สารออกฤทธิ์ทางชีวภาพจากลำต้นและเปลือกมะสัง *Feroniella lucida*

นายสิทธิเดช สมบุญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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BIOACTIVE COMPOUNDS FROM THE STEMS AND STEM BARK OF *Feroniella lucida*

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry

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Chulalongkorn University

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สิทธิเดช สมบุญ : สารออกฤทธิ์ทางชีวภาพจากลำต้นและเปลือกมะสัง *Feroniella lucida* (BIOACTIVE COMPOUNDS FROM THE STEMS AND STEM BARK OF *Feroniella lucida*) อ. ที่ปรึกษา : รศ. ดร.สันติ ทิพยางค์ อ.ที่ปรึกษาร่วม : ผศ. ดร.ปรีชา ภูวไพรศิริศาล 63 หน้า.

ในการศึกษาสารออกฤทธิ์ทางชีวภาพจากพืชสมุนไพรไทยในวงศ์ Rutaceae จึงได้เลือกสิ่งสกัดไดคลอโรมีเทนจากลำต้นมะสังมาแยก ทำให้บริสุทธิ์ และหาสูตรโครงสร้าง จากการแยกสารจากสิ่งสกัดไดคลอโรมีเทนนี้โดยวิธีทางโครมาโทกราฟี ได้สารฟิวแรโนคูมารินชนิดใหม่ 1 ชนิดคือ feroniellin A (**7**) และคูมารินที่มีรายงานมาก่อน 8 ชนิด คือ psoralen (**1**), bergapten (**2**), isopimpinellin (**3**), anisolactone (**4**), 2′,3′-epoxyanisolactone (**5**), marmesin (**6**), feroniellin B (**9**) และ feroniellin C (**8**) นอกจากนี้ยังสกัดส่วนเปลือกของมะสังด้วยไดคลอโรมีเทนและแยกสารจากสิ่งสกัดนี้ ได้สารประเภท triterpenoid 7 ชนิด ซึ่ง 3 ชนิด เป็นสารใหม่ ได้แก่ feroniellide C (**13**), feroniellide D (**15**), และ feroniellide E (**16**) และอีก 4 ชนิดเป็นสารที่มีรายงานแล้วคือสารในกลุ่มของ *N*-methylanthranilic ester triterpenoid (**10-12**) และ isovaleric acid ester triterpenoid (**14**) พิสูจน์สูตรโครงสร้างของสารทั้งหมดที่แยกได้ โดยวิธีทางสเปกโทรสโกปี และเปรียบเทียบกับข้อมูลที่มีรายงานแล้วนำสารทั้งหมดที่แยกได้ไปทดสอบฤทธิ์ในการยับยั้งเอนไซม์ acetylcholinesterase และ ความเป็นพิษต่อเซลล์มะเร็งชนิด HeLa และ KB พบว่า สาร **3**, **6**, **7**, **8**, **9**, **11**, และ **12** แสดงฤทธิ์ยับยั้งเอนไซม์ acetylcholinesterase ที่ความเข้มข้น 0.500 mg/mL นอกจากนั้น พบว่า สาร **14** แสดงความเป็นพิษต่อเซลล์มะเร็งชนิด HeLa ที่ IC_{50} = 8.2 μg/mL ขณะที่สาร **10**, **15**, และ **16** มีความเป็นพิษต่อเซลล์มะเร็งชนิด KB สูงที่ IC_{50} = 5.2, 4.1, และ 3.4 μg/mL ตามลำดับ

ภาควิชา.....เคมี..... ลายมือชื่อนิสิต.....สิทธิเดช สมบุญ.....

สาขาวิชา.....เคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....สันติ ทิพยางค์.....

ปีการศึกษา 2549 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....ปรีชา ภูวไพรศิริศาล.....

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SITTHIDESCH SOMBUND : BIOACTIVE COMPOUNDS FROM THE STEMS AND STEM BARK OF *Feroniella lucida*. THESIS ADVISOR : ASSOC. PROF. SANTI TIP-PYANG, Ph.D., THESIS COADVISOR : ASST. PROF. PREECHA PHUWAPRAISIRISAN, Ph.D., 63 pp.

In phytochemical investigation for bioactive compounds from Thai medicinal plants in the family Rutaceae, dichloromethane crude extract from the stems of *Feroniella lucida* was selected for isolation, purification and structure elucidation. The chromatographic separation of dichloromethane crude extract led to the isolation of a novel furanocoumarin, feroniellin A (**7**), along with eight known coumarins, psoralen (**1**), bergapten (**2**), isopimpinellin (**3**), anisolactone (**4**), 2′,3′-epoxyanisolactone (**5**), marmesin (**6**), feroniellin B (**9**), and feroniellin C (**8**). On the other hand, the dried stem bark of this plant was macerated in dichloromethane. This extract was isolated to afford seven triterpenoids. They were three new triterpenoids; feroniellide C (**13**), feroniellide D (**15**), and feroniellide E (**16**) and four known triterpenoids; *N*-methyl anthranilic ester triterpenoid (**10-12**) and isovaleric acid ester triterpenoid (**14**). The structures of all isolated compounds were elucidated by spectroscopic methods as well as comparison with previous literature data. All of the isolated compounds were tested for inhibitory activity of acetylcholinesterase and cytotoxicity on HeLa and KB cell lines. Compounds **3**, **6**, **7**, **8**, **9**, **11**, and **12** showed inhibitory activity of acetylcholinesterase with MIC value of 0.500 mg/mL. In addition, compound **14** exhibited significant cytotoxicity activity against HeLa cell line with IC_{50} value of 8.2 μg/mL, while compounds **10**, **15**, and **16** also showed potent cytotoxicity against KB cell line with IC_{50} value of 5.2, 4.1, and 3.4 μg/mL, respectively.

Department.....Chemistry..... Student's signature.....Sitthidesch Sombund
Field of study.....Chemistry.....Advisor's signature.....Santi Tip-pyang
Academic year.....2006..... Co-advisor's signature.....P. Phuwapraisirisan

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^{13}C NMR	carbon 13 nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
° C	degree Celsius
brs	broad singlet (NMR)
BSA	bovine serum albumin
c	concentration
calcd	calculated
cat. No.	catalogue number
$CDCl_3$	deuterated chloroform
CH_2Cl_2	dichloromethane, methylene chloride
COSY	correlated spectroscopy
d	doublet (NMR)
dd	doublet of doublet (NMR)
ddd	doublet of doublet of doublet (NMR)
EtOAc	ethyl acetate
e.g.	for example
ESIMS	electrospray ionization mass spectrometry
g	gram (s)
H_2O	water
h	hour
HMBC	heteronuclear multiple bond correlation experiment
HPLC	high performance liquid chromatography
HRESIMS	high resolution electrospray ionization mass spectrometry
HSQC	heteronuclear single quantum correlation
Hz	Hertz
IC_{50}	concentration that is required for 50% inhibition in vitro
J	coupling constant

L	liter (s)
m	multiplet (NMR)
M	molar
MeOH	methanol
mg	milligram (s)
MHz	Megahertz
MIC	minimum inhibitory concentration
min	minute
mL	milliliter (s)
mult	multiplicity
NMR	nuclear magnetic resonance
TMS	tetramethylsilane
U	unit
UV	ultraviolet
δ	chemical shift
δ_C	chemical shift of carbon
δ_H	chemical shift of proton
ε	molar extinction coefficient
µg	microgram (s)
µL	microliter (s)
µm	micrometer (s)
λ_{max}	maximum wavelength
2D NMR	two dimentional nuclear magnetic resonance
$[\alpha]_D^{28}$	specific optical rotation

CHAPTER I

INTRODUCTION

Thailand has many parts; which have different temperature and landscapes thus making this area one of the richest floristic regions of the world. It has been estimated that the vascular plants are not less than 10,000 species from 245 families. Many plants in Thailand are potential medicinal plants. It has been estimated that plants are the most important source of medicine for more than 80% of the world's population. Medicinal plants are a vital source of medication in developing countries. Despite the wealth of human experience and folklore concerning the medicinal uses of plants, proper scientific investigation has only been applied to a small fraction of the world's plants.

Rutaceae or Citrus is one of the interesting families. Many *Ruta* species contain diverse classes of secondary metabolites, including essential oil, flavonoids, coumarins (notably furano and pyranocoumarins), alkaloids, and limonoids[(1)]. These are bioactive natural products which are used as therapeutic agents in the treatment of many diseases. Description of the plants in the Rutaceae is as follows.

Herbs, shrubs or trees have simple or compound leaves dotted with pellucid glands and abounding in a pungent or bitter-aromatic acid volatile oil, producing hypogenous mostly regular 3-5 merous flowers. The stamens of twice as many as the sepals (rarely more numerous); the 2-5 pistils are separate or combined into a compound ovary of as many locules, raised on a prolongation of the receptacle (gymnophore) or glandular disc. Embryo is large, usually in fleshy albumen. Style is commonly united or cohering. Fruits are various.

Among plants in family Rutaceae, *Feroniella lucida* or Ma Saang is the attractive plant. It has never been claimed about its activity. As for the study of chemical constituents, the first study has been reported by our research group. The interesting biological activities of chemical constituents from this plant were attractive reasons for further investigation.

1.1*Feroniella lucida*

Feroniella lucida (Rutaceae) (Thai name; มะสัง)[2,3]is a medium sized tree distributed widely in the Northeast of Thailand. The genus *Feroniella* is categorized into the subtribe Balsamocitrinae, which includes the genera *Swinglea, Aegle, Afraegle, Aeglopsis, Balsamocitrus* and *Feronia.*[4] Although the family *Feroniella* comprises 3 species, *F. lucida* is the only species found in Thailand. Leaves are small and combined 2-5- (usually 3-4-) paired. Fruits are very thick woody epicarp. Seeds are immersed in glutinous pulp arising from the placenta and the endocarp. In Thailand, this species is popularly cultivated as an ornamental plant, the leaves and fruit are used as foods and medicines. In Cambodia and Java this species is occasionally cultivated as fruit tree. The pulp of the raw fruits is eaten as vegetable. The pericarp is used medicinally. Other related genera include *Feronia* and *Aegle*. The study of phytochemical investigations for *F.lucida* has been first reported by our research group[5].

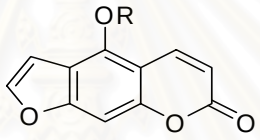
Figure 1.1 *Feroniella lucida*

1.2 Chemical constituents from *Feroniella lucida* and related species *Feronia limonia* and *Aegle marmelos*

1.2.1 Coumarins

Coumarins, a large group of natural occurring compounds possessing a 2*H*-1-benzopyran-2-one, are commonly found in plants in the families Rutaceae and Umbelliferae and sporadically reported in Leguminosae, Moraceae, and Miliaceae[6].

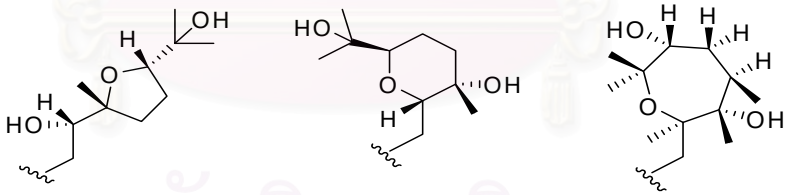
The chemical constituents from the roots and stems of *Feroniella lucida* have been reported three novel furanocoumarins[5] that having an oxolane, oxane, and oxypane moieties. They were feroniellin A(**1**), B(**2**) and C(**3**).



1 R = a.

2 R = b.

3 R = c. .



a. **b.** **c.**

Figure 1.2 Three novel furanocoumarins from *Feroniella lucida*

Feroniellin A and B showed cytotoxic activity against KB cell lines with IC_{50} = 0.13 and 0.23 μM/mL and against HeLa cell lines with IC_{50} = 0.14 and 0.19 μM/mL. On the other hand, the coumarins from the related genus in subtribe Balsamocitrinae; *Feronia* and *Aegle* have been reported since 1970s. There were *Feronia limonia* and *Aegle marmelos*. Coumarins have been isolated from the root bark[7], roots[8], stem bark[9], and leaves[10] of *Feronia limonia*. They were psolaren, bergapten, isopimpinellin, xanthotoxin, marmesin, demethylsuberosin,

aurapten and 6-methoxy-7-geranyloxycoumarin. The structures of reported coumarins from *Feronia limonia* were shown in Figure 1.3.

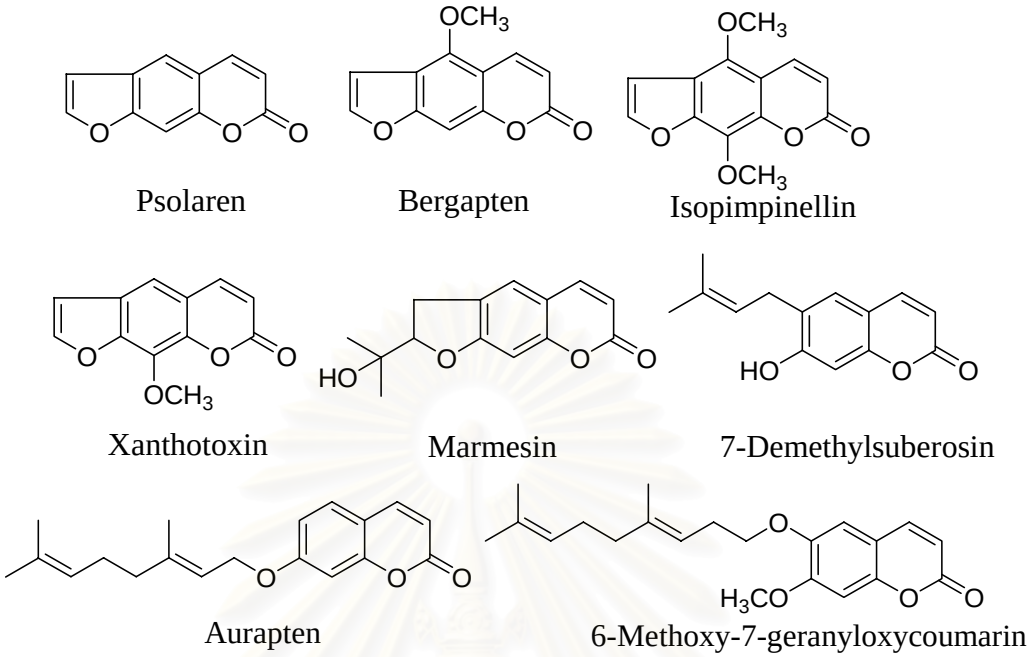


Figure 1.3 Coumarins from *Feronia limonia*.

Many coumarins were also isolated from the roots[11,12,13] and the stems[13] of *Aegle marmelos*. They were 6,7-dimethoxycoumarin, xanthotoxin, scopoletin, marmesin, anhydromarmesin, decursinol, aegelinol, skimmin and marmin. The structures of isolated coumarins from *Aegle marmelos* were exhibited in Figure 1.4.

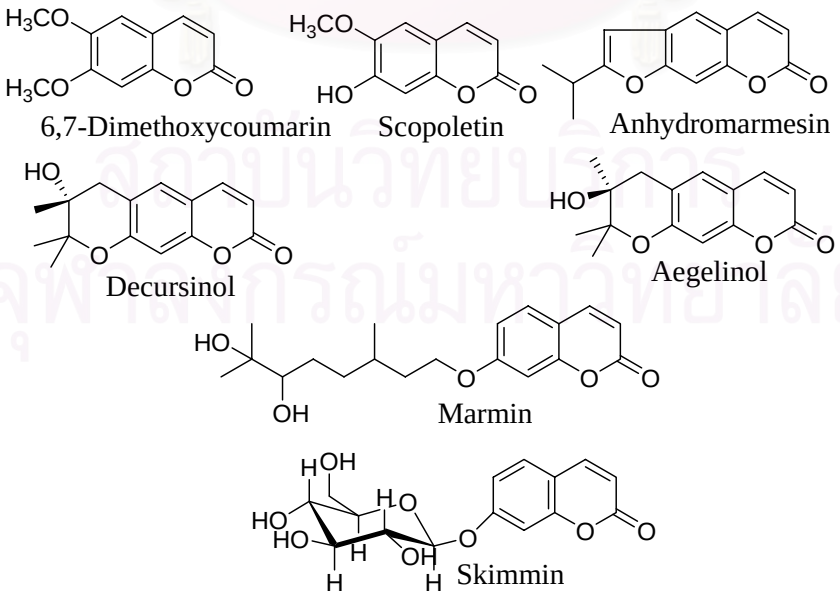
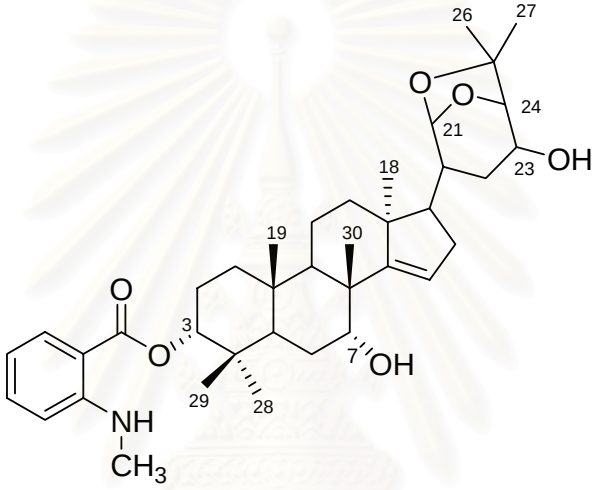


Figure 1.4 Coumarins from *Aegle marmelos*.

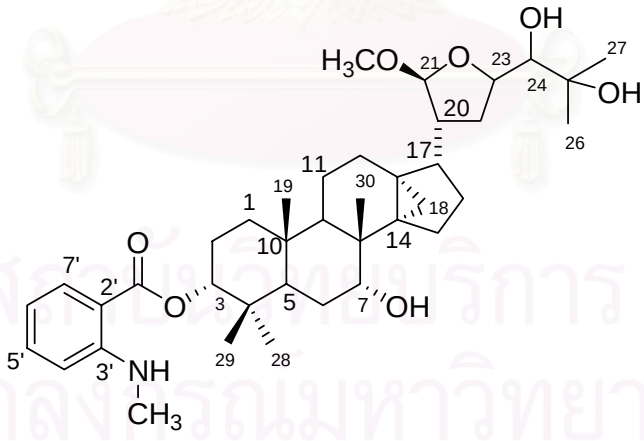
Coumarins are biologically active compounds. Their activities have been studied extensively. Coumarins showed antiplatelet aggregation activity[14], anti human leukaemic HL-60[15], anti-tumor promoting activity[16] and acetylcholinesterase inhibition[17].

1.2.2 Triterpenoids

Apotirucallane-triterpenoids have been found in plants of families Simoroubaceae, Rutaceae, and Meliaceae. Two anthranilate triterpenoids were isolated from the roots of *Feroniella lucida*[18]. Their names were feroniellides A and B.



Feroniellide A



Feroniellide B

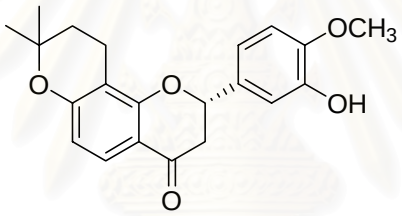
Figure 1.5 Anthranilate apotirucallane triterpenoids from *Feroniella lucida*.

Feroniellide A and B showed cytotoxic activity against KB cell line with IC_{50} = 60 and 49 μg/mL and against HeLa cell line with IC_{50} = 46 and 40 μg/mL.

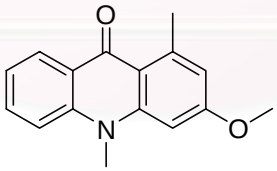
Previous studies revealed that many triterpene glucosides with a 14,15-cycloapotirucallane skeleton exhibited cytotoxicity against leukemia and colon tumor cell lines[19]. The anthranilate triterpenoids also showed antimicrobial and brine shrimp lethality[20]. *Feronia limonia* and *Aegle marmelos* have never been reported for triterpenoids.

1.2.3 Miscellaneous

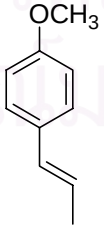
Although *Feroniella lucida* have been reported only coumarins and triterpenoids, other chemical constituents were found from *Feronia limonia* and *Aegle marmelos* were alkaloids, flavonoids, and essential oil. The alkaloid[9]; 1-hydroxy-3-methoxy-*N*-methyl-acridan-9-one and flavanone[9]; (-)-(2*S*)-5,3'-hydroxy-4-methoxy-6",6"-dimethylcromenol(7,8,2",3")-flavanone have been isolated from the root of *Feronia limonia*. On the other hand, anethol[21] was the major component isolated from the essential oil of this plant. (Figure 1.6)



(-)-(2S)-5,3'-hydroxy-4-methoxy-6",6"-dimethylcromenol(7,8,2",3")-flavanone



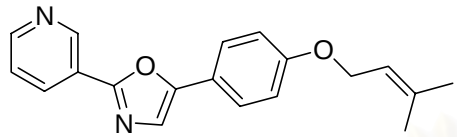
1-Hydroxy-3-methoxy-*N*-methyl-acridan-9-one



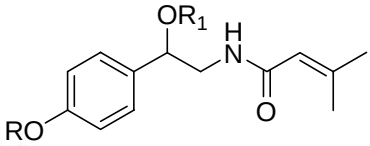
Anethol

Figure 1.6 Alkaloid, flavanone and anethol from *Feronia limonia*

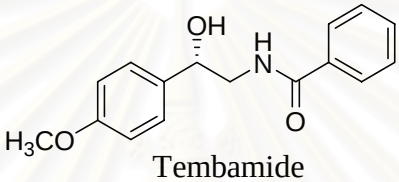
Alkaloids[22] were also isolated from leaves and fruits of *Aegle marmelos*; *O*-(3,3-dimethylallyl)-halfodinol, *N*-2-ethoxy-2-(4-methoxyphenyl)ethyl cinnamamide (**a**), and *N*-2-methoxy-2-[4-3,3-dimethylallyloxy)phenyl] ethylcinnamamide (**b**). Tembamide was also isolated from the root of this plant[11](Figure 1.7).



O-(3,3-dimethylallyl)-halfodinol



	R	R_1
(**a**)	Me	Et
(**b**)	$CH_2CH=CMe_2$	Me



Tembamide

Figure 1.7 Alkaloid from *Aegle marmelos*

From the attractive biological activities of compounds from the previous reports of related species *Feronia limonia* and *Aegle marmelos*, *F. lucida* (Ma Sang) was selected for further investigation of phytochemical constituents and their biological activities. The objectives of this research can be summarized as followed:

1. To extract and isolate compounds from the stems and stem bark of *F. lucida*
2. To elucidate the structures of all isolated compounds.
3. To determine the biological activities of the isolated compounds

CHAPTER II

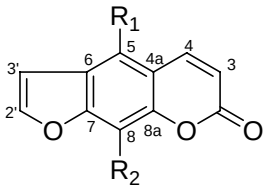
FERONIELLIN A, A NOVEL BIOACTIVE FURANOCOUMARIN WITH HIGHLY OXYGENATED C_{10} MOIETY FROM THE STEMS OF *Feroniella lucida*

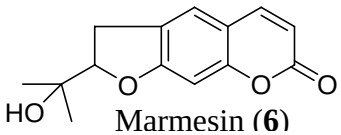
2.1 Extraction and purification

Feroniella lucida stems (3.2 kg), were collected from Nakhon Phanom in October 2004, and extracted with methanol by soxhlet apparatus, yielding a methanolic extract (42 g). This extract was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 layer was evaporated, yielding 20 g of crude extract (Scheme 2.1). The extract was subjected on vacuum column chromatography eluted with a gradient system (hexane, CH_2Cl_2, EtOAc and MeOH) to afford six fractions.

Fraction 5 (100% EtOAc) was fractionated on a Sephadex LH-20 column [MeOH-CH_2Cl_2-hexane (2:3:5)], followed by a separation using a Chromatotron with 50% EtOAc: hexane, to afford a novel furanocoumarin named feroniellin A (**7**). The purification of the other eight known coumarins (**1**-**6** and **8**-**9**) was summarized in Scheme 2.2.

The extraction and purification of all coumarins from CH_2Cl_2 crude extract from the stems of *Feroniella lucida* were briefly summarized in Schemes 2.1 and 2.2.



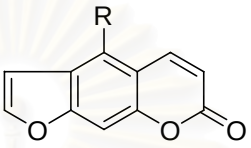


Marmesin (**6**)

Psolaren (**1**): R_1= H, R_2 = H

Bergapten (**2**): R_1= OMe, R_2 = H

Isopimpinellin (**3**): R_1= OMe, R_2 = OMe



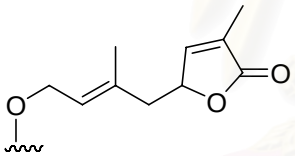
Anisolactone (**4**): R= a

2′,3′-Epoxyanisolactone (**5**): R = b

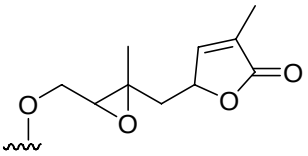
Feroniellin A (**7**): R = c (new compound)

Feroniellin B (**9**): R = d

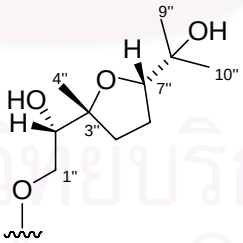
Feroniellin C (**8**): R = e



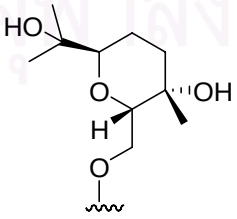
a



b



c



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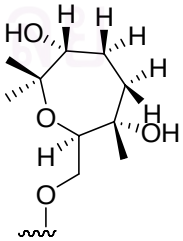
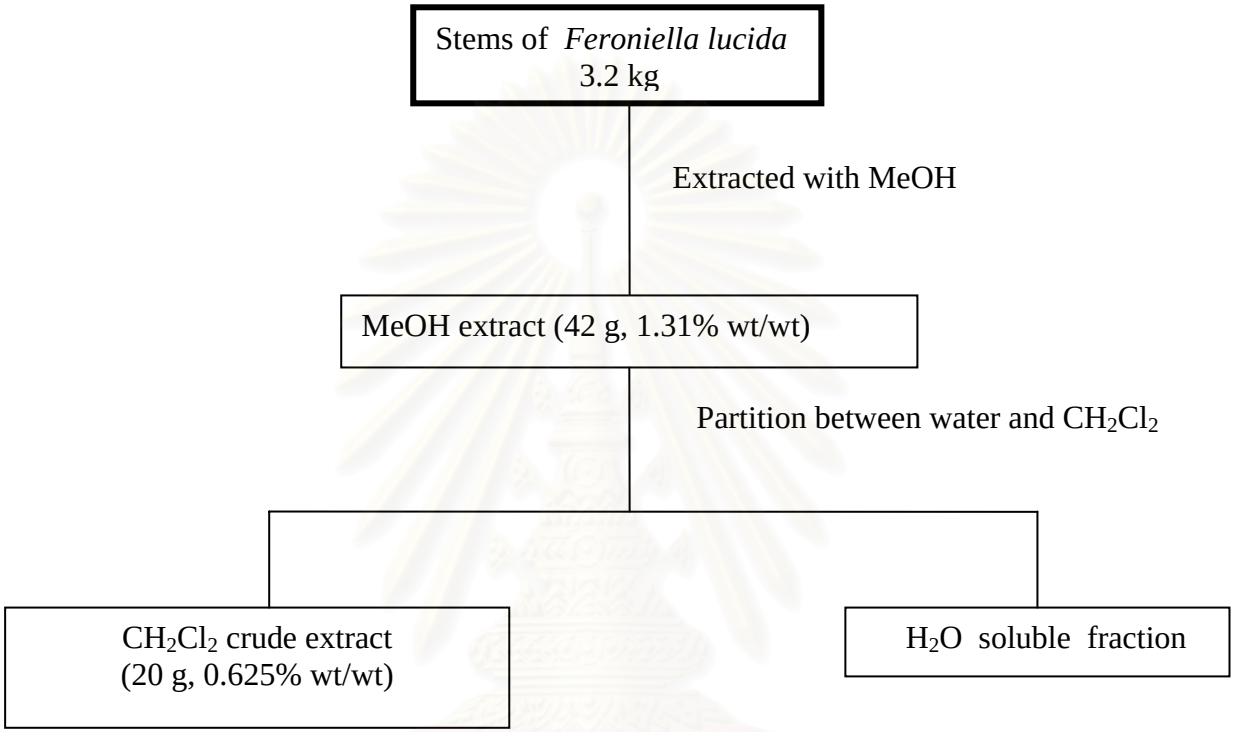
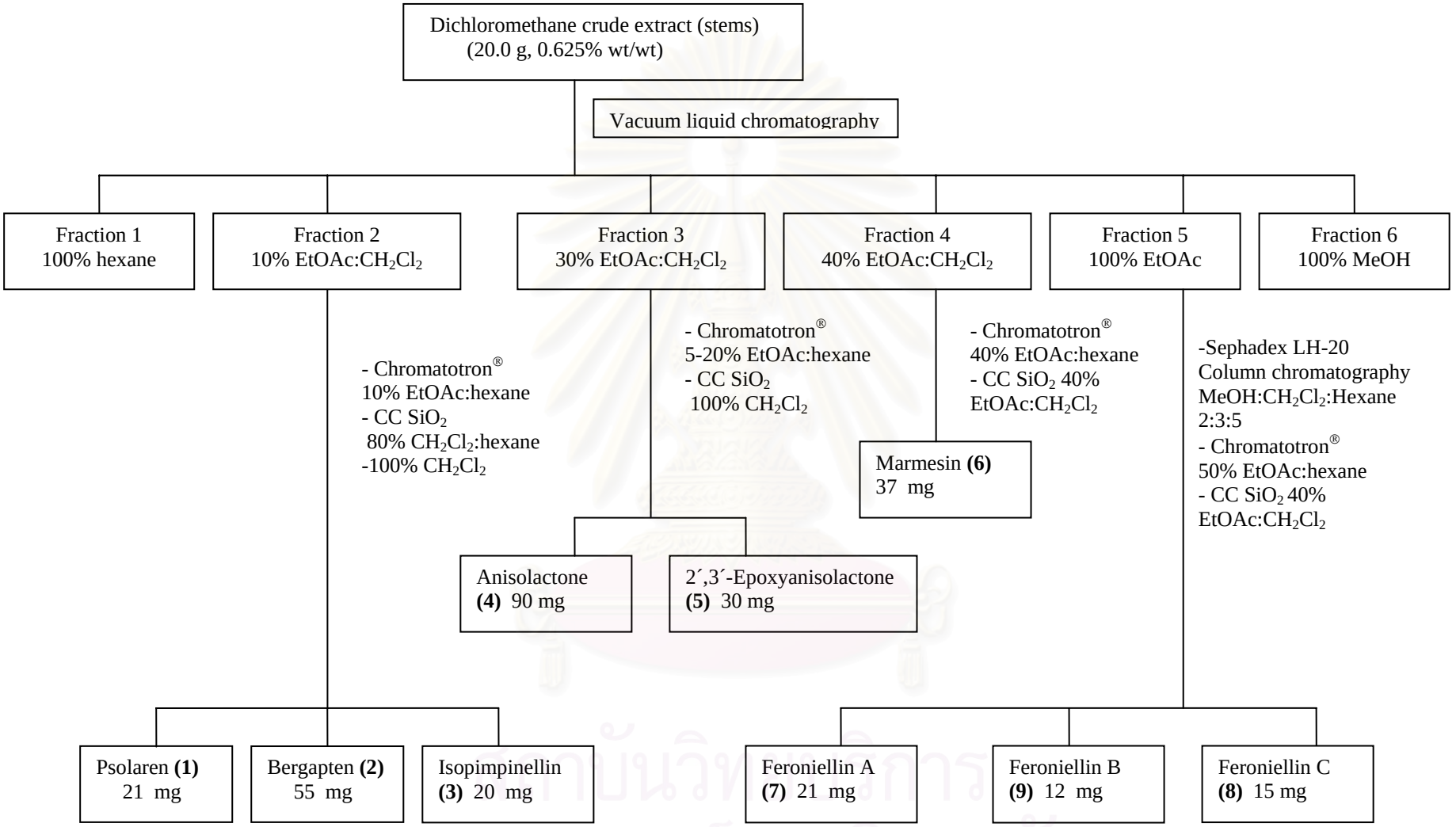


e



Scheme2.1 Extraction of *Feroniella lucida* stems



Scheme 2.2 Isolation procedure of the dichloromethane crude extract (stems)

2.2 Structure elucidation of feroniellin A

Feroniellin A (**7**) was obtained as a pale yellow powder and had molecular formula of $C_{21}H_{24}O_7$ as established by HRESIMS. The UV absorbance at 252 and 309 nm suggested the presence of the coumarin moiety[23].

The ^{1}H NMR data showed signals of H-3 (δ 6.29 d, *J* = 9.6 Hz) and H-4 (δ 8.22 d, *J* = 10.0 Hz) indicating the presence of 3,4-unsubstituted coumarin. Moreover, the presence of a pair of furan protons at H-2' (δ 7.01 d, *J* = 1.6 Hz) and H-3' (δ 7.60 d, *J* = 2.0 Hz) and cross peaks from H-2' and H-3' to C-6 and C-7 indicated that the coumarin was fused with a furan ring at C-6 and C-7 (Table 2.1).

The ^{13}C NMR spectrum revealed twenty-one signals; eleven of which were accounted for by a furanocoumarin nucleus. The remaining proton and carbon signals were ascribable to a geranyl derived portion on the basis of 2D NMR data analysis.

The COSY spectrum of **7** displayed two spin systems, OCH_2-CH-O and C-CH_2-CH_2-CH-O, which were flanked by oxygenated quarternary C-3"(δ 83.9), as suggested by the cross peaks of H-2"(δ 4.04)/C-3" and H-5"(δ 1.75)/C-3". Two singlet methyls, δ 1.26(Me-9") and δ 1.16 (Me-10"), were accommodated at C-8" (δ 70.5), which was in turn connected to C-7". The remaining singlet methyl (δ 1.24) was placed at C-3" (δ 83.9) on the basis of HMBC cross peaks between these methyl (4") protons and C-2", 3" and 5" (Figure 2.1).

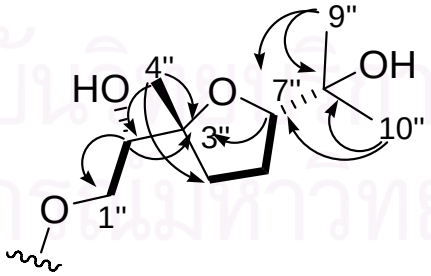


Figure 2.1 Selected HMBC (arrowed curves) and COSY (solid line) correlations of geranyl derived portion of **7**.

The slightly down field shift of oxygenated C-3″ and C-7″ coupled with the HMBC cross peak between H-7″ and C-3″ allowed us to construct a tetrahydrofuran or oxolane part of this portion. According to the NMR data, this

portion was similar to those of dehydrovenustatriols, which were isolated from the red algae *Laurencia viridis*[24]. Although chemical shifts of carbons and protons were not the same as those of algal polyethers, but they gave an information on carbons, which located on the ether linkage in the furan ring (Figure 2.2).

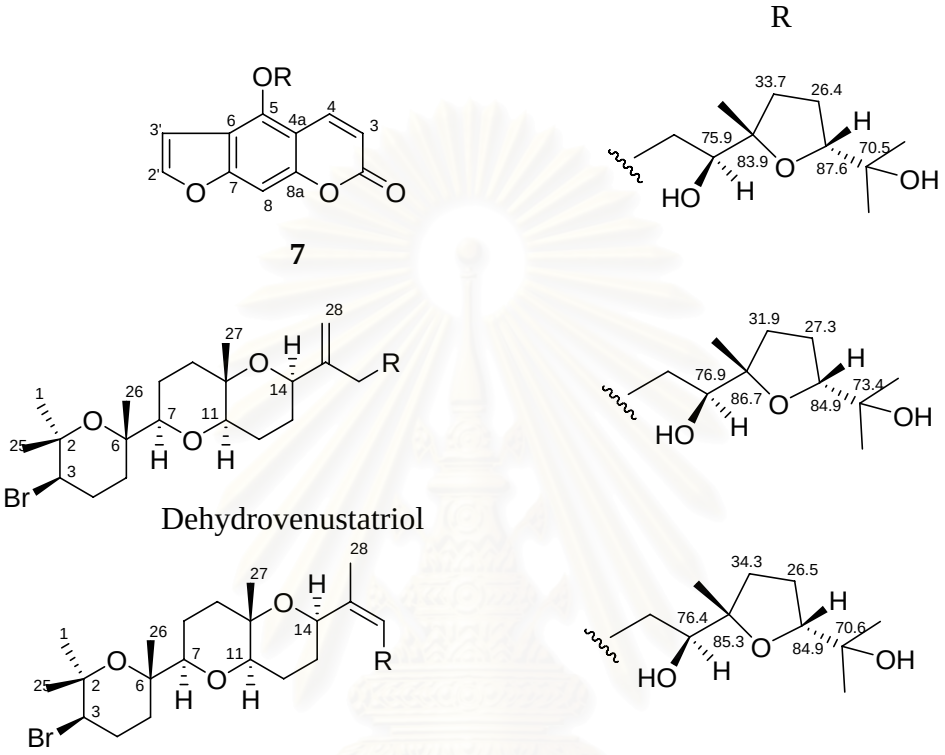


Figure 2.2 Structure of **7,** dehydrovenustatriol and 15-16-dehydrovenustatriol demonstrating carbon chemical shifts around tetrahydrofuran moieties.

The C_{10} subunit was linked to the coumarin nucleus at C-5, as shown by the HMBC correlation of H_2-1"(δ 4.37)/C-5(δ 148.6) and the resonance typical of an unsubstituted C-8 (δ_H 7.17 and δ_C 94.7), thus completing the overall structure of **7**.

The relative configuration of feroniellin A was accomplished by NOESY data analysis. The cross peaks between H-7″ and CH_3-4″ and CH_3-4″ and H-2″ indicated that they were on the same face of five-membered ring. (Figure 2.3)

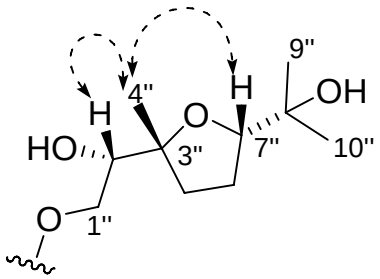


Figure 2.3 NOESY correlations of tetrahydrofuran moiety of **7.**

The complete assignment protons and carbons for feroniellin A was shown in Table 2.1.

Table 2.1 ^{1}H and ^{13}C NMR data for feroniellin A (**7**) in $CDCl_3$

position	δ_C	δ_H mult (*J* in Hz)	HMBC
2	161.2		
3	112.9	6.29 d (9.6)	C-2, 4a
4	139.3	8.22 d (10.0)	C-2, 5, 8a
4a	107.3		
5	148.6		
6	114.1		
7	158.1		
8	94.7	7.17 s	C-4a, 6, 7, 8a
8a	152.5		
2′	145.1	7.60 d (2.0)	C-6, 7, 3′
3′	104.8	7.01 d (1.6)	C-6, 7, 2′
1″	74.3	a 4.37 dd (8.0, 10.0)	C-5, 2″
		b 4.58 dd (2.8, 10.0)	C-5, 2″
2″	75.9	4.04 dd (2.4, 8.0)	C-1″, 3″
3″	83.9		
4″	27.6	1.24 s	C-2″, 3″, 5″
5″	33.7	α 1.75 m	C-3″, 6″
		β 2.16 m	
6″	26.4	1.91 m	C-5″, 7″
7″	87.6	3.84 m	C-3″, 9″, 10″
8″	70.5		
9″	23.3	1.26 s	C-7″, 8″, 10″
10″	24.0	1.16 s	C-7″, 8″, 9″

Furanocoumarins having modified C_{10} side chain have been encountered in plants of the family Rutaceae; however highly oxygenated C_{10} subunit are rare. Feroniellin A is the first report of coumarin containing an oxolane from terrestrial plants.

2.3 Bioactivity of isolated coumarins

The cytotoxic activity against HeLa and KB cell lines of coumarins were determined using MTT assay and evaluated for acetylcholinesterase inhibitory effect and the result showed in Tables 2.2 and 2.3.

Table 2.2 Cytotoxic activity of compounds **1-9** against HeLa and KB cell lines

Compound	HeLa IC_{50} (μg/mL)	KB IC_{50} (μg/mL)
Psolaren (**1**)	70	75
Bergapten (**2**)	>100	>100
Isopimpinellin (**3**)	>100	>100
Anisolactone (**4**)	>100	>100
2′,3′-Epoxyanisolactone (**5**)	>100	>100
Marmesin (**6**)	55	55
Feroniellin A (**7**)	50	55
Feroniellin B (**9**)	90	75
Feroniellin C (**8**)	>100	>100

Note: Standard agent (Adriamycin IC_{50} = 0.018 μg/mL)

Table 2.3 Acetylcholinesterase inhibitory effect of compounds **1-9**

Compound	Concentration (mg/mL)			
	1.000	0.500	0.250	0.125
Psolaren (**1**)	+	-	-	-
Bergapten (**2**)	+	-	-	-
Isopimpinellin (**3**)	+	+	-	-
Anisolactone (**4**)	+	-	-	-
2′,3′-Epoxyanisolactone (**5**)	+	-	-	-
Marmesin (**6**)	+	+	-	-
Feroniellin A (**7**)	+	+	-	-
Feroniellin B (**9**)	+	+	-	-
Feroniellin C (**8**)	+	+	-	-

Note: Standard acetylcholinesterase inhibitor = Phytostigmine (Eserine) +, 0.125 mg/mL (+: inhibit, -: not inhibit)

From Table 2.2, marmesin (**5**) and feroniellin A (**7**) showed mild cytotoxic activity against HeLa and KB cell lines. Marmesin showed cytotoxic against HeLa and KB cell lines with IC_{50} = 55 μg/mL. And feroniellin A showed cytotoxic activity against HeLa cell line higher than KB cells line with IC_{50} = 50 and 55 μg/mL, respectively when compared with reference, Adriamycin (IC_{50} = 0.018 μg/mL). According to an acetylcholinesterase inhibitor assay, all coumarins are powerful inhibited at MIC value of 1 mg/mL. In addition, **3**, **6**, **7**, **8** and **9** showed higher inhibitory affect against acetylcholinesterase with MIC values of 0.5 mg/mL when compared with standard inhibitor, Phytostigmine (Eserine).

2.4 Experiment section

2.4.1 Plant material

The stems of *Feroniella lucida* were collected in Tah-Utane Nakhon Phanom, Thailand in October 2004.

2.4.2 Equipments

NMR spectra were recorded with a Varian model Mercury+ 400 which operated at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei. Most solvents used in this research were commercial grade and were distilled prior to use. Adsorbents such as silica gel 60 Merck cat. No. 7731, 7734, and 7749 were used for quick column chromatography, open column chromatography, chromatotron, respectively. Thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F254 plates (0.25 mm thick layer). Chromatotron (model 7924 T, Harrison Research) on silica gel plate of 1 mm thickness was used for centrifugal thin layer chromatography.

2.4.3 The cytotoxic activity against HeLa and KB cell lines by MTT assay

All compounds (1 mg) were examined for cytotoxic activity against HeLa and KB cell lines by MTT assay. This assay was performed at Natural Products Research Section, Research Division, National Cancer Institute, Thailand. The results were shown in Table 2.2.

2.4.4 Acetylcholinesterase inhibitor assay [25]

Acetylcholinesterase (1000 U) was dissolved in 150 mL of 0.005 M Tris-HCl acid buffer at pH 7.8; bovine serum albumin (BSA) (150 mg) was added to the solution in order to stabilize the enzyme during the bioassay. The stock solution was kept at 4 °C. The sample was dissolved in MeOH (1mg/ml) and spotted on TLC plate. After removal of solvent, the plate was then sprayed with enzyme stock solution and dried again. For incubation of the enzyme, the plate was laid flat on plastic plugs in a plastic tank containing a small amount of water; by this means, water was not directly contact with the plate. The cover was placed on the tank to maintain the level of humidity and incubation was performed at 37 °C for 20 min. The enzyme had satisfactory stability under these conditions. Solutions of 1-naphthyl acetate (250 mg) in ethanol (100 mL) and Fast Blue B salt (400 mg) in distilled water (160 mL) were prepared immediately before use (in order to prevent decomposition) for detection of enzymatic activity. After the incubation of the TLC plate, 10 mL of the naphthyl acetate solution and 40 mL of the Fast Blue B salt solution were mixed and sprayed onto the plate to produce a purple coloration after 1-2 min. Region of the TLC plate which contain acetylcholinesterase inhibitors showed up as whites spots against the purple background.

Psoralen (1): white amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (1H, d, *J* = 9.6 Hz, H-4), 7.71 (1H, d, *J*= 2.4 Hz, H-3′), 7.69 (1H, s, H-5), 7.48 (1H, s, H-8), 6.83 (1H, d, *J* = 1.2 Hz, H-2′), 6.38 (1H, d, *J* = 9.6 Hz, H-3).

Bergapten (2): white amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (1H, d, *J* = 9.6 Hz, H-4), 7.59 (1H, d, *J* = 2.0 Hz, H-3′), 7.14 (1H, s, H-8), 7.01 (1H, d, *J* = 1.6 Hz, H-2′), 6.27 (1H, d, *J* = 9.6 Hz, H-3), 4.27 (3H, s, 5-OCH_3)**.**

Isopimpinellin (3): yellow amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.12 (1H, d, *J* = 10.0 Hz, H-4), 7.63 (1H, d, *J* = 2.0 Hz, H-3′), 7.02 (1H, d, *J* = 1.6 Hz, H-2′), 6.29 (1H, d, *J* = 9.6 Hz, H-3), 4.16 (6H, s, 5-OCH_3 and 8-OCH_3).

Anisolactone (4): colourless crystal solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (1H, d, *J* = 10.0 Hz, H-4), 7.61 (1H, d, *J* = 2.0 Hz, H-2′), 7.17 (1H, s, H-8), 6.98

(1H, s, H-6″), 6.95 (1H, d, *J* = 1.2 Hz, H-2′), 6.29 (1H, d, *J* = 9.6 Hz, H-3), 5.67 (1H, t, *J* = 6.4 Hz, H-2″), 5.50 (1H, m, H-5″), 4.96 (2H, d, *J* = 6.8 Hz, H-1″), 2.46 (1H, dd, *J* = 10.4, 5.0 Hz, H-4″b), 2.35 (1H, dd, *J* = 14.4, 8.2 Hz, H-4″a), 1.92 (3H, s, CH_3-10″),1.79 (3H, s, CH_3-9″).

2′,3′-Epoxyanisolactone (5): colourless crystaline solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (1H, d, *J* = 9.6 Hz, H-4), 7.62 (1H, d, *J* = 2.0 Hz, H-3′), 7.61 (1H, brs, H-2′), 7.18 (1H, *s*, H-8), 7.08 (1H, s, H-6″), 6.32 (1H, d, *J* = 9.6 Hz, H-3), 5.08 (1H, m, H-5″), 4.70 (1H, dd, *J* = 11.4, 4.6 Hz, H-1″b), 4.44 (1H, dd, *J* = 11.0, 6.6 Hz, H-1″a), 3.29 (1H, t, *J* = 8.4 Hz, H-2″), 1.93 (3H, s, CH_3-10″), 1.66 (2H, m, H-4″), 1.49 (3H, s, CH_3-9″).

Marmesin (6): colourless flakes; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (1H, d, *J* = 9.6 Hz, H-4), 7.22 (1H, s, H-5), 6.75 (1H, s, H-8), 6.22 (1H, d, *J* = 9.6 Hz, H-3), 4.74 (1H, t, *J* = 8.8 Hz, H-2′), 3.21 (2H, m, H-3′), 1.37 (3H, s, CH_3-1″), 1.23 (3H, s, CH_3-1″).

Feroniellin A (7): white amorphous solid; $[\alpha]_D^{23}$ +18.8(*c* 0.85 MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.45), 252 (4.40), 309 (3.52) ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR (100 MHz) see table 2.1 HRESIMS *m/z* $[M+Na]^+$ 411.1422 (calcd for $C_{21}H_{24}O_7Na$, 411.4008)

Feroniellin B (9): yellow liquid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (1H, d, *J* = 9.6 Hz H-4), 7.62 (1H, d, *J* = 2.0 Hz H-2′), 7.17 (1H, s, H-8), 6.95 (1H, d, *J* = 2.0 Hz H-3'), 6.29 (1H, d, *J* = 10.0 Hz H-3), 4.72 (1H, dd, *J* = 8.4, 10.4 Hz H-1"b), 4.49 (1H, dd, *J* = 4.4, 10.4 Hz H-1"a), 4.12 (1H, dd, *J* = 4.4, 8.0 Hz H-2′′), 3.44 (1H, dd, *J* = 2.4, 11.2 Hz H-7′′), 1.83 (1H, m, H-6′′a), 1.79(1H, m, H-5′′a), 1.68 (1H, m, H-5′′b), 1.61 (1H, m, H-6′′b), 1.24 (3H, s, 4′′-CH_3), 1.21 (3H, s, 10′′-CH_3), 1.19 (3H, s, 9′′-CH_3).

Feroniellin C (8): brown liquid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (1H, d, *J* = 9.6 Hz H-4), 7.48 (1H, d, *J* = 2.0 Hz H-2′), 7.02 (1H, s, H-8), 7.02 (1H, brs, H-3'), 6.18 (1H, d, *J* = 9.6 Hz H-3), 4.68 (1H, brd, *J* = 8.4 Hz H-1"b), 4.21 (1H, m, H-1"a), 4.16 (1H, m, H-2′′), 3.72 (1H, brd, *J* = 7.6 Hz H-7′′), 2.04 (1H, brt, *J* = 12.4 Hz H-5′′a), 1.86 (1H, m, H-6′′a), 1.76 (1H, m, H-6′′b), 1.59 (1H, m, H-5′′b), 1.28 (3H, s, 9′′-CH_3), 1.22 (3H, s, 4′′-CH_3), 1.14 (3H, s, 10′′-CH_3),

Supporting information

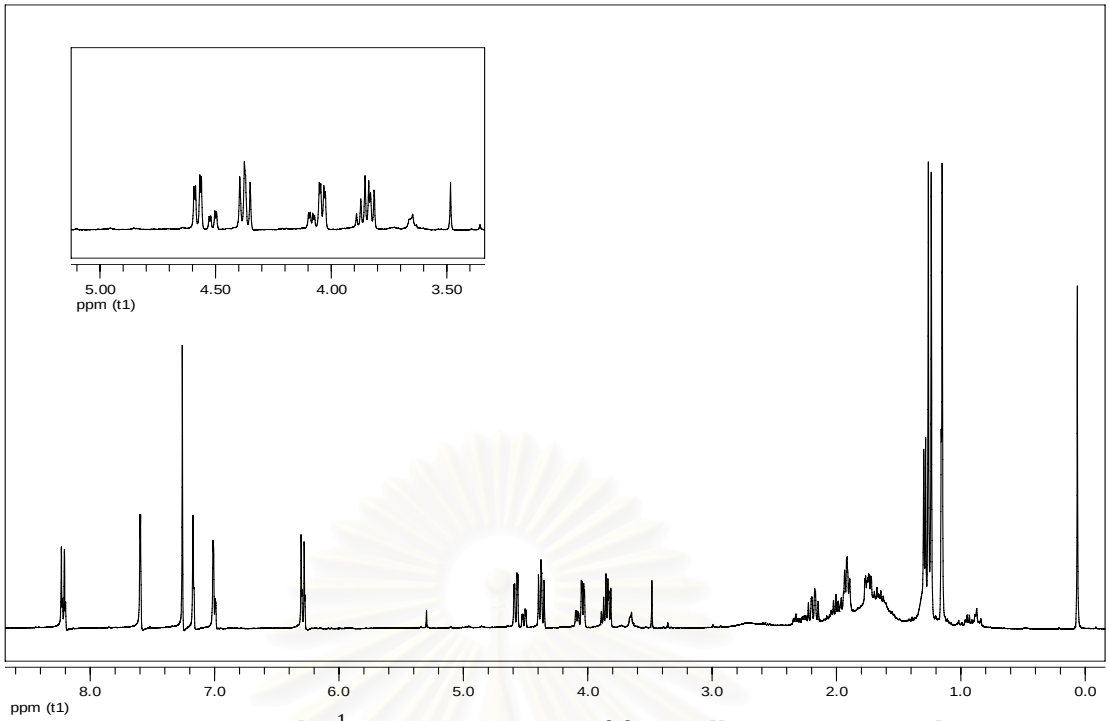


Figure S-2.1 The ^{1}H NMR spectrum of feroniellin A (**7**) in $CDCl_3$.

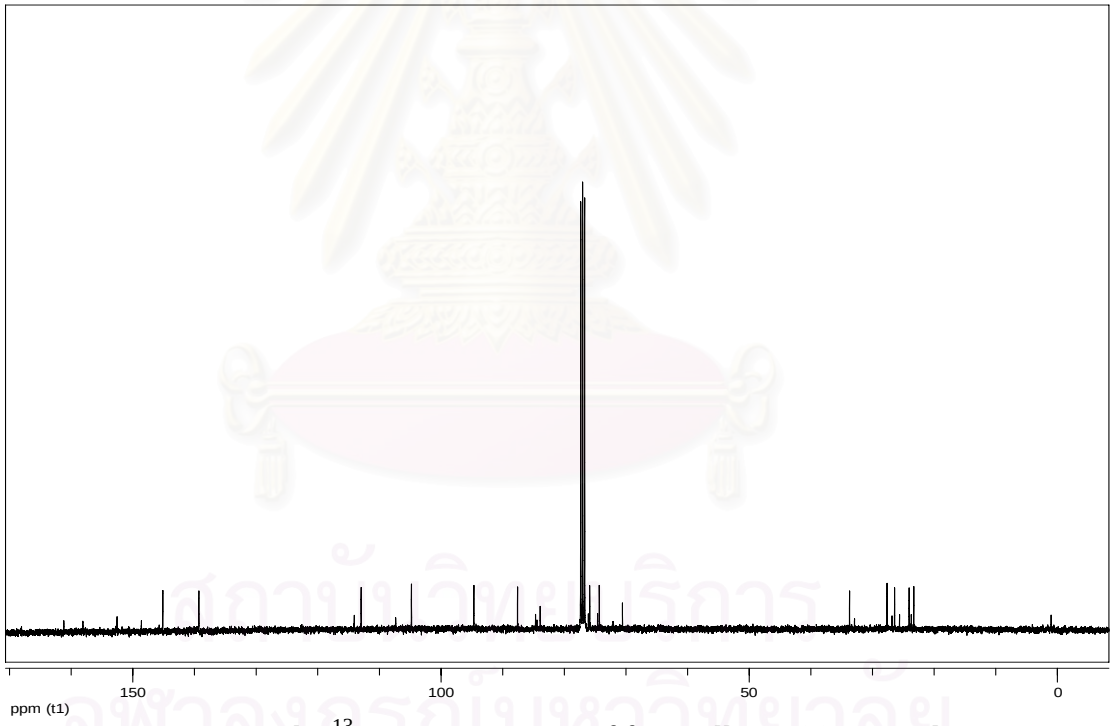


Figure S-2.2 The ^{13}C NMR spectrum of feroniellin A (**7**) in$CDCl_3$.

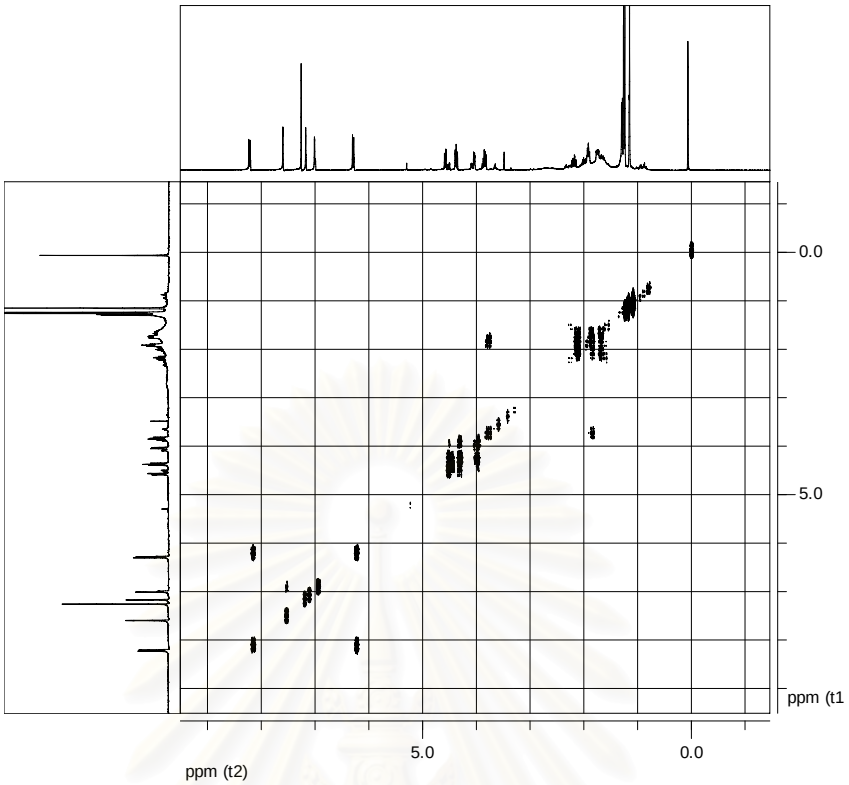


Figure S-2.3 The COSY spectrum of feroniellin A (**7**) in $CDCl_3$.

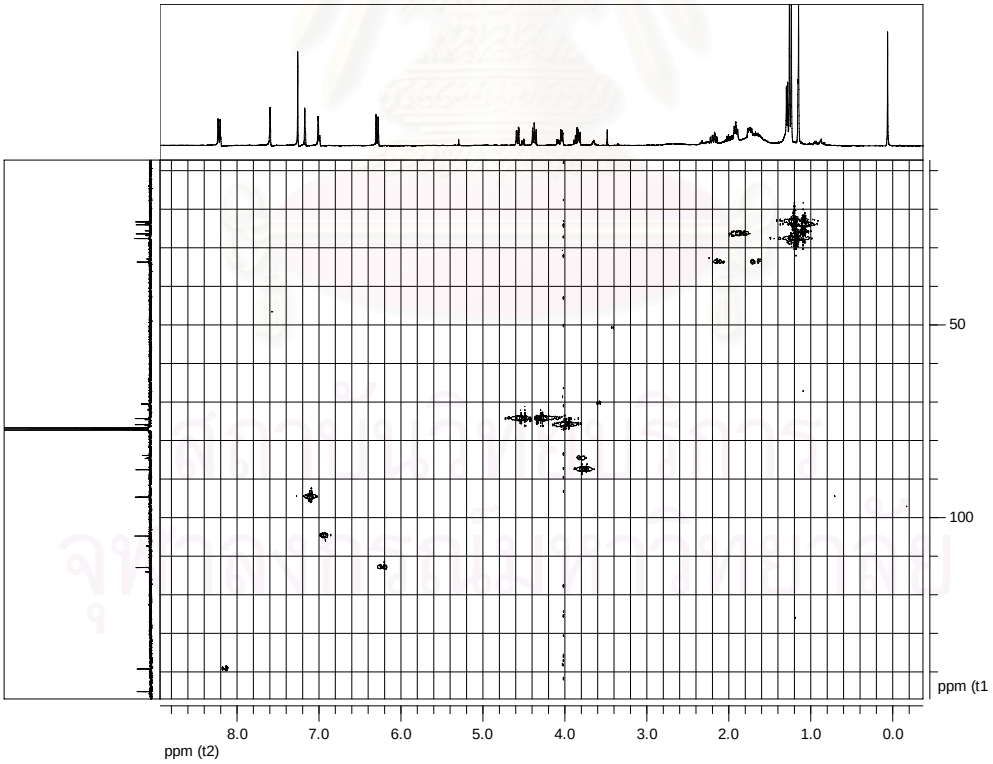


Figure S-2.4 The HSQC spectrum of feroniellin A (**7**) in $CDCl_3$.

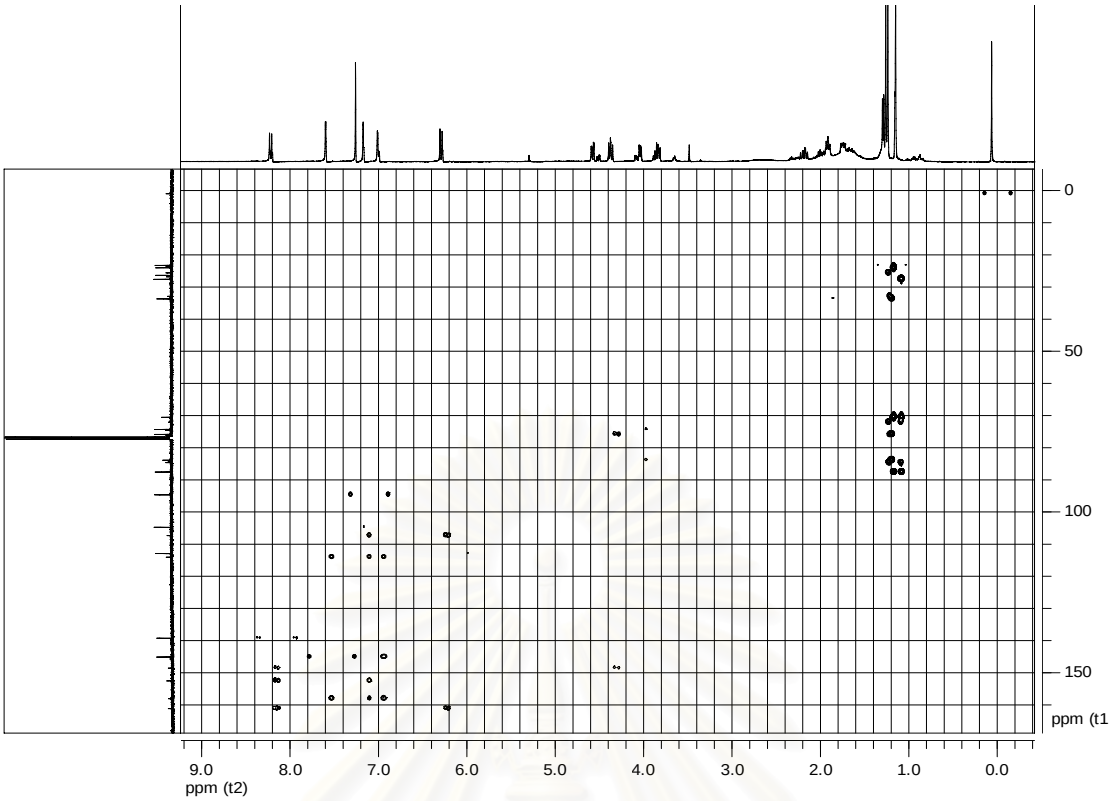


Figure S-2.5 The HMBC spectrum of feroniellin A (**7**) in $CDCl_3$.

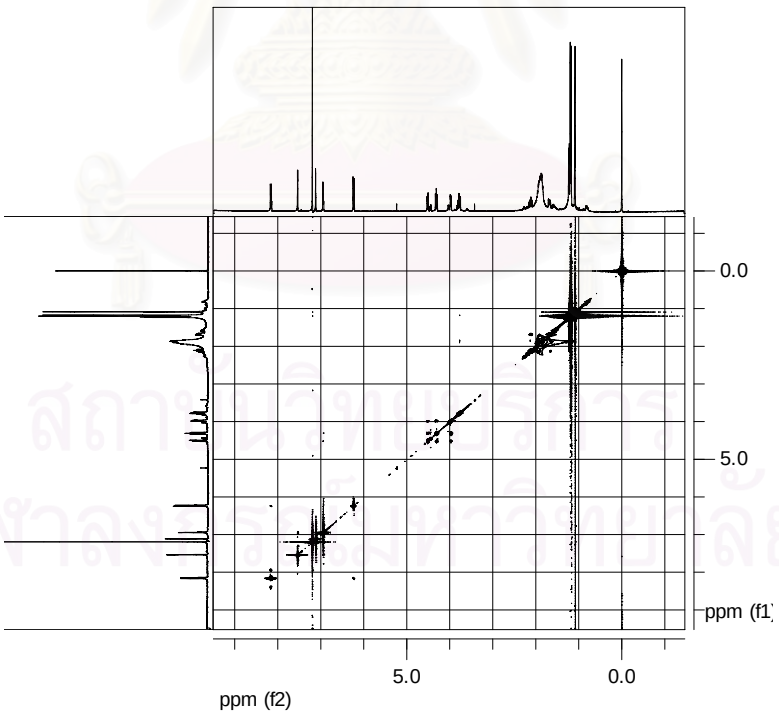


Figure S-2.6 The NOESY spectrum of feroniellin A (**7**) in $CDCl_3$

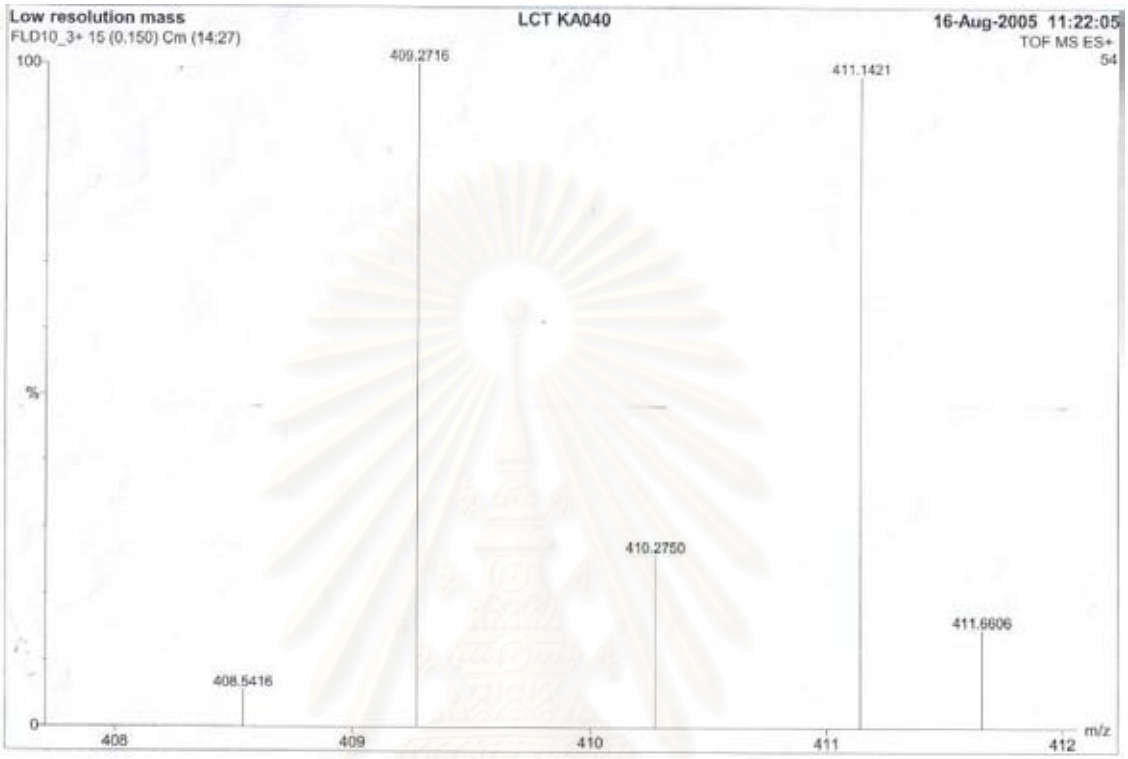


Figure S- 2.7 The high resolution mass spectrum of feroniellin A (**7**).

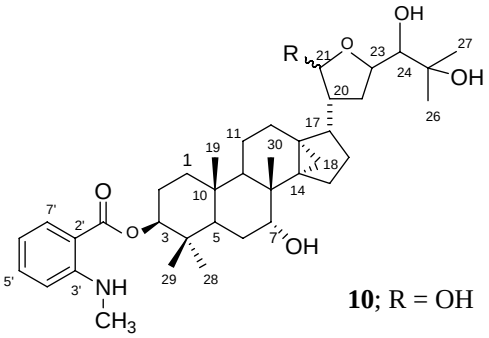
CHAPTER III
FERONIELLIDES C-E, THREE NEW BIOACTIVE APOTIRUCALLANE TRITERPENOIDS FROM THE STEM BARK OF *Feroniella lucida*

3.1 Extraction and isolation

The stem bark of *Feroniella lucida*, collected from Nakhon Phanom in March 2005, was macerated in dichloromethane for two weeks. The solution of dichloromethane was evaporated to obtain dichloromethane crude extract (36 g). The extract from the stem bark was subjected to vacuum column chromatography, eluting with gradient CH_2Cl_2, EtOAc, and MeOH.

Fraction 4 (100% EtOAc) was fractioned on Sephadex LH-20[MeOH-CH_2Cl_2-hexane (2:3:5)] followed by Chromatotron, eluting with 50% EtOAc:hexane, to yield 3 fractions (4.1-4.3). Fraction 4.2 was purified by HPLC (97% MeCN:H_2O) yielded new terpenoid feroniellide D (**15**). Fraction 4.3 was purified by HPLC (99% MeCN: H_2O), to yield **10**, **12** and fraction 4.31. Fraction 4.31 was isolated again by HPLC (99% MeCN:H_2O) and followed by silica gel purification, to furnish two new triterpenoids namely feroniellide C (**13**) and feroniellide E (**16).** In addition, fraction 4.1 and fraction 4.3 were also isolated by ODS HPLC, to afford four known triterpenoids; **10**, **11**, **12**, and **14**. Other fractions were chromatographed, to yield four known coumarins **2**, **4**, **5**, and **6**. Then triterpenoids were elucidated by NMR data. The structure of known triterpenoids and coumarins were confirmed by comparison with their NMR data in the previous reports in literatures.

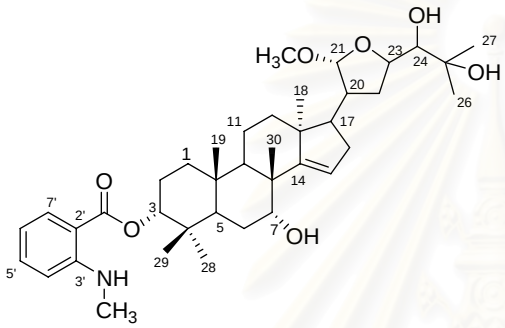
The extraction and purification of all compounds from dichloromethane crude extract from the stem bark of *Feroniella lucida* were summarized in Scheme 3.1.



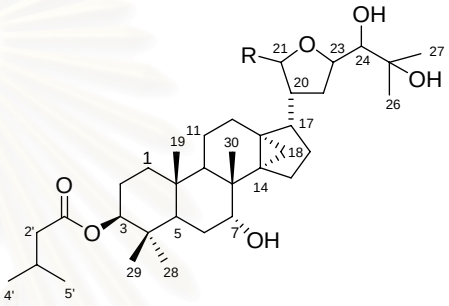
10; R = OH

11; R = α-OMe

12; R = β-OMe

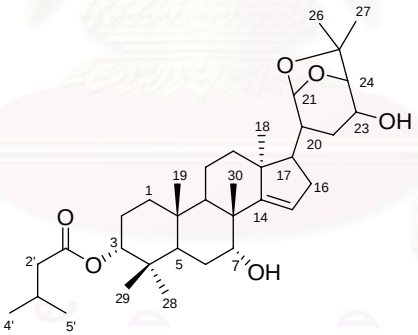


Feroniellide C (**13**) (new compound)

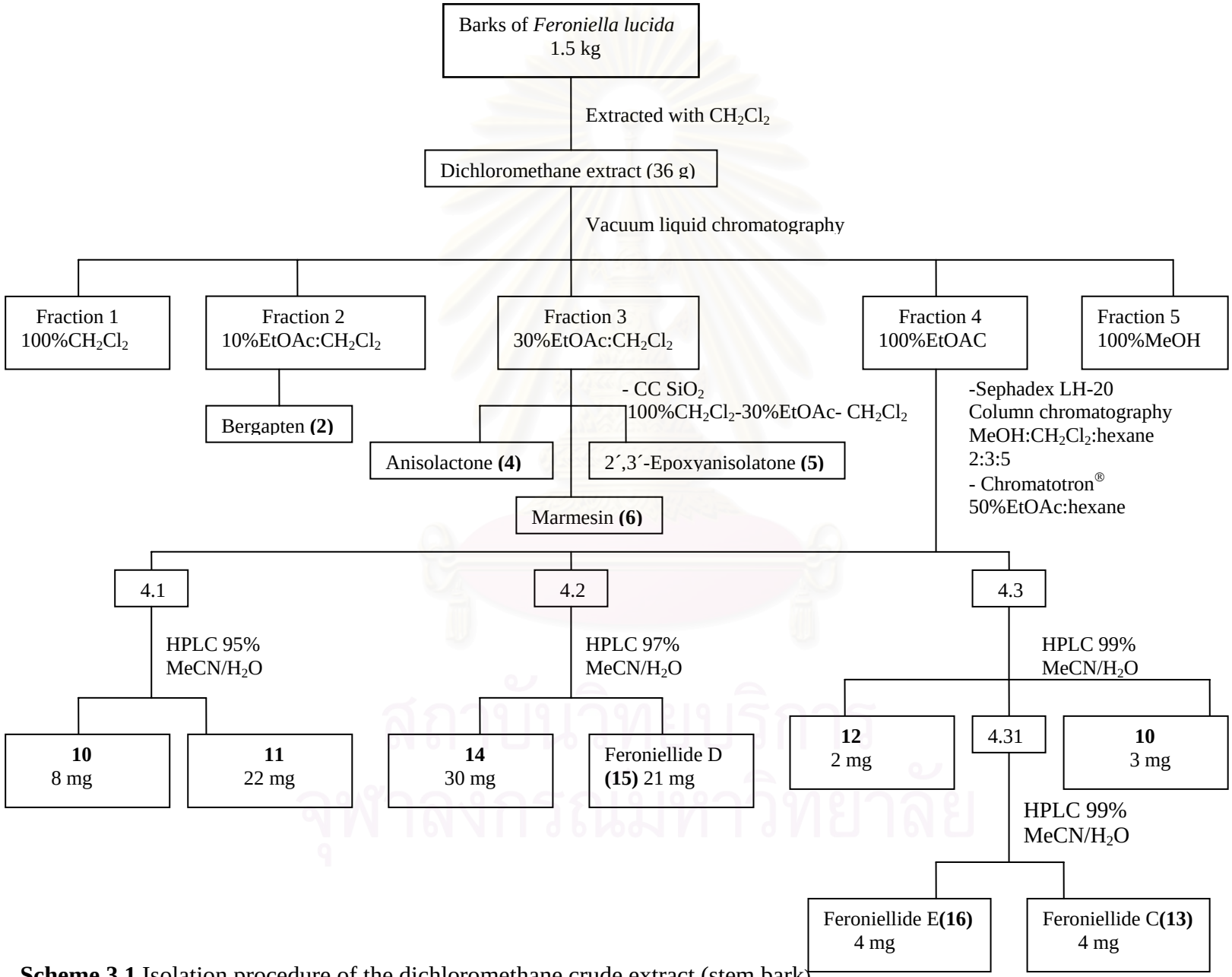


14; R = β-OMe

Feroniellide D (**15**); R= OH (new compound)



Feroniellide E (**16**) (new compound)



Scheme 3.1 Isolation procedure of the dichloromethane crude extract (stem bark)

27

3.2 Structure Elucidation of New Triterpenoids.

3.2.1 Feroniellide C (13)

Feroniellide C was obtained as a yellow liquid. The molecular formula $C_{39}H_{59}NO_7$ was determined by HRESIMS (*m/z* 654.4360 $[M+H]^+$). The ^1H NMR data displayed signals in three notable regions; disubstituted benzene (δ 6.8-8.0, 4H), oxygenated protons (δ 4.70, 4.66, 4.28, 3.86 and 3.58), and olefinic proton (δ 5.45). The resonances in the down field region [δ 6.97 (H-4'), 7.42 (H-5'), 6.79 (H-6') and 7.89 (H-7')] and the singlet methyl at δ 2.90 indicated the existence of an *N*-methyl anthranilate residue[20].

The ^1H NMR showed seven methyl singlets at δ 1.29, 1.21, 1.08, 1.04, 0.96, 0.94, and 0.89, in addition to overlapped resonances of the methine and methylene protons. The correlations of methyls in core structure suggested that the structure of feroniellide C possessed an apotirucallane triterpenoid (Figure 3.1)[19].

The *N*-methyl anthranilic unit was connected to C-3 as evident from downfield shifts of 81.8 (C-3) and 4.70 (H-3) along with HMBC cross peaks of H-7' (δ 7.89) to carbonyl C-1' (δ_C 168.4) and H-3 to C-1'. The location of the double bond at C-14 was determined from HMBC correlations of olefinic proton H-15 (δ 5.45) to C-13 (δ_C 47.0) and C-16 (δ_C 38.0) (Figure 3.1).

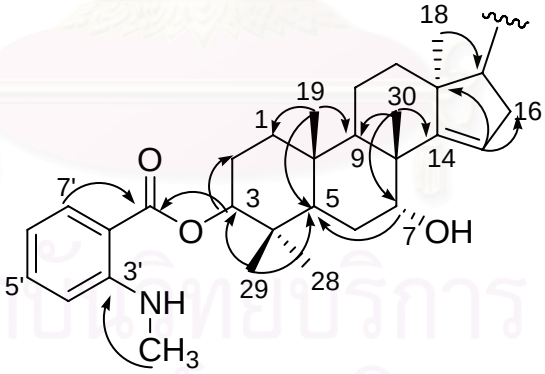


Figure 3.1 Selected HMBC correlations (curve arrows) of feroniellide C.

Five-membered acetal part was established by 2D NMR experiments. 21-OMe location was clarified by cross peaks between methoxy proton (δ 3.31) and C-21 (δ_C 109.2). Two methyls, 26-Me (δ 1.13) and 27-Me (δ 1.21) linked to quaternary oxygenated C-25 (δ_C 72.7) and C-24 (δ_C 77.8). H-21 correlated with C-22 and C-23 by cross-signal between δ 1.29 and δ_C 28.0 and δ_C 79.8. COSY spectrum demonstrated the correlation of protons in acetal. There were cross

peaks between H-20 to H-21, H-22 to H-23 and H-23 to H-24 and H-17 to H-20 (Figure 3.2).

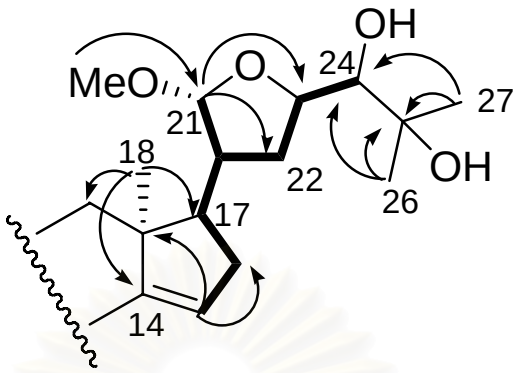


Figure 3.2 Selected HMBC (curve arrows) and COSY correlations (bold lines) in acetal part of **13.**

The relative configuration of **13** was determined from NOESY data. The cross peaks between H-3/CH_3-29, CH_3-29/CH_3-19, CH_3-19/CH_3-30, CH_3-30/H-7 H-17/H-21 and the correlations among H-5/CH_3-28, H-20/CH_3-18 and H-5/H-9, showed that H-3, CH_3-29, CH_3-19, CH_3-30, H-7 and H-21 were β-orientated while H-5, H-9, CH_3-18, and CH_3-28 were α-orientated (Figure 3.3).

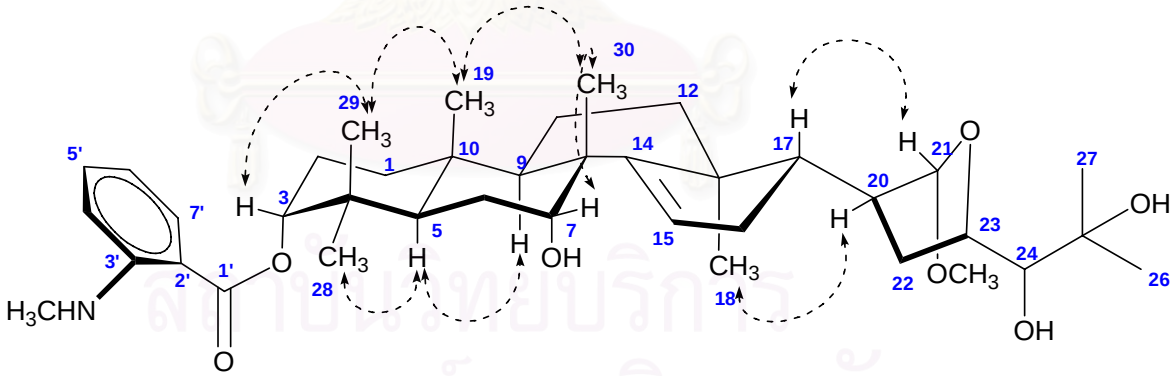


Figure 3.3 Key NOESY correlations for **13.**

The complete assignment of protons and carbons for feroniellide C was shown in Table 3.1.

Table 3.1 ^{1}H and ^{13}C NMR data for feroniellide C (**13**) in $CDCl_3$

position	δ_C	δ_H mult (*J* in Hz)	HMBC
1	38.3	1.18/1.37 m	
2	27.5	1.60/1.90 m	
3	80.8	4.70 brs	C-1', C-2
4	36.8		
5	46.1	1.86 m	
6	37.0	1.72 m	
7	75.2	3.86 brs	C-5, C-8
8	43.5		
9	44.8	2.00 m	
10	42.5		
11	16.4	1.55/1.75 m	
12	24.1	1.46/1.80 m	
13	47.0		
14	161.5		
15	119.8	5.40 br s	C-13, C-16
16	38.0	2.12 m	
17	57.6	1.75 m	
18	13.6	1.08 s	C-14, C-17
19	15.8	0.94 s	C-1, C-5, C-9
20	46.5	2.02 m	C-16
21	109.2	4.66 m	C-22, C-23
22	28.0	1.82 m	
23	79.8	4.28 m	
24	77.8	3.58 m	C-23
25	72.7		
26	27.0	1.13 s	C-24, C-25, C-27
27	25.2	1.21 s	C-24, C-25, C-26
28	27.5	0.87 s	C-3, C-4, C-5, C-29
29	17.5	0.96 s	C-3, C-4, C-5, C-28
30	20.0	1.04 s	C-7, C-9, C-14
1'	168.4		
2'	111.2		
3'	149.8		
4'	111.3	6.97 brd (8.0)	C-2'
5'	135.0	7.42 ddd (1.2, 7.2,8.0)	C-3', C-7'
6'	115.0	6.79 ddd (1.2, 7.2, 8.0)	C-2'
7'	132.4	7.89 d (8.0)	C-1', C-3', C-5'
21-OMe	54.9	3.31 s	C-21
N-Me	33.0	2.90 s	C-3'

3.2.2 Feroniellide D (15)

Feroniellide D was isolated as a colourless liquid. The molecular formula $C_{35}H_{58}O_7$ was determined by HRESIMS (*m/z* 613.4053 $[M+Na]^+$). The ^1H NMR data exhibited resonances similar to those of **13** except for the absence of aromatic proton signal and singlet methoxy signal. There were two doublet methylene signals in the upfield region [δ 0.74 (1H, d, *J* = 3.6 Hz) and 0.45 (1H, d, *J* = 4.4 Hz)]. The data suggested that methyl group at C-13 in **13** was replaced by cyclopropane ring in **15**. The HMBC correlations of H-18/C-13 and H-18/C-14 allowed connection of the cyclopropane moiety at C-13 (δ_C 28.1) and C-14 (δ_C 39.5) (Figure 3.4). The data demonstrated that **15** was the triterpenoid of 14,18-cycloapotirucallane-type[19].

The ^1H NMR showed eight singlet methyl peaks; six of them were accounted for as triterpenoid skeleton. The remaining two methyl groups suggested that the triterpenoid contained dimethyl side chain. From NMR data, the overall structure of **15** was nearly identical to triterpene **14**[19]. Two methyl groups (4′- and 5′- Me) were assigned in isovaleryl ester side chain (δ 0.94 and 0.95) by HMBC. These methyls had correlations with methine carbon, C-3′ (δ_C 31.0) and methylene carbon C-2′ (δ_C 43.9). COSY data demonstated the relation of H-4' (δ 0.94) and H-5' (δ 0.95) with H-3'(δ 2.10), H-2′ (δ 2.20) with H-3′. H-3′, H-2′ and H-3 (δ 4.53) showed cross peaks with carbonyl, C-1′ (δ_C 172.6) in HMBC. The data indicated that isovaleryl ester was connected to C-3 (Figure 3.4).

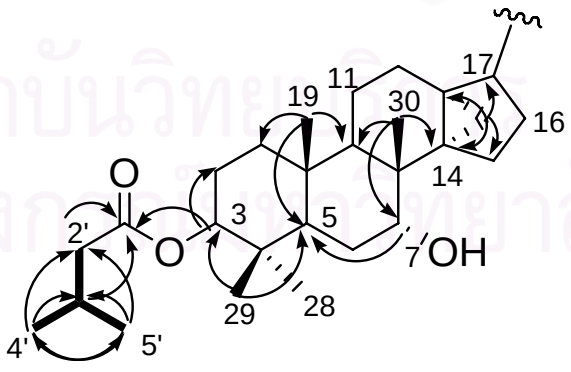


Figure 3.4 Selected HMBC (curve arrows) and COSY correlations (bold lines) of feroniellide D.

Five membered hemiacetal part was recognized from the signal of δ_H 5.30 and δ_C 96.9 for C-21 and the absence of a singlet methoxy at C-21. The data suggested that this part was similar to that **10**. Interpretation of HSQC, HMBC and COSY data led to the assignment of hemiacetal for these remaining resonances (Figure3.5).

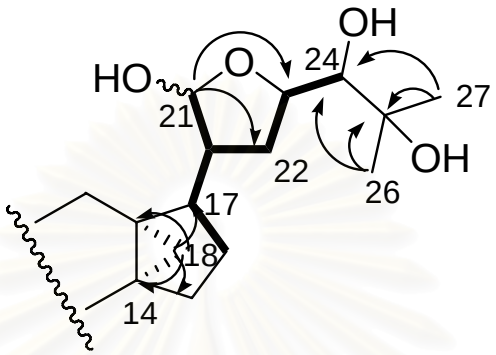


Figure 3.5 Selected HMBC (curve arrows) and COSY correlations (bold lines) of hemiacetal part of **15**.

The relative configuration of **15** was determined from NOESY data. The cross peaks between H-3/H5, H-5/H-9, H-9/H-18 and H-3/CH_3-29, CH_3-29/CH_3-19, CH_3-19/CH_3-30 showed that H-3, H-5, H-9 and H-18 were α-orientated while CH_3-29, CH_3-19 and CH_3-30 were β-orientated (Figure 3.6).

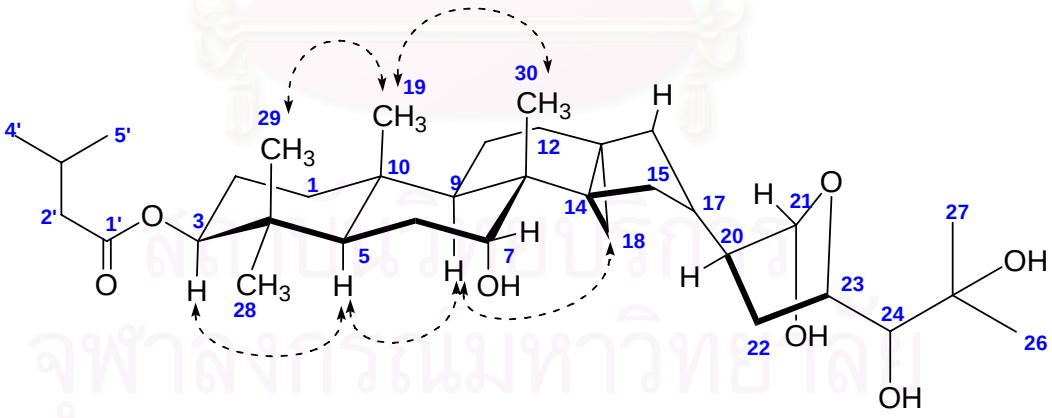


Figure 3.6 Key NOESY correlations for **15.**

The complete assignment protons and carbons for feroniellide D was shown in Table 3.2.

Table 3.2 ^{1}H and ^{13}C NMR data for feroniellide D (**15**) in $CDCl_3$

position	δ_C	δ_H mult (*J* in Hz)	HMBC
1	38.8	1.18/1.38 m	
2	24.0	1.58/1.84 m	
3	80.3	4.50 dd (4.8, 11.2)	C-1', C-2, C-28
4	34.0		
5	45.0	1.55 m	
6	26.0	1.70 m	
7	74.0	3.75 brs	C-5, C-8, C-9
8	41.0		
9	44.8	1.85 m	
10	38.0		
11	16.2	1.32 m	
12	24.2	1.84 m	
13	28.1		
14	39.5		
15	25.9	1.92 m	
16	27.7	1.67 m	C-13, C-14
17	48.8	2.16 s	
18	13.6	0.45 d (4.4), 0.74 d (3.6)	C-13, C-14, C-15, C-17
19	15.9	0.87 s	C-1, C-5, C-9, C-10
20	49.6	1.80 m	C-17
21	96.9	5.30 brs	C-20, C-22, C-23
22	26.4	1.60 m	C-21, C-23
23	77.6	4.23 m	C-22
24	74.7	3.62 m	C-23
25	72.0		
26	25.7	1.17s	C-24, C-25, C-27
27	27.1	1.23 s	C-24, C-25, C-26
28	27.7	0.83 s	C-3, C-5,C-29
29	16.9	0.85 s	C-3, C-5,C-28
30	19.2	1.02 s	C-7,C-8, C-10, C-14
1'	172.6		
2'	43.9	2.20 d (6.0)	C-1', C-3'
3'	25.8	2.10 m	C-1', C-4'
4'	21.9	0.94 s	C-2', C-3', C-5'
5'	21.9	0.95 s	C-2', C-3', C-4'

3.2.3 Feroniellide E (16)

Feroniellide E was obtained as a yellow liquid. Its molecular formular was determined by HRESIMS (*m/z* 595.3969 $[M+Na]^+$) to be $C_{35}H_{36}O_6$. The NMR spectra of **16** were quite similar to those of **15**, suggesting that **16** had the same gross structure. The major difference observed between **16** and **15** was the presence of olefinic proton H-15 (δ_H 5.40) in **16**. The location of double bond at C-14 and C-15 was determined by HMBC correlations of olefinic proton H-15 to C-13 (δ_C 46.6) and C-16 (δ_C 27.7). The data suggested that feroniellide E was a triterpenoid of the $\Delta^{14,15}$-apotirucallane-type.

The occurrence of a cyclic acetal was inferred from characteristic signals at δ_H 5.45 and δ_C 103.0. A careful comparison of the NMR data of **16** and **15** indicated a noticeable difference in the resonance of C-21, in which that of **16** experienced more downfield shift (δ_C 103.0) than **15** (δ_C 96.9) (Figure 3.6).

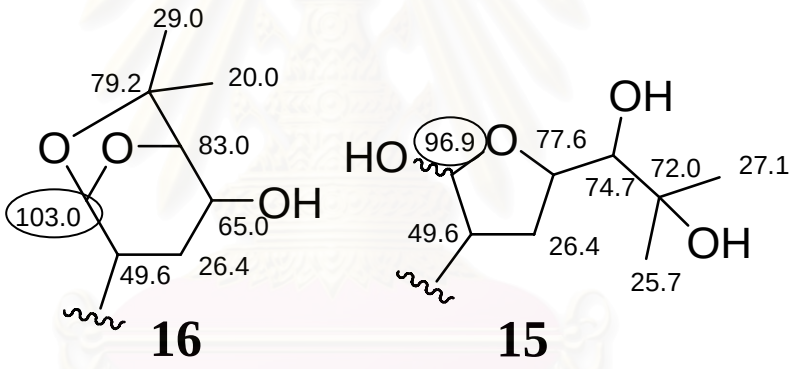


Figure 3.7 Carbon chemical shifts of in cyclic acetals in **16** and **15**.

The HMBC correlation of H-21 to C-24 and C-25 indicated that an additional ether bridge was formed between C-21 and C-25. COSY data showed contiguous correlations from H-21 to H-24, thus completing the dioxabicyclic [3.2.1]octane moiety. The formation of dioxabicyclic possibly arises from the intramolecular nucleophilic attack of the hydroxyl (25-OH) at the cyclic acetal carbon (C-21). The COSY correlation between H-20 with H-17 suggested that cyclic acetal was linked to tetracyclic at C-17 (Figure 3.8). In fact, the carbon chemical shifts of this portion were similar to those of feroniellide A[18], anthranilic triterpenoid, which was previously isolated from the roots of *Feroniella lucida* (Figure 3.9).

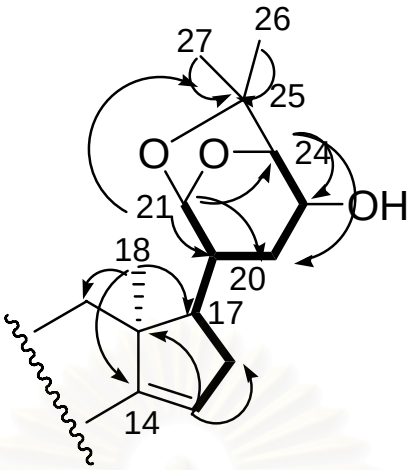


Figure 3.8 Selected HMBC (curve arrows) and COSY correlations (bold lines) of **16**.

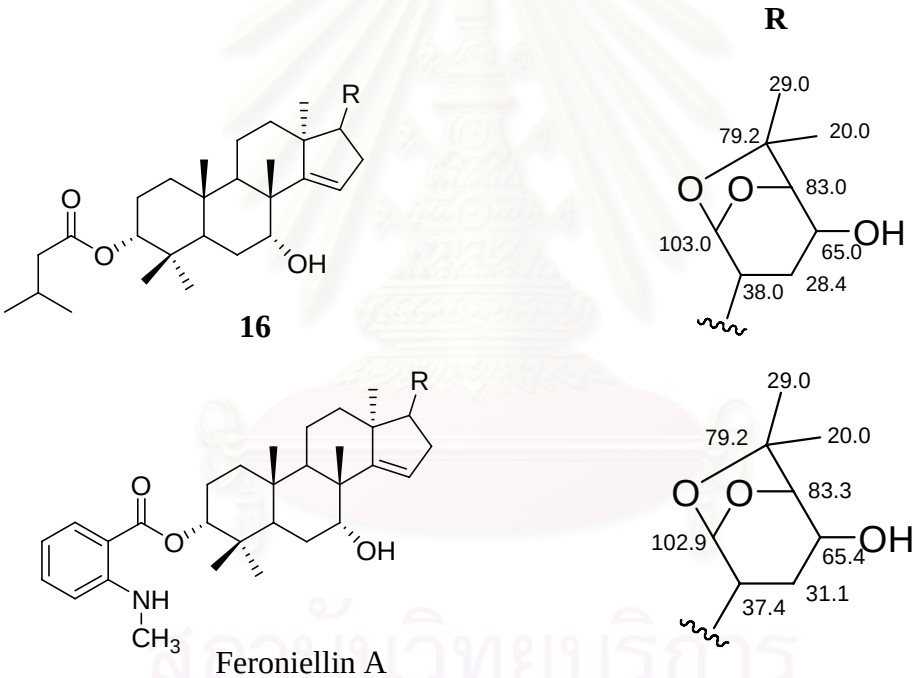


Figure 3.9 Structure of **16** and feroniellide A demonstrating carbon chemical shifts around dioxabicyclic [3.2.1] octane moiety.

The relative configuration of **16** was determined from NOESY data. The cross peaks between H-3/CH_3-29, CH_3-19/CH_3-30, CH_3-30/H-7, showed that H-3, CH_3-29, CH_3-19, CH_3-30 were β-orientated. The correlations of H-21/H-24 and H-23/CH_3-26 affirmed the configurations of the dioxabicyclic moiety (Figure 3.10). The complete assignment of protons and carbons for feroniellide E was shown in Table 3.3.

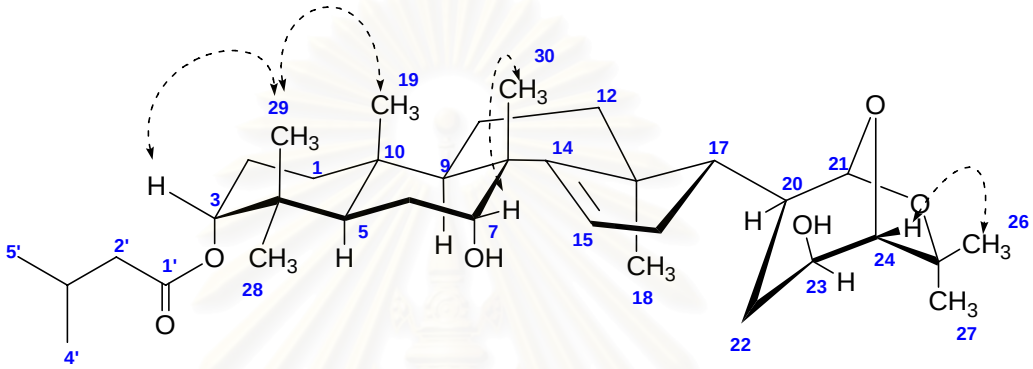


Figure 3.10 Key NOESY correlations for **16**.

Table 3.3 ^{1}H and ^{13}C NMR data for feroniellide E (**16**) in $CDCl_3$

position	δ_C	δ_H mult (*J* in Hz)	HMBC
1	38.8	1.18/1.38 m	
2	24.0	1.58/1.84 m	
3	78.8	4.65 brs	C-1', C-2, C-4
4	34.0		
5	45.0	2.00 m	
6	34.2	1.70 m	
7	74.0	3.85 brs	C-9
8	44.0		
9	41.5	2.15 m	
10	38.0		
11	16.2	1.32/1.75 m	
12	24.1	1.46/1.84 m	
13	46.6		
14	162.4		
15	115.7	5.40 brs	C-13, C-16
16	38.0	2.12 m	
17	55.2	1.75 m	
18	13.6	1.05 s	C-12, C-13, C-14, C-17
19	15.9	0.84 s	C-1, C-5, C-9, C-10
20	38.0	1.80 m	C-17
21	103.0	5.45 brs	C-20, C-22, C-24, C25
22	28.4	1.66 m	
23	65.0	3.70 m	
24	83.0	3.77 m	C-21, C-22, C-23
25	79.2		
26	29.0	1.17 s	C-24, C-25, C-27
27	20.0	1.23 s	C-24, C-25, C-26
28	27.5	0.79 s	C-3, C-4, C-5
29	21.5	0.83 s	C-3, C-4, C-5
30	19.2	1.02 s	C-7,C-8, C-9, C-14
1'	172.6		
2'	43.9	2.18 d (6.0)	C-1', C-3', C-4', C-5′
3'	25.8	2.10 m	C-1', C-4'
4'	22.1	0.89 s	C-2', C-3', C-5'
5'	22.1	0.91 s	C-2', C-3', C-4'

The occurrence of an *N*-methyl anthranilic acid moiety in triterpenoids is rare. The recent examples were reported from *R. echinata*[20] and from the root of *F. lucida*[18]. Feroniellide C is the first report of $\Delta^{14,15}$-apotirucallane triterpenoid containing *N*-methyl anthranilic acid moiety. On the other hand, many triterpenoids having isovaleric acid moiety was isolated from *C. sinensis*[19]. Most of them contained cyclopropyl group between C-13, 14 and methoxy group at C-21. Feroniellide D is the first report of isovalerate triterpenoid having hydroxyl group at C-21 and feroniellide E is the first report for containing dioxabicyclo [3.2.1] octane moiety isolated from the plants. This compound was similar to those *N*-methyl anthranilic triterpenoid; feroniellide A. Compounds bearing similar heterobicyclic systems have been encountered particularly in insect pheromones such as brevicomin[26], frontalin[27] and soridin[28].

3.3 Bioactivity of isolated triterpenoids

The cytotoxic activity against HeLa and KB cell lines of all compounds were determined using MTT assay and evaluated for acetylcholinesterase inhibitory effect. The results were shown in Tables 3.4 and 3.5.

Table 3.4 Cytotoxic activity of **10-16** against HeLa and KB cell lines

Compound	HeLa IC_{50} (μg/mL)	KB IC_{50} (μg/mL)
10	14	5.2
11	18.6	14.1
12	28	19.5
14	8.2	12
Feroniellide C (**13**)	27.5	25.5
Feroniellide D (**15**)	10	4.1
Feroniellide E (**16**)	14	3.4

Note: Standard agent (Adriamycin IC_{50} = 0.018 μg/mL)

Table 3.5 Acetylcholinesterase inhibitory effect of **10-16**

Compound	Concentration (mg/mL)			
	1.000	0.500	0.250	0.125
10	-	-	-	-
11	+	+	-	-
12	+	+	-	-
14	-	-	-	-
Feroniellide C (**13**)	-	-	-	-
Feroniellide D (**15**)	-	-	-	-
Feroniellide E (**16**)	-	-	-	-

Note: Standard acetylcholinesterase inhibitor = Phytostigmine (Eserine) +, 0.125 mg/mL (+: inhibit, -: not inhibit)

From Table 3.4, **14** showed strong cytotoxic activity against HeLa cell lines with IC_{50} = 8.2 μg/mL. For the KB cell lines, **14** showed cytotoxic activity against the cell lines lower than HeLa cells lines with IC_{50} = 12 μg/mL. **10**, **15**, and **16** showed strong cytotoxic activity against KB cell lines with IC_{50} = 5.2, 4.1, and 3.4 μg/mL, respectively. According to an acetylcholinesterase inhibitory assay of two triterpenoids, **11** and **12** are powerful inhibitors at MIC value of 0.5 mg/mL when compared with standard inhibitor, phytostigmine (eserine).

3.4 Experiment section

3.4.1 Plant material

The stem barks of *Feroniella lucida* were collected in Tah-Utane Nakhon Phanom, in March 2005.

3.4.2 Equipments

NMR spectra were recorded with a Varian model Mercury+ 400 which operated at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei. HPLC was performed on Water® 600 controllers equipped with a Water® 2996 dual UV wavelength detector (USA). Econosphere 5C18-ARII (25 × 250 mm) reversed phase column (Alltech Associates, IL, USA) was used for separation (UV λ 220 and 254 nm). Most solvents used in this research for column chromatography were commercial grade and were distilled prior to use. Adsorbents such as silica gel 60 Merck cat. No. 7731, 7734, and 7749 were used for quick column chromatography, open column chromatography, chromatotron, respectively. Thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F254 plates (0.25 mm thick layer). Chromatotron (model 7924 T, Harrison Research) with silica gel plate of 1 mm thickness was used on centrifugal thin layer chromatography.

3.4.3 The cytotoxic activity against HeLa and KB cell lines by MTT assay

All compounds (1 mg) were examined for cytotoxic activity against HeLa and KB cell lines by MTT assay. This assay was performed at Natural Products Research Section, Research Division, National Cancer Institute, Thailand. The results were shown in Table 3.4.

3.4.4 Acetylcholinesterase inhibitor assay [25]

Acetylcholinesterase inhibitory effect of isolated triterpenoids was evaluated by the same procedure as in Chapter II. The results were shown in Table 3.5.

(21,23*S*)-Epoxy-7α,21,24,25-tetrahydroxy-4α,4*β*,8*β*,10*β*-tetramethyl-25-dimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3*β*-*N*-methylanthranilic acid ester (10): pale yellow liquid ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (1H, d, *J* = 8 Hz H-7'), 7.30 (1H, t, *J* = 8.4 Hz H-5'), 6.59 (1H, d, *J* = 8.4 Hz H-4'), 6.52 (1H, t, *J* = 8.8 Hz H-6'), 5.27 (1H, brs, H-21), 4.66 (1H, m, H-3), 4.25 (1H, m, H-23), 3.71 (1H, d, *J* = 6.8 H-7), 3.56 (1H, s, H-24), 2.83 (3H, s, NCH_3), 1.20 (3H, s, 27-CH_3), 1.19 (3H, s, 26-CH_3), 1.12 (3H, s, 30-CH_3), 0.98 (3H, s, 19-CH_3), 0.95 (3H, s, 29-CH_3), 0.89 (3H, s, 28-CH_3), 0.62 (1H, d, *J* = 3.6 Hz H-18), 0.41 (1H, d, *J* = 4.8 Hz H-18).

(21*R*,23*S*)-Epoxy-21α-methoxy-7α,24,25-trihydroxy-4α,4*β*,8*β*,10*β*-tetra methyl-25-dimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3*β*-*N*-methyl anthranilic acid ester (11): yellow liquid ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (1H, d, *J* = 8 Hz H-7'), 7.31 (1H, t, *J* = 8.4 Hz H-5'), 6.63 (1H, d, *J* = 8.4 Hz H-4'), 6.53 (1H, t, *J* = 8.8 Hz H-6'), 4.70 (1H, s, H-21), 4.66 (1H, m, H-3), 4.25 (1H, m, H-23), 3.71 (1H, s, H-7), 3.52 (1H, s, H-24), 3.33 (3H, s, 21-OCH_3), 2.84 (3H, s, NCH_3), 1.20 (3H, s, 27-CH_3), 1.18 (3H, s, 26-CH_3), 1.12 (3H, s, 30-CH_3), 0.98 (3H, s, 19-CH_3), 0.93 (3H, s, 29-CH_3), 0.87 (3H, s, 28-CH_3), 0.63 (1H, d, *J* = 3.6 Hz H-18), 0.41 (1H, d, *J* = 4.8 Hz H-18).

(21*R*,23*S*)-Epoxy-21*β*-methoxy-7α,24,25-trihydroxy-4α,4*β*,8*β*,10*β*-tetra methyl-25-dimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3*β*-*N*-methyl anthranilic acid ester (12): colourless liquid ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (1H, d, *J* = 8 Hz H-7'), 7.37 (1H, t, *J* = 8.4 Hz H-5'), 6.69 (1H, d, *J* = 8.4 Hz H-4'), 6.59 (1H, t, *J* = 8.8 Hz H-6'), 4.84 (1H, *d*, *J* = 3.2 Hz H-21), 4.64 (1H, brs, H-3), 4.05 (1H, m, H-23), 3.75 (1H, brs, H-7), 3.52 (1H, d, *J* = 5.0 Hz H-24), 3.35 (3H, s, 21-OCH_3), 2.90 (3H, s NCH_3), 1.28 (3H, s, 27-CH_3), 1.19 (3H, s, 26-CH_3),

1.00 (3H, s, 30-CH_3), 0.95 (3H, s, 19-CH_3), 0.93 (3H, s, 29-CH_3), 0.84 (3H, s, 28-CH_3), 0.70 (1H, d, *J* = 3.6 Hz H-18), 0.47 (1H, d, *J* = 4.4 Hz H-18).

(21*R*,23*S*)-epoxy-21β-methoxy-7α,24,25-trihydroxy-4α,4*β*,8*β*,10*β*-tetramethyl-25-dimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3α-isovaleric acid ester (14): colourless liquid ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.46 (1H, s, H-3), 4.28 (1H, s, H-23), 3.74 (1H, s, H-7), 3.46 (3H, s, 21-OCH_3), 3.34 (1H, s, H-24), 2.20 (2H, d, *J* = 6.6 H-2'), 2.10 (1H, m, H-3'), 1.25 (3H, s, 27-CH_3), 1.17 (3H, s, 26-CH_3) 1.02 (3H, s, 30-CH_3), 0.95 (3H, s, 5'-CH_3), 0.94 (3H, s, 4'-CH_3) 0.87 (3H, s, 19-CH_3), 0.86 (3H, s, 29-CH_3), 0.83 (3H, s, 28-CH_3) 0.69 (1H, d, *J* = 3.6 Hz H-18), 0.46 (1H, d, *J* = 4.4 Hz H-18).

Feroniellide C (13): yellow liquid $[\alpha]_D^{24.8}$ -12.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 223.0 (3.83) , 256.0 (2.82), 352.0 (3.38); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 3.1; HRESIMS *m/z* $[M+H]^+$ 654.4360 (calcd for $C_{39}H_{60}NO_7$, 654.4370).

Feroniellide D (15): yellow liquid $[\alpha]_D^{24.8}$ +8.19 (*c* 0.62, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 3.2; HRESIMS *m/z* $[M+Na]^+$ 613.4053. (calcd for $C_{35}H_{58}O_7Na$, 613.4075).

Feroniellide E (16): brown liquid $[\alpha]_D^{24.8}$ -16.9 (*c* 0.20, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 3.3; HRESIMS *m/z* $[M+Na]^+$ 595.3955 (calcd for $C_{35}H_{36}O_6Na$, 595.3969).

Supporting information

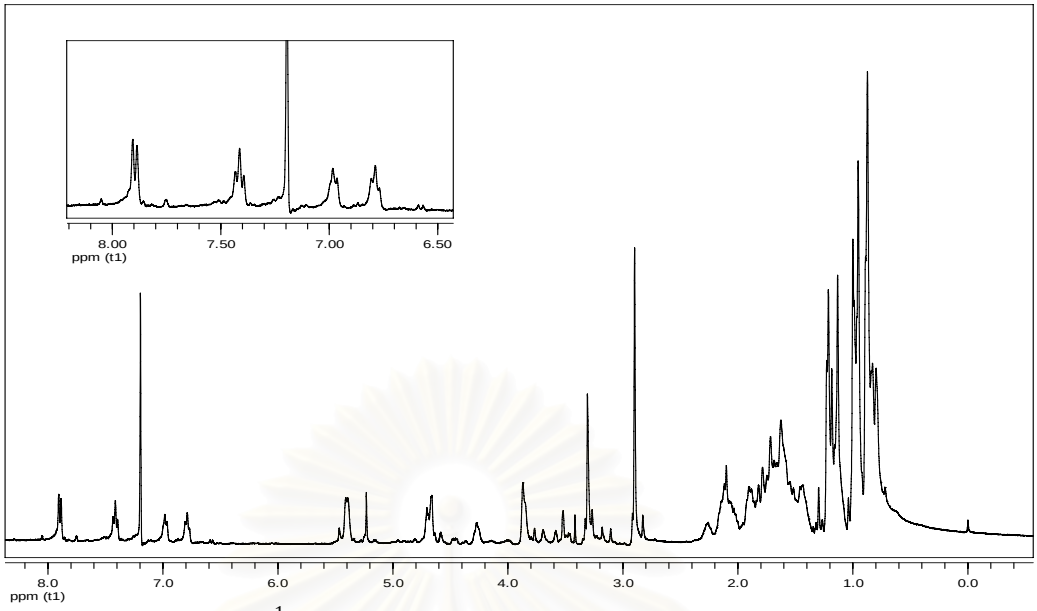


Figure S-3.1 The ^{1}H NMR spectrum of feroniellide C (**13**) in $CDCl_3$.

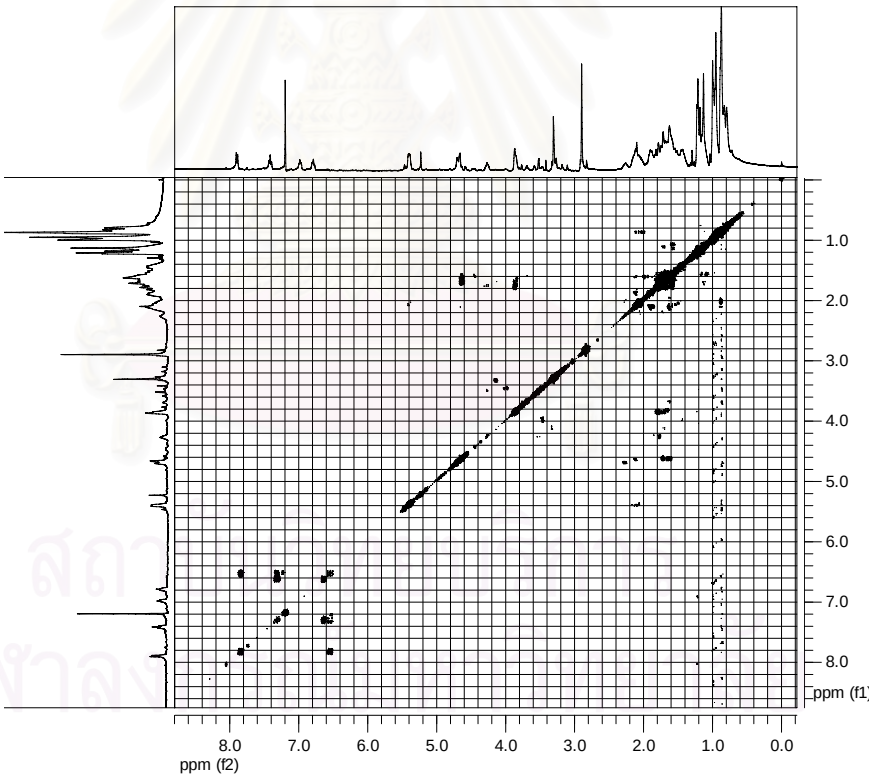


Figure S-3.2 The COSY spectrum of feroniellide C (**13**) in $CDCl_3$.

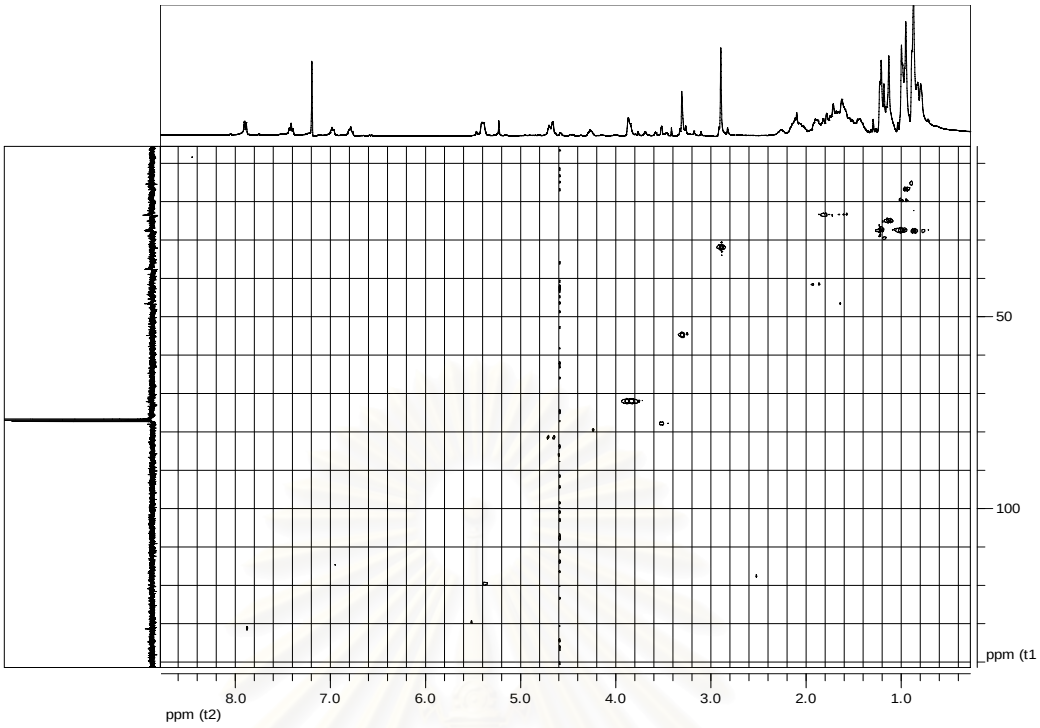


Figure S-3.3 The HSQC spectrum of feroniellide C (**13**) in $CDCl_3$.

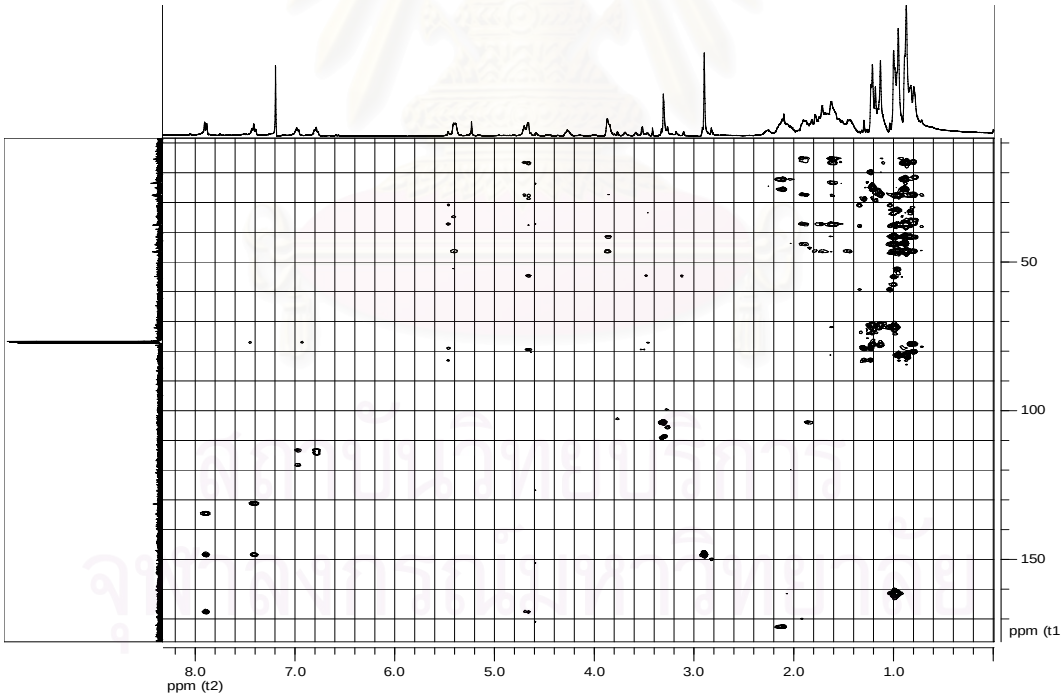


Figure S-3.4 The HMBC spectrum of feroniellide C (**13**) in $CDCl_3$.

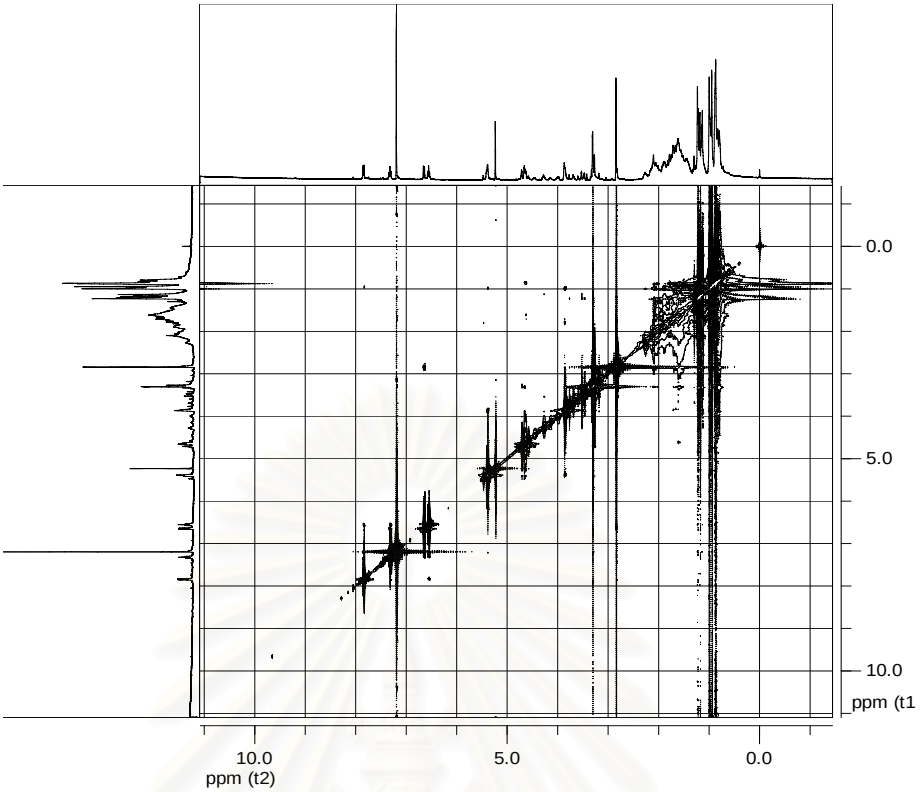


Figure S-3.5 The NOESY spectrum of feroniellide C (**13**) in $CDCl_3$

Figure S-3.6 The high resolution mass spectrum of feroniellide C (**13**)

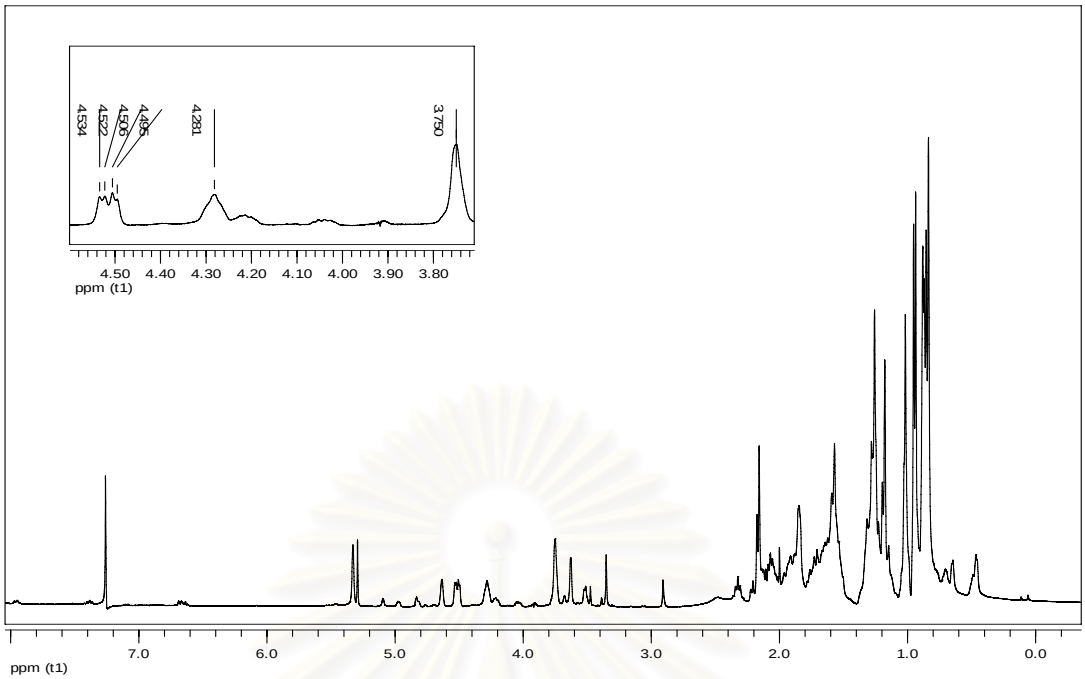


Figure S-3.7 The ^{1}H NMR spectrum of feroniellide D (**15**) in $CDCl_3$

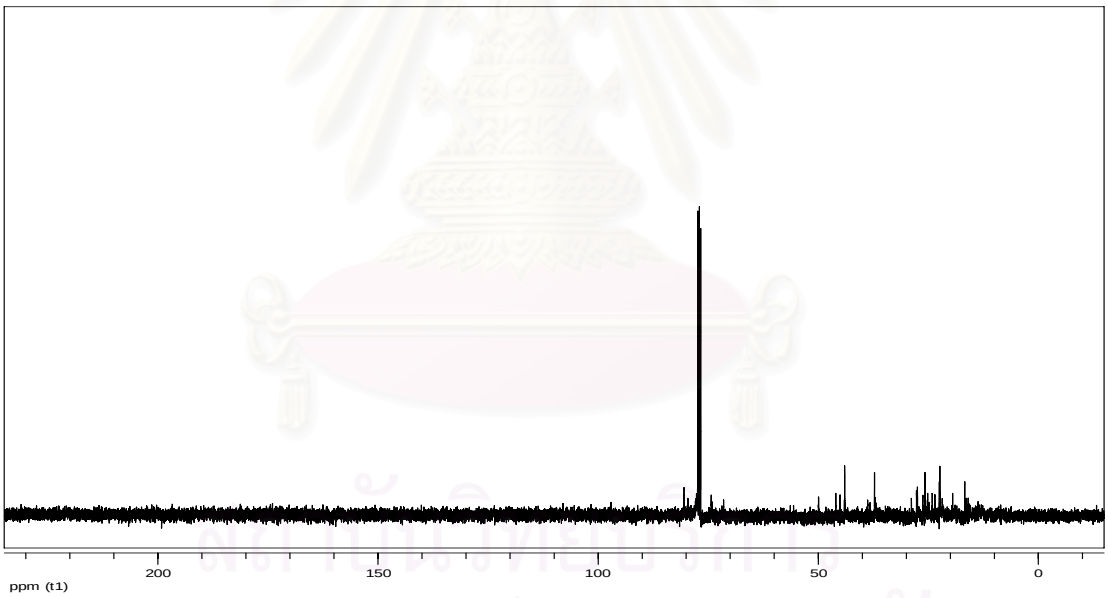


Figure S-3.8 The ^{13}C NMR spectrum of feroniellide D (**15**) in $CDCl_3$

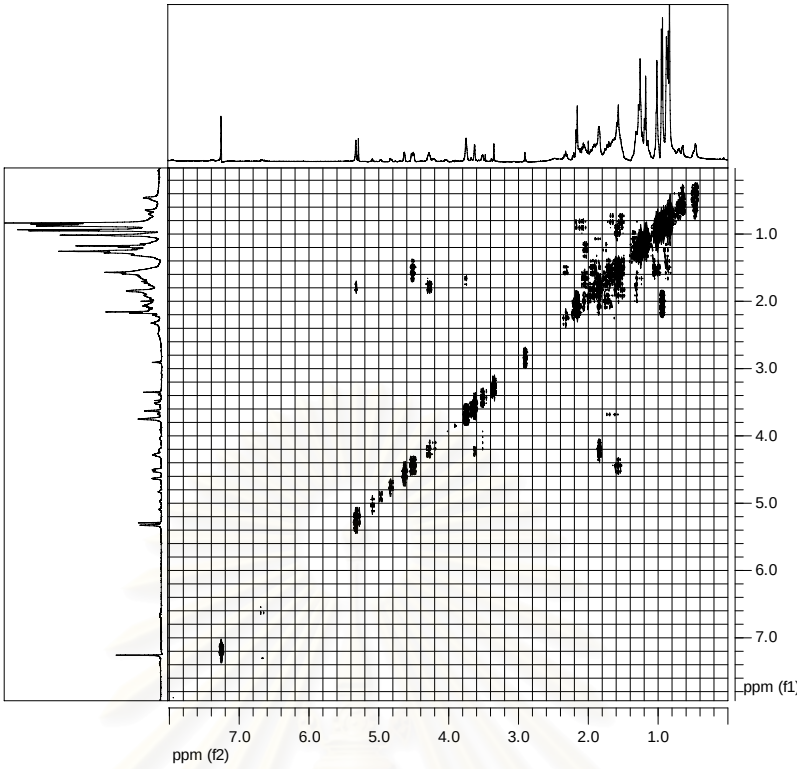


Figure S-3.9 The COSY spectrum of feroniellide D (**15**) in $CDCl_3$

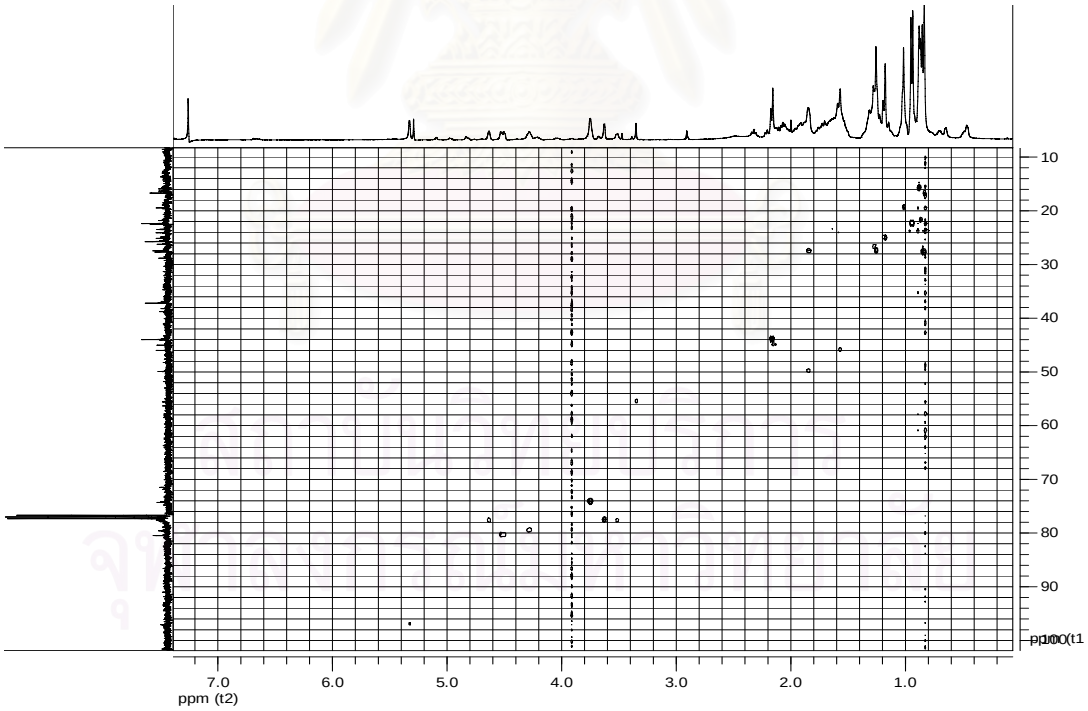


Figure S-3.10 The HSQC spectrum of feroniellide D (**15**) in $CDCl_3$

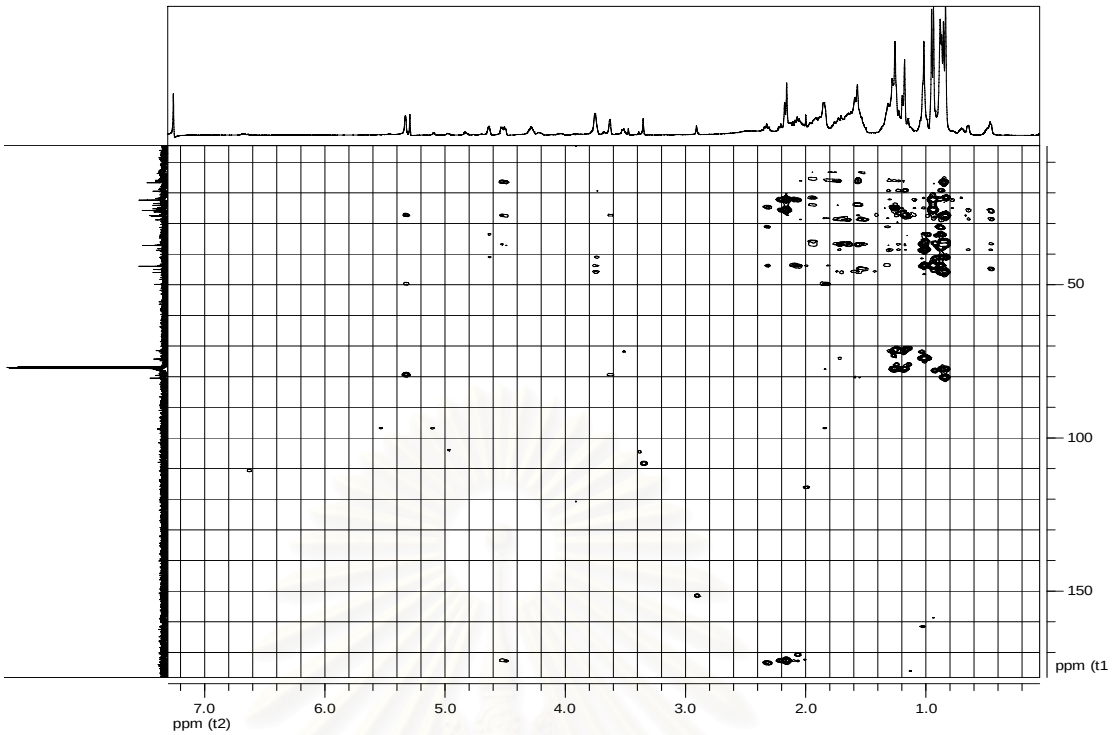


Figure S-3.11 The HMBC spectrum of feroniellide D (**15**) in $CDCl_3$.

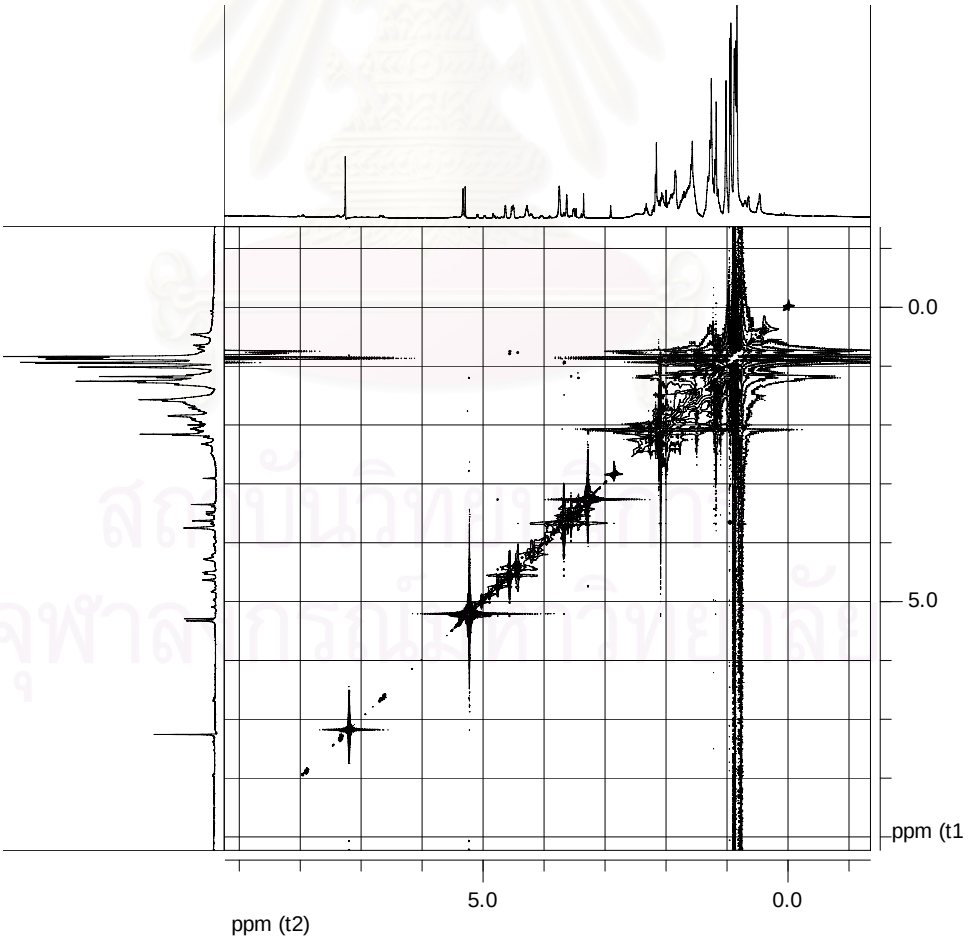


Figure S-3.12 The NOESY spectrum of feroniellide D (**15**) in $CDCl_3$

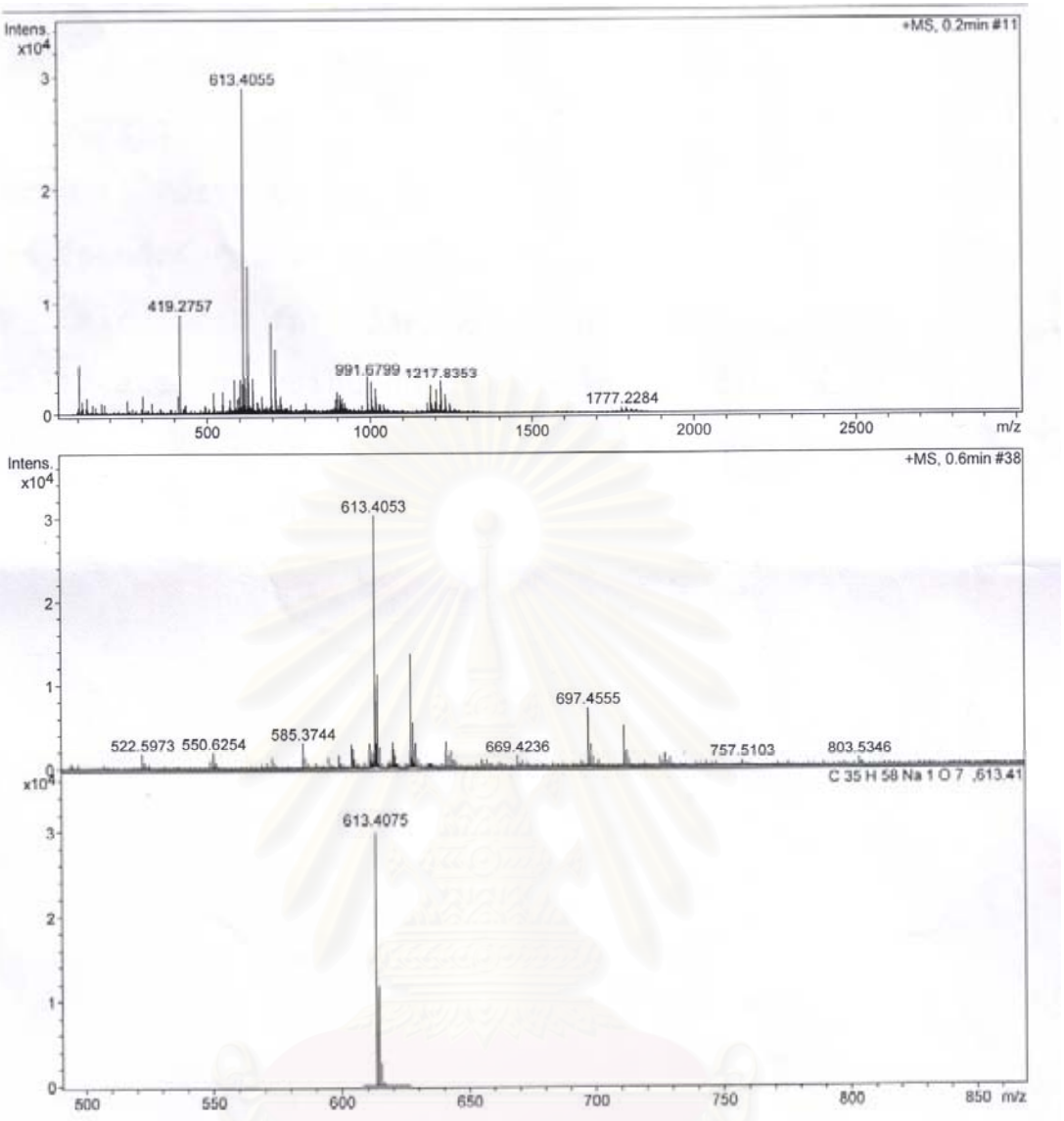


Figure S-3.13 The high resolution mass spectrum of feroniellide D (**15**).

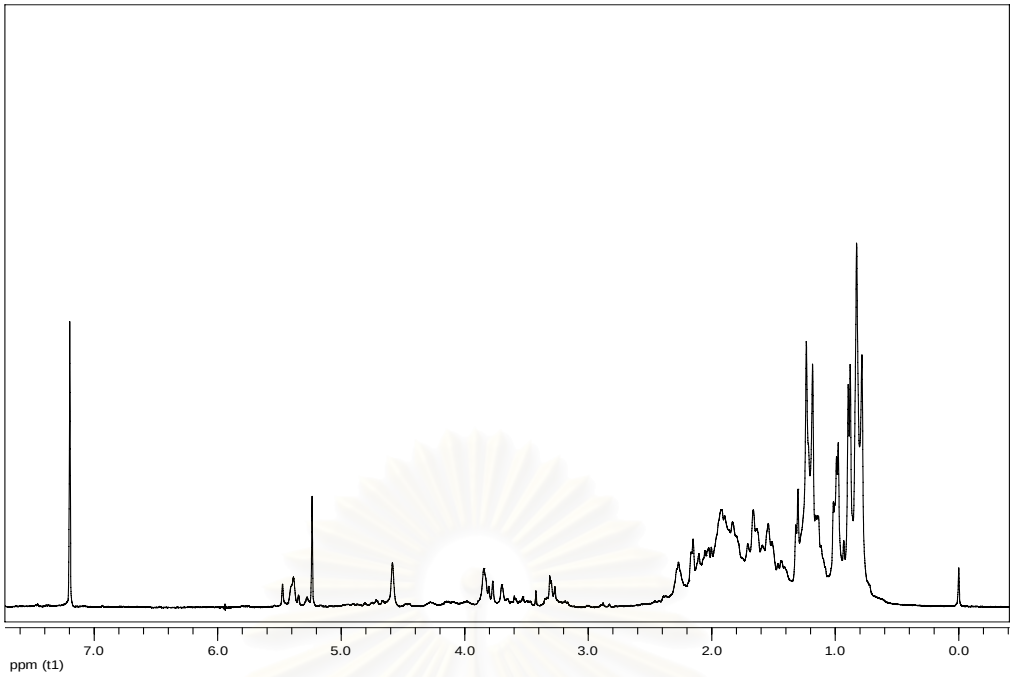


Figure S-3.14 The ^{1}H NMR spectrum of feroniellide E (**16**) in $CDCl_3$.

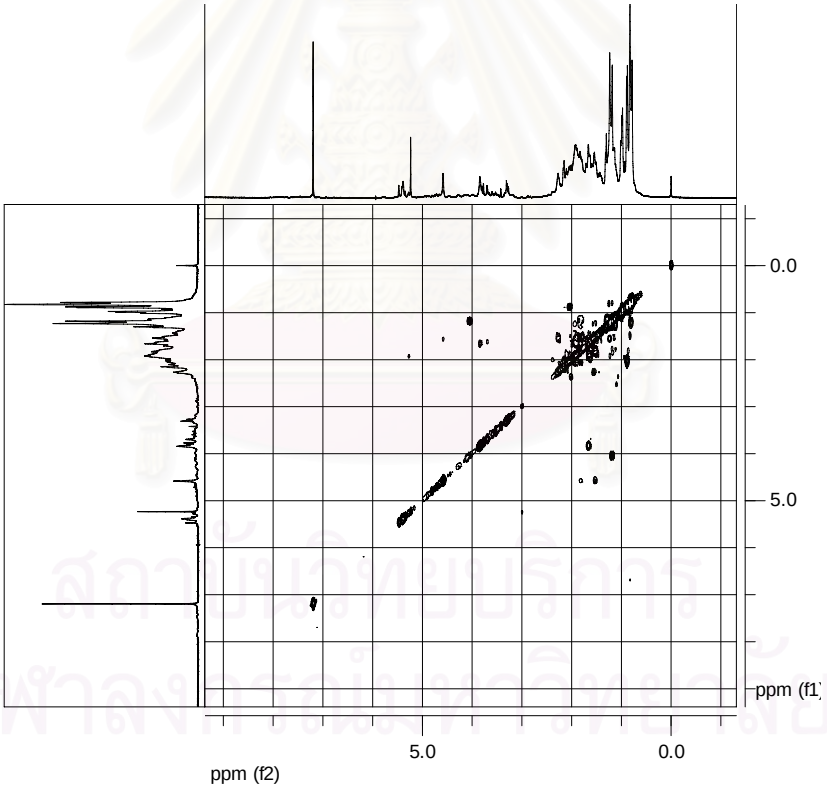


Figure S-3.15 The COSY spectrum of feroniellide E (**16**) in $CDCl_3$.

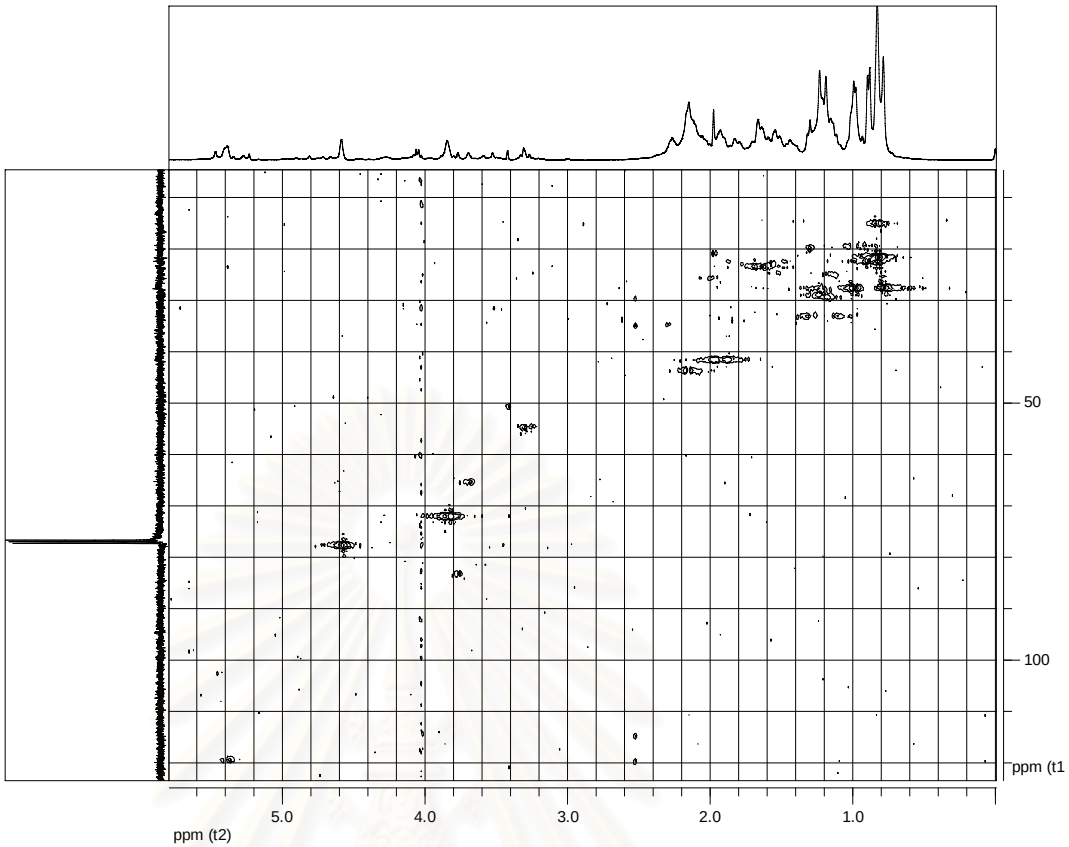


Figure S-3.16 The HSQC spectrum of feroniellide E (**16**) in $CDCl_3$

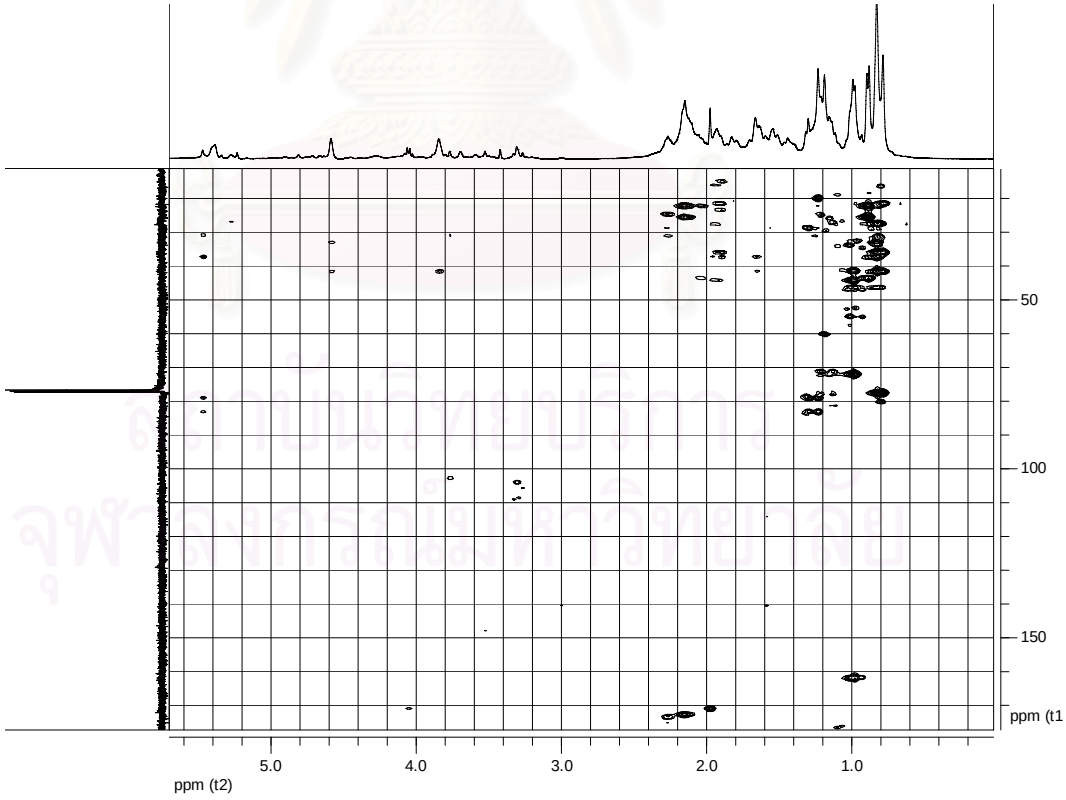


Figure S-3.17 The HMBC spectrum of feroniellide E (**16**) in $CDCl_3$

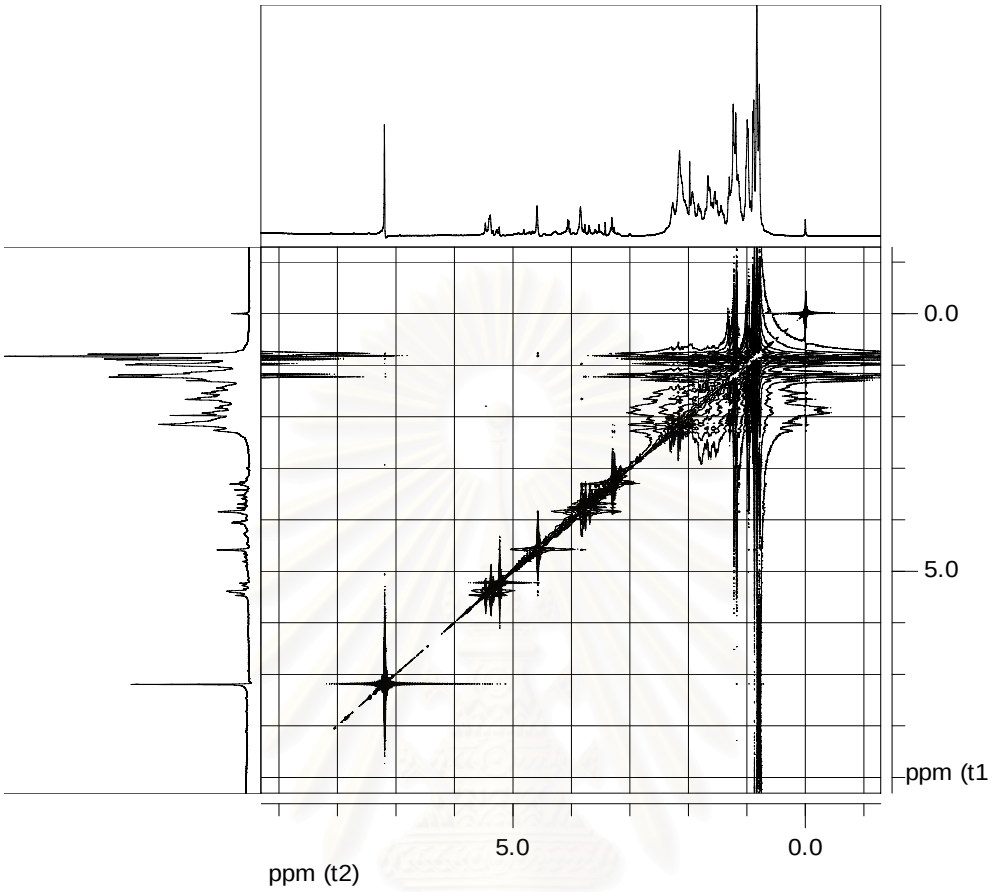


Figure S-3.18 The NOESY spectrum of feroniellide E (**16**) in $CDCl_3$

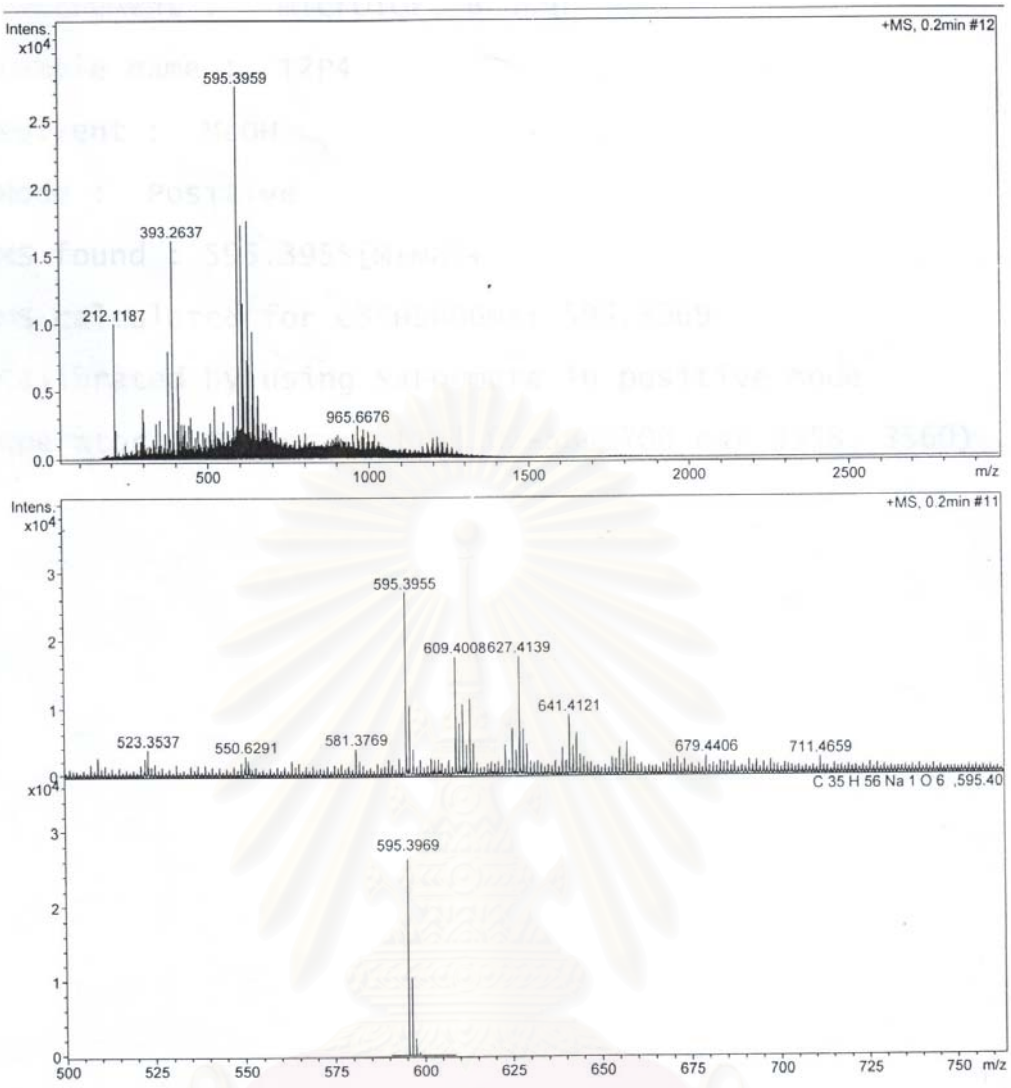
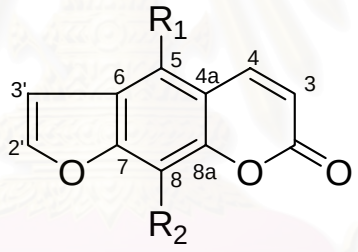


Figure S-3.19 The high resolution mass spectrum of feroniellide E (**16**)

CHAPTER IV

CONCLUSION

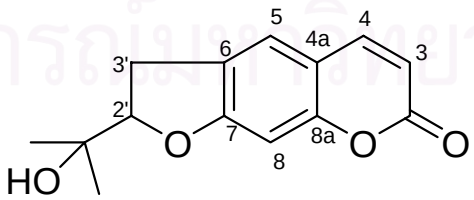
In conclusion, the isolation of the dichloromethane crude extract from the stems and stem barks of *Feroniella lucida* yielded sixteen compounds, which could be divided into two groups; coumarins and triterpenoids. The novel furanocoumarin, feroniellin A (**7**) along with eight known coumarins; psoralen (**1**), bergapten (**2**), isopimpinellin (**3**), anisolactone (**4**), 2′,3′-epoxyanisolactone (**5**), marmesin (**6**), feroniellin B (**9**) and feroniellin C (**8**) were obtained from the stems. From the stem bark, there were three new triterpenoids; feroniellide C (**13**), D (**15**) and E (**16**) and four known compounds (**10**-**12** and **14**). The chemical structures of all isolated compounds were characterized by means of NMR and MS experiments and compared with previous reports.



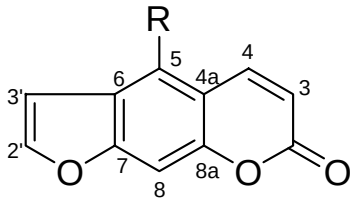
Psolaren (**1**): R_1= H, R_2 = H

Bergapten(**2**): R_1= OMe, R_2 = H

Isopimpinellin (**3**): R_1= OMe, R_2 = OMe



Marmesin(**6**)



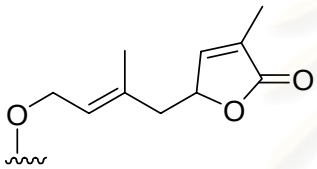
Anisolactone (**4**): R= a

2′,3′-Epoxyanisolactone (**5**): R = b

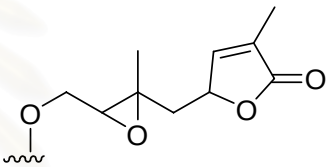
Feroniellin A (**7,** new compound): R = c

Feroniellin B (**9**): R = d

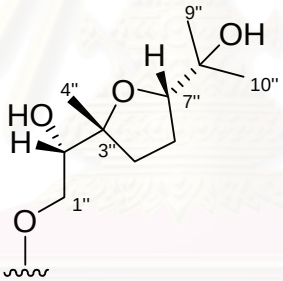
Feroniellin C (**8**): R = e



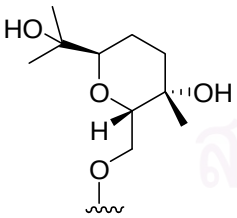
a.



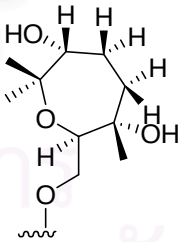
b.



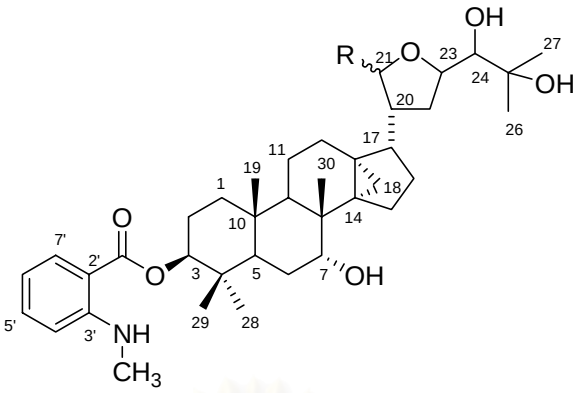
c.



d.



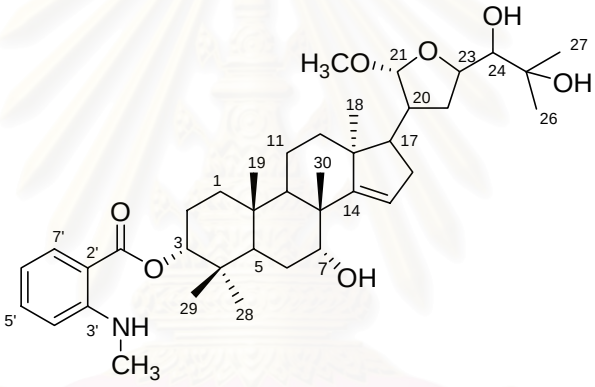
e.



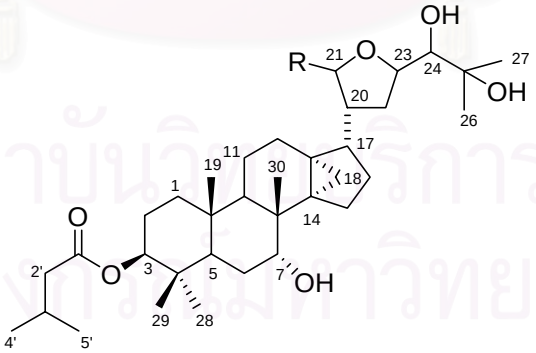
10; R = OH

11; R = α OMe

12; R = β OMe

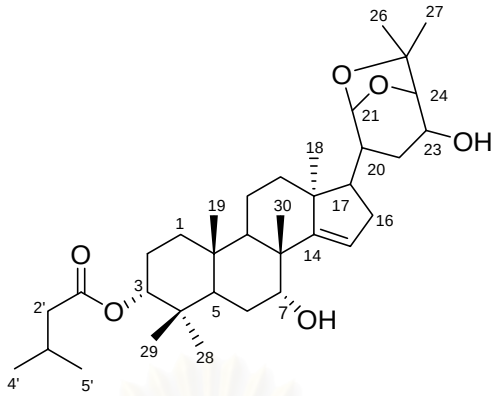


Feroniellide C (**13**, new compound)



14; R = β OMe

Feroniellide D (**15,** new compound); R= OH



Feroniellide E (**16,** new compound)

As for the chemical investigation from the stems and stem bark of *Feroniella lucida*, we were firstly reported isovaleryl apotirucallane triterpenoids **14** and **16** from the subtribe Balsamocitrinae, tribe Citrinae, as reported form Meliaceae, *Cedrela sinensis*[19]. Moreover, we found that psoralen (**1**), bergapten (**2**), isopimpinellin (**3**) and marmesin (**6**) were also previously reported from *Feronia limonia*[7,8,9,10] and psoralen (**1**) and marmesin (**6**) were also reported from *Aegle marmelos*[11]. Anisolactone (**4**) and 2′,3′-epoxyanisolactone (**5**) were reported from *Clausena anisata*[29]. Most importantly, feroniellin A (**7**), feroniellide C (**13**), feroniellide D (**15**) and feroniellide E (**16**) were reported as new compounds.

The investigation and evaluation for cytotoxic activity against HeLa cell lines of all compounds indicated that **14** was the most effective compound. For KB cell lines, triterpenoid **10**, feroniellide D (**15**) and feroniellide E (**16**) showed the stong cytotoxic activity.

According to an acetylcholinesterase inhibitor assay, isopimpinellin (**3**), marmesin (**6**), feroniellin A (**7**), feroniellin B (**9**), feroniellin C (**8**), **11**, and **12** showed inhibitory affect against acetylcholinesterase with MIC values of 0.5 mg/mL. Acetylcholine is a neuronal transmitter. It is metabolized by the enzyme acetylcholinesterase (AChE). The three drugs donepezil, rivastigmine and galantamine inhibit AChE, and they raise the concentration of acetylcholine at sites of neurotransmission. Alzheimer's disease is the most common cause of senile dementia in later life. It is estimated that up to 4 million people are affected this disease in the

USA. Inhibitors of acetylcholinesterase are currently from the basis of the newest drugs available for the management of this disease [25].

The future work may involve the synthesis of isolated coumarins and triterpenoids for increasing quantity of active compounds that could be developed the new drugs. Novel active compounds will afford the target for future synthesis and structure relationship studies as well. This will lead to better understanding in the interaction between active compounds and diseases.

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