CHAPTER III

RESULTS AND DISCUSSION

The Results of Extraction

Various parts of *X. granatum* such as fruits, branches, heartwoods, seeds and leaves were collected from Tum Bon Bang Ta Boon, Phetchaburi province, Thailand during November 1994. Each specimen was extracted for preliminarily biological screening test according to the procedure described in Chapter II. The results of extraction are shown in Table 3.1.

Table 3.1 The results of extraction of several parts of *X. granatum*.

Plant parts	Solvent	Weight (g) and Percentage (% wt/wt)
branches (1 kg)	dichloromethane	12.5 (1.25)
heartwoods (1.5 kg)	dichloromethane	18.1 (1.21)
fruits (1 kg)	dichloromethane	17.6 (1.76)
	95% ethanol	147.3 (14.73)
seeds (1 kg)	dichloromethane	40.2 (4.02)
	95% ethanol	136.4 (13.64)
leaves (1kg)	hexane	50.0 (5.00)
	dichloromethane	38.3 (3.83)
	95% ethanol	160.0 (16.00)

The Results of Biological Activity Screening Tests

Each crude extract of *X. granatum* as mentioned above was preliminarily screened for antifeedant activity against the Greater Wax Moth, *Galleria mellonella*. The results are shown in Table 3.2.

Table 3.2 The antifeedant activity of the crude extracts of *X. granatum* and the ethanolic crude extract of neem, *Azadirachta indica*, seeds against Greater Wax Moth, *Galleria mellonella*.

Entry	Plant part	Solvent	Antifeedant activity
1.	friuts	dichloromethane	+++
		butanol	+++
2.	seeds	dichloromethane	+++
		butanol	++
3.	heartwoods	dichloromethane	++
4.	branches	dichloromethane	++
5.	leaves	hexane	-
		dichloromethane	+
		butanol	-
6.	neem seeds	ethanol	+++

Note: +++ (71-100 %), ++ (41-70 %), + (11-40 %), - (no activity)

From the results of antifeedant preliminary screening test, the most potent one was the dichloromethane extract of fruits, seeds and the butanolic extract of fruits. The dichloromethane extract of heartwoods and leaves gave moderate antifeedant activity results and the others was found to be weak.

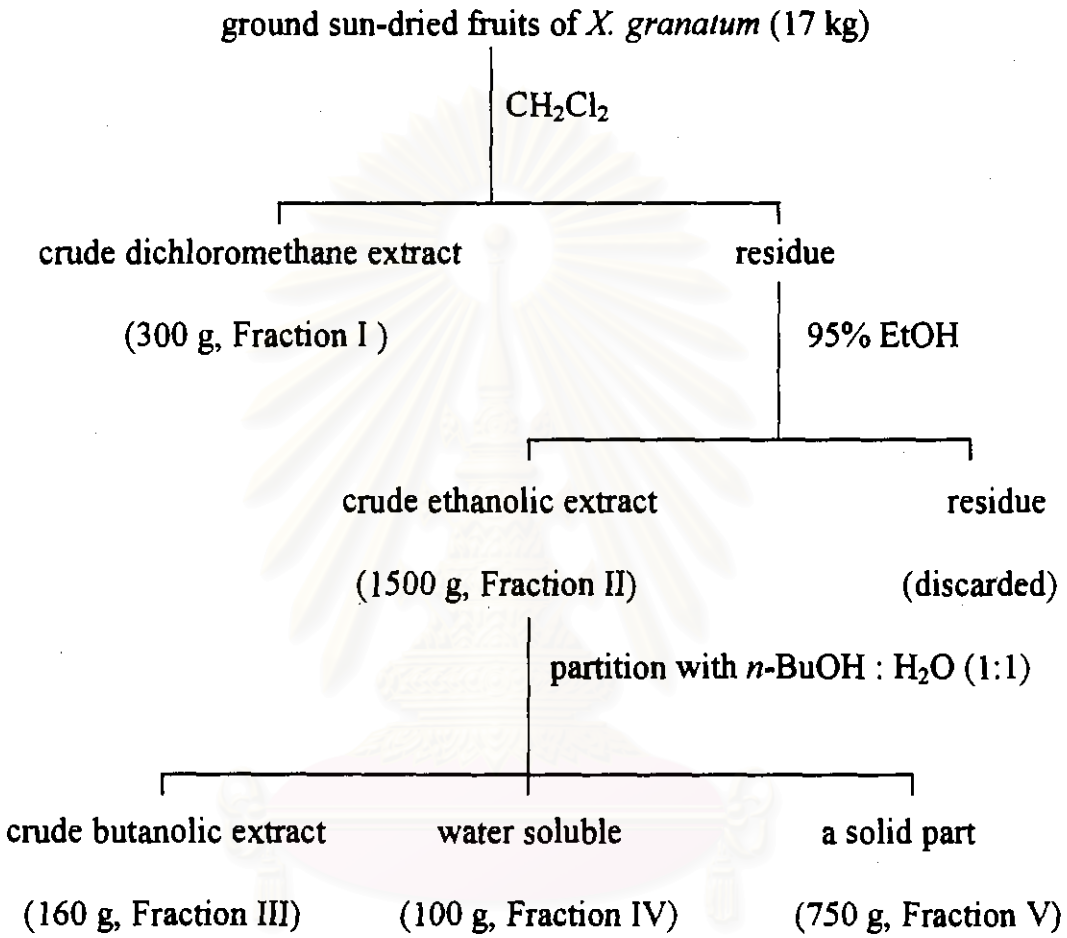
There was, however, no significantly different in antifeeding activity (with significant level 0.05) between the fruits and the seeds crude extract. Compared with the ethanolic extract of neem seeds, which has been long known to exhibit antifeedant activity[9], the crude extracts of fruits and seeds found to reveal the nearly potent antifeedant activity. Thus, the fruit and the seed crude extracts were selected for further examination.

Chemical Constituents of the Fruits of *X. granatum*

Extraction

The ground sun-dried fruits (17 kg) were extracted following the general extraction procedure as described in Chapter II. The crude dichloromethane extract as brown-yellow material, Fraction I, (300 g, 1.76 % wt. by wt. of dried fruits) and the dark brown ethanolic crude extract, Fraction II, (1500 g, 8.82 % wt. by wt.) were obtained. Fraction II was then partitioned with *n*-butanol and water (1:1) providing a butanolic fraction as red-brownish sticky material, Fraction III, (160 g, 0.94 % wt. by wt.), a water soluble fraction as a red-brownish crude, Fraction IV (100 g, 0.59 % wt. by wt.) and a solid part, Fraction V (750 g, 4.41% wt. by wt.). The results of extraction are summarized as shown in Scheme 3.1.

Scheme 3.1 The results of extraction and initial fractionations for the fruits of *X. granatum*



Separation

Separation of Fraction I

The crude dichloromethane extract (Fraction I) was roughly examined by TLC. The results revealed that there were at least four components in this crude (silica gel, solvent: 2% methanol in dichloromethane). This crude 70 g was then separated by column chromatography using silica gel as an adsorbent. The column was initially eluted with *n*-hexane and gradually changed to a mixture of dichloromethane and hexane, dichloromethane, a mixture of dichloromethane and methanol and finally was stripped with methanol. Eluting solvent was collected for each fraction approximately 800 mL and then concentrated to about 30 mL. Each one was investigated for the similarity by using TLC plate (silica gel). The equivalent fractions were combined. The results of separation Fraction I are shown in Table 3.3

Table 3.3 The results of separation of Fraction I by silica gel column chromatography.

Eluents	Fraction No. (800 mL)	Remarks	Weights (g)
n-hexane	1-6	pale yellow oil	0.45
10% CH_2Cl_2-hexane	7-11	pale yellow oil	0.68
20% CH_2Cl_2-hexane	12-21	yellow oil+white ppt (Mixture 1)	2.57
40% CH_2Cl_2-hexane	22-31	yellow oil+white ppt (Mixture 2)	3.50
	32-36	yellow oil	2.25
60% CH_2Cl_2-hexane	27-44	yellow oil+needle ppt (Mixture 3)	7.02
	45-53	yellow oil+white ppt (Mixture 4)	4.85
80% CH_2Cl_2-hexane	54-63	orange oil+white ppt (Compound 5)	8.76
100% CH_2Cl_2	64-75	orange oil+cubic crystal (Compound 6)	15.46
2% MeOH-CH_2Cl_2	76-80	orange oil+cubic crystal (Compound 6)	21.50
	81-85	orange oil	10.10
5% MeOH-CH_2Cl_2	86-94	orange oil	12.35
10% MeOH-CH_2Cl_2	95-101	orange oil	6.80
20% MeOH-CH_2Cl_2	102-107	orange oil	5.15
50% MeOH-CH_2Cl_2	108-112	orange oil	3.56
100% MeOH	113-115	orange oil	2.10

Separation of Fraction III

The thin-layer chromatography of crude butanolic extract presented at least four spots (silica gel, solvent: 10% methanol in dichloromethane). This crude 80.0 g was separated by column chromatography using silica gel as an adsorbent. The eluents used were the same as those for the separation of Fraction I. The results of separation are shown in Table 3.4

Table 3.4 The results of separation of Fraction III by silica gel column chromatography

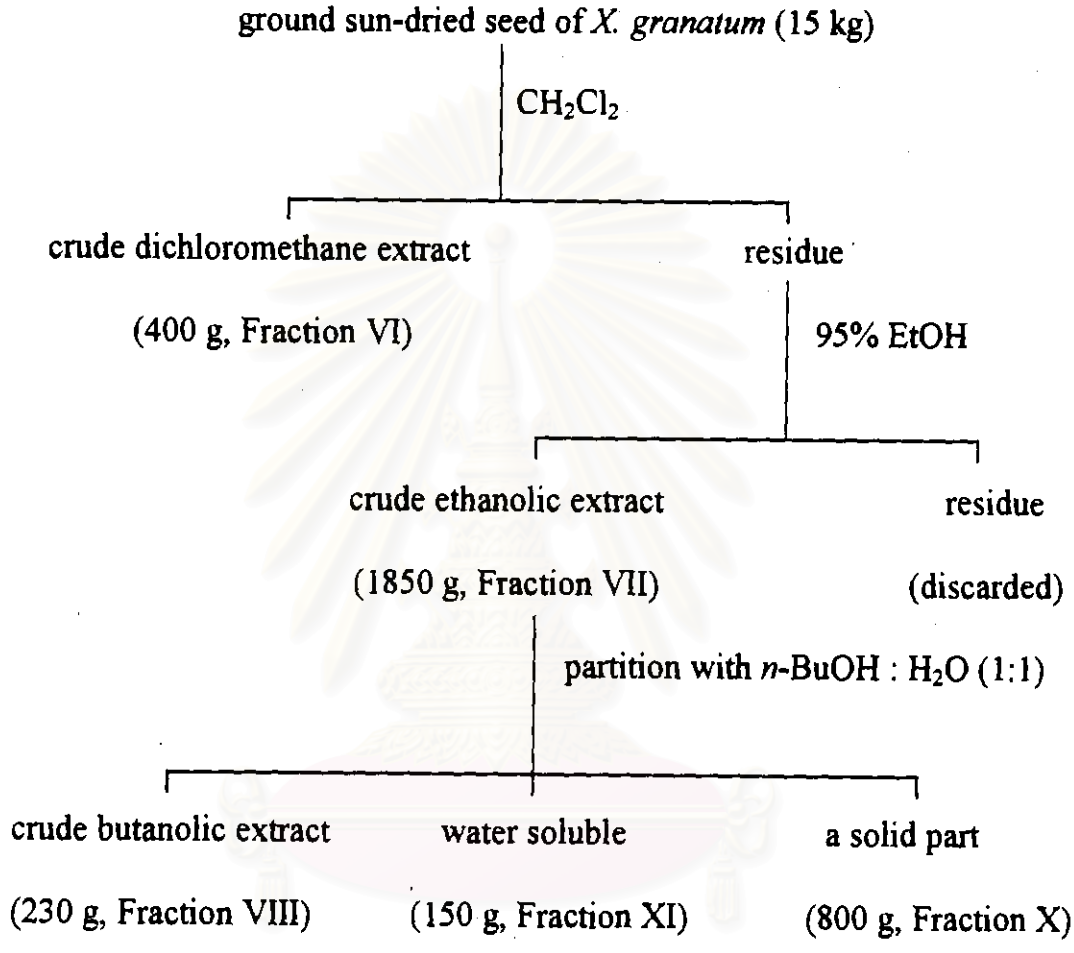
Eluent	Fraction No. (800 mL)	Remarks	Weights (g)
100%CH_2Cl_2	1	colourless oil	trace
	2	orange oil	"
	3	yellow oil	"
2% MeOH-CH_2Cl_2	4-7	yellow oil+solid (Mixture **3**)	25.0
	8	yellow oil+solid	0.5
5% MeOH-CH_2Cl_2	9	yellow oil+solid (Compound **6**)	1.2
	10	orange oil	5.4
	11	orange oil+solid (Compound **6**)	30.0
	12-14	brown-red oil	6.0
10% MeOH-CH_2Cl_2	15-16	brown-red oil+solid (Mixture **8**)	5.2
	17-18	brown-red oil+solid	5.8
20% MeOH-CH_2Cl_2	19-22	brown-red oil+solid	10.4
40% MeOH-CH_2Cl_2	23-25	brown solid	10.1
50% MeOH-CH_2Cl_2	26-28	brown solid	5.5
80% MeOH-CH_2Cl_2	29-31	brown solid	5.0

Chemical Constituents of the Seeds of *X. granatum*.

Extraction

The same procedure used for the extraction of the fruits was applied for ground sun-dried seeds (15 kg), yielding the yellow dichloromethane crude extract 400 g (2.67 % wt. by wt.) and 1850 g (12.33 % wt. by wt. of dried seeds) of ethanolic extract. After partition the ethanolic crude extract with *n*-butanol and water, the brown-reddish butanolic crude extract 230 g (1.53 % wt. by wt.), the water soluble 150 g (1.00 % wt. by wt.) and the solid parts 800 g (5.33 % wt. by wt.) were obtained, respectively. The results of extraction are summarized in Scheme 3.2.

Scheme 3.2 The results of extraction and initial fractionations for the seeds of *X. granatum*



Separation of Fraction VI

The thin-layer chromatography of this crude showed at least 5 spots (silica gel, solvent:10% methanol in dichloromethane). The crude dichloromethane extract of the seeds of *X. granatum* as brown-yellowish solid, 80.0 g, was separated by column chromatography using silica gel as an adsorbent. The results of separation are presented in Table 3.5.

Table 3.5 The results of separation Fraction VI by column chromatography.

Eluent	Fraction No. (800 mL)	Remarks	Weights (g)
30% CH_2Cl_2-hexane	1-6	pale yellow oil	1.5
40% CH_2Cl_2-hexane	7-15	pale yellow oil+white ppt	2.8
60% CH_2Cl_2-hexane	16-19	yellow oil	3.0
	20-28	yellow oil+needle ppt (Mixture **3**)	4.8
80% CH_2Cl_2-hexane	29-44	yellow oil+white bright ppt (Compound **5**,7)	12.5
100%CH_2Cl_2	45-64	yellow oil+white bright ppt (Compound **5**,7)	6.8
2% MeOH-CH_2Cl_2	65-69	yellow oil+white bright ppt (Compound 6)	11.5
	70-76	yellow oil+cubic crystal (Compound 6)	8.2
5% MeOH-CH_2Cl_2	77-83	yellow oil+cubic crystal (Compound 6)	9.3
	84-93	orange oil	4.2
10% MeOH-CH_2Cl_2	94-100	orange oil	3.6
20% MeOH-CH_2Cl_2	101-110	orange oil+white ppt (Mixture 8)	5.0
50% MeOH-CH_2Cl_2	111-113	orange oil	2.2
100%MeOH	114-116	orange oil	1.5

Reseparation of Fraction 29-64 of Fraction VI (Table 3.5)

After combination fraction 29-64, the white bright solid in yellow oil were obtained. The yellow oil was separated by dissolving with methanol. The remaining solid was recrystallized by methanol several times. However, there still showed 2 spots on TLC (silica gel, solvent system: 50% ethyl acetate in hexane). This mixture, 250 mg, was then reseparated by flash column using 20% ethyl acetate in hexane as an eluent. Each fraction was collected approximately 8 mL and was monitored by TLC. The similar fractions were combined. The results of separation this fraction are shown in Table 3.6

Table 3.6 The results of separation fraction 29-64 (Fraction IV) by flash chromatography (using column 2.5 cm X 35.0 cm)

No. of fraction (8.0 mL.)	Remarks	Weight (mg)
1-11	-	-
12-22	white bright solid (Compound 7)	170
23-45	white bright solid (Compound 5, 7)	60

Separation of Fraction VIII

The TLC of Fraction VIII showed at least 2 spots in this crude extract (solvent: 10% methanol-dichloromethane). This crude, 65 g, was separated by column chromatograghy using hexane, dichloromethane and methanol and their appropriate mixtures as mentioned above. The results of separation Fraction VIII are presented in Table 3.7.

Table 3.7 The results of separation Fraction VIII by column chromatography.

Eluent	Fraction No. (800 mL)	Remarks	Weights (g)
50% CH_2Cl_2-hexane	1-5	yellow oil	2.0
70% CH_2Cl_2-hexane	6-14	yellow oil+needle ppt (Mixture 3)	4.5
100% CH_2Cl_2	15-19	yellow oil	10.0
2% MeOH-CH_2Cl_2	20-23	orange oil+cubic crystal (Compound 6)	18.2
5% MeOH-CH_2Cl_2	24-27	orange oil+cubic crystal (Compound 6)	11.5
10% MeOH-CH_2Cl_2	28-31	brown oil	8.6
15% MeOH-CH_2Cl_2	32-35	brown oil+white ppt (Mixture 8)	6.5
20% MeOH-CH_2Cl_2	36-45	brown oil	4.3
50% MeOH-CH_2Cl_2	46-48	brown oil	1.2
	49-50	brown oil	0.8

Purification, Properties and Structural Elucidation

Purification, Properties and Structural Elucidation of Mixture 1

The combined fractions no.12-21 contained white amorphous solid and pale yellow oil (see Table 3.2). The yellow oil was removed with methanol. The remaining white solid was recrystallized from a mixture of hexane and dichloromethane yielding Mixture **1**, 30 mg (0.06 % wt. by wt. of Fraction I), m.p. 69-71 °C. The R_f value of this compound revealed one spot at 0.8 (silica gel, solvent system: 10% dichloromethane-hexane). This compound was soluble in dichloromethane but slightly soluble in ethyl acetate, hexane, acetone and methanol.

The IR spectrum of Mixture **1** (Fig 3.1) indicated the absorption band of C=O stretching vibration of ester at 1735 cm^{-1} (s) and C-O stretching vibration at 1170 cm^{-1} (m). The C-H stretching vibration and C-H bending vibration of $-CH_2-$, $-CH_3$ presented at 2850 cm^{-1} and 1465 cm^{-1}, respectively. The additional absorption at 720 cm^{-1}(w) due to $-CH_2-$ (for chain, 4 carbons).[36-37] Therefore, this mixture should be a saturated long chain aliphatic esters.

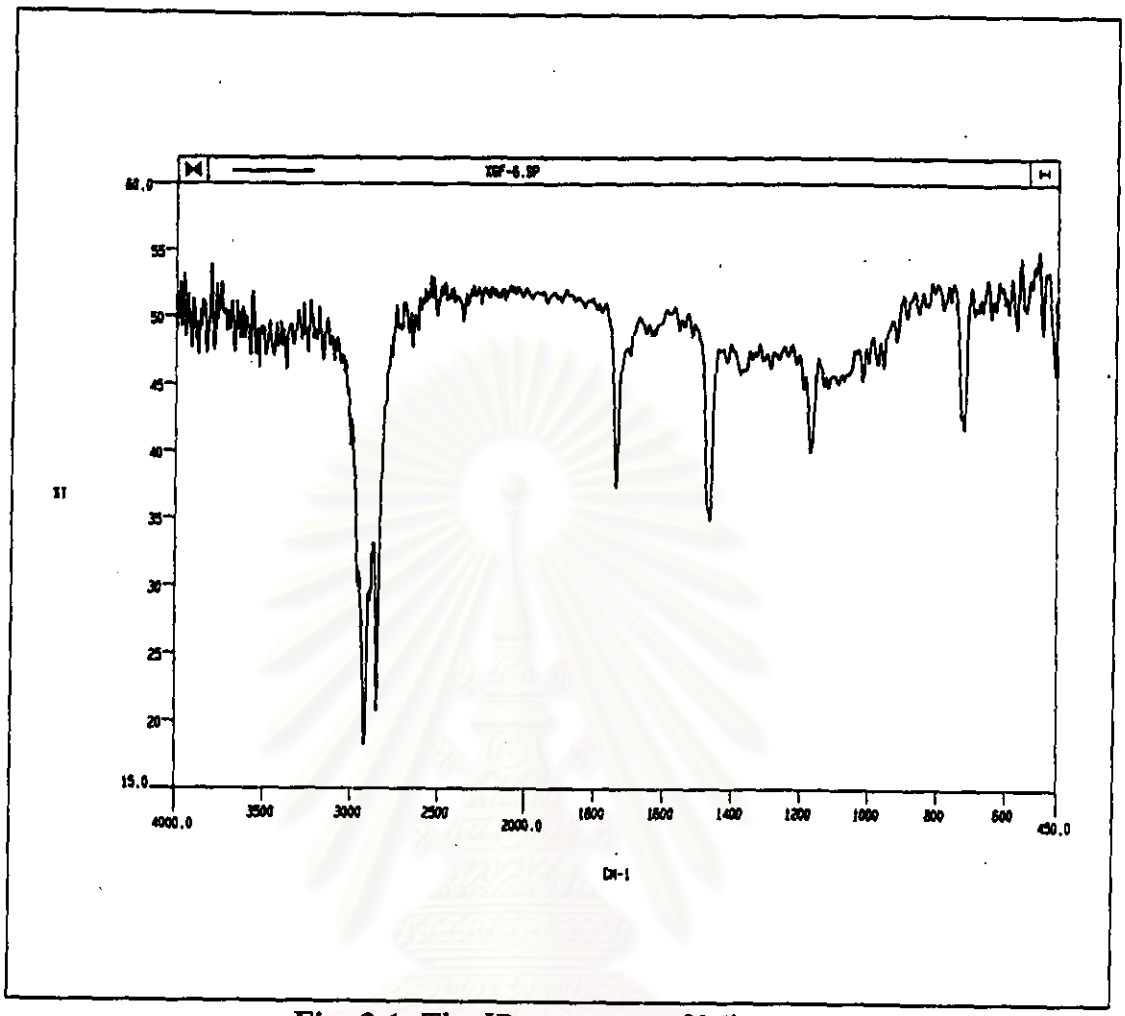


Fig. 3.1 The IR spectrum of Mixture 1

Purification, Properties and Structural Elucidation of Mixture 2

The solid in yellow oil was obtained after combination of fraction no. 22-31 (see Table 3.2). The yellow oil was removed with methanol. The remained solid was recrystallized from acetone for several times to afford white amorphous solid designated as Mixture 2, 1.2 g (4.57 % wt. by wt. of Fraction I), m.p.81-82°C. The TLC plate (silica gel) displayed R_f 0.35 (solvent: dichloromethane). This compound was soluble in dichloromethane, but slightly soluble in hexane.

The IR spectrum of this mixture (Fig.3.2) gave the major absorption bands at 3425-3150 cm^{-1}(s,b) and 1061 cm^{-1}(m) attributed to C=O and C-O stretching vibration[36-37], respectively. This implied that Mixture 2 contained O-H as its functional group. Other absorption bands were similar to those of Mixture 1, *i.e.*,-CH_3 and -CH_2- stretching, bending vibration and C-H rocking mode of -CH_2- (for carbon > 4) which suggested to be an aliphatic compound.

In order to disclose the composition of this mixture, the GLC analysis was used (column OV-1, column temperature 250 °C, injection temperature 290 °C and flow rate of mobile phase, N_2, 50 mL/min with FID detector).

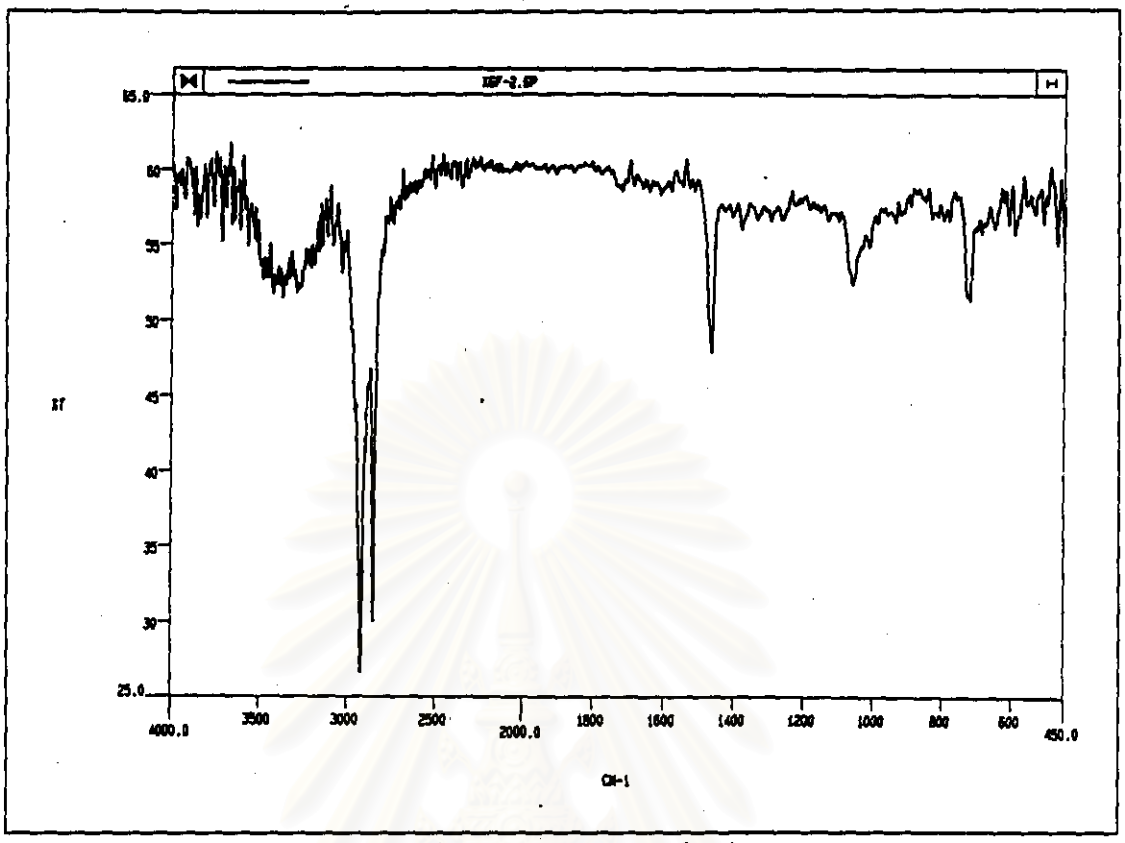


Fig 3.2 The IR spectrum of Mixture 2

The GLC chromatogram of standard saturated long chain aliphatic primary alcohols showed 6 peaks of retention time at 0.79, 1.06, 1.55, 2.41, 3.85 and 31.32 min which were corresponded to the number of carbon 14, 16, 18, 20, 22 and 32, respectively (Fig.3.3). The linear standard correlation curve of logarithm of retention time *versus* the number of carbon in the authentic long chain alcohol sample was plotted in Fig. 3.5.

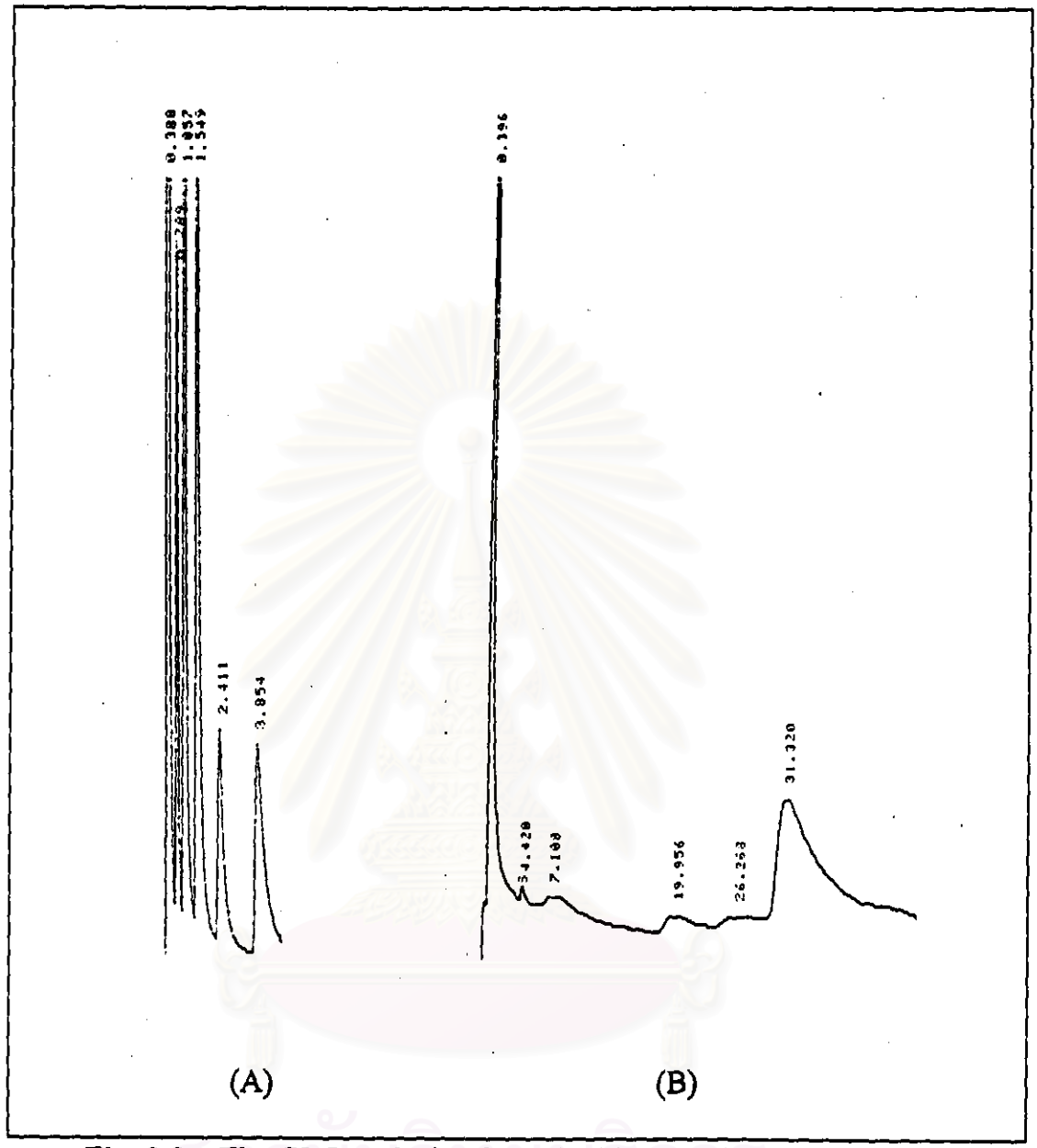


Fig. 3.3 The GLC analysis of standard long chain primary alcohol

(A) C_{14}, C_{16}, C_{18}, C_{20}, C_{22}

(B) C_{32}

The GLC analysis result of Mixture 2 revealed 5 peaks on gas chromatogram (Fig.3.4) at retention time 11.27, 15.15, 18.70, 26.01 and 30.83 min.,respectively, which were corresponded to the number of carbon 27, 29, 30, 31 and 32, respectively.

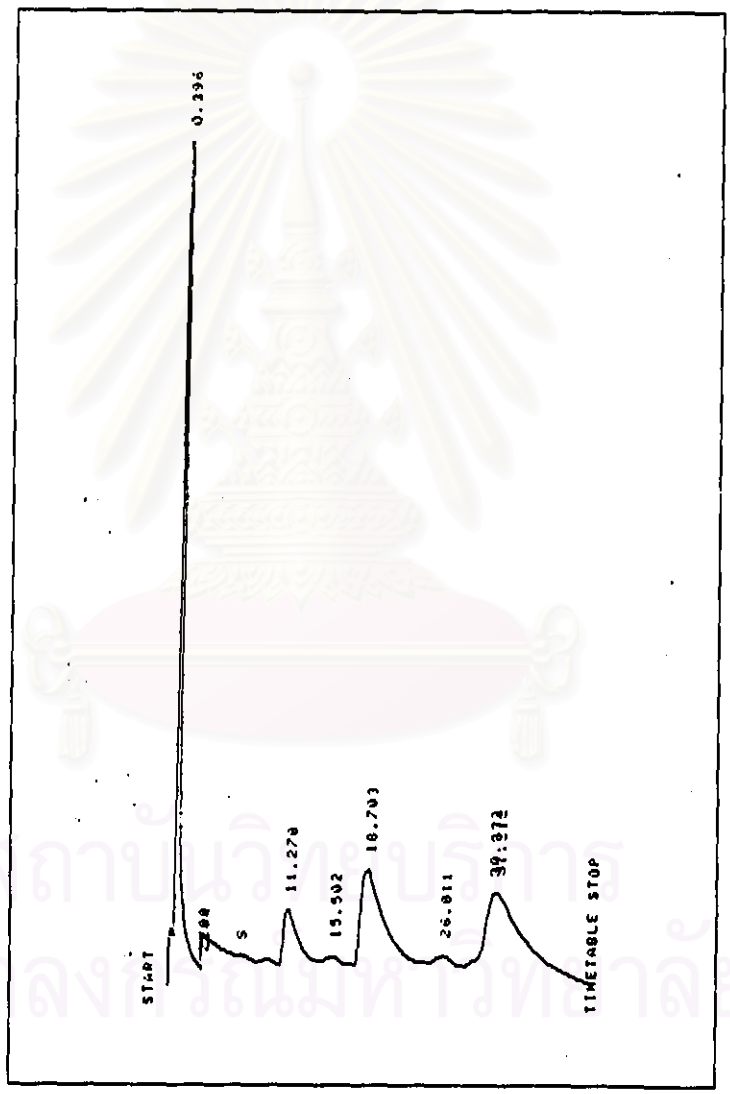


Fig. 3.4 The GLC analysis of Mixture 2

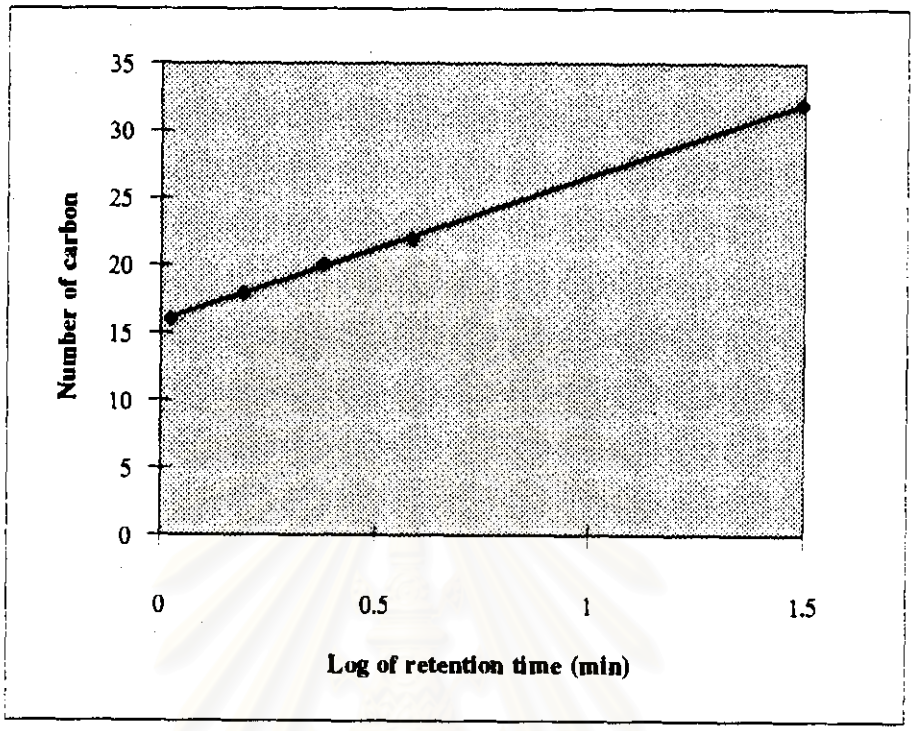


Fig. 3.5 The standard calibration curve of long chain primary alcohol

Hence, Mixture 2 was in fact a mixture of saturated long chain aliphatic primary alcohol which consisted of heptaicosanol ($C_{27}H_{55}OH$), nonaicosanol ($C_{29}H_{59}OH$), triacontanol ($C_{30}H_{61}OH$), hentriacontanol ($C_{31}H_{63}OH$) and dotriacontanol ($C_{32}H_{65}OH$).

The composition of each compound is presented in Table 3.8. The major component in this mixture is triacontanol ($C_{30}H_{61}OH$).

Table 3.8 The composition of saturated long chain aliphatic primary alcohols contained in Mixture 2

Compound	%Composition
heptaicosanol ($C_{27}H_{55}OH$)	12.62
nonaicosanol ($C_{29}H_{59}OH$)	3.09
triacontanol ($C_{30}H_{61}OH$)	35.82
hentriacontanol ($C_{31}H_{63}OH$)	9.55
dotriacontanol ($C_{32}H_{65}OH$)	33.37

The structure of Mixture 2 is shown below:

CH_3-$(CH_2)_n$-CH_2OH n=25, 27, 28, 29 and 30

Mixture 2

Purification, Properties and Structural Elucidation of Mixture 3

The Mixture **3** was obtained as yellow oil and a needle crystal from the separation of all Fractions I, II, III and IV (see Table 3.1, 3.2, 3.4 and 3.5). After removing yellow oil by washing with methanol and recrystallizing the solid with hexane for several times, Mixture **3** as white bright needle, m.p.143-145°C, 1.3 g (1.86 % wt. by wt. of Fraction I), 0.8 g (1.14 % wt by wt. of Fraction III), 1.2 g (1.5 % wt by wt. of Fraction VI), and 0.5 g (0.63 % wt. by wt. of Fraction VIII) were gained. The TLC (silica gel) exhibited only one spot at R_f 0.40 (solvent system : dichloromethane). Mixture **3** was soluble in dichloromethane or acetone, but slightly soluble in hexane, methanol .

After monitoring by colour tests, this substance gave a deep green colour with Liebermann-Burchard's reagent which suggested the appearance of a steroidal moiety.

The IR spectrum (Fig. 3.6) exhibited the eminent absorption band of O-H stretching vibration at 3500-3430 cm^{-1} (s,b) and C-O stretching vibration at 1050 and 1020 cm^{-1}(m). In addition, the band at 1650 cm^{-1}(w) revealed the presence of C=C stretching vibration in this structure. The C-H stretching and bending vibration of $-CH_3$ and $-CH_2-$ were showed at 2960-2840 cm^{-1} and 1460 cm^{-1}, respectively. The absorption bands at 840 and 800 cm^{-1}(C-H out of plane bending) were contributed to Δ^5-3β-hydroxy steroids.[37]

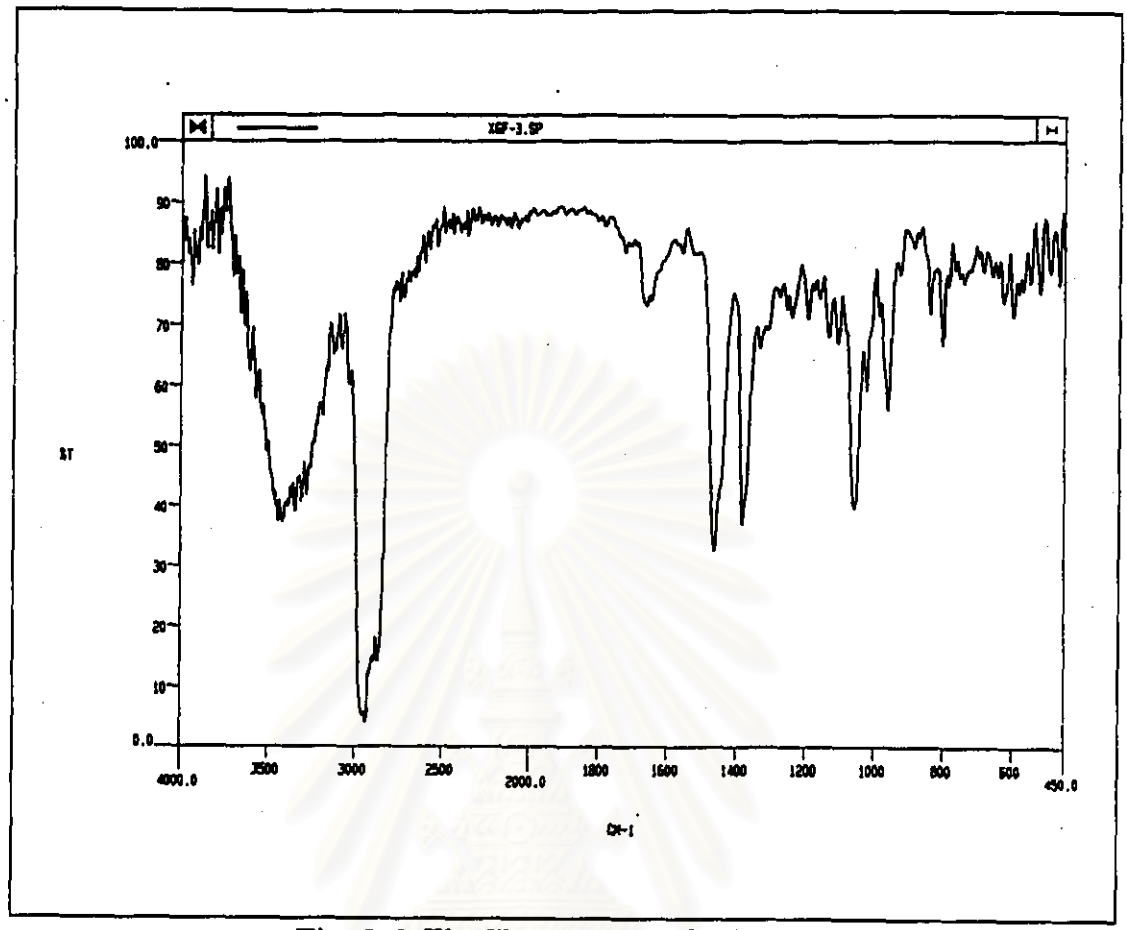


Fig. 3.6 The IR spectrum of Mixture 3

According to its physical properties, a colour test and IR spectrum, this substance was found to be close to those of steroids.[38] Generally, steroidal compounds are widely distributed in higher plants. Previous literatures remarked that the presence of steroids in plant normally occurred as a mixture of steroid such as stigmasterol, β-sitosterol and campesterol.[38] The analysis method selected for further study on the composition of this mixture was GLC. The results of GLC analysis of standard steroids namely cholesterol, campesterol, stigmasterol and β-sitosterol was showed at retention time 13.06,

17.61, 18.76 and 21.00 min, respectively. (Fig.3.7) (condition: column temperature 260°C, injection temperature 290°C and flow rate of carrier gas (N_2), 50 mL/min).

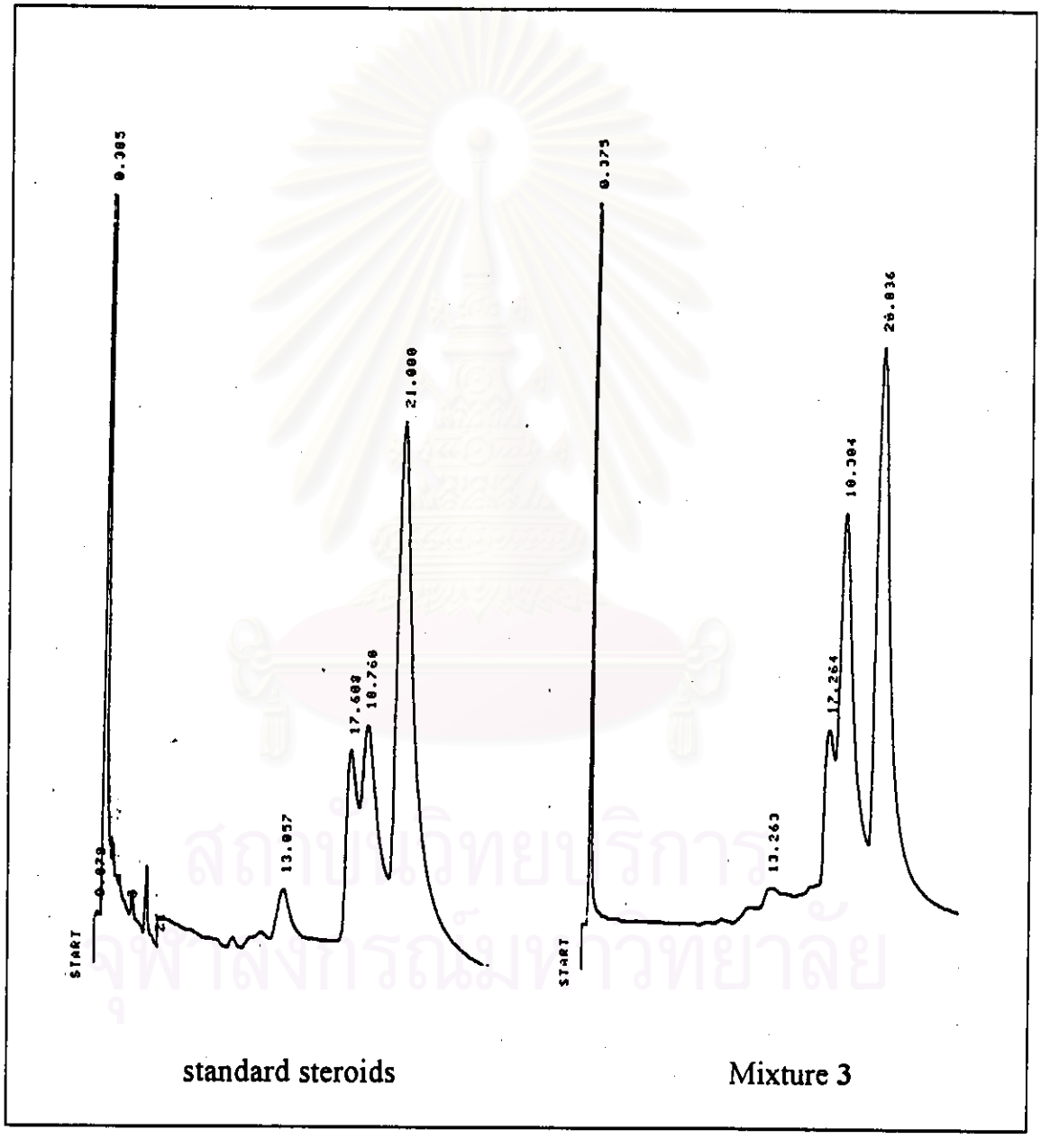


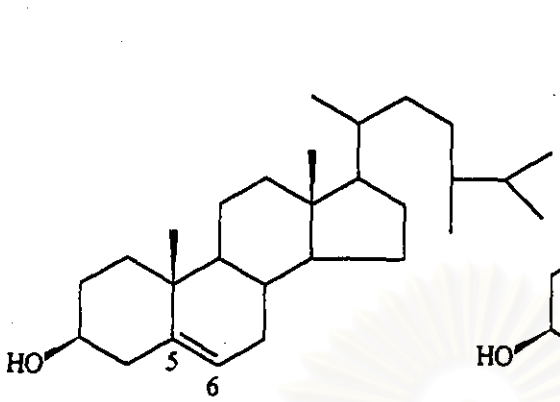
Fig. 3.7 The GLC analysis of standard steroid and Mixture 3

The GLC chromatogram of Mixture 3 (Fig.3.8) showed 4 peaks at retention time 17.26, 18.30 and 20.84 min, respectively which were in fact corresponded to the authentic sample of campesterol, stigmasterol and β-sitosterol, respectively..

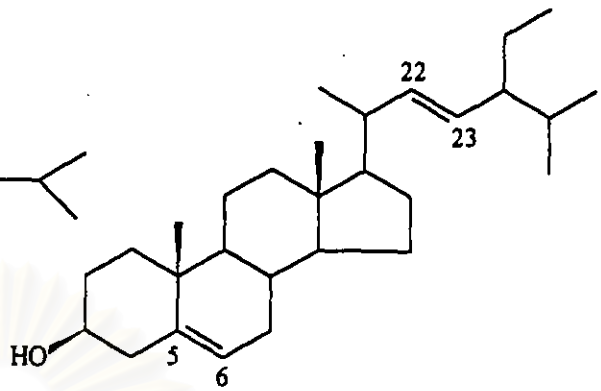
Hence, it could be concluded that Mixture 3 was a mixture of steroid β-sitosterol, campesterol and stigmasterol. β-Sitosterol was found to be the major component. The composition of steroid in Mixture 3 is presented in Table 3.9.

Table 3.9 The composition of steroids in Mixture 3

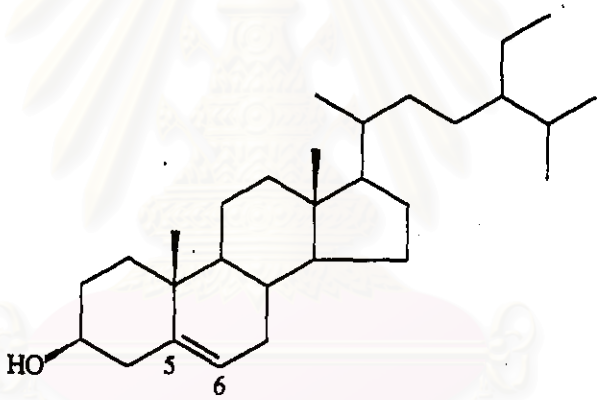
Name	Retention time (min)	% Composition
campesterol	17.26	12.89
stigmasterol	18.30	34.73
β-sitosterol	20.84	48.21



campesterol



stigmasterol



β-sitosterol

Mixture 3 mixture of steroids

Purification, Properties and Structural Elucidation of Mixture 4

Mixture 4 contained white amorphous solid and yellow oil was obtained by combination of fractions no. 45-53 (see Table 3.3). The yellow oil was removed with methanol, the remaining solid was purified by recrystallization with acetone for several times to afford white amorphous solid, 0.8 g (1.14 % wt. by wt. of Fraction I), m.p. 71-74°C , R_f 0.58 (solvent system: dichloromethane). This compound was soluble in dichloromethane but was not soluble in hexane, ethyl acetate, acetone, methanol and ethanol.

The IR spectrum (Fig.3.8) showed a very broad absorption band at 3500-2600 cm^{-1}(s) due to the O-H stretching vibration of carboxylic acid and a strong peak at 1710 cm^{-1} ought to be C=O stretching vibration of carboxylic acid.[37] Other bands at 2929-2500 and 1460 cm^{-1} specified the methyl and methylene vibration modes. Besides, the C-H rocking mode of -CH_2-(for chain>4 carbons) was found at 720 cm^{-1}.

The information obtained from the IR spectrum pointed out that this substance should be saturated long chain aliphatic carboxylic acid.

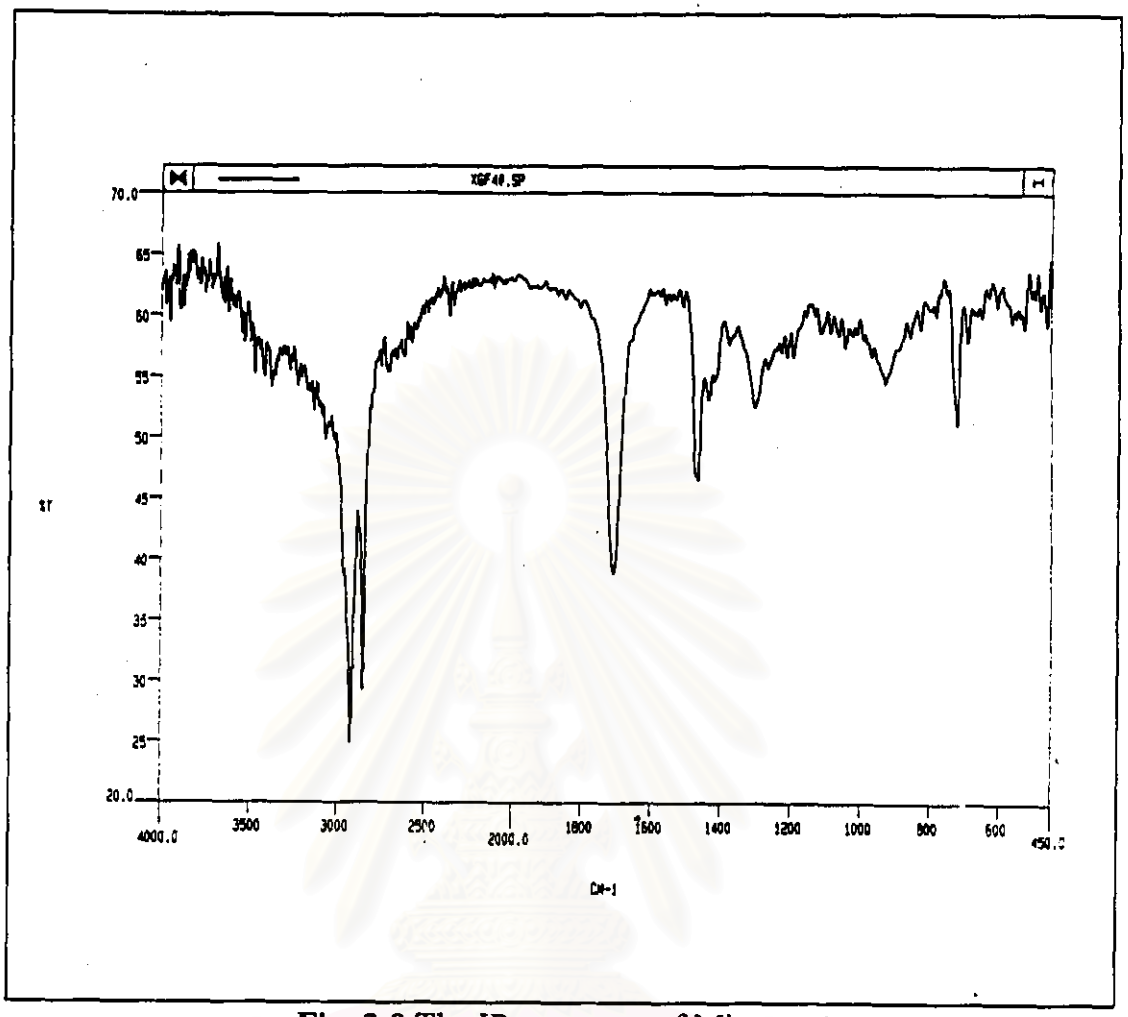


Fig. 3.8 The IR spectrum of Mixture 4

To analyse saturated long chain aliphatic carboxylic acid, its methyl ester derivative was prepared with diazol reagent and analysed by GC-MS. The GC analysis (Fig. 3.9) exhibited 10 peaks which revealed that this mixture was composed of 10 components. The mass spectrum (Fig.3.10-3.19) of each component was searched from a library search[39] and found to coincide with several methyl esters as shown in Table 3.10.

Table 3.10 Methyl ester derivatives of Mixture 4

Compound	Structural formula	Retention time	m/z
Ecosanoic acid, methyl ester	$C_{21}H_{42}O_2$	15.54	326
Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	16.07	348
Docosanoic acid, methyl ester	$C_{23}H_{26}O_2$	16.63	354
Tricosanoic acid, methyl ester	$C_{24}H_{48}O_2$	17.25	368
Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	17.96	382
Pentacosanoic acid, methyl ester	$C_{26}H_{52}O_2$	18.79	396
Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$	19.79	410
Heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	20.96	424
Octacosanoic acid, methyl ester	$C_{29}H_{58}O_2$	22.43	438
Tricontanoic acid, methyl ester	$C_{31}H_{62}O_2$	26.46	466

According to the spectral data, this mixture was clearly a mixture of long chain aliphatic carboxylic acids as shown in Table 3.11.

Table 3.11 The long chain aliphatic carboxylic acid components of Mixture 4

compound	Molecular formula	MW
Ecosanoic acid	$C_{20}H_{40}O_2$	312
Heneicosanoic acid	$C_{21}H_{42}O_2$	326
Docosanoic acid	$C_{22}H_{44}O_2$	340
Tricosanoic acid	$C_{23}H_{46}O_2$	354
Tetracosanoic acid	$C_{24}H_{48}O_2$	368
Pentacoanoic acid	$C_{25}H_{50}O_2$	382
Hexacosanoic acid	$C_{26}H_{53}O_2$	396
Hepacosanoic acid	$C_{27}H_{54}O_2$	410
Octacosanoic acid	$C_{28}H_{56}O_2$	424
Tricontanoic acid	$C_{30}H_{60}O_2$	452

$$CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}COOH$$

n = 17, 18, 19, 20, 21, 22, 23, 24, 25,27

Mixture 4

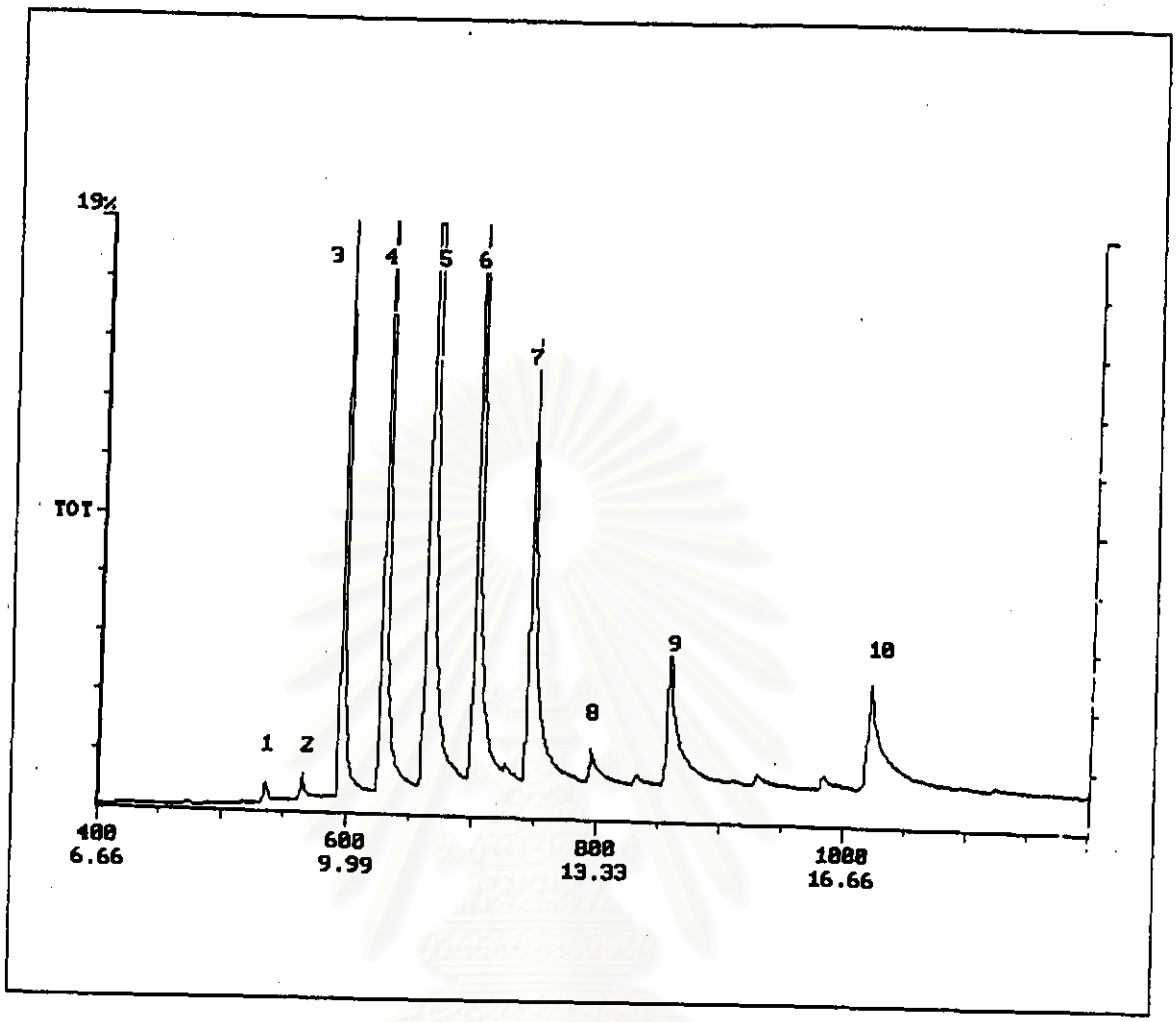


Fig 3.9 The GLC analysis of Mixture 4

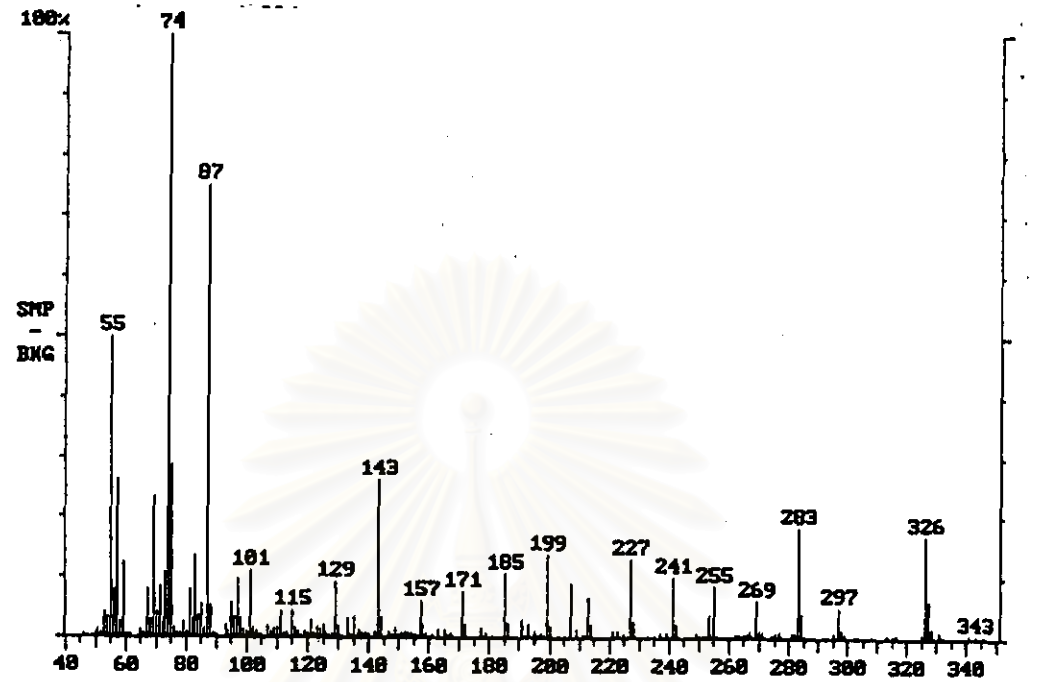


Fig. 3.10 The mass spectrum of component 1 in Mixture 4

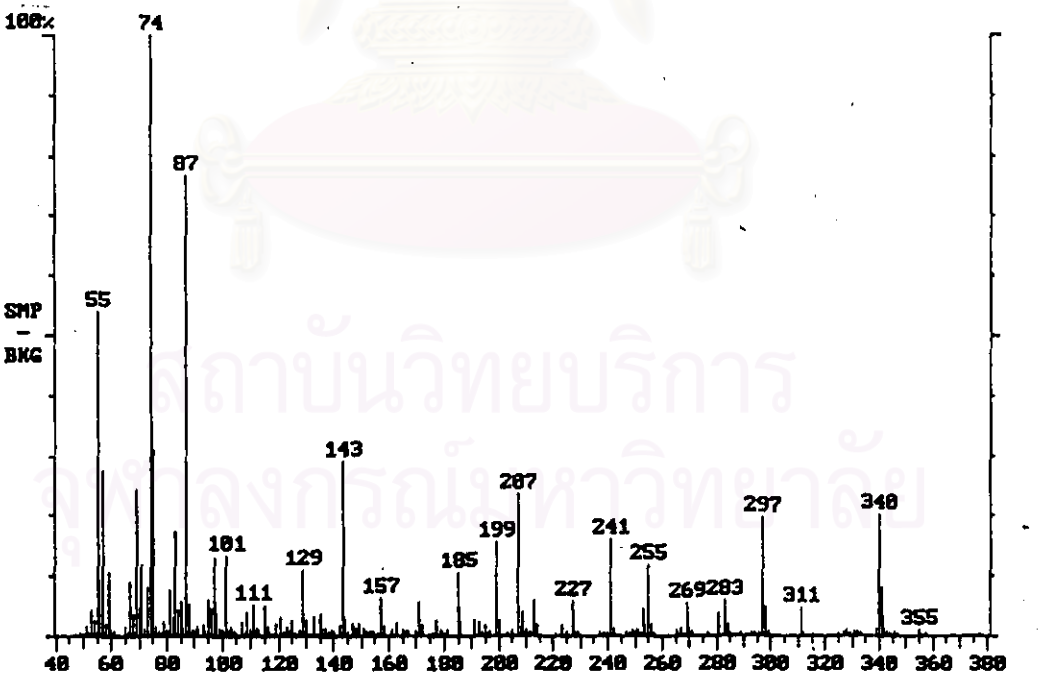


Fig. 3.11 The mass spectrum of component 2 in Mixture 4

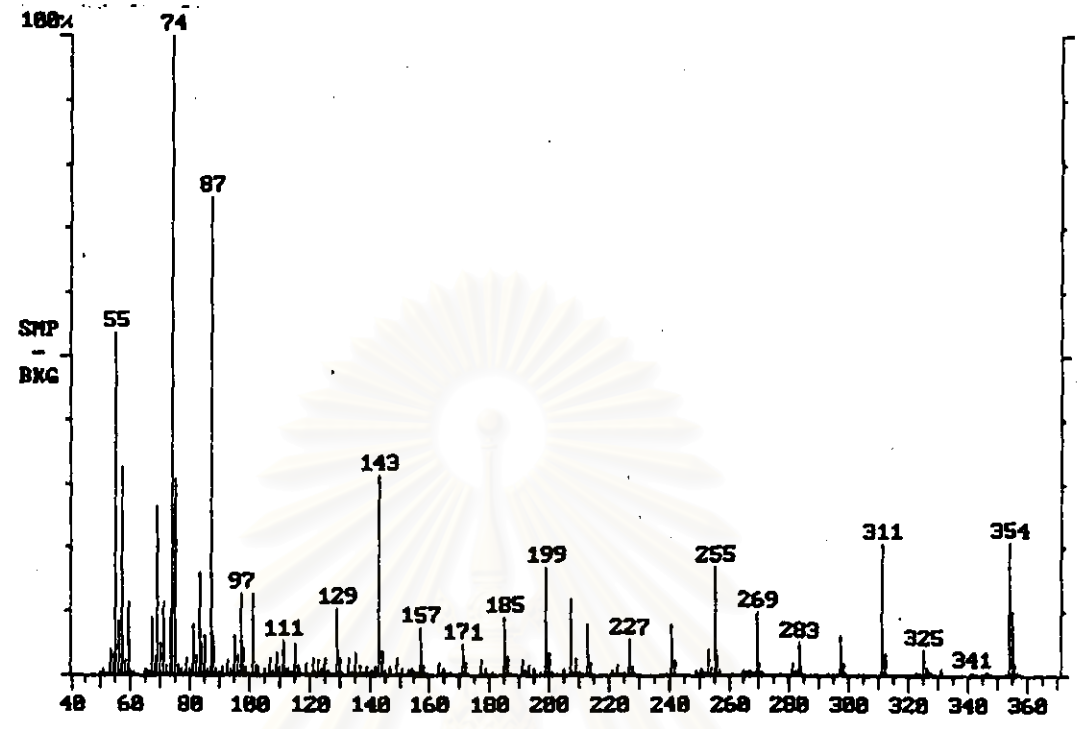


Fig. 3.12 The mass spectrum of component 3 in Mixture 4

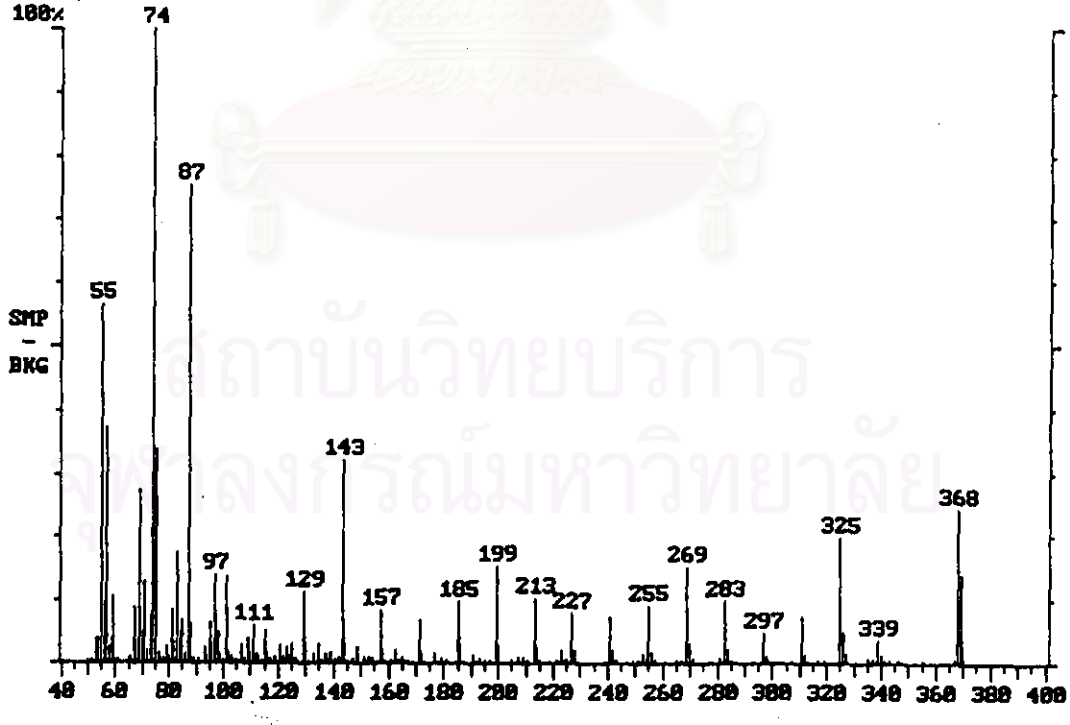


Fig. 3.13 The mass spectrum of component 4 in Mixture 4

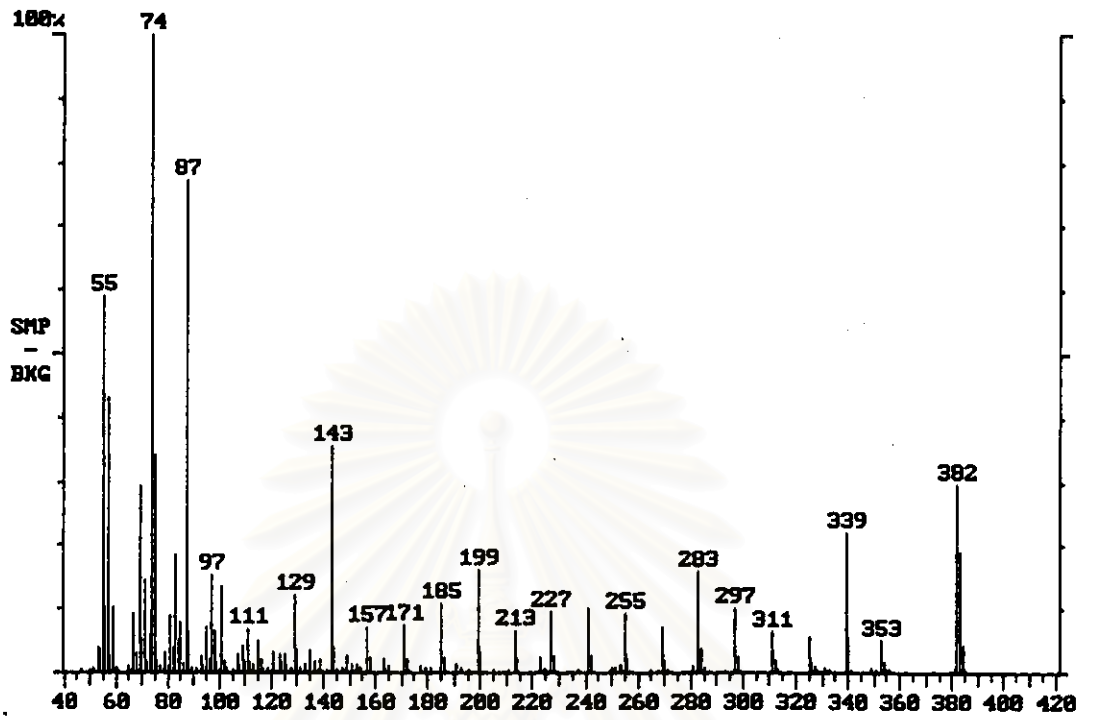


Fig 3.14 The mass spectrum of component 5 in Mixture 4

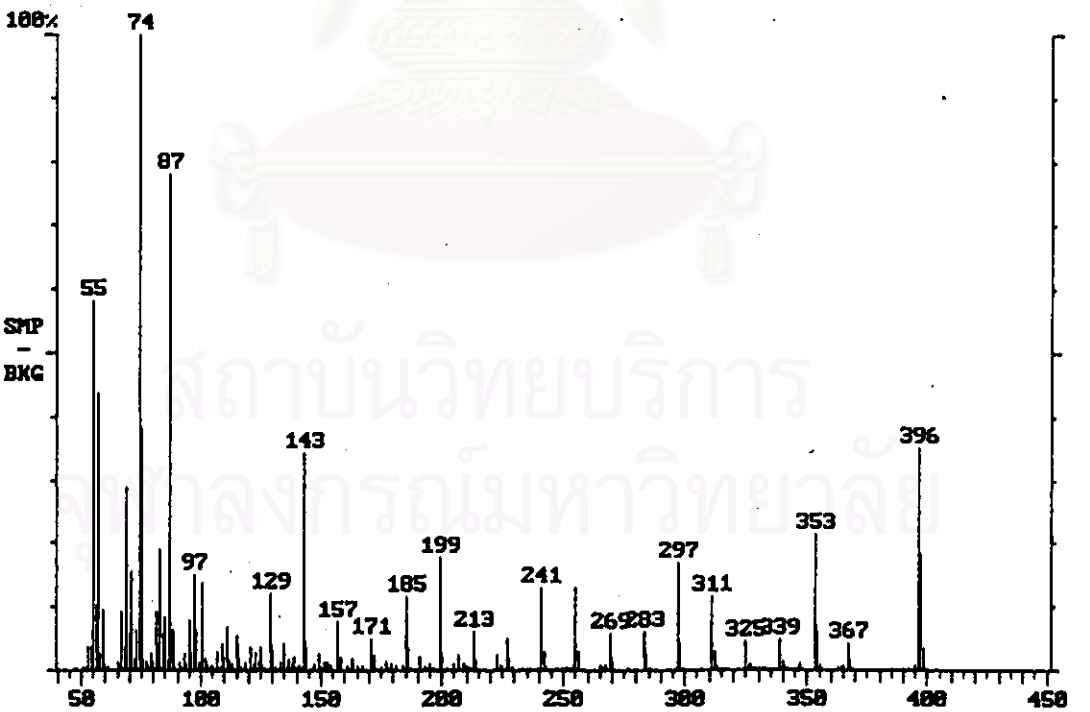


Fig 3.15 The mass spectrum of component 6 in Mixture 4

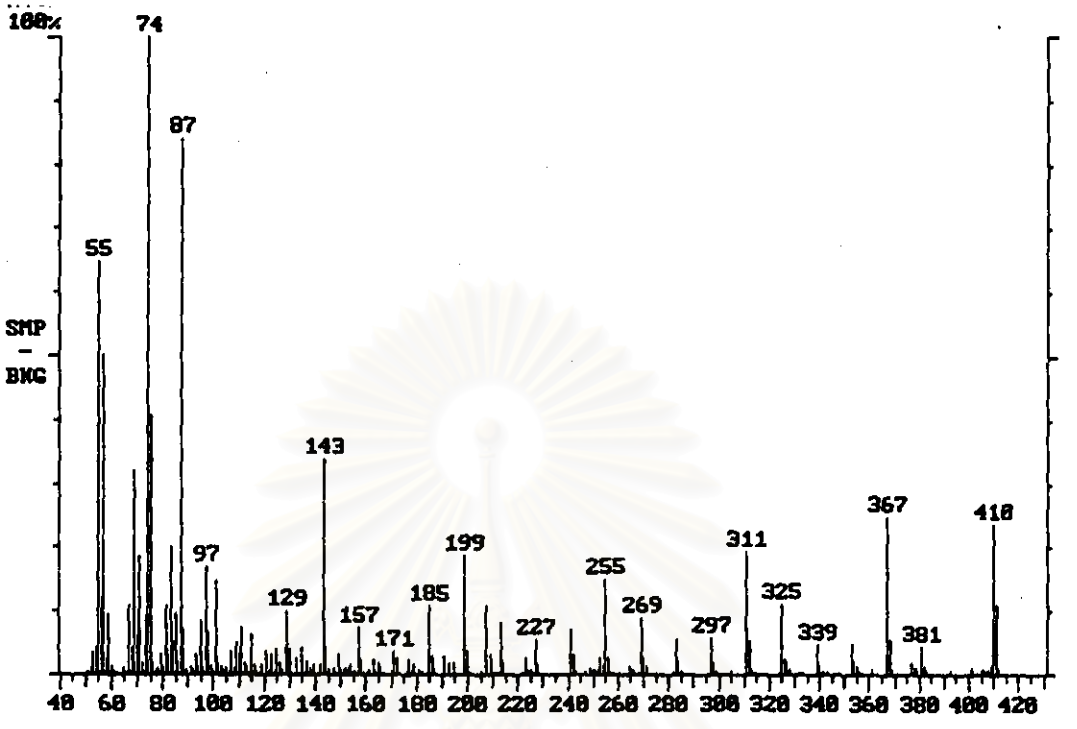


Fig. 3.16 The mass spectrum of component 7 in Mixture 4

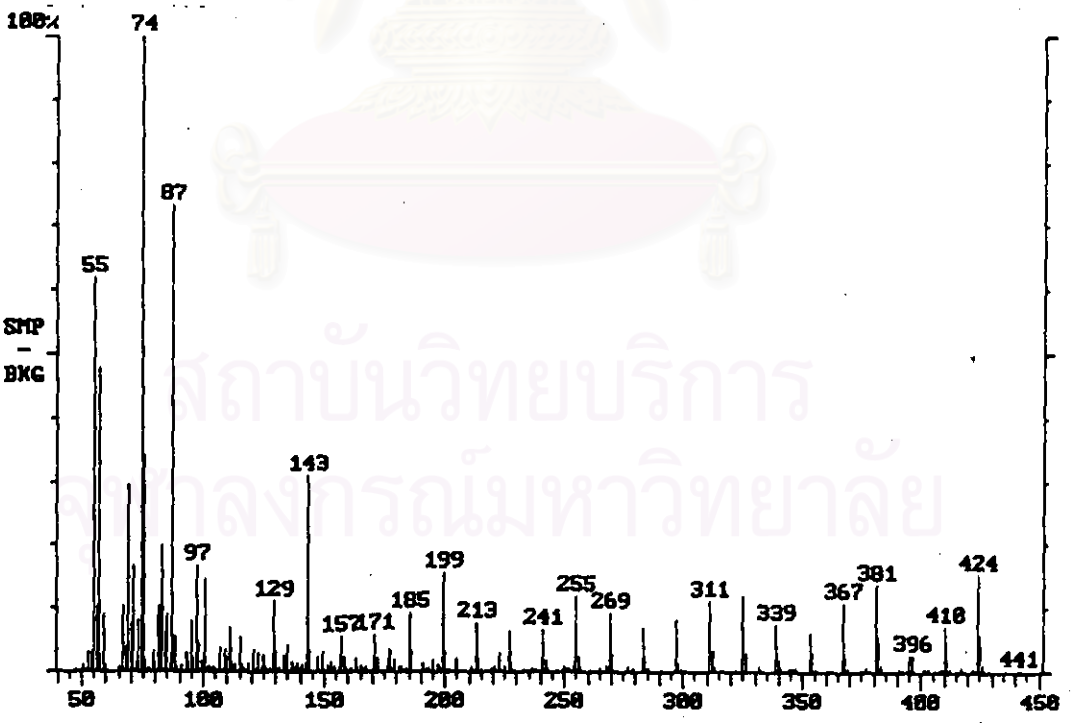


Fig. 3.17 The mass spectrum of component 8 in Mixture 4

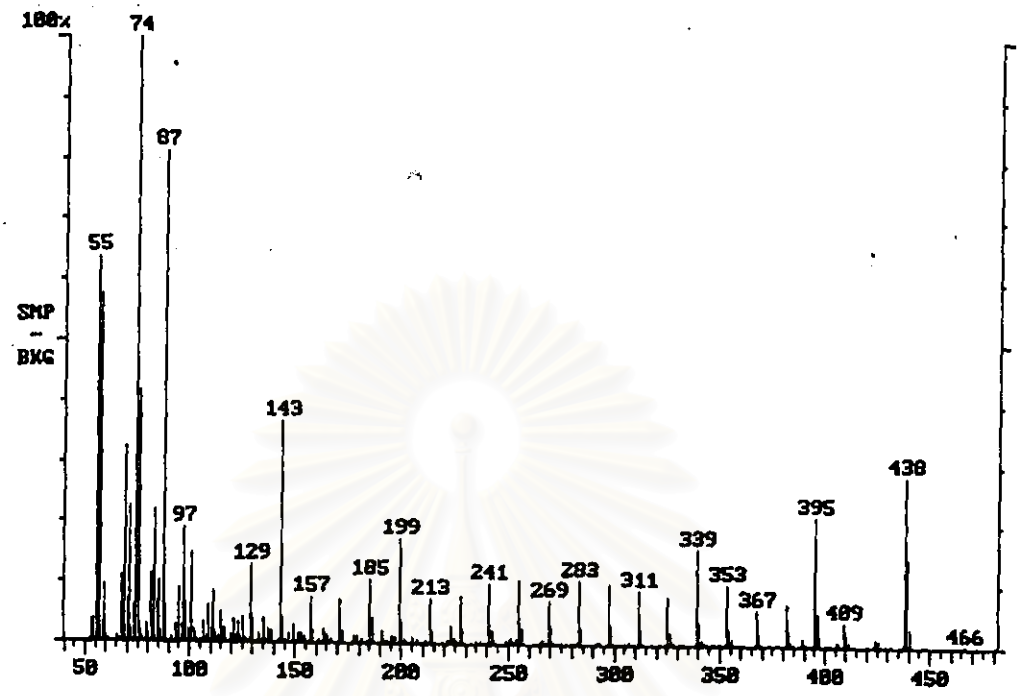


Fig. 3.18 The mass spectrum of component 9 in Mixture 4

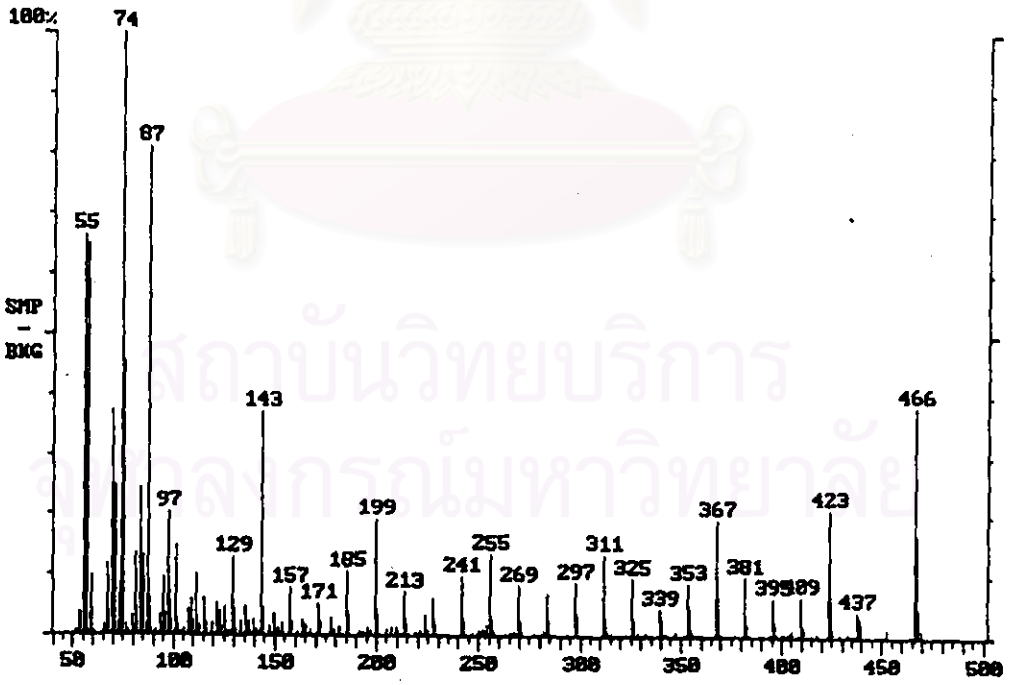


Fig 3.19 The mass spectrum of component 10 in Mixture 4

Purification, Properties and Structural Elucidation of Compound 5

The white bright solid in yellow oil was obtained from the combination of fraction no.54-63 (Fraction I:see Table 3.2). The yellow oil was removed by washing with methanol for several times, the remaining product was recrystallized from methanol twice to yield white bright solid, 50 mg (0.08 % wt. by wt. of Fraction I), m.p. 258-260 °C, R_f 0.50 (silica gel, solvent: 5% hexane in ethyl acetate). In addition, this compound was gained approximately 15 mg as a mixture with Mixture **3** from Fraction III (see Table 3.3) and was a minor component as a mixture with Compound **7** from Fraction IV (see Table 3.4). This compound was soluble in dichloromethane and ethyl acetate, but slightly soluble in methanol.

The IR spectrum of Compound **5** (Fig 3.20) revealed C-H stretching vibration of alkene at 3120-3020 cm^{-1}(m). The strong absorption band at 1740 cm^{-1}, 1710 cm^{-1} and 1670 cm^{-1} were compatible with C=O stretching vibration of conjugated carbonyl moiety, possibly an ester (lactone), ketone and α,β-unsaturated carbonyl group, respectively.[40] Other absorption bands in the range of 1000-1290 cm^{-1} corresponded to the C-O stretching vibration.[37]

The UV spectrum (Fig 3.21) gave the maximum absorption peak (λ_{max}) at 232 nm (log ε = 4.61) indicated the occurance of α,β-unsaturated carbonyl moiety in the molecule.[41]

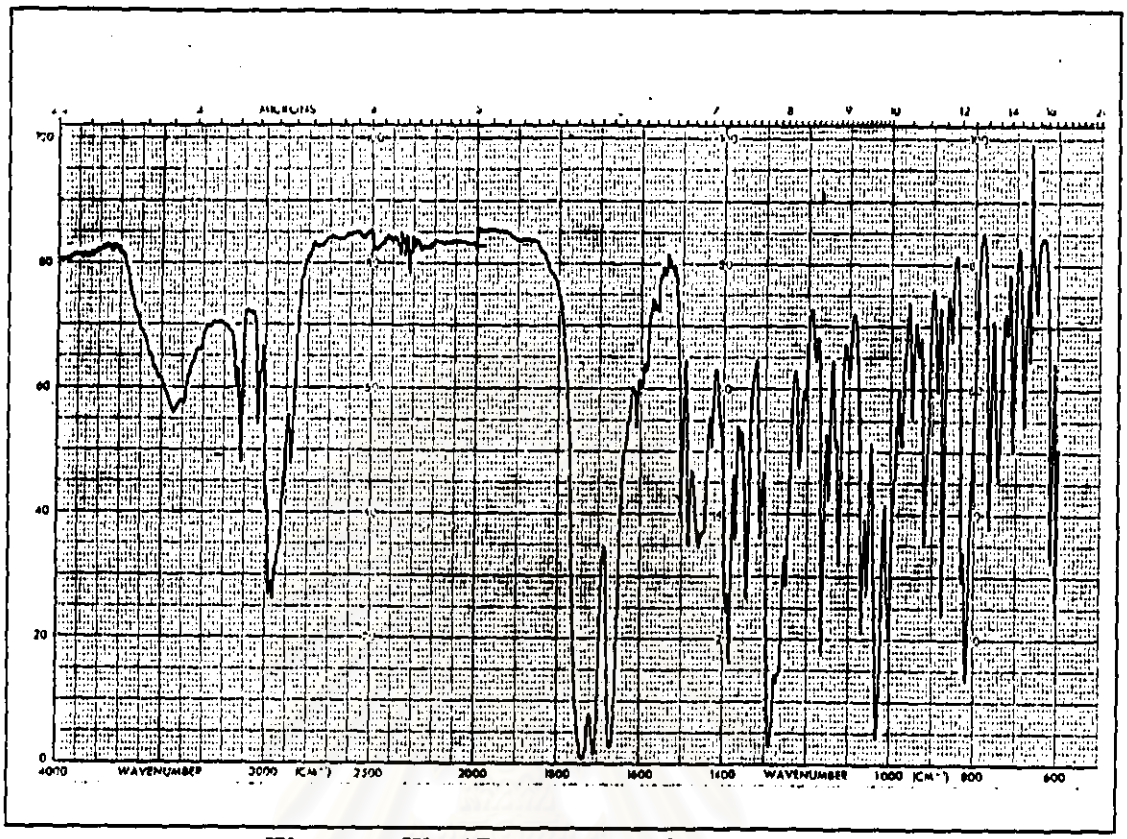


Fig. 3.20 The IR spectrum of Compound 5

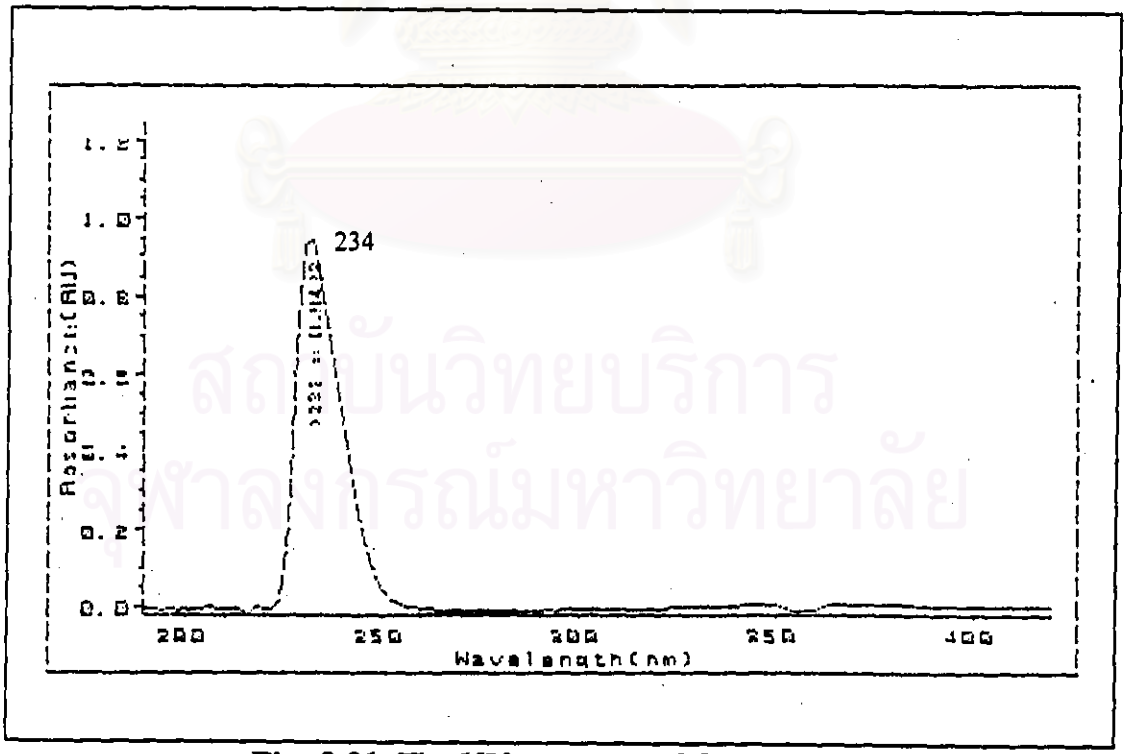


Fig. 3.21 The UV spectrum of Compound 5

The ^{1}H NMR spectrum (Fig 3.22) showed the signals with 1H each at 7.42 ppm (s), 7.39 ppm (d, J=1.5 Hz) and 6.37 ppm (d, J=1.2 Hz) of aromatic protons. This set of signal was particularly characteristic of a furan nucleus.[42] Two doublet signals centered at 7.1 and 5.92 (J= 10.07 and 10.1 Hz, respectively) with 1H each were matched with olefinic protons. The signals at 5.47 ppm (1H, s) and 3.88 ppm (1H, s) could be assigned for methine protons which attached to a carbon adjacent to an oxygen atom. Two doublet of doublet signals with a relative intensity of 1 proton each centered at 2.93 ppm (J=14.6 and 4.8 Hz, respectively) and 2.41 ppm (J=13.7 and 3.1 Hz, respectively) were typical for methylene protons. The signals at δ (ppm) 2.20 and 1.84 (m, 2 H each), 2.01 and 1.48 (m, 1H each) were corresponded to methylene and methine protons. A set of singlet methyl groups was observed at δ (ppm) : 1.40, 1.27, 1.16, 1.14 and 1.13 (s , 3H each).

The ^{13}C NMR spectrum of this compound (Fig 3.23) exhibited a total of 26 signals. From DEPT 90 (Fig. 3.14) showed signals for 9 methine carbons at δ (ppm) : 155.92, 143.07, 140.96, 126.34, 109.72, 77.95, 54.50, 53.56 and 47.54, respectively.

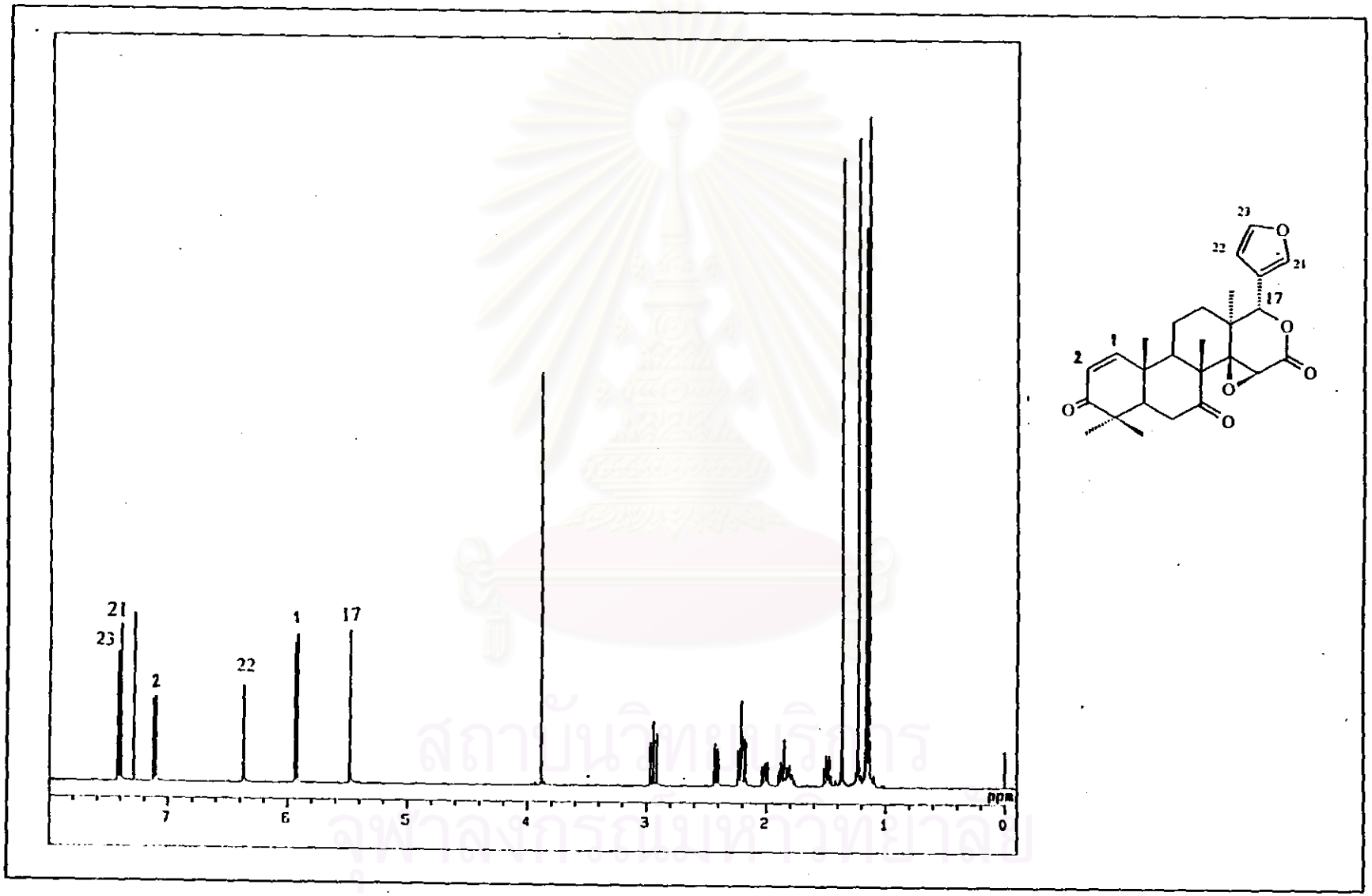


Fig. 3.22 The ^{1}H NMR spectrum of Compound 5

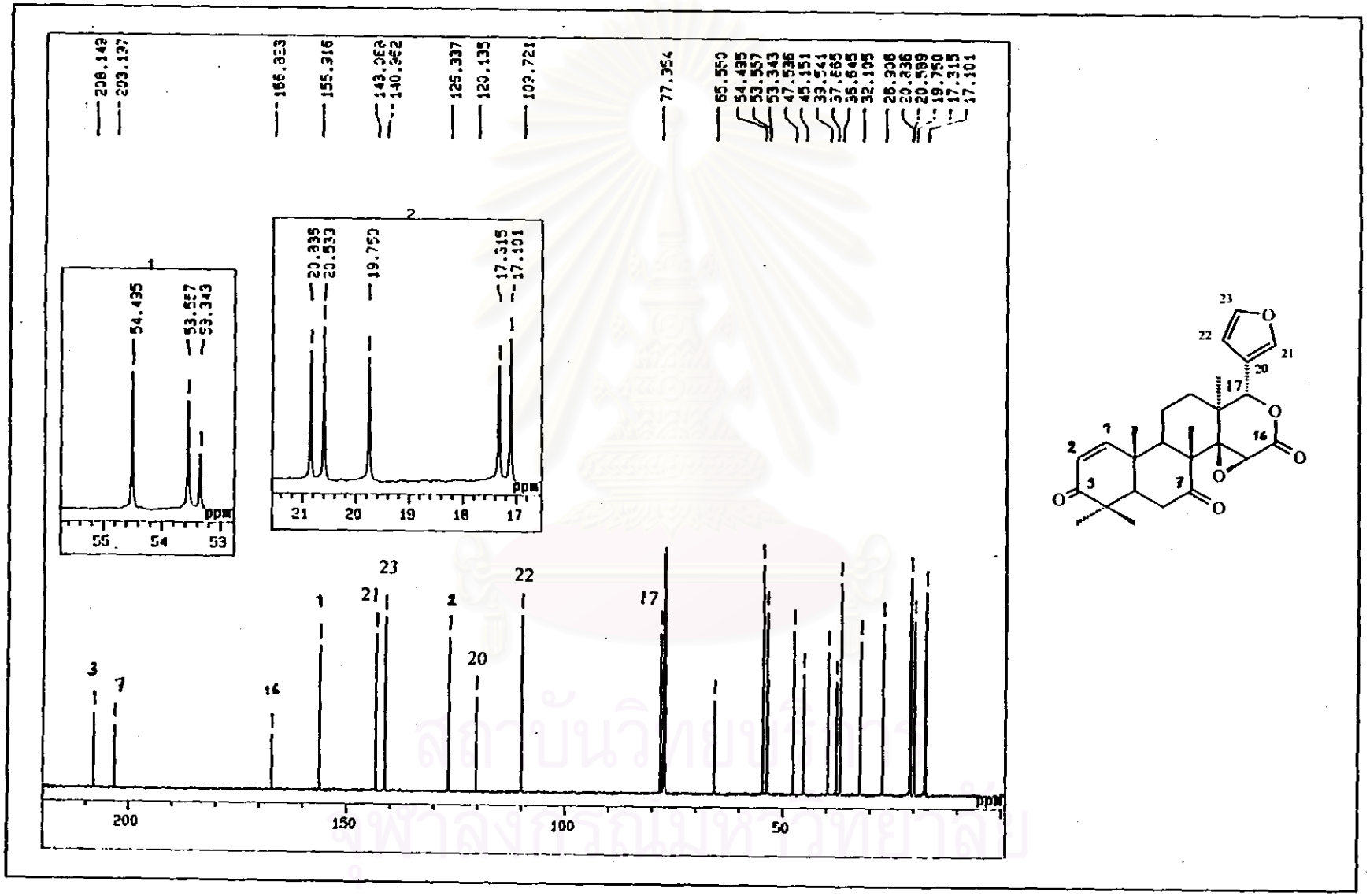


Fig. 3.23 The ^{13}C NMR spectrum of Compound 5

DEPT 135 (Fig 3.24) displayed 14 signals of -CH<, $-CH_3$ which were assigned for five methyl carbon signals at δ (ppm) : 26.9, 20.8, 20.6, 19.8 and 17.3, respectively, and for three signals of $-CH_2-$ at δ (ppm): 36.7, 32.1 and 17.1. The rest signals were compatible with 9 quarternary carbons. Two signals at 208.1 and 203.1 ppm could be assinged for 2 ketonic carbons. The signal at 166.8 ppm was likely a carbonyl carbon of ester. Others were obsreved at 120.1, 65.6, 53.3, 47.5, 39.5 and 37.7 ppm.

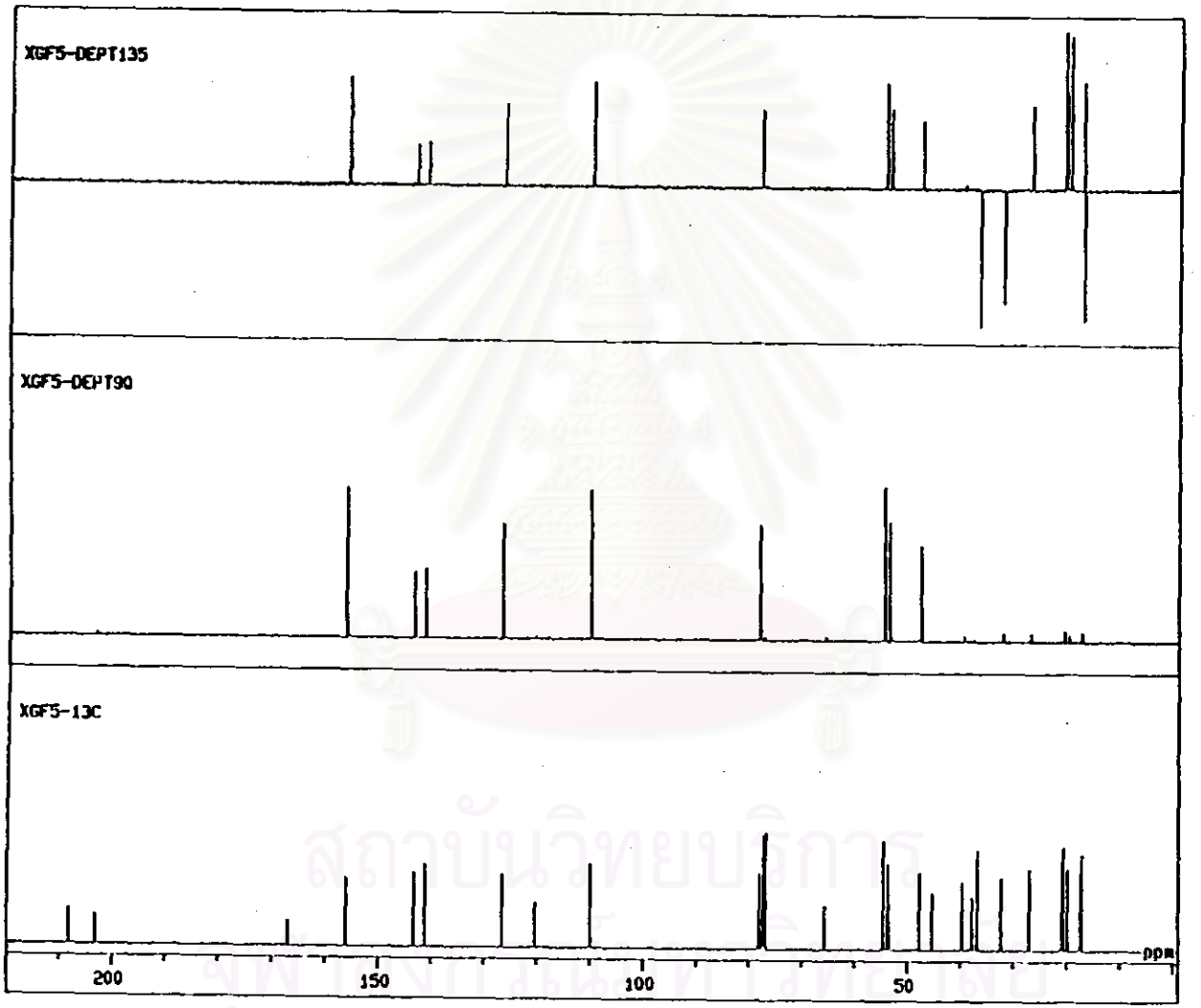
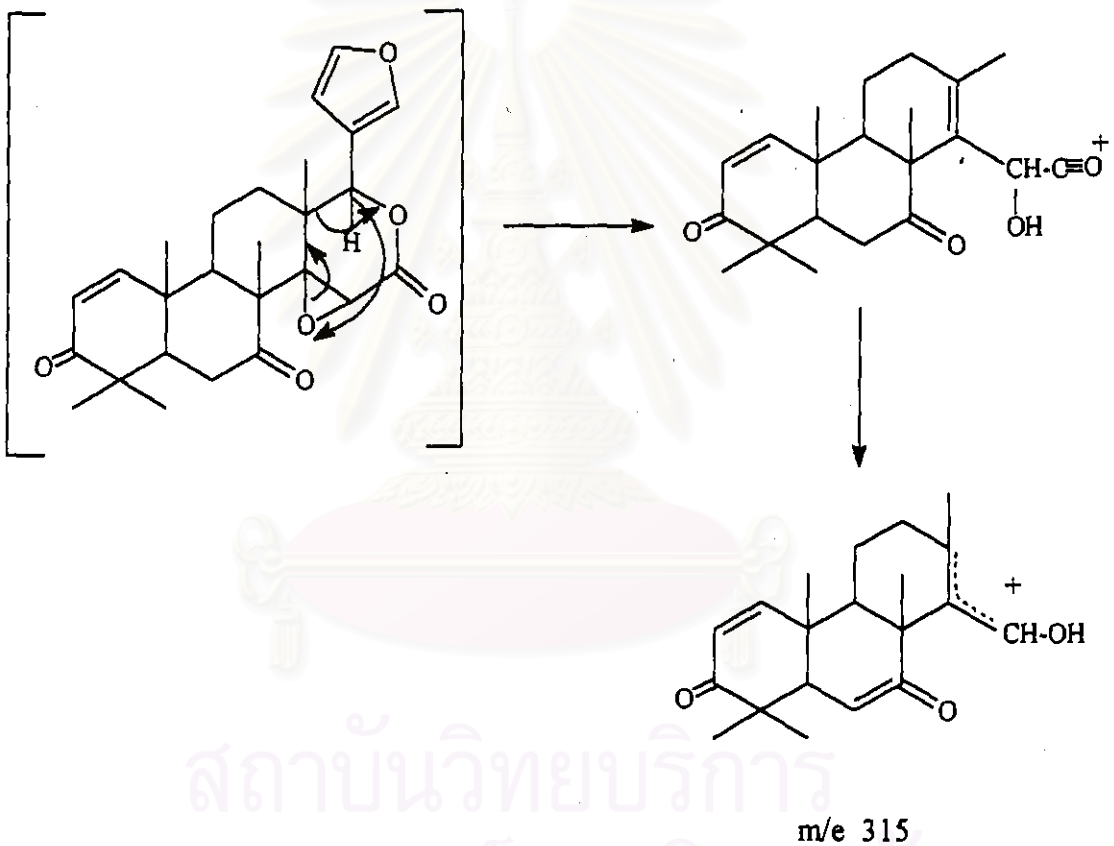


Fig. 3.24 The DEPT 90 and DEPT 135 of Compound 5

The mass spectrum (Fig 3.25) did not show the molecular ion peak (M^+), but exhibited the important fragmentation peaks at m/z 315, 232, 189, 163, 161, 149, 137, 121 and 95. From the literature search,[43] there was no report of the mass spectrum of this compound. The fragmentation for the base peak at m/z 315 was, therefore, proposed as shown below:



m/e 315

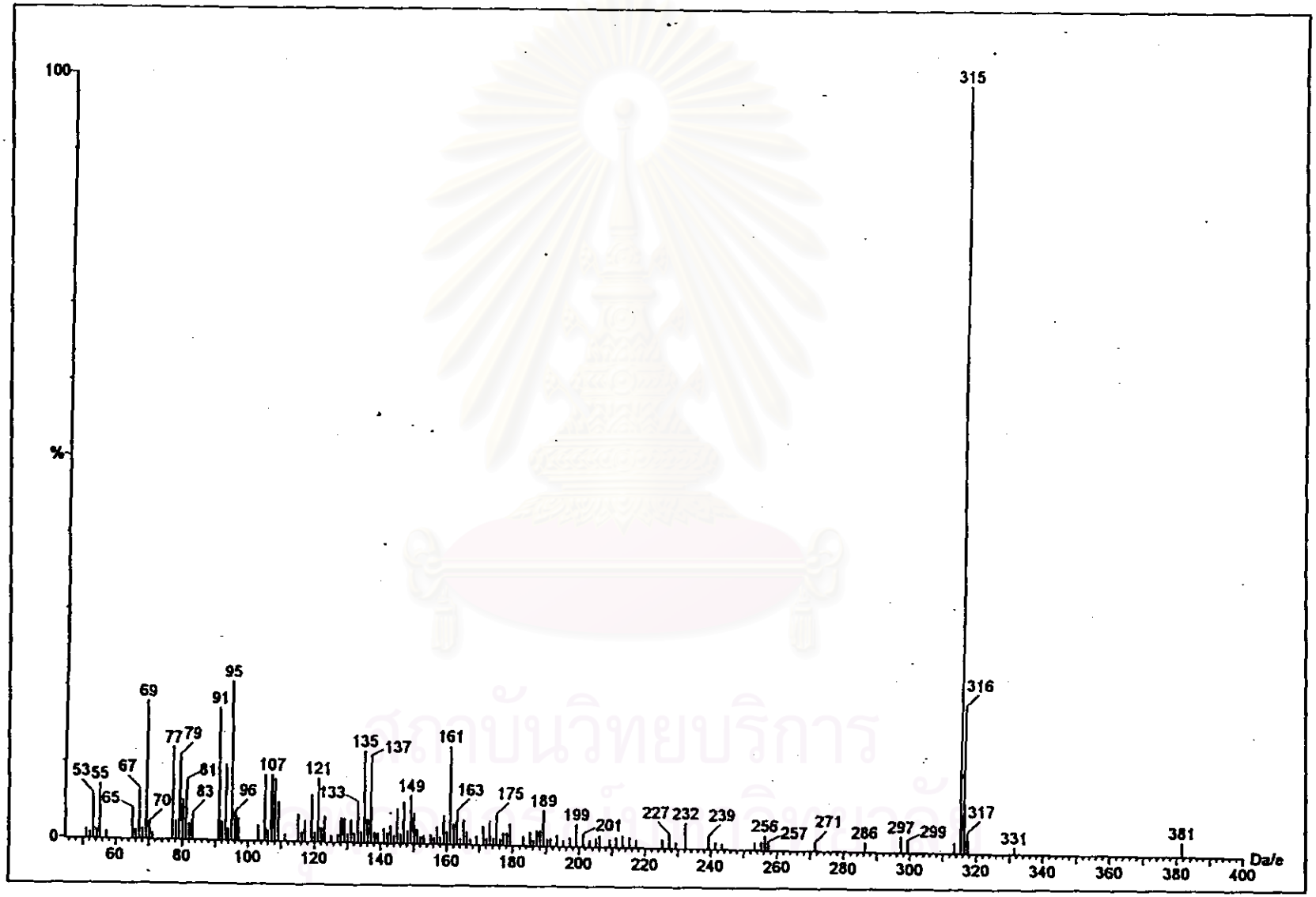
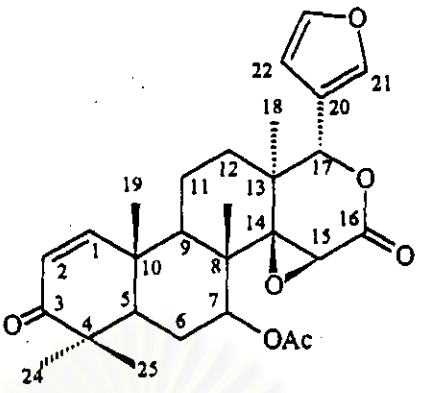


Fig. 3.25 The mass spectrum of Compound 5

From the above mentioned NMR data, this compound was composed of 26 carbon atoms (5-CH_3, 3-CH_2-, 9 -CH< and 9-C<), 30 hydrogen atoms and 5 oxygen atoms (2 for ketone, 2 for ester , possibly lactone, and 1 for furan). The carbon signal at 65.6 ppm located in down feild range of alkane which implied that this carbon should adjacent to the electronegative atom such as ether linkage or epoxide ring. Thus, this compound should possess the formular structural formular $C_{26}H_{30}O_6$. The degree of unsaturation was 12.

The assignment of ^{1}H-NMR spectrum was in good agreement with the data derived from the ^{13}C-NMR spectrum. To illustrate this, four signals observed at δ 143.1, 140.9, 120.1 and 109.7 ppm were corresponded to the signals of C-21, C-23, C-20 and C-22 of a furan ring, respectively (see structure below). From the ^{1}H-NMR spectrum, the signals at δ (ppm) 7.42, 7.39 and 6.37 ppm were assigned for H-21, H-23 (α-furan) and H-22 (β-furan), respectively. These sets of signals were found to be typical signals for β-substituted furan nucleus in limonoid-type structures.[44] Moreover, the pattern of ^{13}C NMR signals of Compound **5** as presented in Table 3.12 was found to be closely related to that of a well-known limonoid, gedunin. The structure of Compound **5** was , therefore, elucidated by comparison with the ^{1}H NMR and ^{13}C NMR data with those of reported gedunin.[45]



gedunin

The similarity of both compounds was the presence of a ketonic carbon at C-3 , an epoxide ring at C-14 and C-15 and unsaturated methine carbon at C-1 and C-2. The occurance of α, β-unsaturated carbonyl moiety was also coincided with the data obtained from the UV spectrum (λ_{max} = 234 nm, log ε = 4.61). (Fig.3.21)[41] The signals corresponded to an acetyl group , *i.e*, the signal for C-7 at 169.8 ppm and the ^{1}H signal for H-7 at 4.50 ppm was not found for Compound **5**. There is, however, another signal at 208.14 ppm which could be assigned for the presence of another ketonic group in Compound **5**. One of the most characteristic features of the spectra of the meliacin is the proton signal of H-17 position . This signal always appearred at 5.47 ppm in gedunin as a sharp singlet. However, when C-7 was replaced by a keto group, this signal would shift upfeild in the range of 5.37-5.44 ppm and the signal of H-15 would shift downfeild in the range of 3.60-3.85 ppm.[44]

After making a comparison of the NMR data of both compounds, it was found that Compound **5** could possibly be a derivative of gedunin: 7-oxo-7-deacetoxy gedunin.

Based upon the information obtained from homonuclear bidimensional technique including ^{13}C-^{1}H (COSY), ^{1}H-^{1}H (COSY), C-H correlation, long-range COSY, the assingment for C-9 and C-15 in 7-oxo-7-deacetoxy gedunin was found to be improper. The observed correlation between the ^{13}C and ^{1}H signal determinded for the ^{13}C signal could easily be distinguished for C-9 and C-15. From previous literature[45], the ^{13}C signals of C-9 and C-15 were presented at δ 53.6 and 54.5 ppm, respectively. The ^{13}C-^{1}H COSY (Fig 3.25) showed that the correlation between the ^{13}C signal at δ 53.6 ppm and the ^{1}H singlet signal at δ 3.88 ppm indicated that this proton had no adjacent proton. Moreover, the correlation between the ^{13}C signal at 54.5 ppm and the ^{1}H signal at 2.18 ppm (dd, 2H, J = 3.4, 14.7 Hz) clearly showed two unequivalent protons adjacented to this proton. According to the formular structure of Compound **5**, it was found that the C-15 connected to C-14 and C-16 which were quarternary carbons. Hence, H-15 had no adjacent proton. The C-9 connected to C-11 which was a methylene carbon; H-9 had, therefore, two adjacent protons. These observation were corresponded to the above spectral data. Hence, the chemical shift assingment for both C-9 and C-15 had to be switched as presented in Table 3.12.

From ^{1}H-^{1}H COSY (Fig 3.26), H-9 was adjacent to H-11 emerged from the correlation between ^{1}H at δ 2.18 (H-9) and 1.80 (H-11). Moreover, the NOESY technique (Fig 3.27) strongly indicated that both protons had the same stereochemistry. Thus, 2D-NMR information enstrengthed the assignment for signals at δ 53.6 and 54.5 ppm must be C-15 and C-9, respectively.

The doubtful 5 methyl signals of C-24, C-18, C-9, C-25 and C-26 could be assigned by using ^{13}C-^{1}H long-range COLOC (correlation spectroscopy via long-range coupling (Fig 3.28) which is presented in the Table 3.12.

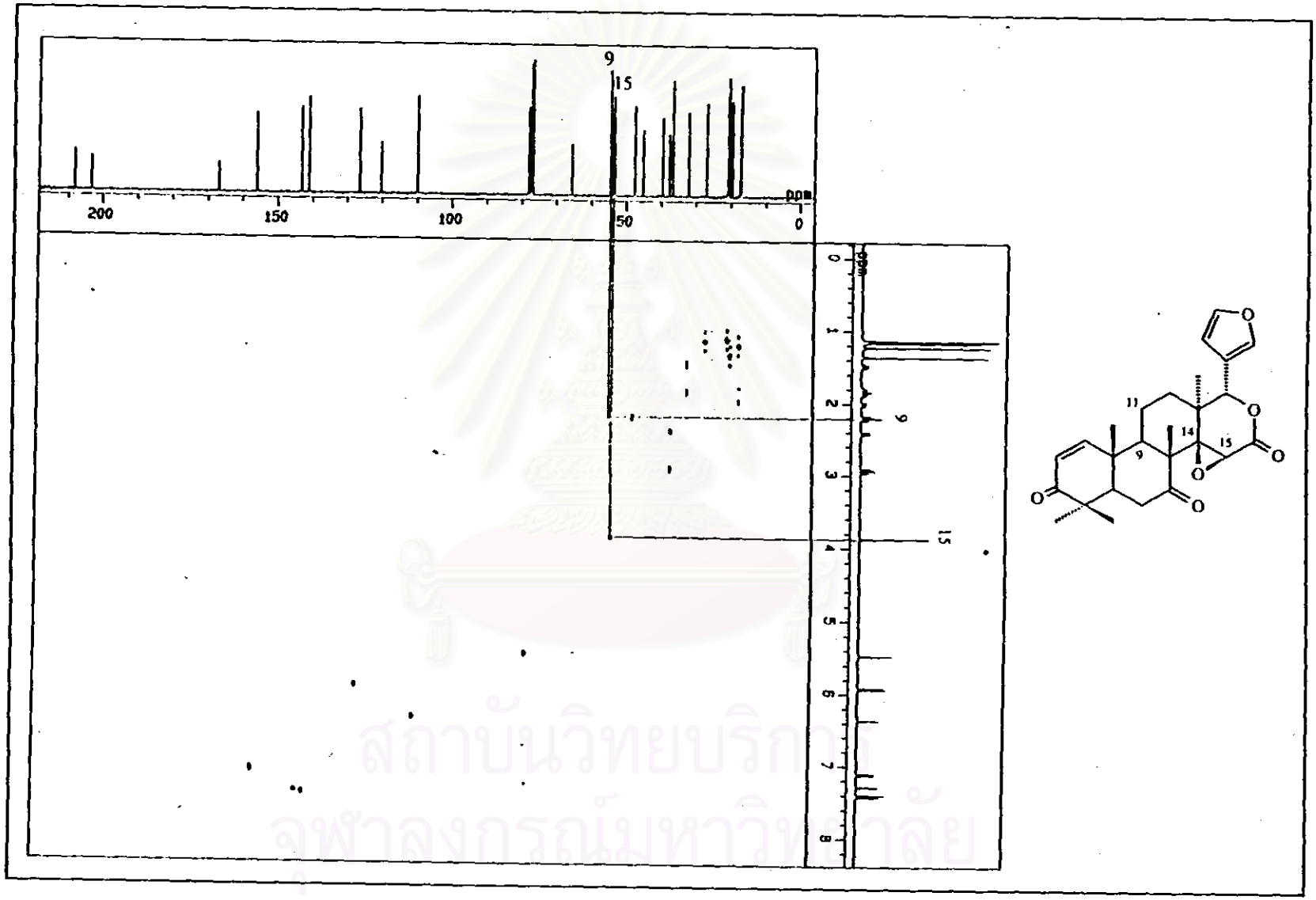


Fig. 3.26 The ^{13}C-^{1}H COSY spectrum of Compound 5

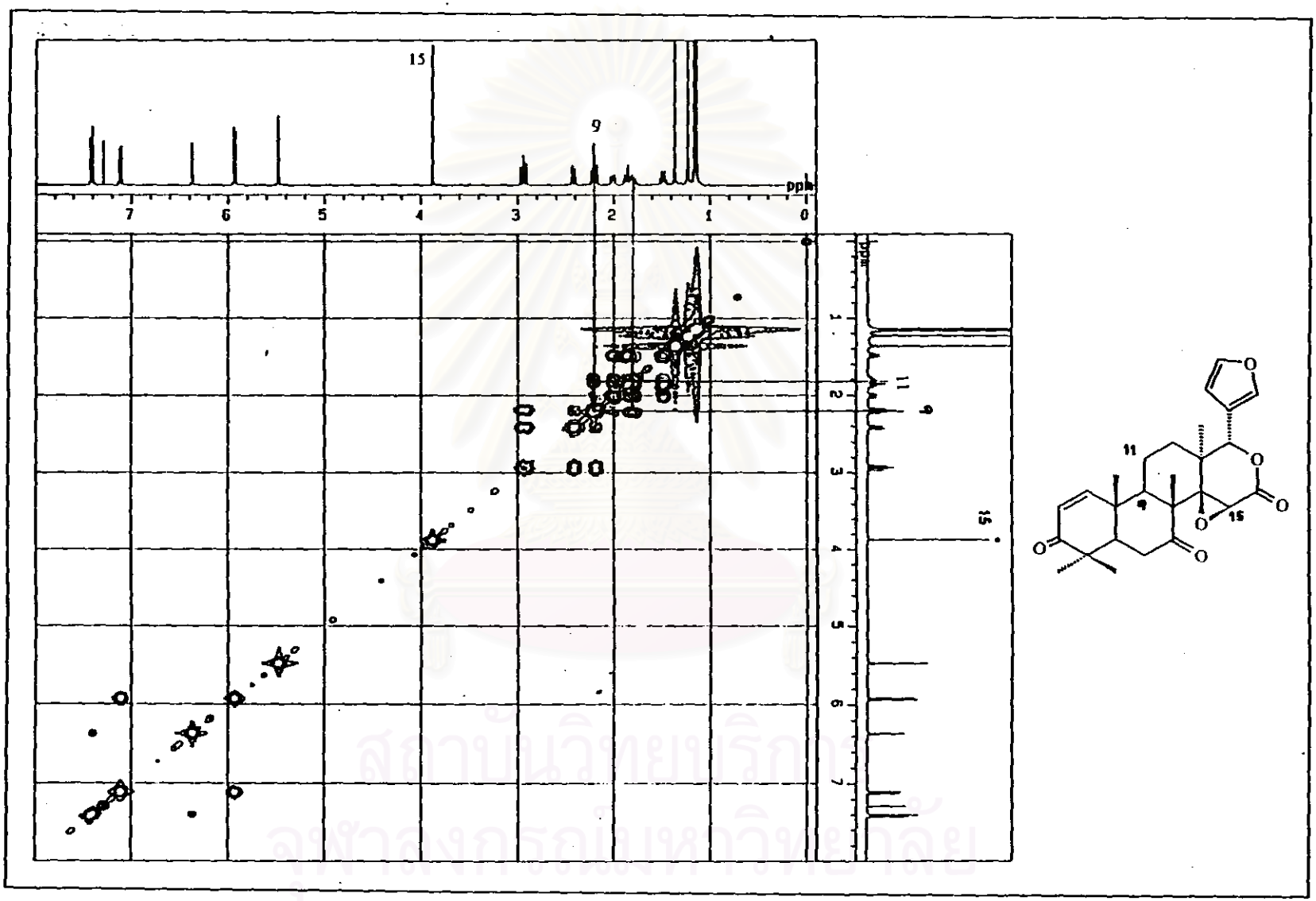


Fig. 3.27 The ^{1}H-^{1}H COSY spectrum of Compound 5

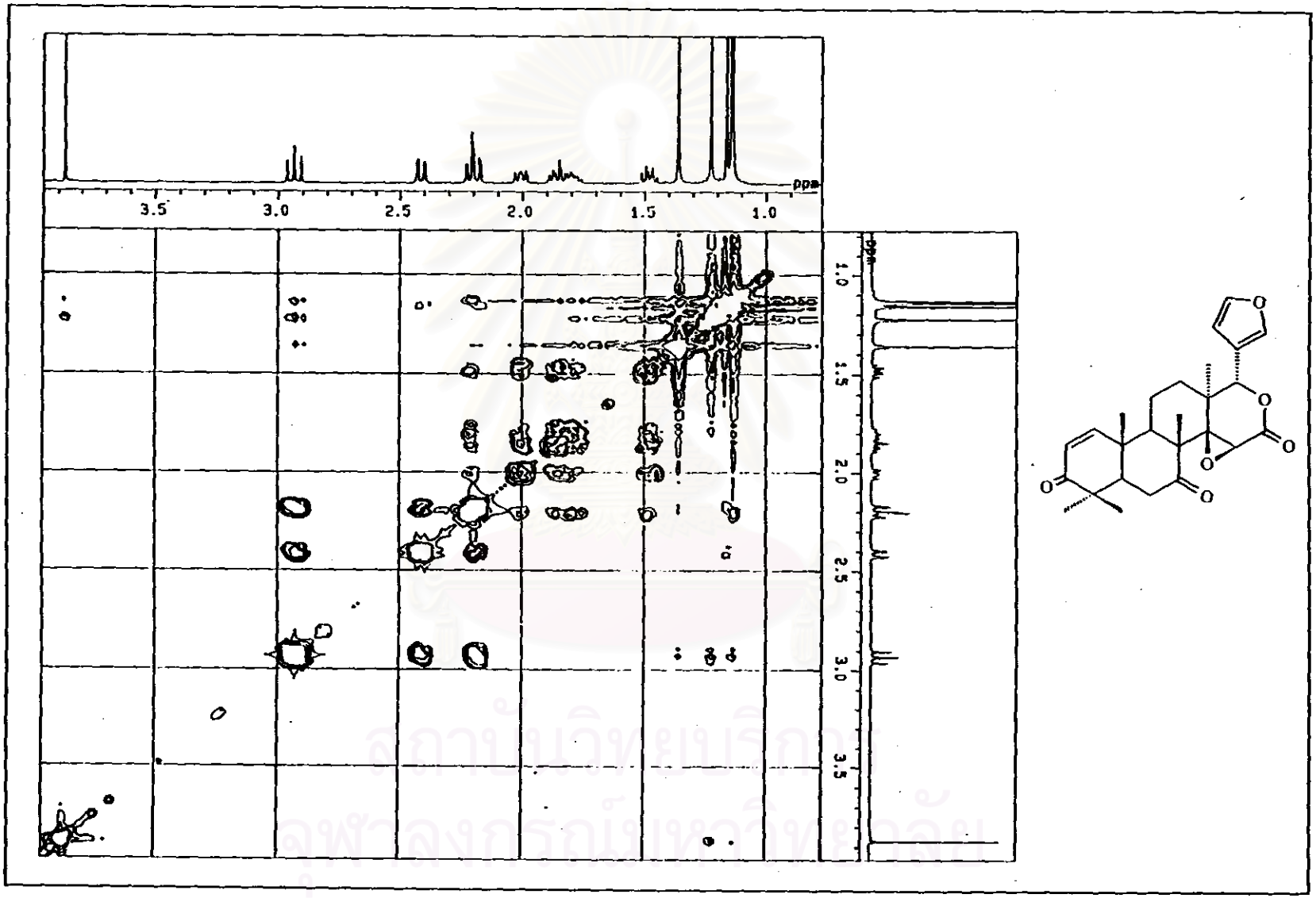


Fig. 3.28 The ^{1}H-^{1}H NOSEY spectrum of Compound 5

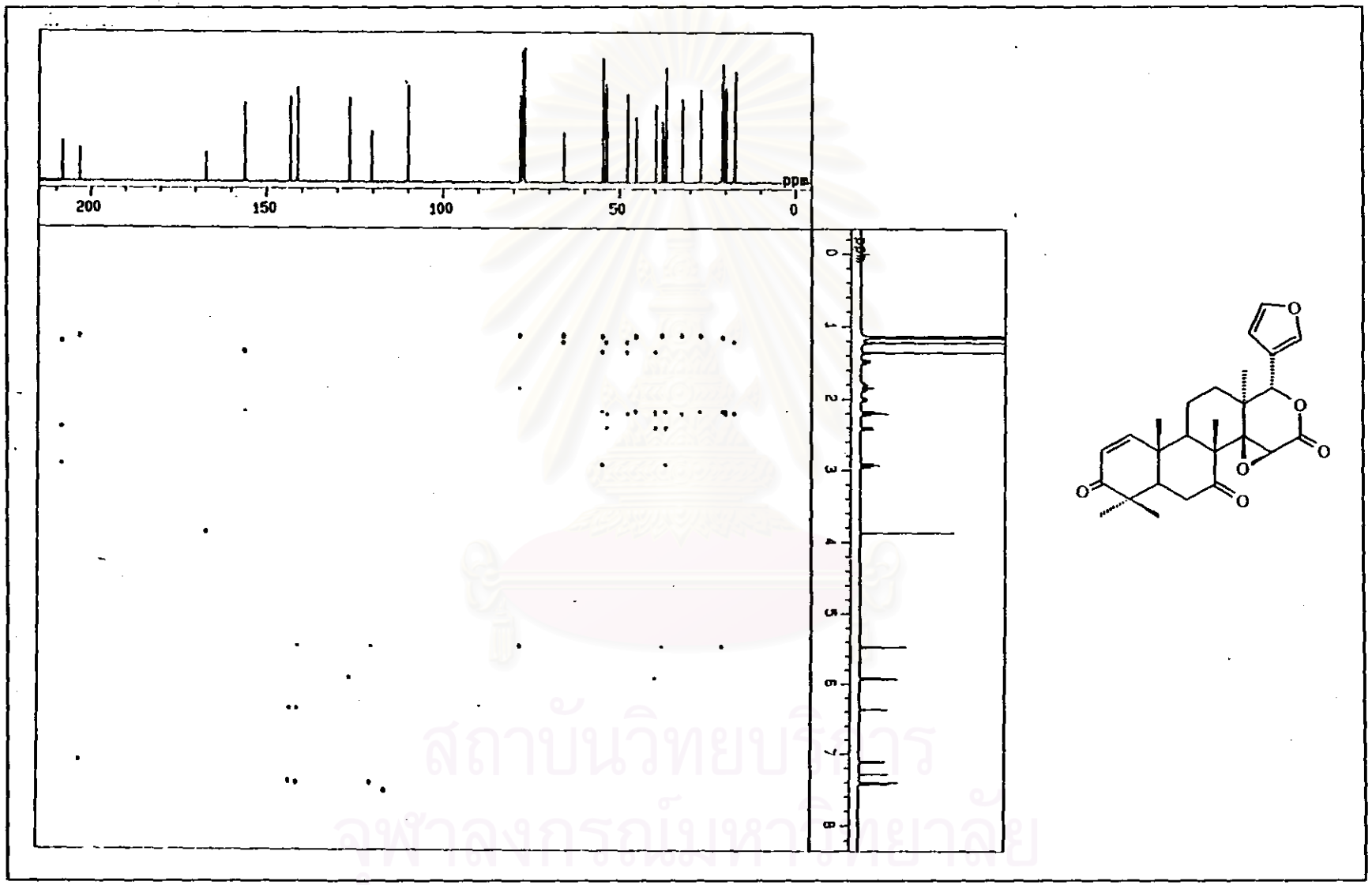


Fig. 3.29 The long-range COSY spectrum of Compound 5

The NMR data of gedunin, 7-oxo-7-deacetoxy gedunin and Compound **5** are presented in Table 3.12.

Table 3.12 The comparison of the ^{13}C and ^{1}H NMR chemical shift of 7-oxo-7-deacetoxy gedunin, gedunin and Compound **5**

Position	Chemical Shift (ppm)					
	7-oxo-7-deacetoxy gedunin [44, 45]		gedunin [44, 45]		Compound **5**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	156.0	7.09	157.0	7.07	155.9	7.14
2	126.3	5.88	125.9	5.81	126.3	5.92
3	203.2	-	203.8	-	203.2	-
4	45.2	-	44.0	-	45.6	-
5	47.6	*	39.5	*	47.5	2.23
6	36.7	*	14.9	*	36.7	2.93, 2.41
7	208.3	-	73.2	4.50	208.2	-
8	53.4	-	42.6	-	53.3	-
9**	53.7	*	46.0	-	54.6	2.18
10	40.0	-	40.0	-	39.5	-
11	17.1	*	17.7	-	17.1	2.01, 1.80
12	32.1	*	18.3	-	32.1	1.85,1.50
13	37.7	-	38.7	-	37.7	-
14	65.7	-	69.8	-	65.6	-
15**	54.5	3.85	56.9	3.50	53.6	3.88
16	166.9	-	167.4	-	166.8	-
17	78.0	5.44	78.2	5.57	77.9	5.47
20	120.3	-	120.5	-	120.1	-

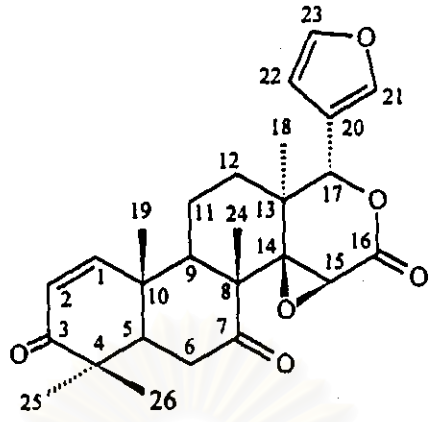
Table 3.12 (Continued)

Position	Chemical Shift (ppm)					
	7-oxo 7-deacetoxy gedunin [44, 45]		gedunin [44, 45]		Compound **5**	
21	143.1	7.37	143.0	7.37	143.1	7.39
22	109.8	6.34	109.8	6.30	109.7	6.37
23	141.0	7.37	141.4	7.37	140.9	7.42
$CH_3\underline{C}O$	-	-	169.8	-	-	-
CH_3	17.4	1.22	27.2	1.04	17.3(C-18)	1.08
	20.7	1.14	26.0	1.04	20.6(C-19)	1.06
	27.0	1.14	23.3	1.13	26.9(C-24)	1.16
	19.7	1.35	21.7	1.20	19.8(C-25)	1.39
	20.9	1.14	21.0	1.23	20.8(C-26)	1.06
Ac	-	-	19.7	2.07	-	-

* not reported

**not properly assinged

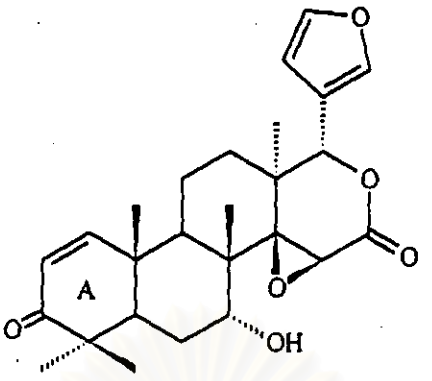
Confirmed by both physical properties and spectroscopic data, Compound **5** should be 7-oxo-7-deacetoxy gedunin. Its IUPAC name is 14,15:21,23-diepoxy-4,4,8-trimethyl-D-Homo-24-nor-17-oxachola-1,20,22-triene-3,7,16-trione and the structure is shown below:



7-oxo-7-deacetoxy gedunin

From previous literature related to the bioactivity,[46] 7-oxo-7-deacetoxy gedunin and other limonoids from Meliaceae were examined for the insect ecdysis inhibiting activity. However, 7-oxo-7-deacetoxy gedunin did not give an effective result. In addition, other limonoids from Meliaceae and Compound **5** were investigated for a structure/activity relationship against the murine P-388 lymphocytic leukemia cell line. Some limonoids gave promising activity, but this compound was found to be inactive.[47]

Furthermore, the antifeedant activity of isolated compounds from neem oil, *Azadirachta indica* was estimated against the Hermite, *Reticulitermes speratus*.[48] The most potent antifeedant was deacetyl gedunin. The structure is shown below.



deacetyl gedunin

Based on the characteristic of insect antifeedant reported, the active compound considered to be an antifeedant agent had some common structural features such as a furan ring moiety and an α,β-unsaturated ketone in ring A. The particular feature for deacetyl gedunin over among the isolated compounds was the presence of a C-7 hydroxyl group which seemed to be the main feature determining the antifeedant activity. Thus, Compound **5** which was a derivative of gedunin and closely related to deacetoxy gedunin was probably an active ingredient of this fraction.

Purification, Properties and Structural Elucidation of Compound 6

Compound **6** was obtained in Fraction I, III, VI and VIII (Table 3.2, 3.3, 3.4 and 3.6), respectively, as the white bright solid in yellow oil. The yellow oil was eliminated by washing with methanol, the precipitate was recrystallized twice by hot methanol to yield cubic crystal, 6.5 g (5.43 % wt. by wt. of Fraction I), 5.5 g (6.90 % of Fraction III), 7.3 g (8.75 % of Fraction VI) and 3.6 g (4.05 % of Fraction VIII), respectively.

This compound has a melting range of 235-237°C and R_f 0.44 (solvent system: 8 % methanol in dichloromethane). The optial rotation was +10.74 (CH_2Cl_2, c = 7.35 %). Compound **6** was soluble in dichloromethane and acetone but slightly soluble in ethyl acetate and methanol. It gave a negative result with 2,4-DNP. This result indicated that this compound was absence of a carbonyl functional group.

The IR spectrum (Fig. 3.30) showed the presence of O-H stretching vibration at 3570 cm^{-1} (s) and C=O stretching vibration of ester at 1735 cm^{-1}. The C-H stretching vibration of aromatic was observed at 3120 cm^{-1} (s). The absorption peak at 1500 cm^{-1} (w) and 880 cm^{-1}(m) were indicative of the presence of β-substituted furan ring.[40] Other absorption bands at 1300-1100 cm^{-1}(m) were due to C-O stretching mode of either a carbonyl or hydroxyl group. The UV spectrum (Fig. 3.31) exhibited λ_{max} (CH_2Cl_2) at 232 nm, log ε = 3.21, this data clearly showed the absence of cojugated system as α,β-

unsaturated carbonyl moiety in this molecule.[37]

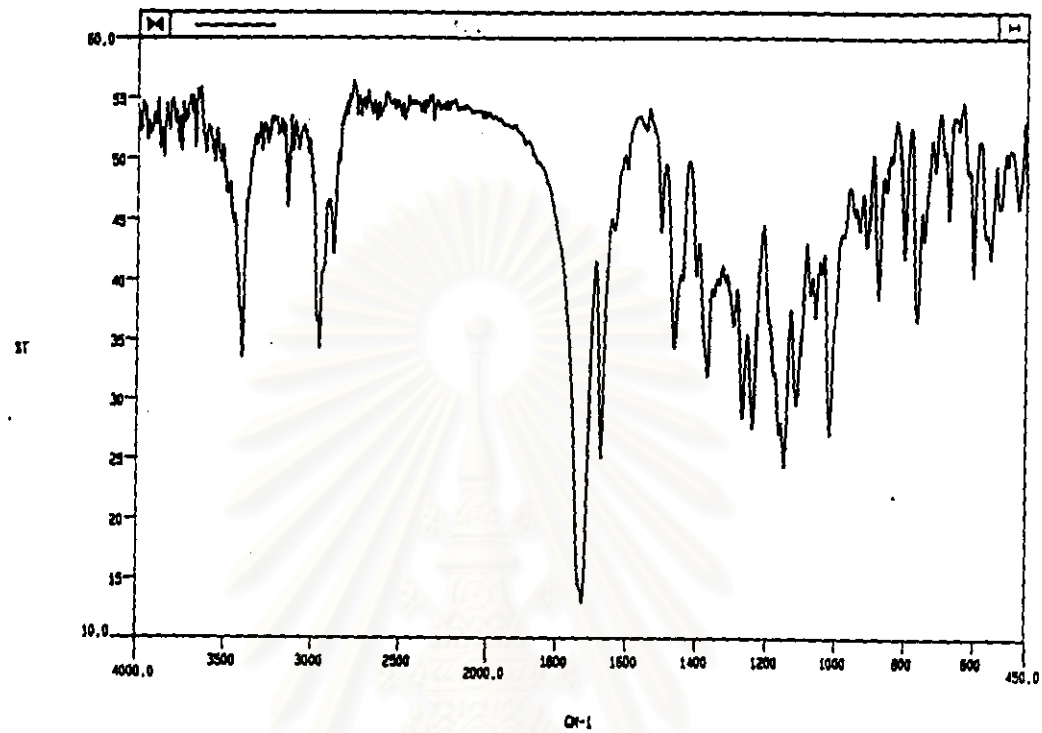


Fig 3.29 The IR spectrum of Compound 6

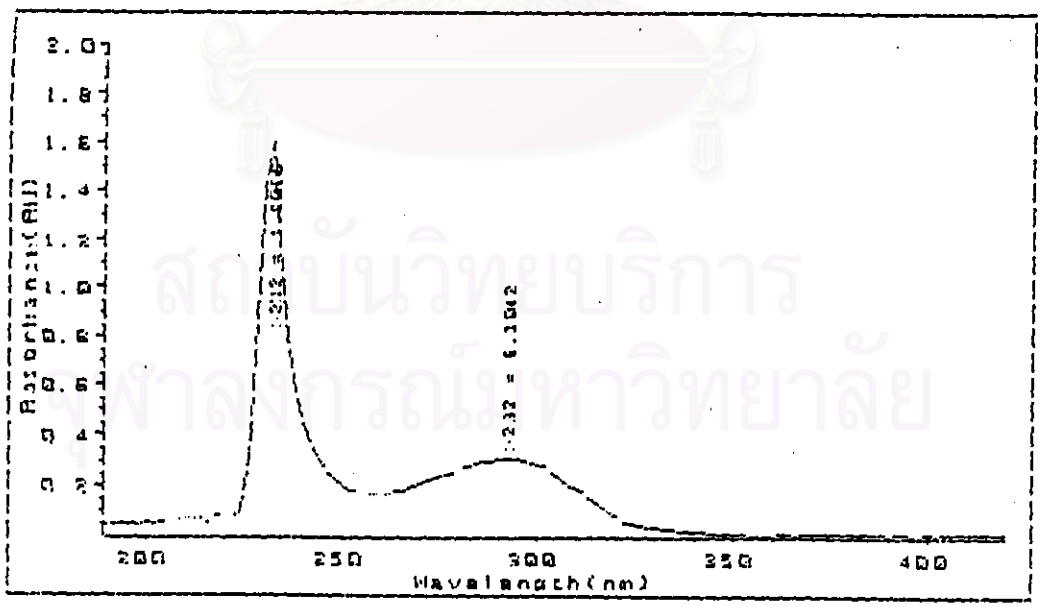
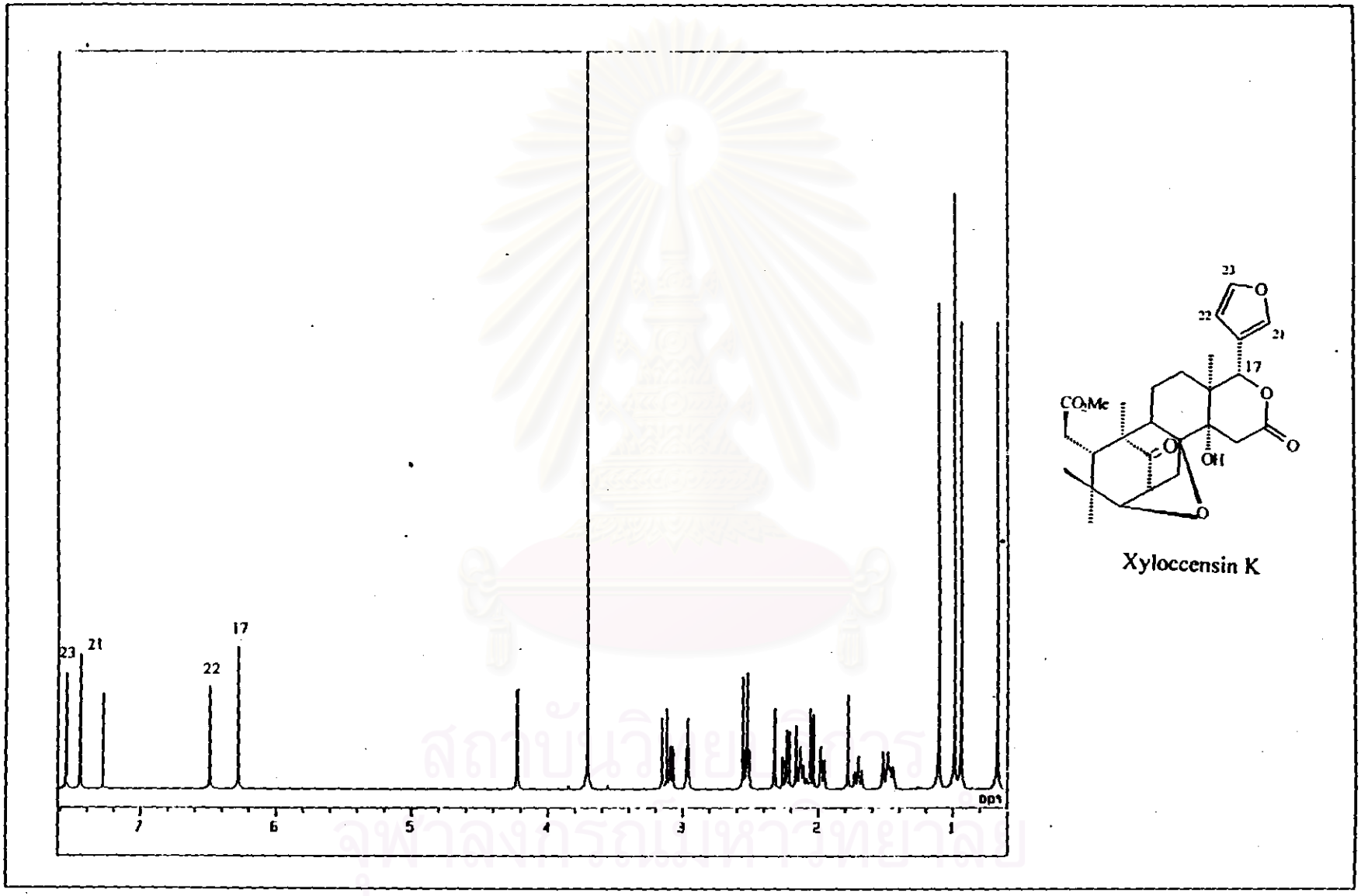


Fig. 3.30 The UV spectrum of Compound 6

The ^{1}H NMR spectrum (Fig 3.32) showed the important proton signals at δ (ppm): 7.55 (1H, s), 7.45 (1H, dd, J = 2.0, 2.0 Hz) and 6.48 (1H, d, J = 2.0 Hz) characteristic of a furan ring.[42] Two signals at δ 6.28 (1 H, s) and 4.22 (1H, d, J = 5.8 Hz) indicated the proton attached to a carbon adjacent to an oxygen atom. The singlet signal at δ 3.70 (3 H) was assinged for a methoxy group. A set of other siganls at δ 1.11, 0.99, 0.94 and 0.66 (3 H each, s) were indicative of four methyl groups. The signal at δ 1.77 was assigned for a hydroxy proton.

In addition, the ^{1}H NMR spectrum displayed the signal centered at δ (ppm) as follows: 3.13 (1H, d, J = 17.7 Hz), 3.08 (1H, dd, J = 2.0, 11.0 Hz), 2.96 (3 H, dd, J = 6.0, 12.2 Hz), 2.54 (1H, d, J = 18.0 Hz), 2.53 (1H, d, J = 17.7 Hz), 2.52 (1H, dd, J = 7.0, 12.0 Hz), 2.24 (1H, dd, J = 11.0, 17.0 Hz), 2.14 (1H, dd, J = 2.0, 17.0 Hz), 2.10 (1H, m), 2.04 (1H, d, J = 12 Hz), 1.97 1H, dd, J = 5.0, 12.5 Hz), 1.70 (1H, ddd, J = 4.0, 14.0, 14.0 Hz), 1.50 (1H, ddd, J = 1.5, 1.5, 14.0 Hz) and 1.46 (1H, m).

The ^{13}C NMR spectrum (Fig 3.33) and DEPT 90, 135 (Fig 3.34) showed 27 signals at δ (ppm) as follows: methyl carbons at 51.9, 27.9, 19.9 and 16.0, methylene carbons at 42.4, 36.8, 32.5, 28.58 and 17.8, methine carbons at 142.9, 140.7, 109.9, 91.3, 76.7, 51.9, 48.9, 42.9 and 19.9 and quarternary carbons at 215.2, 174.2, 170.7, 120.6, 85.4, 74.1, 50.9, 39.9 and 37.1.



Xyloccensin K

Fig. 3.32 The ^{1}H NMR spectrum of Compound 6

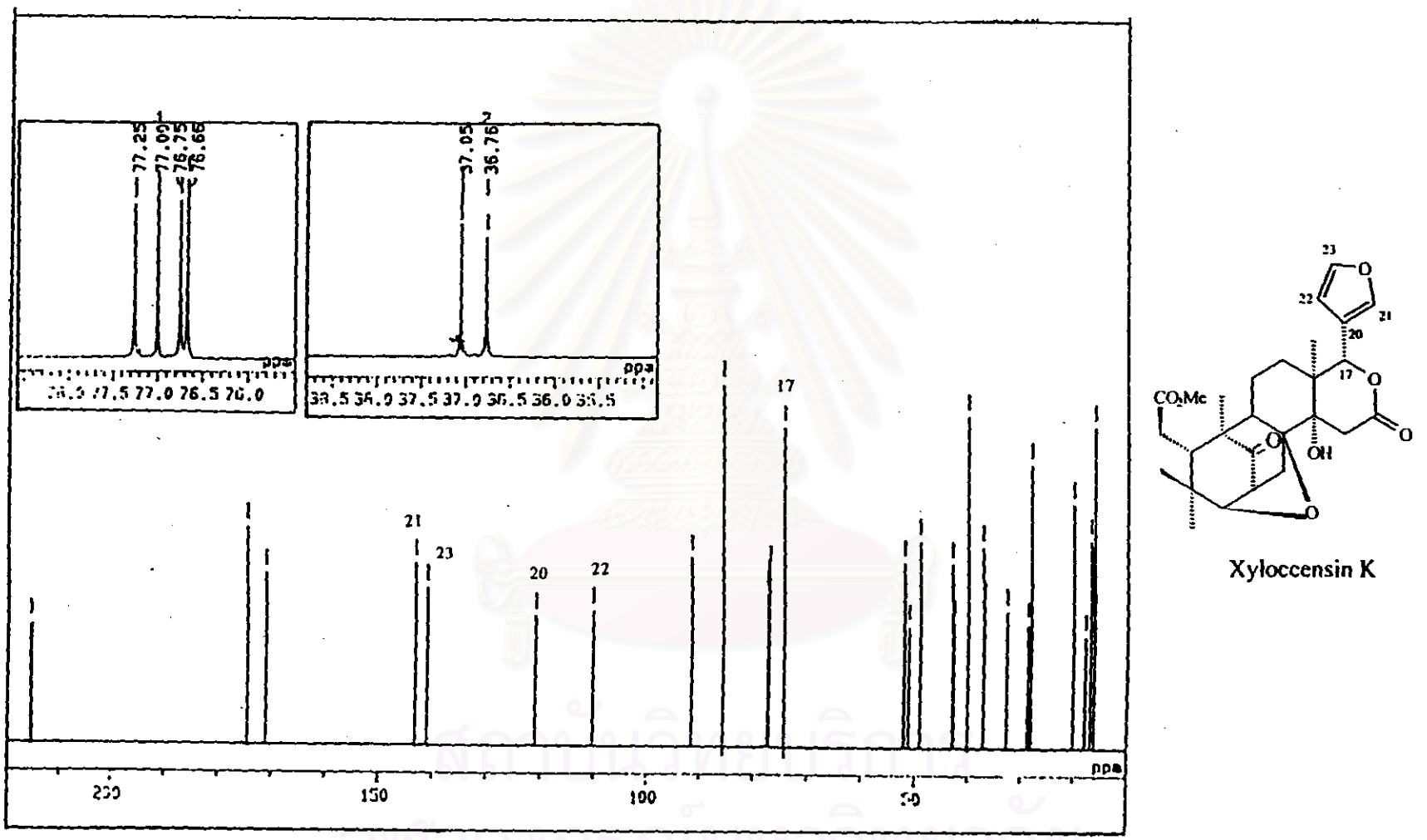


Fig. 3.33 The ^{13}C NMR spectrum of Compound 6

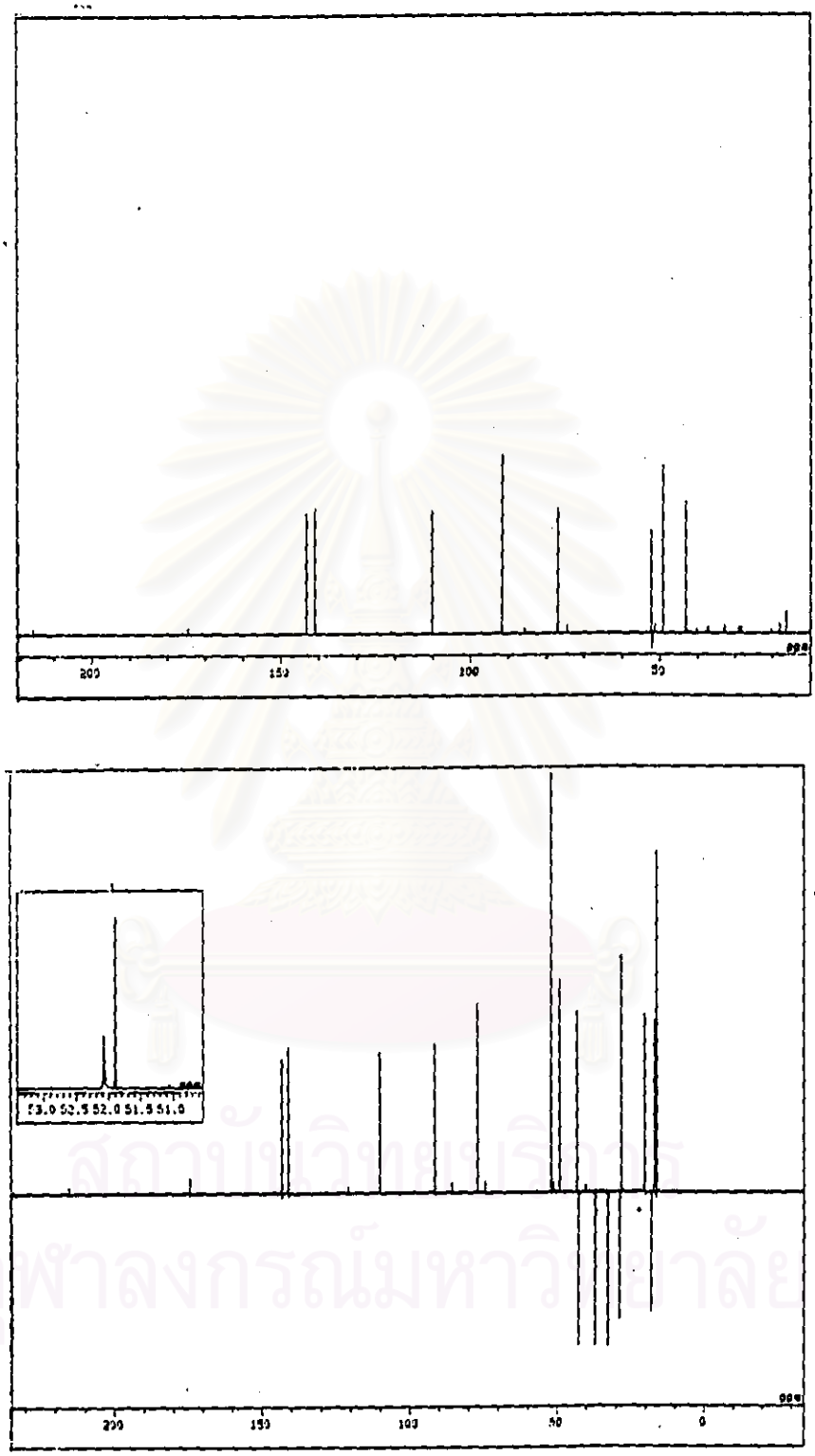
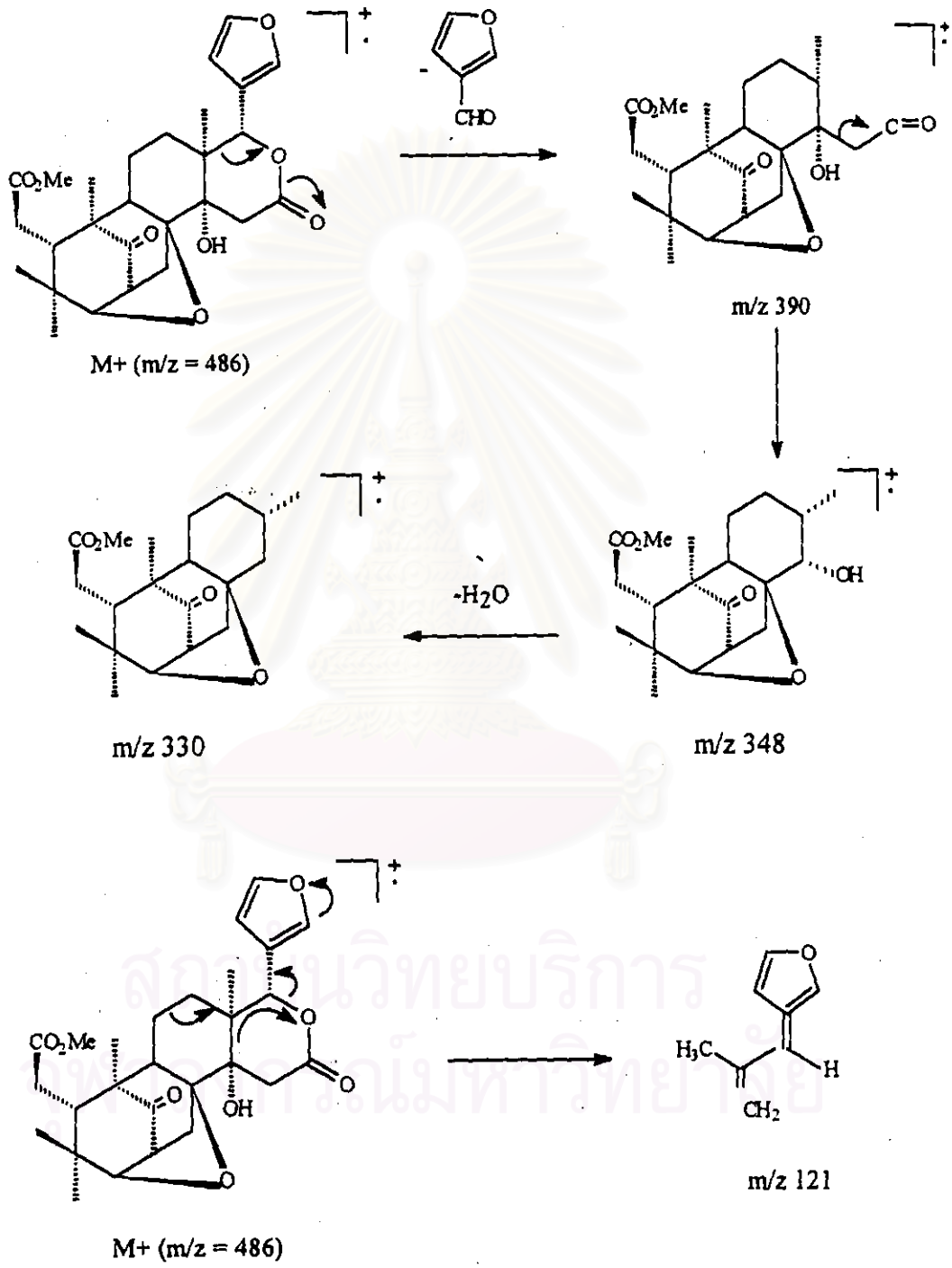


Fig. 3.34 The DEPT 90 and DEPT 135 of Compound 6

The mass spectrum of this compound (Fig 3.35) showed the molecular ion peak (M^+) at m/z (% rel.int) 486 (29.0). Other fragmentation peaks were found at m/z (% rel.int.) 440 (2.0), 348 (100.0), 330 (19.4), 209 (15.3) and 121 (30.0). The fragmentation at m/z 390 can be attributed to the structural of β-substituted furan. The loss of 42 led to m/z 348 which can attribut to the loss of $COCH_2$. The ion at m/z 330 resulted from the loss of H_2O from m/z 348. The fragment at m/z 121 was corresponded to th loss of furan-C_4H_6. The possible mass fragmentation pattern of this compound is shown in Scheme 3.3.

Scheme 3.3 The possible mass fragmentation pattern of Compound 6



According to the spectral data, it clearly revealed the presence of 5 methyl, 5 methylene, 8 methine, 9 quaternary carbons and 1 hydroxyl group. Thus, Compound 6 composed of 27 carbons, 34 protons and 1 oxygen atom. The molecular ion of m/z 486 was found to fit with the elemental analysis result. (Found: %C 66.86, %H 7.00 calcd. for $C_{27}H_{34}O_8$; MW 486.56: %C 66.65, %H 7.04). Therefore, the molecular of Compound 6 had to be $C_{27}H_{34}O_8$. The degree of unsaturation was calculated for 11.

From the ^1H and ^{13}C NMR spectra, it was clearly showed the presence of the typical signals for β-substituted furan nucleus which were generally found in limonoid compounds. The ^1H signals at δ 7.55, 7.45 and 6.28 showed the characteristic signals of protons in furan ring which could be definitely assigned for H-21, H-22 and H-23, respectively. Moreover, the ^{13}C NMR spectrum exhibited the signal at δ 120.6, 142.9, 109.9 and 140.7 which could be assigned for C-20, C-21, C-22 and C-23, respectively.[44,45]

In addition, the significant carbon signals revealed the presence of a ketonic carbon at δ 215.21 and 2 ester carbons at δ 174.2 and 170.7. The ^{13}C signals at 91.3, 85.4, 76.7 and 74.1 were indicated for carbon atoms which adjacent to an electronegative atom. The rest signals were found to be corresponded to sp^3 carbons.

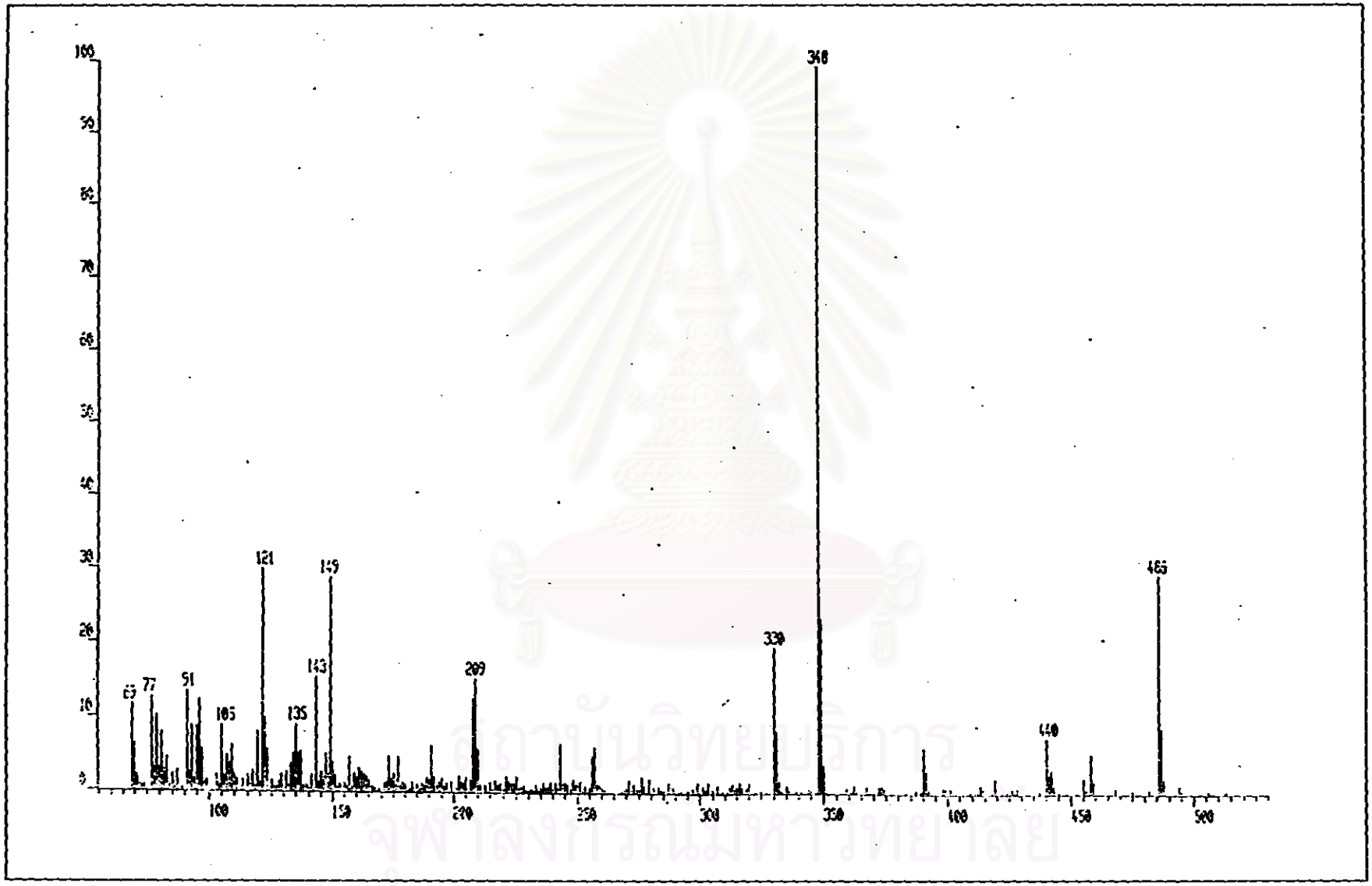
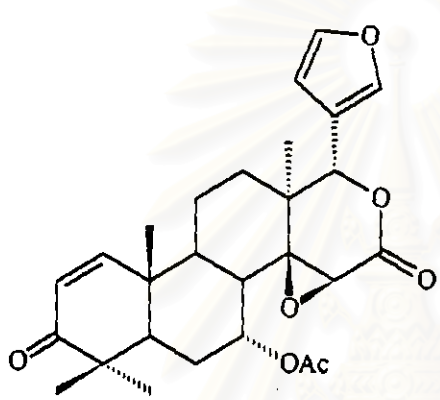
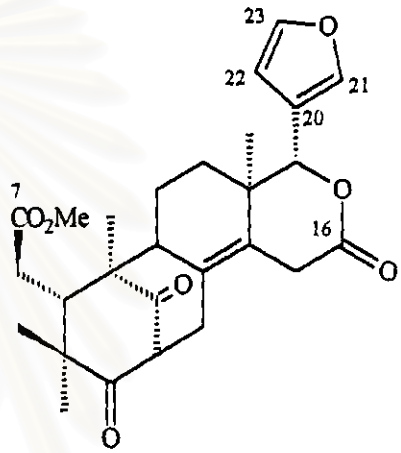


Fig. 3.35 The mass spectrum of Compound 6

The structural elucidation of this compound was initially compared with the previous reported NMR data of some limonoid compounds. The limonoid type which probably closely related to this compound was belonging to either a gedunin group, such as Compound **5**, or a mexicanolide group such as methyl 3β-*n*-butyryloxy-1-oxomeliac-8(14)-enate (**48**).

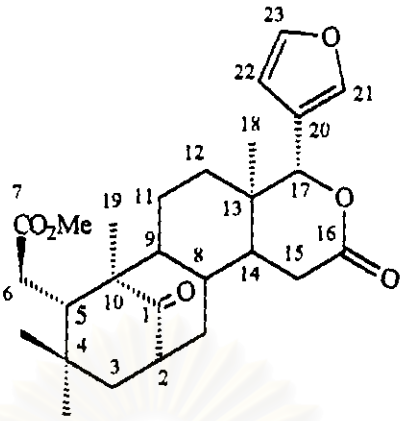


Gedunin (**5**)



Mexicanolide(**48**)

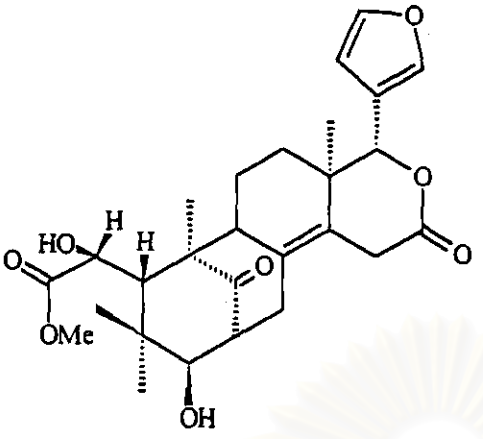
There were, in fact, some difference between limonoids in gedunin and mexicanolide groups. It was reported that at least two ester carbons at C-16 and a methoxy group were generally found in the ^{13}C-NMR pattern of the limonoid in a mexicanolide group. The observed ^{13}C signals of Compound **6** showed ester signals at δ 170.7 and 174.2, a methoxy group at δ 51.9. In addition to the elucidation of compound **6**, the NMR data of this compound was compared with that of (**48**). Hence, this compound was probably a limonoid belonging to a mexicanolide group which perhaps was a derivative of methyl meliacate. The basic skeleton is shown below.



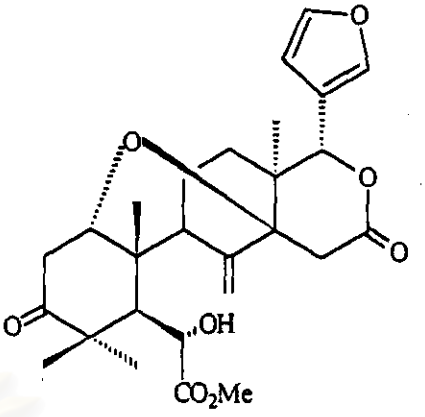
methyl meliacate

Compound **6** was composed of 8 oxygen atoms. After subtracting oxygen atoms as follows: 1 for OH, 2 for ester (C-7) , 2 for lactone (C-16) , 1 for a furan ring and 1 for a ketonic carbonyl, 1 oxygen atom was left. As mentioned above, the signals of 3 carbons at δ 91.3, 85.4 and 76.7 suggested that 1 carbon should attach to a hydroxyl group and the other two should connect to 1 oxygen atom as an epoxide ring or as an ether linkage.

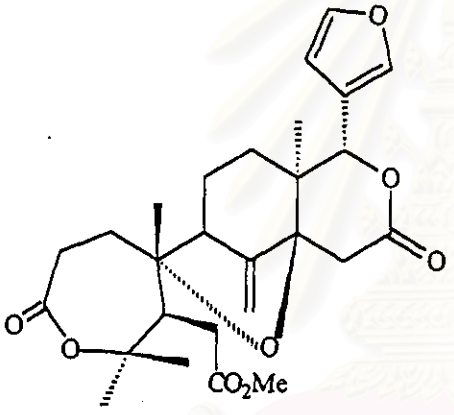
From the literature survey, it was found that some limonoids were of the same molecular formular of $C_{27}H_{34}O_8$ such as sweitenolide **(44)**, 6-methyl-hydroxy angolensate **(45)** and methyl angolensate **(46)**. The other reported structure **(47)** which was resemble to Compound **6** was 3,8-epoxy linkage but its formular structure was $C_{27}H_{32}O_8$. This compound was derived by a selenium dioxide oxidation of either carapin or mexicanolide.[21]



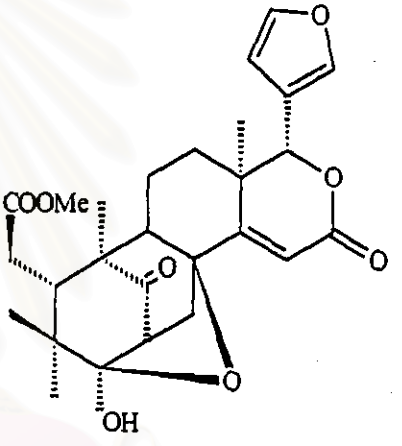
(44)



(45)



(46)



(47)

For furthur information, 2D-NMR, C-H correlation (Fig. 3.37) spectra was used to correlate the ^{13}C and ^{1}H signal. The spectra showed that the proton at δ 6.28 (1 H, s) was attached to the carbon at δ 74.07. This signal was the characteristic feature of a limonoid compound which could be assigned for H-17 and C-17 position. The proton at δ 4.22 (1H, d, J = 5.8 Hz) was found to attach to the carbon at 91.27. It indicated that the proton of this carbon had one adjacent proton. The correlation data between ^{13}C and ^{1}H signal is shown in Table 3.13.

Table 3.13 The ^{1}H and ^{13}C NMR correlation from the C-H COSY (2D-NMR)

^{13}C (ppm)	attached ^{1}H (ppm)	^{13}C (ppm)	attached ^{1}H (ppm)
19.97	0.67 (s)	42.44	2.04 (d) , 2.52 (dd)
16.78	0.94 (s)	32.52	2.14 (dd), 2.24 (dd)
16.03	0.98 (s)	36.76	2.54 (d) , 3.13 (d)
27.94	1.03 (s)	48.91	2.96 (dd)
17.81	1.46 (m) , 2.10 (m)	42.90	3.08 (dd)
28.58	1.70 (ddd), 1.50 (m)	51.81	3.70 (s)
51.98	1.97 (dd)		

The ^{1}H-^{1}H COSY (Fig. 3.36) showed the correlation between the proton at δ 4.22 (d, J= 5.8 Hz, H-3) and 2.96 (dd, J= 6.0, 12.2 Hz, H-2), these at δ 2.96 and 2.52 (dd, J= 7.0, 12.0 Hz, H-30), those at δ 2.52 and 2.04 (d, H-30). The correlation between the protons at δ 1.99 (dd, H-9) and 1.46 (m, H-11), those at δ 1.46 (m, H-11) and 1.50 (ddd, H-12) which colud be attributed to the sequence of H-9-H-11-H-12, while the proton at δ 2.24 (dd, H-6) was correlated to the proton at δ 3.08 (dd, H-5) which emerged the sequence of H-6-H-5.

The observed correlation indicated that the carbon at δ 91.27 (CH) was connected to the carbon at δ 48.9 (CH) and that at δ 48.9 was connected to the carbon at δ 42.4 (CH_2). This data clearly supported the presecne of the sequence of -CH-CH-CH_2 in the molecule.

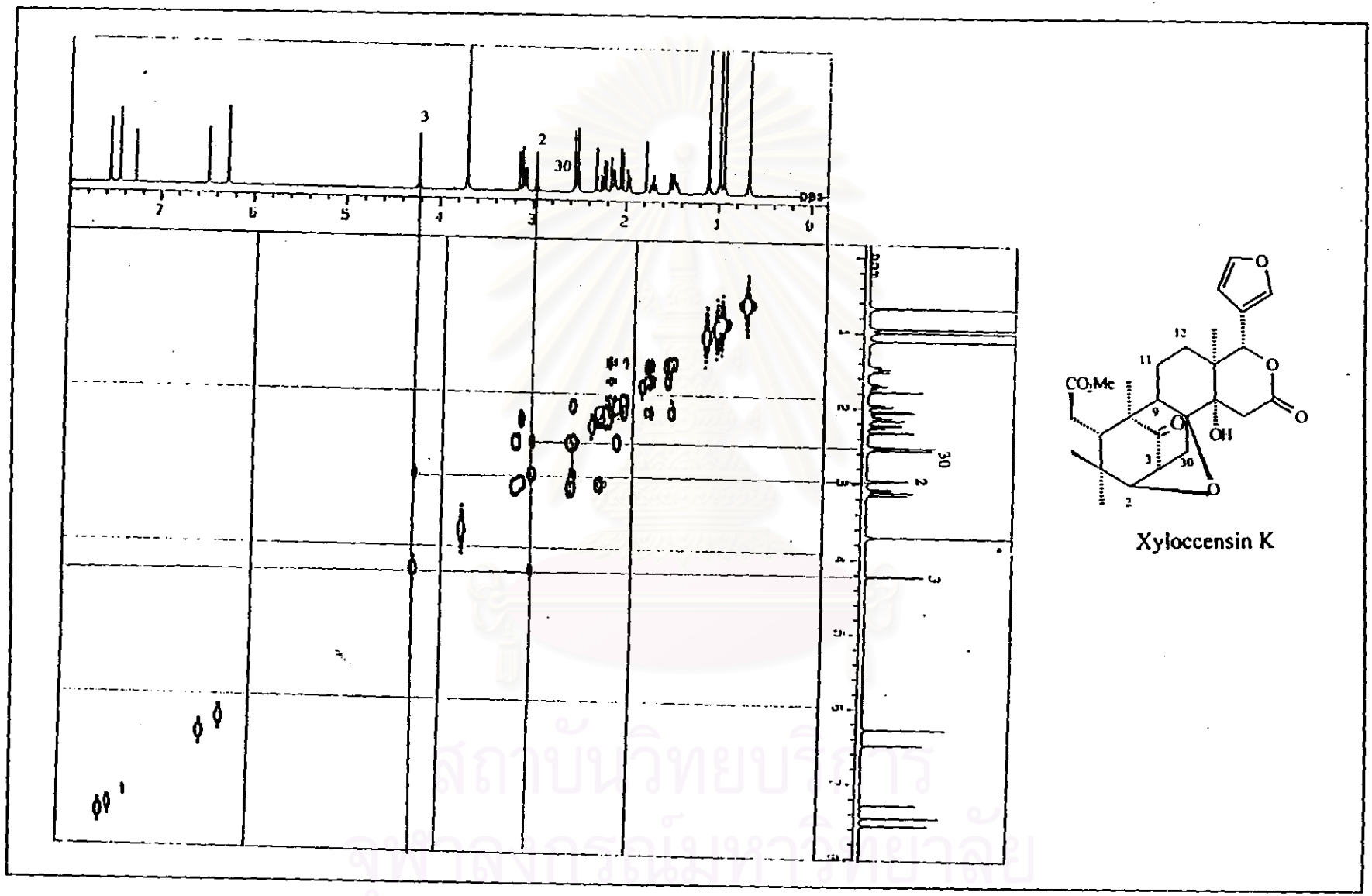


Fig. 3.36 The ^{1}H-^{1}H COSY spectrum of Compound 6

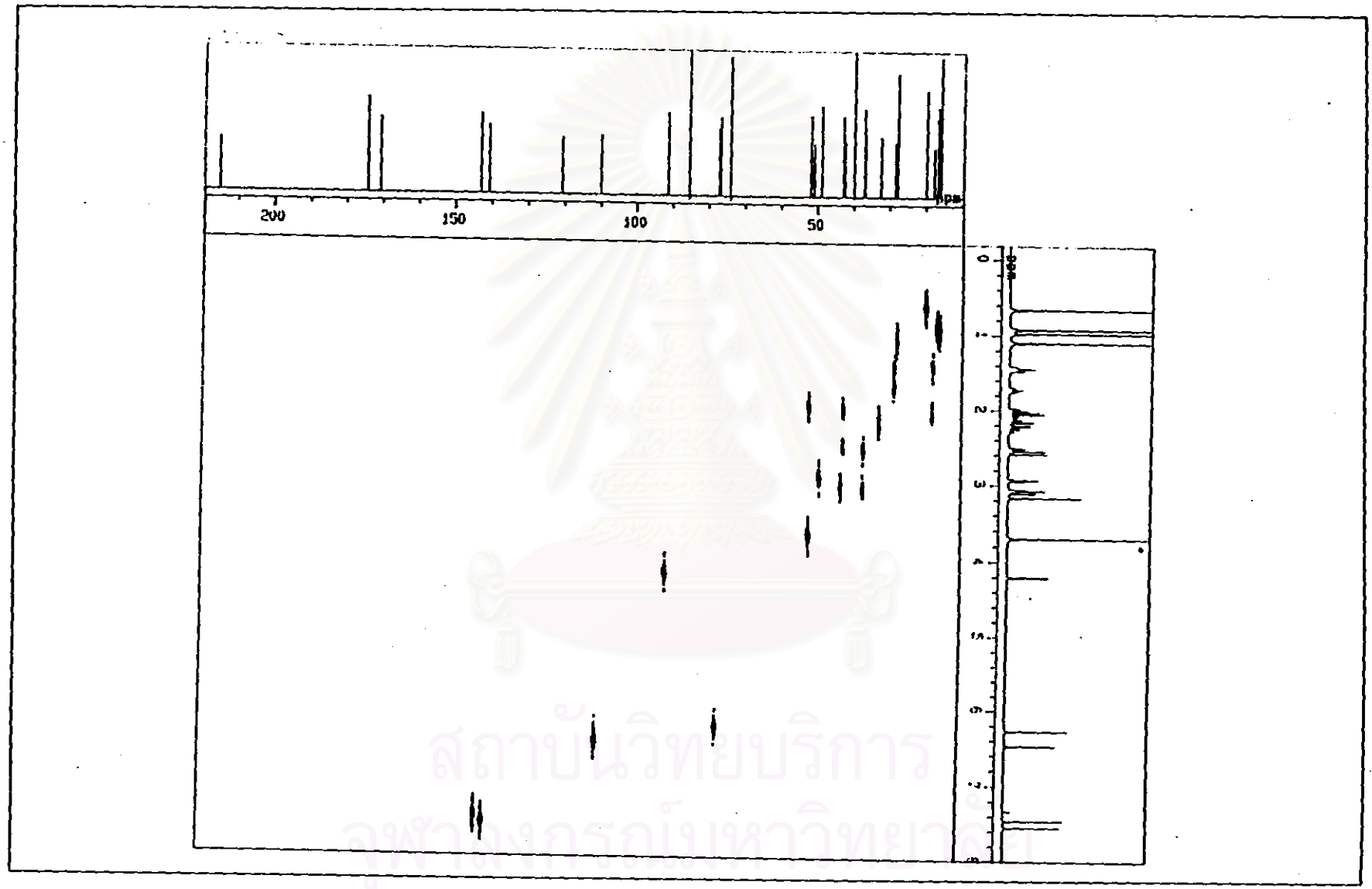
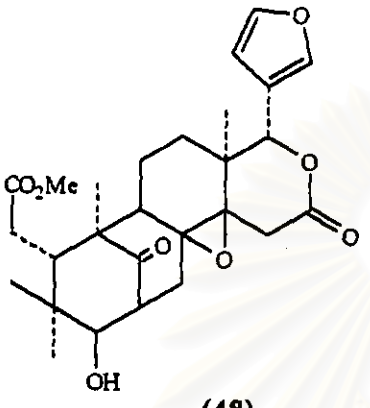
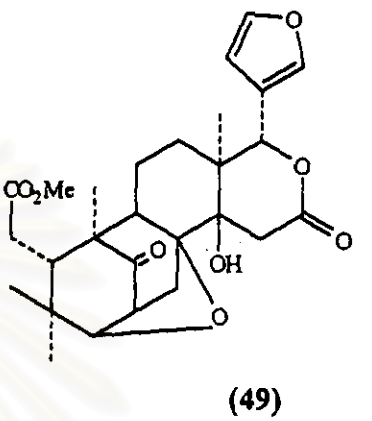


Fig. 3.37 The ^{13}C-^{1}H COSY spectrum of Compound 6

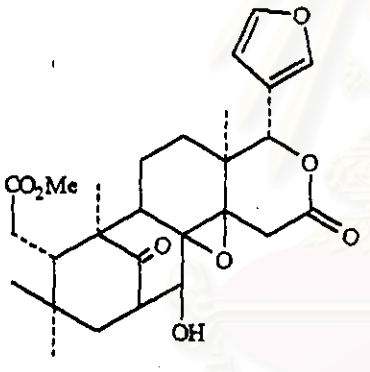
According to the gathered spectroscopic data, the possible structures could be depicted as shown below.



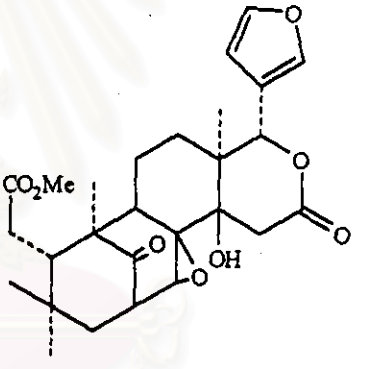
(48)



(49)



(50)



(51)

These related structures (48), (49), (50) and (51) are diffrent only in the presence of either ether linkage or epoxide moeity in the molecule. The data from literature search indicated that the ^{13}C NMR signals of carbons which connected to epoxide was generally observed around δ (ppm) 50-70, while the signals of carbons connected to ether linkage should occur in more down feild region.[45] The carbon signals of Compound 6 which corresponded to the carbons

adjacent to oxygen atom were found at 85.51 and 91.27, respectviely. Thus the most possible structure should be (49).

Moreover, the structure of Compound 6 was confirmed by X-ray crystallography (Fig. 3.38). The tentative assignment of ^{1}H and ^{13}C NMR is presented in Table 3.14.

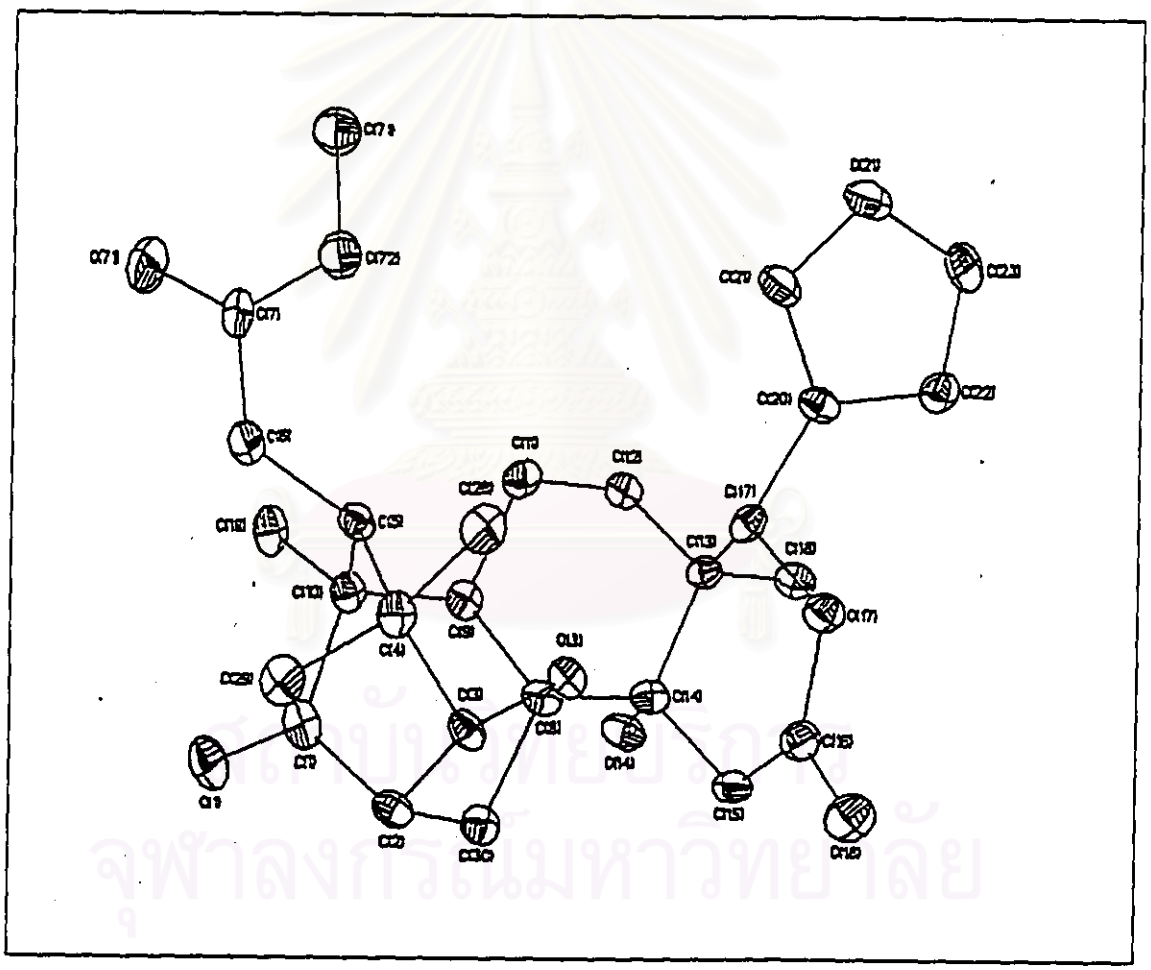
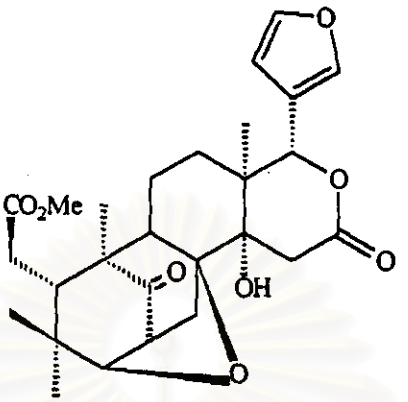


Fig. 3.38 ORTEP of Compound 6

The structure of Compound 6 is shown below:



Xyloccensin K

To our knowledge, there is no report of Compound 6 in the chemical literature. Hence, this compound is a novel naturally occuring limonoid. According to the type of limonoid belonging to a mexicanolide group which have been found such as xyloccensin A-J[26], this compound was ,therefore, named as xyloccensin K, by the Natural Products Research Unit, Chemistry Department, Chulalongkorn University.

Table 3.14 The tentative assignment of ^{1}H and ^{13}C spectral data of Compound **6** compared with 3β-*n* butyryl oxy-1-oxo meliae-8(14)-enate (**48**)

Position	Chemical shift (ppm)			
	3β-*n* butyryl oxy-1-oxo meliae-8(14)-enate (**48**)		Compound **6**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	-	218.1(s)	-	215.2(s)
2	3.13	48.0(d)	2.96	48.9(d)
3	4.95	78.1(d)	4.22	91.3(d)
4	-	38.2(s)	-	39.9(s)
5	3.18	40.8(d)	3.08	42.9(d)
6	2.38	33.4(t)	2.14,2.24	32.5(t)
7	-	174.1(s)	-	174.2(s)
8	-	127.7(s)	-	85.4(s)
9	2.03	52.1(d)	1.97	51.9(d)
10	-	52.9(s)	-	50.9(s)
11	1.67	18.7(t)	2.10,1.46	17.8(t)
12	1.05, 1.75	29.0(t)	1.50,1.70	28.6(t)
13	-	38.1(s)	-	37.1(s)
14	-	131.7(s)	-	74.1(s)
15	3.45,3.70	23.3(t)	2.54,3.13	36.8(t)
16	-	169.9(s)	-	170.7(s)
17	5.65	80.7(d)	6.28	76.7(s)
18	1.08	17.7(q)	0.67	19.9(q)
19	1.15	16.7(q)	0.98	16.9(q)
20	-	120.6(s)	-	120.6(s)

Table 3.14 (cont.)

Position	Chemical shift (ppm)			
	3β-*n* butyryl oxy-1-oxo meliae-8(14)-enate (**48**)		Compound **6**	
	^{1}H	^{13}C	^{1}H	^{13}C
21	7.52	141.7(d)	7.45	142.9(d)
22	6.45	109.9(d)	6.49	109.9(d)
23	7.38	142.8(d)	7.56	140.7(d)
24	-	173.1(s)	-	-
25	2.38	36.3(t)	-	-
26	1.66	18.5(t)	-	-
27	0.95	13.8(q)	-	-
28	0.78	20.5(q)	1.03	27.9(q)
29	0.68	23.2(q)	0.98	16.0(q)
30	2.10,2.75	33.3(t)	2.04,2.52	42.4(t)
31(OMe)	3.66	52.1(q)	3.70	51.8(q)

Purification, Properties and Structural Elucidation of Compound 7a,7b

A mixture of Compound **5**, **7a** and **7b** was obtained from fraction 29-64 (Fraction VI: see Table 3.5) as white bright solid in yellow oil. After washing with methanol, the yellow oil was removed and the remaining solid was recrystallized with methanol. The solid was monitored the purity by using TLC (silica gel) plate and showed the R_f 0.42 and 0.49 (solvent system: 50% ethyl acetate in hexane). The result showed that there were at least two components in this mixture. The attempt to separated this mixture was conducted by using flash column chromatography, yielding two components (see Table 3.5). The compound with R_f 0.42 was found to be exactly the same as Compound **5**. The others were separated and purified to elucidate by NMR spectroscopy.

The ^{1}H and ^{13}C spectra of the latter suggested that this component did not contain a single compound. The HPLC technique was consequently selected to analyse this mixture and found that this mixture was composed of 2 components, designated as Compound **7a** and **7b**. The suitable conditions (by analytical column) was mobile phase 40 % water in acetonitrile, flow rate 1 mL/min . For separation, the semi-prep HPLC was performed and the optimal condition was found to be 50% water in acetonitrile and flow rate 2 mL/min. The retention time using semi-prep column were 4.27 and 5.16 min, respectively.

After separation, both compounds were reexamined for the purity by HPLC, anaytical column, and the observed chromatograms are shown in Fig 3.39 and 3.40

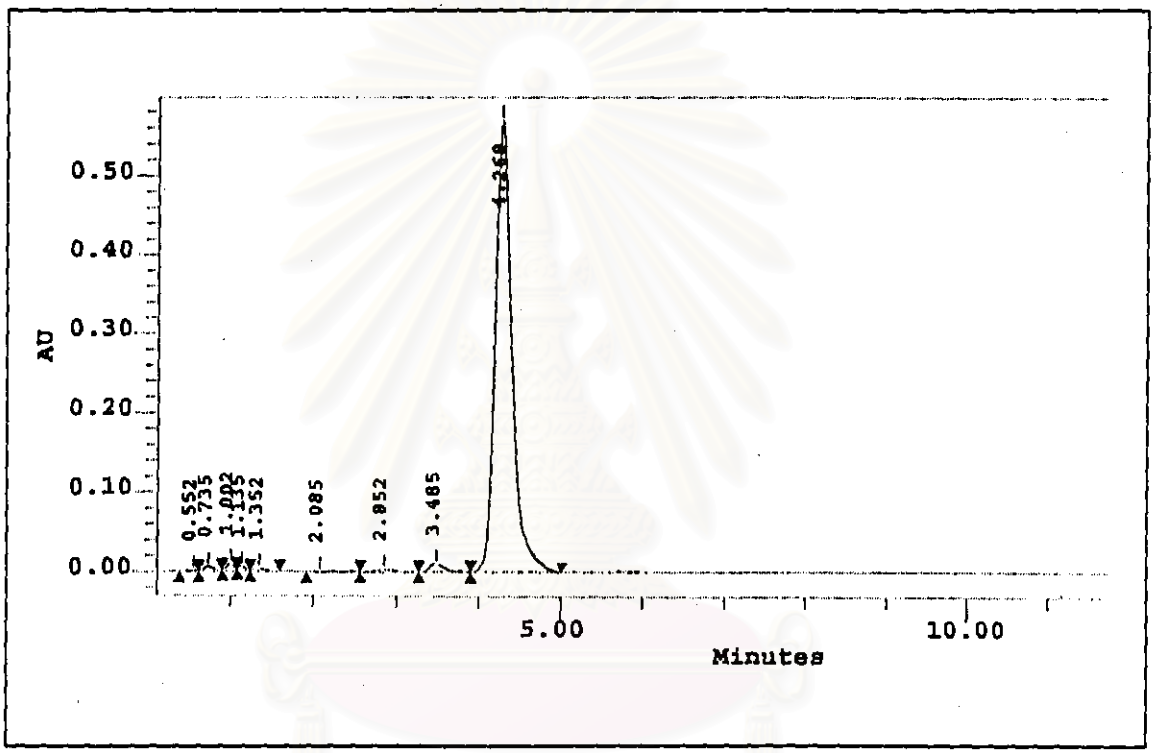


Fig. 3.39 The HPLC chromatogram of Compound 7a

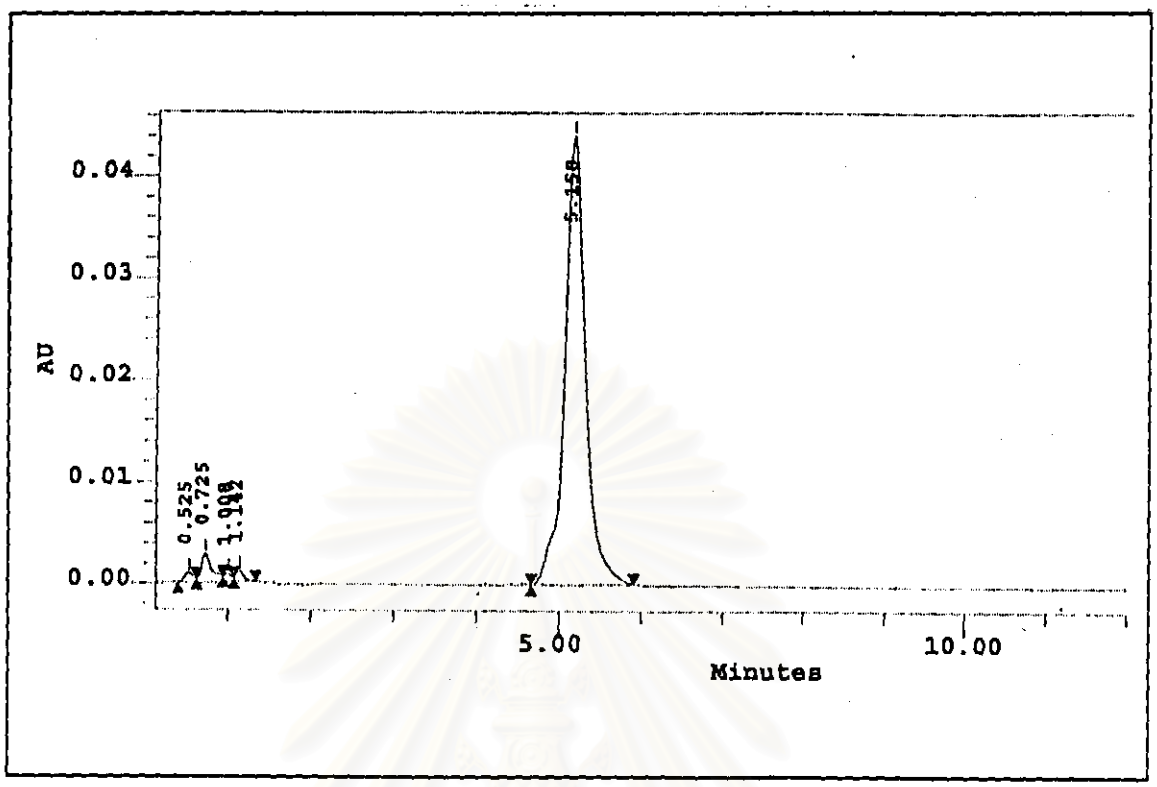


Fig. 3.40 The HPLC chromatogram of Compound **7b**

The IR spectra of both compounds as shown in Fig. 3.41 and 3.42 revealed the similar functional group. They showed important absorption bands as follows: O-H stretching vibration at 3450-3350 cm^{-1} (m), C-H stretching vibration of alkene at 3120 cm^{-1} (w), C-H stretching vibration of CH_3-, -CH_2- at 2950, 2850 cm^{-1}(s) and C=O stretching vibration of carbonyl moiety at 1730, 1710 cm^{-1} (s).

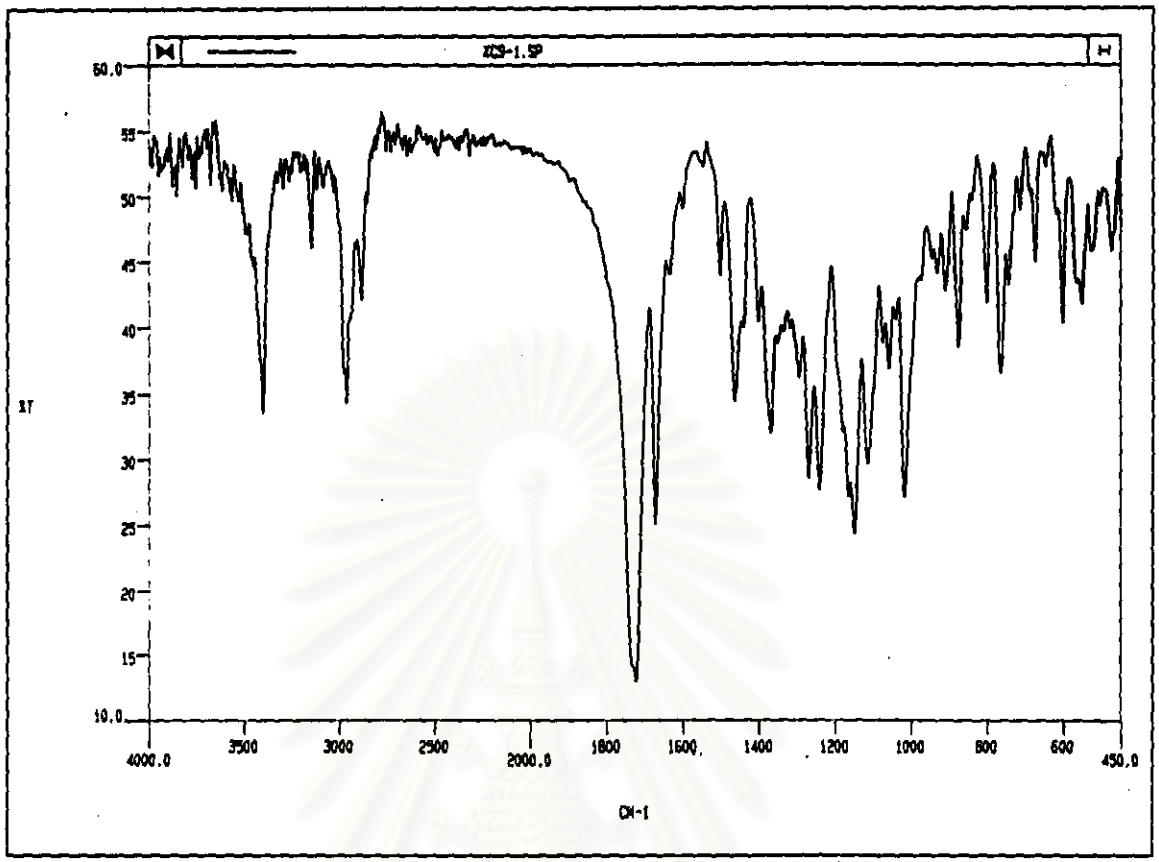


Fig. 3.41 The IR spectrum of Compound 7a

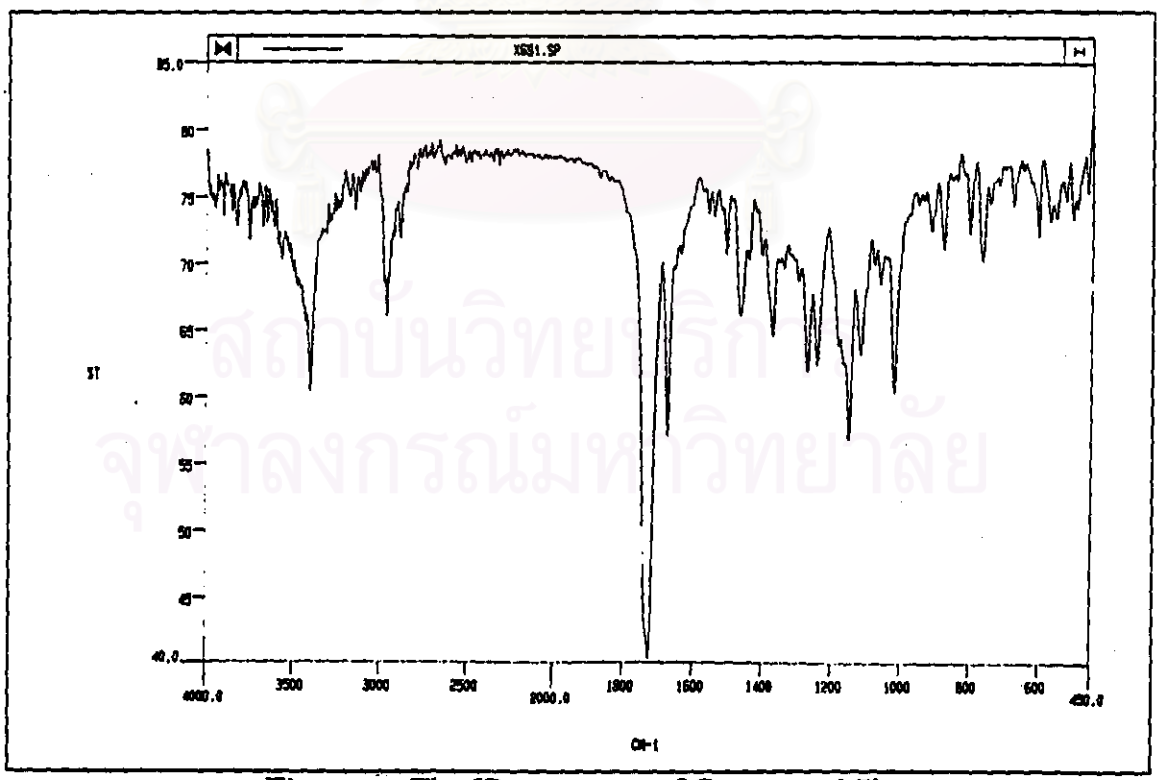


Fig. 3.42 The IR spectrum of Compound 7b

Compound 7a

The ^{1}H NMR spectrum (Fig 3.43) indicated the presence five methyl groups at δ 0.95 (s), 1.00 (d, J = 5.5 Hz), 1.09 (s), 1.13 (s) and 1.15 (s), a singlet single of a methoxy group at δ 3.62, a singlet signals of proton attached to a carbon adjacent to an oxygen atom at δ 5.25, three singlet siganls down field attributed to a β-substituted furan at δ 7.52, 7.41 and 6.42 and two singlet signals of olefinic proton at δ 6.19 and 6.98.

The ^{13}C NMR spectrum (Fig 3.44) and DEPT 90 and 135 (Fig 3.45) displayed totally 28 signals as follows: five methyl carbon signals at δ 11.5, 18.6, 19.1, 20.3 and 27.9, three methylene carbon signals at δ 25.6, 32.9 and 34.6, ten methine carbon signals at δ 34.1, 45.2, 42.8, 66.9, 80.0, 109.7, 118.5, 141.41, 143.2 and 161.8, nine quaternary carbon signals at δ 36.8, 38.4, 119.6, 128.7, 163.6. 173.4, 175.1, 198.7 and 208.7 and a signal of methoxy group at δ 51.9.

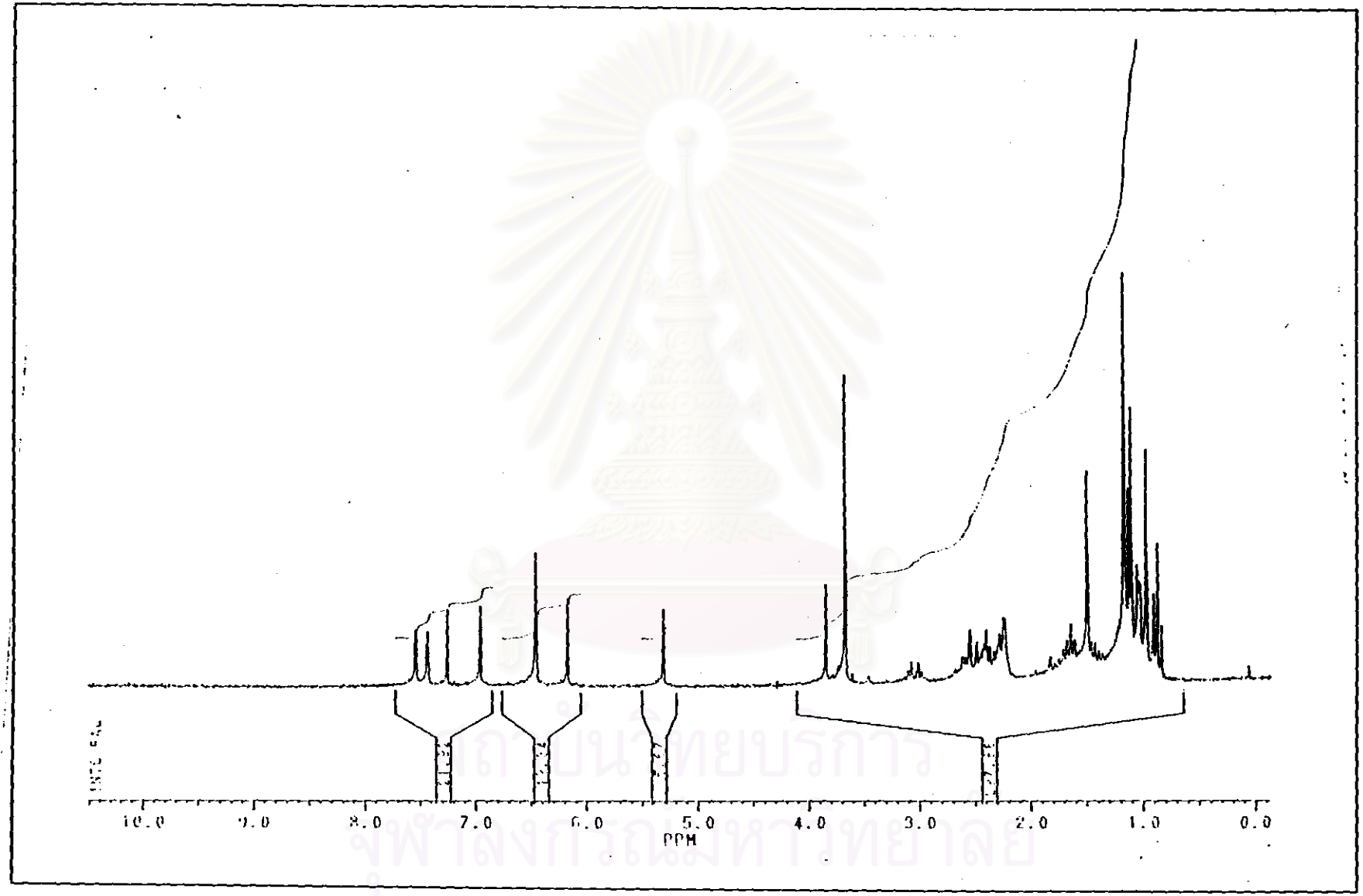


Fig. 3.43 The ^{1}H NMR spectrum of Compound **7a**

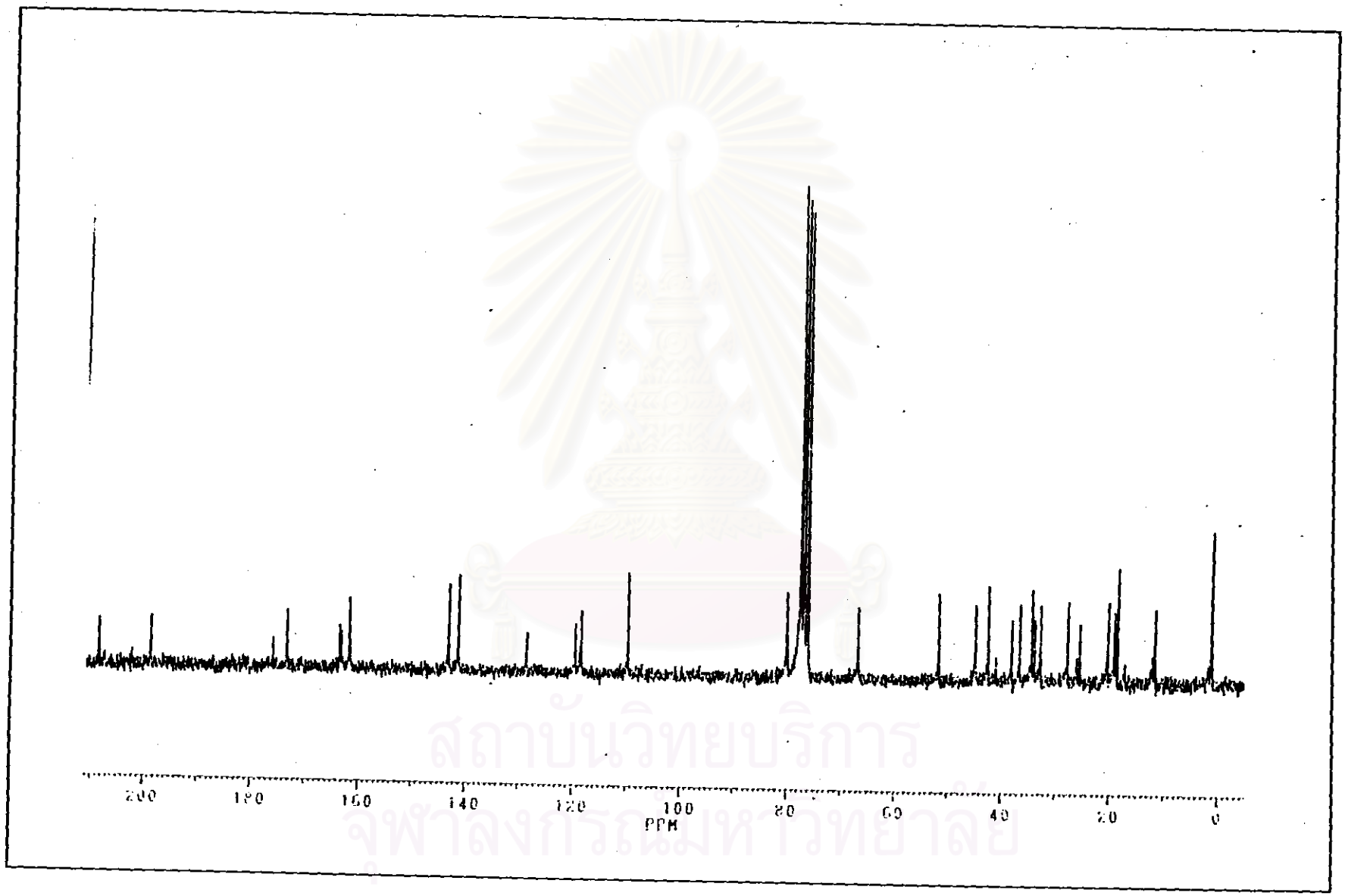


Fig. 3.44 The ^{13}C NMR spectrum of Compound **7a**

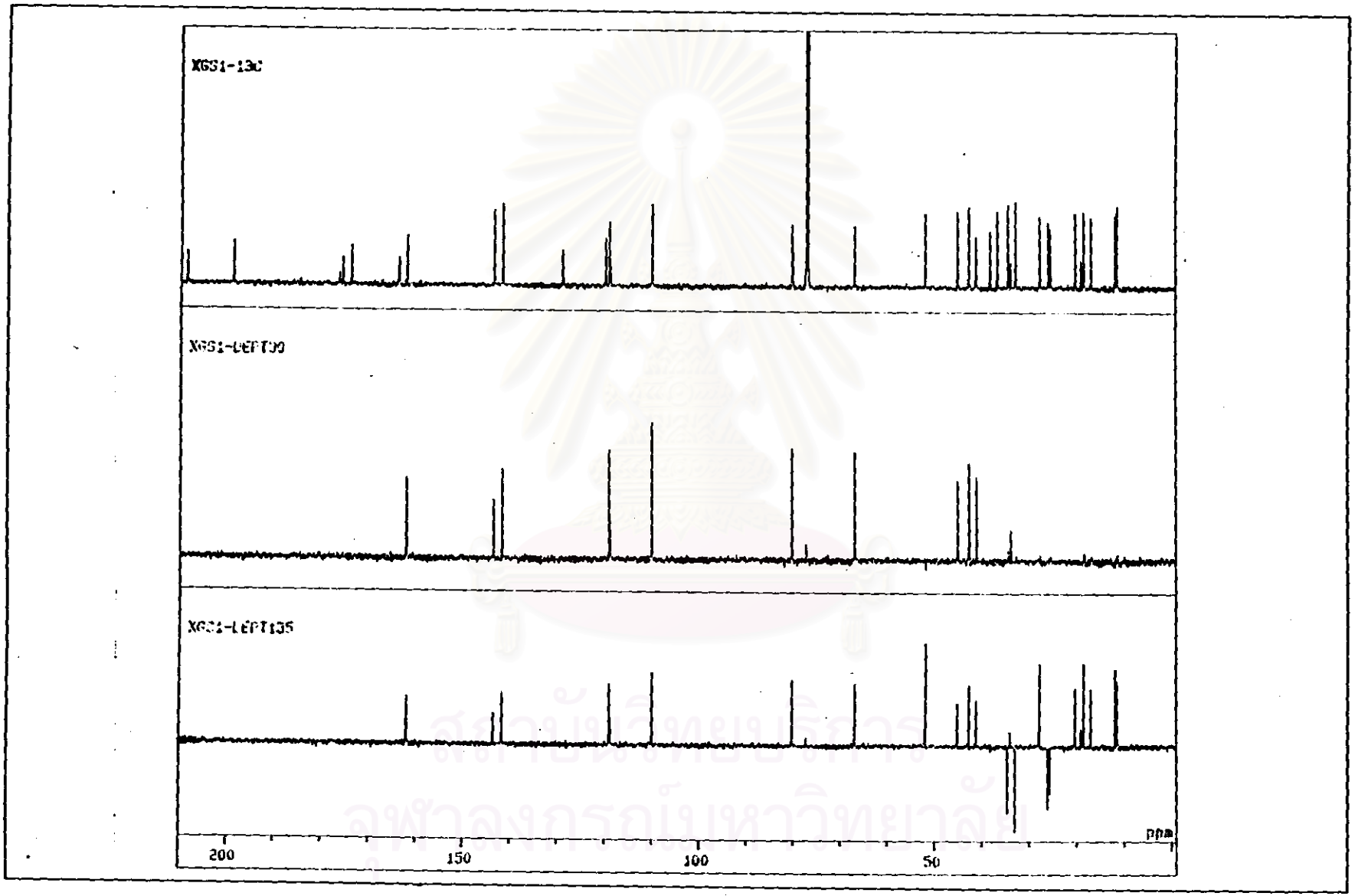


Fig. 3.45 The DEPT 90 and DEPT 135 of Compound 7a

Compound 7b

The ^{1}H NMR spectrum (Fig. 3.46) of Compound **7b** was closely similar to compound **7a**. The diferrence was only a triplet methyl signal of this compound appeared at δ 0.84 (t, J = 7.3 Hz).

The ^{13}C NMR spectrum (Fig.3.47) gave very close signal to that of Compound **7a**, *i.e.*, six methyl carbon signals at δ 11.46, 11.9, 17.0, 18.6, 20.4 and 27.9, four methylene carbon signals at δ 26.1, 25.7, 34.6 and 32.9, nine methine carbon signals at δ 41.3, 42.8, 45.2, 66.9, 109.8, 119.6, 141.4, 143.2 and 161.6, nine quaternary carbon signals at δ 36.8, 38.3, 119.58, 128.7, 163.3, 173.4, 175.3, 198.7 and 208.7 and a methoxy carbon at δ 51.9.

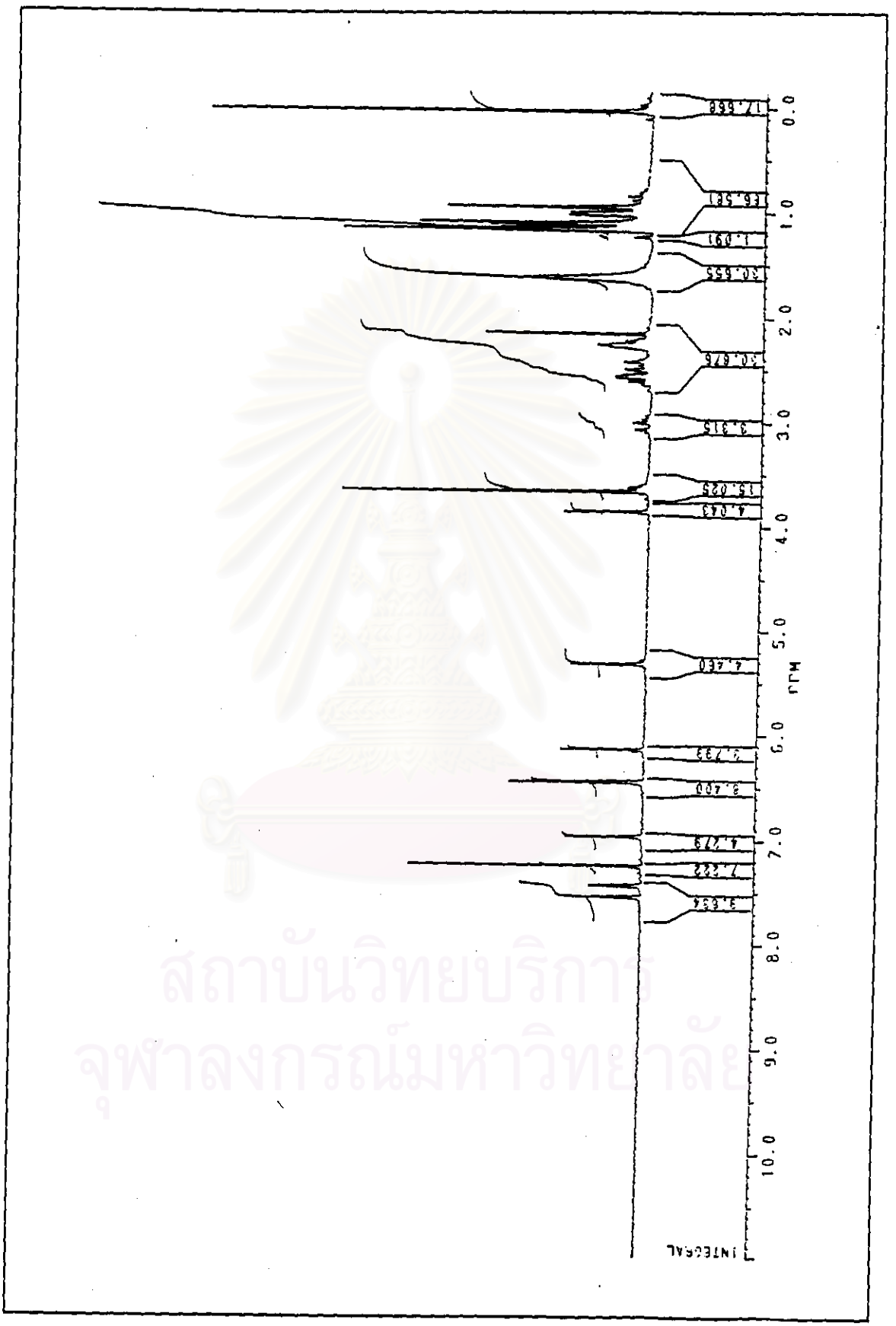


Fig. 3.46 ^{1}H NMR spectrum of Compound 7b

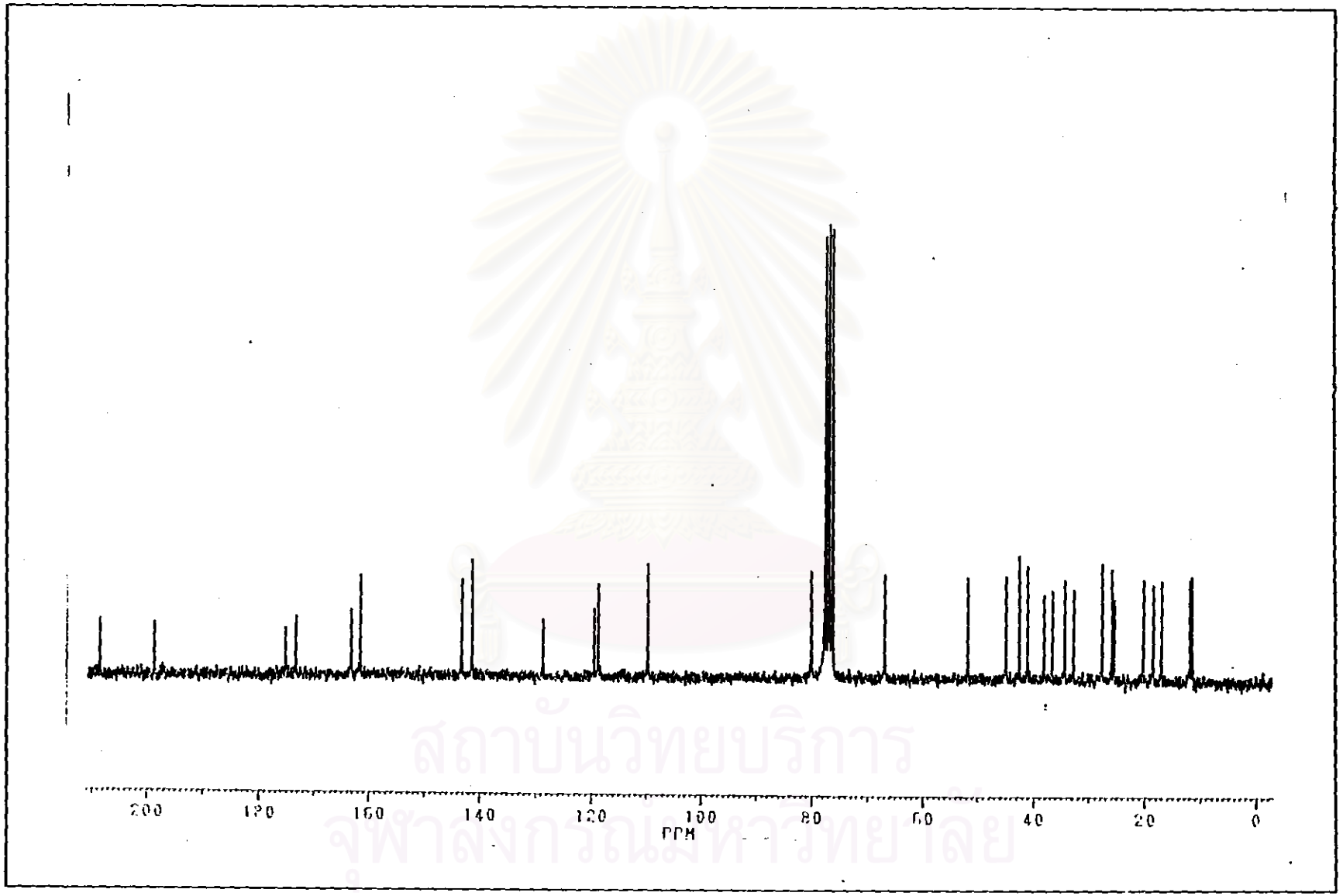


Fig. 3.47 The ^{13}C NMR spectrum of Compound **7b**

There are two additional of methylene and methyl signals in the ^{13}C spectrum of Compound **7b** comparing with that of Compound **7a**. The ^{13}C NMR data of both compounds indicated the presence of carbon signals of furan ring in a limonoid compound at 143.2 (C-21), 141.4 (C-23), 119.6 (C-20) and 109.8 (C-22).[44, 45] The methylene carbon signal which could be assigned for a carbon adjacent to an oxygen atom in a lactone ring was detected at δ 80.2 (C-17) like those in Compound **5** and Compound **6**. In addition, the methoxy signal at 51.9 ppm was also observed.

Both compounds was unfortunately obtained in a small amount. Therefore, the fully structural elucidation of these two compounds was not plausible. However, the pattern of ^{1}H and ^{13}C NMR spectra were clearly pointed out that both compounds were limonoids.

Purification, Properties and Structure Elucidation of Mixture 8

The solid and oil were obtained from the combination of fraction No. 15-16, Fraction III (see Table 3.4), Fraction VI (see Table 3.5), Fraction VIII (see Table 3.7). After removing the red-brownish oil by washing with methanol and the remaining material was recrystallized by hot ethanol for several times to yield white amorphous solid, 1.10 g (2.45 % wt. by wt.of Fraction III), 0.12 g (1.07 % wt. by wt.of Fraction VI), 1.30 g (2.50 % wt. by wt. of Fraction VIII). This compound designated as Mixture 8 decomposed at 270-275°C and gave R_f value on TLC plate (silica gel) at 0.34 using 15% methanol in dichloromethane as a developing solvent.

The IR spectrum (Fig 3.48) showed the important absorption band of O-H stretching vibration at 3400 cm^{-1} (b). The presence of C-O stretching vibration particularly for glycosidic linkage was observed around 1075-1025 cm^{-1}. The additional band at 890 cm^{-1}(w) was compatible with anomeric axial C-H deformation of β-sugar.[37]

By means of chemical and physical properties and IR spectrum, Mixture 8 might likely be a steroidal glycoside.

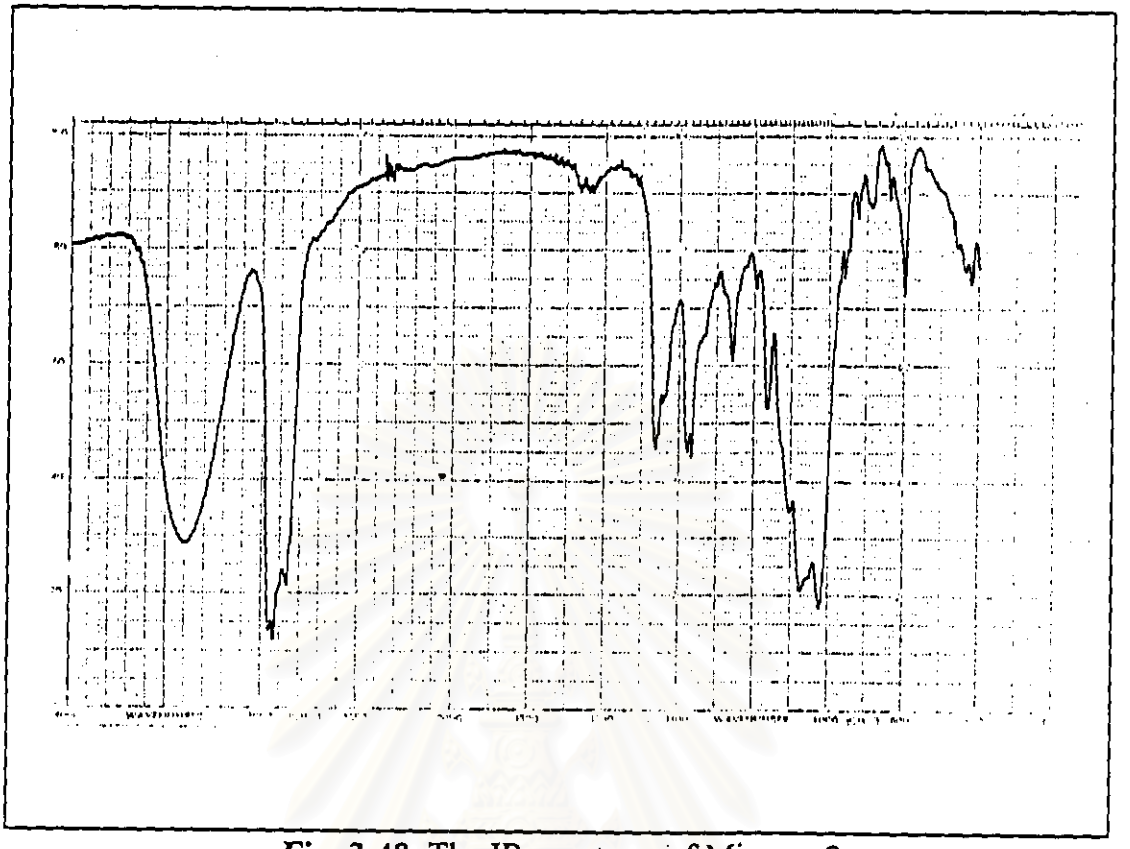


Fig. 3.48 The IR spectrum of Mixture 8

An acetyl derivative of Mixture 8 was prepared according to the reported procedure[51], yielding coarse needle crystalline product, m.p.132-134 °C. The R_f value was 0.7 using chloroform as a developing solvent.

The IR spectrum (Fig 3.49) showed the important absorption bands of acetate functional group at 1750 cm^{-1}(C=O) and at 1220 cm^{-1}(C-O). The others were similar to those of Mixture 8 except for the absence of O-H peak.

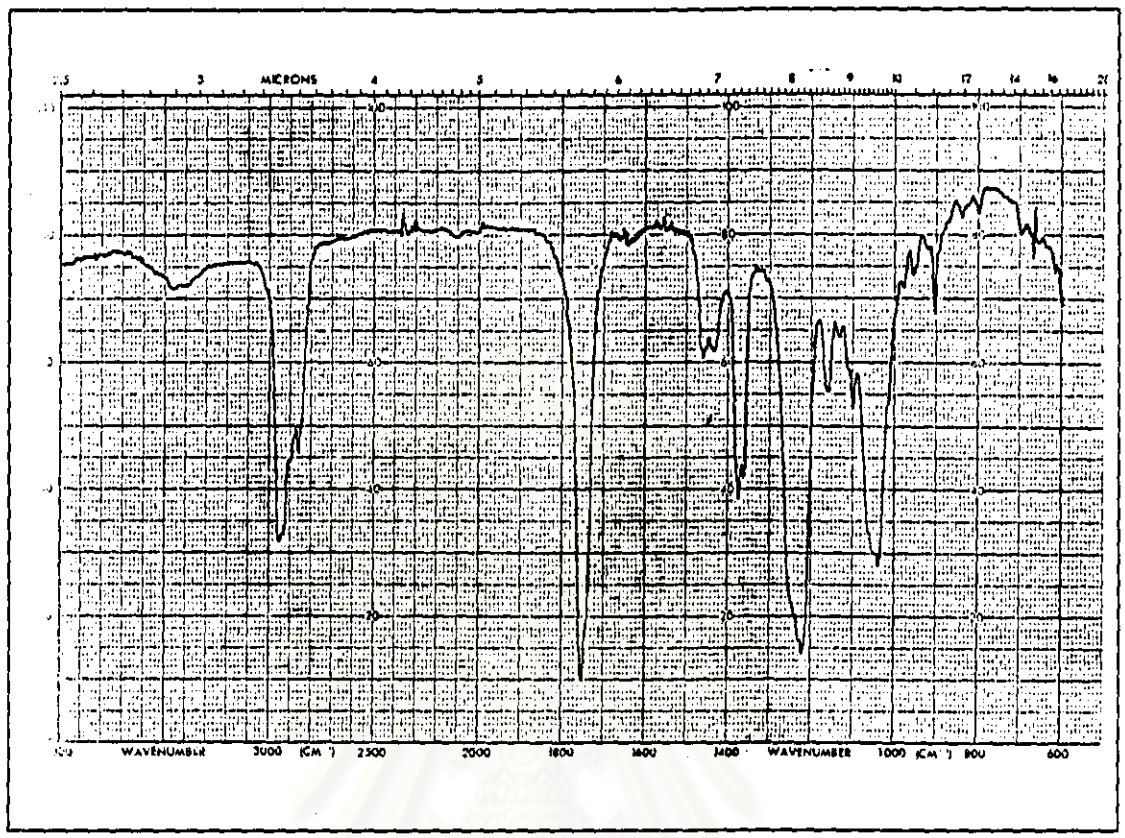


Fig. 3.49 The IR spectrum of Mixture 8 tetraacetate

The ^{1}H NMR of Mixture 8 acetate showed the signals around 3.49 to 0.65 ppm with 48 proton integration which was typical for a steroidal compound.[5] The signal at 3.49 indicated the proton on carbon adjacent to oxygen atom of a sugar molecule. Four singlet signals of tetraacetate revealed at 2.00, 2.02, 2.04 and 2.07 ppm. The signal at 5.35 ppm should be an olefinic proton of -CH=C< of steroid. The signals between 5.20 to 3.68 with 7 H integration were assigned for the protons on a sugar moiety. The doublet signal at 4.59 ppm (1H, J=8.24 Hz) attributed to an anomeric proton was significant to state the presence of glycosidic linkage.

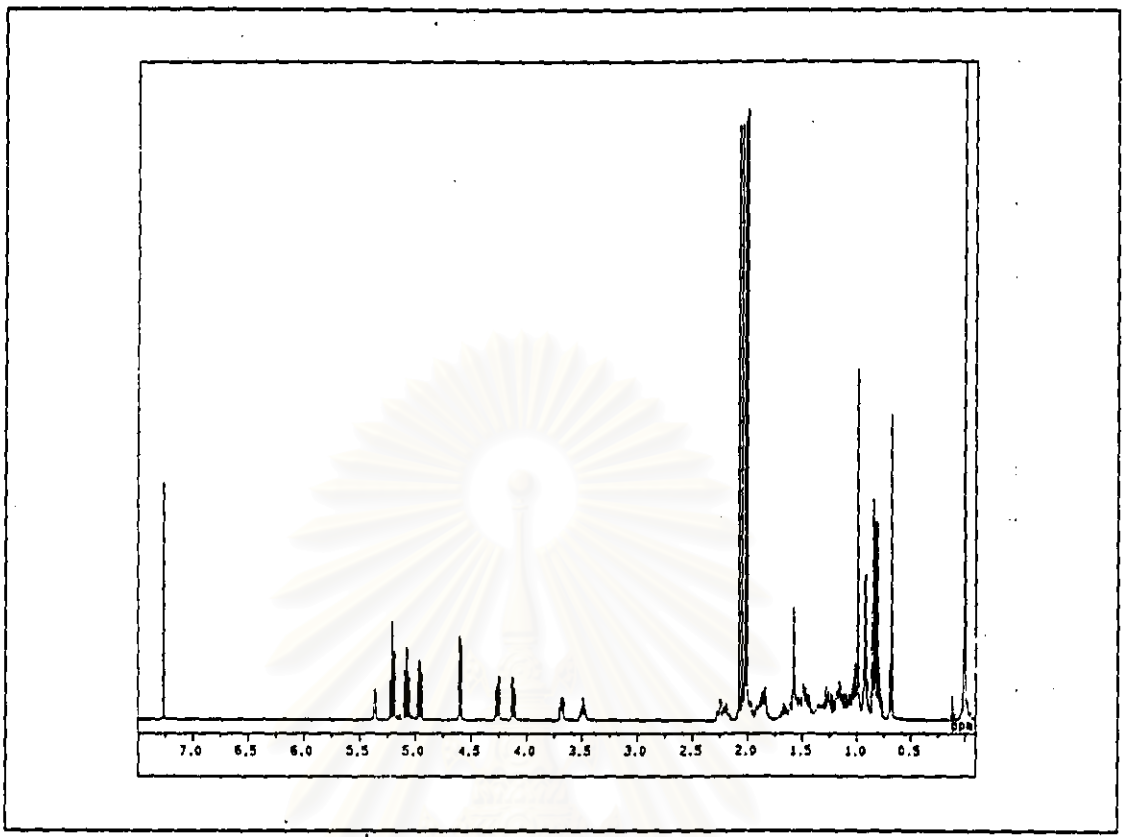
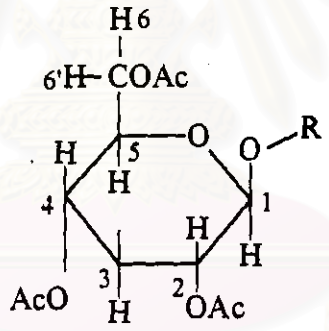


Fig.3.50 The ^{1}H NMR spectrum of Mixture **8** tetraacetate

The ^{1}H NMR spectrum of the acetate of Mixture **8** (Fig 3.50) gave completely coincided with that of the reported β-sitosteryl-3-O-β-D-glucopyranoside tetraacetate[52], which revealed seven signals belonging to the protons on sugar moiety. Thus, it could be concluded that this sugar molecule may be glucose.[5] The ^{1}H NMR signals of both compounds were compared as shown in Table 3.15 .

Table 3.15 The ^{1}H NMR chemical shifts of sugar moiety of Mixture 8 tetraacetate compared with β-sitosteryl-3-O-β-D glucopyranoside tetraacetate

Substance	Chemical shift (ppm)						
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
β-sitosteryl-3-O-β-D gluco-pyranoside tetraacetate	4.59	4.93	5.21	5.06	3.68	4.27	4.13
Mixture 8 tetraacetate	4.59	4.90	5.20	5.06	3.68	4.27	4.12



The ^{13}C NMR spectrum (Fig 3.51) gave four signals of four acetoxy carbonyl group at 169.3, 169.4, 170.4 and 170.7 ppm, respectively. Two olefinic ^{13}C signals were shown at 140.4 and 122.2 ppm and six signals belonging to a sugar molecule were observed at 99.7, 72.9, 71.7, 71.5, 68.6 and 62.1. The signal at 80.1 ppm was assigned for C-3 of steroid attached to the oxygen of a sugar moiety. The other signals were compatible with those of

with those of β-sitosterol.[5] The comparison of ^{13}C NMR signals of Mixture 8 acetate and β-sitosteryl glycoside is shown in Table 3.16.

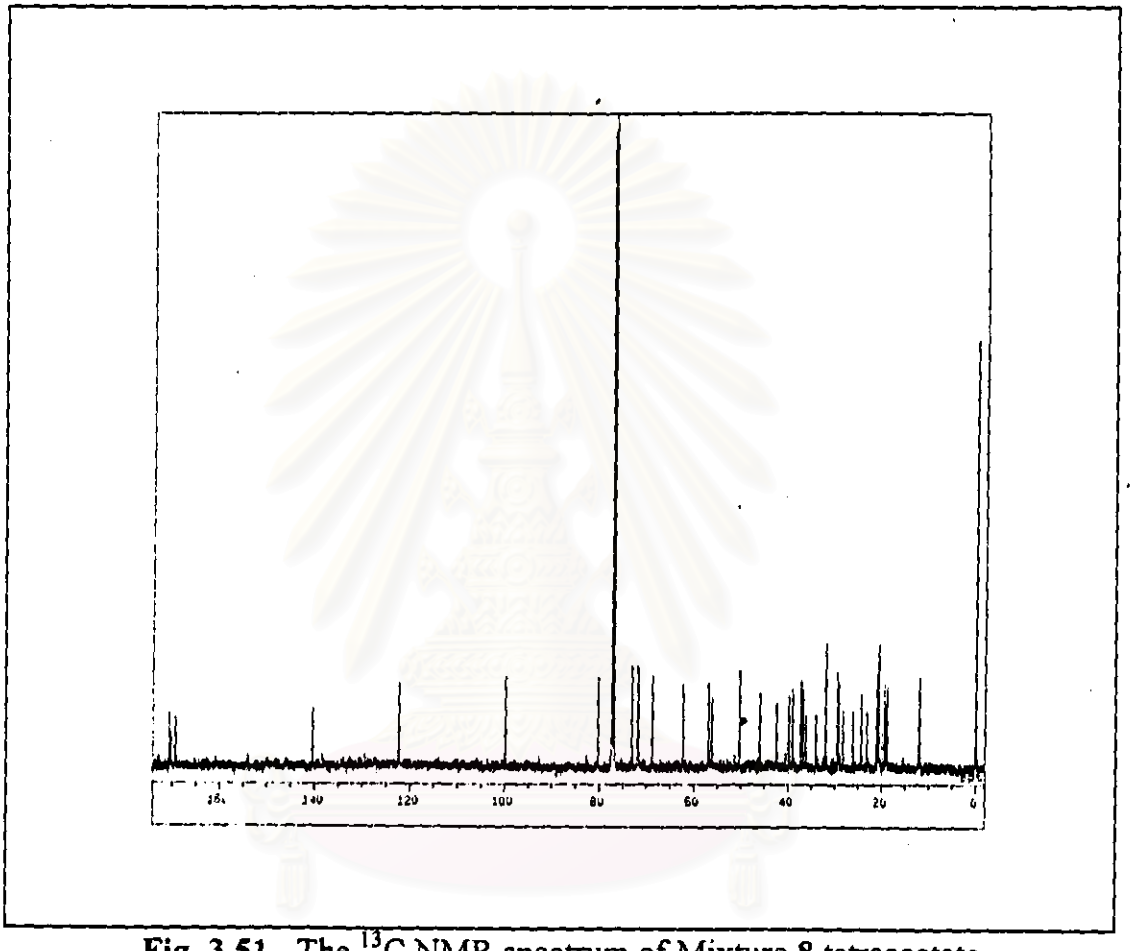


Fig. 3.51 The ^{13}C NMR spectrum of Mixture 8 tetraacetate

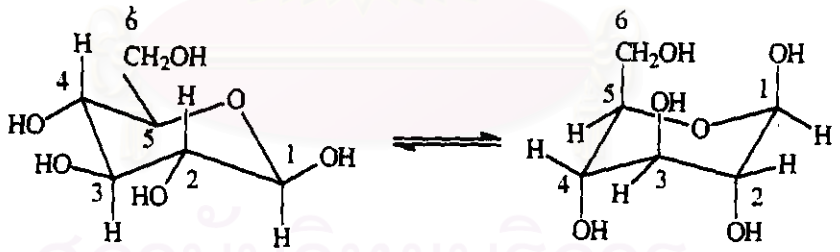
Table 3.16 The ^{13}C NMR chemical shift of Mixture **8** tatraacetate compared with β-sitosteryl glycoside (acetate).

Carbons	Chemical shift (ppm)		Carbons	Chemical shift (ppm)	
	β-sitosteryl glycoside (acetate)	Mixture **8** tetraacetate		β-sitosteryl glycoside (acetate)	Mixture **8** tatraacetate
1	37.2	37.2	19	19.1	19.0
2	29.5	29.5	20	33.9	33.9
3	80.1	80.1	21	18.8	18.8
4	38.8	38.9	22	36.1	36.1
5	140.4	140.4	23	23.1	23.1
6	122.2	122.2	24	42.4	42.3
7	31.9	31.9	25	28.2	28.2
8	31.9	31.8	26	21.1	21.1
9	50.2	50.2	27	19.8	19.8
10	36.7	37.8	28	26.1	26.1
11	19.3	19.3	29	11.9	11.9
12	39.8	39.8	C-1′	99.7	99.7
13	45.1	45.9	C-2′	72.9	72.9
14	56.8	56.8	C-3′	71.5	71.7
15	24.3	24.3	C-4′	71.5	71.5
16	28.8	29.2	C-5′	68.6	68.5
17	56.7	56.1	C-6′	62.1	62.1
18	11.9	11.9	$\underline{C}H_3COO$	20.6 20.8 20.5 20.5	20.6 20.8 20.1 20.6

Table 3.16 (Cont.)

Carbons	Chemical shift (ppm)		Carbons	Chemical shift (ppm)	
	β-sitosteryl glycoside (acetate)	Mixture **8** tetraacetate		β-sitosteryl glycoside (acetate)	Mixture **8** tatraacetate
			$CH_3\underline{C}OO$	173.4	170.7
				170.7	170.3
				170.4	169.4
				169.4	169.3

The anomeric proton of sugar at 4.58 ppm (1H , d, J=8.42 Hz) indicated that the β-glucosidic linkage has two possible chair conformation as shown below.[52]



(1) (2)

These two conformations were differed at an anomeric proton, H-1 and proton at C-2. The first one exhibited the coupling constant (J) 2-4 Hz if both protons were diaxial (1). The other showed J=7-10 Hz when they both were

equatorial (**2**). The observed value of coupling constant of this compound acetate (J= 8.4 Hz) was found to be in good agreement with the second form.

The mass spectrum (Fig 3.62) of Mixture **8** tetraacetate did not give a molecular ion peak (M^+), but it showed the prominent fragmentation ion peaks at m/z 396, 394, 382, 331, 255, 229, 213 and 169 which were the characteristic of glycoside tetraacetate.[5] The other fragmentation pattern was corresponded to β-sitosterol. The peaks at m/z 396, 394, 382 were clearly pointed out that in fact there was a mixture of steroids: β-sitosterol, stigmasterol and compesterol, respectively.

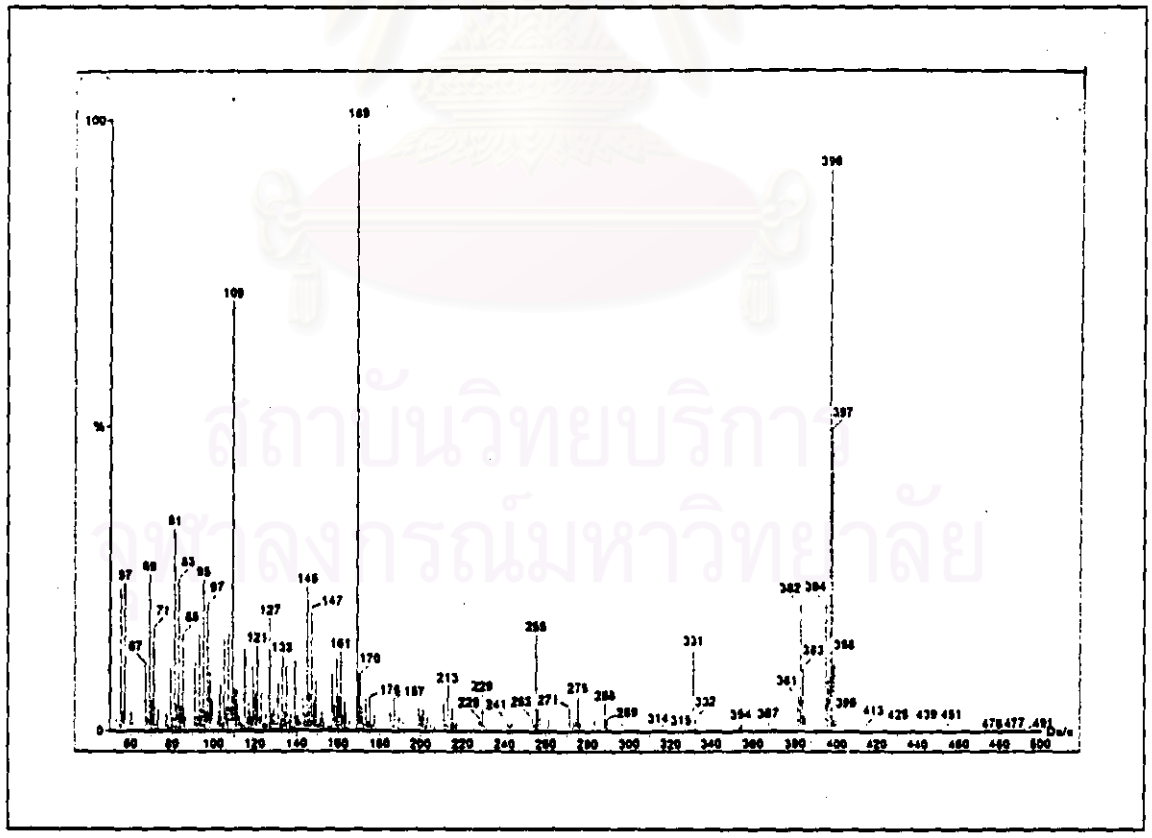
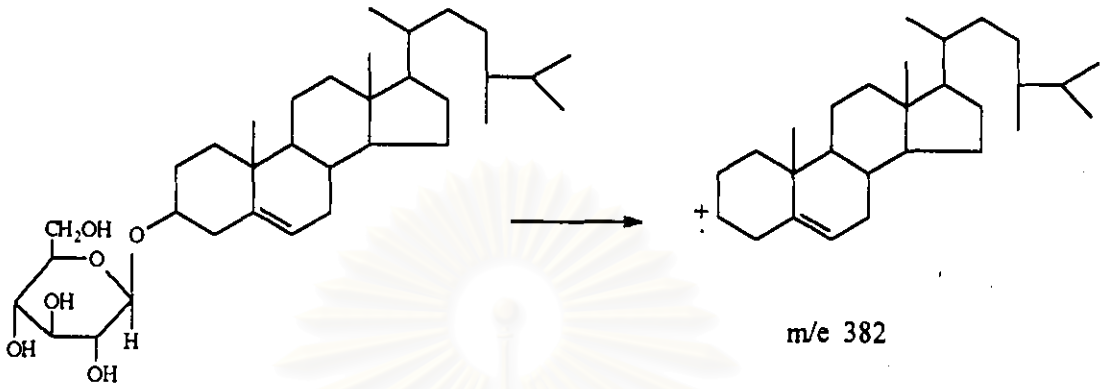
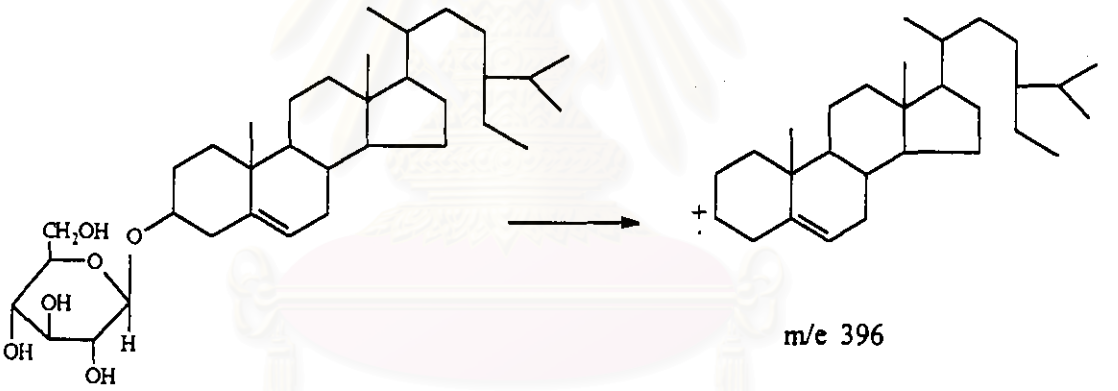


Fig.3.52 The mass spectrum of Mixture **8** tetraacetate

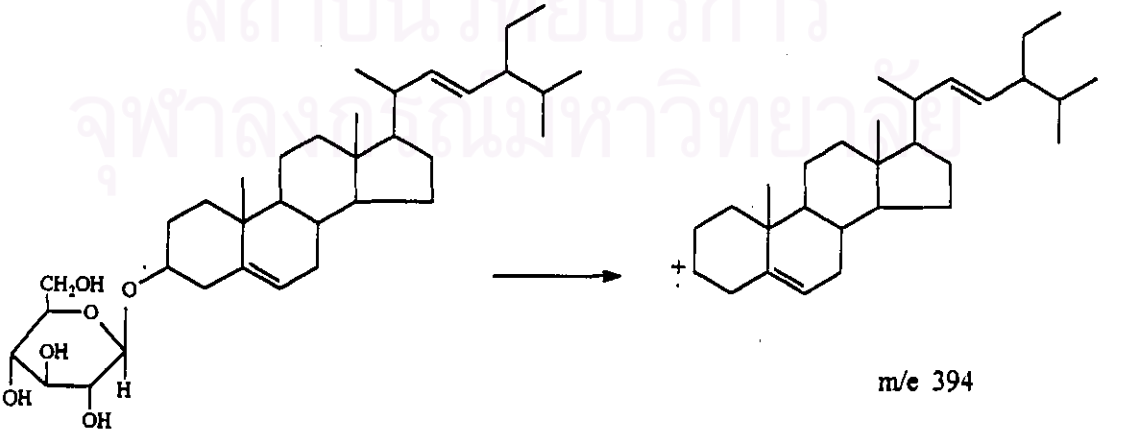
The structure of each steroidal glucoside their fragmentation are shown below.



campesteryl-3-O-β-D-glucopyranoside



β-sitosteryl-3-O-β-D-glucopyranoside

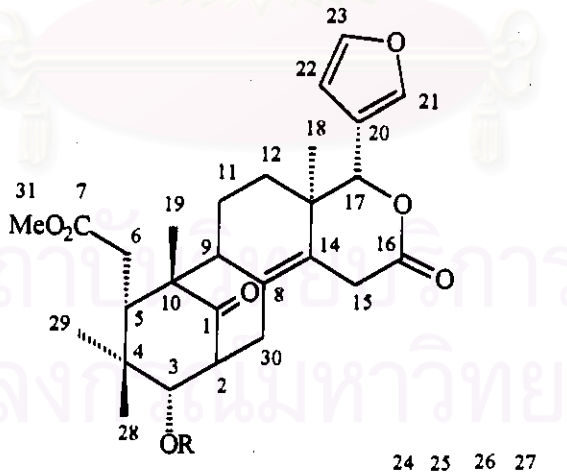


stigmasteryl-3-O-β-D-glucopyranoside

Hence, it could be concluded that this mixture was a mixture of three steroidal glycoside-3-O-β-D-glucopyranosides.

Study on the Biological Activity of Isolated Compounds.

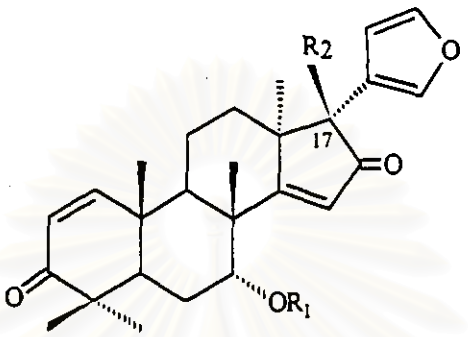
It has been reported for a long while that most limonoids were active as insect antifeedant.[9] These eminant properties were mainly due to their bitterness. Among active limonoids reported, those belonging to mexicanolide type were shown to possess of insect antifeedant activity. For instance the active compound 3β-*n*-butyryloxy-1-oxo-meliac-8(14)-enate **(48)** showed a good antifeedant activity against the larvae of the lepidopteran, *Agrotis segetum*.[48]



R = $OCOCH_2CH_2CH_3$ (24 25 26 27)

(48)

Some limonoids were considered as insect growth inhibitory such as 7-deacetyl-17β-hydroxy azadiradione (**49**), azadiradione (**50**) and 7-deacetyl azadiradione (**51**) against *Heliothis virescens*.[42]



(**49**) R_1 = H, R_2 = OH
(**50**) R_1 = Ac, R_2 = H
(**51**) R_1 = H, R_2 = H

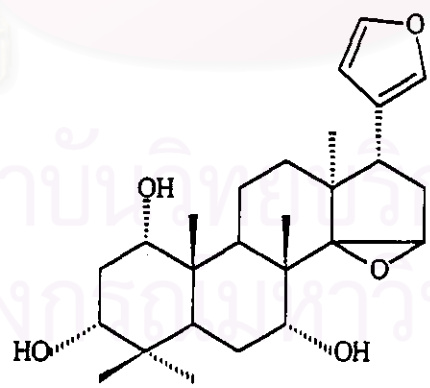
It seemed that the presence of the hydroxyl group in those reported molecules affected activity. Corresponding to this observation, (**49**) was reported to be more active than (**50**) which had no hydroxyl group. Apparently, a hydroxyl group at C-7 reduced the insect growth inhibitory activity, while a hydroxyl group at C-17 increased the activity.[42]

Hydroxyl groups in other types of limonoids were also found to influence biological activity. For example, acetylation or ketonization of the C-7 or C-12 hydroxyl group in the trichilins rendered them inactive as antifeedants against larvae of the southern armyworm (*Spodoptera eridania*). On the other hand, deacetylation of the C-1 acetate group in nomillin rendered

it inactive as a growth inhibitor against larvae of the fall armyworm and the corn earworm.[48] Thus, there are still some exceptions that it sometimes is not facile to predict for the effects of these compounds. Therefore, the thoroughly studies on this effect as an structure activity relationship (SAR) were clearly essential, especially for each particular insect.

Xyloccensin K (Comopund 6) was also composed of a hydroxyl group besides a furan ring. Eventhough it could not definitely fortell how excellent its biological activity is, it could predict that this compound perhaps reveals the antifeedant activity according to its structure and the tendency of other active limonoids reported.

Besides the insect antifeedant activity, some limonoids have also been reported to be active compounds against some types of cancer[9] such as havanensin and prieurianin group.



Havanensin

Xyloccensin K was also assayed for anticancer activity and it was found that this compound exhibited moderat activity against leukemia P388 cell line.[53]

From the former report, not only isolated limonoids were found to be bioactive compounds, but other compounds also showed biological activity. For instance, saturated long chain aliphatic primary alcohols, saturated long chain aliphatic carboxylic acids and mixture of steroids. These compounds were isolated from *Rhizophora apiculata* Bl.

The saturated long chain aliphatic alcohols are widely distributed in the waxy fraction of the plant extract. The triacontanol ($C_{30}H_{61}OH$) had been reported to be widely used as a plant growth regulator (PGR).[54, 55] 10-20 mg./L. of triacontanol used as the plant growth stimulating agent on tomato plants had been illustrated.[56] In addition, the saturated long chain aliphatic alcohols was reported to show the antifeeding activity to insect, boll weevil. A mixture of long chain aliphatic carboxylic acids was also reported to exhibit the 100% feeding inhibition against insect boll weevil at dose level 7 mg.

The co-occurance of three steroids (β-sitosterol, campesterol and stigmasterol) are widely distributed in the plant kingdom. The plant steroids were well known to be used as precursors for preparing steroid hormones. For instance, stigmasterol had been reported to be used as precusors to synthesize progesterone hormone.[57] Moreover, the steroids had been proved to have a

barely significant antihypercholesterolemic effect while exhibiting no obvious effect on the heart or liver.[58] From a previous bioassay report, a mixture of steroids displayed the antifeedant activity 79% inhibition against boll weevil at dose level 4.5 mg.[5]

The Results of Antifeedant Activity against *Galleria mellonella* of Isolated Compounds.

According to the preliminary antifeedant activity screening test (see Table 3.2), the seeds and the fruits crude extracts were selected for further study and searching for the active compounds against *Galleria mellonella*. All samples were prepared using the same dose, 4.0 mg/box (0.4% of larvae food) or otherwise specified. The results are shown in Table 3.17.

Table 3.17 The percentage of antifeedant activity of isolated compounds from *X. granatum* against Greater Wax Moth (*Galleria mellonella*).

Isolated compound	Percentage antifeedant activity
Mixture 1 (mixture of esters)	0
Mixture 2 (mixture of alcohols)	0
Mixture 3 (mixture of stroids)	0
Compound 5 (7-oxo-7-deacetoxy gedunin)	80.69
Compound 6 (xyloccensin K)	66.29
Mixture 9 (steroidal glycoside tetraacetate)	0

According to the bioassay results, the two active compounds were limonoids, xyloccensin K and 7-oxo-7-deacetoxy gedunin while the others were weak and not active. These limonoids were then conducted antifeeding activity test compared with other available limonoids, *i.e.*, angustidienolide (**41**) isolated from the heartwoods of *Xylocarpus moluccensis*[59] and very well-known commercially available antifeedant compound, azadirachtin (**7**). All isolated limonoids were prepared using the same dose 4.0 mg/box while the dose of azadirachtin was used only 0.05 mg/box. The results of percentage antifeedant activity are presented in the Table 3.18.

Table 3.18 The percentage antifeedant activity of isolated limonoids and azadirachtin against Greater Wax Moth (*Galleria mellonella*)

Limonoids	Dose (mg)	Percentage antifeedant activity
Compound **5** (7-oxo-7-deacetoxy gedunin)	4.0	80.69
Compound **6** (xyloccensin K)	4.0	66.29
angustidienolide	4.0	64.70
azadirachtin	0.05	53.29

The isolated limonoids were found to be active at dose 4.0 mg/box. However, azadirachtin seemed to be more potent than isolated limonoids from *Xylocarpus* genus.

From the bioassay results it may conclude that both 7-oxo-7-deacetoxy gedunin and xyloccensin K were active components in the seed and the fruit

crude extracts of *X. granatum*. Xyloccensin K was a major product while 7-oxo-7-deacetoxy gedunin was a minor component. Thus, it could believe that the active principle of the fruits and the seeds of *Xylocarpus granatum* was xyloccensin K.

Xyloccensin K was further investigated the optimized inhibitory dose by varying its concentration from 2.0 mg/box to 30.0 mg/box. The results are shown in the Table 3.19.

Table 3.19 The results of effective antifeeding dose of xyloccensin K against Greater Wax Moth (*Galleria mellonella)*

Dose (mg/1 g of larvae food)	Percentage antifeedant activity
2.0 mg/box (0.2 % w/w of food larvae)	37.49
4.0 mg/box (0.4 % w/w of food larvae)	66.29
10.0 mg/box (1 % w/w of food larvae)	73.76
20.0 mg/box (2 % w/w of food larvae)	79.29
30.0 mg/box (3 % w/w of food larvae)	87.95

The results of effective antifeeding dose of xyloccensin K against Greater Wax Moth were ploted as shown in the Fig. 3.53

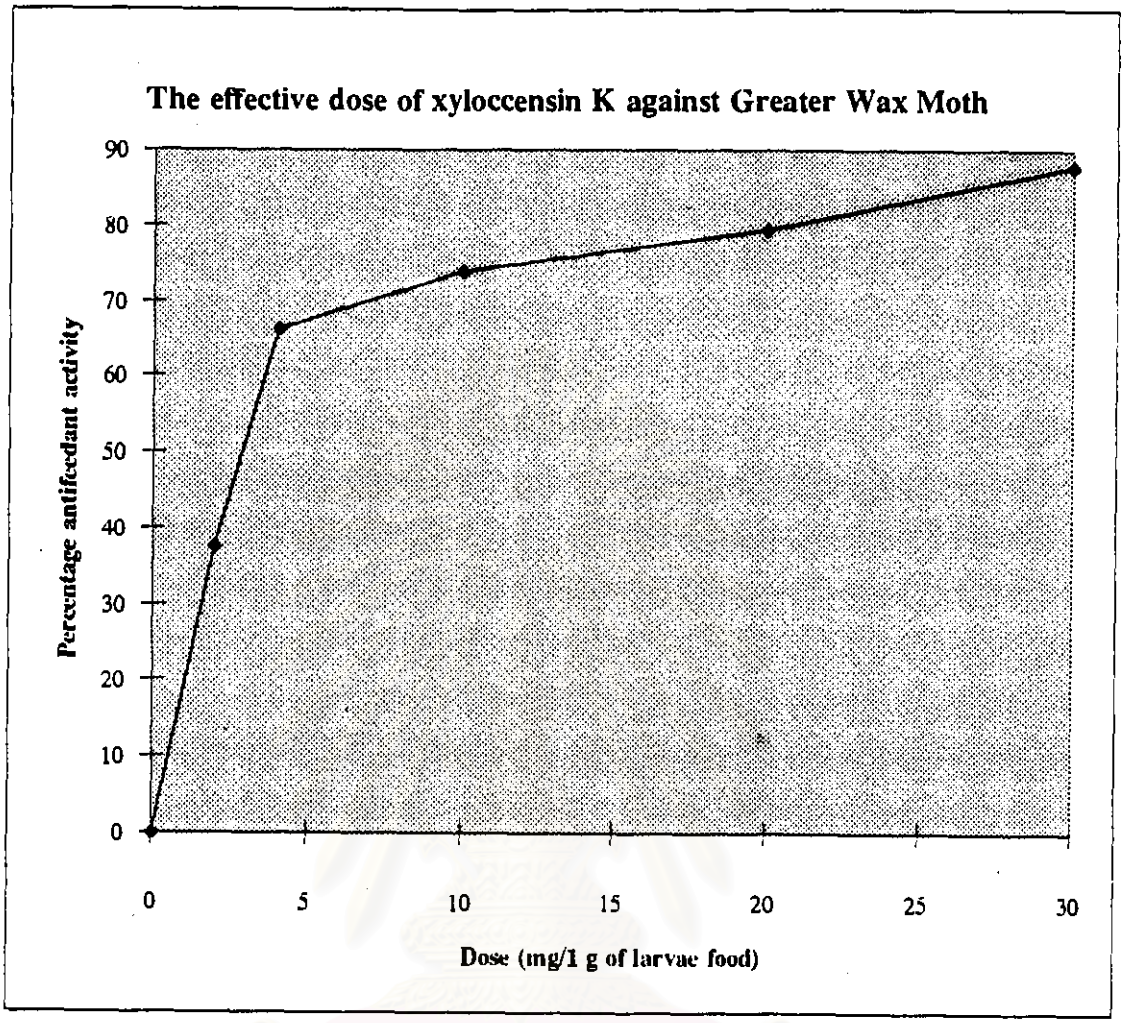


Fig 3.53 The results of effective antifeeding dose of xyloccensin K against Greater Wax Moth

Moreover, xyloccensin K was kindly tested for antifeedant activity against the Australian carpet beetle, *Anthrenocerus australis* by the Plant Extract Research Unit, Otago University.[35] The results as shown in Table 3.20 gave the same trend of the antifeedant activity against Greater Wax Moth.

Table 3.20 The results of Larval survival and wool consumption (weight loss) by *A. australis* larvae.

Substances	Concentration (% w/w)	Weight loss (mg)	Larvae survival ± SE
xyloccesin K	0.24	7.3	100
azadirachtin	0.01	8.9	100
controls			100
untreated	-	27.6	100
dichloromethane	-	28.1	100
Methylated spirit	-	32.50	100

Xyloccensin K turned out to be an active compound compared with the controls. Moreover, there was no significant difference in wool consumption between 0.01 % w/w azadirachtin and xyloccensin K 0.24 % w/w. Therefore, this compound showed antifeedant activity.