CHAPTER IV

RESULT AND DISCUSSION

Dried stem bark of *Moringa pterygosperma* Gaertn(7.5 kg). was macerated with ethanol. The ethanol extract was partitioned by the process shown in previous chapter (page27) Structure elucidation of the compounds isolated from methanol extract will be discussed as follow.

Structure elucidation of the isolated compounds

1. Structure elucidation of PPT8

Compound PPT8 appeared as white flakes that gave violet-pink colour with Liebermann-Burchard reagent. The IR spectrum of PPT8 (Figure 6) showed the pattern of the functional group as Table 2.

Table 2 The IR spectrum assignment of PPT8.

Range of absorption (cm^{-1})	Assignment
3600 - 3100 (broad, strong)	O-H stretching
3050 - 2800 (strong)	C-H stretching
1640 (weak)	C=C stretching
1480 (medium)	C-H bending of CH_3, CH_2
1080 (strong)	C-O stretching
800 (weak)	C-H bending out of plane

From above implied that PPT8 was steroid. The ^{13}C NMR spectrum of PPT8 was shown in Figure 10 and its chemical shifts (Iribarren, and Pomilio.,1983) is shown in table 3.

Table 3 The ^{13}C NMR chemical shifts (in ppm) of β-sitosterol glucoside and PPT8 in pyridine-d_5.

No. of carbon	β-sitosterol glucoside	PPT8
C-1′	102.6	102.6
C-2′	75.3	75.3
C-3′	78.5[c]	78.5
C-4′	71.7	71.7
C-5′	78.6[c]	78.6
C-6′	62.9	62.9
C-1	37.6	37.5
C-2	30.4	30.3
C-3	78.1	78.2
C-4	40.0	40.0
C-5	140.8	141.0
C-6	121.9	121.9
C-7	32.2	32.1
C-8	32.2	32.2
C-9	50.4	50.4
C-10	37.0	37.0
C-11	21.4	21.3
C-12	39.4	39.4
C-13	42.6	42.5
C-14	56.9	56.9
C-15	24.6	24.5
C-16	28.6	28.6
C-17	56.3	56.3

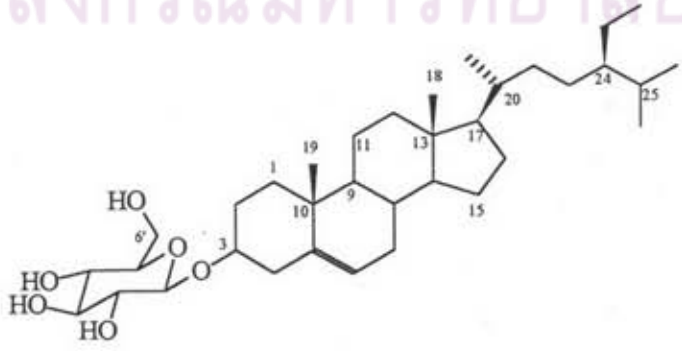
Table 3 (continued) The ^{13}C NMR chemical shifts (in ppm) of β-sitosterol glucoside and PPT8 in pyridine-d_5.

No. of carbon	β-sitosterol glucoside	PPT8
C-18	12.1 [a]	12.0
C-19	19.5	19.4
C-20	36.5	36.4
C-21	19.1 [b]	19.0
C-22	34.3	34.3
C-23	26.5	26.5
C-24	46.1	46.5
C-25	29.6	29.5
C-26	19.3 [b]	19.2
C-27	20.1	20.0
C-28	23.5	23.4
C-29	12.3 [a]	12.2

Note : [a,b,c] These assigments may be interchanged

The EIMS spectrum of PPT8 , shown in Figure 5,exhibited the molecular ion peak at m/z = 577. And when it was loss of sugar part , then showed the ion peak at m/z=398.

All data indicated that PPT8 was sitosterol 3-*O*-β-D-glucoside.



2. Structure elucidation of J1

J1 was obtained as sticky yellow compound and it was deliquescent when exposed to air. The IR spectrum of J1 (Figure 12) showed the pattern of the functional group as Table 4.

Table 4 The IR spectrum assignment of J1

Range of absorption (cm^{-1})	Assignment
3700-3100 (broad, strong)	O-H and N-H stretching
3000,2980 (medium)	C-H stretching
1700, 1690 (medium)	C=S and C=N stretching
1620 (medium)	C=C stretching
1550, 1540 (strong)	C=C aromatic and C=S stretching
1400 (medium)	O-H in plane bending
1150 (strong)	C-N stretching
1040 (strong)	C-O-C stretching
900 (weak)	O-H bending out of plane
830 (medium)	para substituted of aromatic benzene

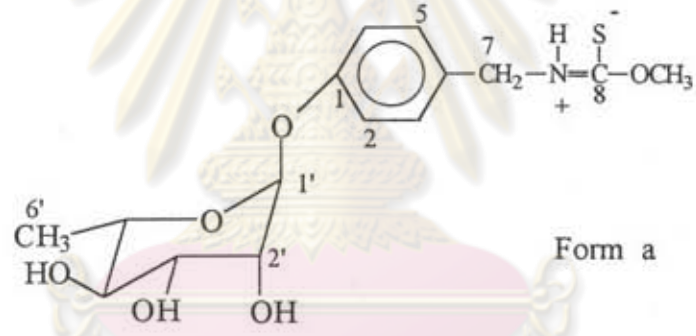
J1 were isolated by means of chromatographic and gel filtration technique then was examined by TLC using 4 solvent systems it was showed only single spot in TLC silica gel plate.

But ^{1}H-NMR spectrum (Figure 13-16) showed J1 was a mixture. That showed only column chromatography and gel filtration technique was not able to separate any of the mixtures since they were all expected to have the nearly identical physical property.

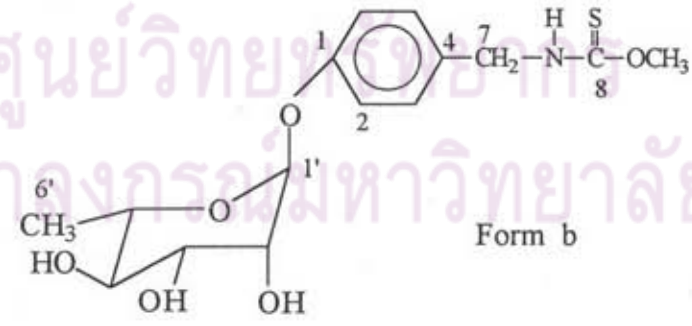
IR spectrum showed the characteristic of many functional groups; -OH, -NH, -CS, -CN and para substituted benzene. These were guidelines to elucidate this mixture.

Furthermore Shaheen Faizi and his collaborators had published their researches in 1992, 1994 and 1995. Their works about thiocarbamate glycoside and cyanogenic glycoside were valuable suggestion.

For their researches, the first structure that proposed was niazinin A. This compound had two tautomers. They are {[[4-[(6-deoxy-α-L-manopyranosyl)oxy] phenyl]methyl]-*O*-methyland Carbonimidothioate(*E*)} ; Form a and Carbamothioate (*E*);Form b



Form a



Form b

The proton assignments of niazinin A and J1 are shown in Table 5. (Faizi, S., et al., 1992)

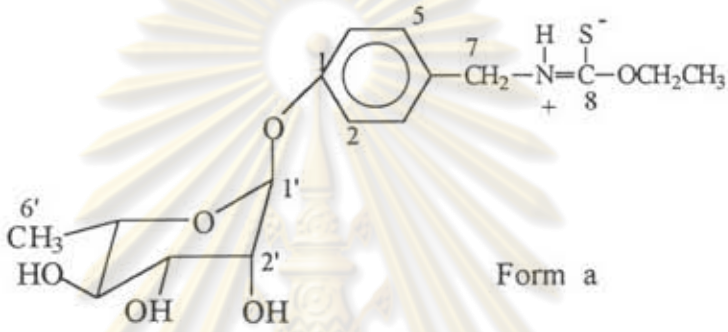
Table 5 ^{1}H NMR data for niazinin A and J1; coupling constants (*J*/Hz) are in parenthesis.

Position	niazinin A		J1	
	a	b	a	b
2-, 6-H	6.97 d (8.6)	6.96 d (8.6)	6.98 d(8.6)	6.98 d (8.6)[1]
3-, 5-H	7.21 d (8.6)	7.18 d (8.6)	7.21 d (?.?)[1]	7.16 d (8.6)
7-H	4.56 d (6.1)	4.17 d (5.7)	4.57 d (6.1)	4.24 d (6.4)
OMe	3.87 s	3.89 s	3.87 s	3.89 s
1′-H	5.33 d (1.8)		5.34 d (1.2?)[1]	
2′-H	3.81 ddd (3.9, 2.9, 1.8)		3.82 m	
3′-H	3.63 ddd (9.2, 5.9, 2.9)		3.65 ddd (9.2, 6.1, 3.0)	
4′-H	3.26 dt (9.2, 5.6)		3.29 dt (9.2, 5.8)	
5′-H	3.46 qd (9.2, 6.1)		3.47qd (9.2, 6.1)[1]	
6′-H_3	1.09 d (6.1)		1.09 d (6.1)[1]	
2′-OH	4.99 d (3.9)		5.02 d (4.6)	
3′-OH	4.66 d (5.9)		4.71 d (6.1)	
4′-OH	4.82 d (5.6)		4.85 d (5.8)	
NH	9.55 t (6.1)		9.56 t (6.1)	9.60 t (6.4)

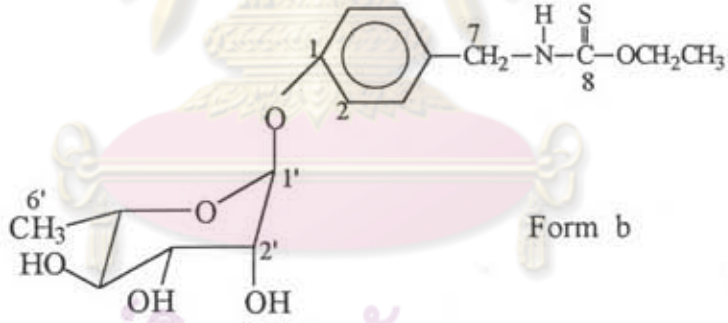
Note : [1] This peak had overlapped. It caused approximate value.

And niazinin A,was E-isomer also exist along the ester bond which cause minor methoxy singlet to appear. It occurs in the ester with larger alkyl groups the terminal methyls, far removed from the nitrogenthiocarbonyl bond, still gave separate signals for each isomer, although not in all solvent. (Bauman, R.A., 1967).And the minor methoxy singlets were present at δ 3.54 and 3.56 ppm on report discussion,but this study was noted at δ 3.531 ppm only. Both structural evident were in DMSO -d_6. Niazinin A from the 300 MHz but J1 from the 500 MHz.

Beside niazinin A, the J1 spectrum has an additional methylene group which could be rationalized as a methylene of an ethoxy group from the ^{1}H NMR spectrum which further inidcated that other compound in J1 besides niazinin A was niacimicin. And liked niazinin A, it had two tautomers ; {[[4-[(6-deoxy-α-L-manopyranosyl)oxy] phenyl]methyl]-*O*-ethyl Carbonimidothioate(*E*)} ; Form a and Carbamothioate(*E*); Form b



Form a



Form b

The proton assignments of niacimicin and J1 are shown in Table 6. (Faizi, S., et al., 1992)

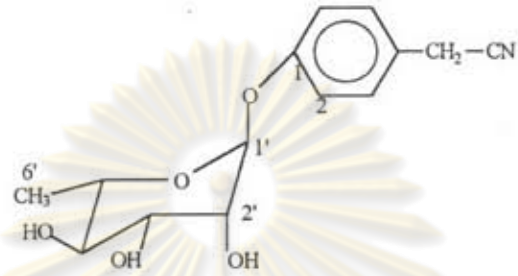
Table 6 ^{1}H NMR data for niacimicin and J1; coupling constants (*J*/Hz) are in parenthesis.

Position	niazimicin		J1	
	a	b	a	b
2-, 6-H	6.97 d (8.6)	6.98 d (8.6)	6.98 d (8.6)	6.986 d (8.6)
3-, 5-H	7.21 d (8.6)	7.16 d (8.6)	7.21 d (?.?)[1]	7.160 d (8.6)
7-H	4.55 d (6.0)	4.22 d (6.0)	4.56 d (6.1)	4.245 d (6.4)
$O\underline{CH_2}CH_3$	4.38 q (7.0)	4.37 q (7.0)	4.39 q (7.0)	4.381 q (7.0)
$OCH_2\underline{CH_3}$	1.24 t (7.0)	1.22 t (7.0)	1.24 t (7.0)	1.19 t (7.0)
1′-H	5.33 d (1.9)		5.34 d (1.2?)[1]	
2′-H	3.80 ddd (4.3, 3.4, 1.9)		3.82 m	
3′-H	3.63 ddd (9.2, 5.9, 3.4)		3.65 ddd (9.2, 6.1, 3.0)	
4′-H	3.27 dt (9.2, 5.6)		3.29 dt (9.2, 5.8)	
5′-H	3.48 qd (9.2, 6.1)		3.48 qd (9.2, 6.1)	
6′-H_3	1.09 d (6.1)		1.09 d (6.1)	
2′-OH	5.01 d (4.3)		5.03 d (4.6)	
3′-OH	4.69 d (5.9)		4.71 d (6.1)	
4′-OH	4.84 d (5.6)		4.85 d (5.8)	
NH	9.52 t (6.1)		9.52 t (6.1)	9.54 t (6.4)[1]

Note : [1] This peak had overlapped. It caused approximate value.

Because of E-isomer also exist along the ester bond it makes minor conformers of tautomer. Like niazinin A case, -$O\underline{CH_2}CH_3$ minor conformers were present in weak quartets at δ 4.00 (*J*=7.1) and 4.09 (*J*=7.1) on report discussion, but this study was noted at δ 3.98 quartet (*J*=7.0) only. The complementary pair of triplets for the ethoxy methyl protons was hidden under the methyl signals of the major contributors.

If note the ^{1}H NMR spectrum on the sugar region (δ 4.70-5.38) a para disubstituted benzene region (δ 7.02-7.28) and methylene proton of benzyl part (δ 3.92) will see weak signal, but its relative of coupling constant and chemical shift guide to the structure of are 4-[(6-deoxy-α-L-manopyranosyl)oxy] Benzeneacetonitrile; niazirin.



The proton assignments of niazirin A and J1 are shown in Table 7. (Faizi, S., et al., 1994)

Table 7 ^{1}H NMR data for niazirin and J1; coupling constants (*J*/Hz) are in parenthesis.

Position	niazirin	J1
2-, 6-H	7.04 d (8.8)	7.04 d (8.6)
3-, 5-H	7.26 d (8.8)	7.26 d (8.6)
7-H	3.94 s	3.93 s
1′-H	5.37 d (1.8)	5.37 d (1.8)
2′-H	3.81 m	3.82 m[2]
3′-H	3.62 ddd (9.2, 5.9, 3.2)	3.64 ddd (9.2, 6.1, 3.0)[2]
4′-H	3.54 t (9.1)	3.29 t (9.2)[2]
5′-H	3.75 m	3.48 m[2]
6′-CH_3	1.27 d (6.2)	1.09 d (6.1)[2]
2′-OH	5.01 d (4.3)	5.03 d (4.3)
3′-OH	4.69 d (5.9)	4.73 d (??)[1]
4′-OH	4.84 d (5.6)	4.86 d (??)[1]

Note : [1] This peak had overlapped. It caused approximate value.

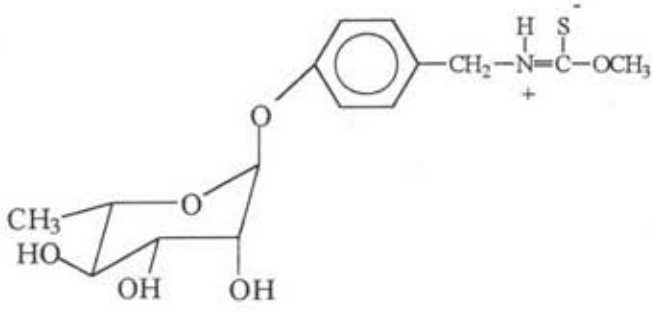
[2] This peak buried in the major peak.

Besides ^{1}H NMR spectrum which assigned proton of these structures, J1 has ^{13}C NMR spectra; proton noise decoupled and DEPT. The carbon assignments of J1 are shown in Table 8.

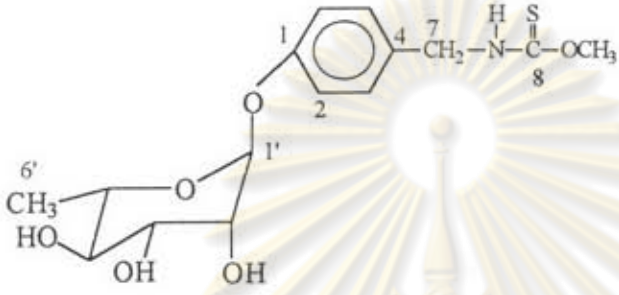
Table 8 ^{13}C NMR chemical shift data for niazinin A (1), niacimicin (2), niazirin (3) and J1.

Carbon	1a	1b	2a	2b	3	J1
1	154.88	155.01	155.00	-	156.03	155.12, 155.25, 155.51
2,6	116.29	-	116.08	116.13	116.65	116.33, 116.92
3,5	128.50	128.64	128.46	-	129.23	128.25,128.65,128.76, 129.34
4	132.29	132.71	131.18	131.36	132.64	131.36,131.46,131.51, 131.63
7	47.40	41.52	47.96	44.88	22.90	21.67,45.18,47.23,47.50, 47.63
8	190.72	-	189.84	187.81	123.72	124.39,187.99,190.08, 190.93
1′	98.49		98.31		97.96	98.40,98.54
2′	70.14		69.96		70.87	70.25
3′	70.42		70.25		71.70	70.51
4′	71.78		71.60		73.48	71.84
5′	69.34		69.17		68.83	69.47
6′	17.78		17.59		17.49	17.80,17.88,17.98
OCH_3	56.43	57.10	-		-	56.56,57.32
$O\underline{CH_2}CH_3$	-		65.06	65.86	-	65.33,66.20
$OCH_2\underline{CH_3}$	-		13.97	13.77	-	14.07,14.26

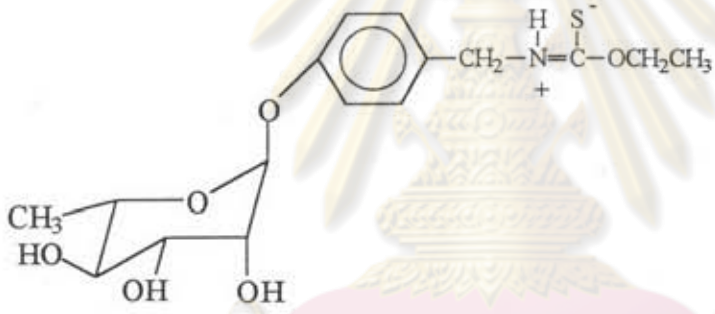
Note : All sample used DMSO-d_6 as solvent except 3 used $CDCl_3$.



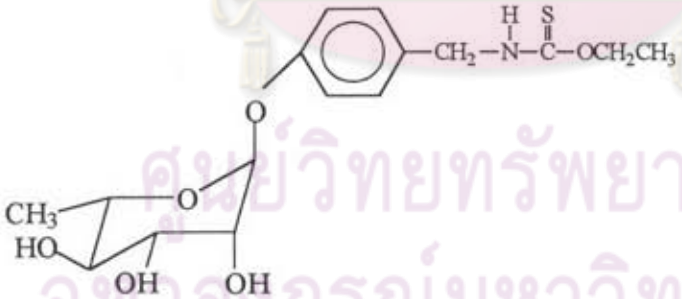
niazinin A form a



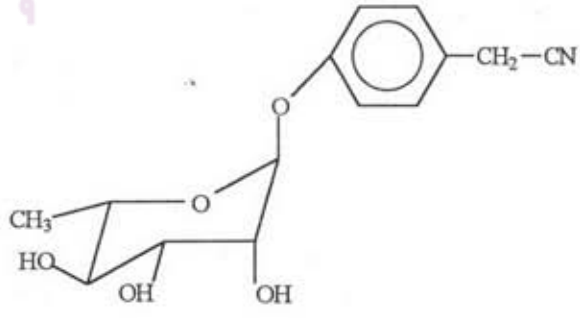
niazinin A form b



niacimicin form a



niacimicin form b



niazirin

The EIMS spectrum of pure mixture is shown in Figure 19. The peak at m/z 279 represent the molecular ion of niazirin. Loss of deoxyrhamnose (m/z 147) from the molecular ion yields a 4-hydroxy-benzylcyanide (m/e 133). Loss of a neutral molecule of HCN gives the prominent peak at m/z 91 ($C_6H_5CH_2^+$) is indicative of an alkyl substituted benzene ring that each have in three proposed structure compound. Like the case of peak at m/z 77 which the peak of phenyl cation ($C_6H_5^+$).

The intense peak at m/z 107 is indicative of an -O-C_6H_5-CH_2-part which in all three proposed structure compound. But the peak at m/z 182 is not fragment peak of niazirin but for niazinin A and niazimicin. Because it is HO-C_6H_4-CH_2-NH=CS-O- which not contain in the molecule of niazirin.

The reversed phase high performance liquid chromatogram (figure 20) showed one minor and two major peaks. The minor peak was unable to collect. The two major peak were collecteded in order to confirm the proposed molecular structure.

The EIMS spectra of the second peak of HPLC chromatogram was niazininA (Figure 21) and the second peak was niazimicin (Figure 22). Figure 23-25 showed the fragmentation pathway of niazirin, niazirinin A and niazimicin, respectively.

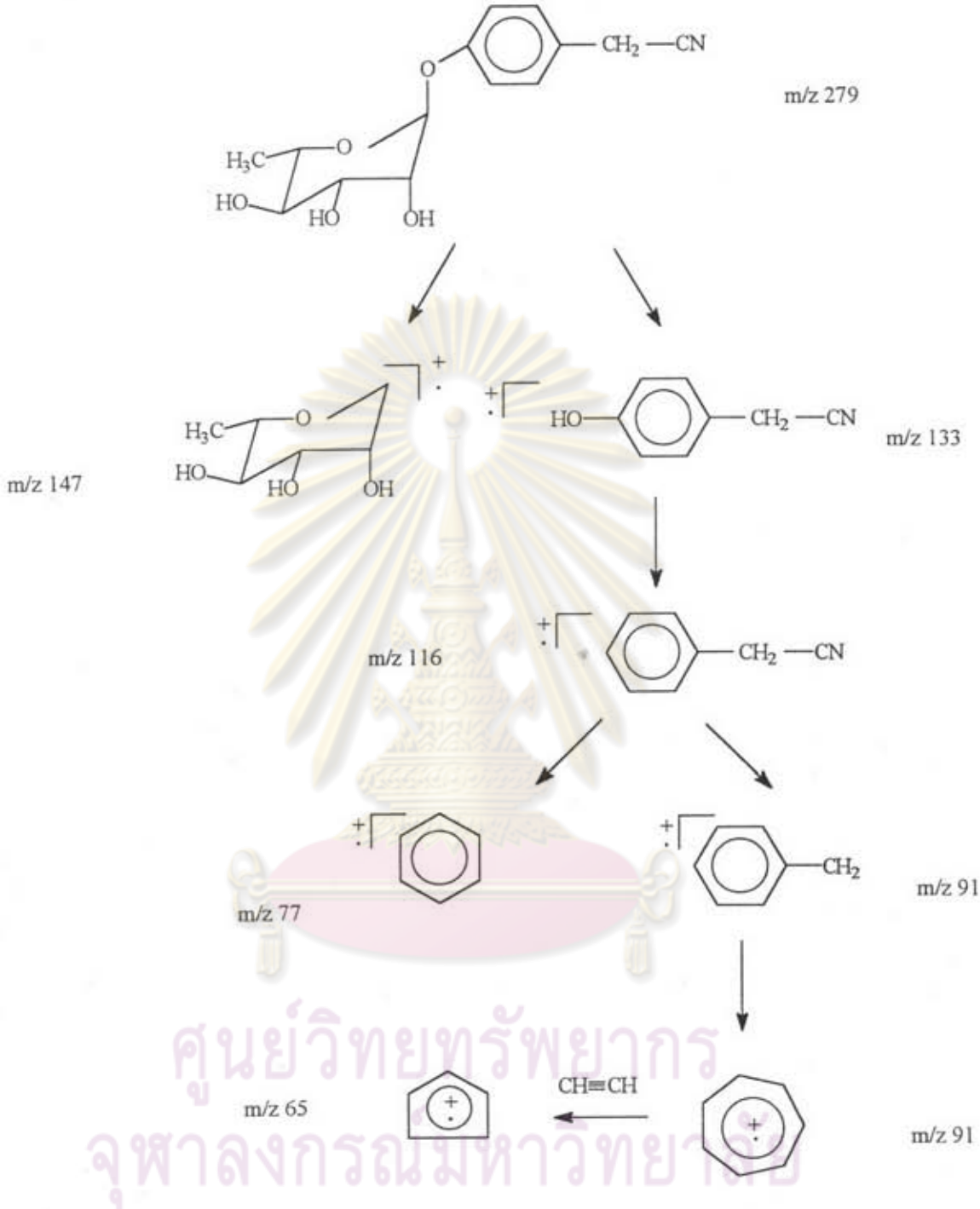


Figure 23 Proposed mass fragmentations of niazirin.

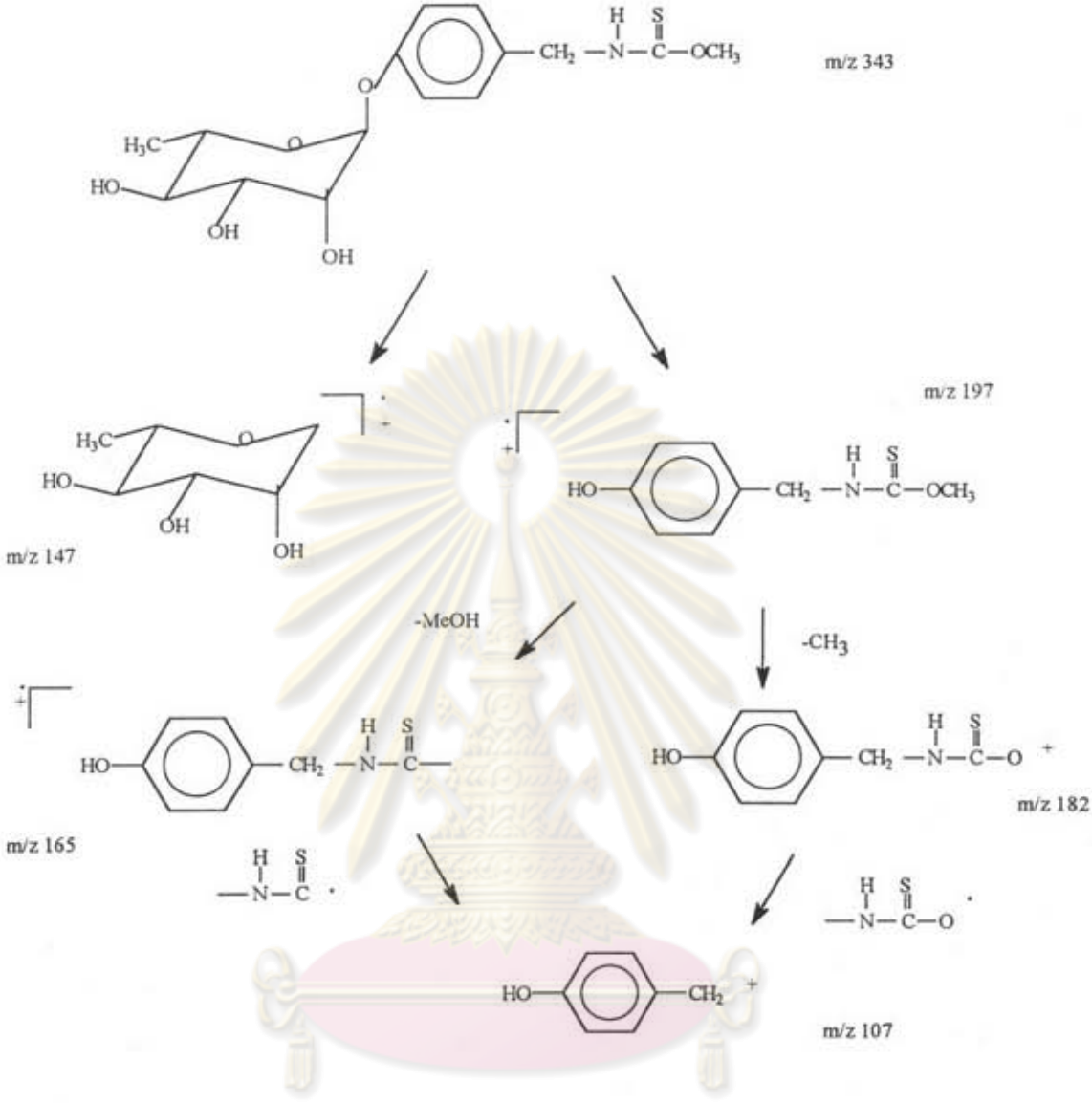


Figure 24 Proposed mass fragmentations of niazinin A.

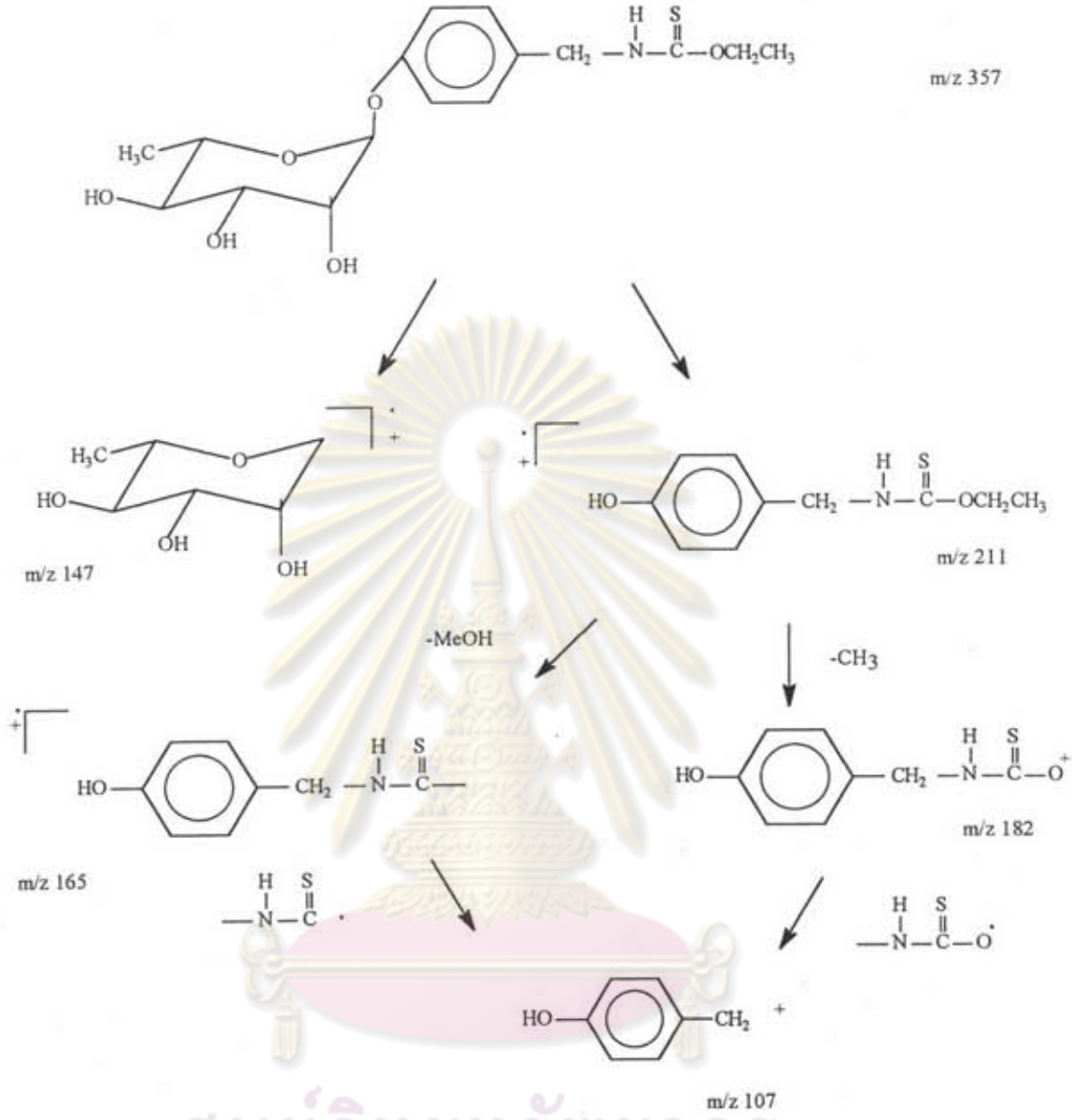


Figure 25 Proposed mass fragmentations of niazimicin.

^{1}H NMR spectrum showed signal of NH proton (δ 9.49-9.61 ppm) separate to four signals but in cited reference had two signals. In both the hybrids, the NH proton appeared at δ 9.56 and 9.516 ppm for form a of niazininA and niazimicin, respectively. That implies NH proton signals appearing at δ 9.60 and 9.54 ppm to be those of form b of these respective compounds.

To confirm those four -NH- triplet signals at chemical shift 9.49-9.61 ppm were correlatd with their neighbour protons. Nuclear Overhauser Effect (NOE) was the method to solve this problem. The NOE difference spectrum shows correlation among protons through space, by increasing intensity of proton signal which are correlated to the irradiated protons.

From the proposed structure, the methylene proton were near the -NH- proton then the methylene proton had selected to irradiated. The signal of methylene protons were complete isolated between a form and its tautomer, b form. It was simple to irradiate for noted the increase intensity of -NH- proton signal of each form.

At chemical shift 4.21-4.26 ppm was the signal of methylene protons of form b of both niazinin A and niazimicin, selected to irradiate at 4.23 ppm. The spectrum showed -NH- proton sinal of the b form increased and -NH- signal of methylene protons of form a of both compounds when selected to irradiated at 4.56 ppm,the-NH- proton signal of form a increased and -NH- signal of b form decreased.

Then this mode of NMR experient was confirmed the proposed structure, and confirmed the existence of all four -NH- in this spectrum.