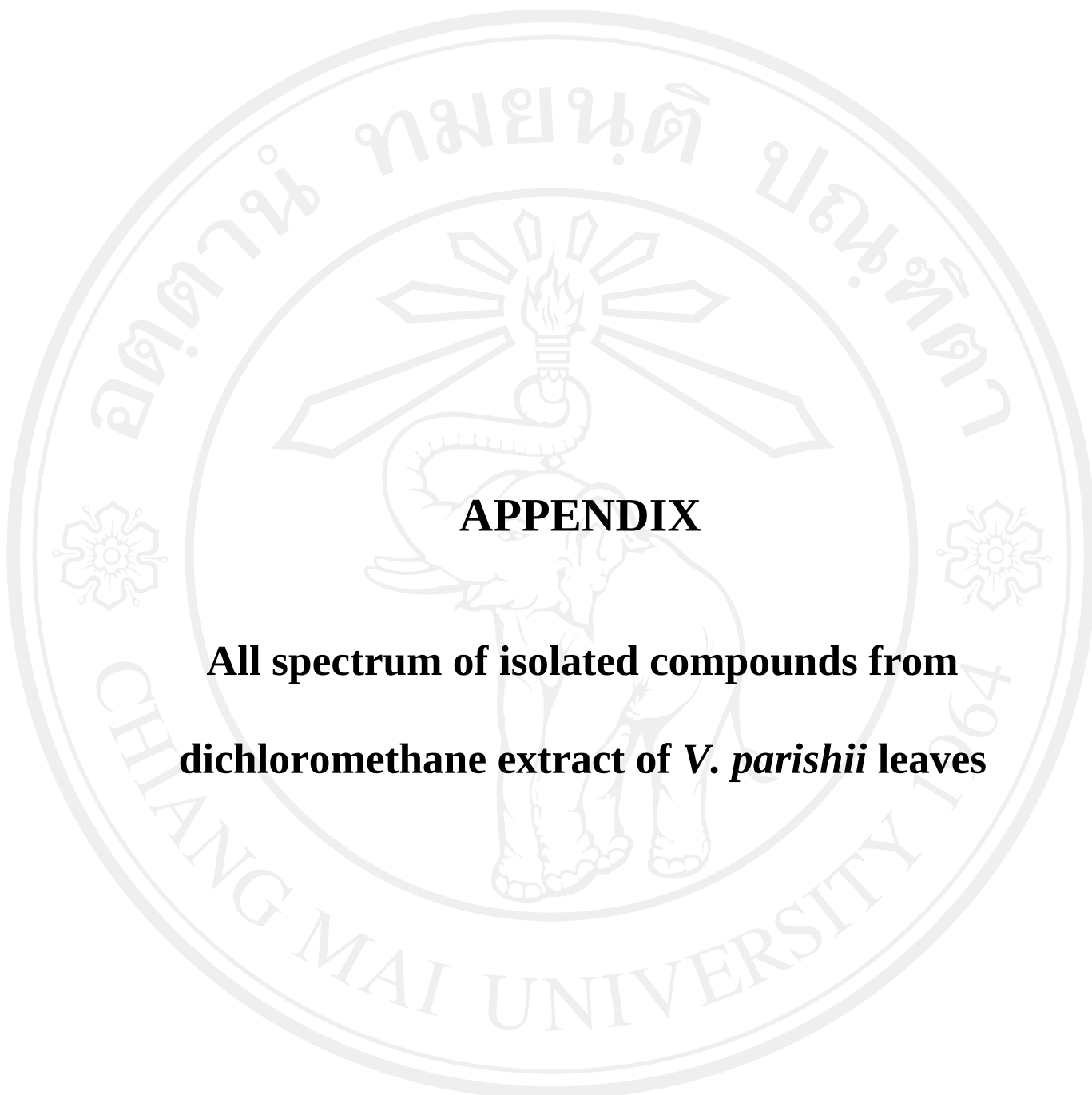


The background of the page features a large, faint watermark of the Chiang Mai University seal. The seal is circular and contains an elephant standing under a five-tiered umbrella. Above the elephant is a flame-like symbol. The Thai text "มหาวิทยาลัยเชียงใหม่" (Mahavithayalai Chiang Mai) is written in a circle around the central image. At the bottom of the seal, the English text "CHIANG MAI UNIVERSITY 1964" is visible.

APPENDIX

**All spectrum of isolated compounds from
dichloromethane extract of *V. parishii* leaves**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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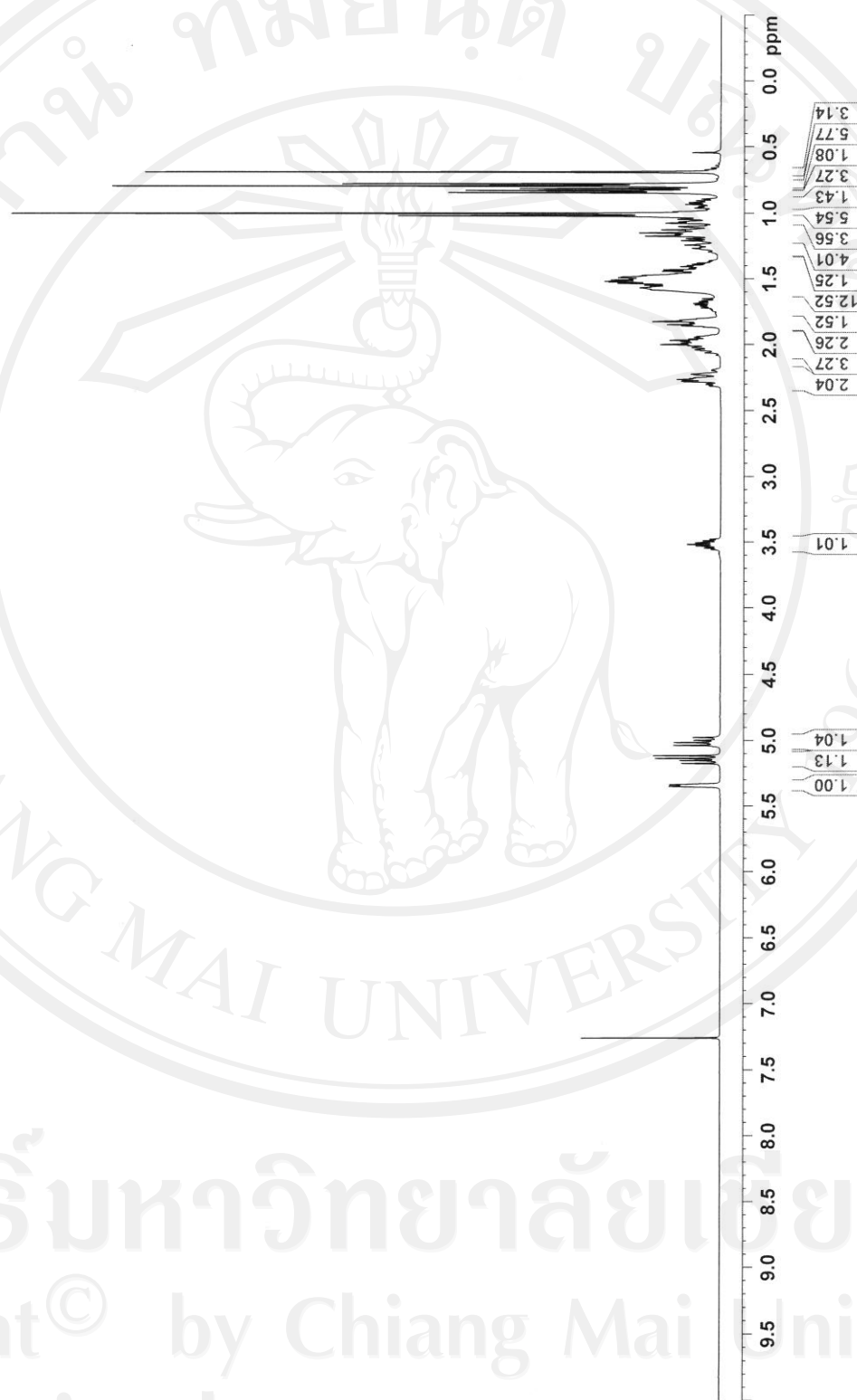


Figure 81 ^1H NMR spectrum of stigmasterol (58)

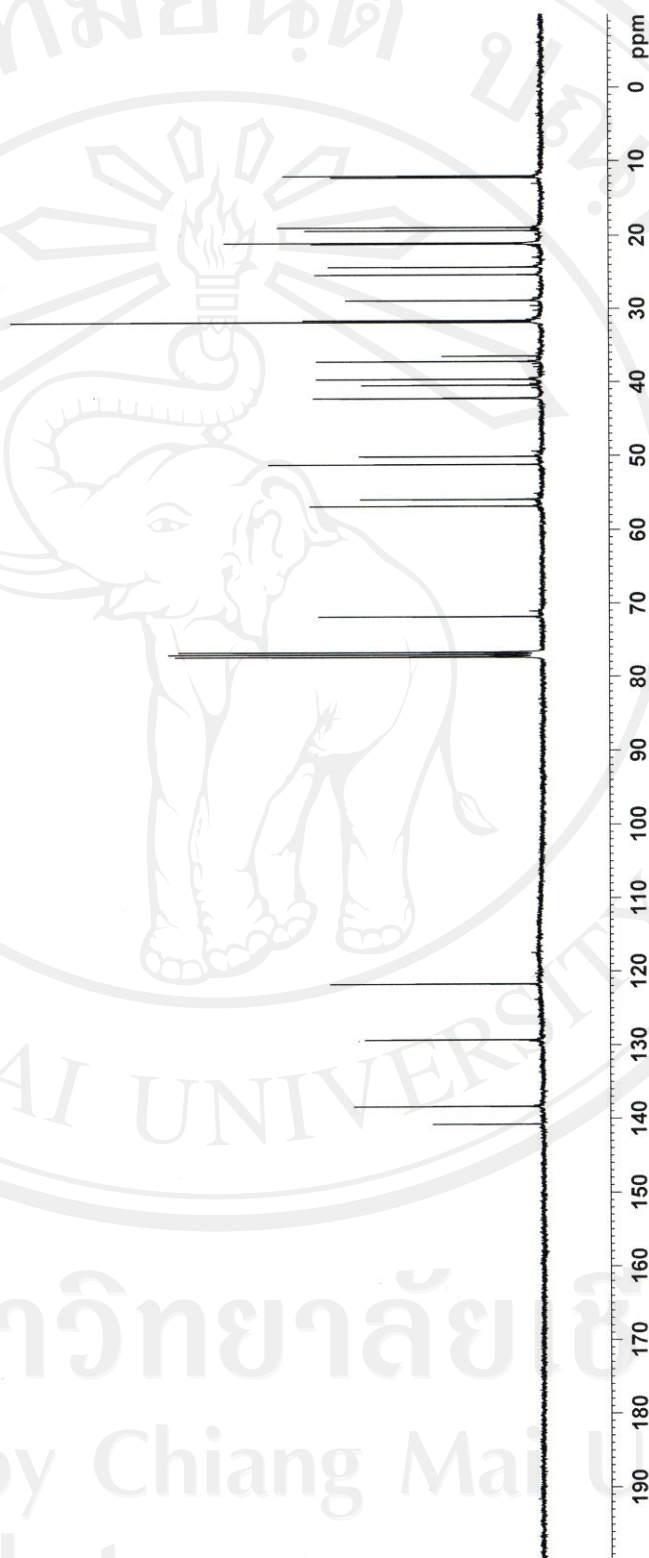


Figure 82 ^{13}C NMR spectrum of stigmasterol (58)

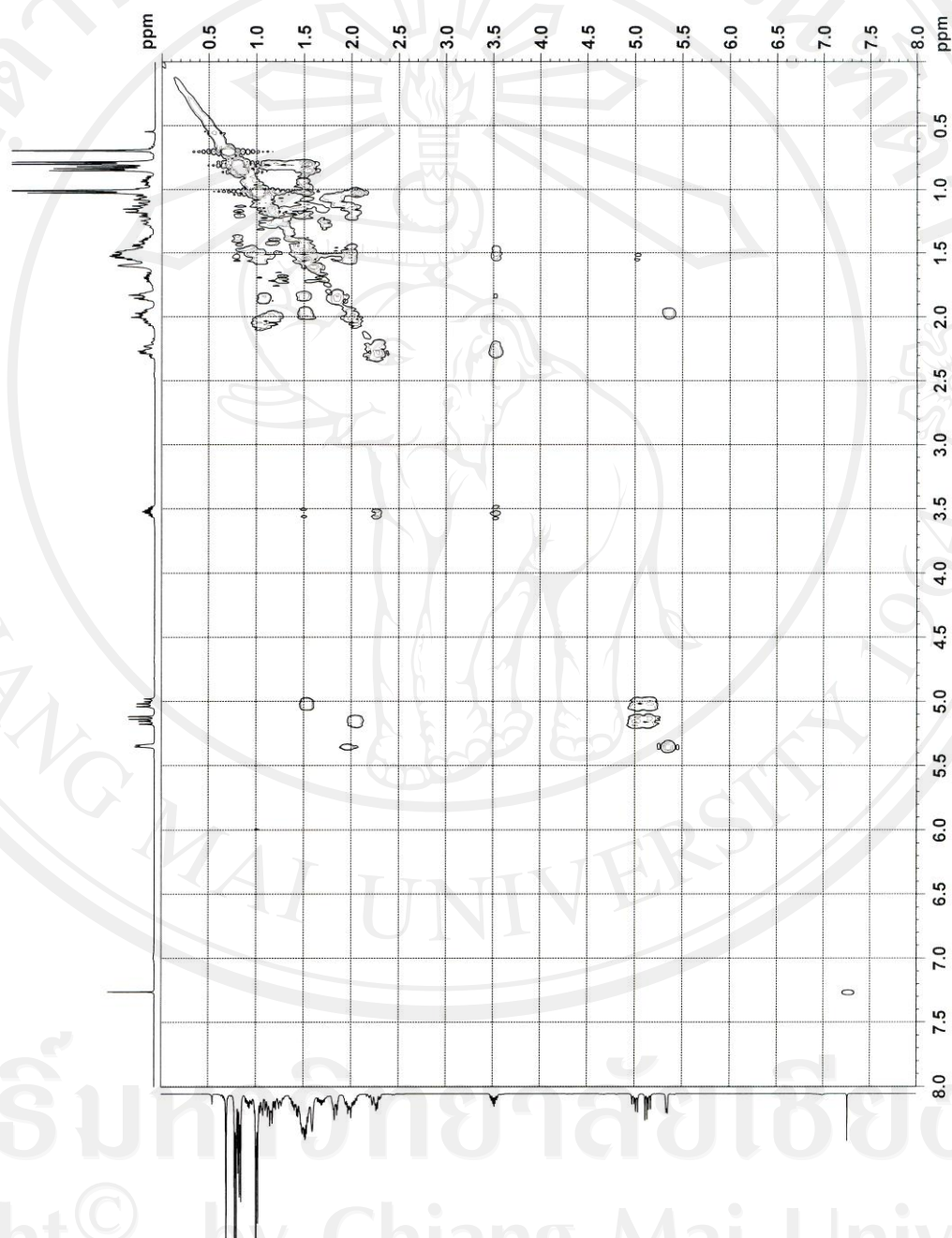


Figure 83 COSY of stigmasterol (58)

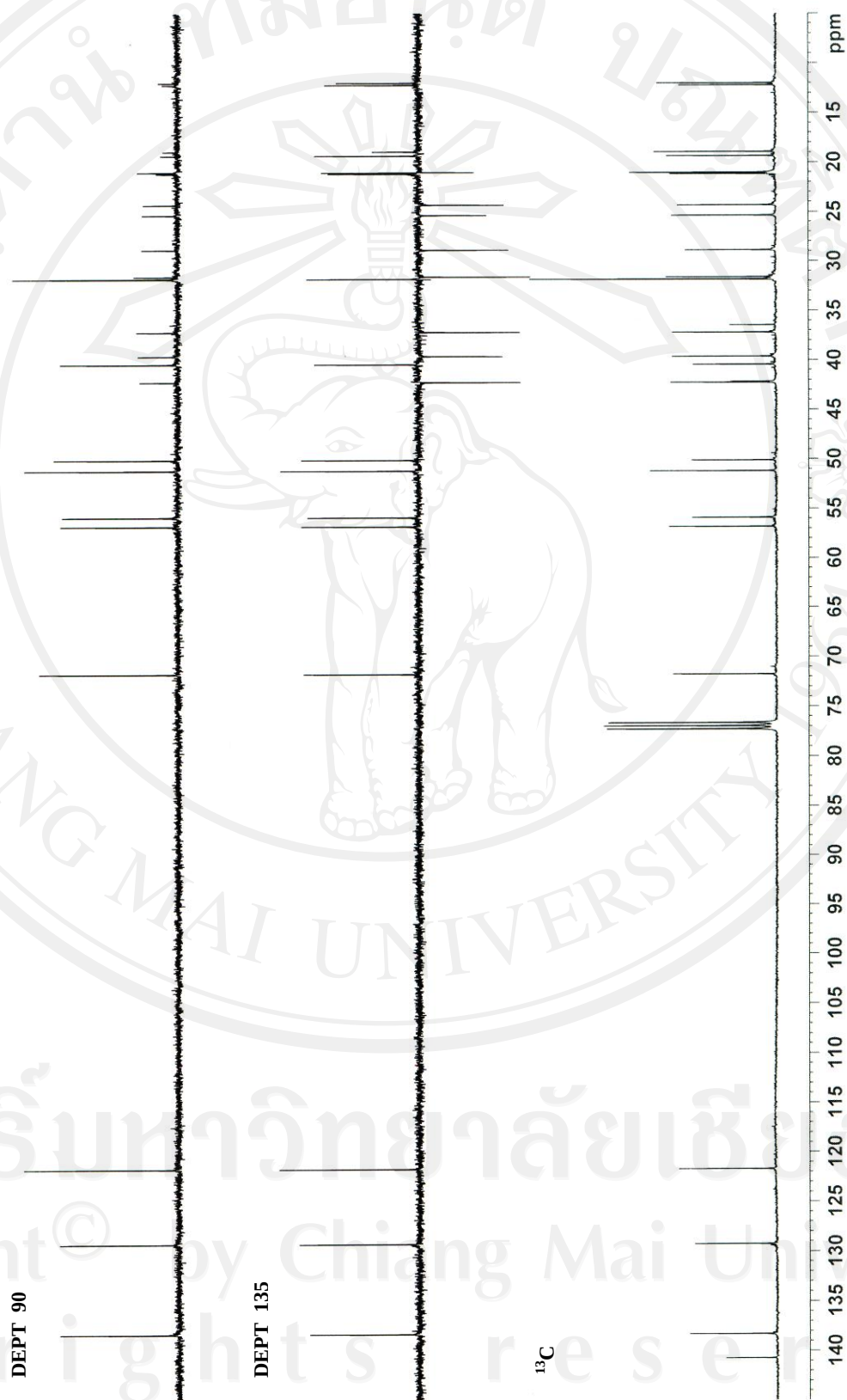


Figure 84 Comparison between DEPT 90, DEPT 135, and ^{13}C of stigmasterol (58)

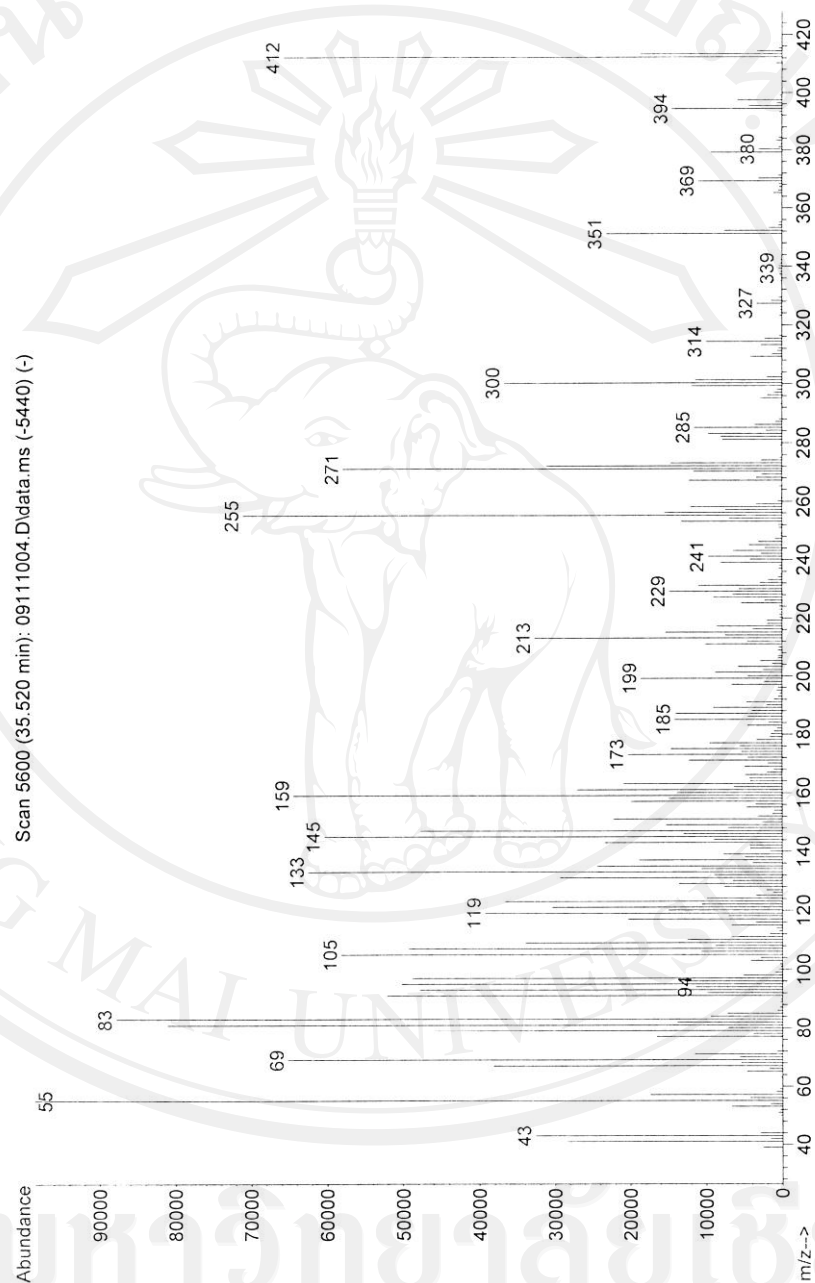


Figure 85 Mass spectrum of stigmasterol (58)



140

120

100

80

60

40

20

0

Transmittance [%]

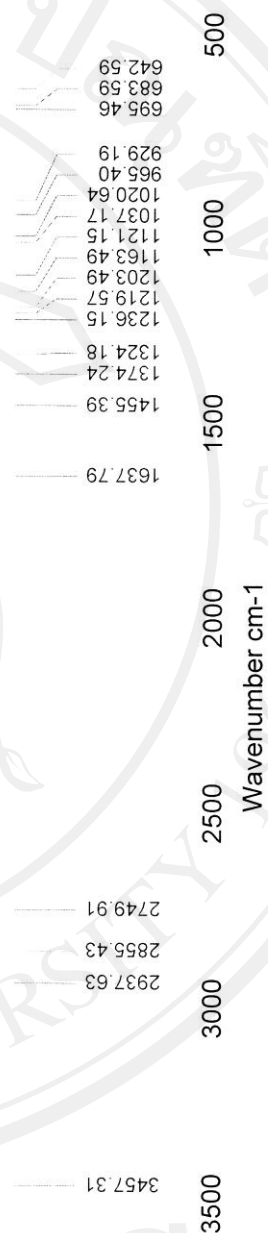


Figure 86 IR spectrum of stigmasterol (58)

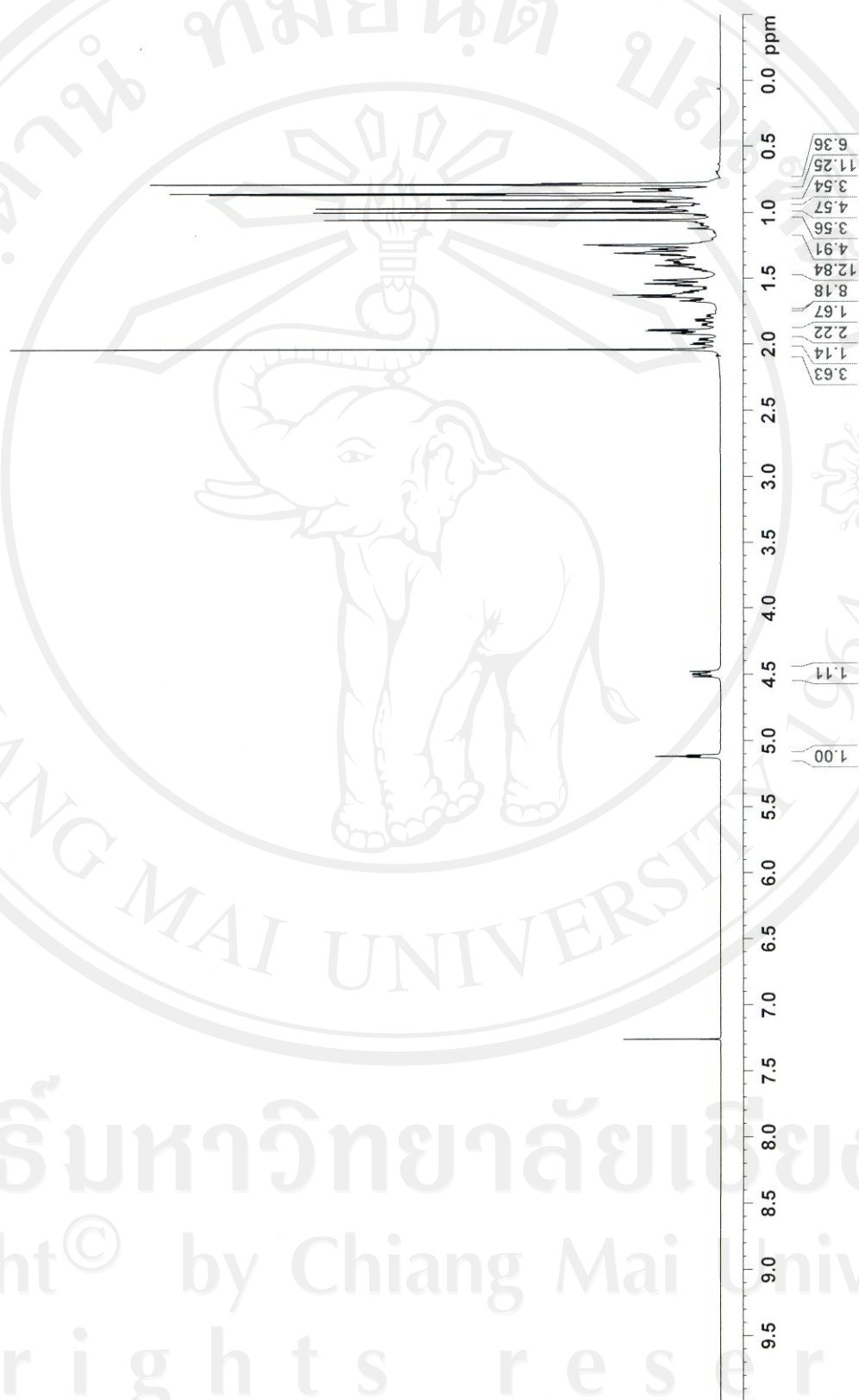


Figure 87 ¹H NMR spectrum of α -amyirin acetate (9)

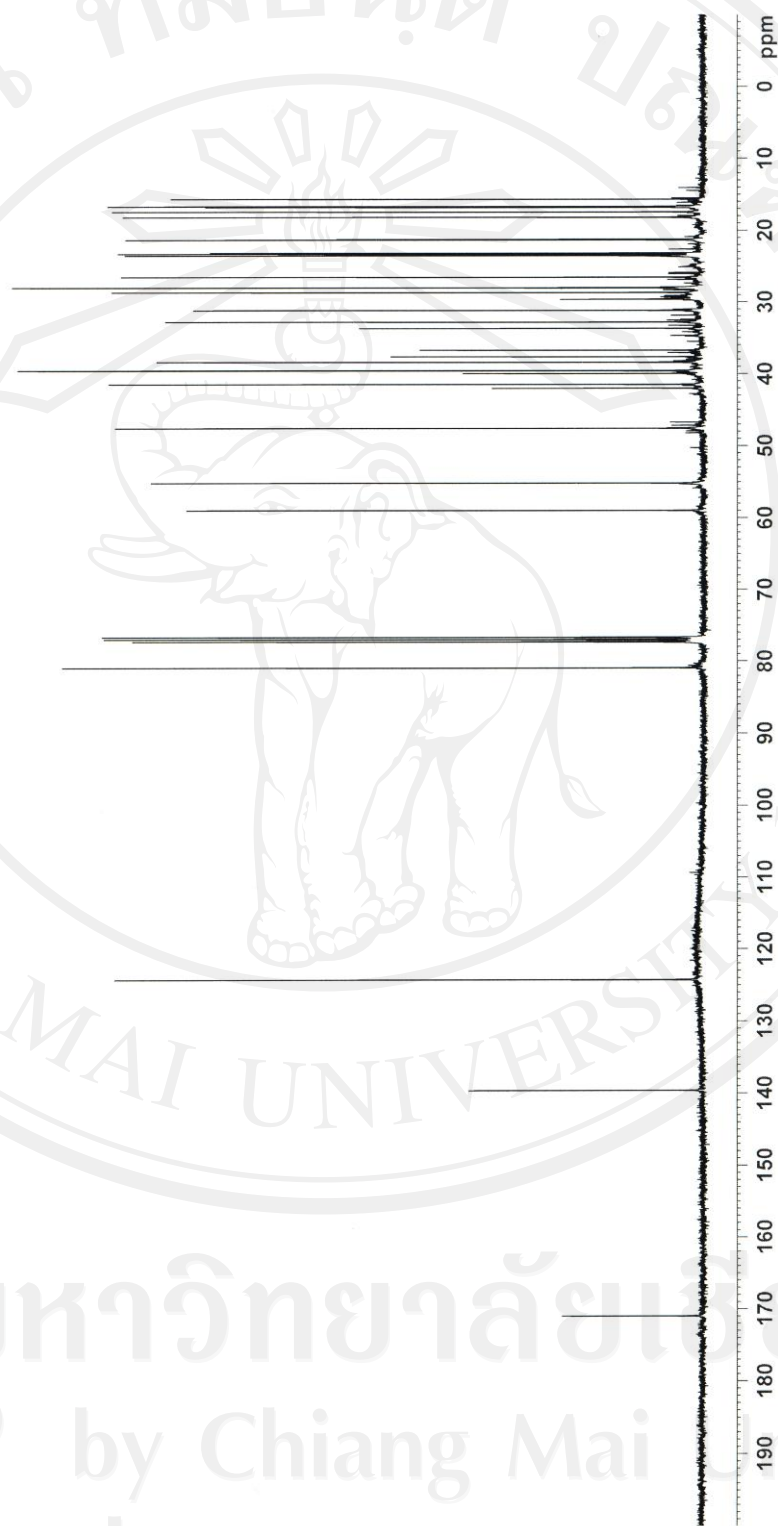


Figure 88 ^{13}C NMR spectrum of α -amyrin acetate (9)

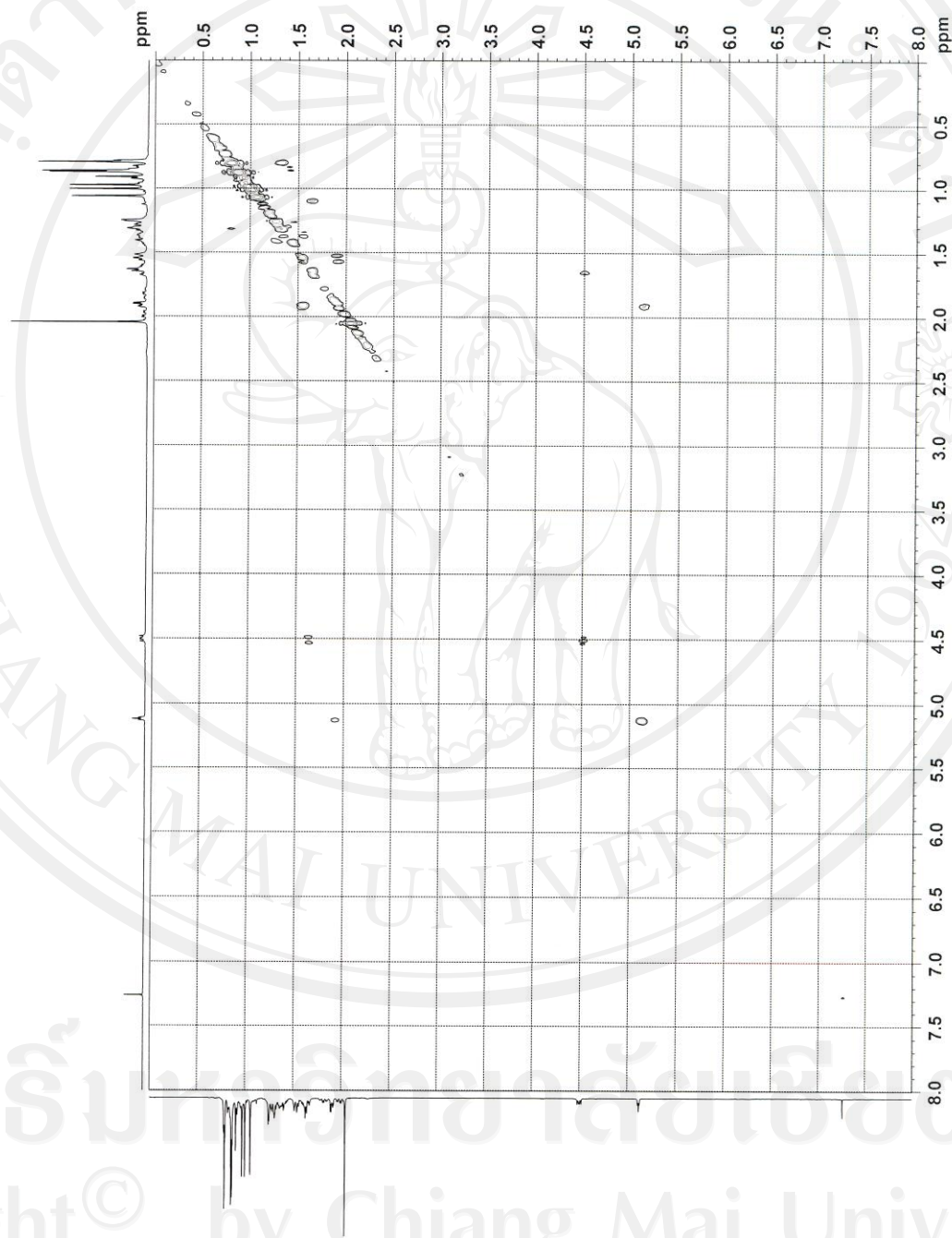


Figure 89 COSY of α -amyrin acetate (9)

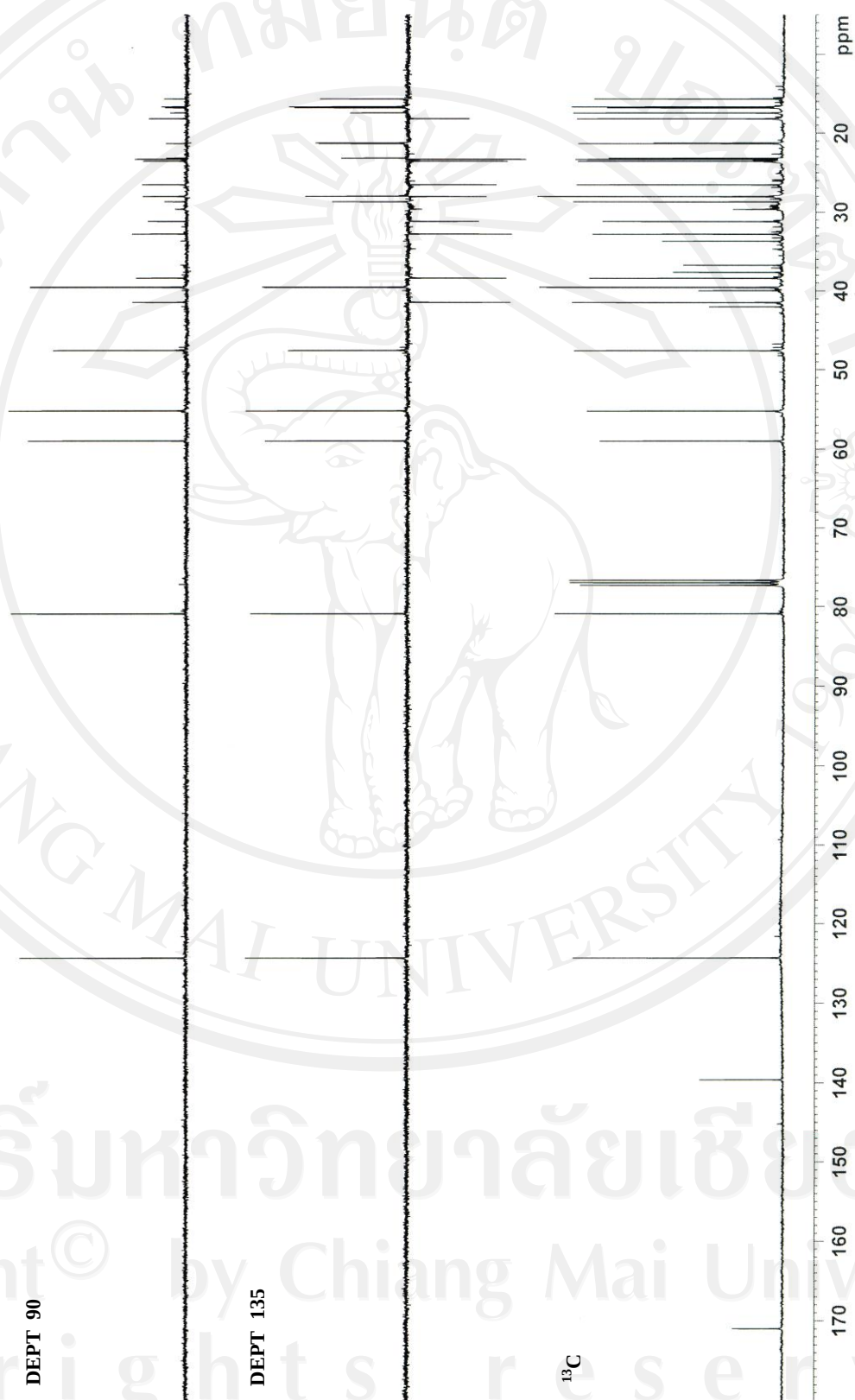


Figure 90 Comparison between DEPT 90, DEPT 135, and ^{13}C of α -myrrin acetate (9)

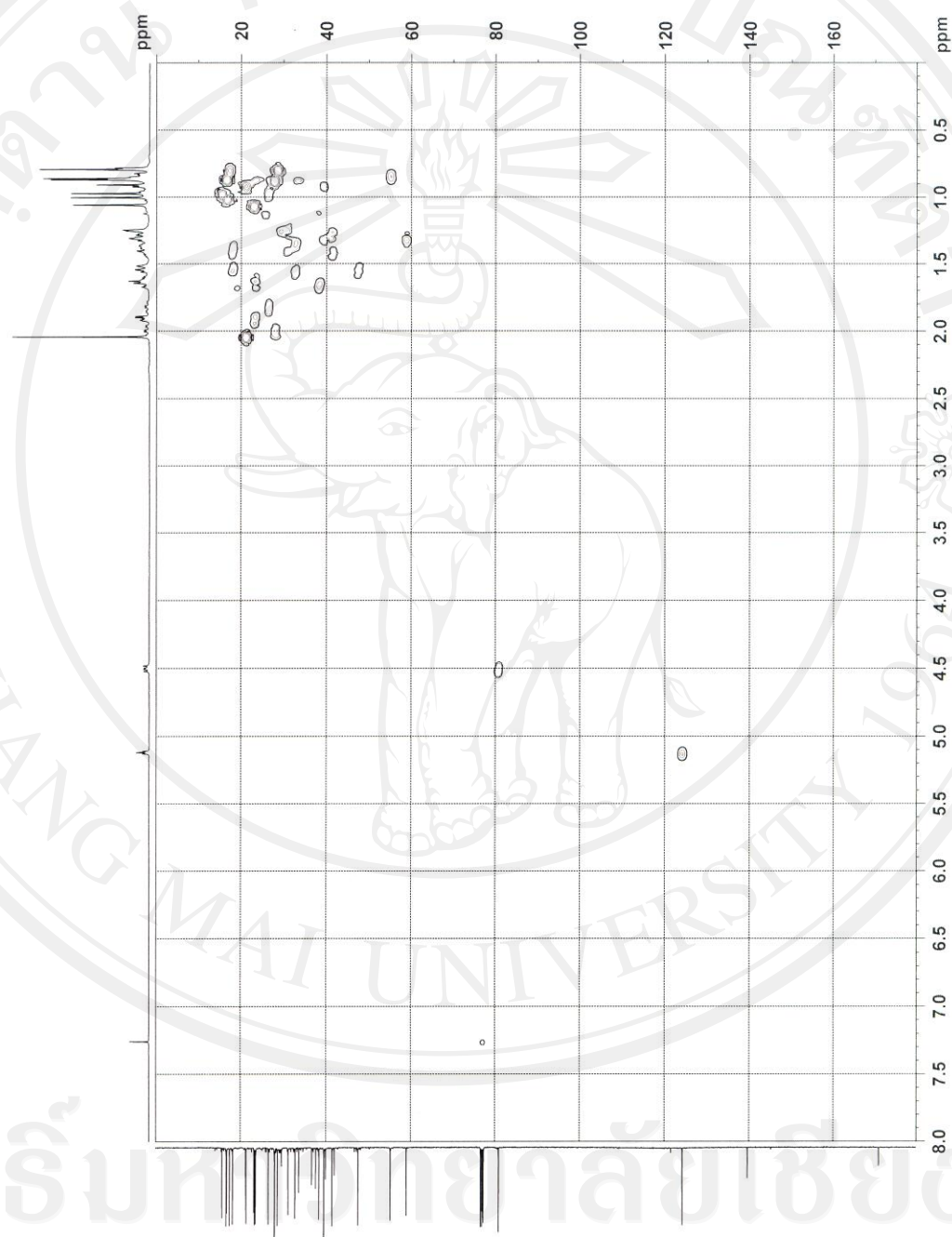


Figure 91 HMQC of α -amyirin acetate (9)

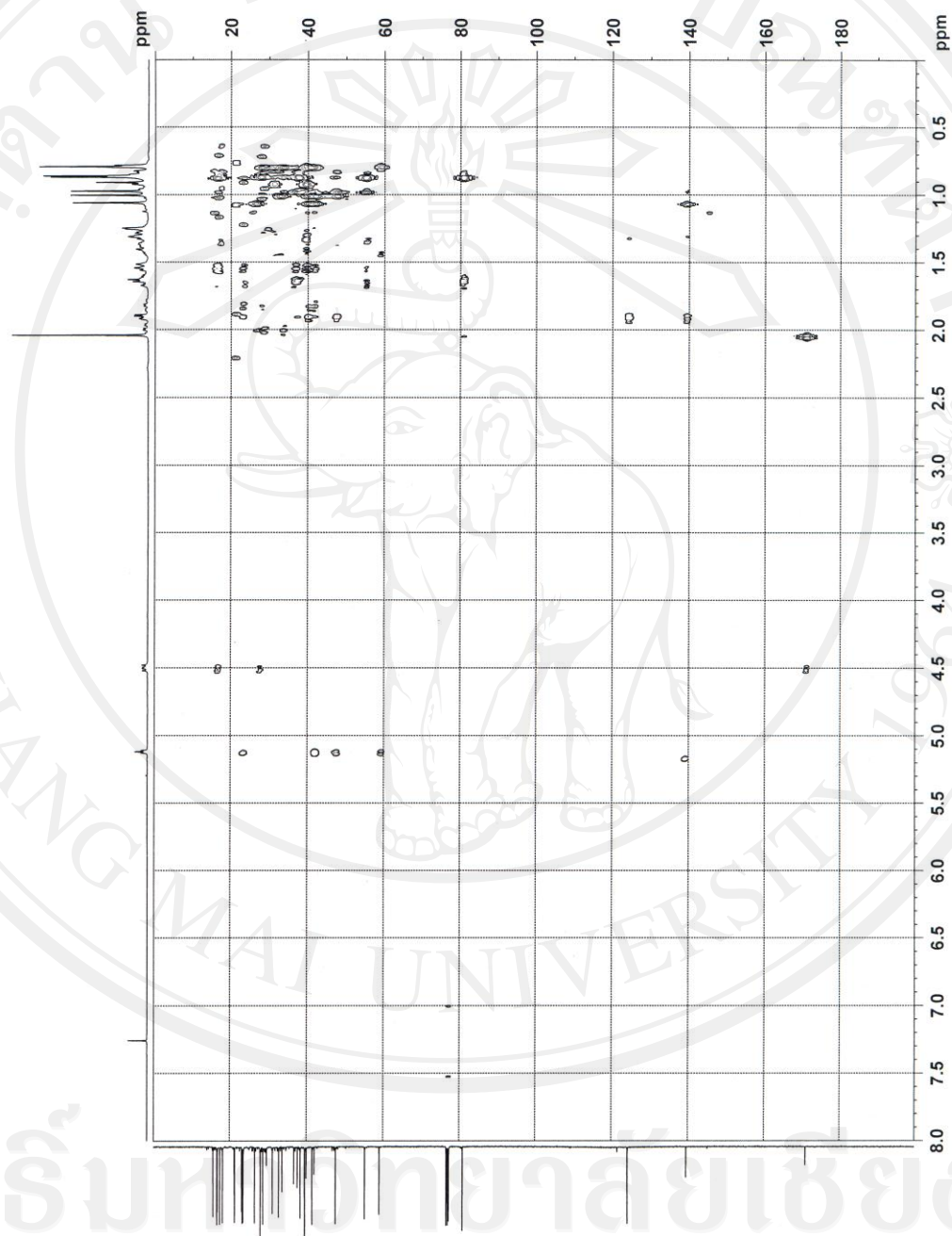


Figure 92 HMBC of α -amyryn acetate (9)

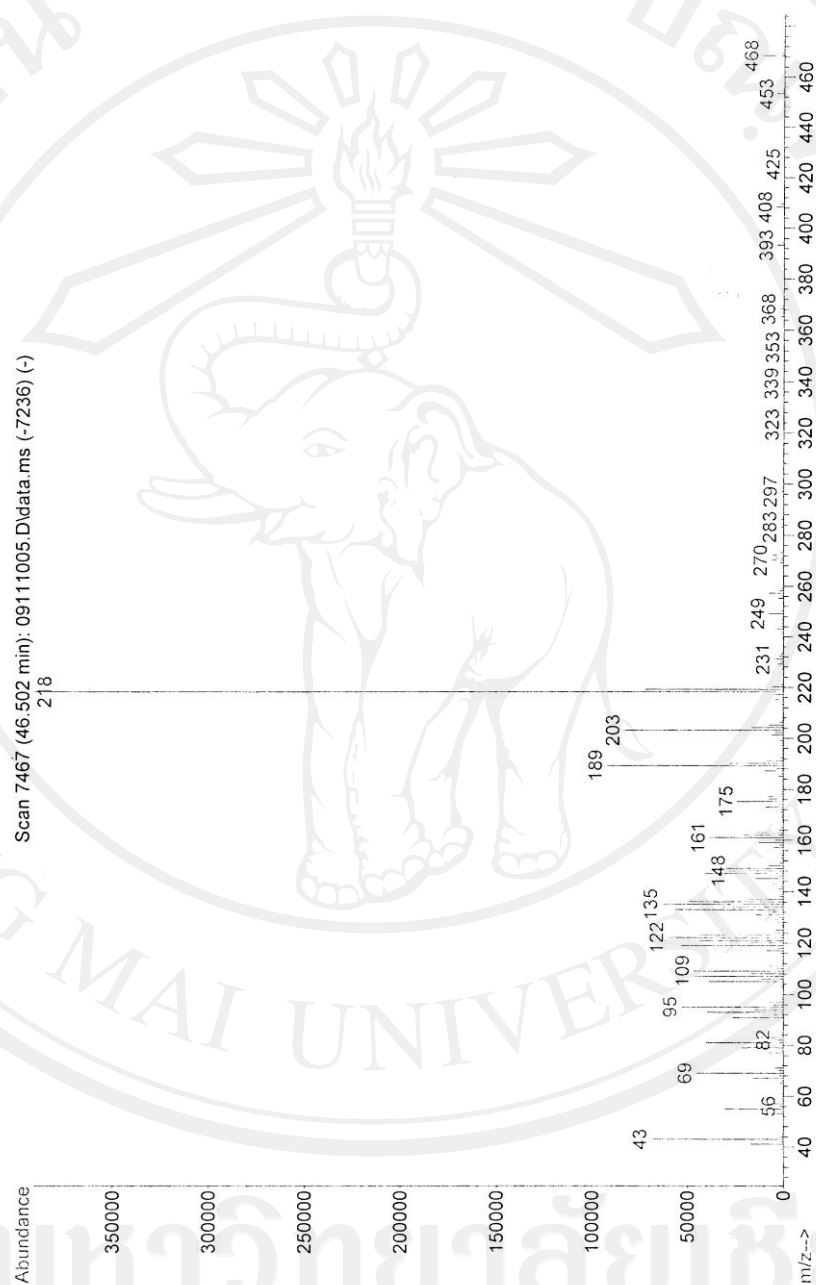


Figure 93 Mass spectrum of α -amyrin acetate (9)

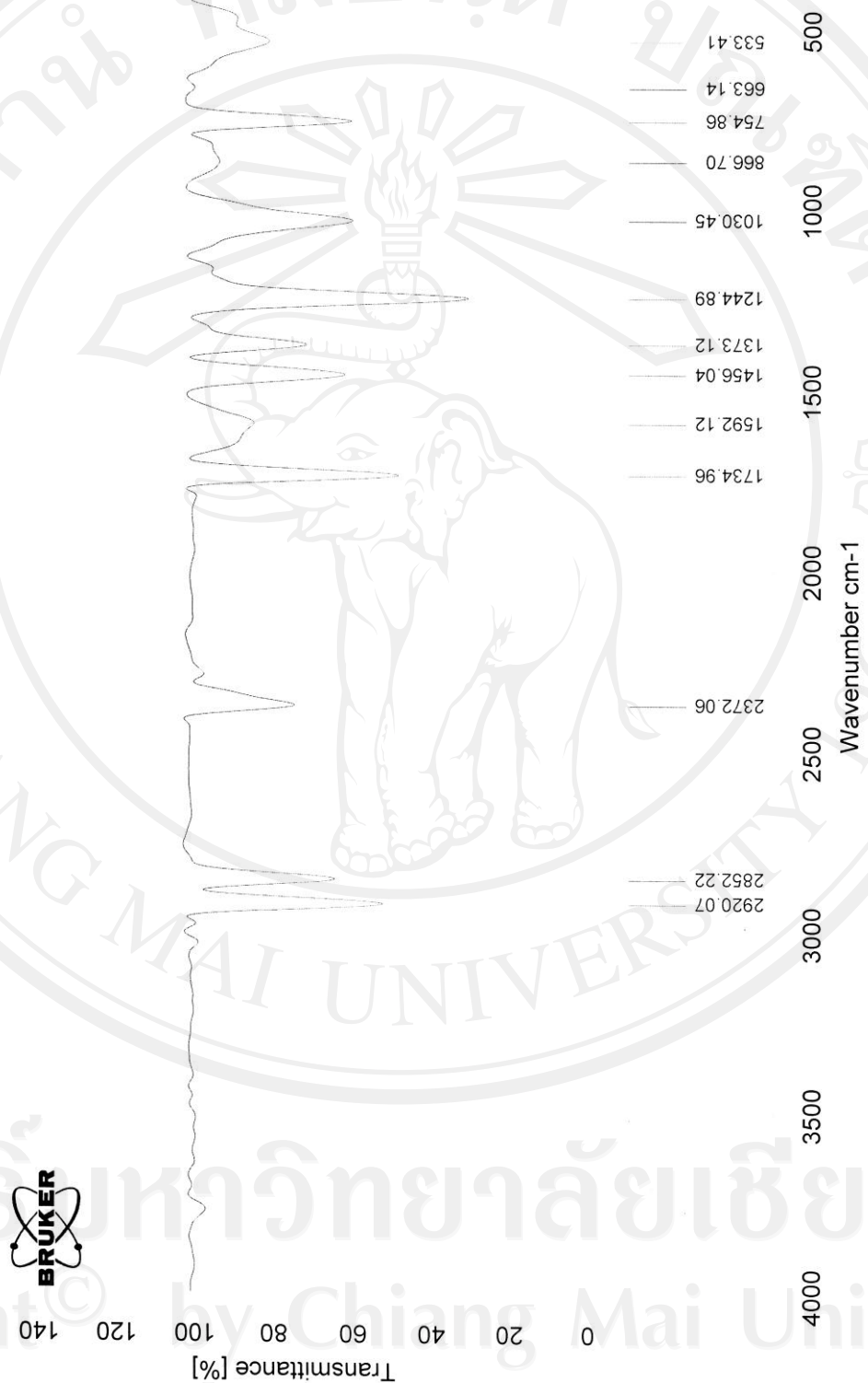


Figure 94 IR spectrum of α -amyrin acetate (9)

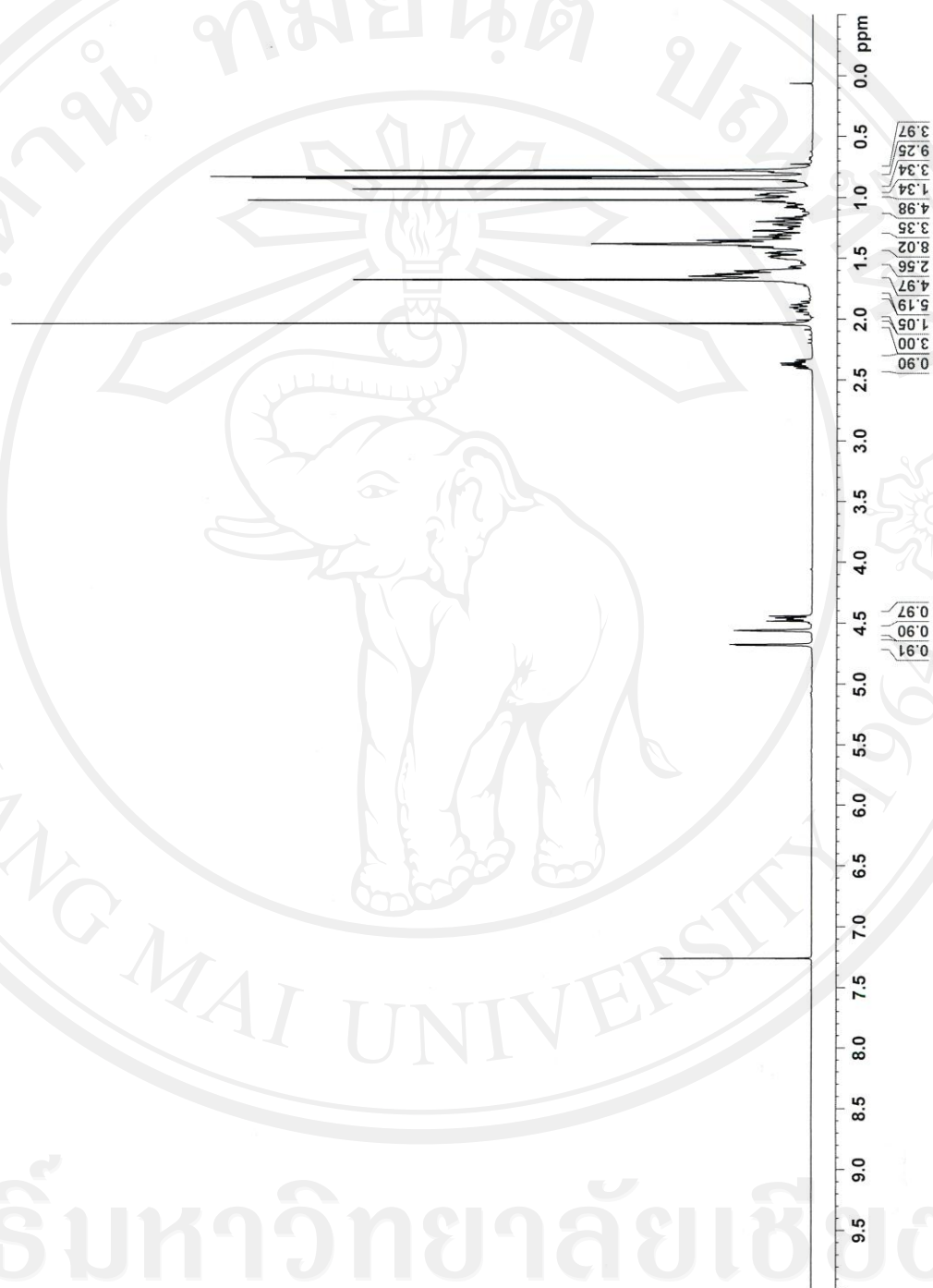


Figure 95 ^1H NMR spectrum of lupeol acetate (59)

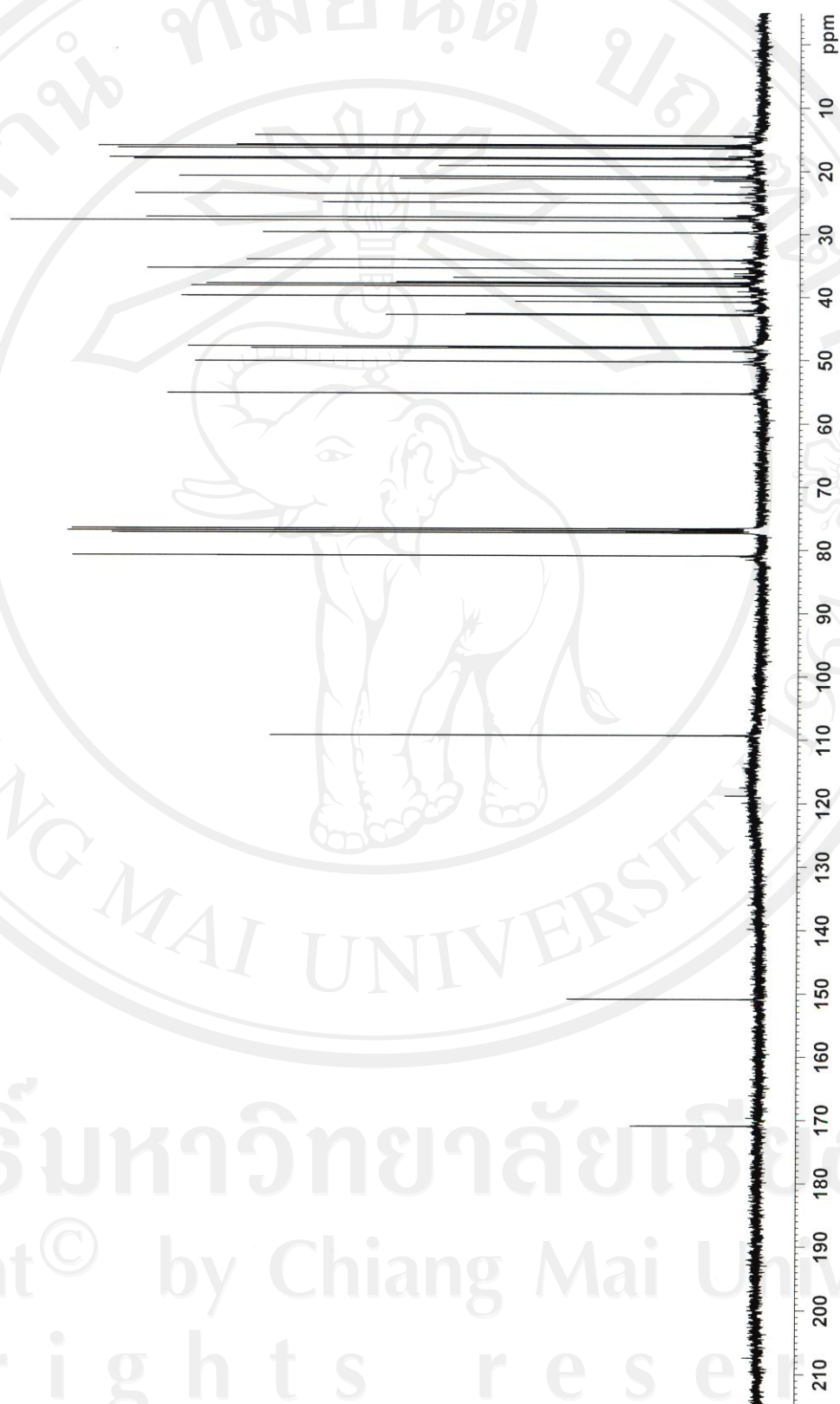


Figure 96 ^{13}C NMR spectrum of lupeol acetate (59)

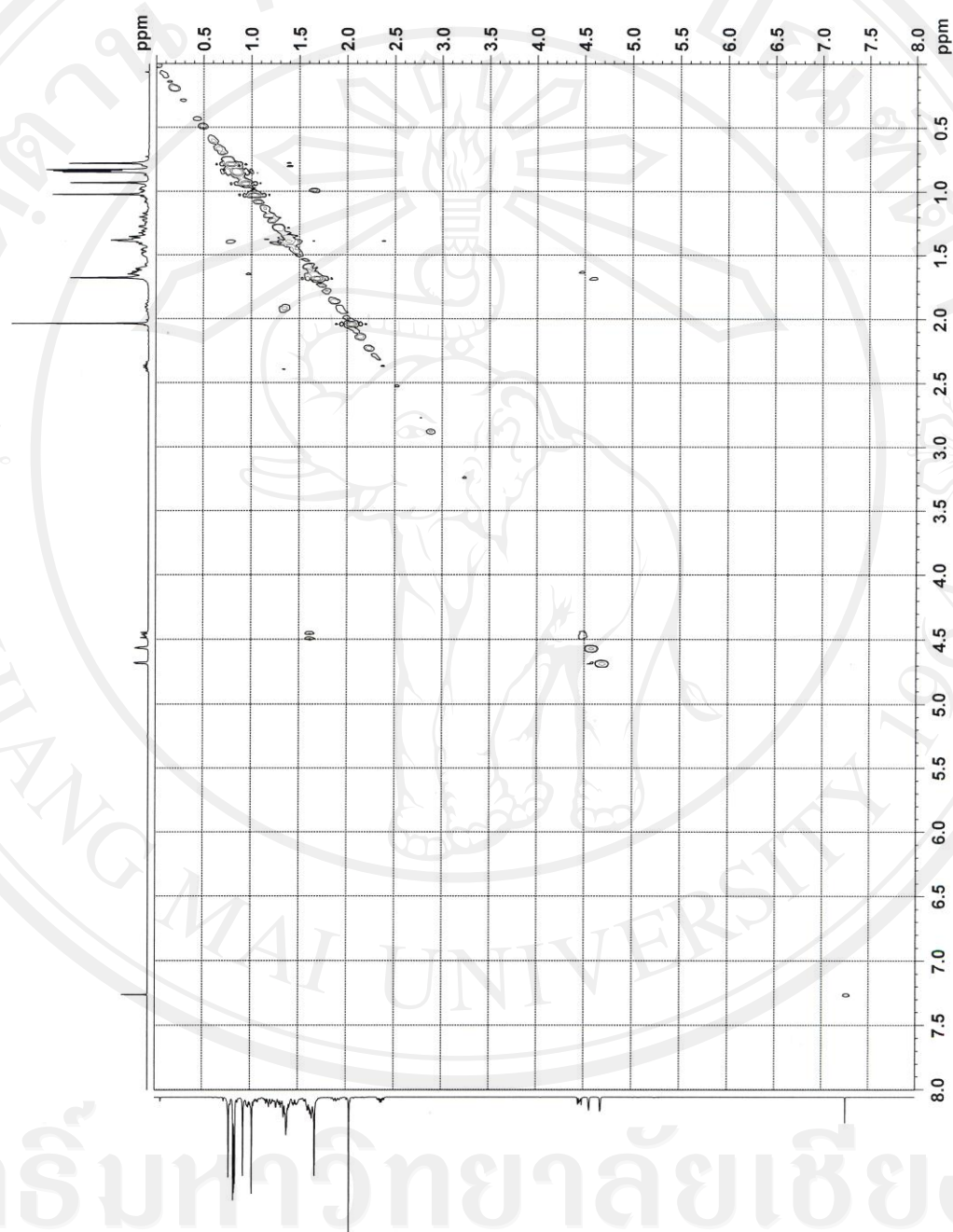


Figure 97 COSY of lupeol acetate (59)

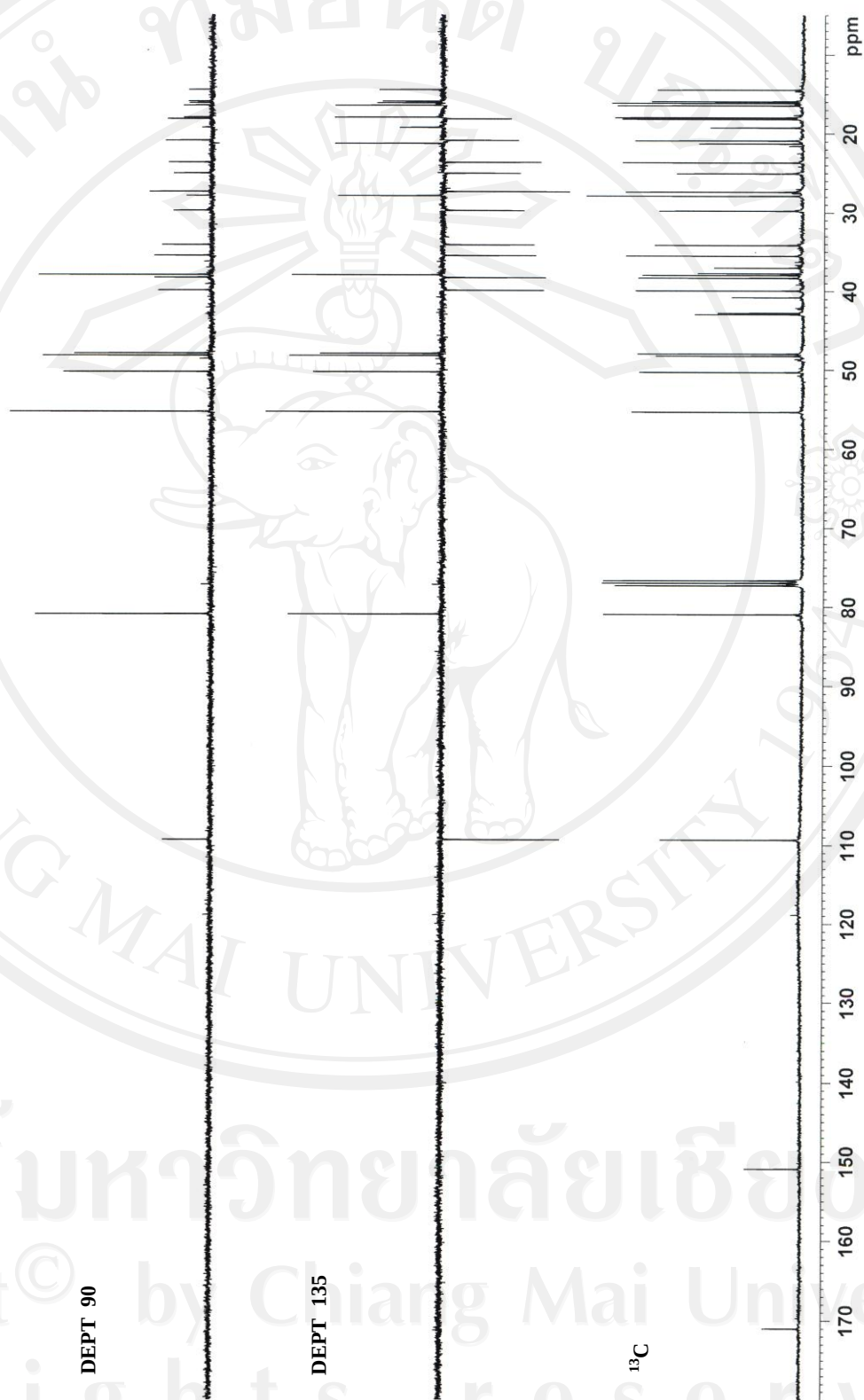


Figure 98 Comparison between DEPT 90, DEPT 135, and ^{13}C of lupeol acetate (59)

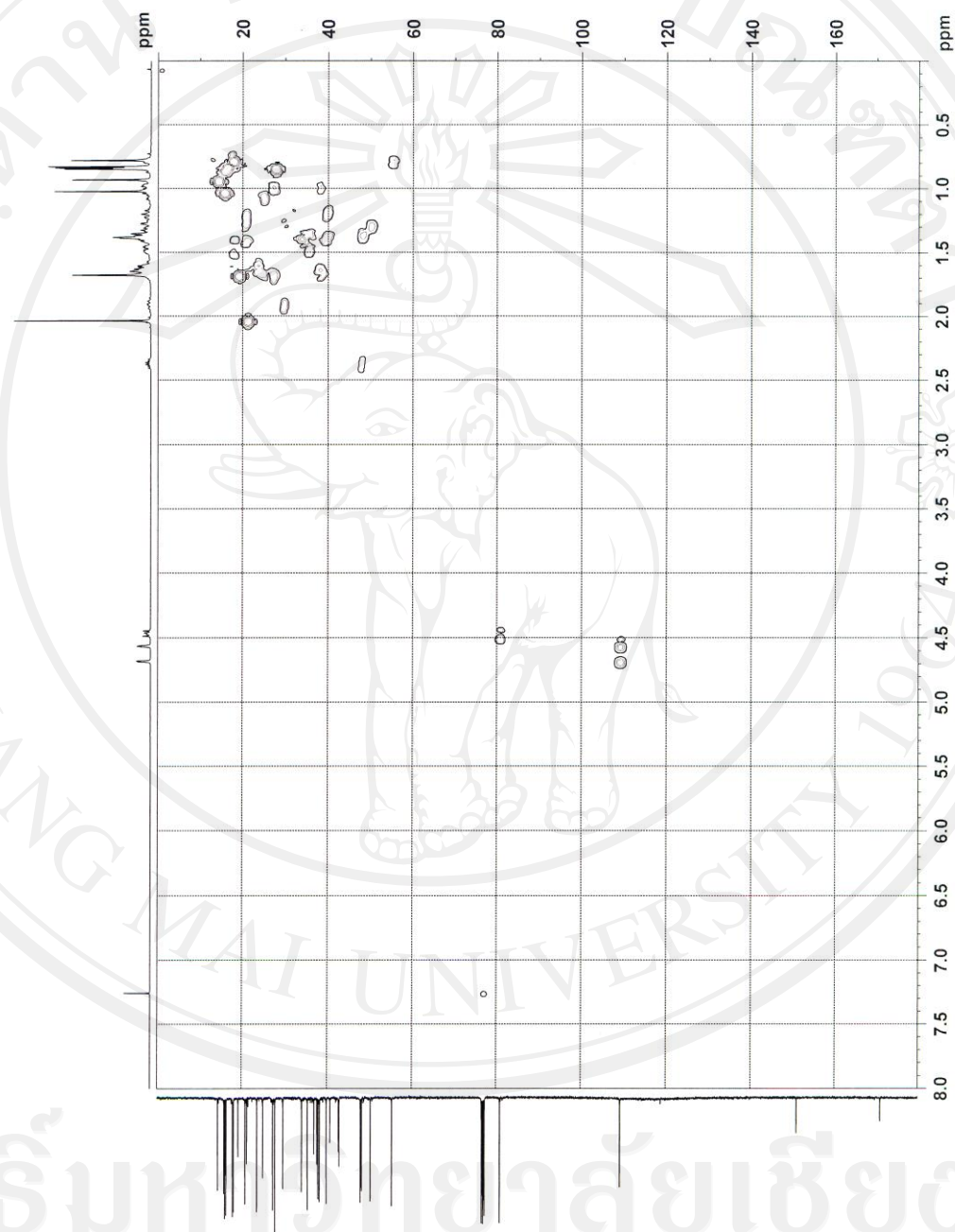


Figure 99 HMQC of lupeol acetate (59)

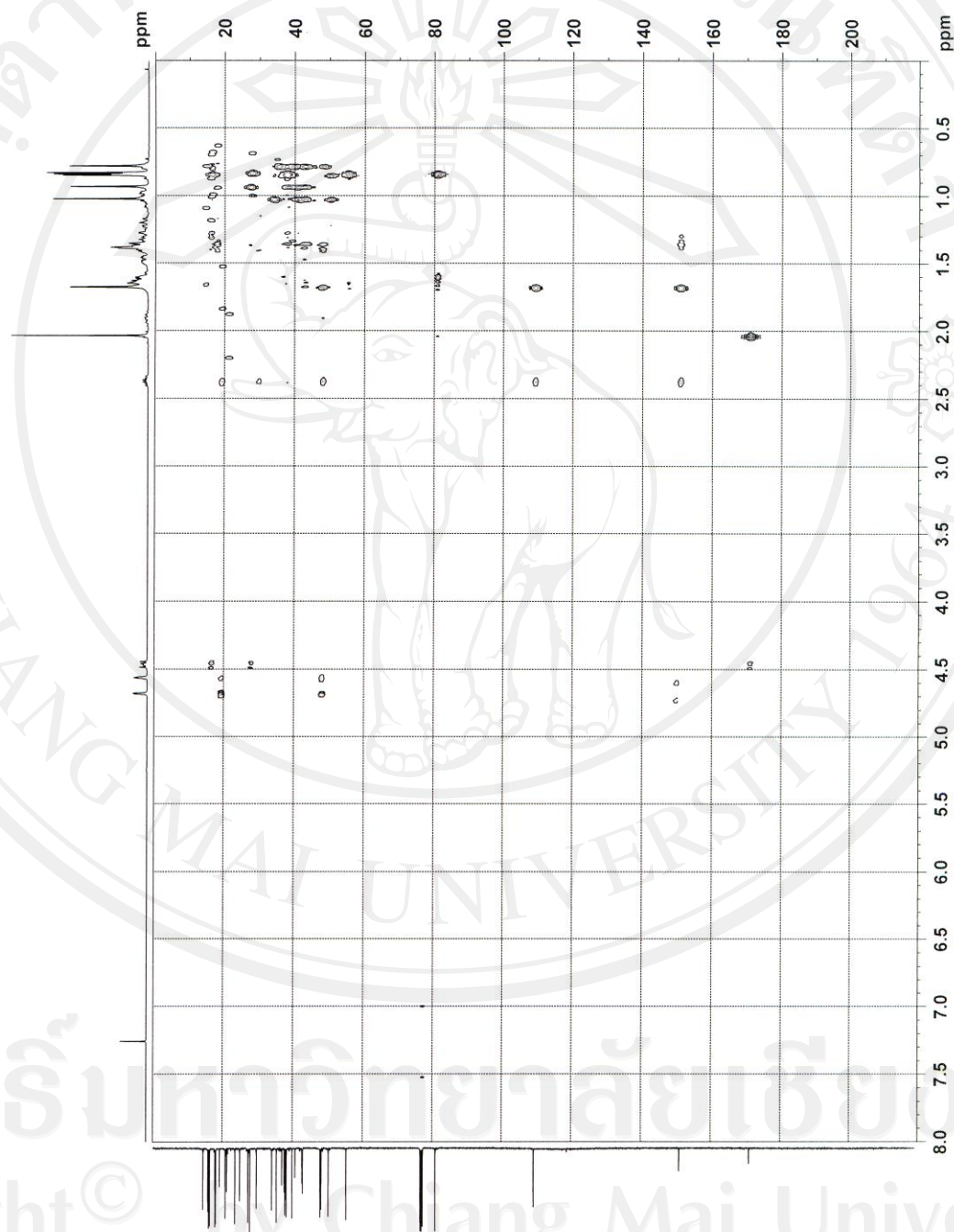


Figure 100 HMBC of lupeol acetate (59)

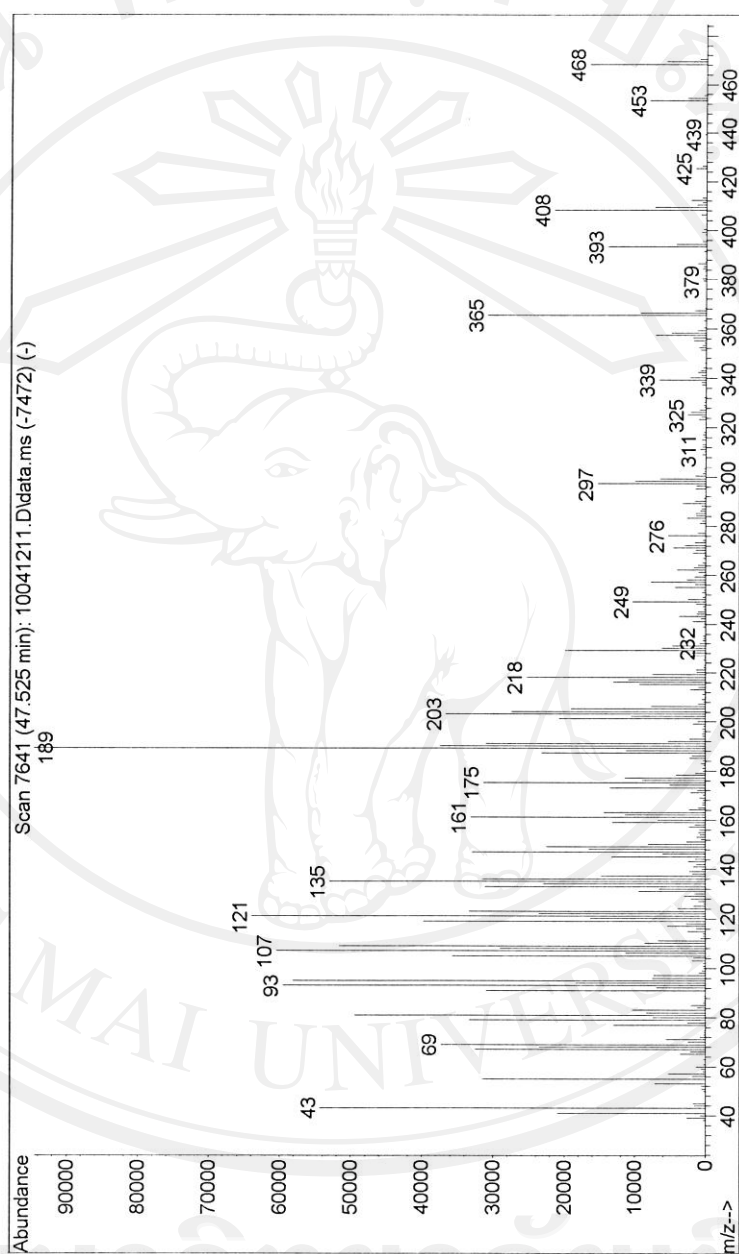


Figure 101 Mass spectrum of lupeol acetate (59)



Transmittance [%]

4000

3500

3000

2500

2000

1500

1000

500

Wavenumber cm⁻¹

2940.20
2856.58

1796.43
1734.31
1643.21

1456.89
1374.43

1244.80
1153.23
1107.71
1027.40
974.56
884.92
789.58
662.13
600.51
547.80

Figure 102 IR spectrum of lupeol acetate (59)

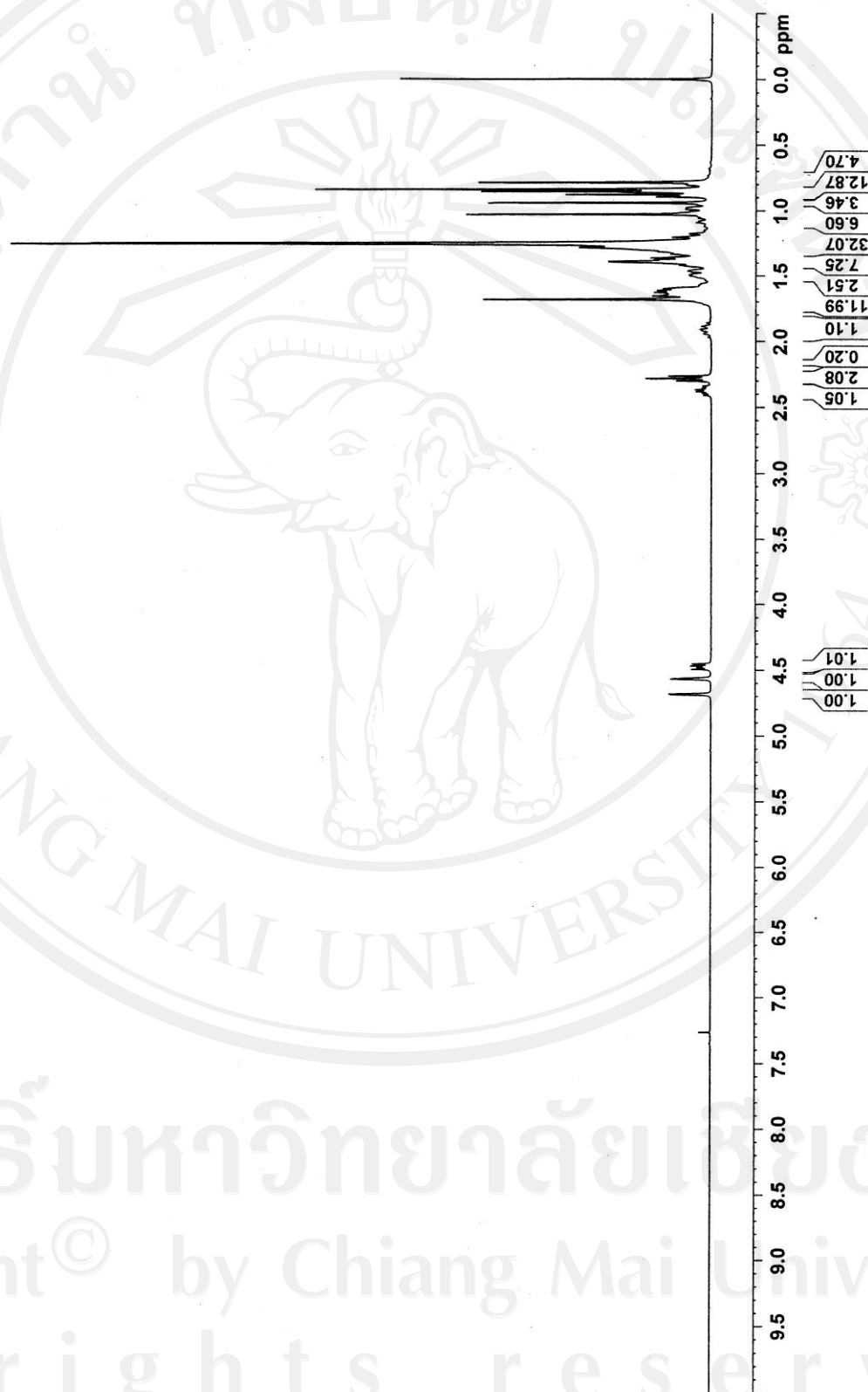


Figure 103 ^1H NMR spectrum of lupeol palmitate (60)

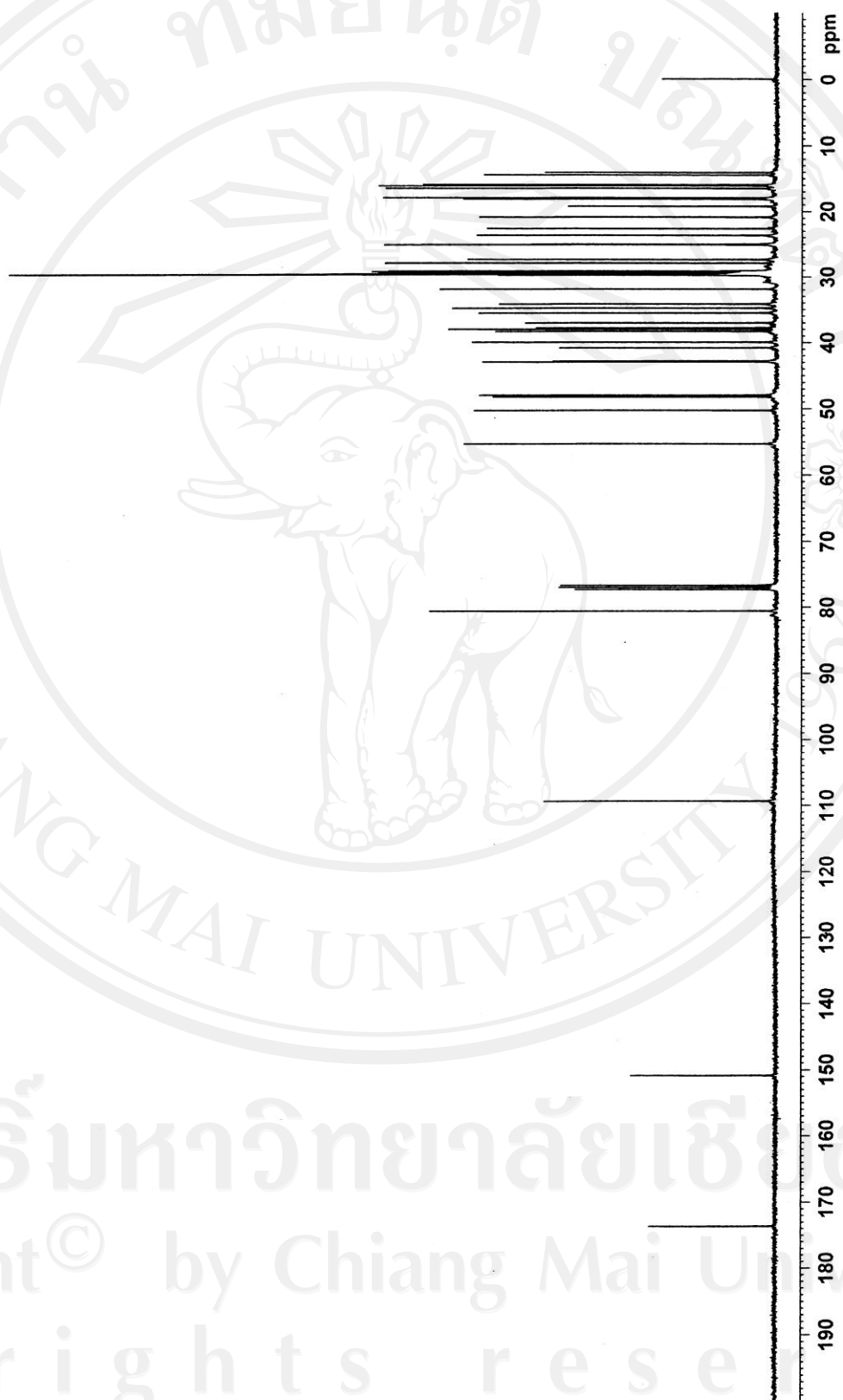


Figure 104 ^{13}C NMR spectrum of lupeol palmitate (60)

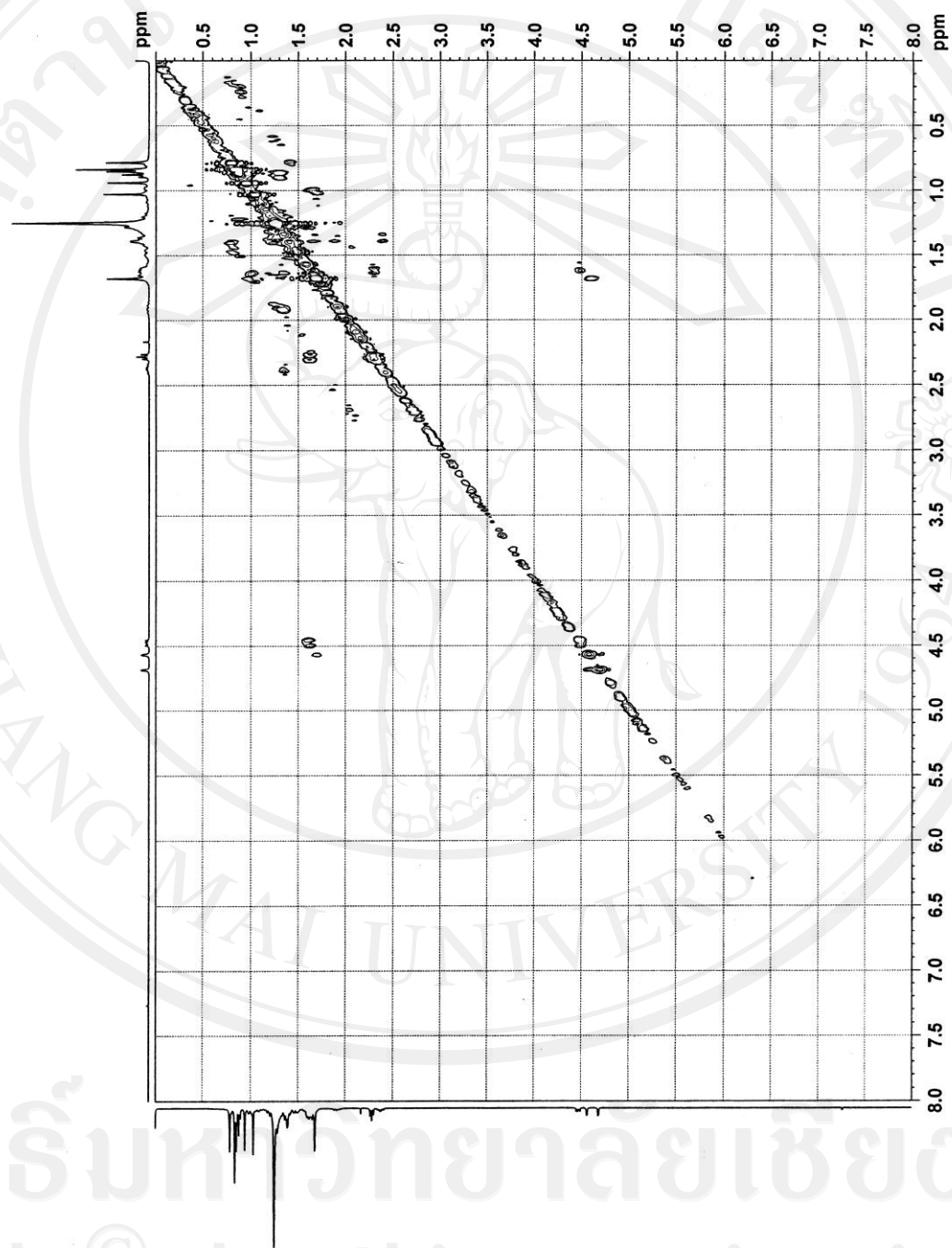


Figure 105 COSY of lupeol palmitate (60)

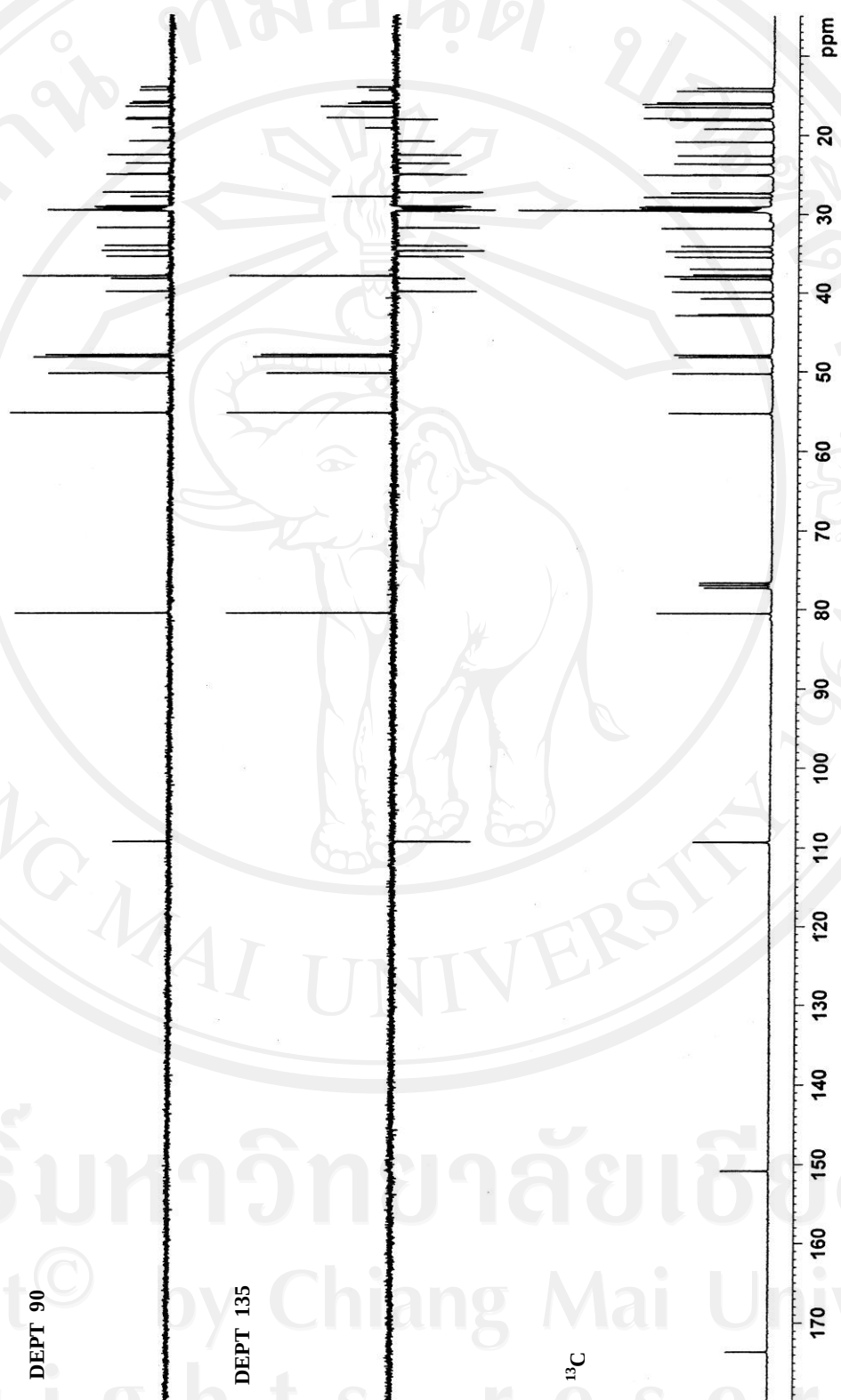


Figure 106 Comparison between DEPT 90, DEPT 135, and ^{13}C of lupeol palmitate (60)

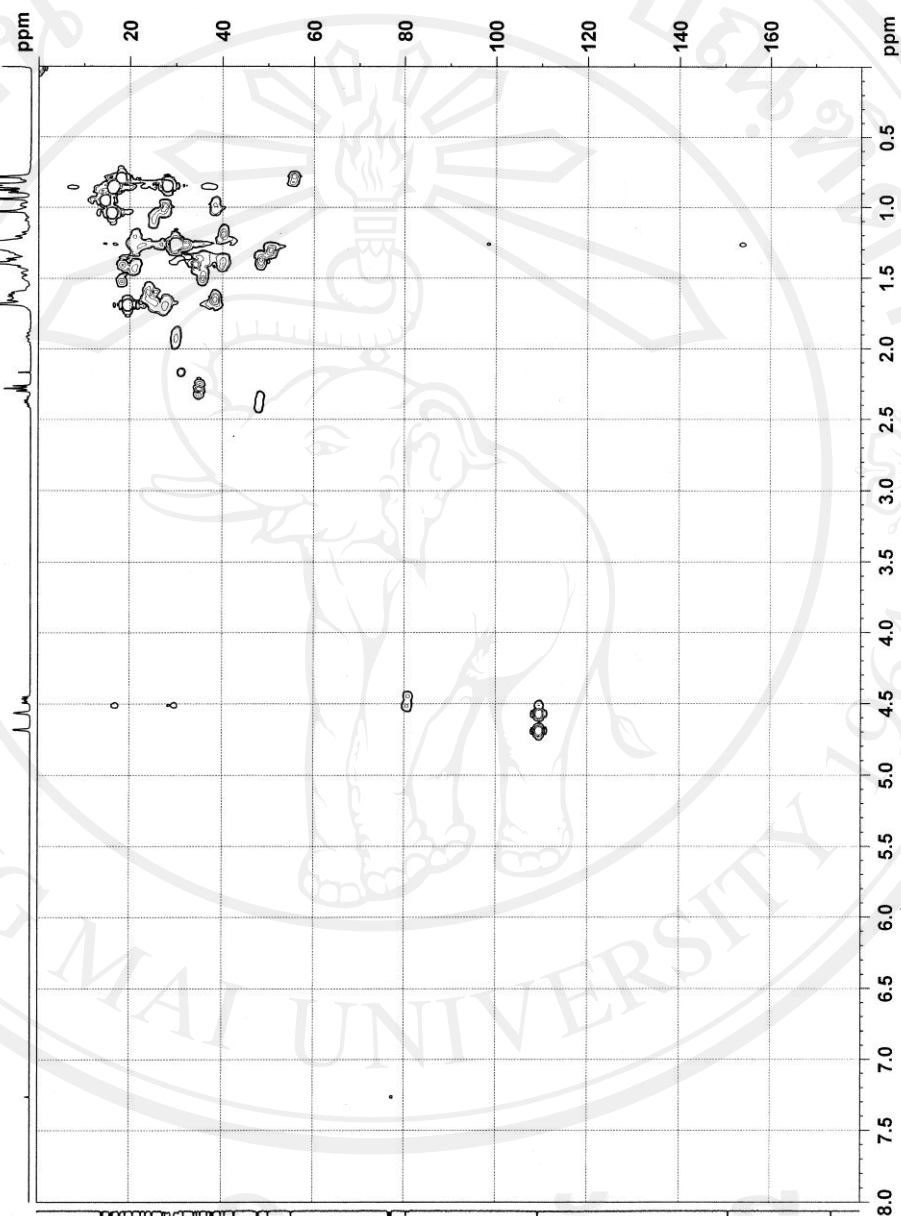


Figure 107 HMQC of lupeol palmitate (60)

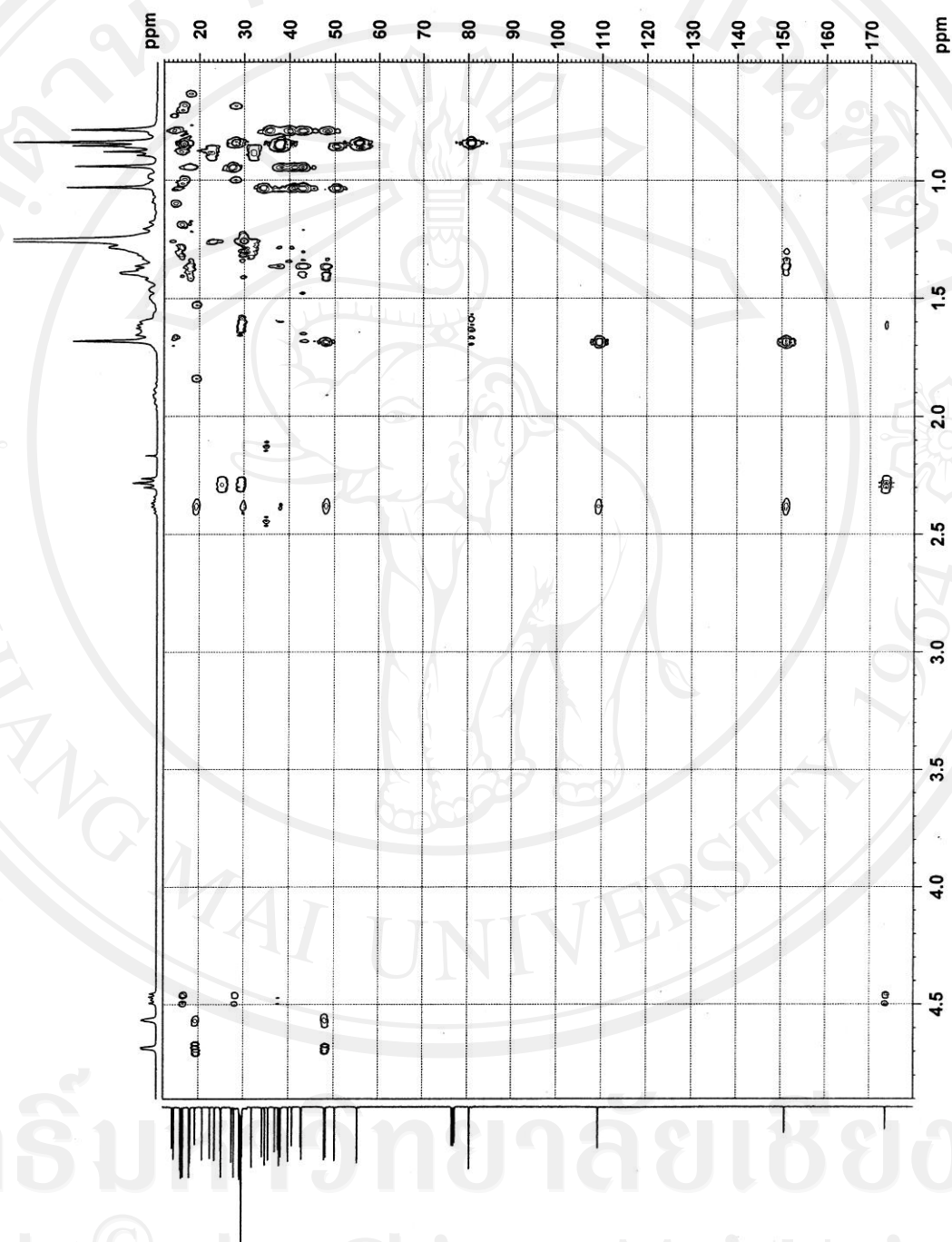


Figure 108 HMBC of lupeol palmitate (60)

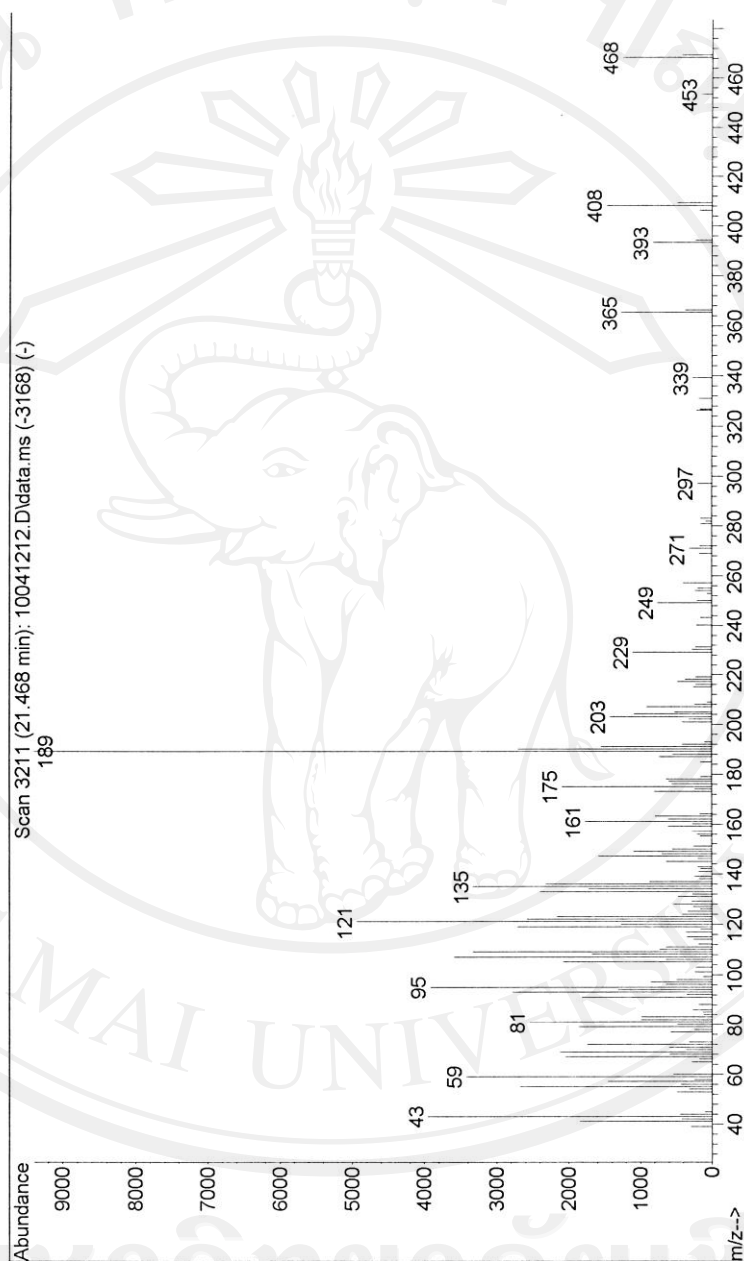


Figure 109 Mass spectrum of lupeol palmitate (60)



Transmittance [%]

4000

3500

3000

2500

2000

1500

1000

500

Wavenumber cm⁻¹

2925.85
2852.31

1727.57

1639.14

1529.13

1457.91

1376.96

1254.18

1177.91

1105.60

1024.30

973.37

878.25

647.54

533.13

457.63

Figure 110 IR spectrum of lupeol palmitate (60)

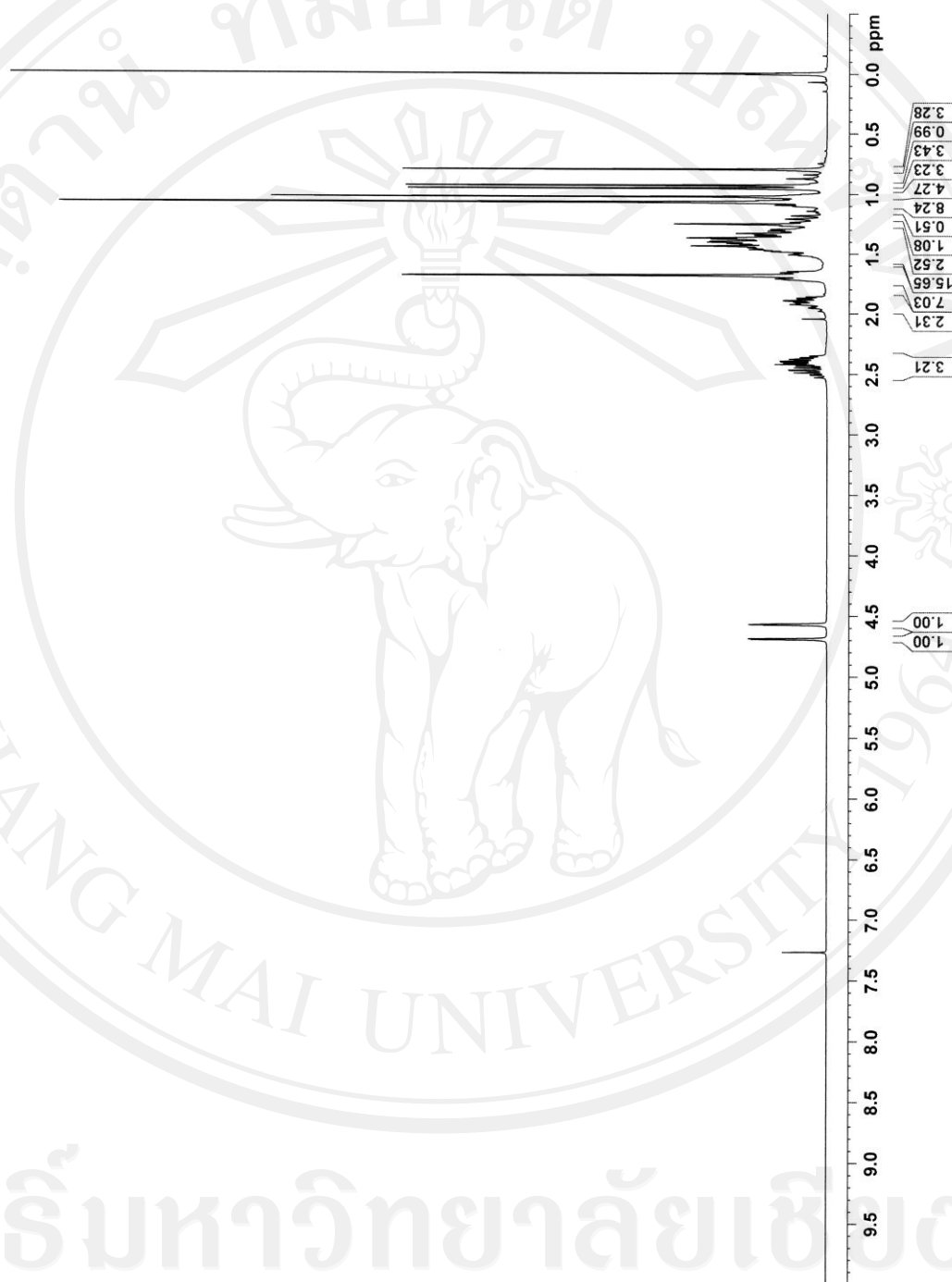


Figure 111 ^1H NMR spectrum of lupenone (61)

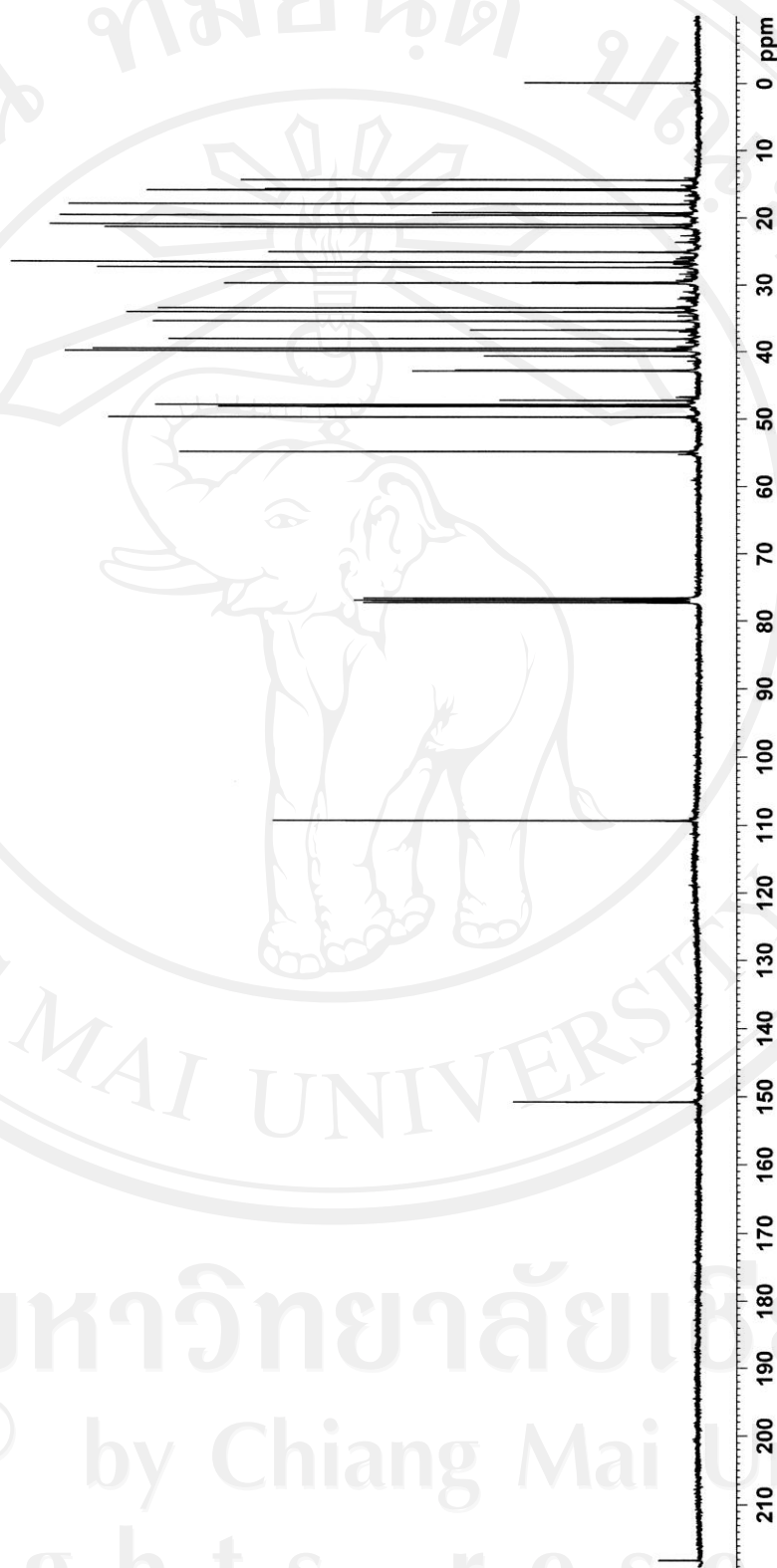


Figure 112 ^{13}C NMR spectrum of lupenone (61)

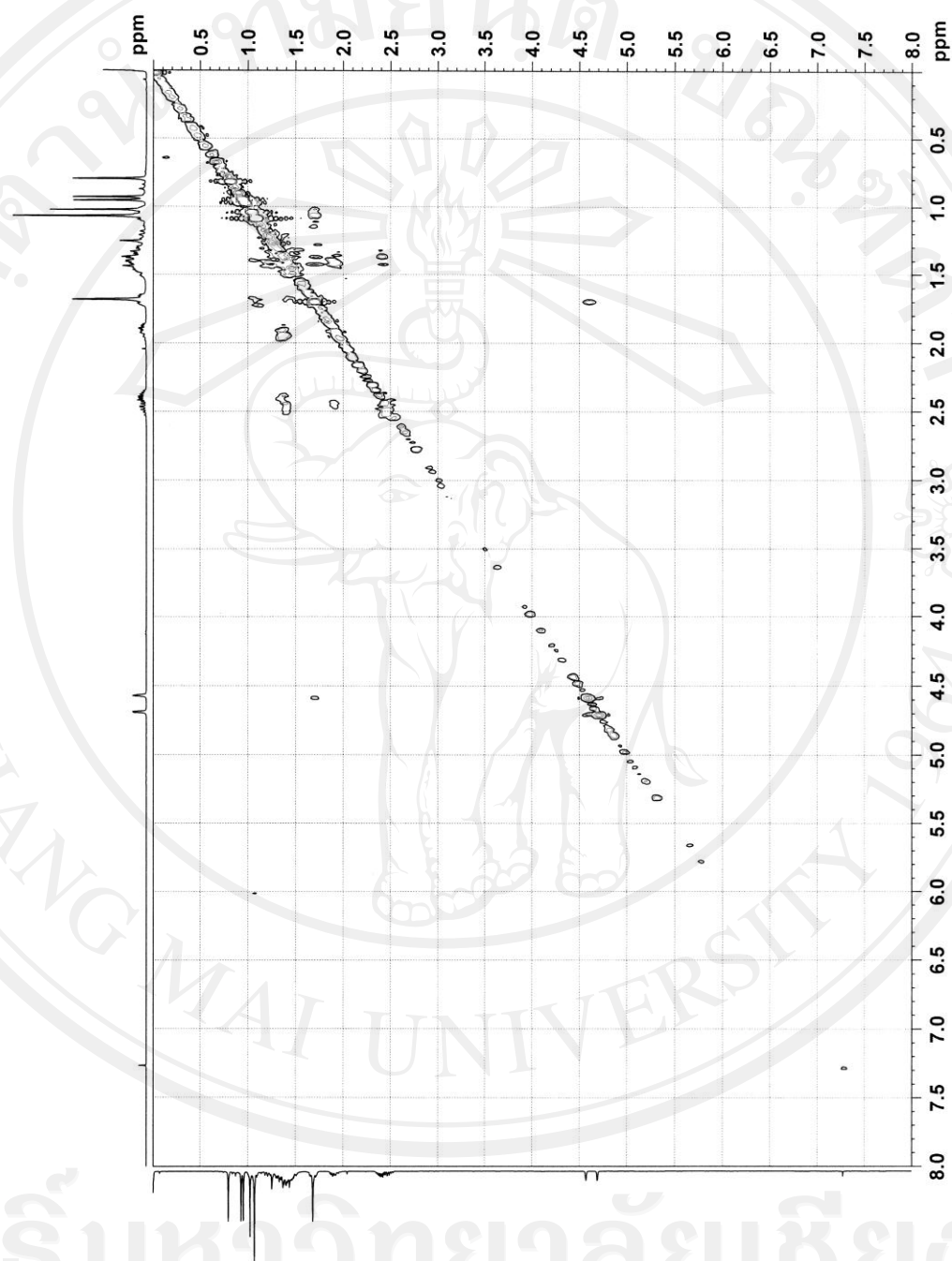


Figure 113 COSY of lupenone (61)

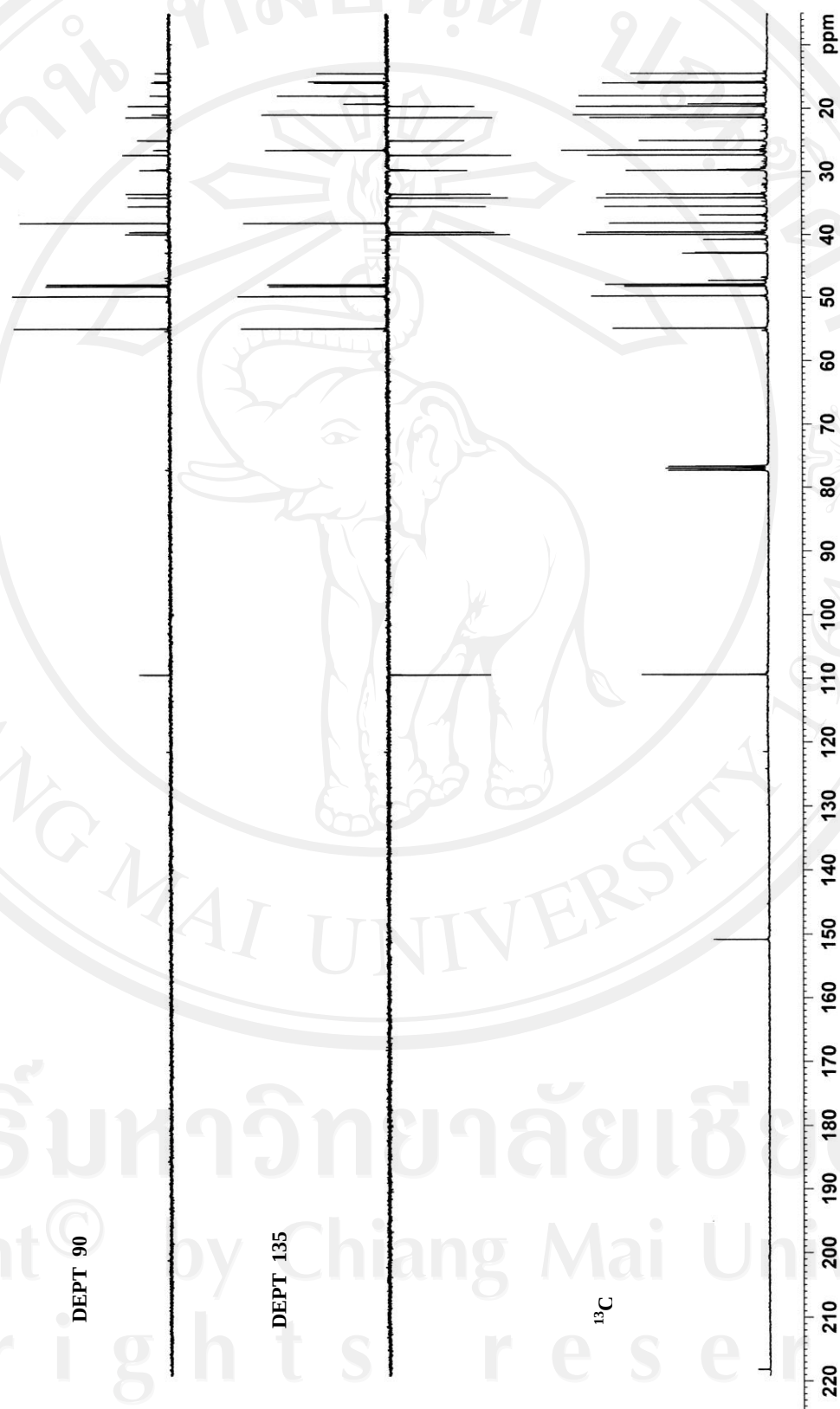


Figure 114 Comparison between DEPT 90, DEPT 135, and ^{13}C of lupenone (61)

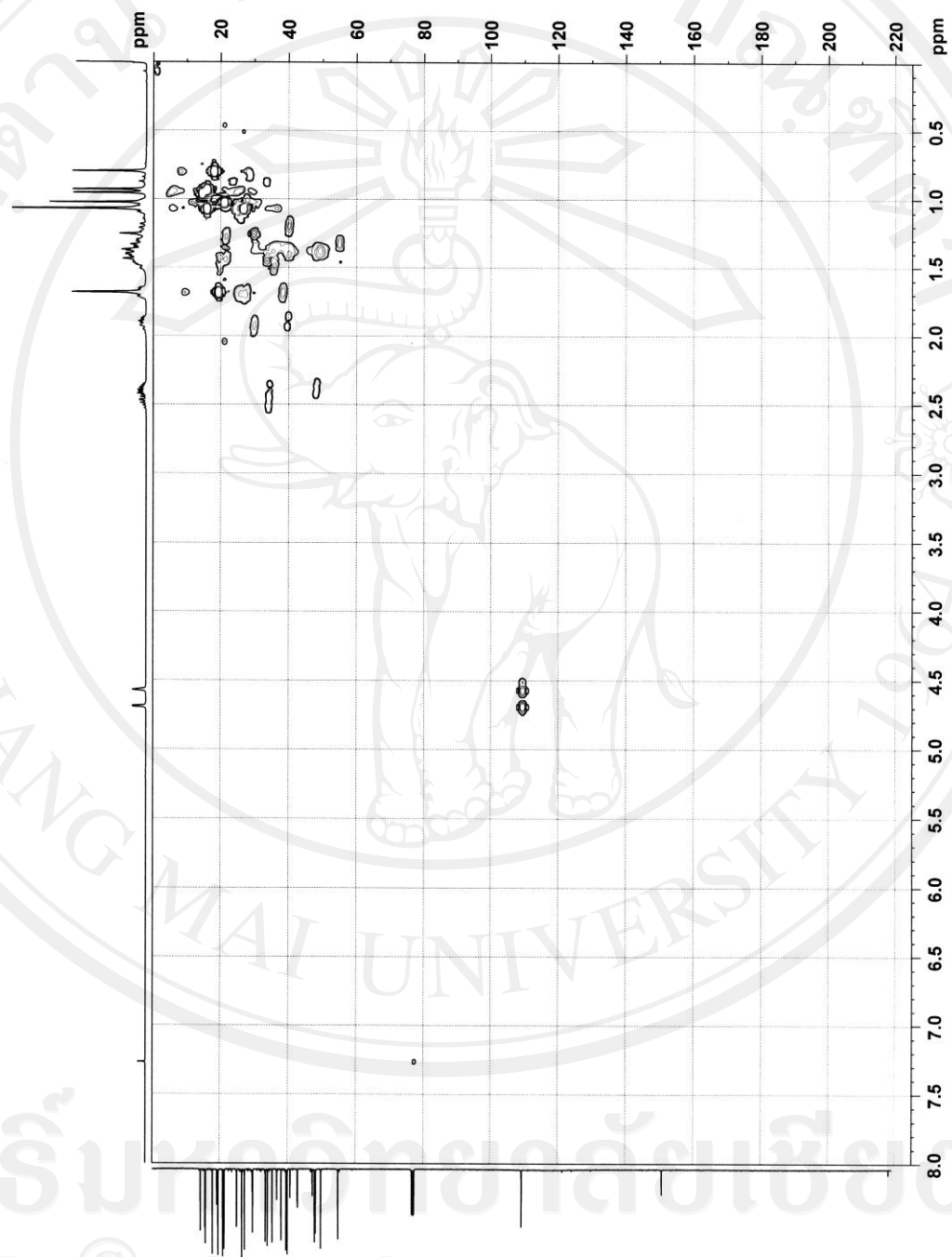


Figure 115 HMQC of lupenone (61)

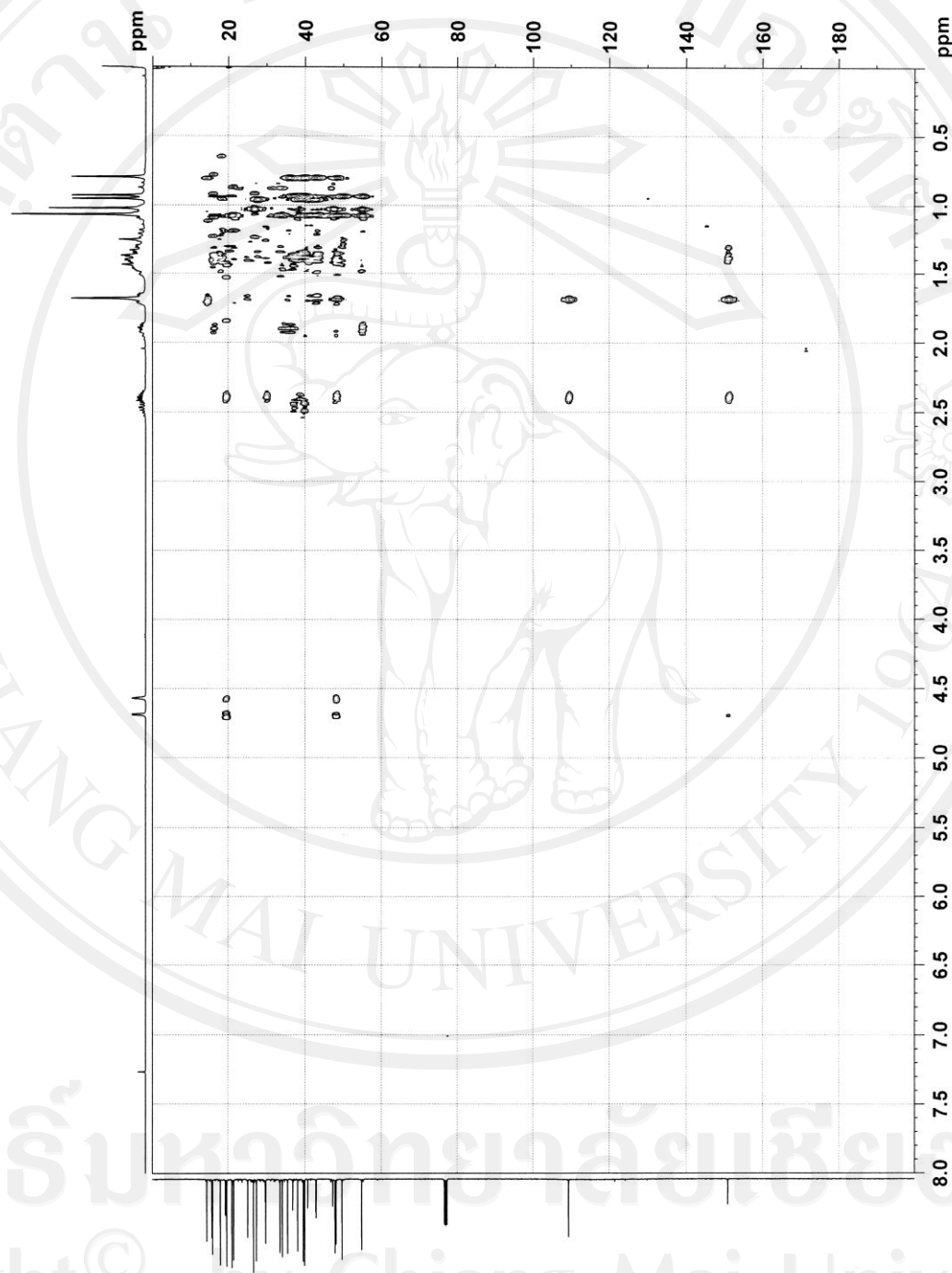


Figure 116 HMBC of lupenone (61)

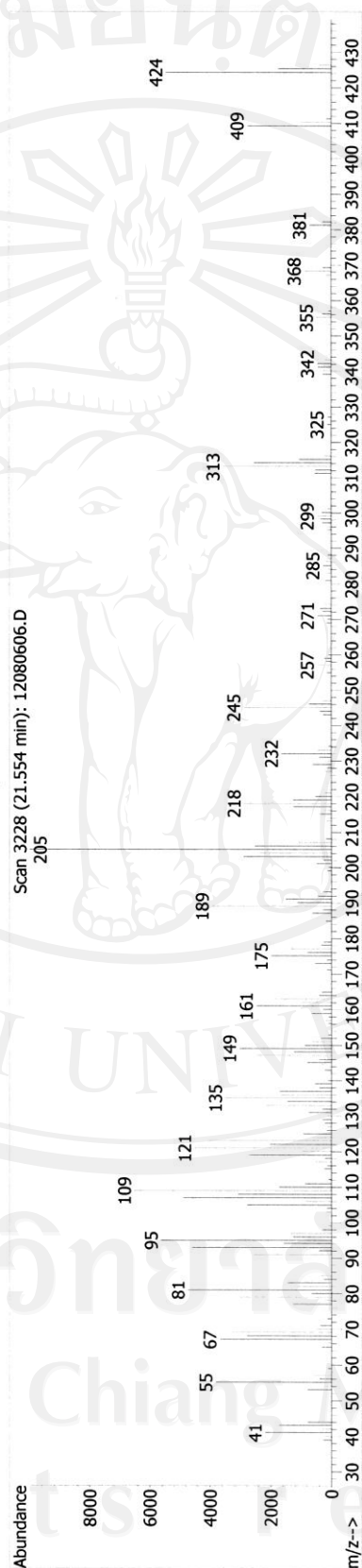


Figure 117 Mass spectrum of lupenone (61)

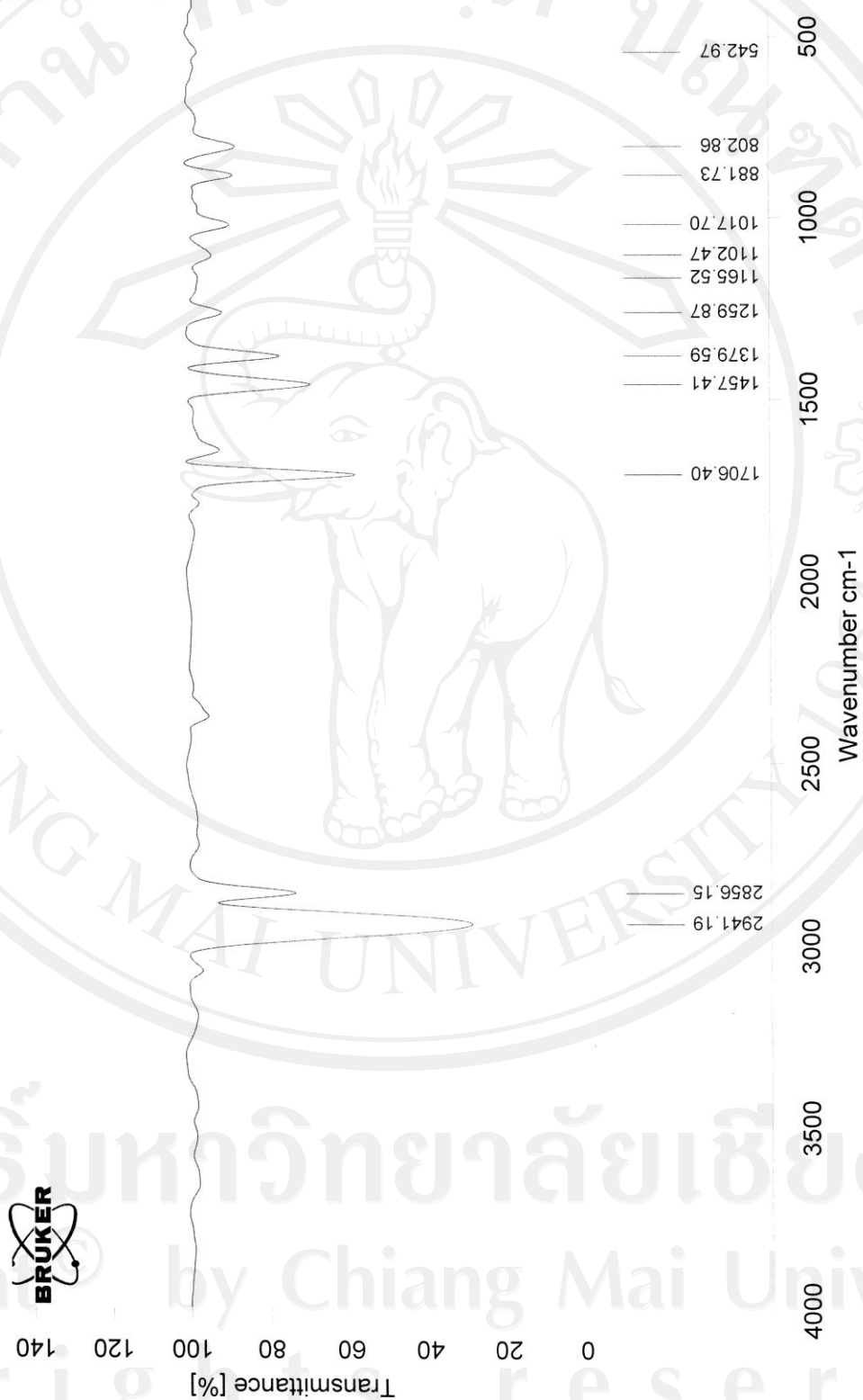


Figure 118 IR spectrum of lupenone (61)

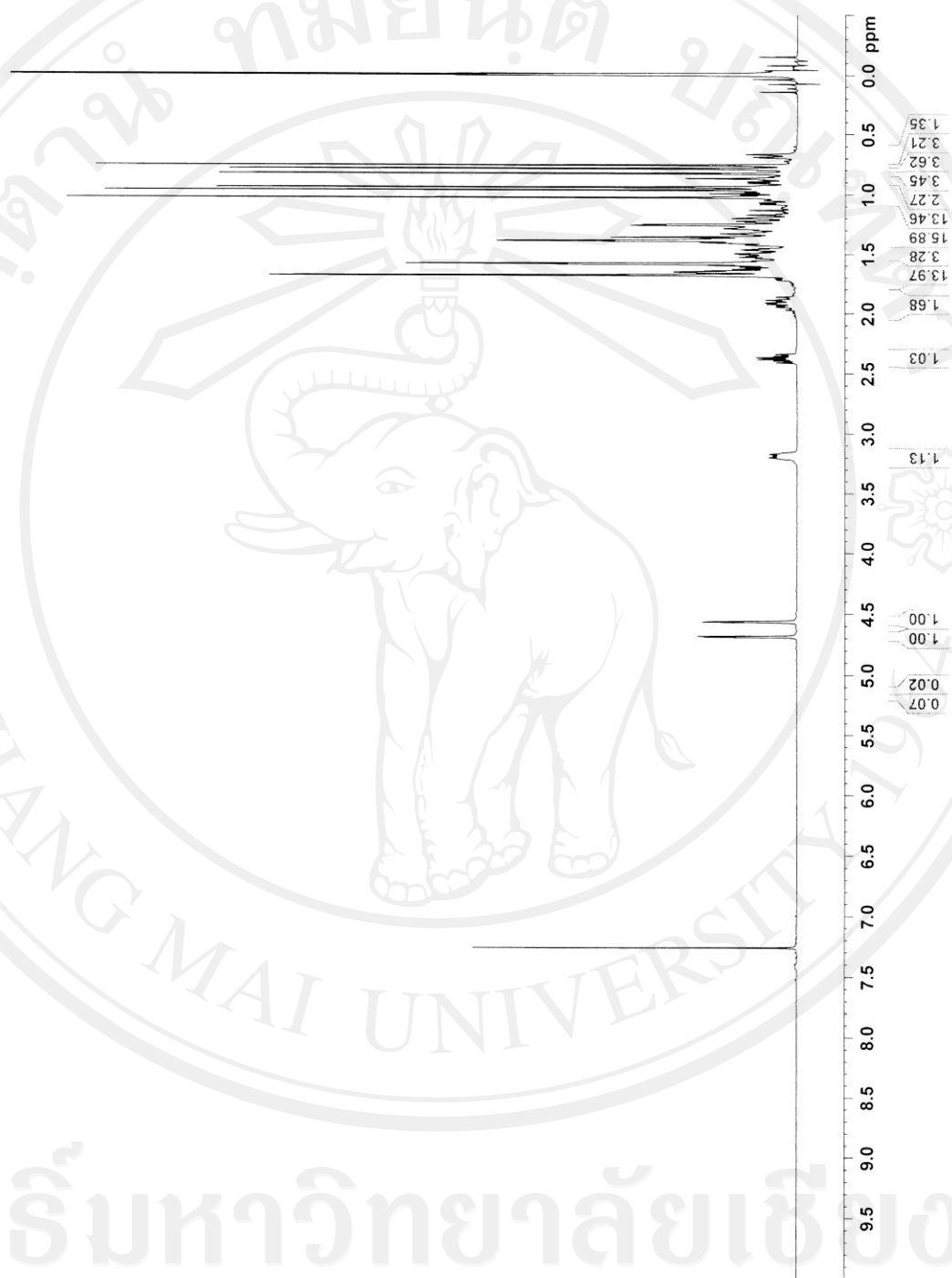


Figure 119 ^1H NMR spectrum of lupeol (41)

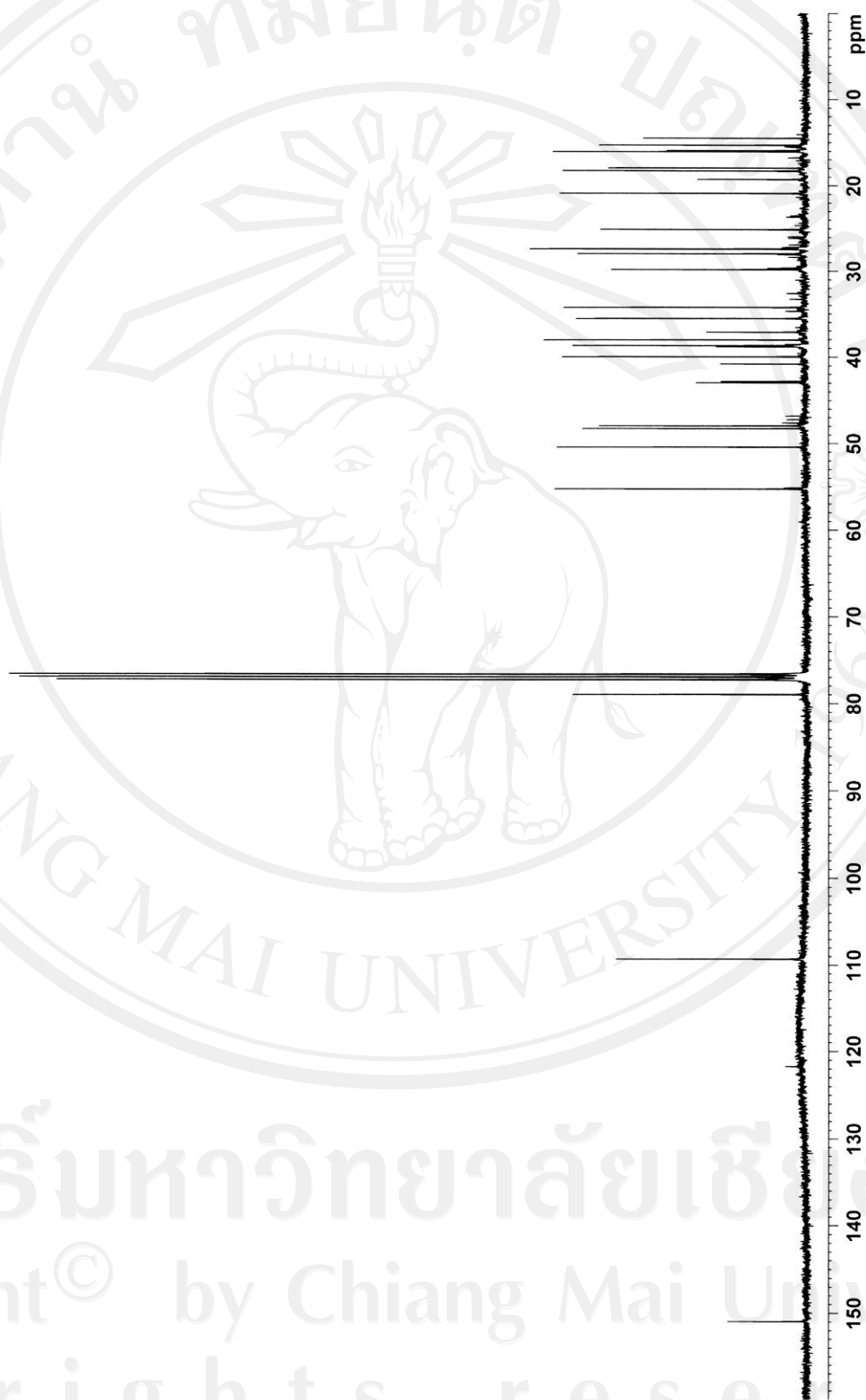


Figure 120 ^{13}C NMR spectrum of lupeol (41)

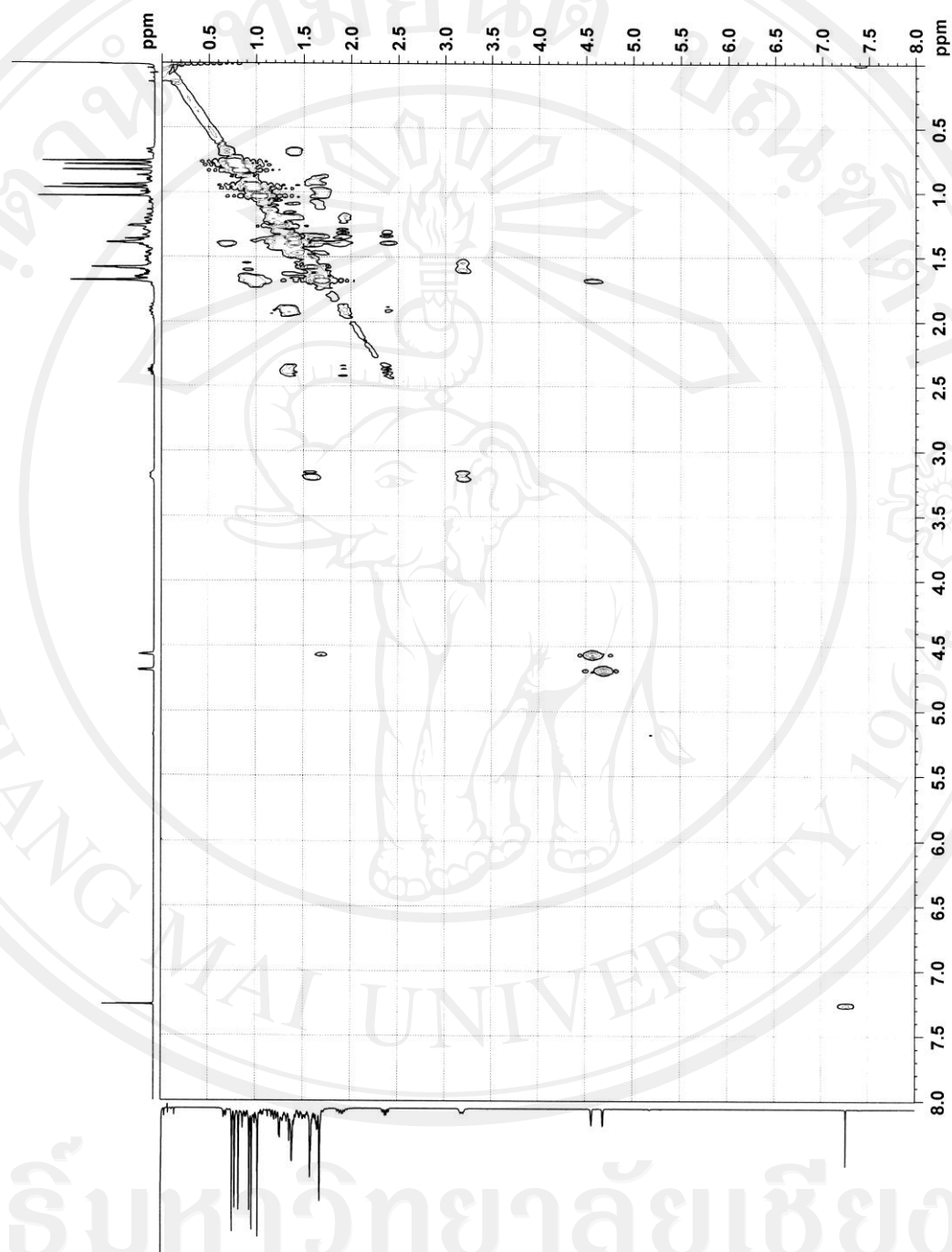


Figure 121 COSY of lupeol (41)

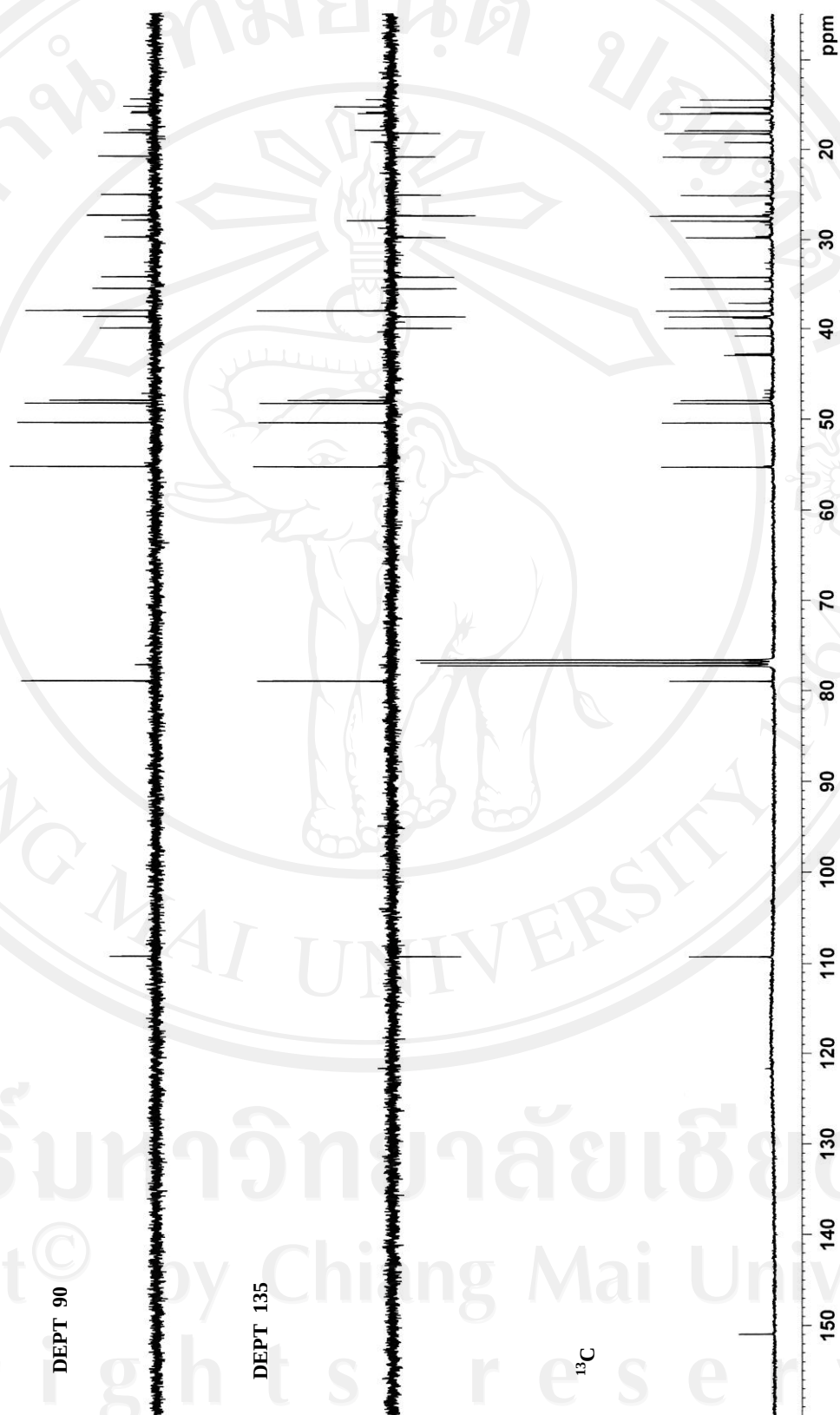


Figure 122 Comparison between DEPT 90, DEPT 135, and ^{13}C of lupeol (41)

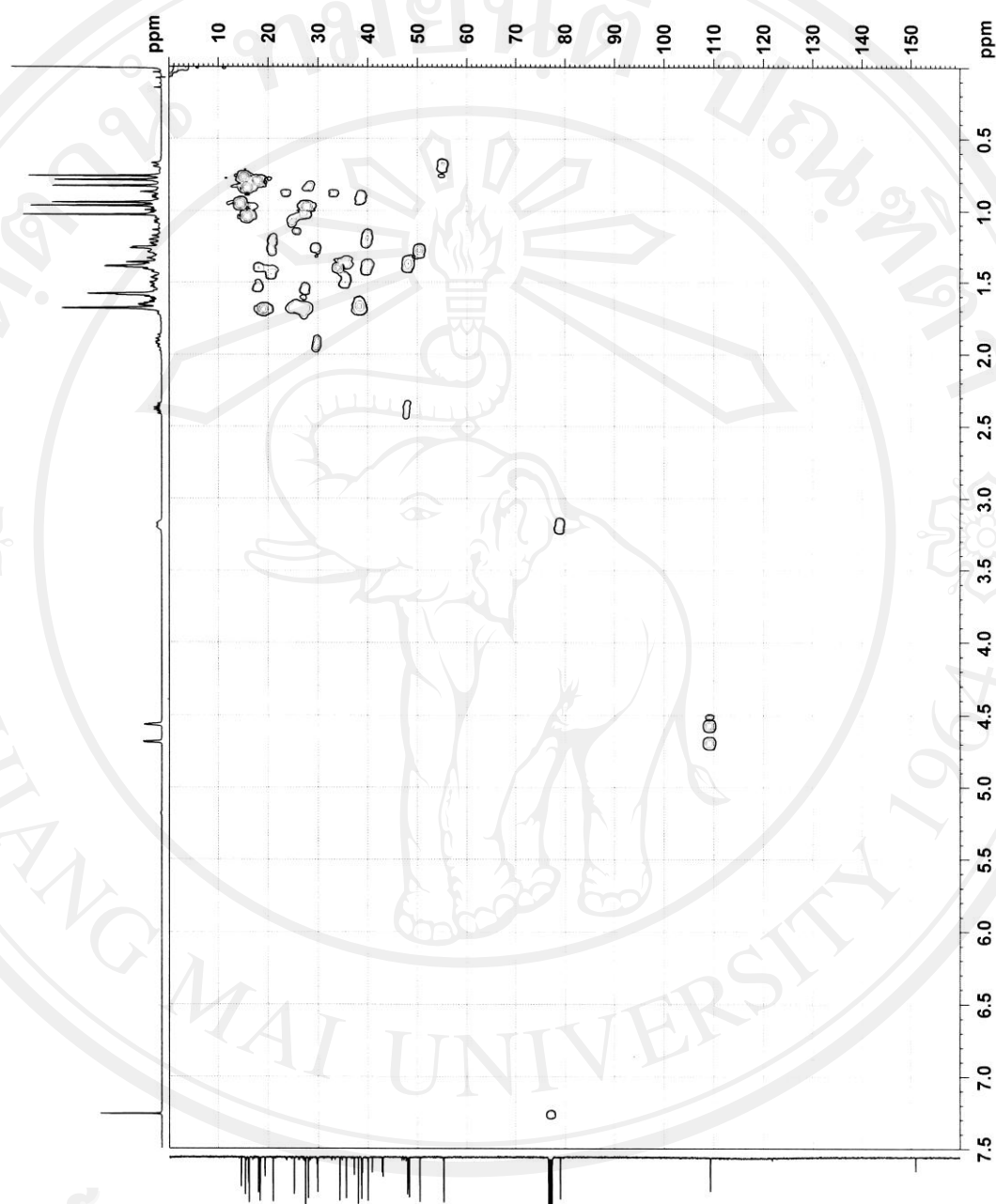


Figure 123 HMQC of lupeol (41)

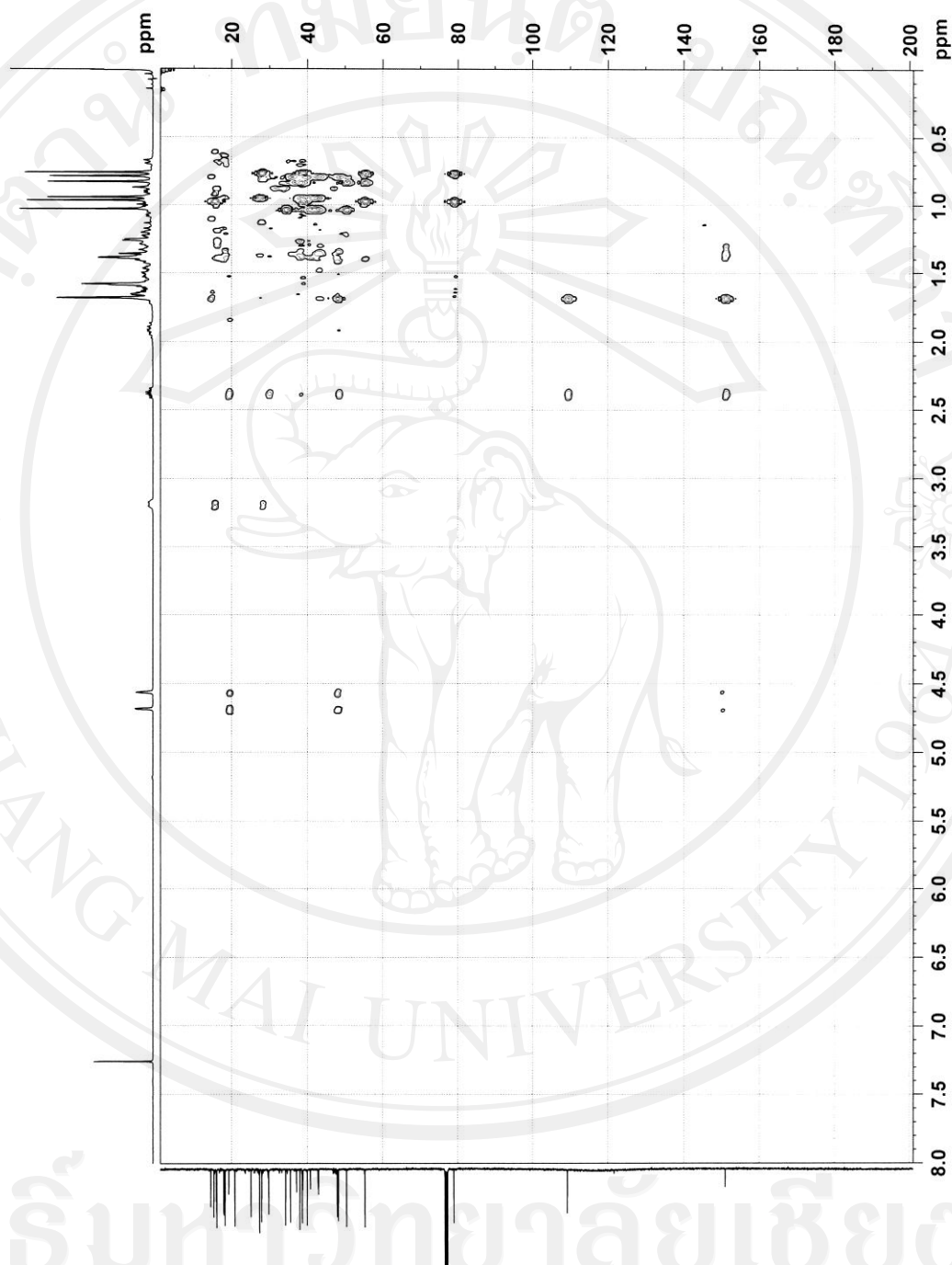


Figure 124 HMBC of lupeol (41)

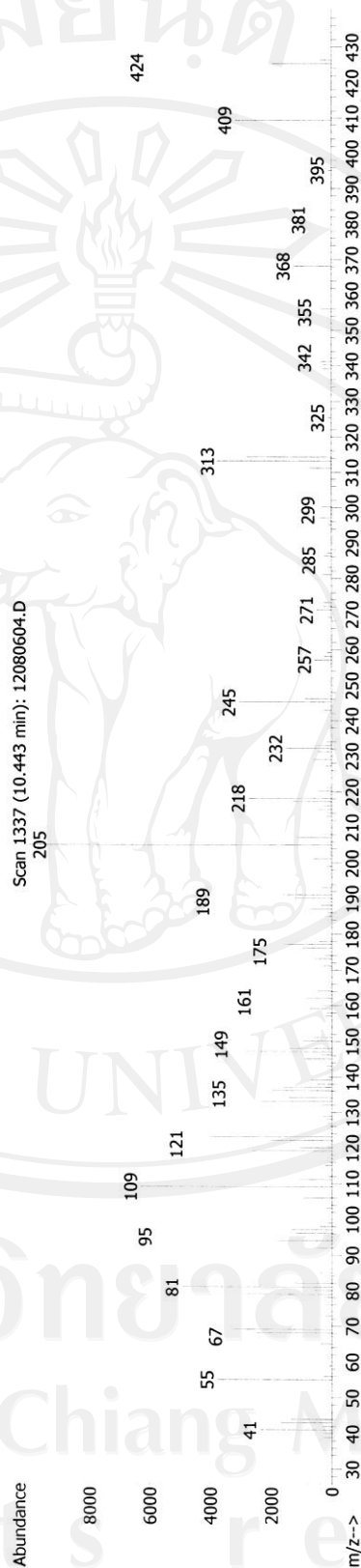


Figure 125 Mass spectrum of lupeol (41)

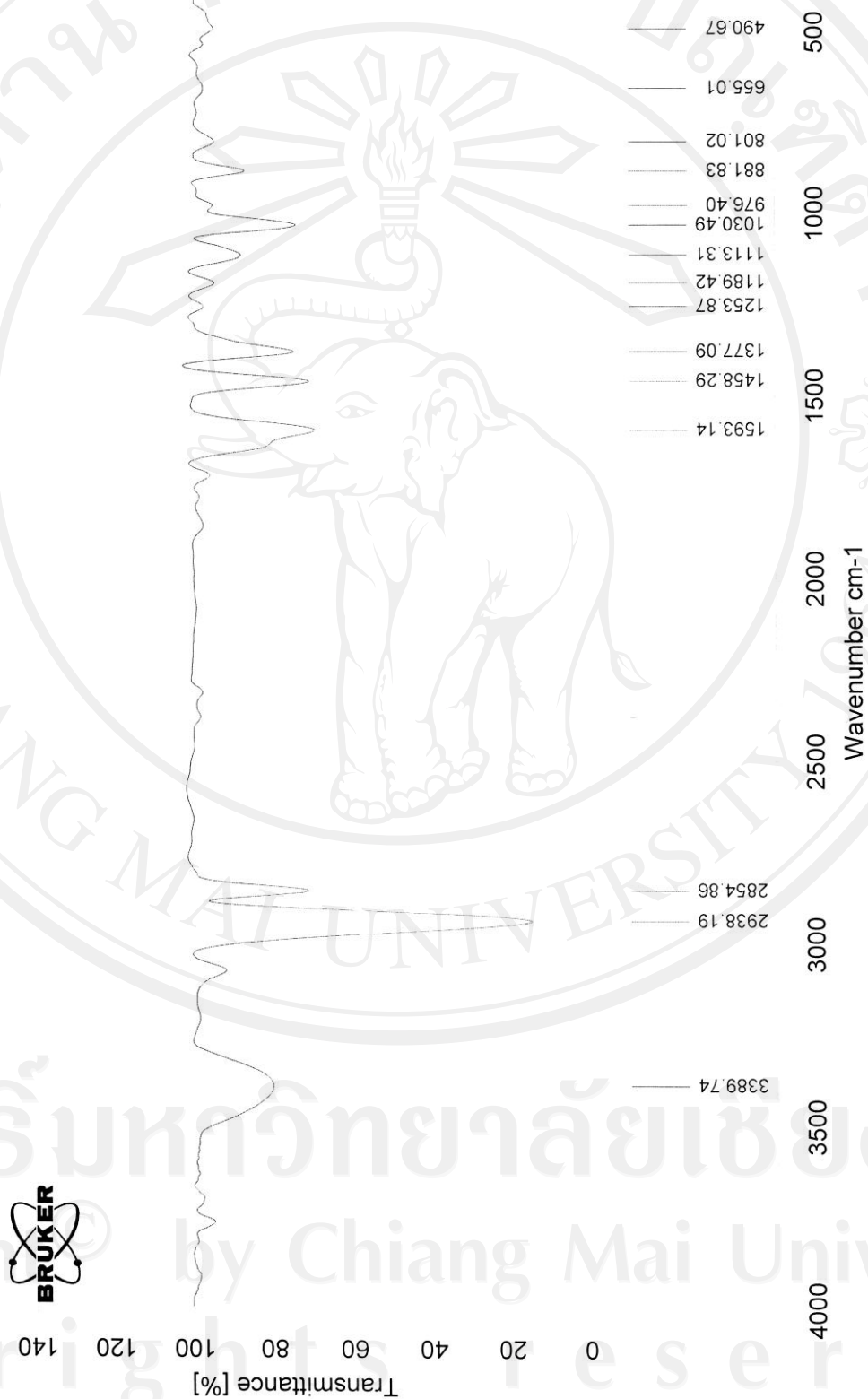


Figure 126 IR spectrum of lupeol (41)

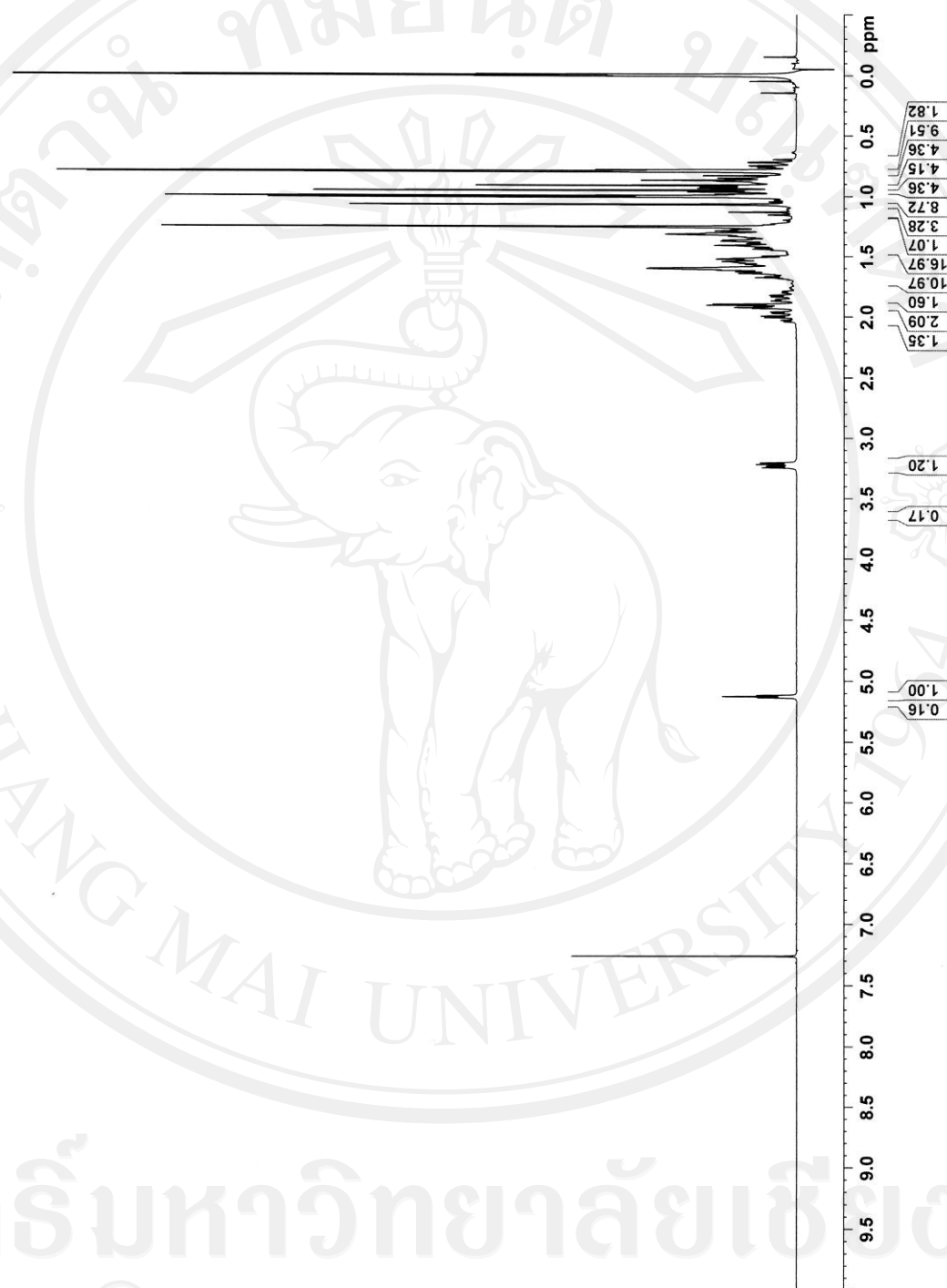


Figure 127 ^1H NMR spectrum of α -amyrin (13)

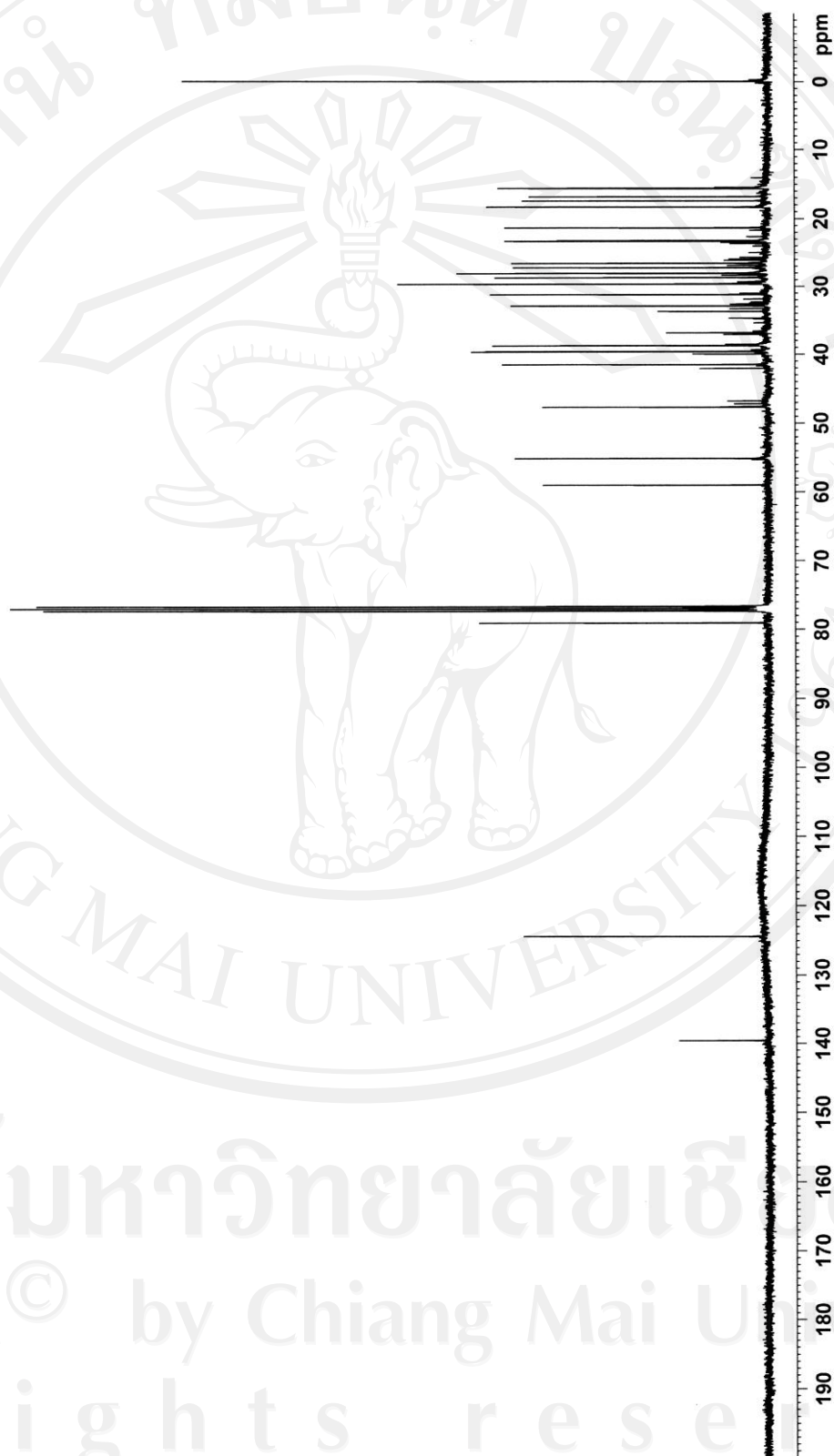


Figure 128 ^{13}C NMR spectrum of α -amyrin (13)

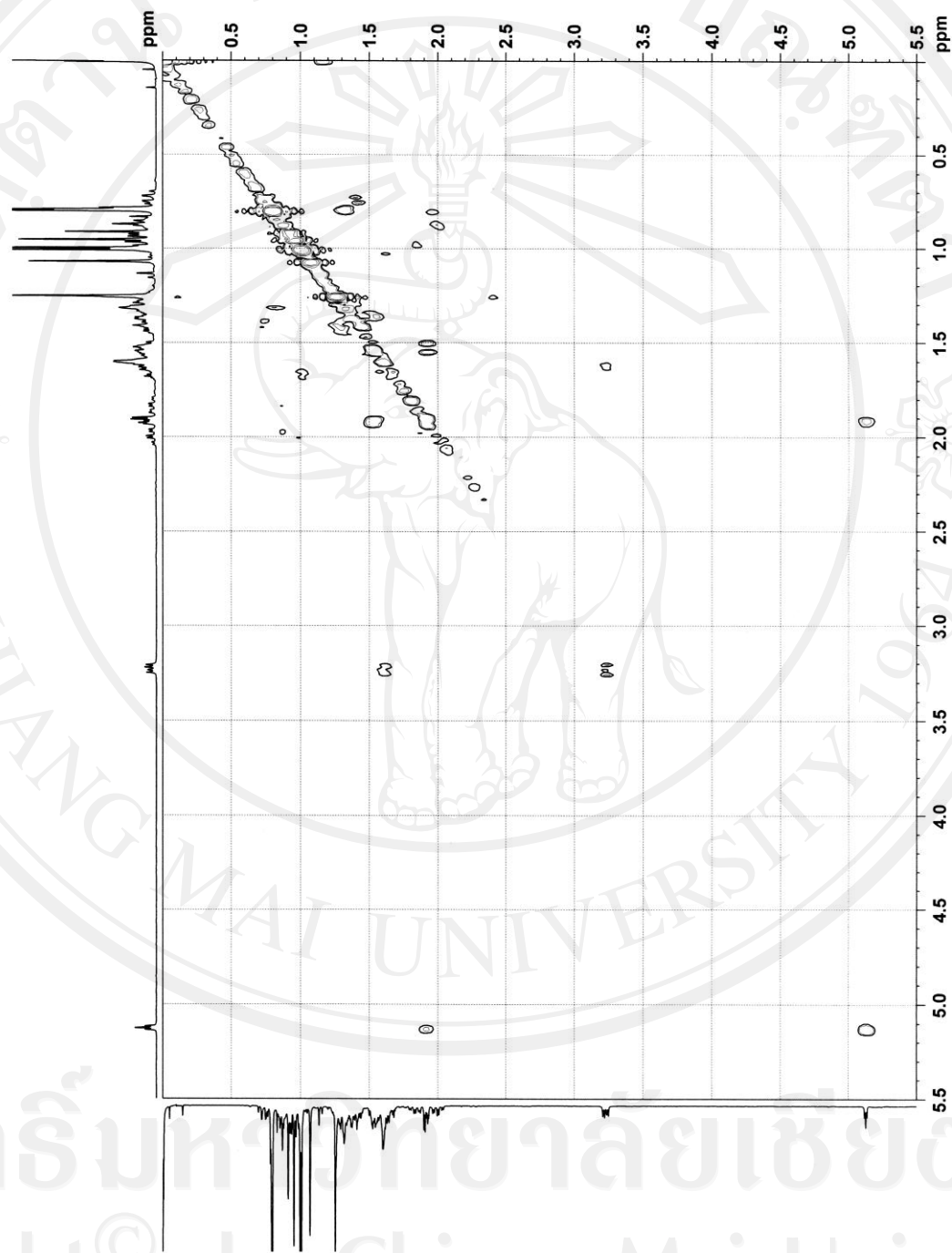


Figure 129 COSY of α -amyryn (13)

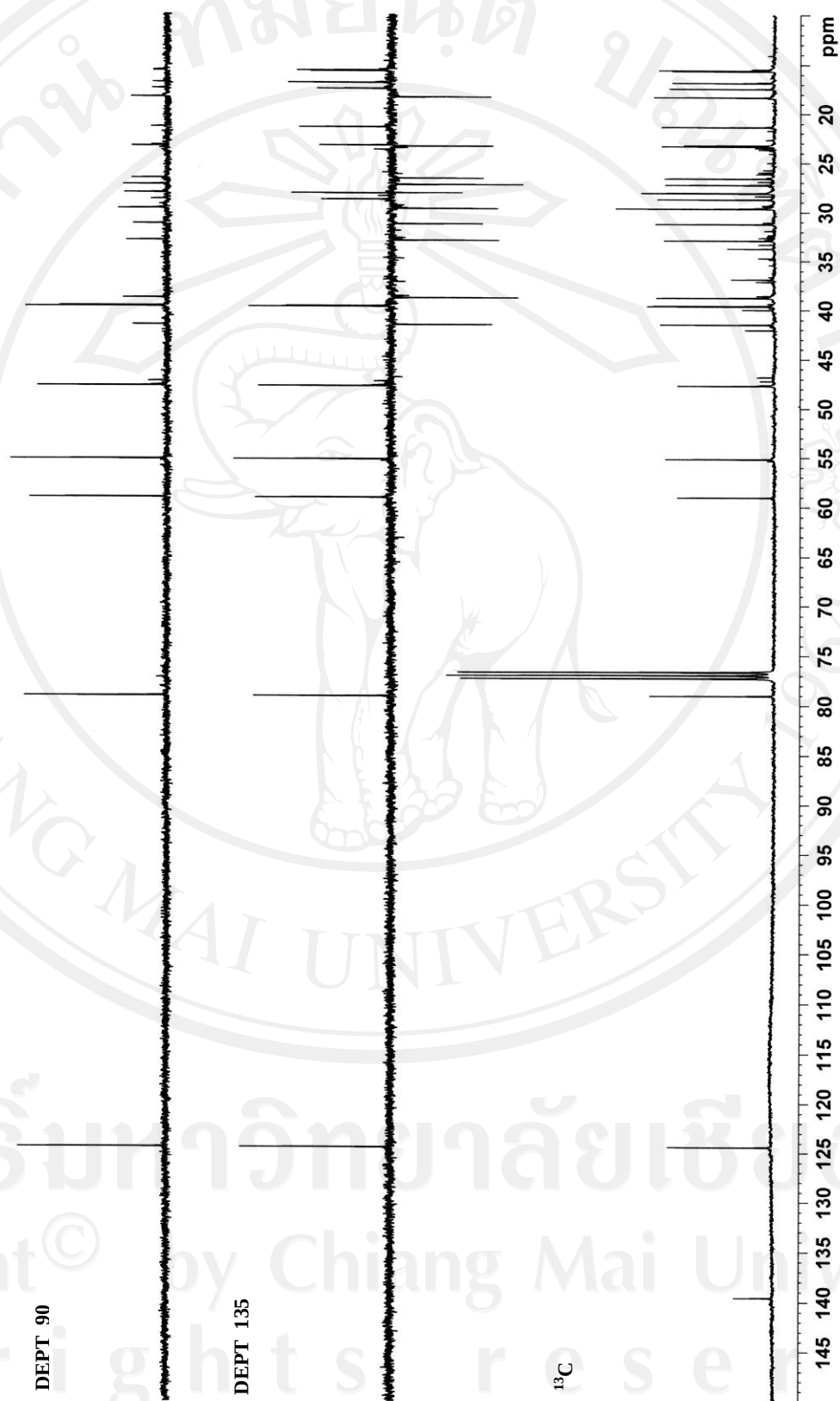


Figure 130 Comparison between DEPT 90, DEPT 135, and ^{13}C of α -amyrin (13)

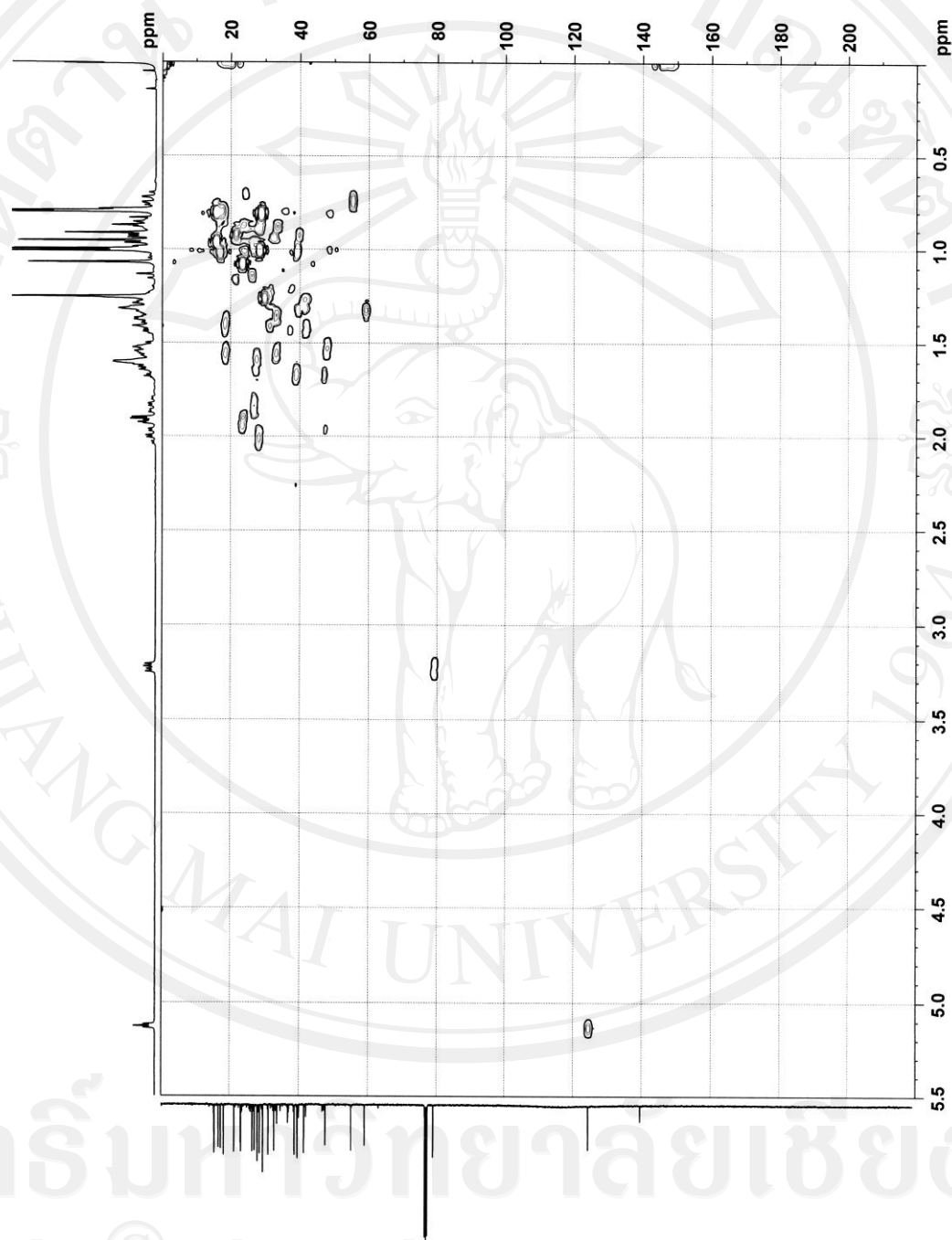


Figure 131 HMQC of α -amyrin (13)

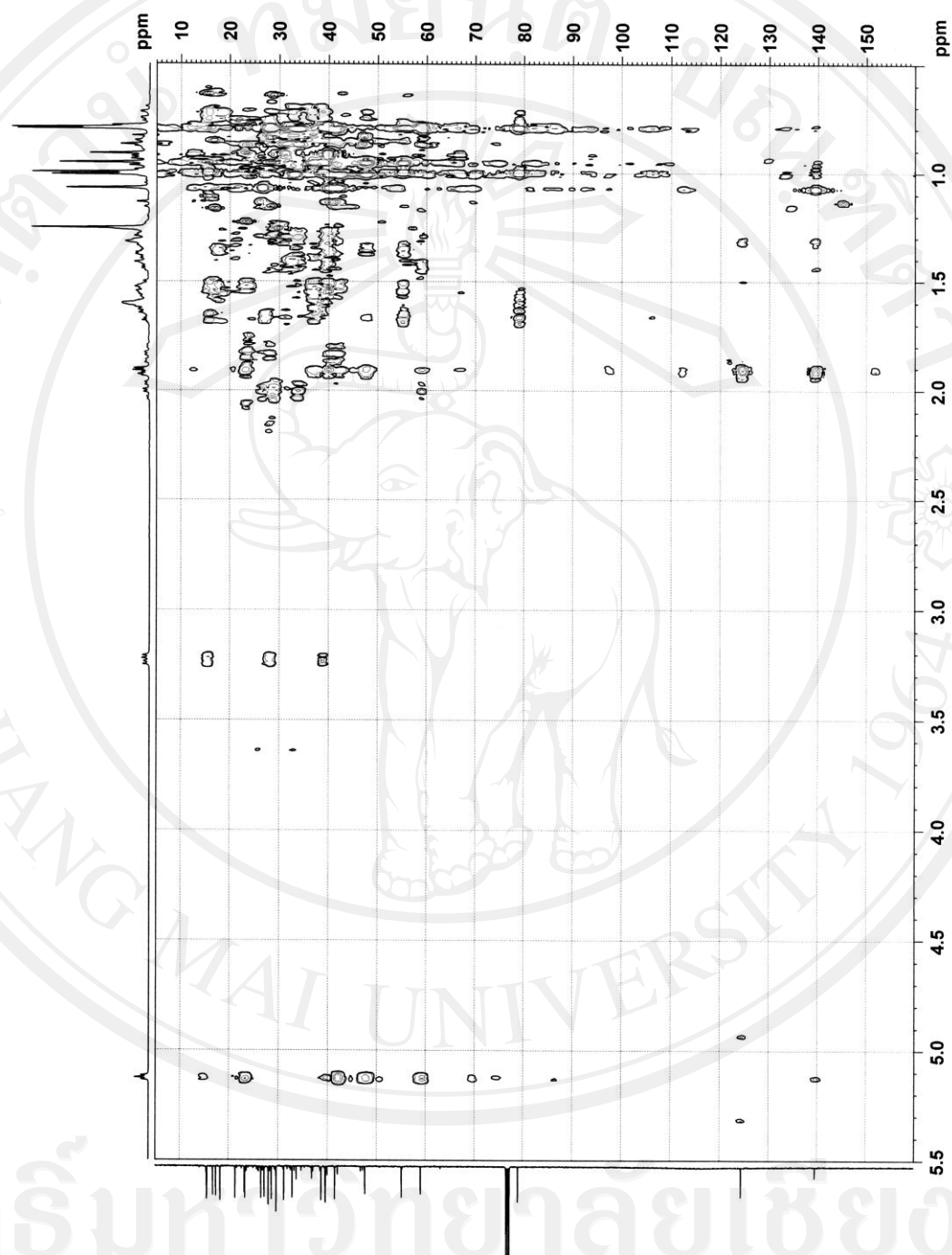


Figure 132 HMBC of α -amyrin (13)

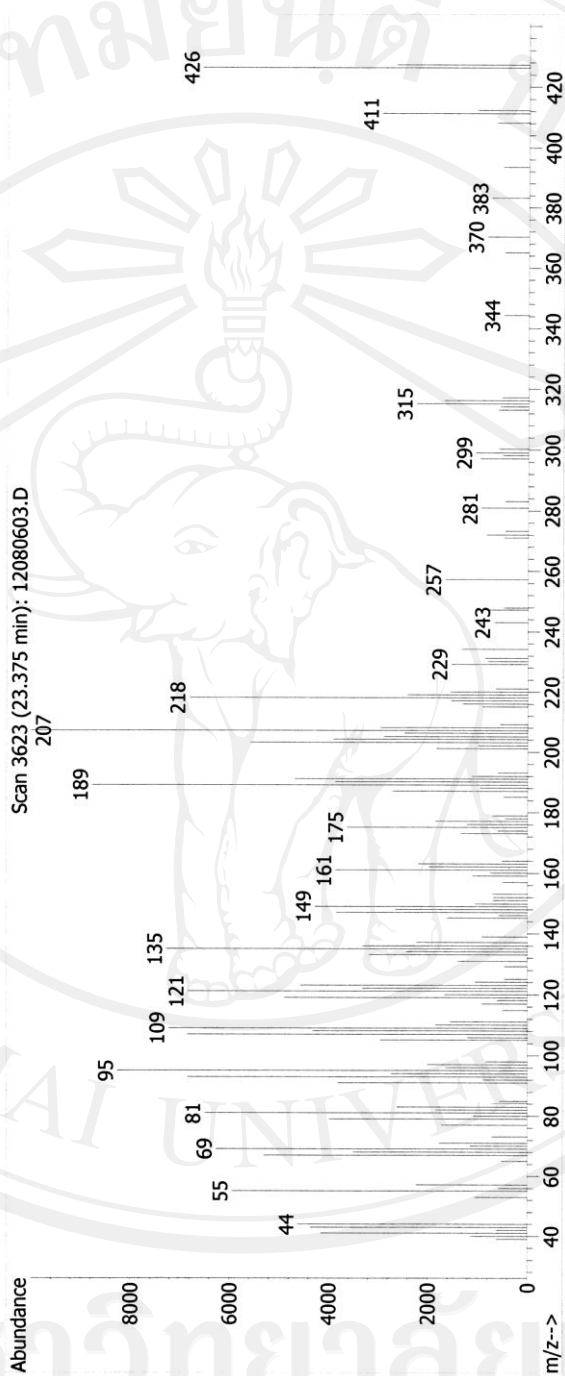


Figure 133 Mass spectrum of α -amyrin (13)

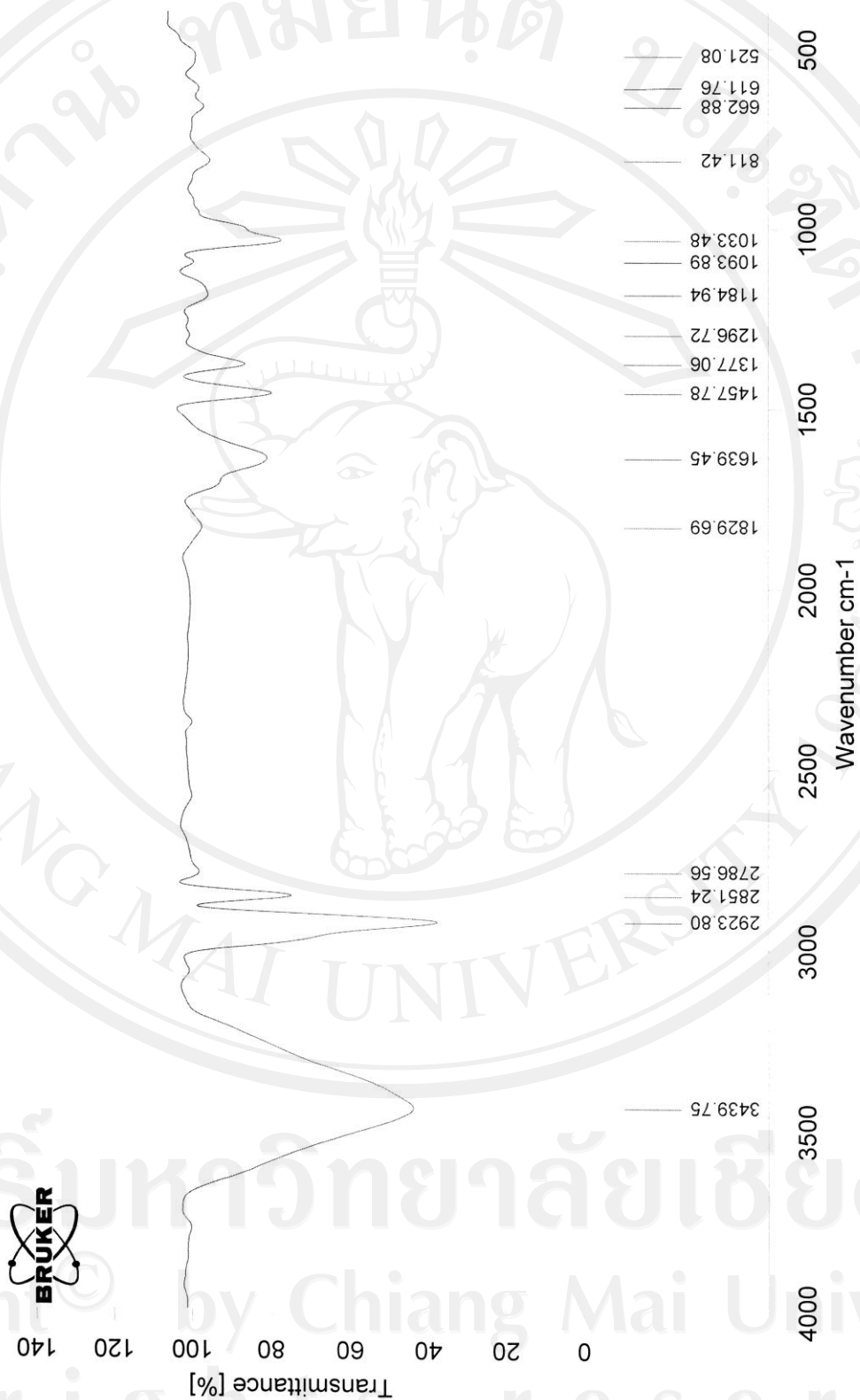


Figure 134 IR spectrum of α -amyrin (13)

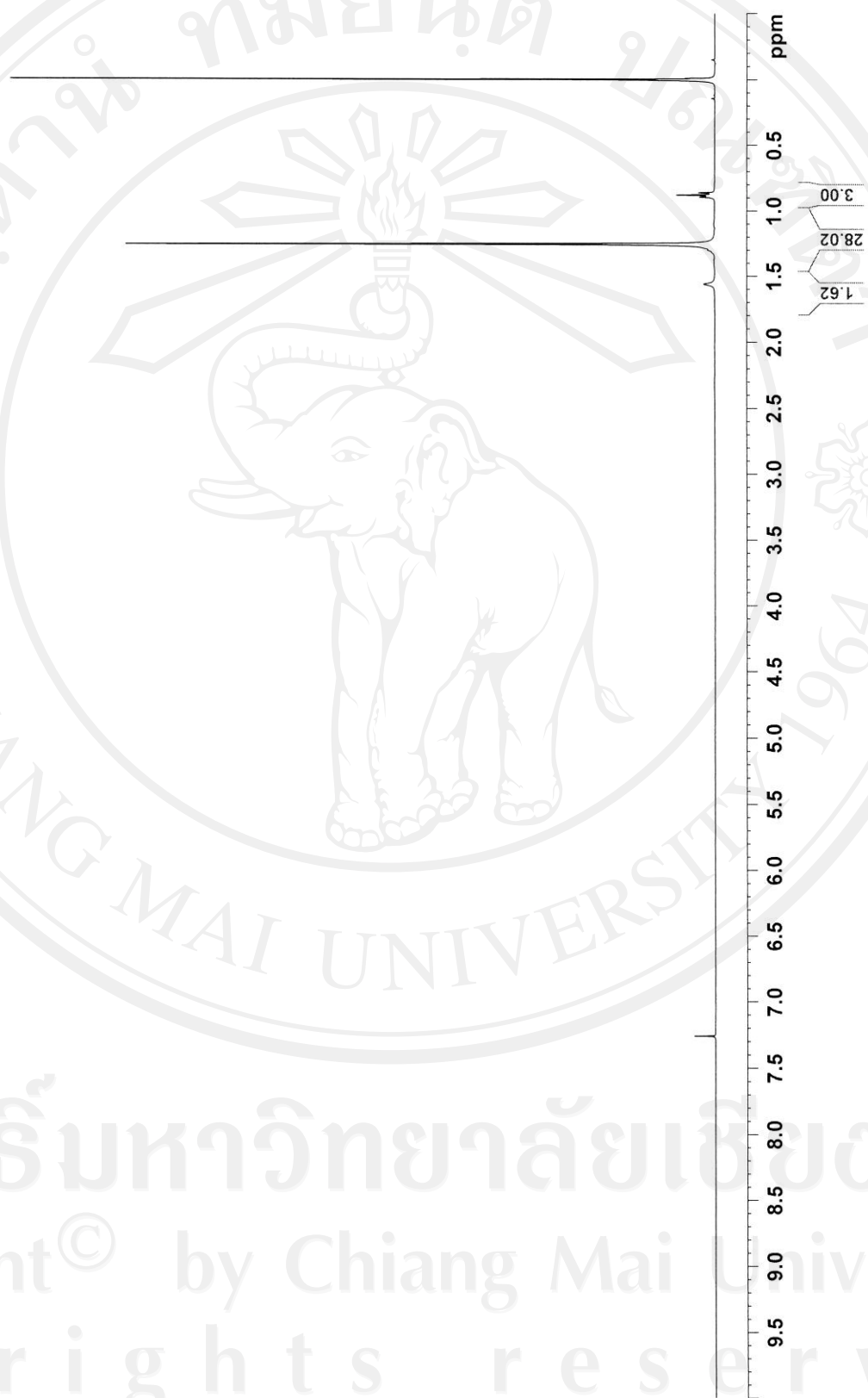


Figure 135 ^1H NMR spectrum of hentriacontane (62)

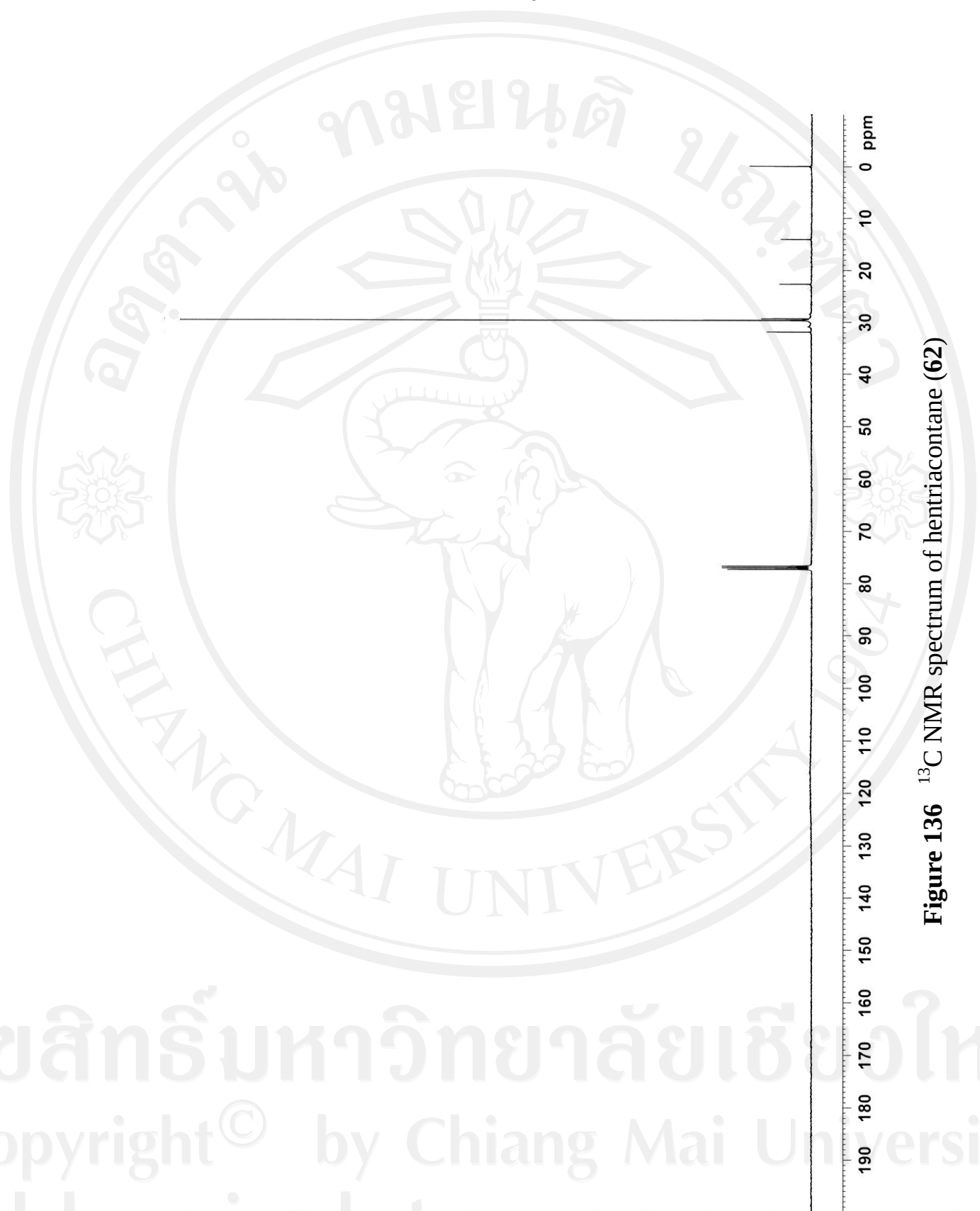


Figure 136 ^{13}C NMR spectrum of hentriacontane (62)

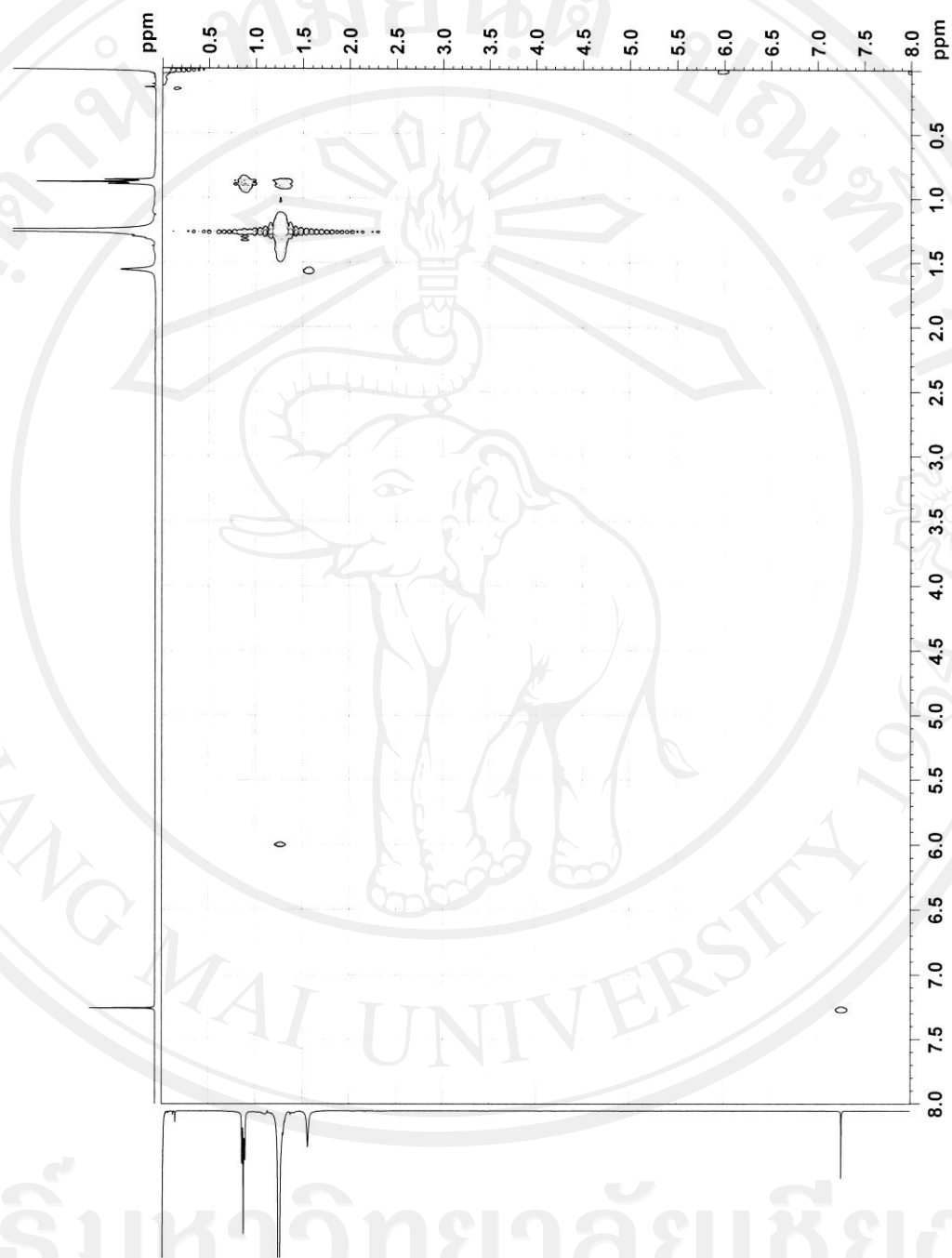


Figure 137 COSY of hentriacontane (62)

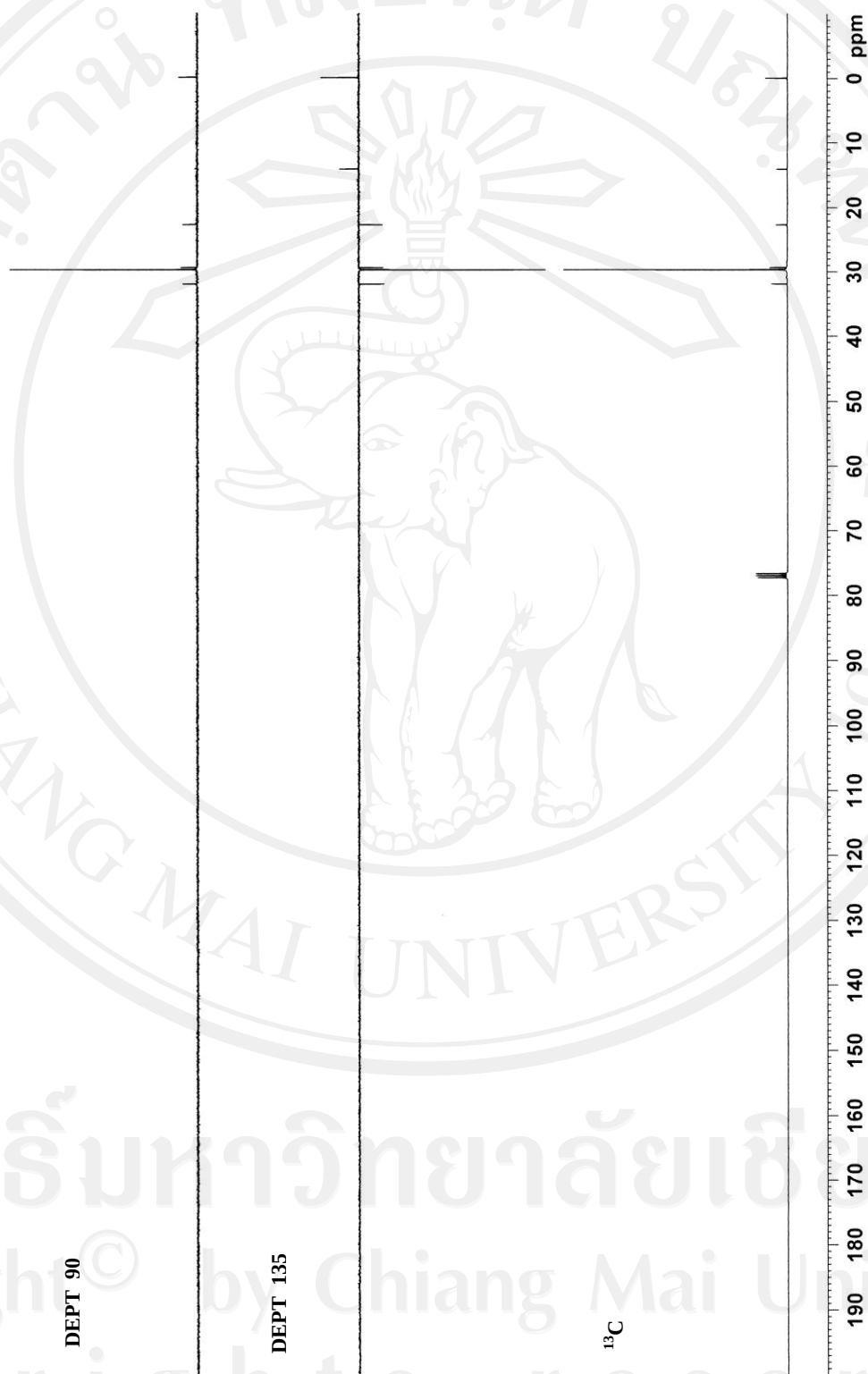


Figure 138 Comparison between DEPT 90, DEPT 135, and ^{13}C of hentriacontane (62)

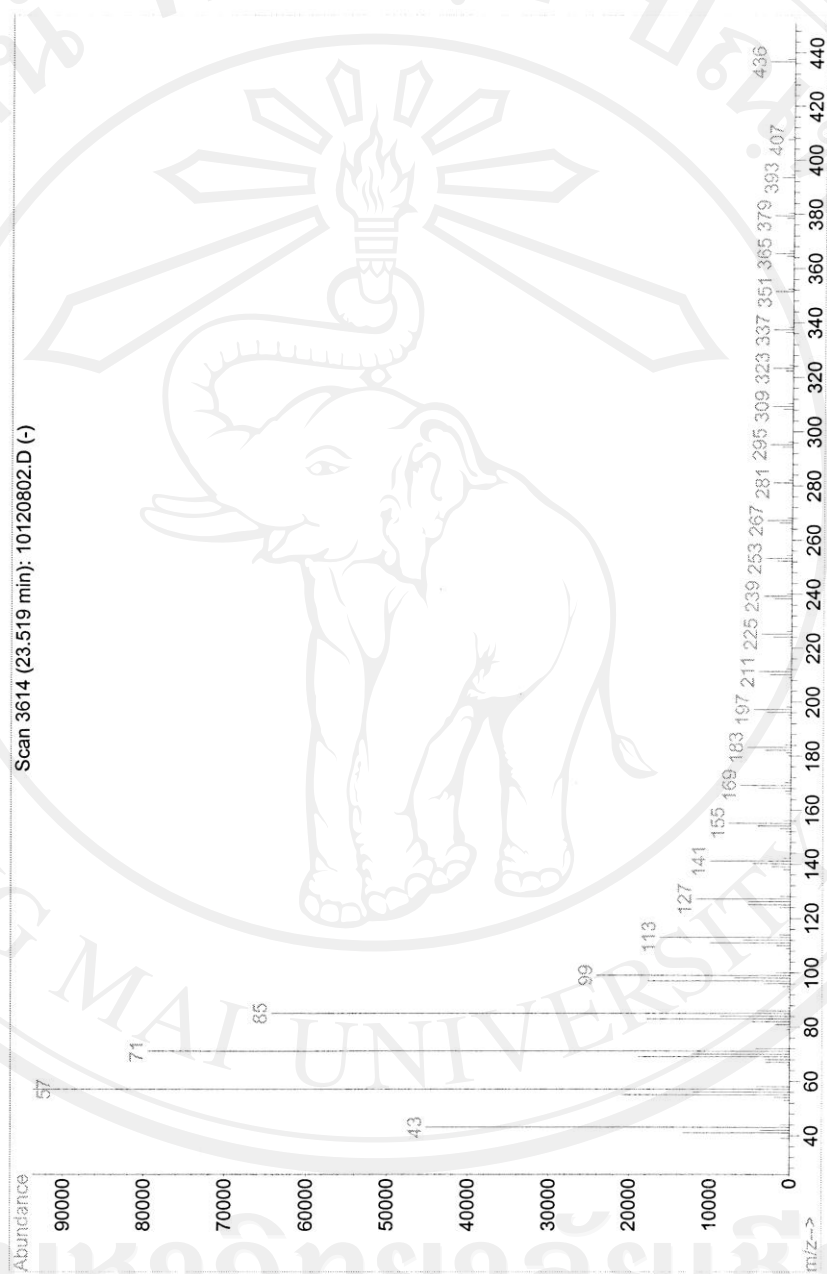


Figure 139 Mass spectrum of hentriacontane (62)



Transmittance [%]

4000 3500

3055.56
2985.33
2924.51
2851.10
2404.76
2307.74
1427.29
1265.15
896.01
739.94

Wavenumber cm⁻¹

Figure 140 IR spectrum of hentriacontane (62)

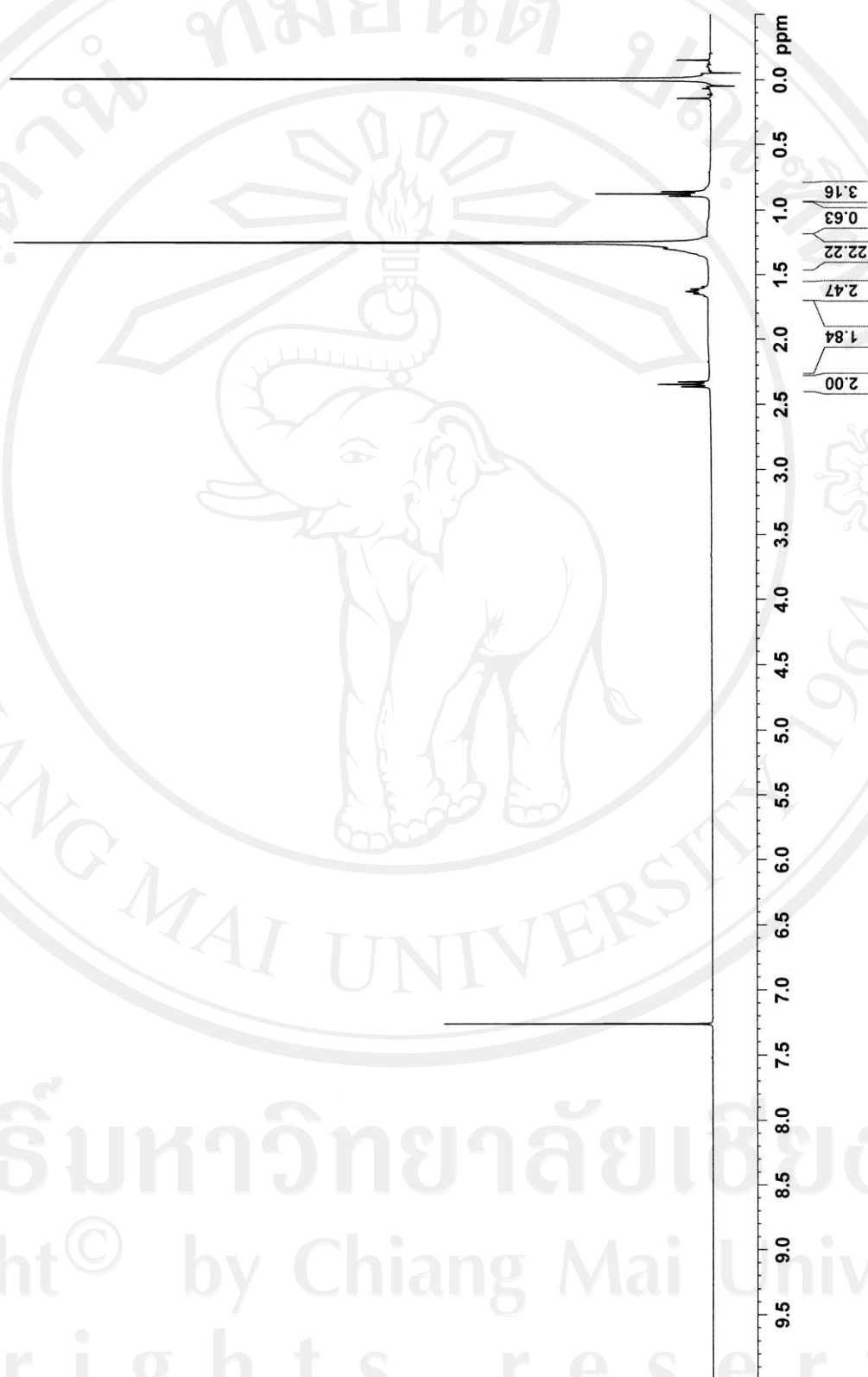


Figure 141 ^1H NMR spectrum of palmitic acid (63)

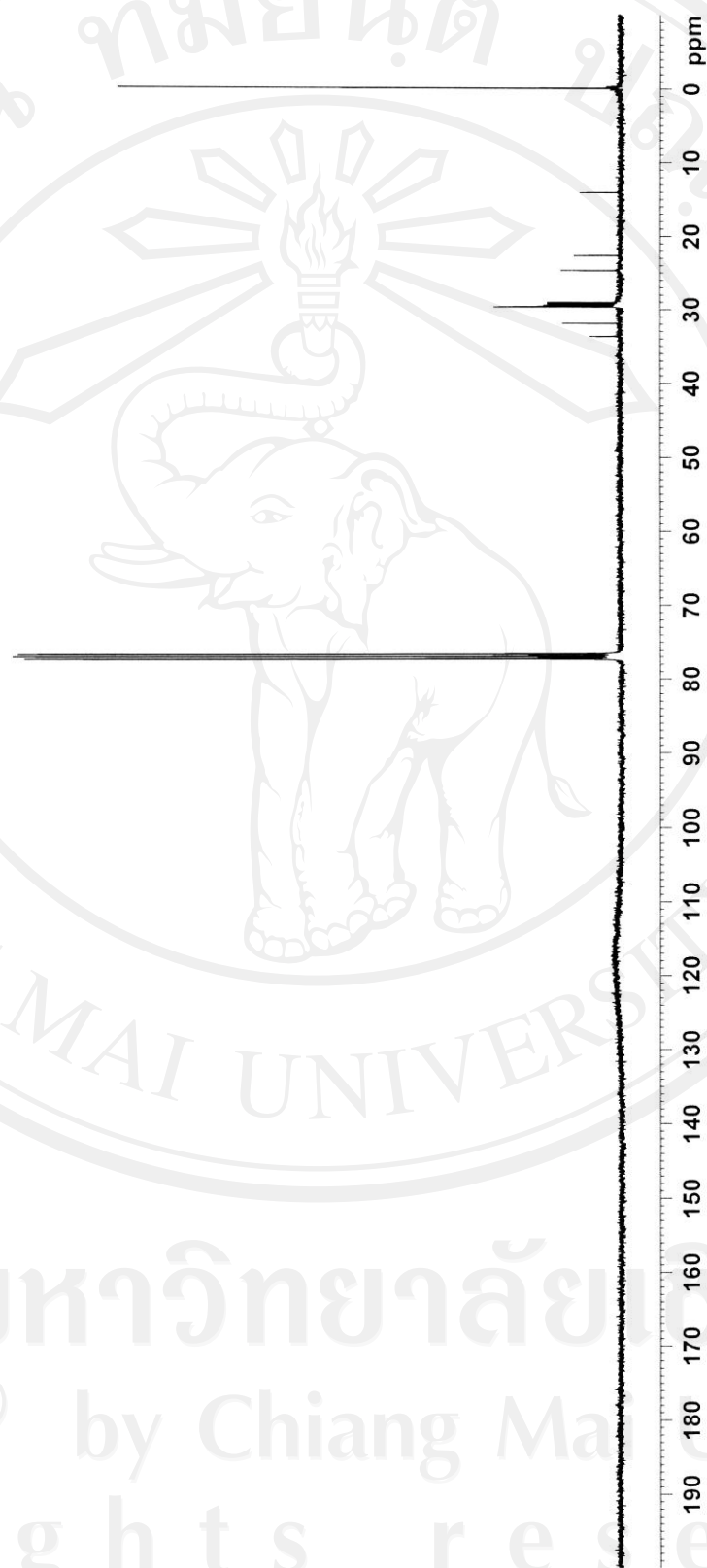


Figure 142 ^{13}C NMR spectrum of palmitic acid (63)

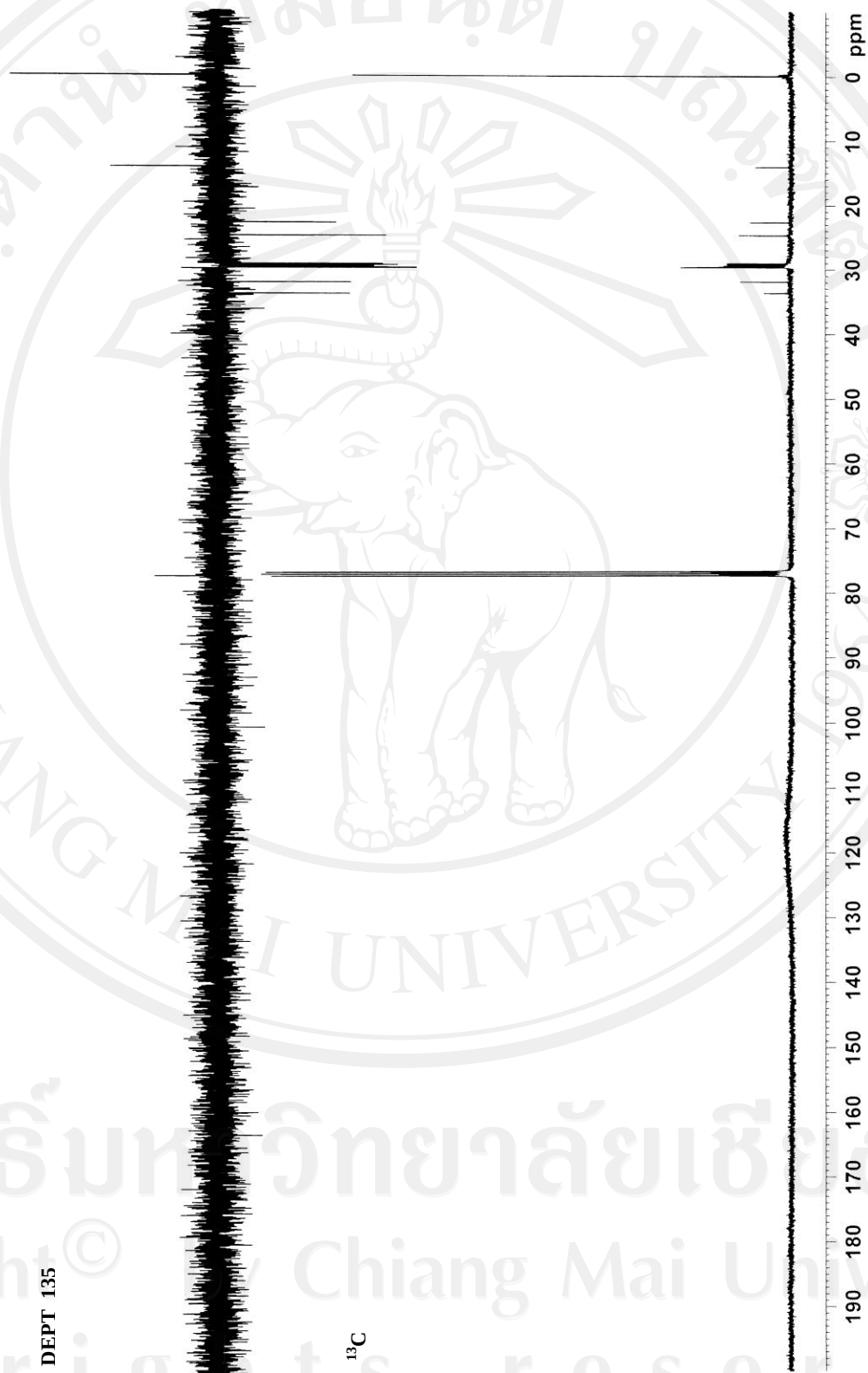


Figure 143 Comparison between DEPT 135 and ¹³C of palmitic acid (63)

DEPT 135

¹³C

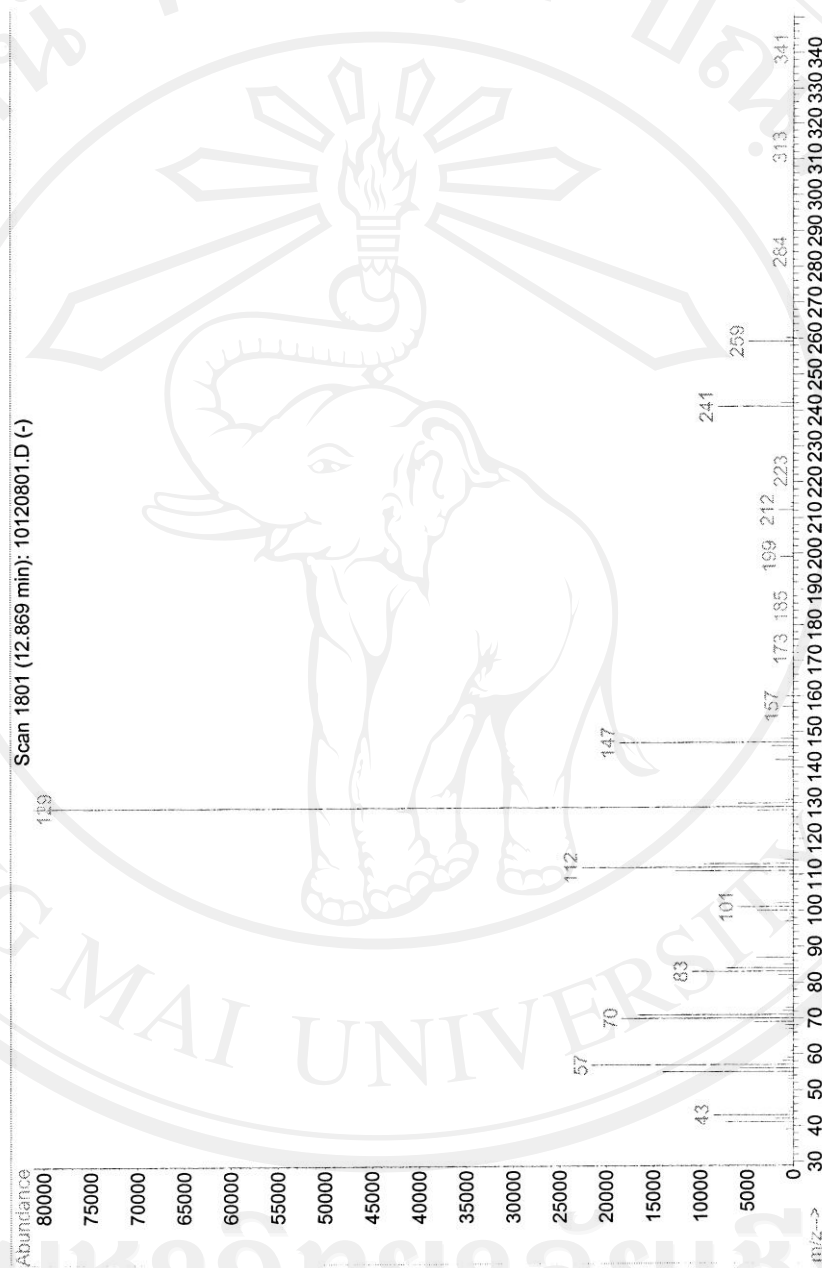


Figure 144 Mass spectrum of palmitic acid (63)

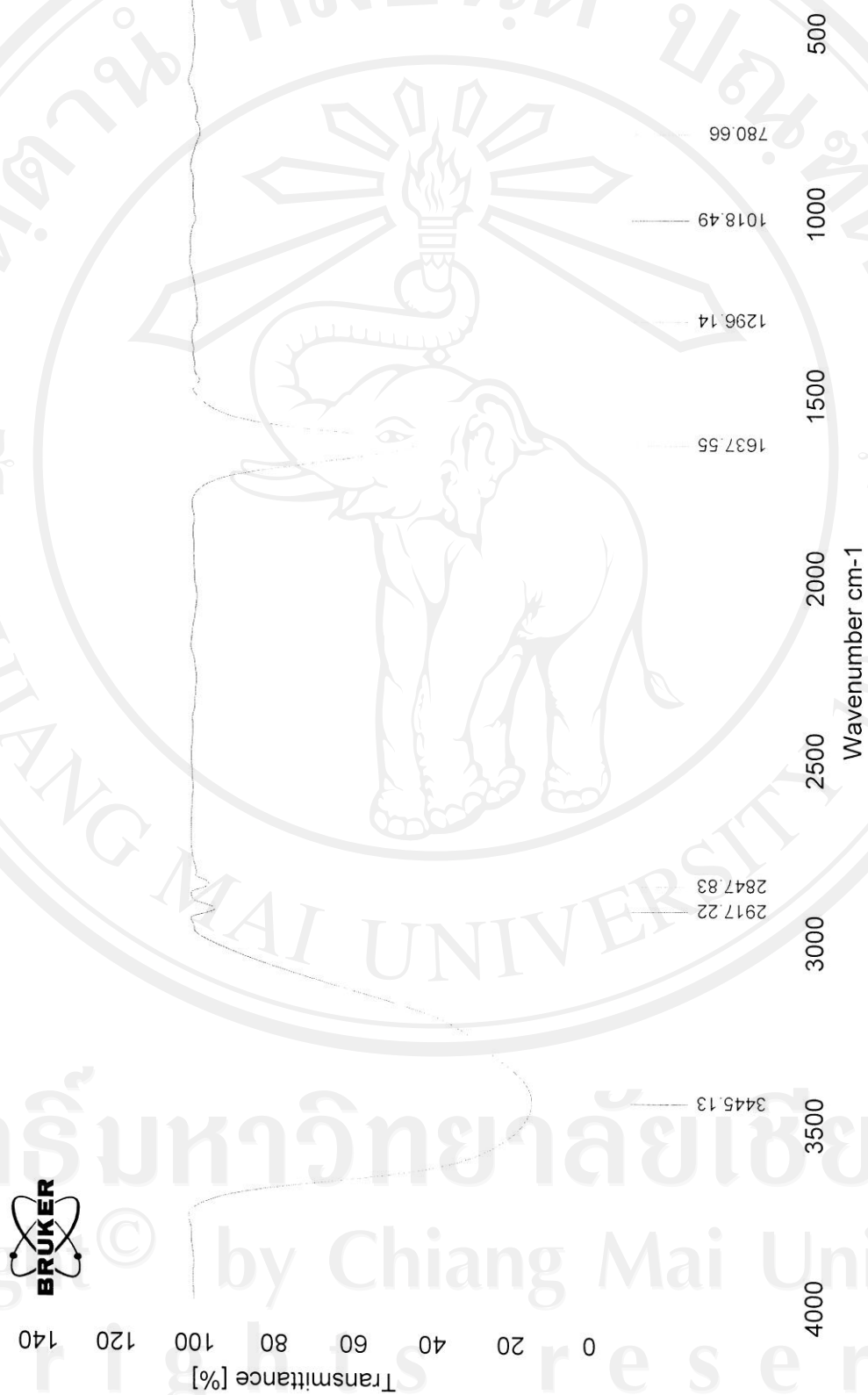


Figure 145 IR spectrum of palmitic acid (63)

CURRICULUM VITAE

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Institution attended

- 2010 B.S., Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.
- 2012 M.S., Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

Presentations and Conferences:

National Conference:

Patcharapong Thangsunan and Puttinan Meepowpan*, “*Chemical Constituents of Dichloromethane Extract of Vernonia parishii Hook f. Leaves*”, PERCH-CIC Congress VII, at Pattaya, Chonburi, Thailand (2011).

International Conference:

Patcharapong Thangsunan, Puttinan Meepowpan*, and Thiti Junpirom, “*Chemical Constituents of Dichloromethane Extract of Vernonia parishii Hook f. Leaves*”, The Sixth Pure and Applied Chemistry International Conference (PACCON VI), at Chiang Mai, Thailand (2012), pp. 760–763.

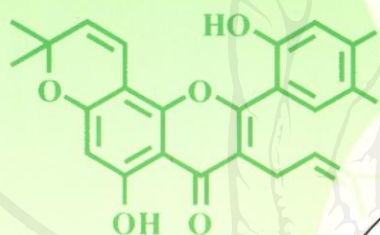
PERCH-CIC

CONGRESS VII

Theme: Chemistry, Environment and Society

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Chemical Constituents of *Vernonia parishii* Hook f. Leaves of Dichloromethane Extract



Patcharapong Thangsunan and Puttinan Meepawpan

Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Chiang Mai University, Huaykaew Road, Chiang Mai 50200, Thailand.

E-mail address : tbscience@gmail.com, puttinan@chiangmai.ac.th

Abstract : The chemical constituents of *Vernonia parishii* found out four terpenoids, e.g. stigmasterol (1), β -amyrin 3-acetate (2), lupeol 3-acetate (3) and lupeol 3-palmitate (4), there were isolated from the dichloromethane extract from leaves of *V. parishii*. The structures of isolated compounds (1–4) were elucidated by chemical and spectral analyses such as ^1H NMR, ^{13}C NMR, DEPT, COSY, HMQC and HMBC which were compared with the previous reports.

Keywords : *Vernonia*, *Vernonia parishii*, stigmasterol, β -amyrin 3-acetate, lupeol 3-acetate, lupeol 3-palmitate

Introduction

Vernonia, one of genera in family Compositae, consists of over 1,000 species of shrubs. From the previous studies, several compounds including terpenoids, alkaloids, flavonoids, steroids and cardiac glycosides were found and categorized into this genus. Furthermore, some compounds in the crude extracts from *Vernonia* also showed efficiently biological active properties such as anti-malarial, anti-tumor, anti-inflammatory, anti-bacterial and anti-fungal.^{1,2,3}

Vernonia parishii Hook. f. (Khang Hang Lek), a shrubby plant, has already been discovered in the forests of northern Thailand. In this study, we will investigate the chemical compositions in *Vernonia parishii* Hook f. leaves.

Method, Results and Discussion

Air-dried leaves of *V. parishii* were extracted by CH_2Cl_2 and MeOH (3 days / time x3), respectively, at room temperature and then solvents were removed under reduce pressure to give the CH_2Cl_2 crude extract 57.79 g (0.76%) and methanol crude extract 43.33 g (0.57%). The CH_2Cl_2 crude extract was added into column chromatography (silica gel 60H). All compounds in the extract were isolated by gradient mixture system of n-hexane, dichloromethane, ethyl acetate and methanol. The isolated results obtained four terpenoids including stigmasterol (1), β -amyrin 3-acetate (2), lupeol 3-acetate (3) and lupeol 3-palmitate (4) from the CH_2Cl_2 extract of *V. parishii* leaves. The structures (Shown as Figure 1) were identified using spectroscopic techniques showed as table 1

Table 1 ^1H -NMR (400 MHz) Data of Compound 1-4

Carbon Position	1	2	3	4
3	3.51 [m, 1H]	4.50 [dd, 1H]; $J = 10.0, 6.1$ Hz	4.47 [dd, 1H]; $J = 10.4, 5.4$ Hz	4.47 [dd, 1H]; $J = 10.6, 5.6$ Hz
5	-	-	0.79 [m, 1H]	-
6	5.34 [m, 1H];	-	-	-
12	2.00 [m, 1H]	5.12 [t, 1H]; $J = 3.6$ Hz	-	-
19	1.10 [s, 3H]	-	2.37 [dt, 1H]; $J = 11.1, 5.8$ Hz	2.37 [dt, 1H]; $J = 11.0, 5.8$ Hz
21	1.02 [m, 3H]	-	1.38 [m, 1H]	-
22	5.14 [dd, 1H]; $J_{21,23} = 15.2$ Hz	-	1.86-1.96 [m, 1H]	-
23	5.01 [dd, 1H]; $J_{22,24} = 8.8$ Hz	0.86 [s, 3H]	0.84 [s, 3H]	0.88 [s, 3H]
24	1.53 [m, 1H]	0.87 [s, 3H]	0.84 [s, 3H]	0.79 [s, 3H]
25	1.55 [m, 1H]	0.97 [s, 3H]	0.84 [s, 3H]	0.85 [s, 3H]
26	0.84 [d, 3H]	1.00 [s, 3H]	1.02 [s, 3H]	1.03 [s, 3H]
27	0.79 [d, 3H]	1.54 [s, 3H]	0.93 [s, 3H]	0.94 [s, 3H]
28	1.43 [m, 1H]	0.79 [s, 3H]	0.83 [s, 3H]	0.84 [s, 3H]
29	0.81 [t, 3H]	0.87 [s, 3H]	4.50 [dd, 1H]; $J = 2.4, 1.3$ Hz	4.57 [m, 1H]; 4.68 [br d, 1H]; $J = 2.1$ Hz
30	-	0.87 [s, 3H]	1.68 [s, 3H]	1.68 [s, 3H]
32	-	2.04 [s, 3H]	2.04 [s, 3H]	2.28 [t, 2H]; $J = 7.4$ Hz
46	-	-	-	1.20 [s, 3H]

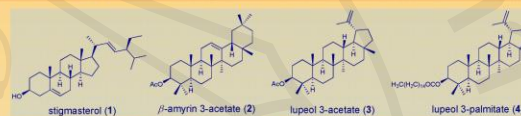


Figure 1 Structure of terpenoids from dichloromethane crude extract

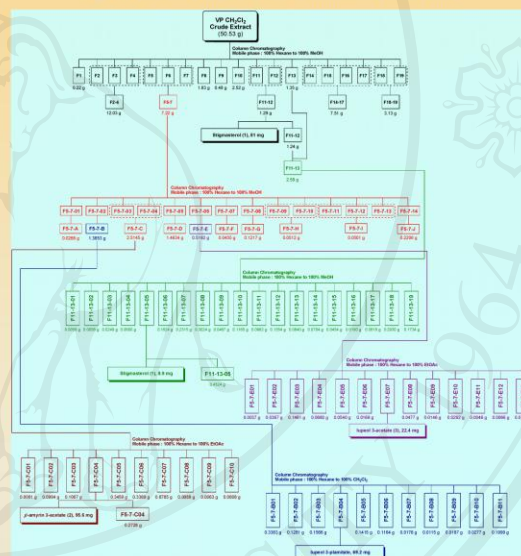


Figure 2 Isolation diagram of dichloromethane crude extract

Conclusion

Stigmasterol, β -amyrin 3-acetate, lupeol 3-acetate and lupeol 3-palmitate can be successfully isolated by column chromatography from the crude CH_2Cl_2 extract of *V. parishii* leaves.

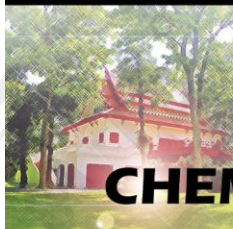
Acknowledgements

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& THE CHEMICAL SOCIETY OF THAILAND UNDER THE PATRONAGE OF
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CHEMICAL CONSTITUENTS OF DICHLOROMETHANE EXTRACT OF *VERNONIA PARISHII* HOOK F. LEAVES

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Abstract: *Vernonia* plants indicated many bioactivities with the microorganisms causing some diseases. In this study, *Vernonia parishii* Hook f. (Compositae), which was found in the forests of northern Thailand, has been investigated for its chemical constituents. It was found that three terpenoids, stigmasterol (1), lupeol 3-acetate (2) and lupeol 3-palmitate (3), have been isolated from the dichloromethane extract of leaves of *V. parishii*. The structures of isolated compounds (1–3) were elucidated by chemical and spectral analysis such as ^1H NMR, ^{13}C NMR, DEPT, COSY, HMQC and HMBC which were comparable with the previous reports.

1. Introduction

The large genus *Vernonia* in Compositae family comprises of more than 1000 species found all regions in the world [1]. There were numerous distinct subgenera and subsections distributing in the tropical and subtropical regions of Asia, Africa and America. Although *Vernonia* consists a number of species, some plants were investigated the phytochemistry and bioactive compounds, for example, vernolide and vernodaline which were isolated from *V. colorata* leaves resisted the two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* [2]. These compounds also showed antiparasitic activity [3]. In addition, three sesquiterpene lactones, 8 α -tigloyloxy-hirsutinolide-13-*O*-acetate, 8 α -tigloyloxy-hirsutinolide and 8 α -(4-hydroxymethacryloyloxy)-tigloyloxy-hirsutinolide-13-*O*-acetate, were active against chloroquine resistant *Plasmodium falciparum* strain (W2) [4]. Seven new stigmasterane-type steroidal glycosides, vernocuminsides A-G, isolated from the stem barks of *V. cumingiana* were reported anti-inflammatory activities and cytotoxicities as well [5]. Moreover, many papers about this genus showed the bioactivities of crude extracts of plants in *Vernonia* such as ethanol extract from *V. amygdalina* leaves exhibited an antiparasitic activity [6] and dichloromethane extract of *V. lasiopus* root and bark highly showed antiparasitic activity [7].

Vernonia parishii Hook f., or “Khang Hang Lek” in Thai, was discovered in India and Southeast Asia between 250–1400 meters from sea level. In Thailand, this plant which was the shrub was found in forest of northern region.

In this report, we indicate three terpenes, stigmasterol (1), lupeol 3-acetate (2) and lupeol 3-palmitate (3), these compounds were found in the dichloromethane extract of *V. parishii*. Their

structures were elucidated by chemical and spectral analyses

2. Materials and Methods

2.1 General Experimental Procedures

All NMR spectra, ^1H and ^{13}C NMR spectra, were recorded on Bruker DRX 400 MHz in CDCl_3 and chemical shifts and chemical shifts which were given in ppm downfield from tetramethylsilane (TMS) were reported as δ -values in parts per million (ppm) relative to residue CHCl_3 as internal reference (^1H : δ 7.26, ^{13}C : δ 77.00) and coupling constants (J values) were reported in hertz (Hz). Peak multiplicities are indicated as follows: *s* (singlet), *d* (doublet), *t* (triplet), *dd* (doublet of doublets), *dt* (doublet of triplets), *br d* (broad doublets) and *m* (multiplet). Infrared spectra were taken with a FT-IR model TENSER 27 (Bruker) spectrometer and absorption frequencies were reported in reciprocal centimeters (cm^{-1}). Melting points of all compounds could measure with Sanyo model Gallenkamp. Flash column chromatography was performed employing Merck silica gel 60H. Preparative thin layer chromatography (PLC) plates used Merck silica gel 60 PF₂₅₄. Analytical thin layer chromatography was performed with Merck silica gel 60 F₂₅₄ aluminum plates.

2.2 Plant Material

Leaf material was collected from a *V. Parishii* in Mae Jaem district, Chiang Mai, Thailand. A voucher specimen (MAXWELL 08-54) was deposited in the herbarium of Department of Biology, Faculty of Science, Chiang Mai University, Thailand.

2.3 Extraction and Isolation

Air-dried powdered leaves (1,265 g) were extracted successively with dichloromethane at room temperature to give crude extract (57.79 g). The extract was isolated for investigating the chemical compositions by flash column chromatography with eluents which consisted of hexane, dichloromethane, ethyl acetate and methanol to receive 10 fractions (VPD-01 to 10). Then all fractions were sought out characteristics with ^1H NMR as a guide to select fractions to be purified further.

Fraction VPD-07 (1.29 g) as yellow solid was recrystallized with Hexane- CH_2Cl_2 (1:1) and then washed with hexane to give compound **1** that was compared data with previous reports.

Fraction VPD-03 (7.22 g) was chromatographed by silica gel 60H and eluting with hexane, dichloromethane, ethyl acetate and methanol, respectively. Fractions were combined using TLC technique [Hexane-CH₂Cl₂-EtOAc (10:0.2:0.1) as solvent system]. And then, all fractions were characterized by ¹H NMR spectroscopy and IR spectrometry. From those techniques, we obtained the two compounds, compounds **2** and **3**, were compared with previous studies.

Compound **1** (51.0 mg) was obtained as colorless solid, mp 167.3–168.5 °C, ¹H NMR is shown in table 1 and ¹³C NMR is shown in table 2.

Compound **2** (22.4 mg) was obtained as white solid, mp 210.0–212.0 °C, ¹H NMR is shown in table 1 and ¹³C NMR is shown in table 2 as well.

Compound **3** (69.2 mg) was obtained as white solid, mp 72.0–74.5 °C, ¹H NMR is also showed in table 1 and ¹³C NMR is showed on table 2

Table 1: ¹H NMR spectral data for compounds **1**, **2** and **3** (CDCl₃) at 400 MHz (*J* in Hz)

Proton	1	2	3
3	3.51 [<i>m</i> , 1H]	4.47 [<i>dd</i> , 1H]; <i>J</i> = 10.4, 5.4 Hz	4.47 [<i>dd</i> , 1H]; <i>J</i> = 10.6, 5.6 Hz
5	-	0.79 [<i>m</i> , 1H]	0.79 [<i>m</i> , 1H]
6	5.34 [<i>m</i> , 1H]	0.84 [<i>s</i> , 1H]	0.85 [<i>s</i> , 1H]
12	1.18, 2.00 [<i>m</i> , 2H]	1.65 [<i>m</i> , 2H]	1.65 [<i>m</i> , 2H]
19	1.10 [<i>s</i> , 3H]	2.37 [<i>dt</i> , 1H]; <i>J</i> = 11.1, 5.8 Hz	2.37 [<i>dt</i> , 1H]; <i>J</i> = 11.0, 5.8 Hz
21	1.02 [<i>m</i> , 3H]	1.38 [<i>m</i> , 1H] 1.86-1.96 [<i>m</i> , 1H]	1.60 [<i>m</i> , 2H]
22	5.14 [<i>dd</i> , 1H]; <i>J</i> _{22,23} = 15.2 Hz	1.63 [<i>m</i> , 2H]	1.61 [<i>m</i> , 2H]
23	5.01 [<i>dd</i> , 1H]; <i>J</i> _{23,24} = 8.6 Hz	0.84 [<i>s</i> , 3H]	0.88 [<i>s</i> , 3H]
24	1.53 [<i>m</i> , 1H]	0.84 [<i>s</i> , 3H]	0.79 [<i>s</i> , 3H]
25	1.55 [<i>m</i> , 1H]	0.84 [<i>s</i> , 3H]	0.85 [<i>s</i> , 3H]
26	0.84 [<i>d</i> , 3H]	1.02 [<i>s</i> , 3H]	1.03 [<i>s</i> , 3H]
27	0.79 [<i>d</i> , 3H]	0.93 [<i>s</i> , 3H]	0.94 [<i>s</i> , 3H]
28	1.18 [<i>m</i> , 1H] 1.43 [<i>m</i> , 1H]	0.83 [<i>s</i> , 3H]	0.84 [<i>s</i> , 3H]
29	0.81 [<i>t</i> , 3H]	4.56 [<i>dd</i> , 1H]; <i>J</i> = 2.4, 1.3 Hz 4.68 [<i>d</i> , 1H]; <i>J</i> = 2.1 Hz <i>J</i> = 2.3 Hz	4.57 [<i>m</i> , 1H] 4.68 [<i>br d</i> , 1H]; <i>J</i> = 2.1 Hz
30	-	1.68 [<i>s</i> , 3H]	1.68 [<i>s</i> , 3H]
32	-	2.04 [<i>s</i> , 3H]	2.28 [<i>t</i> , 2H]; <i>J</i> = 7.4 Hz
46	-	-	1.20 [<i>s</i> , 3H]

Table 2: ¹³C NMR spectral data for compound **1**, **2** and **3** (CDCl₃, 100 MHz)

Carbon	1	2	3
1	31.3	38.3	37.8
2	31.6	23.7	23.7
3	71.8	80.9	80.5
4	42.3	37.7	38.0
5	140.7	55.3	50.3
6	121.7	18.1	18.0
7	31.6	34.2	31.9
8	31.9	40.8	38.4
9	50.2	50.3	40.8
10	36.5	37.0	37.0
11	21.1	20.9	20.9
12	39.7	25.5	25.2
13	42.2	38.0	35.6
14	56.9	42.8	42.8
15	24.4	27.4	27.4
16	28.9	35.5	34.8
17	55.9	42.8	42.9
18	12.0	48.0	48.2
19	19.4	48.2	47.9
20	40.2	150.9	150.9
21	21.2	29.8	38.0
22	138.3	39.9	39.9
23	129.3	27.9	27.9
24	51.2	15.9	15.9
25	31.9	16.1	16.5
26	21.2	16.4	16.1
27	19.0	14.5	14.5
28	25.4	17.9	18.2
29	12.2	109.3	109.4
30	-	19.2	19.3
31	-	171.0	173.6
32	-	21.6	34.2
33	-	-	25.0
34-43	-	-	29.2 – 29.7
44	-	-	29.7
45	-	-	22.7
46	-	-	14.1

Assignments based on HMQC and HMBC experiments

3. Results and Discussion

Compound **1** was identified as stigmasterol which was recrystallized from fraction VPD-07 with mp 167.3–168.5 °C. It was compared with mp 163.0–164.0 °C from the previous study [8]. Their ¹H NMR spectra showed significant peaks, H-3 proton of hydroxyl group indicated signal at 3.51 ppm which was a multiplet, H-6 proton of methine group of endocyclic double bond showed peak at 5.34 ppm that was a multiplet, and H-22 and H-23 protons of exo double bond showed two doublet of doublets peaks at 5.14 (*J* = 15.2) and 5.01 (*J* = 8.6) ppm, respectively. Then, the ¹³C NMR spectra of this compound displayed important peaks, C-3 carbon that had hydroxyl group showed signal at 71.8 ppm, C-5 and C-6 carbons of endocyclic double bond indicated peaks at 140.7 and 121.7 ppm, and C-22 and C-23 of exo double bond showed signals at 138.3 and 129.3 ppm, respectively. All spectral data of compound **1** are identical with spectral data of stigmasterol from previous report [9].

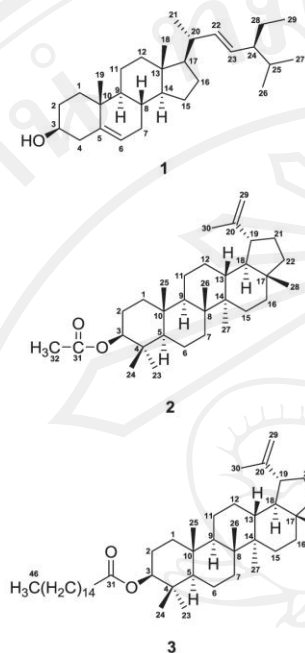


Figure 1. Structures of the isolated compound from *V. parishii* leaves.

Compound **2** was identified as lupeol 3-acetate that was isolated from fraction VPD-03 with mp 210.0–212.0 °C. It compared with mp 216.0–217.0 °C from previous investigation [10]. The ^1H NMR spectra of this compound from the experiment displayed significant peaks, for example, H-3 proton that adhered on the C-3 carbon showed doublet of doublet peak ($J = 10.4, 5.4$ Hz) at 4.47 ppm, H-19 proton that adjoined on the C-19, which bonded with tertiary carbon of exo double bond, indicated doublet of triplet peak ($J = 11.1, 5.8$ Hz) at 2.37 ppm, H-29 had two protons, which was methylene protons, performed two signals at 4.56 ppm that was doublet of doublet ($J = 2.4, 1.3$ Hz) and 4.68 ppm which was doublet ($J = 2.3$ Hz) respectively, and H-32 was identified as three protons of methyl group of acetate group displayed at 2.04 ppm. Their ^{13}C NMR spectra also showed especial signals such as C-3 carbon that adjoined acetate carbon indicated peak at 80.9 ppm, C-20 carbon was identified as tertiary carbon of exo double bond showed peak at 150.9 ppm, C-29 carbon, which was methylene carbon of exo double bond, displayed signal at 109.3 ppm, and C-31 carbon, carbonyl carbon of acetate group, showed obvious peak at 171.0 ppm. The correlation between ^1H and ^{13}C spectra were confirmed by HMQC. From this data, they showed results, for instance, H-3 proton was coupled with C-3 carbon, H-19 which accorded with C-19, two protons (H-29) of methylene group correlated with C-29 carbon, The methyl protons (H-32 protons) of acetate group were linked with C-32 carbon. Therefore, we could deduce all protons, which were identified, were

bonded with all correlated carbon. All spectral data of compound **2** are identical with previous report [11].

Compound **3** was identified as lupeol 3-plamitate which was separated from fraction VPD-03 as well as lupeol 3-acetate with mp 72.0–74.5 °C. There was compared with mp 80.0–81.0 °C from previous study [12]. The ^1H NMR spectra of lupeol 3-plamitate from isolating method indicated characteristic signals like compound **2** such as H-3 proton, which adjoined C-3 carbon, showed doublet of doublet peak at 4.47 ppm ($J = 10.6, 5.6$ Hz), H-19 proton adhered on C-19 carbon, which associated with tertiary carbon of exo double bond, displayed doublet of triplet signal ($J = 11.0, 5.8$ Hz) at 2.37 ppm, and H-29 protons that had two protons were identified as methylene protons of exo double bond showed peaks at 4.57 ppm, multiplet signal, and 4.68 ppm, broad doublet signal ($J = 2.1$ Hz), respectively. The ^{13}C NMR spectra of this compound also performed significant signals, for example, C-3 carbon that bonded with carboxylate group showed peak at 37.8 ppm, C-20 carbon, which was tertiary carbon of exo double bond, indicated peak at 150.9 ppm, C-29 carbon that was methylene group of exo double bond displayed peak at 109.4 ppm, C-31 carbon identified as carbonyl carbon of carboxylate group showed evident signal at 173.6 ppm, and C-34 to 43 carbons that were side chain of alkyl branch of carboxylate group indicated between 29.2–29.7 ppm. To confirm the evidence, HMQC was used to explain correlation between ^1H and ^{13}C NMR spectra of this compound. In the result, it displayed the correlation such as H-3 proton was coupled by C-3 carbon, H-19 proton associated with C-19 carbon, two methylene protons (H-29) showed correlation with C-29 that was methylene carbon of exo double bond. All spectral data are identical with previous investigations of lupeol 3-plamitate [13–14].

4. Conclusions

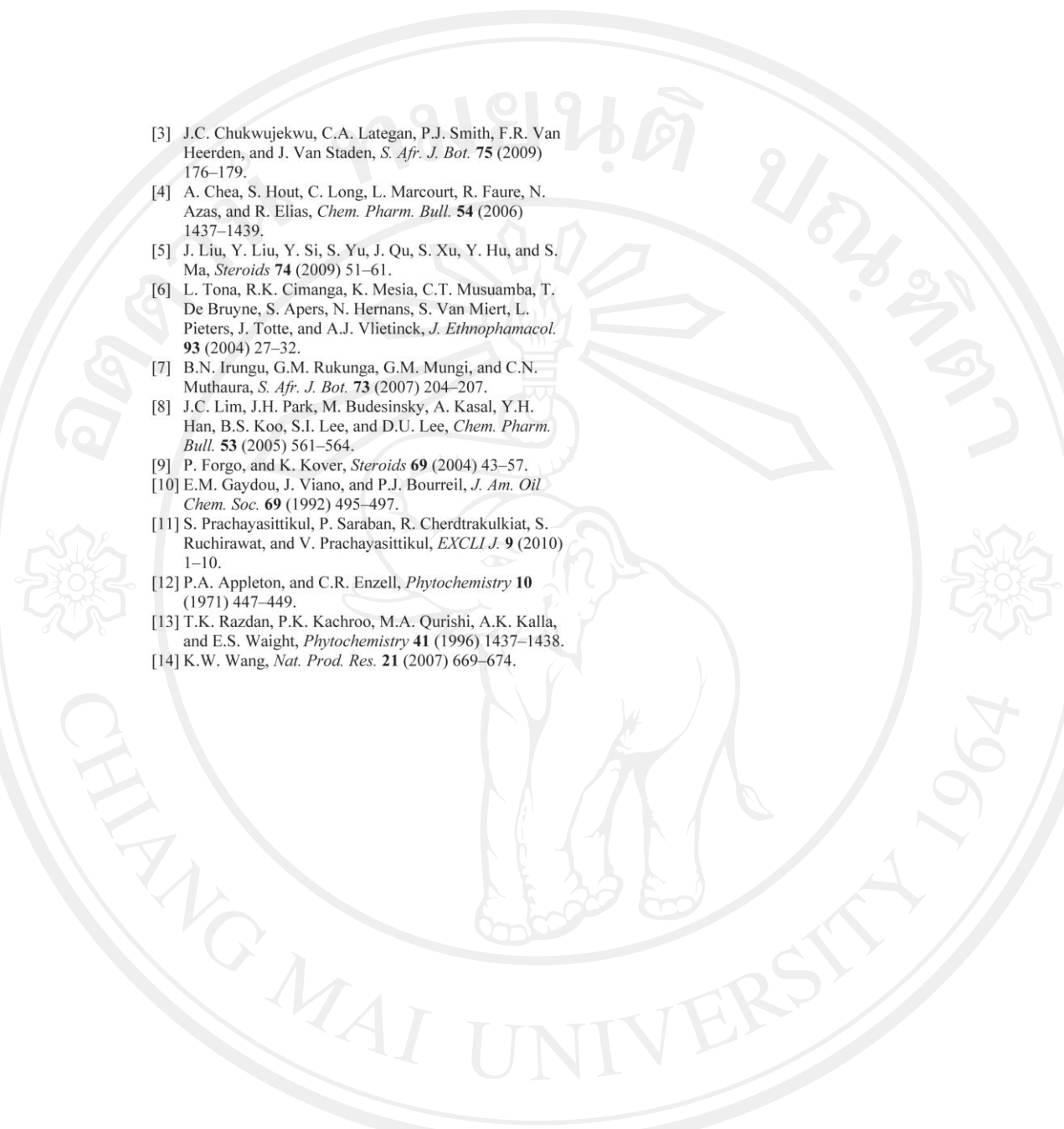
In this study, we isolated three terpenoids, stigmasterol (**1**), lupeol 3-acetate (**2**) and lupeol 3-plamitate (**3**) from leaves of *V. parishii*. All compounds were identified for their structures using a number of spectroscopic techniques such as IR and NMR.

Acknowledgements

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