CHAPTER IV

RESULTS AND DISCUSSION

Three compounds were isolated from the ethyl acetate extract of the aerial parts of *Coleus amboinicus* (Lour.). Identification of their chemical structures will be discussed.

Identification of compounds isolated from *Coleus amboinicus*

To identify the chemical structures of these compounds, spectroscopic techniques, e.g. UV, IR, MS and extensive NMR experiments were employed.

1. Identification of compound RAT 1

RAT 1 was obtained as dark-brown oil (48.9 mg) from fraction F017. The molecular formula of $C_6H_6O_3$ was suggested for this compound based on its ^1H and ^{13}C NMR spectra and $[M]^+$ peak in the EI mass spectrum (Figure 7) at *m/z* 126, A mass fragment at *m/z* 95 also suggested the presence of a hydroxymethyl group. The presence of the alcohol functionality in the molecule was also confirmed by a broad OH bonded peak at 3380 cm^{-1} in the IR spectrum. In addition, IR absorption bands at 1670 cm^{-1} (C=O stretching), 2850 and 2930 cm^{-1} (C-H stretching) suggested the presence of aldehyde moiety (Figure 8).

The ^{13}C NMR spectrum of RAT 1 (Figure 10) exhibited 6 carbon signals, identified by DEPT and ^1H-^{13}C-HETCOR experiments (Figure 12) as those of one

methylene carbons attached to a heteroatom at δ 57.5 ppm, two olefinic methine carbons at δ 110.3 and 123.9 ppm, two downfield quaternary carbons at δ 153.5 and 163.0 ppm, and one formyl carbonyl carbon at δ 178.2 ppm.

In the ^{1}H NMR spectrum (Figure 13), the methylene protons could be recognized from the most upfield signals at δ 4.65 ppm, while that of the formyl group appeared as the most downfield at δ 9.59 ppm. The ^{1}H-^{1}H COSY spectrum (Figure 15) displayed the correlation between one olefinic proton doublet at δ 6.58 ppm (J = 3.6 Hz, H-2) to another at δ 7.37 ppm (J = 3.6 Hz, H-3).

Therefore, a furan structure with a formyl and a hydroxymethyl substituent was proposed as compound RAT 1. ^{1}H-^{13}C HMBC experiment (Figure 16-18) was performed in order to confirm the positions of these substituents. Correlations could be observed between H-4 (δ 6.58 ppm) and C-2 (δ 153.5 ppm), C-3 (δ 123.9 ppm) and C-5 (δ 163.0 ppm), while the nearby H-3 (δ 7.37 ppm) showed cross peaks with C-2 (δ 153.5 ppm), C-4 (δ 110.3 ppm) and C-5 (δ 163.0 ppm). Cross peaks between H_2-7 methylene protons and C-4 and C-5 could also be observed. Major HMBC correlations in the structure of compound RAT 1 can be summarized as shown in Figure 19. Compound RAT 1 was therefore assigned as the known structure of 5-(hydroxymethyl)-2-furaldehyde shown in Figure 20. This compound has never been reported as a constituent of this plant species. Wubert, Oster, and Rudiger (1997) have isolated and identified this compound from bulbs of *Gladiolus* spp., the natural inhibitor of chlorophyll biosynthesis. It is of interest as potential herbicide, result in

low toxicity to animals and man. In addition, it can be used as a reagent in the synthesis of dialdehydes, glycols, ethers, amino alcohols, acetals acid catalyzed ring opening.

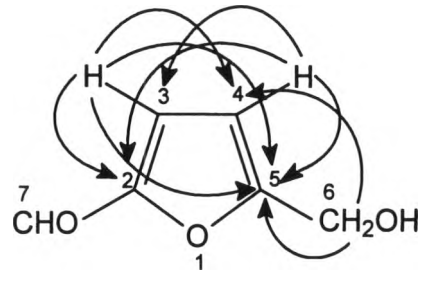


Figure 19. Major HMBC correlations of RAT 1

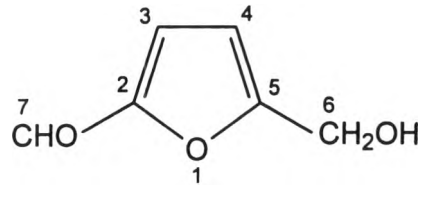


Figure 20. Structure of 5-(hydroxymethyl)-2-furaldehyde

Table 13. ^{1}H and ^{13}C NMR data for RAT 1 (5-(hydroxymethyl)-2-furaldehyde)

Position	$^{\delta}C$	$^{\delta}H$	HMBC correlations
2	153.5	-	
3	123.9	7.37, *d*, *J* = 3.6 Hz	C-2, C-4, C-5
4	110.3	6.58, *d*, *J* = 3.6 Hz	C-2, C-3, C-5
5	163.0	-	
6	57.5	4.65, *s*	C-4, C-5
7	178.2	9.59, *s*	

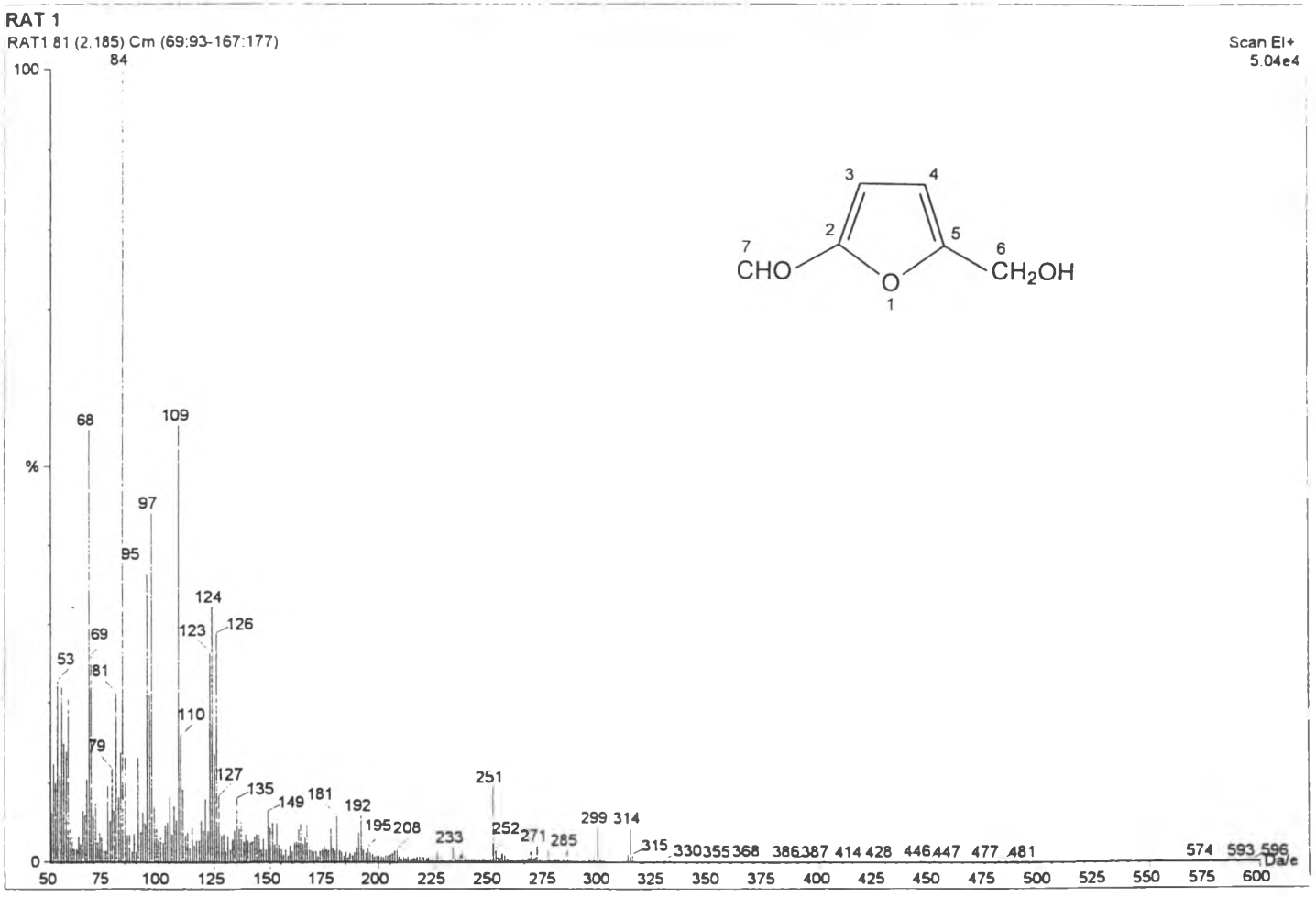
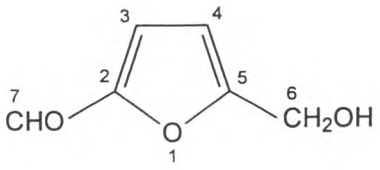


Figure 7. EIMS spectrum of RAT 1



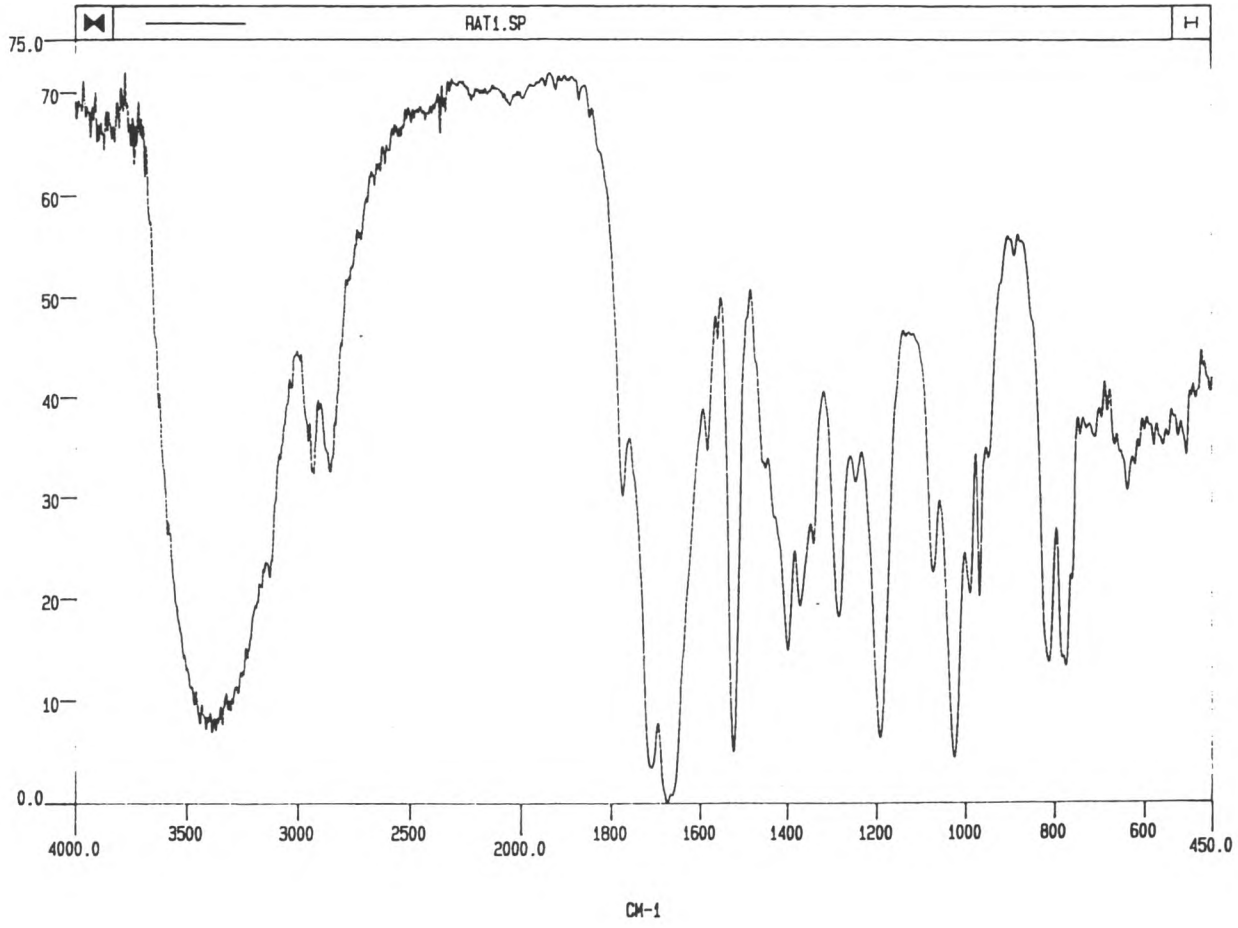
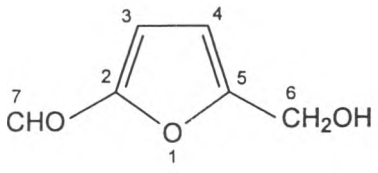


Figure 8. IR spectrum of RAT 1



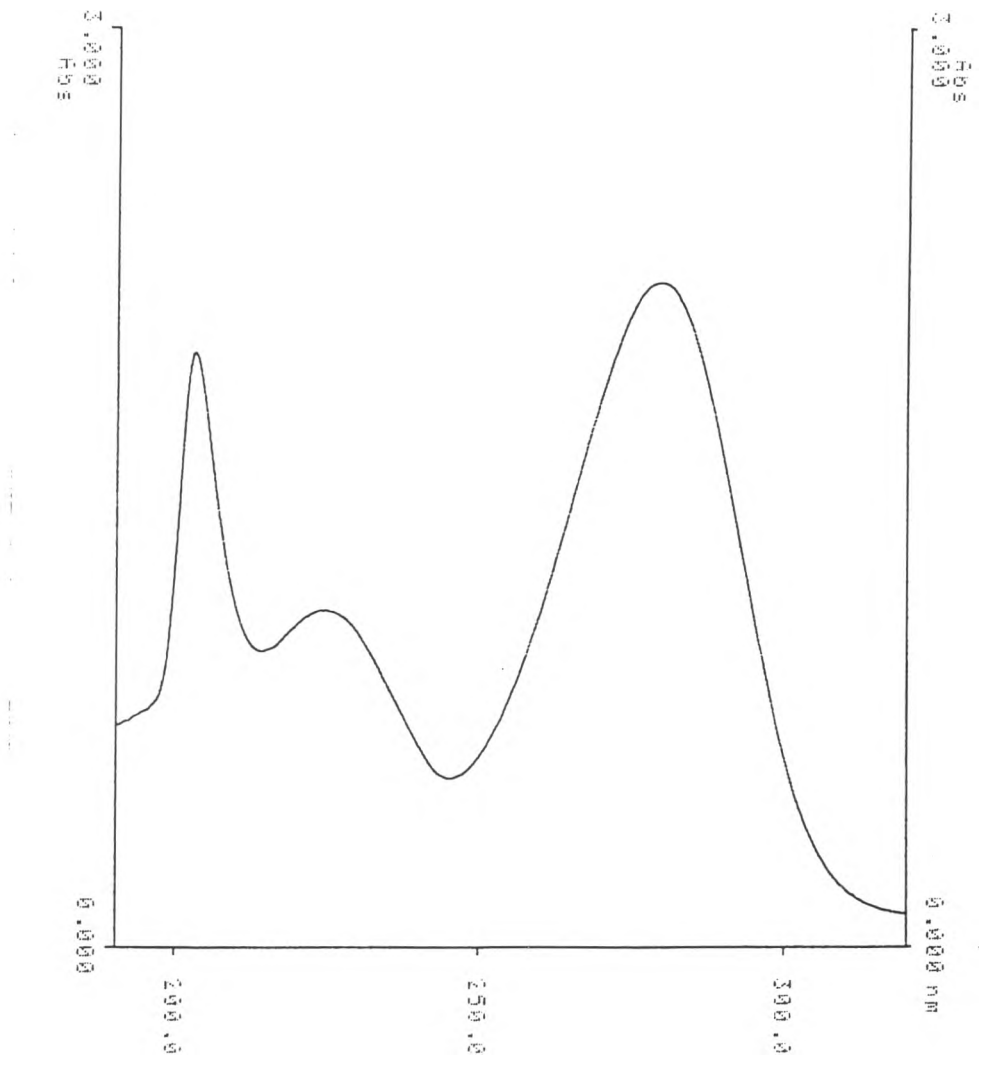
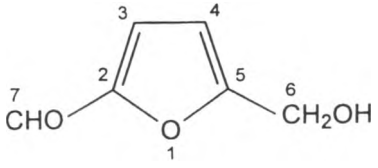


Figure 9. UV spectrum of RAT 1 (in MeOH)



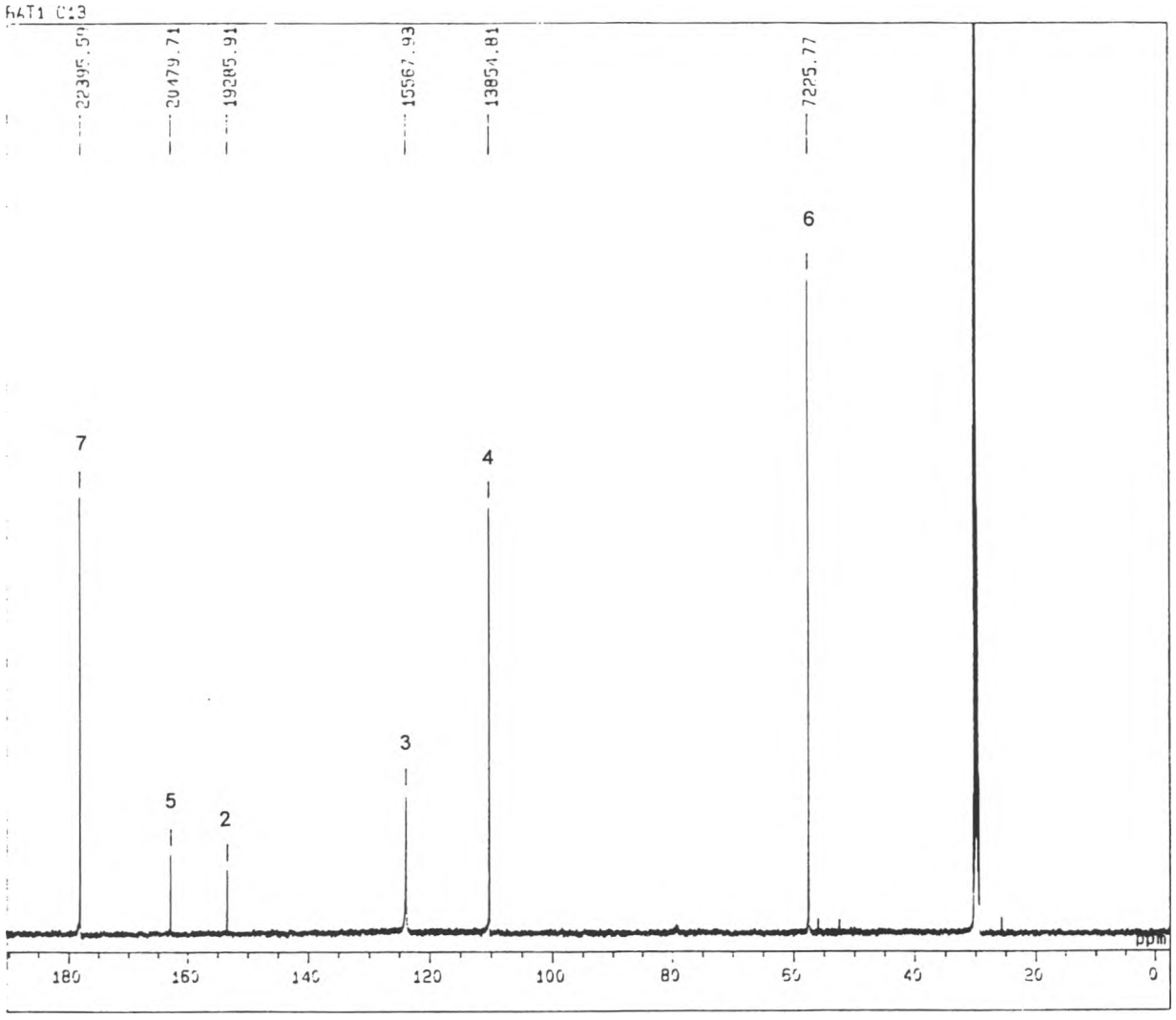
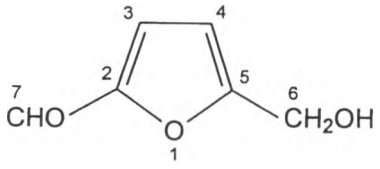


Figure 10. The 125 MHz ^{13}C NMR spectrum of RAT 1 (in acetone-d_6)



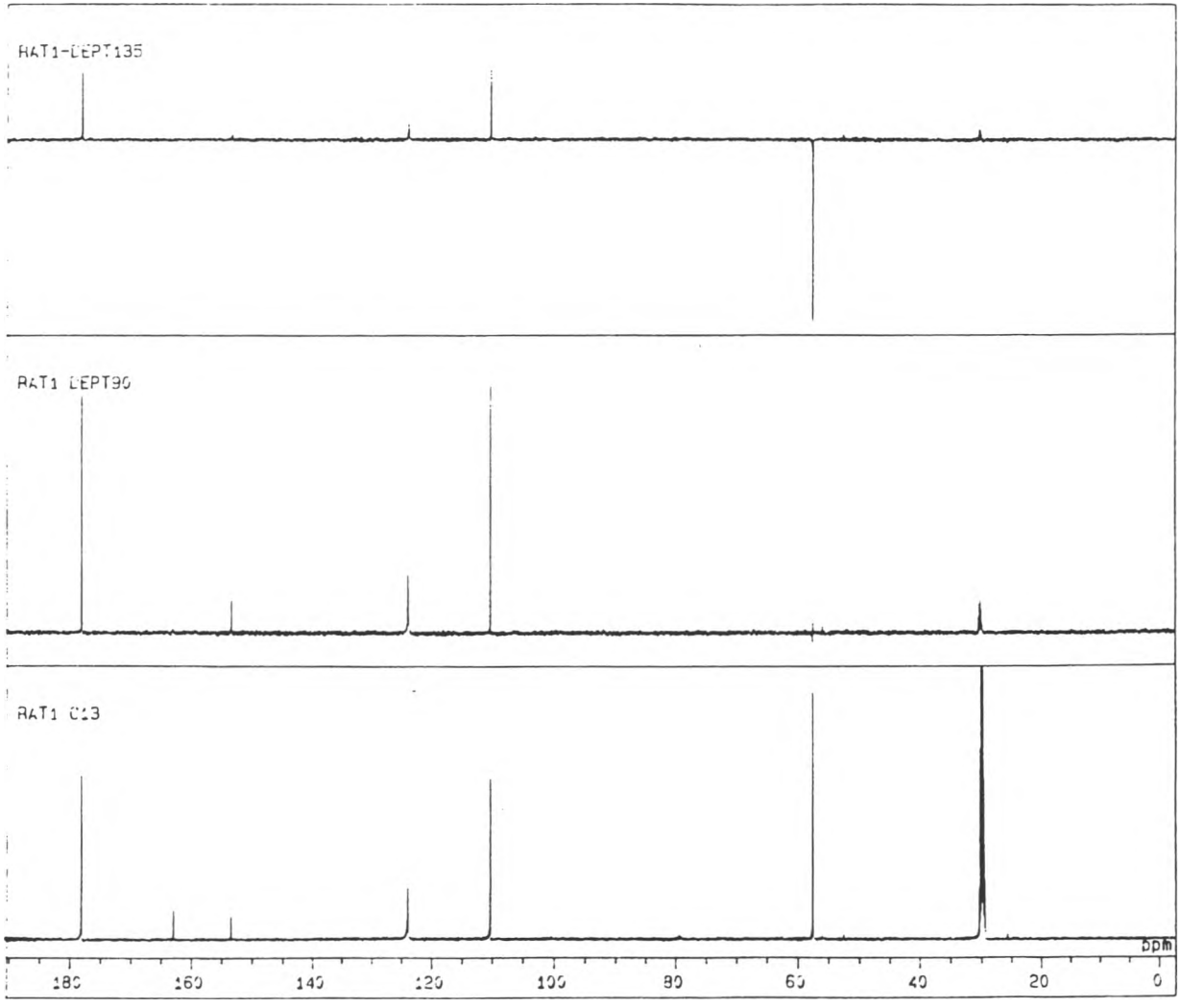
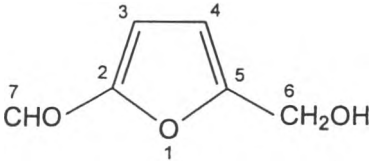


Figure 11. The ^{13}C DEPT spectrum of RAT 1 (in acetone-d_6)



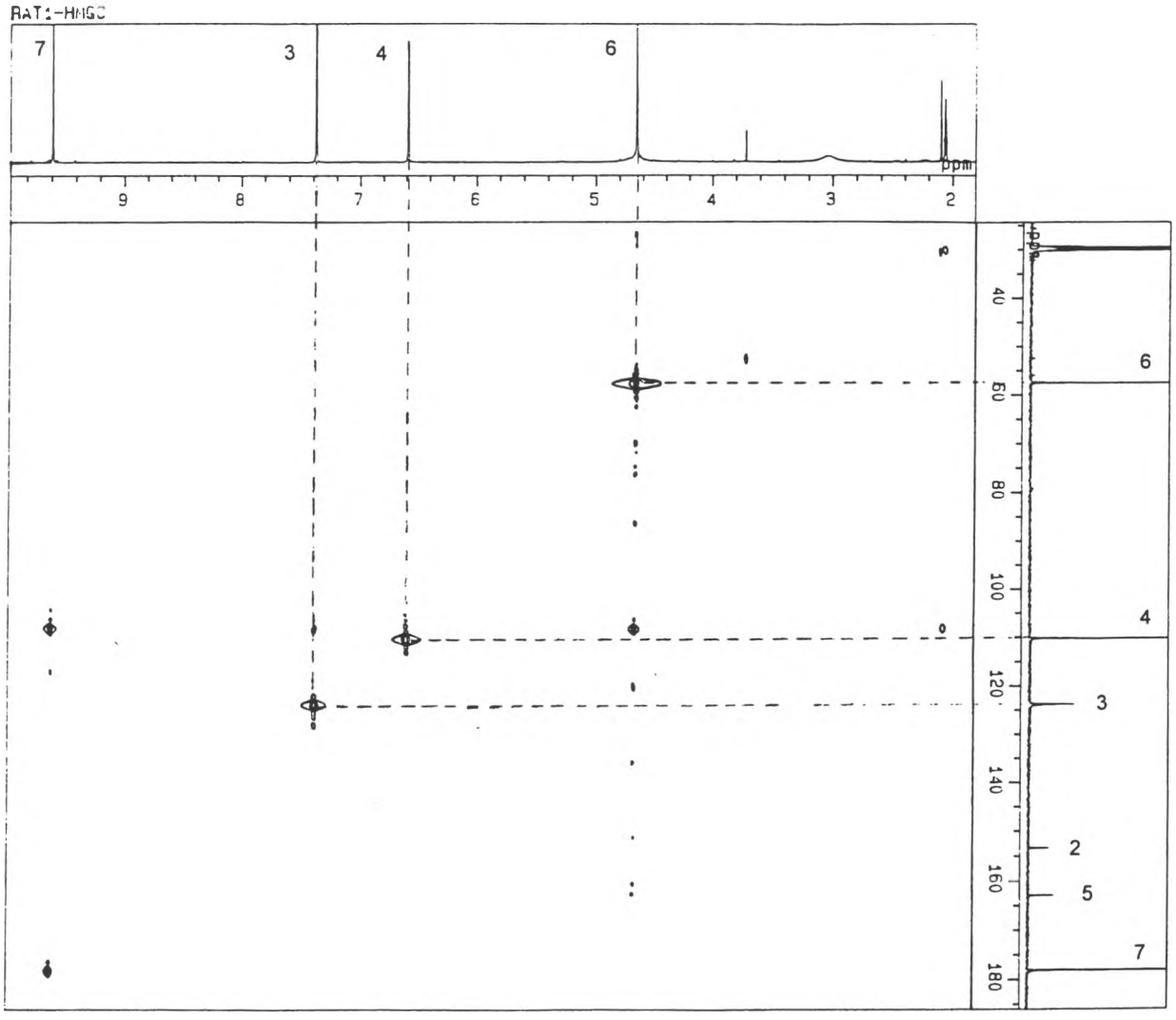


Figure 12. ^{1}H-^{13}C HETCOR spectrum of RAT 1 (in acetone-d_6)

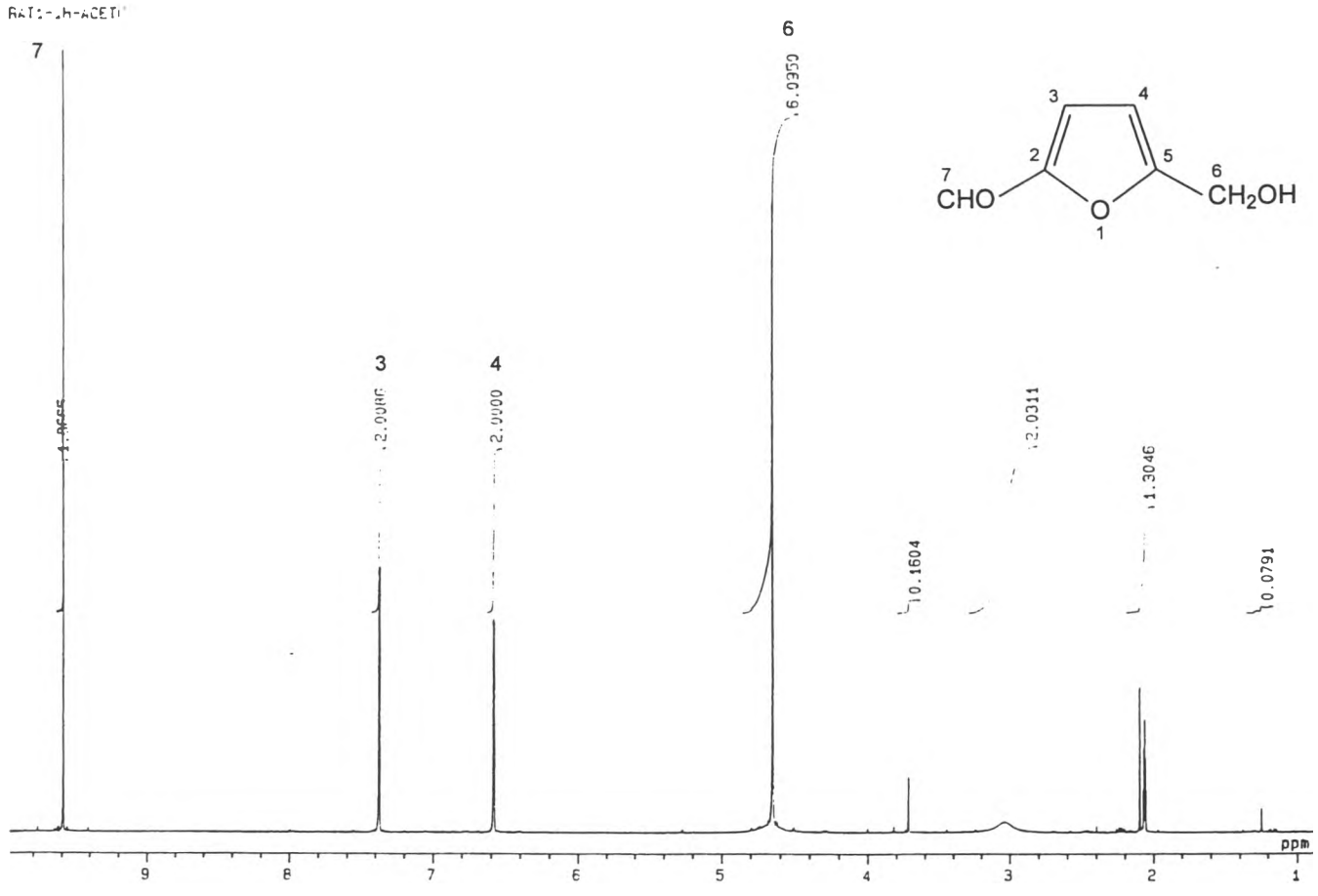


Figure 13. The 500 MHz ^{1}H NMR spectrum of RAT 1 (in acetone-d_6)

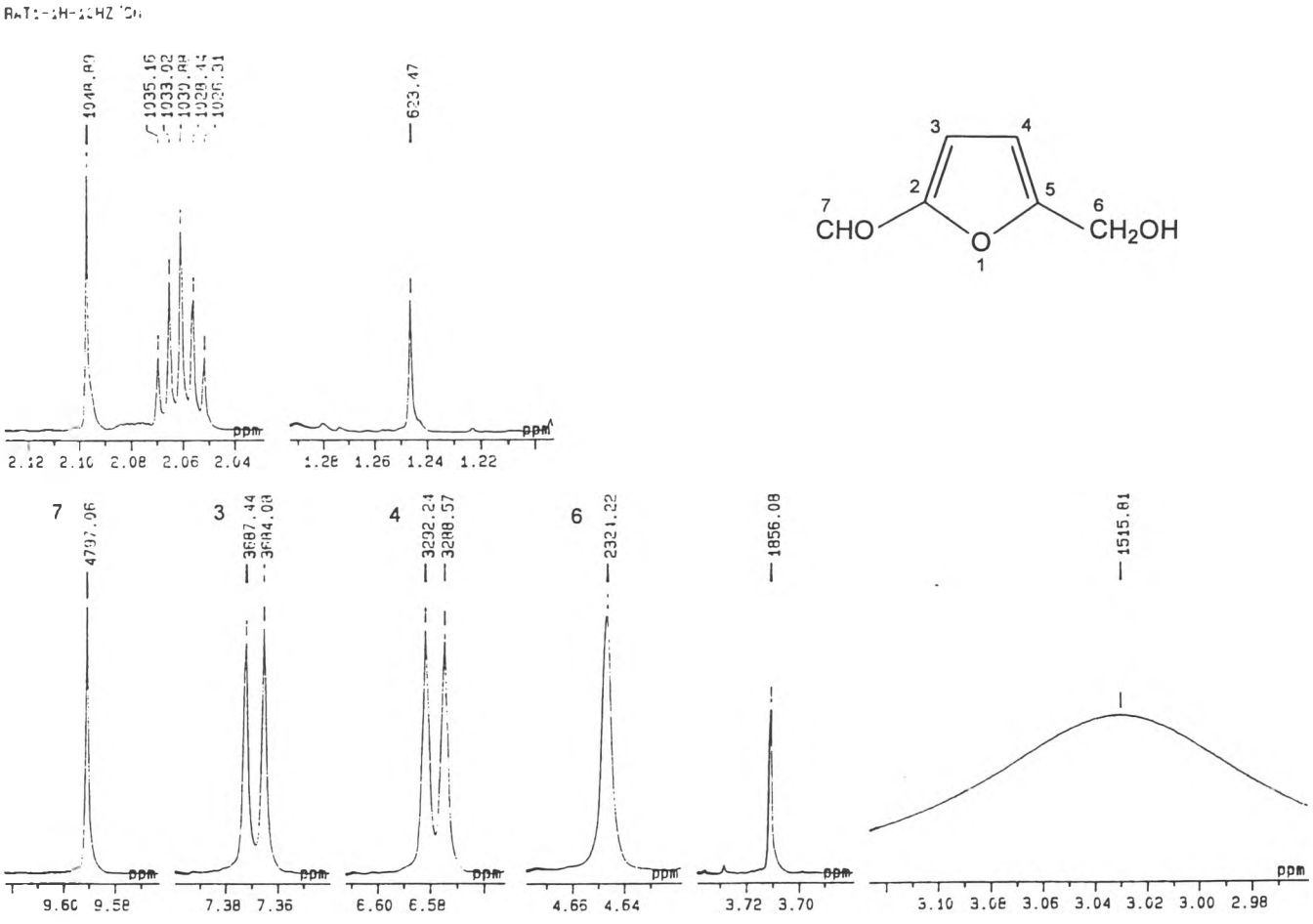
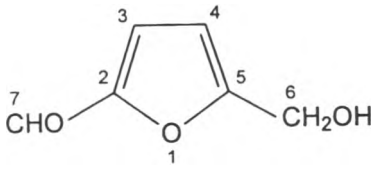


Figure 14. The 500 MHz ^{1}H NMR spectrum of RAT 1 (in acetone-d_6)

(expanded in the range of δ 1.20-3.92 ppm)



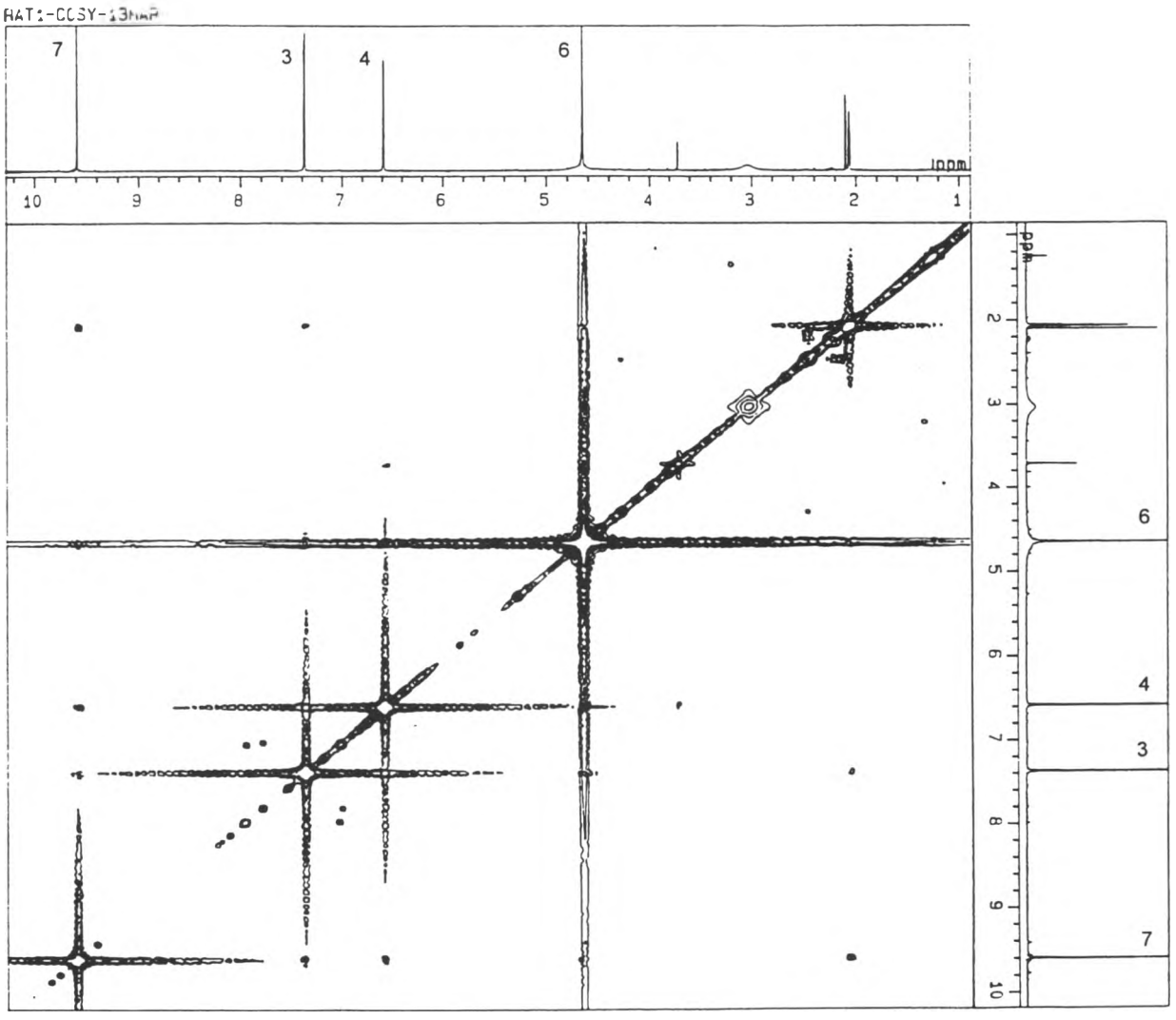
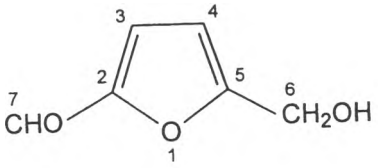


Figure 15. ^{1}H-^{1}H COSY spectrum of RAT 1 (in acetone-d_6)



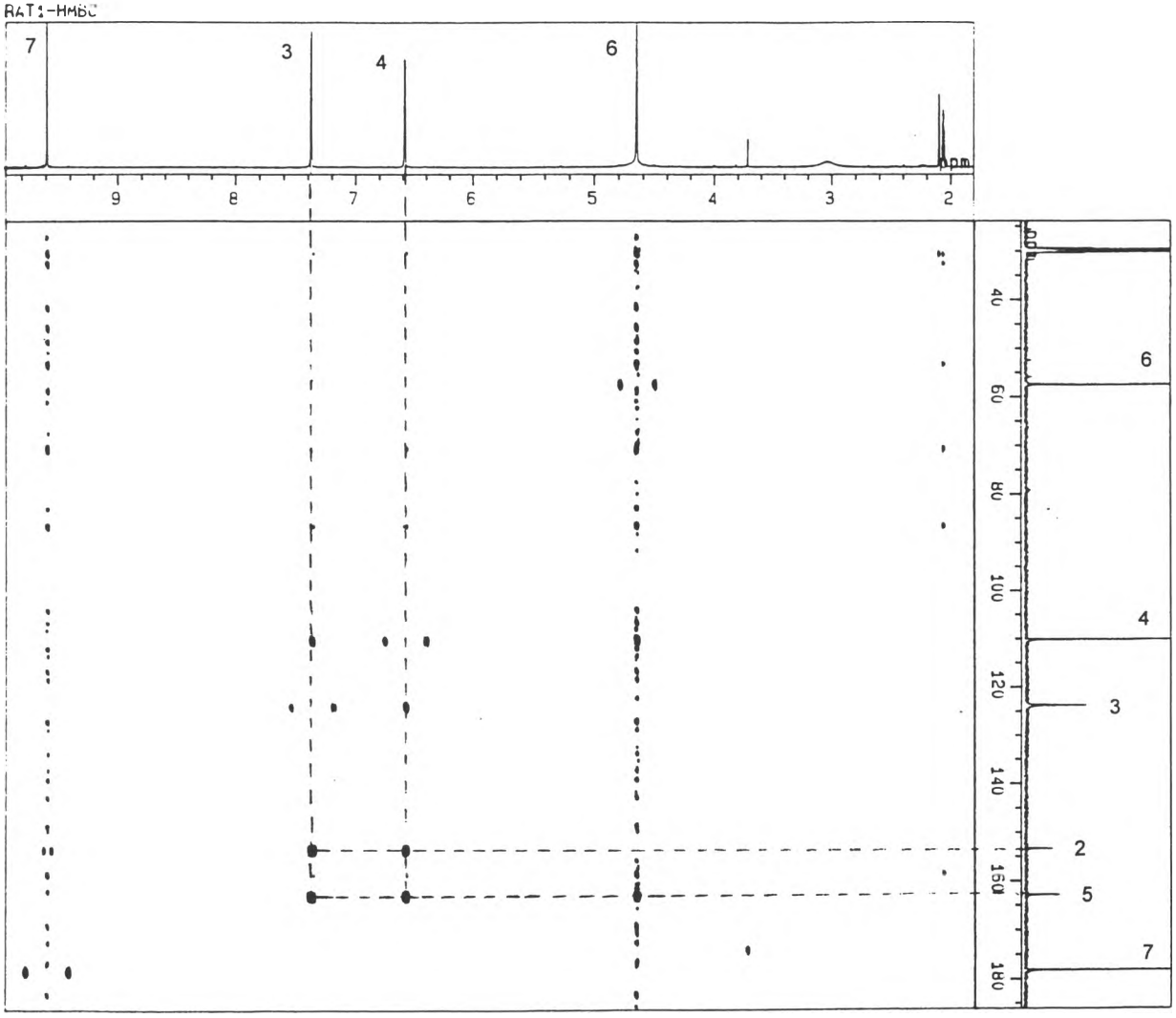
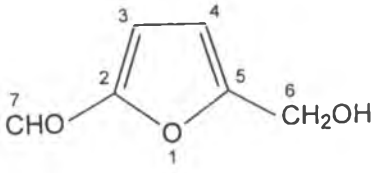


Figure 16. ^{1}H-^{13}C HMBC spectrum of RAT 1 (in acetone-d_6)



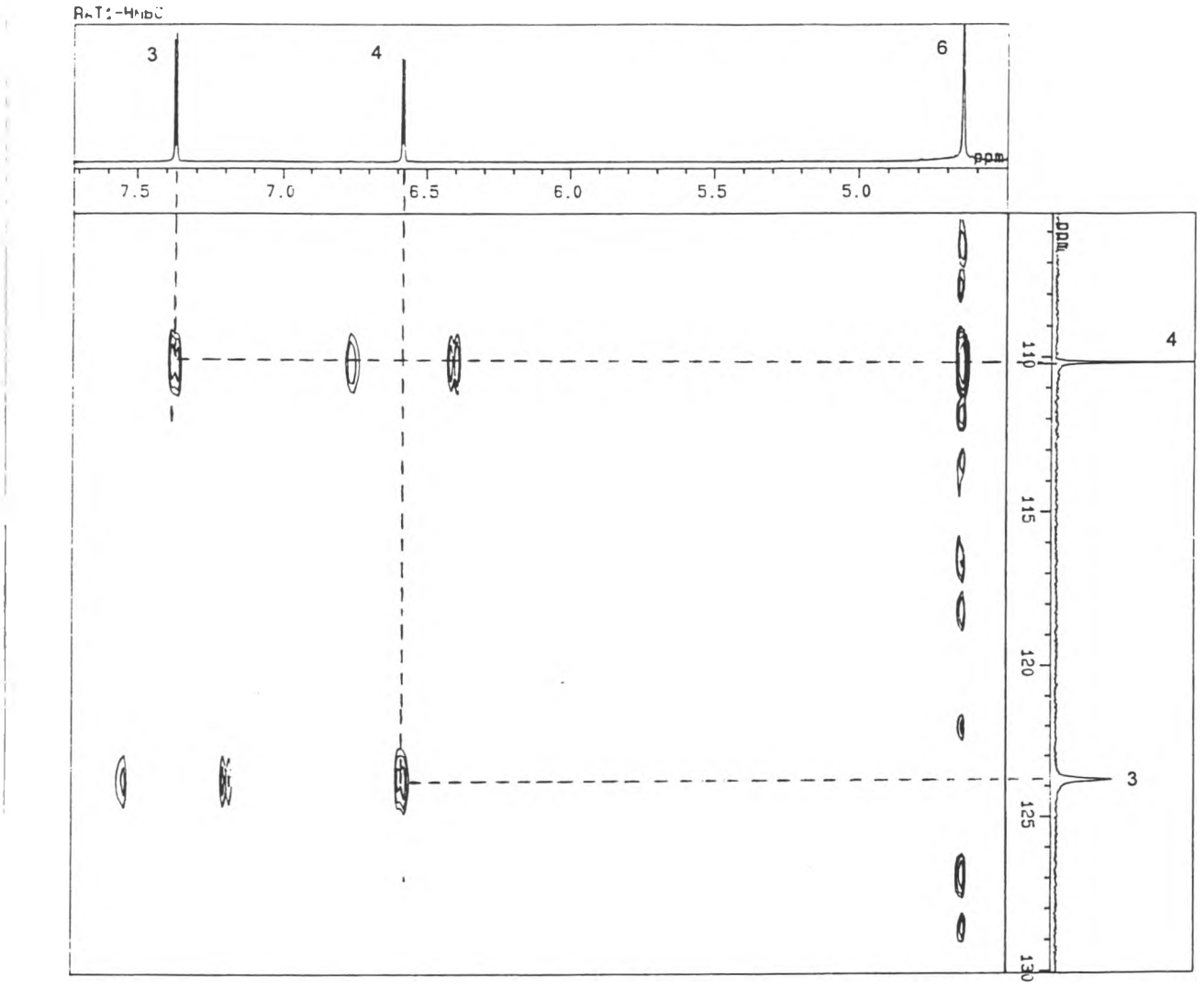
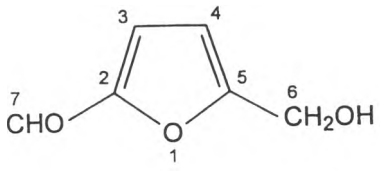


Figure 17. ^{1}H-^{13}C HMBC spectrum of RAT 1 (in acetone-d_6)

(expanded in the range of δ ^{1}H 4.6-7.7 ppm and δ ^{13}C 106-130 ppm)



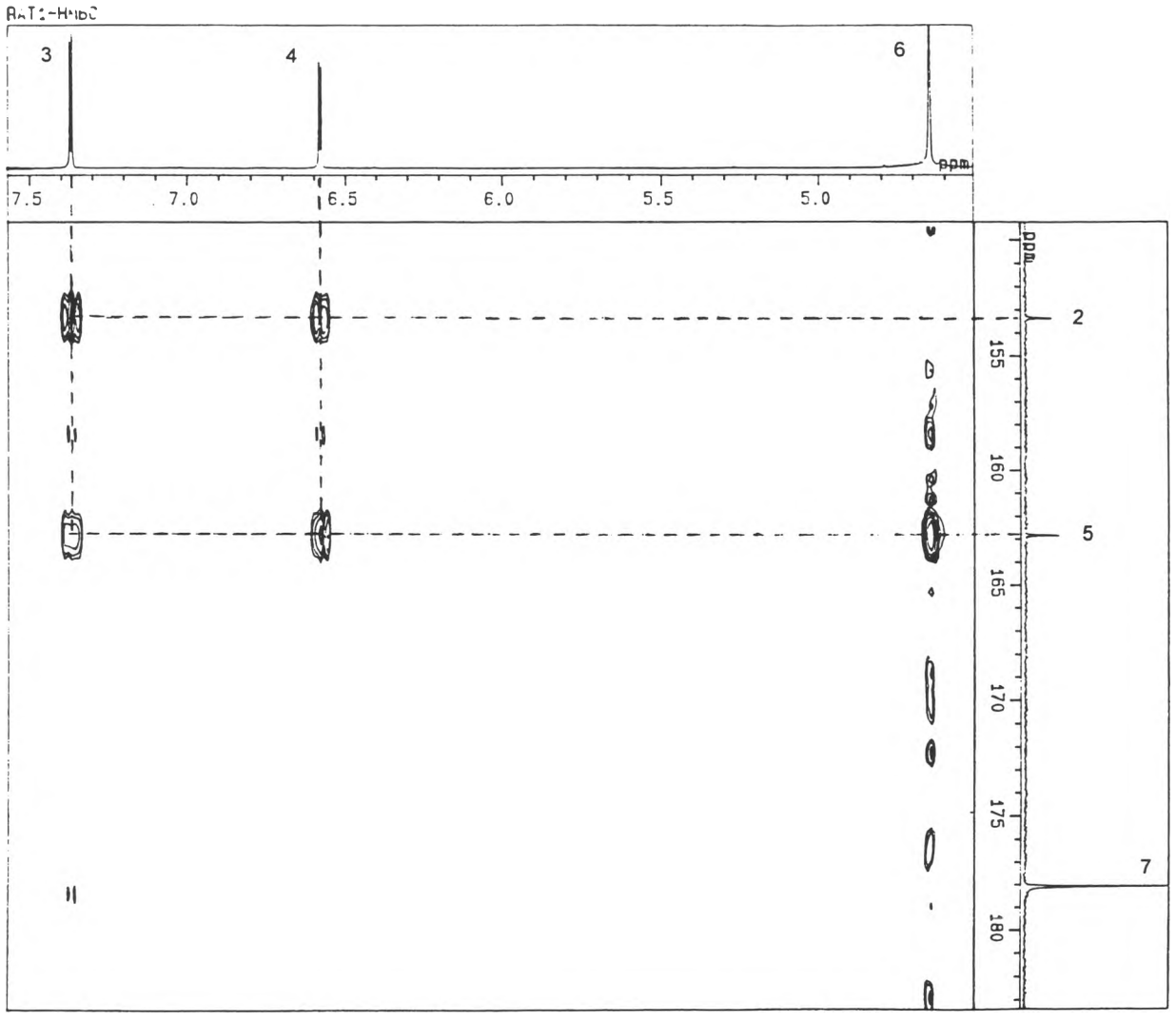


Figure 18. ^{1}H-^{13}C HMBC spectrum of RAT 1 (in acetone-d_6)

(expanded in the range of δ ^{1}H 4.6-7.5 ppm and δ ^{13}C 150-183 ppm)

2. Identification of compound RAT 2

RAT 2 was obtained as light yellow solid (540 mg) from fraction F028. The molecular formula of $C_{16}H_{24}O_7$ was suggested for this compound based on 16 carbon signals observed in its ^{13}C NMR spectrum and its $[M]^+$ peak in the EIMS (Figure 21) at *m/z* 328. Mass fragment peaks at m/z 116 and 151 were indicative of successive loss of a sugar moiety and a methyl group. The presence of the alcohol functionality in the molecule was proven by a very intense IR absorption peak at 3390 cm^{-1} (Figure 22).

Of the sixteen carbon signals in the ^{13}C NMR spectrum of RAT 2 (Figure 24), six (δ 62.7, 71.4, 74.9, 77.4, 78.1 and 104.0 ppm) were reminiscent of a β-glucopyranosyl unit while the other ten of which identified the aglycone portion as an aryl-terpenoid possessing isopropyl, methyl and hydroxyl substitutents. DEPT and ^1H-^{13}C-HETCOR experiments (Figure 27-28) were employed to classify these signals into those of three methyl carbons at δ 16.0, 23.4 and 23.5 ppm, one methylene carbon at δ 62.7 ppm, eight methine carbons at δ 26.5, 71.4, 74.9, 77.4, 78.1, 104.0, 112.9 and 120.2 ppm, and four quaternary carbons at δ 122.5, 137.6, 148.9 and 151.5 ppm.

In the ^1H NMR spectrum (Figure 29), the isopropyl methyl groups could be recognized as the most upfield doublet (6H, J = 7.0 Hz) at δ 1.13 ppm and an aromatic methyl appeared as a singlet at 2.13 ppm. Two aromatic protons appeared as singlets at δ 6.68 and 6.92 ppm, suggestive of their *para* position. The spectrum also

showed an anomeric proton signal at δ 4.75 ppm as a doublet (J = 7.3 Hz), indicating β-configuration of the sugar moiety. The ^{1}H-^{1}H COSY spectrum (Figure 31) displayed the correlation of the two isopropyl methyl protons at δ 1.13 ppm to their vicinal methine proton at δ 3.47 ppm (*septet*, J = 7.0 Hz).

The aglycone of RAT 2 was assigned the monocyclic monoterpene structure of thymoquinol (2-isopropyl-5-methyl-1,4-benzenediol). ^{1}H-^{13}C HMBC experiment (Figure 32-36) was performed in order to confirm the structure. Correlations could be observed between methyl protons at positions 8 and 9 (δ 1.13 ppm) and C-2 (δ 137.6 ppm), whereas the H-3 aromatic methine proton (δ 6.68 ppm) showed correlations to C-1 (δ 148.9 ppm), C-4 (δ 151.5 ppm), C-5 (δ 122.5 ppm) and C-7 (δ 26.5 ppm). Another aromatic proton (δ 6.92 ppm) displayed correlations to C-1, C-2, C-4 and C-10 (δ 16.0 ppm). The position of the aromatic methyl group was confirmed by correlations of its proton signal to C-4, C-5 and C-6 (δ 120.2 ppm). Major HMBC correlations in the structure of RAT 2 can be summarized as shown in Figure 37.

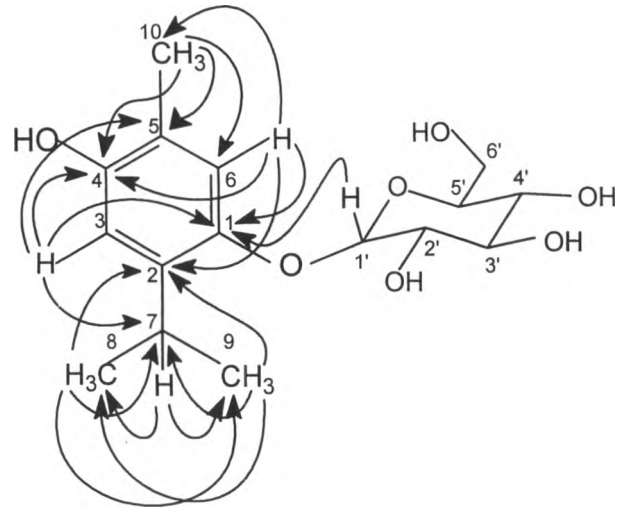


Figure 37. Major HMBC correlations of RAT 2

NOESY experiment (Figure 38) established the glycoside linkage as at position 1, according to the cross peaks observed between the anomeric glucosyl proton signal (H-1′, 4.75 ppm, *d*, $J = 7.3$ Hz) and H-6 (δ 6.92 ppm), H-3′ (δ 3.40 ppm) and H-5′ (δ 3.50 ppm). HMBC correlation observed between H-1′ and C-1 also supported this location. Major NOESY correlations in the structure of RAT 2 are shown in Figure 39. Therefore, compound RAT 2 was assigned the structure 1,4-dihydroxy-2-*iso*-propyl-5-methylphenyl-1-0-β-D-glucopyranoside (thymoquinol-β-D- glucopyranoside), shown in Figure 40. The compound was initially reported as minor component of the mixture of phenolic glycosides from *Geum japonicum* Thunberg (Rosaceae) (Shigenaga, Kouno and Kawano, 1985). Recently, it was isolated from the fresh fronds of *Pteridium aquilinum* var. *caudatum* (Pteridaceae) (Castillo *et al.*, 1995). This constitutes are the first isolation of this compound from *Coleus amboinicus*.

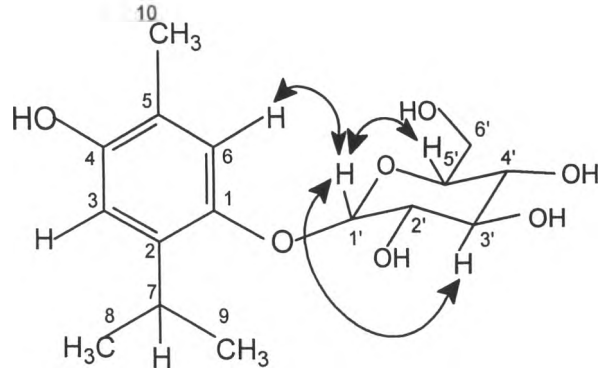


Figure 39. Major NOESY correlations of RAT 2

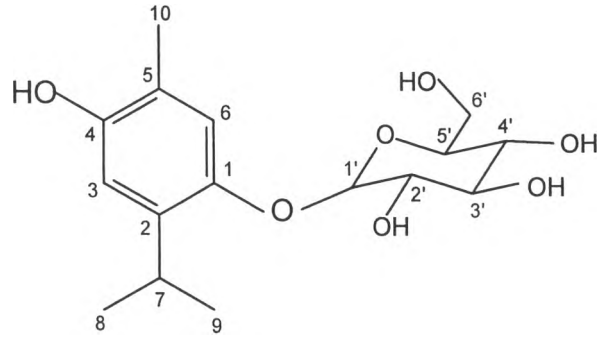


Figure 40. Structure of 1,4-dihydroxy-2-*iso*-propyl-5-methylphenyl-1-0-β-D-glucopyranoside (thymoquinol-β-D- glucopyranoside)

Table 14. Comparison of carbon chemical shift assignments of RAT 2 and 1,4-dihydroxy-2-*iso*-propyl-5-methylphenyl-1-0-β-D-glucopyranoside (Castillo *et al.*, 1995)

	Chemical shift (δ) ppm	
Carbon	Literature value *	RAT 2 **
Aglycone		
1	148.9	148.9
2	137.3	137.6
3	113.0	112.9
4	152.2	151.5
5	123.2	122.5
6	120.3	120.2
7	26.6	26.5
8	23.4	23.4
9	23.7	23.5
10	16.5	16.0
Glucosyl		
1′	104.8	104.0
2′	75.3	74.9
3′	78.8	77.4
4′	71.1	71.4
5′	78.6	78.1
6′	62.6	62.7

* in C_5D_5N

** in acetone-d_6

Table 15. ^{1}H and ^{13}C NMR data for RAT 2 (thymoquinol-β-D-glucopyranoside)

Position	$^{\delta}C$	$^{\delta}H$	HMBC correlations
Aglycone			
1	148.9	-	
2	137.6	-	
3	112.9	6.68, *s*	C-1, C-4, C-5, C-7
4	151.5	-	
5	122.5	-	
6	120.2	6.92, *s*	C-1, C-2, C-4, C-10
7	26.5	3.47, *septet*	C-8, C-9
8	23.4	1.12, *d*, *J* = 7.0	C-2, C-7, C-9
9	23.5	1.13, *d*, *J* = 7.0	C-2, C-7, C-8
10	16.0	2.13, *s*	C-4, C-5, C-6
Glucosyl			
1′	104.0	4.75, *d*, *J* = 7.3	C-1
2′	74.9	3.42, *m*	
3′	77.4	3.40, *m*	
4′	71.4	3.45, *m*	
5′	78.1	3.50, *m*	
6′	62.7		
6′ a		3.88, *dd*, *J* = 10.9, 1.9	
6′ b		3.73, *dd*, *J* = 10.9, 4.9	

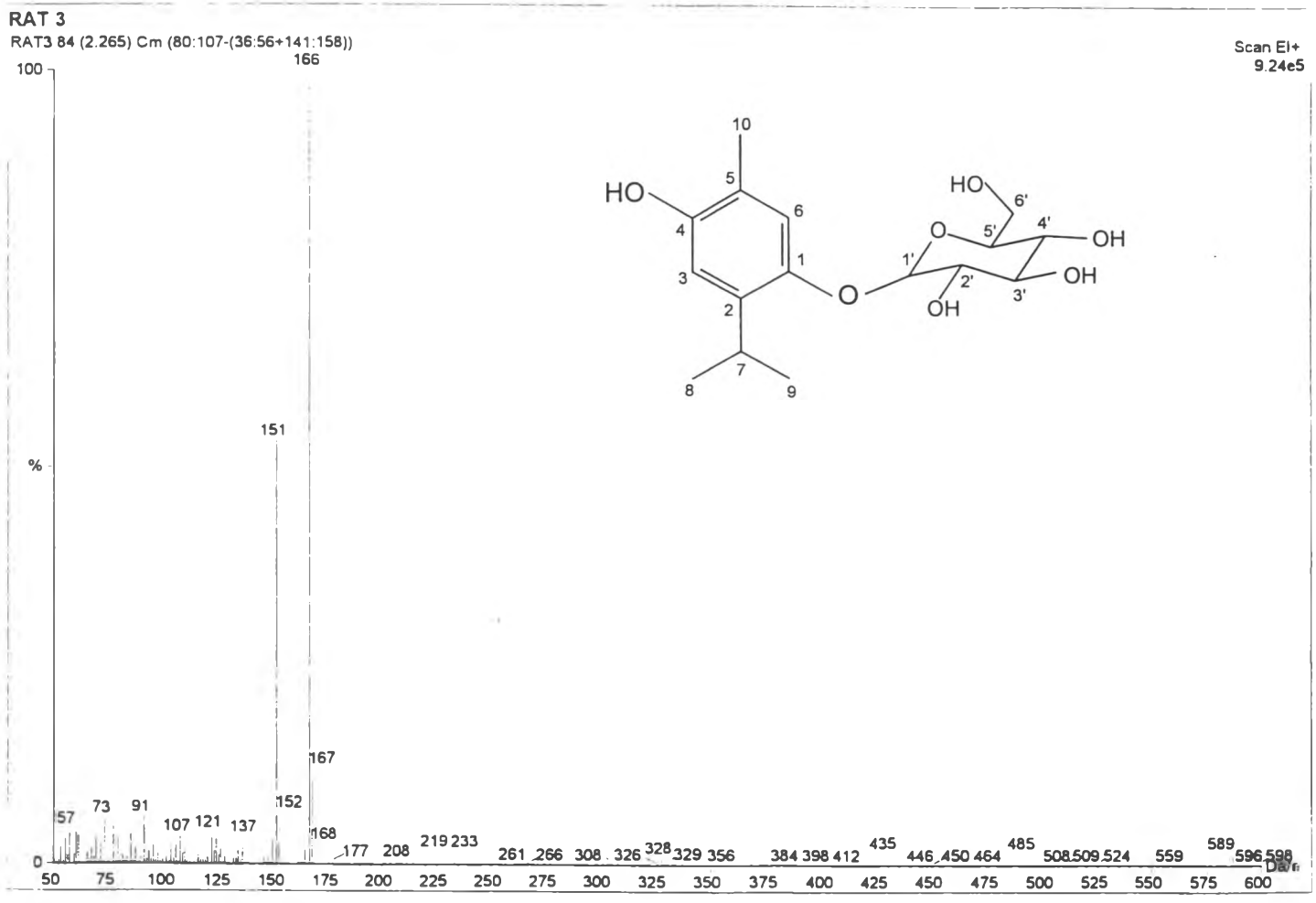


Figure 21. EIMS spectrum of RAT 2

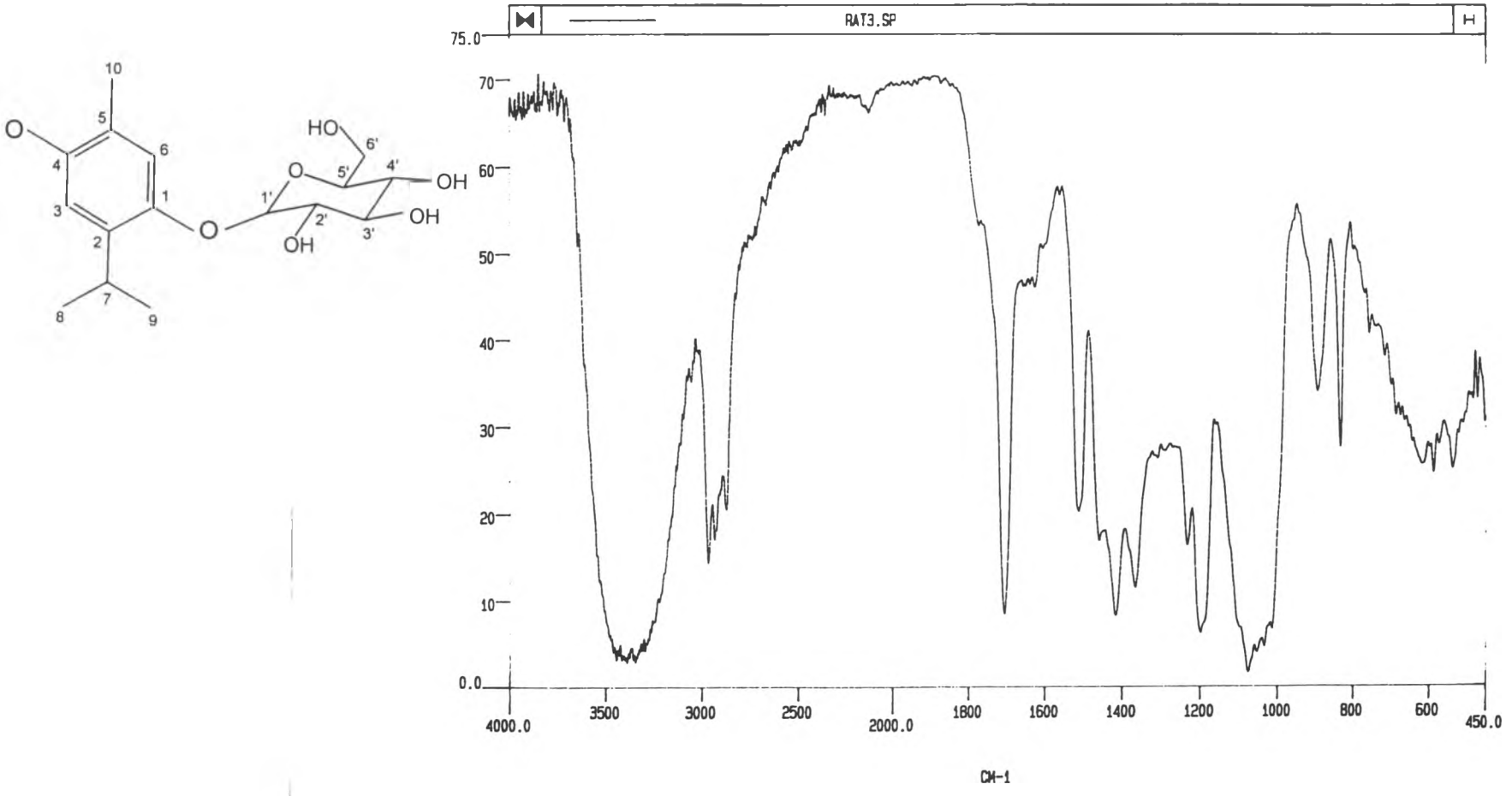
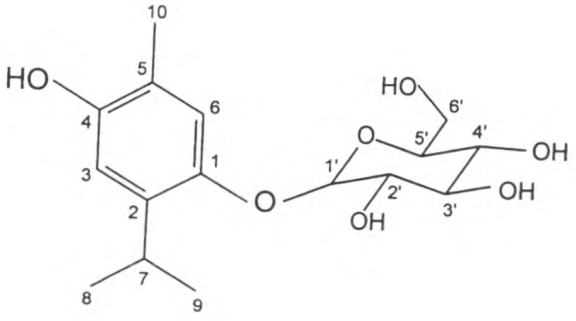


Figure 22. IR spectrum of RAT 2



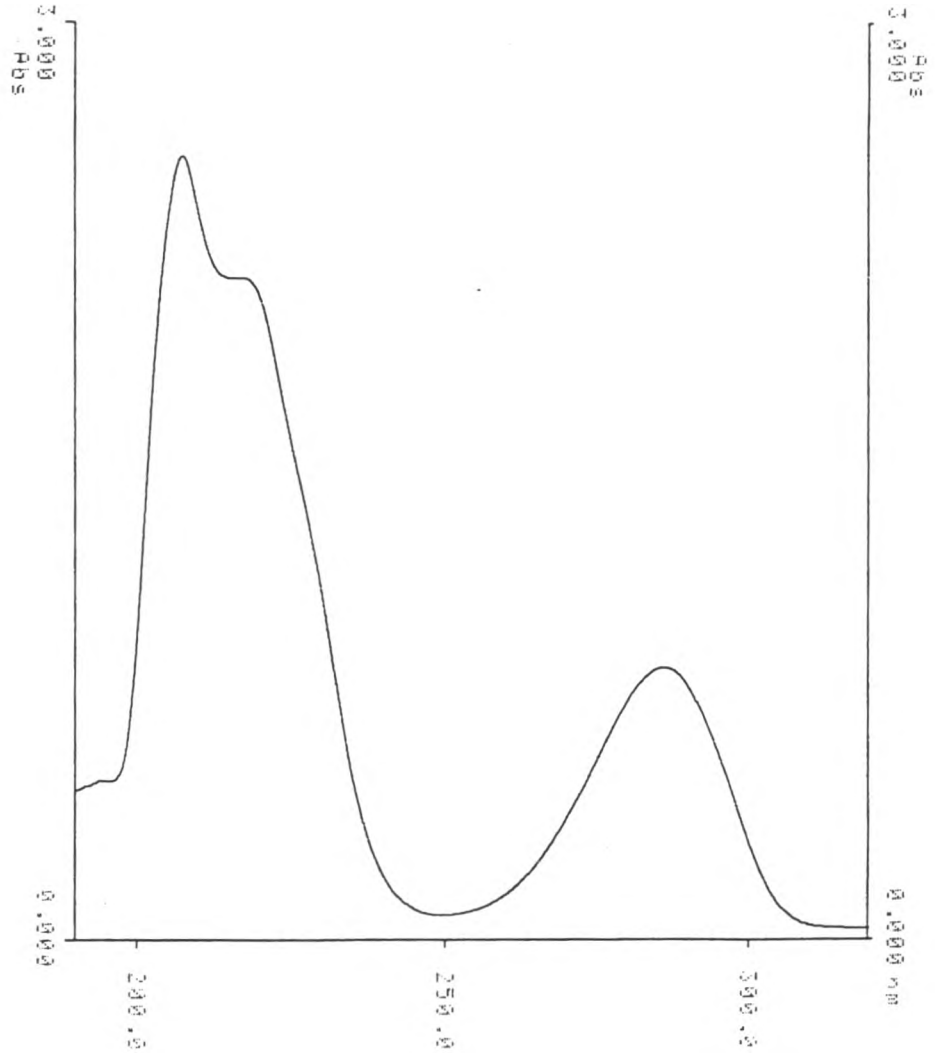


Figure 23. UV spectrum of RAT 2 (in MeOH)

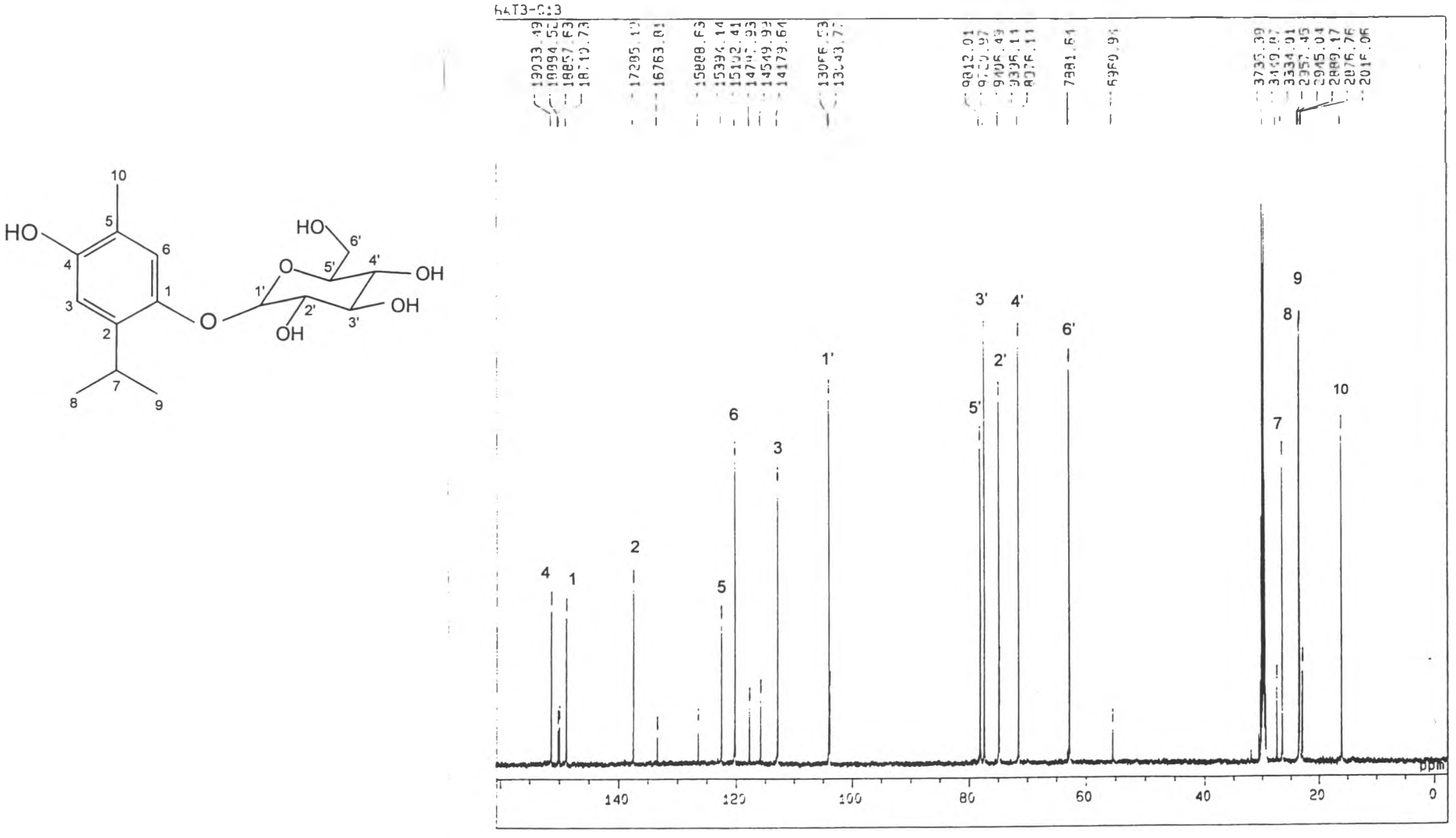


Figure 24. The 125 MHz ^{13}C NMR spectrum of RAT 2 (in acetone-d_6)

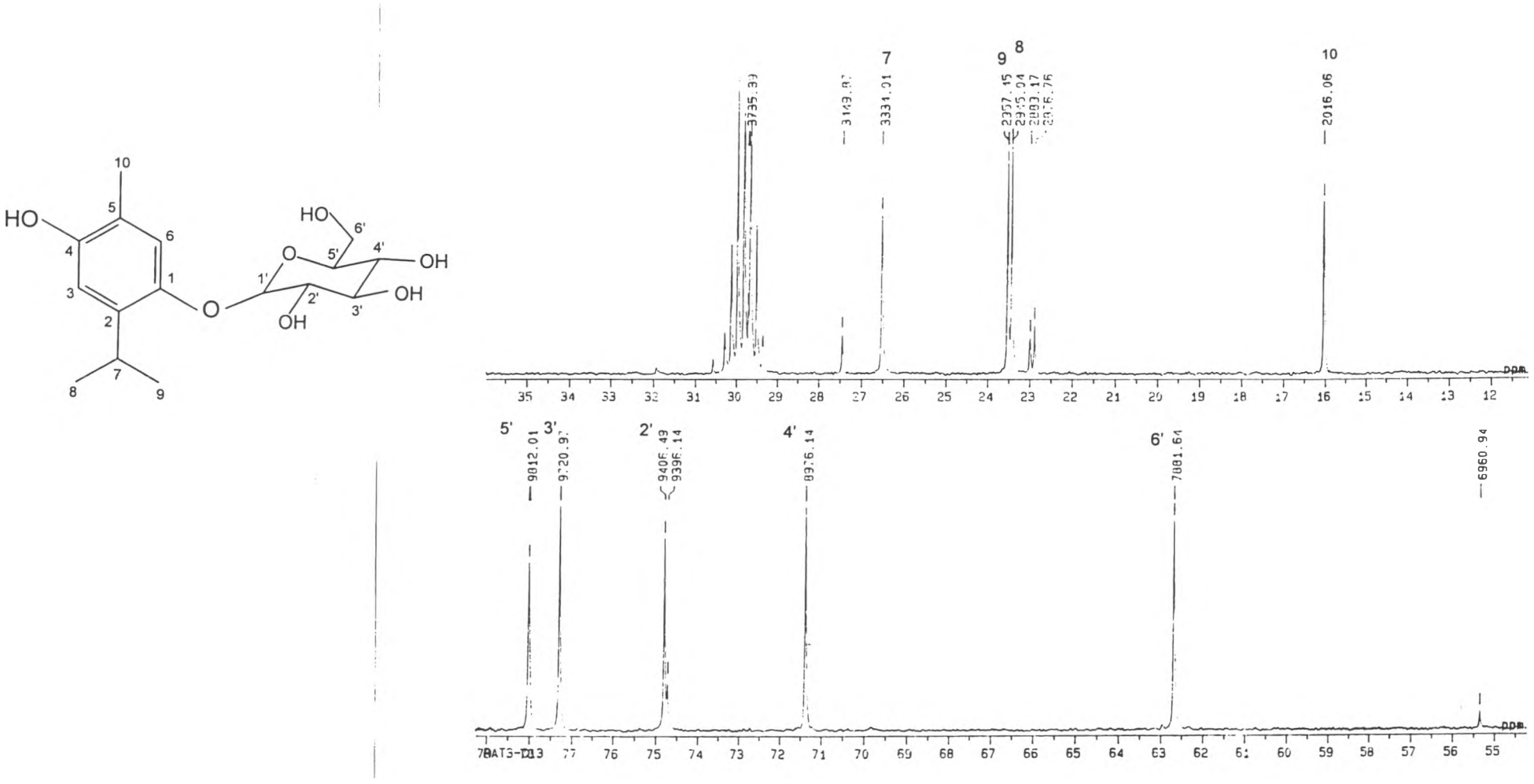


Figure 25. The 125 MHz ^{13}C NMR spectrum of RAT 2 (in acetone-d_6)

(expanded in the range of δ 11-79 ppm)

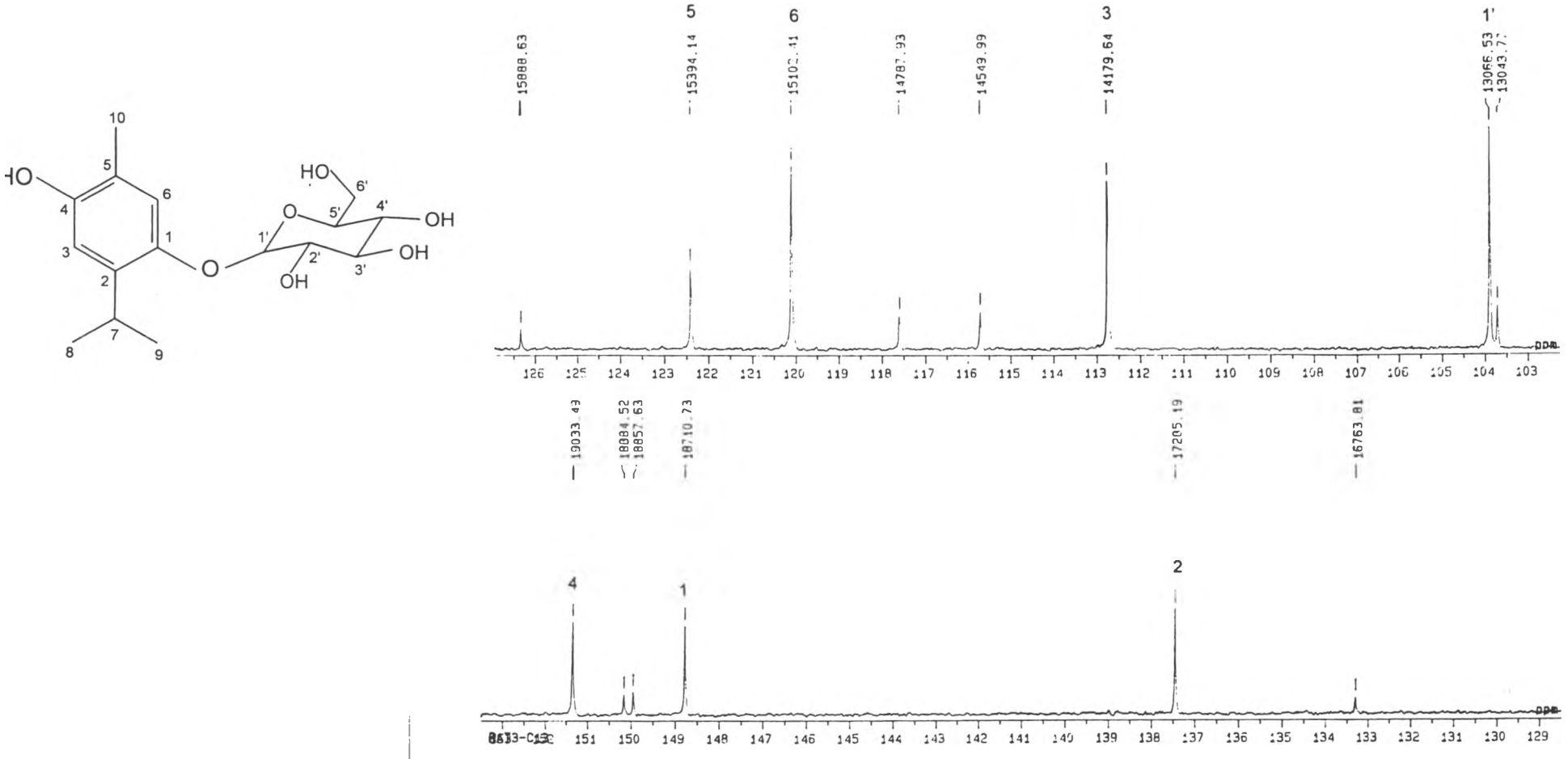


Figure 26. The 125 MHz ^{13}C NMR spectrum of RAT 2 (in acetone-d_6)

(expanded in the range of δ 103-153 ppm)

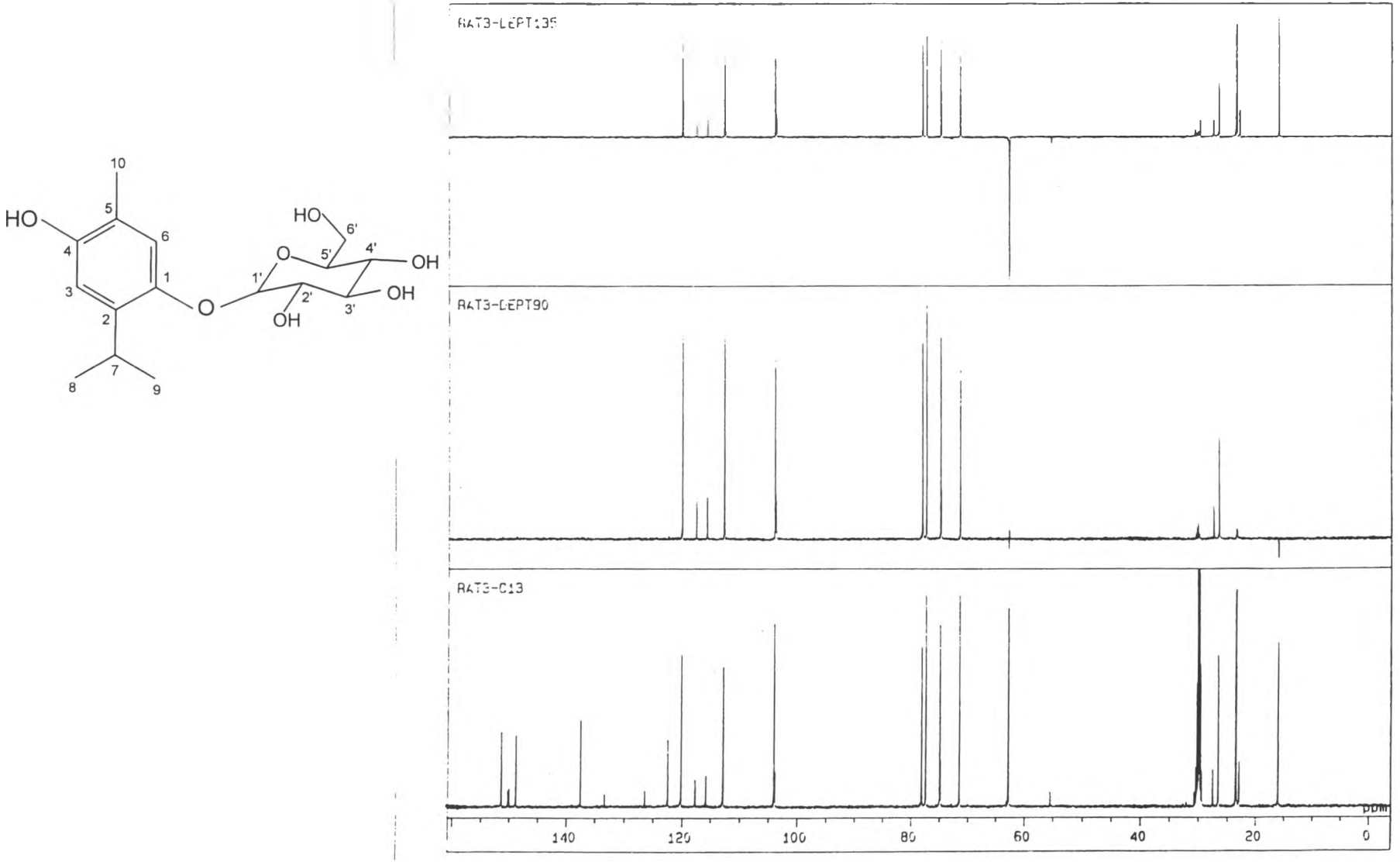
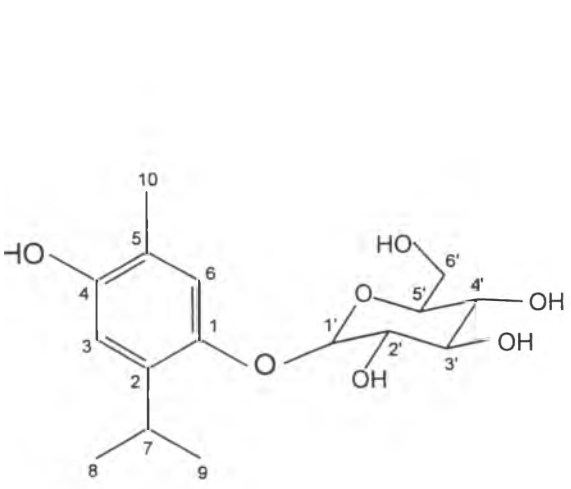


Figure 27. The ^{13}C DEPT spectrum of RAT 2 (in acetone-d_6)



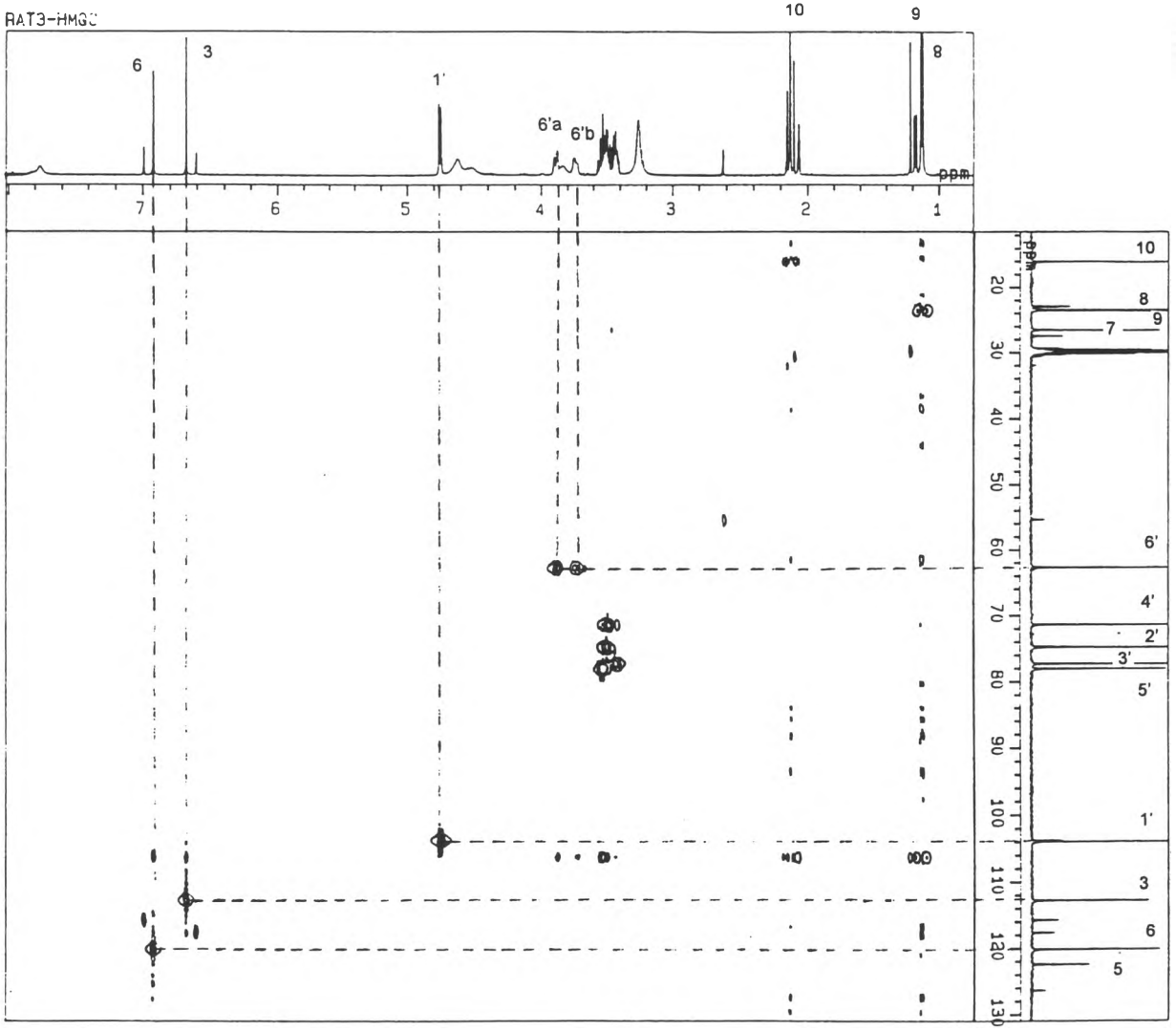


Figure 28. ^{1}H-^{13}C HETCOR spectrum of RAT 2 (in acetone-d_6)

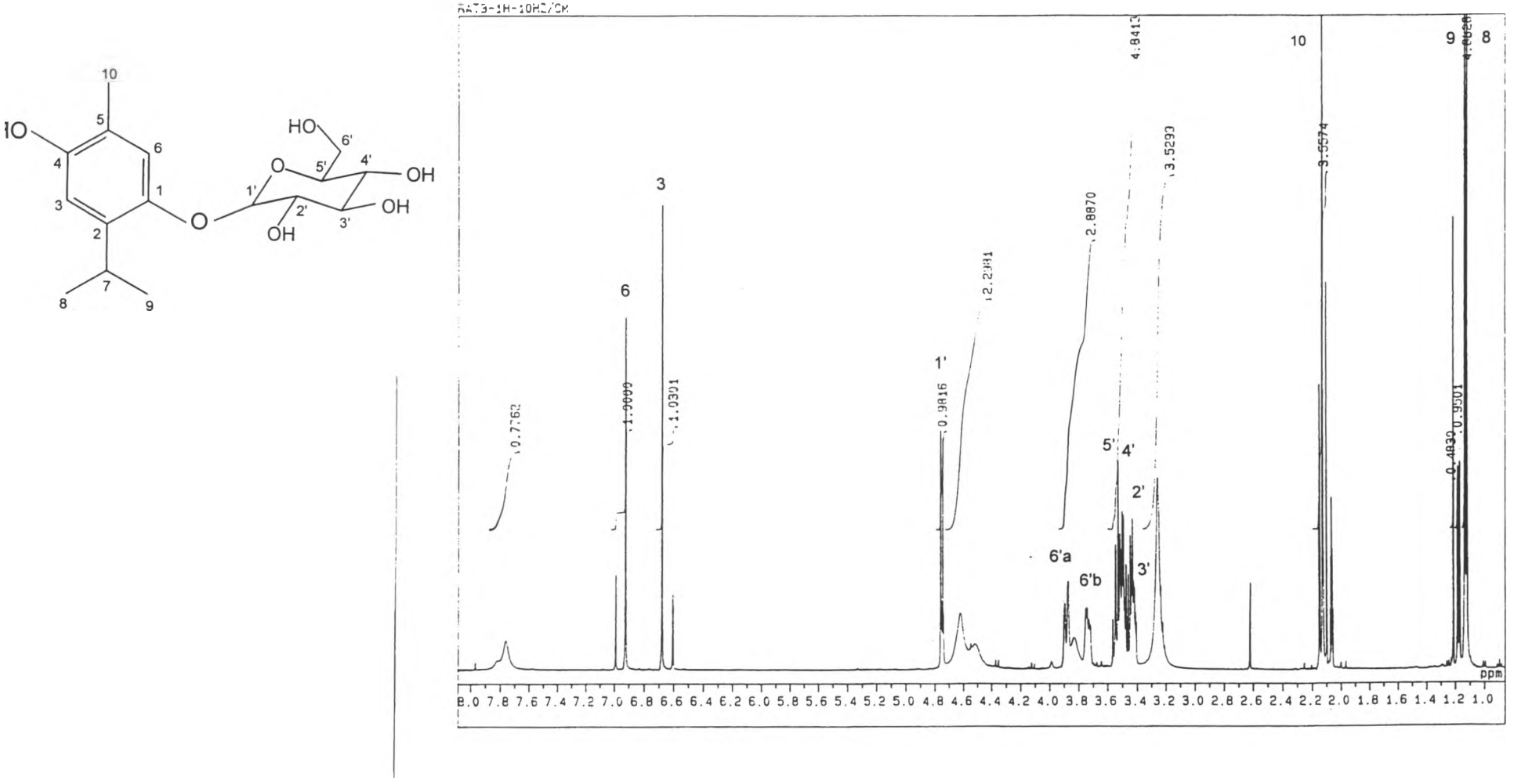


Figure 29. The 500 MHz ^{1}H NMR spectrum of RAT 2 (in acetone-d_6)

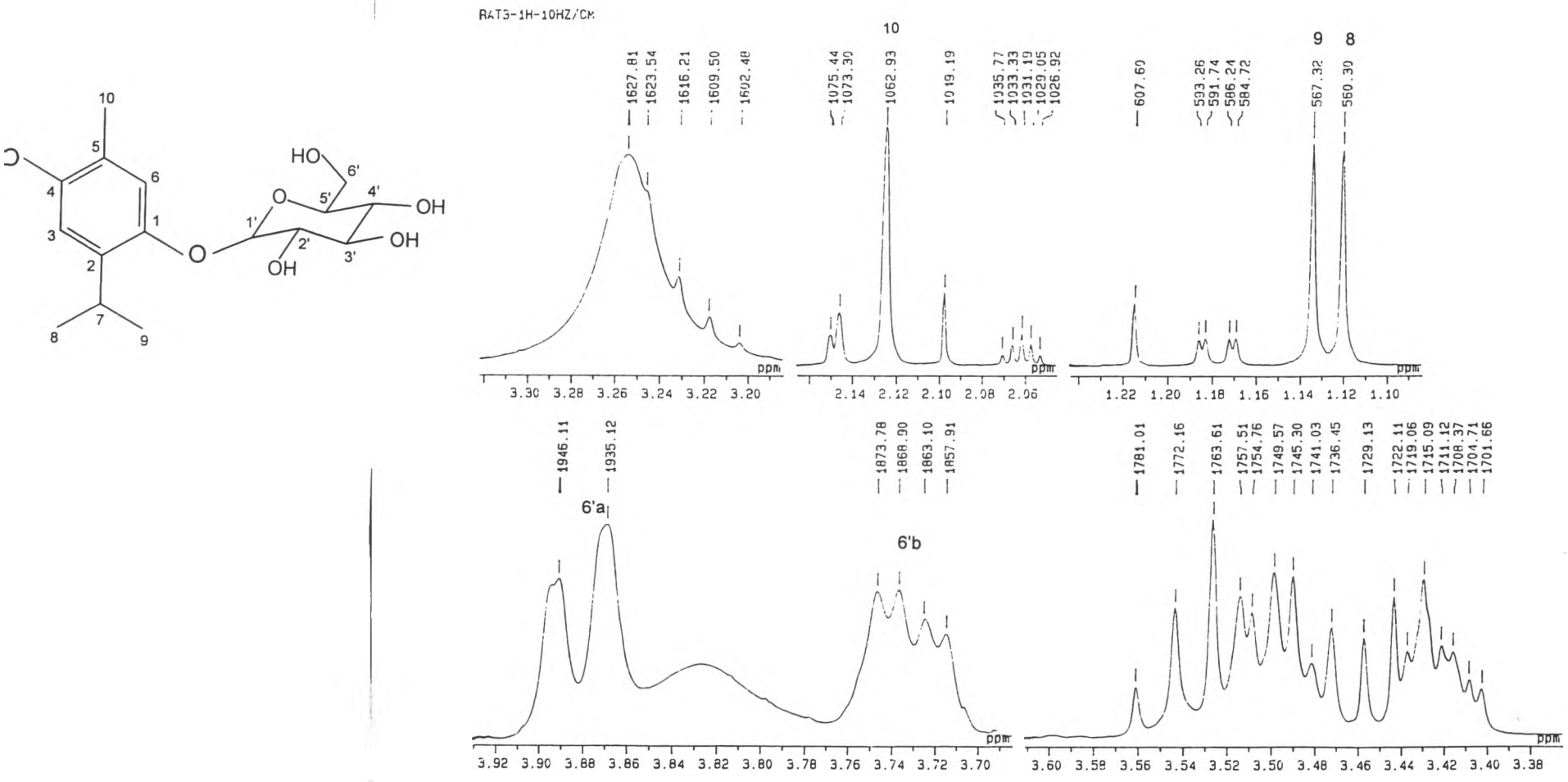


Figure 30. The 500 MHz ^{1}H NMR spectrum of RAT 2 (in acetone-d_6)

(expanded in the range of δ 1.10-3.92 ppm)

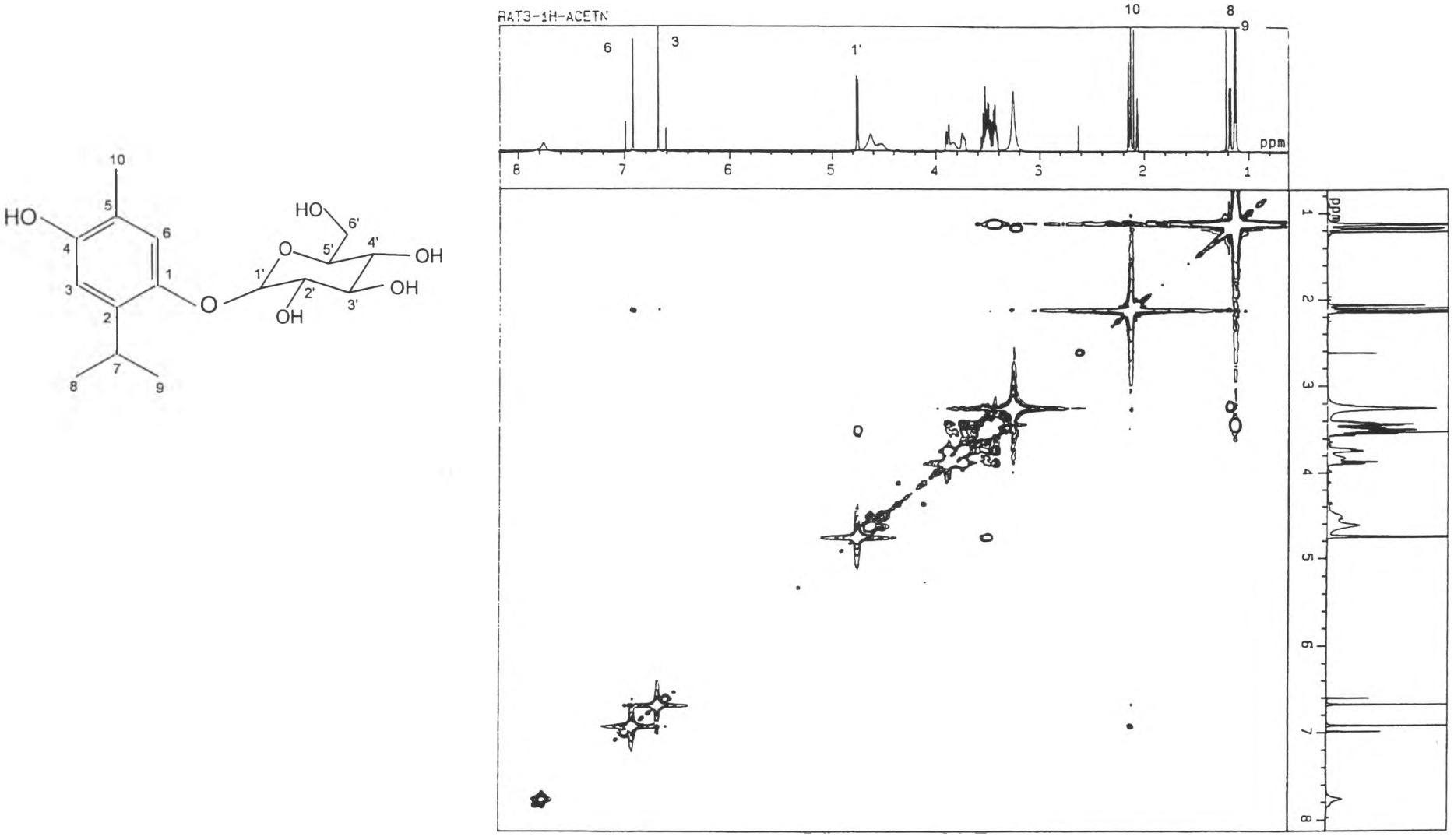


Figure 31. ^{1}H-^{1}H COSY spectrum of RAT 2 (in acetone-d_6)

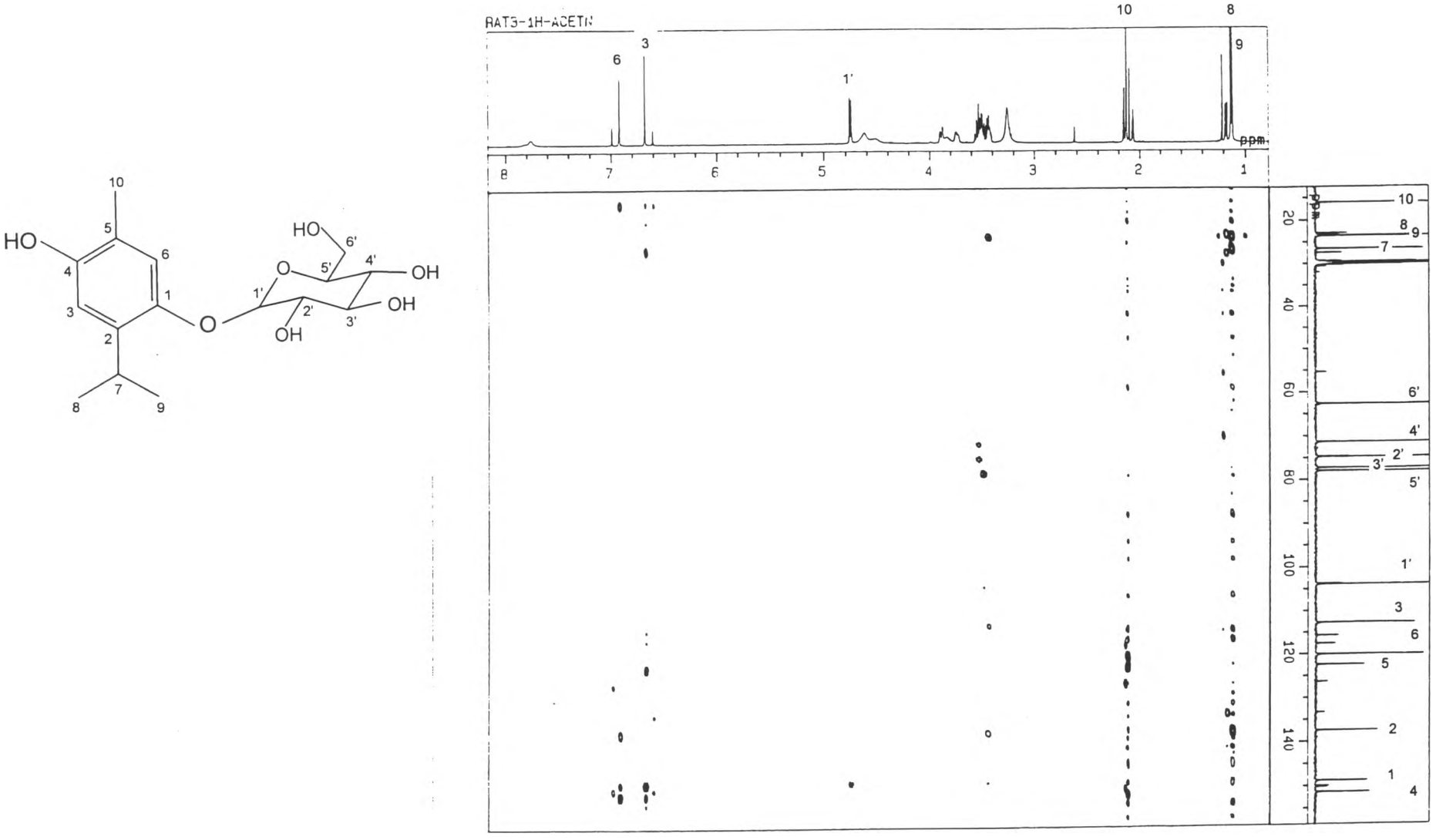


Figure 32. ^{1}H-^{13}C HMBC spectrum of RAT 2 (in acetone-d_6)

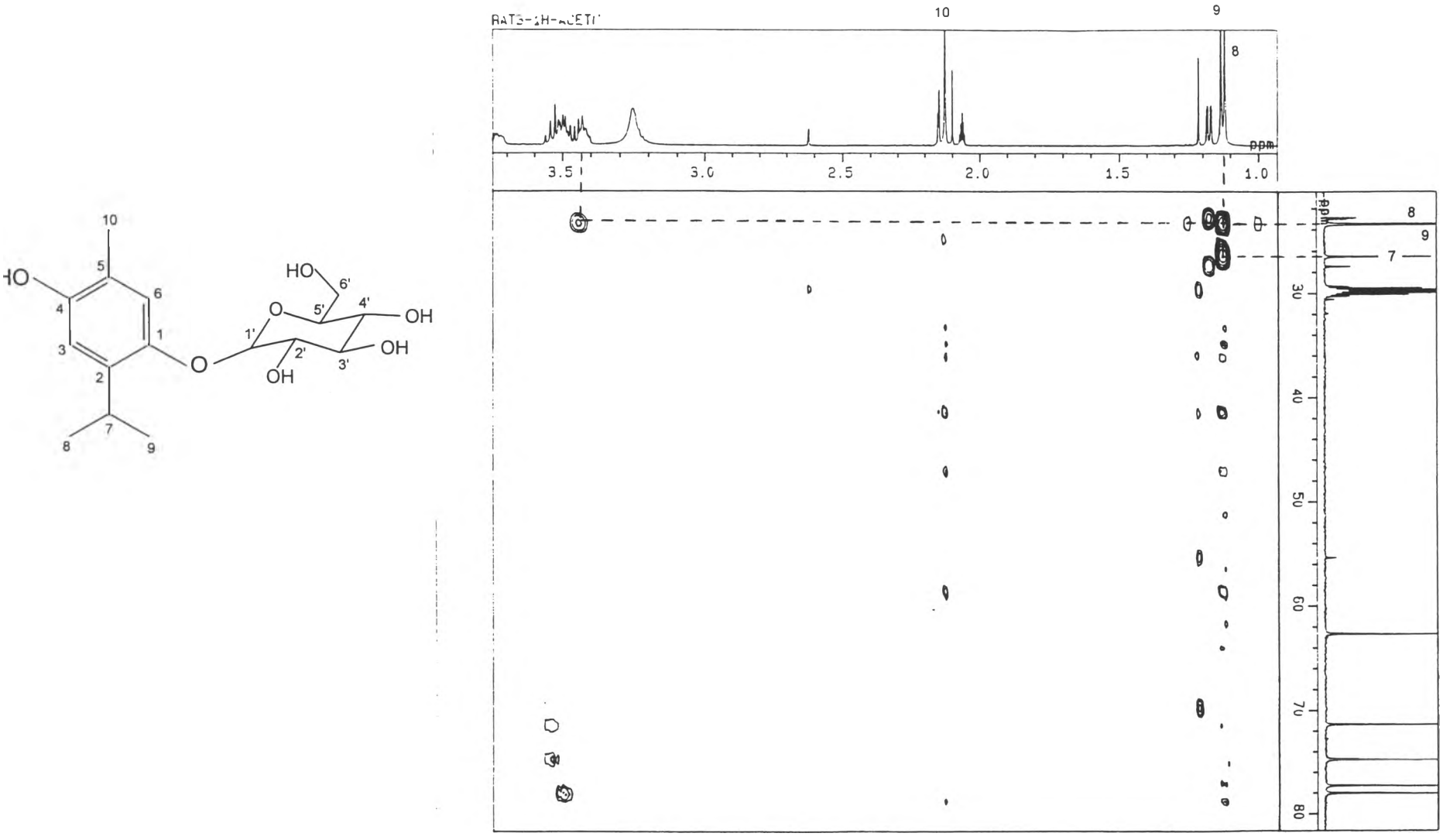


Figure 33. ^{1}H-^{13}C HMBC spectrum of RAT 2 (in acetone-d_6)

(expanded in the range of δ ^{1}H 1.0-3.7 ppm and δ ^{13}C 22-80 ppm)

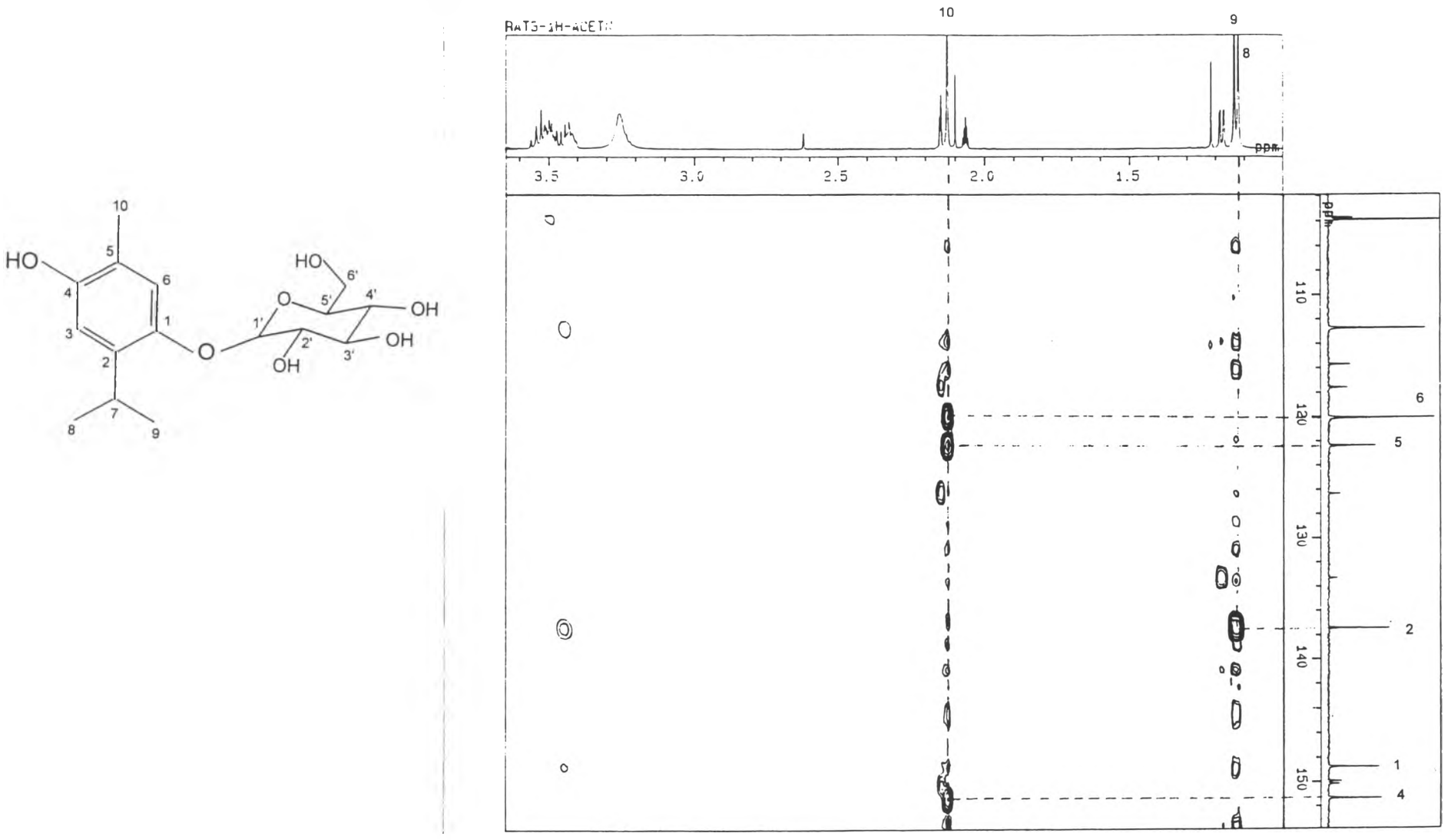


Figure 34. ^{1}H-^{13}C HMBC spectrum of RAT 2 (in acetone-d_6)

(expanded in the range of δ ^{1}H 1.0-3.6 ppm and δ ^{13}C 107-151 ppm)

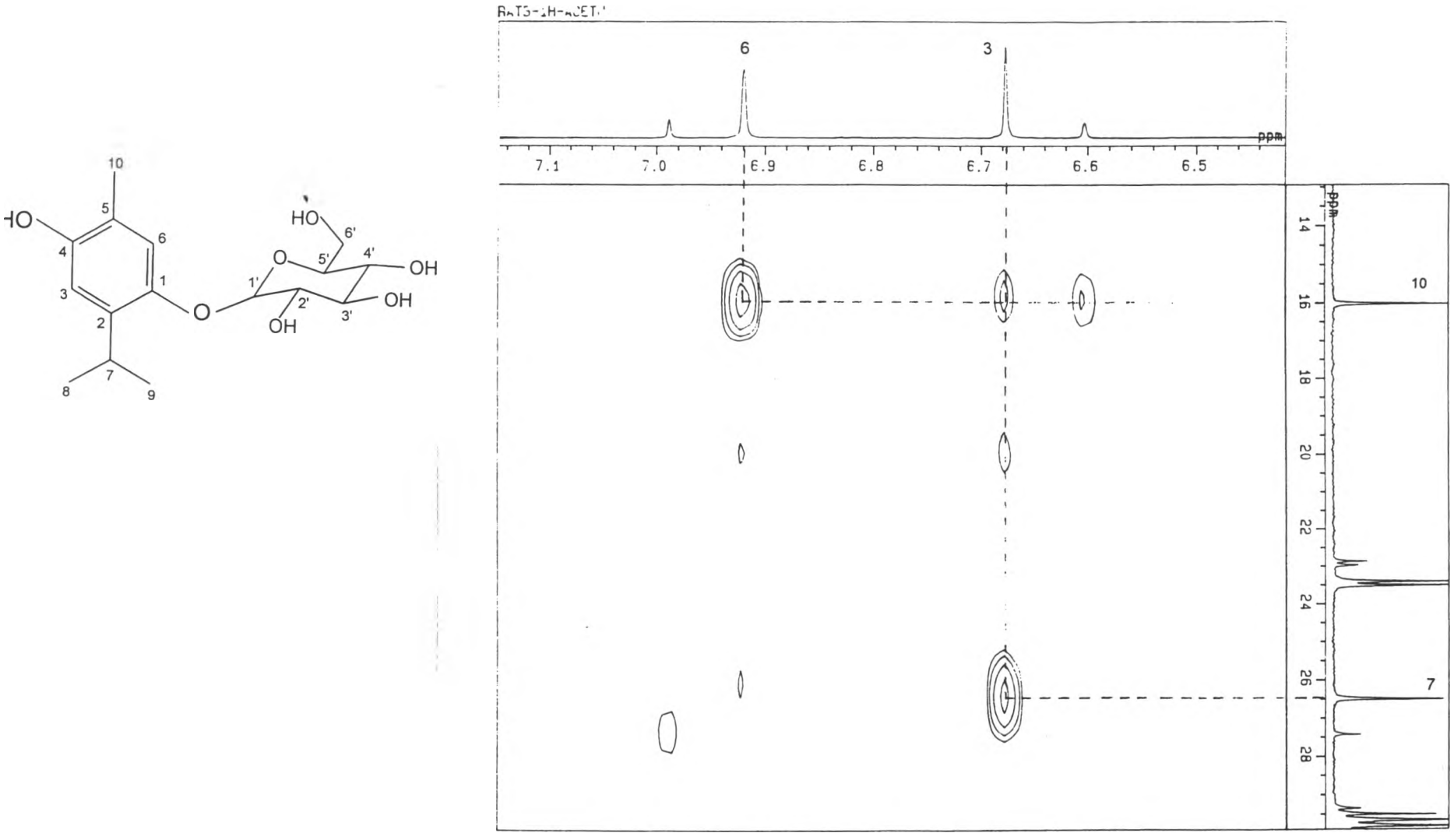


Figure 35. ^{1}H-^{13}C HMBC spectrum of RAT 2 (in acetone-d_6)

(expanded in the range of δ ^{1}H 6.5-7.1ppm and δ ^{13}C 14-29 ppm)

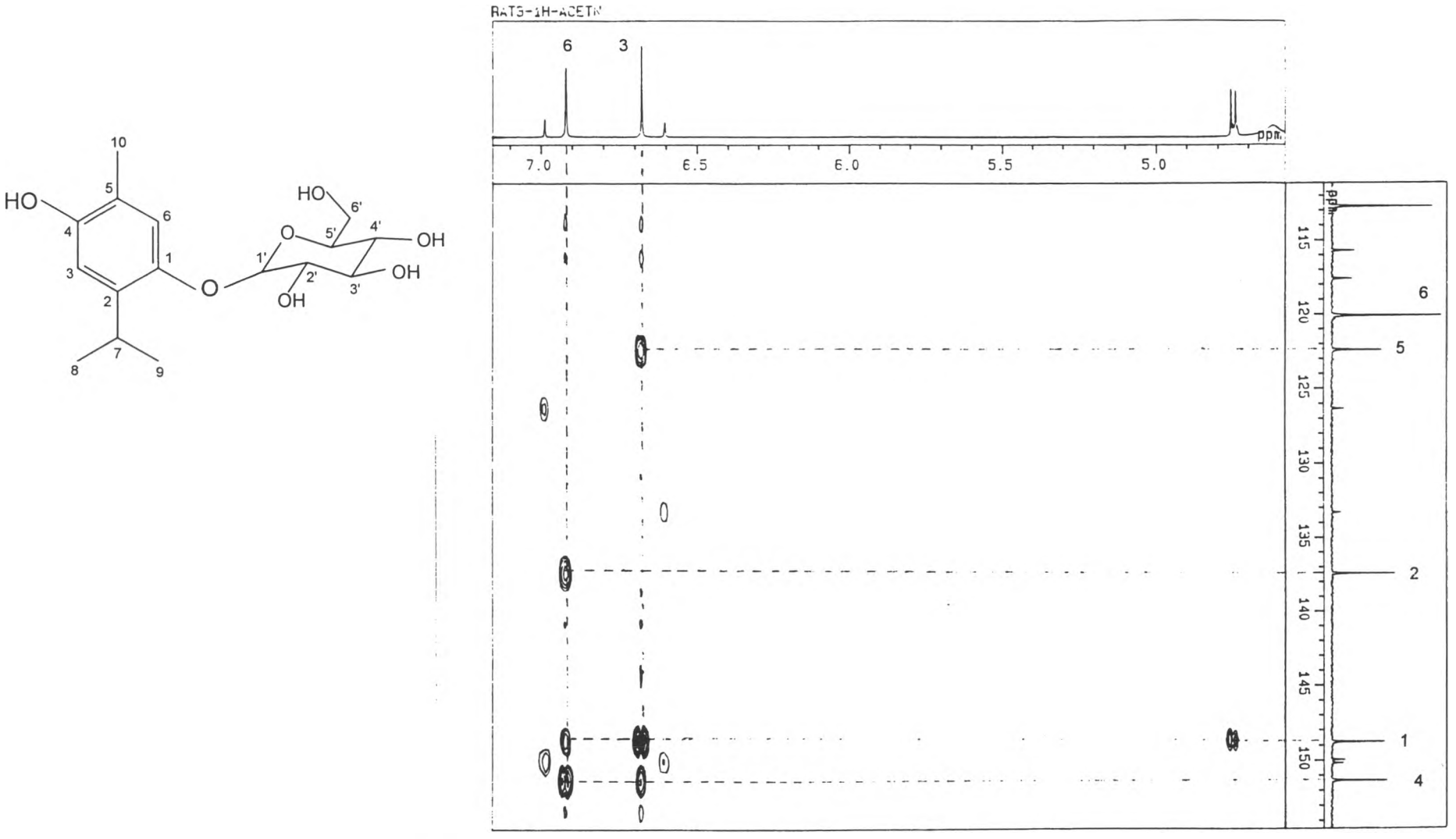
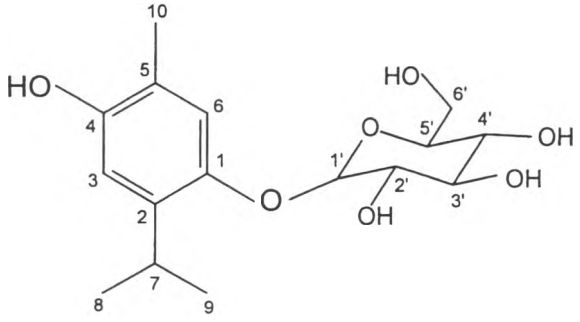


Figure 36. ^{1}H-^{13}C HMBC spectrum of RAT 2 (in acetone-d_6)

(expanded in the range of δ ^{1}H 4.7-7.1 ppm and δ ^{13}C 112-154 ppm)



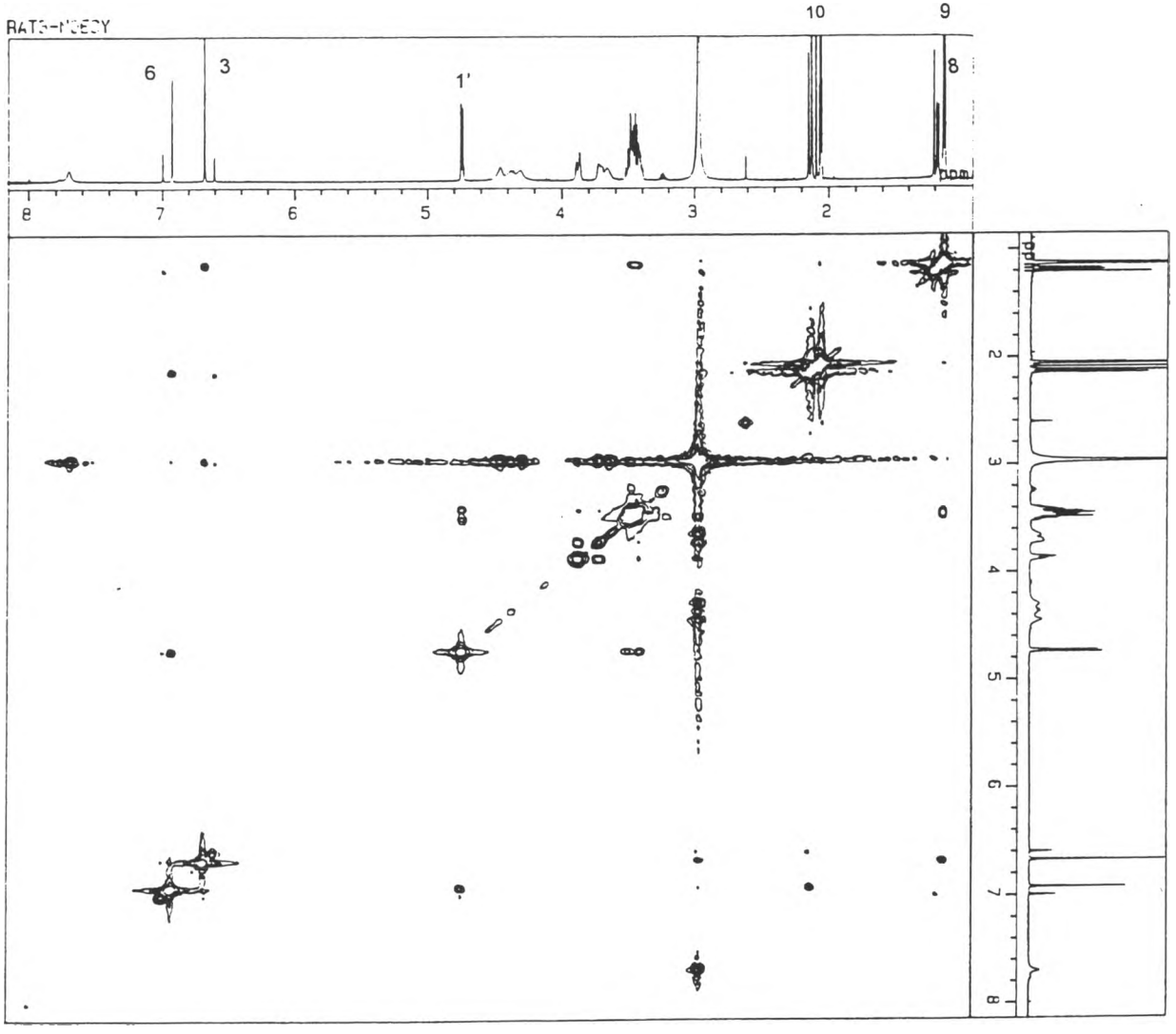


Figure 38. NOESY spectrum of RAT 2 (in acetone-d_6)

3. Identification of compound RAT 3

RAT 3 was obtained as a light yellow solid (190 mg) from fraction F035. The molecular formula of $C_{16}H_{24}O_6$ was suggested for this compound based on its proton spectrum, the number of carbon signals in ^{13}C NMR spectrum and its $[M+H-C_6H_{12}O_6]^+$ peak in the EIMS (Figure 41) at m/z 150. The presence of the alcohol functionality in the molecule could be observed from a broad IR absorption peak at 3390 cm^{-1} (Figure 42).

Similar to previous compound (RAT 2), ^{13}C NMR spectrum of RAT 3 (Figure 44) exhibited 16 carbon signals, six of which should belong to a β-glucopyranosyl unit (at δ 62.6, 71.4, 74.7, 77.5, 78.1 and 102.1 ppm). The other ten could be assigned to an aglycone portion of aryl-terpenoid possessing isopropyl, methyl and hydroxyl substitutent groups, although, according to its molecular formula, with one less hydroxy group. DEPT and ^1H-^{13}C-HETCOR experiments (Figure 47-48) were performed to classify these signals into those of three methyl carbons at δ 16.0, 24.2 and 24.3 ppm, one methylene carbon at δ 62.6 ppm, nine methine carbons at δ 34.5, 71.4, 74.7, 77.5, 78.1, 102.1, 114.0, 120.5 and 131.0 ppm, and three quaternary carbons at δ 125.2, 148.5 and 156.7 ppm.

In the ^1H NMR spectrum (Figure 49), the isopropyl methyl groups could be recognized from the most upfield signal at δ 1.20 ppm (6H, *d*, J = 6.7 Hz). The ^1H-^1H COSY spectrum (Figure 51) displayed the correlation of this signal to a one-proton septet at δ 2.84 ppm (H-7). The meta-coupling of an aromatic proton at

δ 7.02 ppm (*d*, *J* = 1.8 Hz, H-6) to a doublet of doublets at δ 6.78 ppm (*J* = 7.5, 1.8 Hz, H-4), which further ortho-coupling to a doublet at δ 7.02 ppm (*J* = 7.5 Hz, H-3), could also be observed, suggesting 1, 2, 4-substitution of the aromatic ring.

The aglycone of RAT 3 was thus determined as carvacrol (4) (2-hydroxy-4-isopropyl-1-methylbenzene), a monoterpene previously reported from this plant (Skopp and Horster, 1976). ^{1}H-^{13}C HMBC experiment (Figure 52-55) was performed in order to confirm the aglycone structure. Correlations could be observed between both isopropyl methyl protons at δ 1.13 ppm and C-5 (δ 148.5 ppm) and C-7 (δ 34.5 ppm), while the isopropyl methine proton (H-7, δ 2.84 ppm) showed correlations to C-4 , C-5 , C-6, C-8 and C-9 at δ 120.5, 148.5, 114.0, 24.2 and 24.3 ppm, respectively, establishing the isopropyl substituent at position 5. An aromatic proton (δ 6.78 ppm, H-4) displayed correlations to C-2 (δ 125.2 ppm), C-6 and C-7 (δ 34.5 ppm), while its neighbour (H-3) gave cross peaks with C-1 (δ 156.7 ppm), C-5 and C-10 (δ 16.0 ppm). A methyl group could be placed at position 2, according to the correlations of this methyl proton (H3-10) to C-1, C-2 and C-3 at δ 156.7, 125.2 and 131.0 ppm, respectively. Major HMBC correlations in the structure of compound RAT 3 can be summarized as shown in Figure 56.

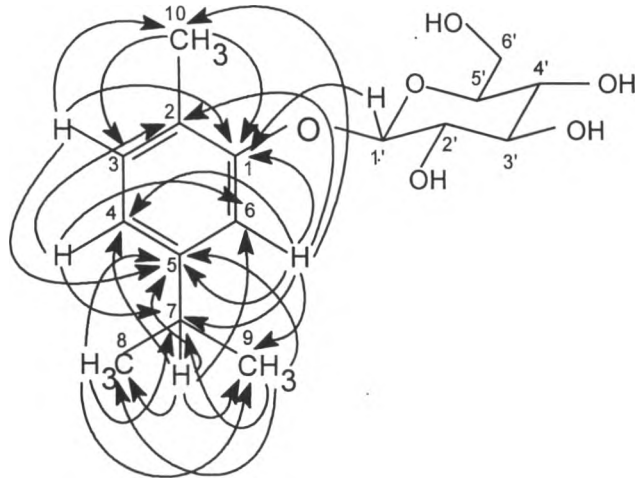


Figure 56. Major HMBC correlations of RAT 3

The β-glucose unit was placed at position 1, according to HMBC correlation between H-1′ (δ 4.93 ppm, *d*, *J* = 7.6 Hz) and C-1. This was supported by NOESY experiment (Figure 57), in which the correlations between H-1′ proton signal and H-6 (δ 7.02 ppm) and H-5′ (δ 3.54 ppm) was readily observable. Major NOESY correlations in the structure of RAT 3 are shown in figure 58. Therefore, RAT 3 was identified as 1-hydroxy-5-*iso*-propyl-2-methylphenyl-1-0-β-D-glucopyranoside (carvacrol-β-D- glucopyranoside), shown in Figure 59. This compound was first isolated from *Thymus vulgaris,* another plant in the Labiatae (Skopp and Horster, 1976). However, this is the first report of this monoterpene glucoside from *Coleus amboinicus.*

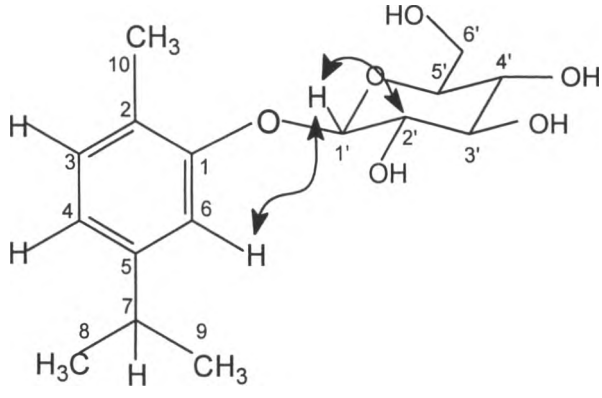


Figure 58. Major NOESY correlations of RAT 3

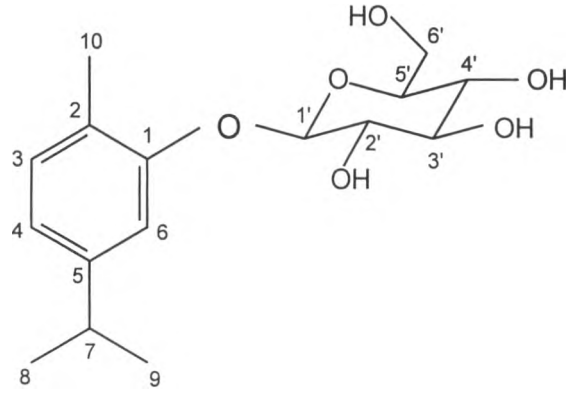


Figure 59. Structure of 1-hydroxy-5-*iso*-propyl-2-methylphenyl-1-0-β-D-glucopyranoside (carvacrol-β-D- glucopyranoside)

Table 16. ^{1}H and ^{13}C NMR data for RAT 3 (carvacrol-β-D-glucopyranoside)

Position	$^{\delta}C$	$^{\delta}H$	HMBC correlations
Aglycone			
1	156.7	-	
2	125.2	-	
3	131.0	7.02, *d*, *J* = 7.5	C-1, C-5, C-10
4	120.5	6.78, *dd*, *J* = 7.5, 1.8	C-2, C-6, C-7
5	148.5	-	
6	114.0	7.02, *d*, *J* = 1.8	C-2, C-4, C-7
7	34.5	2.84, *septet*, *J* = 6.7	C-4, C-5, C-6, C-8, C-9
8	24.2	1.20, *d*, *J* = 6.7	C-5, C-7, C-9
9	24.3	1.21, *d*, *J* = 6.7	C-5, C-7, C-8
10	16.0	2.20, *s*	C-1, C-2, C-3
Glucosyl			
1′	102.1	4.93, *d*, *J* = 7.6	C-1
2′	74.7	3.42, *m*	
3′	77.5	3.40, *m*	
4′	71.4	3.45, *m*	
5′	78.1	3.54, *dd*, *J* = 7.6, 1.8	
6′	62.6		
6′ a		3.72, *dd*, *J* = 11.5, 4.9	
6′ b		3.89, *dd*, *J* = 11.5, 1.8	

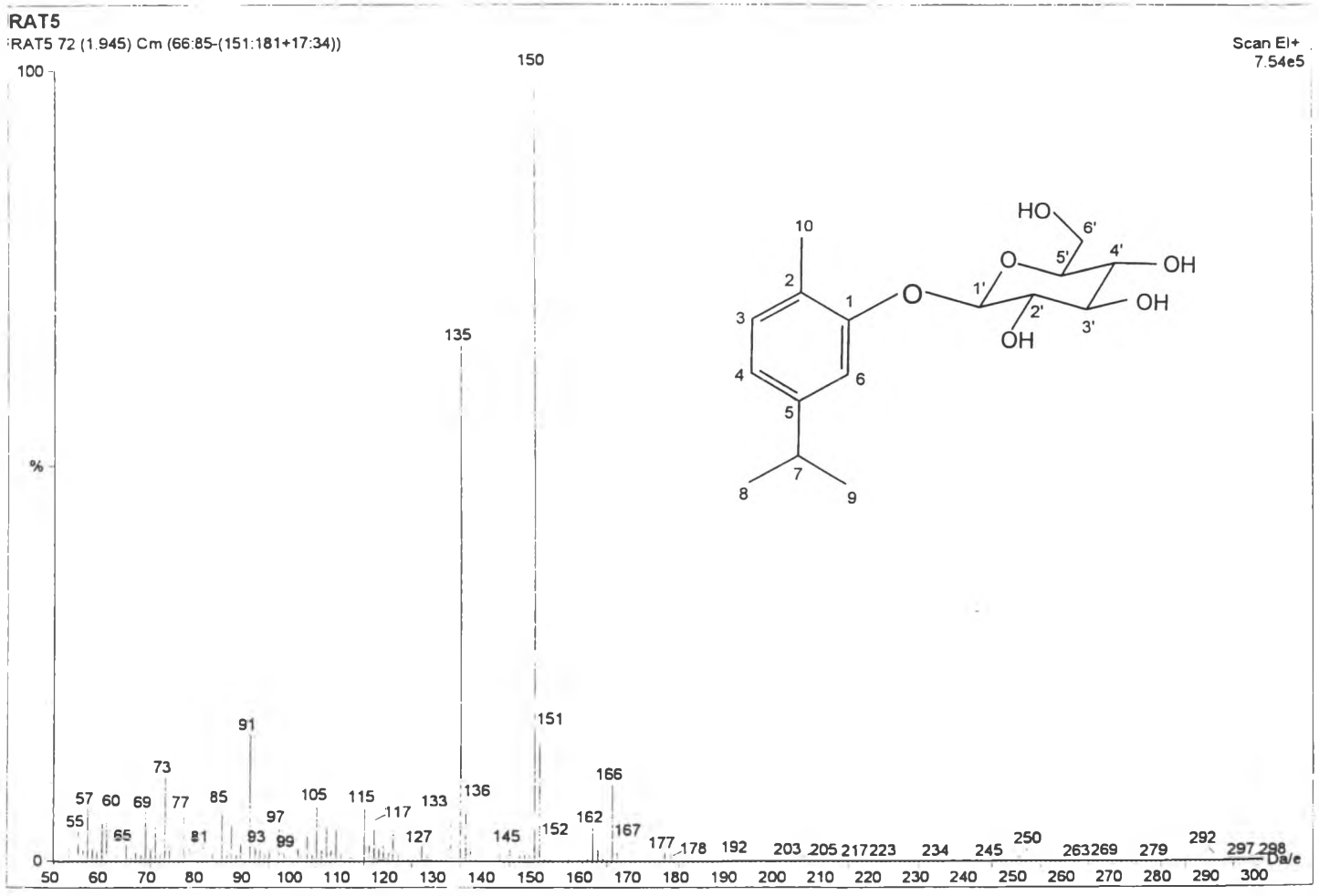


Figure 41. EIMS spectrum of RAT 3

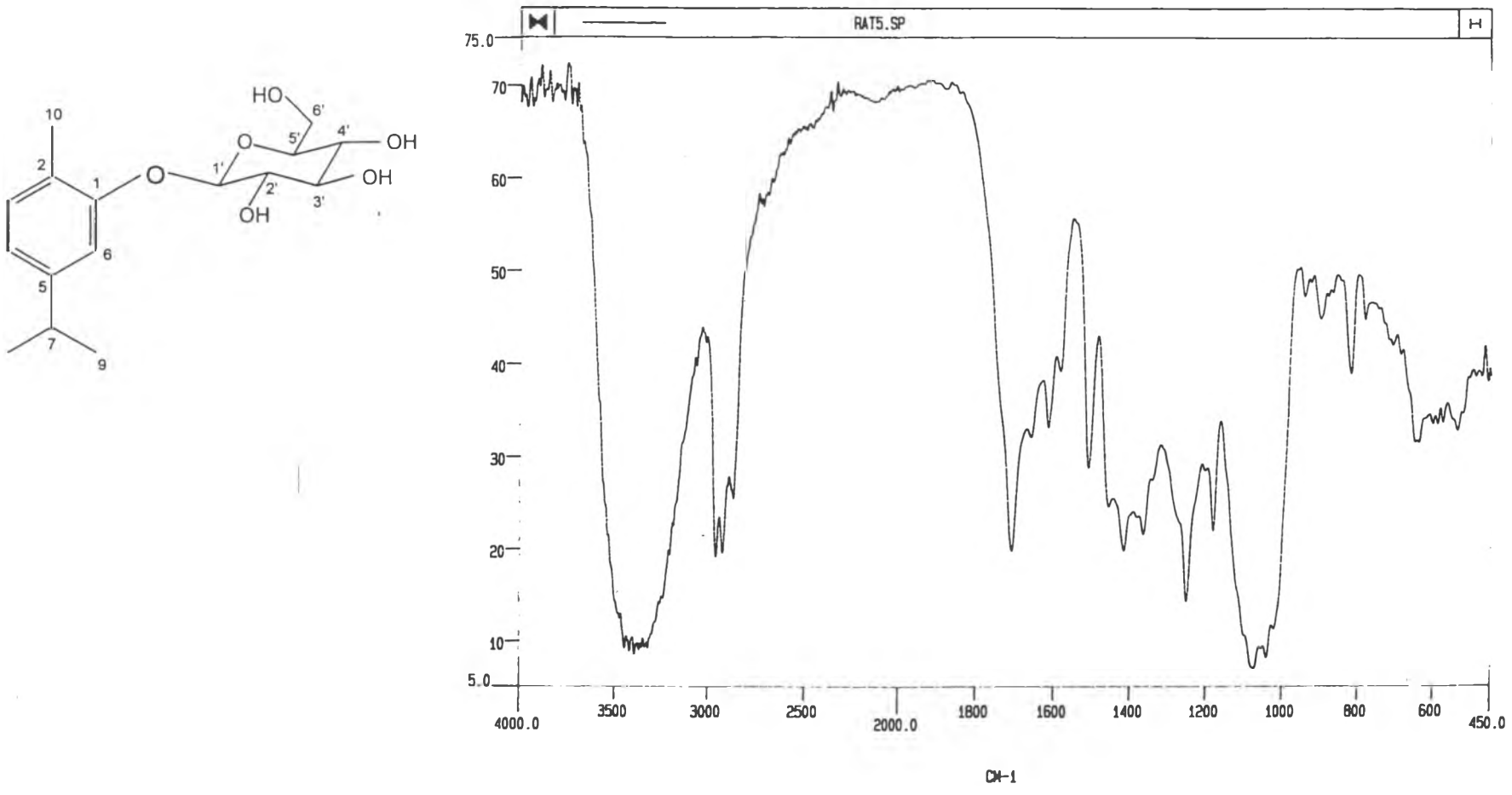
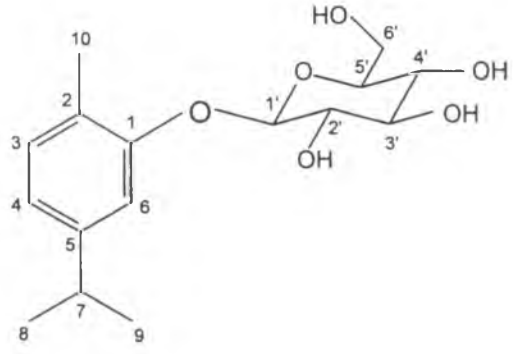


Figure 42. IR spectrum of RAT 3



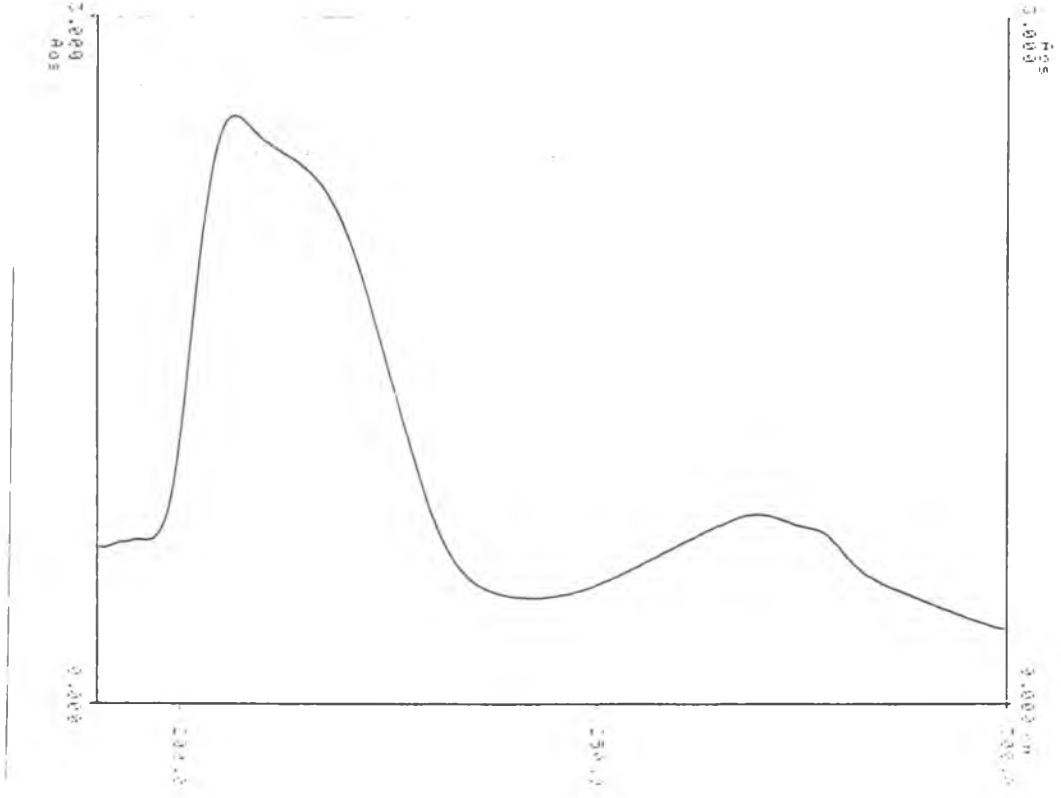
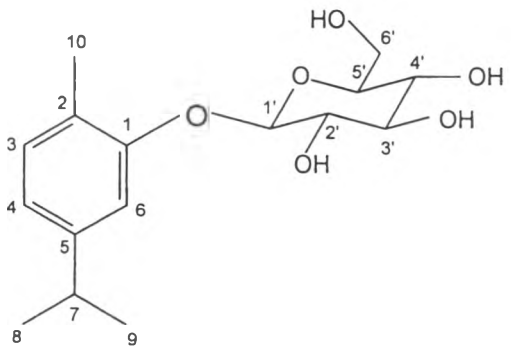


Figure 43. UV spectrum of RAT 3 (in MeOH)



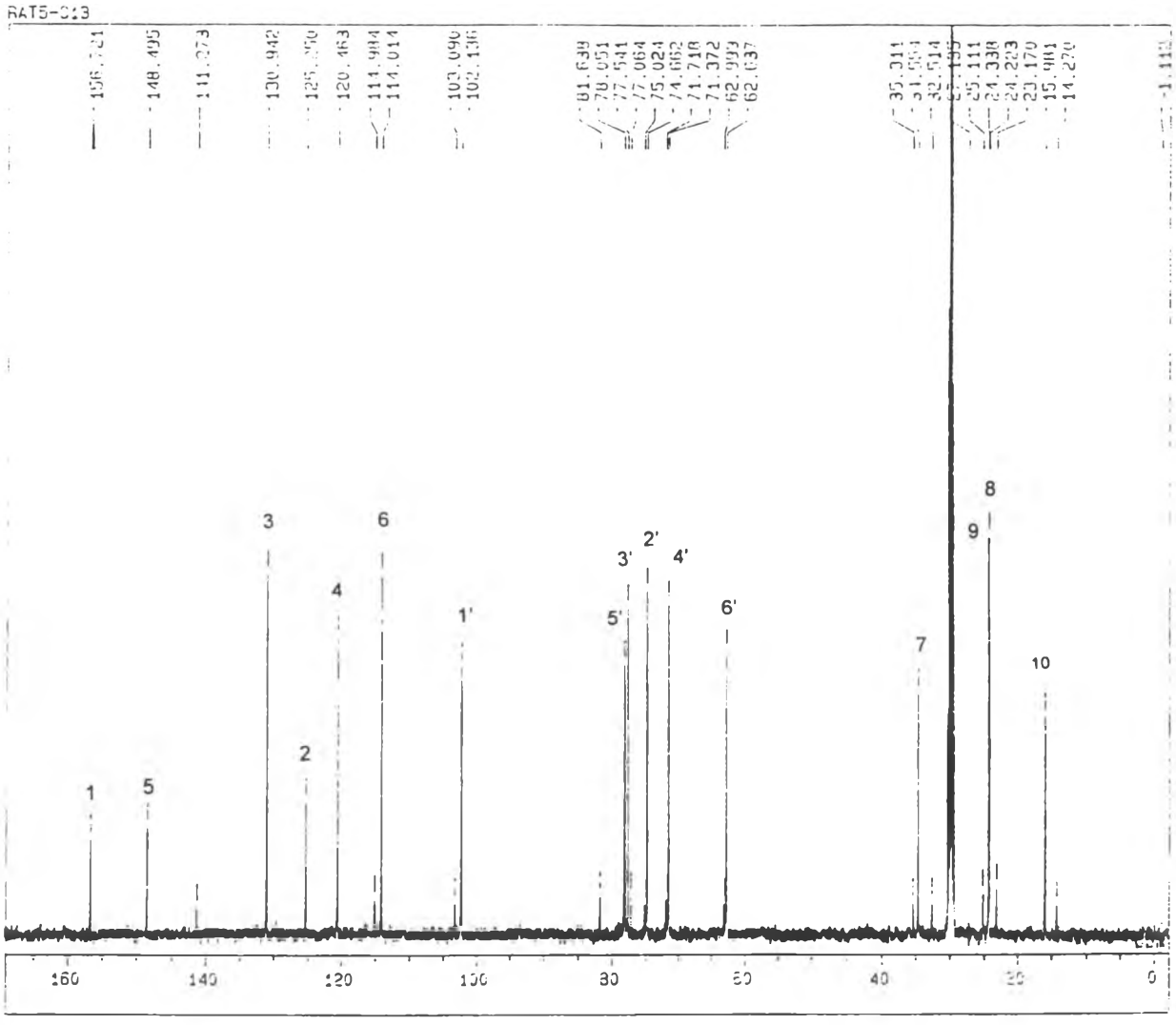


Figure 44. The 125 MHz ^{13}C NMR spectrum of RAT 3 (in acetone-d_6)

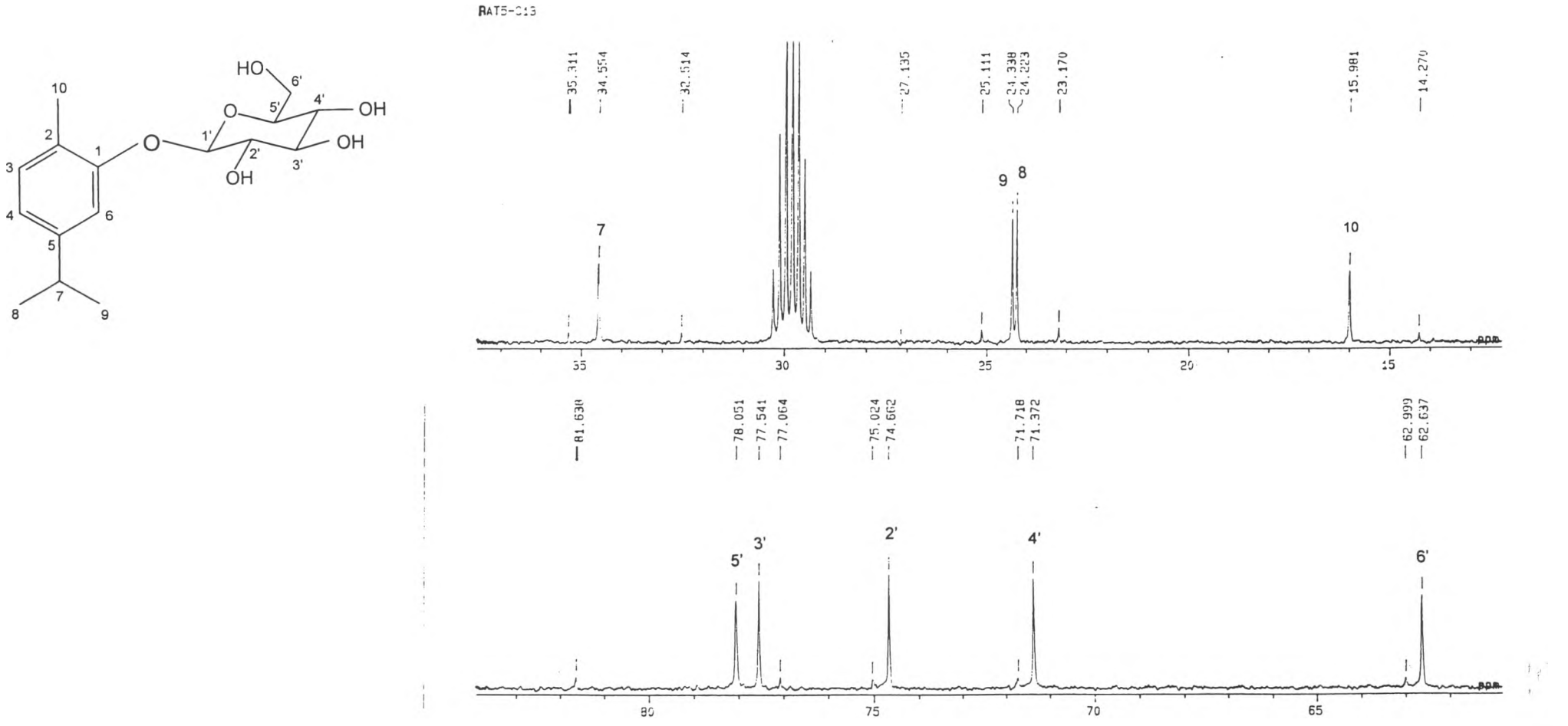


Figure 45. The 125 MHz ^{13}C NMR spectrum of RAT 3 (in acetone-d_6)

(expanded in the range of δ 13-83 ppm)

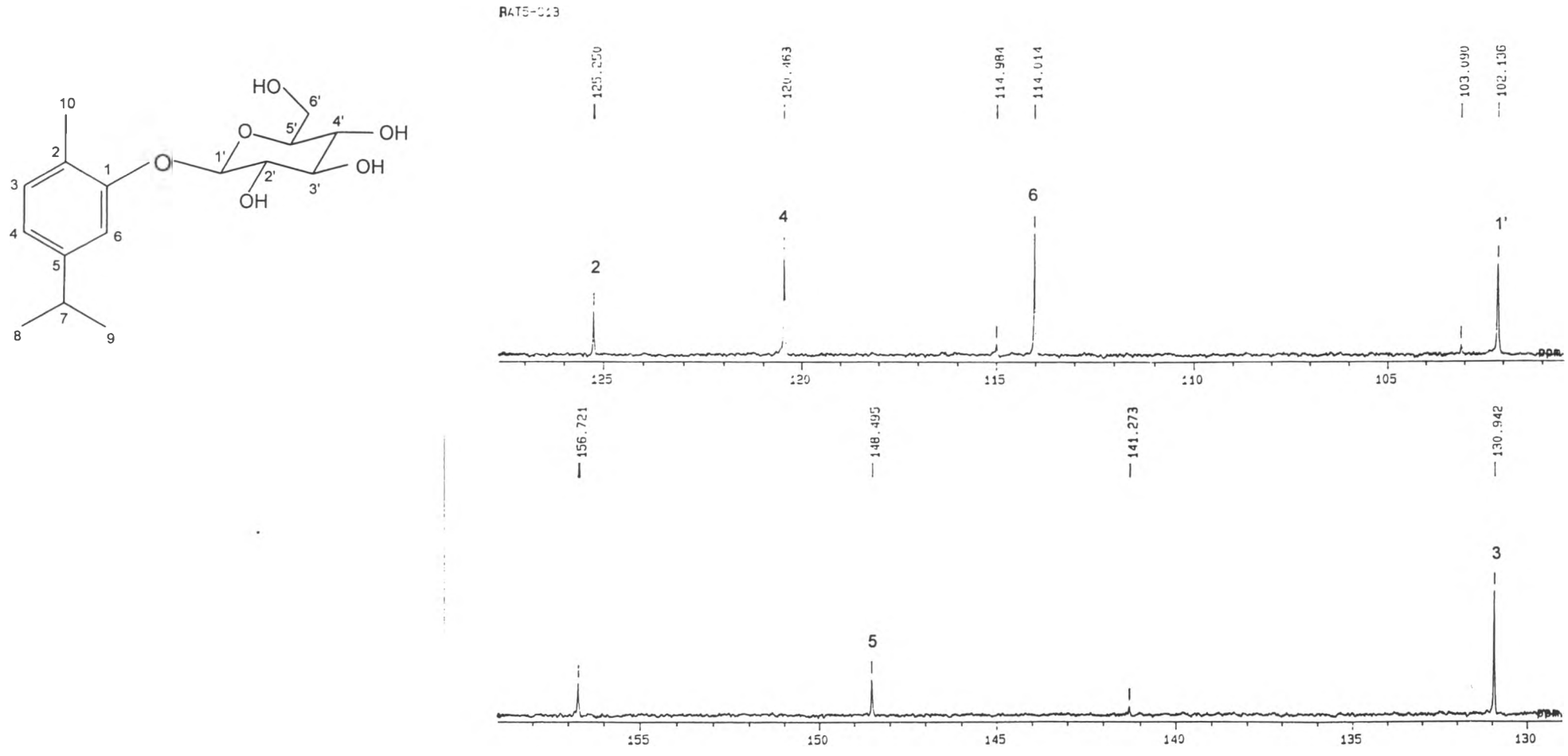
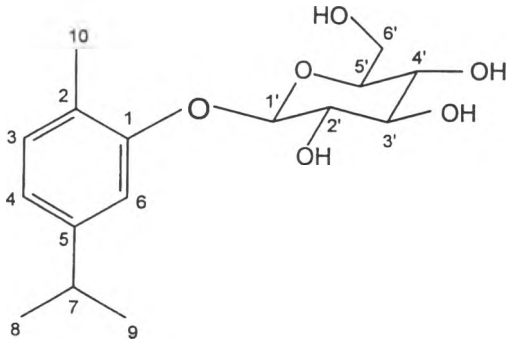


Figure 46. The 125 MHz ^{13}C NMR spectrum of RAT 3 (in acetone-d_6)
(expanded in the range of δ 101-158 ppm)



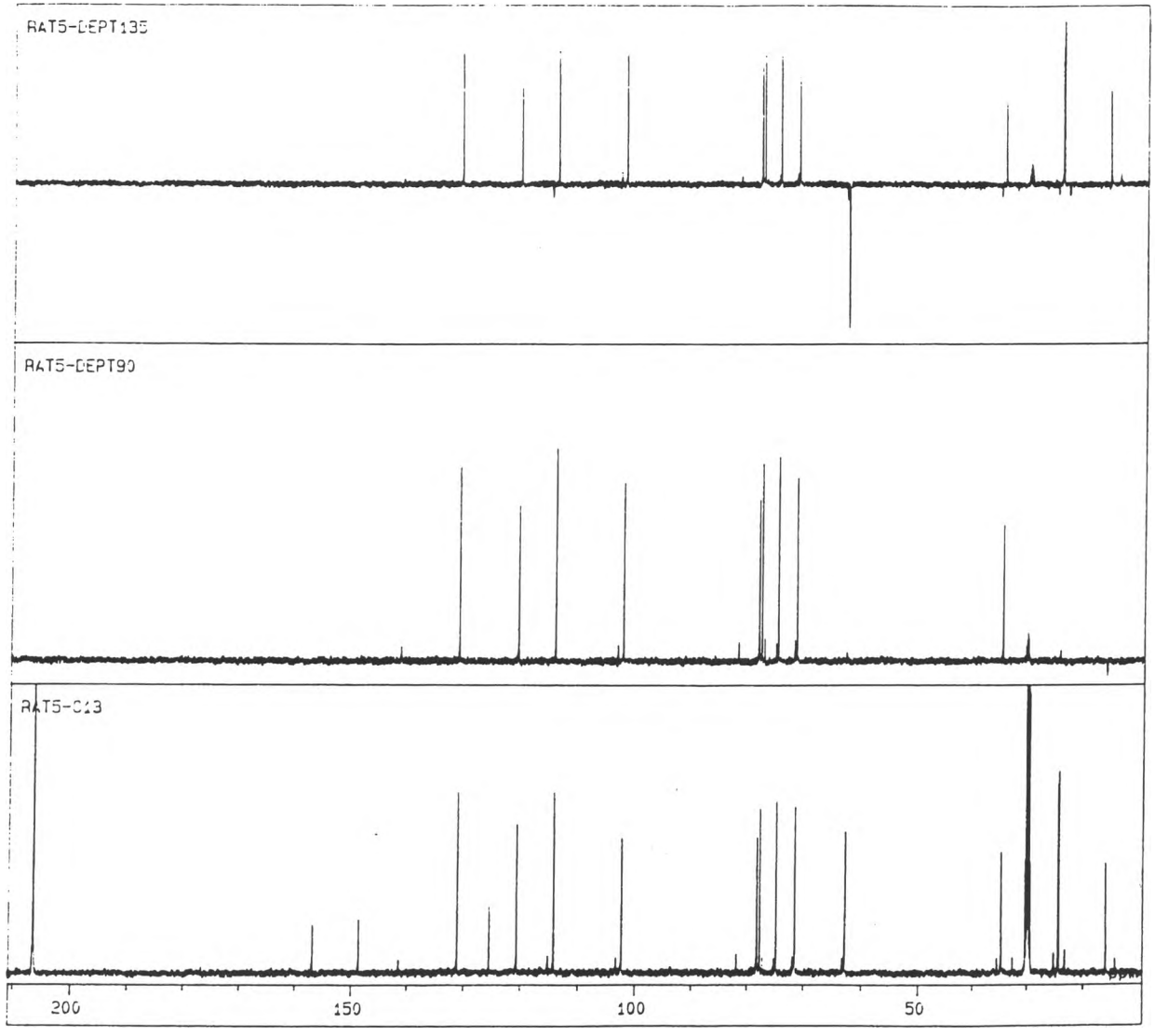
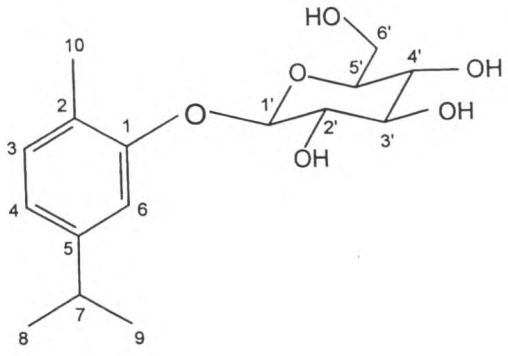


Figure 47. The ^{13}C DEPT spectrum of RAT 3 (in acetone-d_6)



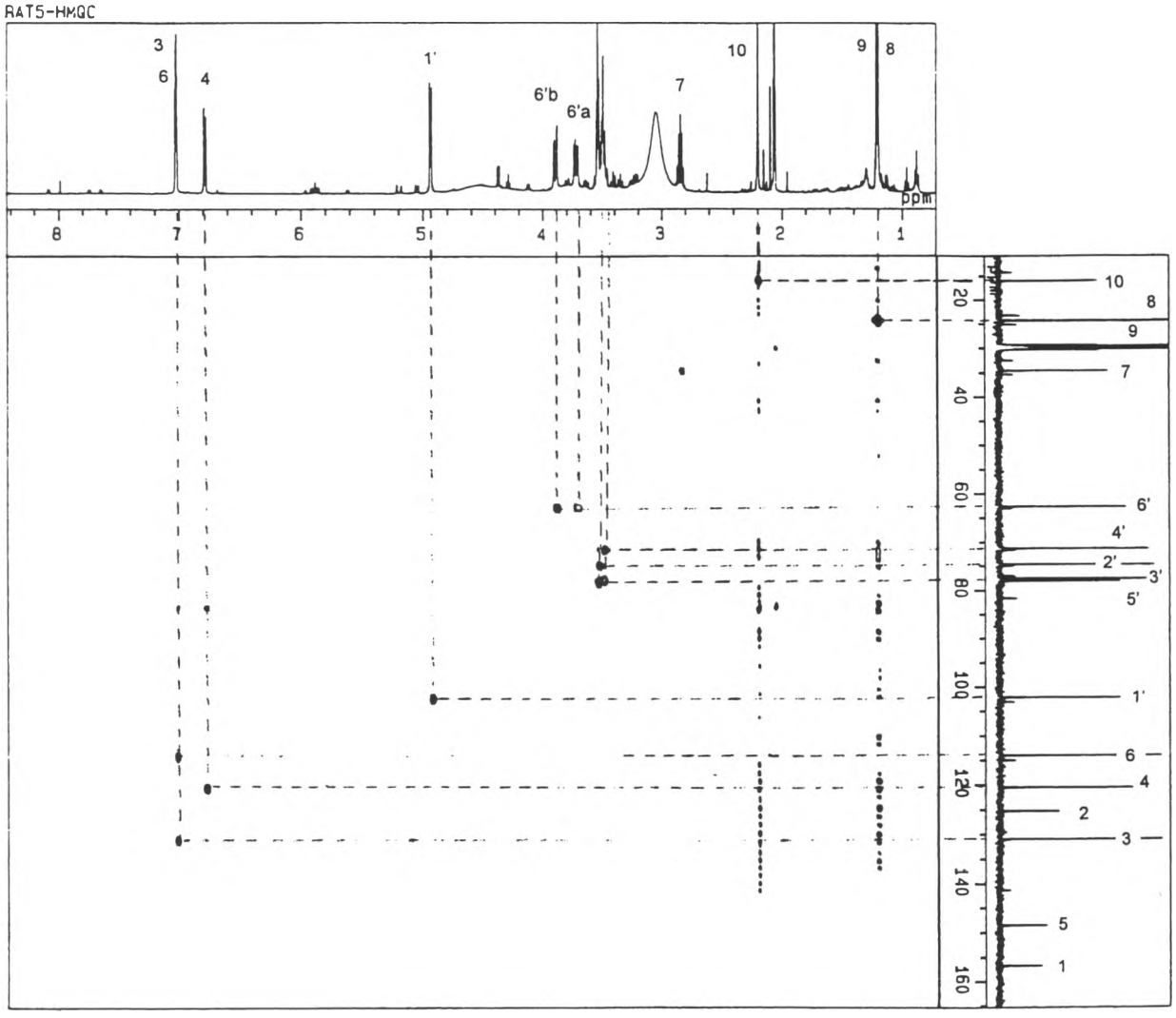


Figure 48. ^{1}H-^{13}C HETCOR spectrum of RAT 3 (in acetone-d_6)

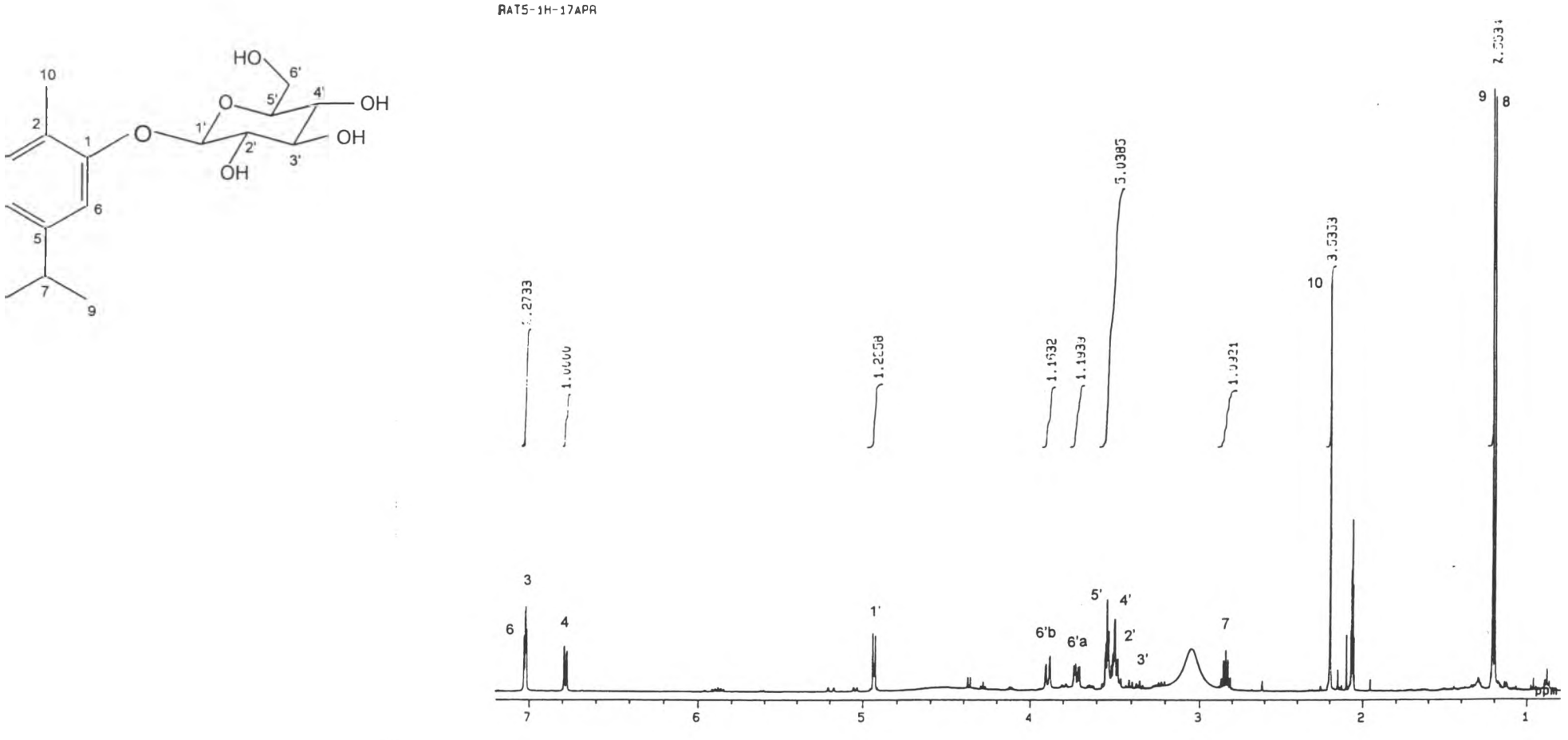


Figure 49. The 500 MHz ^{1}H NMR spectrum of RAT 3 (in acetone-d_6)

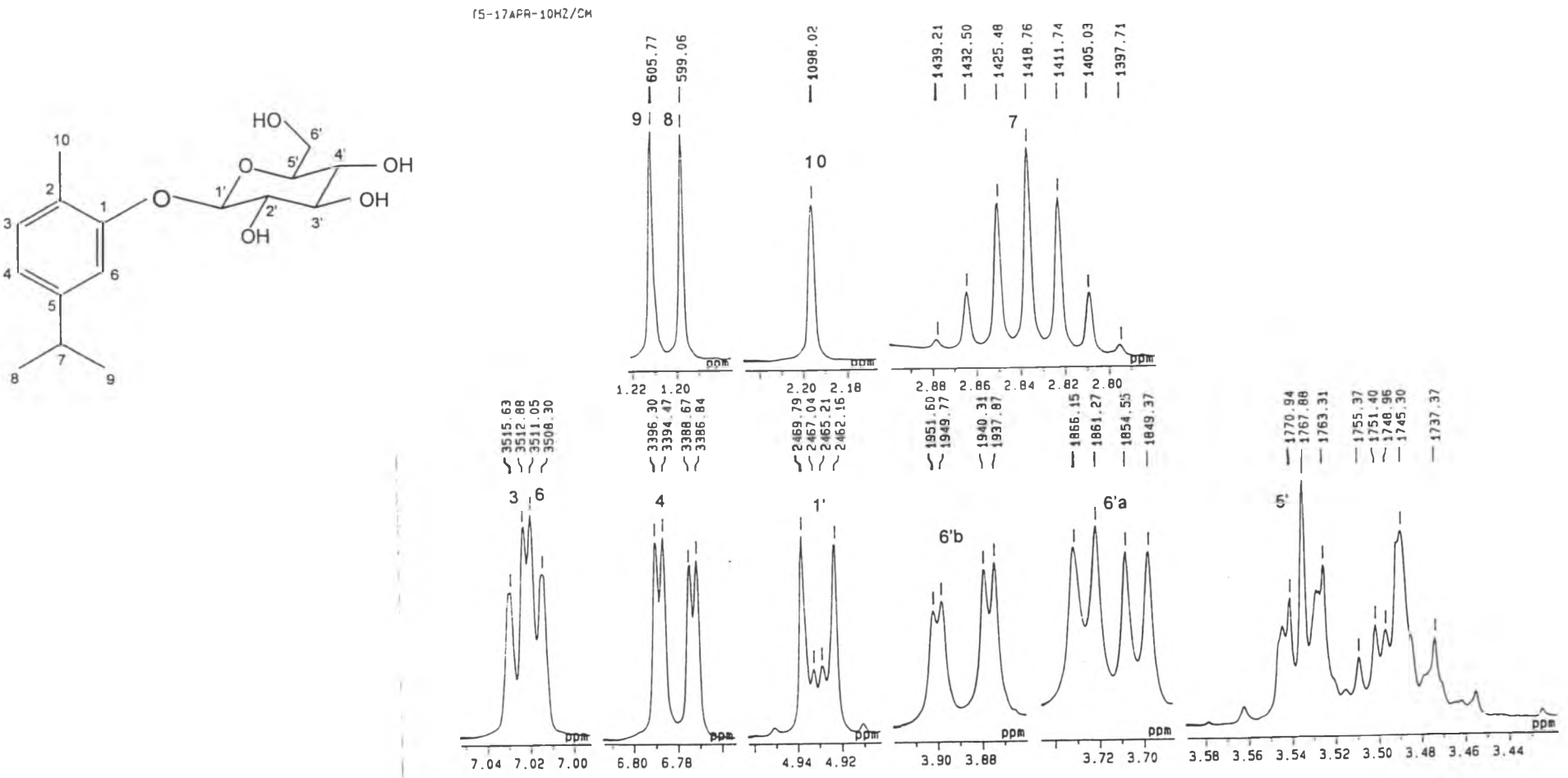
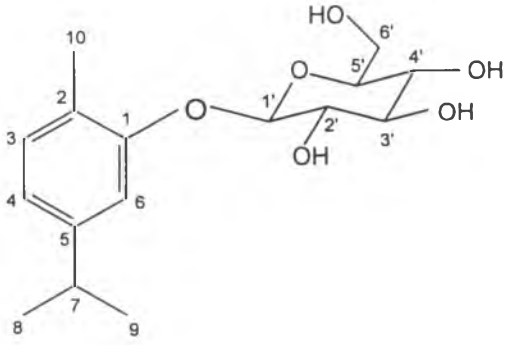


Figure 50. The 500 MHz ^{1}H NMR spectrum of RAT 3 (in acetone-d_6)

(expanded in the range of δ 1.20-7.04 ppm)



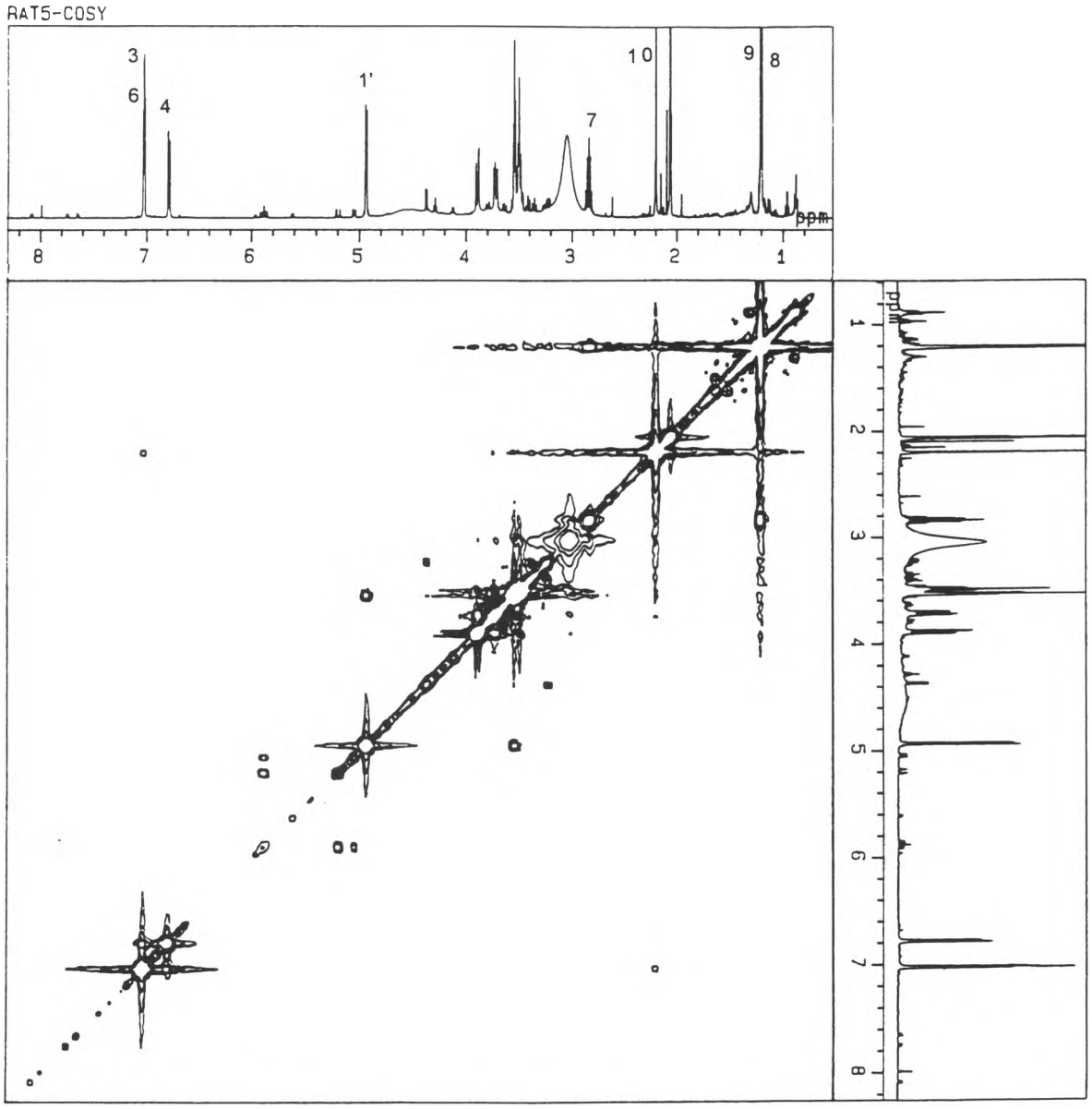
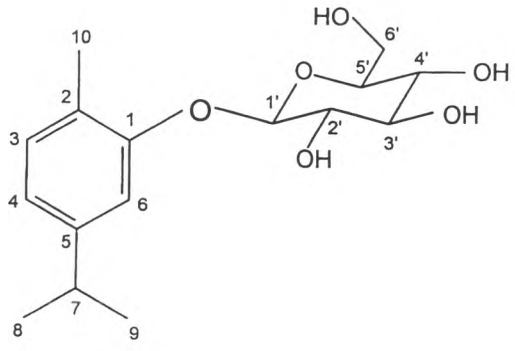


Figure 51. ^{1}H-^{1}H COSY spectrum of RAT 3 (in acetone-d_6)



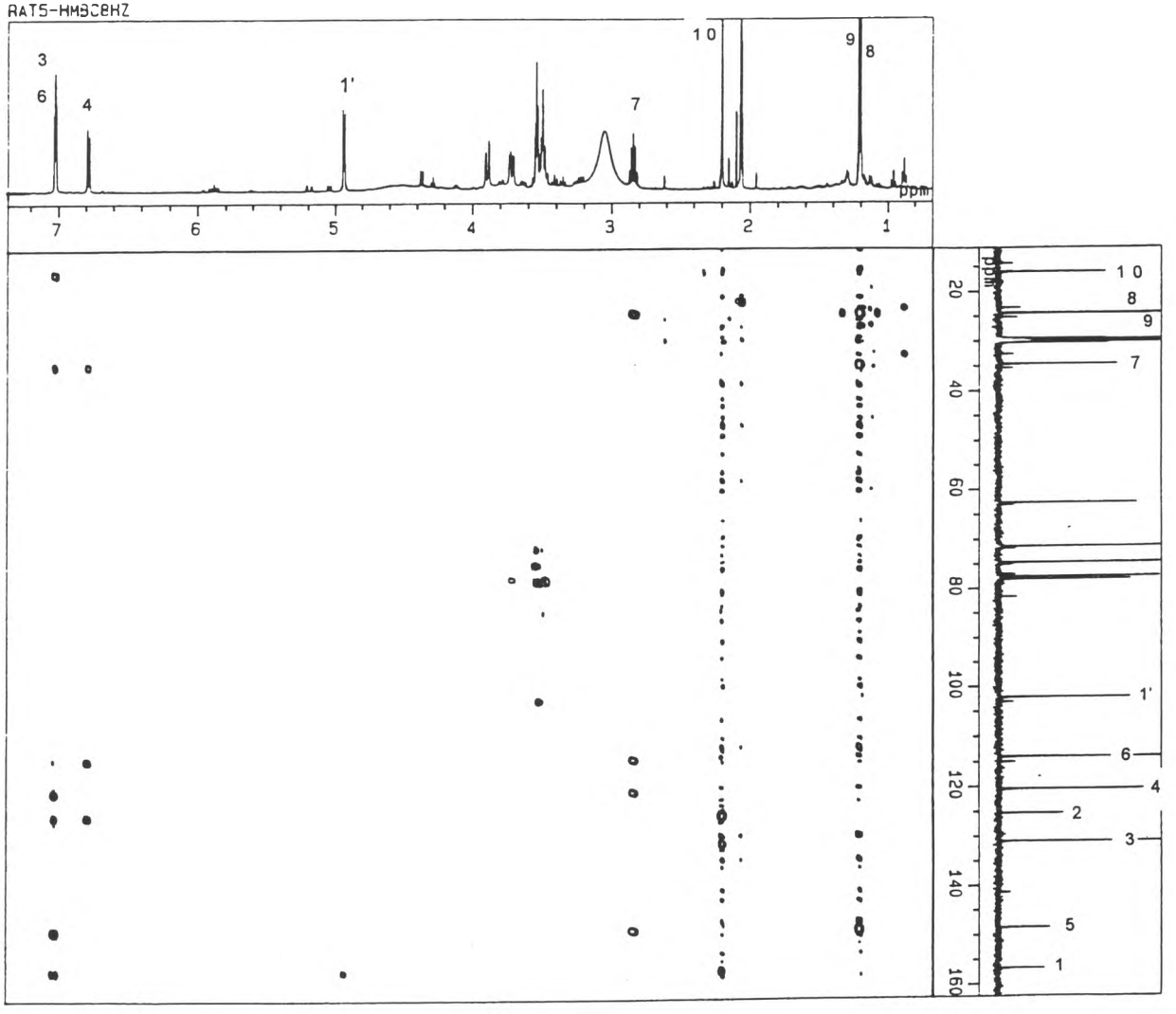


Figure 52. ^{1}H-^{13}C HMBC spectrum of RAT 3 (in acetone-d_6)

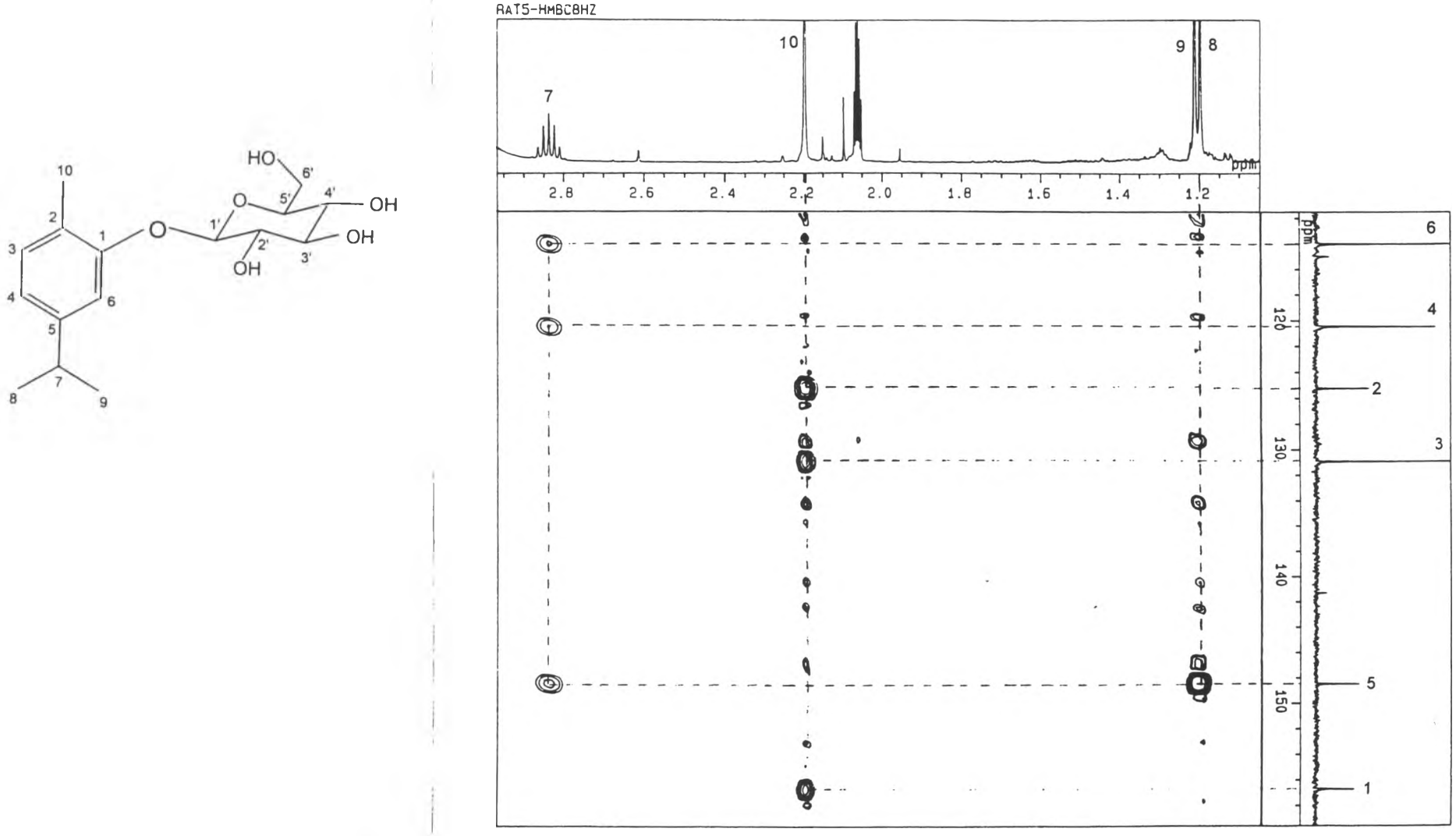


Figure 53. ^{1}H-^{13}C HMBC spectrum of RAT 3 (in acetone-d_6)

(expanded in the range of δ ^{1}H 1.1-2.9 ppm and δ ^{13}C 112-158 ppm)

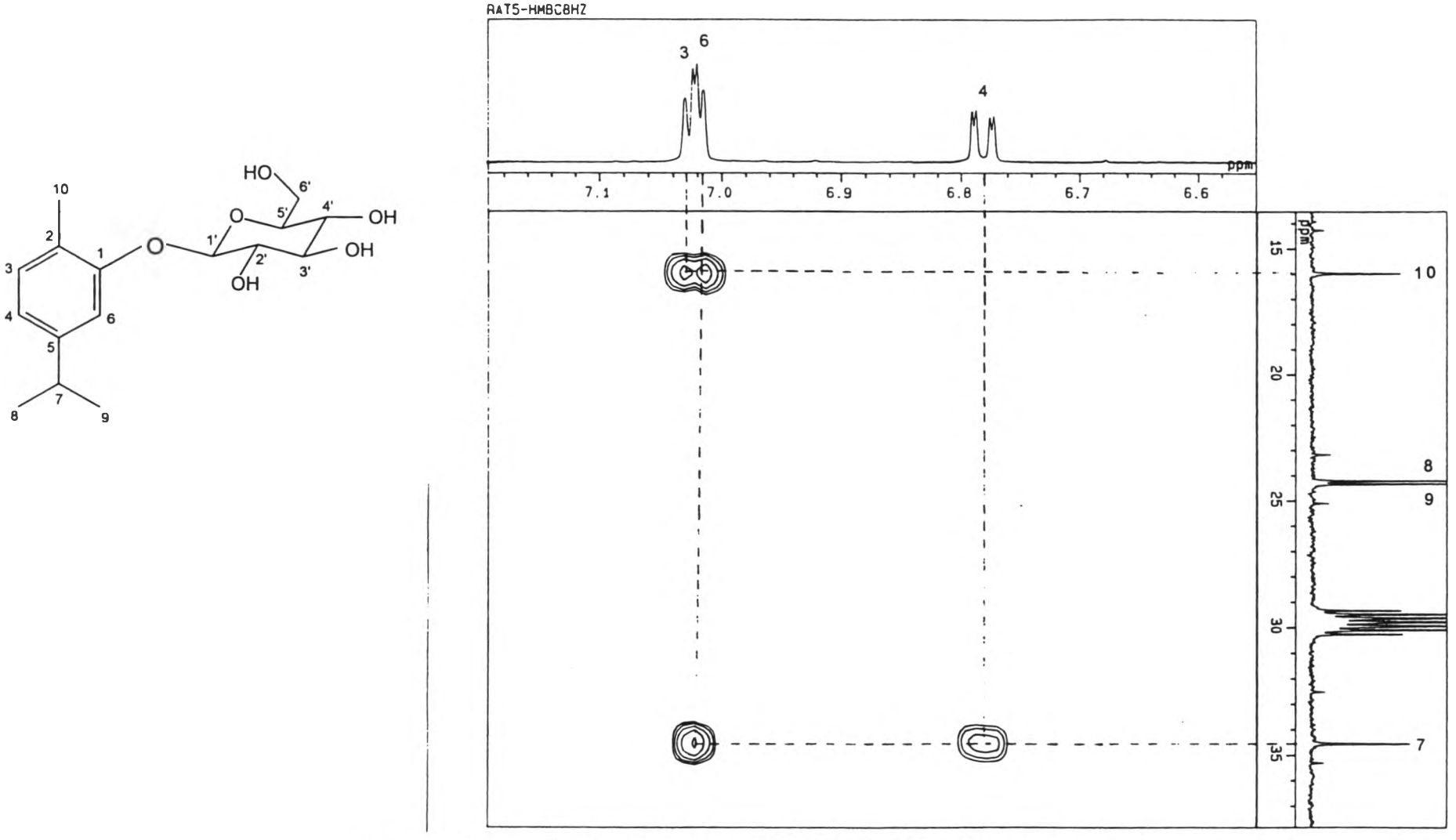


Figure 54. ^{1}H-^{13}C HMBC spectrum of RAT 3 (in acetone-d_6)

(expanded in the range of δ ^{1}H 6.6-7.1ppm and δ ^{13}C 14-37 ppm)

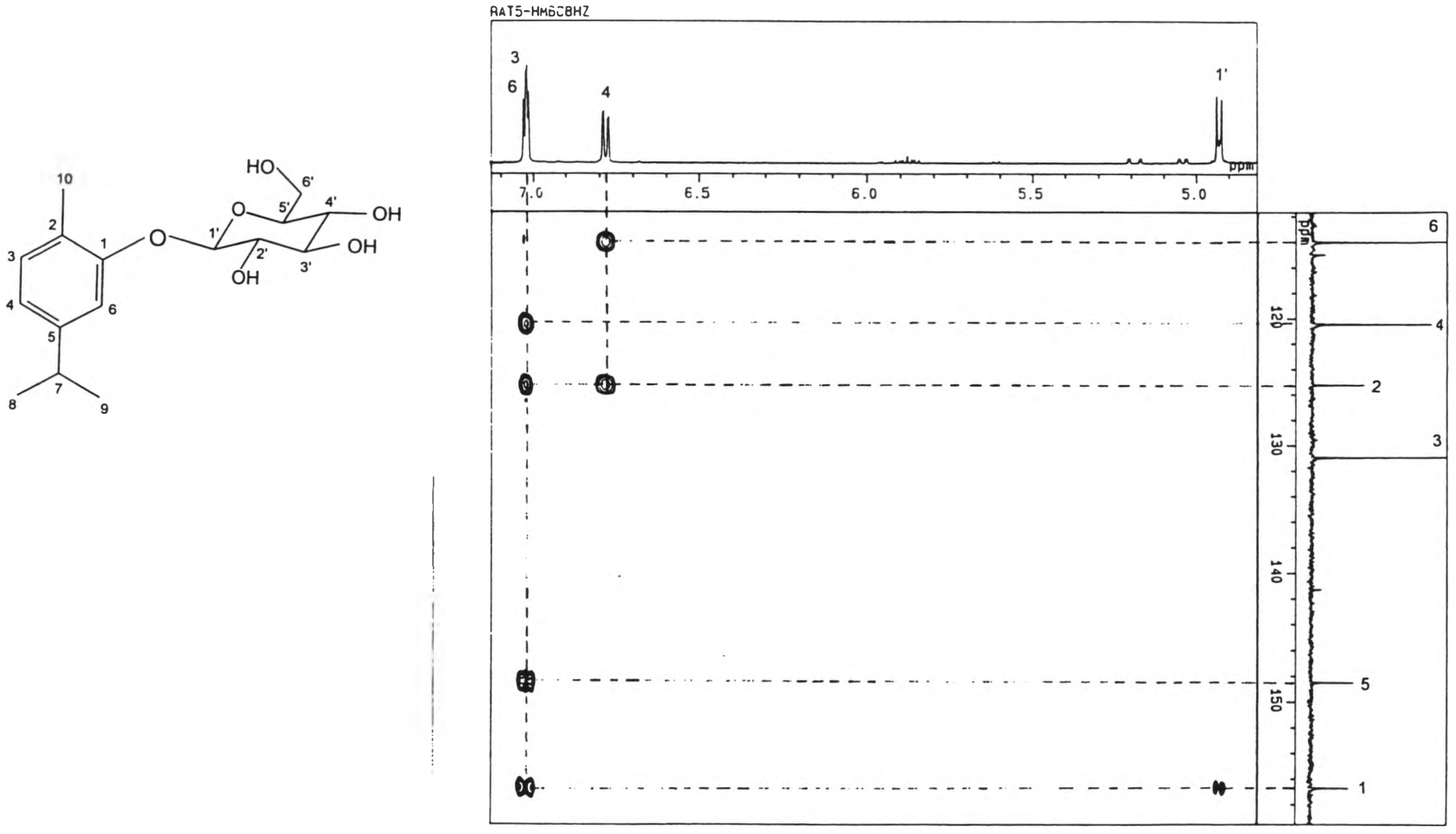


Figure 55. ^{1}H-^{13}C HMBC spectrum of RAT 3 (in acetone-d_6)

(expanded in the range of δ ^{1}H 4.9-7.1 ppm and δ ^{13}C 112-158 ppm)

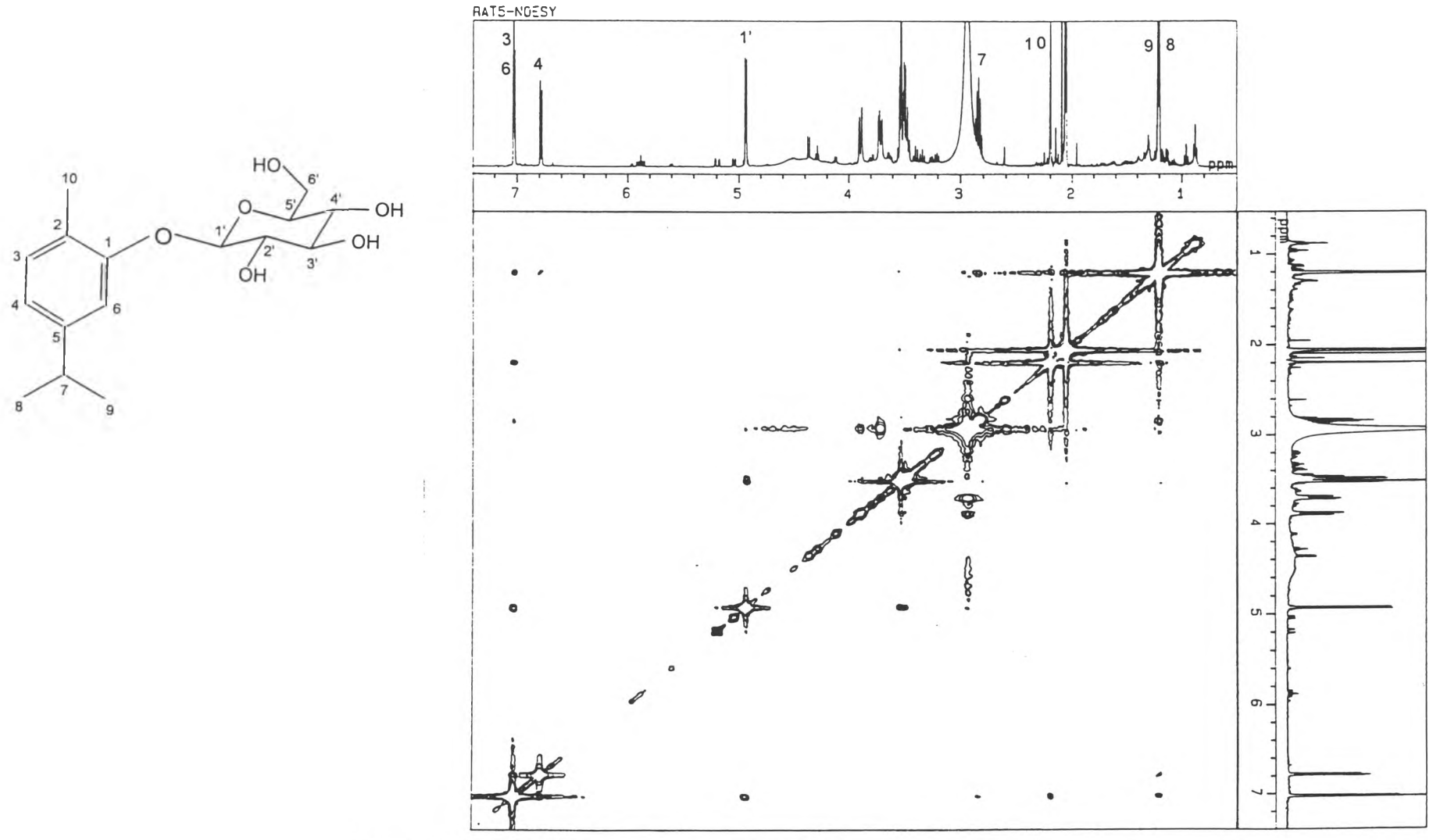


Figure 57. NOESY spectrum of RAT 3 (in acetone-d_6)