องค์ประกอบทางเคมีของเปลือกต้นเปล้าใหญ่ *Croton oblongifolius* จากอำเภอพนัสนิคม จังหวัดชลบุรี

นายชัยวัฒน์ ขำละเอียด

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CHEMICAL CONSTITUENTS OF STEM BARK OF *Croton oblongifolius* FROM AMPHOE PHANAT NIKHOM, CHON BURI PROVINCE

Mr. Chaiwat Komlaiad

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ได้สกัดแยกสารประกอบเคลอโรเดนไดเทอร์ปีนอยด์สามชนิด คือ (-)-hardwickiic acid (**1**), (-)-patagonic acid (**2**) และ nasimalun A (**3**) จากเปลือกต้นเปล้าใหญ่ (*Croton oblongifolius*) จากอำเภอพนัสนิคม จังหวัดชลบุรี และทำการเตรียมอนุพันธุ์ 1 ชนิดจากสารประกอบ **1** คือ (-)-hardwickiic acid methyl ester (**1a**) และทำการเตรียมอนุพันธุ์ 1 ชนิดจากสารประกอบ **2** คือ (-)-patagonic acid methyl ester (**2a**) และทำการเตรียมอนุพันธุ์ 3 ชนิดจากสารประกอบ **3** คือ methyl-15,16-epoxy-3-methoxy-12-oxo-13(16),14-clerodadien-18,19-olide-17-carboxylate (**3a**), methyl-12,17-dihydroxy-15,16-epoxy-13(16),14-clerodadien-18,19-olide (**3b**) และ methyl-12,17-dihydroxy-15,16-epoxy-3,13(16),14-clerodatrien-18,19-olide (**3c**) ซึ่งทำการพิสูจน์โครงสร้างของสารเหล่านี้โดยอาศัยสมบัติทางกายภาพและข้อมูลทางสเปกโทรสโกปี นอกจากนี้นำสารประกอบทั้งหมดมาทดสอบการยับยั้งเซลล์มะเร็งในหลอดทดลองกับเซลล์มะเร็ง 6 ชนิดได้แก่ Hep-G2 (มะเร็งตับ), Chago (มะเร็งปอด), SW 620 (มะเร็งลำไส้ใหญ่), Kato-3 (มะเร็งกระเพาะอาหาร), BT 474 (มะเร็งเต้านม) และ HuCCA-1 (มะเร็งท่อน้ำดี) ซึ่งสารประกอบ **1** และ **1a** มีฤทธิ์ยับยั้งเซลล์มะเร็งทั้ง 3 ชนิดได้ในระดับต่ำ และเป็นครั้งแรกในการรายงานผลการทดสอบการยับยั้งเซลล์มะเร็งของ (-)-hardwickiic acid methyl ester (**1a**), (-)-patogonic acid (**2**), (-)-patagonic acid methyl ester (**2a**), nasimalun A (**3**) และอนุพันธุ์ของ nasimalun A (**3a**, **3b** และ **3c**)

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

สาขาวิชา.....เคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

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Three clerodane compounds, (-)-hardwickiic acid (**1**), (-)-patagonic acid (**2**) and nasimalun A (**3**) were isolated from the stem bark of *Croton oblongifolius* from Amphoe Phanat Nikhom, Chon Buri Province. One derivative of compound **1** was synthesized as (-)-hardwickiic acid methyl ester (**1a**) and a derivative of compound **2** was synthesized as (-)-patagonic acid methyl ester (**2a**) and three derivatives of compound **3** were synthesized as methyl-15,16-epoxy-3-methoxy-12-oxo-13(16),14-clerodadien-18,19-olide-17-carboxylate (**3a**), methyl-12,17-dihydroxy-15,16-epoxy-13(16),14-clerodadien-18,19-olide (**3b**) and methyl-12,17-dihydroxy-15,16-epoxy-3,13(16),14-clerodatrien-18,19-olide (**3c**). The structure of these compounds were established by physical properties and spectroscopic data. All of the compounds were tested for cytotoxicity against six human tumor cell lines *in vitro* including Hep-G2 (hepatoma), Chago (lung), SW 620 (colon), Kato-3 (gastric), BT 474 (breast) and HuCCA-1 (human bile duct epithelial carcinoma). Compound **1** and **1a** showed weak activity against three tumor cell lines. Moreover, this is the first report of cytotoxicity test of (-)-hardwickiic acid methyl ester (**1a**), (-)-patagonic acid (**2**), (-)-patagonic acid methyl ester (**2a**), nasimalun A (**3**) and nasimalun A derivatives (**3a, 3b** and **3c**).

Department……...Chemistry.... Student's signature…….....
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Finally, I wish to express my deepest gratitude to my parents for all things that they have endured and sacrificed for my success.

CONTENTS

Page

CONTENTS (continued)

Page

CONTENTS (continued)

Page

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (continued)

Figure **Page**

LIST OF FIGURES (continued)

Figure **Page**

LIST OF TABLES

LIST OF TABLES (continued)

Table **Page**

LIST OF SCHEMES

Scheme **Page**

LIST OF ABBREVIATIONS AND SYMBOLS

anh	anhydrous
c	concentration
$CDCl_3$	duterated chloroform
cm	centimeter
cm^3	cubic centimeter
δ	chemical shift
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
gCOSY	gradient Correlated Spectroscopy
J	coupling constant
d	doublet (for NMR spectrum)
dd	doublet of doublet (for NMR spectrum)
ddd	doublet of doublet of doublet (for NMR spectrum)
dddd	doublet of doublet of doublet of doublet (for NMR spectrum)
DBE	Double Bond Equivalent
°C	degree Celsius
EI MS	Electron Impact Mass Spectrum
EST MS	Electrospray Ionization Mass Spectrum
eV	electron volt
g	gram
gHMBC	gradient Heteronuclear Multiple Bond Coherence
gHSQC	gradient Heteronuclear Single Quantum Coherence
Hz	Hertz
h	hour
IC_{50}	Inhibitory Concentration 50%
IR	Infrared spectrum
m	multiplet (for NMR spectrum)
mg	milligram
min	minute
ml	milliliter
mp	melting point

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

m/z	mass to charge ratio
M	molar
M^+	molecular ion
MHz	megahertz
MS	Mass spectrometry
MW	molecular weight
NMR	Nuclear Magnetic Resonance
gNOESY	gradient Nuclear Overhauser Enhancement Spectroscopy
ppm	part per million
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
TLC	Thin Layer Chromatography
wt	weight
ν_{max}	the reciprocating wavelength (IR spectrum)
λ_{max}	the wavelength at maximum absorption (UV-VIS)

CHAPTER I

INTRODUCTION

As continuation of our search for natural compounds, Plao-yai (*Croton oblongifolius*) belongs to the Euphorbiaceae family. It is perennial herb widely distributed in Thailand. It is used in Thai folk medicine as tonic, inhibit chronic enlargements of livers, dysmenorrhea, purgative and dysentery [1].

C. oblongifolius from various locations in Thailand have been investigated for their chemical constituents and biological activities. It was found that the main components of each specimen were different. Two cembranoids, crotocembraneic acid and neocrotocembraneic acid, isolated from the stem bark of *C. oblongifolius* collected from Phetchabun Province [2]. Moreover, four labdane compounds including labda-7,12(*E*),14-diene; labda-7,12(*E*),14-triene-17-al; labda-7,12(*E*),14-triene-17-ol and labda-7,12(*E*),14-triene-17-oic acid were discovered in Amphoe Pran Buri, Prachuap Khiri Khan Province [3]. Moreover, from Amphoe Wangsapung, Loei Province, three labdane compounds were discovered, 3-acetoxy-labda-8(17),12(*E*),14-triene-2-ol; 2-acetoxy-labda-8(17),12(*E*),14-triene-3-ol and labda-8(17),12(*E*),14-triene-2,3-diol [4].

Some diterpene compounds found in *C. oblongifolius*, they have been shown to possess cytotoxic activities against P388 cells line and five tumor cell lines including Hep-G2 (hepatoma), Chago (lung), SW620 (colon), Kato-3 (gastric) and BT 474 (breast) [5].

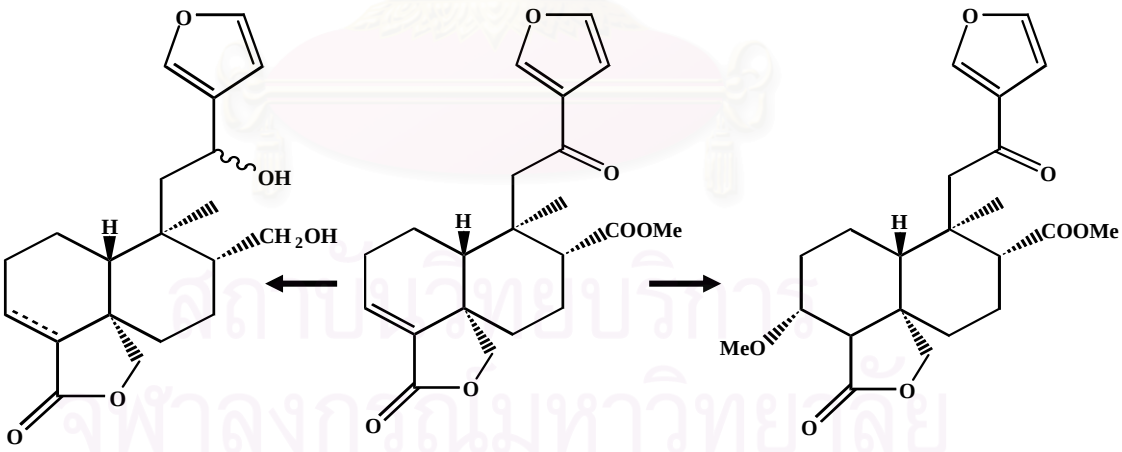
The chemical constituents found in the stem bark of *C. oblongifolius* from Amphoe Phanat Nikhom, Chon Buri Province, were found to be different from other locations in ^{1}H-NMR screening of hexane crude extract. Therefore, it is interesting to reinvestigate the diterpenoid compounds of the stem bark of *C. oblongifolius* from this location as well as their physical and chemical properties.

1.1 The proposed chemical transformation of Nasimalun A from *C. oblongifolius*.

Some natural clerodanes have been useful as starting materials for chemical transformations into other natural products [6].

Methyl-15,16-epoxy-12-oxo-3,13(16),14-neo-clerodatrien-18,19-olide-17-carboxylate (Nasimalun A, **3**) is a clerodane diterpene from the roots of *Barringtonia racemosa* affored novel *neo*-clerodane-type diterpenoids [7]. In addition, nasimalun A (**3**) was found in *C. oblongifolius* from Amphoe Phurua, Loei Province [8]. From the literatures, nasimalun A has never been used as starting material for chemical transformation and derivatization.

In this work, Nasimalun A (**3**) was selected as a starting material due to large quantity of this compound. The modifications of Nasimalun A (**3**) were classified into two types. First, Nasimalun A (**3**) was converted to methoxy derivative by addition of the alcohol to an α, β-unsaturated lactone moiety. Second, it was reduced to diol derivative. The modification of Nasimalun A (**3**) was proposed as shown in Scheme 1.1.



Diol derivative Nasimalun A (**3**) Methoxy derivative

Scheme 1.1 The modification of Nasimalun A (**3**).

Thus, the objective of this research is summarized as follows:

1. To extract, isolate and purify the chemical constituents of stem bark of *C. oblongifolius* from Amphoe Phanat Nikhom, Chon Buri Province.
2. To investigate the chemical properties of (-)-hardwickiic acid (**1**), (-)-patagonic acid (**2**) and nasimalun A (**3**).
3. To examine the cytotoxic activity against human cancer cell lines of isolated compounds and modified compounds.

CHAPTER II

LITERATURE REVIEW

2.1 General characterization of the plants in the Genus Croton [10].

The genus Croton comprises 700 species of trees or shrubs. Leaves are usually alternate with 2-grandular stipule at the base. Their flowers are solitary or clustered in the rhachis of a terminal raceme and bracts are small. Male flowers contain 5-calyx and 5-petals. There are many stamens inserted on a hairy receptacle. In female flowers, sepals are usually more ovate than the male, petals are smaller than the sepals of missing and disk annular of 4-6 glands are opposite the sepals. There are three ovary with solitary ovule in each cell. Seeds are smooth, albumen copious and broad cotyledons.

2.2 General characterization of *C. oblongifolius* [11].

C. oblongifolius is a medium sized tree. Its calyx and ovary are clothed with minute orbicular silvery scales. Leaves are simple, alternate, oblong, elliptic-oblong, ovate or lanceolate, 5-10 cm wide and 9-30 cm long. Young leave is brownish. Inflorescence in terminal raceme or panicle, unisexual, monoecious or dioecious. Flowers are pale greenish yellow and solitary in the axials of minute bracts on long erect racemes. The male flowers locate in the upper part of the raceme and the females flowers in the lower part. Male flowers are slender and have the length of pedicels of 4.0 mm. Calyx is more than 6.0 mm long and segments are ovate, obtuse and more than 2.5 mm long. Petals are 3.0 mm long, elliptic-lanceolate and woolly. The twelve stamens are inflexed in bud and the length of filaments are 3.0 mm. The pedicels are short and stout in female flowers. Its sepals are more acute than in the male with densely ciliated margins. Diameter of fruit is less than 1.3 cm, slightly 3-lobed and clothed with small orbicular scales. In each fruit, the number of seeds are eight which are 6.0 mm long rounded and quite smooth on the back.

The picture of flowers, leaves and stem bark of *C. oblongifolius* are shown in Figure 2.1.

Figure 2.1 The flowers, leaves and stem bark of *C. oblongifolius*.

2.3 The chemical constituents of *C. oblongifolius*.

The chemical constituents of *C. oblongifolius* have been widely studied and found many diterpene compounds.

In 1968, Rao and coworkers found diterpene alcohol, oblongifoliol together with β-sitosterol from the bark of *C. oblongifolius* [12].

In 1969, Aiyar and Seshadri found deoxyoblongifoliol from the stem bark of *C. oblongifolius* [13].

In 1970, Aiyar and Seshadri studied the structure of oblongifolic acid, the major diterpene acid component of the bark, it was assigned as (+)-isopimara-7(8),15-diene-19-oic acid [14].

In 1971, Aiyar and Seshadri found three new minor components from the stem bark, *ent*-isopimara-7,15-diene; 19-hydroxy-*ent*-isopimara-7,15-diene and *ent*-isopimara-7,15-diene-19-aldehyde [15]. In the same year, they also found oblongifoliol and deoxyoblongifoliol which assigned as *ent*-isopimara-7,15-diene-3β-ol and *ent*-isopimara-7,15-diene-3β,19-diol, respectively [16]. Moreover, Acetyl aleuritolic acid and 3β-acetoxy-olean-14(15)-ene-28-oic acid were found from the stem bark [17].

In 1972, Aiyar and Seshadri found two closely related furanoid diterpenes from the bark. One was *ent*-15,16-epoxy-3,11,13(16),14-clerodatetraen-19-oic acid which given the trivial name 11-dehydro-(-)-hardwickiic acid and the second was (-)-hardwickiic acid [18]. In the same year, they studied other parts of *C. oblongifolius* including the root-bark which were obtained in poor yield from wood, while the leaves gave only waxy materials [19].

In 1998, Roengsumran and coworkers found two cembranoids, crotocembraneic acid and neocrotocembraneic acid, isolated from the stem bark of *C. oblongifolius* collected from Phetchabun Province [2].

In 1999, Roengsumran and coworkers found labdane diterpene compounds, labda-7,12(*E*),14-triene; labda-7,12(*E*),14-triene-17-al; labda-7,12(*E*),14-triene-17-ol and labda-7,12(*E*),14-triene-17-oic acid, isolated from *C. oblongifolius* from Amphoe Pran Buri, Prachuap Khiri Khan Province. These compounds gave effective cytotoxicity against cancer cell lines [3]. In the same year, they found cembranoid diterpene, neocrotocembranal, isolated from the stem bark of *C. oblongifolius* from

Amphoe Bungsamphan, Phetchabun Province. This compound showed inhibitory activity on platelet aggregation induced by thrombin and exhibited cytotoxicity against P-388 cell line [20].

In 2001, Roengsumran and coworkers reported three labdane diterpenoids, 2-acetoxy-3-hydrosy-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, isolated from the stem bark of *C. oblongifolius* from Amphoe Wangsapung, Loei Province. 2,3-Dihydroxy-labda-8(17),12(*E*),14-triene showed moderate cytotoxicity against human cancer cell lines; whereas the first two compounds were less active [4].

In 2003, Roengsumran and coworkers found three halimane-type diterpenoids, crotohalimaneic acid; crotohalimoneic acid and 12-benzoyloxycrotohalimaneic acid, from the stem bark of *C. oblongifolius* from Amphoe Pakchong, Nakornratchasima Province [21].

Many diterpenoid compounds of *C. oblongifolius* can be classified into 9 groups, which are shown in the following Table 2.1.

Table 2.1 The chemical constituents of *C. oblongifolius*.

	Compounds	Reference
	Pimarane diterpenoids	
Bark and	Oblongifoliol	[12]
Wood	19-Deoxyoblongifoliol	[13]
	Oblongifolic acid	[14]
	ent-Isopimara-7,15-diene	[15]
	ent-Isopimara-7,15-diene-19-aldehyde	[16]
	19-Hydroxy-*ent*-isopimara-7,15-diene	[16]
	3-Deoxyoblongifoliol	[15]
	(-)-Pimara-9(11),15-diene-19-oic acid	[22]
	(-)-Pimara-9(11),15-diene-19-ol	[22]
	Clerodane diterpenoids	
Bark and	(-)-Hardwickiic acid	[5, 8, 18, 23, 24]
Modified	11-Dehydro-(-)-hardwickiic acid	[18]
	(-)-20-Benzyloxyhardwickiic acid	[23, 24]
	Crovatin	[5]
	Isokolavenol	[5]
	Nasimalun A	[7, 8]
	Cembrane diterpenoids	
Bark and	Crotocembraneic acid	[2, 5, 22, 25]
Modified	Neocrotocembraneic acid	[2, 5, 22, 30]
	Neocrotocembranal	[20, 5]
	Poilaneic acid	[26]
	Halimane diterpenoids	
Bark and	Crotohalimaneic acid	[20, 5]
Modified	Benzoyl crotohalimaneic acid	[20, 5]
	Crotohalimoneic acid	[5]

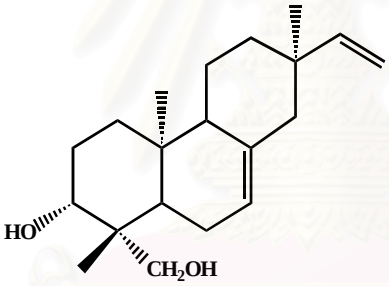
Table 2.1 The chemical constituents of *C. oblongifolius* (continued).

Plant parts	Compounds	Reference
	Labdane diterpenoids	
Bark and	Labda-7,12(*E*),14-triene	[3, 27]
Modified	Labda-7,12(*E*),14-triene-17-al	[3, 27]
	Labda-7,12(*E*),14-triene-17-ol	[3, 27]
	Labda-7,12(*E*),14-triene-17-oic acid	[3, 24, 27]
	3-Acetoxy-labda-8,(17),12(*E*),14-triene-2-ol	[4, 24, 27]
	2-Acetoxy-labda-8,(17),12(*E*),14-triene-3-ol	[4, 24, 27]
	Labda-8(17),12(*E*),14-triene-2,3-diol	[24, 28]
	Labda-7,13(*Z*)-diene-17,12-olide	[23]
	Labda-7,13(*Z*)-diene-17,12-olide-15-ol	[23]
	6-Acetoxy-12(*E*),14-labdadiene-7,8-diol	[26]
	12(*E*),14-Labdadiene-6,7,8-triol	[26]
	Nidorellol	[5, 26]
	Cleistanthane diterpenoids	
Bark and	Cleistantha-4,13(17),15-triene-3-oic acid	[29]
Modified	Methyl- cleistantha-4(18),13(17),15-triene-3-oate	[29]
	Cleistantha-4(18),13(17),15-trien-3-ol	[29]
	Cleistantha-4(18),12,15-triene-3-oic acid	[29]
	Kaurane diterpenoids	
Bark and	Kaur-16-en-19-oic acid	[31]
Modified	Methyl-kaur-16-en-19-oate	[31]
	Kaur-16-en-19-ol	[31]
	16,17-Epoxy-kaur-19-oic acid	[31]
	17-Hydroxykaur-15-en-19-oic acid	[31]

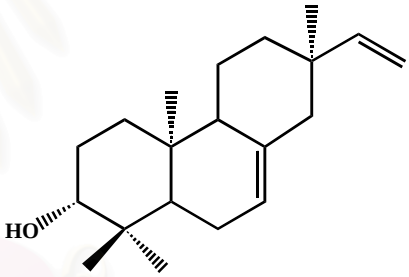
Table 2.1 The chemical constituents of *C. oblongifolius* (continued).

Plant parts	Compounds	Reference
Bark	**Abeitane diterpenoids** Aeita-7,13-diene-3-one	[32]
Bark	**Trachylobane diterpenoids** Trachyloban-19-oic acid	[26]

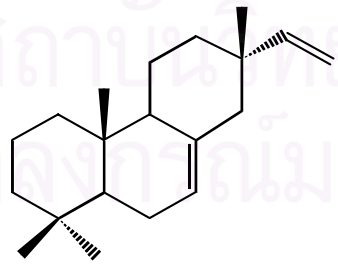
Pimarane diterpenoids



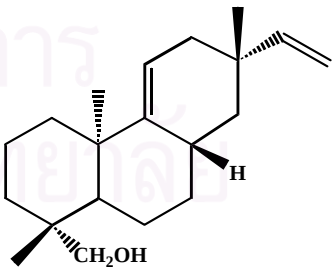
Oblongifoliol



19-Deoxyoblongifoliol



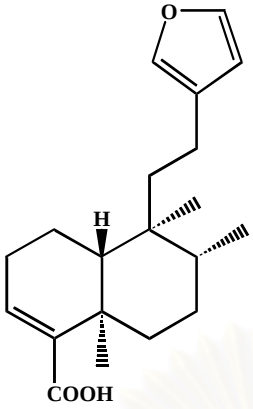
ent-Isopimara-7,15-diene



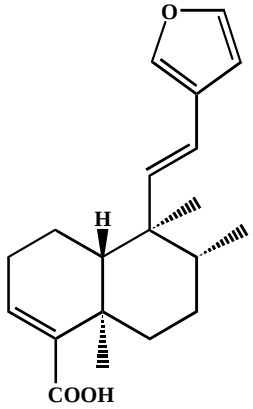
(-)-Pimara-9(11),15-diene-19-ol

Figure 2.2 Structure of compounds isolated from *C. oblongifolius*.

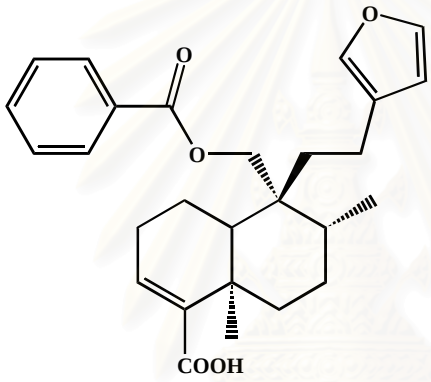
Clerodane diterpenoids



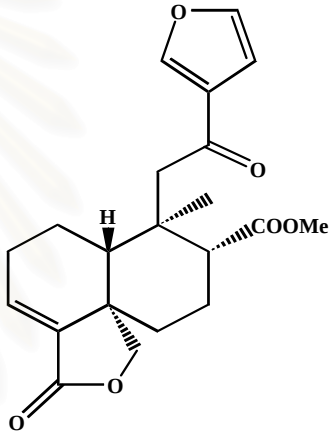
(-)-Hardwickiic acid



11-Dehydro-(-)-hardwickiic acid

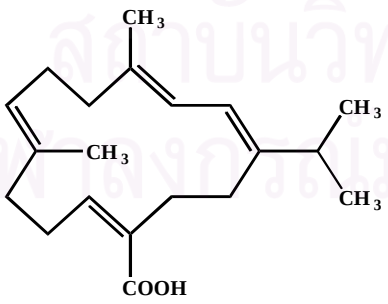


(-)-20-benzyloxyhardwickiic acid

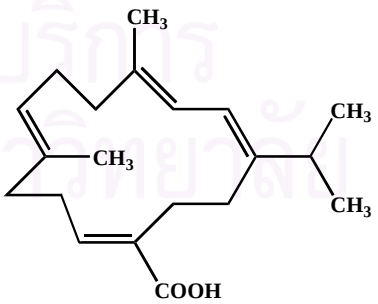


Nasimalun A

Cembrane diterpenoids



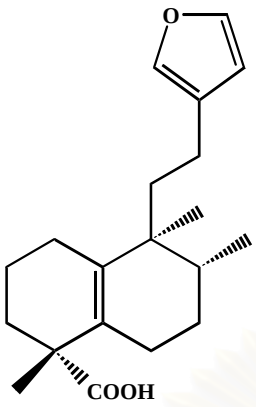
Crotocembraneic acid



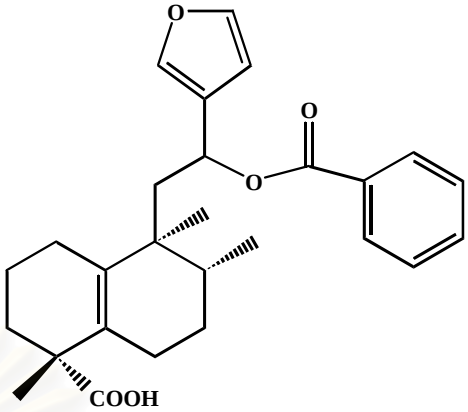
Neocrotocembraneic acid

Figure 2.2 Structure of compounds isolated from *C. oblongifolius* (continued).

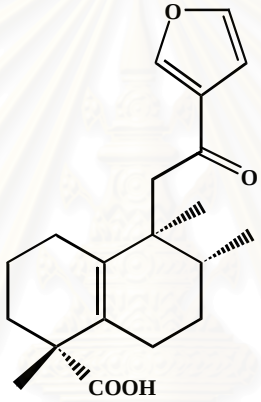
Halimane diterpenoids



Crotohalimaneic acid

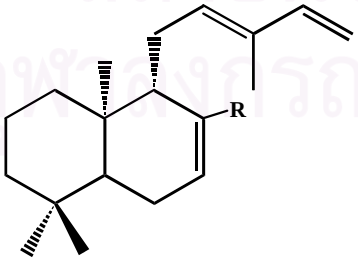


Benzoyl crotohalimonolic acid



Crotohalimoneic acid

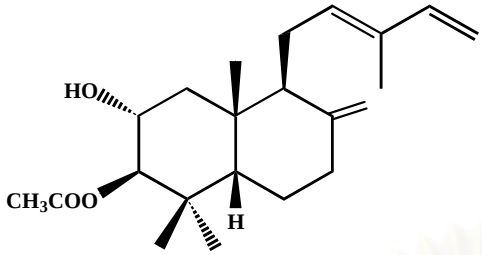
Labdane diterpenoids



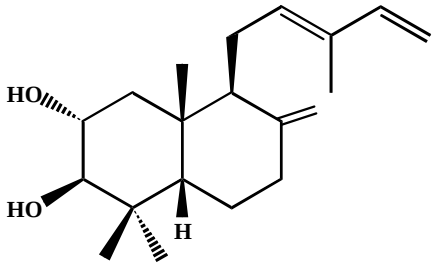
R	
CH_3	Labda-7,12(*E*),14-triene
CHO	Labda-7,12(*E*),14-triene-al
CH_2OH	Labda-7,12(*E*),14-triene-ol
COOH	Labda-7,12(*E*),14-triene-oic acid

Figure 2.2 Structure of compounds isolated from *C. oblongifolius* (continued).

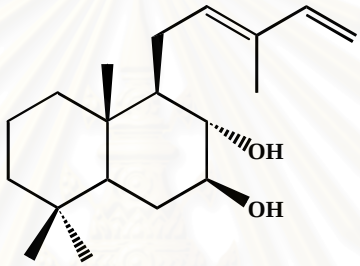
Labdane diterpenoids (continued)



3-Acetoxy-labda-8(17),12(*E*),14-triene-2-ol

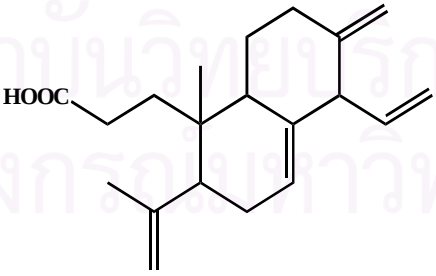


Labda-8(17),12(*E*),14-triene-2,3-ol



Nidorellol

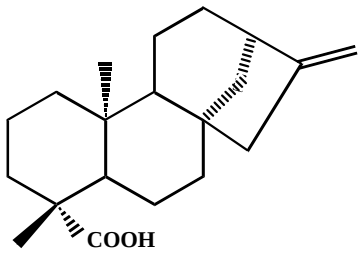
Cleistanthane diterpenoids



Cleistantha-4,13,15-triene-3-oic acid

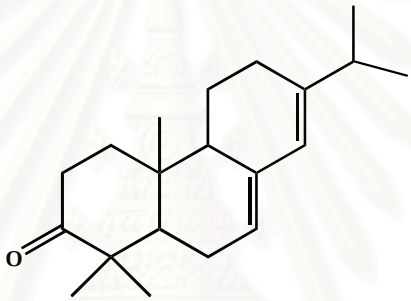
Figure 2.2 Structure of compounds isolated from *C. oblongifolius* (continued).

Kaurane diterpenoids



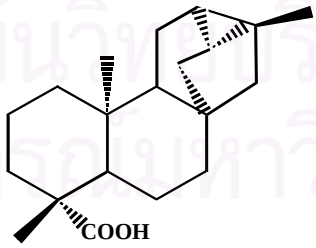
Kaur-16-en-19-oic acid

Abeitane diterpenoids



Aeita-7,13-diene-3-one

Trachylobane diterpenoids



Trachyloban-19-oic acid

Figure 2.2 Structure of compounds isolated from *C. oblongifolius* (continued).

2.4 Cytotoxic activity of diterpene compounds isolated from *C. oblongifolius*.

Some diterpene compounds found in *C. oblongifolius*, have been shown to possess cytotoxic activity against 5 tumor cell lines including Hep-G2 (hepatoma), Chago (lung), SW 620 (colon), Kato-3 (gastric) and BT 474 (breast) (Table 2.1).

Table 2.2 Cytotoxic activity against cancer cell lines of some diterpene compounds from *C. oblongifolius* [2-32].

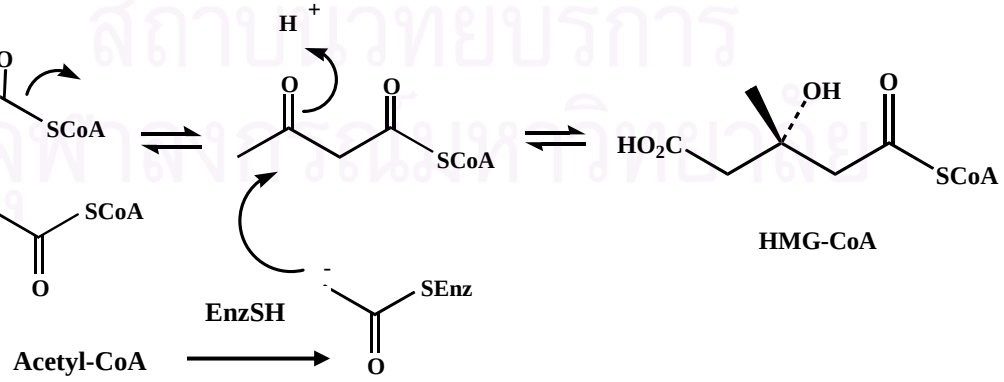
Compounds	% survival				
	BT474	Chago	HepG2	Kato	SW620
Crotocembraneic acid	7	3	71	6	6
Neocrotocembraneic acid	95	97	37	90	96
Neocrotocembranal	46	12	71	10	8
Crotohalimaneic acid	11	82	7	6	3
Crotohalimoneic acid	0	0	86	70	0
(-)-20-Benzyloxyhardwickiic acid	109	111	74	64	84
(-)-Hardwickiic acid	115	104	79	67	112
Labda-7,12(*E*),14-triene	75	72	61	47	73
Labda-7,12(*E*),14-triene-17-al	13	3	7	7	3
Labda-7,12(*E*),14-triene-17-ol	11	82	7	6	3
Labda-7,12(*E*),14-triene-17-oic acid	91	59	57	70	88
(-)-Pimara-9(11),15-diene-19-oic acid	117	85	95	77	95
(-)-Pimara-9(11),15-diene-19-ol	43	66	14	16	62
Poilaneic acid	98	98	64	95	83
Trachyloban-19-oic acid	72	78	58	74	50
Nidorellol	16	27	21	30	12
12(*E*),14-Labdadiene-6,7,8-triol	27	24	8	9	4
6-Acetoxy-12(*E*),14-labdadiene-7,8-diol	75	45	38	30	25
2-Acetoxy-labda-8(17),12(*E*),14-triene-3-ol	42	23	13	15	18

Table 2.1 Cytotoxic activity against cancer cell lines of some diterpene compounds from *C. oblongifolius* (continued) [2-32].

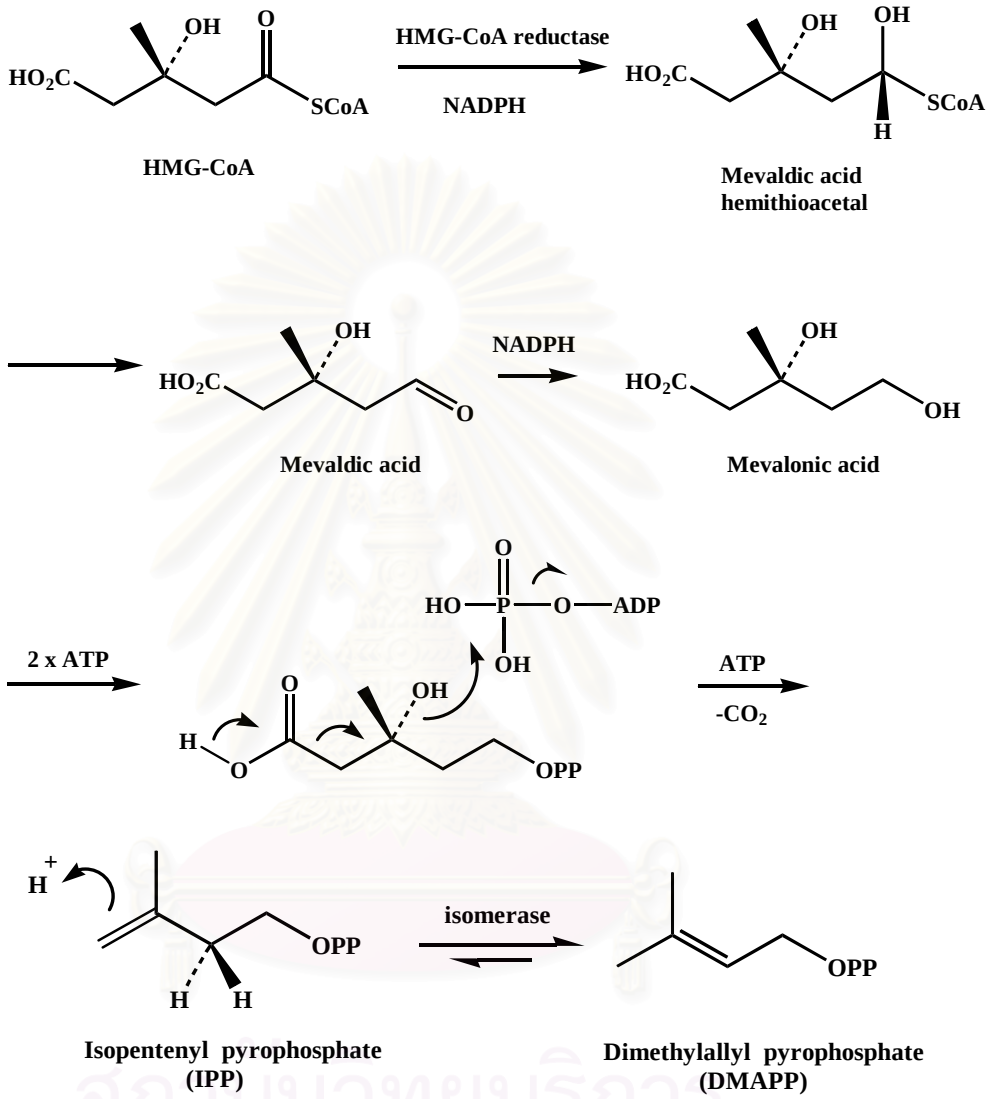
Compounds	% survival				
	BT474	Chago	HepG2	Kato	SW620
3-Acetoxy-labda-8(17),12(*E*),14-triene-2-ol	95	97	62	65	95
Crovatin	16	0	29	30	8
Isokolavenol	89	18	93	94	97
Kaur-16-en-19-oic acid	80	52	77	73	42
Abieta-7,13-diene-3-one	107	96	84	113	98
Cleistantha-4,13(17),15-triene-3-oic acid	20	4	4	6	3

2.5 Biosynthesis of Diterpene compounds.

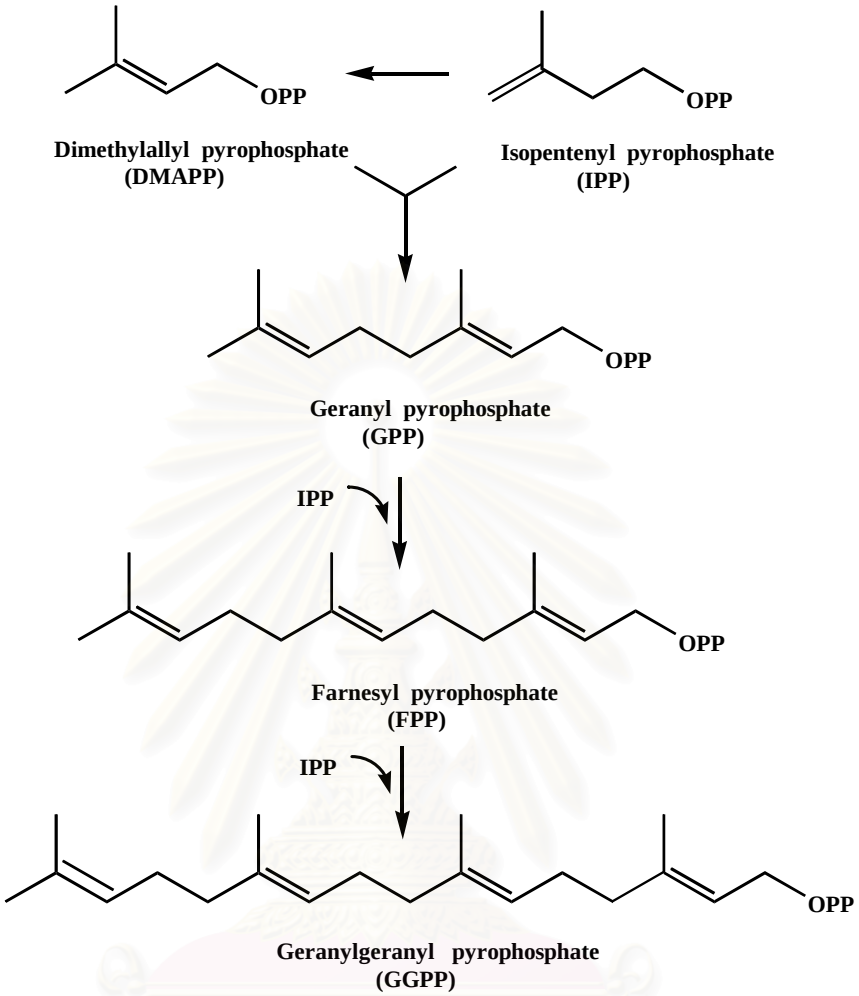
The diterpenes arise from geranylgeranyl pyrophosphate (GGPP), which is formed by addition of a further isopentenyl pyrophosphate (IPP) molecule to farnesyl diphosphate, shown in Scheme 2.1-2.3 [33].



Scheme 2.1 Biosynthesis of β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA).



Scheme 2.2 Origin of the isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP).



Scheme 2.3 Biosynthesis of geranylgeranyl pyrophosphate (GGPP).

All of the presently known diterpenoids are considered to be derived, as a result of the biogenetic isoprenoid rule, from geranylgeranyl pyrophosphate by cyclization to many diterpenoid compounds such as labdane, pimarane, abietane, clerodane, kaurane, trachylobane, cembrane, cassane, beyerane and atisane compounds.

CHAPTER III

EXPERIMENTS

3.1 Plant Material.

The plant material of *C. oblongifolius* (Plao-yai) was collected from Amphoe Phanat Nikhom, Chon Buri Province, Thailand, in August 2002.

3.2 Instruments and Equipments.

3.2.1. Fourier Transform Infrared Spectrophotometer (FT-IR).

The FT-IR spectra were recorded on a Nicolet Impact 410 Spectrophotometer. Spectra of solid samples were recorded as KBr pellets and liquid samples were recorded as thin film on NaCl cells.

3.2.2. Ultraviolet-Visible Spectrometer (UV-VIS).

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

3.2.3. Optical rotation.

The optical rotation were recorded on a Perkin-Elmener 341 polarimeter in chloroform.

3.2.4. Nuclear Magnetic Resonance Spectrometer (NMR).

The ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectra were recorded at 400 and 100 MHz, respectively, on a Varian Mercury + 400 NMR Spectrometer. Chemical shifts are expressed in parts per million (ppm) using residual protonated solvents as reference. COSY, NOESY, HSQC and HMBC experiments were performed on the Varian Mercury + 400 NMR Spectrometer.

3.2.5. Mass Spectrometer (MS).

The mass spectra were recorded on a Fisons Instrument Mass Spectrometer Model Trio 2000 in EI mode at 70 eV.

3.3 Chemicals.

3.3.1. Solvents.

All solvents used in this research such as hexane, ethyl acetate, methanol were commercial grade and were purified prior to use by distillation.

3.3.2. Other chemicals.

3.3.2.1 Merck's silica gel 60 Art. 1.07734.1000 (70-230 mesh ASTM) and Merck's silica gel 60 Art. 1.09385.1000 (230-400 mesh ASTM) were used as adsorbent for column chromatography.

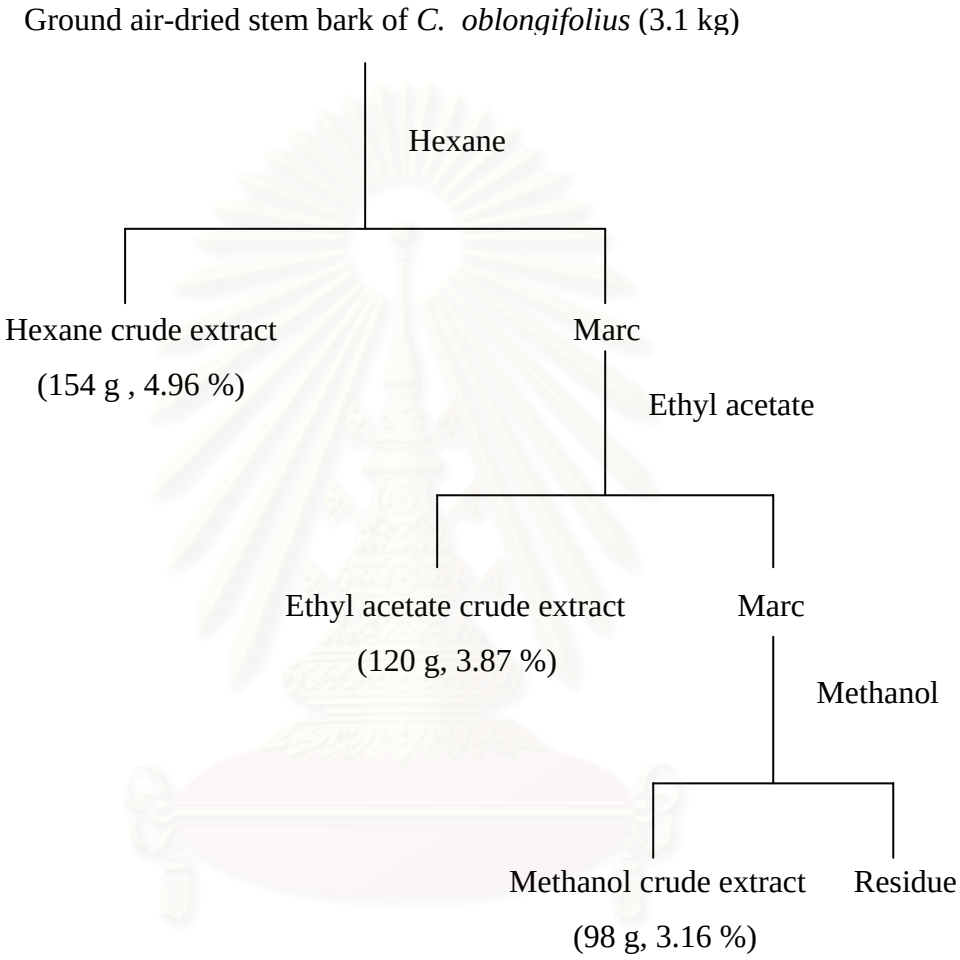
3.3.2.2 Merck's TLC aluminium sheet, silica gel 60F 254 precoated 25 sheets, 20x20 cm^2, layer 0.2 mm. was used to identify the identical fractions.

3.4 Extraction and isolation.

The powdered, sun-dried stem bark (3.1 kg) of *C. oblongifolius* was extracted with hexane (3x10 liters), ethyl acetate (3x10 liters) and methanol (2x10 liters) at room temperature, respectively. The solution was filtered and evaporated under reduced pressure until dry to obtain hexane crude extract (154 g), ethyl acetate crude extract (120 g) and methanol crude extract (78 g), respectively. The crude extracts of the stem bark of *C. oblongifolius* are shown in Table 3.1 and the extraction procedure are shown in Scheme 3.1.

Table 3.1 The solvent extracted of the stem bark of *C. oblongifolius*.

Solvent extracted	Appearance	Weight (g)	%wt by wt of the dried stem bark
hexane	yellowish green oil	154	4.96
ethyl acetate	dark brown oil	120	3.87
methanol	dark brown oil	98	3.16



Scheme 3.1 The procedure of extraction of the stem bark of *C. oblongifolius*.

3.5 Separation of crude extract of the stem bark of *C. oblongifolius*.

3.5.1 Separation of hexane crude extract.

The hexane crude extract was obtained as a yellowish green oil after evaporation. The hexane crude extract (40.0 g) was fractionated by silica gel column chromatography using Merck's silica gel 60 Art. 1.07734.1000 (70-230 mesh ASTM) (390.0 g) as absorbent. The column was eluted with an increasing gradient of hexane, hexane in ethyl acetate, ethyl acetate and finally methanol in ethyl acetate (approximately 50 ml per fraction) for 120 fractions. Each fraction was analyzed by TLC. The separation of hexane crude extract gave compounds **1-3** shown in Table 3.2.

Table 3.2 The results of separation of hexane crude extract by column chromatography.

Compounds	Physical appearance	Weight (g)
1	white solid	6.0
2	white solid	0.012
3	white solid	0.9

3.5.2 Separation of ethyl acetate crude extract.

The ethyl acetate crude extract (30.0 g) was separated on silica gel 60 Art. 1.07734.1000 (70-230 mesh ASTM) (370.0 g) using the column chromatography technique. The column was an increasing gradient of hexane in ethyl acetate, ethyl acetate and finally methanol in ethyl acetate (approximately 50 ml per fraction) for 80 fractions. Each fraction was analyzed by TLC. The separation of ethyl acetate crude extract gave Compounds **2** (12.1 g).

3.5.3 Separation of methanol crude extract.

The methanol crude extract (98.0 g) was gummy residues. It was insoluble in all solvent and this crude extract was not separated by column chromatography.

3.6 Purification and properties of the isolated compounds from *C. oblongifolius*.

3.6.1 Purification and properties of Compound 1.

The compound **1** was eluted with 10% ethyl acetate in hexane on silica gel chromatography. This compound was a white crystalline solid having melting point at 104-105 ºC. It was soluble in various solvents such as hexane, ethyl acetate, chloroform and methanol.

Compound **1** was a white solid (6.0 g, 0.19%), $[\alpha]_D^{25}$ -113° ($CHCl_3$; *c* 0.2), UV ($CHCl_3$) λ_{max}(log ε): 249 (3.10).

FT-IR spectrum (KBr) (Fig. 5, Table 4.1) ν_{max} (cm^{-1}): 3500-2400, 2959, 2856, 1682, 1625, 1456, 1409, 1258, 1164 and 871.

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.) (Fig. 6, Table 4.2) δ (ppm): 7.36 (H-15, s), 7.21 (H-16, s), 6.89 (H-3, m), 6.27 (H-14, s), 2.34 (H-2, m), 2.20 (H-12, m), 1.87 (H-1, m), 1.66 (H-11, m), 1.59 (H-8, m), 1.47 (H-7, m), 1.41 (H-10, d, *J* = 12.8 Hz), 1.29 (Me-19, s), 1.18 (H-6, m), 0.87 (Me-17, d, *J* = 6.4 Hz) and 0.79 (Me-20, s).

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.) (Fig. 7, Table 4.3) δ (ppm): 172.5 (s), 142.7 (d), 141.5 (s), 140.4 (d), 138.4 (d), 125.6 (s), 111.0 (d), 46.7 (d), 38.8 (s), 38.6 (t), 37.6 (s), 36.2 (d), 35.8 (t), 27.5 (t), 27.3 (t), 20.5 (q), 18.3 (q), 18.2 (t), 17.5 (t) and 16.0 (q).

EI MS spectrum (Fig. 8) *m/z*: 316 [M^+], 299 (17), 283 (15), 221 (52), 203 (70), ,175 (25), 137 (40), 125 (100), 95 (35) and 81 (60).

3.6.2 Purification and properties of Compound 2.

Compound **2** was eluted with 25% ethyl acetate in hexane on silica gel chromatography. This compound was a white solid (12.0 mg, $3.87x10^{-4}$% wt by wt) having melting point at 106-107 ºC. It was soluble in organic solvents such as ethyl acetate, chloroform, methanol and ethanol.

Compound **2** was a white solid (12.0 mg, $3.87x10^{-4}$%), $[\alpha]_D^{20}$ -67.0 ($CHCl_3$; *c* 0.1), UV ($CHCl_3$) λ_{max}(log ε): 248 (3.06).

FT-IR spectrum (KBr) (Fig. 9, Table 4.4) ν_{max} (cm^{-1}): 3800-3000, 2912, 2846, 1743, 1682, 1456, 1381, 1254, 1070 and 828.

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.) (Fig. 10, Table 4.5) δ (ppm): 7.12 (H-14, s), 6.88 (H-3, s), 4.80 (H-15, s), 2.45 (H-6b, d, *J* = 13.2 Hz), 2.28 (H-2, m), 2.21

(H-12a, m), 2.07 (H-12b, m), 1.69 (H-1a, m) , 1.65 (H-11b, m), 1.55 (H-8, m), 1.48 (H-7b, m), 1.46 (H-7a, m), 1.38 (H-10, d, *J* = 12.4 Hz), 1.27 (Me-19, s), 1.18 (H-6a, m), 0.85 (Me-17, d, *J* = 6.4 Hz) and 0.79 (Me-20, s).

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.) (Fig. 11, Table 4.6) δ (ppm): 174.4 (s), 171.8 (s), 143.5 (d), 141.2 (d), 140.3 (d), 134.9 (s), 70.2 (t), 46.6 (d), 38.7 (s), 37.5 (s), 36.2 (d), 35.9 (t), 35.7 (t), 27.4 (t), 27.2 (t), 20.5 (q), 19.0 (t), 18.2 (q), 17.3 (t) and 15.9 (q).

EI MS spectrum (Fig. 16) *m/z*: 314 $[M\text{-}H_2O]^+$, 299 (17), 271 (10), 203 (25), 175 (50), 125 (23), 105 (30) and 93 (20).

3.6.3 Purification and properties of Compound 3.

Compound **3** was eluted with 40% ethyl acetate in hexane on silica gel chromatography. This compound was a white crystalline solid having melting point at 157-158 ºC. It was soluble in organic solvents such as ethyl acetate, chloroform and methanol.

Compound **3** was a white solid (12.5 g, 0.40%), $[\alpha]_D^{25}$ -103° ($CHCl_3$; *c* 0.2), UV ($CHCl_3$) λ_{max} (log ε): 253 (3.25), 207 (4.34).

FT-IR spectrum (KBr) (Fig. 17, Table 4.7) ν_{max} (cm^{-1}): 3146, 2950, 1771, 1729, 1663, 1555, 1508, 1366, 1320, 1282, 1197, 1150, 1037 and 770.

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.) (Fig. 18, Table 4.8) δ (ppm): 8.06 (H-16, s), 7.47 (H-15, s), 6.79 (H-3, s), 6.77 (H-14, s), 4.38 (H-19b, d, *J* = 8.8 Hz), 3.97 (H-19a, d, *J* = 8.8 Hz), 3.64 (COOMe, s), 3.26 (H-8ax, dd, *J* = 13.2, 4.8 Hz), 3.07 (H-11b, d, *J* = 18.0 Hz), 2.88 (1H, d, *J* = 18.0 Hz), 2.77 (H-10ax, d, *J* = 12.4 Hz), 2.32 (H-2eq, m), 2.24 (H-2ax, m), 2.08 (H-7ax, m), 2.00 (H-6eq, m), 1.92 (H-7eq, m), 1.68 (H-1eq, dd), 1.39 (H-6ax, td), 1.11 (H-1ax , qd, *J* = 12.0, 12.0, 12.0, 4.4 Hz) and 0.86 (Me-20, s).

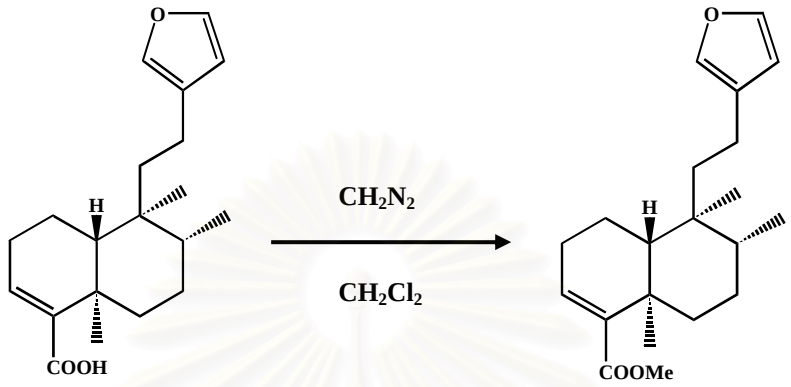
^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.) (Fig. 19, Table 4.9) δ (ppm): 193.6 (s), 174.0 (s), 169.0 (s), 147.1 (d), 144.3 (d), 137.8 (s), 136.2 (d), 128.5 (s), 108.5 (d), 71.4 (t), 51.4 (q), 48.7 (d), 46.7 (d), 46.4 (t), 45.1 (s), 39.5 (s), 33.1 (t), 27.3 (t), 22.0 (t), 20.0 (t) and 19.2 (q).

EI MS spectrum (Fig. 20) *m/z*: 372 $[M^+]$, 341 (13), 263 (24), 245 (30), 145 (50), 110 (65), 95 (100) and 91 (19).

3.7 Modification of isolated compounds from *C. oblongifolius.*

3.7.1 Modification of Compound 1.

The pathway of modification of Compound **1** is shown in Scheme 3.2.



(-)-Hardwickiic acid (**1**) Methyl ester derivative (**1a**)

Scheme 3.2 Methylation of Compound **1**.

Compound **1** (30.2 mg, 0.09 mmol) was methylated with diazomethane in dichloromethane to give methyl ester, Compound **1a** as a viscous transparent oil (28.1 mg, 0.08 mmol, 88.88% yield), $[\alpha]_D^{20}$ -130 ($CHCl_3$; *c* 0.1), UV ($CHCl_3$) λ_{max} (log ε): 230 (3.42).

FT-IR spectrum (neat) (Fig. 21, Table 4.10) ν_{max} (cm^{-1}): 2950, 2865, 1710, 1456, 1432, 1249, 1197 and 779.

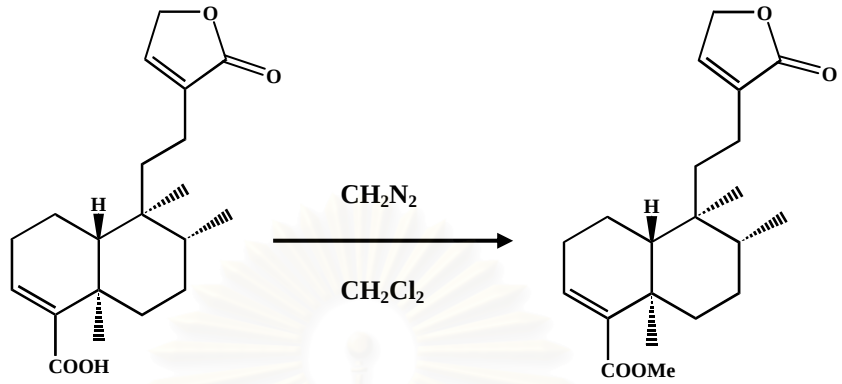
^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.) (Fig. 22) δ (ppm): 7.37 (H-15, bs), 7.23 (H-16, bs), 6.63 (H-3, t, *J* = 3.6 Hz), 6.28 (H-14, bs), 3.71 (COOMe, s), 2.00-2.40 (6H, m), 1.00-1.80 (8H, m), 1.30 (Me-19, s), 0.85 (Me-17, d, *J* = 6 Hz), 0.78 (Me-20, s).

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.) (Fig. 23, Table 4.11) δ (ppm): 167.9 (s), 142.7 (d), 142.6 (s), 138.4 (d), 136.9 (d), 125.6 (s), 111.0 (d), 51.2 (q), 46.5 (d), 38.8 (s), 38.6 (t), 37.6 (s), 36.2 (d), 35.9 (t), 27.3 (t), 27.2 (t), 20.7 (q), 18.3 (q), 18.1 (t), 17.5 (t) and 16.0 (q).

EI MS spectrum (Fig. 24) *m/z*: 330 [M^+], 315 (8), 299 (15), 283 (20), 235 (45), 203 (43), 139 (100), 96 (70), 82 (40) and 81 (85).

3.7.2 Modification of Compound 2.

The pathway of modification of Compound **2** is shown in Scheme 3.3.



(-)-Patagonic acid (**2**) Methyl ester derivative (**2a**)

Scheme 3.3 Methylation of Compound **2**.

Compound **2** (10.1 mg, 0.030 mmol) was methylated with diazomethane in dichloromethane to give methyl ester, Compound **2a** as a viscous transparent oil (9.8 mg, 0.028 mmol, 93.33% yield), $[\alpha]_D^{25}$ -61 ($CHCl_3$; *c* 0.2), UV ($CHCl_3$) λ_{max} (log ε): 490 (0.88).

FT-IR spectrum (neat) (Fig. 25, Table 4.12) ν_{max} (cm^{-1}): 2921, 2855, 1754, 1711, 1458, 1380, 1248, 1232 and 1073.

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.) (Fig. 26) δ (ppm): 7.12 (H-14, s), 6.63 (H-3, s), 4.80 (H-15, s), 3.71 (COOMe, s), 2.45 (H-6b, d, *J* = 13.2 Hz), 2.28 (H-2, m), 2.21 (H-12a, m), 2.07 (H-12b, m), 1.69 (H-1a, m) , 1.65 (H-11b, m), 1.55 (H-8, m), 1.48 (H-7b, m), 1.46 (H-7a, m), 1.38 (H-10, d, *J* = 12.4 Hz), 1.28 (Me-19, s), 1.18 (H-6a, m), 0.85 (Me-17, d, *J* = 6.4 Hz) and 0.80 (Me-20, s).

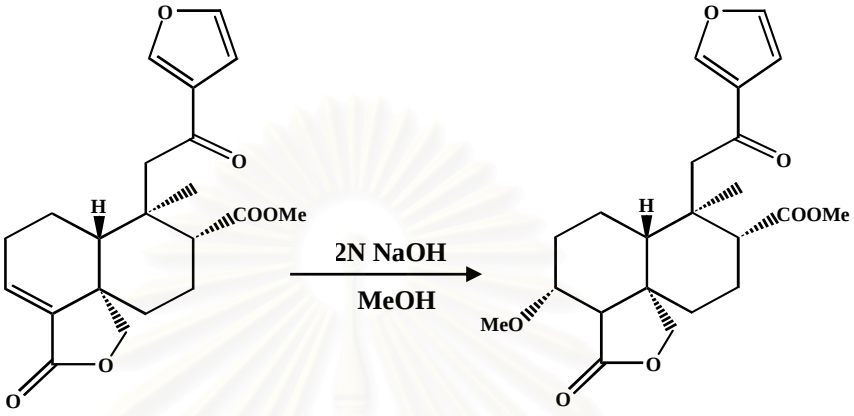
^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.) (Fig. 27, Table 4.13) δ (ppm): 174.6 (s), 167.2 (s), 143.9 (d), 141.2 (s), 136.1 (d), 134.2 (s), 70.3 (t), 51.2 (q), 46.5 (d), 38.7 (s), 37.6 (s), 36.3 (d), 36.3 (t), 35.9 (t), 27.1 (t), 27.1 (t), 20.7 (q), 19.0 (t), 18.1 (q), 17.6 (t) and 15.7 (q).

EI MS spectrum (Fig. 28) *m/z*: 315 $[M\text{-}OMe]^+$ (30), 314 $[M\text{-}MeOH]^+$ (100), 299 $[314\text{-}Me]^+$ (10), 271 $[299\text{-}CO]^+$ (12), 203 (20), 175 (60), 139 (25), 107 (32), 105 (55) and 91 (95).

3.7.3 Modification of Compound 3.

3.7.3.1 Preparation of Methyl-15,16-epoxy-3-methoxy-12-oxo-13(16),14-clerodadien-18,19-olide-17-carboxylate (3a).

The pathway of alcohol addition of Compound **3** is shown in Scheme 3.4.



Nasimalun A (**3**) Methoxy derivative (**3a**)

Scheme 3.4 Alcohol addition of Compound **3**.

Compound **3** (100.3 mg, 0.269 mmol) was treated with 2N NaOH in methanol (5 ml) and stirred at room temperature for 3 h. The methanol was evaporated and the residue was acidified with 1N HCl (3 ml) and extracted with ethyl acetate. The ethyl acetate was washed (H_2O), dried (anh. Na_2SO_4), and evaporated to give product. The crude product was purified by silica gel column chromatography with 80% EtOAc in hexane to obtain compound **3a** (58.4 mg, 0.144 mmol, 53.53 % yield).

Compound **3a** was a white solid crystal, $[\alpha]_D^{25}$ +10° ($CHCl_3$; *c* 0.1), UV ($CHCl_3$) λ_{max}(log ε) : 253 (3.52), mp 194-195 °C.

FT-IR spectrum (KBr) (Fig. 29, Table 4.14) ν_{max} (cm^{-1}): 3431, 2945, 2855 1777, 1723, 1668, 1458, 1372, 1147, 1100, 1011 and 777.

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.) (Fig. 30, Table 4.15) δ (ppm): 8.03 (H-16, s), 7.46 (H-15, d, *J* = 1.6 Hz), 6.76 (H-14, d, *J* = 1.6 Hz), 4.25 (H-19b, d, *J* = 9.6 Hz), 4.21 (H-19a, d, *J* = 9.6 Hz), 3.60 (COOMe, s), 3.36 (OMe, s), 3.35 (H-3, m), 3.25 (H-8, dd, *J* = 12.8, 3.6 Hz), 2.94 (H-11b, d, *J* = 18.0 Hz), 2.87 (H-11a, d, *J* = 18.0 Hz), 2.57 (H-10, dd, *J* = 11.6, 3.2 Hz), 2.17 (H-2b, m), 2.10 (H-2a, m), 1.96 (H-

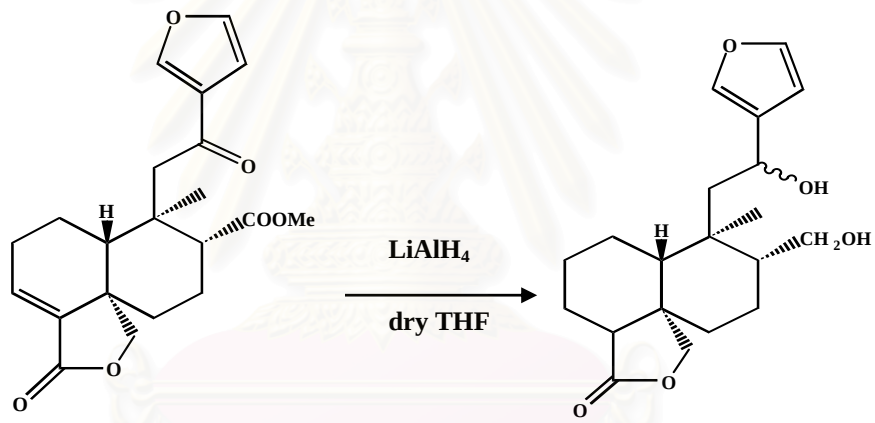
7b, m), 1.95 (H-6b, m), 1.81 (H-7a, m), 1.73 (H-1b, m), 1.36 (H-6a, m), 1.34 (H-2a, m), 1.32 (H-1a, m) and 0.83 (Me-20, s).

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.) (Fig. 31, Table 4.15) δ (ppm): 193.5 (s), 176.3 (s), 174.1 (s), 146.9 (d), 144.3 (d), 128.4 (s), 108.5 (d), 77.0 (q), 70.2 (t), 56.8 (d), 55.0 (d), 51.4 (q), 48.4 (d), 45.9 (t), 43.7 (s), 43.4 (d), 39.5 (s), 35.5 (t), 29.3 (t), 21.4 (t), 19.4 (t) and 18.8 (q).

EI MS spectrum (Fig. 36) *m/z*: 404 [M^+], 373 (6), 295 (27), 249 (20), 157 (25), 110 (57), 95 (100) and 91 (21).

3.7.3.2 Preparation of Methyl-12,17-dihydroxy-15,16-epoxy-13(16),14-clerodadien-18,19-olide (3b).

The pathway of reduction of Compound **3** is shown in Scheme 3.5.



Nasimalun A (**3**) Diol derivative (**3b**)

Scheme 3.5 Reduction of Compound **3** with $LiAlH_4$.

Compound **3** (325.3 mg, 0.874 mmol) in THF (10 ml) was reduced with $LiAlH_4$ (99.5 mg, 2.622 mmol). The mixture was stirred at room temperature for 40 min. The reaction was quenched with water, extracted with ethyl acetate, dried (anh. Na_2SO_4), and on evaporation of the solvent gave a mixture of products. The mixture was purified by silica gel column chromatography with 50% EtOAc in hexane to obtain major product as compound **3b** (72.1 mg, 0.207 mmol, 23.68% yield). The other compounds were not separated and purified.

Compound **3b** was a white solid, $[\alpha]_D^{25}$ -36° ($CHCl_3$; *c* 0.1), UV ($CHCl_3$) λ_{max} (log ε): 249 (2.25), mp 55-56 ºC.

FT-IR spectrum (KBr) (Fig. 37, Table 4.16) ν_{max} (cm^{-1}): 3800-3100, 2932, 2856, 1757, 1188 and 1005.

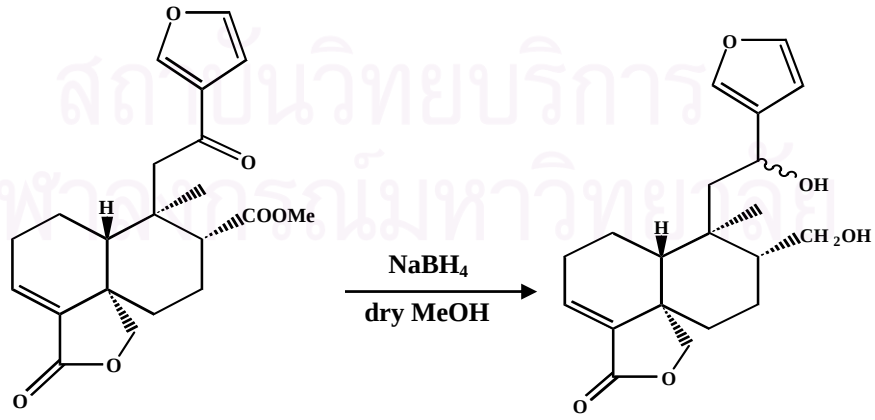
^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.) (Fig. 38, Table 4.17) δ (ppm): 7.42 (H-16, s), 7.41 (H-15, s), 6.45 (H-14, d, *J* = 1.2 Hz), 4.93 (H-12, dd, *J* = 8.4, 3.2 Hz), 4.36 (H-19b, d, *J* = 9.2 Hz), 4.24 (H-19a, d, *J* = 9.2 Hz), 3.74 (H-17b, dd, *J* = 10.8, 4.0 Hz), 3.32 (H-17a, dd, *J* = 10.8, 8.4 Hz), 2.08 (H-4, m), 2.04 (H-11b, m), 2.02 (H-2b, m), 2.01 (H-1b, m), 1.93 (H-3b, m), 1.90 (H-10, m), 1.87 (H-7b, m), 1.84 (H-6b, m), 1.76 (H-8, m), 1.72 (H-11a, dd, *J* = 3.2, 3.2 Hz), 1.44 (H-2a, m), 1.41 (H-7a, m), 1.36 (H-3a, m), 1.18 (H-1a, m), 1.15 (H-6a, m) and 0.64 (Me-20, s).

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.) (Fig. 39, Table 4.17) δ (ppm): 179.3 (s), 143.6 (d), 138.6 (d), 130.7 (s), 108.3 (d), 70.5 (t), 63.6 (t), 63.3 (d), 49.9 (d), 46.5 (d), 45.1 (d), 44.0 (t), 43.1 (s), 38.8 (s), 36.2 (t), 25.7 (t), 25.0 (t), 22.0 (t), 21.3 (t) and 18.1 (q).

EI MS spectrum (Fig. 44) *m/z*: 348 [M^+], 330 (25), 315 (10), 253 (17), 222 (15), 179 (27), 111 (35), 97 (100) and 95 (40).

3.7.3.3 Preparation of Methyl-12,17-dihydroxy-15,16-epoxy-3,13(16),14-clerodatrien-18,19-olide (3c).

The pathway of reduction of Compound **3** is shown in Scheme 3.6.



Nasimalun A (**3**) Diol derivative (**3c**)

Scheme 3.6 Reduction of Compound **3** with $NaBH_4$.

Compound **3** (300.6 mg, 0.808 mmol) in MeOH (5 ml) was reduced with $NaBH_4$ (152.8 mg, 4.040 mmol). The mixture was stirred at room temperature for 5 h. The reaction was quenched with water, extracted with ethyl acetate, dried (anh. Na_2SO_4), and on evaporation of the solvent gave a mixture of products. The mixture was purified by silica gel column chromatography with 70% EtOAc in hexane to obtain major product as compound **3c** (145.6 mg, 0.421 mmol, 52.10 %). The other compounds were not separated and purified.

Compound **3c** was a white solid, $[\alpha]_D^{25}$ -69° ($CHCl_3$; *c* 0.1), UV ($CHCl_3$) λ_{max} (log ε): 340 (3.04), mp 70-71ºC.

FT-IR spectrum (KBr) (Fig. 45, Table 4.18) ν_{max} (cm^{-1}): 3800-3100, 2925, 2855, 1771, 1658, 1564, 1423, 1183, 1032 and 871.

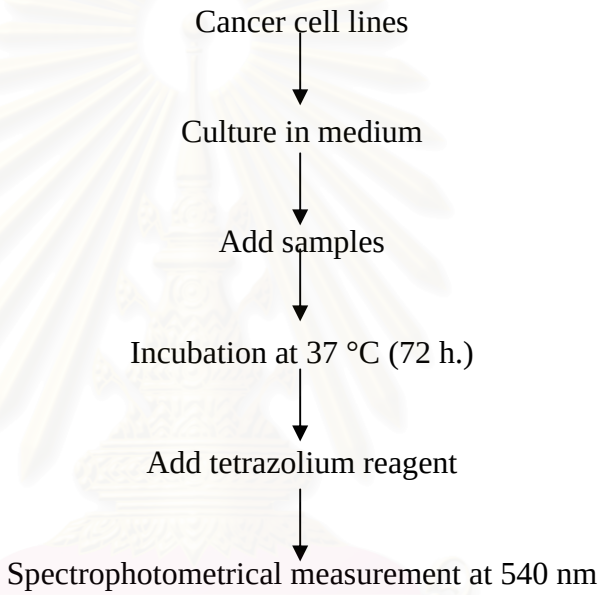
^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.) (Fig. 46, Table 4.19) δ (ppm): 7.43 (H-16, s), 7.40 (H-15, s), 6.80 (H-3, d, *J* = 7.2 Hz), 6.43 (H-14, s), 4.77 (H-12, d, *J* = 9.2 Hz), 4.31 (H-19b, d, *J* = 8.4 Hz), 3.95 (H-19a, d, *J* = 8.0 Hz), 3.85 (H-17b, dd, *J* = 10.4, 8.0 Hz), 3.50 (H-17a, dd, *J* = 10.4, 4.0 Hz), 2.42 (H-11b, m), 2.39 (H-2b, m), 2.34 (H-10, d, *J* = 10.0 Hz), 2.20 (H-2a, m), 2.07 (H-8, d, *J* = 13.6 Hz), 2.02 (H-6b, m), 1.84 (H-11a, d, *J* = 16.8 Hz),1.77 (H-7b, m), 1.64 (H-1eq, m), 1.46 (H-7a, m), 1.37 (H-6a, m), 1.08 (H-1ax, qd, *J* = 12.0, 12.0, 12.0, 4.0 Hz), and 0.72 (Me-20, s).

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz.) (Fig. 47, Table 4.19) δ (ppm): 169.3 (s), 143.7 (d), 138.4 (s), 138.2 (d), 135.8 (d), 130.7 (s), 108.2 (d), 71.7 (t), 65.0 (t), 63.2 (d), 48.4 (d), 45.8 (t), 45.7 (s), 45.3 (d), 39.0 (s), 34.2 (t), 27.4 (t), 23.4 (t), 19.4 (t) and 18.2 (q).

ESI MS spectrum (Fig. 52) *m/z*: 347$[M+H]^+$.

3.8 Cytotoxicity test.

The bioassay of cytotoxic activity against 6 tumor cell lines *in vitro*, which were Hep-G2 (hepatoma), Chago (lung), SW620 (colon), Kato-3 (gastric), BT 474 (breast) and HuCCA-1 (human bile duct epithelial carcinoma). The cytotoxicity test was performed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method [4, 34-36].



Scheme 3.7 The procedure of cytotoxicity test.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Structure elucidation of the isolated compounds from the stem bark of *C. oblongifolius*.

4.1.1 Structure elucidation of Compound 1.

The IR spectrum of compound **1** is shown in Fig 5. and the absorption peaks were assigned as shown in Table 4.1. Its IR spectrum showed important absorption bands at 3500-2400 cm^{-1} of the O-H stretching vibration of acid, the absorption bands at 2959 and 2856 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1682 cm^{-1} of the C=O stretching vibration of carbonyl group and the absorption bands at 1625, 1409 and 871 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.1.

Table 4.1 The IR absorption bands assignment of Compound **1**.

Wave number (cm^{-1})	Intensity	Vibration
3500-2400	broad	O-H stretching vibration of acid
2959, 2856	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1682	strong	C=O stretching vibration of carbonyl group
1625, 1409, 871	medium	C=C stretching vibration of alkene

The ^{1}H-NMR spectrum (Fig. 5, Table 4.2) of compound **1** indicated that it possesses three methyl groups at 0.87, 0.79 and 1.29 ppm, three olefinic protons of furanoid group at 7.38, 7.24 and 6.29 ppm and one vinylic proton at 6.90 ppm.

The ^{13}C-NMR spectrum (Fig. 6, Table 4.3) showed 20 lines. Six signals of olefinic carbons appeared at δ 142.7 (d), 141.5 (s), 140.4 (d), 138.4 (d),125.6 (s) and 111.0 (d) ppm. The signal at δ 172.5 (s) ppm should be the carbonyl of carboxylic acid. There were thirteen sp^3 carbon signals at δ 46.7 (d), 38.8 (s), 38.6 (t), 37.6 (s),

36.3 (d), 35.8 (t), 27.5 (t), 27.3 (t), 20.5 (q), 18.3 (q), 18.2 (t), 17.5 (t) and 16.0 (q) ppm.

Its molecular formula was established as $C_{20}H_{28}O_3$, which was confirmed by observing molecular ion at *m/z* 316 (Fig. 8). The molecular formula, $C_{20}H_{28}O_3$, of compound **1** defined a DBE of 7, therefore, compound **1** must consisted of one ring of furan (DBE = 3) in addition to one double bond, two rings and one carbonyl group of carboxylic acid. These data indicated that compound **1** could be identical to hardwickiic acid.

It could be concluded that compound **1** exhibited the ^{13}C-NMR chemical shifts identical to (-)-hardwickiic acid [5, 37]. The ^{13}C-NMR chemical shifts of compound **1** and (-)-hardwickiic acid could be compared as shown in Table 8.

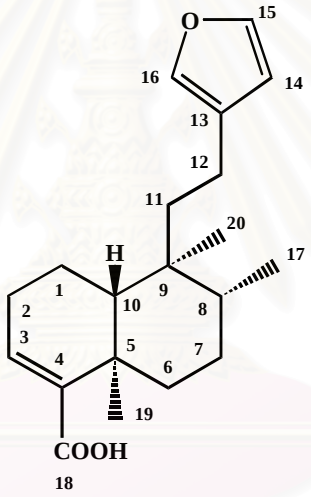


Figure 4.1 Structure of Compound **1**.

Table 4.2 ^{1}H-NMR spectra of Compound **1** and (-)-Hardwickiic acid.

Position	Chemical shifts (ppm)	
	(-)-Hardwickiic acid [5, 37]	Compound **1**
H-1	1.87 (1H, m)	1.70 (1H, m)
H-2	2.33 (1H, m)	2.34 (1H, m)
H-3	6.90 (1H, m)	6.90 (1H, m)
H-4	-	-
H-5	-	-
H-6	1.13 (1H, m)	1.18 (1H, m)
H-7	1.41 (1H, m)	1.47 (1H, m)
H-8	1.55 (1H, m)	1.59 (1H, m)
H-9	-	-
H-10	1.49 (1H, m)	1.41 (1H, d, *J* = 12.8 Hz)
H-11	1.72 (1H, m)	1.66 (1H, m)
H-12	2.30 (1H, m)	2.20 (1H, m)
H-13	-	-
H-14	6.25 (1H, s)	6.29 (1H, s)
H-15	7.37 (1H, bs)	7.38 (1H, s)
H-16	7.23 (1H, s)	7.24 (1H, s)
H-17	0.85 (3H, d, *J* = 6.0 Hz)	0.87 (3H, d, *J* = 6.4 Hz)
H-18	-	-
H-19	1.27 (3H, s)	1.29 (3H, s)
H-20	0.80 (3H, s)	0.79 (3H, s)

Table 4.3 ^{13}C-NMR spectra of Compound **1** and (-)-Hardwickiic acid.

Position	Chemical shifts (ppm)	
	(-)-Hardwickiic acid [5, 37]	Compound **1**
C-1	18.6t	17.5t
C-2	28.0t	27.5t
C-3	138.1d	140.4d
C-4	142.8s	141.5s
C-5	38.3s	37.6s
C-6	37.0t	35.8t
C-7	27.1t	27.3t
C-8	36.6d	36.2d
C-9	39.7s	38.8s
C-10	47.6d	46.7d
C-11	39.5t	38.6t
C-12	18.6t	18.2t
C-13	126.6s	125.6s
C-14	111.8d	111.0d
C-15	139.4d	138.4d
C-16	143.5d	142.7d
C-17	16.2q	16.0q
C-18	173.1s	172.5s
C-19	20.9q	20.5q
C-20	18.2q	18.3q

4.1.2 Structure elucidation of Compound 2.

Compound **2** was obtained as a white solid. The IR spectrum of compound **2** is shown in Fig 9. Its IR spectrum showed important absorption bands at 3800-3000 cm^{-1} of the O-H stretching vibration of acid, the absorption bands at 2912 and 2846 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption bands at 1743 and 1682 cm^{-1} of the C=O stretching vibration of carbonyl group and the absorption band at 1456 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.4.

Table 4.4 The IR absorption bands assignment of Compound **2**.

Wave number (cm^{-1})	Intensity	Vibration
3800-3000	broad	O-H stretching vibration of acid
2912, 2846	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1743, 1682	strong	C=O stretching vibration of carbonyl group
1456	medium	C=C stretching vibration of alkene

The ^{1}H-NMR spectrum (Fig. 10, Table 4.5) of compound **2** indicated that it possesses three methyl groups at 0.79, 0.85 and 1.27 ppm and one vinylic proton at 6.88 ppm.

The ^{13}C-NMR spectrum (Fig. 11, Table 4.6) showed 20 signals. Four signals of olefinic carbons appeared at δ 143.5 (d), 141.2 (s), 140.3 (d) and 134.9 (s) ppm. The signal at δ 171.8 (s) ppm should be the carbonyl of carboxylic acid. The signal at δ 174.4 (s) ppm should be the carbonyl of lactone. Three were sp^3 fourteen carbon signals at δ 70.2 (t), 46.6 (d), 38.7 (s), 37.5 (s), 36.2 (d), 35.9 (t), 35.7 (t), 27.4 (t), 27.2 (t), 20.5 (q), 19.0 (s), 18.2 (q), 17.3 (t) and 15.9 (q) ppm.

gCOSY-NMR spectrum is shown in Fig. 12, gNOESY-NMR spectrum is shown in Fig. 13, gHMBC-NMR spectrum is shown in Fig. 14 and gHSQC-NMR spectrum is shown in Fig. 15.

The mass spectrum of compound **2** (Fig. 16) did not exhibit a molecular ion or ions corresponding to loss of CO_2H or CO_2, but it did show a prominent signal at 314 $[M-H_2O]^+$. The molecular formula, $C_{20}H_{28}O_4$, of compound **2**, defined a DBE of 7, therefore, compound **2** must consisted of one ring of lactone (DBE = 2) in addition to

one carbonyl group of lactone, one double bond, two ring and one carbonyl group of carboxylic acid. These data indicated that compound **2** could be identical to 3,13-Clerodadien-16,15-olid-18-oic acid.

It could be concluded that Compound **2** exhibited the ^{1}H-NMR and ^{13}C-NMR chemical shifts similar to those of the 3,13-Clerodadien-16,15-olid-18-oic acid or (-)-Patagonic acid, previously isolated from *Baccharis magellanica*. [38- 39] as shown in Table 4.5 and 4.6, respectively.

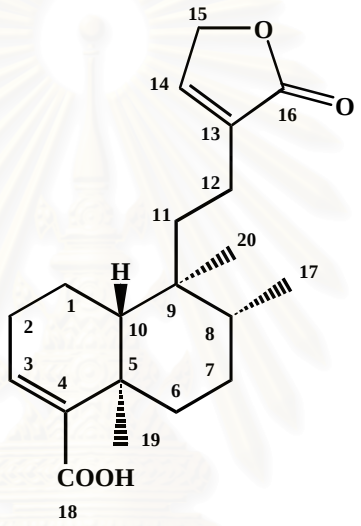


Figure 4.2 Structure of Compound **2.**

Table 4.5 ^{1}H-NMR spectra of Compound **2** and (-)-Patagonic acid

Position	Chemical shifts (ppm)	
	(-)-Patagonic acid [38-39]	Compound **2**
H-1a	1.49 (1H, m)	1.69 (1H, m)
H-1b	-	-
H-2	2.23 (1H, m)	2.28 (1H, m)
H-3	6.87 (1H, t, *J* = 3.7 Hz))	6.88 (1H, s)
H-4	-	-
H-5	-	-
H-6a	1.20 (1H, m)	1.18 (1H, m)
H-6b	2.93 (1H, dd, *J* = 3.0, 10.0 Hz)	2.45 (1H, d, *J* = 13.2 Hz)
H-7a	1.41 (1H, m)	1.46 (1H, m)
H-7b	1.51 (1H, m)	1.48 (1H, m)
H-8	1.59 (1H, m)	1.55 (1H, m)
H-9	-	-
H-10	1.32 (1H, m)	1.38 (1H, d, *J* = 12.4 Hz)
H-11a	-	-
H-11b	1.66 (1H, m)	1.65 (1H, m)
H-12a	2.17 (1H, m)	2.21 (1H, m)
H-12b	2.07 (1H, m)	2.07 (1H, m)
H-13	-	-
H-14	7.12 (1H, t, *J* = 1.4 Hz)	7.12 (1H, s)
H-15	4.78 (2H, d, *J* = 1.4 Hz)	4.80 (2H, s)
H-16	-	-
H-17	0.83 (3H, d, *J* = 6.0 Hz))	0.85 (3H, d, *J* = 6.4 Hz)
H-18	-	-
H-19	1.25 (3H, s)	1.27 (3H, s)
H-20	0.78 (3H, s)	0.79 (3H, s)

Table 4.6 ^{13}C-NMR spectra of Compound **2** and (-)-Patagonic acid.

Position	Chemical shifts (ppm)	
	(-)-Patagonic acid [38-39]	Compound **2**
C-1	17.6t	17.3t
C-2	27.4t	27.4t
C-3	140.2d	140.3d
C-4	141.6s	141.2s
C-5	37.8s	37.5s
C-6	36.0t	35.7t
C-7	27.5t	27.2t
C-8	36.5d	36.2d
C-9	39.0s	38.7s
C-10	46.9d	46.6d
C-11	36.3t	35.9t
C-12	19.2s	19.0s
C-13	135.1s	134.9s
C-14	143.6d	143.5d
C-15	70.2t	70.2t
C-16	174.3s	174.4s
C-17	16.0q	15.9q
C-18	174.4s	171.8s
C-19	20.7q	20.5q
C-20	18.2q	18.2q

4.1.3 Structure elucidation of Compound 3.

Compound **3** was obtained by column chromatography using 40% ethyl acetate in hexane and recrystallization technique. The structure of compound **3** was elucidated by FT-IR, NMR and Mass spectroscopic data as follows.

The IR spectrum of compound **3** (Fig. 17) showed the presence of a three carbonyl groups with corresponding to the strong absorption bands at 1771, 1729 and 1663 cm^{-1} and the absorption bands at 1555 and 1508 cm^{-1} of the C=C stretching vibration of alkene. The IR spectral data of compound **3** are summarized in Table 4.7.

Table 4.7 The IR absorption bands assignment of Compound **3**.

Wave number(cm^{-1})	Intensity	Vibration
3146	medium	=C-H stretching vibration
2950	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1771, 1729, 1663	strong	C=O stretching vibration of carbonyl group
1555, 1508	medium	C=C stretching vibration of alkene
1282	weak	C-O stretching vibration

The ^{1}H-NMR spectrum (Fig. 18, Table 4.8) of compound **3** possessed methyl group at 0.86 ppm, methyl group of ester at 3.64 ppm, three olefinic protons of furanoid groups at 8.06, 7.47 and 6.77 ppm and one vinylic proton at 6.79 ppm.

The ^{13}C-NMR spectrum (Fig. 19, Table 4.9) showed 21 lines, which the carbonyl group of ester corresponded to the signal at 174.0 ppm.

The MS spectrum showed the fragmentation as follows, m/z (EI MS) (Fig. 20): 372 [M^+], 341 (13), 263 (24), 245 (30), 145 (50), 110 (65), 95 (100) and 91 (19).

Compound **3** showed a molecular ion with m/z = 372 ($C_{21}H_{24}O_6$), which indicated a DBE of 10. Compound **3** should be consisted of one carbonyl group of ester, one carbonyl group of lactone, one ring of furan (DBE = 3), one carbonyl group of ketone, in addition to one double bond and three rings.

It could be concluded that Compound **3** exhibited the ^{1}H-NMR and ^{13}C-NMR chemical shifts similar to those of the neo-clerodane-type diterpenoids, methyl-15,16-epoxy-12-oxo-3,13(16),14-neo-clerodatrien-18,19-olide-17-carboxylate or Nasimalun

A, previously isolated from *Barringtonia racemosa* [7] as shown in Table 4.8 and 4.9, respectively.

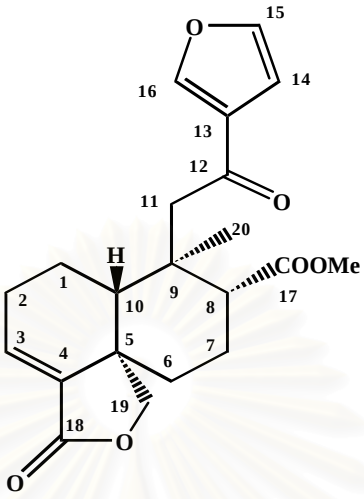


Figure 4.3 Structure of Compound **3**.

Table 4.8 ^{1}H-NMR spectra of Compound **3** and Nasimalun A.

Position	Chemical shifts (ppm)	
	Nasimalun A [7]	Compound **3**
H-1eq	1.64 (1H, dddd, *J* = 11.0, 2.0, 2.0, 2.0 Hz)	1.68 (1H, dd)
H-1ax	1.09 (1H, dddd, *J* = 11.0, 11.0, 11.0, 4.0 Hz)	1.11 (1H, qd, *J* = 12.0, 12.0, 12.0, 4.4 Hz)
H-2eq	2.28 (1H, m)	2.32 (1H, m)
H-2ax	2.22 (1H, m)	2.24 (1H, m)
H-3	6.74 (1H, dd, *J* = 8.0, 2.0 Hz)	6.79 (1H, s)
H-4	-	-
H-5	-	-
H-6eq	1.98 (1H, ddd, *J* = 13.5, 4.5, 3.0 Hz)	2.00 (1H, m)
H-6ax	1.36 (1H, dddd, *J* = 13.5, 13.5, 4.5, 2.0 Hz)	1.39 (1H, td)
H-7eq	1.87 (1H, dddd, *J* = 13.5, 4.5, 4.5, 3.0 Hz)	1.92 (1H, m)
H-7ax	2.03 (1H, dddd, *J* = 13.5, 13.5, 13.5, 4.5 Hz)	2.08 (1H, m)
H-8ax	3.21 (1H, dd, *J* = 13.5, 4.5 Hz)	3.26 (1H, dd, *J* = 13.2, 4.8 Hz)
H-9	-	-
H-10ax	2.73 (1H, d, *J* = 11.0, 2.0 Hz)	2.77 (1H, d, *J* = 12.4 Hz)
H-11a	2.83 (1H, d, *J* = 18.0 Hz)	2.88 (1H, d, *J* = 18.0 Hz)
H-11b	3.04 (1H, d, *J* = 18.0 Hz)	3.07 (1H, d, *J* = 18.0 Hz)
H-12	-	-
H-13	-	-
H-14	6.73 (1H, d, *J* = 2.0 Hz)	6.77 (1H, s)
H-15	7.42 (1H, d, *J* = 2.0 Hz)	7.47 (1H, s)
H-16	8.01 (1H, s)	8.06 (1H, s)
H-17	-	-
H-18	-	-
H-19a	3.93 (1H, dd, *J* = 8.0, 2.0 Hz)	3.97 (1H, d, *J* = 8.8 Hz)
H-19b	4.33 (1H, d, *J* = 8.0 Hz)	4.38 (1H, d, *J* = 8.8 Hz)
H-20	0.82 (3H, s)	0.86 (3H, s)
COOMe	3.60 (3H, s)	3.64 (3H, s)

Table 4.9 ^{13}C-NMR spectra of Compound **3** and Nasimalun A.

Position	Chemical shifts (ppm)	
	Nasimalun A [7]	Compound **3**
C-1	20.1t	20.0t
C-2	27.3t	27.3t
C-3	136.2d	136.3d
C-4	137.8s	137.7s
C-5	45.1s	45.1s
C-6	33.2t	33.1t
C-7	22.1t	22.0t
C-8	48.7d	48.6d
C-9	39.6s	39.5s
C-10	46.7d	46.6d
C-11	46.5t	46.4t
C-12	193.6s	193.7s
C-13	128.6s	128.5s
C-14	108.5d	108.5d
C-15	144.3d	144.3d
C-16	147.1d	147.1d
C-17	174.0s	174.0s
C-18	169.0s	169.1s
C-19	71.4t	71.4t
C-20	19.2q	19.2q
COOMe	51.4q	51.4q

4.2 Modification of isolated compounds from *C. oblongifolius*.

4.2.1 Modification of Compound 1.

4.2.1.1 Purification and properties of Compound 1a.

Methylation of Compound 1.

Compound **1** was methylated with diazomethane in dichloromethane to give compound **1a** as a transparent oil. The IR spectrum of compound **1a** was shown in Fig 21. Its IR absorption bands at 2950 and 2865 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1710 cm^{-1} of the C=O stretching vibration of methyl ester carbonyl group and the absorption band at 1432 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.10.

Table 4.10 The IR absorption bands assignment of Compound **1a**.

Wave number (cm^{-1})	Intensity	Vibration
2950, 2865	strong	C-H stretching vibration of $-CH_3$,$-CH_2-$
1710	strong	C=O stretching vibration of methyl ester
1432	medium	C=C stretching vibration of alkene

The ^{1}H-NMR spectrum (Fig. 22) of compound **1a** indicated that it possesses three methyl groups at 0.78, 0.85 and 1.30 ppm, three olefinic protons of furanoid group at 7.37, 7.23 and 6.28 ppm, one methyl ester group at 3.71 ppm and one vinylic proton at 6.63 ppm.

The ^{13}C-NMR spectrum (Fig. 23, Table 4.11) showed 21 signals. Six signals of olefinic carbons appeared at δ 142.7 (d), 142.6 (s), 138.4 (d), 136.9 (d), 125.6 (s) and 111.0 (d) ppm. The signal at δ 167.9 (s) ppm should be the carbonyl of ester. One signal of methoxy carbon appeared at δ 51.2 (q) ppm. There were sp^3 thirteen carbon signals at δ 46.5 (d), 38.8 (s), 38.6 (t), 37.6 (s), 36.2 (d), 35.9 (t), 27.3 (t), 27.2 (t), 20.7 (q), 18.3 (q), 18.1 (t), 17.5 (t) and 16.0 (q) ppm.

Its molecular formula was established as $C_{21}H_{30}O_3$, which was confirmed by observing molecular ion at *m/z* 330 (Fig. 24). The molecular formula, $C_{21}H_{30}O_3$, of compound **1a** defined a DBE of 7, therefore, compound **1a** must consisted of one ring of furan (DBE = 3) in addition to one double bond, two rings and one carbonyl group

of ester. These data indicated that compound **1a** could be identical to (-)-hardwickiic acid methyl ester.

It could be concluded that Compound **1a** exhibited the ^{13}C-NMR chemical shifts identical to (-)-hardwickiic acid methyl ester [40]. The ^{13}C-NMR chemical shifts of Compound **1a** and (-)-hardwickiic acid methyl ester could be compared as in Table 4.11.

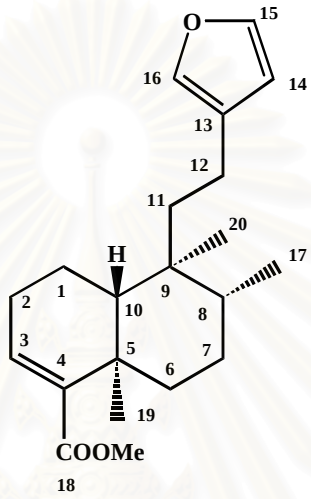


Figure 4.4 Structure of Compound **1a.**

Table 4.11 ^{13}C-NMR spectra of Compound **1a** and (-)-Hardwickiic acid methyl ester

Position	Chemical shifts (ppm)	
	(-)-Hardwickiic acid methyl ester [40]	Compound **1a**
C-1	18.3t	17.5t
C-2	27.5t	27.2t
C-3	136.7d	136.9d
C-4	142.7s	142.6s
C-5	37.7s	37.6s
C-6	36.1t	35.9t
C-7	27.1t	27.3t
C-8	36.5d	36.2d
C-9	39.0s	38.8s
C-10	46.8d	46.5d
C-11	38.9t	38.6t
C-12	17.1t	18.1t
C-13	125.7s	125.6s
C-14	111.0d	111.0d
C-15	138.5d	138.4d
C-16	142.7d	142.7d
C-17	16.0q	16.0q
C-18	167.8s	167.9s
C-19	20.5q	20.7q
C-20	18.3q	18.3q
OMe	51.1q	51.2q

4.2.2 Modification of Compound 2.

4.2.2.1 Purification and properties of Compound 2a.

Methylation of Compound 2.

Compound **2** was methylated with diazomethane in dichloromethane to give compound **2a** as a transparent oil. The IR spectrum of compound **2a** was shown in Fig 25. Its IR absorption bands at 2921 and 2855 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption bands at 1754 and 1711 cm^{-1} of the C=O stretching vibration of carbonyl group and the absorption band at 1458 cm^{-1} of the C=C stretching vibration of alkene. The absorption bands were assigned as shown in Table 4.12.

Table 4.12 The IR absorption bands assignment of Compound **2a**.

Wave number (cm^{-1})	Intensity	Vibration
2921, 2855	strong	C-H stretching vibration of $-CH_3$,$-CH_2-$
1754, 1711	strong	C=O stretching vibration of carbonyl group
1458	medium	C=C stretching vibration of alkene

The ^{1}H-NMR spectrum (Fig. 26) of compound **2a** indicated that it possesses three methyl groups at 0.80, 0.85 and 1.28 ppm, one methyl ester group at 3.71 and one vinylic proton at 6.63 ppm.

The ^{13}C-NMR spectrum (Fig. 27, Table 4.13) showed 21 signals. Four signals of olefinic carbons appeared at δ 143.9 (d), 141.2 (s), 136.1 (d), 134.2 (s) ppm. The signal at δ 174.6 (s) ppm should be the carbonyl of lactone. The signal at δ 167.2 (s) ppm should be the carbonyl of ester. One signal of methoxy carbon appeared at δ 51.2 (q) ppm. There were sp^3 fourteen carbon signals at δ 70.3 (t), 46.5 (d), 38.7 (s), 37.6 (s), 36.3 (d), 36.3 (t), 35.9 (t), 27.1 (t), 27.1 (t), 20.7 (q), 19.0 (t), 18.1 (q), 17.6 (t) and 15.7 (q) ppm.

The mass spectrum of compound **2a** (Fig. 28) did not exhibit a molecular ion or ions corresponding to loss of CO_2H or CO_2, but it did show a prominent signal at 314 $[M\text{-}MeOH]^+$. The molecular formula, $C_{21}H_{30}O_4$, of compound **2**, defined a DBE of 7, therefore, compound **2** must consisted of one ring of lactone (DBE = 2) in

addition to one carbonyl group of lactone, one double bond, two rings and one carbonyl group of ester. These data indicated that compound **2** could be identical to (-)-patogonic acid methyl ester.

It could be concluded that Compound **2a** exhibited the ^{13}C-NMR chemical shifts identical to (-)-patagonic acid methyl ester or 16-Oxo-15,16-hardwickiic acid [40-41]. The ^{13}C-NMR chemical shifts of Compound **2a** and (-)-patagonic acid methyl ester could be compared as in Table 4.13.

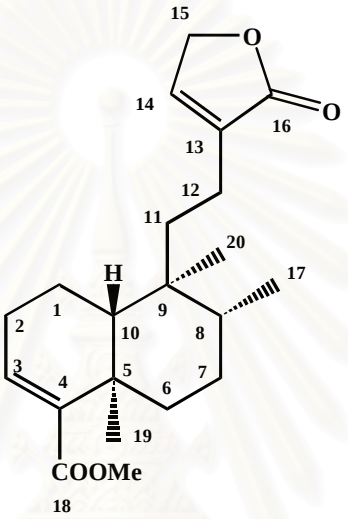


Figure 4.5 Structure of Compound **2a.**

Table 4.13 ^{13}C-NMR spectra of Compound **2a** and (-)-Patagonic acid methyl ester

Position	Chemical shifts (ppm)	
	(-)-Patagonic acid methyl ester [40-41]	Compound **2a**
C-1	17.5t	17.6t
C-2	27.2t	27.1t
C-3	136.9d	136.1d
C-4	142.3s	141.2s
C-5	37.6s	37.6s
C-6	36.3t	36.3t
C-7	27.1t	27.1t
C-8	36.3d	36.3d
C-9	38.7s	38.7s
C-10	46.6d	46.5d
C-11	36.0t	35.9t
C-12	19.0t	19.0t
C-13	135.0s	134.2s
C-14	143.4d	143.9d
C-15	70.1t	70.3t
C-16	174.2s	174.6s
C-17	15.4q	15.7q
C-18	167.7s	167.2s
C-19	20.7q	20.7q
C-20	18.1q	18.1q
OMe	51.1q	51.2q

4.2.3 Chemical Transformation of Compound 3.

4.2.3.1 Purification and properties of Compound 3a.

Alcohol addition of Compound 3.

The hydroxyl derivative of Nasimalun A (**3**) (100.3 mg, 0.269 mmole) was hydrolyzed with NaOH-methanol to obtained methoxy derivative (**3a)** (58.4 mg, 0.144, 53.53% yield) by alcohol addition to an α,β-unsaturated lactone moiety [42].

The IR spectrum of compound **3a** was shown in Fig 29. Its IR absorption bands at 2945 and 2855 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption bands at 1777, 1723 and 1668 cm^{-1} of the C=O stretching vibration of three carbonyl groups and the absorption band at 1458 cm^{-1} of the C=C stretching vibration of olefin. The absorption bands were assigned as shown in Table 4.14.

Table 4.14 The IR absorption bands assignment of Compound **3a**.

Wave number(cm^{-1})	Intensity	Vibration
3431	medium	=C-H stretching vibration
2945, 2855	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1777, 1723, 1668	strong	C=O stretching vibration of carbonyl group
1458	medium	C=C stretching vibration of olefin

The ^{1}H-NMR spectrum (Fig. 30, Table 4.15) of Compound **3a** possessed methyl group at 0.83 ppm, methyl group of ester at 3.60 ppm, methyl group of methoxy at 3.36 ppm, and three olefinic protons of furanoid group at 8.03, 7.46 and 6.76 ppm.

The ^{13}C-NMR spectrum (Fig. 31, Table 4.15) showed 22 signals, which the carbonyl group of ester corresponded to the signal at 174.1 ppm.

The information of 2D-NMR including gCOSY correlations (Fig. 32, Table 4.15), gNOESY correlations (Fig. 33), gHMBC correlations (Fig. 34, Table 4.15) and gHSQC correlations (Fig. 35) supported the structural elucidation of compound **3a**.

The configuration of the methoxy group follows from the gNOESY-NMR spectrum (Fig. 4.7 and Fig 33) which shows the H-4 with H-3 resonance (2.10, d, J = 9.2 Hz) expected for diaxial coupling [43].

The MS spectrum showed the fragmentation as follows, m/z (EI MS) (Fig. 36): 404 [M^+], 373 (6), 295 (27), 249 (20), 157 (25), 110 (57), 95 (100) and 91 (21).

Compound **3a** showed a molecular ion with m/z = 404 ($C_{22}H_{28}O_7$), which indicated a DBE of 9. Compound **3a** should be consisted of one carbonyl group of ester, one carbonyl group of lactone, one ring of furan (DBE = 3), one carbonyl group of ketone, in addition to three rings.

From all this spectroscopic data, the new compound (**3a**), named Methyl-15,16-epoxy-3-methoxy-12-oxo-13(16),14-clerodadien-18,19-olide-17-carboxylate. The structure of Compound **3a,** is shown in Figure 4.6.

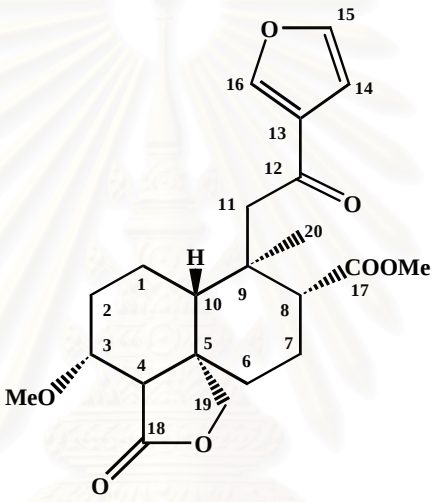


Figure 4.6 Structure of Compound **3a.**

Table 4.15 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Compound **3a**.

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.	gCOSY correlations	gHMBC correlations
1	19.4t	1a 1.32 (1H, m)	H-10, H-2b, H-2a, H-1b	C-10, C-4, C-1,-OMe,
		1b 1.73 (1H, m)	H-10, H-2b, H-2a, H-1a	C-5, -OMe,
2	29.3t	2a 1.34 (1H, m)	H-3, H-2b, H-1b, H-1a	C-10, C-1, -OMe
		2b 2.17 (1H, m)	H-3, H-2a, H-1b, H-1a	C-10, C-4, -OMe
3	56.8d	3.35 (1H, m)	H-4, H-2b, H-2a	-OMe
4	55.0d	2.10 (1H, d, *J* = 9.2 Hz)	H-3	C-19, C-18, C-6, C-5, C-2, -OMe
5	43.7s	-	-	-
6	35.5t	6a 1.36 (1H, m)	H-19a, H-19b, H-7a, H-7b, H-6a	C-19, C-10, C-7, C-5, C-4
		6b 1.95 (1H, m)	H-19a, H-19b, H-7a, H-7b, H-6a	C-8
7	21.4t	7a 1.81 (1H, m)	H-8, H-7b, H-6a, H-6b	C-8, C-5
		7b 1.96 (1H, m)	H-6a, H-6b, H-7a	C-8, C-6
8	48.4d	3.25 (1H, dd, *J* = 12.8, 3.6 Hz)	H-7a, H-7b	C-20, C-9, C-7
9	39.5s	-	-	-
10	43.4d	2.57 (1H, dd, *J* = 11.6, 3.2 Hz)	H-1a, H-1b	C-19, C-9, C-5, C-1
11	45.9t	11a 2.87 (1H, d, *J* = 18.0 Hz)	H-20	C-20, C-12, C-10, C-9, C-8
		11b 2.94 (1H, d, *J* = 18.0 Hz)	H-20	C-20, C-12, C-10, C-9, C-8
12	193.5s	-	-	-
13	128.4s	-	-	-
14	108.5d	6.76 (1H, d, *J* = 1.6 Hz)	H-15	C-16, C-15, C-13
15	144.3d	7.46 (1H, d, *J* = 1.6 Hz)	H-14	C-16, C-14, C-13
16	146.9d	8.03 (1H, s)	H-15, H-14	C-15, C-13, C-14
17	174.1s	-	-	-
18	176.3s	-	-	-
19	70.2t	19a 4.21 (1H, d, *J* = 9.6 Hz)	H-19b, H-6a	C-18, C-10, C-6, C-5, C-4
		19b 4.25 (1H, d, *J* = 9.6 Hz)	H-19a. H-6a	C-18, C-10, C-6, C-5, C-4
20	18.8q	0.83 (3H, s)	H-11a, H-11b	C-12, C-11, C-10, C-9, C-8
OMe	77.0q	3.36 (3H, s)	H-4, H-3, H-2a, H-2b	-OMe
COOMe	51.4q	3.60 (3H, s)	H-8	-

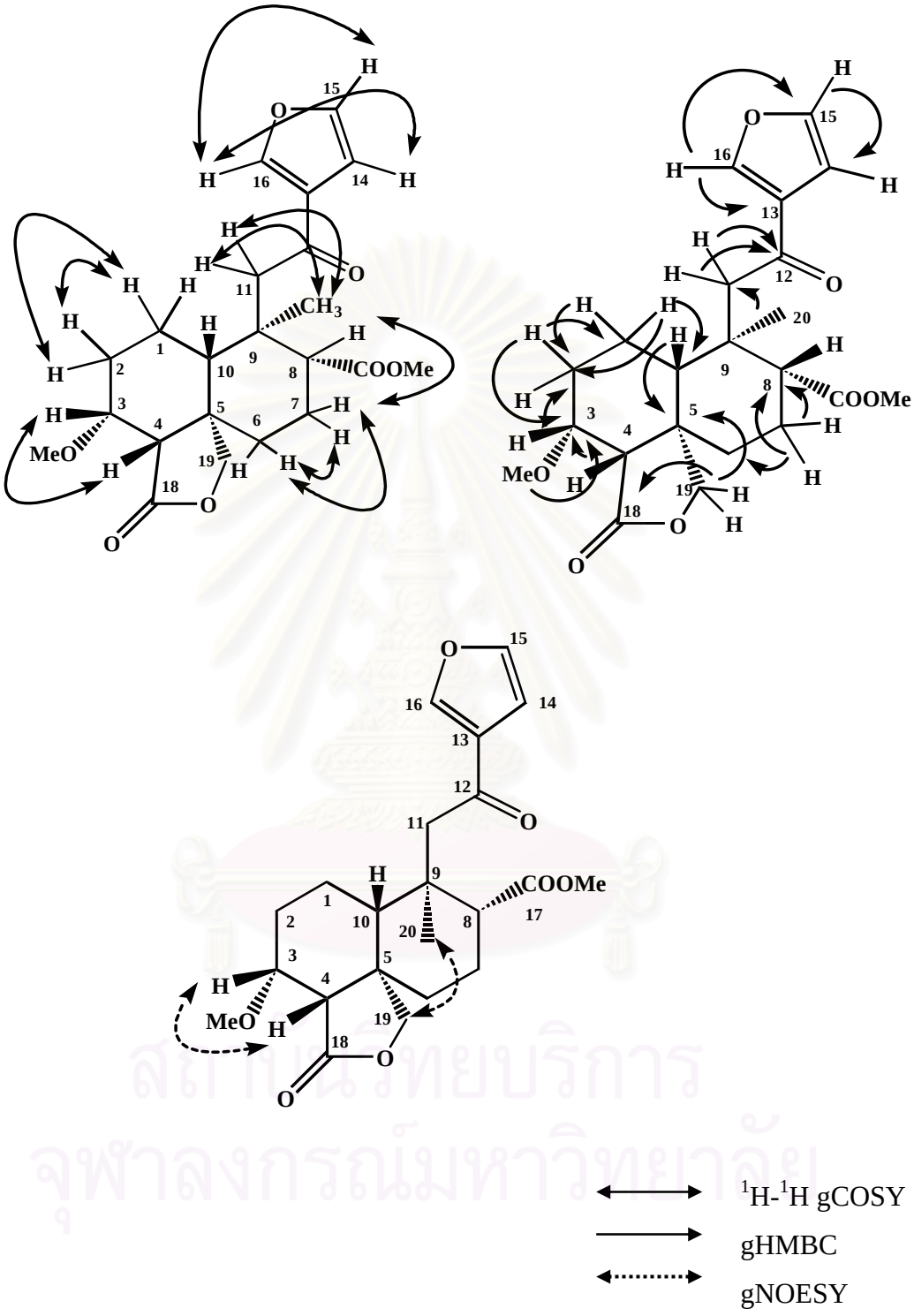


Figure 4.7 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Compound **3a**.

4.2.3.2 Purification and properties of Compound 3b.

Reduction of Compound 3.

Compound **3** (325.3 mg, 0.874 mmol) was reduced with lithium aluminium hydride in THF to give a mixture of products. The mixture was purified by silica gel column chromatography to obtain major product as compound **3b** (72.1 mg, 0.207 mmol, 23.68 %). The other compounds were not separated and purified.

Compound **3b** was reduced with $LiAlH_4$ at ketone and methyl ester group, and hydrid addition to the conjugated double bond.

The IR spectrum of compound **3b** is shown in Fig. 37. Its IR absorption bands at 3800-3100 cm^{-1} of the O-H stretching vibration of alcohol, the absorption bands at 2932 and 2856 cm^{-1} of the C-H stretching vibration of methyl and methylene group and the absorption band at 1757 cm^{-1} of the C=O stretching vibration of carbonyl group. The absorption bands were assigned as shown in Table 4.16.

Table 4.16 The IR absorption bands assignment of Compound **3b**.

Wave number (cm^{-1})	Intensity	Vibration
3800-3100	broad	O-H stretching vibration of alcohol
2932, 2856	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1757	strong	C=O stretching vibration of lactone

The ^{1}H-NMR spectrum (Fig. 38, Table 4.17) of Compound **3b** showed a possessed methyl group at 0.64 ppm, primary alcohols at 3.74 and 3.32 ppm, secondary alcohol at 4.93 ppm and three olefinic protons of furanoid group at 7.42, 7.41 and 6.45 ppm.

The ^{13}C-NMR spectrum (Fig. 39, Table 4.17) showed 20 signals, which the carbonyl group of lactone corresponded to the signal at 179.3 ppm, methylene carbon of primary alcohol at 63.6 ppm and methine carbon of secondary alcohol at 63.3 ppm

The information of 2D-NMR including gCOSY correlations (Fig. 40, Table 4.17), gNOESY correlations (Fig. 41), gHMBC-NMR correlations (Fig. 42, Table 4.17) and gHSQC correlations (Fig. 43) supported the structural elucidation of compound **3b**.

The MS spectrum showed the fragmentation as follows, m/z (EI MS) (Fig. 44): 348 [M^+], 330 (25), 315 (10), 253 (17), 222 (15), 179 (27), 111 (35), 97 (100) and 95 (40). Compound **3b** showed a molecular ion with m/z = 348 ($C_{20}H_{28}O_5$), which indicated a DBE of 7. Compound **3b** should be consisted of one ring of furan (DBE=3), one carbonyl group of lactone, in addition to three rings.

From all this spectroscopic data, the new compound (**3b**), named Methyl-12,17-dihydroxy-15,16-epoxy-13(16),14-clerodadien-18,19-olide. The structure of Compound **3b** is shown in Figure 4.8.

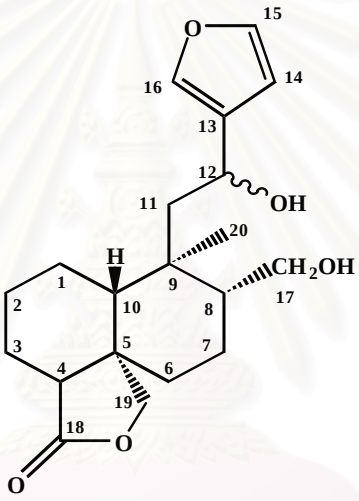


Figure 4.8 Structure of Compound **3b.**

Table 4.17 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Compound **3b**.

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.	gCOSY correlations	gHMBC correlations
1	21.3t	1a 1.18 (1H, m)	H-10, H-2a, H-2b, H-1b	C-10, C-3, C-2
		1b 2.01 (1H, m)	H-10, H-2a, H-2b, H-1a	C-5, C-4
2	25.7t	2a 1.44 (1H, m)	H-4, H-3a, H-3b, H-2b H-1a, H-1b	C-3
		2b 2.02 (1H, m)	H-4, H-3a, H-3b, H-2a H-1a, H-1b	C-5, C-4, C-1
3	25.0t	3a 1.36 (1H, m)	H-4, H-3a, H-2a, H-2b	C-2
		3b 1.93 (1H, m)	H-4, H-3b, H-2a, H-2b	C-19, C-5, C-4, C-1
4	49.9d	2.08 (1H, m)	H-3a, H-3b	C-19, C-18, C-6, C-2
5	43.1s	-	-	
6	36.2t	6a 1.15 (1H, m)	H-7a, H-7b,H-6b	C-19
		6b 1.84 (1H, m)	H-7a, H-7b,H-6a	C-10, C-8, C-7
7	22.0t	7a 1.41 (1H, m)	H-8, H-7b, H-6a, H-6b	C-6
		7b 1.87 (1H, m)	H-8, H-7a, H-6a, H-6b	C-20, C-10, C-9, C-8, C-5
8	45.1d	1.76 (1H, m)	H-17a, H-17b, H-7a, H-7b	C-20, C-10, C-9
9	38.8s	-	-	-
10	46.5d	1.90 (1H, m)	H-11b, H-1a, H-1b	C-20, C-19, C-9, C-8, C-5, C-4, C-1
11	44.0t	11a 1.72 (1H, d, *J* = 3.2 Hz)	H-12, H-11b	C-20, C-17, C-13, C-12, C-10, C-9, C-8
		11b 2.04 (1H, m)	H-12, H-11a	C-20, C-17, C-13, C-12, C-10, C-9, C-8
12	63.3d	4.93 (1H, dd, *J* = 8.4, 3.2 Hz)	H-11a, H-11b	C-15, C-13, C-14, C-11, C-9
13	130.7s	-	-	-
14	108.3d	6.45 (1H, d, *J* = 1.2 Hz)	H-16, H-15	C-16, C-15, C-13
15	138.6d	7.41 (1H, s)	H-16, H-14	C-16, C-14, C-13
16	143.6d	7.42 (1H, s)	H-15, H-14	C-15, C-14, C-13
17	63.6t	17a 3.32 (1H, dd, *J* = 10.8, 8.4 Hz)	H-17b, H-8	C-9, C-8, C-7
		17b 3.74 (1H, dd, *J* = 10.8, 4.0 Hz)	H-17a, H-8	C-9, C-8, C-7
18	179.3s	-	-	-
19	70.5t	19a 4.24 (1H, d, *J* = 9.2 Hz)	H-19b	C-18, C-6, C-5, C-4
		19b 4.36 (1H, d, *J* = 9.2 Hz)	H-19a	C-10, C-6, C-5
20	18.1q	0.64 (3H, s)	-	C-17, C-12, C-11, C-10, C-9, C-8

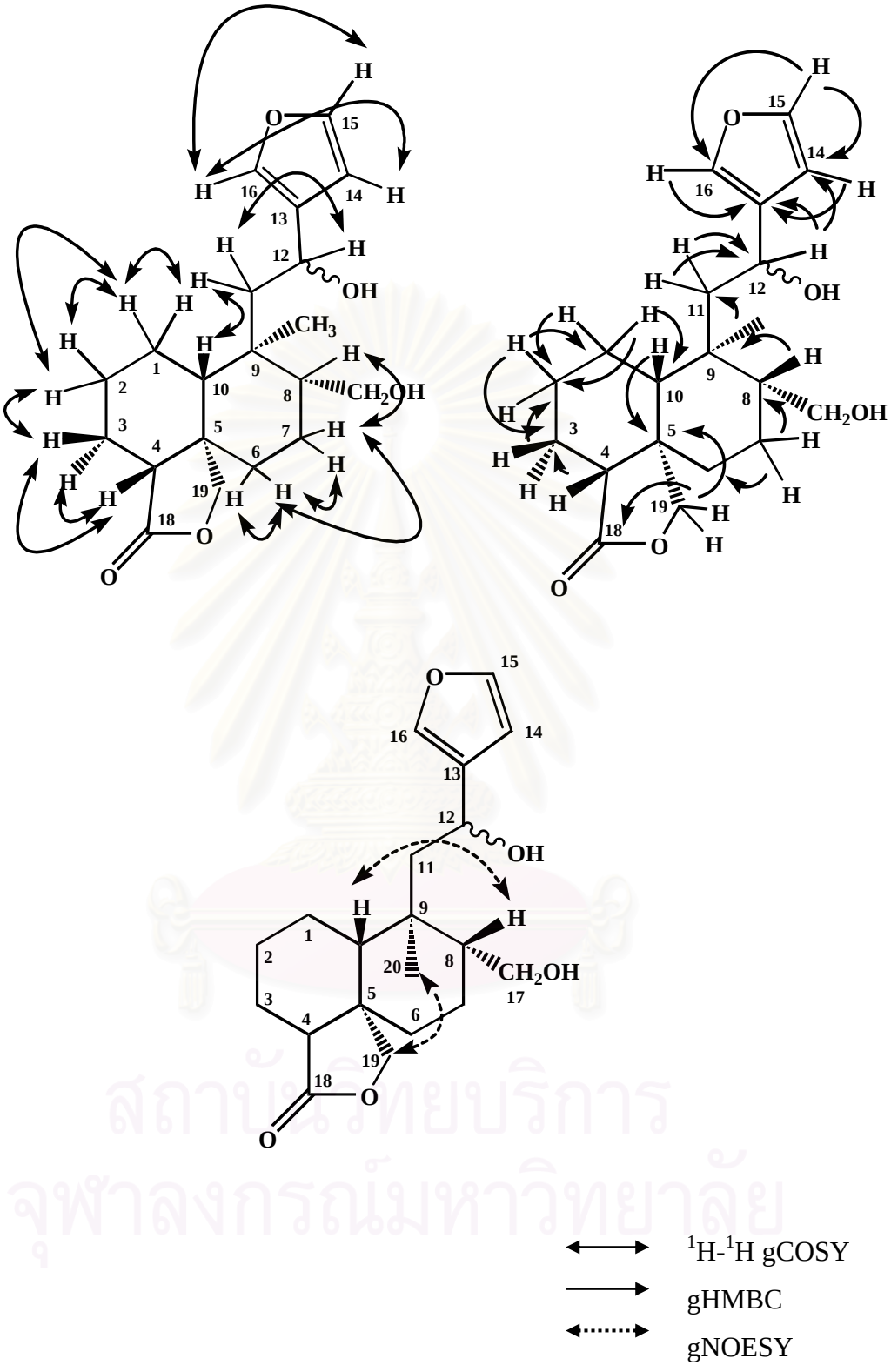


Figure 4.9 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Compound **3b**.

4.2.3.3 Purification and properties of Compound 3c.

Reduction of Compound 3.

Compound **3** (300.6 mg, 0.808 mmol) was reduced with sodium borohydride in MeOH to give a mixture of products. The mixture was purified by silica gel column chromatography to obtain major product as compound **3c** (145.6 mg, 0.421 mmol, 52.10% yield). The other compounds were not separated and purified.

Compound **3c** was reduced with $NaBH_4$ at ketone and methyl ester group.

The IR spectrum of compound **3c** is shown in Fig. 45. Its IR absorption bands at 3800-3100 cm^{-1} of the O-H stretching vibration of alcohol, the absorption bands at 2925 and 2855 cm^{-1} of the C-H stretching vibration of methyl and methylene group, the absorption band at 1771 cm^{-1} of the C=O stretching vibration of carbonyl group and the absorption bands at 1658 and 1423 cm^{-1} of the C=C stretching vibration of alkene The absorption bands were assigned as shown in Table 4.18.

Table 4.18 The IR absorption bands assignment of Compound **3c**.

Wave number (cm^{-1})	Intensity	Vibration
3800-3100	broad	O-H stretching vibration of alcohol
2925, 2855	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1771	strong	C=O stretching vibration of lactone
1658, 1423	medium	C=C stretching vibration of alkene

The ^{1}H-NMR spectrum (Fig. 46, Table 4.19) of Compound **3c** showed a possessed methyl group at 0.72 ppm, primary alcohols at 3.85 and 3.50 ppm, secondary alcohol at 4.77 ppm and three olefinic protons of furanoid group at 7.43, 7.40 and 6.43 ppm.

The ^{13}C-NMR spectrum (Fig. 47, Table 4.19) showed 20 signals, which the carbonyl group of lactone corresponded to the signal at 169.3 ppm, methylene carbon of primary alcohol at 65.0 ppm and methine carbon of secondary alcohol at 63.2 ppm.

The information of 2D-NMR including gCOSY correlations (Fig. 48, Table 4.19), gNOESY correlations (Fig. 49), gHMBC correlations (Fig. 50, Table 4.19) and gHSQC correlations (Fig. 51) supported the structural elucidation of compound **3c**.

The MS spectrum showed the fragmentation as follows, m/z (ESI MS) (Fig. 52): 347 $[M+H]^+$. Compound **3c** showed a molecular ion with m/z = 347 $[M+H]^+$ ($C_{20}H_{27}O_5$), which indicated a DBE of 8. Compound **3c** should be consisted of one ring of furan (DBE=3), one carbonyl group of lactone, in addition to one double bond and three rings.

From all this spectroscopic data, the new compound (**3c**), named Methyl-12,17-dihydroxy-15,16-epoxy-3,13(16),14-clerodatrien-18,19-olide. The structure of Compound **3c** is shown in Figure 4.10.

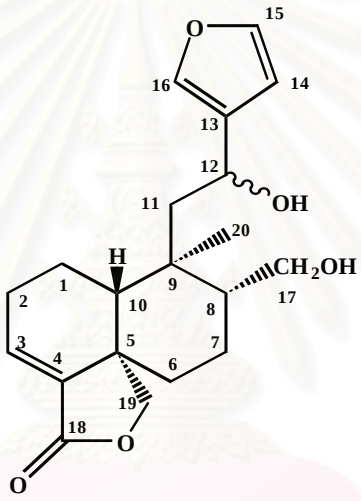


Figure 4.10 Structure of Compound **3c.**

Table 4.19 The ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC spectral data of Compound **3c**.

Position	^{13}C-NMR δ, ppm.	^{1}H-NMR δ, ppm.	gCOSY correlations	gHMBC correlations
1	19.4t	1eq 1.64 (1H, m)	H-10, H-2a, H-1ax	C-10, C-3, C-2
		1ax 1.08 (1H, qd, *J* = 12.0, 12.0, 12.0, 4.0 Hz)	H-10, H-2a, H-2b, H-1eq	C-2
2	27.4t	2a 2.20 (1H, m)	H-3, H-2b, H-1ax,H-1eq	C-10, C-9
		2b 2.39 (1H, m)	H-3, H-2a, H-1ax, H-1eq	C-9, C-4, C-3
3	135.8d	6.80 (1H, d, *J* = 7.2 Hz)	H-2a, H-2b	C-18, C-5, C-2, C-1
4	138.4s	-	-	-
5	45.7s	-	-	-
6	34.2t	6a 1.37 (1H, m)	H-19a, H-7a, H-7b,H-6b	C-7
		6b 2.02 (1H, m)	H-17b, H-7a, H-7b, H-6a	C-19, C-10, C-9, C-8, C-7, C-2, C-1
7	23.4t	7a 1.46 (1H, m)	H-8, H-7b, H-6a, H-6b	C-6
		7b 1.77 (1H, m)	H-8, H-7a, H-6a, H-6b	C-20, C-9, C-8, C-5
8	48.4d	2.07 (1H, d, *J* = 13.6 Hz)	H-17b, H-7a, H-7b	C-20, C-10, C-9, C-6, C-1
9	39.0s	-	-	-
10	45.8d	2.34 (1H, d, *J* = 10.0 Hz)	H-12, H-11a, H-1ax, H-8, H-2a	C-20, C-12, C-9, C-8, C-5
11	45.3t	11a 1.84 (1H, d, *J* = 16.8 Hz)	H-12, H-11b, H-10	C-20, C-13, C-12, C-11b, C-10, C-8,
		11b 2.42 (1H, m)	H-11a, H-10	C-20, C-12, C-9, C-8
12	63.2d	4.77 (1H, d, *J* = 9.2 Hz)	H-11a	C-15, C-13, C-14, C-11, C-9
13	130.7s	-	-	-
14	108.2d	6.43 (1H, s)	H-16, H-15	C-16, C-15, C-13
15	138.2d	7.40 (1H, s)	H-16, H-14	C-16, C-14, C-13
16	143.7d	7.43 (1H, s)	H-15, H-14	C-15, C-14, C-13
17	65.0t	17a 3.50 (1H, dd, *J* = 10.4, 4.0 Hz)	H-17b	C-11, C-9, C-7
		17b 3.85 (1H, dd, *J* = 10.4, 8.0 Hz)	H-17a	C11, C-9
18	169.3s	-	-	-
19	71.7t	19a 3.95 (1H, d, *J* = 8.0 Hz)	H-19b, H-6a	C-6
		19b 4.31 (1H, d, *J* = 8.4 Hz)	H-19a	C-18, C-6, C-5, C-4
20	18.2q	0.72 (3H, s)	-	C-19, C-12, C-11, C-10, C-9

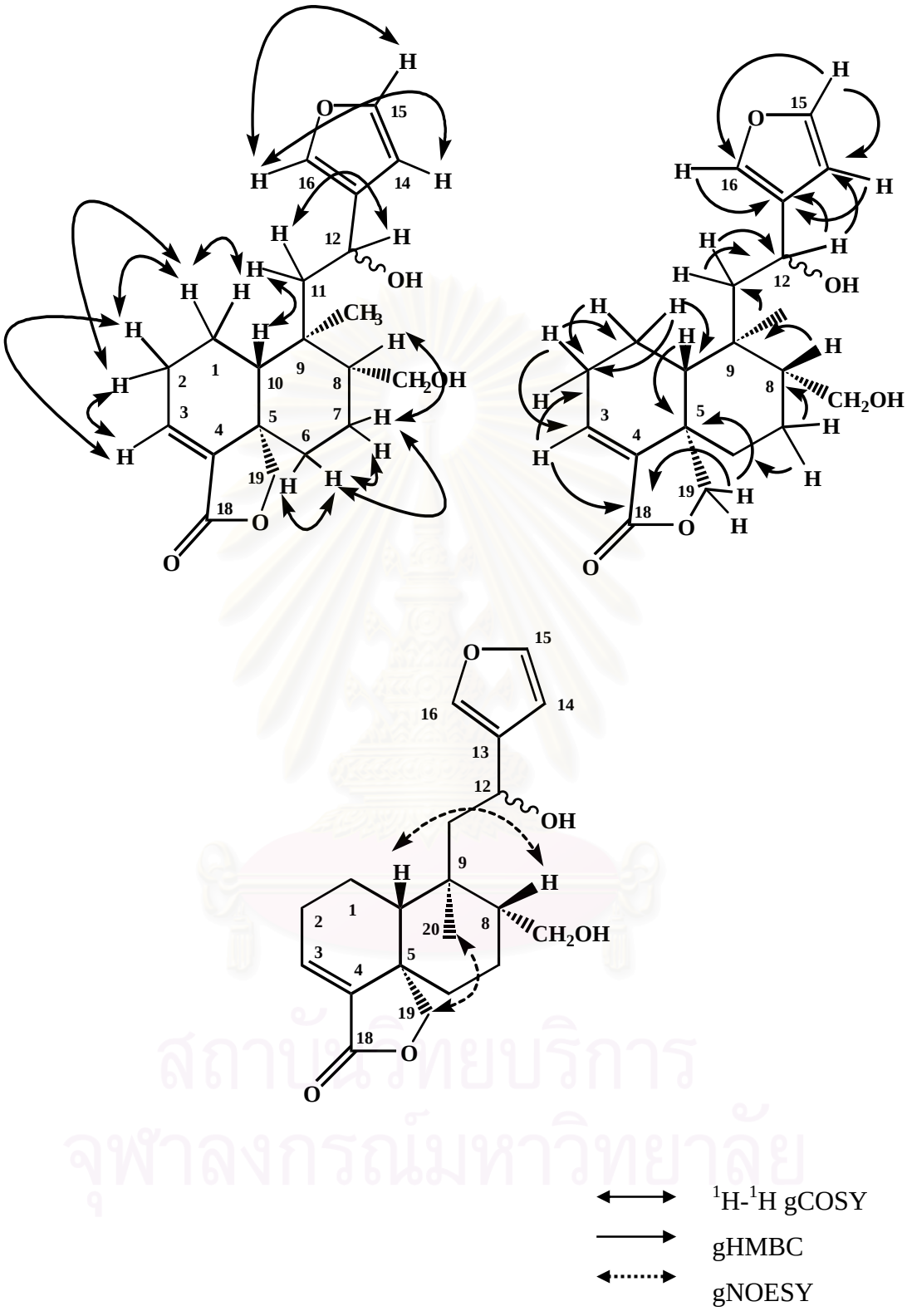


Figure 4.11 Selected ^{1}H-^{1}H gCOSY, gHMBC and gNOESY correlations for Compound **3c**.

4.3 Results of cytotoxicity test.

The *in vitro* activity of some compounds IC_{50} from *C. oblongifolius* against the 6 cell lines, for example, BT 474 (breast), Chago (lung), Hep-G2 (hepatoma), Kato (gastric), SW 620 (colon) and HuCCA-1 (human bile duct epithelial carcinoma) cancer are reported in Table 4.20.

Table 4.20 Cytotoxic activity against 6 cell lines of natural compounds and synthesized compounds.

Compound	IC_{50} (µg/ml) for cell lines					IC_{50} (µM)
	BT 474	Chago	Hep-G2	Kato	SW 620	HuCCA-1
1	>10	7.4	>10	7.1	7.6	NT
2	>10	>10	>10	>10	>10	NT
3	>10	>10	>10	>10	>10	NT
1a	>10	7.4	>10	6.6	6.4	NT
2a	NT	NT	NT	NT	NT	>10
3a	NT	NT	NT	NT	NT	>10
3b	NT	NT	NT	NT	NT	>10
3c	NT	NT	NT	NT	NT	>10

NT is expressed not test

As summarized in Table 4.20, all compounds were evaluated against a panel of human tumor cell lines. Compound **1** and **1a** showed weak cytotoxicity against Chago (lung), Kato (gastric) and SW 620 (colon) cancer cell lines. Compound **2** and **3** were inactive against all cell lines. This was the first report of the cytotoxicity test of (-)-hardwickiic acid methyl ester (**1a**), (-)-patagonic acid (**2**), (-)-patagonic acid methyl ester (**2a**), nasimalun A (**3**) and nasimalun A derivatives (**3a**, **3b** and **3c**) against BT 474, Chago, Hep-G2, Kato, SW 620 and HuCCA-1 cancer cell lines.

4.4 Literature reviews in biological activity of (-)-hardwickiic acid.

Furthermore, (-)-hardwickiic acid (**1**) which was the main product of the hexane crude extract has been widely studied for its biological activity such as antimicrobial activity, insecticidal activity and anti-tumor activity. The bioactive properties of (-)-hardwickiic acid are shown below.

In 1987, Bandara and coworkers isolated (-)-hardwickiic acid from the roots of *C. aromaticus* and found that. (-)-Hardwickiic acid showed insecticidal activity against *Aphis craccivora* [44].

In 1991, McChesney an Clark reported that (-)-hardwickiic acid which was isolated from *C. sonderianus* showed significant qualitative antibacterial activity against the Gram-positive bacteria (*B. subtilis*, *St. auerus*) and *M. smegmatis* [45].

In 1994, Chen and coworkers isolated (-)-hardwickiic acid from the sap of *C. lechleri*. In that studied, they have shown that (-)-hardwickiic acid posses cytotoxicity against human oral epidermoid carcinomar (IC_{50} = 21.90 ± 3.50 μg/ml), using emetine hydrochloride, as a control substance (IC_{50} = 0.2 μg/ml) [46].

CHAPTER V

CONCLUSION

In this research work, the stem bark of *C. oblongifolius* from Amphoe Phanat Nikhom, Chon Buri Province was investigated. The hexane crude extract and ethyl acetate crude extract were separated on silica gel column chromatography and three clerodane diterpenoids, (-)-hardwickiic acid (**1**), patagonic acid (**2**) and nasimalun A (**3**) were obtained as shown in Table 5.1.

The derivative of compound **1**, (-)-hardwickiic acid methyl ester (**1a**) and the derivative of compound **2**, (-)-patagonic acid methyl ester (**2a**) and the derivatives of compound **3**, methyl-15,16-epoxy-3-methoxy-12-oxo-13(16),14-clerodadien-18,19-olide-17-carboxylate (**3a**), methyl-12,17-dihydroxy-15,16-epoxy-13(16),14-Cleroda dien-18,19-olide (**3b**) and methyl-12,17-dihydroxy-15,16-epoxy-3,13(16),14-cleroda trien-18,19-olide (**3c**) were synthesized. The derivatives are presented in Table 5.2.

Table 5.1 Isolated substances from the stem bark of *C. oblongifolius***.**

Compounds	Name of compound	Weight* (g)
1	(-)-Hardwickiic acid	6.0
2	(-)-Patagonic acid	0.012
3	Nasimalun A	12.5

* From dried stem bark 3,100 g.

Table 5.2 The modification of isolated compounds from *C. oblongifolius*.

Compounds	Name of compound	Weight (mg)	% yield from starting material
1a	(-)-Hardwickiic acid methyl ester	28.1	88.88
2a	(-)-Patagonic acid methyl ester	9.8	93.33
3a	Methyl-15,16-epoxy-3-methoxy-12-oxo-13(16),14-clerodadien-18,19-olide-17-carboxylate	58.4	53.53
3b	Methyl-12,17-dihydroxy-15,16-epoxy-13(16),14-clerodadien-18, 19-olide	72.1	23.68
3c	methyl-12,17-dihydroxy-15,16-epoxy-3,13(16),14-clerodatrien-18,19-olide (**3b**)	145.6	52.10

The isolated compounds and their synthesized derivatives showed cytotoxic activity against 6 cell lines as shown in Table 4.20. From the results, Compound **1** and **1a** exhibited weak cytotoxicity against Chago, Kato and SW 620 human tumor cell lines. Moreover, this research represents the first report of cytotoxicity test of (-)-hardwickiic acid methyl ester (**1a**), (-)-patagonic acid (**2**), (-)-patagonic acid methyl ester (**2a**), nasimalun A (**3**) and nasimalun A derivatives (**3a**, **3b** and **3c**).

Suggestion for the future work

1. The investigation of chemical constituents of *C. oblongifolius* should be continued in order to find new sources of diterpenoids and better understanding of the biodiversity of this species.
2. The chemistry of Nasimalun A shoud be explored in order to find the possible application of this compound and its derivatives.
3. Nasimalun A shoud be widely studied for its biological activity such as anti-feedant activity, antimicrobial activity and others.

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APPENDICES

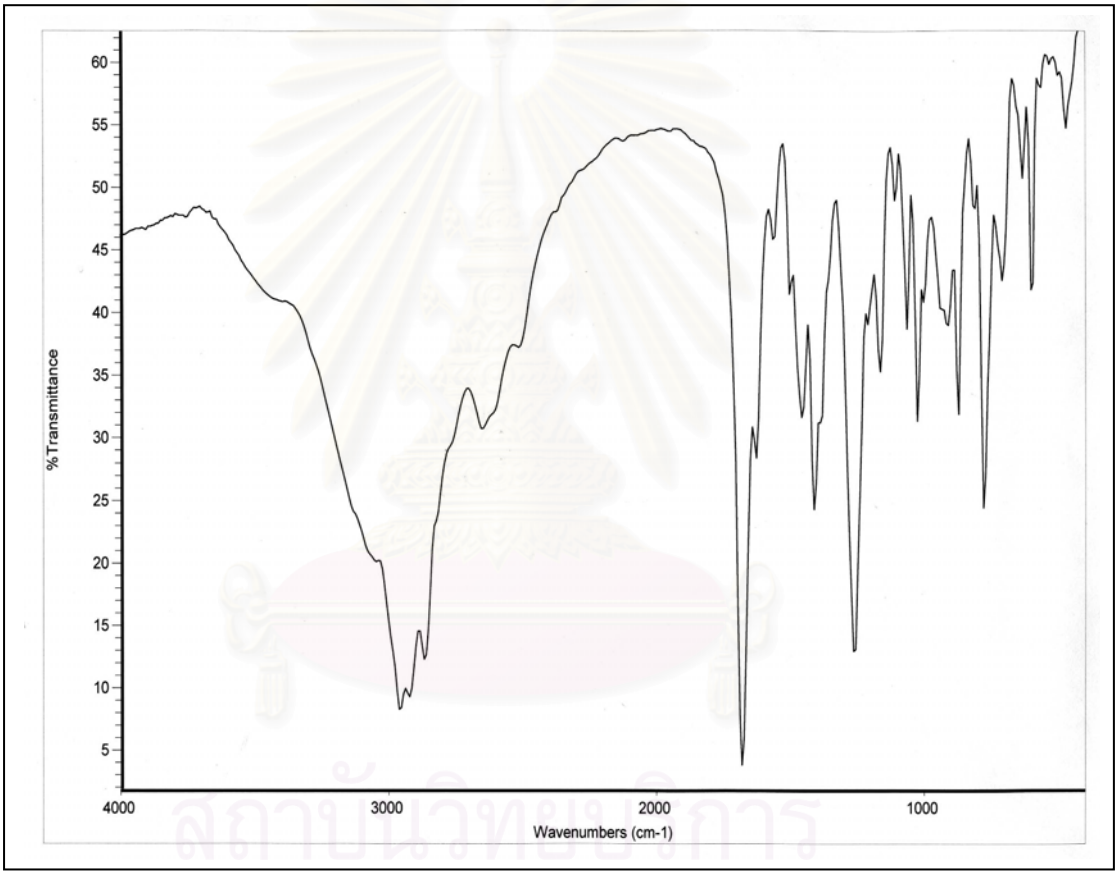


Figure 5. The IR spectrum of Compound **1**.

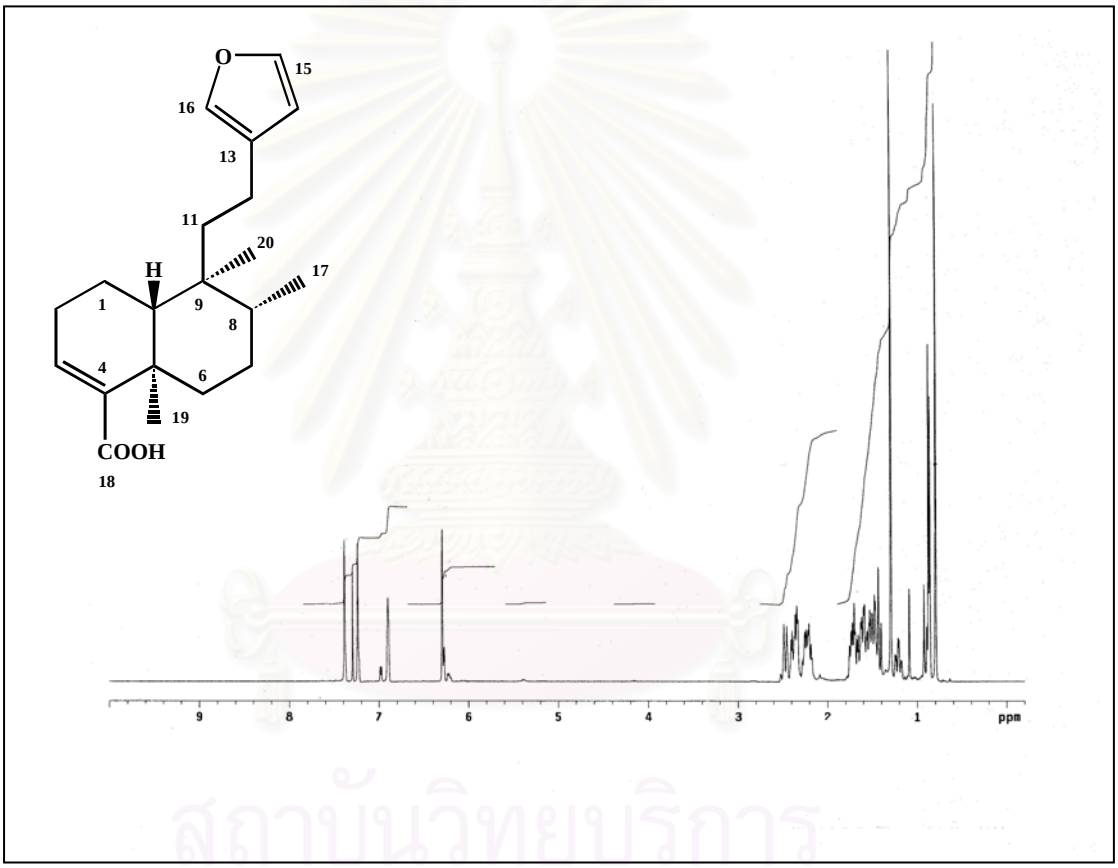


Figure 6. The ^{1}H-NMR spectrum of Compound **1**.

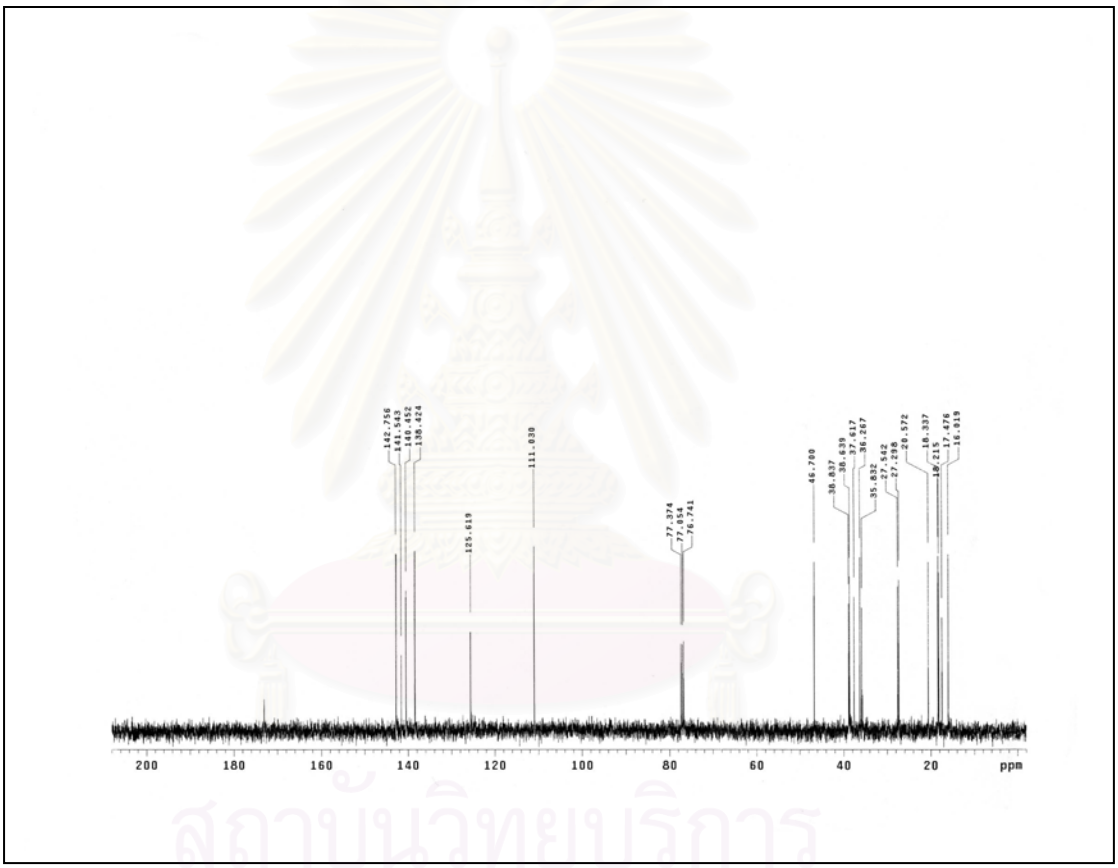


Figure 7. The ^{13}C-NMR spectrum of Compound **1**.

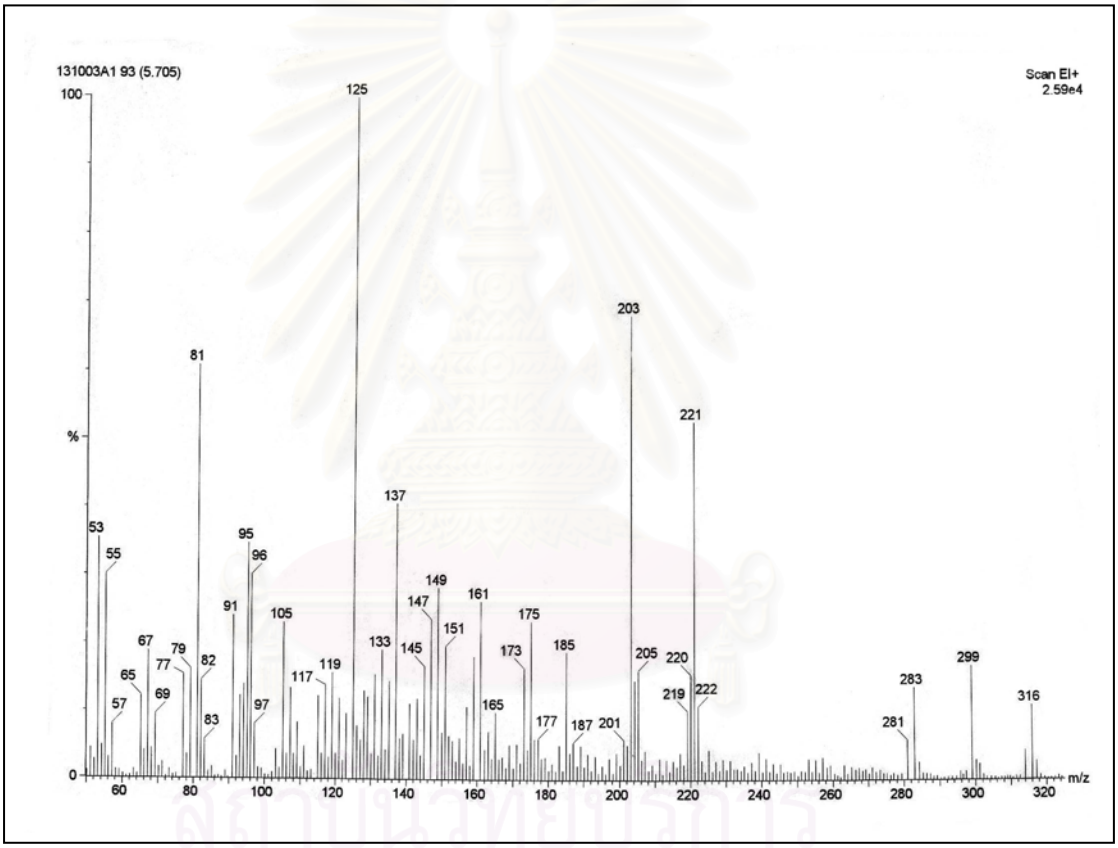


Figure 8. The EI MS spectrum of Compound **1**.

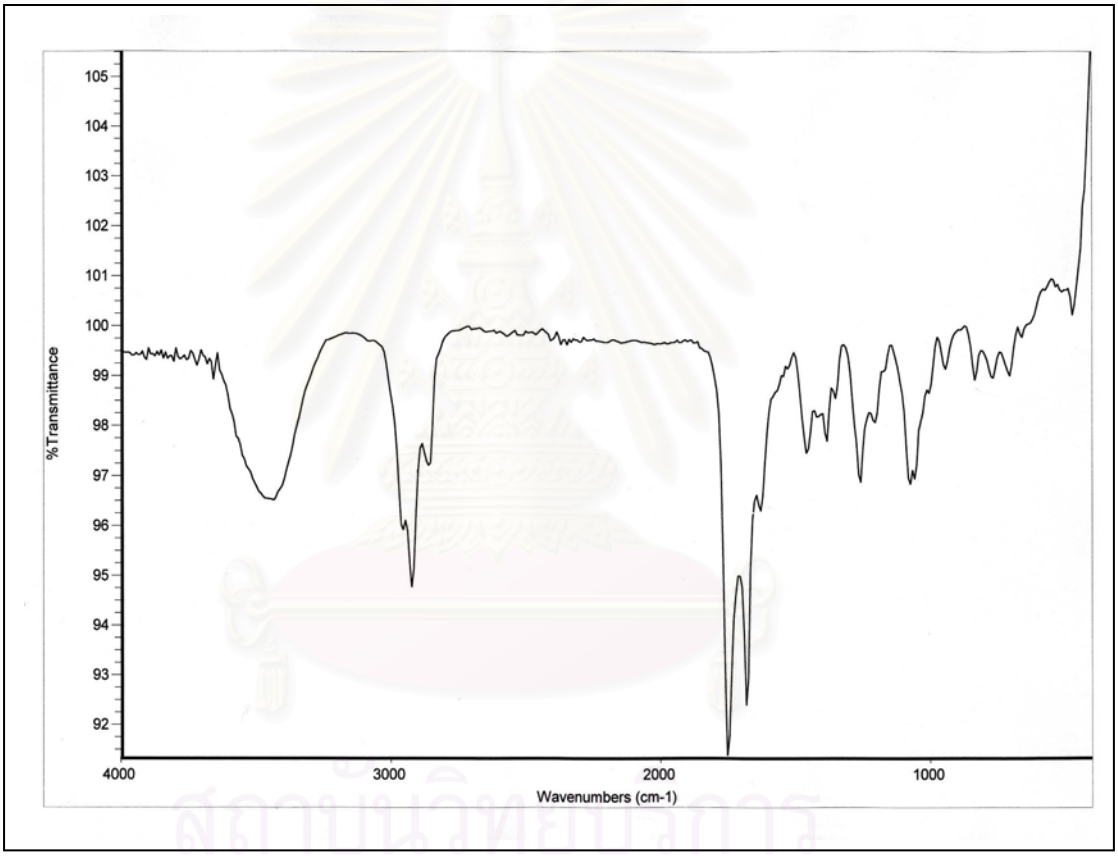


Figure 9. The IR spectrum of Compound **2**.

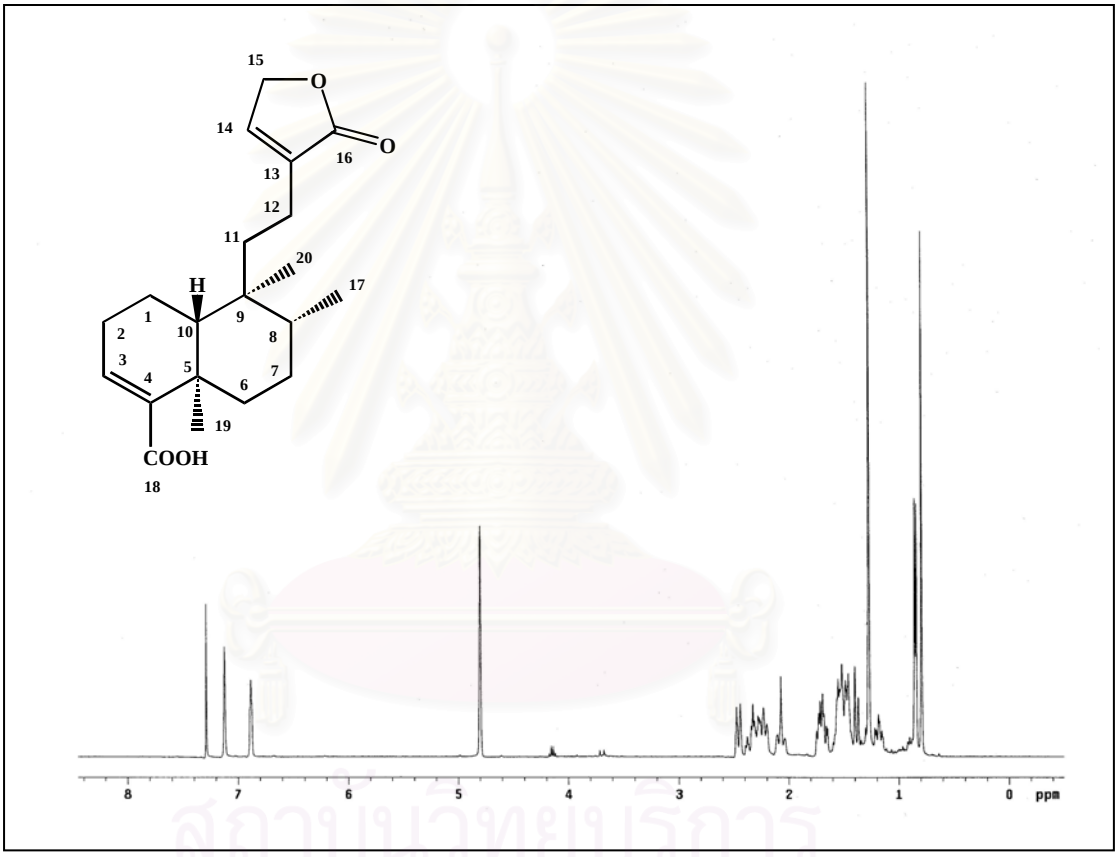


Figure 10. The ^{1}H-NMR spectrum of Compound **2**.

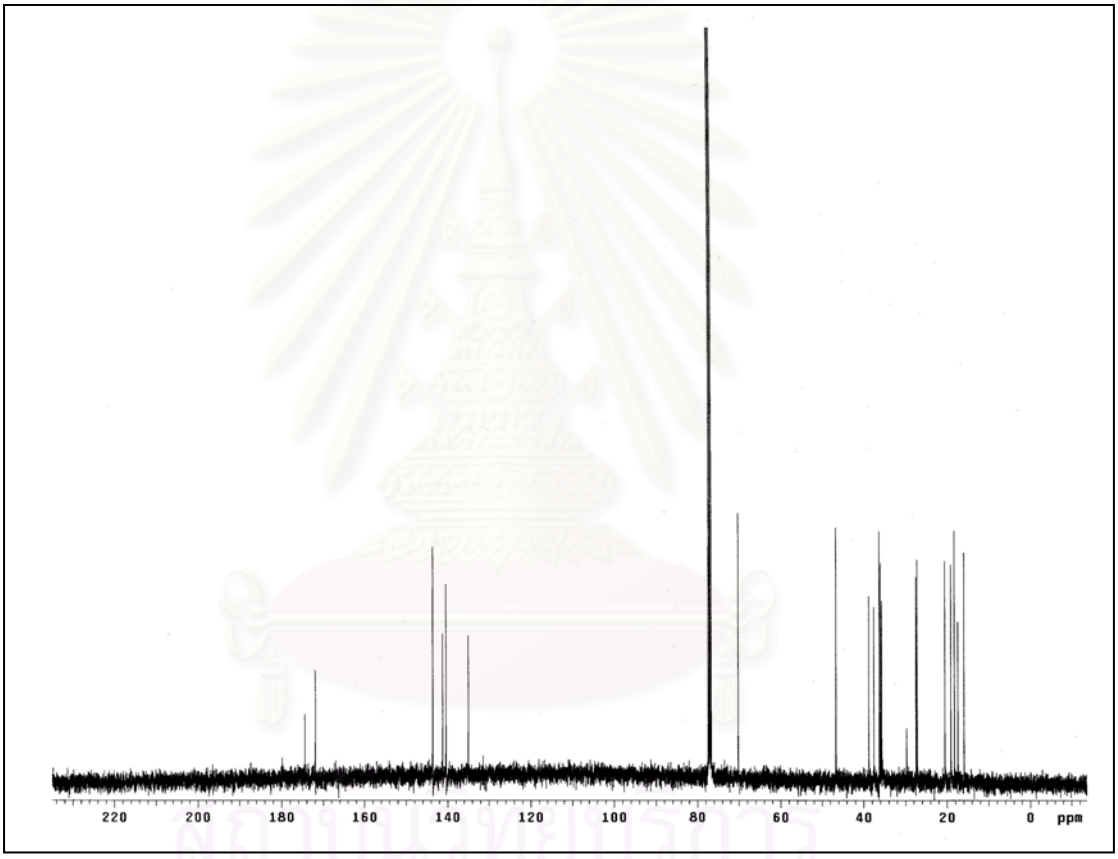


Figure 11. The ^{13}C-NMR spectrum of Compound **2**.

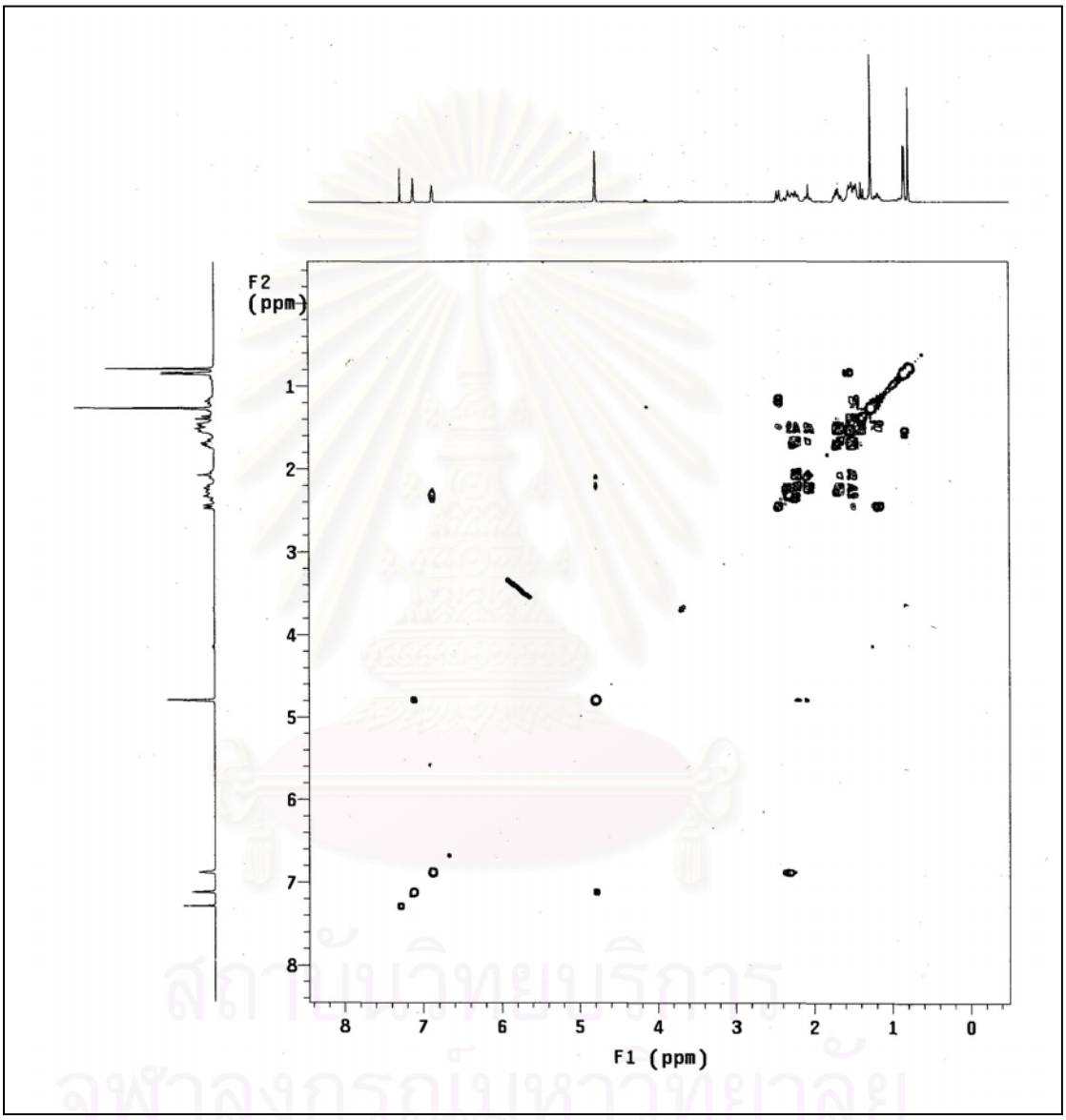


Figure 12. The gCOSY-NMR spectrum of Compound **2**.

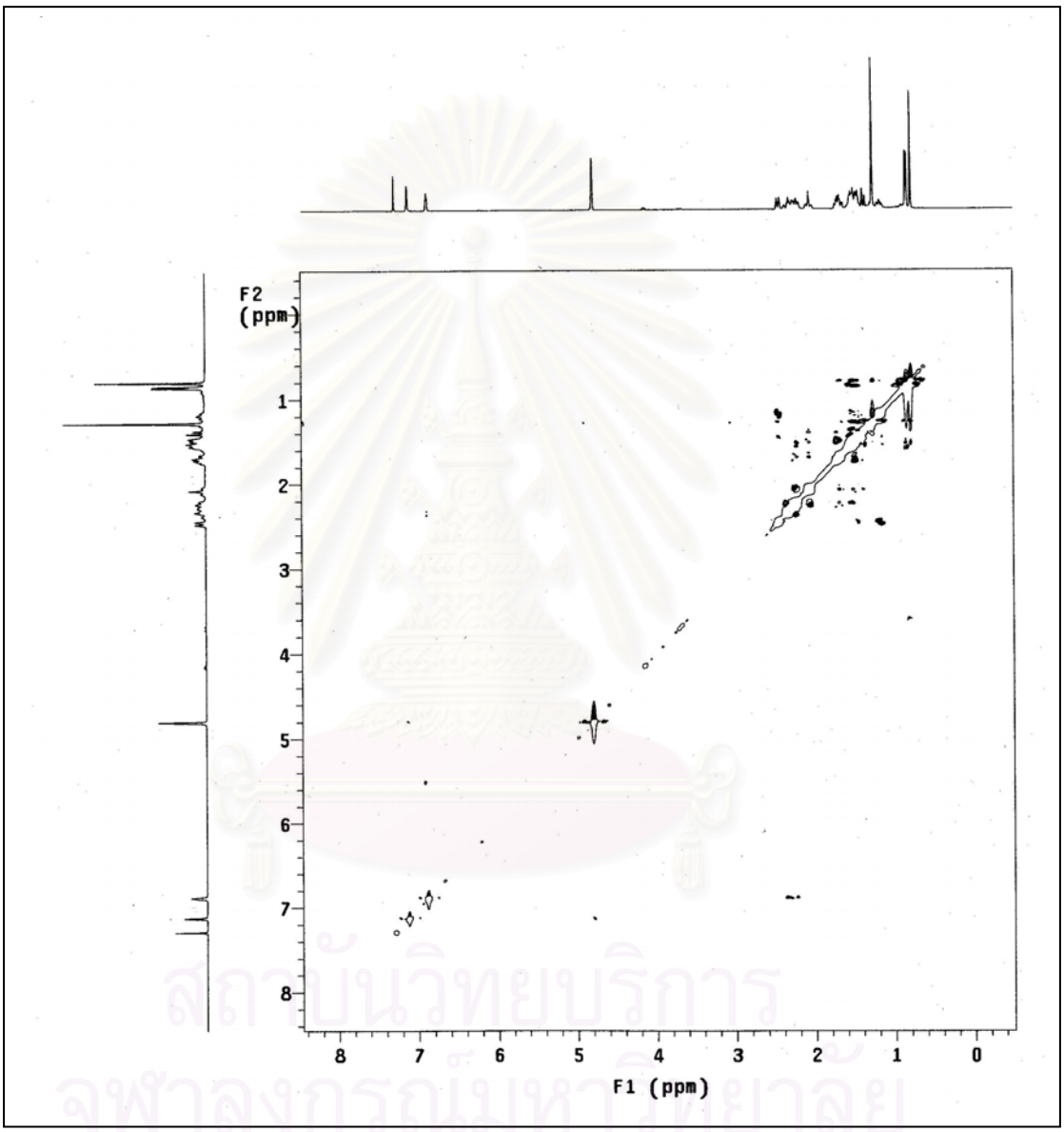


Figure 13. The gNOESY-NMR spectrum of Compound **2**.

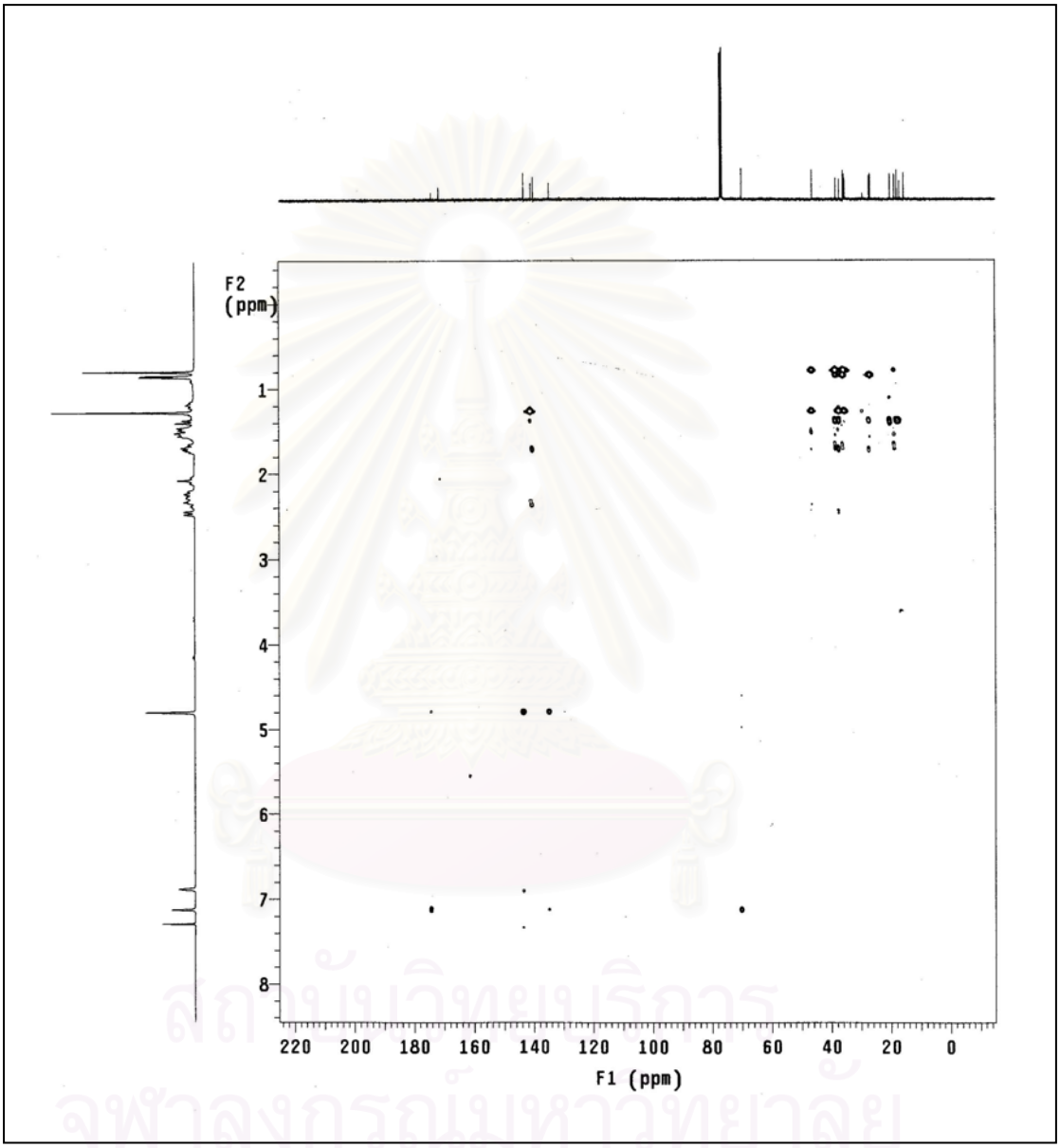


Figure 14. The gHMBC-NMR spectrum of Compound **2**.

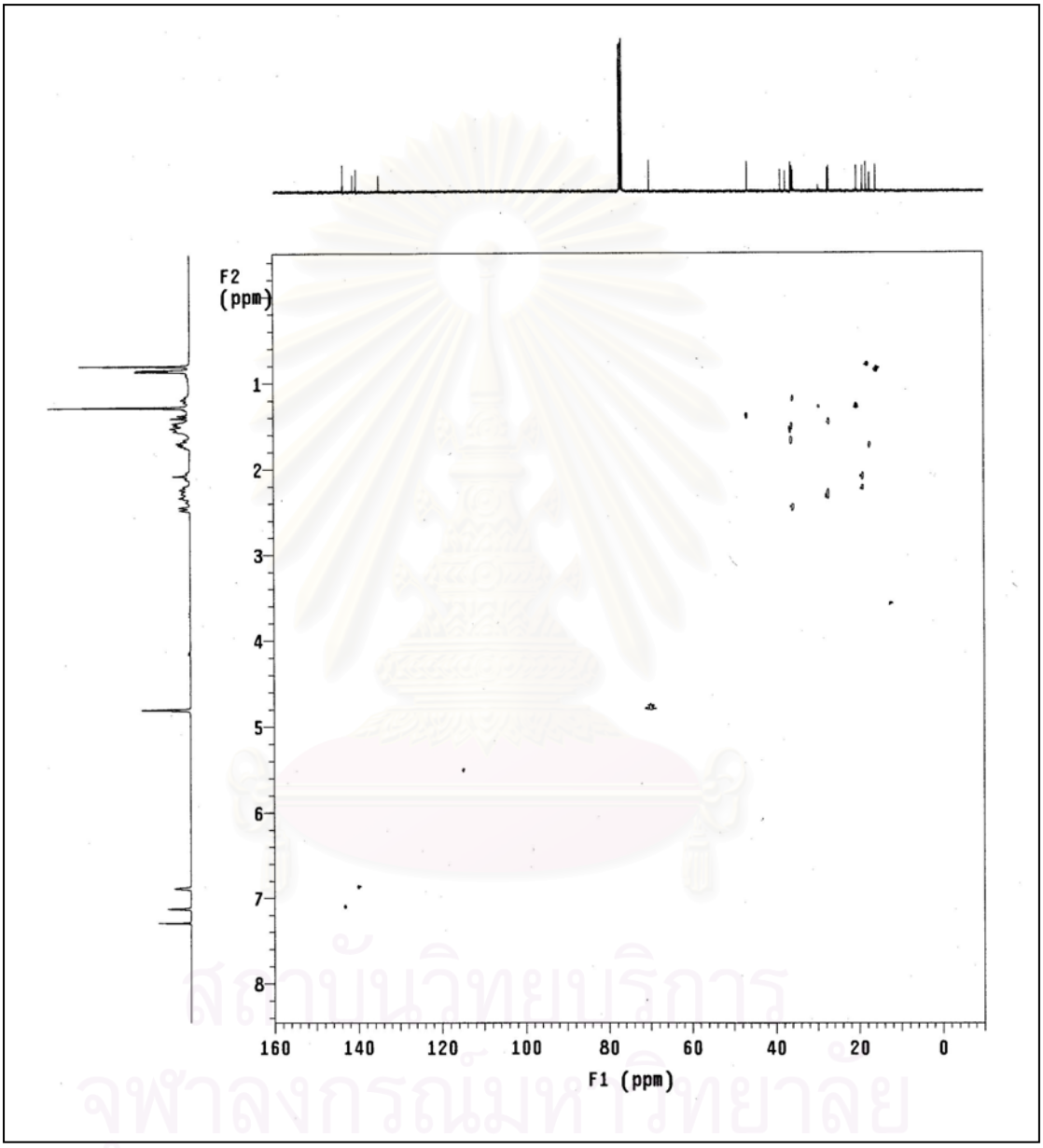


Figure 15. The gHSQC-NMR spectrum of Compound **2**.

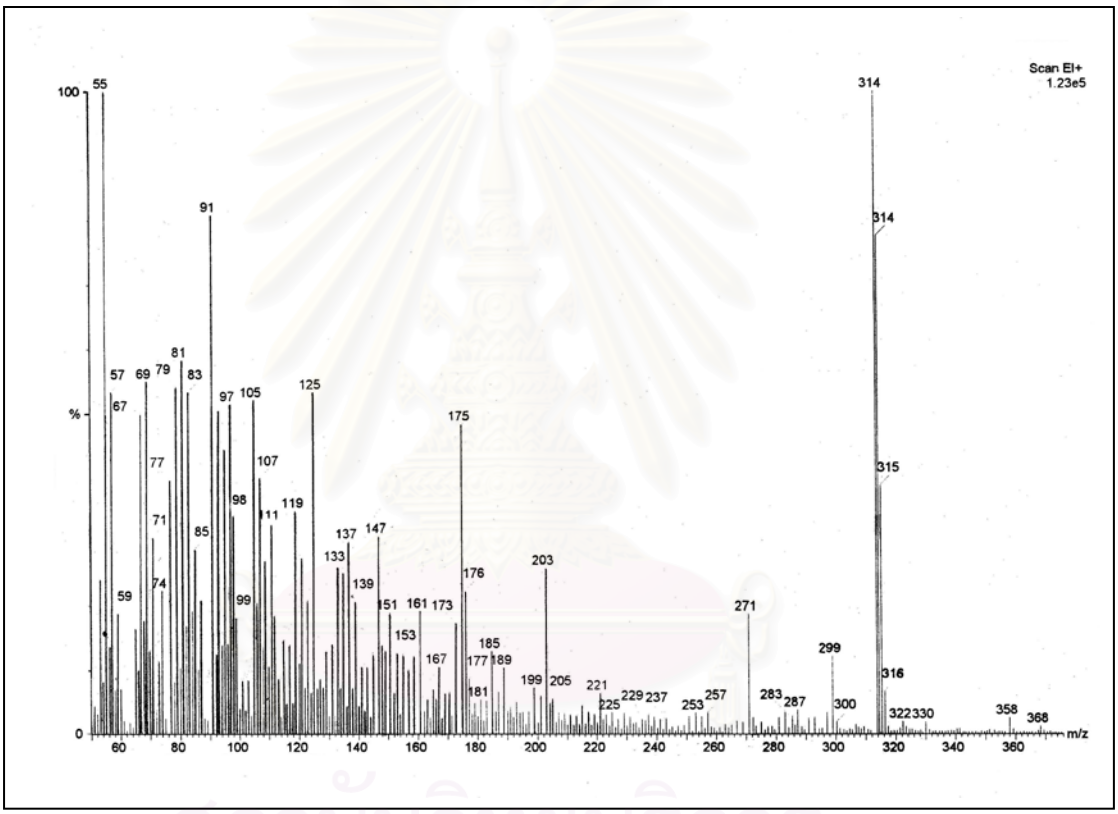


Figure 16. The EI MS spectrum of Compound **2**.

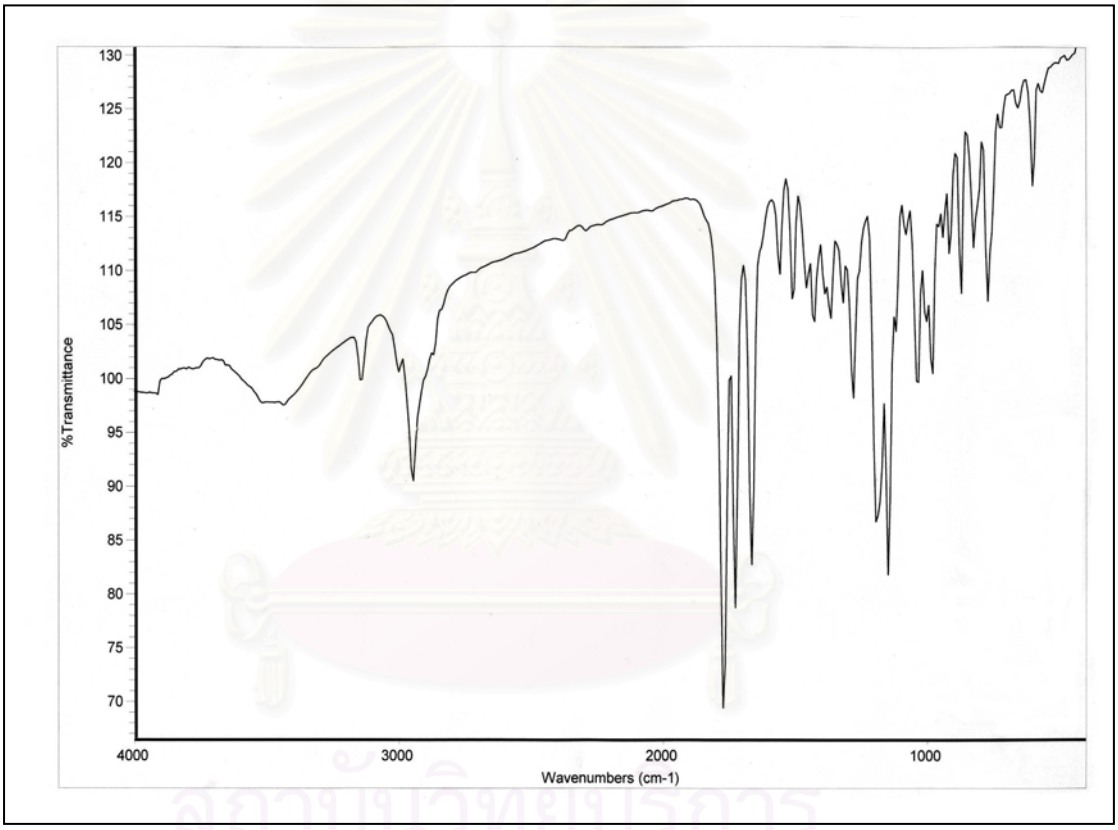


Figure 17. The IR spectrum of Compound **3**.

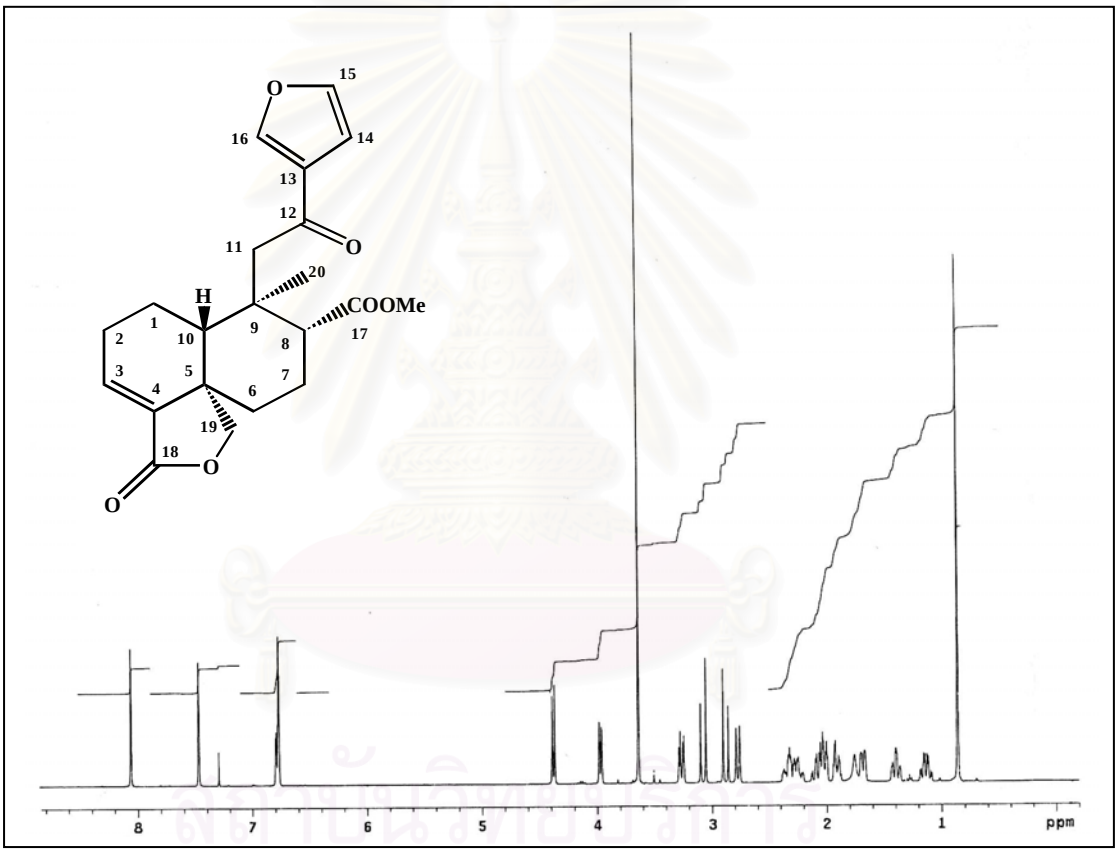


Figure 18. The ^{1}H-NMR spectrum of Compound **3**.

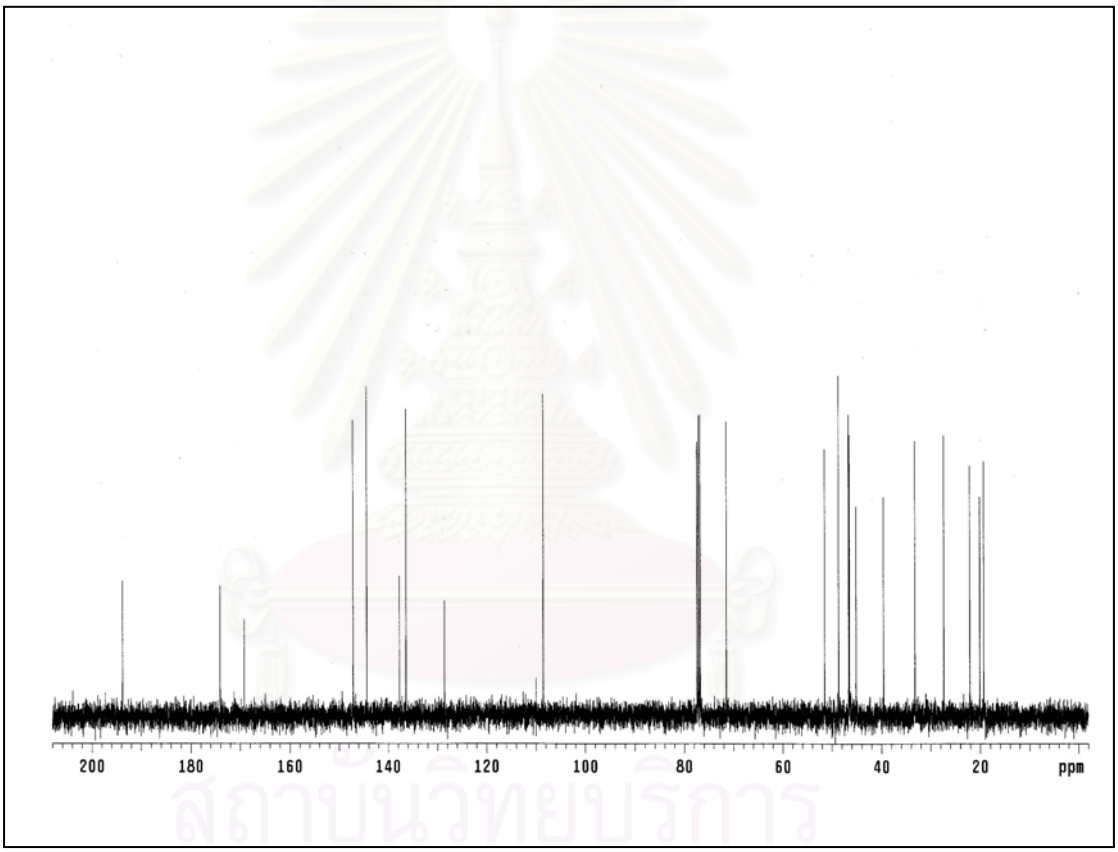


Figure 19. The ^{13}C-NMR spectrum of Compound **3**.

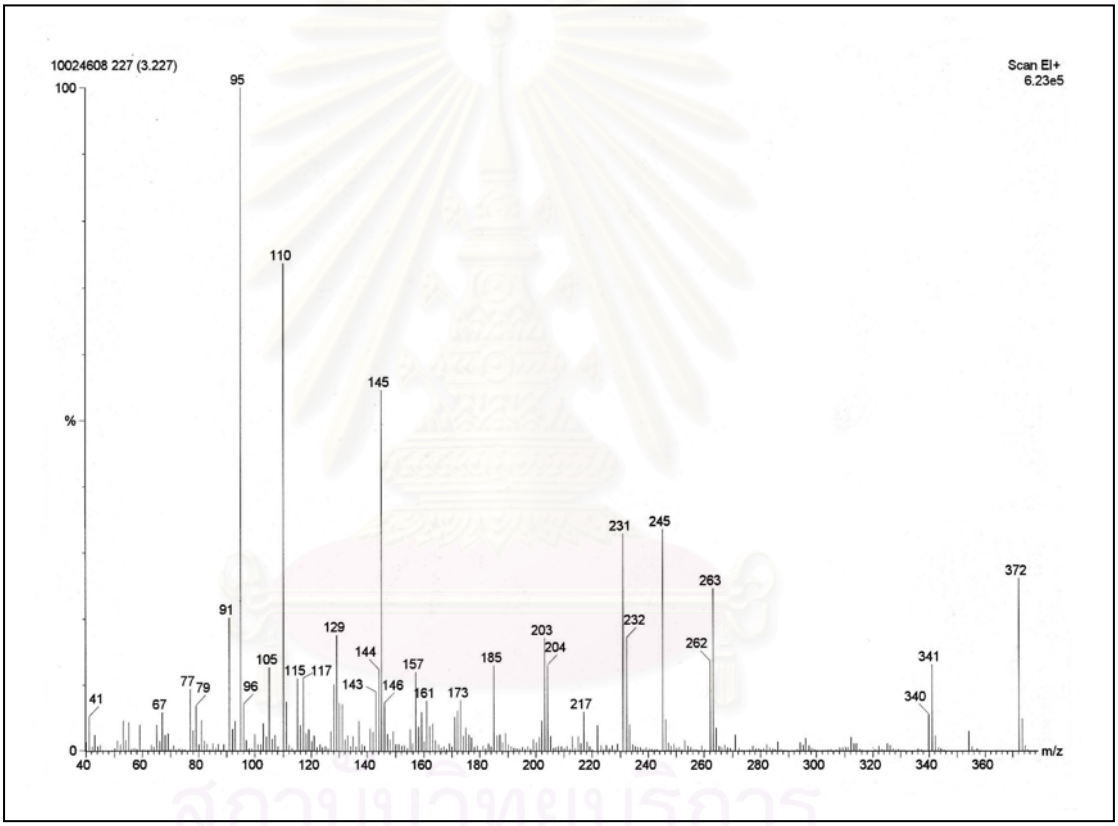


Figure 20. The EI MS spectrum of Compound **3**.

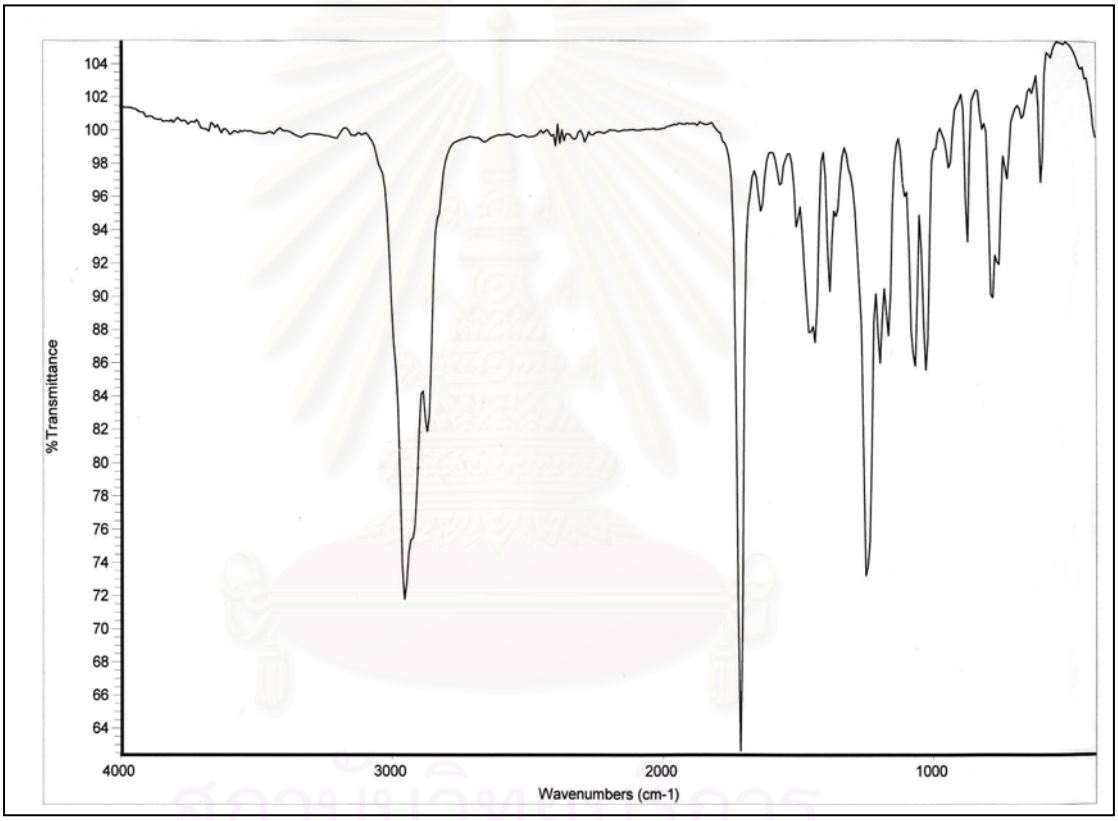


Figure 21. The IR spectrum of Compound **1a**.

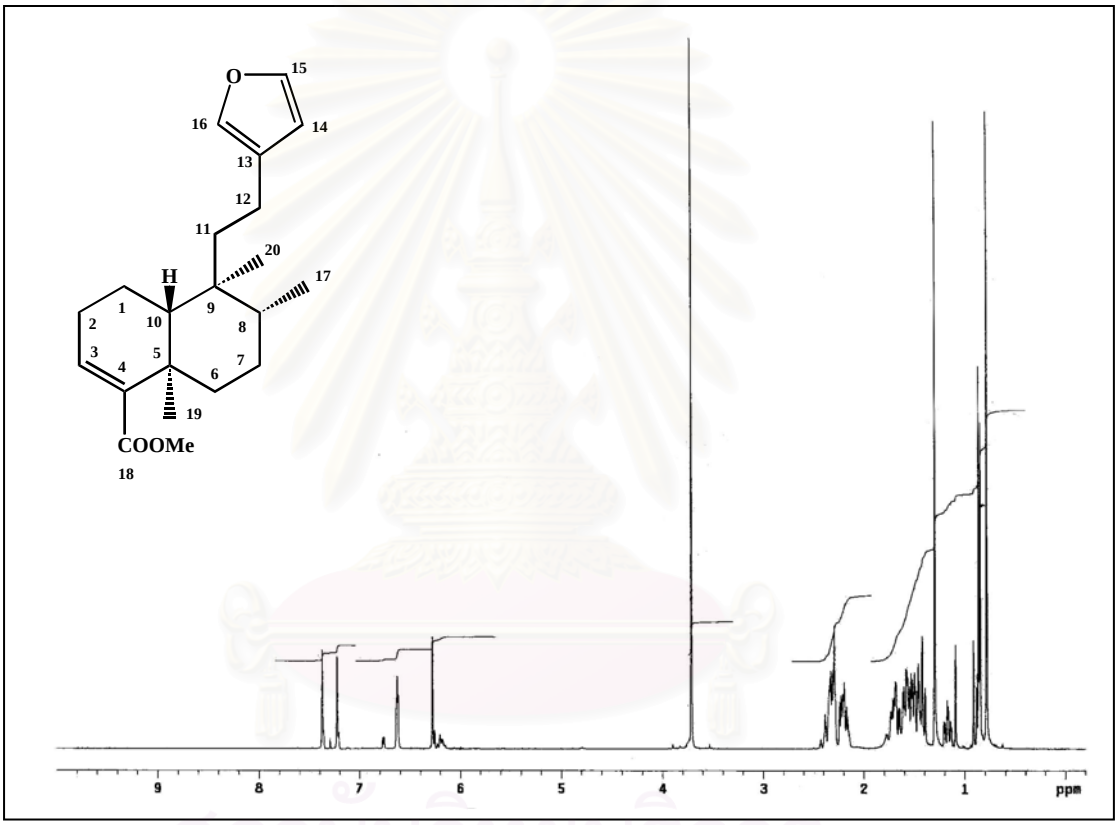


Figure 22. The ^{1}H-NMR spectrum of Compound **1a**.

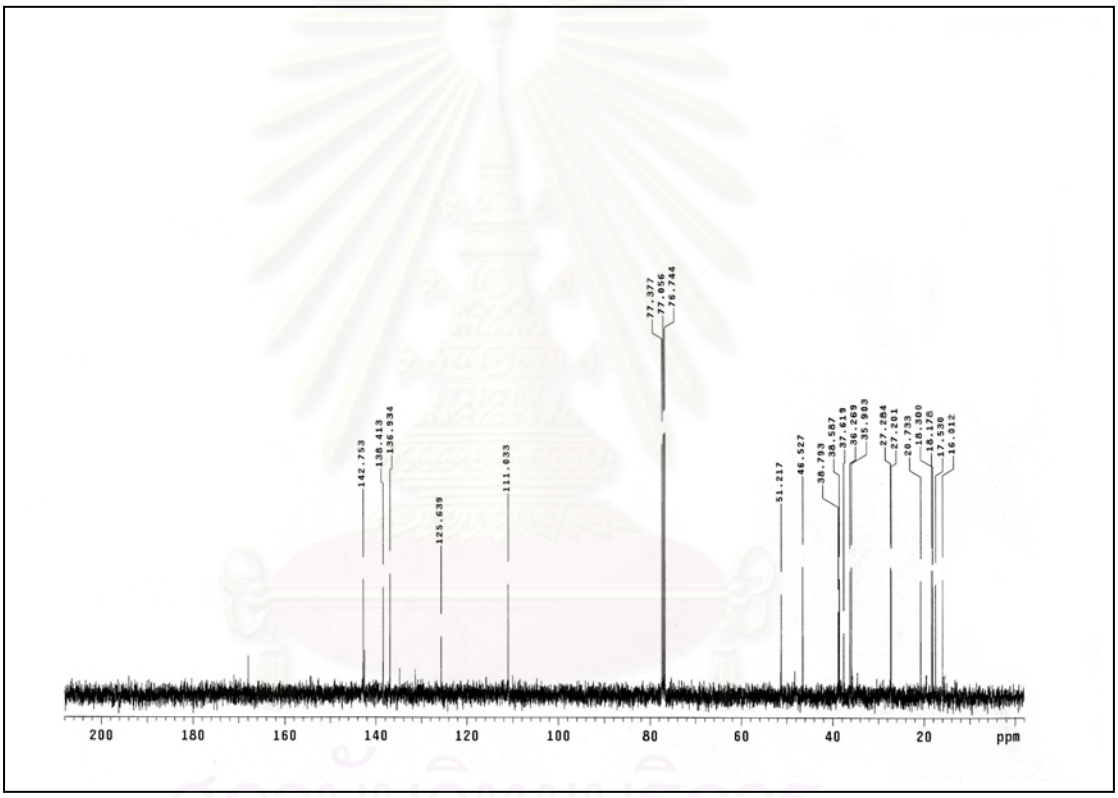


Figure 23. The ^{13}C-NMR spectrum of Compound **1a**.

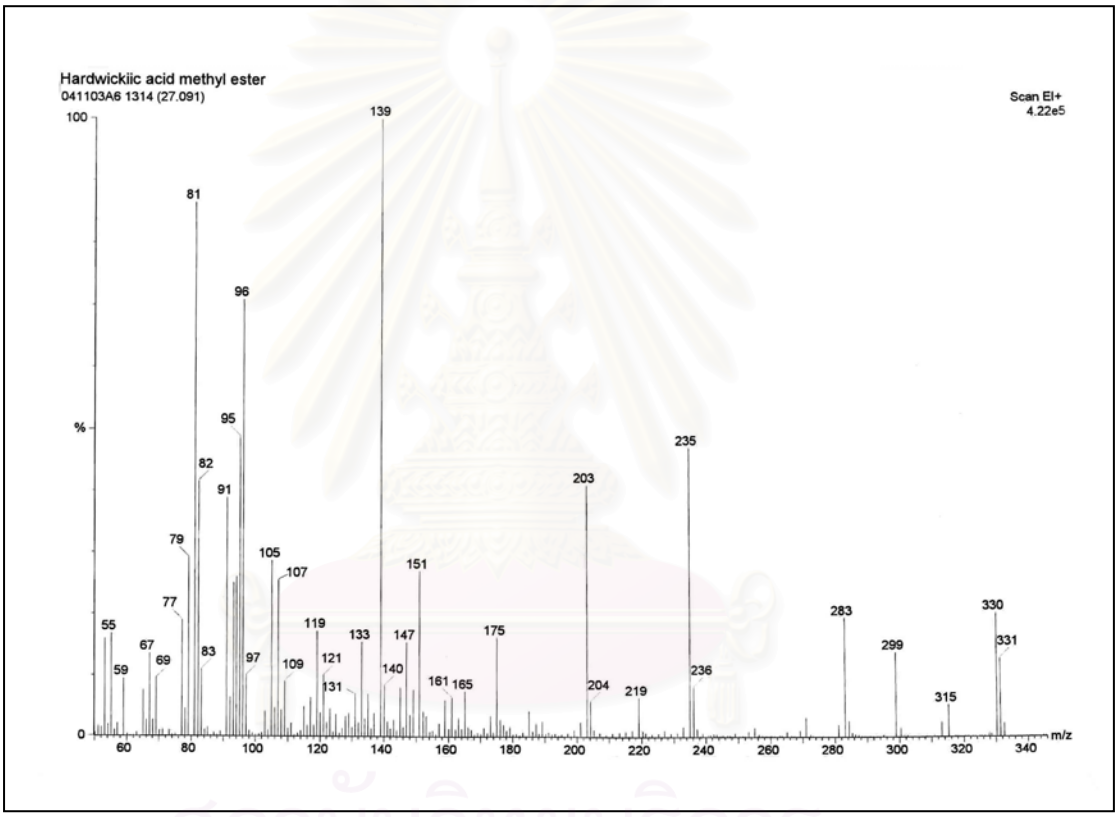


Figure 24. The EI MS spectrum of Compound **1a**.

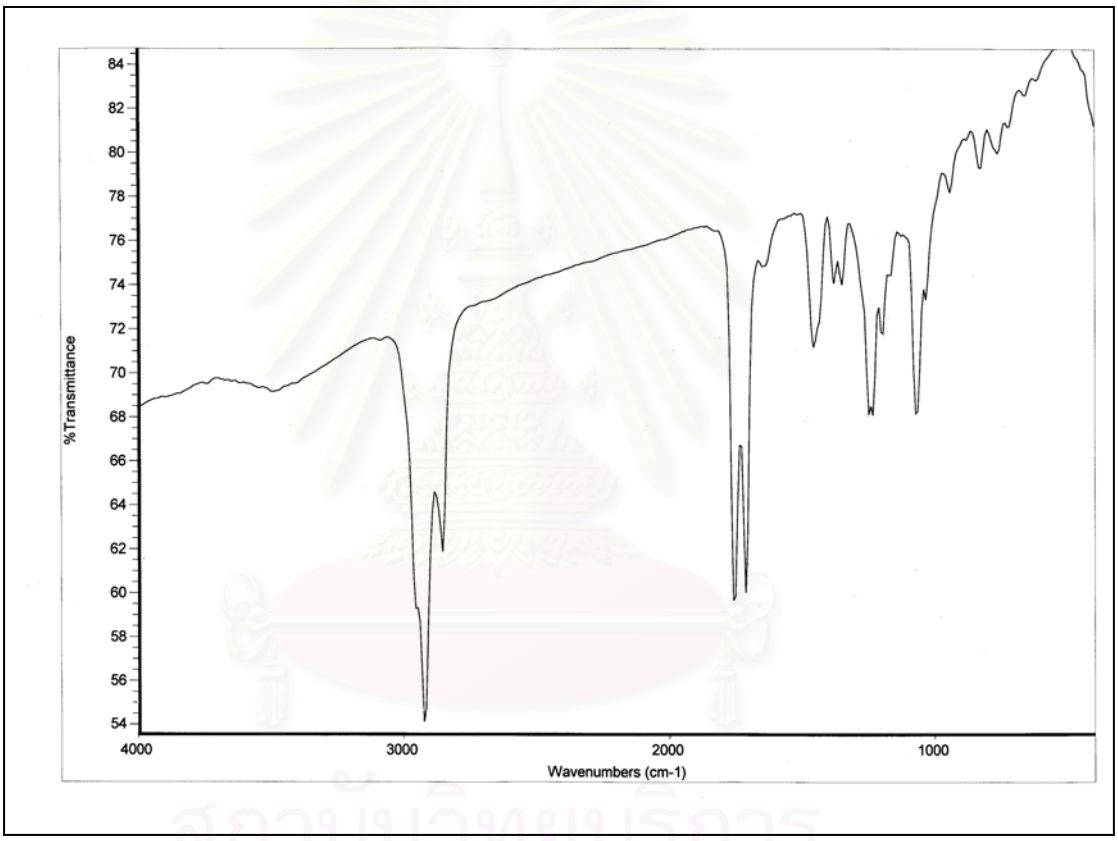


Figure 25. The IR spectrum of Compound **2a**.

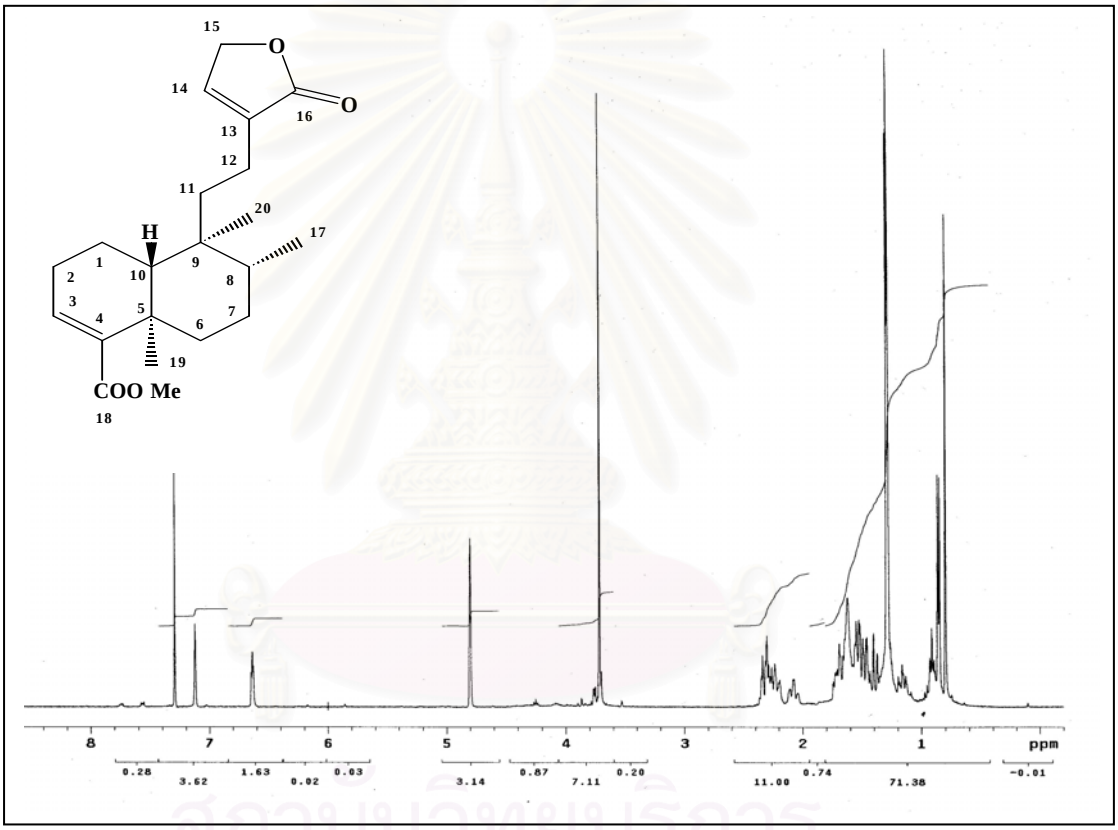


Figure 26. The ^{1}H-NMR spectrum of Compound **2a**.

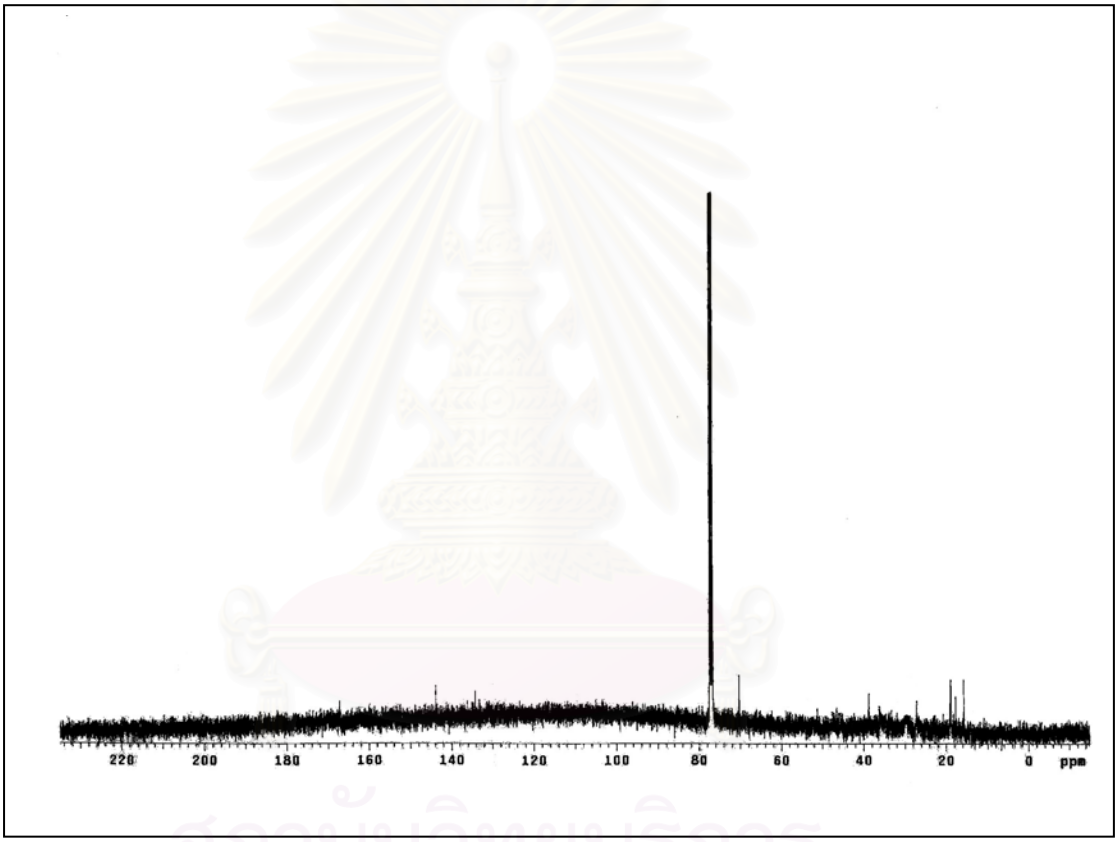


Figure 27. The ^{13}C-NMR spectrum of Compound **2a**.

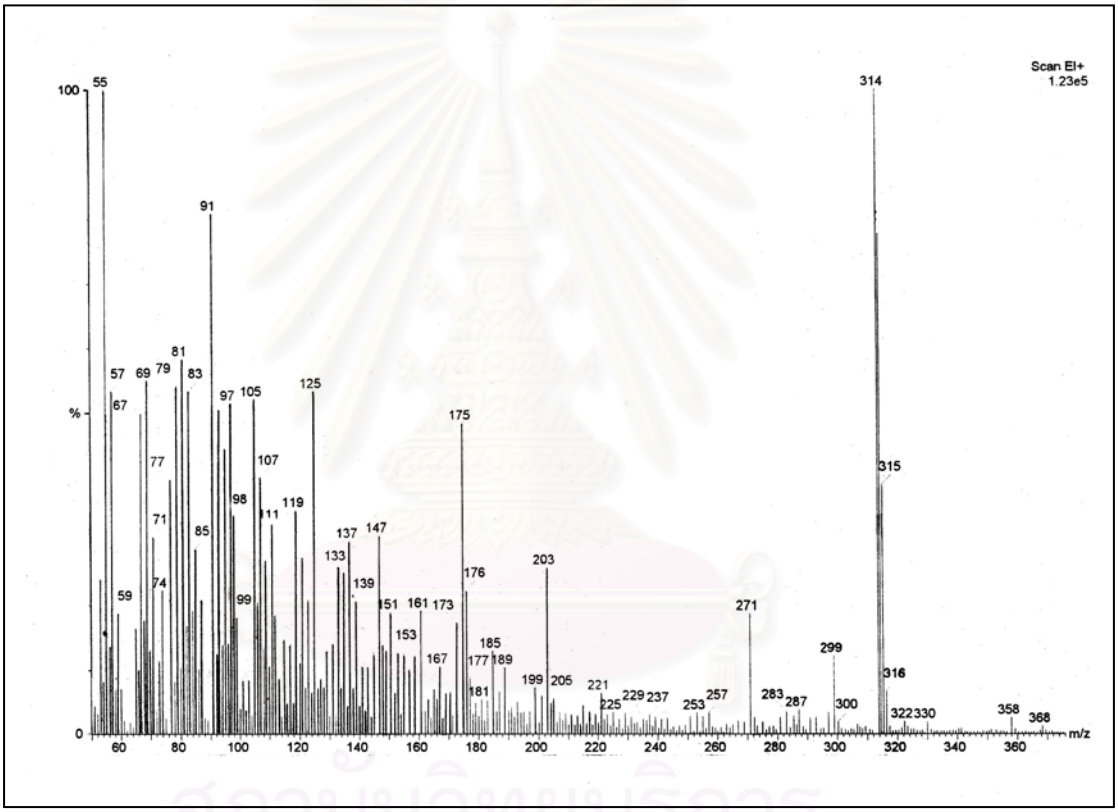


Figure 28. The EI MS spectrum of Compound **2a**.

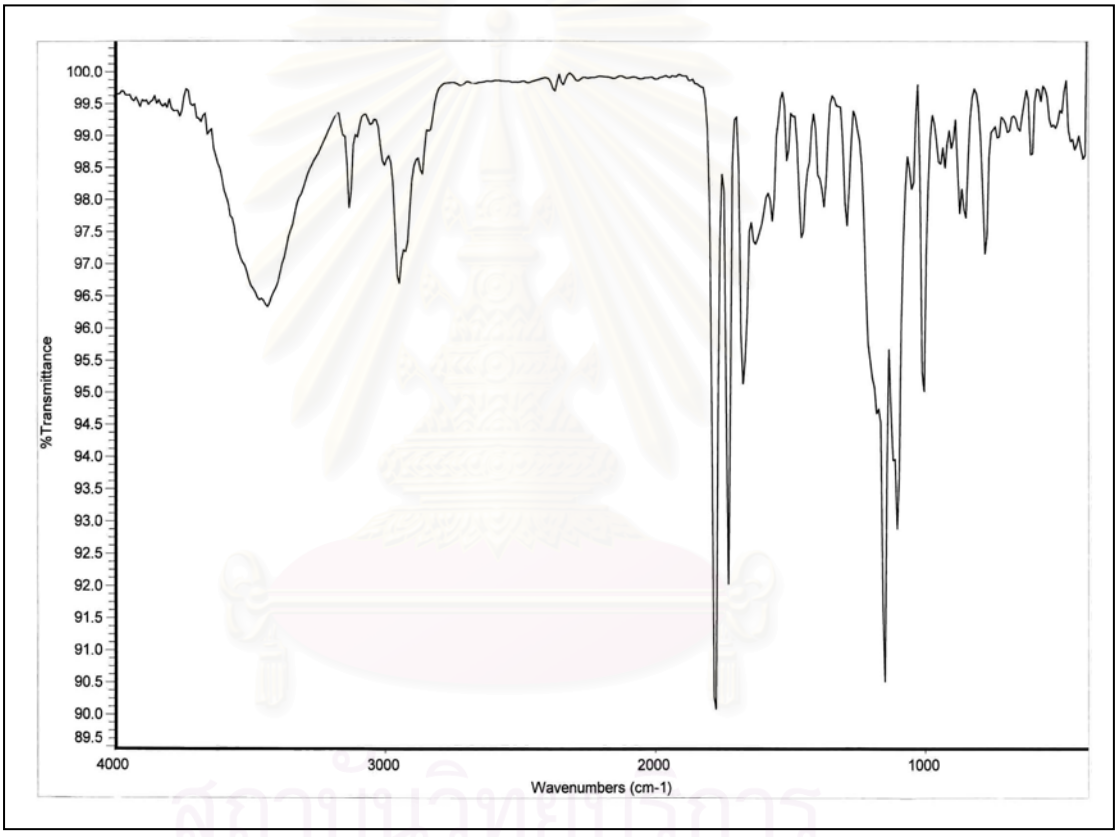


Figure 29. The IR spectrum of Compound **3a**.

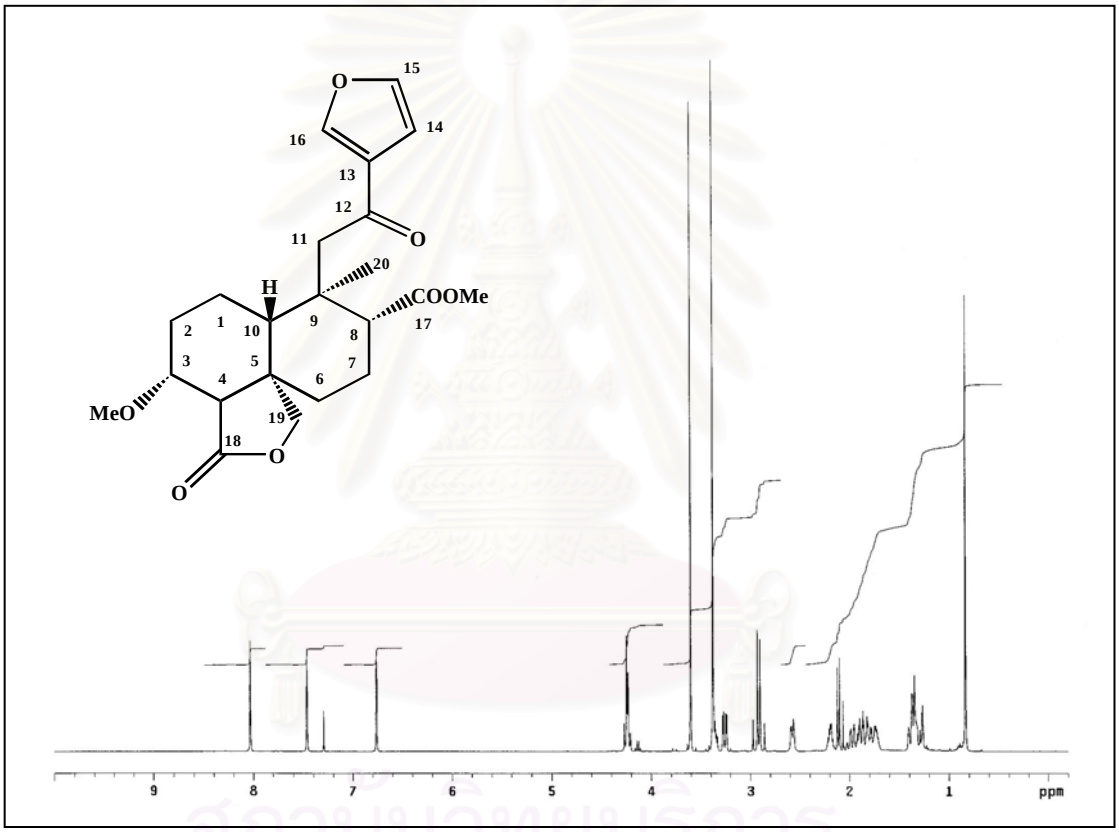


Figure 30. The ^{1}H-NMR spectrum of Compound **3a**.

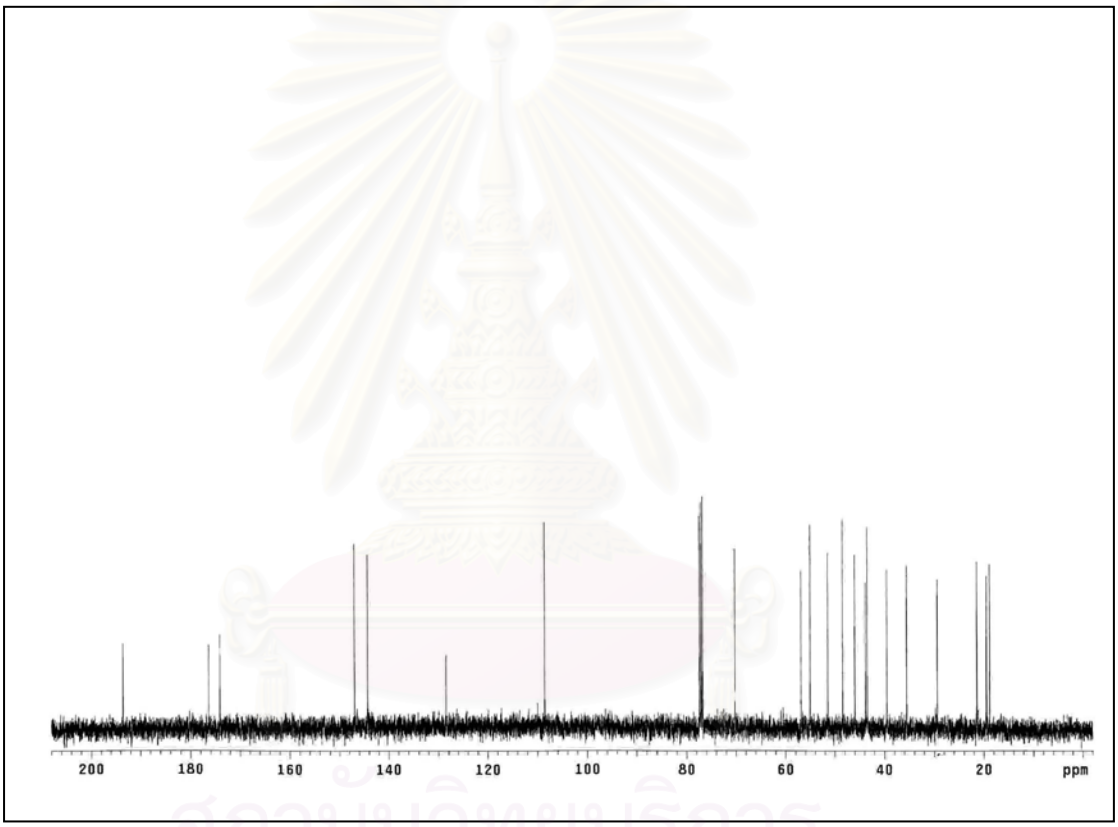


Figure 31. The ^{13}C-NMR spectrum of Compound **3a**.

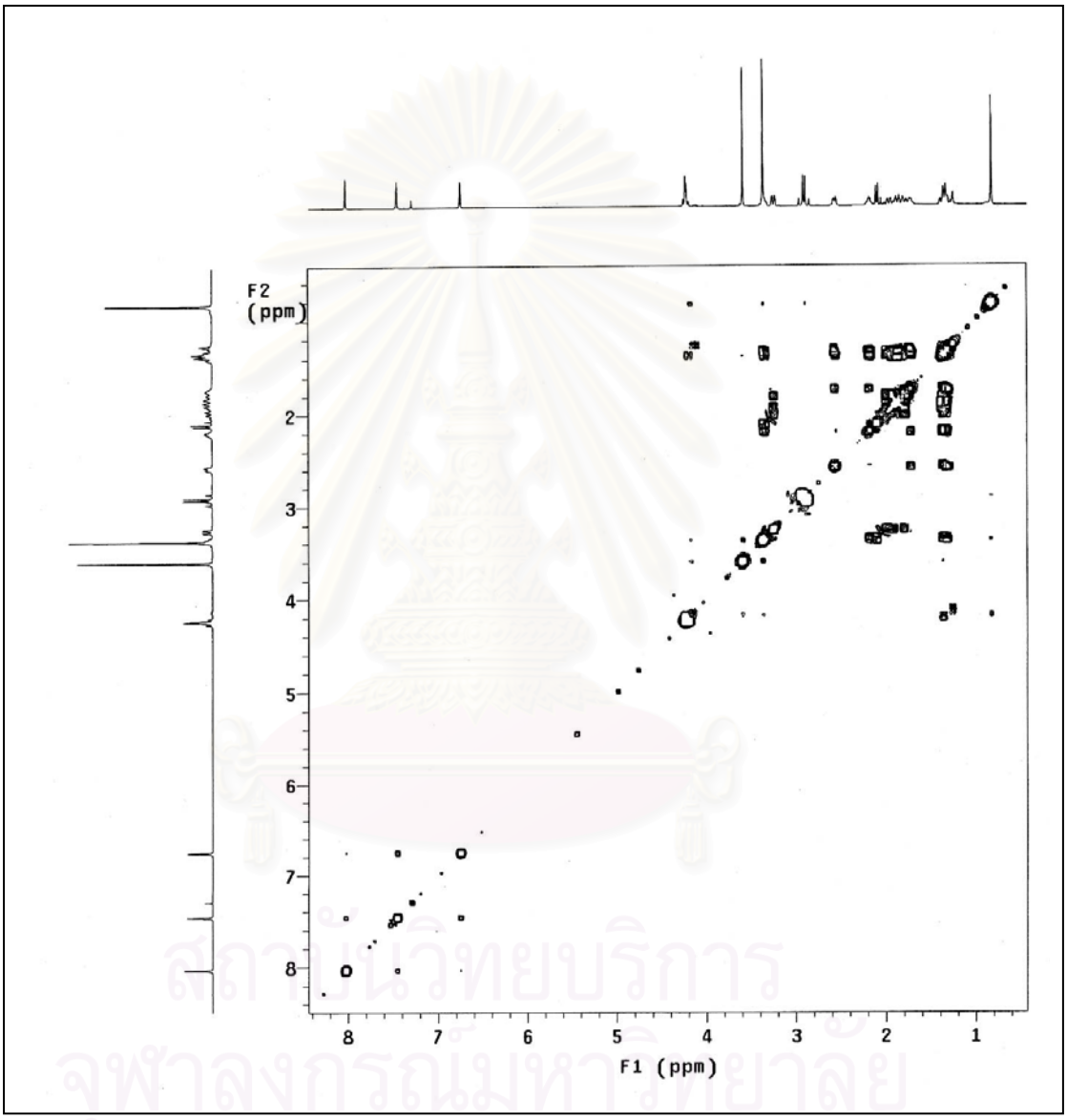


Figure 32. The gCOSY-NMR spectrum of Compound **3a**.

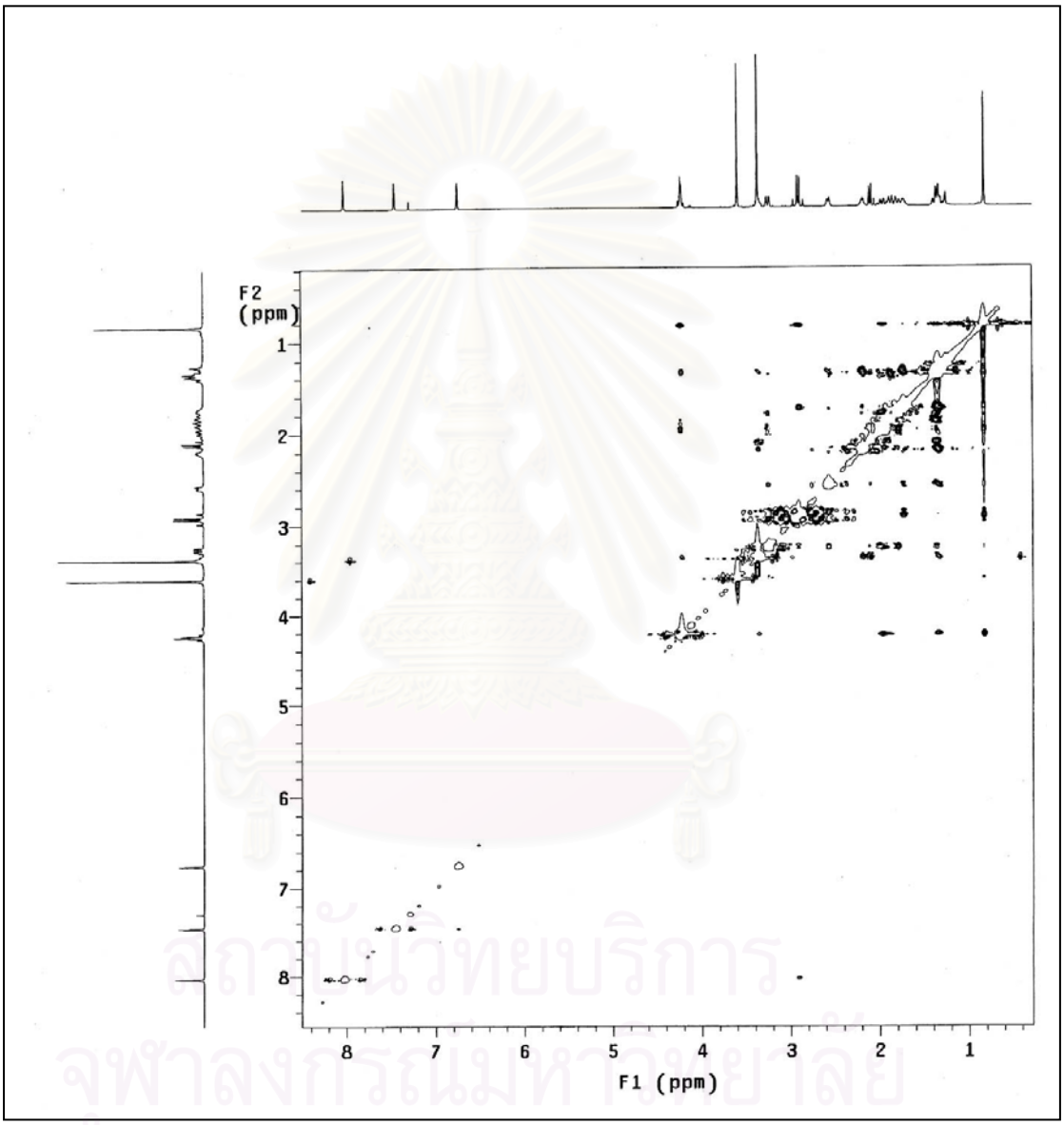


Figure 33. The gNOESY-NMR spectrum of Compound **3a**.

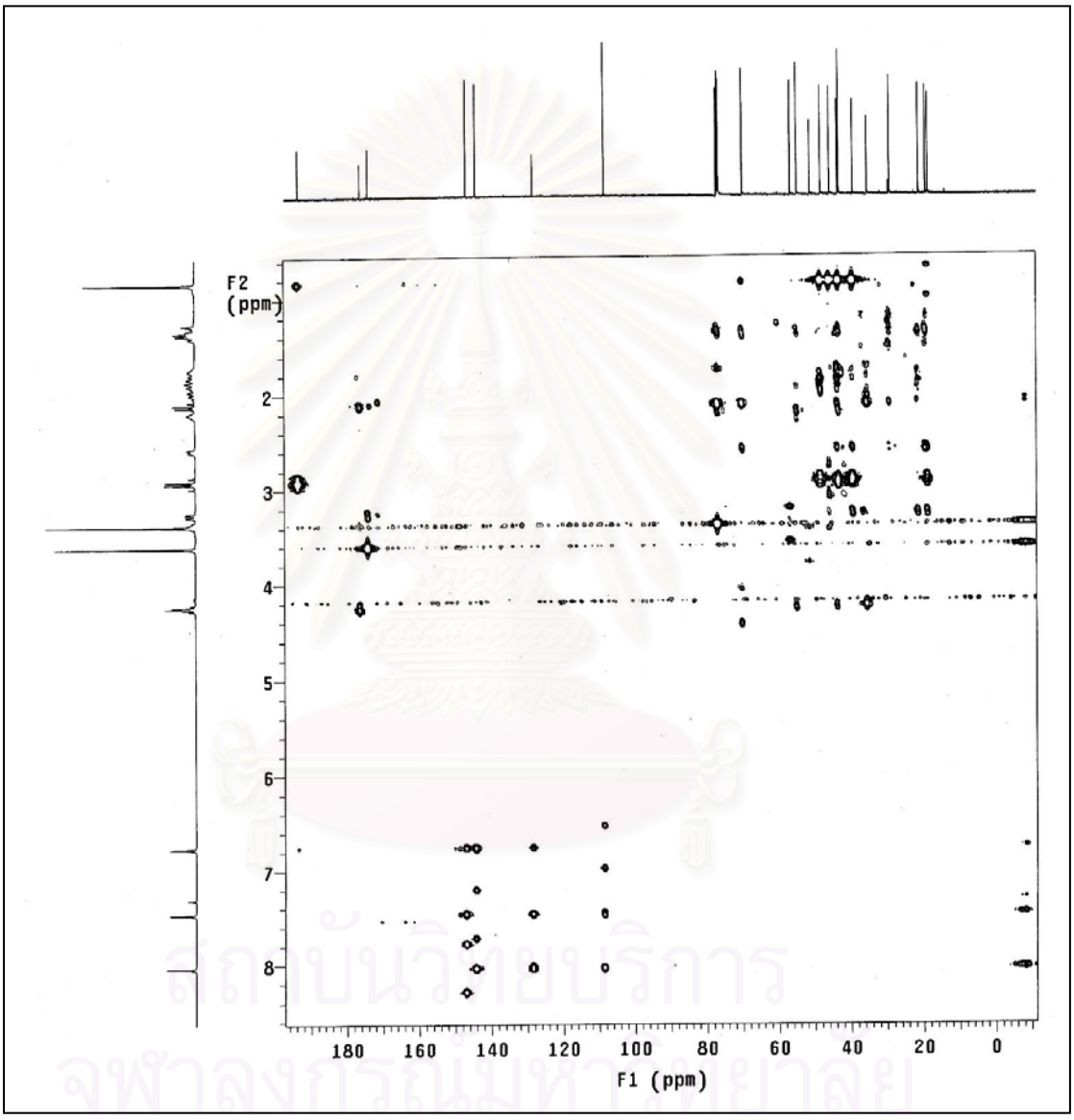


Figure 34. The gHMBC-NMR spectrum of Compound **3a**.

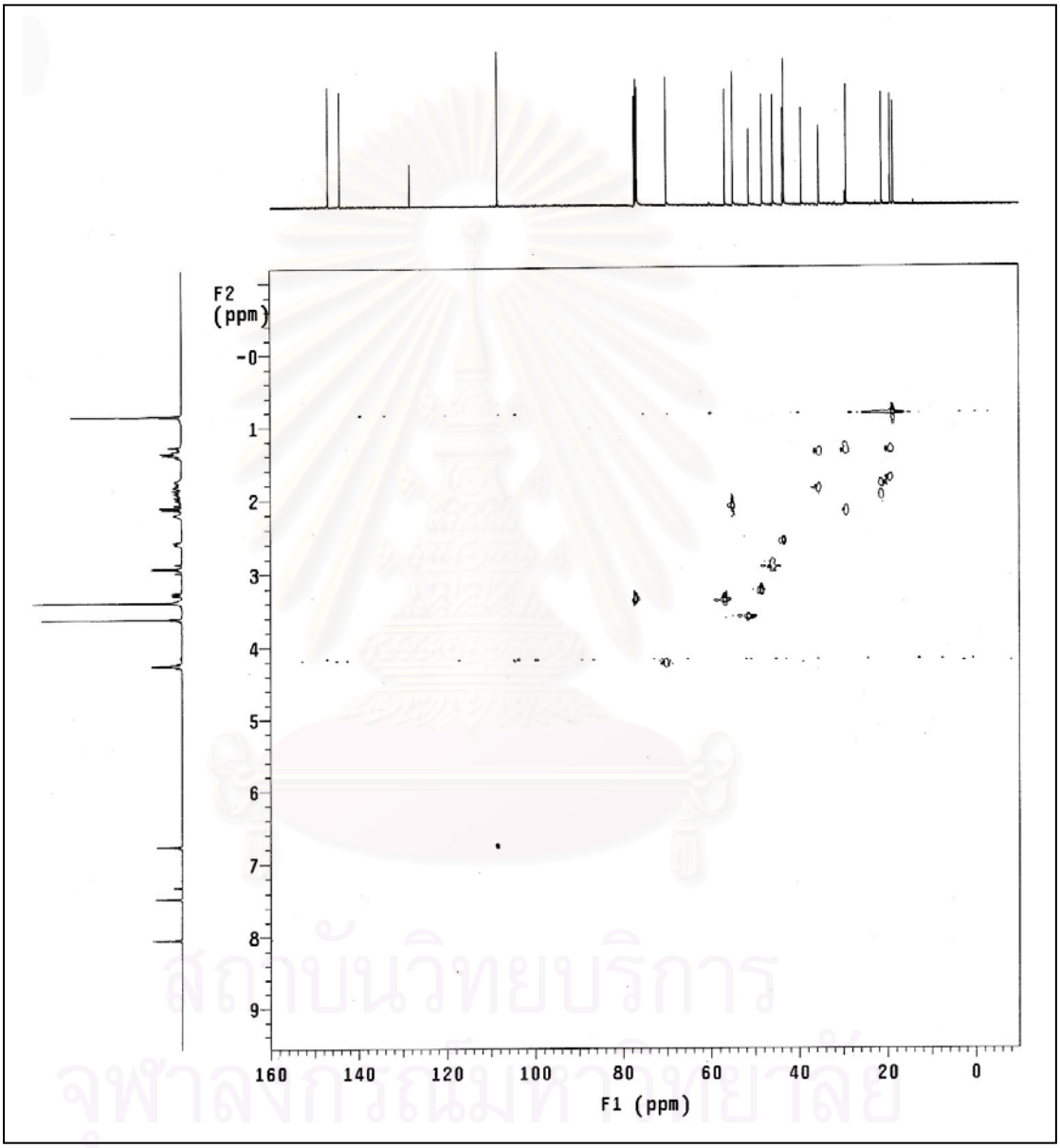
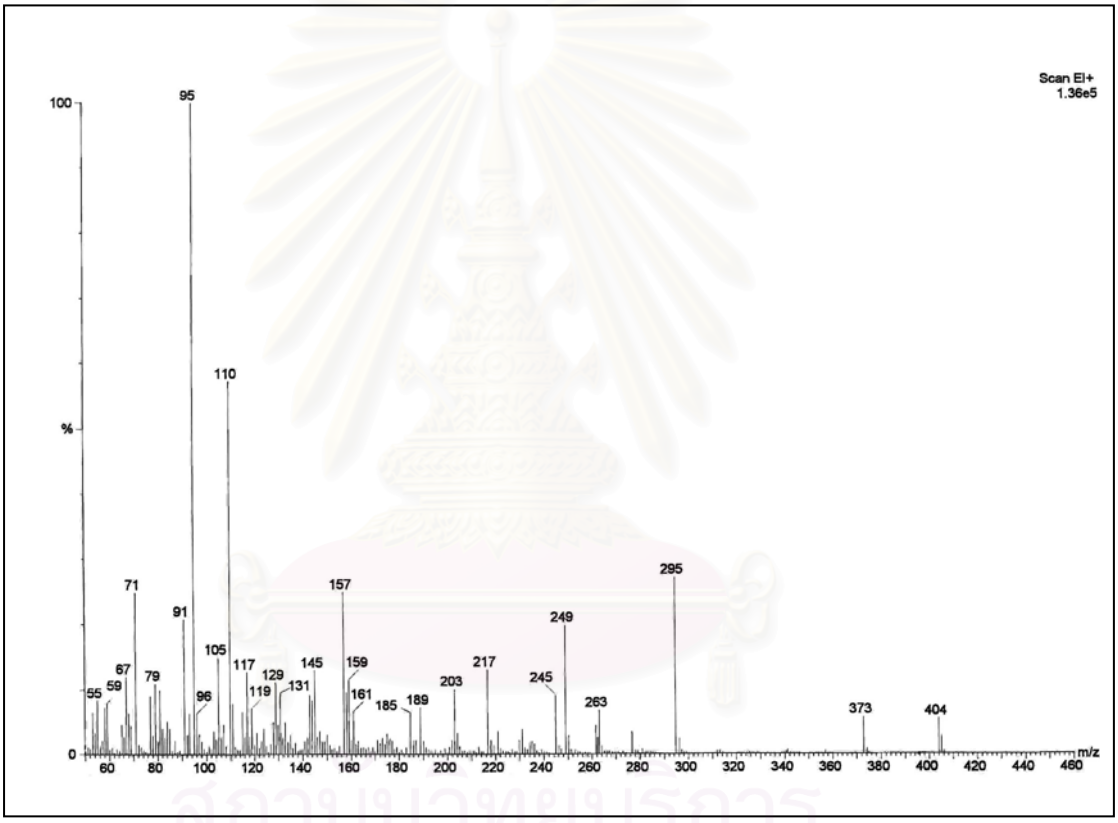


Figure 35. The gHSQC-NMR spectrum of Compound **3a**.



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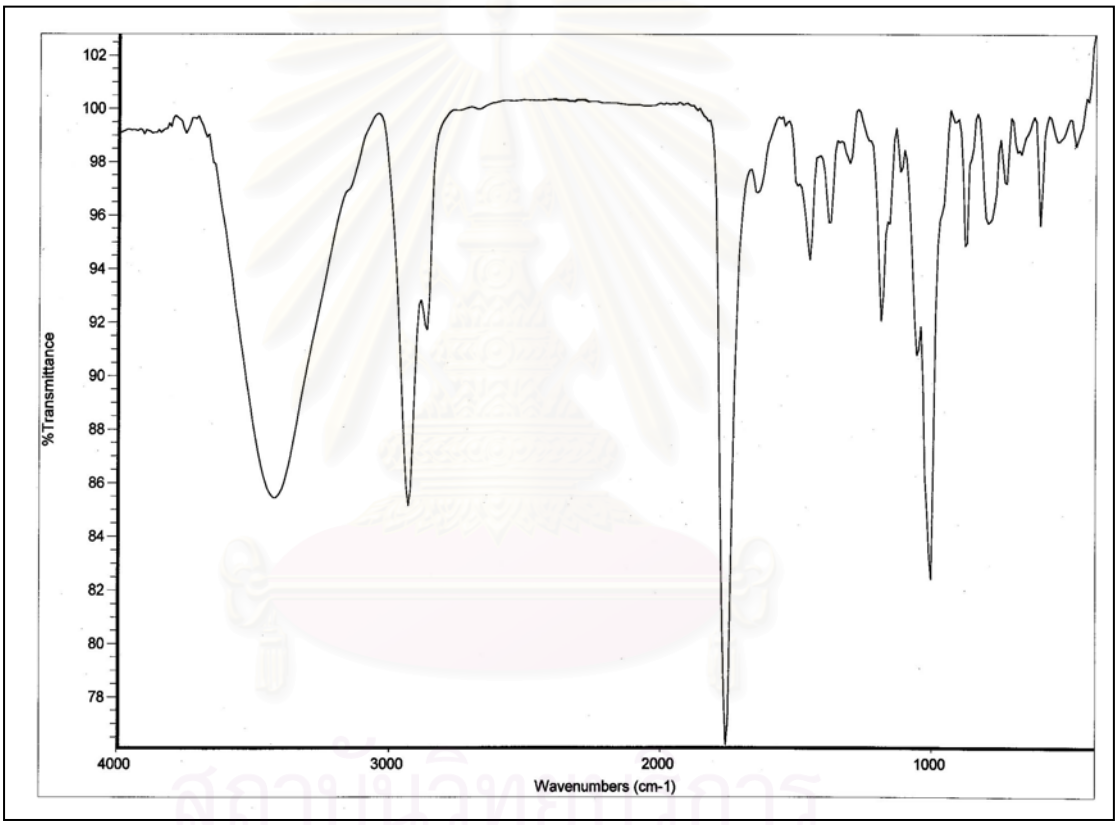

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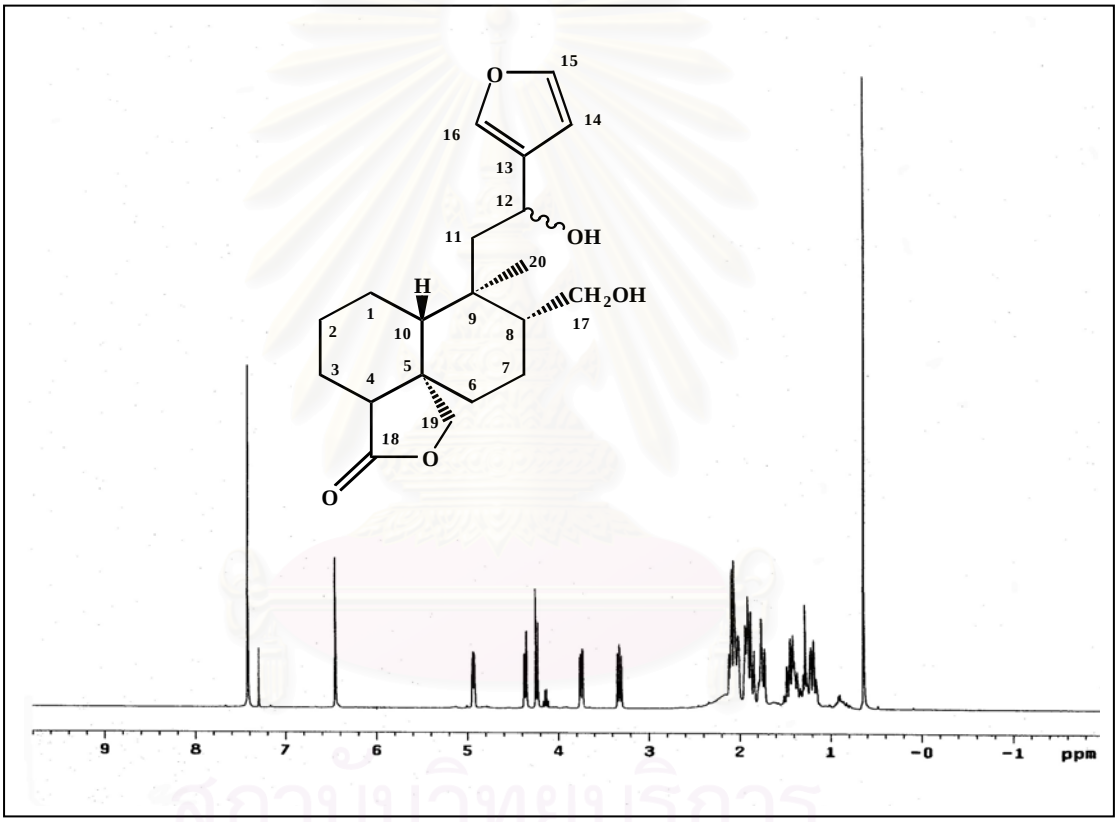


Figure 38. The ^{1}H-NMR spectrum of Compound **3b**.

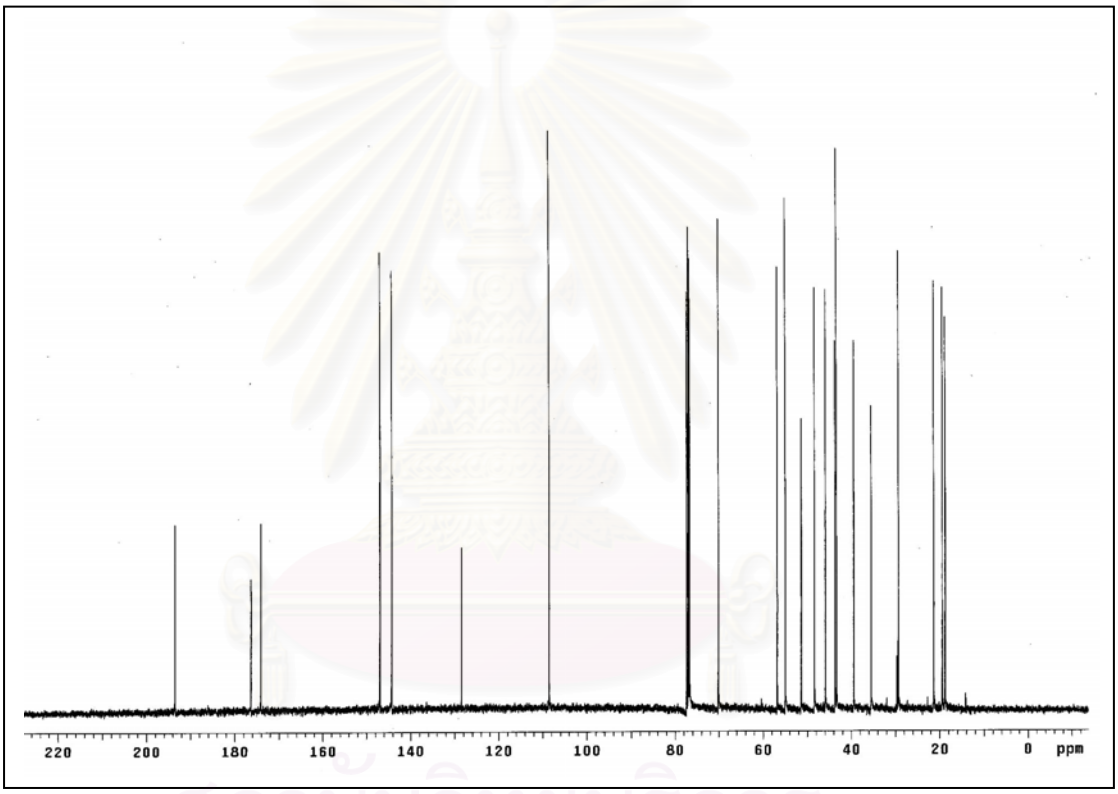
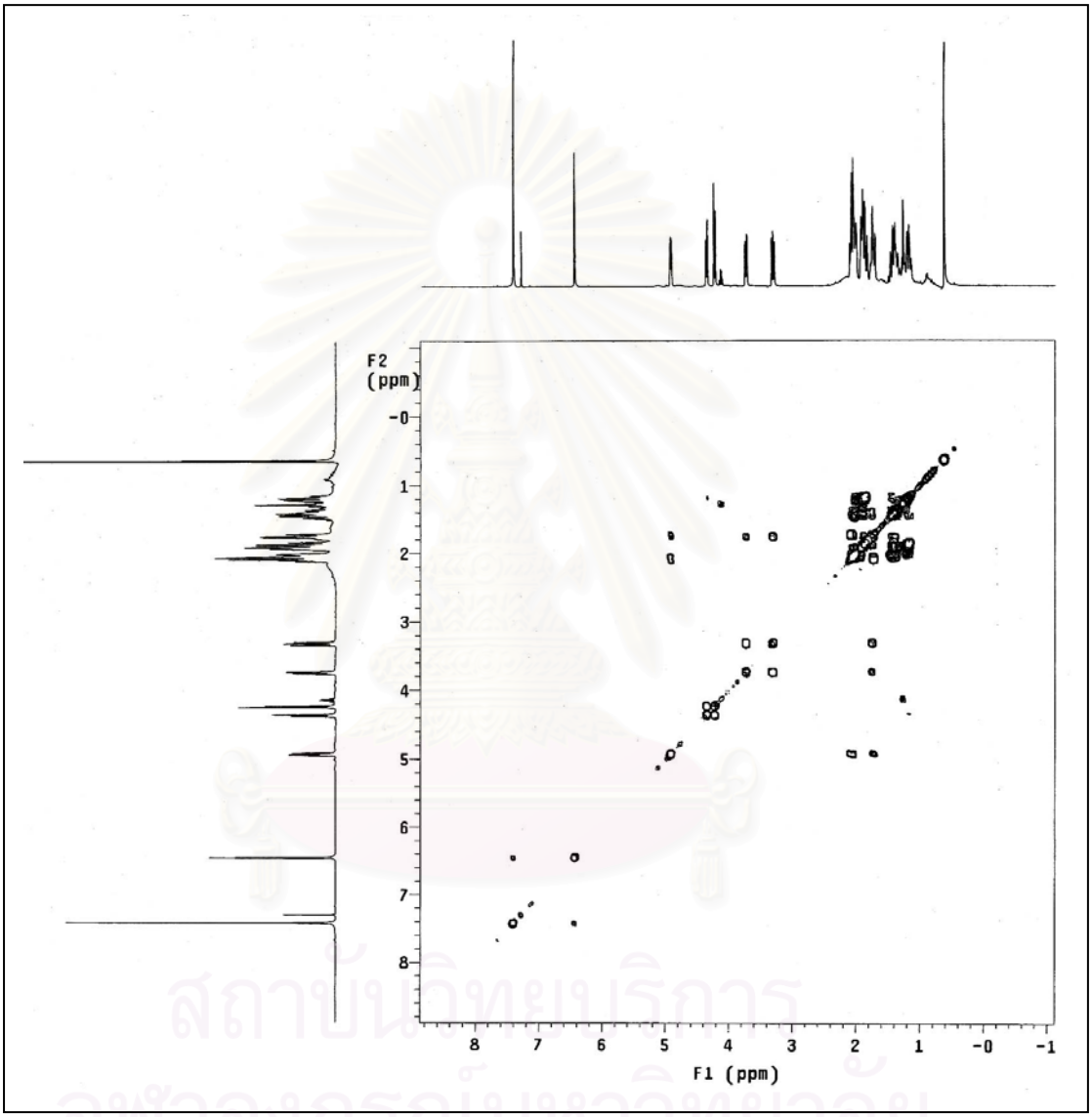


Figure 39. The ^{13}C-NMR spectrum of Compound **3b**.



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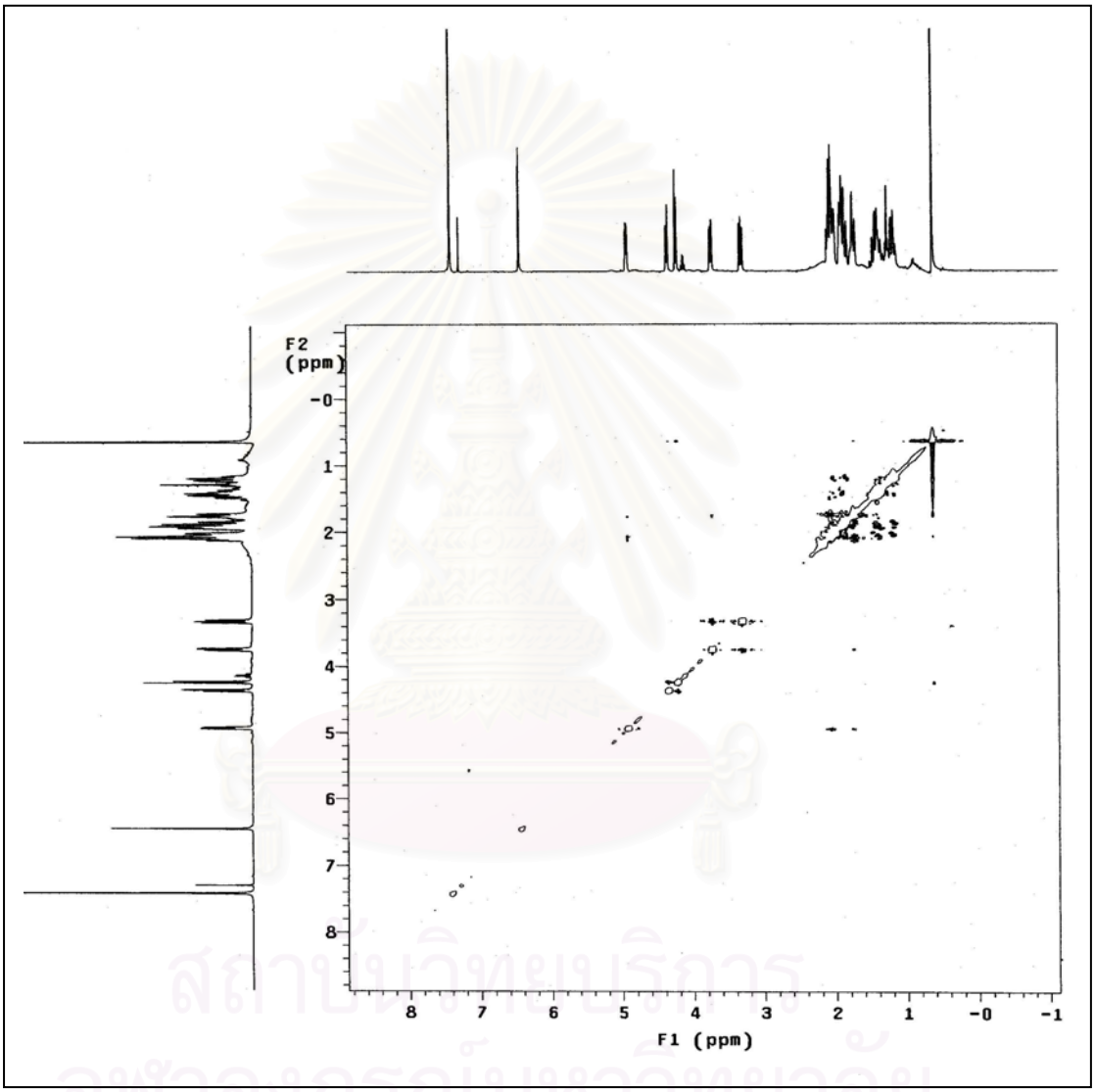

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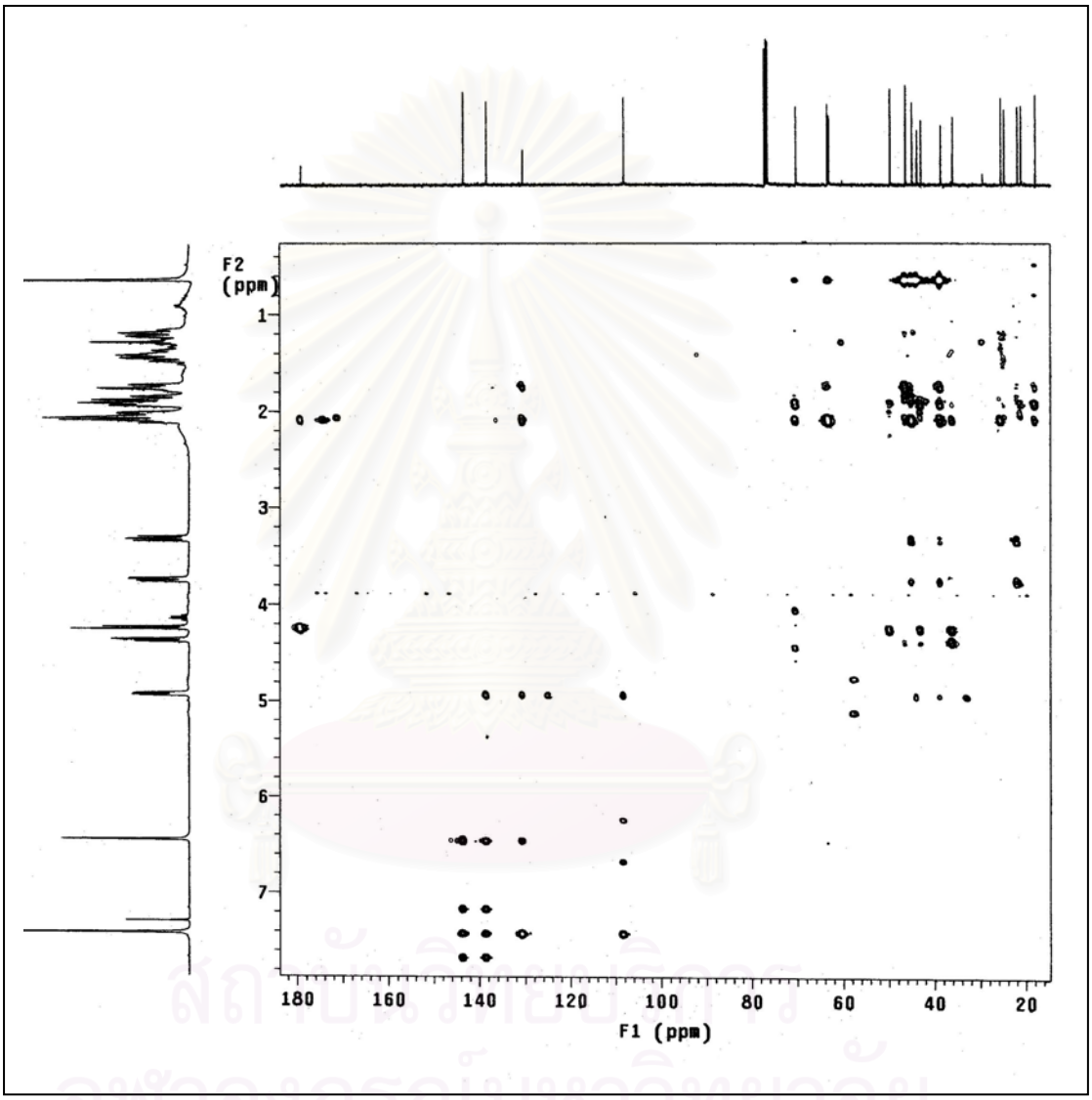


Figure 42. The gHMBC-NMR spectrum of Compound **3b**.

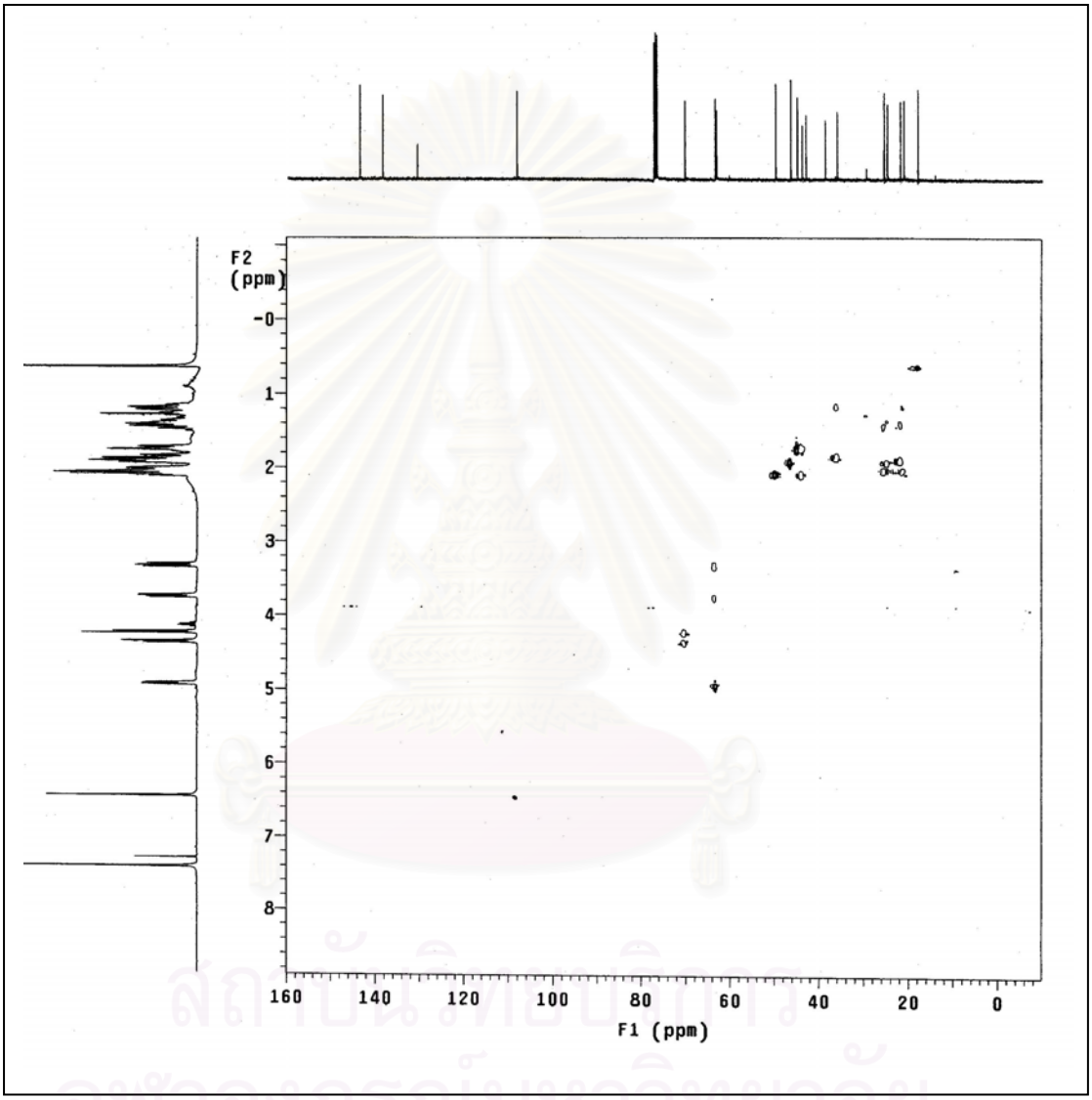


Figure 43. The gHSQC-NMR spectrum of Compound **3b**.

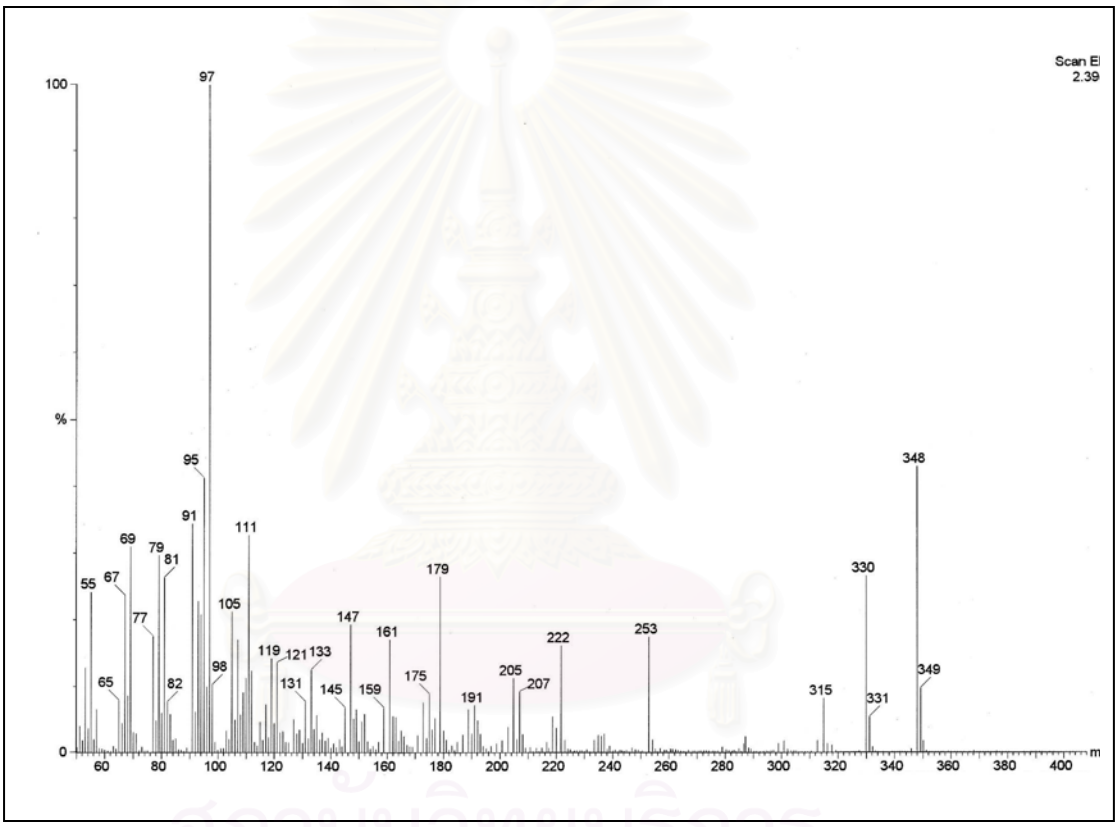
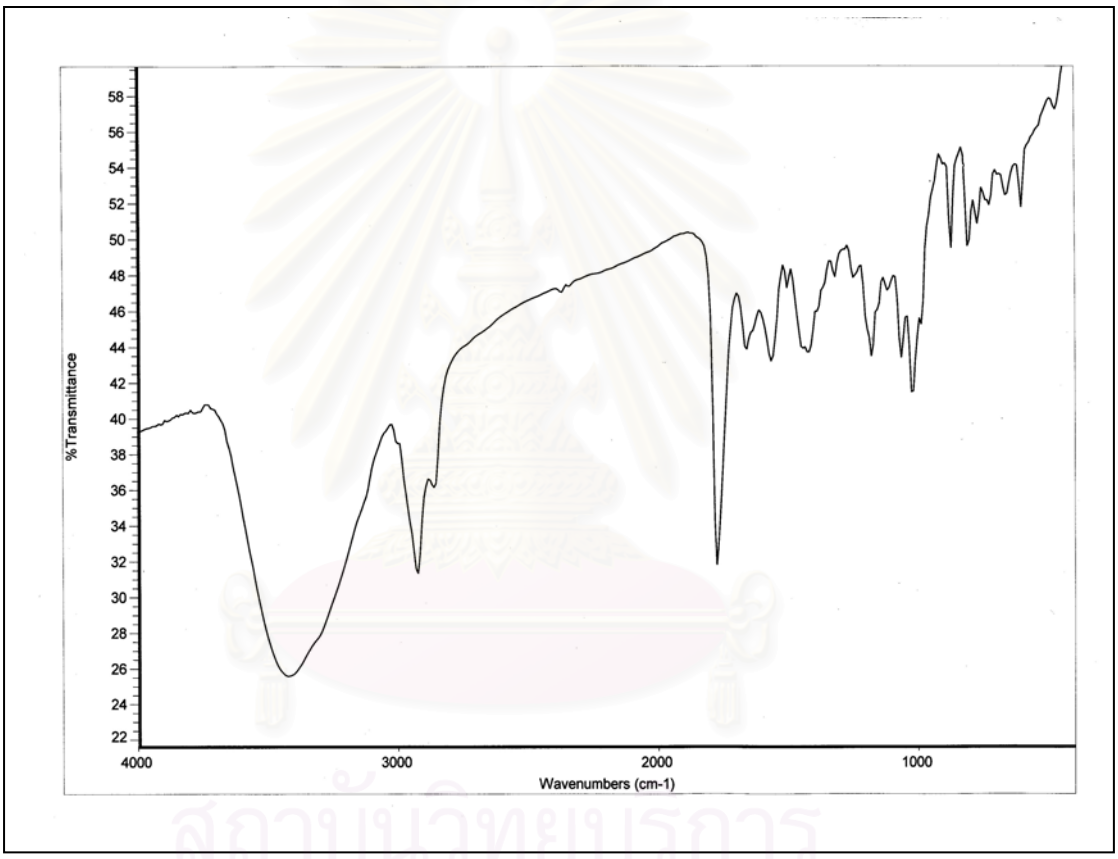


Figure 44. The EI MS spectrum of Compound **3b**.



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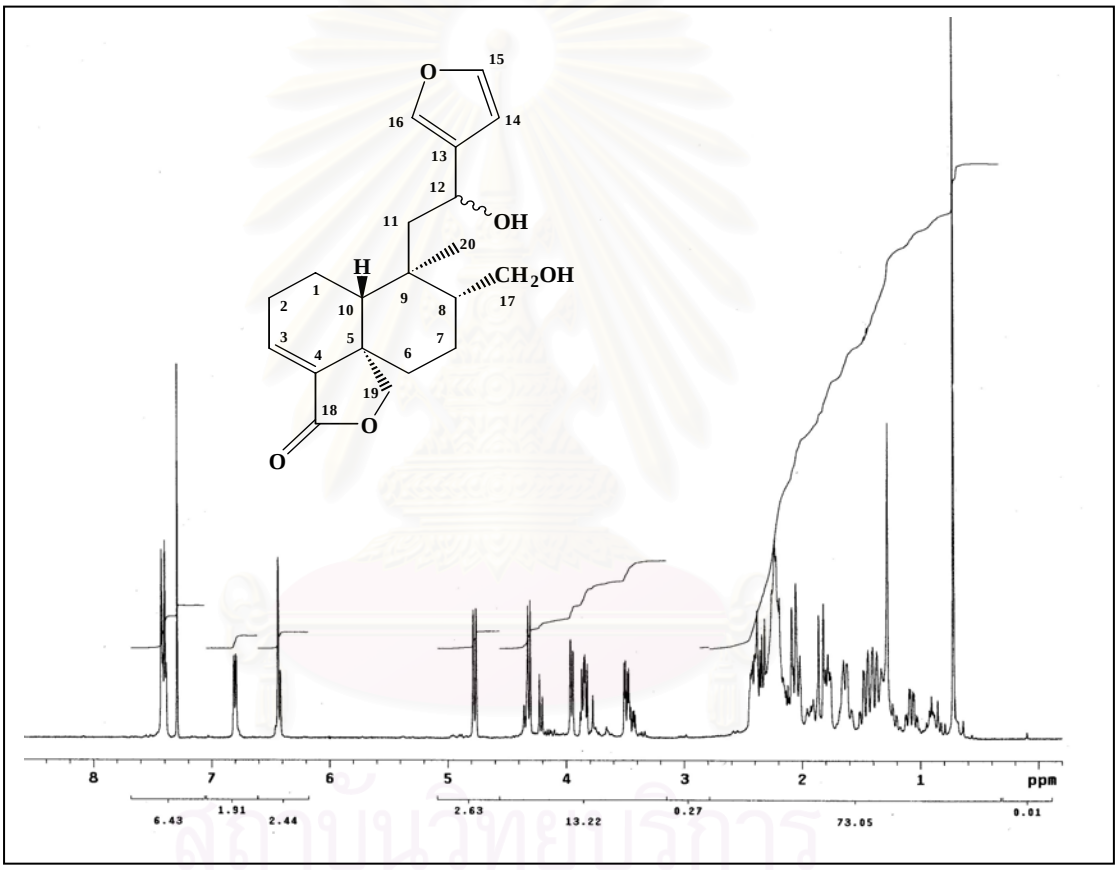

Figure 46. The ^{1}H-NMR spectrum of Compound **3c**.

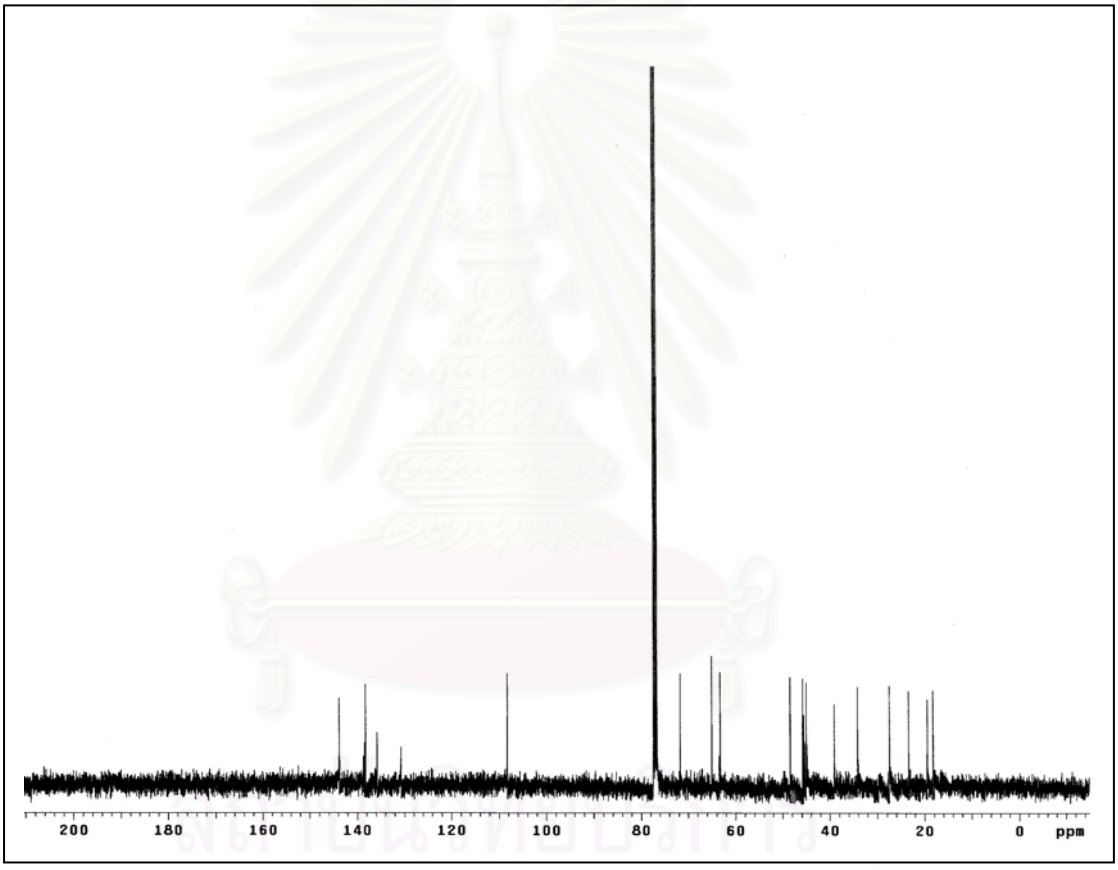

Figure 47. The ^{13}C-NMR spectrum of Compound **3c**.

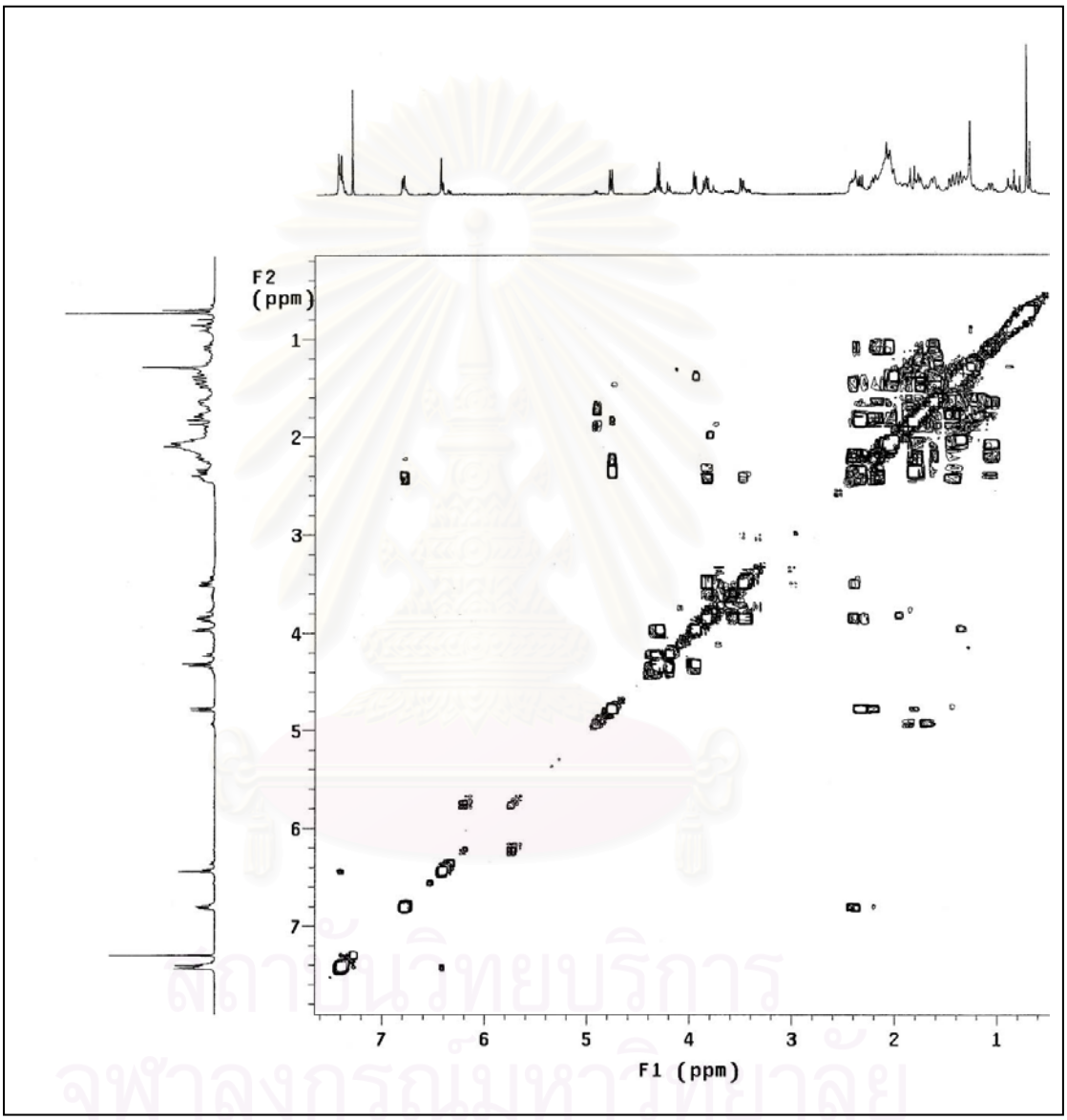

Figure 48. The gCOSY-NMR spectrum of Compound **3c**.

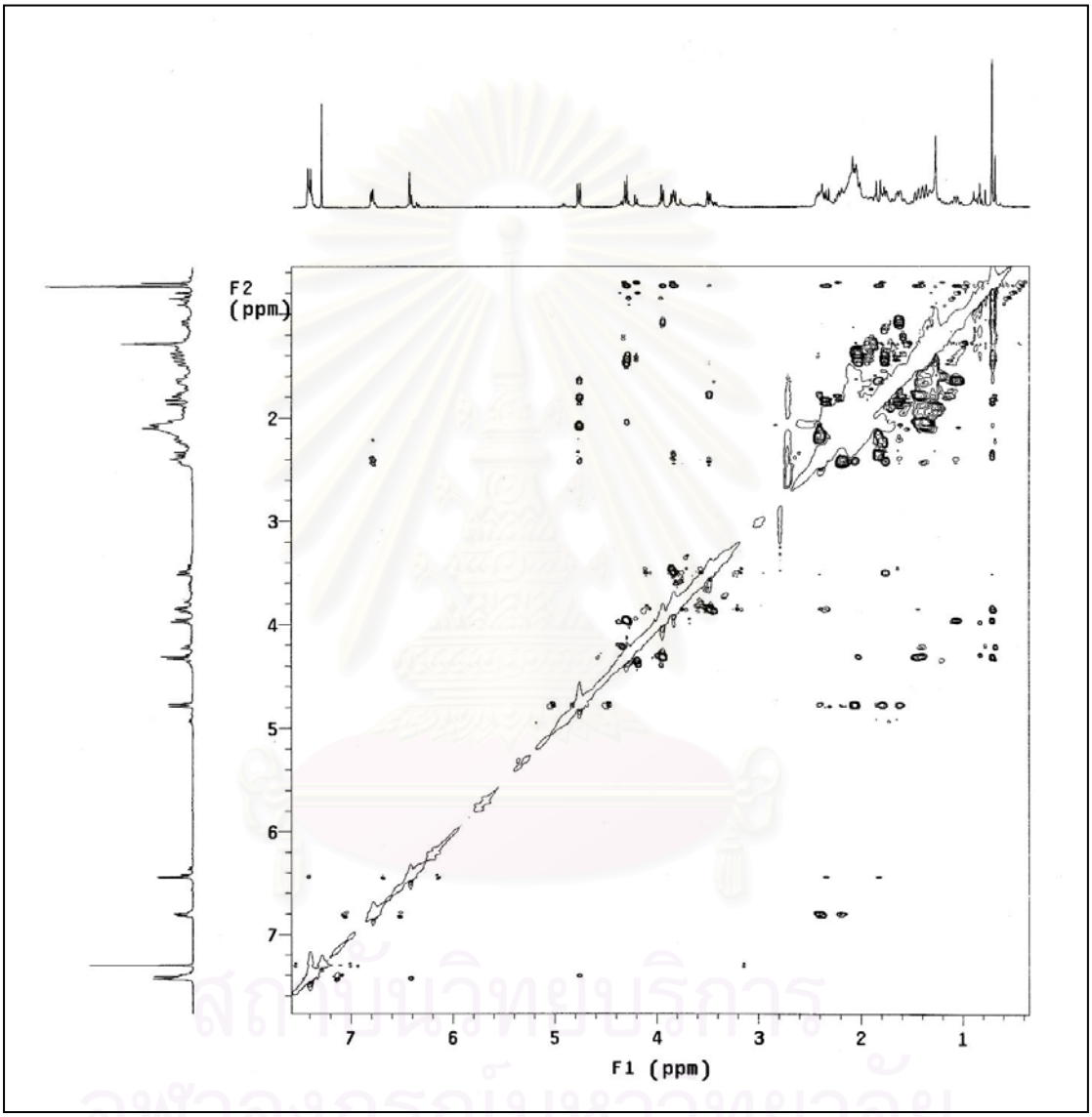

Figure 49. The gNOESY-NMR spectrum of Compound **3c**.

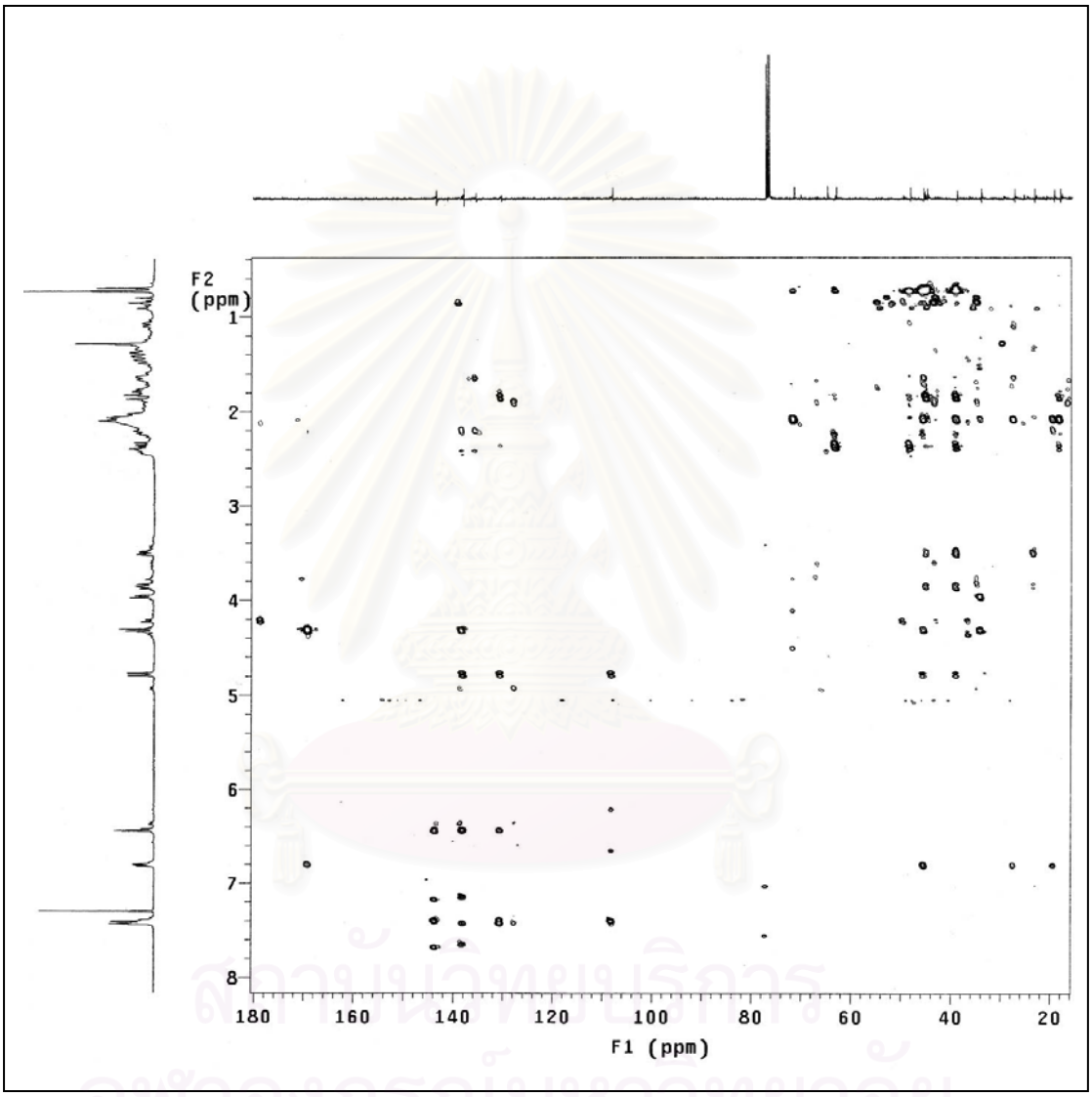

Figure 50. The gHMBC-NMR spectrum of Compound **3c**.

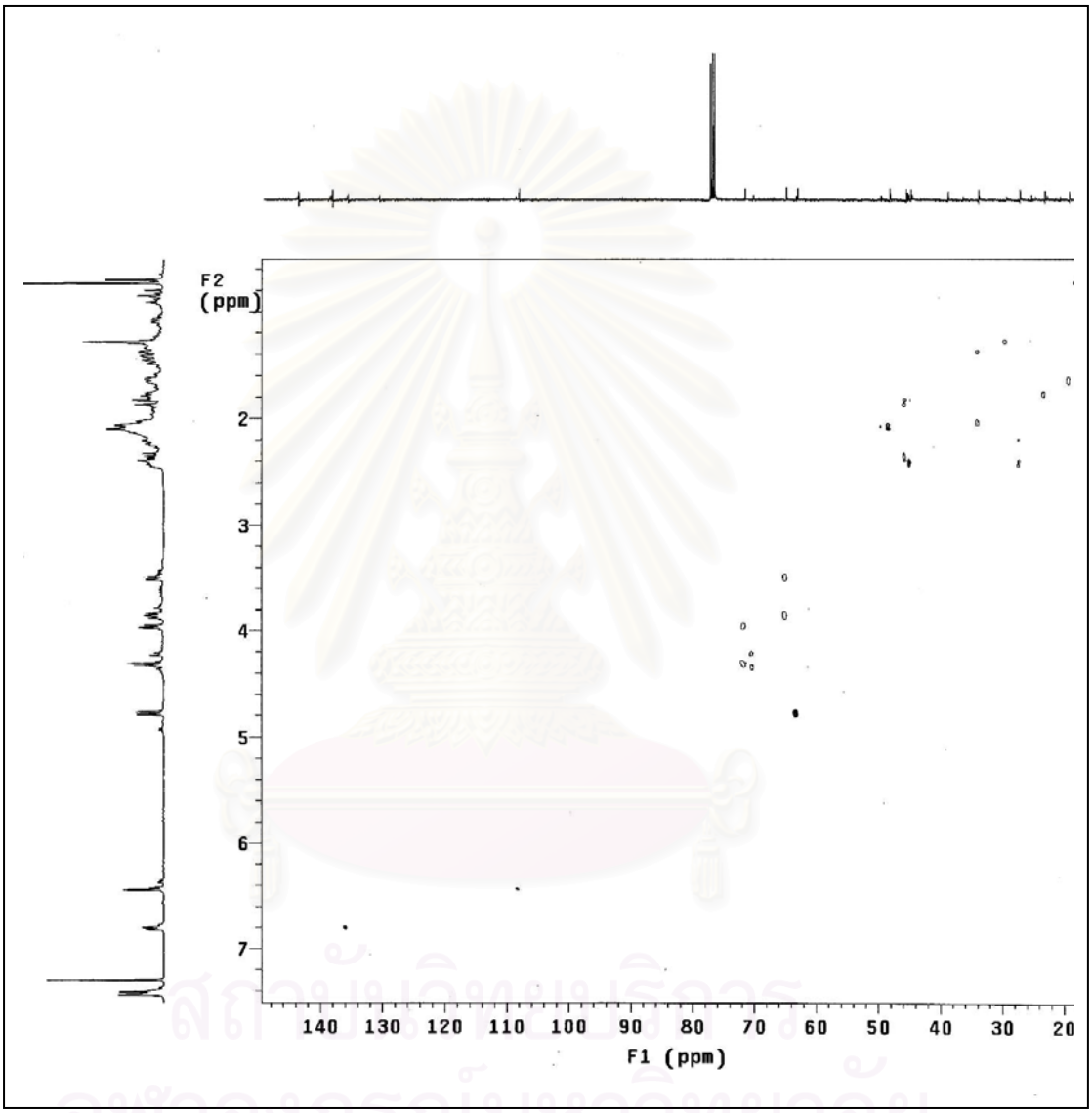

Figure 51. The gHSQC-NMR spectrum of Compound **3c**.

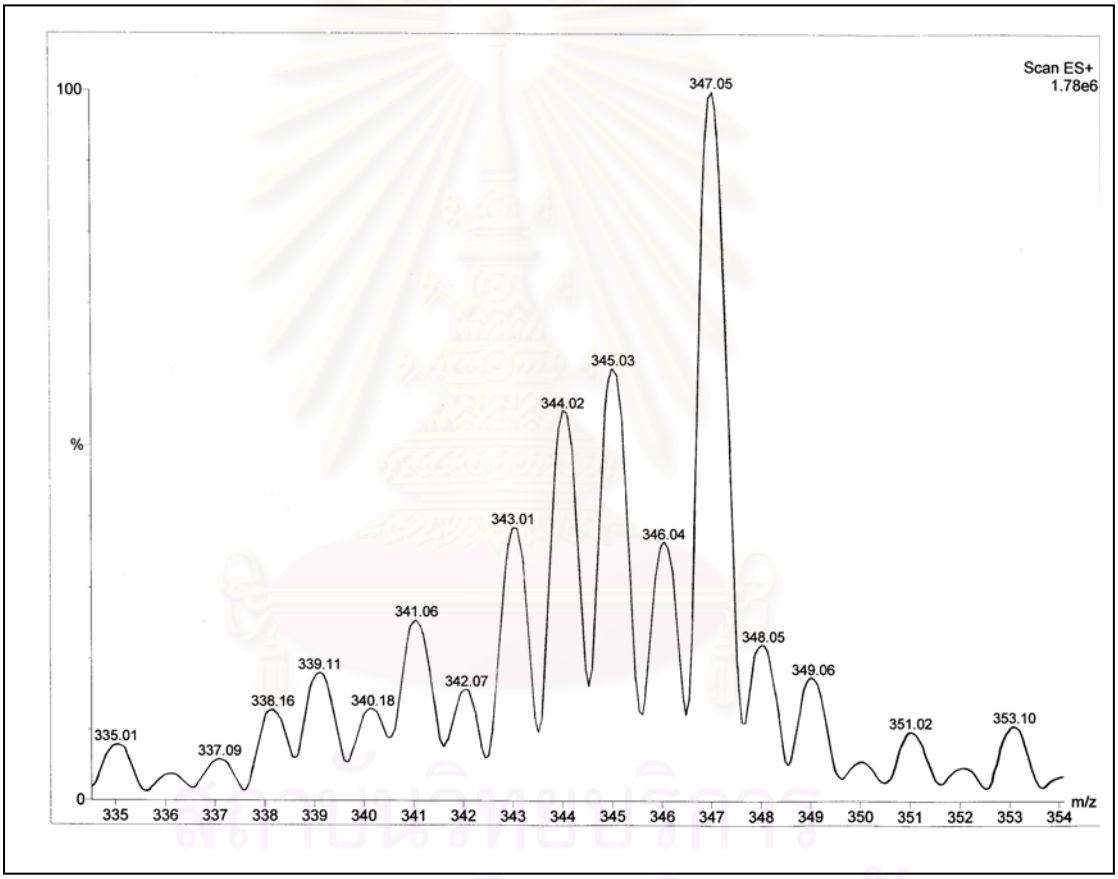

Figure 52. The ESI MS spectrum of Compound **3c**.

VITA

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