สารออกฤทธิ์ทางชีวภาพจากเมล็ดของตะบัน *Xylocarpus rumphii* (Kostel.) Mabb.

นายชนินทร์ สาริกภูติ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

BIOACTIVE COMPOUNDS FROM THE SEED KERNELS OF *Xylocarpus rumphii* (Kostel.) Mabb.

Mr. Chanin Sarigaputi

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biotechnology
Faculty of Science
Chulalongkorn University
Academic Year 2009

Thesis Title	BIOACTIVE COMPOUNDS FROM THE SEED KERNELS OF *Xylocarpus rumphii* (Kostel.) Mabb.
By	Mr. Chanin Sarigaputi
Field of study	Biotechnology
Thesis Advisor	Assistant Professor Khanitha Pudhom, Ph.D.
Thesis Co-Advisor	Associate Professor Somchai Pengprecha, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

S. Hannongbua

.....Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

.....Chairman

(Associate Professor Somkiat Piyatiratitivorakul, Ph.D.)

Khanitha Pudhom

.....Thesis Advisor

(Assistant Professor Khanitha Pudhom, Ph.D.)

Somchai Pengprecha

.....Thesis Co-Advisor

(Associate Professor Somchai Pengprecha, Ph.D.)

N. Ngamrojanavanich

.....Examiner

(Associate Professor Nattaya Ngamrojanavanich, Ph.D.)

Damrong Sommit

.....External Examiner

(Damrong Sommit, Ph.D.)

ชนินทร์ สาริกภูติ : สารออกฤทธิ์ทางชีวภาพจากเมล็ดของตะบัน *Xylocarpus rumphii* (Kostel.) Mabb. อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ. ดร. ขนิษฐา พุดหอม, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ดร. สมใจ เพ็งปรีชา, 133 หน้า.

การศึกษาสารออกฤทธิ์ทางชีวภาพจากเมล็ดของตะบัน *Xylocarpus rumphii* (Kostel.) Mabb. โดยนำสารสกัดหยาบเอธิลอะซีเตทจากเมล็ดตะบัน มาทำการแยกสารบริสุทธิ์โดยอาศัยเทคนิคโครมาโทกราฟี สามารถแยกสารลิโมนอยด์ได้ 7 ชนิด เป็นสารใหม่ 4 ชนิด คือ xylorumphiins A-D (**3**, **4**, **1** และ **7**) และสารที่มีการรายงานมาก่อนอีก 3 ชนิด คือ methyl angolensate (**2**), xyloccensins E (**5**) และ K (**6**) การพิสูจน์ทราบโครงสร้างทางเคมีของสารที่แยกได้ทำโดยอาศัยวิธีการทางสเปกโทรสโกปี นอกจากนี้ยังเป็นการรายงานข้อมูล NMR ที่สมบูรณ์และข้อมูล X-ray ของ xyloccensin E (**5**) เป็นครั้งแรก เมื่อนำสารบริสุทธิ์ที่แยกได้ทั้งหมดมาทำการทดสอบฤทธิ์ต้านแบคทีเรียและฤทธิ์ต้านเซลล์มะเร็ง พบว่า สารทั้งหมดไม่แสดงฤทธิ์ดังกล่าว

สาขาวิชา.....เทคโนโลยีชีวภาพ..... ลายมือชื่อนิสิต.....

ปีการศึกษา.....2552..... ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

##5172255023: MAJOR BIOTECHNOLOGY
KEYWORDS: limonoids/ *Xylocarpus rumphii* / Antibacterial / Anticancer

CHANIN SARIGAPUTI: BIOACTIVE COMPOUNDS FROM THE SEED KERNELS OF *Xylocarpus rumphii* (Kostel.) Mabb. THESIS ADVISOR : ASST. PROF. KHANITHA PUDHOM, Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. SOMCHAI PENGPRECHA, Ph.D., 133 pp.

The objective of this study was to search for bioactive compounds from the seed kernels of *Xylocarpus rumphii* (Kostel.) Mabb. The ethyl acetate crude extract of *X. rumphii* was purified by chromatographic techniques to afford four new limonoids, xylorumphiins A-D (**3**, **4**, **1** and **7**), along with three known limonoids namely, methyl angolensate (**2**), xyloccensins E (**5**) and K (**6**). The chemical structures of all isolated compounds were established on the basis of chemical and spectroscopic methods. In addition, this is the first report of the complete assignments for NMR and X-ray data of xyloccensin E (**5**). However, all isolated compounds showed to be inactive for both antibacterial and anticancer activity assays.

Field of Study:.....Biotechnology.....Student's Signature.....C. Sarigaputi.....
Academic Year:.....2009..... Advisor's Signature.....Khanitha Pudhom.....
Co-Advisor's Signature.....Somchai Pengprecha.....

ACKNOWLEDGEMENTS

The author would like to express his sincere appreciation to his advisor, Assistant Professor Dr. Khanitha Pudhom, and co-advisor, Associate Professor Dr. Somchai Pengprecha, Department of Chemistry, Faculty of Science, Chulalongkorn University, for excellent instruction, great valuable advice, kind guidance and support throughout this thesis.

Many thanks to the Chairperson: Associate Professor Dr. Somkiat Piyatiratitivorakul, Department of Marine Science, Faculty of Science, Chulalongkorn University; the thesis examiners: Associate Professor Dr. Nattaya Ngamrojnavanich, Department of Chemistry, Faculty of Science, Chulalongkorn University and Dr. Damrong Sommit, Mahanakorn University of Technology, for their invaluable discussion and suggestion.

I am grateful to Associate Professor Dr. Nongnuj Muangsin, Department of Chemistry, Faculty of Science, Chulalongkorn University and Mr. Thapong Teerawatananond, Center for Petroleum, Petrochemicals, and Advanced Materials, Chulalongkorn University who kindly performed the X-ray crystallographic analysis.

Sincere thanks are extended to the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, for tumor cell lines and facilitating use of the cytotoxicity assays throughout the entire study. The author also thanks Miss Phunlap Pompeng and Mr. Ruengrit Suppapan for antibacterial and cytotoxicity assays, respectively. Many thanks to Mr. Thanesuan Nuanyai for his assistance about NMR spectra and kindness.

Special thanks to my friends in the Laboratory of Research Centre for Bioorganic Chemistry (RCBC), Department of Chemistry, Faculty of Science, Chulalongkorn University for their kindness and encouragement.

I wish to thank The 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) for granting in partial financial support to conduct this research.

Finally, I am deeply grateful to my family for their love and encouragement throughout my M.Sc. study.

CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

Figure **Page**

LIST OF SCHEMES

LIST OF ABBREVIATIONS

J	Coupling constant
δ	Chemical shift
δ_H	Chemical shift of proton
δ_C	Chemical shift of carbon
s	Singlet (for NMR spectra)
d	Doublet (for NMR spectra)
dd	Doublet of doublet (for NMR spectra)
ddd	Doublet of doublet of doublet (for NMR spectra)
dddd	Doublet of doublet of doublet of doublet (for NMR spectra)
t	Triplet (for NMR spectra)
m	Multiplet (for NMR spectra)
q	Quartet (for NMR spectra)
brs	Broad singlet (for NMR spectra)
brd	Broad doublet (for NMR spectra)
qC	Quaternary carbon
calcd.	Calculated
^{1}H NMR	Proton nuclear magnetic resonance
^{13}C NMR	Carbon-13 nuclear magnetic resonance
2D NMR	Two dimensional nuclear magnetic resonance
^{1}H-^{1}H COSY	Homonuclear (proton-proton) correlation spectroscopy
NOESY	Nuclear overhauser effect spectroscopy
HSQC	Heteronuclear single quantum coherence
HMBC	Heteronuclear multiple bond correlation
ORTEP	Oak ridge thermal ellipsoid plot
HPLC	High performance liquid chromatography
HRESIMS	High resolution electrospray ionization mass spectrometry
ESIMS	Electrospray ionization mass spectrometry
CC	Column chromatography
TLC	Thin layer chromatography

MIC	Minimum inhibitory concentration
IC_{50}	Half maximal inhibitory concentration
$CDCl_3$	Deuterated chloroform
MeOH	Methanol
EtOH	Ethanol
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane
EtOAc	Ethyl acetate
DMSO	Dimethylsulfoxide
KBr	Potassium bromide
$(NH_4)_6Mo_7O_{24}$	Ammonium molybdate
H_2SO_4	Sulfuric acid
SiO_2	Silicon dioxide
g	Gram (s)
mg	Milligram (s)
mL	Milliliter (s)
μg	Microgram (s)
μL	Microliter (s)
μM	Micromolar
mM	Millimolar
L	Liter (s)
M	Molar
min	Minute
h	Hour
rpm	Round per minute
m	Meter (s)
mm	Millimeter (s)
cm	Centimeter (s)
nm	Nanometer
Hz	Hertz
MHz	Megahertz

cm^{-1}	Reciprocal centimeter (unit of wave number)
ppm	part per million
NMR	Nuclear magnetic resonance
MS	Mass spectrometry
IR	Infared
UV	Ultraviolet
m.p.	Melting point
α	Alpha
β	Beta
Δ	Delta
m/z	Mass to charge ratio
$[M+H]^+$	Protonated molecule
$[M+Na]^+$	Pseudomolecular ion
$[\alpha]_D^{20}$	Specific rotation at 20 ^oC and sodium D line (589 nm)
λ_{max}	Wavelength of maximum absorption
c	Concentration
ε	Molar extinction coefficient
Å	Angstrom
°C	Degree celcius
deg.	Degree
sp.	Species
No.	Number
ATCC	American type culture collection
UCLA	University of California, Los Angeles
ESBL	Extended-spectrum beta-lactamase
BT-474	Breast ductal carcinoma
CHAGO	Undifferentiated lung carcinoma
KATO-3	Gastric carcinoma
SW-620	Colon adenocarcinoma
CH-Liver	Liver cell line

CHAPTER I

INTRODUCTION

Natural products are chemical compounds or substances produced by living organisms that can be found in nature. Generally, they possess a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. These small molecules provide the source or incentive for the majority of FDA approved agents and continue to be one of the major sources of inspiration for drug discovery. In particular, these compounds are important in the treatment of life threatening conditions (Newman and Cragg, 2007).

At the present time, drugs are the most important things for human being. However, the continuing threat disease such as cancer, AIDS, SARS, influenza, etc., and increasing drug resistance provided impetus in the world to find alternatives, for example, by modifying structure of existing drugs or search for novel compounds from the natural sources. Natural products have been the source of therapeutics since the arrival of traditional medicine and healing, and remain a dominant source to date (Donnelly, 2009).

Thai medicinal plants are one of the important sources for bioactive compounds that are applicable in various fields. Especially, using in pharmaceutical because Thailand is located in the tropical areas which have a great biodiversity of plant species. Furthermore, the metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs, because they must accumulate within living cells (El-Shemy, 2007). In addition, the use of traditional medicine based on plants have received considerable interest (Han *et al*., 2002).

For this reason, the finding of new drugs from medicinal plants might be one of the ways to obtain effective candidates for treating a variety of diseases in humans and animals.

1.1 The plants in the genus *Xylocarpus*

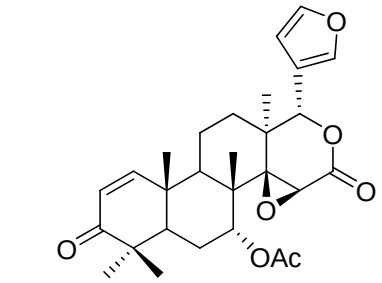
The genus *Xylocarpus* belongs to the order Geraniales of the family Meliaceae (Sastri, 1950). The family Meliaceae comprises of the 50 genera including *Xylocarpus* and 1400 other species distributed all over the world (Banerji and Nigam, 1984). In general, *Xylocarpus* species are widely spreaded along the seacoast of southeastern Asia, Australia, East Africa and Polynesia (Wu *et al*., 2006). The *Xylocarpus* genus is a small genus comprising only three species, *Xylocarpus granatum* Koenig, *Xylocarpus moluccensis* (Lam.) M. Roem. and *Xylocarpus runphii* (Kostel.) Mabb. All three species are found in mangrove, swamp or coastal scrub of the old world, all in Thailand (Ximu and Pongumphai, 1994). Particularly, *X. granatum* and *X. moluccensis* are the most popular plants in empirical study (Yin *et al*., 2009). In addition, the plants in the genus *Xylocarpus* are reported to contain a special class of bitter substances termed as limonoids (Taylor *et al*., 1984).

Limonoid examination of the Meliaceae family (meliacins) is of growing interest due to a range of biological activities, such as insect antifeedants and growth regulators, and antibacterial, antifungal, antimalarial, anticancer, and antiviral activities in humans (Koul *et al*., 2004; Nakagawa *et al*., 2001). Moreover, several types of compounds have been isolated from *Xylocarpus*, and can be classified as monoterpene, triterpene, flavonone, sterol glycoside, phenolic acid and alkaloid compounds. Obviously, limonoids are the main secondary metabolites of this genus (Wu *et al*., 2008).

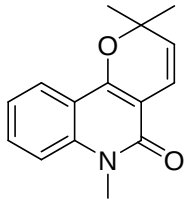
Morphologically, *X. granatum* is a large spreading mangrove, with rounded coriaceous leaves, smooth thin bark, and an abundant red heartwood, which furnishes a useful, if rather hard, timber of the characteristic mahogany type. The fruit is grape fruit sized, hard and heavy. *X. moluccensis* is a smaller, less branched mangrove, with pointed leaves, deeply serrated bark and an undistinguished timber. The fruit is the size of a mandarin orange. *X. rumphii* is a rare plant on the East African coast, similar to *X. granatum*, but having the small fruit typical of *X. moluccensis*, which has been considered as a possible hybrid of these two species (Mullholand and Taylor, 1992).

All the species of *Xylocarpus* have similar medicinal uses. All parts are used as astringent (Sastri, 1950), but the bark and root are more widely used. The bark is also used in dysentery, diarrhea and other abdominal troubles and febrifuge (Sastri, 1950; Chopra *et al*., 1956). Seed ash is mixed with sulphur and coconut oil and applied as ointment for itch (Chopra *et al*., 1956). The root is used to treat cholera from Burma to Phillipines. Traditionally, the bark pressings of *X. granatum* are used in the treatment of cholera, fever and malaria and that of *X. moluccensis* is used in cholera and fever (Bandarnayake, 1998). The fruits of *X. moluccensis* are also used as a anaphrodisiac and a cure in elephantiasis (Chopra *et al*., 1956). The kernels are used in tonics and in relieving colic. The seeds or peels of the fruits are utilized to poultice swellings and ash of the seeds is applied to itch. The bark pressings are used to treat fevers including those caused by malaria (Bandarnayake, 1998).

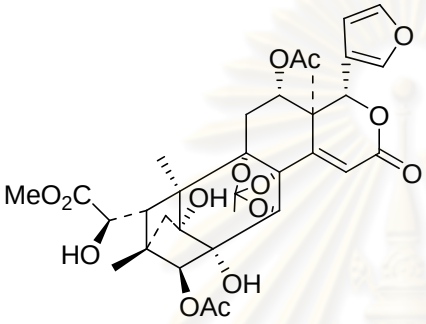
Various biological activities have reported in the extracts and compounds of the genus *Xylocarpus.* Antidiarrhoeal activity of methanol extract of the barks of *X. moluccensis* in castor oil and magnesium sulphate induced diarrhea models in mice have been studied in 2005 (Uddin *et al*., 2005). Antibacterial activity of the extract of *X. granatum* has also been reported in 2005 (Choudhary *et al*., 2005). It showed inhibition of the growth of six virulent strains of bacteria pathogenic to fish viz. *Edwardsiella tarda*, *Vibrio alginolyticus*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Aeromonas hydrophila*. The compound gedunin from *X. granatum* showed significant *in vitro* antimalarial activity but poor *in vitro* activity (Omar *et al*., 2003). *N*-methylflindersine from *X. granatum* has antifeedant, insect repellant, antimicrobial, antiyeast and antifungal (Chou *et al*., 1977; Bandarnayake, 2002). Xyloccensins Q-V from *X. granatum* have been reported to have antifeedant activity (Wu *et al*., 2005). The structures of these compounds are shown in Figure 1.1.



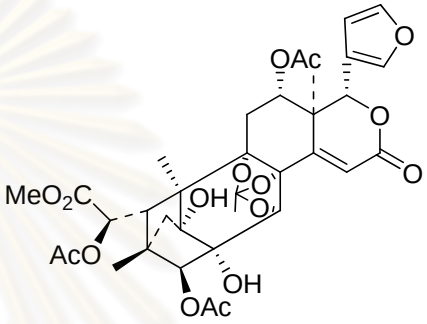
Gedunin



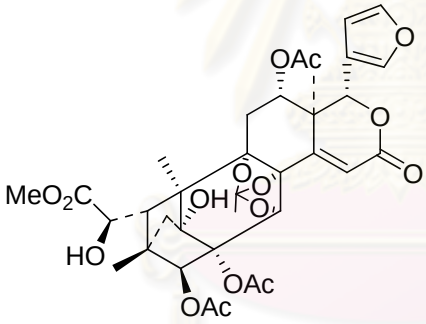
N-methylflindersine



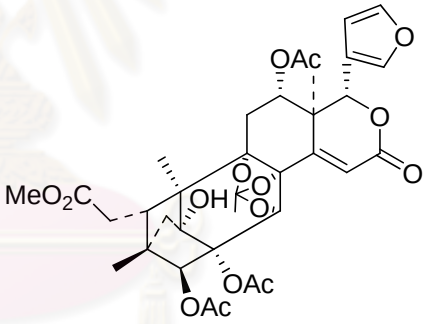
Xyloccensin Q



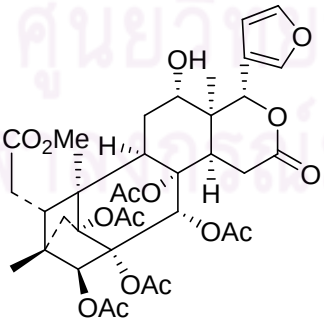
Xyloccensin R



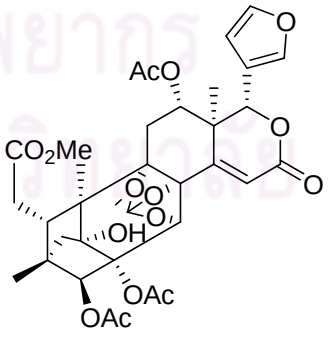
Xyloccensin S



Xyloccensin T



Xyloccensin U



Xyloccensin V

Figure 1.1 Bioactive compounds isolated from the genus *Xylocarpus*

1.2 Taxonomical and Botanical characteristics of *Xylocarpus rumphii* (Kostel.) Mabb.

Taxonomy of *Xylocarpus rumphii* (Kostel.) Mabb. is categorized as

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Rutales

Family : Meliaceae

Genus : *Xylocarpus*

Species : *Xylocarpus rumphii* (Kostel.) Mabb.

X. rumphii is a tree up to 4-12 m with neither conspicuous buttresses nor pneumatophores; bole usually solitary, to 50 cm diameters, frequently of poor form. Bark lenticellate to finely fissured, grayish; inner bark bright pink to red. Leaf rachis and petiole to 22 cm with terminal spike to 1 mm. Leaflets in 2-4 pairs, 5-10 by 3-5 cm, ovate to cordate, sometimes falcate, base broadly cuneate or rounded to truncate or cordate, asymmetric, apex acute to acuminate; venation prominent on both surfaces in sicco, conspicuous in vivo; petiolule 1-3 mm. Thyrses 10-18 cm long, lax, pendent, main axis distinct; lateral branches to 8 cm; bracts and bracteoles 0.5 mm, narrowly triangular, persistent; pedicles 3-8 mm, not conspicuously swollen near calyx. Calyx lobes 1-1.1.5 mm long. Petals 3.5-6 by 2-2.5 mm, elliptic-oblong, creamy white. Staminal tube 2-2.5 mm diameters, lobes apiculate or bifid to retuse. Fruit 6-8 cm diameters, globose. Seeds 8-16, 3.6-7 cm long (Mabberley *et al*., 1995)

In general, *X. rumphii* is found in South-Eastern part of Thailand as follow Chon Buri, Rayong, Ranong and Krabi province which scattered along rocky seashores and headlands. Furthermore, it also found in East Africa to Tonga; throughout Malaysia but so far unrecorded from the Bornean (or New Caledonian) mainland and rare in Sumatra. In addition, the vernacular names of this plant are “Niri” or “Nyireh” and local name in Thailand is “Ta Ban” (Mabberley *et al*., 1995).

The pictures of *X. rumphii* are shown in Figure 1.2.

Figure 1.2 *Xylocarpus rumphii* (Kostel.) Mabb.

Preliminary investigation on the chemical constituents of this plant was performed by using NMR spectroscopic technique and its spectrum displayed characteristic signals for limonoids.

Therefore, the objectives of this research are summarized as follow;

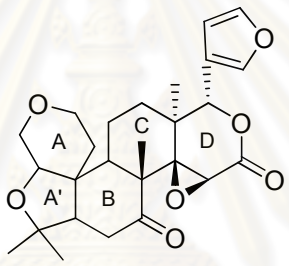
1. To extract, isolate and purify chemical constituents from the seed kernels of *Xylocarpus rumphii* (Kostel.) Mabb.
2. To elucidate structures of the isolated compounds by spectroscopic technique.
3. To evaluate biological activity of pure compounds such as antibacterial activity and anticancer activity.

CHAPTER II

LITERATURE REVIEWS

2.1 Limonoids

The investigation for limonoids started long back when scientists started looking for the factor responsible for bitterness in citrus. The term limonoids was derived from limonin, the first tetranortriterpenoid obtained from citrus bitter principles (Roy and Saraf, 2006).



Limonin

Figure 2.1 Citrus limonoid (limonin)

Continuing studies show that limonoids are highly oxygenated, modified terpenoids and have recently attracted attention because compounds belonging to this group have displayed a range of biological activities like insecticidal, insect antifeedant and growth regulating activity on insects as well as antibacterial, antifungal, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans (Koul *et al*., 2004; Nakagava *et al*., 2001).

Although hundreds of limonoids have been isolated from various plants but, their incident in the plant kingdom is confined to only plant families of order Rutales and that too more plentifully in Meliaceae and Rutaceae, and less frequently in Cneoraceae and *Harrisonia* sp. of Simaroubaceae (Lakshmi and Gupta, 2008).

Table 2.1. Prominent sources of limonoids

Family	Plant species	Plant part	References
Meliaceae	*Agalia andamanica*	Leaves	Puripattanavong *et al*., 2000
	Astrotrichilia vomatata	Stem bark	Mulholland *et al*., 2000
	Azadirachta indica	Seeds	Hallur *et al*., 2002
	Azadirachta indica	Kernels	Malathi *et al*., 2002
	Azadirachta indica	Leaves	Siddiqui *et al*., 2000
	Azadirachta indica	Seeds	Koul *et al*., 2004
	Carapa granatum	Fruits	Saxena and Babu, 2001
	Cedrela montana	Fruits and Seeds	Castellanos *et al*., 2002
	Cedrela salvadorensis	Leaves	Cespedes *et al*., 2001
	Cedrela sinensis	Leaves	Mitsui *et al*., 2004
	Chukrasia tabularis	Root bark	Nakatani *et al*., 2004
	Cipadessa fruticosa	Fruits	Leite *et al*., 2004
	Khaya anthotheca	Stem bark	Tchimene *et al*., 2005
	Khaya grandifolia	Bark and Seeds	Bickii *et al*., 2000
	Khaya ivorensis	Stem bark	Abdelgaleil *et al*., 2005
	Khaya senegalensis	Stem bark	Nakatani *et al*., 2002
	Khaya senegalensis	Fruits	Abdelgaleil *et al*., 2004
	Melia azedarach	Leaves	Alche *et al*., 2003
	Melia azedarach	Ripe fruits	Zhou *et al*., 2004
	Melia azedarach	Kernels	Wandscheer *et al*., 2004
	Melia azedarach	Fruits	Carpinella *et al*., 2003
	Melia azedarach	Kernels	Carpinella *et al*., 2002
	Melia dubia	Barks	Koul *et al*., 2002
	Munronia henryi	Whole plant	Zhang *et al*., 2004
	Neobeguea leandreana	Stem bark	Coombes *et al*., 2003
	Pterorhachis zenkeri	Stem	Vardamides *et al*., 2001
	Quivisia papinae	Seeds	Coobes *et al*., 2004
	Quivisia papinae	Seeds	Coobes *et al*., 2005

Table 2.1. Prominent sources of limonoids (continued)

Family	Plant species	Plant part	References
Meliaceae	*Sandoricum koetjape*	Leaves	Ismail *et al*., 2003
	Sandoricum koetjape	Leaves	Ismail *et al*., 2004
	Swietenia mahogany	Stem bark	Saad *et al*., 2003
	Teucrium tomentosum	Aerial parts	Soundarya *et al*., 2003
	Trichilia emetica	Roots	Germano *et al*., 2005
	Trichilia estipulata	Stem bark	Cortez *et al*., 2000
	Trichilia havanensis	Seeds	Maria *et al*., 2003
	Trichilia pallida	Roots	Simmonds *et al*., 2001
	Trichilia rubescens	Leaves	Krief *et al*., 2004
	Turraea floribunda	Seeds	McFarland *et al*., 2004
	Turraea wakefieldii	Root bark	Ndung'u *et al*., 2004
	Turraea floribunda	Root bark	Ndung'u *et al*., 2004
	Xylocarpus granatum	Stem bark	Wu *et al*., 2004
Rutaceae	*Citrus reticulata*	Seeds	Khalil *et al*., 2003
	Citrus sudachi	Seeds	Nakagawa *et al*., 2001
	Citrus unshiu	Peels	Sawabe *et al*., 1999
	Dictamnus dasycarpus	Root bark	Zhao *et al*., 1998
	Hortia colombiana	Wood	Suarez *et al*., 2002
	Raulinoa echinata	Stems and Leaves	Biavatti *et al*., 2001
	Bouchardatia neurococca	Aerial parts	Wattanapiromsakul *et al*., 2003
	Clausena excavate	Rhizomes and Roots	Sunthitikawinsakul *et al*., 2003
Simaroubaceae	*Harrisonia abyssinica*	Root bark	Rugutt *et al*., 2001
	Harrisonia perforate	Leaves	Khuong-Huu *et al*. 2001; Chiaroni *et al*., 2000

2.1.1 Chemistry and biosynthesis of limonoids

Limonoids, which have been found only plants of the order Rutales, are triterpene derivatives from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton (Zhou *et al*., 2006). All naturally occurring limonoids contain a furan ring attached to the D-ring, at C-17, as well as oxygen containing functional groups at C-3, C-4, C-7, C-16 and C-17 (Somrutai *et al*., 2005).

These compounds are moderately polar, insoluble in water, but soluble in alcohols and ketones (Aliero, 2003). Limonoids are appearance in neutral (noncarboxylated/aglycone) as well as acidic (carboxylated/glucoside) forms, the formers are insoluble and bitter while latter are soluble and tasteless. Chemically they are highly oxygenated triterpenes in which the side chain has become a furan ring by the loss of four carbons, therefore alternatively called as tetranortriterpenoids (Lakshmi and Gupta, 2008).

The biosynthesis of limonoids shows that limonoids are synthesized via terpenoid biosynthetic pathway, starting with cyclization of squalene (as shown in Figure 2.2), which results into a tetracyclic ion, euphane and tirucallane (as shown in Figure 2.3), two chemically corresponding compounds may be the ultimate biogenetic precursors. Oxidative degradation at the C-17 side chain of either of these nucleus results in loss of four carbon atoms and formation of β-substituted furan, further oxidations and skeletal rearrangements in one or more of the four rings, which are designated as A, B, C and D (as shown in Figure 2.1), give rise to different groups of limonoids are shown in Figure 2.4 (Endo *et al*., 2002; Suarez *et al*., 2002). However, the oxidations are either epoxidations of double bonds or Baeyer Villiger attacks on ketones and are all of the types to be expected from a biological peracid equivalent, presumably a peroxidase (Lakshmi and Gupta, 2008).

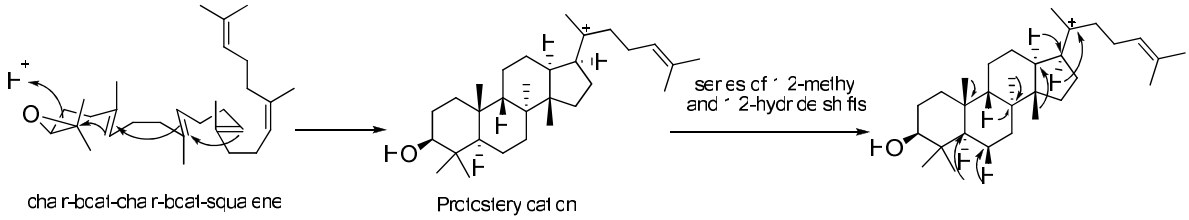
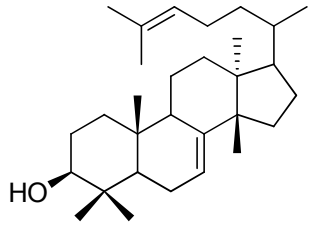
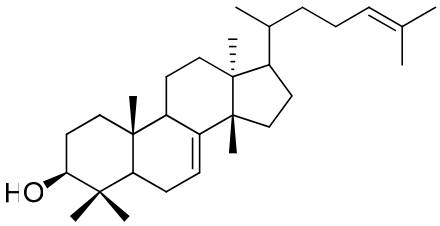


Figure 2.2 Squalene epoxide leading to different intermediate triterpene cations

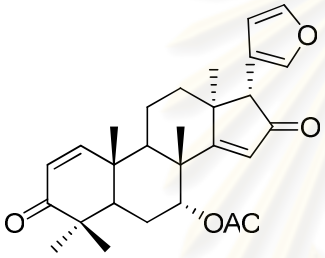


Euphane

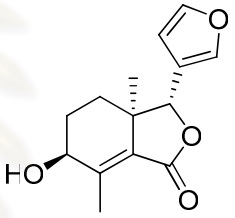


Tirucallane

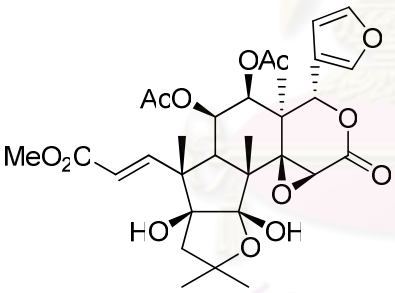
Figure 2.3 Precursors of limonoids



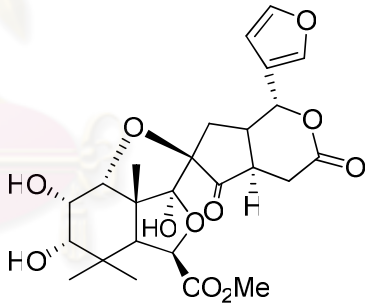
Intact limonoid



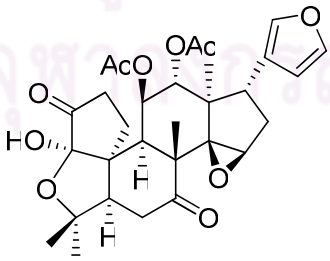
Degraded limonoid



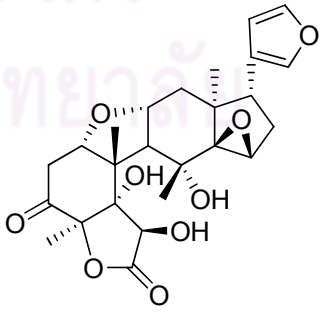
Highly modified limonoid



Highly cleaved limonoid

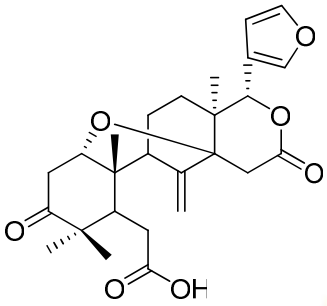


Ring A-*seco* limonoid

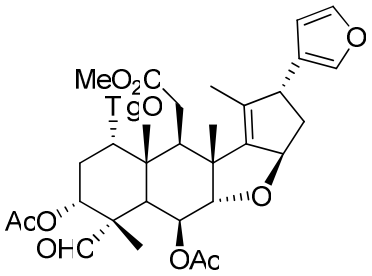


Ring-A,C,D-intact-ring-B-*seco* limonoid

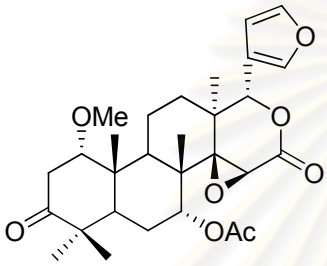
Figure 2.4 Example of limonoids showing different degree of oxidation and skeleton arrangement



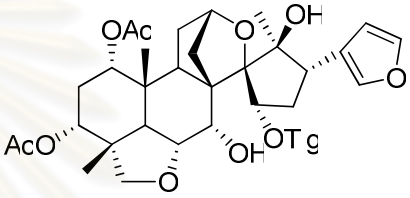
Ring-B,D-*seco* limonoid



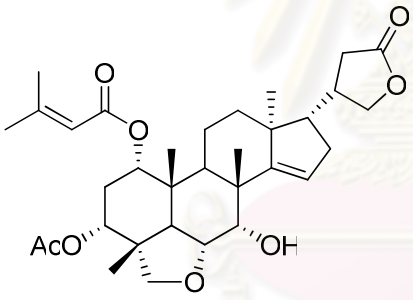
Ring-C cleaved limonoid



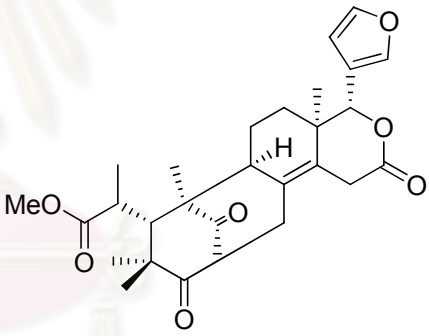
Ring-D-lactone-limonoid



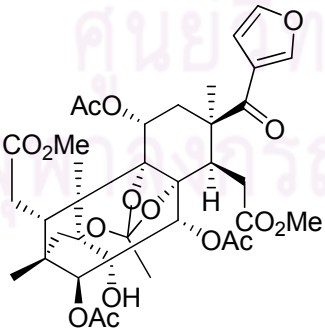
Ring-C-*seco* limonoid



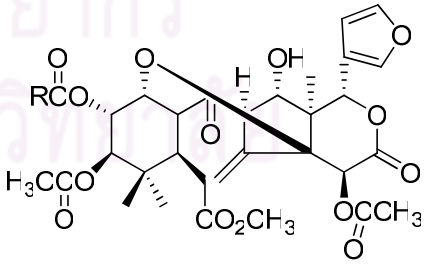
Gamma-lactone side chain limonoid



Mexicanolide

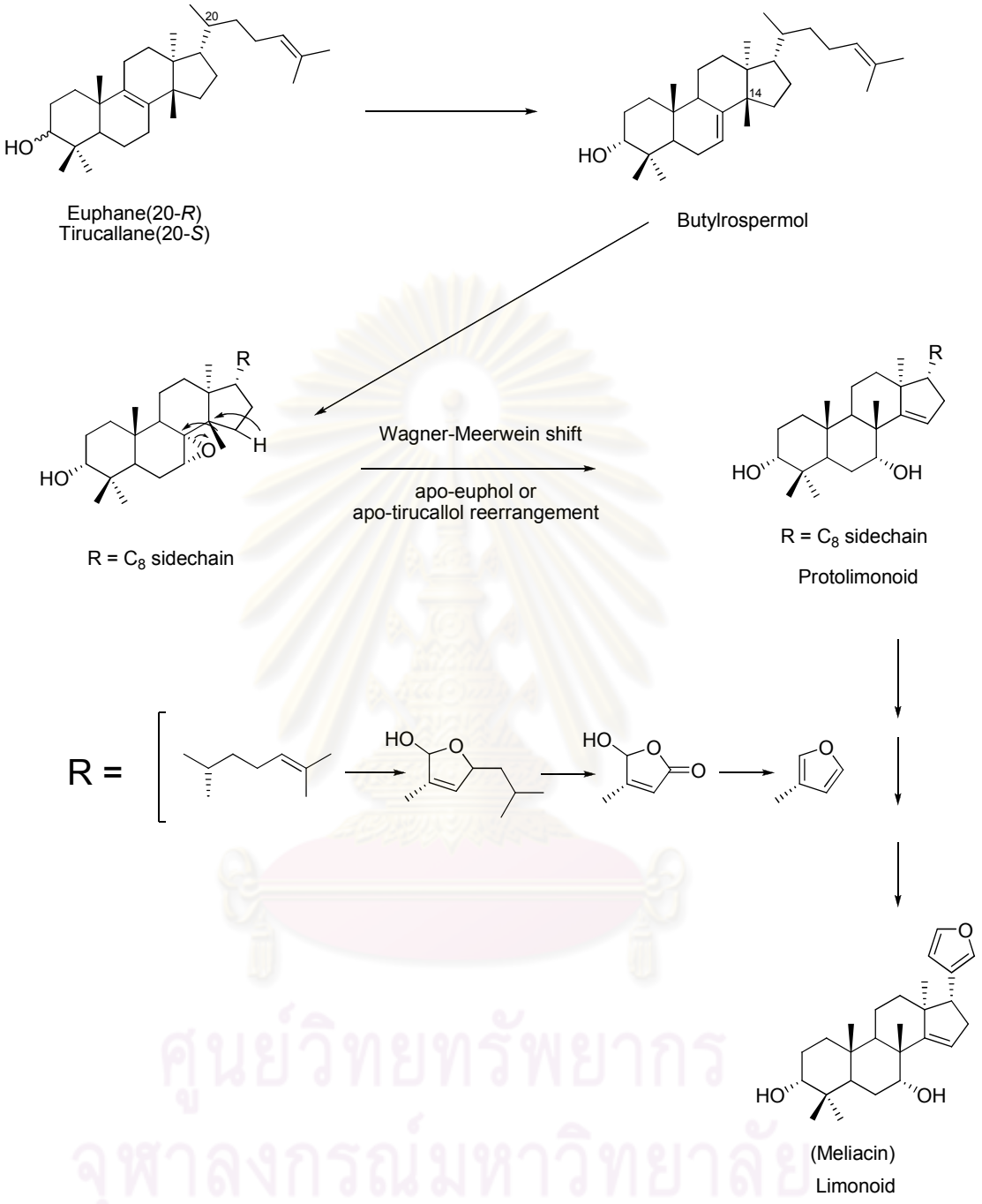


Phragmalin

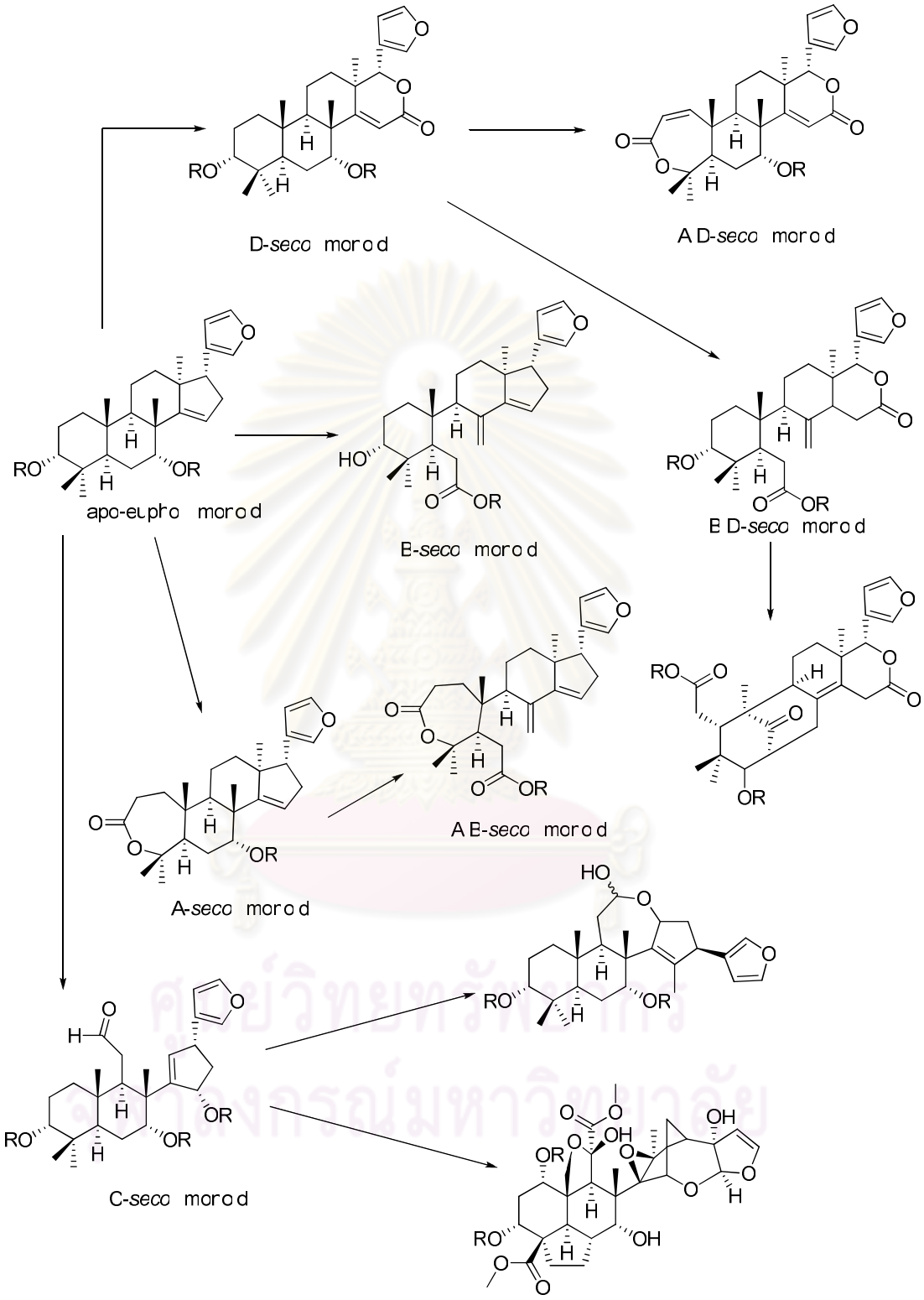


Trijugin-type-limonoid

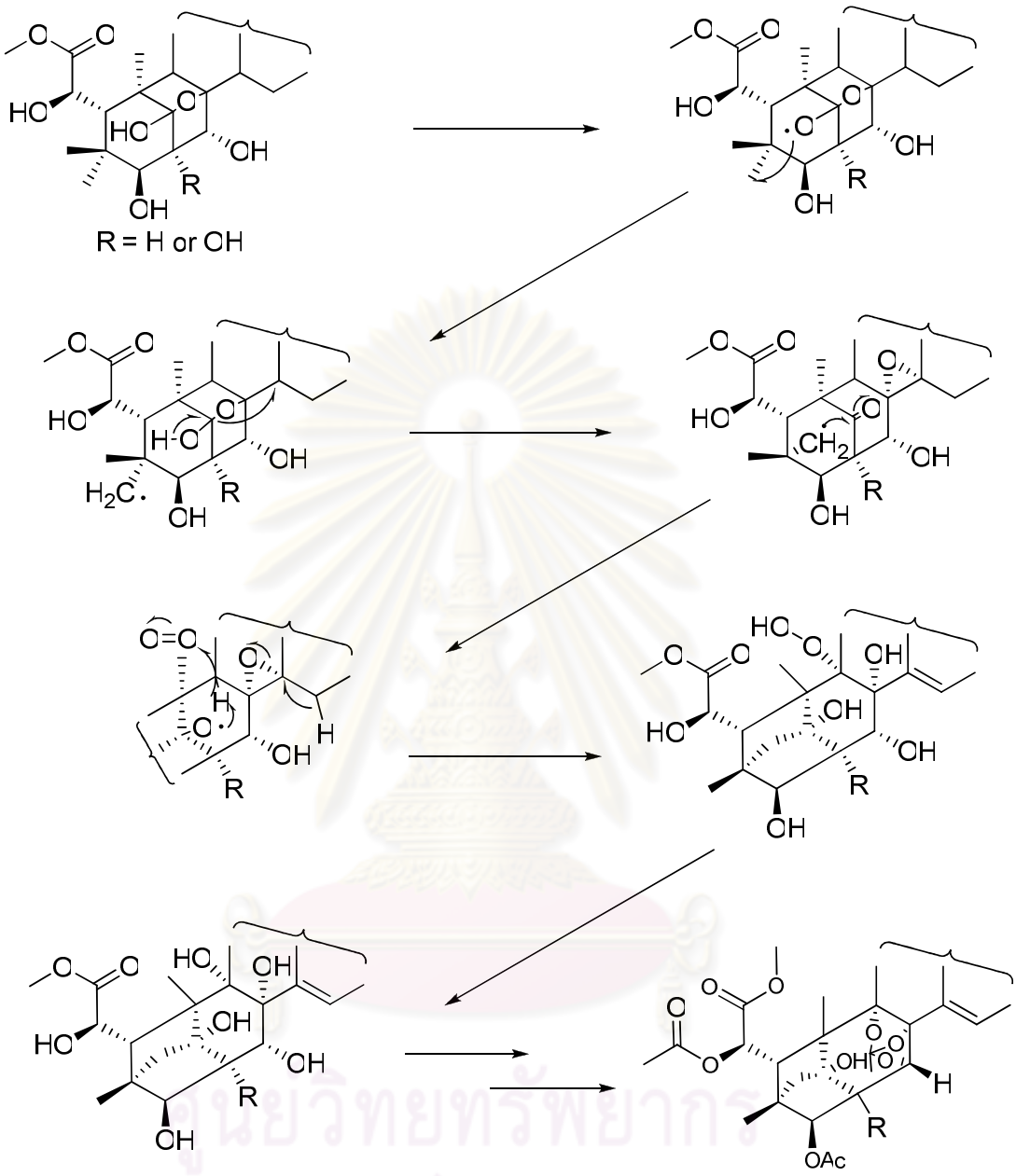
Figure 2.4 Example of limonoids showing different degree of oxidation and skeleton arrangement (continued)



Scheme 2.1 Biosynthetic pathway leading to the formation of a simple limonoid (Champagne *et al*., 1992)



Scheme 2.2 Major biosynthetic routes of limonoids (Champagne *et al*., 1992)



Scheme 2.3 Proposed biosynthetic pathway to 8,9,30-phragmalin *ortho* esters from a mexicanolide (Wu *et al*., 2004)

2.1.2 Biological activities of limonoids

2.1.2.1 Anticancer activity

Many probative evidences have exposed that limonoids present in citrus fruits and their juice have cancer chemopreventive property, they have been shown to inhibit the growth estrogen receptor-negative and -positive human breast cancer cells in culture, and also found to target and stop neuroblastoma cells (Jacob *et al*., 2000; Poulose *et al*., 2005; Miller *et al*., 2004; Tian *et al*., 2001). Moreover, significant cytotoxic activity has also been exhibited by limonoids isolated from *Melia azedarach, Melia toosendan* and azadirachtin A (Okamura *et al*., 1997; Tada *et al*., 1999; Akudugu *et al*., 2002). In addition, the citrus limonoid, obacunone, was found to enhance the cytotoxicity of vincristine against L1210 cells by approximately 10 folds. Furthermore, it was found that the cytotoxicity of other microtubule inhibitors such as vinblastine and taxol in drug sensitive KB-3-1 cells as well as in multidrug-resistant KB-V1 cells was improved greatly in the presence of obacunone (Jung *et al*., 2000).

2.1.2.2 Antimalarial activity

Gedunin, nimbin, nimbolide and many more limonoids isolated from *Azadirachta indica, Cedrela odorata, Guarea mltiflora* and *Khaya grandifoliola* have been identified for their *in vitro* antimalarial activity on *Plasmodium falciparum* (Kayser *et al*., 2003; Saxena *et al*., 2003). Gedunin was found to be most effective, against *Plasmodium falciparum,* out of several limonoids isolated from *Khaya grandifoliola* and it also exhibited additive effect in combination with chloroquine (Bickii *et al*., 2000). Novel antimalarial limonoids were isolated following a veterinary and self-medicative behavioral survey of wild chimpanzees in Uganda, from leaves of *Trichilia rubescens* (Krief *et al*., 2004).

2.1.2.3 Antimicrobial activity

The presence of limonoids in *Trichilia emetica* can be considered to be responsible for activity against many clinically, isolated bacterial strains (Germano *et al*., 2005). In addition, limonoids obtained from some *Khaya* species, showed good antibacterial and antifungal activity (Abdelgaleil *et al*., 2005; Abdelgaleil *et al*., 2004). In another study limonoids from several plants belonging to Meliaceae as well as Rutaceae family were reported to have significant antifungal activity (Abdelgaleil *et al*., 2005; Govindachari *et al*., 2000; Govindachari *et al*., 1999).

2.1.2.4 Anti HIV activity

Limonin and nomilin have shown to inhibit the replication of HIV-1 in a number of cellular systems (Battinelli *et al*., 2003). A novel limonoid isolated from *Clausena excavate* have also shown HIV-1 inhibitory activity (Sunthitikawinsakul *et al*., 2003).

2.1.2.5 Other miscellaneous activities

Limonoid, 1-cinnamoyl-3,11-dihydroxymeliacarpin, isolated from *Melia azedarach* showed IC_{50} values of 6 μmL and 20 μmL for vesicular stomatitis and herpes simplex viruses (HSV-1), respectively (Alche *et al*., 2003). Furthermore, limonoids in *Trichilia emetica* were considered to be responsible for hepatoprotective activity on CCl_4 induced damage in rat hepatocytes. In an *in vitro* study, limonoids isolated from *Swietenia humilis* have exhibited a concentration dependant and non-reversible spasmogenic and uterotonic activity (Perusquia *et al*., 1997). In their review, it has also reported a number of other pharmacological activities of limonoids derived from neem tree, like anti-inflammatory, anti-arthritic, antipyretic, hypoglycemic, anti-gastric ulcer, spermicidal and diuretic.

2.1.3 Classes of limonoids

The tetranortriterpenoids or limonoids are grouped according to the oxygenation of ring A to D and cyclization to modified skeleton (Taylor, 1984). The limonoids have been classified on the basis of which of the four rings in the triterpene nucleus has been oxidized.

Generally, the limonoids from only Meliaceae can be classified into 12 groups as follows;

1. Protolimonoids and related compounds triterpenoids
2. Havanensin group (all rings intact)
3. Gedunin group (Ring D opened)
4. Limonoids with ring B and D opened
5. Mexicanolide group (modified ring B opened and recyclised)
6. Phragmalin group (modified, ring B opened and recyclised)
7. Methyl ivorensate group (Rings A, B and D opened)
8. Obacunol group (rings A and D opened)
9. Nimbin group (ring C opened)
10. Toonafolin group (ring B opened)
11. Evodulone group (ring A opened)
12. Prieurianin group (ring A and B opened)

In case of the genus *Xylocarpus* is reported to have compounds belonging to some of the above-mentioned groups of limonoids from its different species. The classes of limonoids which have been isolated from the genus *Xylocarpus* are as follows;

1. Gedunin group
2. Andirobin group
3. Mexicanolide group
4. Phragmalin group
5. Obacunol group
6. Protolimonoid group

2.2 Chemical constituents of the genus *Xylocarpus*

Xylocarpus species have been proved to be the important sources of limonoids (Table 2.2) and limonoid derivatives have been found in all *Xylocarpus* plants studied.

Table 2.2. Limonoids isolated from *Xylocarpus* species

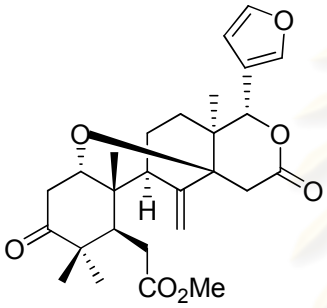
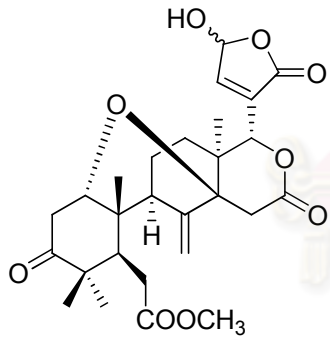
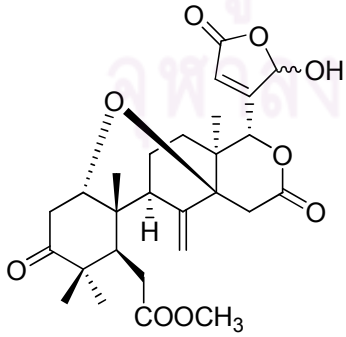
Compound	Category	Plant (part)	References
Methyl angolensate 	Andirobin	*X. moluccensis* (Timber)	Connolly *et al*., 1976
Moluccensin N 	Andirobin	*X. moluccensis* (Seed)	Wu *et al*., 2010
Moluccensin O 	Andirobin	*X. moluccensis* (Seed)	Wu *et al*., 2010

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

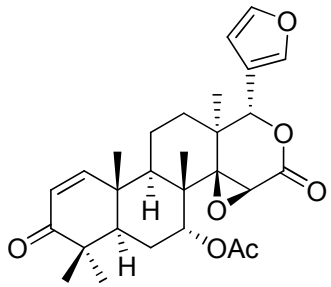
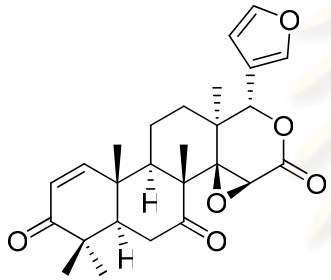
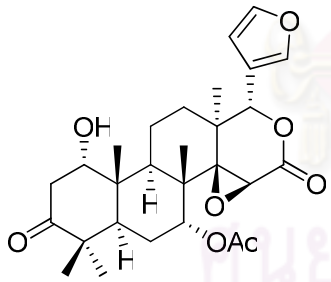
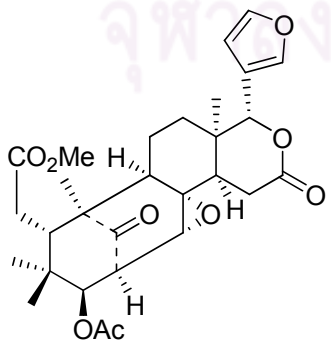
Compound	Category	Plant (part)	References
Gedunin 	Gedunin	*X. granatum* (Timber)	Taylor, 1965; Akisanya *et al*., 1961
7-Oxogedunin 	Gedunin	*X. granatum* and *X. moluccensis* (Timber and Seed)	Taylor, 1983; Mulholland and Taylor, 1992
1α-Hydroxy-1,2-dihydrogedunin 	Gedunin	*X. granatum* (bark)	Uddin *et al*., 2007
Xylocarpin 	Mexicanolide	*X. granatum* and *X. moluccensis* (Timber and Seed)	Okorie and Taylor, 1970

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

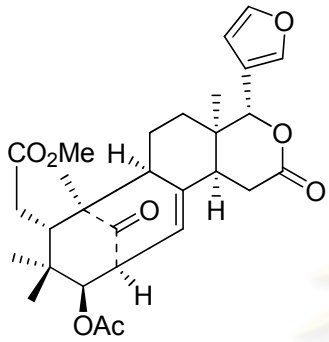
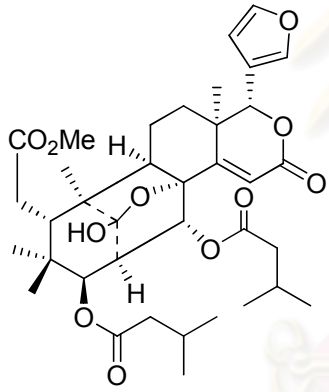
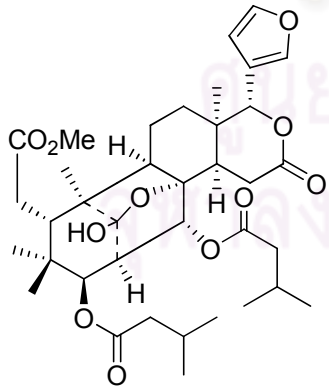
Compound	Category	Plant (part)	References
3β-Acetoxy-6-deoxy-swietenine 	Mexicanolide	*X. granatum* and *X. moluccensis* (Timber and Seed)	Okorie and Taylor, 1970
Xyloccensin A 	Mexicanolide	*X. moluccensis* (Timber)	Connolly *et al*., 1976
Xyloccensin B 	Mexicanolide	*X. moluccensis* (Timber)	Connolly *et al*., 1976

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

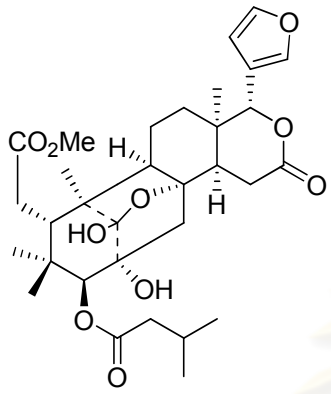
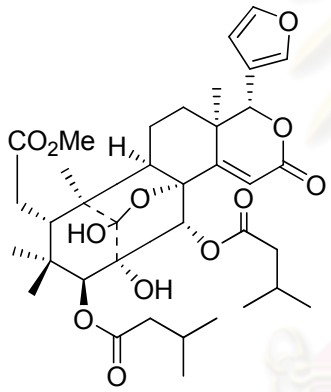
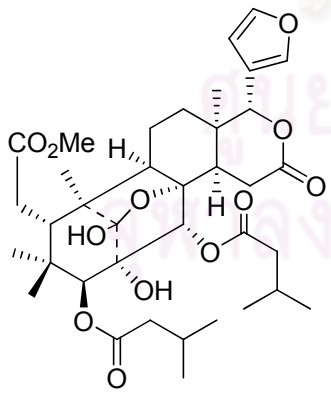
Compound	Category	Plant (part)	References
Xyloccensin C 	Mexicanolide	*X. moluccensis* (Timber)	Connolly *et al*., 1976
Xyloccensin D 	Mexicanolide	*X. moluccensis* (Timber)	Connolly *et al*., 1976
Xyloccensin F 	Mexicanolide	*X. moluccensis* (Timber)	Connolly *et al*., 1976

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

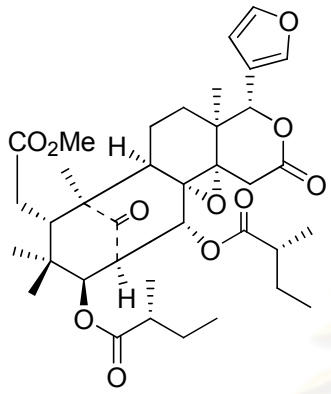
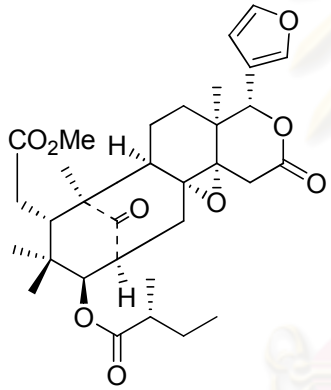
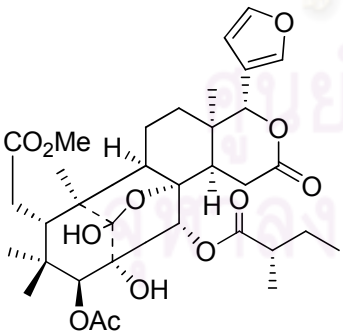
Compound	Category	Plant (part)	References
Xyloccensin G 	Mexicanolide	*X. moluccensis* (Timber)	Taylor, 1983
Xyloccensin H 	Mexicanolide	*X. moluccensis* (Timber)	Taylor, 1983
Xyloccensin I 	Mexicanolide	*X. moluccensis* (Timber)	Taylor, 1983

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

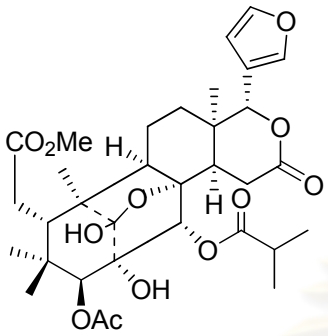
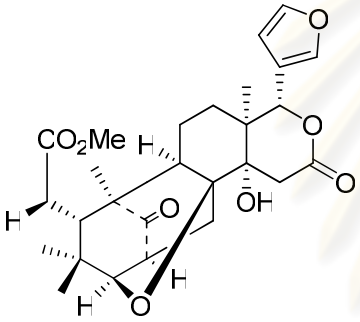
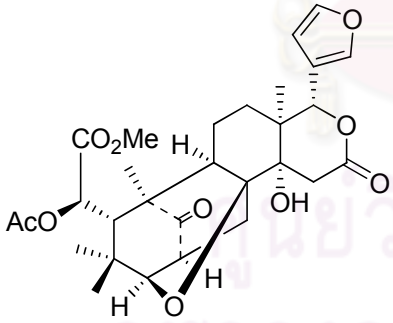
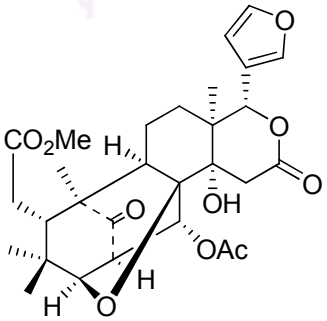
Compound	Category	Plant (part)	References
Xyloccensin J 	Mexicanolide	*X. granatum* and *X. moluccensis* (Fruit)	Alvi *et al*., 1991
Xyloccensin K 	Mexicanolide	*X. granatum* (Seed)	Kokpol *et al*., 1996
6-Acetoxycedrodorin 	Mexicanolide	*X. granatum* (Fruit)	Wu *et al*., 2006
Xyloccensin W 	Mexicanolide	*X. granatum* (Fruit)	Wu *et al*., 2006

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

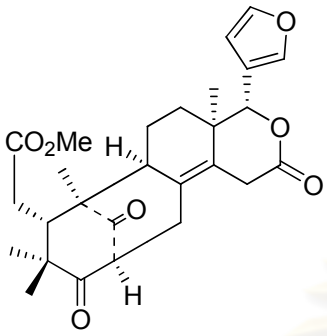
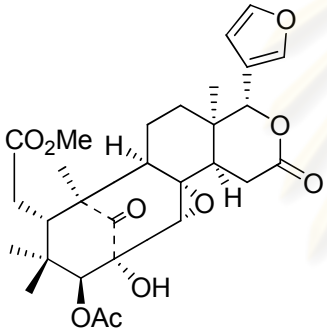
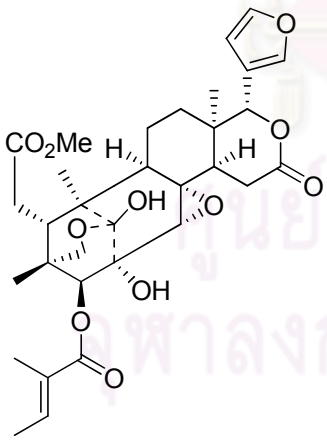
Compound	Category	Plant (part)	References
Mexicanolide 	Mexicanolide	*X. granatum* and *X. moluccensis* (Timber and Seed)	Ng and Fallis, 1979; Taylor, 1983
Humilin B 	Mexicanolide	*X. moluccensis* (Seed)	Mulholland and Taylor, 1992
Xyloccensin L 	Phragmalin	*X. granatum* (Stem bark)	Wu *et al*., 2004

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

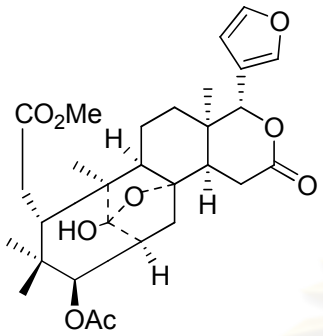
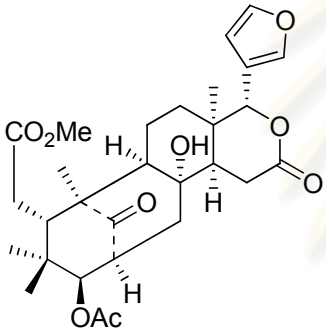
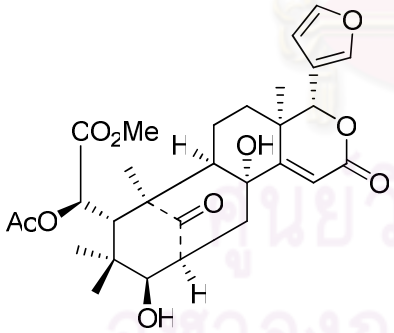
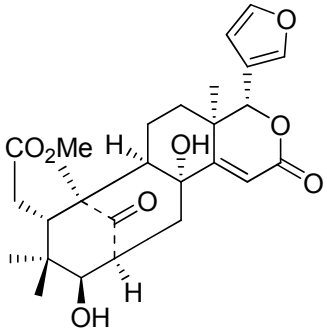
Compound	Category	Plant (part)	References
Xyloccensin M 	Mexicanolide	*X. granatum* (Stem bark)	Wu *et al*., 2003
Xyloccensin N 	Mexicanolide	*X. granatum* (Stem bark)	Wu *et al*., 2003
Xyloccensin X_1 	Mexicanolide	*X. granatum* (Fruit)	Cheng *et al*., 2006
Xyloccensin X_2 	Mexicanolide	*X. granatum* (Fruit)	Cheng *et al*., 2006

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

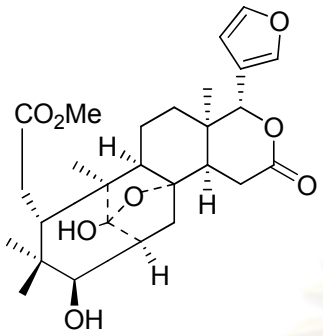
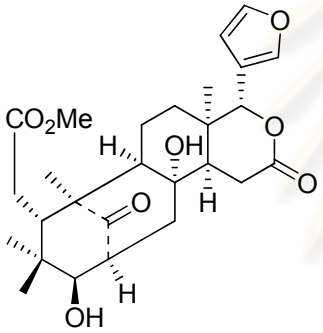
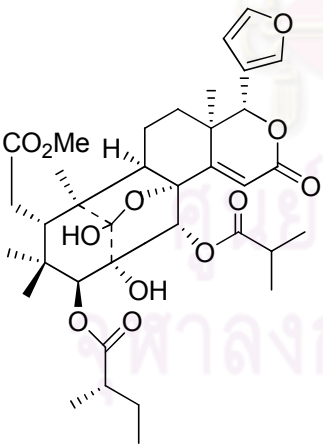
Compound	Category	Plant (part)	References
3-Deacetyl-xyloccensin M 	Mexicanolide	*X. granatum* (Fruit)	Wu *et al*., 2005
3-Deacetyl-xyloccensin N 	Mexicanolide	*X. granatum* (Fruit)	Wu *et al*., 2005
Xyloccensin X 	Mexicanolide	*X. moluccensis* (Fruit)	Roy *et al*., 2006

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

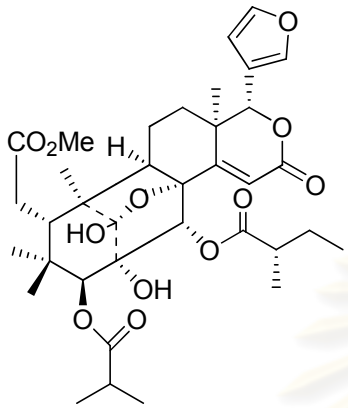
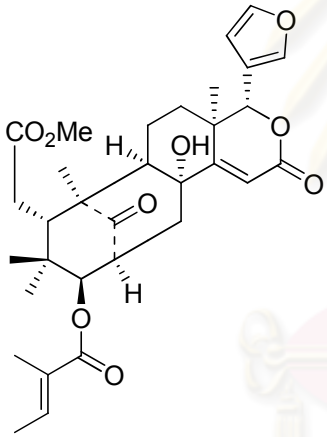
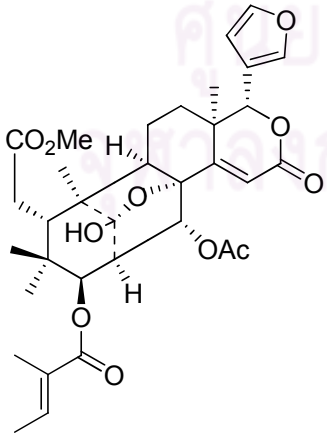
Compound	Category	Plant (part)	References
Xyloccensin Y 	Mexicanolide	*X. moluccensis* (Fruit)	Roy *et al*., 2006
Xylogranatin A 	Mexicanolide	*X. granatum* (Fruit)	Wu *et al*., 2006
Xylogranatin B 	Mexicanolide	*X. granatum* (Fruit)	Wu *et al*., 2006

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

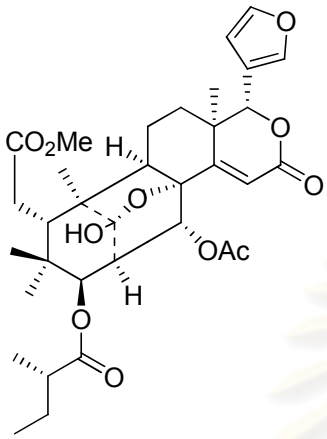
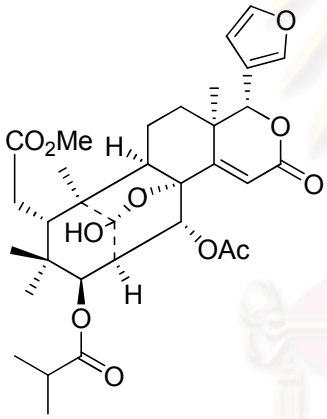
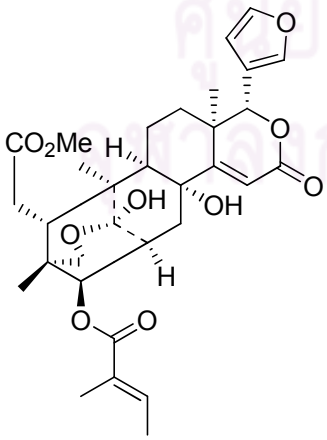
Compound	Category	Plant (part)	References
Xylogranatin C 	Mexicanolide	*X. granatum* (Fruit)	Wu *et al.*, 2006
Xylogranatin D 	Mexicanolide	*X. granatum* (Fruit)	Wu *et al.*, 2006
Xylogranatin E 	Phragmalin	*X. granatum* (Fruit)	Wu *et al.*, 2007

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

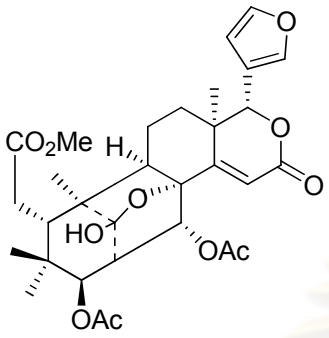
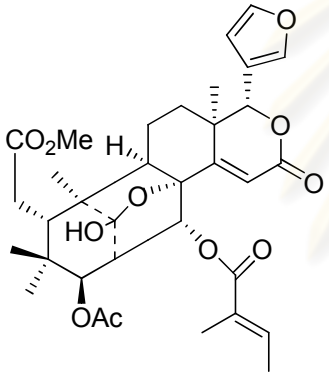
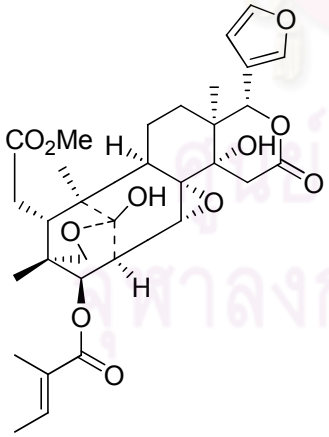
Compound	Category	Plant (part)	References
Xylocarpin F 	Mexicanolide	*X. granatum* (Fruit and Seed)	Cui *et al*., 2007
Xylocarpin G 	Mexicanolide	*X. granatum* (Fruit and Seed)	Cui *et al*., 2007
Granaxylocarpin C 	Phragmalin	*X. granatum* (Fruit and Seed)	Yin *et al*., 2007

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

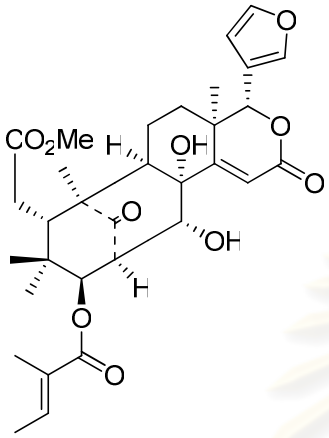
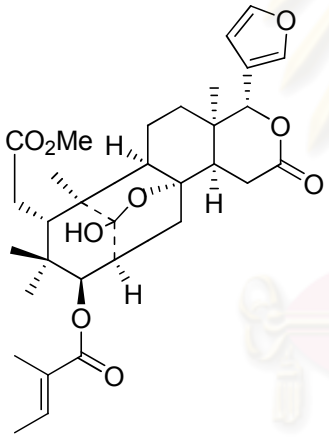
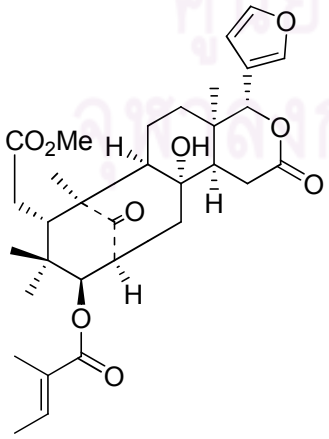
Compound	Category	Plant (part)	References
30α-Hydroxyl-xylogranatin A 	Mexicanolide	*X. granatum* (Fruit and Seed)	Wu *et al*., 2007
Xylocarpin A 	Mexicanolide	*X. granatum* (Fruit and Seed)	Li *et al*., 2007
Xylocarpin B 	Mexicanolide	*X. granatum* (Fruit and Seed)	Li *et al*., 2007

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

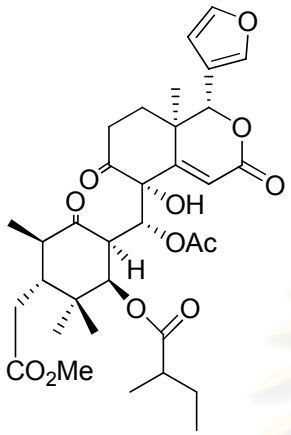
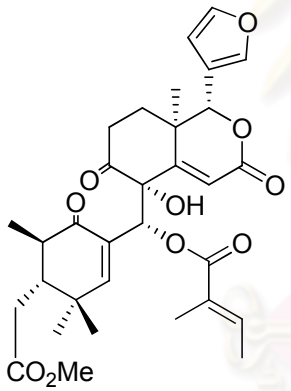
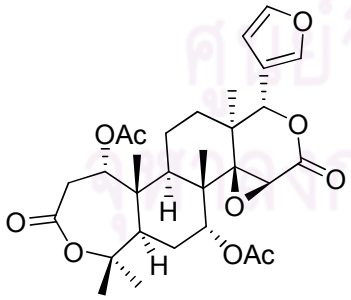
Compound	Category	Plant (part)	References
Granaxylocarpin A 	Mexicanolide	*X. granatum* (Seed)	Yin *et al*., 2007
Granaxylocarpin B 	Mexicanolide	*X. granatum* (Seed)	Yin *et al*., 2007
7α-Acetoxydihydronomilin 	Obacunol	*X. granatum* (Seed)	Ng and Fallis, 1979; Ahmed *et al*., 1978

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

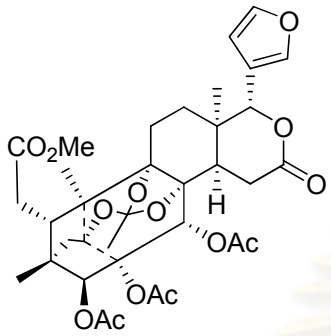
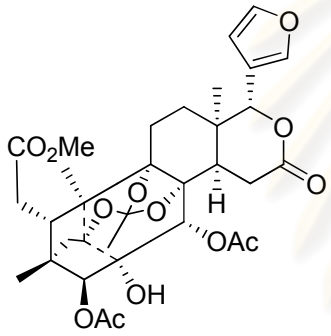
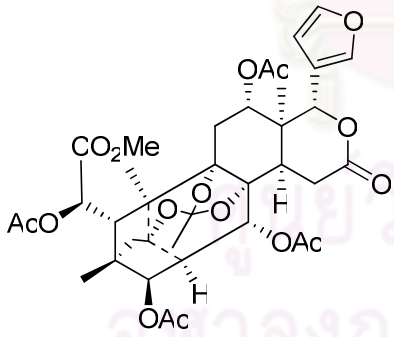
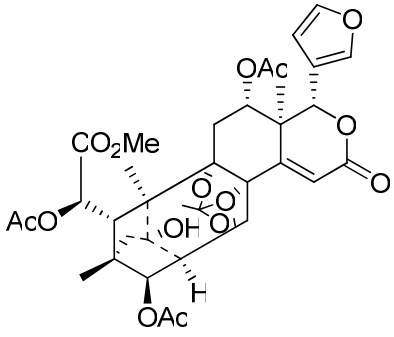
Compound	Category	Plant (part)	References
Xyloccensin E 	Phragmalin	*X. moluccensis* (Timber)	Connolly *et al*., 1976
3β, 30α-Diacetyl-Phragmalin 	Phragmalin	*X. moluccensis* (Timber)	Mulholland and Taylor, 1992
Xylocarpin I 	Phragmalin	*X. granatum* (Fruit)	Cui *et al*., 2007
Xyloccensin O 	Phragmalin	*X. granatum* (Stem bark)	Wu *et al*., 2004; Wu *et al*., 2005; Cui *et al*., 2005; Wu *et al*., 2006

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

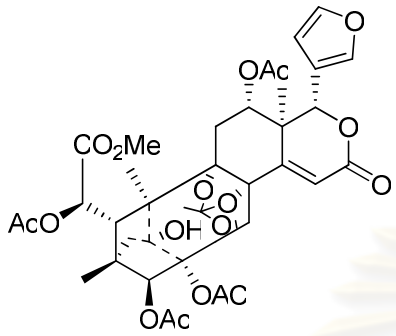
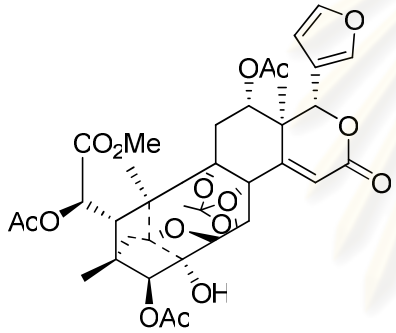
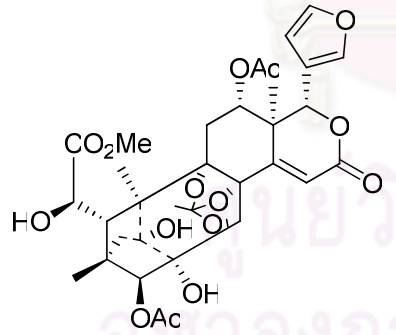
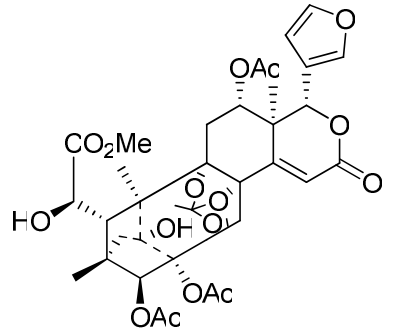
Compound	Category	Plant (part)	References
Xyloccensin P 	Phragmalin	*X. granatum* (Stem bark)	Wu *et al*., 2004; Wu *et al*., 2005; Cui *et al*., 2005; Wu *et al*., 2006
Xyloccensin Q 	Phragmalin	*X. granatum* (Stem bark)	Wu *et al*., 2004; Wu *et al*., 2005; Cui *et al*., 2005; Wu *et al*., 2006
Xyloccensin R 	Phragmalin	*X. granatum* (Stem bark)	Wu *et al*., 2004; Wu *et al*., 2005; Cui *et al*., 2005; Wu *et al*., 2006
Xyloccensin S 	Phragmalin	*X. granatum* (Stem bark)	Wu *et al*., 2004; Wu *et al*., 2005; Cui *et al*., 2005; Wu *et al*., 2006

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

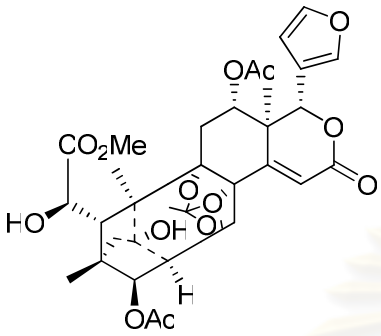
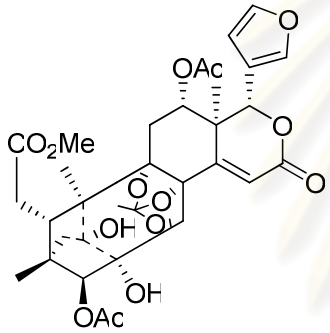
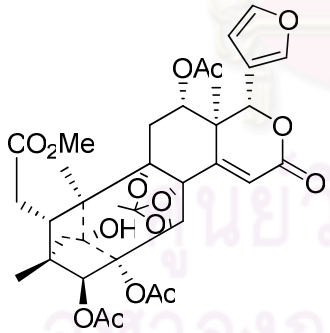
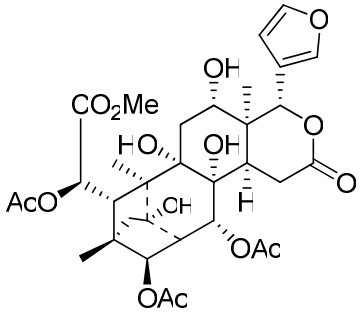
Compound	Category	Plant (part)	References
Xyloccensin T 	Phragmalin	*X. granatum* (Stem bark)	Wu *et al*., 2004; Wu *et al*., 2005; Cui *et al*., 2005; Wu *et al*., 2006
Xyloccensin U 	Phragmalin	*X. granatum* (Stem bark)	Wu *et al*., 2004; Wu *et al*., 2005; Cui *et al*., 2005; Wu *et al*., 2006
Xyloccensin V 	Phragmalin	*X. granatum* (Stem bark)	Wu *et al*., 2004; Wu *et al*., 2005; Cui *et al*., 2005; Wu *et al*., 2006
Xyloccensin Y 	Phragmalin	*X. granatum* (Stem bark, Fruit and Seed)	Zhou *et al*., 2006

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

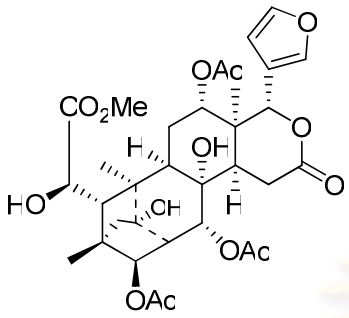
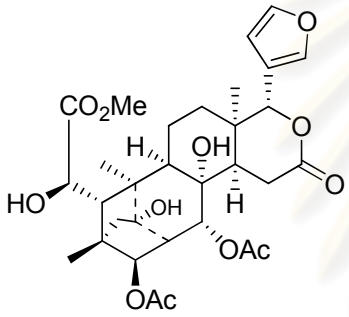
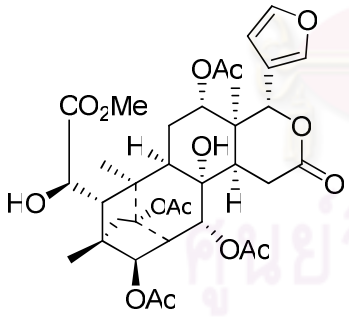
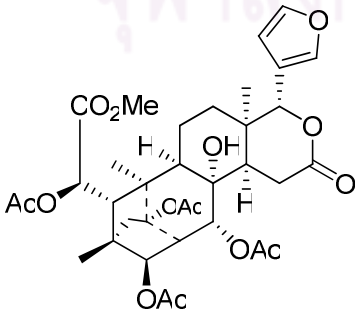
Compound	Category	Plant (part)	References
Xyloccensin Z_1 	Phragmalin	*X. granatum* (Stem bark, Fruit and Seed)	Zhou *et al*., 2006
Xyloccensin Z_2 	Phragmalin	*X. granatum* (Stem bark, Fruit and Seed)	Zhou *et al*., 2006
Granaxylocarpin D 	Phragmalin	*X. granatum* (Stem bark, Fruit and Seed)	Yin *et al*., 2007
Granaxylocarpin E 	Phragmalin	*X. granatum* (Stem bark, Fruit and Seed)	Yin *et al*., 2007

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

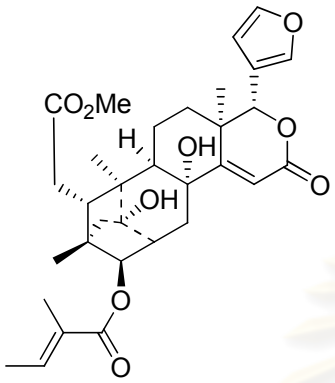
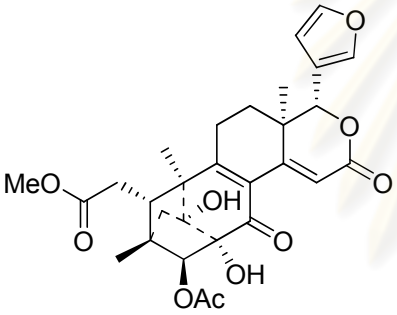
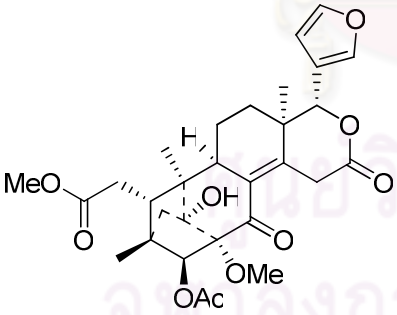
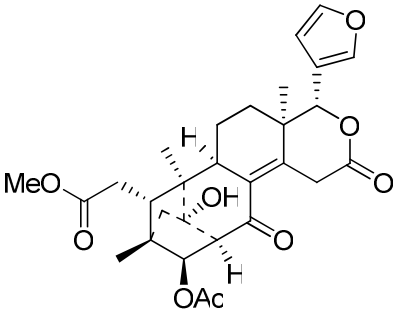
Compound	Category	Plant (part)	References
Xylogranatin E_2 	Phragmalin	*X. granatum* (Stem bark, Fruit and Seed)	Wu *et al*., 2007
Moluccensin H 	Phragmalin	*X. moluccensis* (Seed)	Pudhom *et al*., 2010
Moluccensin I 	Phragmalin	*X. moluccensis* (Seed)	Pudhom *et al*., 2010
Moluccensin J 	Phragmalin	*X. moluccensis* (Seed)	Pudhom *et al*., 2010

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

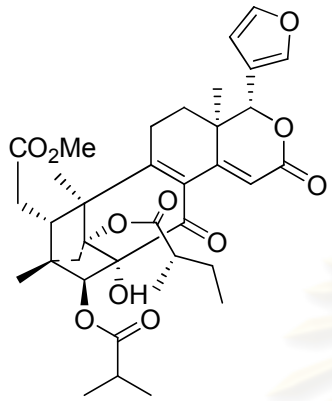
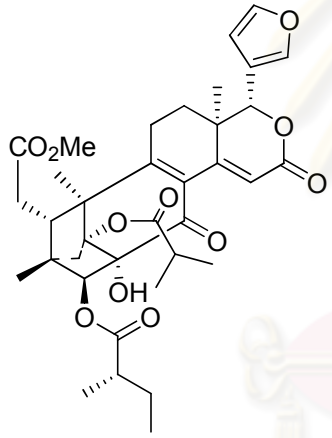
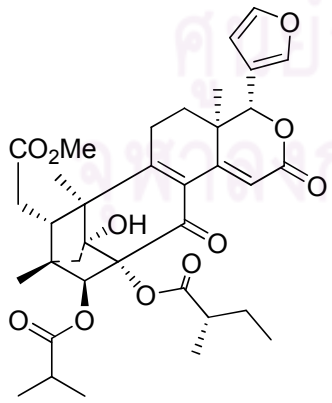
Compound	Category	Plant (part)	References
Moluccensin H 	Phragmalin	*X. moluccensis* (Seed)	Wu *et al*., 2010
Moluccensin I 	Phragmalin	*X. moluccensis* (Seed)	Wu *et al*., 2010
Moluccensin J 	Phragmalin	*X. moluccensis* (Seed)	Wu *et al*., 2010

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

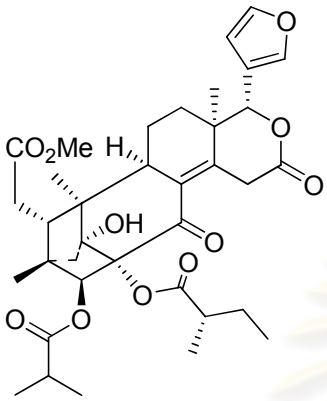
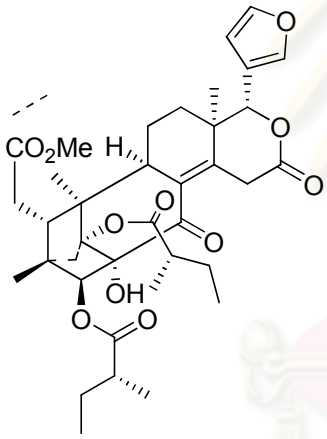
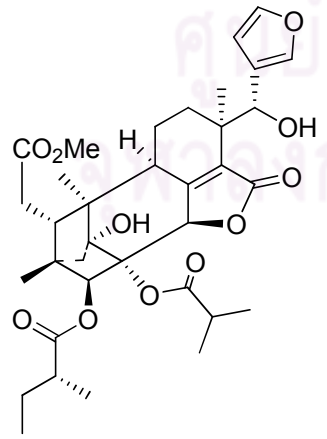
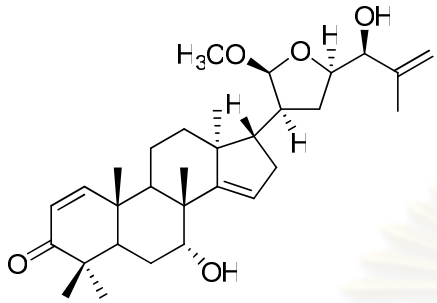
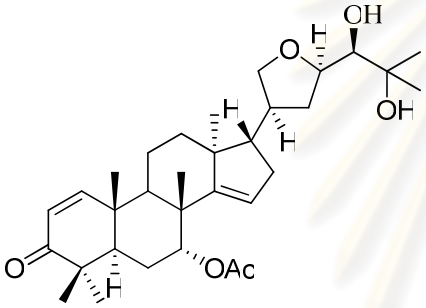
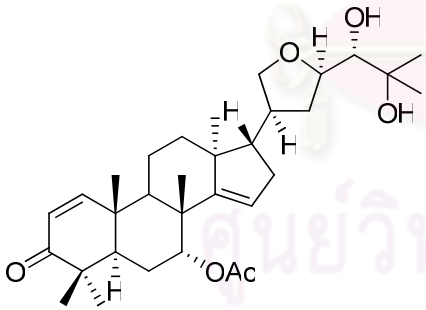
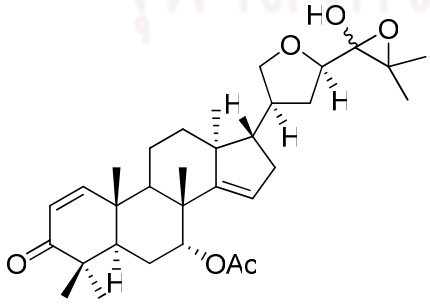
Compound	Category	Plant (part)	References
Moluccensin K 	Phragmalin	*X. moluccensis* (Seed)	Wu *et al*., 2010
Moluccensin L 	Phragmalin	*X. moluccensis* (Seed)	Wu *et al*., 2010
Moluccensin M 	Phragmalin	*X. moluccensis* (Seed)	Wu *et al*., 2010

Table 2.2. Limonoids isolated from *Xylocarpus* species (continued)

Compound	Category	Plant (part)	References
Protoxylogranatin A 	Protolimonoid	*X. granatum* (Fruit and seed)	Li *et al*., 2008
Protoxylocarpin F 	Protolimonoid	*X. granatum* (Seed)	Pudhom *et al*., 2009
Protoxylocarpin G 	Protolimonoid	*X. granatum* (Seed)	Pudhom *et al*., 2009
Protoxylocarpin H 	Protolimonoid	*X. granatum* (Seed)	Pudhom *et al*., 2009

2.3 Biolgical activities of chemical costituents from *Xylocarpus* species

In 2005, Wu and coworkers found six new 8,9,30-phragmalin *ortho* esters, named xyloccensins Q-V, which were isolated from the stem bark of a Chinese mangrove *Xylocarpus granatum*. Xyloccensin Q was exhibited potent antifeedant activity against the third instar larvae of *Mythimna separata* (Walker) at a concentration of 500 ppm (Wu *et al*., 2005).

In 2007, Yin and coworkers reported five new limonoids, granaxylocarpins A-E, were isolated from the seeds of the Chinese marine mangrove *Xylocarpus granatum*. Granaxylocarpins A and B showed weak cytotoxic activities against the P-388 cell line with IC_{50} values of 9.3 and 4.9 μM, respectively (Yin *et al*., 2007).

In 2009, Cui and coworkers isolated five new protolimonoids, protoxylocarpins A-E, and two new limonoids, xylocarpins J and K, together with xyloccensins M and Y from the fruits of a Chinese mangrove plant *Xylocarpus granatum*. These compounds exhibited moderate to weak activity against HCT-8, Bel-7402, BGC-823 and A2780 cell lines (Cui *et al*., 2009).

In 2009, Du and coworkers found a new lactone, named 3-(1-hydroxyethyl)-4,4-dimethyl-4-butyrolactone, isolated from the leaves of *Xylocarpus granatum*. At a concentration of 20 μg/mL, this lactone gave a 67.4% inhibition rate against wheat powdery mildew (Du *et al*., 2009).

In 2009, Li and coworkers reported khayasin T, a limonoid from the seeds of an Indian mangrove *Xylocarpus granatum*. This compound exhibited moderate insecticidal activity against fifth instar larvae of *Brontispa longissima* (Gestro) at a concentration of 20 mg/L. Its lethal rates against the fifth instar larvae of *B. longissima* at exposure times of 48, 72 and 96 h were 17.4%, 27.8% and 41.5%, respectively (Li *et al*., 2009).

In 2009, Pudhom and coworkers found xylogranatin C and 7-oxo-7-deaxetoxygedunin isolated from seed kernels of *Xylocarpus granatum*. Xylogranatin C was active against CHAGO cells with an IC_{50} value of 9.16 μM, while 7-oxo-7-deacetoxygedunin was cytotoxic toward Hep-G2 cells with an IC_{50} value of 16.17 μM (Pudhom *et al*., 2009).

In 2010, Pudhom and coworkers found three new phragmalin limonoids, moluccensins H-J, which were isolated from seed kernels of the cedar mangrove, *Xylocarpus moluccensis*. Only moluccensin I displayed weak antibacterial activity against *Staphylococcus hominis* ATCC 27844 and *Enterococcus faecalis* ATCC 29212, with a MIC at 256 μg/mL (Pudhom *et al*., 2010)

In 2010, Wu and coworkers reported moluccensins H and I from the seeds of an Indian mangrove, *Xylocarpus moluccensis*. These compounds showed moderate insecticidal activity against the fifth instar larvae of *Brontispa longissima* (Gestro) at a concentration of 100 mg/L. The lethal rates of moluccensin H at exposure times of 72 and 96 h were 20.7% and 27.6%, respectively, while those of moluccensin I were 10.7% and 28.7%, respectively (Wu *et al*., 2010).

Limonoid derivatives have been found in all *Xylocarpus* plants studied, but their distribution and content varies between different species and between parts, or geocultivars, of the same species. These unique characteristics prompted us to investigate another plant in this genus, *Xylocarpus rumphii* (Kostel.) Mabb. due to a few reports on its chemical constituents.

CHAPTER III

EXPERIMENTS

3.1 Plant material

The seed kernels of *Xylocarpus rumphii* (Kostel.) Mabb. were collected from Rayong Province, Thailand, in April 2009. Plant materials were identified by Royal Forest Department, Bangkok, Thailand. A voucher specimen (BKF No. 163884) was deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

3.2 General Experimental Procedures

3.2.1 Nuclear magnetic resonance spectrometer (NMR)

The NMR spectra were recorded in $CDCl_3$ using a Bruker AV400 spectrometer at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR using TMS (Tetramethylsilane) as internal standard.

3.2.2 Mass spectrometer (MS)

HRESIMS spectra were obtained with a Bruker micrOTOF.

3.2.3 Ultraviolet-visible spectrophotometer (UV-vis)

UV data were recorded on a CARY 50 Probe UV-visible spectrophotometer.

3.2.4 Fourier transform infrared spectrophotometer (FT-IR)

The FT-IR spectra were recorded on a Perkin-Elmer Model 1760X Fourier Transform Infrared Spectrophotometer. Solid samples were formally examined by incorporating the sample with potassium bromide (KBr) to form a pellet.

3.2.5 Optical rotation

Optical rotations were measured on a Perkin-Elmer 341 polarimeter at 589 nm.

3.2.6 Melting point

Melting points were recorded on a Fisher-Johns melting point apparatus.

3.2.7 High performance liquid chromatography (HPLC)

Preparative HPLC was performed on a Water system (Waters 600 HPLC pump and Waters 2996 Photodiode array detector). GL Science column C18 (20 × 250 mm, 3 μm) was used for separation.

3.2.8 X-ray crystallography

The crystal structure was solved by direct methods and using the SHELXS97 program. Crystallographic data, excluding structure factors, have been deposited at the Cambridge Crystallographic Data Centre.

3.3 Chemicals used

3.3.1 Solvent

All commercial grade solvents, used in this research such as hexane, chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), acetone and methanol (MeOH), were purified by distillation prior to use.

The deuterated solvent for NMR experiments is $CDCl_3$.

3.3.2 Other chemicals

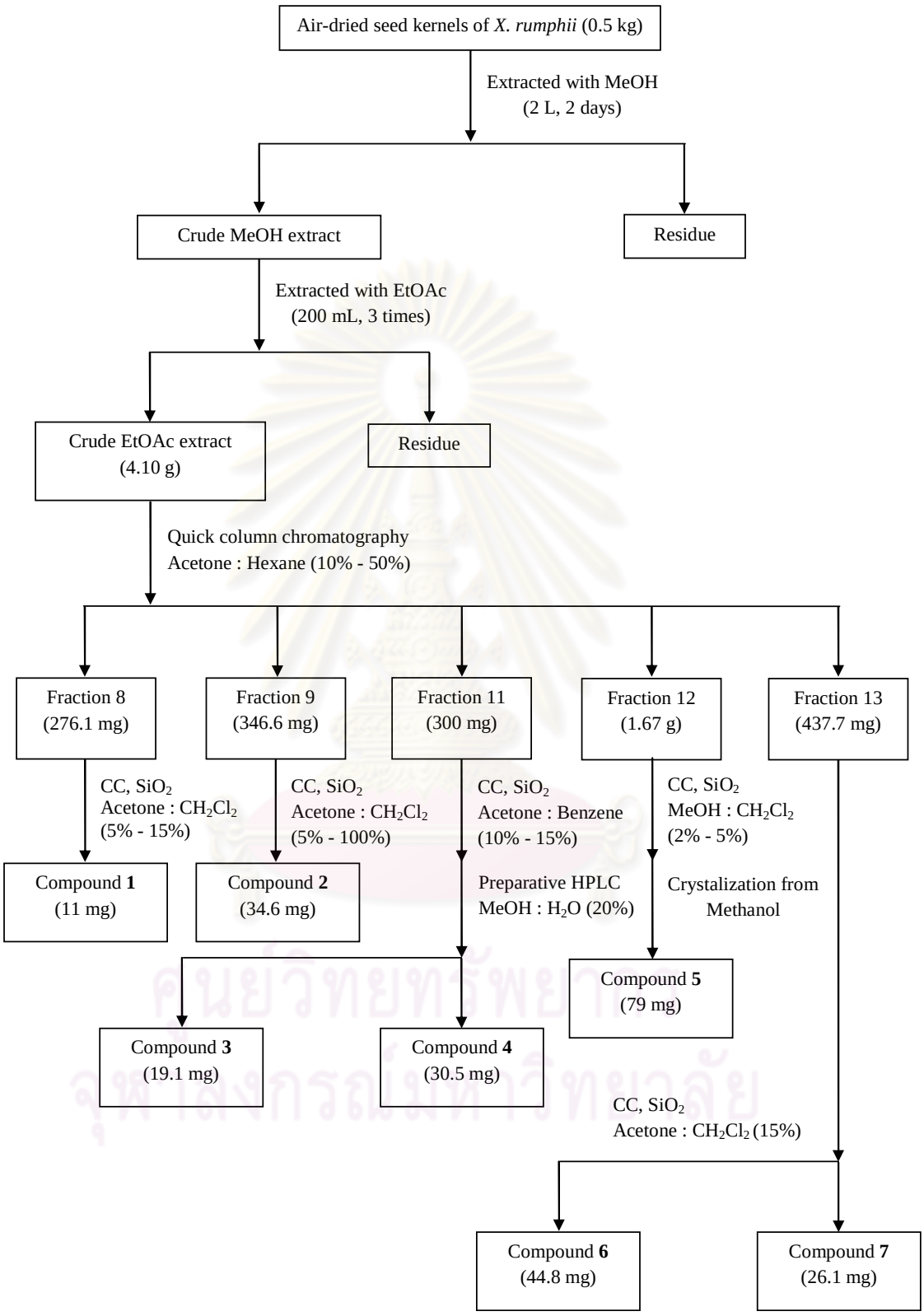
- Merck's silica gel 60 No. 7734 and No. 9385 were used as adsorbents for open column chromatography.

- Merck's Thin layer chromatography (TLC) aluminum and glass sheets, silica gel 60 F_{254} precoated 25 sheets, 20x20 cm, layer thickness 0.2 mm were used for TLC analysis. Detection was visualized under ultraviolet light at wavelengths of 254 and 356 nm and dipped with $(NH_4)_6Mo_7O_{24}$ solution in 5% H_2SO_4 /EtOH.

3.4 Extraction and Isolation

Air-dried and powder seed kernels of *X. rumphii* (0.5 kg) were extracted with MeOH (2 L x 3, each 2 days) at room temperature. After removing the solvent in vaccuo, the combined MeOH crude extract was suspened in H_2O (250 mL), then partitioned with EtOAc (200 mL x 3) to afford the crude EtOAc extract (4.10 g). The EtOAc extract was chromatographed on a silica gel column eluted with a gradient of acetone-hexane (10%-50%) to yield fifteen major fractions. Each fraction was analyzed by TLC and ^{1}H NMR spectrum. Fraction eighth was subjected to column chromatography over silica gel eluting with acetone-CH_2Cl_2 (5%-15%) to give compound **1** (11 mg). Fraction ninth was chromatographed on a silica gel column chromatography using acetone-CH_2Cl_2 (5%-100%) to obtain compound **2** (34.6 mg). Fraction eleventh was subjected to silica gel column chromatography eluting with acetone-benzene (10%-15%), and further purified by preparative HPLC (C18) silica gel using a mixture of MeOH-H_2O (80%) to afford compound **3** (19.1 mg) and compound **4** (30.5 mg), respectively. Fraction twelfth was separated on a silica gel column eluting with MeOH-CH_2Cl_2 (2%-5%) and then crystallized from MeOH to obtain compound **5** (79 mg). Fraction thirteenth was chromatographed on a silica gel column using acetone-CH_2Cl_2 (15%) to furnish compound **6** (44.9 mg) and compound **7** (26.1 mg), respectively.

The extraction and isolation of the ethyl acetate crude extract of *X. rumphii* is summarized in Scheme 3.1.



Scheme 3.1 The extraction and isolation procedure of *X. rumphii* seed kernels

3.5 Evaluation of biological activities

The pure compounds were evaluated for their antibacterial and anticancer activity.

3.5.1 Antibacterial activity

A total of 12 strains of gram-positive and gram-negative bacteria (Table 3.1) were selected for *in vitro* antimicrobial assay. The test was performed by using microdilution assays as follows:

Table 3.1 Gram-positive and gram-negative bacteria tested

Gram-positive bacteria	Gram-negative bacteria
1. *Enterococcus faecalis* ATCC 29212	1. *Escherichia coli* ATCC 35218
2. *Enterococcus faecalis* ATCC 51299 (vancomycin resistant)	2. *Klebsiella pneumoniae* ATCC 27736
3. *Enterococcus faecium* UCLA 192	3. *Klebsiella pneumoniae* (ESBL producing) ATCC 700603
4. *Salmonella typhimurium* ATCC 13311	4. *Pseudomonas aeruginosa* ATCC 27853
5. *Staphylococcus aureus* ATCC 25923	5. *Proteus vulgaris* ATCC 13315
6. *Staphylococcus epidermidis* ATCC 12228	
7. *Staphylococcus hominis* ATCC 27844	

3.5.1.1 Preparation of bacterial inocula

Bacteria were grown on Mueller Hinton agar (MHA) for 24 h at 37 °C. Selected fresh single colonies were inoculated into 10 mL of Mueller Hinton broth (MHB) and incubated in shaking incubator for 2-3 h at 37 °C. The turbidity of the bacterial suspension was adjusted with sterile normal saline solution to match the turbidity of 0.5 McFarland standard (OD 0.1 at 625 nm). Then, the suspension was diluted 1:100 with Mueller Hinton broth (MHB) to contain $1x10^6$ CFU/mL.

3.5.1.2 Determination of minimum inhibitory concentration (MIC)

Solution of a test compound in DMSO (25.6 mg/mL) was diluted with MHB. The test compound was prepared at the concentration ranges of 0.5 to 256 μg/mL. MIC is defined as the lowest concentration that inhibits growth of test microorganisms.

A 50 μL of MHB containing the test compound was dispensed into each well of microtiter plates (96-flat-bottom wells) for the evaluation of antibacterial activities. Sterile compound-free medium containing the corresponding amount of DMSO was dispensed in the growth control wells. The final adjusted bacterial suspensions were inoculated into each well with volumes of 50 μL. Compound-free MHB in volumes of 100 μL were used as the sterility control. The experiments were done in duplicate. After incubation at 37 °C for 24 h, a 20 μL of *p*-iodonitrotetrazolium (INT) solution (1 mg/mL) was added into each well. The antibacterial assay plates were further incubated for 1 h. Growth in each well was indicated by a color change from colorless to violet. Compounds that inhibit microbial growth would prevent the development of a violet color. The well that shows no change in color indicates antimicrobial activity of the test compound.

3.5.2 Anticancer activity

Cytotoxicity assay was carried out at the institute of Biotechnology and Genetic Engineering, Chulalongkorn University. All isolated compounds were tested for their cytotoxic activity towards five human cancer cell lines including HEP-G2 (hepatocarcinoma), SW-620 (colon adenocarcinoma), CHAGO (undifferentiated lung carcinoma), KATO-3 (gastric carcinoma), BT-474 (breast ductal carcinoma) cancer cell lines and CH-Liver (liver cell line) used as control cell. Herein, the *in vitro* cytotoxicity was determined by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphynyltrazolium bromide) calorimetric method (Carmichael *et al*., 1987). In principle, the viable cell number/well was directly proportional to the production of formazan, followed by solubilization, and could be measured spectrophotometrically.

The human cancer cell line was harvested from exponential-phase maintenance cultures (T-25 cm^2 flask), counted by trypan blue exclusion, seed cells in a 96-well culture plates at a density of $1x10^5$ cells/well in 200 μL of culture medium without compounds to be tested. Cells were cultured in a 5% CO_2 incubator at 37 ^oC, 100% relative humidity for 24 h. Culture medium containing the sample was dispensed into the appropriate wells (control cells group, N = 3; each sample treatment group, N = 3). Peripheral wells of each plate (lacking cells) were utilized for sample blank (N = 3) and medium/DMSO blank (N = 3) “background” determination. Culture plates were then incubated for 3 days prior to the addition of tetrazolium reagent. MTT stock solution in a concentration of 5 mg/ml in PBS was sterilized by filtering through 0.45 μL filter units. MTT working solution was prepared just prior to culture application by dilution of MTT stock 1:5 (v/v) in prewarmed standard culture medium. The freshly prepared MTT reagent in a volume of 10 μL was added into each well and mixed gently for 1 minute on an orbital shaker. The cells were further incubated for 4 h at 37 ^oC in a 5% CO_2 incubator. After incubation, the formazan produced in the cells will capture as dark crystals in the bottom of the wells. All of the culture medium supernatant were removed from wells and 150 μL of DMSO was added to dissolve the resulting formazan. Samples in the culture plate were mixed for 5 minutes on an orbital shaker. Subsequently, 25 μL of 0.1 M Glycine pH 10.5 was

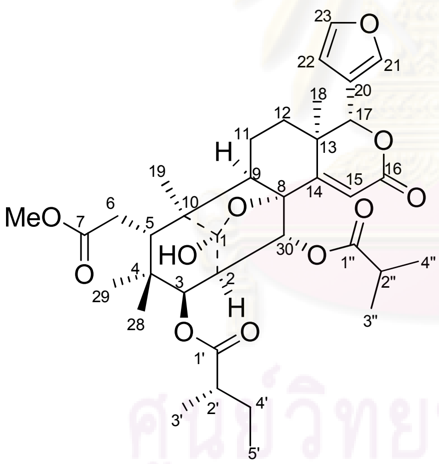
added and the culture plate was shaken for 5 minutes. Following formazan solubilization, the absorbance was measured using a microculture plate reader at 540 nm (single wavelength, calibration factor = 1.00).

CHAPTER IV

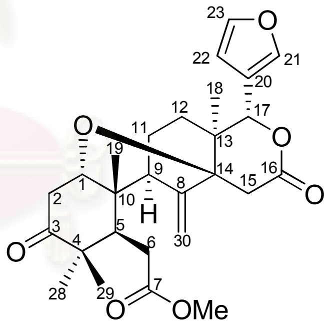
RESULTS AND DISCUSSION

4.1 The isolated compounds from *Xylocarpus rumphii* (Kostel.) Mabb.

The ethyl acetate crude extract of the seed kernels of *Xylocarpus rumphii* (Kostel.) Mabb. was separated by chromatographic techniques to obtain seven limonoids including four new limonoids, xylorumphiins A-D (compounds **3**, **4**, **1** and **7**) and three known limonoids namely methyl angolensate (compound **2**), xyloccensins E (compound **5**) and K (compound **6**). Their structures are shown in Figure 4.1.

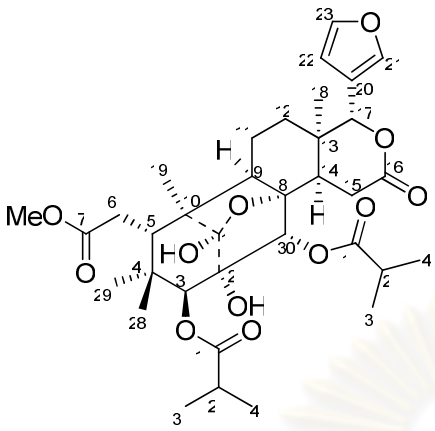


Compound **1**

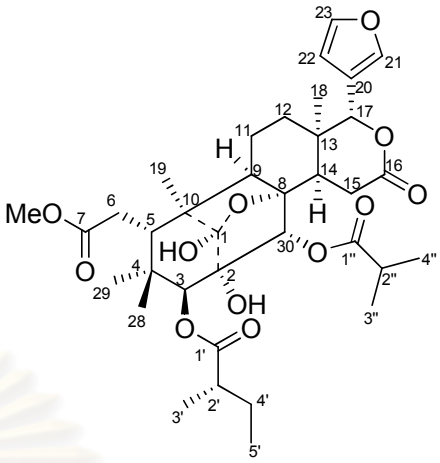


Compound **2**

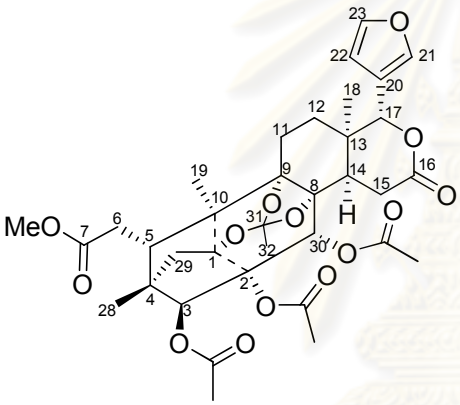
Figure 4.1 The chemical structures of isolated compounds from *X. rumphii*



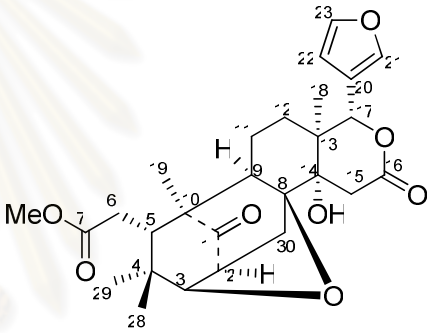
Compound **3**



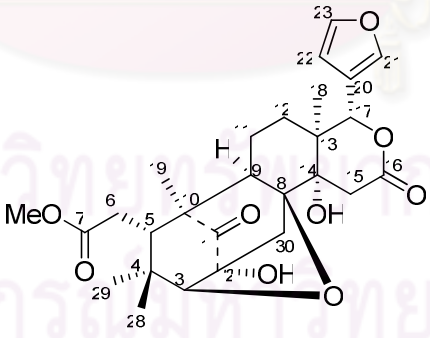
Compound **4**



Compound **5**



Compound **6**



Compound **7**

Figure 4.1 The chemical structures of isolated compounds from *X. rumphii* (continued)

4.1.1 Structure elucidation of compound 1

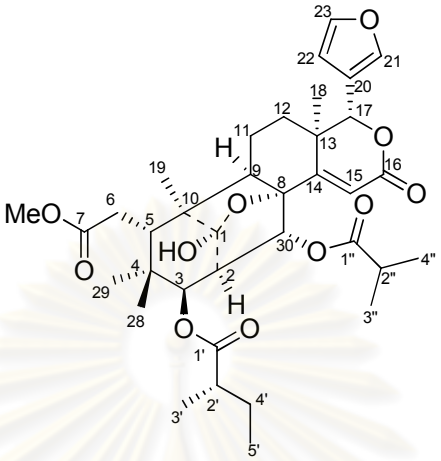


Figure 4.2 Compound **1**

Molecular formula	$C_{36}H_{48}O_{11}$
Appearance	White amorphous solid
m.p.	180.5-182.5 ^{o}C
$[\alpha]_D^{20}$	-13 (*c* 0.1 $CHCl_3$)
UV ($CHCl_3$) λ_{max} (log ε)	245 nm (3.48)
IR (KBr)	3393, 2973, 2945, 2372, 1723, 1461, 1380, 1294, 1256, 1189 and 1151 cm^{-1}
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 4.1
HRESIMS *m/z*	679.3095 $[M+Na]^+$, calcd. 679.3094

Compound **1** had the molecular formula $C_{36}H_{48}O_{11}$ as established by HRESIMS (*m/z* 679.3095 $[M+Na]^+$, calcd. 679.3094). The ^1H, ^{13}C (Table 4.1) and 2D NMR (HSQC, COSY, HMBC) data revealed the presence of four methyl groups [δ_H 1.20 s, 1.07 s, 1.25 s, 0.78 s; δ_C 19.7, 20.5, 21.9, 26.4], a 2-methylbutyryl group [δ_H 2.28 (m, 1H), 1.13 (m, 3H), 1.64 (m, 2H), 0.88 (t, J = 7.4 Hz, 3H); δ_C 41.4 CH, 16.1 CH_3, 26.2 CH_2, 11.5 CH_3, 175.8 qC], an isobutyryl group [δ_H 2.47 (m, 1H), 1.10 (m, 6H); δ_C 34.1 CH, 18.8 CH_3, 18.9 CH_3, 176.4 qC], a methoxy carbonyl [δ_H 3.68 s; δ_C 51.9 CH_3, 173.8 qC], a sp^2 methine group [δ_H 6.00 s; δ_C 117.6], as well as three oxygenated methine [δ_H 5.11 (d, J = 9.1 Hz), 5.02 s, 5.54 (d, J = 4.2 Hz); δ_C 73.8, 81.3, 76.2]. The downfield shifted proton resonances at δ_H 7.41 (s, 1H), 6.42 (s, 1H) and 7.49 (s, 1H) were characteristic of a β-substituted furan ring found in all known limonoids. These NMR data strongly suggested that **1** was a maxicanolide type limonoid. The sp^2 methine proton at δ_H 6.00 showed HMBC correlation (Figure 4.3) between C-8, C-13, C-14, C-16 and C-18, thus this proton was assigned to H-15. The doublet oxymethine proton at δ_H 5.11 (d, J = 9.1 Hz) was assigned to H-3 through HMBC correlations from this proton to the carbon at C-4, C-5, C-28, C-30 and C-1′. In addition, the proton at δ_H 5.54 (d, J = 4.2 Hz) showed HMBC correlations to C-1, C-2, C-3, C-8 and C-1″, were indicated as H-30. A quaternary carbon at δ_C 107.2 was assignable to C-1, a hemiketal group related to that of xylogranatin A (Wu *et al*., 2006), xylocarpin F and G (Cui *et al*., 2007). The HMBC correlation between H-3 and an acetyl carbonyl at δ_C 177.8 clarified the acetyl substitution. Moreover, 2-methylbutyryl and isobutyryl groups were located at C-3 and C-30 through HMBC correlations from H-3 to carbonyl at δ_C 175.8 and from H-30 to another carbonyl at δ_C 176.4, respectively. The relative configuration of **1** was elucidated by the NOE correlations of H-9/H_3-18, H_3-18/H_3-19, H-17/H-30, H-2/H_3-29 (Figure 4.4). Thus, compound **1** was assigned to be xylorumphiin C as shown in Figure 4.2.

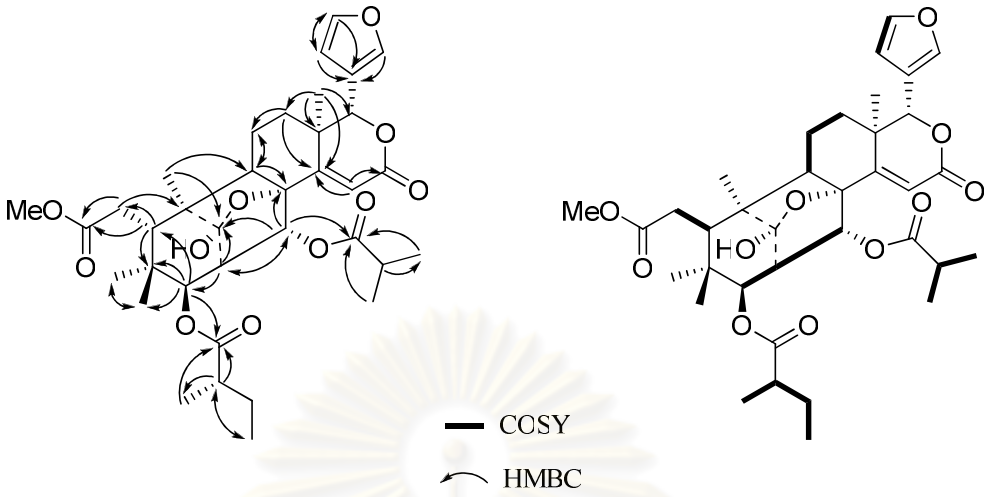


Figure 4.3 Key HMBC and COSY correlations of compound **1**

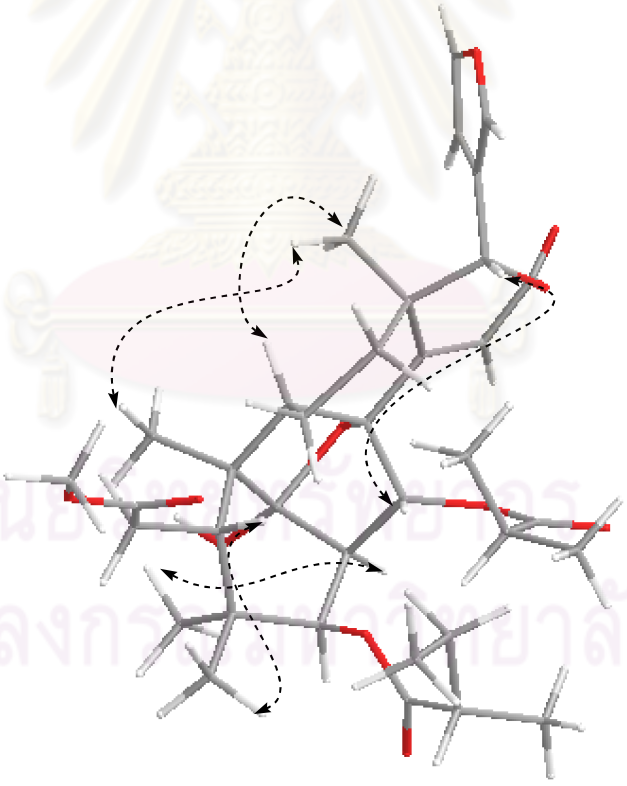


Figure 4.4 Key NOE correlations of compound **1**

Table 4.1. The NMR data of compound **1**

Position	^{1}H	^{13}C	COSY	HMBC
1		107.5		
2	2.91 (dd, *J* = 9.1, 4.3 Hz, 1H)	53.2		C- 1, C-3, C-4, C-10
3	5.11 (d, *J* = 9.1 Hz, 1H),	73.8		C-4, C-5, C-28, C-30, C-1′
4		37.8		-
5	2.62 (d, *J* = 10.2 Hz, 1H)	40.6	H-6a, H-6b	C-4, C-7, C-9, C-10, C-28
6	2.16 (m, 1H) 2.35 (m, 1H)	32.1	H-5	C-4, C-7
7		173.8		
8		81.5		
9	2.14 (m, 1H)	51.5	H-11a, H-11b	C-8, C-11
10		42.9		
11	1.81 (m, 1H) 2.36 (m, 1H)	15.0	H-9, H-12a, H-12b	C-9
12	2.19 (m, 2H)	24.9	H-11a, H-11b	C-11, C-14
13		38.9		
14		159.7		
15	6.00 (s, 1H),	117.6		C-8, C-13, C-14, C-16, C-18
16		163.7		
17	5.02 (s, 1H),	81.3		C-13, C-18, C-20, C-22, C-23
18	1.20 (s, 3H)	19.7		C-1, C-9, C-10, C-12, C-13, C-14, C-17
19	1.07 (s, 3H)	20.5		
20		120.0		
21	7.41 (s, 1H),	142.9		C-20
22	6.42 (s, 1H),	109.9	H-23	C-20, C-23
23	7.49 (s, 1H),	141.2	H-22	C-20, C-22
28	1.25 (s, 3H)	21.9		C-3, C-4, C-5, C-29
29	0.78 (s, 3H)	24.6		C-3, C-4, C-5, C-28
30	5.54 (d, *J* = 4.2 Hz, 1H)	76.2		C-1, C-2, C-3, C-8, C-1″
3-*Acyl*				
1′		175.8		
2′	2.28 (m, 1H)	41.1	H-3′, H-4a′, H-4b′	C-1′, C-3′, C-4′, C 5′
3′	1.13 (m, 3H)	16.1	H-2′	C-1′, C-2', C-4′
4′	1.64 (m, 2H)	26.2	H-2′, H-5′	
5′	0.88 (t, *J* = 7.4 Hz, 3H)	11.5	H-4a′, H-4b′	C-2', C-4′
30-*Acyl*				
1″		176.4		
2″	2.47 (m, 1H)	34.1	H-3′, H-4′	C-1", C-3″
3″	1.10 (m, 3H)	18.8	H-2′	C-1″
4″	1.10 (m, 3H)	18.9	H-2′	C-1"
7-*OMe*	3.68 (s, 3H)	51.9		C-7

4.1.2 Structure elucidation of compound 2

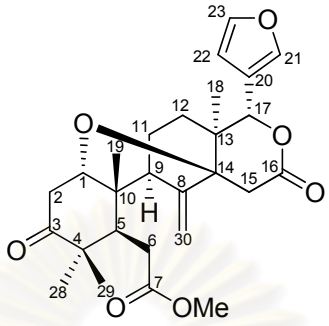


Figure 4.5 Compound **2**

Molecular formula	$C_{27}H_{34}O_7$
Appearance	White amorphous solid
m.p.	184.5-186.5 ^{o}C
$[\alpha]_D^{20}$	-37 (*c* 0.1 $CHCl_3$)
UV ($CHCl_3$) λ_{max} (log ε)	270 nm (2.14)
IR (KBr)	3426, 3116, 2964, 2358, 2339, 1719, 1456, 1390, 1242, 1170, 1127 and 1022 cm^{-1}
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 4.2
ESIMS *m/z*	471.54 $[M+H]^+$, calcd. 471.56

Compound **2** had the molecular formula $C_{27}H_{34}O_7$, as established by ESIMS (*m/z* 471.54 $[M+H]^+$, calcd. 471.56). The NMR data of **2** (Table 4.2) and its 2D NMR showed signals for a methoxy ester [δ_H 3.70 s; δ_C 52.1 CH_3, 173.8 qC], a ketone carbonyl [δ_C 212.8 qC], an ester carbonyl [δ_C 170.1 qC], four methyls [δ_H 0.85 s, 0.93 s, 1.03 s, 1.18 s; δ_C 13.7, 21.6, 25.8, 21.6], two sp^2 methylenes [δ_H 4.88 s, 5.14 s; δ_C 111.5 CH_2, 145.6 qC], two oxygenated methines [δ_H 3.51 (dd, J = 6.1, 4.0 Hz), 5.65 s; δ_C 77.2, 79.5], together with a β-furyl ring [δ_H 7.42 s, 6.37 (d, J = 1.0 Hz), 7.36 (t, J = 1.6 Hz); δ_C 120.8 qC, 140.7 CH, 109.9 CH, 142.7 CH]. The aforementioned data indicated that **2** is andirobin type limonoid. The location of $\Delta^{8,30}$ double bond was confirmed by HMBC correlations (Figure 4.6) from methylene protons at δ_H 4.88 and 5.14 to C-8 and C-14. An observed HMBC correlation from H-1 to C-14 allowed the assignment of the oxygen bridge between C-1 and C-14. The relative configuration of **2** was elucidated by the NOE correlations (Figure 4.7) at H-9/H_3-18, H-5/H_3-28, H-1/H_3-19, H_3-19/H_3-29, H-17/H_3-29. Based on these findings and comparison of its NMR data with those reported in the literatures (Table 4.2), it has proved that compound **2** was assigned to be methyl angolensate as shown in Figure 4.5 (Kadota *et al*., 1990).

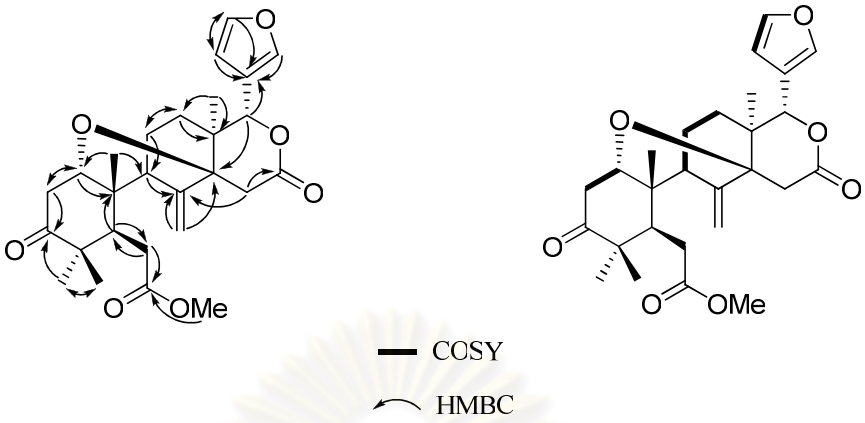


Figure 4.6 Key HMBC and COSY correlations of compound **2**

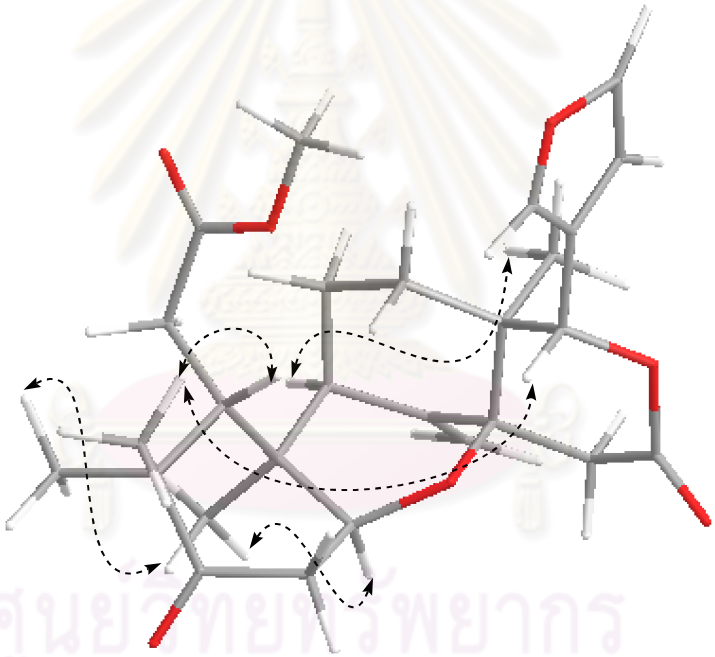


Figure 4.7 Key NOE correlations of compound **2**

Table 4.2. The ^{1}H and ^{13}C NMR data of methyl angolensate and compound **2**

Position	Methyl angolensate		Compound **2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	3.52 (dd, *J* = 6.5,4.0 Hz, 1H)	77.2	3.51 (dd, *J* = 6.1, 4.0 Hz, 1H)	77.2
2	2.51 (dd, *J* = 14.5,4.0 Hz, 1H)	39.5	2.48 (dd, *J* = 14.3, 4.0 Hz, 1H)	39.3
	2.90 (dd, *J* = 14.5,6.0 Hz, 1H)		2.91 (m, 1H)	
3		212.6		212.8
4		48.0		48.0
5	2.88 (d, *J* = 10.5 Hz, 1H)	43.0	2.87 (m, 1H)	42.8
6	2.25 (d, *J* = 16.5 Hz, 1H)	33.6	2.26 (m, 1H)	33.7
	2.61 (dd, *J* = 16.5,10.5 Hz, 1H)		2.60 (m, 1H)	
7		173.8		173.8
8		145.9		145.6
9	2.17 (dd, *J* = 5.0,1.5 Hz, 1H)	50.0	2.15 (m, 1H)	49.8
10		44.1		43.9
11	1.57 (t, *J* = 14.5 Hz, 1H)	23.8	1.56 (m, 1H)	23.7
	2.20 (m, 1H)		2.22 (m, 1H)	
12	1.14 (ddd, *J* = 16.5 Hz, 1H)	29.3	1.12 (m, 1H)	29.3
	2.61 (dd, *J* = 16.5,10.5 Hz, 1H)		2.61 (m, 1H)	
13		41.5		41.4
14		80.2		80.2
15	2.91(d, *J* = 18.0 Hz, 1H)	33.8	2.91 (m, 1H)	33.7
	2.58 (d, *J* = 18.0 Hz, 1H)		2.59 (m, 1H)	
16		169.9.		170.1
17	5.67 (s, 1H)	79.6	5.65 (s, 1H)	79.5
18	0.84 (s, 3H)	13.8	0.85 (s, 3H)	13.7
19	0.95 (s, 3H)	21.7	0.93 (s, 3H)	21.6
20		120.9		120.8
21	7.44 (dd, *J* = 1.5, 0.8, 1H)	140.8	7.42 (s, 1H)	140.7
22	6.39 (dd, *J* = 1.5, 0.8 Hz, 1H)	109.9	6.37 (d, *J* = 1.0 Hz, 1H)	109.9
23	7.38 (t, *J* = 1.5 Hz, 1H)	142.7	7.36 (t, *J* = 1.6 Hz, 1H)	142.7
28	1.05 (s, 3H)	26.0	1.03 (s, 3H)	25.8
29	1.19 (s, 3H)	21.5	1.18 (s, 3H)	21.6
30	4.90 (s, 1H)	111.5	4.88 (s, 1H)	111.5
	5.15 (s, 1H)		5.14 (s, 1H)	
7-*OMe*	3.72 (s, 3H)	52.1	3.70 (s, 3H)	52.1

4.1.3 Structure elucidation of compound 3

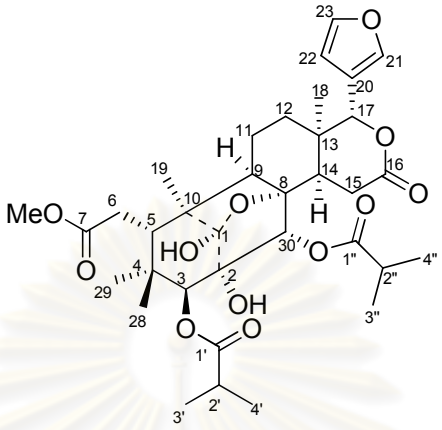


Figure 4.8 Compound **3**

Molecular formula	$C_{35}H_{48}O_{12}$
Appearance	White amorphous solid
m.p.	124.5-126.5 ^{o}C
$[\alpha]_{D}^{20}$	-115 (*c* 0.1 $CHCl_3$)
UV ($CHCl_3$) λ_{max} (log ε)	243 nm (3.10)
IR (KBr)	3460, 2978, 2940, 2363, 1733, 1456, 1385, 1294, 1189, 1151, 1060 and 1017 cm^{-1}
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 4.3
HRESIMS *m/z*	683.3038 $[M+Na]^{+}$, calcd. 683.3043

Compound **3**, a white, amorphous solid, possessed a molecular formula of $C_{35}H_{48}O_{12}$ as established by the HRESIMS (*m/z* 683.3038 $[M+Na]^+$, calcd. 683.3043). The NMR data (Table 4.3) of **3** and the information from its 2D NMR studies (^1H-^1H COSY, HSQC, HMBC) indicated the presence of the following functional groups; a methoxycarbonyl [(δ_H 3.69 s; δ_C 51.9 CH_3, 173.9 qC)], two isobutyryl groups [δ_H 2.64 m, 1.08 (d, J = 6.7 Hz), 1.10 m; δ_C 17.8 CH_3, 19.6 CH_3, 33.8 CH, 174.4 qC; δ_H 2.64 m, 1.10m, 1.21 (d, J = 7.1 Hz); δ_C 18.3 CH_3, 20.1 CH_3, 33.9 CH, 177.8 qC], and a β-furanyl ring [δ_H 6.38 (d, J = 0.9 Hz), 7.39 (t, J = 1.6 Hz), 7.53 br s; δ_C 109.9 CH, 120.8 qC, 141.6 CH, 143.0 CH]. A δ-lactone ring D characterized by NMR data [δ_H 5.19 s, 2.73 m, 3.14 (d, J = 19.7 Hz), 2.22 m; δ_C 77.1 CH, 29.0 CH_2, 46.4 CH, 36.2 qC, 169.7 qC], was corroborated by HMBC correlations between H-17/C13, H-17/C14, H-14/C13, H-14/C15, H-14/C16, H-15/C13, H-15/C14, H-15/C16. The 1D and 2D NMR data strongly suggested that **3** was a mexicanolide type limonoid. Protons of a tertiary methyl group [δ_H 104 s; δ_C 22.2 CH_3] showing HMBC correlations to C-13, C-14 and C-17, were assigned to H_3-18. Protons of the second tertiary methyl group [δ_H 1.12 m; δ_C 21.0 CH_3], exhibiting HMBC correlation to C-1 and C-9, were identified as H_3-19. Protons of the third tertiary methyl group [δ_H 0.73 s; δ_C 24.2 CH_3], displaying HMBC correlations to C-3, C-4, C-5 and C-29, were indicated as H_3-28, and those of the fourth tertiary methyl group [δ_H 1.24 s; δ_C 22.1 CH_3], showing HMBC correlations to C-3, C-4, C-5 and C-8, were assigned to H_3-29. The singlet oxymethine proton at δ_H 4.85 was assigned to H-3 through HMBC correlations from this proton to the carbons at C-2, C-4, C-5 and C-1″. In addition, the proton at δ_H 6.18 (δ_C 75.6 CH) showing HMBC correlations to C-1, C-3, C-9 and C-1′, were indicated as H-30. The remaining quaternary carbon at δ_C 107.2 was attributed to C-1, a hemiketal group related to that of **1**. Two isobutyryl groups were located at C-3 and C-30 through HMBC correlatins of H-3 to a carbonyl at δ_C 177.8 and of H-30 to another carbonyl at δ_C 177.8. The relative stereochemistry of **3** was established by analysis of 1D NOE data. The significant NOE interactions (Figure 4.10) observed from H-30 to H-5 and H-17 helped to establish this 30β-H and the corresponding 30α-isobutyl group. Moreover, the lack of NOE interaction from H-30 to H-3

indicated α-orientation of H-3. Therefore, the structure of **3**, named xylorumphiin A, was established as shown in Figure 4.8.

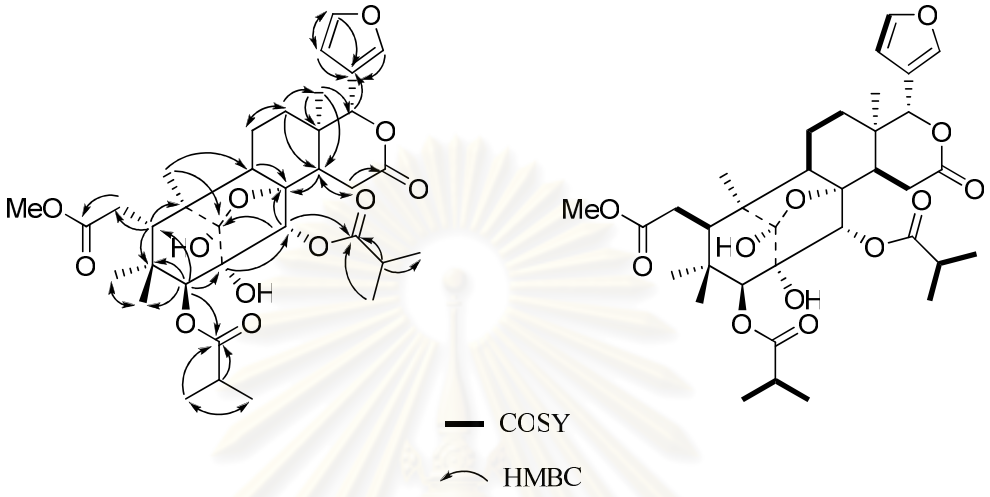


Figure 4.9 Key HMBC and COSY correlations of compound **3**

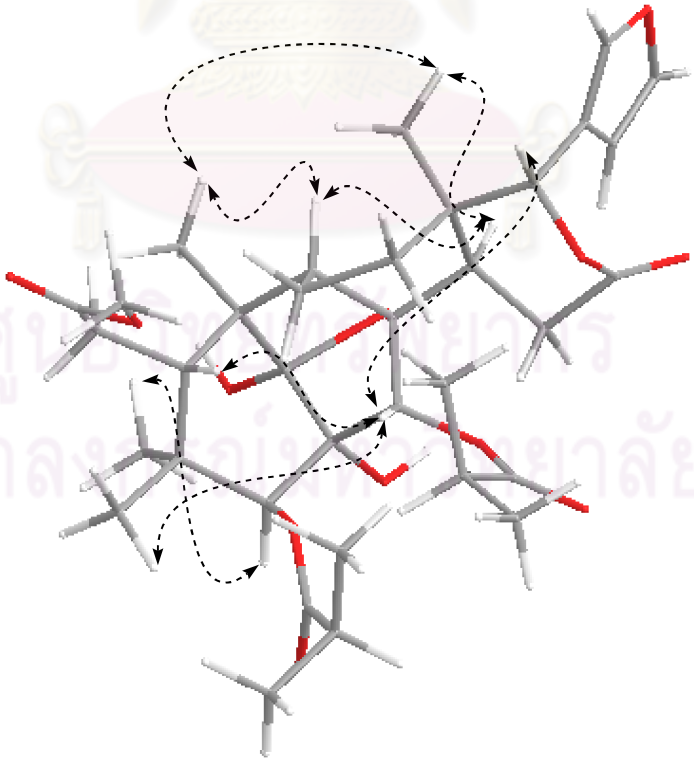


Figure 4.10 Key NOE correlations of compound **3**

Table 4.3. The NMR data of compound **3**

Position	^{1}H	^{13}C	COSY	HMBC
1		107.2		
2		82.2		
3	4.85 (s, 1H)	80.6		C-2, C-4, C-5, C-30, C-29, C-1′
4		38.9		
5	2.63 (m, 1H)	40.3	H-6a, H-6b	C-4, C-6, C-10
6	2.33 (m, 1H) 2.27 (m, 1H)	32.3	H-5	C-4, C-7
7		173.9		
8		81.0		
9	1.48 (m, 1H)	63.2	H-11a, H-11b	C-5, C-8, C-10, C-14, C-30
10		42.6		
11	1.88 (m, 1H) 1.68 (m, 1H)	19.7	H-9, H-12a, H-12b	C-12, C-13
12	1.83 (m, 1H) 1.32 (m, 1H)	35.8	H-11a, H-11b	C-11, C-13, C-14, C-18
13		36.2		
14	2.22 (m, 1H)	46.4	H-15a, H-15b	C-8, C-9, C-13, C-15, C-16, C-17, C-18, C-30
15	3.14 (d, *J* = 19.7 Hz, 1H) 2.73 (m, 1H)	29.0	H-14	C-13, C-14, C-8, C-16
16		169.7		
17	5.19 (s, 1H),	77.1		C-18, C-13, C-14, C-20, C-21, C-23
18	1.04 (s, 3H)	22.2		C-13, C-14, C-17
19	1.12 (m, 3H)	21.0		C-9, C-1
20		120.8		
21	7.53 (s, 1H),	141.6		C-22, C-20, C-23
22	6.38 (d, *J* = 0.9 Hz, 1H),	109.9	H-23	C-20, C-21
23	7.39 (t, *J* = 1.6 Hz, 1H),	143.0	H-22	C-20, C-21
28	0.73 (s, 3H)	24.2		C-29, C-4, C-5, C-3
29	1.24 (s, 3H)	22.1		C-28, C-4, C-5, C-3
30	6.18 (s, 1H),	75.6		C-9, C-3, C-1, C-1″
3-*Acyl*				
1′		177.8		
2′	2.64 (m, 1H)	33.9	H-3′, H-4′	C-1′, C-3′
3′	1.10 (m, 3H)	18.3	H-2′	C-2′, C-4′
4′	1.22 (d, *J* = 7.1 Hz, 3H)	20.1	H-2′	C-1′, C-2′, C-3′
30-*Acyl*				
1″		174.4		
2″	2.64 (m, 1H)	33.8	H-3″, H-4″	C-1″, C-4″
3″	1.10 (m, 3H)	19.6	H-2″	C-1″, C-2″, C-4″
4″	1.08 (d, J= 6.7 Hz, 3H)	17.8	H-2″	C-2″ C-3″
7-*OMe*	3.69 (s, 3H),	51.9		C-7

4.1.4 Structure elucidation of compound 4

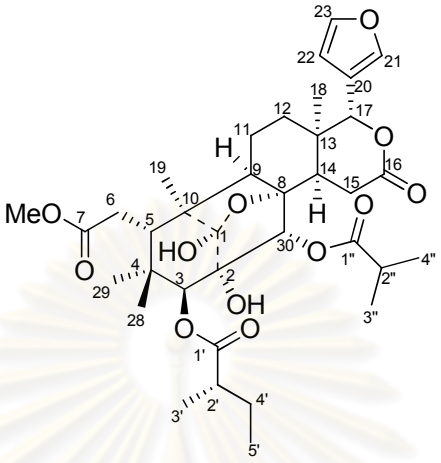


Figure 4.11 Compound **4**

Molecular formula	$C_{36}H_{50}O_{12}$
Appearance	White amorphous solid
m.p.	115.5-117.5 ^{o}C
$[\alpha]_{D}^{20}$	-38 (*c* 0.1 $CHCl_3$)
UV ($CHCl_3$) λ_{max} (log ε)	240.9 nm (2.66)
IR (KBr)	3445, 2965, 2934, 2878, 2356, 2330, 1730, 1630, 1460, 1386, 1291, 1195 and 1147 cm^{-1}
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 4.4
HRESIMS *m/z*	697.3194 $[M+Na]^{+}$, calcd. 697.3200

Compound **4** had a molecular formula of $C_{36}H_{50}O_{12}$ as established by the HRESIMS (*m/z* 697.3194 $[M+Na]^+$, calcd. 697.3200). The NMR data (Table 4.4) of **4** and its 2D NMR studies (^1H-^1H COSY, HSQC, HMBC) indicated the presence of a methoxy carbonyl group [(δ_H 3.68 s, δ_C 51.9 CH_3, 173.8 qC)], a 2-methyl butyryl group [δ_H 0.89 (t, *J* = 7.5 Hz), 1.41 m, 1.67 m, 1.20 (d, *J* = 7.1 Hz), 2.37 m; δ_C 11.2 CH_3, 25.3 CH_2, 16.8 CH_2, 40.5 CH, 177.3 qC], an isobutyryl group [(δ_H 1.08 (d, *J* = 6.7 Hz), 1.11 (d, *J* = 7.1 Hz), 2.67 m; δ_C 17.8 CH_3, 19.7 CH_3, 33.7 CH, 174.3 qc)], and a β-furanyl ring [δ_H 7.53 s, 6.38 (d, *J* = 1.0 Hz), 7.39 (t, *J* = 1.7 Hz); δ_C 141.6, 109.9, 143.0, 120.8]. The NMR data of compound **4** were virtually identical to those of **1** with the only difference being the appearance of an additional methine and methylene instead of $\Delta^{14,15}$ double bond in **1**. Both compound **1** and **4** shared the same configuration as confirmed by similarities between the NOE correlations (Figure 4.13). Thus, the structure of **4** named xylorumphiin B, was established as shown in Figure 4.11.

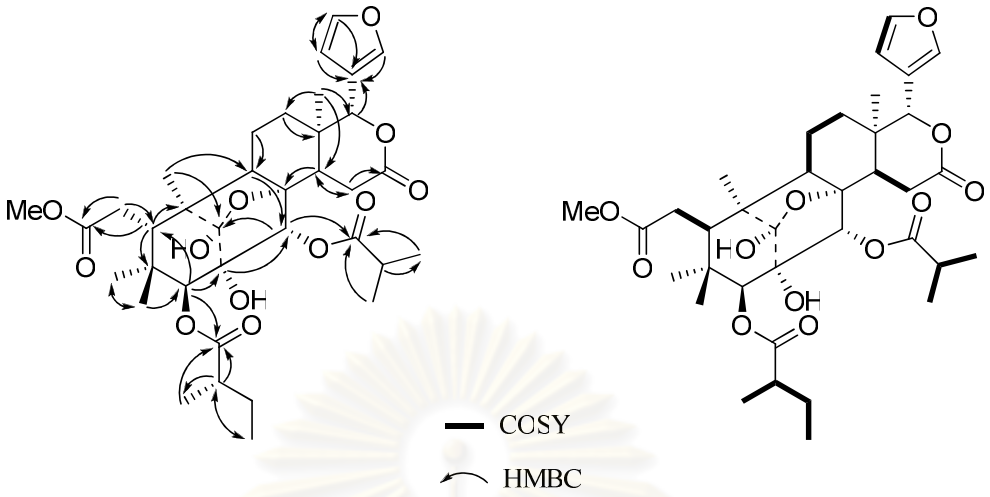


Figure 4.12 Key HMBC and COSY correlations of compound **4**

Figure 4.13 Key NOE correlations of compound **4**

Table 4.4. The NMR data of compound **4**

Position	^{1}H	^{13}C	COSY	HMBC
1		107.2		
2		82.1		
3	4.86 (s, 1H)	80.5		C-2, C-5, C-30, C-1′
4		38.9		
5	2.60 (m, 1H)	40.4	H-6a, H-6b	C-4, C-9, C-7, C-10,
6	2.27 (m, 1H)	32.3	H-5	C-5, C-7
	2.31 (m, 1H)			
7		173.8		
8		81.0		
9	1.48 (m, 1H)	63.2	H-11a, H-11b	C-30
10		42.6		
11	1.67 (m, 1H)	19.5	H-9, H-12a,	C-9, C-14
	1.87 (m, 1H)		H-12b	
12	1.32 (m, 1H)	35.8	H-11a, H-11b	C-13, C-17
	1.83 (m, 1H)			
13		36.2		
14	2.20 (m, 1H)	46.4	H-15a, H-15b	C-8, C-9, C-13, C-15, C-18 C-16, C-20
15	3.14 (d, *J* = 19.7 Hz, 1H)	29.0	H-14	C-8, C-13, C-14, C-16
	2.75 (m, 1H)			
16		169.8		
17	5.19 (s, 1H)	77.1		C-13, C-14, C-18, C-20, C-21, C-22
18	1.03 (s, 3H)	22.1		C-12, C-14, C-17
19	1.10 (s, 3H)	20.9		C-1, C-9
20		120.8		
21	7.53 (s, 1H),	141.6		C-20, C-22, C-23
22	6.38 (d, *J* = 1.0 Hz, 1H)	109.9	H-23	C-20, C-23
23	7.39 (t, *J* = 1.7 Hz, 1H)	143.0	H-22	C-20, C-21
28	0.72 (s, 3H)	24.2		C-3, C-4, C-18
29	1.23 (s, 3H)	22.0		C-3, C-4, C-28
30	6.20 (s, 1H)	75.5		C-1, C-8, C-9, C-1″
3-*Acyl*				
1′		177.3		
2′	2.37 (m, 1H)	40.5	H-3′, H-4a′, H-4b′	C-1', C-4′
3′	1.20 (d, *J* = 7.1 Hz, 3H)	16.8	H-2′	C-1′, C-2′, C -4′,
4′	1.41 (m, 1H)	25.3	H-2′, H-5′	C-1′,C-2′, C 5′, C-3′,
	1.67 (m, 1H)			
5′	0.89 (t, *J* = 7.5 Hz, 3H)	11.2	H-4a′, H-4b′	C-2′,,C-4′
30-*Acyl*				
1″		174.3		
2″	2.67 (m, 1H)	33.7	H-3′, H-4′	C-1", C-3″
3″	1.11 (d, J = 7.1 Hz, 3H)	19.7	H-2′	C-1", C-2″, C -4″
4″	1.08 (d, J = 6.7 Hz, 3H)	17.8	H-2′	C-1", C-2″
7-*OMe*	3.68 (s, 3H)	51.9		C-7

4.1.5 Structure elucidation of compound 5

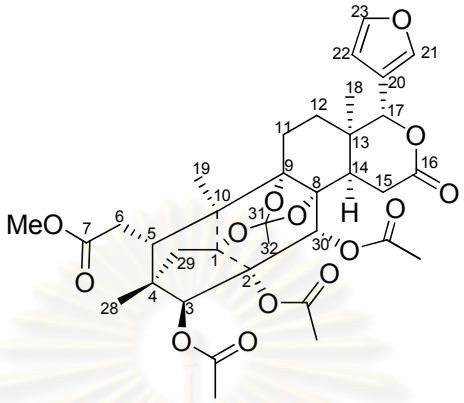


Figure 4.14 Compound **5**

Molecular formula	$C_{35}H_{42}O_{14}$
Appearance	Colorless crystals
m.p.	141.5-143.5 ^{o}C
$[\alpha]_{D}^{20}$	-50 (*c* 0.1 $CHCl_3$)
UV ($CHCl_3$) λ_{max} (log ε)	239.1 nm (2.70)
IR (KBr)	3531, 3469, 2959, 1747, 1366, 1237, 1094, 1056 and 1022 cm^{-1}
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 4.6
ESIMS *m/z*	687.27 $[M+H]^{+}$, calcd. 687.26

Compound **5**, colorless crystals, had a molecular formula $C_{35}H_{42}O_{14}$ determined by ESIMS (*m/z* 687.27 $[M+H]^+$, calcd. 687.26). The ^1H, ^{13}C data and 2D NMR data (Table 4.6) of **5** indicated the presence of the following functional groups; a carbomethoxy [δ_H 3.69 s, δ_C 52.1 CH_3, 172.7 qC], three oxygenated methines [δ_H 5.10 s, 6.30 s, 5.54 s; δ_C 81.1 CH, 69.3 CH, 78.6 CH], an orthoacetate [δ_H 1.66 s; δ_C 21.0 CH_3, 119.0 qC], two sp^3 methines [δ_H 2.96 (d, J = 8.5 Hz), 2.06 m; δ_C 35.5, 43.1], ten sp^3 methylenes [δ_H 2.47 m, 2.24 m, 2.07 m, 1.65 m, 1.54 m, 1.30 m, 3.28 (d, J = 20.3 Hz), 2.70 m, 1.98 m, 1.67 m; δ_C 33.3, 25.4, 29.1, 26.5, 40.2], three methyls [δ_H 1.06 s, 1.14 s, 0.89 s; δ_C 19.9, 16.5, 14.6], three acetyls [δ_H 1.94 s, 2.25 s, 2.15 s; δ_C 21.6 CH_3, 168.6 qC; 21.1 CH_3, 170.2 qC; 21.1 CH_3, 170.2 qC]. The ^1H and ^{13}C NMR data of **5** were characteristic of a phragmalin type limonoid. The quaternary carbon at δ_C 119.0 (C-31) showing a HMBC correlation (Figure 4.15) to H-32 suggested the presence of an orthoacetate group. In addition, the nature of oxygenated carbons assigned for C-1 (δ_C 86.9), C-8 (δ_C 86.0) and C-9 (δ_C 85.3) was comparable to xyloccensin E. This suggested the position of the orthoacetate at C-1, C-8 and C-9. Three acetoxy groups were assigned to C-2, C-3 and C-30 according to HMBC correlations of H-3 and H-30 to both acetyl carbonyls and its molecular formula. The structure and relative stereochemistry of **5** were confirmed by the single-crystal X-ray diffraction analysis as shown in Figure 4.16. In addition, the crystal data and structure refinement for compound **5** are shown in Table 4.5.

From these results, the structure of **5** was identified as xyloccensin E (Connolly *et al.*, 1978). Furhtermore, this is the first report for the complete assignment of NMR data for xyloccensin E.

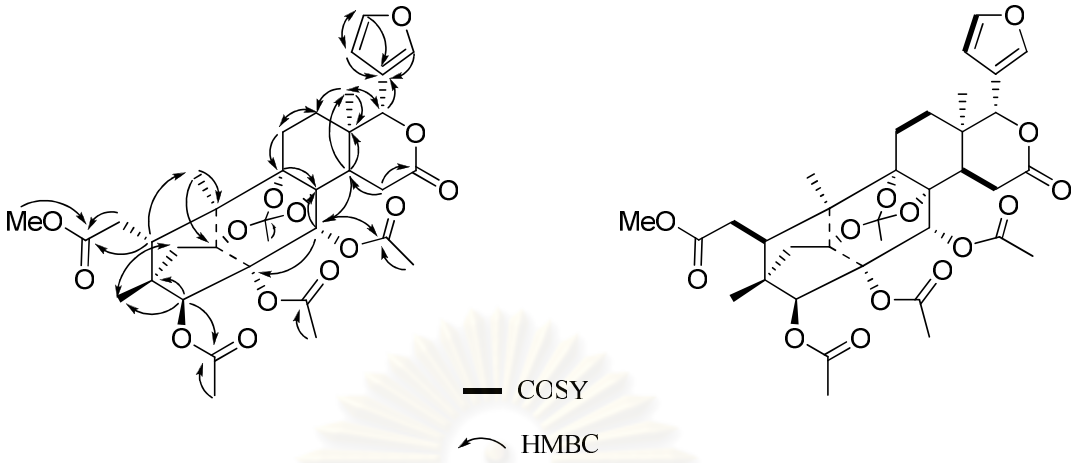


Figure 4.15 Key HMBC and COSY correlations of compound **5**

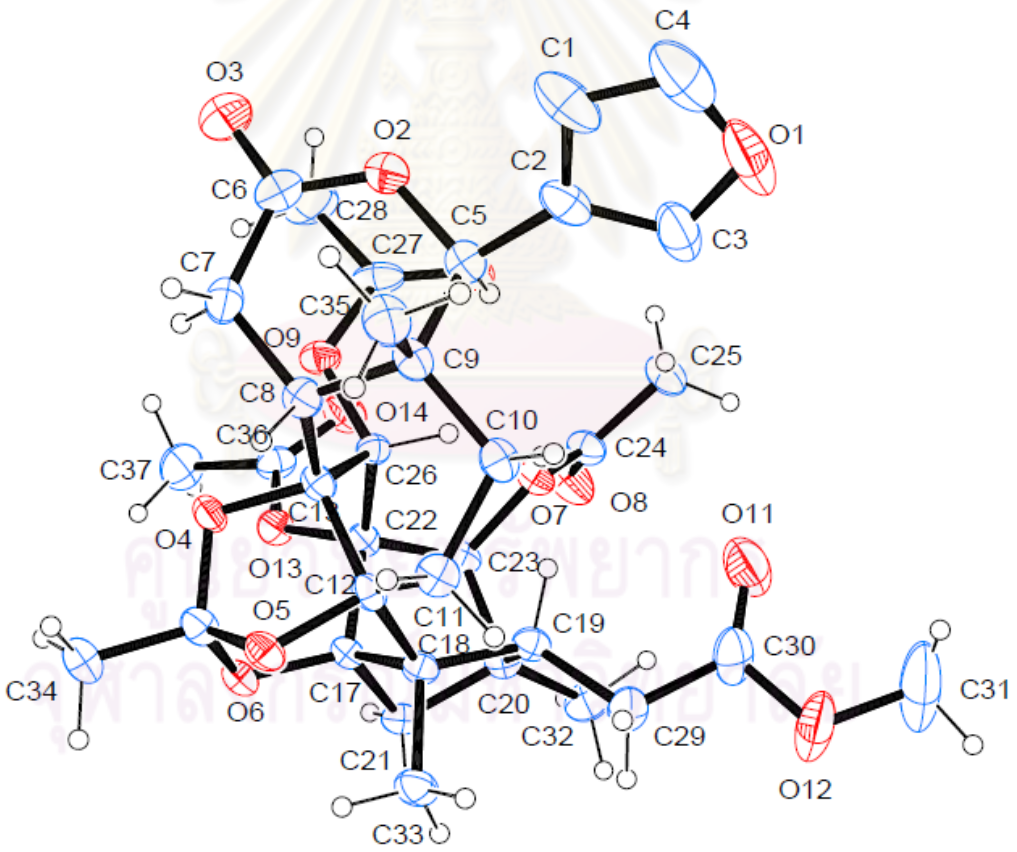


Figure 4.16 ORTEP diagram of compound **5**

Table 4.5. Crystal data and structure refinement for compound **5**

Identification code	Xyloccensin E
Empirical formula	C_{35} H_{42} O_{14}
Formula weight	686.69
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	hexagonal, P6
Unit cell dimensions	a = 17.8937(4) Å alpha = 90 deg.
	b = 17.8937(4) Å beta = 90 deg.
	c = 19.7758(4) Å gamma = 120 deg.
Volume	5483.6(2) $Å^3$
Z, Calculated density	6, 1.248 Mg/m^3
Absorption coefficient	0.097 mm^{-1}
F(000)	2184
Crystal size	? x ? x ? mm
Theta range for data collection	2.44 to 23.83 deg.
Limiting indices	-16<=h<=20, -20<=k<=20,-20<=l<=22
Reflections collected / unique	26863 / 2879 [R_{int} = 00295]
Completeness to theta = 23.83	98.70 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2879 / 1 / 456
Goodness-of-fit on F^2	1.029
Final R indices [I>2sigma(I)]	R_1 = 0.0420, *wR2* = 0.1115
R indices (all data)	R_1 = 0.0506, *wR2* = 0.1191
Absolute structure parameter	-10(10)
Largest diff. peak and hole	0.412 and -0.172 $e.Å^3$

Table 4.6. The ^{1}H and ^{13}C NMR data of xyloccensin E and compound **5**

Position	Xyloccensin E		Compound **5**	
	^{1}H	^{13}C	^{1}H	^{13}C
1		86.9		86.8
2		85.3		85.2
3	5.09	81.1	5.10 (s, 1H)	81.1
4		46.2		46.2
5		35.5	2.96 (d, *J* = 8.5 Hz, 1H)	35.5
6		33.3	2.47 (m, 1H)	33.3
			2.24 (m, 1H)	
7		172.9		172.7
8		86.0		85.9
9		85.3		85.3
10		45.8		45.7
11		25.4	2.07 (m, 1H)	25.4
			1.66 (m, 1H)	
12		29.2	1.54 (m, 1H)	29.1
			1.30 (m, 1H)	
13		34.4		34.3
14		43.2	2.06 (m, 1H)	43.1
15		26.2	3.28 (d, *J* = 20.3 Hz, 1H)	26.5
			2.70 (m, 1H)	
16		170.3		170.4
17	5.53	78.6	5.54 (s, 1H)	78.6
18		19.6	1.06 (s, 3H)	19.9
19		16.6	1.14 (s, 3H)	16.5
20		121.2		121.1
21	7.50	140.8	7.51 (s, 1H)	140.8
22	6.42	109.8	6.44 (s, 3H)	109.7
23	7.38	143.0	7.40 (s, 1H)	143.0
28		14.6	0.89 (s, 3H)	14.6
29		40.2	1.98 (m, 1H)	40.2
			1.67 (m, 1H)	
30	6.29	69.3	6.30 (s, 1H)	69.3
31		119.1		119.0
32		21.1	1.66 (s, 3H)	21.0
2-*OAc*		21.1	2.25 (s, 3H)	21.1
		170.3		170.2
3-*OAc*		21.7	2.15 (s, 3H)	21.7
		170.3		170.2
30-*OAc*		21.7	1.94 (s, 3H)	21.6
		168.6		168.6
7-*OMe*		52.1	3.69 (s, 3H)	52.1

4.1.6 Structure elucidation of compound 6

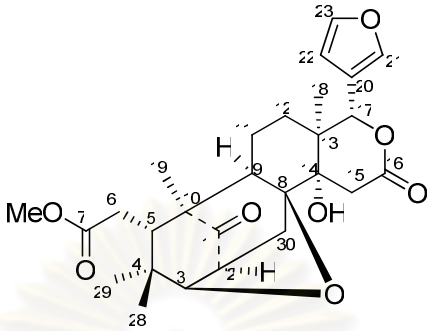


Figure 4.17 Compound **6**

Molecular formula	$C_{27}H_{34}O_8$
Appearance	White amorphous solid
m.p.	236.5-238.5 ^{o}C
$[\alpha]_D^{20}$	-12 (*c* 0.1 $CHCl_3$)
UV ($CHCl_3$) λ_{max} (log ε)	242 nm (2.76)
IR (KBr)	3531, 3445, 3469, 2968, 2959, 2358, 2329, 1747, 1733, 1466, 1375, 1366, 1237, 1170, 1094, 1056, 1027, and 1022 cm^{-1}
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 4.7
ESIMS *m/z*	487.23 $[M+H]^+$, calcd. 487.22

Compound **6** was isolated as a white, amorphous solid and its molecular formula was determined as $C_{27}H_{34}O_8$ on the basis of ESIMS (*m/z* 487.23 $[M+H]^+$, calcd. 487.22) and NMR data (Table 4.7). The ^1H NMR data of **6** exhibited typical signals for four methyls [δ_H 0.66s, 0.94 s, 1.09 s, 0.99 s], two oxymethines [δ_H 6.28 s, 4.22 (d, J = 5.6 Hz)], a methoxy [δ_H 3.69 s], together with a β-furyl ring [δ_H 7.45 br s, 6.49 s, 7.57 (d, J = 0.5 Hz)]. The ^{13}C NMR and HSQC experiment revealed the presence of a ketone carbonyl [δ_C 215.1], two ester carbonyls [δ_C 175.0, 170.3], a β-furyl ring [δ_C 121.0 qC, 141.3 CH, 110.3 CH, 143.3 CH], two oxymethine carbons [δ_C 76.8, 91.7] and two oxygenated quaternary carbons [δ_C 85.8, 74.8]. The above NMR studies suggested that **6** was a mexicanolide type limonoid. On the basis of HMBC correlations (Figure 4.18), oxymethine proton at δ_H 4.22 (d, J = 5.6 Hz) showing correlations with C-8, C-5 and C-29, was attributed to H-3. The oxygen bridge between C-3 and C-8 was corroborated by the HMBC cross-peak from H-3 to H-8. On the basis of the above results, compound **6** was identified as xyloccensin K. The structure of this compound was finally confirmed by comparing its NMR data with those previously reported as shown in Table 4.7 (Kokpol *et al*. 1996). Additionally, its relative stereochemistry was confirmed by NOE analysis (Figure 4.19) and found to be identical to that of xyloccensin K as reported.

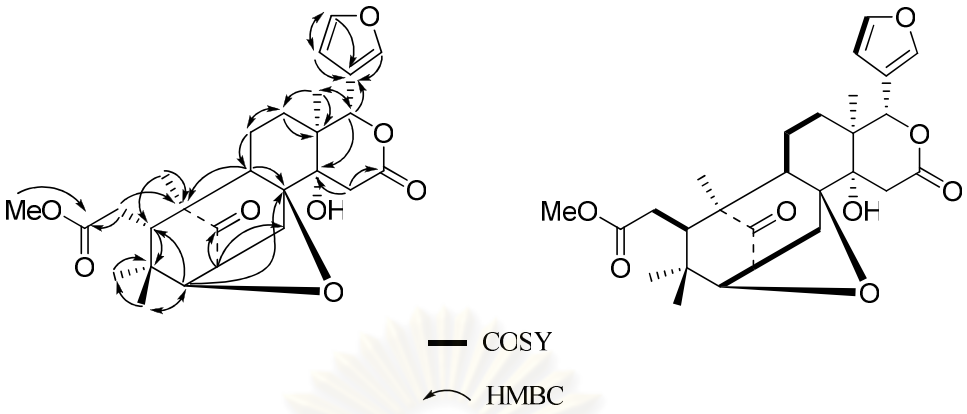


Figure 4.18 Key HMBC and COSY correlations of compound **6**

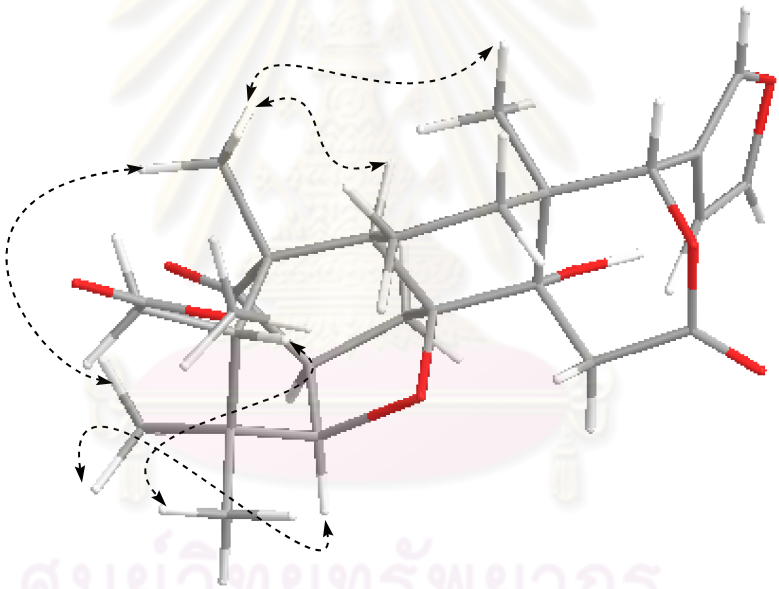


Figure 4.19 Key NOE correlations of compound **6**

Table 4.7. The ^{1}H and ^{13}C NMR data of xyloccensin K and compound **6**

Position	Xyloccensin K		Compound **6**	
	^{1}H	^{13}C	^{1}H	^{13}C
1		215.2		215.1
2	2.96 (dd, *J* = 6.0 Hz, 1H)	48.9	2.97 (t, *J* = 6.0 Hz, 1H)	49.3
3	4.22 (d, *J* = 6.0 Hz, 1H)	91.3	4.22 (d, *J* = 5.6 Hz, 1H)	91.7
4		37.5		37.3
5	3.08 (dd, *J* = 2.0, 11.0, 1H)	42.9	3.07 (m, 1H)	43.3
6	2.14 (dd, *J* = 2.0, 17.0 Hz, 1H) 2.24 (dd, *J* = 11.0, 17.0 Hz, 1H)	32.6	2.11 (m, 1H) 2.23 (m, 1H)	32.9
7		174.2		175.0
8		85.4		85.8
9	1.97 (dd, *J* = 5.0, 12.5 Hz, 1H)	52.0	1.95 (dd, *J* = 12.6, 4.0 Hz, 1H)	52.4
10		51.0		51.5
11	1.46 (m, 1H) 2.10 (m, 1H)	17.8	1.47 (m, 1H) 2.11 (m, 1H)	18.0
12	1.50 (ddd, *J* = 1.5, 14.0 Hz, 1H) 1.70 (ddd, *J* = 1.5, 14.0 Hz, 1H)	28.6	1.53 (m, 1H) 1.69 (m, 1H)	29.1
13		40.0		40.4
14		74.1		74.8
15	2.54 (d, *J* = 17.0 Hz. 1H) 3.13 (d, *J* = 17.0 Hz, 1H)	36.8	2.52 (m, 1H) 3.15 (d, *J* = 17.7 Hz, 1H)	37.4
16		170.7		170.3
17	6.28 (br s, 1H)	76.7	6.28 (s, 1H)	76.8
18	0.67 (s, 3H)	16.0	0.66 (s, 3H)	16.4
19	0.94 (s, 3H)	16.8	0.94 (s, 3H)	17.2
20		120.6		121.0
21	7.45 (dd, *J* = 2.0 Hz, 1H)	142.9	7.45 (br s, 1H)	141.3
22	6.49 (br d, *J* =2.0 Hz, 1H)	109.9	6.49 (s, 1H)	110.3
23	7.56 (br s, 1H)	140.7	7.55 (d, *J* = 0.5 Hz, 1H)	143.3
28	1.03 (s, 3H)	20.0	1.09 (s, 3H)	20.4
29	0.98 (s, 3H)	27.9	0.99 (s, 3H)	28.4
30	2.04 (d, *J* = 12 Hz, 1H) 2.52 (dd, *J* = 7.0,12.0 Hz, 1H)	42.4	2.05 (m, 1H)	42.8
7-*OMe*	3.70 (s, 3H)	51.8	3.69 (s, 3H)	52.2

4.1.7 Structure elucidation of compound 7

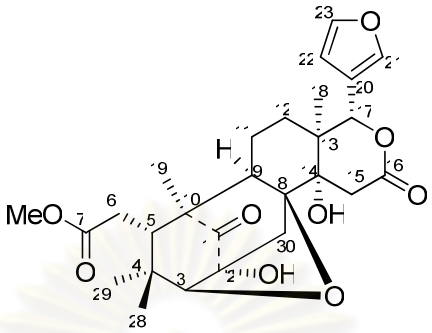


Figure 4.20 Compound **7**

Molecular formula	$C_{27}H_{34}O_9$
Appearance	White amorphous solid
m.p.	208.0-210.0 ^{o}C
$[\alpha]_D^{20}$	+4 (*c* 0.1 $CHCl_3$)
UV ($CHCl_3$) λ_{max} (log ε)	242 nm (2.32)
IR (KBr)	3531, 3469, 3455, 3445, 2968, 2959, 2949, 2372, 2358, 2329, 1747, 1733, 1723, 1466, 1452, 1423, 1375, 1366, 1270, 1237, 1165, 1170, 1099, 1094, 1056, 1041, 1022 and 1027 cm^{-1}
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 4.8
HRESIMS *m/z*	525.2101 $[M+Na]^+$, calcd. 525.2101

Compound **7** had a molecular formula of $C_{27}H_{34}O_9$ established by HRESIMS (*m/z* 525.2101 $[M+Na]^+$, calcd. 525.2101). It was larger than that of xyloccensin K (**6**) by 16 mass units. Its NMR studies also revealed the characteristics for mexicanolide type limonoid including four methyls [δ_H 1.00 s, 1.06 s, 0.61 s, 1.06 s; δ_C 16.2, 28.2, 19.1, 16.8], a β-furyl ring [δ_H 7.52 s, 6.45 s, 7.43 s; δ_C 140.9 CH, 110.0 CH, 143.1 CH, 120.6 qC], a carbomethoxy [δ_H 3.69 s; δ_C 52.0 CH_3, 174.2 qC], a ketone carbonyl (δ_C 214.8) and a lactone carbonyl (δ_C 169.7). In addition, the NMR data of **7** (Table 4.8) were very similar to those of xyloccensin K (**6**), except for the presence of an oxygenated quaternary carbon (δ_C 85.5) and the absence of a methine group in **6**. This quaternary carbon with a hydroxyl group was assigned as C-2 by HMBC correlations of this carbon with H-3 and H-30 (Figure 4.21). The relative configuration of **7** was established as the same as that of **6** on the basis of the NOE correlations (Figure 4.22). Therefore, the structure of **7,** named xylorumphiin D, was identified as 2-hydroxy-xyloccensin E as shown in Figure 4.20.

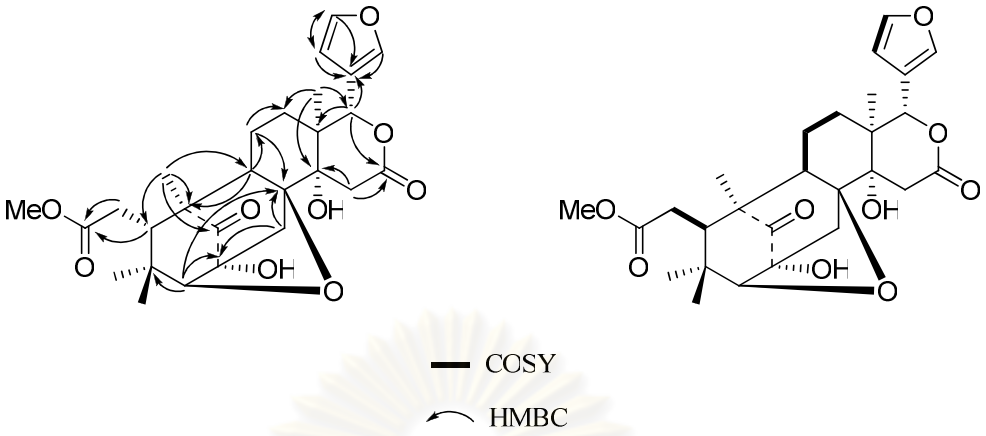


Figure 4.21 Key HMBC and COSY correlations of compound **7**

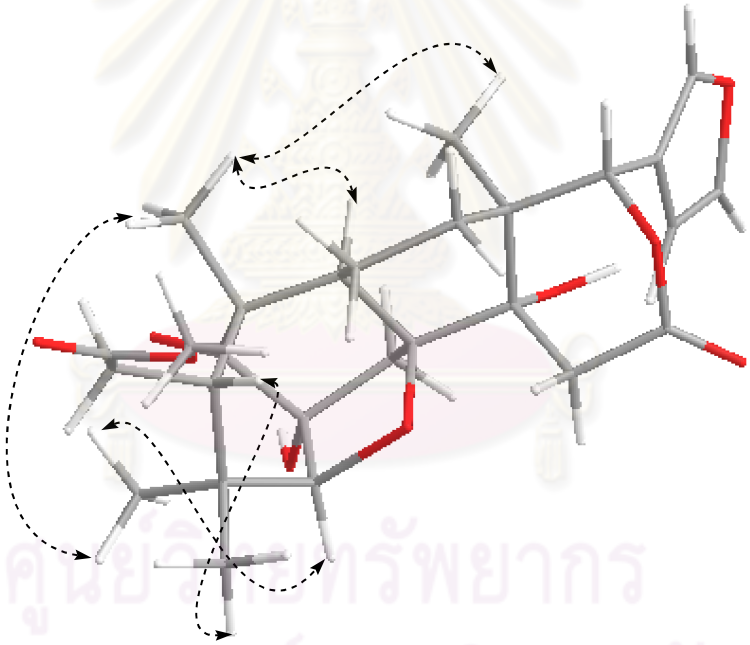


Figure 4.22 Key NOE correlations of compound **7**

Table 4.8. The NMR data of compound **7**

Position	^{1}H	^{13}C	COSY	HMBC
1		214.8		
2		85.5		
3	3.95 (s, 1H)	93.1		C-2, C-8
4		37.2		
5	2.19 (m, 1H)	32.6	H-6a, H-6b	C-7
6	3.11 (dd, *J* = 10.6, 2.2 Hz, 2H)	43.7	H-5	C-2, C-7
7		174.2		
8		80.7		
9	2.00 (m, 1H)	52.3	H-11a, H-11b	C-10, C-11
10		50.2		
11	1.51 (m, 1H) 2.16 (m, 1H)	18.0	H-9, H-12a, H-12b	C-8, C-12
12	1.53 (m, 1H) 1.68 (m, 1H)	28.8	H-11a, H-11b	C-11, C-13
13		40.1		
14		74.4		
15	2.53 (d, *J* = 17.9 Hz, 1H) 3.25 (d, *J* = 17.9 Hz, 1H)	37.5		C-14, C-16
16		169.7		
17	6.16 (s, 1H)	76.4		C-13, C-14, C-18, C-20, C-21, C-22
18	1.00 (s, 3H)	16.2		C-12, C-13, C-14, C-17
19	1.07 (s, 3H)	28.2		
20		120.6		
21	7.52 (s, 1H),	140.9		C-20, C-22, C-23
22	6.45 (s, 1H),	110.0	H-23	C-20, C-21
23	7.42 (m, 1H)	143.1	H-22	C-20
28	0.61 (s, 3H)	19.1		C-3, C-4, C-6, C-19
29	1.06 (s, 3H)	16.8		C-3, C-4, C-6 , C-28, C-30
30	1.96 (d, *J* = 11.9 Hz, 1H) 2.70 (d, *J* = 11.9 Hz, 1H)	48.7		C-2, C-8, C-14
7-*OMe*	3.69 (s, 3H)	52.0		C-7

4.2 Biological activities of isolated compounds

4.2.1 Antibacterial activities

All isolated compounds were evaluated for their antibacterial effects using microdilution assay against seven Gram-positive bacteria; *Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis* ATCC 51299 (vancomycin resistant), *Enterococcus faecium* UCLA 192, *Salmonella typhimurium* ATCC 13311, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus hominis* ATCC 27844 and five Gram-negative bacteria; *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 27736, *Klebsiella pneumoniae* (ESBL producing) ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, at a single concentration of 256 μg/mL for screening. Unfortunately, all compounds showed to be inactive to all tested bacteria.

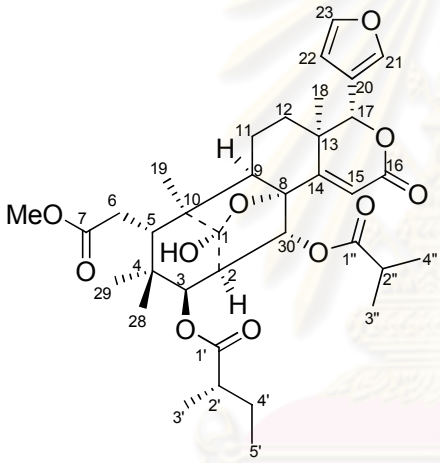
4.2.1 Anticancer activities

All compounds isolated were also assessed for their cytotoxicity toward five human cancer cell lines including Hep-G2 (hepatocarcinoma), SW-620 (colon adenocarcinoma), CHAGO (undifferentiated lung carcinoma), KATO-3 (gastric carcinoma), BT-474 (breast ductal carcinoma) and CH-Liver (liver cell line), at a concentration of 1 mg/mL by MTT colorimetric method. All compounds were not cytotoxic to any of the cell lines tested.

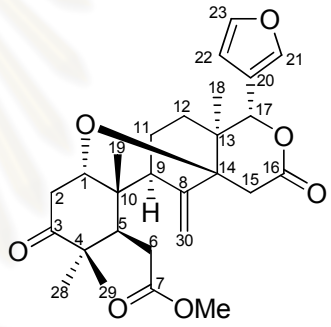
CHAPTER V

CONCLUSION

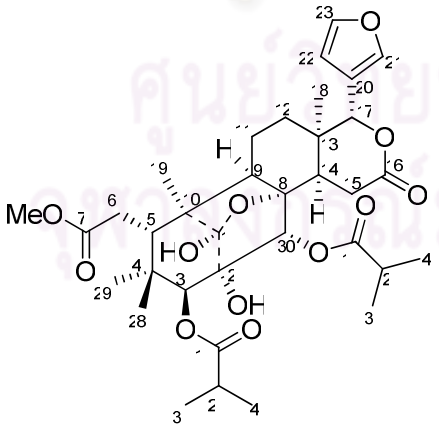
Chemical examination of the seed kernels of *Xylocarpus rumphii* (Kostel.) Mabb. led to the isolation of seven limonoids. These isolated compounds included four new limonoids, xylorumphiins A-D (Compound **3**, **4**, **1** and **7**), and three known limonoids namely methyl angolensate (compound **2**), xyloccensins E (compound **5**) and K (compound **6**).



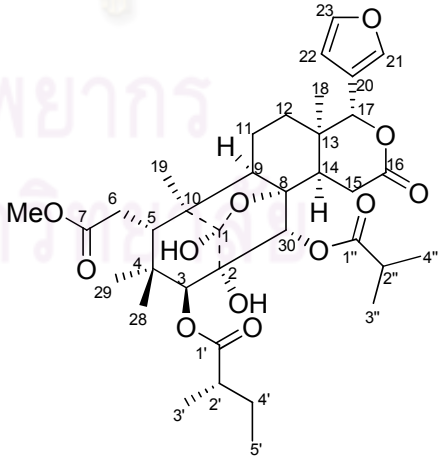
Compound **1**



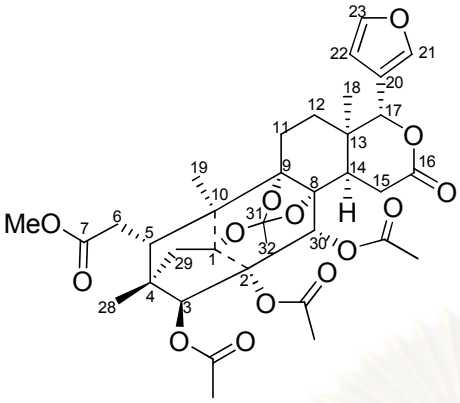
Compound **2**



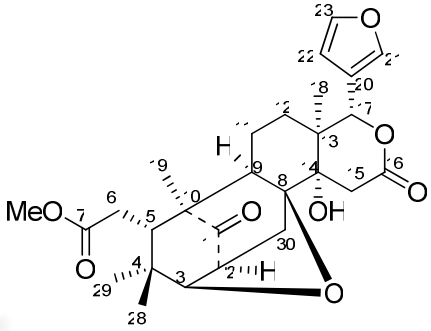
Compound **3**



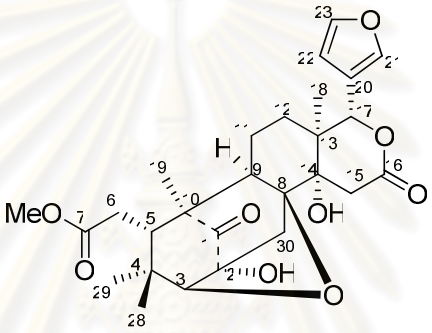
Compound **4**



Compound **5**



Compound **6**



Compound **7**

The isolated compounds were subjected to antibacterial and anticancer activity assays. Unfortunately, all of them showed to be inactive in both assays.

As can be seen in the activity results, all isolated compounds did not exhibit both antibacterial and anticancer activity, this might be because these activity are not appropriate for this type compound. However, the compounds should be further subjected to the insecticidal and anti-inflammatory according to the literature reviews.

REFERENCES

Abdelgaleil, S. A. M., Iwagawa, T., Doe, M. and Nakatani, M. 2004. Antifungal limonoids from the fruits of *Khaya senegalensis*. Fitoterapia. 75: 566-572.

Abdelgaleil, S. A. M., Hashinaga, F. and Nakatani, M. 2005. Antifungal activity of limonoids from *Khaya ivorensis*. Pest. Manag. Sci. 61: 186-190.

Ahmed, F. R., Ng, A. S. and Fallis, A. G. 1978. 7α-Acetoxydihydronomilin: isolation, spectra, and crystal structure. Can. J. Chem. 56: 1020-1025.

Akisanya, A., Bevan, C. W. L., Halsall, T. G., Powell, J. W. and Taylor, D. A. H. 1961. West african timbers. Part IV. Some reactions of gedunin. J. Chem. Soc. (resumed): 3705 - 3708.

Akudugu, J., Gade, G. and Bohm, L. 2001. Cytotoxicity of azadirachtin A in human glioblastoma cell lines. Life Sci. 68: 1153-1160.

Alche, L. E., Ferek, G. A., Meo, M., Coto, C. E. and Maier, M. S. 2003. An antiviral meliacarpin from leaves of *Melia azedarach* L. J Biosci. 58: 215-219.

Aliero, B. L. 2003. Larvaecidal effects of aqueous extracts of *Azadirachta indica* (neem) on the larvae of *Anopheles mosquito.* Afr. J. Biotechnol. 2: 325-327.

Alvi, K. A., Crews, P., Aalbersberg, B. and Prasad, R. 1991. Limonoids from the Fijian medicinal plant Dabi (*Xylocarpus*). Tetrahedron. 47: 8943-8948.

Arisdason, W., Magesh, C. R. and Venu, P. 2008. The Genus *Xylocarpus* J. Koeing (Meliaceae) in India. Rheedea. 18: 43-52.

Bandaranayake, W. M. 1998. Traditional and medicinal uses of mangroves. Mangroves and Salt Marshes. 2: 133-148.

Bandarnayake, W. M. 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecol. Manage. 10: 421-452.

Banerji, B. and Nigam, S. K. 1984. Wood constituents of Meliaceae: A review Fitoterapia. 55: 3-36.

Battinelli, L., Mengoni, F., Lichtner, M., Mazzanti, G., Saija, A., Mastroianni, C. M. and Vullo, V. 2003. Effect of Limonin and Nomilin on HIV-1 Replication on Infected Human Mononuclear Cells. Planta Med. 69: 910-913.

Biavatti, M. W., Vieira, P. C., Da-Silva, M. F. G. F., Fernandes, J. B. and Albuquerque, S. Limonoids from the endemic Brazilian species *Raulinoa echinata*. J. Biosci. 56: 570-574.

Bickii, J., Njifutie, N., Ayafor-Foyere, J., Basco, L. K. and Ringwald, P. 2000. *In vitro* antimalarial activity of limonoids from *Khaya grandifoliola* C.D.C. (Meliaceae). J. Ethnopharmacol. 69: 27-33.

Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D. and Mitchell, J. B. 1987. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res. 47: 936-942.

Carpinella, M. C., Defago, M. T., Valladares, G. and Palacios, S. M. 2003. Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management. J. Agric. Food Chem. 51: 369-374.

Carpinella, C., Ferrayoli, C., Valladares, G., Defago, M. and Palacios, S. 2002. Potent limonoid insect antifeedant from *Melia azedarach*. Biosci. Biotechnol. Biochem. 66: 1731-1736.

Castellanos, L., De Correa, R. S., Martinez, E. and Calderon, J. S. 2002. Oleanane triterpenoids from *Cedrela montana* (Meliaceae). J. Biosci. 57: 575-578.

Cespedes, C. L., Calderon, J. S., Salazar, J. R., Lotina-Hennsen, B. and Segura, R. 2001. Plant-growth inhibitory activity of cedrelanolide from *Cedrela salvadorensis*. J. Chem. Ecol. 27: 137-149.

Champagne, D. E., Koul, O., Isman, M. B., Scudder, G. G. E. and Neil Towers, G. H. 1992. Biological activity of limonoids from the rutales. Phytochemistry. 31: 377-394.

Cheng, F., Zhou, Y., Wu, J. and Zou, K. 2006. Xyloccensins X_1 and X_2, two new mexicanolides from the fruit of a Chinese mangrove *Xylocarpus granatum* J. Chem. Sci. 61: 626-628.

Chiaroni, A., Riche, C., Khuong-Huu, Q., Nguyen-Ngoc, H., Nguyen-Viet, K. and Khuong-Huu, F. 2000. New limonoids from *Harrisonia perforata* (Blanco) Merr. Acta. Crystallogr. C. 56: 711-713.

Chopra, R. N., Nayar, S. L. and Chopra, I. C. 1956. Glossary of Indian Medicinal Plants. Ed.V, New Delhi: CSIR.

Chou, F. Y., Hosttmann, K., Kubo, I., Nakanishi, K. and Taniguchi, M. 1977. Isolation of an insect antifeedant *N*-methylflindersine and several benz[c]phenanthridine alkaloids from East African plants; A comment on chelerythrine Heterocycle. 7: 969-977.

Choudhary, S., Sree, A., Mukherjee, S. C., Patnaik, P. and Bapuji, M. 2005. *In Vitro* Antibacterial Activity of Extracts of Selected Marine Algae and Mangroves against Fish Pathogens. Asian Fisheries Sci. 18: 285.

Connolly, J. D., Labbe, C. and Rycroft, D. S. 1978. Tetranortriterpenoids and Ralated Substance, Part 20: New Tetranortriterpenoids from the Seeds of *Chukrasia tabularis* (Meliaceae); Simple Esters of Phragmalin and 12α-Acetoxyphragmalin. J. Chem. Soc. 1: 285-288.

Connolly, J. D., MacLellan, M., Okorie, D. A. and Taylor, D. A. H. 1976. Limonoids from *Xylocarpus moluccensis* (Lam.) M. Roem. J. Chem. Soc. 1: 1993-1996.

Coombes, P. H., Mulholland, D. A. and Randrianarivelojosia, M. 2003. Phragmalin limonoids from the Madagascan Meliaceae *Neobeguea leandreana*. J. Nat. Prod. 66: 735-738.

Coombes, P. H., Mulholland, D. A. and Randrianarivelojosia, M. 2004. Quivisianthone, an evodulone limonoid from the Madagascan Meliaceae *Quivisia papinae*. Phytochemistry. 65: 377-380.

Coombes, P. H., Mulholland, D. A. and Randrianarivelojosia, M. 2005. Mexicanolide limonoids from the Madagascan Meliaceae *Quivisia papinae*. Phytochemistry. 66: 1100-1107.

Cortez, D. A. G., Fernandes, J. B., Vieira, P. C., Fatima-Das G. F., Da-Silva, M. and Ferreira, A. G. 2000. A limonoid from *Trichilia estipulata*. Phytochemistry. 55: 711-713.

Cui, J., Deng, Z., Li, J., Fu, H., Proksch, P. and Lin, W. 2005. Phragmalin-type limonoids from the mangrove plant *Xylocarpus granatum.* Phytochemistry. 66: 2334-2339.

Cui, J., Deng, Z., Xu, M., Proksch, P., Li, Q. and Lin, W. 2009. Protolimonoids and limonoids from the chinese mangrove plant *Xylocarpus granatum.* Helv. Chem. Acta. 92: 139-150.

Cui, J., Wu, J., Deng, Z., Proksch, P. and Lin, W. 2007. Xylocarpins A-I, limonoids from the Chinese mangrove plant *Xylocarpus granatum.* J. Nat. Prod. 70: 772-778.

Donnelly, A. C. Marine Natural Products as Anticancer Agents: Therapeutic Treasures from the Deep. Nat. Prod. Res. [Online]. 2009. Available from : http://www.organicdivision.org/ama/orig/Fellowship/2009_2010_Awardees/Essays/Donnelly.pdf [2009, April 26]

Doyle, A. and Griffiths, J. B. 1997. Mammalian Cell Culture: Essential Techniques, John Wiley and Sons, New York.

Du, S., Wang, M., Zhu, W. and Qin, Z. 2009. A new fungicidal lactone from *Xylocarpus granatum* (Meliaceae). Nat. Prod. Res. 23: 1316-1321.

El-Shemy, H. A., Aboul-Enein, A. M., Aboul-Enein, K. M. and Fujita, K. 2007. Willow Leaves Extracts Contain Anti-Tumor Agents Effective against Three Cell Types. PLoS One. 2: 178.

Endo, T., Kita, M., Shimada, T., Moriguchi, T., Hidaka, T., Matsumoto, R., Hasegawa, S. and Omura, M. 2002. Modification of Limonoid Metabolism in Suspension Cell Cultures of Citrus. Plant Biotechnol. 19: 397-403.

Germano, M. P., D Angelo, V., Sanogo, R., Catania, S., Alma, R., Pasquale, R. D. and Bisignano, G. 2005. Hepatoprotective and antibacterial effects of extracts from *Trichilia emetica* Vahl. (Meliaceae). J. Ethnopharmacol. 96: 227-232.

Govindachari, T. R., Suresh, G., Banumathy, B., Masilamani, S., Gopalakrishnan, G. and Krishna Kumari, G. N. 1999. Antifungal activity of some B,D-*seco* limonoids from two meliaceous plants. J. Chem. Ecol. 25: 923-933.

Govindachari, T. R., Suresh, G., Gopalakrishnan, G., Masilamani, S. and Banumathi, B. 2000. Antifungal activity of some tetranortriterpenoids. Fitoterapia. 71: 317-320.

Hallur, G., Sivramakrishnan, A. and Bhat, S. V. 2002. Three new tetranortriterpenoids from neem seed oil. J. Nat. Prod. 65: 1177-1179.

Han, S. S., Keum, Y. S., Seo, H. J. and Surh, J. 2002. Curcumin suppresses activation of NF-*k*B and AP-1 induced by phorbol ester in cultured human promyelocytic Leukemia cells. J. Biochem. Mol. Biol. 35: 337–342.

Ismail, I. S., Ito, H., Hatano, T., Taniguchi, S. and Yoshida, T. 2003. Modified limonoids from the leaves of *Sandoricum koetjape*. Phytochemistry. 64: 1345-1349.

Ismail, I. S., Ito, H., Hatano, T., Taniguchi, S. and Yoshida, T. 2004. Two new analogues of trijugin-type limonoids from the leaves of *Sandoricum koetjape*. Chem. Pharm. Bull. 52: 1145-1147.

Jacob, R., Hasegawa, S. and Manners, G. 2000. The potential of Citrus Limonoids as anticancer agents. Perishables Handling. 102: 6-8.

Jung, H., Sok, D. E., Kim, Y., Min, B., Lee, J. and Bae, K. 2000. Potentiating effect of obacunone from *Dictamnus dasycarpus* on cytotoxicity of microtuble inhibitors, vincristine, vinblastine and taxol. Planta Med. 66: 74-76.

Kadota, S., Marpaung, L., Kikuchi, T. and Ekimoto, H. 1990. Constituents of the Seeds of *Swietenia mahogany* JACQ. I. Isolation , Structures, and ^{1}H- and ^{13}C-Nuclear Magnetic Resonance Signal Assignments of New Tetranortriterpenoids Related to Swietenine and Swietenolide. Chem. Pharm. Bull. 38: 639-651.

Kayser, O., Kiderlen, A. F. and Croft, S. L. 2003. Natural products as antiparasitic drugs. Parasitol. Res. 90: 55-62.

Khalil, A. T., Maatooq, G. T. and El-Sayed, K. A. 2003. Limonoids from *Citrus reticulate*. J. Biosci. 58: 165-170.

Khuong-Huu, Q., Chiaroni, A., Riche, C., Nguyen-Ngoc, H., Nguyen-Viet, K. and Khuong-Huu, F. 2001. New rearranged limonoids from *Harrisonia perforata*. III. J. Nat. Prod. 64: 634-637.

Kokpol, U., Chavasiri, W., Tip-pyang, S., Veerachato, G., Zhao, F., Simpson, J. and Weavers, R. T. 1996. A limonoid from *Xylocarpus granatum.* Phytochemistry. 41: 903-905.

Koul, O., Singh, G., Singh, R., Singh, J., Daniewski, W. M. and Berlozecki, S. 2004. Bioefficacy and mode-of-action of some limonoids of salannin group from *Azadirachta indica* A. Juss and their role in a multicomponent system against lepidopteran larvae. J. Biosci. 29: 409-416.

Koul, O., Multani, J. S., Singh, G. and Wahab, S. 2002. Bioefficacy of toosendanin from *Melia dubia* (syn. *M. azedarach*) against gram pod-borer, *Helicoverpa armigera* (Hubner). Curr. Sci. 83: 1387-1391.

Krief, S., Martin, M. T., Grellier, P., Kasenene, J. and Sevenet, T. 2004. Novel antimalarial compounds isolated in a survey of self-medicative behavior of wild chimpanzees in Uganda. Antimicrob. Agents Chemother. 48: 3196-3199.

Lakshmi, V. and Gupta, P. 2008. An overview of the genus *Xylocarpus*. Nat. Prod. Res. 22: 1197-1224.

Li, M., Wu, J., Zhang, S., Xiao, Q. and Li, Q. 2007. Xylocarpins A and B, two new mexicanolides from the seeds of a Chinese mangrove *Xylocarpus granatum*: NMR investigation in mixture. Magn. Reson. Chem. 45: 705-709.

Li, M. Y., Wu, J., Zhang, S., Xiao, Q. and Li, Q. X. 2008. The absolute stereochemistry of protoxylogranatin A - A new protolimonoid from the seeds of Chinese mangrove *Xylocarpus granatum*. J. Asian Nat. Prod. Res. 10: 503-508.

Li, M. Y., Yang, X. B., Pan, J. Y., Feng, G., Xiao, Q., Sinkkonen, J., Satyanandamurty, T. and Wu, J. 2009. Granatumins A-G, Limonoids from the Seeds of a Krishna Mangrove, *Xylocarpus granatum*. J. Nat. Prod. 72: 2110–2114.

Leite, A. C., Oliveira, C. G., Fernandes, J. B., Vieira, P. C., Da-Silva, M. F. G. F., Hebling, M. J. A., Pagnocca, F. C. and Bueno, O. C. 2004. Extracts containing limnoids from *Cipadessa fruticosa* and their activity in ant control. (Universidade Federal de Sao Carlos, Chemistry Department, C.P. 676-13565-905- Sao Carlos, SP, Brazil) IUPAC International Conference on Biodiversity and Natural Products Chemistry and Medical Applications, New Delhi: 26-31.

Mabberley, D. J., Pannell, C. M. and Sing, A. M. 1995. Flora Malesiana. vol. 12. pp. 407-408. Leiden, The Netherland: Rijksherbarium/Hortus Botanicus.

McFarland, K., Mulholland, D. A. and Fraser, L. A. 2004. Limonoids from *Turraea floribunda* (Meliaceae). Phytochemistry. 65: 2031-2037.

Malathi, R., Rajan, S. S., Gopalakrishnan, G. and Suresh, G. 2002. Azadirachtol, a tetranotriterpenoid from neem kernels. Acta. Crystallogr. C. 58: 708-710.

Maria C. C., Maria T. D., Graciela V., and Sara M. P. 2003. Antifeedant and Insecticide Properties of a Limonoid from *Melia azedarach* (Meliaceae) with Potential Use for Pest Management. J. Agric. Food Chem. 51: 369-374.

Miller, E. G., Porter, J. L., Binnie, W. H., Guo, I. Y. and Hasegawa, S. 2004. Further studies on the anticancer activity of citrus limonoids. J. Agric. Food Chem. 52: 4908-4912.

Mitsui, K., Maejima, M., Fukaya, H., Hitotsuyanagi, Y. and Takeya, K. 2004. Limonoids from *Cedrela sinensis*. Phytochemistry. 65: 3075-3081.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 65: 55-63.

Mulholland, D. A. and Taylor, D. A. H. 1992. Limonoids from Australian members of the meliaceae. Phytochemistry. 31: 4163-4166.

Mulholland, D. A., Randrianarivelojosia, M., Lavaud, C., Nuzillard, J. M. and Schwikkard, S. L. 2000. Limonoid derivatives from *Astrotrichilia voamatata* Phytochemistry. 53: 115-118.

Nakagawa, H., Duan, H. and Takaishi, Y. 2001. Limonoids from *Citrus sudachi* Chem. Pharm. Bull. 49: 649-651.

Nakatani, M., Abdelgaleil, S. A. M., Kassem, S. M. I., Takezaki, K., Okamura, H., Iwagawa, T. and Doe, M. 2002. Three new modified limonoids from *Khaya senegalensis*. J. Nat. Prod. 65: 1219-1221.

Nakatani, M., Abdelgaleil, S. A. M., Saad, M. M. G., Huang, R. C., Doe, M. and Iwagawa, T. 2004. Phragmalin limonoids from *Chukrasia tabularis*. Phytochemistry. 65 : 2833-2841.

Ndung'u, M., Torto, B., Knols, B. G. J. and Hassanali, A. 2004. Laboratory evaluation of some eastern African Meliaceae as sources of larvicidal botanicals for *Anopheles gambiae*. J. Trop. Insect Sci. 24: 311-318.

Ndung'u, M. W., Kaoneka, B., Hassanali, A., Lwande, W., Hooper, A. M., Tayman, F., Zerbe, O. and Torto, B. 2004. New mosquito larvicidal tetranortriterpenoids from *Turraea wakefieldii* and *Turraea floribunda.* J. Agric. Food Chem. 52: 5027-5031.

Newman, D. J. and Cragg, G. M. 2007. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70: 461-477.

Ng, A. S. and Fallis, A. G. 1979. 7α-Acetoxydihydronomilin and mexicanolide: limonoids from *Xylocarpus gralzatum* (Koenig). Can. J. Chem. 57: 3088.

Okamura, H., Yamauchi, K., Miyawaki, K., Iwagawa, T. and Nakatani, M. 1997. Synthesis and biological activities of degraded limonoids, (±)-fraxinellonone and its related compounds. Tetrahedron Lett. 38: 263-266.

Okorie, D. A., Taylor, D. A. H. 1970. Limonoids from *Xylocarpus granatum* Koenig. J. Chem. Soc. C: Org. 2: 211-213.

Omar, S., Zhang, J., MacKinnon, S., Leaman, D., Durst, T., Philogene, B. J., Arnason, J. T. and Pezzuto, J. M. 2003. Traditionally-used antimalarials from the Meliaceae. Curr. Top Med. Chem. 3: 133-139.

Perusquia, M., Hernandez, R., Jimenez, M. A., Pereda-Miranda, R. and Mata, R. 1997. Contractile response induced by a limonoid (Humilinolide A) on spontaneous activity of isolated smooth muscle. Phytother Res. 11: 354-357.

Poulose, S. M., Harris, E. D. and Patil, B. S. 2005. Citrus limonoids induce apoptosis in human neuroblastoma cells and have radical scavenging activity. J. Nutr. 135: 870-877.

Pudhom, K., Sommit, D., Nuclear, P., Ngamrojanavanich, N. and Petsom, A. 2010. Moluccensins H-J, 30-Ketophragmalin Limonoids from *Xylocarpus moluccensis*. J. Nat. Prod. 73: 263-266.

Pudhom, K., Sommit, D., Nuclear, P., Ngamrojanavanich, N. and Petsom, A. 2009. Protoxylocarpins F-H, Protolimonoids from Seed Kernels of *Xylocarpus granatum*. J. Nat. Prod. 72: 2188-2191.

Puripattanavong, J., Weber, S., Brecht, V. and Frahm, A. W. 2000. Phytochemical investigation of *Aglaia andamanica*. Planta Med. 66: 740-745.

Roy, A. D., Kumar, R., Gupta, P., Khaliq, T., Narender, T., Aggarwal, V. and Roy, R. 2006. Xyloccensin X and Y, two new limonoids from *Xylocarpus molluccensis*: NMR investigation in mixture. Magn. Reson. Chem. 44: 1054-1057.

Roy, A. and Saraf, S. 2006. Limonoids: Overview of Significant Bioactive Triterpenes Distributed in Plants Kingdom. Biol. Pharm. Bull. 29: 191-201.

Rugutt, J. K., Rugutt, K. J. and Berner, D. K. 2001. Limonoids from Nigerian *Harrisonia abyssinica* and Their Stimulatory Activity against *Striga hermonthica* Seeds. J. Nat. Prod. 64: 1434–1438

Saad, M. M. G., Iwagawa, T., Doe, M. and Nakatani, M. 2003. Swietenialides, novel ring D opened phragmalin limonoid orthoesters from *Swietenia mahogani* JACQ. Tetrahedron. 59: 8027-8033.

Sastri, B. N. 1950. The wealth of India; Raw Materiales, Vol. 2(C), pp. 74–75, New Delhi, Publication and Information Directorate, CSIR

Sawabe, A., Morita, M., Kiso, T., Kishine, H., Ohtsubo, Y., Minematsu, T., Matsubara, Y. and Okamoto, T. 1999. Isolation and characterization of new limonoid glycosides from *Citrus unshiu* peels. Carbohydr. Res. 315: 142-147.

Saxena, E. and Babu, U. V. 2001. Constituents of *Carapa granatum* fruits. Fitoterapia. 72: 186-187.

Saxena, S., Pant, N., Jain, D. C. and Bhakuni, R. S. 2003. Antimalarial agents from plant sources. Curr. Sci. 85: 1314-1329.

Siddiqui, B. S., Afshan, F., Ghiasuddin, Faizi, S., Naqvi, S. N. H. and Tariq, R. M. 2000. Two insecticidal tetranortriterpenoids from *Azadirachta indica.* Phytochemistry. 53: 371-376.

Simmonds, M. S. J., Stevenson, P. C., Porter, E. A. and Veitch, N. C. 2001. Insect antifeedant activity of three new tetranortriterpenoids from *Trichilia pallida.* J. Nat. Prod. 64: 1117-1120.

Somrutai, J., Chantachum, S., Ratanaphan, A. and Chantrapromma, K. 2005. Stability of limonin from lime seeds. Electron. J. Environ. Agric. Food Chem. 4: 938-944.

Soundarya, D. S., Malathi, R., Rajan, S. S., Aravind, S., Krishnakumari G. N. and Ravikumar, K. 2003. A new clerodane diterpene with antifeedant activity from *Teucrium tomentosum*. Acta. Cryst. 59: 530-532.

Suarez, L. E. C., Menichini, F. and Monache, F. D. 2002. Tetranortriterpenoids and Dihydrocinnamic Acid Derivatives from *Hortia colombiana.* J. Braz. Chem. Soc. 13: 339-344

Sunthitikawinsakul, A., Kongkathip, N., Kongkathip, B., Phonnakhu, S., Daly, J. W., Spande, T. F., Nimit, Y. and Yoosook, C. 2003. Anti-HIV-1 Limonoid: First Isolation from *Clausena excavate.* Phytother. Res. 17: 1101-1103.

Tada, K., Takido, M. and Kitanaka, S. 1999. Limonoids from fruit of *Melia toosendan* and their cytotoxic activity. Phytochemistry. 51: 787-791.

Taylor, D. A. H. 1965. Extractives from East African timbers. Part I. J. Chem. Soc. (Resume): 3495-3496.

Taylor, D. A. H. 1983. Limonoid extractives from *Xylocarpus moluccensis*. Phytochemistry. 22: 1297-1299.

Taylor, D. A. H. 1984. The chemistry of the limonoids from Meliaceae. *Progress in the Chemistry of Organic Natural Products*. 45: 1-102.

Tchimene, M. K., Tane, P., Ngamga, D., Connolly, J. D. and Farrugia, L. J. 2005. Four tetranortriterpenoids from the stem bark of *Khaya anthotheca* Phytochemistry. 66: 1088-1093.

Tian, Q., Miller, E. G., Ahmad, H., Tang, L. and Patil, B. S. 2001. Differential inhibition of human cancer cell proliferation by citrus limonoids. Nutr. Cancer. 40: 180-184.

Tominaga, H., Ishiyama, M., Ohseto, F., Sasamoto, K., Hamamoto, T., Suzuki, K. K., and Watanabe, M. 1999. A water-soluble tetrazolium salt useful for colorimetric cell viability assay. Anal. Commun. 36: 47-50.

Uddin, S. J., Nahar, L., Shilpi, J. A., Shoeb, M., Borkowski, T., Gibbons, S., Middleton, M. and Sarker, S. D. 2007. Gedunin, a limonoid from *Xylocarpus granatum*, inhibits the growth of CaCo-2 colon cancer cell line *in vitro*. Phytother. Res. 21: 757-761.

Uddin, S. J., Shilpi, J. A., Alam, S. M. S., Alamgir, M., Rahman, M. T. and Sarker, S.D. 2005. Antidiarrhoeal activity of the methanol extract of the barks of *Xylocarpus moluccensis* in castor oil- and magnesium sulphate-induced diarrhoea models in mice. J. Ethnopharmacol. 101: 139-143.

Vardamides, J. C., Dongo, E., Nkengfack, A. E., Fomum, Z. T., Ngando, T. M., Vogler, B. and Kraus, W. 2001. Diterpenoid and limonoids from the stem of *Pterorhachis zenkeri*. Fitoterapia. 72: 386-393.

Wandscheer, C. B., Duque, J. E., Da-Silva, M. A. N., Fukuyama, Y., Wohlke, J. L., Adelmann, J. and Fontana, J. D. 2004. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. Toxicon. 44: 829-835.

Wattanapiromsakul, C., Forster, P. I. and Waterman, P. G. 2003. Alkaloids and limonoids from Bouchardatia neurococca: Systematic significance. Phytochemistry. 64: 609-615.

Wu, J., Ding, H., Li, M. and Zhang, S. 2007. Xylogranatin E, a new phragmalin with a rare oxygen bridge between C_1 and C_{29}, from the fruit of a Chinese mangrove *Xylocarpus granatum*. J. Chem. Sci. 62: 569-572.

Wu, J., Li, M., Zhang, S., Xiao, Q. and Li, Q. 2007. Two new limonoids with a 3-*O*-*β*-tigloyl group from the seeds of the Chinese mangrove *Xylocarpus granatum*. J. Chem. Sci. 62: 859-862.

Wu, J., Xiao, Q., Huang, J., Xiao, Z., Qi, S., Li, Q. and Zhang, S. 2004. Xyloccensins O and P, Unique 8,9,30-Phragmalin *Ortho* Esters from *Xylocarpus granatum*. Org. Lett. 6: 1841-1844.

Wu, J., Xiao, O. and Li, Q. 2006. Limonoids from the Mangrove *Xylocarpus granatum*. Biochem. Syst. Ecol. 34: 838-841.

Wu, J., Xiao, Q., Xu, J., Li, M. Y., Pan, J. Y. and Yang, M. H. 2008. Natural products from true mangrove flora: source, chemistry and bioactivities. Nat. Prod. Rep. 25: 955-981.

Wu, J., Xiao, Q., Zhang, S., Li, X., Xiao, Z., Ding, H. and Li, Q. 2005. Xyloccensins Q-V, six new 8,9,30-phragmalin *ortho* ester antifeedants from the Chinese mangrove *Xylocarpus granatum*. Tetrahedron. 61: 8382-8389.

Wu, J., Xiao, Z., Song, Y., Zhang, S., Xiao, Q., Ma, C., Ding, H. and Li, Q. 2006. Complete assignments of ^{1}H and ^{13}C NMR data for two 3*β*,8*β*-epoxymexicanolides from the fruit of a Chinese mangrove *Xylocarpus granatum.* Magn. Reson. Chem. 44: 87-89.

Wu, J., Yang, S. X., Li, M. Y., Feng, G., Pan, J. Y., Xiao, Q., Sinkkonen, J. and Satyanandamurty, T. 2010. Limonoids and Tirucallane Derivatives from the Seeds of a Krishna Mangrove, *Xylocarpus moluccensis*. J. Nat. Prod. 73: 644-649.

Wu, J., Zhang, S., Xiao, Q., Li, Q., Huang, J., Xiao, Z. and Long, L. 2003. Xyloccensin M and N, Two New B, D-*seco* Limonoids from *Xylocarpus granatum.* J. Chem. Sci. 58: 1216-1219.

Wu, J., Zhang, S., Xiao, Q., Li, Q., Huang, J., Long, L. and Huang, L. 2004. Xyloccensin L, a novel limonoid from *Xylocarpus granatum.* Tetrahedron Lett. 45: 591-593.

Wu, J., Zhang, S., Song, Y., Xiao, Z., Xiao, Q. and Li, Q. 2005. Two new mexicanolides from the fruit of the Chinese mangrove *Xylocarpus granatum.* J. Chem. Sci. 60: 1291-1294.

Wu, J., Zhang, S., Li, M., Zhou, Y. and Xiao, Q. 2006. Xylogranatins A-D, new mexicanolides from the fruit of a Chinese mangrove *Xylocarpus granatum*. Chem. Pharm. Bull. 54: 1582-1585.

Ximu, C. and Pongumphai, S. 1994. Preliminary revision of Swietenioideae (Meliaceae) in Thailand. Thai J. For. 13: 1-9.

Yin, B., Huo, C., Shen, L., Wang, C., Zhoa, L. and Wang, Y. 2009. Protolimonoids from the seeds of *Xylocarpus granatum*. Biochem. Syst. Ecol. 37: 218-220.

Yin, S., Wang, X. N., Fan, C. Q., Lin, L. P., Ding, J. and Yue, J. M. 2007. Limonoids from the seeds of the marine mangrove *Xylocarpus granatum.* J. Nat. Prod. 70: 682-685.

Zhao, W., Wolfender, J. L., Hostettmann, K., Xu, R. and Qin, G. 1998. Antifungal alkaloids and limonoid derivatives from *Dictamnus dasycarpus*. Phytochemistry. 47: 7-11.

Zhou, Y., Cheng, F. and Zou, K. 2006. Polyhydroxylated Phragmalins from the Fruit of a Chinese Mangrove, *Xylocarpus granatum.* J. Nat. Prod. 69: 1083-1085.

Zhou, H., Hamazaki, A., Fontana, J. D., Takahashi, H., Esumi, T., Wandscheer, C. B., Tsujimoto, H. and Fukuyama, Y. 2004. New ring C-*seco* limonoids from Brazilian *Melia azedarach* and their cytotoxic activity. J. Nat. Prod. 67: 1544-1547.

Zhang, H. P., Bao, G. H., Wang, H. B. and Qin, G. W. 2004. Two new limonoids from *Munronia henryi.* Nat. Prod. Res. 18: 415-419.

APPENDIX

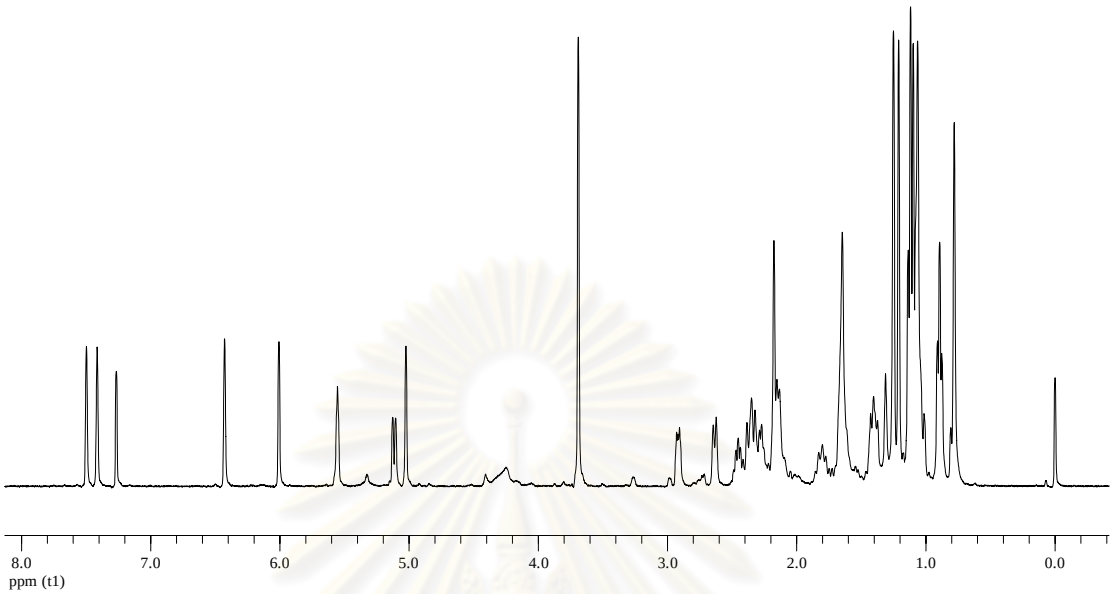


Figure S-1 ^{1}H NMR (400 MHz) spectrum of compound **1** ($CDCl_3$)

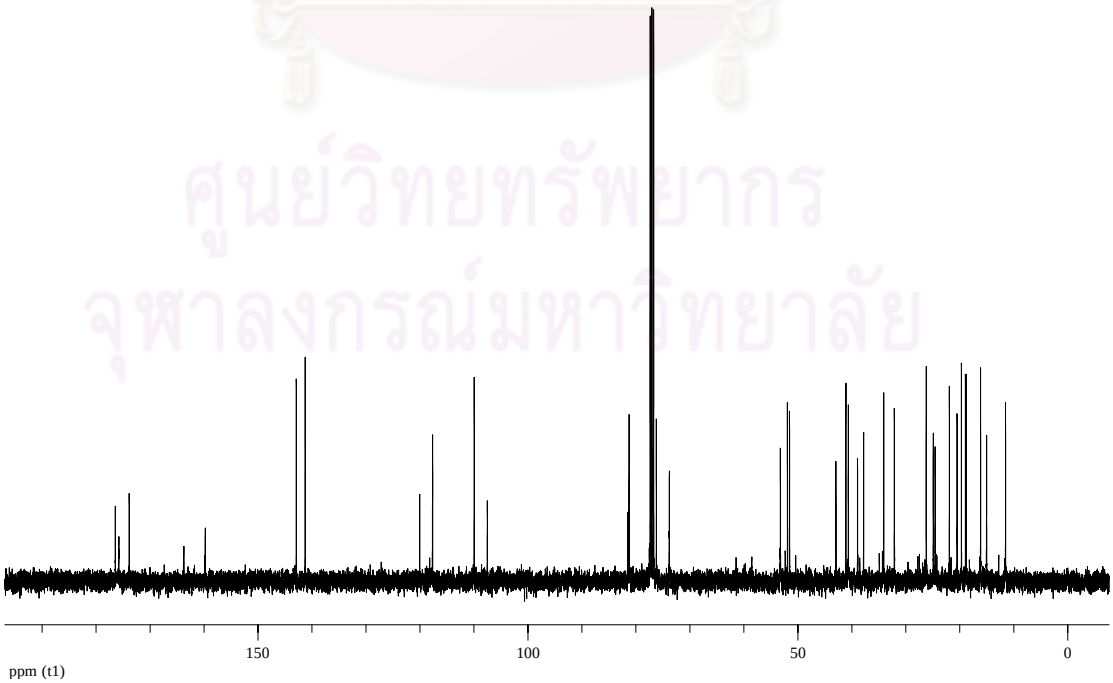


Figure S-2 ^{13}C NMR (100 MHz) spectrum of compound **1** ($CDCl_3$)

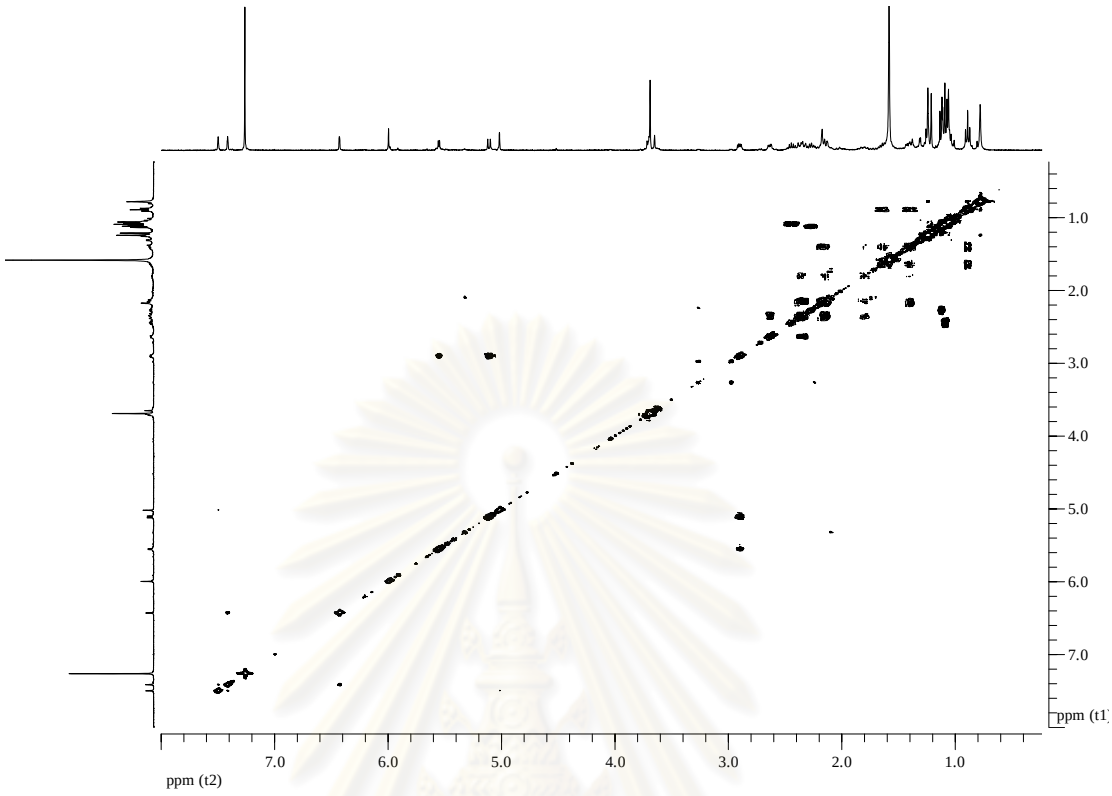


Figure S-3 ^{1}H-^{1}H COSY spectrum of compound **1** ($CDCl_3$)

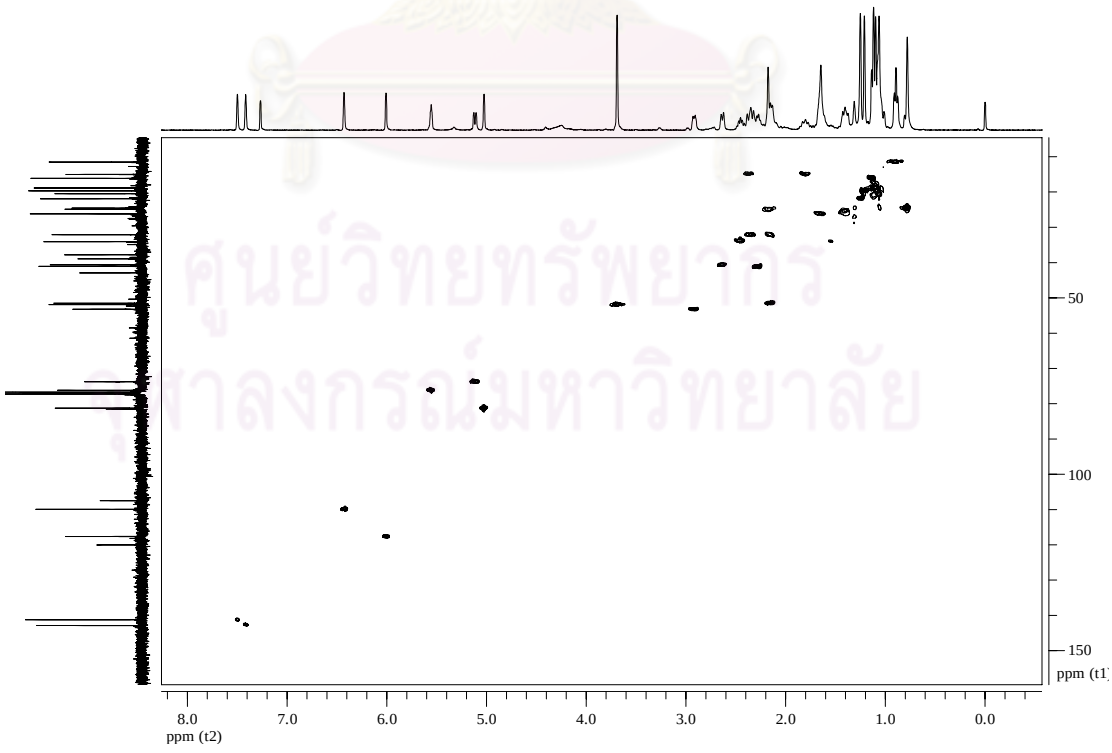


Figure S-4 HSQC spectrum of compound **1**

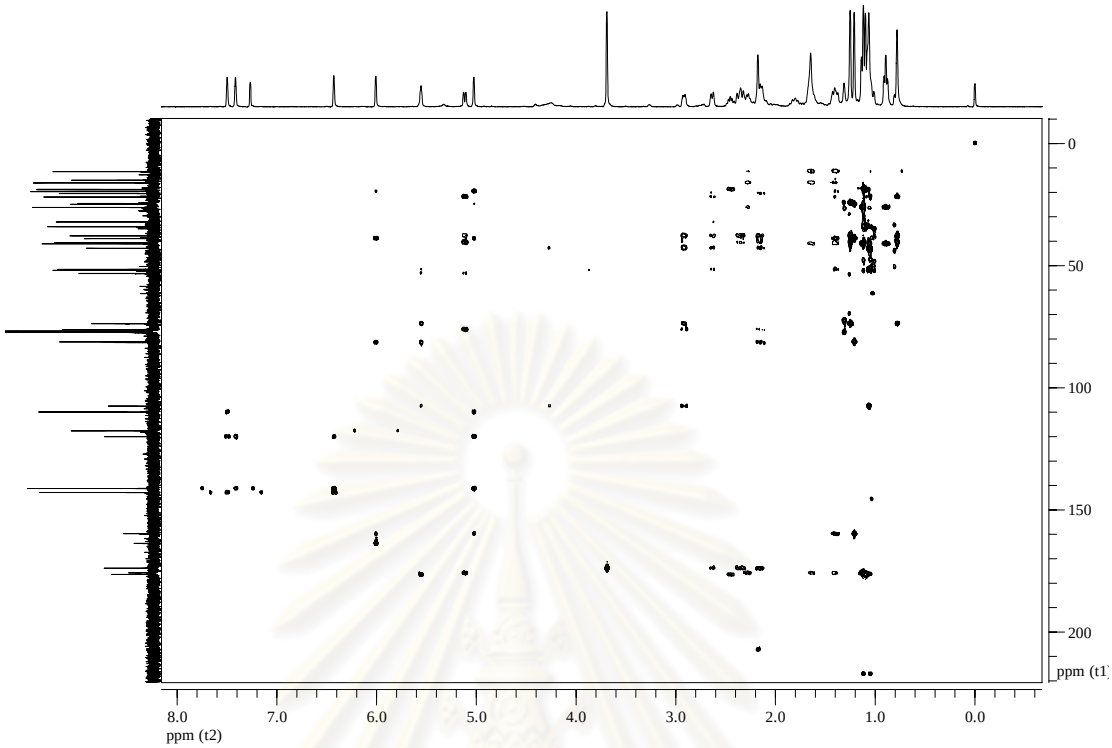


Figure S-5 HMBC spectrum of compound **1**

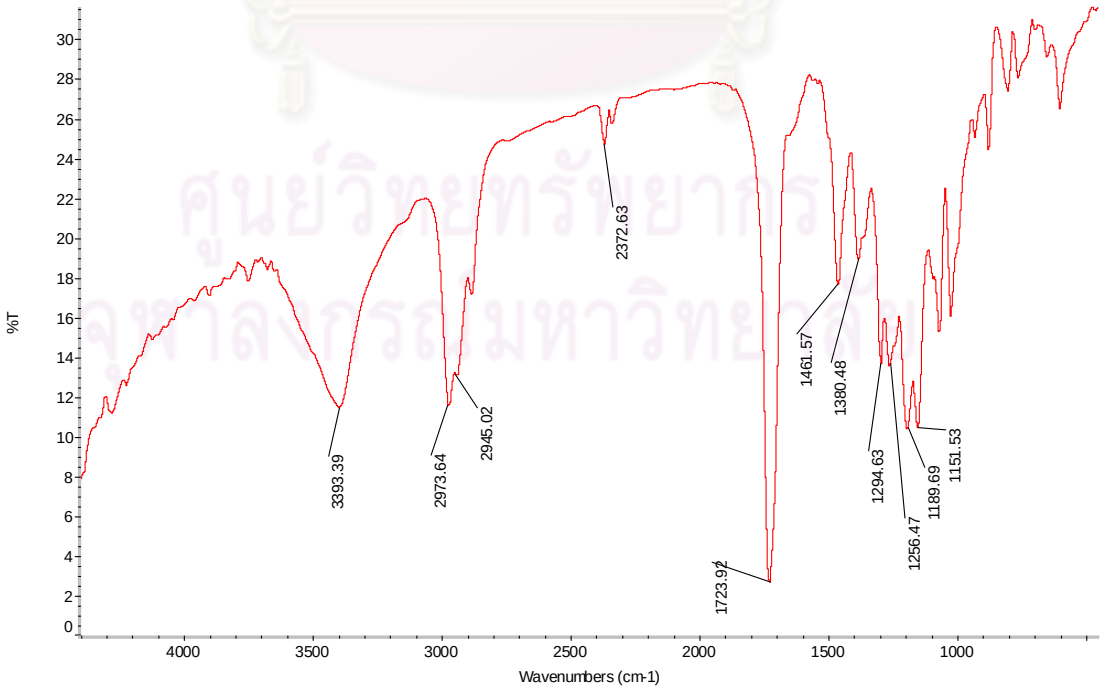


Figure S-6 IR spectrum of compound **1** (KBr)

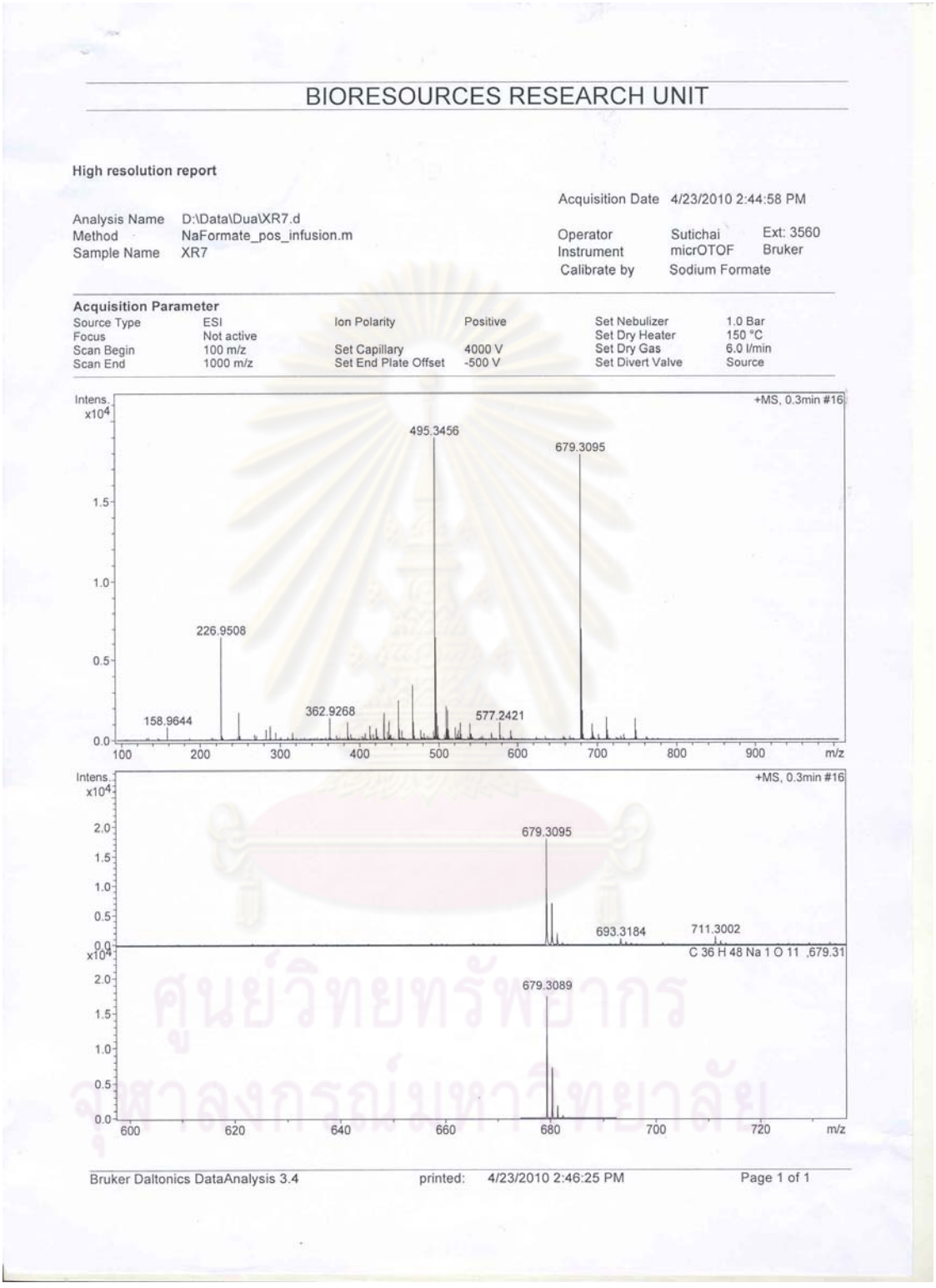


Figure S-7 HRESIMS Mass spectrum of compound **1**

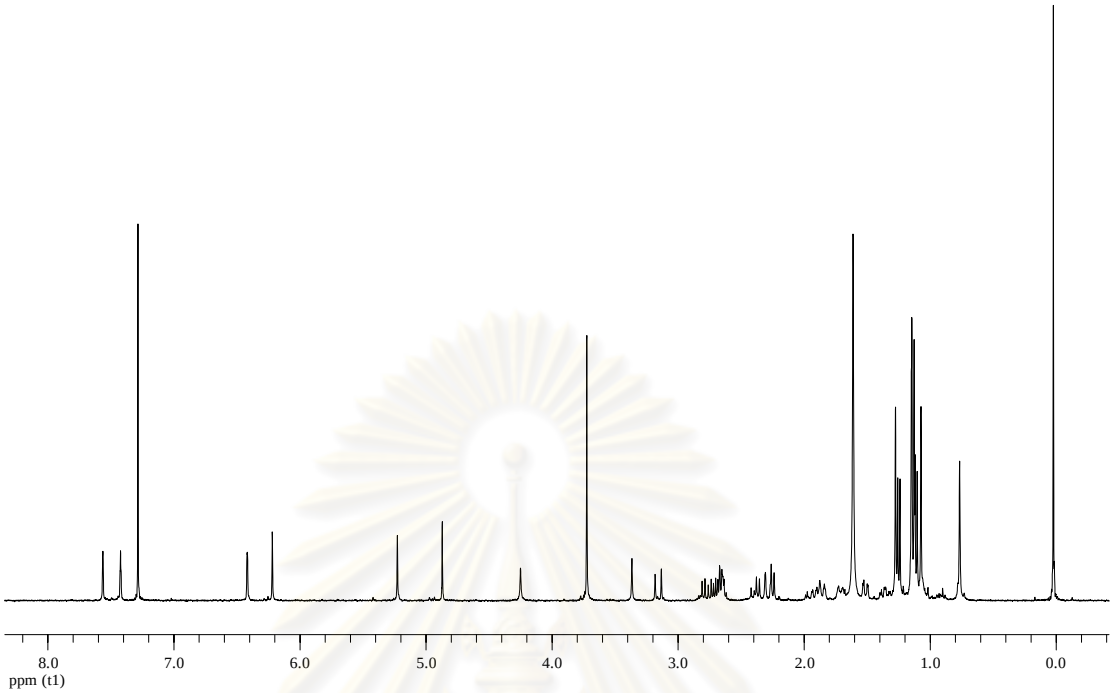


Figure S-8 ^{1}H NMR (400 MHz) spectrum of compound **2** ($CDCl_3$)

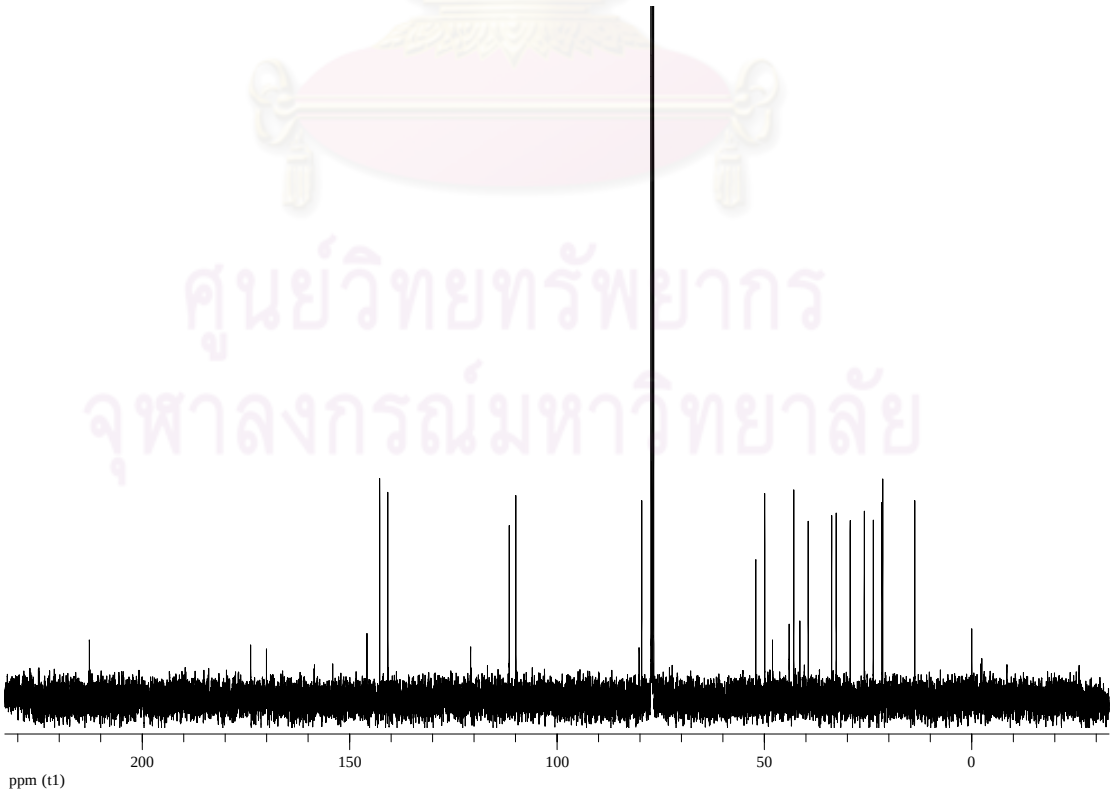


Figure S-9 ^{13}C NMR (100 MHz) spectrum of compound **2** ($CDCl_3$)

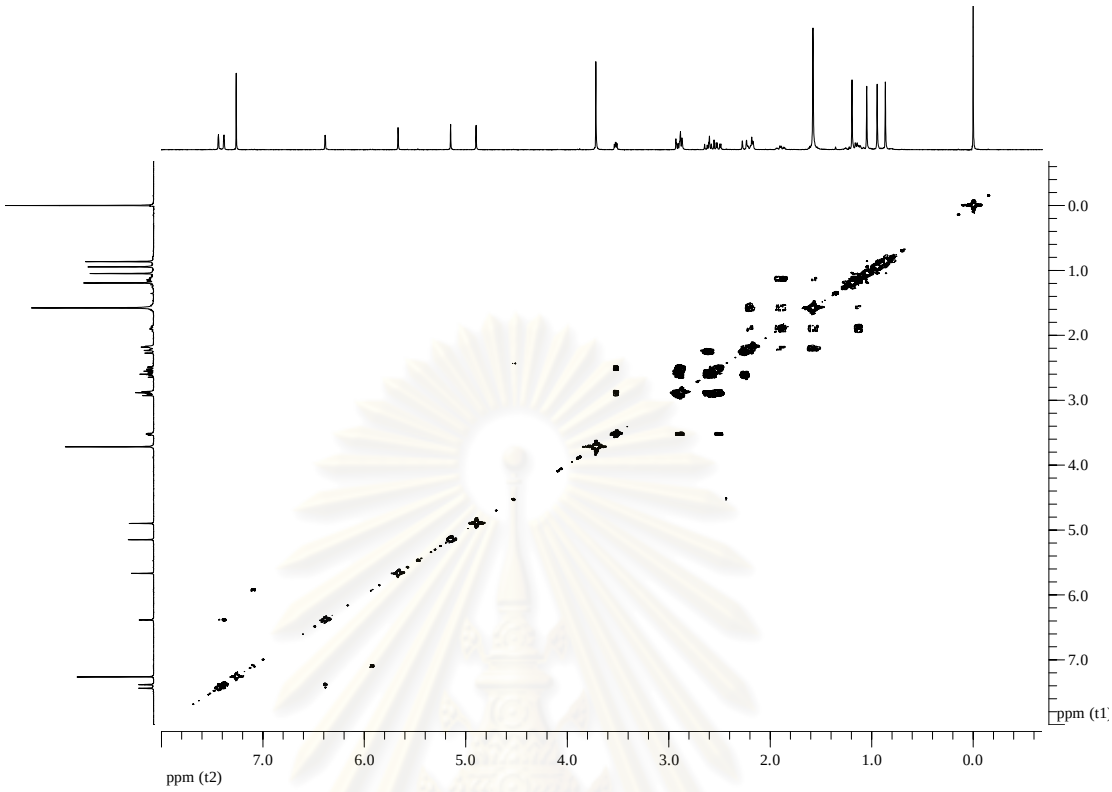


Figure S-10 ^{1}H-^{1}H COSY spectrum of compound **2** ($CDCl_3$)

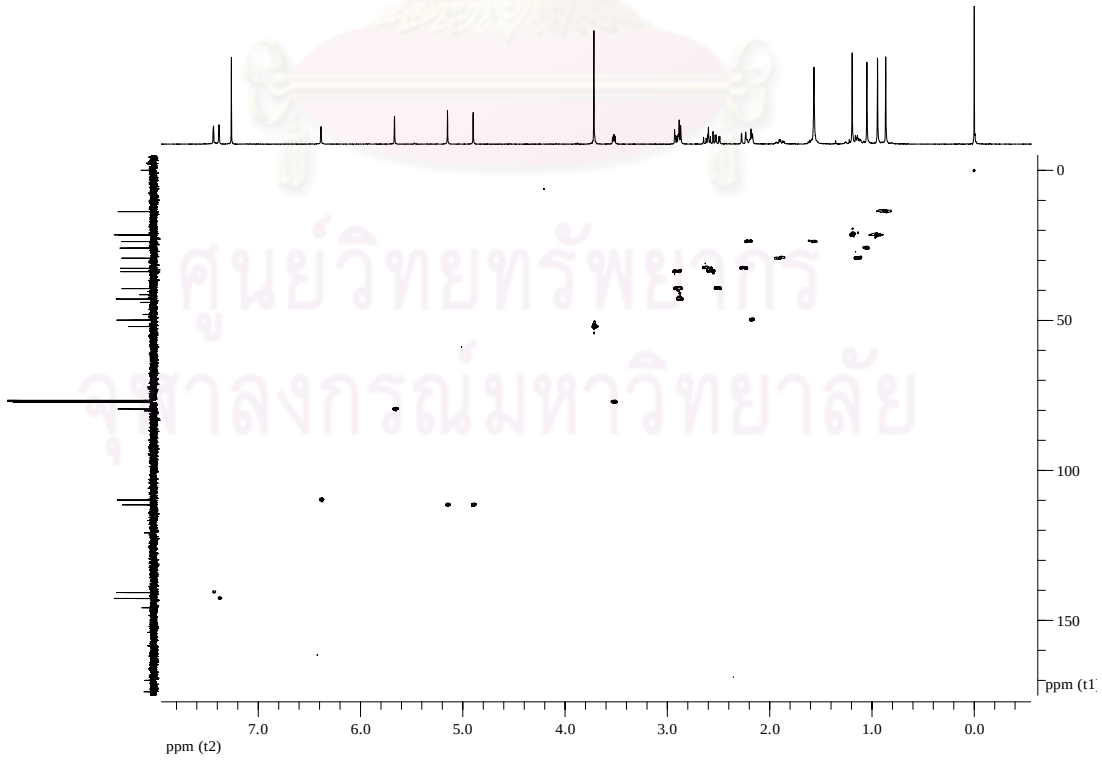


Figure S-11 HSQC spectrum of compound **2**

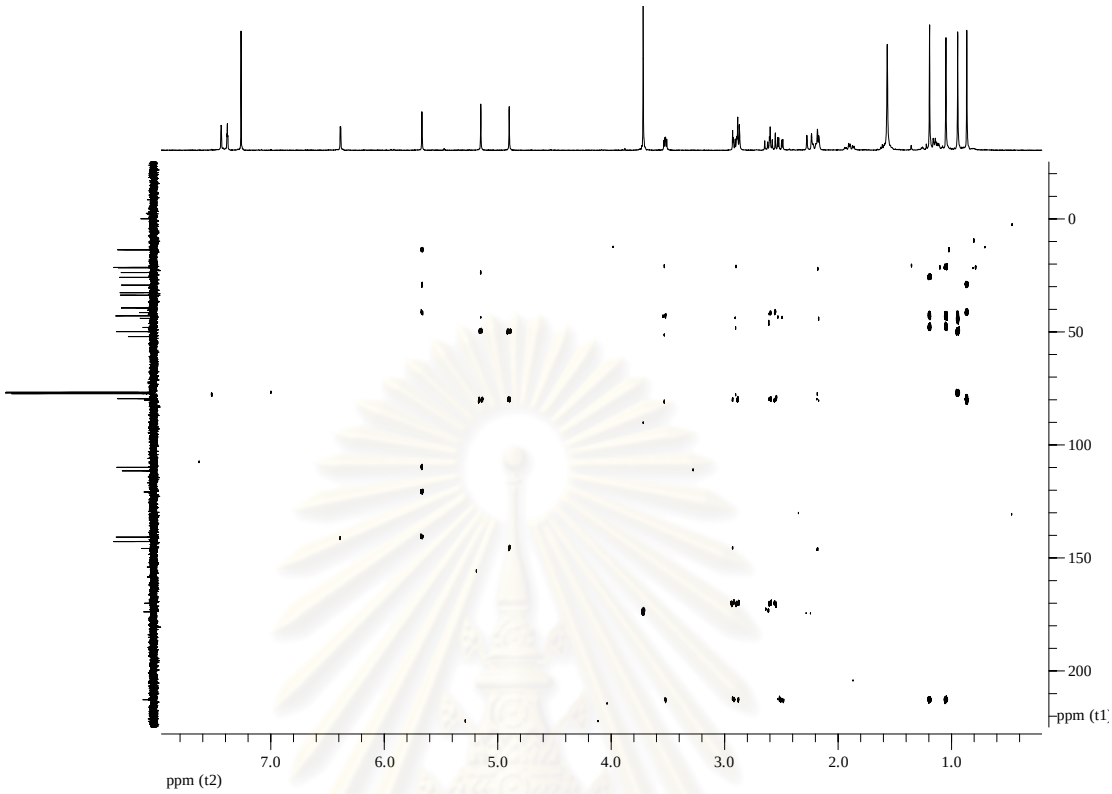


Figure S-12 HMBC spectrum of compound **2**

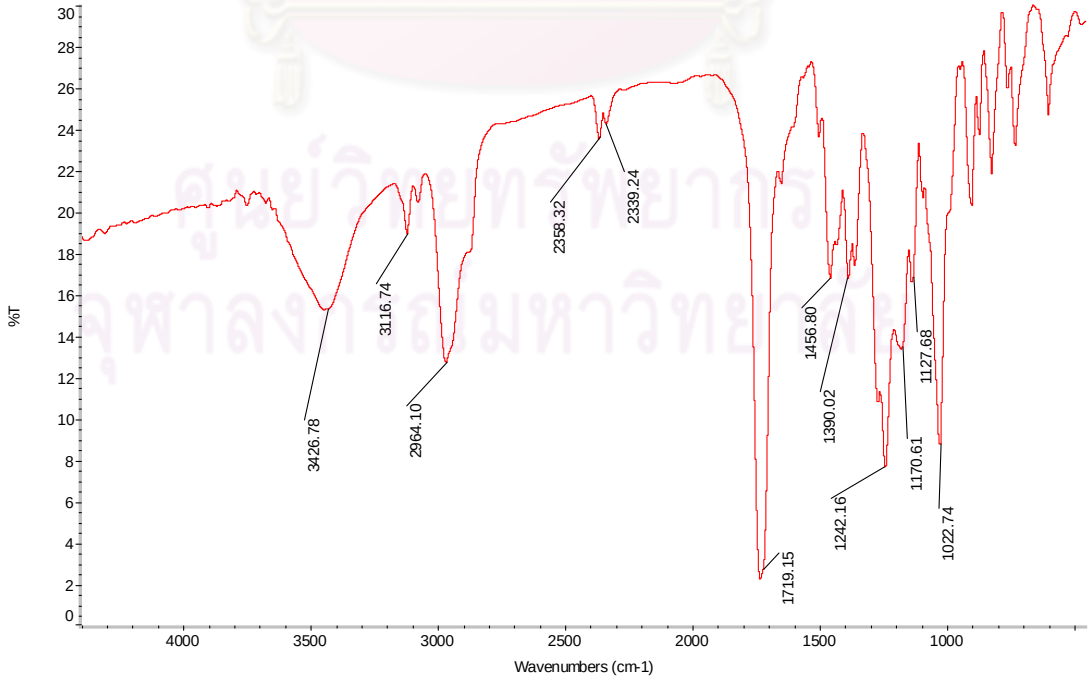


Figure S-13 IR spectrum of compound **2** (KBr)

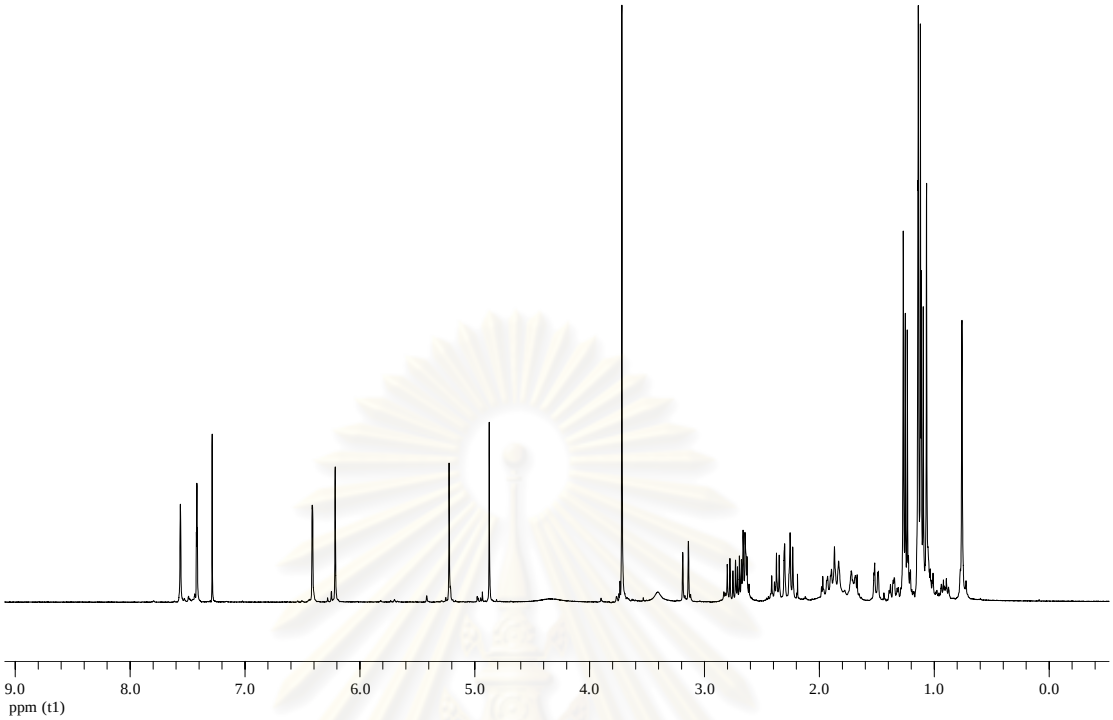


Figure S-14 ^{1}H NMR (400 MHz) spectrum of compound **3** ($CDCl_3$)

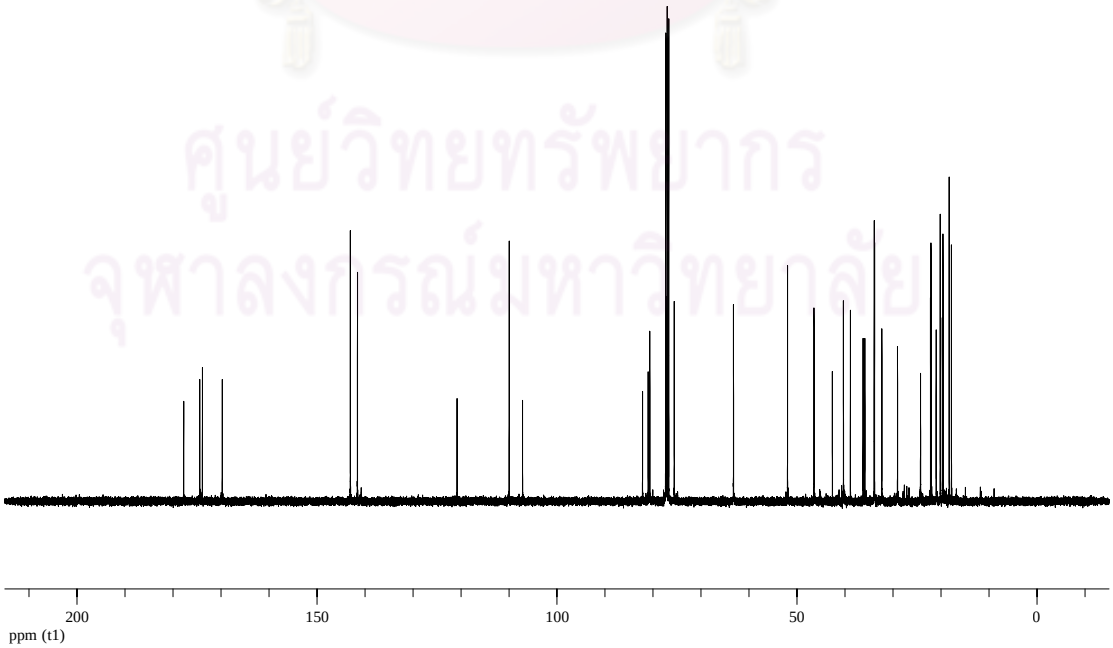


Figure S-15 ^{13}C NMR (100 MHz) spectrum of compound **3** ($CDCl_3$)

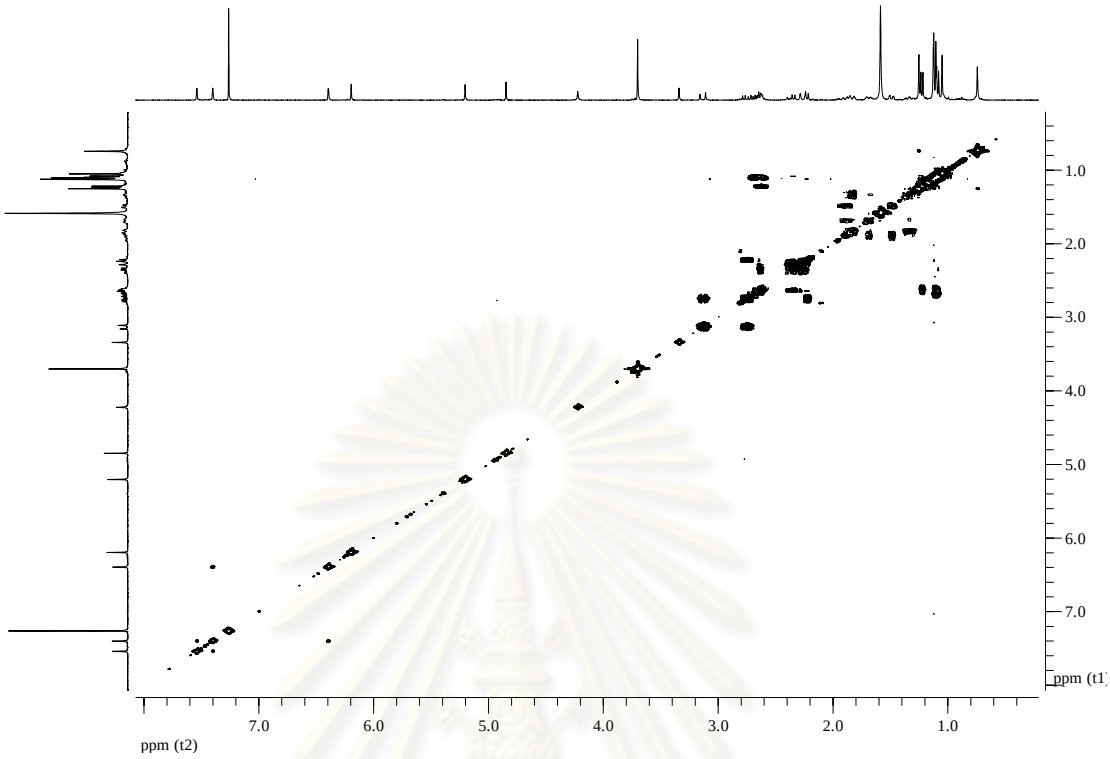


Figure S-16 ^{1}H-^{1}H COSY spectrum of compound **3** ($CDCl_3$)

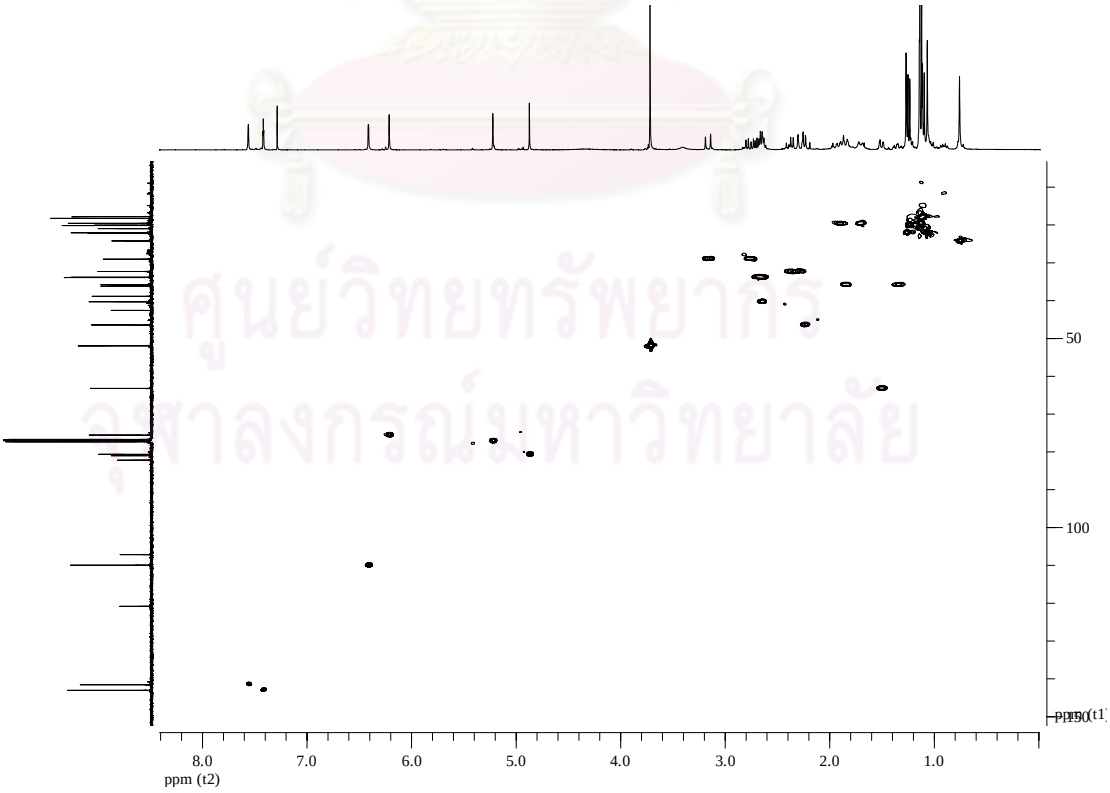


Figure S-17 HSQC spectrum of compound **3**

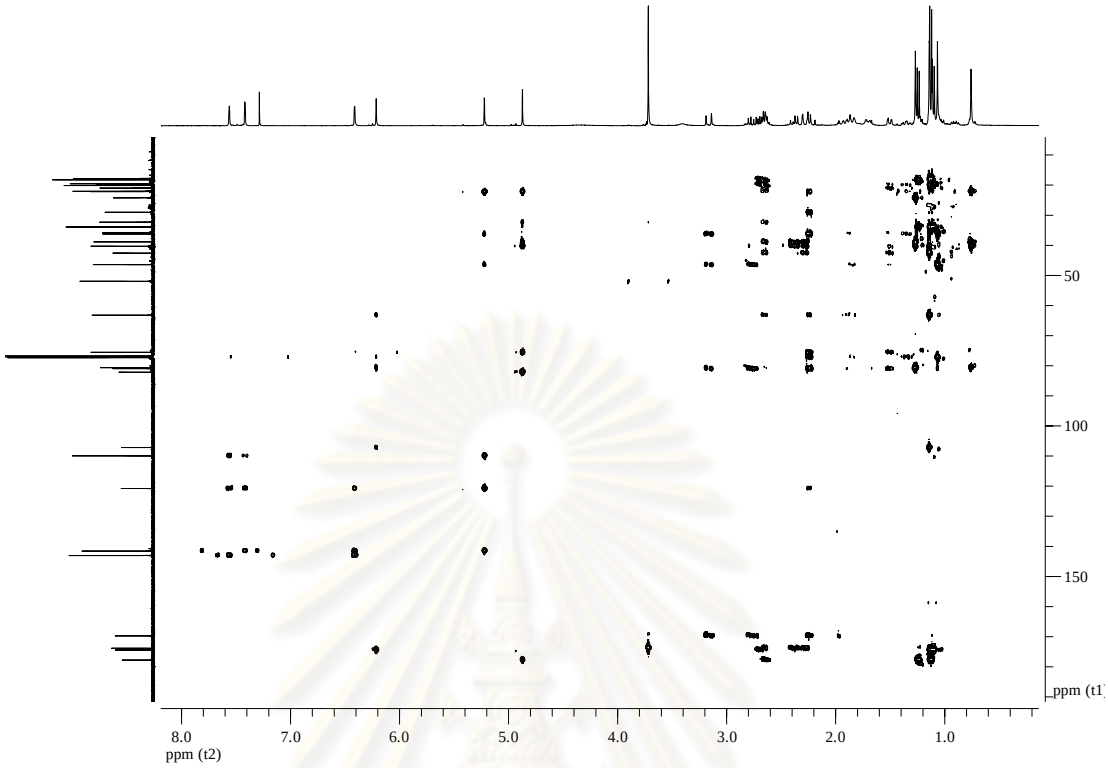


Figure S-18 HMBC spectrum of compound **3**

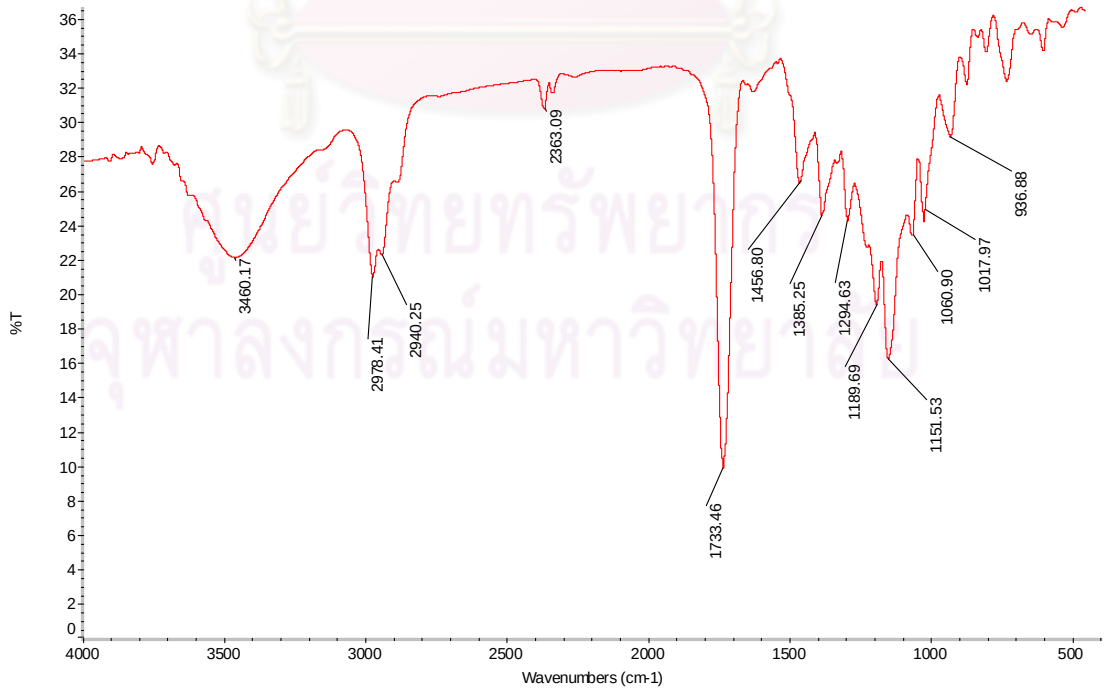


Figure S-19 IR spectrum of compound **3** (KBr)

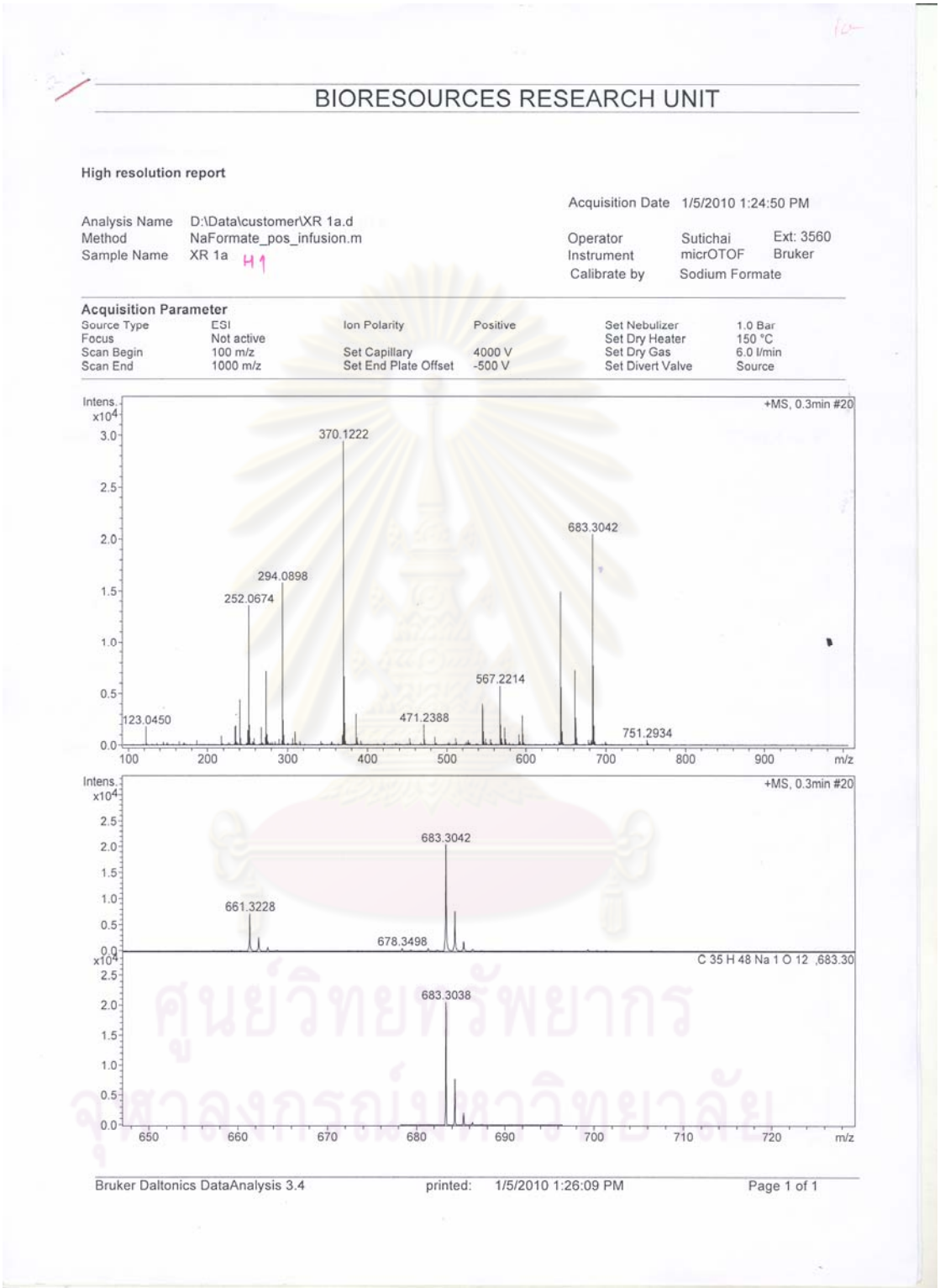


Figure S-20 HRESIMS Mass spectrum of compound **3**

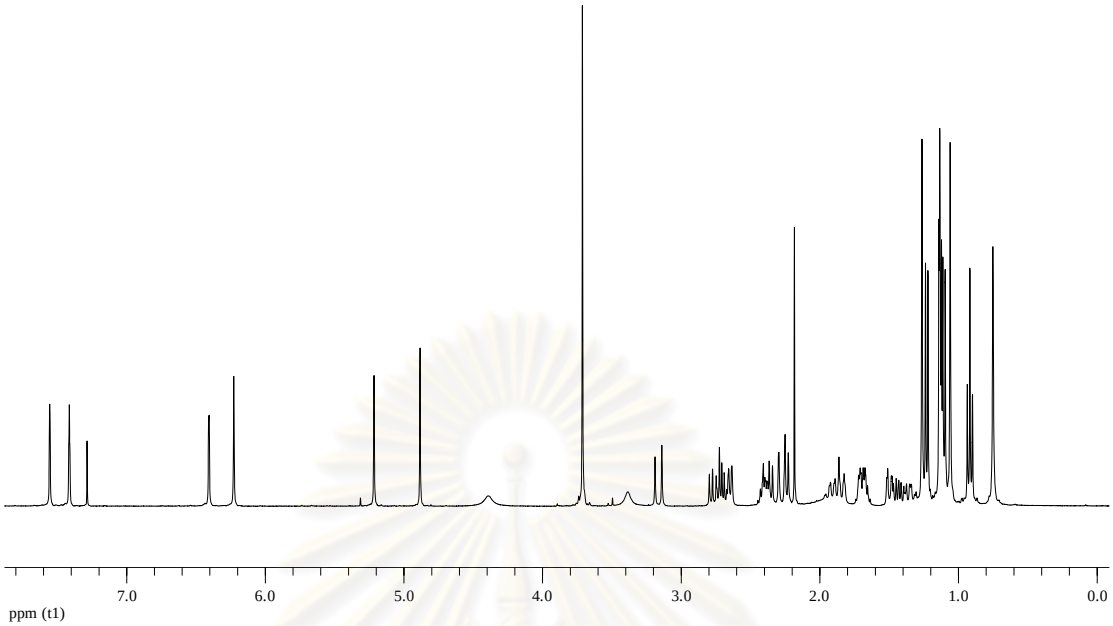


Figure S-21 ^{1}H NMR (400 MHz) spectrum of compound **4** ($CDCl_3$)

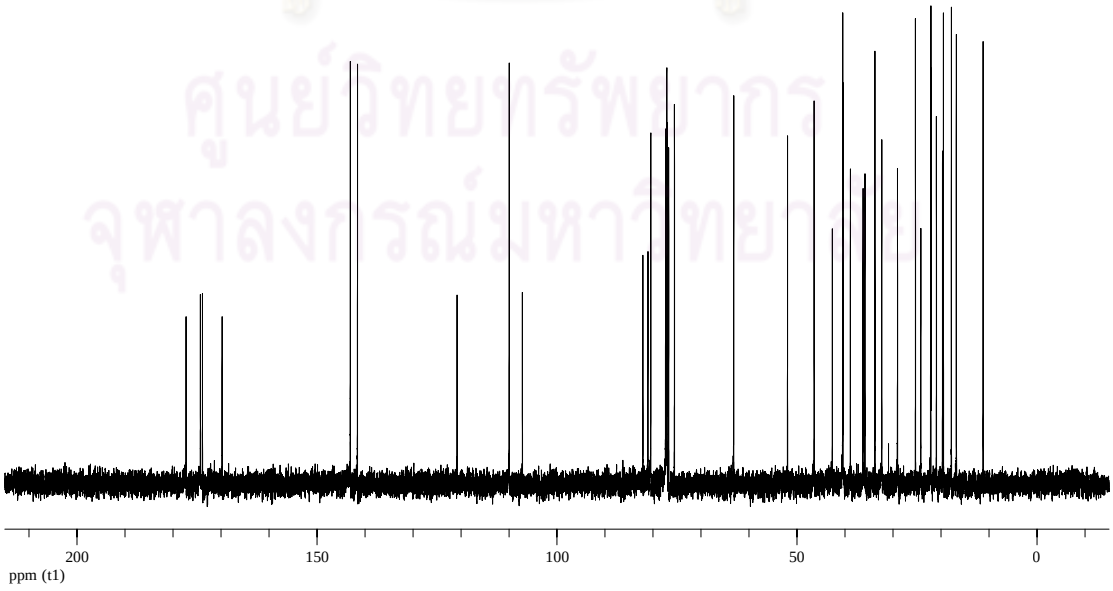


Figure S-22 ^{13}C NMR (100 MHz) spectrum of compound **4** ($CDCl_3$)

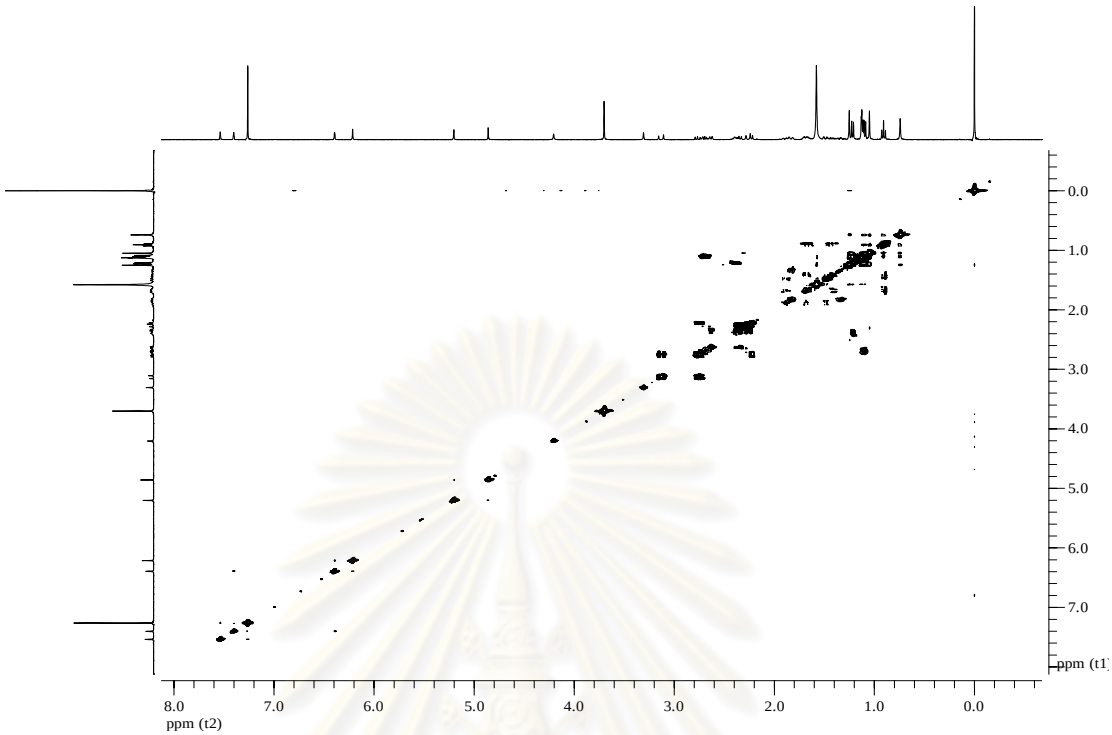


Figure S-23 ^{1}H-^{1}H COSY spectrum of compound **4** ($CDCl_3$)

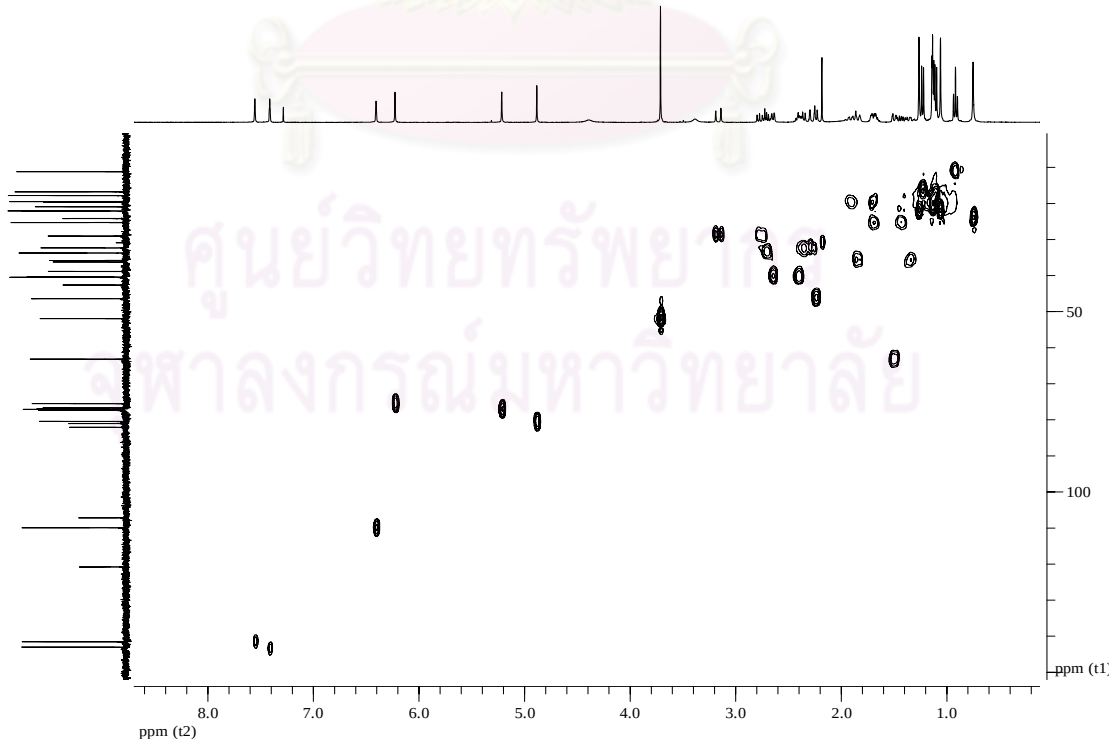


Figure S-24 HSQC spectrum of compound **4**

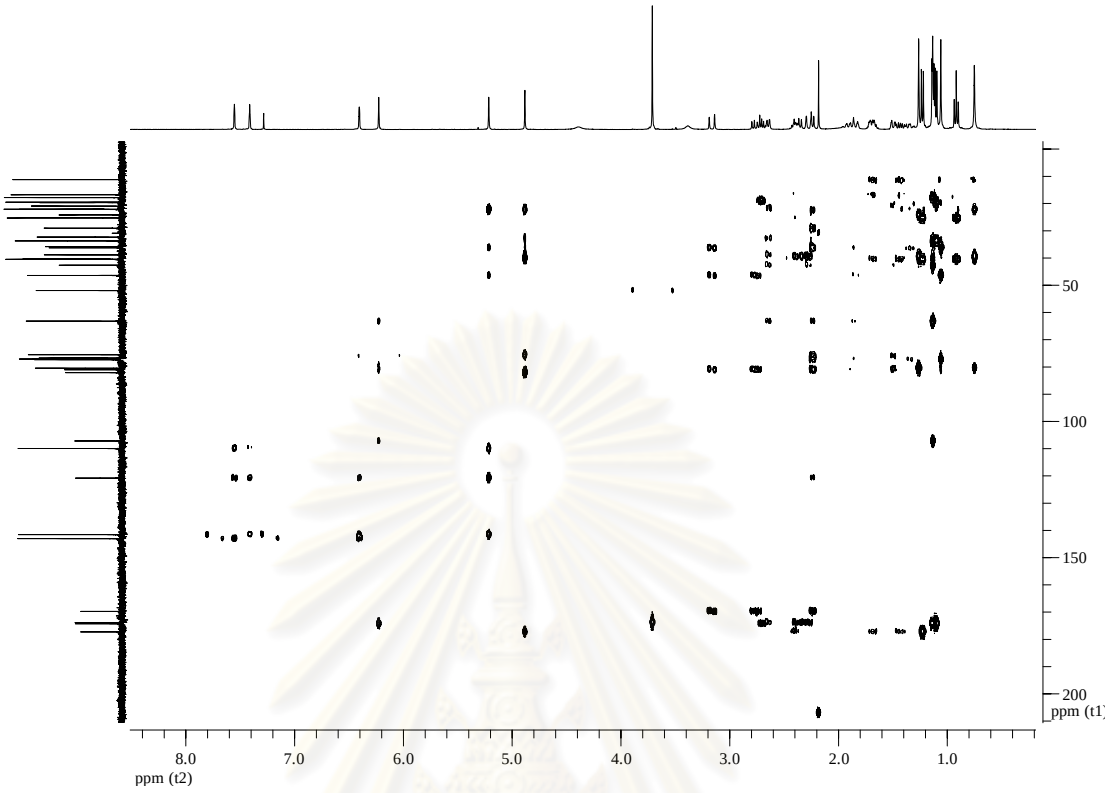


Figure S-25 HMBC spectrum of compound **4**

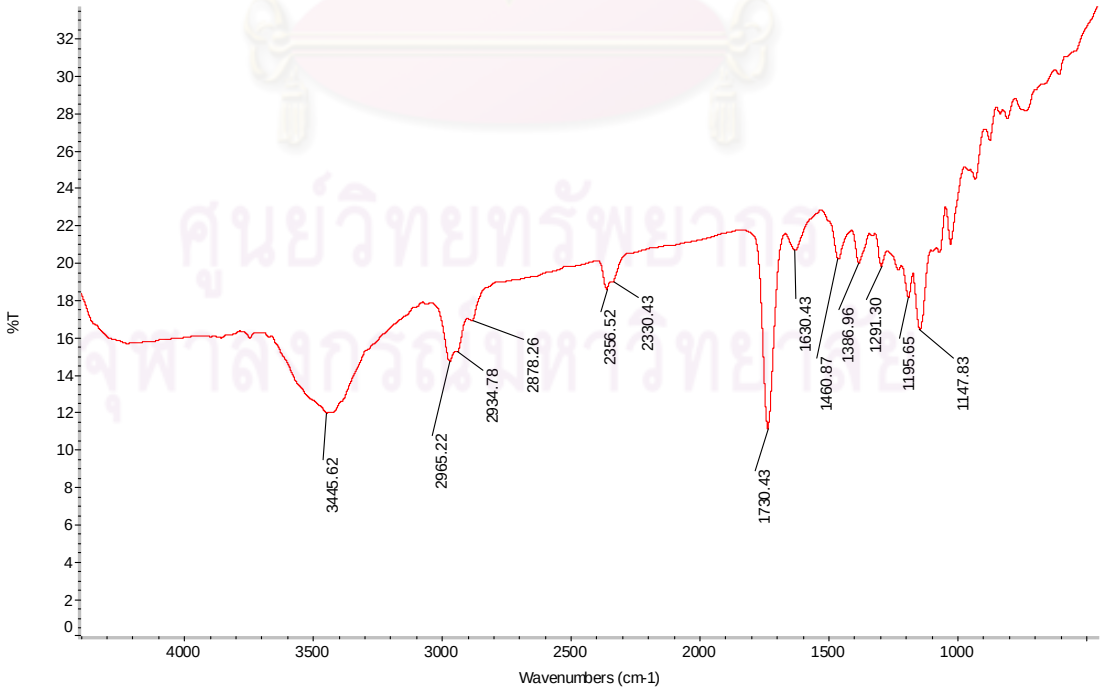


Figure S-26 IR spectrum of compound **4** (KBr)

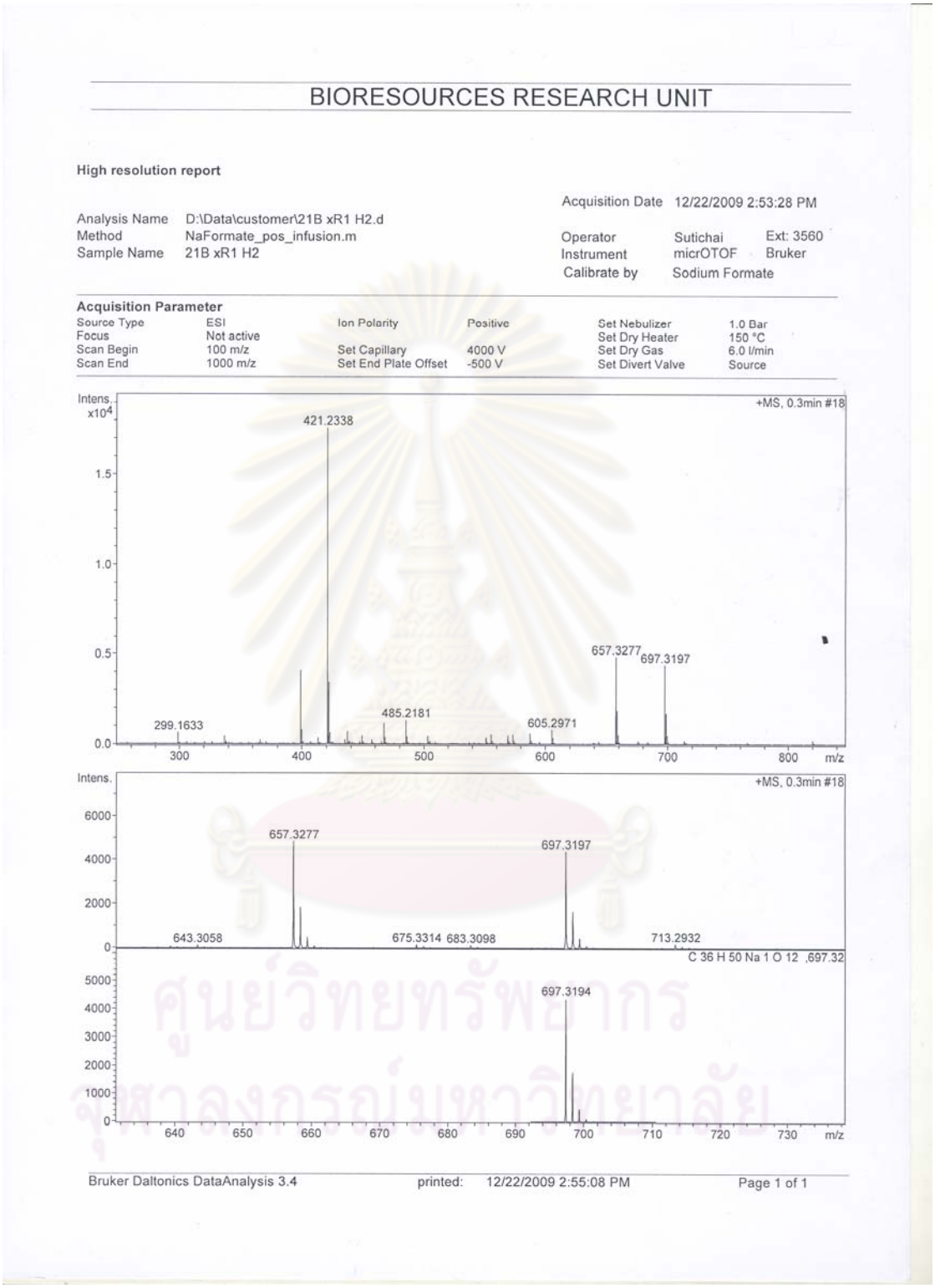


Figure S-27 HRESIMS Mass spectrum of compound **4**

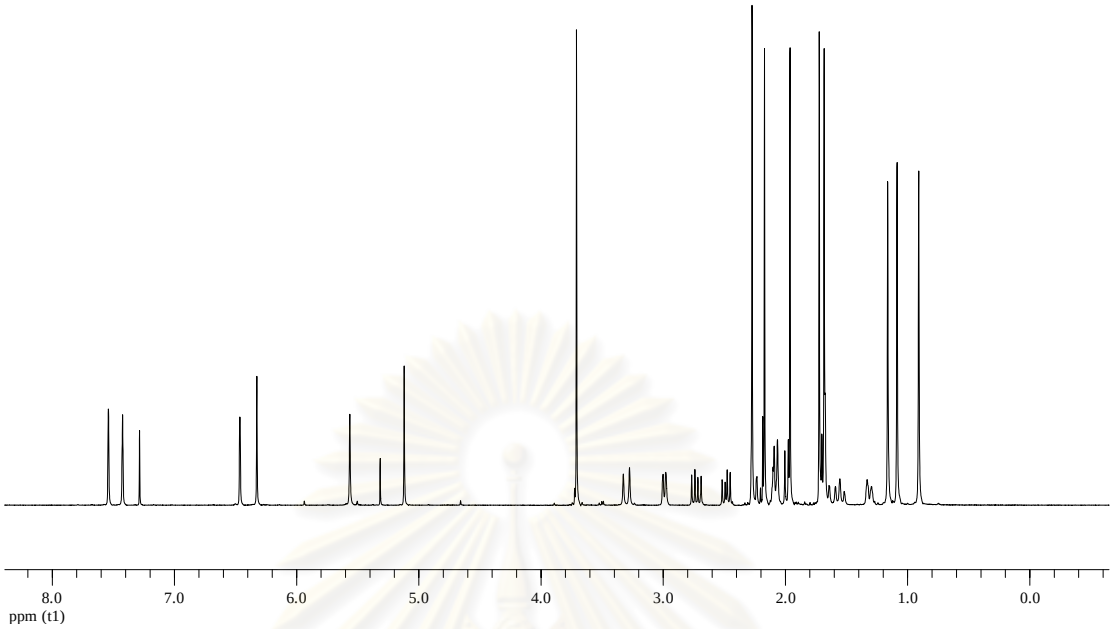


Figure S-28 ^{1}H (400 MHz) NMR spectrum of compound **5** ($CDCl_3$)

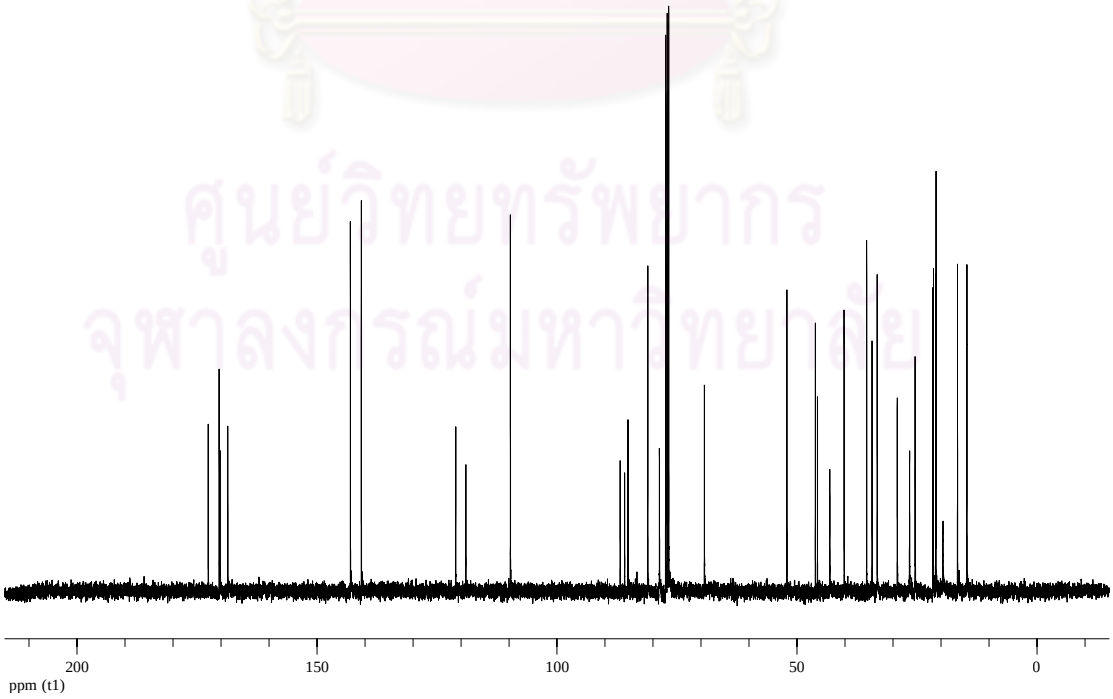


Figure S-29 ^{13}C NMR (100 MHz) spectrum of compound **5** ($CDCl_3$)

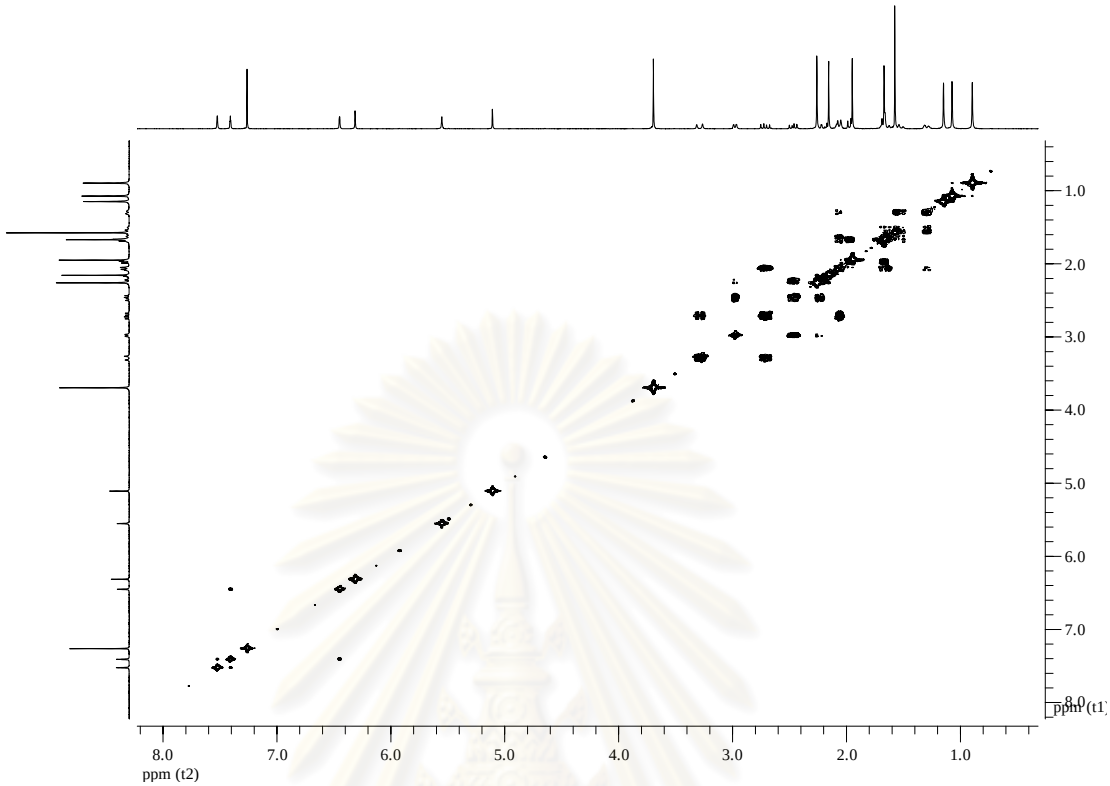


Figure S-30 ^{1}H-^{1}H COSY spectrum of compound **5** ($CDCl_3$)

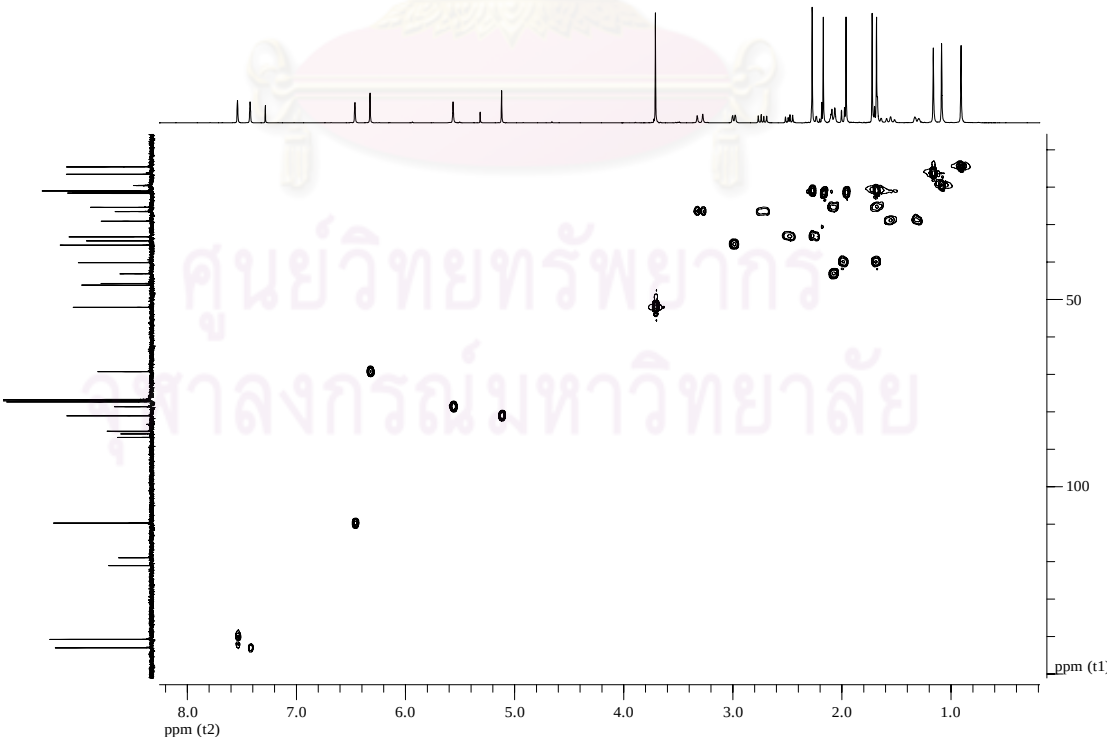


Figure S-31 HSQC spectrum of compound **5**

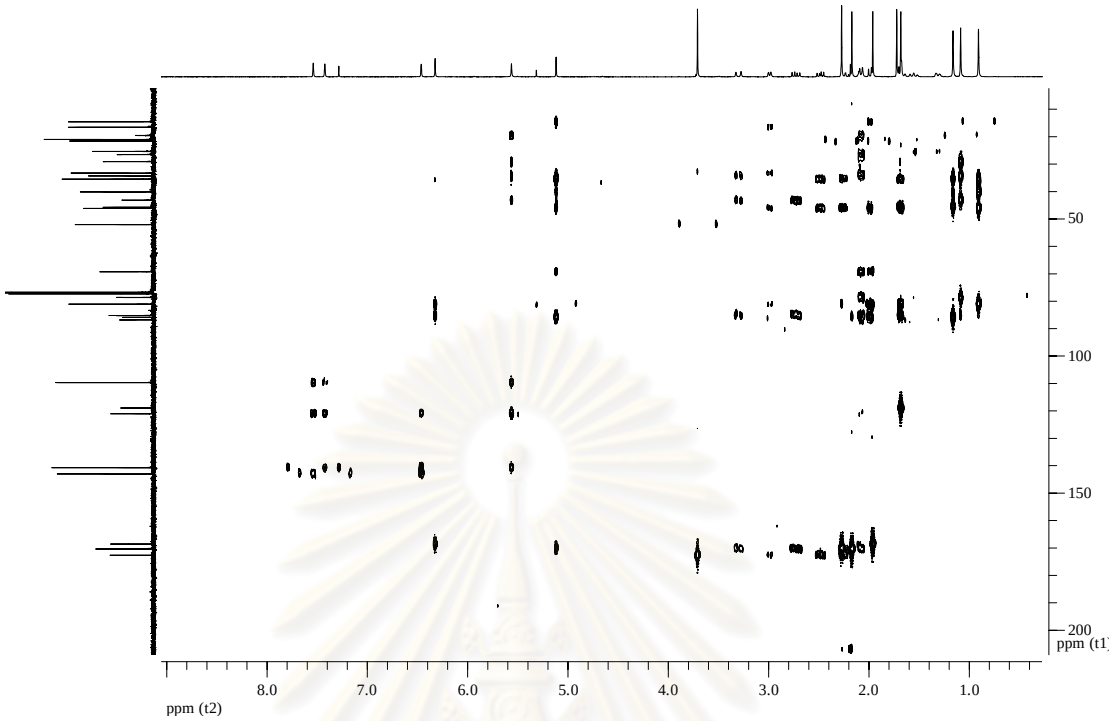


Figure S-32 HMBC spectrum of compound **5**

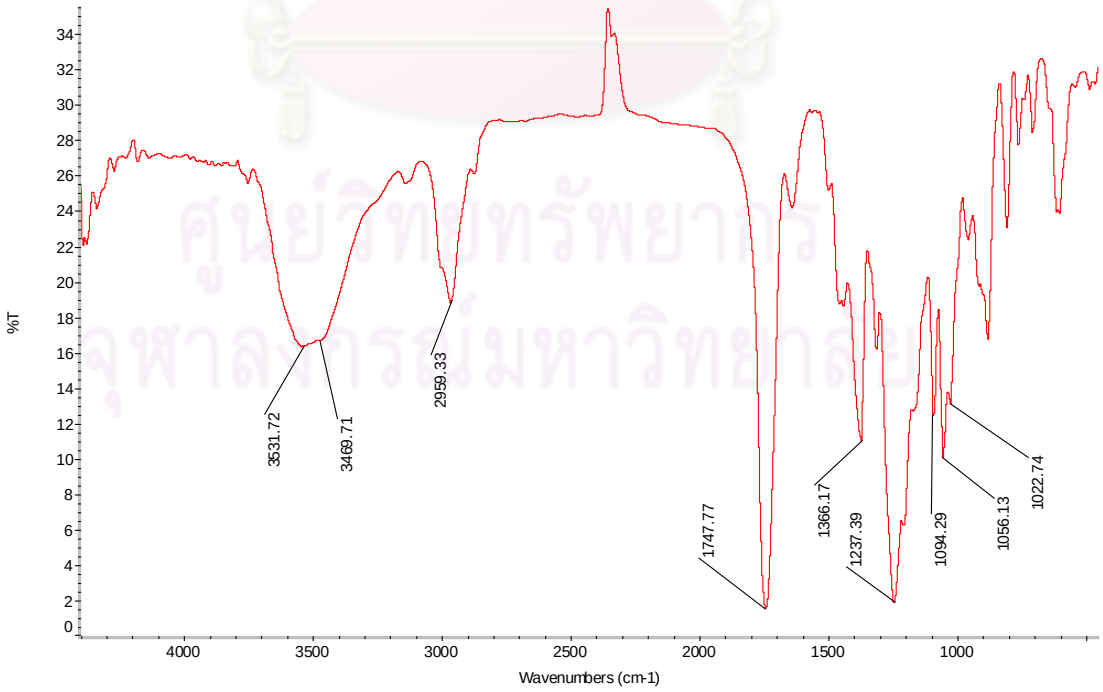


Figure S-33 IR spectrum of compound **5** (KBr)

Table E-1. Crystal data and structure refinement for compound **5**

Identification code	Xyloccensin E
Empirical formula	$C_{35}\ H_{42}\ O_{14}$
Formula weight	686.69
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	hexagonal, P6
Unit cell dimensions	a = 17.8937(4) Å alpha = 90 deg.
	b = 17.8937(4) Å beta = 90 deg.
	c = 19.7758(4) Å gamma = 120 deg.
Volume	5483.6(2) $Å^3$
Z, Calculated density	6, 1.248 Mg/m^3
Absorption coefficient	0.097 mm^{-1}
F(000)	2184
Crystal size	? x ? x ? mm
Theta range for data collection	2.44 to 23.83 deg.
Limiting indices	-16<=h<=20, -20<=k<=20,-20<=l<=22
Reflections collected / unique	26863 / 2879 [R_{int} = 00295]
Completeness to theta = 23.83	98.70 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2879 / 1 / 456
Goodness-of-fit on F^2	1.029
Final R indices [I>2sigma(I)]	R_1 = 0.0420, *wR2* = 0.1115
R indices (all data)	R_1 = 0.0506, *wR2* = 0.1191
Absolute structure parameter	-10(10)
Largest diff. peak and hole	0.412 and -0.172 $e.Å^3$

Table E-2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for compound **5** is defined as one third of the trace of the orthogonalized Uij tensor

	x	y	z	U(eq)
C(1)	-673(5)	7446(5)	10972(3)	95(2)
C(2)	-467(3)	6839(3)	10702(2)	55(1)
C(3)	-270(4)	6529(4)	11253(3)	80(2)
C(4)	-561(5)	7470(7)	11635(4)	110(3)
C(5)	-477(3)	6586(3)	9985(2)	44(1)
C(6)	-337(3)	7389(3)	8943(3)	58(1)
C(7)	-991(3)	6583(3)	8584(2)	46(1)
C(8)	-1322(2)	5735(2)	8962(2)	37(1)
C(9)	-1348(2)	5845(3)	9729(2)	39(1)
C(10)	-1517(2)	5001(2)	10085(2)	28(1)
C(11)	-2057(2)	4211(3)	9648(2)	39(1)
C(12)	-1572(2)	4224(2)	9012(2)	34(1)
C(13)	-958(2)	5157(2)	8730(2)	32(1)
C(14)	-1630(2)	4187(2)	7873(2)	38(1)
C(17)	-623(2)	3860(2)	8380(2)	32(1)
C(18)	-1181(2)	3617(2)	9027(2)	32(1)
C(19)	-516(2)	3689(2)	9569(2)	35(1)
C(20)	184(2)	3629(2)	9117(2)	35(1)
C(21)	-237(2)	3280(2)	8429(2)	39(1)
C(22)	200(2)	4789(2)	8476(2)	31(1)
C(23)	799(2)	4567(2)	8895(2)	32(1)
C(24)	1992(2)	5385(2)	9635(2)	39(1)
C(25)	2207(3)	5871(3)	10277(2)	54(1)
C(26)	-3(2)	5447(2)	8831(2)	30(1)
C(27)	1191(2)	6917(2)	8875(3)	50(1)
C(28)	1682(3)	7687(3)	8441(3)	75(2)
C(29)	-883(2)	3051(3)	10154(2)	46(1)
C(30)	-332(3)	3344(4)	10773(2)	62(1)
C(31A)	-111(19)	2990(40)	11842(11)	128(13)
C(31B)	-90(30)	2720(50)	11840(30)	180(30)
C(32)	654(3)	3206(3)	9436(3)	51(1)
C(33)	-1935(2)	2672(2)	8983(2)	44(1)
C(34)	-2138(3)	4045(3)	7240(2)	56(1)
C(35)	-2090(3)	6018(3)	9897(2)	51(1)
C(36)	1321(2)	5661(2)	7655(2)	40(1)
C(37)	1437(3)	5794(3)	6924(2)	65(1)
O(1)	-327(3)	6898(4)	11824(2)	109(2)
O(2)	-180(2)	7377(2)	9600(2)	57(1)
O(3)	32(3)	8069(2)	8653(2)	97(1)
O(4)	-1091(2)	5064(2)	8008(1)	37(1)
O(5)	-2170(1)	3878(2)	8443(1)	39(1)
O(6)	-1129(2)	3775(2)	7798(1)	38(1)

Table E-2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for compound **5** is defined as one third of the trace of the orthogonalized Uij tensor (continued)

	x	y	z	U(eq)
O(7)	1173(1)	5128(2)	9457(1)	33(1)
O(8)	2457(2)	5217(2)	9309(2)	68(1)
O(9)	494(2)	6288(2)	8528(2)	40(1)
O(10)	1353(2)	6849(2)	9449(2)	59(1)
O(11)	221(3)	4046(3)	10908(2)	105(2)
O(12)	-560(3)	2686(3)	11197(2)	97(2)
O(13)	499(2)	5050(2)	7795(1)	37(1)
O(14)	1868(2)	6032(2)	8076(2)	51(1)

Table E-3. Bond lengths [Å] and angles [deg.] for compound **5**

C(1)-C(4)	1.325(11)	C(24)-C(25)	1.477(6)
C(1)-C(2)	1.414(8)	C(26)-O(9)	1.441(4)
C(2)-C(3)	1.347(8)	C(27)-O(10)	1.193(6)
C(2)-C(5)	1.487(6)	C(27)-O(9)	1.374(5)
C(3)-O(1)	1.338(8)	C(27)-C(28)	1.482(7)
C(4)-O(1)	1.337(11)	C(29)-C(30)	1.492(7)
C(5)-O(2)	1.453(5)	C(30)-O(11)	1.177(6)
C(5)-C(9)	1.541(6)	C(30)-O(12)	1.333(6)
C(6)-O(3)	1.201(6)	C(31A)-O(12)	1.46(3)
C(6)-O(2)	1.333(6)	C(31B)-O(12)	1.50(5)
C(6)-C(7)	1.505(7)	C(36)-O(14)	1.201(5)
C(7)-C(8)	1.521(6)	C(36)-O(13)	1.353(5)
C(8)-C(9)	1.533(6)	C(36)-C(37)	1.463(6)
C(8)-C(13)	1.543(5)	C(4)-C(1)-C(2)	107.7(8)
C(9)-C(35)	1.542(6)	C(3)-C(2)-C(1)	103.5(5)
C(9)-C(10)	1.554(6)	C(3)-C(2)-C(5)	127.7(5)
C(10)-C(11)	1.520(6)	C(1)-C(2)-C(5)	128.7(5)
C(11)-C(12)	1.520(5)	O(1)-C(3)-C(2)	112.3(6)
C(12)-O(5)	1.461(4)	C(1)-C(4)-O(1)	110.7(7)
C(12)-C(18)	1.558(5)	O(2)-C(5)-C(2)	105.0(3)
C(12)-C(13)	1.572(5)	O(2)-C(5)-C(9)	112.7(3)
C(13)-O(4)	1.444(4)	C(2)-C(5)-C(9)	115.4(4)
C(13)-C(26)	1.531(5)	O(3)-C(6)-O(2)	117.9(5)
C(14)-O(4)	1.397(4)	O(3)-C(6)-C(7)	121.1(5)
C(14)-O(5)	1.404(5)	O(2)-C(6)-C(7)	120.9(4)
C(14)-O(6)	1.426(5)	C(6)-C(7)-C(8)	116.9(4)
C(14)-C(34)	1.493(6)	C(7)-C(8)-C(9)	112.3(3)
C(17)-O(6)	1.424(4)	C(7)-C(8)-C(13)	116.0(3)
C(17)-C(21)	1.509(5)	C(9)-C(8)-C(13)	115.5(3)
C(17)-C(18)	1.545(5)	C(8)-C(9)-C(5)	110.6(3)
C(17)-C(22)	1.589(5)	C(8)-C(9)-C(35)	108.9(3)
C(18)-C(33)	1.551(5)	C(5)-C(9)-C(35)	111.0(3)
C(18)-C(19)	1.558(5)	C(8)-C(9)-C(10)	109.1(3)
C(19)-C(29)	1.525(5)	C(5)-C(9)-C(10)	108.0(3)
C(19)-C(20)	1.585(5)	C(35)-C(9)-C(10)	109.2(3)
C(20)-C(32)	1.522(6)	C(11)-C(10)-C(9)	111.8(3)
C(20)-C(21)	1.529(6)	C(12)-C(11)-C(10)	111.3(3)
C(20)-C(23)	1.541(5)	O(5)-C(12)-C(11)	109.8(3)
C(22)-O(13)	1.438(4)	O(5)-C(12)-C(18)	102.0(3)
C(22)-C(23)	1.554(5)	C(11)-C(12)-C(18)	115.2(3)
C(22)-C(26)	1.560(5)	O(5)-C(12)-C(13)	98.7(3)
C(23)-O(7)	1.422(4)	C(11)-C(12)-C(13)	113.6(3)
C(24)-O(8)	1.203(5)	C(18)-C(12)-C(13)	115.3(3)
C(24)-O(7)	1.346(4)	O(4)-C(13)-C(26)	105.0(3)

Table E-3. Bond lengths [Å] and angles [deg.] for compound **5** (continued)

O(4)-C(13)-C(8)	105.7(3)	O(8)-C(24)-C(25)	126.9(4)
C(26)-C(13)-C(8)	120.6(3)	O(7)-C(24)-C(25)	110.1(3)
O(4)-C(13)-C(12)	104.0(3)	O(9)-C(26)-C(13)	107.5(3)
C(26)-C(13)-C(12)	112.5(3)	O(9)-C(26)-C(22)	110.2(3)
C(8)-C(13)-C(12)	107.6(3)	C(13)-C(26)-C(22)	109.1(3)
O(4)-C(14)-O(5)	104.0(3)	O(10)-C(27)-O(9)	123.9(4)
O(4)-C(14)-O(6)	110.0(3)	O(10)-C(27)-C(28)	125.7(4)
O(5)-C(14)-O(6)	111.6(3)	O(9)-C(27)-C(28)	110.3(5)
O(4)-C(14)-C(34)	111.8(3)	C(30)-C(29)-C(19)	113.5(4)
O(5)-C(14)-C(34)	111.4(3)	O(11)-C(30)-O(12)	121.4(5)
O(6)-C(14)-C(34)	108.1(3)	O(11)-C(30)-C(29)	128.0(5)
O(6)-C(17)-C(21)	117.6(3)	O(12)-C(30)-C(29)	110.5(4)
O(6)-C(17)-C(18)	110.6(3)	O(14)-C(36)-O(13)	124.1(4)
C(21)-C(17)-C(18)	101.9(3)	O(14)-C(36)-C(37)	125.8(4)
O(6)-C(17)-C(22)	114.6(3)	O(13)-C(36)-C(37)	110.1(4)
C(21)-C(17)-C(22)	102.0(3)	C(3)-O(1)-C(4)	105.8(5)
C(18)-C(17)-C(22)	109.0(3)	C(6)-O(2)-C(5)	123.2(3)
C(17)-C(18)-C(33)	110.4(3)	C(14)-O(4)-C(13)	107.4(3)
C(17)-C(18)-C(12)	104.0(3)	C(14)-O(5)-C(12)	103.7(2)
C(33)-C(18)-C(12)	108.0(3)	C(14)-O(6)-C(17)	113.0(3)
C(17)-C(18)-C(19)	100.8(3)	C(24)-O(7)-C(23)	119.6(3)
C(33)-C(18)-C(19)	109.9(3)	C(27)-O(9)-C(26)	118.8(3)
C(12)-C(18)-C(19)	123.0(3)	C(30)-O(12)-C(31B)	125(3)
C(29)-C(19)-C(18)	115.8(3)	C(30)-O(12)-C(31A)	111(2)
C(29)-C(19)-C(20)	115.8(3)	C(31B)-O(12)-C(31A)	20(4)
C(18)-C(19)-C(20)	101.7(3)	C(36)-O(13)-C(22)	122.1(3)
C(32)-C(20)-C(21)	116.5(3)		
C(32)-C(20)-C(23)	112.9(3)		
C(21)-C(20)-C(23)	97.4(3)		
C(32)-C(20)-C(19)	117.0(3)		
C(21)-C(20)-C(19)	106.5(3)		
C(23)-C(20)-C(19)	104.2(3)		
C(17)-C(21)-C(20)	94.5(3)		
O(13)-C(22)-C(23)	113.0(3)		
O(13)-C(22)-C(26)	111.4(3)		
C(23)-C(22)-C(26)	113.8(3)		
O(13)-C(22)-C(17)	103.1(3)		
C(23)-C(22)-C(17)	101.1(3)		
C(26)-C(22)-C(17)	113.6(3)		
O(7)-C(23)-C(20)	111.9(3)		
O(7)-C(23)-C(22)	112.2(3)		
C(20)-C(23)-C(22)	102.9(3)		
O(8)-C(24)-O(7)	123.0(3)		

Symmetry transformations used to generate equivalent atoms:

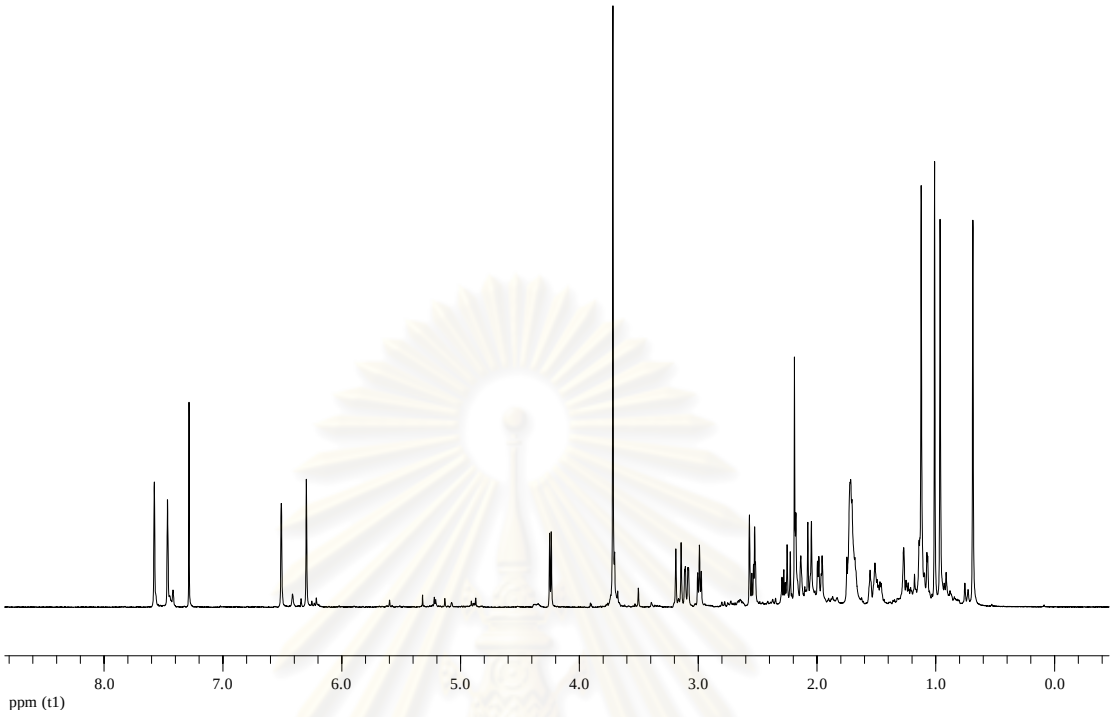


Figure S-34 ^{1}H NMR (400 MHz) spectrum of compound **6** ($CDCl_3$)

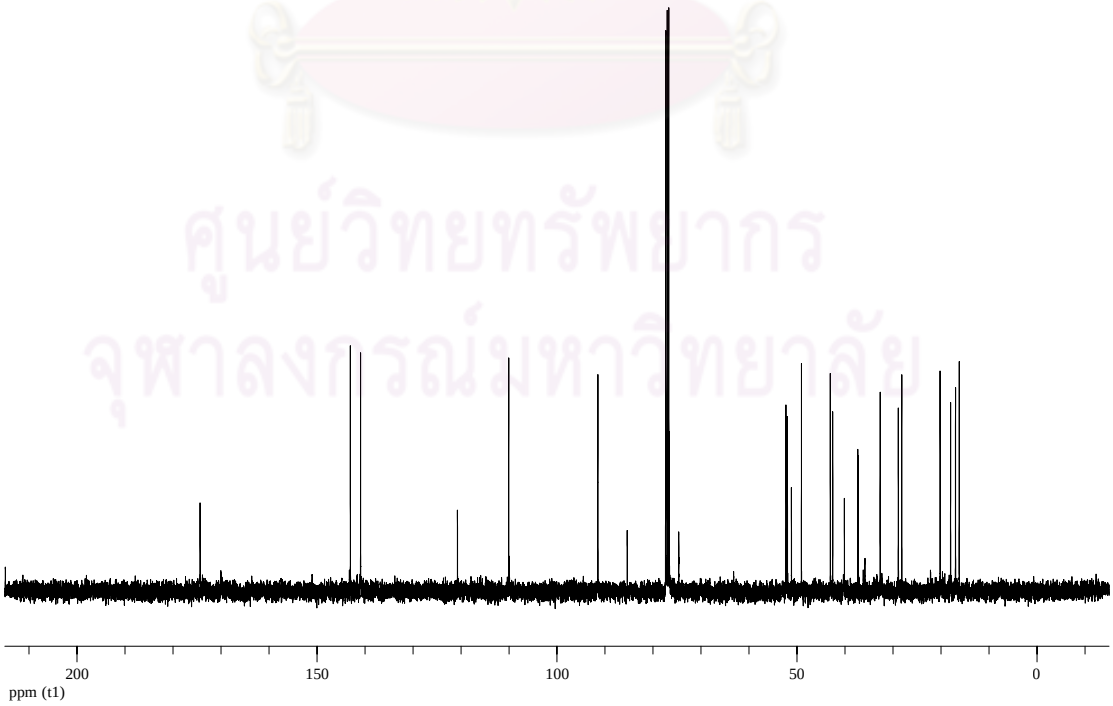


Figure S-35 ^{13}C NMR (100 MHz) spectrum of compound **6** ($CDCl_3$)

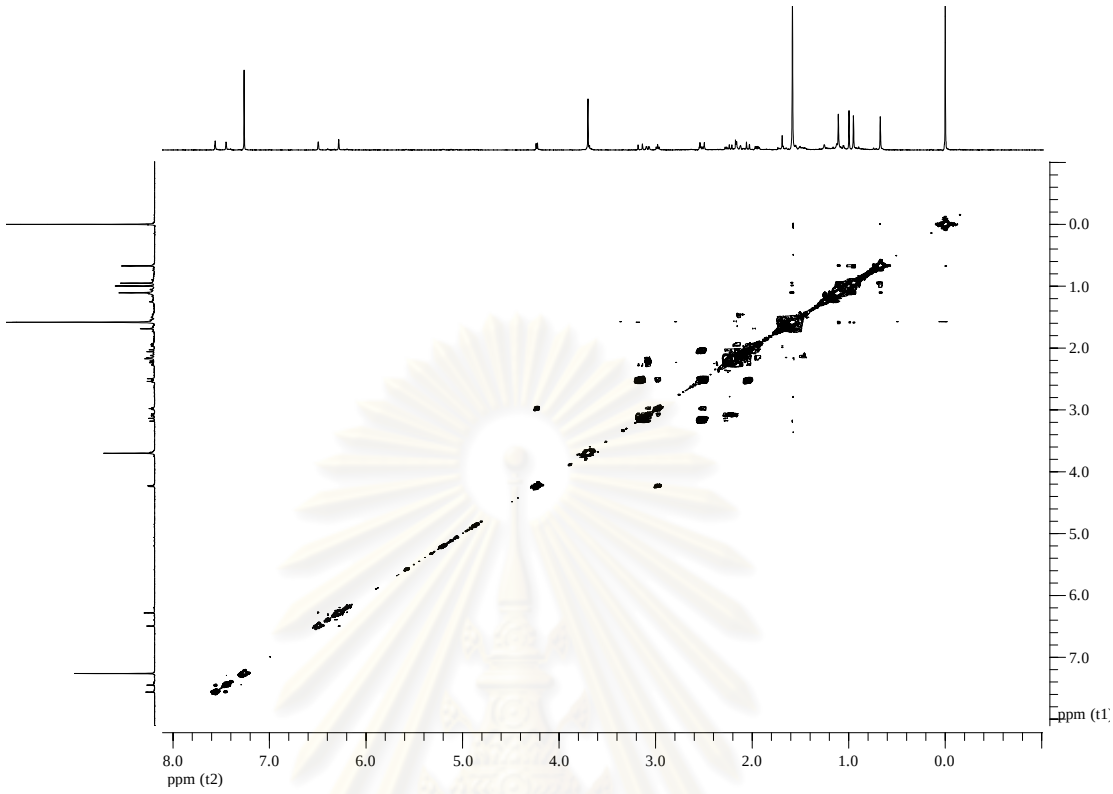


Figure S-36 ^{1}H-^{1}H COSY spectrum of compound **6** ($CDCl_3$)

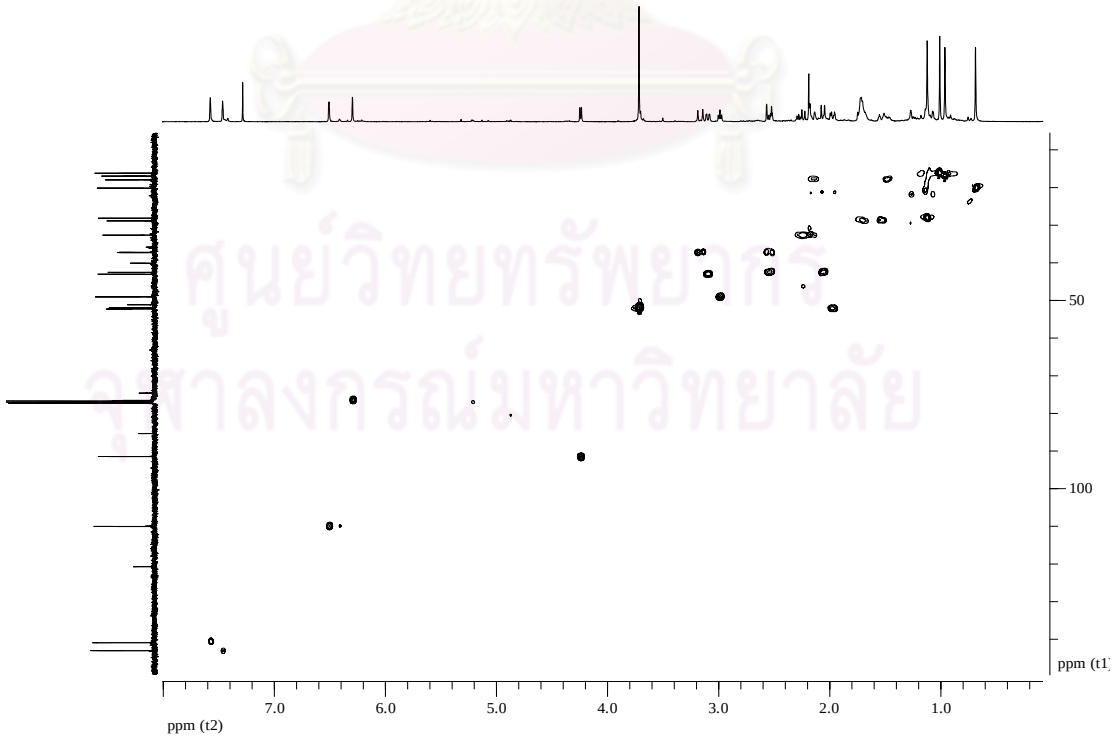


Figure S-37 HSQC spectrum of compound **6**

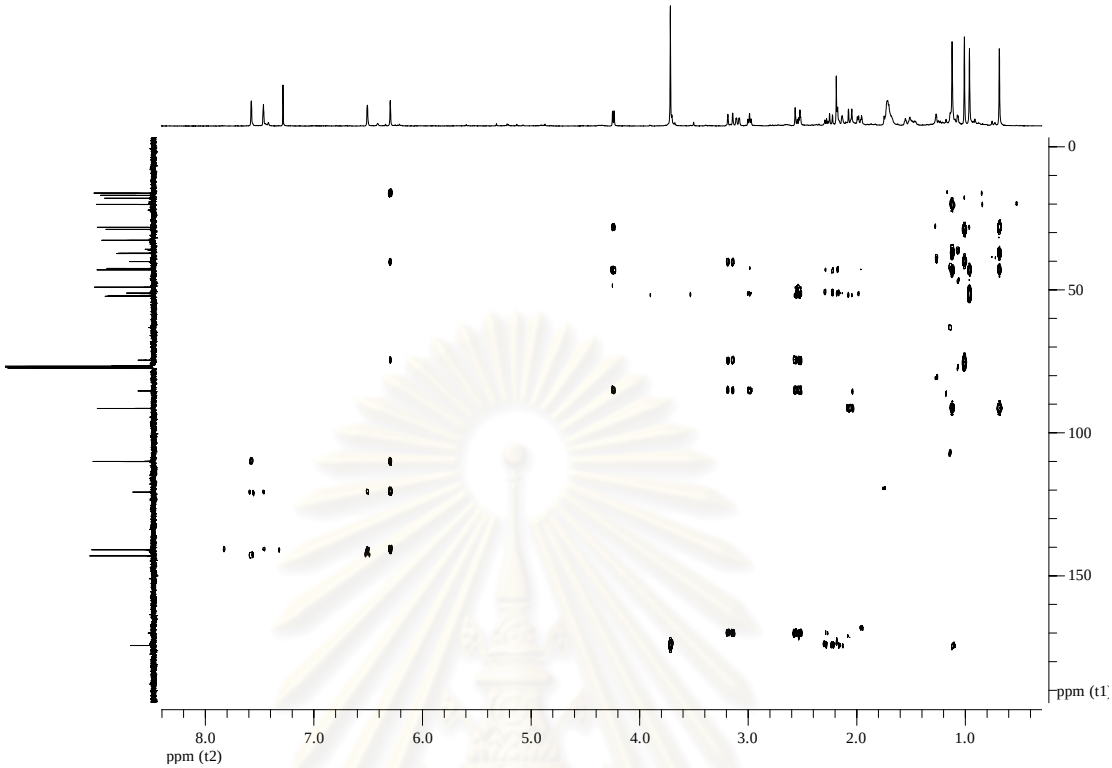


Figure S-38 HMBC spectrum of compound **6**

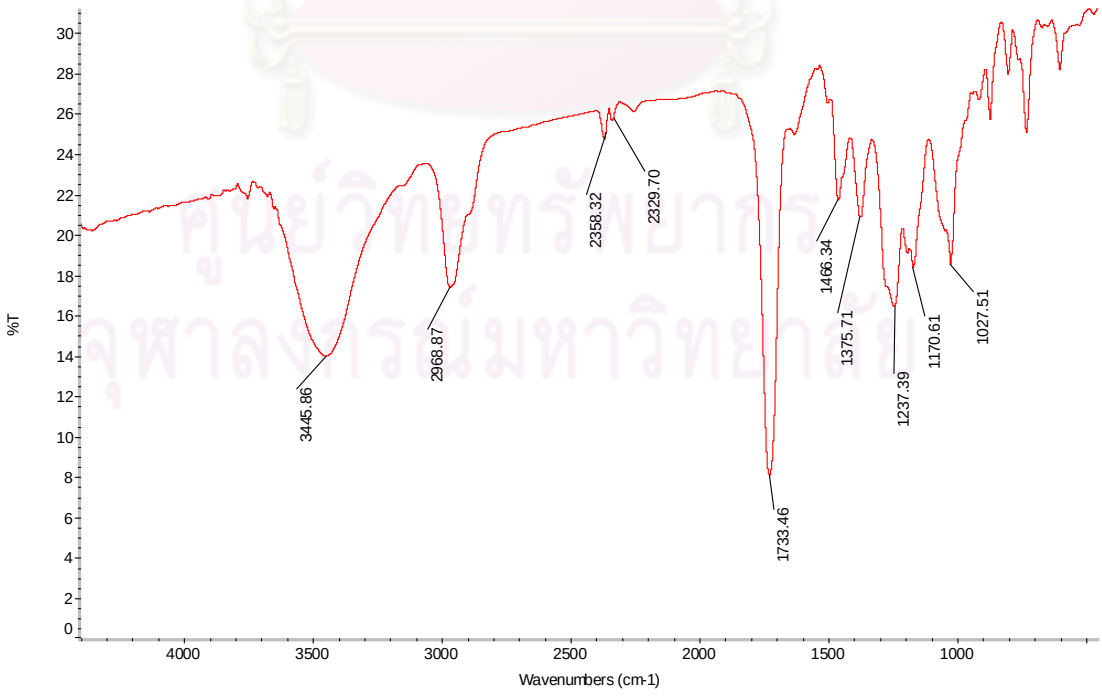


Figure S-39 IR spectrum of compound **6** (KBr)

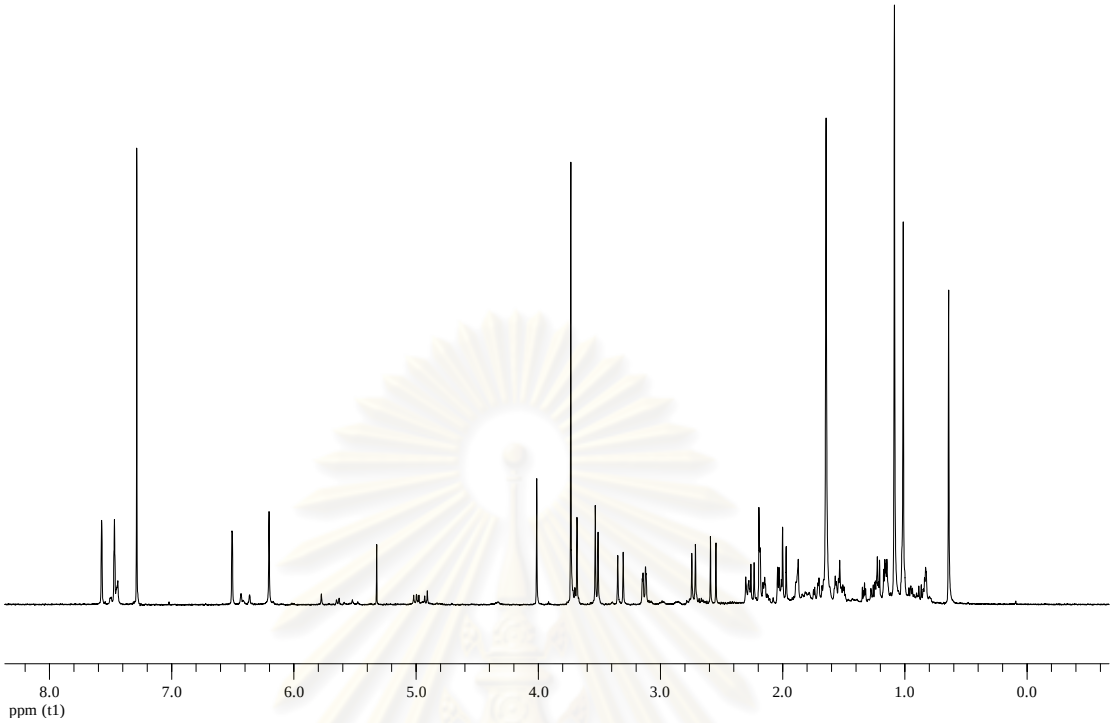


Figure S-40 ^{1}H NMR (400 MHz) spectrum of compound **7** ($CDCl_3$)

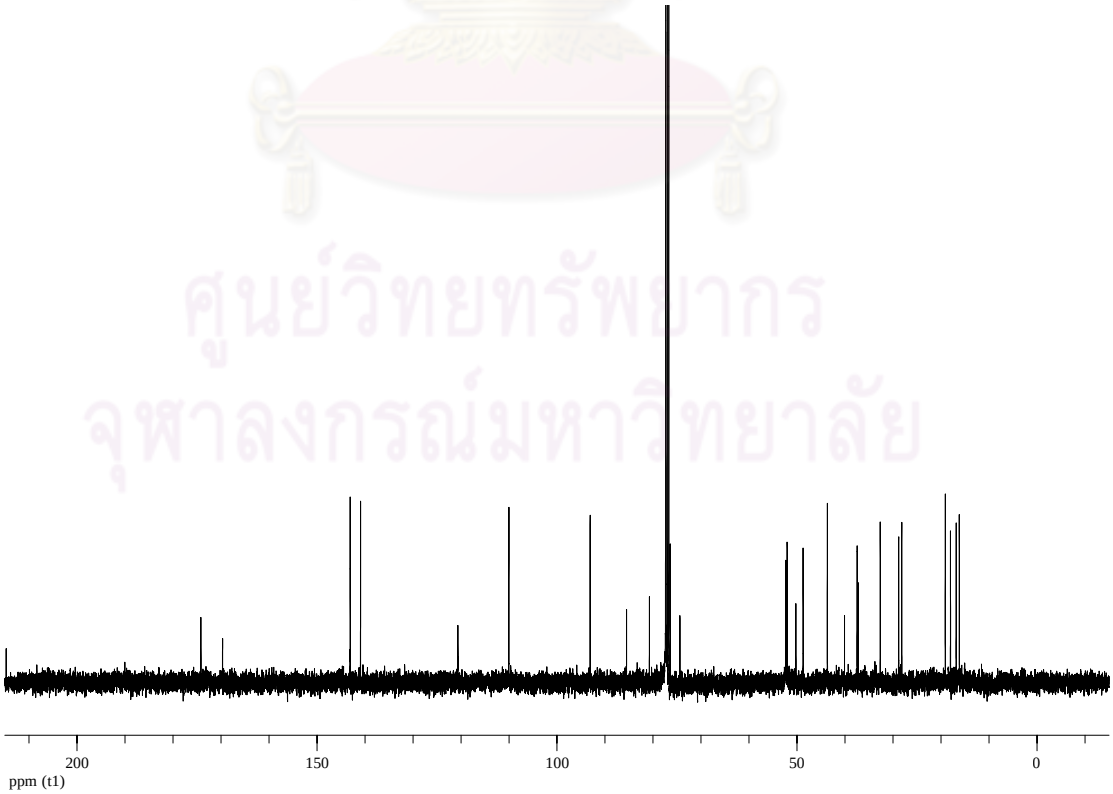


Figure S-41 ^{13}C NMR (100 MHz) spectrum of compound **7** ($CDCl_3$)

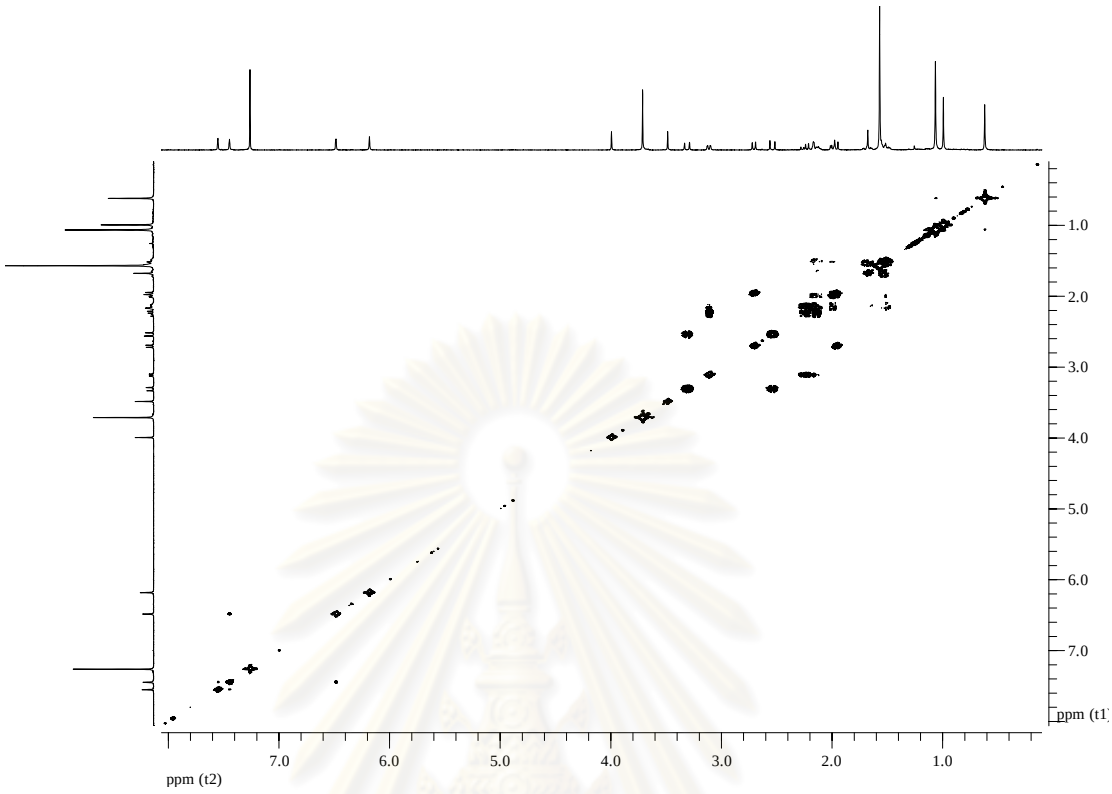


Figure S-42 ^{1}H-^{1}H COSY spectrum of compound **7** ($CDCl_3$)

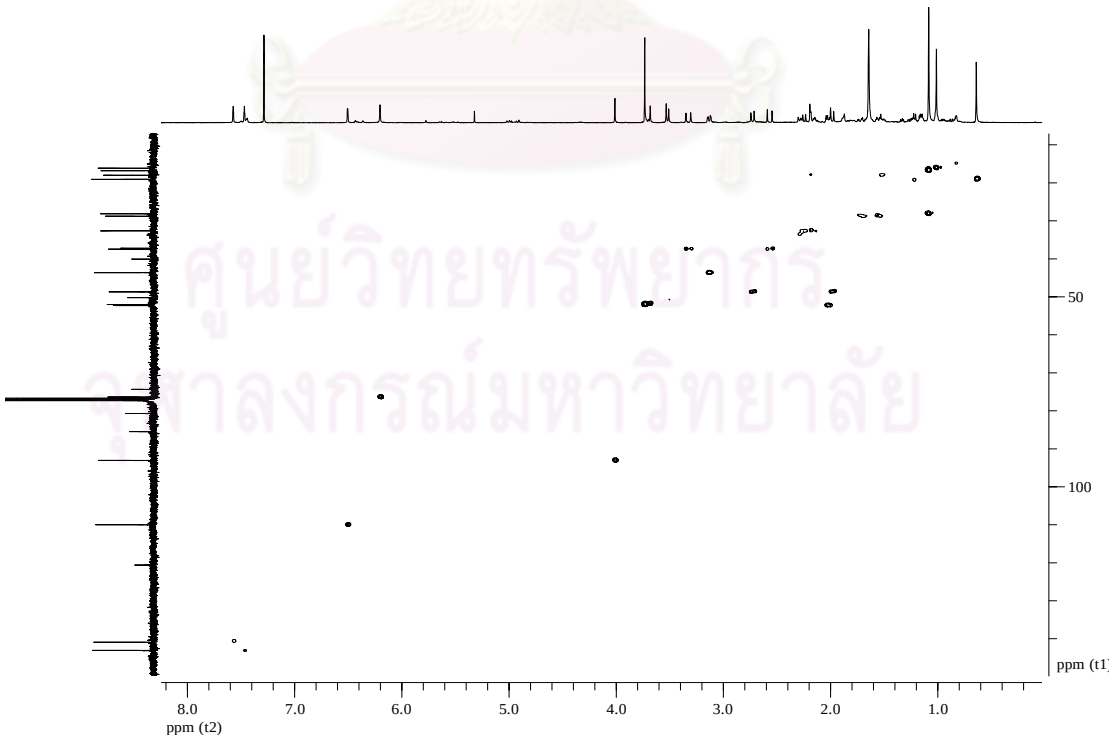


Figure S-43 HSQC spectrum of compound **7**

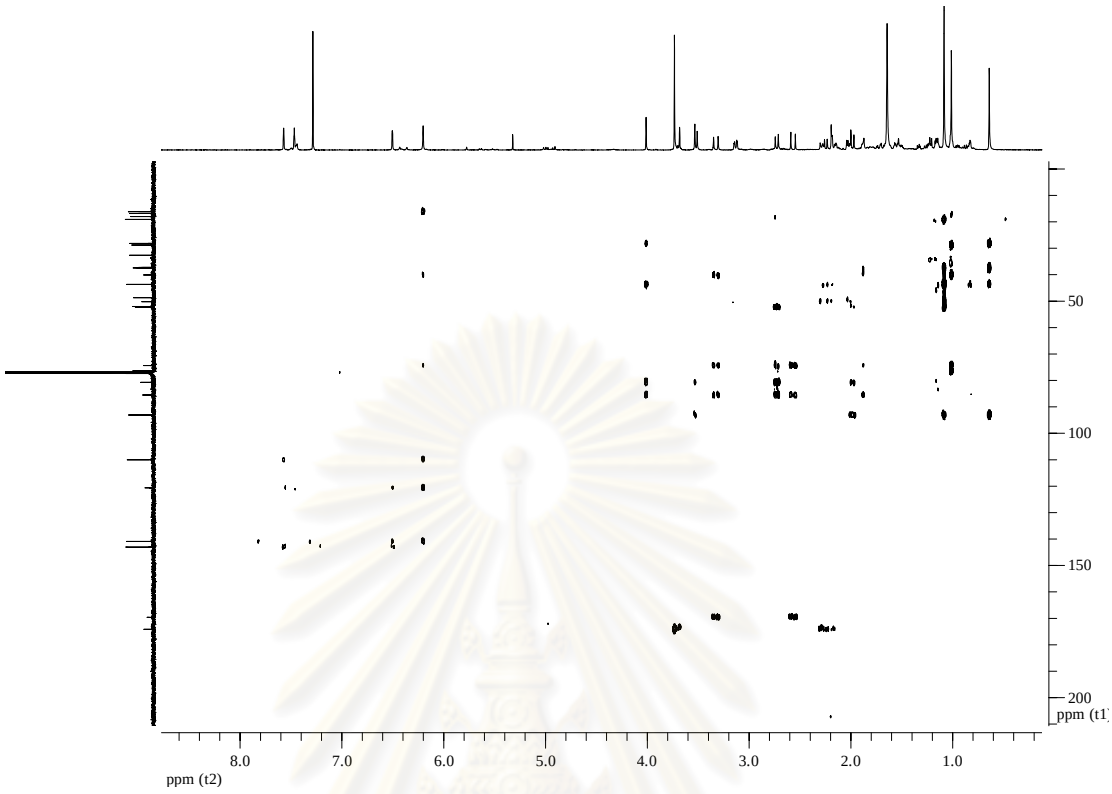


Figure S-44 HMBC spectrum of compound **7**

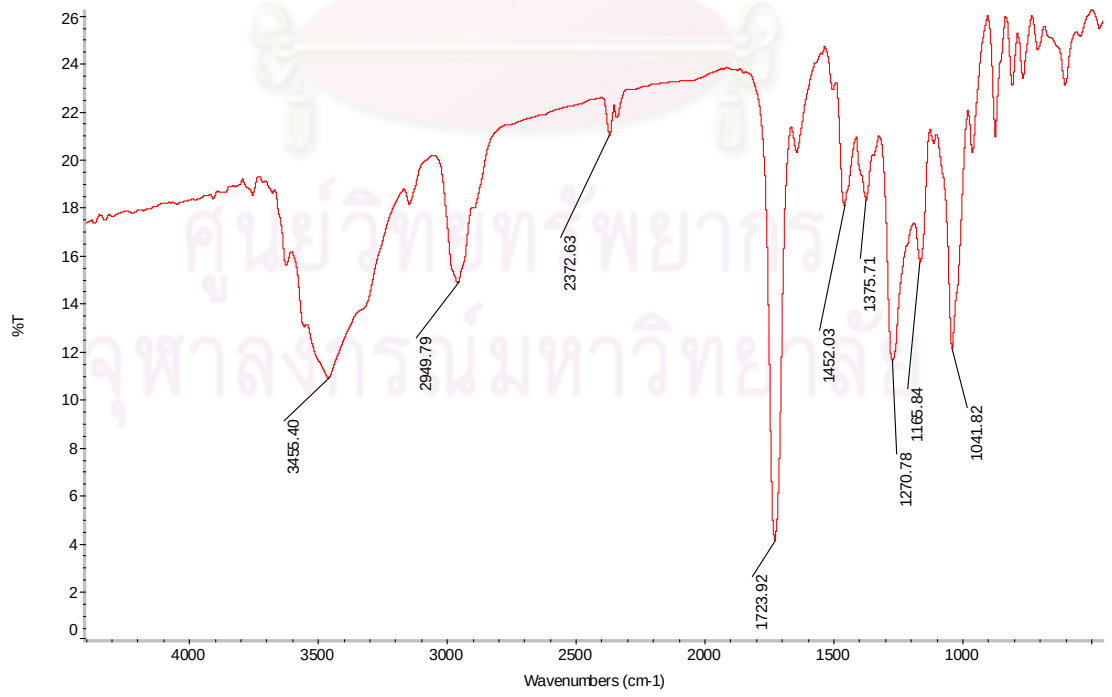


Figure S-45 IR spectrum of compound **7** (KBr)

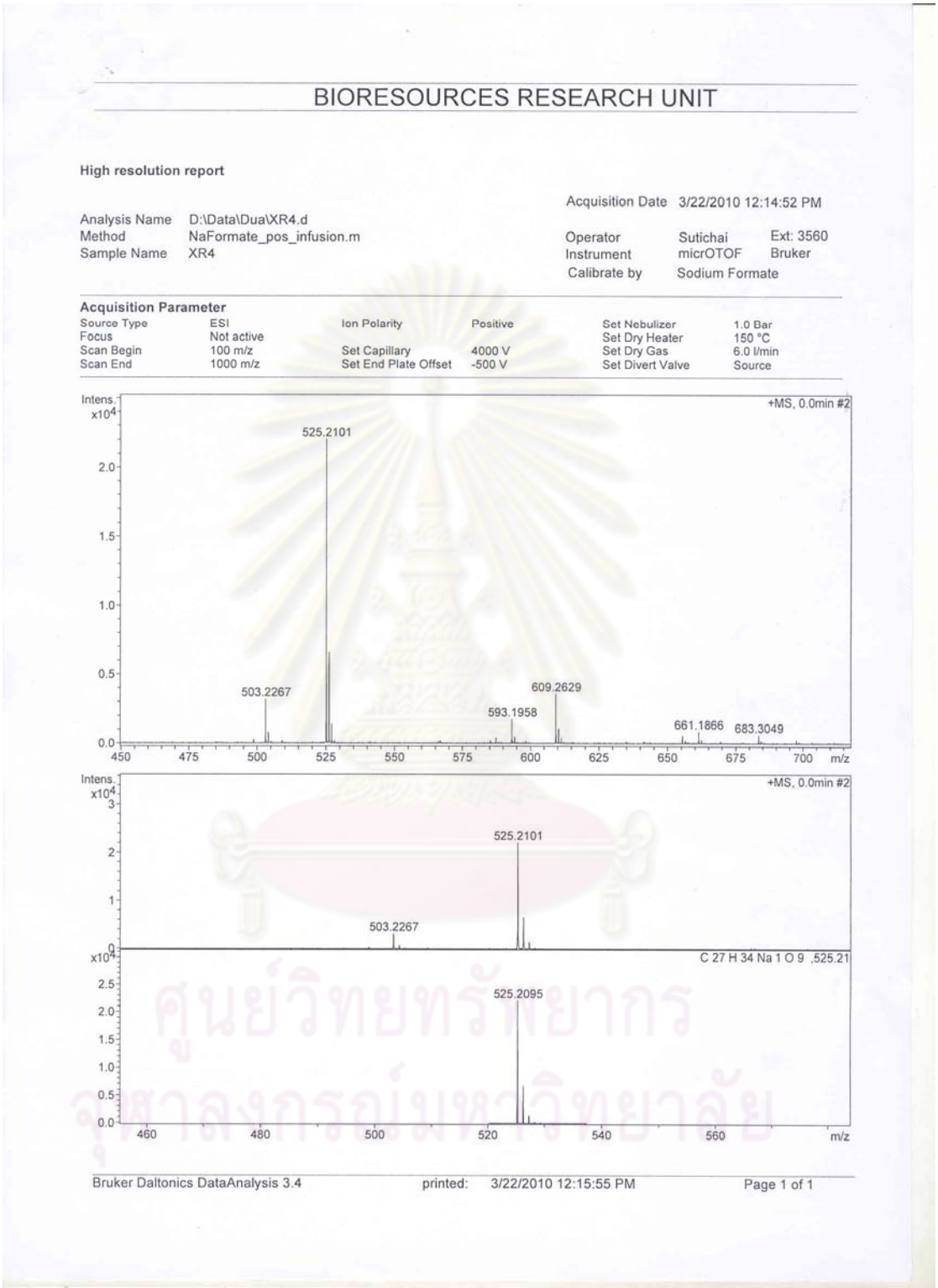


Figure S-46 HRESIMS Mass spectrum of compound **7**

VITAE

Mr. Chanin Sarigaputi was born on January 5, 1986 in Bangkok, Thailand. He graduated with Bachelor's Degree of Science in Biology from Faculty of Science, Kasetsart University, in 2007. During the time he was studying in the Master Degree in Biotechnology program, he received the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) for supporting his research project.

His present address is 310/887 Soi Songprapa 14, Songprapa Rd. Seekan, Donmuang, Bankok, Thailand, 10210, Tel: 081-7772977.