องค์ประกอบทางเคมีของเปลือกต้นเปล้าใหญ่จากจังหวัดเลย

นางสาว อัชชยา อิศรางกูร ณ อยุธยา

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CHEMICAL CONSTITUENTS OF THE STEM BARK OF *CROTON OBLONGIFOLIUS* FROM LOEI PROVINCE

Miss Achaya Israngkura Na Ayutthaya

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy
Department of Pharmacognosy
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By	Miss Achaya Israngkura Na Ayutthaya
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อัชชยา อิศรางกูร ณ อยุธยา : องค์ประกอบทางเคมีของเปลือกต้นเปล้าใหญ่จากจังหวัดเลย (CHEMICAL CONSTITUENTS OF THE STEM BARK OF *CROTON OBLONGIFOLIUS* FROM LOEI PROVINCE) อาจารย์ที่ปรึกษา : รศ. ชัยโย ชัยชาญทิพยุทธ, 117 หน้า, ISBN 974-17-0885-8

จากการศึกษาองค์ประกอบทางเคมีของเปลือกต้นเปล้าใหญ่ (*Croton oblongifolius* Roxb.) จากจังหวัดเลย สามารถสกัดแยกสารบริสุทธิ์ได้สองชนิด ได้แก่สารในกลุ่มนีโอ-เคลอโรเดนไดเทอร์ปีน คือ methyl-15,16-epoxy-12-oxo-3,13(16),14-*neo*-clerodatrien-18,19-olide-17-carboxylate และสารในกลุ่มไคลสแทนเธน ไดเทอร์ปีน คือ 3,4-seco-cleistantha-4(18),13(17),15-trien-3-oic acid การพิสูจน์เอกลักษณ์และสูตรโครงสร้างทางเคมีของสารทั้งสองชนิดกระทำโดยการวิเคราะห์ข้อมูลจากสเปคตรัมของ UV, IR, MS, 1-D NMR และ 2-D NMR ร่วมกับการเปรียบเทียบข้อมูลที่ได้กับสารที่มีการรายงานในอดีต

ภาควิชาเภสัชเวท
สาขาวิชาเภสัชเวท
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ลายมือชื่อนิสิต..อัชชยา อิศรางกูร ณ อยุธยา.....
ลายมือชื่ออาจารย์ที่ปรึกษา...ชัยโย ชัยชาญทิพยุทธ

##4276612033 PHARMACOGNOSY

KEY WORD: *CROTON OBLONGIFOLIUS*/ DITERPENE/ *NEO*-CLERODANE/ CLEISTANTHANE

ACHAYA ISRANGKURA NA AYUTTHAYA : CHEMICAL CONSTITUENTS OF THE STEM BARK OF *CROTON OBLONGIFOLIUS* FROM LOEI PROVINCE. THESIS ADVISOR : ASSOCIATE PROFESSOR CHAIYO CHAICHANTIPYUTH, M.Sc. in Pharm. 117 pp. ISBN 974-17-0885-8

In the course of the investigation for chemical constituents of the stem bark of *Croton oblongifolius* Roxb., a *neo*-clerodane-type diterpene, methyl-15,16-epoxy-12-oxo-3,13(16),14-*neo*-clerodatrien-18,19-olide-17-carboxylate, and a cleistanthane-type diterpene, 3,4-seco-cleistantha-4(18),13(17),15-trien-3-oic acid, have been isolated. The structure elucidation and identification of the isolated compounds were established by extensive UV, IR, MS, 1-D NMR and 2-D NMR spectral data analyses, as well as comparison with reported data.

Department of Pharmacognosy Student's signature Achaya Israngkura Na Ayutthaya

Field of study Pharmacognosy Advisor's signature Chaiyo Chaichantipyuth

Academic year 2002

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LIST OF ABBREVIATIONS

$[\alpha]^{25}_{D}$	=	Specific Rotation at 25°C and Sodium D line (589 nm)
br	=	Broad (for NMR spectral data)
c	=	Concentration
°C	=	Degree Celcius
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chloroform
cm	=	Centimeter
cm^{-1}	=	reciprocal centimeter (unit of wave number)
^{13}C-NMR	=	Carbon-13 Nuclear Magnetic Resonance
^{1}H-^{1}H COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
2D	=	Two Dimentional
d	=	doublet (for NMR spectral data)
dd	=	doublet of doublets (for NMR spectral data)
ddd	=	doublet of doublets of doublets (for NMR spectral data)
dddd	=	doublet of doublets of doublets of doublets (for NMR spectral data)
diam.	=	Diameter
DEPT	=	Distortionless Enhancement by Polarization Transfer
ε	=	Molar Absorptivity
δ	=	Chemical Shift
EIMS	=	Electron Impact Mass Spectroscopy
EtOAc	=	Ethyl Acetate
g	=	Gram
^{1}H-NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	^{1}H-detected Heteronuclear Multiple Bond Coherence
HMQC	=	^{1}H-detected Heteronuclear Multiple Quantum Coherence
Hz	=	Hertz
IR	=	Infrared
J	=	Coupling Constant

LIST OF ABBREVIATIONS (Cont.)

KBr	=	Potassium bromide
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at Maximal Absorption
m	=	Multiplet (for NMR spectral data)
MeOH	=	Methanol
mg	=	Milligram
ml	=	Milliliter
mm	=	Millimeter
MS	=	Mass Spectroscopy
m/z	=	mass-to-charge ratio
M^+	=	Molecular Ion
No.	=	Number
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Enhancement Spectroscopy
ppm	=	part per million
ν_{max}	=	Wave number at maximum absorption
q	=	Quartet (for NMR spectral data)
s	=	Singlet (for NMR spectral data)
t	=	Triplet (for NMR spectral data)
TLC	=	Thin Layer Chromatography
UV-VIS	=	Ultraviolet and Visible Spectrophotometry

CHAPTER I

INTRODUCTION

Croton oblongifolius Roxb. is a medium-sized tree belonging to the Spurge family (Euphorbiaceae), a large family with over 7,000 currently recognized species (Clapham, Tutin, and Warburg, 1962). According to Tem Smitinand (1980), this plant is known in Thailand as เปล้าใหญ่ Plao yai (Central), เปล้าหลวง Plao luang (Northern), คาะวู Khwa-wuu (Karen-Kanchanaburi), เซ่งเค่คัง Seng-khe-khang, สะกาวา Sa-kaa-waa, ส่ากุวะ Saa-kuu-wa (Karen-Mae Hong Son), เปาะ Poh (Kamphaeng Phet), ห้าเยิ่ง Haa-yoeng (Shan-Mae Hong Son).

For many centuries *Croton oblongifolius* Roxb. has been regarded as being among the most efficacious of medicinal plants in Asia. It is often used in combination with *Croton sublyratus* Kurz to treat gastric ulcer and gastric cancer. The seeds and fruits are known to have a purgative effect. The flowers are believed to be parasiticide. The bark is used in India as a remedy for chronic liver enlargement and remittent fever, whereas in Thailand it is used to cure biliary diseases and to reduce phlegm. The Santals use the bark and root as a purgative and as an alterative in dysentery. The root bark is given in small doses as a purge; a larger quantity is poisonous. The sapwood is used for dyspepsia, while the heartwood is recommended for flatulence. The leaves are used externally in Cambodia for liver complaints and scabies (Blatter, Caius and Mhaskar, 1975; Kittikhajorn, 1983).

1. Botanical aspects of *Croton oblongifolius* Roxb.

A medium-sized tree; young shoots, branchlets, inflorescence, calyx, and ovary clothed with minute orbicular silvery scales. Leaves 12.5-25 by 5.7-11.5 cm., crowded towards the ends of the branchlets, oblong-lanceolate, subacute, glabrous when fully grown, more or less crenate or serrate, penninerved, base usually acute with no apparent glands above the petioles; main nerves numerous, slender; petioles 2-3.2 cm. Long. Flowers pale yellowish green, solitary or fascicled in the axils of minute bracts on long erect often fascicled racemes, the males in the upper part of the raceme, the

females in the lower part. Male flowers: Pedicels variable in length, reaching 4 mm. long, slender. Calyx more than 6 mm. across when flattened out, divided about ¾ the way down; segments more than 2.5 mm. long, ovate, obtuse. Petals 3 mm. long, elliptic-lanceolate, obtuse, woolly. Stamens 12, inflexed in bud; filaments 3 mm. long, the lower half hairy. Female flowers: Pedicels short, stout. Sepals more acute than in the male, with densely ciliate margins. Petals 2 mm. long, obovate, with densely woolly margins. Styles 3, nearly 4 mm. long, each again subdivided into 2 long slender curled branches 3 mm. long. Capsules less than 1.3 cm. diam., subglobose, a little depressed, slightly 3-lobed, clothed with small orbicular scales. Seeds 8 by 6 mm., ellipsoid, rounded and quite smooth on the back. (Blatter, Caius and Mhaskar, 1975)

2. The objectives of this research.

Previous phytochemical studies of *Croton oblongifolius* Roxb. carried out by Seshadri *et al*. have revealed the presence of many diterpene compounds in the stem bark of the plant. It was later discovered that this same plant collected in various parts of Thailand contains different chemical constituents, which is possibly due to geographic variations (Roengsumran *et al*., 1998). Further phytochemical studies of this plant have thereafter been conducted, with a view to complete the chemotaxonomic knowledge of this species. In this study, as a continuation of the investigation of chemical substances from *Croton oblongifolius* Roxb., the plant specimen collected from Loei province has been examined. The TLC pattern and NMR spectral data showed the plant to have constituents unlike those collected elsewhere. The plant was therefore considered to be very interesting, and selected to be investigated.

The main objectives of this investigation are as follows :

1. to isolate and purify chemical compounds from the stem bark of *Croton oblongifolius* Roxb. collected from Loei province, Thailand

2. to determine the chemical structure of each isolated compound.

Figure 1 : *Croton oblongifolius* Roxb.

CHAPTER II
HISTORICAL

1. Chemical constituents of *Croton oblongifolius* Roxb.

According to previous phytochemical studies, *Croton oblongifolius* Roxb. has been found to be a rich source of diterpenes. Up until now, six different types of the main diterpene skeletons have been found in this plant, namely Pimarane, Clerodane, Cembrane, Labdane, Kaurane, and Cleistanthane. In addition to these diterpenes, steroids and several other chemical compounds are also presented, as summarized in the table below.

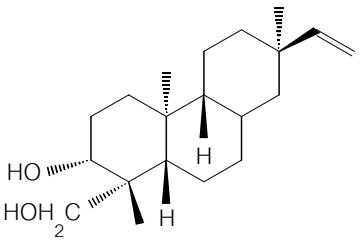
Table 1. The chemical constituents of *Croton oblongifolius* Roxb.

Chemical Compounds	Plants Parts	References
Diterpenes		
1. Pimarane Diterpenes		
• oblongifoliol [1]	stem bark root bark, wood	Rao *et al*., 1972 Aiyar and Seshadri, 1972b
• 19-deoxyoblongifoliol [2]	stem bark root bark, wood	Aiyar *et al*., 1969 Aiyar and Seshadri, 1972b
• 3-deoxyoblongifoliol [3]	stem bark root bark, wood	Aiyar and Seshadri, 1971a Aiyar and Seshadri, 1972b
• oblongifolic acid [4]	stem bark root bark, wood	Aiyar and Seshadri, 1970 Aiyar and Seshadri, 1972b
• *ent*-isopimara-7,15-diene [5]	stem bark root bark, wood	Aiyar and Seshadri, 1971b Aiyar and Seshadri, 1972b
• *ent*-isopimara-7,15-diene-19 -aldehyde [6]	stem bark root bark, wood	Aiyar and Seshadri, 1971b Aiyar and Seshadri, 1972b
• 19-hydroxy-*ent*-isopimara-7,15-diene [7]	stem bark	Aiyar and Seshadri, 1971b

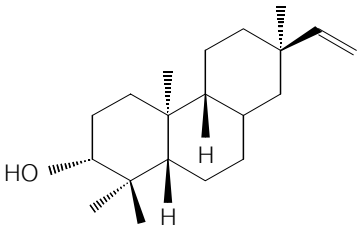
Chemical compounds	Plant Parts	References
• (-)- pimara-9(11),15-diene-19-oic acid **[8]**	stem bark	Tanwattanakun, 1999
• (-)- pimara-9(11),15-diene-19-ol **[9]**	stem bark	Tanwattanakun, 1999
2. Clerodane Diterpenes • (-)-hardwickiic acid **[10]**	stem bark root bark, wood	Aiyar and Seshadri, 1972a Aiyar and Seshadri, 1972b
• 11-dehydro-(-)-hardwickiic acid **[11]**	stem bark root bark, wood	Aiyar and Seshadri, 1972a Aiyar and Seshadri, 1972b
• (-)-20-benzyloxyhardwickiic acid **[12]**	stem bark	Baiagern, 1999
• methyl-15,16-epoxy-12-oxo-3,13(16),14-clerodatriene-20,19-olide-17-oate **[13]**	stem bark	Tanwattanakun, 1999
3. Cembrane Diterpenes • crotocembraneic acid **[14]**	stem bark	Roengsumran *et al*., 1998
• neocrotocembraneic acid **[15]**	stem bark	Roengsumran *et al*., 1998
• neocrotocembranal **[16]**	leaves stem bark	Achayindee, 1996 Roengsumran *et al*., 1999b
• 1-isopropyl-4,8-dimethylcyclotetradeca-1,4,8-triol- 2*E*,6*Z*,11*E*-triene-12-carboxylic acid **[17]**	stem bark	Tanwattanakun, 1999
4. Labdane Diterpenes • labda-7,12(*E*),14-triene **[18]**	stem bark	Roengsumran *et al*., 1999a
• labda-7,12(*E*),14-triene-17-al **[19]**	stem bark	Roengsumran *et al*., 1999a
• labda-7,12(*E*),14-triene-17-ol **[20]**	stem bark	Roengsumran *et al*., 1999a
• labda-7,12(*E*),14-triene-17-oic acid **[21]**	stem bark	Roengsumran *et al*., 1999a
• *ent*-8(17),12*E*,14-labdatrien-18-oic acid **[22]**	stem bark	Pattamadilok, 1998

Chemical Compounds	Plant Parts	References
• 12,15-epoxy-8(17),12,14-labdatriene **[23]**	stem bark	Pattamadilok, 1998
• labda-7,13(*Z*)-diene-17,12-olide **[24]**	stem bark	Baiagern, 1999
• labda-7,13(*Z*)-diene-17,12-olide – 16-ol **[25]**	stem bark	Baiagern, 1999
• 2-acetoxy-3-hydroxy-labda-8(17),12(*E*)-14-triene **[26]**	stem bark	Roengsumran *et al*., 2001
• 3-acetoxy-2-hydroxy-labda-8(17),12(*E*)-14-triene **[27]**	stem bark	Roengsumran *et al*., 2001
• 2,3-dihydroxy-labda-8(17),12(*E*)-14 -triene **[28]**	stem bark	Roengsumran *et al*., 2001
5. **Kaurane Diterpene** • *ent*-kaur-16-en-19-oic acid **[29]**	stem bark	Pattamadilok, 1998
6. **Cleistanthane Diterpene** • 3,4-seco-cleistantha-4(18), 13(17),15-trien-3- oic acid **[30]**	stem bark	Siriwat, 1999
<u>Triterpene</u> • acetyl aleuritolic acid **[31]**	stem bark	Aiyar and Seshadri, 1971c
<u>Steroids</u> • campesterol **[32]**	wood stem bark	Chaicharoenpong, 1996 Pattamadilok, 1998
• stigmasterol **[33]**	wood leaves stem bark	Chaicharoenpong, 1996 Achayindee, 1996 Pattamadilok, 1998
• β-sitosterol **[34]**	stem bark wood leaves	Rao *et al*., 1968 Chaicharoenpong, 1996 Achayindee, 1996

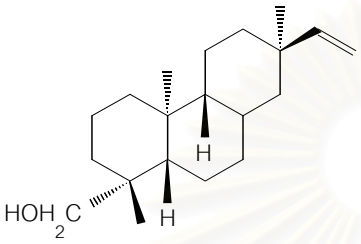
Chemical Compounds	Plant Parts	References
<u>Steroid Glycosides</u> • stigmasteryl-3-O-β-D-glucopyranoside **[35]**	wood	Chaicharoenpong, 1996
• β-sitosteryl-3-O-β-D-glucopyranoside **[36]**	wood	Chaicharoenpong, 1996
• campesteryl-3-O-β-D-glucopyranoside **[37]**	wood	Chaicharoenpong, 1996
<u>Coumarin</u> • 7-hydroxy-6-methoxycoumarin (Scopoletin) **[38]**	wood	Chaicharoenpong, 1996
<u>Miscellaneous</u> • long chain aliphatic hydrocarbons (C_{27}-C_{33})	wood leaves	Chaicharoenpong, 1996 Achayindee, 1996
• long chain carboxylic acids (C_{18}, C_{22}-C_{34})	wood	Chaicharoenpong, 1996
• long chain alcohols (C_{28}-C_{29}, C_{31}-C_{32}, C_{34})	leaves	Achayindee, 1996
• 6,10,14-trimethyl-2-pentadecanone ($C_{18}H_{36}O$) **[39]**	leaves	Achayindee, 1996
• potassium chloride	leaves	Achayindee, 1996



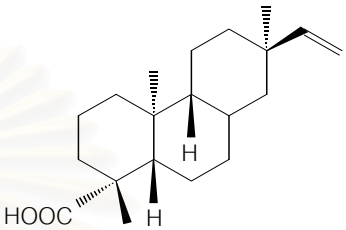
oblongifoliol **[1]**



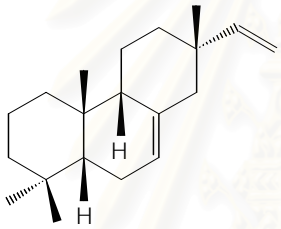
19-deoxyoblongifoliol **[2]**



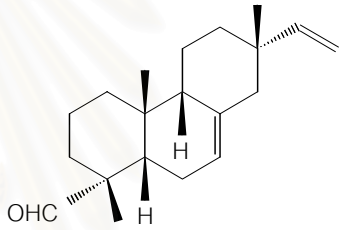
3-deoxyoblongifoliol **[3]**



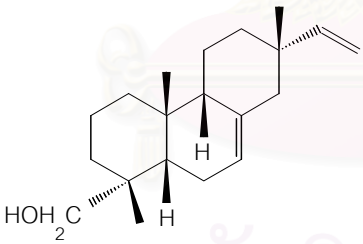
oblongifolic acid **[4]**



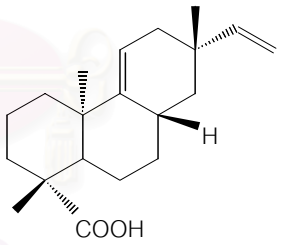
ent-isopimara-7,15-diene **[5]**



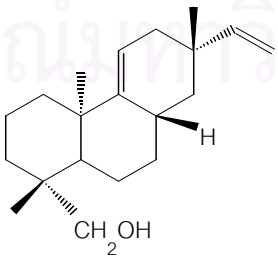
ent-isopimara-7,15-diene-19-aldehyde **[6]**



19-hydroxy-*ent*-isopimara-7,15-diene **[7]**

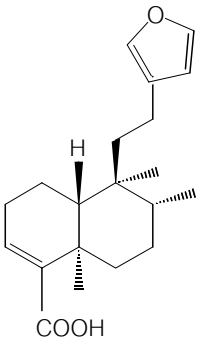


(-)-pimara-9(11),15-diene-19-oic acid **[8]**

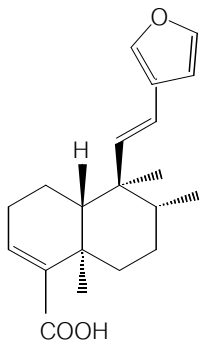


(-)-pimara-9(11),15-diene-19-ol **[9]**

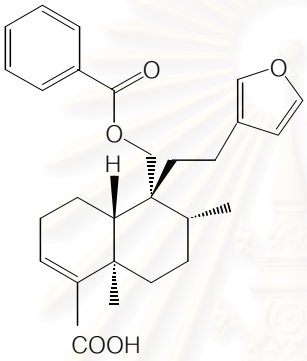
Figure 2 : Structures of chemical constituents of *Croton oblongifolius*



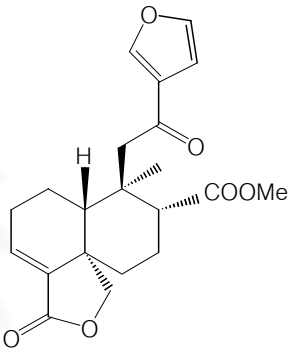
(-)-hardwickiic acid **[10]**



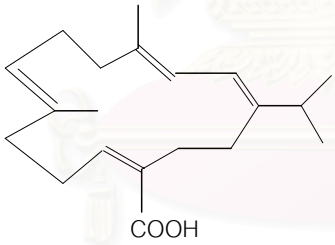
11-dehydro-(-)-hardwickiic acid **[11]**



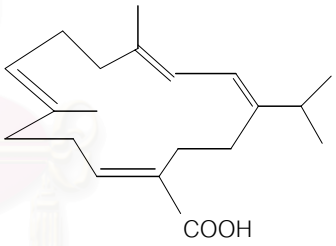
(-)-20-benzyloxyhardwickiic acid **[12]**



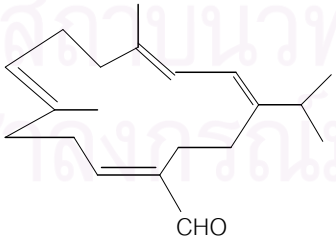
methyl-15,16-epoxy-12-oxo-3,13(16),
14-clerodatriene-20,19-olide-17-oate **[13]**



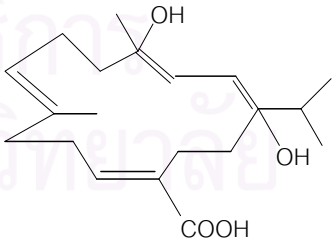
crotocembraneic acid **[14]**



neocrotocembraneic acid **[15]**

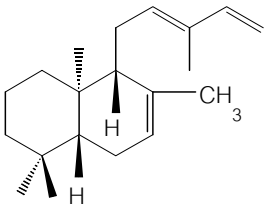


neocrotocembranal **[16]**

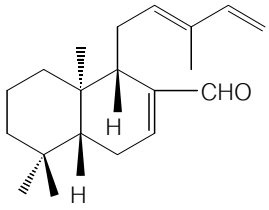


1-isopropyl-4,8-dimethylcyclotetradeca-1,4,8
-triol-2*E*,6*Z*,11*E*-triene-12-carboxylic acid **[17]**

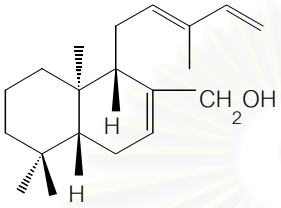
Figure 2 : Structures of chemical constituents of *Croton oblongifolius* (continue)



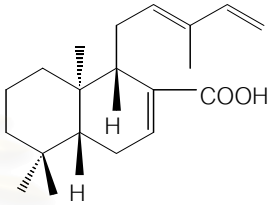
labda-7,12(*E*),14-triene **[18]**



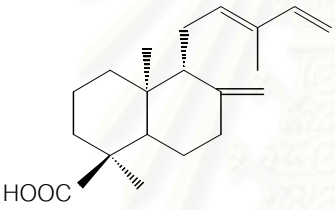
labda-7,12(*E*),14-triene-17-al **[19]**



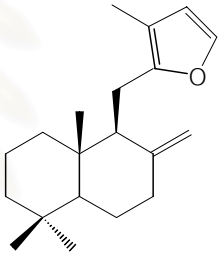
labda-7,12(*E*),14-triene-17-ol **[20]**



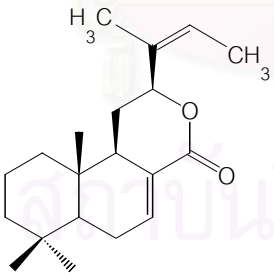
labda-7,12(*E*),14-triene-17-oic acid **[21]**



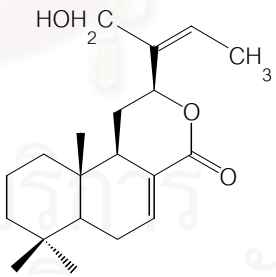
ent-8(17),12*E*,14-labdatrien-18-oic acid **[22]**



12,15-epoxy-8(17),12,14-labdatriene **[23]**

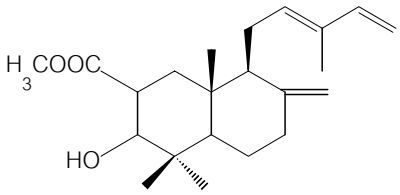


labda-7,13(*Z*)-diene-17,12-olide **[24]**

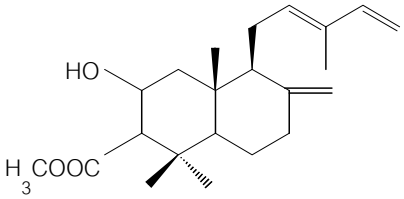


labda-7,13(*Z*)-diene-17,12-olide-16-ol **[25]**

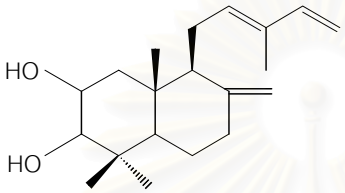
Figure 2 : Structures of chemical constituents of *Croton oblongifolius* (continue)



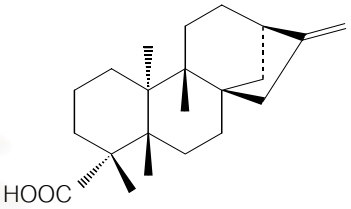
2-acetoxy-3-hydroxy-labda-8(17),12(*E*)-14-triene **[26]**



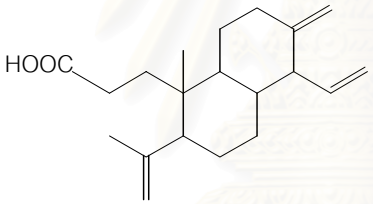
3-acetoxy-2-hydroxy-labda-8(17),12(*E*)-14-triene **[27]**



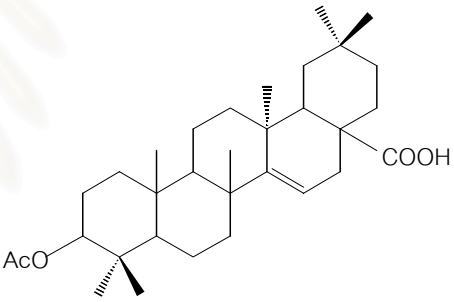
2,3-dihydroxy-labda-8(17),12(*E*),14-triene **[28]**



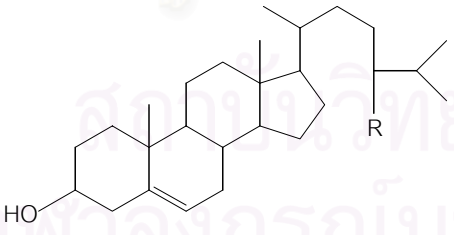
ent-kaur-16-en-19-oic acid **[29]**



3,4-seco-cleistantha-4(18),13(17),15-trien-3-oic acid **[30]**

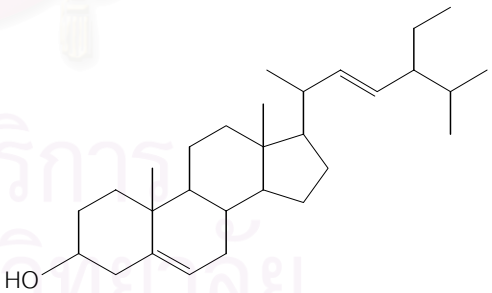


acetyl aleuritolic acid **[31]**



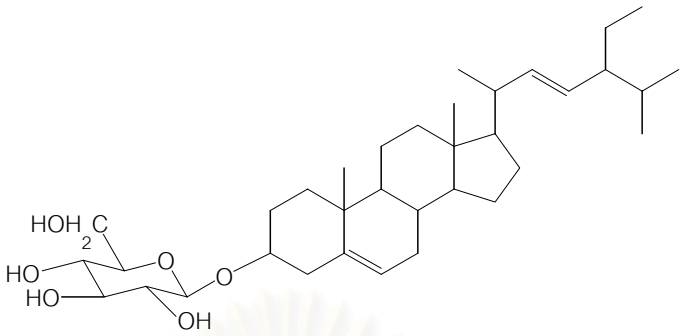
campesterol **[32]** : R = CH_3

β - sitosterol **[34]** : R = C_2H_5

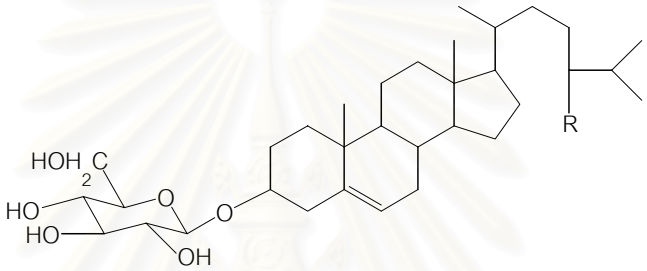


stigmasterol **[33]**

Figure 2 : Structures of chemical constituents of *Croton oblongifolius* (continue)

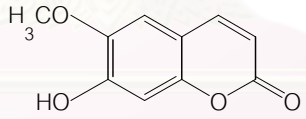


stigmasteryl-3-O-β-D-glucopyranoside **[35]**

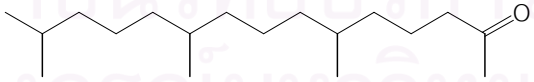


β - sitosteryl-3-O-β-D-glucopyranoside **[36]** : R = C_2H_5

campesteryl-3-O-β-D-glucopyranoside **[37]** : R = CH_3



scopoletin (7-hydroxy-6-methoxycoumarin) **[38]**



6,10,14-trimethyl-2-pentadecanone ($C_{18}H_{36}O$) **[39]**

Figure 2 : Structures of chemical constituents of *Croton oblongifolius* (continue)

Some of the diterpene compounds listed in **Table 1** have been shown to exhibit *in vitro* cytotoxicity against many human tumor cell lines, as summarized below.

Table 2. Cytotoxicity data of the diterpenes from *Croton oblongifolius* Roxb.

Compounds	IC_{50} (μg/ml)					References
	KATO-3	SW620	BT474	HEP-G2	CHAGO	
[9]	6.5	5.9	> 10	6.7	6.1	Tanwattanakun, 1999
[25]	7.1	6.5	> 10	5	6.4	Baiagern, 1999
[26]	5.7	7.1	> 10	> 10	> 10	Roengsumran *et al.*, 2001
[27]	3.3	> 10	5.9	> 10	> 10	Roengsumran *et al.*, 2001
[28]	2.2	2.7	4.6	3.7	3.3	Roengsumran *et al.*, 2001

[9] = (-)-pimara-9(11),15-diene-19-ol

[25] = labda-7,13(*Z*)-diene-17,12-olide-16-ol

[26] = 2-acetoxy-3-hydroxy-labda-8(17),12(*E*)-14-triene

[27] = 3-acetoxy-2-hydroxy-labda-8(17),12(*E*)-14-triene

[28] = 2,3-dihydroxy-labda-8(17),12(*E*)-14-triene

Tumor Cell Lines:

KATO-3 = human gastric carcinoma

SW620 = human colon adenocarcinoma

BT474 = human breast ductol carcinoma

HEP-G2 = human liver hepatoblastoma

CHAGO = human undifferentiated lung carcinoma

From the data in **Table 2** it is very interesting to see that, among the three structurally related labdane diterpenes **[26-28]**, **[26]** and **[27]** were less active but more selective than **[28]**. The presence of the acetyl group is believed to be the cause of this, since it is likely that an acetylation of these compounds could render their ability to form hydrogen bond with certain receptor on tumor cells and made them more selective but less active. (Roengsumran *et al.,* 2001)

Another notable compound derived from this plant, apart from those already mentioned in **Table 2**, is neocrotocembranal **[16]**. This compound inhibited platelet aggregation induced by thrombin with an IC_{50} value of 47.21 μg/ml., and exhibited cytotoxicity against P-388 cells (lymphoid neoplasm) *in vitro* with an IC_{50} value of 6.48 μg/ml. However, two other tested cembranoid diterpenes, crotocembraneic acid **[14]** and neocrotocembraneic acid **[15]**, have been proved to show no inhibitory effect on platelet aggregation. Thus arise a hypothesis that the reactive aldehyde functionality plays an important part in this effect. (Roengsumran *et al.,* 1999b)

Furthermore, (-)-hardwickiic acid **[10]**, a well-known clerodane diterpene, has been reported as having insecticidal activity against *Aphis craccivora* (Aphididae). The compound at a dose of 5 ppm./insect caused 62% mortality of adult female aphids after 24 hours. (Bandara *et al*., 1987)

Since the bioactivity of diterpenes from *Croton oblongifolius* Roxb. has not yet been thoroughly investigated, the large majority of the compounds left unsaid are simply untested or unreported. This, therefore, shows a tendency for this plant to be another source of interesting bioactive compounds, which need to be studied further on.

2. Biogenetic pathway of diterpenoids in *Croton oblongifolius* Roxb.

The diterpenes are C_{20} compounds biogenetically derived from geranylgeranyl pyrophosphate. The notable feature of diterpene structures is the fascinating variation encountered in their skeletons, which accounts for the division of these compounds into several types. The following correlation chart shows the main diterpene skeletons found in *Croton oblongifolius* Roxb. (Devon and Scott, 1972)

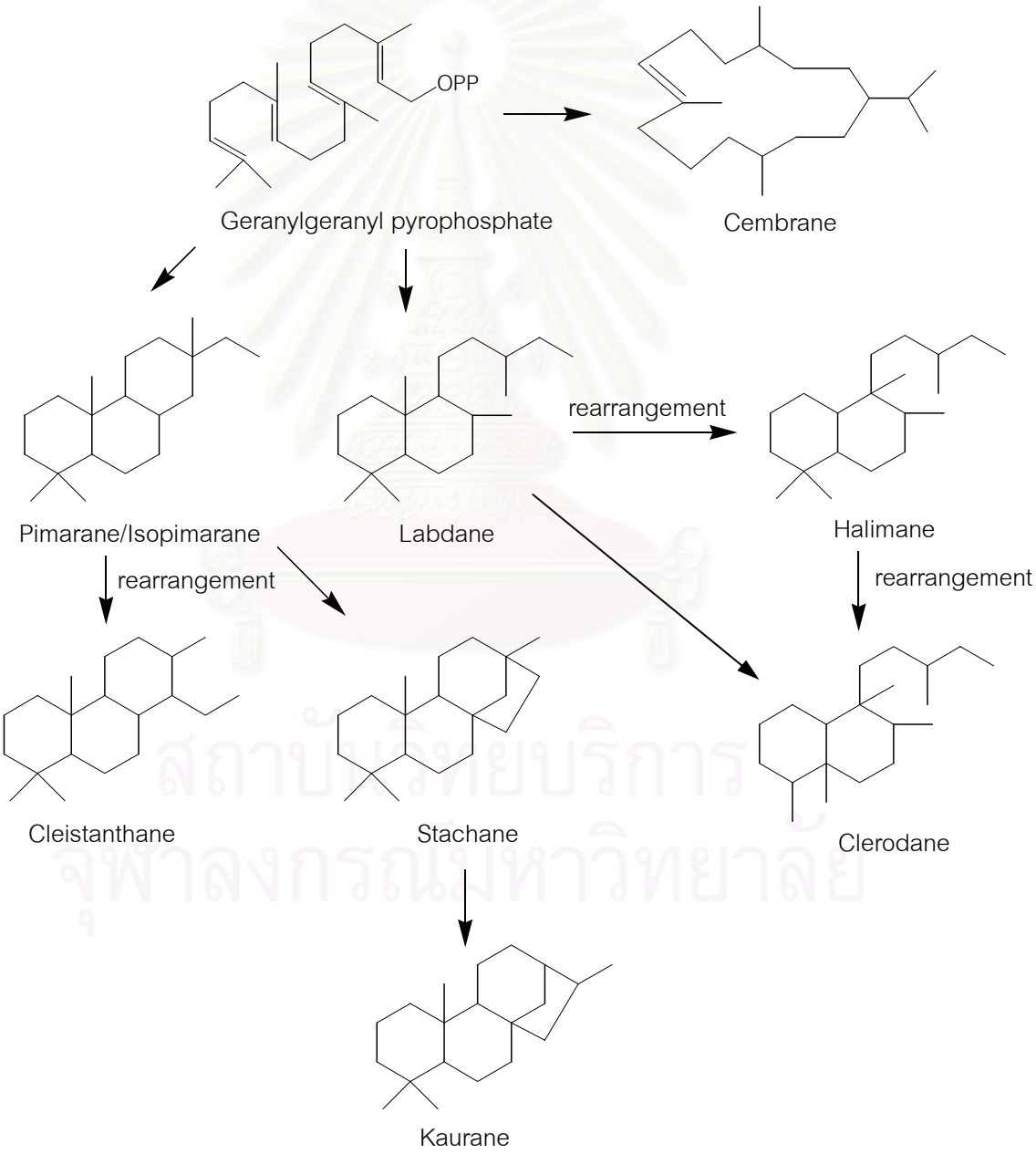


Figure 3 : Biogenetic pathway of diterpenoids in *Croton oblongifolius* Roxb.

3. Cleistanthane diterpenes

Cleistanthane diterpenes are secondary metabolites uncommon in nature. Most of the members of this series were isolated from species of the family Euphorbiaceae, Compositae, and Velloziaceae. All the diterpenes isolated from Euphorbiaceae and Compositae possess the (5*R*,10*R*) – absolute configuration while the cleistanthane diterpenes isolated from Vellociaceae belong mostly to the normal series (5*S*,10*S*) (Pinto and Macaira, 1988). Recent studies also show the presence of cleistanthane diterpenes in other sources, like in plants of the family Potamogetonaceae and Lamiaceae, and in the fungus (*Zythiostroma* sp.).

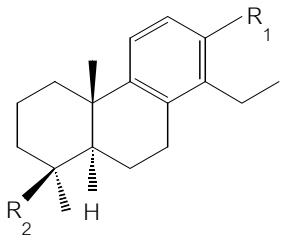
Table 3. Distribution of cleistanthane diterpenes in nature.

Sources	Chemical Compounds	References
Family Velloziaceae		
• *Vellozia flavicans* Martius ex. Schultz	• veadeirol **[40]**	Pinchin *et al*., 1978
	• veadeiric acid **[41]**	
(stem, roots and leaves)	• (4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-ol **[42]**	Pinto *et al*., 1987
	• (4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-oic acid **[43]**	
	• (4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-al **[44]**	
	• 8,11,13-cleistanthatrien-7-one-17-oic acid **[45]**	Pinto *et al*., 1995
	• 8,11,13-cleistanthatrien-7-one-19-oic acid **[46]**	
	• 8,11,13-cleistanthatrien-17,19-dioic acid **[47]**	

Sources	Chemical Compounds	References
• *V. nivea* L.B. Smith & Ayensu (stem, roots and leaves)	• 11-hydroxycleistantha-8,11,13-trien-7-one **[48]** • 7,11-diketo-14α-hydroxy-cleistantha-8,12-diene **[49]**	Pinto and Macaira, 1988
• *V. compacta* Martius ex. Schultz (stem, roots and leaves)	• 8,11,13-cleistanthatrien-7-one-19,20β-olide **[50]**	Riehl and Pinto, 2000
• *V. declinans* Goethart & Henrard (stem, roots and leaves)	• 7,16-epoxy-20-nor-5,7,9,11,13-cleistanthapentaen-3-one **[51]** • 7,16-epoxy-20-nor-1,5,7,9,11, 13-cleistanthahexaen-3-one **[52]** • 20-hydroxycleistantha-8,11,13-trien-7-one **[53]** • 20-carboxaldehyde-cleistantha-8,11,13-trien-7-one **[54]** • 7,16-epoxy-20-nor-5,7,9,11,13-cleistanthapentaene **[55]** •(5*S*,10*S*)-8,11,13-cleistanthatrien-7-one **[56]** • (5*S*,7*S*,10*R*)- 7α,16,7β,20-diepoxycleistantha-1,8,11,13-tetraen-3-one **[57]** • (5*S*,7*S*,10*R*)- 7α,16,7β,20-diepoxycleistantha-8,11,13-trien-3-one **[58]** • 8,11,13-cleistanthatriene **[59]** • 6,8,11,13-cleistanthatetraene **[60]** • (5*S*,7*S*,10*S*)-7β-hydroxy-8,11,13-cleistanthatriene **[61]**	Pinto *et al*., 1979 Pinto *et al*., 1991 Pinto *et al*., 1992

Sources	Chemical Compounds	References
	• (3S,5S,7*S*,10*R*)-3β-hydroxy-7α,16,7β,20-diepoxycleistantha-8,11,13-triene **[62]**	Pinto *et al*., 1992
• *V. phalocarpa* Pohl (stem, roots and leaves)	• 20-hydroxycleistantha-8,11,13-trien-7-one **[53]** • 20-carboxaldehyde-cleistantha-8,11,13-trien-7-one **[54]**	Pinto *et al*., 1991
• *V. pusilla* Pohl (stem, roots and leaves)	• 20-hydroxycleistantha-8,11,13-trien-7-one **[53]**	Pinto *et al*., 1991
• *V. stipitata* L.B.Smith et Ayensu (stem, roots and leaves)	• 7,16-epoxy-20-nor-5,7,9,11,13-cleistanthapentaen-3-one **[51]**	Pinto *et al*., 1979
Family Compositae • *Brickellia eupatoriedes* (L.) Shinner (roots)	• 15,16-epoxycleistanth-12-en-11-one **[63]** • 15,16-epoxy-11-oxo-cleistanth-12-en-17-al **[64]** • 17-acetoxy-15,16-epoxy cleistanth-12-en-11-one **[65]** • 3α-angeloyloxy-15,16-epoxy cleistanth-12-en-11-one **[66]** • 17-acetoxy-14β-hydroxy-15,16-epoxycleistanth-12-en-11-one **[67]** • 17-acetoxy-3α- angeloyloxy – 15,16-epoxycleistanth-12-en-11-one **[68]** • 11-oxo-8,9,15,16-diepoxy cleistanth-12-en-17-al **[69]**	Bohlmann *et al*., 1982

Sources	Chemical Compounds	References
	• 17-acetoxy-15,16-epoxy isocleistanth-12-en-11-one **[70]** • 17-acetoxy-3,4,15,16-diepoxy isocleistanth-12-en-11-one **[71]** • 14β,15β-dihydroxy-16,17-oxido cleistanth-12-en-11-one **[72]**	Bohlmann *et al*., 1982
Family Euphorbiaceae • *Croton sonderianus* Muell. Arg. (heartwood)	• sonderianol **[73]** • 3,4-seco-sonderianol **[74]**	Craveiro and Silveira, 1982
Family Potamogetonaceae • *Amphibolis antartica* Aschers. (leaves)	• (5α,8β,9α,10β)-4,4,10-trimethyl phenanthrene **[75]**	Dunlop, 1985
Family Lamiaceae • *Pogostemon auricularis* Hassk (whole plant)	• cleistanth-13,15-dien-18-oic acid **[76]** • 7-hydroxycleistanth-13,15-dien-18-oic acid **[77]** • 7-acetoxycleistanth-13,15-dien-18-oic acid **[78]**	Hussaini, 1988
Fungus • *Zythiostroma* sp. (liquid cultures)	• zythiostromic acid A **[79]** • zythiostromic acid B **[80]** • zythiostromolid **[81]**	Ayer and Khan, 1996



veadeirol **[40]** : R_1=CH_2OH, R_2= CH_3

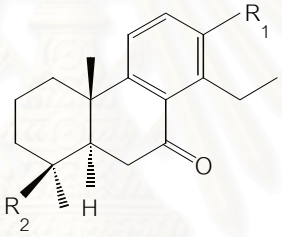
veadeiric acid **[41]** : R_1=COOH, R_2= CH_3

(4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-ol **[42]** : R_1=CH_3, R_2= CH_2OH

(4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-oic acid **[43]** : R_1=CH_3, R_2= COOH

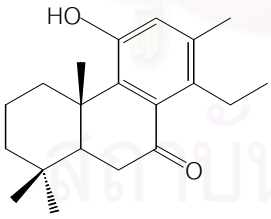
(4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-al **[44]** : R_1=CH_3, R_2= CHO

8,11,13-cleistanthatrien-17,19-dioic acid **[47]** : R_1=COOH, R_2= COOH

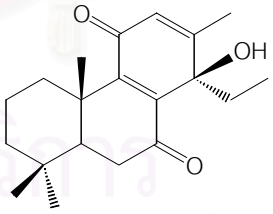


8,11,13-cleistanthatrien-7-one-17-oic acid **[45]** : R_1=COOH, R_2= CH_3

8,11,13-cleistanthatrien-7-one-19-oic acid **[46]** : R_1=CH_3, R_2= COOH

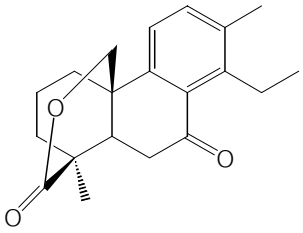


11-hydroxycleistantha-8,11,13-trien-7-one **[48]**

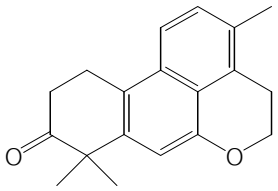


7,11-diketo-14α-hydroxy-cleistantha-8,12-diene **[49]**

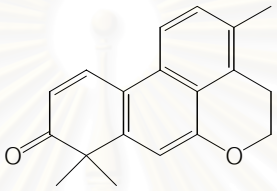
Figure 4 : Structures of cleistanthane diterpenes.



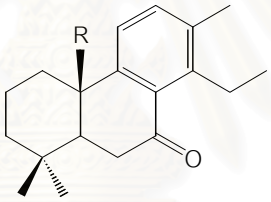
8,11,13-cleistanthatrien-7-one-19,20β-olide **[50]**



7,16-epoxy-20-nor-5,7,9,11,13-cleistanthapentaen-3-one **[51]**

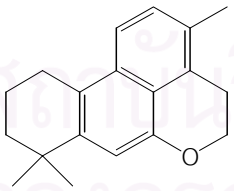


7,16-epoxy-20-nor-1,5,7,9,11,13-cleistanthahexaen-3-one **[52]**

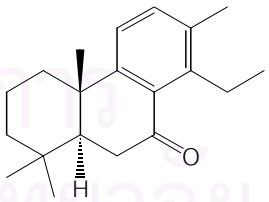


20-hydroxycleistantha-8,11,13-trien-7-one **[53]** : R = CH_2OH

20-carboxaldehyde-cleistantha-8,11,13- trien-7-one **[54]** : R = CHO

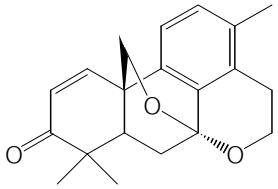


7,16-epoxy-20-nor-5,7,9,11,13-cleistanthapentaene **[55]**

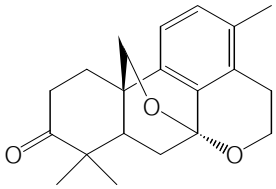


(5*S*,10*S*)-8,11,13-cleistanthatrien-7-one **[56]**

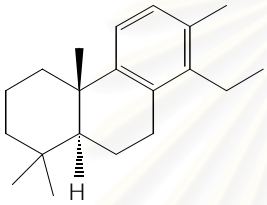
Figure 4 : Structures of cleistanthane diterpenes (continue).



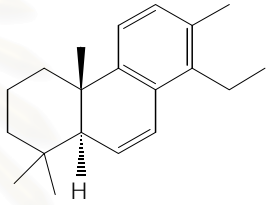
(5*S*,7*S*,10*R*)- 7α,16,7β,20-diepoxycleistantha -1,8,11,13-tetraen-3-one **[57]**



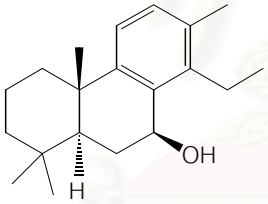
(5*S*,7*S*,10*R*)- 7α,16,7β,20-diepoxycleistantha-8,11,13-trien-3-one **[58]**



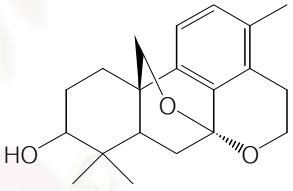
8,11,13-cleistanthatriene **[59]**



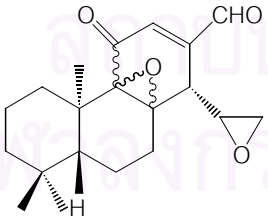
6,8,11,13-cleistanthatetraene **[60]**



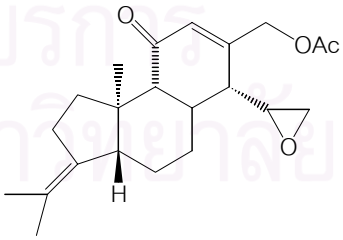
(5*S*,7*S*,10*S*)-7β-hydroxy-8,11,13-cleistanthatriene **[61]**



(3S,5S,7*S*,10*R*)-3β-hydroxy-7α,16,7β,20-diepoxycleistantha- 8,11,13-triene **[62]**

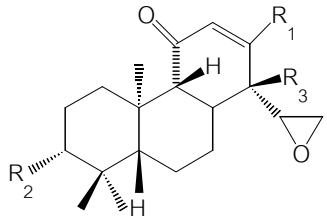


11-oxo-8,9,15,16-diepoxy cleistanth-12-en-17-al **[69]**



17-acetoxy-15,16-epoxy isocleistanth-12-en-11-one **[70]**

Figure 4 : Structures of cleistanthane diterpenes (continue).



15,16-epoxycleistanth-12-en-11-one **[63]** : R_1= CH_3, R_2= H, R_3= H

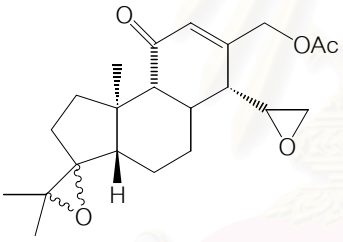
15,16-epoxy-11-oxo-cleistanth-12-en-17-al **[64]** : R_1= CHO, R_2= H, R_3= H

17-acetoxy-15,16-epoxycleistanth-12-en-11-one **[65]** : R_1= CH_2OAc, R_2= H, R_3= H

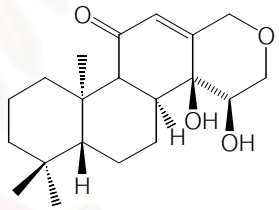
3α-angeloyloxy-15,16-epoxycleistanth-12-en-11-one **[66]** : R_1= CH_3, R_2= OAng, R_3= H

17-acetoxy-14β-hydroxy-15,16-epoxycleistanth-12-en-11-one **[67]** : R_1= CH_2OAc, R_2= H, R_3= OH

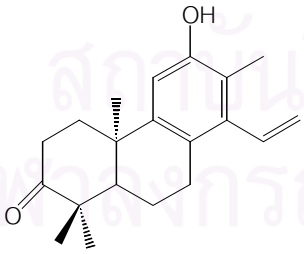
17-acetoxy-3α- angeloyloxy–15,16-epoxycleistanth-12-en-11-one **[68]** : R_1= CH_2OAc, R_2= OAng, R_3= H



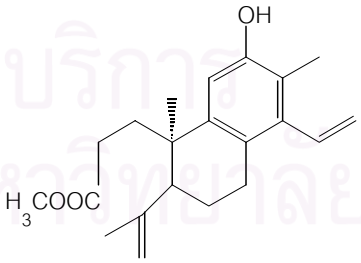
17-acetoxy-3,4,15,16-diepoxy isocleistanth-12-en-11-one **[71]**



14β,15β-dihydroxy-16,17-oxido cleistanth-12-en-11-one **[72]**

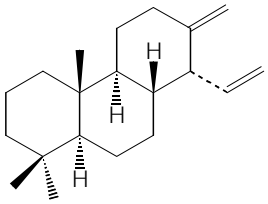


sonderianol **[73]**

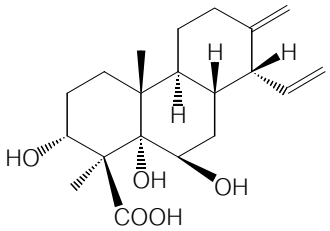


3,4-seco-sonderianol **[74]**

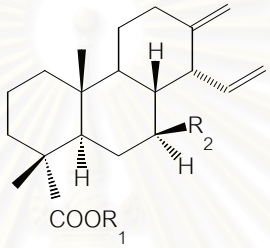
Figure 4 : Structures of cleistanthane diterpenes (continue).



(5α,8β,9α,10β)-4,4,10-trimethyl phenanthrene **[75]**



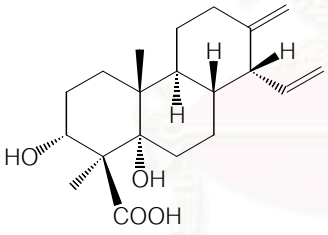
zythiostromic acid A **[79]**



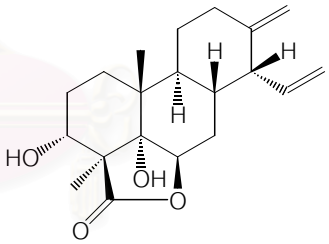
cleistanth-13,15-dien-18-oic-acid **[76]** : R_1= H, R_2= H

7-hydroxycleistanth-13,15-dien-18-oic-acid **[77]** : R_1= H, R_2= OH

7-acetoxycleistanth-13,15-dien-18-oic-acid **[78]** : R_1= H, R_2= OAc



zythiostromic acid B **[80]**



zythiostromolid **[81]**

Figure 4 : Structures of cleistanthane diterpenes (continue).

4. Clerodane diterpenes

Up to the present, over a thousand of diterpenoids with the clerodane carbon skeleton have been isolated. The first member of the clerodane series is clerodin **[82]**, isolated from *Clerodendron infortunatum* Linn. (Barton *et al*., 1961). The vast majority of clerodanes have been isolated from dicotyledonous plants, only a limited range of these compounds can be produced from monocotyledonous species, fungi, and bacteria (Merritt and Ley, 1992). From previous studies, various biological activities of these compounds have been reported, as described below.

4.1. Clerodane diterpenes with insect antifeedant activity

The clerodane diterpenes, especially those obtained from plants of the family Verbenaceae and Labiatae, are best known for their insect antifeedant properties, as listed in **Table 4**.

Table 4. Distribution of natural insect antifeeding clerodane diterpenoids.

Plants	Chemical Compounds	References
Family Verbenaceae • *Clerodendron infortunatum* L. (leaves and twigs)	• clerodin **[82]**	Barton *et al*., 1961
• *C. colebrookianum* Walp. (roots)	• clerodin **[82]**	Joshi *et al*., 1979
• *C. phlomoides* Willd.	• clerodin **[82]** • clerodendrin A **[83]**	Joshi *et al*., 1979
• *C. tricotomum* Thunb. (leaves)	• clerodendrin A **[83]** • clerodendrin B **[84]**	Kato *et al*., 1972
• *C. cryptophyllum*	• clerodendrin A **[83]**	Merritt and Ley, 1992
• *C. fragrans* Willd.	• 3-epicaryoptin **[85]**	Merritt and Ley, 1992
• *C. calamitosum* L. (leaves)	• 3-epicaryoptin **[85]**	Hosozawa *et al*., 1974a
• *C. inerme* Gaertn.	• 3-epicaryoptin **[85]**	Merritt and Ley, 1992

Plants	Chemical Compounds	References
• *Caryopteris divaricata* Maxim (leaves and stems)	• clerodin [82] • 3-epicaryoptin [85] • dihydroclerodin-I [86] • caryoptin [87] • dihydrocaryoptin [88] • caryoptinol [89] • dihydrocaryoptinol [90]	Hosozawa *et al*., 1973 Merritt and Ley, 1992 Hosozawa *et al*., 1973 Hosozawa *et al*., 1974b
Family Labiatae • *Ajuga remota* Benth. (leaves)	• ajugarin I [91] • ajugarin II [92] • ajugarin III [93] • clerodin [82]	Camps and Coll, 1993
• *A. iva* Schreber (whole plants)	• ivain I [94] • ivain II [95] • ivain III [96] • ivain IV [97]	Camps *et al*., 1982
• *A. reptan* L. (whole plants)	• ajugareptansin [98] • 14,15-dehydro ajugareptansin [99]	Camps and Coll, 1993 Bremner *et al*., 1997
• *A. chamaepitys* Schreber (whole plants)	• ajugapitin [100] • dihydroajugapitin [101]	Camps and Coll, 1993 Hernandez *et al*., 1982
• *A. chamaepitys* var. pseudochia (whole plants)	• ajugapitin [100] • dihydroajugapitin [101]	Camps and Coll, 1993
• *A. pseudoiva* (L.) Schreber (whole plants)	• acetylivain I [102] • dihydroajugapitin [101]	Camps *et al*., 1984
• *A. decumbens* Thunb. (whole plants)	• ajugacumbin A [103] • ajugacumbin B [104] • ajugacumbin C [105]	Zhi-da *et al*., 1989
• *Teucrium turredanum*	• eriocephalin [110]	Merritt and Ley, 1992

Plants	Chemical Compounds	References
• *Teucrium montanum* L. sub *skorpilii*	• teucjaponin A **[108]**	Simmonds *et al*., 1989
• *T. japonicum* Houtt. (aerial parts)	• teucjaponin A **[108]** • teucjaponin B **[109]**	Miyase *et al*., 1981 Simmonds *et al*., 1989
• *T. polium* L. sub *capitatum* (L.) (aerial parts)	• teucjaponin B **[109]**	Fernandez *et al*., 1986
• *T. polium* L. sub *vincentinum* (Rouy) D. Wood (aerial parts)	• eriocephalin **[110]**	Simmonds *et al*., 1989
• *T. massiliense* L.	• teucjaponin A **[108]** • 6,19-diacetyl teumassilin **[111]**	Simmonds *et al*., 1989
• *T. creticum* L. (aerial parts)	• 6,19-diacetyl teumassilin **[111]** • teucjaponin B **[109]**	Simmonds *et al*., 1989 Savona *et al*., 1987
• *T. africanum* Thunb. (leaves and stems)	• tafricanin A **[106]** • tafricanin B **[107]**	Hanson *et al*., 1982
• *T. eriocephalum* Willk. (aerial parts)	• eriocephalin **[110]**	Fayos *et al*., 1979
• *T. chartaginense* Lange sub *homotrichum* Font Quer (aerial parts)	• eriocephalin **[110]**	Bruno *et al*., 1985
• *T. lanigerum* Lag. (aerial parts)	• eriocephalin **[110]**	Fernandez-Gadea *et al*., 1984
• *T. flavum* L. sub *glaucum* (Jordan & Fourr.) Ronniger (aerial parts)	• 12-epiteucvin **[112]**	Savona *et al*., 1984
• *Scutellaria woronowii* Juz. (whole plants)	• jodrellin A **[113]** • jodrellin B **[114]**	Anderson *et al*., 1989
• *S. violacea* Heyne ex Wall. (whole plants)	• clerodin **[82]** • jodrellin A **[113]**	Cole *et al*., 1991

Plants	Chemical Compounds	References
Family Caesalpiniaceae		
• *Hardwickia pinnata* Roxb. (oleoresin)	• kolavenol **[115]** • hardwickiic acid **[10]**	Misra *et al*., 1979
Family Aristolochiaceae		
• *Aristolochia galeata* Mart. et Zucc. (roots)	• kolavenol **[115]**	Lopes and Bolzani, 1988
Family Compositae		
• *Solidago altissima* L. (rhizomes)	• kolavenol **[115]**	Merritt and Ley, 1992
• *Baccharis macraei* Hook. et Arn. (aerial parts)	• hardwickiic acid **[10]**	Gambaro *et al*., 1986
• *Melampodium divaricatum* DC. (leaves)	• kolavenol **[115]**	Hubert and Wiemer, 1985
• *Plazia daphnoides* Wedd. (aerial parts)	• kolavenol **[115]**	Zdero *et al*., 1988
• *Grangea maderaspatana* Poir. (aerial parts)	• hardwickiic acid **[10]**	Singh *et al*., 1988
Family Euphorbiaceae		
• *Croton californicus* Muell. Arg. (whole plants)	• hardwickiic acid **[10]**	Merritt and Ley, 1992
• *C. oblongifolius* Roxb. (stem bark, wood)	• hardwickiic acid **[10]** • 11-dehydro-(-)-hardwickiic acid **[11]** • (-)-20-benzyloxy hardwickiic acid **[12]** • methyl-15,16-epoxy-12-oxo-3,13(16),14-clerodatriene-20,19-olide-17-oate **[13]**	Aiyar and Seshadri, 1972a Aiyar and Seshadri, 1972b Baiagern, 1999 Tanwattanakun, 1999
• *C. aromaticus* L. (roots)	• hardwickiic acid **[10]**	Bandara *et al*., 1987

Plants	Chemical Compounds	References
Family Annonaceae • *Polyalthia longifolia* Benth. et **Hook. f. ex Hook. f. (leaves)**	• 16α-hydroxy-3,13(14)*Z*-dien-15,16-olide **[116]** • 16-oxocleroda-3,13(14)*E*-dien-15-oic acid **[117]**	Phadnis *et al*., 1988

4.2. Clerodane diterpenes with cytotoxic activity

Casearins A-R **[118-135]**, isolated from the leaves of *Casearia sylvestris* Sw. (Flacourtiaceae), have shown cytotoxic activity against cloned chinese hamster V-79 cells *in vitro* (Morita *et al*., 1991)

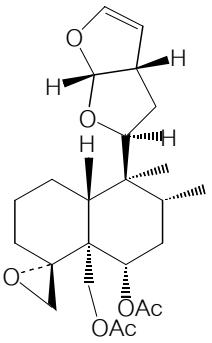
Table 5 Casearins and their cytotoxic activities against V-79 cells.

casearins	IC_{50} (μmol/l)
casearin A **[118]**	1.0
casearin B **[119]**	8.5
casearin C **[120]**	0.77
casearin D **[121]**	1.8
casearin E **[122]**	4.7
casearin F **[123]**	29
casearin G **[124]**	0.17
casearin H **[125]**	0.37
casearin I **[126]**	0.51
casearin J **[127]**	1.1
casearin K **[128]**	0.52
casearin L **[129]**	1.6
casearin M **[130]**	1.8
casearin N **[131]**	5.9
casearin O **[132]**	6.0
casearin P **[133]**	7.8
casearin Q **[134]**	4.3
casearin R **[135]**	5.4

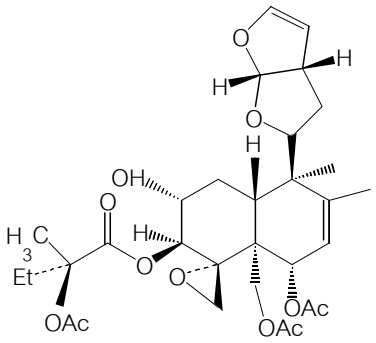
In addition, articulin acetate **[136]**, isolated from aerial parts of *Baccharis gaudichaudiana* DC. (Compositae), exhibited significant cytotoxic activity against P-388 (murine lymphoid neoplasm) cell, with an ED_{50} value of 1.7 μg/ml (Fullas *et al*., 1994)

4.3. Clerodane diterpenes with anti-peptic ulcer activity

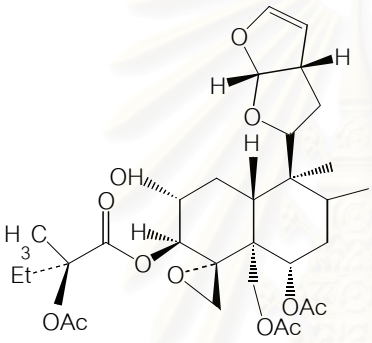
From stems of a well-known Thai medicinal plant, *Croton sublyratus* Kurz (Euphorbiaceae), many clerodane diterpenes have been isolated, some of which have shown significant anti-peptic ulcer activity. As reported in 1980, plaunols B-E **[137-140]** inhibited Shay ulcers in rats with 55, 36, 44, 52% inhibition respectively at a dose of 3 mg./kg., and 85, 88, 61, 82% inhibition respectively at a dose of 10 mg./kg. (Kitazawa *et al*., 1980)



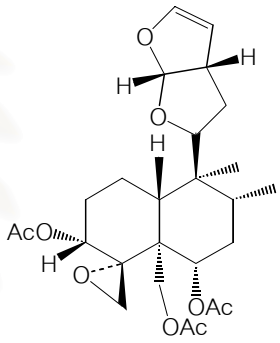
clerodin **[82]**



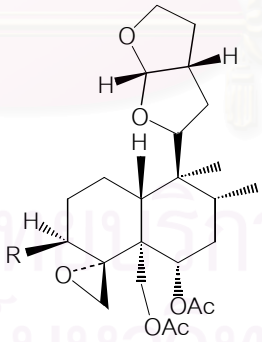
clerodendrin A **[83]**



clerodendrin B **[84]**



3-epicaryoptin **[85]**

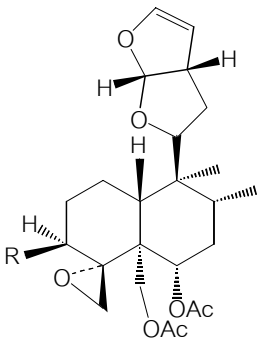


dihydroclerodin-I **[86]** : R = H

dihydrocaryoptin **[88]** : R = OAc

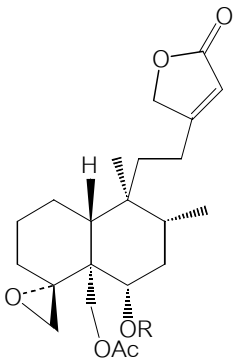
dihydrocaryoptinol **[90]** : R = OH

Figure 5 : Structures of clerodane diterpenes



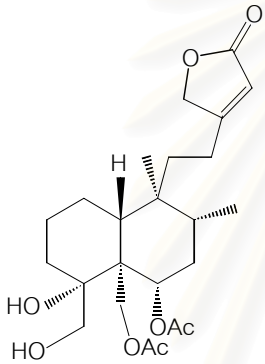
caryoptin **[87]** : R = OAc

caryoptinol **[89]** : R = OH

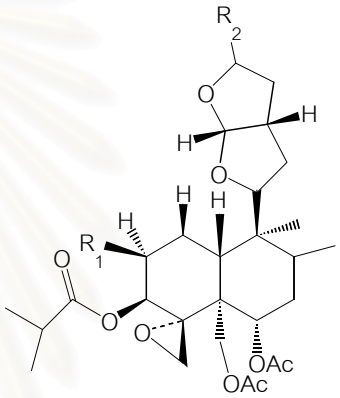


ajugarin I **[91]** : R = Ac

ajugarin II **[92]** : R = H



ajugarin III **[93]**

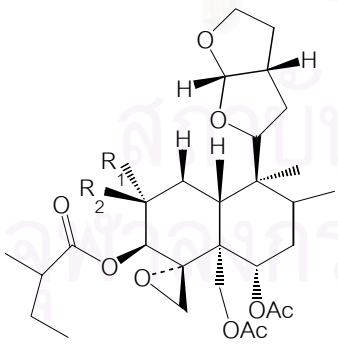


ivain I **[94]** : R_1 = OH, R_2 = H

ivain II **[95]** : R_1 = H, R_2 = H

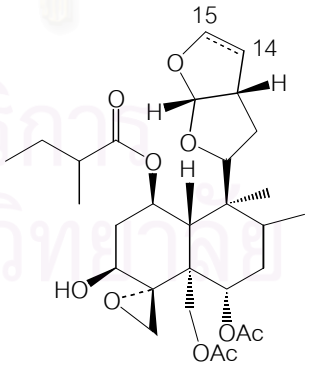
ivain III **[96]** : R_1 = OH, R_2 = OEt

acetylivain I **[102]** : R_1 = OAc, R_2 = H



ivain IV **[97]** : R_1 = H, R_2 = OH

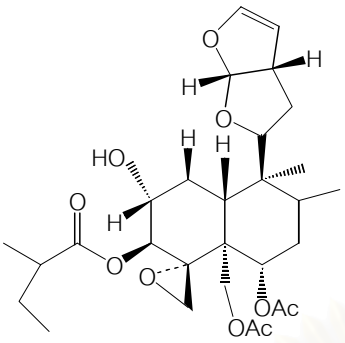
dihydroajugapitin **[101]** : R_1 = OH, R_2 = H



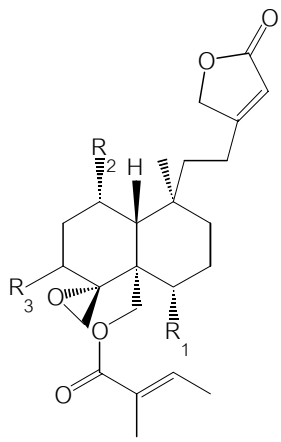
ajugareptansin **[98]** : 14-15 = single

dehydroajugareptansin **[99]** : 14-15 = double

Figure 5 : Structures of clerodane diterpenes (continue)



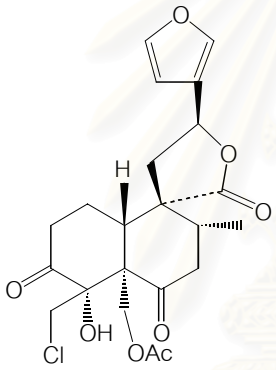
ajugapitin **[100]**



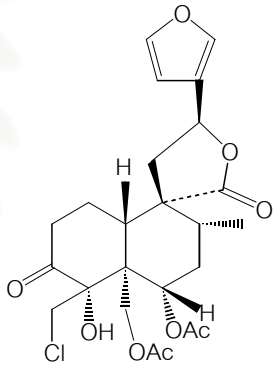
ajugacumbin A **[103]** : R_1 = OAc, R_2 = R_3 = H

ajugacumbin B **[104]** : R_1 = OH, R_2 = R_3 = H

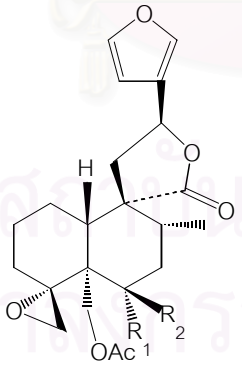
ajugacumbin C **[105]** : R_1 = R_2 = R_3 = OAc



tafricanin A **[106]**

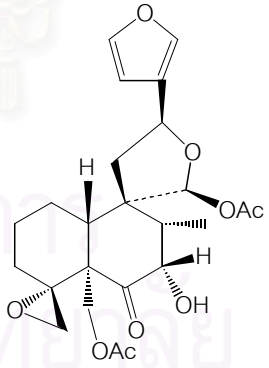


tafricanin B **[107]**



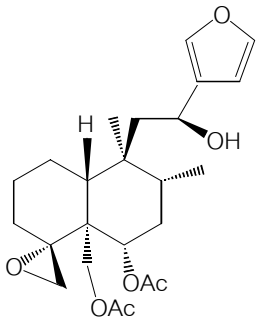
teucjaponin A **[108]** : R_1 = H, R_2 = OH

teucjaponin B **[109]** : R_1 = OH, R_2 = H

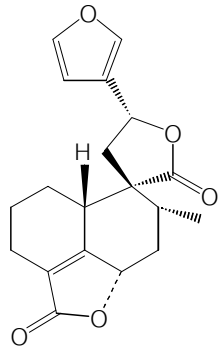


eriocephalin **[110]**

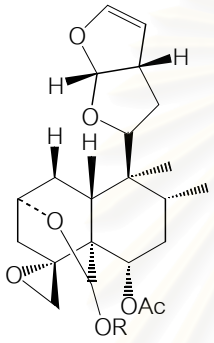
Figure 5 : Structures of clerodane diterpenes (continue)



6,19-diacetylteumassilin **[111]**



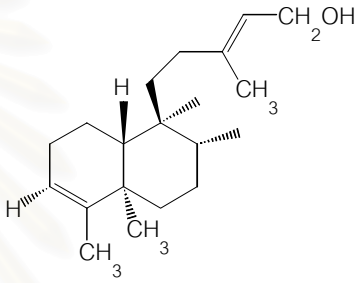
12-epiteucvin **[112]**



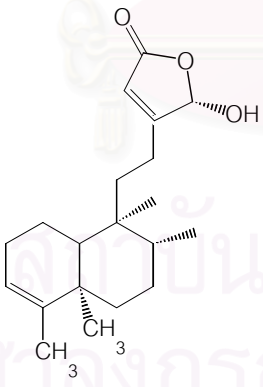
jodrellin A **[113]** : R = Ac

jodrellin B **[114]** : R = CO ^{i}Pr

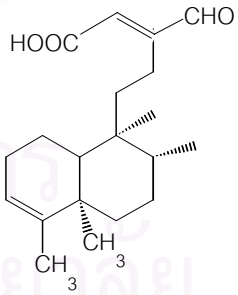
(CO ^{i}Pr = isobutyrate ester)



kolavenol **[115]**

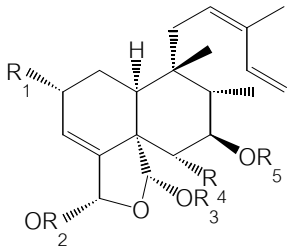


16α-hydroxy-3,13(14)*Z*-dien-15,16-olide **[116]**



16-oxo-cleroda-3,13(14)*E* -dien-15-oic acid **[117]**

Figure 5 : Structures of clerodane diterpenes (continue)



casearin A **[118]** : R_1 = OMe, R_2 = Ac, R_3 = Ac, R_4 = OH, R_5 = Bu

casearin B **[119]** : R_1 = OMe, R_2 = Ac, R_3 = Ac, R_4 = OAc, R_5 = Bu

casearin C **[120]** : R_1 = OH, R_2 = Ac, R_3 = Ac, R_4 = OAc, R_5 = Dc

casearin D **[121]** : R_1 = OH, R_2 = Bu, R_3 = Ac, R_4 = OH, R_5 = Bu

casearin E **[122]** : R_1 = OH, R_2 = Et, R_3 = Ac, R_4 = OH, R_5 = Dc

casearin F **[123]** : R_1 = OH, R_2 = Et, R_3 = Ac, R_4 = OH, R_5 = Bu

casearin G **[124]** : R_1 = OMe, R_2 = Ac, R_3 = Ac, R_4 = H, R_5 = Bu

casearin H **[125]** : R_1 = OH, R_2 = Ac, R_3 = Ac, R_4 = H, R_5 = Bu

casearin I **[126]** : R_1 = OH, R_2 = Ac, R_3 = Bu, R_4 = H, R_5 = Bu

casearin J **[127]** : R_1 = OMe, R_2 = Bu, R_3 = Ac, R_4 = OH, R_5 = Bu

casearin K **[128]** : R_1 = OAc, R_2 = Ac, R_3 = Ac, R_4 = OH, R_5 = Bu

casearin L **[129]** : R_1 = OMe, R_2 = Bu, R_3 = Ac, R_4 = OAc, R_5 = H

casearin M **[130]** : R_1 = OH, R_2 = Bu, R_3 = Bu, R_4 = OAc, R_5 = H

casearin N **[131]** : R_1 = OMe, R_2 = Ac, R_3 = Bu, R_4 = OAc, R_5 = Bu

casearin O **[132]** : R_1 = OMe, R_2 = Bu, R_3 = Ac, R_4 = OAc, R_5 = Bu

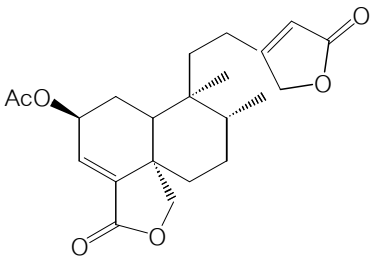
casearin P **[133]** : R_1 = OMe, R_2 = Ac, R_3 = Ac, R_4 = OAc, R_5 = Ac

casearin Q **[134]** : R_1 = OH, R_2 = Ac, R_3 = Ac, R_4 = OAc, R_5 = Bu

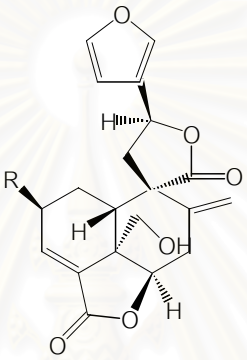
casearin R **[135]** : R_1 = =O, R_2 = Ac, R_3 = Ac, R_4 = OH, R_5 = Bu

(Bu = butylate, Dc = decanoate)

Figure 5 : Structures of clerodane diterpenes (continue)

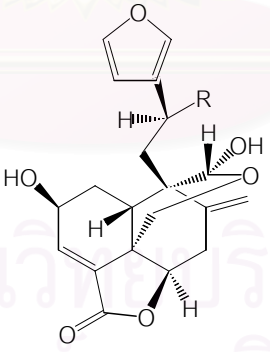


articulin acetate **[136]**



plaunol B **[137]** : R = H

plaunol C **[138]** : R = OH



plaunol D **[139]** : R = OH

plaunol E **[140]** : R = OAc

Figure 5 : Structures of clerodane diterpenes (continue)

CHAPTER III
EXPERIMENTAL

1. Source of Plant Material

The stem bark of *Croton oblongifolius* Roxb. was collected from amphur Dansai, Loei province, Thailand (N 17° 15' 17.0" E 101° 09' 21.3") in October 2000. The plant material was authenticated by comparison with the voucher specimen no. BKF 084729, deposited in the herbarium of the Royal Forest Department of Thailand.

2. General Techniques

2.1 Analytical Thin Layer Chromatography (TLC)

Technique : One dimension, ascending

Adsorbent : Silica gel 60 F_{254} precoated plate (E. Merck)

Layer thickness : 0.2 mm.

Developing distance : 6.0 cm.

Temperature : Laboratory room temperature (30-35°C)

Detection : 1. Ultraviolet light at wavelength of 254 nm

2. Iodine vapour

3. Anisaldehyde-H_2SO_4 reagent

2.2 Column Chromatography

2.2.1. <u>Conventional Column Chromatography</u>

Adsorbent : 1. Silica gel 60 (No. 7734) (E. Merck)
particle size 0.063-0.200 nm (70-230 mesh ASTM)

2. Silica gel 60 (No. 9385) (E. Merck)
particle size 0.040-0.063 nm (230-400 mesh ASTM)

Packing method : Wet packing

Sample loading : The sample was dissolved in a small amount of eluant, then applied gently on top of the column.

Detection : Fractions were examined using TLC technique. In order to detect the compounds in each of the fractions, the TLC plate was observed under UV light at wavelength of 254 nm, and then exposed to iodine vapour and anisaldehyde- H_2SO_4 reagent respectively.

2.2.2. Flash Column Chromatography

Adsorbent : 1. Silica gel 60 (No. 7734) (E. Merck)
particle size 0.063-0.200 nm (70-230 mesh ASTM)
2. Silica gel 60 (No. 9385) (E. Merck)
particle size 0.040-0.063 nm (230-400 mesh ASTM)

Packing method : Wet packing

Sample loading : The sample was dissolved in a small amount of eluant, then applied gently on top of the column.

Detection : Fractions were examined in the same manner as described in section 2.2.1.

2.3. Spectroscopic Techniques

2.3.1. Ultraviolet (UV) Absorption Spectra

UV spectra were obtained from a Shimadzu UV-160A UV/VIS spectrophotometer at the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2.3.2. Infrared (IR) Absorption Spectra

IR spectra were recorded on a Perkin-Elmer FT-IR 1760X spectrometer at the Scientific and Technological Research Equipment Center, Chulalongkorn University.

2.3.3. Mass Spectra (MS)

Electron Impact Mass Spectrum (EIMS) of compound COL-1 was obtained on a Micromass Platform II mass spectrometer at 70 eV. at the Department of Medicinal Organic Chemistry, Faculty of Pharmaceutical Sciences, Chiba University.

EIMS of compound COL-2 was obtained on a FISONS VG TRIO 2000 mass spectrometer at 70 eV. at the Department of Chemistry, Faculty of Sciences, Chulalongkorn University.

2.3.4. Nuclear Magnetic Resonance (NMR) Spectra

^{1}H NMR spectrum of compound COL-2 were recorded on a JEOL JMN-A500 (Alpha series) 500 MHz NMR Spectrometer at the Scientific and Technological Research Equipment Center, Chulalongkorn University.

^{1}H NMR spectra of compound COL-1 and COL-3, and ^{13}C NMR spectra of the three compounds were recorded at 300 and 75 MHz respectively, on a Bruker ADVANCE DPX-300 FT-NMR spectrometer at the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Deuterated chloroform was used as the NMR solvent throughout this study. Spectral data were reported in ppm scale using the solvent chemical shift as the reference frequency.

2.4. Physical Property Measurement Apparatus

2.4.1. Melting Points

Melting points were determined on a Gallenkamp Melting Point Apparatus at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2.4.2. Optical Rotations

Optical rotations were measured on a Perkin-Elmer Polarimeter model 341 at the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2.4.3. Elemental Components

Elemental components were analyzed on a Perkin-Elmer PE2400 Series II CHNS/O Analyzer (option CHN) at the Scientific and Technological Research Equipment Center, Chulalongkorn University, by the method of pyrolysis in high-purity oxygen (static-state oxidation) and quantitatively detected by thermal conductivity detector.

2.5. Solvents

Organic solvents used in extraction were of commercial grade. In column chromatography, solvents were redistilled prior to use.

3. Extraction and Isolation

3.1. Extraction of the stem bark of *Croton oblongifolius* Roxb.

The dried-powdered stem bark of *Croton oblongifolius* (4.5 kg.) was macerated twice with hexane (2×6L.) and then twice with ethyl acetate (2×6L.) successively, each time for three days. The obtained extract was evaporated under reduced pressure at a temperature of approximately 40°C to give 380 g of hexane extract (8.44%of dry weight of the stem bark), and 670 g of ethyl acetate extract (14.89% of dry weight of the stem bark).

3.2. Isolation

3.2.1. Isolation of compound COL-1

The crude hexane extract (380 g) was chromatographed on a conventional silica gel column (silica gel 60, No. 7734, 500g), eluted with hexane-ethyl acetate mixtures of increasing polarity to yield 85 fractions of 300 ml Each. Fractions showing similar TLC profiles in hexane-ethyl acetate, 3:2 were combined to give a total of eight fractions, as shown in **Table 6**. A white amorphous crystal of compound COL-1 (0.2074 g, 0.055% of hexane extract) was obtained from fraction F.

Table 6. Combination of fractions from conventional column chromatography of the crude hexane extract (380 g)

Eluants	Fraction code	Number of fraction	Weight (g)
100% hexane	A	1-4	20.71
5% EtOAc in hexane	B	5-17	135.12
10% EtOAc in hexane	C	18-49	36.52
15% EtOAc in hexane	D	50-62	21.11
20% EtOAc in hexane	E	63-72	18.54
25% EtOAc in hexane	F	73-77	6.46
30% EtOAc in hexane	G	78-80	6.91
100% EtOAc	H	81-85	82.20

3.2.2. Isolation of compound COL-2

Fraction B (135.12 g) was further chromatographed on a silica gel 60 (No.7734) column and eluted with hexane-ethyl acetate mixtures in a polarity gradient manner. Fractions (approximately 75 ml each) were combined according to their TLC patterns (hexane-ethyl acetate, 3:2) to yield a total of eleven fractions, as shown in **Table 7**.

Table 7. Isolation of fraction B (135.12 g) by column chromatography.

Eluants	Fraction code	Number of fraction	Weight (g)
100% hexane	B1	1-9	2.85
1% EtOAc in hexane	B2	10-16	2.24
2% EtOAc in hexane	B3	17-25	3.39
3% EtOAc in hexane	B4	26-45	3.24
5% EtOAc in hexane	B5	46-55	6.44
8% EtOAc in hexane	B6	56-63	11.08
12% EtOAc in hexane	B7	64-117	25.70
16% EtOAc in hexane	B8	118-127	6.02
20% EtOAc in hexane	B9	128-166	26.80
40% EtOAc in hexane	B10	167-179	14.70
100% EtOAc	B11	180-200	18.94

Fraction B4 (3.24 g) was subjected to flash column chromatography over silica gel 60 (No. 9385) using hexane-ethyl acetate mixtures as a mobile phase. Eluate was collected approximately 50 ml per fraction, and then examined by TLC using hexane-ethyl acetate, 3:1 as a developing solvent. Fractions showing similar TLC patterns were combined to give a total of eight fractions.

Table 8. Isolation of fraction B4 (3.24 g) by flash column chromatography.

Eluants	Fraction code	Number of fraction	Weight (g)
100% hexane	B41	1-5	0.2105
100% hexane	B42	6-13	0.3200
1% EtOAc in hexane	B43	14-19	0.2181
2% EtOAc in hexane	B44	20-53	1.0699
3% EtOAc in hexane	B45	54-57	0.4447
4% EtOAc in hexane	B46	58-67	0.4850
5% EtOAc in hexane	B47	68-99	0.2781
100% EtOAc	B48	100-110	0.2125

Fraction B47 (0.2781 g) was rechromatographed on a flash silica gel column (silica gel 60, No. 9385) using gradient elution technique starting with 100% hexane to 10% ethyl acetate in hexane. Fractions (approximately 50 ml each) were combined according to their TLC patterns (hexane-ethyl acetate, 3:1) to give a total of six fractions. A colorless semi-solid fraction B473 (0.0233 g, 0.006% of hexane extract) gave a single spot on TLC and therefore was coded as compound COL-2.

Table 9. Isolation of fraction B47 (0.2781 g) by flash column chromatography.

Eluants	Fraction code	Number of fraction	Weight (g)
100% hexane	B471	1-29	0.0479
2% EtOAc in hexane	B472	30-44	0.1004
4% EtOAc in hexane	B473	45-54	0.0233
6% EtOAc in hexane	B474	55-67	0.0109
8% EtOAc in hexane	B475	68-73	0.0026
10% EtOAc in hexane	B476	74-90	0.0103

Physical and Spectral Data of the Isolated Compounds

1.Compound COL-1

Compound COL-1 was obtained as white crystal (0.2074 g.)

Elemental Components : C:H:O = 67.72:6.50:25.78

Melting Point: 167-168°C

$[\alpha]^{25}_{D}$: -93° ($CHCl_3$; *c* 0.54)

UV : λ_{max} nm (log ε), in MeOH; **Figure 10**

251 (3.60), 203 (4.30)

IR : ν_{max} cm^{-1}, KBr disc; **Figure 11**

3146, 3000, 2952, 2899, 2867, 2840, 1773, 1729, 1665, 1560, 1509, 875

EIMS : *m/z* (% relative intensity); **Figure 12**

372 [M^+] (16), 341 (7), 263 (15), 95 (100)

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; **Figure 13**

8.00 (1H, s), 7.41 (1H, br.s), 6.72 (1H, t, *J* = 2.4 Hz, partly overlapping), 6.70 (1H, br.s), 4.31 (1H, d, *J* = 8.1 Hz), 3.92 (1H, br.d, *J* = 7.4Hz), 3.60 (3H, s), 3.20 (1H, dd, *J* = 12.6, 4.5 Hz), 3.02 (1H, d, *J* = 17.7 Hz), 2.82 (1H, d, *J* = 18.0 Hz) , 2.70 (1H, br.d, *J* = 12.0 Hz), 2.26 (1H, m, overlapping), 2.20 (1H, m overlapping), 2.02 (1H, m, overlapping), 1.95 (1H, m, overlapping), 1.85 (1H, m), 1.62 (1H, br.d, *J* = 12.3), 1.32 (1H, br.dd, *J* = 13.1, 13.1 Hz), 1.07 (1H, dddd, *J* = 11.1, 11.7, 12.3, 4.5 Hz), 0.79 (3H, s)

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; **Figure 14**

193.6 (s), 173.9 (s), 169.0 (s), 147.0 (d), 144.2 (d), 137.7 (s), 136.2 (d), 128.5 (s), 108.4 (d), 71.3 (t), 51.3 (q), 48.6 (d), 46.6 (d), 46.4 (t), 45.0 (s), 39.5 (s), 33.1(t), 27.2 (t), 22.0, (t), 20.0 (t), 19.1 (q)

2.Compound COL-2

Compound COL-2 was obtained as a colorless semi-solid (0.0233 g.)

$[\alpha]^{25}_{D}$: +21° ($CHCl_3$; *c* 0.49)

UV : λ_{max} nm (log ε), in MeOH; **Figure 20**

204 (3.83)

IR : ν_{max} cm^{-1}, KBr disc; **Figure 21**

3073, 2932, 2872, 1710, 1729, 1649, 1449, 1416, 1395

EIMS : *m/z* (% relative intensity); **Figure 22**

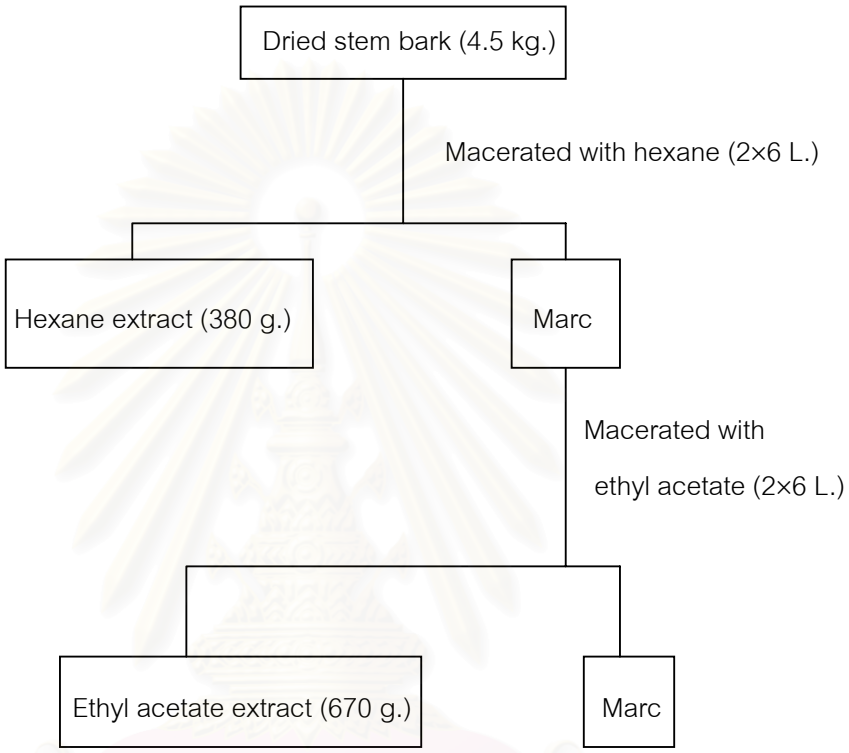
302 [M^+] (16), 287 (9), 259 (20), 201 (69), 145 (83), 91 (98)

^{1}H-NMR : δ ppm, 500 MHz, in $CDCl_3$; **Figure 23**

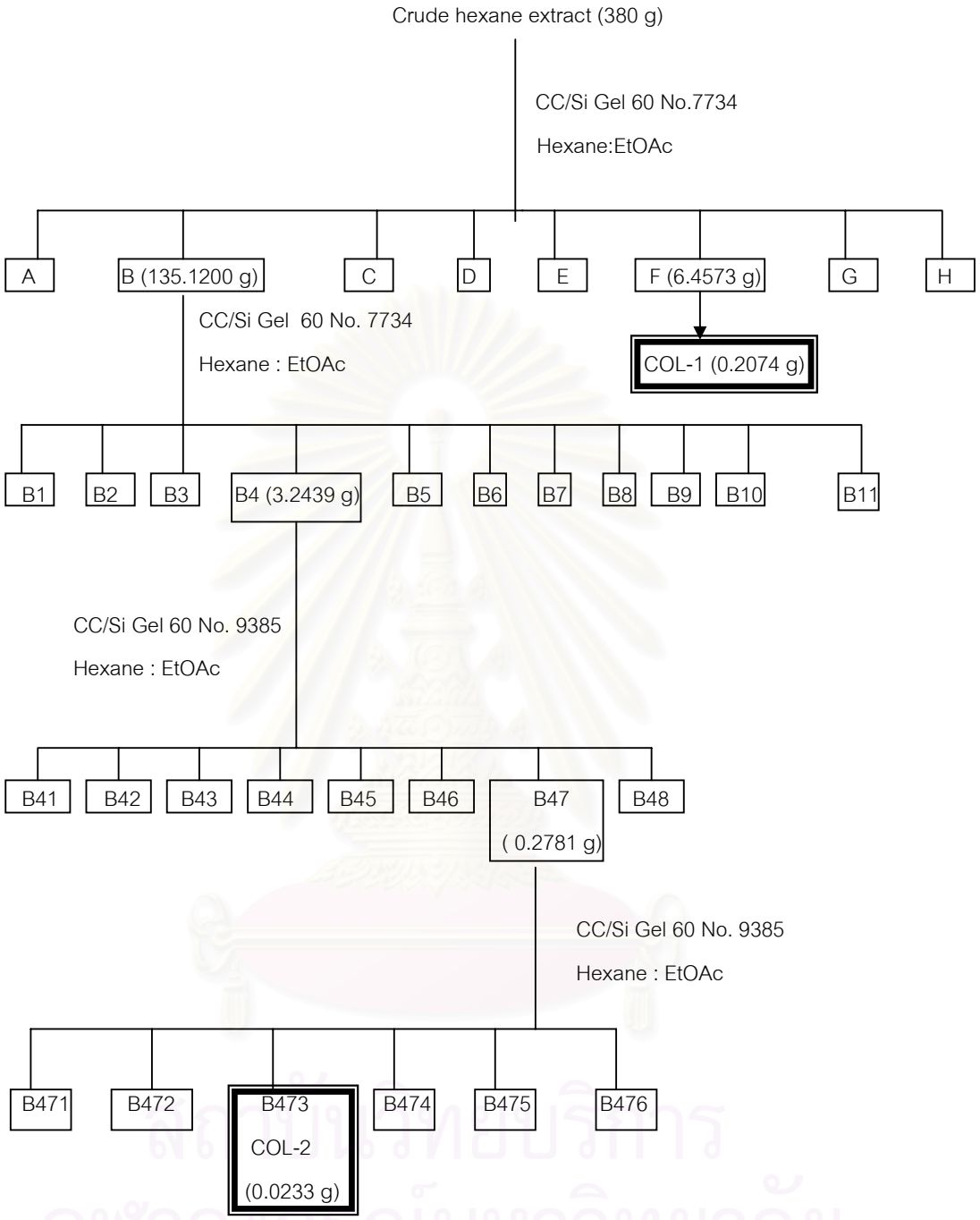
6.00 (1H, ddd, *J* = 16.6, 12.1, 9.3 Hz), 5.03 (1H, dd, *J* = 8.4, 1.7 Hz), 5.00 (1H, br.s.), 4.84 (1H, br.s.), 4.65 (2H, br.s), 4.57 (1H, br.s), 2.81 (1H, dd, *J* = 9.2, 4.6 Hz), 2.40 (1H, ddd, *J* = 13.3, 8.0, 7.2 Hz), 2.27 (1H, ddd, *J* = 13.3, 8.0, 7.2 Hz), 2.17 (2H, m), 1.94 (1H, dd, *J* = 12.7, 2.9 Hz), 1.72 (3H, s), 1.71 (1H, m), 1.68 (1H, m), 1.63 (2H, m), 1.56 (1H, dddd, *J* = 15.6, 12.2, 4.0, 4.0 Hz), 1.44 (1H, m), 1.41 (1H, m), 1.26 (1H, m), 1.23 (1H, m), 1.16 (1H, m), 0.81 (3H, s)

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; **Figure 24**

180.1 (s), 151.9 (s), 147.4 (s), 137.3 (d), 116.1 (t), 113.6 (t), 106.7 (t), 54.6 (d), 50.7 (d), 41.0 (d), 40.3 (d), 38.9 (s), 32.1 (t), 31.4 (t), 31.3 (t), 28.0 (t), 27.6 (t), 27.3 (t), 23.8 (q), 16.7 (q)



Scheme 1. Extraction scheme of the stem bark of *Croton oblongifolius* Roxb.



Scheme 2. Isolation scheme of the hexane extract of *Croton oblongifolius* Roxb.

CHAPTER IV
RESULTS AND DISCUSSION

By means of several chromatographic techniques, two compounds: COL-1 and COL-2, were isolated from crude hexane extract of the stem bark of *Croton oblongifolius* Roxb.

Spectroscopic data (UV-VIS, IR, MS, and NMR) were used to determine the chemical structures of the two compounds. The structures were confirmed by comparative analysis using previous reports as references.

Structure Determination of the Isolated Compounds

1. Structure determination of compound COL-1

Compound COL-1 was obtained as white crystal (0.2074 g.) with a melting point of 167-168°C

The FT-IR spectrum of compound COL-1 (**Figure 11**) displayed bands indicating a furan ring (ν 1560, 1509, 875 cm^{-1}), and three carbonyl groups (ν 1773, 1729, 1665 cm^{-1}).

Table 10. The IR absorption band assignments of compound COL-1

Wave number (cm^{-1})	Tentative assignments
3146	alkene C-H stretch
3000-2840	alkane C-H stretch
1560, 1509, 875	furan ring
1773, 1729, 1665	C=O stretch

The ^{1}H-NMR spectrum (**Figure 13**) of compound COL-1 showed one methyl group attached to a quaternary carbon at δ_H 0.79 (3H, s; H-20), one methoxy group at δ_H 3.60 (3H, s; H-21), and four olefinic protons at δ_H 6.70 (1H, br.s; H-14), 6.72 (1H, t, *J* = 2.4 Hz, partly overlapping; H-3), 7.41 (1H, br.s; H-15), and 8.00 (1H,s; H-16) .

The ^{13}C-NMR spectrum (**Figure 14**) showed twenty-one carbon resonances, six of which are olefinic carbons (δ_c 108.4, 128.5, 136.2, 137.7, 144.2, and 147.0). The presence of two ester carbonyls (δ_c 169.0, 173.9), and one conjugated ketone carbonyl (δ_c 193.6) was also observed.

In a DEPT-90 experiment (**Figure 15**), four sp^2 methine carbon signals (δ_c 108.4, 136.2, 144.2, and 147.0) were shown, together with two saturated methine carbons (δ_c 46.6 and 48.6). In addition, the DEPT-135 spectrum (**Figure 15**) showed six methylene carbon signals at δ_c 20.0, 22.0, 27.2, 33.1, 46.4, and 71.3. The downfield δ_c 71.3 signal implied that this methylene carbon should be attached to an oxygen atom. Two methyl signals were shown at δ_c 19.1 and 51.3. The downfield signal at δ_c 51.3 confirmed the presence of a methoxy group in the molecule. According to the ^{13}C-NMR, DEPT-90, and DEPT-135 data, it was concluded that there were seven quaternary carbons (δ_c 39.5, 45.0, 128.5, 137.7, 169.0, 173.9, and 193.6) in this structure.

In the EI-MS (**Figure 12**), compound COL-1 gave a molecular ion peak $[M]^+$ at *m/z* 372, consistent with the molecular formula $C_{21}H_{24}O_6$. The degree of unsaturation calculated for this molecular formula is ten. The characteristic ion peak at *m/z* 341 indicated the loss of a methoxy group from the molecule.

Several 2D-NMR techniques were then used to assist the interpretation of the structure of this compound. All of the proton-proton spin systems were traced by using data from a COSY-45 experiment (**Figure 17**). Heteronuclear correlation experiments, HMQC (**Figure 16**) and HMBC (**Figure 18**) allowed unambiguous assignment of all ^{1}H and ^{13}C NMR resonances in compound COL-1.

The ^{1}H-NMR spectrum of compound COL-1 showed signals for an α,β-unsaturated-18,19-clerodanolide at δ_H 6.72 (H-3), 3.92 (H-19a) and 4.31 (H-19b). HMBC correlations from H-3 to the carbons at δ_c 45.0 (C-5), and 169.0 (C-18), and from H-19b to the carbons at δ_c 137.7 (C-4), 45.0 (C-5), 33.1 (C-6) and 169.0 (C-18) confirmed the 18,19-olide functionality in compound COL-1. The HMQC spectrum indicated that the protons at δ_H 2.20 and 2.26 were geminal protons (H_2-2). In HMBC data, long-range correlations from these two protons to the carbons at δ_c 20.0 (C-1), 136.2 (C-3), and 46.6 (C-10) were observed, together with correlations from H-19a and H-19b to the carbon at δ_c 46.6 (C-10).

The AB-protons quartet centered at δ_H 2.82 and 3.02 (H_2-11) demonstrated connectivities over two bonds to the carbons at δ_c 39.5 (C-9) and 193.6 (C-12), and over three bonds to the carbons at δ_c 48.6 (C-8), 46.6 (C-10), and 19.1(C-20) in the HMBC spectrum. The last correlation placed the single methyl group resonating at δ_H 0.79 on the carbon at δ_c 39.5 (C-9). The HMBC spectrum further showed a correlation between δ_H 3.60 (H-21) and δ_c 173.9 (C-17). Thus concluded that the methoxy group (δ_H 3.60, δ_c51.3) was a part of a carboxymethyl substituent. The HMBC correlation from δ_H 3.20 (H-8) to the ester carbonyl at δ_c 173.9 (C-17) placed this substituent on δ_c 48.6 (C-8). Long-range correlations from δ_H 1.95 (H-6eq) to the carbons at δ_c 48.6 (C-8) and 22.0 (C-7) were also observed.

Typical low-field signals in the ^{1}H-NMR spectrum of compound COL-1 at δ_H 6.70 (H-14), 7.41 (H-15) and 8.00 (H-16) suggested the presence of a 3-substituted furan ring. The downfield resonance of H-16 revealed that the furan ring was conjugated with a carbonyl group (δ_c 193.6; C-12). The EI-MS ion peak at 95 confirmed the presence of this furan-carbonyl moiety in the structure. The COSY spectrum showed correlation between H-14 and H-15. The HMBC spectrum showed long-range correlation from H-15 to the carbons at δ_c 108.4 (C-14), 128.5 (C-13) and 147.0 (C-16).

From NOESY spectrum (**Figure 19**), the relative stereochemistry of the chiral centers in compound COL-1 could be determined. Correlations from δ_H 2.70 (H-10ax) to δ_H 1.32 (H-6ax) and δ_H 3.20 (H-8ax) could be observed. Moreover, the protons at

δ_H 0.79 (H_3-20) showed strong NOESY interactions with the protons at δ_H 1.07 (H-1ax), δ_H 2.03 (H-7ax), δ_H 3.92 (H-19a), δ_H 4.31 (H-19b), and both of the H-11 (δ_H 2.82, 3.02) methylene proton resonances. This established a *cis* relationship between H-8 and H-10 and between the methyl group at δ_c 39.5 (C-9) and H_2-19. Therefore, the configuration of the side chain at C-9 was assigned to be equatorial. All NOESY correlations were consistent with the X-ray structure (**Figure 7**).

By direct comparison of the ^{1}H- and ^{13}C-NMR spectra of compound COL-1 with those of the previously reported structure (Hasan *et al*., 2000), the compound was identified as nasimalun A or methyl-15,16-epoxy-12-oxo-3,13(16),14-*neo*-clerodatrien-18,19-olide-17-carboxylate. This is the first time the complete stereochemistry of this compound is clarified by the x-ray structure. (**Figure 7**)

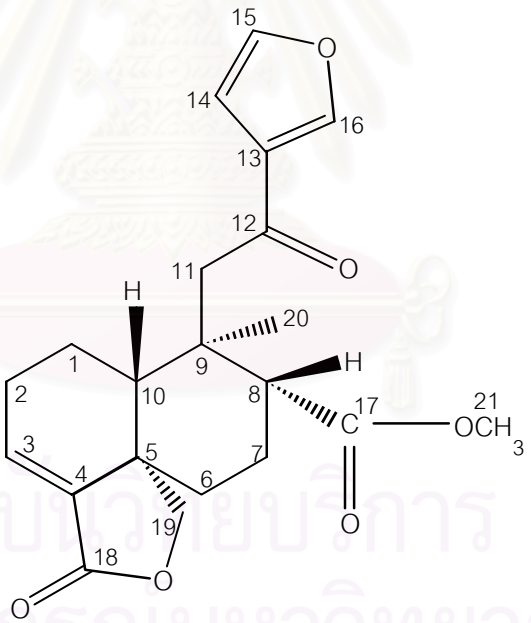


Table 11. ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY and HMBC spectral data of compound COL-1

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_c [a] (ppm)	^{1}H-^{1}H COSY	HMBC
1ax 1eq	1.07 (dddd, 11.1, 11.7, 12.3, 4.5) 1.62 (br.d, 12.3)	20.0 t	H_2-2, H-10	C-2, C-5, C-10 C-3, C-5
2ax 2eq	2.20 (m, overlapping) 2.26 (m, overlapping)	27.2 t	H_2-1, H-3	C-1, C-3, C-10
3	6.72 (2.4, partly overlapping)	136.2 d	H_2-2	C-1, C-2, C-5, C-18
4	-	137.7 s	-	-
5	-	45.0 s	-	-
6ax 6eq	1.32 (br.dd, 13.1, 13.1) 1.95 (m, overlapping)	33.1 t	H_2-7	C-5, C-7, C-19 C-5, C-7, C-8, C-10
7eq 7ax	1.85 (m) 2.03 (m, overlapping)	22.0 t	H_2-6, H-8ax	C-5, C-6, C-8, C-9 C-5, C-6, C-8
8ax	3.20 (dd, 12.6, 4.5)	48.6 d	H_2-7	C-7, C-9, C-10, C-11, C-17, C-20
9	-	39.5 s	-	-
10ax	2.70 (br.d, 12.0)	46.6 d	H_2-1	C-5, C-9, C-19, C-20
11a 11b	2.82 (d, 18.0) 3.02 (d, 17.7)	46.4 t	H-11b H-11a	C-8, C-9, C-10, C-12, C-20 C-9, C-10, C-12, C-20
12	-	193.6 s	-	-
13	-	128.5 s	-	-
14	6.70 (br.s)	108.4 d	H-15	C-15, C-16
15	7.41 (br.s)	144.2 d	H-14	C-13, C-14, C-16
16	8.00 (s)	147.0 d	-	C-13, C-14, C-15
17	-	173.9 s	-	-
18	-	169.0 s	-	-
19a 19b	3.92 (br.d, 7.4) 4.31 (d, 8.1)	71.3 t	H-19b H-19a	C-6 C-4, C-5, C-6, C-18
20	0.79 (s)	19.1 q	-	C-8, C-9, C-10, C-11
21	3.60 (s)	51.3 q	-	C-17

[a] Carbon type as determined by DEPT experiments

Table 12. ^{1}H-NMR (300 MHz in $CDCl_3$) and ^{13}C-NMR (75 MHz in $CDCl_3$) spectral data of compound COL-1, and ^{1}H-NMR (600 MHz in $CDCl_3$) and ^{13}C-NMR (150 MHz in $CDCl_3$) spectral data of nasimalun A (Hasan *et al*., 2000)

Position	Compound COL-1		Nasimalun A	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_c (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_c (ppm)
1ax 1eq	1.07 (dddd, 11.1, 11.7, 12.3, 4.5) 1.62 (br.d, 12.3)	20.0	1.08 (dddd, 11.0, 11.0, 11.0, 4.0) 1.64 (dddd, 11.0, 2.0, 2.0, 2.0)	20.1
2ax 2eq	2.20 (m) 2.26 (m)	27.2	2.22 (m) 2.28 (m)	27.3
3	6.72 (t, 2.4, partly overlapping)	136.2	6.74 (dd, 8.0, 2.0)	136.2
4	-	137.7	-	137.8
5	-	45.0	-	45.1
6ax 6eq	1.32 (br.dd, 13.1, 13.1) 1.95 (m, overlapping)	33.1	1.36 (dddd, 13.5, 13.5, 4.0, 2.0) 1.98 (ddd, 13.5, 4.5, 3.0)	33.2
7eq 7ax	1.85 (m) 2.03 (m, overlapping)	22.0	1.87 (dddd, 13.5, 4.5, 4.5, 3.0) 2.03 (dddd, 13.5, 13.5, 13.5, 4.5)	22.1
8ax	3.20 (dd, 12.6, 4.5)	48.6	3.21 (dd, 13.5, 4.5)	48.7
9	-	39.5	-	39.6
10ax	2.70 (br.d, 12.0)	46.6	2.73 (dd, 11.0, 2.0)	46.7
11a 11b	2.82 (d, 18.0) 3.02 (d, 17.7)	46.4	2.83 (d, 18.0) 3.04 (d, 18.0)	46.5
12	-	193.6	-	193.6
13	-	128.5	-	128.6
14	6.70 (br.s)	108.4	6.73 (d, 2.0)	108.5
15	7.41 (br.s)	144.2	7.42 (d, 2.0)	144.3
16	8.00 (s)	147.0	8.01 (s)	147.1
17	-	173.9	-	174.0
18	-	169.0	-	169.0
19a 19b	3.92 (br.d, 7.4) 4.31 (d, 8.1)	71.3	3.93 (dd, 8.0, 2.0) 4.33 (d, 8.0)	71.4
20	0.79 (s)	19.1	0.82 (s)	19.2
21	3.60 (s)	51.3	3.60 (s)	51.4

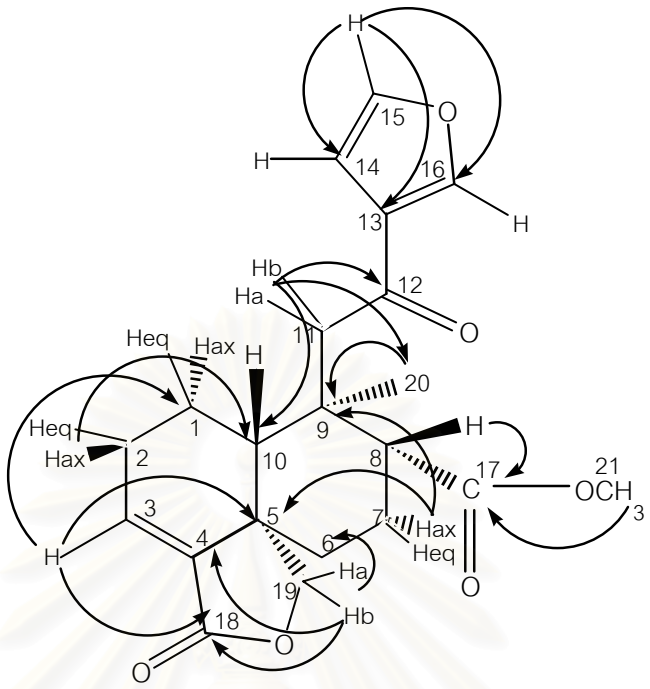


Figure 6. Long-range correlation from HMBC spectrum of compound COL-1

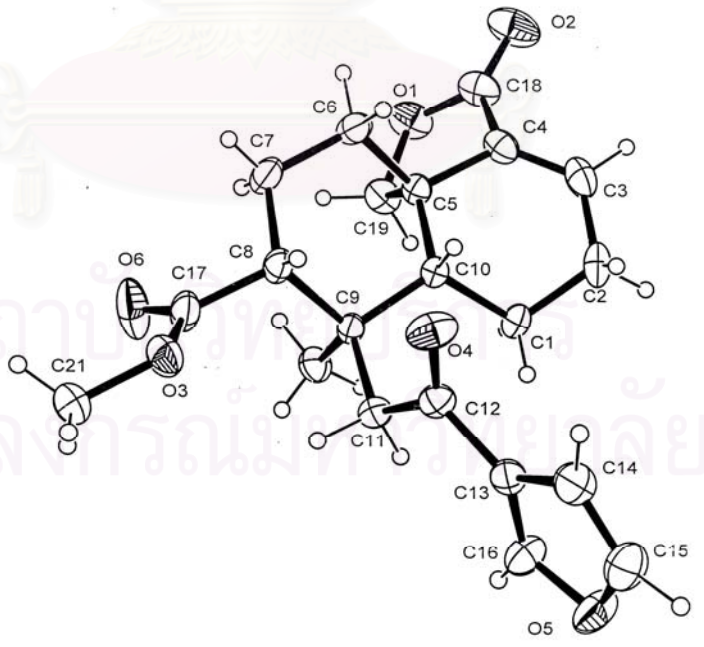


Figure 7. The X-ray structure of compound COL-1

2. Structure determination of compound COL-2

Compound COL-2 was obtained as a colorless semi-solid substance (0.0233 g.)

The FT-IR spectrum of compound COL-2 (**Figure 21**) displayed significant absorption bands at 2500-3500 cm^{-1} and 1710 cm^{-1}, indicating the presence of a carboxylic group in the molecule.

Table 13. The IR absorption band assignments of compound COL-2

Wave number (cm^{-1})	Tentative assignments
2500-3500	carboxylic O-H stretch
3073	alkene C-H stretch
2932, 2872	alkane C-H stretch
1710	C=O stretch
1649	C=C stretch

The ^{1}H-NMR spectrum of compound COL-2 (**Figure 23**) showed seven olefinic protons at δ_H 4.57 (1H, br.s; H-17), 4.65 (2H, br.s; H-17, H-18), 4.84 (1H, br.s; H-18), 5.00 (1H, br.s; H-16), 5.03 (1H, dd, *J* = 8.4, 1.7 Hz; H-16) and 6.00 (1H, ddd, *J* = 16.6, 12.1, 9.3 Hz; H-15), and two methyl groups at δ_H 0.81 (3H, s; H-20) and 1.72 (3H, s; H-19).

The ^{13}C-NMR spectrum (**Figure 24**) showed twenty carbon signals including six olefinic carbons at δ_C 106.7, 113.6, 116.1, 137.3, 147.4, 151.9 and one carboxyl carbon signal at δ_C 180.1.

From DEPT-90 and DEPT-135 experiments (**Figure 25**), one sp^2 methine carbon was shown at δ_C 137.3, together with four saturated methine carbons at δ_C 40.3, 41.0, 50.7, and 54.6. Two methyl carbons were presented at δ_C 16.7 and 23.8, and three sp^2 methylene carbon signals appeared at δ_C 106.7, 113.6, and 116.1. The signals at

δ_C 27.3, 27.6, 28.0, 31.3, 31.4 and 32.1 were of saturated methylene carbons. Four quaternary carbon signals δ_C 38.9, 147 .4, 151.9, and 180.1 could then be detected from the ^{13}C-NMR spectrum.

The EI-MS of compound COL-2 (**Figure 22**), with a molecular ion peak $[M]^+$ at *m/z* 302, gave strong support for a diterpene of molecular formula $C_{20}H_{30}O_2$. The degree of unsaturation calculated for this molecular formula is six.

A combination of several 2D-NMR techniques was used to assist the interpretation of the structure of compound COL-2. The protons directly attached to carbons were assigned by HMQC experiment (**Figure 26**). Crucial long-range ^{1}H-^{13}C correlation was obtained from HMBC spectrum (**Figure 28**). And the ^{1}H-^{1}H correlation was observed from COSY spectrum (**Figure 27**).

The ^{13}C-NMR spectrum further showed signals from an exocyclic methylene (δ_C 106.7; CH_2-17 : δ_C 151.9; C-13) and a vinyl group (δ_C 137.3; CH-15 : δ_C 116.1, CH_2-16). The presence of these groups was also apparent in the ^{1}H-NMR spectrum, which showed signals at δ_H 4.57 (1H, br.s; H-17) and 4.65 (1H, br.s; H-17) for the methylene, and at δ_H 6.00 (1H, ddd, *J* = 16.6, 12.1, 9.3 Hz; H-15), 5.00 (1H, br.s; H-16), 5.03 (1H, dd, *J* = 8.4, 1.7 Hz; H-16) for the vinyl group.

The ^{1}H-NMR spectrum also showed singlets at δ_H 0.81 and 1.72 for two methyl groups. The latter signal was downfield, indicative of its attachment to a double-bond quaternary carbon atom (δ_C 147.4; C-4) which linked to the methylene carbon at δ_C 113.6, δ_H 4.65, 4.84 (CH_2-18)

Of the six degree of unsaturation required for this spectrum, one was accounted for by the carboxyl group, and three by carbon-carbon double bonds. Therefore, there should be two ring systems in the structure. The functionality at C-4, C-13, C-14, together with the degree of unsaturation, suggested the strong possibility that

compound COL-2 might be a diterpene of the cleistanthane type with only two six-membered rings presented.

The exact positions of the side chains mentioned above were clarified by ^{1}H-^{1}H-COSY and HMBC correlation. The proton resonance at δ_H 6.00 (H-15) showed correlation with H_2-16 (δ_H 5.00, 5.03) and H-14 (δ_H 2.81). The H-14 proton showed correlation with H-15 (δ_H 6.00) and H-8 (δ_H 1.56). From the HMBC spectrum, correlation over three bonds from H_2-16 to C-14 (δ_C 54.6) were observed. Therefore, the vinylic side chain was placed on C-14.

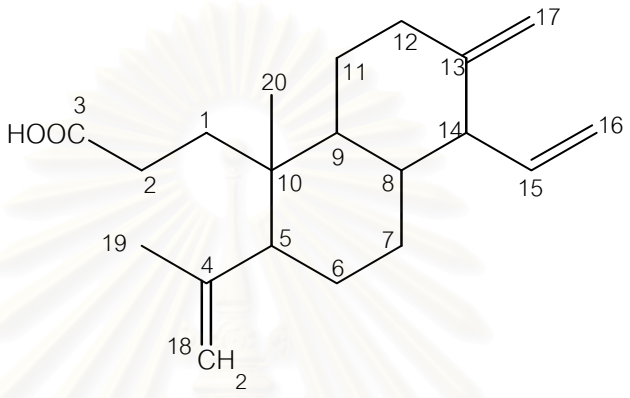
The long-range correlation from H_2-17 (δ_H 4.57, 4.65) to C-14 (δ_C 54.6) and C-12 (δ_C 31.3) placed the exocyclic methylene on C-13 (δ_C 151.9) .

The HMBC spectrum further showed three-bond correlation from the methyl group at H_3-19 (δ_H 1.72) to C-5 (δ_C 50.7) and C-18 (δ_C 113.6), and from H-6 (δ_H 1.68) to C-4 (δ_C 147.4). The ^{1}H-^{1}H COSY correlation between H-5 (δ_H 1.94) and H_2-6 (δ_H 1.41, 1.68) could also be detected. Therefore, the position of the propylene side chain was assigned.

The protons at δ_H 1.63 (H-1) showed HMBC correlation over three bonds with carbons at δ_C 180.1 (C-3; -COOH), 50.7 (C-5) and 41.0 (C-9), and showed correlation over two bonds with carbons at δ_C 28.0 (C-2) and 38.9 (C-10). Long-range correlation from H_3-20 (δ_H 0.81) to C-1 (δ_C 32.1), C-5 (δ_C 50.7), C-9 (δ_C 41.0) and C-10 (δ_C 38.9) were observed. From these data, it was concluded that the methyl group (CH_3-20) and the propionic moiety (CH_2-1, CH_2-2, COOH-3) were both placed on C-10.

The assignments of the two six-membered rings were completed by the ^{1}H-^{1}H COSY correlation from H-8 (δ_H 1.56) to H_2-7 (δ_H 1.23, 1.44), H-9 (δ_H 1.26) and H-14 (δ_H 2.81), and from H_2-11 (δ_H 1.71, 1.16) to H-9 (δ_H 1.26) and H_2-12 (δ_H 2.17). The interpretation of this structure by far was in agreement with A ring opening in the cleistanthane skeleton.

By comparing the spectral data of compound COL-2 to those of the compounds previously isolated from this plant (Siriwat, 1999), compound COL-2 was proposed to be 3,4-seco-cleistantha-4(18),13(17),15-trien-3-oic acid **[30]**.



[30]

The HMQC spectrum of compound COL-2, however, clearly showed signals of H_2-6 at δ_H 1.41 and 1.68, and H_2-7 at δ_H 1.23 and 1.44, inconsistent with the data reported for 3,4-seco-cleistantha-4(18),13(17),15-trien-3-oic acid **[30]**, as shown in **Table 15**. Moreover, the H_2-11 HMQC signals of compound COL-2 appeared at δ_H 1.16 and 1.71, while for compound **[30]** the protons in this position were both assigned at δ_H 1.72. By consulting the data of structurally related compounds zythiostromic acid A **[79]** (H_2-11; δ_H 1.16, 1.76), zythiostromic acid B **[80]** (H_2-11; δ_H 1.06, 1.76), and zythiostromolide **[81]** (H_2-11; δ_H 1.16, 1.70) (Ayer and Khan, 1996), it was most likely that the signals of the two protons (H_2-11) appeared separately in ^{1}H-NMR spectrum. All the evidence lead to the conclusion that the assignments of protons at position 6,7 and 11 should be revised according to this present data.

The stereochemistry of the molecule was established by the coupling constants in the ^{1}H-NMR spectrum and by NOESY experiment (**Figure 29**). In the ^{1}H-NMR spectrum of compound COL-2, H-14 (δ_H 2.81) showed a 9.2 Hz coupling with H-15 (δ_H 6.00) and a 4.6 Hz coupling with H-8 (δ_H 1.56), suggesting that H-14 is equatorial. This

assignment was confirmed by NOESY spectrum which displayed correlation from H-14 (δ_H 2.81) to H-17 (δ_H 4.65) and H-8 (δ_H 1.56), and from H-15 (δ_H 6.00) to H-9 (δ_H 1.26), showing the β-axial orientation of H-9. NOESY correlation between C-10 methyl (CH_3-20; δ_H 0.81) and Hax-6 (δ_H 1.68), H-8 (δ_H 1.56), Hax-11 (δ_H 1.71) indicated that the methyl group was in α-axial orientation. Therefore the propionic moiety (C-1, C-2, C-3) must be in equatorial position, confirmed by NOESY correlation from H-2 (δ_H 2.27) to H-9 (δ_H 1.26) and from H-2 (δ_H 2.40) to H-5 (δ_H 1.94).

The HMBC and NOESY correlation of compound COL-2 are summarized in **Figure 8** and **Figure 9**, respectively.

Table 14. ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY and HMBC spectral data of compound COL-2

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_c [a] (ppm)	^{1}H-^{1}H COSY	HMBC
1	1.63 (m)	32.1 t	H_2-2	C-2, C-3, C-5, C-9, C-10
2	2.40 (ddd, 13.3, 8.0, 7.2) 2.27 (ddd, 13.3, 8.0, 7.2)	28.0 t	H_2-1	C-3
3	-	180.1 s	-	-
4	-	147.4 s	-	-
5	1.94 (dd, 12.7, 2.9)	50.7 d	H_2-6	C-10, C-19
6eq 6ax	1.41 (m) 1.68 (m)	27.6 t	H-5, H_2-7	- C-4, C-8, C-10
7ax 7eq	1.23 (m) 1.44 (m)	31.4 t	H_2-6, H-8	C-8 -
8	1.56 (dddd, 15.6, 12.2, 4.0, 4.0)	40.3 d	H_2-7, H-9, H-14	-
9	1.26 (m)	41.0 d	H-8, H_2-11, H_2-12	C-8
10	-	38.9 s	-	-
11eq 11ax	1.20 (m) 1.71 (m)	27.3 t	H-9, H_2-12	C-8 C-8, C-10
12	2.17 (m)	31.3 t	H_2-11, H-9	C-9
13	-	151.9 s	-	-
14	2.81 (dd, 9.2, 4.6)	54.6 d	H-15, H-8	C-8, C-13, C-17
15	6.00 (ddd, 16.6, 12.1, 9.3)	137.3 d	H-14, H_2-16	C-14
16	5.00 (br.s) 5.03 (dd, 8.4, 1.7)	116.1 t	H-15	C-14
17	4.57 (br.s) 4.65 (br.s)	106.7 t	H_2-12 -	C-12, C-14
18	4.65 (br.s) 4.84 (br.s)	113.6 t	H_3-19	C-5, C-19
19	1.72 (s)	23.8 q	H_2-18	C-5, C-18
20	0.81 (s)	16.7 q	-	C-1, C-5, C-9, C-10

[a] Carbon type as determined by DEPT experiments

Table 15. ^{1}H-NMR (500 MHz in $CDCl_3$) and ^{13}C-NMR (75 MHz in $CDCl_3$) spectral data of compound COL-2, and ^{1}H-NMR (500 MHz in $CDCl_3$) and ^{13}C-NMR (150 MHz in $CDCl_3$) spectral data of cleistantha-4(18),13(17),15-trien-3-oic acid (Siriwat, 1999)

Position	Compound COL-2		Cleistantha-4(18),13(17),15-trien-3-oic acid	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_c ppm	δ_H (ppm) (multiplicity, *J* in Hz)	δ_c ppm
1	1.63 (m)	32.1	1.64 (m)	32.1
2	2.40 (ddd, 13.3, 8.0, 7.2) 2.27 (ddd, 13.3, 8.0, 7.2)	28.0	2.40 (ddd, 10.68, 7.02, 7.02) 2.3 (ddd, 10.68, 7.02, 7.02)	28.1
3	-	180.1	-	180.8
4	-	147.4	-	147.4
5	1.94 (dd, 12.7, 2.9)	50.7	1.95 (dd, 12.82, 2.75)	50.7
6eq 6ax	1.41 (m) 1.68 (m)	27.6	1.45 (m) 1.70 (m)	27.5
7ax 7eq	1.23 (m) 1.44 (m)	31.4	1.24 (m) 1.42 (m)	31.4
8	1.56 (dddd, 15.6, 12.2, 4.0, 4.0)	40.3	1.56 (dddd, 16.17, 12.51, 4.27, 4.27)	40.3
9	1.26 (m)	41.0	1.26 (m)	41.0
10	-	38.9	-	38.9
11eq 11ax	1.16 (m) 1.71 (m)	27.3	1.72 (m)	27.3
12	2.17 (m)	31.3	2.17 (m)	31.3
13	-	151.9	-	151.8
14	2.81 (dd, 9.2, 4.6)	54.6	2.82 (dd, 8.85, 4.58)	54.6
15	6.00 (ddd, 16.6, 12.1, 9.3)	137.3	6.00 (ddd, 16.18, 10.38, 8.85)	137.3
16	5.00 (br.s) 5.03 (dd, 8.4, 1.7)	116.1	5.00 (br.s) 5.02 (m)	116.1
17	4.57 (br.s) 4.65 (br.s)	106.7	4.56 (br.s) 4.65 (br.s)	106.7
18	4.65 (br.s) 4.84 (br.s)	113.6	4.65 (br.s) 4.85 (br.s)	113.6
19	1.72 (s)	23.8	1.73 (s)	23.8
20	0.81 (s)	16.7	0.82 (s)	16.7

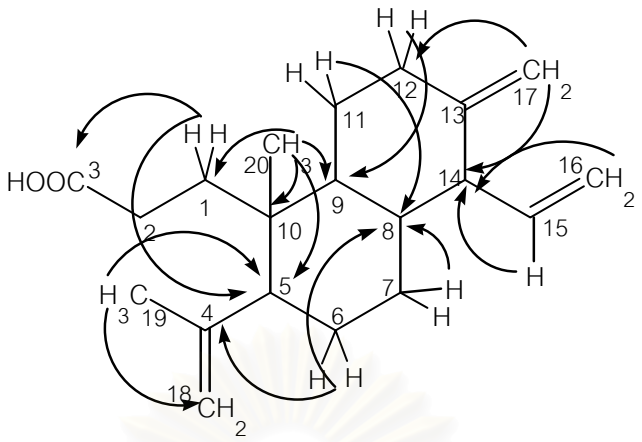


Figure 8. Long-range correlation from HMBC spectrum of compound COL-2

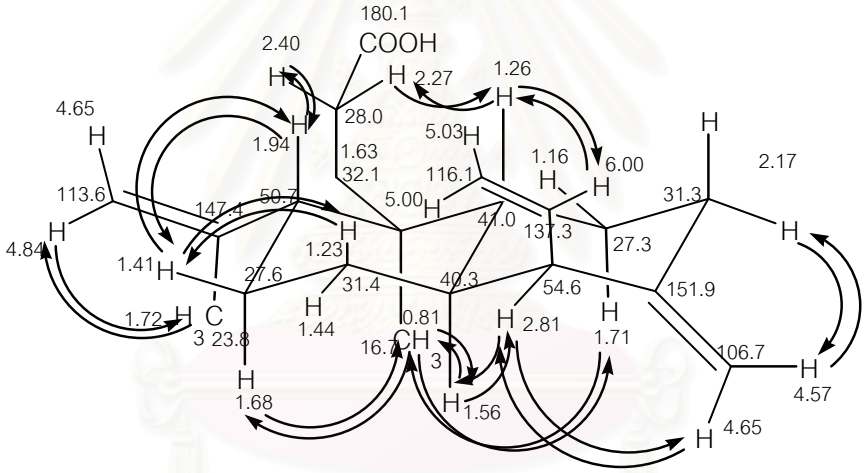


Figure 9. The NOESY correlation of compound COL-2

CHAPTER V
CONCLUSION

From the stem bark of *Croton oblongifolius* Roxb. (Euphorbiaceae) collected from Loei, Thailand, two diterpene compounds have been isolated. Their chemical structures were elucidated and identified by several spectroscopic techniques and comparison with previous reports. Compound COL-1 was identified as methyl-15,16-epoxy-12-oxo-3,13(16),14-*neo*-clerodatrien-18,19-olide-17-carboxylate, or Nasimalun A, a *neo*-clerodane type diterpene previously found in the roots of *Barringtonia racemosa* Blume. (Lecythidaceae). In this study the X-ray structure of Nasimalun A has been provided, therefore the stereochemistry is now unambiguous. Compound COL-2 was iidentified as a known cleistanthane-type diterpene, 3,4-seco-cleistantha-4(18),13(17),15-trien-3-oic acid, with the assignment revisions on position 6,7 and 11. This study has provided additional chemotaxonomic information of *Croton oblongifolius* Roxb. It is highly recommended that further studies concerning the chemical constituents of this plant should be conducted, for the completion of the phytochemical data of this specie.

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Appendix

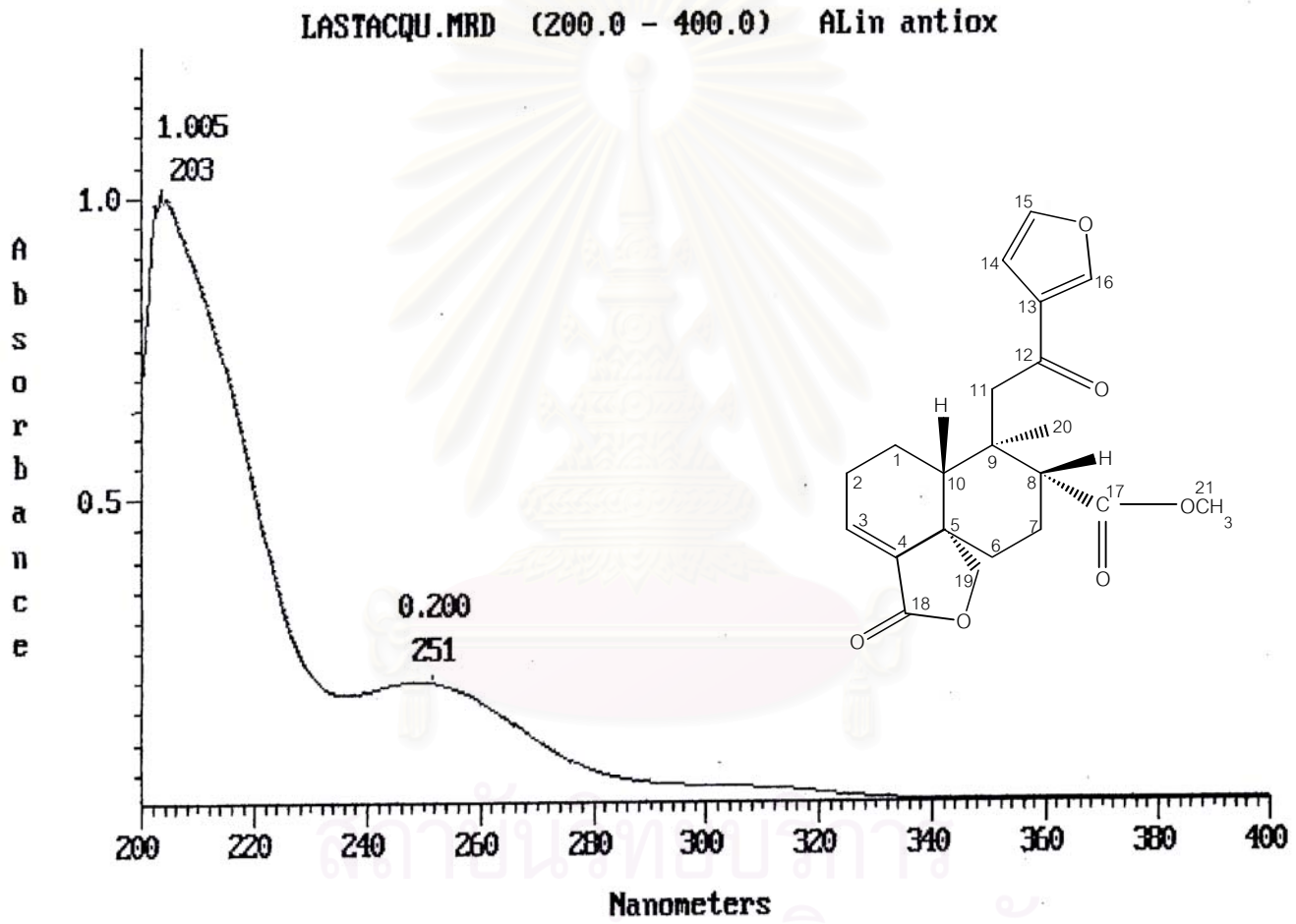


Figure 10. The UV spectrum of compound COL-1 (in MeOH)

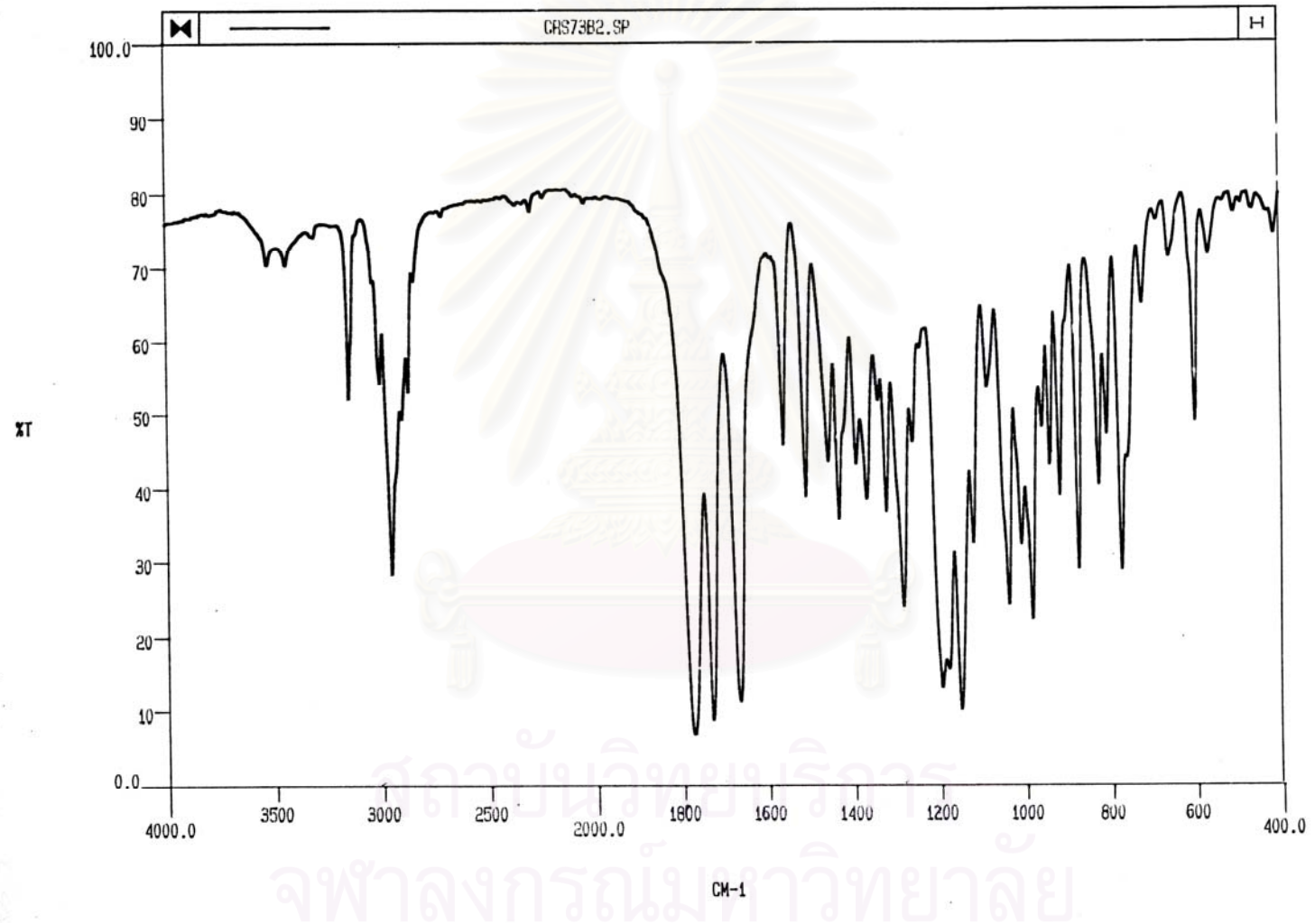


Figure 11. The IR spectrum of compound COL-1 (KBr disc)

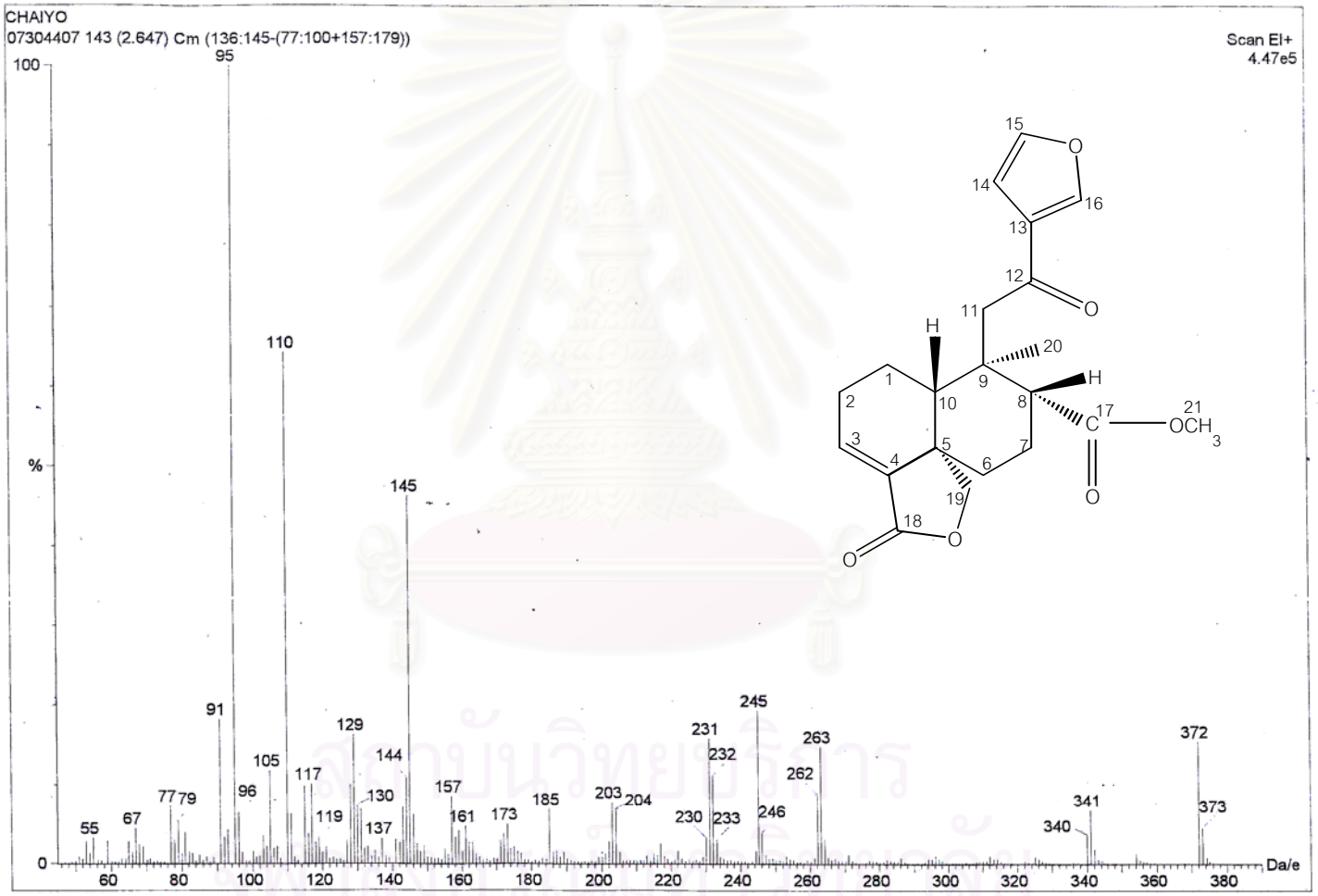
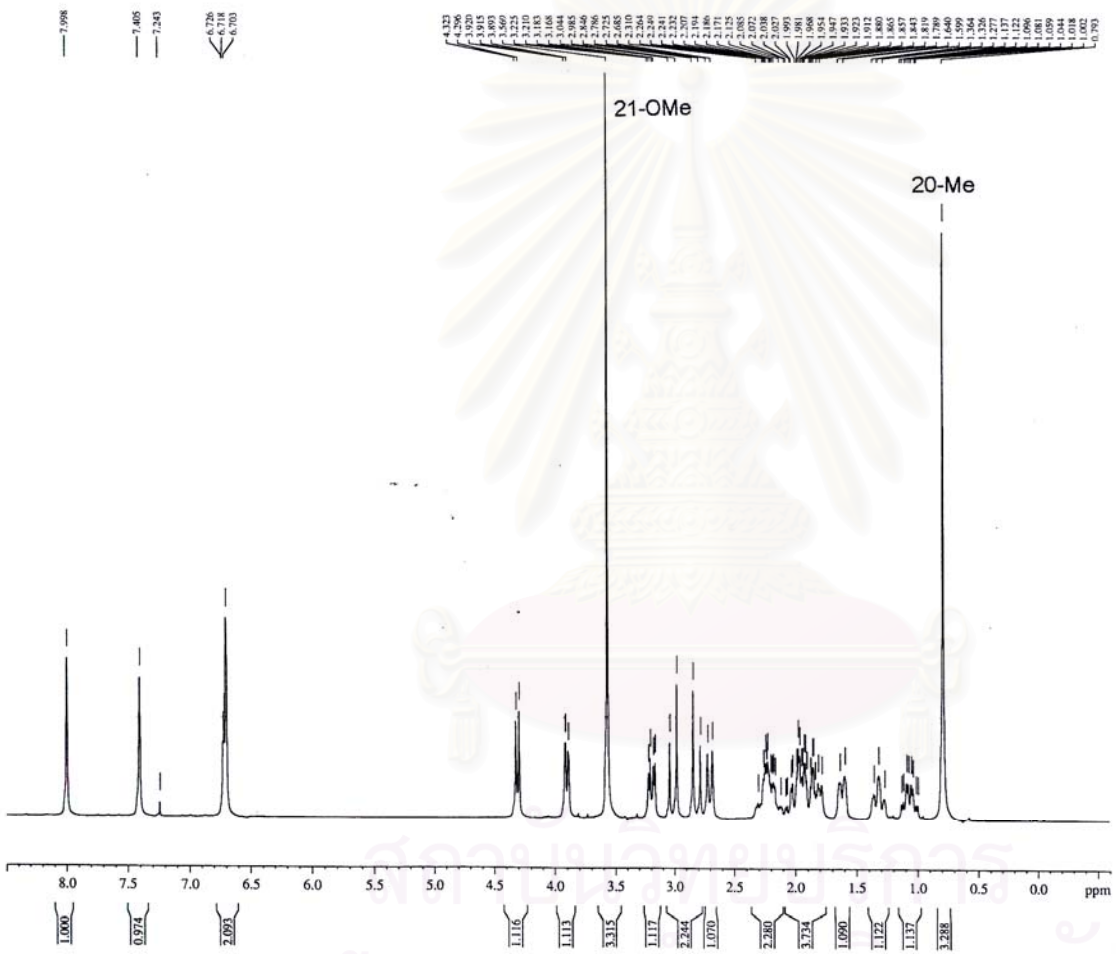


Figure 12. The EIMS spectrum of compound COL-1



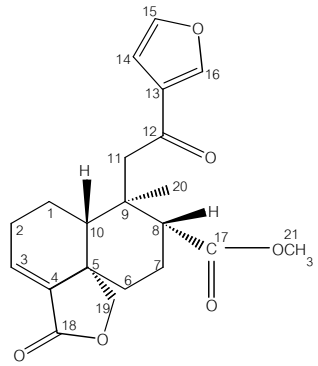


Figure 13a. The 300 MHz ^{1}H-NMR spectrum of compound COL-1 (in $CDCl_3$)

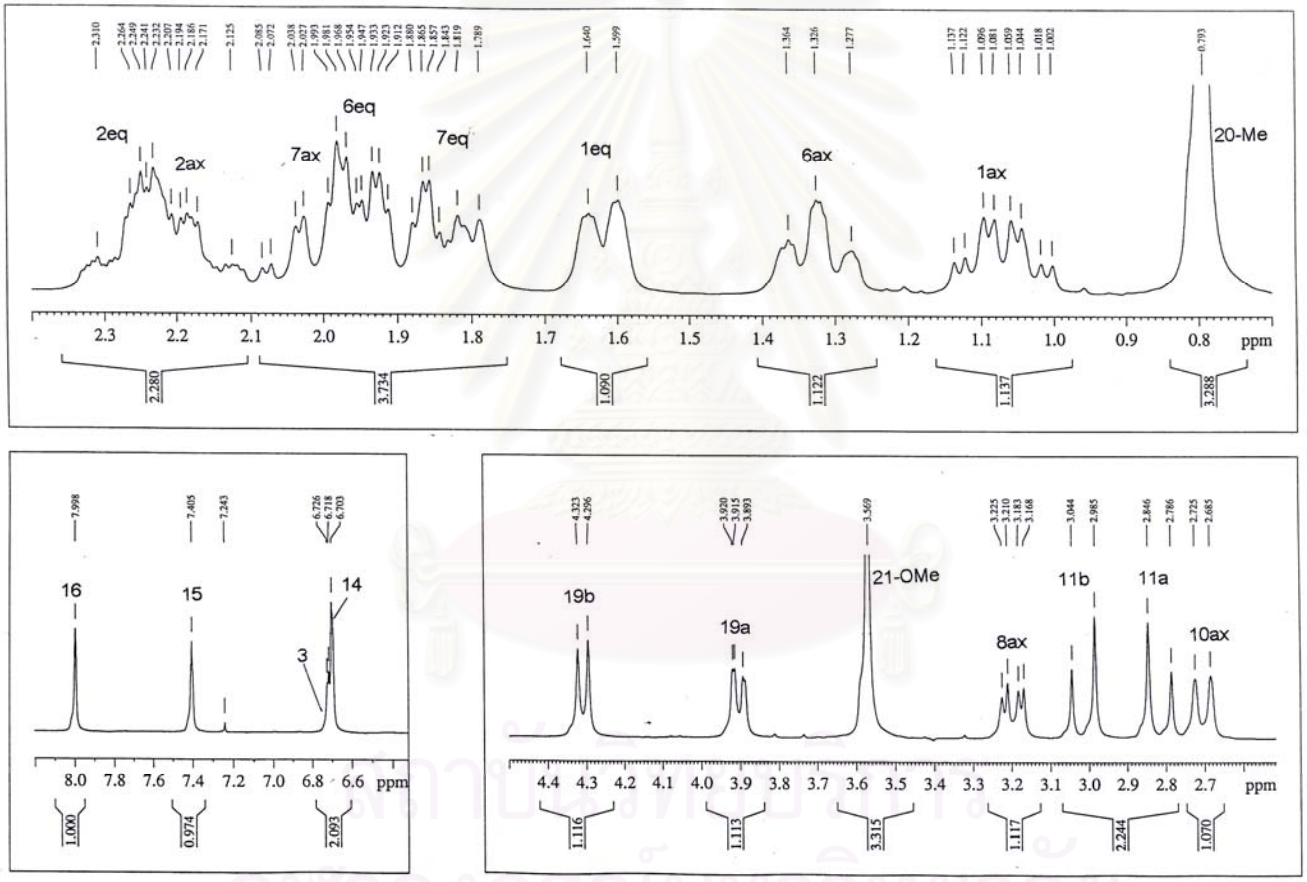
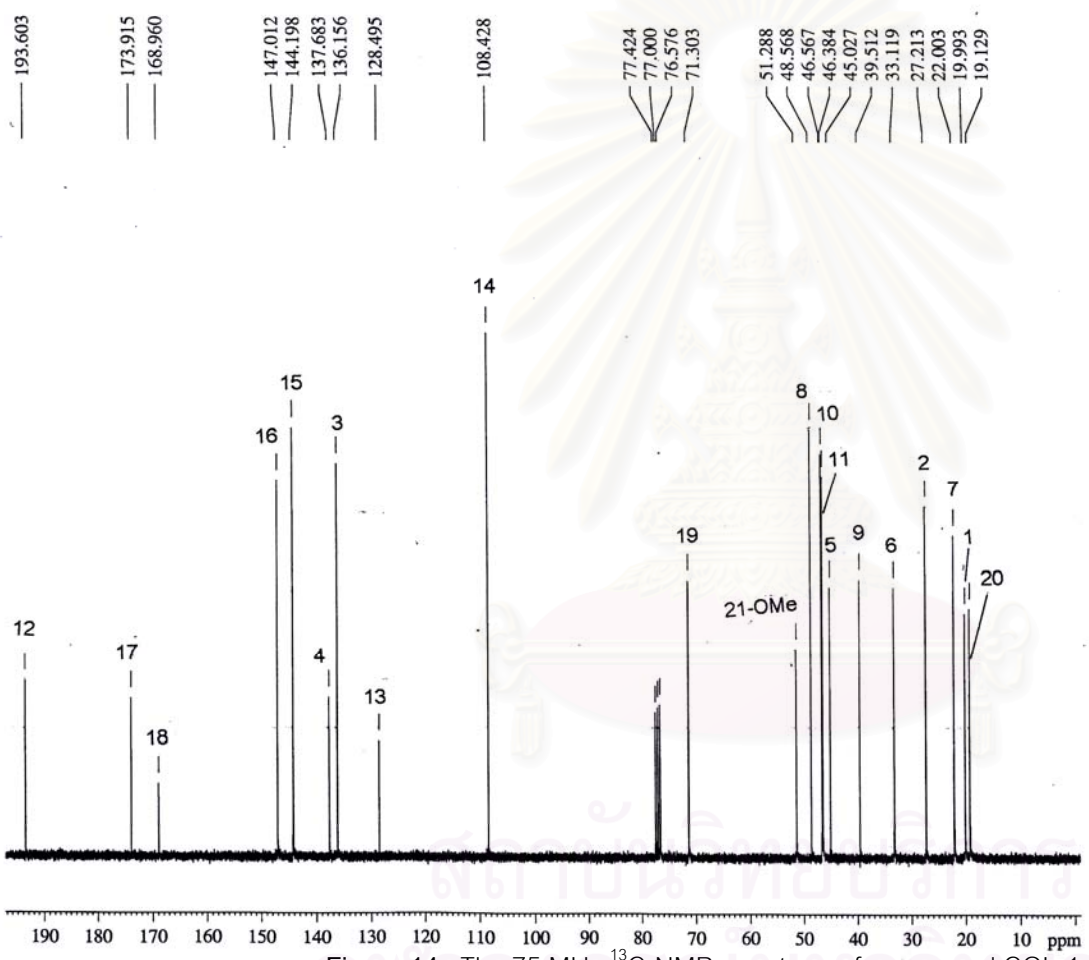


Figure 13b. The expanded 300 MHz ^{1}H-NMR spectrum of compound COL-1 (in $CDCl_3$)



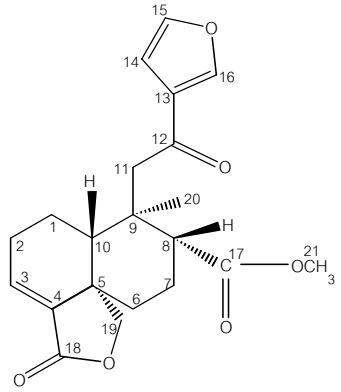
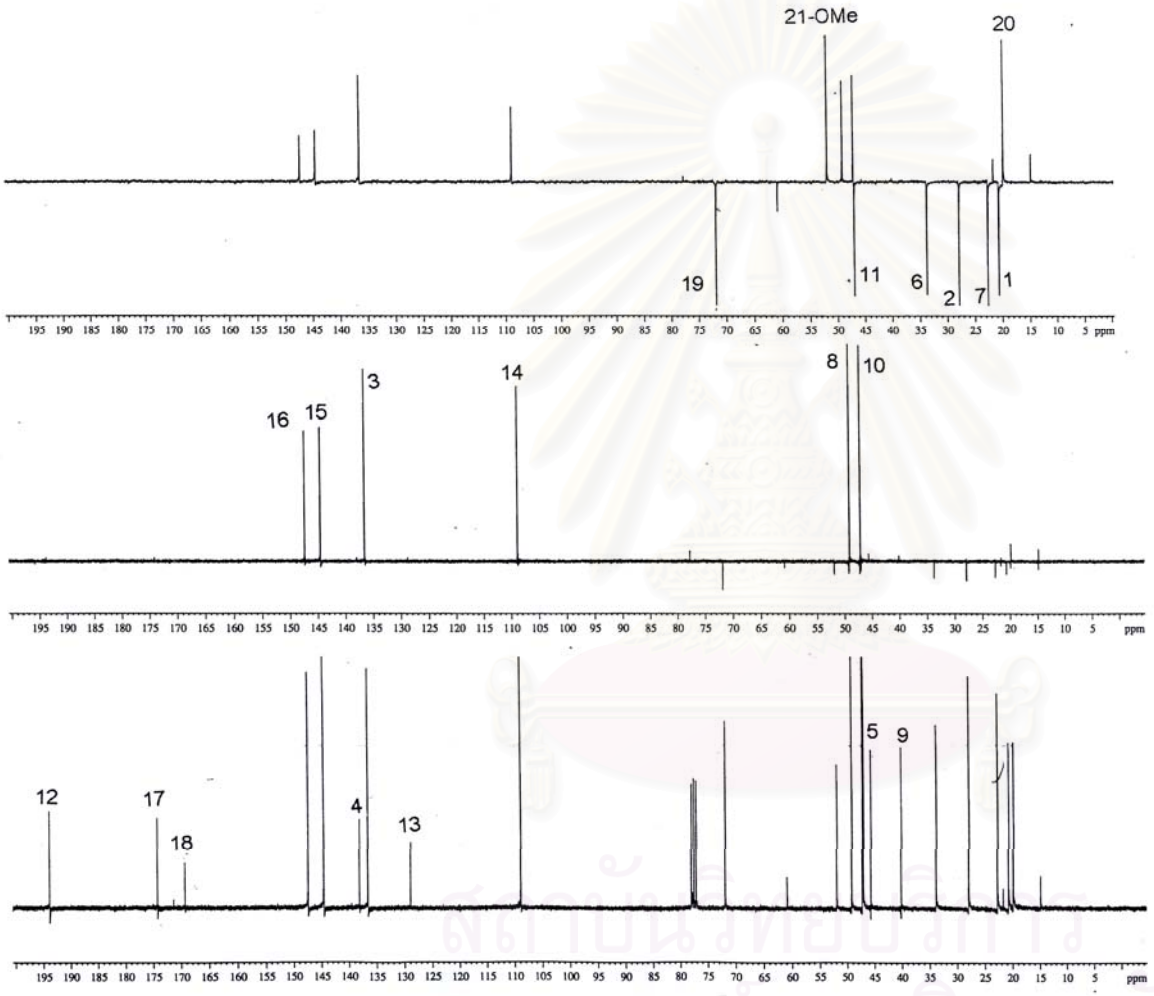


Figure 14. The 75 MHz ^{13}C-NMR spectrum of compound COL-1 (in $CDCl_3$)



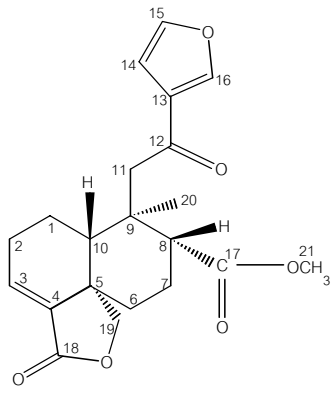
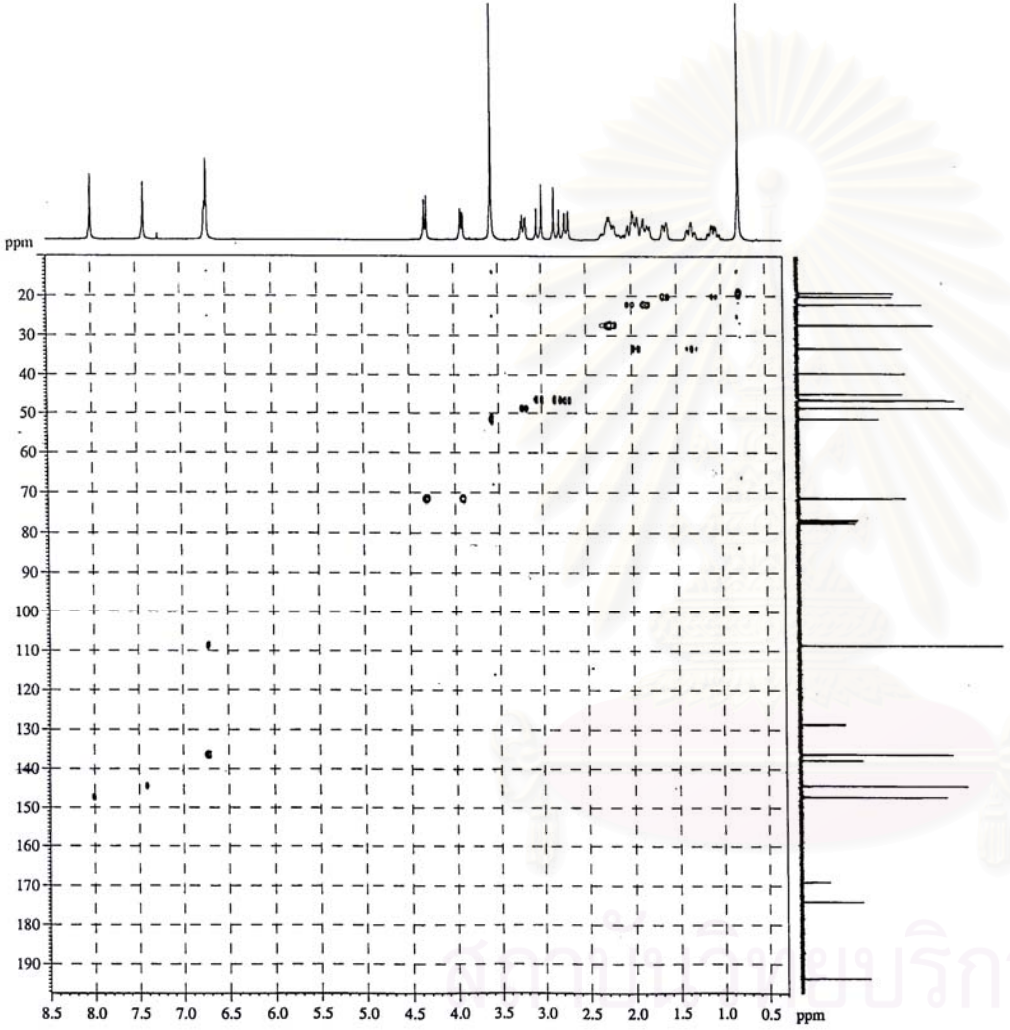


Figure 15. The 75 MHz ^{13}C-NMR, DEPT-90 and DEPT-135 spectra of compound COL-1(in $CDCl_3$)



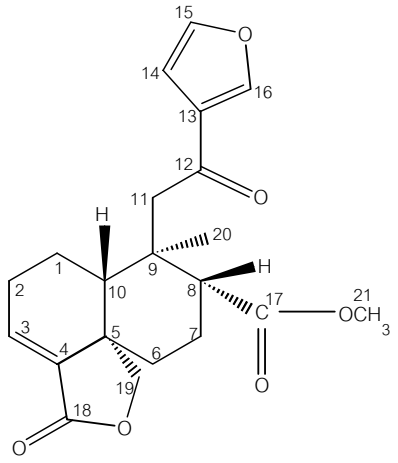


Figure 16a. The 300 MHz HMQC spectrum of compound COL-1 (in $CDCl_3$)

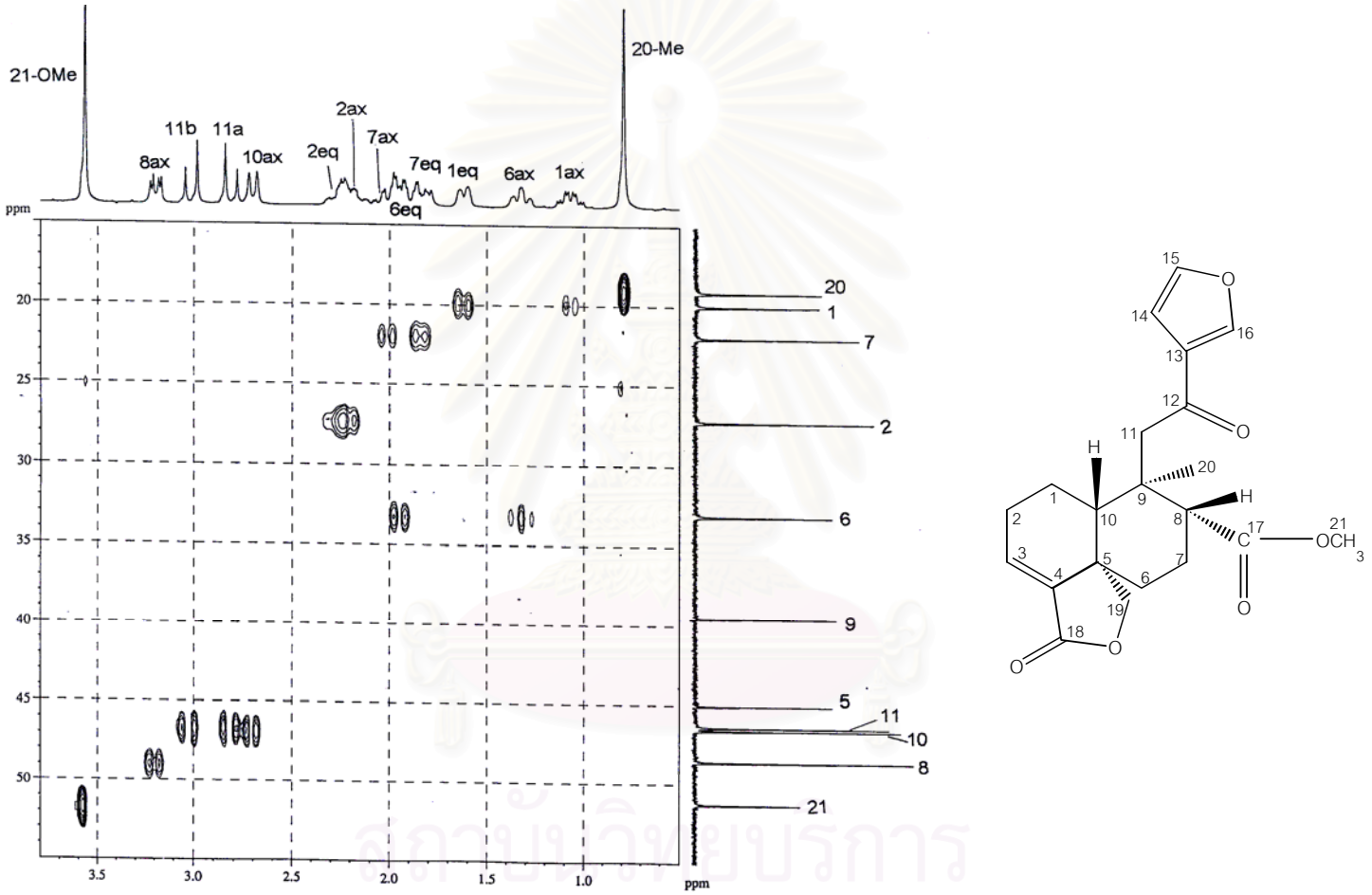
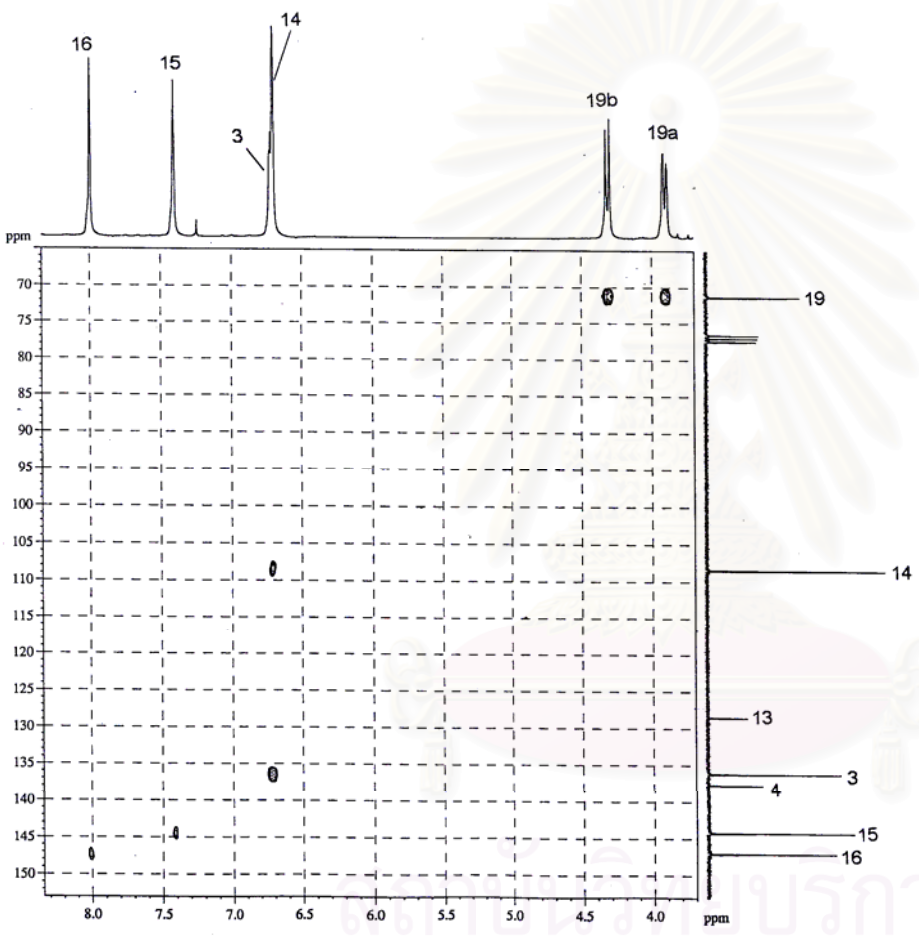


Figure 16b. The expanded 300 MHz HMQC spectrum of compound COL-1 (in $CDCl_3$)

(δ_H 0.5-3.7 ppm, δ_C 15.0-55.0 ppm)



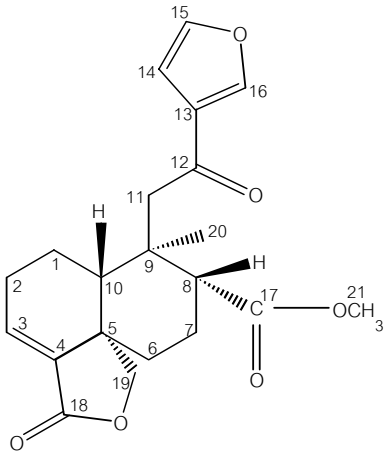
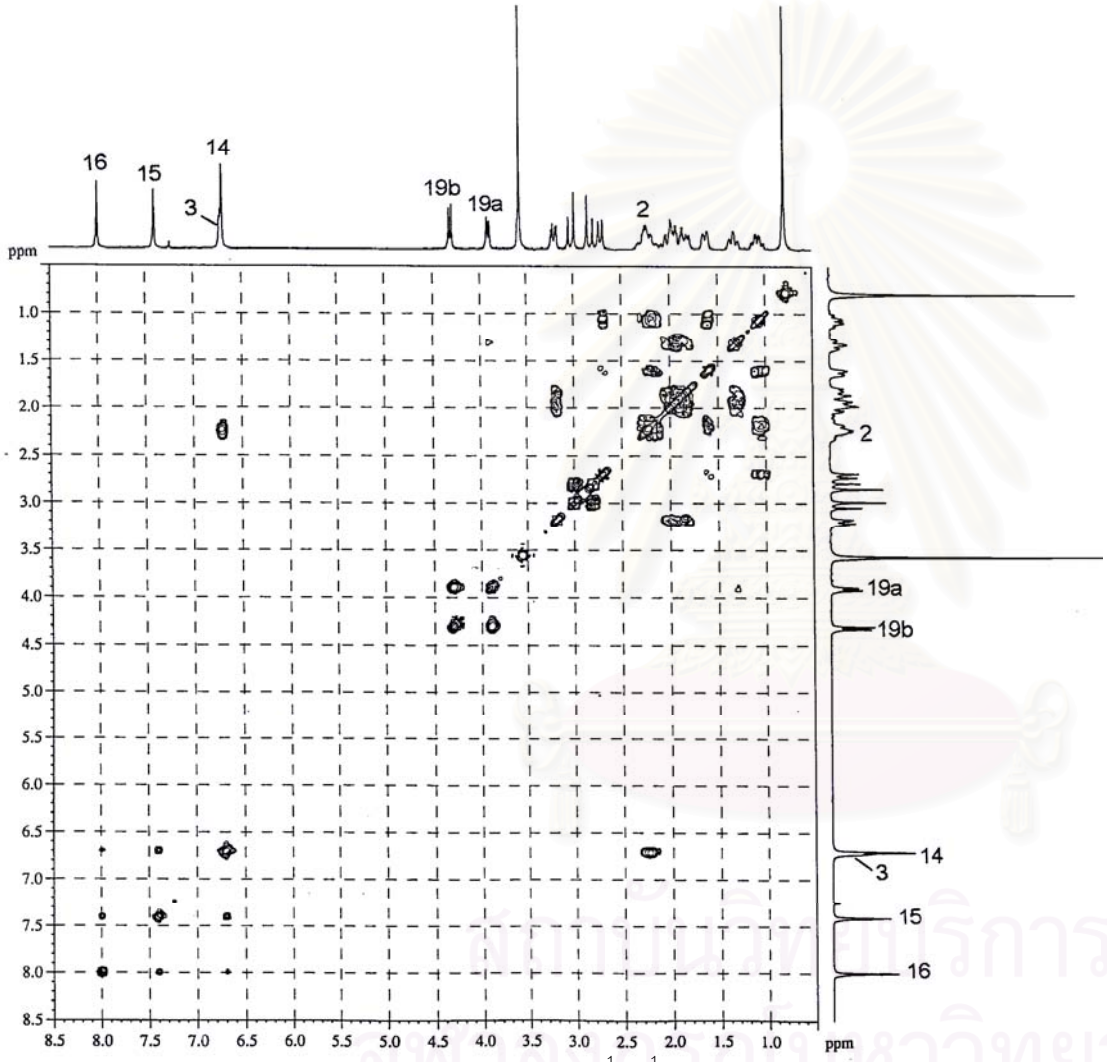


Figure 16c. The expanded 300 MHz HMQC spectrum of compound COL-1 (in $CDCl_3$)

(δ_H 3.7-8.5 ppm, δ_C 65.0-150.0 ppm)



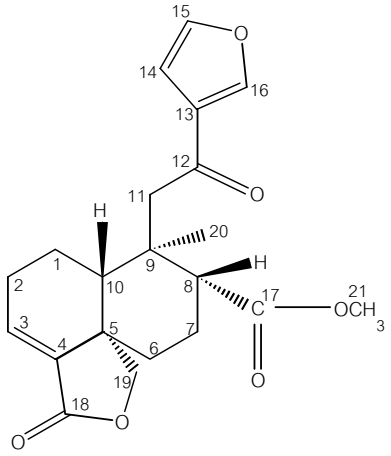


Figure 17a. The 300 MHz ^{1}H- ^{1}H COSY NMR spectrum of compound COL-1 (in $CDCl_3$)

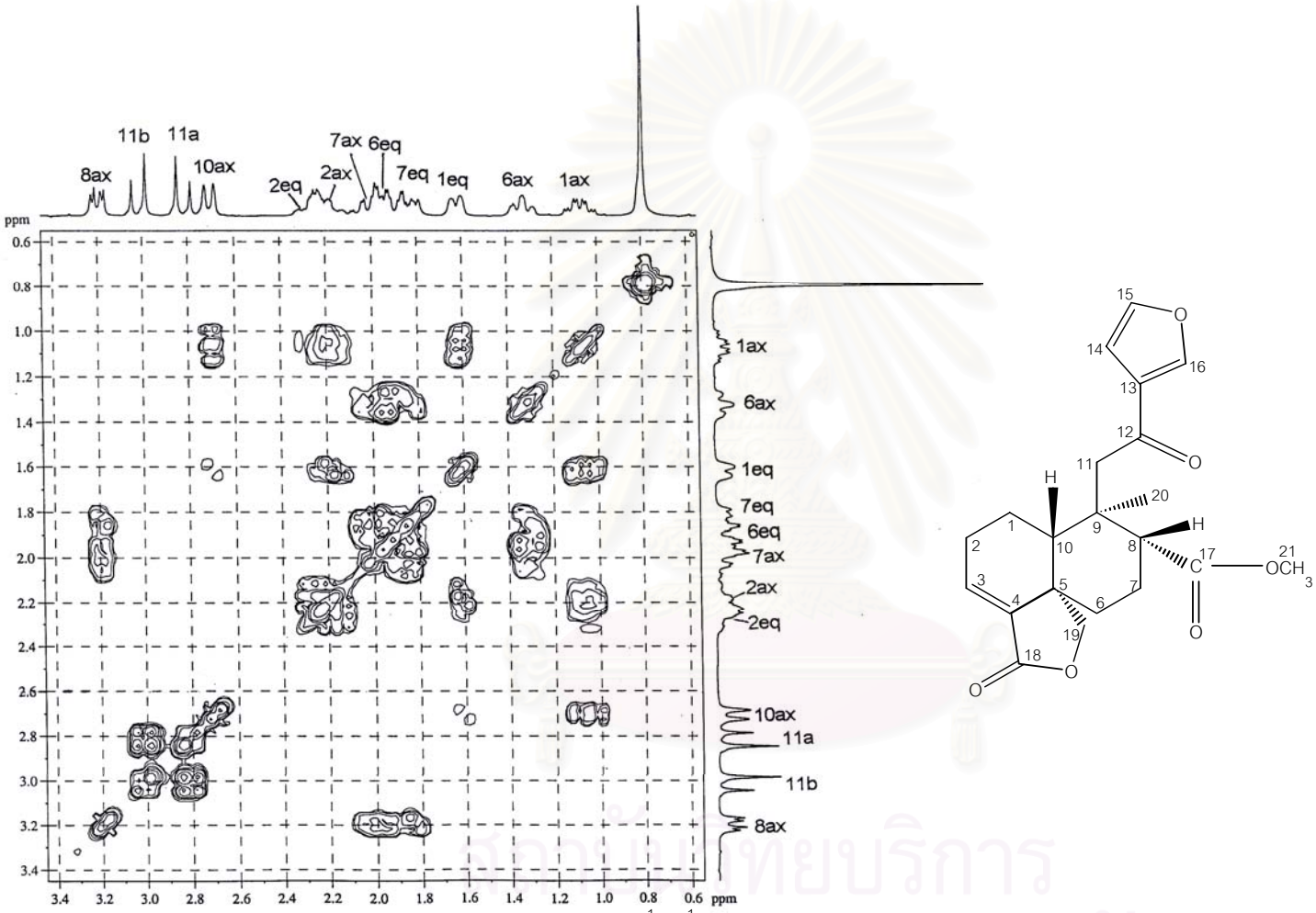
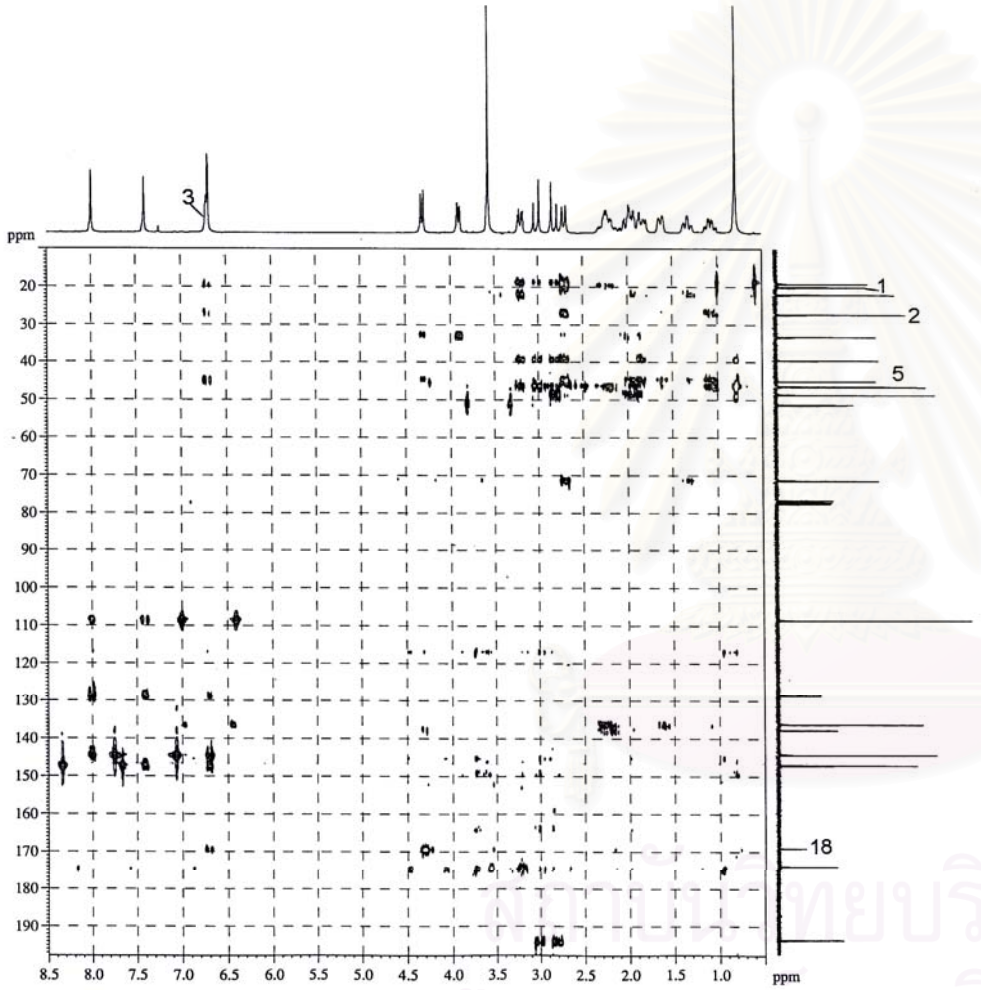


Figure 17b. The expanded 300 MHz ^{1}H- ^{1}H COSY NMR spectrum of compound COL-1 (in $CDCl_3$)

(δ_H 0.6-3.4 ppm)



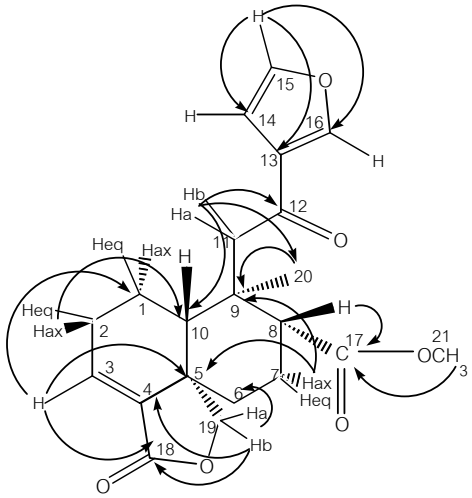


Figure18a. The 300 MHz HMBC ($^{n}J_{CH}$= 8 Hz) spectrum of compound COL-1 (in $CDCl_3$)

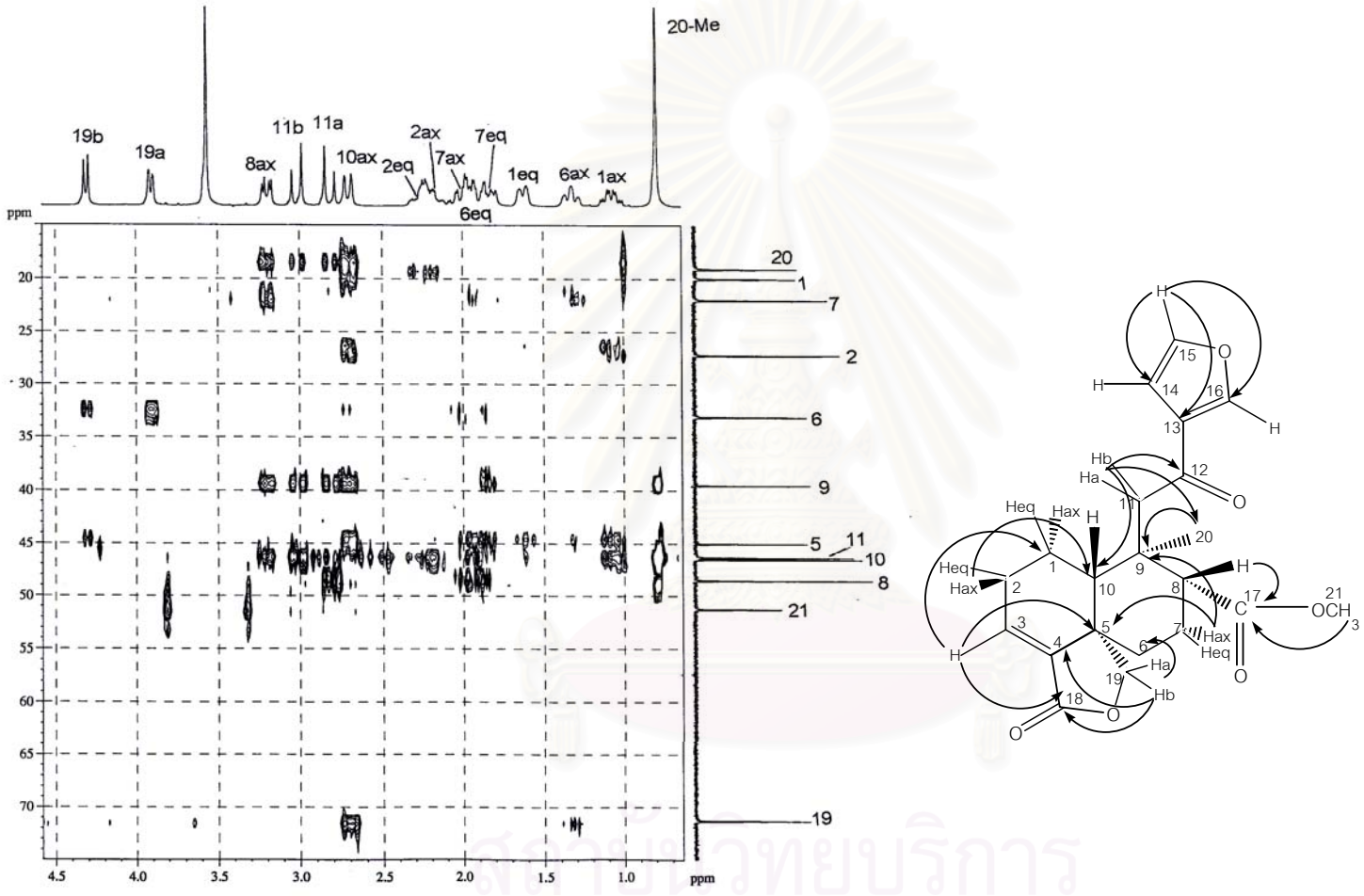


Figure 18b. The expanded 300 MHz HMBC ($^{n}J_{CH}$= 8 Hz) spectrum of compound COL-1 (in $CDCl_3$)

(δ_H 0.5-4.5 ppm, δ_C 15.0-75.0 ppm)

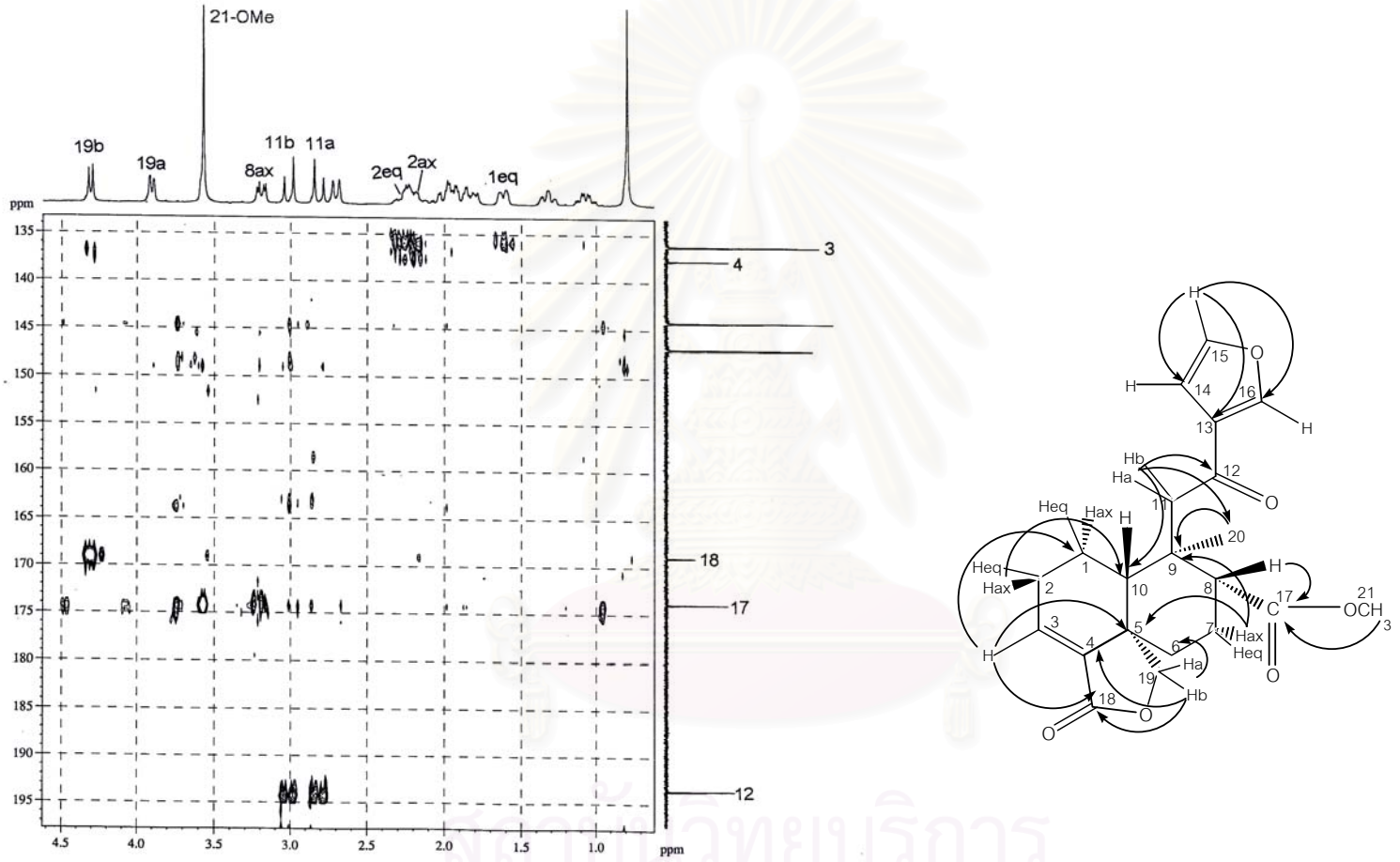


Figure 18c. The expanded 300 MHz HMBC ($^{n}J_{CH}$= 8 Hz) spectrum of compound COL-1 (in $CDCl_3$)

(δ_H 0.5-4.5 ppm, δ_C 135.0-195.0 ppm)

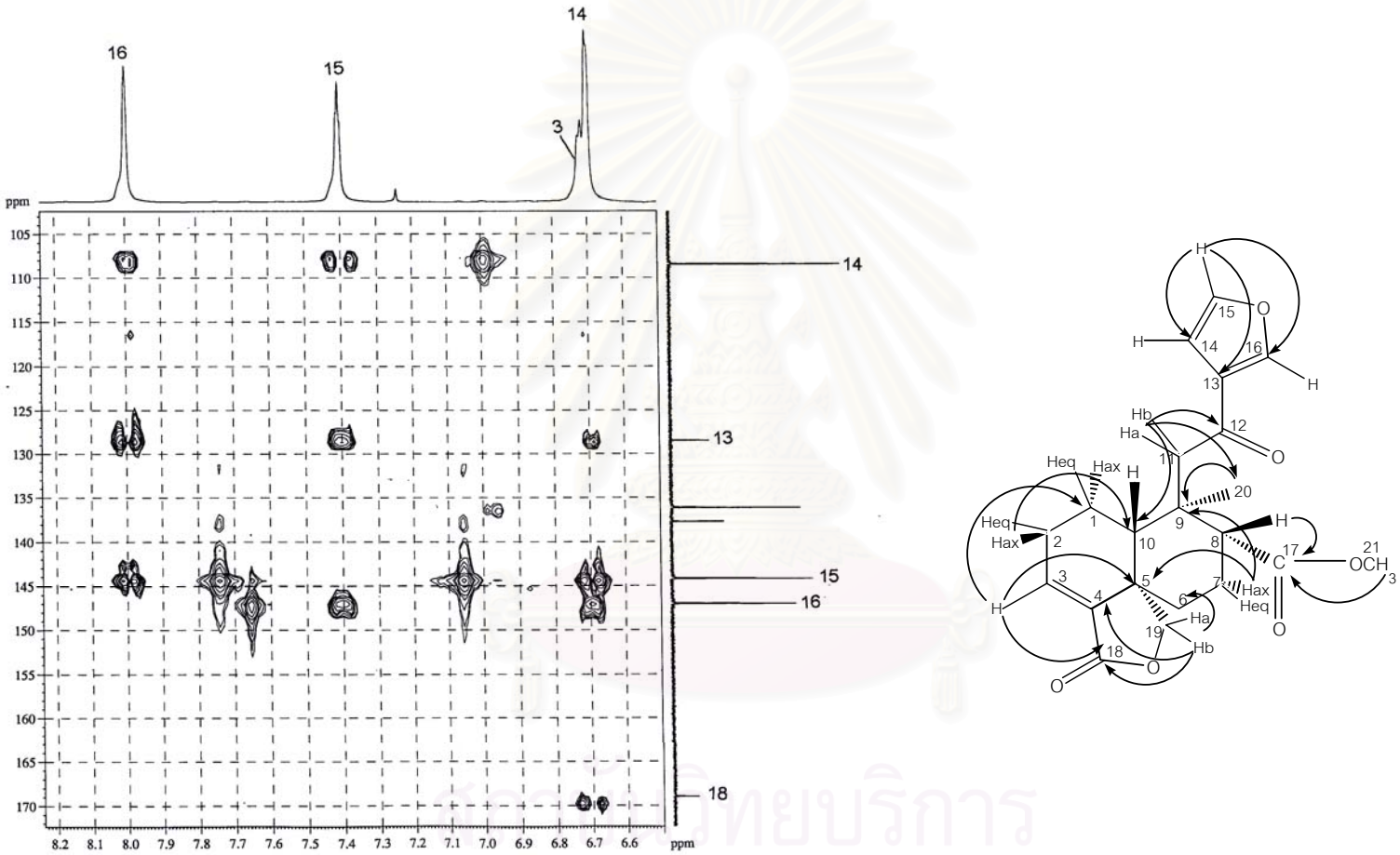
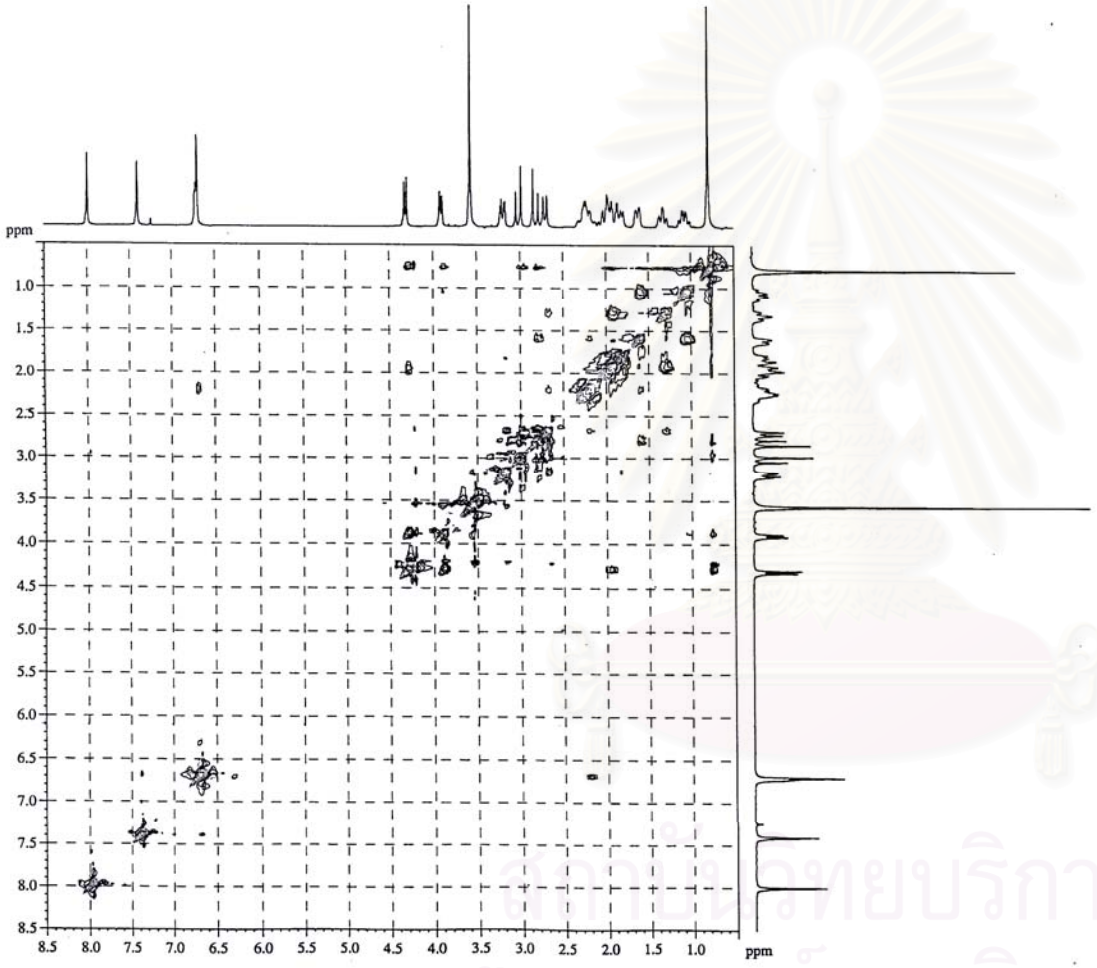


Figure 18d. The expanded 300 MHz HMBC ($^{n}J_{CH}$= 8 Hz) spectrum of compound COL-1 (in $CDCl_3$)

(δ_H 0.5-8.2 ppm, δ_C 105.0-175.0 ppm)



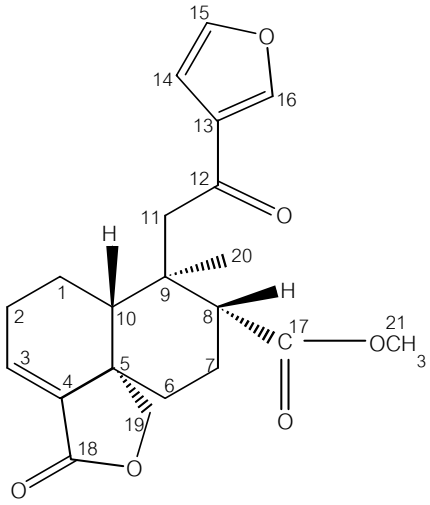
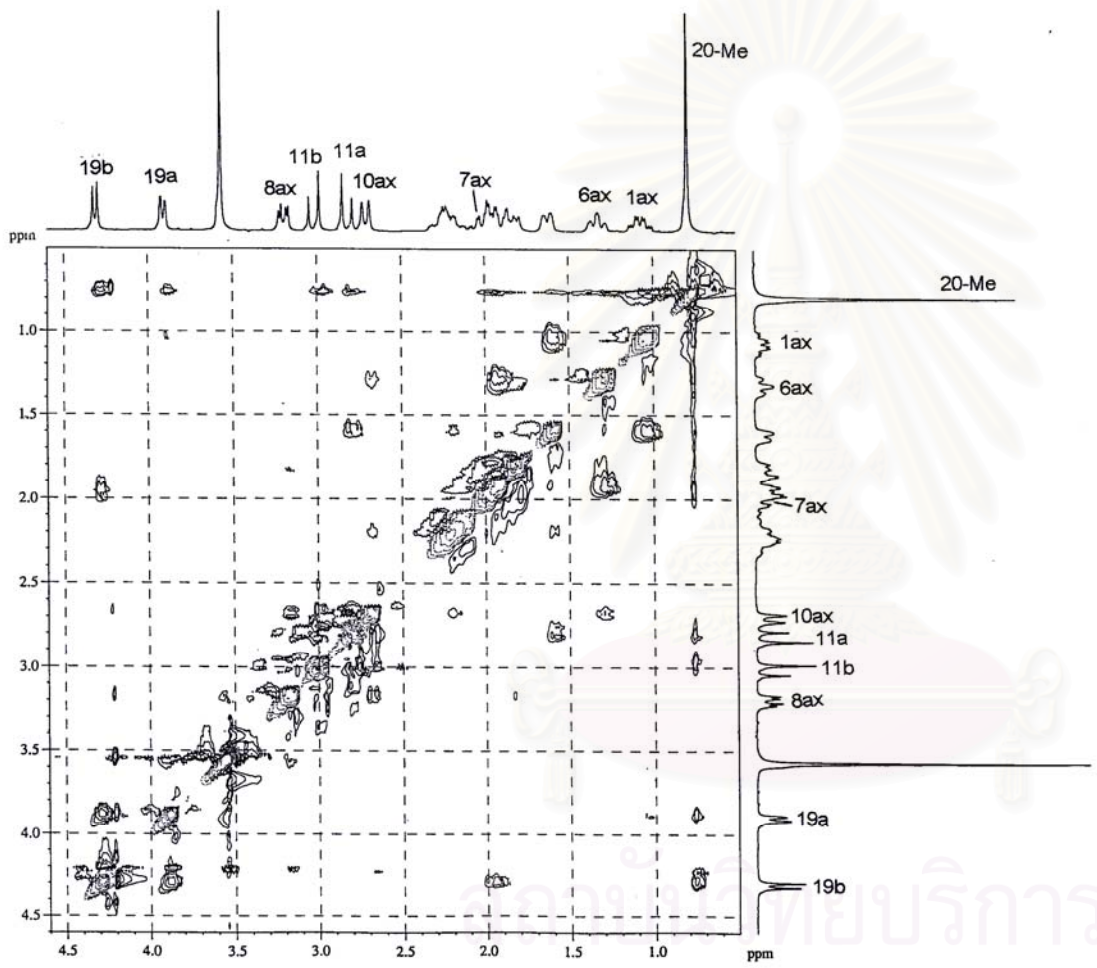


Figure 19a. The 300 MHz NOESY spectrum of compound COL-1 (in $CDCl_3$)



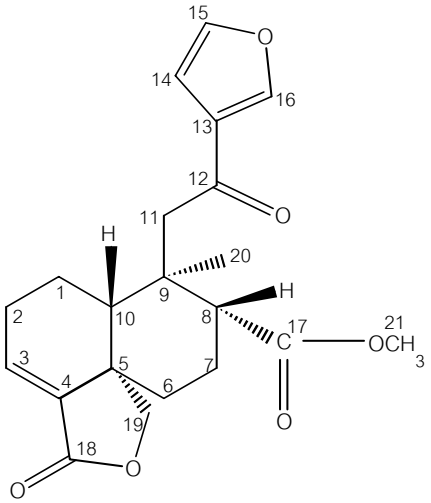


Figure 19b. The expanded 300 MHz NOESY spectrum of compound COL-1 (in $CDCl_3$)

(δ_H 0.5-4.5 ppm)

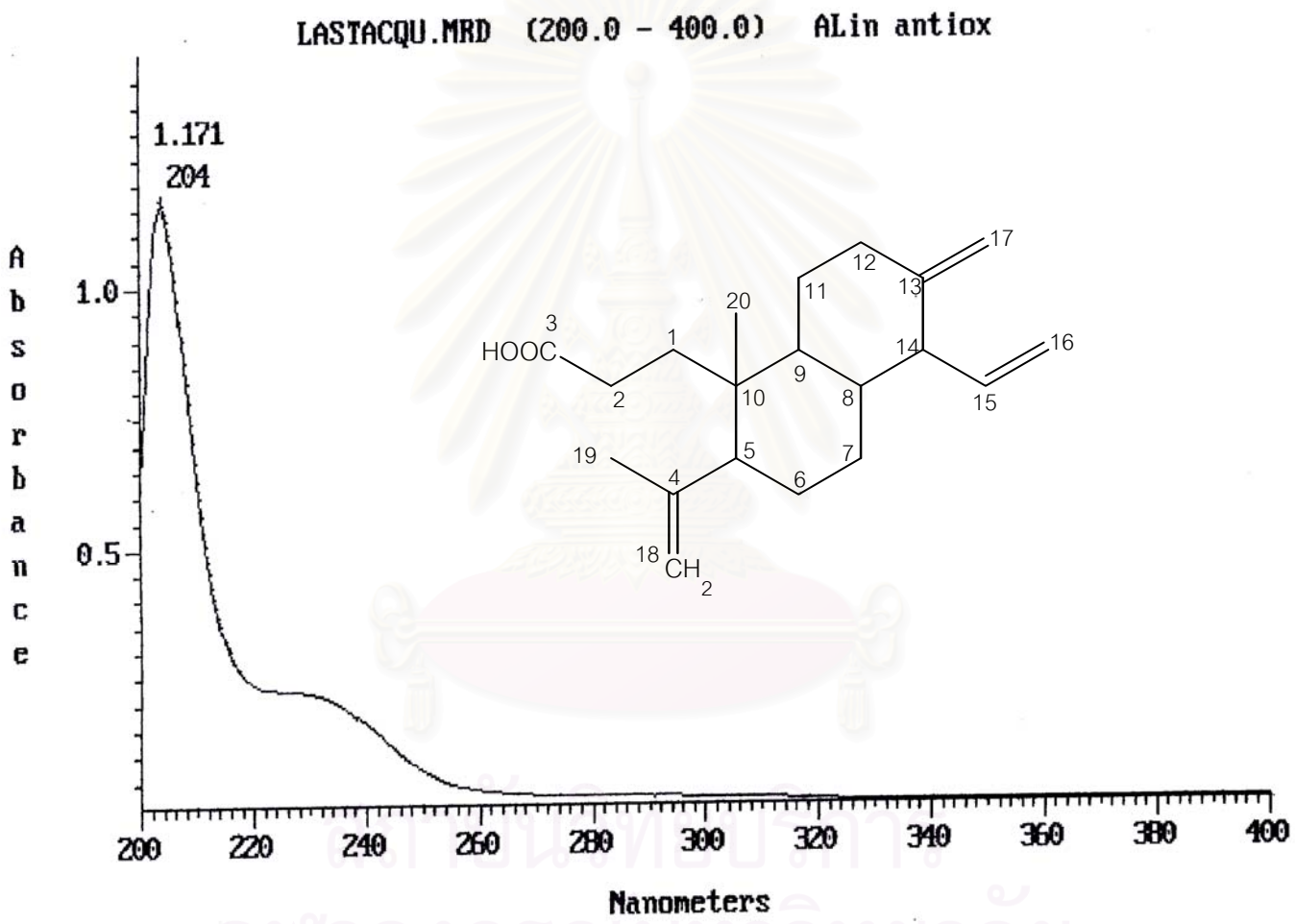


Figure 20. The UV spectrum of compound COL-2 (in MeOH)

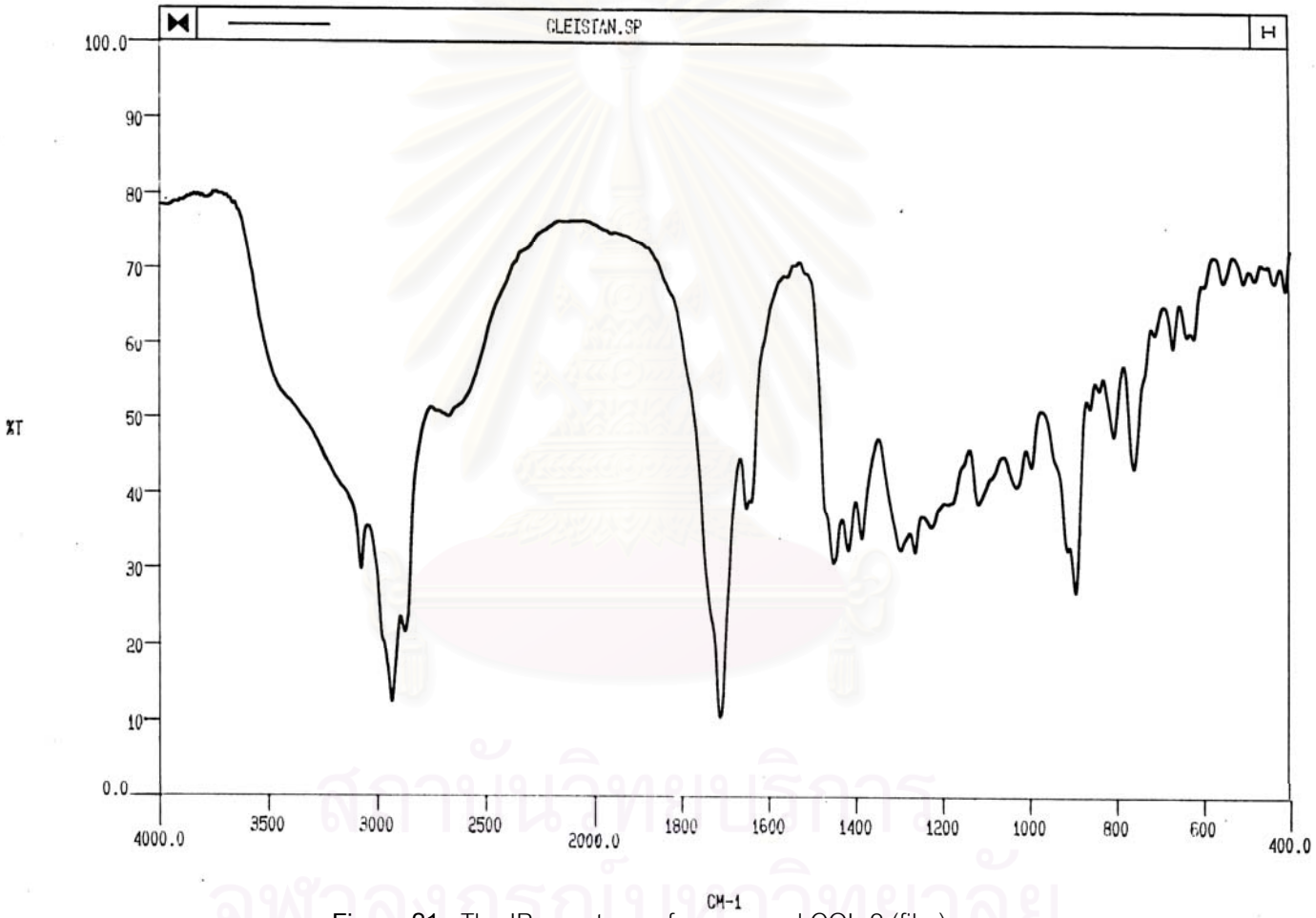


Figure 21. The IR spectrum of compound COL-2 (film)

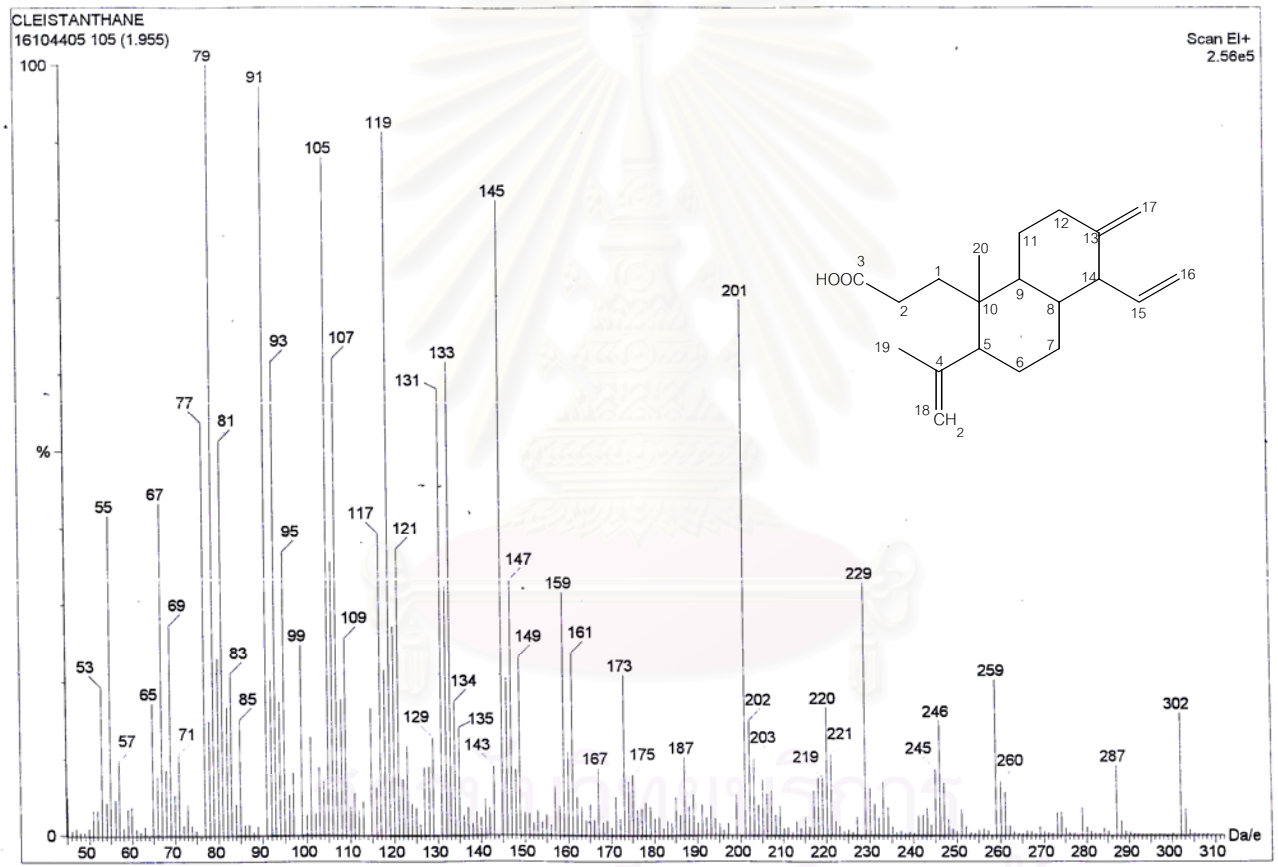


Figure 22. The EIMS spectrum of compound COL-2

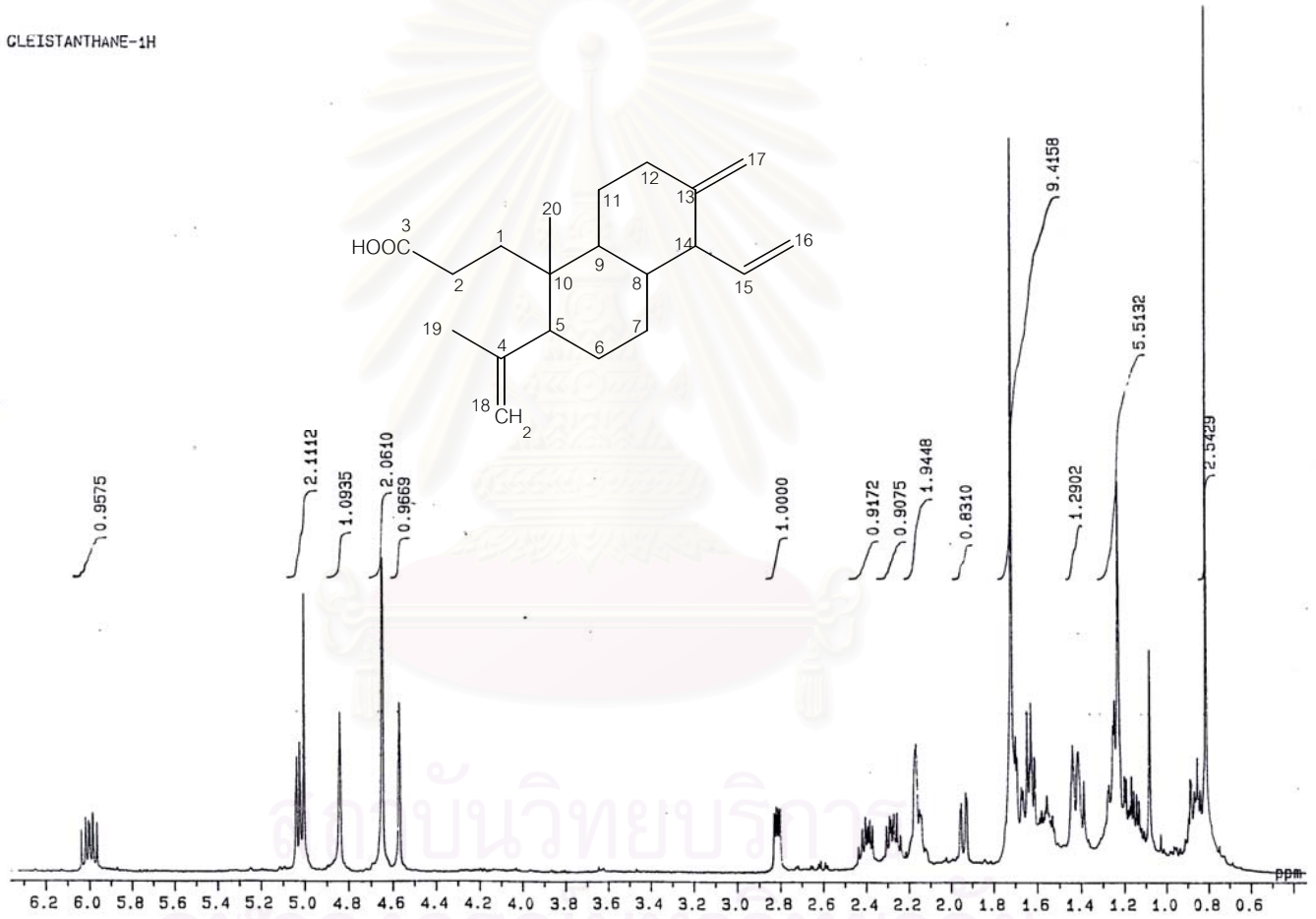


Figure 23a. The 500 MHz ^{1}H-NMR spectrum of compound COL-2 (in $CDCl_3$)

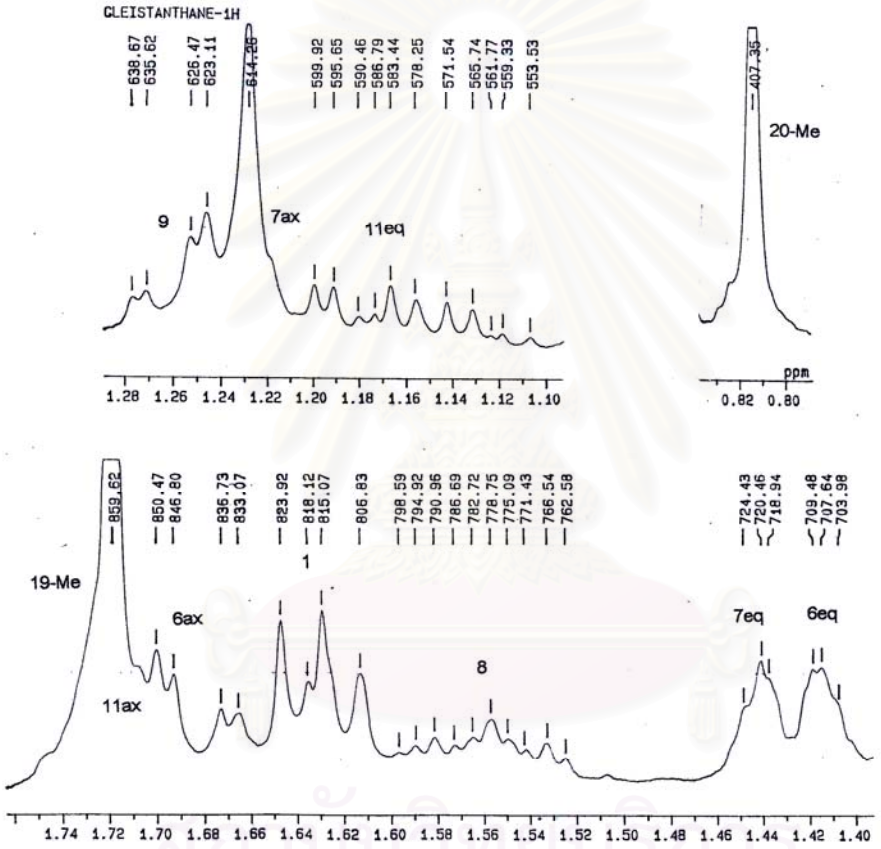


Figure 23b. The expanded 500 MHz ^{1}H-NMR spectrum of compound COL-2 (in $CDCl_3$)

(δ_H 0.8-1.8 ppm)

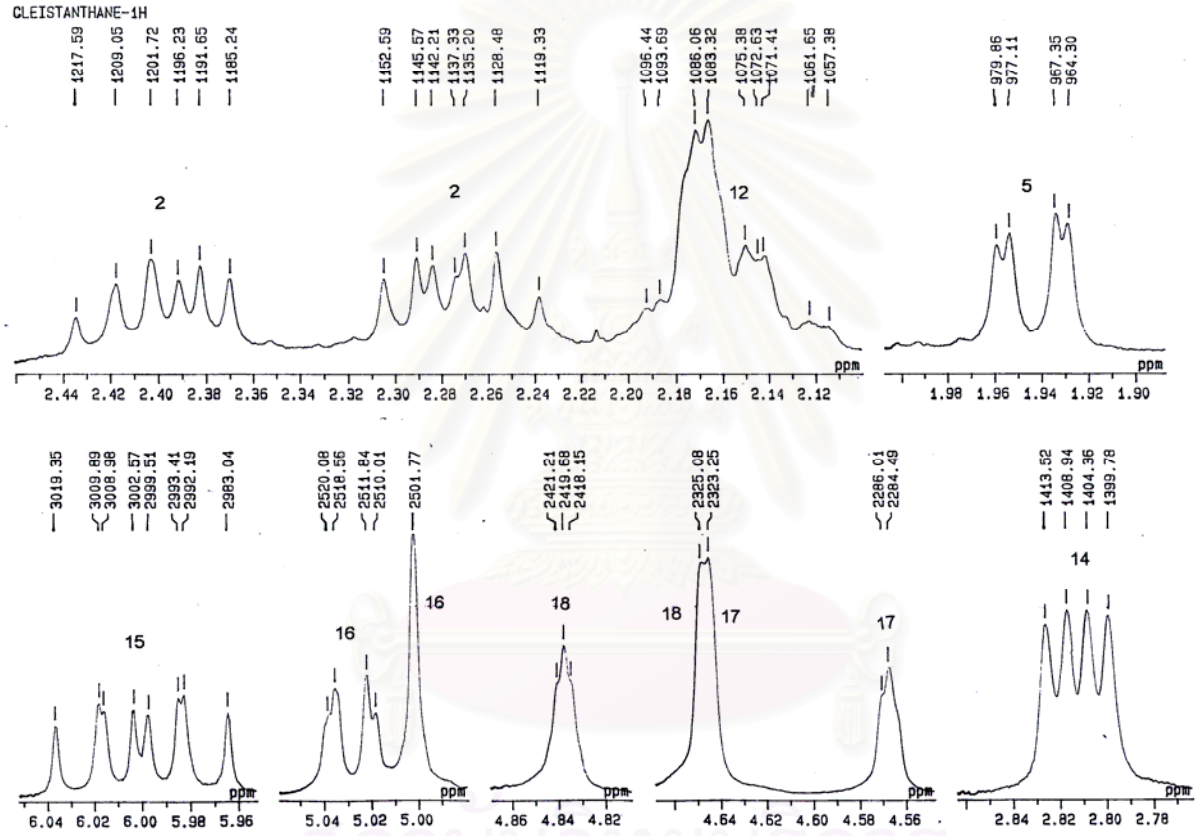
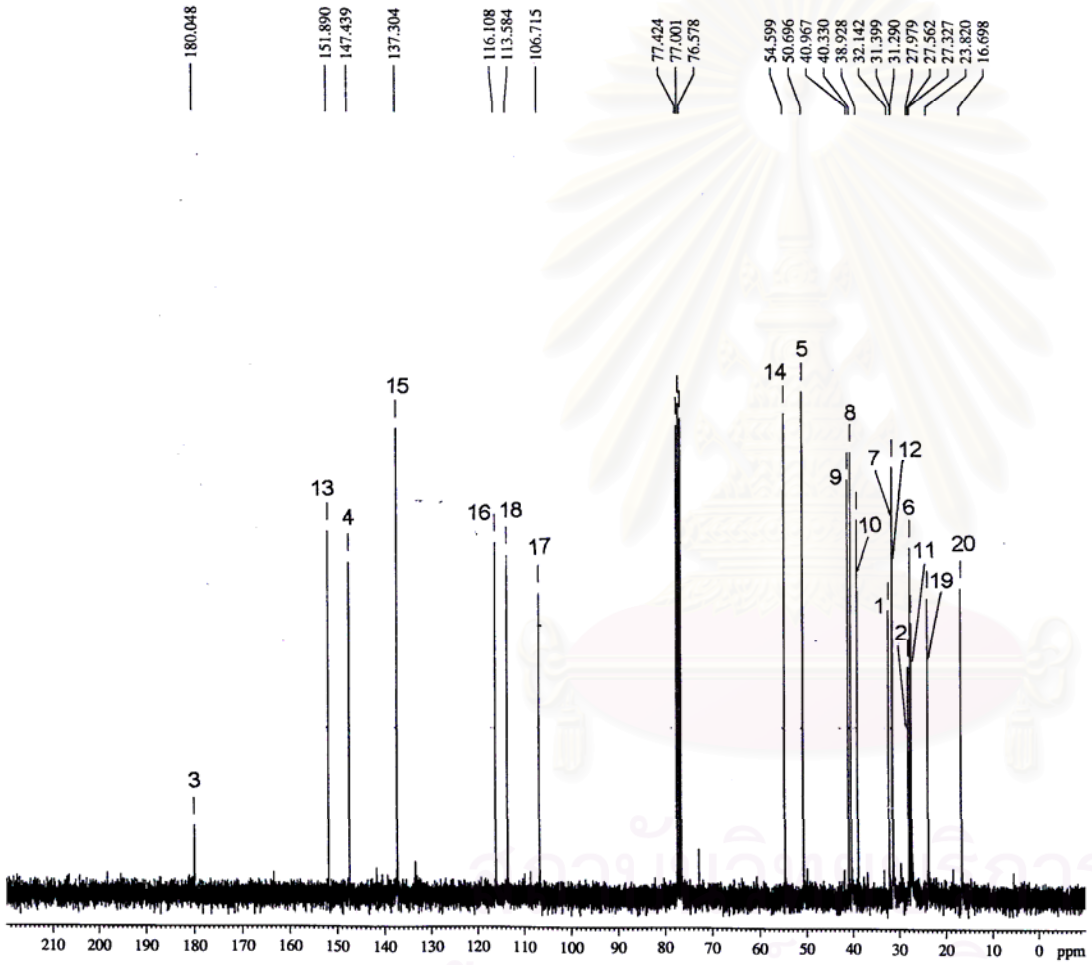


Figure 23c. The expanded 500 MHz ^{1}H-NMR spectrum of compound COL-2 (in $CDCl_3$)

(δ_H 1.9-6.1 ppm)



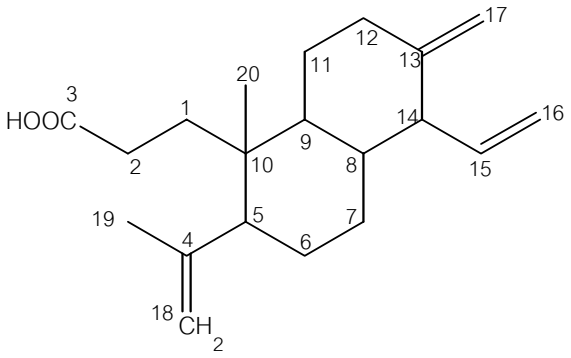
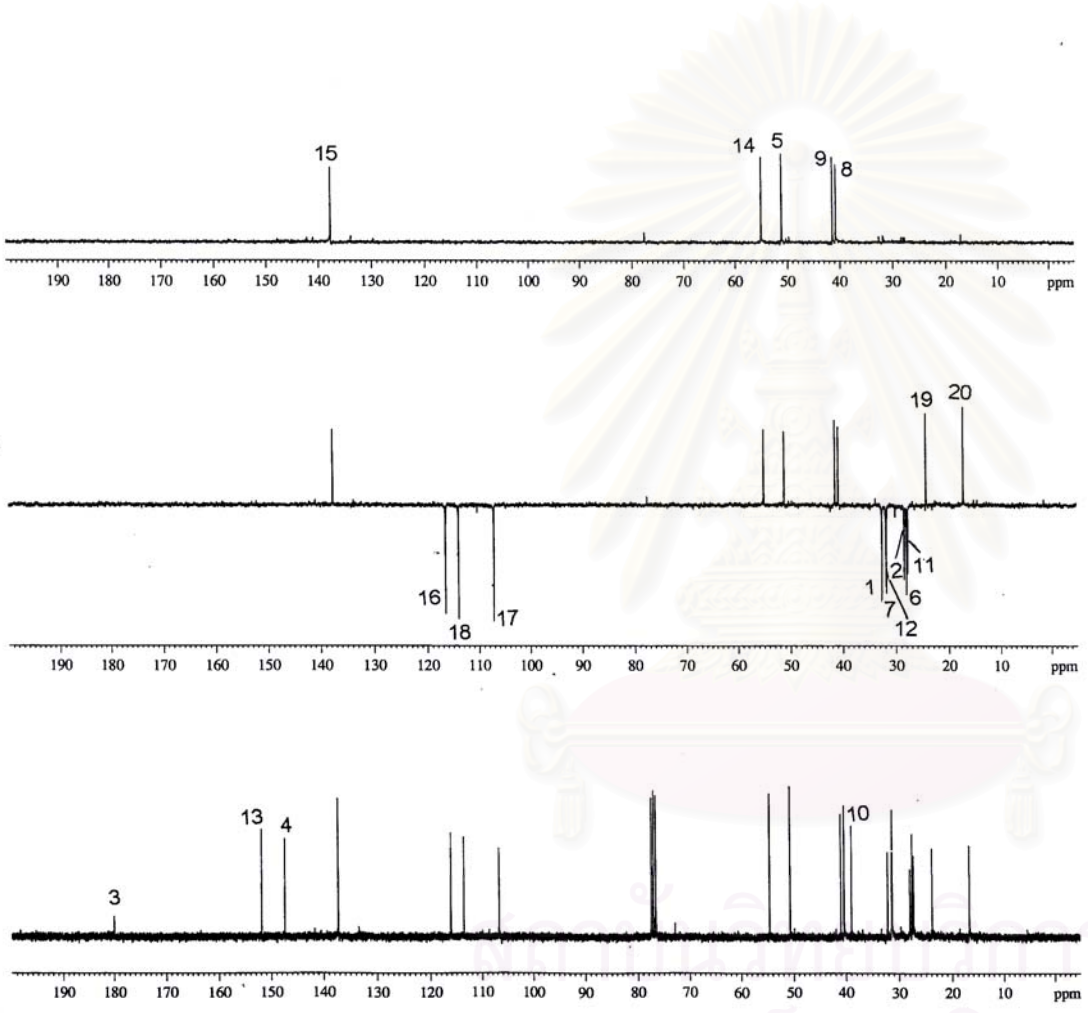


Figure 24. The 75 MHz ^{13}C-NMR spectrum of compound COL-2 (in $CDCl_3$)



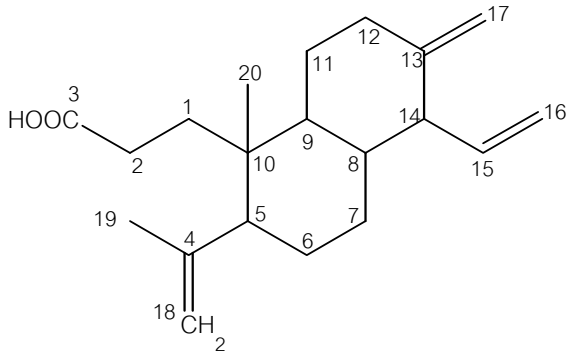
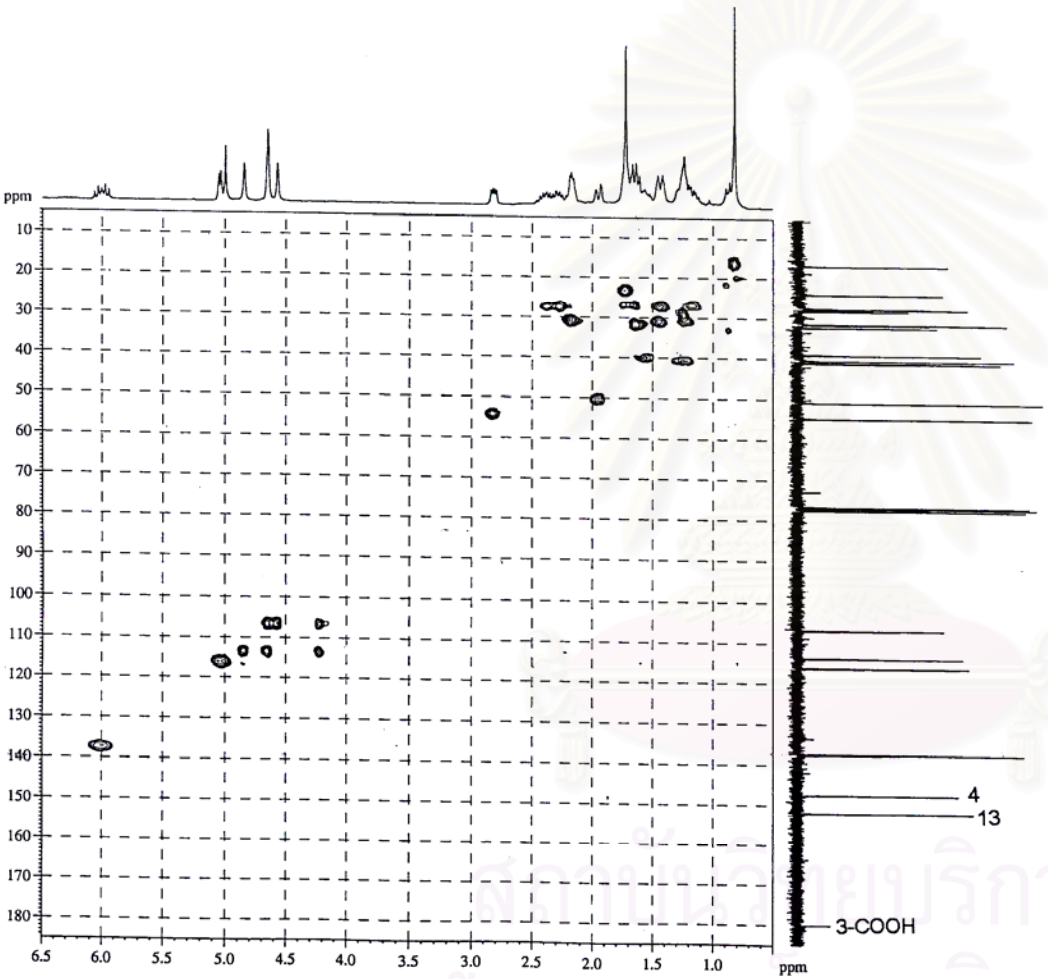


Figure 25. The 75 MHz ^{13}C-NMR, DEPT-90 and DEPT-135 spectra of compound COL-2 (in $CDCl_3$)



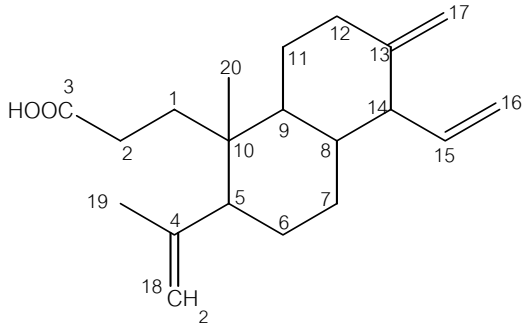


Figure 26a. The 300 MHz HMQC spectrum of compound COL-2 (in $CDCl_3$)

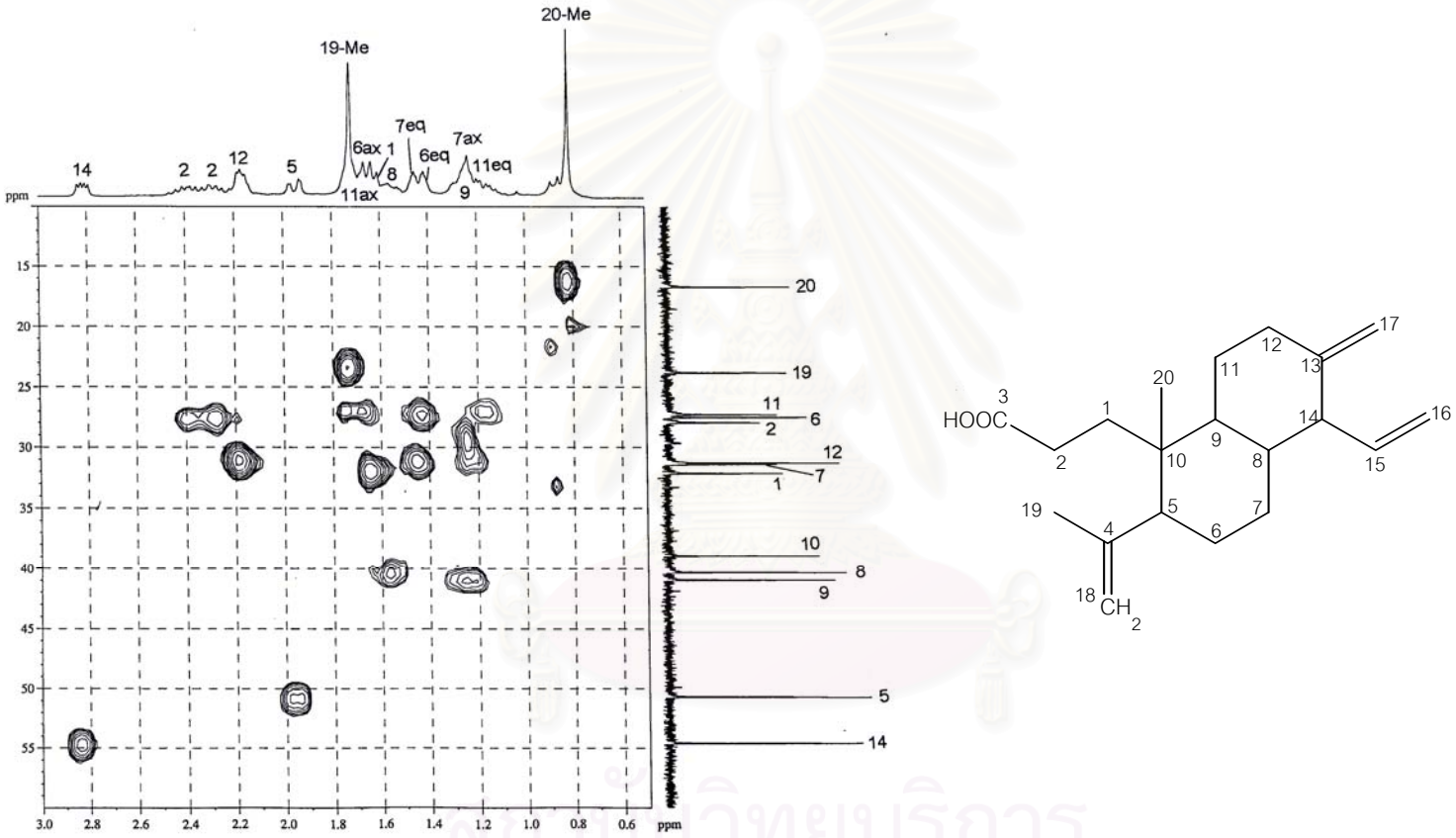


Figure 26b. The expanded 300 MHz HMQC spectrum of compound COL-2 (in $CDCl_3$)

(δ_H 0.6-3.0 ppm, δ_C 10.0-60.0 ppm)

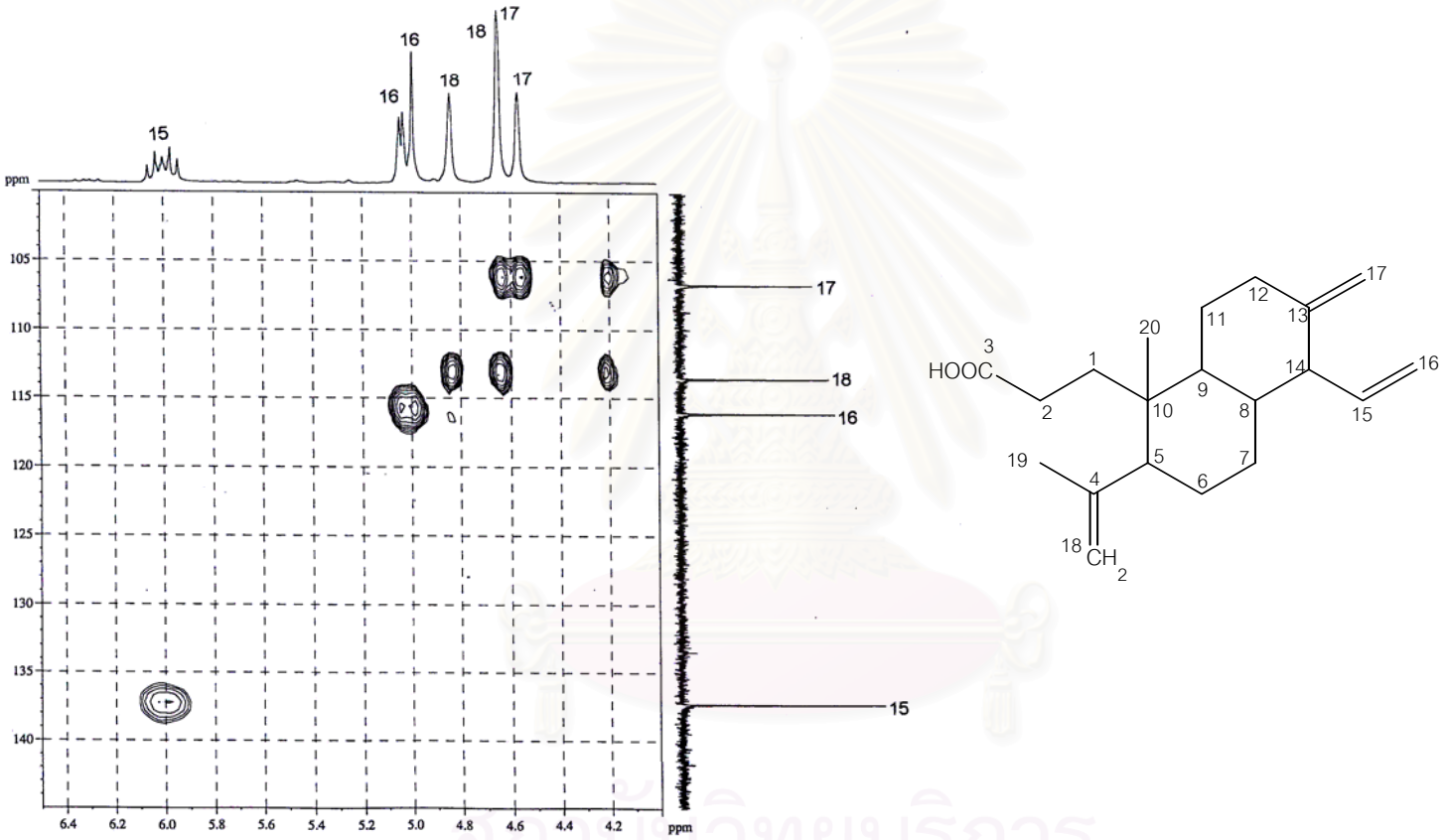


Figure 26c. The expanded 300 MHz HMQC spectrum of compound COL-2 (in $CDCl_3$)

(δ_H 4.1-6.4 ppm, δ_C 100.0-145.0 ppm)

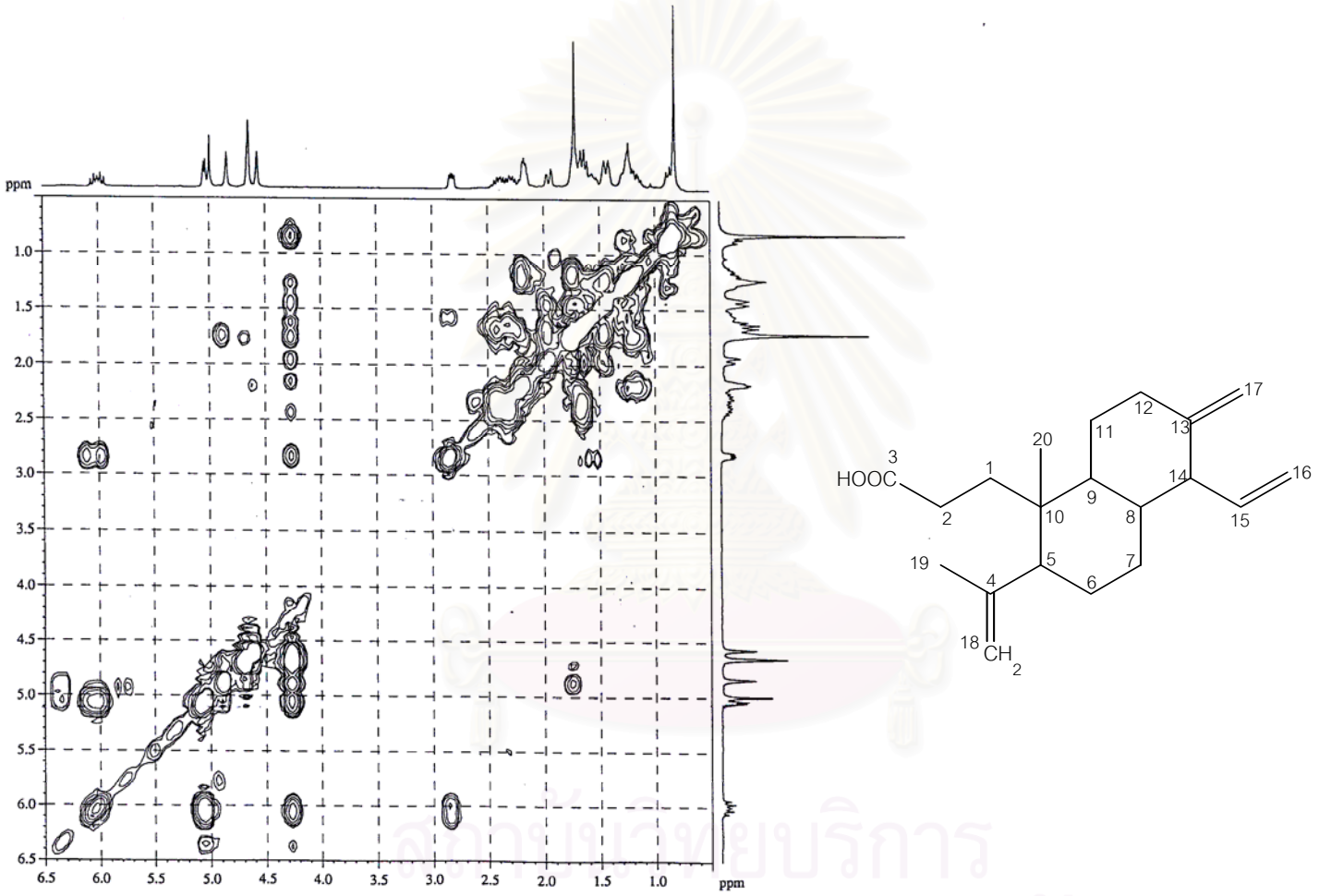


Figure 27a. The 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound COL-2 (in $CDCl_3$)

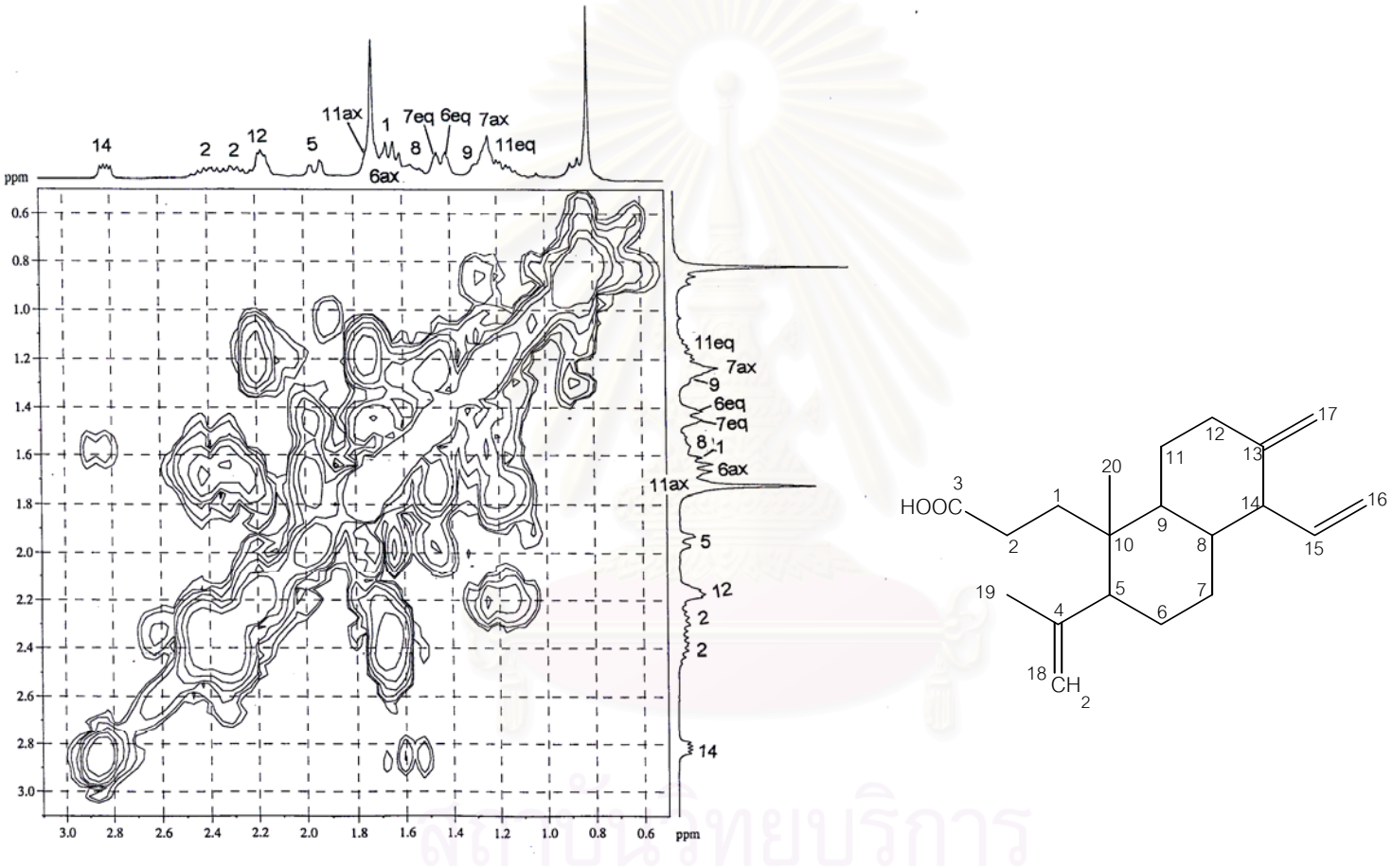


Figure 27b. The expanded 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound COL-2 (in $CDCl_3$)

(δ_H 0.6-3.0 ppm)

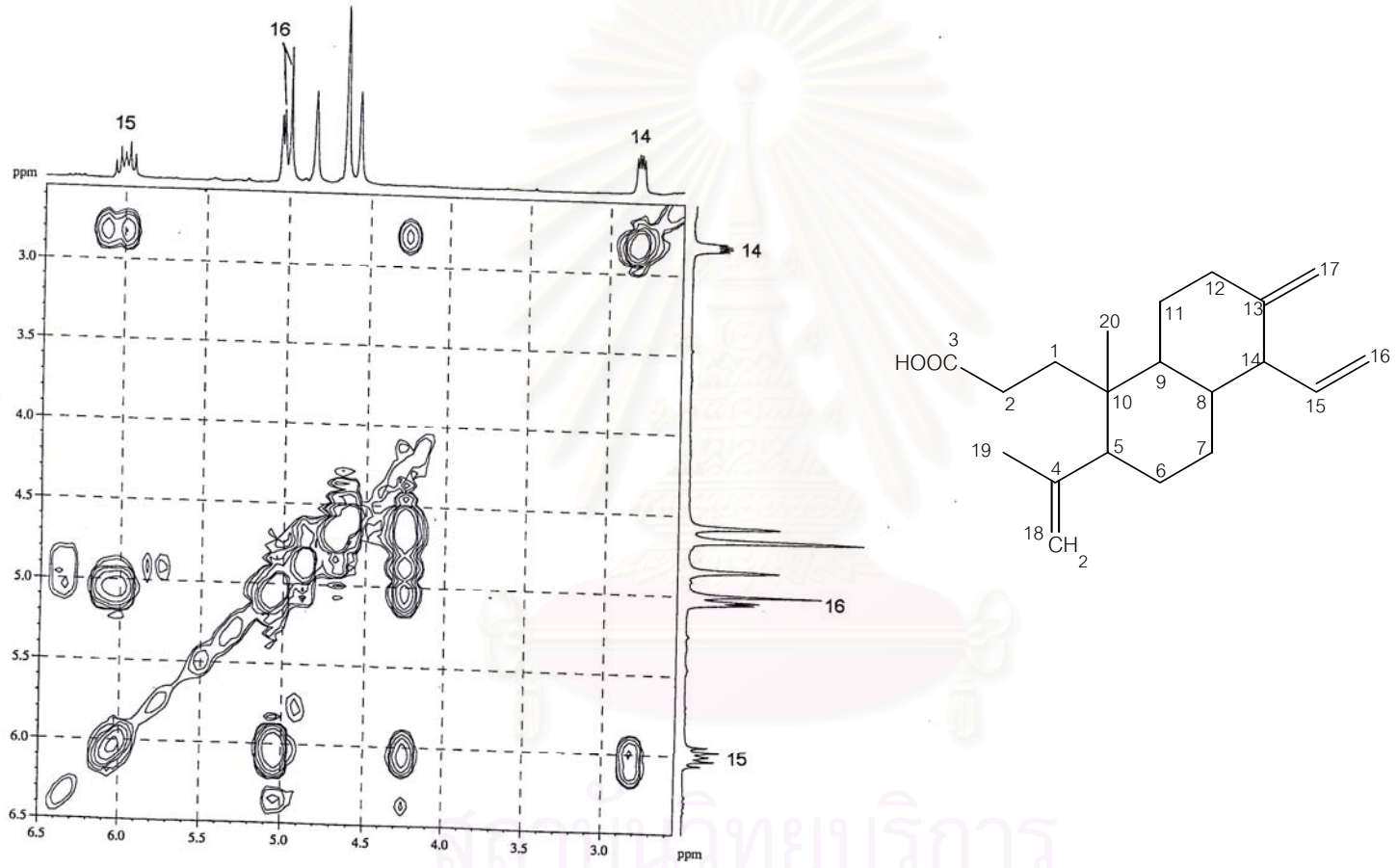


Figure 27c. The expanded 300 MHz ^{1}H-^{1}H COSY NMR spectrum of compound COL-2 (in $CDCl_3$) (δ_H 2.5-6.5 ppm)

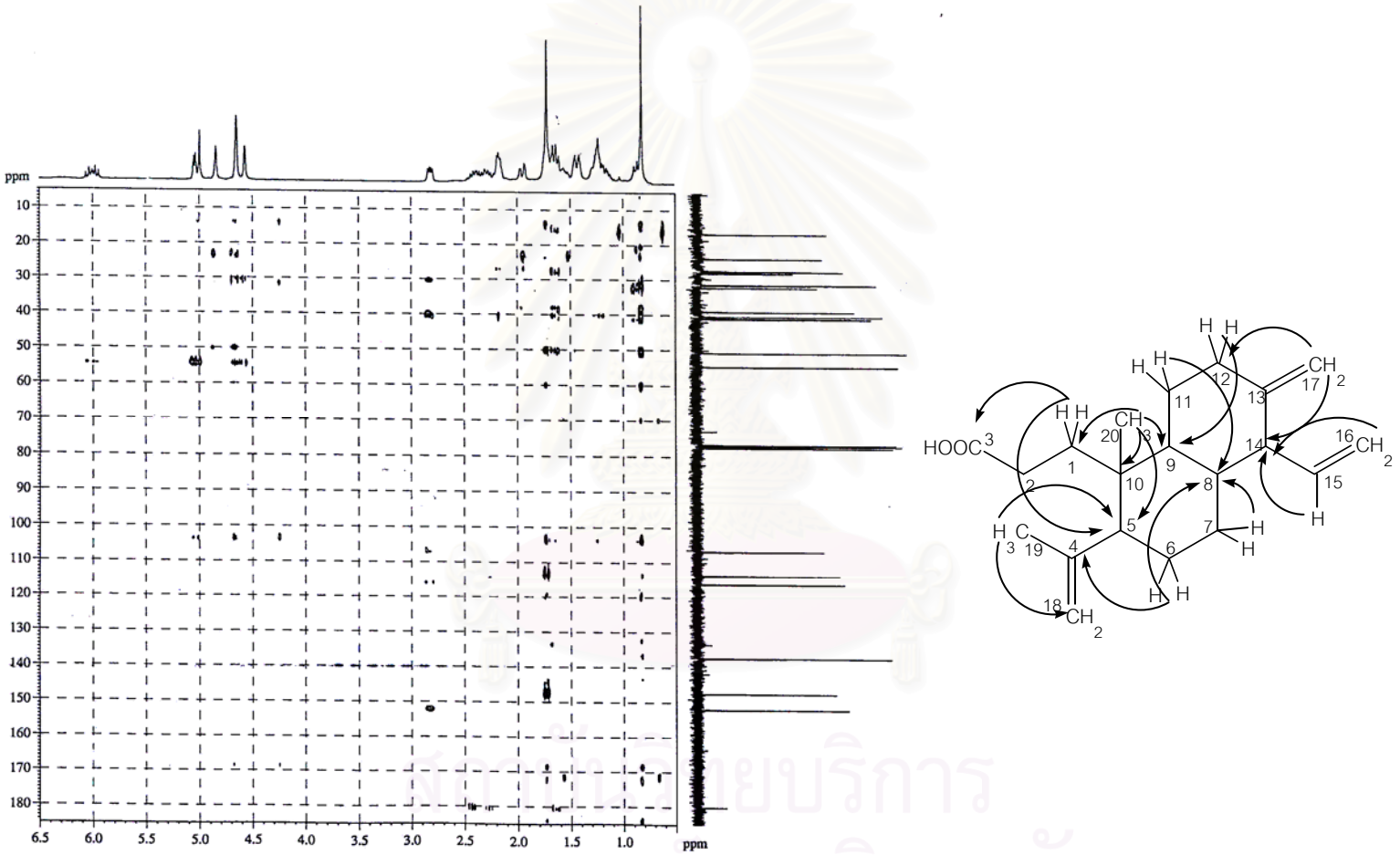


Figure 28a. The 300 MHz HMBC ($^{n}J_{CH}$= 8Hz) spectrum of compound COL-2 (in $CDCl_3$)

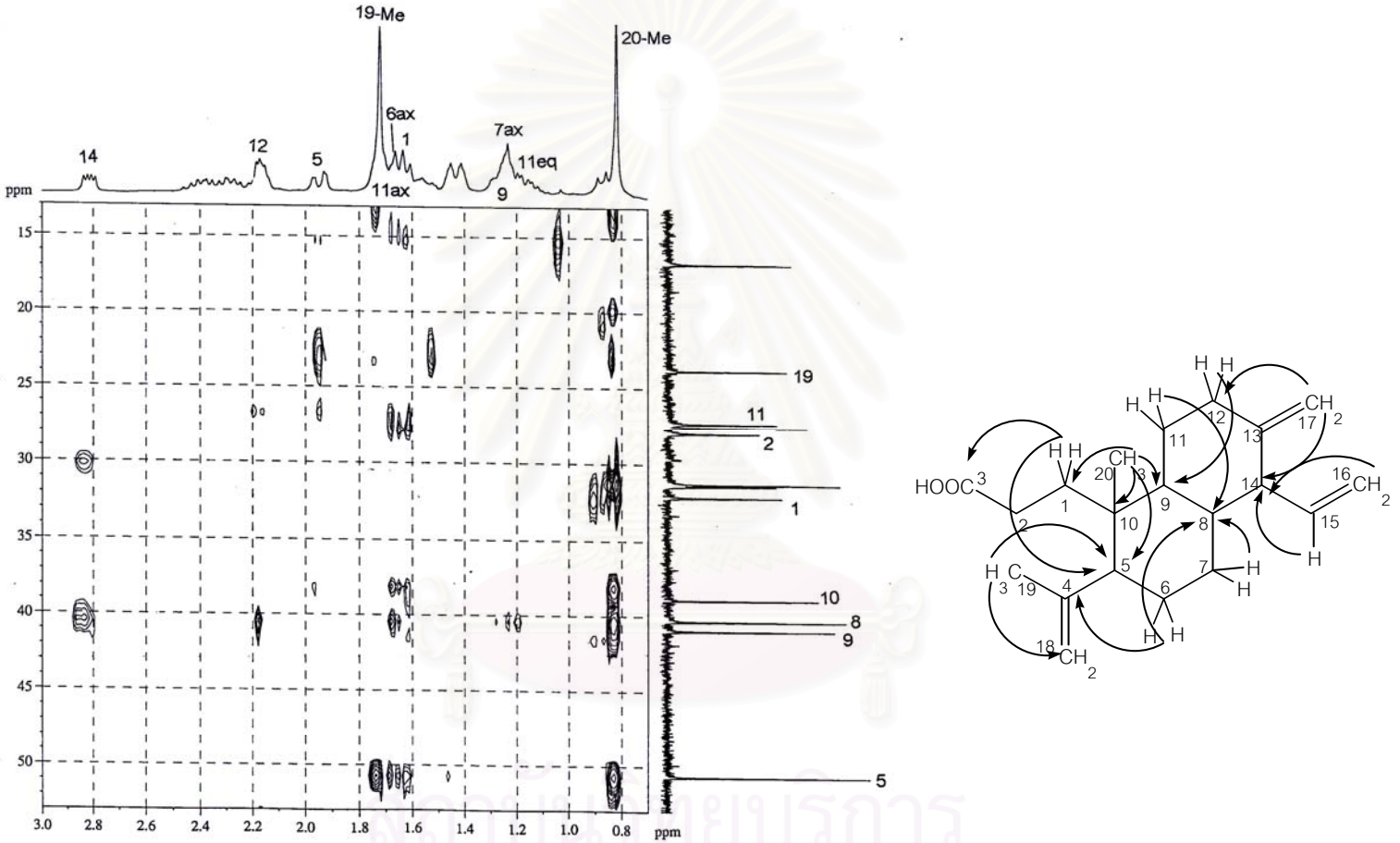


Figure 28b. The expanded 300 MHz HMBC ($^{n}J_{CH}$= 8Hz) spectrum of compound COL-2 (in $CDCl_3$)

(δ_H 0.8-3.0 ppm, δ_C 15.0-53.0 ppm)

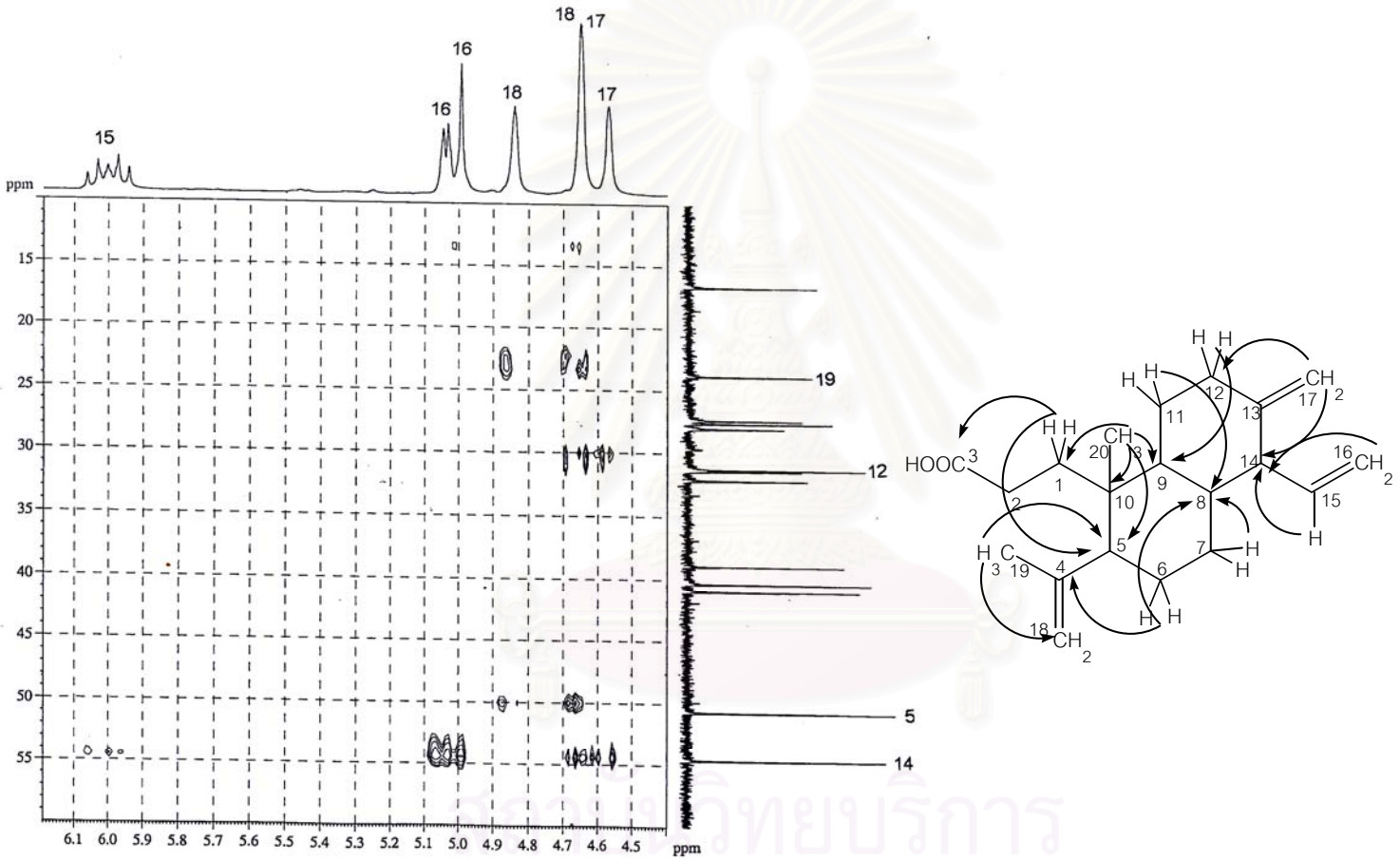


Figure 28c. . The expanded 300 MHz HMBC ($^{n}J_{CH}$= 8Hz) spectrum of compound COL-2 (in $CDCl_3$)

(δ_H 4.5-6.1 ppm, δ_C 15.0-55.0 ppm)

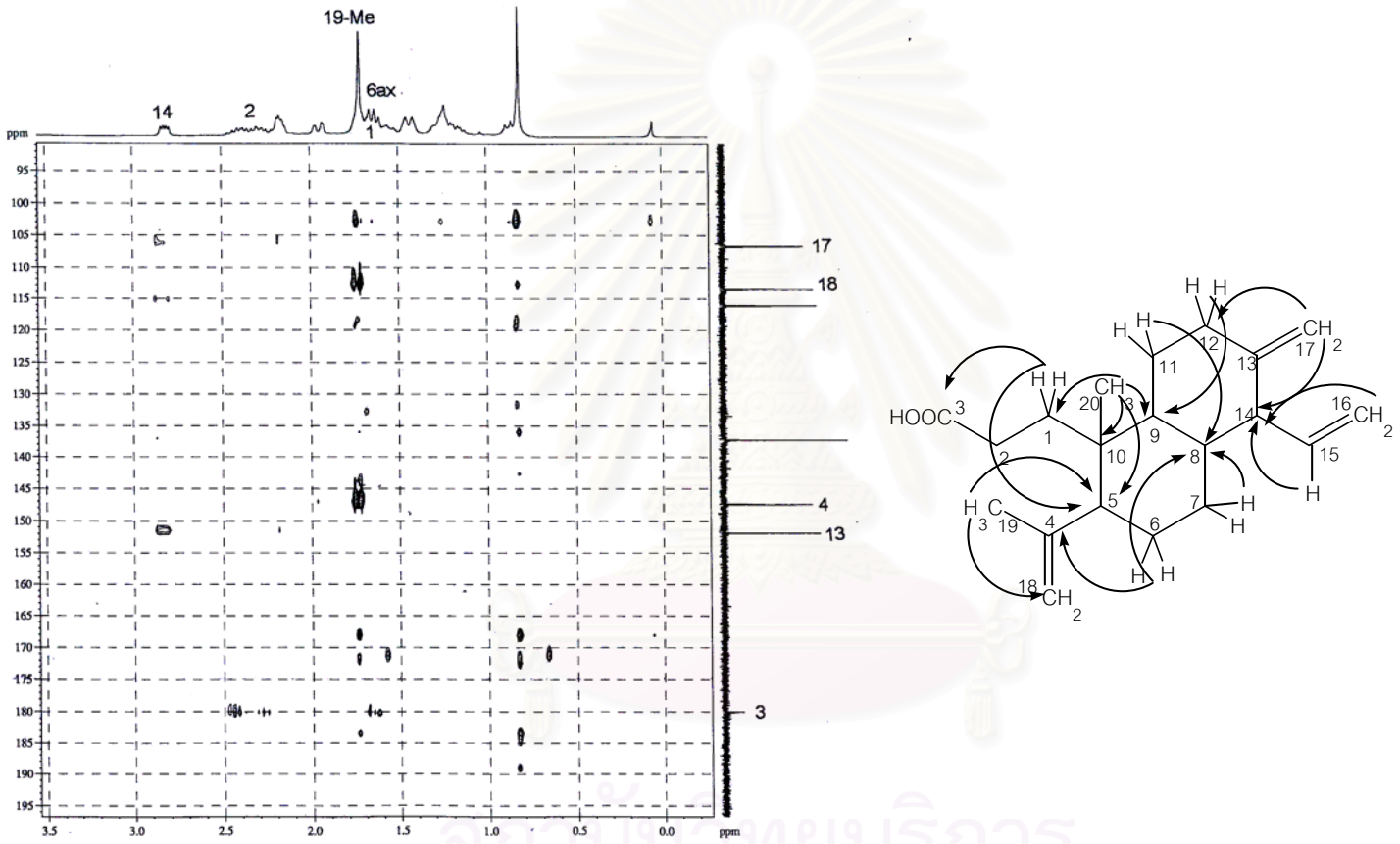


Figure 28d. The expanded 300 MHz HMBC ($^{n}J_{CH}$= 8Hz) spectrum of compound COL-2 (in $CDCl_3$)

(δ_H -0.3-3.5 ppm, δ_C 95.0-195.0 ppm)

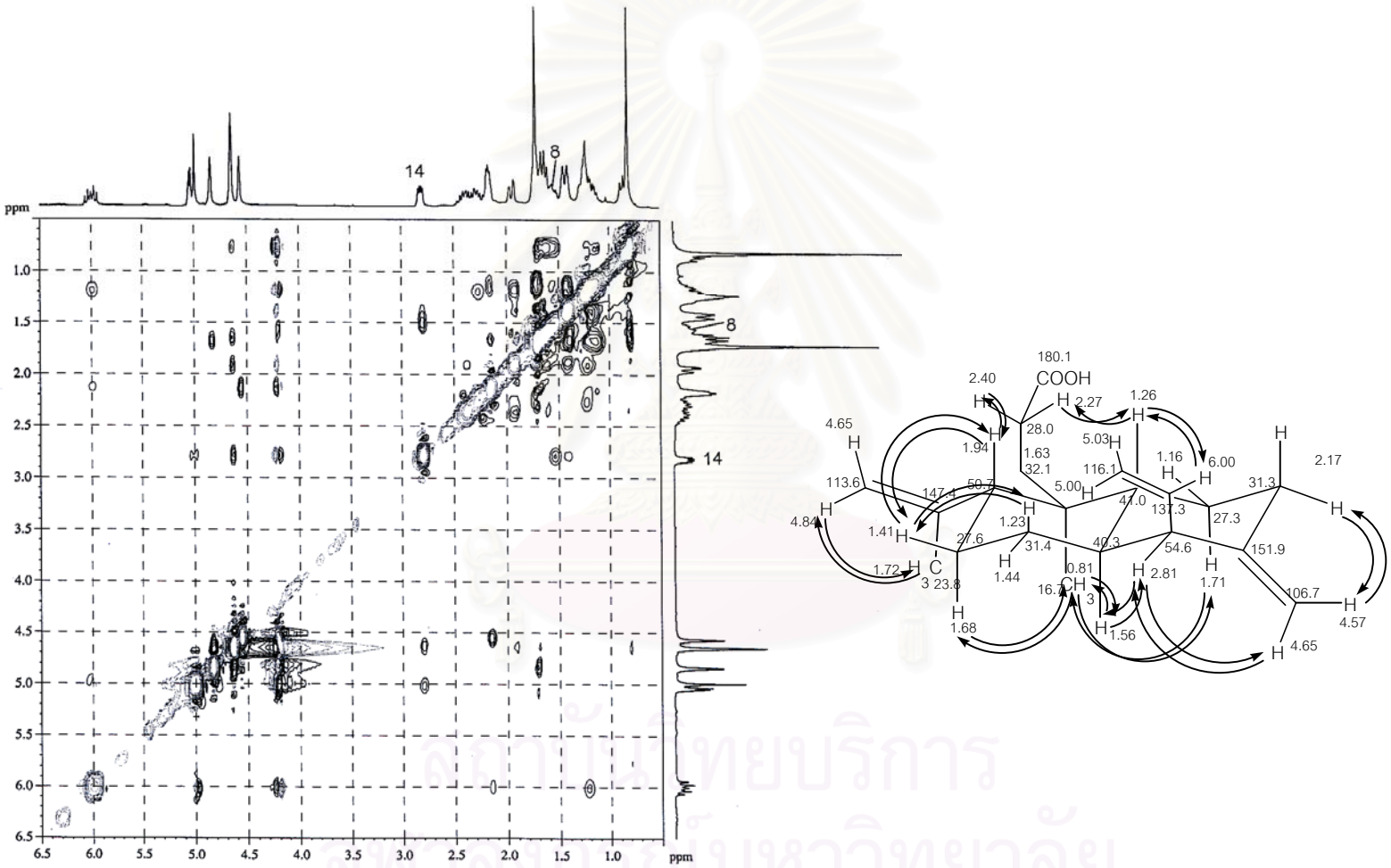


Figure 29a. The 300 MHz NOESY spectrum of compound COL-2 (in $CDCl_3$)

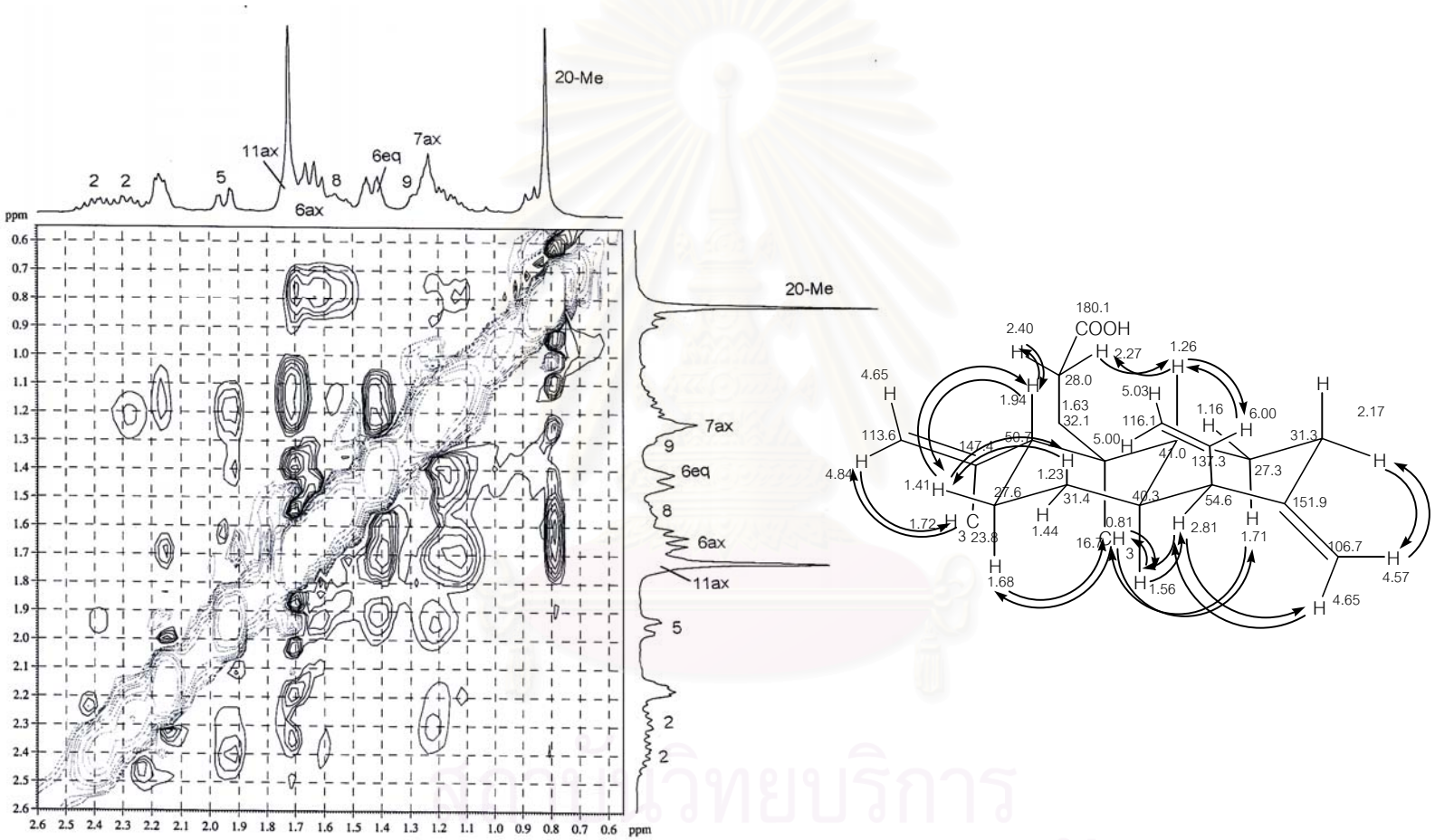
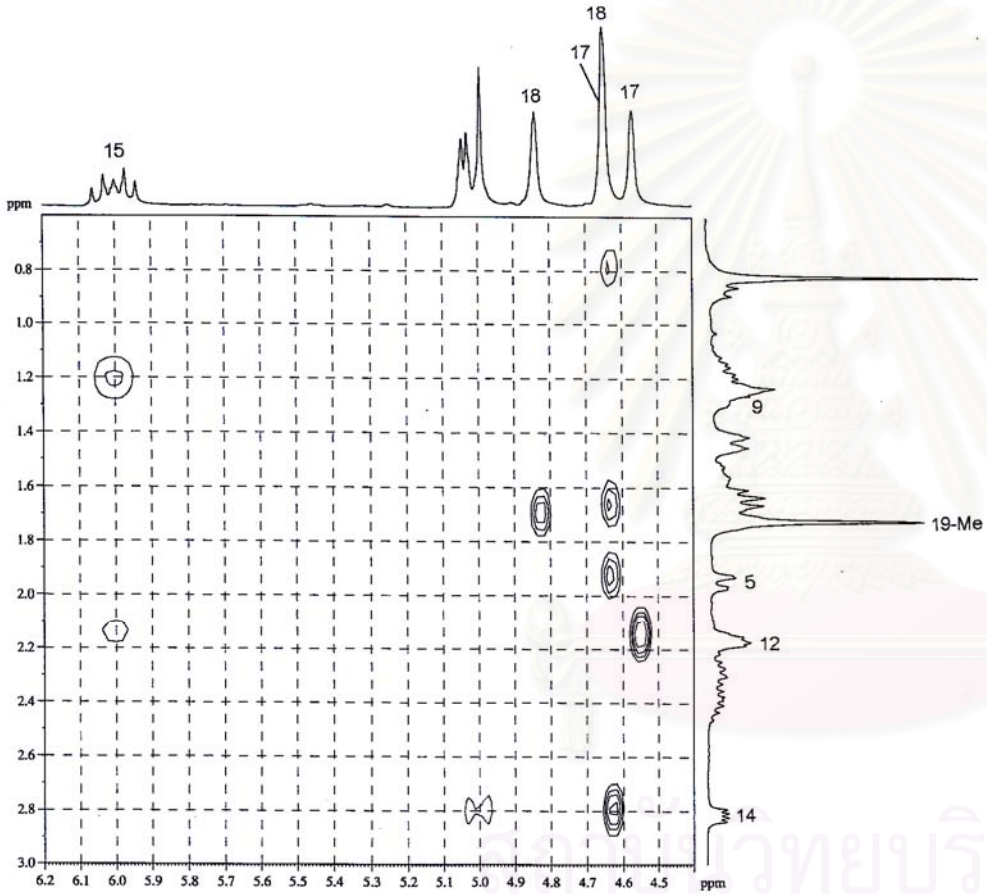


Figure 29b. The expanded 300 MHz NOESY spectrum of compound COL-2 (in $CDCl_3$)

(δ_H 0.6-2.6 ppm)



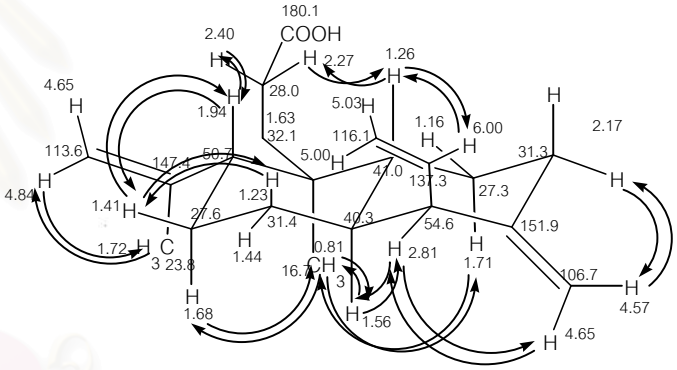


Figure 29c. The expanded 300 MHz NOESY spectrum of compound COL-2 (in $CDCl_3$)

(δ_H 0.6-3.0 ppm, 4.4-6.2 ppm)

Crystal data and structure refinement for Compound COL-1.

Empirical formula $C_{21}H_{24}O_6$

Formula weight 372.40

Temperature 293(2) K

Wavelength 0.71073 A^0

Crystal system, space group Orthorhombic, P2(1)2(1)2(1)

Unit cell dimensions a = 10.10890(10) A^0 alpha = 90 deg.

b = 10.14330(10) A^0 beta = 90 deg.

c = 18.2541(2) A^0 gamma = 90 deg.

Volume 1871.73(3) A^{03}

Z, Calculated density 4, 1.322 Mg/m^3

Absorption coefficient 0.096 m^{-1}

F(000) 792

Theta range for data collection 2.23 to 30.46 deg.

Limiting indices $-14 \leq h \leq 14$, $-14 \leq k \leq 11$, $-25 \leq l \leq 20$

Reflections collected / unique 13816 / 5403 [R(int) = 0.0181]

Completeness to theta = 30.46 97.3 %

Refinement method Full-matrix least-squares on F^2

Data / restraints / parameters 5403 / 0 / 341

Goodness- of –fit on F^2 0.873

Final R indices [I>2 sigma(I)] R1 = 0.0364, wR2 = 0.1126

R indices (all data) R1 = 0.0425, wR2 = 0.1203

Absolute structure parameter 0.3(8)

Extinction coefficient 0.022(4)

Largest diff. Peak and hole 0.208 and –0.120 $e.A^{-3}$

Table 16. Atomic coordinates (x104) and equivalent isotropic displacement parameters (A2 x 103) for Compound COL-1.

U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
C(1)	41(1)	-205(1)	2578(1)	44(1)
C(2)	-1185(1)	537(2)	2845(1)	63(1)
C(3)	-1003(1)	1008(2)	3617(1)	59(1)
C(4)	188(1)	1157(1)	3906(1)	46(1)
C(5)	1492(1)	915(1)	3526(1)	37(1)
C(6)	2433(1)	2110(1)	3585(1)	48(1)
C(7)	3734(1)	1813(2)	3200(1)	54(1)
C(8)	3565(1)	1422(1)	2389(1)	42(1)
C(9)	2529(1)	284(1)	2265(1)	35(1)
C(10)	1267(1)	663(1)	2700(1)	33(1)
C(11)	1294(1)	151(1)	1438(1)	41(1)
C(12)	1341(1)	1207(1)	1082(1)	42(1)
C(13)	393(1)	799(1)	509(1	42(1)
C(14)	-424(2)	1668(2)	79(1)	58(1)
C(15)	-1111(2)	912(2)	-377(1)	69(1)
C(16)	127(2)	-433(2)	256(1)	56(1)
C(17)	4915(1)	1031(2)	2093(1)	53(1)
C(18)	452(2)	1008(2)	4702(1)	58(1)
C(19)	1987(2)	-200(2)	4036(1)	50(1)
C(20)	3078(1)	-1068(1)	2502(1)	47(1)
C(21)	6392(2)	1128(3)	1095(1)	69(1)
O(1)	1514(1)	192(1)	4767(1)	65(1)
O(2)	-124(2)	1428(2)	5233(1)	83(1)
O(3)	5129(1)	1468(1)	1417(1)	58(1)
O(4)	1423(2)	2376(1)	1240(1)	62(1)
O(5)	-796(2)	-394(1)	-284(1)	73(1)
O(6)	5721(1)	395(2)	2424(1)	93(1)

Table 17. Bond lengths [A^0] for Compound COL-1.

	Bond lengths [A^0]		Bond lengths [A^0]
C(1)-C(2)	1.530(2)	C(9)-C(11)	1.5527(15)
C(1)-C(10)	1.5359(15)	C(11)-C(12)	1.5409(17)
C(2)-C(3)	1.500(3)	C(12)-O(4)	1.2228(18)
C(3)-C(4)	1.322(2)	C(12)-C(13)	1.4782(17)
C(4)-C(18)	1.484(2)	C(13)-C(16)	1.359(2)
C(4)-C(5)	1.5102(16)	C(13)-C(14)	1.440(2)
C(5)-C(10)	1.5452(14)	C(14)-C(15)	1.329(3)
C(5)-C(6)	1.5445(17)	C(15)-O(5)	1.373(3)
C(5)-C(19)	1.5485(17)	C(16)-O(5)	1.3582(17)
C(6)-C(7)	1.521(2)	C(17)-O(6)	1.203(2)
C(7)-C(8)	1.5420(17)	C(17)-O(3)	1.3285(18)
C(8)-C(17)	1.5203(17)	C(18)-O(2)	1.2083(19)
C(8)-C(9)	1.5750(16)	C(18)-O(1)	1.361(2)
C(9)-C(20)	1.509(17)	C(19)-O(1)	1.4709(17)
C(9)-C(10)	1.5516(14)	C(21)-O(3)	1.4468(19)

Table 18. Bond angles [deg] for Compound COL-1.

Angles	[A^0]	Angles	[A^0]
C((2)-C(1)-(C10)	108.97(11)	C(1)-C(10)-C(5)	110.85(9)
C(3)-C(2)-C(1)	110.93(12)	C(1)-C(10)-C(9)	116.56(9)
C(4)-C(3)-C(1)	121.48(13)	C(5)-C(10)-C(9)	114.76(8)
C(3)-C(4)-C(18)	122.81(13)	C(12)-C(11)-C(9)	118.59(10)
C(3)-C(4)-C(5)	126.38(12)	O(4)-C(12)-C(13)	118.86(12)
C(18)-C(4)-C(5)	106.02(12)	O(4)-C(12)-C(11)	122.91(12)
C(4)-C(5)-C(10)	110.25(9)	C(13)-C(12)-C(11)	118.22(11)
C(4)-C(5)-C(6)	112.22(10)	C(16)-C(13)-C(14)	105.36(13)
C(10)-C(5)-C(6)	106.83(8)	C(16)-C(13)-C(12)	128.77(12)
C(4)-C(5)-C(19)	97.13(10)	C(14)-C(13)-C(12)	125.85(13)
C(10)-C(5)-C(19)	120.91(10)	C(15)-C(14)-C(13)	106.74(16)
C(6)-C(5)-C(19)	109.35(11)	C(14)-C(15)-O(5)	110.95(14)
C(7)-C(6)-C(5)	110.15(11)	O(5)-C(16)-C(13)	110.80(14)
C(6)-C(7)-C(8)	113.52(11)	O(6)-C(17)-O(3)	122.35(14)
C(17)-C(8)-C(7)	108.06(10)	O(6)-C(17)-C(8)	124.67(13)
C(17)-C(8)-C(9)	110.76(11)	O(3)-C(17)-C(8)	112.98(12)
C(7)-C(8)-C(9)	113.57(9)	O(2)-C(18)-O(1)	121.59(16)
C(20)-C(9)-C(10)	111.89(9)	O(2)-C(18)-C(4)	131.51(18)
C(20)-C(9)-C(11)	105.90(10)	O(1)-C(18)-C(4)	106.83(12)
C(10)-(9)-C(11)	109.84(8)	O(1)-C(19)-C(5)	104.11(11)
C(20)-C(9)-C(8)	111.86(10)	C(18)-O(1)-C(19)	109.93(11)
C(10)-C(9)-C(8)	106.99(9)	C(17)-O(3)-C(21)	116.22(15)
C(11)-C(9)-C(8)	110.40(9)	C(16)-O(5)-C(15)	106.15(13)

VITA

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