		
6a	2.99 m	62.6	1b, 7
7	α 2.51(d,3.6)	34.3	11a,8,1b,7a,6a
	β 2.95 (d,4.1)		
7a		130.2	
8	6.87 s	113.9	11a,10,7
9		144.8	
10		145.3	
11	8.09 s	111.2	9,7a,1a,11a
11a		124.0	
1-OMe	3.63 s	60.2	1
2-OMe	3.87 s	55.8	2
10-OMe	3.90 s	56.1	10
N-Me	2.54 s	44.0	6a

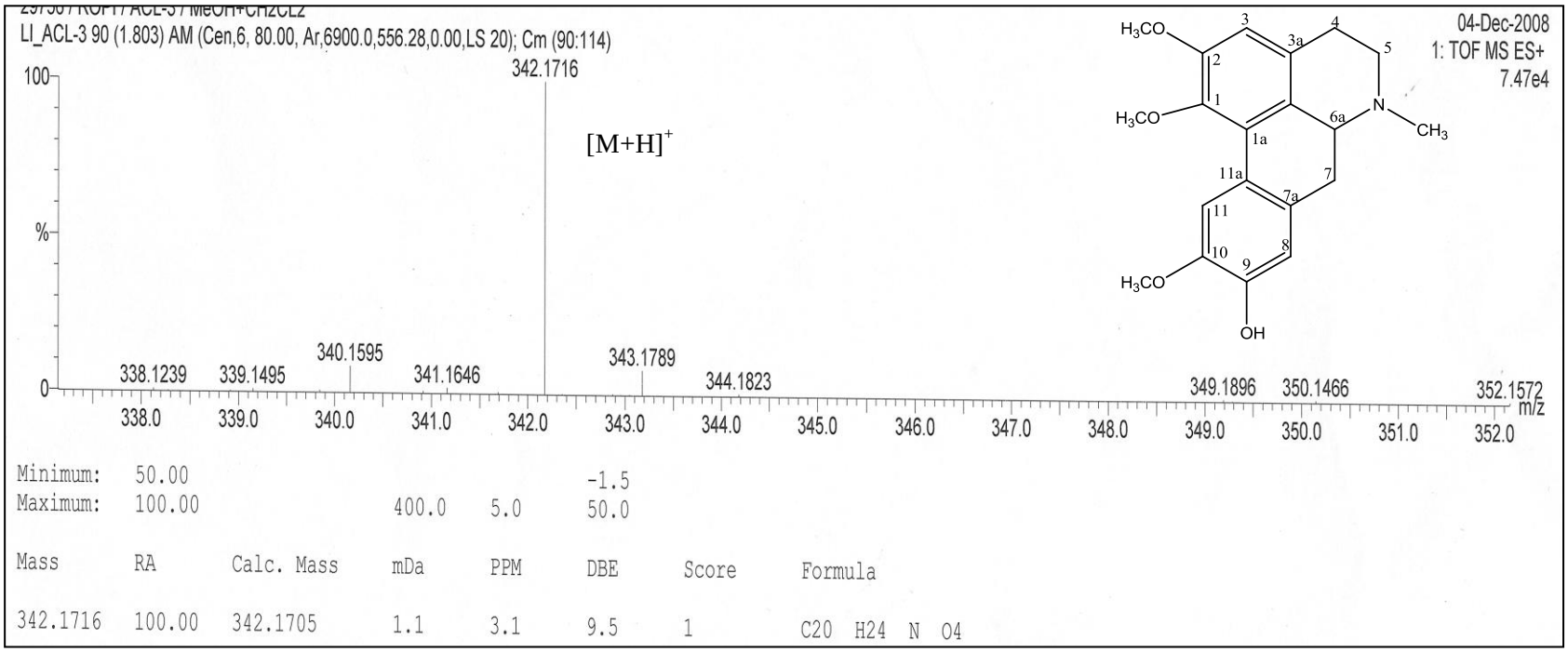


Figure 3.18: HREIMS Spectrum of Alkaloid **46**

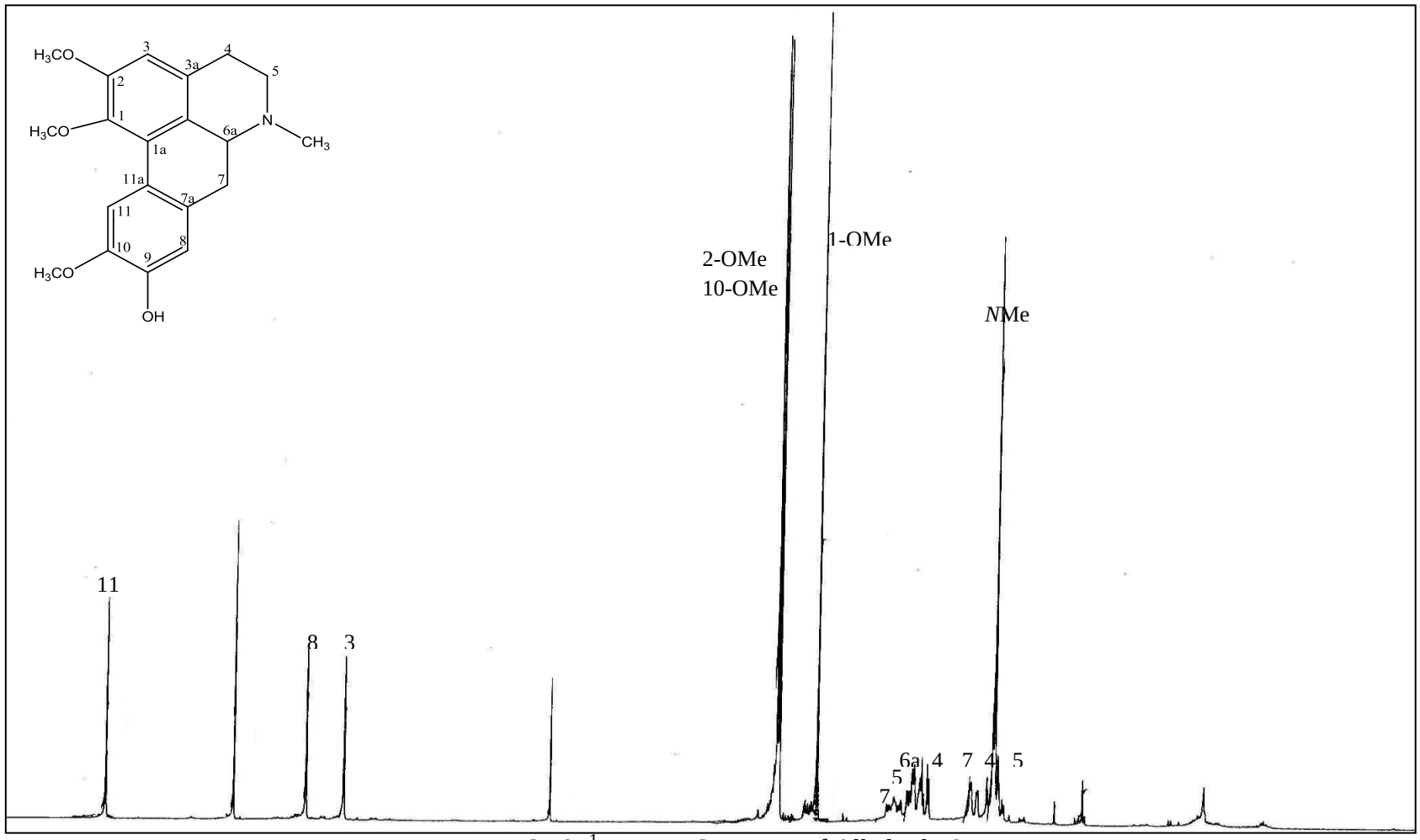


Figure 3.19: ^{1}H-NMR Spectrum of Alkaloid **46**

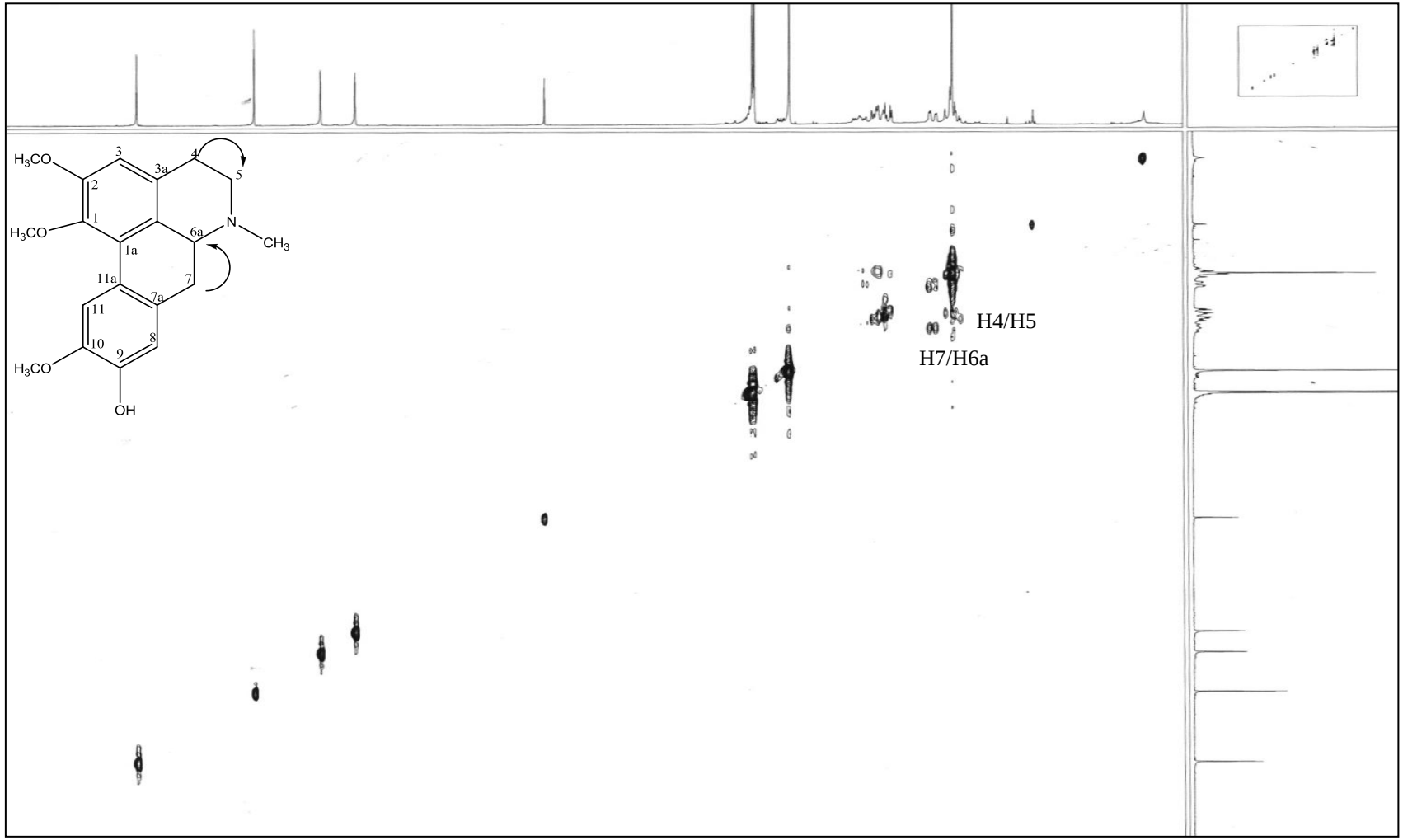


Figure 3.20: COSY Spectrum of Alkaloid **46**

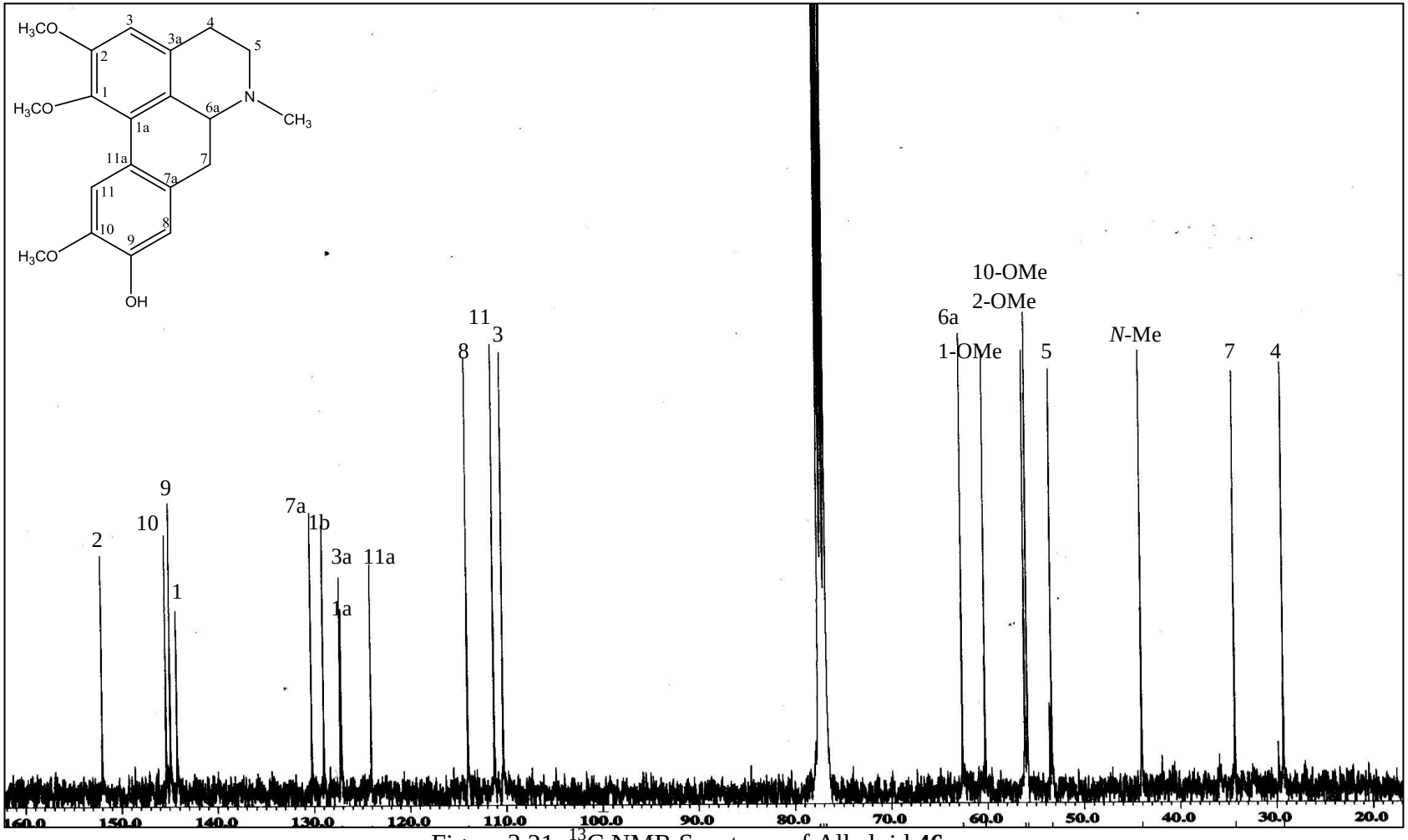


Figure 3.21: ^{13}C NMR Spectrum of Alkaloid **46**

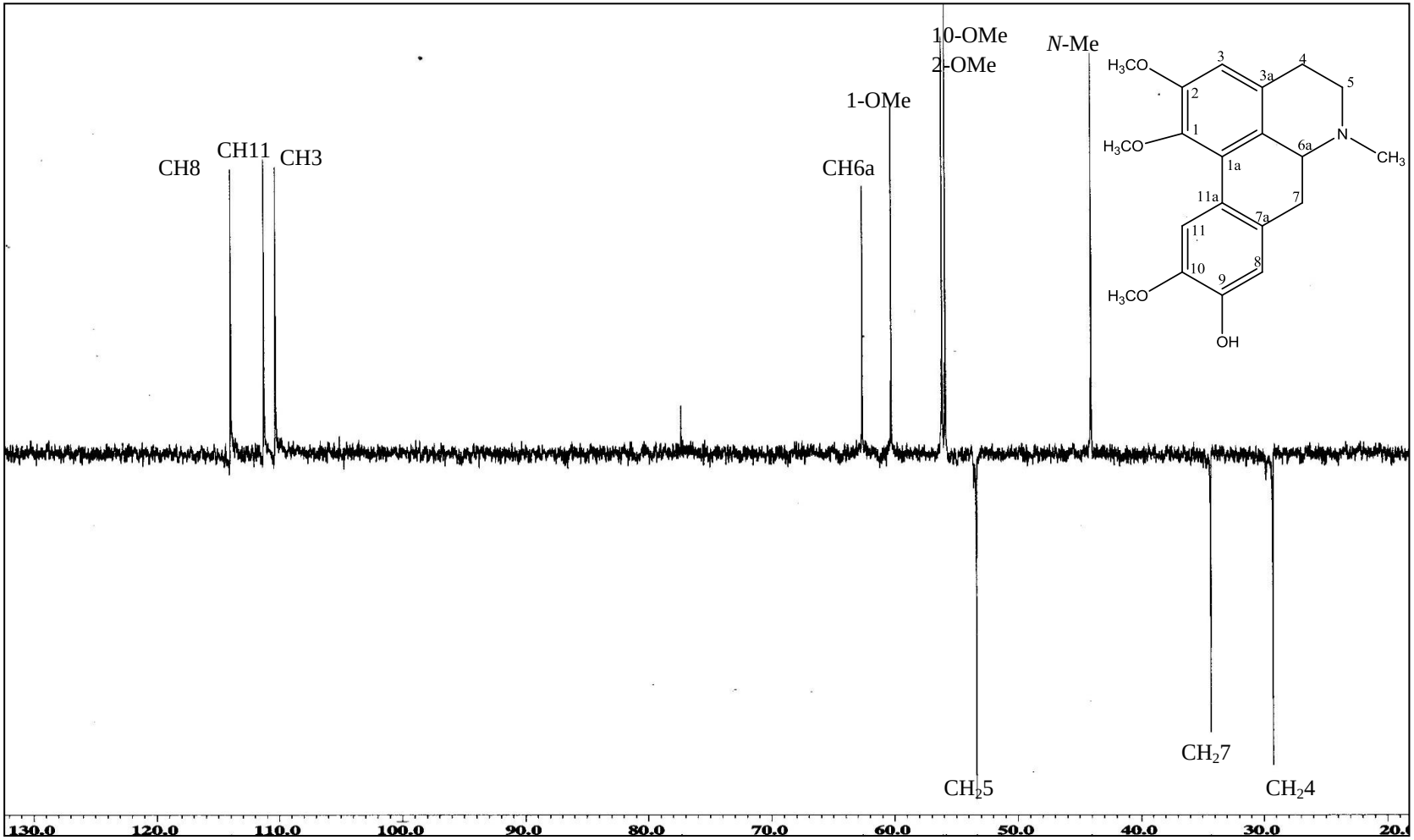


Figure 3.22: DEPT Spectrum of Alkaloid **46**

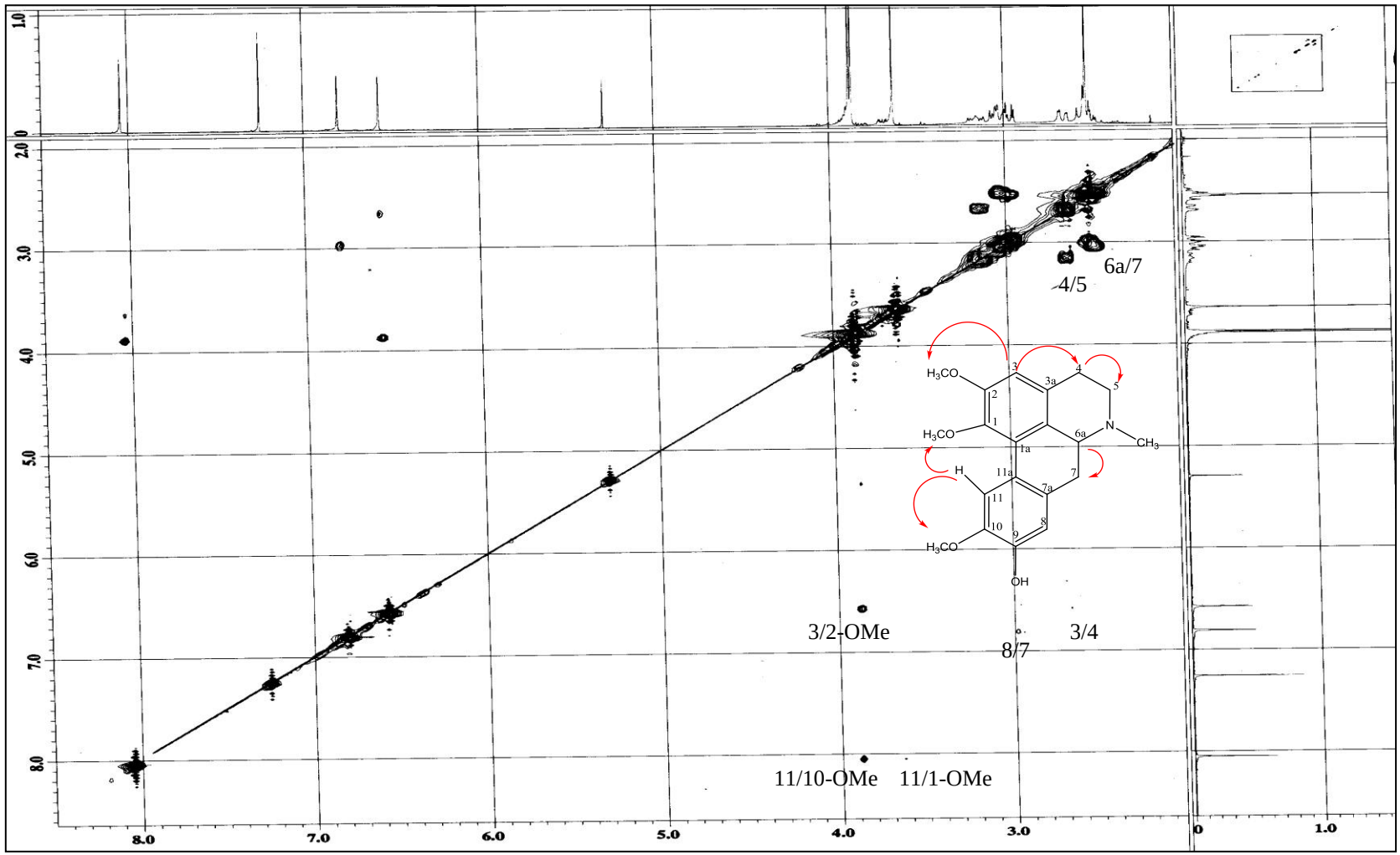


Figure 3.23: NOESY Spectrum of Alkaloid **46**

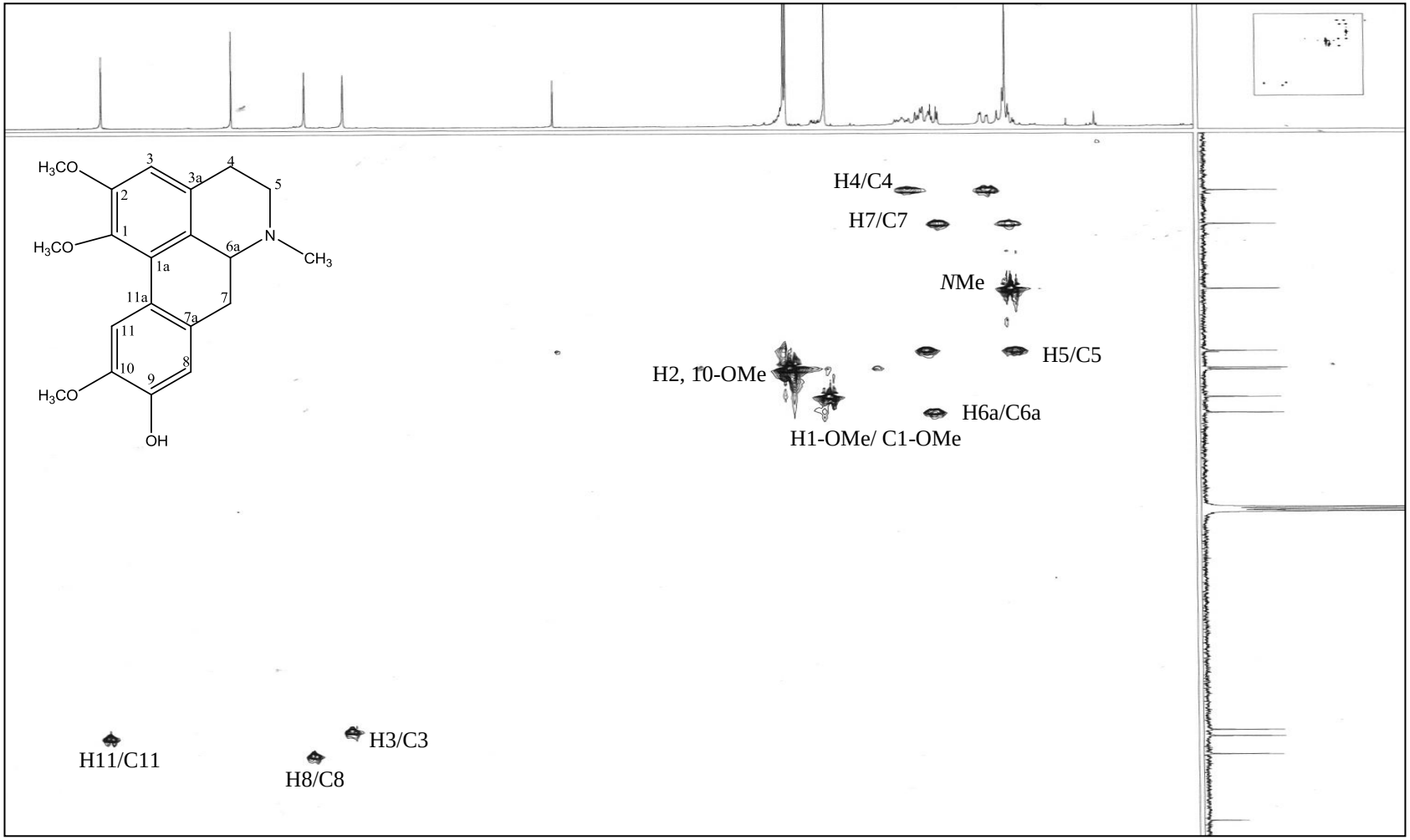


Figure 3.24: HSQC Spectrum of Alkaloid **46**

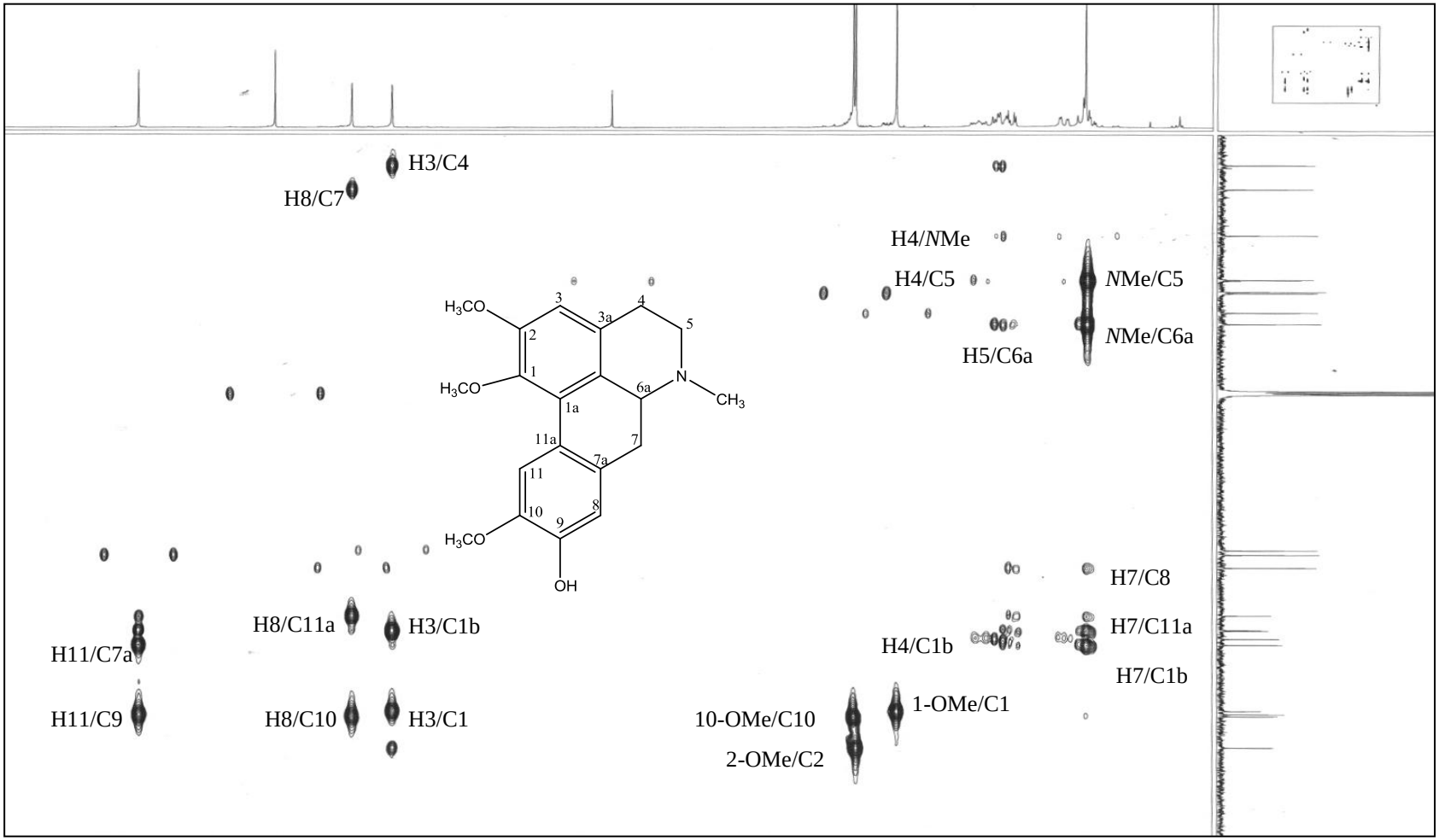
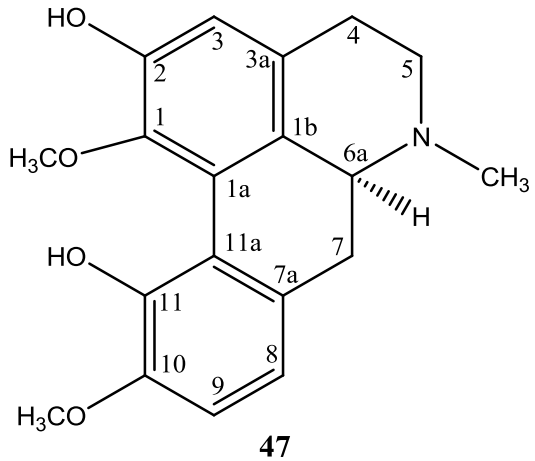


Figure 3.25: HMBC Spectrum of Alkaloid **46**

3.1.4 *N*-methyl lindcarpine, 47



47

Alkaloid **47** was isolated as a yellowish amorphous solid with $[\alpha]_D^{25}$ +160.0 (c=0.21, MeOH) indicating the absolute configuration at C-6a as *S*.[78, 79] It was unstable and tends to darken when exposed to air. Alkaloid **47** showed OH absorption band at 3336 cm^{-1}. The EIMS showed a molecular ion peak at m/z 327 $[M]^+$ giving a possible molecular formula of $C_{19}H_{21}O_4N$.

^{1}H-NMR spectrum showed the presence of a singlet peak at δ 2.45 which was due to *N*-CH_3 group. The deshielded value of 10-OMe (δ 3.85, s) compared to 1-OMe group is due to the anistropic character of benzene ring D. The C-1 methoxy signal at δ 3.58 was rather shielded compared to the C-10 aromatic methoxy since the former protons were forced to place themselves on top of ring A where electron density was high, thus the signal of 1-OMe is sterically hindered.

H-3 was found to resonate at δ 6.69, indicating that C-1 and C-2 in ring A are disubstituted with methoxy and hydroxyl groups. In addition, the vicinal proton H-8 and H-9 signals appeared as doublet at δ 6.78 with a coupling constant of 8.0Hz. In the

aliphatic region, H-5 appeared at deshielded area (δ 2.99-3.06) compared to H-4 (δ 2.41, 2.97) due to the withdrawing effect of the neighboring electronegative *N*-atom.

The aliphatic protons were assigned based on the COSY spectrum. The COSY spectrum showed cross peaks between CH_2-5/CH_2-4, CH_2-7/CH-6a and CH-8/CH-9 as shown in Figure 3.28.

The ^{13}C-NMR spectrum showed 19 carbon signals which showed three methyls, three methylenes, four methines and nine quaternary carbon signals. The signal for quaternary carbons revealed at δ 149.5, 143.6, 148.2 and 141.2 which could be assigned to oxygenated C-2, C-11, C-10 and C-1 respectively.

From the analysis of the spectroscopic data obtained and comparison with literature values, alkaloid **47** was identified as *N*-methyllindcarpine.[78, 79]

Table 3.4: ^{13}C-NMR and ^{1}H-NMR Data for Alkaloid **47**

Position	^{1}H- NMR (δ (Hz))	^{13}C- NMR (δ)
1		141.2
1a		124.8
1b		129.8
2		149.5
3	6.69 s	114.7
3a		130.5
4	2.41 m	29.7
	2.97 m	
5	2.99-3.06 m	45.5
6a	3.60 m	52.3
7	2.36-2.42 m	38.7
	2.60 m	
7a		130.0
8	6.78 d (8.0)	119.5
9	6.78 d (8.0)	111.3
10		148.2
11		143.6
11a		120.0
1-OMe	3.58 s	62.4
10-OMe	3.85 s	56.2
N-Me	2.45 s	31.1

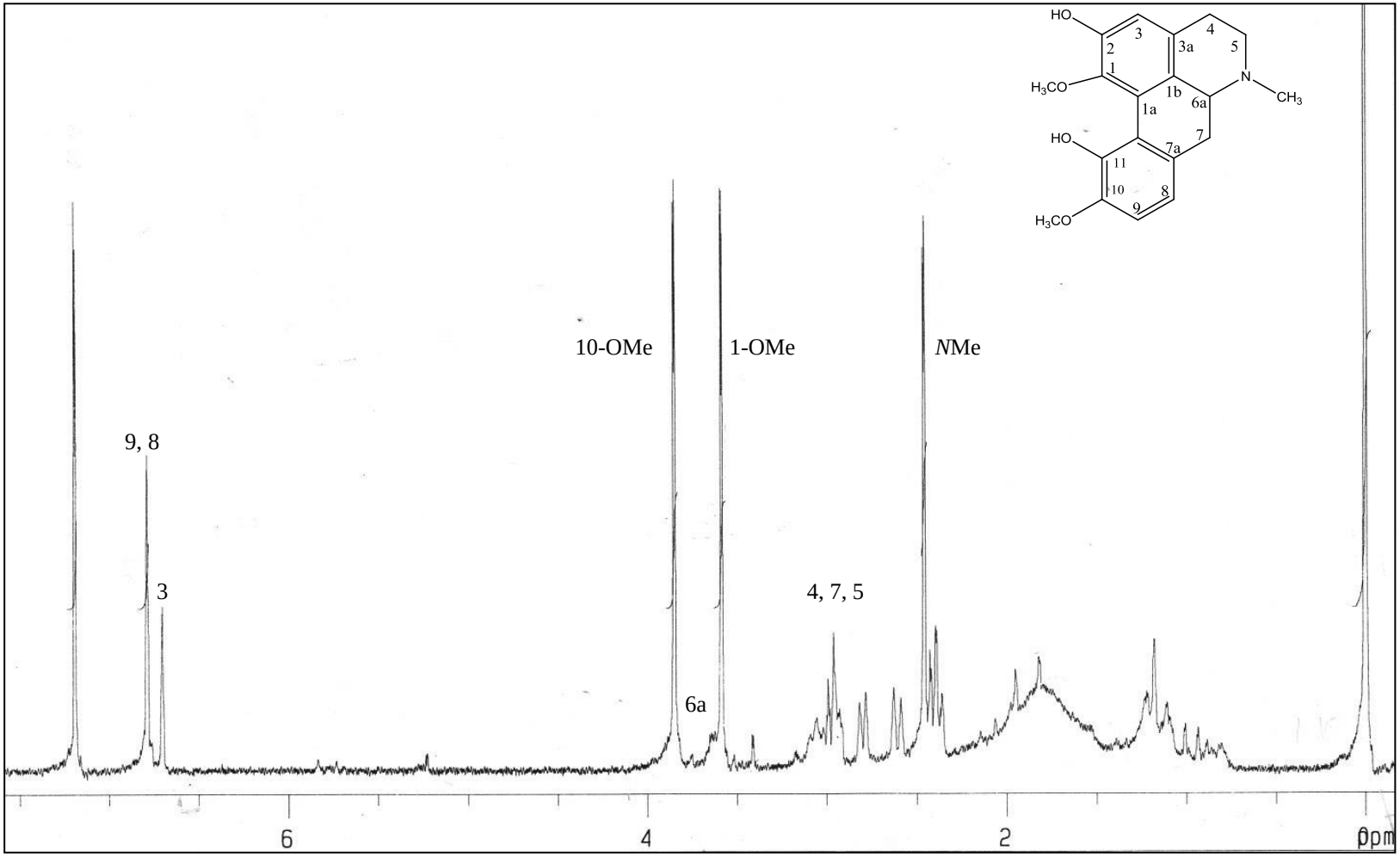


Figure 3.26: [1] H-NMR Spectrum of Alkaloid **47**

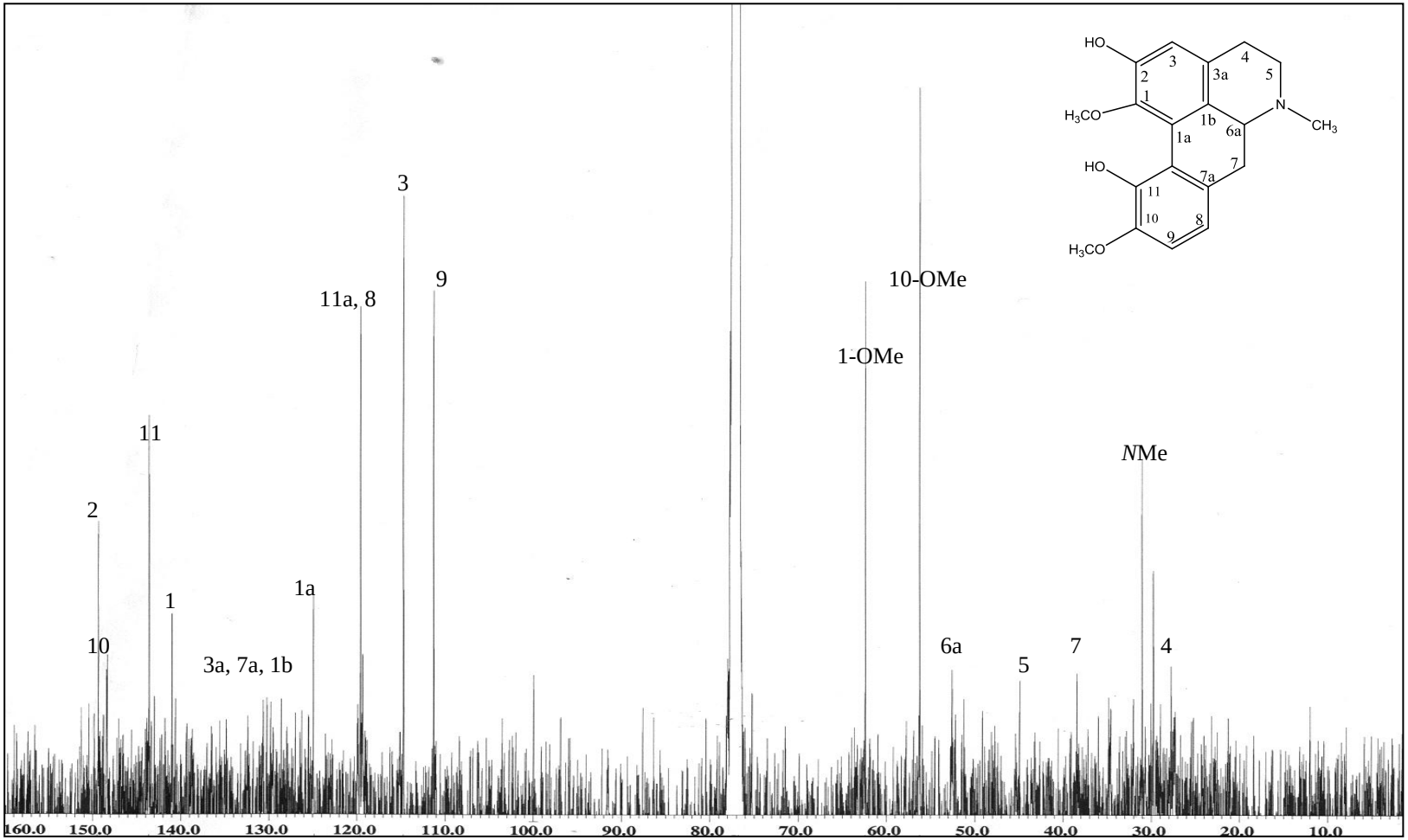


Figure 3.27: ^{13}C-NMR Spectrum of Alkaloid **47**

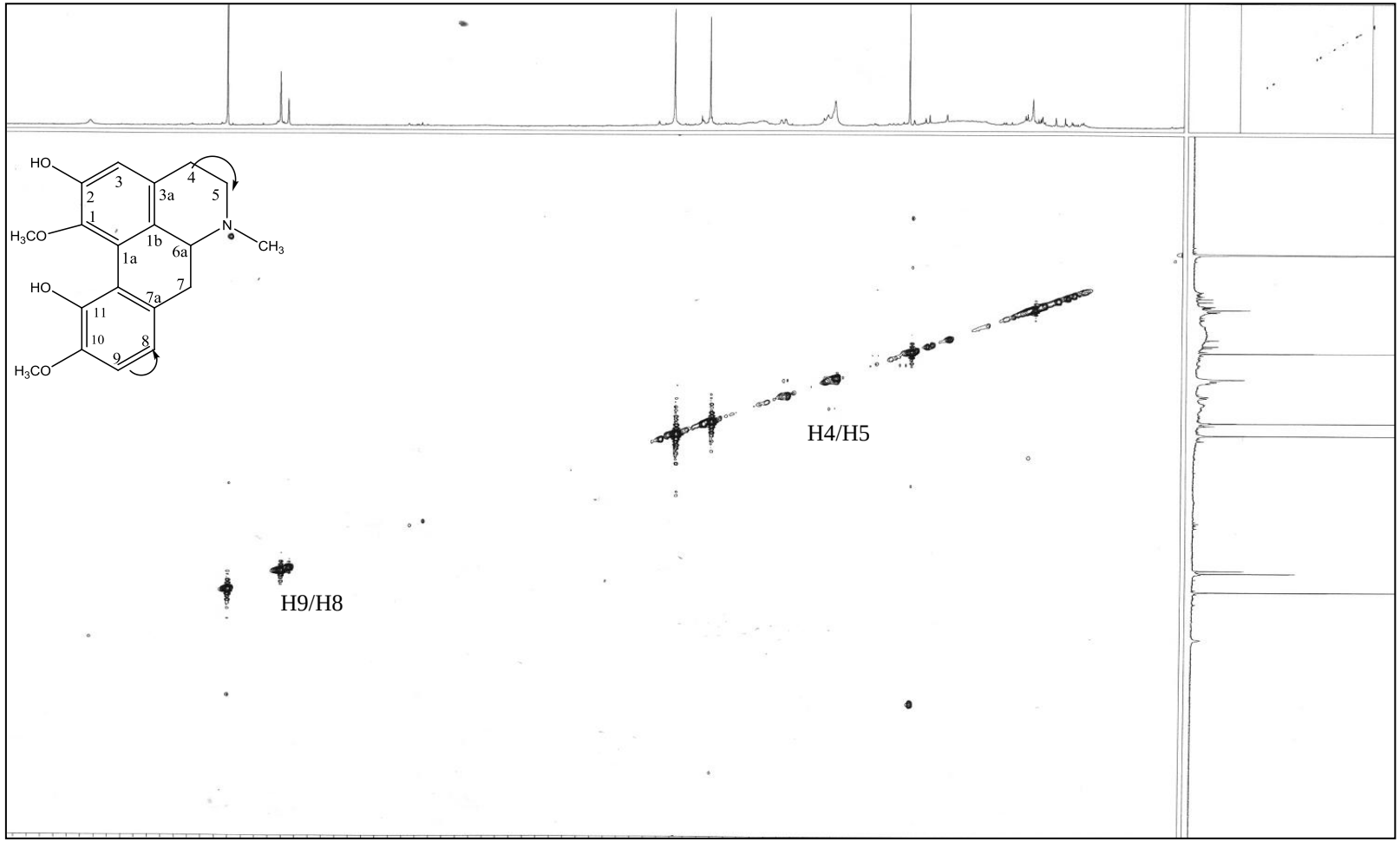
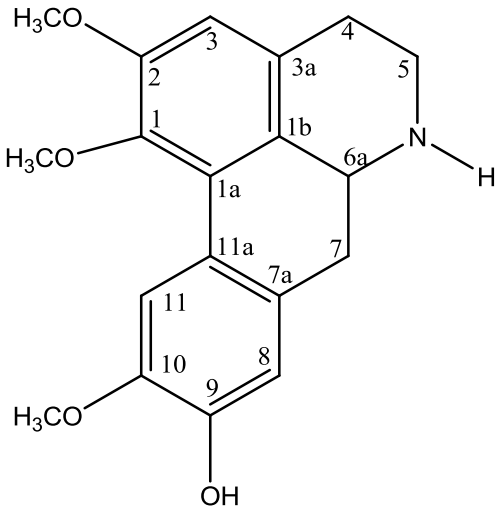


Figure 3.28: COSY Spectrum of Alkaloid **47**

3.1.5 Laurotetanine, 48



48

Alkaloid **48** was isolated as a dark brown amorphous with $[\alpha]_D^{25}$ +125° (c=2.28, MeOH) indicating the absolute configuration at C-6a as *S* as in the known alkaloids[14-16]. The UV spectrum showed absorption bands at 220, 281, 302 and 312 nm, thus suggesting a 1,2,9,10-tetrasubstituted aporphine skeleton[36,77]. Moreover the IR spectrum exhibited the presence of a highly conjugated hydroxyl group at about 3429 cm^{-1}.

The ESI^+ mass spectrum showed a pseudomolecular ion peak at m/z 328.1566, $[M+H]^+$ corresponding to the molecular formula of $C_{19}H_{21}NO_4$.

The ^1H NMR and ^{13}C NMR spectra of alkaloid **48** were almost similar to that of alkaloid **46**, which suggested a close structural relationship between these two compounds. Indeed, the same substituent could be characterized in both compounds namely methoxy groups (C-1,C-2,C-10) and hydroxyl group at position C-9. However, alkaloid **48** exhibited the absent of *N*-Methyl signal in ^1H NMR and ^{13}C NMR spectra in the range of δ 2.00-2.50 and δ 44.0-45.0 respectively.

The HMBC experiments clearly proved that the methoxyl substitutents are located at C-1 (δ 144.3), C-2 (δ 152.2), C-9 (δ 145.3) and C-10 (δ 144.9) through the following observed correlations; H3/C1, H11/C9 and H8/C10 as shown below.

Extensive analysis of all spectroscopic data established the complete assignment of all the ^{1}H and ^{13}C signals of alkaloid **48**, which eventually lead to the identification of the compound as laurotetanine.[14-16] The ^{1}H NMR, ^{13}C NMR and HMBC data are summarized in table 3.5.

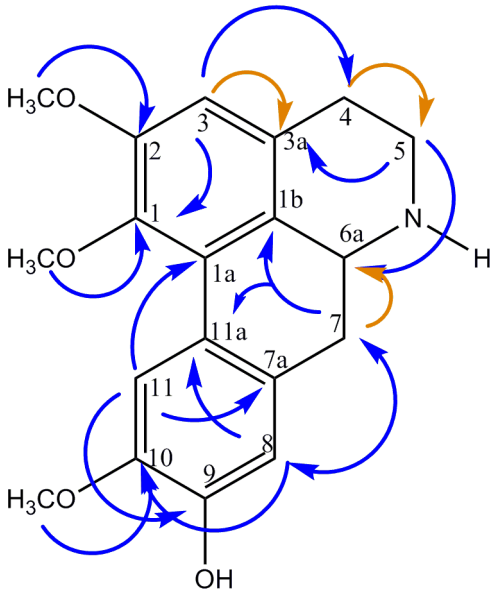


Figure 3.29: ^{1}H-^{13}C Correlations observed in HMBC Spectrum of Alkaloid **48**

Table 3.5: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **48**

Position	^{1}H- NMR (δ,(Hz))	^{13}C- NMR (δ)	HMBC
1		144.3	
1a		126.8	
1b		127.4	
2		152.2	
3	6.57s	110.8	1,2,4,1b
3a		129.0	
4	2.74(dd,4.6,13.6)	29.0	5,3a
5	3.01 (dd, 4.1, 12.9)	43.1	3a,6a
	3.65 m		
6a	3.80 (dd.4.4,13.2)	53.7	1b, 7
7	2.64 (d,13.6)	36.5	6a,8,1b
7a		129.7	
8	6.77 s	113.9	11a,10,7
9		145.3	
10		144.9	
11	8.06 s	111.3	9,7a,1a,11a
11a		124.0	
1-OMe	3.64 s	60.2	1
2-OMe	3.86 s	55.9	2
10-OMe	3.87 s	56.1	10

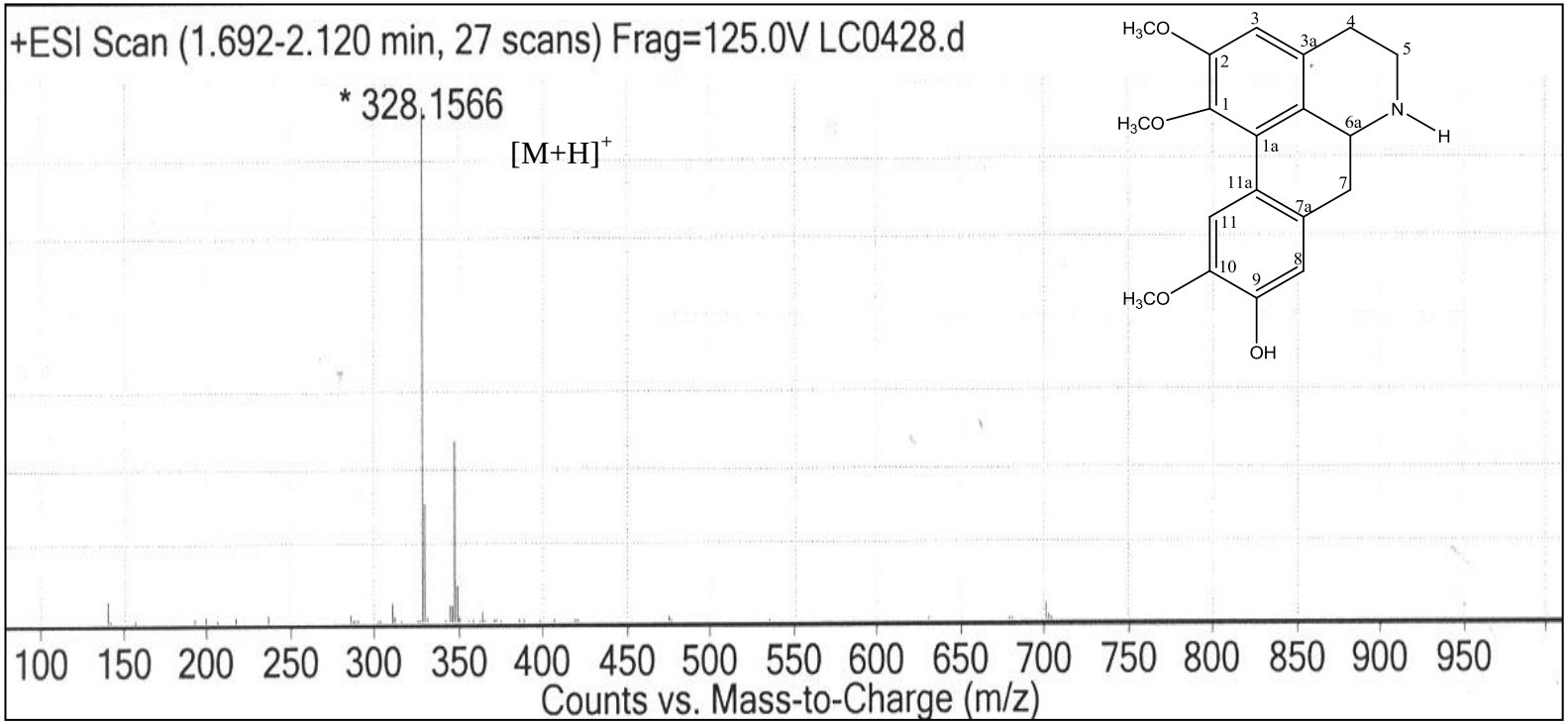


Figure 3.30: EIMS Spectrum of Alkaloid **48**

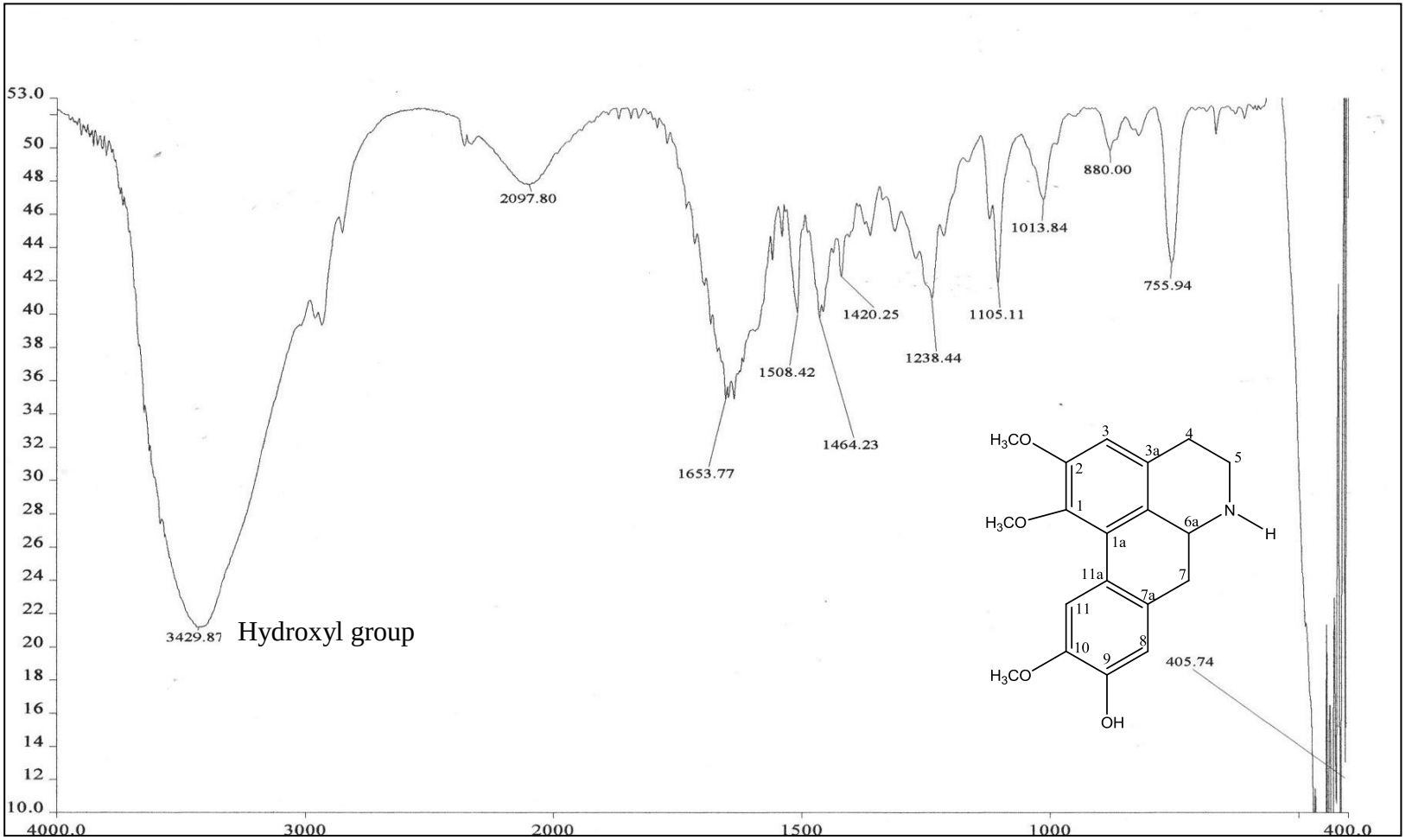


Figure 3.31: Infrared Spectrum of Alkaloid **48**

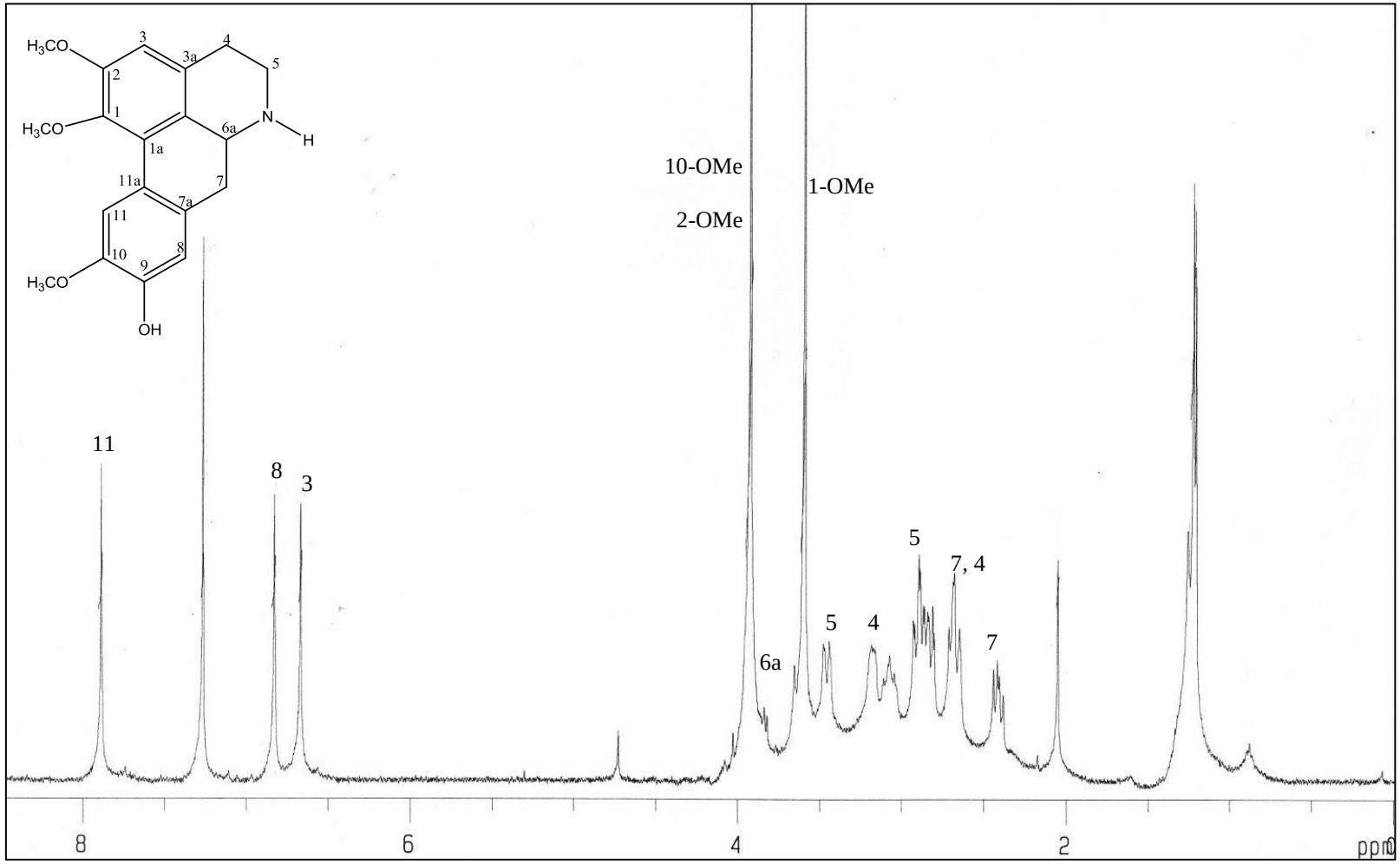


Figure 3.32: 1H NMR Spectrum of Alkaloid **48**

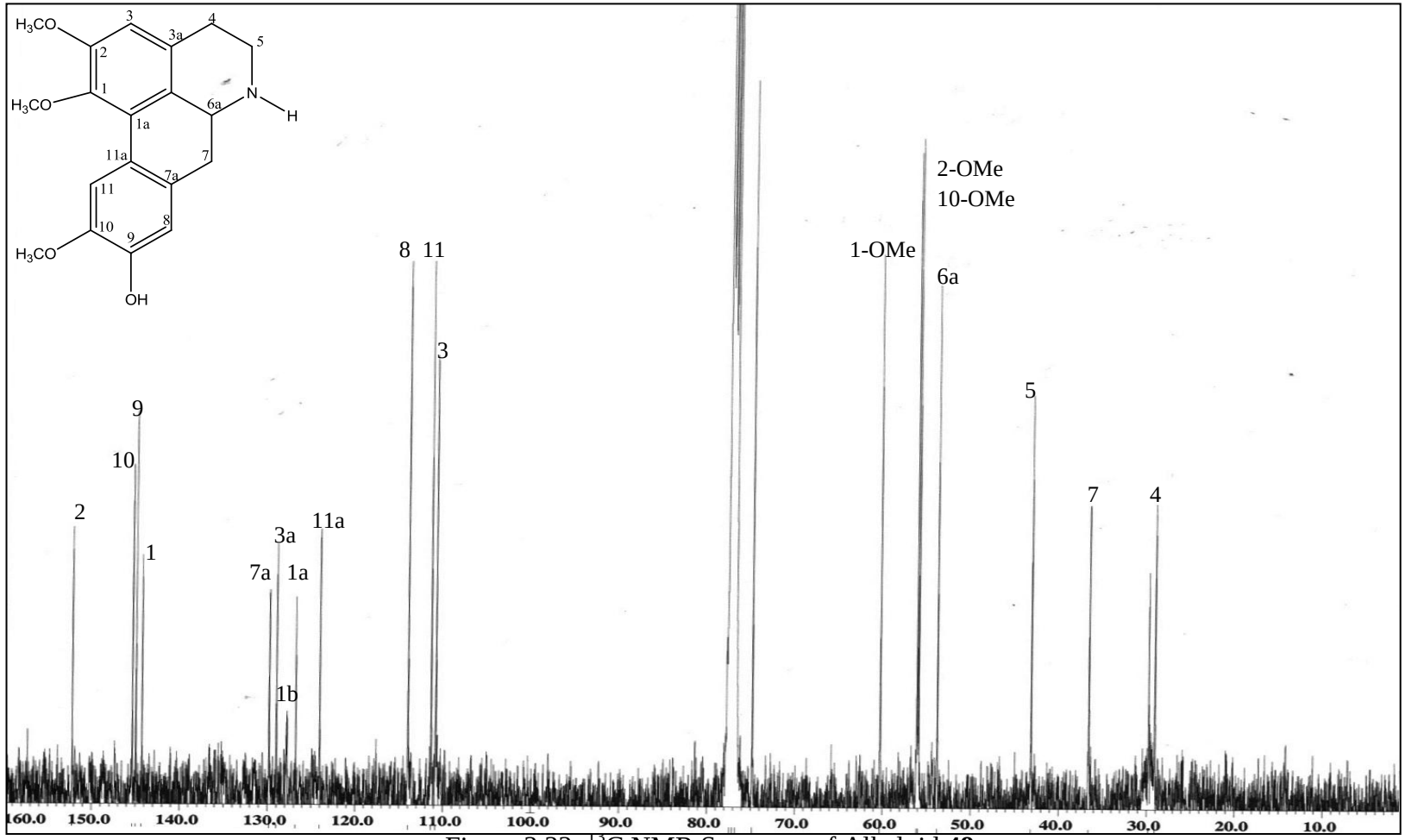


Figure 3.33: ^{13}C NMR Spectrum of Alkaloid **48**

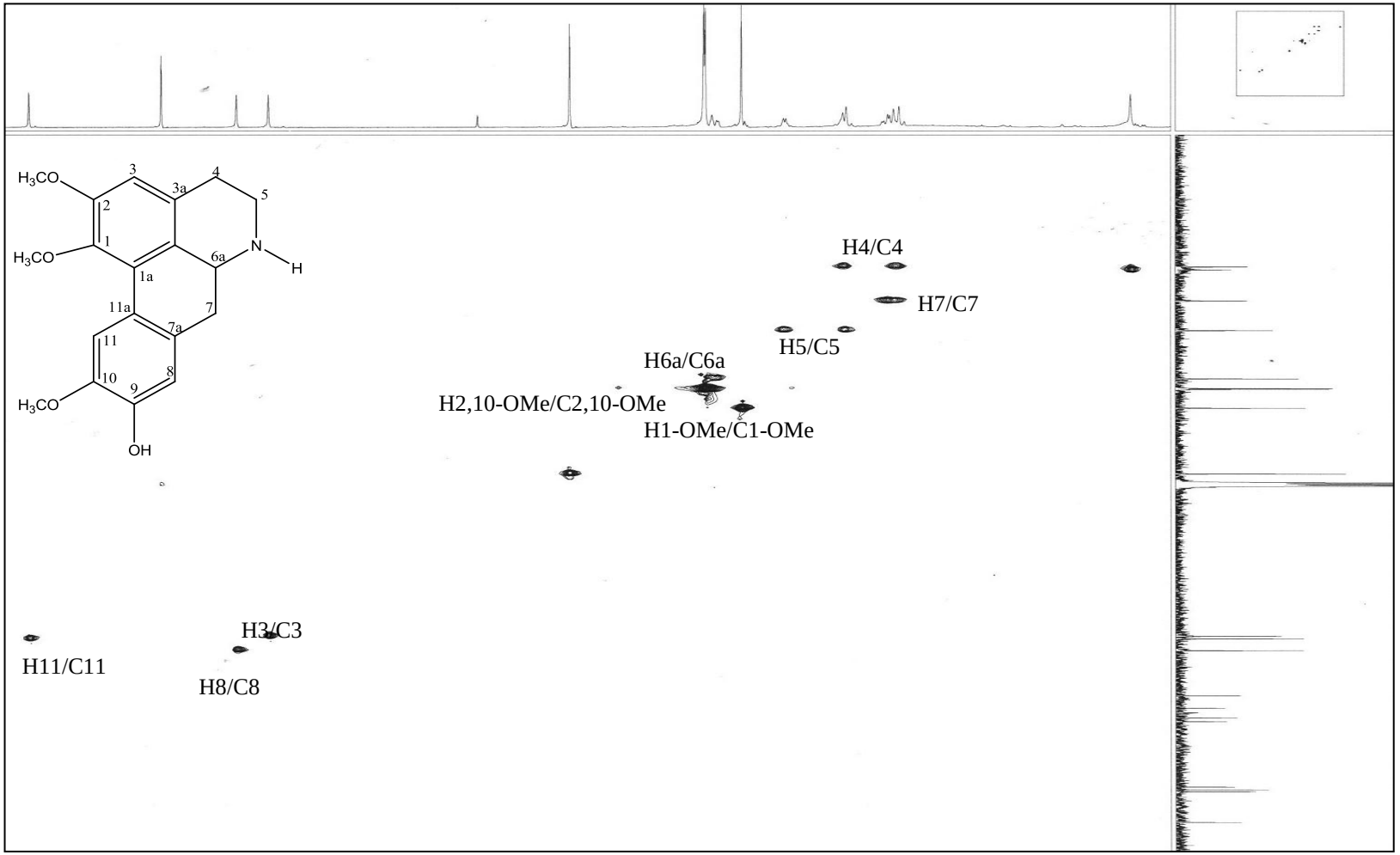


Figure 3.34: HSQC Spectrum of Alkaloid **48**

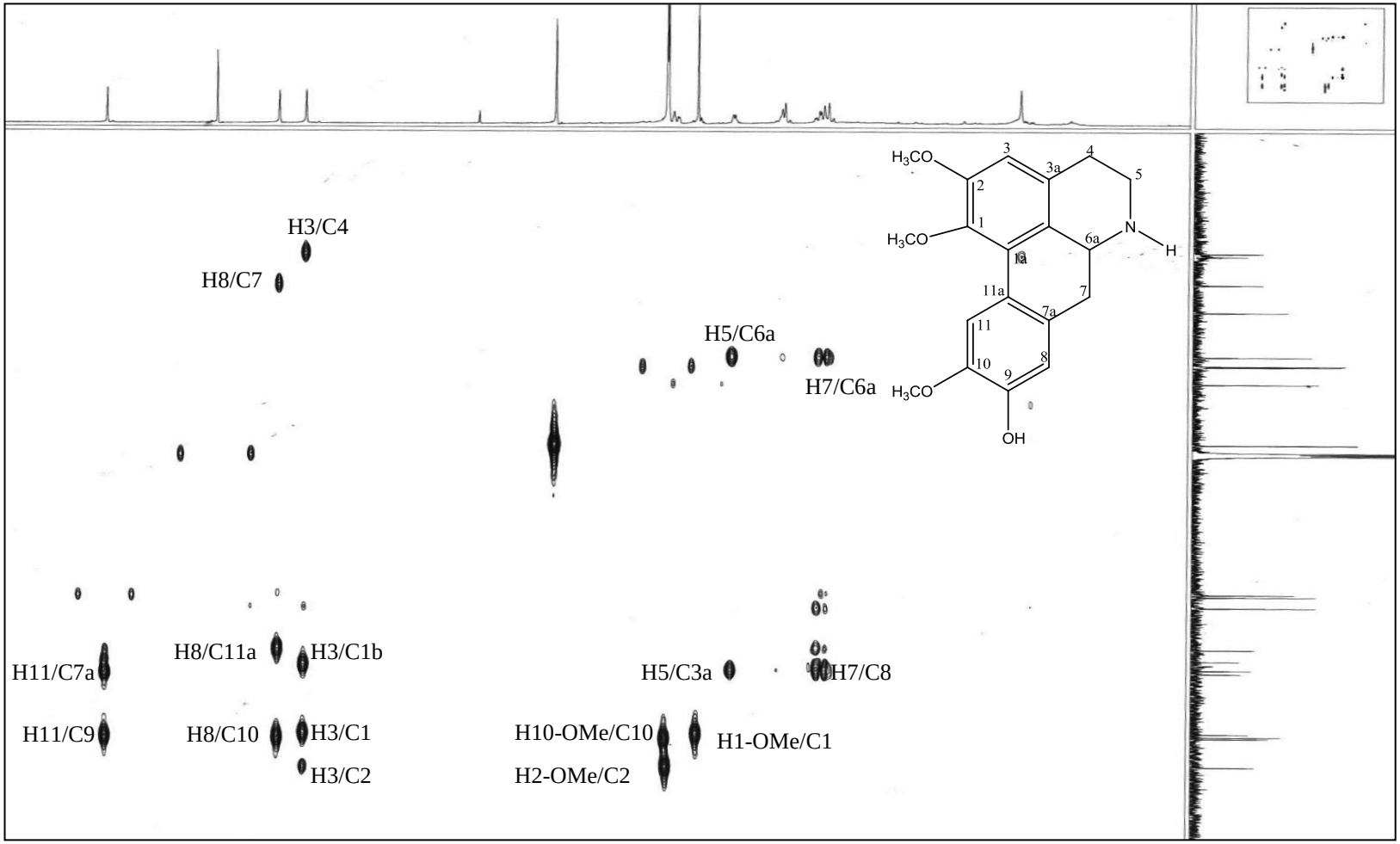
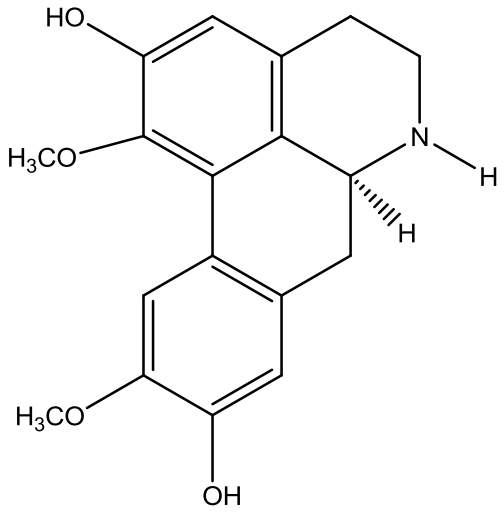


Figure 3.35: HMBC Spectrum of Alkaloid **48**

3.1.6 Norboldine, 49



49

Alkaloid **49** was isolated as brown amorphous solid with $[\alpha]_D^{25}$ +102.5 (c=0.01, MeOH) indicating the absolute configuration at C-6a as *S* as in the known alkaloids[80-83] . The UV spectrum showed absorption bands at 283 and 304 nm due to the degree of resonance in the biphenyl system and characteristic of 1,2,9,10-tetrasubstituted aporphine.[36, 77] The IR spectrum showed broad bands at 3584 and 3162 cm^{-1} due to the presence of OH and NH functional groups in the structure.

The ESI^+ mass spectrum showed a pseudomolecular ion peak at (*m/z*: 314.1446) $[M-H]^+$ corresponding to the molecular formula of $C_{18}H_{19}NO_4$.

The ^{1}H-NMR spectrum, showed the existence of two methoxyls by revealing two singlets at δ 3.58 and 3.85. These methoxyl groups belonged most probably to C-1 and C-10. The position of methoxy groups was proven by NOESY correlation between H11/10-OMe and H11/1-OMe. The respective C10-OMe and C1-OMe were further confirmed by analyzing the HMBC spectrum. The correlations between 10-OMe/C-10, H8/C10 for 10-OMe were observed in the spectrum. Three singlet signals representing

three aromatic protons revealed at δ 6.60, 6.73 and 7.90 which can be assigned to H-3, H-8 and H-11, respectively. The cross correlation deduced from COSY spectrum revealed the exact position of aliphatic proton between H4/H5, H6a/H7.

The ^{13}C-NMR spectrum established 20 carbon resonances which closely resembled to those reported for norboldine. [80-83] In addition, the signals are two methyl, three methylenes, four methines and nine quaternary carbon signals. Four oxygenated aromatic quaternary carbon signals were observed at δ 142.28, 145.28, 148.51 and 145.87 indicating the presence of hydroxyl groups at C-2 and C-9, and methoxyl groups at C-1 and C-10 respectively. The ^{1}H NMR, ^{13}C NMR and HMBC data are summarized in table 3.6.

On the basis of these spectral evidences and comparison with those literature values, Alkaloid **49** was characterized as norboldine **49.**[80-83]

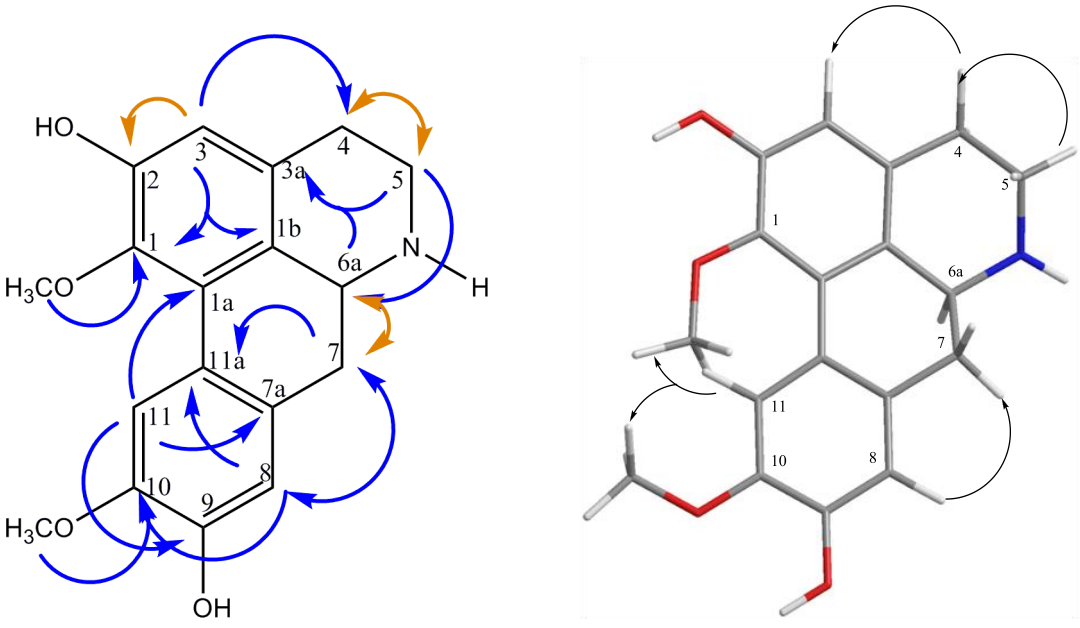


Figure 3.36: HMBC and NOESY Correlations of Alkaloid **49**

Table 3.6: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **49**

Position	^{1}H- NMR δ,(Hz)	^{13}C- NMR (δ)	HMBC
1		148.51	
1a		125.87	
1b		127.35	
2		142.28	
3	6.60s	113.98	1,2,4,1b
3a		129.75	
4	α 2.60 (m)	28.70	5
	β 2.90 (m)		
5	α 2.95 (m)	42.98	4,3a,6a
	β 3.31 (m)		
6a	3.74 (dd,4.12,13.72)	53.60	3a,7
7	α 2.68 (m)	36.30	11a,8,1b,6a
7a		129.75	
8	6.73 s	114.50	11a,10,7
9		145.28	
10		145.87	
11	7.90 s	110.61	9,7a,1a
11a		123.58	
1-OMe	3.58 s	60.20	2
10-OMe	3.85 s	56.10	10

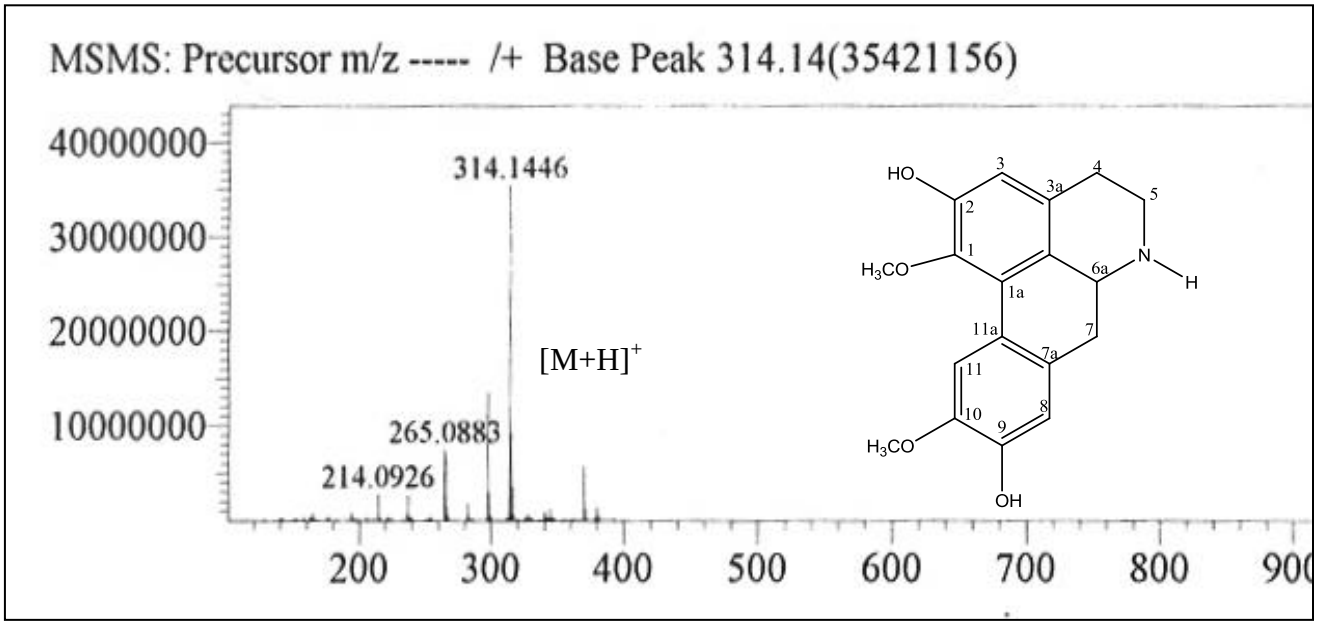


Figure 3.37: LCMS Spectrum of Alkaloid **49**

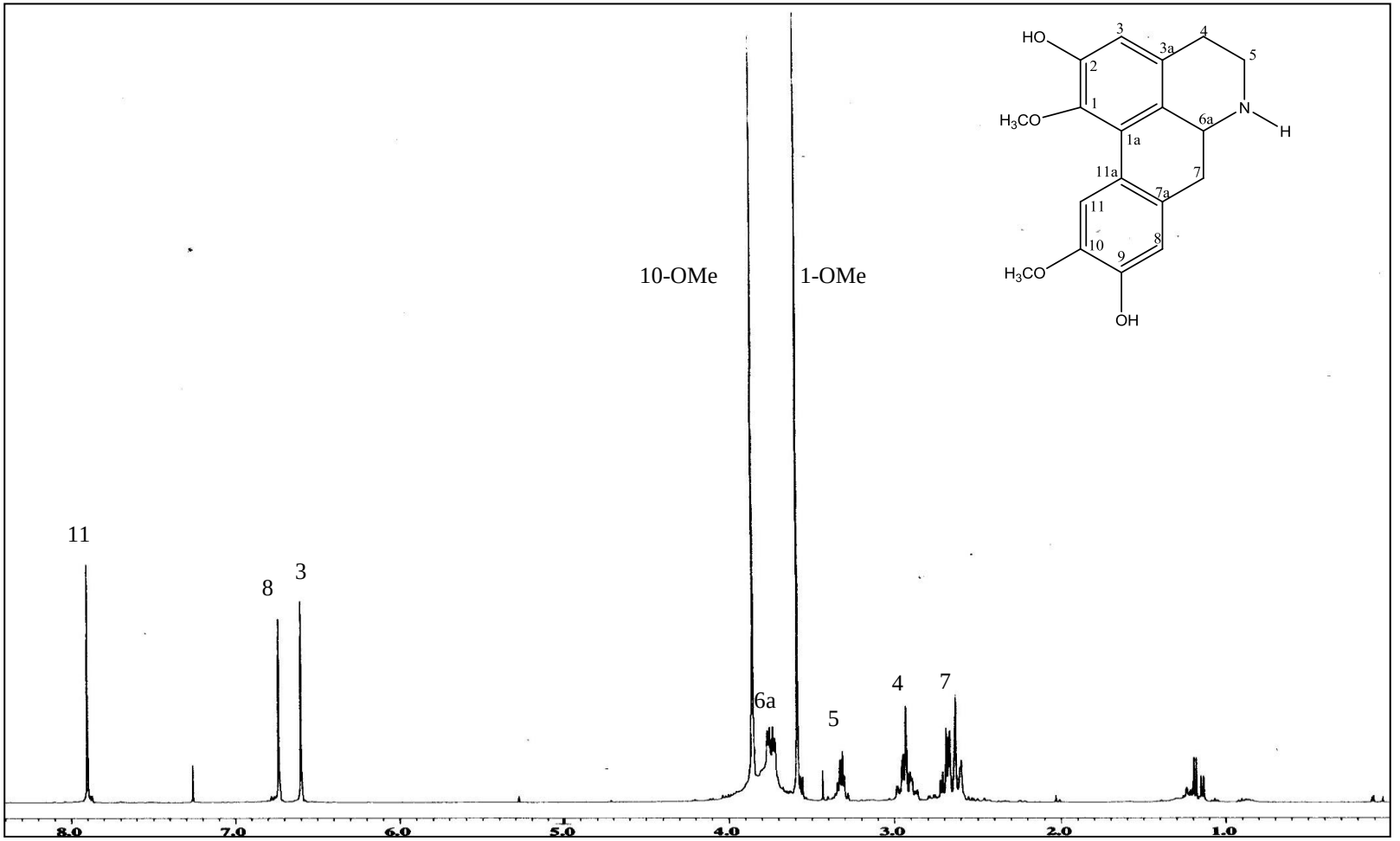


Figure 3.38: ^{1}H NMR Spectrum of Alkaloid **49**

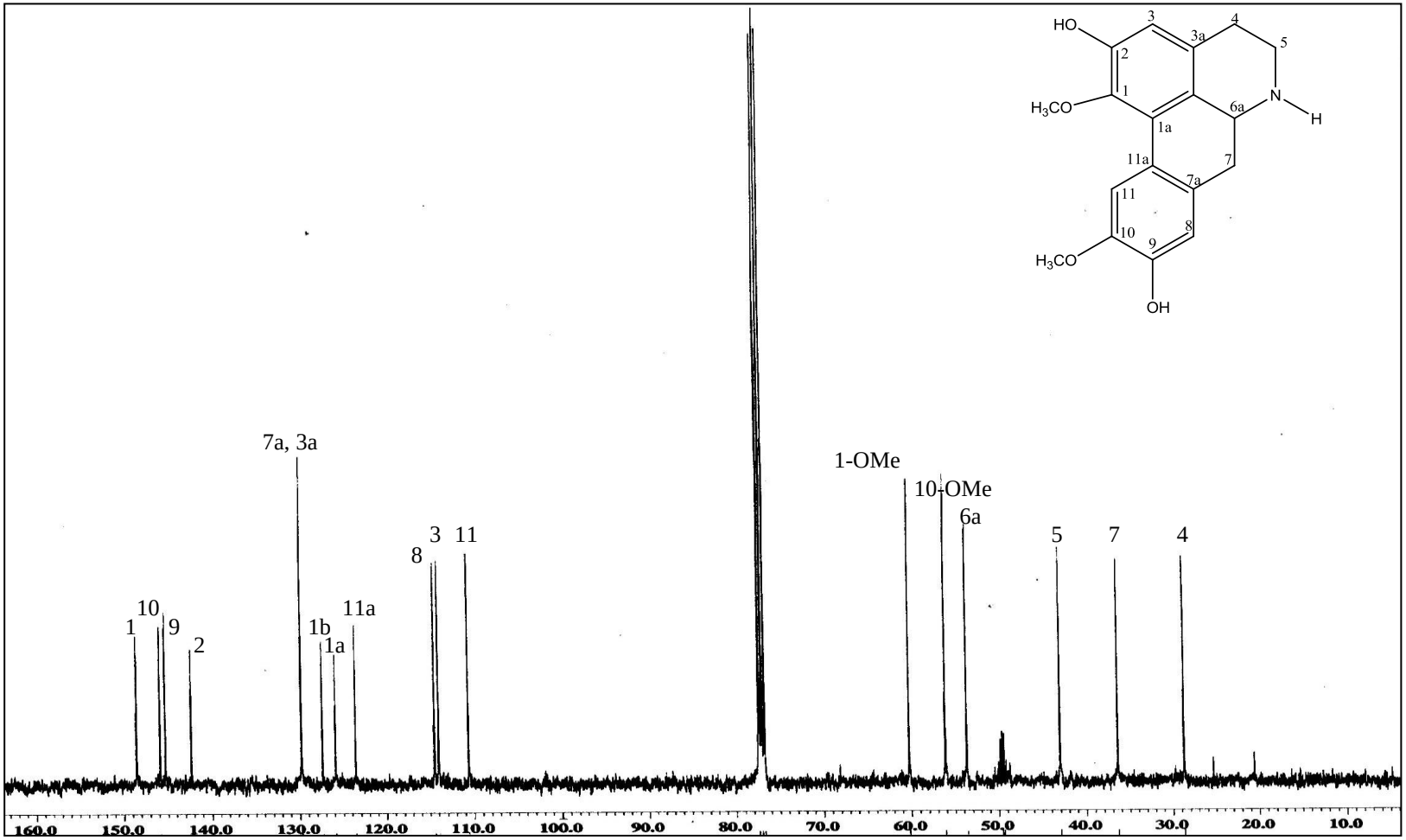


Figure 3.39: ^{13}C NMR Spectrum of Alkaloid **49**

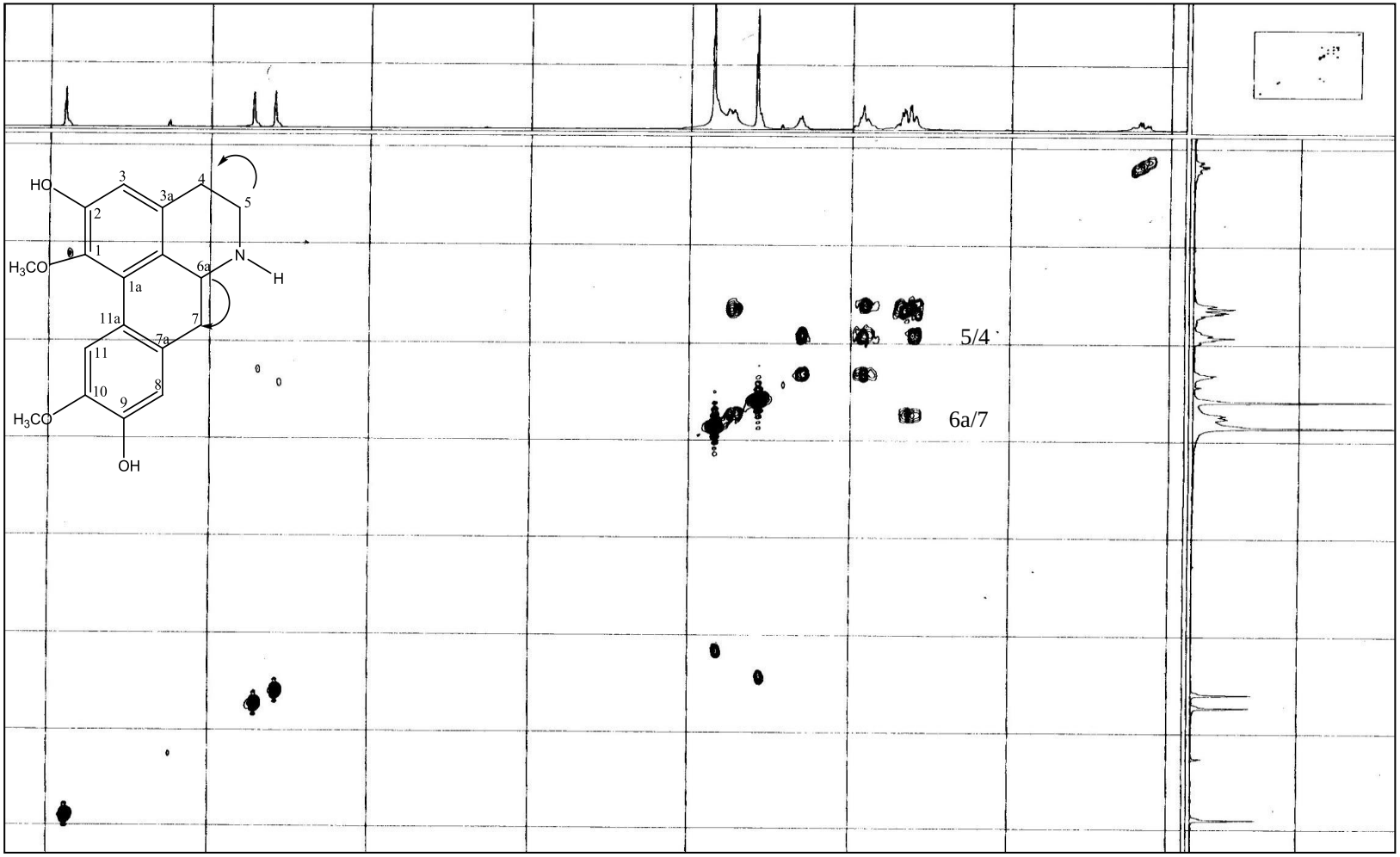


Figure 3.40: COSY Spectrum of Alkaloid **49**

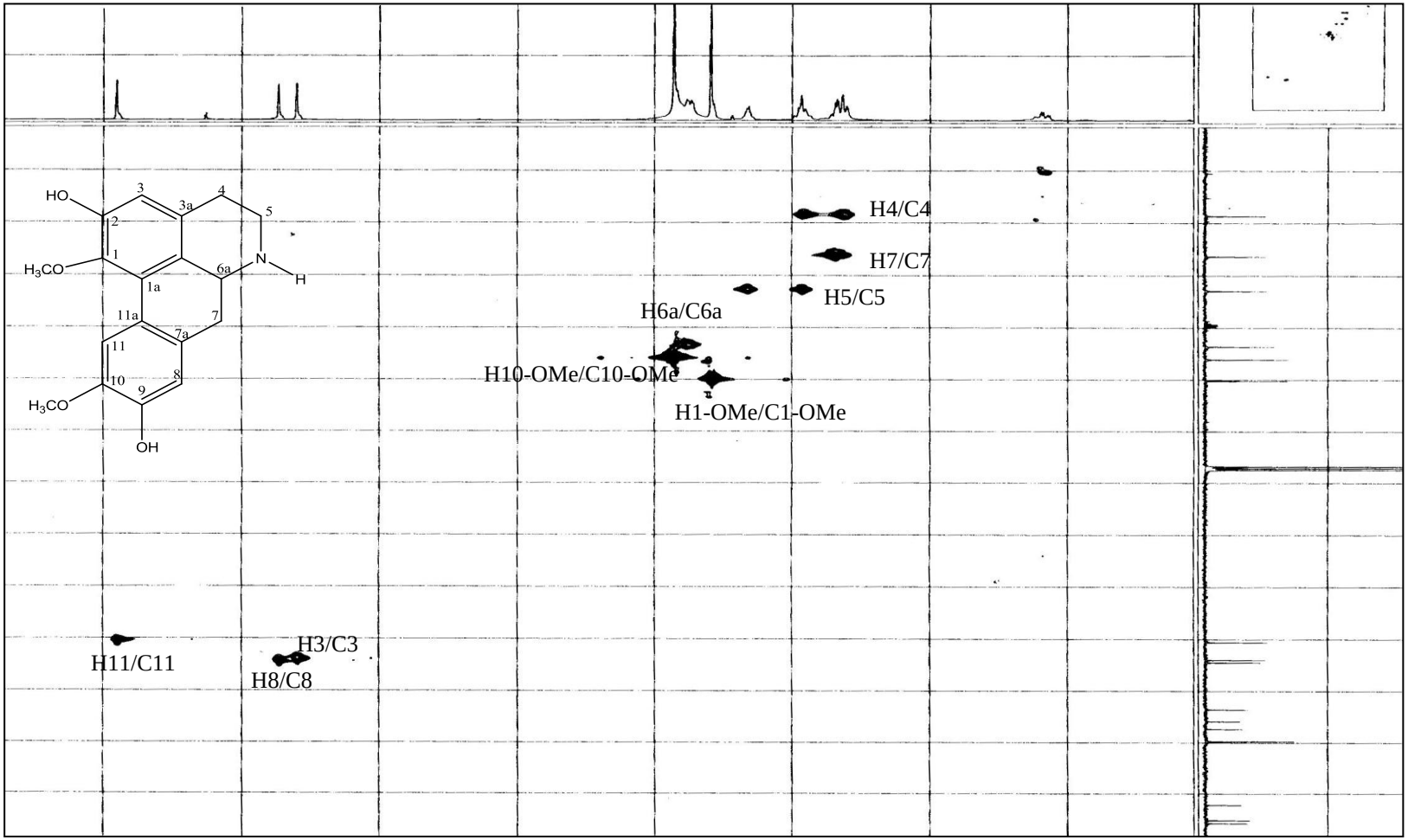


Figure 3.41: HSQC Spectrum of Alkaloid **49**

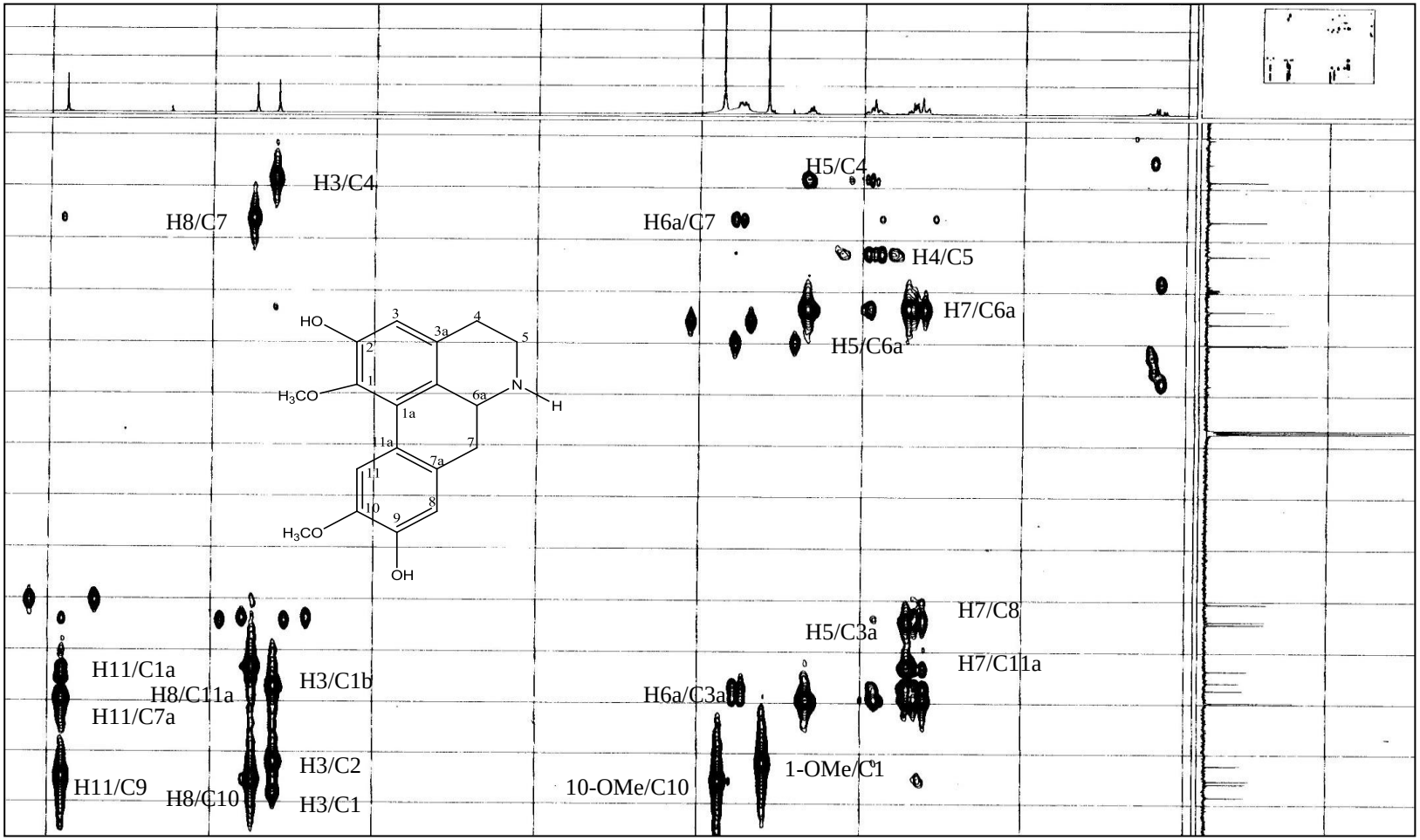


Figure 3.42: HMBC Spectrum of Alkaloid **49**

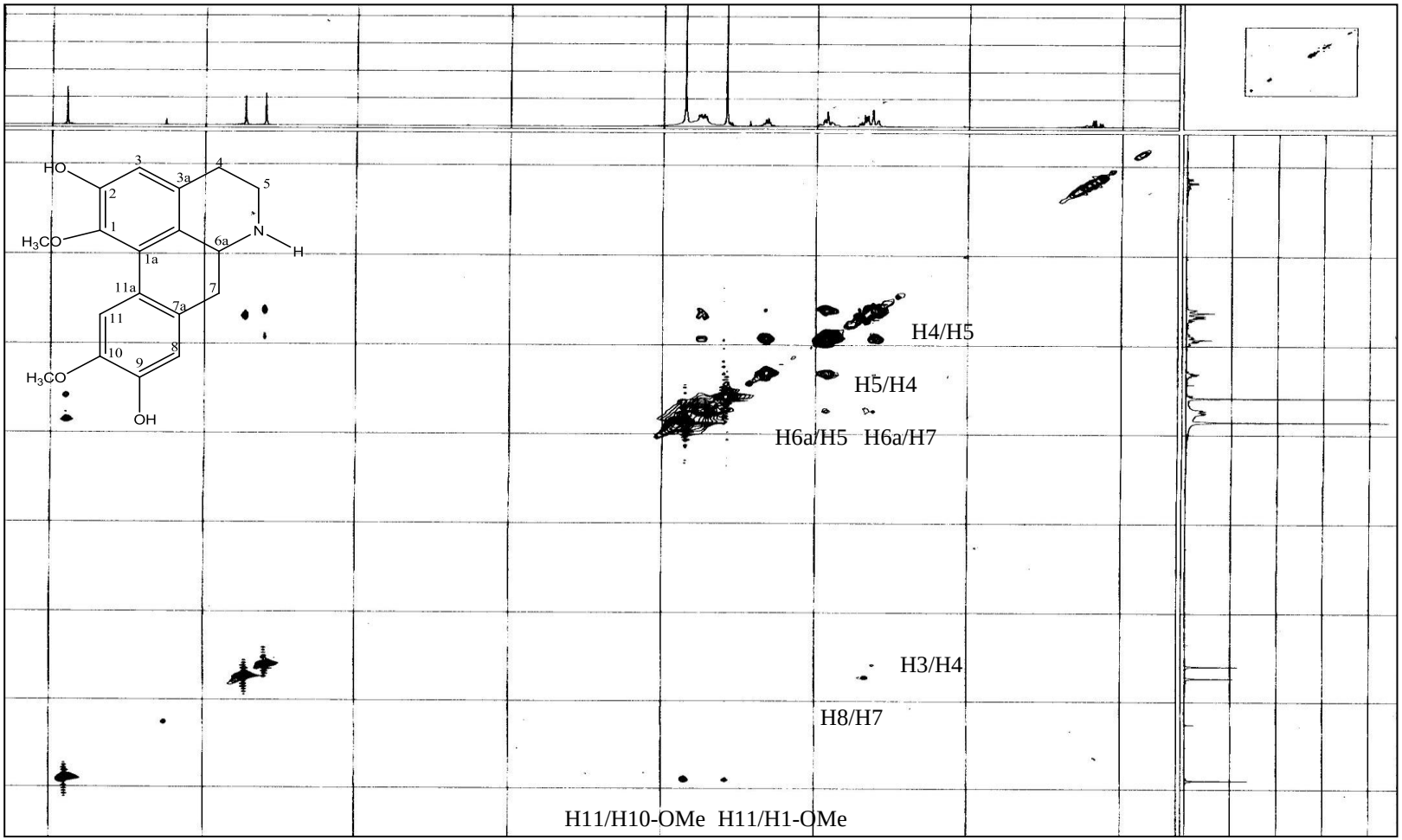
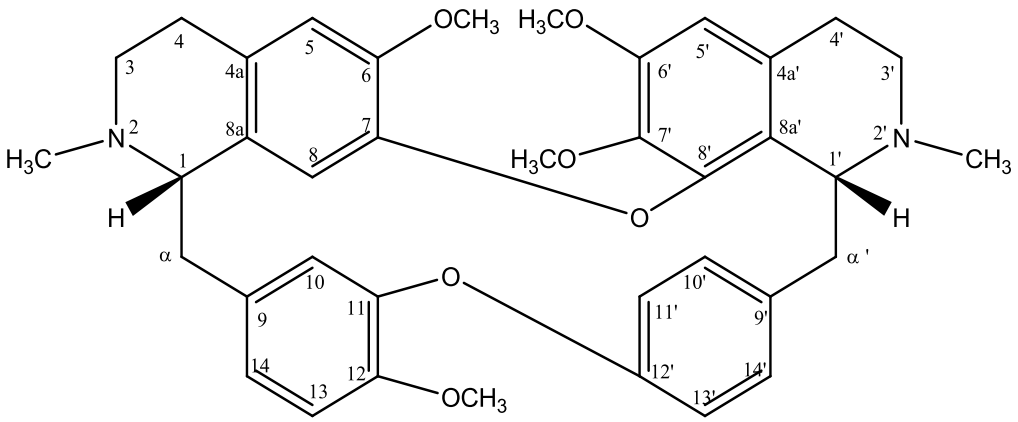


Figure 3.43: NOESY Spectrum of Alkaloid **49**

3.1.7 Gyrolidine, 50



50

Alkaloid **50** was obtained as yellow amorphous solid with $[\alpha]_D^{25}$ -115.0 (c = 1.1, MeOH). The UV spectrum displayed maxima absorptions at 244 and 286 nm which suggested the presence of bisbenzylisoquinoline moiety[36, 84]. Its IR spectrum revealed absorption bands due to phenyl ether groups (1070, 1126, 1231, and 1268 cm^{-1}) and aromatic ring (1511 and 1606 cm^{-1}). The ESIMS spectrum revealed the $[M]^+$ peak at m/z 622 suggesting a molecular formula of $C_{38}H_{42}N_2O_6$

The ^1H NMR spectrum showed two *N*-methyls resonated as a singlet at δ 2.52 and 2.65 assignable to *N*-2 and *N*-2' methyl protons respectively. H-1 and H-1' signals were observed at δ 4.19 and 3.31. Four methoxy groups displayed four singlets in the spectrum at δ 3.19 (C-7'), 3.63 (C-6), 3.79 (C-6'), and 3.84 (C-12) respectively. The singlet for 7'-methoxyl was observed further up field at δ 3.19 due to the presence of the bulky substituents near C-7' methoxy group.

The ^1H NMR spectrum displayed 10 proton signals at the aromatic region, including three singlets belong to H-5 (δ 6.31), H-5' (δ 6.35), H-8 (δ 6.63) and typical type VI bisbenzylisoquinoline of H-10 was also observed at (δ 5.44). This spectrum also

displayed four doublet of doublets attributable to H10', H11', H13' and H14' were present at δ 6.93, 6.37, 6.95 and 7.42 respectively. In addition, H-13, H-14 appeared as a broad doublet at δ 6.76 and 6.80 respectively with the coupling constant of 8.0 Hz.

^{13}C NMR spectrum showed, signals at δ 63.9 and 61.5 belonged to the chiral centers of C-1 and C-1'. The signals of *N*-methyls was observed at δ 43.7 and 42.1 while four methoxy carbons resonated at δ 54.9, 55.9, 56.0 and 60.5 which corresponded to C-6, C-12, C-6' and C-7' respectively. Carbon in aromatic regions resonated at lower chemical shift, δ 110.9, 105.7, 110.7, 116.7, and 116.4 may belonged to C-5, C-5', C-13, C-8 and C-10 whereas the signals of substituted aromatic carbon atoms were observed at the higher chemical shifts for C-11, C-6, C-6',C-7' and C-12 at δ 149.0, 148.3, 151.6, 137.0 and 146.6 respectively. The higher chemical shifts carbons were affected by the presence of electronegative substituents effect. The methylene carbons for C-*α* and C-*α'* were observed at δ 37.5 and 39.5 typical for methylene positions.

The COSY spectrum showed the correlations of vicinal proton between H-13'/ H-14', H-10'/11' and H-3/H-4.

In the HMBC spectrum these methylene proton belongs to H*α* and H*α*' correlated with C1 and C1', suggesting that benzyl substituent attached at position C1 and C1'. In addition the HMBC correlations clearly proved that the presence of two diaryl ether bridges were located between C7-C8' and C11-C12' by exhibiting the following correlation; H5/C7, H1'/C8', H13/C11 and H10'/C12'.

Based on the above analysed data, it can be proposed that alkaloid **50** has structure as shown below**.** Through comparison of all obtained data with the literatures values confirmed that alkaloid **50** is gyrolidine.[85]

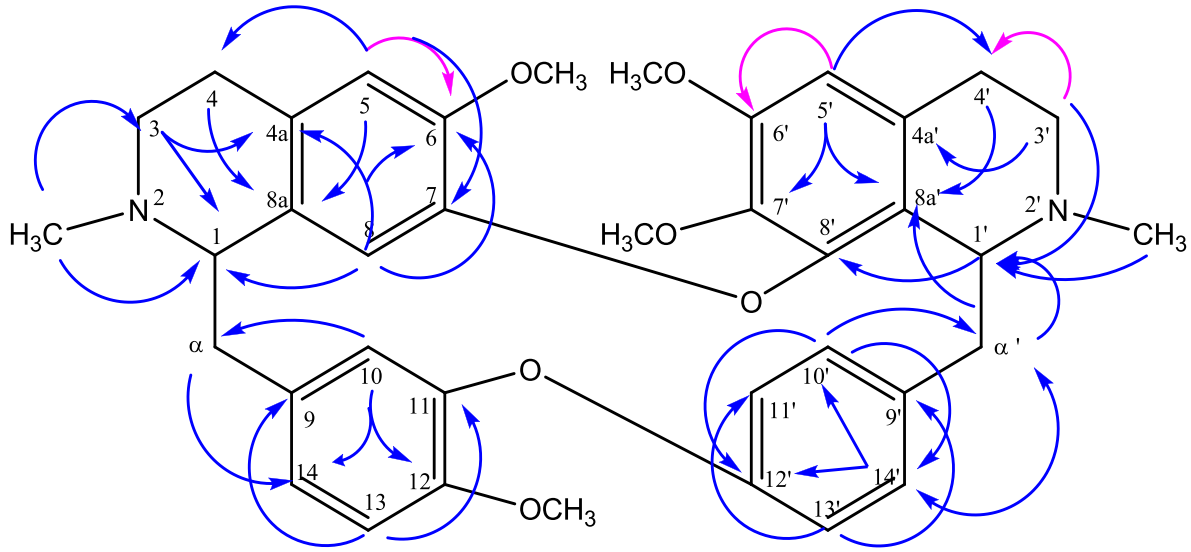


Figure 3.44: ^{1}H-^{13}C Correlations observed in HMBC Spectrum of Alkaloid **50**

Table 3.7: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **50**

Position	^{1}H- NMR (δ(Hz))	^{13}C- NMR(δ)	HMBC
1	3.64 m	63.9	*N*-CH_3, 4a
N-Me	2.57 s	43.7	
3	2.76 m	50.8	
	2.42 m		
4	2.34 m	28.4	
4a		130.7	
5	6.31 s	110.9	4,6,7,8a
6		148.3	
6-OMe	3.62 s	54.9	
7		143.8	
8	6.63 s	116.7	1,4a,6,7
8a		127.3	
α	3.15 m	37.5	
	2.86 (dd,14.6,3.6)		
9		130.7	
10	5.44 brs	116.4	*α* , 11,12,14
11		149.0	
12		146.6	
12-OMe	3.88 s	55.9	
13	6.76 (d 8.0)	110.7	9,11
14	6.80 (d 8.0)	123.5	*α* , 11
1’	4.21 (brd,5.4)	61.5	3’,8’,8a’, *α* ’9’
N’-Me	2.66 s	42.1	1’,3’
3’	3.21 m	45.3	4’,4a’
	2.93 m		
4’	3.03 m	25.4	
	2.70 m		
4a’		127.2	
5’	6.35 s	105.7	4’,6’,7’,8a’
6’		151.6	
6’-OMe	3.78 s	56.0	
7’		137.0	
7’-OMe	3.18 s	60.5	
8’		147.5	
8’a		138.9	
α ’	3.35 m	39.5	1’,8a’, *α* ’,9’,10’,14’
	2.80 (dd,14.6,5.9)		
9’		127.7	
10’	6.93 (dd,8.72,2.7)	131.4	*α* ’,9’,11’,12’,14’
11’	6.37 (dd,8.24,2.2)	121.1	10’
12’		152.2	
13'	6.95 (dd,8.72,2.7)	122.3	9’,11’
14’	7.42 (dd,8.72,1.8)	127.8	*α* ’,10’,12’

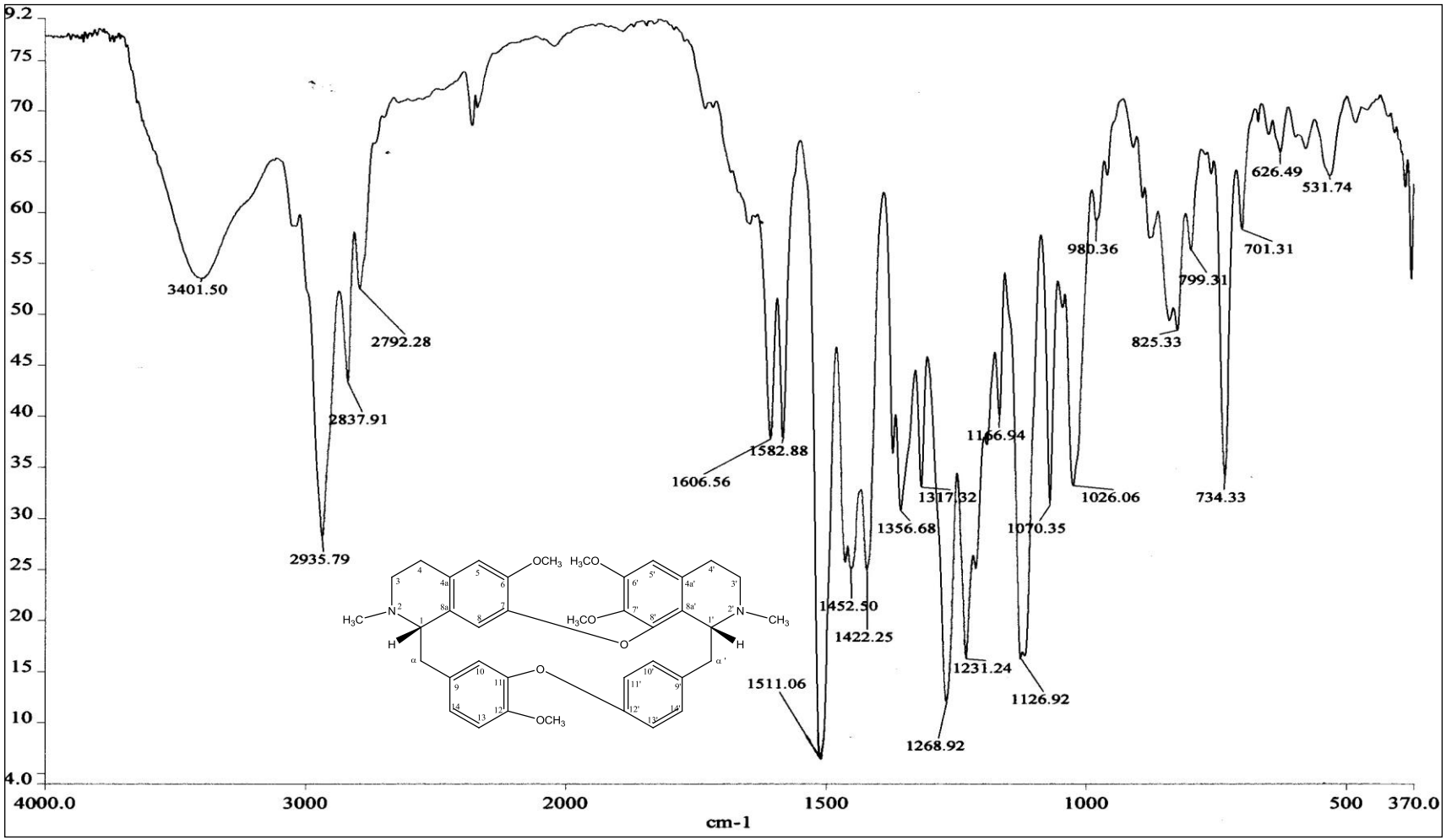


Figure 3.45: Infrared Spectrum of Alkaloid **50**

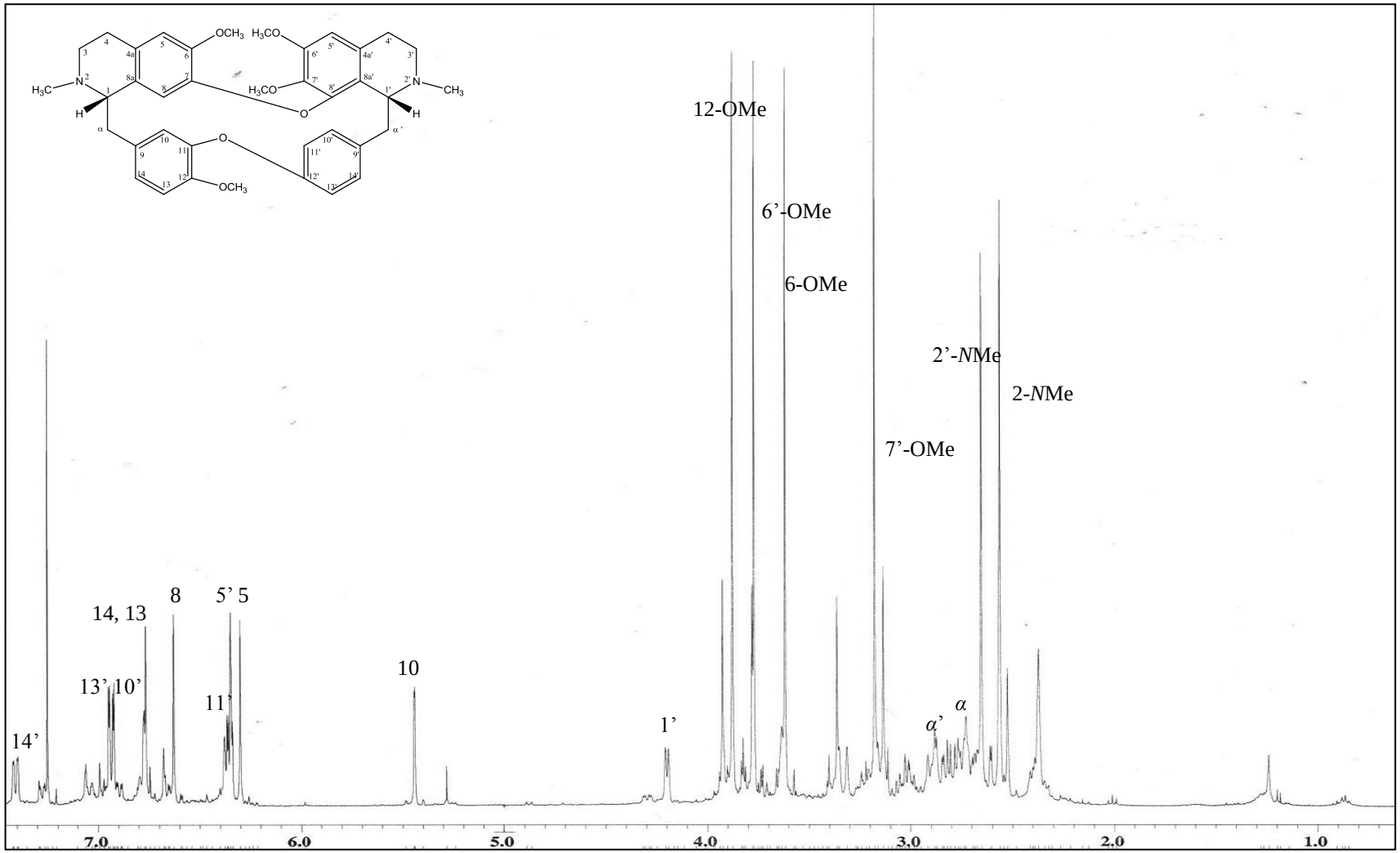


Figure 3.46: ^{1}H NMR Spectrum of Alkaloid **50**

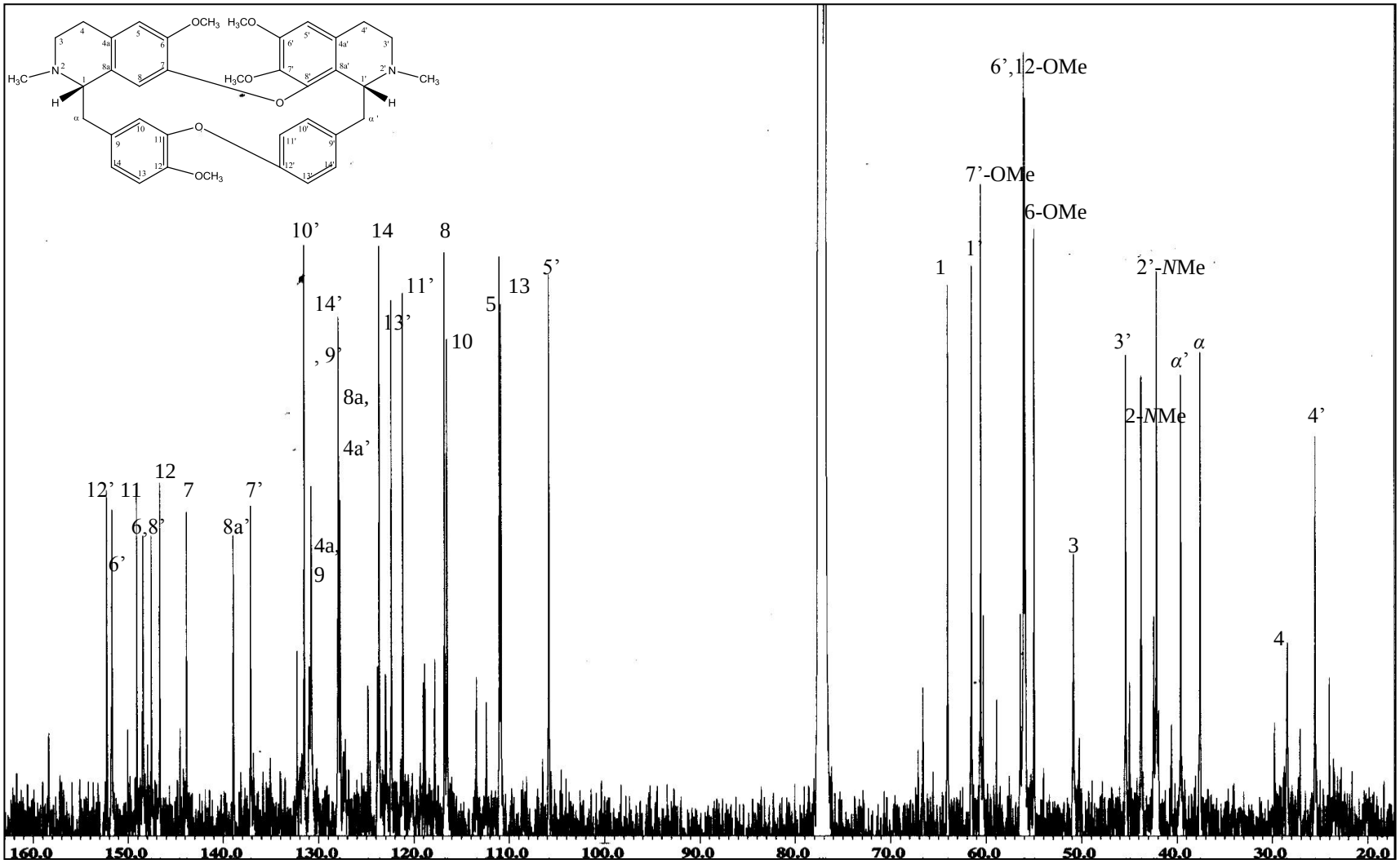


Figure 3.47: ^{13}C NMR Spectrum of Alkaloid **50**

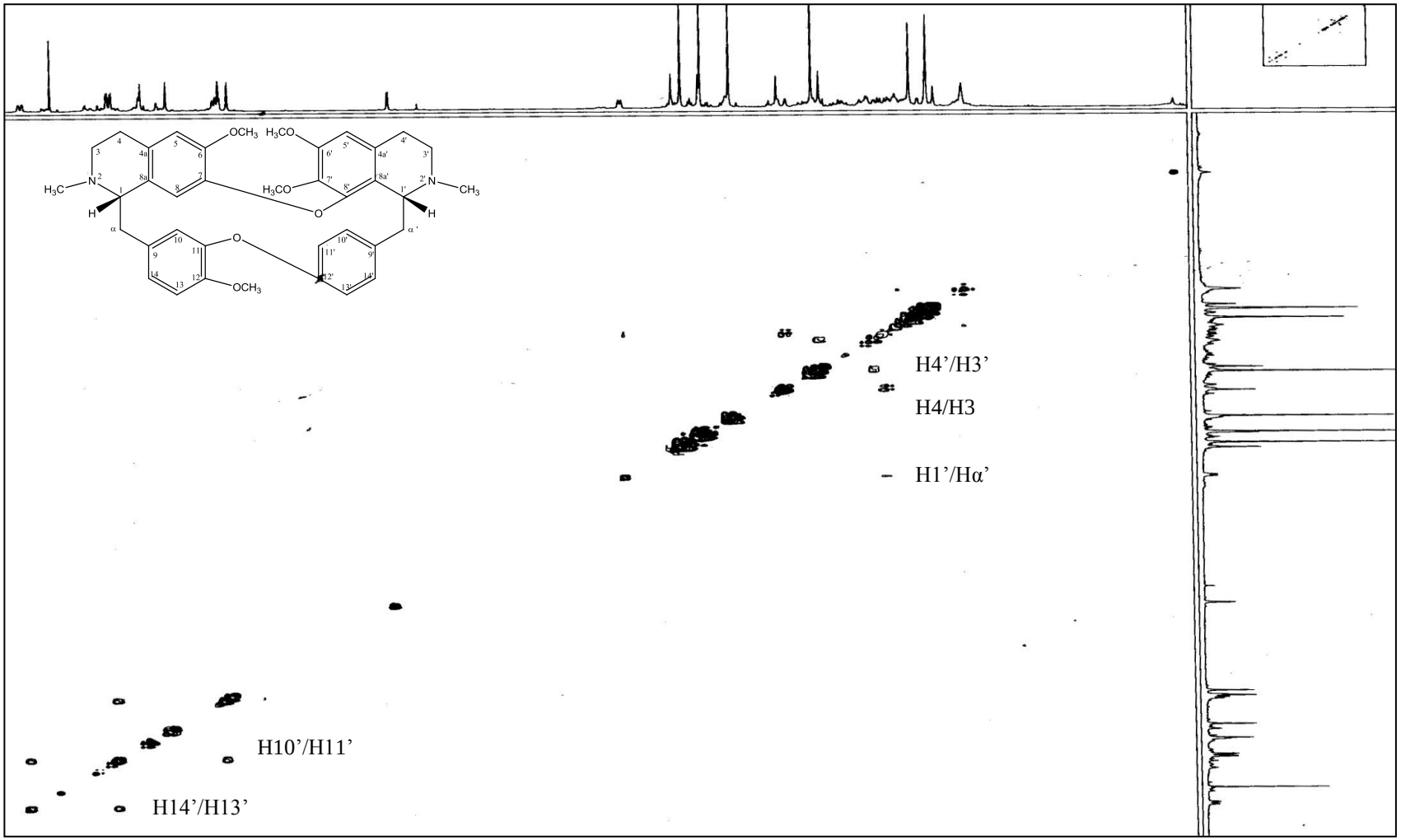


Figure 3.48: COSY Spectrum of Alkaloid **50**

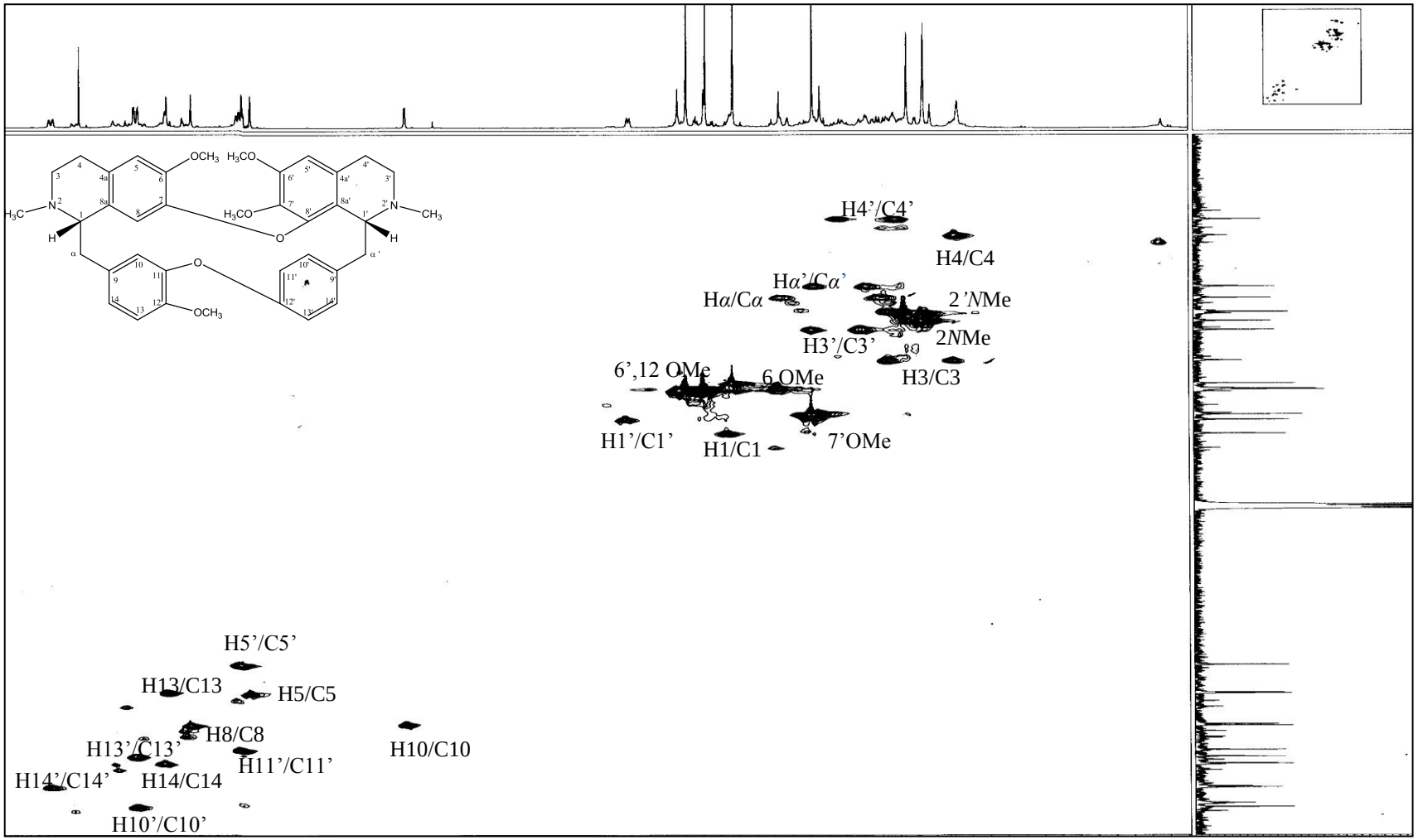


Figure 3.49: HSQC Spectrum of Alkaloid **50**

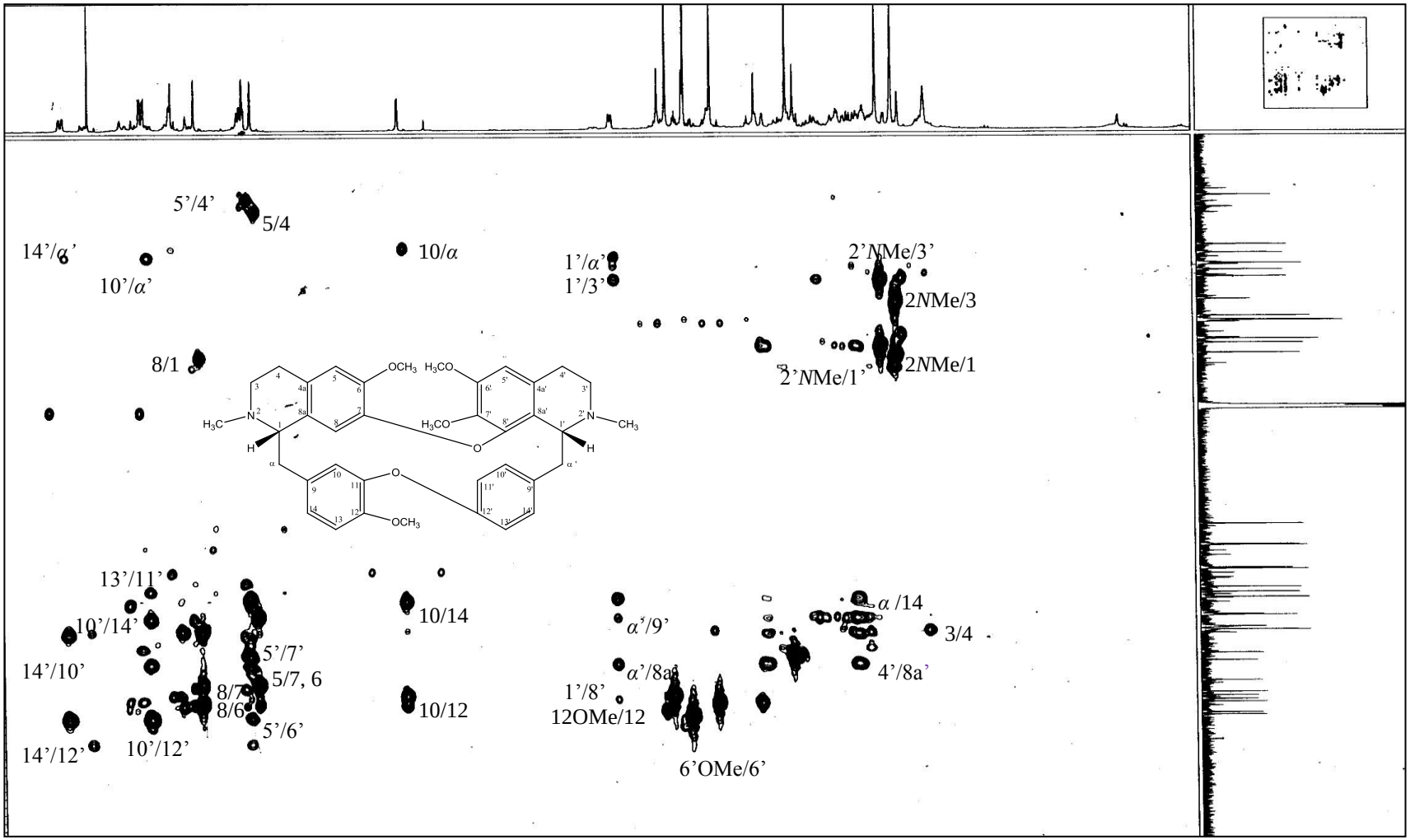
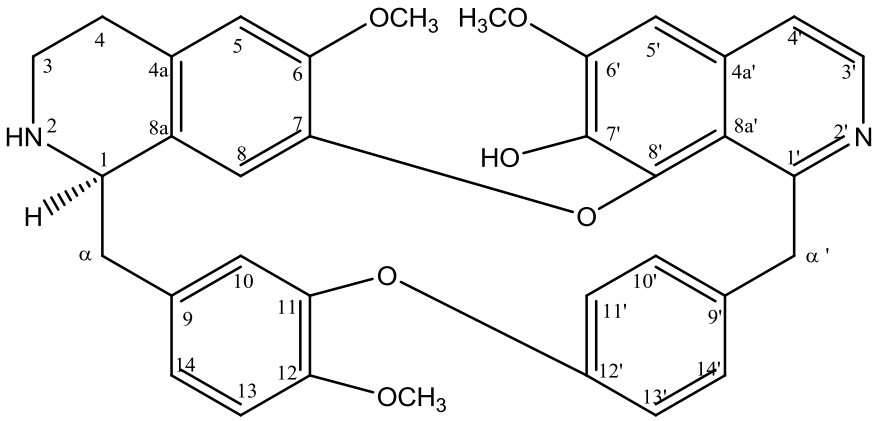


Figure 3.50: HMBC Spectrum of Alkaloid **50**

3.1.8 Norstephasubine, 51



51

Alkaloid **51** was isolated as a pale yellow amorphous solid with $[\alpha]_D^{25}$ +309 (c=1.0, MeOH) indicating the absolute configuration at C-1' as *R* as in the known alkaloids[86]. The UV absorption bands at 240, 286 and 338 nm were indicative of a bisbenzylisoquinoline structure.[36] Moreover, the IR spectrum confirmed this evidence by showing the imine and hydroxyl absorptions at 1606 and 2929 cm^{-1}. The ESI^+ mass spectrum exhibited a molecular ion peak at m/z 576 suggesting a molecular formula of $C_{35}H_{32}N_2O_6$.

The ^1H NMR spectrum showed two doublets at δ 8.43 and 7.47 with a coupling constant of 5.6 Hz corresponding to H3' and H4' respectively. The former peak was assigned in the downfield region due to the fact that it was attached to the carbon adjacent to nitrogen atom and as result it was deshielded compared to the latter. In addition, the presence of deshielding germinal methylene protons belong to Hα' with a large coupling constant of 13.6 Hz is due to the benzylic methylene adjacent to the pyridine ring. Alkaloid showed the absence of an upfield *N*-methyl singlet which normally appeared at δ 2.56. Moreover, this compound bears three aromatic methoxy groups as shown by three singlets at δ 4.05, 3.88 and 4.04 which most probably

belonged to C6, 12 and 6'. The presence of hydroxyl group, as indicated by the IR absorption and the absence of the relatively upfield shifted 7'-methoxy proton signal suggested the presence of 7'-hydroxyl group in the structure. This was confirmed by the HMBC correlation between H5' and the quaternary carbon signal at δ 135.7 (C7'). This hypothesis was further supported by NOESY spectrum, showing that the proton belongs to H 5', 5 and 13 only correlates with three methoxy proton attributed to 6, 6' and 12 methoxyl groups.

The COSY spectrum showed cross peaks between CH-14'/CH-13', CH10'/CH11'and CH-4'/CH-3', as shown in Figure 3.55.

Analysis of the ^{13}C NMR and HMQC spectra of alkaloid **51** indicated the presence of thirty five carbons atoms. A downfield signal at δ 157.0 was belonged to an imine carbon located at C-1'.

Comparison of the observed data with the literature values confirmed alkaloid **51** is indeed (+)-norstephasubine.[86]

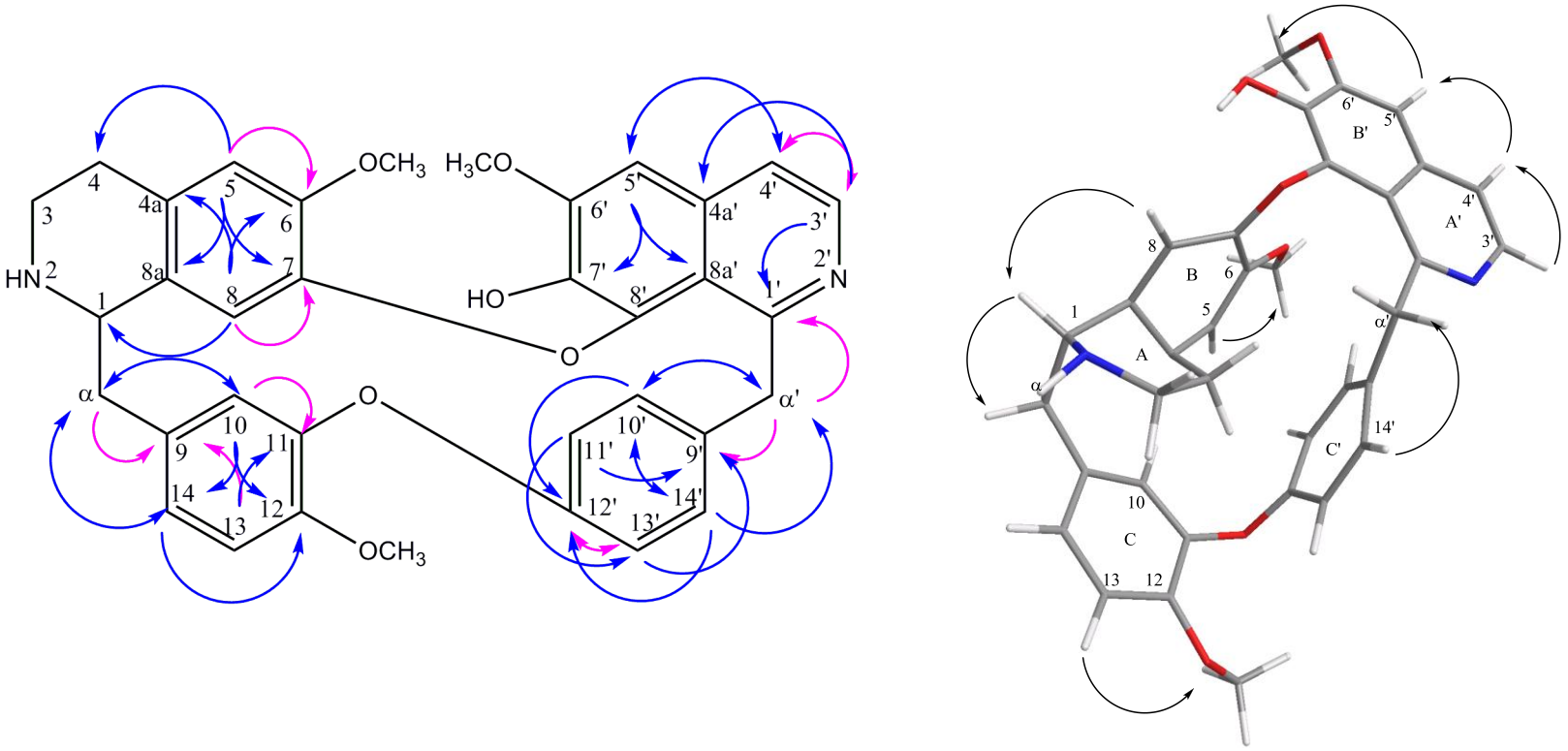


Figure 3.51: HMBC and NOESY Correlations of Alkaloid **51**

Table 3.8: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **51**

Position	^{1}H- NMR (δ (Hz)) alkaloid **51**	^{1}H- NMR (δ (Hz)) Norstephasubine[86]	^{13}C-NMR(δ)	HMBC
1	4.09 m	4.02 m	54.6	
3	2.90 m		41.5	
	2.55 m			
4	2.38 m		29.7	
	2.20 m			
4a			129.8	
5	6.56 s	6.53 s	112.4	4,6,7,8a
6			147.5	
6-OMe	4.05 s	4.02 s	56.1	
7			144.6	
8	6.04 s	6.02 s	110.9	1,4a,6,7
8a			127.9	
α	2.73 m		38.6	10, 14, 9
	2.70 m			
9			127.9	
10	4.88 d (1.7)	4.87 d	116.6	*α*,11,12,14
11			150.4	
12			146.9	
12-OMe	3.88 s	3.88 s	55.9	
13	6.72 d (8.3)	6.73 d	110.8	9, 11, 12
14	6.65 dd (8.2,2.0)	6.71 dd	122.6	*α*, 12, 9
1’			157.0	
3’	8.43 d (5.6 Hz)	8.41 d (5.6)	140.4	1’, 4’,4a’
4’	7.47 d (5.6 Hz)	7.46 d (5.6)	119.0	4a’,3’, 5’
4a’			133.4	
5’	6.97 s	6.93 s	101.6	4’,6’,8a’
6’			151.3	
6’-OMe	4.04 s	4.03 s	56.3	
7’			135.7	
8’			145.2	
8’a			137.0	
α’	5.36 d (13.6)	5.35 d (13.7)	45.3	1’, 9’, 10’
	4.51 d (13.6)	4.50 d (13.7)		
9’			137.6	
10’	7.09 dd (8.0)	7.09 dd	129.2	*α* ’,12’,14’
11’	6.65 dd (8.2,1.9)	6.66 dd	122.6	9’, 12’, 13’
12’			152.3	
13'	6.43 dd (8.2,2.4)	6.43 dd	121.9	9’,11’,12’
14’	7.37 dd (7.1)	7.37dd (2.1,8.4)	127.8	*α* ’,10’,12’

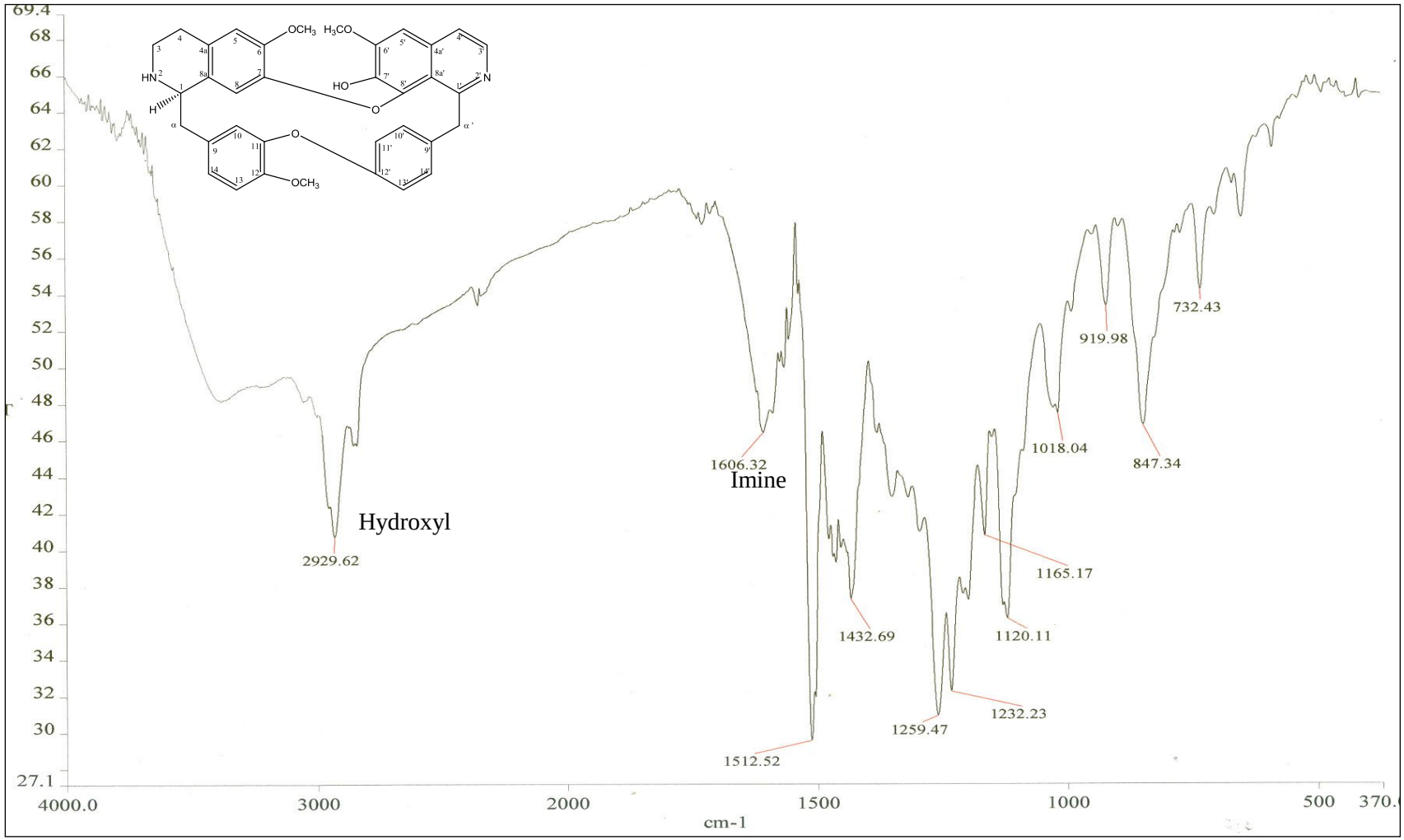


Figure 3.52: Infrared Spectrum of Alkaloid **51**

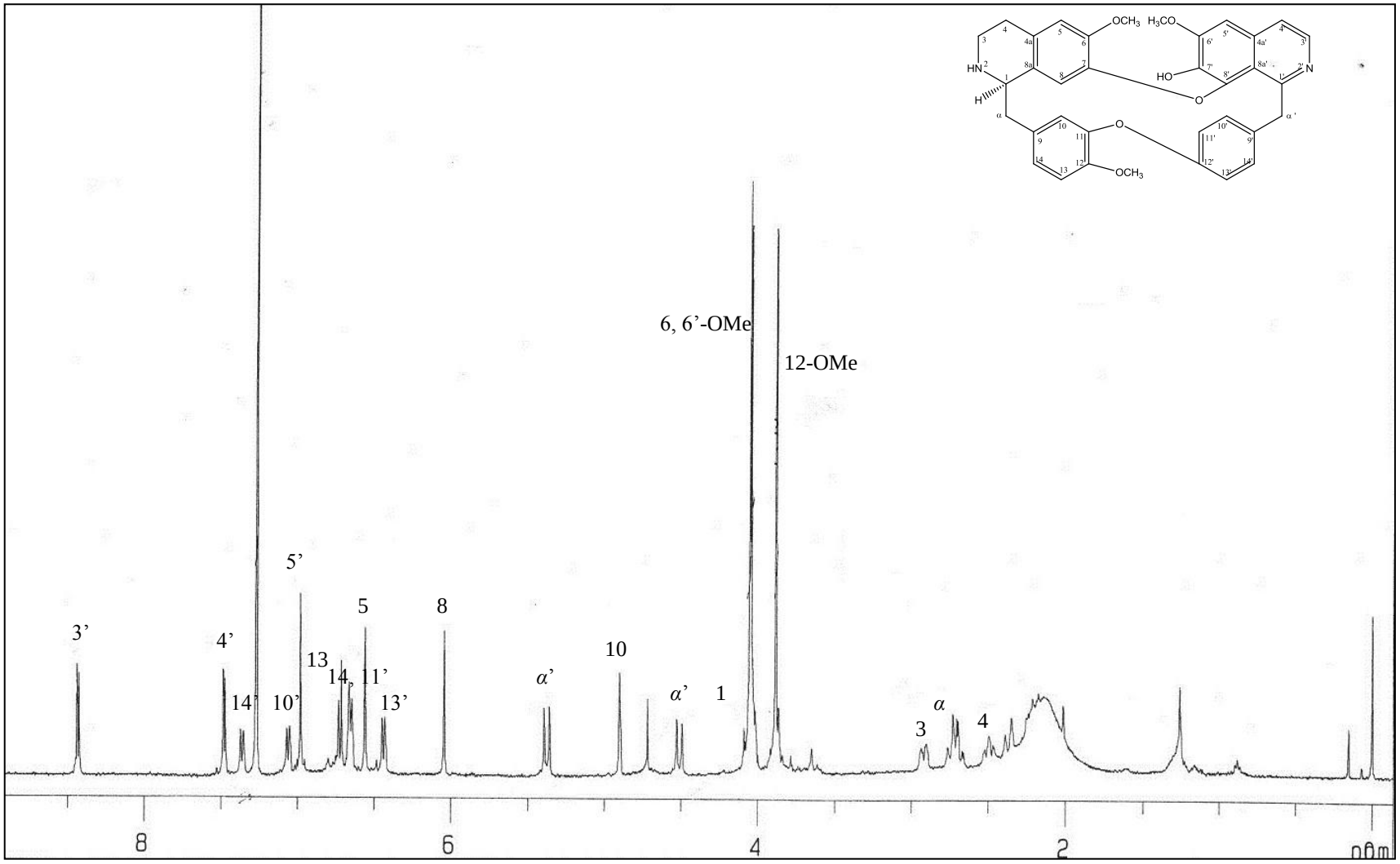


Figure 3.53: ^{1}H NMR Spectrum of Alkaloid **51**

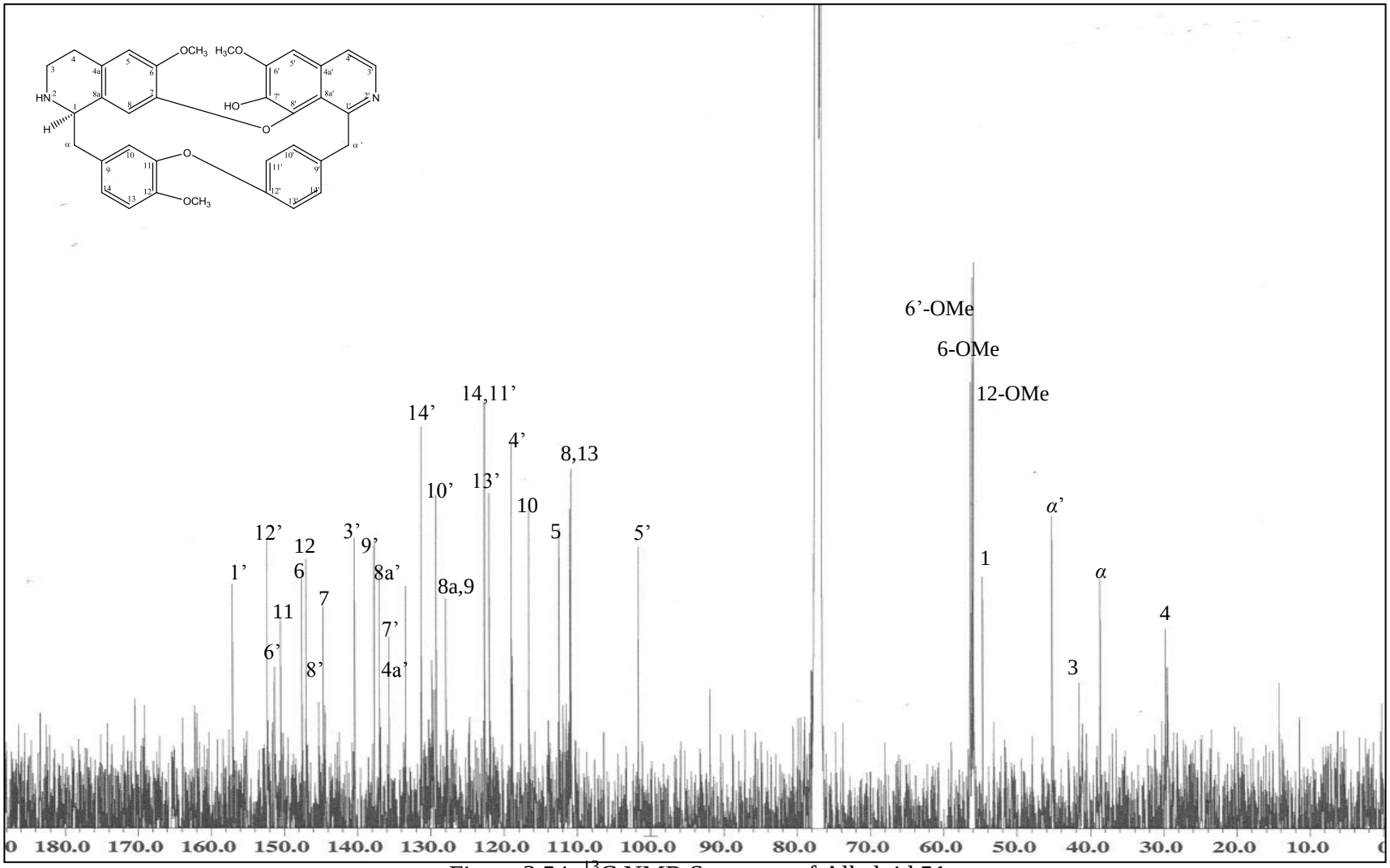


Figure 3.54: ^{13}C NMR Spectrum of Alkaloid **51**

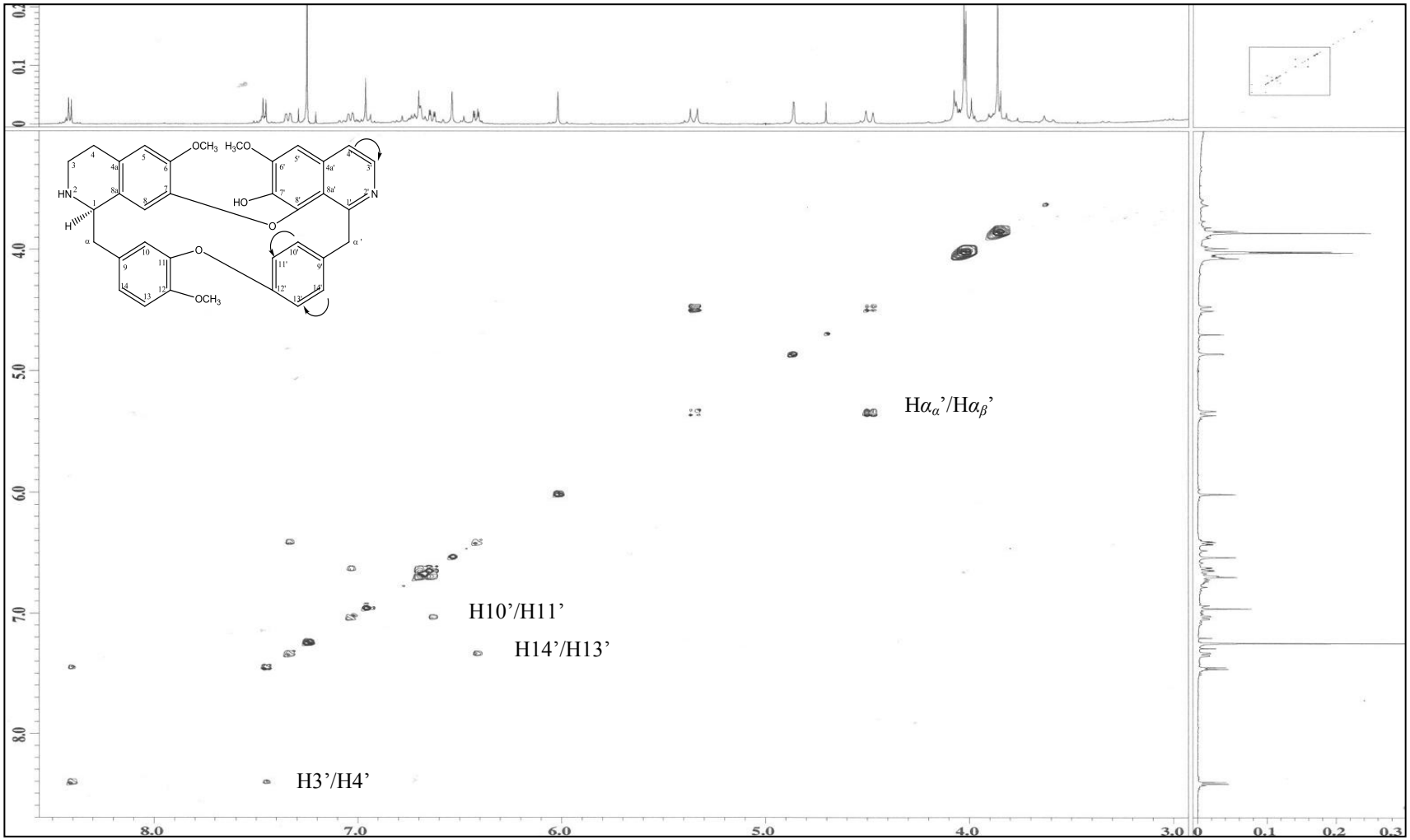


Figure 3.55: COSY Spectrum of Alkaloid **51**

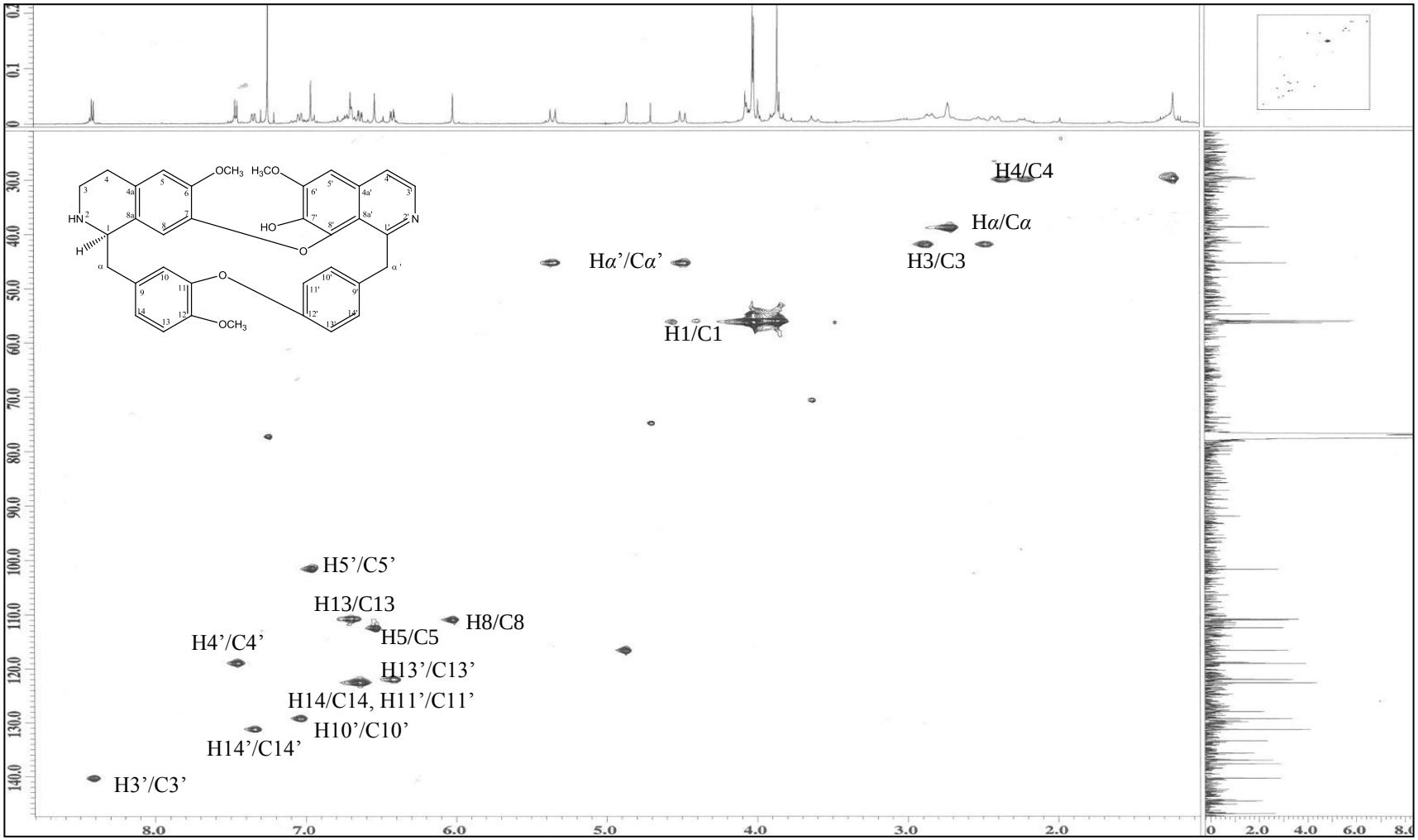


Figure 3.56: HMQC Spectrum of Alkaloid **51**

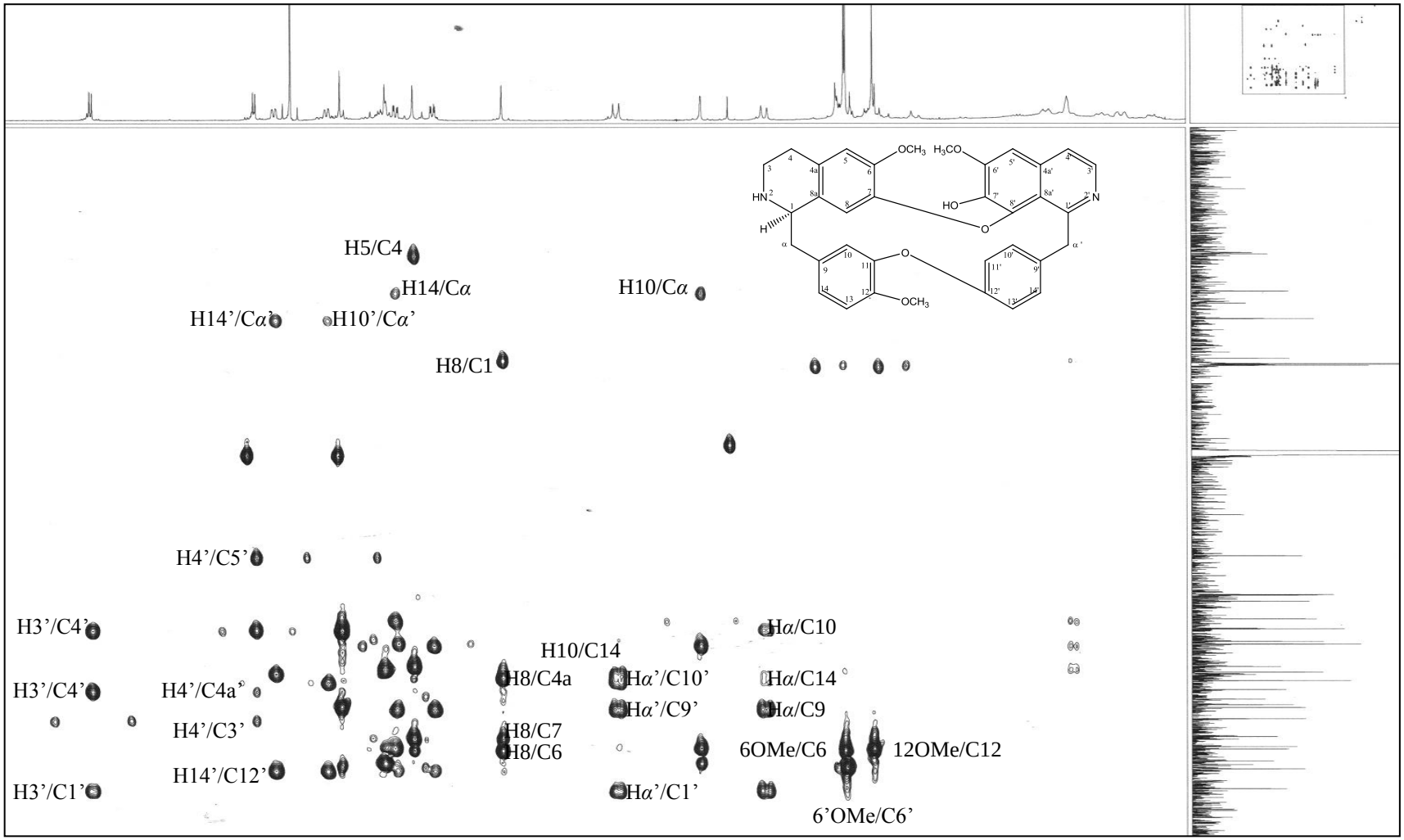


Figure 3.57: HMBC Spectrum of Alkaloid **51**

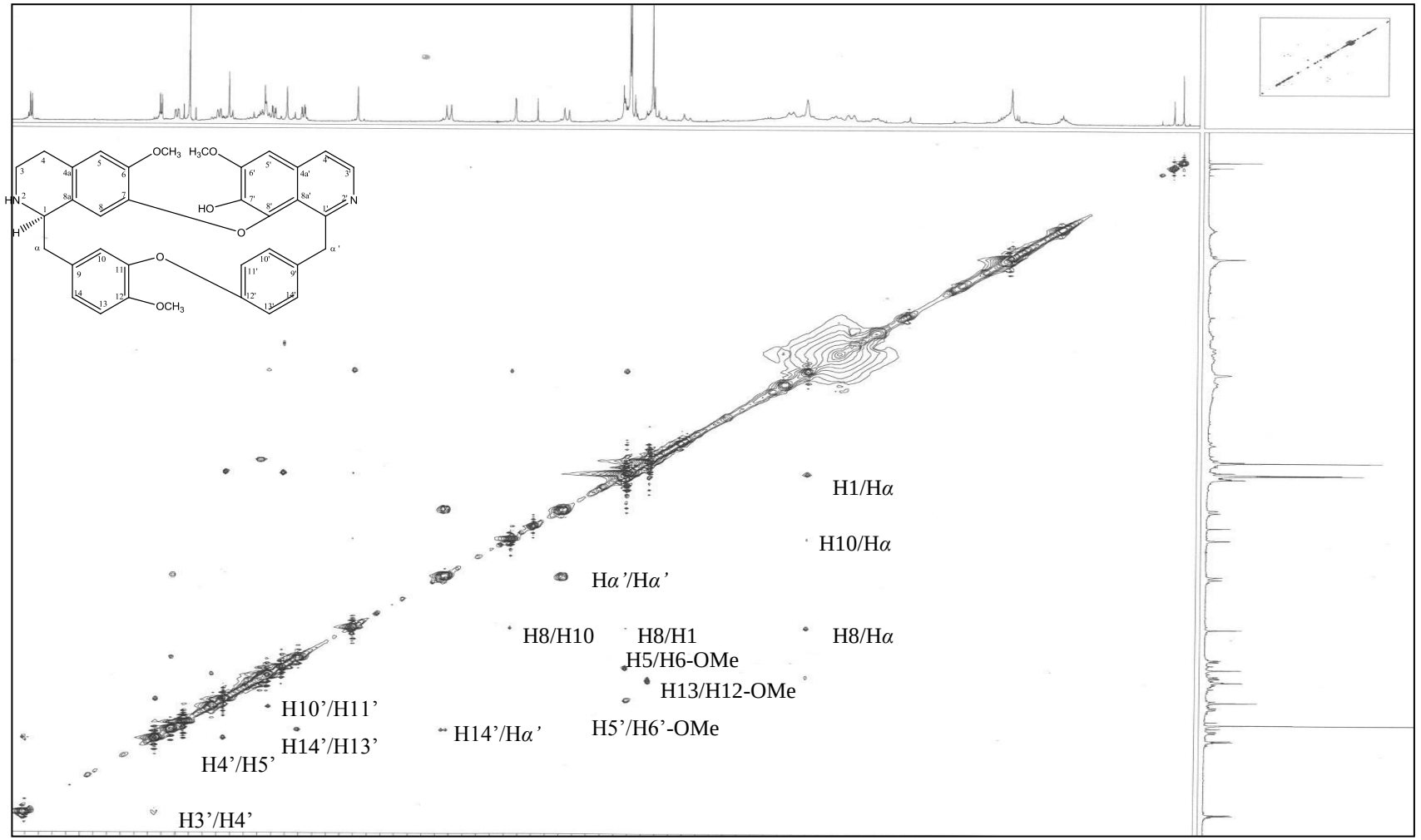
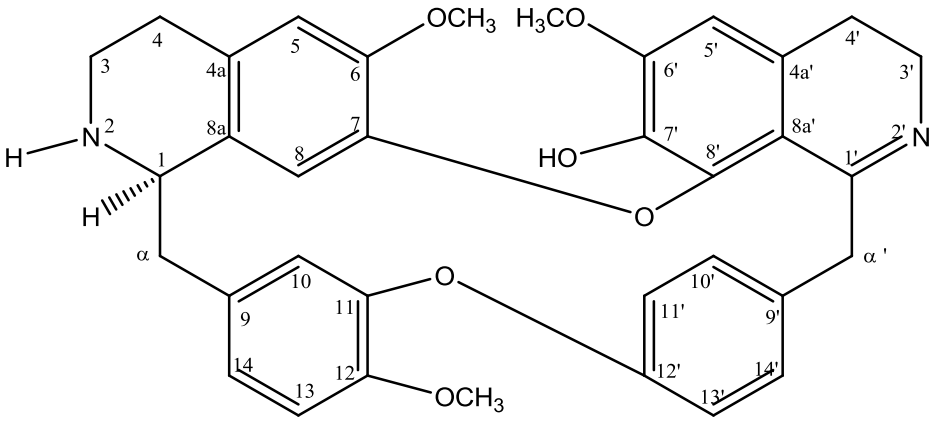


Figure 3.58: NOESY Spectrum of Alkaloid **51**

3.1.9 3′, 4′-dihydronorstephasubine, 52



52

Alkaloid **52** was isolated as a brown amorphous solid with $[\alpha]_D^{25}$ +22 (c=0.5, MeOH). The HRESIMS spectrum of alkaloid **52** showed a pseudomolecular ion peak at *m/z* 579.2535 $[M+H]^+$ corresponding to the molecular formula of $C_{35}H_{34}N_2O_6$ (calcd 579.2495). An absorption band at 1604 and 2934 cm^{-1} in the IR spectrum is typical of an imine and hydroxyl absorption band. [20]

The gross features of the ^{1}H and ^{13}C-NMR spectra of alkaloid **51** and **52** confirmed a close structural relationship between these two compounds. Indeed, the same substitutents could be characterized in both compounds through HMBC and NOESY experiment. However, one distinct different feature was apparent in the ^{1}H-NMR spectrum, the signal corresponding to the C3' and C4' at deshielding area was absent in the^{1}H-NMR of alkaloid **52**. In the ^{1}H-NMR spectrum, signals for ten aromatic protons, three methoxy groups, two CH_2-CH_2-N groups, and a set of isolated none equivalent methylene protons were observed. Among the ten aromatic proton signals, three singlets at δ 6.43, 6.52, and 6.10 were attributed to H-5, H-5′, and H-8, respectively. The upfield signal of H-10 (δ 4.95, broad singlet) was a characteristic peak of head to head and tail to tail bisbenzylisoquinoline alkaloid (two ether linkages between 7-8′, 11-12′).[90] H-10

signal appeared as a broad singlet because it was *meta*-coupled with H-14 (δ 6.68, d), which was placed vicinal to H-13 (δ 6.72, dd), indicating that ring C was trisubstituted. Other prominent peaks of an AX spin system were observed at δ 3.96 and 4.50 (J = 13.7 Hz), supporting the presence of two geminal protons of the methylene adjacent to the imine function (2H-α'). Three broad doublet signals of H-10′ (δ 7.27), H-13′ (δ6.74), and H-14′ (δ 7.41), and one doublet of doublets of H-11′ (δ 6.40), indicating that ring C′ was *para* disubstituted (AA′BB′) ring system.

The ^{13}C NMR spectrum showed 35 carbon resonances, which were in agreement with the molecular formula. The signals at δ 54.5 and 165.1 could be assigned to the chiral (C-1) and imines (C-1′) carbons, respectively.[87]

The position of $\Delta^{1'\text{-}N'}$ double bond on the right side of the dimer was confirmed by the HMBC correlations of H-3′ to C-1′ (δ 165.1) and H-α' to C-1′ and C-8a′ (δ 116.3). The position of the three methoxy groups, was assigned by detecting the cross-peaks in the NOESY spectrum between H-5 (δ 6.43)/6-OCH_3 (δ 3.91), H-5' (δ 6.52)/6'-OCH_3 (δ 3.86), and H-13 (δ 6.72)/12-OCH_3 (δ 3.86) respectively. Furthermore, the COSY spectrum, showed the correlation of vicinal proton between CH14'/CH13', CH10'/CH11', CH3'/CH4', CH3/ CH4.

Based on the spectroscopic findings alkaloid **52** could be proposed a new compound, namely 3′, 4′-dihydronorstephasubine. [61, 86]

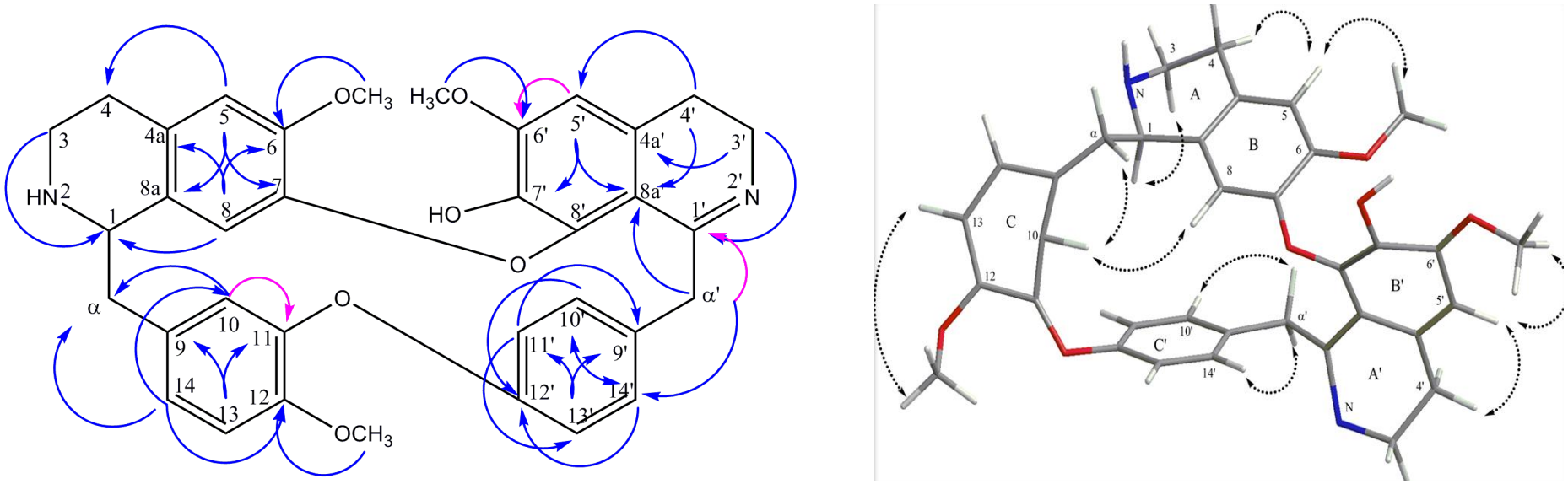


Figure 3.59: HMBC and NOESY Correlation of alkaloid **52**

Table 3.9: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **52**

Position	^{1}H- NMR (δ (Hz))	^{13}C- NMR(δ)	HMBC
1	3.90 m	54.5	
3	2.12 m	41.2	1, 4a
	2.77 m		
4	2.14 m	29.4	4a
	2.28 m		
4a		130.2	
5	6.43 s	112.7	4, 6, 7, 8a
6		148.0	
6-OMe	3.91 s	55.9	6
7		145.5	
8	6.10 s	112.5	1,4a,6,7
8a		127.0	
α	2.77-2.82 m	38.6	
9		127.9	
10	4.95 br s	116.9	α, 11, 12, 14
11		150.5	
12		147.0	
12-OMe	3.86 s	56.2	12
13	6.72 dd (2.0, 8.7)	110.8	9,11
14	6.68 d (8.7)	122.6	α , 10, 12
1’		165.1	
3’	3.60 m	46.7	1’, 4a’
	3.84 m		
4’	2.65 m	27.0	4a’,8a’
4a’		131.5	
5’	6.52 s	105.9	4’,6’,7’,8a’
6’		150.9	
6’-OMe	3.86 s	56.2	6’
7’		136.1	
8’		142.0	
8’a		116.3	
α’	3.96 d (13.7)	44.7	1’, 8a’ ,9’,
	4.50 d (13.7)		10’, 14’
9’		135.3	
10’	7.27 d (8.2)	132.1	12’, 14’, α’
11’	6.40 dd (2.0, 8.2)	121.9	9’, 12’, 13’
12’		152.6	
13'	6.74 d (8.6)	122.4	9’, 11’
14’	7.41 d (8.6)	128.8	α’, 10’, 12’

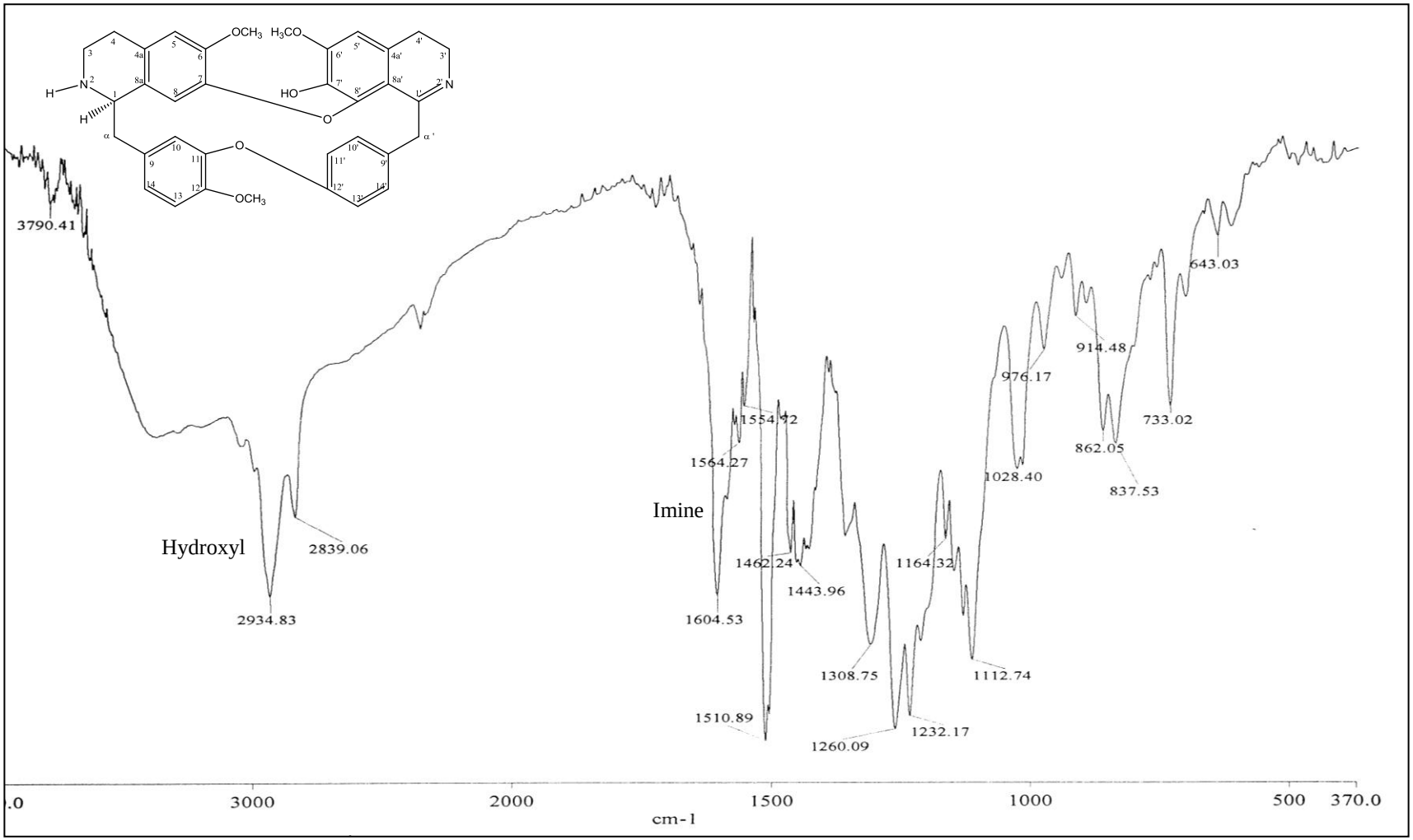


Figure 3.60: Infrared Spectrum of Alkaloid **52**

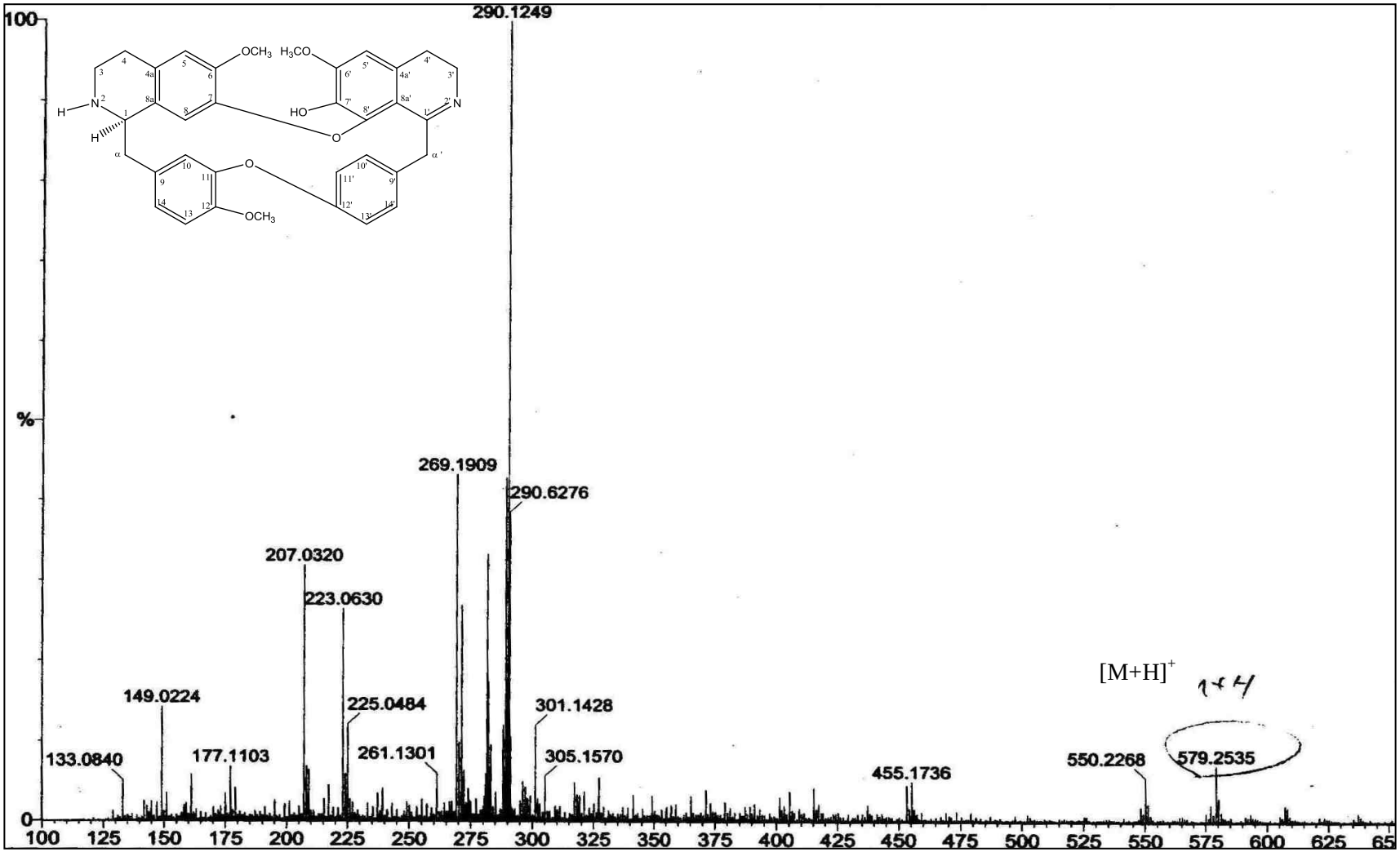


Figure 3.61: HRMS Spectrum of Alkaloid **52**

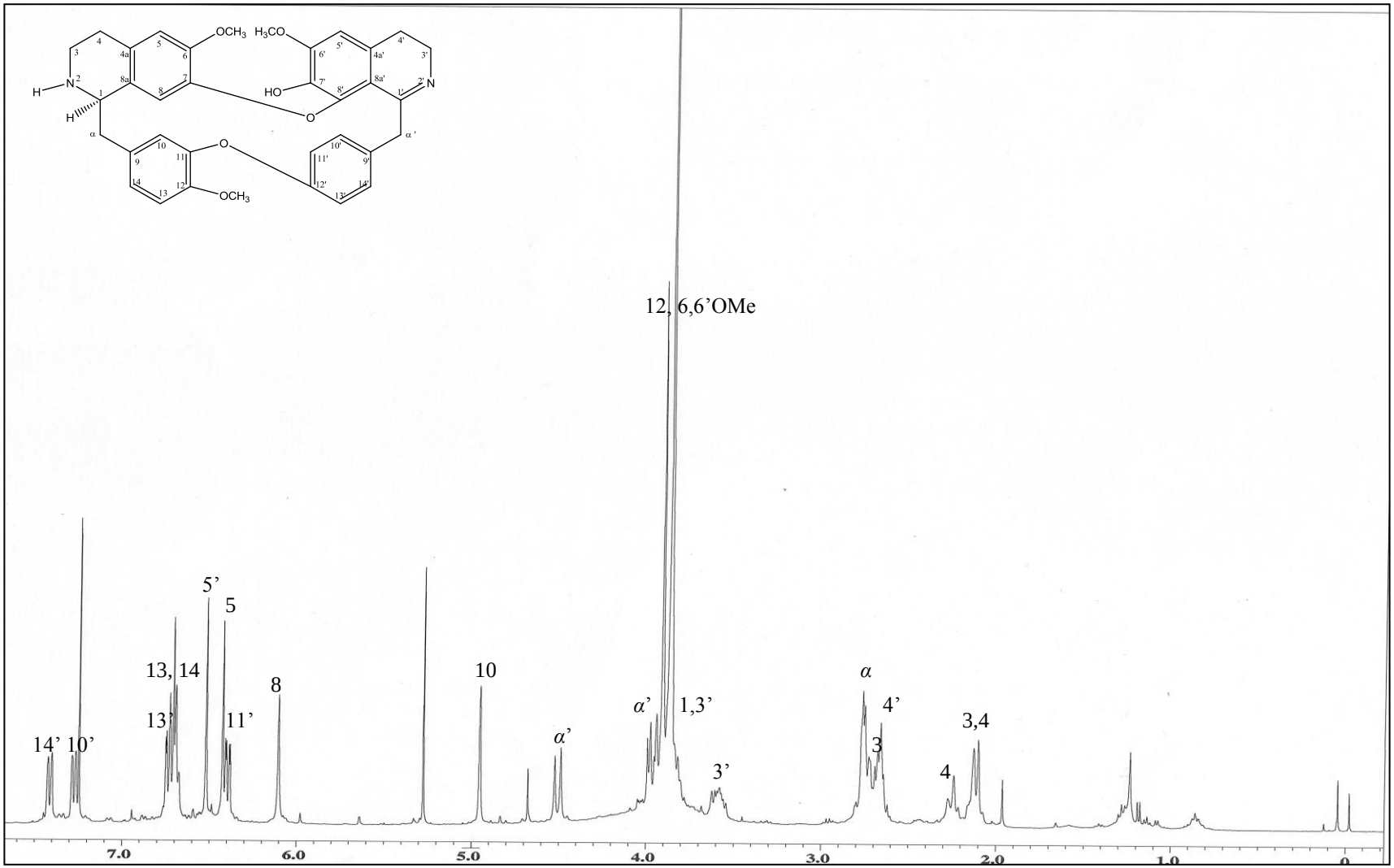


Figure 3.62: ^{1}H NMR Spectrum of Alkaloid **52**

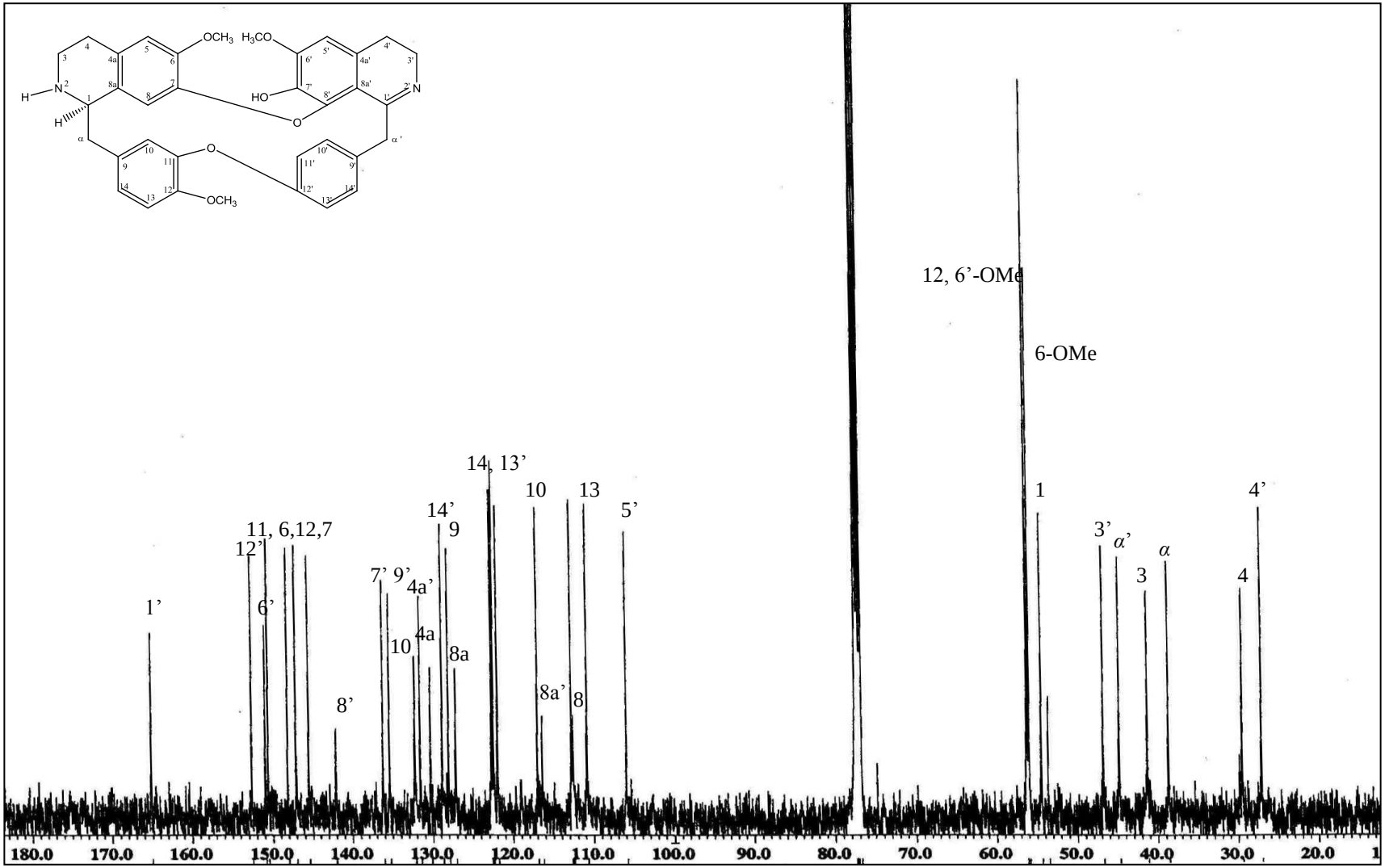


Figure 3.63: ^{13}C NMR Spectrum of Alkaloid **52**

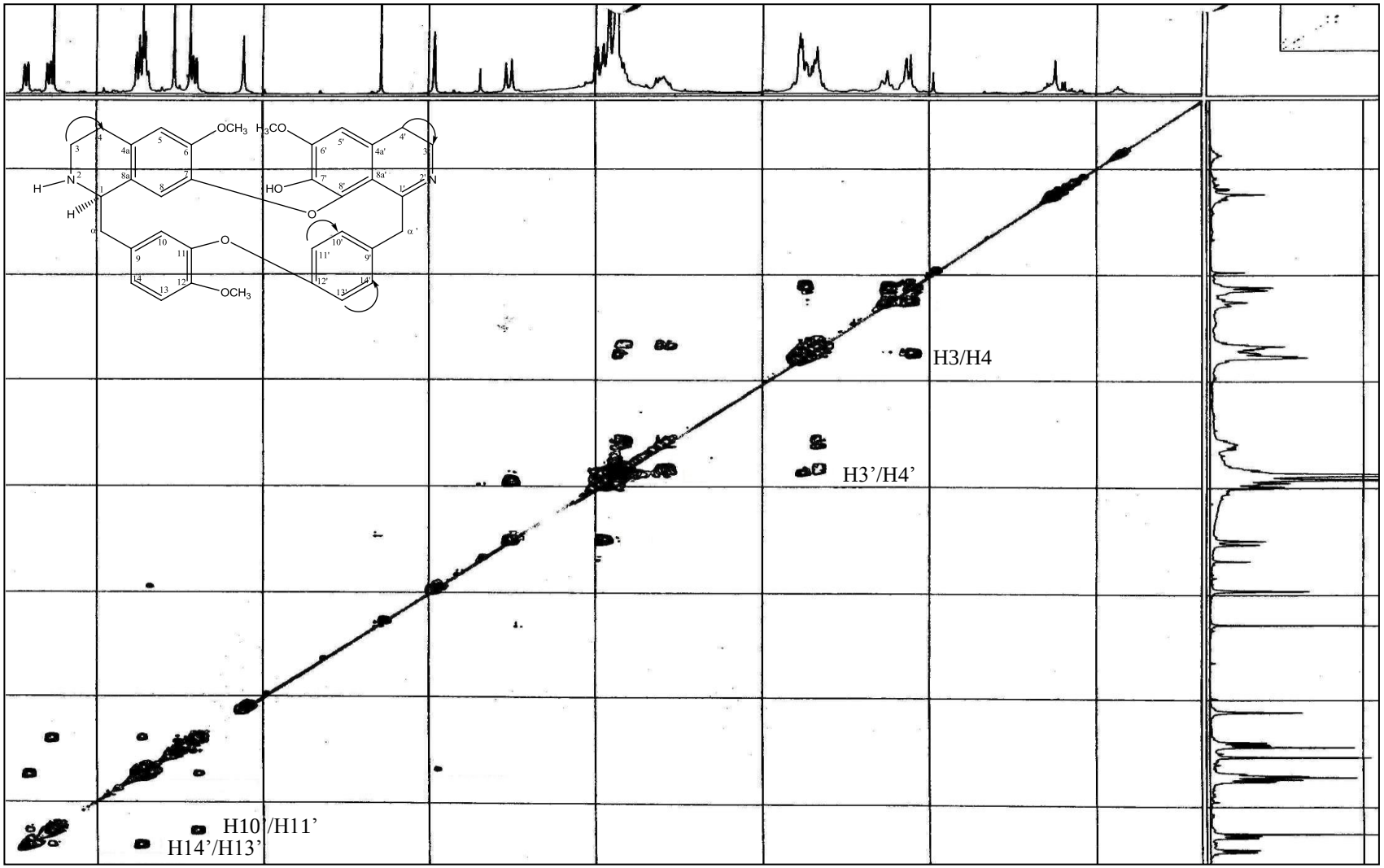


Figure 3.64: COSY Spectrum of Alkaloid **52**

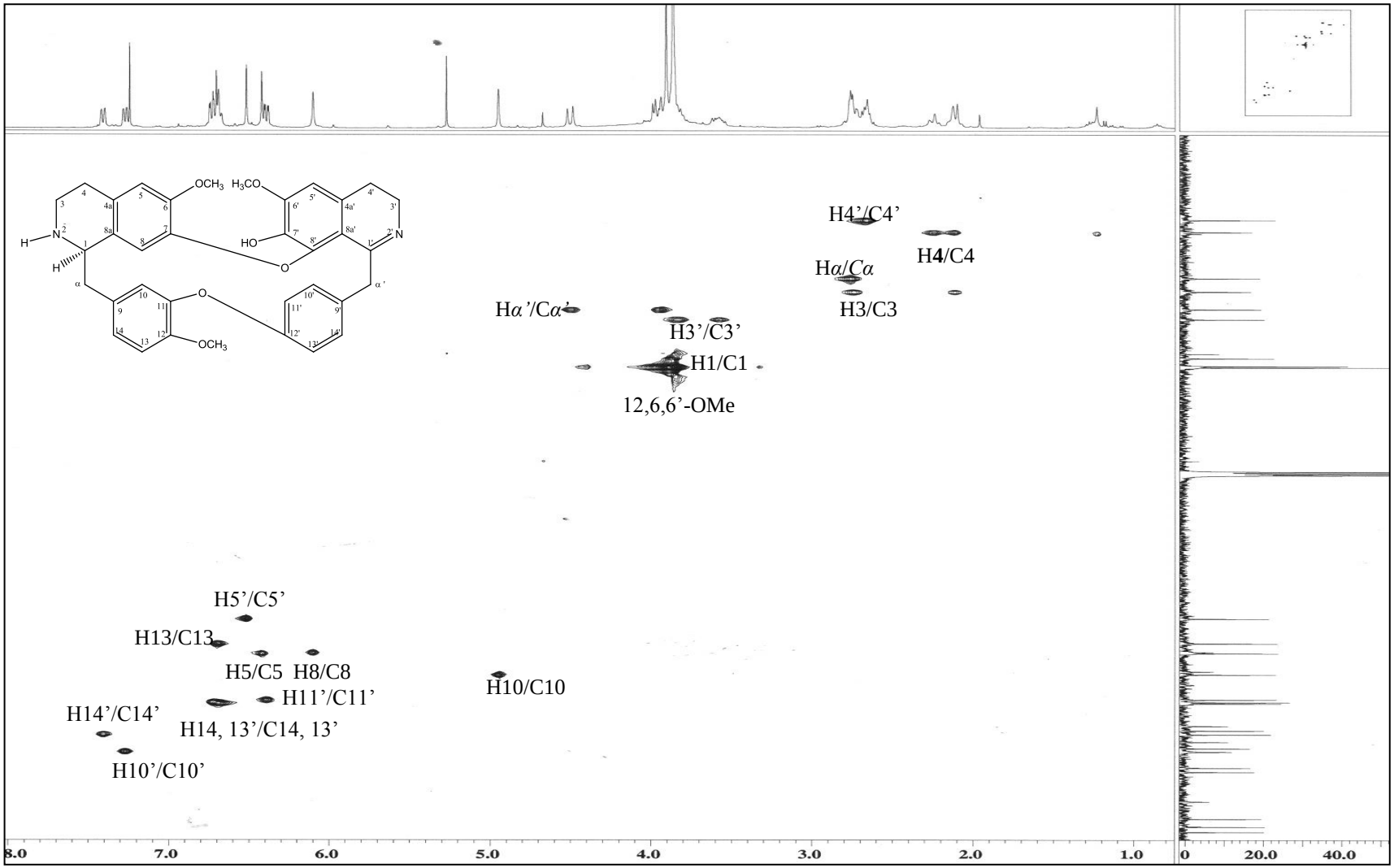


Figure 3.65: HSQC Spectrum of Alkaloid **52**

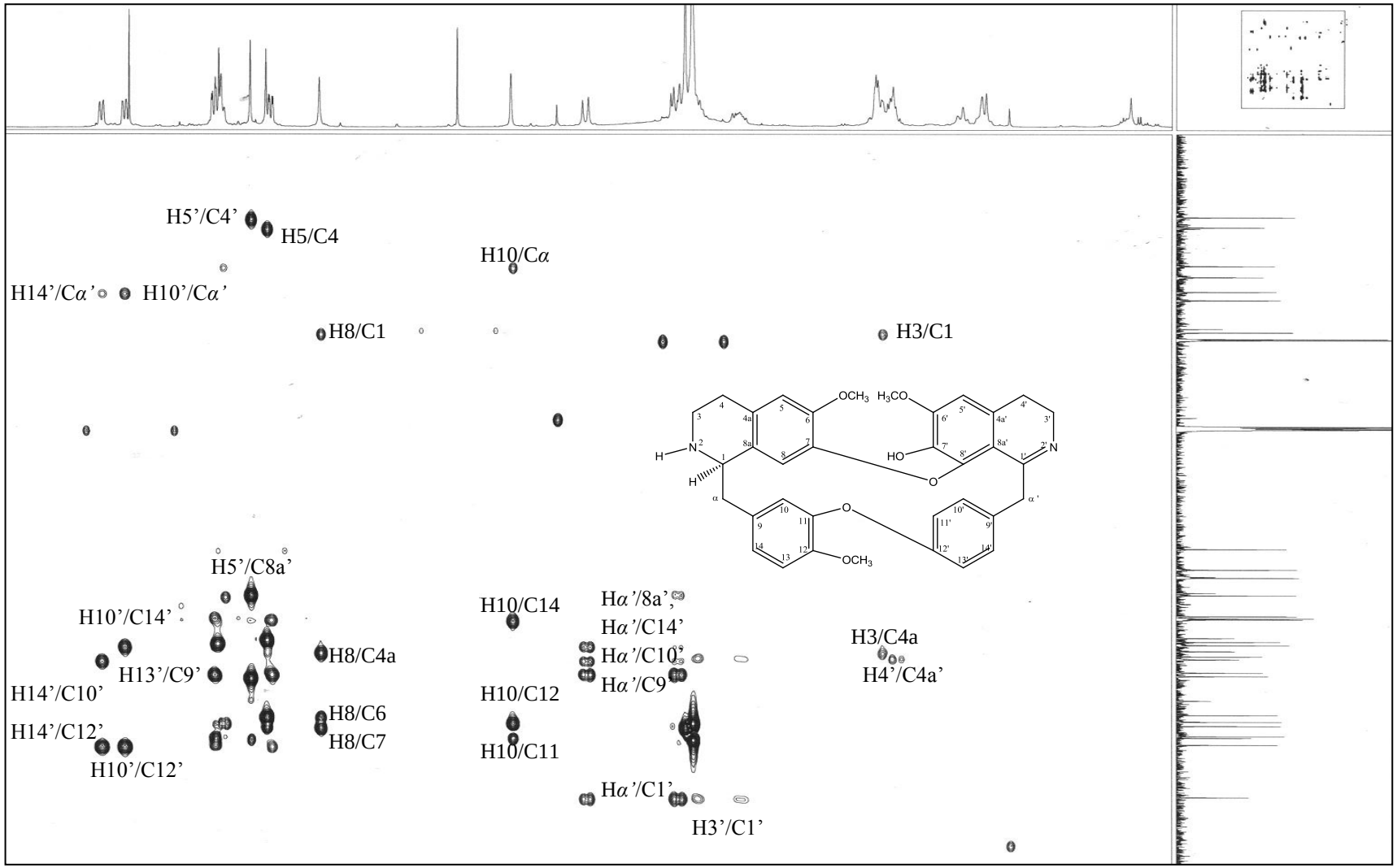


Figure 3.66: HMBC Spectrum of Alkaloid **52**

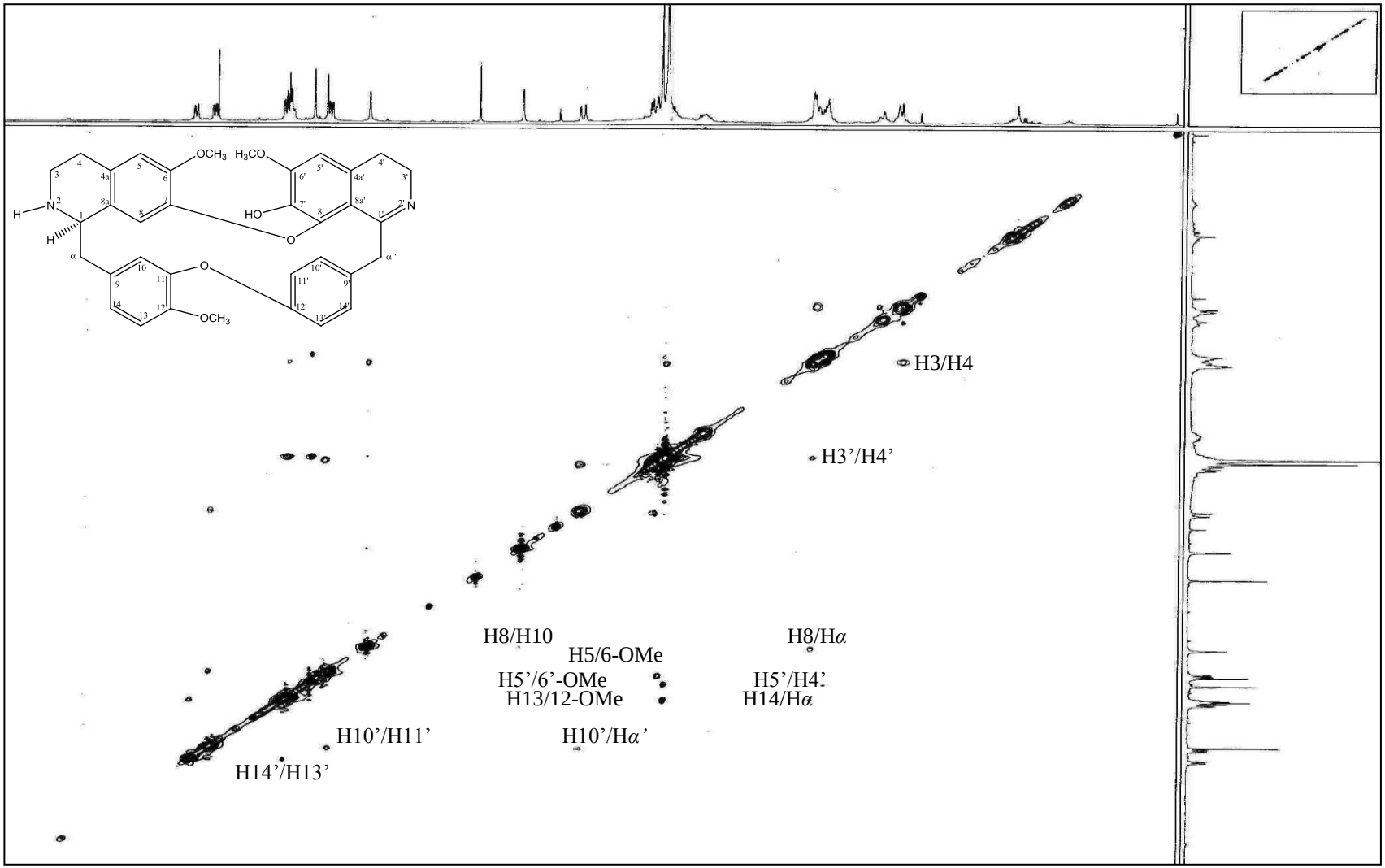
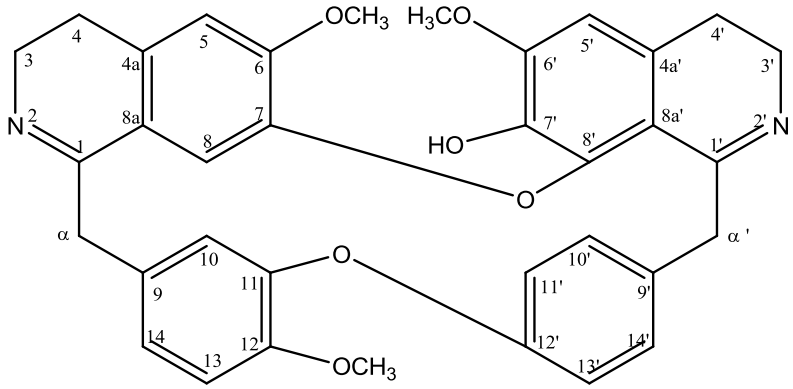


Figure 3.67: NOESY Spectrum of Alkaloid **52**

3.1.10 7'-*O*-demethylstebisimine, 53



53

Alkaloid **53** was obtained as white amorphous solid. The ESI^+ mass spectrum revealed a pseudomolecular ion peak at m/z 577.20 $[M+H]^+$ corresponding to the molecular formula of $C_{35}H_{32}N_2O_6$. The IR spectrum showed a broad band of hydroxyl and imine groups at 3583 and 2200 cm^{-1} respectively.

The ^1H NMR spectrum displayed ten proton signals in the aromatic region suggesting the same type VI of bisbenzylisoquinoline alkaloids. Two doublets at δ 7.22 and 6.78 with a coupling constant of 8.24 Hz corresponding to two protons were assigned to H14' and H10' respectively. In addition, double of doublets at δ 6.38 and 6.90 were assigned to H11' and H13' respectively thus, indicating ring C' was para disubstituted (AA'BB' system) benzene ring. The spectrum also showed three singlets at δ 6.73, 6.61 and 6.55 were assignable to H5, H8 and H5'. The correlation between vicinal protons was further confirmed by COSY experiment. In the COSY spectrum correlations of H3/H4, H13/H14, H10'/H11', H13'/H14' and H3'/H4' were observed.

The ^{13}C NMR spectrum of alkaloid **53** is reminiscent with that of alkaloid **52**, However it showed presence of another quaternary carbon belonged to C1 at δ 167.1,

suggesting that alkaloid **53** has two imine functional groups. The C1' signal appeared at δ 164.4. The ^{13}C NMR spectrum of alkaloid **53** showed thirty five carbons in which ten aromatic, sixteen are quaternary and nine are aliphatic carbons in accordance with the proposed structure. The chemical shifts of the C7 (δ143.6), C8' (139.8), C11 (147.0), C12' (153.4) in ^{13}C NMR spectrum further proved the presence of two diaryl ether bridges between C7 and C8', C11 and C12', significant signals of head to head and tail to tail bisbenzylisoquinoline alkaloids. [21, 45]

The HMBC correlations for 7'-O-demethylstebisimine were shown in Figure 3.74. the positions of $\Delta^{1'\text{-}N'}$ and $\Delta^{1\text{-}N}$ double bonds were confirmed by the HMBC correlations of H3' (δ 3.83) and Hα' (δ 4.72) to C1' (δ 164.4) on one hand and H8 (δ 6.61) and Hα (δ 3.76) to C1 on the other hand. Therefore peak at 167.1 was assignable to C1. In addition, three methoxy groups at δ 3.99, 3.88 and 3.86 were assignable to 6-OMe (δ 3.99), 12-OMe (δ 3.88) and 6'-OMe (δ 3.86) by analyzing the cross peaks in the HMBC and HMQC spectra.

Finally, comparison of spectroscopic data obtained with literature data, established alkaloid **53** is 7'-*O*-demethylstebisimine. This is the first report of the occurrence of alkaloid **53** in *Alseodaphne* species.[88]

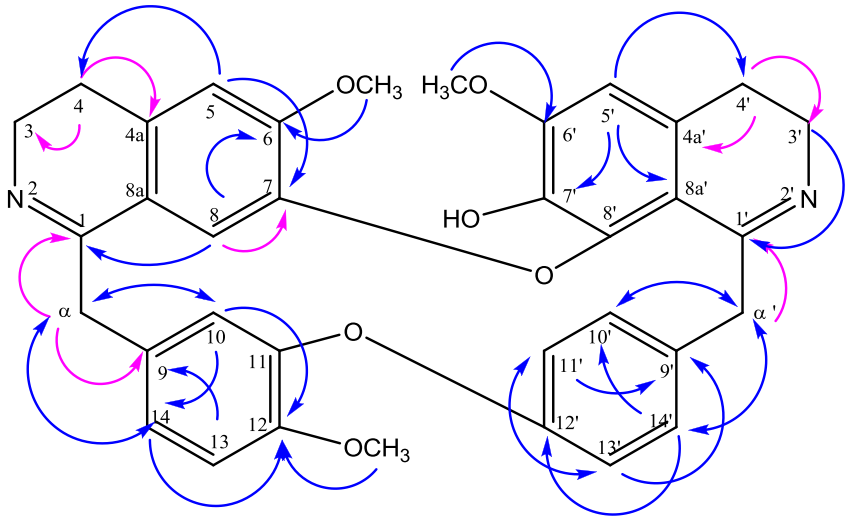


Figure 3.68: ^{1}H-^{13}C Correlations observed in HMBC Spectrum of Alkaloid **53**

Table 3.10: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **53**

Position	^{1}H- NMR δ (Hz)	^{13}C- NMR(δ)	HMBC
1		167.1	
3	3.16 m	46.5	
4	2.48 m	26.3	3,4a
4a		134.3	
5	6.73 s	110.7	4,7,8a
6		151.43	
6-OMe	3.99 s	56.3	6
7		143.7	
8	6.61 s	114.7	1,4a,6,7
8a		121.0	
α	3.76(d,13.7) 3.29 (d,12.8)	41.3	
9		130.2	
10	5.67 (d,2.0)	116.1	α,11,12,14
11		147.0	
12		150.1	
12-OMe		56.2	12
13	6.76 (m)	112.0	9
14	6.83 (dd, 2.7, 8.7)	121.7	α ,10,12
1’		164.4	
3’	3.83 m 3.58 m	46.9	1’
4’	2.76 m	27.5	3’,4a’
4a’		132.4	
5’	6.55 s	105.2	4’,7’,8a’
6’		149.1	
6’-OMe	3.86 s	56.1	
7’		135.0	
8’		139.8	
8’a		115.5	
α’	4.72 (dd,1.4, 15.1) 3.97 m	45.5	1’,8a’, 9’,10’,14’
9’		135.0	
10’	6.78 (d,8.2)	130.9	α’,12’,14’
11’	6.38 (dd,8.9,2.7)	122.3	9’,13’
12’		153.4	
13'	6.90 (dd,8.6,2.7)	122.3	9’,11’,12’
14’	7.22 (brd,8.2)	128.2	α’,10’,12’

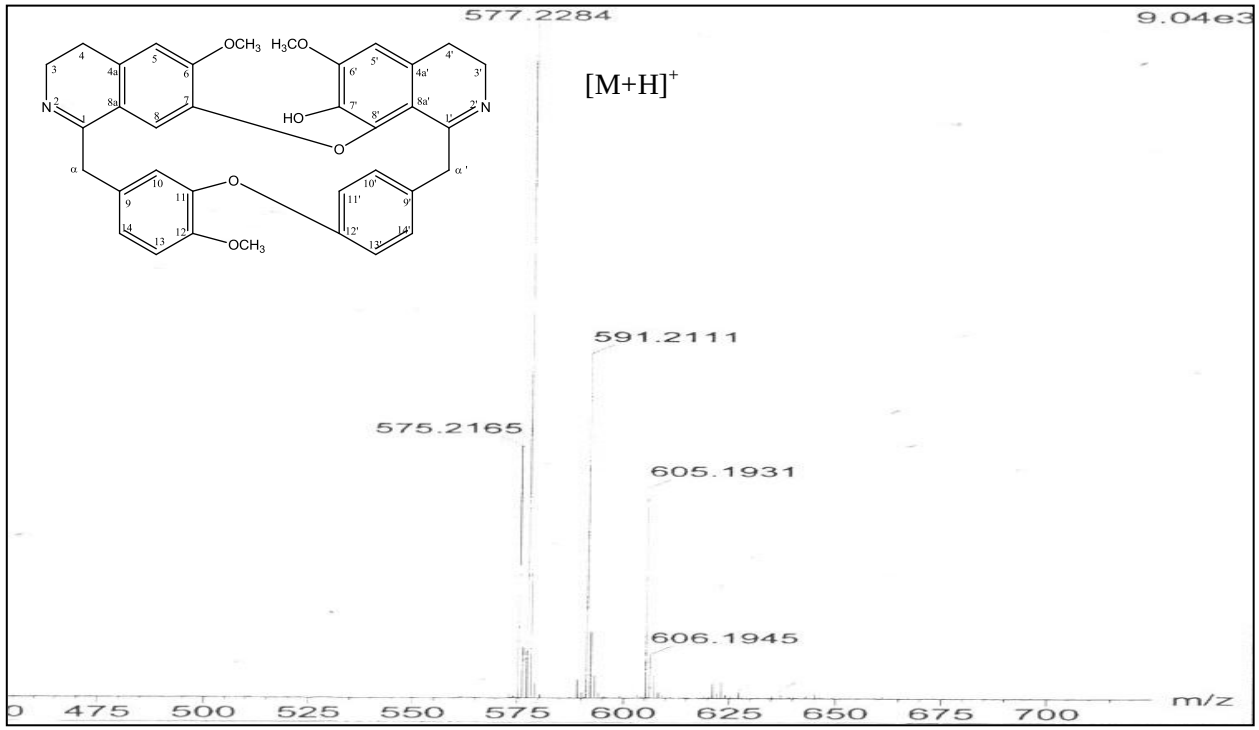


Figure 3.69: HRMS Spectrum of Alkaloid **53**

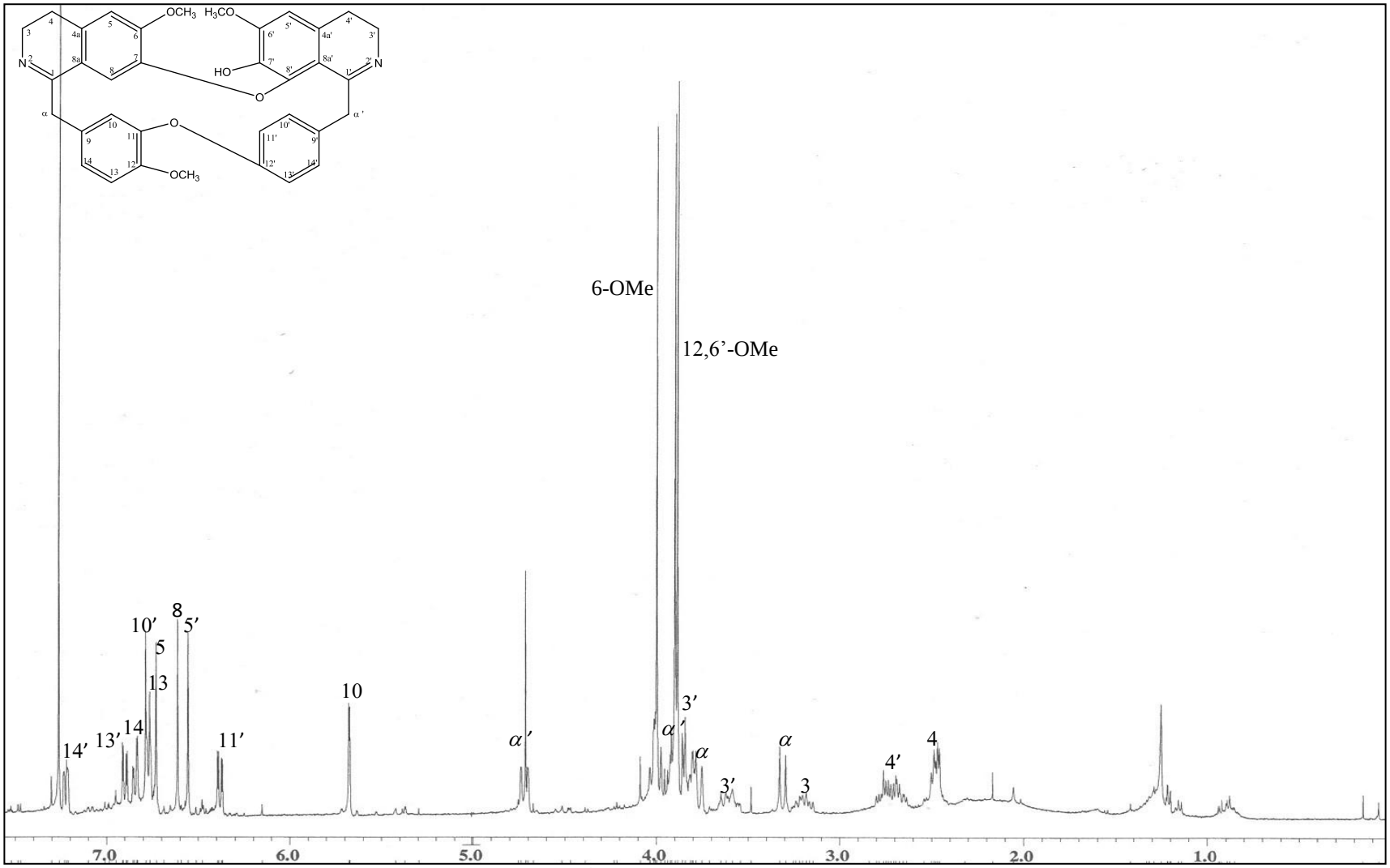


Figure 3.70: ^{1}H NMR Spectrum of Alkaloid **53**

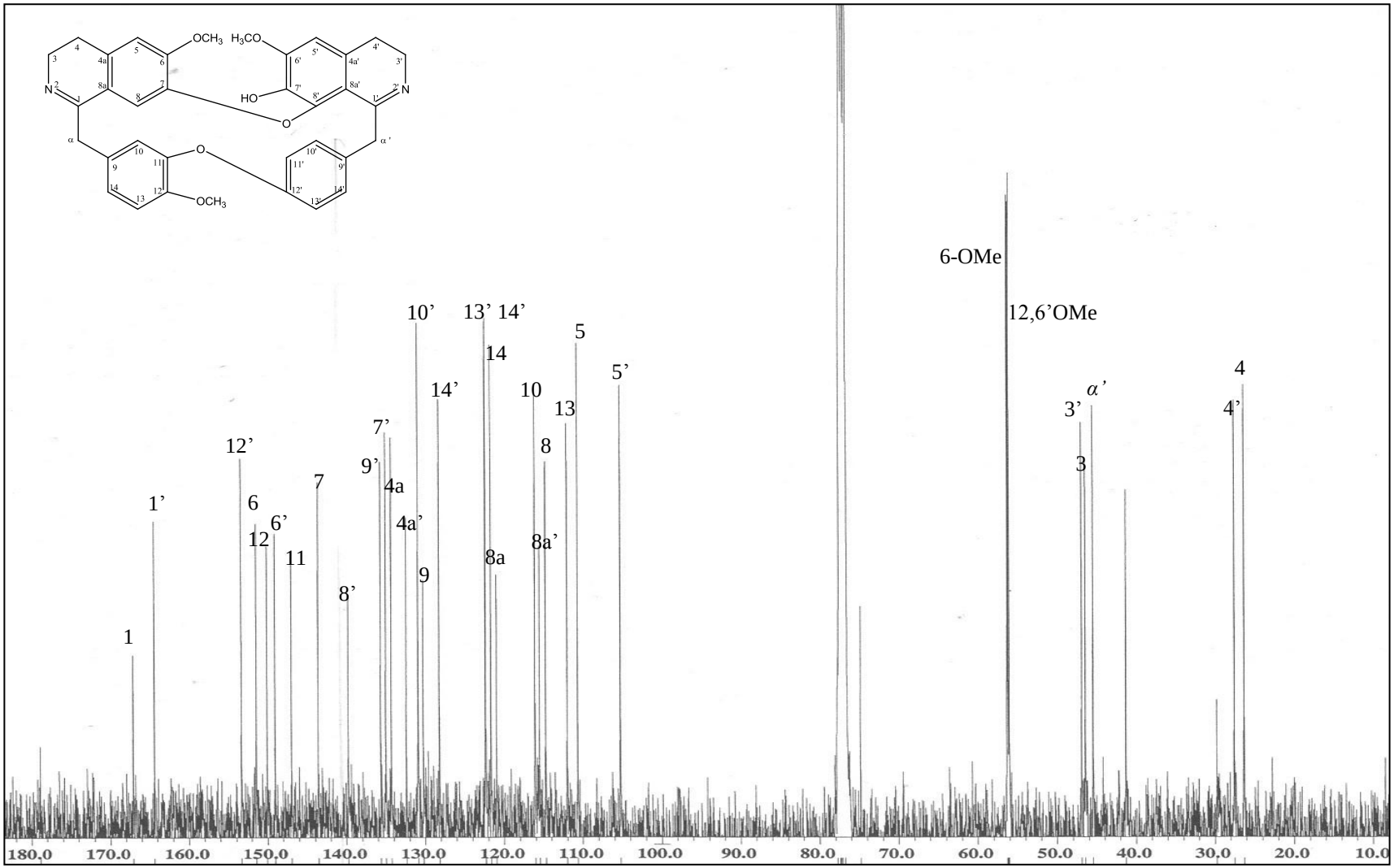


Figure 3.71: ^{13}C NMR Spectrum of Alkaloid **53**

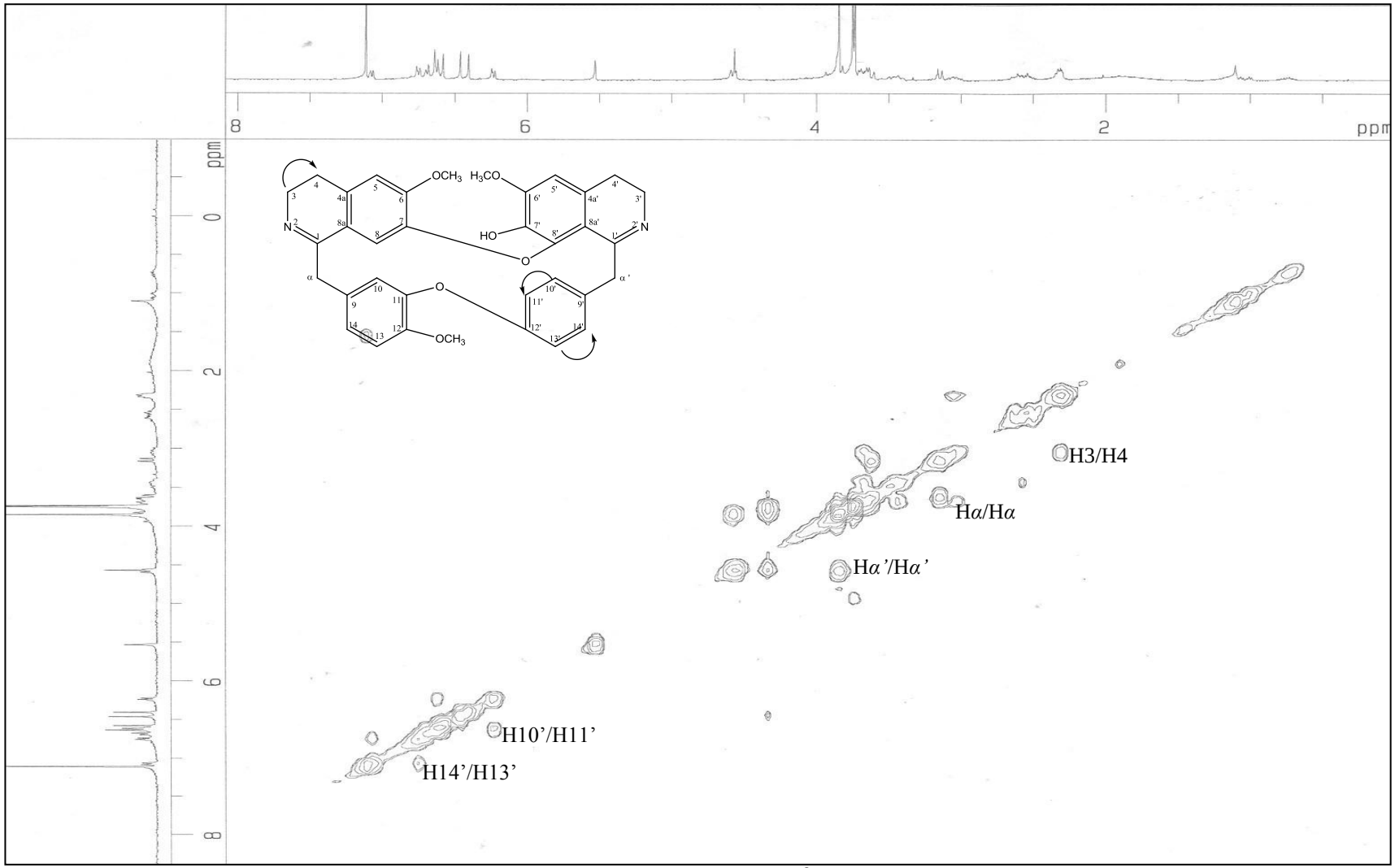


Figure 3.72: COSY Spectrum of Alkaloid **53**

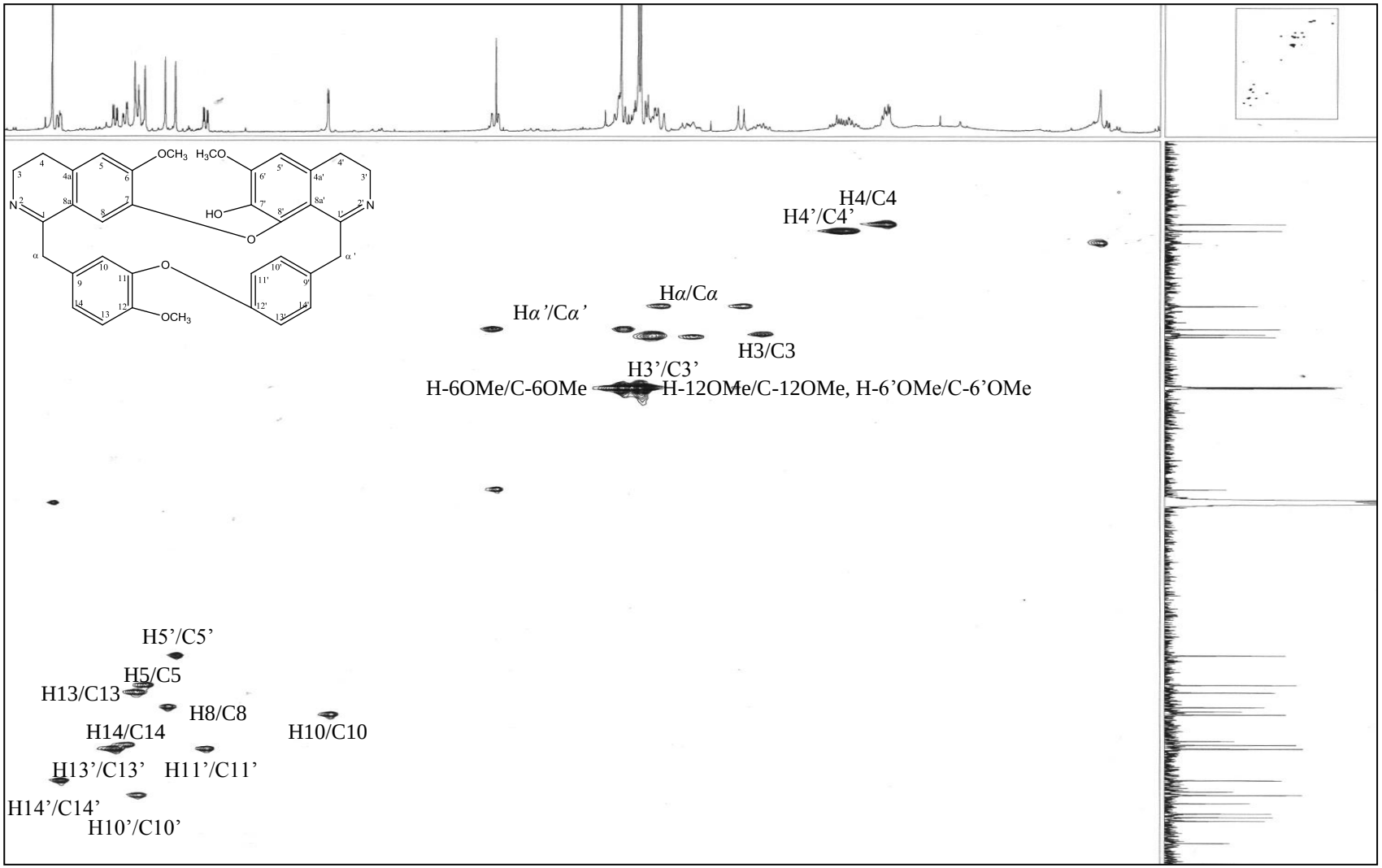


Figure 3.73: HSQC Spectrum of Alkaloid **53**

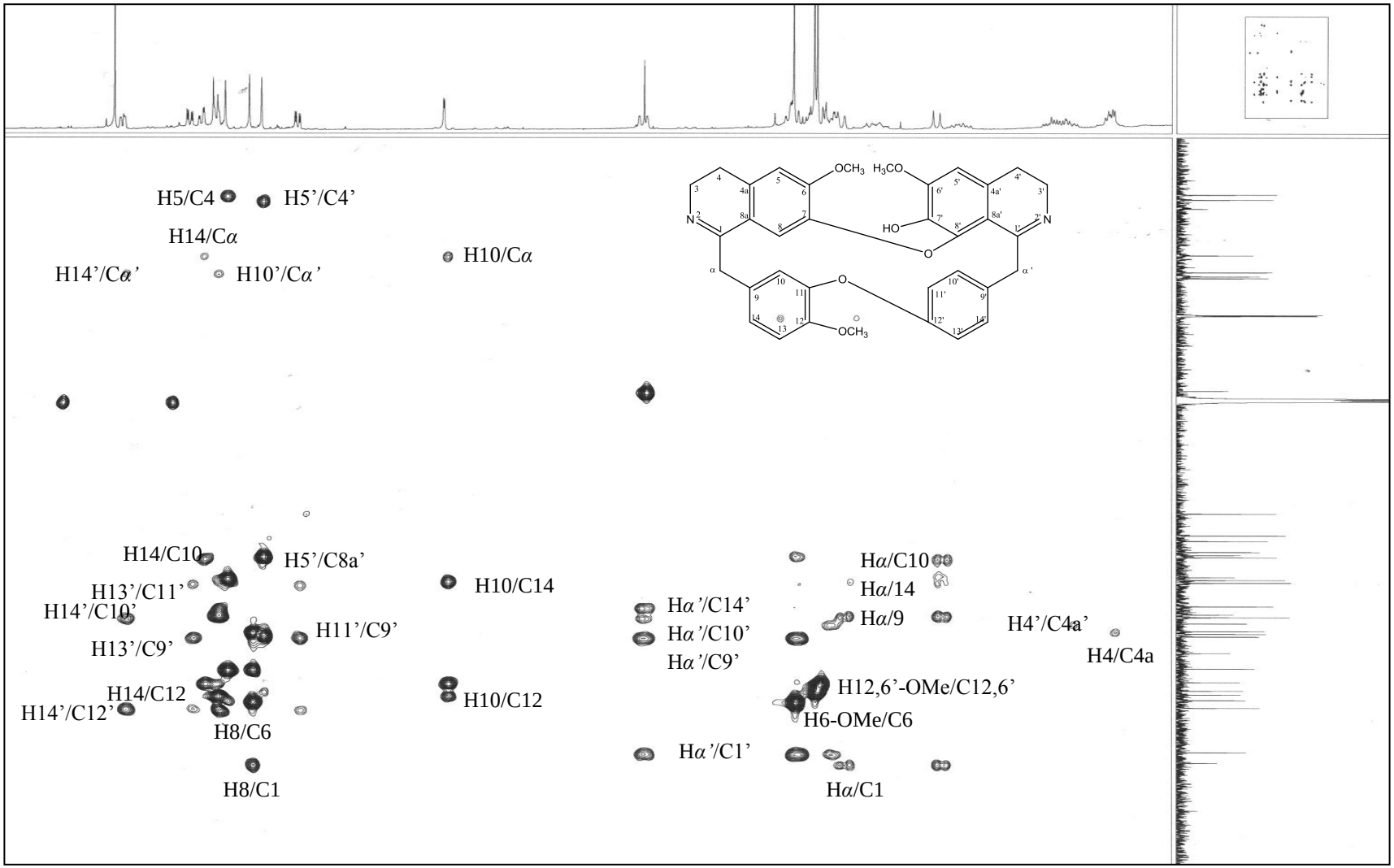
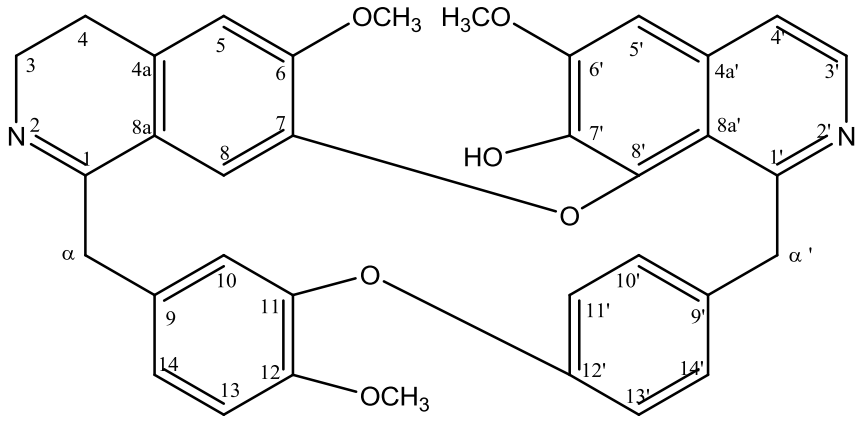


Figure 3.74: HMBC Spectrum of Alkaloid **53**

3.1.11 Stephasubimine, 54



54

Alkaloid **54** was isolated as a brown amorphous solid. UV absorptions at 248, 281, 323 nm were indicative of a bisbenzylisoquinoline structure[36]. The HRESIMS spectrum of alkaloid **54** showed a pseudomolecular ion peak at *m/z* 575.2164 $[M+H]^+$ corresponding to the molecular formula of $C_{35}H_{31}N_2O_6$ (calcd 575.2182). The absorption bands at 1602 and 3392 cm^{-1} in the IR spectrum are typical of an imine and hydroxyl absorption band. [20]

The gross features of the ^1H and ^{13}C-NMR spectra of alkaloid **51** and **54** showed a close structural relationship between these two compounds. In fact, the same substitution groups could be characterized in both compounds through HMBC experiment. However, one distinct difference was apparent in the ^1H-NMR spectrum of alkaloid **54**, the signal corresponding to the C1 at shielded area was absent in the ^1H NMR spectrum of alkaloid **54**. Furthermore, signals for ten aromatic protons, three methoxyl groups, one CH_2-CH_2-N group, and two sets of isolated none equivalent methylene protons were observed. Among the ten aromatic proton signals, three singlets at δ 6.82, 6.98, and 6.52 were attributed to H-5, H-5′, and H-8, respectively. The upfield signal of H-10 (δ 5.33, broad singlet) was a characteristic peak of head to

head and tail to tail bisbenzylisoquinoline alkaloid (two ether linkages between 7-8′, 11-12′).[90]H-10 signal appeared as a broad singlet because it was *meta*-coupled with H-14 (δ 6.96, m), which was placed vicinal to H-13 (δ 6.76, d), indicating that ring C was trisubstituted. Other prominent peaks of an AX spin system were observed at δ 4.53 and 5.41 (J = 14.0 Hz) and δ 3.33 and 3.63 (J = 12.0 Hz) supporting the presence of two geminal protons of the methylene adjacent to the imine function (H-α') and (H- α) respectively. Two broad doublet signals of H-10′ (δ 6.97), H-11′ (δ 6.74), and two doublet of doublets of H-13′ (δ 6.42), and H-14′ (δ 7.08), indicating that ring C′ was *para* disubstituted (AA′BB′) ring system.

The ^{13}C NMR spectrum showed 35 carbon resonances, which were in agreement with the molecular formula. The signals at δ 152.0 and δ 157.41 could be assigned as the imines (C-1 and C-1′) carbons, respectively.[87]

The positions of $\Delta^{1'\text{-}N'}$and $\Delta^{1\text{-}N}$ double bonds of dimer were confirmed by the HMBC correlations of H-3′ to C-1′ (δ 157.41) and H-α' to C-14′ (δ 130.9) on one hand, and H-8 to C-1 (δ 152.0) on the other hand. Furthermore, the COSY spectrum, showed the correlation of vicinal proton between CH13'/CH14', CH10'/CH11', CH14/ CH13, CH3'/CH4' and CH3/CH4. Based on the literatures search of known compounds it could be proposed that alkaloid **54** is a known compound, stephasubimine.[86]

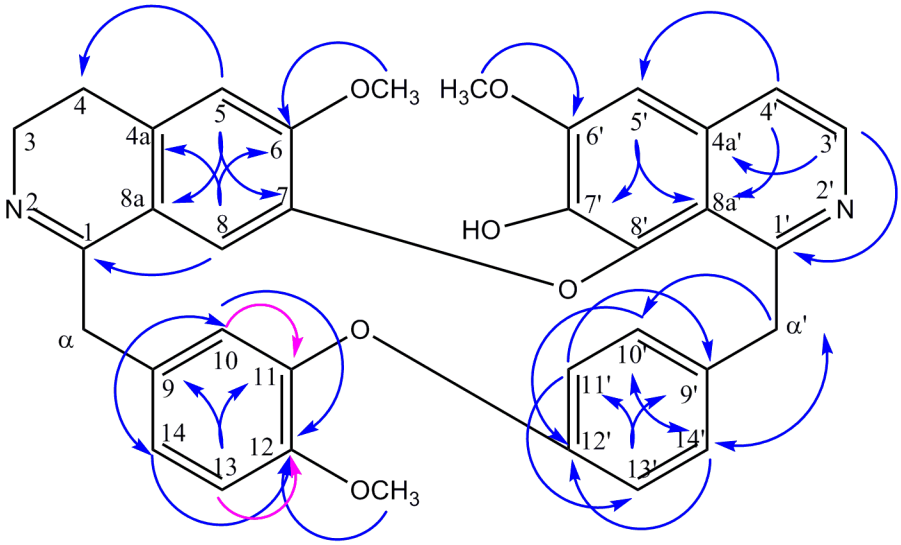


Figure 3.75: ^{1}H-^{13}C Correlations observed in HMBC Spectrum of Alkaloid **54**

Table 3.11: ^{13}C-NMR, ^{1}H-NMR and HMBC Data for Alkaloid **54**

Position	^{1}H- NMR (δ (Hz))	^{13}C-NMR(δ)	HMBC
1		152.0	
3	3.80 m	45.6	
	3.05 m		
4	2.41 m	26.5	
4a		133.6	
5	6.82 s	110.5	4,7,8a
6		144.9	
6-OMe	3.87 s	55.9	6
7		144.5	
8	6.52 s	114.1	1,4a,6
8a		121.0	
α	3.62 m	40.7	
	3.32 m		
9		129.6	
10	5.33 s	115.5	11,12,14
11		146.9	
12		150.1	
12-OMe	4.05 s	56.5	12
13	6.76 d	112.0	9,11,12
14	6.96 m	121.7	12
1’		157.41	
3’	8.44 (d,5.9)	140.6	1’,4’,4a’
4’	7.48 (d,5.5)	119.1	5’,8a’
4a’		133.0	
5’	6.98 s	101.2	7’,8a’
6’		150.7	
6’-OMe	4.08 s	56.5	6’
7’		134.7	
8’		140.6	
8’a		128.9	
α’	5.41 (dd,3.3, 13.7)	45.6	1’, 9’, 14’
	4.53(d,14.0)		
9’		137.9	
10’	6.97 (d,8.2)	129.6	12’,14’
11’	6.74 brs	122.8	9’,13’
12’		152.9	
13'	6.42 (dd,8.6,2.8)	122.2	9’,11’
14’	7.08 (dd,8.6)	130.9	10’,12’

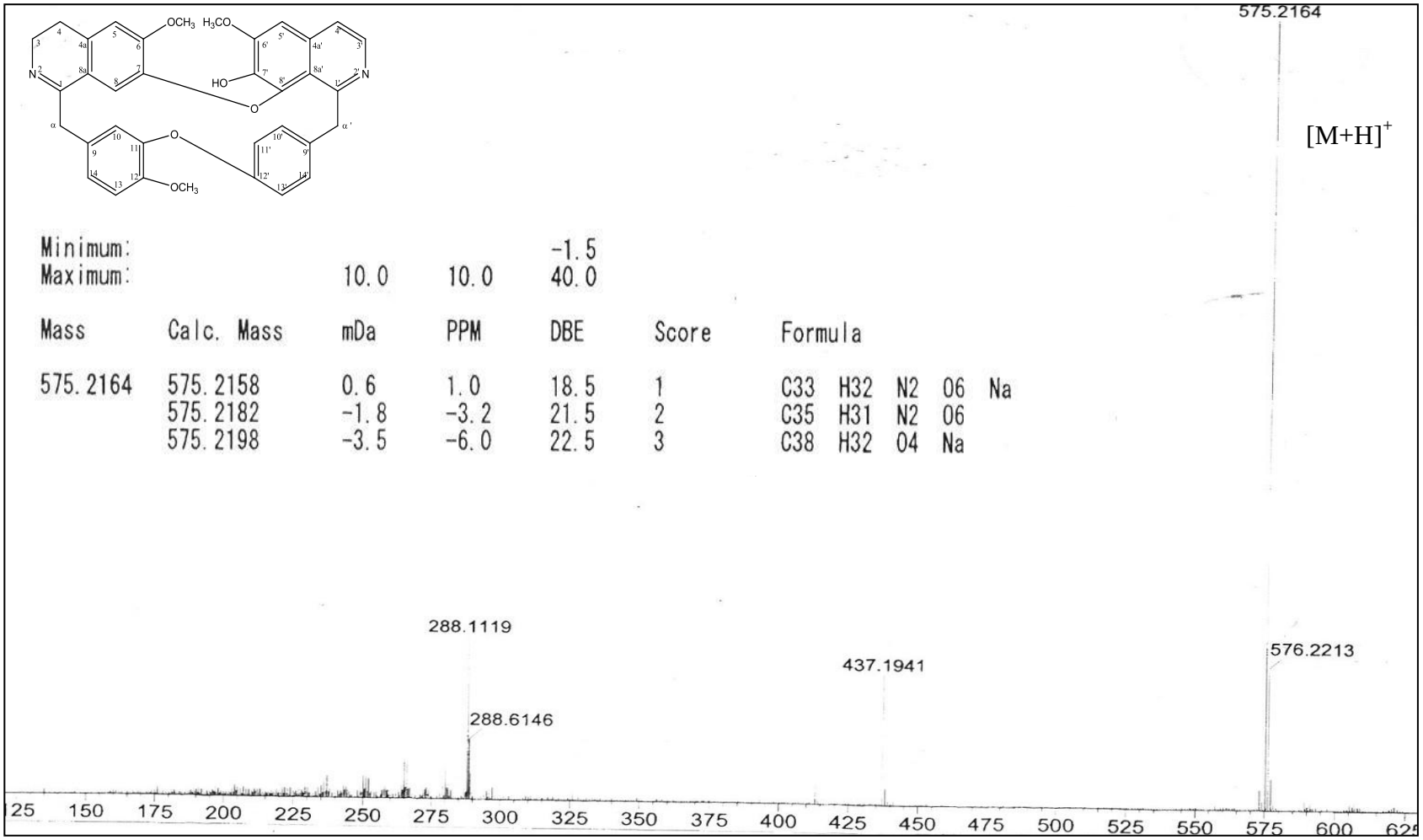


Figure 3.76: HRMS Spectrum of Alkaloid **54**

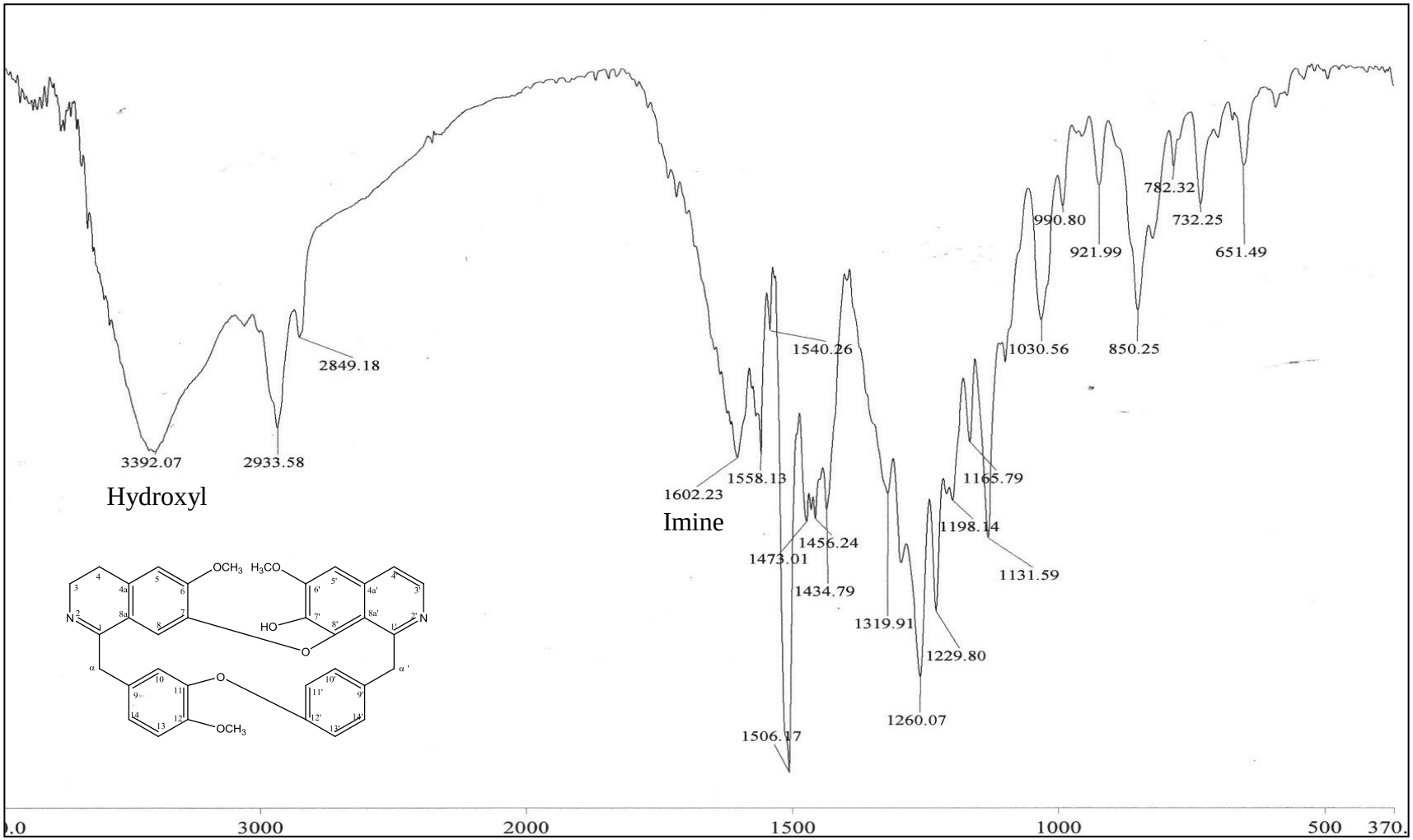


Figure 3.77: Infrared Spectrum of Alkaloid **54**

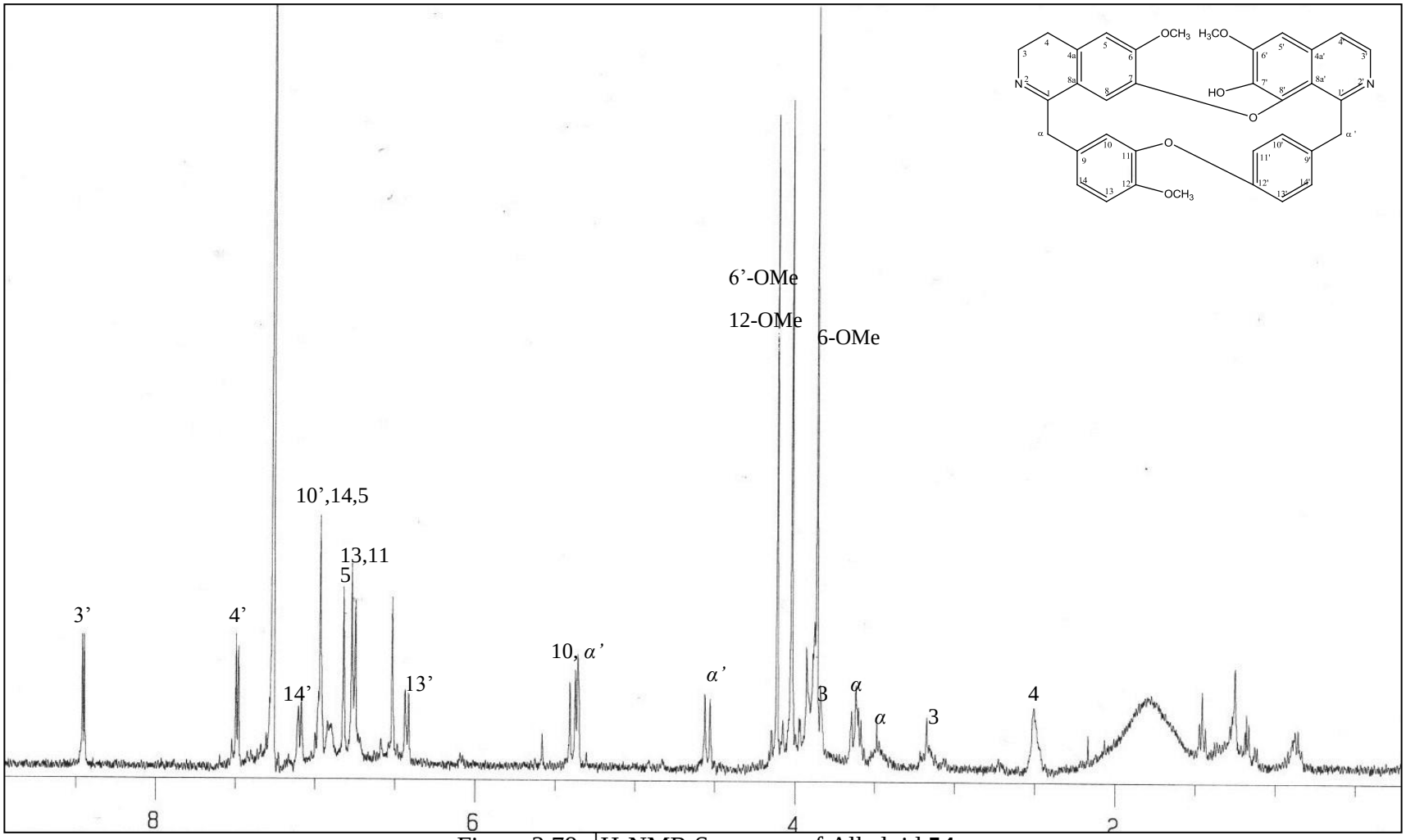


Figure 3.78: ^{1}H-NMR Spectrum of Alkaloid **54**

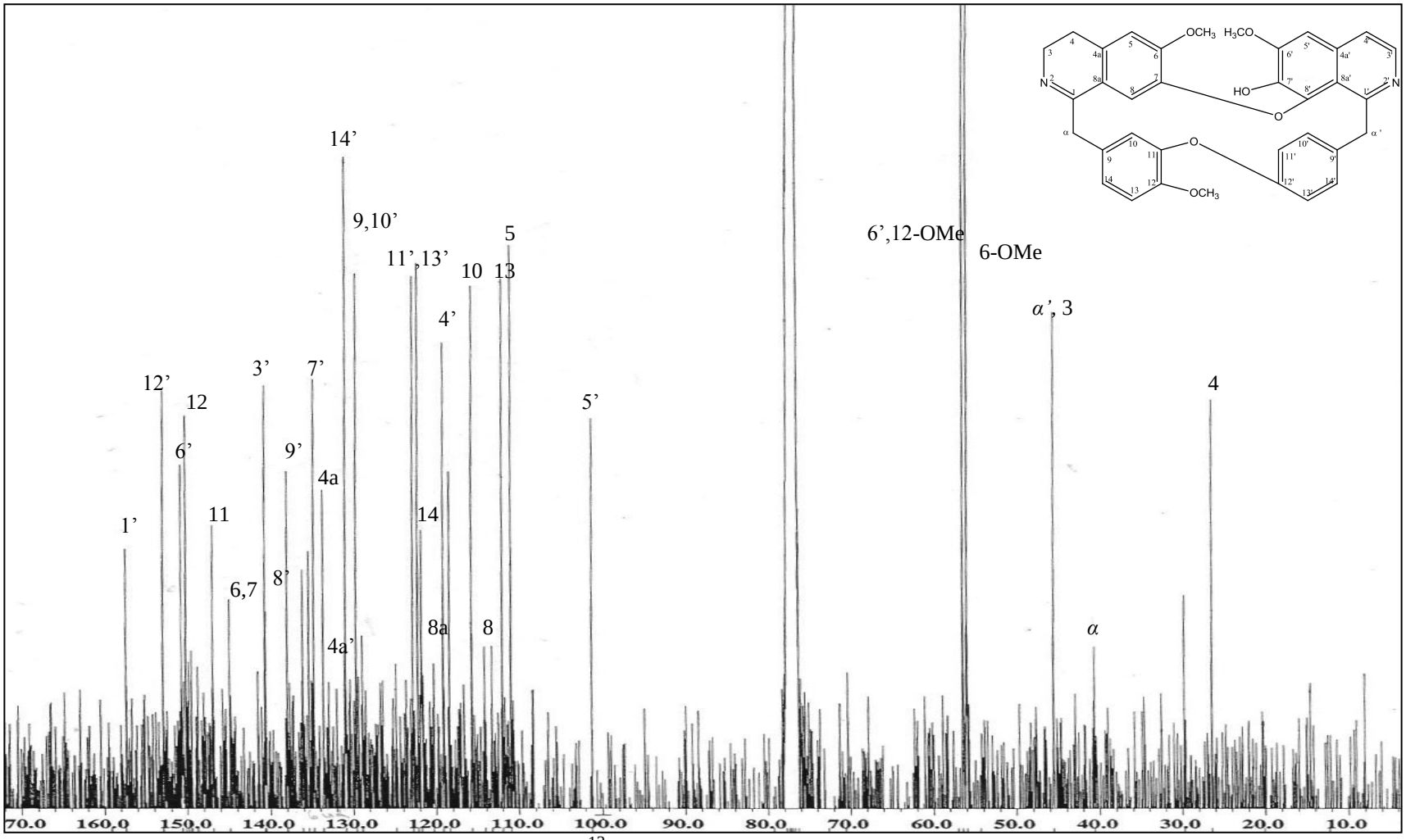


Figure 3.79: ^{13}C-NMR Spectrum of Alkaloid **54**

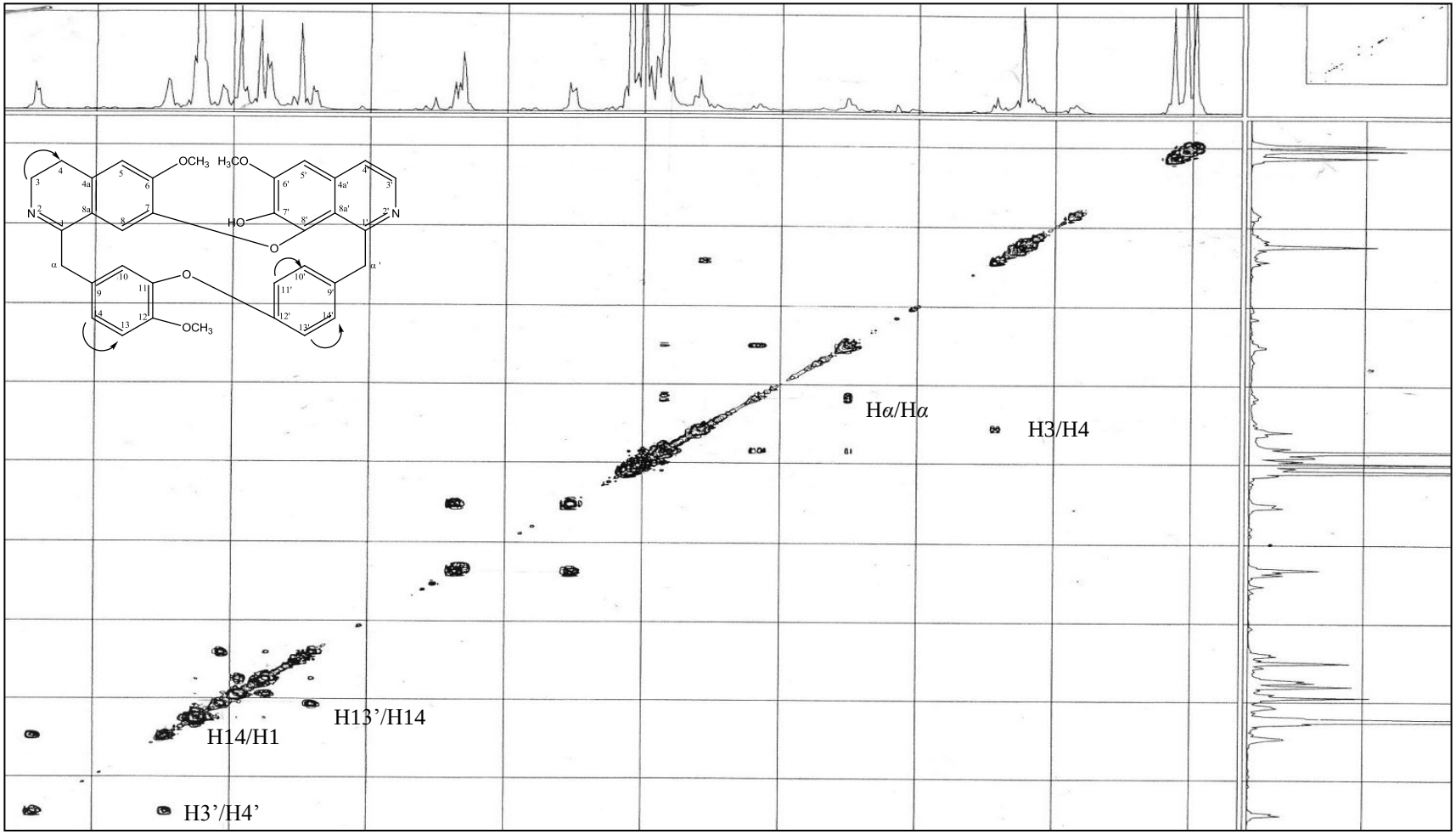


Figure 3.80: COSY Spectrum of Alkaloid **54**

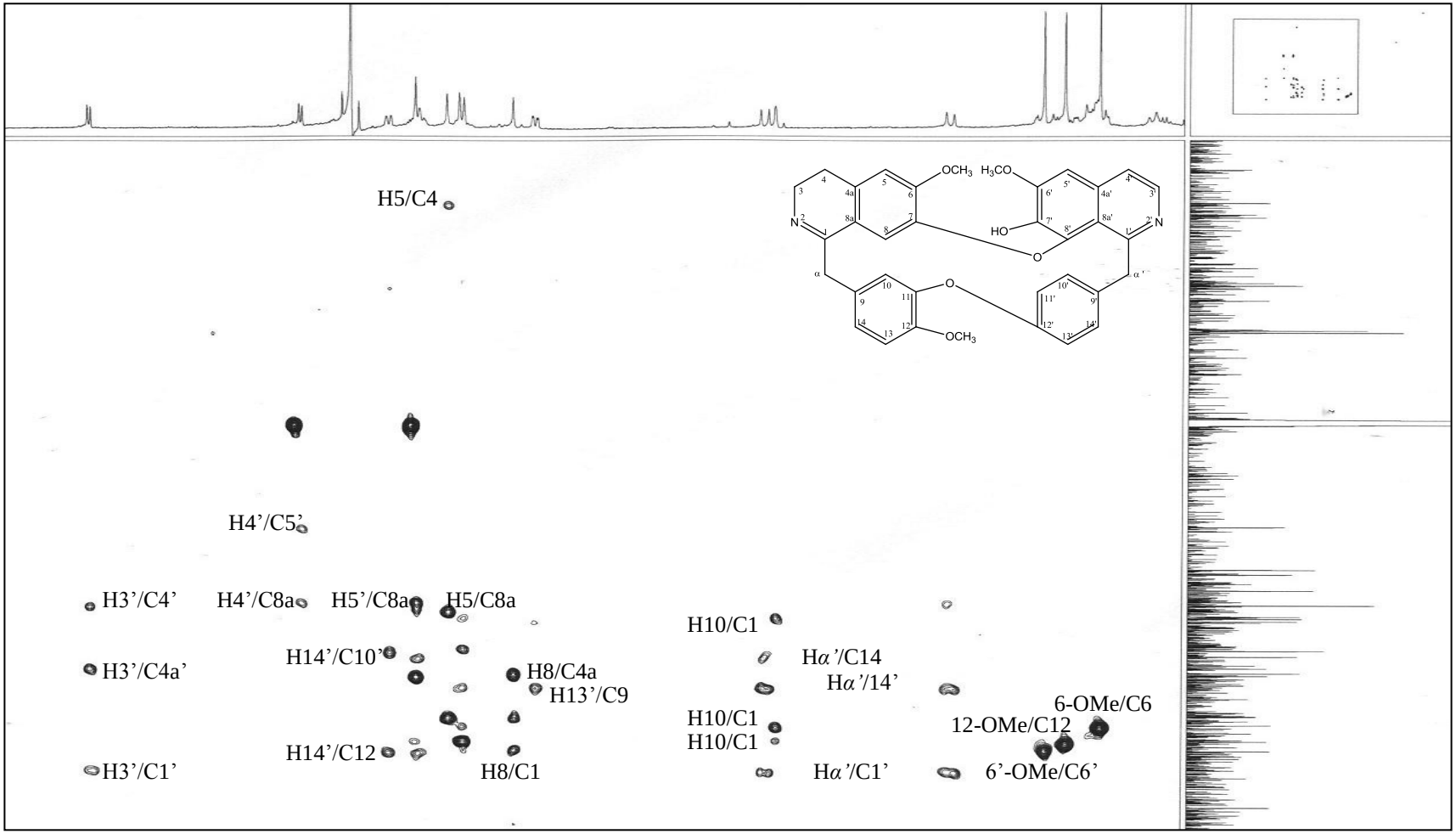


Figure 3.81: HMBC Spectrum of Alkaloid **54**

3.2. Biological Activities

3.2.1 Antiplasmodial Activities against *Plasmodium falciparum* Strains

With over 300 millions of new cases each year, resulting in more than one million deaths annually, malaria remains one of the most important infectious diseases of the developing world. Fatal cases are generally caused by the most virulent human malaria parasite, *Plasmodium falciparum*. Symptoms of malaria include fever, shivering, joint pain, vomiting and retinal damage.[89]Current clinical treatments involve the use of inexpensive antimalarial drugs such as chloroquine and their derivatives. This protocol for assessing compounds efficiency as a marker for inhibition of parasite growth. [90, 91]

The antiplasmodial activity of alkaloid **44-47** was evaluated by their ability to inhibit the growth of *P. falciparum* following the method of Desjardins et al. (1979). The anti-plasmodial activity against the chloroquine-resistant strain of *P. falciparum* FcB1 and the cytotoxicity on the MRC-5 cell line are summarized in Table 3.12. The results demonstrated that alkaloid **46**, *N*-methyllaurotetanine exhibited potent antiplasmodial activity with an IC_{50} value of 8.4µM. The hydroxyl groups on the aromatic ring seem to play an important role for the antiplasmodial activity. In contrast, alkaloid **44, 45** and **47** showed moderate antiplasmodial activities with IC_{50} over 10 µM.

Table 3.12: Results of *P.falciparum* and MRC-5 Cell Line Inhibition Screening Assay

Compound	*P. falciparum* FcB1	Human fibroblast cell line MRC-5
	IC_{50} µM	IC_{50} µM
Standard (Chloroquine)	0.078	
(Taxatore)		0.010
Alkaloid **44**; Isocorydine	51.3	>100
Alkaloid **45**; Norisocorydine	19.8	>100
Alkaloid **46**; *N*-methyl laurotetanine	8.4	79.3
Alkaloid **47**; *N*-methyl lindcarpine	27.6	>100

3.2.2 Vasorelaxant Effects on Isolated Rat Aorta[61]

The American Heart Association estimates high blood pressure affects approximately one in three adults in the United State of America and about 73 million peoples were affected. Hypertension is clearly a major public health problem. High blood pressure or hypertension means high pressure (tension) in the arteries. Vasodilators are useful for treatment of cerebral vasopasm and hypertension, and for improvement of peripheral circulation.[92] An experiment was done to study vasorelaxant effect of the alkaloids on the isolated aorta. Phenylepherine was used to increase blood pressure. Phenylephrine (PE) 3×10^{-7} M was applied to thoracic aortic rings with endothelium in this experiment. After achieving a maximal response, alkaloid **50-52** were added. Alkaloid **50** and **52** showed a moderate and slow vasorelaxant activity on isolated rat aorta (65% relaxation at 3×10^{-5}M), whereas alkaloid **51** did not show any vasorelaxant activity (20% relaxation at 3×10^{-5}M). Vasodilation seems to be influenced by aromaticity of the ring A′ in the bisbenzylisoquinoline structure. The mode of actions