สารประกอบไดเทอร์พีนอยด์จากรากเปล้าใหญ่ *Croton oblongifulius* Roxb.

นายวิทูรย์ ยวงสะอาด

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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DITERPENOIDS FROM THE ROOTS OF *Croton oblongifolius* Roxb.

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วิทูรย์ ยวงสะอาด : สารประกอบไดเทอร์พีนอยด์จากรากเปล้าใหญ่ *Croton oblongifolius* Roxb. (DITERPENOIDS FROM THE ROOTS OF *Croton oblongifolius* Roxb.) อ.ที่ปรึกษา : รศ. ดร.นาตยา งามโรจนวณิชย์ อ.ที่ปรึกษาร่วม ดร.ประสาท กิตตะคุปต์, 107 หน้า

การศึกษาสารออกฤทธิ์ทางชีวภาพจากรากเปล้าใหญ่ (*Croton oblongifolius*) โดยนำสารสกัดไดคลอโรมีเทนจากรากเปล้าใหญ่ (*C. oblongifolius*) มาทำการแยกสารบริสุทธิ์โดยเทคนิคโครมาโทกราฟี สามารถแยกสารไดเทอร์พีนอยด์ได้ 9 ชนิด ซึ่งเป็นสารใหม่ 2 ชนิด คือ 19,20-dimethyl-15,16-epoxy-3,13(16),14-clerodatriene-17,18-oate-12-one และ methyl-15,16-epoxy-3,13(16),14-clerodatriene-18,19-olide-17-oate นอกจากนี้ยังพบสารที่มีรายงานมาแล้วอีก 7 ชนิด ได้แก่ levatin, crovatin, nasimalun A, nasimalun B, 15 hydroxy-cis-*ent*-cleroda-3,13(*E*)-diene, patchoulenone และ (-)-hardwickiic acid พิสูจน์ทราบสูตรโครงสร้างทางเคมี ของสารที่แยกได้ โดยอาศัยวิธีการทางเคมีและสเปกโทรสโกปี เมื่อนำสารบริสุทธิ์ที่แยกได้ไปทดสอบฤทธิ์ ทางชีวภาพ พบว่า patchoulenone มีฤทธิ์ยับยั้งเซลล์มะเร็ง P388 ด้วยค่า IC_{50} เท่ากับ 1.06 µg/ml ในขณะที่ 15 hydroxy-cis-*ent*-cleroda-3,13(*E*)-diene, nasimalun B, and methyl-15,16-epoxy-3,13(16),14-clerodatriene-18,19-olide-17-oate มีฤทธิ์ในการยับยั้งเซลล์มะเร็ง T47D ด้วยค่า IC_{50} เท่ากับ 17.0, 13.0, และ 10.0 µg/ml ตามลำดับ

ภาควิชา.....เคมี.....
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WITOON YOUNGSA-AD: DITERPENOIDS FROM THE ROOTS OF *Croton oblongifolius* Roxb. THESIS ADVISOR: ASSOC. PROF. NATTAYA NGAMROJANAVANICH, Ph.D, THESIS CO-ADVISOR: PRASAT KITTAKOOP, Ph.D., 107 pp.

The objective of this study was to search for bioactive compounds from the roots of *Croton oblongifolius*. The dichloromethane crude extract of the roots of *C. oblongifolius* was purified by chromatographic techniques to afford two new compounds, 19,20-dimethyl-15,16-epoxy-3,13(16),14-clerodatriene-17,18-oate-12-one and methyl-15,16-epoxy-3,13(16),14-clerodatriene-18,19-olide-17-oate, and seven known compounds namely levatin, crovatin, nasimalun A, nasimalun B, 15 hydroxy-cis-*ent*-cleroda-3,13(*E*)-diene, patchoulenone and (-)-hardwickiic acid. The chemical structures of all isolated compounds were established on the basis of chemical and spectroscopic methods. The isolated compounds were also subjected to bioactivity tests. Patchoulenone exhibited cytotoxicity against P388 cell line with IC_{50} value of 1.06 µg/ml. 15 Hydroxy-cis-*ent*-cleroda-3,13(*E*)-diene, nasimalun B, and methyl-15,16-epoxy-3,13(16),14-clerodatriene-18,19-olide-17-oate showed weak cytotoxicity against the T47D cell line with IC_{50} values of 17.0, 13.0, and 10.0 µg/ml, respectively.

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Field of Study... Chemistry..... Advisor's Signature N. Ngamrojanavanich

Academic Year .....2006..... Co-Advisor's Signature P. K.

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LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]_D^{23}$	=	Specific rotation at 23^0C and sodium D line (589 nm)
α	=	Alpha
β	=	Beta
δ	=	Chemical shift
A549	=	Human lung cancer, non-small cell
APCIMS	=	Atmospheric pressure chemical ionization
br	=	Broad
^{0}C	=	Degree Celsius
C	=	Carbon
ca	=	Approximate
calcd	=	Calculated
$CDCl_3$	=	Deuterated chloroform
cm	=	Centimeter
cm^{-1}	=	Reciprocal centimeter (unit of wave number)
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
DBE	=	Double Bond Equivalent
d	=	Doublet (for NMR spectra)
dd	=	Doublet of doublet (for NMR spectra)
ddd	=	Doublet of doublet of doublet (for NMR spectra)
dddd	=	Doublet of doublet of doublet of doublet (for NMR spectra)
ddt	=	Doublet of doublet of triplet (for NMR spectra)
dq	=	Doublet of quartet (for NMR spectra)
dt	=	Doublet of triplet (for NMR spectra)
DMAPP	=	Dimethylally pyrophosphate
DEPT	=	Distortionless Enhancement by Polarization Transfer
EIMS	=	Electron Impact Mass Spectrometry
EtOAc	=	Ethyl acetate

g	=	Gram
GGPP	=	Geranylgeranyl pyrophosphate
H	=	Proton
H69AR	=	Lung cancer, small cell, multidrug resistance
HeLa	=	Human cervical carcinoma
HepG2	=	Human hepatocellular carcinoma
HL-60	=	Human promyelocytic leukemia cell
^{1}H NMR	=	Proton Nuclear Magnetic Resonance
^{1}H-^{1}H COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
HMBC	=	^{1}H-detected Heteronuclear Multiple Bond Coherence
HMQC	=	^{1}H-detected Heteronuclear Multiple Quantum Coherence
HuCCA-1	=	Human cholangiocarcinoma
Hz	=	Hertz
IC_{50}	=	Median Inhibitory Concentration
IPP	=	Isopentenyl pyrophosphate
IR	=	Infrared Spectrum
J	=	Coupling constant
KB	=	Human epidermoid carcinoma in mouth
KBr	=	Potassium bromine
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
m	=	multiplet (for NMR spectra)
µg	=	Microgram
MeOH	=	Methanol
MDA-MB-231	=	Hormone-independent breast cancer
mg	=	Milligram
$[M+H]^+$	=	Protonated molecule
MHz	=	Megahertz
m.p.	=	Melting point

m/z	=	Mass to charge ratio
MS	=	Mass Spectrometry
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
No.	=	Number
NOESY	=	Nuclear Overhauser Effect Spectroscopy
n.r.	=	not resolved due to overlapping of signals.
P388	=	Mouse lymphoid neoplasm
ppm	=	part per million
ν_{max}	=	Wave number at maximal absorption
s	=	Singlet (for NMR spectra)
S102	=	Human liver cancer
spp.	=	Species
t	=	Triplet (for NMR spectra)
T47D	=	Hormone-dependent breast cancer
TLC	=	Thin Layer Chromatography
UV	=	Ultraviolet
UV-vis	=	Ultraviolet and Visible Spectruphotometry
wt/wt	=	weight by weight

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CHAPTER I
INTRODUCTION

The genus ***Croton*** belongs to the family Euphorbiaceae. They are a big genus and comprise of approximately 800 species. They are pantropical with the center of diversity in America and in Asia slightly less than 100 species. In Thailand there are about 30 species and they are a big genus in the North forest of Thailand [1,2]. The plants in the genus *Croton* contain various types of diterpenes that exhibit interesting biological activities such as anti-inflammatory [3], antimycobacterial, antimalarial [4], and cytotoxic activity [5]. In addition, several types of compounds have been isolated from *Croton*, and can be classified as sesquiterpenes, diterpenes, triterpenes and alkaloid compounds.

Croton are tree, shrubs or herbs. The followings are the descriptions of *Croton*: *Leaves* alternate to crowded, sometimes pseudo-verticillate; petiole distinct; blade simple, symmetric, variable in shape and margin, always with a pair of glands on the upper or lower base of blade or midrib or on the petiole apex. *Inflorescences* unbranched, a basal part pistillate with 1 flower per bract, more rarely completely pistillate or staminate. *Flowers* actinomorphic, pedicellate; sepals 5, ovate-elliptic; petals 5. *Staminate flowers*: stamens 10-20, inflexed in bud. *Pistillate flowers* petals much smaller than the sepals or more often absent; staminodes absent; ovary 3-locular, with 1 ovule per carpel. *Fruits* 3-locular, smooth or shallowly muriculate. *Seeds* 3 per fruit, dry, ellipsoid, brown, glabrous or (rarely) with scattered hairs, smooth, with or without a caruncle[1,2].

Various common names of *Croton oblongifolius* Roxb. have been called in each part of Thailand: Plao (เปล้า), Plao Yai (เปล้าใหญ่) (general); Chi-Mae-Chi-Cha, Chi-Mia-Chi-Yat-Apa (Akha-Chiang Rai); Say-Ga-Wa (Karen-Lamphun); Plao Luang (เปล้าหลวง) (Northern); Khwa-Wu (คะวู) (Karen-Kanchanaburi); Plaohua Kwan (เปล้าหัวขวาน) (Prachuap Khiri Khan); Po (เปาะ) (Kamphaeng Phet); Seng-Khe-Khang (เซ่งเค่คัง), Sa-Ka-Wa (สะกาวะ), Sa-Ku-Wa (ส่ากูวะ) (Karen-Mae Hong Son); Ha-Yoeng (ห้าเยิ่ง) (Shan-Mae Hong Son) [1].

Botanical characteristics of *Croton oblongifolius* Roxb. [1,2]

C. oblongifolius is a shrub or a tree up to 10(15) m tall. *C. oblongifolius* can be described as follow: *Bark* thin, smoot, cracked with age, grayish to tan, inner bark pink. *Leaves*: alternate (sometimes only in an apical whorl); petiole 1.0-1.2 cm; blade elliptic, 10-32 by 4-12 cm, margin distinctly serrate, apex acute to rounded, very sparsely pubescent to glabrous below. *Inflorescences*: greenwish-whithish, often several in an apical leafless whorl, 9-36 cm long, with 9-23 pistillate flowers, sometime completely staminate, without bisexual bracts. *Staminate flowers*: pedicel 2.5-5 mm long, densely pubescent; sepals 2.5-3 by 1.5 mm; petal 3 by 1 mm; stamens 10-12, glabrous or variously pubescent (rarely even on the anthers). *Fruits:* 6-7 mm, sulcate, surface sparsely but distinctly pubescent; pericarp quite thick. *Seeds*: 6 by 4mm, with a small caruncle.

The picture of *C. oblongifolius* Roxb. is shown in Figure 1.

C. oblongifolius is found in Thailand in the following areas. NORTHERN: Mea Hong Son, Chiang Mai, Chiang Rai, Phayao, Nan, Lamphun, Lampang, Phrae, Tak, Sukothai, Phitsanulok, Kampaeng Phet, Nakhon Sawan; NORTH-EASTERN: Phetchaboon, Loei, Nong Khai, Sakon Nakhon, Nakhon Phanom, Mukdahan; EASTERN: Chaiyaphum, Nakhon Ratchasima; SOUTH-WESTERN: Uthai Thani, Kanchanaburi, Phetchaburi, Prachuap Khiri Khan; CENTRAL: Saraburi, Nakhon Nayok; SOUTH-EASTERN: Sa Kaeo, Chanthaburi.

C. oblongifolius is also found in other parts of Asia, e.g. India, Nepal, Bhutan, Bangladesh, Burma, Laos, Cambodia, and Vietnam.

Our preliminary evaluation for biological activity revealed that the crude extract of *Croton oblongifolius* showed cytotoxicity against HuCCA-1, KB, HeLA, MDA-MB231, and T47D cell lines with IC_{50} values of 37.0, 30.0, 22.0, 18.0, and 17.0 µg/ml, respectively.

The objectives of this research are summarized as follow:

1. To extract, isolate, and purify chemical constituents from the roots of *C. oblongifolius*.

2. To elucidate structure of the isolated compounds by analysis of spectroscopic data.

3. To evaluate cytotoxic activity of the isolated compounds.

Figure 1: *Croton oblongifolius* Roxb.

CHAPTER II
LITERATURE REVIEW

2.1 Chemical constituents of the genus *Croton*.

Croton species have been shown to be sources of terpenoids, alkaloids, and flovanoids (Table 1).

Table 1: Compounds isolated from *Croton* species.

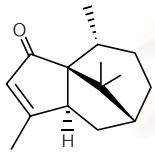
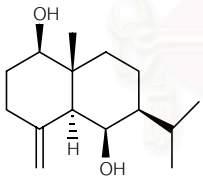
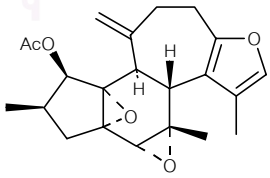
Compound	Category	Plant part	References
C. arboreous	Sesquiterpene	Aerial	[6]
5α,7α,10βH-3-Patchoulen-2-one 			
5α,10β-4(15)-Eudesmen-1β,6β-diol 	Sesquiterpene	Aerial	[6]
C. Haumanianus	Diterpene	Trunk bark	[7]
Crotohaumanoxide 			

Table 1: Compounds isolated from *Croton* species (continued).

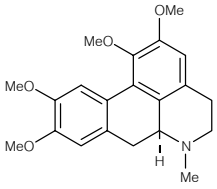
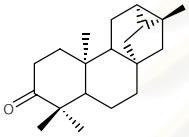
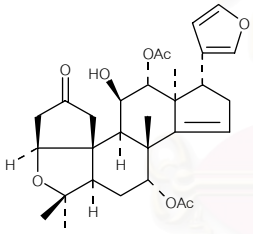
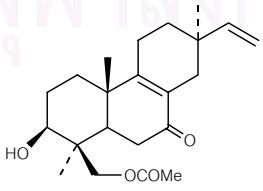
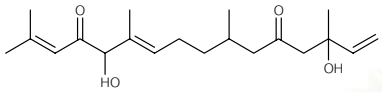
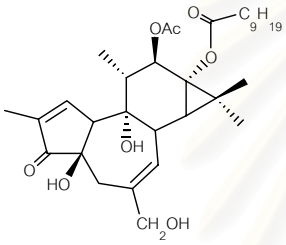
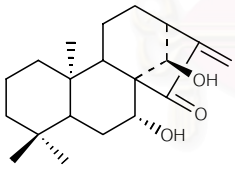
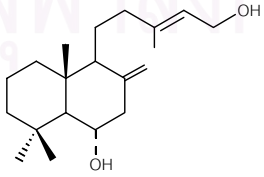
Compound	Category	Plant part	References
C. hemiargyreus Glaucine 	Alkaloid	Leaves and stem	[8]
C. insularis *ent*-Trachyloban-3-one 	Diterpene	Leaves	[9]
C. jatrophoides Zumsenol 	Triterpene	Roots	[10]
C. joufra 3β-Hydroxy-19-O-acetyl-pimara-8(9),15-dien-7-one 	Diterpene	Leaves	[11]

Table 1: Compounds isolated from *Croton* species (continued).

Compound	Category	Plant part	References
C. salutaris (10E)-3,12-Dihydroxy-3,7,11,15-tetramethyl-1,10,14-hexadecatrien-5,13-dione 	Diterpene	Twig	[12]
C. tiglium 12-O-Acetylphorbol-13-decanoate 	Diterpene	Seed	[13]
C. tonkinensis *ent*-7α,14β-Dihydroxykaur-16-en-15-one 	Diterpene	Leaves	[14]
C. zambesicus Crotonadiol 	Diterpene	Stem bark	[15]

2.2 Chemical constituents of *C. oblongifolius*.

In 1968, Rao and coworkers isolated a diterpene alcohol, oblongifoliol, together with β-sitosterol from the barks of *C. oblongifolius* [16].

In 1969, Aiyar and Seshadri found a deoxyoblongifoliol from the stem barks of *C. oblongifolius* [17].

In 1970, Aiyar and Seshadri studied the structure of oblongifolic acid, the major diterpene acid component of the barks, which was assigned as (+)-isopimara-7(8),15-diene-19-oic acid [18].

In 1971, Aiyar and Seshadri found three new minor components from the stem barks, including *ent*-isopimara-7,15-diene, 19-hydroxy-*ent*-isopimara-7,15-diene, and *ent*-isoimara-7,15-diene-19-aldehyde [19]. In the same year, they also isolated oblongifoliol and deoxyoblongifoliol which were assigned as *ent*-isopimara-7,15-diene-3β-ol, and *ent*-isopimara-7,15-diene-3β,19-diol, respectively [20]. Moreover, acetyl aleuritolic acid and 3β-acetoxy-olean-14(15)-ene-28-oic acid were found from the stem barks of this plant [21].

In 1972, Aiyar and Seshadri found two closely related furanoid diterpenes from the barks. One was *ent*-15,16-epoxy-3,11,13(16),14-clerodateraen-19-oic acid which given the trivial name 11-dehydro-(-)-hardwickiic acid and the second was (-)-hardwickiic acid [22]. In the same year, they studied other parts of *C. oblongifolius* including the roots, woods, and leaves. Most compounds reported were isolated from the stem barks, not from the woods (poor yield), while the leaves gave only waxy materials [23].

In 1998, Roengsumran and coworkers isolated two new cembranoids, crotocembraneic acid and neocrotocembraneic acid from the stem barks of *C. oblongifolius* [24].

In 1999, Roengsumran and coworkers isolated four new labdane diterpenoids, labda-7,12(E),14-triene, labda-7,12(E),14-triene-17-al, labda-7,12(E),14-triene-17-ol, and labda-7,12(E),14-triene-17-oic acid from the stem barks of *C. oblongifolius* [25]. In the same year, they also found a new cembranoid diterpene and neocrotocembranal

in the stem barks of *C. oblongifolius*. These compounds inhibited platelet aggregation induced by thrombin, and also exhibited cytotoxicity against P-388 cells [26].

In 2001, Roengsumran and coworkers reported the presence of three labdane diterpenes, 2-acetone-3-hydroxy-labda-8(17),12(E),14-triene, 3-acetoxy-2-hydroxy-labda-8(17),12(E)-14-triene, and 2,3-dihydroxy-labda-8(17),12(E)-14-triene in the stem barks of *C. oblongifolius*. These compounds showed significant cytotoxicity against various human tumor cell lines [27].

In 2002, Roengsumran and coworkers found a new furoclerodane, croblongifolin, together with a known clerodane, crovatin, and a known labdane, nodorellol, in the stem barks of *C. oblongifolius*. These diterpenes exhibited significant cytotoxicity against various human tumor cell lines, including HEP-G2, SW620, CHAGO, KATO3, and BT474 [5].

In 2004, Roengsumran and coworkers found three new halimane-type diterpenoids, 12-benzoyloxycrotohalimaneic acid, crotohalimaneic acid, and crotohalimonelic acid in the stem barks of *C. oblongifolius*. The isolated compounds showed significant cytotoxicity against various human tumor cell lines [28].

The diterpenes isolated from *C. oblongifolius* can be classified into 10 groups [44], as shown in the Table 2 and Figure 2

Table 2: Diterpenes isolated from *C. oblongifolius*.

Compound	Plant part	Reference
1. Cembrane diterpenoids		
crotochembraneic acid	Stem Bark	[24,29,30,31]
neocrotocembraneic acid	Stem Bark	[24,30,31]
	Leaves	[32]
neocrotocembranal	Stem Bark	[26,30]
polianeic acid	Stem Bark	[33]
(2E,7E,11E)1-isopropyl-1,4-dihydroxy-4,8-dimethylcyclotetradeca-2,7,11-triene-12-carboxilic acid	Stem Bark	[31]
2. Clerodane diterpenoids		
crovatin	Stem Bark	[30,34]
croblongifolin	Stem Bark	[5]
isokolavenol	Stem Bark	[30]
nasimalun A	Stem Bark	[35,44]
(-)-hardwickiic acid	Stem Bark	[22,29,30,35, 36,37,38,39,44]
	Roots	[23]
(-)-20 benxyloxyhardwickiic acid	Stem Bark	[36,38]
11-dehydro-(-)-hardwickiic acid	Stem Bark	[22]
	Roots	[23]
methyl-15,16-epoxy-12-oxo-3,13(16),14-clerodatriene-20,19-olide-17-oate	Stem Bark	[31]
3. Cleistanthane diterpenoids		
cleistantha-4,13(17),15-triene-3-oic acid	Stem Bark	[34]
cleistantha-4(18),13(17),15-triene-3-ol	Stem Bark	[34]
cleistantha-4(18),12,15-triene-3-oic acid	Stem Bark	[34]
methyl-cleistantha-4(18),13(17),15-triene-3-oate	Stem Bark	[34]
3,4-*seco*-cleistantha-4(18),13(17),15-triene-3-oic acid	Stem Bark	[34,37,45]

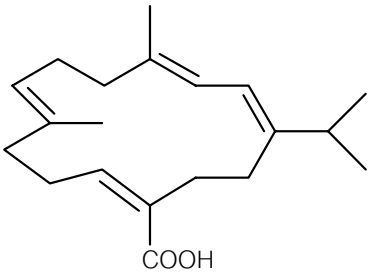
Table 2: Diterpenes isolated from *C. oblongifolius* (continued).

Compound	Plant part	Reference
3. Cleistanthane diterpenoids (continued)		
cleistantha-4,13(17),15-triene-3-oic acid	Stem Bark	[37]
3,4-*seco*-13,17-epoxycleistantha-4(18),15-diene-3-oic acid	Stem Bark	[45]
3,4-*seco*-8,14-epoxypimara-4(18),15-diene-3-oic acid	Stem Bark	[45]
3-hydroxycleistantha-13(17),15-diene	Stem Bark	[45]
4. Kaurane diterpeneoids		
kaur-16-en-19-oic acid	Stem Bark	[39]
kaur-16-en-19-ol	Stem Bark	[39]
methyl-kaur-16-en-19-oate	Stem Bark	[39]
16,17-epoxy-kaur-19-oic acid	Stem Bark	[39]
17-hydroxykaur-15-en-19-oic acid	Stem Bark	[39]
5. Labdane diterpenoids		
nidorellol	Stem Bark	[5,30,33]
labda-7,12(E),14-triene	Stem Bark	[25,40]
labda-7,12(E),14-triene-17-al	Stem Bark	[25,40]
labda-7,12(E),14-triene-17-ol	Stem Bark	[25,40]
labda-7,12(E),14-triene-17-oic acid	Stem Bark	[25,38,40]
3-acetoxy-labda-8(17),12(E),14-triene-2-ol	Stem Bark	[27,38,40,41]
2-acetoxy-labda-8(17),12(E),14-triene-3-ol	Stem Bark	[17,38,40,41]
labda-8(17),12(E),14-triene-2,3-diol	Stem Bark	[38,41]
labda-7,13(Z)-diene-17,12-olide	Stem Bark	[36]
labda-7,13(Z)-diene-17,12-olide-15-ol	Stem Bark	[36]
6-acetoxy-12(E),14-labdadiene-7,8-diol	Stem Bark	[33]
12(E),14-labdadiene-6,7,8-triol	Stem Bark	[33]
12(E),14-labdadiene-6,7-diol	Stem Bark	[33]
12,15-epoxy-8(17),12,14-labdatriene	Stem Bark	[42]
ent-8(17),12(E),14-labdatriene-18-oic acid	Stem Bark	[42]

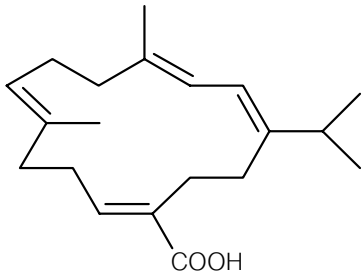
Table 2: Diterpenes isolated from *C. oblongifolius* (continued).

Chemical compound	Plant part	Reference
5. Labdane diterpenoids (continued)		
(5S,8S,9S,10R,12S,13S)-8,13-epoxy-12-hydroxy-labda-1,14-dien-3-ol	Stem Bark	[43]
(5S,8S,9S,10R,12S,13S)-8,13-epoxy-12-hydroxy-labda-1,14-dien-3-one	Stem Bark	[43]
6. Halimane diterpenoids		
crotohalimaneic acid	Stem Bark	[26,30]
benzoyl crotohalimaneic acid	Stem Bark	[26,30]
crotohalimoneic acid	Stem Bark	[30]
7. Pimarane diterpenoids		
oblongifoliol	Stem Bark	[16]
	Roots	[23]
oblongifolic acid	Stem Bark	[18]
	Roots	[23]
(-)-pimara-9(11),15-diene-19-oic acid	Stem Bark	[31]
(-)-pimara-9(11),15-diene-19-ol	Stem Bark	[31]
3-deoxyoblongifoliol	Stem Bark	[19]
	Roots	[23]
19-deoxyoblongifoliol	Stem Bark	[17]
	Roots	[23]
8. Isopimarane diterpenoids		
ent-isopimara-7,15-diene	Stem Bark	[20]
ent-isopimara-7,15-diene-19-aldehyde	Stem Bark	[19]
9. Abitane diterpene		
aebita-7,13-diene-3-one	Stem Bark	[37]
10. Trachylobane diterpenoid		
trachylobane-19-oic acid	Stem Bark	[33]

1. Cembrane diterpenoids

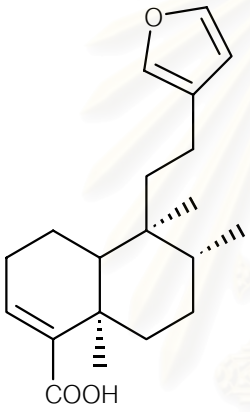


Crotocembraneic acid

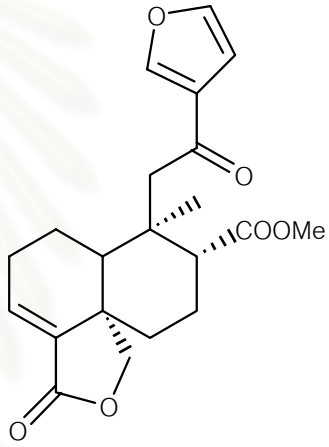


Neocrotocembraneic acid

2. Clerodane diterpenoids

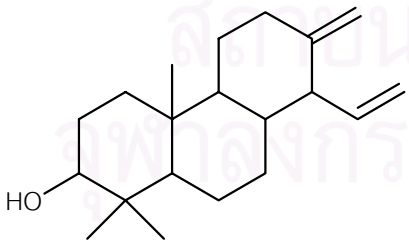


(-)-Hardwickiic acid

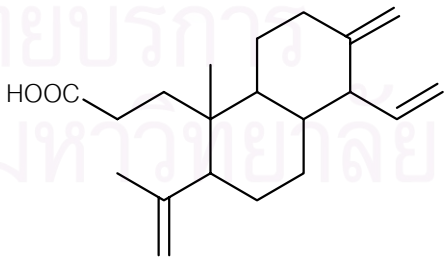


Nasimalun A

3. Cleistanthane diterpenoids



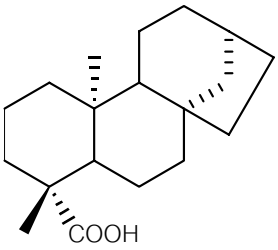
3-Hydroxycleistantha-13(17),15-diene



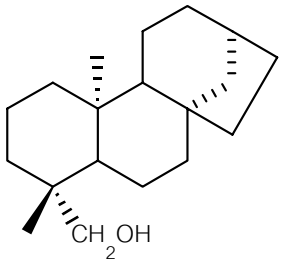
3,4-*seco*-Cleistantha-4(18),13(17),15-triene-3-oic acid

Figure 2: The structures of diterpenes isolated from *C. oblongifolius*.

4. Kaurane diterpeneoids

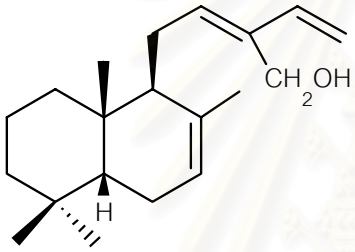


Kaur-16-en-19-oic acid

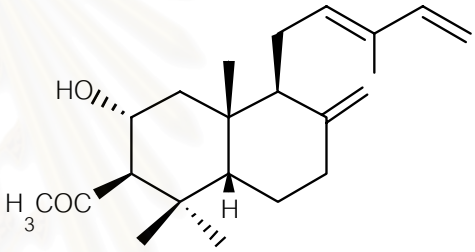


Kaur-16-en-19-ol

5. Labdane diterpenoids

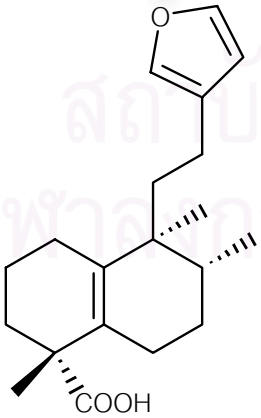


Labda-7,12(E),14-triene-17ol

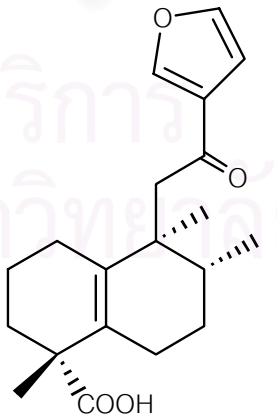


2-Acetoxy-labda-8(17),12(E),14-triene-3-ol

6. Halimane diterpenoids



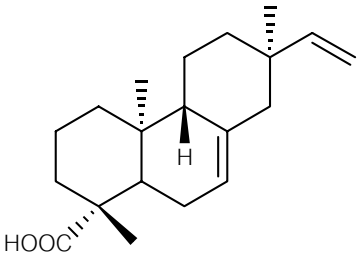
Crotohalimaneic acid



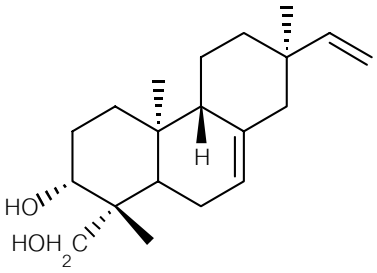
Crotohalimoneic acid

Figure 2: The structures of diterpenes isolated from *C. oblongifolius*.

7. Pimarane diterpenoids

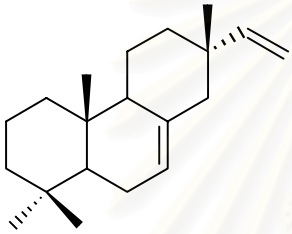


Oblongifolic acid

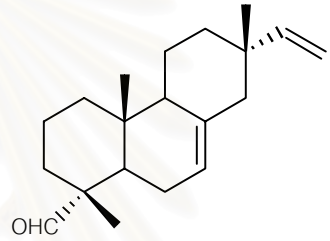


Oblongifoliol

8. Isopimarane diterpenoids

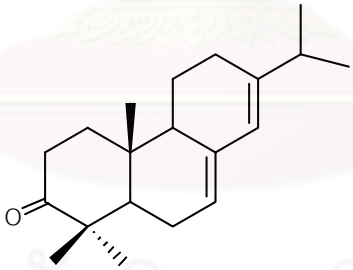


ent-isopimara-7,15-diene



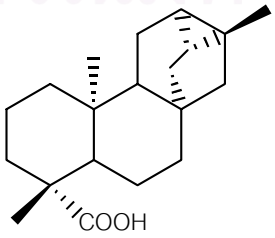
ent-isopimara-7,15-diene-19-aldehyde

9. Abitane diterpene



Aeita-7,13-diene-3-one

10. Trachyloban diterpenoids

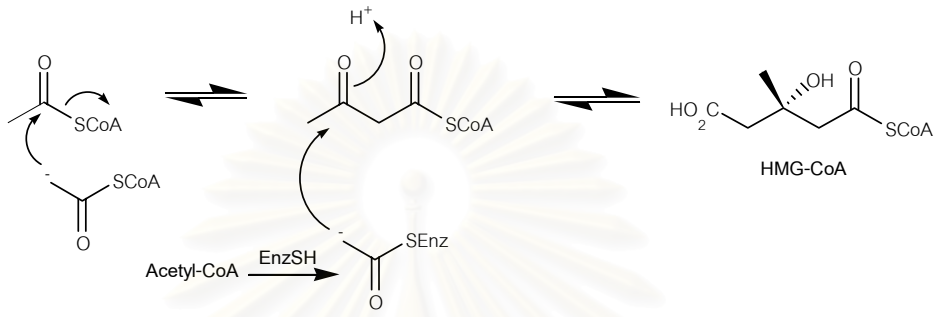


Trachyloban-19-oic acid

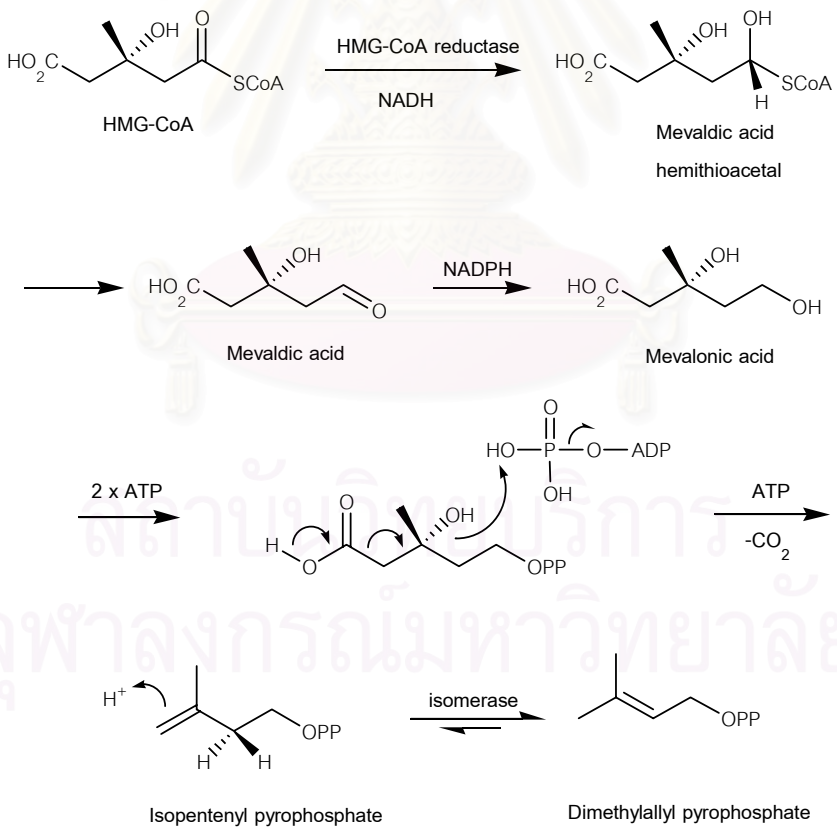
Figure 2: The structures of diterpenes isolated from *C. oblongifolius*.

2.3 Biosynthesis of diterpene compounds.

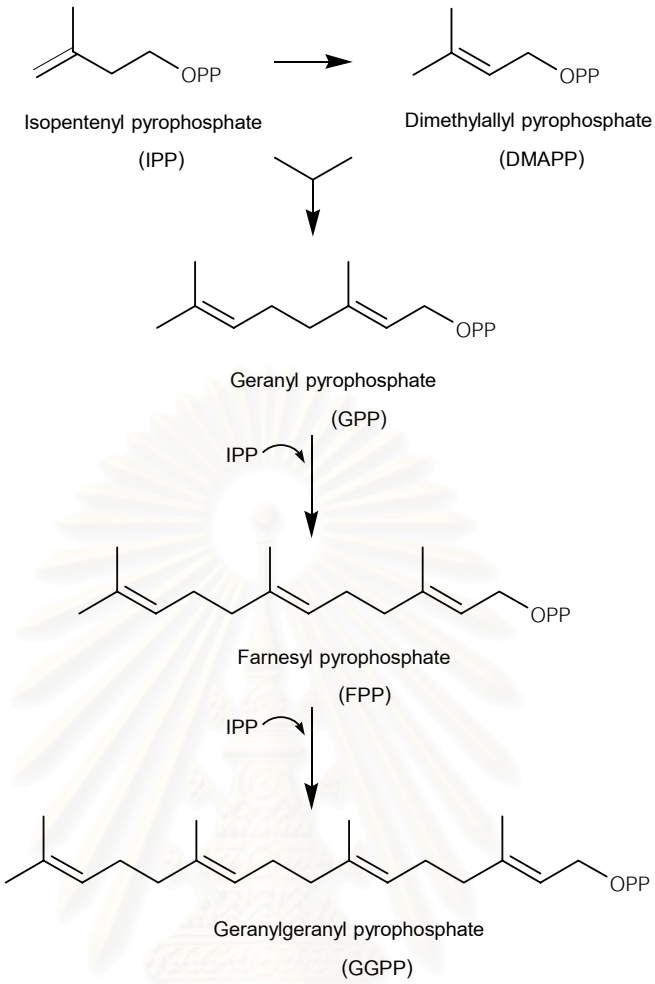
The diterpenes prosess twenty carbon atoms in their molecule. They are biogenetically derived from geranylgeranyl pyrophosphate (GGPP) by the addition of a futher isopentenyl pyrophosphate (IPP) molecule to farnesyl diphosphate, as shown in Schemes 1-3 [46].



Scheme 1: Biosynthesis of 3-hydroxy-3-methylglutaroyl coenzyme A.



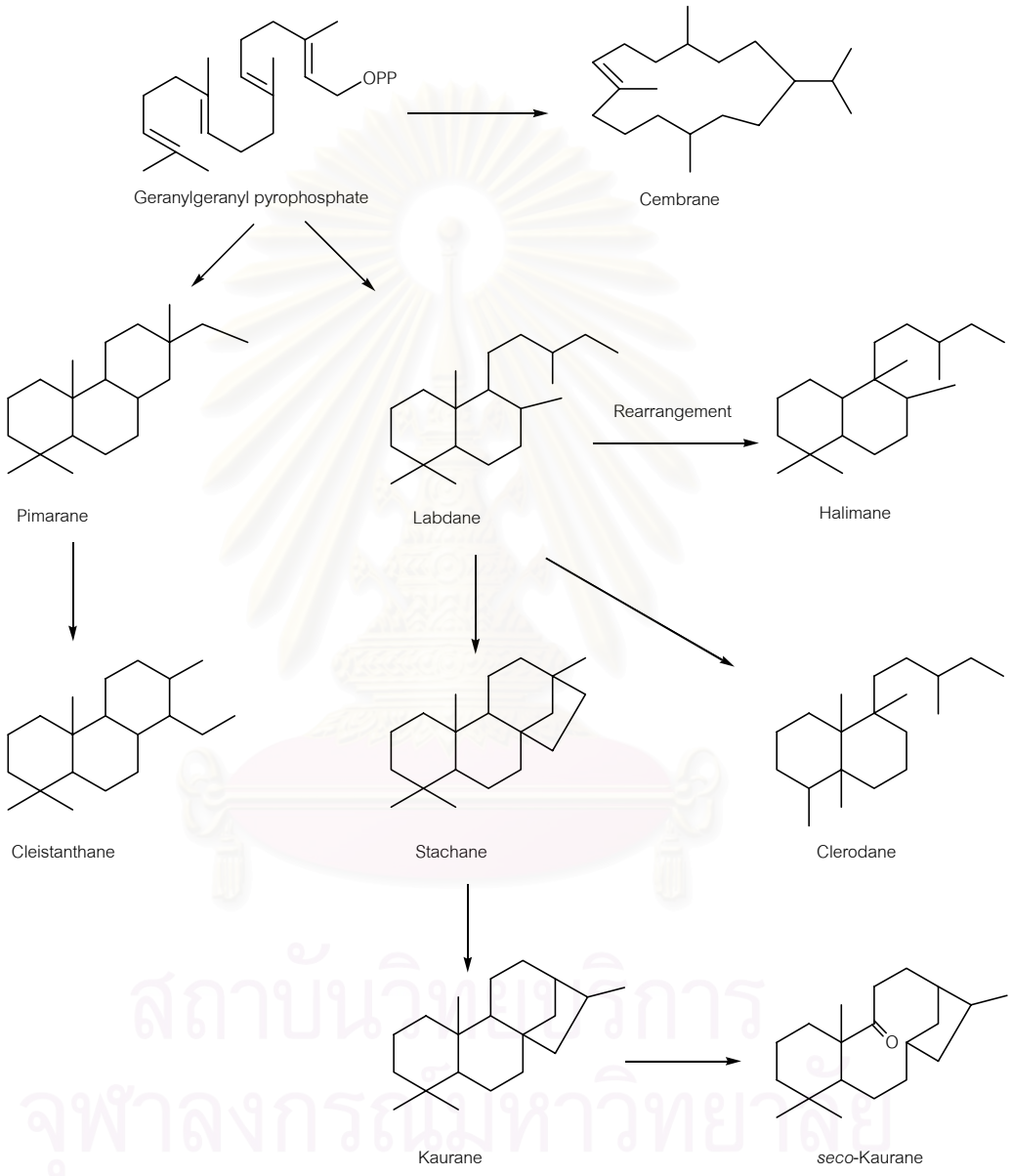
Scheme 2: Origin of the isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP).



Scheme 3: Biosynthetic of geranylgeranyl pyrophosphate (GGPP).

2.4 Biosynthetic relationship of diterpene compounds.

The typical diterpenes in *Croton* species are casbane, cembrane, cleisthathane, clerodane, kaurane, labdane, pimarane, and halimane. The biosynthetic relationships among diterpenes are displayed in Scheme 4 [47].



Scheme 4: Biosynthetic relationships of diterpenes in *Croton* species.

CHAPTER III

EXPERIMENTS

3.1 Plant material.

The roots of *Croton oblongifolius* Roxb. (Plao-Yai) were collected from Nakhon Sawan Province, Thailand, in August 2005.

3.2 Instruments and equipments.

3.2.1 Nuclear magnetic resonance spectrometer (NMR).

NMR spectra were recorded on a Bruker AM-400 (400 MHz) spectrometer operated at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

3.2.2 Mass spectrometer (MS).

EI-MS spectra were obtained from Finnigan Mat GCQ mass spectrometer.

Accurate mass was obtained from the time of flight (TOF) technique, using a Micro TOF, Bruker daltoincs by APCI ionization mode or ESI mode.

3.2.3 Fourier transform infrared spectrophotometer (FT-IR).

FT-IR spetra were recorded on a Perkin Elmer Spectrum One spectrophotometer.

3.2.4 Ultraviolet-visible spectrometer (UV-vis).

UV-vis spectra were recorded on a Shimadzu UV-vis 2001s spectrophotometer.

3.2.5 Optical rotation.

Optical rotations were recorded with a sodium D line, using a JASCO DPI-370 digital polarimeter.

3.2.6 Melting point

Melting points were recorded on a Büchi 535.

3.3 Chemicals.

3.3.1 Solvents.

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

3.3.2 Other chemicals.

3.3.2.1 Sephadex LH-20 (No. 17-009-01)

3.3.2.2 Merck's silica gel 60 Art. 1.07734.1000 (70-230 mesh ASTM) was used as adsorbent for column chromatography.

3.3.2.3 Merck's TLC aluminium sheet, silica gel 60F 254 precoated 25 sheets, 20x20 cm, layer 0.2 mm was used for thin layer chromatography.

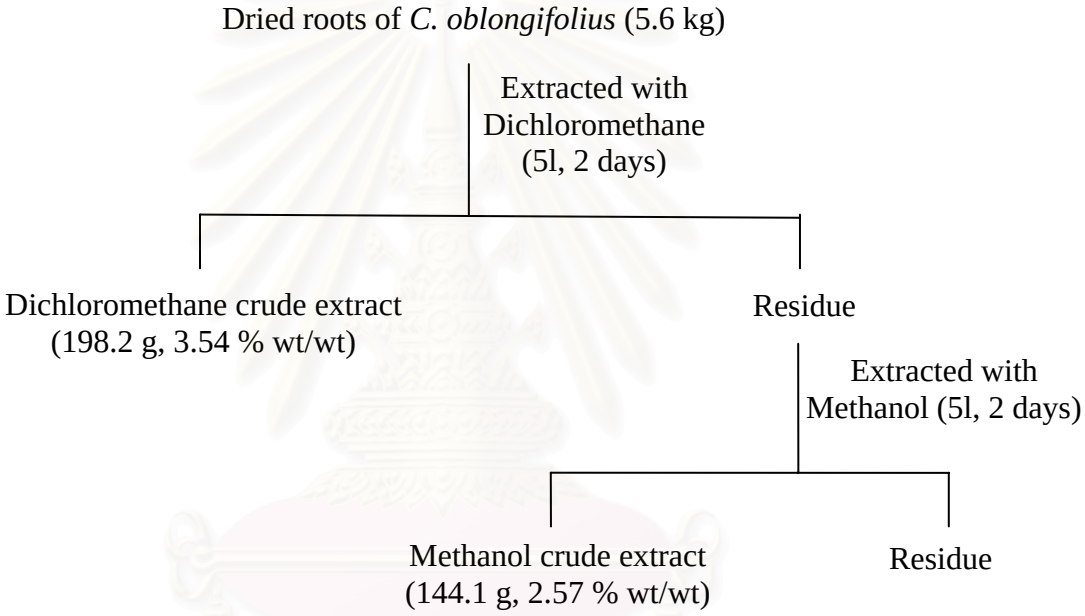
3.4 Extraction and Separation.

3.4.1 Extraction.

Dried roots (5.6 kg) of *C. oblongifolius* was extracted sequentially with dichloromethane (2x15 liters) and methanol (2x15 liters) at room temperature for 2 days. The solution was filtered and evaporated under reduced pressure to obtain dichloromethane crude extract (198.2 g) and methanol crude extract (144.1 g), respectively. Yields and appearance of crude extracts obtained from the roots of *C. oblongifolius* are shown in Table 3, and the extraction processes are shown in Scheme 5.

Table 3: The crude extracts of *C. oblongifolius* roots.

Solvent	**Appearance**	**Weight (g)**	**%Yield (wt/wt of dried roots)**
Dichloromethane	Yellowish green oil	198.2	3.54
Methanol	Dark brown oil	144.1	2.57



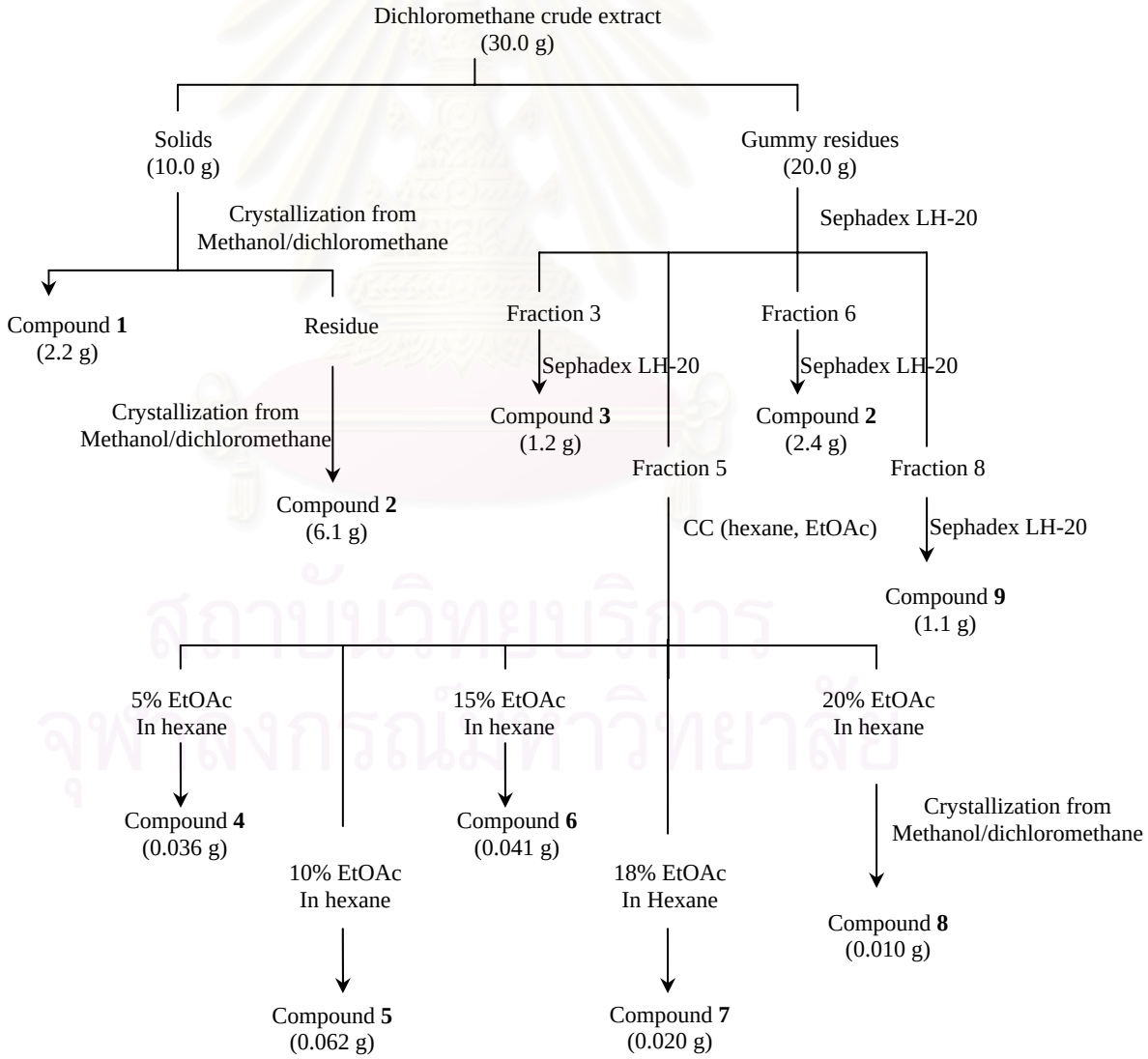
Scheme 5: The extraction procedure of the roots of *C. oblongifolius*.

3.4.2 Separation of crude extracts of *C. oblongifolius.*

The dichloromethane crude extract (30.0 g) was divided into two parts, solids (10.0 g) and gummy residues (20.0 g). The solids was re-crystallized from dichloromethane : methanol (ratio *ca* 1:2) to give compound **1** (2.2 g) and compound **2** (6.1 g), respectively. The gummy residues was separated by Sephadex LH-20 column chromatography, eluted with 100% methanol (approximately 50 ml per

fraction), to provide 8 fractions. Each fraction was analyzed by TLC and ^{1}H NMR spectrum. Fraction 3 was separated by Sephadex LH-20 eluted with methanol again to give compound **3** (1.2 g). Fraction 5 was separated by silica gel and eluted by a gradient mixture of n-hexane and EtOAc to EtOAc (100%), providing compound **4** (36 mg), compound **5** (62 mg), compound **6** (41 mg), compound **7** (10 mg), and compound **8** (20 mg); compound **8** was obtained with crystallization from methanol/dichloromethane. Fractions 6 and 8 were separated by Sephadex LH-20 column eluted with methanol to give compound **2** (2.4 g) and compound **9** (1.1 g), respectively.

The isolation of the dichloromethane crude extract of *C. oblongifolius* is briefly summarized in Scheme 6.



Scheme 6: The isolation procedure of a dichloromethane crude extract.

3.5 Physio-chemical properties of the isolated compounds from *C. oblongifolius.*

3.5.1 Physio-chemical properties of compound 1.

Compound **1** was obtained as a white needle crystal (2.2 g, $39.3x10^{-5}$ %wt/wt); m.p. 197 ^{o}C; $[\alpha]_D^{23}$ $+100^0$ (c=0.53, $CHCl_3$); APCITOF MS *m/z* $[M+H]^+$ 329.1384 (calcd 329.1386 for $C_{19}H_{21}O_5$); UV (MeOH) λ_{max} (log ε) 221.5 (3.46).

EIMS *m/z* (%relative intensity) (Figure 10) : 328 [M^+] (30), 310 (64), 282 (56), 237 (100), 197 (59), 129 (61), 91 (49)

FT-IR spectrum (KBr) (Figure 11, Table 5) ν_{max} cm^{-1} : 3142, 2922, 2853, 1771, 1736, 1638, 1454, 1155, 873

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 12, Table 6) δ ppm : 1.47 (1H, td, *J*=13.5, 13.5, and 3.9 Hz), 1.67 (1H, m), 1.69 (1H, m), 1.81 (1H, m), 1.81 (1H, m), 2.05 (1H, dt, *J*= 13.3, 3.8, and 3.8 Hz), 2.11 (1H, m), 2.19 (1H, m), 2.29 (1H, dt, *J*=13.7, 3.8, and 3.8 Hz), 2.34 (1H, ddd, *J*=12.0, 6.0 and 2.0 Hz), 2.47 (1H, dd, *J*=14.3 and 8.6 Hz), 2.86 (1H, dd, *J*=14.2 and 7.0 Hz), 3.67 (1H, td, *J*=14.0 and 0.8 Hz), 4.67 (1H, t, *J*=4.9 Hz), 5.03 (1H, d, *J*=1.4 Hz), 5.46 (1H, dd, *J*=7.1 and 8.5 Hz), 6.33 (1H, t, *J*=1.5 Hz), 7.43 (1H, d, *J*=1.5 Hz), 7.43 (1H, d, *J*=1.5 Hz), 4.82 (1H, s)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 14, Table 6) δ ppm : 22.4 (t), 28.6 (t), 29.1 (t), 32.4 (t), 40.5 (t), 44.5 (s), 46.9 (t), 51.5 (d), 52.6 (s), 71.0 (d), 73.8 (d), 107.9 (d), 110.9 (d), 125.6 (s), 139.5 (d), 144.1 (t), 146.9 (s), 173.7 (s), 176.5 (s)

3.5.2 Physio-chemical properties of compound 2.

Compound **2** was obtained as a white crystal (8.5 g, $151.8x10^{-5}$ %wt/wt); m.p. 172 ^{o}C; $[\alpha]_D^{23}$ -106^0 (c=0.77, $CHCl_3$); APCITOF MS *m/z* $[M+H]^+$ 373.1646 (calcd 373.1646 for $C_{21}H_{25}O_6$); UV (MeOH) λ_{max} (log ε) 221 (3.94), 252 (3.74).

EIMS *m/z* (%relative intensity) (Figure 15) : 372 [M^+] (56), 263 (34), 245 (76), 231 (73), 203 (36), 185 (60), 157 (58), 145 (100), 129 (44), 95 (65)

FT-IR spectrum (KBr) (Figure 16, Table 7) ν_{max} cm^{-1} : 3135, 2924, 1765, 1727, 1672, 1276, 1177, 1173

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 17, Table 8) δ ppm : 1.12 (1H, ddd, *J*=11.2, 4.3, and 4.3 Hz), 1.38 (1H, ddt, *J*=5.8, 3.7, and 1.9 Hz), 1.67 (1H, m), 1.90 (1H, m), 2.00 (1H, m), 2.06 (1H, m), 2.25 (1H, m), 2.30 (1H, m), 2.75 (1H, d, *J*=11.5 Hz), 2.86 (1H, d, *J*=17.8 Hz), 3.07 (1H, d, *J*=17.8 Hz), 3.24 (1H, dd, *J*=4.4 and 12.8 Hz), 3.62 (3H, s), 3.95 (1H, dd, *J*=2.0 and 8.1 Hz), 4.36 (1H, d, *J*=8.1 Hz), 6.75 (1H, dd, *J*=0.8 and 2.0 Hz), 6.76 (1H, dd, *J*=2.4 and 7.2 Hz), 7.45 (1H, t, *J*=1.8 Hz), 8.04 (1H, dd, *J*=1.3 and 1.3 Hz), 0.85 (3H, s)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 18, Table 8) δ ppm : 19.2 (q), 20.0 (t), 22.0 (t), 27.2 (t), 33.2 (t), 39.5 (s), 45.1 (s), 46.4 (t), 46.6 (d), 48.6 (d), 51.3 (q), 71.3 (t), 108.5 (d), 128.5 (s), 136.2 (d), 137.7 (s), 144.2 (d), 147.0 (d), 174.0 (s), 169.0 (s), 193.6 (s)

3.5.3 Physio-chemical properties of compound 3.

Compound **3** was obtained as a colorless oil (1.2 g, $21.4x10^{-5}$ %wt/wt); $[\alpha]_D^{23}$ -29^0 (c=2.0, $CHCl_3$); APCITOF MS *m/z* $[M+H-H_2O]^+$ 273.2572 (calcd 273.2577 for $C_{20}H_{35}O$); UV (MeOH) λ_{max} (log ε) 239 (3.35).

EIMS *m/z* (%relative intensity) (Figure 19) :290 [M^+] (5), 272 (12), 257 (5), 229 (3), 189 (40), 175 (27), 133 (42), 121 (96), 107 (94), 95 (72), 81 (57), 69 (15), 55 (28)

FT-IR spectrum (Figure 20, Table 9) ν_{max} cm^{-1} : 3000-3600, 2937, 1265, 862

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 21, Table 10) δ ppm : 0.77 (3H, d, *J*=6.8 Hz), 0.81 (3H, s), 1.06-1.10 (1H, m), 1.03-1.05 (1H, m), 1.20-1.30 (1H, m), 1.28-1.38 (1H, m), 1.33-1.40 (1H, m), 1.4-1.5 (1H, m), 1.55-1.63 (1H, m), 1.03 (3H, s), 1.67 (3H, dd, *J*=2.1 and 3.6 Hz), 1.70 (3H, br s), 1.83-1.92 (2H, m), 1.90-2.00 (2H, m), 2.00 (1H, m), 1.95-2.15 (2H, m), 4.15 (2H, d, *J*=6.9 Hz), 5.28 (1H, br s), 5.42 (1H, tq, *J*=1.1, 2.3, and 6.9 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 23, Table 10) δ ppm : 15.9 (q), 16.5 (q), 17.2 (q), 17.7 (t), 19.7 (q), 24.0 (t), 28.7 (t), 32.7 (t), 33.0 (q), 36.4 (t), 36.8 (s), 37.3 (d), 37.7 (t), 40.0 (s), 44.6 (d), 59.4 (t), 122.8 (d),123.1 (d), 139.8 (s), 141.0 (s)

3.5.4 Physio-chemical properties of compound 4.

Compound **4** was obtained as a colorless oil (36 mg, 6.43x10^{-6} %wt/wt); $[\alpha]_D^{23}$ -30^0 (c=1.0, $CHCl_3$); APCITOF MS *m/z* $[M+H]^+$ 219.1743 (calcd 219.1742 for $C_{15}H_{23}O$).

EIMS *m/z* (%relative intensity) (Figure 14) : 218 $[M^+]$ (54), 203 (23), 189 (54), 175 (47), 161 (49), 147 (39), 133 (34), 105 (26), 91 (34), 77 (13)

FT-IR spectrum (Figure 25, Table 11) ν_{max} cm^{-1} : 2919, 1709, 1656, 1461, 1374, 802

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 26, Table 12) δ ppm : 0.84 (3H, d, *J*=6.4 Hz), 0.88 (3H, s), 1.01 (3H, s), 1.15 (1H, ddt, *J*=12.0, 7.1, and 14.1 Hz), 1.57 (1H, dt, *J*=14.0 and 6.7 Hz), 1.65 (1H, ddd, *J*=13.6, 8.3, and 1.4 Hz), 1.73 (1H, br ddd, *J*=13.2, 6.7, and 3.8 Hz), 1.86 (1H, dt, *J*=13.5 and 9.7 Hz), 1.95 (1H, br ddt, *J*=6.6, 3.3, and 13.6 Hz), 2.05 (1H, t, *J*=3.5 Hz), 2.07 (3H, br s), 2.17 (1H, dq, *J*=12.1 and 6.4 Hz), 2.44 (1H, br dd, *J*=18.2 and 10.1 Hz), 2.78 (1H, br dt, *J*=18.1 and 9.7 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 28, Table 12) δ ppm : 15.2 (q) , 17.8 (q), 18.9 (q), 25.9 (t), 26.2 (t), 26.3 (q), 28.0 (t), 34.6 (d), 41.4 (s), 43.4 (t), 63.0 (d), 63.6 (s), 139.6 (s), 148.5 (s), 207.0 (s)

3.5.5 Physio-chemical properties of compound 5.

Compound **5** was obtained as a colorless oil (62 mg, 11.07x10^{-6} %wt/wt); $[\alpha]_D^{23}$ +222^0 (c=0.35, $CHCl_3$); APCITOF MS *m/z* $[M+H]^+$ 375.2169 (calcd 375.2166 for $C_{22}H_{31}O_5$); UV (MeOH) λ_{max} (log ε) 221 (3.85).

EIMS *m/z* (%relative intensity) (Figure 29) : 374 [M^+] (3), 299 (13), 283 (35), 255 (20), 219 (65), 187 (49), 159 (63), 145 (32), 131 (35), 119 (38), 105 (55), 91 (87), 79 (62), 67 (35)

FT-IR spectrum (Figure 30, Table 13) ν_{max} cm^{-1} : 3053, 2951, 2866, 1750, 1712, 1434, 1242, 1149, 733

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 31, Table 14) δ ppm : 0.86 (3H, s), 1.05 (1H, td, *J*=3.6 and 13.3 Hz), 1.23 (3H, s), 1.35 (1H, m), 1.37 (1H, m), 1.57 (1H, m), 1.61 (1H, m), 1.68 (1H, m), 1.70 (1H, m), 1.97 (1H, ddd, *J*=3.4, 13.9, and 13.9 Hz), 2.10 (1H, m), 2.13 (1H, m), 2.25 (1H, dt, *J*=19.8, 4.9, and 4.9 Hz), 2.35 (1H, dt, *J*=3.4 and 13.2 Hz), 2.45 (1H, td, *J*=3.6 and 13.3 Hz), 2.52 (1H, dd, *J*=3.6 and 12.9 Hz), 3.59 (3H, s), 3.63 (3H, s), 6.19 (1H, d, *J*=0.9 Hz), 6.57 (1H, t, *J*=4.3 Hz), 7.13 (1H, s), 7.26 (1H, t, *J*=1.6 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 33, Table 14) δ ppm : 17.1 (t), 18.3 (t), 19.88 (q), 20.8 (q), 21.5 (t), 26.9 (t), 34.8 (t), 37.2 (s), 38.7 (s), 40.8 (t), 46.4 (d), 49.0 (d), 51.1 (q), 51.2 (q), 110.9 (d), 125.1 (s), 137.2 (d), 138.5 (d), 141.7 (s), 167.0 (s), 172.7 (d), 174.9 (s)

3.5.6 Physio-chemical properties of compound 6.

Compound **6** was obtained as a yellowish oil (41 mg, $7.32x10^{-6}$ %wt/wt); $[\alpha]_D^{23}$ $+90^0$ (c=0.83, $CHCl_3$); APCITOF MS *m/z* $[M+H]^+$ 389.1959 (calcd 389.1962 for $C_{22}H_{29}O_6$); UV (MeOH) λ_{max} (log ε) 222 (3.85).

EIMS *m/z* (%relative intensity) (Figure 34) : 388 [M^+] (5), 357 (22), 325 (7), 279 (16), 246 (92), 231 (100), 219 (13), 187 (30), 171 (24), 159 (34), 143 (25), 131 (9), 91 (12)

FT-IR spectrum (Figure 35, Table 15) ν_{max} cm^{-1} : 3133, 2915, 1710, 1434, 1230, 1150, 735

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 36, Table 16) δ ppm : 1.01 (3H, s), 1.20 (1H, td, *J*=3.7, 13.6, and 13.6), 1.31 (3H, s), 1.53 (1H, m), 1.68 (1H, m), 1.97

(2H, td, *J*=3.2, 13.3, and 13.3 Hz), 2.02 (1H, m), 2.08 (1H, m), 2.22 (1H, dt, *J*=4.9, 4.9, and 19.7 Hz), 2.40 (1H, dt, *J*=3.4, 3.4, and 13.2 Hz), 2.93 (1H, s), 3.19 (2H, dd, *J*=3.9 and 13.0 Hz), 3.62 (3H, s), 3.67 (3H, s), 6.60 (1H, dd, *J*=2.8 and 4.5 Hz), 6.75 (1H, dd, *J*=0.6 and 1.8 Hz), 7.36 (1H, t, *J*=1.6 Hz), 8.06 (1H, s)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 38, Table 16) δ ppm : 18.3 (t), 19.5 (q), 20.6 (q), 20.6 (t), 21.5 (t), 34.2 (t), 37.4 (s), 40.2 (s), 46.1 (d), 47.6 (t), 49.0 (d), 51.1 (q), 51.1 (q), 108.7 (d), 129.2 (s), 137.6 (d), 141.1 (s), 144.1 (d), 147.1 (d), 167.0 (s), 175.0 (s), 194.2 (s)

3.5.7 Physio-chemical properties of compound 7.

Compound **7** was obtained as a colorless oil (20 mg, $3.57x10^{-6}$ %wt/wt); $[\alpha]_D^{23}$ $+169^0$ (c=0.47, $CHCl_3$); APCITOF MS *m/z* $[M+H]^+$ 359.1853 (calcd 359.1858 for $C_{21}H_{27}O_5$).

EIMS *m/z* (%relative intensity) (Figure 42) : 358 [M^+] (15), 341 (18), 301 (53), 264 (21), 246 (58), 214 (51), 204 (46), 186 (38), 159 (35), 145 (47), 131 (36), 117 (32), 105 (36), 91 (71), 81 (57), 77 (30), 67 (52)

FT-IR spectrum (Figure 43, Table 17) ν_{max} cm^{-1} : 3140, 2926, 2870, 1767, 1728, 1452, 1263, 1192, 872, 734

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 44, Table 18) δ ppm : 0.79 (3H, s), 1.11 (1H, dq, *J*=3.7,12.2,12.2,12.2, and 12.2 Hz), 1.23 (1H, m), 1.57 (1H, m), 1.68 (1H, m), 1.75 (1H, m), 1.79 (1H, m), 1.85 (1H, m), 2.03 (1H, dt, *J*=3.1,3.1, and 13.2 Hz), 2.11 (1H, m), 2.18 (1H, m), 2.20 (1H, m), 2.43 (1H, m), 2.63 (1H, td, *J*=4.2, 13.4, and 13.4 Hz), 2.73 (1H, dd, *J*=4.0 and 13.5 Hz), 3.70 (3H, s), 3.95 (1H, dd, *J*=2 and 8.1 Hz), 4.40 (1H, d, *J*=8.2 Hz), 6.27 (1H, s), 6.79 (1H, dd, *J*=2 and 7.4 Hz), 7.23 (1H, s), 7.37 (1H, t, *J*=1.6 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 46, Table 18) δ ppm : 18.3 (t), 19.2 (t), 19.3 (q), 22.0 (t), 27.4 (t), 33.4 (t), 38.8 (s), 39.8 (t), 45.2 (s), 47.7 (d), 48.8 (d), 51.4 (q), 71.9 (t), 110.9 (d), 124.5 (s), 135.9 (d), 137.8 (s), 138.0 (s), 138.5 (d), 142.9 (d), 168.9 (s)

3.5.8 Physio-chemical properties of compound 8.

Compound **8** was obtained as a white solid (10 mg, $1.78x10^{-6}$ %wt/wt); $[\alpha]_D^{23}$ -45 (c=0.10, $CHCl_3$); APCITOF MS *m/z* $[M+H]^+$ 375.1804 (calcd 375.1802 for $C_{21}H_{27}O_6$).

EIMS *m/z* (%relative intensity) (Figure 50) : 374 [M^+] (5), 358 (8), 328 (19), 269 (9), 235 (12), 219 (14), 187 (10), 159 (16), 145 (28), 131 (17), 117 (17), 105 (18), 91 (25)

FT-IR spectrum (KBr) (Figure 51, Table 18) ν_{max} cm^{-1} : 3133, 1914, 1739, 1436, 1276, 1160, 801, 775

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 52, Table 20) δ ppm : 0.95 (3H, d, *J*=6.4 Hz), 1.35 (1H, m), 1.45 (1H, m), 1.58 (1H, m), 1.73 (1H, m), 1.73 (1H, m), 1.85 (1H, m), 1.95 (1H, m), 2.05 (1H, m), 2.15 (1H, *J*=7.3 Hz), 2.25 (1H, *J*=9.3 Hz), 2.30 (1H,m), 2.40 (1H, m), 2.85 (1H, dd, *J*=0.9 and 5.7 Hz), 3.71 (3H, s), 4.51 (1H, br t, *J*=4.9 Hz), 5.14 (1H, d, *J*=1.1 Hz), 5.28 (1H, s), 5.33 (1H, dd, *J*=7.4 and 9.0 Hz), 6.39 (1H, br d, *J*=0.9 Hz), 7.38 (1H, br t, *J*=1.7 Hz), 7.40 (1H, br s),

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 54, Table 20) δ ppm : 16.8 (q), 20.1 (t), 26.4 (t), 30.4 (t), 31.4 (t), 37.4 (d), 38.5 (t), 38.8 (d), 44.2 (s), 50.2 (s), 51.6 (q), 54.0 (d), 75.7 (t), 74.9 (d), 100.6 (d), 104.3 (d), 108.6 (d), 127.1 (s), 139.3 (d), 143.4 (d), 170.2 (s)

3.5.9 Physio-chemical properties of compound 9.

Compound **9** was obtained as a white solid (1.1 g, $1.96x10^{-4}$ %wt/wt); $[\alpha]_D^{23}$ -114 (c=0.5, $CHCl_3$); APCITOF MS *m/z* $[M+H]^+$ 317.2111 (calcd 317.2116 for $C_{21}H_{27}O_5$); UV (MeOH) λ_{max} (log ε) 221 (3.97).

EIMS *m/z* (%relative intensity) (Figure 55) : 316 [M^+] (10), 299 (21), 283 (29), 221 (61), 203 (100), 175 (26), 137 (15), 125 (66), 95 (35), 81 (68)

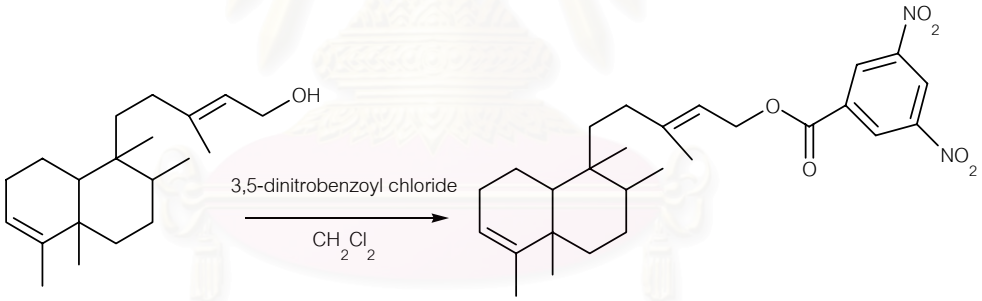
FT-IR spectrum (KBr) (Figure 56, Table 19) ν_{max} cm^{-1} : 2921, 1678, 1453, 1383, 1263, 1159, 1023, 872, 737

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 57, Table 22) δ ppm : 0.71 (3H, s), 0.77 (3H, d, J=6.5 Hz), 1.1 (1H, td, J=5.2, 12.9 and 12.9 Hz), 1.19 (3H, s), 1.33 (1H, m), 1.37 (1H, m), 1.47 (1H, m), 1.47 (1H, m), 1.52 (1H, m), 1.54 (1H, m), 1.57 (1H, m), 1.63 (1H, m), 2.13 (1H, m), 2.13 (1H, m), 2.23 (1H, m), 2.25 (1H, m), 2.37 (1H, dt, J=3.2, 3.2, and 13.0 Hz), 6.19 (1H, dd, *J*=0.8 and 1.7 Hz), 6.79 (1H, dd, *J*=3.0 and 4.4 Hz), 7.14 (1H, br s), 7.28 (1H, t, J=1.7 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 59, Table 20) δ ppm : 15.9 (q), 17.4 (t), 18.1 (t), 18.2 (q), 20.5 (q), 27.2 (t), 27.5 (t), 35.8 (t), 36.2 (d), 37.5 (s), 38.6 (t), 38.8 (s), 46.6 (d), 111.0 (d), 125.5 (s), 138.4 (d), 140.3(d), 141.4 (s), 142.7 (d), 172.5 (s)

3.6 Derivatization of compound 3.

3.6.1 Esterification of compound 3 with 3,5-dinitrobenzoyl chloride yielded the ester 3a as shown below.



3a

Compound **3** (60 mg) was treated with 3,5-dinitrobenzoyl chloride in dichloromethane and stirred at room temperature for 4 hours. The reaction mixture was washed with water 5-6 times. The dichloromethane layer was evaporated to obtain a crude product, which was purified by silica gel column chromatography eluted with 20% EtOAc in hexane to obtain compound **3a** (20 mg, 33% yield).

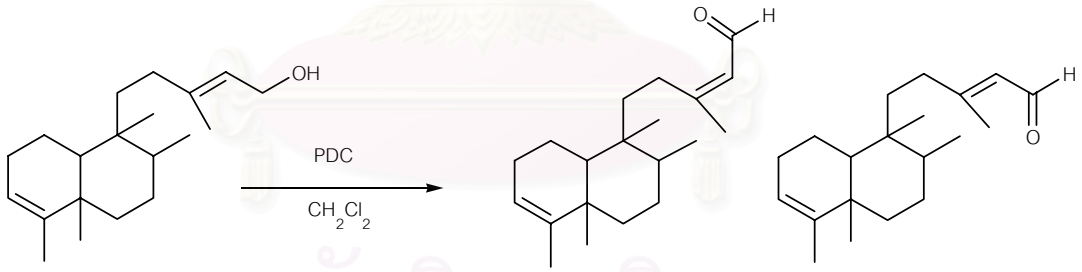
Compound **3a** was a yellowish oil APCITOF MS *m/z* $[M+H]^+$ 485.2872 (calcd 485.2876 for $C_{27}H_{37}O_6N_2$).

EIMS *m/z* (%relative intensity) (Figure 60) : 484 [M^+] (2), 257 (14), 190 (100), 175 (30), 161 (17), 147 (19), 121 (29), 107 (44), 91 (35), 79 (29)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 62) δ ppm : 0.79 (3H, d, *J*=6.8 Hz), 0.83 (3H, s), 1.00-1.10 (H, m), 1.01-1.04 (H, m), 1.04 (3H, s), 1.20-1.30 (H, m), 1.30-1.40 (H, m), 1.33-1.39 (H, m), 1.40-1.48 (H, m), 1.55-1.65 (H, m), 1.68 (3H, br d, *J*=1.5 Hz), 1.83 (3H, d, *J*=1.0 Hz), 1.90-2.00 (H, m), 1.93-2.05 (H, m), 1.95-2.05 (H, m), 1.97-2.05 (H, m), 4.70 (H, d, *J*=7.3 Hz), 5.27 (H, br s), 5.50 (H, dt, *J*=1.2 and 7.3 Hz), 9.18 (2H), 9.22 (H, t, *J*=2.5 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 64) δ ppm : 15.9 (q), 16.9 (q), 17.2 (q), 17.7 (t), 19.7 (q), 24.0 (t), 28.7 (t), 32.8 (t), 33.0 (q), 36.1 (t), 36.8 (s), 37.3 (d), 37.7 (t), 40.1 (s), 44.6 (d), 63.7 (t), 116.5 (d), 122.2 (d), 123.1 (d), 129.2 (s), 129.4 (d), 129.4 (d), 134.2 (s), 139.8 (s), 145.7 (s), 148.6 (s), 162 (s)

3.6.2 Oxidation of compound 3 with piridinium dichromate (PDC) afforded compound 3b and 3c as shown below.



Compound **3** was treated with PDC (peridinium dichromate) in dichloromethane and stirred at room temperature for 4 hours. The solution was filtered with celite and evaporated to obtain a crude product. The crude product was purified by silica gel column chromatography eluted with 20% EtOAc in hexane to obtain compound **3b** (5 mg, 10% yield) and compound **3c** (5 mg, 10% yield)

Compound **3b** was a yellowish oil APCITOF MS *m/z* $[M+H]^+$ 289.2526 (calcd 289.2526 for $C_{20}H_{33}O$).

EIMS *m/z* (%relative intensity) (Figure 60) : 288 $[M^+]$ (8), 277 (100), 243 (93), 205 (27), 189 (54), 161 (29), 145 (31), 135 (40), 121 (61), 107 (59), 91 (74), 79 (50), 67 (38)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 66) δ ppm : 0.77 (3H, d, *J*=6.8 Hz), 0.84 (3H, s), 1.05 (3H, s), 1.10-1.12 (H, m), 1.20-1.30 (2H, m), 1.30-1.40 (H, m), 1.40-1.48 (H, m), 1.60-1.70 (2H, m), 1.68 (3H, m), 1.70-1.80 (H, m), 1.95-2.10 (H, m), 1.97-2.05 (H, m), 2.00-2.13 (2H, m), 2.03-2.10 (2H, m), 2.20 (3H, d, *J*=1.2 Hz), 5.3 (H, br s), 5.9 (H, qd, *J*=1.1, 2.2, and 8.1 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 68) δ ppm : 15.9 (q), 17.1 (q), 17.7 (t), 17.8 (q), 19.7 (q), 23.9 (t), 28.6 (t), 33.0 (q), 34.2 (t), 35.7 (t), 36.8 (s), 37.3 (d), 37.7 (t), 40.2 (s), 44.6 (d), 127.1 (d), 123.1 (d), 139.7 (s), 165.6 (s), 191.3 (s)

Compound **3c** was a yellowish oil APCITOF MS *m/z* $[M+H]^+$ 289.2528 (calcd 289.2526 for $C_{20}H_{33}O$).

EIMS *m/z* (%relative intensity) (Figure 69) : 288 $[M^+]$ (8), 277 (42), 243 (35), 205 (67), 189 (100), 161 (57), 145 (38), 135 (67), 121 (76), 107 (39), 91 (71), 79 (57), 67 (44)

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) (Figure 70) δ ppm : 0.81 (3H, d, *J*=6.8 Hz), 0.84 (3H, s), 1.02-1.10 (H, m), 1.05 (3H, s), 1.20-1.30 (H, m), 1.35-1.40 (H, m), 1.40 (H, m), 1.40 (H, m), 1.64-1.74 (H, m), 1.68 (3H, m), 1.75-1.78 (H, m), 1.98-2.00 (3H, d, *J*=1.3 Hz), 2.00-2.12 (H, m), 2.05 (2H, m), 2.06 (H, m), 2.40-2.50 (H, dq, *J*=5.3 and 12.3 Hz), 5.28 (H, m), 5.85 (H, dd, *J*=1.0 and 8.2 Hz)

^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) (Figure 72) δ ppm : 15.9 (q), 17.6 (t), 17.8 (q), 19.7 (q), 23.9 (t), 25.4 (q), 26.4 (t), 28.6 (t), 33.0 (q), 36.0 (s), 37.5 (t), 37.6 (t), 37.4 (d), 40.6 (s), 44.7 (d), 123.1 (d), 127.1 (d), 139.6 (s), 166.2 (s), 190.6 (s)

3.7 Cytotoxic assay

Cytotoxic assay was performed using the colorimetric method as previously described. Briefly, cell lines suspended in RPMI 1640 containing 10% FBS were seeded at $1x10^4$ cells (100µl) per well in 96-well plate, and incubated in humidified atmosphere, 95% air and 5% CO_2 at 37 ^{0}C. After 24 h, additional medium (100µl) containing the test compound and vehicle was added to a final concentration of 50 µg/ml, 0.2% DMSO, and future incubated for 3 days. Cell were subsequently fixed with 95% EtOH, stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH, after which absorbance was measured at 550 nm. The number of surviving cells was determined from the absorbance. Results were expressed as percent survival compared with contral. Epothoside was used as the reference compound [55].

CHAPTER IV
RESULTS AND DISCUSSION

The dichloromethane crude extract of the roots of *C. oblongifolius* was separated by chromatographic techniques to obtain seven compounds as shown in Table 4.

Table 4: Compounds isolated from the dichloromethane crude extract of the roots of *C. oblongifolius* by chromatographic techniques.

Compounds	Physical appearance	Weight (g)
1	White solid	2.20
2	White solid	8.50
3	Colorless oil	1.20
4	Colorless oil	0.036
5	Colorless oil	0.062
6	Colorless oil	0.041
7	Yellowish oil	0.020
8	White solid	0.010
9	White solid	1.10

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

4.1.1 Structure elucidation of compound 1.

The IR spectrum of compound **1** is shown in Figure 11 and the absorption peaks were assigned as displayed in Table 5.

Table 5: Assignments of the IR absorption bands of compound **1**.

Wave number (cm^{-1})	Intensity	Tentative assignment
3142	Weak	C-H stretching vibration of 5-ring-heteroaromatic
2922, 2853	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1771, 1736	Strong	C=O stretching vibration of carbonyl group
1638	Weak	$C=CH_2$ stretching vibration of alkene
1454	Medium	C-H bending vibration of $-CH_3$, $-CH_2$
1155	Medium	C-O stretching vibration of ester
873	Medium	C=CH out of plane vibration

The ^{1}H NMR spectrum of compound **1** (Figure 12, Table 6) indicated that it possessed three olefinic protons of a furanoid group at 6.33 (1H, t, *J*=1.5 Hz) and 7.43 (2H, d, *J*=1.5 Hz) ppm, two vinylic protons at 5.03 (1H, d, *J*=1.4 Hz) and 4.82 (1H, s, 4.82) ppm, two downfield triplets at 4.67 and 5.46 (deshielded by ester linkage), and a number of methine and methylene protons resonanced at 1.4-2.8 ppm.

The ^{13}C NMR spectrum (Figure 14, Table 6) and DEPT experiments of compound **1** revealed the presence of 19 carbons, of which 11 carbons are sp^3 (six methylene carbons at 22.4, 28.6, 29.1, 32.4, 40.5, and 46.9 ppm; three methine carbons at 51.5, 71.0, and 73.8 ppm; and two quaternary carbons at 44.5 and 52.6 ppm) and 8 carbons are sp^2 (one methylene carbon at 110.9 ppm; three methine carbons at 107.9, 139.5, and 144.1 ppm; and four quaternary carbons at 125.6, 146.9, 173.7, and 176.5 ppm) hybridized carbons, together with carbonyl carbons of ester.

The molecular formula of compound **1** was established as $C_{19}H_{20}O_5$ by the APCITOF MS, showing the peak at *m/z* 329.1384 (calcd for $[C_{19}H_{20}O_5+H]^+$,

329.1386). It therefore contains a degree of unsaturated of 10, thus consisting of one ring of furanoid (DBE=3), in addition to one double bond, two carbonyl groups and three ring closers.

The coupling correlations of compound **1** were observed from the ^{1}H-^{1}H COSY spectrum. A methylene proton (H-1, δ_H 1.80 and 2.10 ppm) splitted into multiplet resulting from coupling to a methine proton (H-10, δ_H 1.80 ppm) and methylene proton (H-2, δ_H 1.70 and 2.20 ppm); an oxygenated methine proton (H-3, δ_H 4.67 ppm) showed correlations to a methylene (H-2) and methylene (H-4, δ_H 1.67 and 2.35 ppm); a methylene proton (H-6, δ_H 1.47 and 2.05 ppm) revealed correlations with methylene proton (H-7, δ_H 2.27 and 3.67 ppm); an oxygenated methine proton (H-12, δ_H 5.45 ppm) illustrated correlations to methylene proton (H-11, δ_H 2.47 and 2.85 ppm); and a methine proton (H-16, δ_H 7.40 ppm) showed allylic correlations to methine proton (H-14, δ_H 6.35 ppm) and methine proton (H-15, δ_H 7.40 ppm). In addition, the HMBC spectra confirmed the structure of compound **1**, showing correlations of H-10, H-4, and H-6 to C-5 (δ_C 44.5 ppm) and C-19 (δ_C 176.5 ppm); H-7 to C-8 (δ_C 146.9 ppm) and C-17 (δ_C 110.9 ppm); H-17 to C-6 (δ_C 32.4 ppm), C-9 (δ_C 52.6 ppm), and C-20 (δ_C 173.7 ppm); H-11 to C-9, C-20, C12 (δ_C 70.1 ppm), and C-13 (δ_C 125.6 ppm); and H-14 and H-16 to C-12.

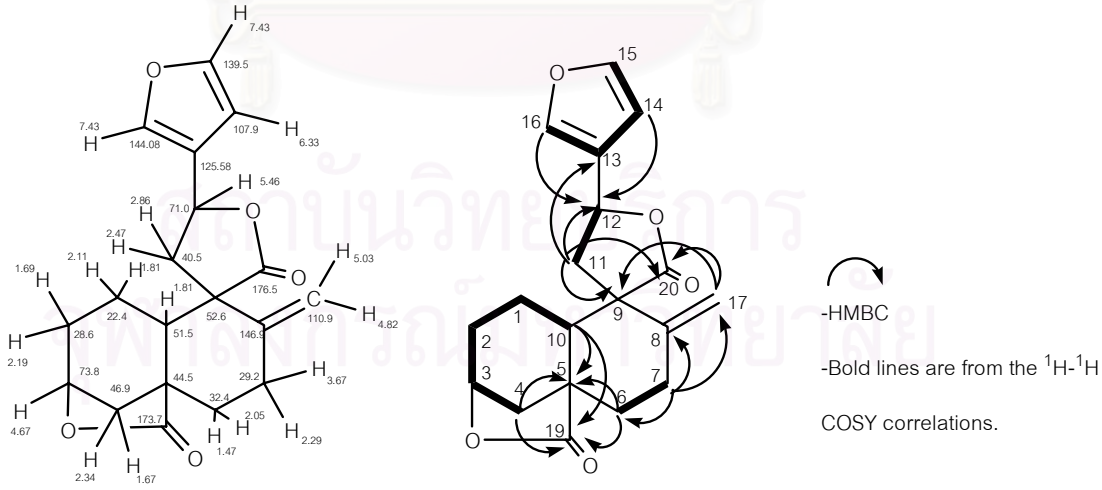
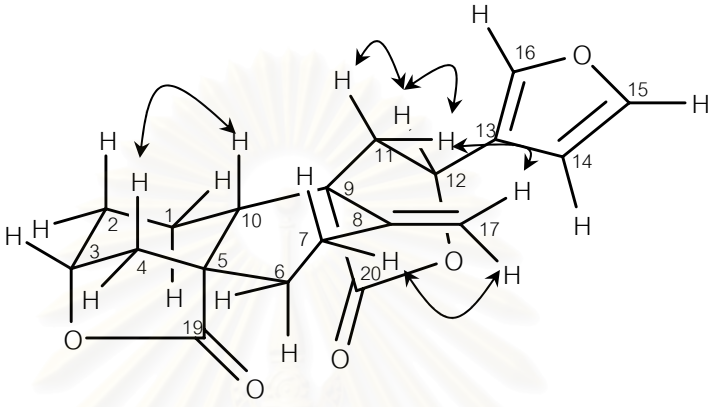


Figure 3: HMBC and ^{1}H-^{1}H COSY correlations of compound **1**

The relative configuration was established by analyses of the NOESY spectrum, showing the correlations of H_{eq}-7 to H_b-17; H_a-17 to H-11; and H-12 to H-11. The configuration at the C-10 was also confirmed by cross peaks between H-10 to H_{ax}-4. The relative configurations of compound **1** are shown below.



A literature search revealed that compound **1** is a known compound, levatin, which is norclerodan diterpene group. Levatin was previously isolated from the trunk bark of *Croton levatii* [48], however, its biological activity has not been investigated todate.

The ^{1}H and ^{13}C spectral data of levatin [48] and compound **1** are shown in Table 6.

Table 6: The ^{1}H and ^{13}C spectral data of levatin and compound **1**.

Levatin [48]			Compound **1**	
Position	δ_c	δ_H multiplicity, *J* in Hz	δ_c	δ_H multiplicity, *J* in Hz
1	22.48	1.73, m, 11.8, 3	22.4	1.81, m
		2.12, m, 11.8		2.11, m
2	28.62	1.71, m, 10.5	28.6	1.69, m
		2.17, m, 10.5, 4.2, 2.14		2.19, m
3	74.00	4.64, dd, 6, 4.2, 4	73.8	4.67, t, 4.9
4	46.93	1.67, m, 11.7, 4	46.9	1.67, m
		2.31, ddd, 11.7, 6,2		2.34, ddd, *J*=12, 6, 2
5	44.60	-	44.5	-
6	32.50	1.45, td, 13.5, 13.5, 3.8	32.4	1.47, td, 13.5, 13.5, 3.9
		2.03, dt, 13.5, 3.8, 3.8		2.05, dt, 13.3, 3.8, 3.8
7	29.23	2.26, dt, 13.7, 3.8, 3.8	29.1	2.29, dt, 13.7, 3.8, 3.8
		3.65, ddd, 13.7, 13.5, 3.8, 0.8		3.67, dt, 14.0, 0.8
8	146.93	-	146.9	-
9	52.76	-	52.6	-
10	51.53	1.79, m	51.5	1.81, m
11	40.62	2.47, dd, 14.3, 8.6	40.5	2.47, dd, 14.3, 8.6
		2.48, dd, 14.3, 7.1		2.86, dd, 14.2, 7.0
12	71.11	5.44, dd, *J*=7.1, 8.6	71.0	5.46, dd, 7.1, 8.5
13	125.66	-	125.6	-
14	107.97	6.35, t, 1.6	107.9	6.33, t, 1.5
15	139.65	7.41, d, 1.6	139.5	7.43, d, 1.5
16	144.17	7.41, d, 1.6	144.1	7.43, d, 1.5
17	111.00	4.80, bs	110.9	4.82, s
		5.01, d, 1.6		5.03, d, 1.4
19	173.88	-	173.7	-
20	176.75	-	176.5	-

4.1.2 Structure elucidation of compound 2.

The IR spectrum of compound **2** is shown in Figure 16 and the absorption peaks were assigned as shown in Table 7.

Table 7: Assignments of the IR absorption bands of compound **2**.

Wave number (cm^{-1})	Intensity	Vibration
3135	Weak	C-H stretching vibration of 5-ring-heteroaromatic
2924	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1765, 1727	Strong	C=O stretching vibration of carbonyl group
1672	Strong	C=CH stretching vibration of alkene
1276	Medium	C-H bending vibration of $-CH_3$, $-CH_2$
1177, 1173	Medium	C-O stretching vibration of ester
872	Medium	C=CH out of plane vibration

The ^{1}H NMR spectrum of compound **2** (Figure 17, Table 8) indicated that it possessed three olefinic protons of a furanoid group at 6.75 (1H, dd, *J*=0.8 and 2.0 Hz), 7.45 (1H, t, *J*=1.8 Hz), and 8.04 (1H, dd, *J*=1.3 and 1.3 Hz) ppm, one vinylic proton at 6.76 (1H, dd, *J*=2.4 and 7.2 Hz) ppm, one methyl group at 0.85 (1H, s), one methoxy group at 3.62 (3H, s) ppm, and a number of methine and methylene protons resonanced at 1.0-2.4 ppm.

The ^{13}C NMR (Figure 18, Table 8) and DEPT spectral data of compound **2** revealed the presence of 21 carbons, of which 12 carbons are sp^3 (two methyl carbons at 19.2 and 51.3 ppm; six methylene carbons at 20.0, 22.1, 27.3, 33.2, 46.6, and 71.4 ppm; two methine carbons at 20.0 and 48.6 ppm; and two quaternary carbons at 39.6 and 45.1 ppm), and 9 carbons are sp^2 (four methine carbons at 108.5, 136.2, 144.2, and 147.0 ppm and five quaternary carbons at 128.5, 137.7, 169.0, 174.0, and 193.6 ppm) hybridized carbons, together with ketone and carbonyl carbons of ester.

The APCITOF MS indicated the molecular formula of compound **2** as $C_{21}H_{24}O_6$. Analyses of the ^{1}H-^{1}H COSY spectrum data showed correlations of a methylene proton (H-1, δ_H 1.12 and 1.67 ppm) to methine proton (H-10, δ_H 2.75 ppm) and methylene proton (H-2, δ_H 2.25 and 2.30 ppm); a methylene proton (H-2) to

methine proton (H-3, δ_H 6.76 ppm); a methylene proton (H-7, δ_H 1.90 and 2.06 ppm) to methylene proton (H-6, δ_H 1.38 and 2.00 ppm) and methine proton (H-8, δ_H 3.24 ppm); and a methine proton (H-14, δ_H 6.75 ppm) to methine proton (H-15, δ_H 7.45 ppm) and methine proton (H-16, δ_H 8.04 ppm). The information observed from HMBC spectrum illustrated correlations of H-3 to C-5 (δ_C 45.1 ppm), C-18 (δ_C 169.0 ppm), C-2 (δ_C 27.3 ppm), and C-1 (δ_C 20.0 ppm); H-6 to C-19 (δ_C 71.4 ppm), C-5, and C-7 (δ_C 22.1 ppm); H-8 to C-17 (δ_C 174.0 ppm), C-9 (δ_C 39.6 ppm), and C-20 (δ_C 19.2 ppm); H-10 to C-8 (δ_C 48.6 ppm) ; H-11 to C-12, C-9, C-10 (δ_C 46.6 ppm), and C-20 (δ_C 19.2 ppm); H-20 to C-9, C-12, and C-8; and H-14 to C-12, C-13 (δ_C 128.5 ppm), C-15 (δ_C 144.2 ppm), and C-16 (δ_C 147.0 ppm).

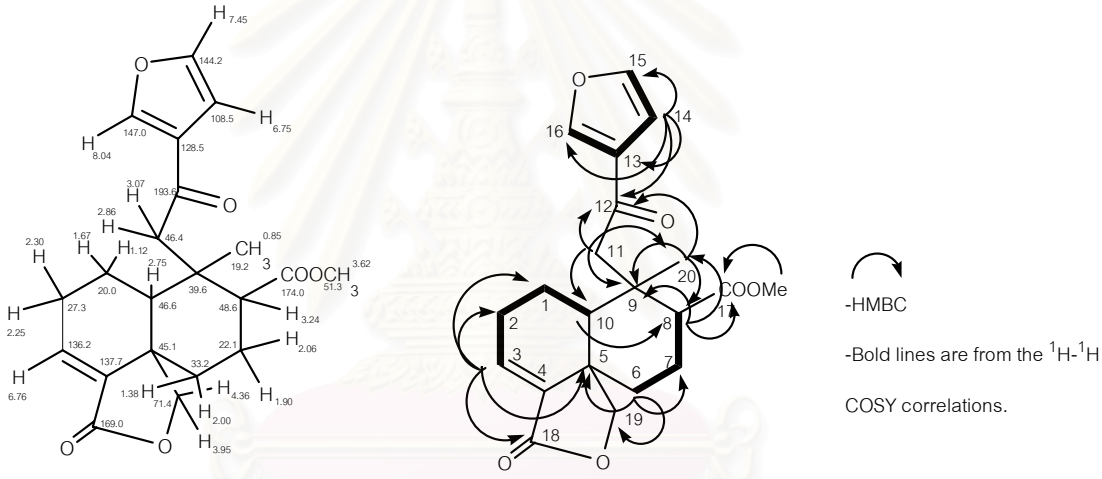
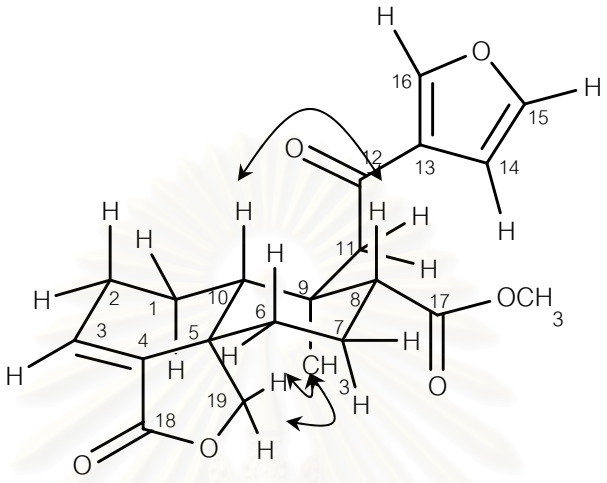


Figure 4: HMBC and ^{1}H-^{1}H COSY correlations of compound **2**.

The relative configuration was established as a *trans* relationship between H-10 and H-19 methylene because the NOESY showed the correlations of H-20 to H-19 and H-10 to H-8. The relative configurations of compound **2** are shown below.



Based upon these spectroscopic data, the structure of compound **2** was secured. Compound **2** was a known diterpene, namely nasimalun A, previously isolated from the roots of *Barringtonia racemosa* [49] and the stem barks of *C. oblongifolius* [35,44]. Comparision of ^{1}H and ^{13}C NMR data of nasimalun A and compound **2** is shown in Table 8. Nasimalun A exhibited antibacterial activity [54].

Table 8: The ^{1}H and ^{13}C spectral data of nasimalun A and compound **2**.

Nasimalun A [49]			Compound **2**	
position	δ_c	δ_H multiplicity, *J* in Hz	δ_c	δ_H multiplicity, *J* in Hz
1	20.1	1.09, dddd, 11.0, 11.0 11.0, 4.0	20.0	1.12, ddd, 11.2, 4.3, 4.3
		1.64, dddd, 11.0, 2.0, 2.0, 2.0		1.67, m
2	27.3	2.22, m	27.3	2.25, m
		2.28, m		2.30, m
3	136.2	6.74, dd, 8.0, 2.0	136.2	6.76, dd, 2.4, 7.2
4	137.8	-	137.7	-
5	45.1	-	45.1	-
6	33.2	1.36, dddd, 13.5, 13.5, 4.5, 2.0	33.2	1.38, ddt, 5.8, 3.7, 1.9
		1.98, ddd, 13.5, 4.5, 3.0		2.00, m
7	22.1	1.87, dddd, 13.5, 4.5, 4.5, 3.0	22.1	1.90, m
		2.03, dddd, 13.5, 13.5, 13.5, 4.5		2.06, m
8	48.7	3.21, dd, 13.5, 4.5	48.6	3.24, dd, 4.4, 12.8
9	39.6	-	39.6	-
10	46.7	2.73, dd, 11.0, 2.0	46.6	2.75, d, 11.5
11	46.5	2.83, d, 18.0	46.4	2.86, d, 17.8
		3.04, d, 18.0		3.07, d, 17.8
12	193.6	-	193.6	-
13	128.6	-	128.5	-
14	108.5	6.37, d, 2.0	108.5	6.75, dd, 0.8, 2.0
15	144.3	7.42, d, 2.0	144.2	7.45, t, 1.8
16	147.1	8.01, s	147.0	8.04, dd, 1.3, 1.3
17	174.0	-	174.0	-
18	169.0	-	169.0	-
19	71.4	3.93, dd, 8.0, 2.0	71.4	3.95, dd, 2.0, 8.1
		4.33 ,d, 8.0		4.36, d, 8.1
20	19.2	0.82, s	19.2	0.85, s
21	51.4	3.60, s	51.3	3.62, s

4.1.3 Structure elucidation of compound 3.

The IR spectrum of compound **3** is shown in Figure 20 and the absorption peaks are assigned in Table 9.

Table 9: Assignments of the IR absorption bands of compound **3**.

Wave number (cm^{-1})	Intensity	Vibration
3000-3600	Medium	O-H stretching vibration of hydroxyl group
2937	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1651, 1700	Strong	C=CH stretching vibration of alkene
862	Medium	C=CH out of plane vibration

The ^{1}H NMR spectrum of compound **3** (Figure 21, Table 10) revealed the presence of five methyl groups at 0.77 (3H, d, *J*=6.8 Hz), 0.81 (3H, s), 1.03 (3H, s), 1.67 (3H, dd, *J*=2.1 and 3.6 Hz), and 1.70 (3H, br s) ppm, two olefinic protons at 5.28 (1H, br s) and 5.42 (1H, tq, *J*=1.1, 2.3, and 6.9 Hz) ppm, and a number of methine and methylene proton resonanced at 0.7-2.2 ppm.

The ^{13}C NMR (Figure 23, Table 10) and DEPT spectral data of compound **3** revealed the presence of 20 carbons, including four signals of olefinic carbons at 122.8, 123.1, 139.8, and 141.1 ppm, five signals of methyl group at 15.9, 16.5, 17.2, 19.7, and 33.0 ppm, seven signals of methylene carbons at 17.7, 24.0, 28.8, 32.7, 36.5, 37.7, and 59.4 ppm, two signals of methine carbons at 37.3 and 44.6 ppm, and two quaternary carbons at 36.9 and 36.9 ppm.

The molecular formula of $C_{20}H_{34}O$ for compound **3** was deduced from the APCITOF MS. Analyses of the ^{1}H-^{1}H COSY spectrum demonstrated correlations of H-1 (δ_H 1.90-2.00 ppm) to H-2 (δ_H 1.95-2.15 ppm) and H-10 (δ_H 1.33-1.40 ppm); H-2 to H-3 (δ_H 5.28 ppm); H-7 (δ_H 1.03-1.05 and 1.20-1.30 ppm) to H-6 (δ_H 1.06-1.10 and 2.00 ppm) and H-8 (δ_H 1.4-1.5 ppm); H-8 to H-17 (δ_H 0.77 ppm); H-11 (δ_H 1.28-1.38 and 1.55-1.63 ppm) to H-12 (δ_H 1.83-1.92 ppm); and H-14 (δ_H 5.42 ppm) to H-15 (δ_H 4.15 ppm), while the HMBC spectrum revealed correlations of H-10 to C-1 (δ_C 17.7 ppm) and C-2 (δ_C 24.0 ppm); H-18 to C-3 (δ_C 123.1 ppm), C-4 (δ_C 139.8 ppm), and C-5 (δ_C 36.8 ppm); H-19 to C-4, C-6 (δ_C 37.7 ppm), and C-10 (δ_C 44.6 ppm); H-17 to C-7 (δ_C 28.7 ppm) and C-9 (δ_C 40.0 ppm); H-20 to C-10 and C-11

(δ_C 36.4 ppm); H-14 to C-12 (δ_C 32.7 ppm), C-15 (δ_C 59.4 ppm), and C-16 (δ_C 16.5 ppm); and H-16 to C-12 and C-13 (δ_C 141.0 ppm).

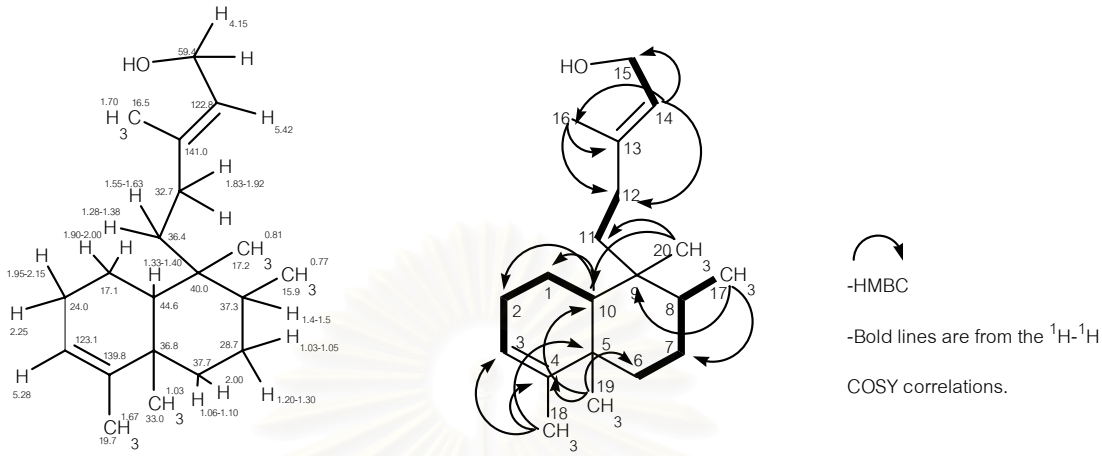
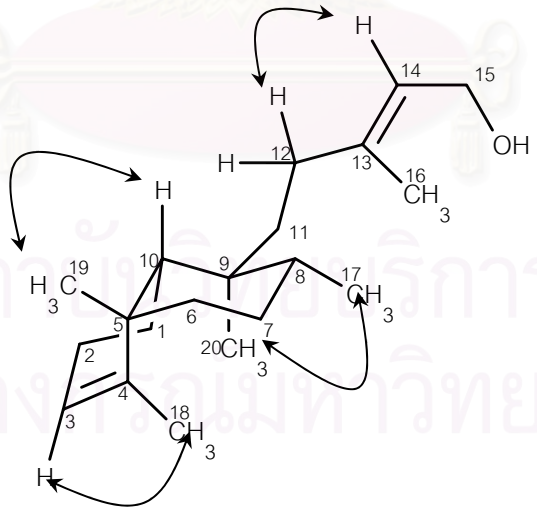


Figure 5: HMBC and ^{1}H-^{1}H COSY correlations of compound **3.**

The NOESY correlation between H-12 and H-14 indicated an *E* geometry of a C-13/C-14 double bond in compound **3**, while the correlation of H-10 and H-19 methyl suggested a *cis* relationship between the methyl and H-10. It should be noted that clerodane diterpene normally adopts *trans* relationship between H-19 methyl and H-10.



Based on the information of ^{1}H, ^{13}C NMR spectral data and literature data comparision (Table 10), compound **3** was identified as 15-hydroxy-cis-*ent*-cleroda-3-13(*E*)-diene, which was previously isolated from the liverwort *Aldelanthus lindenbergianus* [50]. The biological activity of compound **3** has not been investigated todate.

Table 10: The ^{1}H and ^{13}C spectral data of 15-hydroxy-cis-*ent*-cleroda-3,13(*E*)-diene and compound **3**.

15-Hydroxy-cis-*ent*-cleroda-3,13(*E*)-diene [50]			Compound **3**	
position	δ_c	δ_H multiplicity, *J* in Hz	δ_c	δ_H multiplicity, *J* in Hz
1	17.7	1.70, n.r.	17.7	1.90-2.00, m
		1.95, n.r.		-
2	24.0	1.96, n.r.	24.0	1.95-2.15, m
		2.11, n.r.		-
3	123.1	5.26, br s	123.1	5.28, br s
4	139.8	-	139.8	-
5	36.9	-	36.8	-
6	37.7	1.01, n.r.	37.7	1.06-1.10, m
		2.01, n.r.		2.00, m
7	28.8	1.03, n.r.	28.7	1.03-1.05, m
		1.18, n.r.		1.20-1.30, m
8	37.3	1.43, n.r.	37.3	1.4-1.5, m
9	40.1	-	40.0	-
10	44.6	1.34, n.r.	44.6	1.33-1.40, m
11	36.5	1.20, n.r.	36.4	1.28-1.38, m
		1.55, n.r.		1.55-1.63, m
12	32.7	1.86, n.r.	32.7	1.83-1.92, m
13	141.1		141.0	-
14	122.8	5.41, t, 7.0	122.8	5.42, br dt, 1.1, 6.9
15	59.4	4.13, d, 7.0	59.4	4.15, d, 6.9
16	16.5	1.67, s	16.5	1.70, br s
17	15.9	0.76, d, 6.6	15.9	0.77, d, 6.8
18	19.7	1.66, s	19.7	1.67, dd, 2.1, 3.6
19	33.0	1.01, s	33.0	1.03, s
20	17.2	0.76, s	17.2	0.81, s

n.r. = not resolved due to overlapping of signals.

4.1.4 Structure elucidation of compound 4.

The IR spectrum of compound **4** is shown in Figure 25 and the absorption peaks are in Table 11.

Table 11: Assignments of the IR absorption bands of compound **4**.

Wave number (cm^{-1})	Intensity	Vibration
2924	Weak	C-H stretching vibration of $-CH_3$, $-CH_2$
1709	Strong	C=O stretching vibration of carbonyl group
1657	Strong	C=CH stretching vibration of alkene
1263	Medium	C-H bending vibration of $-CH_3$, $-CH_2$
804	Medium	C=C out of plane vibration

The ^{1}H NMR spectrum of compound **4** (Figure 26, Table 12) revealed the presence of three methyl groups at 0.84 (3H, d, *J*=6.5 Hz), 0.88 (3H, s), and 1.01 (3H,s) one vinylic methyl group at 2.07 (3H, br s), and a number of methine and methylene protons resonanced at 0.7-2.8 ppm.

The ^{13}C NMR (Figure 28, Table 12) and DEPT spectral of compound **4** revealed the presence of 15 carbons, including twelve sp^3 carbons (four methyl carbons at 15.2, 17.8, 18.9, and 26.4; four methylene carbons at 25.9, 26.2, 28.0, and 43.4 ppm; two methine carbons at 34.6 and 63.0 ppm; and two quaternary carbons at 41.4 and 63.6 ppm), three sp^2 carbons (one methine carbon at 148.5 ppm and two quaternary carbons at 139.6 and 207.0 ppm), and a carbonyl ketone.

The molecular formula of compound **4**, $C_{15}H_{22}O$, was established by the APCITOF MS showing the pseudomolecular ion peak at *m/z* 219.1743 (calcd for $[C_{15}H_{22}O+H]^+$, 219.1742). The ^{1}H-^{1}H COSY spectrum of compound **4** established the partial structure from H-2 (δ_H 1.62 and 1.86 ppm) to H-3 (δ_H 2.44 and 2.78 ppm); H-8 (δ_H 1.73 and 1.95 ppm) to H-7 (δ_H 2.05 ppm) and H-9 (δ_H 1.15 and 1.57 ppm); and H-9 to H-10 (δ_H 2.16 ppm), while the HMBC spectrum revealed correlations of H-2 to C-1 (δ_C 63.7 ppm), C-10 (δ_C 34.6 ppm), and C-5 (δ_C 139.7 ppm); H-10 to C-15 (δ_C 17.8 ppm), C-11 (δ_C 41.0 ppm), C-8 (δ_C 25.9 ppm), and C-5; H-8 to C-11, C-7 (δ_C 63.0 ppm), and C-6 (δ_C 207.0 ppm); H-12 to C-13 (δ_C 26.4 ppm), C-11, C-1, and C-7; H-14 to C-1 and C-9 (δ_C 28.0 ppm); and H-14 to C-4, C-5, C-3, and C-6.

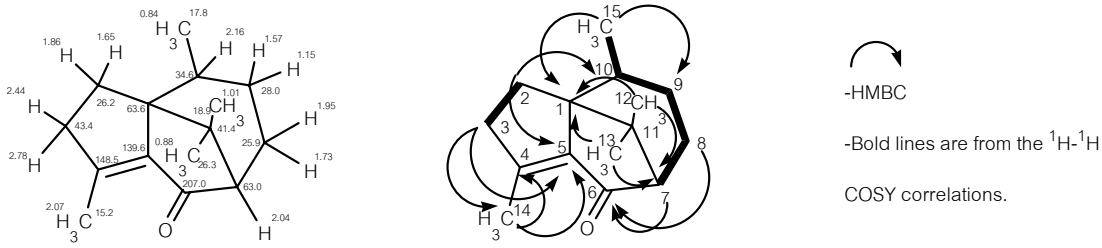
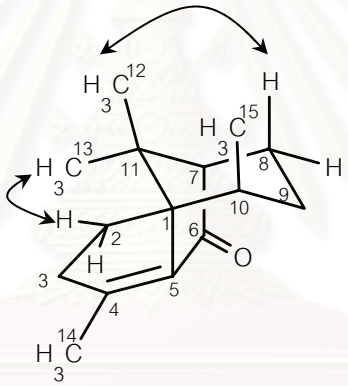


Figure 6: HMBC and ^{1}H-^{1}H COSY correlations of compound **4.**

The relative configuration of compound **4** could be established by analyses of the NOESY correlations, showing correlations from H-12 to H-8 and H-13 to H-2. The relative configurations of compound **4** are shown below.



On the basis of these spectral evidence, as well as data comparison of compound **4** with those in the literature (Table 12), compound **4** was identified as patchoulenone, which was previously isolated from *Cyperus rotundes*. Patchoulenone was found to be an antimalarial again [51].

Table 12: The ^{1}H and ^{13}C spectral data of patchoulenone and compound **4**.

Patchoulenone [51]			Compound **4**	
position	δ_c	δ_H multiplicity, *J* in Hz	δ_c	δ_H multiplicity, *J* in Hz
1	63.7	-	63.6	-
2	26.2	1.64, ddd, 13.5, 9.5, 1.4	26.2	1.65, ddd, 13.6, 8.3, 1.4
		1.89, dt, 13.5, 9.5		1.86, m
3	43.5	2.44, br dd, 18.0, 9.5	43.4	2.44, br dd, 18.2, 10.1
		2.78, br dt, 18.0, 9.5		2.78, br dt, 18.1, 9.7
4	148.5	-	148.5	-
5	139.7	-	139.6	-
6	207.3	-	207.0	-
7	63.0	2.05, t, 3.5	63.0	2.05, t, 3.5
8	25.9	1.73, ddd, 14.0, 7.0, 3.5	25.9	1.73, br ddd, 13.2, 6.7, 3.8
		1.93, ddt, 6.5, 3.5, 14.0		1.95, br ddt, 13.6, 6.6, 3.3
9	28.1	1.15, ddt, 12.0, 7.0, 14.0	28.0	1.15, ddt, 12.0, 7.1, 14.1
		1.57, dt, 14.0, 6.5		1.57, br dt, 14.0, 6.7
10	34.6	2.16, ddq, 12.0, 6.5, 6.5	34.6	2.16, dq, 12.0, 6.4
11	41.4	-	41.4	-
12	19.0	1.01, s	18.9	1.01, s
13	26.4	0.88, s	26.3	0.88, s
14	15.2	2.07, q like	15.2	2.07, br s
15	17.9	0.84, d, 6.5	17.8	0.84, d, 6.4

4.1.5 Structure elucidation of compound 5.

The IR spectrum of compound **5** is shown in Figure 30 and the absorption peaks are listed in Table 13.

Table 13: Assignments of the IR absorption bands of compound **5**.

Wave number (cm^{-1})	Intensity	Vibration
3053	Weak	C-H stretching vibration of 5-ring-heteroaromatic
2951, 2866	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1750, 1712	Strong	C=O stretching vibration of carbonyl group
1242	Medium	C-H bending vibration of $-CH_3$, $-CH_2$
1198, 1149	Medium	C-O stretching vibration of ester
872	Medium	C=CH out of plane vibration

The ^{1}H NMR spectrum of compound **5** (Figure 31, Table 14) revealed the presence of two methyl groups at 0.68 (3H, s) and 1.25 (3H, s) ppm, two methoxy groups at 3.59 (3H, s) and 3.69 (3H, s) ppm, three olefinic protons of a furanoid group at 6.19 (1H, d, *J*=0.9 Hz), 7.13 (1H, s), and 7.26 (1H, t, *J*=1.6 Hz) ppm, one vinylic proton at 6.57 (1H, t, *J*=4.3 Hz) ppm, and a number of methine and methylene protons resonanced at 0.7-2.6 ppm.

The ^{13}C NMR (Figure 33, Table 14) and DEPT spectral data of compound **5** revealed the presence of 22 lines. Six lines of olefinic carbons appeared at 111.0, 125.1, 137.2, 138.5, 141.7, and 142.7 ppm, while two lines at 167.0 and 174.9 ppm should be of the carbonyl ester. Two lines at 51.1 and 51.2 ppm were of the methoxy carbons; two lines at 19.9 and 20.8 ppm were of the methyl group; six lines at 17.1, 18.3, 21.5, 26.9, 34.8, and 40.8 ppm were of the methylene carbons; two lines at 40.4 and 49.0 ppm were of the methine carbons; and two lines were quaternary carbons at 37.2 and 38.7 ppm.

The APCITOF MS indicated the molecular formula of compound **5** as $C_{22}H_{30}O_5$. The ^{1}H-^{1}H COSY spectrum revealed correlations of H-1 (δ_H 1.57 and 1.70 ppm) to H-10 (δ_H 1.35 ppm) and H-2 (δ_H 2.13 and 2.25 ppm); H-2 to H-3 (δ_H 6.57 ppm); H-7 (δ_H 1.61 and 1.97 ppm) to H-6 (δ_H 1.05 and 2.35 ppm) and H-8 (δ_H 2.52

ppm); C-11 (δ_H 1.37 and 1.68 ppm) to H-12 (δ_H 2.10 and 2.45 ppm); and H-14 (δ_H 6.19 ppm)to H-15 (δ_H 7.26 ppm) and H-16 (δ_H 7.13 ppm). In addition, the HMBC spectrum also confirmed the structural connectivity, showing correlations of H-10 to C-2 (δ_C 26.9 ppm), C-5 (δ_C 37.2 ppm), and C-9 (δ_C 38.7 ppm); H-3 to C-4 (δ_C 111.0 ppm) and C-18 (δ_C 167.0 ppm); H-19 to C-4, C-6 (δ_C 34.8 ppm), and C-10 (δ_C 46.4 ppm); H-7 to C-5 (δ_C 37.2 ppm); H-8 to C-9 (δ_C 38.7 ppm) and C-17 (δ_C 174.9 ppm); H-20 to C-9, C-8 (δ_C 49.0 ppm), and C-11 (δ_C 40.8 ppm); H-12 to C-13 (δ_C 125.1 ppm), C-14 (δ_C 111.0 ppm), and C-16 (δ_C 138.5 ppm); and H-14 to C-15 (δ_C 172.7 ppm) and C-16.

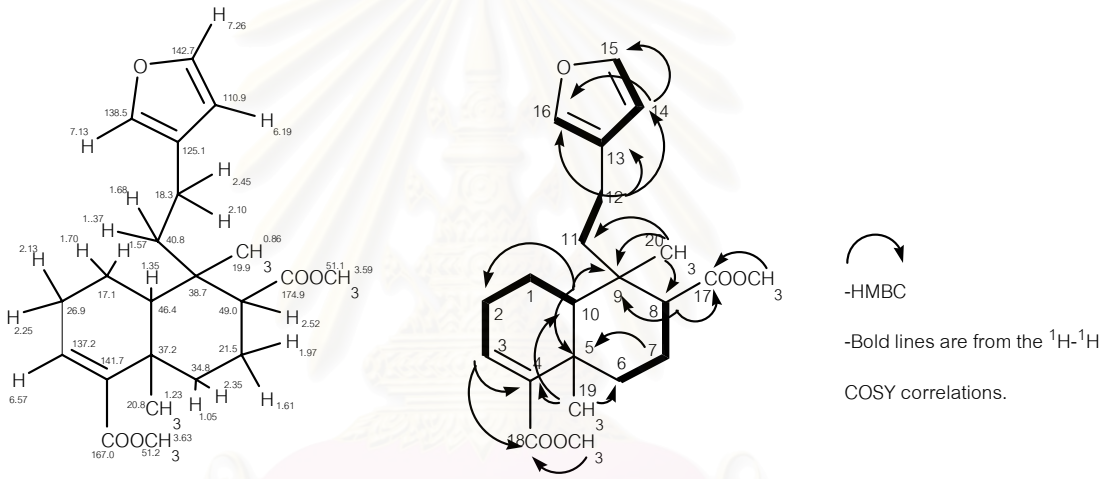
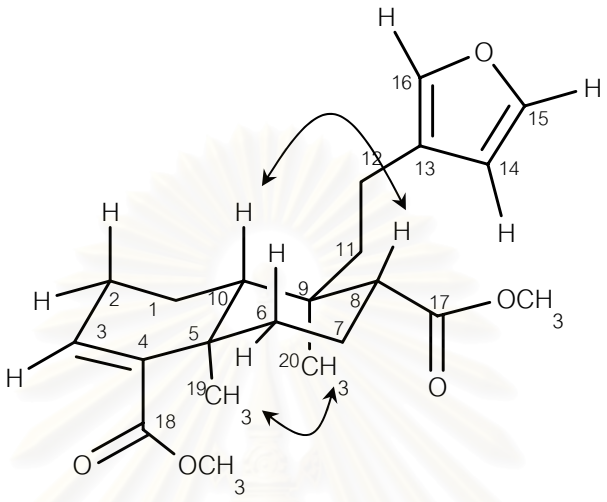


Figure 7: HMBC and ^{1}H-^{1}H COSY correlations of compound **5.**

The relative configuration was established by analyses of the NOESY spectrum, which also demonstrated the proximities of H-8 to H-10, and H-19 to H-20. The relative configurations of compound **5** are shown below.



Based upon these spectroscopic data, the structure of compound **5** was secured. Compound **5** was a known diterpene, namely nasimalun B, previously isolated from the roots of *Barringtonia racemosa* [49]. Comparison of ^{1}H and ^{13}C NMR data of nasimalun B and compound **5** is shown in Table 14. Nasimalun B exhibited antibacteria activity [54].

Table 14: The ^{1}H and ^{13}C spectral data of Nasimalun B and compound **5**.

Nasimalun B [49]			Compound **5**	
position	δ_c	δ_H multiplicity, *J* in Hz	δ_c	δ_H multiplicity, *J* in Hz
1	17.2	1.53, dddd, 13.5, 13.5, 13.5, 5.0	17.1	1.57, m
		1.70, dddd, 13.5, 5.5, 5.0, 5.0		1.70, m
2	26.9	2.19, m	26.9	2.13, m
		2.33, ddd, 20.0, 5.0, 5.0		2.25, dt, 19.8, 4.9, 4.9
3	137.2	6.65, dd, 5.0, 3.0	137.2	6.57, t, 4.3
4	141.8	-	141.8	-
5	37.3	-	37.2	-
6	34.9	1.13, ddd, 13.5, 13.5, 4.0	34.8	1.05, td, 3.6, 13.3
		2.44, ddd, 13.5, 3.0, 3.0		2.35, dt, 3.4, 13.2
7	21.6	1.66, m	21.5	1.61, m
		2.05, dddd, 13.5, 13.5, 13.5, 3.0		1.97, ddd, 3.4, 13.9, 13.9
8	49.1	2.59, dd, 12.0, 6.0	49.0	2.52, dd, 3.6, 12.9
9	38.7	-	38.7	-
10	46.4	1.43, dd, 12.0, 6.0	46.4	1.35, m
11	40.9	1.45, m	40.8	1.37, m
		1.77, ddd, 18.0, 13.5, 5.0		1.68, m
12	18.1	2.21, m	18.3	2.10, m
		2.53, ddd, 13.5, 13.5, 3.5		2.45, td, 3.6, 13.3
13	125.1	-	125.1	-
14	111.0	6.28, d, 2.0	111.0	6.19, d, 0.9
15	142.7	7.30, dd, 2.0, 1.0	142.7	7.26, t, 1.6
16	138.5	7.22, s	138.5	7.13, s
17	174.9	-	174.9	-
18	167.5	-	167.0	-
19	20.8	1.34, s	20.8	1.23, s
20	19.9	0.95, s	19.9	0.86, s
21	51.2	3.71, s	51.2	3.63, s
22	51.1	3.68, s	51.1	3.59, s

4.1.6 Structure elucidation of compound 6.

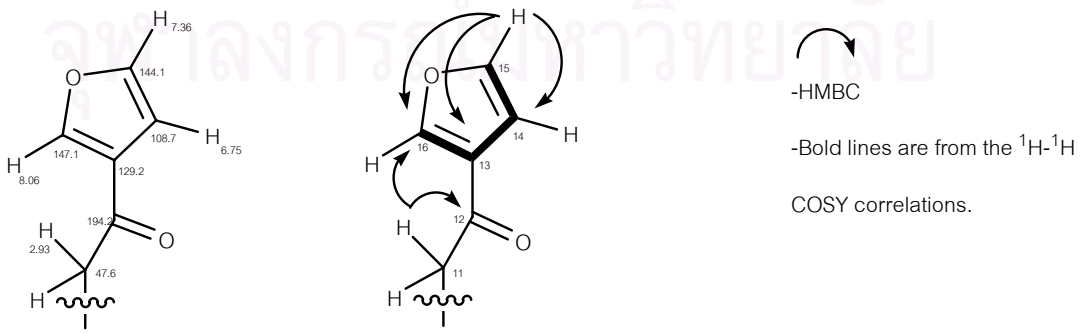
The IR spectrum of compound **6** is shown in Figure 35 and the absorption peaks are in Table 15.

Table 15: Assignments of the IR absorption bands of compound **6**.

Wave number (cm^{-1})	Intensity	Vibration
3133	Weak	C-H stretching vibration of 5-ring-heteroaromatic
2951	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1710	Strong	C=O stretching vibration of carbonyl group
1230	Medium	C-H bending vibration of $-CH_3$, $-CH_2$
1193, 1150	Medium	C-O stretching vibration of ester
873	Medium	C=CH out of plane vibration

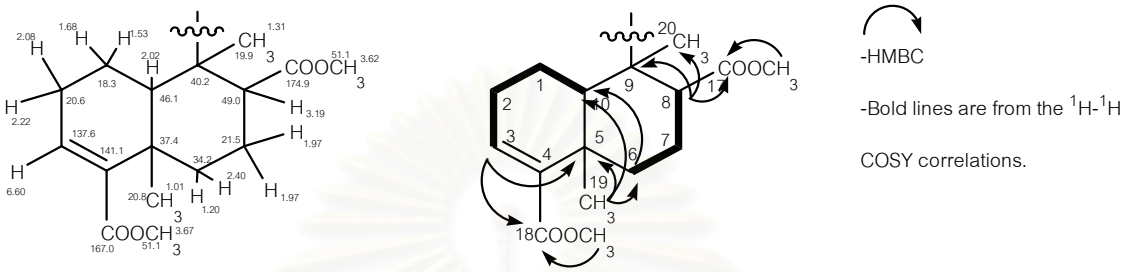
The ^{1}H NMR and ^{13}C NMR spectra compound **6** (Figure 36 and 38, Table 16) shows a great deal of similarity to that compound **5** except the replacement of a methylene C-12 by a ketone in compound **6**.

The molecular of compound **6** was established as $C_{22}H_{28}O_6$ by the APCITOF MS, showing the peak at *m/z* 389.1959 (calcd for $[C_{22}H_{28}O_6+H]^+$, 389.1962). The ^{1}H NMR spectrum showed the furan signals (δ_H 6.75, dd, *J*=0.6 and 1.8 Hz, δ_H 7.36, t, *J*=1.6 Hz, and δ_H 8.06, s). The ^{1}H-^{1}H COSY spectrum (Fig. 39) of compound **6** established the partial structure from H-14 to H-16, while the HMBC spectra (Fig. 40) revealed correlations of H-15 to C-13, C-14, and C-15; and H-11 to C-12 and C-16.



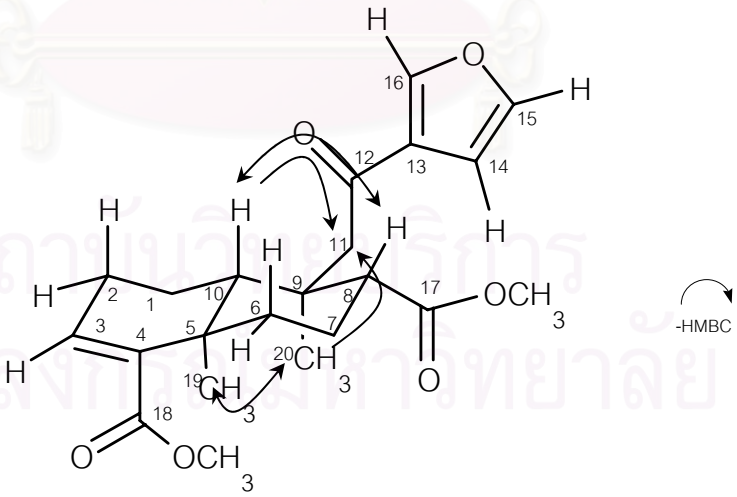
Partial structure A was elucidated by analyses of the ^{1}H-^{1}H COSY and HMBC spectral data.

Partial structure B was obtained by analyses of the ^{1}H-^{1}H COSY and HMBC spectral data. The ^{1}H-^{1}H COSY spectrum allowed the assignment from H-10 to H-3 and H-6 to H-8. In addition, the HMBC showed correlations from H-3 to C-18 and C-5; H-19 to C-5, C-6, and C-10; and H-8 to C-9, C-17, and C-20



Partial structure B was elucidated by analyses of the ^{1}H-^{1}H COSY and HMBC spectral data.

The connectivity of two substructures was established with the help of the HMBC spectrum, showing correlation of H-10 and H-20 to C-11. The relative configuration could be established by analyses of the NOESY spectrum (Fig. 41), showing the correlations of H-8 to H-10 and H-19 to H-20.



The ^{1}H and ^{13}C NMR spectral data of compound **6** are displayed in Table 16.

Table 16: The ^{1}H and ^{13}C spectral data of compound **6**.

Compound **6**				
Position	δ_c	δ_H multiplicity, *J* in Hz	COSY	HMBC
1	18.3	1.53, m	H-10, H-2, H-1b	C-2, C10, C-5
		1.68, m	H-10, H-2, H-1a	C-2, C-10, C-5, C-9
2	26.8	2.08, m	H-1, H-3, H-2b	C-1, C-3, C-4
		2.22, dt, 4.9, 4.9, 19.7	H-1, H-3, H-2a	C-1, C-3, C-4, C-10
3	137.6	6.60, dd, 2.8, 4.5	H-2	C-1, C-5, C-18
4	141.1	-		
5	37.4	-		
6	34.2	1.20, td, 3.7, 13.6, 13.6	H-7, H-6b	C-5
		2.40, dt, 3.4, 3.4, 13.2	H-7, H-6a	C-5, C-8, C-10
7	21.5	1.97, td, 3.2, 13.3, 13.3	H-6, H-8	C-6
8	49.0	3.19, dd, 3.9, 13.0	H-7	C-7, C-9, C17
9	40.2	-		
10	46.1	2.02, m	H-11	C-1, C-2, C-6, C-9, C-11
11	47.6	2.93, s	H-10	C-8, C-9, C-10, C-12
12	194.2	-		
13	129.2	-		
14	108.7	6.75, dd, 0.6, 1.8	H-15, H16	C-12, C-13, C-15, C-16
15	144.1	7.36, t, 1.6	H-14, H16	C-13, C-14, C-16
16	147.1	8.06, s	H-14, H-15	C-13, C-14, C-15
17	175.0	-		
18	167.0	-		
19	26.9	1.01, s		C-4, C-6, C-10
20	19.5	1.31, s		C-8, C-9, C-10, C-12
17’	51.1	3.62, s		C-17
18’	51.1	3.67, s		C-18

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4.1.7 Structure elucidation of compound 7.

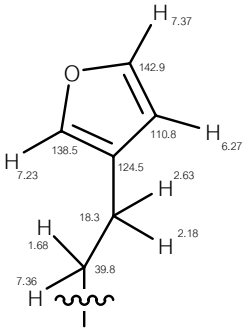
The IR spectrum of compound **7** is shown in Figure 43 and the absorption peaks are assigned in Table 17.

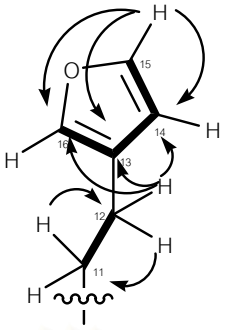
Table 17: Assignments of the IR absorption bands of compound **7**.

Wave number (cm^{-1})	Intensity	Vibration
3140	Weak	C-H stretching vibration of 5-ring-heteroaromatic
2926, 2870	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1767, 1728	Strong	C=O stretching vibration of carbonyl group
1663	Strong	C=CH stretching vibration of alkene
1263	Medium	C-H bending vibration of $-CH_3$, $-CH_2$
1192, 1146	Medium	C-O stretching vibration of ester
872	Medium	C=CH out of plane vibration

The ^{1}H NMR and ^{13}C NMR spectra of compound **7** (Figure 44 and 46, Table 16) demonstrated general features very similar to those compound **2**. The significant differences between compounds **2** and **7** were the absence of the carbonyl ketone (C-12, 193.6 ppm) and replacement of the methylene carbon (H-3_a=2.18, m; H-3_b=2.63, td, *J*=4.2, 13.4m, 13.4 Hz).

The molecular formula of $C_{21}H_{26}O_5$ for compound **7** was deduced from the APCITOF MS. The ^{1}H-^{1}H COSY spectrum (Fig. 47) of compound **7** revealed correlations of H-14 to H-16, while the HMBC spectrum (Fig. 48) showed correlations of H-11 to C-12; H-12 to C-13, C-14, and C-16; and H-15 to C-13, C-14, and C-16.



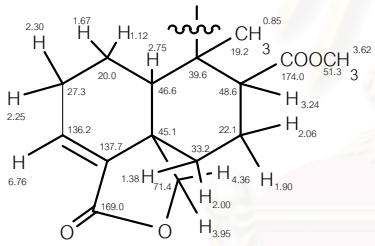


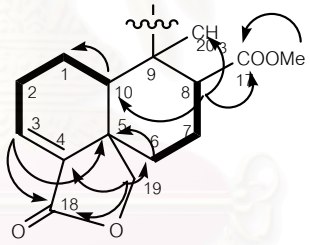
-HMBC

-Bold lines are from the ^{1}H-^{1}H COSY correlations.

Partial structure A was elucidated by analyses of the ^{1}H-^{1}H COSY and HMBC spectral data.

Partial structure B was obtained by analyses of the ^{1}H-^{1}H COSY and HMBC spectral data. The ^{1}H-^{1}H COSY spectrum showed the correlations from H-10 to H-3 and H-6 to H-8. In addition, the HMBC showed correlations from H-3 to C-18 and C-5; H-19 to C-4, C-6, and C-18; and H-8 to C-10, C-17, and C-20



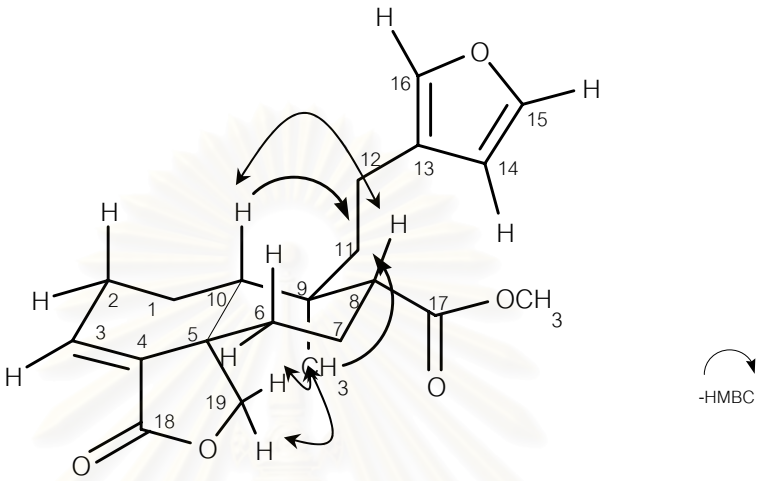


-HMBC

-Bold lines are from the ^{1}H-^{1}H COSY correlations.

Partial structure B was elucidated by analyses of the ^{1}H-^{1}H COSY and HMBC spectral data.

The connectivity of two substructures was established with the help of the HMBC spectrum, showing correlation of H-10 and H-20 to C-11. The relative configuration was established by analyses of the NOESY (Fig. 49) spectrum where the correlations between H-8 to H-10 and H-19 to H-20 are shown below.



The ^{1}H and ^{13}C NMR spectral data of compound **7** are shown in Table 18.

Table 18: The ^{1}H and ^{13}C spectral data of compound **7**.

Compound **6**				
position	δ_c	δ_H multiplicity, *J* in Hz	COSY	HMBC
1	19.2	1.11, dq, 3.7,12.2,12.2,12.2, 12.2	H-10, H-2, H-	C-3, C-9, C-10
		1.75, m	1b	C-3
2	27.5	2.20, m	H-10, H-2, H-	C-4
		2.43, m	1a	C-4, C-10
3	135.9	6.79, dd, 2, 7.4	H-1, H-3, H-2b	C-1, C-2, C-5, C-18
4	138.0	-	H-1, H-3, H-2a	
5	45.2	-	H-2	
6	33.4	1.23, m		C-4, C-5, C-7, C-19
		2.03, dt, 3.1, 3.1, 13.2		C-7
7	22.0	1.85, m	H-7, H-6b	C-9
		2.11, m	H-7, H-6a	C-8
8	48.8	2.73, dd, 4.0, 13.5	H-6, H-7b, H-8	C-7, C-9, C-17
9	38.8	-	H-7a, H-8	
10	47.7	1.79, m	H-7	C-2, C-5, C-6, C-9, C19
11	39.8	1.57, m		C-9, C-10
		1.68, m	H-1	C-9, C-10
12	18.3	2.18, m	H-11b, H-12	C-9, C-13, C-14, C-16
		2.63, td, 4.2, 13.4, 13.4	H-11a, H-12	C-9, C-13, C-14, C-16
13	124.5	-	H-11, H-12b	
14	110.8	6.27, s	H-11, H-12a	C-13, C-15, C-16
15	142.9	7.37, t, 1.6		C-13, C-14, C-16
16	138.5	7.23, s	H-15, H16	C-13, C-14, C-15
17	173.8	-	H-14, H16	
18	169.0	-	H-14, H-15	
19	71.9	3.95, dd, 2.0, 8.1		C-6
		4.40, d, 8.2		C-5, C-6, C-18
20	19.3	0.79, s		C-8, C-9, C-10, C-11
17’	51.4	3.70, s		C-17

4.1.8 Structure elucidation of compound 8.

The IR spectrum of compound **8** is shown in Figure 51 and the absorption peaks are in Table 19.

Table 19: Assignments of the IR absorption bands of compound **8**.

Wave number (cm^{-1})	Intensity	Vibration
3133	Weak	C-H stretching vibration of 5-ring-heteroaromatic
2960, 2889	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1739	Strong	C=O stretching vibration of carbonyl group
1276	Medium	C-H bending vibration of $-CH_3$, $-CH_2$
1198	Medium	C-O stretching vibration of ester
1160, 1133, 1113	Medium	C-O stretching vibration of ether
801	Medium	C=CH out of plane vibration

The ^{1}H NMR spectrum of compound **8** (Figure 52, Table 18) revealed the presence of one methyl group at 0.95 (3H, d, *J*=6.4 Hz); two oxygenated methine groups at 4.51 (1H, dd, *J*=0.9 and 5.7 Hz) and 5.33 (1H, dd, *J*=7.4 and 9.0 Hz) ppm; two ketal methine groups at 5.14 (1H, d, *J*=1.1 Hz) and 5.28 (1H, s) ppm; three olefinic protons of furanoid groups at 6.39 (1H, br d, *J*=0.9 Hz), 7.38 (1H, br t, *J*=1.7 Hz), and 7.40 (1H, br s) ppm, one methoxy group at 3.71 (3H, s) ppm; and a number of methine and methylene protons resonanced at 1.7-2.5 ppm.

The ^{13}C NMR (Figure 54, Table 18) and DEPT spectral of compound **8** revealed the presence of 15 carbons, including sixteen sp^3 carbons (two methyl carbons at 16.8 and 51.6 ppm; five methylene carbons at 20.2, 26.5, 30.5, 31.5, and 38.6 ppm; seven methine carbons at 37.4, 38.8, 54.0, 74.9, 75.7, 100.6, and 104.3 ppm; and two quaternary carbons at 44.3 and 50.4 ppm), three sp^2 carbons (three methine carbons at 108.6, 139.3, and 143.4 ppm), and a carbonyl ester.

The molecular formula of compound **8** was established as $C_{21}H_{26}O_6$ by the APCITOF MS showing the peak at *m/z* 375.1804 (calcd for $[C_{21}H_{26}O_6+H]^+$, 375.1802). The ^{1}H-^{1}H COSY spectrum of compound **8** established the partials structure from H-1 (δ_H 1.85 and 2.30 ppm) to H-10 (δ_H 2.40 ppm) and H-2 (δ_H 1.58

and 1.95 ppm); H-3 (δ_H 4.50 ppm) to H-2 and H-4 (δ_H 2.85 ppm); and H-7 (δ_H 1.45 and 2.05 ppm) to H-6 (δ_H 1.35 and 1.73 ppm) and H-8 (δ_H 1.73 ppm), H-8 to H-17 (δ_H 0.95 ppm), H-11 (δ_H 2.15 and 2.25 ppm) to H-12 (δ_H 5.33 ppm), and H-14 (δ_H 6.39 ppm) to H-15 (δ_H 7.38 ppm) and H-16 (δ_H 7.40 ppm), while the HMBC spectrum revealed correlations of H-4 to C-5 (δ_C 44.2 ppm), C-18 (δ_C 170.2 ppm), and C-19 (δ_C 104.3 ppm); H-10 to C-1 (δ_C 20.1 ppm), C-9 (δ_C 50.2 ppm), and C-20 (δ_C 100.6 ppm); H-19 to C-10 (δ_C 38.8 ppm)and C-20; H-17 to C-9 and C-7 (δ_C 31.4 ppm); H-12 to C-13 (δ_C 127.1 ppm), C-14 (δ_C 108.6 ppm), and C-16 (δ_C 139.3 ppm); and H-14 to C-15 (δ_C 143.4 ppm) and C-16.

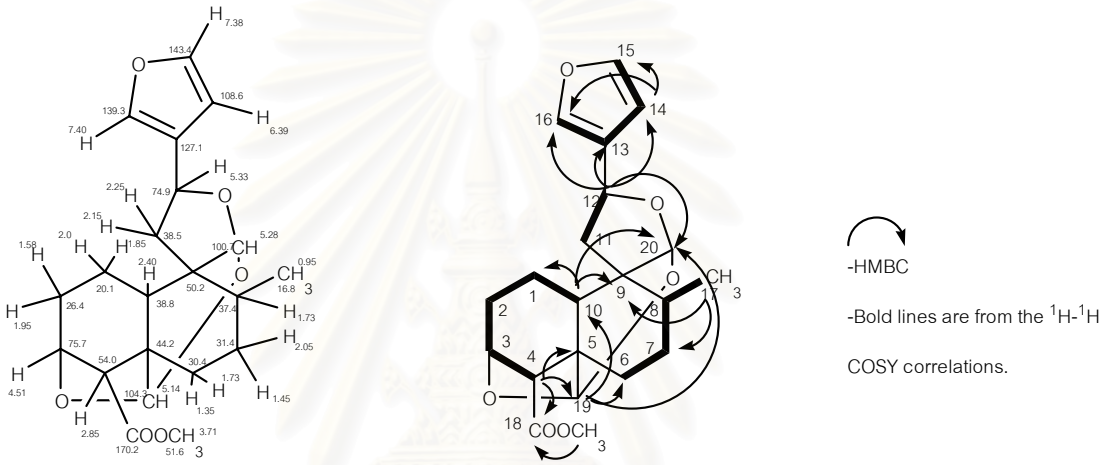
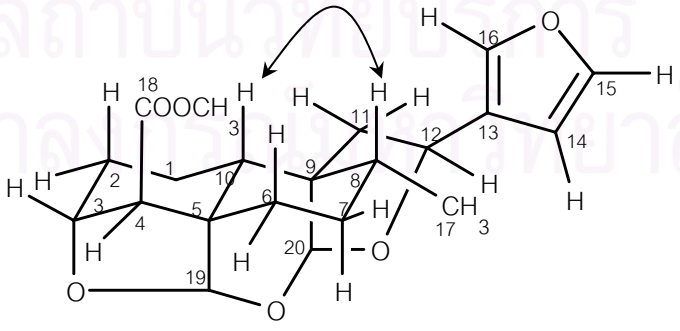


Figure 8: HMBC and ^{1}H-^{1}H COSY correlations of compound 8.

The relative configuration was established by analyses of the NOESY spectrum, demonstrating the proximities of H-8 and H-10. The relative configurations of compound **8** are shown below.



Comparison of spectroscopic data to those published in the literature (Table 20) confirmed that compound **8** was crovatin. Crovatin was previously isolated from

the stem barks of *Croton levatii* [53], however, its biological activity has not been investigated todate.

Table 20: The ^{1}H and ^{13}C spectral data of crovatin and compound **8**.

Crovatin [53]			Compound **8**	
position	δ_c	δ_H multiplicity, *J* in Hz	δ_c	δ_H multiplicity, *J* in Hz
1	20.2	1.9, m	20.1	1.85, m
		2.3, m		2.30, m
2	26.5	1.6, m	26.4	1.58, m
		1.9, m		1.95, m
3	75.8	4.48, ddd, 5.6, 4.3, 1.2	75.7	4.51, br t, 4.9
4	54.0	2.83, dd, 1.0, 5.6	54.0	2.85, dd, 0.9, 5.7
5	44.3	-	44.2	-
6	30.5	1.35, m	30.4	1.35, m
		1.70, m		1.73, m
7	31.5	1.45, m	31.4	1.45, m
		2.00, m		2.05, m
8	37.5	1.7, m	37.4	1.73, m
9	50.4	-	50.2	-
10	38.9	2.4, m	38.8	2.40, m
11	38.6	2.22, 13.4, 7.3	38.5	2.15, 7.3
		2.13, 9.2		2.25, 9.3
12	75.0	5.31, dd, 7.3, 9.2	74.9	5.33, dd, 7.4, 9.0
13	127.2	-	127.1	-
14	108.7	6.37, dd, 1.7, 0.8	108.6	6.39, br d, 0.9
15	143.5	7.63, dd, 1.7, 1.7	143.4	7.38, br t, 1.7
16	139.3	7.37, dd, 1.7, 0.8	139.3	7.40, br s
17	16.9	0.93, d, 6.5	16.8	0.95, d, 6.4
18	170.2	-	170.2	-
19	104.4	5.11, d, 1.0	104.3	5.14, d, 1.1
20	100.7	5.26, s	100.6	5.28, s
21	51.7	3.69, s	51.6	3.71, s

4.1.9 Structure elucidation of compound 9.

The IR spectrum of compound **9** is shown in Figure 56 and the absorption peaks were assigned as shown in Table 21.

Table 21: Assignments of the IR absorption bands of compound **9**.

Wave number (cm^{-1})	Intensity	Vibration
3135	Weak	C-H stretching vibration of 5-ring-heteroaromatic
2924	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1765, 1727	Strong	C=O stretching vibration of carbonyl group
1672	Medium	C=CH stretching vibration of alkene
1276	Medium	C-H bending vibration of $-CH_3$, $-CH_2$
1177, 1173	Medium	C-O stretching vibration of ester
872	Medium	C=CH out of plane vibration

The ^{1}H NMR spectrum of compound **9** (Figure 57, Table 20) revealed the presence of three methyl groups at 0.70 (3H, s), 0.77 (3H, d, *J*=6.5 Hz), and 1.19 (3H, s) ppm; three olefinic protons of furanoid groups at 6.19 (1H, s), 7.14 (1H, s), and 7.28 (1H, t, *J*=1.6 Hz) ppm; one vinylic proton at 6.79 (1H, t, *J*=3.0 Hz) ppm; and a number of methine and methylene protons resonanced at 1.0-2.4 ppm.

The ^{13}C NMR (Figure 59, Table 20) and DEPT spectral data of compound **9** revealed the presence of 20 signals, of which 13 carbons are sp^3 (three methyl carbons at 15.9, 18.3, and 20.5, six methylene carbons at 17.4, 18.1, 27.2, 27.5, 35.8, and 38.6 ppm; two methine carbons at 36.2 and 46.6 ppm; and two quaternary carbons at 37.5 and 38.8 ppm) and 7 carbons are sp^2 (four methine carbons at 111.0, 138.4, 140.3, and 142.7 ppm and three quaternary carbons at 125.6, 141.4, and 172.5 ppm) hybridized carbons, together with carbonyl carbon of a carboxylic acid.

The APCITOF MS indicated the molecular formula of compound **9** as $C_{20}H_{28}O_3$. The ^{1}H-^{1}H COSY spectrum revealed correlations of H-1 (δ_H 1.54 and 1.63 ppm) to H-10 (δ_H 1.33 ppm) and H-2 (δ_H 2.13 and 2.25 ppm); H-2 to H-3 (δ_H 6.79 ppm); H-7 (δ_H 1.37 and 1.47 ppm) to H-6 (δ_H 1.10 and 2.37 ppm) and H-8 (δ_H 1.52 ppm); H-8 to H-17 (δ_H 0.77 ppm); H-11 (δ_H 1.47 and 1.57 ppm) to H-12 (δ_H 2.13 and

2.23 ppm); and H-14 (δ_H 6.19 ppm)to H-15 (δ_H 7.14 ppm) and H-16 (δ_H 7.28 ppm). In addition, the HMBC spectrum fully established the structure of compound **9**, showing correlations of H-3 to C-4 (δ_C 141.3 ppm) and C-18 (δ_C 172.5 ppm); H-19 to C-4, C-10 (δ_C 46.6 ppm), and C-6 (δ_C 35.8 ppm); H-6 to C-5 (δ_C 37.5 ppm) and C-19 (δ_C 20.5 ppm); H-17 to C-7 (δ_C 27.2 ppm), C-8 (δ_C 36.2 ppm), and C-9 (δ_C 38.8 ppm); H-20 to C-10, C-7, and C-12 (δ_C 18.1 ppm); H-12 to C-11 (δ_C 38.6 ppm), C-13 (δ_C 125.6 ppm), and C-14 (δ_C 111.0 ppm); H-14 to C-15 (δ_C 138.4 ppm) and C-16 (δ_C 142.7 ppm); and H-16 to C-13.

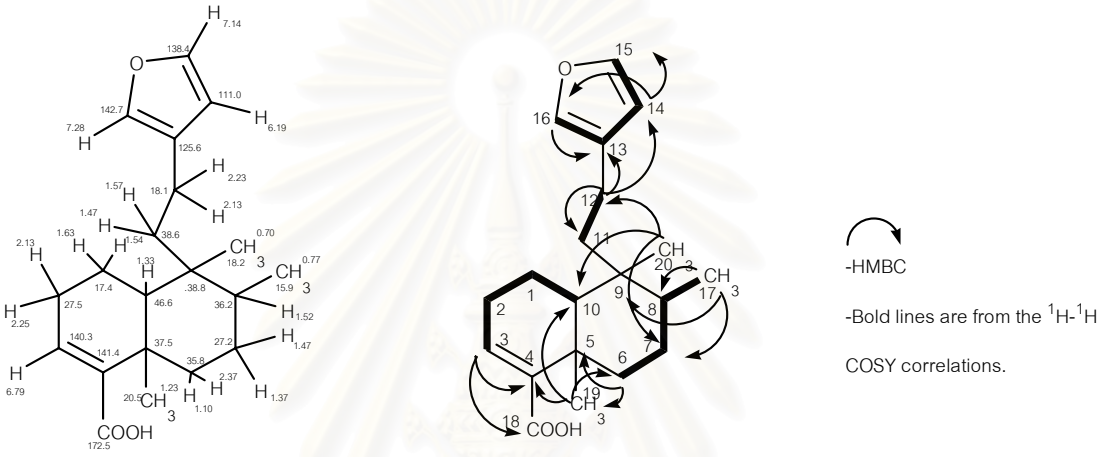
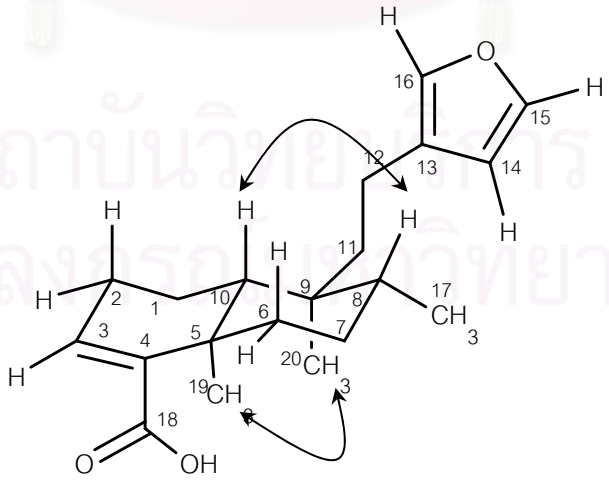


Figure 9: HMBC and ^{1}H-^{1}H COSY correlations of compound **9**.

The relative configuration of compound **9** was established by analyses of the NOESY spectrum, showing the proximities of H-8 and H-10; and H-19 and H-20. The relative configurations of compound **9** are shown below.



Based on these NMR spectral data and literature data comparison (Table 22), compound **9** was identified as (-)-hardwickiic acid, previously isolated from

genus *Croton*[37], *Salvia*[56], *Solidago*[57], *and Clerodendrum*[58]. (-)-Hardwickiic acid was reported to possess cytotoxicity.

Table 22: The ^{1}H and ^{13}C spectral data of (-)-hardwickiic acid and compound **9**.

(-)-Hardwickiic acid [30]			Compound **9**	
Position	δ_c	δ_H multiplicity, *J* in Hz	δ_c	δ_H multiplicity, *J* in Hz
1	18.6	1.87, m	17.4	1.54, m
				1.63, m
2	28.0	2.33, m	27.5	2.13, m
				2.25, m
3	138.1	6.90, m	140.3	6.79, dd, 3.0, 4.4
4	142.8	-	141.4	-
5	38.3	-	37.5	-
6	37.0	1.13, m	35.8	1.1, td, 5.2, 12.9, 12.9
				2.37, dt, 3.2, 3.2, 13.0
7	27.1	1.41, m	27.2	1.37, m
				1.47, m
8	36.6	1.55, m	36.2	1.52, m
9	39.5	-	38.8	-
10	47.6	1.49, m	46.6	1.33, m
11	39.5	1.72, m	38.6	1.47, m
				1.57, m
12	18.6	2.30, m	18.1	2.13, m
				2.23, m
13	126.5	-	125.6	-
14	111.8	6.25, s	111.0	6.19, dd, 0.8, 1.7
15	139.4	7.37, br s	138.4	7.14, br s
16	143.5	7.23, s	142.7	7.28, t, 1.7
17	16.2	0.85, d, 6.0	15.9	0.77, d, 6.5
18	173.2	-	172.5	-
19	20.9	1.27 s	20.5	1.19, s
20	18.2	0.80	18.3	0.71, s

4.2 Biological activity.

The compounds investigated for cytotoxic activity were 15-hydroxy-cis-*ent*-cleroda-3,13(*E*)-diene (compound **3**), patchoulenone (compound **4**), nasimalun B (compound **5**), and methyl-15,16-epoxy-3,13(16),14-clerodatriene-18,19-olide-17-oate (compound **7**). Compound **3** showed weak cytotoxicity against T47D, HepG2, and A549 cell lines with the IC_{50} values of 17.0, 14.5, and 7.4 µg/ml, respectively. Compound **4** showed weak cytotoxicity against HL-60 cell line at respective IC_{50} value of 15.38 µg/ml and exhibited strong cytotoxicity against P388 cell line with IC_{50} value of 1.06 µg/ml. Compound **5** showed weak cytotoxicity against HuCCA-1 and T47D cell lines with IC_{50} values of 18.0 and 13.0 µg/ml. Compound **7** displayed non-specific weak cytotoxicity against T47D, HL-60, and P388 cell lines at respective IC_{50} values of 10.0, 16.38, and 16.52 µg/ml. Compounds **1**, **2**, **6**, **8**, and **9** were inactive against all cell lines tested. The bioactivity results of compounds **1-9** are summarized in Table 23.

Table 23: Cytotoxic activity against cell lines of compounds **1-9**

Sample	IC_{50} (μg/ml)										
	HuCCA-1	KB	HeLA	MDA-MB231	T47D	H69AR	HepG2	A549	S102	HL-60	P388
Compound **1**	Inactive	Inactive	Inactive	Inactive	50.0	Inactive	>50	>50	>50	ND	ND
Compound **2**	Inactive	50.0	47.0	40.0	43.0	50.0	>50	>50	>50	ND	ND
Compound **3**	28.0	28.0	28.0	25.0	17.0	25.0	14.5	7.4	27.5	ND	ND
Compound **4**	Inactive	44.0	Inactive	46.0	32.0	Inactive	ND	ND	ND	15.38	1.065
Compound **5**	18.0	20.0	27.0	26.0	13.0	39.0	ND	ND	ND	ND	ND
Compound **6**	39.0	27.0	29.0	27.0	25.0	Inactive	ND	ND	ND	ND	ND
Compound **7**	36.0	26.0	30.0	29.0	10.0	41.0	ND	ND	ND	16.38	16.52
Compound **8**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Compound **9**	45.0	30.0	32.0	28.0	35.0	Inactive	38.5	35.0	>50	ND	ND
Etoposide	4.8	0.3	0.3	0.23	0.02	27	0.2	0.5	0.83	1.41	0.21

ND = not determine

Type of cell lines

HuCCA-1	=	Human cholangiocarcinoma
KB	=	Human epidermoid carcinoma in mouth
HeLa	=	Human cervical carcinoma
MDA-MB-231	=	Hormone-independent breast cancer
T47D	=	Hormone-dependent breast cancer
H69AR	=	Lung cancer, small cell, multidrug resistance
HepG2	=	Human hepatocellular carcinoma
A549	=	Human lung cancer, non-small cell
S102	=	Human liver cancer
HL-60	=	Human promyelocytic leukemia cell
P388	=	Mouse lymphoid neoplasm

CHAPTER V

CONCLUSION

Chemical examination of the roots of *Croton oblongifolius* Roxb. led to the isolation of nine clerodane diterpenoids. These isolated compounds included two new compounds, 19,20-dimethyl-15,16-epoxy-3,13(16),14-clerodatriene-17,18-oate-12-one (compound **6**) and methyl-15,16-epoxy-3,13(16),14-clerodatriene-18,19-olide-17-oate (compound **7**), and seven known compounds namely levatin (compound **1**), crovatin (compound **8**), nasimalun A (compound **2**), nasimalun B (compound **5**), 15 hydroxy-cis-*ent*-cleroda-3,13(*E*)-diene (compound **3**), patchoulenone (compound **4**) and (-)-hardwickiic acid (compound **9**). The derivatives of compound **3**, 15-(3,5-dinitrobenzoyl)-cis-*ent*-cleroda-3,13(*E*)-diene (compound **3a**), cis-*ent*-cleroda-3,13(*E*)-diene-15-al (compound **3b**), and cis-*ent*-cleroda-3,13(*Z*)-diene-15-al (compound **3c**) were synthesized.

The isolated compounds from *C. oblongifolius* were tested for their cytotoxicity against human tumor cell line HuCCA-1 (Human cholangiocarcinoma), KB (Human epidermoid carcinoma in mouth), HeLa (Human cervical carcinoma), MDA-MB-231 (Hormone-independent breast cancer), T47D (Hormone-dependent breast cancer), H69AR (Lung cancer, small cell, multidrug resistance), HepG2 (Human hepatocellular carcinoma), A549 (Human lung cancer, non-small cell), S102 (Human liver cancer), HL-60 (Human promyelocytic leukemia cell), and P388 (Mouse lymphoid neoplasm). Compound **3** showed weak cytotoxicity towards T47D and HepG2 cell lines at IC_{50} values of 17.0 and 14.5 µg/ml and showed strong cytotoxicity against A547 cell line at IC_{50} value of 7.4 µg/ml; compound **4** exhibited strong cytotoxicity against P388 cell line with IC_{50} value of 1.06 µg/ml, but weak cytotoxicity towards HL-60 cell line with IC_{50} value of 15.38 µg/ml; compound **5** showed weak cytotoxicity against HuCCA-1 and T47D cell lines at IC_{50} values of 18.0 and 13.0 µg/ml; and compound **7** showed weak cytotoxicity activity against T47D, HeLa, and P388 cell lines at respective IC_{50} values of 10.0, 16.38, and 16.52 µg/ml. Compounds **1**, **2**, **6**, **8**, and **9** were inactive against all cell lines.

REFERENCES

1. Available online from :
http://nationaalherbarium.nl/ThaiEuph/ThCspecies/ThCroton.htm

2. ไซมอน การ์ดเนอร์, พินดา สิทธิสุนทร และ วิไลวรรณ อนุสารสุทร. **ต้นไม้เมืองเหนือ.** พิมพ์ครั้งที่ 1. กรุงเทพมหานคร : โครงการจัดพิมพ์คบไฟ, 2543.

3. Icihari, Y., Takeya, K., Hitotsuyanagi, Y., Morita, H., Okuyama, S., Suganuma, M., Fujiki, H., Motidome, M. and Itokawa, H. Anti-infammatory diterpenes from *Croton cajucara*. Planta Med., **1991**, *58*, 549-551.

4. Thongtan, J., Kittakoop, P., Ruangrungsi, N., Saenboonrueng, J and Thebtaranonth, Y. New antimycobacterial and antimalarial 8,9-secokaurane diterpenes from *Croton kongensis*. J. Nat. Prod., **2003**, *66*, 868-870.

5. Roengsumran, S., Musikul, K., Petsom, A., Vilaivan, T., Sangvanich, P., Pornpakakul, S., Putong, S., Chaichantipyuth, C., Jaiboon, N. and Chaichit, N. Crobolngifolin, a new anticancer clerodan from *Croton oblongigolius*. Planta Med., **2002**, *68*, 274-277.

6. Aguilar, A. B., Guadarrama, A. B. and Rios, M. Y. Three new sesquiterpenes from *Croton arboreous.* J. Nat. Prod., **2004**, *67*, 914-917.

7. Tchissambou, L., Chaiaroni, A., Riche, C. and Khuong-Huu, F. Crotocorylifuran and crotohaumanoxide, new diterpenes from *Croton haumanianus* J. Leonard. Tetrahedron., **1990**, *46*, 5199-5202.

8. Amaral, A. C. F. and Barnes, R. A. A tetrahydroprotoberberine alkaloid from *Croton hemiargyreus.* Phytochemistry., **1998**, *47*, 1445-1447.

9. Graikou, K., Aligiannis, N., Skaltsounis, A. L., Chinou, I., Michel, S., Tillequin, F. and Litaudon, M. New diterpenes from *Croton insularis.* J. Nat. Prod., **2004**, *67*, 685-688.

10. Nihei, K. I., Asaka, Y., Mine, Y. and Kubo, I. Insect antifeedants from *Croton jatrophoides*: Structures of zumketol, zumsenin and zumsenol. J. Nat. Prod., **2005**, *68*, 244-247.

11. Sutthivaiyakit, S., Nareeboon, P., Ruangrungsi, N., Ruchirawat, S., Pisutjaroenpong, S. and Mahidol, C. Labdane and pimarane diterpenes from *Croton joufra.* Phytochemistry., **2001**, *56*, 811-814.

12. Itokawa, H., Ichihara, Y., Takeya, K., Morita, H. and Motidome, M. Diterpenes from *Croton salutaris*. Phytochemistry., **1991**, *30*, 4071-4073.

13. El-Mekkawy, S., Meselhy, M. R.., Nakamura, N., Hattori, M., Kawahata, T. and Otake, T. Anti-HIV-1 phorbol esters from the seeds of *Croton tiglium*. Phytochemistry., **2000**, *53*, 457-464

14. Giang, P. M., Jin, H. Z., Son, P. T., Lee, J. H., Hong, Y. S. and Lee, J. J. *ent*-Kaurane diterpenes from *Croton tonkinensis* inhibit LPS-Induced NF-kB Activation and NO Production. J. Nat. Prod., **2003**, *66*, 1217-1220.

15. Ngadjui, B. T., Folefoc, G. G., Keumedjio, F., Dongo, E., Sondengam, B. L. and Connolly, J. D. Crotonadiol, a labdane diterpenoid from the stem bark of *Croton zambesicus*. Phytochemistry., **1999**, *51*, 171-174.

16. Rao, P. S., Sachdev, T. R. and Singh, H. B. Isolation and constitution of oblongifoliol, a new diterpene of *Croton oblongifolius*. Tetrahedron Letter., **1968**, *45*, 4695.

17. Aiyar, V. N., Rao, P. S., Sachdev, T. R. and Singh, H. B. Isolation and constitution of deoxyoblongifoliol. Indian J. Chem., **1969**, *7*, 838.

18. Aiyar, V. N. and Seshadri, T. R. Components of *Croton oblongifolius* Roxb. III constitution of oblongifolic acid. Tetrahedron., **1970**, *26*, 5275-5279.

19. Aiyar, V. N. and Seshadri, T. R. Chemical components of *Croton oblongifolius* Roxb. Part IV Constitution of oblongifoliol and deoxyoblongifoliol. *Indain J. Chem*., **1971**, *9*, 1055-1059.

20. Aiyar, V. N. and Seshadri, T. R. Chemical components of *Croton oblongifolius* Roxb. Part V. Indain J. Chem., **1971**, *9*, 613-614.

21. Aiyar, V. N. and Seshadri, T. R. Isolation of acetyl aleuritolic acid from *Croton oblongifolius* Roxb. Indiam J. Chem., **1971**, *9*, 1028-1029.

22. Aiyar, V. N. and Seshadri, T. R. 11-dehydro-hardwickiic acid from *Croton oblongifolius* Roxb. Phytochemistry., **1972**, *11*, 1473-1746.

23. Aiyar, V. N. and Seshadri, T. R. Chemical components of *Croton oblongifolius* Roxb. Part V. Curr. Sci., **1972**, *41*, 839.

24. Roengsumram, S., Achayindee, S., Petom, A., Pudhom, K., Singtothong, P., Surachetapan, C. and Vilaivan, T. Two new cembranoids from *Croton oblongifolius* Roxb. J. Nat. Prod., **1998**, *61*, 652.

25. Roengsumran, S., Petsom, A., Sommit, D. and Vilaivan, T. Labdane diterpenoids from *Croton oblongifolius* Roxb. Phytochemistry., **1999**, *50*(3), 449-453.

26. Reongsumran, S., Singtotong, P., Pudhom, K., Ngamrojanavanich, N., Petsom, A. and Chaichantipyuth, C. Neocrotocembranal from *Croton oblongifolius* Roxb. J. Nat. Prod., **1999**, *62*, 1163-1164.

27. Reongsumran, S., Petsom, A., Kuptiyanuwat, N., Vilaivan, T., Ngamrojanavanich, C., Chaichantiyuth, C. and Phuthong, S. Cytotoxic labdane diterpenoids from *Croton oblongifolius* Roxb. Phytochemistry., **2001**, *56*(1), 103-107.

28. Reongsumran, S., Pornpakakul, S., Muangsin, N., Sangvanich, P., Nhujak, T., Singtotong, P., Chaichit, N., Puthong, S. and Petsom, A. New halimane diterpenoids from *Croton oblongifolius* Roxb. Planta Med., **2004**, *7*, 1-3.

29. Surachethapan, C. **Chemical constituents of the stem bark of *Croton oblongifolius Roxb*.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 1996.

30. Singtotong, P. **Chemical and biological activity of deterpenoid from *Croton oblongifolius* Roxb.** Ph. D. Dissertation, Department of chemistry, Faculty of science, Chulalongkorn University, 1999.

31. Tanwattanakul, T. **Chemical constituents and their biological activity of stem bark of *Croton oblongifolius* Roxb. From Amphoe Muang, Uttaradit Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 1999.

32. Achayindee, S. **Chemical constituents form the leaves of *Croton oblongifolius* Roxb.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 1996.

33. Boontha, B. **Cytotoxicity of chemical constituents of the stem bark of *Croton***

oblongifolius **Roxb. from Amphoe Muang, Prachuap Khiri Khan Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 2000.

34. Siriwat, K. **Chemical constituents abd biological activity from stem bark of *Croton oblongifolius* Roxb. from Amphoe Dan Sai, Loei Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 2000.

35. Ruangraweewat, N. **Chemical constituents and biological activity from stem bark of *Croton oblongifolius* Roxb. from Amphoe Phurua, Loei Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 2001.

36. Baigern, S. **Chemical constituents and biological activity from stem bark of *Croton oblongifolius* Roxb. from Amphoe Muang, Udon Thani Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 1999.

37. Sriyanok, S. **Chemical constituents and biological activity from stem bark of *Croton oblongifolius* Roxb. from Amphoe Pran Buri, Prachuap Khiri Khan Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 2000.

38. Bunyamanee, P. **Cytotoxicity to cancer cell lines of diterpenoid compounds from stem barks of *Croton oblongifolius* Roxb. from Chachoengsao Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 2000.

39. Sirimongkhon, S. **Kauranne diterpenes from stem bark of *Croton oblongifolius* Roxb. from Kui Buri, Prachuap Khiri Khan Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 2000.

40. Sommit, D. **Structure analysis of diterpenoid compounds from stem barks of *Croton oblongifolius* Roxb.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 1996.

41. Kuptiyanuwat, N. **Chemical constituents of stem barks of *Croton oblongifolius***

Roxb. and their biological activity from Amphoe Wangsapung, Loei Province. M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 1999.

42. Pattamadilok, D. **Chemical constituents of *Croton oblongifolius* stem bark from Chainat.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 1998.

43. Permpanya, L. **Chemical constituents of the stem barks of *Croton roxburghii* from Nahaew district, Loei Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 2003.

44. Khamlaeid, C. **Chemical constituents of stem barks of *Croton oblongifolius* from Amphoe Phanat Nikhom, Chonburi Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 2003.

45. Pata, P. **Chemical constituents with cytotoxicity of *Croton oblongifolius* from Nan Province.** M. Sc. Thesis, Department of chemistry, Faculty of science, Chulalongkorn University, 2004.

46. Dewick, P. M. **Medicanal natural product a biosynthetic approach. 2nd de**. John Wiley & Sons, 2001, 169-191.

47. Devon, T.K. and Scott, A. L. **Handbook of naturally occurring compounds.** New York and London : Academic press, 1972.

48. Moulis, C. and Fouraste, I. Levatin, an 18-norclerodane diterpene from *Croton levatii*. J. Nat. Prod., **1992**, *55*, 445-449.

49. Hansan, C.M., Khan, S., Jabbar, M. A., and Rashid, A.M. Nasimaluns A and B: neoclerodan diterpenes from *Barringtonia racemosa*. J. Nat. Prod., **2000**, *63*, 410-411.

50. Bläs, B., Zapp, J., and Becker, H. *ent*-Clerodane diterpenes and other constituents from the liverwort *Adelanthus lindenbergianus* (Lehm.) Mitt. Phytochemistry, **2004**, *65*, 127-137.

51. Thebtaranonth, C., Tebhtaranonth, Y., Wanauppathamkul, S., and Yuthavong, Y. antimalarial sesquiterpenes from tubers of *Cyperus rotundus*: structure of 10,12-peroxycalamenene, asesquiterpene endoperoxide. Phytochemistry,

1995, *40*, 125-128.

52. Monti, H., Tiliacos, N., and Faure, R. Copaiba oli : Isolation and characterization of a new diterpenoid with the dinorlabdane skeleton. Phytochemistry., **1999**, *51*, 1013-1015.

53. Moulis, C., Bon, M.,Joel, J., and Fouraste, I. Crovatin, a furanoid diterpene from *Croton levatii*. Phytochemistry., **1992**, *31*, 1421-1423.

54. Khan, S., Jabbar, A., Hasan, C.M., and Rashid, M.A. Antibacterial activity of *Barringtonia racemosa*. Fitoterapia., **2001**, *72*, 162-164.

55. Tenchaisri, T., Chawengkirttikul, R., Rachaphaew, N., Reutrakul, V., Sangsuwan, R., and Sirisinha, S. //. Cancer Letters., **1998**, *133*, 169-175.

56. Ortega, A., Cardenas, J., Gage, D.A., and Maldonado, E. Abietane and clerodane diterpenes from *Salvia regia*. Phytochemistry., **1995**, *39*, 931-933.

57. Lu, T., Vargas, D., Franzblau, S.G., and Fischer, N. H.Diterpenes from *Solidago rugosa*. Phytochemistry., **1995**, *38*, 451-456.

58. Ganapaty, S., and Rao, D. V. Cleroflavone, a new C-methylflavone from the leaves of *Clerodendrum neriifolium*. JCS. Section B., **1990**, *29*, 289-290

APPENDIX

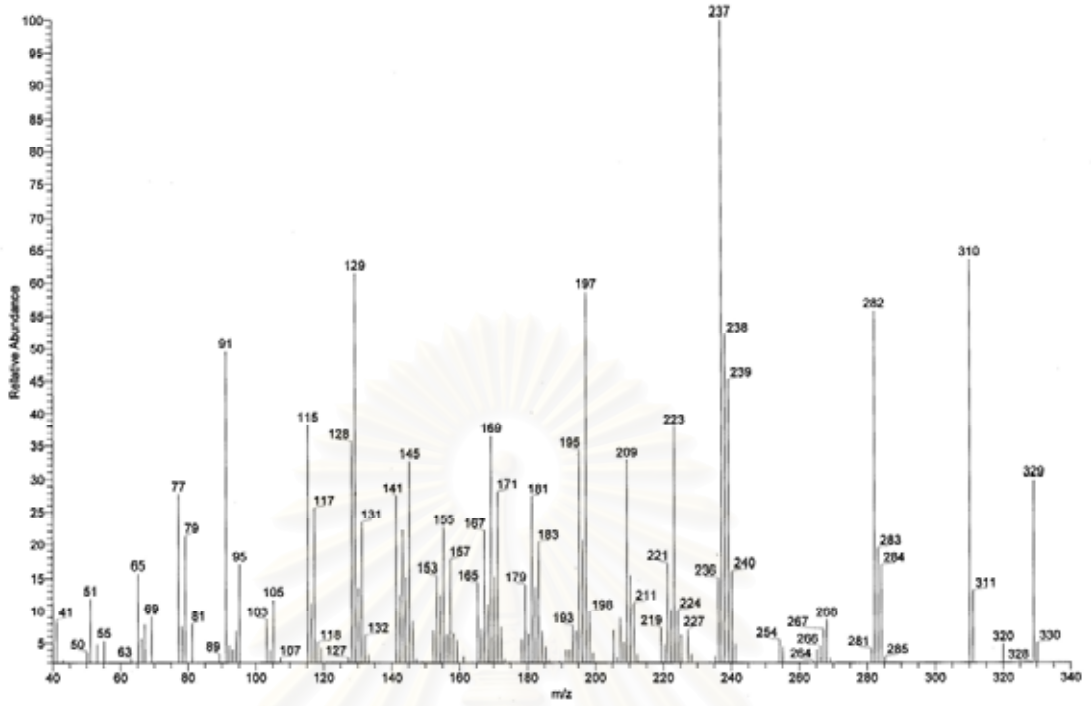


Figure 10: EIMS Mass spectrum of compound 1

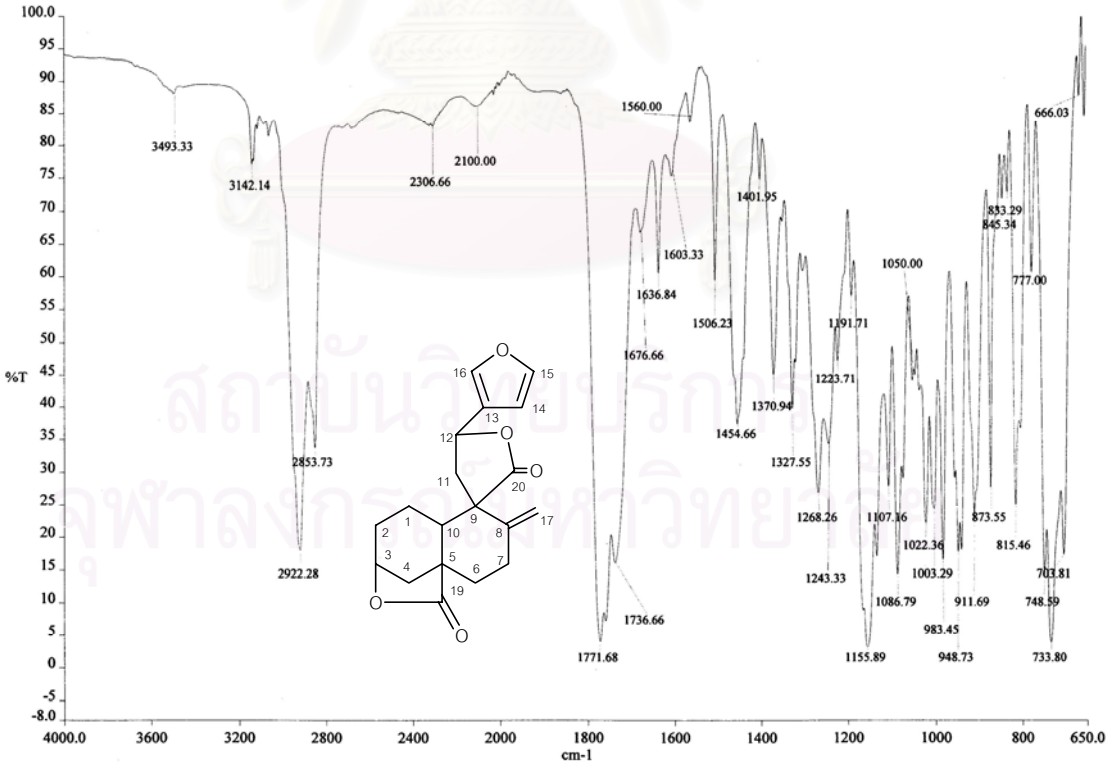


Figure 11: IR Spectrum of compound 1

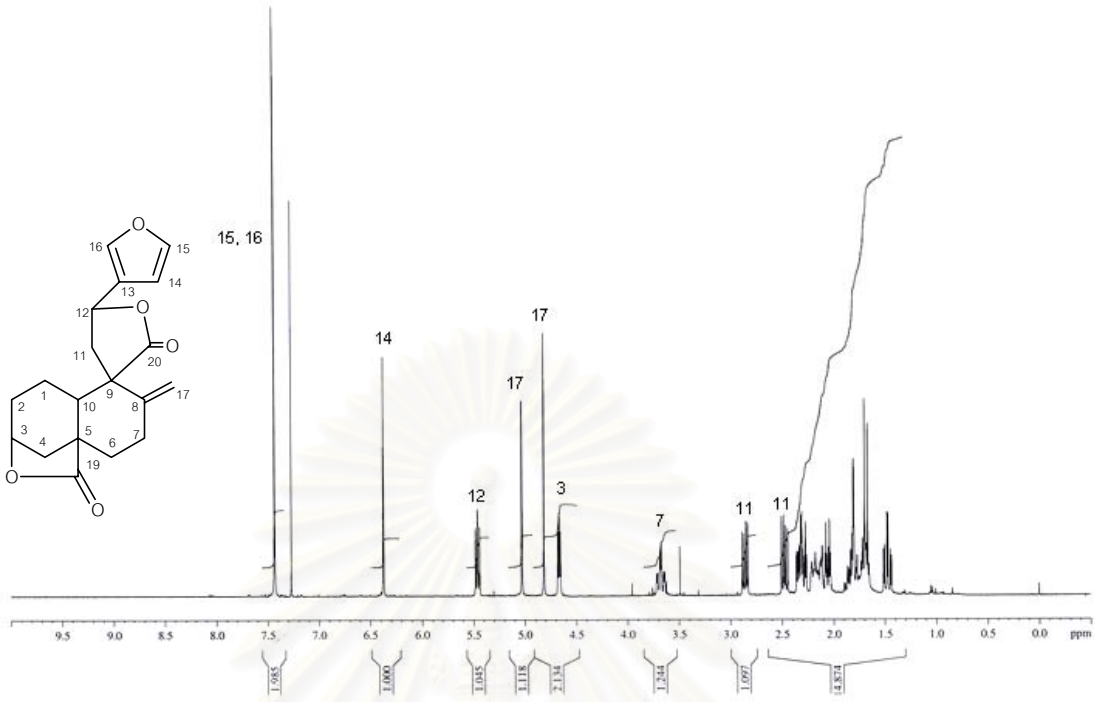


Figure 12: ^{1}H NMR (400 MHz) Spectrum of compound **1** ($CDCl_3$)

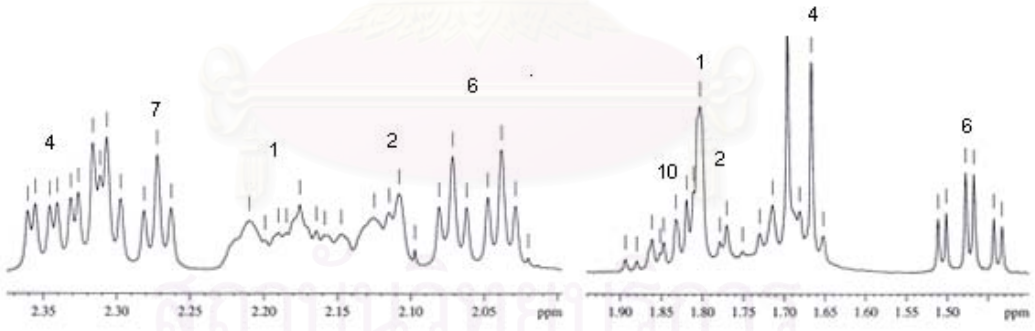


Figure 13: Expansion of ^{1}H NMR spectrum of compound **1**

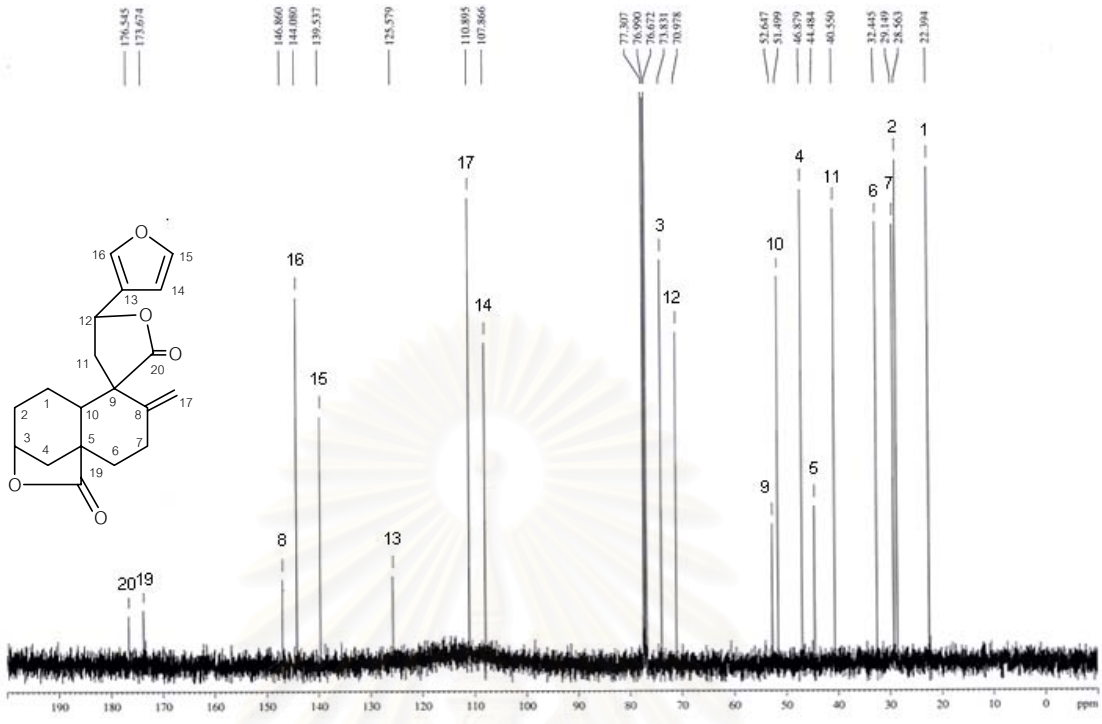


Figure 14: ^{13}C NMR (100 MHz) Spectrum of compound **1** ($CDCl_3$)

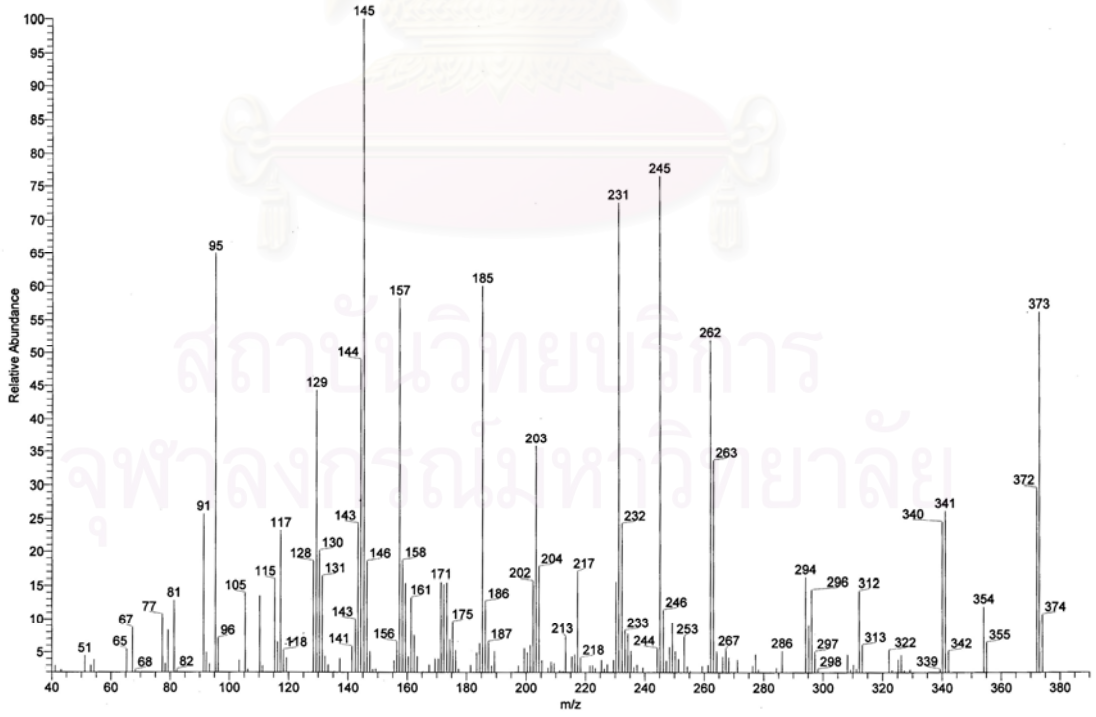


Figure 15: EIMS Mass spectrum of compound **2**

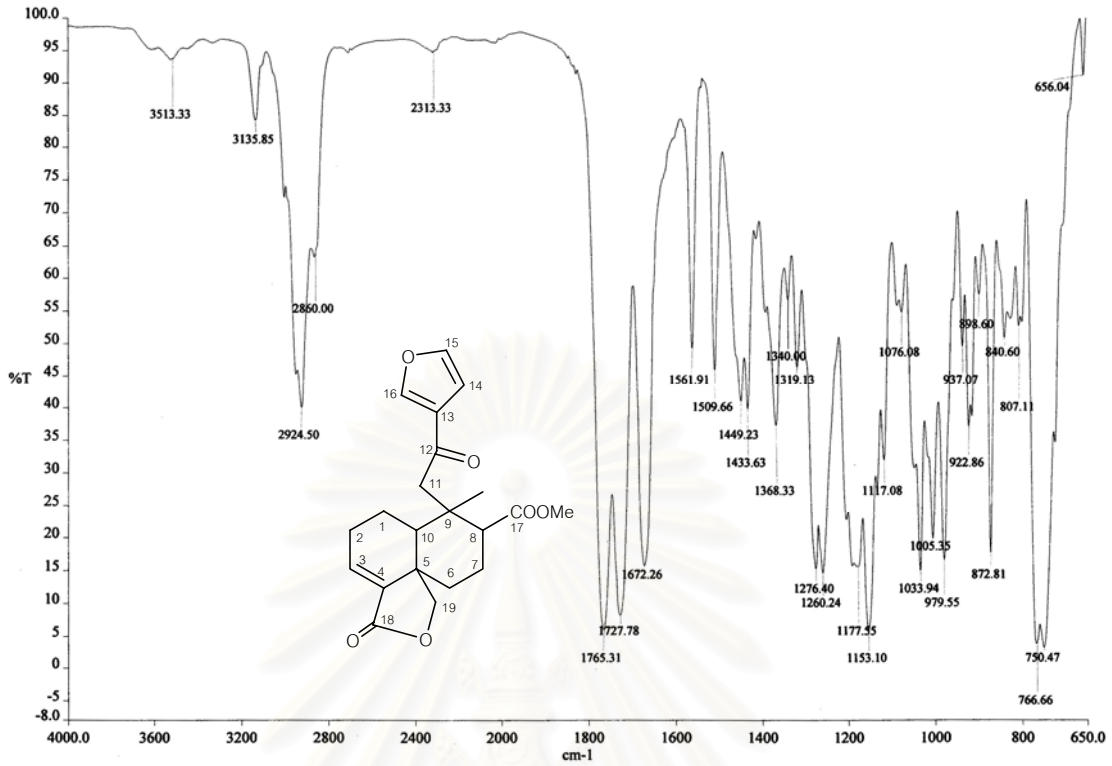


Figure 16: IR Spectrum of compound 2

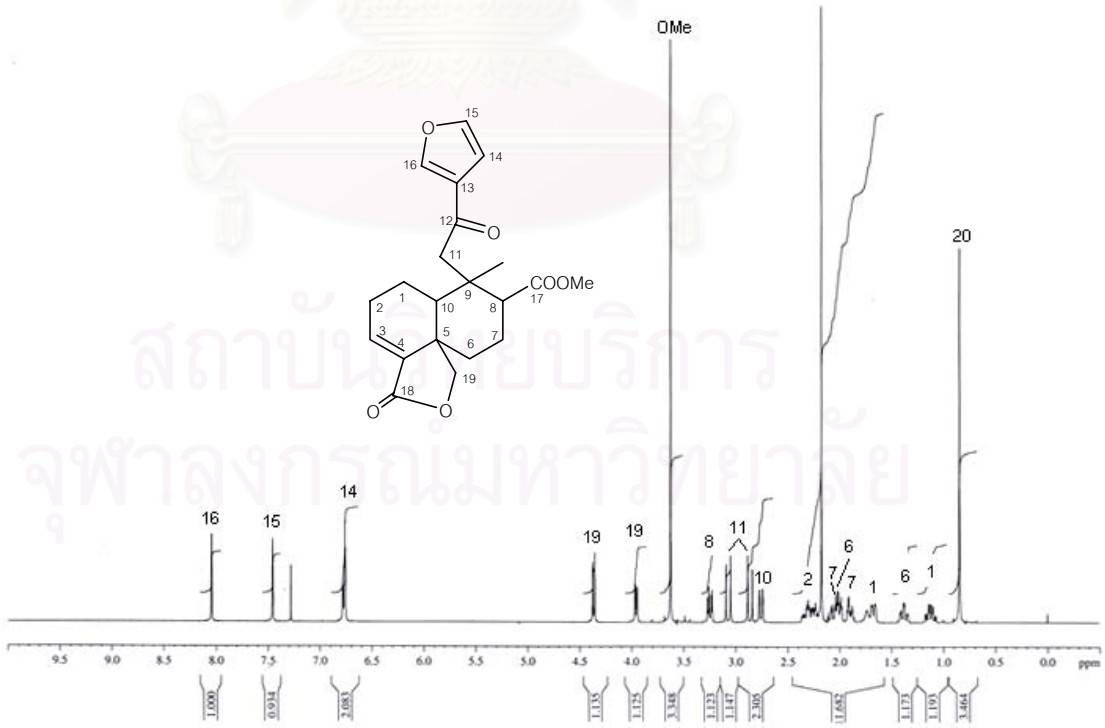


Figure 17: ^{1}H NMR (400 MHz) Spectrum of compound 2 ($CDCl_3$)

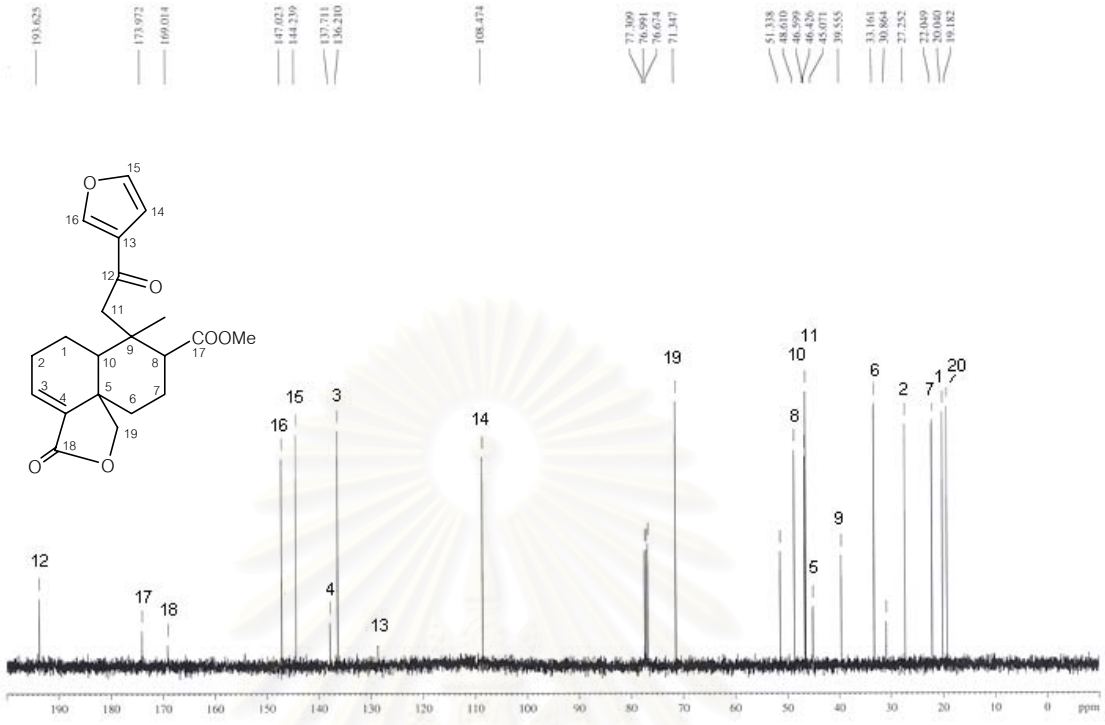


Figure 18: ^{13}C NMR (100 MHz) Spectrum of compound **2** ($CDCl_3$)

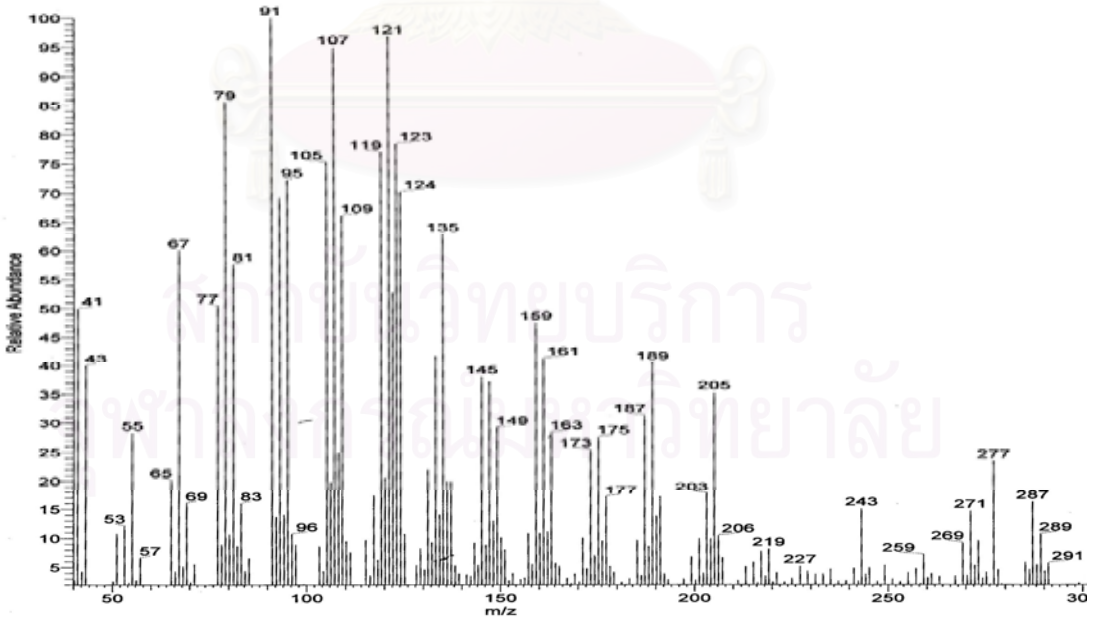


Figure 19: EIMS Mass spectrum of compound **3**

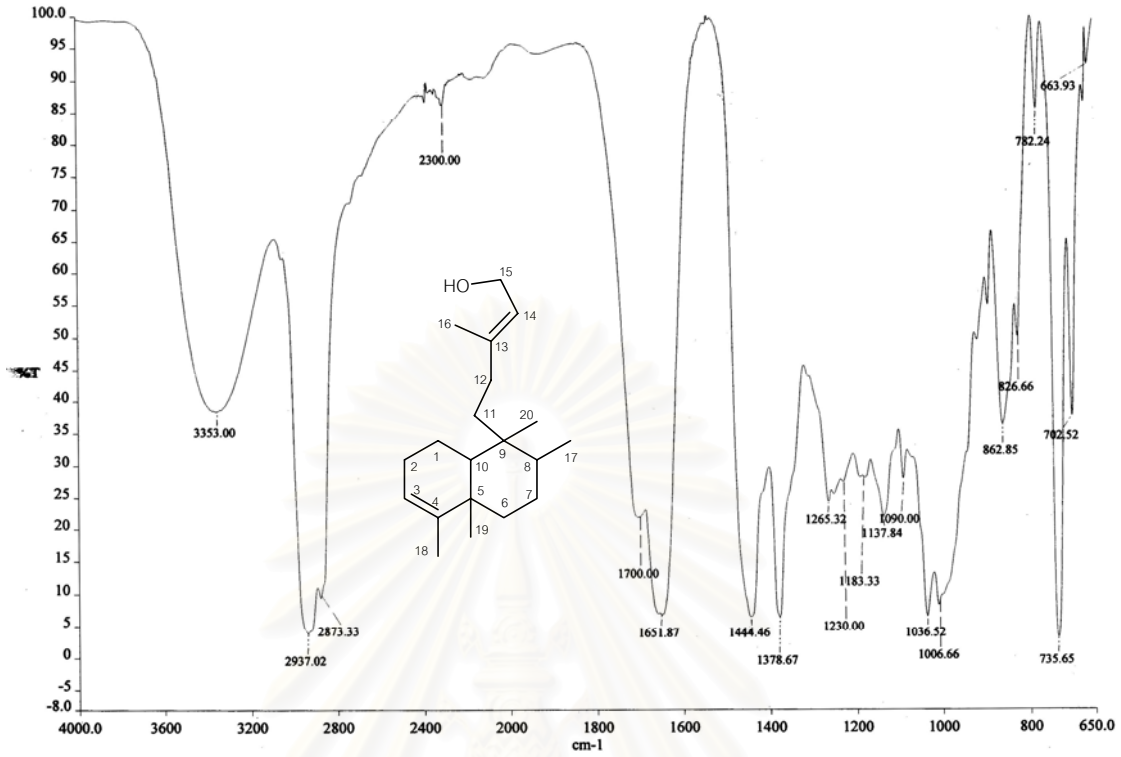


Figure 20: IR Spectrum of compound **3**

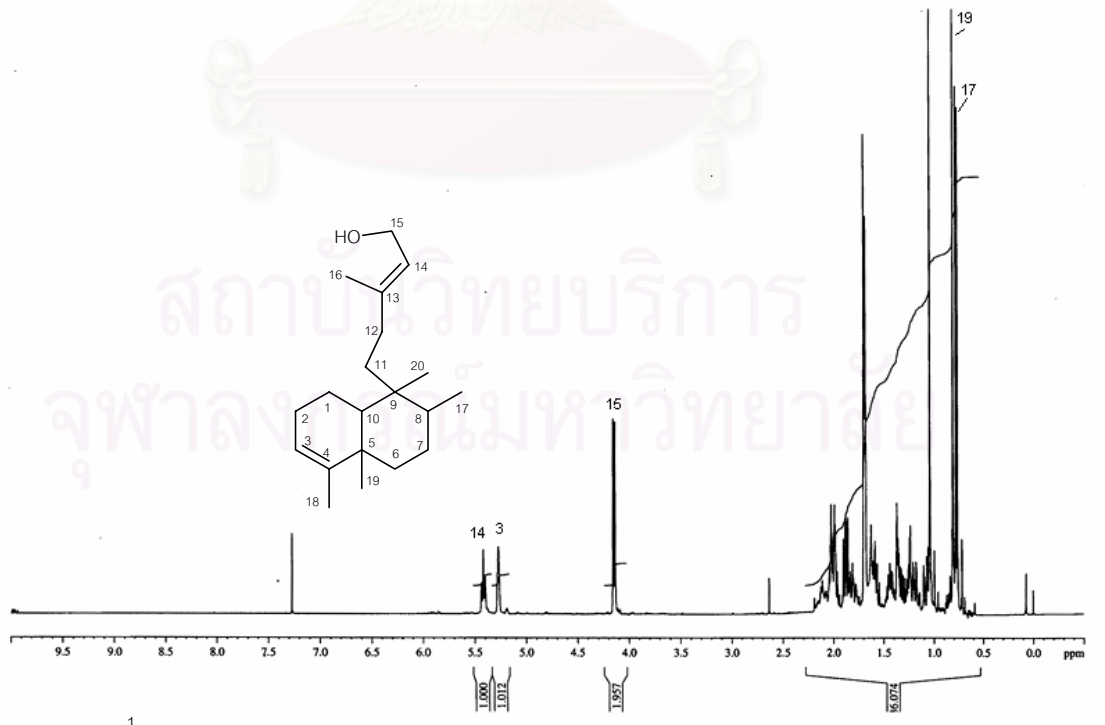


Figure 21: ^{1}H NMR (400 MHz) Spectrum of compound **3** ($CDCl_3$)

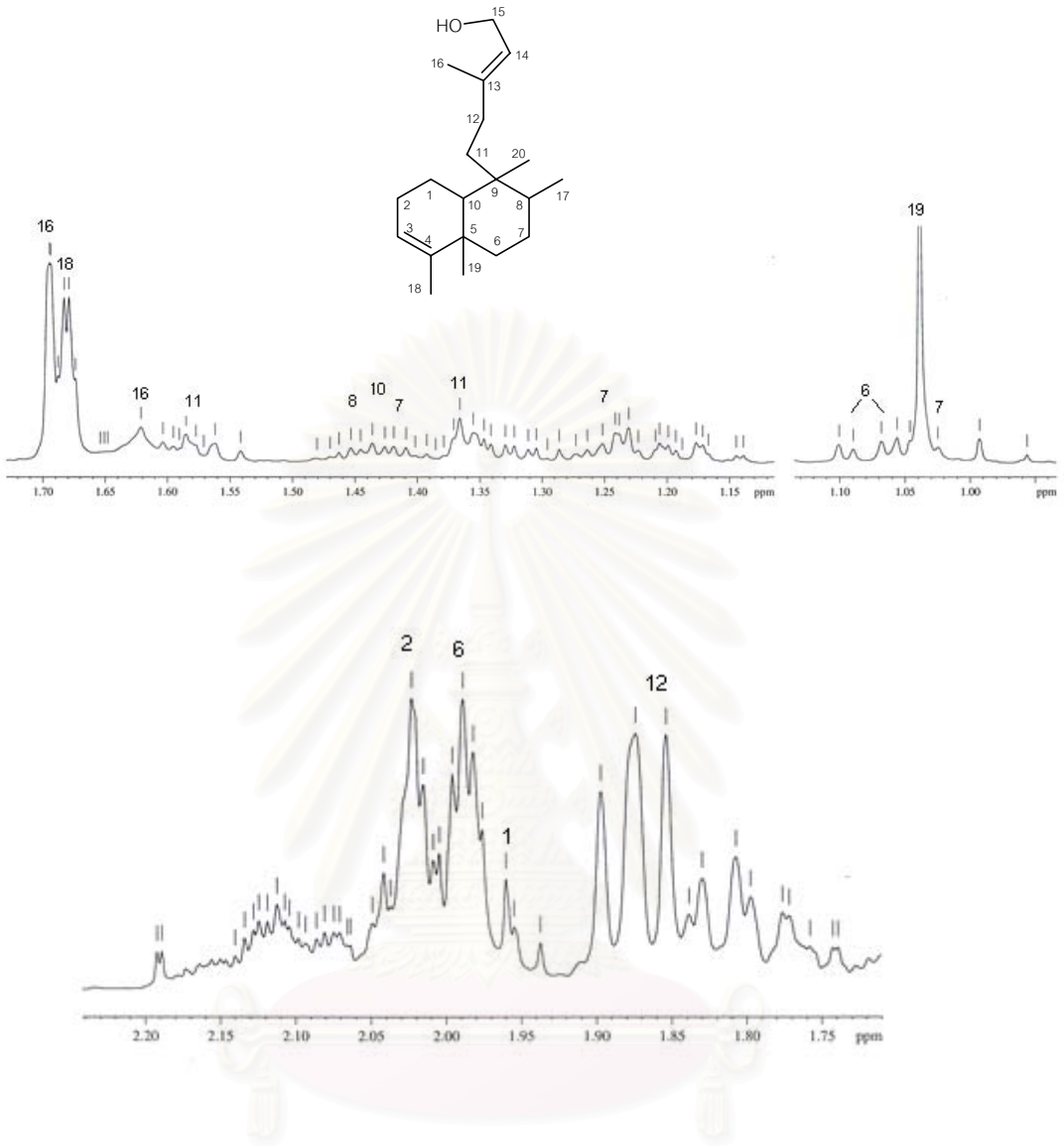


Figure 22: Expansion of ^{1}H NMR spectrum of compound **3**

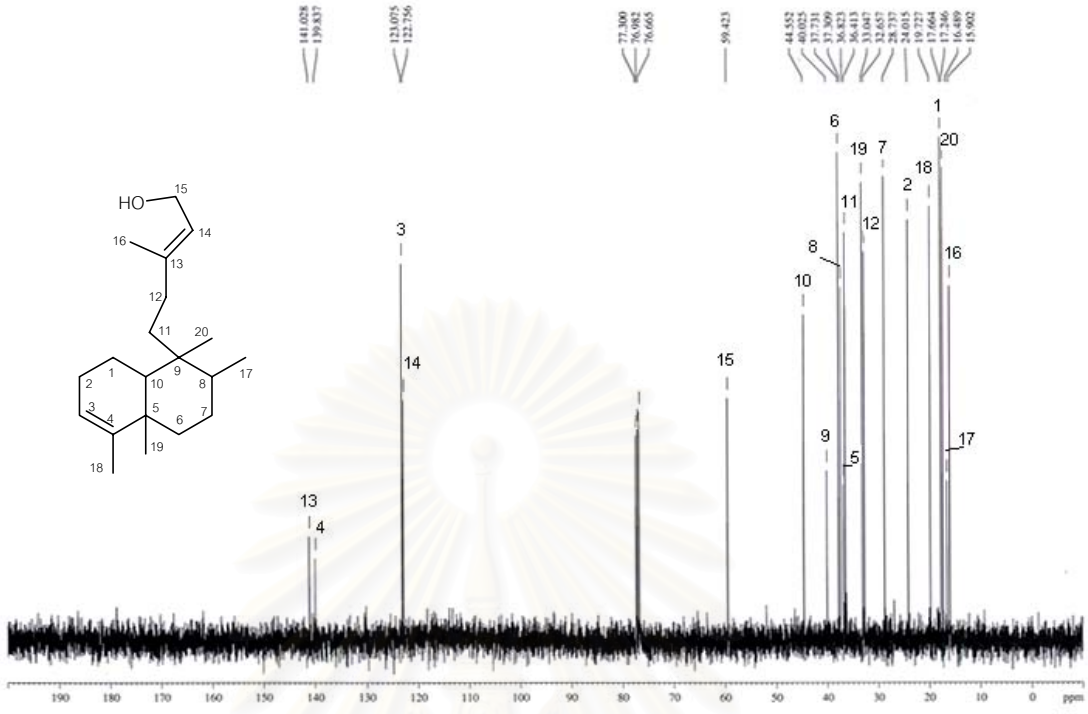


Figure 23: ^{13}C NMR (100 MHz) Spectrum of compound **3** ($CDCl_3$)

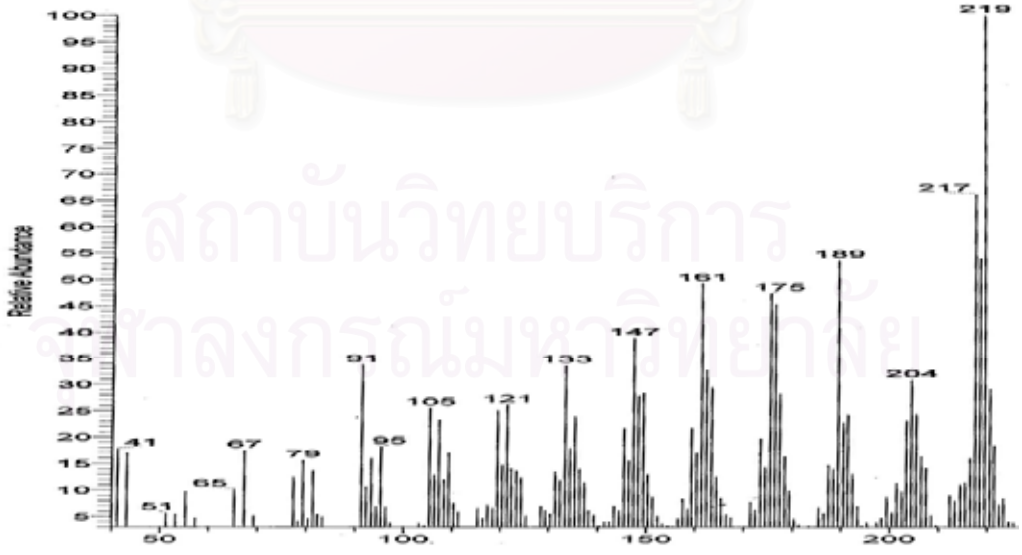


Figure 24: EIMS Mass spectrum of compound **4**

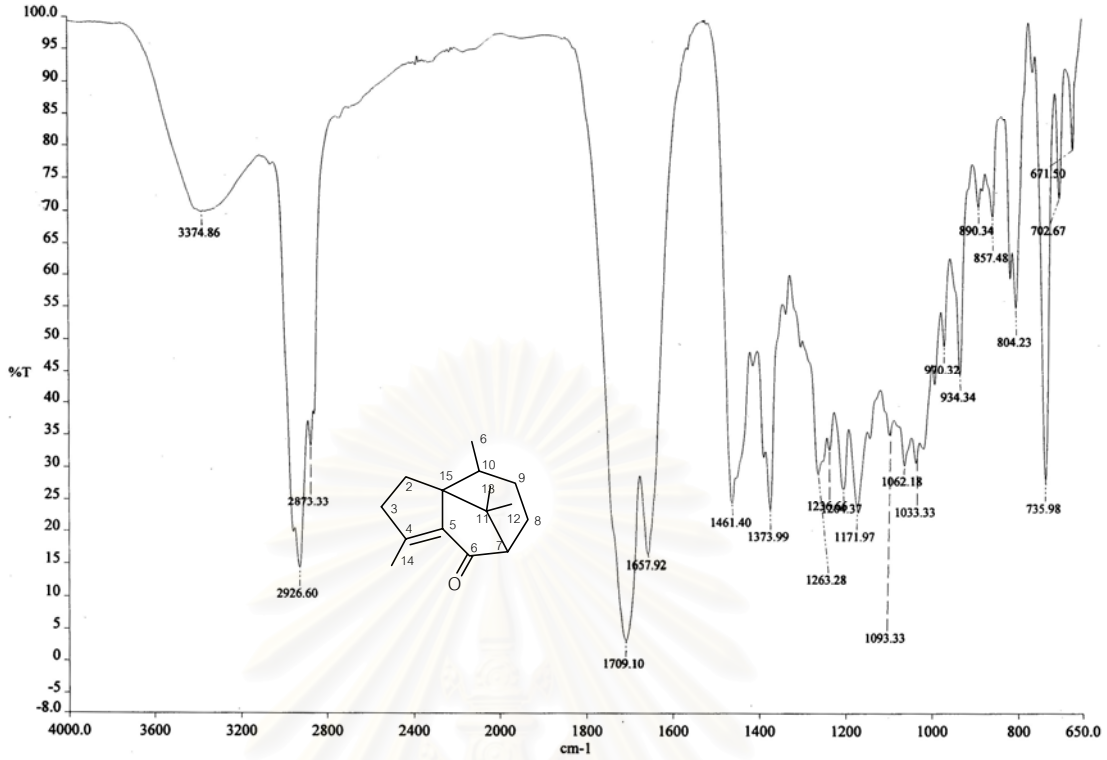


Figure 25: IR Spectrum of compound **4**

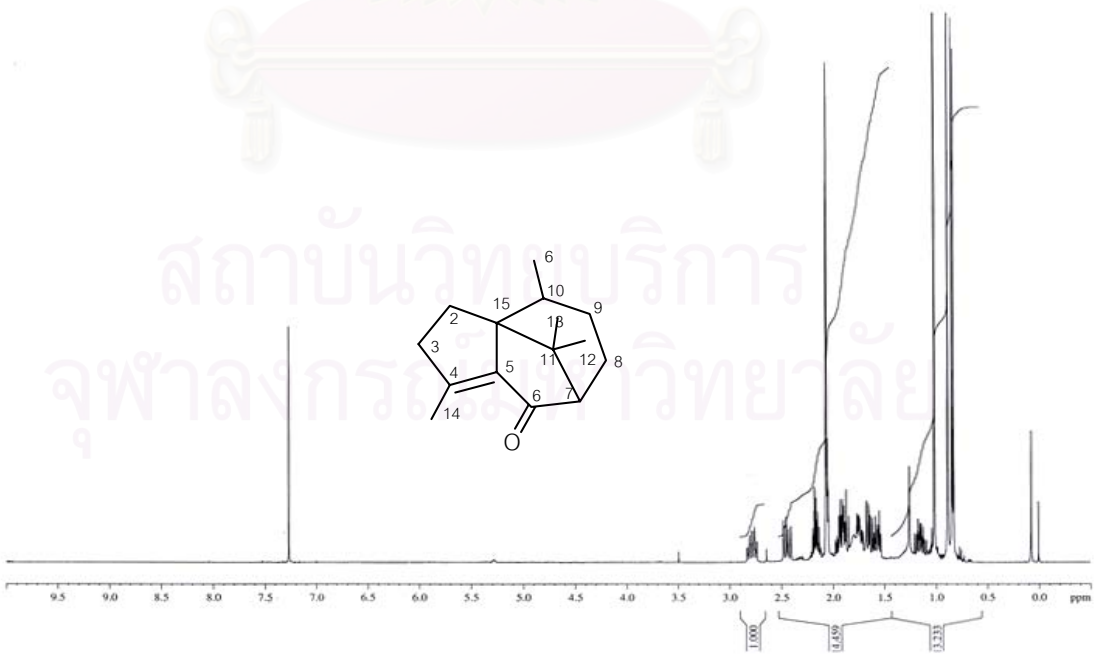


Figure 26: ^{1}H NMR (400 MHz) Spectrum of compound **4** ($CDCl_3$)

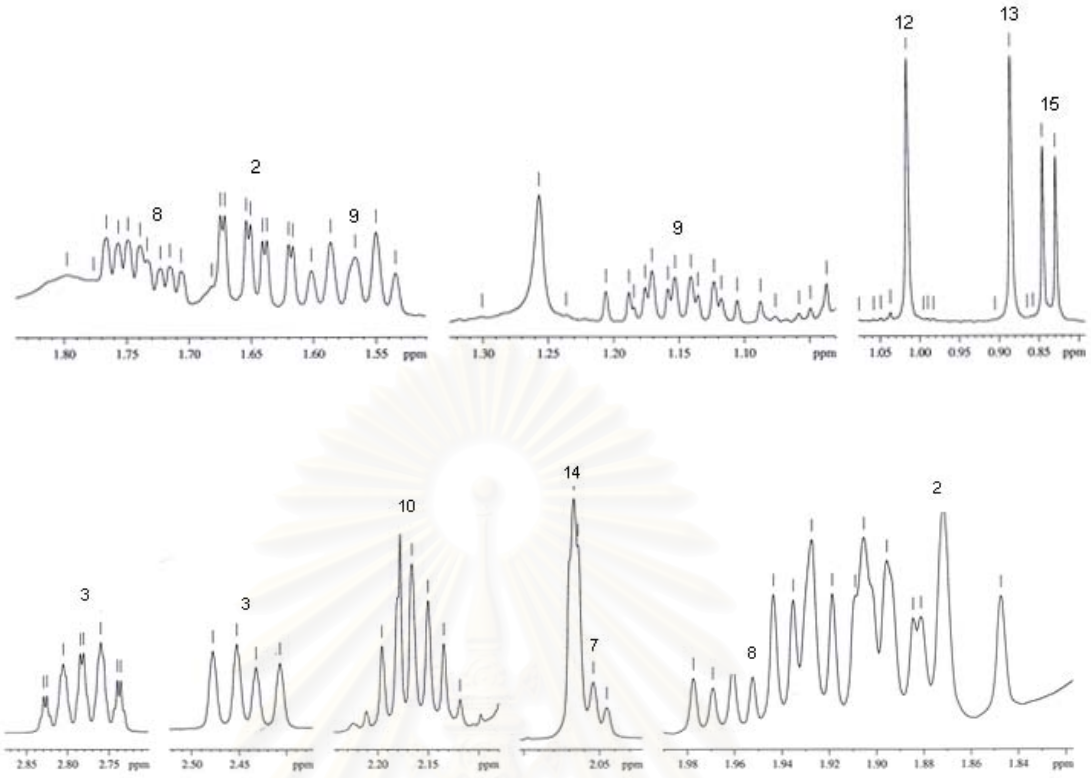


Figure 27: Expansion of ^{1}H NMR spectrum of compound **4**

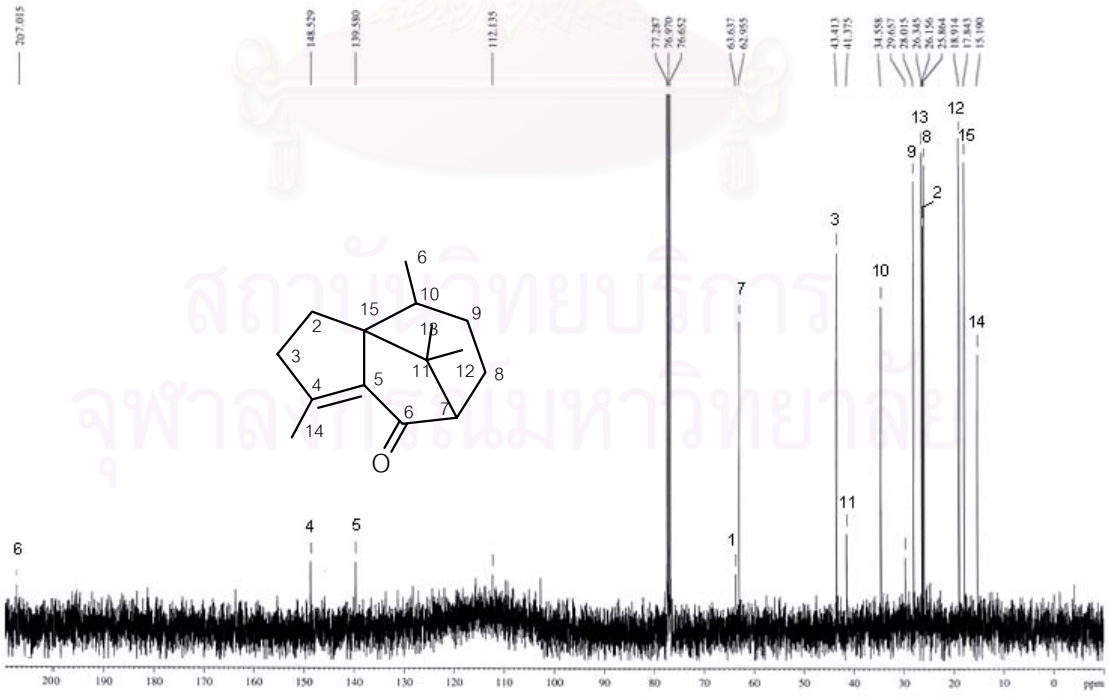


Figure 28: ^{13}C NMR (100 MHz) Spectrum of compound **4** ($CDCl_3$)

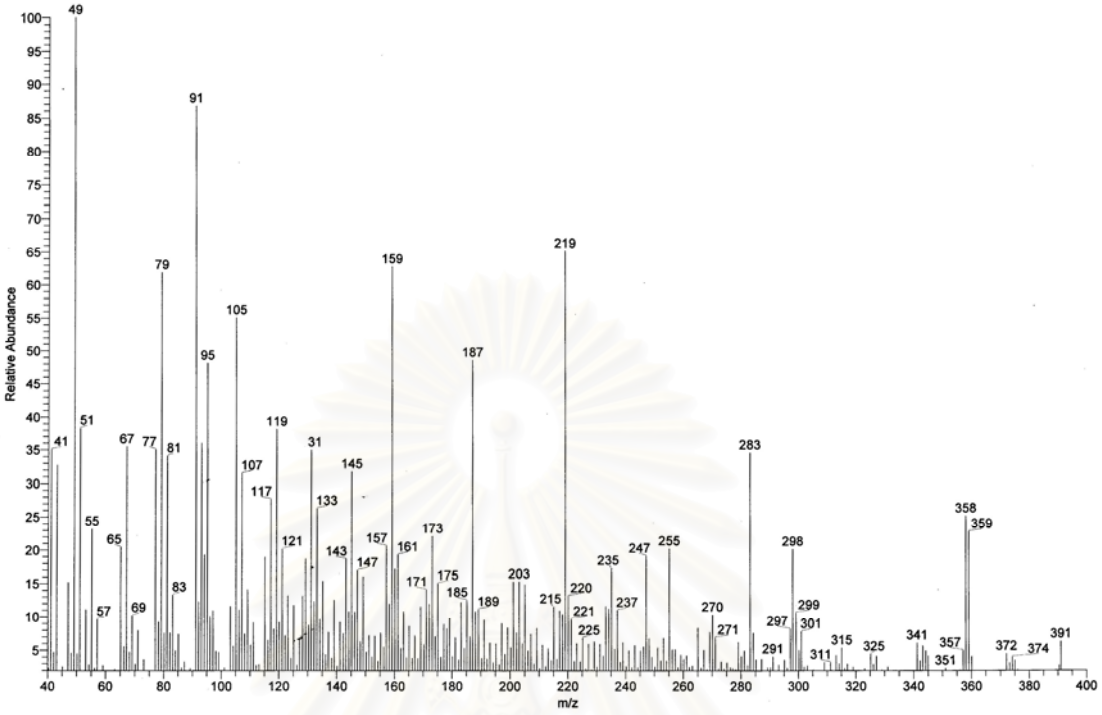


Figure 29: EIMS Mass spectrum of compound 5

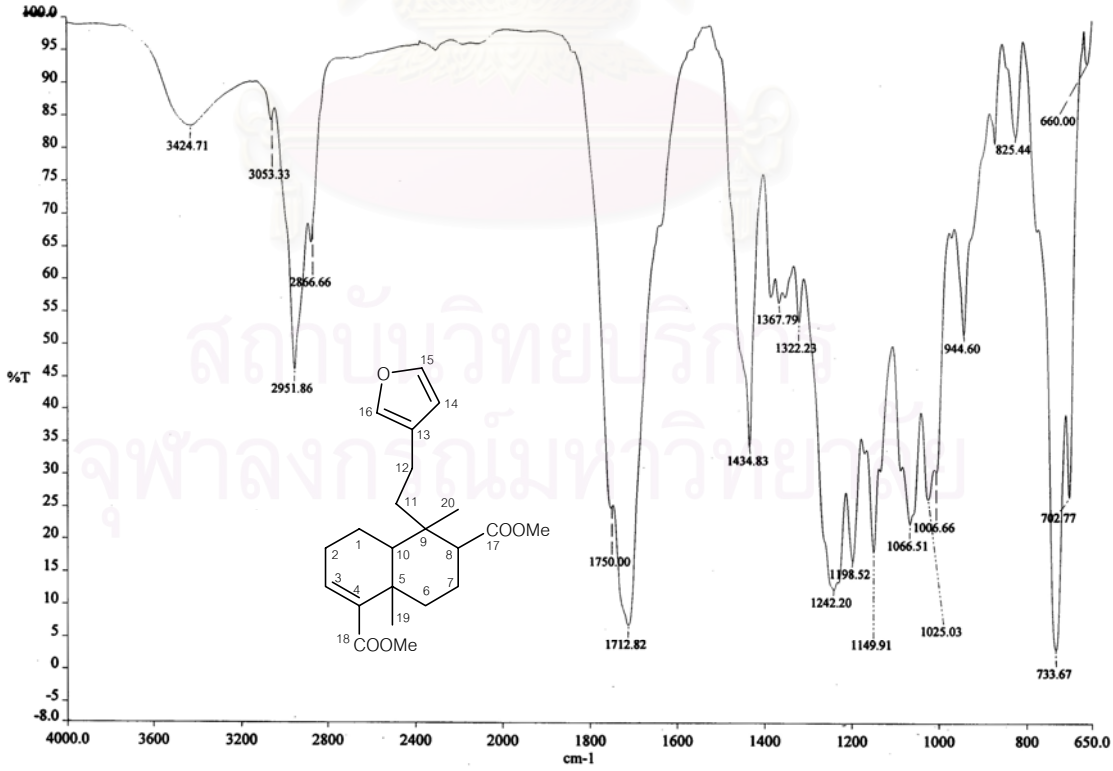


Figure 30: IR Spectrum of compound 5

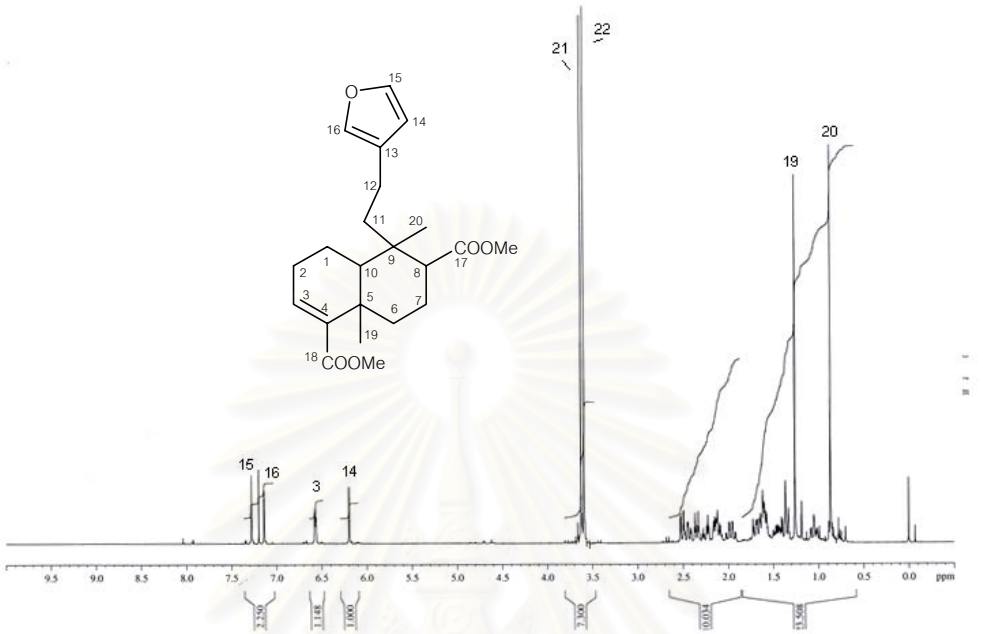


Figure 31: ^{1}H NMR (400 MHz) Spectrum of compound **5** ($CDCl_3$)

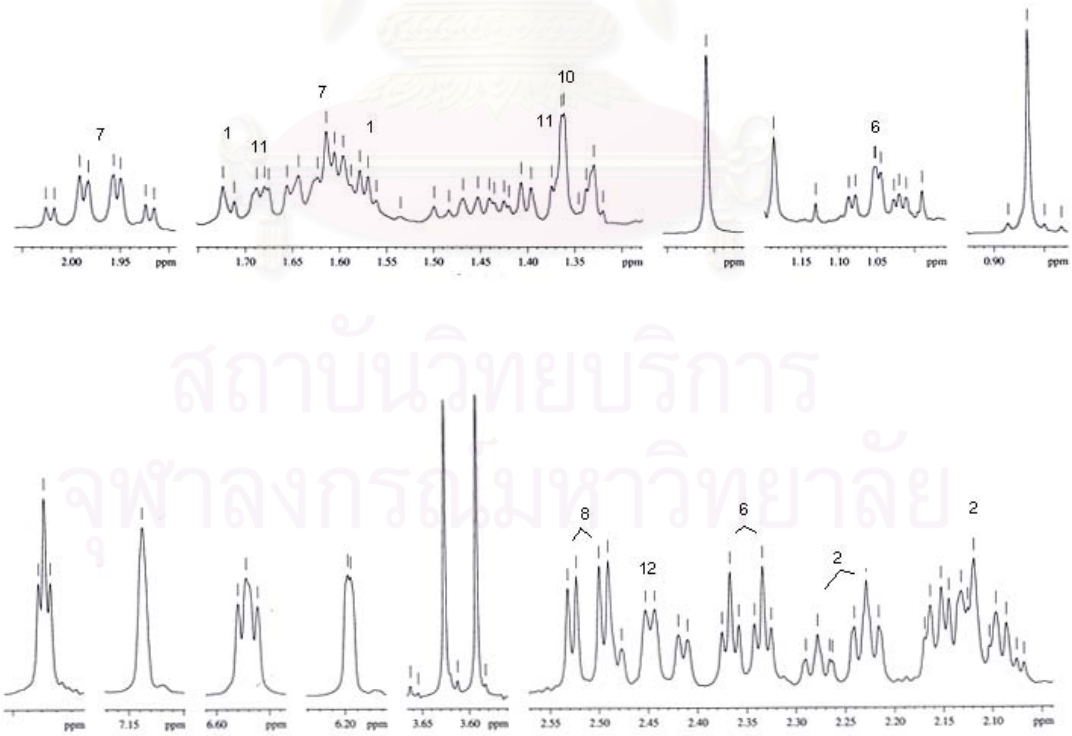


Figure 32: Expansion of ^{1}H NMR spectrum of compound **5**

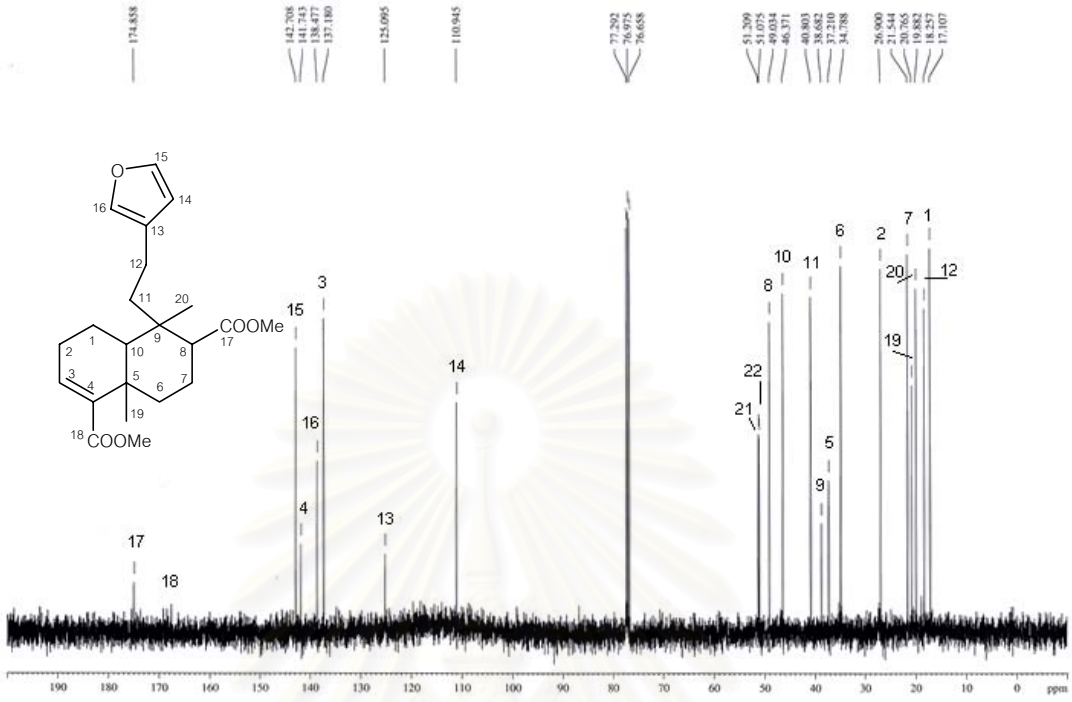


Figure 33: ^{13}C NMR (100 MHz) Spectrum of compound **5** ($CDCl_3$)

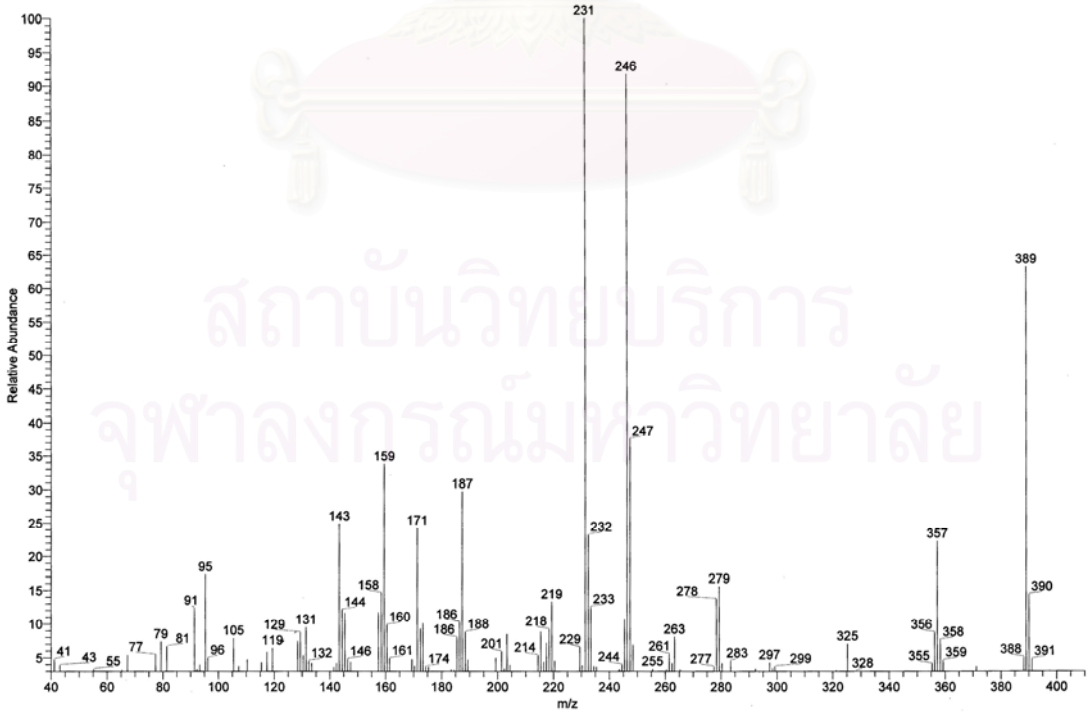


Figure 34: EIMS Mass spectrum of compound **6**

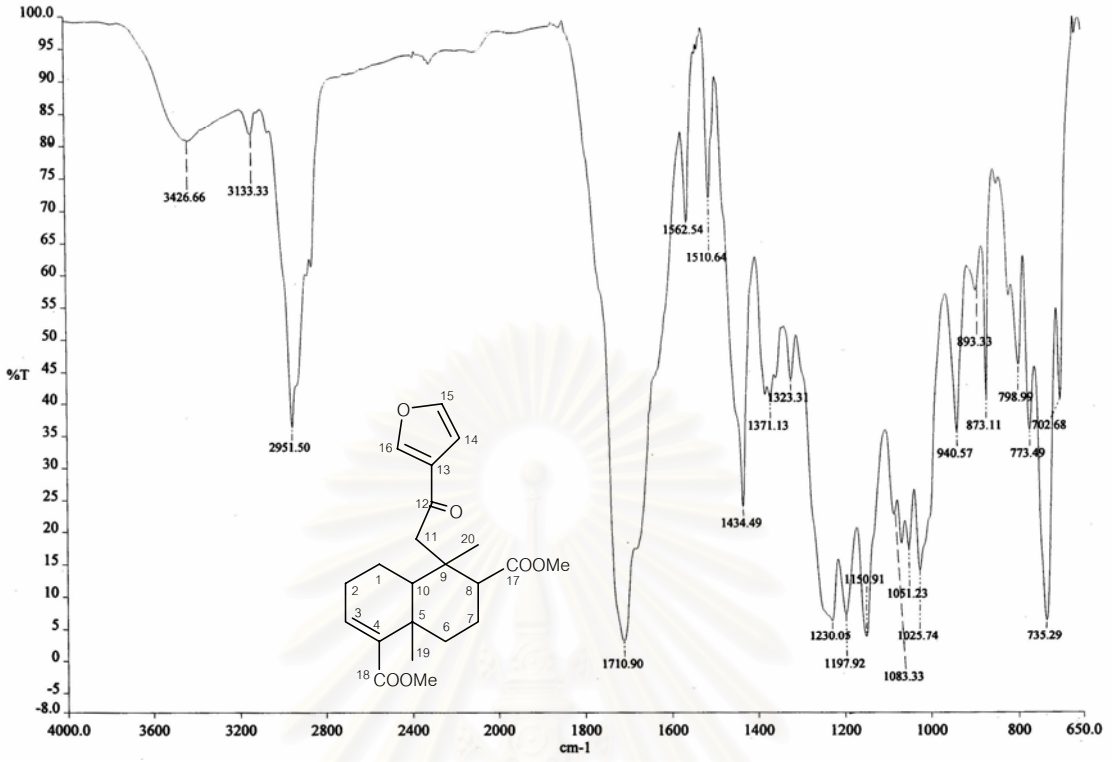


Figure 35: IR Spectrum of compound **6**

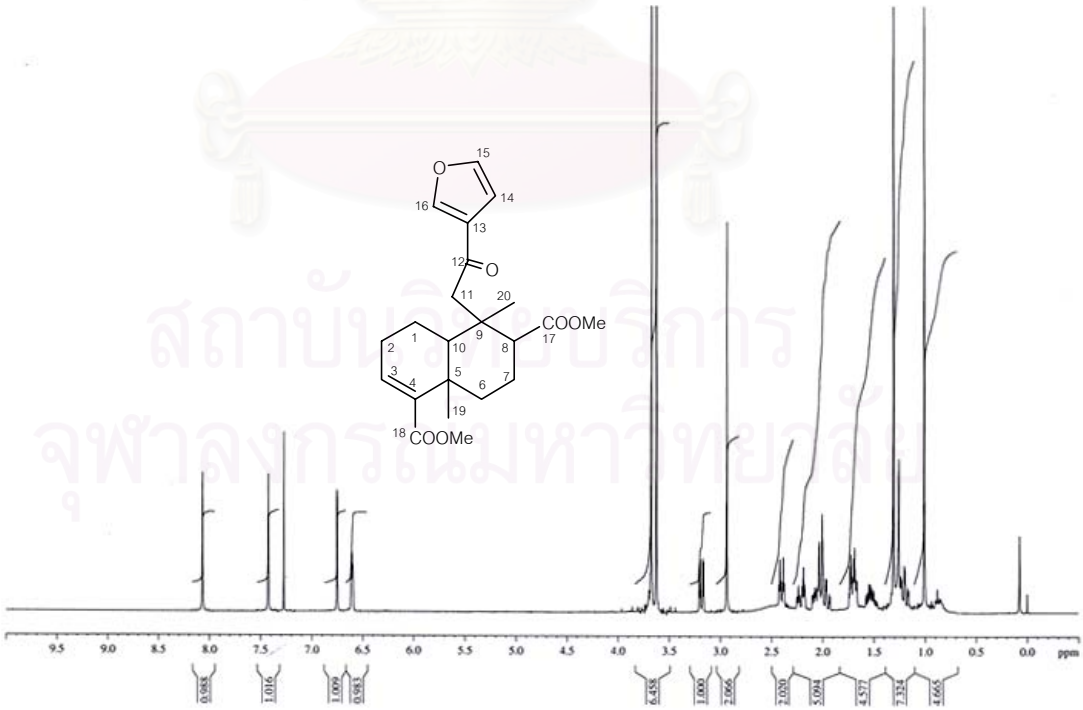


Figure 36: ^{1}H NMR (400 MHz) Spectrum of compound **6** ($CDCl_3$)

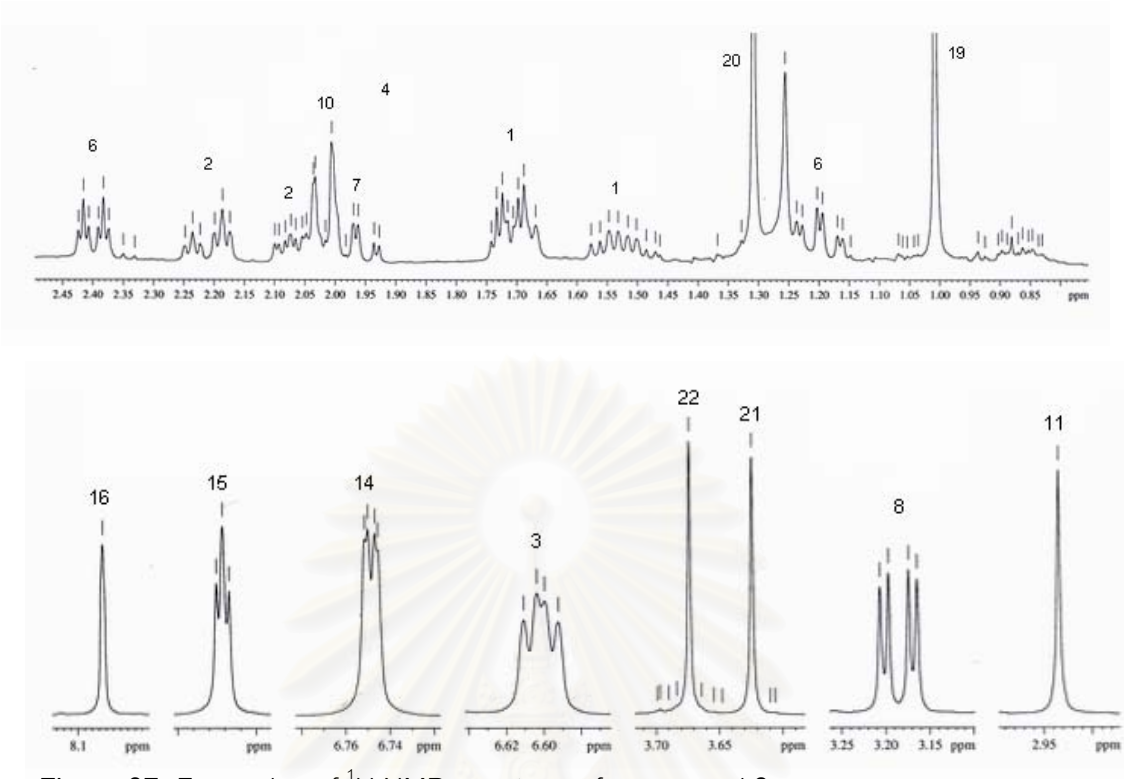


Figure 37: Expansion of ^{1}H NMR spectrum of compound **6**

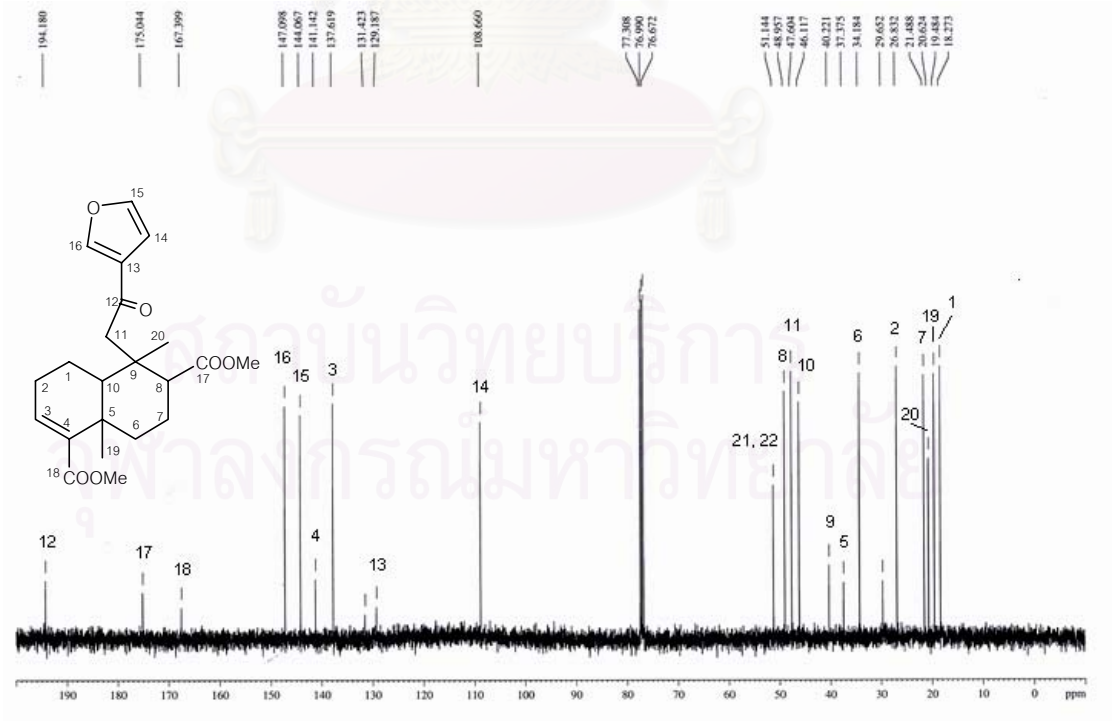


Figure 38: ^{13}C NMR (100 MHz) Spectrum of compound **6** ($CDCl_3$)

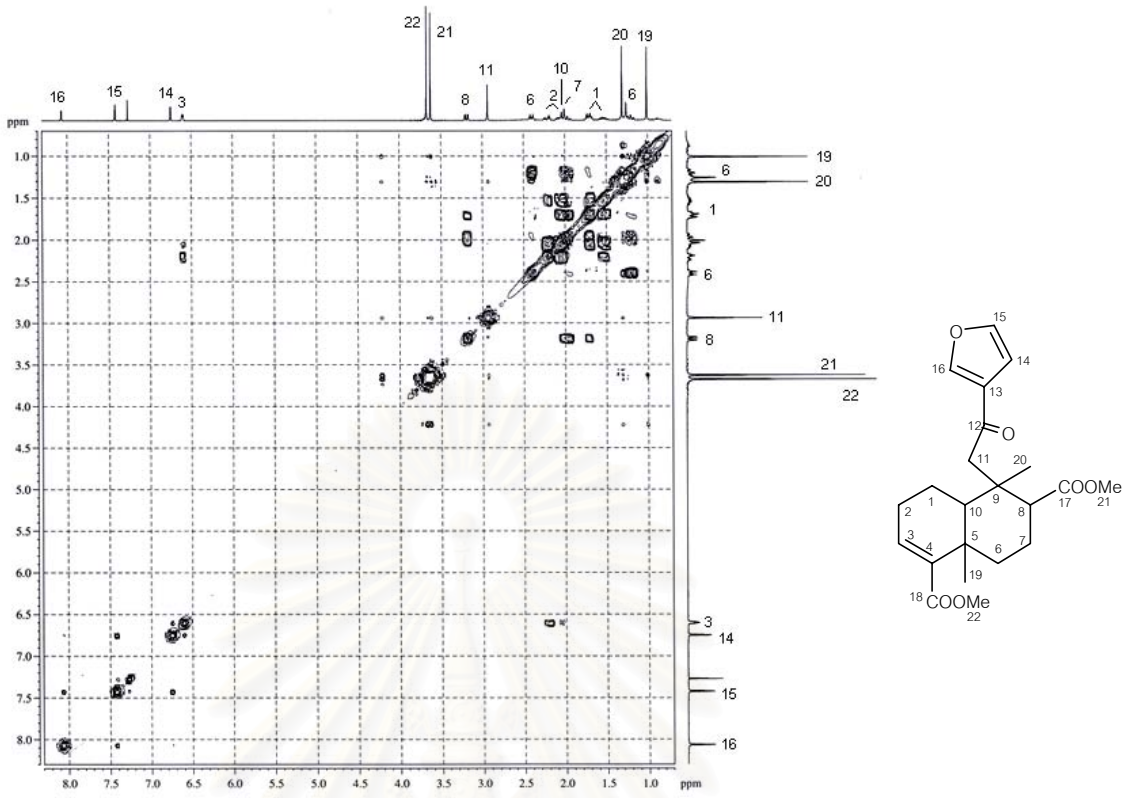


Figure 39: ^{1}H-^{1}H COSY Spectrum of compound **6** ($CDCl_3$)

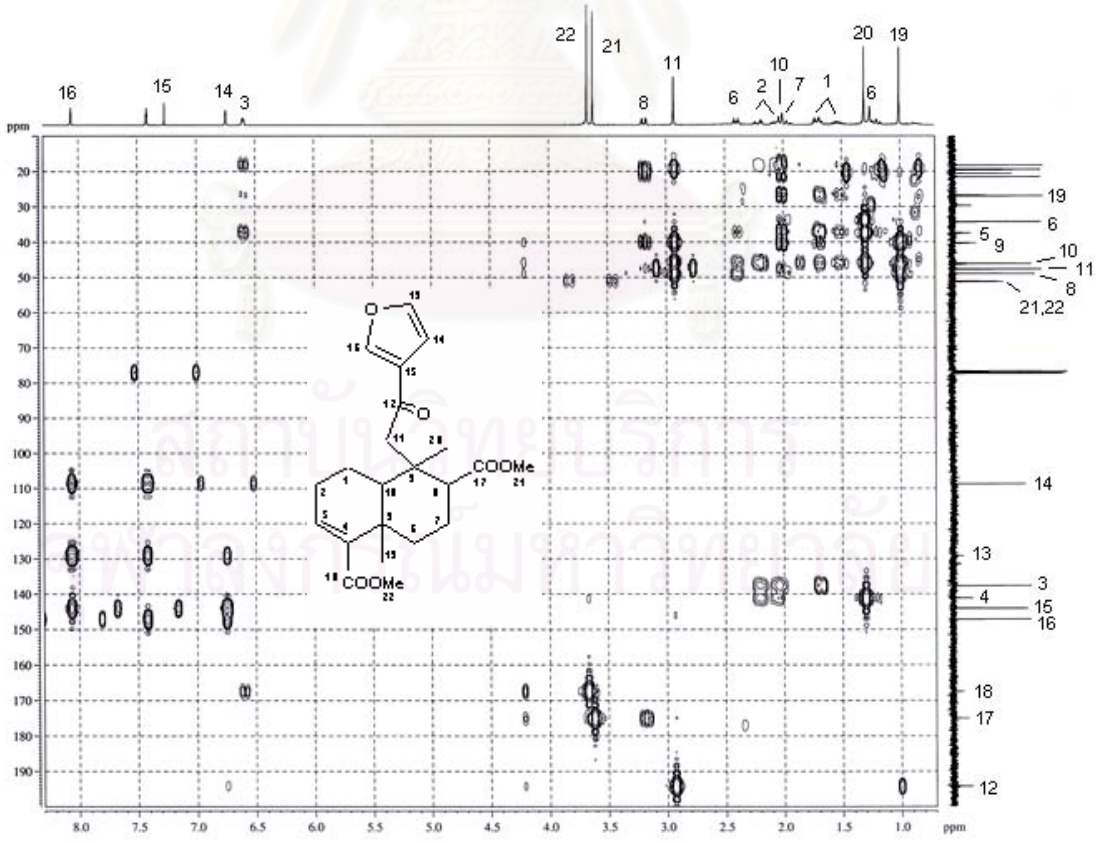


Figure 40: HMBC Spectrum of compound **6** ($CDCl_3$)

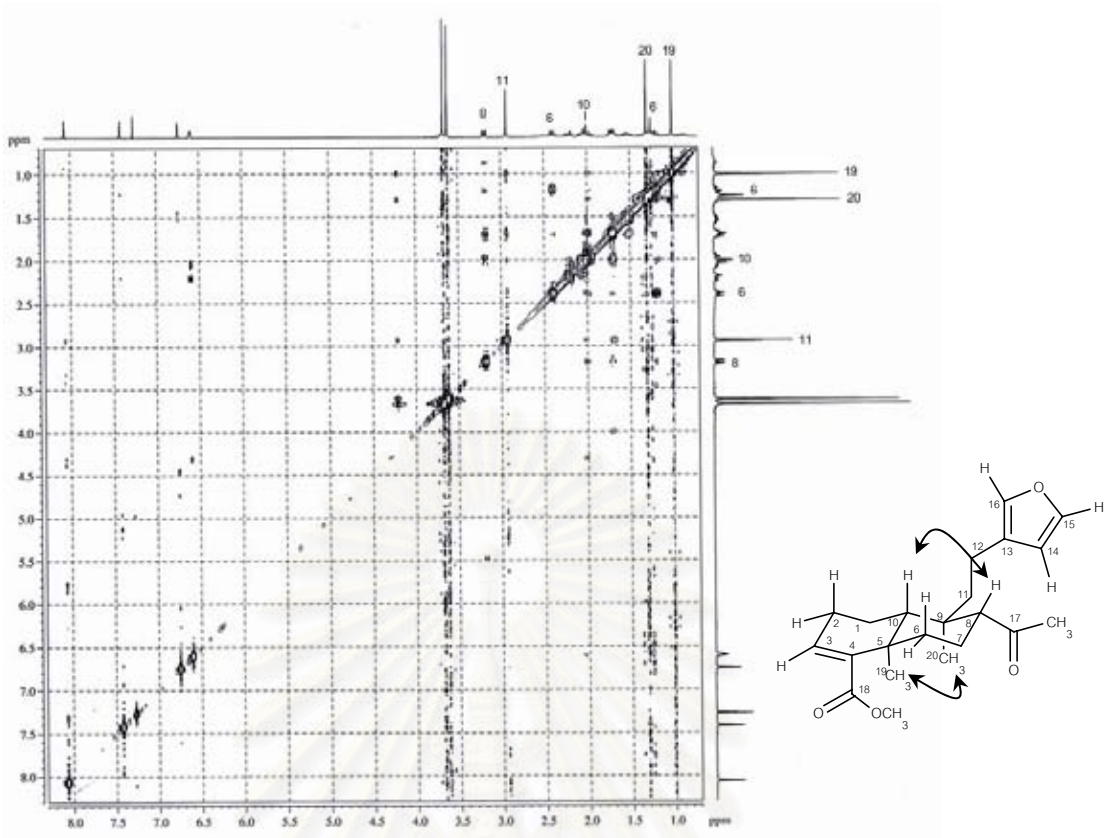


Figure 41: NOESY Spectrum of compound **6** ($CDCl_3$)

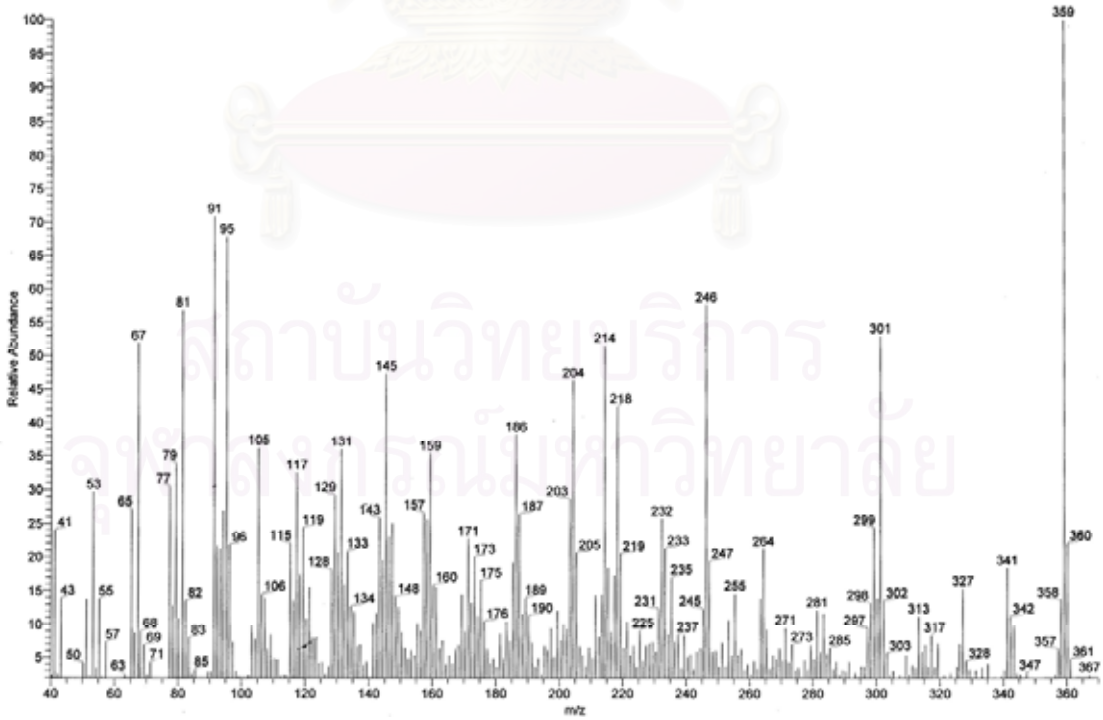


Figure 42: EIMS Mass spectrum of compound **7**

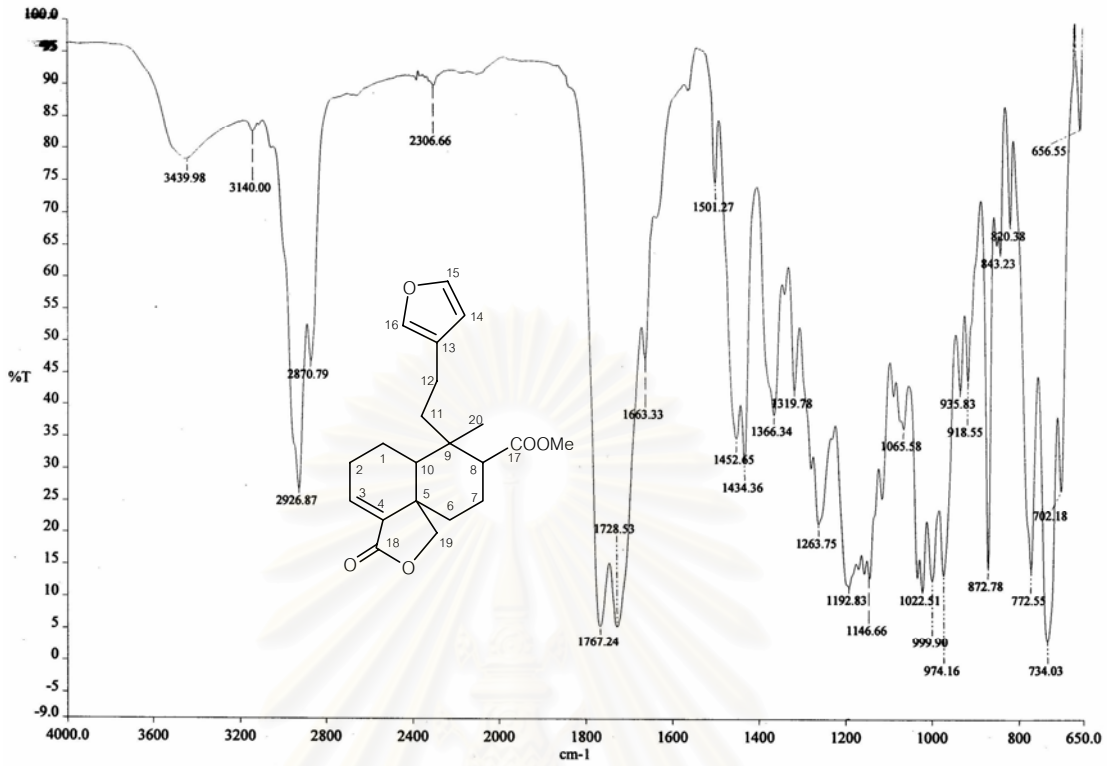


Figure 43: IR Spectrum of compound 7

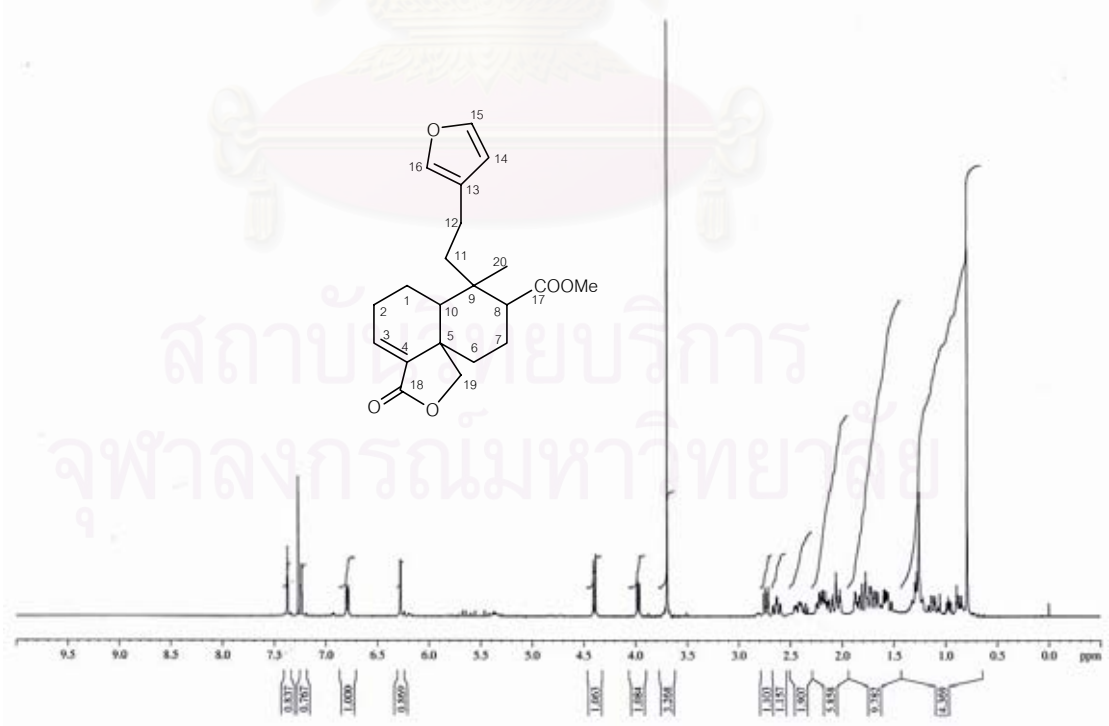


Figure 44: ^{1}H NMR (400 MHz) Spectrum of compound 7 ($CDCl_3$)

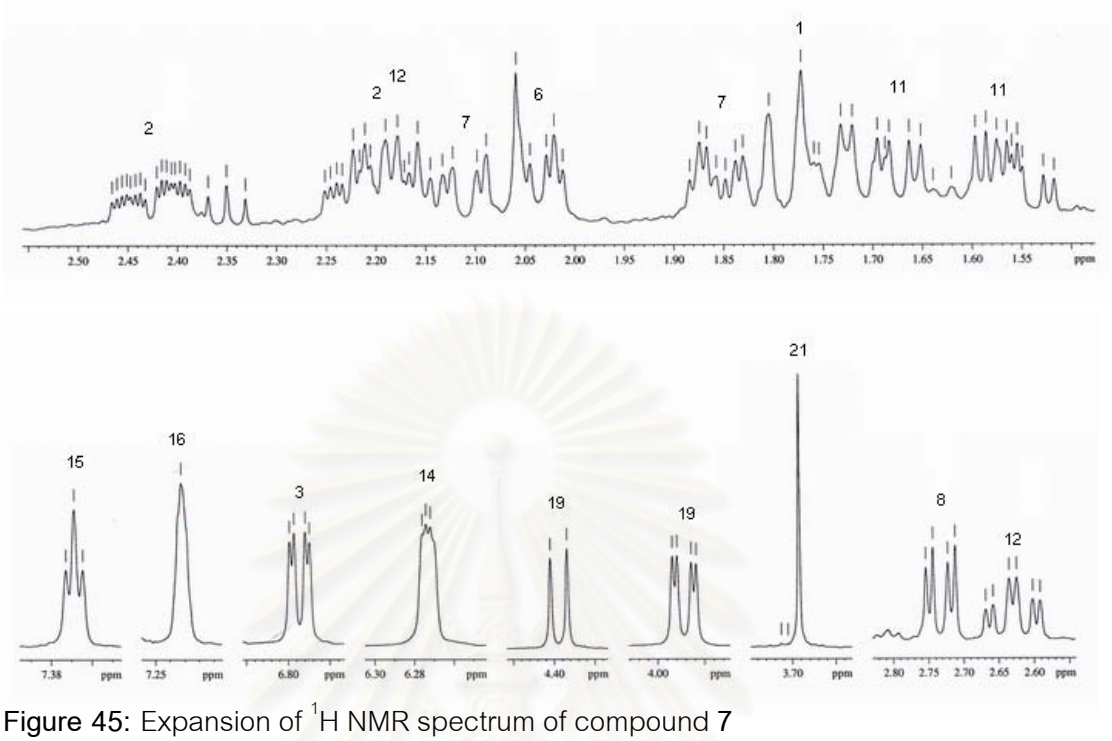


Figure 45: Expansion of ^{1}H NMR spectrum of compound 7

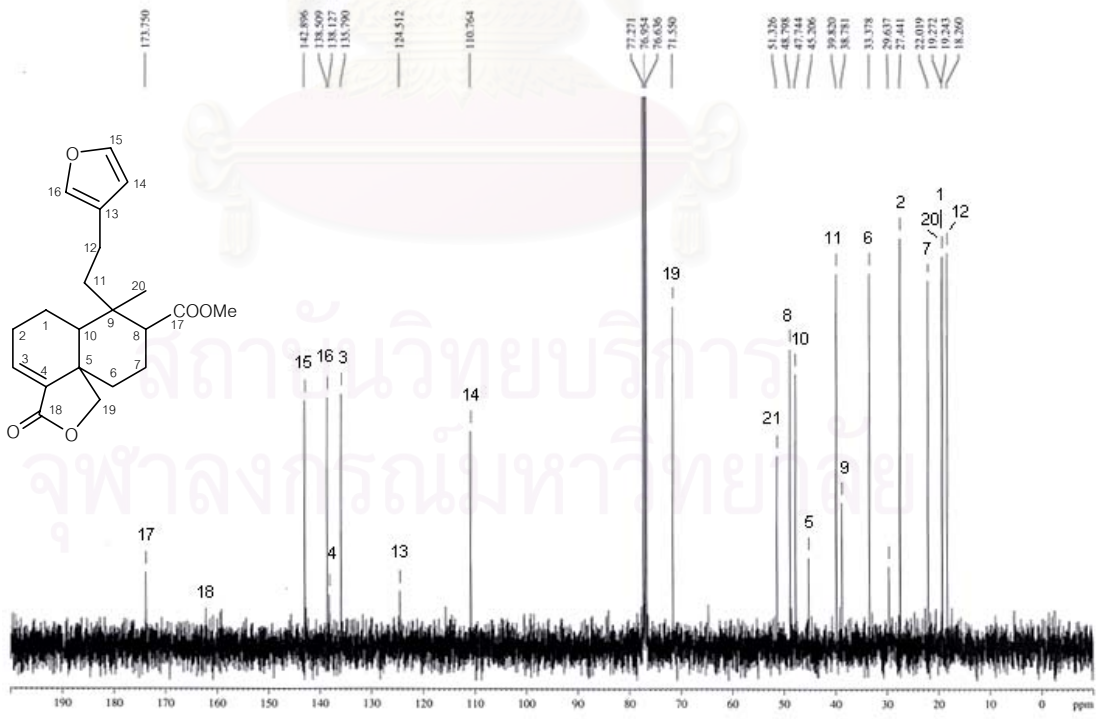


Figure 46: ^{13}C NMR (100 MHz) Spectrum of compound 7 ($CDCl_3$)

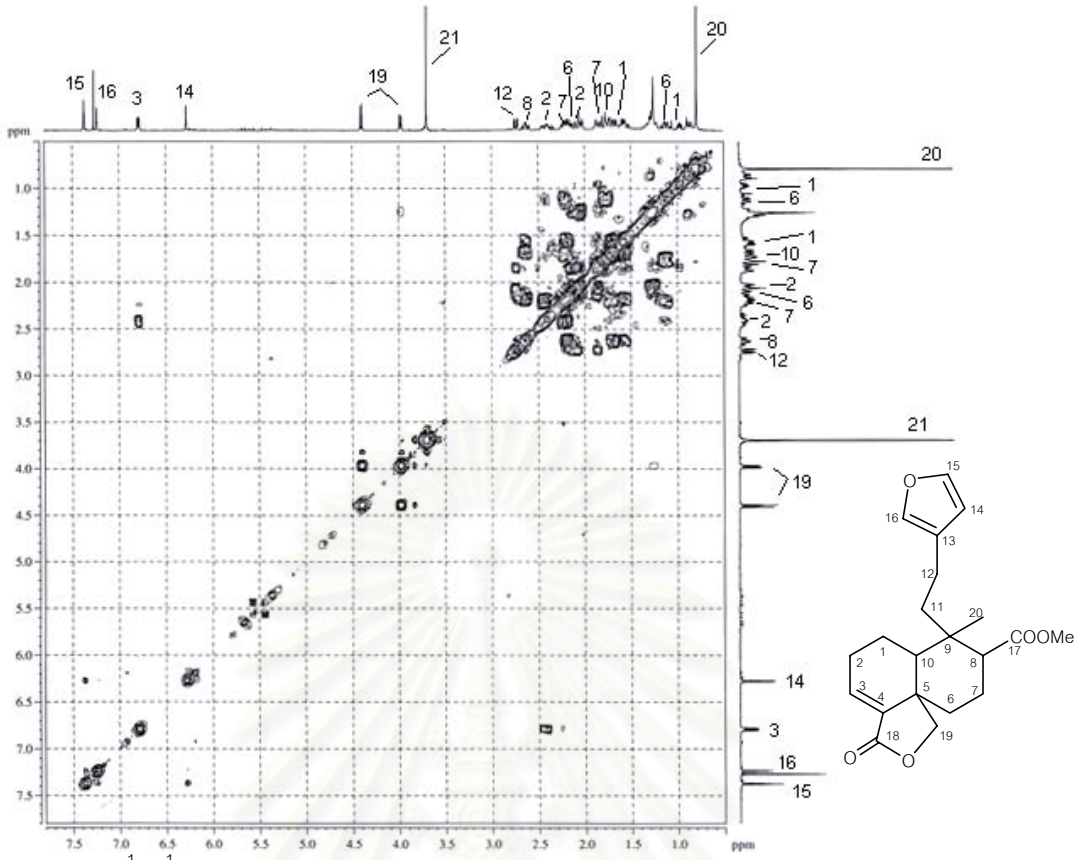


Figure 47: ^{1}H-^{1}H COSY Spectrum of compound **7** ($CDCl_3$)

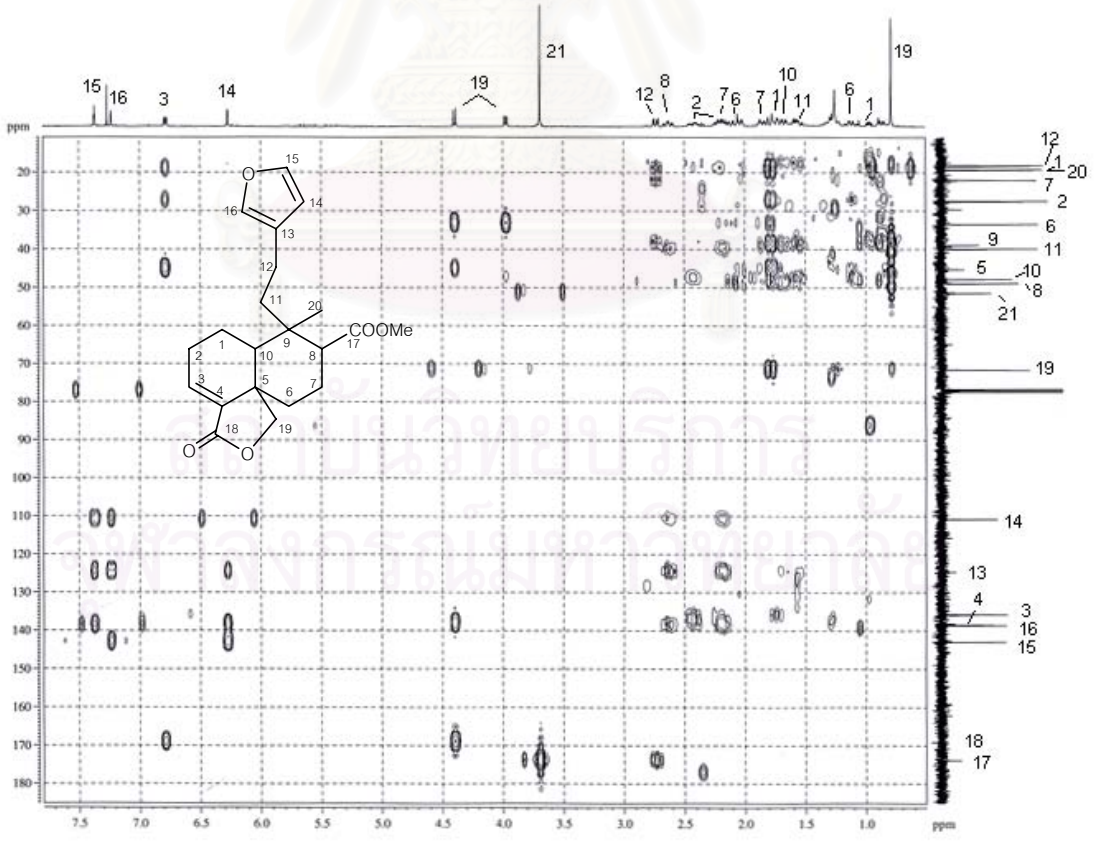


Figure 48: HMBC Spectrum of compound **7** ($CDCl_3$)

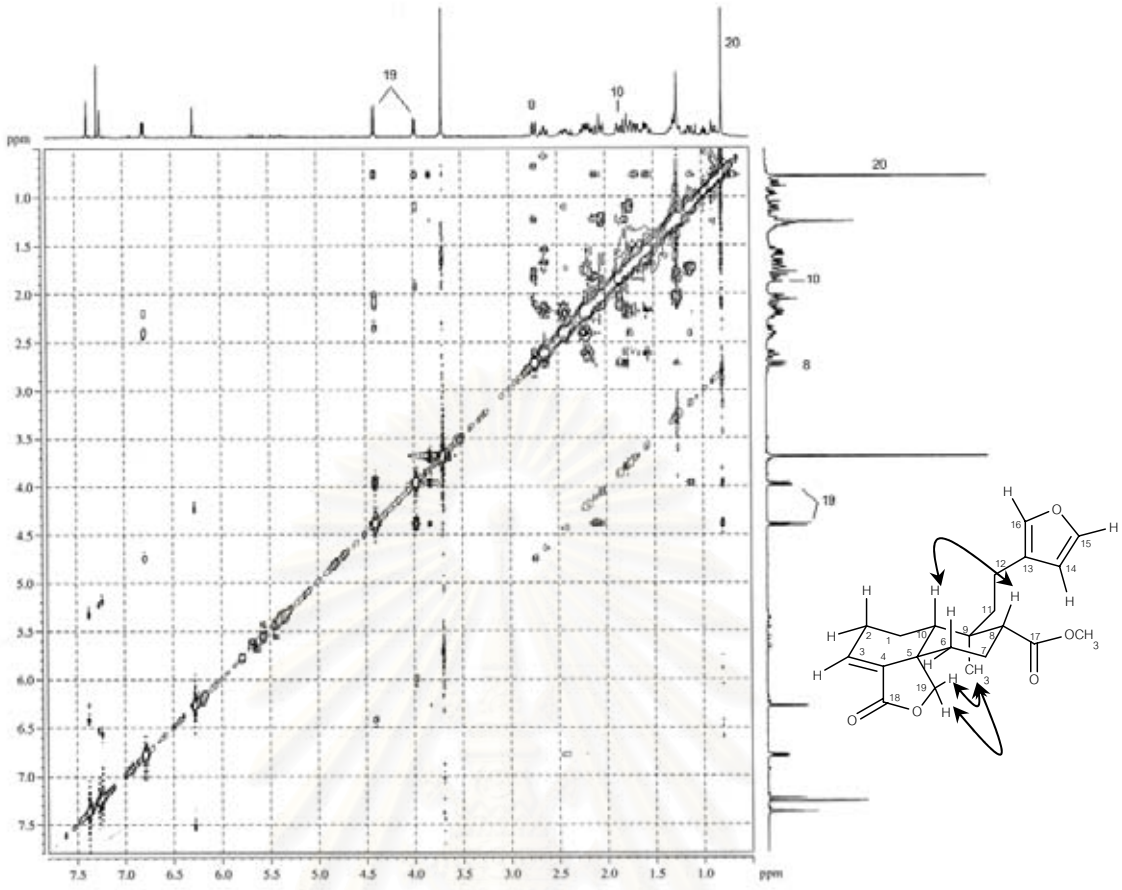


Figure 49: NOESY Spectrum of compound **7** ($CDCl_3$)

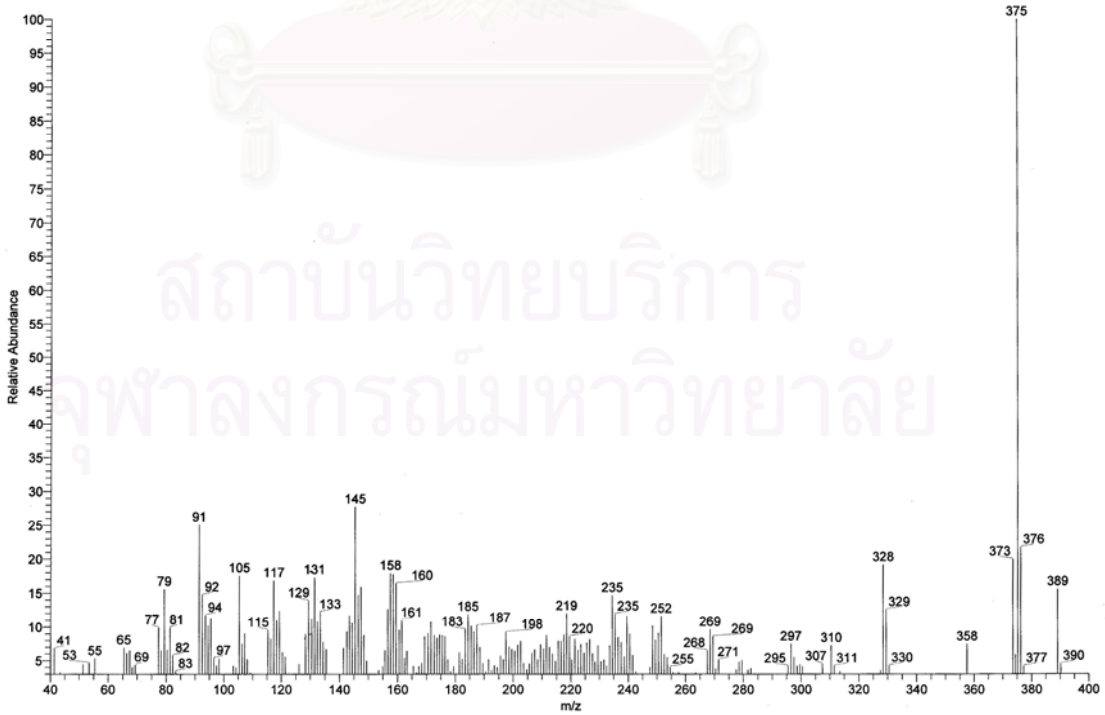


Figure 50: EIMS Mass spectrum of compound **8**

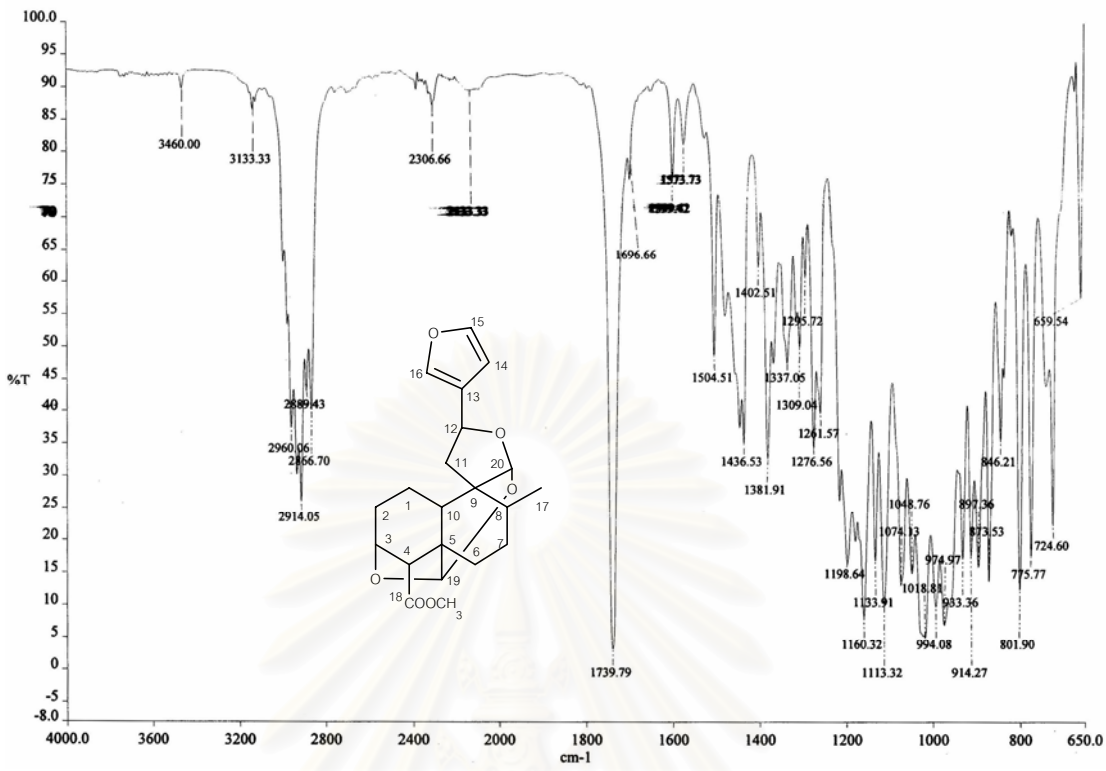


Figure 51: IR Spectrum of compound **8**

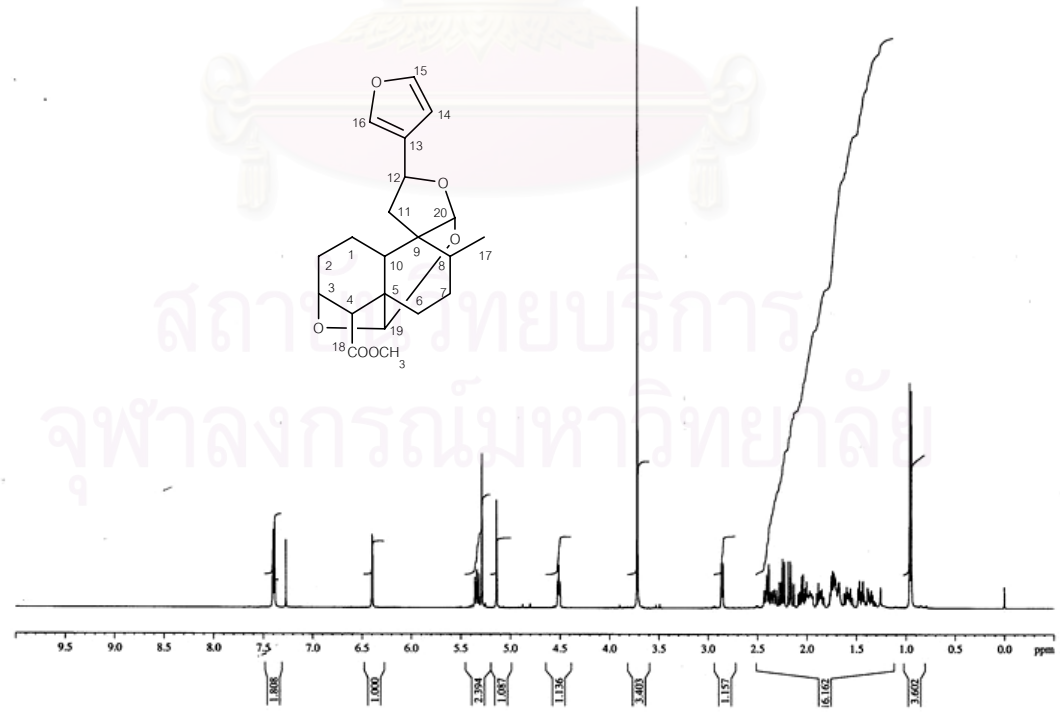


Figure 52: ^{1}H NMR (400 MHz) Spectrum of compound **8** ($CDCl_3$)

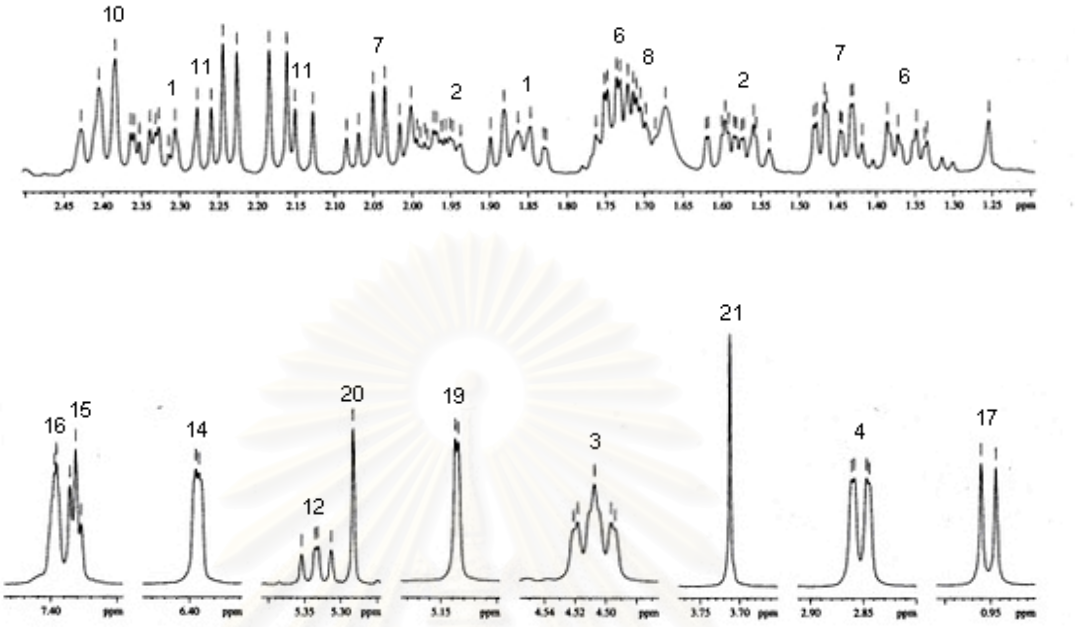


Figure 53: Expansion of ^{1}H NMR spectrum of compound **8**

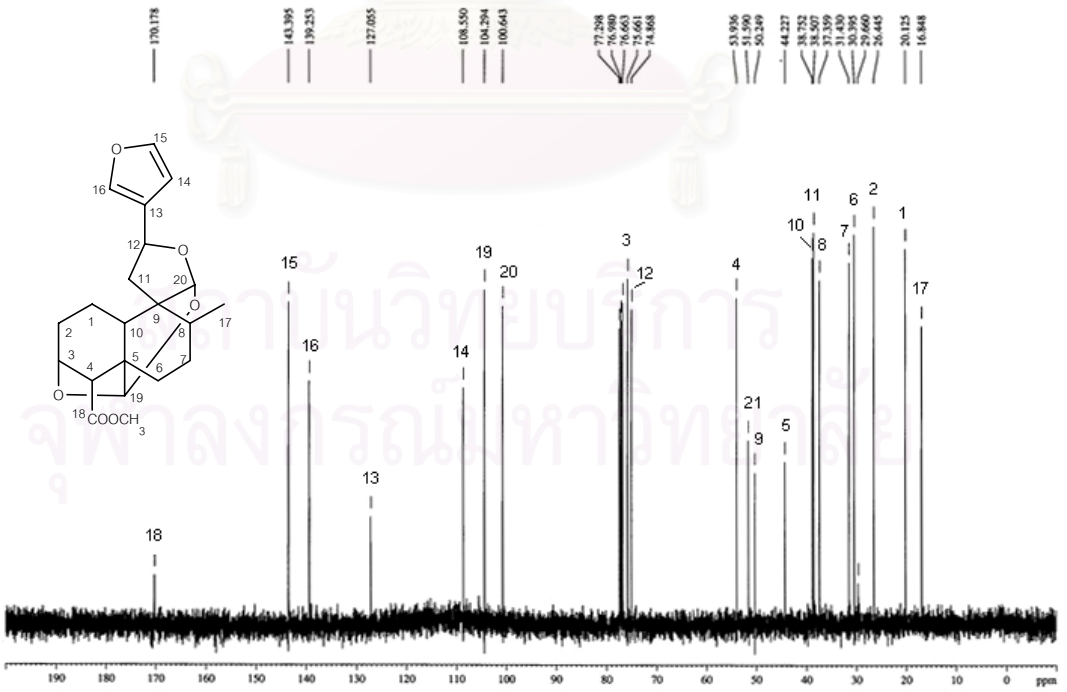


Figure 54: ^{13}C NMR (100 MHz) Spectrum of compound **8** ($CDCl_3$)

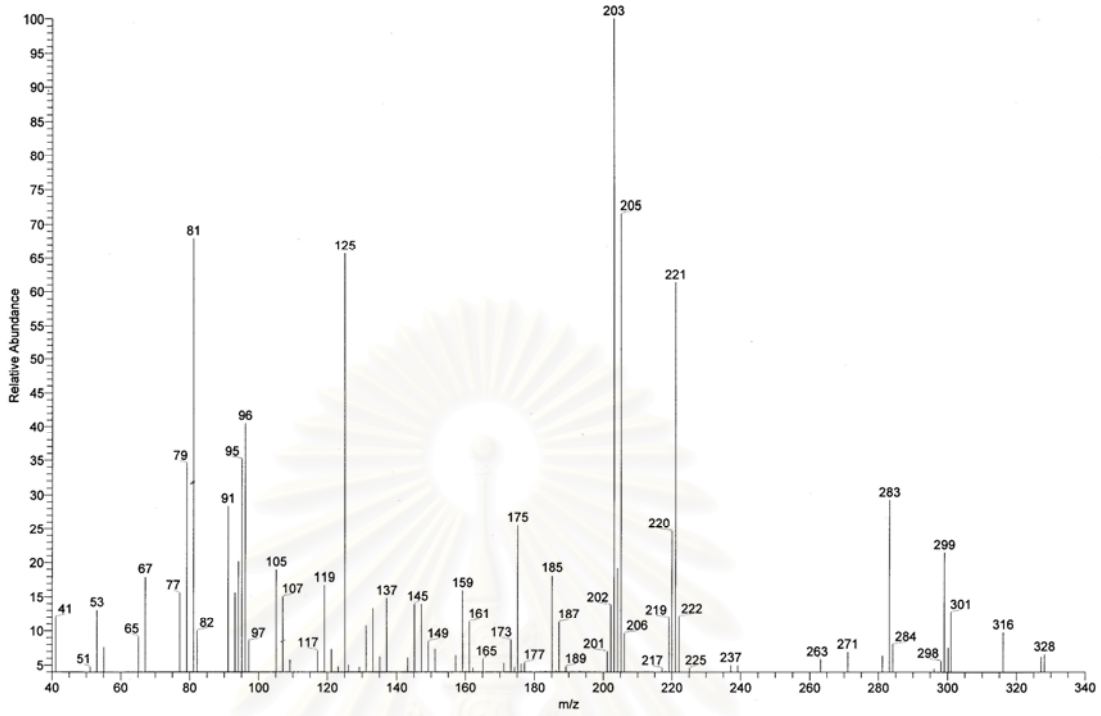


Figure 55: EIMS Mass spectrum of compound 9

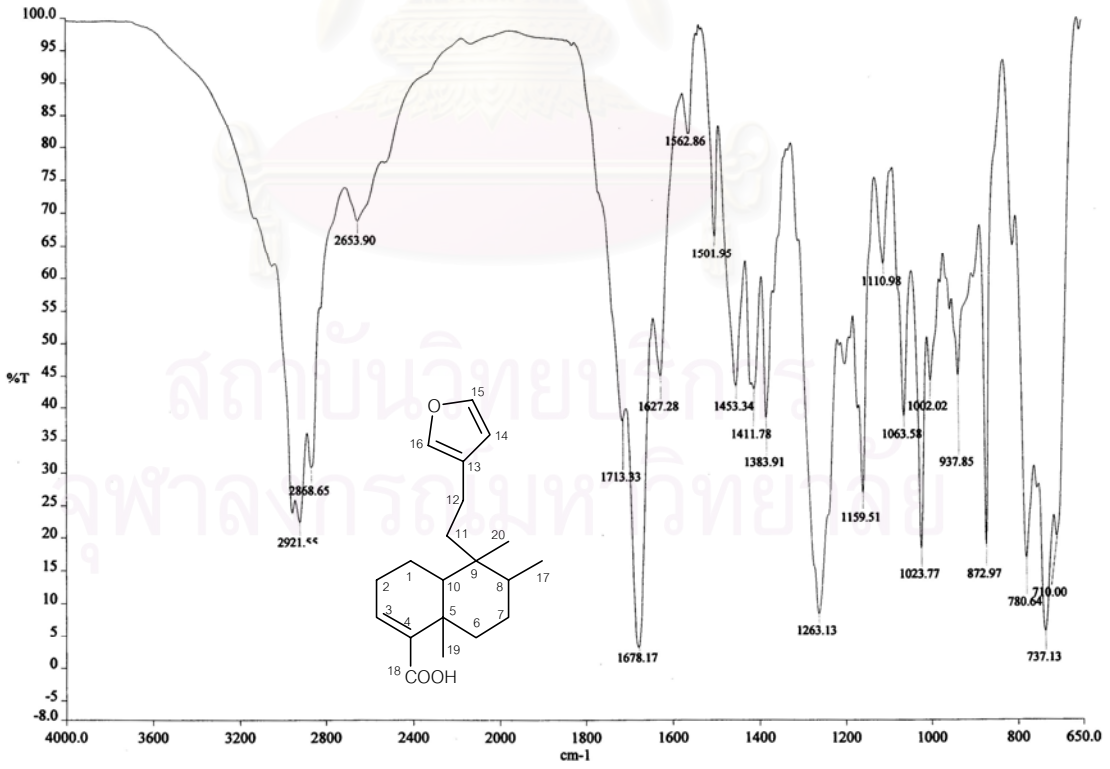


Figure 56: IR Spectrum of compound 9

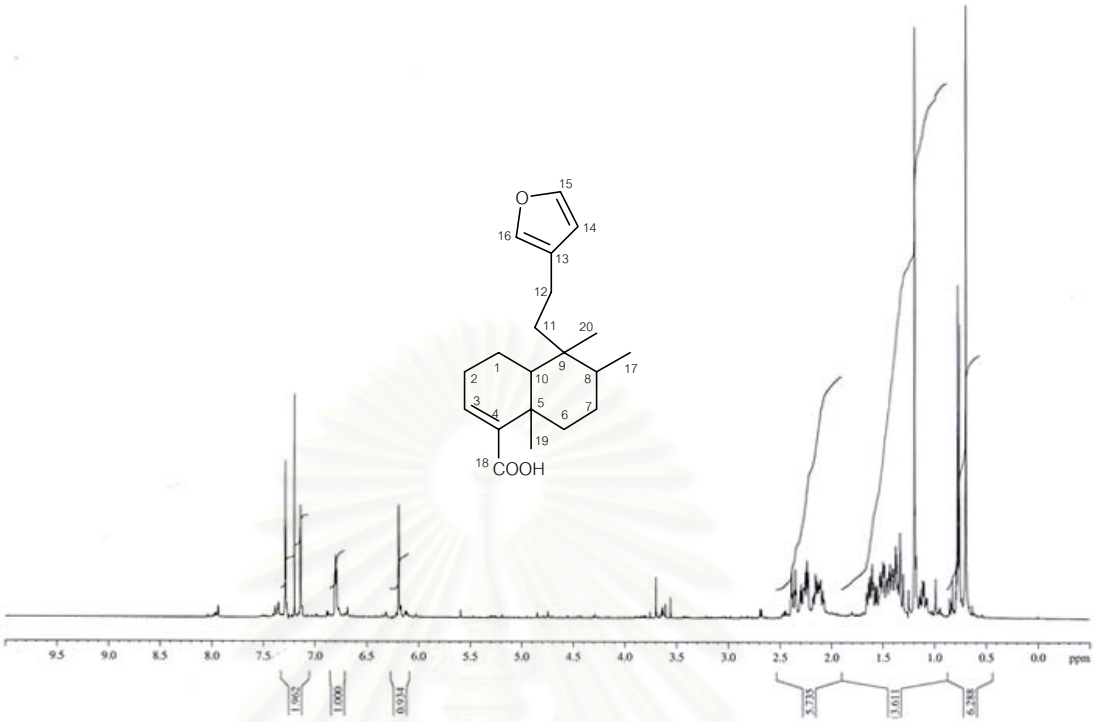


Figure 57: ^{1}H NMR (400 MHz) Spectrum of compound **9** ($CDCl_3$)

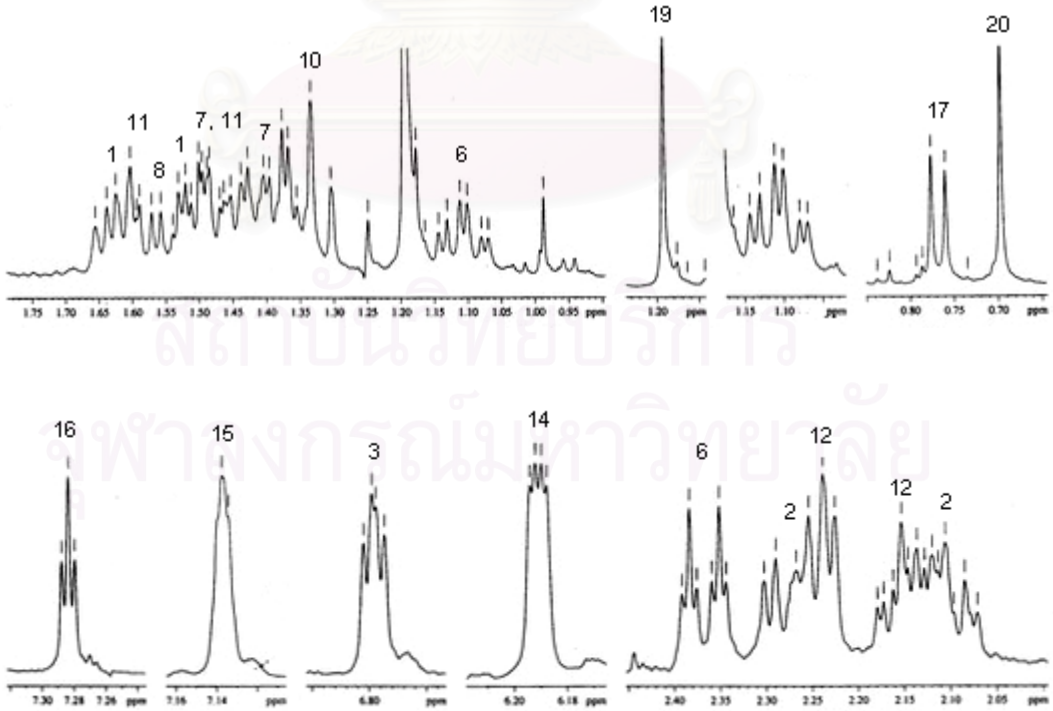


Figure 58: Expansion of ^{1}H NMR spectrum of compound **9**

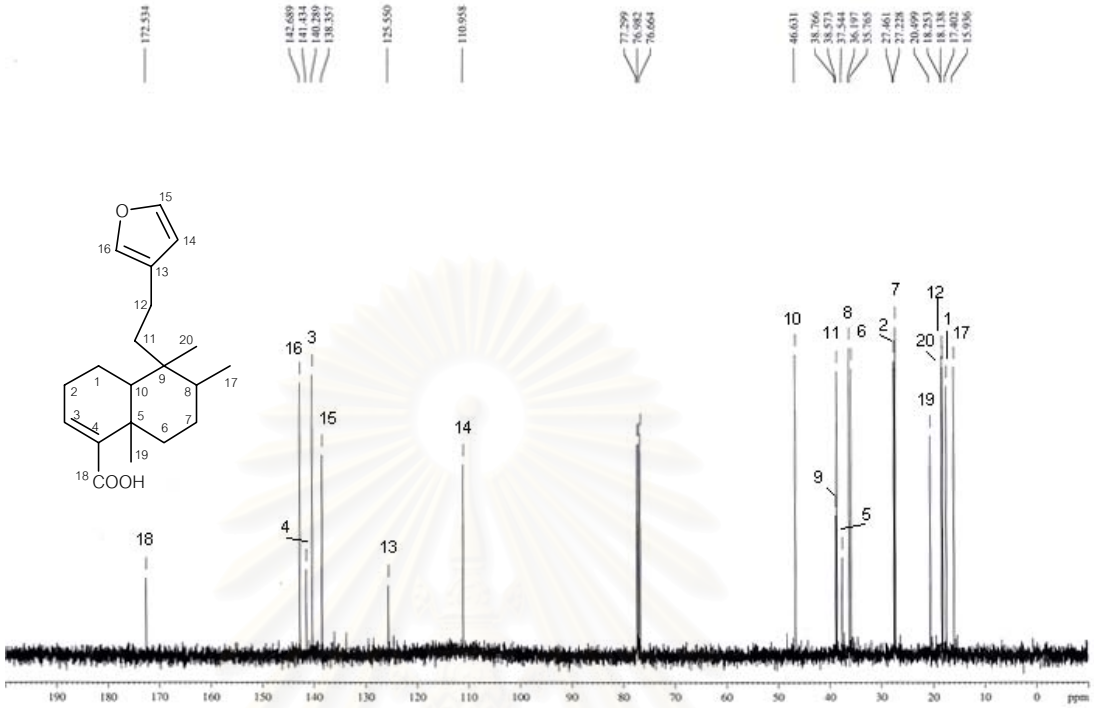


Figure 59: ^{13}C NMR (100 MHz) Spectrum of compound **9** ($CDCl_3$)

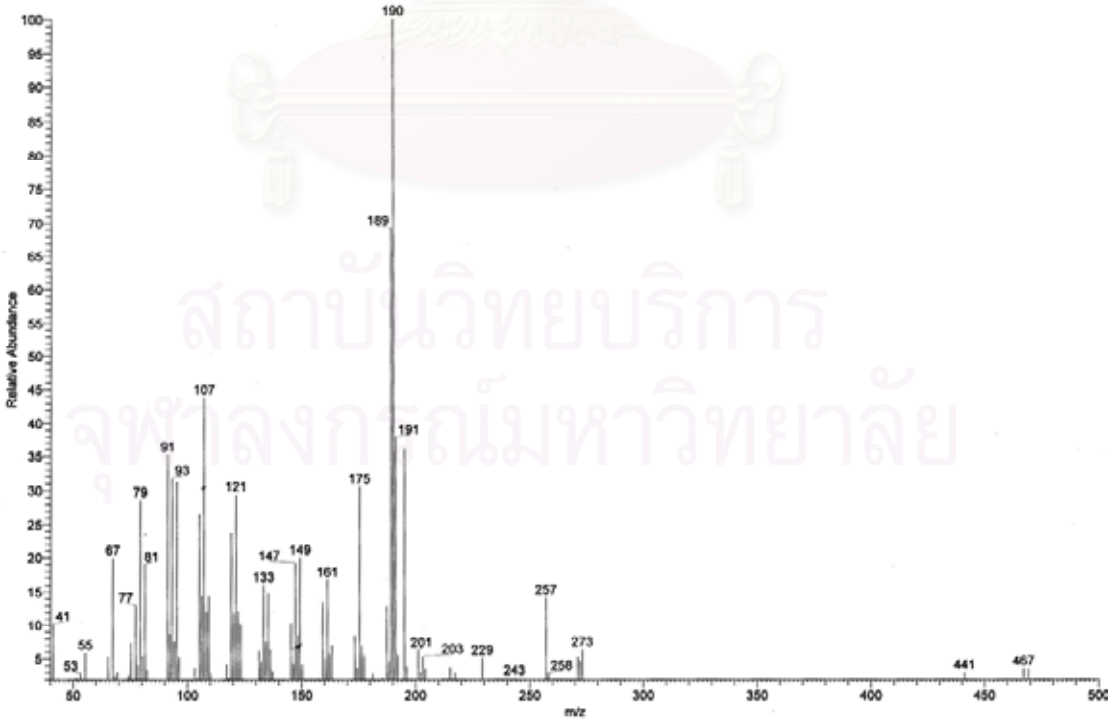


Figure 60: EIMS Mass spectrum of compound **3a**

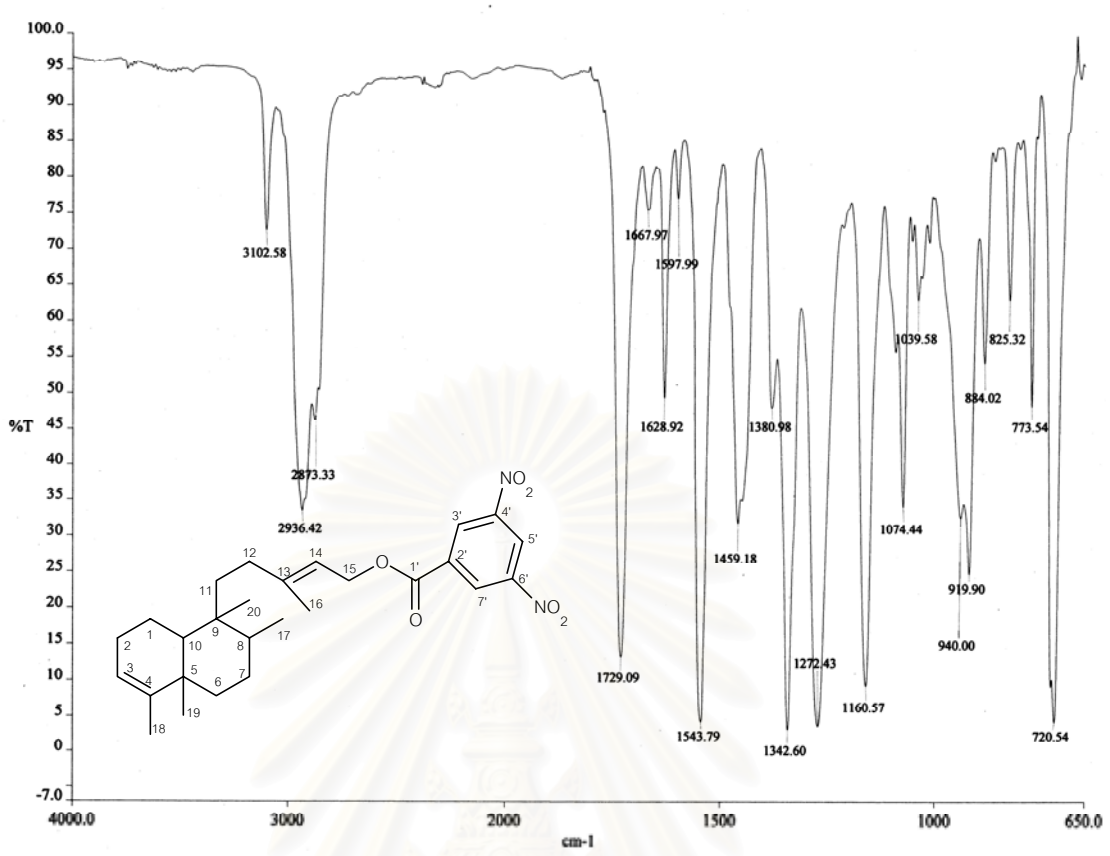


Figure 61: IR Spectrum of compound **3a**

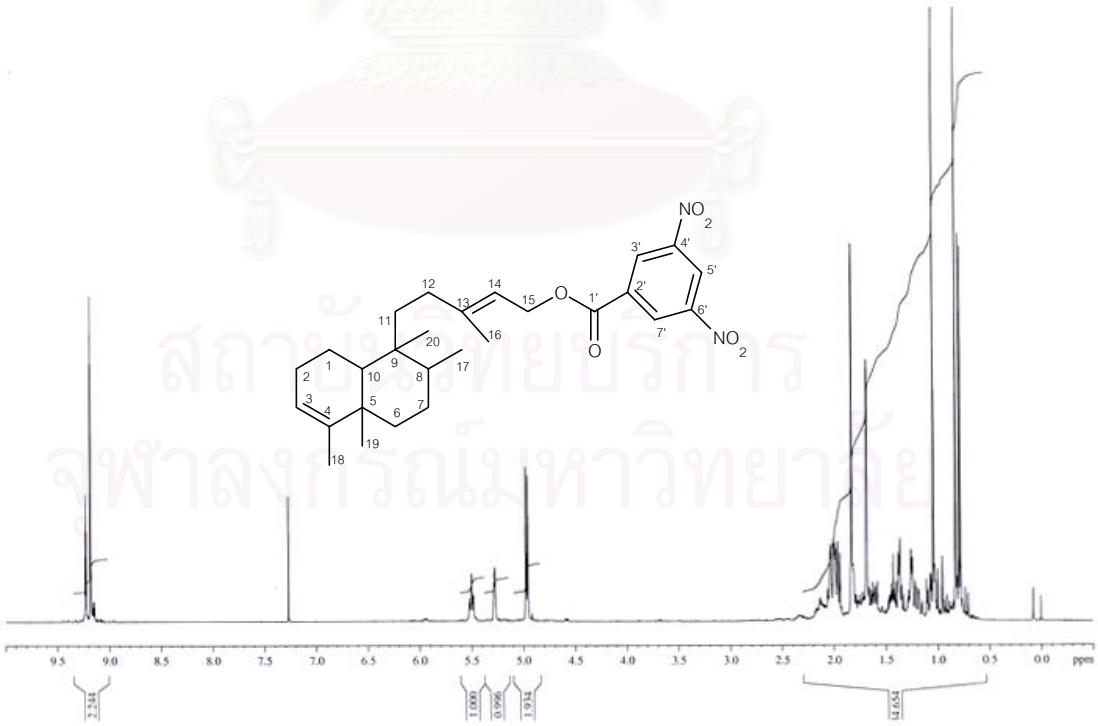


Figure 62: ^{1}H NMR (400 MHz) Spectrum of compound **3a** ($CDCl_3$)

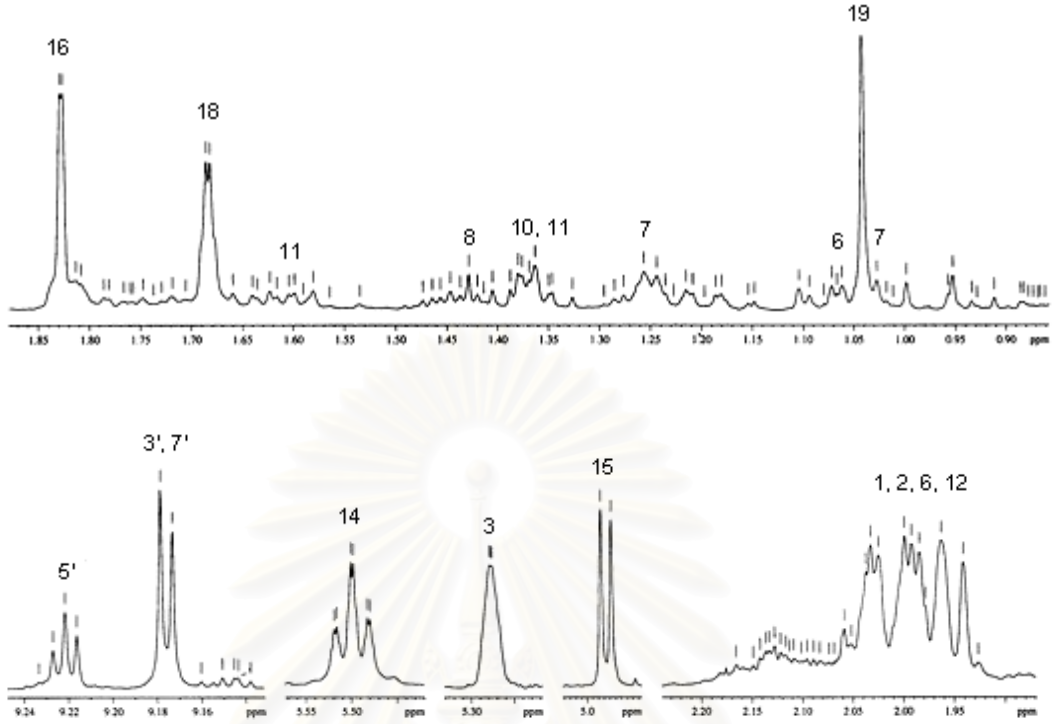


Figure 63: Expansion of ^{1}H NMR spectrum of compound **3a**

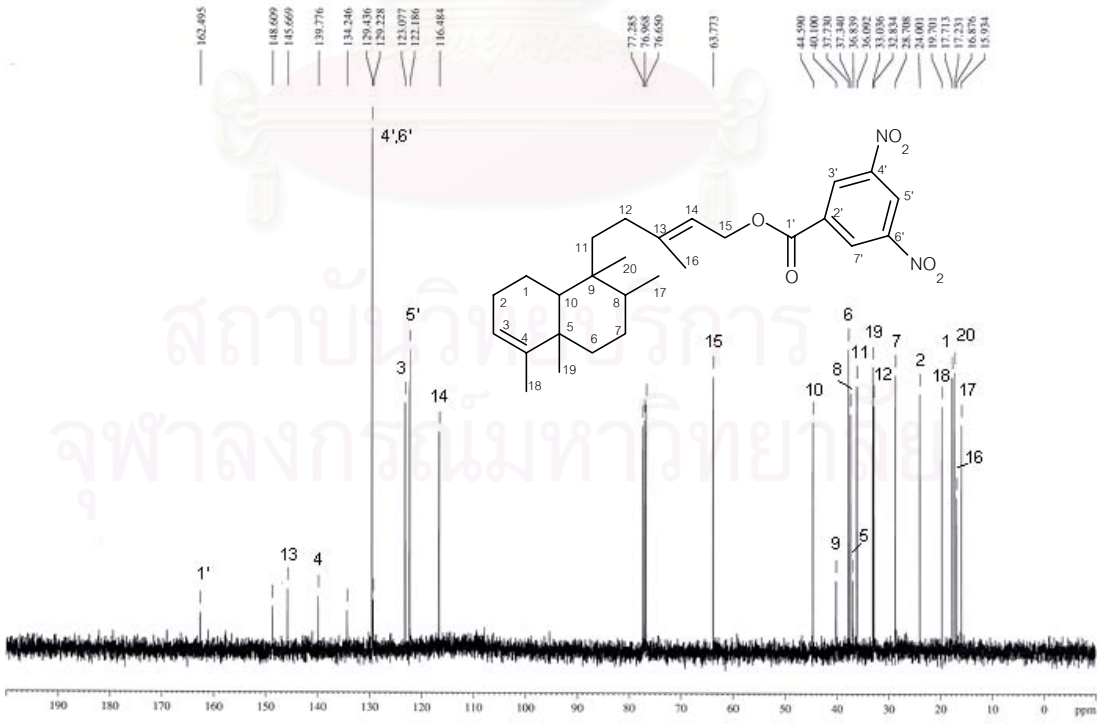


Figure 64: ^{13}C NMR (100 MHz) Spectrum of compound **3a** ($CDCl_3$)

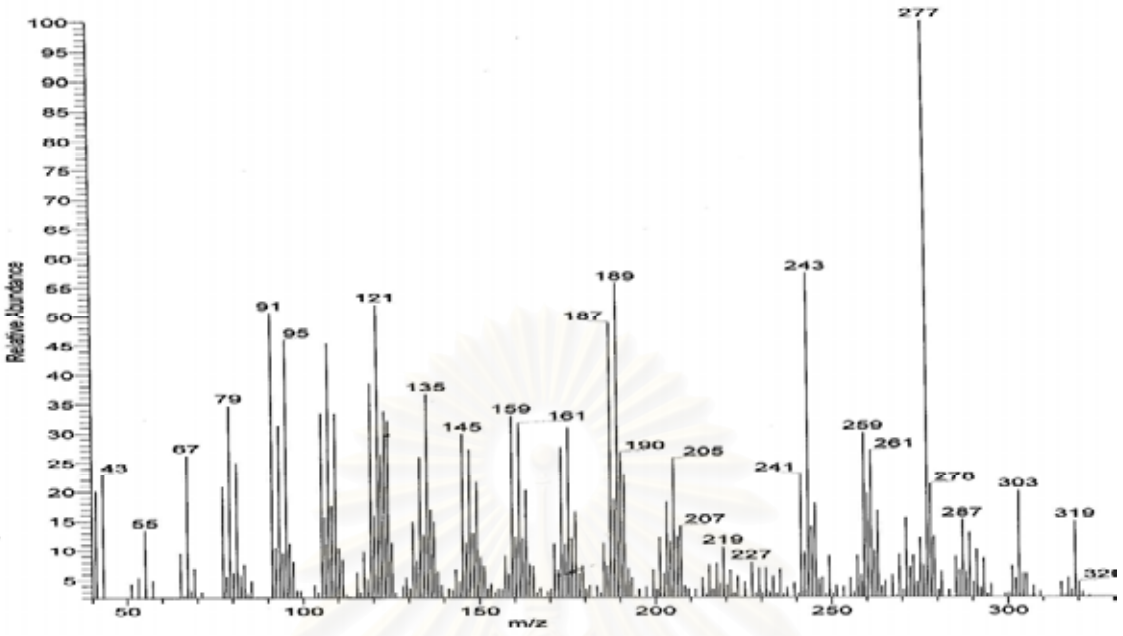


Figure 65: EIMS Mass spectrum of compound **3b**

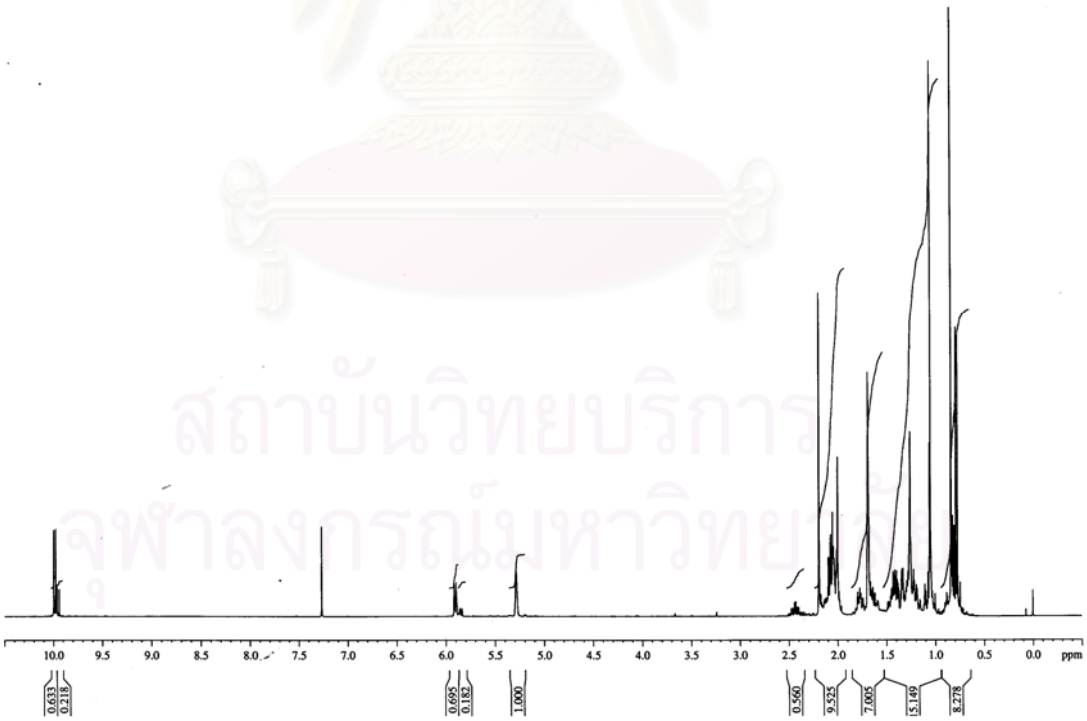


Figure 66: ^{1}H NMR (400 MHz) Spectrum of compound **3b** ($CDCl_3$)

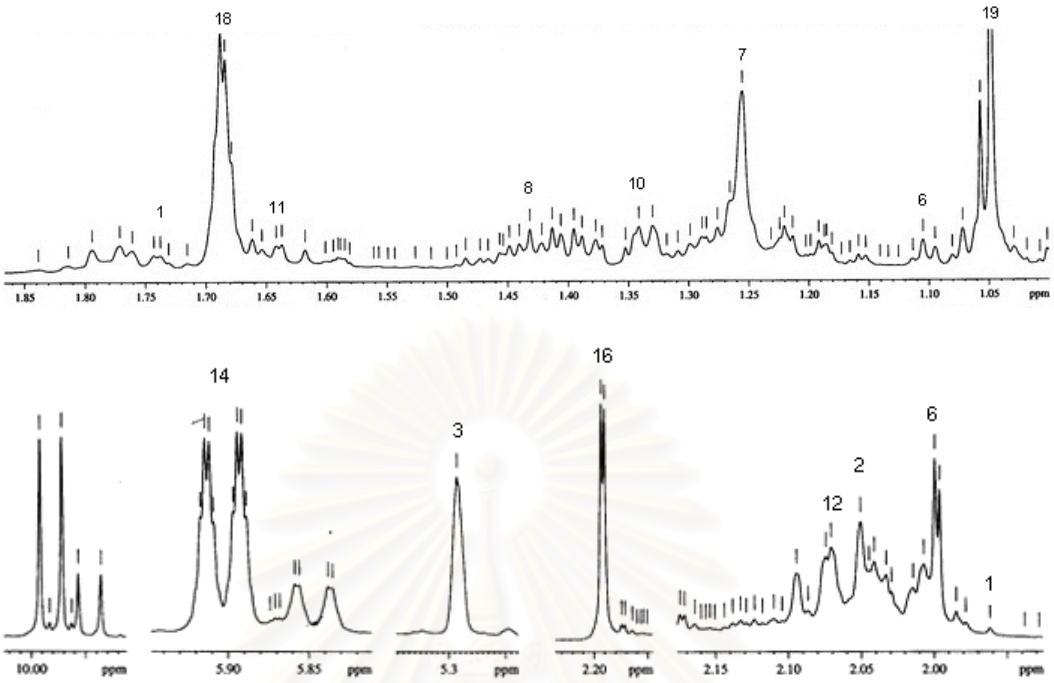


Figure 67: Expansion of ^{1}H NMR spectrum of compound **3b**

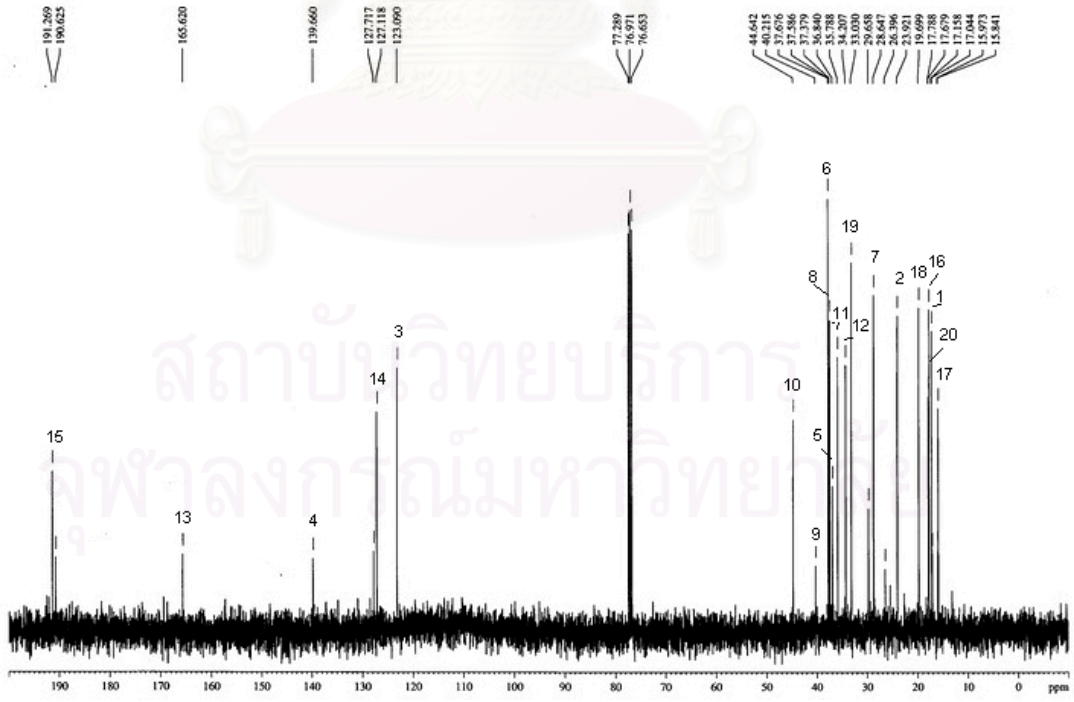


Figure 68: ^{13}C NMR (100 MHz) Spectrum of compound **3b** ($CDCl_3$)

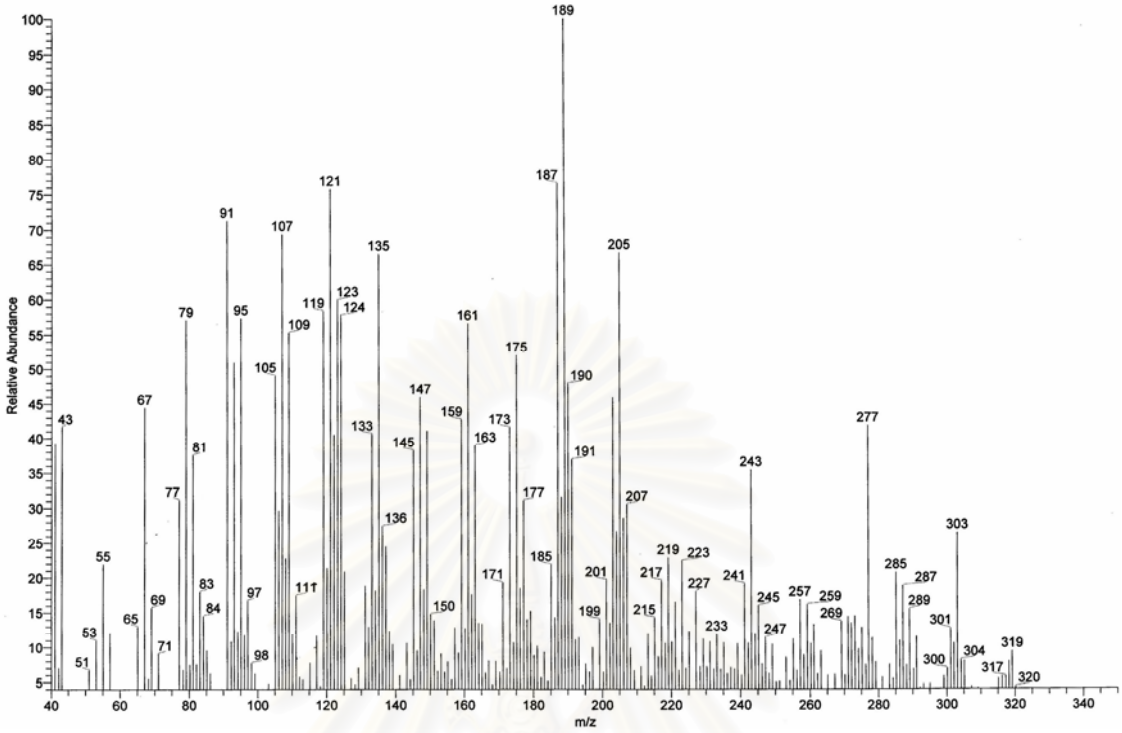


Figure 69: EIMS Mass spectrum of compound **3c**

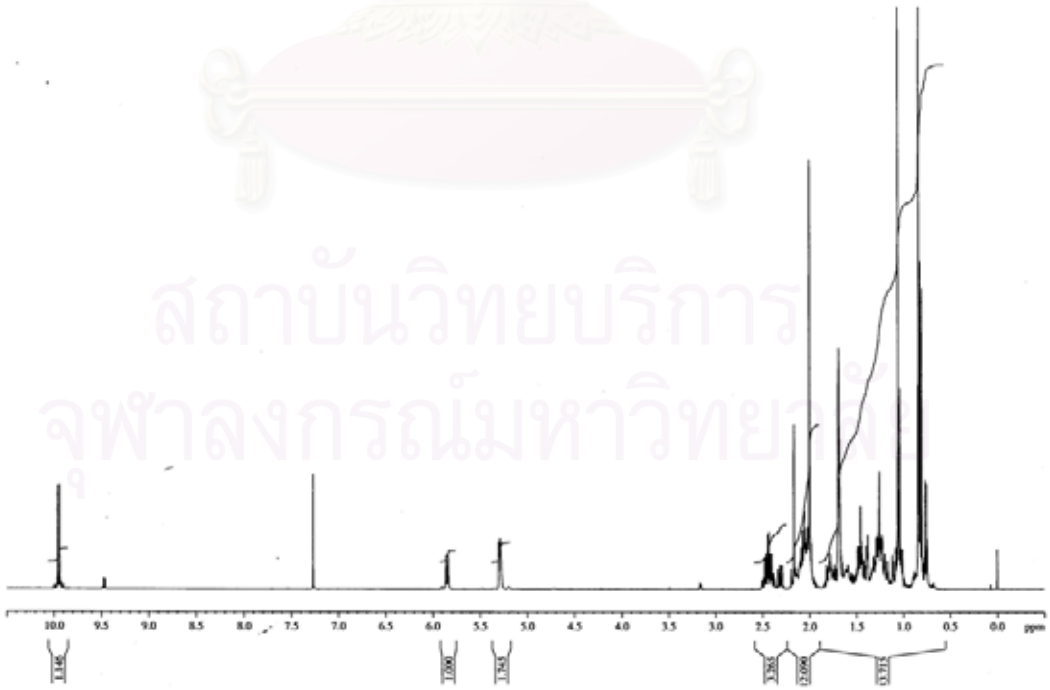


Figure 70: ^{1}H NMR (400 MHz) Spectrum of compound **3c** ($CDCl_3$)

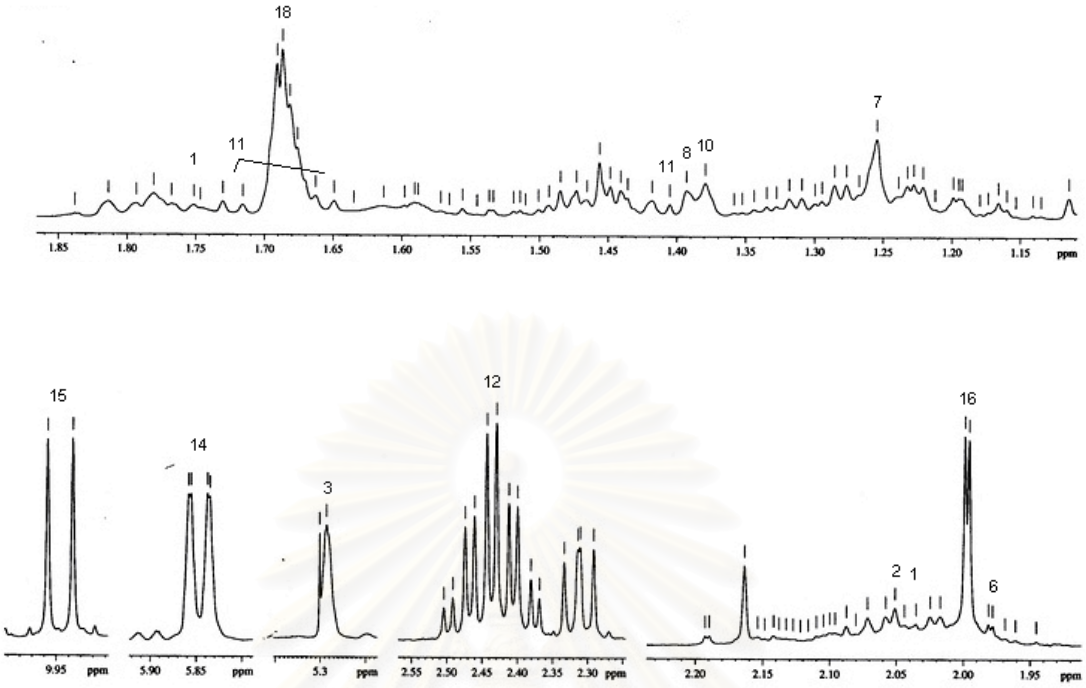


Figure 71: Expansion of ^{1}H NMR spectrum of compound **3c**

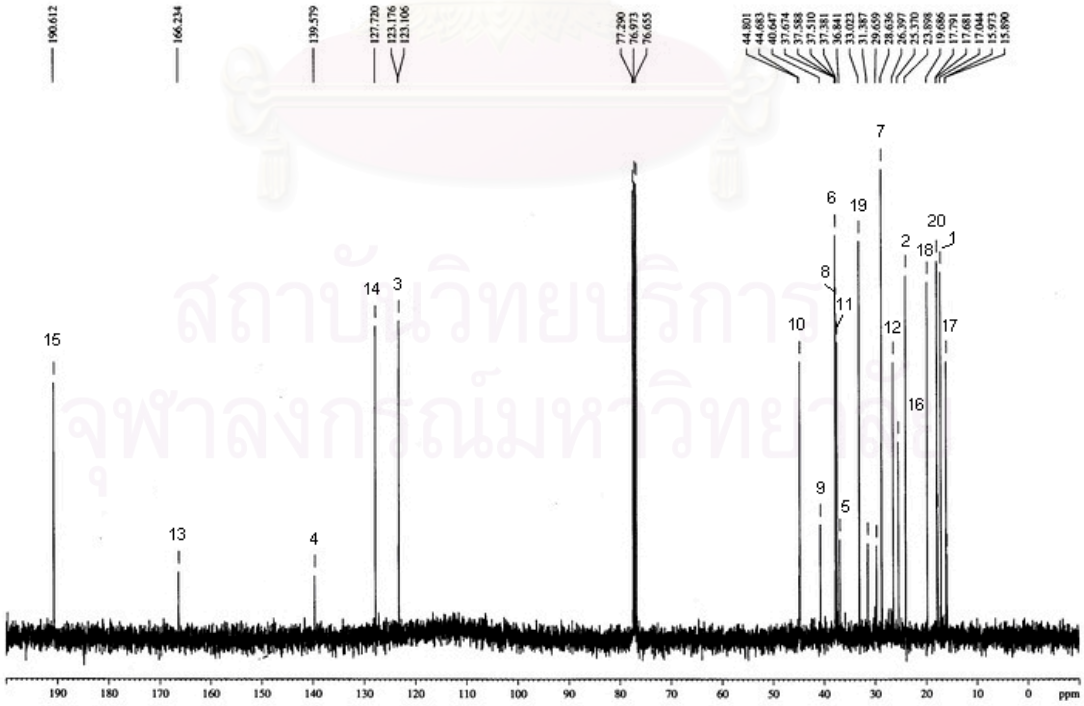


Figure 72: ^{13}C NMR (100 MHz) Spectrum of compound **3c** ($CDCl_3$)

VITA

Mr. Witoon Youngsa-ad was born on March 19th, 1982 in Nakhon Nayok, Thailand. He received his Bachelor's degree of Education in Chemistry from the Faculty of Education, Srinakharinwirot University in 2004. In the same year, he was admitted into a Master Degree program in organic chemistry at Department of Chemistry, Faculty of Science Chulalongkorn University and completed the program in 2006.