

CHAPTER IV

RESULTS AND DISCUSSION

Three compounds were isolated from the hexane extract of the sponge *Acanthella cavernosa* Dendy (Figure 3). Identification and elucidation of their chemical structures will be discussed.

Identification and structure elucidation of isolated compounds from *Acanthella cavernosa*

To elucidate the chemical structures of the isolated compounds, UV, IR, MS and extensive NMR experiments were employed.

1. Structure elucidation of compound K020

Compound K020 was obtained as white amorphous powder (16.2 mg) from fraction F020. The molecular formula of $C_{22}H_{35}ClN_2O_3S$ was suggested for this compound based on its $[M+H]^+$ peak in the APCI-MS (Figure 4) at m/z 443, and the $[M+H-H_2O]^+$, $[M+H-HCl]^+$ and $[M+H-HCl-NCS]^+$ peaks at m/z 425, 407.5 and 348, respectively. The presence of the isothiocyanate functionality in the molecule was also confirmed by a very intense IR absorption band at 2093 cm^{-1} (Figure 5) and a UV maximum absorption at 244 nm (Figure 6) (Alvi *et al.*, 1991), while a carbonyl absorption band at 1683 cm^{-1} and two N-H stretching bands at 3464 and 3414 cm^{-1} indicated the presence of the formamide group (Figure 3). A broad band at 3553 cm^{-1} further suggested hydroxyl group(s) as a part of the structure.

The ^{13}C NMR spectrum of K020 (Figures 7-8) exhibited 22 carbon signals, suggestive of a diterpenoid structure with two carbon atoms in the substituents. DEPT and ^1H - ^{13}C -HETCOR (Figures 9-10) experiments were employed to classify these signals into those of five methyl carbons at δ 19.7, 23.5, 27.7, 28.8 and 31.4 ppm, six methylene carbons at δ 22.4, 22.4, 27.6, 33.3, 38.0 and

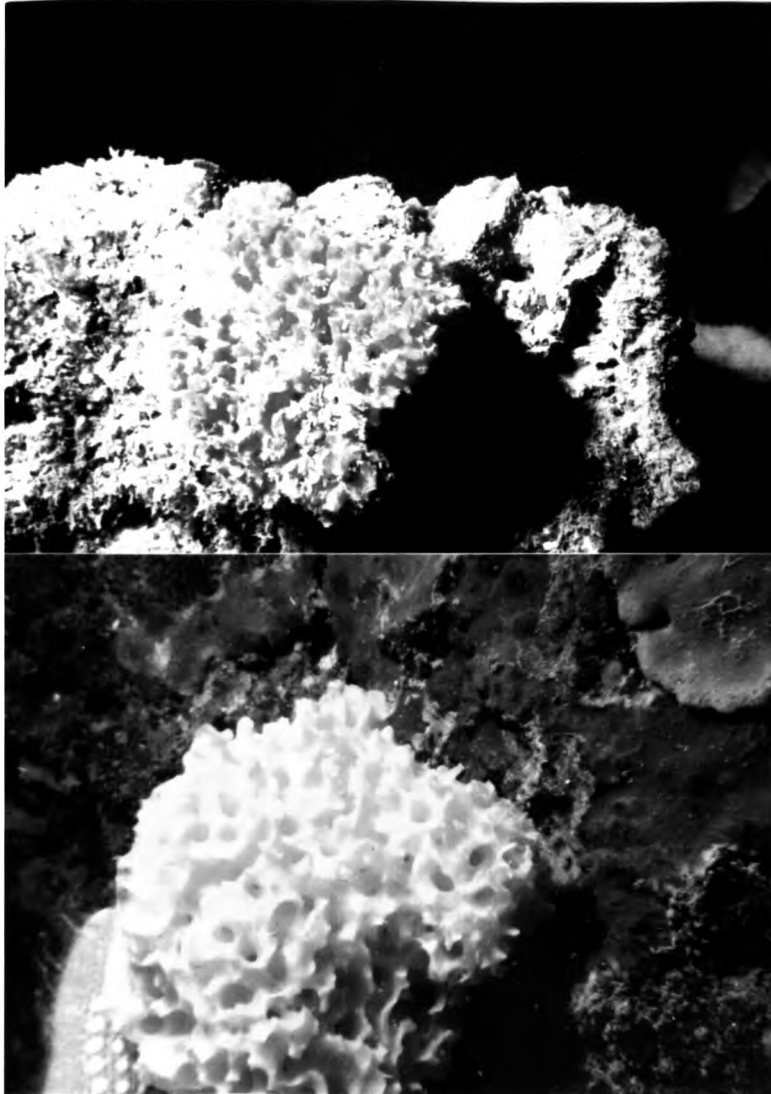


Figure 3. The marine sponge *Acanthella cavernosa* Dendy

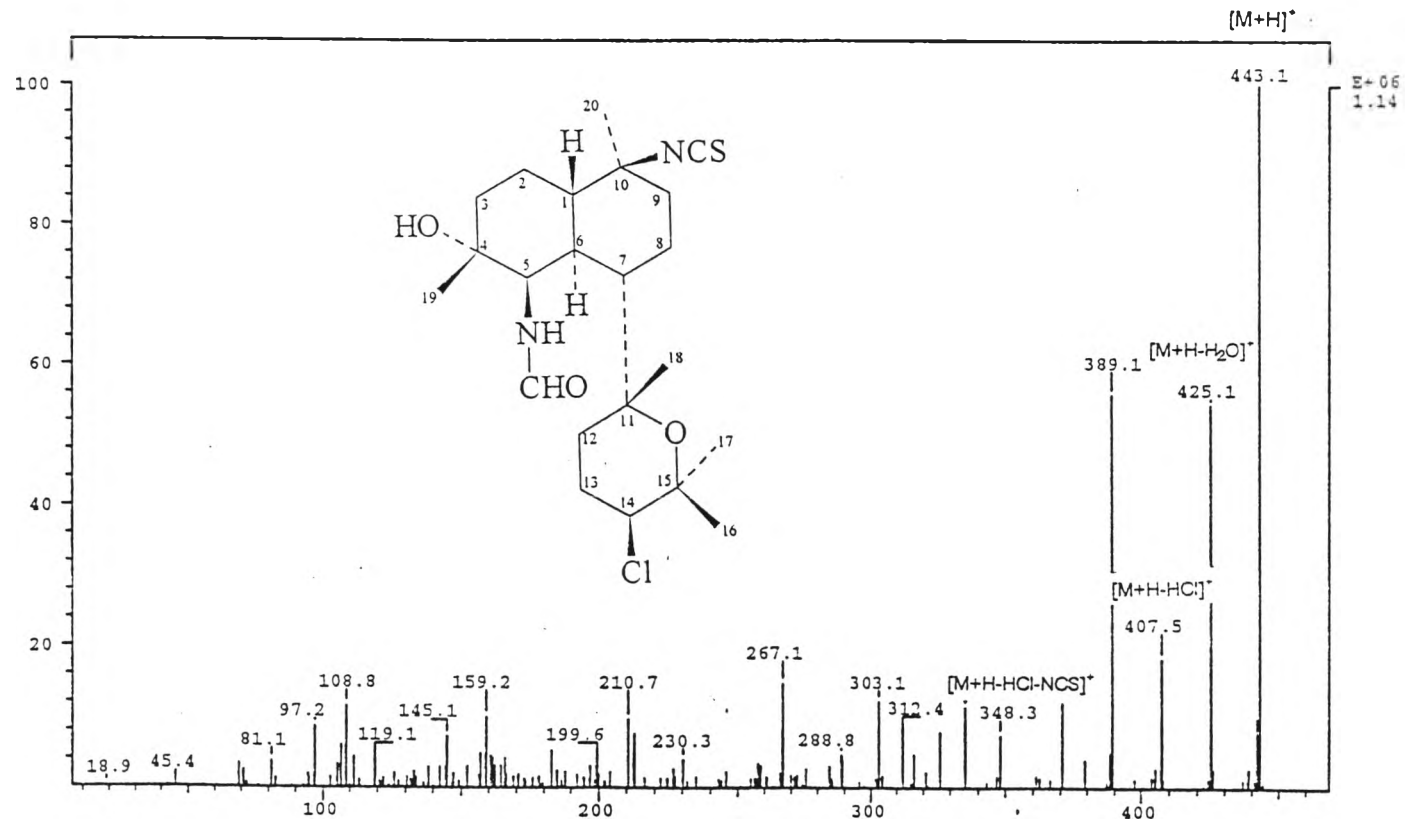
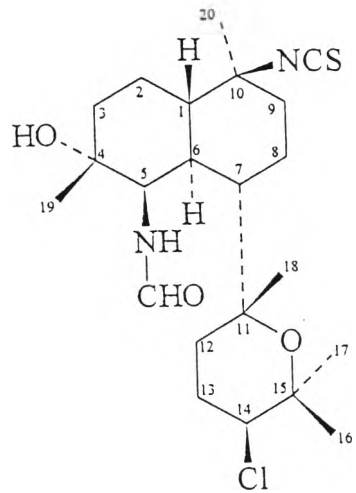


Figure 4. APCI-MS spectrum of compound K020



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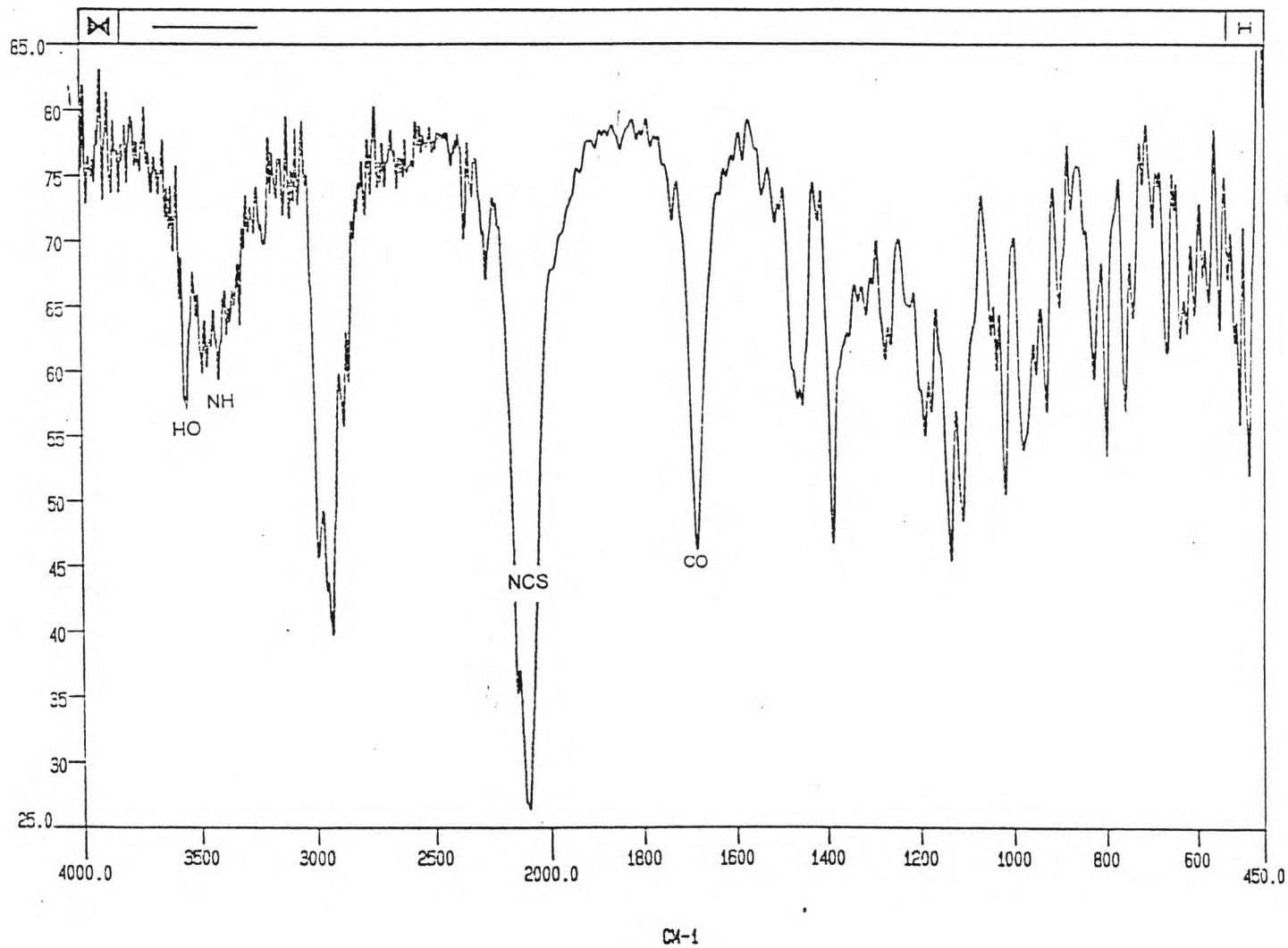


Figure 5. IR spectrum of compound K020

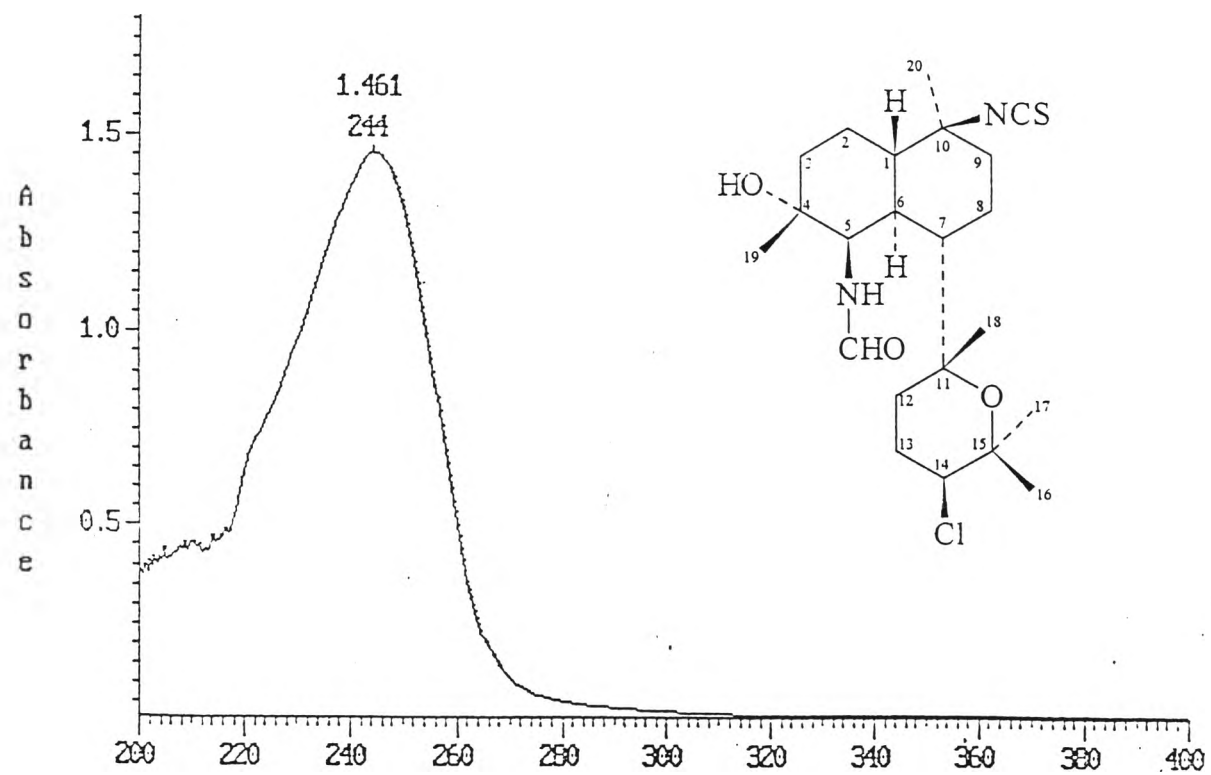


Figure 6. UV spectrum of compound K020 (in MeOH)

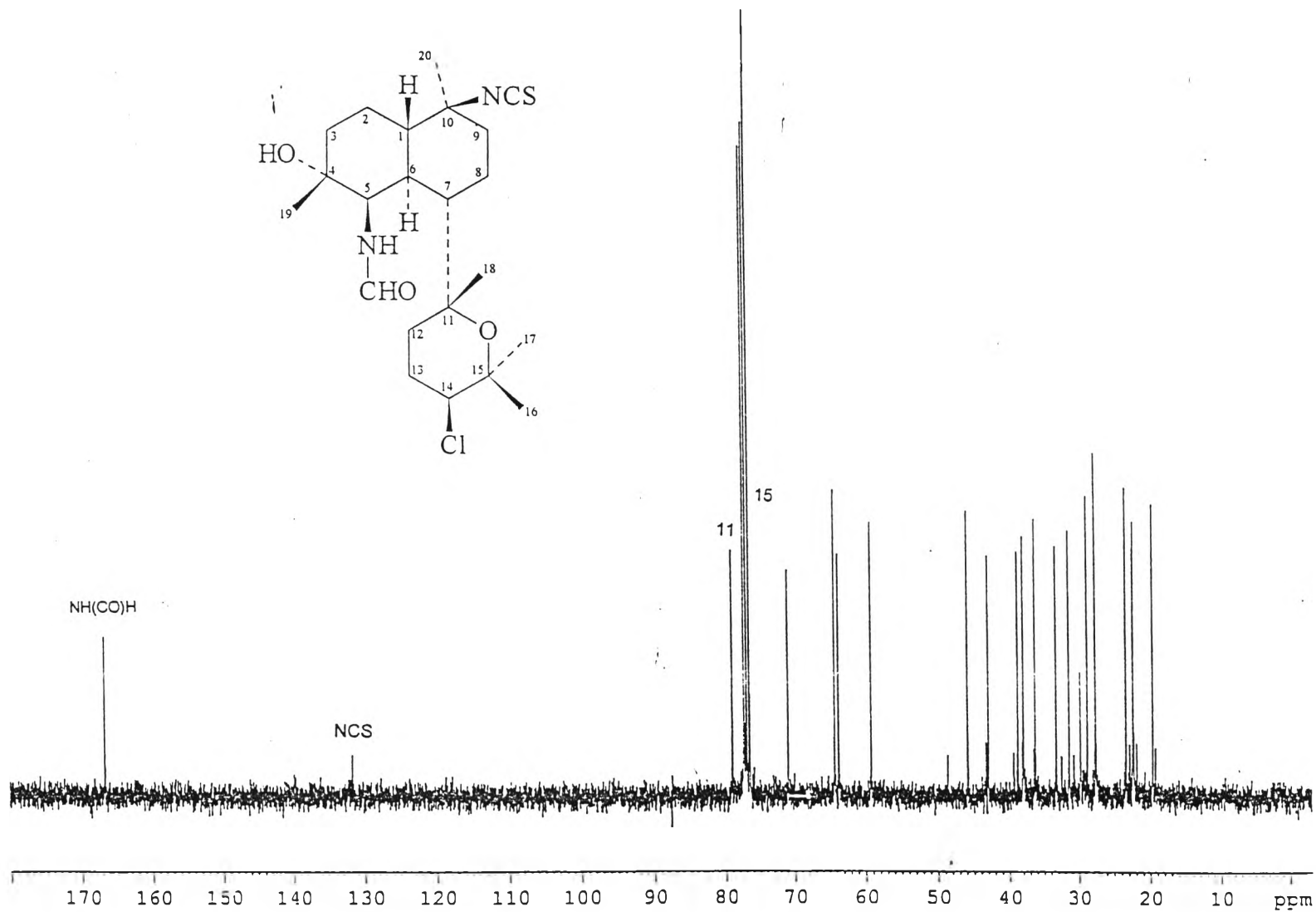


Figure 7. The 75 MHz ^{13}C NMR spectrum of compound K020 (in CDCl_3)

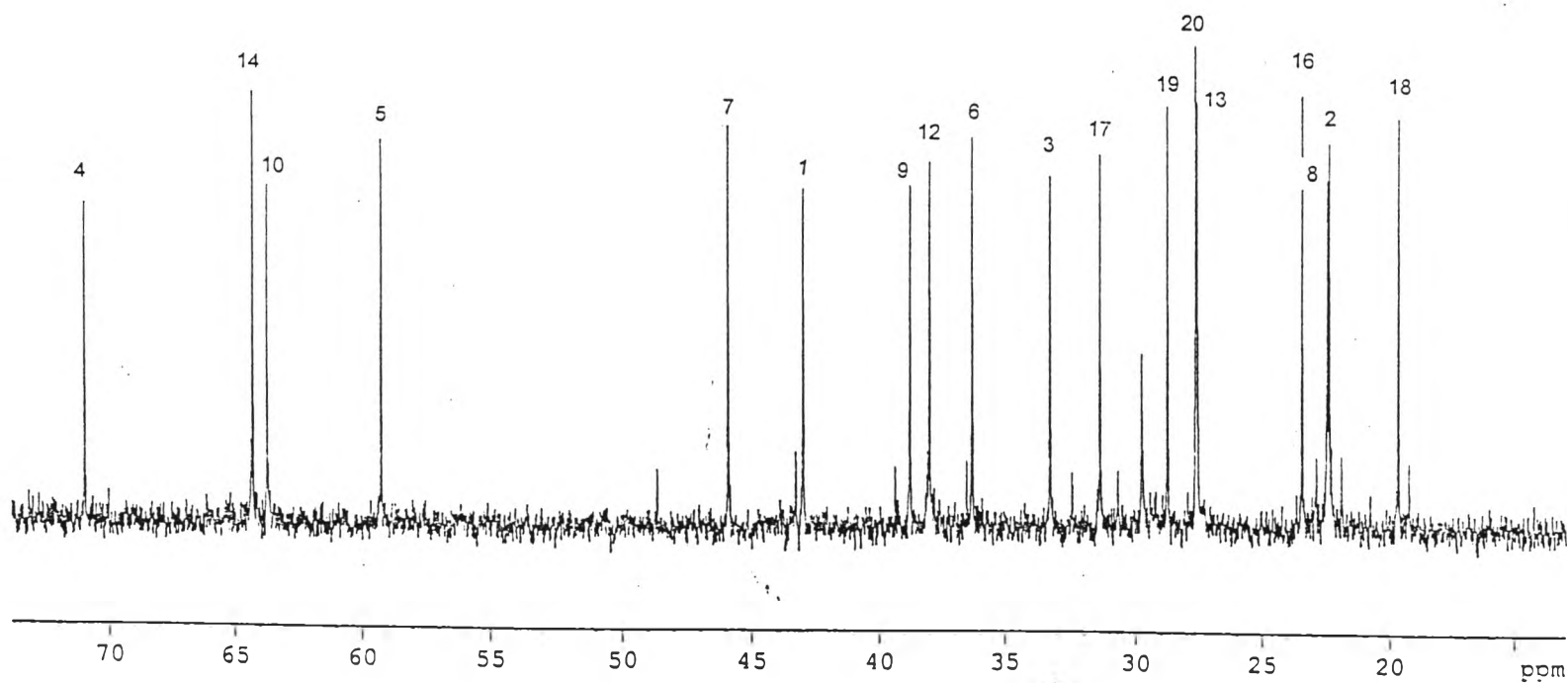
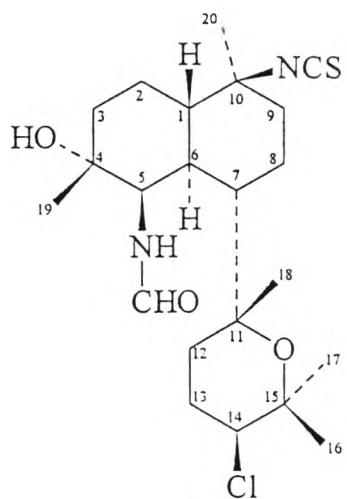


Figure 8. The 75 MHz ^{13}C NMR spectrum of compound K020 (in CDCl_3)
 (expanded in the range of δ 15-75 ppm)

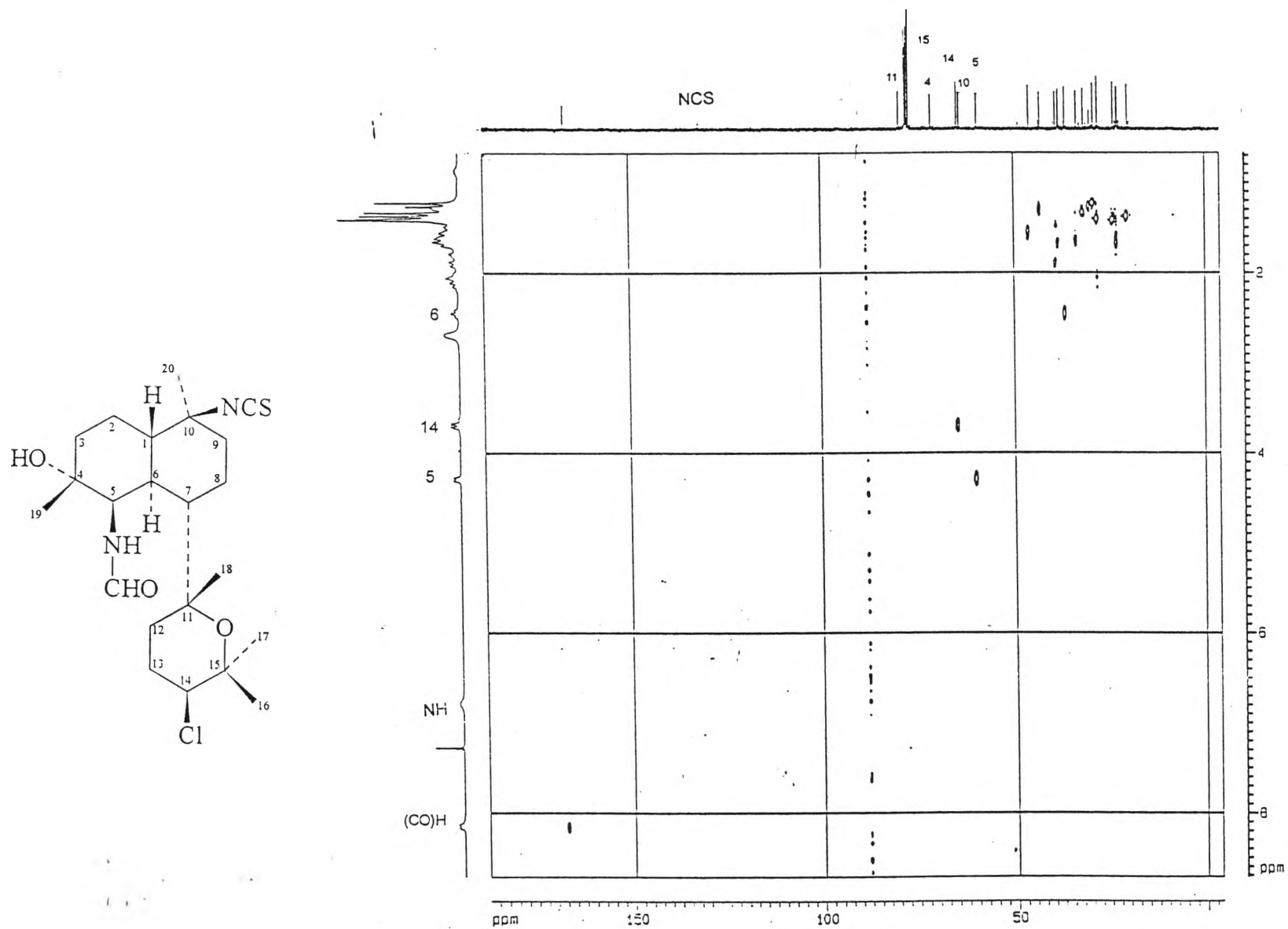


Figure 9. ^1H - ^{13}C HETCOR spectrum of compound K020 (in CDCl_3)

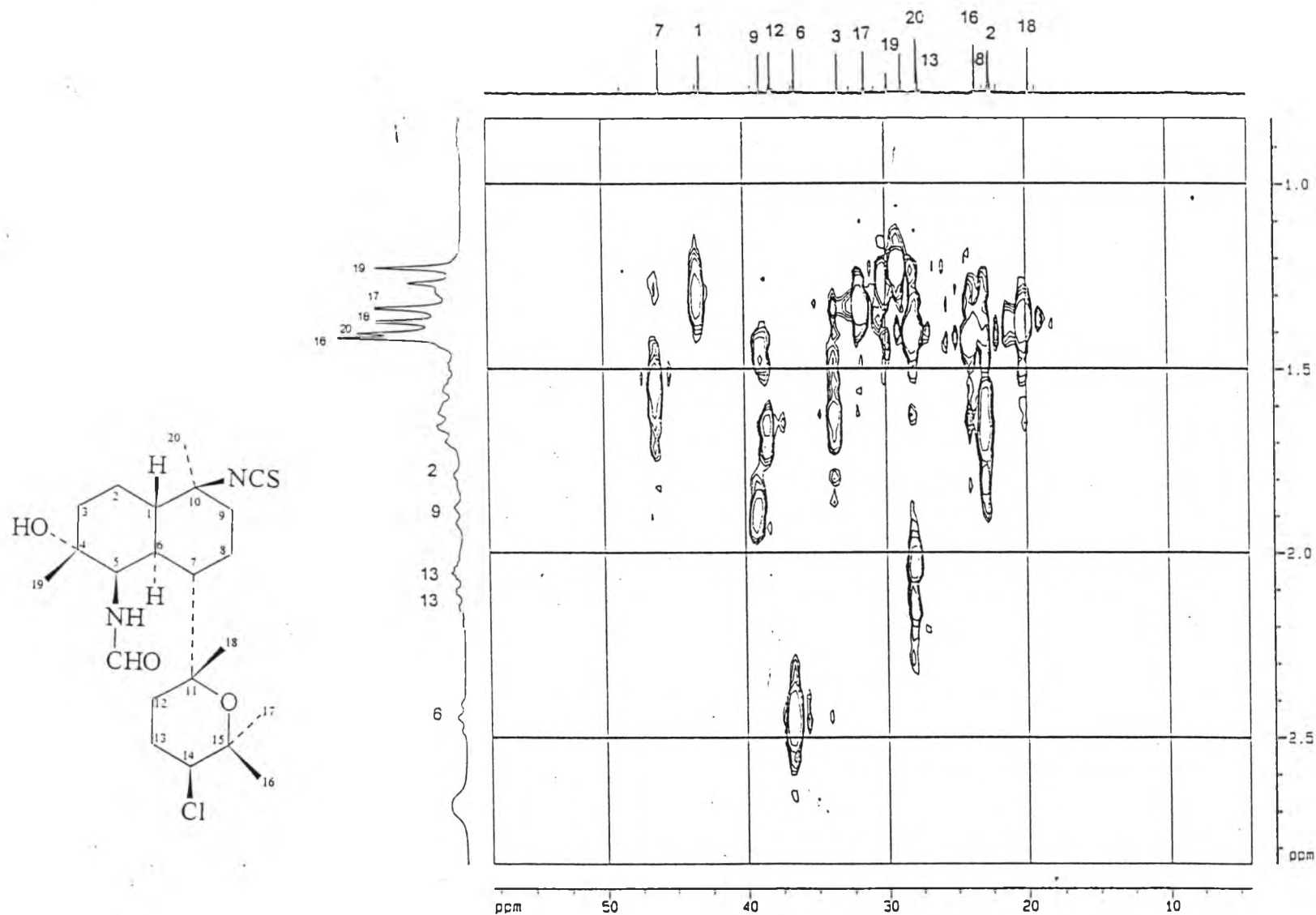


Figure 10. ^1H - ^{13}C HETCOR spectrum of compound K020 (in CDCl_3)
 (expanded in the range of δ ^1H 0.9-2.8 ppm and δ ^{13}C 5-60 ppm)

38.8 ppm, five methine carbons at δ 36.3, 43.0, 45.9, 59.3 and 64.4 ppm, five quaternary carbons at δ 63.7, 71.0, 77.2, 79.1 and 132.0 ppm, and one formyl carbonyl carbon at δ 166.7 ppm.

In the ^1H NMR spectrum (Figures 11-12), the formamide group could be recognized from the two most downfield signals at δ 8.12 (d, $J = 11.0$ Hz, (CO)H) and 6.55 (br t, $J = 11.0$ Hz, NH). The ^1H - ^1H COSY spectrum (Figures 13) displayed the correlation of the NH proton of the formamide to a one-proton doublet at δ 4.26 ppm ($J = 10.1$ Hz, H-5), which, alternately, coupled to a one-proton doublet of triplets at δ 2.41 ppm ($J = 11.4, 1.7$ Hz, H-6). Signals of a methyl, as well as a *gem*-dimethyl protons geminal to an ether oxygen appeared at δ 1.34 (18-Me), 1.38 (16-Me) and 1.30 (17-Me) ppm, respectively. These methyl groups are parts of a chlorotetrahydropyranyl ring, which could be identified by a key mass fragment peak at m/z 161, the doublet of doublets at δ 3.65 (1H, $J = 12.4, 4.5$ Hz, H-14) and the ^{13}C NMR resonance of α -carbons of pyran ring at δ 79.1 (C-11) and 77.2 (C-15) ppm (Chang *et al.*, 1987; Alvi *et al.*, 1991). Two other methyl singlets at δ 1.19 and 1.36 ppm could be assigned to a methyl (19-Me) geminal to a hydroxyl group and a methyl (20-Me) geminal to an isothiocyano group, respectively.

Assignments of the ^{13}C NMR data of K020 were made by comparison with those of kalihinol J (Alvi *et al.*, 1991) and other related multifunctional diterpenes in the kalihinol series previously reported from marine sponges of this genus. ^1H - ^{13}C HMBC experiment (Figures 14-15) was performed in order to confirm the positions of these substituents. Correlations could be observed between 19-methyl protons at δ 1.19 and C-3 (δ 33.3 ppm), C-4 (δ 71.0 ppm) and C-5 (δ 59.3 ppm), while the H-5 methine proton (δ 4.26 ppm) showed further correlations to C-1 (δ 43.0 ppm), C-6 (δ 36.3 ppm) and the formamide carbonyl (δ 116.7 ppm), establishing the position of the methyl geminal to a hydroxy group and the formamide group to be at C-4 and C-5, respectively. Another methyl geminal to an isocyano group (δ 1.36 ppm) displayed two- and three-bond correlations to C-1, C-9 (δ 38.8 ppm) and C-10 (δ 63.7 ppm), confirming its position to be at C-10. The chlorotetrahydropyranyl ring could be placed at position 7, according to the correlations between 18-methyl proton signals to C-7 (δ 45.9 ppm), C-11 (δ 79.1 ppm) and C-12 (δ 38.0 ppm) and between H-6 (δ 2.41 ppm) to C-7 and C-11. Major HMBC correlations in the structure of compound K020 can be summarized as shown in Figure 16.

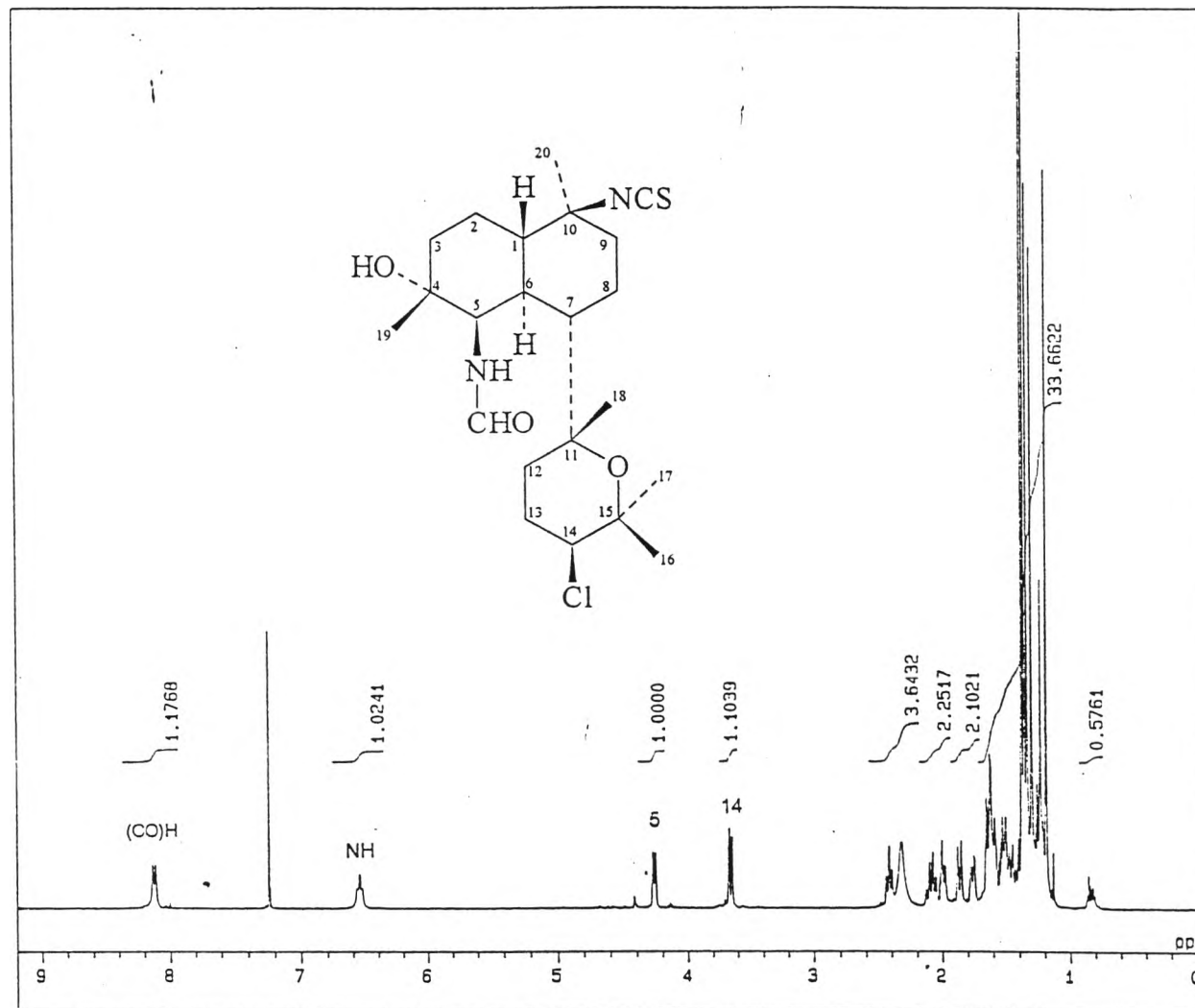


Figure 11. The 500 MHz ^1H NMR spectrum of compound K020 (in CDCl_3)

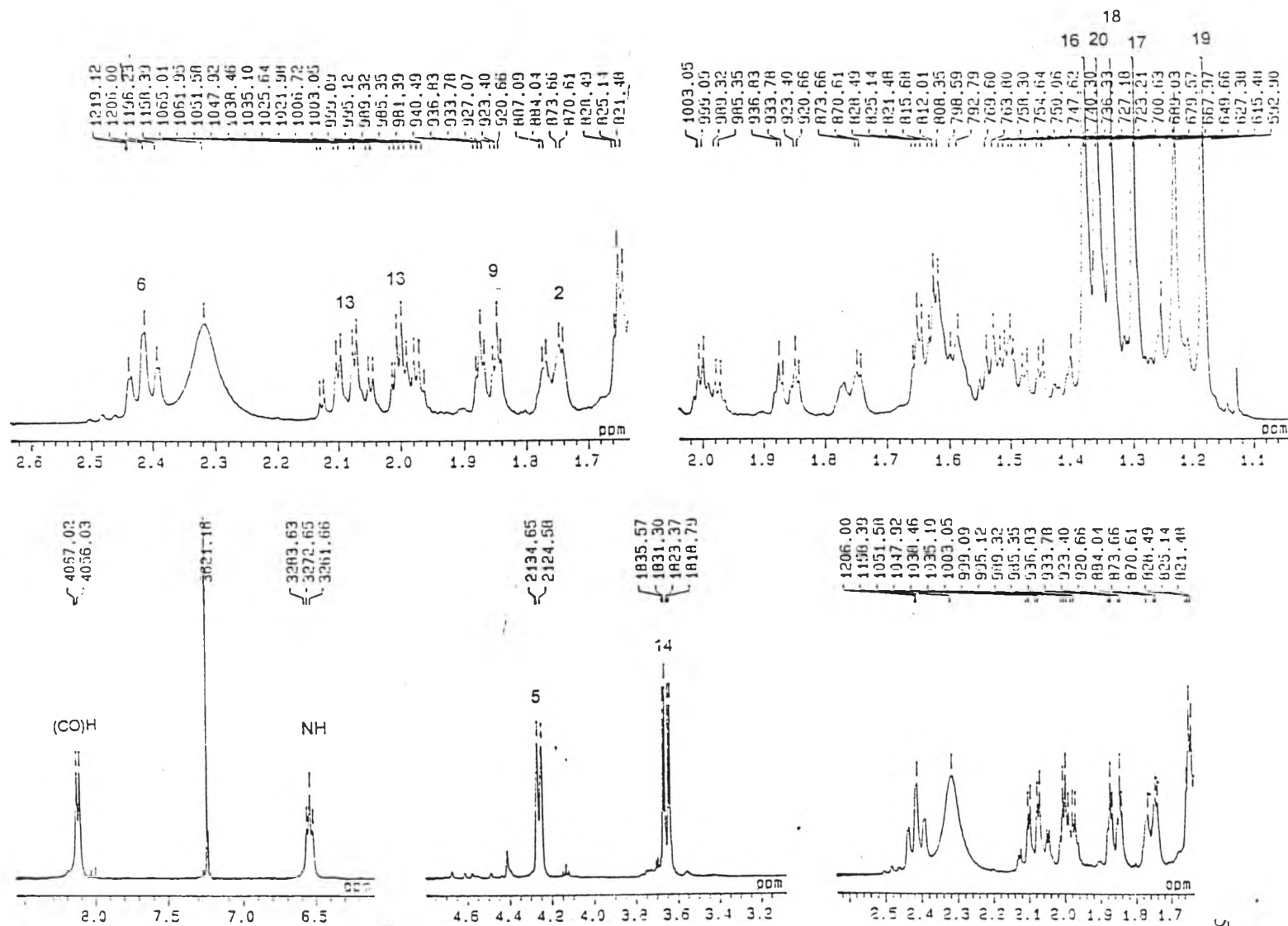
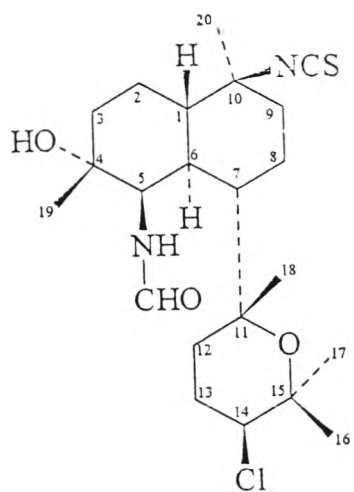


Figure 12. The 500 MHz ^1H NMR spectrum of compound K020 (in CDCl_3)(expanded)

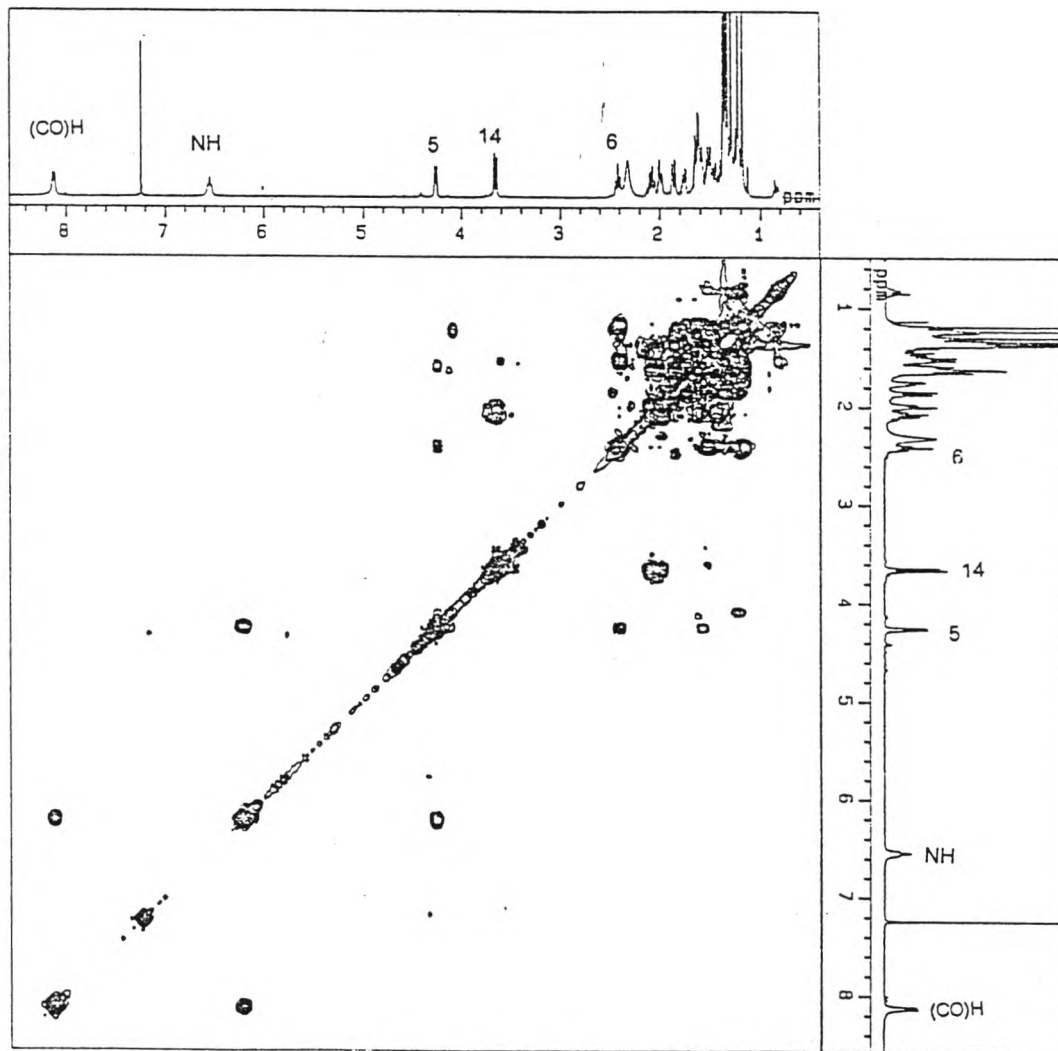
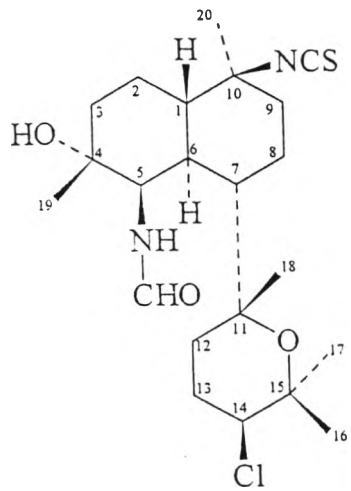


Figure 13. ^1H - ^1H COSY spectrum of compound K020 (in CDCl_3)

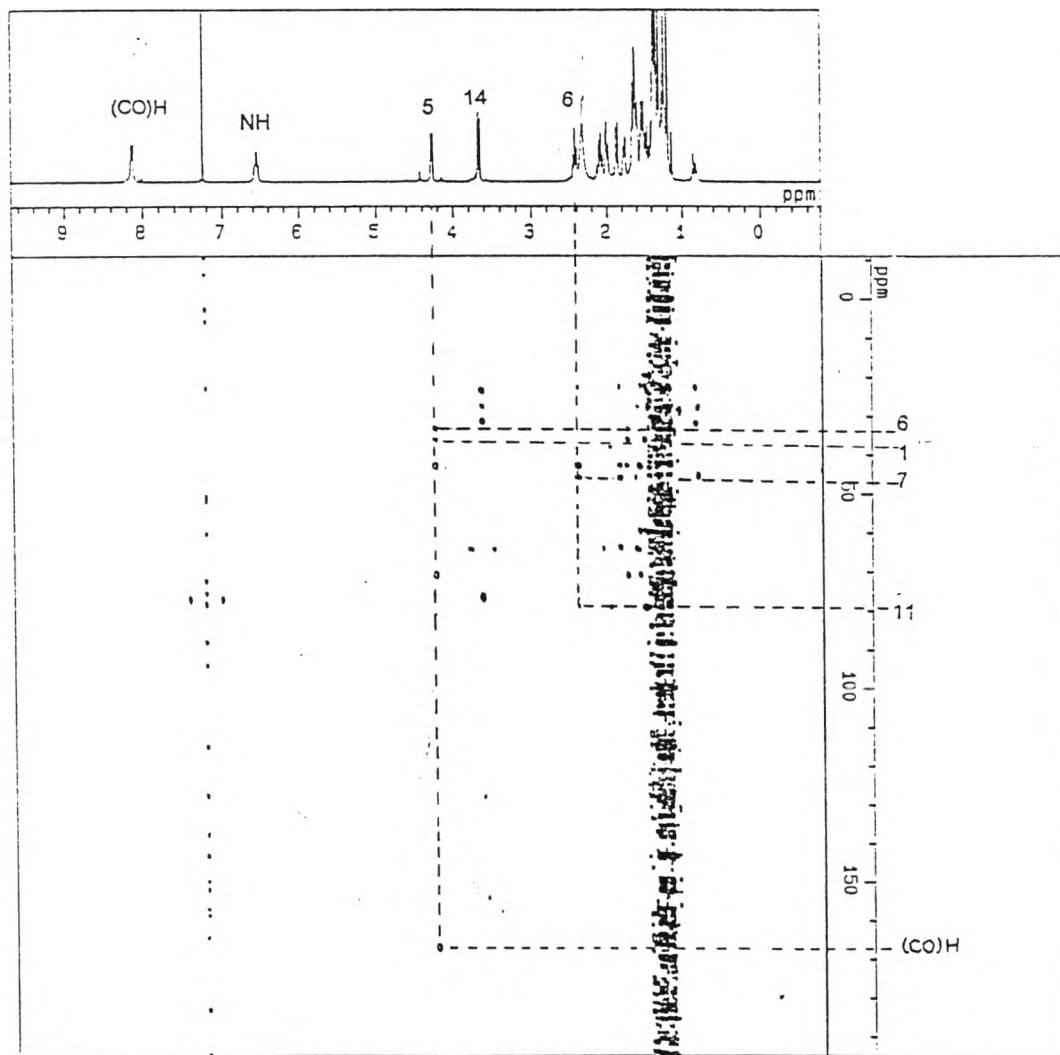
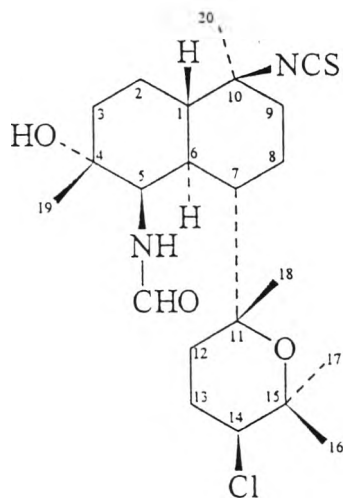


Figure 14. ^1H - ^{13}C HMBC spectrum of compound K020 (in CDCl_3)

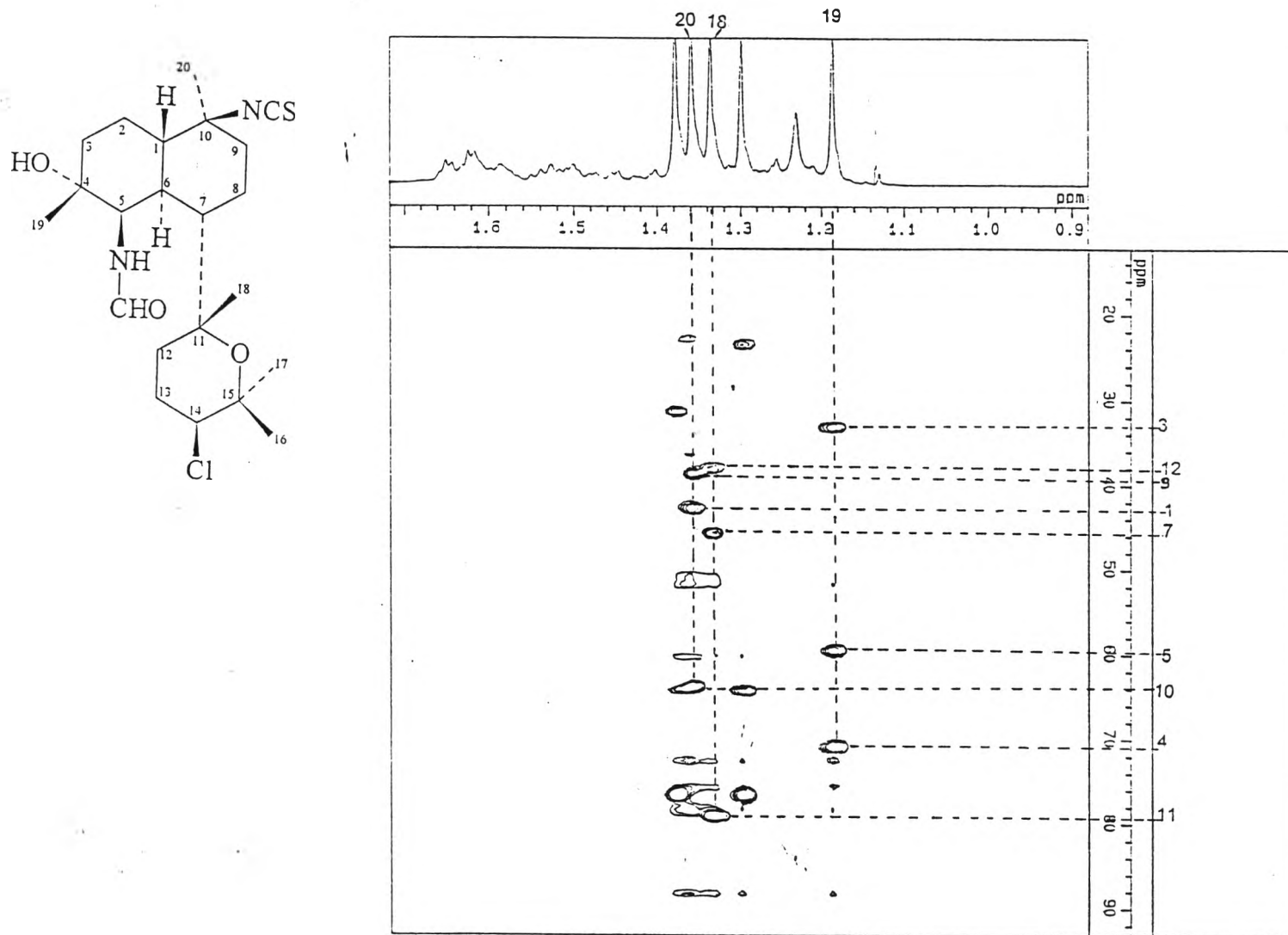


Figure 15. ^1H - ^{13}C HMBC spectrum of compound K020 (in CDCl_3)
 (expanded in the range of δ ^1H 0.9-1.7 ppm and δ ^{13}C 20-90 ppm)

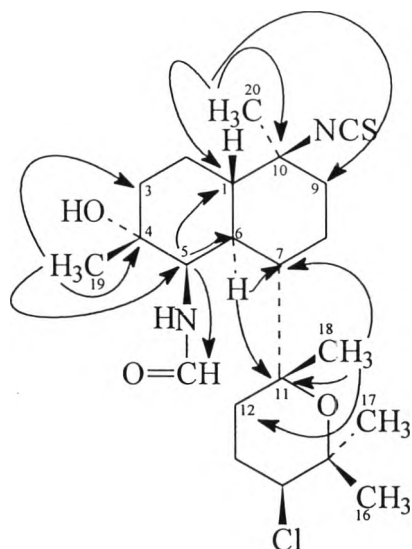


Figure 16. Major HMBC correlations of compound K020

Although most of carbon signals of K020 were nearly identical to those of kalihinol J (45), a major difference in chemical shift was observed for C-10 (δ 63.7 ppm for K020 and δ 76.5 ppm for kalihinol J) (Table 9). The C-10 chemical shift of K020 was comparable with that of the previously reported kalihinol H (37) (Chang *et al.*, 1987), in which the chemical shift of the same position was 63.3 ppm and the conformation of the methyl group at that particular position was axial. NOESY experiment (Figures 17-18), in which correlation between 20-Me and H-6 could be observed, also supported such conformation. Major NOESY correlations in the structure of K020 are shown in Figure 19. Therefore, compound K020 was assigned the structure shown in Figure 20 and named 10-*epi*-kalihinol J.

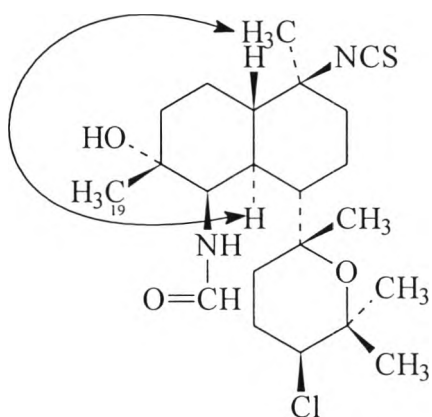


Figure 19. Major NOESY correlations of compound K020

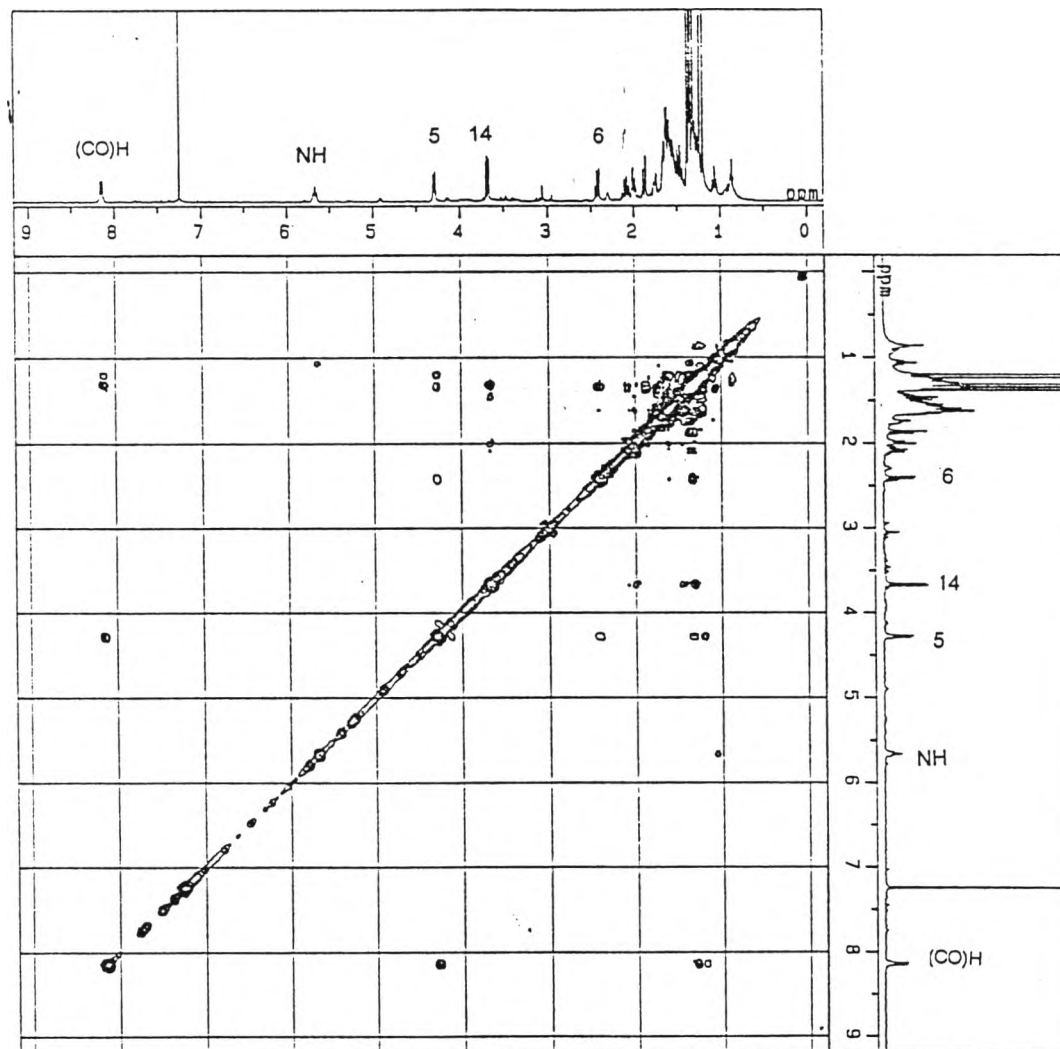
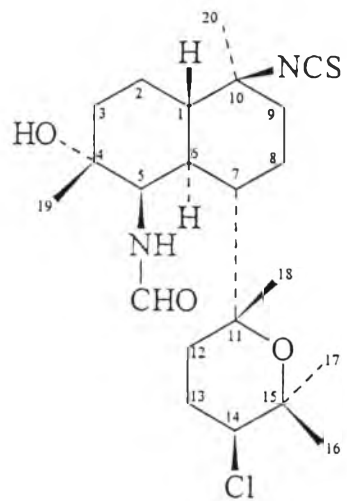


Figure 17. NOESY spectrum of compound K020 (in CDCl₃)

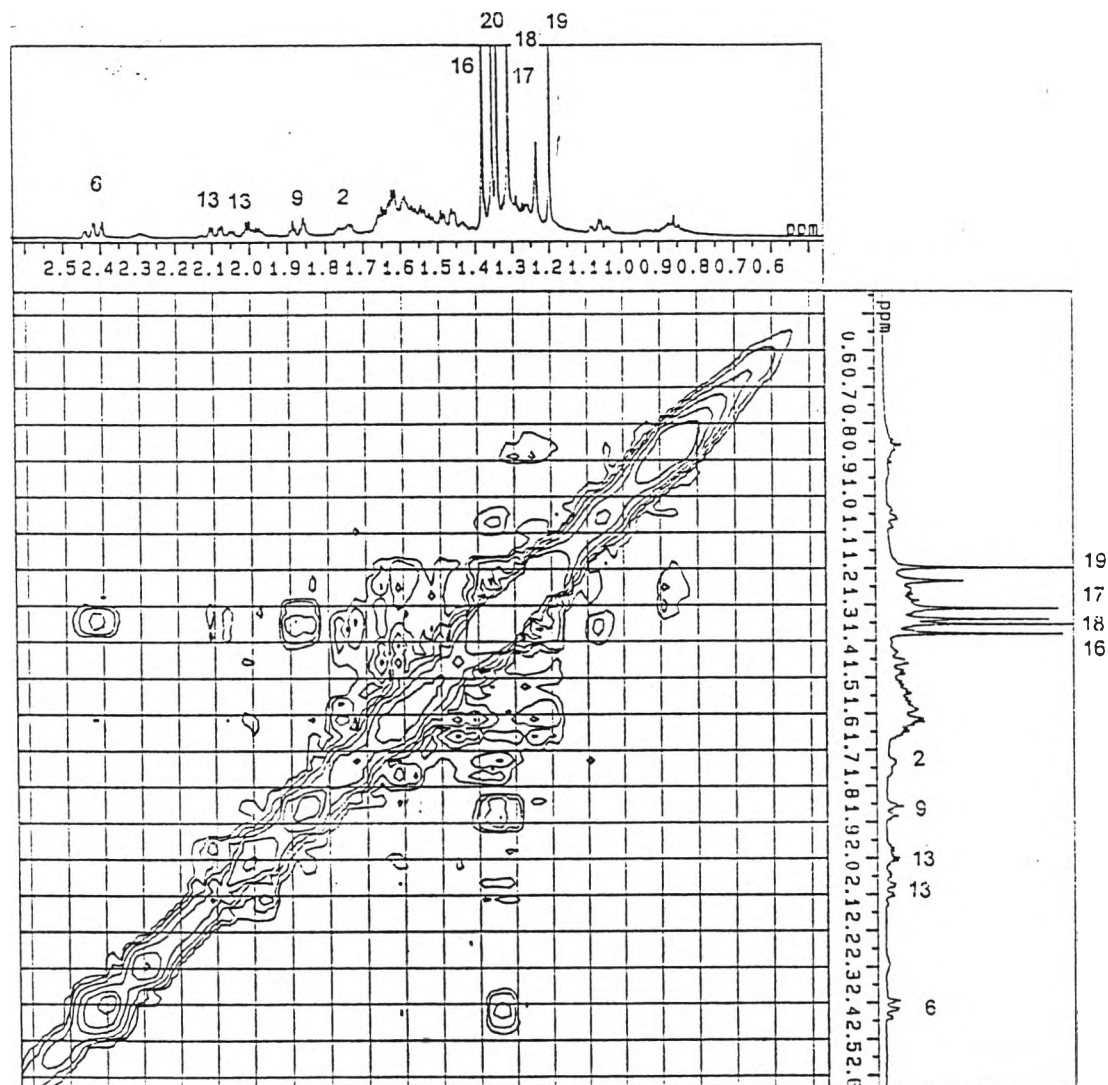
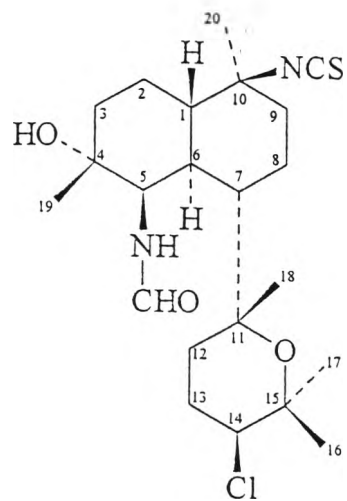


Figure 18. NOESY spectrum of compound K020 (in CDCl₃)
(expanded in the range of δ 0.6-2.5 ppm)

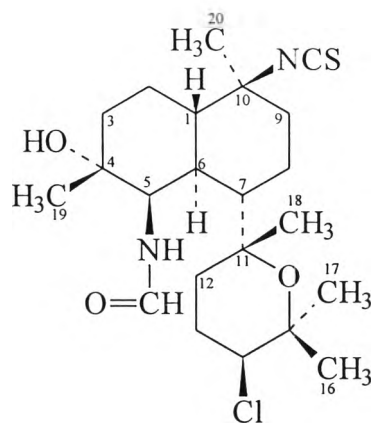


Figure 20. Structure of 10-*epi*-kalihinol J

Table 9. Carbon chemical shift assignments of kalihinol J (45) K020 and kalihinol H (37)

Carbon	Chemical shift (δ) ppm, in CDCl ₃		
	Kalihinol J (45)	K020	Kalihinol H (37)
1	43.1	43.0	42.2
2	22.4	22.4	21.6
3	33.3	33.3	32.7
4	71.1	71.0	70.8
5	58.9	59.3	63.2
6	36.3	36.3	36.0
7	45.9	45.9	46.3
8	22.4	22.4	24.3
9	38.8	38.8	39.9
10	76.5	63.7	63.3
11	79.1	79.1	87.3
12	38.0	38.0	38.1
13	27.6	27.6	25.1
14	64.4	64.4	83.5
15	71.1	77.2	59.5
16	23.4	23.5	25.5
17	31.3	31.4	24.2
18	19.7	19.7	17.2
19	28.7	28.8	28.8
20	28.8	27.7	20.8
NCS	131.0	132.0	129.9
NHCOH	166.9	166.7	-
NC	-	-	-

Table 10. Proton chemical shift assignments of K020

Proton	Chemical shift (δ) ppm, in CDCl ₃	Multiplicity (J-value, Hz)
1	~1.3	m
2	1.76	m
3	~1.65	m
4	-	-
5	4.26	d (J = 10.1)
6	2.41	dt (J = 11.0, 2.0)
7	~1.55	m
8	~1.65	m
9	1.86	ddd (J = 13.4, 3.7, 3.1)
10	-	-
11	-	-
12	~1.67	m
13	2.09	ddd (J = 13.4, 3.6,)
13	1.99	ddd (J = 13.7, 7.6, 3.7)
14	3.65	dd (J = 12.0, 4.0)
15	-	-
16	1.38	s
17	1.30	s
18	1.34	s
19	1.19	s
20	1.36	s
NH	6.55	br t (J = 11.0)
(CO)H	8.12	d (J = 11.0)

2. Structure elucidation of compound K028

K028, obtained as white amorphous powder (25 mg) from fraction F028, has a molecular formula of $C_{22}H_{33}ClN_2O_3S_2$ which was established by APCI-MS ($[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-HCl]^+$, $[M+H-NCS]^+$ and $[M+H-HCl-2NCS]^+$ peaks at m/z 457, 439, 421, 398 and 303, respectively (Figure 21). The presence of the isothiocyanate functionality in the molecule was again confirmed by a very intense IR band at 2102 cm^{-1} (Figure 22) and a UV maximum at 245 nm (Figure 23). A broad band at 3396 cm^{-1} in its IR spectrum further suggested hydroxyl group(s) as part of the molecule (Figure 22).

The ^{13}C NMR spectrum of K028 (Figures 24-25) showed 21 signals. DEPT and ^1H - ^{13}C HSQC (Figure 26) experiments were utilized to classify these signals into those of five methyl carbons at δ 19.1, 22.8, 27.5, 28.7, and 30.6 ppm, six methylene carbons at δ 21.8, 22.2, 27.4, 32.4, 38.0 and 39.3 ppm, five methine carbons at δ 36.4, 43.3, 48.6, 63.7 and 64.3 ppm, four quaternary carbons at δ 64.3, 70.1, 75.9, and 77.2 ppm, and two isothiocyano carbons at the same chemical shift of 132.1 ppm.

The two most downfield signals in the ^1H NMR spectrum of K028 (Figures 27-28) were those of H-5, appearing as a broad one-proton singlet at δ 4.58 ppm, and H-14 as a one-proton doublet of doublets at δ 3.75 ppm ($J = 12.5, 4.5\text{ Hz}$). Signals of a methyl and gem-dimethyl protons geminal to an ether oxygen appeared at δ 1.25 (18-Me), 1.36 (16-Me) and 1.35 (17-Me) ppm, respectively. These methyl groups are parts of a chlorotetrahydropyranyl ring, which could be identified by a key mass fragment peak at m/z 161.

The ^{13}C NMR spectrum of K028 was assigned by comparison with that of kalihinol I (Alvi *et al.*, 1991) and other related kalihinol diterpenes previously reported from the same genus of marine organism. ^1H - ^{13}C HMBC experiment (Figures 29-31) was very useful in confirming the assignments of these signals. While the methyl proton at δ 1.41 (H-19) showed correlated peaks with three carbon signals at δ 32.4 (C-3), 70.1 (C-4) and 63.7 (C-5) ppm, the downfield methine proton at δ 4.58 ppm (H-5) could be observed as correlating with five carbon signals at δ 43.3 (C-1), 32.4 (C-3), 70.1 (C-4), 36.4 (C-6) and 28.7 (C-19), establishing the position of the methyl geminal to hydroxy group at

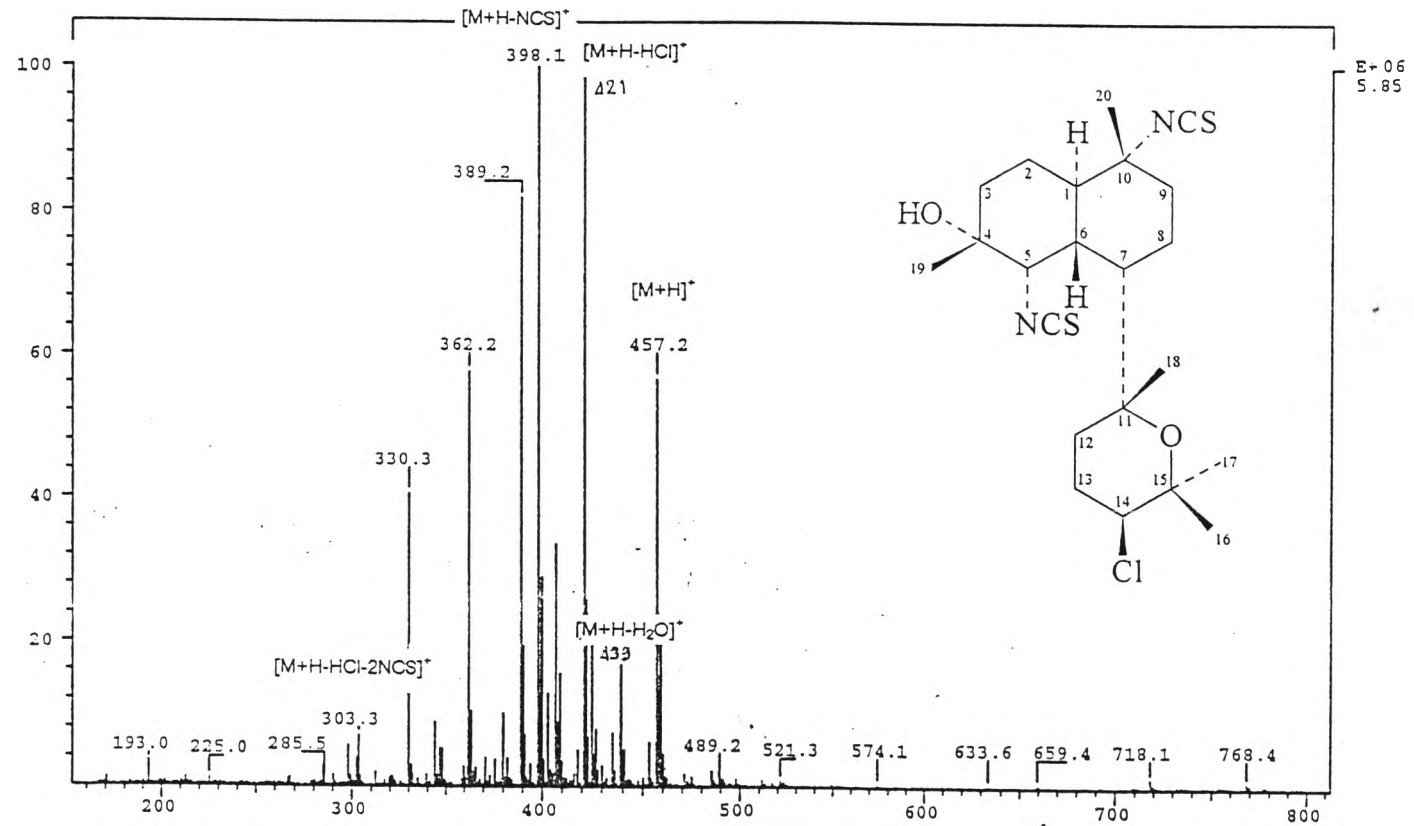


Figure 21. APCI-MS spectrum of compound K028

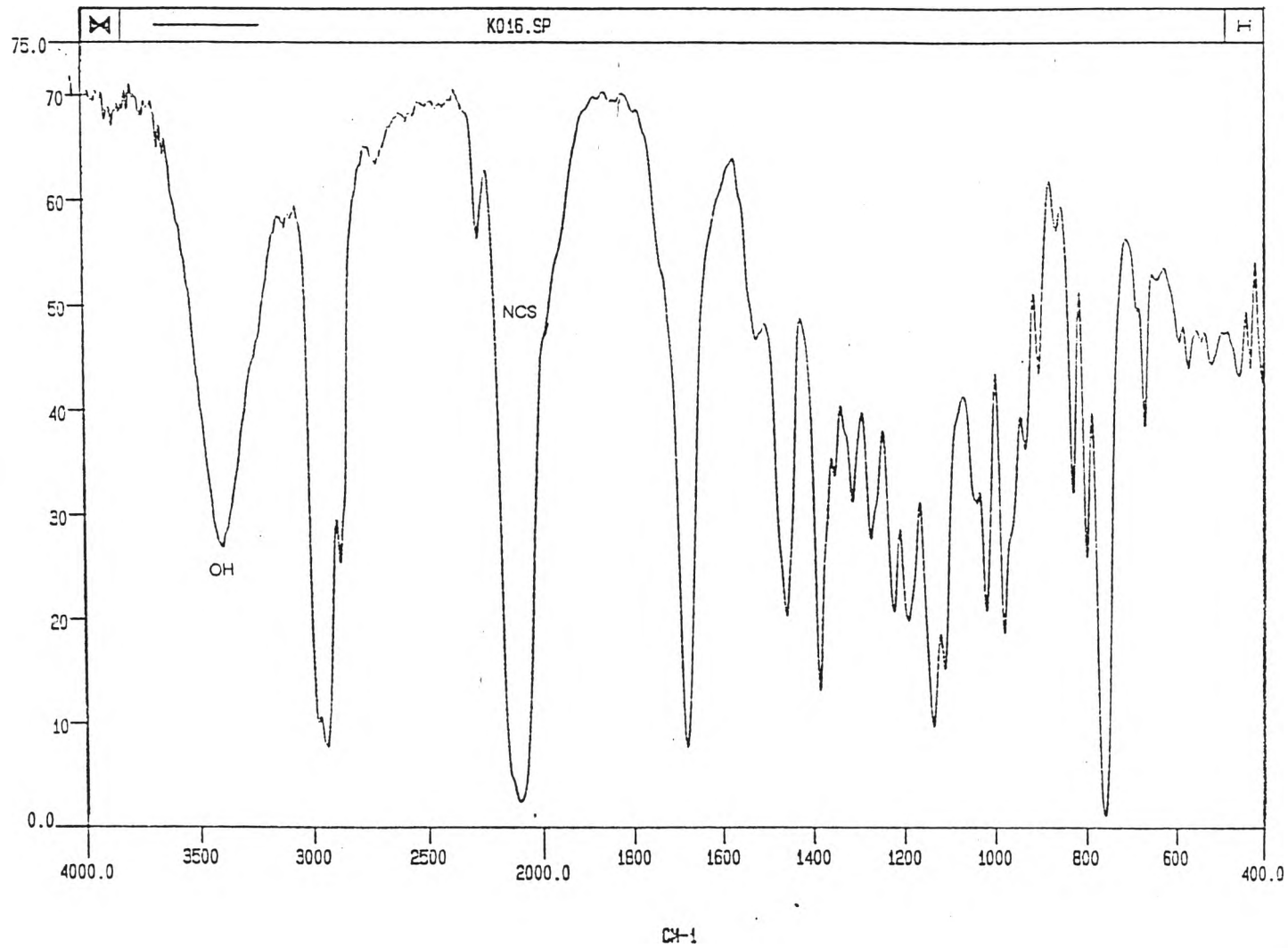
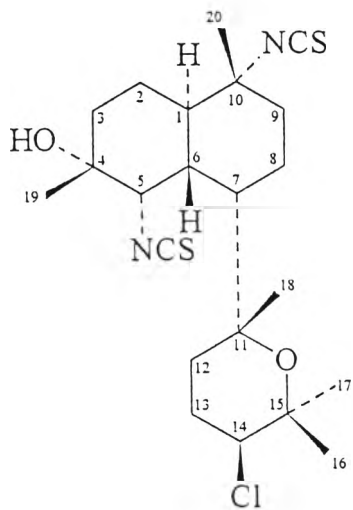


Figure 22. IR spectrum of compound K028

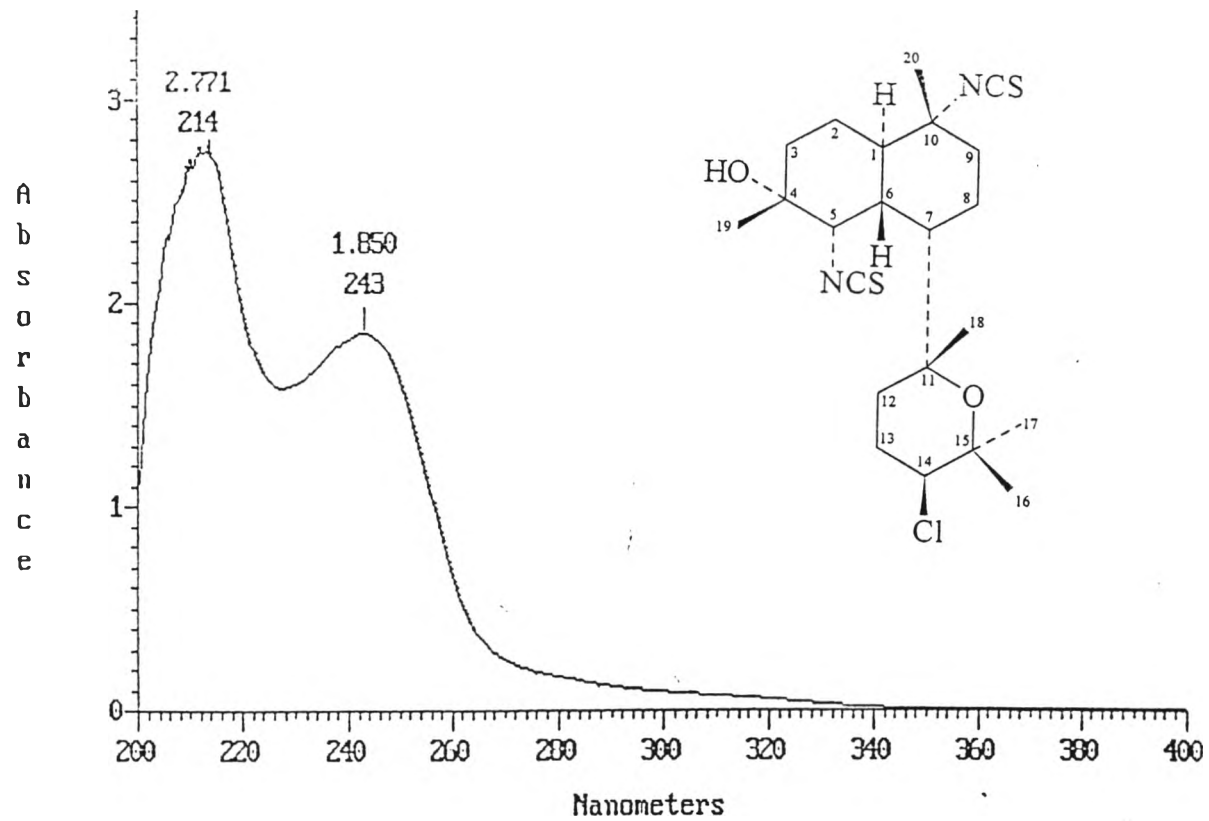


Figure 23. UV spectrum of compound K028 (in MeOH)

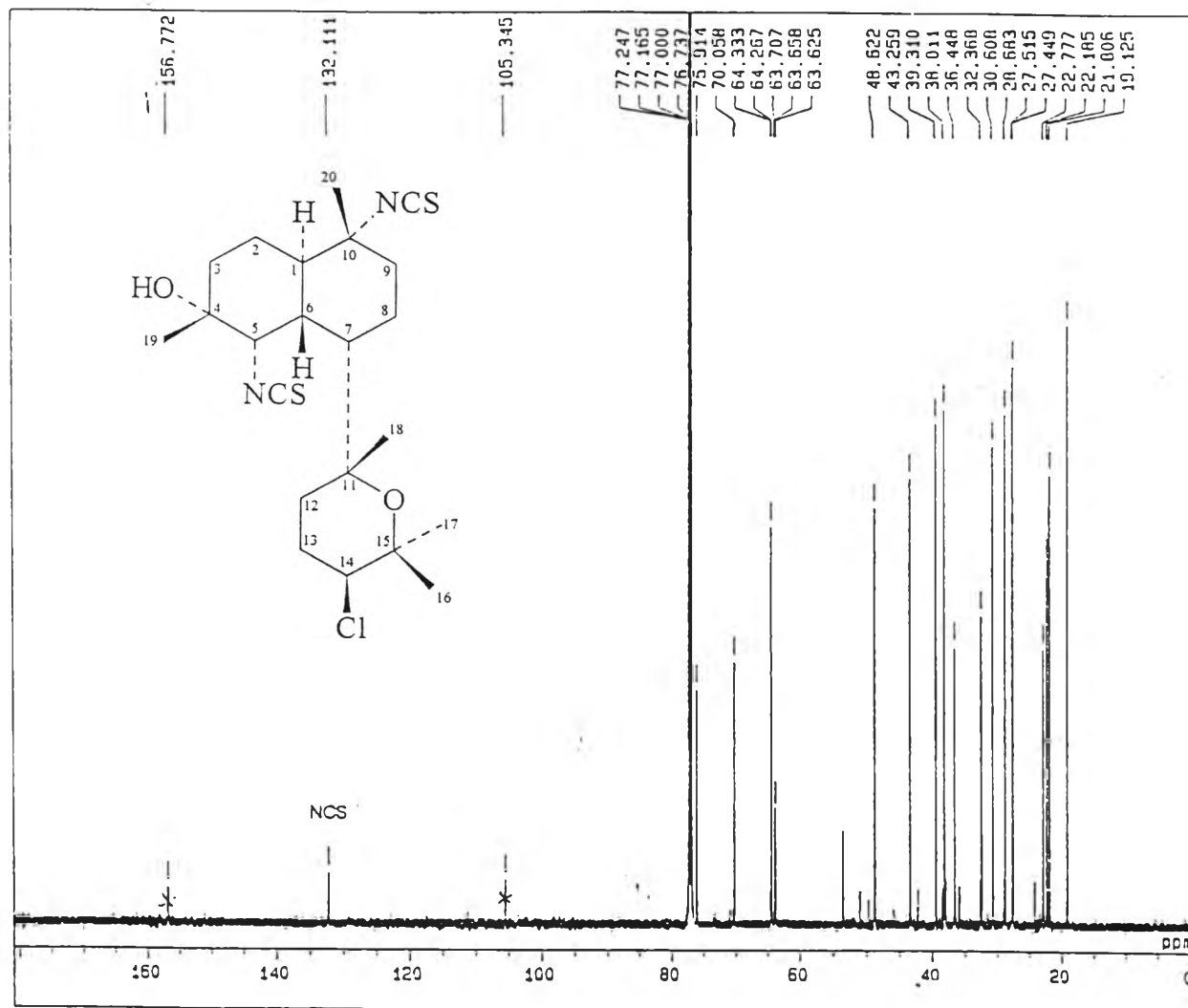


Figure 24. The 125 MHz ^{13}C NMR spectrum of compound K028 (in CDCl_3)

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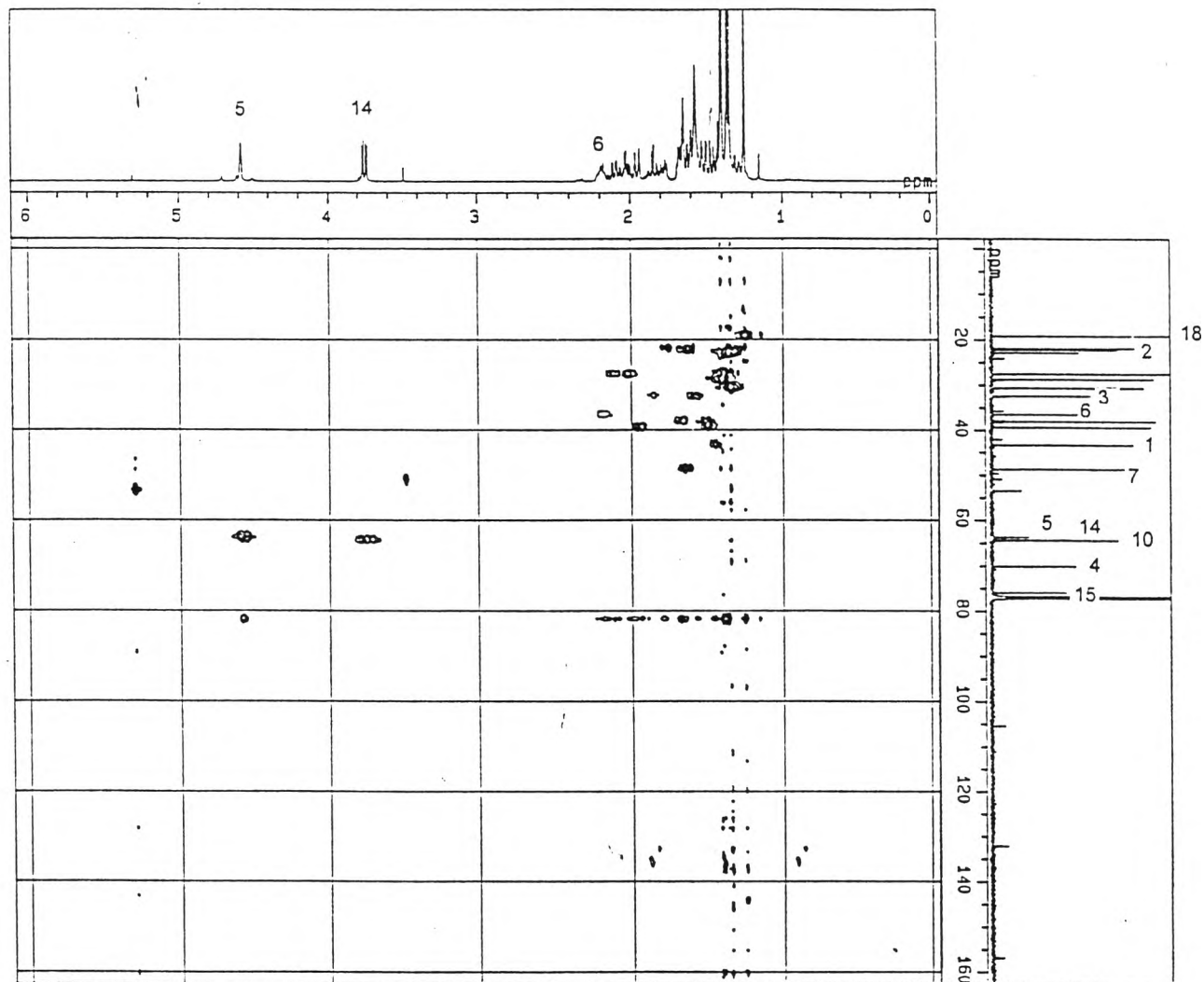
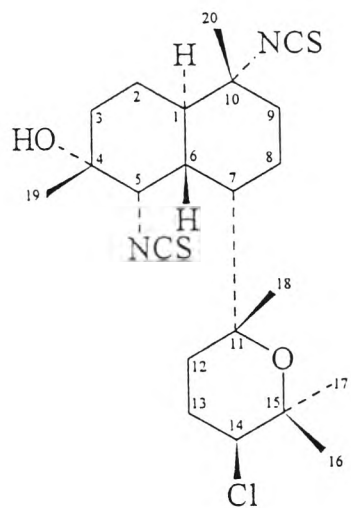


Figure 26. ^1H - ^{13}C HSQC spectrum of compound K028 (in CDCl_3)

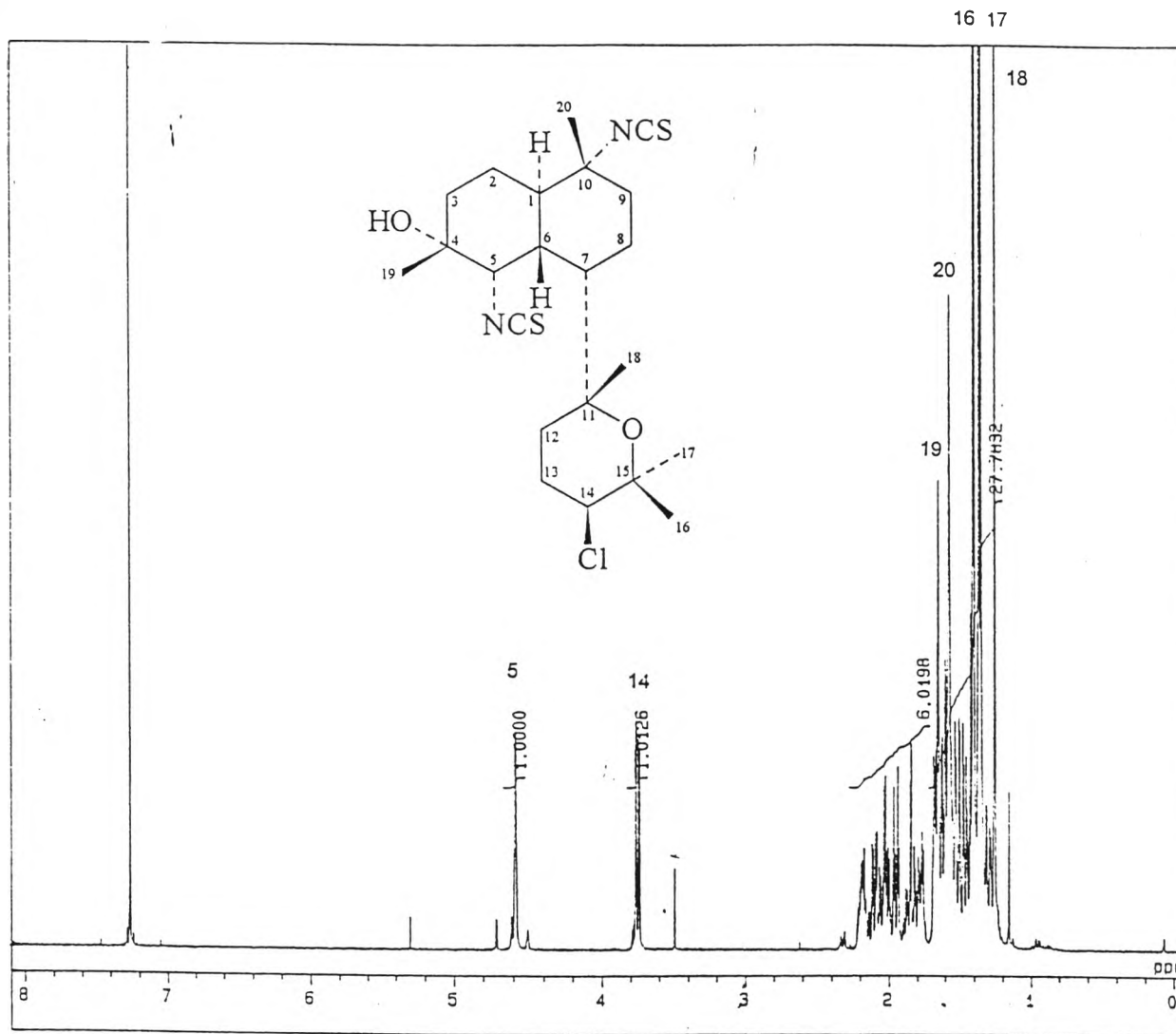


Figure 27. The 500 MHz ^1H NMR spectrum of compound K028 (in CDCl_3)

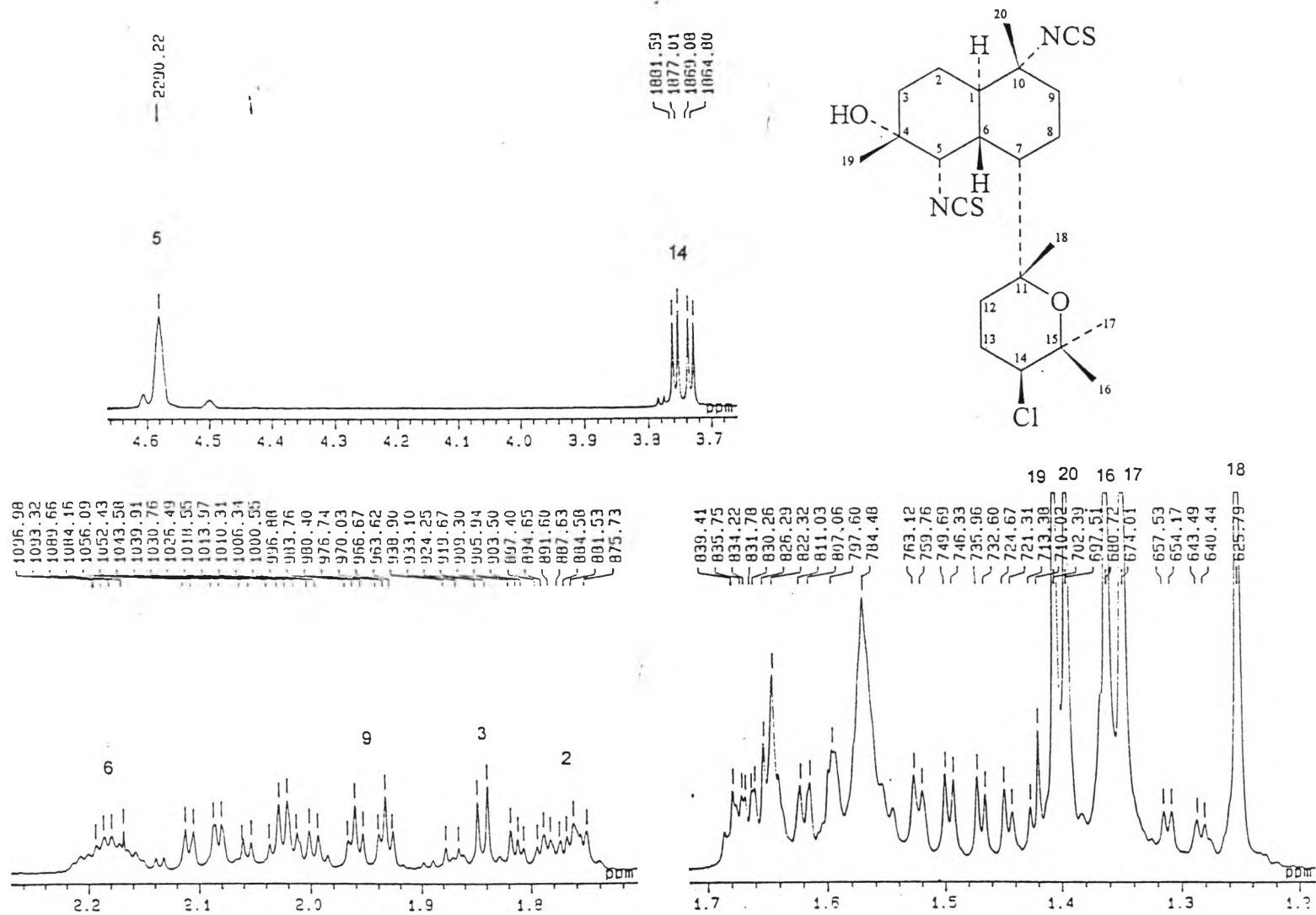


Figure 28. The 500 MHz ¹H NMR spectrum of compound K028 (in CDCl₃)(expanded)

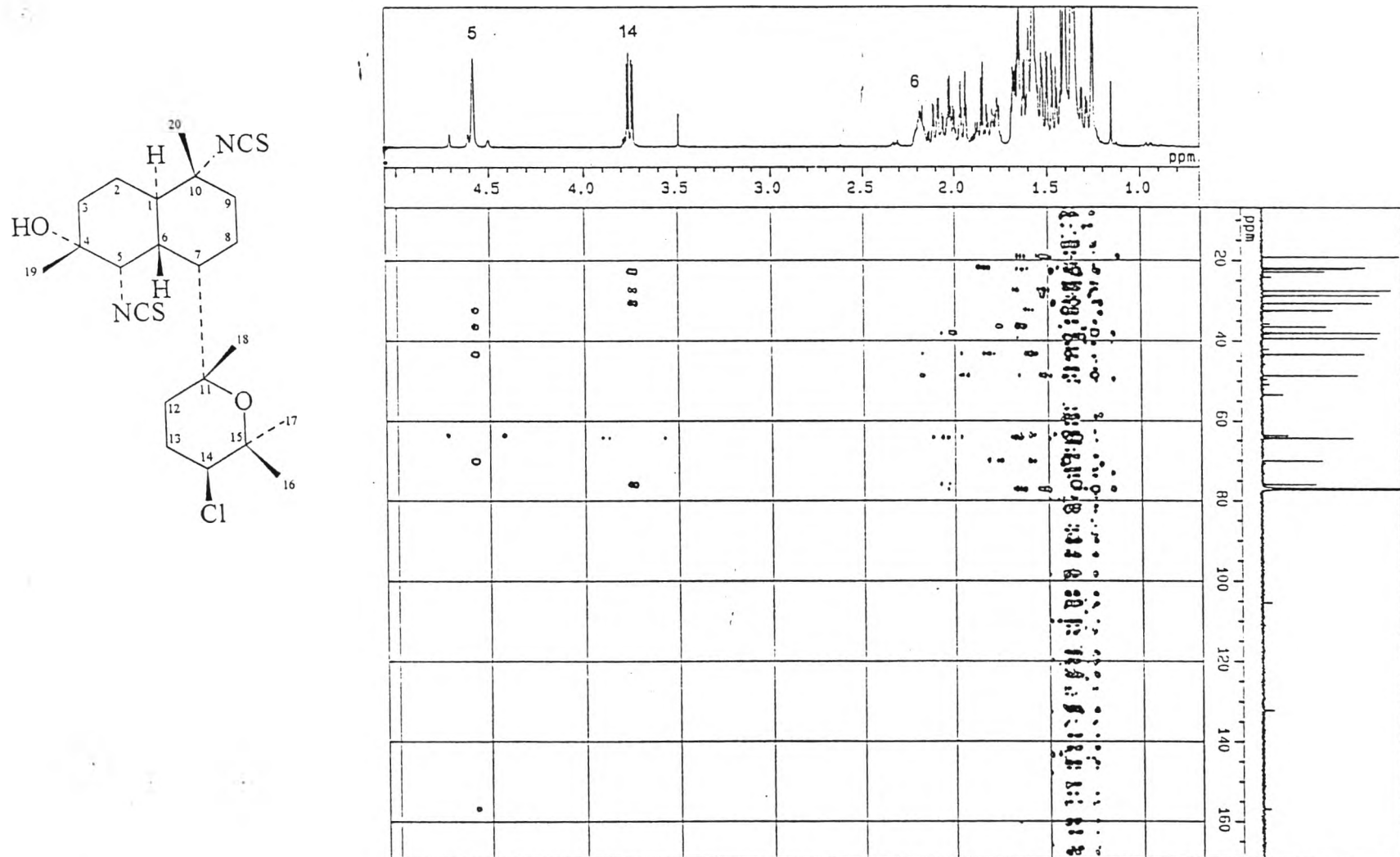


Figure 29. ^1H - ^{13}C HMBC spectrum of compound K028 (in CDCl_3)

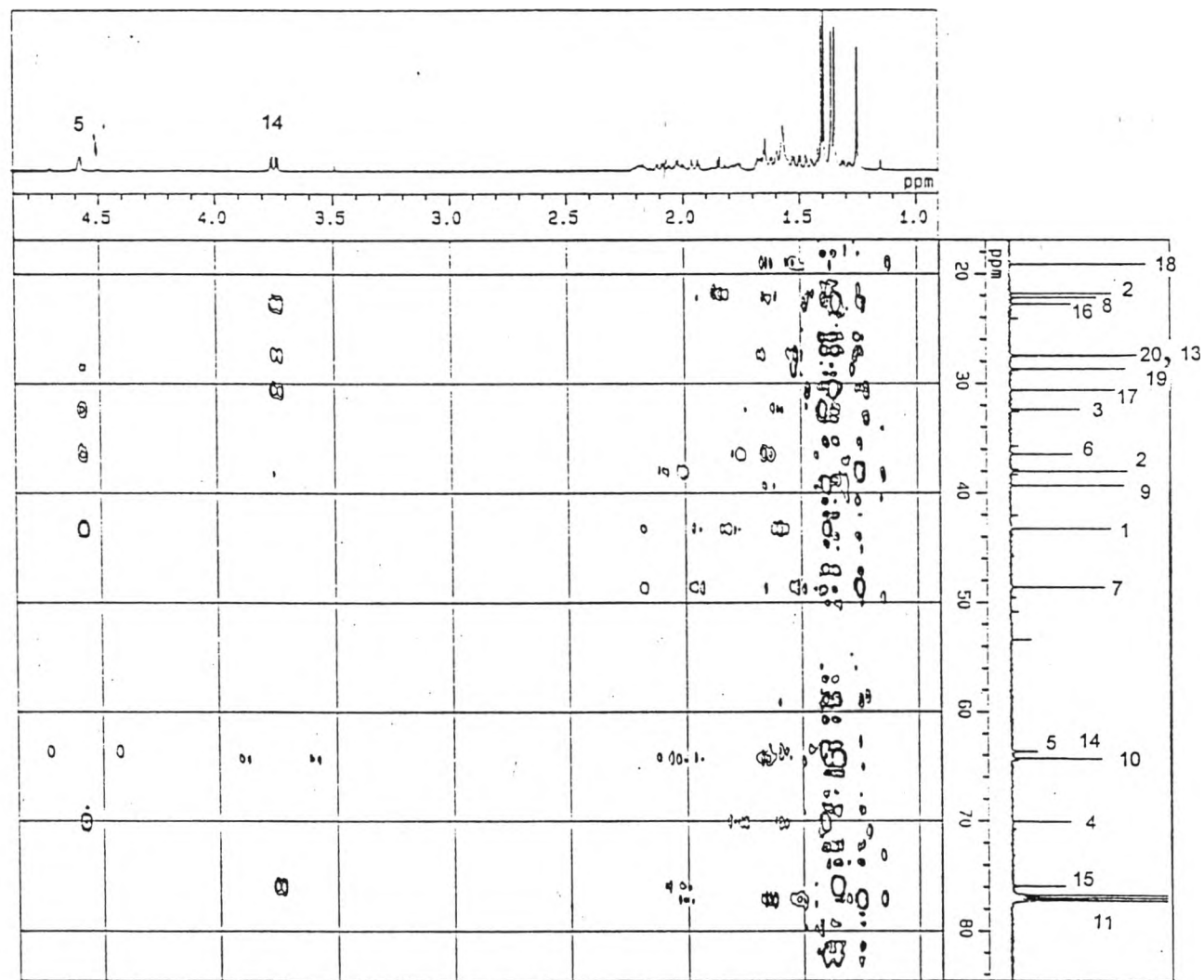
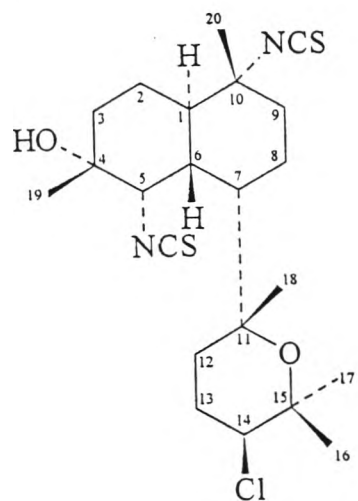


Figure 30. ^1H - ^{13}C HMBC spectrum of compound K028 (in CDCl_3)
 (expanded in the range of δ ^1H 1.0-4.8 ppm and δ ^{13}C 18-84 ppm)

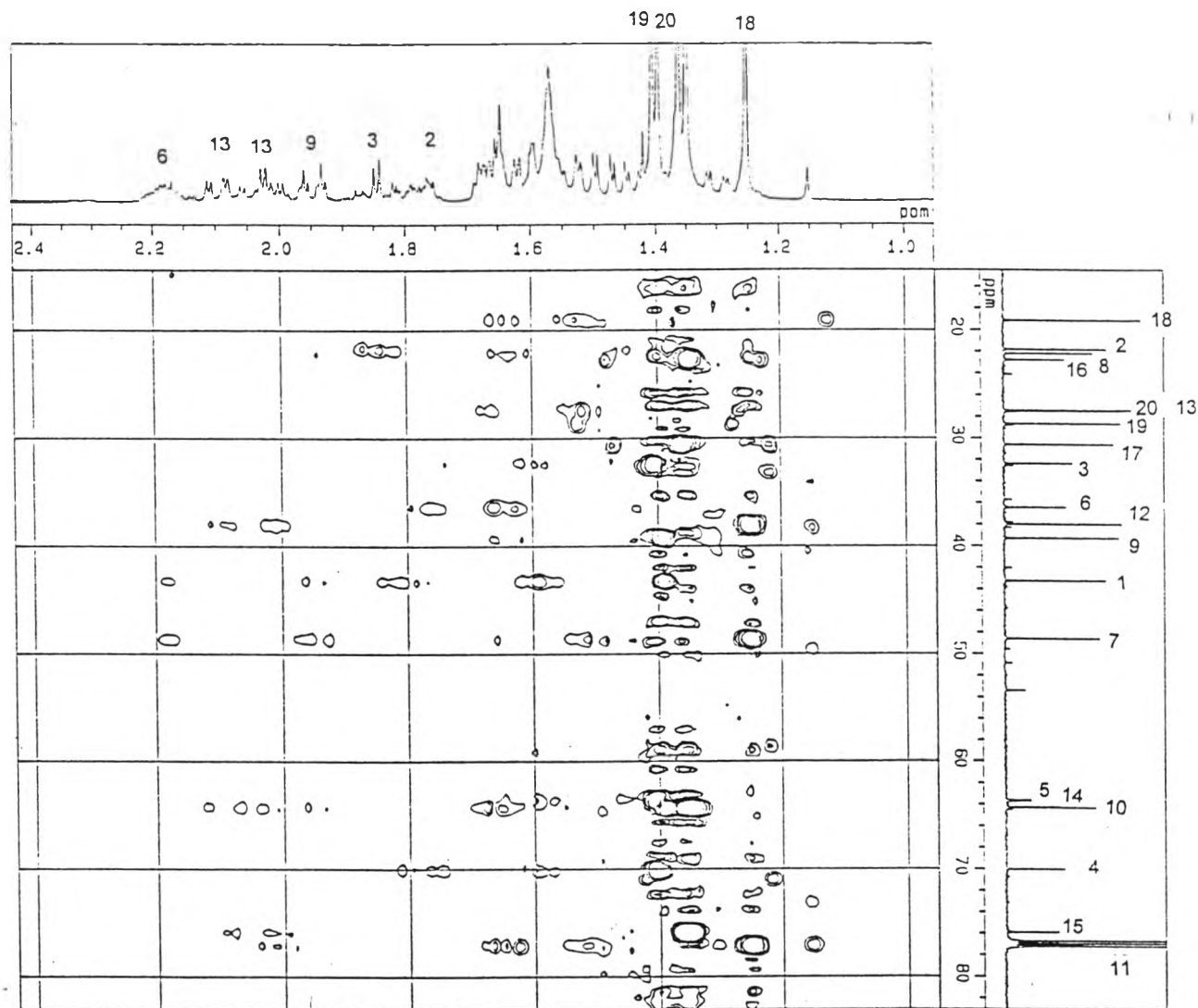
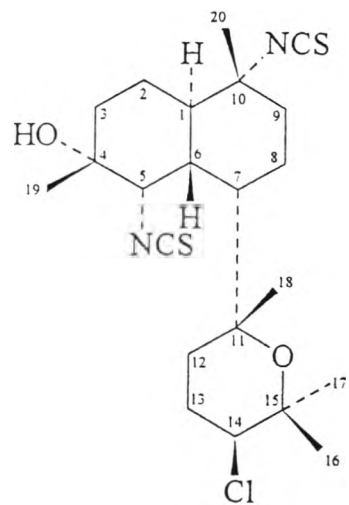


Figure 31. ^1H - ^{13}C HMBC spectrum of compound K028 (in CDCl_3)

(expanded in the range of δ ^1H 1.0-2.4 ppm and δ ^{13}C 16-82 ppm)

C-4. Another methyl geminal to an isothiocyano group (δ 1.40 ppm) displayed correlations to C-1, C-9 (δ 39.3 ppm) and C-10 (δ 64.3 ppm), confirming its position as at C-10. The chlorotetrahydropyranyl ring could again be placed at position 7, according to the correlations between the signal of 18-methyl protons to C-7 (δ 48.6 ppm), C-11 (δ 77.2 ppm) and C-12 (δ 38.0 ppm). Major ^1H - ^{13}C HMBC correlations in the structure of compound K028 are shown in Figure 32.

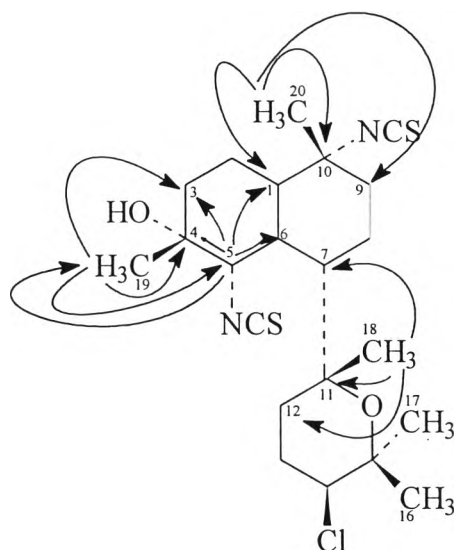


Figure 32. Major ^1H - ^{13}C HMBC correlations of compound K028

Although most carbon chemical shifts of K028 were nearly identical to those of kalihinol I (Alvi *et al.*, 1991) (Table 11), a big discrepancy could be observed for the position 10 (δ 64.3 ppm for K028 and 76.0 ppm for kalihinol I). Difference in splitting pattern of the H-6 signal (multiplet for K028 and doublet of triplets for kalihinol I) was also noticed. The carbon chemical shift of K028 was found to be in agreement with another previously reported compound, 10-*epi*-isokalihinol H (47) (Trimurtula and Faulkner, 1994), of which the conformation of H-1, H-6 and isothiocyano group at C-10 were axial, axial and equatorial, respectively. NOESY experiment (Figures 33-34), in which correlations of 19-Me and 20-Me to H-6 could be observed, also supported the conformation of H-6 as axial. Major NOESY correlations of compound K028 are displayed in Figure 35. Therefore, compound K028 was assigned the structure shown in Figure 36 and named 1,5,6-*tri-epi*-kalihinol I.

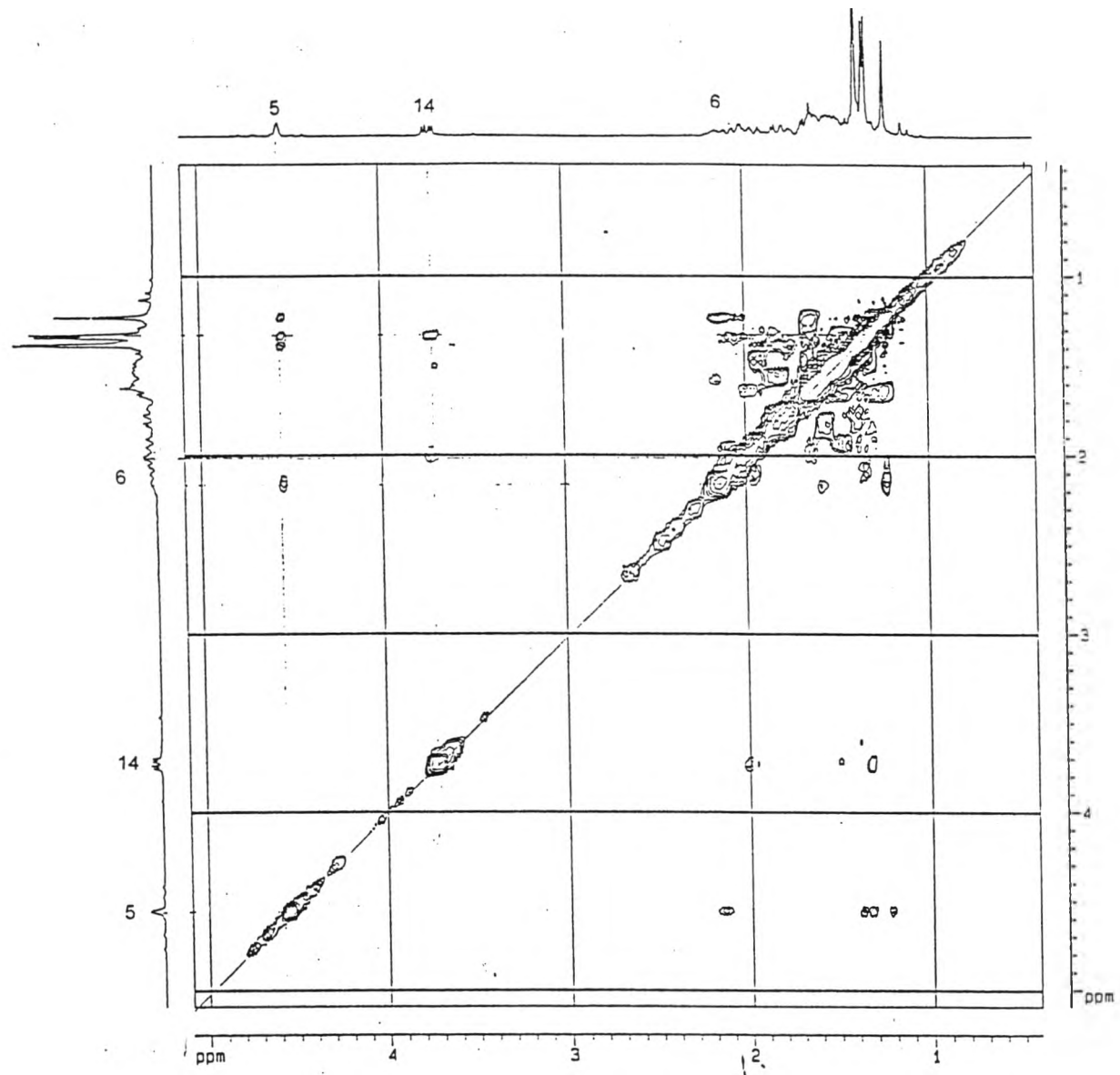
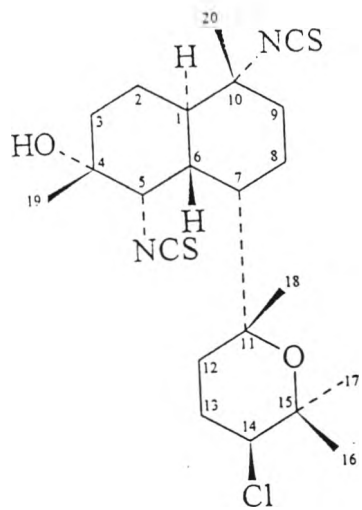


Figure 33. NOESY spectrum of compound K028 (in $CDCl_3$)

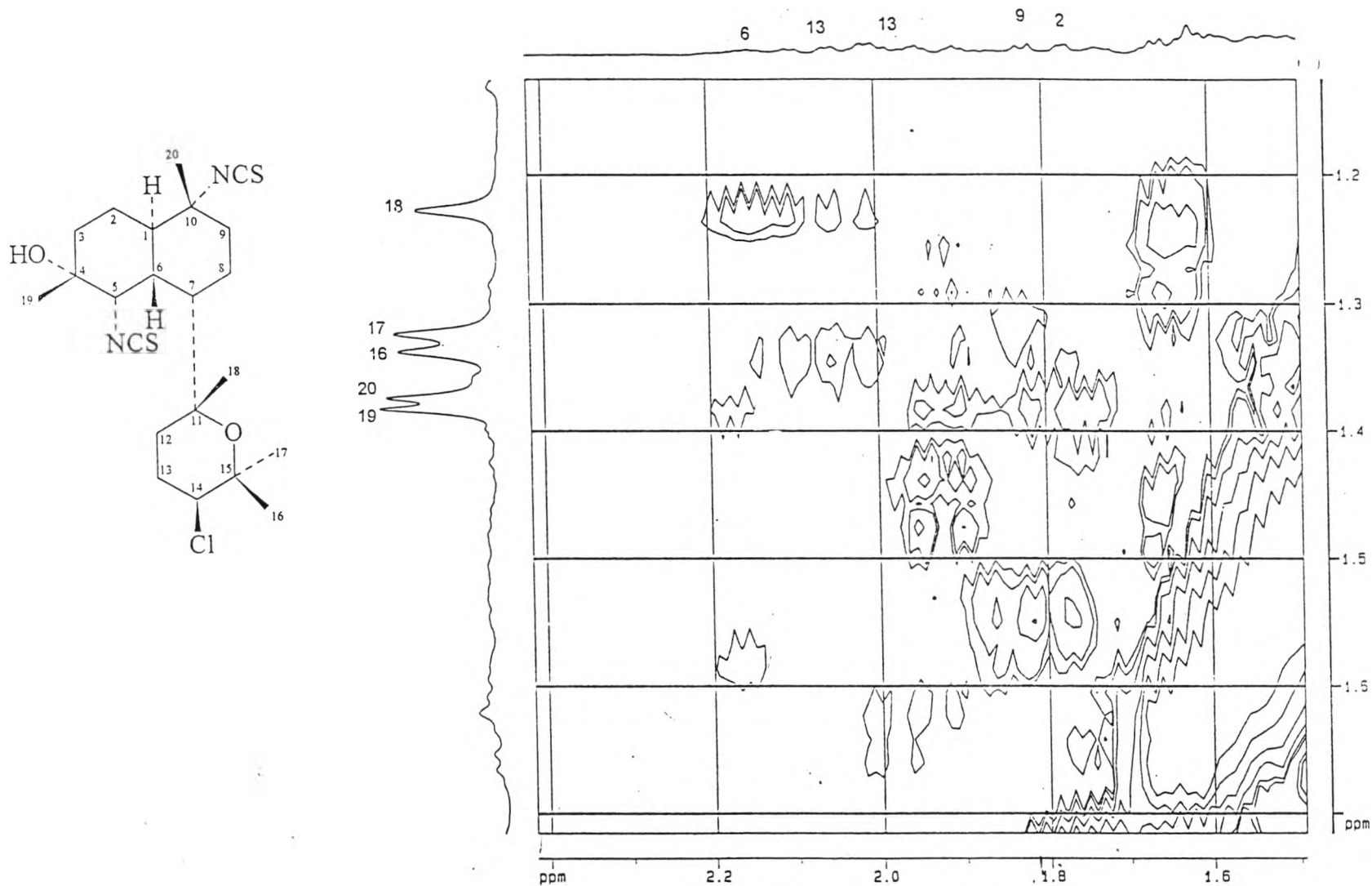


Figure 34. NOESY spectrum of compound K028 (in CDCl₃)
 (expanded in the ranges of δ 1.2-1.7 and 1.5-2.4 ppm)

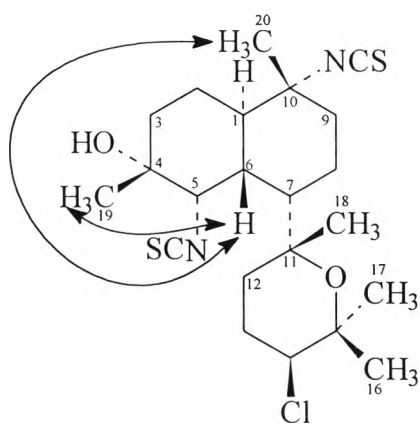


Figure 35. Major NOESY correlations of compound K028

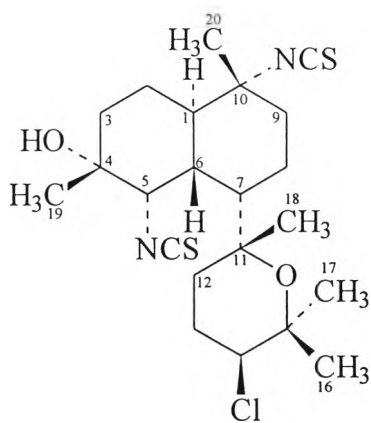


Figure 36. Structure of 1,5,6-Tri-*epi*-kalihinol I

Table 11. Carbon chemical shift assignments of kalihinol I (44), K028 and 10-*epi*-isokalihinol H (47)

Carbon	Chemical shift (δ) ppm, in CDCl ₃		
	Kalihinol I (44)	K028	10- <i>Epi</i> -sokalihinol H (47)
1	44.0	43.3	48.7
2	21.9	21.8	21.3
3	33.0	32.4	36.9
4	70.6	70.1	-
5	65.9	63.7	76.8
6	38.3	36.4	43.6
7	49.3	48.6	53.8
8	22.4	22.2	26.9
9	39.2	39.3	39.8
10	76.0	64.3	64.2
11	77.7	77.2	87.9
12	38.1	38.0	38.9
13	27.5	27.4	25.0
14	64.4	64.3	82.3
15	77.3	75.9	-
16	23.0	22.8	25.5
17	30.9	30.6	25.8
18	19.4	19.1	18.3
19	29.1	28.7	19.7
20	27.6	27.5	27.3
NCS	129.9	132.1	-
NC	-	-	-

Table 12. Proton chemical shift assignments of K028

Proton	Chemical shift (δ) ppm, in CDCl ₃	Multiplicity (J-value, Hz)
1	~1.44	m
2	1.78	m
3	~1.62	m
4	-	-
5	4.58	br s
6	2.18	m
7		
8	~1.30	dd (J = 13.8, 3.3)
9	1.95	dt (J = 13.7, 3.4)
9	1.46	dd (J = 11.3, 3.4)
10	-	-
11	-	-
12	1.51	dd (J = 13.4, 3.4)
13	2.10	dd (J = 12.5, 3.7)
13	2.01	dd (J = 13.4, 3.7)
14	3.75	dd (J = 12.5, 4.5)
15	-	-
16	1.36	s
17	1.35	s
18	1.25	s
19	1.41	s
20	1.40	s

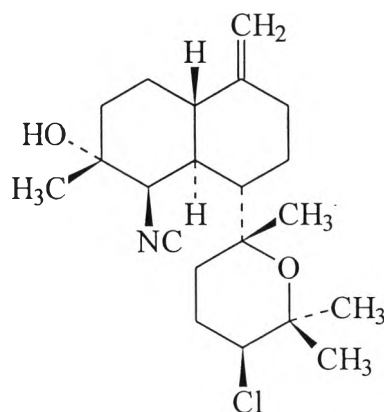


3. Identification of compound K023

Compound K023 was obtained as white needle crystal (18 mg) from fraction F023. Its APCI-MS spectrum (Figure 37) exhibited the $[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-HNC]^+$ and $[M+H-HCl]^+$ peaks at m/z 366, 348, 339 and 330, respectively, establishing the tentative molecular formula of $C_{21}H_{32}ClNO_2$. The presence of the isocyanate functionality in the molecule was also confirmed by an IR absorption band at 2107 cm^{-1} (Figure 38). A strong IR band at 759 cm^{-1} and a broad band at 3353 cm^{-1} suggested the presence of exomethylene and hydroxyl group(s) as parts of the structure.

The ^{13}C NMR spectrum of K023 (Figure 40) displayed 21 carbon signals, suggestive of a diterpenoid structure with one carbon atom in the substituent. The two most downfield signals in the ^1H NMR spectrum of K023 (Figure 39) were two exomethylene protons of the position 20, appearing as two broad singlets at δ 4.69 and 4.59 ppm. The other two downfield signals were those of H-5 (δ 4.48 ppm, br s) and H-14 (δ 3.75 ppm, dd, $J = 11.8, 4.8$ Hz). The four upfield methyl proton singlets at δ 1.40, 1.35, 1.33 and 1.33 represented 19-Me, 16-Me, 17-Me and 18-Me, respectively.

When compared with the previously reported kalihinol Y (39) (Chang *et al.*, 1984), the carbon (Table 13) and proton chemical shifts of K023 and 39 were highly similar. Therefore, compound K023 was identified as kalihinol Y.



kalihinol Y (39)

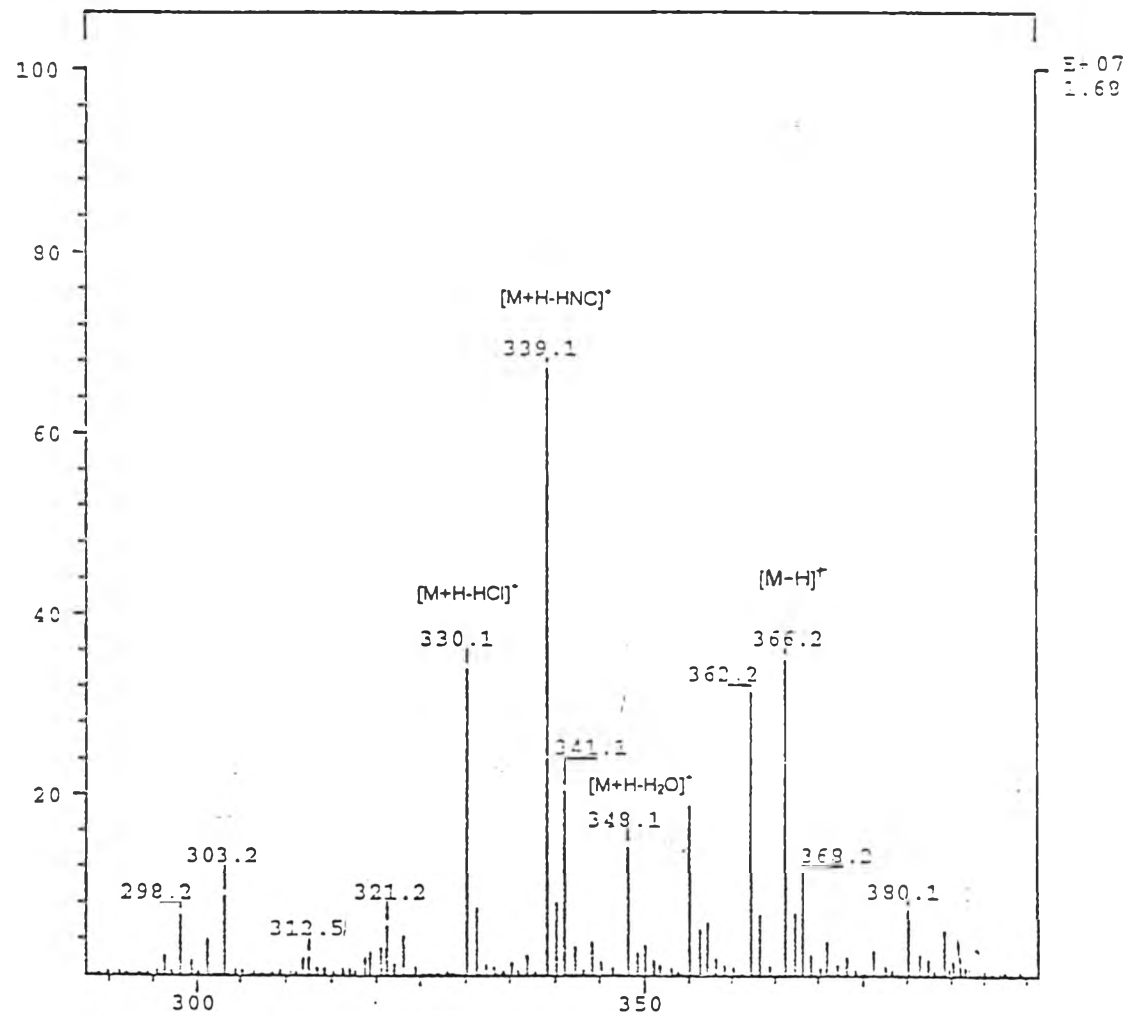
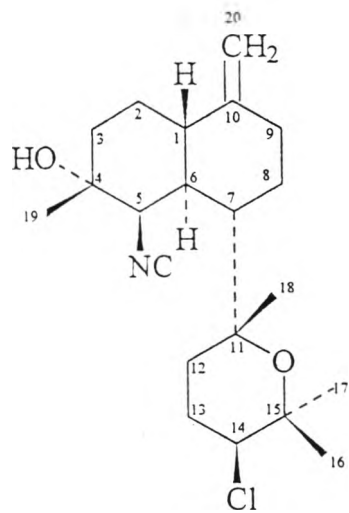
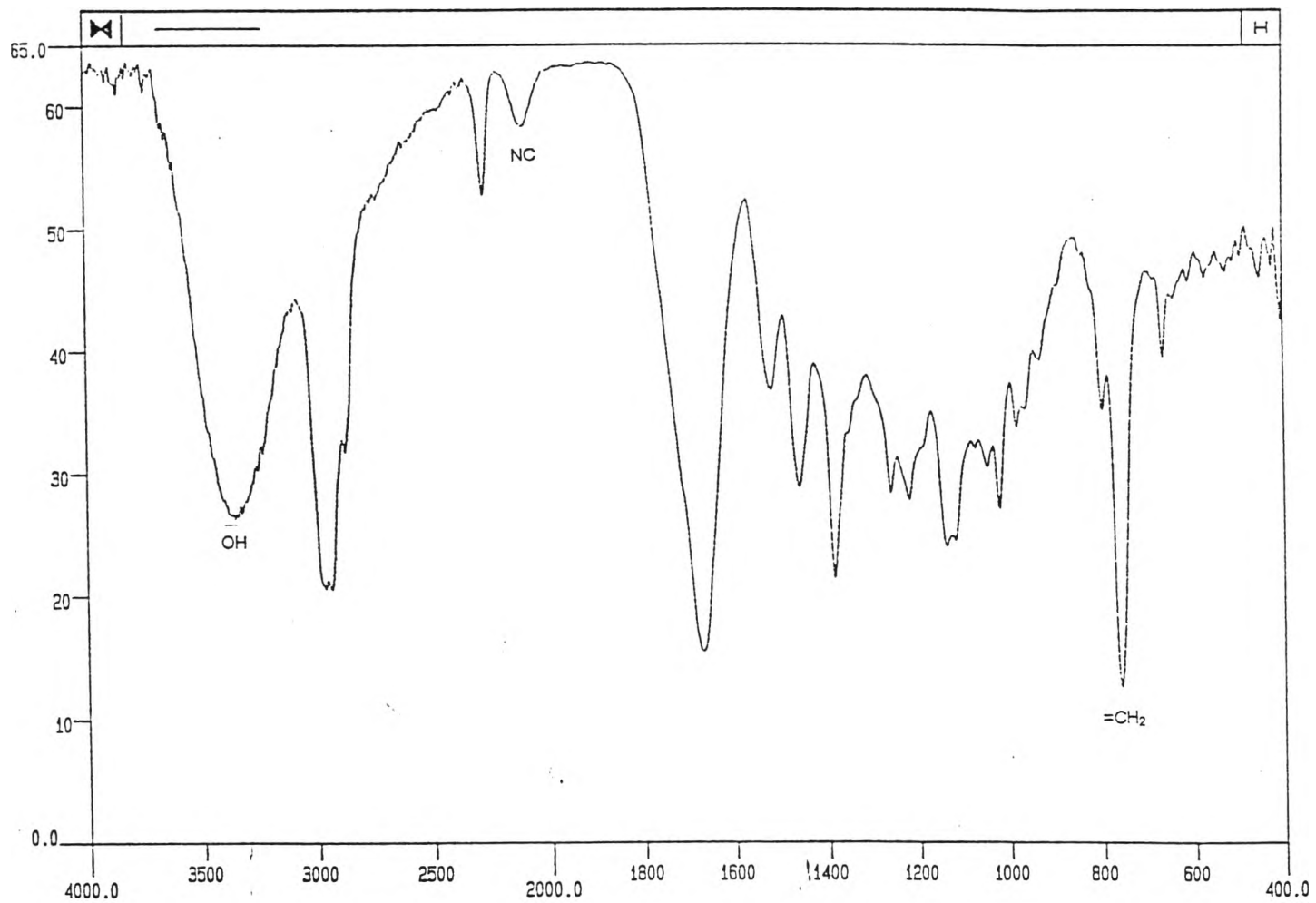
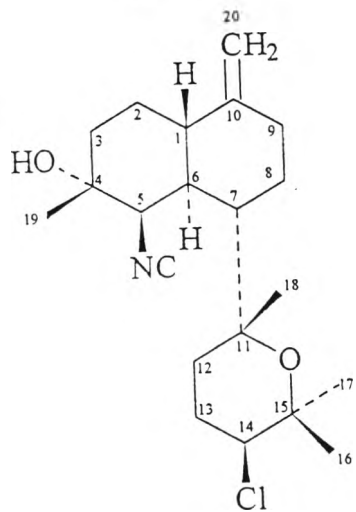


Figure 37. APCI-MS spectrum of compound K023



CM-1

Figure 38. IR spectrum of compound K023

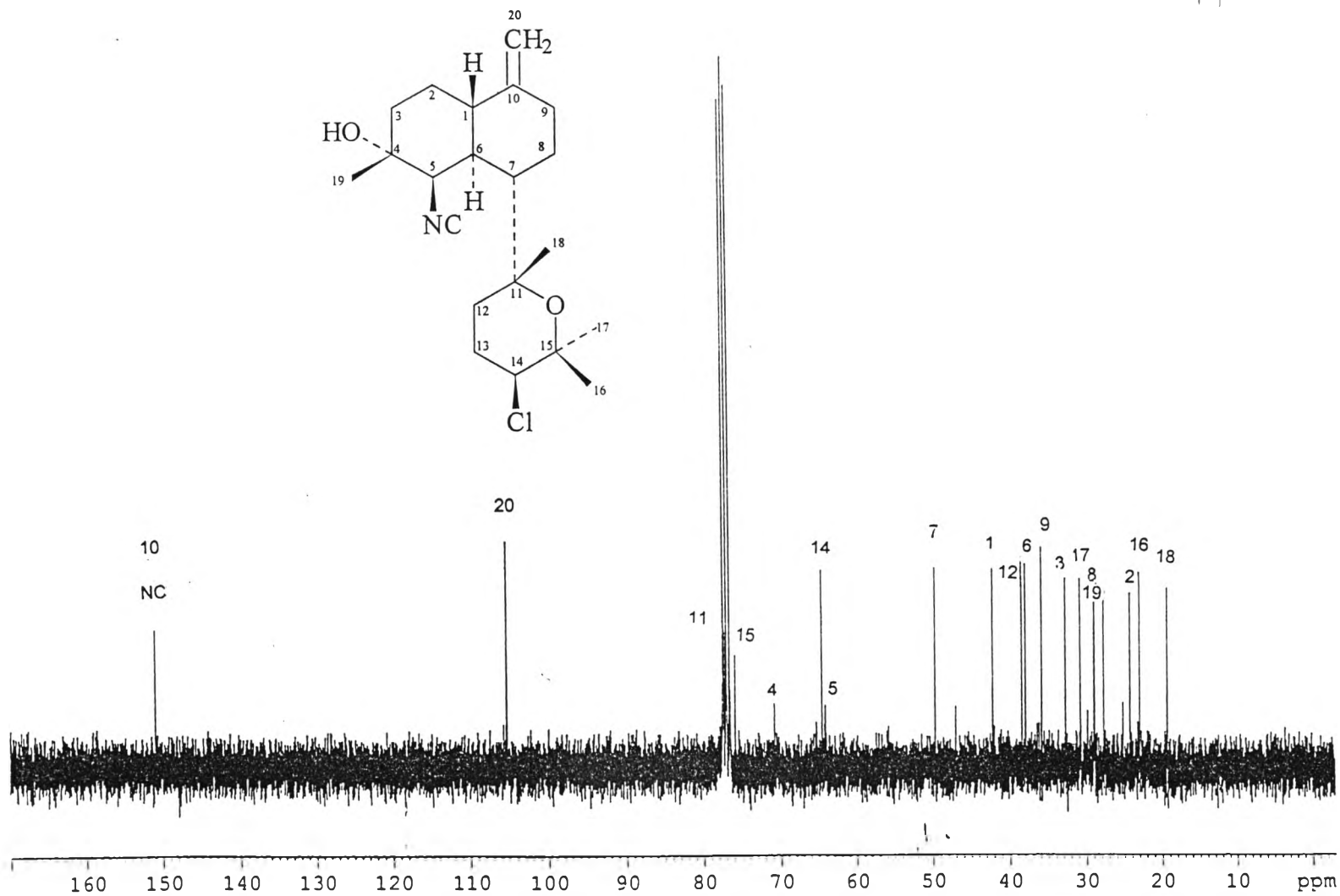


Figure 39. The 75 MHz ¹³C NMR spectrum of compound K023 (in CDCl₃)

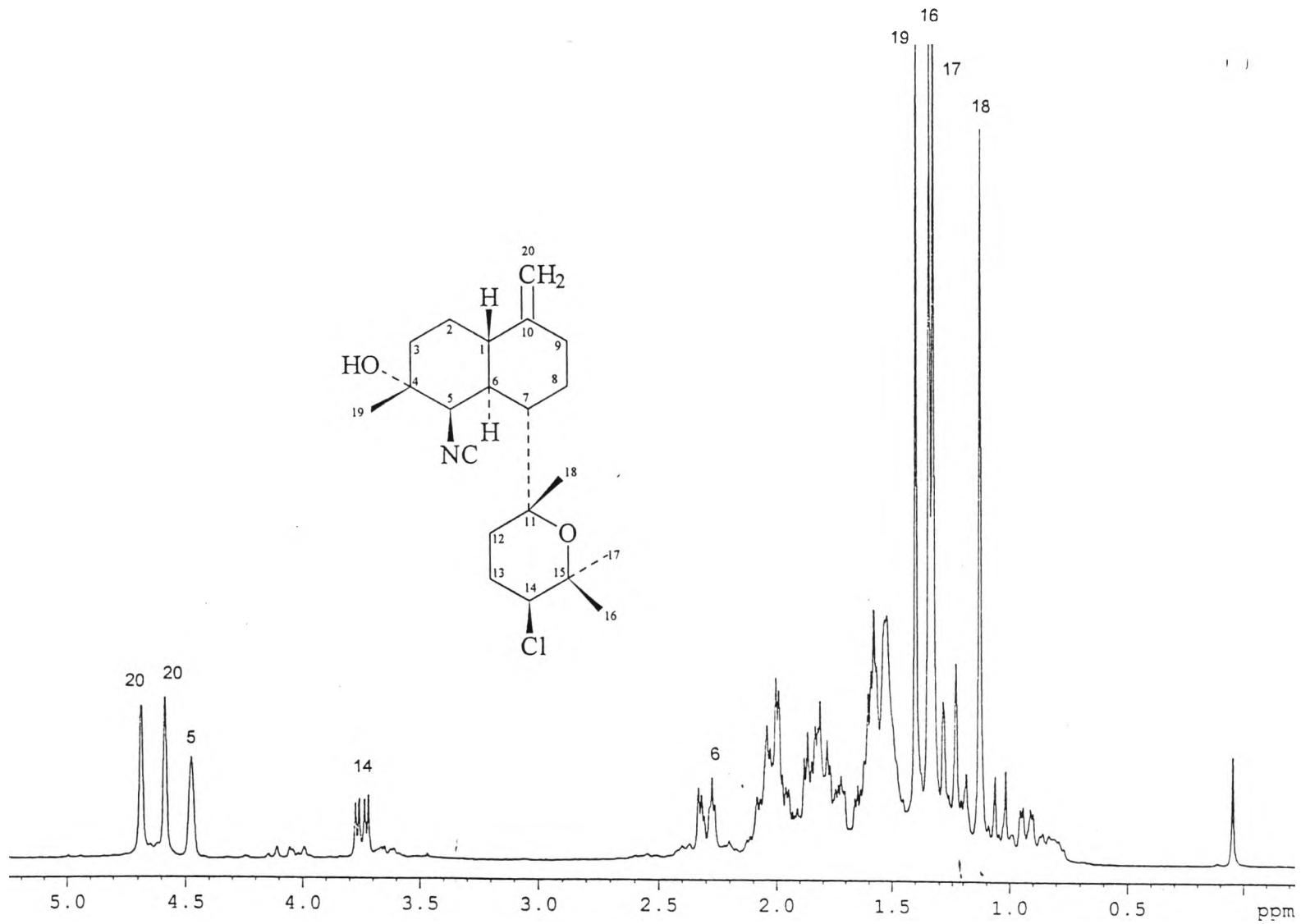


Figure 40. The 300 MHz ¹H NMR spectrum of compound K023 (in CDCl₃)

Table 13. Carbon chemical shift assignments of kalihinol Y and K023

Carbon	Chemical shift (δ) ppm, in CDCl_3	
	Kalihinol Y	K023
1	41.9	42.1
2	24.0	24.2
3	32.4	32.7
4	70.6	70.8
5	63.9	64.1
6	37.7	37.9
7	49.5	49.7
8	28.7	28.9
9	35.6	35.8
10	150.9	150.9
11	77.0	77.1
12	38.2	38.4
13	27.5	27.7
14	64.4	64.5
15	76.5	75.8
16	22.7	22.9
17	30.6	30.8
18	19.1	19.3
19	28.7	29.0
20	105.3	105.3
NC		150.9