องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของรากและใบจันทน์ชะมด *Mansonia gagei* Drumm.

นางสาวพิมลพร เที่ยงธรรม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547

ISBN 974-53-1480-3

CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITIES OF THE ROOTS AND THE LEAVES OF *Mansonia gagei* Drumm.

Miss Pimonporn Tiengtham

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

Department of Chemistry
Faculty of Science
Chulalongkorn University

Academic Year 2004

ISBN 974-53-1480-3

Thesis Title	Chemical Constituents and Their Biological Activities of the Roots and the Leaves of *Mansonia gagei* Drumm.
By	Miss Pimonporn Tiengtham
Field of Study	Chemistry
Thesis Advisor	Assistant Professor Warinthorn Chavasiri, Ph.D.
Thesis Co-advisor	Pattara Sawasdee, Ph.D.

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

…… Deputy Dean for Administrative Affairs,
Acting Dean, The Faculty of Science
(Associate Professor Tharapong Vitidsant, Ph.D.)

THESIS COMMITTEE

…….. Chairman
(Professor Sophon Roengsumran, Ph.D.)

…… Thesis Advisor
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

…….. Thesis Co-advisor
(Pattara Sawasdee, Ph.D.)

…….. Member
(Associate Professor Nattaya Ngamrojanavanich, Ph.D.)

…….. Member
(Fuangfa Unob, Ph.D.)

พิมลพร เที่ยงธรรม : องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของรากและใบจันทน์ชะมด (CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITIES OF THE ROOTS AND THE LEAVES OF *Mansonia gagei* Drumm.) อ.ที่ปรึกษา : ผศ. ดร. วรินทร ชวศิริ, อ.ที่ปรึกษาร่วม : อ. ดร. พัฒทรา สวัสดี, 85 หน้า ISBN 974-53-1480-3

ผลการศึกษาฤทธิ์ต้านการเกิดฮีสทามีนเบื้องต้นของต้นจันทน์ชะมดโดยใช้เซลล์ RBL-2H3 ชี้ให้เห็นว่าสิ่งสกัดไดคลอโรมีเทนจากรากและใบ แสดงฤทธิ์ต้านการเกิดอาการแพ้ในระดับสูง จากการศึกษาองค์ประกอบทางเคมีของรากจันทน์ชะมด พบสารบริสุทธิ์ 9 ชนิด สามารถหาโครงสร้างของสารบริสุทธิ์ที่แยกได้โดยอาศัยสมบัติทางกายภาพและข้อมูลทางสเปกโทรสโกปีได้ 8 ชนิด ได้แก่ 2,5-dimethoxy-1,4-benzoquinone, mansonone G, vanillic acid, mansonone H, mansonone C, mansonone E, mansonone T และ stigmastane-3β,6α-diol สาร mansonone T พบว่าเป็นสารใหม่ จากการศึกษาองค์ประกอบทางเคมีของใบจันทน์ชะมดสามารถแยกได้สารบริสุทธิ์เพิ่มอีก 3 ชนิด สามารถหาโครงสร้างโดยอาศัยสมบัติทางกายภาพ และข้อมูลทางสเปกโทรสโกปีได้ 2 ชนิด คือ ได้แก่ 3,11-dioxo-β-amyrin, 11α-hydroxy-β-amyrin และของผสม 2 ชนิด ได้แก่ ของผสมของไฮโดรคาร์บอนโซ่ตรง และ ของผสมของกรดคาร์บอกซิลิกโซ่ตรง mansonones G และ C แสดงฤทธิ์ต้านฮีสทามีนสูงสุดเมื่อเทียบกับสารชนิดอื่น โดยมีค่า IC_{50} 212 μM และ 222 μM ตามลำดับ

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

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

ปีการศึกษา.....2547..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4572422623 : MAJOR CHEMISTRY

KEY WORD: *Mansonia gagei* / CHEMICAL CONSTITUENTS / BIOLOGICAL ACTIVITIES

PIMONPORN TIENGTHAM : CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITIES OF THE ROOTS AND THE LEAVES OF *Mansonia gagei* Drumm. ASSIST. PROF. WARINTHORN CHAVASIRI, Ph.D. AND PATTARA SAWASDEE, Ph.D, 85 pp. ISBN 974-53-1480-3.

The preliminary antihistaminic activity screening using RBL-2H3 cells of *Mansonia gagei* Drumm. revealed that the dichloromethane extracts from the roots and the leaves exhibited high antihistaminic activity. The chemical constituents investigation of the roots disclosed nine pure compounds. By means of physical properties, chemical reactions and spectroscopic evidences, the structures of eight compounds could be elucidated to 2,5-dimethoxy-1,4-benzoquinone, mansonone G, vanillic acid, mansonone H, mansonone C, mansonone E, mansonone T and stigmastane-3β,6α-diol. Mansonone T was disclosed to be a naturally new compound. Other three compounds could be isolated from the leaves of this plant. By means of physical properties, chemical reactions and spectroscopic evidences, the structures of two compounds could be deduced as 3,11-dioxo-β-amyrin, 11α-hydroxy-β-amyrin together with two mixtures: a mixture of long chain hydrocarbon and a mixture of long chain acid. Among isolated substances, mansonones G and C exhibited the highest antihistaminic activity against RBL-2H3 cells at IC_{50} 212 μM and 222 μM, respectively.

Department…..Chemistry……. Student's signature……..

Field of study…Chemistry……. Advisor's signature……..

Academic year…2004……... Co-advisor's signature…….

ACKNOWLEDGEMENTS

The author would like to express her appreciation to her advisor and co-advisor, Assistant Professor Dr. Warinthorn Chavasiri and Dr. Pattara Sawasdee for advice, assistance and opinions during this course of research. She is also grateful to the Thailand Research Fund for a 2004 Master research assistant fellowship (TRF Master Research Grants: TRF-MAG program) which supported her for financial and research expenses. Thanks are also extended to Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University for the support of chemicals and laboratory facilities and to the thesis committee, Professor Dr. Sophon Roengsumran, Associate Professor Dr. Nattaya Ngamrojanavanich and Dr. Fuangfa Unob for their valuable discussion and suggestion.

The author also acknowledged Professor Dr. Hideaki Otsuka, Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University, Japan, for providing the knowledge and practical skill on antihistaminic activity, and accepted the author for visitation to Japan. The author also would like to thank Associate Professor Dr. Tripetch Kanchanapoom, Faculty of Pharmaceutical Sciences, Khon Kaen University, for practical polar parts separation. Appreciation is also extended the Graduate School of Chulalongkorn University for granting partial financial support to conduct this research.

In addition, the author would also like to express her special thanks to Professor Dr. Udom Kokpol's Scholarship for supporting partial research fund to carry on the research at Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University, Japan. Finally, the author would like to express her gratitude to her parents and family members for their inspiration, understanding, great support and encouragement throughout the entire study.

CONTENTS

Page

Contents (Continued)

Page

List of Figures

Figures **Page**

List of Figures (continued)

Figures **Page**

List of Tables

List of Tables (Continued)

Tables **Page**

List of Schemes

Schemes **Page**

List of Abbreviations

br	=	broad
ºC	=	Degree Celsius
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chlorofrom
cm^{-1}	=	Unit of wave number
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
d	=	doublet (NMR)
dd	=	Doublet of doublet (NMR)
DMSO-d_6	=	Deuterated dimethylsulfoxide
EtOAc	=	Ethyl acetate
g	=	Gram (s)
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Single Quantum Coherence
^1H NMR	=	Proton-1 Nuclear Magnetic Resonance
H_2O	=	Water
Hz	=	Hertz (NMR)
IR	=	Infrared
J	=	Coupling constant
Kg	=	Kilogram (s)
m	=	multiplet (NMR)
MeOH	=	Methanol
mg	=	Milligram (s)
min	=	Minute
mL	=	Milliliter (s)
mm	=	Millimeter (s)
m.p.	=	Melting point
MS	=	Mass Spectrometry
MW	=	Molecular weight

List of Abbreviations (continued)

No.	=	Number
PIPES	=	Piperazine 1,4-bis (2-ethanesulfonic acid)
ppm	=	Part per million
R_f	=	Retarding facter in chromatography
RBL	=	Rat Basophilic Leukemia
s	=	Singlet (NMR)
s	=	Strong (IR)
t	=	Triplet (NMR)
TLC	=	Thin Layer Chromatography
w	=	weak (IR)
w/w	=	weight by weight
δ	=	Unit of chemical shift

CHAPTER I

INTRODUCTION

Naturally occurring organic chemical compounds or well-recognized as natural products have always fascinated chemists. Interesting and intriguing points of this field of chemistry involved their *in vivo* production and in their laboratory utilization and their importance as structural materials and biologically active molecules (substrates for life processes, toxins, hormones, drugs, *etc*.) is of unparalleled importance. [*1*]

The rapid growth of the study of natural products in recent years has been accompanied by the publication of numerous specialist monographs on alkaloids, carbohydrates, coumarins, acetylenes, terpenes, *etc*., and several on biosynthesis. [*2*] The important sources for novel drug discovered from terrestrial and marine plant materials, and their potential for application in medicine.

1.1 Botanical characteristics of *Mansonia gagei* Drumm.

The Sterculiaceae family was a large family in the plant kingdom. In Thailand 64 species were found; nonetheless, *Mansonia gagei* Drumm. is the only species belonging to *Mansonia* genus in Sterculiaceae family found in Thailand. [*3*]

M. gagei, a medicinal plant in Thailand, is a large tree and found in dry evergreen forests on the limestone hills. This plant is also known locally as “chan-chamod”, “chan-hom”, “chan-khao”, or “chan-phama” and, according to folklore belief, its heartwood is locally used as a cardiac stimulant, antiemetic, antidepressant and refreshment agent [*3*]. The bark is white-grey and quite smooth. The oblong-lanceolate leaf about 3-6 cm wide and 8-14 cm long. The flower is white and cluster. The pictures of the leaves and flowers of *M. gagei* are shown in Figure 1.1.

Figure 1.1 The leaves and flowers of *Mansonia gagei* Drumm.

1.2 Chemical constituents and literature review of *Mansonia* genus.

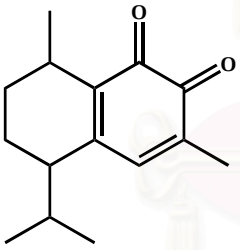
According to the literature review, there were only two species of *Mansonia* genus which have been examined on their chemical constituents: *M. altissima* Chev. and *M. gagei* Drumm.

In 1965 Bettolo *et al*. investigated chemical constituents of *M. altissima* and reported the occurrance of some sesquiterpenoids and mansonones. Based on the structures of isolated compounds, two major characteristics with the C15 empirical formula could be recognized as cadinane sesquiterpenoid skeleton and a quinonic character. [*4*]

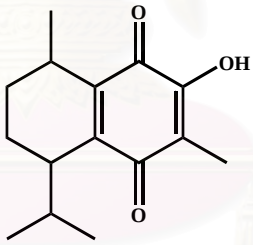
Table 1.1 The isolated compounds from *Mansonia altissima* Chev.

Scientific Name	Plant Parts	Organic Compounds	References
M. altissima	heartwood	mansonone A, mansonone B,	5
	heartwood	mansonone C, mansonone D,	
		mansonone E, mansonone F mansonone	6
		G, mansonone H	
	seed	strophanthidin 2,3-di-*O*-methyl-6-	7
		deoxy-β-D-glucopyranoside,	
		strophanthidin-3-*O*-methyl-6-deoxy-β-	
		D-glucopyranoside	
	heartwood	mansonone I	8
	heartwood	mansonone L	9

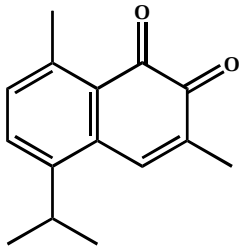
The structures of isolated compounds are shown below.



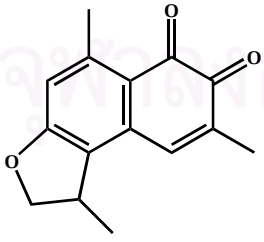
MANSONONE A



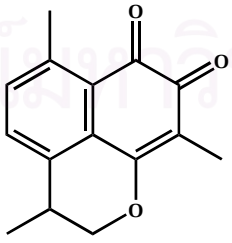
MANSONONE B



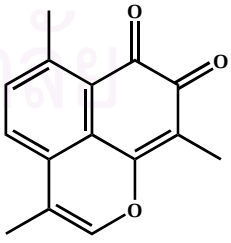
MANSONONE C



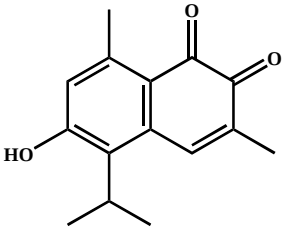
MANSONONE D



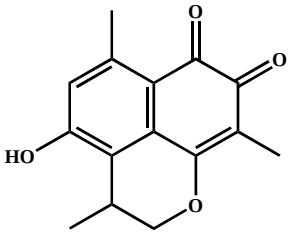
MANSONONE E



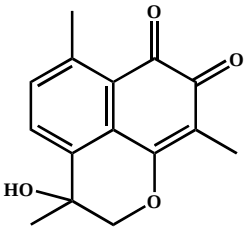
MANSONONE F



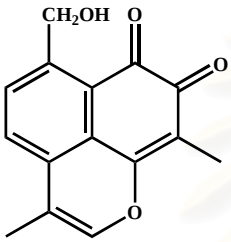
MANSONONE G



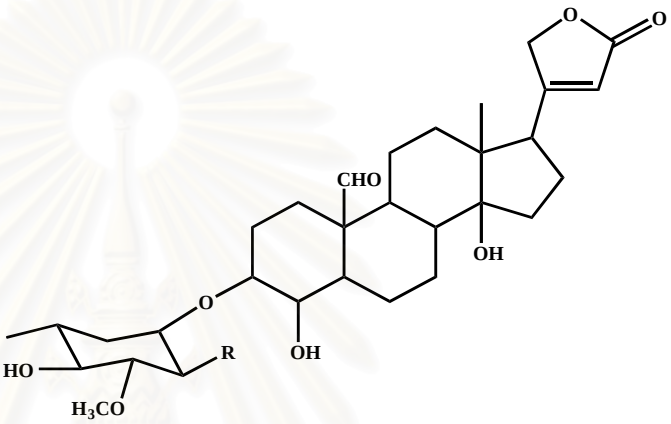
MANSONONE H



MANSONONE I



MANSONONE L



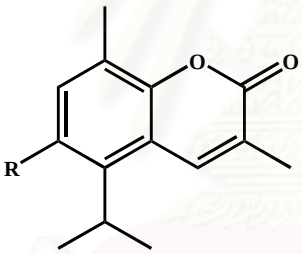
R = OCH_3 : MANSONIN
R = OH : STROPHOTHEVOSIDE

The first report described chemical constituents of the heartwoods of *Mansonia gagei* Drumm. was addressed in 1995. [*11*] Three novel compounds, mansorins A-C and three mansonones, mansonones C, G and H were isolated, but no biological activity was mentioned. Additionally, the document in 2002 reported the presence of other seven novel compounds and nine known compounds. [*4*,*24-27*] The structures of isolated compounds are shown in Table 1.2.

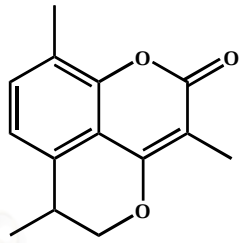
Table 1.2 The isolated compounds from the heartwood of *M. gagei*

Scientific name	Plant part	Organic compounds	References
M. gagei	heartwood	mansorin A, mansorin B, mansorin C, mansonone C, mansonone G and mansonone H	4,11,25
	heartwood	dehydroxoperezinone, 3-methoxy-4,5-dihydroxybenzaldehyde, mansonone N, mansonone O, mansonone P, mansonone Q, mansonone R, mansonone S and mansoxetane	4, 24-27

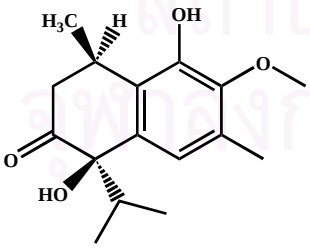
The structures of isolated compounds are shown below.



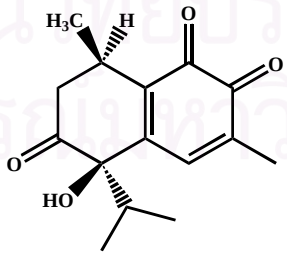
R = OCH_3 : MANSORIN A
R = OH : MANSORIN B



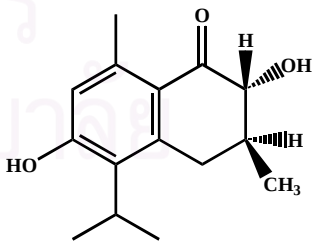
MANSORIN C



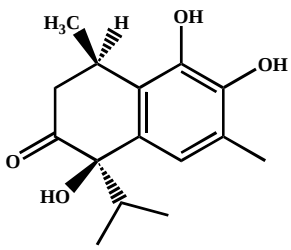
MANSONONE N



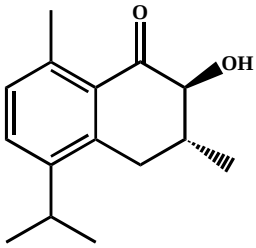
MANSONONE O



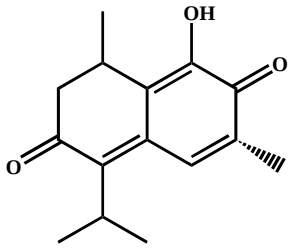
MANSONONE P



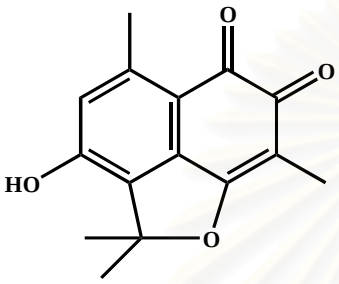
MANSONONE Q



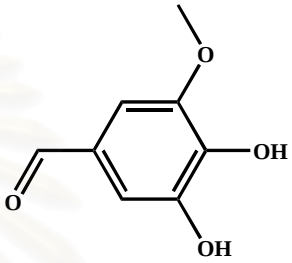
MANSONONE R



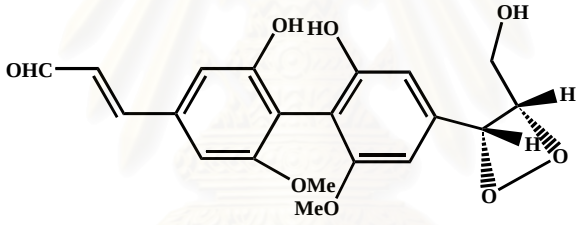
MANSONONE S



Dehydrooxoperezinone



3-methoxy-4,5-dihydroxybenzaldehyde



MANSOXETANE

1.3 Antihistaminic activities in plants

Histamine is a biogenic amine formed by the enzymatic decarboxylation of histidine. In a human organism, histamine is stored in its inactive form in mast cell and basophil granules. The mechanism described of histamine release is shown below.

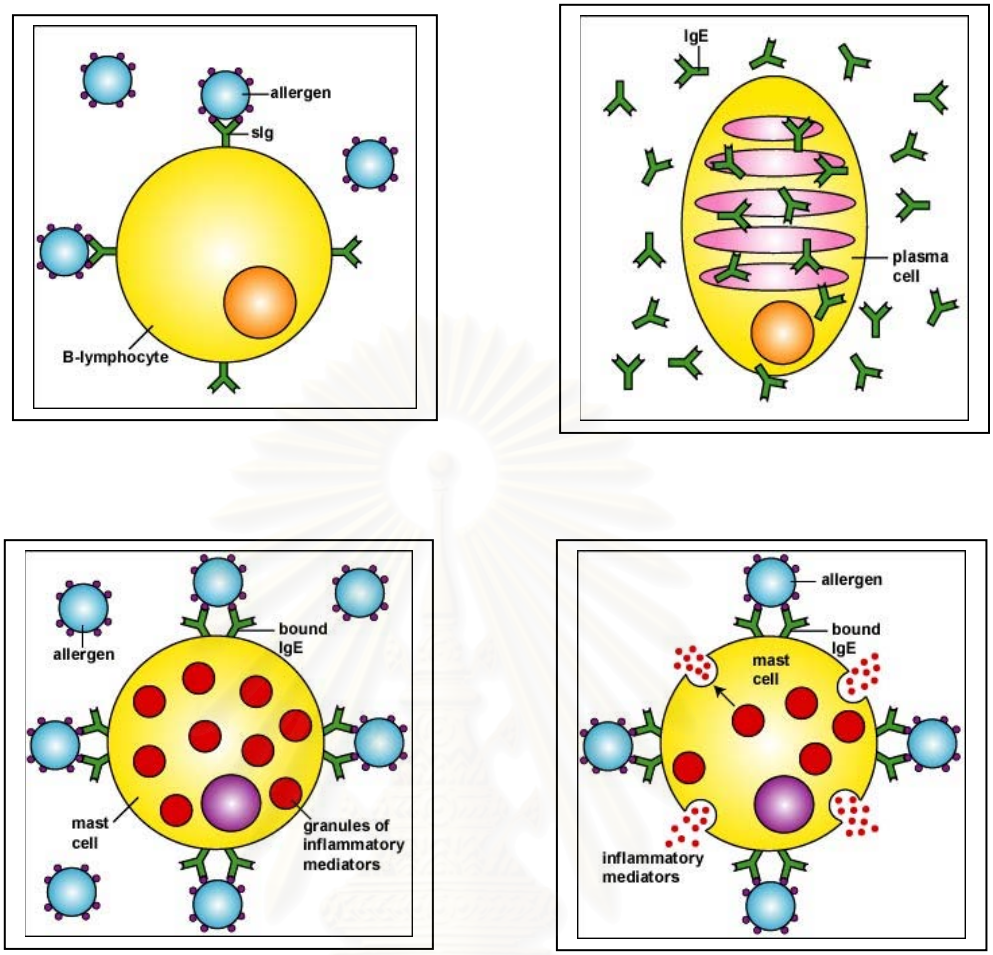


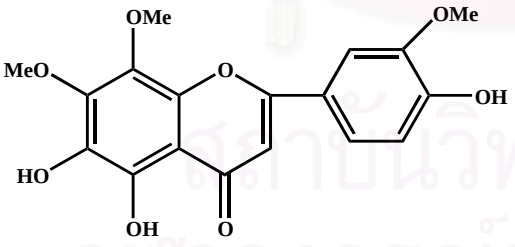
Figure 1.2 The mechanism of histamine release.

The physiological secretion of histamine can be initiated by a number of factors, all of which involve binding of IgE, cross-linked by antigen, to the mast cell or basophil's Fc receptors causing degranulation of these cells. Once released, histamine binds to a number of different target cell receptors causing the symptomatic effects of allergies. [*12-14*] The amount of histamine biological activities cross to effect in human is described in Table 1.3.

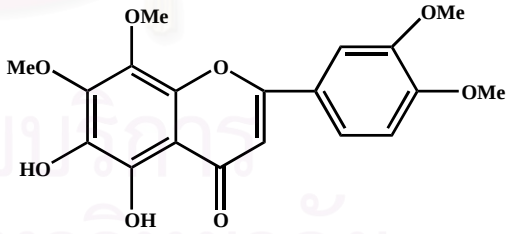
Table 1.3 Effects of histamine release amount in humans.

Histamine (ng/ml)	Biological activities
0-1	none
1-2	enhanced gastric acid secretion
3-5	tachycardia, skin reaction
6-8	decreased arterial pressure
7-12	broncho-spasms
Approx.100	cardiac arrest

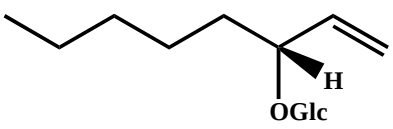
Throughout the literature review, the antihistaminic activity of methanolic extract from the leaves of *Mentha spicata* L. var. *crispa* Benth. was addressed in 1997. [*15*] Yamanura *et al*. reported that three compounds exhibited this biological activity were 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone (thymonin) (IC_{50} = 6.4 μM), 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone (IC_{50} = 56 μM) and (3*R*)-1-octan-3-yl-β-D-glucopyranoside (IC_{50} = 560 μM). In addition, they suggested that, in case of flavonoids, the catechol structure in B-ring is necessary to exhibit activity. The substitution pattern in the A-ring seems to have only little influence on the allergic activity of flavonoids. The structures of active compounds are displayed below.



5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone (thymonin)



5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone



(3*R*)-1-octan-3-yl-β -D-glucopyranoside

Kuwabara *et al.* reported structure-activity relationship (SAR) of synthetic flavonoids. [*16*] Tricin, a flavanoid from the nature, was the major substance that could be isolated from the methanolic extract of the leaves of *Agelaea pentagyna* (Lam.) Baill. (Connaraceae) and synthetic tricins were studied for antihistaminic activity. [*16*] The synthetic tricins were synthesized from the corresponding benzoic acid derivatives and acetophenone derivatives. As judged from the results, it is tentatively concluded that tricin was a 5,7-dihydroxyflavone with the maximal state of activity, but synthetic tricins showed lower activity than tricin.

Based upon the preliminary antihistaminic activity results which were carried out at Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University, Japan under the collaboration project with Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, it was observed that the dichloromethane extract of the roots and the leaves of *M. gagei* exhibited high activity. Thus, these two parts of this target plant were selected for further investigation.

1.4 The aim of this research

According to the literature review, the isolated substances from *M. gagei* previously studied displayed interesting biological activities such as anticancer, antioxidant *etc.*, but none has been reported on antihistaminic activity. Thus, the goal of this research could be summarized as follows:

1. To extract the roots and the leaves of *M. gagei*
2. To separate and isolate chemical constituents from both crude extracts and elucidate the structure of isolated compounds.
3. To perform activity test of crude extracts and pure isolated substances such as antihistaminic and antioxidant activity.

CHAPTER II

EXPERIMENTAL

2.1 Plant materials

Mansonia gagei Drumm. was obtained from Saraburi province, Thailand, in 1997 and was identified by comparing with voucher specimen No. 43281 at the herbarium of the Royal Forestry Department of Thailand.

2.2 Instruments and equipment

The Fourier Transform-Infrared spectrum (FT-IR) was recorded on Nicolet impact 410 spectrophotometer. The ^{1}H and ^{13}C-NMR spectra (in $CDCl_3$, methanol-d_4 and DMSO-d_6) were determined with a nuclear magnetic resonance spectrometer of Varian model Mercury+ 400 and Bruker model ACF 200 spectrometer. Melting points were recorded with Fisher-John melting point apparatus and are uncorrected. Adsorbents used for isolation were silica gel 60 Merck, No. 7734, 9385 and 7731 for column chromatography, flash column chromatography and quick column chromatography, respectively. Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 PF_{254}). The spots on plate were detected under UV light or visualized by spraying with 10% H_2SO_4 in ethanol. Gel filtration chromatography was performed on sephadex LH 20.

2.3 Chemical reagents

All commercial solvents used in this research were distilled prior to use except for those which were reagent grades.

2.4 Chemical reaction

2.4.1 Basic hydrolysis of compound 11 [*17*]

A solution of 10% ethanolic KOH (20.0 mL) was added to compound **11** (100 mg) and the mixture was heated under refluxing in an oil bath for 5 hours (check

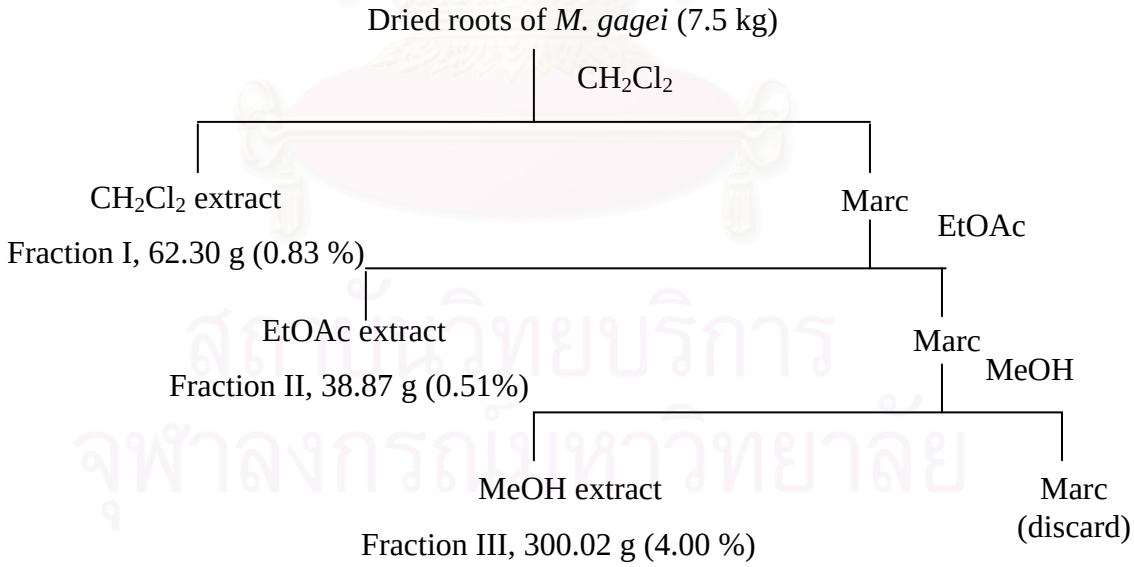
whether the reaction was completed or not by TLC). Evaporation of ethanol gave a solid which was then extracted by diethylether, 100 mL each, three times. The combined diethylether layer was dried over anhydrous sodium sulphate. The solvent was evaporated to gain a mixture of desired product. The mixture was purified by column chromatography to yield bright white needle 24.3 mg (24.3%).

2.5 Extraction procedure

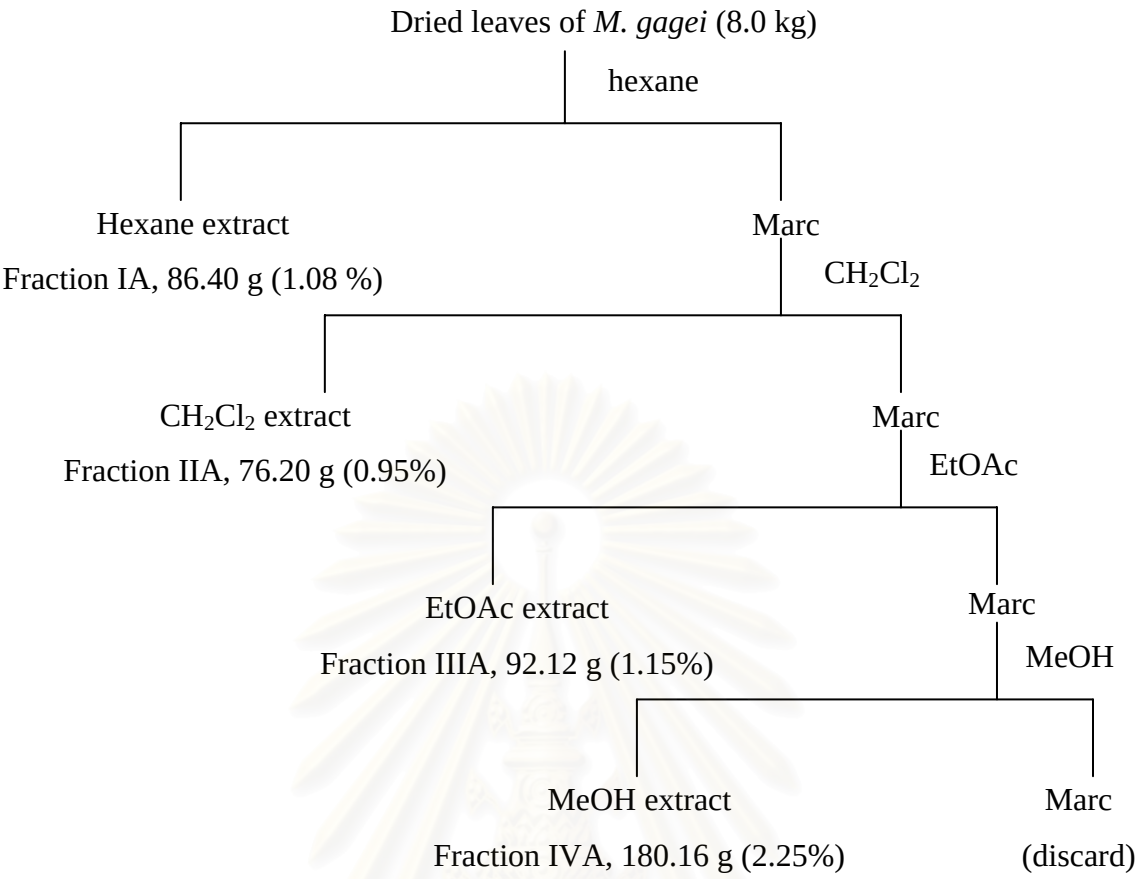
The dried roots (7.5 kg) of *M. gagei* were milled and extracted by soaking with dichloromethane for seven days at room temperature, three times, and then evaporated to furnish a brownish crude designated as fraction I. The residue was similarly extracted with ethyl acetate and methanol produced fractions II and III as dark brownish crudes.

The grinded leaves (8.0 kg) were extracted following the same procedure, except for initially extracting by hexane, to furnish greenish crudes as fractions IA, IIA, IIIA and IVA.

The extraction procedures of the roots and the leaves are summarized as shown in Schemes 2.1 and 2.2, respectively.



Scheme 2.1 Extraction procedure for the dried roots of *M. gagei*.



Scheme 2.2 Extraction procedure for the dried leaves of *M. gagei*.

2.6 Biological screening assay

2.6.1 Scavenging effects on DPPH radicals [*18*]**]**

2,2-Diphenyl-1-picryhydrazyl (DPPH) radical is considered to be very stable radical with a purple color (λ_{max} = 517 nm). Upon reduction by scavenger, the extensive conjugation is disrupted and the compound turns yellow.

2.6.1.1 TLC autographic assay

TLC plates were developed in appropriate solvent systems, dried and sprayed with 0.2% DPPH in methanolic solution. The plates were examined for 5 minutes after spraying. Active compounds appeared as yellow spots against purple background.

2.6.2 Antihistaminic activity assay

2.6.2.1 RBL-2H3 cell culture [*16*]

RBL-2H3 cells were cultured in minimum essential medium (S-MEM, GIBCO-BRL) containing 15 % of fetal bovine serum (FBS, GIBCO-BRL), 100 U/mL penicillin G (GIBCO-BRL), 100 μg/mL streptomycin (GIBCO-BRL) and 0.29 mg/mL L-glutamine (GIBCO-BRL). Culture was done up to 80 % confluency in a 25 cm^2 canter-necked tissue culture flask (FALCON) in a CO_2 incubator (5 % CO_2 and 95 % air, 37 ºC) (SANYO).

The S-MEM was removed, after cultivation and cells were washed twice with fresh S-MEM. Three mL of trypsine-EDTA (GIBCO-BRL) was added into the flask and allowed to stand for 4 min at 37 ºC. The RBL-2H3 cells were then collected and transferred to a 1.5 mL micro tube, which was centrifuged at 6000 rpm for 3 sec. The precipitated cell lump was suspended in S-MEM, a part of which was cultivated in a new flask, where the medium was changed every two days. The rest of the cells were used for experiment.

2.6.2.2 Measurement of β-hexosaminidase secretion for antihistaminic activities [*16*]

For measurement of the secrection of β-hexosaminidase, RBL-2H3 cells were incubated with DNP-specific IgE (0.5 μg/mL) (SIGMA) in complete growth medium in 96-well plates (4X 104 cells/80 μL of medium/well) overnight. Cells were washed twice with a glucosesaline PIPES-buffered medium containing 1 nM Ca^{2+} (Siraganian buffer), and then the medium was replaced with Siraganian buffer. Cells were pre-incubated for 10 min at 37 ºC in 40 μL of Siraganian buffer containing crude extracts or pure compounds, which were prepared in DMSO and diluted to give < 0.1% DMSO solutions. Cells were then stimulated with DNP-BSA (20 ng/mL) (CALBIOCHEM) for 15 min. Aliquots (10 μL) of the medium and cell lysate (in 50uL of 0.1% Triton X-100) were incubated with 10 μL of 1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in 0.1 M sodium citrate buffer (pH 4.5) at 37 ºC for 1 hr. At the end of the incubation, 250 μL of 0.1 M Na_2CO_3-0.1 M $NaHCO_3$ buffer (pH 10) was added. Absorption was measured at 415 nm. Values were calculated as the actual release (percentage of total β-hexosaminidase), after correction for spontaneous release (2-3%), or as a percentage of the maximal response. The inhibitory percentage of substance was calculated by using equation shown below and IC_{50} values were

calculated for at least six independent concentrations ranging from 1 to 500 μM due to the limit of solubility.

$$\% \text{ Inhibition} = \{1- \frac{(A_{sample}-A_{M.control})}{(A_{R.control}-A_{M.control})} \} \text{ X } 100 (\%)$$

Note: A = absorbance, A_{sample} = absorbance of sample and medium culture, $A_{M.\ control}$ = absorbance of medium culture, $A_{R.\ control}$ = absorbance of antigen (DNP-BSA) and medium culture

CHAPTER III

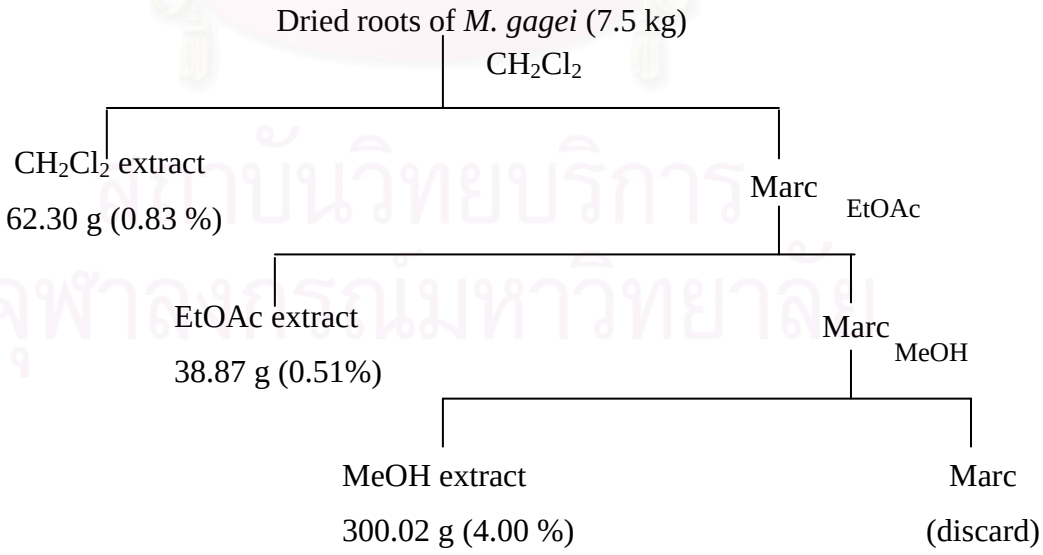
RESULTS AND DISCUSSION

According to the screening test results of antihistaminic activity and scavenging effects on DPPH radical of *M. gagei*, the dichloromethane extracts of the roots and the leaves of this plant showed impressive activity. Based upon the literature review studies, there are only two reports regarding to the chemical constituents of the heartwoods of *M. gagei* [*4,11*] none had been focused on chemical constituents of other parts and antihistaminic activity. Thus, the roots and the leaves of *M. gagei* were chosen for further investigating of their chemical constituents and bioactivities.

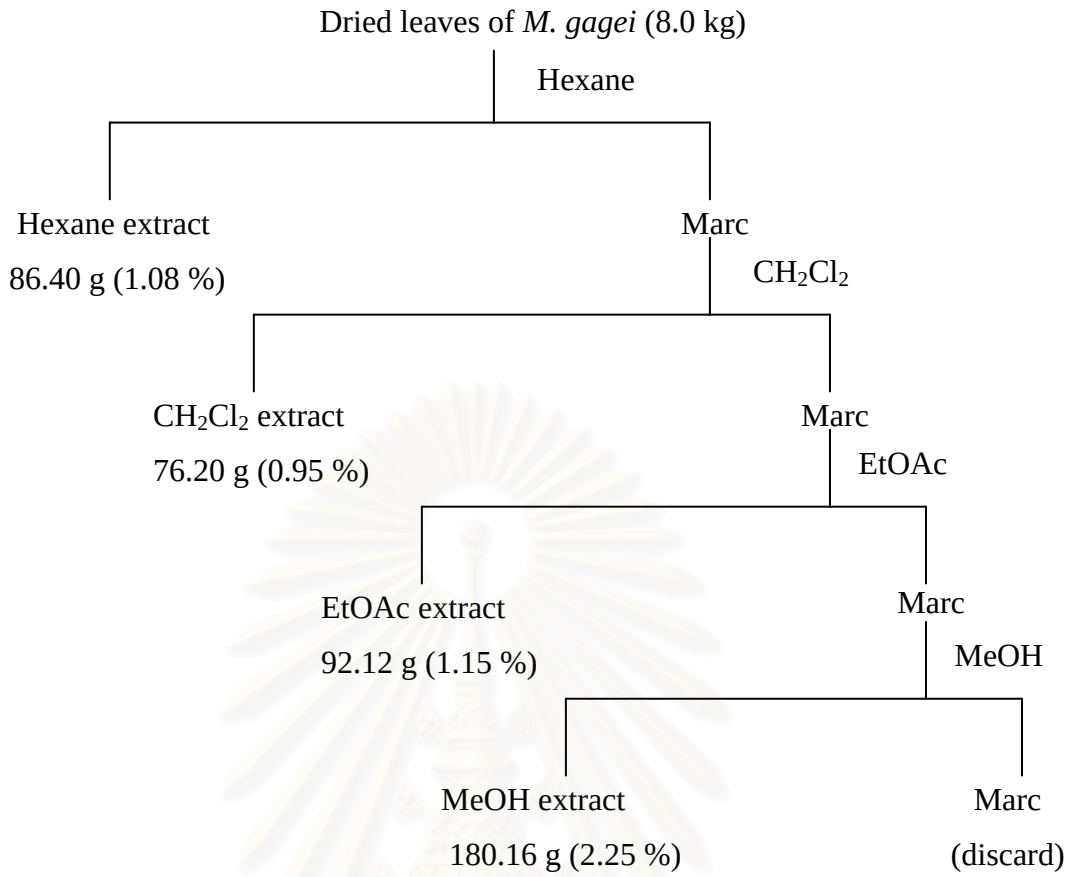
3.1 Results of extraction

General extraction

The crush dried roots and leaves of *M. gagei* were extracted with appropriate solvents according to the procedure described in Chapter II. The results of extraction are presented in Schemes 3.1 and 3.2.



Scheme 3.1 The results of extraction of dried roots of *M. gagei*.



Scheme 3.2 The results of extraction of dried leaves of *M. gagei*.

3.2 The results of preliminary biological screening tests

3.2.1 Antihistaminic activity

The preliminary study on antihistaminic activity was carried out at Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University, Japan under the collaboration project with Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University. The results of antihistaminic activity of all extracts are revealed in Table 3.1.

Table 3.1 The inhibition percentage results of antihistaminic activity of crude extracts of *M. gagei* using RBL-2H3 cells.

Plant part	solvent	% inhibition
		0.1 mg/ mL
Root	CH_2Cl_2	96.50
	MeOH	15.46
Bark	CH_2Cl_2	9.72
	EtOAc	38.90
	MeOH	25.30
Leaf	Hexane	33.80
	CH_2Cl_2	81.00
	EtOAc	57.40
	MeOH	31.20

The results of antihistaminic activity studied included the roots, leaves and barks of *M. gagei* revealed significant activity in dichloromethane extract of the roots and the leaves, but all of crude extracts of the barks did not show good result at 0.1 mg/mL. The ethyl acetate and methanol extracts showed less inhibition percentage than dichloromethane extract. Based on the preliminary test results, the dichloromethane extract of the roots and other crude extract were selected for investigated the chemical constituents. Therefore, this research was concerned with the search for chemical constituents of this plant with an effort to discover new antiallergic agents.

3.2.2 Scavenging effects on DPPH radical [*18*]

Antioxidant test was observed using TLC autographic assay described in Chapter II. The results of antioxidant activity screening test of the roots and the leaves of *M. gagei* are presented in Table 3.2.

Table 3.2 The antioxidant activity screening test of the roots and the leaves of *M. gagei*

Plant parts	Extracts	DPPH radical scavenging activity TLC autographic
Leaves	Hexane	+
	CH_2Cl_2	++
	EtOAc	+++
	MeOH	+++
Roots	CH_2Cl_2	+++
	EtOAc	+++
	MeOH	+++

Note: +++ = strong activity (suddenly detected), ++ = moderate activity (detected at 0-15 minute), + = weak activity (detected at 15-30 minute)

This test was performed on Thin-layer chromatography, plants extract were spot on aluminium-backed silica gel plate. The plates were developed in appropriate solvent before being dried and used 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a spray reagent. The active substances were seen as yellow spots on a purple background.

Table 3.2 demonstrates that all crude extracts, except for hexane and dichloromethane extracts of the leaves, exhibited strong radical scavenging effect against 2,2-diphenyl-1-picryhydrazyl (DPPH).

From the preliminary biological screening test results, Tables 3.1 and 3.2, the dichloromethane extracts of both roots and leaves displayed the most interesting antihistaminic and antioxidant activities. However, in this research all extracts were selected for further study on their chemical constituents and biological activities according to the lack of information on these parts of this particular plant.

3.3 Chemical constituents of the roots of *M. gagei*

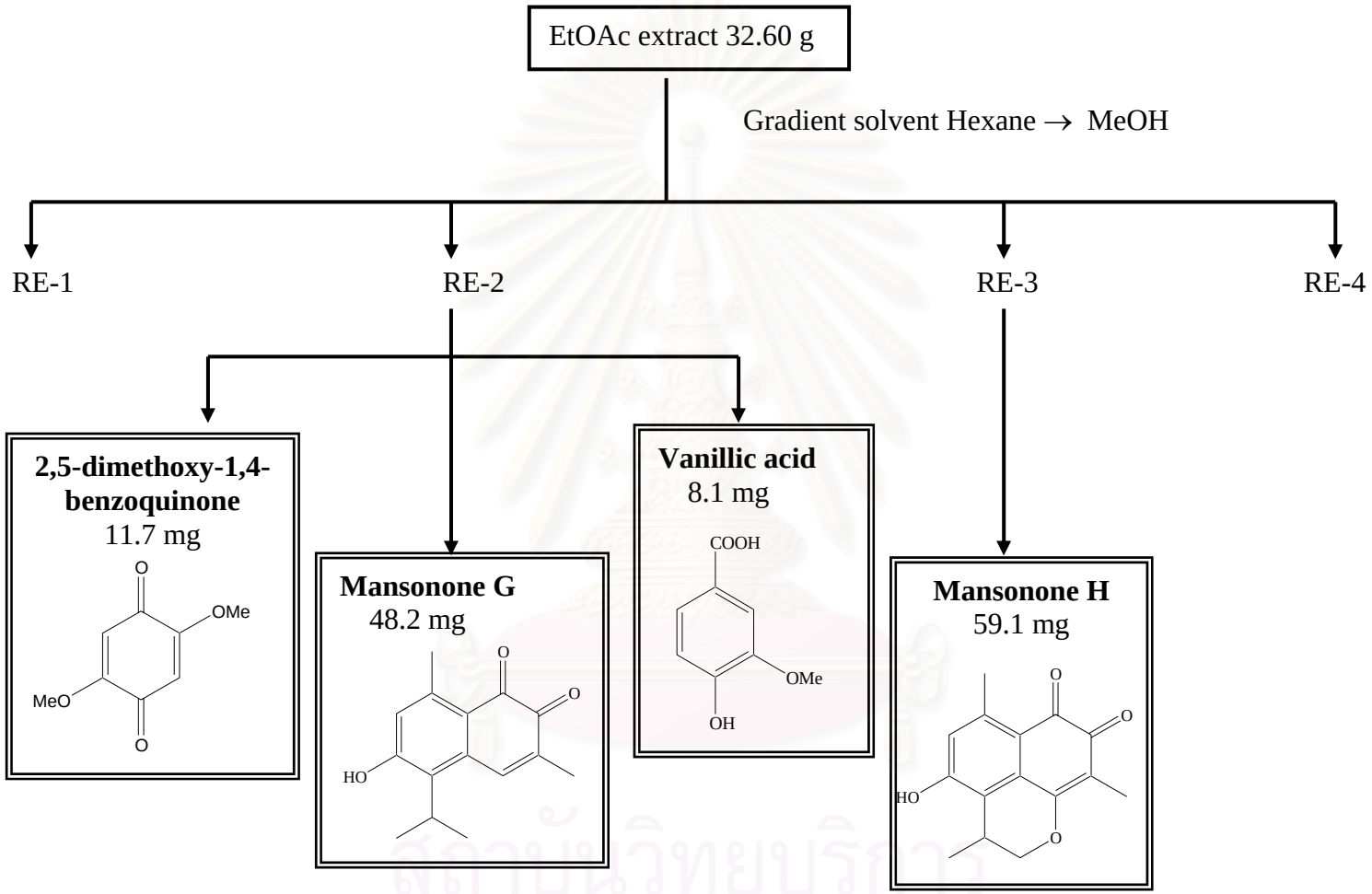
3.3.1 Chemical constituents of ethyl acetate extract

A part of ethyl acetate extract 32.6 g was subjected to silica gel column chromatography using gradient solvent starting from CH_2Cl_2, CH_2Cl_2-EtOAc and EtOAc-MeOH. Each subfraction was monitored by TLC and subfractions which displayed the similar component pattern were combined. Four subfractions, RE1-RE4 were obtained. The results of separation are shown in Table 3.3.

Table 3.3 The separation of ethyl acetate extract of the roots of *M. gagei*

Fraction	Solvent system	Remarks	Weight (g)
RE1	0-5 % CH_2Cl_2-EtOAc	Yellow oil	1.02
RE2	5-10 % CH_2Cl_2-EtOAc	Orange solid	5.10
RE3	15 % CH_2Cl_2-EtOAc	Orange solid	1.68
RE4	15 % CH_2Cl_2-EtOAc	Brown solid	11.79

Fractions RE1, RE2, RE3 and RE4 were re-separated on silica gel column eluted with mixed solvents of hexane-EtOAc and EtOAc-MeOH for several times. 2,5-Dimethoxy-1,4-benzoquinone (**1**), mansonone G (**2**), vanillic acid (**3**) were obtained from subfraction RE2 and mansonone H (**4**), the major compound, was isolated from subfraction RE3. The results of separation are shown in Scheme 3.3.



Scheme 3. 3 The separation of ethyl acetate extract from the roots of *M. gagei*

3.3.2 Structural elucidation of compound 1

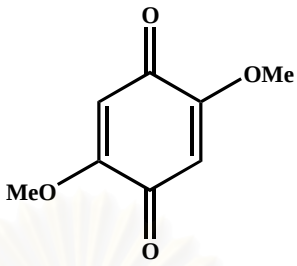
After elution with 50% EtOAc-CH_2Cl_2, compound **1** was isolated as yellow needle, this crystal was melted at 249-250 °C, 11.7 mg, 0.04% w/w of ethyl acetate crude extract and showed a single spot on TLC with R_f value 0.45 in hexane: EtOAc (1:1). The ^{1}H NMR spectrum (Figure 3.1) revealed the important signal at δ 5.89 (s, 2H) which could be assigned for olefinic protons. Two methoxy groups were clearly detected at δ 3.86 (s, 6H).

The ^{13}C-NMR spectrum (Figure 3.2) displayed four carbon signals; at δ 186.9 coinciding with carbonyl carbon of a quinonoid skeleton, at δ 157.3 and 107.4 were consistent with olefinic carbons and that at δ 56.5 was corresponded to methoxy carbon. From the accumulated spectral data, it could be concluded that this compound was 2,5-dimethoxy-1,4-benzoquinone. [*19*] The comparison of ^{1}H and ^{13}C-NMR spectral data of reported 2,5-dimethoxy-1,4-benzoquinone and those of compound **1** is presented in Table 3.4.

Table 3.4 The ^{1}H and ^{13}C-NMR spectral data of 2,5-dimethoxy-1,4-benzoquinone[*19*] and compound **1**.

Position	Chemical shift (ppm)			
	2,5-dimethoxy-1,4-benzoquinone		compound **1**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	188.0	-	186.9	-
2	158.0	-	157.3	-
3	107.5	5.70	107.4	5.89
4	188.0	-	186.9	-
5	158.0	-	157.3	-
6	107.5	5.70	107.4	5.89
OMe	56.0	3.80	56.5	3.86

According to the previous report cited in literature [*19*], it was no doubt to conclude that compound **1** was 2,5-dimethoxy-1,4-benzoquinone. The structure is shown below.



Compound 1: 2,5-dimethoxy-1,4-benzoquinone

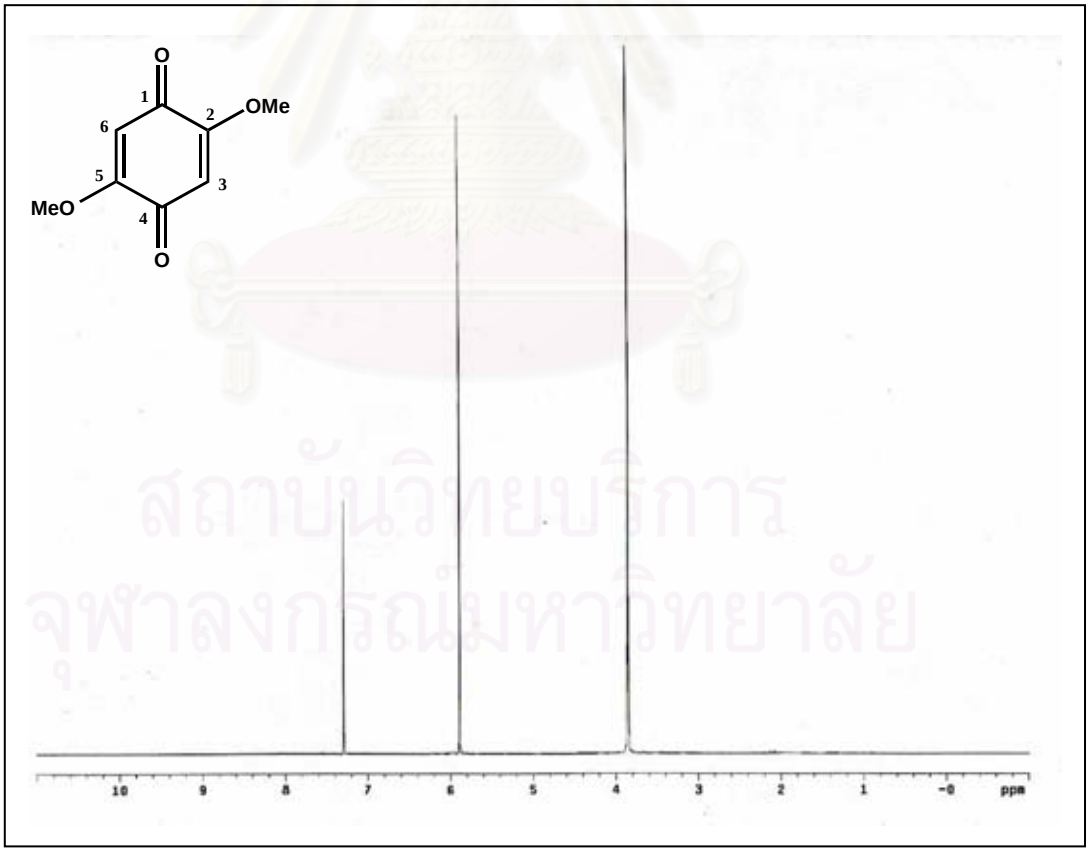


Figure 3.1 The ^{1}H-NMR spectrum of compound **1**.

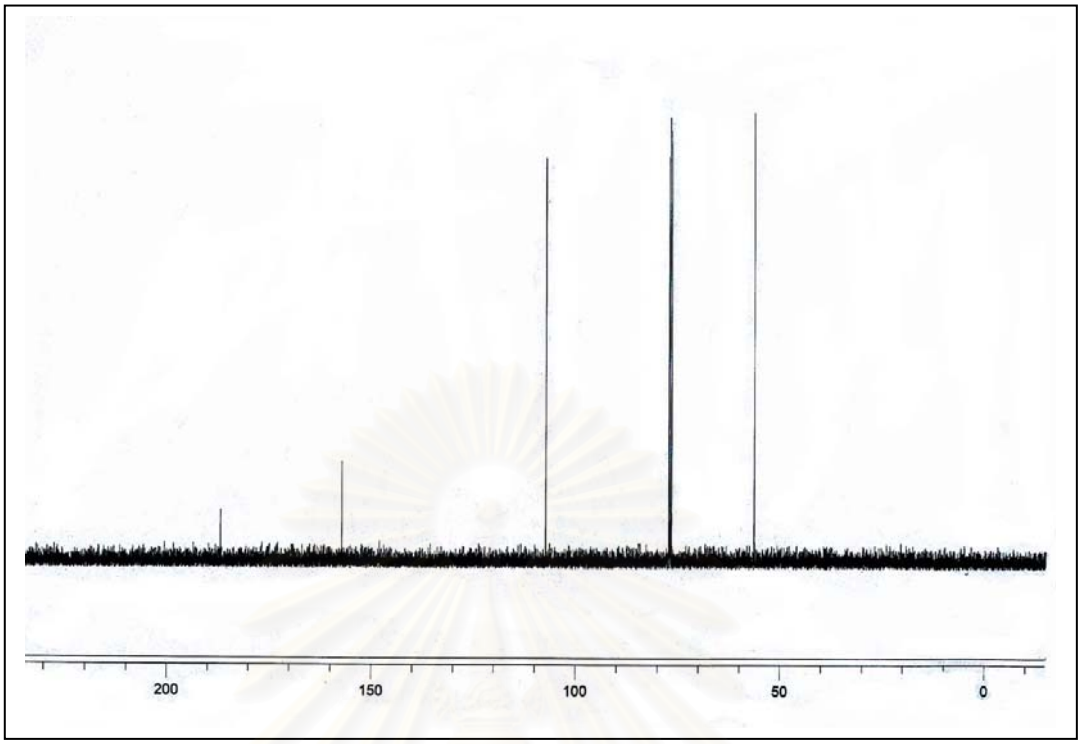


Figure 3.2 The ^{13}C-NMR spectrum of compound **1**.

3.3.3 Structural elucidation of compound 2

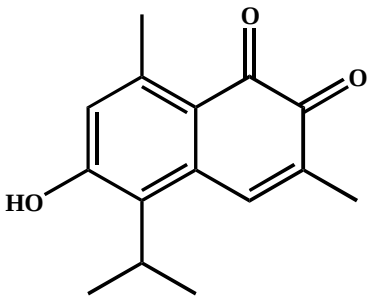
Compound **2** was isolated as orange powder, 48.2 mg, decomposed at 183 °C, 0.15 % w/w of ethyl acetate crude extract and had R_f value 0.53 in hexane: EtOAc (1:1).

The ^{1}H NMR spectrum (Figure 3.3) showed the presence of three methyl protons at δ 2.10 (s, 3H), 2.61 (s, 3H) and 1.49 (d, 3H, *J* = 7.0 Hz), two aromatic protons at δ 6.58 (s, 1H) and 7.74 (s, 1H) and the methine proton at δ 3.61 (m, 1H). The preliminary co-TLC study of this compound with the isolated compounds from *M. gagei* visualized that the R_f of this compound was almost appeared at the same R_f value as mansonone G. The comparison of ^{1}H-NMR spectral data of compound **2** with mansonone G [*4,11*] is thus summarized in Table 3.5.

Table 3.5 The ^{1}H-NMR spectral data of mansonone G [*4,11*] and compound **2**.

Position	Chemical shift (ppm)	
	mansonone G	compound **2**
	^{1}H (*J* in Hz)	^{1}H (*J* in Hz)
1	-	-
2	-	-
3	-	-
4	7.70, s, 1H	7.74, s, 1H
4a	-	-
5	-	-
6	6.50, s, 1H	6.58, s, 1H
7	-	-
8	-	-
8a	-	-
9	3.58, m, 1H	3.61, m, 1H
3-CH_3	2.06, s, 3H	2.10, s, 3H
8-CH_3	2.58, s, 3H	2.61, s, 3H
(9-CH_3)$_2$	1.42, d, 6H (7.0)	1.49, d, 6H (7.0)

According to the literature review concerning 1,2-naphthoquinones, the ^{1}H and ^{13}C-NMR spectral data of compound **2** were compared with those of 1,2-naphthoquinones. The ^{1}H NMR data of compound **2** was identical with mansonone G which was previously isolated from *Mansonia* plants [*4,11*]. Moreover, based on these spectroscopic data and co-TLC with authentic sample previously attained from the heartwood of *M. gagei* [*4,11*], compound **2** was confidently identified as mansonone G.



Compound 2: Mansonone G

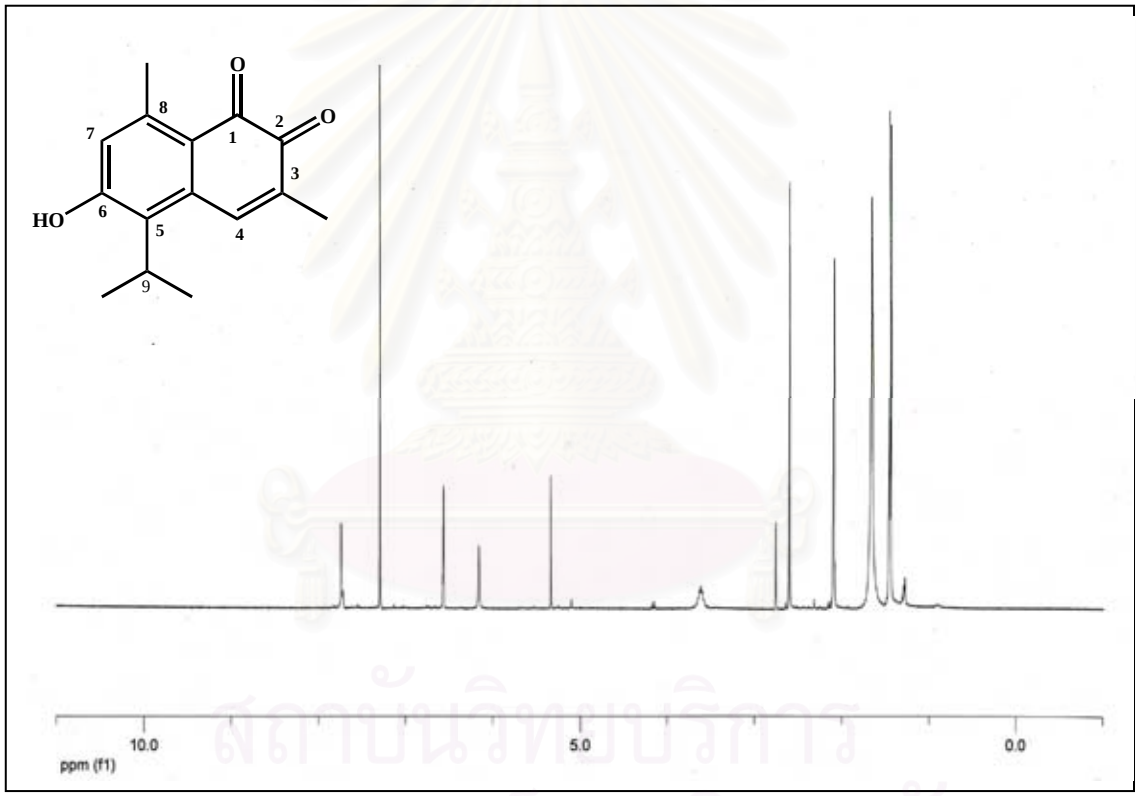


Figure 3.3 The ^{1}H-NMR spectrum of compound **2**.

3.3.4 Structural elucidation of compound 3

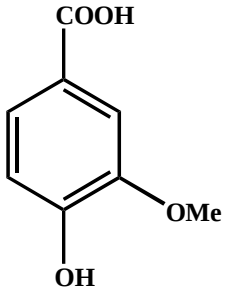
Compound **3** was isolated as pale yellow powder 8.1 mg, m.p. 208-210 °C (0.23 % w/w of ethyl acetate crude extract). This compound showed a single spot at R_f 0.36 in CH_2Cl_2:EtOAc (1:1). Characterization of compound **3** was commenced with the ^{1}H NMR spectrum (Figure 3.4) which displayed the signal at δ 6.86 (1H, d, *J* = 7.8 Hz). That signal could be assigned for aromatic protons, together with another aromatic proton appeared at δ 7.47 (d, 1H, *J* = 7.8 Hz). A methoxy group was detected at δ 3.83 (s, 3H).

The ^{13}C-NMR spectrum (Figure 3.5) displayed six carbon signals at δ 167.3 coinciding with a carbonyl carbon of carboxylic acid, the chemical shift at δ 151.1, 147.3, 123.5 and 121.7 being well consistent with olefinic carbon signals and at δ 55.6 corresponded to a methoxy carbon signal.

According to the spectroscopic data and information derived from literature review, its structure could be deduced as vanillic acid. The comparison of ^{1}H-NMR spectral data of compound **3** and vanillic acid [*19*] is tabulated in Table 3.6.

Table 3.6 The ^{1}H NMR chemical shift assignments of vanillic acid [*19*] and compound **3**

Position	Chemical shift (ppm)			
	vanillic acid		compound **3**	
	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)
1	151.5	-	151.1	-
2	123.6	7.48, 1H	123.6	7.44, s, 1H
3	147.3	-	147.3	-
4	121.7	-	121.7	-
5	112.2	6.85, 1H	112.8	6.86, d, 1H (7.8)
6	115.1	7.48, 1H	115.1	7.47, d, 1H (7.8)
7	167.3	-	167.3	-
OMe	55.6	3.85	55.6	3.82



Compound 3: Vanillic acid

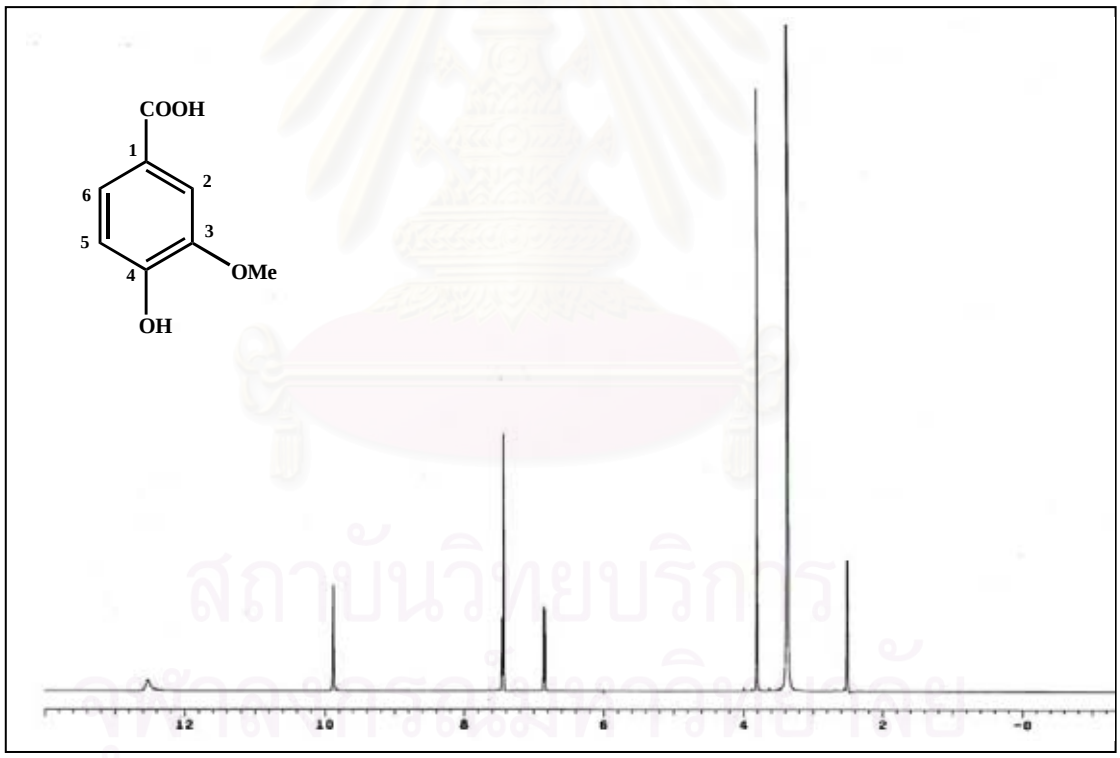


Figure 3.4 The ^{1}H-NMR spectrum of compound **3**.

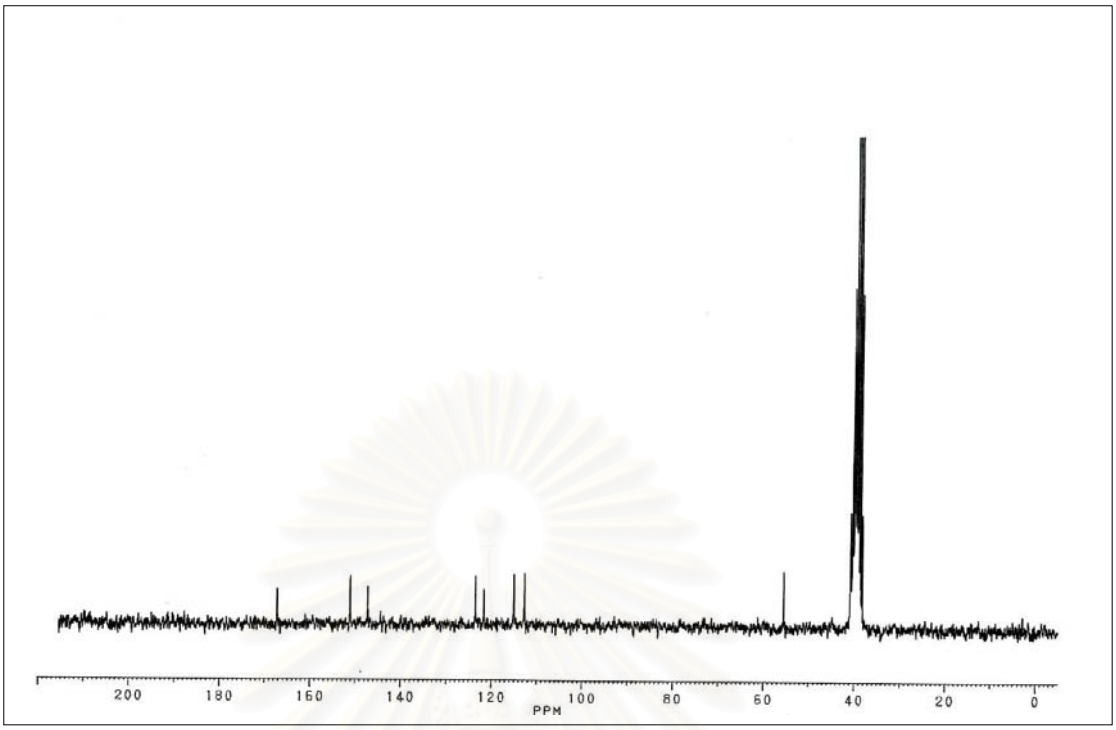


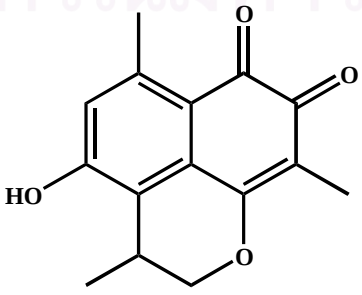
Figure 3.5 The ^{13}C-NMR spectrum of compound **3**.

3.3.5 Structural elucidation of compound 4

The red solid (59.1 mg, 0.15 % w/w of ethyl acetate extract) showed a single spot on TLC at R_f 0.55 in CH_2Cl_2: EtOAc (1:1) and decomposed at 250 °C.

According to the spectroscopic data, the ^{1}H NMR spectrum (Figure 3.6) clearly showed the presence of an aromatic proton signal at δ 6.33 (s, 1H) and three methyl groups at δ 1.92 (s, 3H), 2.62 (s, 3H) and 1.35 (d, 3H, *J* = 7.1 Hz).

Based on these spectroscopic data and co-TLC with an authentic sample previously obtained from the heartwood of *M. gagei* [*4,11*], compound **4** could be concluded as mansonone H. The comparison of ^{1}H-NMR spectral assignment of compound **4** and mansonone H is displayed in Table 3.7.



Compound 4: Mansonone H

Table 3.7 The ^{1}H chemicals shift assignments of mansonone H [*4,11*] and compound **4**

Position	Chemical shift (ppm)	
	mansonone H	compound **4**
	^{1}H (*J* in Hz)	^{1}H (*J* in Hz)
1	-	-
2	-	-
3	-	-
4	-	-
4a	-	-
5	-	-
6	-	-
7	6.33, s, 1H	6.74, s, 1H
8	-	-
8a	-	-
9	3.21, m, 1H	3.34, m, 1H
10	4.40, br d, 1H (10.3) 4.28, dd, 1H (3.5, 10.3)	4.51, dd, 1H (3.1, 10.9) 4.39, dd, 1H (3.1, 10.9)
3-CH_3	1.85, s, 3H	1.92, s, 3H
6-OH	-	-
8-CH_3	2.48, s, 3H	2.62, s, 3H
9-CH_3	1.24, d, 3H (7.3)	1.35, d, 3H (7.3)

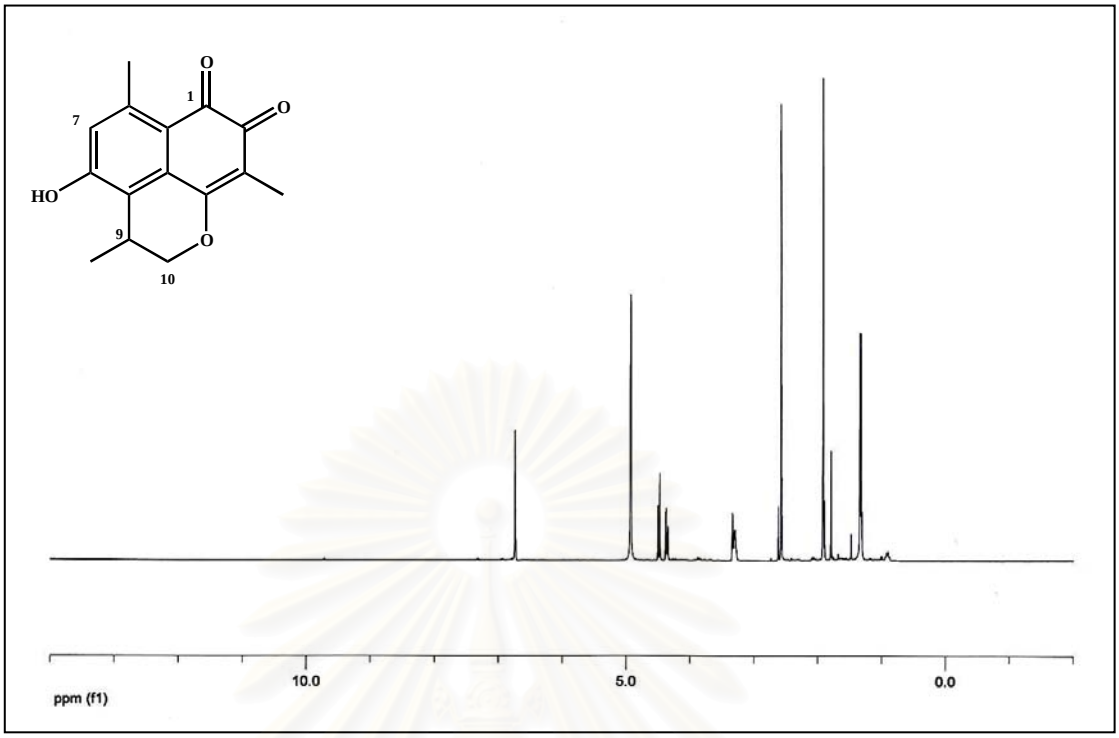


Figure 3.6 The ^{1}H-NMR spectrum of compound **4**.

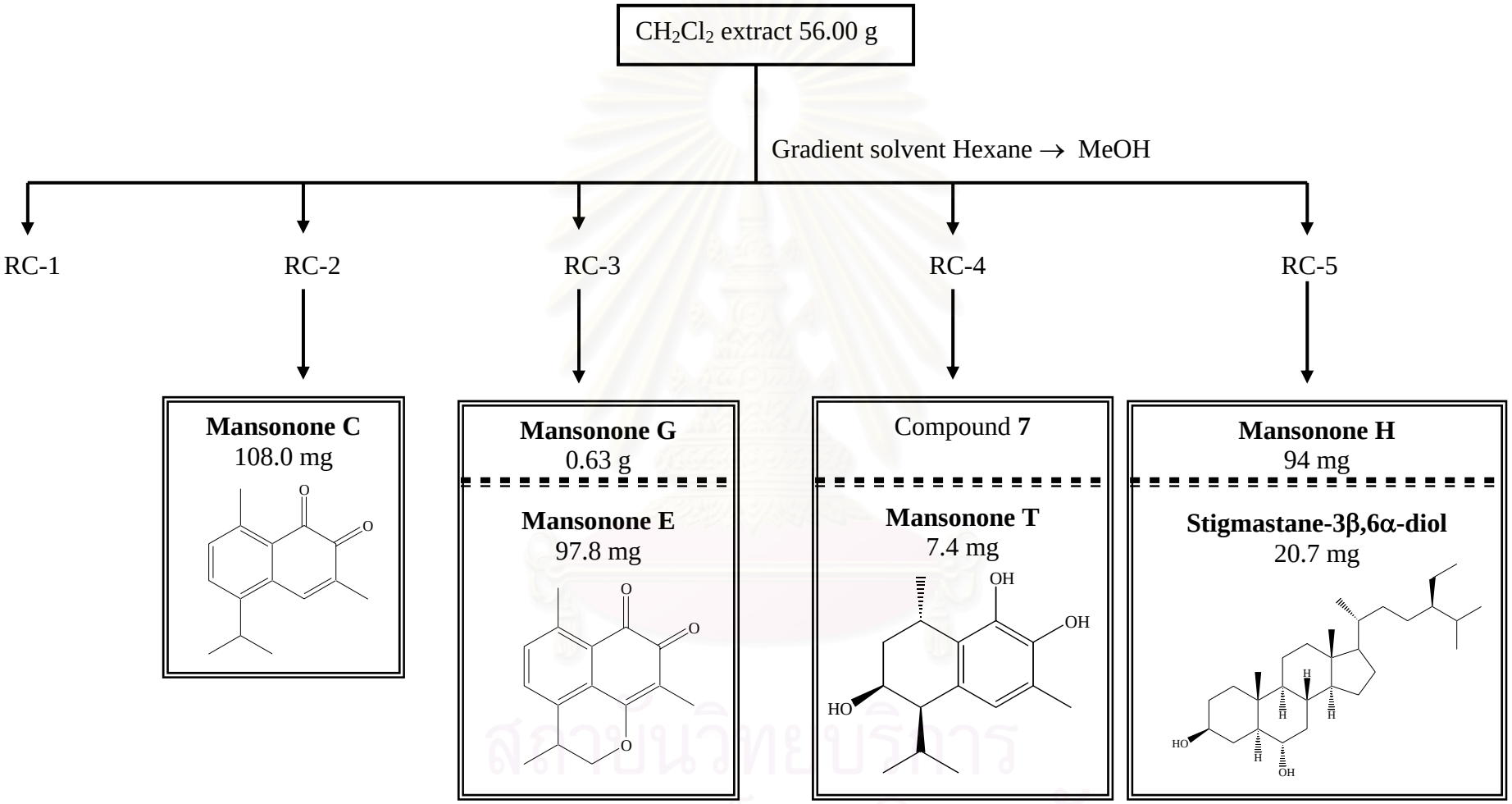
3.3.6 Chemical constituents of dichloromethane extract

The dried roots were extracted with dichloromethane three times to give a brownish extract. A part of dichloromethane extract 56.0 g was subjected to silica gel column chromatography using gradient solvent starting from hexane and increased polarity by mixing with CH_2Cl_2, EtOAc and MeOH. The fractions were collected and combined according to TLC results to obtain five fractions (RC1-5). The results are summarized in Table 3.8.

Table 3.8 The results of the separation of dichloromethane extract.

Fraction	Solvent system	Remarks	Weight (g)
RC1	0-10 % Hexane-CH_2Cl_2	Yellow oil	0.72
RC2	15-60 % Hexane-CH_2Cl_2	Brownish solid	12.84
RC3	80-100 % Hexane-CH_2Cl_2	Brownish solid	3.03
RC4	0-50 % CH_2Cl_2-EtOAc	Brownish solid	2.16
RC5	50-80 % CH_2Cl_2-EtOAc 0-20 % EtOAc-MeOH	Brownish solid	5.56

Subfractions RC1, RC2, RC3, RC4 and RC5 were re-separated on silica gel column chromatography using step gradients of hexane-EtOAc and EtOAc-MeOH. As judged from the results, two compounds were found to be identical with those derived from ethyl acetate extract: mansonone G (**2**) and mansonone H (**4**). Additionally, five compounds including mansonone C (**5**), mansonone E (**6**), compound **7**, mansonone T (**8**) and stigmastane-3β,6α-diol (**9**) were obtained. The results are summarized in Scheme 3.4.



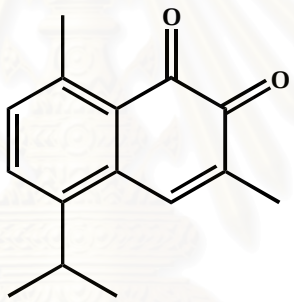
Scheme 3. 4 The separation of CH_2Cl_2 extract of the roots of *M. gagei*

3.3.7 Structural elucidation of compound 5

Compound **5** was isolated as orange needle (108.0 mg, 0.19 % w/w of dichloromethane crude extract), m.p. 133.8-135.1 ºC, R_f 0.7 in hexane: EtOAc (1:1).

The ^{1}H NMR spectrum (Figure 3.7) indicated the presence of two methyl groups at δ 2.67 (s, 3H) and 2.11 (d, 3H, *J* = 2.0 Hz), an isopropyl unit at δ 3.42 (m, 1H) and 1.34 (d, 6H, *J* = 7.0 Hz), two aromatic protons at δ 7.24 (d, 1H, *J* = 8.6 Hz), 7.48 (d, 1H, *J* = 8.6 Hz) and an olefinic proton at δ 7.69 (d, 1H, *J* = 2.5 Hz) ppm.

Based upon the spectroscopic data and co-TLC with authentic sample previously obtained from the heartwood of *M. gagei* [*4,11*], compound **5** was clearly mansonone C. The comparison of ^{1}H- NMR spectral assignment of compound **5** and mansonone C is presented in Table 3.9.



Compound 5: Mansonone C

Table 3.9 The ^{1}H-NMR spectral data of mansonone C [*4*,*11*] and compound **5**

Position	Chemical shift (pm)	
	mansonone C	compound **5**
	^{1}H (*J* in Hz)	^{1}H (*J* in Hz)
1	-	-
2	-	-
3	-	-
4	7.63, d, 1H (1.5)	7.69, d, 1H (1.5)
4a	-	-
5	-	-
6	7.16, d, 1H (8.2)	7.24, d, 1H (8.2)
7	7.40, d, 1H (8.2)	7.48, d, 1H (8.2)
8	-	-
8a	-	-
9	3.36, m, 1H	3.42, m, 1H
3-CH_3	2.02, d, 3H (2.0)	2.11, d, 3H (2.0)
8-CH_3	2.60, s, 3H	2.67, s, 3H
9-$(CH_3)_2$	1.27, d, 1H (7.0)	1.34, d, 1H (7.0)

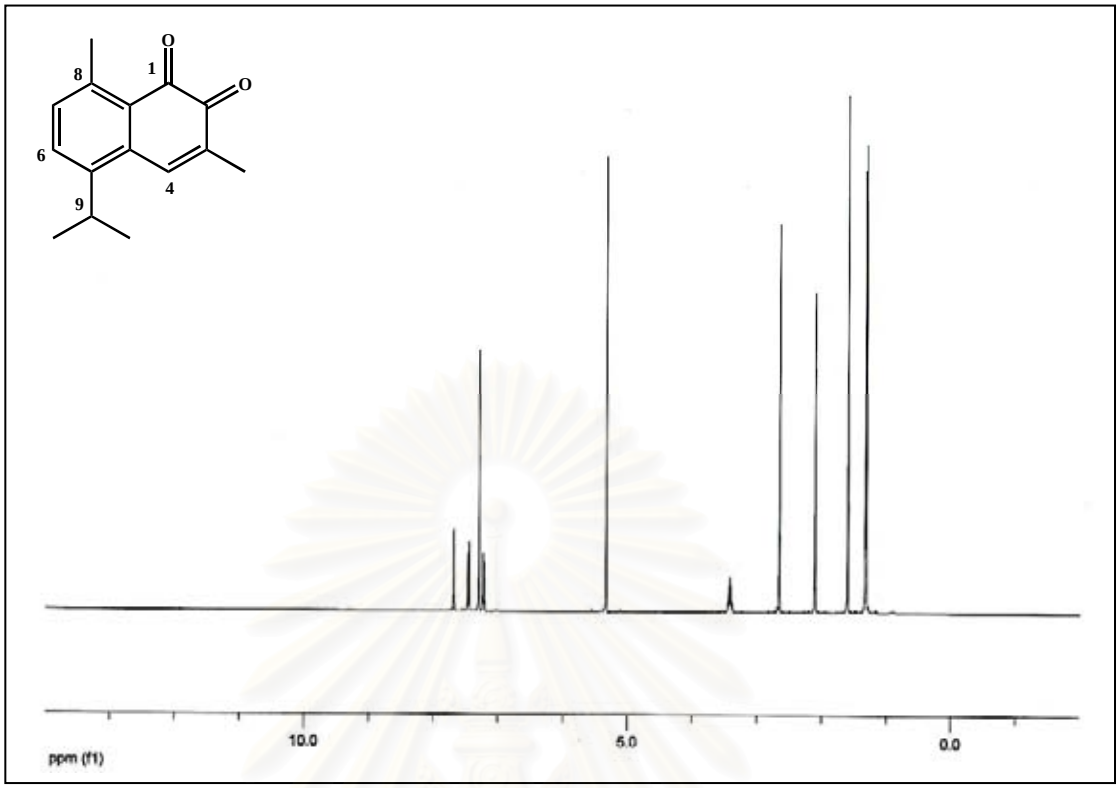


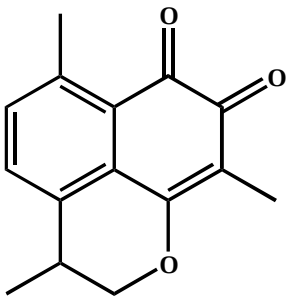
Figure 3.7 The ^{1}H-NMR spectrum of compound **5**.

3.3.8 Structural elucidation of compound 6

Compound **6** was isolated as orange powder from dichloromethane extract subfraction RC-3, 97.8 mg (0.17 % w/w of dichloromethane extract), m.p.161.2-163.2 ºC and revealed a single spot on TLC with R_f 0.45 in hexane: EtOAc (1:1).

Figure 3.8 shows the ^{1}H NMR spectrum of compound **6** exhibiting three methyl groups at δ 1.99 (s, 3H), 2.69 (s, 3H) and 1.44 (d, 3H, *J* = 7.0 Hz), two aromatic protons at δ 7.39 (d, 1H, *J* = 8.6 Hz) and 7.30 (d, 1H, *J* = 8.6 Hz), a methine proton at δ 3.10 (m, 1H) and two methylene protons at δ 4.46 (dd, 1H, *J* = 3.9, 10.1 Hz) and 4.28 (dd, 1H, *J* = 3.9, 10.1 Hz).

According to the spectroscopic data and co-TLC with an authentic sample derived from the heartwood of *M. gagei* [*4,11*], the structure of compound **6** could be deduced as mansonone E. The comparison of ^{1}H-NMR spectral assignment of compound **6** and mansonone E is shown in Table 3.10.



Compound 6: mansonone E

Table 3.10 The ^{1}H-NMR spectral data of mansonone E [*4,11*] and compound **6**

Position	Chemical shift (pm)	
	mansonone E	compound **6**
	^{1}H (*J* in Hz)	^{1}H (*J* in Hz)
1	-	-
2	-	-
3	-	-
4	-	-
4a	-	-
5	-	-
6	7.35, d, 1H (7.8)	7.39, d, 1H (8.6)
7	7.25, d, 1H (7.8)	7.30, d, 1H (8.6)
8	-	-
8a	-	-
9	3.10, m, 1H	3.14, m, 1H
10	4.41, br d, 1H (3.9, 10.7) 4.23, dd, 1H (5.1, 10.3)	4.46, dd, 1H (3.9, 10.1) 4.28, dd, 1H (3.9, 10.1)
3-CH_3	1.94, s, 3H	1.99, s, 3H
8-CH_3	2.63, s, 3H	2.69, s, 3H
9-CH_3	1.37, d, 3H (6.8)	1.44, d, 3H (7.0)

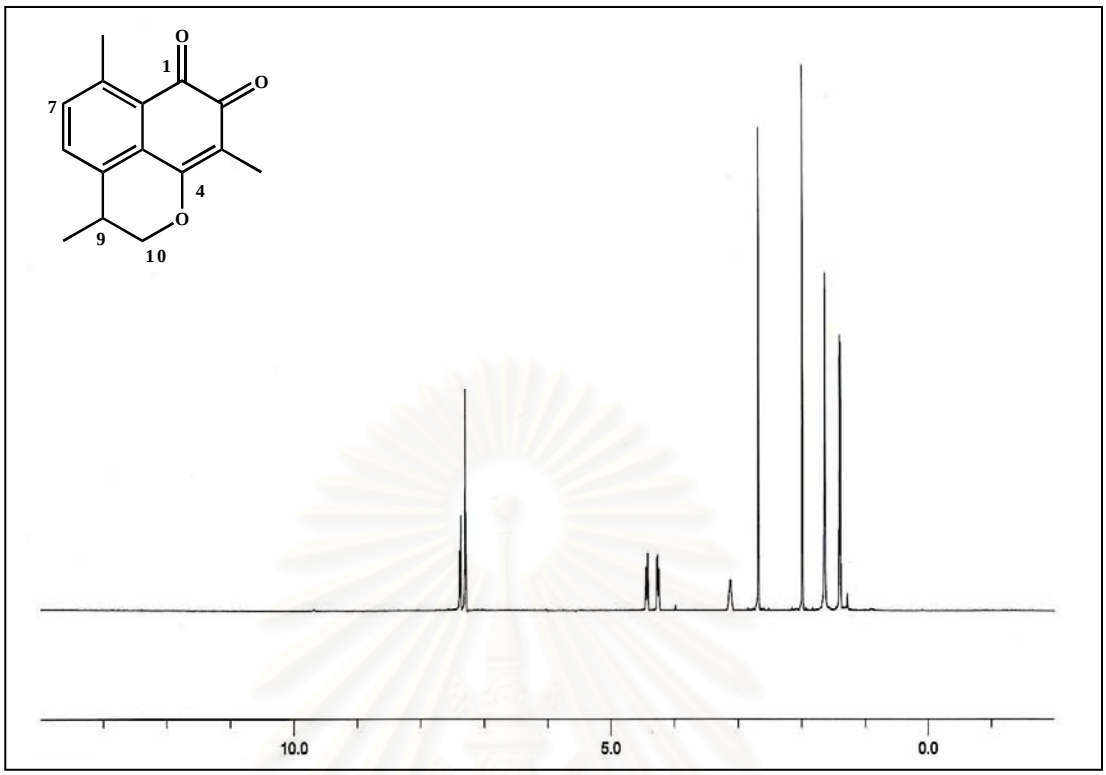
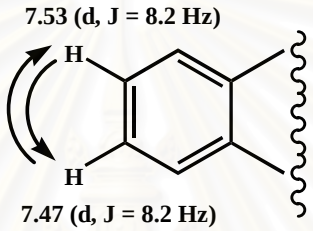


Figure 3.8 The ^{1}H-NMR spectrum of compound **6**.

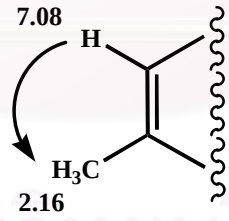
3.3.9 Structural elucidation of compound 7 [*4,11,28*]

Compound **7** was isolated as purple needle. It showed a single spot on TLC with R_f 0.25 in hexane: EtOAc (1:1).

The ^{1}H NMR spectrum of compound **7** (Figure 3.9) displays four methyl groups at δ 1.29 (s, 3H), 2.03 (s, 3H), 2.16 (s, 3H) and 2.77 (s, 3H). The COSY spectrum (Figure 3.10) presents the correlation between two *ortho*-coupled aromatic protons at δ 7.47 (d, 1H, *J* = 8.2 Hz) and 7.53 (d, 1H, *J* = 8.2 Hz). The related coupling constant between two aromatic protons suggested that this compound have *ortho* coupling characteristic as shown below.



The other important characteristic of this compound was an olefinic proton at δ 7.08 (d, 1H, *J* = 1.0 Hz) correlated with a methyl group at δ 2.16 (d, 3H, *J* = 1.2 Hz) ppm.



The ^{1}H-NMR spectrum of this compound was resemble to those of some 1,2-naphthoquinones: mansonones C [*4,11,28*]. Unfortunately, due to the small amount of this isolated compound no further information particularly on the structural elucidation could be drawn.

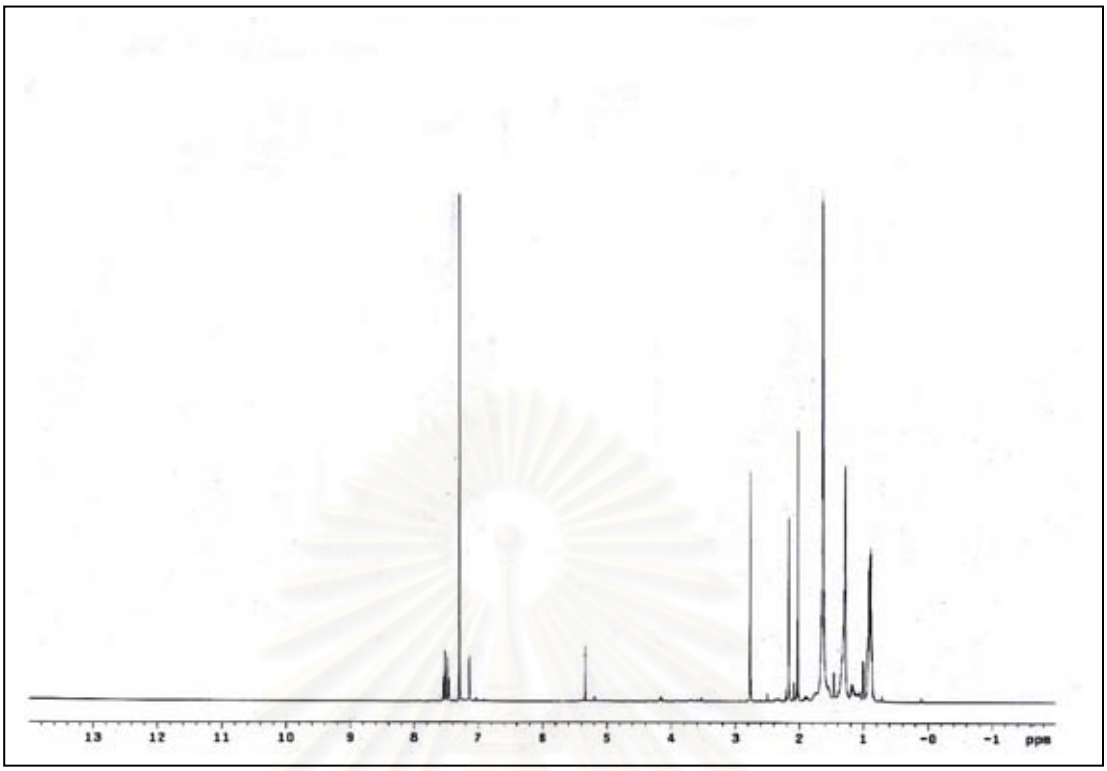


Figure 3.9 The ^{1}H-NMR spectrum of compound **7**.

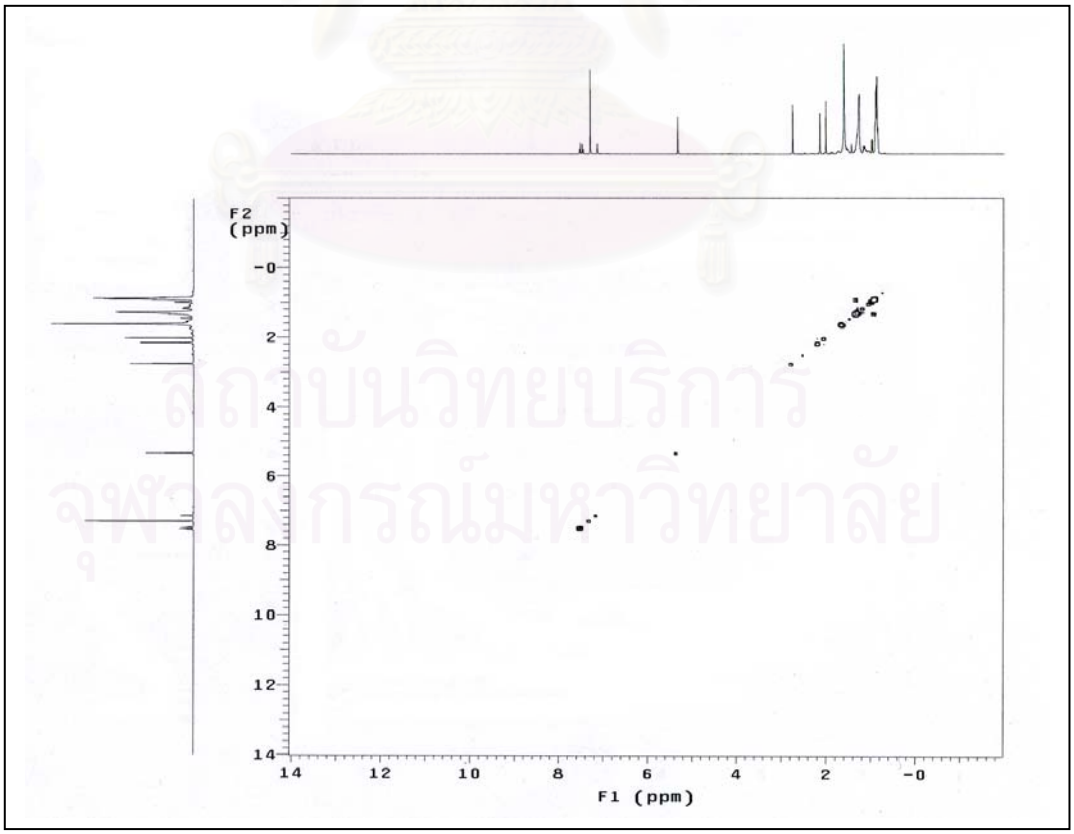
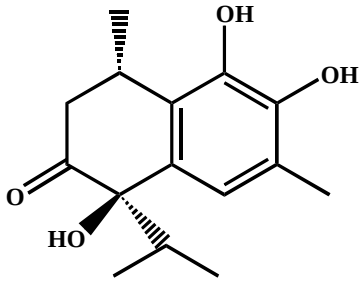


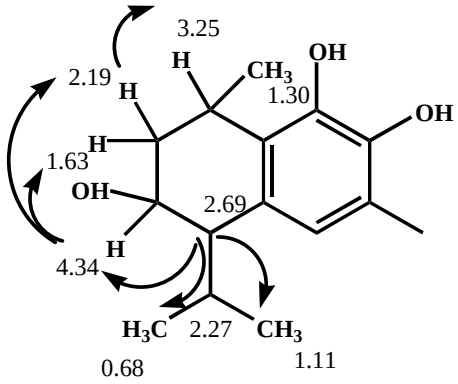
Figure 3.10 The COSY spectrum of compound **7**.

3.3.10 Structural elucidation of compound 8

Compound **8** was obtained as colorless needle, 7.4 mg, decomposed at 150 °C. A molecular formula of $C_{15}H_{22}O_3$ was deduced from the ^1H and ^{13}C NMR spectra. An isopropyl group could be noticable from the ^1H NMR spectrum (Figure 3.11) by the presence of two methyl groups at δ 1.11 (d, 3H, *J* = 6.9 Hz, CH_3-9) and 0.68 (d, 3H, *J* = 6.8 Hz, CH_3-9) which correlated with the NOESY spectrum (Figure 3.13) with the methine proton at δ 2.27 (m, 1H, H-9). This isopropyl unit also showed long-range heteronuclear correlations to the aliphatic group between the protons of the methyl groups located at δ 0.68 (CH_3-9) and δ 1.11 (CH_3-9). Other two methyl signals were presented at δ 2.24 (s, 3H) and 1.30 (d, 3H, *J* = 7.1 Hz). The HMQC spectrum (Figure 3.12) exhibited six resonances between δ 121.1 and 141.5, typical of an aromatic moiety, and other nine signals from δ 15.7 to 67.8. A detail analysis of ^1H and ^{13}C-NMR spectra of compound **8**, aided by HMQC and NOESY 2D experiments, allowed the assignment of all proton and carbon signals (see Table 3.11) by comparison the ^{13}C NMR spectrum with those sesquiterpenoids previously obtained from the heartwood of *M. gagei* [*4,27*]. The structure of compound **8** was suggested to possess sesquiterpenoidal skeleton with 15 carbon signals. The ^1H and ^{13}C NMR spectra of this compound were found to be similar to those of mansonone Q. The NOESY spectrum exhibited the correlation between isopropyl group and the proton signal at δ 2.69. This methine proton showed long range coupling to the proton signal at δ 4.34 (*J* = 3.6 Hz) indicating *cis* arrangement of these two protons. The proton signal at δ 4.34 showed a direct bond with the carbon signal at δ 67.8 which was the carbon attached to oxygen atom. From this point the proton signal also displayed the correlation to the proton signal at δ 2.19 (*J* = 10.2 Hz) and 1.63 (*J* = 3.6 Hz), these two protons exhibited a direct bond with carbon signal at δ 36.2. According to the information attained from the NOE spectrum, the proton signals at δ 4.34 and 2.19 clearly exhibited *trans* arrangement between these two protons. The proton signal at δ 2.19 was related to that at δ 3.25 (*J* = 7.4 Hz). Thus, *cis* arrangement between these two protons was manifestly established. The correlations between all proton signals of compound **8** are shown below.



mansonone Q



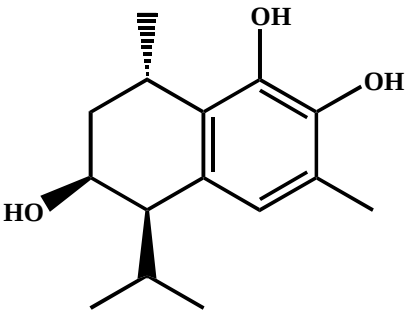
compound 8

Compound **8** was differed from mansonone Q by the absence of the carbonyl signal at δ 212.5 and a carbon bearing a hydroxyl group at δ 80.4 in the ^{13}C NMR spectrum. The carbonyl group in mansonone Q was replaced by a hydroxyl group which could be confirmed by the appearance of a multiplet signal at δ 4.34 in ^{1}H NMR and a carbon bearing a hydroxyl group at δ 80.4 was substituted by a methine carbon detected as a triplet proton at δ 2.69 (J = 3.9 Hz). The assignments immediately permitted by correlation of the proton and carbon signals by HMQC and ^{1}H-^{1}H NOESY as presented in Table 3.11.

Table 3.11 The ^{1}H and ^{13}C-NMR spectral data of mansonone Q [*4,27*] and compound **8**.

Position	Chemical shift (ppm)			
	mansonone Q		compound **8**	
	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C
1	-	141.2	-	141.5
2	-	140.1	-	139.9
3	-	122.1	-	121.1
4	6.88, s, 1H	119.9	6.51, s, 1H	122.7
4a	-	133.0	-	129.6
5	-	80.4	2.69, t, 3H (3.9)	49.7
6	-	212.5	4.34, m, 1H	67.8
7	2.50, dd, 1H (4.6, 16.8) 3.04, dd, 1H (10.1, 16.8)	40.5	2.19, ddd, 1H (13.2, 10.3, 7.4) 1.63, m, 1H	36.2
8	3.60, m, 1H	29.3	3.25, m, 1H	27.6
8a	-	123.7	-	126.0
9	2.10, sept, 1H (6.9)	38.0	2.27, m, 1H	26.7
1-OH	5.46, s, 1H	-	5.25, s, 1H	-
2-OH	4.65, s, 1H	-	4.75, s, 1H	-
3-CH_3	2.25, s, 3H	15.6	2.24, s, 1H	15.7
5-OH	3.88, s, 1H	-	-	-
6-OH	-	-	1.41, s, 1H	-
8-CH_3	1.52, d, 3H (7.3)	23.4	1.30, d, 3H (7.1)	22.6
9-$(CH_3)_2$	0.86, d, 3H (6.7) 0.86, d, 3H (6.7)	16.7 15.6	0.68, d, 3H (6.8) 1.11, d, 3H (6.9)	22.0 24.0

According to spectroscopic data, compound **8** was obviously characterized as 5-isopropyl-3,8-dimethyl-5,6,7,8-tetrahydronaphthlene-1,2,3-triol. Throughout the chemical literatures, this compound had never been reported. Therefore, compound **8** was another new sesquiterpenoid isolated from *M. gagei*, named mansonone T.



Compound 8: Mansonone T

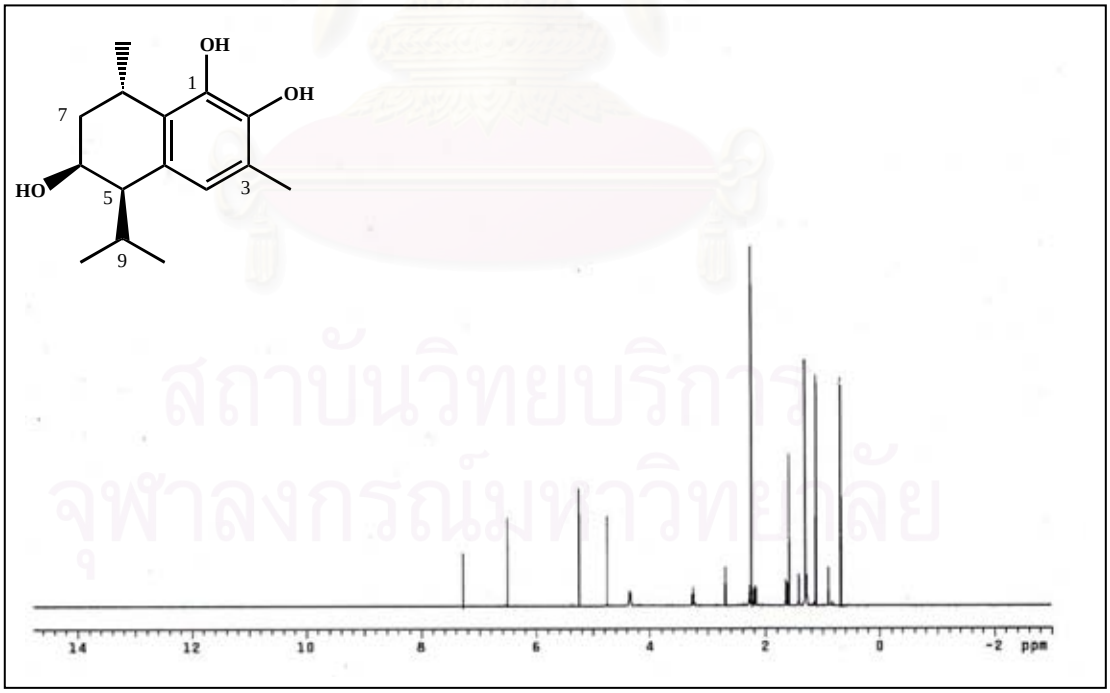


Figure 3.11 The ^{1}H-NMR spectrum of compound **8**.

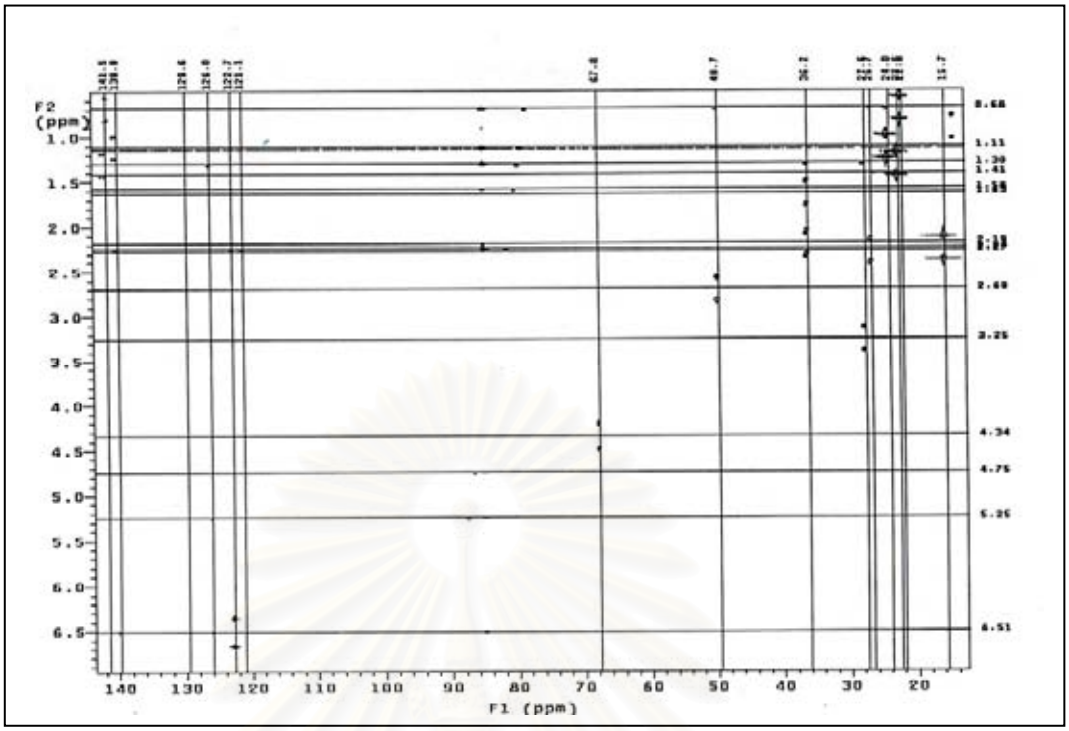


Figure 3.12 The HMQC spectrum of compound **8**.

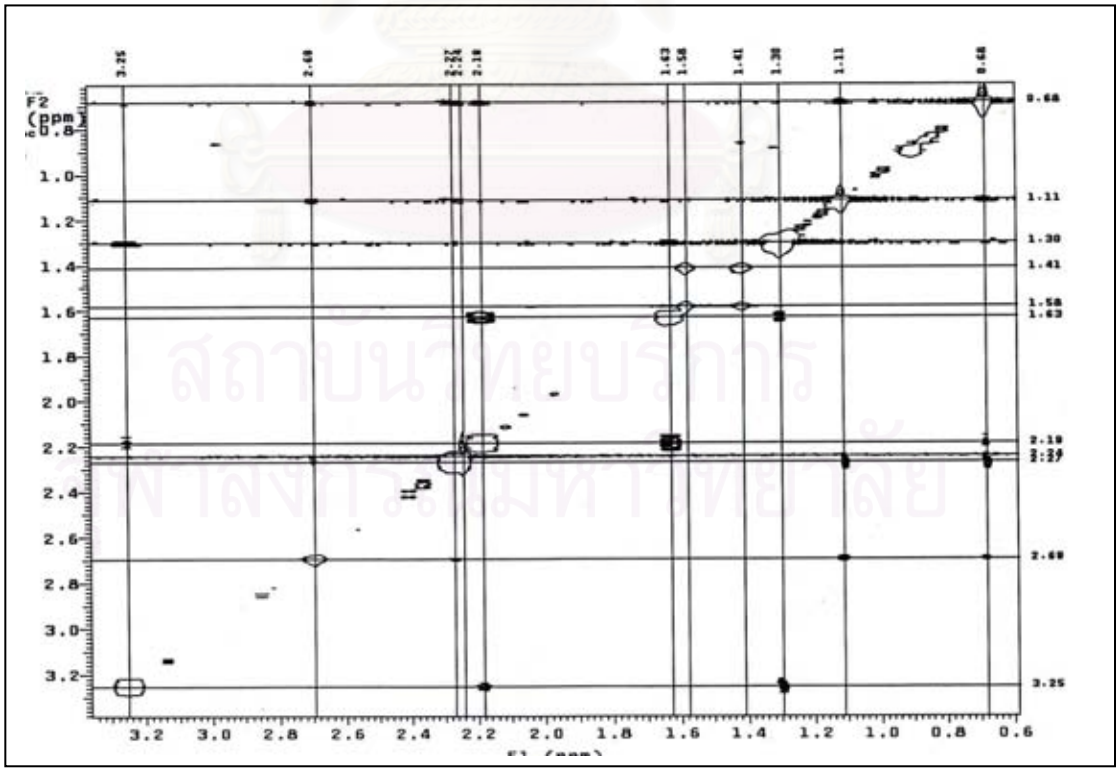


Figure 3.13 The NOESY spectrum of compound **8**.

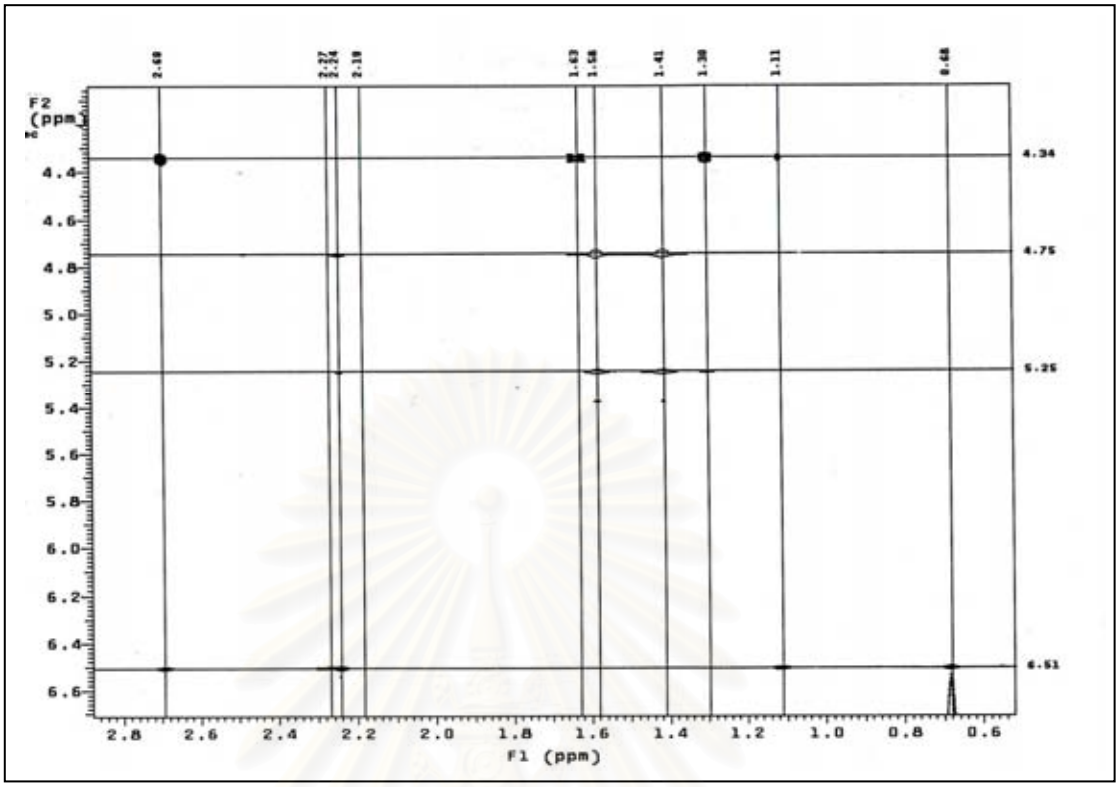


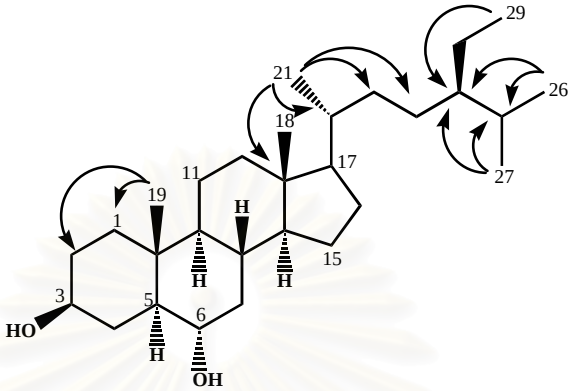
Figure 3.13 (continued)

3.3.11 Structural elucidation of compound 9

Compound **9** was isolated form fraction RC-5 as white platelet, 20.7 mg, m.p. 214.0-215.0 ºC, 0.04 % w/w of dichloromethane crude extract. It showed a single spot on TLC with R_f value 0.23 in hexane: EtOAc (1:1).

The ^{1}H NMR spectrum (Figure 3.14) showed the pattern of saturated 24-ethylcholestane structure with two hydroxyl groups. The proton signals at δ 3.42 (dt, 1H, *J* = 4.4, 11.0 Hz) and 3.58 (m, 1H) could be assigned as hydroxymethine protons containing a large *J* values (11.0 Hz). In addition, six methyl groups were observed at δ 0.63 (s, 3H), 0.80 (s, 3H), 0.91 (d, 3H, *J* = 6.5 Hz), 0.82 (d, 3H, *J* = 7.0 Hz), 0.81 (d, 3H, *J* = 7.0 Hz) and 0.85 (t, 3H, *J* = 7.0 Hz). The ^{13}C NMR (Figure 3.15) spectral data exhibited 29 carbon signals. From the HSQC spectrum (Figure 3.17), the carbon signal at δ 71.3 showed direct bond to hydroxymethine proton at δ 3.58. This proton signal exhibited long range coupling to the carbon signal at δ 32.3 (C-4) and 31.0 (C-2). This was supported that the carbon signal at δ 71.3 was the carbon attached to oxygen atom at C-3 position. The carbon signal at δ 69.3 exhibited direct coupling to

proton signal at δ 3.42. This proton showed long range correlation to carbon signal at δ 41.7 (C-7) and 51.7 (C-5). The long range coupling correlated between methyl proton and their carbon signals was also detected by HMBC spectrum (Figure 3.18).



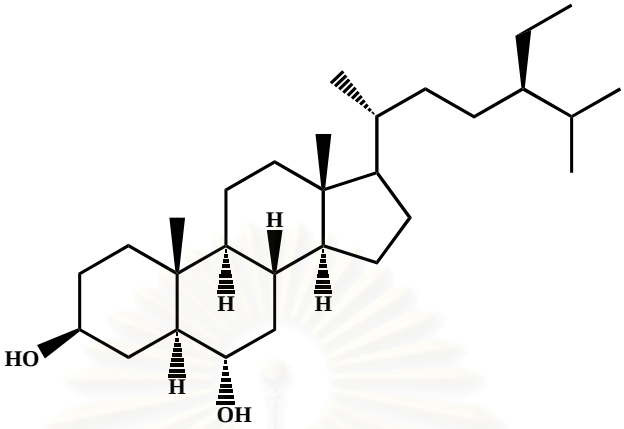
The COSY spectrum (Figure 3.16) exhibited the correlation between the proton signal at δ 3.58 and two methylene protons at δ 2.20 and 1.25. The proton signal at δ 3.42 revealed the correlation to proton signals at δ 2.00, 1.05 and 0.90.

The comparison between ^{13}C NMR chemical shifts of compound **9** with those of relevant compounds [*29-31*] suggested that compound **9** should contain a steroidal skeleton with two hydroxyl groups. Based on the literature review, compound **9** showed the similar physical property and spectroscopic data to those of stigmastane-3β,6α-diol. Table 3.12 shows the ^{13}C NMR spectral data between stigmastane-3β,6α-diol and that of compound **9**.

Table 3.12 The ^{13}C-NMR spectral data of stigmastane-3β,6α-diol [*29-31*], and compound **9**.

Position	Chemical shift (ppm)	
	stigmastane-3β,6α-diol	compound **9**
1	37.3	37.3
2	31.1	31.0
3	71.3	71.3
4	32.3	32.3
5	51.7	51.7
6	69.5	69.6
7	41.7	41.7
8	34.3	34.3
9	53.8	53.8
10	36.3	36.3
11	21.2	21.1
12	39.8	39.8
13	42.6	42.6
14	56.2	56.2
15	24.2	24.2
16	28.2	28.2
17	56.1	56.1
18	12.0	12.0
19	13.5	13.5
20	36.1	36.1
21	18.7	18.7
22	33.9	33.9
23	26.1	26.0
24	45.8	45.8
25	29.1	29.1
26	19.8	19.9
27	19.0	19.0
28	23.1	23.1
29	12.0	12.1

The structure of compound **9** was elucidated as stigmastane-3β,6α-diol.



Compound 9: Stigmastane-3β,6α-diol

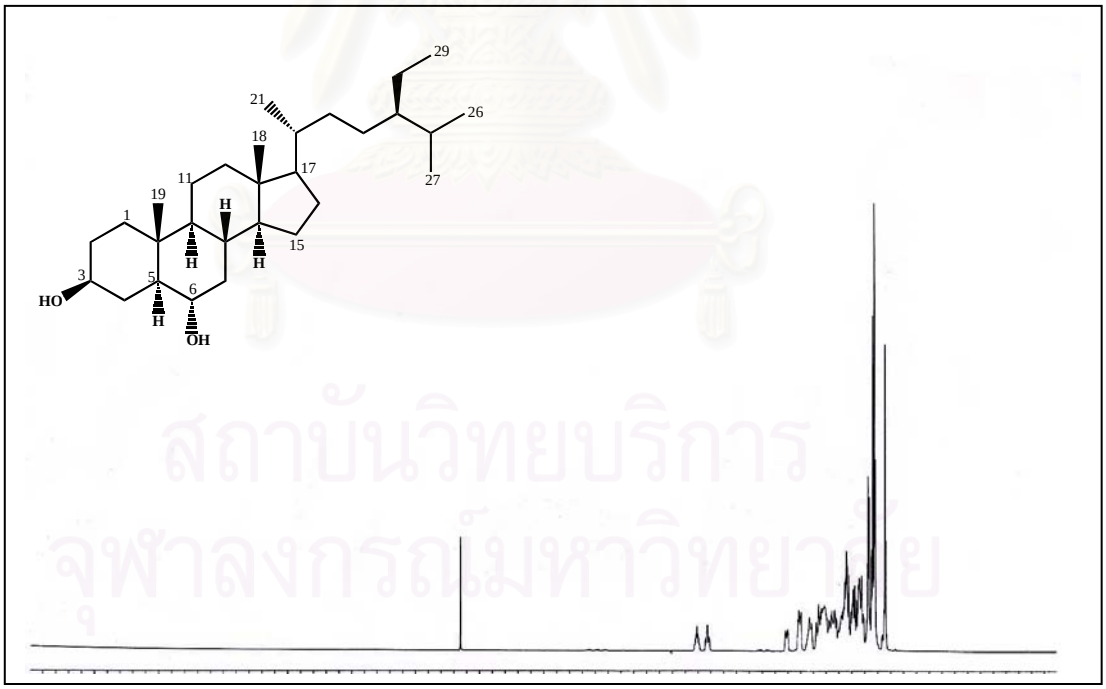


Figure 3.14 The ^{1}H-NMR spectrum of compound **9**.

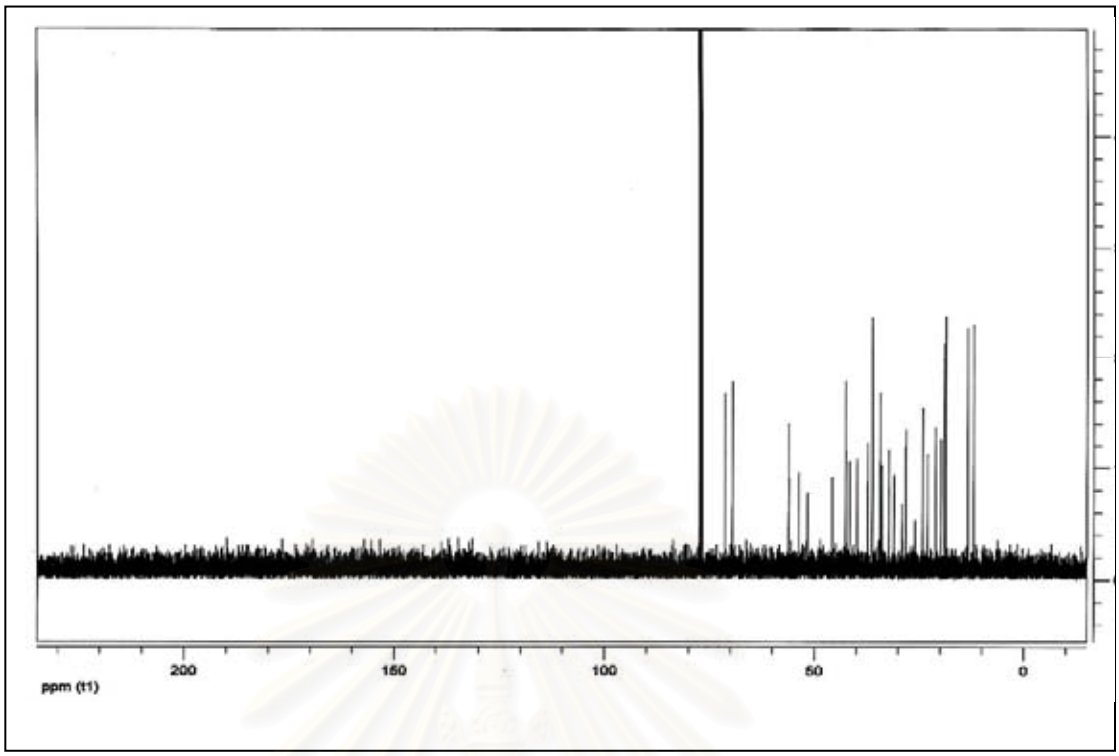


Figure 3.15 The ^{13}C-NMR spectrum of compound **9**.

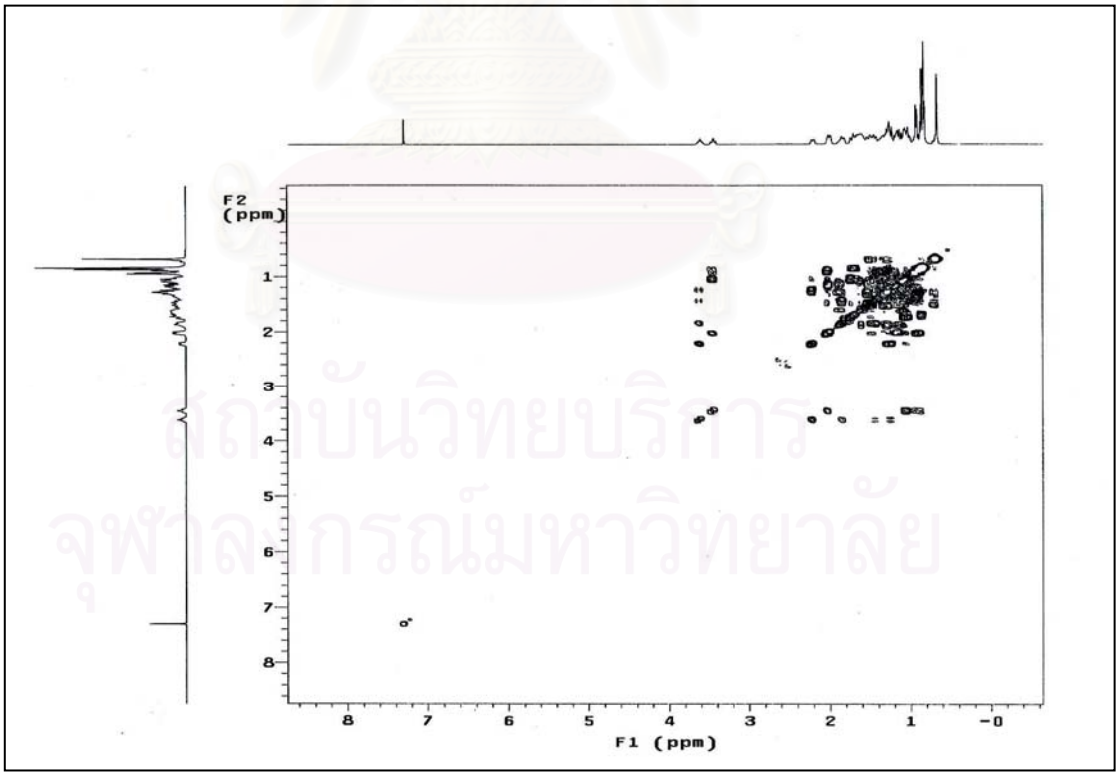


Figure 3.16 The COSY spectrum of compound **9**.

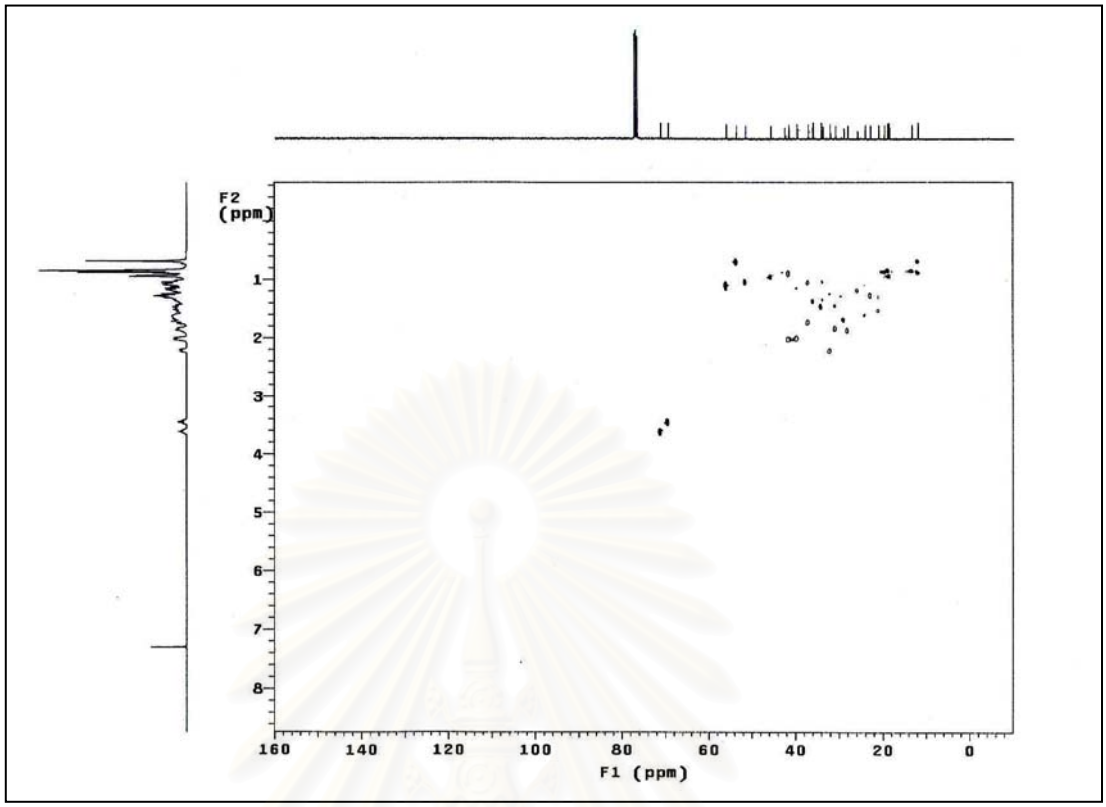


Figure 3.17 The HSQC spectrum of compound **9**.

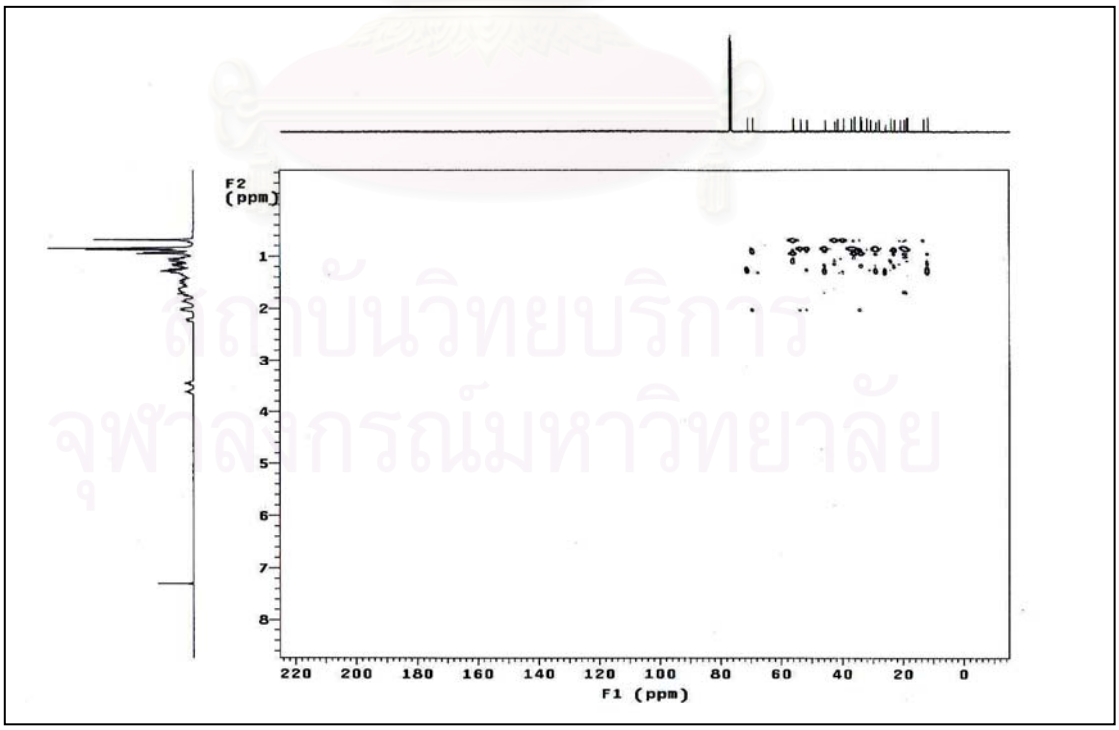


Figure 3.18 The HMBC spectrum of compound **9**.

3.4 Chemical constituents of the leaves of *M. gagei*

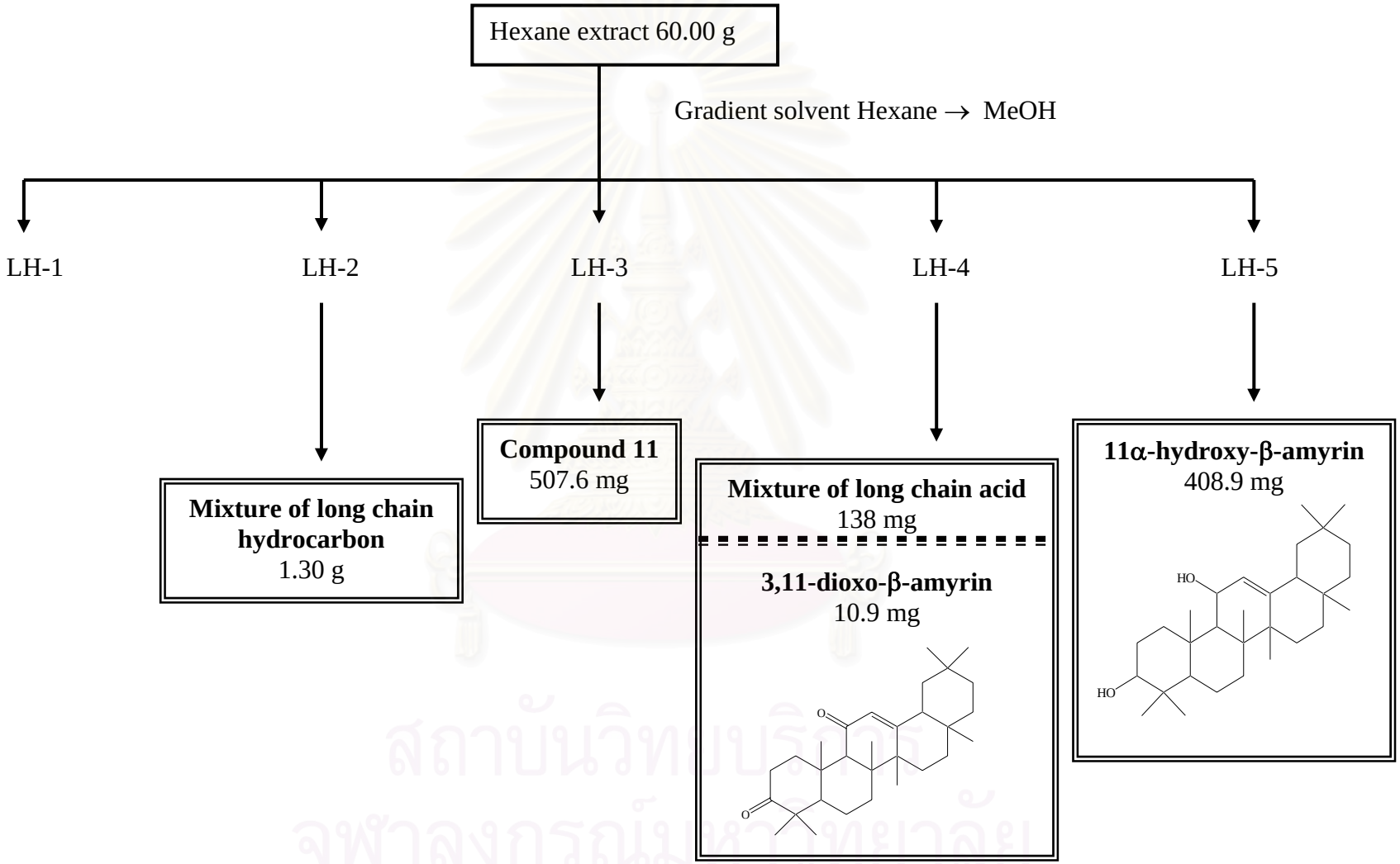
3.4.1 Chemical constituents of hexane extract

The hexane extract (60.0 g), greenish crude, was separated into subfractions by silica gel quick column chromatography technique eluting with CH_2Cl_2, CH_2Cl_2-EtOAc and EtOAc-MeOH. The combined five subfractions, LH1-LH5 were obtained. The results of separation are shown in Table 3.13.

Table 3.13 The results of separation the hexane extract of the leaves of *M. gagei*.

Fraction	Solvent system	Appearance	Weight (g)
LH1	0-5 % hexane-CH_2Cl_2	yellow oil	1.63
LH2	10-20 % hexane-CH_2Cl_2	yellow wax	7.31
LH3	20-100 % hexane-CH_2Cl_2	green -yellow wax	6.42
LH4	0-100 % CH_2Cl_2-EtOAc	green solid	7.12
LH5	0-20 % EtOAc-MeOH	green solid	2.45

Fractions LH1-5 were reseparated using column chromatography. As the results, two mixtures: a mixture of long chain alcohol (**10**), a mixture of long chain acid (**12**) and three compounds: 3,11-dioxo-β-amyrin (**13**), 11α-hydroxy-β-amyrin (**14**) and compound **11** were isolated. The results are summarized in Scheme 3.5.



Scheme 3.5 The separation of hexane extract of the leaves of *M. gagei*

3.4.2 Structural elucidation of mixture 10

Mixture **10** was obtained from subfraction LH-1 as white platelet, 1.30 g (2.33 % w/w of hexane extract), m.p. 63.4-65.5 ºC, R_f value 0.80 in hexane.

The IR spectral data exhibited absorption bands at 2914, 2842 cm^{-1} corresponding to C-H stretching vibration and at 1460 cm^{-1} to C-H bending vibration of CH_3- and -CH_2-. The IR spectrum and the tentative assignments of mixture **10** are shown in Figure 3.19 and Table 3.14.

Table 3.14 The IR absorption band assignments of mixture **10**.

Wave number (cm^{-1})	Band type	Tentative assignments
2914, 2842	s	C-H stretching vibration of CH_3-, -CH_2-
1460	s	C-H bending vibration of CH_3- and -CH_2-
722	s	C-H rocking mode of -CH_2-

The ^{1}H NMR spectrum (Figure 3.20) exhibited high intensity signal at δ 1.23 revealed that several interlinking of methylene groups was present in the molecule. In addition, the signals belong to a methyl group could be detected at δ 0.80, and ethylene protons around δ 1.12 to 1.46. According to all spectroscopic data, mixture **10** could be a saturated long chain hydrocarbon.

$$\mathbf{CH_3\text{-}(CH_2)_n\text{-}CH_3}$$

Mixture 10: saturated long chain hydrocarbon

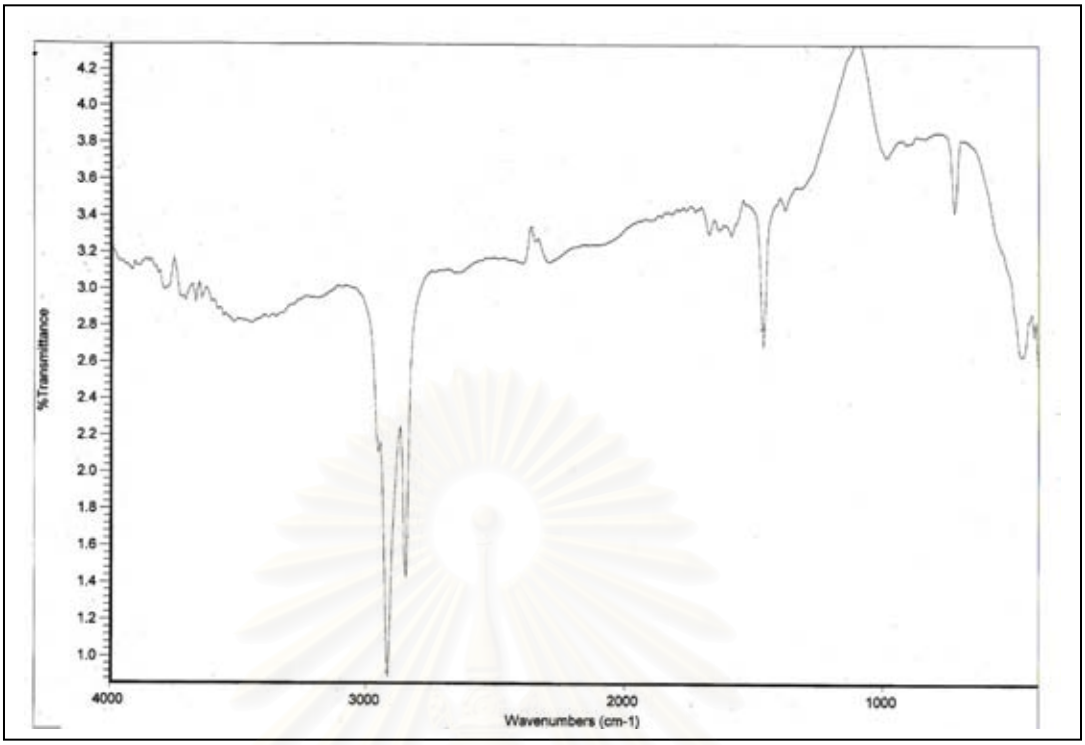


Figure 3.19 The IR spectrum of mixture **10**.

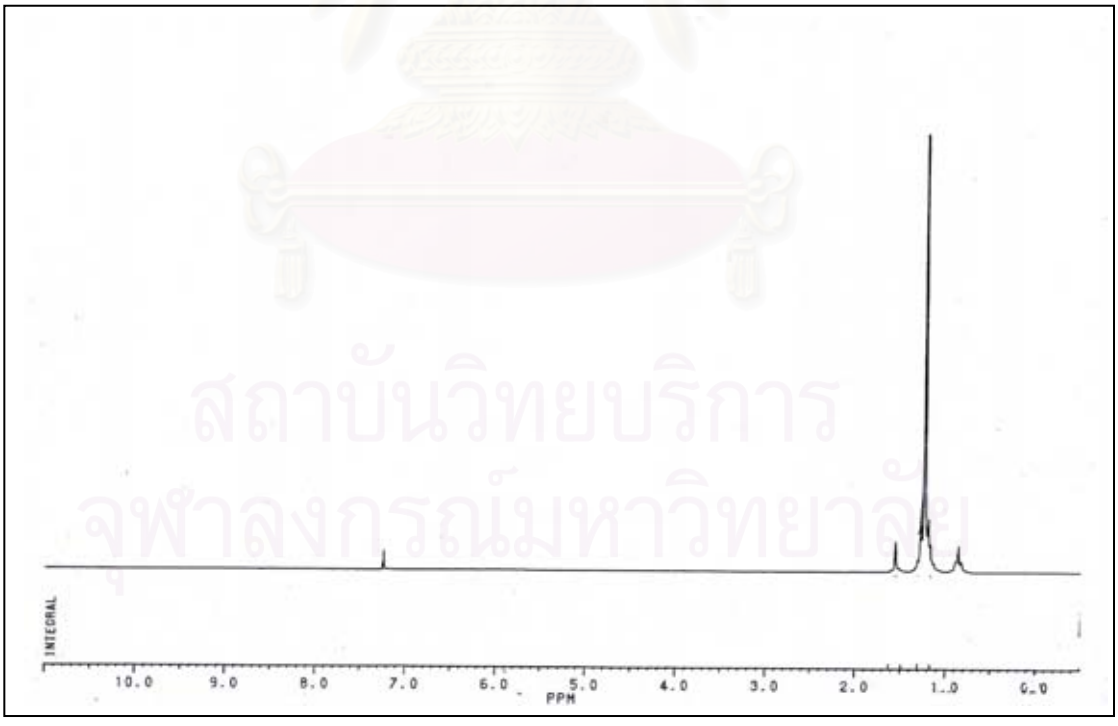


Figure 3.20 The ^{1}H NMR spectrum of mixture **10**.

3.4.3 Structural elucidation of compound 11.

Compound **11** was obtained as white solid, 0.50 g (0.91 % w/w of hexane extract), m.p. 67.5-69.5 ºC, R_f value 0.75 in hexane: EtOAc (3:1). It was soluble in hexane and CH_2Cl_2 but insoluble in EtOAc and MeOH.

The IR spectrum (Figure 3.21) revealed significant absorption peak at 3448 cm^{-1} (O-H stretching) and 1734 cm^{-1} (C=O stretching) carbonyl group. The IR absorption band assignments of this compound are shown in Table 3.15.

Table 3.15 The IR absorption band assignments of compound **11**.

Wave number (cm^{-1})	Band type	Tentative assignments
3448	br	O-H stretching vibration of hydroxyl group
2916, 2849	s	C-H stretching vibration of CH_3-, -CH_2-
1734	s	C=O stretching vibration of carbonyl ester
1472	s	C-H bending vibration of CH_3- and -CH_2-

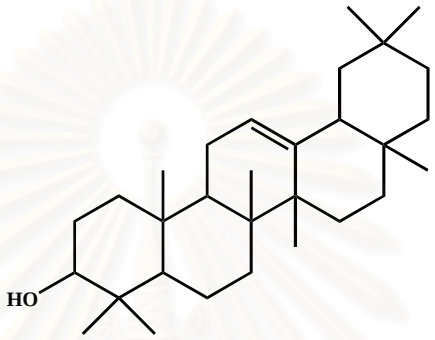
The ^{1}H NMR spectrum (Figure 3.21) exhibited the proton signals at δ 5.21 (s), 4.20 (t, *J* = 6.7 Hz) and other proton signals around δ 0.91 to 2.25. The ^{13}C NMR spectrum (Figure 3.22) revealed two carbonyl carbon signals at δ 173.7 and 174.0 and olefinic carbon signals at δ 121.6 and 145.2. The carbons bearing hydroxyl groups could be detected at δ 64.4 and 80.5. From the spectroscopic data, it was indicative that compound **11** may contain a triterpenoidal moiety joining with saturated component by an ester linkage.

Study on compound **11A**

Compound **11A** was obtained from basic hydrolysis of compound **11** as bright white needle, R_f 0.54 (solvent: hexane: ethyl acetate, 1:1), 24.3 mg, m.p. 195.0-197.0 ºC. It was soluble in CH_2Cl_2, but slightly soluble in EtOAc.

The IR spectrum (Figure 3.24) exhibited absorption band at 3447 cm^{-1} stated the presence of O-H stretching vibration of hydroxyl group, at 2918, 2849 cm^{-1} for C-H stretching vibration of -CH_3, -CH_2- and C=C stretching vibration at 1637 cm^{-1}. The ^{1}H NMR spectrum (Figure 3.25) exhibited an olefinic proton at δ 5.23 (t, 1H, *J* = 3.5 Hz). At δ 3.20 displayed a multiplet proton which could be assigned for the proton bearing hydroxyl group. Other signals around δ 0.70 to 1.90 should be the signals of

methyl, methylene and methine protons. The ^{13}C NMR spectrum (Figure 3.26) exhibited 30 carbon signals. Two signals at δ 121.7 to 145.0 could be assigned for two olefinic carbons and that at δ 78.9 should correspond to a carbon bearing a hydroxyl group. From the literature review [*17*] and co-TLC with authentic sample obtained from *Rhizophora apiculata* Bl. [*17*], compound **11A** was clearly identified as β-amyrin.



Compound 11A: β-amyrin

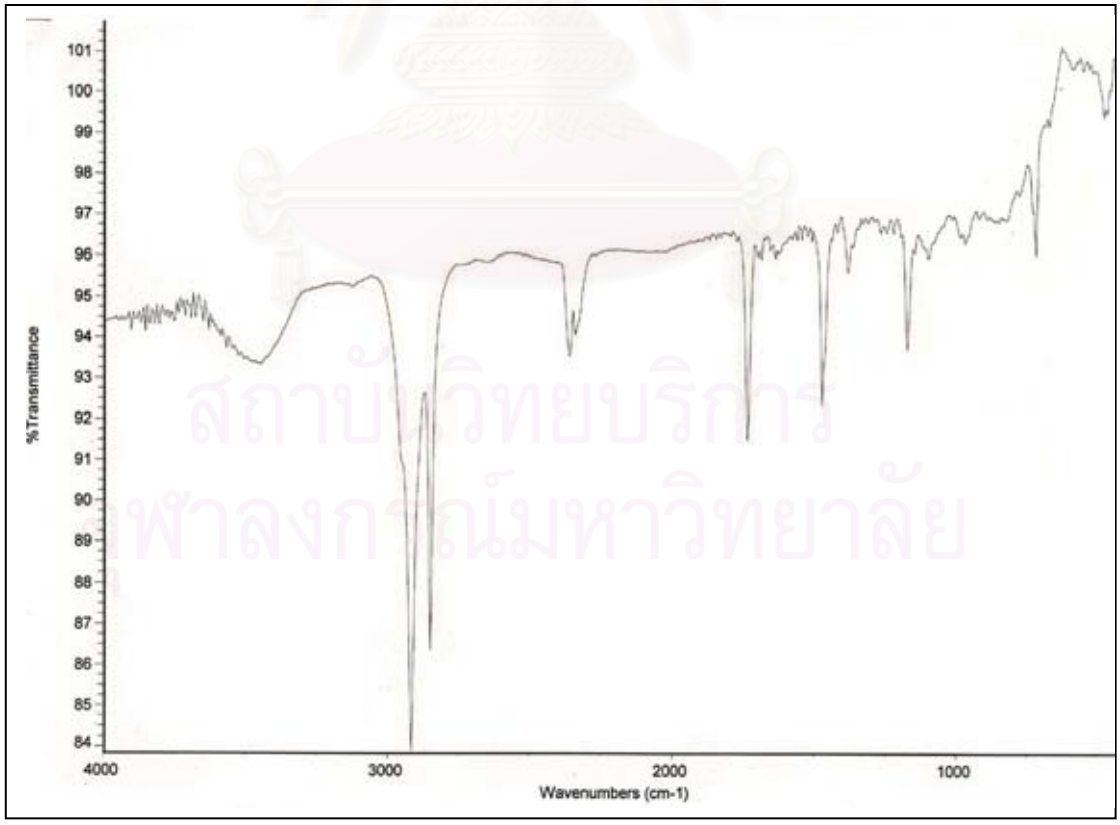


Figure 3.21 The IR spectrum of compound **11**.

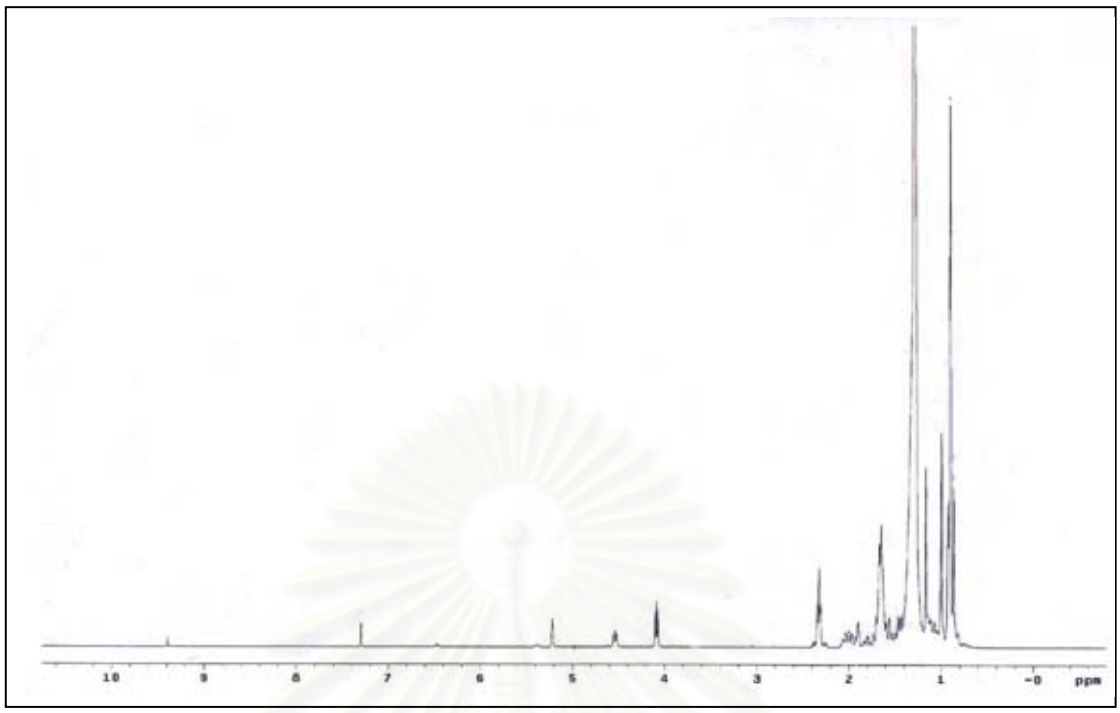


Figure 3.22 The ^{1}H NMR spectrum of compound **11**.

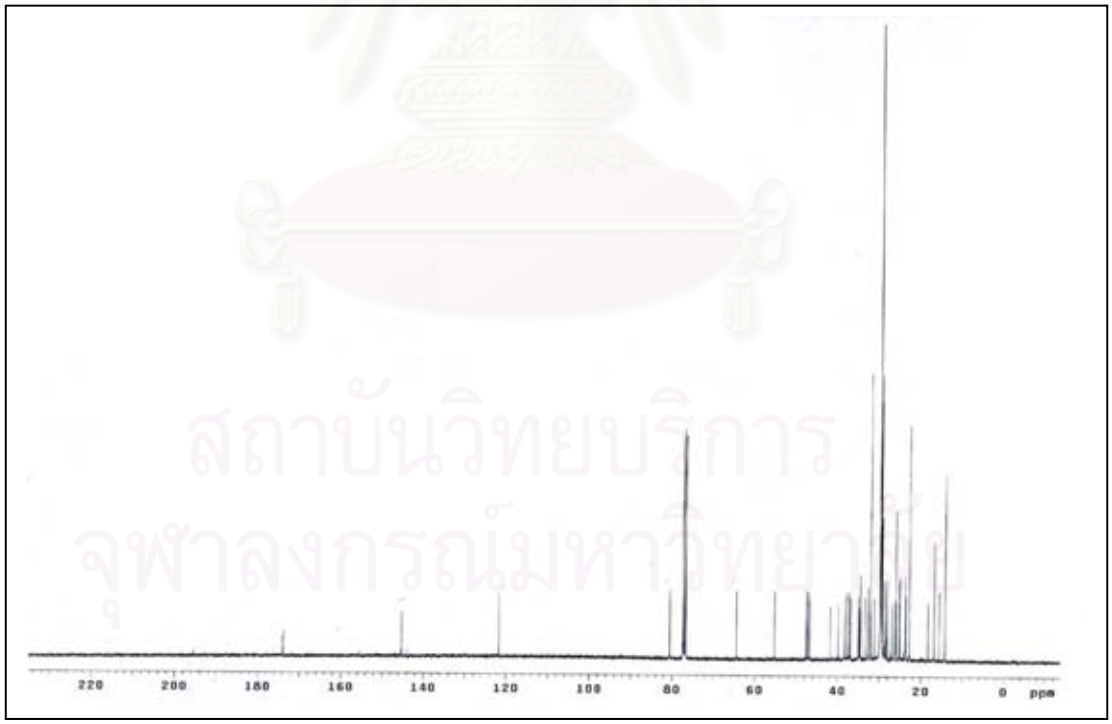


Figure 3.23 The ^{13}C NMR spectrum of compound **11**.

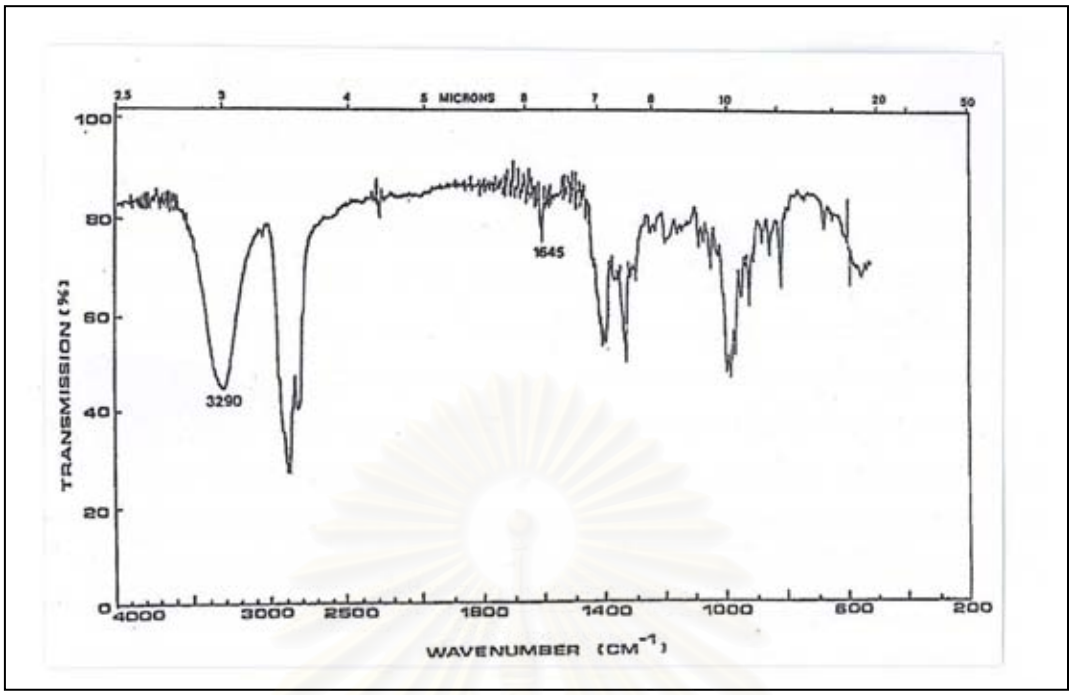


Figure 3.24 The IR spectrum of compound **11A**.

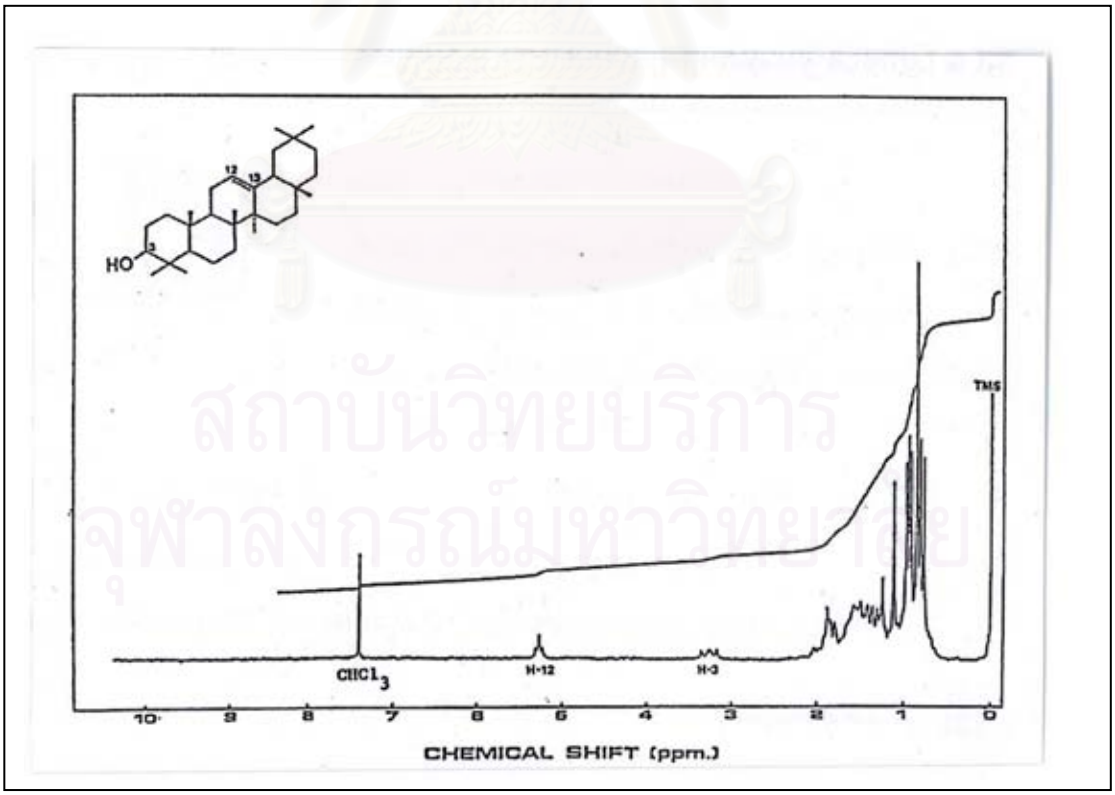


Figure 3.25 The ^{1}H NMR spectrum of compound **11A**.

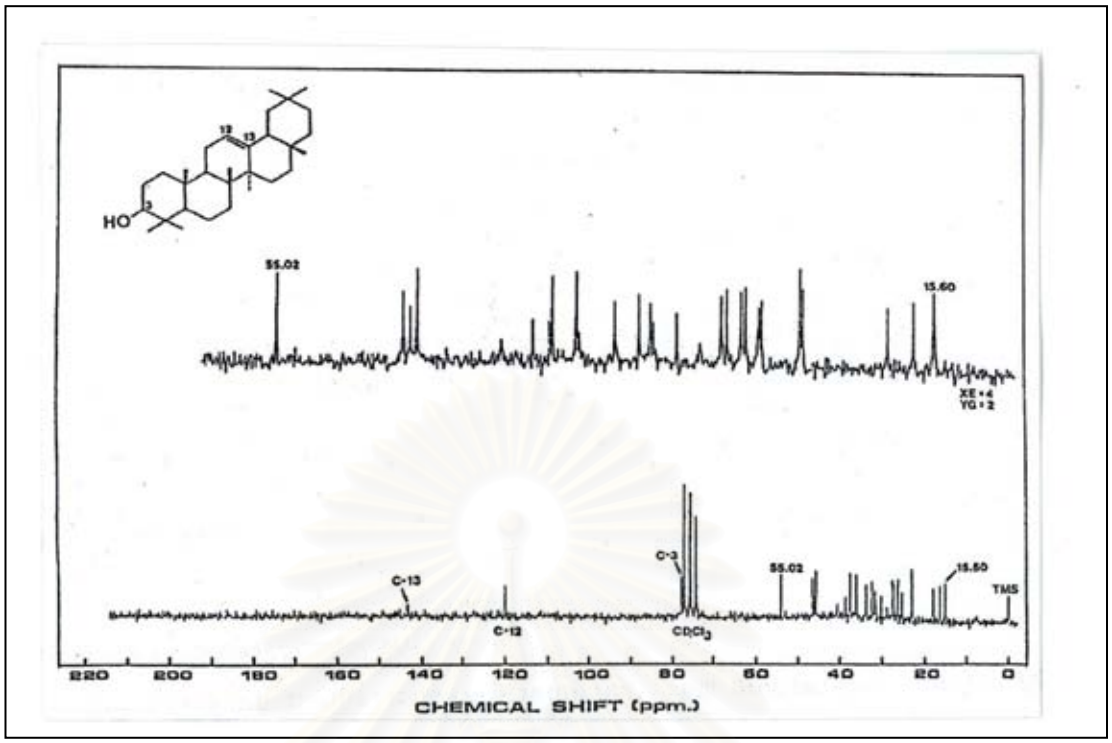
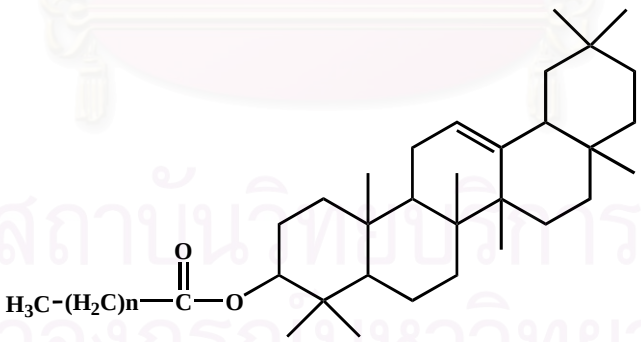


Figure 3.26 The ^{13}C NMR spectrum of compound **11A**.

According to all spectroscopic data, compound **11** should be β-amyrin connecting with a saturated component by an ester linkage as structure shown below.



Compound 11: β-amyrin ester

3.4.4 Structural elucidation of mixture 12.

Mixture **12** was isolated as white solid, 0.14 g, m.p. 70.5-74.0 ºC, 0.25 % w/w of hexane extract. The IR spectrum of this mixture (Figure 3.27) was similar to that of mixture **10** except for the absorption band at 1703 cm^{-1} in the IR spectrum of mixture **12** which was the C=O stretching of carbonyl group. Table 3.16 shows the IR absorption band assignments of this mixture.

Table 3.16 The IR absorption band assignments of mixture **12**.

Wave number (cm^{-1})	Band type	Tentative assignments
2914, 2842	s	C-H stretching vibration of CH_3-, -CH_2-
1703	s	C=O stretching vibration
1460	s	C-H bending vibration of CH_3- and -CH_2-
722	s	C-H rocking mode of -CH_2-

The ^{1}H NMR spectrum (Figure 3.28) exhibited a high intensity signal at δ 1.40 (t, *J* = 3.4 Hz) suggesting several interlinking of methylene groups in the molecule. The signal of a methyl group was detected at δ 0.95 (t, *J* = 2.45 Hz). The ^{13}C NMR spectrum (Figure 3.29) revealed an important signal at δ 180.0 of a carbonyl group, possibly a carboxylic group. According to all spectroscopic data, mixture **12** could be elucidated as saturated long chain carboxylic acid.

$$\mathbf{CH_3\text{-}(CH_2)_n\text{-}COOH}$$

Mixture 12: Saturated long chain carboxylic acid

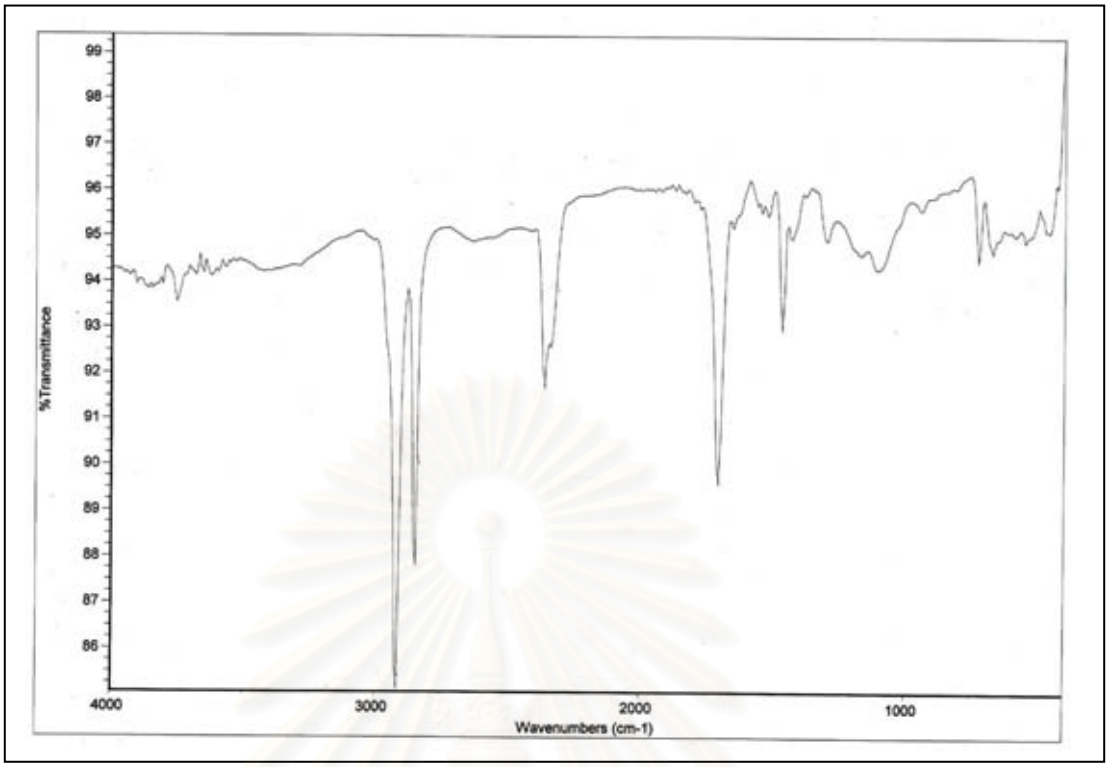


Figure 3.27 The IR spectrum of mixture **12**.

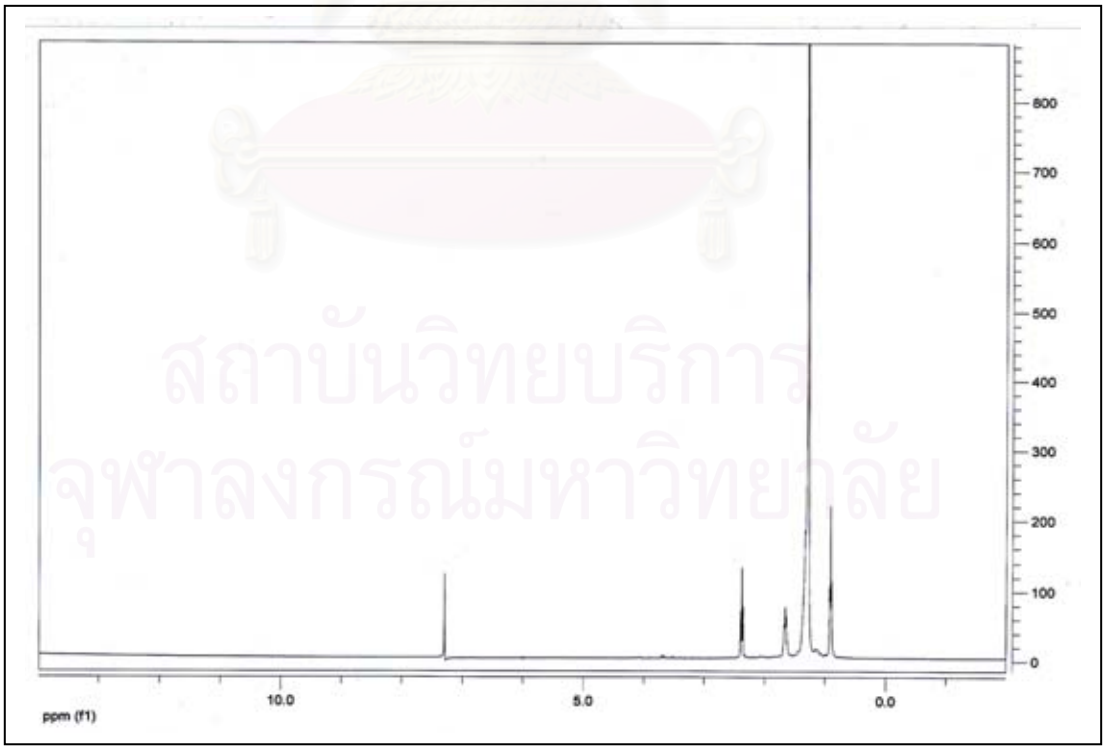


Figure 3.28 The ^{1}H NMR spectrum of mixture **12**.

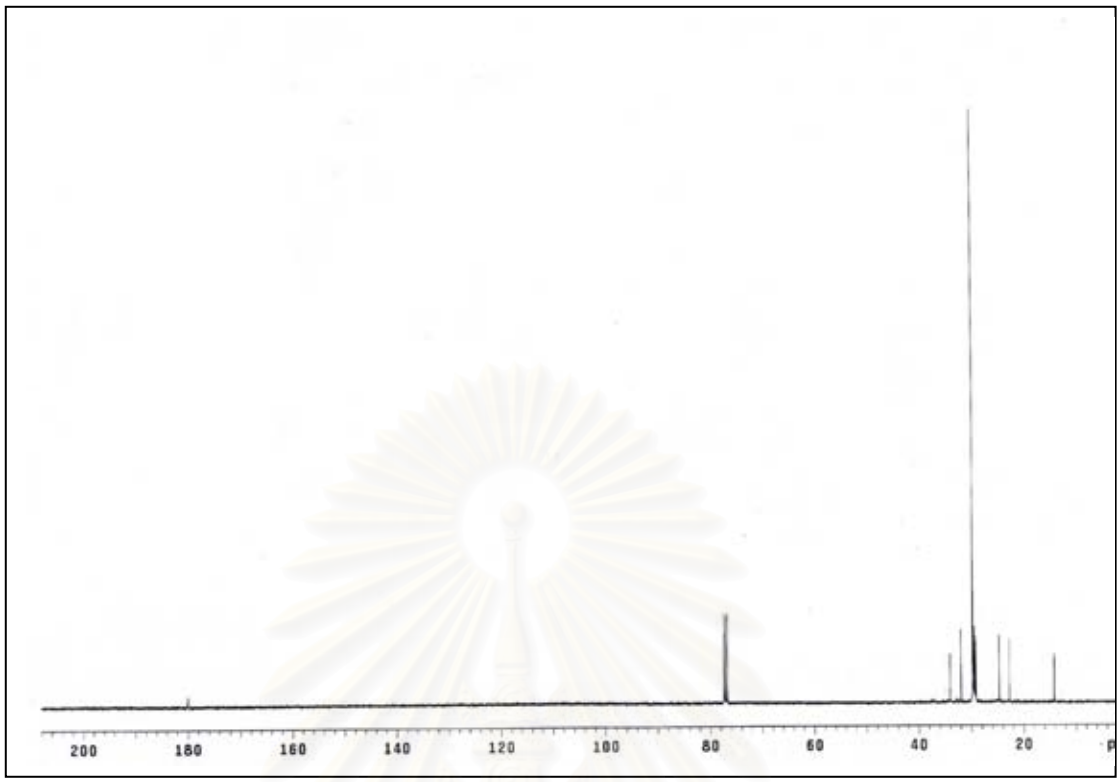
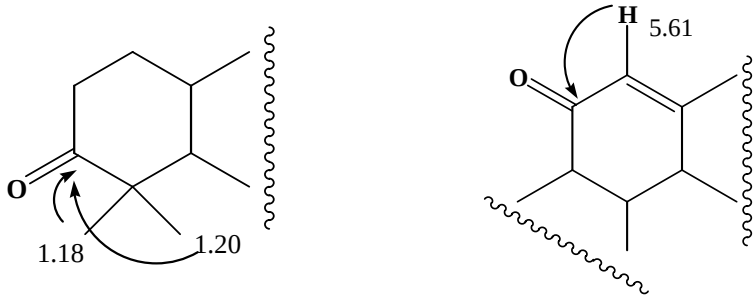


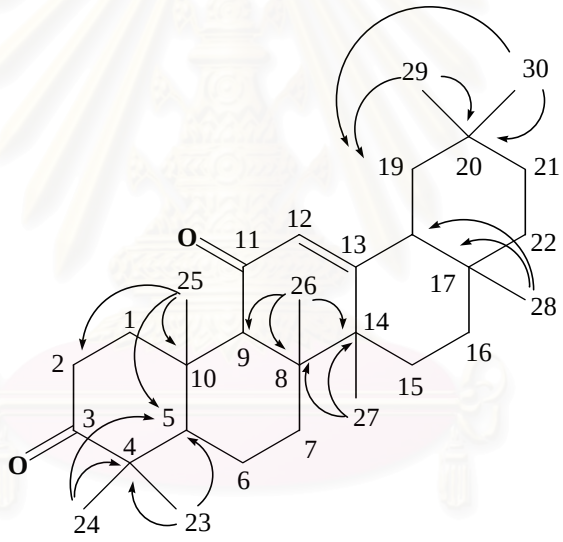
Figure 3.29 The ^{13}C NMR spectrum of mixture **12**.

3.4.5 Structural elucidation of compound 13.

Compound **13** was obtained as white solid, 10.7 mg (0.02 % w/w of hexane extract). This compound showed a single spot on TLC with R_f value 0.80 in hexane: EtOAc (1:1). The ^{1}H NMR spectrum (Figure 3.31) exhibited the proton signals at δ 2.98 (dt, 2H, *J* = 6.32, 7.04 Hz), 2.60 (m, 1H) and other aliphatic signals around δ 0.93 to 2.42. The ^{13}C NMR spectrum (Figure 3.32) exhibited 30 carbon signals. The important signals at δ 217.3 and 199.6 revealed the carbonyl carbons and two signals at δ 171.2 and 128.0 were olefinic carbons. From DEPT and HSQC spectra, eight methyl groups were detected at δ 33.1, 28.8, 26.4, 23.5, 23.4, 21.4, 18.6 and 15.7. Another set of signals around δ 18.8 to 61.0 revealed an aliphatic part of this compound. The COSY spectrum (Figure 3.33) showed the correlation between the proton signals at δ 2.99 and 2.65 and at δ 2.62 and 2.48. The olefinic proton at δ 5.61 (s, 1H) did not show any correlation to other protons. On the other hand, this proton exhibited the long range coupling to the carbonyl carbon at δ 199.6 which was identified as the carbonyl group located at C-11.



The presence of long range coupling of two methyl groups at δ 1.20 (C-23) and 1.18 (C-24) to the carbonyl signal at δ 217.4, suggested that this carbonyl group be located at C-3 position. The long range coupling correlations between eight methyl groups and relavant carbons were obtained from HMBC spectrum (Figure 3.35) as described below.

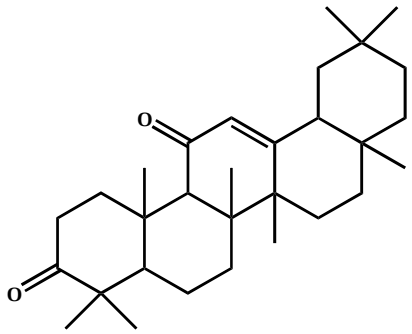


All the spectroscopic data supported that the structure of compound **13** include triterpenoidal skeleton. The ^{13}C NMR spectral data of compound **13** compared with those of β-amyrin and β-amyrenonypalmitate is demonstrated in Table 3.17.

Table 3.17 The ^{13}C NMR chemical shift assignments of β-amyrin, β-amyrenonypalmitate [*32-36*] and compound **13** and compound **14**.

Position	Chemical shift (ppm)			
	β-amyrin	β-amyrenonypalmitate	compound **13**	Compound **14**
1	38.5	38.8	34.4	40.4
2	27.0	23.4	39.8	27.3
3	78.9	80.3	217.4	78.7
4	38.7	38.1	47.8	39.0
5	55.1	55.0	55.4	55.1
6	18.3	18.7	18.8	18.4
7	32.6	32.7	32.1	33.0
8	39.7	43.4	43.5	43.4
9	47.6	61.7	61.0	56.4
10	37.0	36.9	36.7	38.0
11	23.4	200.1	199.6	67.6
12	121.7	128.1	128.0	125.3
13	145.0	170.6	171.2	150.0
14	41.7	45.4	45.2	41.7
15	28.3	26.4	26.5	26.7
16	26.2	26.4	26.3	26.2
17	32.5	32.4	32.4	32.3
18	47.2	45.4	47.6	46.5
19	46.8	45.2	45.2	46.5
20	31.1	31.0	31.1	31.1
21	34.8	34.4	34.3	34.6
22	37.2	36.5	36.5	36.9
23	28.1	28.1	26.4	28.1
24	15.5	16.8	18.6	15.5
25	15.5	16.4	15.7	16.9
26	16.8	17.4	21.4	18.0
27	26.0	23.5	23.5	26.2
28	27.3	28.8	28.8	28.5
29	33.2	33.1	33.1	33.2
30	23.6	23.5	23.4	23.6

From the spectroscopic data accumulated, compound **13** had a basic skeleton similar to β-amyrin and β-amyenonypalmitate [*32-36*]. The important signals appeared at δ 217.4 and 199.6 revealed two carbonyl groups in the molecule. According to all spectroscopic data, compound **13** ought to be 3,11-dioxo-β-amyrin.



Compound 13: 3,11-dioxo-β-amyrin

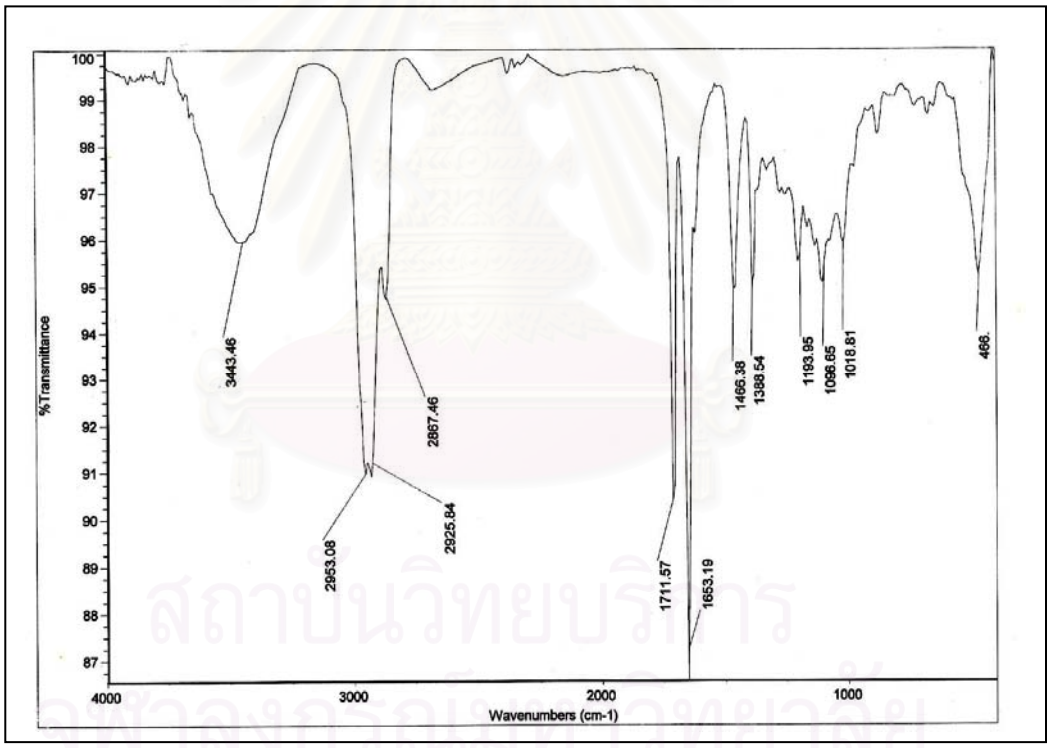


Figure 3.30 The IR spectrum of compound **13**.

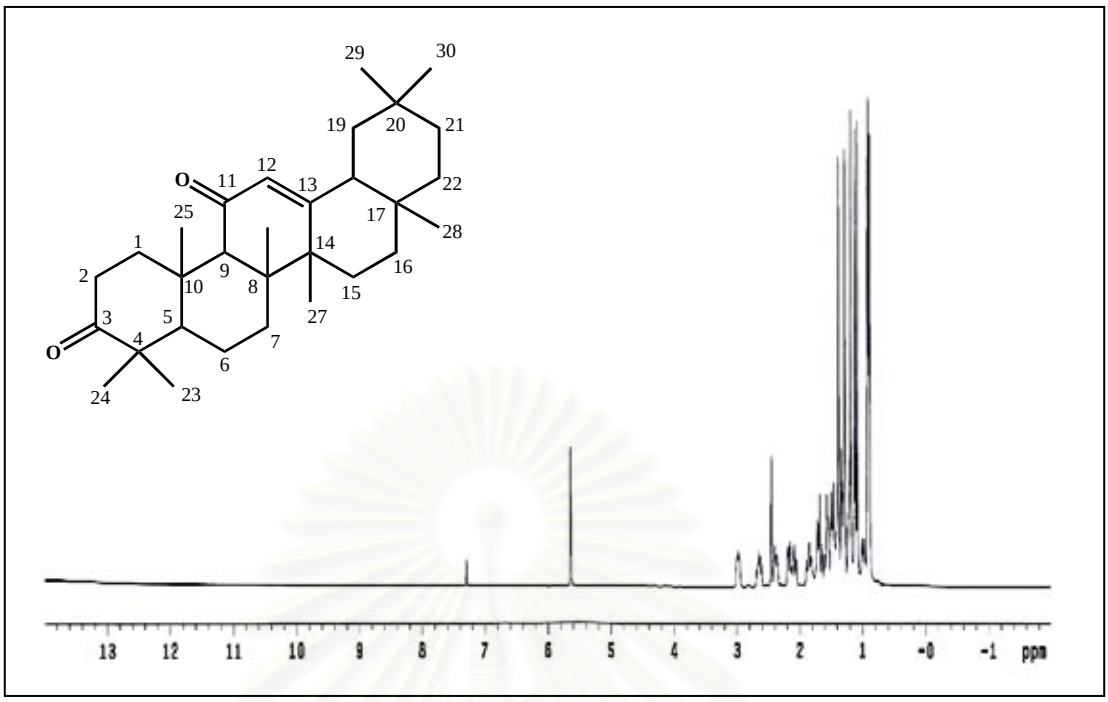


Figure 3.31 The ^{1}H NMR spectrum of compound **13**.

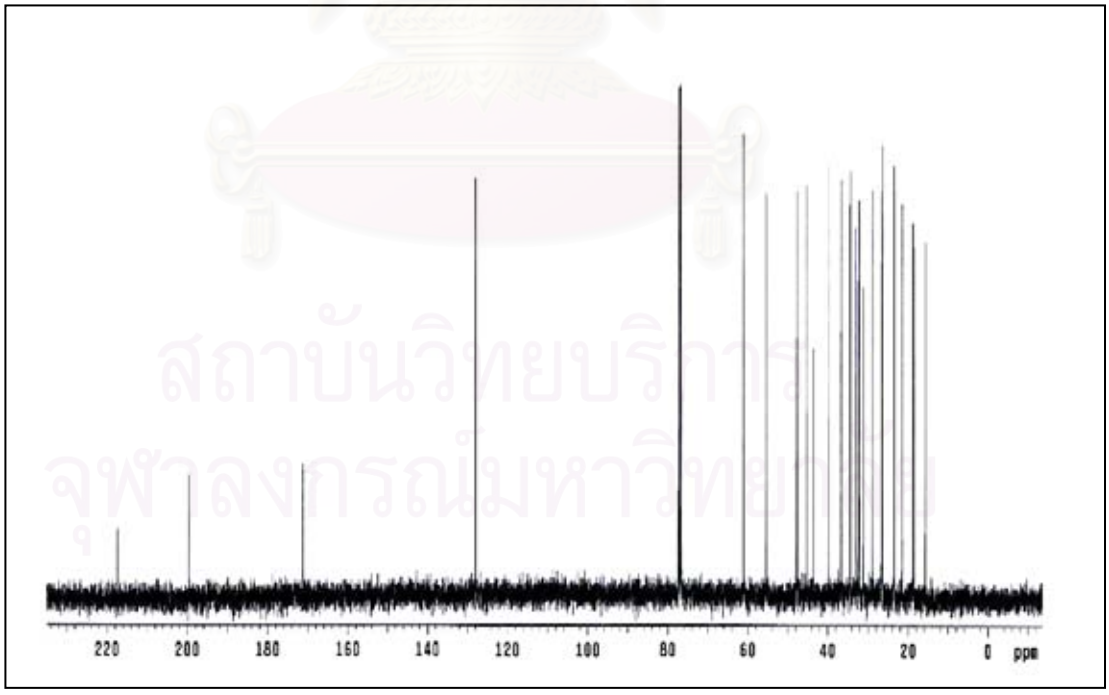


Figure 3.32 The ^{13}C NMR spectrum of compound **13**.

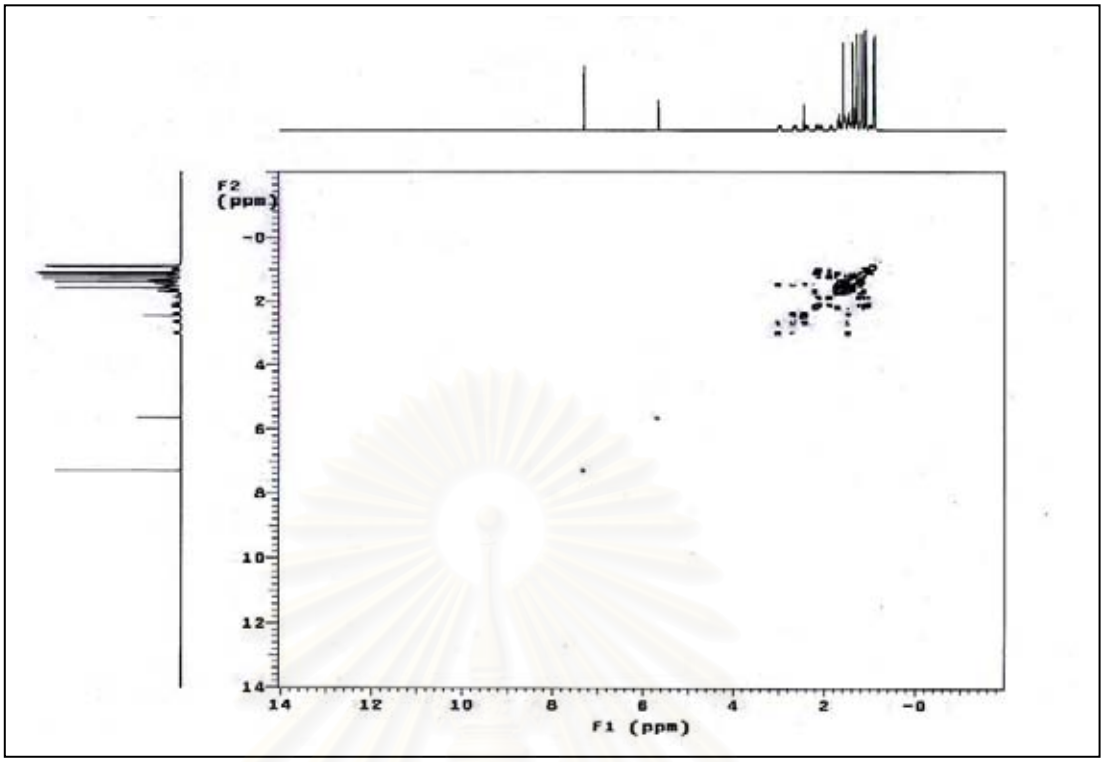


Figure 3.33 The COSY spectrum of compound **13**.

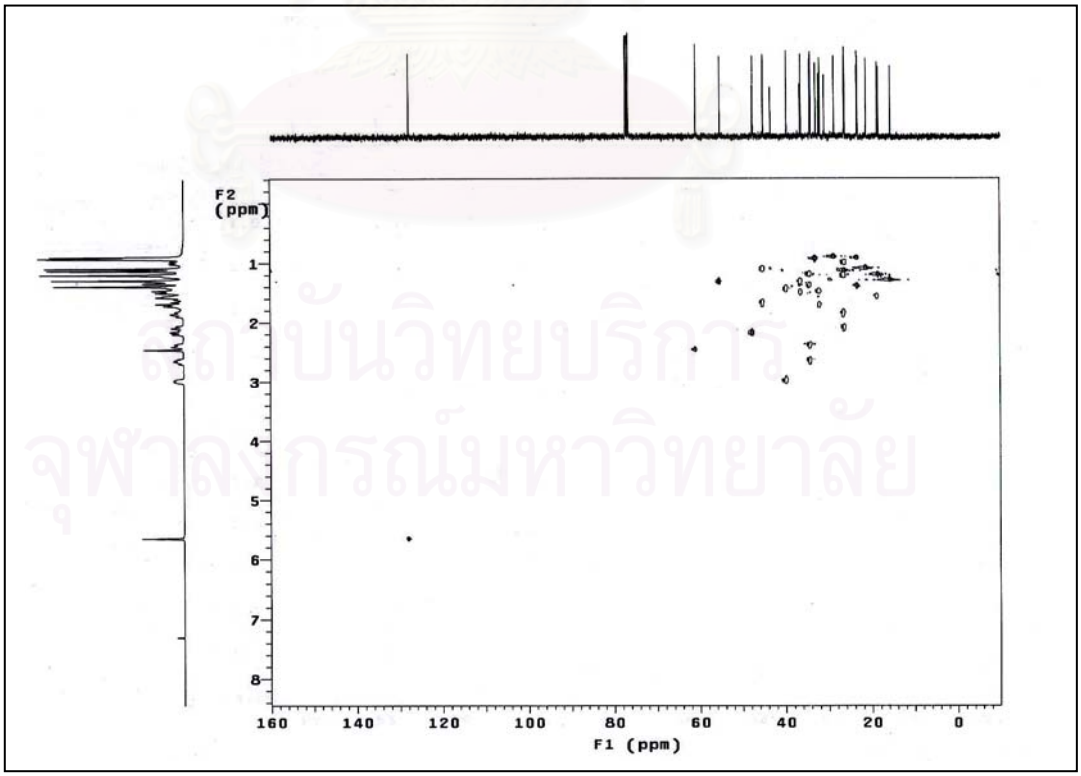


Figure 3.34 The HSQC spectrum of compound **13**.

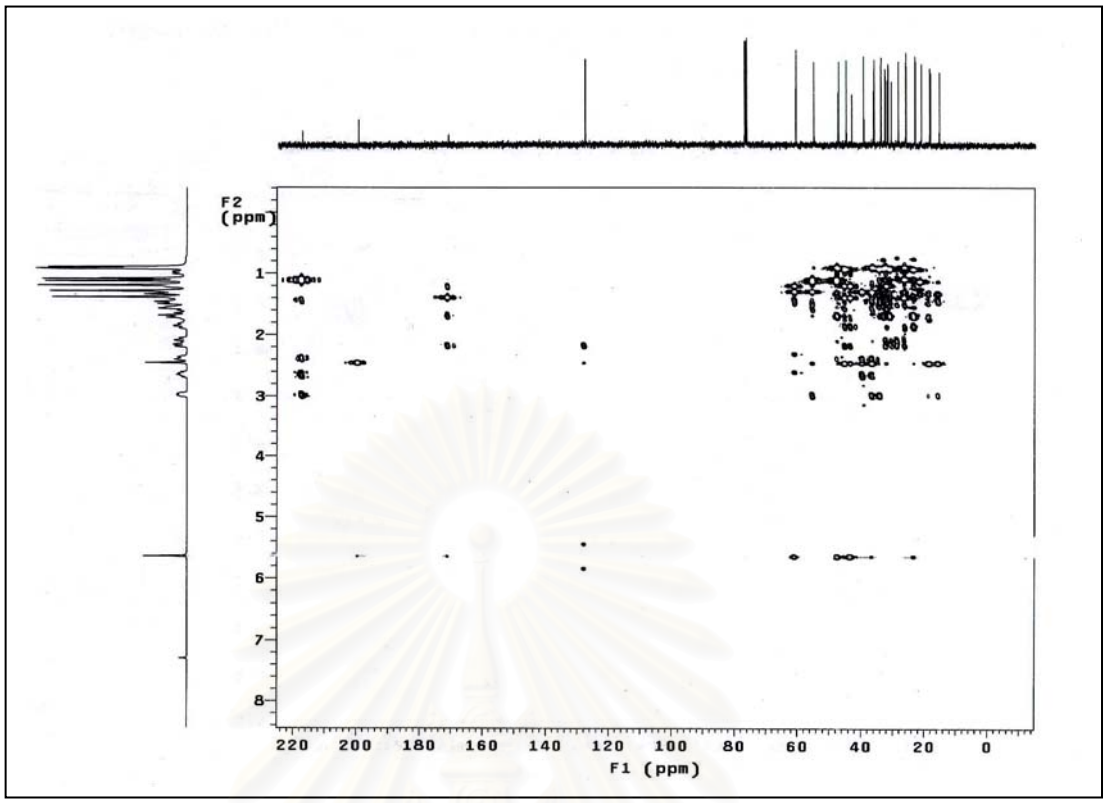


Figure 3.35 The HMBC spectrum of compound **13**.

3.4.6 Structural elucidation of compound 14.

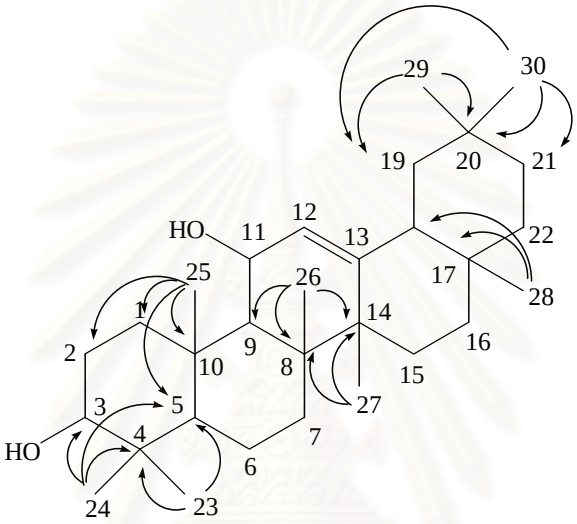
Compound **14** was obtained as pale yellow powder, 408.9 mg (0.73 % w/w of hexane extract), m.p. 207.3-208.5 ºC, R_f = 0.80 in hexane: EtOAc (1:1). It was soluble in hexane and CH_2Cl_2 but insoluble in EtOAc and MeOH.

The IR spectrum (Fig 3.37) revealed significant absorption bands around 3300-3400 cm^{-1} (br, O-H) and that at 1462 cm^{-1} for C-O stretching. Other absorption bands were similar to those of mixture **10**. The ^{1}H NMR spectrum (Figure 3.38) exhibited an olefinic proton at δ 5.12 (d, 1H, *J* = 3.58 Hz), this proton related to the signals appeared at δ 4.20 (dd, 1H, *J* = 3.41, 3.44 Hz) which revealed a proton signal on the carbon bearing a hydroxyl group. Another hydroxyl group was appeared at δ 3.20 (dd, 1H, *J* = 5.13, 4.85 Hz). The signals around δ 0.85 to 2.12 were proton signals of aliphatic parts of this compound.

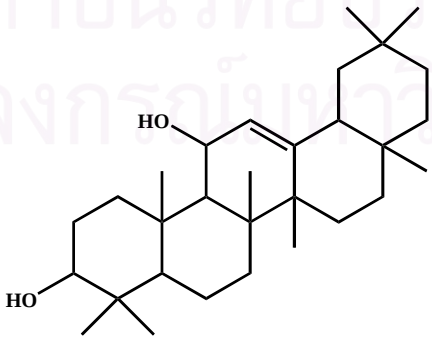
The ^{13}C NMR spectrum (Fig 3.39) exhibited 30 carbon signals; the important signals at δ 150.0 and 125.3 were olefinic carbons; those at δ 78.7 and 67.6 could be assigned for the carbons attached to an oxygen atom. The DEPT and HSQC spectra (Figures 3.39 and 3.41) indicated that this compound contained eight methyl groups at

δ 33.2, 28.5, 28.1, 26.2, 23.6, 18.0, 16.9 and 15.5 and other signals were an aliphatic part of this compound. The ^{13}C NMR spectrum of this compound compared with those of β-amyrin and β-amyrenonypalmitate are demonstrated in Table 3.17 (p. 64).

The ^{13}C NMR spectral data exhibited the important signals at δ 78.7 and 67.6 belong to carbon signals bearing hydroxyl groups. The HMBC spectrum (Figure 3.42) revealed the long range coupling between eight methyl protons and adjacent or 3-bonded carbons as prented below.



Two methyl signals at δ 0.81 and 1.00 displayed the close correlation to the carbon signal at 78.7 where attached to oxygen atom. According to spectroscopic data, it suggests that compound **14** could be β-amyrin derivatives, 11α-hydroxy-β-amyrin.



Compound 14: 11α-hydroxy-β-amyrin

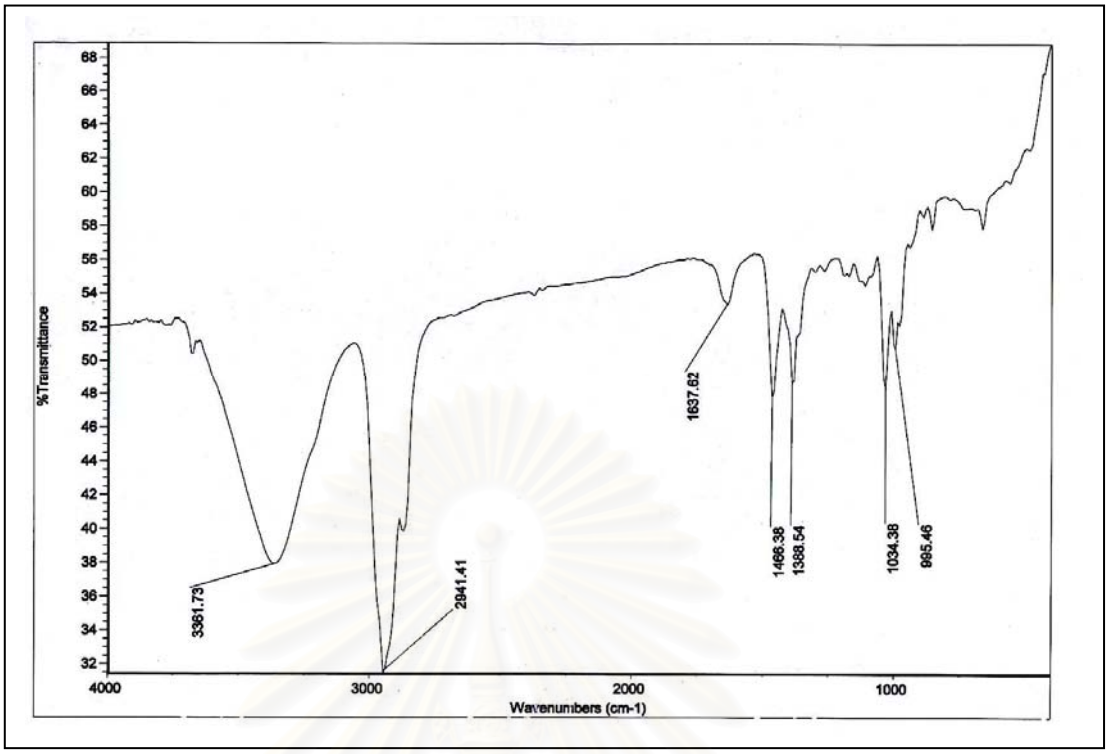


Figure 3.36 The IR spectrum of compound **14**.

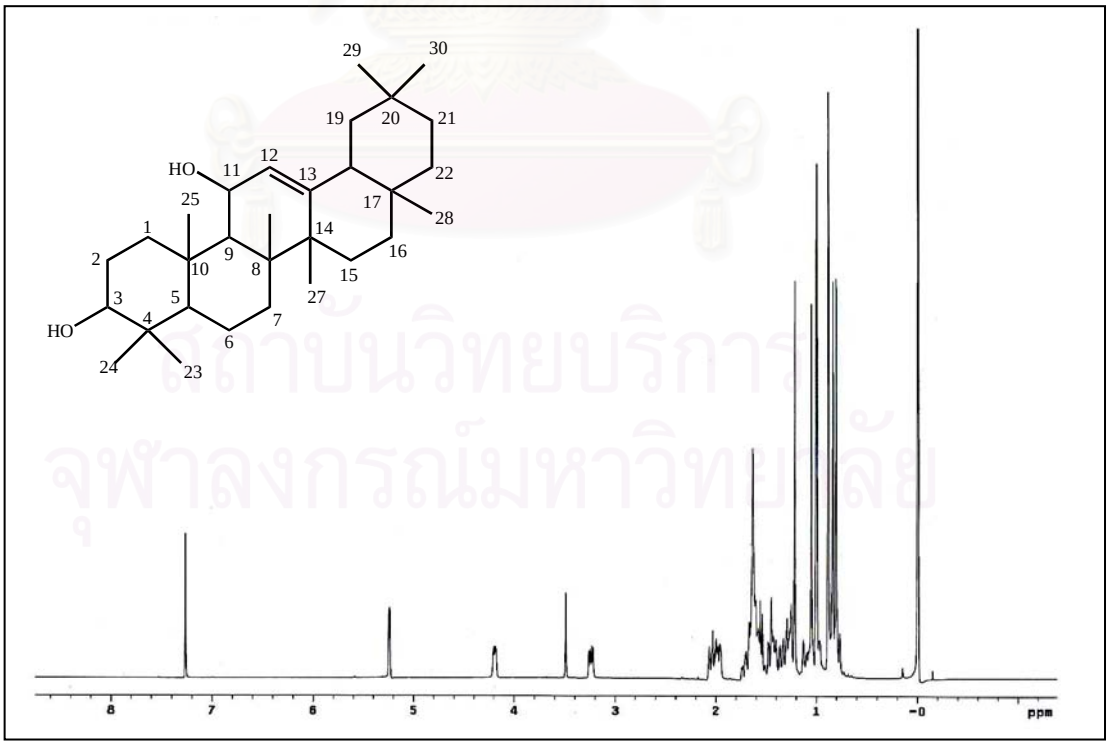


Figure 3.37 The ^{1}H NMR spectrum of compound **14**.

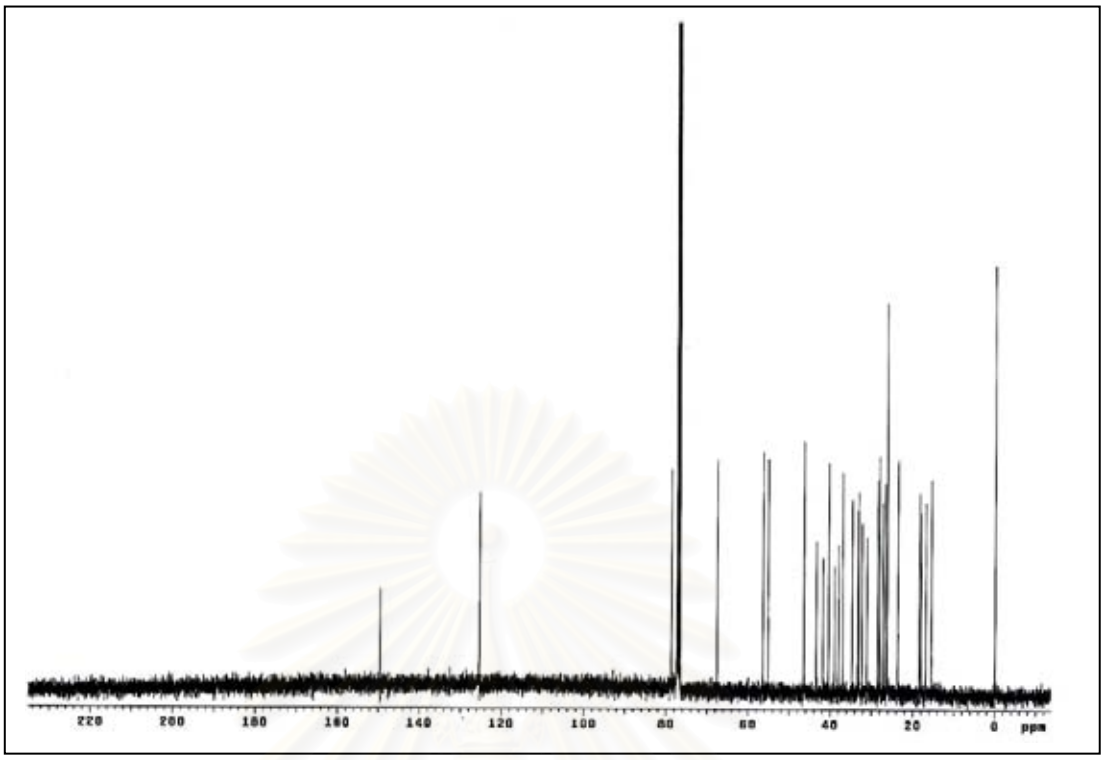


Figure 3.38 The ^{13}C NMR spectrum of compound **14**.

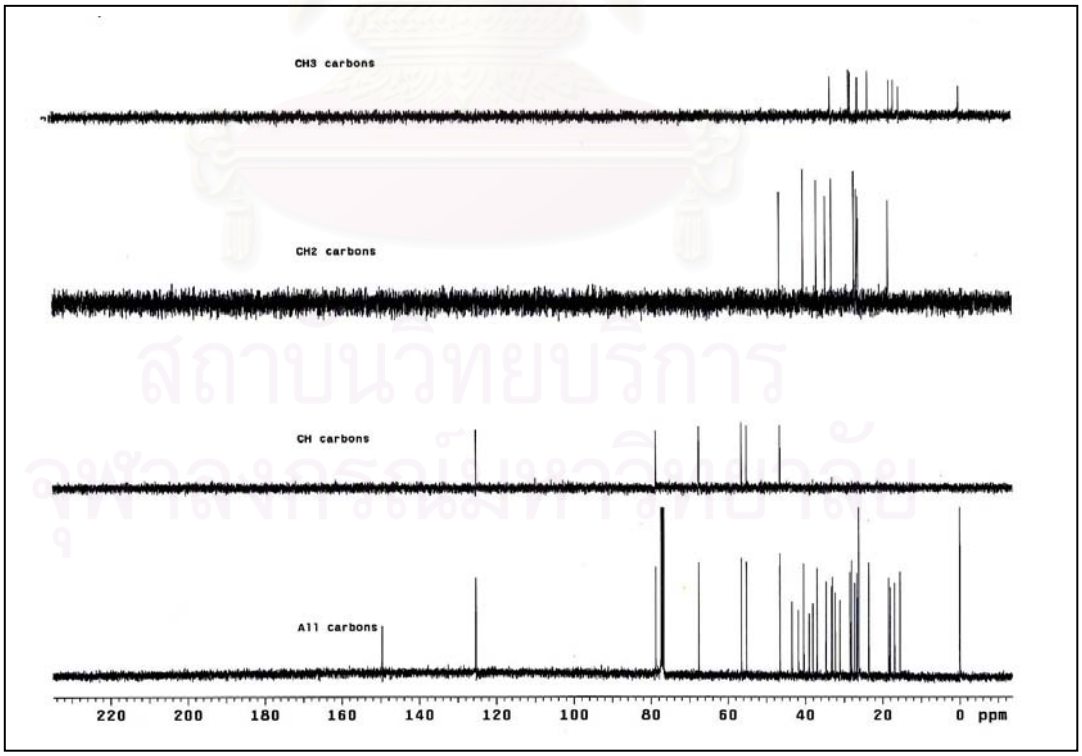


Figure 3.39 The DEPT spectrum of compound **14**.

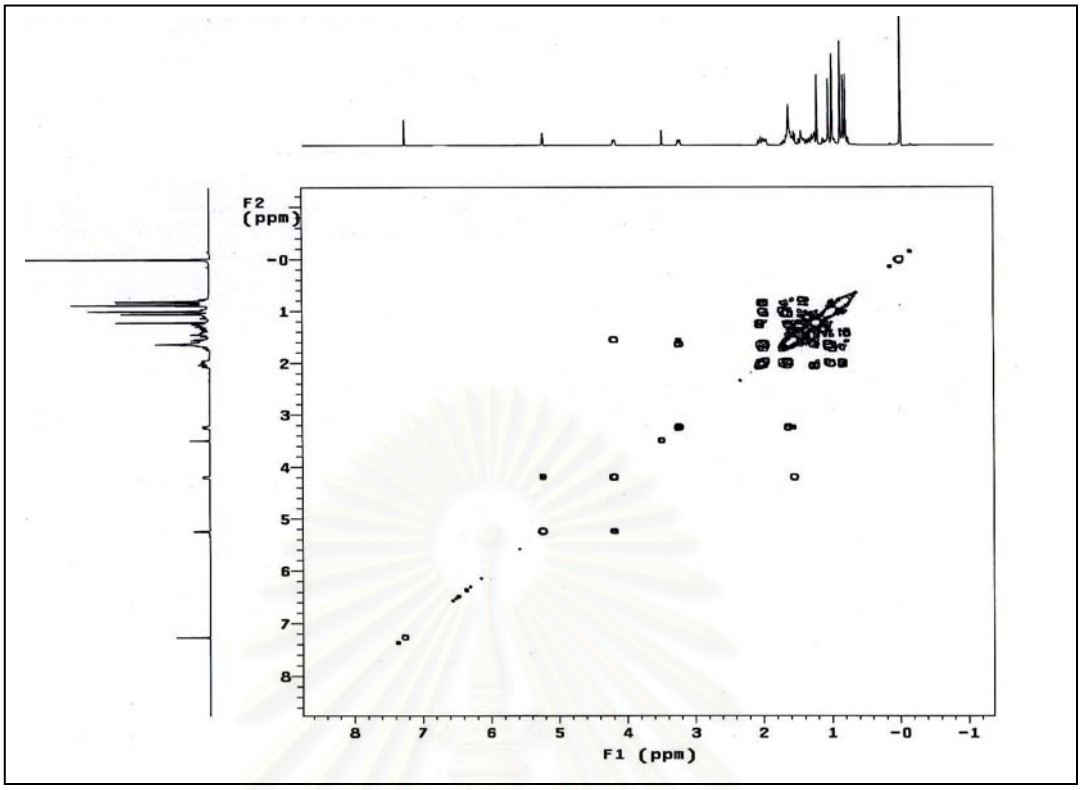


Figure 3.40 The COSY spectrum of compound **14**.

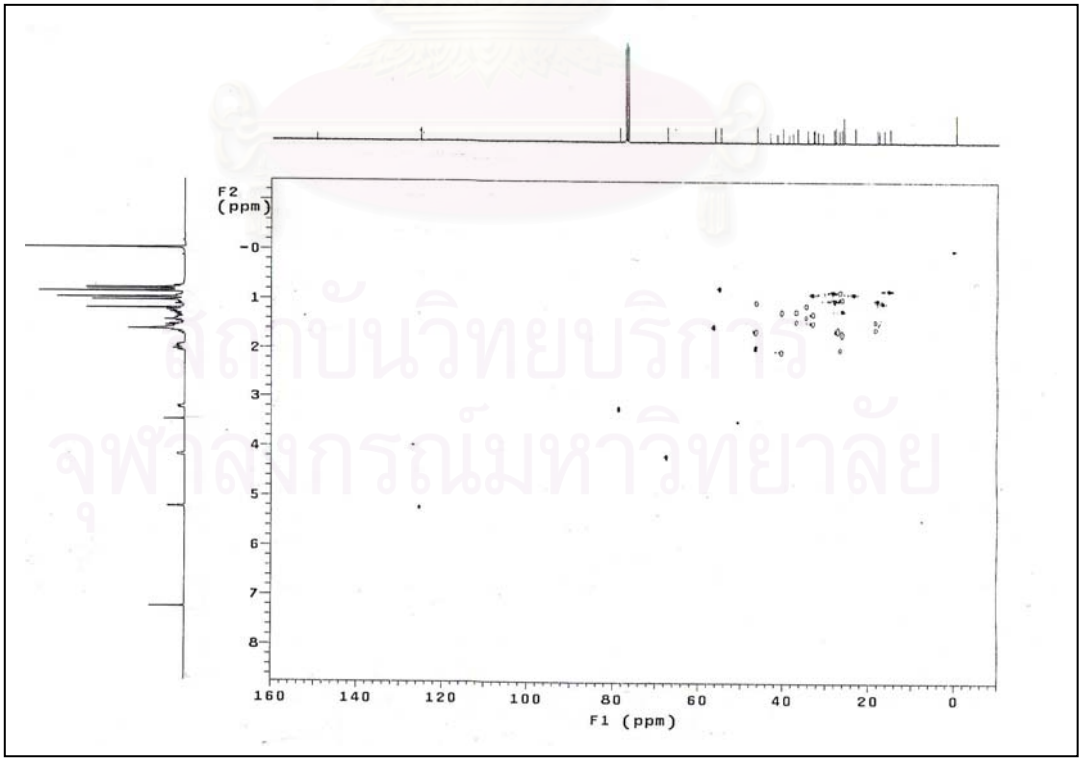


Figure 3.41 The HSQC spectrum of compound **14**.

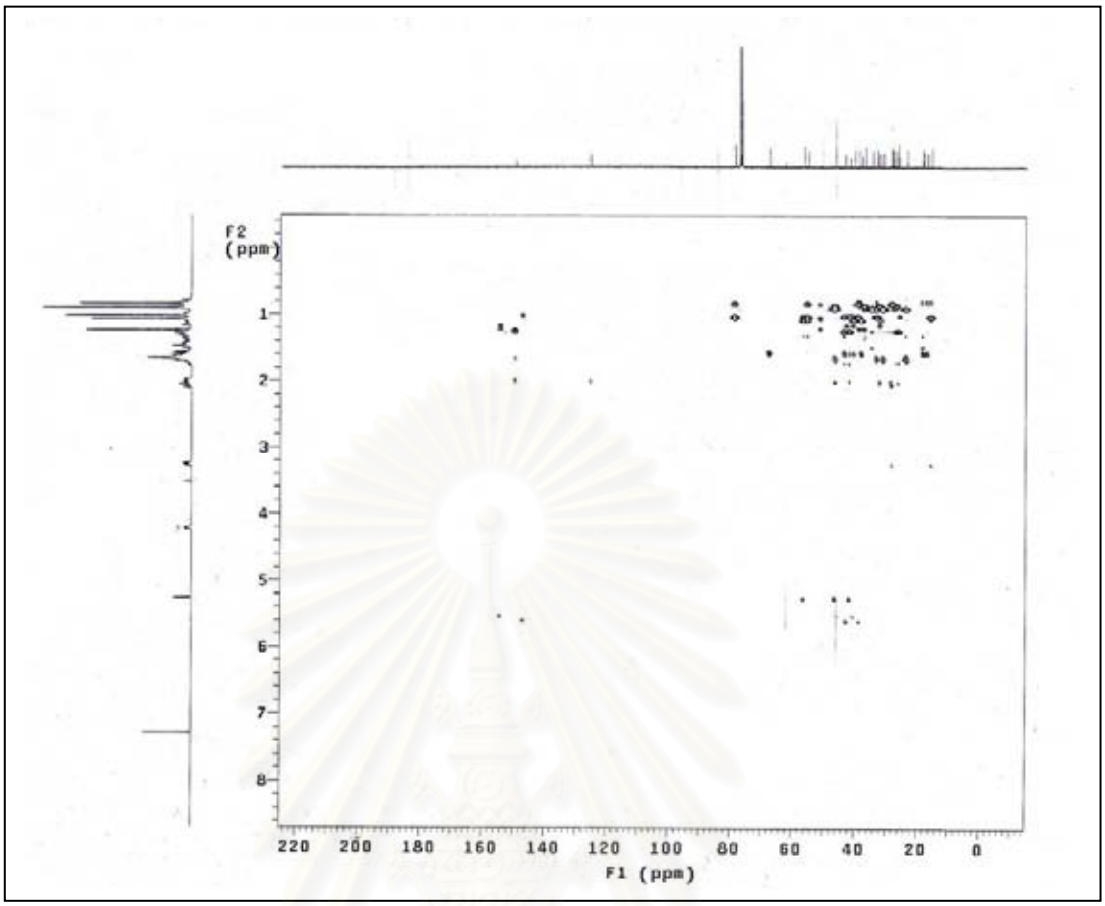


Figure 3.42 The HMBC spectrum of compound 14.

3.5 Antihistaminic activities of isolated compounds using RBL-2H3 cells.

Seven isolated substances from the roots, a subatance from the leaves and four substances from the heartwoods[4] of *M. gagei* were subjected to the test for antihistaminic activities under collaboration work with Prof. Hideaki Otsuka from Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University, Japan. The results of antihistaminic activities are presented in Table 3.18.

Table 3.18 Percent inhibition of isolated compounds from the heartwoods, roots and leaves of *M. gagei* using RBL-2H3 cells.

Compounds	% inhibition	
	0.01 mg/ml	0.1 mg/ml
mansorin A[a]	2	12
mansorin B[a]	3	41
mansorin C[a]	8	20
mansonone C[a,b]	12	92
mansonone G[a,b]	10	99
mansonone H[a,b]	18	62
dehydrooxoperezinone[a]	-	15
mansonone E[a,b]	30	84
2,5-dimethoxy-1,4-benzoquinone[b]	8	100
vanillic acid[b]	8	10
stigmastane-3β,6α-diol	10	20
11α-hydroxy-β-amyrin[c]	-6	-10

[a] = isolated compounds from heartwoods of *M. gagei*, [b] = isolated compounds from roots of *M. gagei*, [c] = isolated compounds from leaves of *M. gagei*

Table 3.18 reveals the inhibitory percentage of antihistaminic activity which measured from the secretion of β-hexosaminidase release and calculated with equation described in chapter II. Disodium cromoglycate (DSCG), clinically used as an antiallergic, was used as a positive control, and its IC_{50} value was comparable to the reported value [*16*]. 2,5-Dimethoxy-1,4-benzoquinone and mansonone G showed the highest activity; mansonones C and E exhibited high activity while mansonone H gave moderate activity. According to the results, the compound possessed a quinonoid character showed high activity, whereas a triterpenoid type such as 11α-hydroxy-β-amyrin was completely inactive toward histamine receptors. Based on the attained results, mansonones G and C were further investigated to find IC_{50} and the results are tabulated in Figure 3.43.

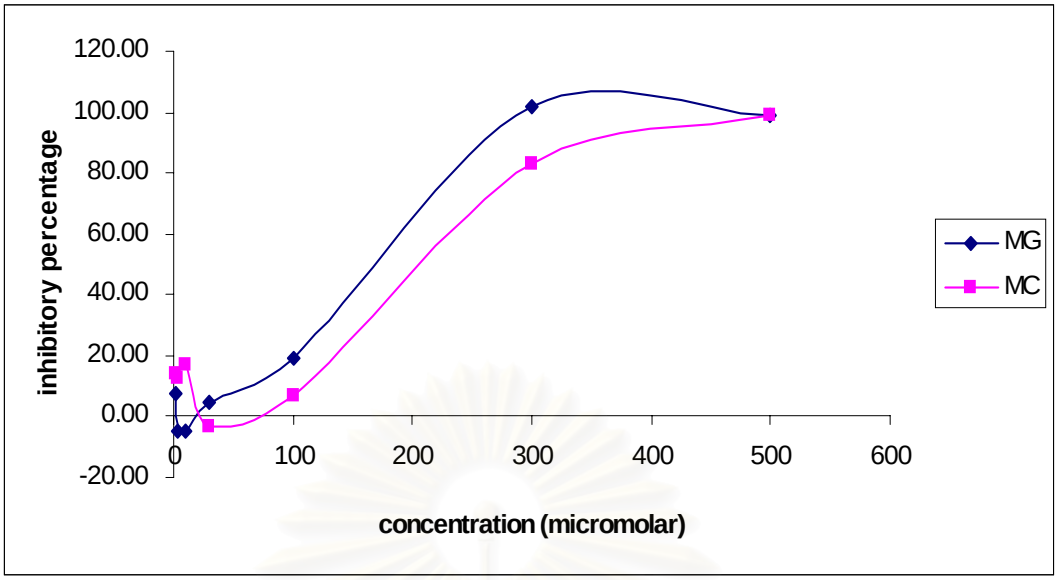


Figure 3.43 Inhibitory activity of mansonones G and C in RBL-2H3.

As judged from the results presented in Table 3.18 and Figure 3.43, it was found that mansonone G gave IC_{50} 212 μM while mansonone C showed IC_{50} 222 μM.

This is the first report to disclose that isolated substances of *M. gagei* were of antihistaminic activity. The results of this research may help to define further study for the components which are responsible for pharmacological actions of *M. gagei*.

CHAPTER IV

CONCLUSION

During the course of this research, chemical constituents and biological activity of the roots and the leaves of *M. gagei* were thoroughly investigated. Nine pure compounds were obtained from the roots and other three pure compounds and two mixtures discovered from the leaves. All isolated substances were elucidated their structures by means of physical properties, chemical reactions and spectroscopic evidences. The summary of isolated substances was accumulated in Table 4.1.

Most of isolated substances were further examined for antihistaminic activity by measurement of β-hexosaminidase secretion using RBL-2H3 cell. As judged from the results, 2,5-dimethoxy-1,4-benzoquinone (**1**), mansonones G (**2**) and C (**5**) exhibited the highest antihistaminic activity. However, only two mansonones: mansonone G and C were attained in appropriate amount for further study and exhibited antihistaminic activity with IC_{50} 212 and 222 μM, respectively. While mansonones H (**4)** and E (**6)** showed moderately activity and the other compounds were inactive.

In conclusion, this is the first report on the chemical constituents and biological activity of the roots and the leaves of *M. gagei.* 2,5-Dimethoxy-1,4-benzoquinone and mansonone G (**2**) exhibited as the most active compound from *M. gagei*. Based on the chemical structures, mansonone T (**8)** was in addition another new sesquiterpenoid compound disclosed. In clusion, it could be clearly seen that the roots of *M. gagei* could be used as a good source of mansonones, the components that possess a variety of biological activity. Moreover, the leaves of this plant produced triterpenoidal moiety in good yield.

Table 4.1 Structures of the isolated compounds from the roots and the leaves of *M. gagei* and their physical properties.

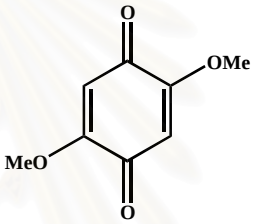
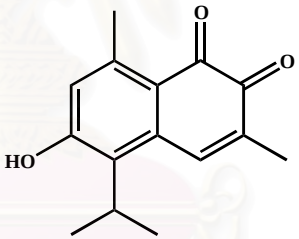
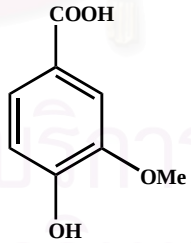
Compounds	Remarks	Molecular formula	Structure	Melting point (°C)	yield
2,5-dimethoxy-1,4-benzoquinone	yellow needle	$C_8H_8O_4$		249-250 °C	11.7 mg
Mansonone G	orange needle	$C_{15}H_{16}O_3$		decomposed at 183 °C	48.2 mg
Vanillic acid	pale yellow powder	$C_8H_8O_4$		208-210 °C	8.1 mg

Table 4.1 (Continued)

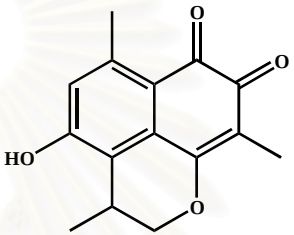
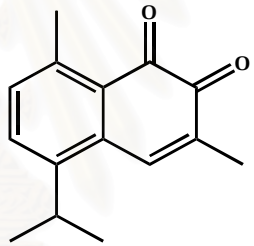
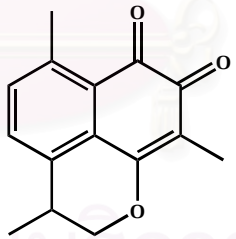
Compounds	Remarks	Molecular formula	Structure	Melting point (°C)	yield
Mansonone H	red platelet	$C_{15}H_{14}O_4$		decomposed at 250 °C	59.1 mg
Mansonone C	orange needle	$C_{15}H_{16}O_2$		133.8-135.1 ºC	108.0 mg
Mansonone E	orange needle	$C_{15}H_{14}O_3$		161.2-163.2 ºC	97.8 mg

Table 4.1 (Continued)

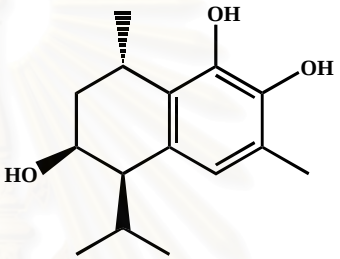
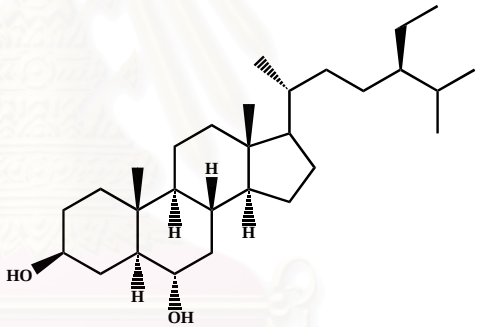
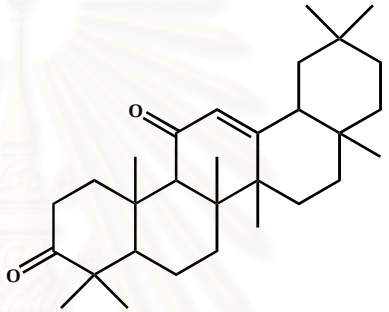
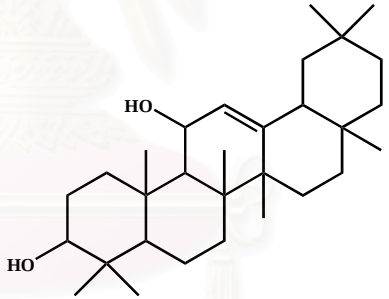
Compounds	Remarks	Molecular formula	Structure	Melting point (°C)	yield
Mansonone T (new compound)	colorless needle	$C_{15}H_{22}O_3$		decomposed at 150 °C	7.4 mg
Stigmastane-3β,6α-diol	white platelet	$C_{29}H_{52}O_2$		214.0-215.0 ºC	20.7 mg
Mixture of long chain Hydrocarbon	white platelet	-	$\mathbf{CH_3\text{-}(CH_2)_n\text{-}CH_3}$	63.4-65.5 ºC	1.30 g
Mixture of long chain acid	white platelet	-	$\mathbf{CH_3\text{-}(CH_2)_n\text{-}COOH}$	67.5-69.5 ºC	138 mg

Table 4.1 (Continued)

Compounds	Remarks	Molecular formula	Structure	Melting point (°C)	yield
3,11-dioxo-β-amyrin	white platelet	$C_{30}H_{43}O_2$		-	10.9 mg
11α-hydroxy-β-amyrin	pale yellow powder	$C_{30}H_{46}O_2$		207.3-208.5 ºC	408.9 mg

Propose for future work

The exploration of substances belonging to the roots and the leaves of *M. gagei* reported in this research provided several intriguing points for future investigation. Isolated substances from this plant exhibited several activities such as antioxidant, anticancer [*4*]. In addition, this is the first study on antihistaminic activity of this plant, 2,5-dimethoxy-1,4-benzoquinone, mansonones G and C which possessed the highest inhibition activity. These two mansonones could therefore be employed as the template for investigation on antihistaminic activity and structure activity relationship between those mansonones. These approaches will confidently lead to the discovery of the potentially active compounds in the future.

REFERENCES

1. Mann, J.; Davidson, R. S.; Hobbs, J. B.; Banthorpe, D.V.; Harborne, J.B. *Natural products: their chemistry and biological significance*. First edition. Printed in Hong Kong, **1994**.
2. Thomson, R.H. *The chemistry of natural products*. First edition. Printed in Great Britain by Thomson Litho Ltd., **1985**.
3. Pongboonrod, S. *Mai Tet Muang Thai,* Bangkok: Kasembunnakich Press, **1976**, 162.
4. Tiew, P. *Bioactive compounds from heartwood of Mansonia gagei* Drumm. Docter's thesis, Department of Chemistry, Chulalongkorn University, **2002**.
5. Marini Bettolo, G. B.; Casinovi, C. G.; Galeffi, C. A new class of quinones; sesquiterpenoid quinones of *Mansonia altissima* Chev., *Tetrahedron Lett*., **1965**, *52*, 4857-4864.
6. Tanaka, N.; Yasue, M.; Imamura, H. The quinonoid pigment from *Mansonia altissima* wood, *Tetrahedron Lett*., **1966,** *24*, 2767-2773.
7. Shimada, K.; Yasue, M.; Imamura, H. A new pigment from *Mansonia altissima* wood, *Mokuzai Gakkaishi*, **1967**, *13*, 126.
8. Galeffi, C.; Miranda delle Monache, E.; Casinovi, C. G.; MariniBettolo, G. B. A new quinine from heartwood of *Mansonia altissima* Chev., *Tetrahedron Lett*., **1969**, *40*, 3583-3584.
9. Allgeier, H.; Weiss, E.; Reichstein, T. Cardenolindes from *Mansonia atissima* seeds, *Helv. Chim. Acta*, **1967**, *50*, 431-455.
10. Allgeier, H.; Weiss, E.; Reichstein, T. Cardenolindes from *Mansonia atissima* seeds 2. Structure of mansonone and strophotheoside, *Helv. Chim. Acta*, **1967**, *50*, 456-462.
11. Puntumchai, A. *Chemical constituents of the heartwoods of Mansonia gagei and their biological activity*. Master's thesis, Department of Chemistry, Chulalongkorn University, **1997**.
12. Anton, A. H.; Sayre, D. F. A Modified fluorometric procedure for tissue histamine and its distribution in various animals. *J. Pharmacol. Exp. Ther*., **1969**, *166*, 285-295.

13. Foreman, J. C., Non-immunological stimuli of mast cells and basophil leucocytes. *Immunopharmacology of Mast Cells and Basophils*. London: Academic Press., **1993**, 57-69

14. Janeway, C. A.; Travers, P., Walport, M.; Shlomchik, M. The humoral immune response. *Immunobiology*. Fifth edition. Newyork/London: Garland Publishing., **2001**, 374-375.

15. Yamamura, S.; Ozawa, K.; Ohtani, K.; Kasai, R.; Yamasaki, K. Antihistaminic flavones and aliphatic glycosides from *Mentha spicata*. *Phytochemistry*., **1998**, *48*, 131-136.

16. Kuwabara, H.; Mouri, K.; Otsuka, H.; Kasai, R.; Yamasaki, K. Tricin from a Malagasy connaraceous plant with potent antihistaminic activity. *J. Nat. Prod*. 2**003**, *66*, 1273-1275.

17. Chavasiri, W. *Constituents and biological activities of Rhizophora apicalata* Bl. Master's Thesis, Department of Chemistry, Chulalongkorn University, **1988**.

18. Prasertsang, P. *Chemical constituents of the heartwoods of Ardisia colorata* Roxb. *fruit and their biological activities*. Master's thesis, Department of Chemistry, Chulalongkorn University, **2002**.

19. Pongrod, K. *Chemical constituents of the stems of Hibiscus tliaceus* Linn. *and their biological activities*. Master's thesis, Department of Chemistry, Chulalongkorn University, **1999**.

20. Saxena, R. S.; Gupta, B.; Lata, S. Tranquilizing, antihistaminic and purgative activity of *Nyctanthes arbor* tristis leaf extract. *J. Ethnopharmacol*., **2002**, *81*, 321-325.

21. Cafieri, F.; Carnuccio, R.; Fattorusso, E.; Scafati, O. T., Vallefuoco, T. Anti-histaminic activity of bromopyrrole alkaloids isolated from Carribbean *Agelas* Sponges. *Bioorg. Med. Chem. Lett*., **1997**, *17*, 2283-2288.

22. Iwata, N.; Wang, N.; Yao, X.; Kitanaka, S. Structures and histamine release inhibitory effectes of prenylated orcinol derivatives from *Rhododendron dauricum*. *J. Nat. Prod*., **2004**, *67*, 1106-1109.

23. Shamsa, F.; Ahmadiani, A.; Khosrokhavar, R. Antihistaminic and anticholinergic activity of barberry fruit (*Berberis vulgaris*) in the guinea-pig ileum. *J. Ethnoplarmacoloy*., **1999**, *64*, 161-166.

24. Tiew, P.; Takayama, H.; Kitajima, M.; Aimi, N.; Kokpol, U.; Chavasiri, W. A novel neolignan, mansoxetane, and two new sesquiterpenes, mansonones R and S, from *Mansonia gagei*. *Tetrahedron lett*., **2003**, *44*, 6759-6761.

25. Tiew, P.; Puntumchai, A.; Kokpol, U.; Chavasiri, W. Coumarins from the heartwoods of *Mansonia gagei* Drumm. *Phytochemistry*., **2002**, *60*, 773-776.

26. Tiew, P.; Ioset, J. R.; Kokpol, U.; Chavasiri, W.; Hostettmann, K. Antifungal, antioxidant, and larvicidal activities of compounds isolated from the heartwoods of *Mansonia gagei*. *Phytother*. *Res*, **2003**, *17*, 190-193.

27. Tiew, P.; Ioset, J.R.; Kokpol, U.; Schenk, K.; Nongnuj, J.; Chaichit, N.; Chavasiri, W.; Hostettmann, K. Four new sesquiterpenoid derivatives from the heartwoods of *Mansonia gagei*. *J. Nat. Prod.*, **2002**, *65*, 1332-1335.

28. Kim, J. P.; Kim, W. G.; Koshino, H.; Jung, J.; Yoo, I. D. Sesquiterpenoid *o*-naphthoquinones from the root bark of *Ulmus davidiana*. *Phytochemistry*., **1996**, *43*, 425-430.

29. Das, B.; Srinivas, K. V. N. S. Dihydroxysterols from the marine red algae, *Grscilaria edulis*. *Phytochemistry*, **1992**, *31*, 4371-4373.

30. Kimura, Y.; Akihisa, T.; Yasukawa, K.; Takido, M.; Tamura, T. Structures of five hydroxylated sterols from the seeds of *Trichosanthes kisilowii* Maxim. *Chem. Pharm. Bull.*, **1995**, *43*, 1813-1817.

31. Chaurasia, N.; Wichtl, M. Sterols and steryl glycosides from *Urtica dioica*. *J. Nat. Prod*., **1987**, *50*, 881-885.

32. Barnes, R. A.; Pereira, A. L.; Scofield, T. C. V.; Filho, R. B.; Pinto, A. C. A new triterpene from *Vellozia compacta*. *Chem. Pharm. Bull*., **1984**, *32*, 3674-3677.

33. Bandaranayake, W. M. Terpenoids of *Canarium zeylanicum*. *Phytochemistry*., **1980**, *19*, 255-257.

34. Ito, K.; Lai, J. Studies on the constituents of *Marsdenia formosana* Masamune. II. Structures of marsformoxide A and marsformoxide B. *Chem. Pharm. Bull*., **1978**, *26*, 1908-1911.

35. Caldwell, C. G.; Franzblau, S. G.; Suarez, E.; Timmermann, B. N. Oleanane triterpenes from *Junellia tridens*. *J. Nat. Prod*., **2000**, *63*, 1611-1614.

36. Chiang, Y. M.; Kuo, Y. H. Taraxastane-type triterpenes from the aerial roots of *Ficus microcarpa*. *J. Nat. Prod*., **2000**, *63*, 898-901.

VITA

Pimonporn Tiengtham was born on November 13, 1980 in Yasothon province, Thailand. She obtained a Bachelor Degree of Science, majoring in Chemistry from Srinakharinwirot University, Bangkok, Thailand in 2001. Since 2002, she has been a graduate student studying Organic Chemistry at Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. During the course of study, she obtained financial support from Thailand Research Fund for a 2004 Master research assistant fellowship and a TRF Master Research Grants: TRF-MAG program and Graduate School Chulalongkorn University.