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BIOLOGICAL ACTIVITY OF CROVATIN AND ITS DERIVATIVES

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ได้เตรียมอนุพันธ์ของโครวาตินเตรียมทั้งหมด 6 ชนิด คือ สารประกอบ *ent*-(8R,10β)-3,19S:15, 16:12S,20R:19,20-tetraepoxy-cleroda-13(16), 14-diene-18β-oic acid **(2)**, *ent*-(8R,10β)-3, 19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18α-oic acid **(3)** , *ent*-(8R,10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18β-ol **(4)**, *ent*-(8R,10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-4(18),13(16),14-clerodatriene **(6)**, *ent* -(8R,10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18β-al **(7)** และ *ent*-(8R,10β)-15,16:12S.20R-diepoxy-18-hydroxy-cleroda-13(16),14-diene-3,19-olide **(8)** ซึ่งอนุพันธ์ทั้งหมดนี้เป็นสารชนิดใหม่ที่ยังไม่พบในธรรมชาติ ปฏิกิริยาที่ใช้ในการเตรียมอนุพันธ์ ได้แก่ ปฏิกิริยาไฮโดรไลซิสในสภาวะกรดและด่าง, ปฏิกิริยารีดักชัน และปฏิกิริยาออกซิเดชัน เมื่อนำโครวาตินและอนุพันธ์ทั้ง 6 ชนิดไปทดสอบฤทธิ์การยับยั้งเอนไซม์3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA), HIV-1 protease, α-glucosidase และเซลล์มะเร็งท่อน้ำดี (HuCCA-1) พบว่าสารประกอบ **3** และ **8** มีความสามารถในการยับยั้งเอนไซม์ 3-hydroxy-3-methylglutaryl CoA reductase ซึ่งสารประกอบทั้งสองชนิดมีค่า IC_{50} 1.45 mM และ สารประกอบ **2** และ **3** มีความสามารถในการยับยั้งเอนไซม์ α-glucosidase โดยมีค่า IC_{50}3.90 และ IC_{50} 1.85 mM ตามลำดับ

ภาควิชา.....เคมี..... **ลายมือชื่อนิสิต.....**

สาขาวิชา.....เคมีอินทรีย์..... **ลายมือชื่ออาจารย์ที่ปรึกษา.....**

ปีการศึกษา.....2546.....

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BIOLOGICAL ACTIVITY OF CROVATIN AND ITS DERIVATIVES. THESIS ADVISOR : PROF. SOPHON ROENGSUMRAN, Ph. D. 126 pp. ISBN 974-17-4400-5

Six new crovatin derivatives were obtained from the modification of crovatin by hydrolysis in acidic and basic conditions, oxidation and reduction. Six compounds were *ent*-(8R,10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18β-oic acid **(2)**, *ent*-(8R,10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18α-oic acid **(3)**, *ent*-(8R,10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18β-ol **(4)**, *ent*-(8R,10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-4(18),13(16),14-clerodatriene **(6)**, *ent*-(8R, 10β)-3, 19S:15, 16:12S, 20R:19, 20-tetraepoxy-cleroda-13(16), 14-diene-18β-al **(7)** and *ent*-(8R,10β)-15,16:12S.20R-diepoxy-18-hydroxy-cleroda-13 (16),14-diene-3,19-olide **(8).** Crovatin and its derivatives were tested for their inhibitory activity against 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA), HIV-1 reductase α-glucosidase and human tumor cell line. The result indicated that compounds **3** and **8** showed inhibitory activities against 3-hydroxy-3-methylglutaryl CoA reductase. Both compounds **3** and **8** showed IC_{50} 1.45 mM. Moreover, compounds **2** and **3** showed inhibitory activities against α-glucosidase with IC_{50} 3.90 and 1.85 mM , respectively. All compounds were inactive against HIV-1 protease and tumor human cell line (HuCCA-1).

Program……Chemistry…… Student's signature……..
Field of study…Organic Chemistry …… Advisor's signature……..
Academic Year……2003…….

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LIST OF SCHEMES

LIST OF ABBREVIATIONS

cm	centimeter
mm	millimeter
wt	weight
eV	electron Volt
MHz	megahertz
TLC	Thin Layer Chromatography
kg	kilogram
g	gram
cm^3	cubic of centimeter
$CHCl_3$	chloroform
MeOH	methanol
EtOAc	ethyl acetate
mg	milligram
mp	melting point
KBr	potassium bromide
ν_{max}	the wave number at maximum absorption
cm^{-1}	unit of wave number
s	strong (IR)
m	medium (IR)
w	weak (IR)
°C	degree clsius
mL	milliter
R_f	rate of flow in chromatography
ppm	part per million
m/e	mass to charge ratio
δ	chemical shift
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
COSY	Correlated Spectroscopy

J	coupling constant
d	doublet (for NMR spectrum)
dd	double of doublet (for NMR spectrum)
EI-MS	Electron Impact Mass Spectrometer
ESI-MS	Electrospay Ionization Mass Spectrometer
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Correlation
M	molar
M^+	molecular ion
m/z	mass to charge ratio
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Enhancement Spectroscopy
^{1}H-NMR	Proton Nuclear Magnetic Resonance
q	quartet (for NMR spectrum)
rt	room temperature
s	singlet (for NMR spectrum)
t	triplet (for NMR spectrum)
THF	tetrahydrofuran
ν_{max}	the reciprocating wavelength (IR spectrum)
λ_{max}	the wavelength at maximum absorption (UV-VIS)
ORTEP	Okridge Termal Ellipse Program

CHAPTER I
INTRODUCTION

Medicinal plants are rich natural resources. They are cheap, effective and have less harmful side-effects than synthetic drugs. Plao Yai (*Croton oblongifolius* Roxb.) in Euphorbiaceae family has been used as a traditional medicine for many applications such as for dysmenorrheal, as a purgative, and to treat dyspepsia and dysenteria. Moreover, this plant had been used as folk-medicine in conjunction with *Croton sublyratus* to treat gastric ulcers and gastric cancers.

In previous work, many chemical constituents from *Croton oblongifolius*. have been investigated. For example, crotocembraneic acid, neocrotocembranic acid and neocrotocembranalm are isolated from the stem bark of *C. oblongifolius* from Petchaboon Province.[1] Four labdane diterpenoids, labda-7,12(*E*),14-triene,labda-7,12 (*E*),14-triene-17-al,labda-7,12(*E*),14-triene-17-ol and labda-17-oic acid are isolated from the stem bark of stem bark of *C. oblongifolius* from Prachuab Kirikhan Province,[2] three labdane diterpenoids, 2-acetoxy-3-hydroxy-labda-8(17),12(*E*)-14-triene, 3-acetoxy-2-hydroxy-labda-8(17),12(*E*)-14-triene and 2,3-dihydroxy-labda-8 (17), 12(*E*),14-triene are isolated from specimen obtained from Loei Province.[3] Moreover, crovatin, a clerodane derivative is isolated from the stem bark of *C. oblongifolius*. collected from Kanchanaburi Province .[4]

Crovatin, major compound was isolated from *C. oblongifolius*., is an interesting compound because of its unique multiple ring system as shown in Figure 1. The molecular formula of crovatin is $C_{21}H_{26}O_6$ and molecular weight is 374. Crovatin contains two methyl, five methylene, ten methine and four quaternary carbon atoms. Crovatin is a neo-furoclerodane diterpenoid with a C-20 →C-12 bridge. They reveal a -CO-OMe group corresponding to C-18–C-21, a secondary methyl group attribute Table to Me-17 and surprisingly two acetal groups with carbon at C-19 and C-20 involving also the C-3 and C-12 in a structural moiety such as CH-O-CH-O-CH-O-CH which accounted for the C-3, C-19, C-20 and C-12 tertiary carbons. It followed that the relative configulations for C-3, C-19 and C-9 must be consistent with this system. The relative configuration for all the nine asymmetric centers of crovatin was established by 2D NMR experiments (COSY and NOESY). ^{1}H-^{1}H COSY showed the ^{3}J interaction between H-3β and H-4α, H-12 and H_a-11, H_a-11 and H-10β and a *W*

type long range coupling between the equatorial protons H-2α and H-4α and between H-19 and H-10. ^{1}H-^{1}H NOESY revealed interactions between H-4α and H-19, H-19 and H-20, H-20 and Me-17α, H-12 and the equatorial proton H-1β, and Me-17 and H-14.

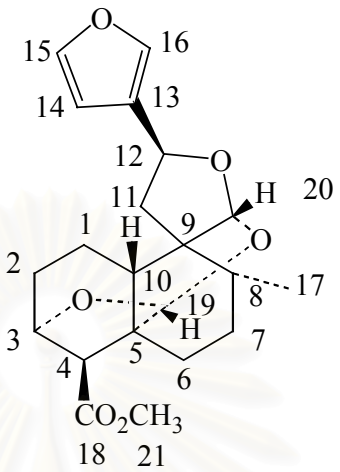


Figure 1 Structure of crovatin **1**.

Since it discovered in 1992 [5] and again in 2002 [4], the chemistry of crovatin has never been investigated. Therefore, this research work is aimed to investigate the chemistry of crovatin in term of acid and base hydrolysis and reduction reaction. The spectroscopic properties as well as the biological activity of crovatin and its derivatives are also studied.

Objective of the research

The objective of this thesis is to investigate the chemical and spectroscopic properties as well as biological activity of crovatin and its derivatives.

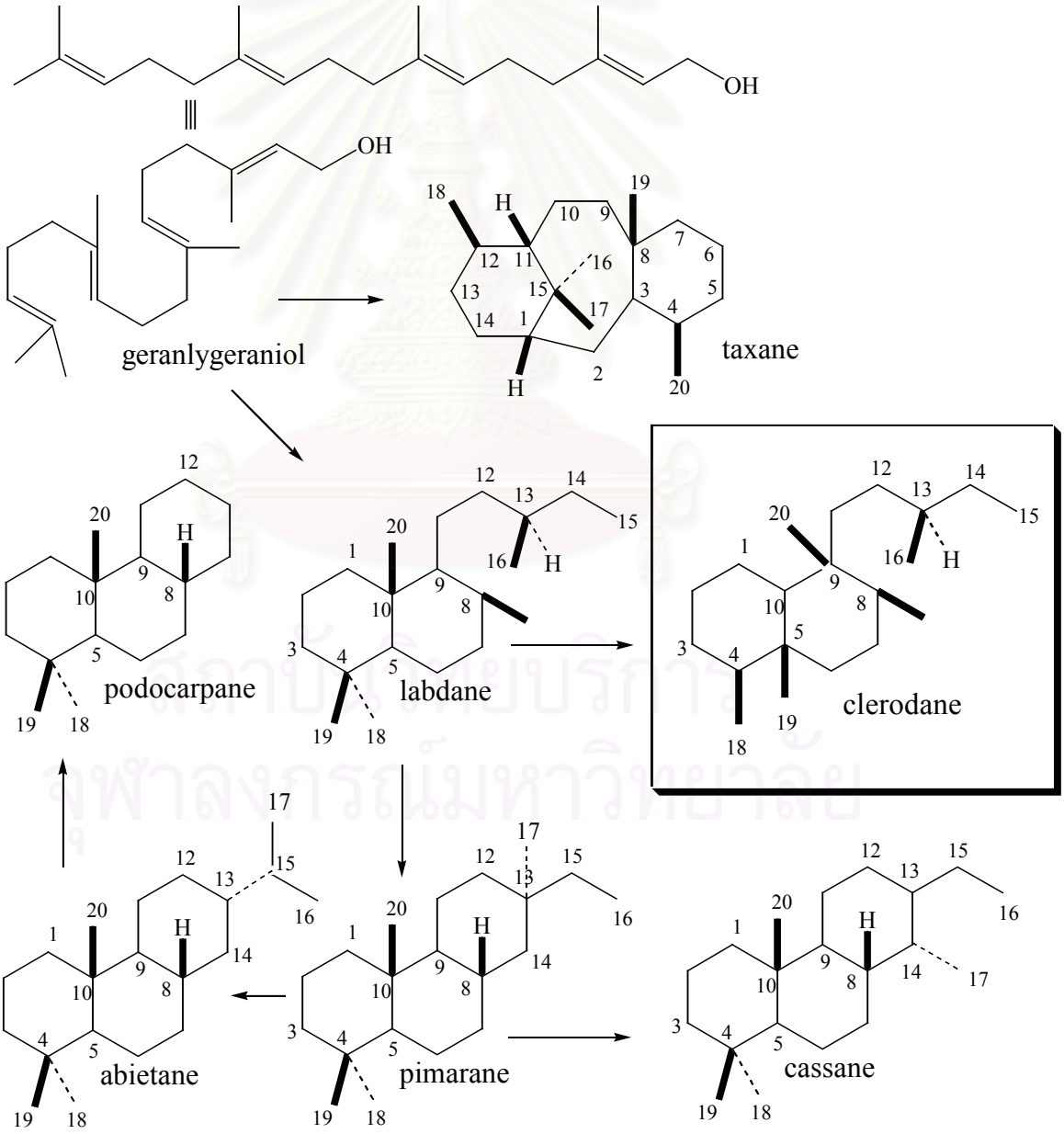
Scope of the research

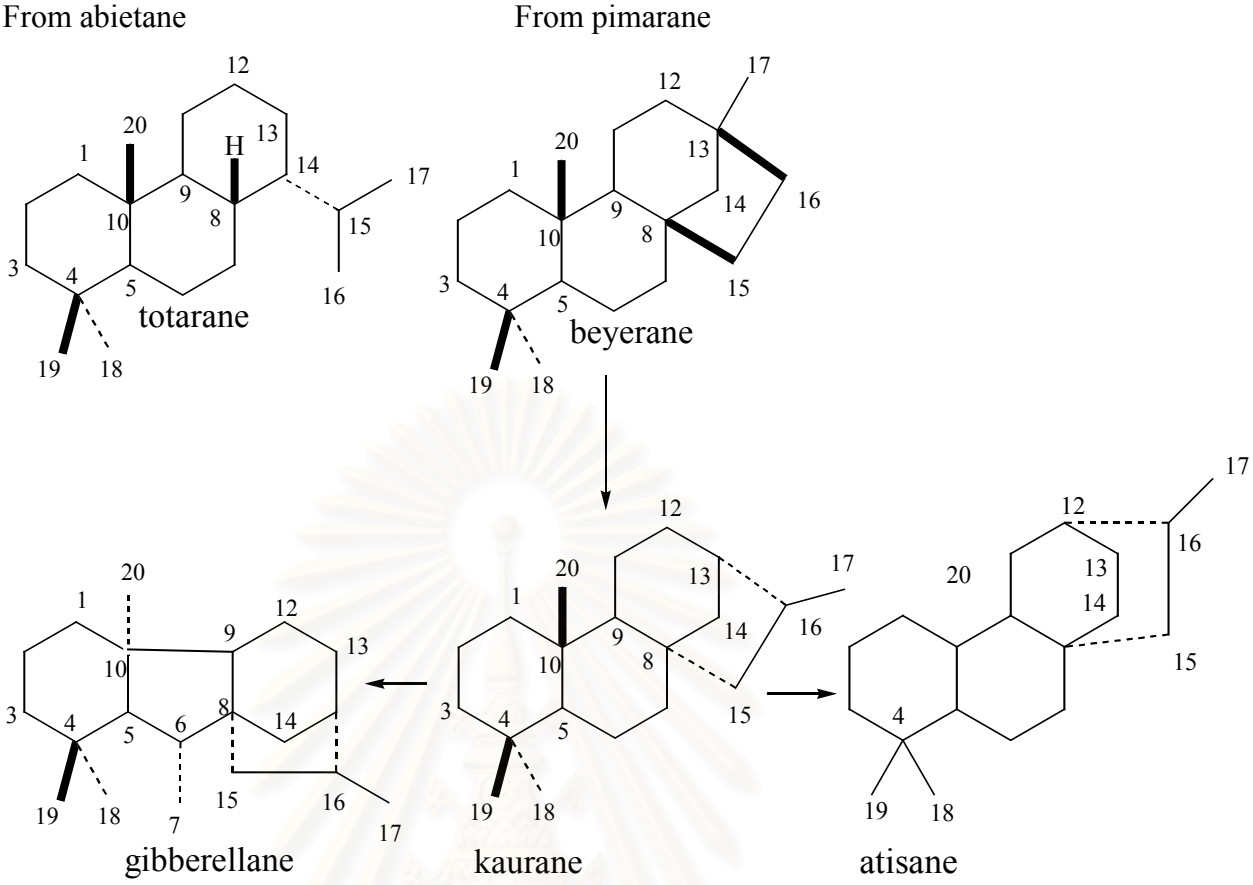
1. Extract and isolate crovatin from stem barks of *Croton oblongifolius*.
2. Hydrolysis under acidic and basic conditions.
3. Reduction with lithium aluminum hydride
   - convert alcohol product of this reaction to exo-cyclic double bond and aldehyde
4. Acetal ring opening under acidic condition.
5. Inhibitory activity against 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, HIV-1 protease, α-glucosidase and tumor human cell line (HuCCA-1)

CHAPTER II
LITERATURE REVIEW

2.1 Biosynthesis of Clerodane

Clerodane is a diterpenes compound. The diterpenes are C_{20} compounds biogenetically derived from geranylgeranyl pyrophosphate. The notable feature of diterpene structures is the fascinating variation encountered in their skeletons and the occurrence in nature of both normal and antipodal stereochemical series. The correlation chart in Figure 2 shows the main diterpenes skeletons according to the classification recommended by Rowe et al.[6)]





Scheme 1

2.2 Previous Studies of Crovatin

In 1992, Crovatin was first isolated from the stem bark of *C. levatii* Guill. collected at Vanuatu near Port-Vila in France by Glaude Mouls and co-workers.[5]

In 2002, Roengsumran S. and co-workers isolated crovatin together with one new furoclerodane, croblongifolin and a known labdane, nidorellol from the stem bark of *C. oblongifolius* Roxb. which colleted from Kanchanaburi Province, Thailand. All compounds were tested for their cytotoxicities against human tumor cell lines. Doxorubicin hydrochloride was used as a positive control, as shown in Table 1. croblongifolin showed high activities against human hepatocarcinoma (HEP-G2), breast carcinoma (BT474), colon carcinoma (SW620), lung carcinoma (CHAGO) and gastric carcinoma (KATO) at 0.35, 0.12, 0.47, 0.24 and 0.35 μM, respectively. Crovatin and nidorellol were inactive against all cell line (IC_{50} › 12 μM).[4]

Table 1 Cytotoxicity data of crovatin, croblongifolin and nidorellol

componud	Cell line				
	KATO3	SW620	BT474	HEP-G2	CHAGO
Crovatin	>12	>12	>12	>12	>12
Croblongifolin	0.35	0.47	0.12	0.35	0.24
Nidorellol	>12	>12	>12	>12	>12
Doxorubicin hydrochloride	3.00	1.94	0.18	1.59	0.53

2.3 Previous Studies for Biological activities of Clerodane derivatives compounds

In 1997, Munoz, D.M. and co-worker reported a new clerodane, 11-deacetylscutalpin D and seven known neo-clerodanes. All compounds were tested for insect antifeedants activity. This result, scutalpins B-D were assessed against larvae of *Spodoptera littoralis* and one of them (scutalpins C) showed very potent activity.[7]

In 1998, Brember, P.D. and co-worker discovered three new neo-clerodane diterpenoids (14, 15-dehydroajugareptansin, 3β-hydroxyajugavensin B and 3α-hydroxy-ajugamarin F4) together with the known compound, ajugareptansin from axrial parts of *Ajuga reptans* cv Catlins Giant. Insect antifeedant testing of all four compounds reveals that 14, 15-dehydroajugareptansin had significant activity against sixth statum larvae of *Spodoptera litoralis.*[8]

In 1999, Bruno, M. and co-worker reported the antifeedant activity of three *neo*-clerodane diterpenoid, fruticolone, isofruticolone and fruticolide from *Teucrium fruticans* was assessed using larvae of *Spodoptera littoralis.* Isofruticolone was one of the most potene of the *Teucrium* derived *neo*-clerodanes. [9]

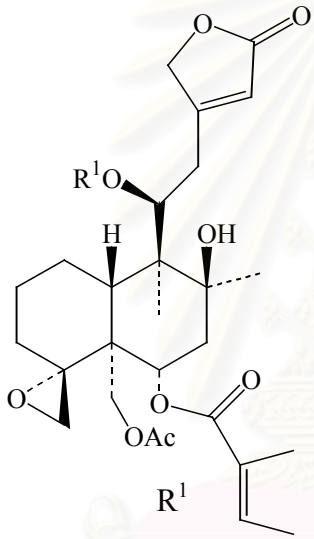
In 2002, Hayashi, K. and co-worker reported four new clerodane diterpenes, bucidarasins A-D from *Bucida buceras*. Bucidarasins A-C showed potent cytotoxicity against human tumor cell lines with IC_{50} values of 0.5-1.9 μM. [10]

In 2003, Kumari, K.G.N. and co-worker reported a new antifeedant diterpenoid teuctosin together with teuflin, teucrin-H_2, 6β-hydroxyteuscordin, 6β-hydroxyteuscor-

din and montanin-D from the acetone extract of *Teucrium tomentosum.* All the compounds showed effective antifeedancy against *Plutella xylostella* and *Spodoptura litura* at 10 μg/cm^2 of leaf area.[11]

In 2003, Salah, M.A. and co-worker reported the hexane and ethyl acetated phases of the methanol extract of *Macaranga monandra* showed fungal growth inhibition. Bioassay-guided fractionation led to the isolation of two active clerodane-type diterpenoids as kolavenic acid and 2-oxo-kolavenic acid. A 96-well microbioassay revealed that kolavenic acid and 2-oxo-kolavanic acid produced moderate growth inhibition in *Phomopsis viticola* and *Botrytis cinerea.* [12]

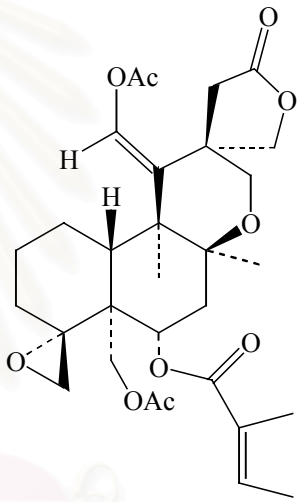
The structure of selected clerodanes is shown in Scheme 3.



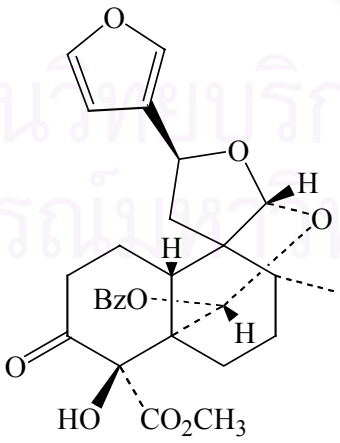
R^1

scutalpins B Ac

scutalpins C H

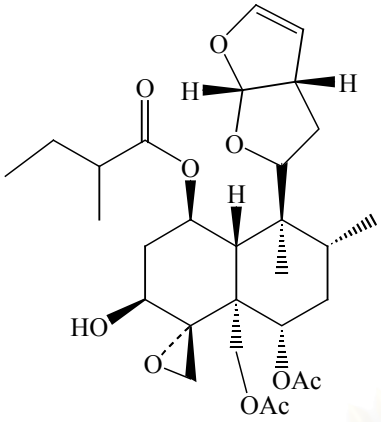


scutalpins D

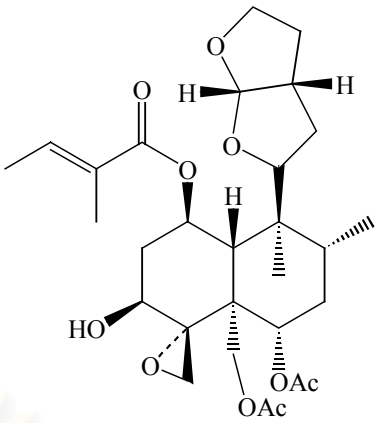


croblongifolin

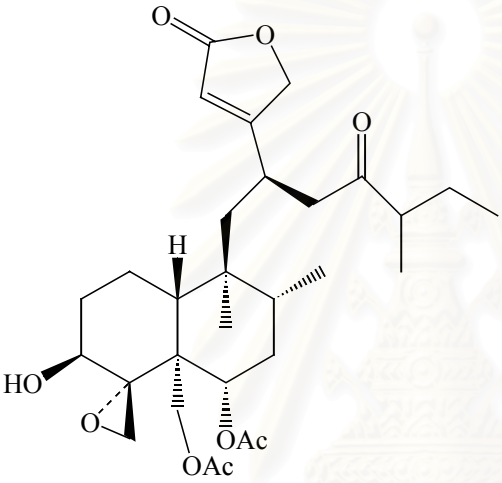
Figure 2 Structures of selected clerodanes**.**



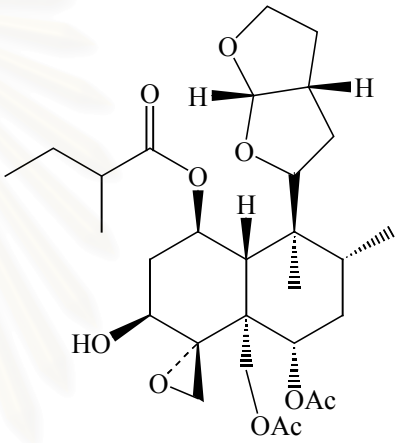
14,15-dehydroajugareptansin



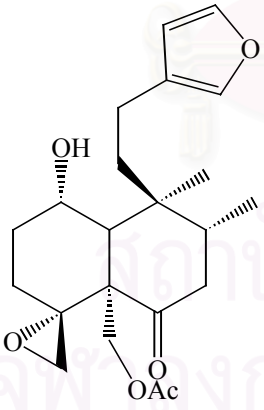
3β-hydroxyajugavensin B



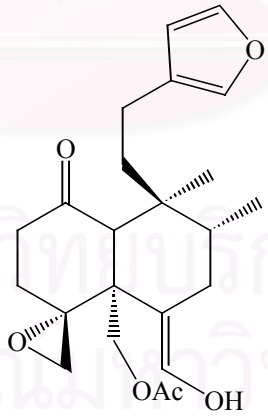
3α-hydroxy -ajugamarin F4



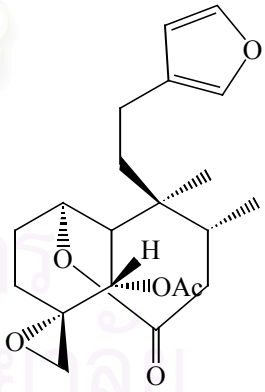
ajugareptansin



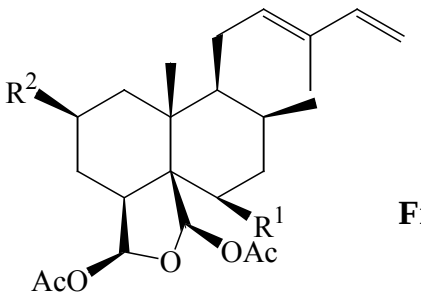
fruticolone



isofruticolone



fruticolide



bucidarasins A; R_1=OH R_2;

bucidarasins B; R_1=OH R_2;

bucidarasins C; R_1=H R_2;

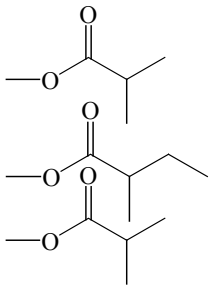
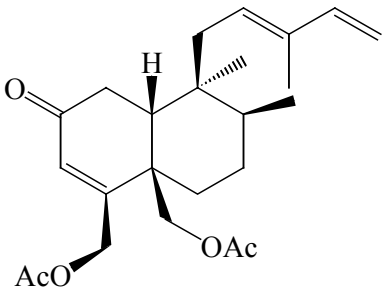
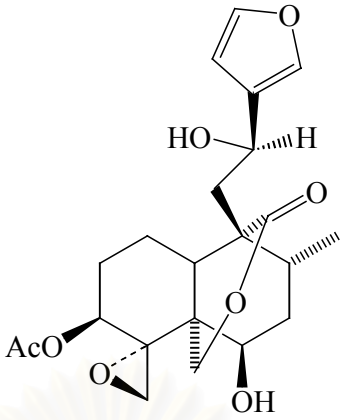


Figure 2 Continued.



bucidarasins D



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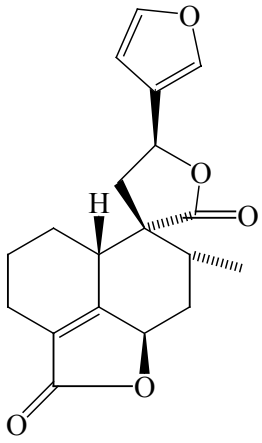
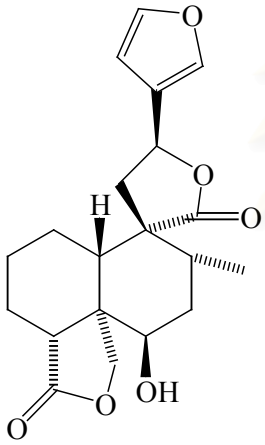
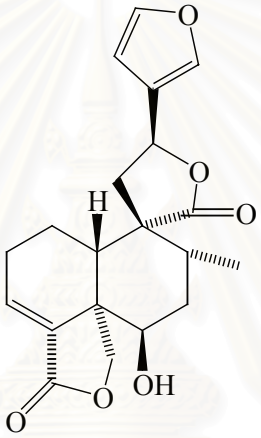


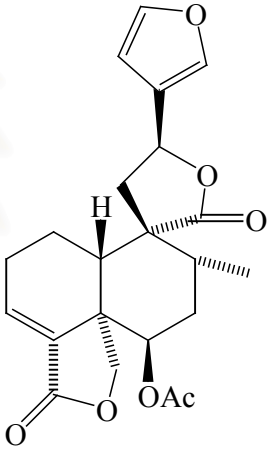
tueflin



teucrin-H_2



6β-hydroxyteuscordin



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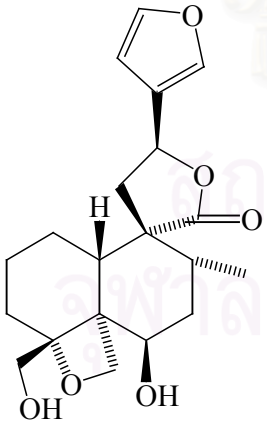
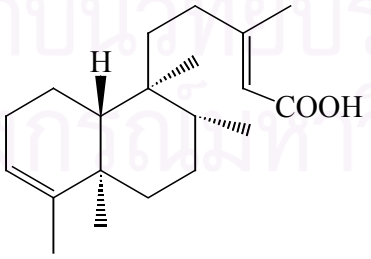
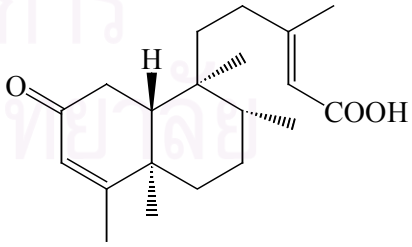


montanin-D



kolavenic acid



2-oxo-kolavenic acid

Figure 2 Continued.

CHAPTER III
EXPERIMENTAL

3.1 Instrument and Equipments

2.1.1 Fourier Transform infrared spectrophotometer (FT-IR)

The infrared spectra were recorded on a Nicolet Impact 410 Fourier Transform Infrared Spectrophotometer. Spectra of solid samples were recorded as KBr pellets and liquid samples as thin film on NaCl cells.

2.1.2 Mass Spectrometer (MS)

The mass spectra were recorded on a Fisons Instruments Mass Spectrometer model Trio 2000 GC-MS in Electron Impact (EI) mode at 70 eV.

2.1.3 Ultraviolet-Visible Spectrophotometer (UV-Vis)

The UV-Vis spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer in chloroform.

2.1.4 ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectrometer (NMR)

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 spectrometer operated at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei. Moreover COSY, NOESY, HSQC and HMBC experiments were performed on this spectrometer.

2.1.5 Polarimeter

The optical rotation was measured on a Perkin-Elmer 341 polarimeter in $CHCl_3$.

3.2 Isolation of crovatin from *C. oblongifolius* Roxb.

3.2.1 Plant material

The stem barks of *C. oblongifolius* were collected from Ampur Sai Yok Kanchanaburi Province, Thailand, in June, 1999. Botanical identification was claimed through comparison with voucher specimen no. BKF084729 in the herbarium of the Royal Forest Department of Thailand.

3.2.2 Extraction and Isolation

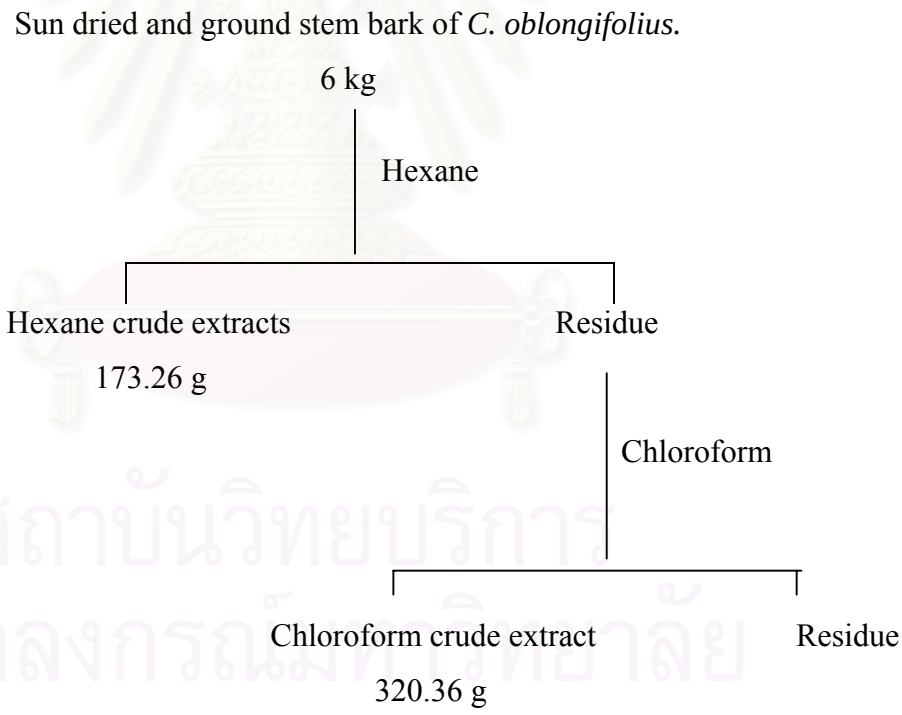
The powder, sun-dried stem barks (6 kg) of *C. oblongifolius* was soaked in hexane (6 liters) at room temperature for 2 weeks. The hexane solution was filtered and the filtrated evaporated under reduced pressure to obtain a dark-red gummy residue. The residue was extracted again with chloroform (6 liters) for 2 weeks. The

extract of chloroform was filtered and then evaporated the solvent under reduced pressure to obtain a dark-red gummy residue.

The different crude extracts of the stem barks of *C. Oblongifolius* are shown in Table 2 and the extraction procedure is shown in Scheme 2.

Table 2 The various extracts of the stem bark of *C. oblongofolius.*

solvent extract	**appearance**	**weight (g)**	**% wt/wt of the dried stem bark**
hexane	dark-red gummy	173.26	2.89
$CHCl_3$	dark-red gummy	320.36	5.34



Scheme 2 The extraction procedure of the stem barks of *C. Oblongifolius*.

3.2.3 Isolation of Crovatin from hexane crude extract

The hexane crude extract (50 g) was isolated by column chromatography. The column was packed with silica gel 60 (Art 7734.1000, 70-230 mesh ASTM, 750 g) as adsorbent and the crude extract was dissolved in a small amount of chloroform and

then mixed with silica gel (20 g) to afford the extract paste. The paste was evaporated to dryness under reduced pressure before being placed on top of column. The column was eluted with hexane:chloroform gradient in a stepwise fashion (1-100% chloroform) followed by final elution a 10 % methanol in chloroform (1000 ml). Each fraction (250 ml) was collected, then checked by TLC in order to combine the fractions which had the same TLC pattern and the solvents were removed by rotary evaporation. Compound **1** was obtained at elution with 20 % chloroform in hexane (Fraction 29-32). Compound **1** was crystallized from chloro and hexane to give 0.2573 g, as colorless crystals. The results of isolation of hexane crude extract were shown in Table 3.

Table 3 The results of isolation of hexane crude extract by column chromatography

Fraction No.	Eluent (% by volume)	Remark	Weight (g)
1-5	100 % hexane	Yellow oil	1.9558
6-8	3 % $CHCl_3$/hexane	Orange oil	2.7013
9-13	5-10 % $CHCl_3$/hexane	Yellow oil	3.7532
14-18	15 % $CHCl_3$/hexane	Yellow oil	1.9586
19-23	20 % $CHCl_3$/hexane	Yellow oil	2.1042
24-28	20 % $CHCl_3$/hexane	Green oil	1.7192
29-32	20 % $CHCl_3$/hexane	Colorless crystal	0.5227
33-37	30 % $CHCl_3$/hexane	Yellow oil	1.3274
38-41	40 % $CHCl_3$/hexane	Yellow oil	9.2593
42-45	50 % $CHCl_3$/hexane	Yellow oil	8.7619
46-48	70 % $CHCl_3$/hexane	Yellow oil	5.6139
49-51	90 % $CHCl_3$/hexane	Yellow tar	1.2638
52-57	100 % $CHCl_3$	Brown tar	2.3674
58-61	10 % MeOH: $CHCl_3$	Brown tar	2.1163

The remained hexane extract crude was purified by the same procedure. The results of isolation of hexane extract crude were shown in Table 4.

Table 4 The results of separation of the rest hexane crude extract by column chromatography

crude hexane (g)	Compound **1** (g)
50	0.2139
50	0.2255

The separation of 150 g of hexane extract crude could give total crovatin 0.6967 g which then was calculated into percent by comparison with the weight of the stem bark of *C. oblongifolius* equal 1.2 x 10^{-4} %

3.2.4 Isolation of Crovatin from chloroform extract crude

Concentrated chloroform crude extract (60 g) was isolated on silica gel 60 (Art 7734.1000, 70-230 mesh ASTM; 800 g) using column chromatography technique. The column was eluted with hexane:chloroform gradient is a stepwise with fashion (1-100% chloroform) followed by a methanol wash. The eluted fraction was collected about 250 ml and then checked by TLC. Similar fractions were combined and the solvents were removed by rotary evaporation. Crovatin was obtained at elution with 20% chloroform in hexane (Fraction 25-28) to give 0.3215 g of crovatin **1**, as colorless crystals. The results of separation of chloroform extract crude were shown in Table 5.

Table 5 The results of separation of chloroform crude extract by column chromatography

Fraction No.	Eluent (% by volume)	Remark	Weight (g)
1-3	100 % hexane	Yellow oil	1.5223
4-7	3 % $CHCl_3$/hexane	Orange oil	2.5113
8-11	5+10 % $CHCl_3$/hexane	Yellow oil	3.5231
12-16	15 % $CHCl_3$/hexane	Yellow oil	1.7882
17-20	20 % $CHCl_3$/hexane	Yellow oil	3.2349
21-24	20 % $CHCl_3$/hexane	Green oil	2.3645
25-28	20 % $CHCl_3$/hexane	Colorless crystal	0.7862
29-33	30 % $CHCl_3$/hexane	Yellow oil	1.6358

34-40	40 % $CHCl_3$/hexane	Yellow oil	13.2013
41-45	50 % $CHCl_3$/hexane	Yellow oil	10.0027
46-49	70 % $CHCl_3$/hexane	Yellow oil	4.9678
50-52	90 % $CHCl_3$/hexane	Yellow tar	2.6574
53-55	100 % $CHCl_3$	Brown tar	3.9823
56-60	10 % MeOH: $CHCl_3$	Brown tar	3.0067

The remained chloroform extract crude was purified by the same procedure. The results of separation of chloroform crude extract are shown in Table 7.

Table 6 The results of isolation of the rest chloroform crude extract by column chromatography

crude hexane (g)	Colorless crystal (g)
60	0.3136
60	0.3255
60	0.3310
60	0.3357

The isolation of 300 g of chloroform extract crude could give 1.6273 g of total crovatin which then calculate into percent by compare with the weight of the stem bark of *C. oblongifolius* equal 2.7 x 10^{-4} %

Compound 1

R_f = 0.63 (siliga gel TLC, EtOAc:hexane (2: 3), $[\alpha]_D^{20}$ -52 ° (c = 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) : 241; mp 160.8 °C

FT-IR spectrum (Figure 1); ν_{max} (neat) / cm^{-1} 2950 (m), 2866 (m), 1734 (C=O) (s), 1442 (m), 1198 (m), 1169 (m), 1023 (m)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.) (Figure 3)δ 0.98 (d, 3H, 17-H), 1.49 (m, 1H, 7-H_a), 1.49 (m, 1H, 6-H_a), 1.62 (m, 1H, 2-H_a), 1.78 (m, 1H, 8-H), 1.75 (m, 1H, 7-H_b), 1.91 (m, 1H, 1-H_a), 1.91 (m, 1H, 2-H_b), 2.02 (m, 1H, 6-H_b), 2.09 (m, 1H, 11-H_a), 2.20 (m, 1H, 11-H_b), 2.31 (m, 1H, 1-H_b), 2.41 (m, 1H, 10-H), 2.88 (d, 1H, 4-H), 4.54 (d, 1H, 3-H), 5.17 (s,1H, 19-H), 5.31 (s, 1H, 20-H), 5.37 (t, 1H, 12-H), 6.43 (s, 1H, 14-H), 7.41 (d, 1H, 16-H), 7.43 (d, 1H, 15-H)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.), (Figure 4); δ 16.94 (CH_3), 20.21 (CH_2), 26.52 (CH_2), 30.46 (CH_2), 31.49 (CH_2), 37.42 (CH), 38.56 (CH_2), 38.56 (CH), 44.29 (C), 50.32 (C), 53.99 (CH), 75.96 (CH-O), 75.74 (CH-O), 100.73 (O-CH-O), 104.36 (O-CH-O), 108.63 (CH), 127.09 (C), 139.35 (CH), 143.50 (CH), 740.26 (C=O)

3.3 The hydrolysis reaction of crovatin 1 under acidic condition

A solution of crovatin **1** in 1.5 ml of 2 M acids in methanol was refluxed at 80 ^{0}C. The resulting solution was monitored by TLC. The resulting solution was quenched with 15 mL of saturated sodium hydrogen carbonate. The reaction mixture was extracted twice with 15 mL of ethyl acetate. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate and filtered. The filtrate solvent was removed the solvent by rotary evaporation. The crude product was purified on column chromatography (silica gel 60 Art 1.09385.1000).

Crovatin (mg)	mmol	Acids (2 M)	Time (h)	Product (mg)
20.6	0.0548	H_2SO_4	8	mixture
25.3	0.0673	HCl	8	mixture
24.3	0.0643	*p*-TsOH	8	mixture
25.0	0.0665	CH_3COOH	8	no reaction

3.4 The hydrolysis reaction of crovatin 1 under basic condition

A solution of crovatin **1** in 2 ml of 2 M bases in methanol was refluxed at 80 ^{O}C. The reaction was monitored by TLC. The resulting solution was added 15 ml of 10 % HCl. The reaction mixture was extracted twice with ethyl acetate and then the combined organic layer was washed with brine, and dried over anhydrous sodium sulfate. The dried organic layer was filtered and the solvent was removed by rotary evaporation. The crude reactions were determined the ratio of product compounds by ^{1}H-NMR integration. The crude product was purified by column chromatography (silica gel Art 1.09385.1000) to afford compound **2** and compound **3,** as a white solid.

Crovatin (mg)	mmol	Base (2 M)	Time (h)	Ratio **2:3**	Product (mg)		% Yield	
					2	**3**	**2**	**3**
43.4	0.1161	NaOH	4.5	1:1	0.0119	0.0179	28	41
50.0	0.1337	KOH	4.5	1:1	0.0183	0.0189	40	41

Compound 2

R_f = 0.50 (siliga gel TLC, chloroform as mobile phase), $[\alpha]_D^{20}$ = -38 ° (c = 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) : 242; mp 247.0 °C

FT-IR spectrum (Figure 9); ν_{max} (neat) / cm $^{-1}$ 3471 (OH) (br), 2922 (m), 2867 (m), 1735 (C=O) (s), 1446 (m), 1385 (m), 1159 (m), 1019 (m)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz), (Figure 11);δ 0.98 (d, 3H, 17-H), 1.41 (m, 1H, 7-H_a), 1.51 (m, 1H, 6-H_a), 1.76 (m, 1H, 2-H_a), 1.77 (m, 1H, 8-H), 1.78 (m, 1H, 7-H_b), 1.93 (m, 1H, 1-H_a), 2.07 (m, 1H, 2-H_b), 2.15 (m, 1H, 6-H_b), 2.18 (m, 1H, 11-H_a), 2.27 (m, 1H, 11-H_b), 2.38 (m, 1H, 1-H_b), 2.40 (m, 1H, 10-H), 2.95 (d, 1H, 4-H), 4.61 (m, 1H, 3-H), 5.19 (s,1H, 19-H), 5.32 (s, 1H, 20-H), 5.38 (dd, 1H, 12-H), 6.43 (s, 1H, 14-H), 7.41 (d, 1H, 16-H), 7.43 (d, 1H, 15-H)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz), (Figure 12); δ 16.93 (CH_3), 20.12 (CH_2), 26.44 (CH_2), 30.42 (CH_2), 31.39 (CH_2), 37.43 (CH), 38.53 (CH_2), 38.81 (CH), 44.19 (C), 50.33 (C), 53.94 (CH), 75.00 (CH-O), 75.70 (CH-O), 100.72 (O-CH-O), 104.39 (O-CH-O), 108.60 (CH), 127.00 (C), 139.40 (CH), 143.56 (CH), 174.79 (C=O)

EI m/z (figure 10) ; 360 [M^+] (38), 315 (17), 314 (69), 285 (14), 257 (34), 239 (32) and 211 (51)

Compound 3

R_f = 0.38 (siliga gel as mobile phase, chloroform); $[\alpha]_D^{20}$= +15 ° (c = 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) : 243 ; mp 233.4 °C

FT-IR spectrum (Figure 17); ν_{max} (neat) / cm $^{-1}$ 3493 (OH) (br), 2922(m), 2875 (m), 1715 (C=O) (s), 1640 (m), 1447 (m), 1023 (m)

^{1}H-NMR spectrum, ($CDCl_3$, 400 MHz.), (Figure 19); δ 0.98 (d, 3H, 17-H), 1.35 (m, 1H, 2-H_a), 1.39 (m, 1H, 7-H_a), 1.60 (m, 1H, 6-H_a), 1.71 (m, 1H, 8-H), 1.81 (m, 1H,7-H_b), 1.97 (m, 1H, 1-H_a), 1.98 (m, 1H, 6-H_b), 2.15 (m, 1H, 2-H_b), 2.09 (m, 1H, 11-H_a), 2.19 (m, 1H, 11-H_b), 2.43 (m, 1H, 1-H_b), 2.49 (s, 1H, 4-H), 4.43 (m, 1H, 3-H), 5.33 (s, 1H, 20-H), 5.38 (dd, 1H, 12-H), 6.42 (d, 1H, 14-H), 7.41 (d, 1H, 15-H), 7.43 (d, 1H, 16-H)

^{13}C-NMR spectrum, ($CDCl_3$, 100 MHz.), (Figure 20); δ 16.98 (CH_3), 21.27 (CH_2), 29.53 (CH_2), 30.31 (CH_2), 30.93 (CH_2), 37.59 (CH), 38.36 (CH_2), 45.82 (C), 46.36 (C), 51.29 (C), 60.81 (CH), 75.24 (CH-O), 76.74 (CH-O), 101.18 (O-CH-O), 103.59 (O-CH-O), 108.60 (CH), 126.79 (CH), 139.40 (CH), 143.52 (CH), 176.45 (C=O)

EI m/z (Figure 18); 360 [M^+] (5), 314 (22), 296 (7) and 268 (9)

3.5 The reduction reaction of crovatin 1 with lithium aluminum hydride ($LiAlH_4$)

A solution of 108.1 mg (0.2875 mmol) of crovatin **1** in 2 ml of dry tetrahydrofuran under Argon was added 14.19 mg (0.3739 mmol) of $LiAlH_4$. The resulting solution was stirred at room temperature for 1 hour. Ethyl acetate was added to dilute the reaction mixture followed by H_2O to quench the reaction. The reaction mixture was extracted twice with 15 ml of ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and removed the solvent by rotary evaporation. The crude product was purified by column chromatography (silica gel Art 1.09385.1000) to afford 100 % (99.41 mg, 0.2873 mmol) yield of compound **4,** as colorless crystals.

Compound 4

R_f = 0.25 (siliga gel TLC, EtOAc:hexane (7: 3) as mobile phase); $[\alpha]_D^{20}$= +16 ° (c=1.0, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) : 241; mp 168.4 ^{O}C

FT-IR spectrum (Figure 25); ν_{max} (neat) / cm $^{-1}$ 3428 (OH) (br), 2922 (m), 2871 (m), 1104 (m), 1447 (m), 1030 (m), 988 (m)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.), (Figure 27) ;δ 0.97 (d, 3H, 17-H), 1.41 (m, 1H, 7-H_a), 1.52 (m, 1H, 6-H_a), 1.60 (m, 1H, 8-H), 1.71 (m, 1H, 2-H_a), 1.72 (m, 1H, 7-H_b), 1.74 (m, 1H, 10-H), 1.75 (m, 1H, 6-H_b), 1.89 (m, 1H, 1-H_a), 1.98 (m, 1H, 2-H_b), 2.16 (m, 1H, 11-H_a), 2.20 (m, 1H, 4-H), 2.25 (m, 1H, 11-H_b), 2.35 (m, 1H, 1-H_b), 3.82 (dd, 1H, 18-H_a), 3.83 (dd, 1H, 18-H_b), 4.33 (m, 1H, 3-H), 5.22 (s, 1H, 19-H), 5.31 (s, 1H, 20-H), 5.37 (dd, 1H, 12-H), 6.42 (d, 1H, 14-H), 7.42 (d, 1H, 16-H), 7.43 (d, 1H, 15-H)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.), (Figure 28);δ 16.95 (CH_3), 20.08 (CH_2), 24.77 (CH_2), 30.59 (CH_2), 30.02 (CH_2), 37.76 (CH), 38.50 (CH_2), 40.05 (CH), 43.73 (C), 50.75 (C), 51.31 (CH), 60.00 (CH_2-O), 75.20 (CH-O), 76.75 (CH-O),

100.86 (O-CH-O), 105.25 (O-CH-O), 108.62 (CH), 127.04 (C), 139.38 (CH), 143.50 (CH)

EI m/z (Figure 26); 346 [M^+] (29), 300 (17), 282 (6), 269 (7), 251 (6) and 179 (53)

3.6 The procedure for preparation of exo-cyclic double bond

2.7.1 Mesylation reaction of crovatin alcohol

` 81.3 mg (0.235 mmol) of compound **4** in 1.5 ml of dry dichloromethine under Argon was added (0.2820 mmol) of pyridine 23 µl, 15 mg (0.1637 mmol) of dimethylamino pyridine (DMAP), 22 µl (0.2820 mmol) of methansulfonyl chloride (MsCl) was stirred at room temperature for 48 hours. The reaction mixture was diluted with 10 ml ethyl acetate and then, was extracted with 15 ml of 10 % HCl. The ethyl acetate layer was washed with 15 ml saturated sodium hydrogen carbonate, followed with 15 ml brine and dried over anhydrous sodium sulfate. The mixture was filtered and the solvent removed by rotary evaporation. The crude product was purified by column chromatography (silica gel Art 1.09385.1000) to afford 98 % (98 mg, 0.2311 mmol) yield of compound **5**, as colorless oil.

Compound 5

R_f = 0.45 (siliga gel TLC, EtOAc:hexane (3:7) as mobile phase); $[\alpha]_D^{20}$ = +20° (c = 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) : 242

FT-IR spectrum (Figure 27);ν_{max} (neat) / cm^{-1} 2931 (m), 2875 (m), 1752 (C=O) (s), 1446 (m), 1352 (m), 1174 (m), 1024 (m), 955 (m), 878 (m)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.), (Figure 29); δ 0.98 (d, 3H, 17-H), 1.42 (m, 1H, 7-H_a), 1.55 (m, 1H, 6-H_a), 1.60 (m, 1H, 8-H), 1.64 (m, 1H, 2-H_a), 1.71 (m, 1H, 7-H_b), 1.72 (m, 1H, 10-H), 1.73 (m, 1H, 6-H_b), 1.89 (m, 1H, 1-H_a), 2.00 (m, 1H, 2-H_b), 2.17 (m, 1H, 11-H_a), 2.20 (m, 1H, 4-H), 2.27 (m, 1H, 11-H_b), 2.37 (m, 1H, 1-H_b), 3.09 (s, 3H, 21-H), 4.32 (m, 1H, 3-H), 4.39 (m, 2H, 18-H), 5.23 (s, 1H, 19-H), 5.31 (s, 1H, 20-H), 5.37 (dd, 1H, 12-H), 6.42 (d, 1H, 14-H), 7.41 (d, 1H, 16-H), 7.43 (d, 1H, 15-H)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz), (Figure 30) ; δ 16.87 (CH_3), 19.81 (CH_2), 24.59 (CH_2), 30.35 (CH_2), 31.93 (CH_2), 37.69 (CH), 38.46 (CH_2), 39.99 (CH), 43.96 (C), 48.00 (C), 50.74 (C), 66.33 (CH_2-O), 75.25 (CH), 76.05 (CH_3), 76.76 (CH), 100.80 (O-CH-O), 104.75 (O-CH-O), 108.56 (CH), 127.34 (C), 139.38 (CH), 143.55 (CH)

ESI m/z (Figure 28); 425 $[M+H]^+$

3.7.1 Elimination reaction of compound **5**

A solution of compound **5** (0.0663 mg, 0.1563 mmol) in 2 M KOH in methanol was refluxed at 80 ^{0}C for 24 hours and added 15 ml of 10 % HCl. The resulting solution was extracted twice with ethyl acetate and then, the combined organic layer was washed with brine, and dried over anhydrous sodium sulfate. The mixture was filtered and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography (silica gel Art 1.09385.1000) to give 95 % (51.1 mg. 0.1485 mmol) yield of compound **6**, as a colorless crystal.

Compound 6

R_f = 0.63 (siliga gel TLC, EtOAc: hexane (3: 7) as mobile phase); $[\alpha]_D^{20}$= +36° (c =0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) : 241; mp 141.8 °C

FT-IR spectrum (Figure 31); ν_{max} (neat) / cm $^{-1}$ 2926 (m), 2871 (m), 1669 (m), 1443 (m), 1158,(m), 1022 (m), 968 (m)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.), (Figure 33); δ 0.99 (d, 3H, 17-H), 1.44 (m, 1H, 7-H_a), 1.57 (m, 1H, 2-H_a), 1.65 (m, 1H, 8-H), 1.71 (m, 1H, 6-H_a), 1.81 (m, 1H, 10-H), 1.83 (m, 1H, 2-H_b), 84 (m, 1H, 7-H_b), 1.90 (m, 1H, 1-H_a), 2.23 (m, 1H, 6-H_b), 2.25 (M, 2H, 11-H), 2.53 (m, 1H, 1-H_b), 4.58 (dd, H, -H), 4.74 (d, 1H, 18-H_a), 4.91 (d, 1H, 18-H_b), 5.18 (d, 1H, 19-H), 5.37 (s, H, 20-H), 5.38 (dd, 1H, 12-H), 6.4 (d, 1H, 14-H), 7.14 (d, 1H, 16-H), 7.42 (d, 1H, 15-H)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.), (Figure 34); δ 17.00 (CH_3), 21.25 (CH_2), 28.15 (CH_2), 30.09 (CH_2), 34.32 (CH_2), 37.68 (CH), 38.59 (CH_2), 45.01 (C), 48.25 (CH), 50.10 (C), 74.07 (CH-O), 78.86 (CH-O), 100.57 (CH_2), 100.87 (O-CH-O), 103.89 (O-CH-O), 108.64 (CH), 127.46 (C), 139.23 (CH), 143.53 (CH), 156.26 (C)

ESI m/z (Figure 32); 329 $[M+H]^+$

3.7 The oxidation reaction of compound 4 with pyridinium chlorocromate (PCC)

38.4 mg (0.1110 mmol) of compound **4** was treated with a solution of 28.7 mg (0.1332 mmol) of pyridinium chlorocromate (PCC) in 1.5 mL of dichloromethine. The mixture was stirred at room temperature for 1 hour, and then worked up by the addition of ethyl acetate, filtration, and evaporation. The blank gummy residue was

purified by column chromatography to afford 94 % (36.1 mg, 0.1043 mmol) yield of compound **7**, as a white solid.

Compound 7

R_f = 0.25 (siliga gel TLC, $CHCl_3$:hexane (2:3); $[\alpha]_D^{20}$ = -26 (c = 1.0, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) : 242, mp; 161.8 ^{O}C

FT-IR spectrum (Figure 39); ν_{max} (neat) / cm $^{-1}$ 2926(m), 2867 (m), 1708 (C=O) (s), 1462 (m), 1381 (m), 1116 (m), 1015 (m)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHZ.), (Figure 41); δ 0.99 (d, 3H, 17-H), 1.42 (m, 1H, 7-H_a), 1.62 (m, 1H, 6-H_a), 1.71 (m, 1H, 8-H), 1.77 (m, 1H, 2-H_a), 1.82 (m, 1H, 7-H_b), 1.89 (m, 1H, 1-H_a), 2.00 (m, 1H, 6-H_b), 2.12 (m, 1H, 2-H_b), 2.20 (m, 1H, 10-H), 2.27 (m, 1H, 11-H), 2.45 (m, 1H, 1-H_b), 2.83 (d, 1H, 4-H), 4.63 (m, 1H, 3-H), 5.19 (s,1H, 19-H), 5.31 (s, 1H, 20-H), 5.38 (dd, 1H, 12-H), 6.42 (s, 1H, 14-H), 7.41 (d, 1H, 16-H), 7.43 (d, 1H, 15-H), 9.88 (d, 1H, 18-H)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.), (Figure 42); δ 16.88 (CH_3), 20.46 (CH_2), 27.16 (CH_2), 30.43 (CH_2), 31.92 (CH_2), 37.53 (CH), 38.48 (CH_2), 40.36 (CH), 45.44 (C), 50.71 (C), 61.21 (CH), 75.24 (CH-O), 75.36 (CH-O), 100.71 (O-CH-O), 104.29 (O-CH-O), 108.57 (CH), 126.79 (C), 139.42 (CH), 143.55 (CH), 200.66 (C=O)

ESI m/z (Figure 40); 347 $[M+H]^+$

3.8 Hydrolysis of compound 4 under acidic condition

Compound **4** in 1.5 ml of acids in methanol, the reaction mixture was refluxed at 80 ^{0} C. Reaction was monitored by TLC. The resulting solution was quenched with 15 mL of saturated sodium hydrogen carbonate. The reaction mixture was extracted twice with 15 mL of ethyl acetate. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate, filtered and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography (silica gel Art 1.09385.1000) to afford compound **8**, as colorless crystals.

Compound **4** (mg)	mmol	Acids	Time (h)	Product **8** (mg)	% Yield
28.3	0.0818	H_2SO_4 (2 M)	5	colorless crystal : 26.1	92
25.8	0.0746	H_2SO_4 (4 M)	3	colorless crystal : 23.0	89
22.7	0.0673	HCl (2 M)	7	colorless crystal : 12.7	56
29.7	0.0858	p-TsOH (2 M)	8	colorless crystal : 16.0	54

Compound 8

R_f = 0.40 (siliga gel TLC, EtOAc:hexane (7:3) as mobile phase); $[\alpha]_D^{20}$ = -20° (c = 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) : 242; mp 190.1 °C

FT-IR spectrum (Figure 47); ν_{max} (neat) / cm^{-1} 3428 (OH) (br), 2922 (m), 1762 (C=O) (s), 1456 (m), 1155 (m), 1028 (m)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.), (Figure 49); δ 0.95 (d, 3H, 17-H), 1.31 (m, 1H, 8-H), 1.44 (m, 2H, 7-H_a), 1.47 (m, 1H, 6-H_a), 1.63 (m, 1H, 6-H_b), 1.74 (m, 1H, 2-H_a), 1.75 (m, 1H, 10-H), 1.81 (m, 1H, 1-H_a), 1.86 (m, 1H, 11-H), 1.88 (m, 1H, 1-H_b), 1.90 (m, 1H, 7-H_b), 2.01 (m, 1H, 2-H_b), 2.27 (m, 1H, 4-H), 3.62 (d, 1H, 20-H_a), 3.76 (d, 1H, 20-H_b), 3.80 (d, 2H, 18-H), 4.54 (dd, 1H, 12-H), 4.68 (t, 1H, 3-H), 6.31 (d, 1H, 14-H), 7.30 (d, 1H, 15-H), 7.31 (d, 1H, 16-H)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.), (Figure 50); δ 17.15 (CH_3), 19.67 (CH_2), 22.39 (CH_2), 28.30 (CH_2), 30.24 (CH_2), 40.84 (CH), 41.87 (CH_2), 45.01 (CH), 45.6 (C), 50.48 (C), 51.29 (CH), 58.55 (CH_2-O), 67.01 (O-CH-O), 73.31 (CH), 76.66 (CH-O), 108.73 (CH), 126.05 (C), 139.30 (CH), 143.35 (CH), 179.43 (C=O)

ESI m/z (Figure 48); 347 $[M+H]^+$

The structure of compound **8** was supported by a single-crystal X-ray determination, as shown in Figure 3.

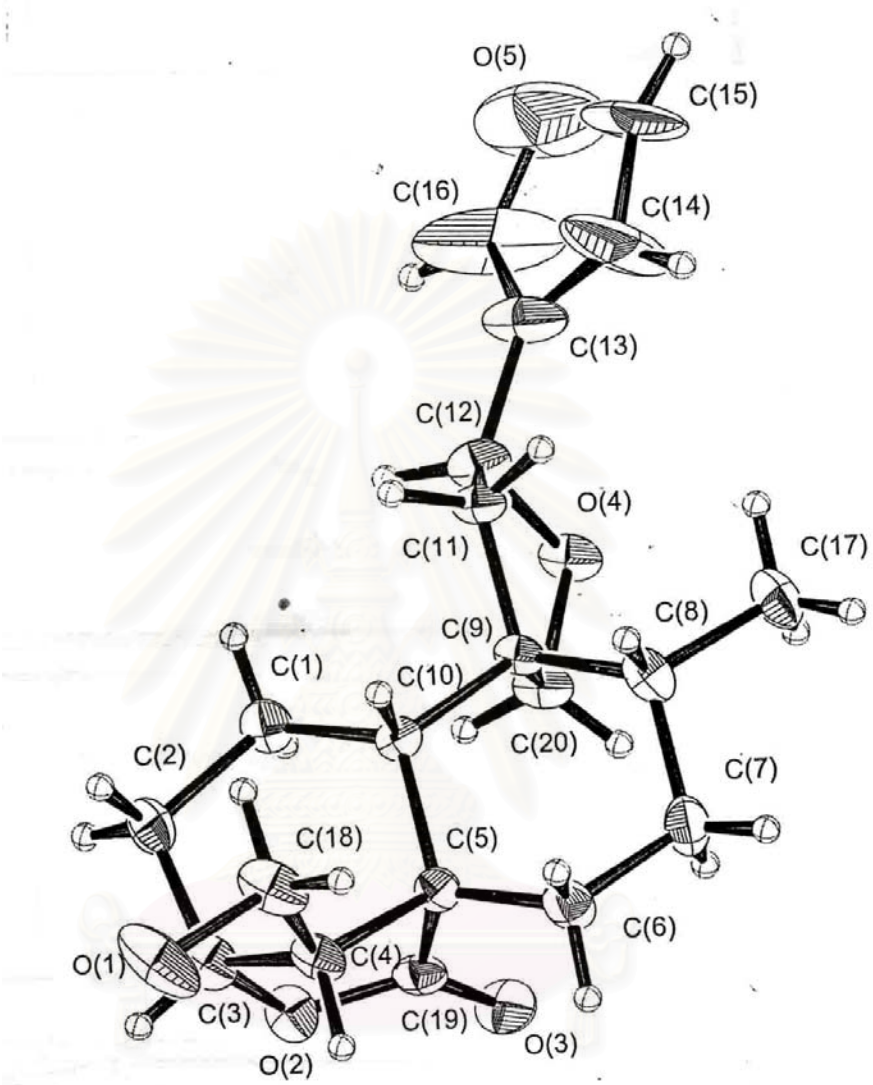


Figure 3 ORTEP diagrams of compound **8.**

CHAPTER IV
RESULTS AND DISCUSSION

The chemistry of crovatin and its derivatives were studied in this thesis. The resulting of reactions was characterized by IR, ^{1}H-NMR and ^{13}C-NMR and MS.

4.1 The structure elucidation of crovatin 1

The IR spectrum of crovatin **1** (Figure 17) is summarized in Table 7. The IR absorption bands at 2950 and 2866 cm^{-1} suggested methyl and methylene groups stretching vibration. The adsorption band at 1734 cm^{-1} indicated the carbonyl group of ester. The medium intensity absorption peak at 1442 cm^{-1} corresponded to C=C stretching vibration of a furan ring. Moreover, the absorption bands at 1198 and 1169 cm^{-1} and 1023 cm^{-1} were assigned as the O-C-O stretching vibration of a acetal group and C-O stretching vibration, respectively. The =C-H out of plane bending of furan occurred at 901 cm^{-1}.

Table 7 The IR absorption band assignment of compound **1**

Tentative assignments	Band type	Wave numbers
C-H stretching vibration of CH, CH_2, CH_3	b	2950, 2866
C=O stretching vibration of acid	s	1734
C=C stretching vibration	m	1442
O-C-O stretching vibration of acetal group	m	1198, 1169
C-O stretching vibration	m	1023
=C-H out-of-plane bending of furan	m	901

The ^{1}H-NMR spectrum (Figure 19, Table 8) of compound **1** showed three doublet of doublet signals at 7.43, 7.41 and 7.30 ppm which indicated the three olefinic protons of a furan ring. Moreover, the doublet of doublet signal was observed at 5.37 ppm which was assigned to a methine proton attaching between the furan ring and an oxygen atom. Two singlet signals at 5.31 and 5.17 ppm indicated two methine protons which attached to two oxygen atoms. The methine proton attaching to oxygen

atom appeared at 4.54 ppm. The signal at 3.74 ppm was assigned as the methoxy group and the methine proton which attached to a carbonyl group, respectively. Twelve protons were observed in the range of 2.46-1.37 ppm, as a multiplet signal.

The ^{13}C-NMR spectrum (Figure 20, Table 8) and HSQC (Figure 23) of compound **1** showed 21 lines. The spectrum illustrated the signal of a carbonyl carbon of ester at 170.26 ppm and the olefinic carbon signals at 143.50, 139.35, 127.09 and 108.63 ppm which indicated carbon atoms of a furan ring. The signal at 104.36 and 100.73 ppm were assigned to be the two methine carbons which attached to two oxygen atoms. The signals of two methine carbons also were found at 75.74 and 74.96 ppm, attached to oxygen atom. The signals at 50.32 and 44.29 indicated the signals of quaternary carbons. Moreover, the signals indicated the three methine carbons at 53.99, 38.81 and 37.42 ppm , five methylene carbons at 38.56, 31.49, 30.46, 26.52 and 20.21 ppm and two methyl groups at .51.70 and 16.94 ppm.

The relative configuration for all the nine asymmetric centers of crovatin was established by 2D NMR experiments (COSY; Figure 21 and NOESY; Figure 22). ^{1}H-^{1}H COSY showed the ^{3}J interaction between H-3 (δ 4.54) and H-4 (δ 2.83), H-12 (δ 5.37) and H $_{B}$-11, H $_{B}$-11 and H-10, H -14 (δ 6.43) and H-15 (δ 7.43) and a *W* type long range coupling between the equatorial protons between H-19 (δ 5.17) and H-10. NOESY revealed interactions between H-4 and H-19, H-19 and H-20 (δ 5.31), H-20 and Me-17 and H-12 and the equatorial proton H-1 (δ 2.31), and Me-17 and H-14 (δ 6.43)

From ^{1}H-NMR and ^{13}C-NMR spectral data, compound **1** was assigned as crovatin ^{5}(*ent*-(8R,10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18β-oate). The structure of crovatin **1** is shown in Figure 4.

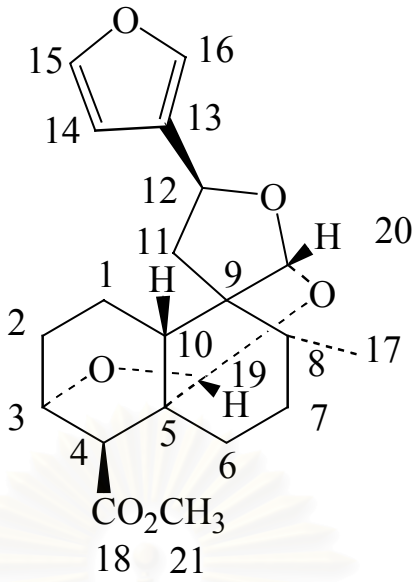


Figure 4 Structure of compound **1.**

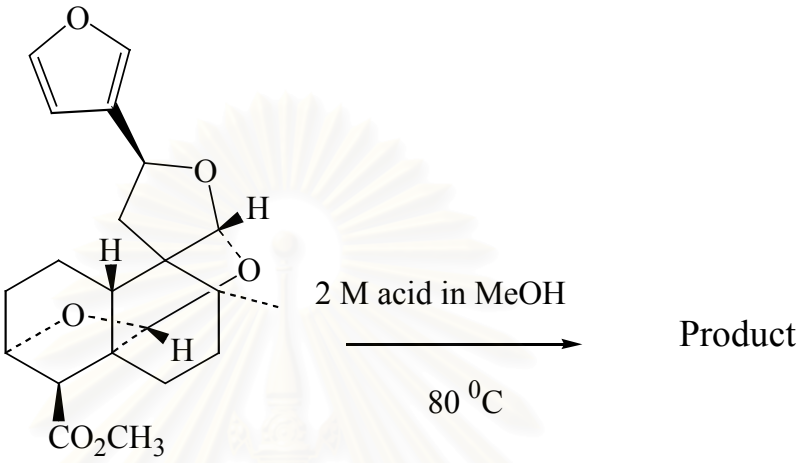
Table 8 The ^{1}H-^{13}C NMR, HMBC, COSY and NOESY spectral data of compound **1**

	δ_C	δ_H	HMBC (H to C)	COSY	NOESY
1	20.21t	A=1.91 (m)	C-2, C-3, C-5, C-10	$H_{A,B}$-2, H-10	H-12
		B=2.31 (m)	C-2, C-10	$H_{A,B}$-2, H-10	-
2	26.52t	A=1.62 (m)	C-1,	$H_{A,B}$-1	-
		B=1.91 (m)	C-3, C-4, C-10	$H_{A,B}$-1, H-4	-
3	75.74d	4.54 (m)	C-2, C-3, C-5, C-6, C-10, C-19	H-4	-
4	53.99d	2.88 (d) (J = 5.2)	C-1, C-4, C-5	H-3,H-2, H-19	H-19
5	44.29s	-	-	-	-
6	31.49t	A=1.49 (m)	C-4, C-5, C-7, C-8, C-10	H_A-7	-
		B=2.02 (m)	C-4, C-5, C-7, C-10	H_B-7	-
7	30.46t	A=1.38 (m)	C-6, C-8, C-17	H_A-6	-
		B=1.75 (m)	C-5, C-6, C-8, , C-9, C-17	H_B-6	-
8	37.42d	1.78 (m)	C-6, C-9, C-10, C-17	H-17	-
9	50.32s	-	-	-	-
10	37.81d	2.41 (m)	C-9, C-11	H_B-1, H-19	-
11	38.56t	A= 2.09 (m)	C-11, C-12	H_A-11	-
		B= 2.20 (m)	C-11, C-12	H-12	-
12	74.96t	5.37 (dd) (J = 8.4, 8.4)	C-11, C-13, C-14, C-16	H_{AB}-11	H-1B
13	127.09s	-	-	-	-
14	108.63d	6.43 (d) (J = 0.8)	C-13, C-15, C-16	H-15	H-17
15	143.50d	7.43 (d) (J = 0.8)	C-13, C-14, C-16	H-14	-
16	139.35d	7.41 (d) (J = 0.8)	C-13, C-14, C-15	-	-
17	16.94q	0.98 (d) (J = 6.4)	C-7, C-8, C-9	H-8	H-20
18	170.26s	-	-	-	-
19	104.36d	5.17 (s)	C-3, C-5, C-6, C-10, C-20	H-10	H-4, H-20
20	100.73d	5.31 (s)	C-8, C-9, C-10, C-12, C-19	-	H-19, H-17
21	51.70	3.74 (s)		-	-

Axial and equatorial conformations are expressed as A and B, respectively.

4.2 Reaction of crovatin 1 under acidic condition

The crovatin was hydrolyzed under acidic condition. The objective of this reaction is to break out the acetal ring by following conditions used by Hsin-Yi Chiu and co-workers.[13] Treatment of crovatin **1** with different acids at 80 ^{0}C for 8 hours.



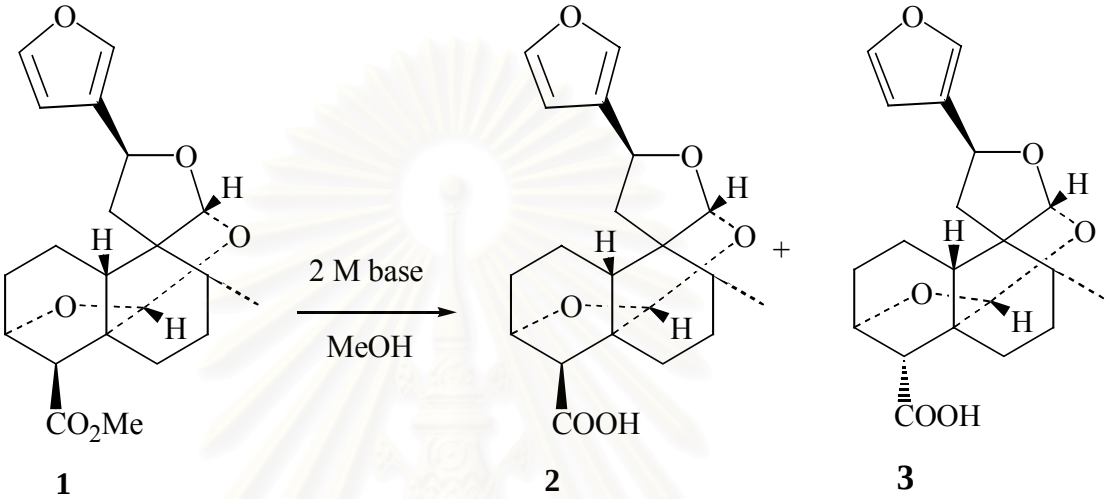
1

entry	Crovatin (mmol)	Acid (2 M)	Time (h)	Product (mg)
1	0.0548	H_2SO_4	8	mixture
2	0.0673	HCl	8	mixture
3	0.0643	*p*-TsOH	8	mixture
4	0.0665	CH_3COOH	8	no reaction

Among the various acids examined, the reaction was carried out in 1.8 M acid in methanol (H_2SO_4, HCl and *p*-TsOH) to afford many products (entry 1-3) which could not be isolated. The hydrolysis of crovatin under acetic acidic condition was not successful (entry 4). Result, indicated that strong acidic conditions gave very low selectivity of products. On the other hand, mind condition did not show any reaction (entry 4).

4.3 Reaction of crovatin under basic condition

The aim of this experiment is to change methyl ester of crovatin **1** to carboxylic acid and opened the acetal ring. The hydrolysis reaction of crovatin **1** was carried out under basic condition. Treatment of crovatin with 2 M NaOH or KOH in methanol at 80 ^{o}C for 4.5 hours afforded compound **2** and **3**.



1 **2** **3**

Crovatin (mg)	mmol	Base (2 M)	Time (h)	Ratio **2:3**	Product (mg)		% Yield	
					2	**3**	**2**	**3**
43.4	0.1161	NaOH	4.5	1:1	0.0119	0.0179	28	41
50.0	0.1337	KOH	4.5	1:1	0.0183	0.0189	40	41

The ratio of isomer mixture **2, 3** showed in equivalent 1:1 by ^{1}H-NMR integration in both NaOH and KOH crude reactions. After purify the crude reactions by column chromatography, the reaction was carried out in 2 M NaOH in methanol to obtained compound **2** in 28 % yield and compound **3** in 41 % yield. However, hydrolysis reaction carried out in 2 M KOH in methanol gave compound **2** in 40 % yield and compound **3** giving 41 % yields. From these reactions, the methyl ester group of crovatin was converted to carboxylic acid in compound **2** and its epimer (compound **3**)**.** The formation of compound **3** involved the epimerization. The carbonyl at C-18 was converted from axial to equatorial configuration.

The spectroscopic data clearly confirmed the structure of compound **2.** The IR spectrum of compound **2** is shown in Figure 25 and the absorption peaks are assigned

in Table 5. The IR spectrum revealed the presence of carboxyl group as evidenced by the absorption band at 1735 cm^{-1} of C=O stretching vibration peak and at 3641-3293 cm^{-1} of O-H stretching vibration of acid. The C-H bending vibration peaks of CH_2 and CH_3 were observed at 2922 and 2867 cm^{-1}. The medium intensity absorption peak at 1447 cm^{-1} corresponed to C-H bending vibration of CH_2 and CH_3. Moreover, the absorption band at 1387 cm^{-1} and 1019 cm^{-1} were assigned as the O-C-O stretching vibration of a acetal group and C-O stretching vibration, respectively. The =C-H out of plane bending of furan occurred at 929 cm^{-1}.

Table 9 The IR absorption band assignment of compound **2**

Tentative assignments	Band type	Wave numbers
O-H stretching vibration of acid	b	3641-3293
C-H stretching vibration of CH_2, CH_3	m	2922, 2867
C=O stretching vibration of acid	s	1735
C-H bending vibration of CH_2,CH_3	m	1447
O-C-O stretching vibration of acetal group	m	1387
C-O stretching vibration	m	1019
=C-H out-of-plane bending of furan	m	929

The ^{1}H-NMR spectrum (Figure 27, Table 10) of compound **2** showed three doublet signals at 7.41, 7.43 and 6.43 ppm which indicated protons of a furan ring. The signal at 5.38 ppm indicated the doublet of doublet of methine proton which attached to a furan ring and an oxygen atom. Two singlet signals at 5.19 and 5.32 ppm represented the two protons of methine groups which attached to two oxygen atom. Methine proton attached to oxygen atom was abserved in the multiplet signal at 4.61 ppm.

The ^{13}C-NMR (Figure 28, Table 10) and HSQC (Figure 31) spectrum of compound **2** showed the carboxyl carbon 174.79 ppm and the olefinic carbon signals at 149.56, 139.40, 127.00 and 108.60 ppm which indicated carbon of a furan ring. The signal at 104.39 and 100.72 ppm were assigned to the two methine carbons which attached to two oxygen atom. The signals of two methine carbons attached to oxygen atom also found at 75.70 and 75.00 ppm.

Comparison of ^{1}H-NMR spectrum of compound **2** with that of compound **1** demonstrated that the signal of a methyl proton at 3.39 ppm of compound **1** disappeared in ^{1}H-NMR spectrum of compound **2**.

Comparison of ^{13}C-NMR spectrum of compound **2** with that of compound **1** indicated that the signal of a methyl group at 51.7 ppm of compound **1** disappeared in ^{3}C-NMR spectrum of compound **2**. Moreover, the signal at 170.2 ppm of compound **1** converted to 174.79 ppm in ^{13}C-NMR spectrum of compound **2.**

The relative configuration for all the nine asymmetric centers of crovatin was established by 2D NMR experiments (COSY; Figure 29 and NOESY; Figure 30). ^{1}H-^{1}H COSY showed the ^{3}J interaction between H-3 (δ 4.61) and H-4 (δ 2.95), H-12 (δ 5.38) and H_B-11 (δ 2.20), H -14 (δ 6.43) and H-15 (δ 7.43), H_B-11 and H-10 (δ 2.40). NOESY revealed interactions between H-3 and H-4, H-4 and H-19 (δ 5.19) and H-4 and H_A-6 (δ 2.15).

The mass spectrum (Figure 26) of compound **2** showed the molecular ion peak at m/z 360, 315, 314, 285, 257, 239 and 211. These ion peaks were assigned as $C_{20}H_{24}O_6^+$, $C_{19}H_{22}O_4^+$, $C_{19}H_{22}O_4^+$, $C_{17}H_{17}O_4^+$, $C_{16}H_{17}O_3^+$, $C_{16}H_{15}O_2^+$ and $C_{15}H_{15}O^+$, respectively.

From the spectroscopic data, it can be concluded that compound **2** was *ent*-(8R, 10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18β-oic acid. The structure of compound **2** is shown in Figure 5.

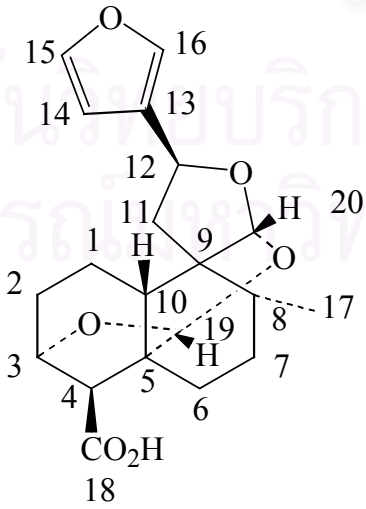


Figure 5 Structure of compound **2.**

Table 10 The ^{1}H-^{13}C NMR, HMBC, COSY and NOESY spectral data of compound **2**

	δ_C	δ_H	HMBC (H to C)	COSY	NOESY
1	20.12t	A=1.93 (m)	C-2, C-5, C-9, C-19	$H_{A,B}$-2, H-10	$H_{A,B}$-2
		B=2.38 (m)	-	H-10, $H_{A,B}$-2	-
2	26.44t	A=1.76 (m)	C-1, C-3, C-4, C-10	$H_{A,B}$-1	-
		B=2.07 (m)	-	H-3, $H_{A,B}$-1	H_B-1
3	75.70d	4.61 (dd) (*J* = 4.4, 0.5)	C-1, C-5, C-19	H-4, $H_{A,B}$-2	H-4
4	53.94d	2.95 (d) (*J* = 6)	C-2, C-3, C-5, C-6, C-10, C-18	H-4	H-3, H-19
5	44.19S	-	-	-	-
6	30.42t	A=1.41 (m)	C-4, C-5, C-7, C-8, C-10, C-19	H_A-7	H-4, H-11
		B=1.78 (m)	-	H_B-7	H_B-7
7	31.39t	A=1.51 (m)	C-5, C-6, C-8, C-9, C-17	H_A-6	-
		B=2.15 (m)	-	H_B-6	H_B-6
8	37.43d	1.77 (m)	C-9, C-10, C-11, C-17, C-20	H-17	H-17
9	50.33s	-	-	-	-
10	38.81d	2.40 (m)	C-1, C-2, C-5, C-9, C-19, C-20	$H_{A,B}$-1	H-19
11	38.58t	A=2.18 (m)	C-8, C-9, C-10, C-12, C-13,	H-12	H_A-6, H-12
		B=2.27 (m)	C-20	-	H-12
12	75.00d	5.38 (dd) (*J* = 7.6, 7.6)	C-9, C-11, C-13, C-14, C-20	$H_{A,B}$-11	$H_{A,B}$-12
13	127.00s	-	-	-	-
14	108.60d	6.43 (d) (*J* = 0.8)	C-13, C-15, C16	H-15	H-15
15	143.56d	7.43 (d) (*J* = 1.6)	C-13, C-14, C-16	H-14	H-14
16	139.40d	7.41 (d) (*J* = 1.6)	C-13, C-14, C-15	-	-
17	16.93q	0.98 (d) (*J* = 6)	C-7, C-8, C-9	H-8	H-8, H-20
18	174.79s	-	-	-	-
19	104.89d	5.19 (s)	C-3, C-5, C-6, C-10, C-20	-	H-4, H-10
20	100.72d	5.32 (s)	C-8, C-9, C-12, C-19	-	H-17

Axial and equatorial conformations are expressed as A and B, respectively.

The spectroscopic data clearly confirmed the structure of compound **3.** The IR spectrum of compound **3** is shown in Figure 33 and the absorption peaks are assigned in Table 11. The IR spectrum indicated hydroxyl group (O-H stretching vibration of acid) at 3688-3309 cm^{-1}, C-H stretching vibration of CH_2 and CH_3 at 2992, 2875 cm^{-1}, C=O stretching vibration of acid at 1715 cm^{-1}, C-H bending vibration vibration of CH_2,CH_3 at 1447 cm^{-1}, O-C-O stretching vibration of acetal group at 1169 cm^{-1}, C-O stretching vibration at 1023 cm^{-1} and =C-H out-of-plane bending of furan at 753 cm^{-1}

Table 11 The IR absorption band assignment of compound **3**

Tentative assignments	Band type	Wave numbers
O-H stretching vibration of acid	b	3688-3309
C-H stretching vibration of CH_2, CH_3	s	2922, 2875
C=O stretching vibration of acid	s	1715
C-H bending vibration vibration of CH_2,CH_3	s	1447
O-C-O stretching vibration of acetal group	m	1169
C-O stretching vibration	s	1023
=C-H out-of-plane bending of furan	m	753

The ^{1}H NMR spectrum (Figure 35, Table 12) of compound **3** showed three doublet signals at 7.33, 7.31 and 6.33 ppm which indicated protons of furan ring. The signal at 5.43 and 5.23 ppm presented the two singlet signals of two methine protons which attached to two oxygen atoms. The doublet of doublet signal at 5.29 ppm indicated the methine proton which attached to a furan ring and oxygen atom. Moreover, the signals at 4.43 and 2.49 ppm were assigned to the two methine protons which attached to oxygen atom and carbonyl carbon, respectively.

The ^{13}C-NMR (Figure 36, Table 12) and HSQC (Figure 39) spectrum of compound **3** showed the carboxyl carbon of acid signal at 176.45 ppm and the olefinic carbon signals at 143.52, 139.40, 126.79, and 108.60 ppm of a furan ring. Two signals appeared at 103.59 and 101.18 were assigned to the two methine carbons which attached to two oxygen atoms. The signals of two methine carbons attached to an oxygen atom also found at 76.74 and 75.24 ppm.

A compairison of ^{1}H-NMR spectrum of compound **3** with that of compound **1** illustrated that the signal at 3.69 ppm of a methyl group of compound **1** was

disappeared in ^{1}H-NMR spectrum of compound **3** and the position of protons singal in compound **1** at 5.31, 5.26, 5.11, 4.48 and 2.83 ppm converted to 5.43, 5.23, 4.43 and 2.95 ppm in spectrum of compound **3**, respectively.

In comparison of ^{13}C-NMR of compound **3** with that of compound **1,** the signal of methyl carbon at 51.7 ppm compound **1** disappeared in spectrum of compound 3**.** The signal of carbonyl carbon at 170.2 ppm in compound **1** was converted to 176.45 ppm in spectrum of compound 3**.** Moreover, the signals of methine carbons at 104.4, 100.7, 75.8 and 54.0 ppm in the spectrum of compound 1 was converted to 103.59, 101.18, 76.74 and 60.81 ppm in the spectrum of compound **3,** respectively.

The relative configuration for all the nine asymmetric centers of crovatin was established by 2D NMR experiments (COSY; Figure 37, HBMC; Figure 40 and NOESY; Figure 38). ^{1}H-^{1}H COSY showed the ^{3}J interaction between H-12 (δ 5.29) and H_A-11 (δ 2.09), H-12 and H_B-11 (δ 2.19), and H -14 (δ 6.33) and H-15 (δ 7.31). The correlations in HMBC spectrum (Figure 40) showed the interaction of compound **3**, as shown in Table 8 and Figure 6. NOESY revealed the interactions between H-4 (δ 2.49) and H_A-2 (1.35), H-4 and H-10 (δ 2.43) and H_A-7 (1.39) and H-20 (δ 5.33), as shown in Figure 7.

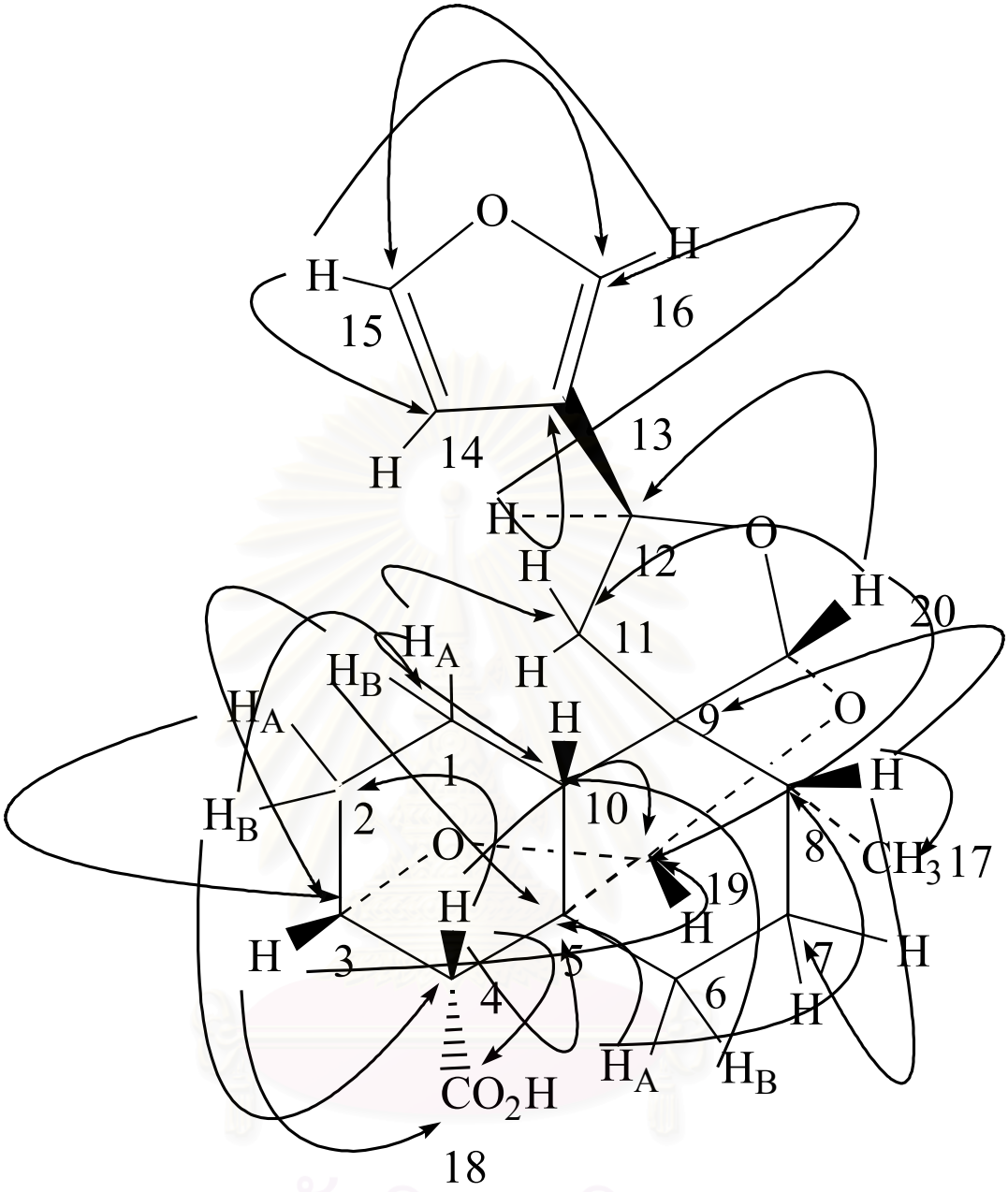


Figure 6 The HMBC correlation of compound **3.**

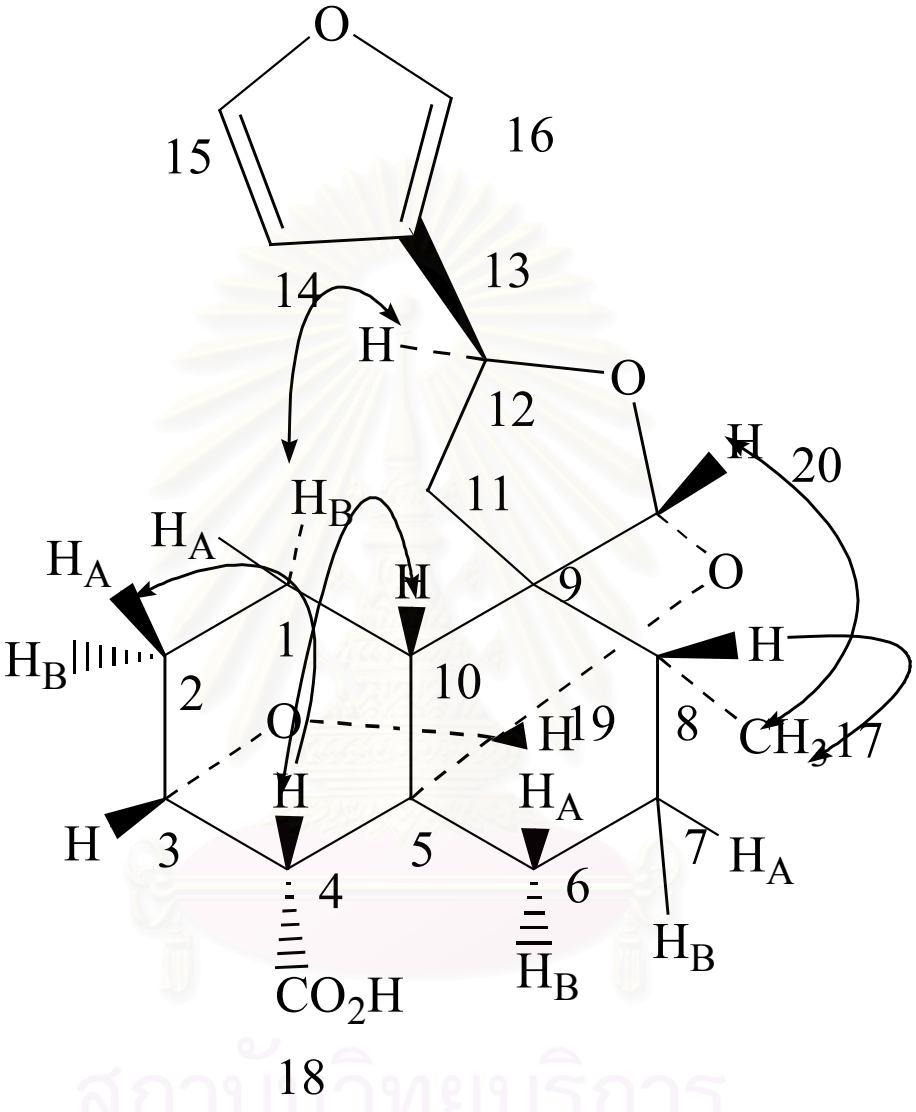


Figure 7 The NOESY correlation of compound **3.**

The mass spectrum (Figure 34) of compound **3** showed the molecular ion peak at m/z 360, 315, 314, 296 and 268. These ion peaks were assigned as $C_{20}H_{24}O_6^+$, $C_{19}H_{23}O_4^+$, $C_{19}H_{22}O_4^+$, $C_{18}H_{20}O_3^+$ and $C_{17}H_{20}O^+$, respectively

From the spectroscopic data, it can be concluded that compound **3** was *ent*-(8R, 10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18α-oic acid. The structure of compound **3** is shown in Figure 8.

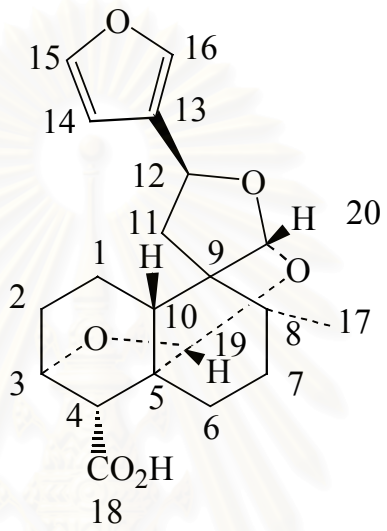


Figure 8 Structure of compound **3.**

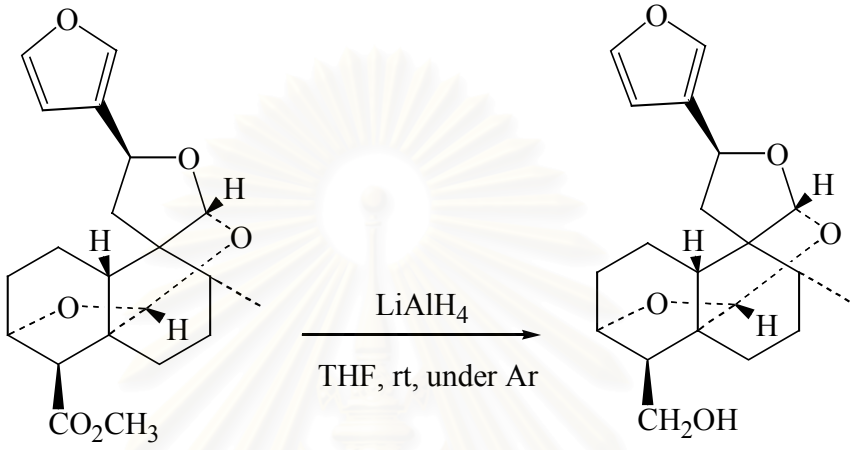
Table 12 The ^{1}H-^{13}C NMR, HMBC, COSY and NOESY spectral data of compound **3**

	δ_C	δ_H	HMBC (H to C)	COSY	NOESY
1	21.27t	A= 1.97 (m)	C-2, C-5, C-10	$H_{A,B}$-2, H-10	-
		B= 2.43 (m)	C-101	$H_{A,B}$-2, H-10	H-12
2	30.93t	A= 1.35 (m)	C-1, C-4	$H_{A,B}$-1	-
		B= 2.15 (m)	C-1, C-4	H-3	H-4
3	76.74 d	4.43 (m)	C-1, C-5, C-18, C-19	H_B-2	-
4	60.81d	2.49 (s)	C-2, C-5, C-18, C-19	-	H_A-2, H-10
5	46.36s	-	-	-	-
6	30.31t	A= 1.60 (m)	C-5, C-8	H_A-7	H-17
		B= 1.98 (m)	C-5, C-8, C-10	H_B-7	-
7	29.56t	A= 1.39 (m)	C-6, C-8, C-17	H_A-6	-
		B= 1.81 (m)	C-5, C-6, C-8, C-9	H_B-6	H-17, H-20
8	37.59d	1.71 (m)	C-7, C-9, C-10, C-20	H_A-7, H-17	-
9	51.29s	-	-	-	-
10	45.82d	2.43 (m)	C-5, C-11, C-19	$H_{A,B}$-1	H-4
11	38.36t	A= 2.09 (m)	C-8, C-9, C-10	H-12	H_B-11
		B= 2.19 (m)	C-8, C-9, C-10, C-12, C-13, C-20	H-12	H_A-11
12	75.24d	5.29 (dd) (J = 8.4, 8.4)	C-13, C-14, C-16	$H_{A,B}$-11	$H_{A,B}$-11
13	126.79s	-	-	-	-
14	108.60d	6.33 (d) (J = 1.2)	C-13, C-15, C16	H-15	-
15	143.52d	7.31 (d) (J = 0.8)	C-13, C-14, C-16	H-16, H-14	-
16	139.40d	7.33 (d) (J = 0.8)	C-13, C-14, C-15	-	-
17	19.98q	0.89 (d) (J = 6)	C-7, C-8, C-9	H-8	H-20
18	176.45s	-	-	-	-
19	103.59d	5.51 (s)	C-3, C-5, C-6, C-20	H-10	-
20	101.18d	5.33 (s)	C-8, C-9, C-10, C-12, C-19	-	H-17

Axial and equatorial conformations are expressed as A and B, respectively.

4.5 Reduction of crovatin 1

Compound **4** could be synthesized by reduction of crovatin **1**. The reaction between crovatin (1 equiv) and $LiAlH_4$ (1.2 equiv) was carried out in dry tetrahydrofuran under Argon at room temperature for 1 hour to afforded quantitative yield of compound **4**.



1 **4**

The spectroscopic data clearly confirmed the structure of compound **4.** The IR spectrum of compound **4** is shown in Figure 41 and the absorption peaks are assigned in Table 13. The IR spectrum revealed the presence of O-H at 3631-3165 cm^{-1}, C-H stretching vibration of CH_2, CH_3 at 2922 and 2871cm^{-1}, C-H bending vibration vibration of CH_2, CH_3 at 1469 cm^{-1}, O-C-O stretching vibration of an acetal group at 1326 cm^{-1}, C-O stretching vibration at 1104 cm^{-1} and =C-H out-of-plane bending of furan at 871 cm^{-1}.

Table 13 The IR absorption band assignment of compound **4**

Tentative assignments	Band type	Wave numbers
O-H stretching vibration of alcohol	b	3631-3165
C-H stretching vibration of CH_2, CH_3	s	2922, 2871
C-H bending vibration of CH_2,CH_3	s	1469
O-C-O stretching vibration of acetal group	m	1326
C-O stretching vibration	m	1104
=C-H out-of-plane bending of furan	m	871

^{1}H-NMR spectrum (Figure 43, Table 14) of compound **4** showed three doublet signals at 7.43, 7.42 and 6.42 ppm which indicated protons of a furan ring. The signal at 5.37 ppm assigned as the methine proton which attached to a furan ring and an oxygen atom. The signals of methine protons attached to the two oxygen atoms were observed at 5.31 and 5.22 ppm, respectively. The multiplet signal at 4.33 ppm indicated the methine proton which was attached to an oxygen atom.

The ^{13}C-NMR spectrum (Figure 44, Table 14) and HSQC (Figure 31) of compound **4** showed three methine carbons and a quaternary carbon at 143.5, 139.38, 108.62 and 127.40 ppm, respectively, indicating the carbons of a furan ring. The signal at 105.25 and 100.86 ppm indicated the two methine carbons, which attached to two carbon atoms. The signals of two methine carbons at 76.75 and 75.20 ppm were assigned to be the two carbons which attached to oxygen atoms.

In comparison of ^{1}H-NMR spectrum of compound **4** with that of compound **1**, the signal of methyl group at 3.69 ppm of compound **1** disappeared in ^{1}H-NMR spectrum of compound **4**. The signal of methylene proton at 6.82 ppm was increased in the spectrum of compound **4.**

In comparison of ^{13}C-NMR spectrum of compound **4** with that of compound **1,** the signal of carbonyl carbon at 170.2 ppm disappeared in the spectrum of compound **4** and the signal of methylene carbon at 59.99 ppm was observed.

The relative configuration for all the nine asymmetric centers of crovatin was established by 2D NMR experiments (COSY; Figure 45 and NOESY; Figure 46). ^{1}H-^{1}H COSY showed the ^{3}J interaction between H-3 (δ 4.33) and H-4 (δ 2.20), H-12 (δ 5.37) and H_B-11 (δ 2.25), H -14 (δ 6.43) and H-15 (δ 7.43) and H_B-11 and H-10 (δ 1.74). NOESY revealed interactions between H-4 and H_A-18 and H-4 and H_B-18 (δ 3.82, 3.83)

The mass spectrum (EI MS; Figure 42) of compound **4** showed the molecular ion peak at m/z 346, 300, 282, 269 and 251. These ion peaks were assigned as $C_{20}H_{26}O_5^+$, $C_{19}H_{24}O_3^+$, $C_{19}H_{22}O_2^+$, $C_{18}H_{21}O_2^+$ and $C_{18}H_{19}O^+$, respectively

From the spectroscopic data, it can be concluded that compound **4** was *ent*-(8R, 10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18β-ol.The structure of compound **4** was shown in Figure 9

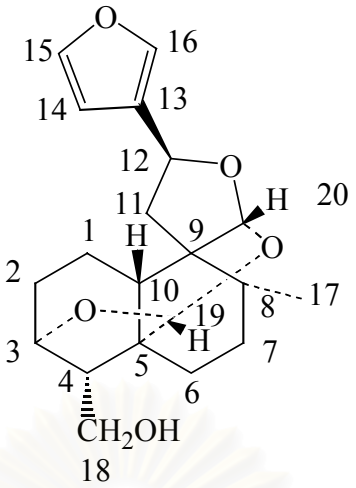


Figure 9 Structure of compound **4.**

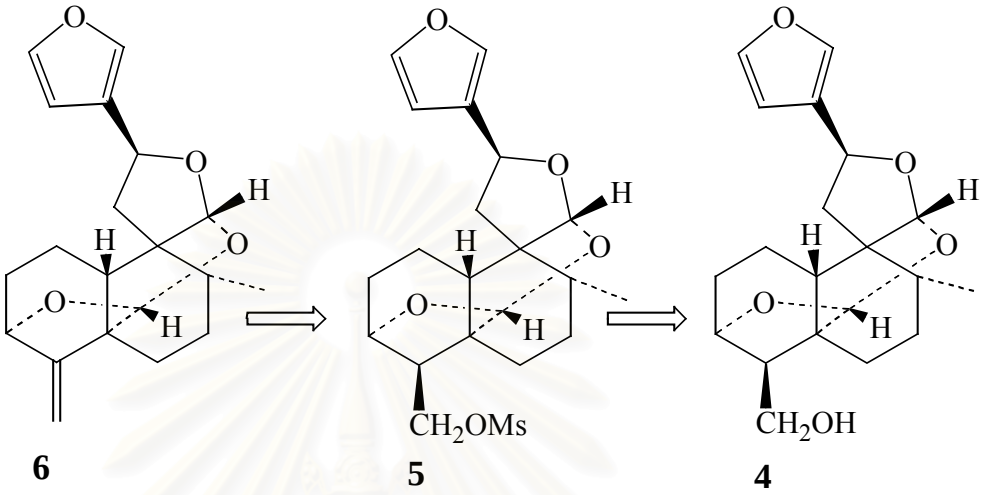
Table 14 The ^{1}H, ^{13}C, HMBC, COSY and NOESY spectral data of compound **4**

	δ_C	δ_H	HMBC (H to C)	COSY	NOESY
1	20.08t	A= 1.89 (m)	C-2, C-3, C-5, C-10	H-10	-
		B= 2.35 (m)	C-10	H_A-2, H-10	H_B-2
2	24.77t	A= 1.71 (m)	C-1, C-4, C-5	H_B-1,H-3	-
		B= 1.98 (m)	C-10	-	H_B-1
3	76.75d	4.33 (m)	C-5	H_B-2, H-4	-
4	51.31d	2.20 (m)	C-3, C-10, C-18	H-3, H-18	H-18
5	43.73s	-	-	-	-
6	32.02t	A= 1.52 (m)	C-4, C-5, C-7, C-8, C-10	-	-
		B= 1.75 (m)	C-4, C-5, C-7, C-8	-	-
7	30.59t	A= 1.41 (m)	C-6, C-17	-	-
		B= 1.72 (m)	C-6, C-9	-	-
8	37.76d	1.60 (m)	C-5, C-7, C-10, C-17	H-17	-
9	50.75s	-	-	-	-
10	40.05d	1.74 (m)	C-4, C-5	H_A-1, H_B-1	-
11	38.50t	A= 2.16 (m)	C-9, C-10	-	H-12
		B= 2.25 (m)	C-9, C-10	H-12	-
12	75.20d	5.37 (dd) (J = 6.8, 6.8)	C-14, C-16, C-20	H_B-11	H_A-11
13	127.40s	-	-	-	-
14	108.62d	6.42 (d) (J = 0.8)	C-13, C-15, C16	H-15	-
15	143.50d	7.43 (d) (J = 2.0)	C-13, C-14, C-16	H-14	-
16	139.38d	7.42 (d) (J = 2.0)	C-13, C-14, C-15	-	-
17	16.95q	0.97 (d) (J = 6.8)	C-7, C-8, C-9	H-8	-
18	56.99t	A= 3.82 (dd) (J = 10.4, 6.0)	C-3, C-4, C-5	H-4	H-4
		B= 3.83 (dd) (J = 10.8, 8.4)	C-3, C-4, C-5	H-4	H-4
19	100.86d	5.22 (s)	C-3, C-5, C-6, C-10	-	-
20	105.25d	5.31 (s)	C-8, C-9, C-12	-	-

Axial and equatorial conformations are expressed as A and B, respectively.

4.6 Preparation of exo-cyclic double bond (compound 6)

Preparation of exo-cyclic double bond (compound **6**) was envisioned that compound **6** cloud be obtained from compound **5** via a mesylation of compound **4,** as shown in Scheme 3.

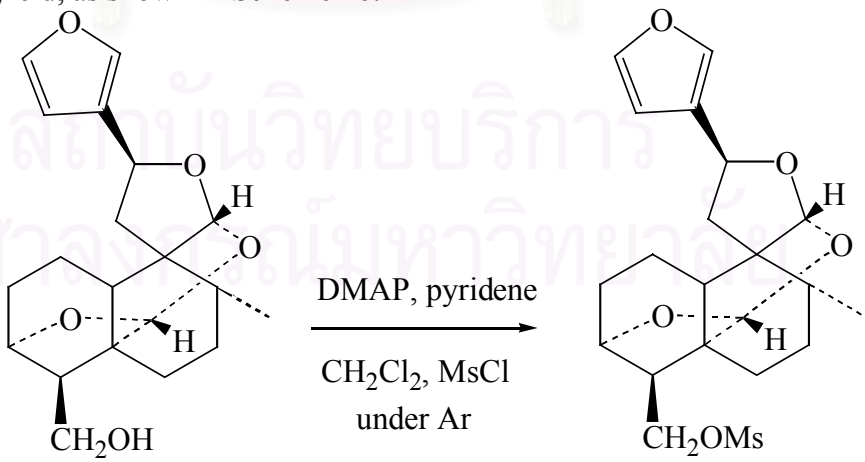


6 **5** **4**

Scheme 3

4.6.1 Mesylation reaction of compound 4

In this step, a hydroxyl group in compound **4** was converted to O-Mesyl group (OMs) in compound **5.** A solution of compound **4** (1 equiv) in dry dichloromethine under argon, was added of pyridine (1.2 equiv), DMAP (catalytic amount) and methinesulfonly chloride (MsCl) (1.2 equiv). The mixture was stirred at room temperature for 48 hours and after worked up was obtained compound **5** in quantitative yield, as shown in Scheme 16.



4 **5**

The spectroscopic data clearly confirmed the structure of compound **5.** The IR spectrum of compound **5** is shown in Figure 48 and the absorption peaks are assigned in Table 15. The IR spectrum at 2929 and 2878 cm^{-1} indicated the C-H stretching

vibration of CH_2, CH_3. The carbonyl group of S=O stretching vibration was observed at 1352 cm^{-1} and C-H bending vibration of CH_2, CH_3 was observed at 1470 cm^{-1}. Moreover, the IR spectrum revealed the presence of O-C-O stretching vibration of acetal group at 1174 cm^{-1}, C-O stretching vibration at 1024 cm^{-1} and the =C-H out-of-plane bending of furan at 958 cm^{-1}.

Table 15 The IR absorption band assignment of compound **5**

Tentative assignments	Band type	Wave numbers
C-H stretching vibration of CH_2, CH_3	b	2929, 2878
C-H bending vibration of CH_2,CH_3	s	1470
S=O asymmetric stretch vibration	s	1352
O-C-O stretching vibration of acetal group	s	1174
C-O stretching vibration	s	1024
=C-H out-of-plane bending of furan	s	958

The ^{1}H-NMR spectrum (Figure 50, Table 16) of compound **5** showed three doublet signals at 7.43, 7.42 and 6.42 ppm which indicated protons of a furan ring. The doublet of doublet signal at 5.37 ppm represented the methine proton which attached to a furan ring and an oxygen atom. Two signals at 5.31 and 5.22 ppm were assigned to the two methine protons which attached to carbon bearing two oxygen atoms. Moreover, the signal at 4.33 ppm indicated the methine proton which attached to carbon bearing an oxygen atom.

The ^{13}C-NMR (Figure 51, Table 16) spectrum of compound **5** showed the three methine carbons and one quaternary carbon of a furan ring at 143.50, 139.38, 127.40 and 108.62 ppm, respectively. Moreover, the signal of a methine carbon attached to an oxygen atom appeared at 76.75 ppm. The signal of 75.20 ppm were indicated the methine group which attached to furan ring and oxygen atom.

In comparison of ^{1}H-NMR spectrum of compound **5** with that of compound **4** indicated that compound **4** differed from compound **5** only in the signal of methylene proton at 3.82 ppm shifted to 4.39 ppm and increased the signal of methyl proton at 3.09 ppm.

In comparison of ^{13}C-NMR spectrum of compound **5** with that of compound **4,** the signal of methylene carbon at 59.99 ppm shifted to 66.33 ppm and appeared the signal of methyl carbon was at 76.05 ppm.

The mass spectrum (ESI MS; Figure 49) of compound **5** showed the molecular ion peak $[M+H]^+$ at m/z 425. The result indicated that the molecular weight of compound **5** was 424.

From the spectroscopic data, it could be concluded that the structure of compound **5** is shown below in Figure 10.

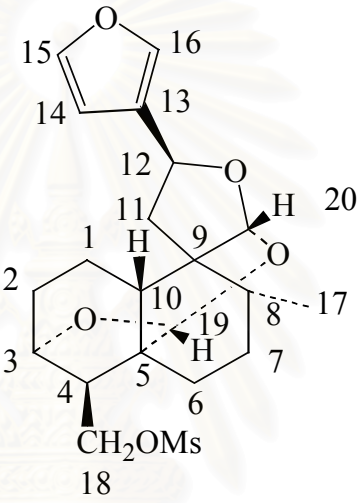


Figure 10 Structure of compound **5.**

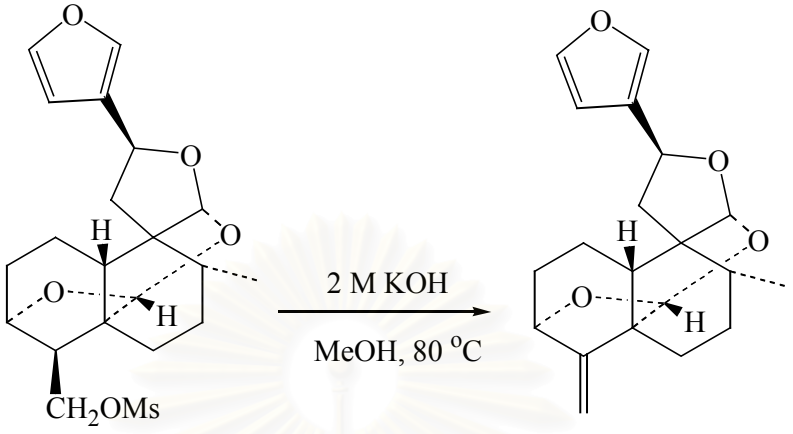
Table 16 The ^{1}H and ^{13}C NMR spectral data of compound **5**

position	δ_C	δ_H
1	19.81t	A=1.89 (m)
		B=2.37 (m)
2	24.59t	A=1.64 (m)
		B=2.00 (m)
3	76.76d	A=4.32 (m)
4	50.74d	B=2.20 (m)
5	43.96s	-
6	31.93t	A=1.55 (m)
		B=1.73 (m)
7	30.35t	A=1.42 (m)
		B=1.71 (m)
8	37.69d	1.60 (m)
9	48.00s	-
10	39.99d	1.72 (m)
11	38.46t	A=2.17 (m)
		B=2.27 (m)
12	75.25d	5.37 (dd) (*J* = 8.8, 8.8)
13	127.04s	-
14	108.56d	6.42 (d) (*J* = 1.7)
15	143.55d	7.43 (d) (*J* = 1.7, 1.7)
16	139.38d	7.41 (d) (*J* = 1.7, 1.7)
17	16.87q	0.98 (d) (*J* = 6.8)
18	66.33t	4.39 (m)
19	104.75d	5.23 (s)
20	100.80d	5.31 (s)
21	76.05q	3.09 (s)

Axial and equatorial conformations are expressed as A and B, respectively.

4.6.2. Elimination reaction of compound 5

The O-Mesyl group in compound **5** was eliminated with methanol containing 2 M KOH at 80 ^{0}C for 24 hours to obtaine compound **6** in quantitative yield.



5 **6**

The spectroscopic data clearly confirmed the structure of compound **6.** The IR spectrum of compound **6** is shown in Figure 52 and the absorption peaks assigned are shown in Table 17. The IR spectrum revealed the absorption band at 2931 and 2875 cm^{-1} of C-H stretching vibration of CH_2, CH_3 and the C=C stretching vibration at 1668 cm^{-1}. The C-H bending vibration peaks of CH_2, CH_3 were observed at 1443 cm^{-1}. Moreover, the absorption band at 1353 cm^{-1} and 1159 cm^{-1} were assigned to be the O-C-O stretching vibration of acetal group and C-O stretching vibration, respectively. The =C-H out of plane bending of furan occurred at 973 cm^{-1}.

Table 17 The IR absorption band assignment of compound **6**

Tentative assignments	Band type	Wave numbers
C-H stretching vibration of CH_2, CH_3	s	2926, 2871
C=C stretching vibration	s	1668
C-H bending vibration vibration of CH_2,CH_3	w	1443
O-C-O stretching vibration of acetal group	m	1353
C-O stretching vibration	s	1159
=C-H out-of-plane bending of furan	s	973

The ^{1}H NMR spectrum (Figure 54, Table 18) of compound **6** showed three doublet signals at 7.42, 7.41 and 6.41 ppm which indicated protons of a furan ring. The doublet of doublet signal at 5.3 ppm represented that methine proton which attached to a furan ring and an oxygen atom. Two signals at 5.37 and 5.18 ppm were assigned to the two methine protons which attached to carbon bearing two oxygen atoms. Moreover, the signal at 4.58 ppm was indicated to be the methine proton which attached to carbon bearing an oxygen atom.

The ^{13}C-NMR spectrum of compound **6** (Figure 55, Table 18) and HSQC (Figure 37) showed three methine carbons and one quaternary carbon of a furan ring, quaternary carbon and methine carbon of double bond according to the signals at 156.26, 143.5, 139.23, 127.4, 108.64and 100.57, respectively. Moreover, the signal of methine carbon attached to oxygen atom, appeared at 78.86 ppm. The signal of 74.07 ppm indicated the methine group which attached to a funran ring and an oxygen atom.

In comparison of ^{1}H-NMR spectrum of compound **6** with that of compound **5**, the signal of methine proton at 2.20 ppm and methylene proton at 4.39 ppm disappeared in the spectrum of compound **5** but the signal of two olefinic protons were obsearved at 4.74 and 4.91 ppm, which indicated the methylene proton of alkene.

In comparison of ^{13}C-NMR spectrum of compound **6** with that of compound **5**, the signal of methylene carbon at 66.33 ppm and methine carbon at 50.74 ppm disappeared in spectrum of compound **6.** The signals at 156.26 and 100.57 ppm in the spectrum of compound **6** were assigned to be olefinic carbons.

The relative configuration for all the nine asymmetric centers of crovatin was established by 2D NMR experiments (COSY; Figure 56 and NOESY; Figure 57). ^{1}H-^{1}H COSY showed the ^{3}J interaction between H-12 (δ 5.38) and H -11 (δ 2.25), H -14 (δ 6.41) and H-15 (δ 7.42), H -8 (δ 1.65) and H-17 (δ 0.99). NOESY revealed interactions between H-8 and H-17 and H-11 and H-12.

The mass spectrum (Figure 53) of compound **6** showed the molecular ion peak at m/z 329. The result indicated that the molecular weight of compound **6** was 328.

From the spectroscopic data, it could be concluded that compound **6** was *ent*-(8R, 10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-4(18),13(16),14-clerodatriene. The structure of compound **6** is shown in Figure 11.

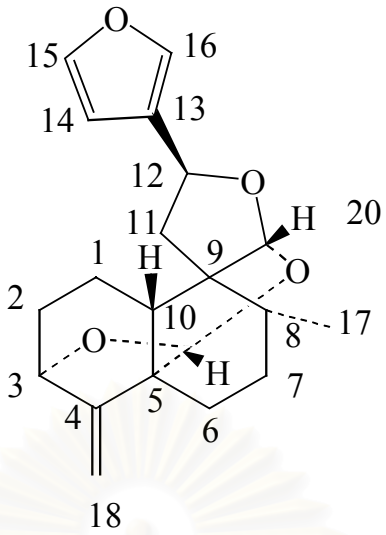


Figure 11 Structure of compound **6.**

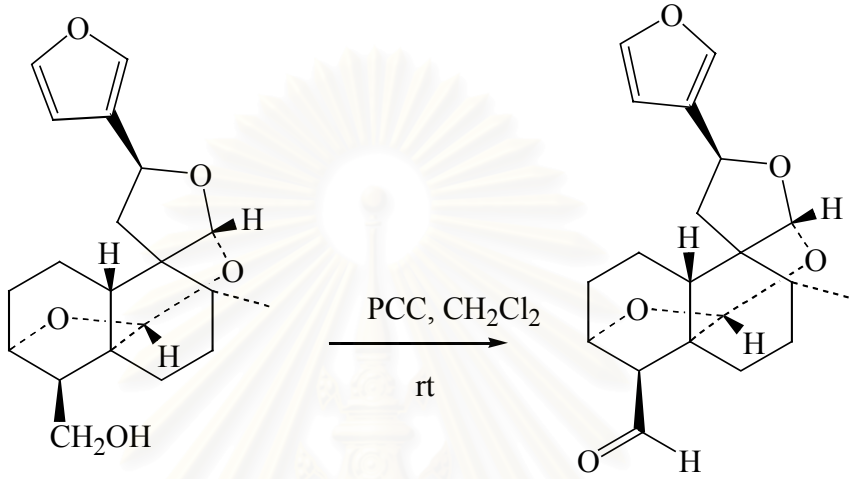
Table 18 The ^{1}H-^{13}C NMR, HMBC, COSY and NOESY spectral data of compound **6**

	δ_C	δ_H	HMBC (H to C)	COSY	NOESY
1	21.25t	A= 1.90 (m)	C-5, C-6, C-9, C-10	H_B-2	-
		B= 2.53 (m)	C-6, C-9, C-10	H_B-2, H-10	H_B-2
2	28.15t	A= 1.57 (m)	C-1, C-5, C-10	H_A-1	-
		B= 1.83 (m)	C-1, C-5, C-10	H_A-1, H_B-1	H_B-1
3	78.86d	4.58 (dd) (*J* = 4.4, 0.5)	C-4, C-18, C-19	H-4	-
4	156.26s	-		H-3, H-2	-
5	45.01s	-		-	-
6	34.32t	A= 1.71 (m)	C-1, C-7	H_A-7	-
		B= 2.23 (m)	C-1, C-8, C-10	H_B-7	-
7	30.09t	A= 1.44 (m)	C-5, C-8, C-9, C-17	H_A-6	-
		B= 1.84 (m)	C-5, C-8, C-9, C-17	H_B-6	-
8	37.68d	1.65 (m)	C-17	H-17	H-17
9	50.10s	-	-	-	-
10	48.25d	1.81 (m)	C-8, C-9, C-19	H_B-1	-
11	38.59t	2.25 (m)	C-8, C-9, C-10	H-12	H-12
12	74.07d	5.38 (dd) (*J* = 5.6, 1.6)	C-13, C-14, C-16, C-20	H-11	H-11
13	127.46s	-	-	-	-
14	108.64d	6.41 (d) (*J* = 1.4)	C-13, C-16	H-15	H-15
15	143.53d	7.42 (d) (*J* = 3.2)	C-13, C-14, C-16	H-14	H-14
16	139.23d	7.41 (d) (*J* = 1.6)	C-14, C-15	-	-
17	17.00q	0.99 (dd) (*J* = 6.8)	C-7, C-8, C-9	H-8	H-8
18	100.57t	A= 4.91(d) (*J* = 0.5)	C-3, C-4, C-5, C-10, C-19	-	-
		B= 4.74 (d) (*J* = 0.5)	C-3, C-4, C-5, C-19	-	-
19	103.89d	5.18 (d) (*J* = 0.8)	C-3, C-10, C-20	-	-
20	100.87d	5.37 (s)	C-10, C-19	-	-

Axial and equatorial conformations are expressed as A and B, respectively.

4.7 Oxidation reaction of compound 4

Scheme 20 illustrated the oxidation reaction of compound **4.** The hydroxyl group of compound **4** was converted to aldehyde group in compound **7**. The reaction between compound **4** (1 equiv) and pyridinium chlorocromate (PCC) (1.2 equiv) in dichloromethine at room temperature for 1 hour gave compound **7** in quantitative yield.



4 **7**

The spectroscopic data clearly confirmed the structure of compound **7.** The IR spectrum of compound **7** is shown in Figure 60 and the absorption peaks assigned are shown in Table 19**.** The IR spectrum revealed the absorption band at 1704 cm^{-1} of C=O stretching vibration of aldehyde, at 2926 and 2867 cm^{-1} of C-H stretching vibration of CH_2, CH_3 and at 1462 cm^{-1} C-H bending vibration of CH_2,CH_3. Moreover, the absorption band at 1372 and 1112 cm^{-1} were assigned as the O-C-O stretching vibration of acetal group and C-O stretching vibration, respectively. The =C-H out of plane bending of furan occurred at 876 cm^{-1}.

Table 19 The IR absorption band assignment of compound **7**

Tentative assignments	Band type	Wave numbers
C-H stretching vibration of CH2, CH_3	s	2926, 2867
C=O stretching vibration of aldehyde	s	1704
C-H bending vibration of CH_2,CH_3	s	1462
O-C-O stretching vibration of acetal group	m	1372
C-O stretching vibration	s	1112
=C-H out-of-plane bending of furan	w	876

The ^{1}H-NMR spectrum (Figure 62, Table 20) of compound **7** showed three doublet of doublet signals at 7.43, 7.41 and 6.42 ppm which indicated the three olefinic protons of a furan ring. Moreover, the doublet of doublet signal was observed at 5.38 ppm which assigned the methine proton attaching between a furan ring and an oxygen atom. Two singlet signals at 5.31 and 5.19 ppm were assigned to the two methine protons which attached to two carbon bearing oxygen atoms. The methine proton attaching to carbon bearing an oxygen atom appeared at 4.63 ppm. The signal at 9.88 ppm was assigned the methine proton of aldehyde.

The ^{13}C-NMR spectrum (Figure 63, Table 20) and HSQC (Figure 45) of compound **4** showed 20 lines. The spectrum showed the signal of carbonyl carbon of aldehyde at 200.66 ppm and olefinic carbon signals at 143.55, 139.42, 126.79 and 108.57 ppm which indicated carbon of a furan ring. The signal at 104.29 and 100.71 ppm were assigned to the two methine carbons which attached to two oxygen atom. The signals of two methine carbons also found at 75.36 and 75.24 ppm, attached to oxygen atom.

In comparison of ^{1}H-NMR spectrum of compound **7** with that of compound **4** demonstrated that the signal of methylene protons at 3.82 and 3.83 ppm of compound **1** disappeared in ^{1}H-NMR spectrum of compound **7**. On the other hand, the ^{1}H-NMR spectrum of compound **7** showed doublet signal at 9.88 ppm.

In comparison of ^{13}C-NMR spectrum of compound **7** with that of compound **4** indicated that the signal of methylene carbon at 59.99 ppm of compound **4** disappeared in ^{3}C-NMR spectrum of compound **7**. Moreover, the signal at 200.66 ppm of compound **1** appeared in the ^{1}H-NMR spectrum of compound **7.**

The relative configuration for all the nine asymmetric centers of crovatin was established by 2D NMR experiments (COSY; Figure 64 and NOESY; Figure 65). ^{1}H-^{1}H COSY showed the ^{3}J interaction between H-3 (δ 4.63) and H-4 (δ 2.83), H-4 and H-18 (δ 9.88) , H-12 (δ 5.38) and H -11 (δ 2.27) and H -14 (δ 6.42) and H-15 (δ 7.43). NOESY revealed interactions between H-3 and H-18 (δ 9.88), H-4 and H-19 (δ 5.19), H-19 and H-20 (δ 5.31)

The mass spectrum (ESI MS; Figure 61) showed the molecular ion peak $[M+H]^{+}$ at m/z 347. The result indicated that the molecular weight of compound **7** was 346.

From the spectroscopic data, it could be concluded that compound **7** was *ent*-(8R, 10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18β-al. The structure of compound **7** is shown in Figure 12.

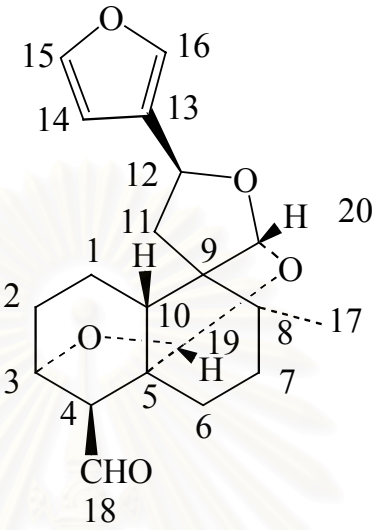


Figure 12 Structure of compound **7.**

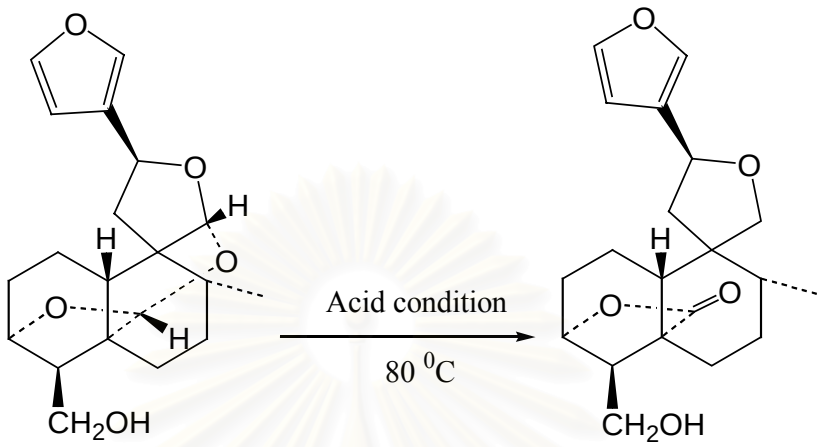
Table 20 The ^{1}H-^{13}C NMR, HMBC, COSY and NOESY spectral data of compound **7**

	δ_C	δ_H	HMBC (H to C)	COSY	NOESY
1	20.46t	A= 1.89 (m)	C-2, C-3, C-5, C-10	$H_{A,B}$-2, H-10	H-11, H_B-1
		B= 2.45 (m)	C-2, C-3, C-5, C-10	$H_{A,B}$-2, H-10	H_B-1-
2	27.16t	A= 1.77 (m)	C-3	$H_{A,B}$-1	H_B-2
		B= 2.12 (m)	C-1, C-3, C-4, C-8	$H_{A,B}$-1, H-10	H_A-2
3	75.36d	4.63 (m)	C-1, C-4, C-5, C-19	H-4	H-18
4	61.21d	2.83 (m)	C-5, C-18, C-19	H-3	H-19
5	45.44s	-	-	-	-
6	31.92t	A= 1.62 (m)	C-19	H_A-7	H-10
		B= 2.00 (m)	C-19	H_B-7	-
7	30.43t	A= 1.42 (m)	C-5, C-6, C-8, C-9, C-17	H_A-6	-
		B= 1.82 (m)	C-5, C-8, C-9, C-17	H_B-6	-
8	37.53d	1.71 (m)	C-20	H-17	-
9	50.71s	-	-	-	-
10	40.36d	2.20 (m)	C-1, C-4, C-10	$H_{A,B}$-1	H_A-6
11	38.48t	2.27 (m)	C-9, C-10, C-12	H-12	H-12
12	75.24d	5.38 (t) (J = 8.8, 7.6)	C-11, C-14, C-16, C-20	H-11	H-11
13	126.79s	-	-	-	-
14	108.57d	6.42 (d) (J = 0.8)	C-12, C-15, C-16	H-15	H-15
15	143.55d	7.43 (d) (J = 1.6)	C-13, C-14, C-16	H-14	H-14
16	139.42d	7.41 (d) (J = 1.6)	C-12, C-13, C-14, C-15	-	-
17	16.88q	0.99 (d) (J = 4.0)	C-7, C-8, C-9	-	H-8, H-19,H-20
18	200.66s	9.88 (d) (J = 2.0)	C-3, C-4	H-4	H-3
19	104.29d	5.19 (s)	C-4, C-6	-	H-4, H-20
20	100.71d	5.31 (s)	C-8, C-9, C-12	-	H-17, H-19

Axial and equatorial conformations are expressed as A and B, respectively.

4.8 Hydrolysis of compound 4 with acidic condition

The hydrolysis reaction of compound **4** was carried out in acidic conditions. The reaction was stirred at 80 ^{0}C to give compound **8**.



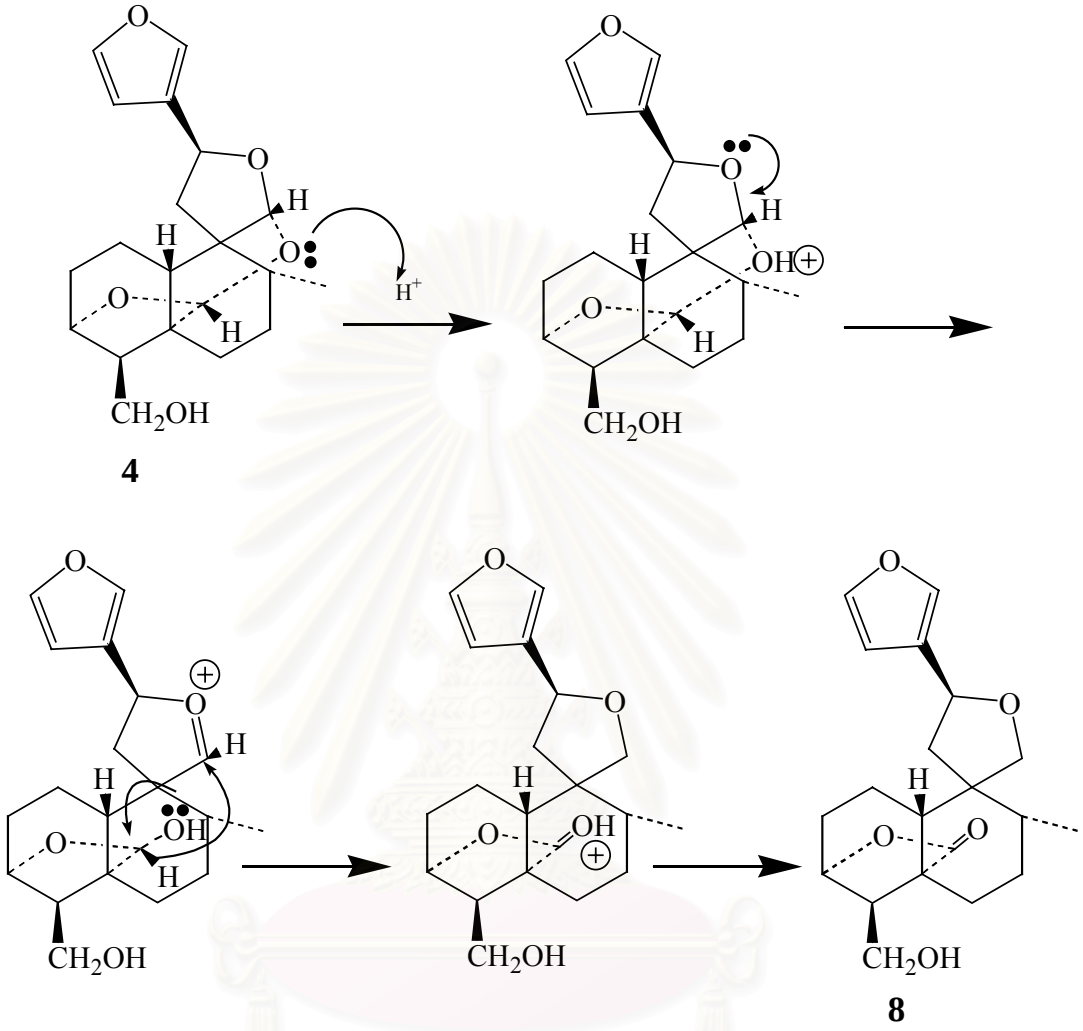
4 **8**

entry	Compound **4** (mg)	Acids	Time (h)	Product **8** (mg)	% Yield
1	28.3	H_2SO_4(2 M)	5	colorless crystal : 26.1	92
2	25.8	H_2SO_4 (4 M)	3	colorless crystal : 23.0	89
3	22.7	HCl(2 M)	7	colorless crystal : 12.7	56
4	29.7	p-TsOH(2 M)	8	colorless crystal : 16.0	54

TLC was used to monitor the result of hydrolysis reaction. For acidic condition in entries 1 and 2, the compound **8** was obtained with excellent selectivity and good yields. However, the reaction of compound **4** carried out in 2 M HCl and 2 M *p*-TsOH in methanol, obtained compound **8** in a moderate yield in entries 3 and 4, respectively. Among the various concentrations of sulfuric acid in entry 1 and 2, the reaction carried out in 4 M H_2SO_4 (entry 2) is faster than that in 2 M H_2SO_4 (entry 1).

The hydrolysis mechanism of compound **4** is proposed for the hydride rearrangement [14] from compound **4** to compound **8.** Protonation to the oxygen atom (O') of compound **4** followed by cleavage of the C (20)-O" and C (19)-O' bonds give the oxocarbenium ion **9,** alcohol and aldehyde. In next step, a hydroxyl group attacked to carbonyl (C-19) of aldehyde, and then followed by nucleophilic addition of the

hydride on the oxocarbenium ion from the inside concave to give the observed products compound **8.** This mechanism was proposed that a hydride rearrangement is an intramolecular process[14], as schown in Scheme 4.



Scheme 4

The spectroscopic data clearly confirmed the structure of compound **8.** The IR spectrum of compound **8** is shown in Figure 68 and the absorption peaks are assigned in Table 21. The IR spectrum indicated hydroxyl group (O-H stretching vibration of alcohol) at 3636-3165 cm^{-1}, C=O stretching vibration of lactone at 1762 cm^{-1}, the C-H stretching vibration of CH_2, CH_3 at 2922 and 2873 cm^{-1} and C-H bending vibration vibration of CH_2,CH_3 at 1456 cm^{-1} . Moreover, the absorption bands at 1150 cm^{-1} and 1028 cm^{-1} were assigned as the O-C-O stretching vibration of acetal group and C-O stretching vibration, respectively. The =C-H out of plane bending of furan occurred at 871 cm^{-1}.

Table 21 The IR absorption band assignment of compound **8**

Tentative assignments	Band type	Wave numbers
O-H stretching vibration of alcohol	b	3636-3165
C-H stretching vibration of CH_2, CH_3	s	2922, 2873
C=O stretching vibration of lactone	s	1762
C-H bending vibration vibration of CH_2,CH_3	w	1456
O-C-O stretching vibration of acetal group	m	1150
C-O stretching vibration	m	1028
=C-H out of plane bending of furan	m	871

The ^{1}H-NMR spectrum (Figure 70, Table 22) of compound **8** showed three doublet signals at 6.31, 7.30 and 7.31 ppm which indicated protons of a furan ring. The triplet signal at 4.68 ppm indicated the methine proton attaching to carbon bearing an oxygen atom. The signal at 4.54 ppm indicated the doublet of doublet of methine proton which attached to a furan ring and an oxygen atom. Moreover, the two doublet signals appeared at 3.62 and 3.76 ppm which indicated methylene protons attaching to carbon bearing an oxygen atom.

The ^{13}C-NMR (Figure 710, Table 22) and HSQC (Figure 53) spectra of compound **8** showed the signal of a carbonyl carbon at 179.43 ppm. The spectrum was indicated the three methine carbons and one quaternary carbon of furan ring, quaternary carbon and methine carbon of double bond according to the signals at 143.35, 139.30, and 108.73 ppm, respectively. Moreover, the signal of methine carbon attached to an oxygen atom, appeared at 76.66 ppm. The signal of 73.31 ppm indicated the methylene carbon which attached to a furan ring and an oxygen atom.

In comparison of ^{1}H-NMR and ^{13}C-NMR spectrum of compound **8** with that compound **4,** the protons signals at 5.22 and 5.31 ppm in the ^{1}H-NMR spectrum and 100.86 and 105.25 ppm were disappeared in the ^{13}C-NMR spectrum of compound **8**, showed the ring opening of some acetal in molecule of compound **4.** The signal of methine proton at 4.33 ppm in spectrum of compound **4** was shifted to 4.8 ppm in spectrum of compound **8.** Moreover, the signal of carbonyl compound at 179.43 ppm appeared in the spectrum of compound **8.**

The relative configuration for all the nine asymmetric centers of crovatin was established by 2D NMR experiments (COSY; Figure 72, HMBC; Figure 75 and NOESY; Figure 73). ^{1}H-^{1}H COSY showed the ^{3}J interaction between H-12 (δ 5.38) and H -11 (δ 2.25), H -14 (δ 6.41) and H-15 (δ 7.42), H -8 (δ 1.65) and H-17 (δ 0.99). The correlation of HMBC spectrum was shown in Table 18 and Figure 13. NOESY revealed the interactions between H_A-7 (δ 1.44) and H-17, H_B-7 (δ 1.90) and H-17, H-8 and H-17, H-11 and H-12, H-14 and H-15, H_A-20 (δ 3.62) and H-17, H_B-20 (δ 3.76) and H-17, as shown in Figure 14.

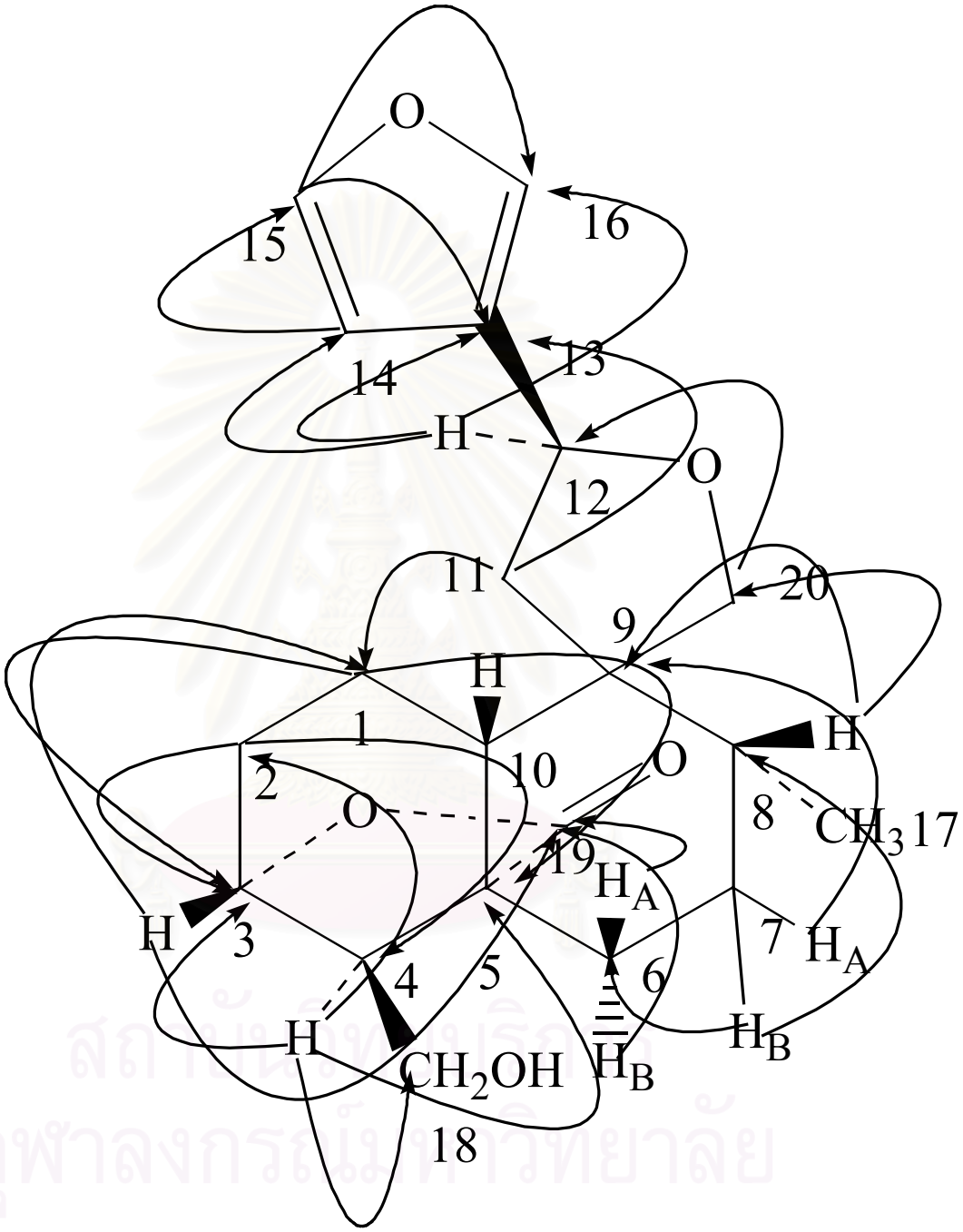


Figure 13 The HMBC correlation of compound **8.**

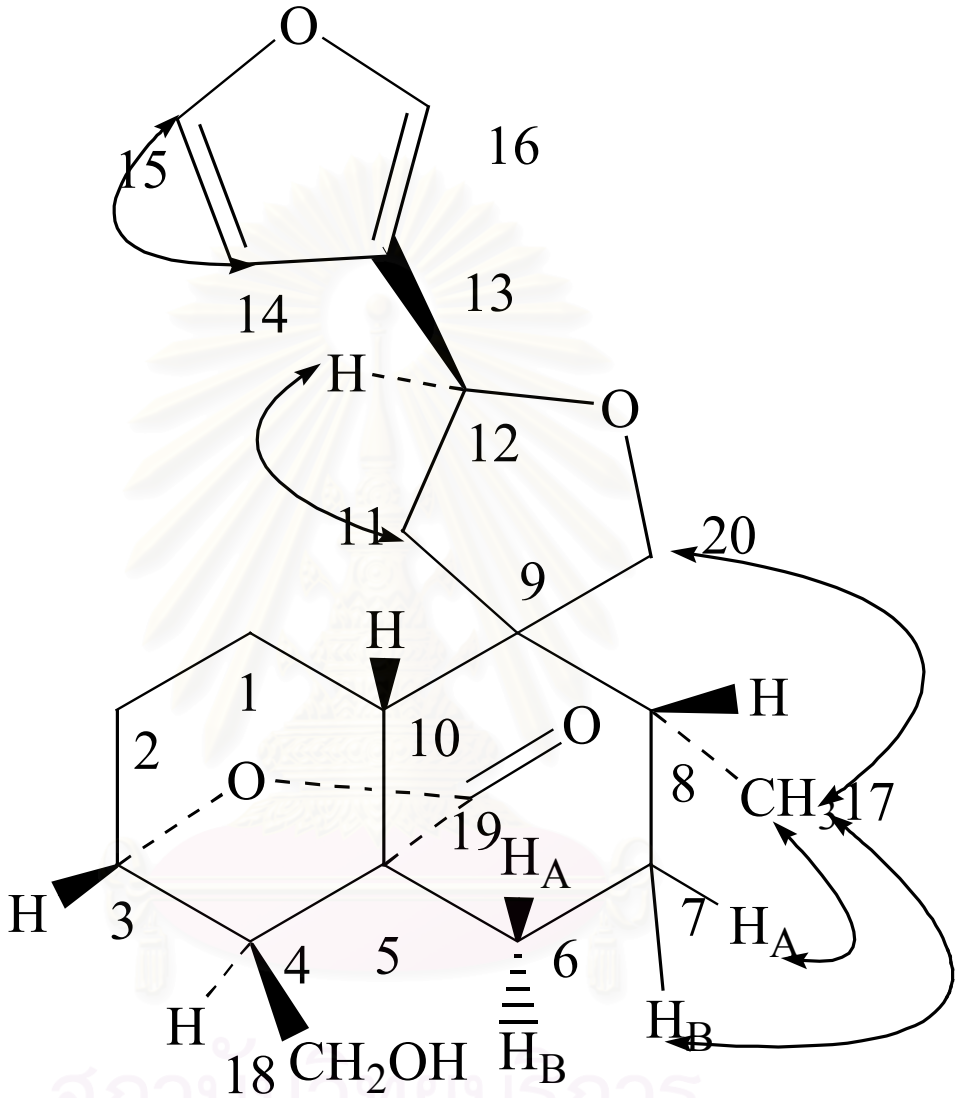


Figure 14 The NOESY correlation of compound **8.**

Accordingly, compound **8** possesses the structure and relative stereochemistry depicted in Scheme 23. The conclusion of this compound was supported by a single-crystal X-ray determination which showed the same relative stereochemistry, as shown in Figure 15.

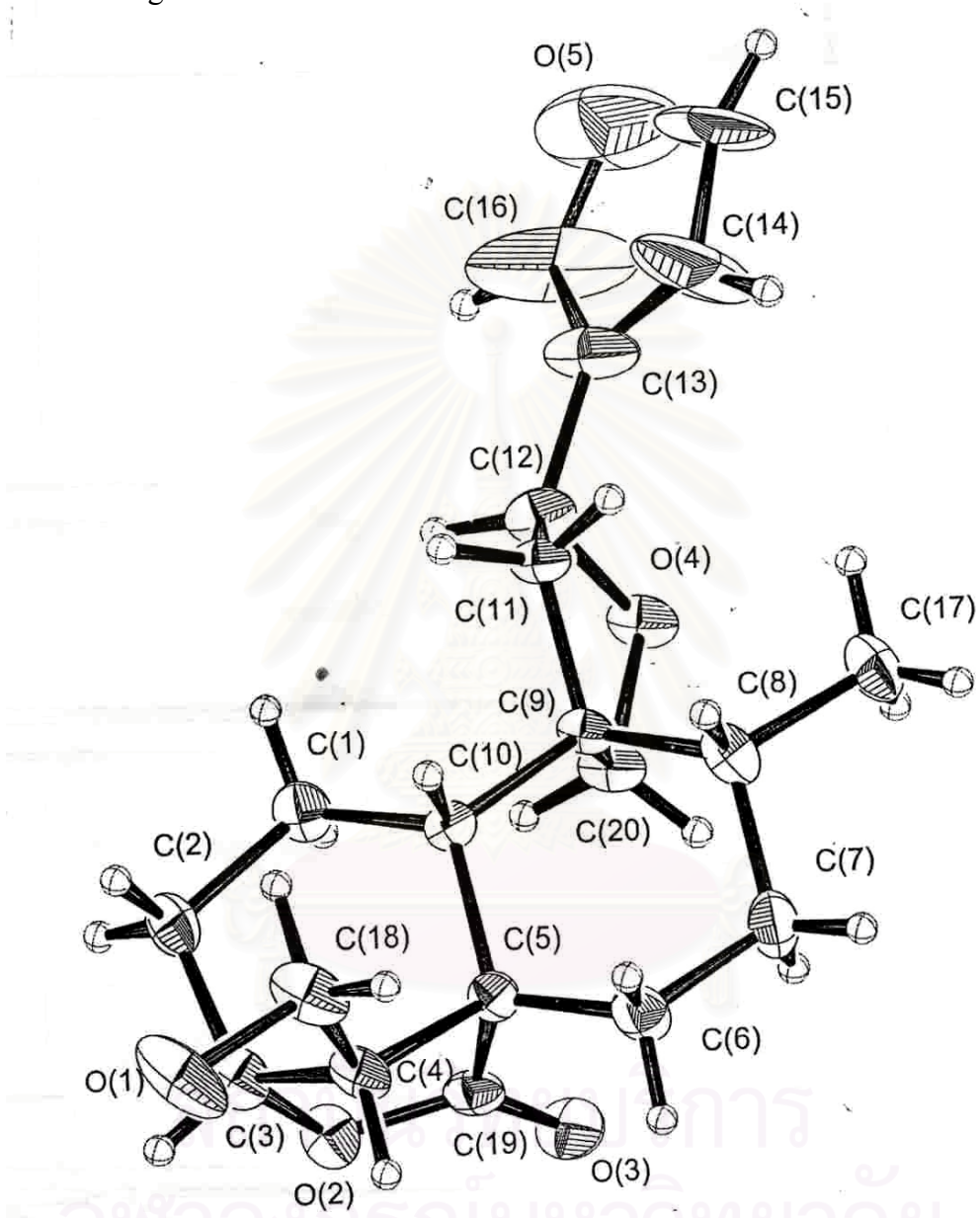


Figure 15 ORTEP of compound **8.**

The mass spectrum (ESI MS; Figure 48) showed the molecular ion peak $[M+H]^+$ at m/z 347. The result indicated that the molecular weight of compound **7** was 346.

From the spectroscopic data, it could be conclude that compound **7** was *ent*-(8R, 10β)-3,19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16),14-diene-18β-al. The structure of compound **7** is shown in Figure 16.

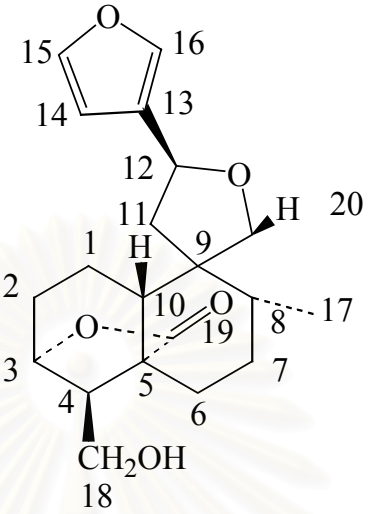


Figure 16 Structure of compound **8.**

Table 22 The ^{1}H, ^{13}C, HMBC, COSY and NOESY spectral data of compound **8**

	δ_C	δ_H	HMBC (H to C)	COSY	NOESY
1	19.67t	1.81 (m)	C-3, C-5	-	-
		1.88 (m)	C-3, C-5	-	-
2	22.39t	1.74 (m)	-	-	-
		2.01 (m)	C-3, C-4	H-3	-
3	76.66d	4.68 (t) (*J* = 4.8)	C-1, C-19	H_B-2, H-4	-
4	51.29d	2.27 (m)	C-2, C-3, C-5, C-18	H-3, H-18	-
5	45.63s	-	-	-	-
6	28.30d	1.47 (m)	C-4, C-5, C-7, C-19	-	-
		1.62 (m)	C-5, C-7, C-19	H_B-7	-
7	30.24d	1.44 (m)	C-5, C-6, C-8, C-9	H-8	H-17
		1.85 (m)	C-5, C-6, C-8, C-9	H_B-6	H-17
8	40.84d	1.31 (m)	C-9, C-17, C-20	H_A-7, H-17	H-17
9	50.48s	-	-	-	-
10	45.01d	1.90 (m)	C-5, C-8, C-9, C-11, C-12, C-20	-	-
11	41.87t	1.69 (m)	C-1, C-13	H-12	H-12
12	73.31d	4.54 (dd) (*J* = 8.8, 8.0)	C-13, C-14, C-16	H-11	H-11
13	126.05s	-	-	-	-
14	108.73d	6.31 (d) (*J* = 1.6)	C-13, C-15, C-16	H-15	H-15
15	143.35d	7.30 (d) (*J* = 1.6)	C-13, C-14, C-16	H-14	H-14
16	139.30d	7.31 (d) (*J* = 1.6)	C-13, C-14, C-15	-	-
17	17.15q	0.95 (d) (*J* = 6.4)	C-7, C-8, C-9	H-17	H_B-20, H-8
18	58.55t	3.80 (d) (*J* = 7.6)	C-3, C-4, C-5	H-4	-
19	179.43s	-	-	-	-
20	67.01t	3.62 (d) (J = 11.2)	C-8, C-12	-	H-17
		3.76 (d) (J = 10.8)	C-8, C-10, C-12	-	H-17

Axial and equatorial conformations are expressed as A and B, respectively.

4.9 Biological Activity of Crovatin and Its Derivatives

In order to study and investigate the structure activity relationship, crovatin **1** and its derivatives (compounds 2-4, 6-8) were tested for 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase , α-glucosidase, HIV-1 protease and tumor human cell line (HuCCA-1). The results are summarized in Table 23.

Table 23 Inhibitory Activity of Crovatin and Its Derivatives

Sample	HMG-CoA reductase IC_{50} (mM)	α-glucosidase IC_{50} (mM)	HIV-1 protease IC_{50} (μM)	Cytotoxicity (HuCCA-1) IC_{50} (μM)
Crovatin **1**	NA	NA	>10	>10
Compound **2**	NA	3.90	>10	>10
Compound **3**	1.45	1.82	>10	>10
Compound **4**	NA	NA	>10	>10
Compound **6**	NA	NA	>10	>10
Compound **7**	NA	NA	>10	>10
Compound **8**	1.45	NA	>10	>10

NA is expressed as no activity.

HuCCA-1; human bile duct epithelial carcinoma cell line

From Table 29 it showed that crovatin itself did not show inhibitory activity on 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, α-glucosidase, HIV-1 protease and tumor human cell line (HuCCA-1). On the other hand, compound **3** and compound **8** showed moderate inhibitory activity on 3-hydroxy-3-methyl glutaryl CoA (HMG-CoA) reductase with IC_{50} 1.45 mM and compound **2** and compound **3** showed inhibitory activity on α-glucosidase with IC_{50} 3.90 and 1.85 mM , respectively.

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CHAPTER IV
CONCLUSION

Crovatin was isolated as a main constituent from crude extracts of stem bark of *Croton oblongifolius* collected from Ampur SaiYok Kanchanaburi Province, Thailand. Compound **2** and **3** were synthesized by basic hydrolysis reaction using 2.0 M NaOH and 2.0 M KOH in methanol. When compare the reaction condition between 2.0 M NaOH and 2.0 M KOH, it was found that a NaOH solution gave a lower yield. Compound **4** was prepared by reduction of crovatin with Lithium Aluminum hydride in quantitative yield. Compound **6** was obtained by mesylation reaction of compound **4** followed by treating it with 2.0 M KOH in methanol. Moreover, compound **7** was obtained from the oxidation of the compound **4** with pyridinium chlorocromate in quantitative yield. Finally, compound **8,** was obtained from hydrolysis of compound **4** in acidic condition using sulfuric acid. The mechanism of this reaction was proposed as an intramolecular the hydride rearrangement. The structure of crovatin and its derivatives were characterized by ^{1}H and ^{13}C-NMR spectroscopy, mass spectrometry and infrared spectroscopy.

Crovatin and its derivatives were tested for the inhibition of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, α-glucosidase, HIV-1 protease and tumor human cell line (HuCCA-1) and it was found that compounds **3** and **8** showed inhibitory activities against 3-hydroxy-3-methylglutaryl CoA reductase. Both compounds **3** and **8** showed IC_{50} 1.45 mM. Moreover, compounds **2** and **3** showed inhibitory activities against α-glucosidase with IC_{50} 3.90 and 1.85 mM , respectively. All compounds were inactive against HIV-1 protease and tumor human cell line (HuCCA-1).

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APPENDEX

Figure 17 IR spectrum of compound **1.**

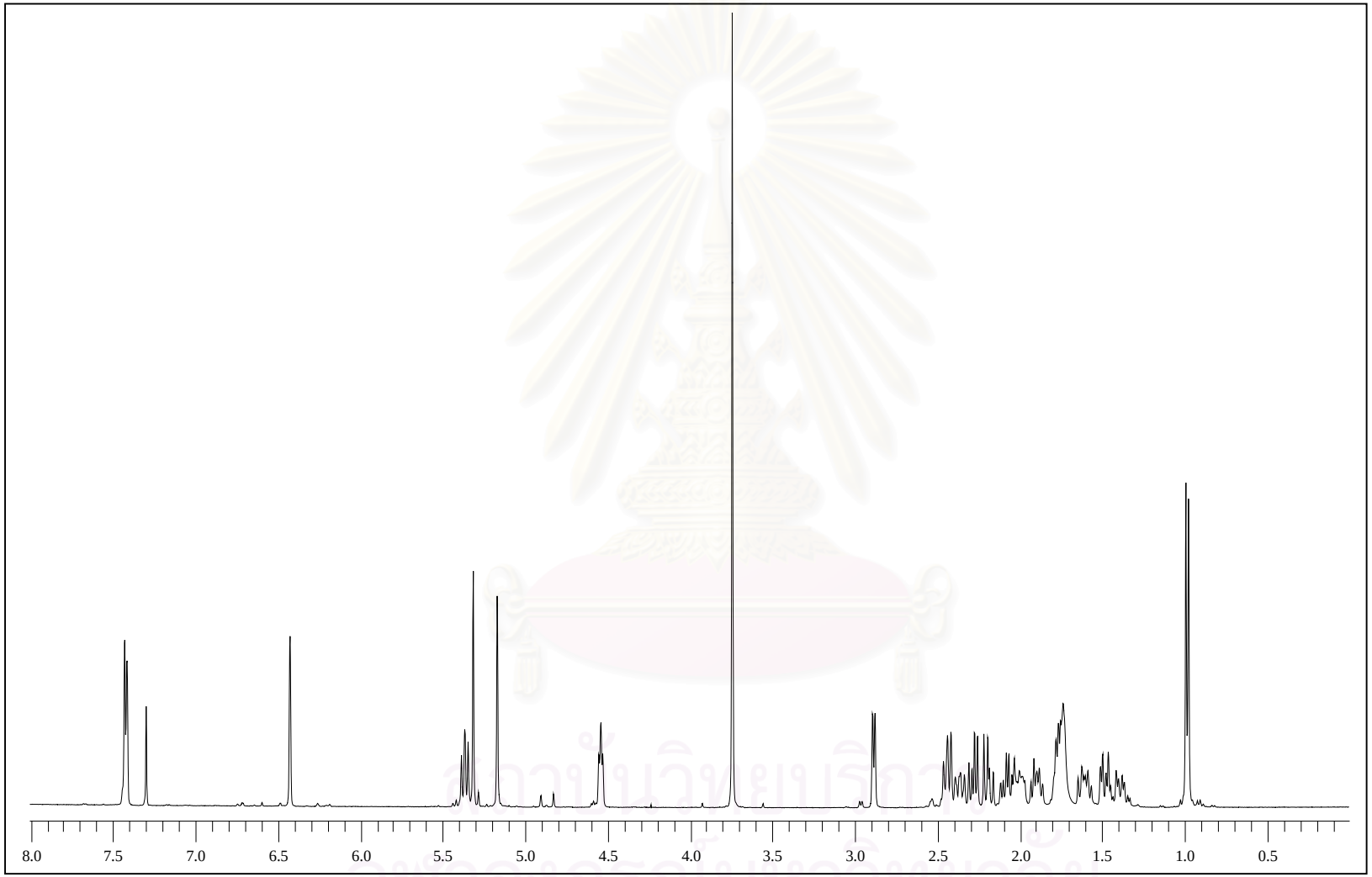
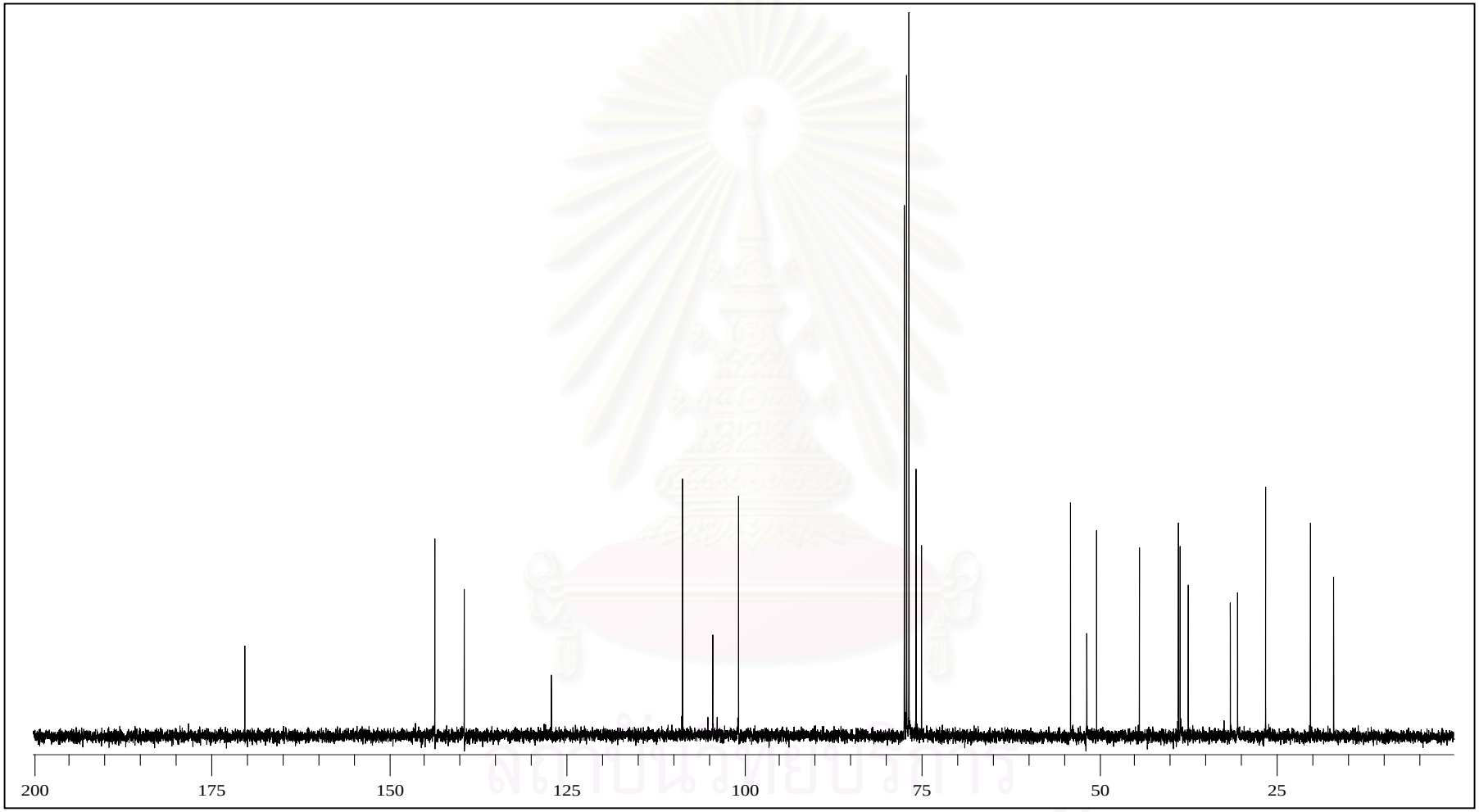


Figure 18 ^{1}H-NMR spectrum of compound **1.**



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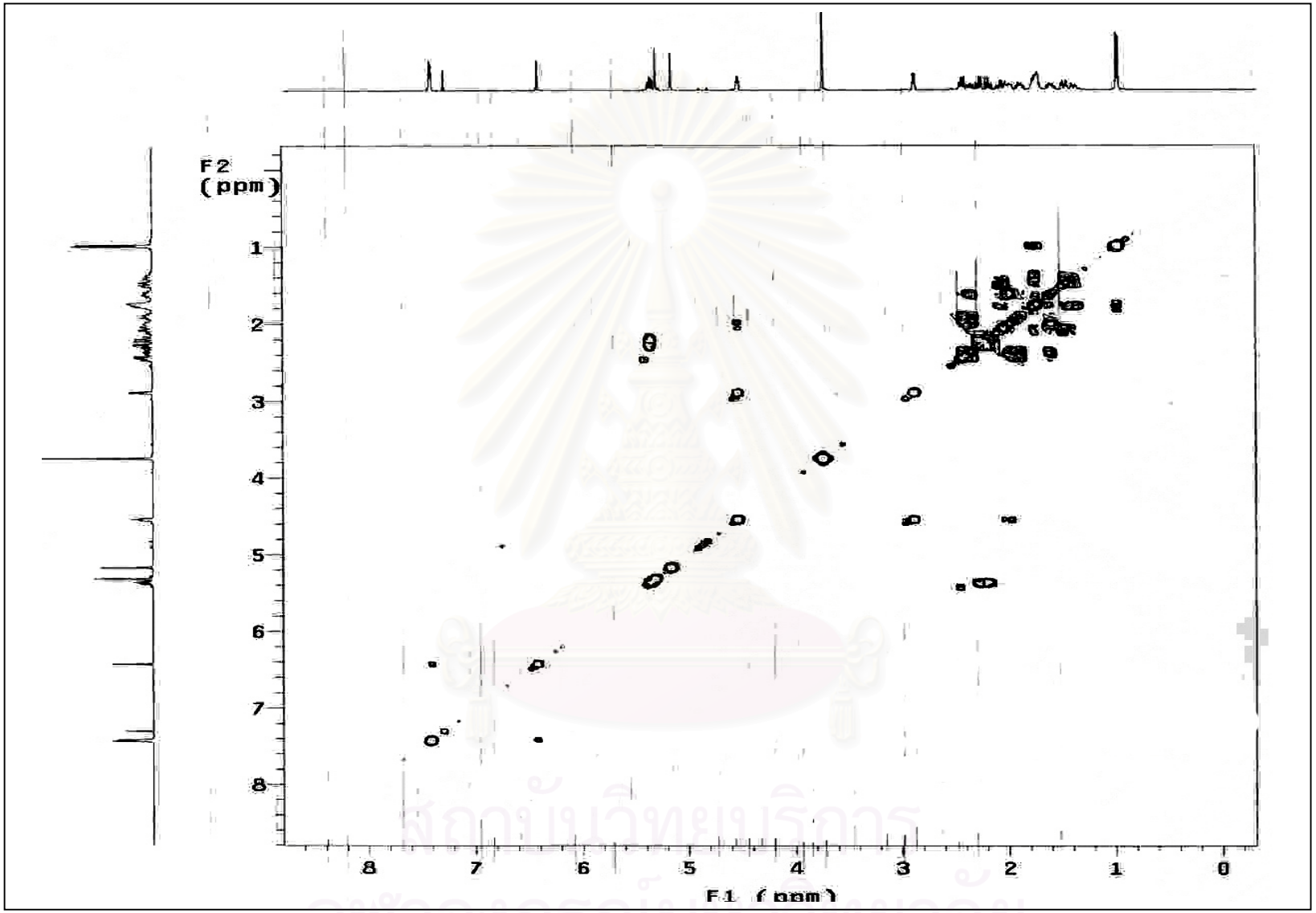

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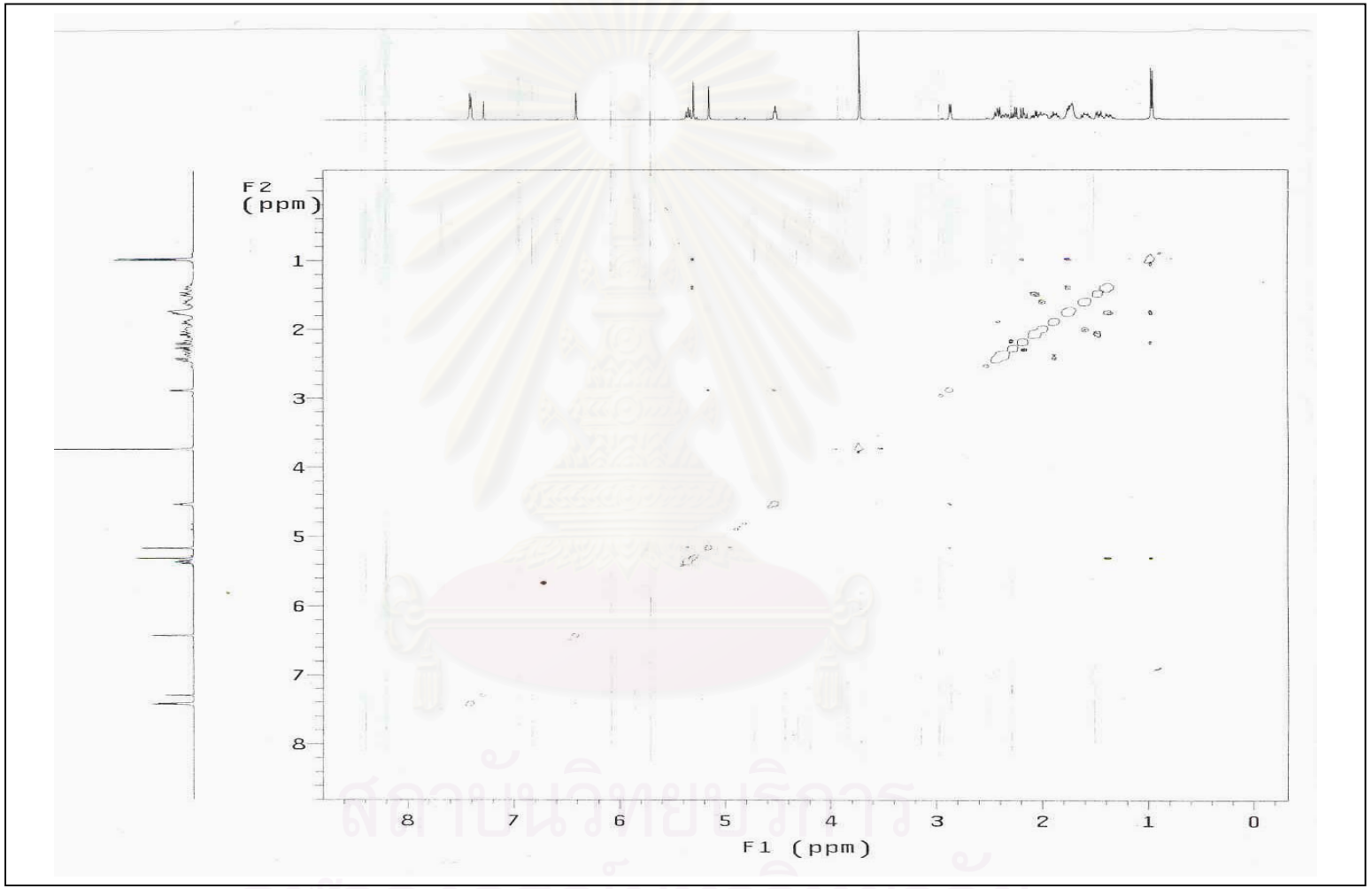


Figure 21 NOESY spectrum of compound **1.**

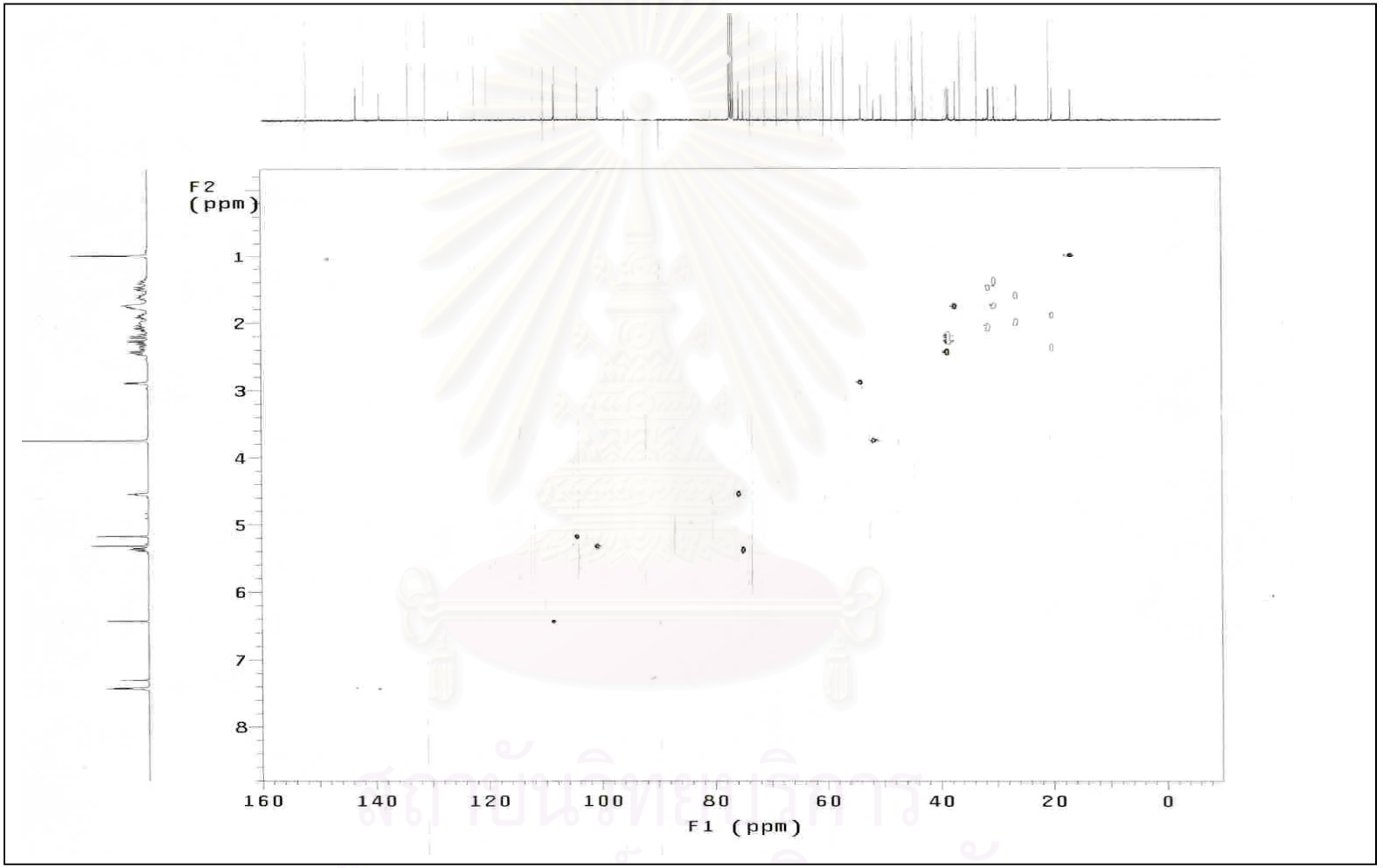


Figure 21 HSQC spectrum of compound **1.**

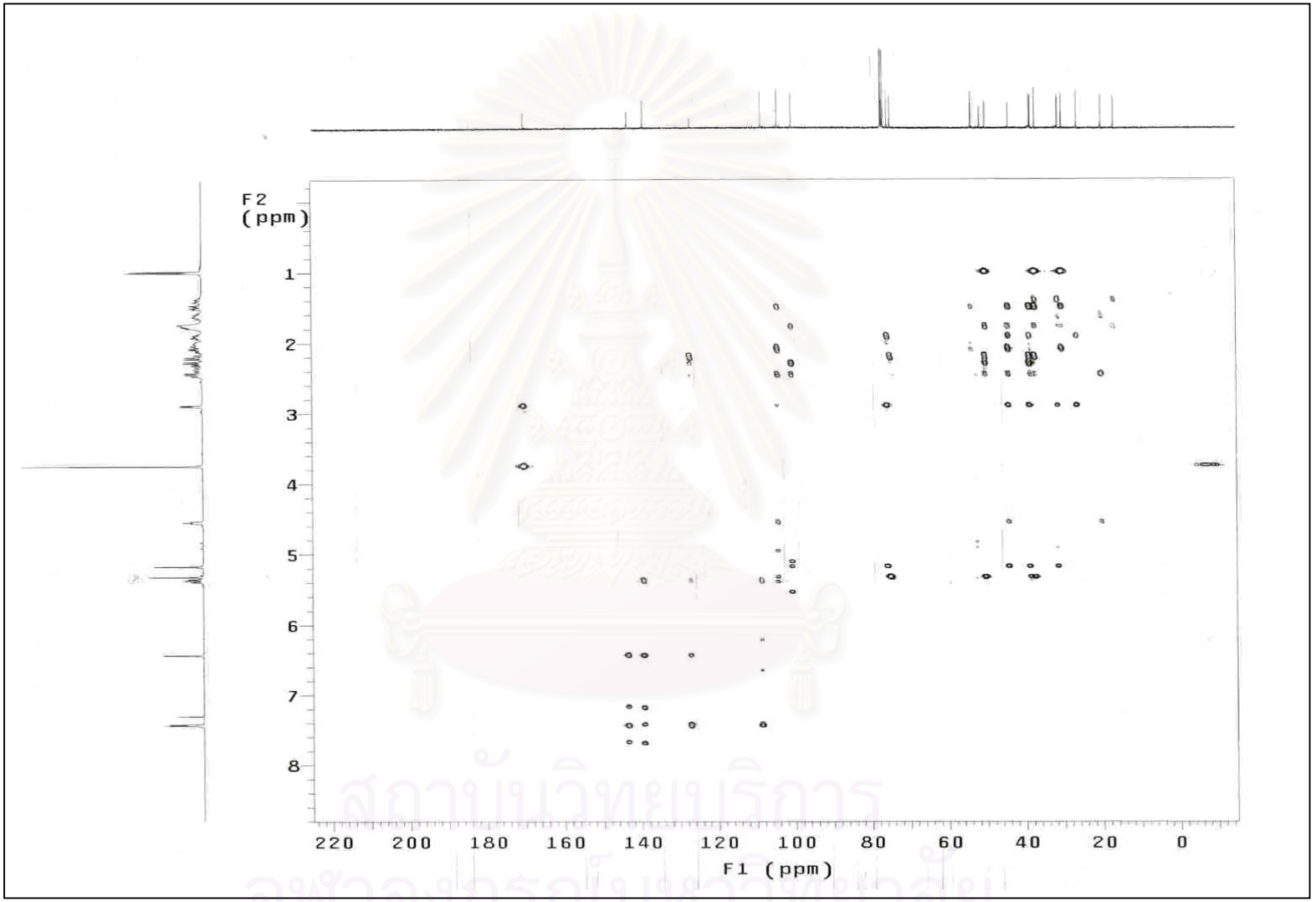
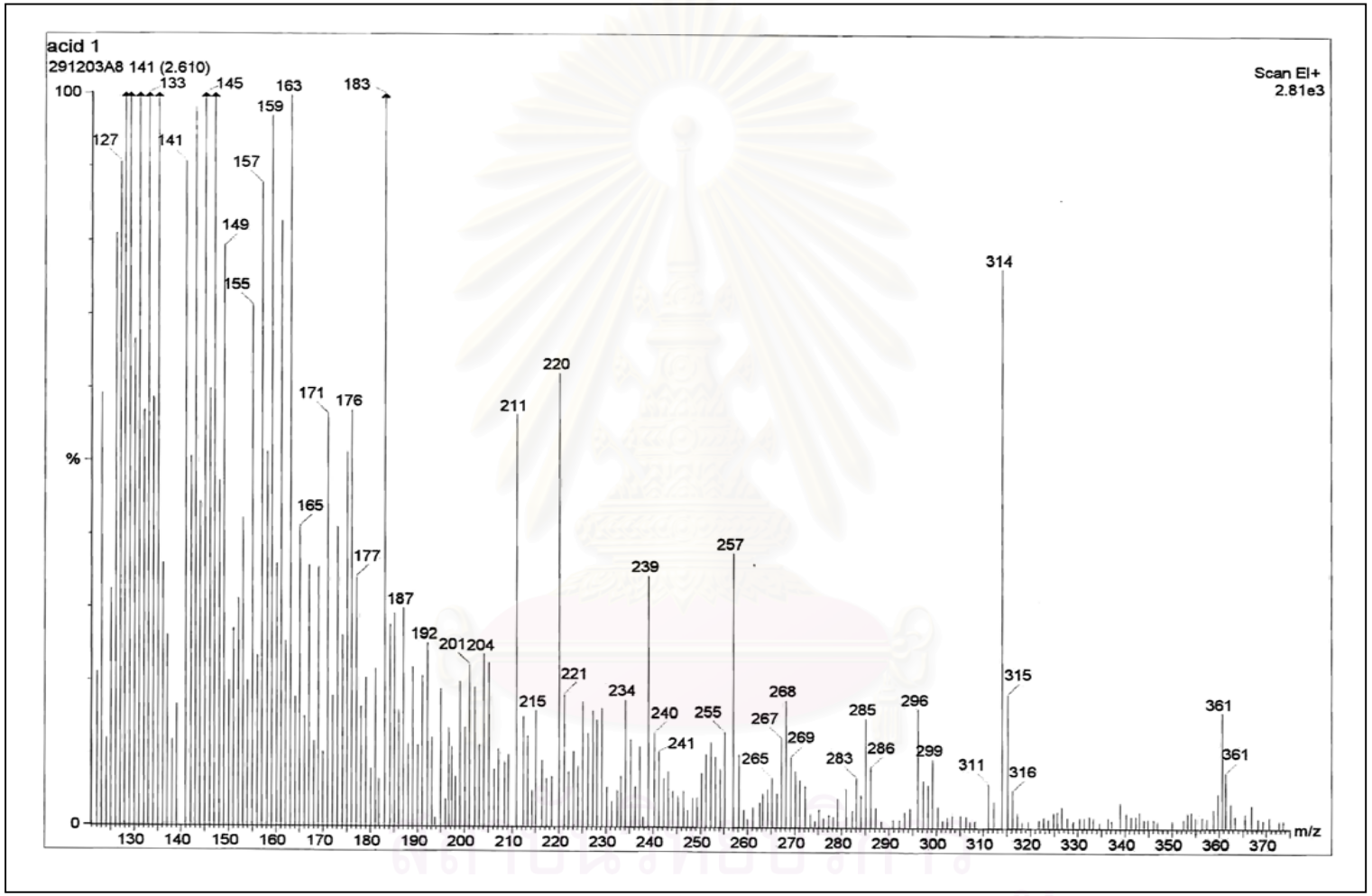


Figure 22 HMBC spectrum of compound **1.**

Figure 23 IR spectrum of compound **2.**



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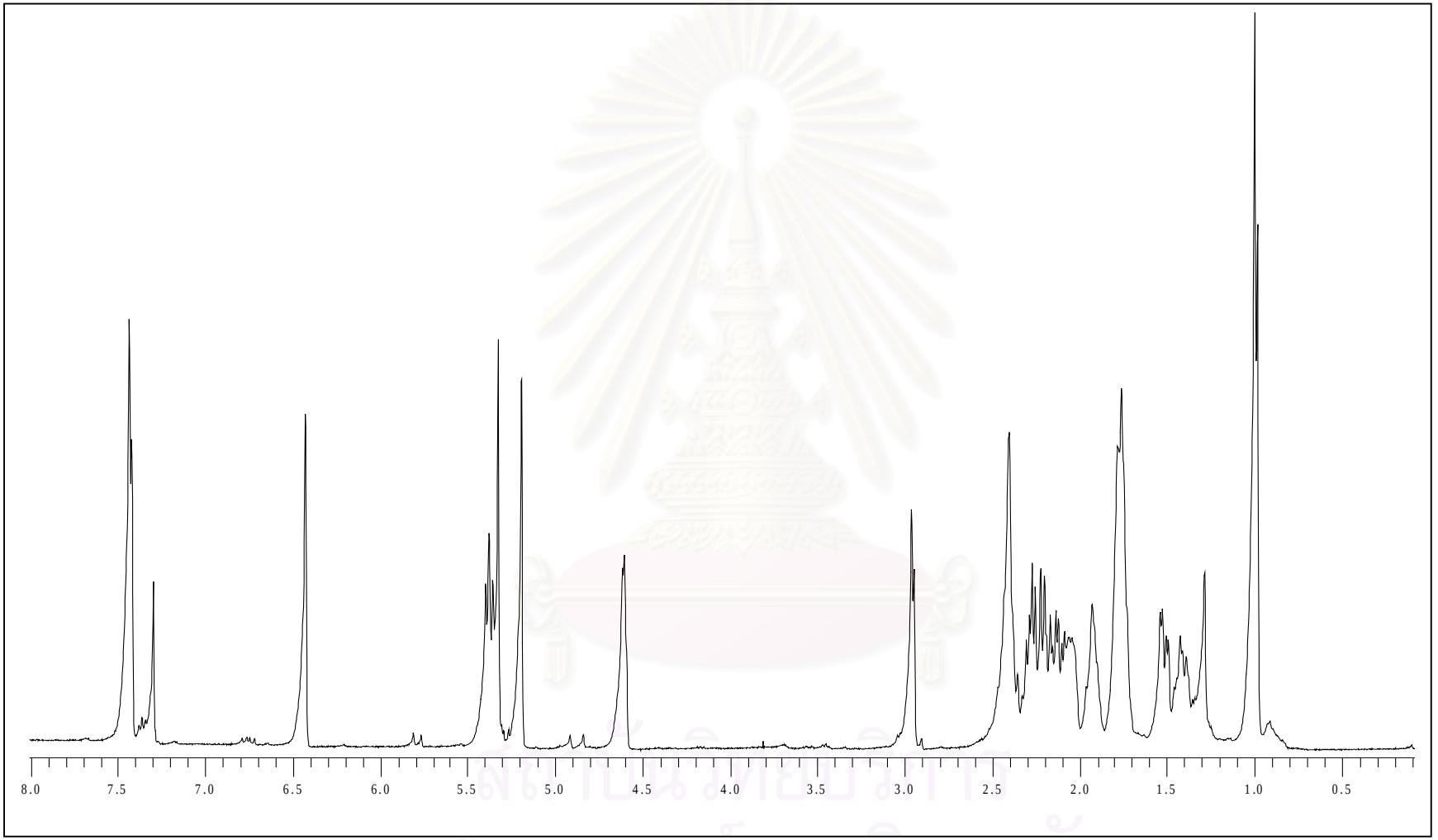

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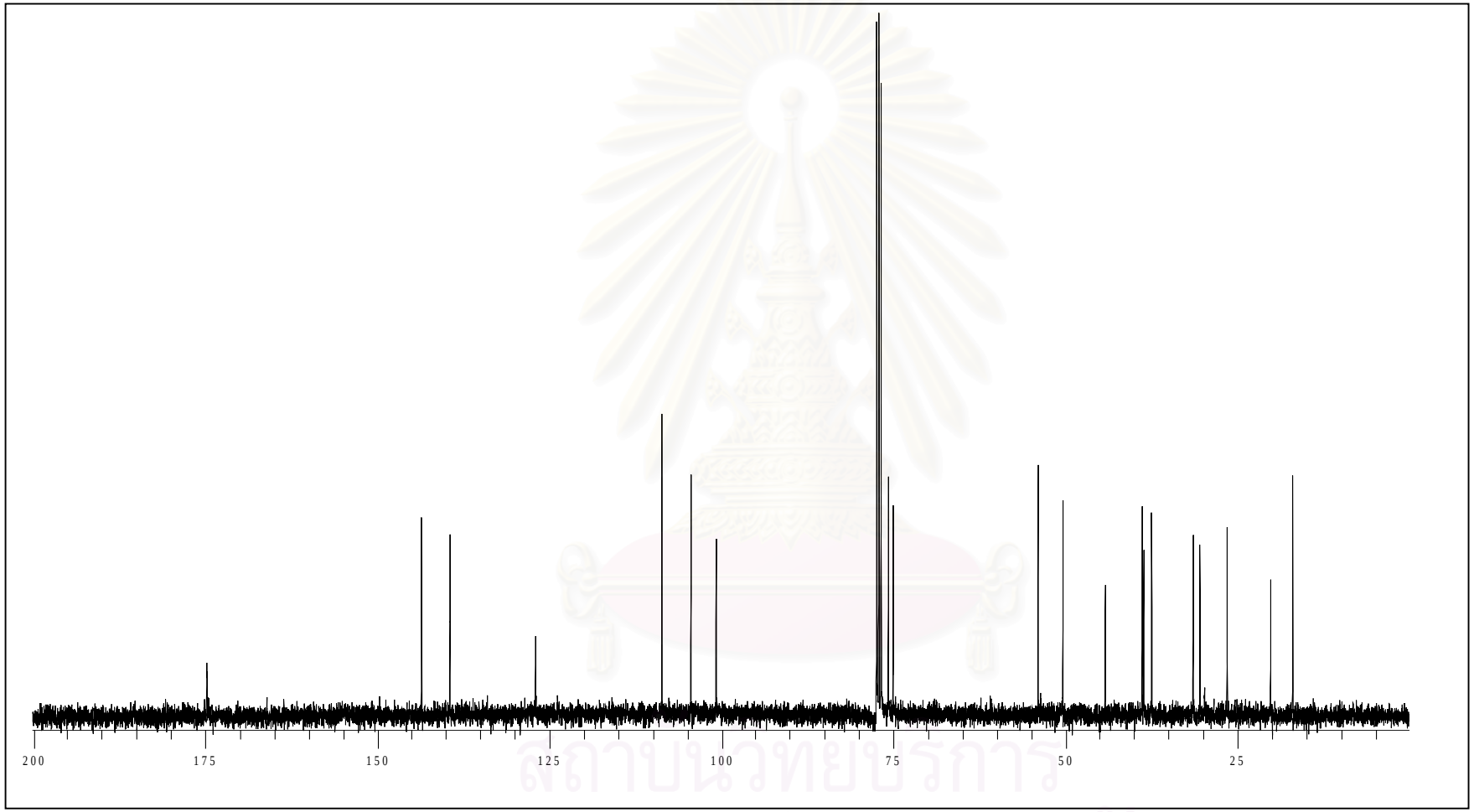

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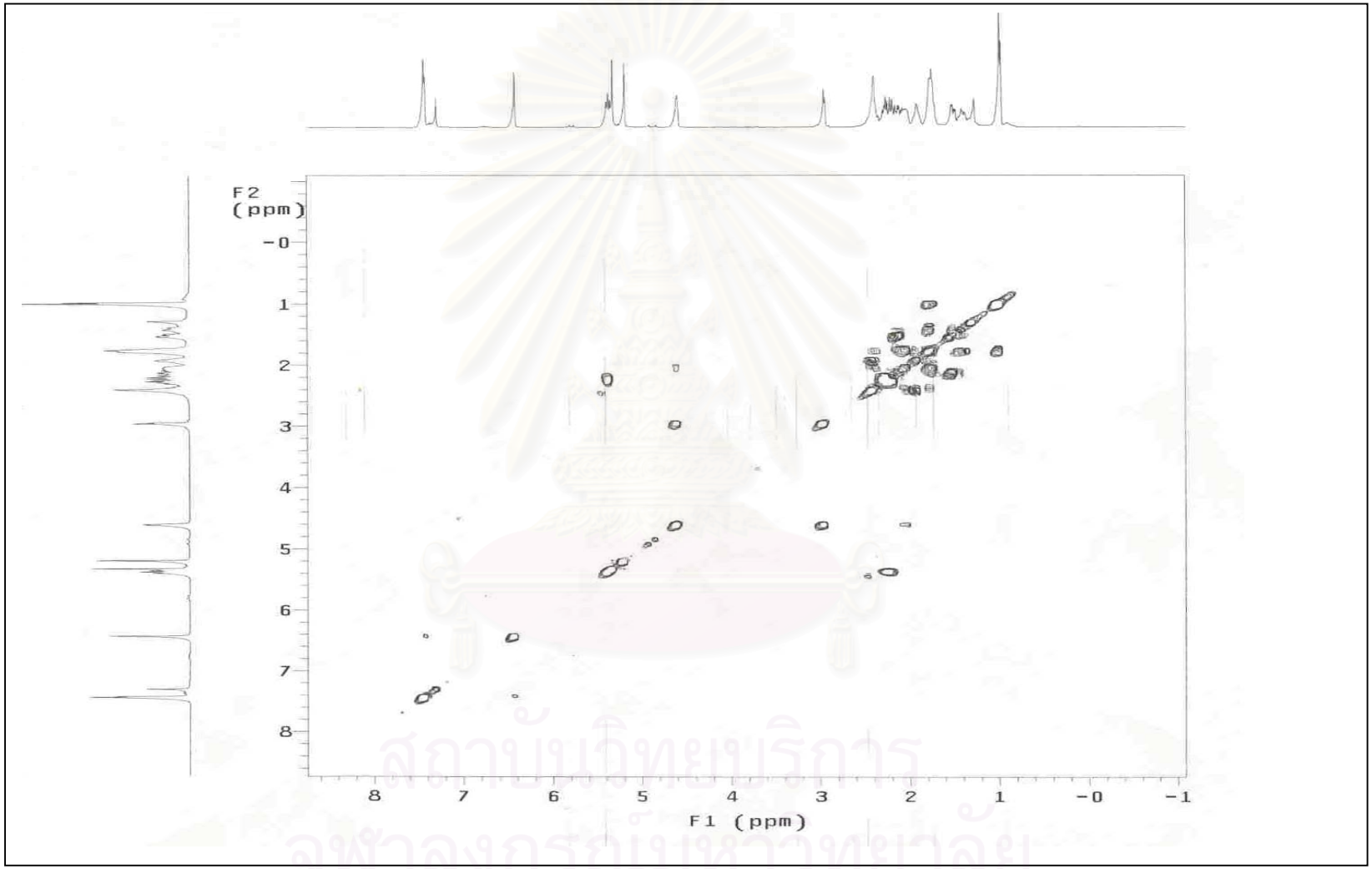

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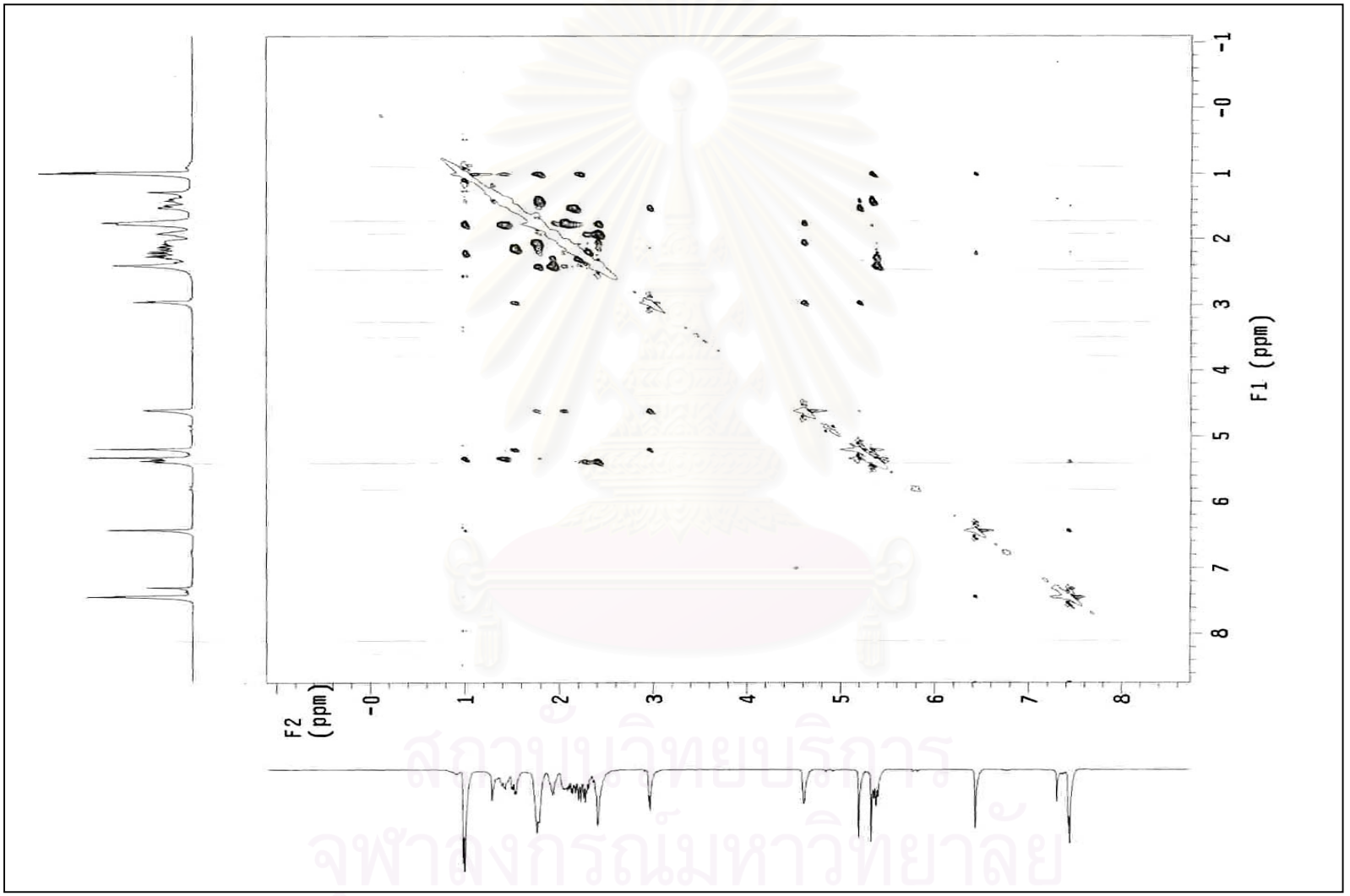

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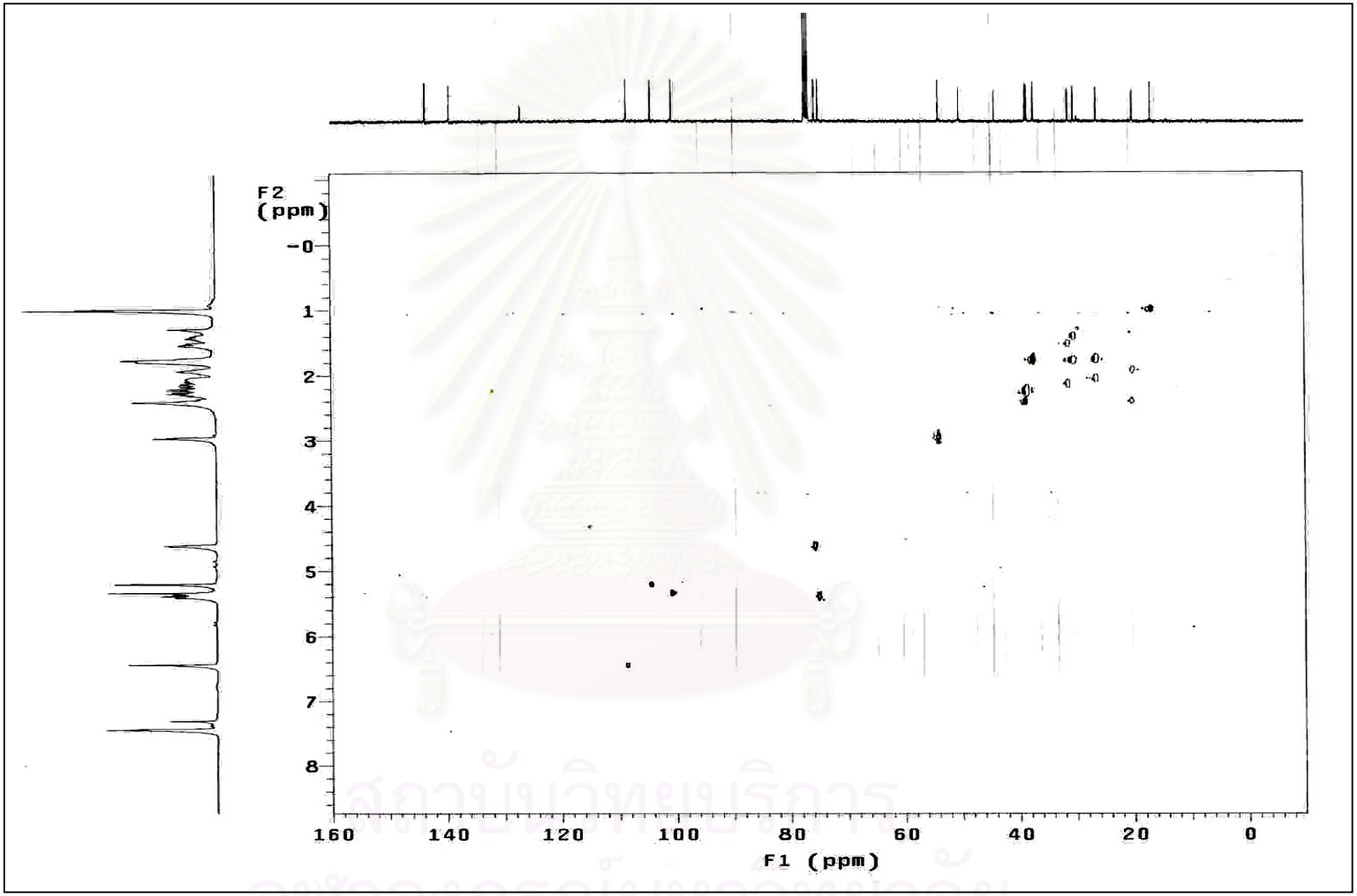
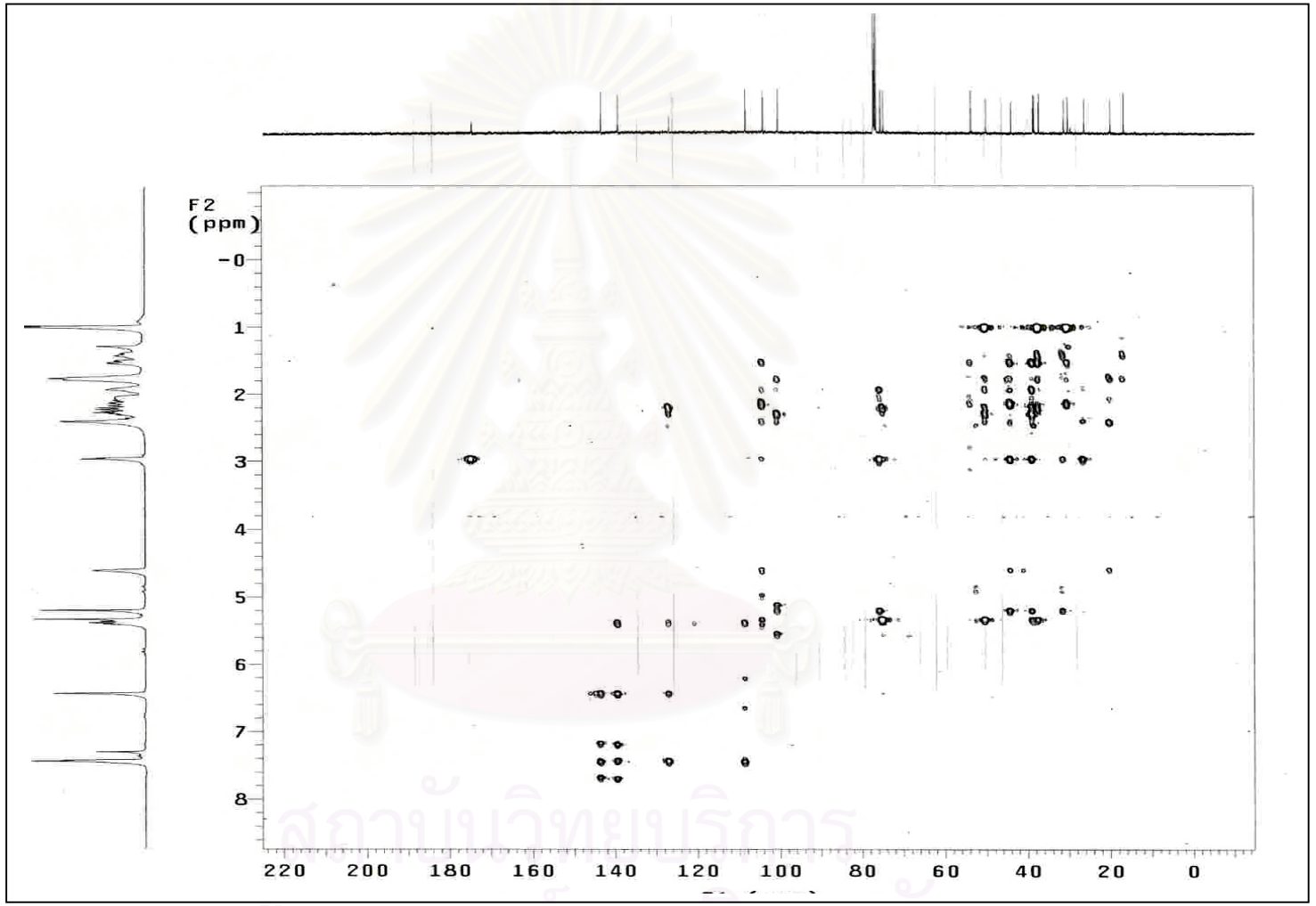


Figure 29 HSQC spectrum of compound **2.**



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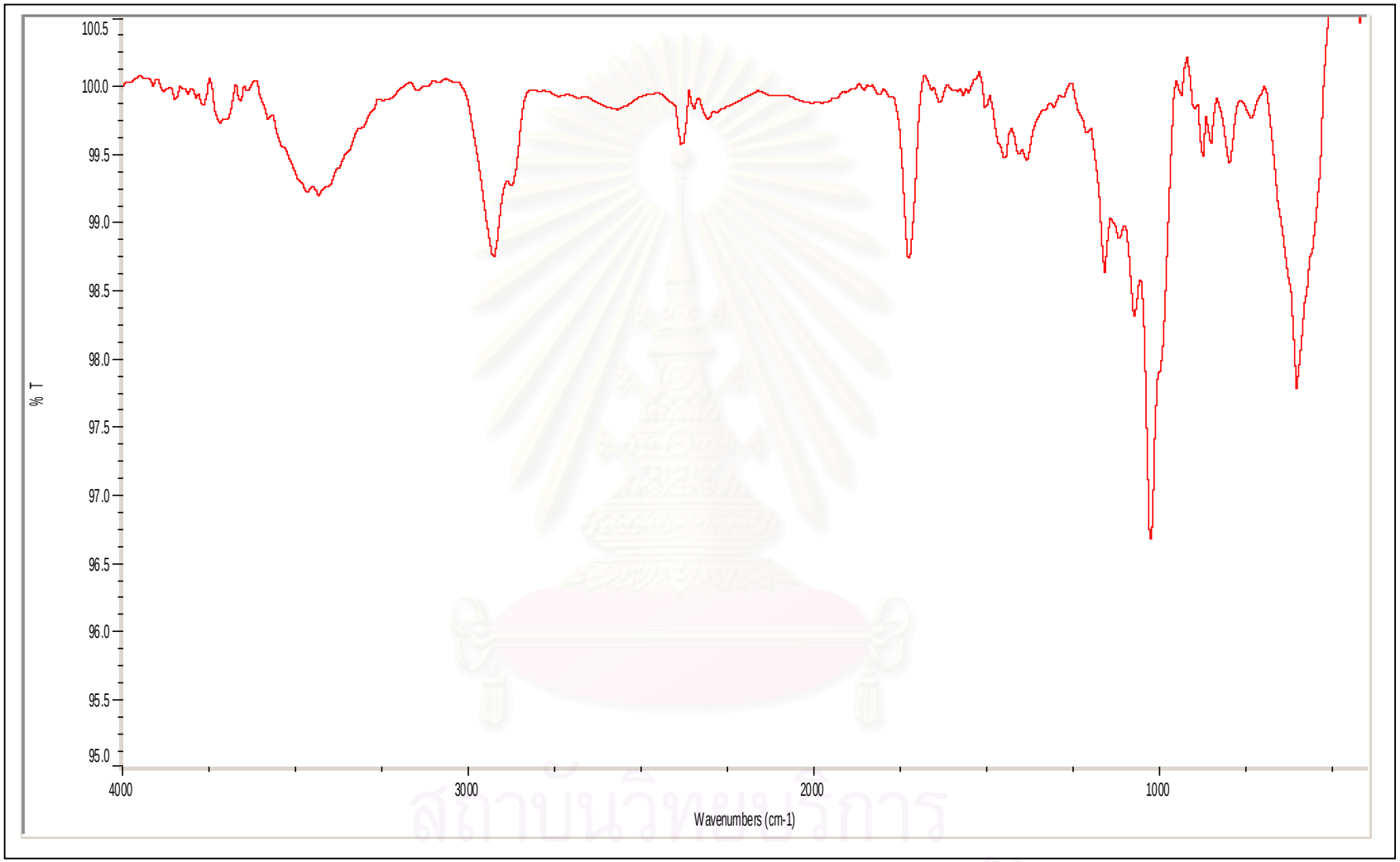
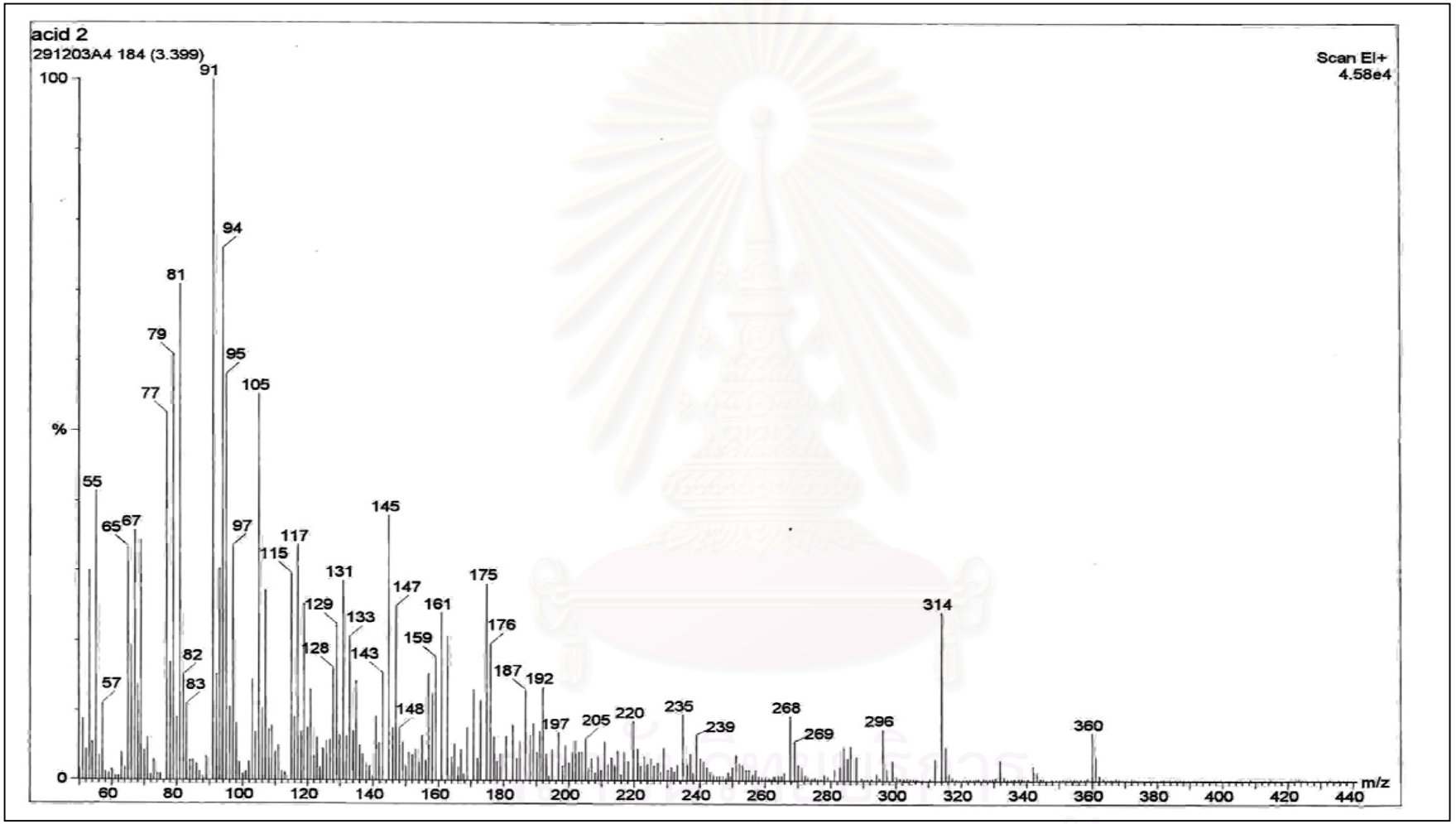


Figure 31 IR spectrum of compound **3.**



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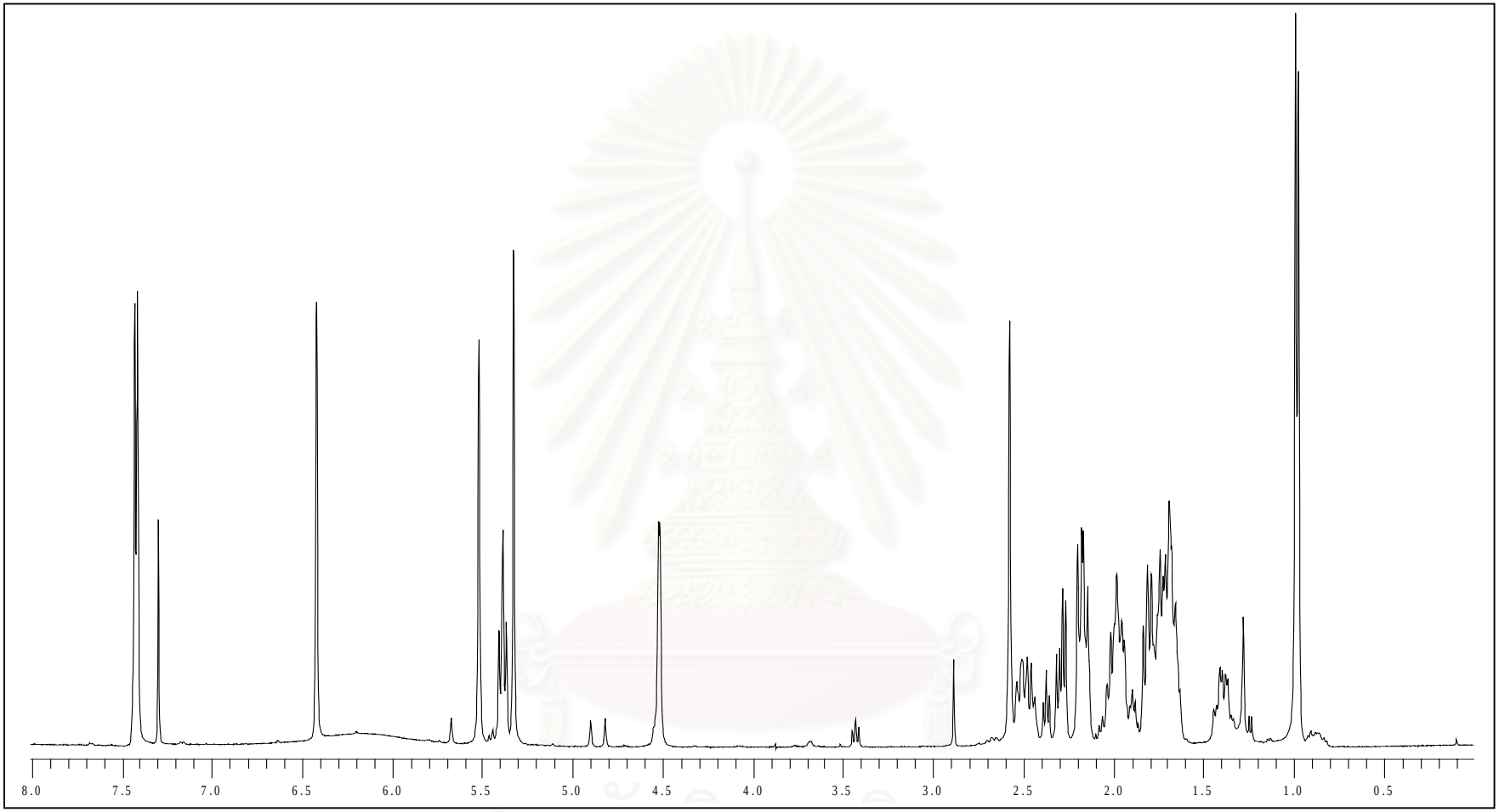

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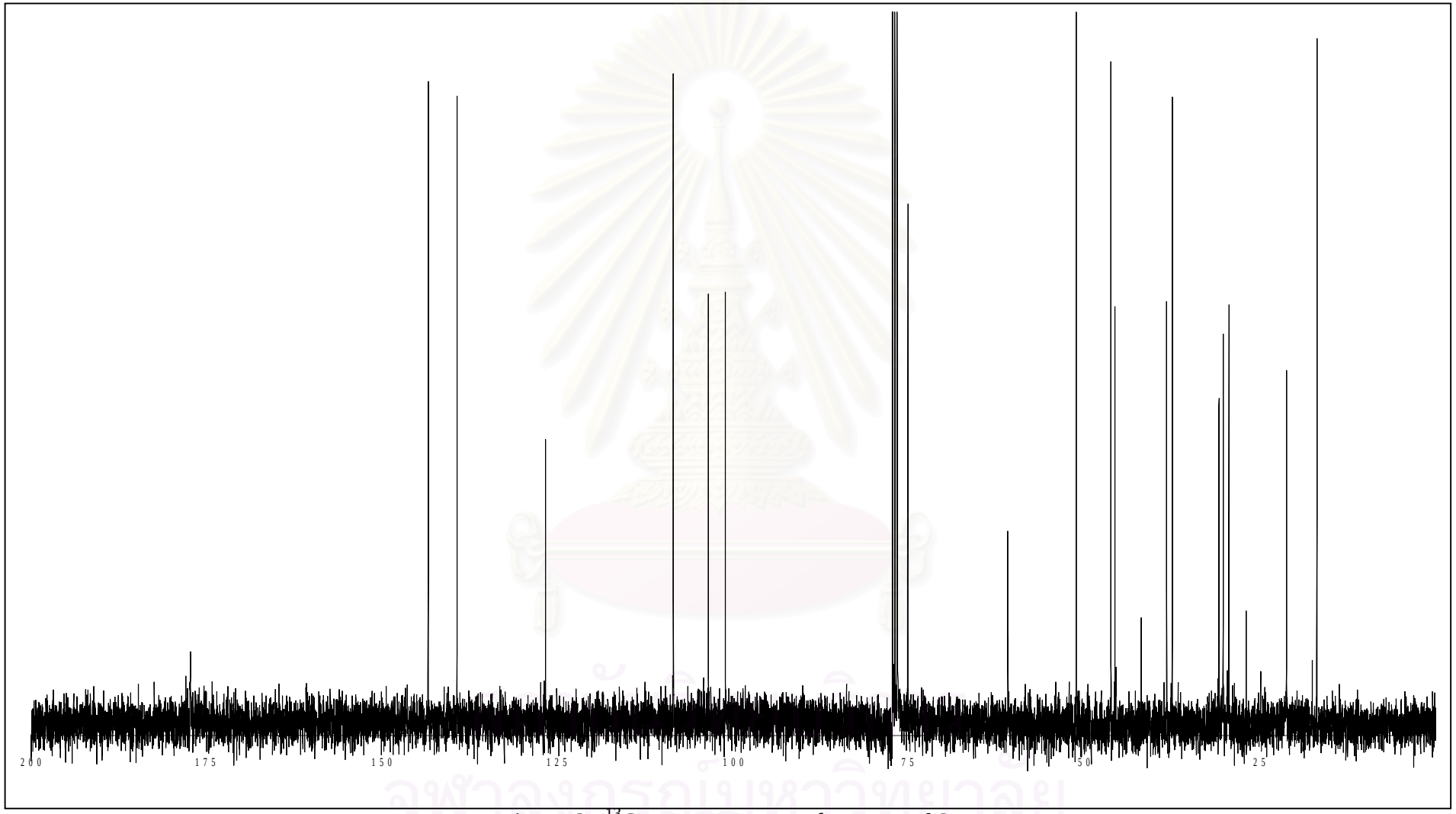

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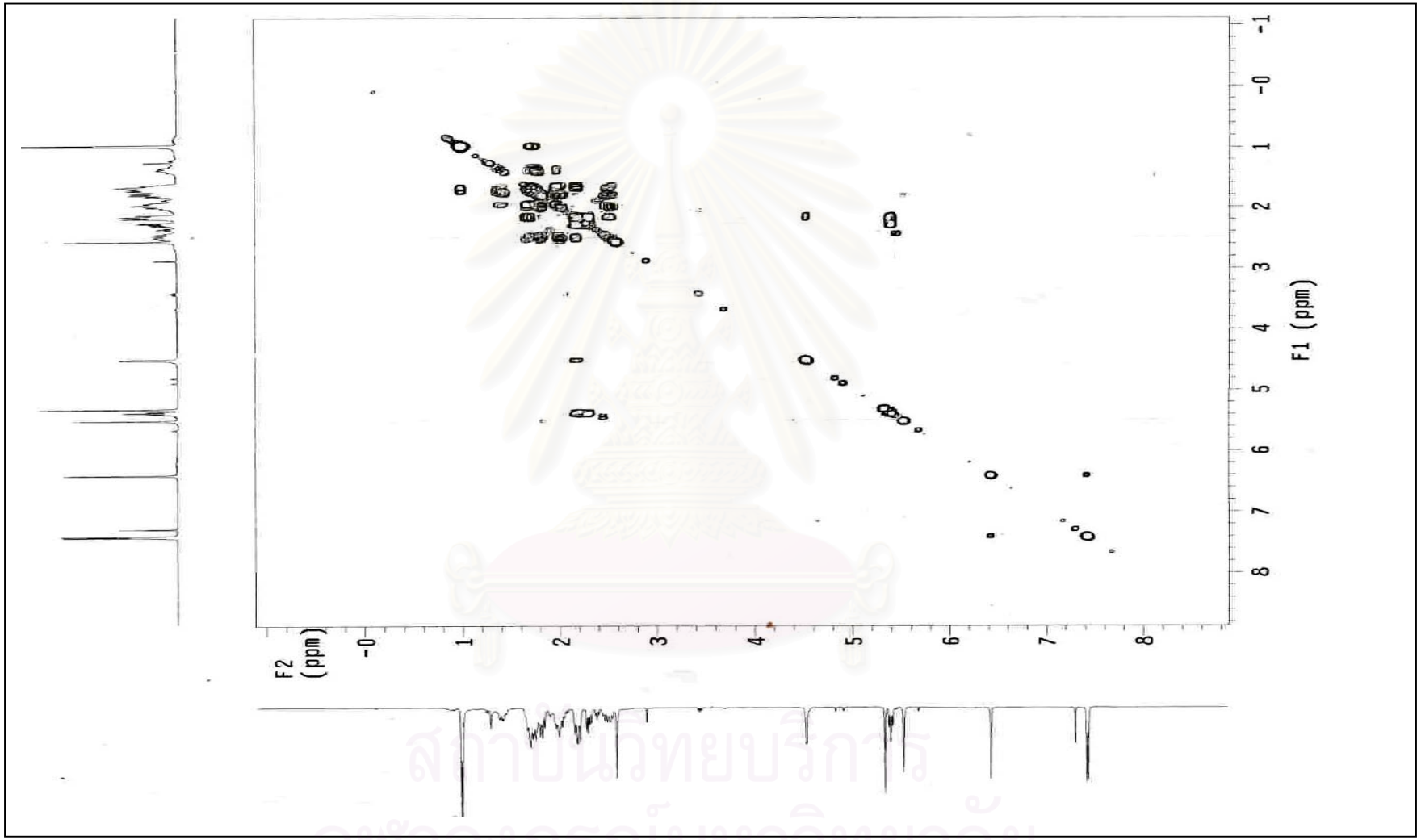

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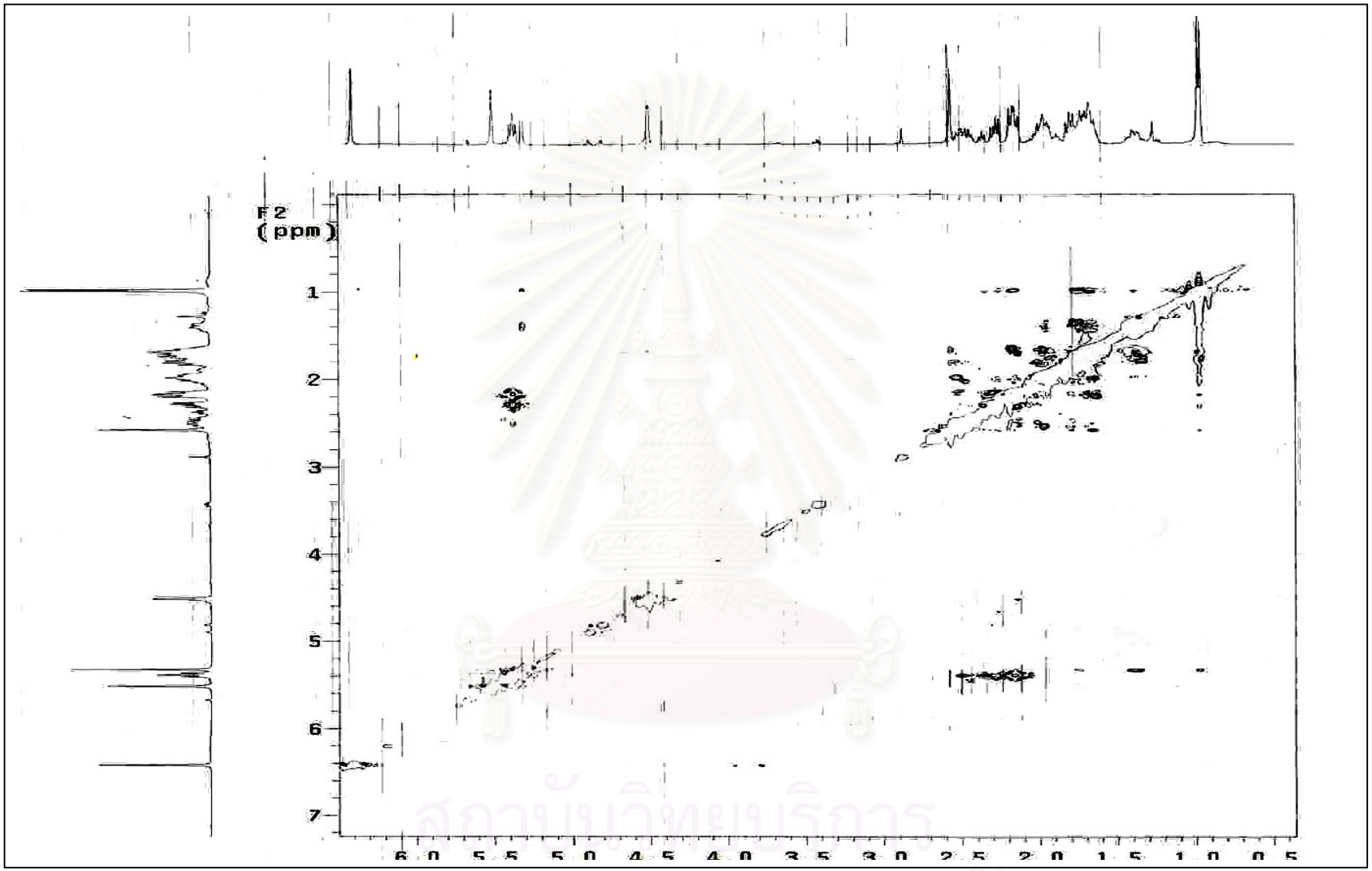

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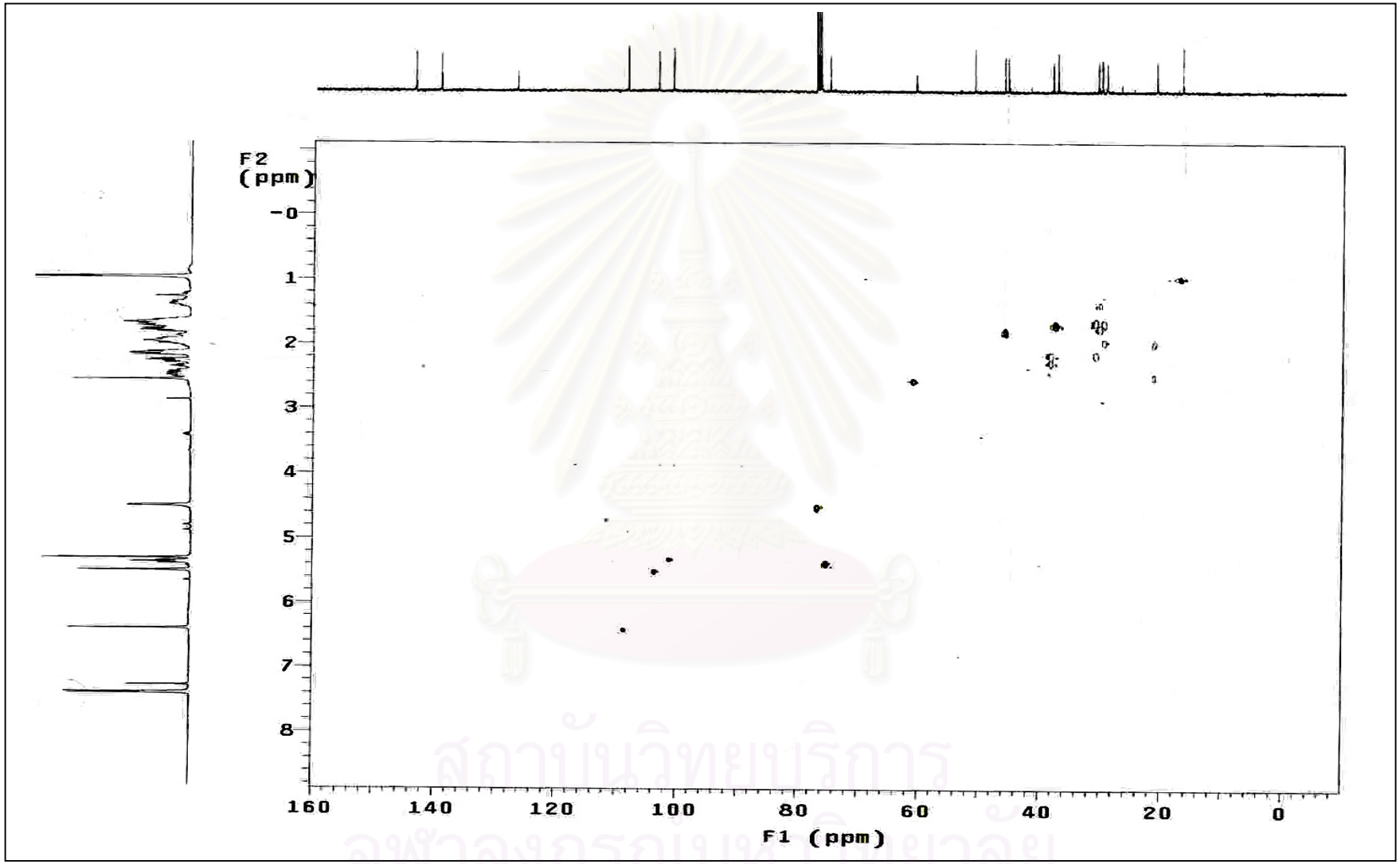

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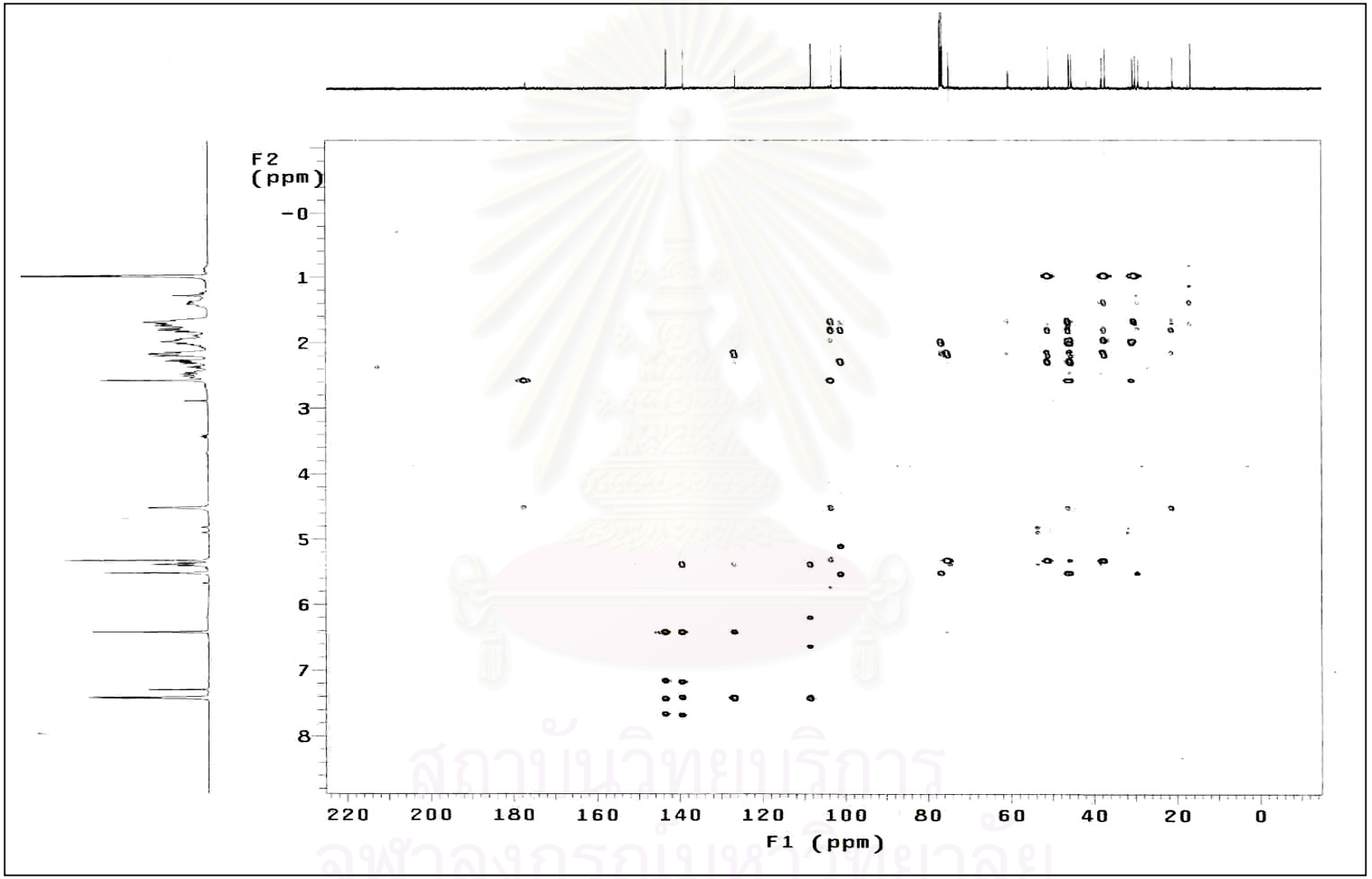

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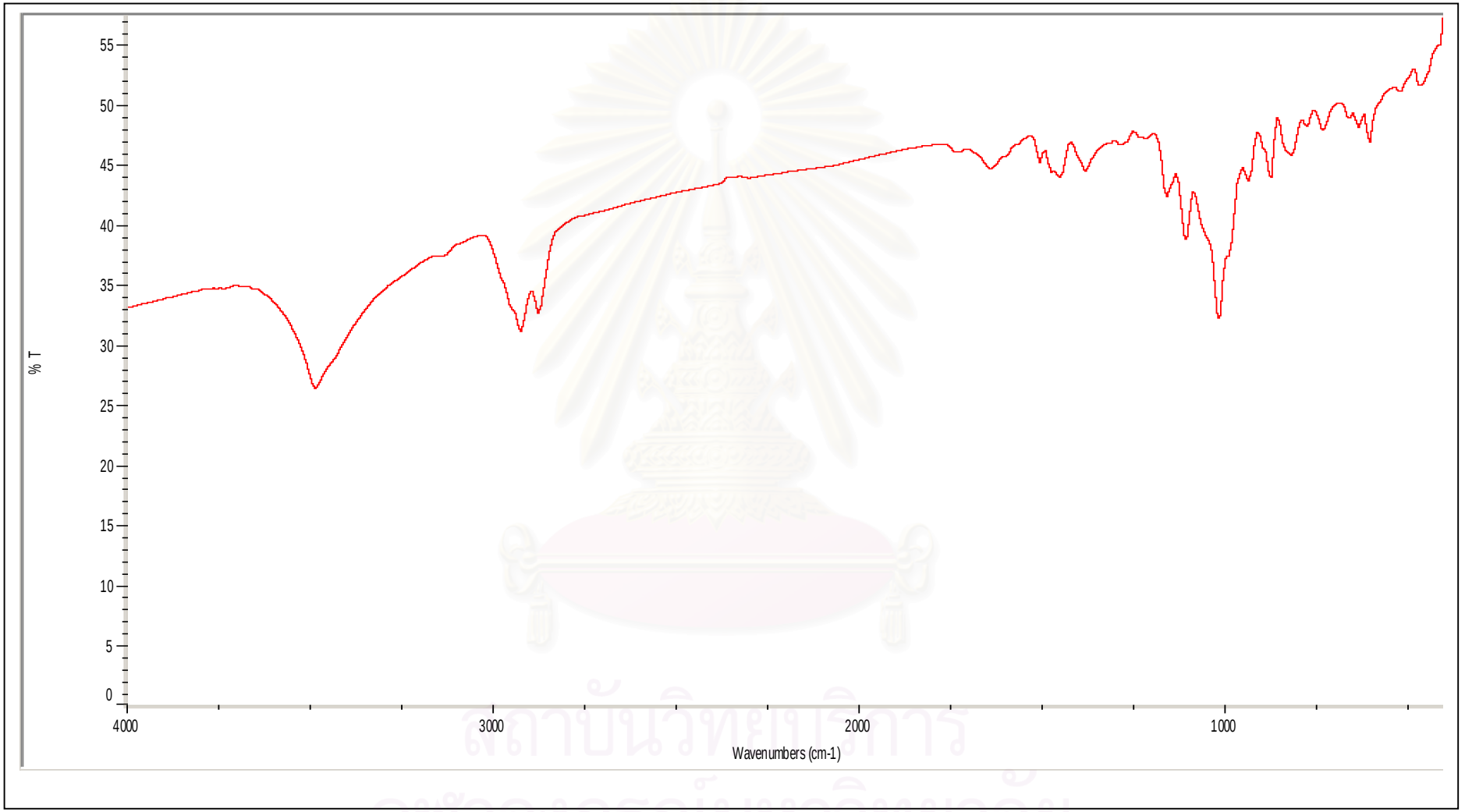

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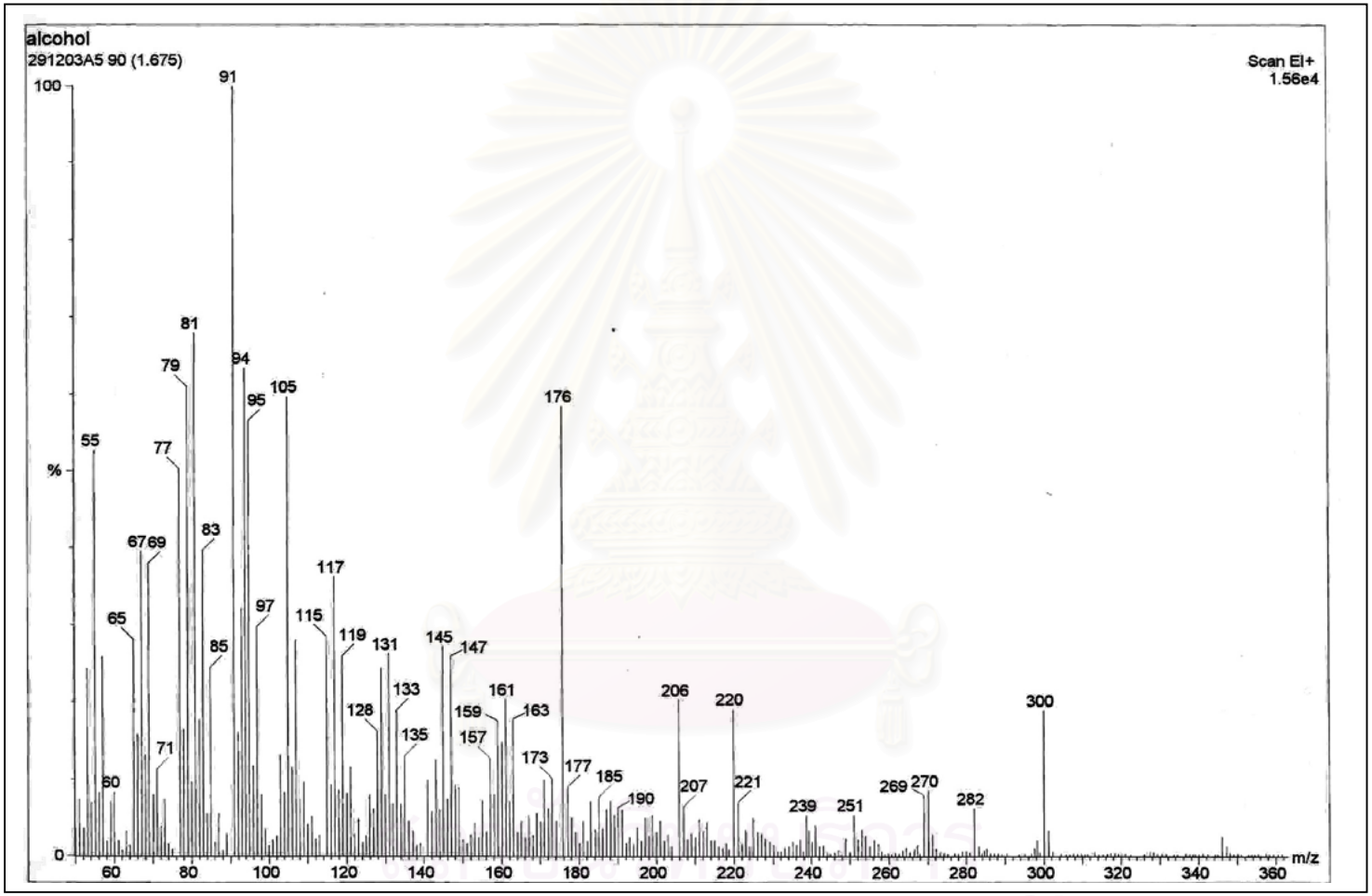

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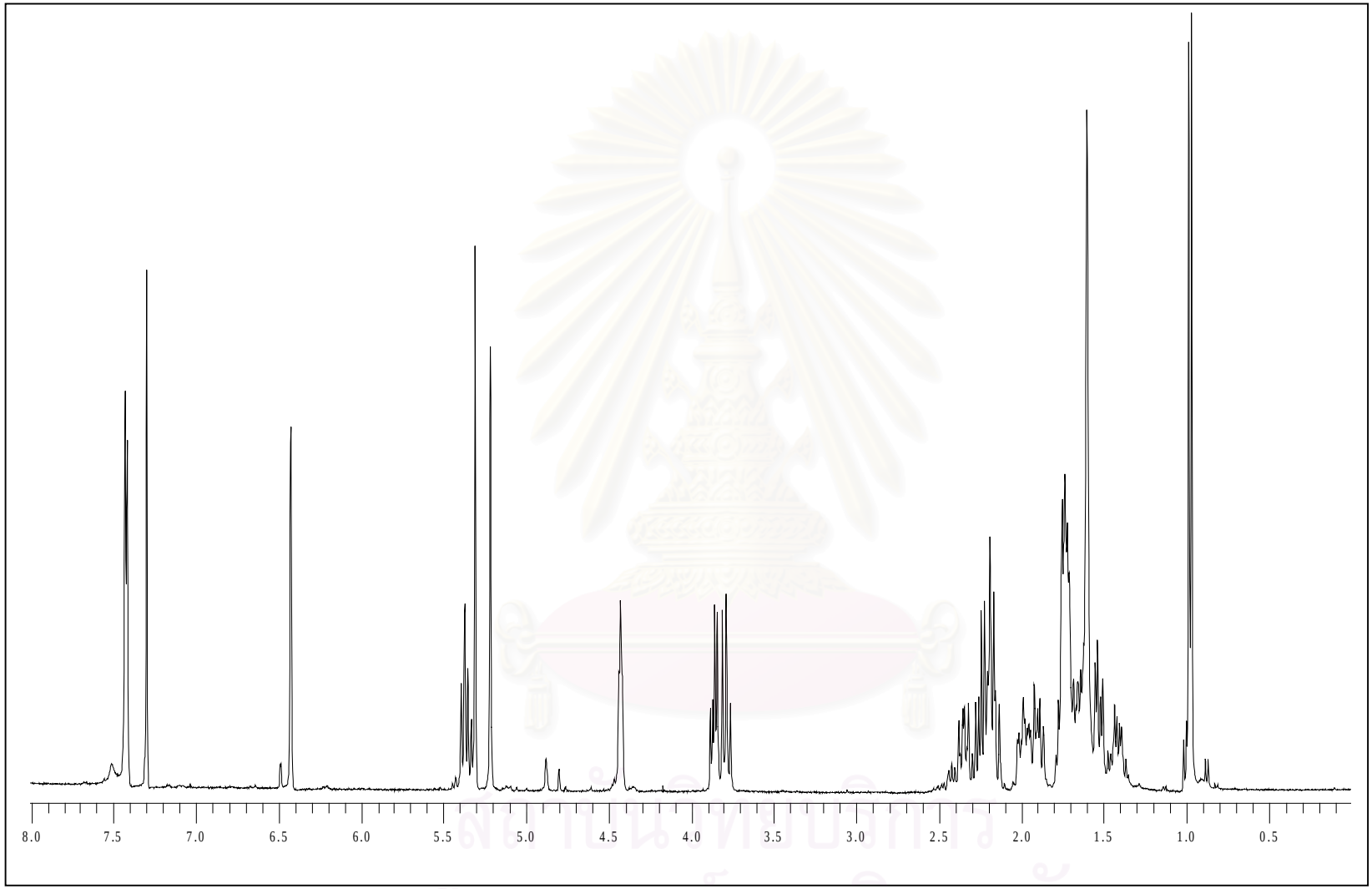

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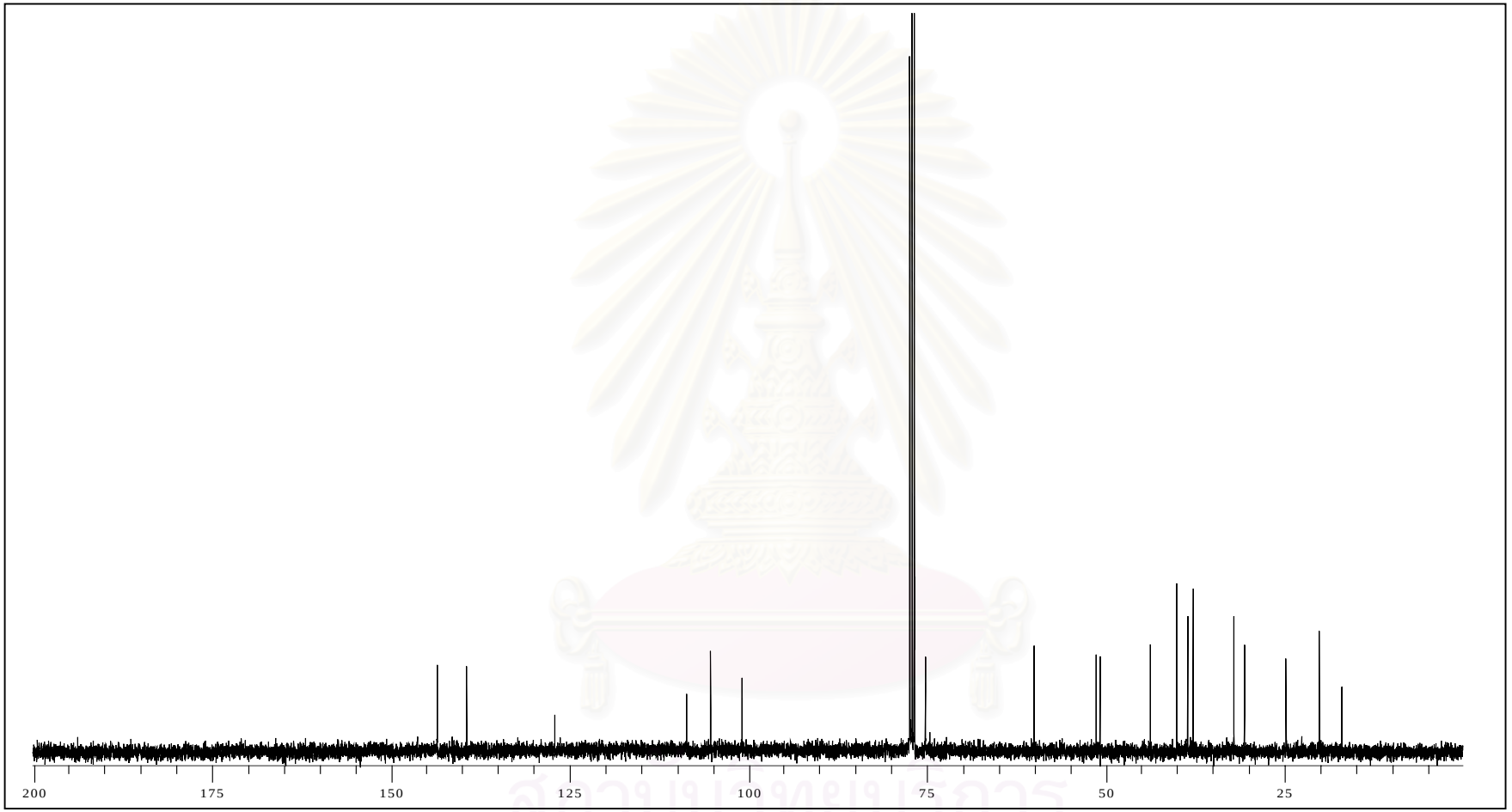

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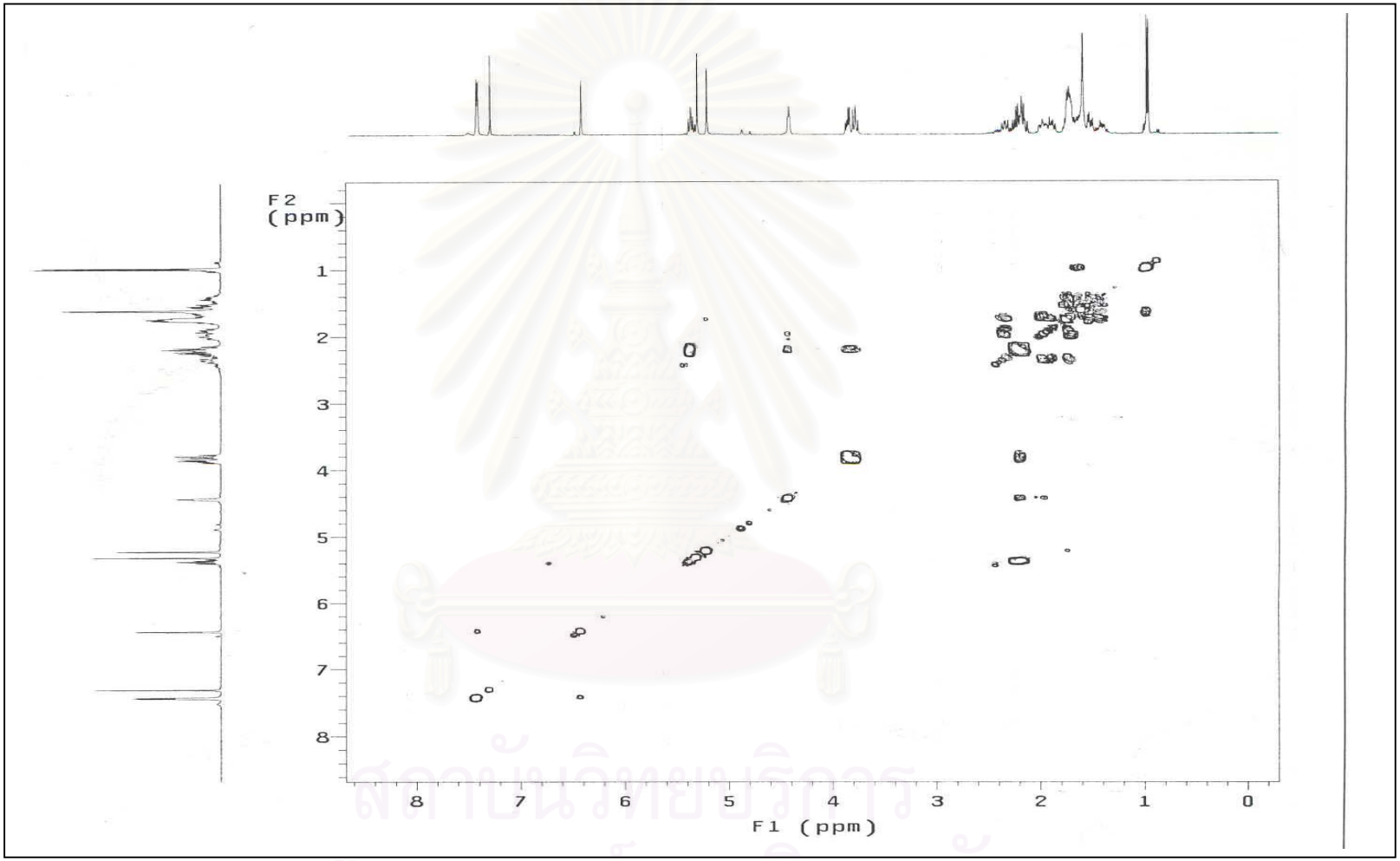

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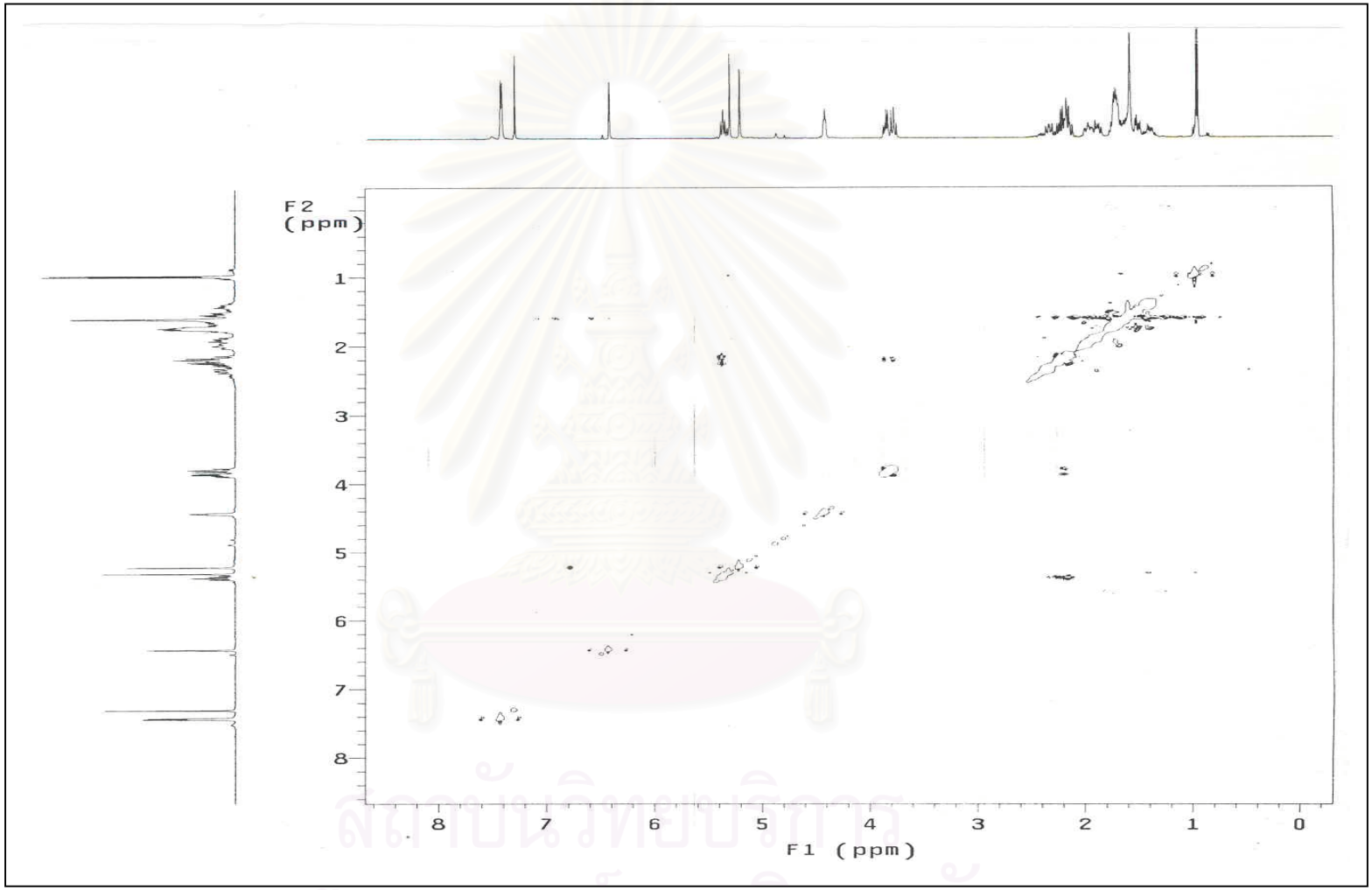

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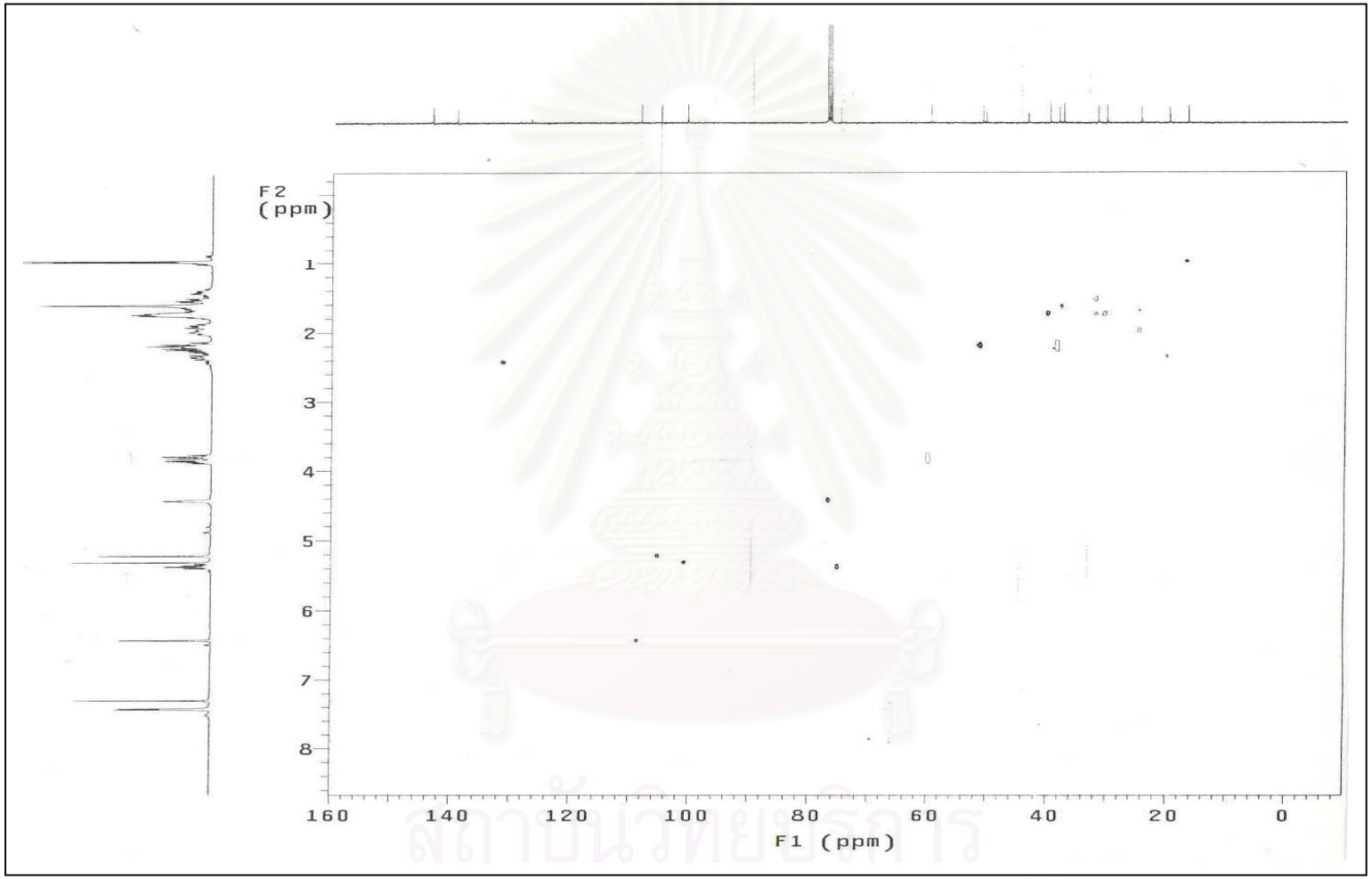

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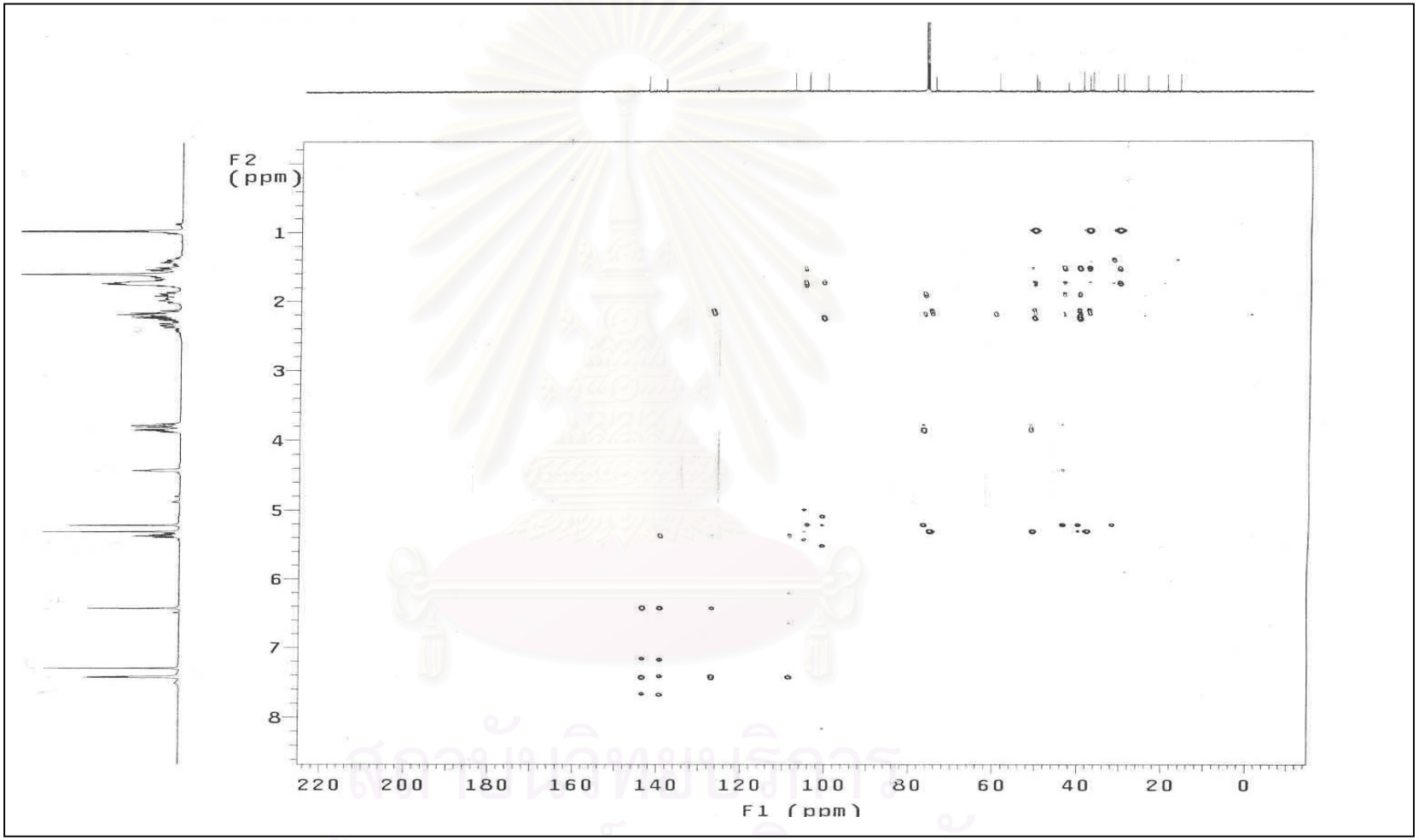

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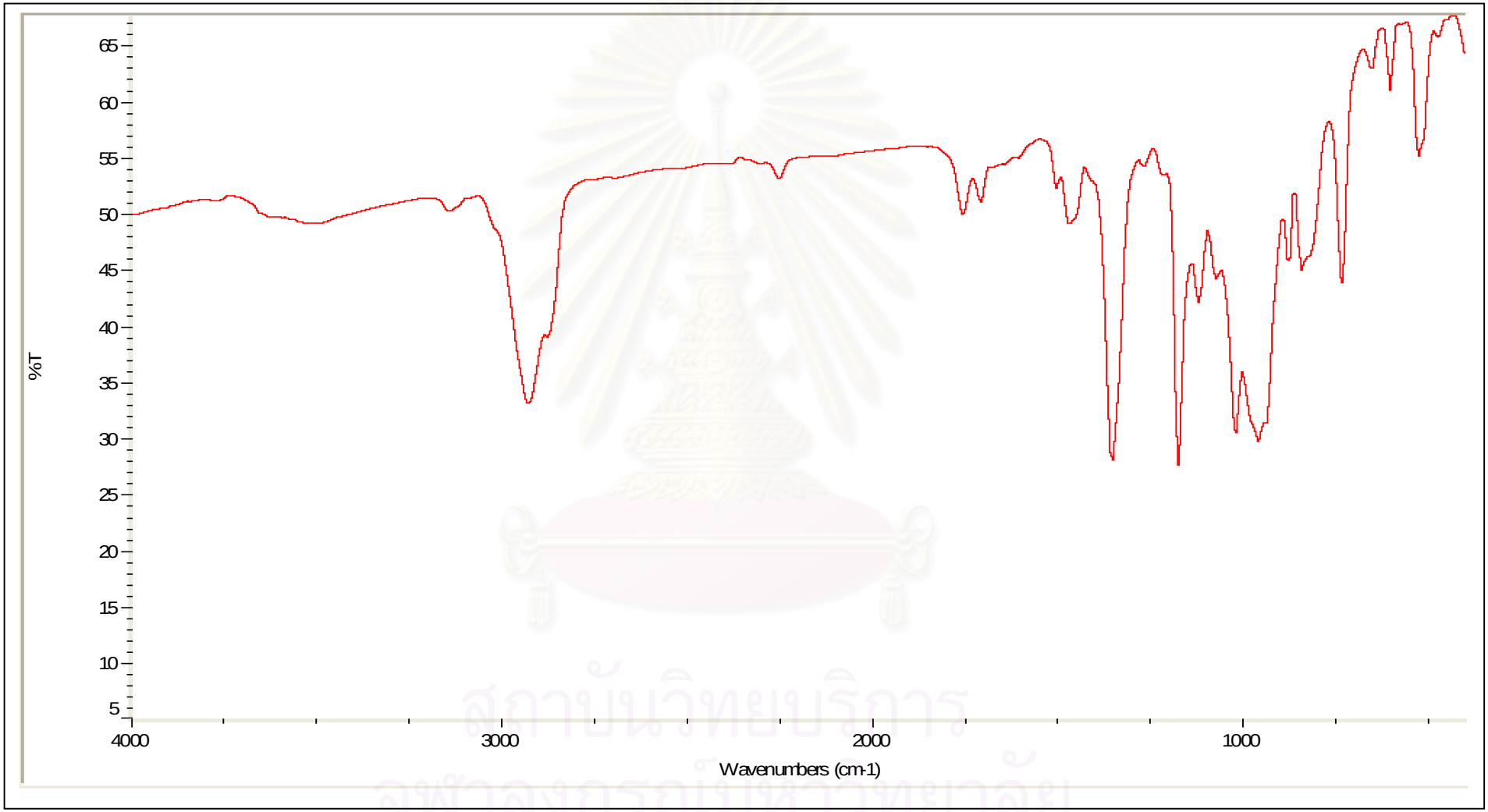

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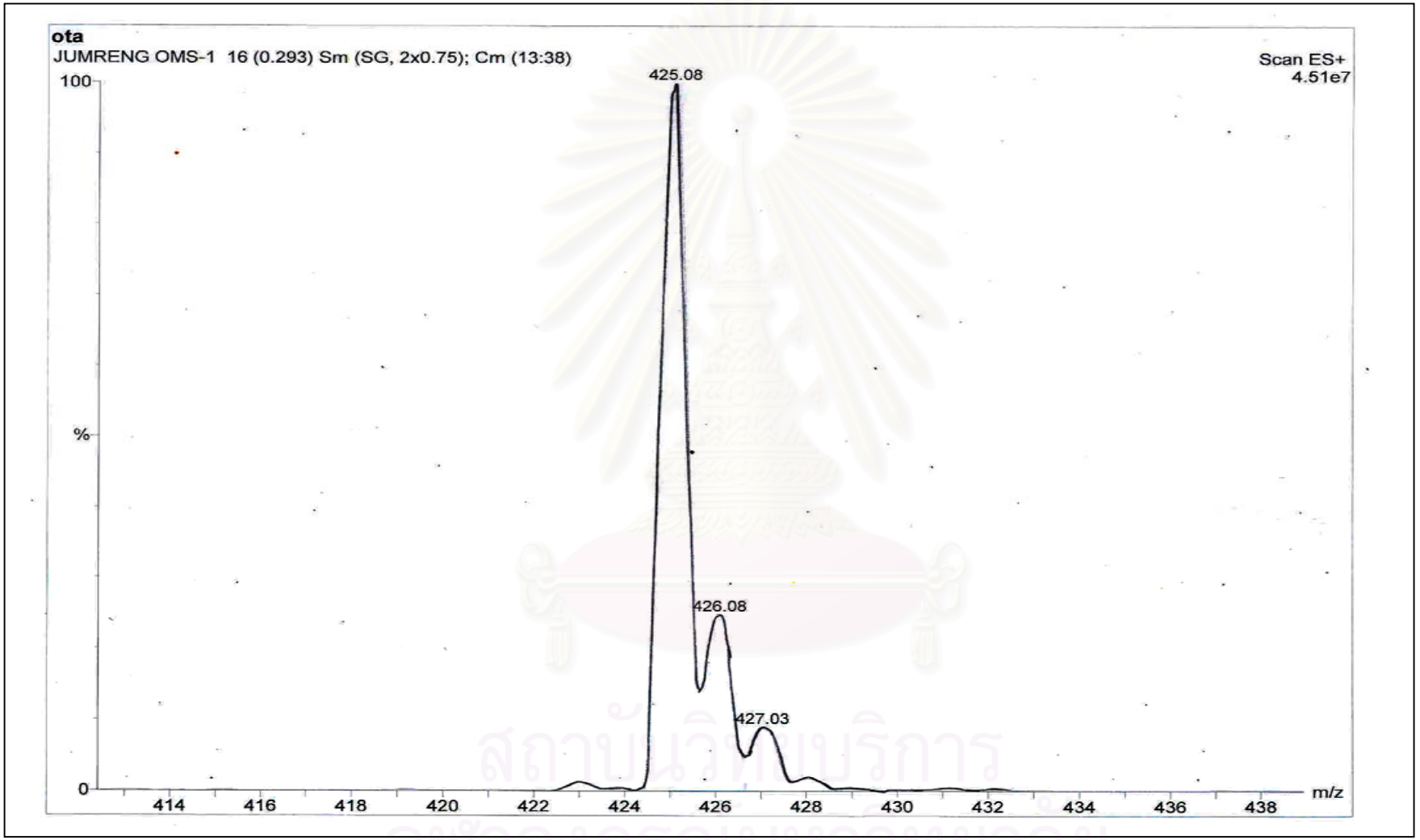

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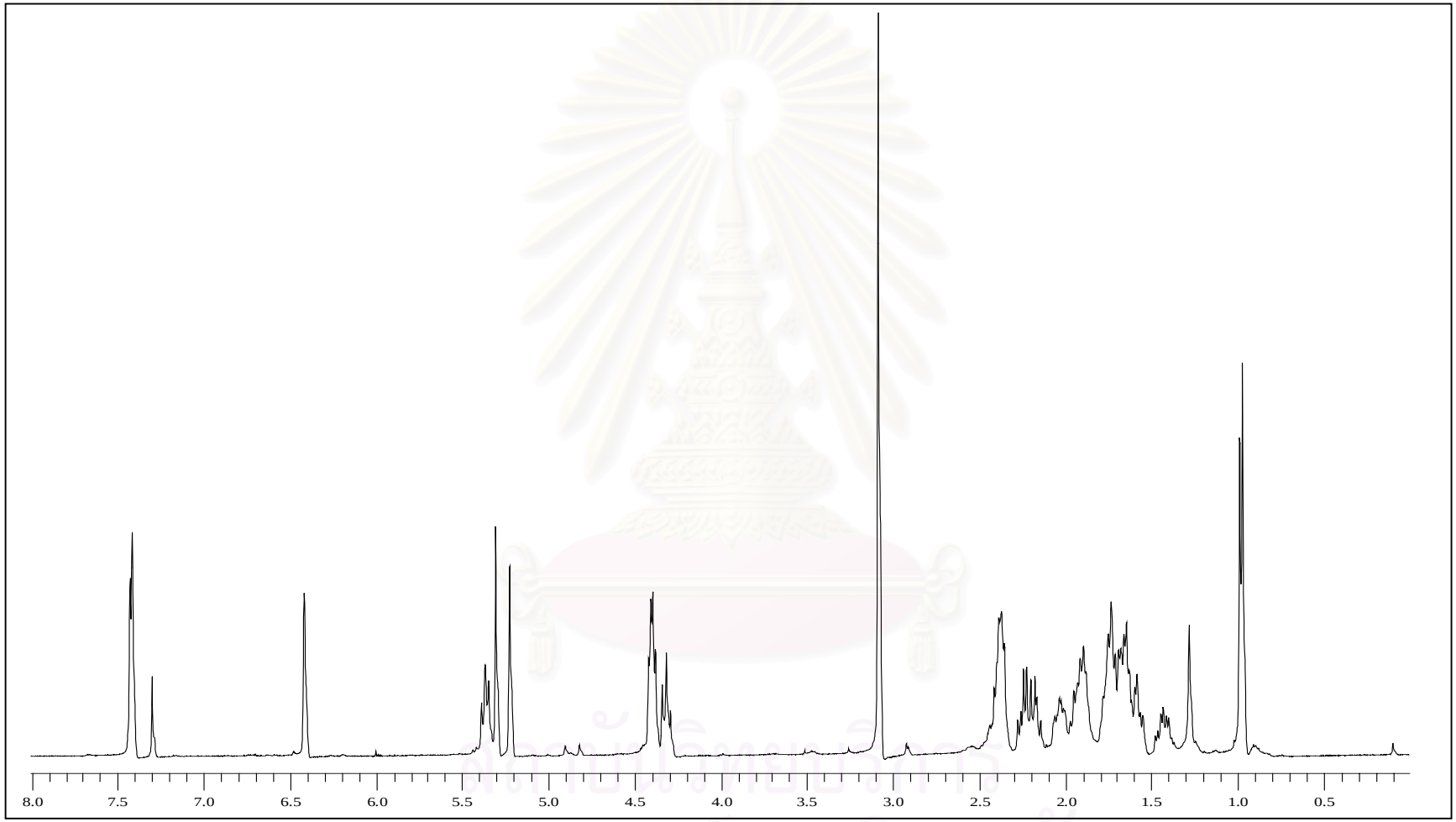

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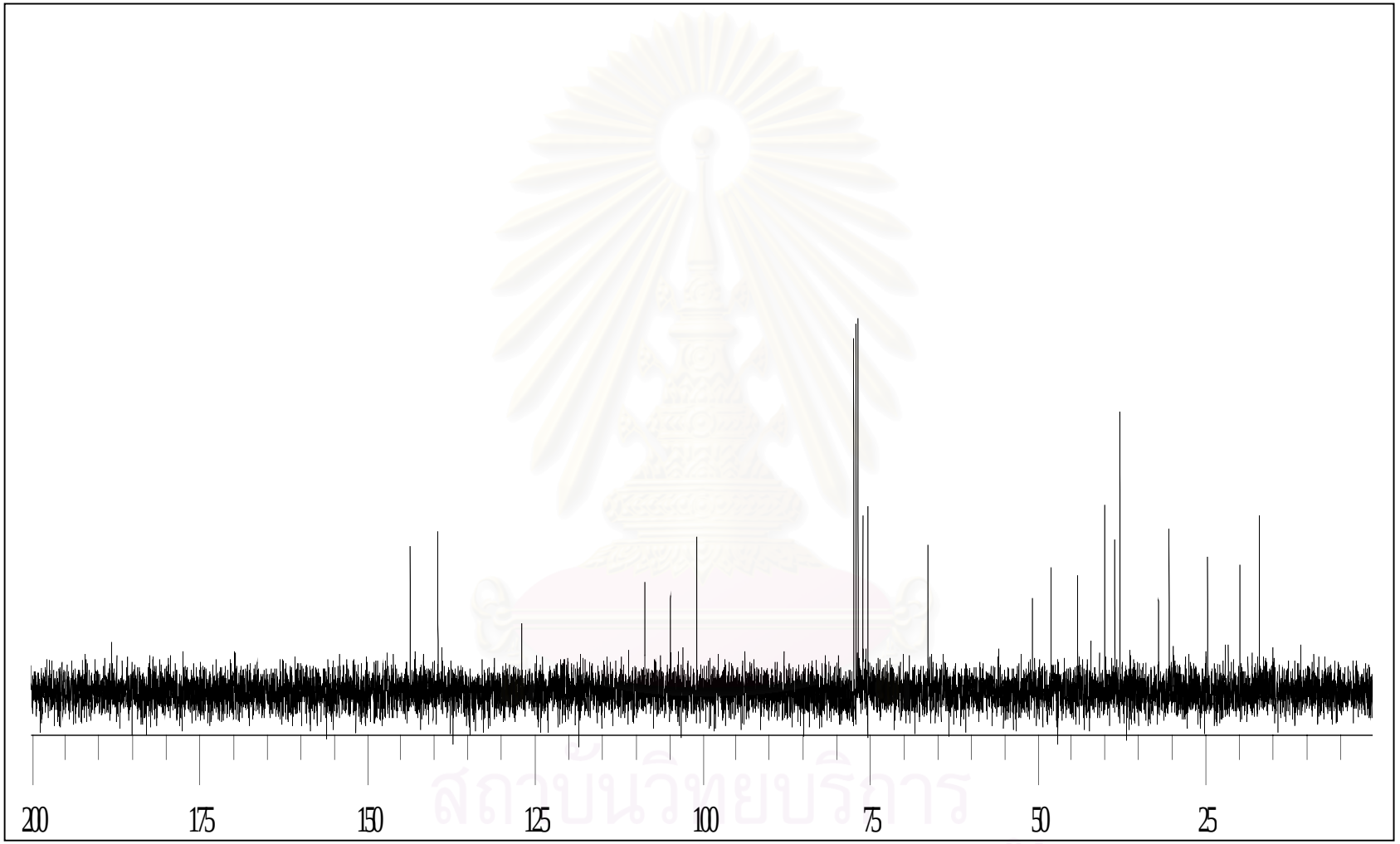

Figure 50 ^{13}C-NMR spectrum of compound **5.**

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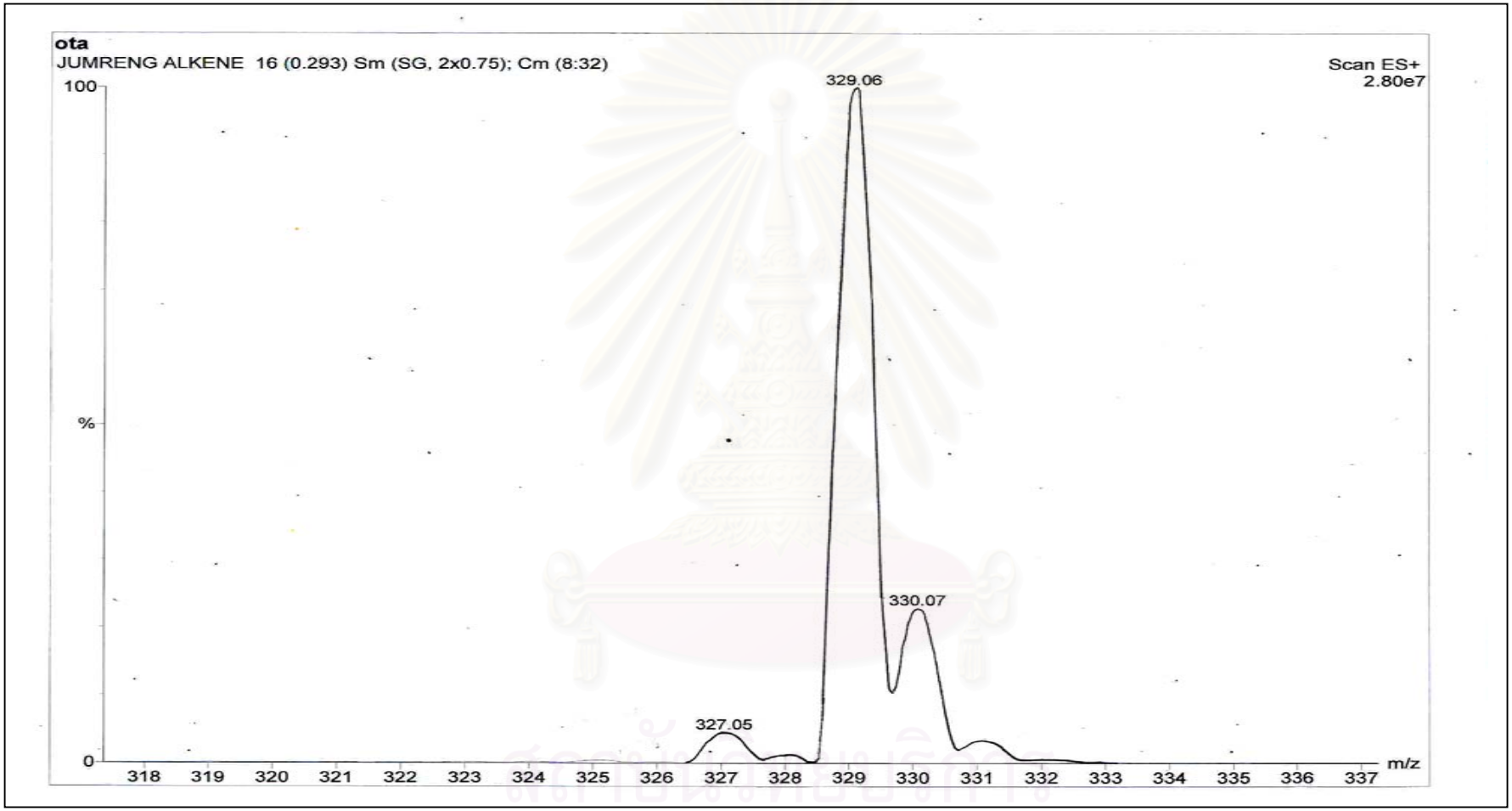

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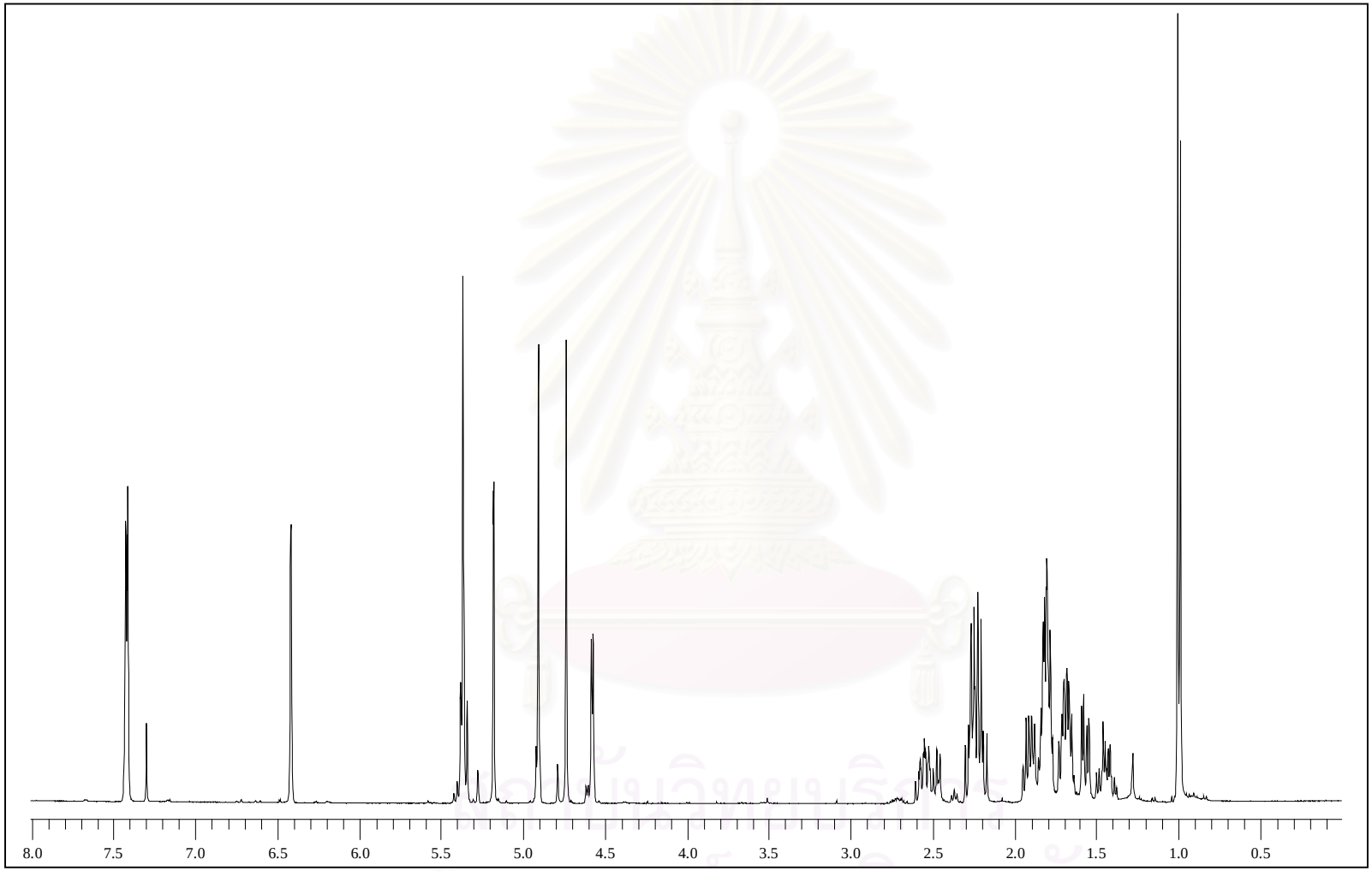

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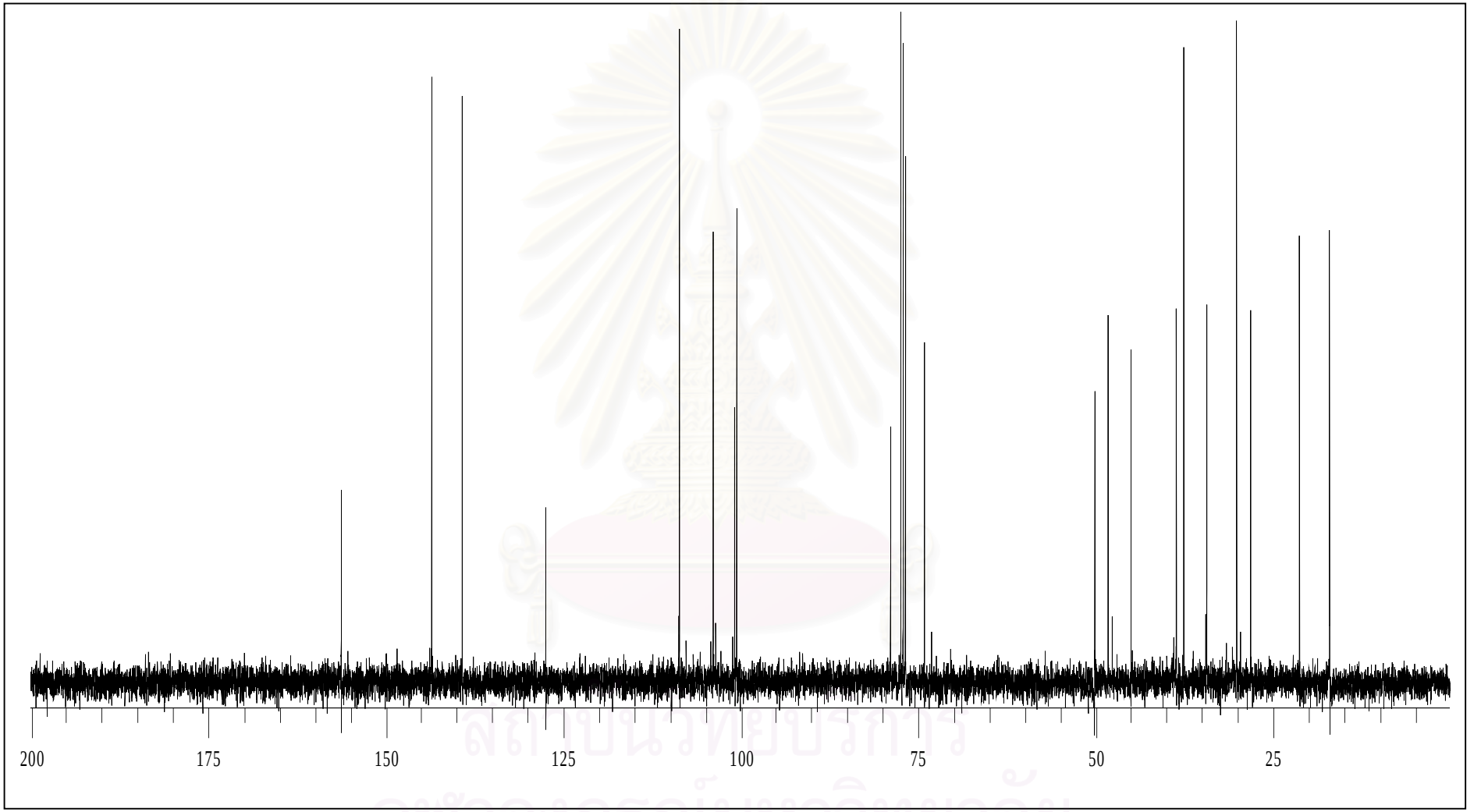

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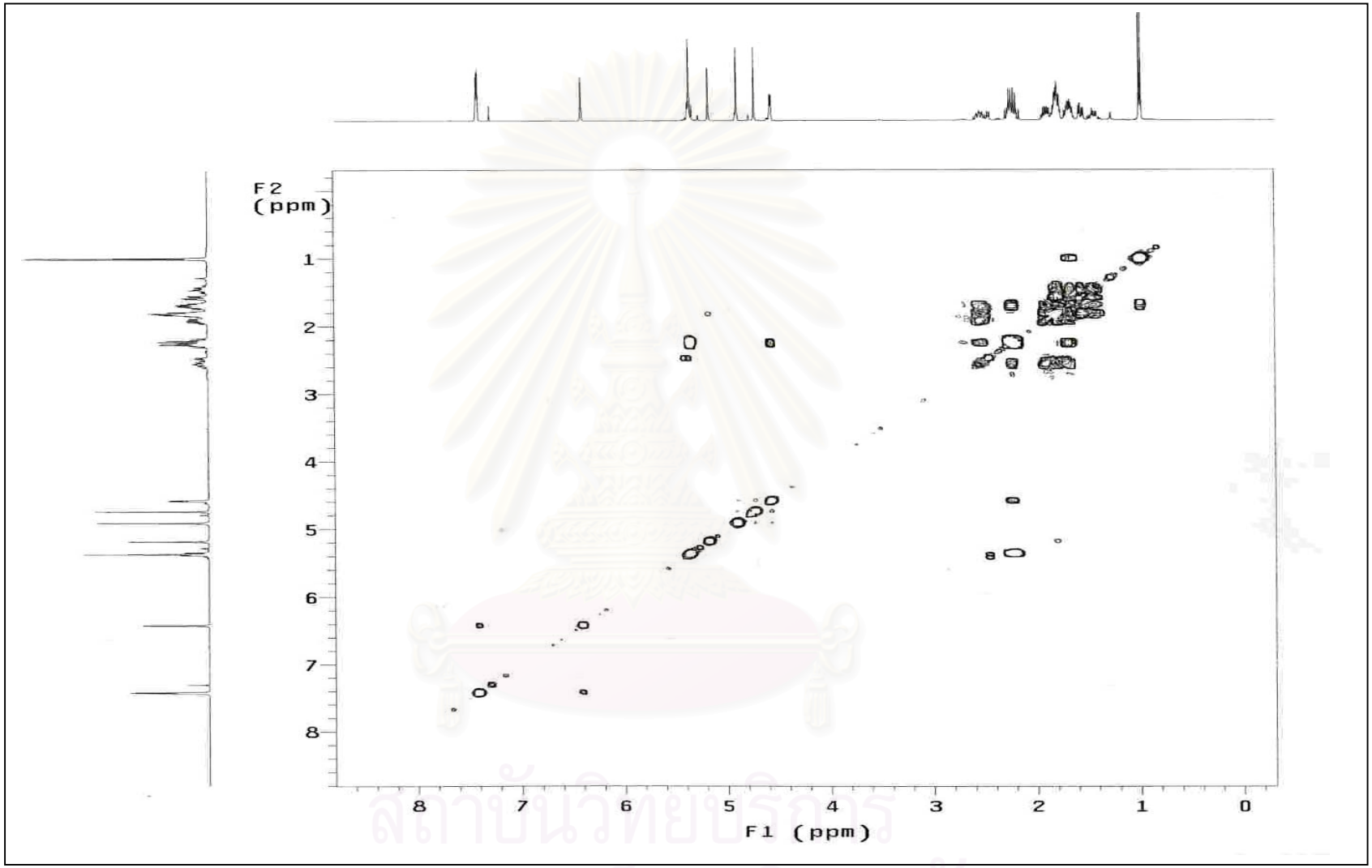

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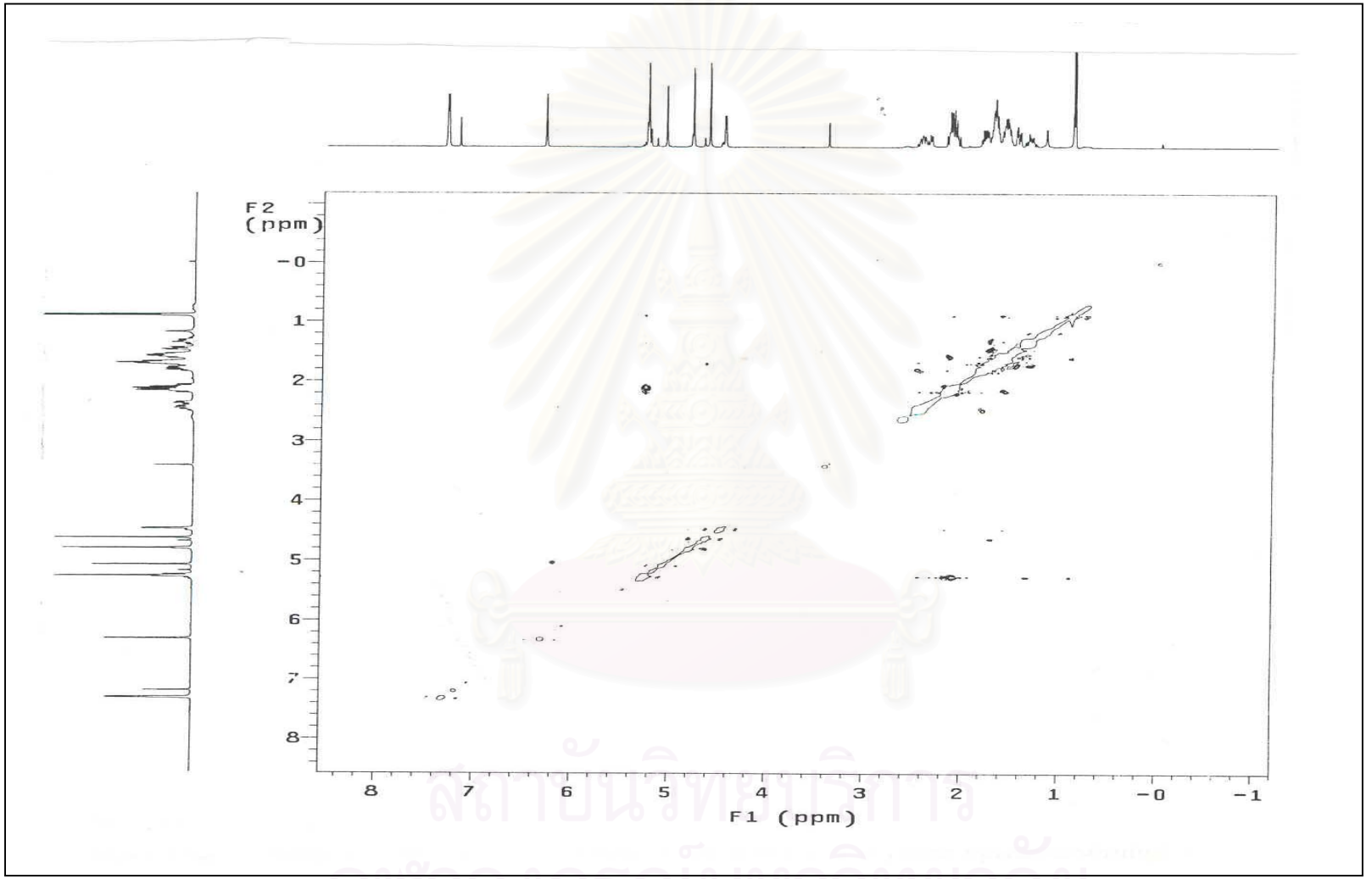

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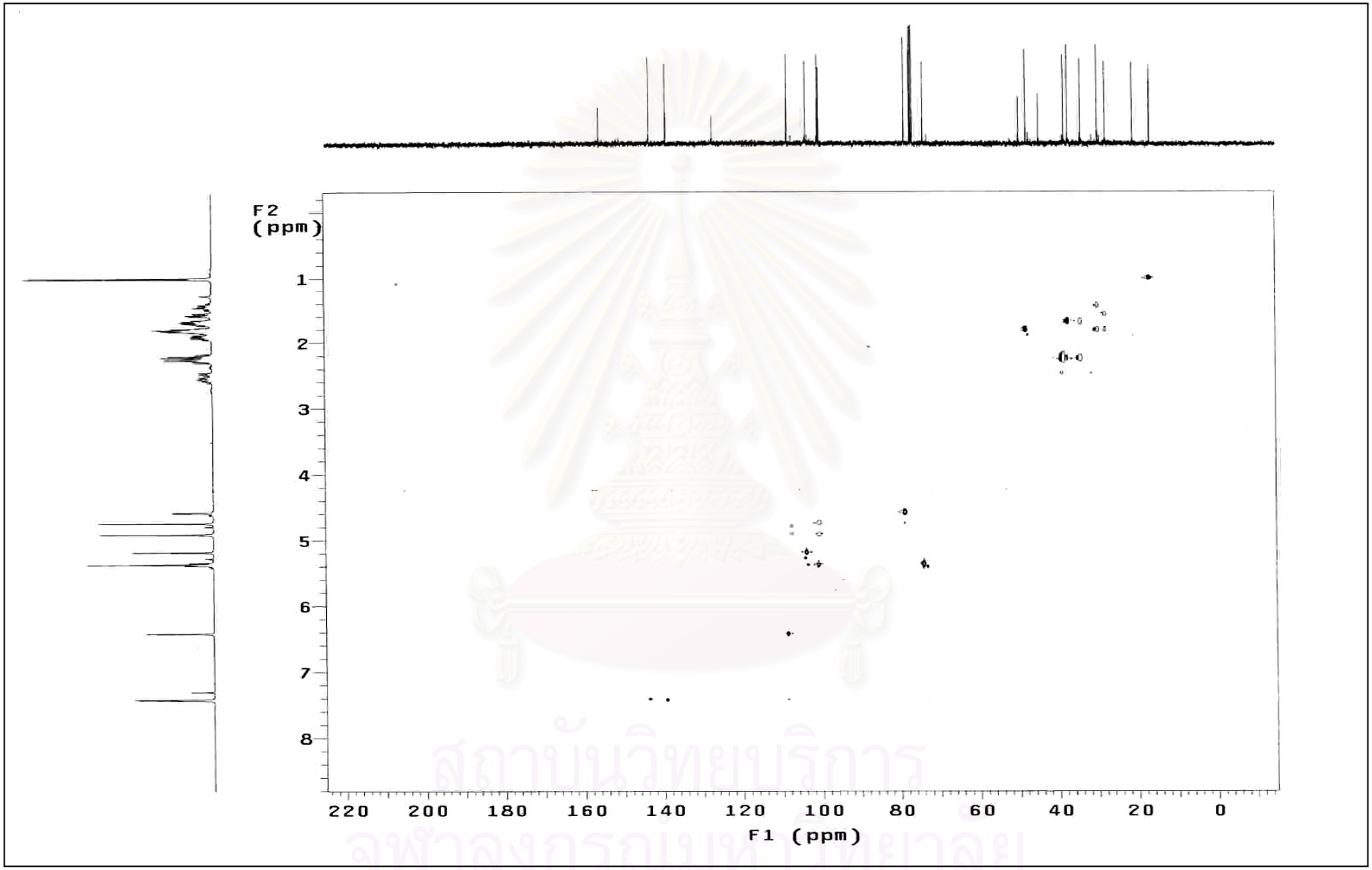

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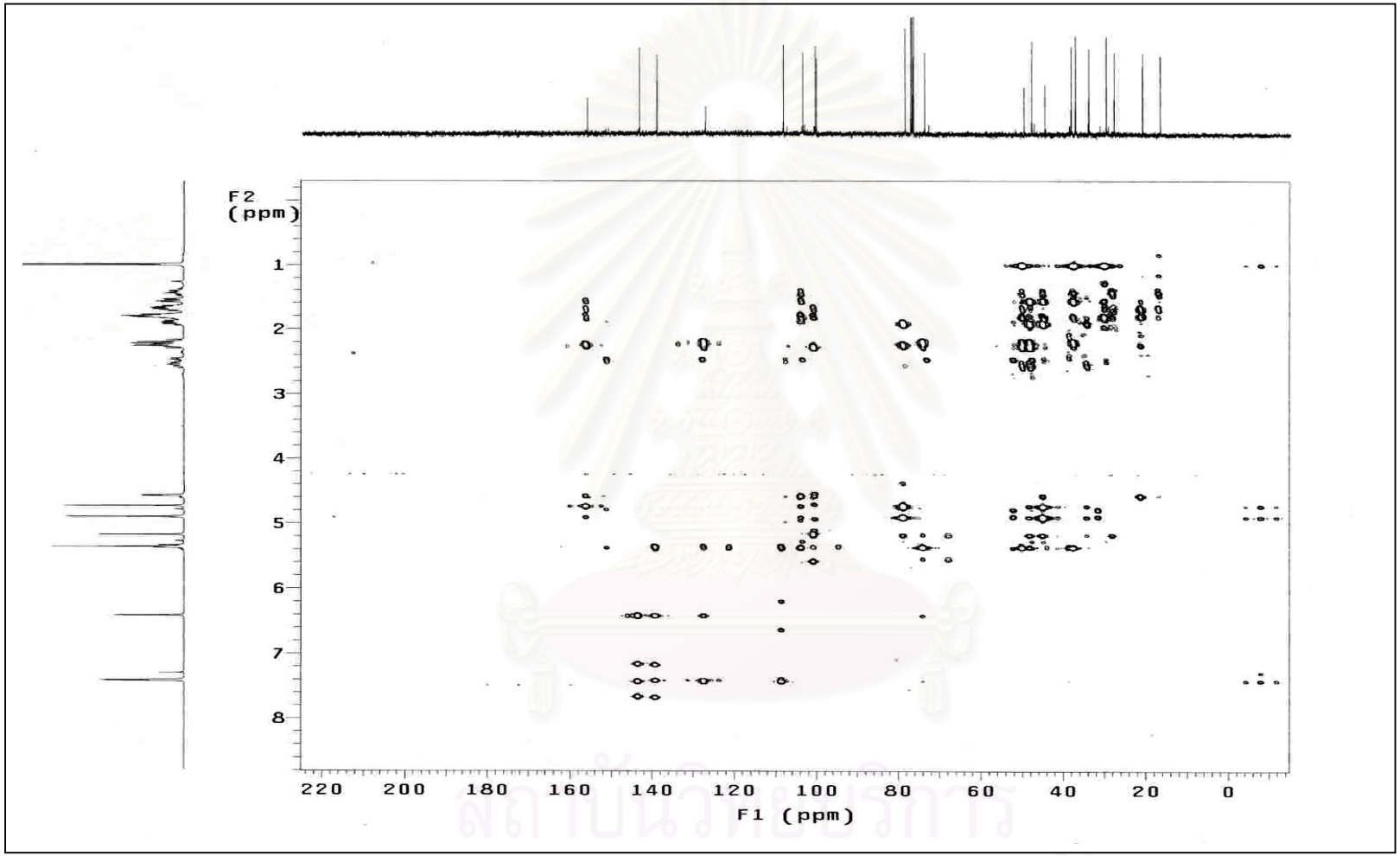
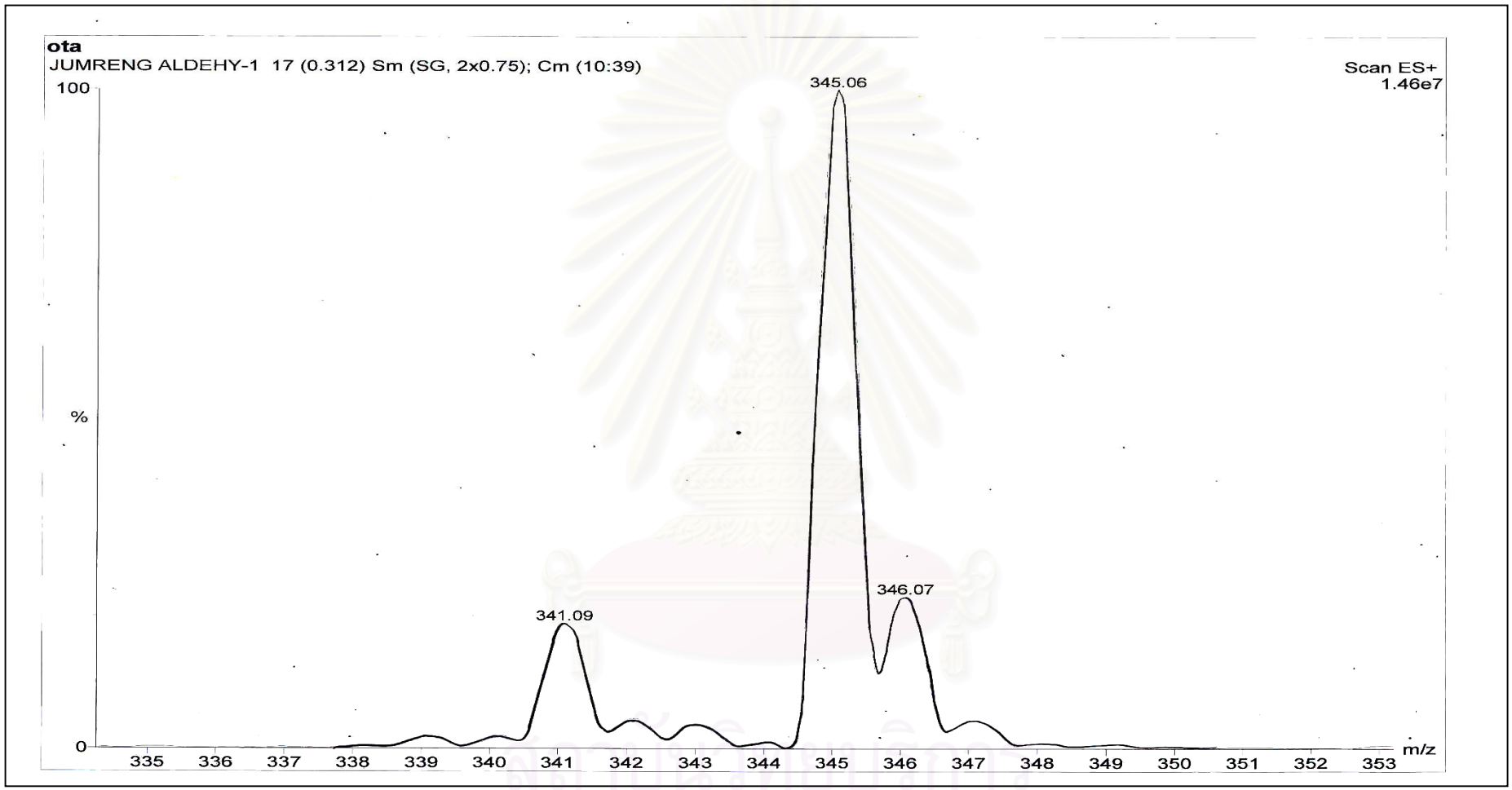


Figure 58 HMBC spectrum of compound **6.**

Figure 59 IR spectrum of compound **7.**



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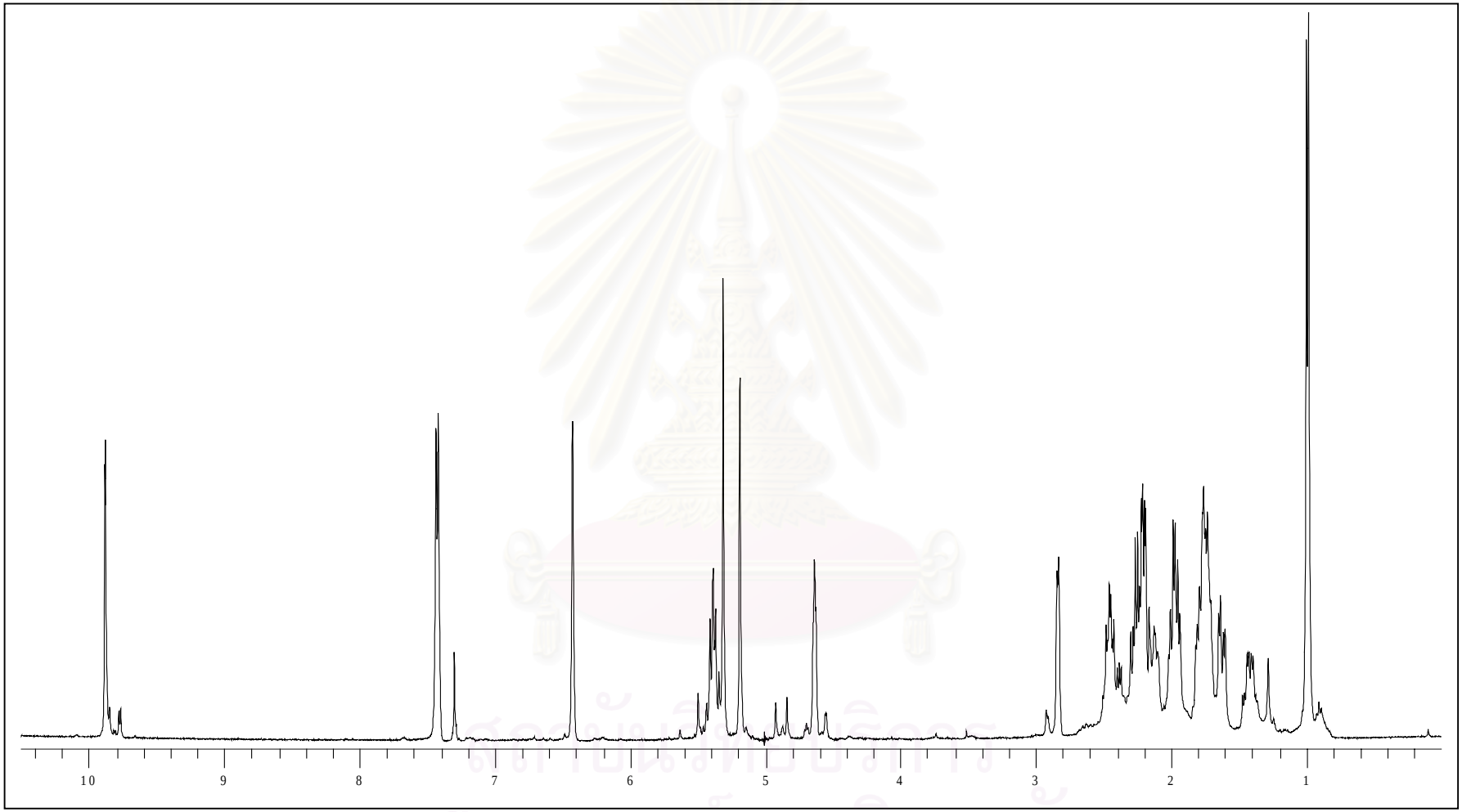
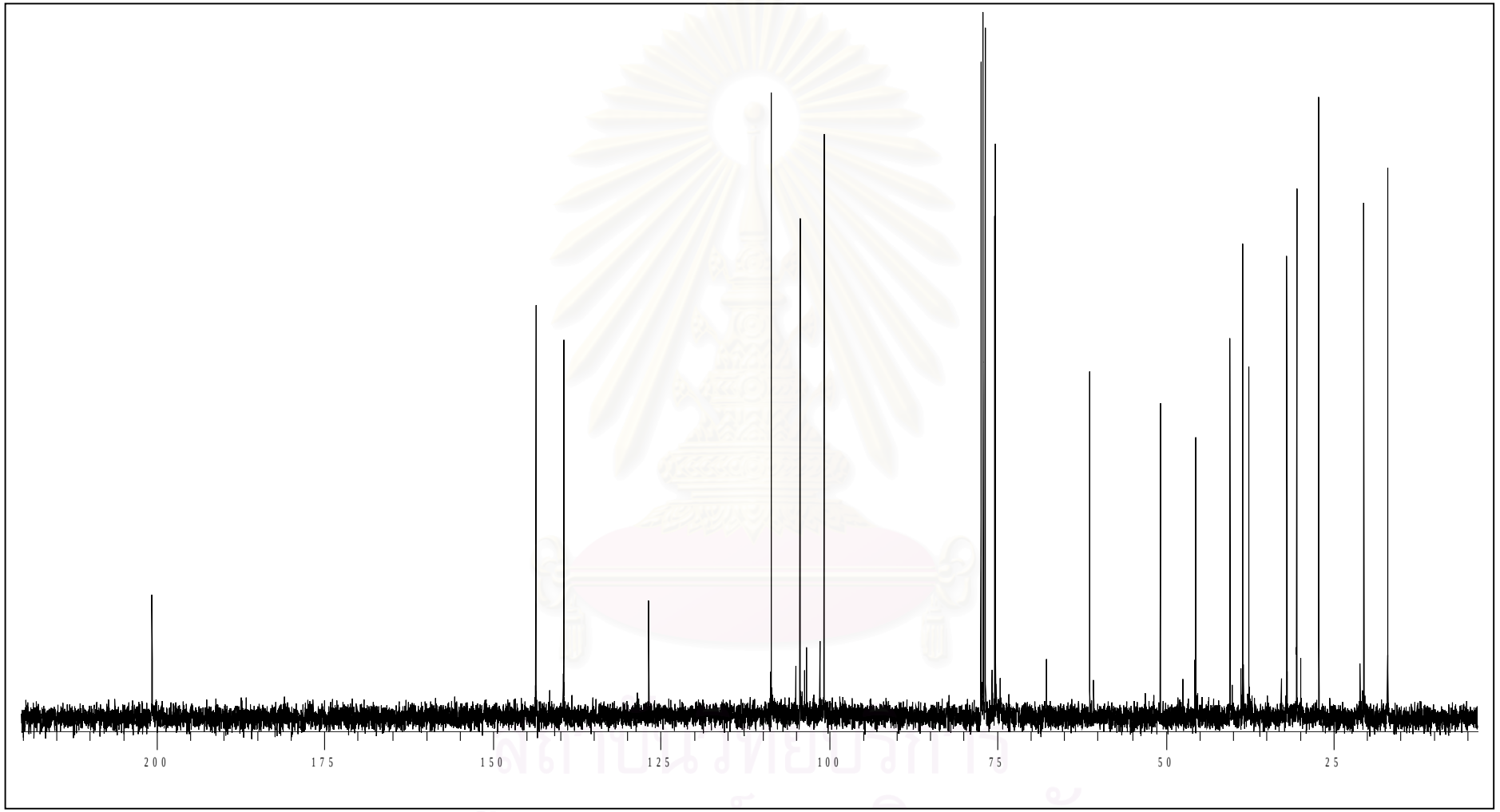


Figure 61 ^{1}H-NMR spectrum of compound **7.**



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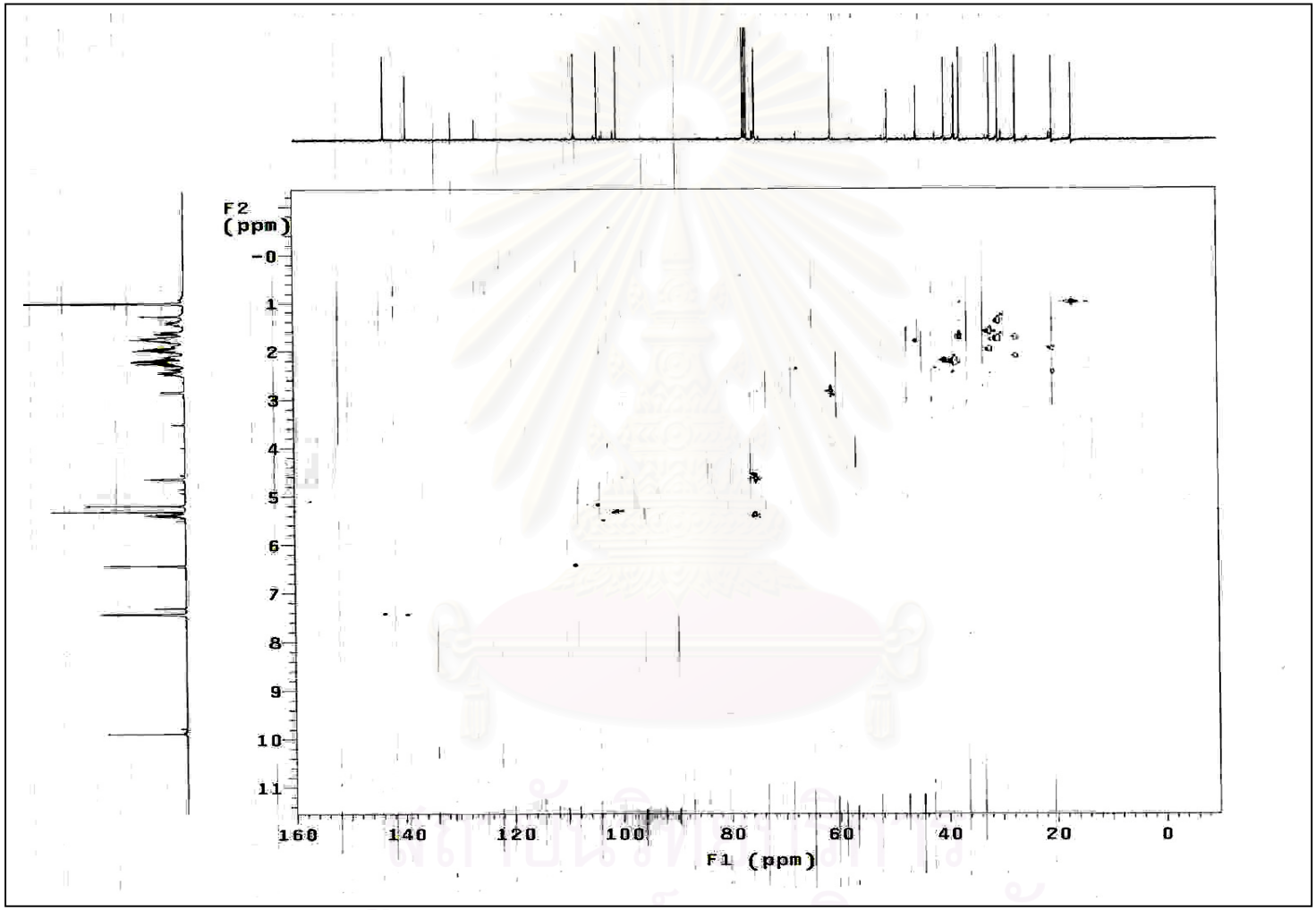

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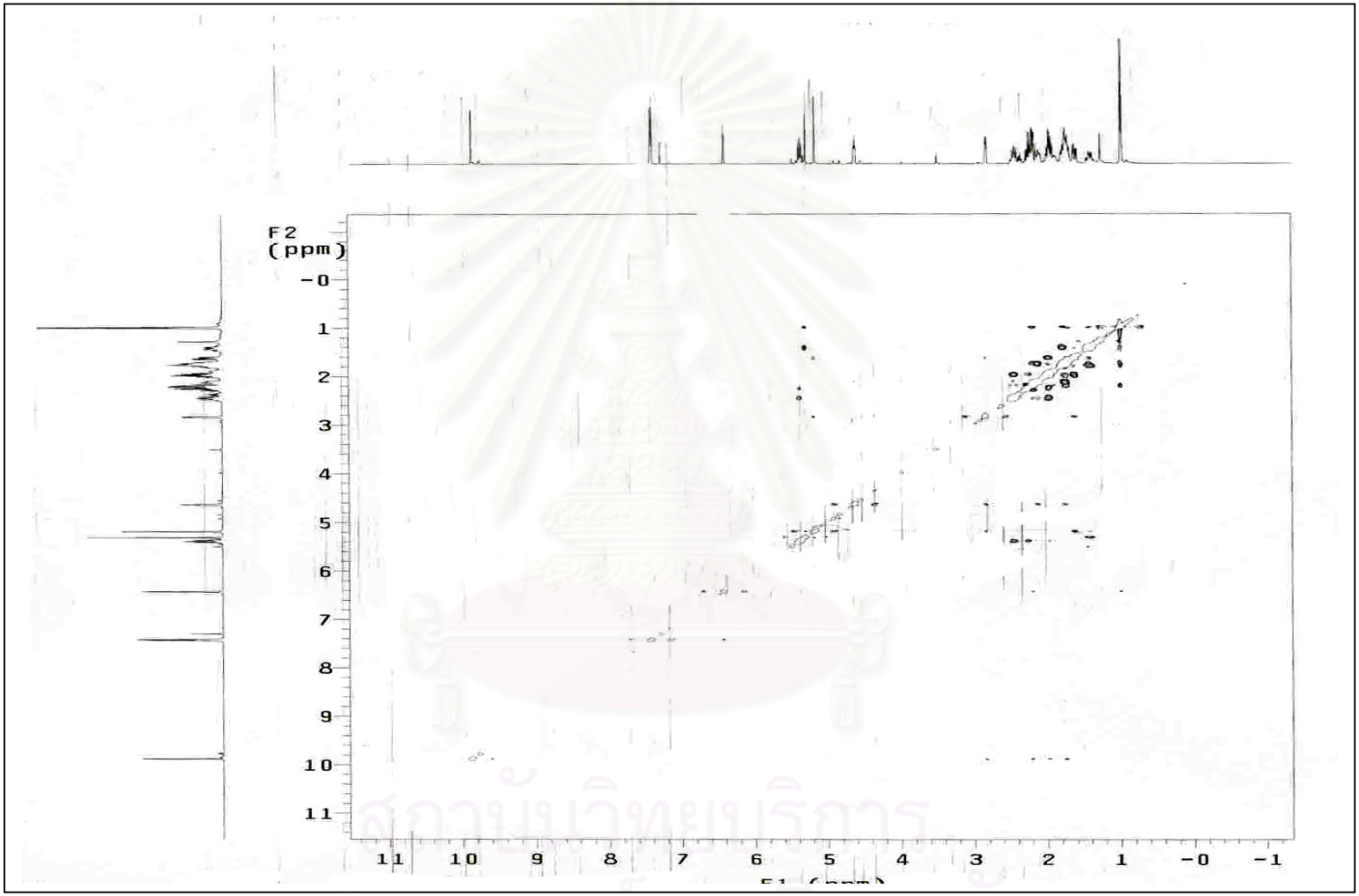

Figure 64 NOESY spectrum of compound **7.**

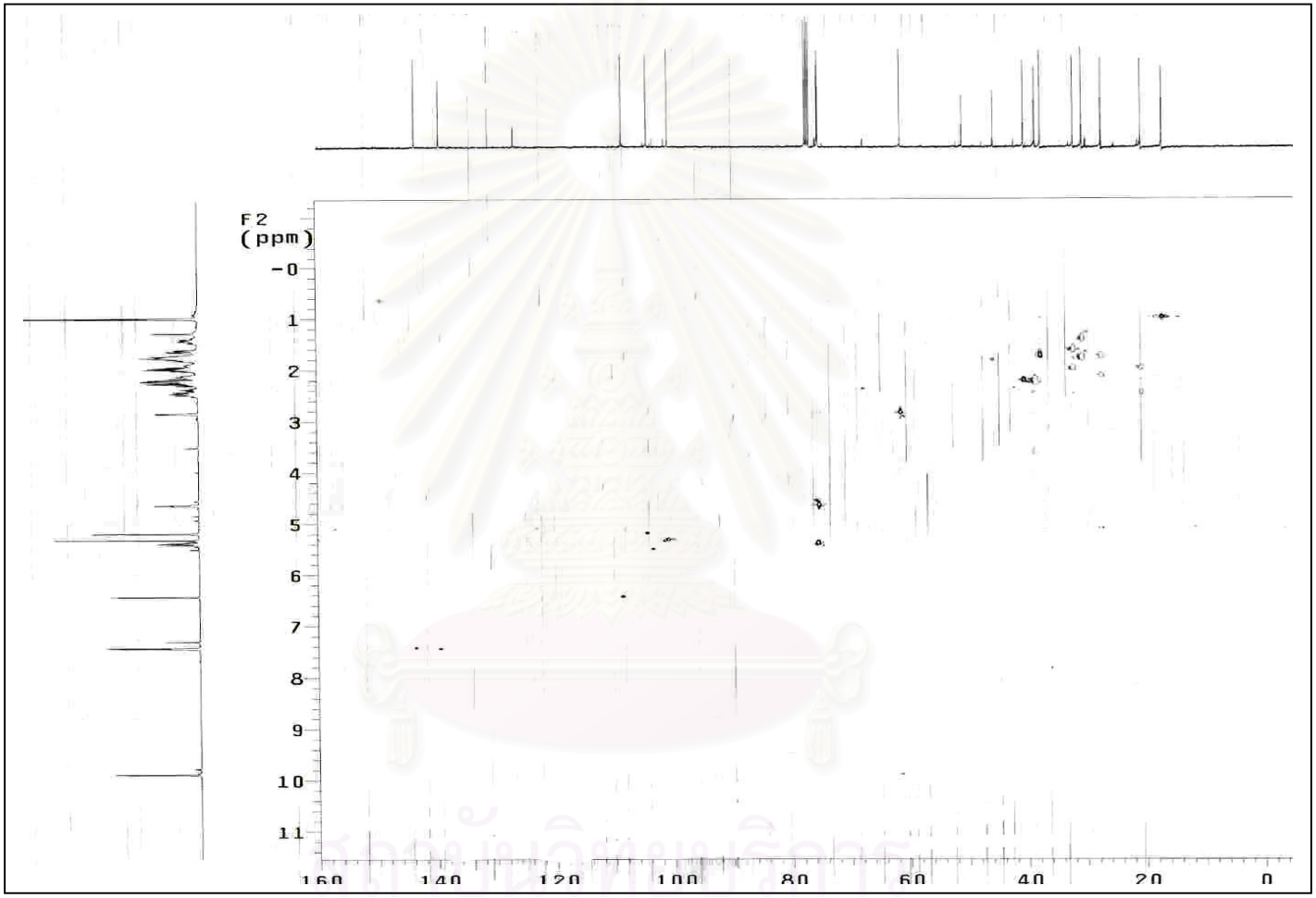

Figure 65 HSQC spectrum of compound **7.**

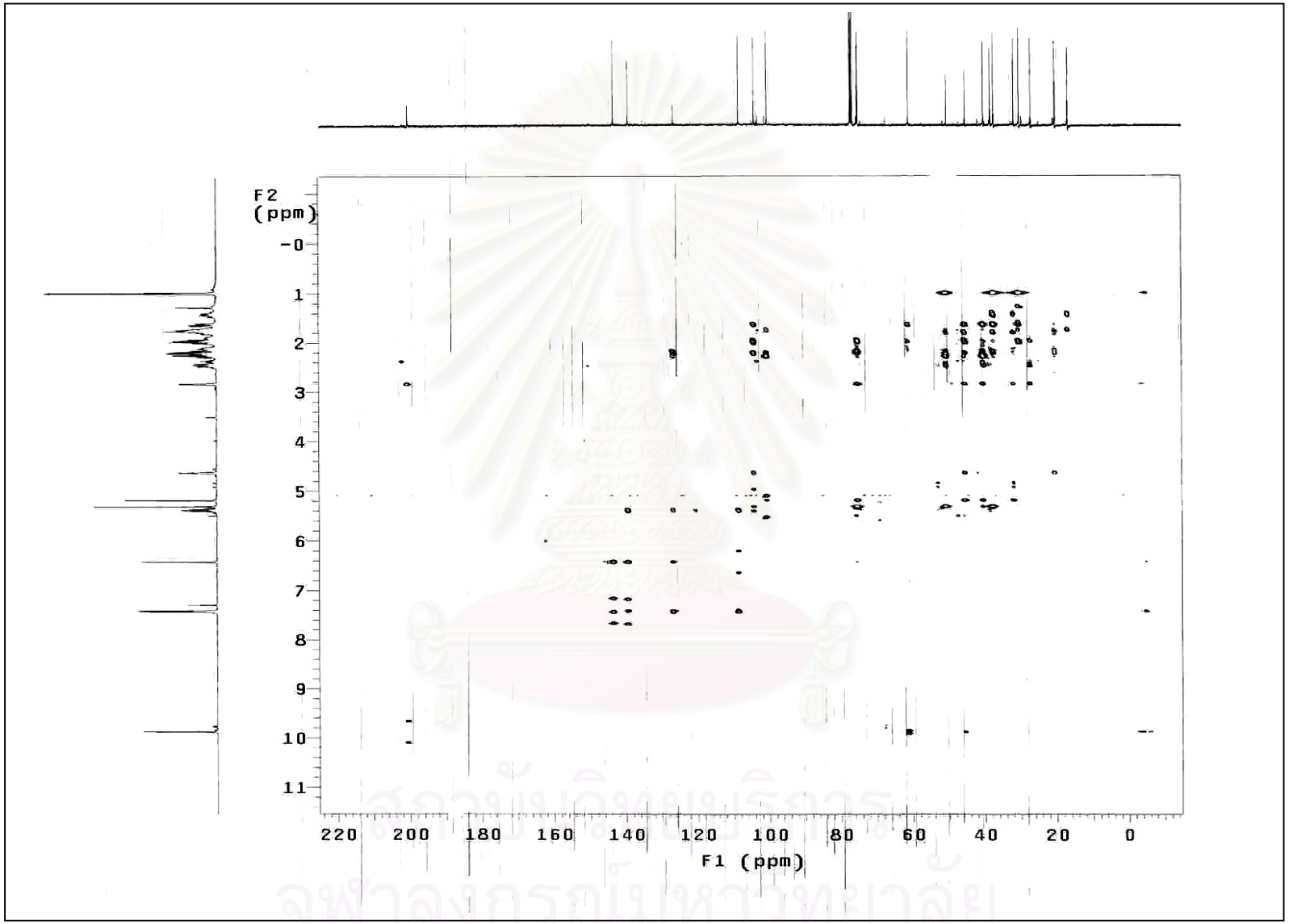

Figure 66 HMBC spectrum of compound **7.**

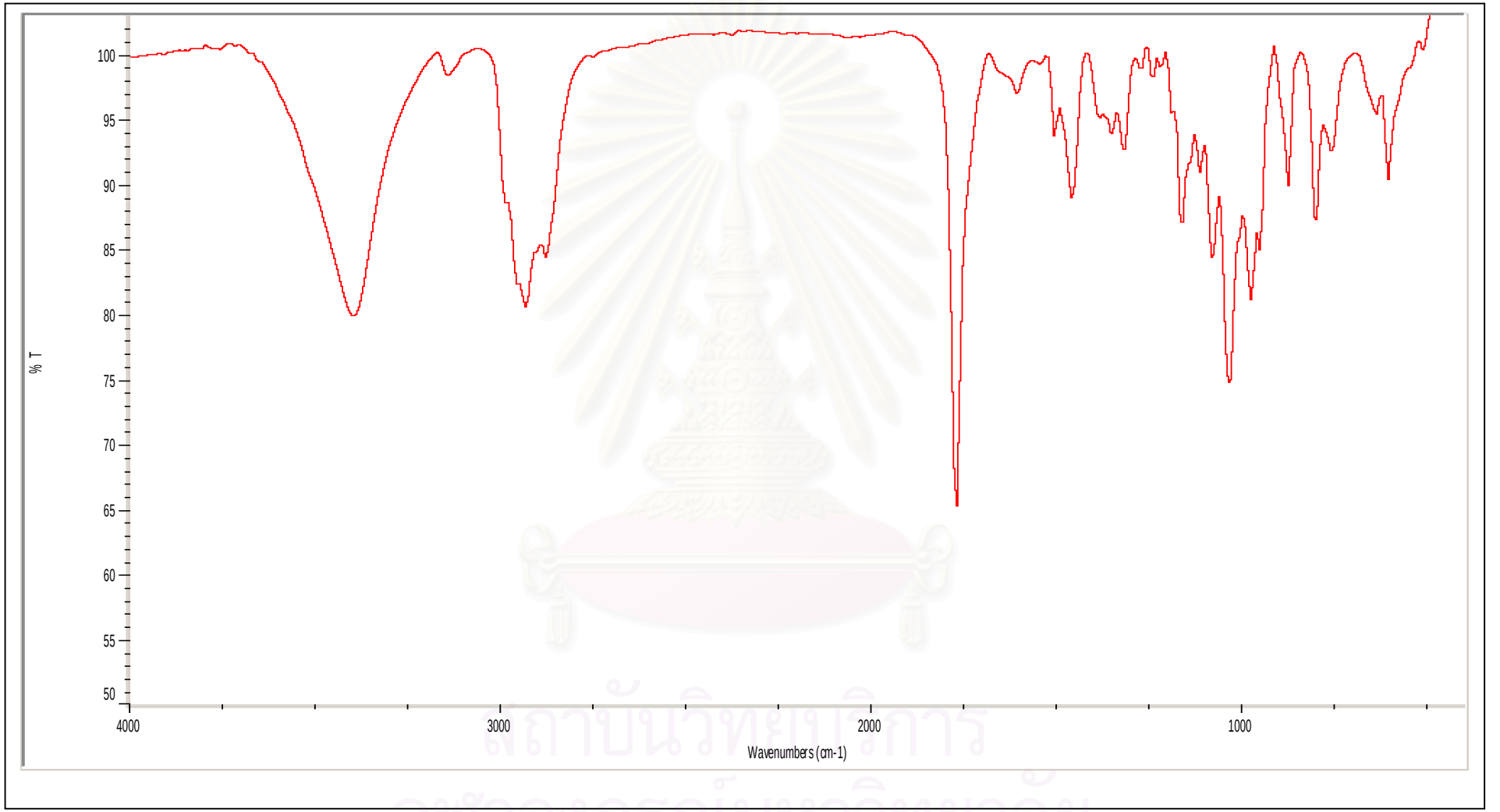

Figure 67 IR spectrum of compound **8.**

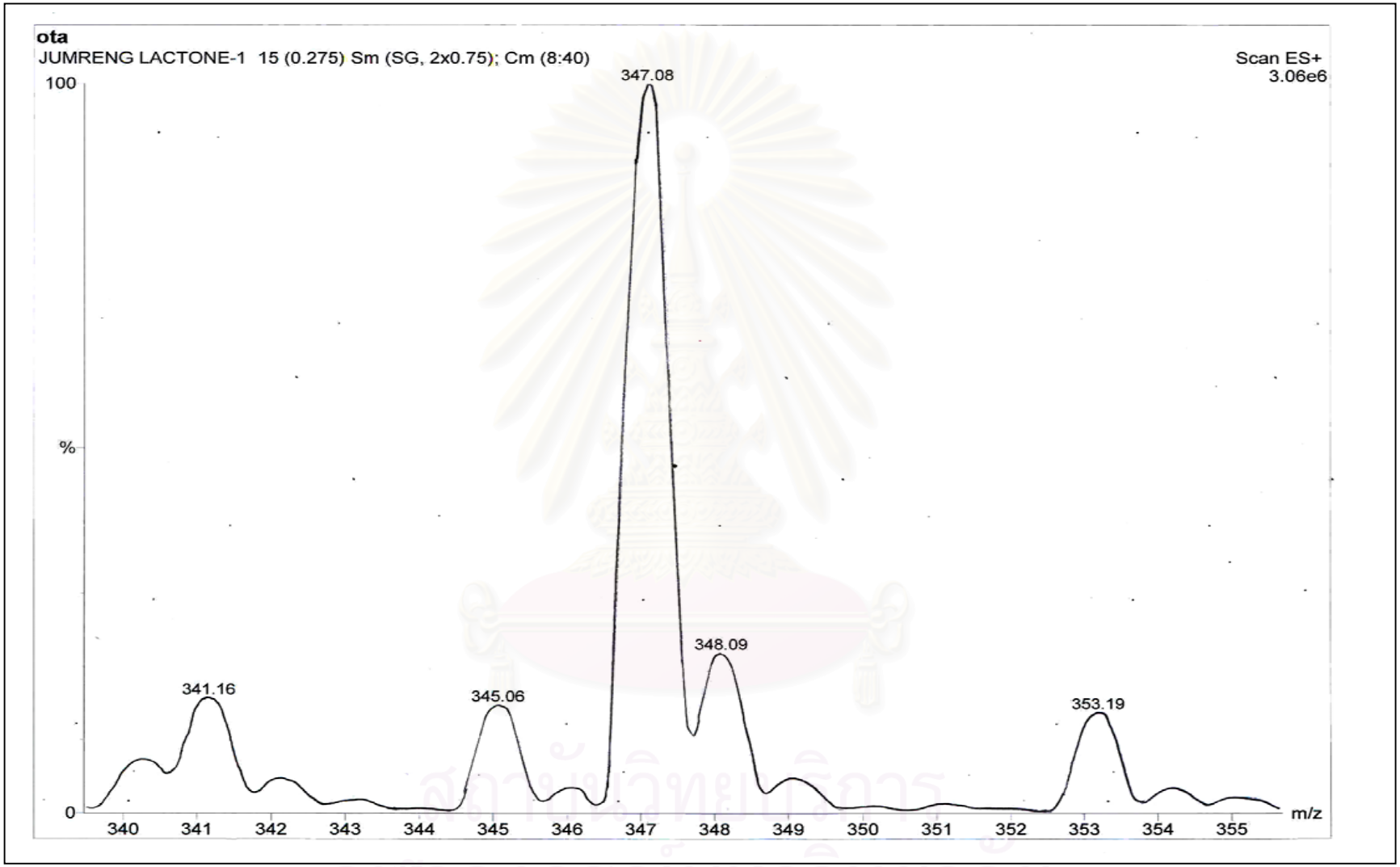

Figure 68 Mass spectrum of compound **8.**

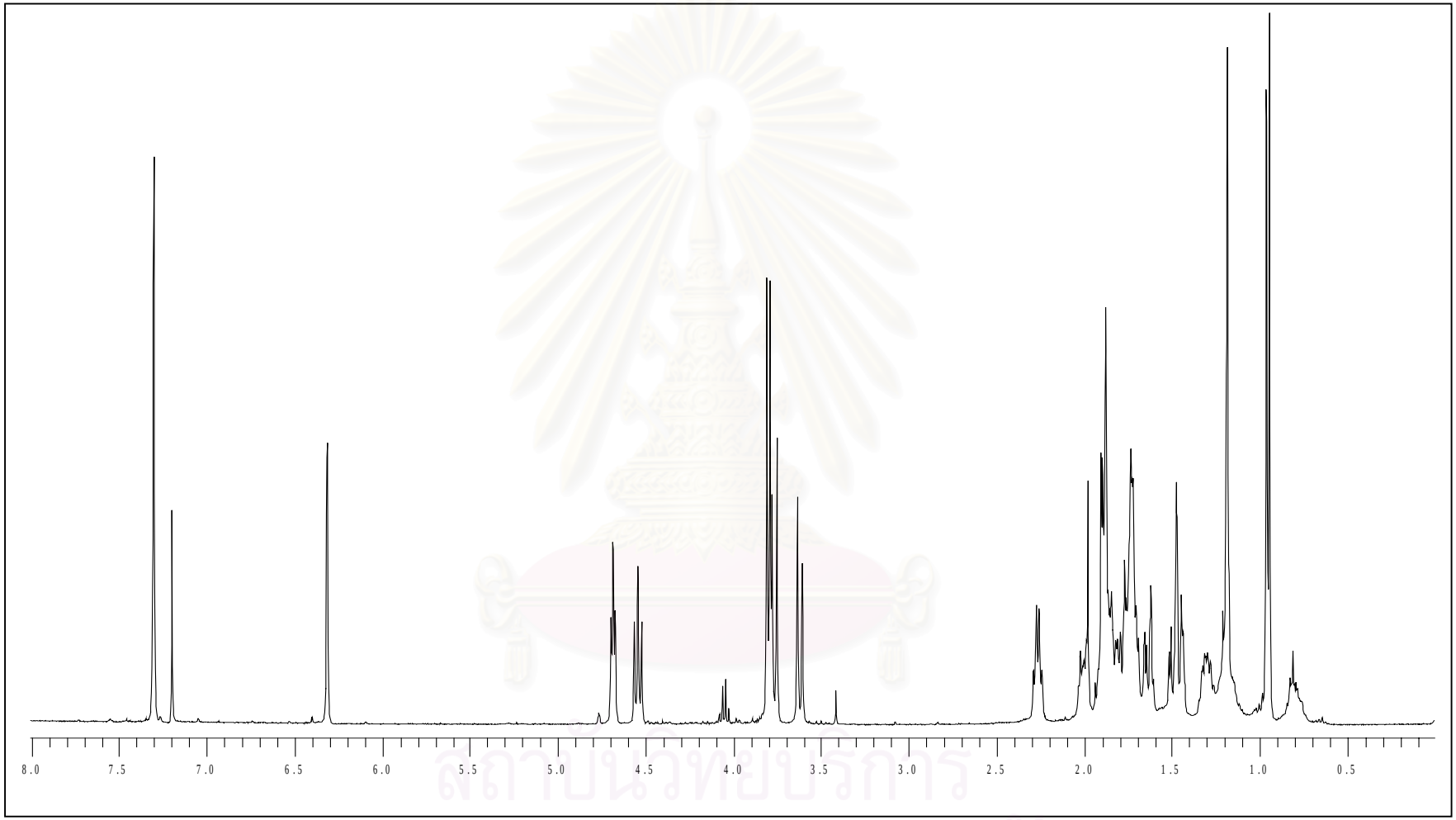

Figure 69 ^{1}H-NMR spectrum of compound **8.**

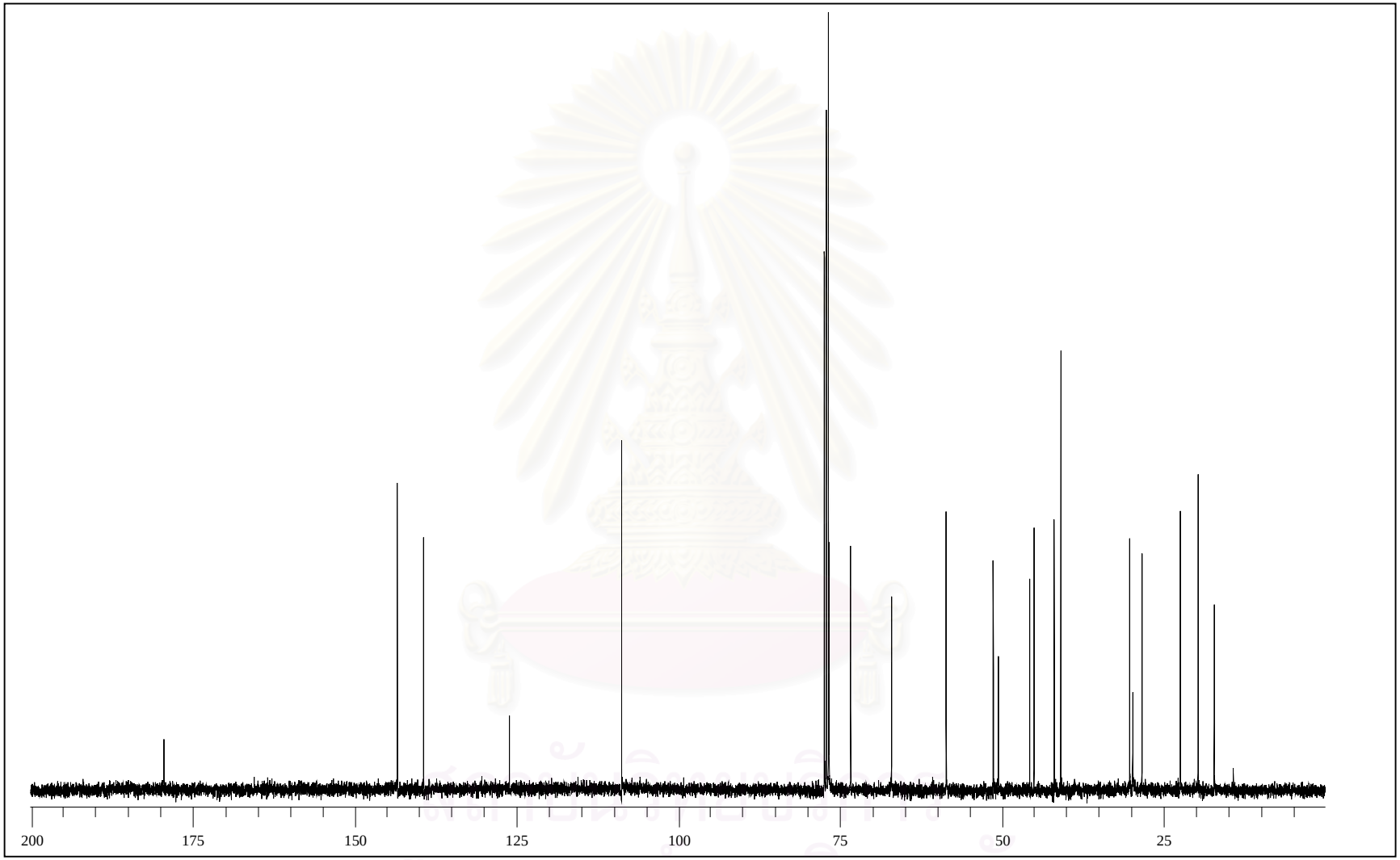

Figure 70 ^{13}C-NMR spectrum of compound **8.**

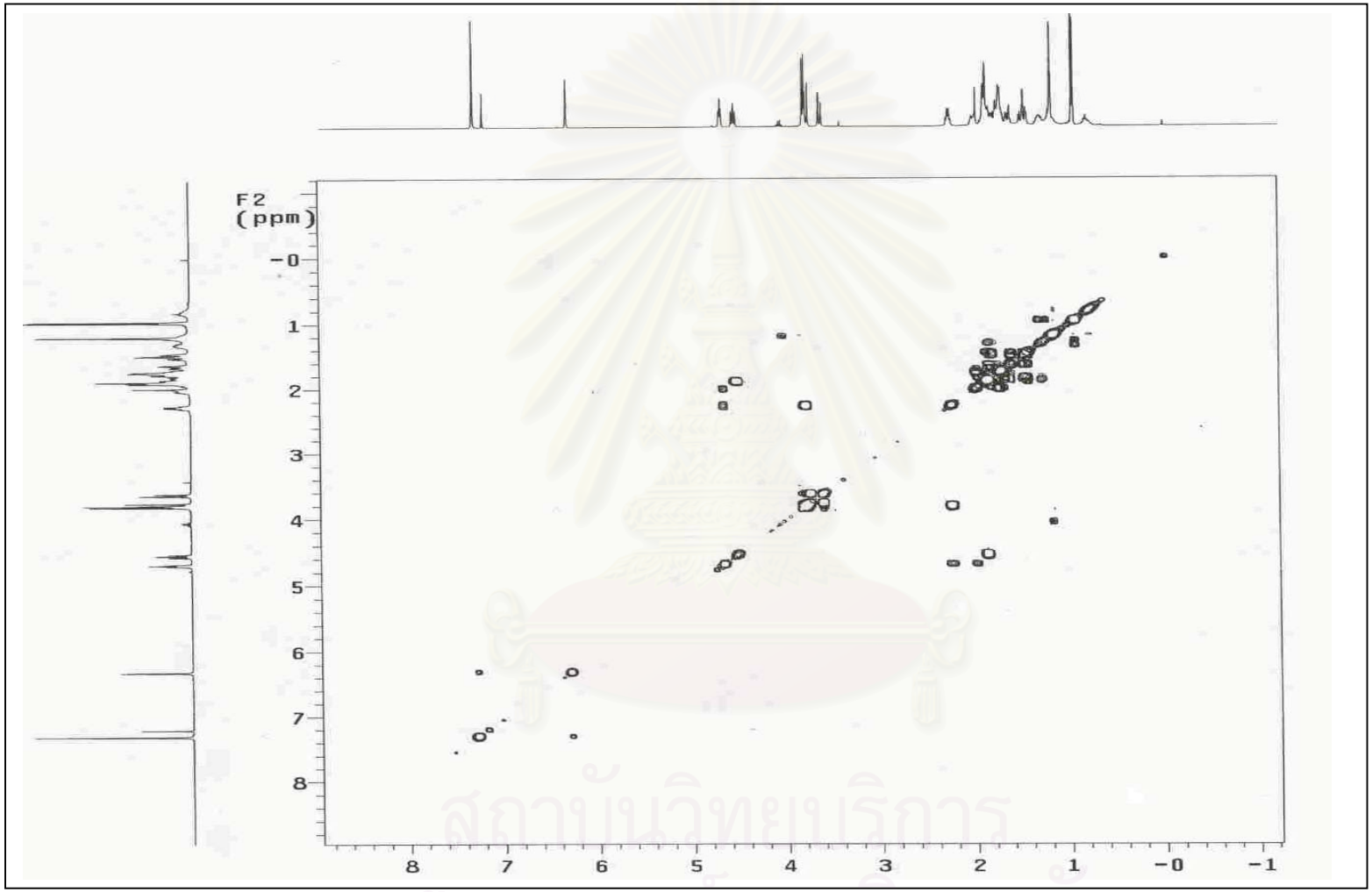

Figure 71 COSY spectrum of compound **8.**

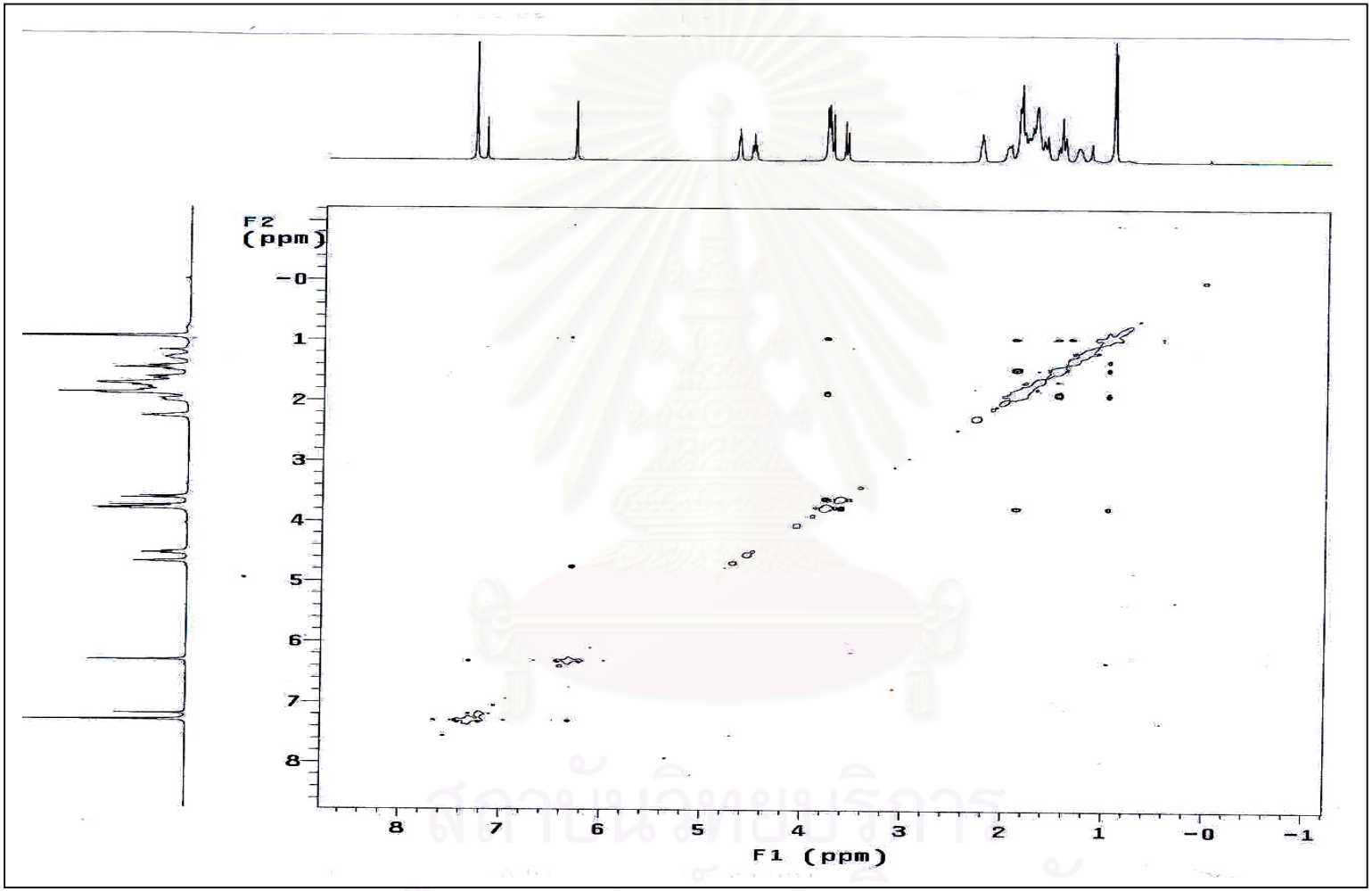

Figure 72 NOESY spectrum of compound **8.**

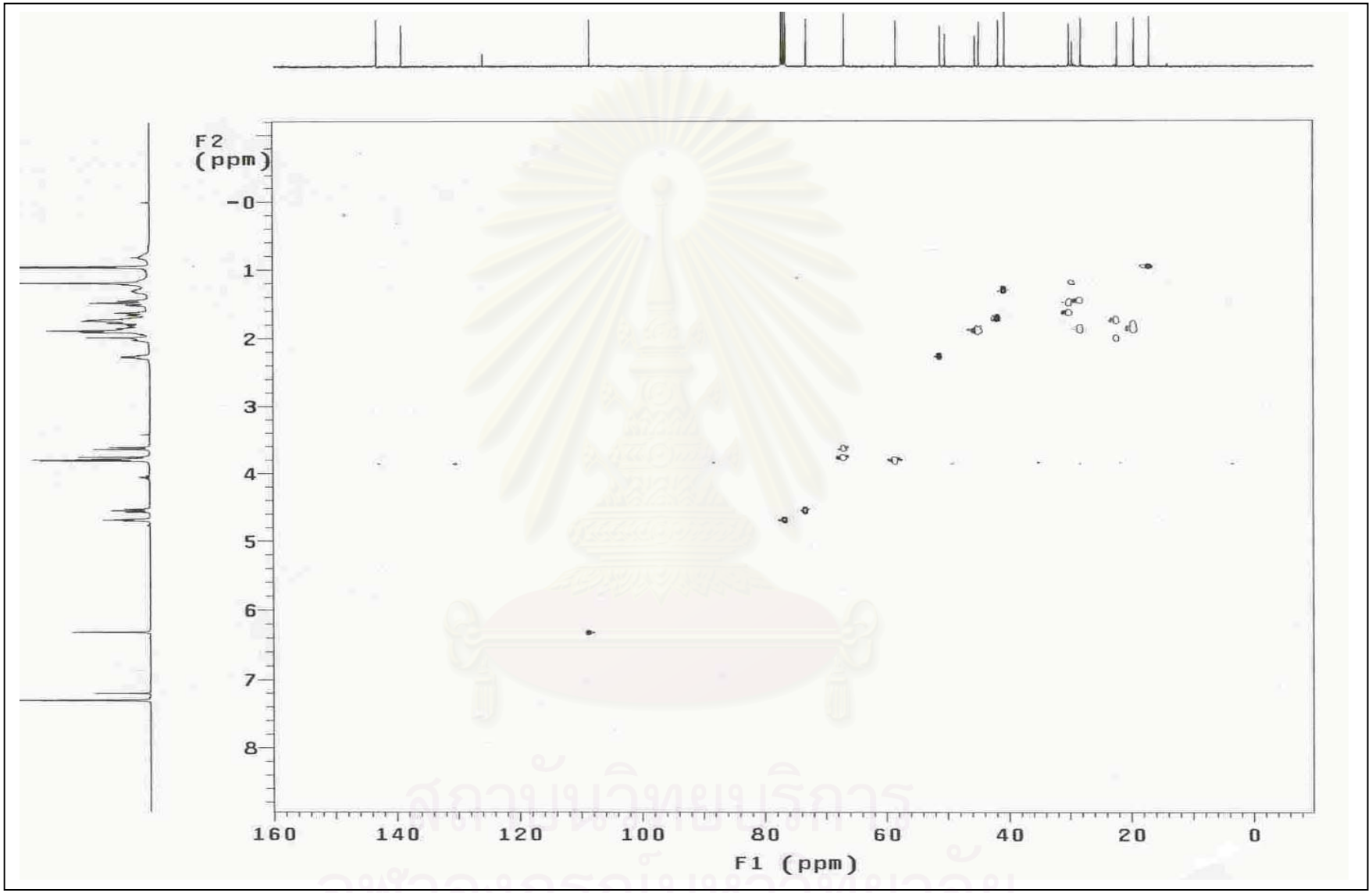

Figure 73 HSQC spectrum of compound **8.**

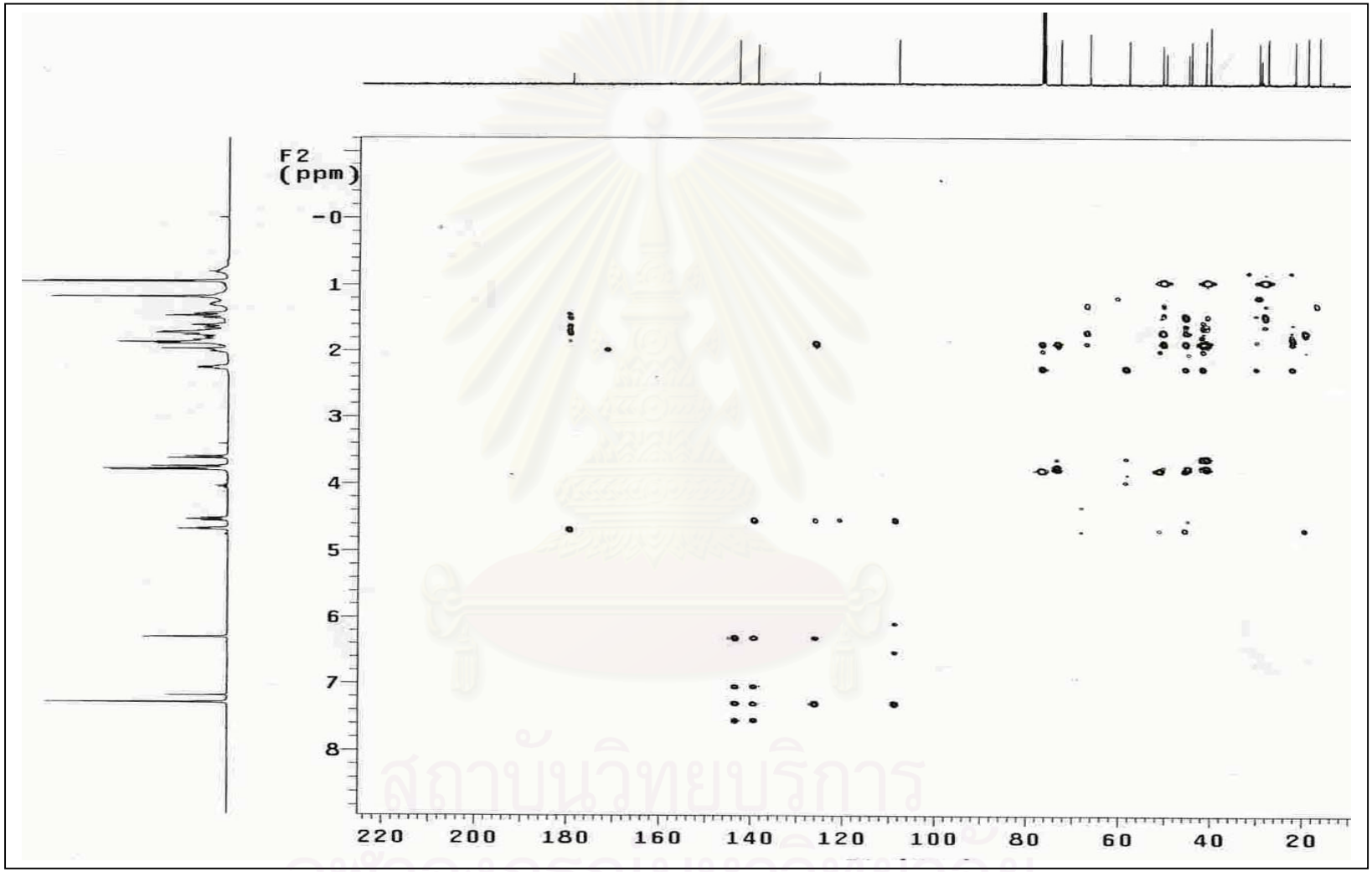

Figure 74 HMBC spectrum of compound **8.**

VITA

Miss Jumreang Tummatorn was born on May 1, 1980 in Chachoengsao Province, Thailand. She graduated with a Bachelor Degree of Science in Chemistry from Burapha University in 2001. In the same year, she was admitted into a Master Degree program in organic chemistry at Department of Chemistry, Faculty of Science Chulalongkorn University.