องค์ประกอบทางเคมีของผลขี้กาแดง

นายวิทวัส มิ่งวานิช

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CHEMICAL CONSTITUENTS IN THE FRUIT OF *Gymnopetalum integrifolium* KURZ

Mr. Withawat Mingvanish

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By	Mr. Withawat Mingvanish
Department	Chemistry
Thesis Advisor	Associate Professor Dr. Sophon Roengsumran

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master ' s Degree/

..... Dean of Graduate School

(Professor Thavorn Vajrabhaya, Ph.D.)

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..... Chairman

(Assistant Professor Amorn Petsom, Ph.D.)

..... Thesis Advisor

(Associate Professor Sophon Roengsumran, Ph.D.)

..... Member

(Assistant Professor Somchai Pengprecha, Ph.D.)

..... Member

(Associate Professor Chaiyo Chaichantipyuth, M.Sc.)

วิทวัส มิ่งวานิช : องค์ประกอบทางเคมีของผลขี้กาแดง (CHEMICAL CONSTITUENTS IN THE FRUIT OF *Gymnopetalum integrifolium* KURZ)
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นำเนื้อและเมล็ดของผลขี้กาแดง (*Gymnopetalum integrifolium* Kurz) มาสกัดด้วยตัวทำละลาย และนำสิ่งสกัดที่ได้จากเมล็ดของขี้กาแดงมาทำการแยกด้วยคอลัมน์โครมาโทกราฟี และแฟลชคอลัมน์โครมาโทกราฟีสามารถแยกสารได้ 4 ชนิด การหาสูตรโครงสร้างของสารเหอาศัยคุณสมบัติทางกายภาพ คุณสมบัติทางเคมีและหลักฐานทางสเปกโตรสโคปี พบว่าสาร 3 ที่ได้จากการแยกและสามารถหาสูตรโครงสร้างได้คือ ของผสมกรดคาร์บอกซิลิกโซ่ตรงยาว, ผสม β-sitosterol-3-O-β-D-glucopyranoside และ stigmasterol-3-O-β-D-glucopyraside สำหรับสารที่เหลืออีกหนึ่งชนิดนั้นยังไม่สามารถหาสูตรโครงสร้างได้

ภาควิชา เคมี ลายมือชื่อนิสิต วิทวัส มิ่งวานิช

สาขาวิชา เคมี ลายมือชื่ออาจารย์ที่ปรึกษา

ปีการศึกษา 2536

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The fleshes and seeds of the fruits of *Gymnopetalum integrifolium* Kurz v extracted by solvents. The crude extracts from the seeds of *Gymnopeta integrifolium* Kurz were subjected to column chromatography and flash col chromatography and four compounds were obtained. The structures of t compounds were established on the basis of physical properties, chemical prope and spectral evidences. Three identified components were isolated as a mixture of chain carboxylic acids, a mixture of β-sitosterol-3-O-β-D-glucopyranoside stigmasterol-3-O-β-D-glucopyranoside. The other compound cannot be identified.

ภาควิชา เคมี ลายมือชื่อนิสิต วิทวัส มิ่งวานิช

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Deeply gratitude is acknowleged to his parents, his young brother, Mr. Vathanyu Mingvanish, his cousins, and his fellows for their understanding and encouragement throughout the entire study.

Withawat Mingvanish

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LIST OF ABBREVIATIONS

TMS	Tetramethylsilane
δ	chemical shift
ppm	part per million
m.p.	melting point
TLC	thin layer chromatography
m.m.	millimeter
g	gram (s)
cm	unit of centrimeter
wt	weight
l	litre(s)
ml	millilitre (s)
^{o}C	degree Celsius
pp	page
R_t	retention time in gas chromatogram
min	minute
mg	milligram (s)
Fig.	Figure
R_f	rate of flow in chromatography
ν_{max}	the wavelength at maximum absorption
cm^{-1}	unit of wavenumber
s	strong (IR)
m	medium (IR)
b	broad (IR)

w	weak (IR)
s	singlet (NMR)
t	triplet (NMR)
m	multiplet (NMR)
M.W.	Molecular weight
M^+	molecular ion in mass spectrum
m/e	mass to charge ratio
CI	chemical ionization technique in mass spectrometry
EI	electron impact technique in mass spectrometry

CHAPTER I

INTRODUCTION

Gymnopetalum integrifolium Kurz (or *Trichosanthes integrifolia* Kurz (1,2)) is a creeping herb in the Cucurbitaceae family. In Thailand it is known as Kheekaa daeng and has been used as herbal medicine in the past. There are 118 genera and 825 species in this family.

General characterization of the plants in the genus Gymnopetalum (3)

The plants in Genus "Gymnopetalum" are usually creeping herbs. Leaves are nearly entire and deeply three- to five-lobed. Flowers have white colour and rather large size. In the fully developed plants, there are two male peduncles from each axil. The former is a flower as while the latter is a flower-cluster. However, both of them are suppressed. Bracts on the flower-cluster are large lanceolate. There are usually one-flowered female peduncles in separated axils. The calyx tube of the male flower is long and contracted near the mouth. It has the limb of five lanceolate segments. The five petals are not fimbriate on margin. There are three stamens and the others form one cluster. In the female flowers, the calyx and corolla resemble those of the male flowers. The ovary is oblong. The style is long. There are three stigmas. Their placentas, three in number, are long and vertical. The fruit of this plant is ovate-oblong and acute at both ends. Many or few seeds are ellipsoid and compressed. They have a margin and are nearly smoothness.

Gymnopetalum integrifolium Kurz (3) is a creeping herb with mono - or bi - fid tendrils as shown in Fig.1. Its leaf is reniform, blunt and 1.75 inches in length and its size of the leaf is 0.8 - 2.25 inches in width. The upper and lower surfaces of the leaf are very harshly scabrous and densely villous, respectively. The margin of the leaf is nearly entire and lobed. The petiole is 0.45 - 0.65 inches in length. The flowers of this plant are all solitary and white. Male and female peduncles are 1.5 and 0.25 inches in length, respectively. The male flower has the hairly calyx-tube. It has five lanceolate teeth. The Corolla is white and 1.35 inches in width. Moreover, it was covered with soft and short hair. Corollas of the female flowers are like those in the male. This plant has orange-red smooth globular fruit. Its diameter is 0.75 inches. Flowers and fruits flourish in September to October. (1)

For medicinal uses of Kheekaa daeng (1,2,4), it was believed that when the roots of Kheekaa daeng were boiled in water, the extract could be used as an anti-pyretic and heart-tonic drug. Dried roots were supposed to be able to treat diseases of the spleen,liver and other internal organs. Its fruits, with very bitter and acrid taste, can be consumed as anthelmintic. The fruits boiled with vegetable oil were used to treat migraine.

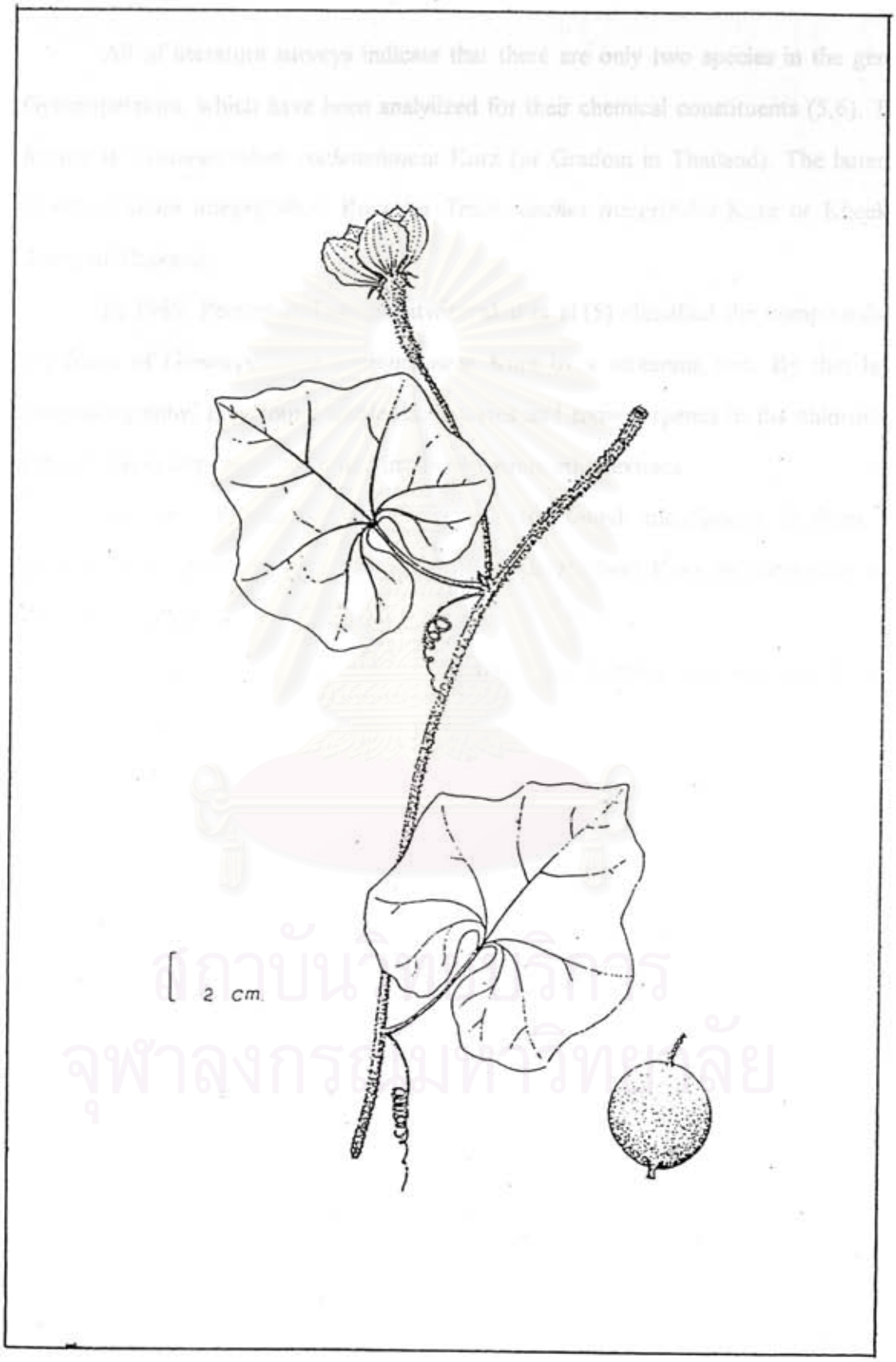


Figure 1 The stem, leaf, flower, and fruit of *Gymnopetalum integrifolium* Kurz.

All of literature surveys indicate that there are only two species in the genus Gymnopetalum, which have been analylized for their chemical constituents (5,6). The former is *Gymnopetalum cochinchinese* Kurz (or Gradom in Thailand). The latter is *Gymnopetalum integrifolium* Kurz (or *Trichosanthes integrifolia* Kurz or Kheekaa daeng in Thailand)

In 1989, Pornphan Tawishchatwitayakul et al.(5) classified the compounds in the fruits of *Gymnopetalum cochinchinese* Kurz by a screening test. By thin layer chromatography, they found alkaloids, lactones and some terpenes in the chloroform extract. Most terpenes were found in the petroleum ether extract.

In 1981, Weena Silipa-Archa et al. (6) found cucurbitacin B from the powdered and dried fruits of *Gymnopetalum integrifolium* Kurz by comparing with TLC of the standard, cucurbitacin B.

The chemical constituents of some Trichosanthes species which were investigated are summarized in Table 1.

Table 1 The chemical constituents of some Trichosanthes species. (for explanation of symbols: see the end of Table 1)

plant / part of plant	A	B		C	D	E			F					G	H		I						J	K	L	M	N	
organic compound	f	s	oth	r	s	s	dvs	f	s	fc	f	frr	ddr	s	f	ddr	s	f	r	rt	oth	pe	r	frr	frr	l	oth	ap
1. amino acids																		+										
2. α - aminobutyric acid									+																			
3. arachidonic acid										+																		
4. arginine									+																			
5. bryonolic acid [1]																			-				+	+				
6. campesterol [2]																	-											
7. Δ^7- campesterol [3]																	-											
8. conjugated dienolic acid										+																		
9. conjugated trienolic acid										+																		
10. cucurbitacin B [4]	+							+															+		+			
11. cucurbitacin D [5]																							+		+			
12. cucurbitacin E [6]								+																				
13. cycloeucalenol [7]																										+		-
14. cyclotrichosantol [8]																										+		
15. α,β - diaminopropionic acid									+																			
16. dihydrocucurbitacin B								-																				
17. dihydrocucurbitacin D								+																-	-			
18. α - eleostearic acid		-				+			+					-														
19. ethanolamine									+																			
20. ethyl - α - L - arabinofranoside																							+					
21. 24 - ethyl - cholesta - 5,25 - diene - 3β -ol																-												
22. 24- ethyl - cholesta - 5,24(25) - diene - 3β - ol																-												
23. 24 - ethyl - cholesta - 7,25 -diene - 3β - ol																-												
24. 24 - $\frac{5}{2}$ - ethyl - 5α - cholesta - 7 - en - 3β - ol								+																				
25. 24 - R - (24 - β -) - ethyl - 5α - cholesta - 7,22 - diene - 3β - ol [9]								+																				

Table 1 (continued)

plant / part of plant	A	B		C	D	E			F					G	H		I						J	K	L	M	N	
organic compound	f	s	oth	r	s	s	dvs	f	s	fc	f	frr	ddr	s	f	ddr	s	f	r	rt	oth	pe	r	frr	frr	l	oth	ap
26. 24 - S - (24 - β -) - ethyl - 5α - cholesta - 7,22,25 - triene - 3β - ol								+									+											
27. γ - guanidinobutyric acid [10]									+																			
28. D - glucose -																							+					
29. glycoprotein			+																									
30. 2 - O - β - D - glucopyranosyl - 23,24 - dihydrocucurbitacin B								+																				
31. 3 - O - β - glucopyranosyl - 24 - ξ - ethyl - 5α - cholesta - 7,22- diene								+																				
32. hexacosanoic																												+
33. histidine									+																			
34. isoleusine									+																			
35. karounidiol [11]																	+											
36. lectin																					+							
37. linoleic acid		+				+	+			+				+														
38. linolenic acid							+			+																		
39. lysine									+																			
40. methyl palmitate												+											-					
41. oleic acid		+				+	+			+				+														
42. palmitic acid		+					+					+											-					
43. phenylalanine									+																			
44. pipecolic acid [12]									+																			
45. punicic acid		+			-	+	+																					
46. santholin [13]																											+	
47. saturated aliphatic alcohol																						+						
48. saturated fatty acid						+				+				+														
49. β - sitosterol [14]																	+											+
50. α - spinasterol [15]											+	+	-		+	+			+				+	+	-			+
51. β - spinasterol																						+						
52. α - spinasterol - 3 - O - β - D - glucopyranoside [16]												+	-			+			+				+	+	-			

Table 1 (continued)

plant / part of plant	A	B		C	D	E			F					G	H		I						J	K	L	M	N	
organic compound	f	s	oth	r	s	s	dvs	f	s	fc	f	frr	ddr	s	f	ddr	s	f	r	rt	oth	pe	r	frr	frr	l	oth	ap
53. stearic acid		+					+																					
54. steryl (mixture of stigmast- 7- en - 3β -													+			+			+									
ol and α - spinasterol) - 6 - fatty acyl																												
(mixture of palmitic acid and																												
(Z,Z) - 9.12 - octadecadienoic acid)- β -																												
D -glucopyranosides [17-20]																												
55. stigmastanol [21]									+																			
56. stigmasterol [22]																	+											
57. stigmast - 7- en - 3β - ol [23]											+	+	+		+	+	+		+			+	+	+	+			
58. stigmast - 7 - en - 3β - ol - 3 - O - β												+	+			+			+			+	+	+	+			
D - glucopyranoside [24]																												
59. 7,22 - stigmastadien - 3 - ol																	+											
60. 7,24 - stigmastadien - 3 - ol																	+											
61. suberic acid [25]																							+					
62. tricholirin																					+							
63. trichosan [26]																												+
64. trichosanthin																			+									
65. trichosanthin - ZG																				+								
66. Radix trichosanthis																			+									
67. 3 triterpenoid glycosides [27-29]												+																
68. trypsin inhibitor																					+							
69. tyrosine									+																			
70. vanillic acid [30]												+																
71. vomifoliol [31]																							+					
72. 19 compounds such as sterols, sterol				+																								
glycosides, cucurbitacin B and D																												
and bryonolic acid																												
reference	6	7,8	9	10	8	8,11	12	13,	15,	18	19	20	21	22	19	21	23,	26	21,	30	31,	34	35	27	27	36	37	38
								14	16,								24,		27,		32,							
									17								25		28,		33							
																			29									

Table 1 (continued)

Symbols

A : *T. integrifolia* Kurz.	B : *T. anguina* L.	C : *T. bracteata* Voigt
D : *T. cordata* Roxb.	E : *T. cucumerina* L.	F : *T. cucumeroides* Maxim.
G : *T. dioica*	H : *T. japonica* Regel	I : *T. kirilowii* Maxim.
J : *T. kirilowii* Maxim. *var japonicum* Kitam.		K : *T. multiloba* Miq.
L : *T. miyagii* Hay.	M : *T. palmata* L.	N : *T. palmata* Roxb.

ap : aerial part	ddr : dried root	dvs : developing seed
f : fruit	fc : fruit coat	frr : fresh root
l : leaf	oth : other	pe : peel
r : root	rt : root tissue	s : seed

\+ : found

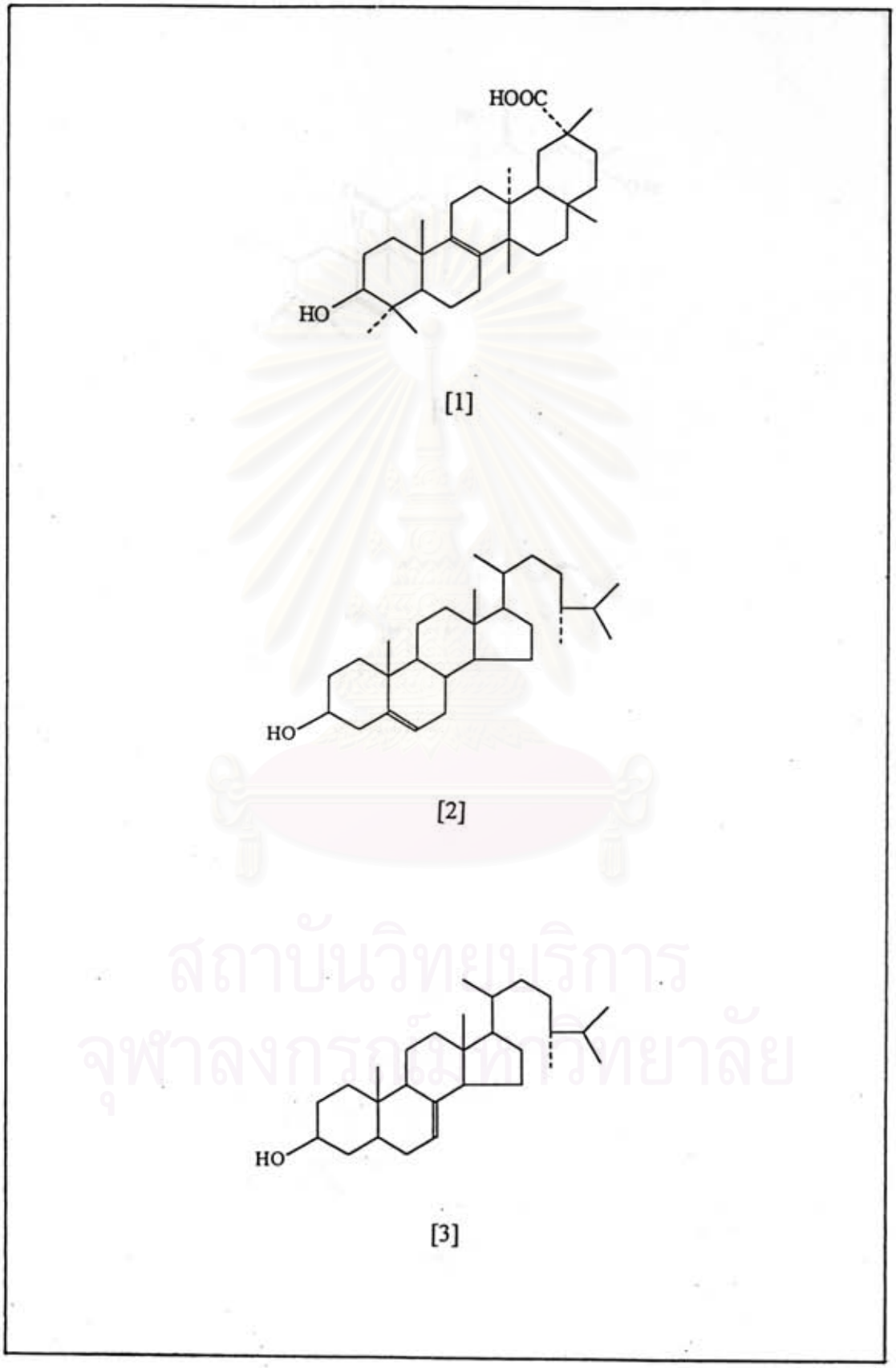
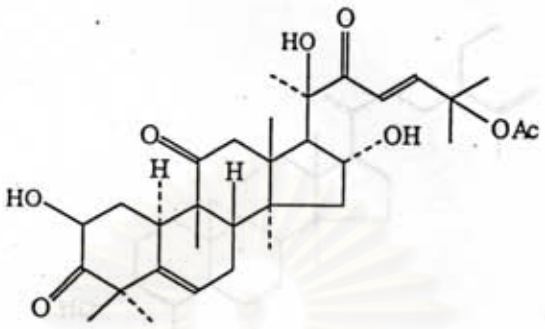


Figure 2 Chemical constituents of some Trichosanthes species.



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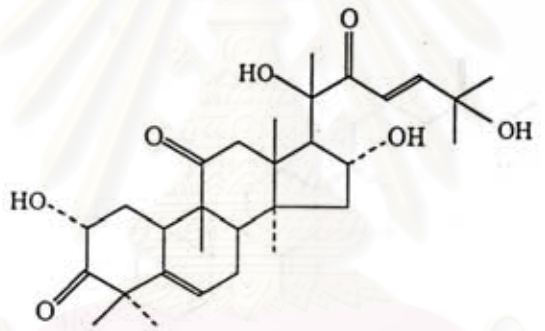

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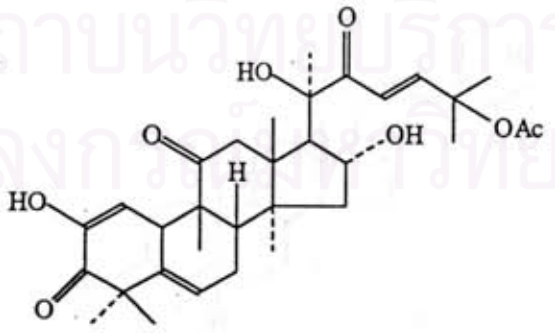

[6]

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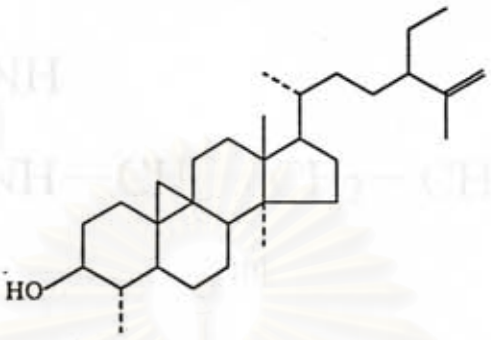

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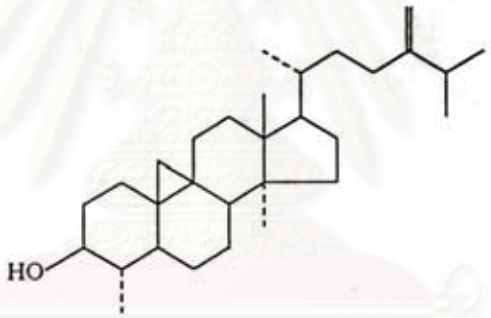

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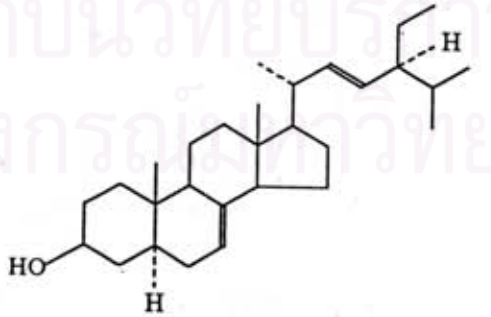

[9]

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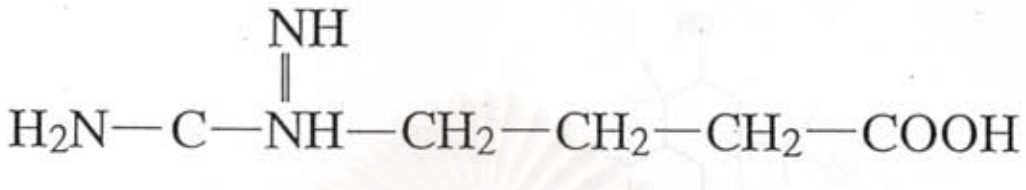
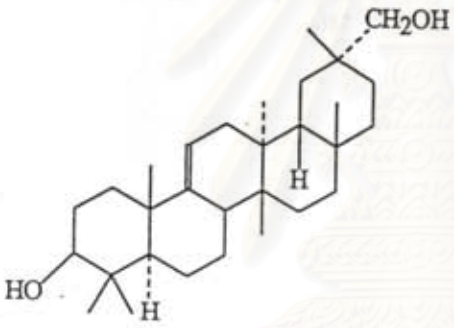
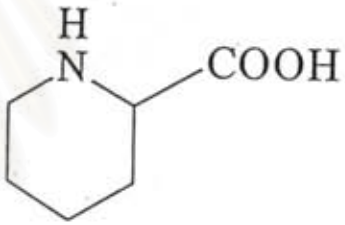


[10]



[11]



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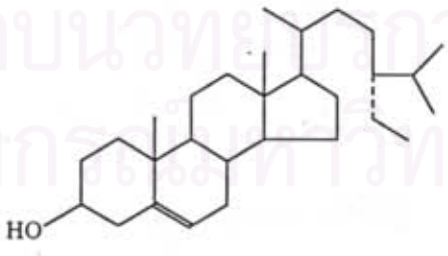

[14]

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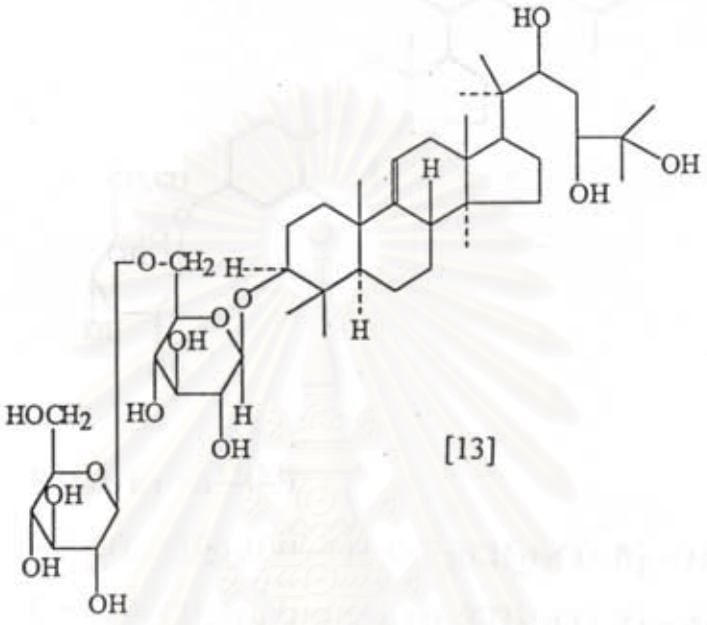

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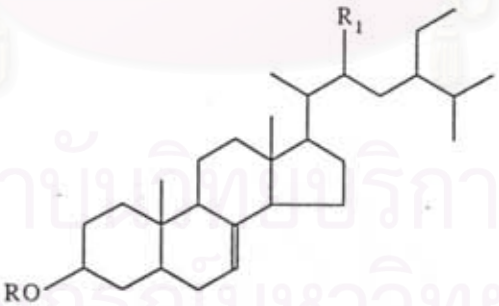
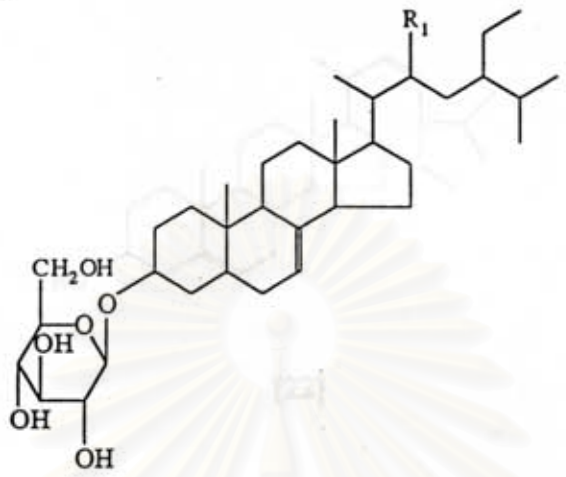


[15] R = H, $R_1 = \Delta^{22(23)}$

[23] $R = R_1 = H$

Figure 2 (continued)



[16] R = H, $R_1 = \Delta^{22(23)}$

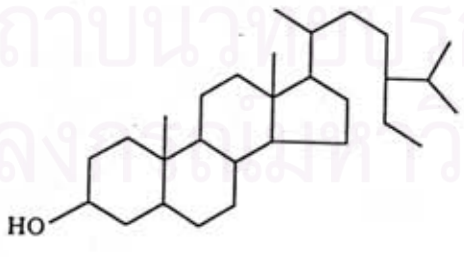
[17] R = $CH_3(CH_2)_3(CH_2\text{-}CH{=}CH)_2(CH_2)_7CO$, R_1 = H

[18] R = $CH_3(CH_2)_3(CH_2\text{-}CH{=}CH)_2(CH_2)_7CO$, $R_1 = \Delta^{22(23)}$

[19] R = $CH_3(CH_2)_{14}CO$, R_1 = H

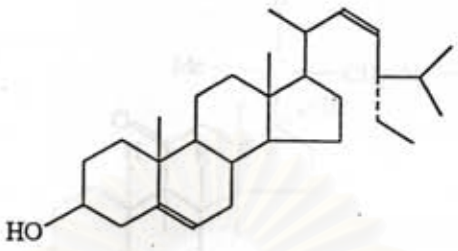
[20] R = $CH_3(CH_2)_{14}CO$, $R_1 = \Delta^{22(23)}$

[24] R = R_1 = H

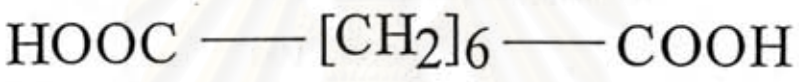


[21]

Figure 2 (continued)



[22]



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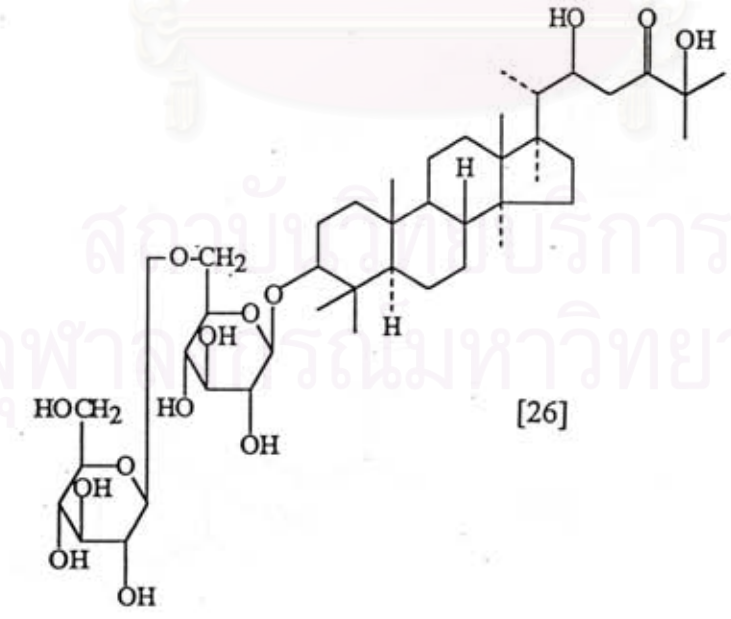


[26]

Figure 2 (continued)

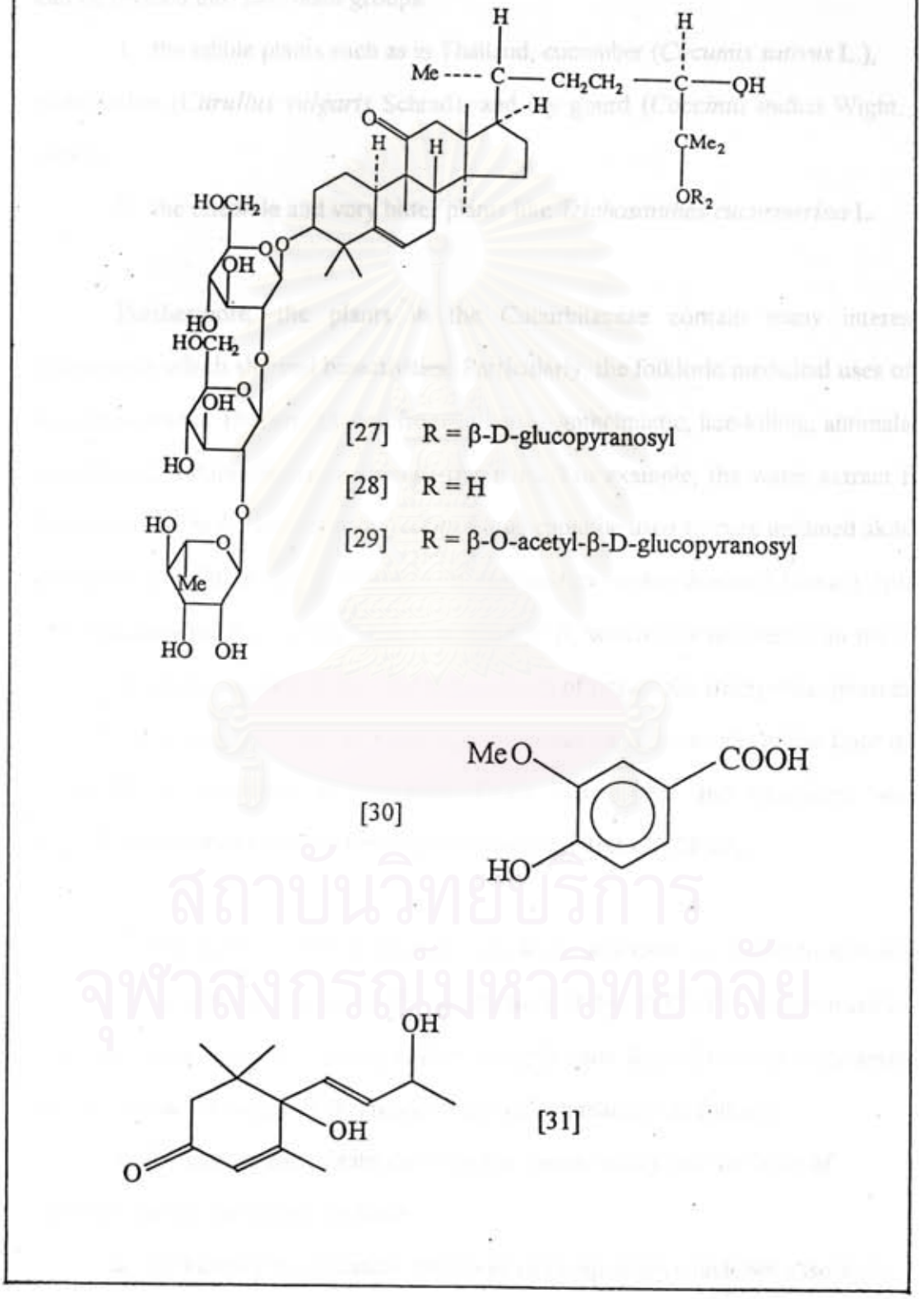


Figure 2 (continued)

Additionally, the references above indicate that the plants in the Cucurbitaceae can be divided into two main groups.

1. the edible plants such as in Thailand, cucumber (*Cucumis sativus* L.), watermelon (*Citrullus vulgaris* Schrad), and ivy gourd (*Coccinia indica* Wight. and Arm.).

2. the unedible and very bitter plants like *Trichosanthes cucurmerina* L.

Furthermore, the plants in the Cucurbitaceae contain many interesting compounds which showed bioactivities. Particularly, the folkloric medicinal uses of the Cucurbitaceae in Thailand ranged from cathartic, anthelmintic, lice-killing, antimalarial, antidiabetic, antiinflammatory to wart-removing. For example, the water extract from the leaves of *Trichosanthes tricuspidata* Lour. could be used to cure inflamed skin and diarrhoea in children (2) or the roots of *Momordica cochinchinensis* (Lour.) Spreng. had efficiency for lice- killing (39). Cucurbitacin B, which was isolated from the fruits of *Trichosanthes cucumerina* L. and was a group of tetracyclic triterpenes, possesses a very strong cytotoxic action, *in vitro*, against human carcinoma cells of the floor of the mouth (6). Furthermore, trichosanthin, Radix trichosanthis and trichokirin isolated from *Trichosanthes kirilowii* have an abortifacient effect (26,28,29).

Due to *Gymnopetalum integrifolium* Kurz was used as an folkloric medicine and found a bioactive compound; cucurbitacin B by TLC (6). Unfortunately, the chemical constituents of *Gymnopetalum integrifolium* Kurz have not been seriously studied. Thus, the target of this research can be summarized as follows:

1. To extract and isolate the chemical constituents from the fruits of *Gymnopetalum integrifolium* Kurz.

2. To identify the chemical structures of compounds which were isolated.

CHAPTER II

EXPERIMENTS AND RESULTS

2.1 Plant Materials.

The fruits of *Gymnopetalum integrifolium* Kurz or Kheekaa daeng were collected by M.L. Charuphant Thongtham from ROYAL PROJECT, Chiang Mai province during November 1991. This specimen was compared with the herbarium No. 073134 in the Royal Forest Department of Thailand.

2.2 Equipments.

2.2.1 Rotatory Evaporator

Eyela type N-N rotatory vacuum evaporator was used for the rapid removal of large amounts of volatile solvents.

2.2.2 Fourier Transform-Infrared Spectrophotometer (FT-IR)

The FT-IR spectra were recorded on a Perkin-Elmer Model 1760X Fourier Transform-Infrared Spectrophotometer. Solid samples were examined by incorporating the sample into a pellet of potassium bromide.

2.2.3 ^{1}H- and ^{13}C- Nuclear Magnetic Resonance Spectrometer

The ^{1}H-NMR and ^{13}C-NMR spectra were obtained by using a Bruker Model ACF 200 Spectrometer operated at 200.13 MHz. for ^{1}H and 50.32 MHz. for ^{13}C-nuclei. The chemical shift in δ (ppm.) was assigned with reference to the signal from the residual proton in deuterated solvent. Accordingly, the signal due to deuterated chloroform was assigned to be 7.24 ppm. with the reference to TMS.

2.2.4 Melting Point (m.p.)

The melting points were obtained on a Fisher - John apparatus.

2.2.5 Gas Chromatography-Mass spectrometry (GC-MS)

The GC-MS analysis was performed by a Fison Gas-Liquid Chromatography Model GC 8000 - Fison Mass Spectrometer Model Trio 2000.

2.3 Chemical Reagents.

2.3.1 All solvents used in this research were purified prior to use by distillation, except solvents that were reagent grade.

2.3.2 Merck's silica gel 60 Art. 7734.1000 (70-230 mesh ASTM) was used as adsorbents for column chromatography.

2.3.3 Merck's silica gel 60G Art. 7731 was applied as adsorbent for flash column chromatography.

2.3.4 Merck's TLC aluminium sheets, silica gel 60 F254 pre-coated 25 sheet, 20*20 $cm.^{2}$, layer 0.2 mm., was used for identifying the identical frations.

2.4 Physical Separation Techniques.

2.4.1 Flash Column Chromatography.

Flash Column Chromatography or Quick Column Chromatography will be explained in Reference No.40.

2.4.2 Column Chromatography (CC).

Column Chromatography will be presented in Reference No.41.

2.4.3 Thin-Layer Chromatography (TLC).

Thin-Layer Chromatography was expounded in Reference No.42,43.

2.5 Extraction.

The fruits of *Gymnopetalum integrifolium* Kurz were washed and its parts of the fleshes and seeds were separated. Its fresh fleshes (4700 g.) were ground with 4 litres of methanol and soaked in methanol 4 litres for 3-4 days at room temperature for 8 times. After each filtration and evaporation of the solvent under reduced pressure, the crude extract was obtained 165.59 g. as a dark-red oil (3.52 % wt. by wt. of the fleshes)

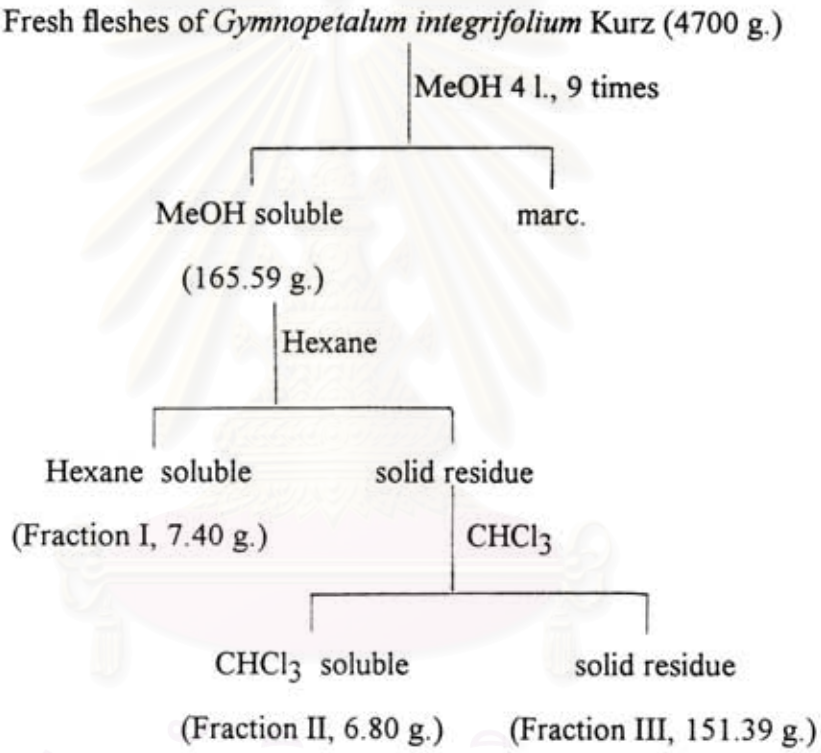
The crude extract was reextracted by hexane until the solution was clear and the filtered solution was evaporated to afford a hexane crude extract as a dark-green oil 7.40 g. (Fraction I, 0.16% % wt. by wt. of the fleshes)

After extraction with hexane, the crude extract was reextracted by chloroform until the solution was clear. The chloroform extract was evaporated and a dark-green oil was obtained 6.80 g. as a chloroform crude extract. (Fraction II, 0.14 % wt. by wt. of the fleshes)

The methanol extract was the hexane and chloroform insoluble part as a dark-red solid 151.39 g. (Fraction III, 3.22 % wt. by wt. of the fleshes)

The procedure of the extractions is shown in Scheme 1.

Sheme 1 Extraction of the fleshes of *Gymnopetalum integrifolium* Kurz.



The seeds of *Gymnopetalum integrifolium* Kurz (900 g.) were separated and sun-dried for 7 days. Then they were ground with 2 litres of methanol and soaked in methanol 1.5 litres for 3-4 days at room temperature for 7 times. After that it was filtered, and the soaking was further repeated until the colour of the solution was pale. After each filtration and evaporation of the solvent under reduced pressure, the crude extract was obtained 172.39 g. as a dark-green oil. (19.15 % wt. by wt. of the seeds)

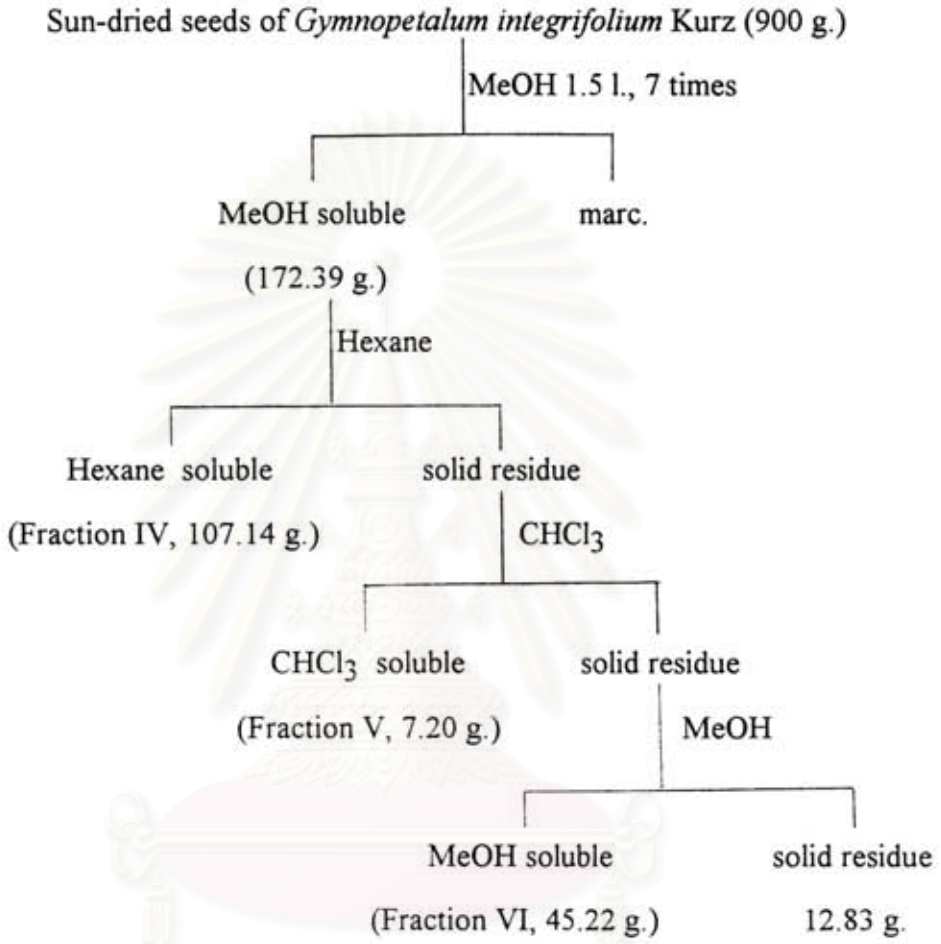
The crude extract was reextracted by hexane until the solution was clear and the filtered solution was evaporated to afford a hexane crude extract 107.14 g. as a dark-green oil. (Fraction IV, 11.90 % wt. by wt. of the seeds)

After extraction with hexane, the crude extract was reextracted by chloroform until the solution was clear. The chloroform extract was evaporated and a dark-green oil was obtained 7.20 g. as a chloroform crude extract. (Fraction V, 0.80 % wt. by wt.of the seeds)

The chloroform insoluble part was redissolved in methanol again until the solution was clear. The methanol soluble part was collected and concentrated by rotatory evaporator under reduced pressure. The methanol soluble part was obtained as the methanol crude extract (Fraction VI), a reddish-brown solid, 45.22 g. (5 % wt. by wt. of the seeds) and the residue, the methanol insoluble part, weighted 12.83 g. (1.43 % wt. by wt.of the seeds)

The procedure of the extractions is shown in Scheme 2.

Sheme 2 Extraction of the seeds of *Gymnopetalum integrifolium* Kurz.



2.6 Isolation of the Chemical Constituents of the Fruits of *Gymnopetalum integrifolium* Kurz.

2.6.1 Separation of Fraction III.

Flash column chromatography technique was used for separating 50.01 g. of the methanol crude extract into fractions. Silica gel Art. 7731 as adsorbent was packed to the height of about 5.5 cms. This column was initially eluted by $CHCl_3$ and taken about 800 ml. Each fraction was concentrated to about 50 ml. They were further removed to 10 ml. with the water bath and checked by TLC plate. The fractions containing similar components were combined together. The results of the separation of Fraction III by flash column chromatography are seen in Table 2.

Table 2 The results of the separation of Fraction III by flash column chromatography.

Eluent	Fraction No.	Remark
100% $CHCl_3$	1-2	yellow oil
	3-4	orange oil
10% MeOH-$CHCl_3$	5-10	black-brown oil
20-30% MeOH-$CHCl_3$	11-21	black oil
40-50% MeOH-$CHCl_3$	22-29	brown oil
50% MeOH-$CHCl_3$	30-31	solid in brown oil
	32-41	solid in brown oil
60% MeOH-$CHCl_3$	46-50	solid in brown oil
	51-53	brown tar
80% MeOH-$CHCl_3$	54-57	brown tar
80% MeOH-$CHCl_3$	58-62	brown tar
and 100% MeOH		
100% MeOH	63-69	brown tar
10% H_2O-MeOH	70-77	brown tar
25% H_2O-MeOH	78-85	brown tar

2.6.2 Separation of Fraction IV.

Concentrated hexane extract (50.02 g.) was chromatographed on silica gel 60G Art.7734 (500 g.) by using a column chromatography of 2.5 cm. Φ i.d.. The column was eluted with hexane, hexane-chloroform, chloroform-methanol, and methanol, respectively. After the eluent was collected (approximately 800 ml. for each fraction), it was concentrated by rotatory evaporator and by distillation to a volume of about 50 ml.. Each fraction was monitored by thin-layer chromatography and the identical fractions were combined. The results of the separation of Fraction IV are presented in Table 3.

Table 3 The results of the separation of Fraction IV by column chromatography.

Eluent	Fraction No.	Remark
100% hexane	1-2	-
	3-5	trace
10% $CHCl_3$-hexane	6-16	yellow oil (Mixt. I)
25% $CHCl_3$-hexane	17-22	yellowish-green oil (Mixt. I)
	23-26	yellowish-green oil (Mixt. I)
40% $CHCl_3$-hexane	27	yellow oil (Mixt. I)
	28-29	yellow oil (Mixt. I)
	30-33	orange oil (Mixt. I)
50% $CHCl_3$-hexane	34-42	white semi-solid in yellow oil
70% $CHCl_3$-hexane	43-51	white semi-solid in yellow oil
80% $CHCl_3$-hexane	52-63	brown oil
80-90% $CHCl_3$-hexane and 100% $CHCl_3$	64-75	brownish-yellow oil
100% $CHCl_3$ and 10% MeOH-$CHCl_3$	76-89	white ppt. in dark green oil
20-50% MeOH-$CHCl_3$	90-105	dark-brown oil
75% MeOH-$CHCl_3$	106-110	brown oil
100% MeOH	111-115	light brown oil
	116-122	red brown oil

2.6.2.1 The separation of the eluted fractions No. 34-51. (Table 3, pp. 27, 3.58 g.)

TLC indicated that fractions No.34-42 and 43-51 should be combined. The combined fractions were chromatographed on 53.7 g. silica gel on 2.1 cm. Φ i.d. column. Approximately 50 ml. fractions were colected and concentrated on a water bath to about 10 ml.. Each portion was monitored by TLC for combinating each identical fraction together. The results of the separation of these fractions are shown in Table 4.

Table 4 The results of the separation of the eluted fractions No. 34-51 by silica gel column chromatography.

Eluent	Fraction No.	Remark
25% $CHCl_3$-hexane	1-2	trace
	3-4	semi-solid in yellow oil (Mixt. II)
	5	semi-solid (Mixt. II)
	6	semi-solid in yellow oil (Mixt. II)
25-30% $CHCl_3$-hexane	7-12	yellow oil
30-60% $CHCl_3$-hexane	13-24	yellow oil
75-85% $CHCl_3$-hexane and 100% $CHCl_3$	25-35	yellow oil
2-5% MeOH-$CHCl_3$	36-46	dark yellow oil
10-50% MeOH-$CHCl_3$	47-55	dark yellow oil
100% MeOH	56-59	trace

2.6.2.2 The separation of the eluted fractions No. 64-75. (Table 3, pp. 27, 5.70 g.)

The eluted fractions No. 64-75, brownish-yellow oil 5.70 g., was further purified by column chromatography (silica gel Art.7734 114.02 g., 3 cm. Φ i.d. column). The initial eluent was 25% chloroform in hexane. About 50 ml. fractions were collected. The method of identifying identical fractions was similar to the method in the topic 2.6.2.1. The results of examination of the eluted fractions No.64-75 are seen below:

Table 5 The results of the separation of the eluted fractions No. 64-75 by silica gel column chromatography.

Eluent	Fraction No.	Remark
25% $CHCl_3$-hexane		-
50% $CHCl_3$-hexane	1-2	yellow oil
75-85% $CHCl_3$-hexane	3-8	yellow oil
and 100% $CHCl_3$	9-21	
2% MeOH-$CHCl_3$		yellow oil
	22-26	trace
5% MeOH-$CHCl_3$	27-32	yellow oil
	34-38	light yellow oil
	39-41	white wax in yellow oil
10% MeOH-$CHCl_3$	42	light yellow oil
	43-45	orange-yellow oil
20% MeOH-$CHCl_3$	46-50	orange-yellow oil
50% MeOH-$CHCl_3$	51-60	orange-yellow oil
75% MeOH-$CHCl_3$	61-68	solid in orange-yellow oil
100% MeOH	69-72	orange-yellow oil
	73-81	

2.6.2.3 The separation of the eluted fractions No. 76-89. (Table 3, pp. 27, 4.63 g.)

The fractions No.76-89 contained a white precipitate in dark green oil when concentrated and were rechromatographed (70.09 g. silica gel Art.7734. 2.1 cm. i.d. column). The fractions were initially eluted by 25% chloroform in hexane. 50 ml. fractions were collected. The method of identifying identical fractions were similar to the method in the topic 2.6.2.1. The results of the separation of these fractions are presented in Table 6.

Table 6 The results of the separation of the eluted fractions No. 76-89 by silica gel column chromatography.

Eluent	Fraction No.	Remark
25% $CHCl_3$-hexane	1-4	trace of yellow oil
40% $CHCl_3$-hexane	5-29	yellow oil
50% $CHCl_3$-hexane	30-40	yellow oil
70% $CHCl_3$-hexane	41-50	yellow oil
85% $CHCl_3$-hexane	51-61	yellow oil
100% $CHCl_3$ and 2% MeOH-$CHCl_3$	62-73	yellow oil
2% MeOH-$CHCl_3$	74-78	green oil
3% MeOH-$CHCl_3$	79-82	white needle crystal in green oil (Mixt.III)
4-5% MeOH-$CHCl_3$	83-95	green oil
10% MeOH-$CHCl_3$	96-98	green oil
	99-101	white ppt. in green oil (Mixt. IV)
10-20% MeOH-$CHCl_3$	102-111	green oil
30-50% MeOH-$CHCl_3$	112-128	green oil

Table 6 (continued)

Eluent	Fraction No.	Remark
75% MeOH-$CHCl_3$ and 100% MeOH	122-128	green oil
100% MeOH	129-130	yellow oil

2.6.2.4 The separation of the eluted fractions No. 90-105. (Table 3, pp. 27, 2.66 g.)

2.66 g. of dark-brown oil fractions No. 90-105 was chromatographed on 53.2 g. silica gel. The column had internal diameter 2.1 cms. and was eluted by various ratios of mixtures of chloroform in hexane, methanol in chloroform. Finally, it was stripped off with methanol. The other steps were performed as the topic 2.6.2.1. About 50 ml. fractions were collected. The results of the separation of these fractions are shown in Table 7.

Table 7 The results of the separation of the eluted fractions No. 90-105 by silica gel column chromatography.

Eluent	Fraction No.	Remark
50% $CHCl_3$-hexane	1	trace
	2	green oil
	3	green oil
	4-8	yellow oil
60% $CHCl_3$-hexane	9-14	greenish-yellow oil
75% $CHCl_3$-hexane	15-21	trace
100% $CHCl_3$ and 2% MeOH-$CHCl_3$	22-26	trace

Table 7 (continued)

Eluent	Fraction No.	Remark
100% $CHCl_3$ and	27-31	trace
2% MeOH-$CHCl_3$		
10% MeOH-$CHCl_3$	36-46	green-brown oil
25% MeOH-$CHCl_3$	47-55	green-brown oil
35-50% MeOH-$CHCl_3$	56-65	green oil
75% MeOH-$CHCl_3$	66-70	brown oil
100% MeOH	71-78	trace

2.6.2.5 The separation of the eluted fractions No. 106-110. (Table 3, pp. 27, 0.74 g.)

0.74 g. of brown oil was chromatographed on 14.8 g. silica gel. The column had internal diameter 1.25 cms. The fractions were initially eluted by 100% $CHCl_3$. About 30 ml. fractions were collected. Other steps were performed as discussed above. The results of the separation of these fractions are shown in Table 8.

Table 8 The results of the separation of the eluted fractions No. 106-110 by silica gel column chromatography.

Eluent	Fraction No.	Remark
100% $CHCl_3$	1	trace
	2	yellow oil
	3-4	yellow oil
1-3% MeOH-$CHCl_3$	5-11	yellow oil
5% MeOH-$CHCl_3$	12-14	greenish-yellow oil
	15-17	greenish-yellow oil
10% MeOH-$CHCl_3$	18-22	yellowish-green oil

Table 8 (continued)

Eluent	Fraction No.	Remark
20% MeOH-$CHCl_3$	23-26	yellowish-green oil
20-35% MeOH-$CHCl_3$	27-33	brown oil
60% MeOH-$CHCl_3$	34-40	brown tar
85% MeOH-$CHCl_3$	41-50	brown tar
and 100% MeOH		

2.6.3 Separation of Fraction V.

Concentrated chloroform extract (7.20 g.) was chromatographed on silica gel Art. 7734 (86.40 g.) by using a column chromatography of 5 cm. Φ i.d.. The column was initially eluted by 50% chloroform in hexane. The polarity of eluent was gradually increased from chloroform in hexane to chloroform and from chloroform to methanol. About 50 ml. of the eluted solution was collected in each time. Fractions were evaporated on the water bath and checked by TLC. The similar fractions were combined . The results of the separation of Fraction V by column chromatography are presented in Table 9.

Table 9 The results of the separation of Fraction V by column chromatography.

Eluent	Fraction No.	Remark
50% $CHCl_3$-hexane	1-5	-
60% $CHCl_3$-hexane	6-17	yellow oil
	18-22	green oil
60-80% $CHCl_3$-hexane	23-40	green oil
100% $CHCl_3$	41-50	green oil
2% MeOH-$CHCl_3$	51-55	green oil
	56-60	green oil
5% MeOH-$CHCl_3$	61-67	brown oil
	68-69	brown oil
	70-73	white ppt. in brown oil
10% MeOH-$CHCl_3$	74-82	red-brown oil
	83-89	red-brown oil
20-30% MeOH-$CHCl_3$	90-102	red-brown oil
30% MeOH-$CHCl_3$	103-108	red-brown oil
50% MeOH-$CHCl_3$	109-116	brown oil
75% MeOH-$CHCl_3$	117-120	brown oil
100% MeOH	121-133	brown oil

2.6.3.1 The separation of the eluted fractions No. 70-73. (Table 9, pp. 34, 0.88 g.)

Fractions No. 70-73, eluted from the silica gel column with 5% methanol-chloroform, consisted of a brown oil together with some white precipitated solid. These fractions were partially purified by silica gel column chromatography again (silica gel : material = 1 : 12). The size of the internal column diameter was 1.4 cms. About 20 ml. of fractions were collected. The results of the separation of these fractions are shown as below:

Table 10 The results of the separation of the eluted fractions No. 70-73 by silica gel column chromatography.

Eluent	Fraction No.	Remark
50% $CHCl_3$-hexane	1-3	yellow oil
	4-6	yellow oil
75% $CHCl_3$-hexane	7-11	trace
100% $CHCl_3$	12-17	-
2% MeOH-$CHCl_3$	18-21	white oil
	22-25	yellow oil
5% MeOH-$CHCl_3$	26-29	yellow oil
10% MeOH-$CHCl_3$	30-32	yellow ppt. in yellow oil (Mixt. IV)
	33-39	yellow oil
20% MeOH-$CHCl_3$	40-46	yellow oil
30% MeOH-$CHCl_3$	47-53	yellow oil
50% MeOH-$CHCl_3$	54-59	brown oil
75% MeOH-$CHCl_3$	60-69	brown oil
100% MeOH	70-77	trace

2.6.3.2 The separation of the eluted fractions No. 74-82. (Table 9, pp. 34, 1.07 g.)

The eluted fractions No. 74-82 ,which were eluted from column with 10% MeOH-$CHCl_3$, contained a red-brown oil. This fraction was purified by the same method as fractions No. 70-73. About 20 ml. fractions were collected. The results of the separation of these fractions are discussed in Table 11.

Table 11 The results of the separation of the eluted fractions No. 74-82 by silica gel column chromatography .

Eluent	Fraction No.	Remark
75% $CHCl_3$-hexane	1	-
	2-4	yellow-brown oil
	5-8	yellow oil
100% $CHCl_3$	9-14	yellow oil
2% MeOH-$CHCl_3$	15-20	yellow oil
5% MeOH-$CHCl_3$	21-22	-
	23-28	trace
	29-31	yellowish brown oil
10% MeOH-$CHCl_3$	32-34	brown oil
	35-44	brown oil
20% MeOH-$CHCl_3$	45-48	brown oil
	49-52	brown oil
35% MeOH-$CHCl_3$	53-55	brown oil
	56-59	brown tar
50% MeOH-$CHCl_3$	60-64	brown tar

Table 11 (continued)

Eluent	Fraction No.	Remark
75% MeOH-$CHCl_3$ and 100% MeOH	65-69	brown oil
100% MeOH	70-72	trace

2.6.4 Separation of Fraction VI.

Flash column chromatography technique was used for separating 22.61 g. of the methanol crude extract into fractions. Silica gel Art. 7731 as adsorbent was packed to the height of about 4 cms. This column was initially eluted by 50% chloroform in hexane and taken about 400 ml. Each fraction was concentrated to about 50ml. They were further removed to 10 ml. with the water bath and checked by TLC plate. The fractions containing similar components were combined together. The results of the separation of the methanol extract by flash column chromatography are seen in Table 12.

Table 12 The results of the separation of Fraction VI by flash column chromatography.

Eluent	Fraction No.	Remark
50% $CHCl_3$-hexane	1-3	transparent oil
	4-6	yellow oil
75-85% $CHCl_3$-hexane	7-19	yellow oil
100% $CHCl_3$	20-26	yellow oil
	27-28	reddish-brown oil
5-10% MeOH-$CHCl_3$	29-41	reddish-brown oil
10% MeOH-$CHCl_3$	42-44	solid in reddish-brown oil
20% MeOH-$CHCl_3$	45-50	reddish-brown oil
30% MeOH-$CHCl_3$	51-55	reddish-brown oil
	56-57	reddish-brown oil
50% MeOH-$CHCl_3$	58-61	solid in reddish-brown oil
	62-69	brown tar
75% MeOH-$CHCl_3$	70-78	brown tar
85% MeOH-$CHCl_3$	79-86	brown tar
100% MeOH	87-99	brown tar

2.7 Purification and Properties of the Eluted Compounds by Column Chromatography.

2.7.1 Purification and Properties of Mixture I.

Mixture I was a yellowish-green oil, which was obtained from the hexane crude extract and eluted by 10% $CHCl_3$-hexane in fractions No. 6-16, by 25% $CHCl_3$ in hexane in fractions No. 17-22 and 23-26 and 40% $CHCl_3$ in hexane in fractions No. 27, 28-29 and 30-33 from silica gel column chromatography (Table 3). Mixture I weighed 18.88 g.(17.68 % wt. by wt. of hexane part). The oil dissolved in hexane and chloroform, but not in methanol.

FT-IR spectrum (neat) $\nu_{max}(cm^{-1})$ 2926(s), 2855(s), 1743(s), 1462(m), 1435(m), 1363(m), 1172(m), 724(m) (Fig. 3)

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm) 0.86(t), 1.23(s), 1.59, 2.00, 2.28(t), 3.64(s), 5.32(t) (Fig. 4)

^{13}C-NMR spectrum ($CDCl_3$) δ (ppm) 13.8, 22.5, 24.7, 27.0, 28.9-29.5, 31.8, 33.8, 51.0, 127.7, 127.8, 129.7, 129.8, 173.8 (Fig. 5)

DEPT-90 ^{13}C-NMR ($CDCl_3$) δ (ppm.) (Fig. 6)

CH signals 129.7, 129.8

DEPT-135 ^{13}C-NMR ($CDCl_3$) δ (ppm.) (Fig. 7)

CH_3, CH signals (down phase) 13.8, 51.0, 129.7, 129.8

CH_2 signals (up phase) 22.5,.24.7, 27.0, 28.9-29.5, 31.8, 33.8

The mixture I was analysed by GC-MS spectrometer. The conditions of GC were as follows:

column : DB-1

injection temperature : 250^{o}C

oven temperature : 70-220^{o}C

rate of heating : 3^{o}C/min.

flow rate of He : 7 Psi column head pressure

The GC-MS spectrum is shown in Figure 8. Retention times were 11.65, 24.64, 29.47, 31.92, 32.59, 33.23, 44.11, 49.31 and 49.63 min., respectively. (Fig. 9-14)

2.7.2 Purification and Properties of Mixture II.

Mixture II was a white semi-solid in yellow oil in fractions No. 34-42 and 43-51. Both of these fractions were isolated with 25% $CHCl_3$-hexane from hexane part and rechromatographed to afford white amorphous solid in fractions No. 3-4, 5, and 6, respectively. (Table 4) After recrystallization from $CHCl_3$-hexane solution, white amorphous solid, 122.4 mg.(0.11% wt. by wt. of hexane part), m.p. 58-59^{o}C, was obtained. This mixtture was soluble in hexane, chloroform, and methanol.

FT-IR spectrum (KBr) ν_{max} (cm^{-1}) 3500-2400(very b.), 2919(s), 2850(s), 1702(s), 1465(m), 1434(m), 1298(m), 941(m), 724(m) (Fig. 15)

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm) 0.88(t), 1.25(s), 1.63(m), 2.00(m), 2.35(t), 5.34 (Fig. 16)

^{13}C-NMR spectrum ($CDCl_3$) δ (ppm) 14.0, 22.6, 24.6, 27.1, 29.0-29.6, 31.8, 34.0, 128.9, 129.2, 129.3, 130.8, 180.0 (Fig. 17)

DEPT-90 ^{13}C-NMR ($CDCl_3$) δ (ppm.) (Fig. 18)

CH signals 128.9, 129.2, 129.3, 130.8

DEPT-135 ^{13}C-NMR ($CDCl_3$) δ (ppm.) (Fig. 19)

CH_3, CH signals (down phase) 14.0, 128.9, 129.2, 129.3, 130.8

CH_2 signals (up phase) 22.6,.24.6, 27.1, 29.0-29.6, 31.8, 34.0

Methylation of carboxylic acid compounds by diazomethane.

The generation of diazomethane involves adding 0.5 g. of N-methyl-N'-nitro-N-nitrosoguanidine gradually to a mixture of 30 ml. of ether and 5 ml. of 40% aqueous potassium hydroxide solution cooled to $0^{o}C$ with shaking. The yellow diazomethane was codistillated with ether and then passed into the solution of carboxylic acid compounds in ether until the solution colour changed to yellow.

The methyl esters which were thus synthesized were analyzed by GC-MS spectrometer. The GC-MS analysis results of Mixture II (Fig. 20) (condition: the same as the conditions which was applied to identify the compounds of the mixture I) displayed the peaks at R_t 24.21, 31.47, 44.38, 49.55 and 50.62 min., respectively. (Fig. 21-24)

2.7.3 Purification and Properties of Mixture III.

Mixture III was obtained from the column chromatography of the hexane crude extract of the fractions No. 76-89 which were eluted with 3% MeOH-$CHCl_3$(Table 6). Mixture III was a mixture of white needle like crystals in a green oil. After the green oil was removed by washing with hexane, the remaining product was recrystallized by hexane-chloroform for twice to yield a white amorphous solid, 2.6 mg.(0.002 % wt. by wt. of hexane part). It had m.p. at 275-277^{o}C and decomposed at 270-280^{o}C. R_f value was 0.59 in 10% MeOH in $CHCl_3$. This compound dissolved in chloroform, and methanol but not in hexane.

FT-IR spectrum (KBr) ν_{max} (cm^{-1}) 3192, 3092, 3057, 2962, 2876, 1663(s), 1452, 1387 (Fig. 25)

2.7.4 Purification and Properties of Mixture IV.

Mixture IV was collected from hexane crude in Fractions No. 99-101 which was eluted by 10% methanol in chloroform by column chromatography (Table 6) and was obtained from chloroform crude in Fractions No. 30-32 which was eluted by the same eluent by column chromatography (Table 10). The precipitate was purified by recrystallization from the mixture of chloroform and methanol for several times. The white amorphous solid so obtained is Mixture IV (9.1 mg., 0.008 % wt. by wt. of hexane and chloroform part). Melting point was 258-260^{o}C. R_f values were 0.14 and 0.51 in 10 and 20% methanol in chloroform systems, respectively. This mixture was slightly soluble in chloroform and methanol and not soluble in hexane. It gave a positive test (green-blue colour) with Liebermann-Burchard's reagent.

FT-IR spectrum (KBr) ν_{max} (cm^{-1}) 3427(b), 2937(s), 2872(s), 1633(m), 1462(m), 1376(m), 1070-1023(s), 890(w) (Fig. 26)

CI MS spectrum m/e 414, 412, 396, 255 (Fig. 27)

EI MS spectrum m/e 414, 412, 396, 394, 382, 329, 327, 273, 255, 213 (Fig. 28)

CHAPTER III

RESULTS AND DISCUSSION

The fruits of *Gymnopetalum integrifolium* Kurz (Kheekaa daeng) studied in this investigation were collected from ROYAL PROJECT, Chiang Mai province, Thailand during November, 1991. Its fleshes and seeds were separated to study their chemical constituents. The fresh flesh part was milled and extracted with methanol. The methanol extract was evaporated by the rotatory evaporator to give dark-red oil. It was then reextracted with hexane and chloroform. Ultimately, the methanol extract was obtained. These methods explained above were shown in Scheme 1. Both of the percent yields of the hexane and chloroform parts were obtained in such a limited amount as while the methanol part had the hightest percent yield. After isolating the methanol extract by flash column chromatography, the brown tar was obtained so it was not further elucidated.

The part of seeds of *Gymnopetalum integrifolium* Kurz was sun-dried, milled and extracted with methanol. The methanol extract was evaporated by the rotatory evaporator to give dark-green oil methanol extract. It was then reextracted with hexane and chloroform. The remaining solid was redissolved in methanol again. The methanol solution was evaporated. These methods explained above were shown in Scheme 2. The hexane part had the highest percent yield. In turn, the chloroform part had the lowest percent yield. All of the three extract parts were separated by the column chromatography as discussed in Chapter II.

3.1 Structural Elucidation of the Eluted Compounds from the Seeds of *Gymnopetalum integrifolium* Kurz.

3.1.1 Structural Elucidation of Mixture I.

Mixture I was obtained as a yellowish-green liquid. Its IR-spectrum, Fig. 3, indicated the presence of one or more ester groups (C=O stretching vibration peak at 1743 cm^{-1} and C-O stretching vibration peak of ester group at 1170 cm^{-1}). The C-H stretching vibration peaks of an aliphatic compound were observed at 2926 and 2855 cm^{-1}. The absorption peak at 1363 cm^{-1} corresponded to C-H symmetric bending vibration mode of CH_3 group. In addition, the absorption peak at 724 cm^{-1} might possibly indicate the presence of one or more saturated long chain of $(-CH_2-)_n$; $n > 4$.

Table 13 The IR absorption band assignments of Mixture I.

Wavenumber (cm^{-1})	Band type	Tentative assignment
2926,2855	s	C-H stretching vibration of CH_3-, -CH_2
1743	s	C=O stretching vibration of ester
1462	m	C-H bending vibration of -CH_2- ,CH_3-
1435,1363	m	C-H symmetric bending vibration of CH_3-
1172	m	C-O stretching vibration of ester
724	m	C-H rocking mode of -CH_2- (for carbon > 4)

The ^{1}H-NMR spectrum, Fig. 4, exhibited the methyl peak at 3.64 ppm. indicating the presence of a methoxy group. This was corroborated by the presence of the signal at 51.0 ppm. in the ^{13}C-NMR spectrum. The signal of α-proton in the acidic portion was observed at 2.28 ppm. The high intensity singlet peak at 1.23 ppm. was consistent with several interlinking methylene protons in this type of compound. The peaks at 1.59 and 2.00 ppm. were likely to correspond to the methylene protons of the carboxylic part of an ester. The protons of an olefinic group appeared as the signal at 5.32 ppm. This was supported by the signals at 129.8 and 129.7 ppm. of the ^{13}C-NMR spectrum.

The ^{13}C-NMR, DEPT-90 ^{13}C-NMR and DEPT-135 ^{13}C-NMR spectra, Fig. 5-7 could be interpreted as follows:

The carbonyl carbon of ester group was located at 173.8 ppm. The olefinic carbons of an olefinic group (2 CH= , 2 C=) appeared as the signals 129.8, 129.7, 127.8 and 127.7 ppm., respectively. The signals of interlinking methylene carbons were observed around 22.5 - 29.5 ppm., while the signal of a methylene carbon connected to an olefinic carbon was observed at 29.5 ppm. The α- and β- carbons next to carboxyl groups were located at 31.8 and 33.8 ppm., respectively. The signal at 13.8 ppm. belonged to a methyl group of the carboxylic part of an ester molecule. The spectral data are summarized in Table 14.

Table 13 Comparison of spectroscopic data of Mixture I.

chemical shift (ppm.)				wavenumber (cm^{-1})	Tentative assignment
^1H	^{13}C	DEPT-135	DEPT-90	IR	
0.86	13.8	13.8^d		1435,1363	CH_3
1.23	22.5-29.5	$22.5\text{-}29.5^u$		724	$(CH_2)_n$
1.59	31.8	31.8^u		1462	$\beta\text{-}CH_2$
2.00	27.0	27.0^u			$\text{-}CH_2CH\text{=}$
2.28	33.8	33.8^u		1462	$\alpha\text{-}CH_2$
3.64	51.0	51.0^d			$\text{-}O\text{-}CH_3$
5.32	129.7-8	$129.7\text{-}8^d$	$129.7\text{-}8^u$		=CH
	127.7-8	$127.7\text{-}8^d$			=C
	173.8			1743,1172	$\text{-}CO_2R$

d : down phase u : up phase

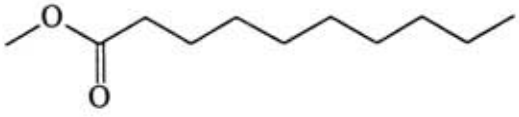
The spectral evidences above indicate that Mixture I is composed of a mixture of unsaturated fatty acid methyl esters. These methyl esters may be generated by methylation as while methanol solvent was removed by distillation from the methanol crude extract by the methanol. To study the chemical constituents of this mixture, Mixture I was chromatographed by GC-MS spectrometer and GC chromatogram, Fig. 8, was obtained. GC chromatogram displayed peaks at retention times (R_t) 11.65, 24.64, 29.47, 31.92, 32.59, 33.23, 44.11, 49.31 and 49.63 min., respectively. Each peak was analyzed by MS spectrometer. From comparing the fragmentation ion pattern of mass spectra of methyl ester wth those substances through library search of GC-MS spectrometer

(NIST database), Mixture I appeared to contain methyl esters of a mixture of carboxylic acids of Mixture I which are discussed below:

The fragmentation ion pattern of methyl ester derivative observed at R_t 11.65 min. was similar to that of octanoic acid methyl ester (Fig.9). Furthermore, fragmentation ions of both methyl esters gave the same heights of relative intensities. Thus, the methyl ester observed at R_t 11.65 min. was probably octanoic acid or caprylic acid methyl ester (M.W. 158) and its structure is

However, the identity of this compound oughts to be comfirmed by comparison with an authentic sample.

The fragmentation ion pattern of methyl ester derivative observed at R_t 24.64 min. was similar to that of 9-oxo-nonanoic acid methyl ester (Fig. 10). Molecular weight of this unknown is 186. However, the relative intensity of fragmentation ion peak at m/e 29 of the unknown was lower than that of 9-oxo-nonanoic acid methyl ester. Moreover, the peak of carbonyl carbon of aldehyde group was not observed in ^{13}C-NMR spectrum so unknown was not 9-oxo-nonanoic acid methyl ester. The sample should be decanoic acid or n-capric acid methyl ester ($C_9H_{19}COOCH_3$, M.W. 186). The structure is suggested to be

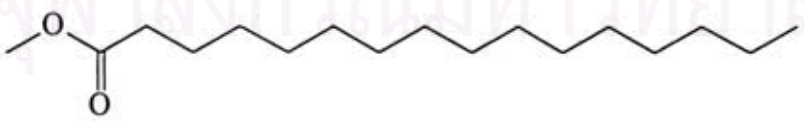


However, the identity of this compound oughts to be confirmed by comparison with an authentic sample.

The fragmentation ion pattern of methyl ester derivative observed at R_t 29.47 min. was similar to that of nonanedioic acid dimethyl ester (Fig. 11). Moreover, the relative intensities of the fragmentation ions of both compounds corresponded well. Thus, methyl ester observed at R_t 29.47 min. should be nonanedioic acid or azelaic acid dimethyl ester (M.W. 216) with the structure :

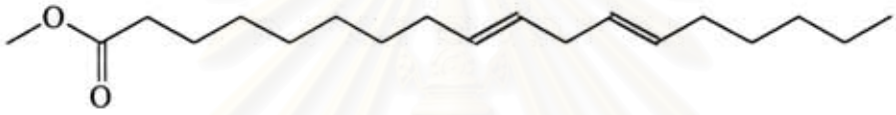
However, the identity of this compound oughts to be confirmed by comparison with an authentic sample.

Molecular ion of methyl ester derivative observed at R_t 44.11 min. was 270. The carbon number (from (M+1) peak) is 17. Molecular formula was $C_{17}H_{34}O_2$. The fragmentation ion pattern of methyl ester derivative observed at R_t 44.11 min. was similar to that of 14-methyl pentadecanoic acid methyl ester (Fig. 12). The relative intensities of the fragmentation ions of both compounds were alike. Methyl ester observed at R_t 44.11 min. should be 14-methyl pentadecanoic acid methyl ester (M.W. 270). From the ^{13}C-NMR spectrum, the carbon peak of C-H group was not observed so unknown is likely to be a long chain methyl ester of hexadecanoic acid or palmitic acid methyl ester and this was corroborated by literature data (7). Its structure is



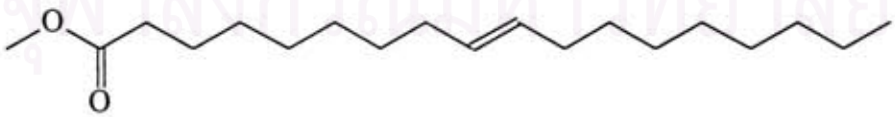
However, the identity of this compound oughts to be confirmed by comparison with an authentic sample

Molecular ion of methyl ester derivative observed at R_t 49.31 min. was 294. The carbon number (from (M+1) peak) is 19. Molecular formula was therefore $C_{19}H_{34}O_2$. The fragmentation ion pattern of methyl ester derivative observed at R_t 49.31 min. was identical to that of (Z,Z)-9,12-octadecadienoic acid methyl ester (Fig. 13). The relative intensities of the fragmentation ions of both compounds were similar. The unknown was possibly (Z,Z)-9,12-octadecadienoic acid or linoleic acid methyl ester (M.W. 294) and this was corroborated by literature data (7,11,22). Its structure is therefore



To establish the position of the double bond, it must be examined by authentic samples since other isomers are also possible.

Molecular ion of methyl ester derivative observed at R_t 49.63 min. was 296. The carbon number (from (M+1) peak) was 19. Molecular formula was $C_{19}H_{39}O_2$. The fragmentation ion pattern of methyl ester derivative observed at R_t 49.63 min. excellently matched that of 9-octadecenoic acid methyl ester (Fig. 14). The relative intensities of the fragmentation ions of both compounds were similar. These indicated that the unknown had high possibility to be 9-octadecenoic acid or elaidic acid methyl ester (M.W. 296). Its structure is



However, this assignment is not certain because the MS spectra of other isomers were not available for comparison.

The components which were located at R_t 31.92, 32.59 and 33.23 min. cannot be identified because these components were not independently isolated. The composition of long chain carboxylic acids contained Mixture I was presented in Table 15.

Table 15 The composition of long chain carboxylic acids contained in Mixture I

R_t (min.)	Formula	Name
11.25	$C_8H_{16}O_2$	octanoic acid or caprylic acid
24.64	$C_{10}H_{19}O_2$	decanoic acid or n-capric acid
29.47	$C_9H_{16}O_4$	nonanedioic acid or azelaic acid
44.11	$C_{16}H_{32}O_2$	hexadecanoic acid or palmitic acid
49.31	$C_{18}H_{32}O_2$	(Z, Z)-9,12-octadecadienoic acid or linoleic acid
49.63	$C_{18}H_{34}O_2$	9-octadecenoic acid or elaidic acid

3.1.2 Structural Elucidation of Mixture II.

Mixture II was isolated from the silica gel column chromatography of Fractions No. 34-42 in Fractions No. 43-51 by 25% $CHCl_3$ in hexane. These compounds were crystallized from methanol to yield Mixture II as a white amorphous solid.

The IR spectrum of Mixture II, Fig. 19, could be assigned in Table 16. Its IR spectrum indicated the presence of one or more carboxylic group (C=O stretching vibration peak(s) at 1702 cm^{-1}, O-H stretching vibration peak of carboxylic group (very b) at 3500-2400 cm^{-1} and C-O stretching vibration mode of carboxylic group at 1298 cm^{-1}). The C-H stretching vibration peaks of an aliphatic compound were observed at 2919 and 2850 cm^{-1}. The moderate absorption peaks at 1465, 1434 cm^{-1} corresponded to methylene and methyl bending vibration modes, respectively. The absorption peak at 724 cm^{-1} might possibly revealed the presence of one or more saturated long chain of $-(CH_2)_n$; $n > 4$.

Table 16 The IR absorption band assignments of Mixture II.

wavenumber (cm^{-1})	Band type	Tentative assignment
3500-2400	very b	O-H stretching vibration of acid
2919,2850	s	C-H stretching vibration of CH_3-, -CH_2
1702	s	C=O stretching vibration of acid
1465,1434	m	C-H bending vibration of -CH_2-, CH_3-
1298	m	C-O stretching vibration of coupled with O-H deformation
941	m	O-H out-of-plane deformation
724	m	C-H rocking mode of -CH_2- (for carbon>4)

The ^{1}H-NMR spectrum (Fig. 16) displayed the protons of an olefinic group at 5.34 ppm. which indicated the possible presence of one or more double bonds in the molecule. These were corroborated by the presence of the signals at 128.9-130.8 ppm. in the ^{13}C-NMR spectrum. The signals of methylene protons adjacent to olefinic system were positioned at 2.00 ppm. The high intensity singlet peak at 1.25 ppm. corresponded to methylene group(s). Other signals were observed and could be assigned as follows: 2.35 (-C$\underline{H}_2$-COOH), 1.63 (methylene protons adjacent to -CH_2-COOH group) and 0.88 (methyl protons).

The ^{13}C-NMR, DEPT-90 and DEPT-135 ^{13}C-NMR spectra, Fig. 17-19, gave important information which corrosponded to the information from ^{1}H-NMR spectrum and could be interpreted as follows:

The carbon signal characteristic of carboxyl carbon was located at 179.96 ppm. The signals about 128.0-131.2 ppm. were corresponded to olefenic carbon (= CH). Other signals were detected at 33.97 and 31.84 (α-, and β- carbon next to carboxyl group, respectively), 28.98-29.27 (interlinking methylene carbons) and 14.01 corresponded to methyl carbon. The 2 carbons adjacent to methyl group were located at 2.60 and 24.60 ppm., respectively. The methylene carbons which jointed with olefinic carbon (= CH) were positioned at 27.13 ppm. The spectral informations were summaried in Table 17.

Table 17 Comparison of spectroscopic data of Mixture II.

chemical shift (ppm.)				wavenumber (cm^{-1})	Tentative assignmemt
^1H	^{13}C	DEPT-135	DEPT-90	IR	
0.88	14.0	14.0^d		1434	CH_3
1.25	22.6-29.6	$22.6\text{-}29.6^u$		724	$(CH_2)_n$
1.63	31.2	31.2^u		1465	$\beta\text{-}CH_2$
2.00	27.1	27.1^u			$\text{-}CH_2CH\text{=}$
2.35	34.0	34.0^u		1465	$\alpha\text{-}CH_2$
5.34	128.9-130.8	128.9-130.8^d	128.9-130.8^u		=CH
	180.0			3500-2400	$\text{-}CO_2H$
				1702,1298	

d : down phase u : up phase

The spectral evidences discussed above proposed that Mixture II was the mixture of unsaturated long chain fatty acids. To analyze the type of these substances, the long chain fatty acids must be converted into their derivatives of methyl esters by using diazomethane reagent and then analyzed by GC-MS spectrometer. The GC-MS analysis results, Fig. 20, displayed 5 peaks on gas chromatogram at R_t 24.21, 31.47, 44.38, 49.55 and 50.62 min., respectively. By using comparison between the mass fragmentation ion pattern of sample and the mass fragmentation ion pattern of standard of library search (in NIST database) of mass spectrometer, it deduced that derivative of methyl esters should be:

The fragmentation ion pattern of methyl ester derivative observed at R_t 24.21 min. matched to that of 9-oxo-nonanoic acid methyl ester from NIST database very well, Fig. 21. This unknown should have M.W. 186. The ^{13}C-NMR spectrum revealed that this unknown did not have an aldehyde group in the molecule. The methyl ester was not 9-oxo-nonanoic acid methyl ester but it should be decanoic acid or n-capric acid methyl ester with the structure :

However, this compound oughts to be compared with an authentic sample.

The fragmentation ion pattern of methyl ester derivative observed at R_t 31.47 min. was the same as that of nonanedioic acid monomethyl ester. The relative intensities of fragmentation ions of sample and standard were related, Fig. 22. The mass spectrum of nonanedioic acid dimethyl ester was not identical with the mass spectrum of unknown due to the absence of the peak at m/e 171. This compound may be nonanedioic acid or azelaic acid monomethyl ester (M.W. 202) with the structure :

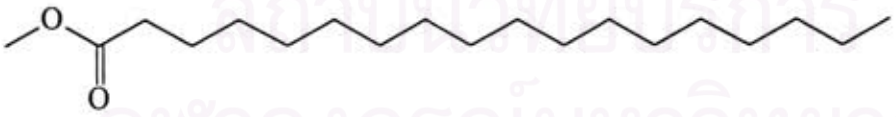
However, this compound oughts to be compared with an authentic sample.

Molecular ion of methyl ester derivative observed at R_t 44.38 min. was 270 The carbon number (from (M+1) peak) is 17. The fragmentation ion pattern of methyl ester derivative observed at R_t 44.38 min. matched well that of 14-methyl pentadecanoic acid methyl ester, Fig 23. The relative intensities of fragmentation ions of unknown and 14-methyl pentadecanoic acid methyl ester were alike. This

compound was therefore 14-methyl pentadecanoic acid methyl ester. However, the signal of the methine carbon was not found in ^{13}C-NMR spectrum. So unknown should be unbranced long chain methyl ester. The derivative of methyl ester observed at R_t 44.38 min. should probably be hexadecanoic acid or palmitic acid methyl ester (M.W. 270) and this was corroborated by literature data (7). Its structure is

However, this structure should be compared with an authentic sample

Molecular ion of methyl ester derivative observed at R_t 50.62 min. was 298. The carbon number (from (M+1) peak) was 19. Molecular formula was $C_{19}H_{38}O_2$. The mass spectrum of methyl ester derivative observed at 50.62 min. matched well that of 16-methyl heptadecanoic acid methyl ester, Fig. 24. Therefore, this methyl ester was possibly 16-methyl heptadecanoic acid methyl ester. Since this mixture did not have the signal of a methine carbon, so this compound is likely to be octadecanoic acid or stearic acid methyl ester (M.W. 270) and this was corroborated by literature data (7). Its structure is



However, this struture should be compared with an authentic sample.

The components which appeared at R_t 49.55 min. cannot be indentified because these components were not separated from each other. The composition of long chain carboxylic acids contained in Mixture II is presented in Table 18.

Table 18 The composition of long chain carboxylic acids contained in Mixture II.

R_f (min.)	Formula	Name
24.21	$C_{10}H_{20}O_2$	decanoic acid or n-capric acid
31.47	$C_9H_{16}O_4$	nonanedioic acid or azelaic acid
44.38	$C_{16}H_{32}O_2$	hexadecanoic acid or palmitic acid
50.62	$C_{18}H_{36}O_2$	octadecanoic acid or stearic acid

The results of Mixture I and II demonstrate that Mixture I and II are composed of the same mixture of components. The original components of Mixture I were probably converted into methyl esters because Mixture I reacted with methanol which was used in the extraction of the seeds of *Gymnopetalum integrifolium* Kurz or while methanol crude extract was evaporated. Mixture I was also a mixture of long chain carboxylic acids. In turn, Mixture II did not react with methanol. Mixture II was a mixture of long chain carboxylic acids.

3.1.3 Structural Elucidation of Mixture III.

Mixture III, 2.6 mg., was obtained from silica gel recolumn chromatography of Fractions No. 76-89 by using the mixture of methanol in chloroform in Fractions No. 79-82. After recrystallization, it has afforded a white amorphous solid, 0.002% wt. by wt.of hexane crude. It melted at 275-277 ^{o}C and immediately decomposed. R_f value was 0.59 in $CHCl_3$.

The IR spectrum of Mixture III (Fig. 25) revealed the presence of NH_2 group at 3192 and 3020 cm^{-1} (N-H stretching) and carbonyl group at 1663 cm^{-1} (C-O stretching). The out-of-plane N-H wagging vibration mode was observed at 849 cm^{-1}. The adsorption peaks at 2962.0 and 2876.0 cm^{-1} corresponded to C-H stretching vibration of CH_3, $-CH_2-$ group. The peak at 1452 cm^{-1} supported the existence of $-CH_2-$ group.

The information obtained from the IR spectrum suggested that Mixture III should have an amine and carbonyl group in molecule. Unfortunately, this compound was obtained in such a limited amount that its structure cannot be further elucidated.

3.4.1 Structural Elucidation of Mixture IV.

Mixture IV was a white amorphous solid which was crystallized by chloroform and methanol and weighted on 9.1 mg. Its melting point was 258-260 ^{o}C. R_f value was 0.14 and 0.51 in 10 and 20% methanol in chloroform systems, respectively. The colour test of this compound indicated the presence of a steroidal nucleus in the molecule since it gave a green-blue colour with Liebermann-Burchard's reagent.

The IR spectrum of Mixture IV is presented in Fig. 26 and the absorption peaks are assigned in Table 16. Its IR spectrum strongly indicated a O-H stretching vibration (b) at 3427 cm^{-1}. The C-O stretching vibrations (s) of glycosidic linkage was observed at 1070-1023 cm^{-1} and an anomeric axil C-H deformation of β-sugar was positioned at 890 cm^{-1}. The additional bands which were compatible with a trisubstituted olefin and gem-dimethyl group were observed at 1633 and 1376 cm^{-1}, respectively.

Table 19 The IR absorption band assignments of Mixture IV.

Frequency	Band type	Tentative assignment
3427	b	O-H stretching vibration
2937,2872	s	C-H stretching vibration of CH_3-, -CH_2
1633	m	C=C stretching vibration
1462	m	C-H bending vibration of -CH_2-, CH_3-
1376	m	C-H symmetric bending vibration of CH_3-
1070-1023	s	C-O stretching vibration of glycosidic linkage
890	w	anomeric axil C-H deformation of β-sugar

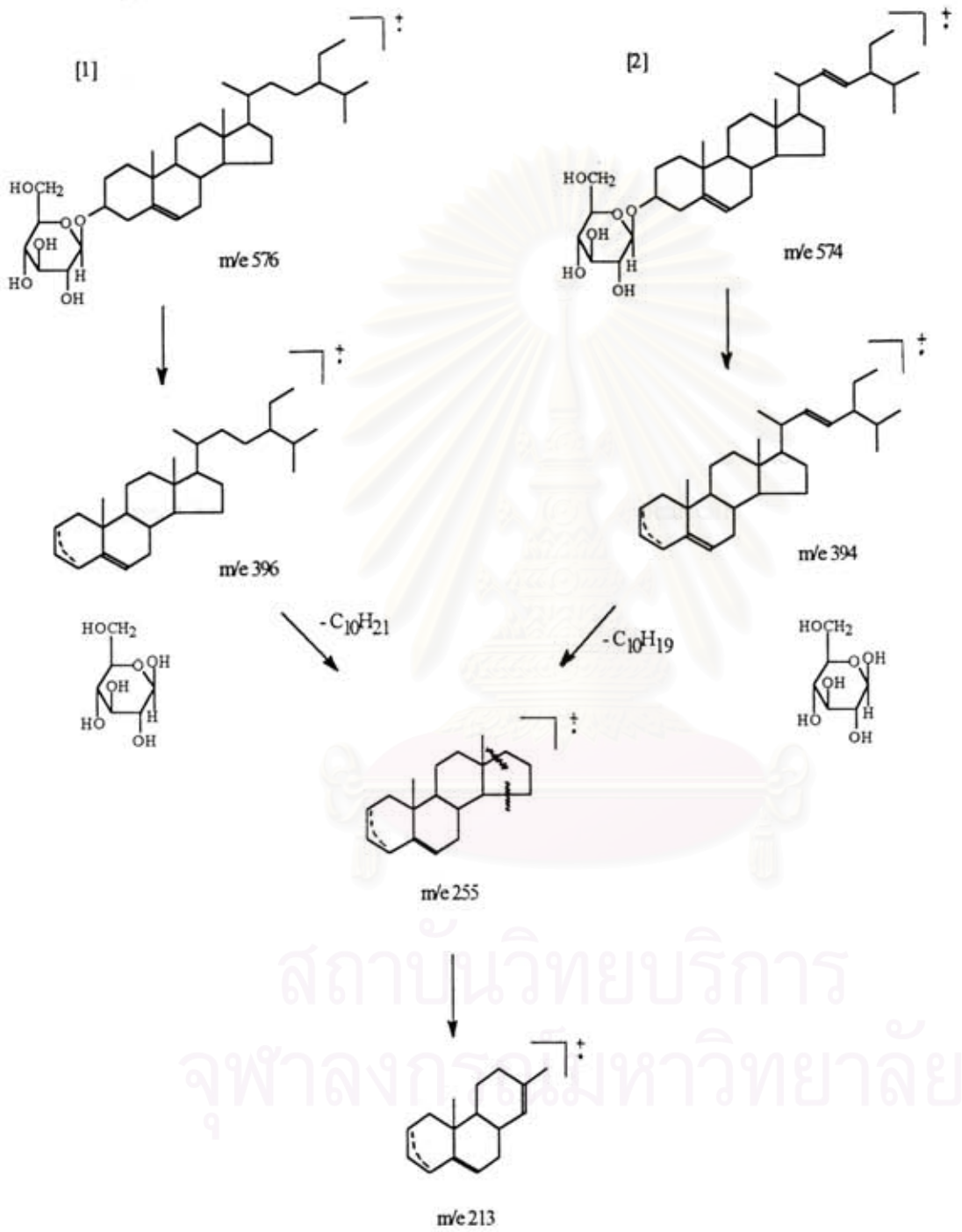
The informations obtained from both of the colour test and IR spectrum hinted that Mixture IV might be a steroid glycoside.

The mass spectrum which was obtained by chemical ionization technique (Fig. 27) did not give the molecular ion peak (M^+), due to the breaking of molecule but it exhibited the dominant fragmentation ion peaks at m/e 414 and 412 indicating that this compound may be a mixture of 2 sterols; β-sitosterol and stigmasterol. After it was analyzed again by electron impact technique of mass spectrometer, its mass spectrum of Mixture IV (Fig. 28) revealed the same fragmentation pattern as that of the mixture of β-sitosterol-3-O-β-D-glucopyranoside and stigmasterol-3-O-β-D-glucopyranoside(44). The series of fragmentation ion peaks, like both sterol glycosides of β-sitosterol and stigmasterol, suggested that this Mixture IV was the mixture of sterol glycosides of β-sitosterol and stigmasterol.

Literature studies suggested that the carbohydrate which was linked to both of these sterols was D-glucose. Thus, Mixture IV consisted of a mixture of β-sitosterol-3-O-β-D-glucopyranoside [1] and stigmasterol-3-O-β-D-glucopyranoside[2].

A suggested fragmentation ion pattern of Mixture IV is given in Scheme 3.

Scheme 3 Suggested mass fragmentation ion pattern of Mixture IV.



CHAPTER IV

CONCLUSION

In this course of research, the fleshes and seeds of the fruits of *Gymnopetalum integrifolium* Kurz, which is a plant in the family Cucurbitaceae, were selected for the investigation of their chemical constituents. In the fleshes, the hexane and chloroform extracts were obtained in such a limited amount that both of them cannot be further elucidated as while the chemical constituents from the methanol extract cannot be isolated by flash column chromatography. For the seeds of *Gymnopetalum integrifolium* Kurz, four compounds isolated from hexane crude extract by column chromatography are shown to be a mixture of long chain fatty acids, a mixture of β-sitosterol-3-O-β-D-glucopyranoside and stigmasterol-3-O-β-D-glucopyranoside and one other which cannot be identified due to the limited amount. Available two compounds are separated from chloroform crude extract by column chromatography and identified as a mixture of β-sitosterol-3-O-β-D-glucopyranoside and stigmasterol-3-O-β-D-glucopyranoside. All isolated substances from the seed part of *Gymnopetalum integrifolium* Kurz are summarized in Scheme 4.

Scheme 4 All isolated substances from the seeds of *Gymnopetalum integrifolium* Kurz.

Seeds of *Gymnopetalum integrifolium* Kurz

hexane crude extract

- Mixt.1+2 a mixt. of long chain carboxylic acids as yellowish-green oil and white amorphous solid, 17.79 % wt. by wt. of hexane part

- Mixt.3 white amorphous solid which could not be identified, 0.002 % wt. by wt. of hexane part, m.p. 275-277°C

- Mixt.4 a mixt. of glycosides of β-sitosterol and stigmasterol as white amorphous solid, 0.007 % wt. by wt. of hexane part m.p. 258-260°C

chloroform crude extract

- Mixt.4 a mixt. of glycosides of β-sitosterol and stigmasterol as white amorphous solid, 0.019 % wt. by wt. of chloroform part, m.p. 258-260°C

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APPENDIX

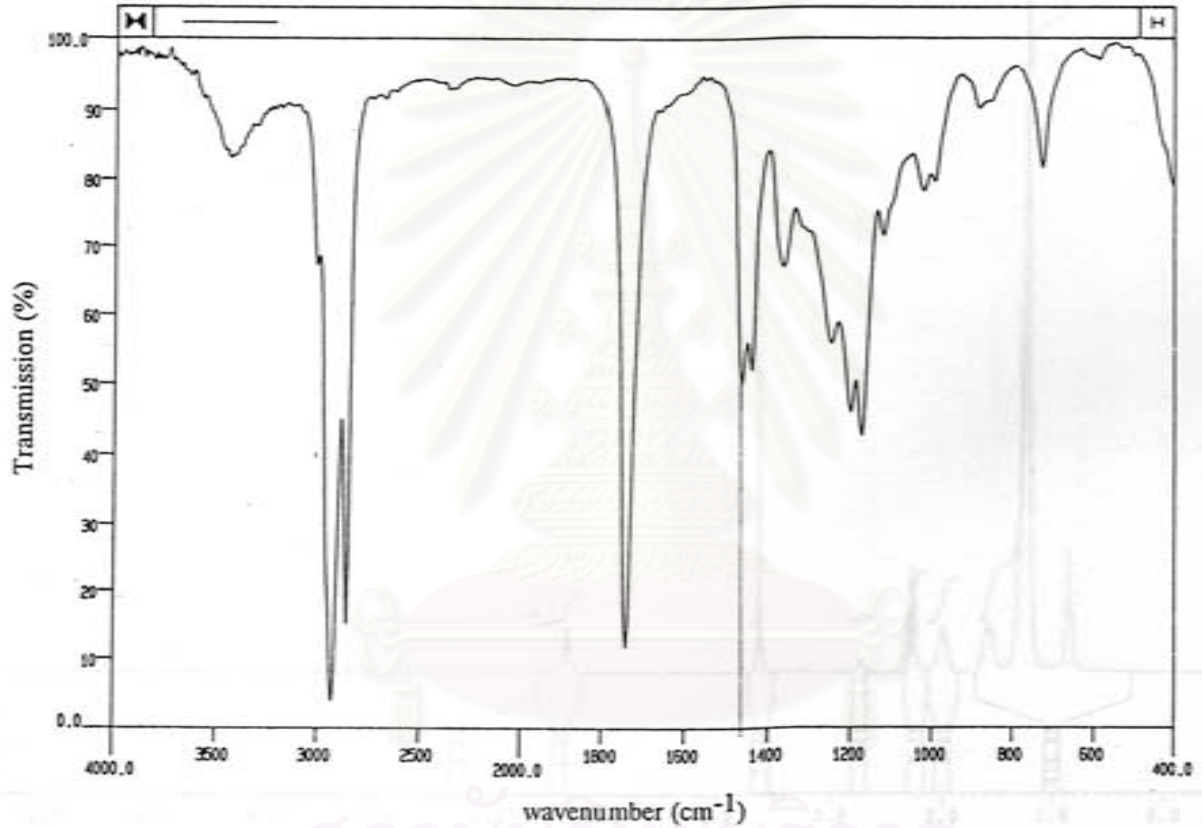

Figure 3 The IR spectrum of Mixture I.

69

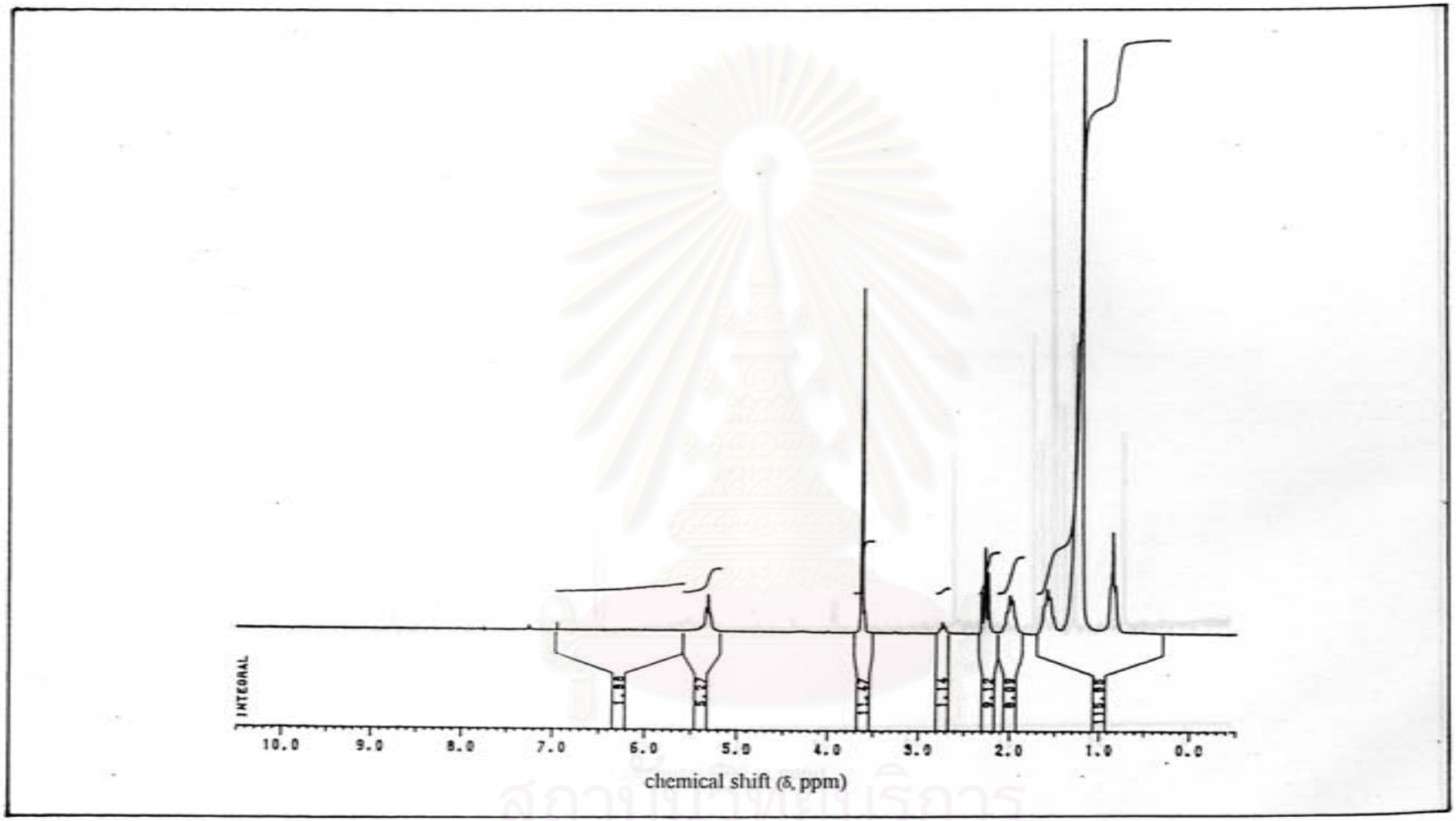

Figure 4 The ^1H - NMR spectrum of Mixture I in $CDCl_3$.

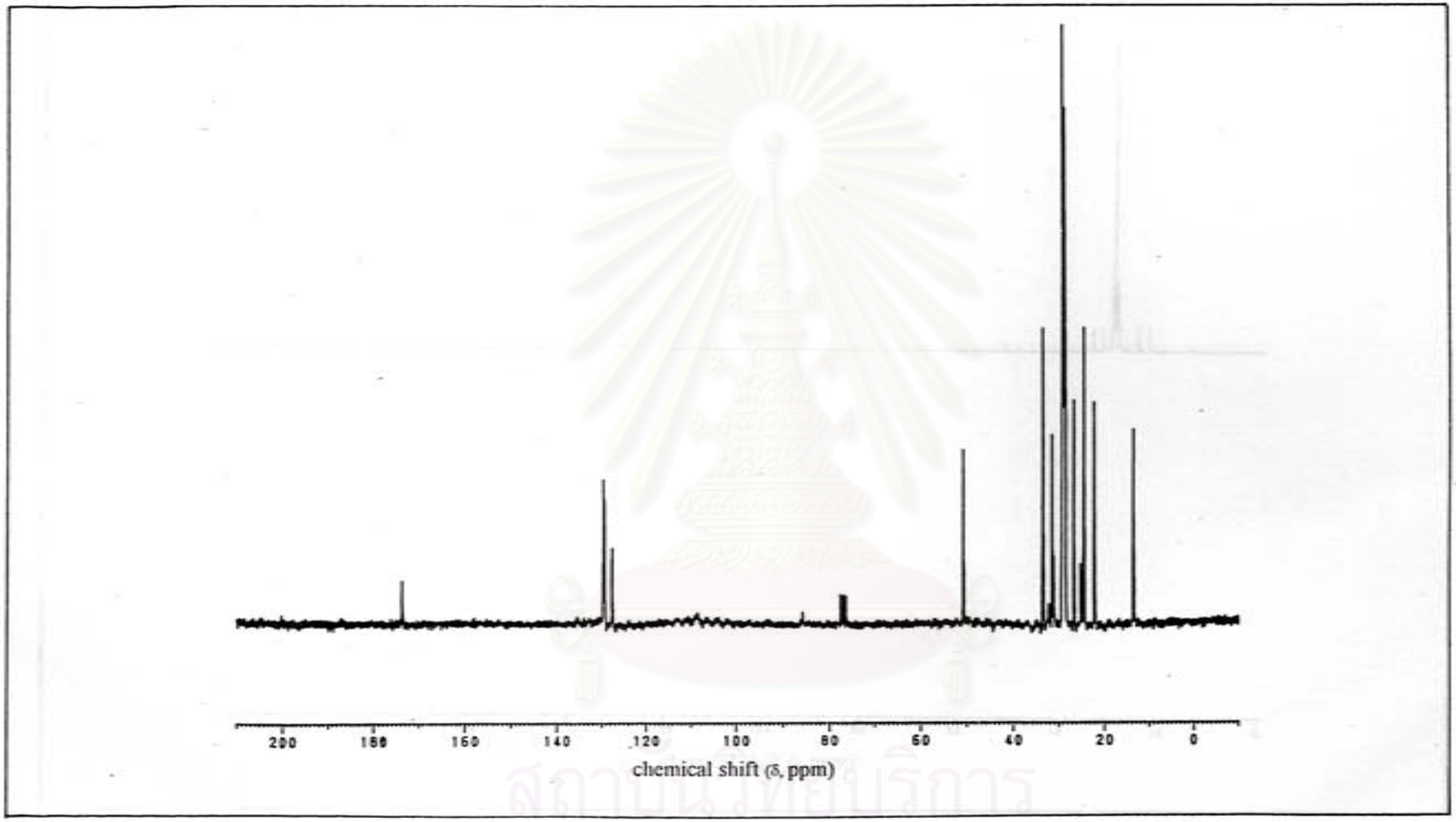

Figure 5 The ^{13}C - NMR spectrum of Mixture I in $CDCl_3$.

71

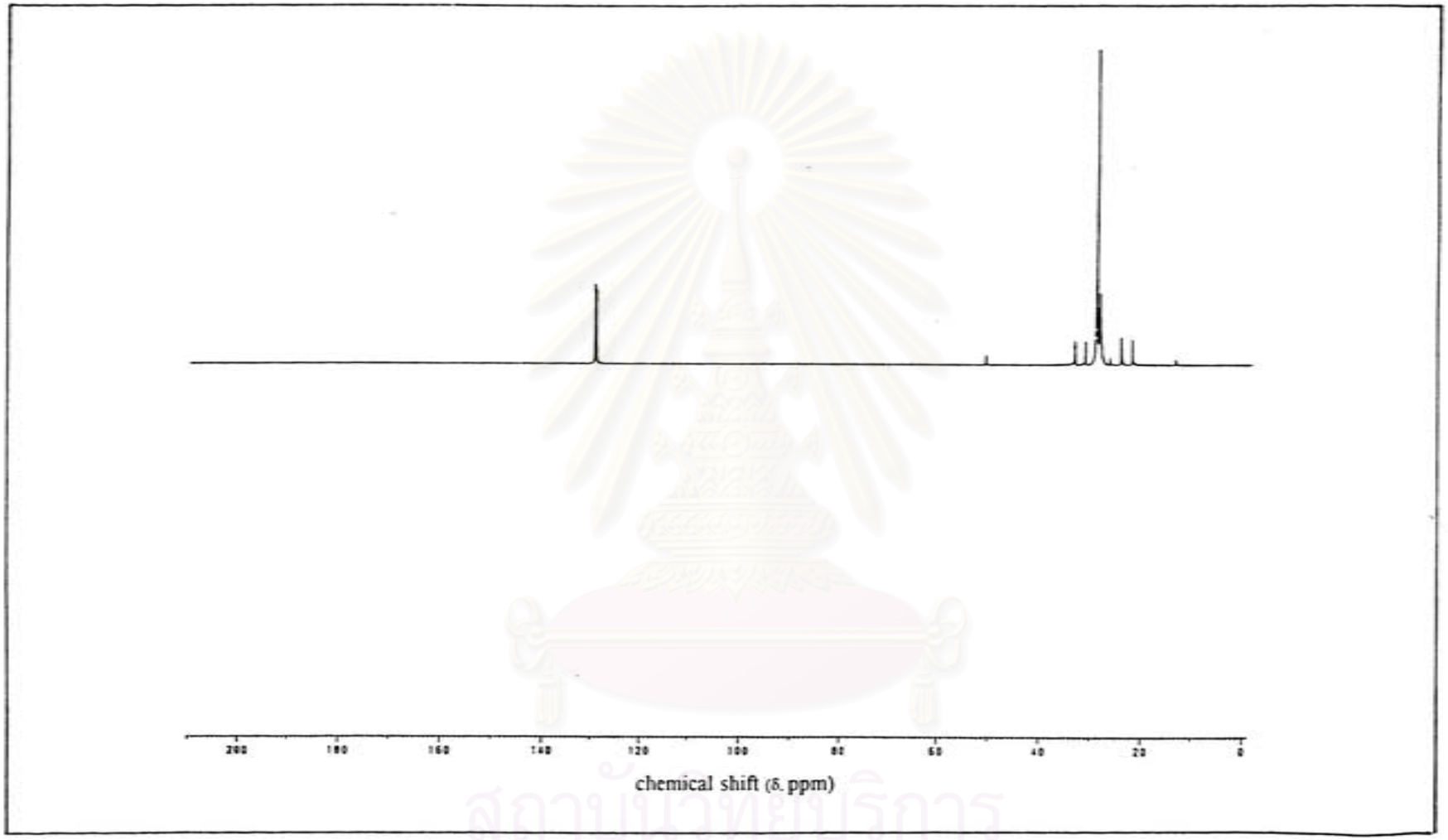

Figure 6 The DEPT - 90 ^{13}C - NMR spectrum of Mixture I in $CDCl_3$.

72

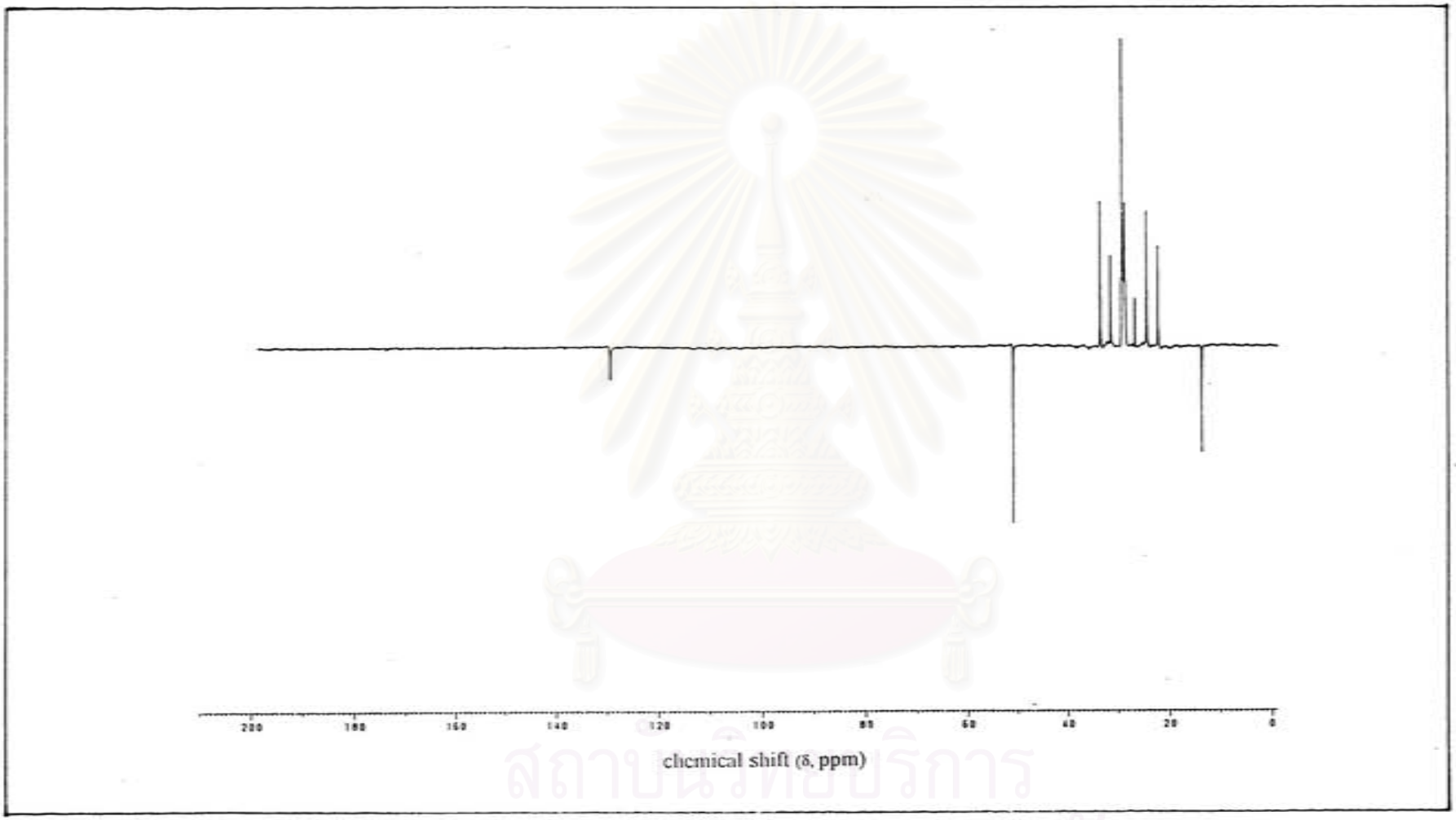

Figure 7 The DEPT - 135 ^{13}C - NMR spectrum of Mixture I in $CDCl_3$.

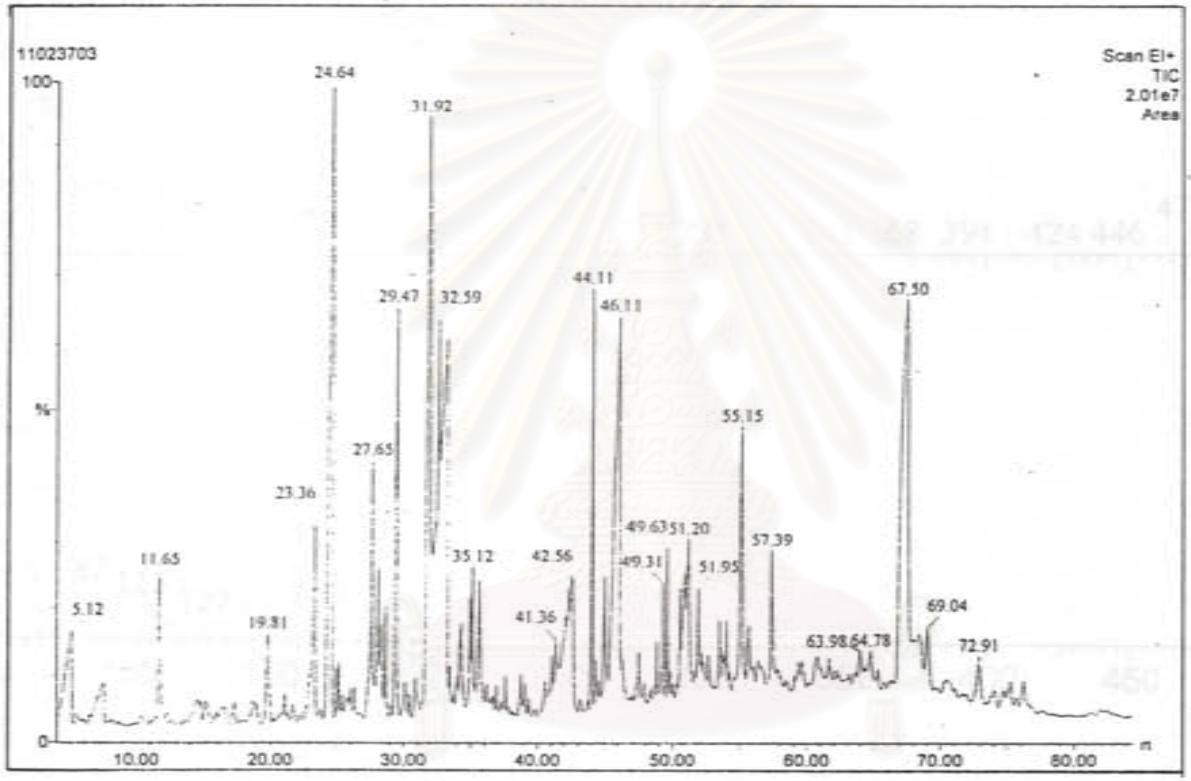

Figure 8 The Gas - Liquid chromatogram of Mixture I.

74

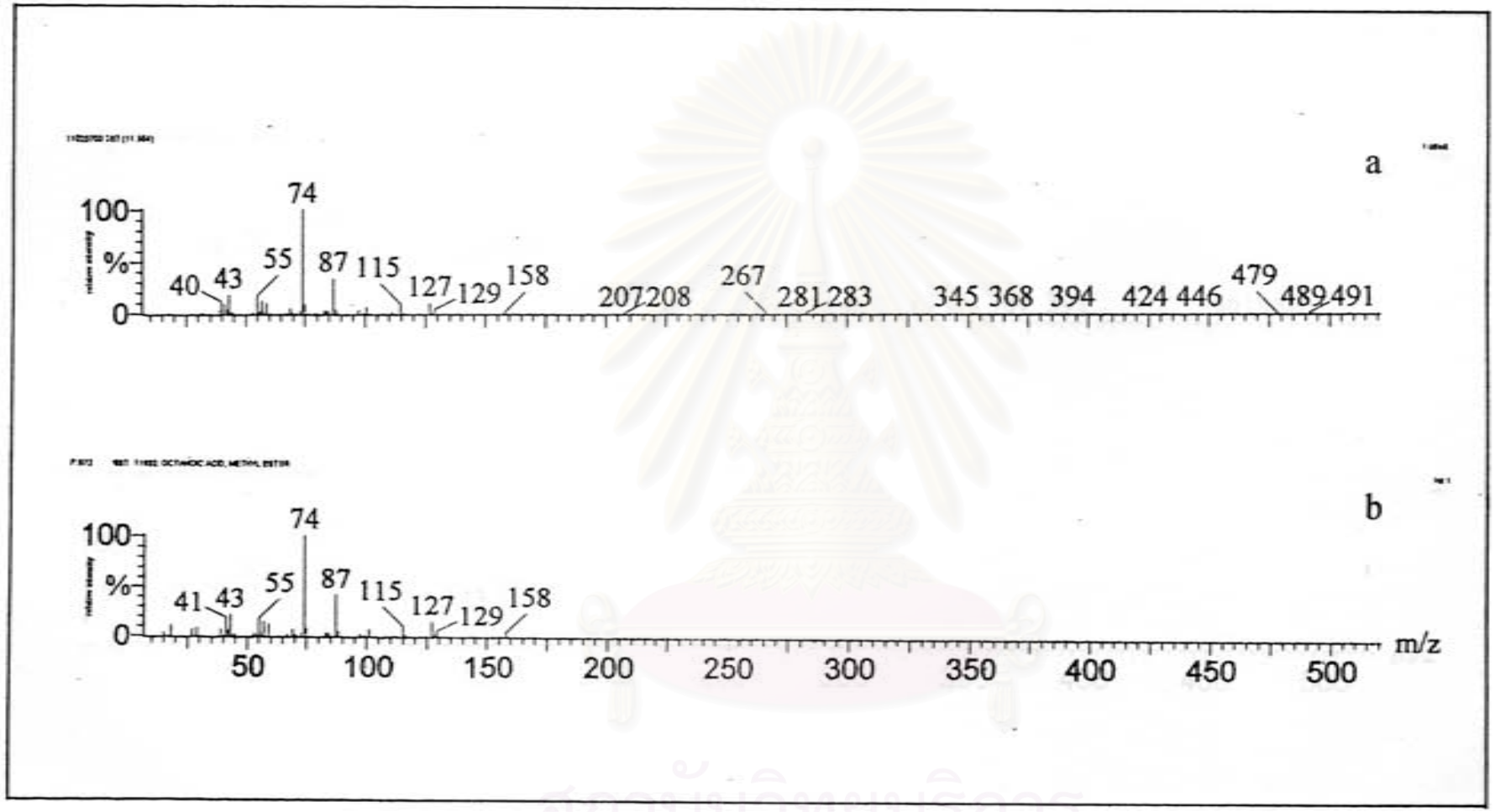

Figure 9 The mass spectrum of a. Mixture I at retention time 11.65 min.

b. octanoic acid methyl ester.

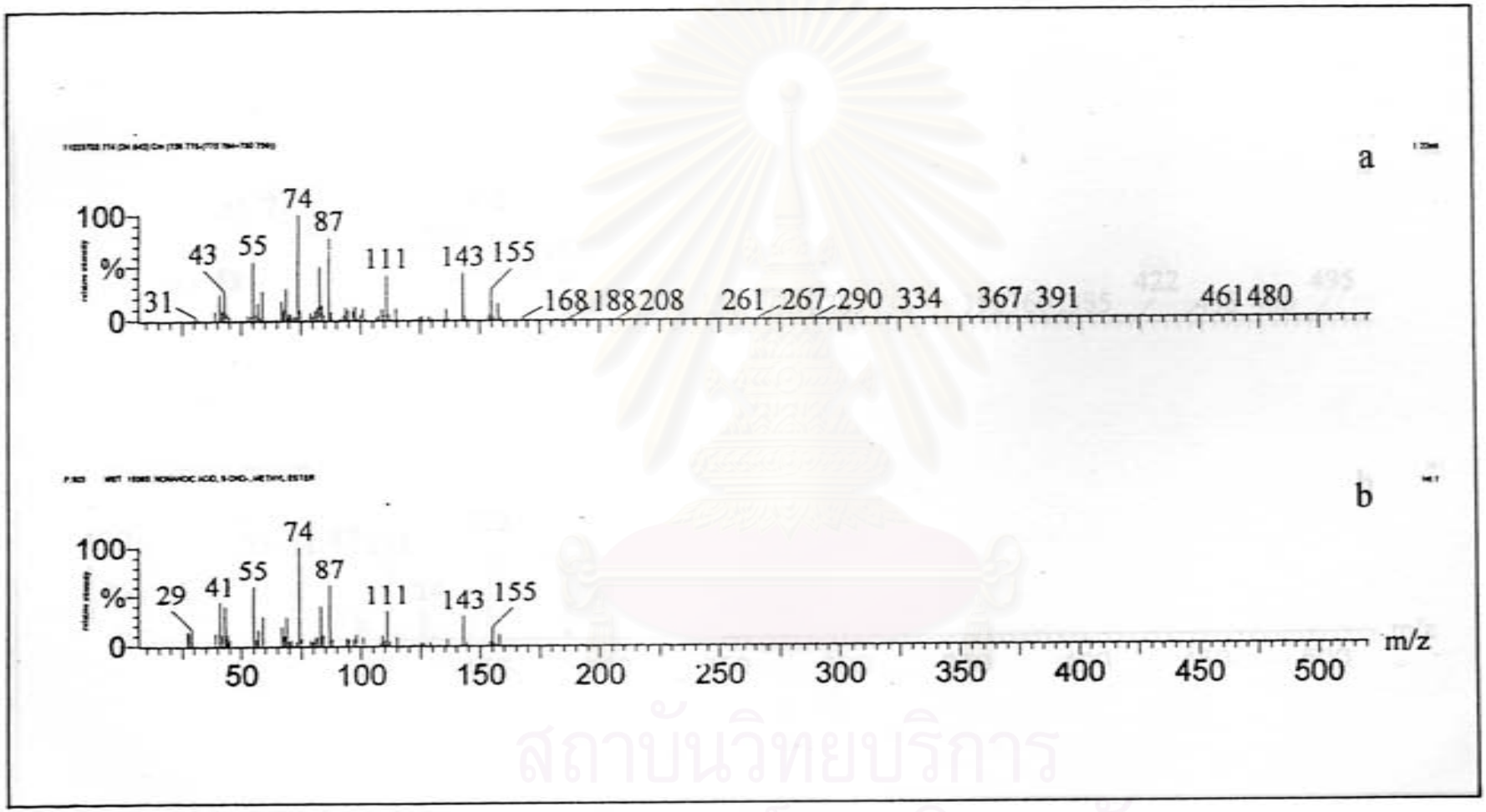

Figure 10 The mass spectrum of a. Mixture I at retention time 24.64 min.

b. 9-oxo-nonanoic acid methyl ester.

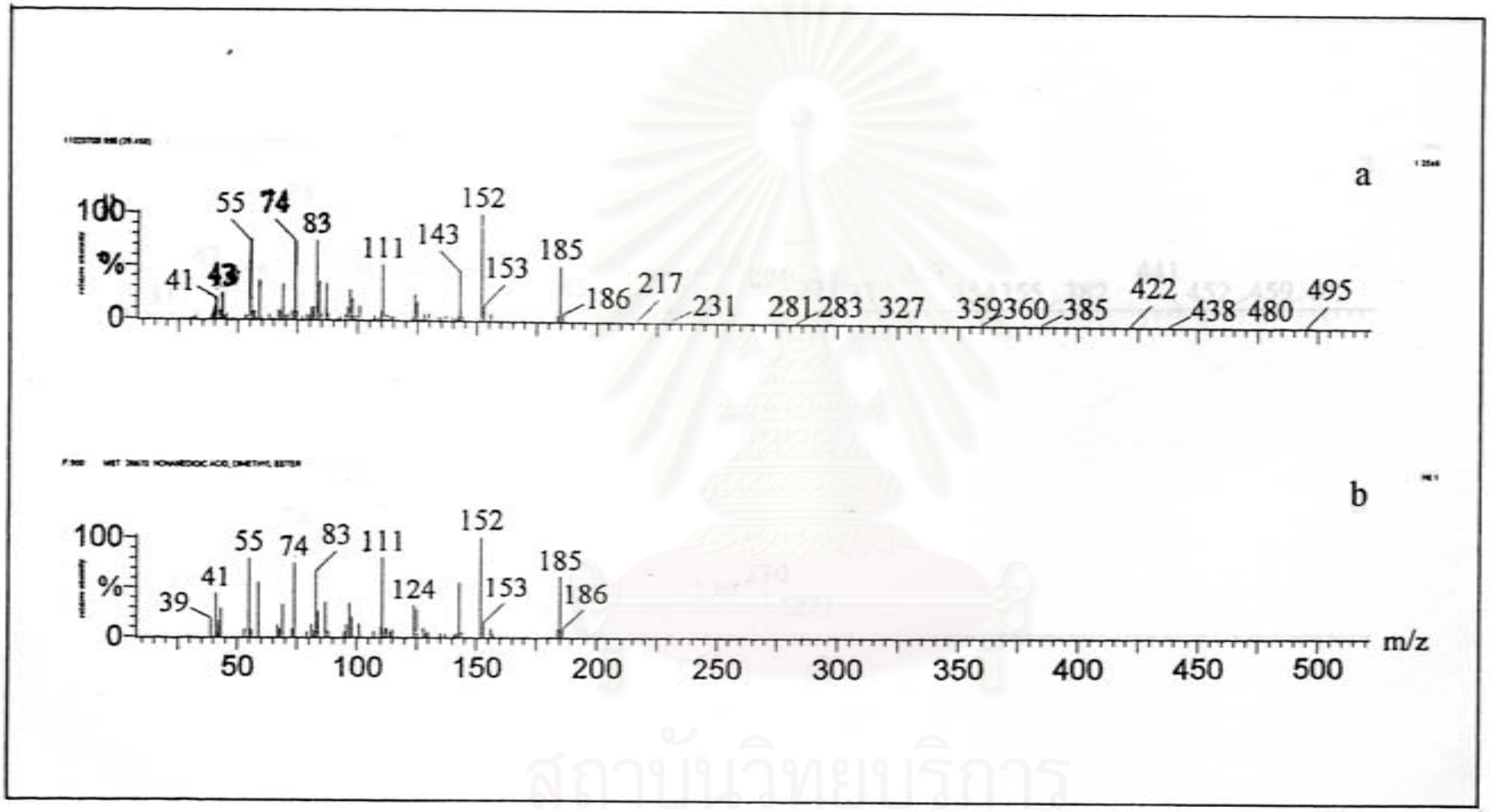

Figure 11 The mass spectrum of a. Mixture I at retention time 29.47 min.
b. nonanedioic acid dimethyl ester.

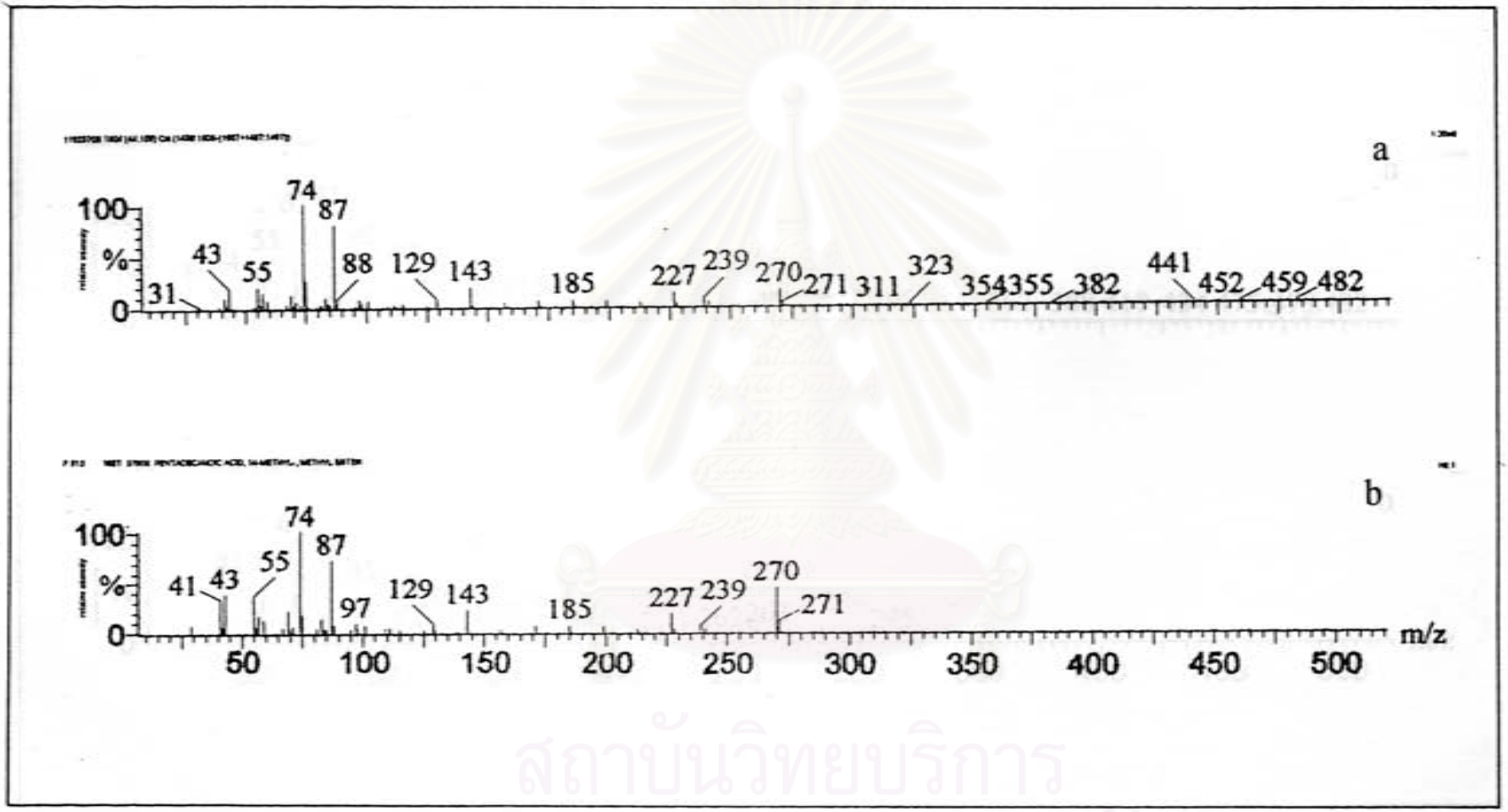

Figure 12 The mass spectrum of a. Mixture I at retention time 44.11 min.

b. 14-methyl pentadecanoic acid methyl ester.

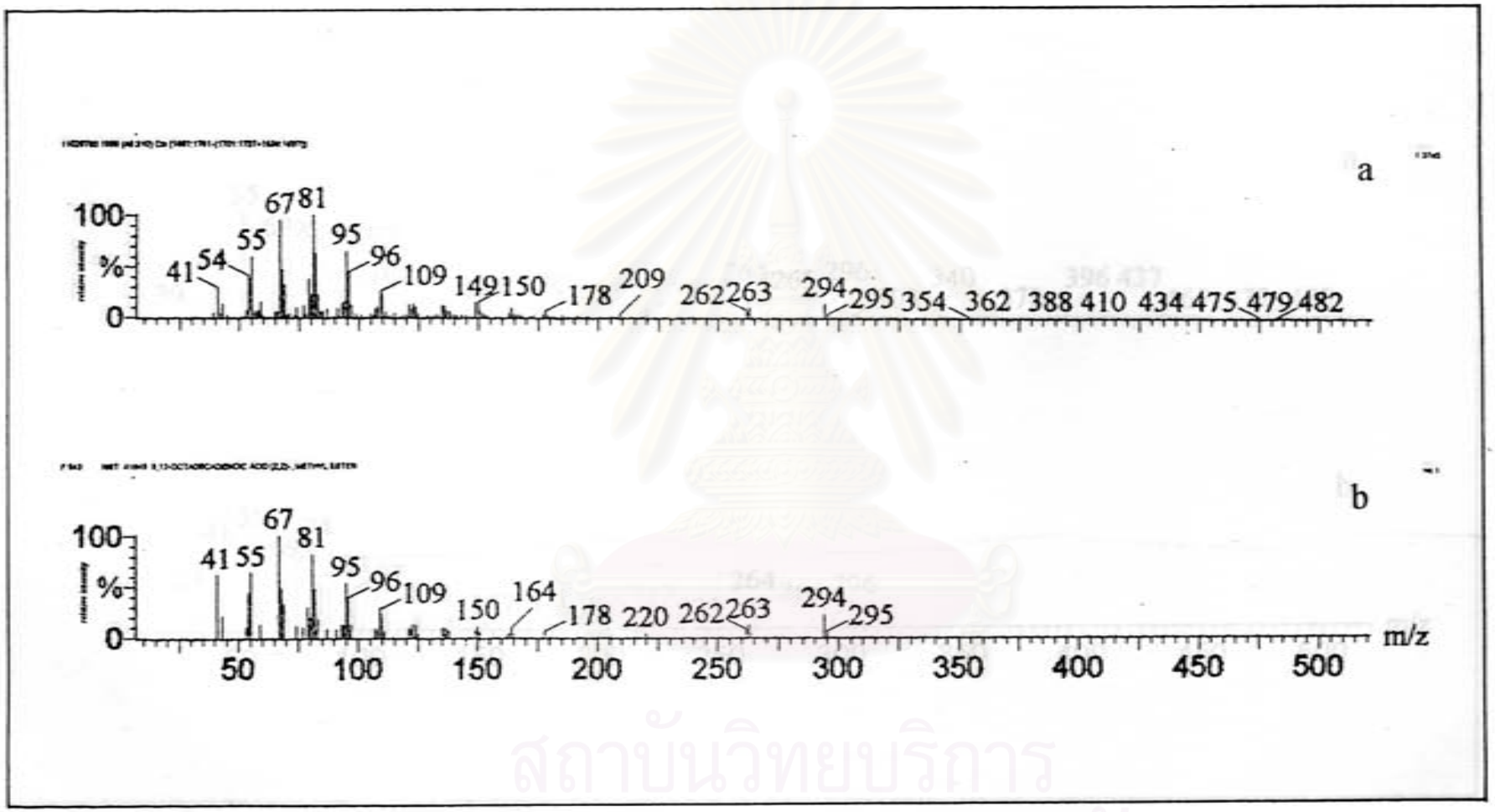

Figure 13 The mass spectrum of a. Mixture I at retention time 49.31 min.

b. (Z,Z)-9,12-octadecadienoic acid methyl ester.

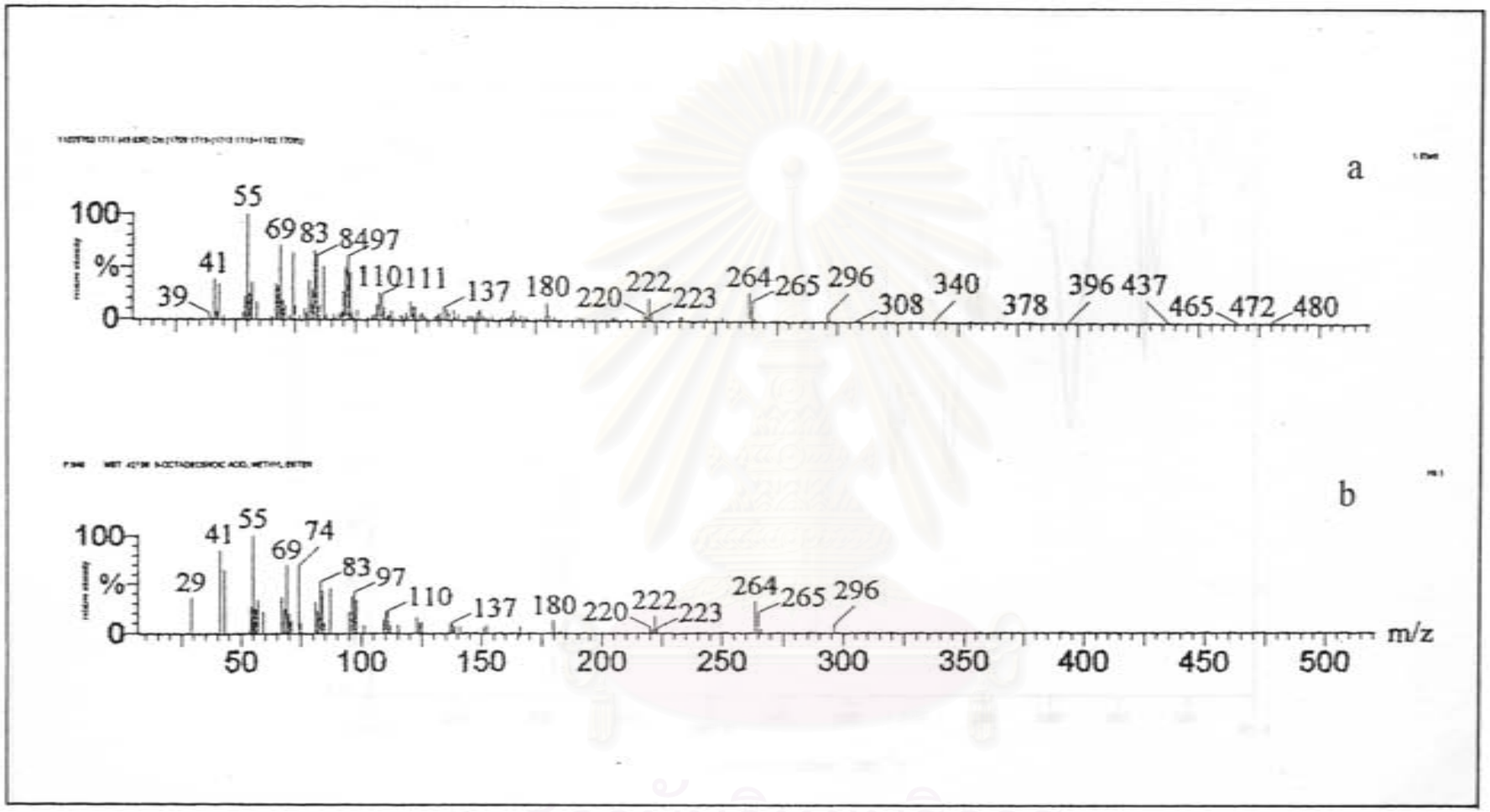

Figure 14 The mass spectrum of a. Mixture I at retention time 49.63 min.

b. 9-octadecenoic acid methyl ester.

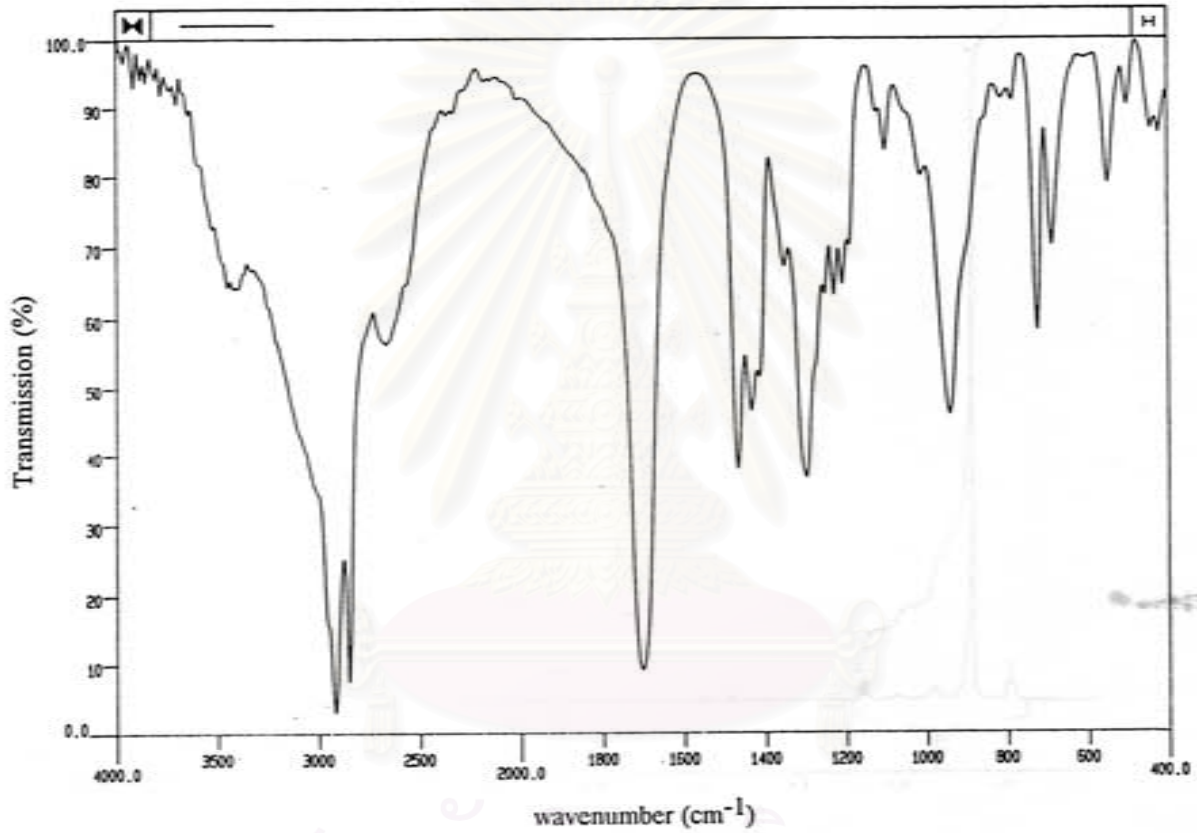

Figure 15 The IR spectrum of Mixture II.

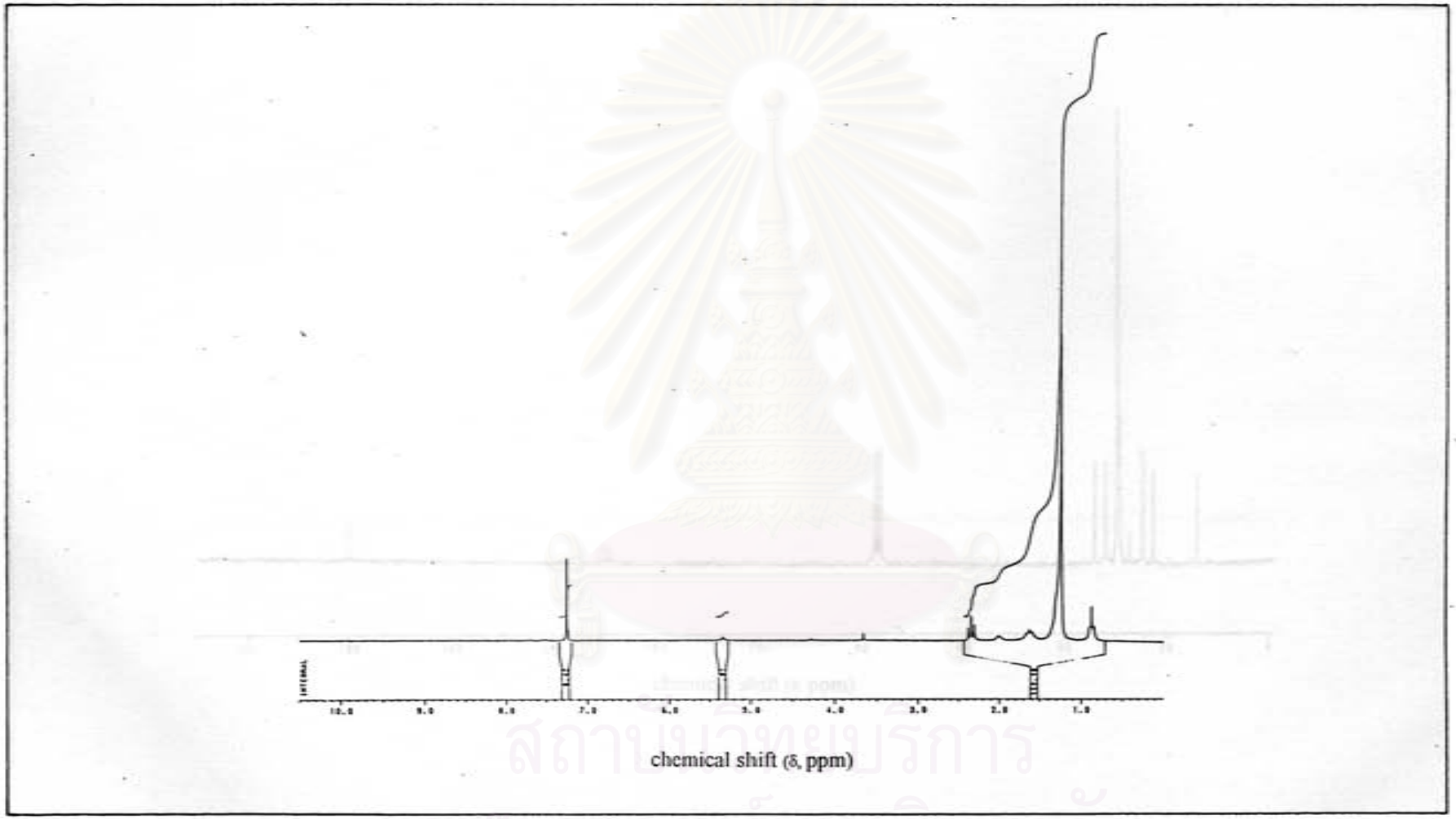

Figure 16 The ^1H - NMR spectrum of Mixture II in $CDCl_3$.

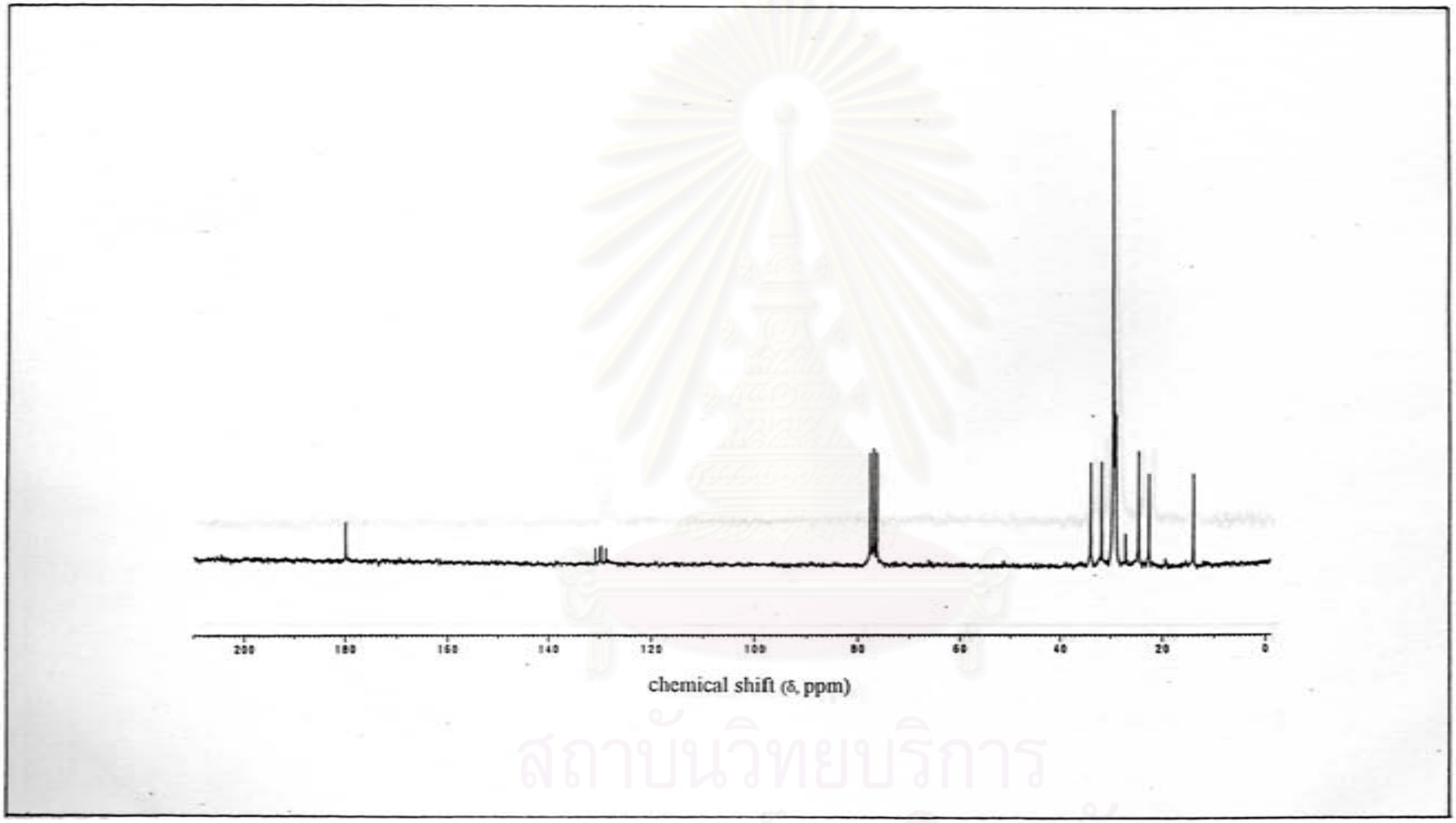

Figure 17 The ^{13}C - NMR spectrum of Mixture II in $CDCl_3$.

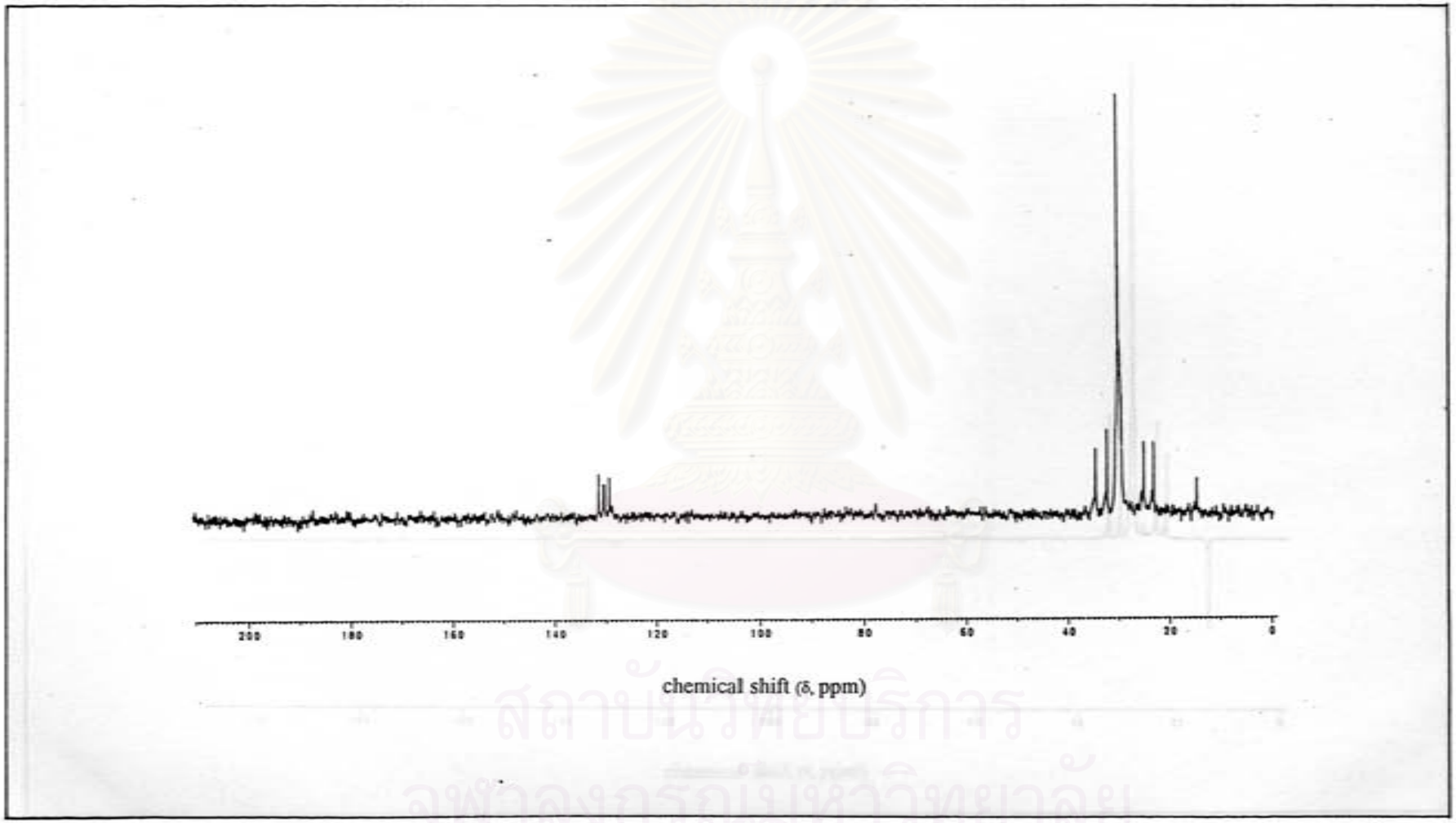

Figure 18 The DEPT - 90 ^{13}C - NMR spectrum of Mixture II in $CDCl_3$.

84

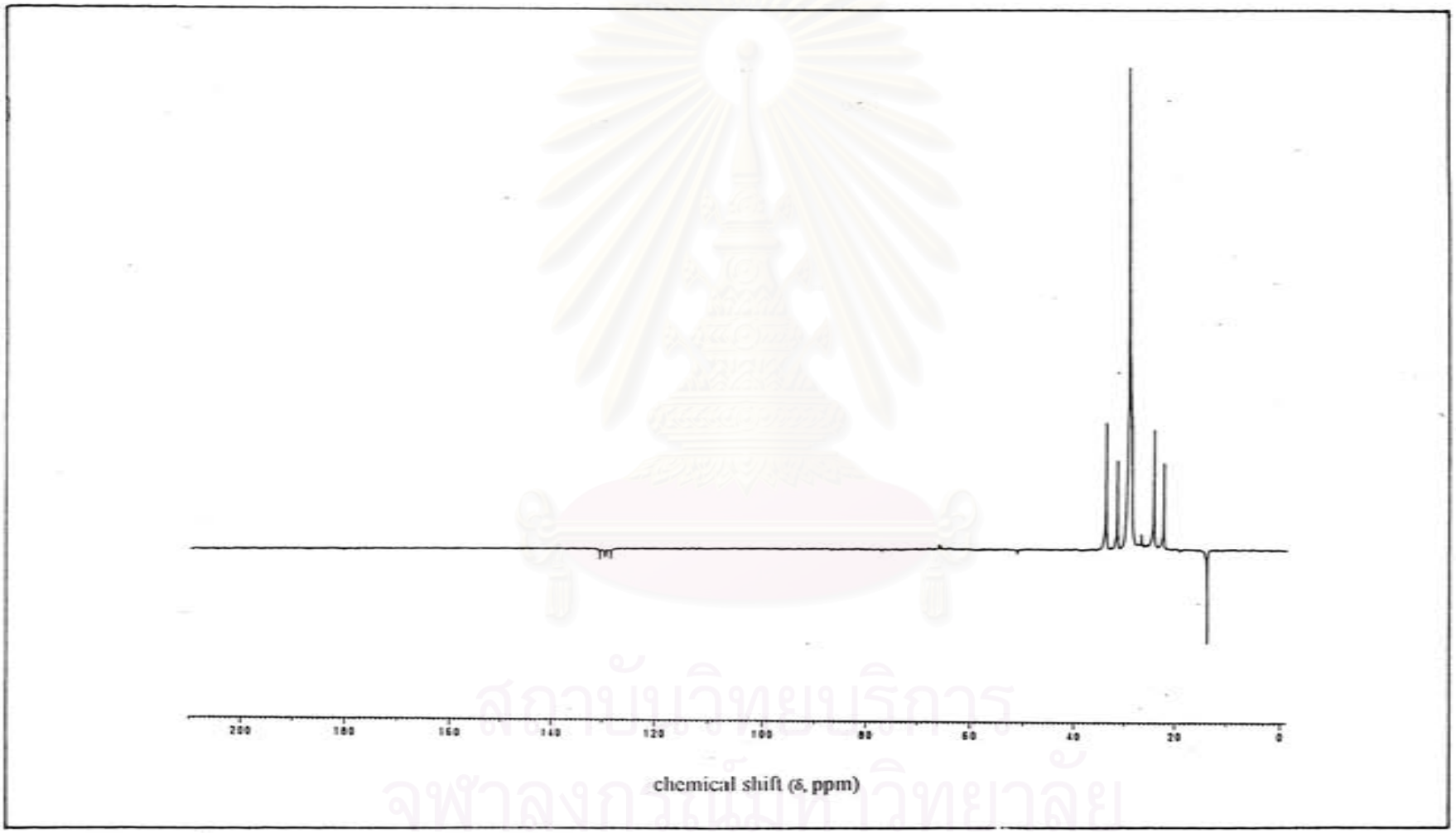

Figure 19 The DEPT - 135 ^{13}C - NMR spectrum of Mixture II in $CDCl_3$.

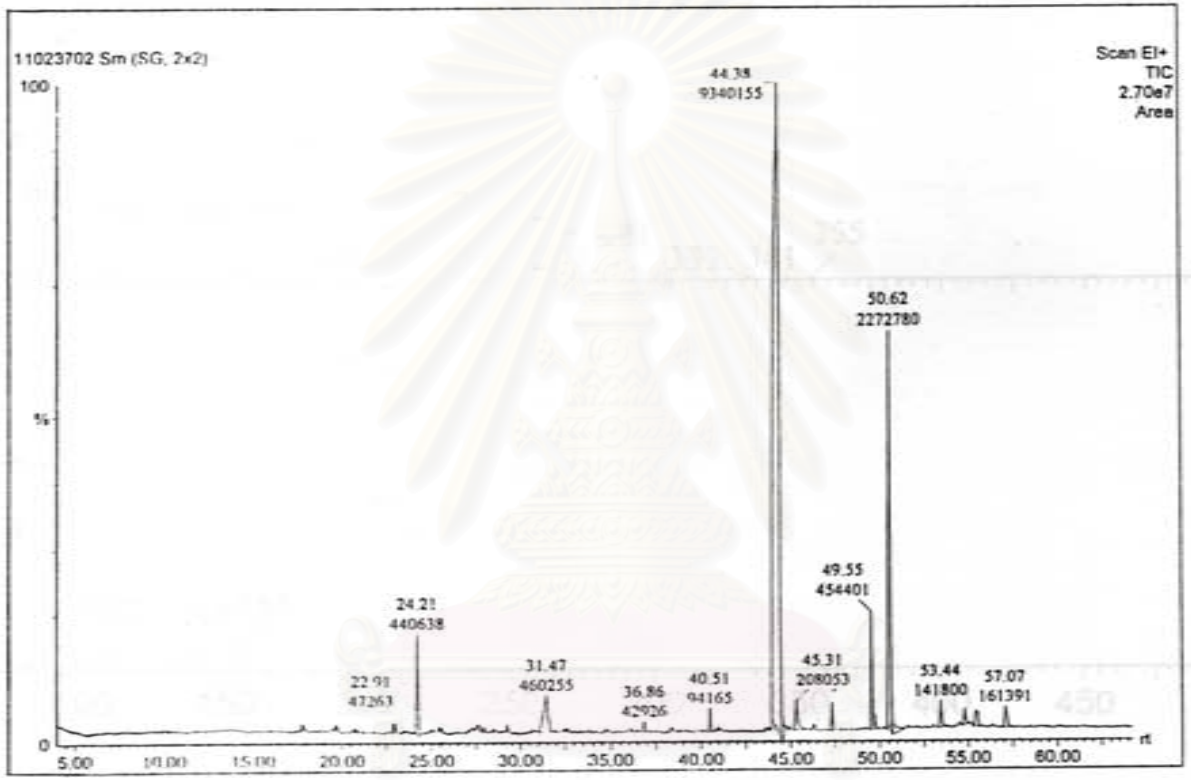

Figure 20 The Gas - Liquid chromatogram of Mixture II.

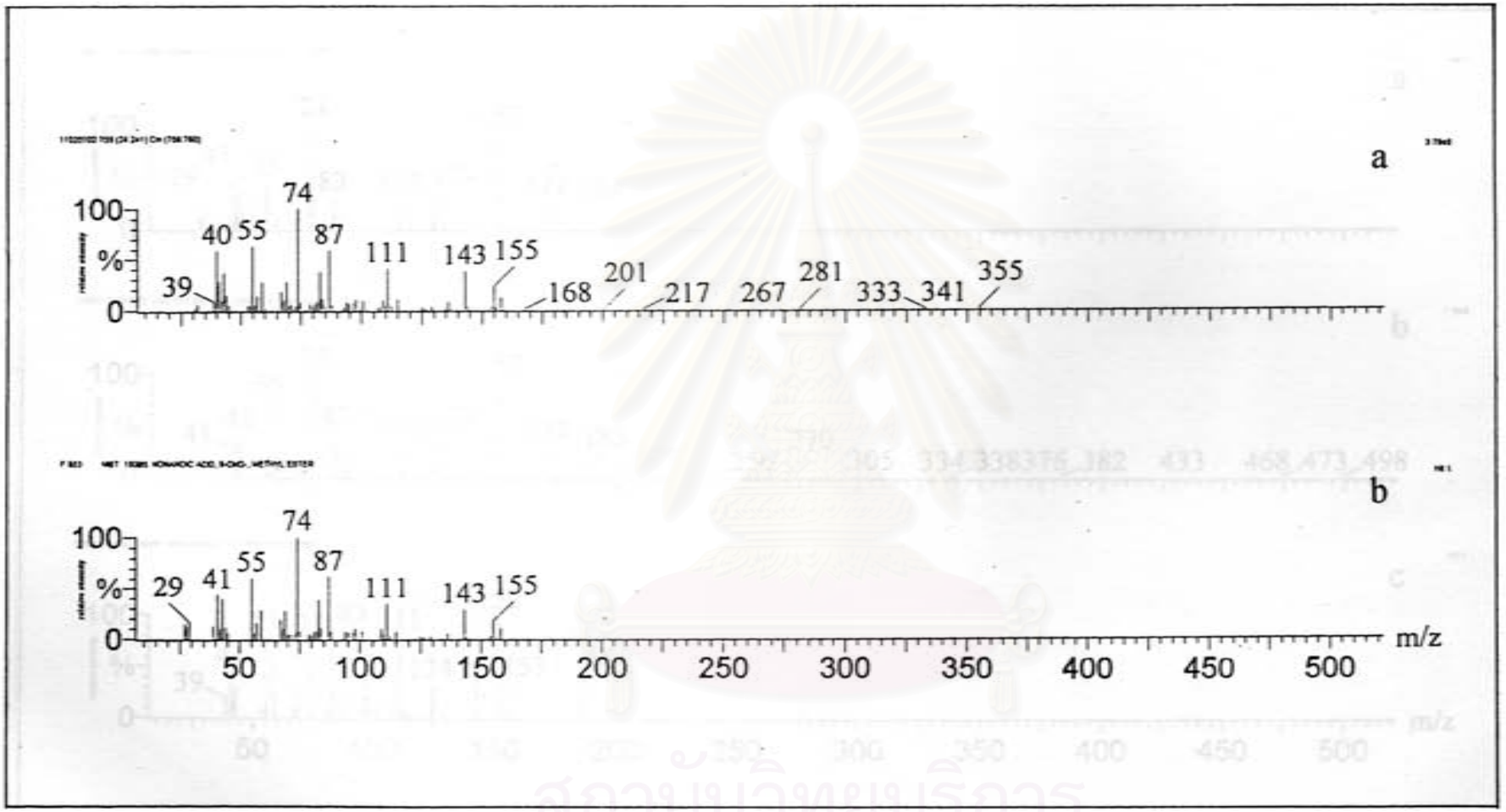

Figure 21 The mass spectrum of a. Mixture II at retention time 24.21min.

b. 9-oxo-nonanoic acid methyl ester.

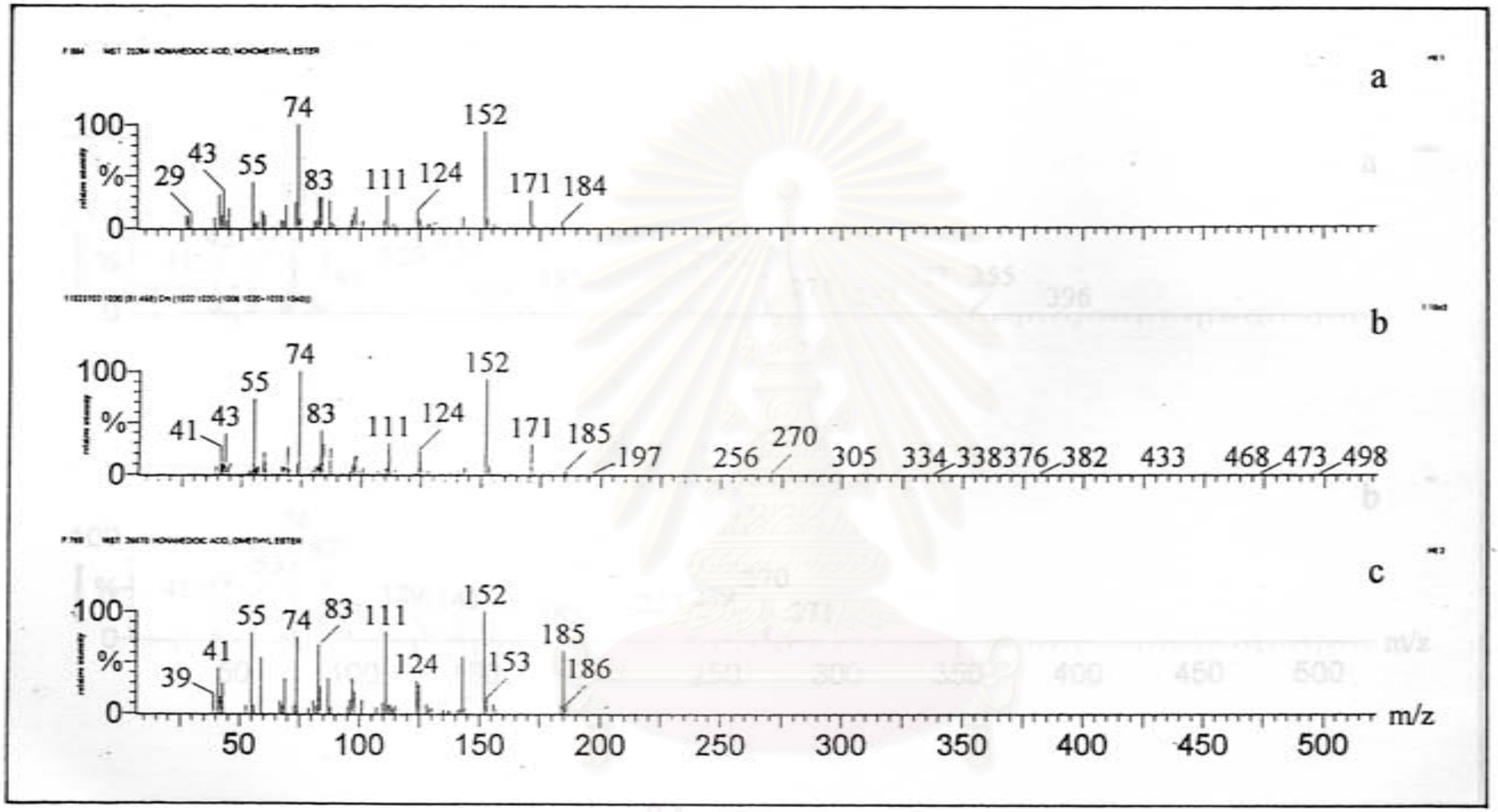

Figure 22 The mass spectrum of a. Mixture II at retention time 31.47 min.

b. nonanedioic acid monomethyl ester.

c. nonanedioic acid dimethyl ester.

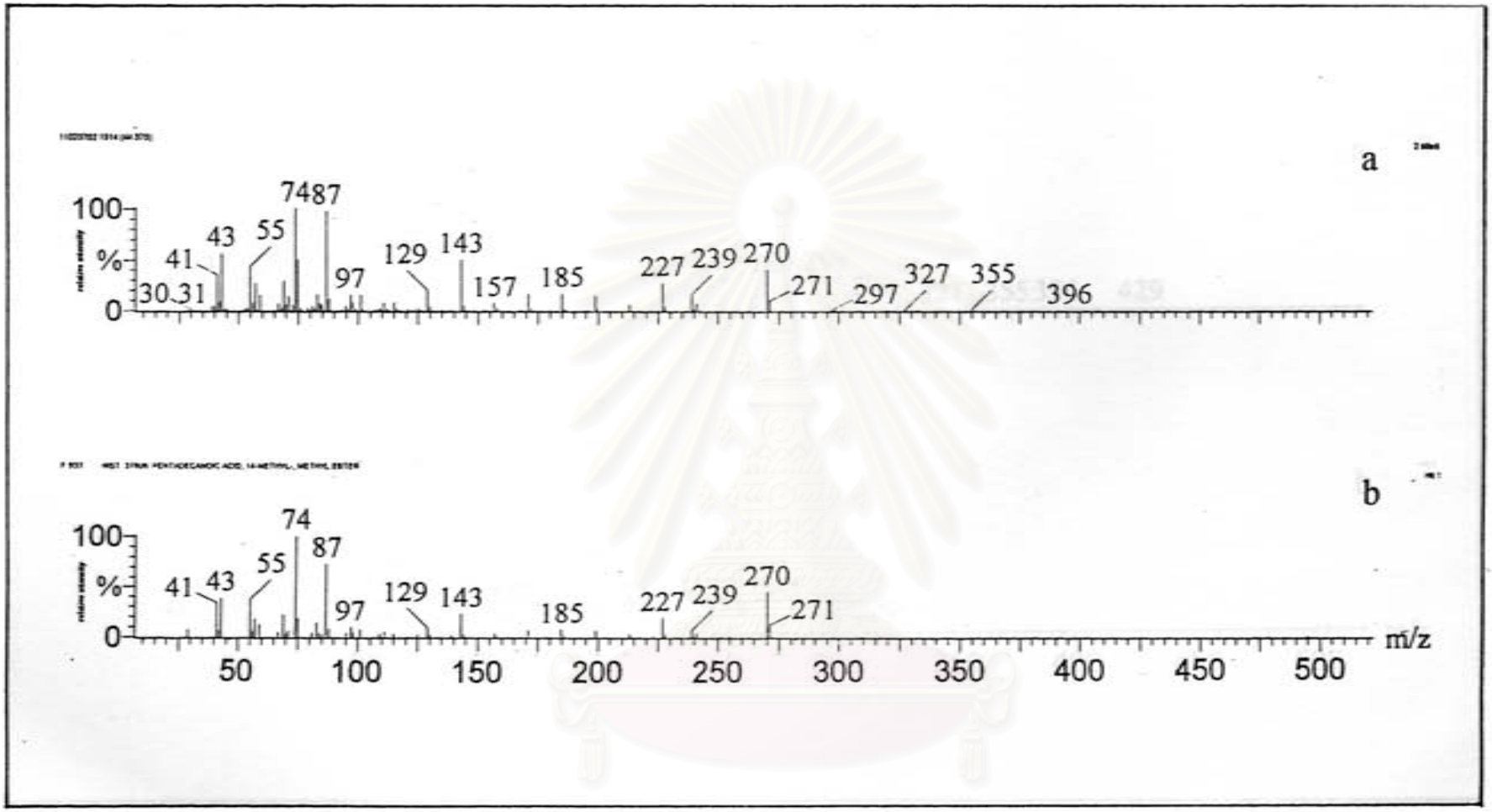

Figure 23 The mass spectrum of a. Mixture II at retention time 44.38 min.

b. 14-methyl pentadecanoic acid methyl ester.

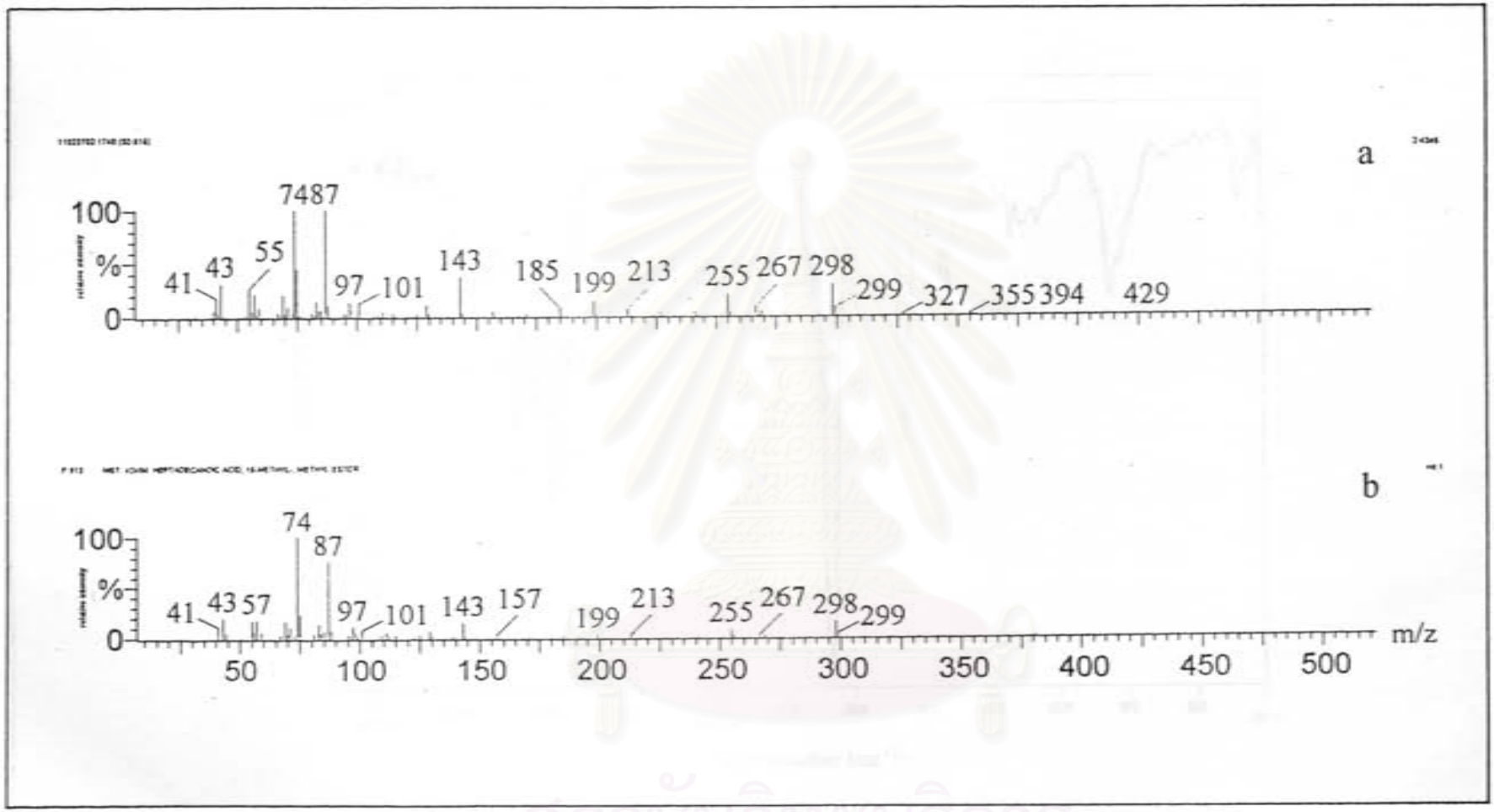

Figure 24 The mass spectrum of a. Mixture II at retention time 50.62 min.

b. 16-methyl heptadecanoic acid methyl ester.

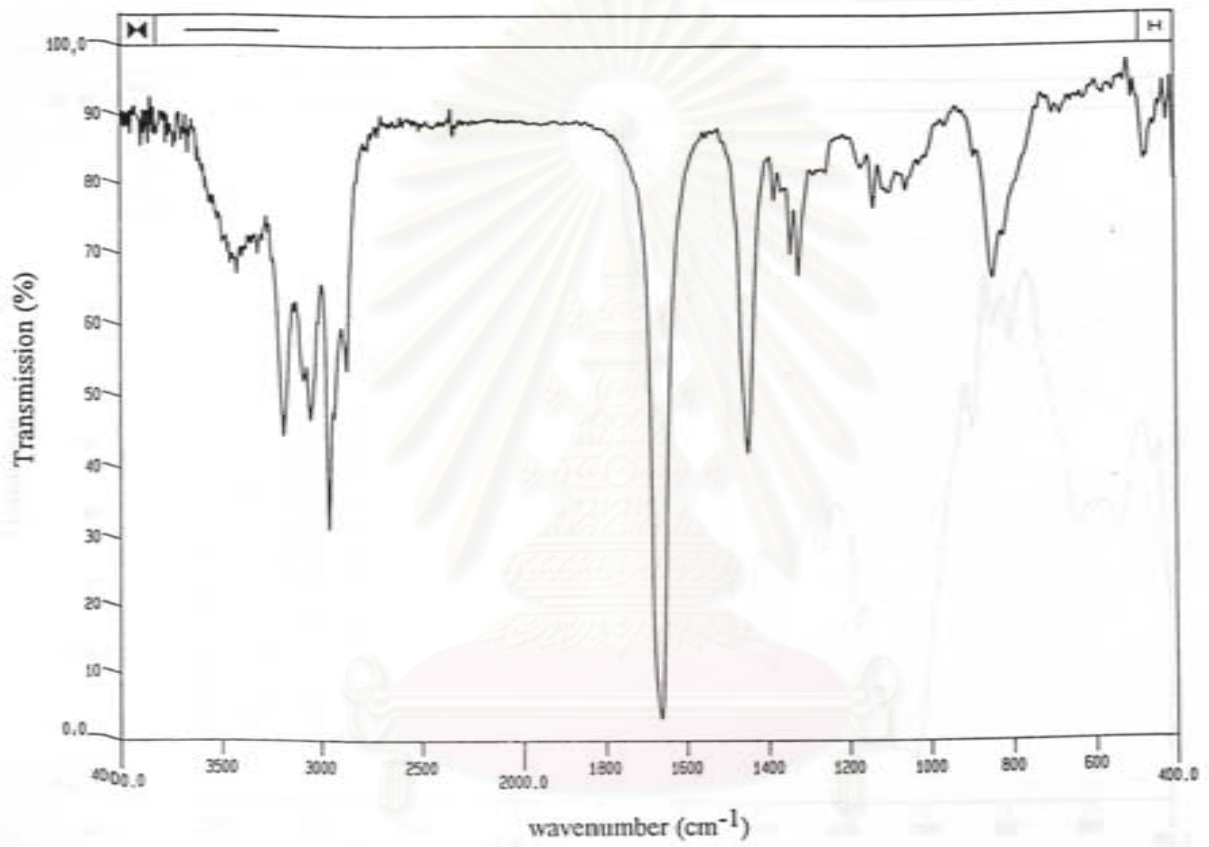

Figure 25 The IR spectrum of Mixture III.

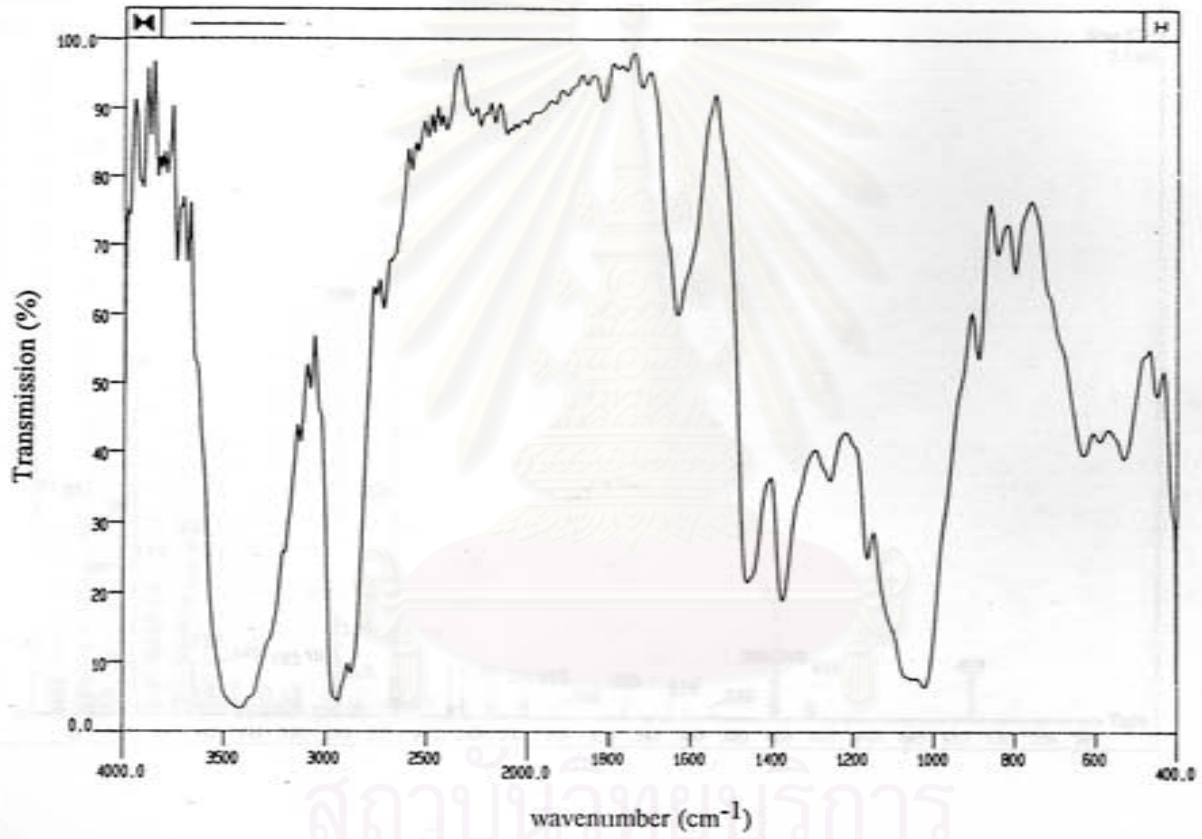

Figure 26 The IR spectrum of Mixture IV.

92

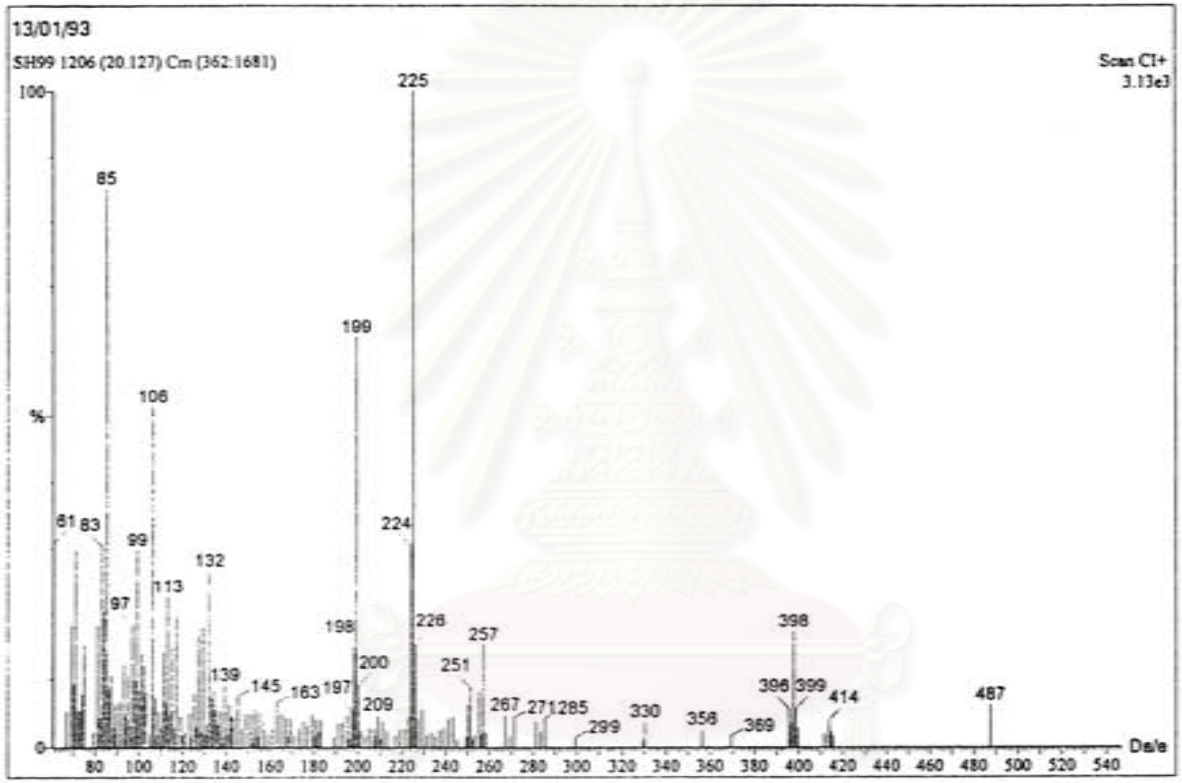

Figure 27 The CI mass spectrum of Mixture IV.

93

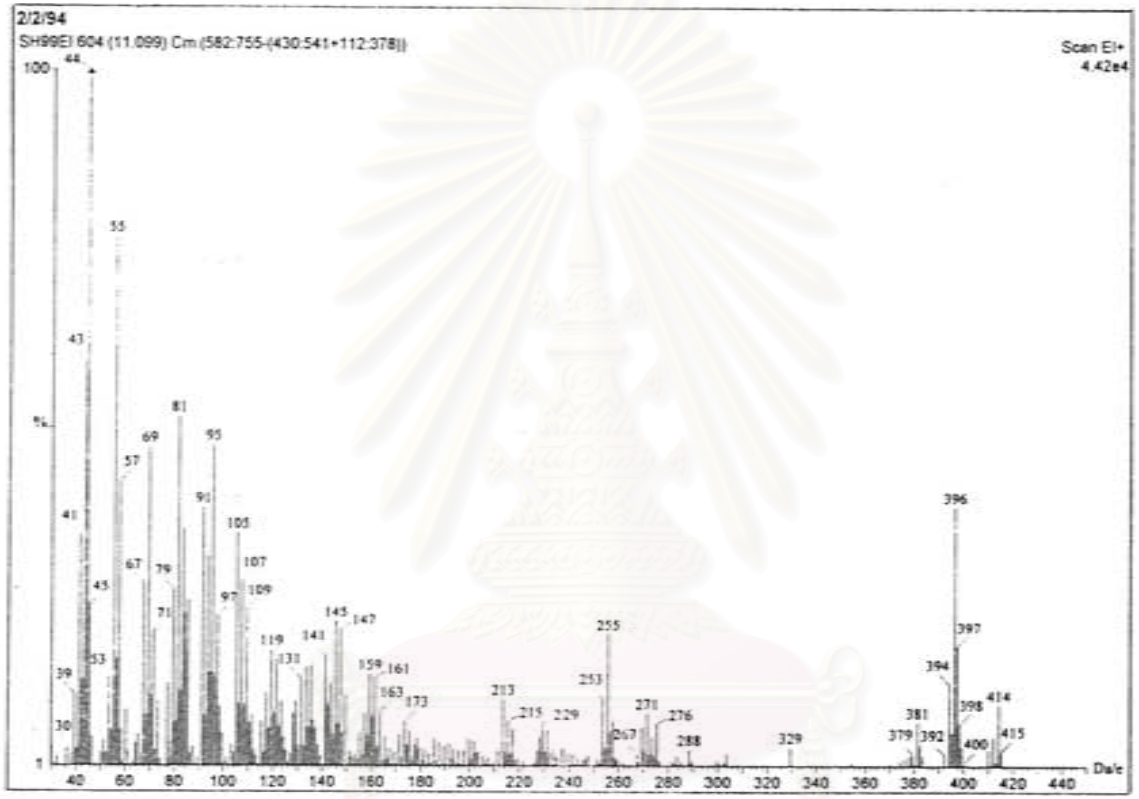

Figure 28 The EI mass spectrum of Mixture IV.

VITA

Mr. Withawat Mingvanish was born on April 30, 1969 in Bangkok, Thailand. He graduated with Bachelor Degree of Science in chemistry from Kasetsart University in 1991. In the same year, he was admitted into a Master Degree program in organic chemistry at Chulalongkorn University. During his study towards the Master's degree, he received financial support from the french government during 1991-1992.