องค์ประกอบทางเคมีและการเตรียมอนุพันธ์ของเมโลโดรินอลจากราก
ลำดวน *Melodorum fruticosum* Lour.

นายสิริวัฒน์ หงษ์นาค

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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CHEMICAL CONSTITUENTS AND DERIVATIZATION OF MELODORINOL FROM THE ROOTS OF *Melodorum fruticosum* Lour.

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	CHEMICAL CONSTITUENTS AND DERIVATIZATION OF MELODORINOL FROM THE ROOTS OF *Melodorum fruticosum* Lour.
By	Mr. Siriwat Hongnak
Field of Study	Chemistry
Thesis Advisor	Associate Professor Santi Tip-pyang, Ph.D.

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สิริวัฒน์ หงษ์นาค : องค์ประกอบทางเคมีและการเตรียมอนุพันธ์ของเมโลโดรินอลจากรากลำดวน *Melodorum fruticosum* Lour. (CHEMICAL CONSTITUENTS AND DERIVATIZATION OF MELODORINOL FROM THE ROOTS OF *Melodorum fruticosum* Lour.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: สันติ ทิพยางค์, หน้า.

การศึกษาองค์ประกอบทางเคมีของสิ่งสกัดไดคลอโรมีเทนและเมทานอลจากรากลำดวน (*Melodorum fruticosum* Lour.) สามารถแยกสารในกลุ่มไตรเทอปีนอยด์ได้ 4 ชนิด คือ **β**-sitosterol (1) stigmasterol (2) polycarpol (3) และ lanosta-7,9(11),24-trien-3**β**,21-diol (12) สารกลุ่มเฮปทีน 4 ชนิด คือ melodorinol (4) acetylmelodorinol (7) (4*Z*)-6-benzoyloxy-7-heptadien-4-olide (8) และ (4Z)-6,7-dihydroxy-2,4-heptadien-4-olide (13) สารในกลุ่มฟลาโวนอยด์ 6 ชนิด ได้แก่ chamanetin (5) chrysin (6) pinocembrin (9) isochamanetin (10) dichamanetin (11) และ catechin (14) สารในกลุ่มไตรเทอปีนอยด์กลัยโคไซด์ 1 ชนิด คือ ampelopsisionoside (15) พิสูจน์สูตรโครงสร้างของสารทั้งหมดที่แยกได้โดยอาศัยวิธีทางสเปกโตรสโกปีร่วมกับเปรียบเทียบข้อมูลที่มีการรายงานมาก่อนหน้านี้ นำสารที่แยกได้มาทดสอบความเป็นพิษต่อเซลล์มะเร็งชนิด KB HeLa MCF-7 และ HepG-2 พบว่าสาร chamanetin (5) แสดงความเป็นพิษต่อเซลล์มะเร็งชนิด KB ได้ดีโดยมีค่า IC_{50} เท่ากับ 0.86 µg/mL ในขณะที่ acetylmelodorinol (4) แสดงความเป็นพิษสูงสุดทั้ง KB และ HeLa เซลล์โดยมีค่า IC_{50} เท่ากับ 0.66 และ 0.66 µg/mL สารในกลุ่มไตรเทอปีนอยด์แสดงความเป็นพิษต่อเซลล์มะเร็งระดับปานกลางจนถึงไม่มีฤทธิ์ในการยับยั้ง จากการทดลองพบว่าสารในกลุ่มเฮปทีนแสดงความเป็นพิษต่อเซลล์มะเร็งได้ดีที่สุด จึงได้นำสาร 7 มาเตรียมเป็นอนุพันธ์ใหม่ 6 ชนิด (7a-7d และ 7f-7g) และอนุพันธ์ที่มีรายงานมาแล้ว 1 ชนิด (7e) นำมาทดสอบความเป็นพิษกับเซลล์มะเร็งทั้งหมด พบว่าอนุพันธ์ propanoylmelodorinol (7b) แสดงความเป็นพิษกับเซลล์มะเร็งได้ดีที่สุด โดยแสดงความเป็นพิษกับเซลล์ชนิด KB HeLa MCF-7 และ HepG-2 โดยมีค่า IC_{50} เท่ากับ 0.64 0.75 0.78 และ 3.57 µg/mL ตามลำดับ ในการศึกษาความสัมพันธ์เบื้องต้นระหว่างสูตรโครงสร้างและการยับยั้งเซลล์มะเร็งของสารในกลุ่มเฮปทีนพบว่าหมู่ฟังก์ชันที่เป็น benzoyl, lactone ring และ hydrophobic ester มีความสำคัญต่อการยับยั้งเซลล์มะเร็ง

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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SIRIWAT HONGNAK: CHEMICAL CONSTITUENTS AND DERIVATIZATION OF MELODORINOL FROM THE ROOTS OF *Melodorum fruticosum* Lour.. ADVISOR: ASSOC. PROF. SANTI TIP-PYANG, Ph.D., pp.

The phytochemical investigation of the CH_2Cl_2 and MeOH crude extracts from the roots of *M. fruticosum* led to the isolation of four triterpenoids, β-sitosterol (1), stigmasterol (2), polycarpol (3) and lanosta-7,9(11),24-trien-3β,21-diol (12), four heptenes, acetylmelodorinol (4), melodorinol (7), (4*Z*)-6-benzoyloxy-7-hydroxy-2,4-heptadien-4-olide (8) and (4*Z*)-6,7-dihydroxy-2,4-heptadien-4-olide (13), six flavonoids, chamanetin (5), chrysin (6), pinocembrin (9), isochamanetin (10), dichamanetin (11) and catechin (14), and one terpenoid glycoside, ampelopsisionoside (15). Their structures were elucidated on basis of spectroscopic data as well as comparison with the previous literature data. All isolated compounds were evaluated on cytotoxicity against KB, HeLa, MCF-7 and HepG-2 cells. Chamanetin (5) showed good selectivity on cytotoxicity against only KB cell with IC_{50} value of 0.86 µg/mL, while acetylmelodorinol (4) showed the highest cytotoxicity against both KB and HeLa with IC_{50} values of 0.66 and 0.66 µg/mL. The terpenoids revealed moderate to inactive cytotoxicity against all cell lines. Based on their cytotoxicity results, heptenes presented the lowest cytotoxic values against all of four cell lines. Melodorinol (7) was selected for further derivertization to yield six new (7a-7d and 7f-7g) and one known (7e) melodorinol derivatives. All of derivatives were tested against four cell lines. The analogue propanoylmelodorinol (7b) exhibited the most active against KB, HeLa, MCF-7 and HepG-2 with IC_{50} values of 0.64, 0.75, 0.78 and 3.57 µg/mL, respectively. Preliminary structure activity relationships analysis of functional groups on cytotoxicity effects were benzoyl, lactone ring, and hydrophobic ester groups in heptene core scaffolds.

Department: Chemistry Student's Signature .....

Field of Study: Chemistry Advisor's Signature .....

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acetone-d_6	deuterated acetone
calcd	calculated
^{13}C NMR	carbon-13 nuclear magnetic resonance
$CDCl_3$	deuterated chloroform
CD_3OD	deuterated methanol
CH_2Cl_2	dichloromethane
COSY	correlated spectroscopy
DMAP	4-(dimethylamino)pyridine
d	doublet (NMR)
dd	doublet of doublet (NMR)
ddd	doublet of doublet of doublet (NMR)
dt	doublet of triplet (NMR)
2D NMR	two dimensional nuclear magnetic resonance
1D NMR	one dimensional nuclear magnetic resonance
equiv	equivalent (s)
ESI-MS	electrospray ionization mass spectrometry
EtOAc	ethyl acetate
g	gram (s)
HeLa	Human cervix adenocarcinoma
HepG-2	Human hepatocellular carcinoma
^{1}H NMR	proton nuclear magnetic resonance
HMBC	heteronuclear multiple bond correlation

HSQC	heteronuclear single quantum correlation
Hz	Hertz
HRESIMS	high resolution electrospray ionization mass spectrum
h	hour (s)
IC_{50}	concentration that required for 50% inhibition *in vitro*
J	coupling constant
KB	Human epidermoid carcinoma
MCF-7	Human breast adenocarcinoma
MeOH	methanol
mg	milligram (s)
mL	milliliter (s)
mmol	millimole (s)
q	quartet (NMR)
s	singlet (NMR)
t	triplet (NMR)
TEA	triethylamine
TLC	thin layer chromatography
VLC	vacuum liquid chromatography
δ	chemical shift
δ_C	chemical shift of carbon
δ_H	chemical shift of proton
μL	microliter (s)

CHAPTER I

INTRODUCTION

1.1 Introduction

Natural products are any naturally occurring substances produced by plants, fungi, bacteria or animals. Natural products generally mean to secondary metabolites, small molecules that are not directly involved in the growth, development, or production but that usually has ecological function. In the past some natural compounds were widely used in several purposes including medicine and poison. Traditional medicines, which were derived predominantly from plants, were the basis of medicines such as aspirin, digitoxin, morphine, quinine, and pilocarpine figure 1.1 [1].

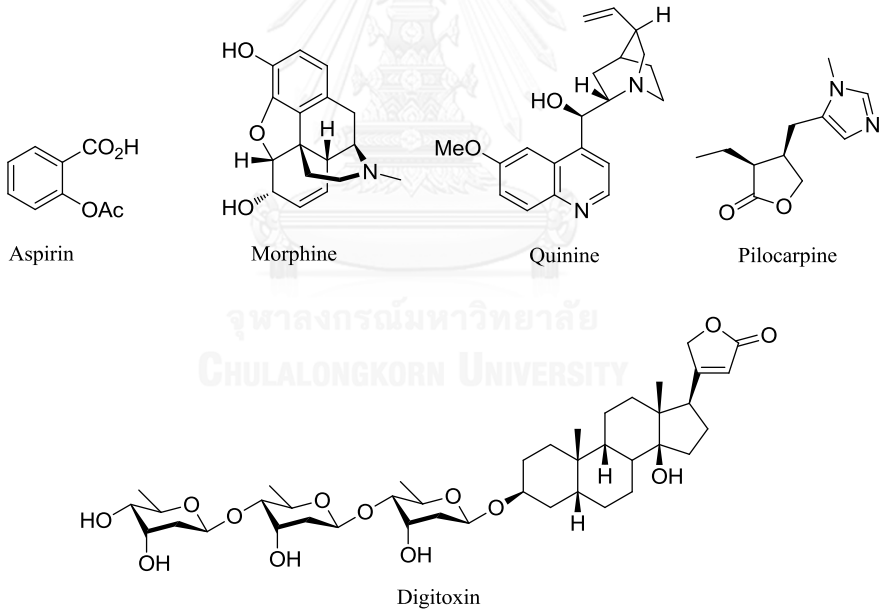


Figure 1.1 Traditional medicines derived from plants

A total of 19 natural product based drugs were approved for marketing worldwide between the year 2005 to 2010, among which 7 are classified as natural products, 10 as semi-synthetic natural products, and 2 as natural product-derived drugs [2]. Although there are many methods in drug discovery, natural products are

still providing their fair share of new clinical candidates and drugs. Nowadays the studies of medicinal plants play an important role in drug discovery.

1.2 Flavonoids: biosynthesis pathway and biological activities

Flavonoids are a group of plant polyphenolic secondary metabolites comprising a common three ring skeletons, C6-C3-C-6, and the rings are referred to as A-, C-, and B-rings, respectively. Flavonoids can be classified by structure into various classes i.e. Flavones, Isoflavones, Flavonols, Flavanones, Flavononols, Leucoanthocyanidins, Anthocyanidins, Flavans, Isoflavans, Flavanols, Neoflavonoids, Chalcones, Dihydrochalcones and Aurones. The major classes of flavonoids are anthocyanins (red to purple pigments), flavonols (colourless to pale yellow pigments) and flavanols (colourless pigments that become brown after oxidation). Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors. It was reported that flavonoids have well known as antioxidant compounds. Flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase and protein kinase C. Flavonoids have been also shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione *S*-transferase, mitochondrial succinoxidase and NADH oxidase, which involved in reactive oxygen species generation [3].

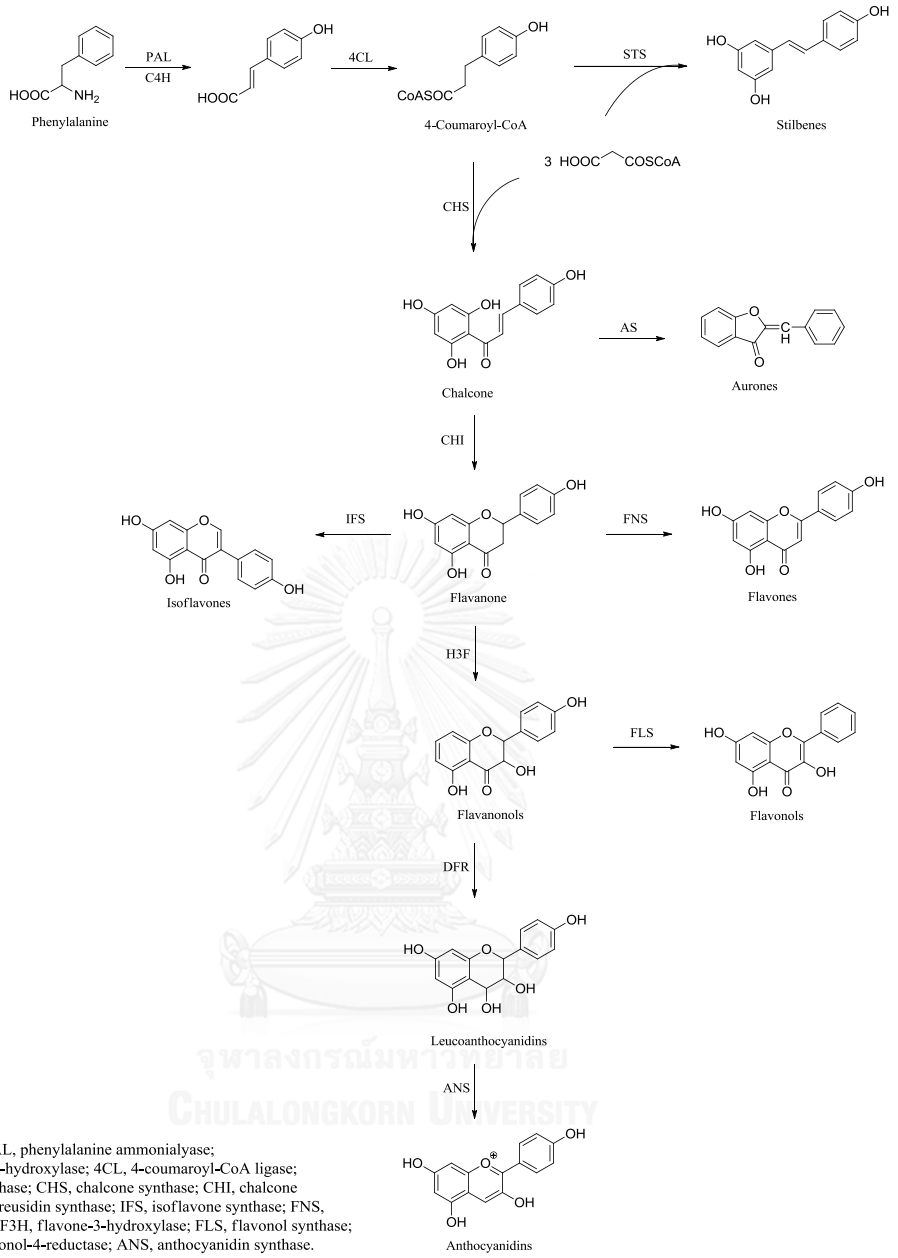


Figure 1.2 Overiew of major flavonois biosynthesis pathway in plants

Natural flavonoids, semi-synthesis flavonoids and synthetic flavonoids were reported results of inhibition of tubulin polymerization and showed inhibition of cell proliferative activity [4-6]. Apigenin, a common plant flavonoid, showed anti-tumor properties in various types including hepatocarcinogenesis, neuroblastoma, breast cancer, esophageal squamous cell carcinoma, colon cancer, lung, prostate cancer cells, cell mitosis impairment and cell apoptosis promotion [7]. Besides anti-oxidant

and anti-cancer activities, flavonoids still have various biological activities such as Anti-inflammation, Anti-diabetes, Anti-HIV, Cardiovascular activity and Anti-platelets/antithrombotic activity [8].

Annonaceae is the largest family in *Magnoliales* comprising *ca* 130 genera and 2106 accepted species. This family is a family of flowering plants and edible fruit. Oils from seeds of some annonaceae plants can be used for the production of edible oils and soap; moreover, some annonaceae woods from annonaceae plants also have been employed for alcohol production. Fragrant flowers of ylang-ylang (*Cananga odorata*) are an important raw material for perfumery [9]. In addition, many members of this family are used in folk medicine for various purposes.

The genus *Melodorum* is a family of Annonaceae and contains 55 species distributed throughout Indo-China and Australia [10]. In Thailand, *Melodorum* was found 3 species comprise of, *Melodorum fruticosum* Lour., *Melodorum hahnii* (Finet & Gagnep.) Ban and *Melodorum siamensis* (Scheff.) Ban [11]. In 2012, *Melodorum fruticosum* was named as *Sphaerocoryne affinis* (Teijsm. & Binn.) Ridl.

Melodorum fruticosum Lour. (synonym: *Sphaerocoryne affinis* (Teijsm. & Binn.) Ridl.) is a shrub, evergreen and 10-15 m height distributed in South East Asia. Leave elliptic or oblong, tip acute, base acute, glabrous above, glacous beneath; main nerves 14-18 pairs, fine the secondary quite as well as marked; reticulations rather lax, fine on both surfaces; length 8-10 cm; breadth 2-3 cm; petiole 0.5-0.7 cm long; pale brown herbarium material. Flower solitary or terminal. Petals 2 cm long, thickened below calyx, 3-3.5 cm long, lengthening in fruit, bearing 2-3 minute bracts at base and another slightly below the middle. Sepals broadly triangular, connate 0.3-0.4 cm long, puberulous or glabrous outside, glabrous inside except the base, concave inside; outer about 1 cm long and 1.1 cm broad, the inner slightly smaller, thicker and more concave. Stamens 0.2 cm long, and connectives flat-topped, pollen grains large, visible under a lens. Tours depressed in centre. Ovaries 0.2 cm long, elongate, tomentose, with short style, grooved on the inner side from the stigmatic portion downwards, stigma small, not thickened, expanded or extinct from style.

Fruits violet, ripe carpels ovoid, slightly apiculate, glabrous, 0.8 cm long and 0.7 cm in diameter; stalks slender, glabrous, 1.8-2.5 cm long [12].

1.3 Chemical constituents from *Melodorum* species and their biological activities

Previous phytochemical study on the genus *Melodorum* revealed various types of secondary metabolites including terpenoids, aromatic compounds, flavonoids and heptenes. In addition, there were also reports several biological activities.

1.3.1 *Melodorum fruticosum* Lour.

There were chemically constituent reports from many parts of this plant include flowers, leaves, branches, barks, seeds and roots. In Thai traditional medicine, flowers were used as ingredient of medicinal recipe known as “Geasorn Thung Gao” which are used as tonic stimulant, mild cardiotonic and reduce fever.

1.3.1.1 Triterpene constituents from *M. fruticosum*

Three triterpenoids, polycarpol, stigmasterol and β-sitosterol, were isolated from ethanol extract of the bark of *M. fruticosum* [13]. Acetylpolycarpol was isolated for the first time from the root of *M. fruticosum* [12]. β-sitosteroyl-3-O-β-D-glucoside was isolated from the leaves of *M. fruticosum*.

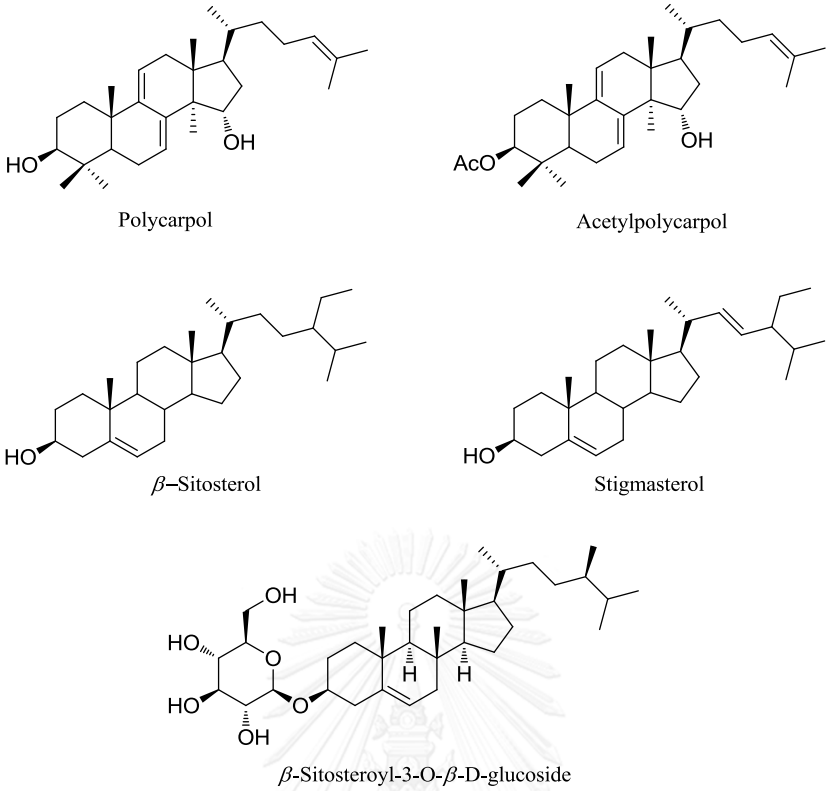


Figure 1.3 Triterpene constituents from *M. fruticosum*

1.3.1.2 Aromatic compounds constituents from *M. fruticosum*

Benzylbenzoate was isolated from the bark of *M. fruticosum*. Melodamide A was isolated for the first time from the leaves of this plant and showed strong inhibition of superoxide anion generation; moreover, benzoic acid was also isolated from these parts.

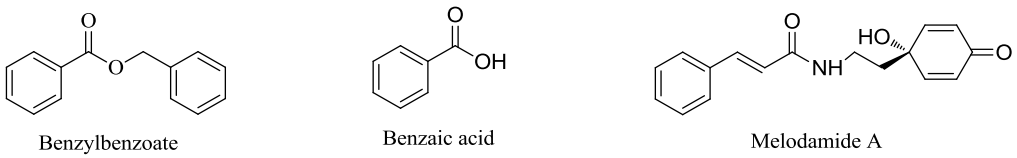


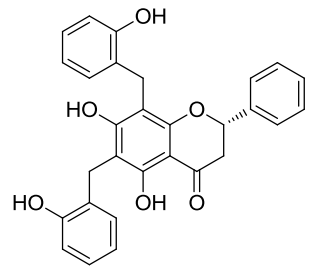
Figure 1.4 Aromatic compound constituents from *M. fruticosum*

1.3.1.3 Flavonoids constituents from *M. fruticosum*

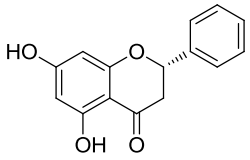
Three flavonoids, dichamanetin, pinocembrin and chrysin, were isolated from the barks of *M. fruticosum* [13]. Eight flavonoids, 5,7 dimethoxyflavone, flavokawain-A, 7,4′-dihydroxy-5-methoxyflavanone, 2′,6′-dihydroxy-4′-methoxy-chalcone, 2′,4′-dihydroxy-4,6′-dimethoxydihydrochalcone, 4′,5-dimethoxy-7-hydroxy-flavanone, ponciretin and kaempferol 3-O-β-D-apiofuranosyl-(1⟶2)-O-[α-L-rhamnopyranosyl-(1⟶6)]-β-D-glucopyranoside, were also isolated from the leaves of this plant [14, 15]. In addition, flavokawain-A and 2′,6′-dihydroxy-4′-methoxychalcone showed strong inhibition of superoxide anion generation [15].

1.3.1.4 Heptenes constituents from *M. fruticosum*

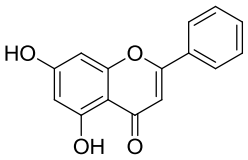
Thirteen heptenes were isolated from *M. fruticosum*. Seven heptenes, melodorinol, acetylmelodorinol, melodienone, homomelodienone, isomelodienone, homoisomelodienone and 6-hydroxy-5-hydromelodienone, were found for the first time from the barks of *M. fruticosum* [13, 16]. In 1991, *Tuchida et al*. reported three heptenes, (4E)-6-acetoxy-7-benzoyloxy-2,4-heptadien-4-olide, (4E)-7-benzoyloxy-6-hydroxy-2,4-heptadien-4-olide and (4Z)-6-benzoyloxy-7-hydroxy-2,4-heptadien-4-olide, which found from the leaves of this plant [14]. In 1998, Tiyaworanan reported three heptenes, melodorinone A, melodorinone B and tautomelodorinone, were isolated for the first time from the flower of this plant. Several heptene compounds showed significant cytotoxicity against several human tumor cell lines.



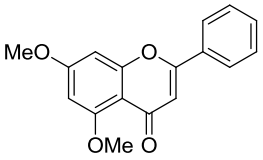
Dichamanetin



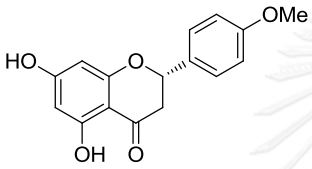
Pinocembrin



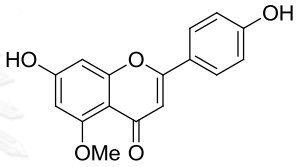
Chrysin



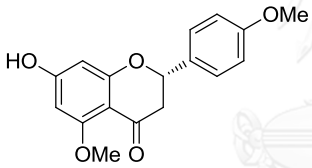
5,7-Dimethoxyflavone



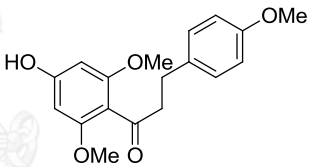
Ponciretin



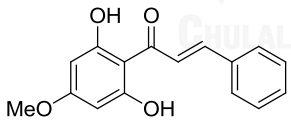
7,4′-Dihydroxy-5-methoxyflavanone



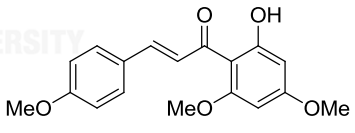
4′,5-Dimethoxy-7-hydroxyflavanone



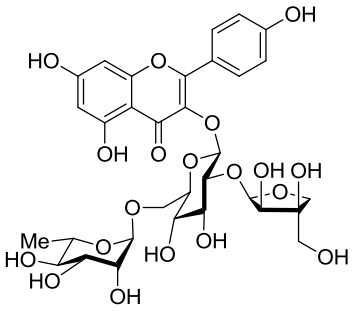
2′,4′-Dihydroxy-4,6′-dimethoxydihydrochalcone



2′,6′-Dihydroxy-4′-methoxychalcone



Flavokawain-A



Kaempferol 3-O-β-D-apiofuranosyl-(1→ 2)-O-
[α-L-rhamnopyranosyl-(1→ 6)]-β-D-glucopyranoside

Figure 1.5 Flavonoids constituents from *M. fruticosum*

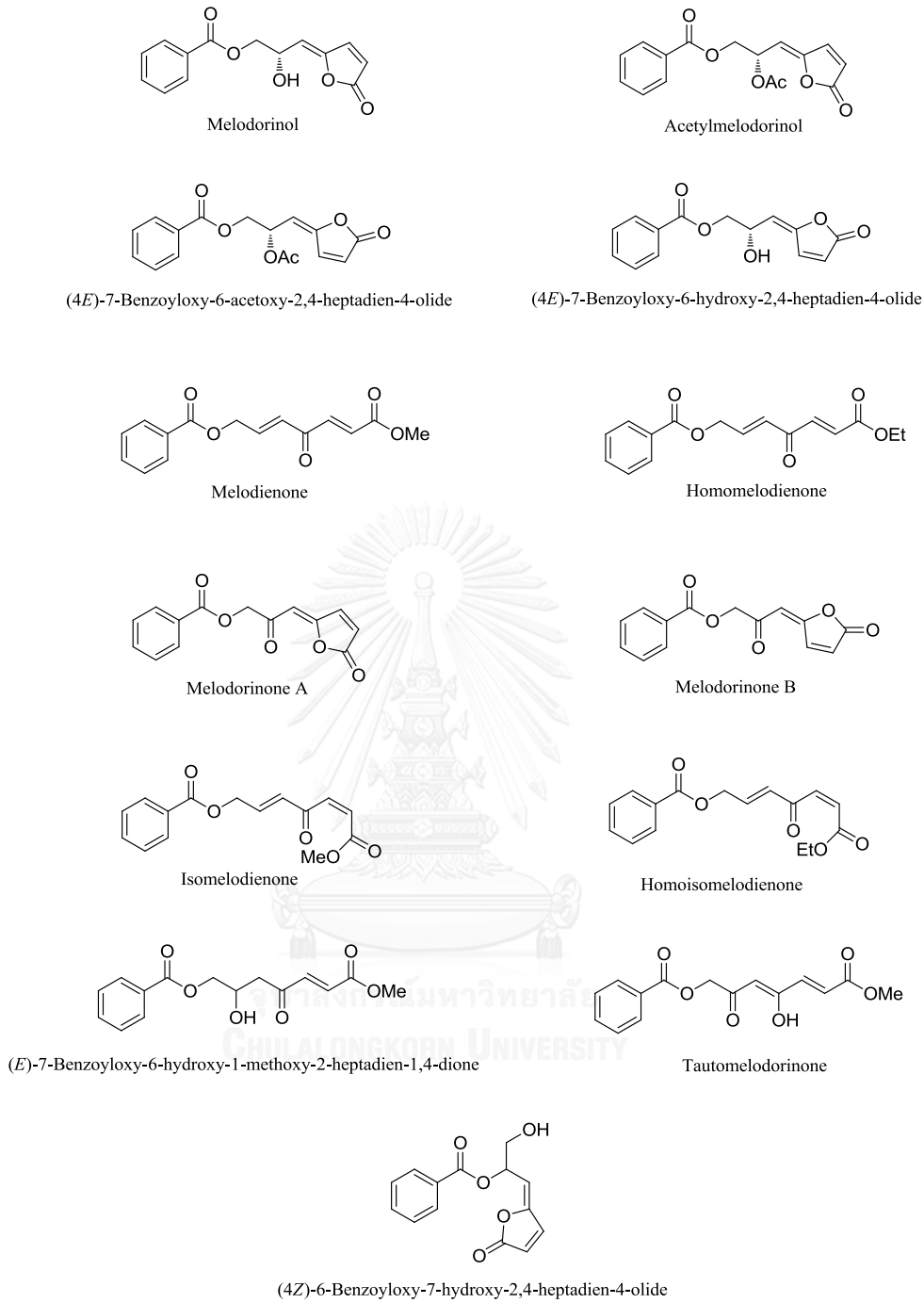


Figure 1.6 Heptenes constituents from *M. fruticosum*

Flowers

Fruits

Stems

Leaves

Figure 1.7 The flowers, fruits, stems and leaves of *M. fruticosum*

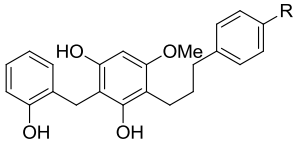
1.3.2 *Melodorum siamensis* (Scheff.) Ban

Melodorum siamensis is a scadent and original in central and southern of Thailand as well as distributed in mixed deciduous forest in South East Asia. The leaves are alternate, lance-shaped, tip acuminate, base rounded, entire edge; length 3-6.5 cm; breadth 10-22.5 cm, flower solitary, axillary, near terminal, fruit 1-2 cm long and 1 cm in diameter, yellow ripe and edible.

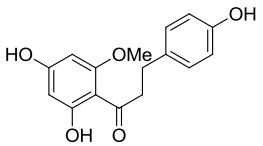
1.3.2.1 Flavonoids constituents from *M. siamensis*

Several flavonoids were isolated from EtOAc extract of the leaves of *M. siamensis*. 4,2′,4′-trihydroxy-6′-methoxy-3′(2″-hydroxybenzyl)dihydrochal-cone and 2′,4′-dihydroxy-4,6′-dimethoxy-3′(2″-hydroxybenzyl) dihydrochalcone were isolated for the first time from EtOAc extract of the leaves of *M. siamensis* [10]. Both compounds

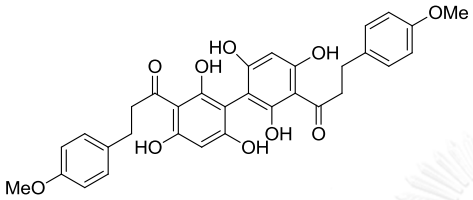
exhibited strong cytotoxicity against human tumor cell lines KB and NCI-H187, with IC_{50} values in the range of 0.66–7.16 μg/mL.



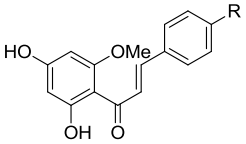
4,2',4'-Trihydroxy-6'-methoxy-3'(2''-hydroxybenzyl)dihydrochalcone R = OH
2',4'-Dihydroxy-4,6'-dimethoxy-3'(2''-hydroxybenzyl)dihydrochalcone R = OMe



4,2',4'-Trihydroxy-6'-methoxydihydrochalcone



3',3''-Bis-2',4',6'-trihydroxy-4-methoxydihydrochalcone

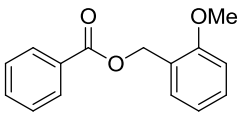


2',4'-Dihydroxy-4,6'-dimethoxychalcone R = OH
2'-Hydroxy-4,4',6'-trimethoxychalcone R = OMe

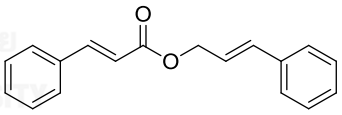
Figure 1.8 Flavonoids constituents from *M. siamensis*

1.3.2.2 Aromatic compounds constituents from *M. siamensis*

2-methoxybenzylbenzoate and 3-phenylpropenyl 3-phenylallylate were isolated for the first time from EtOAc extract of the leaves of *M. siamensis*.



2-Methoxybenzylbenzoate



3-Phenylpropenyl 3-phenylallylate

Figure 1.9 Aromatic compounds constituents from *M. siamensis*

1.4 Biological activity against cancer cells

Several anticancer drugs have origin from natural sources. Nature continues to be the most prolific source of biologically active. Cell-based assays are important tools for contemporary biology and drug discovery because of their predictive potential for *in vivo* applications. However, sometimes cellular complexity gives complicating data to interpret by inherent biological variation. Therefore, researchers often need to duplicate assay to assure that the result is not derive from fallibility.

Cytotoxicity can also be monitored using MTT or MTS assay. This assay involves reducing reaction of viable cells. The viable cells produce reducing compounds, such as NADH or NADPH, pass their electrons to an intermediate electron transfer reagent that can reduce MTT reagent or MTS reagent to formazan product. On the other hand, death cells rapidly lose the ability to reduce MTT reagent or MTS reagent. The production of the colored formazan product, therefore, is proportional to the number of viable cells in culture. The numbers of viable cells were measured by colorimetric method.

The objectives of this research:

The main objectives in this investigation as follows

1. To isolate and purify compounds from the roots of *M. fruticosum*.
2. To identify the chemical structures of all isolated compounds.
3. To evaluate the cytotoxicity against HeLa, KB, MCF-7 and HepG-2 cell lines of the isolated compounds.

CHAPTER II

EXPERIMENTAL

2.1 Plant material

The roots of *Melodorum fruticosum* Lour. were collected from Mahasarakham province of Thailand in November 2012. The plant material was identified by Dr. Suttitra Khumkratok, a botanist at Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (khumkratok no. 1-13) is deposited.

2.2 General experiment procedures

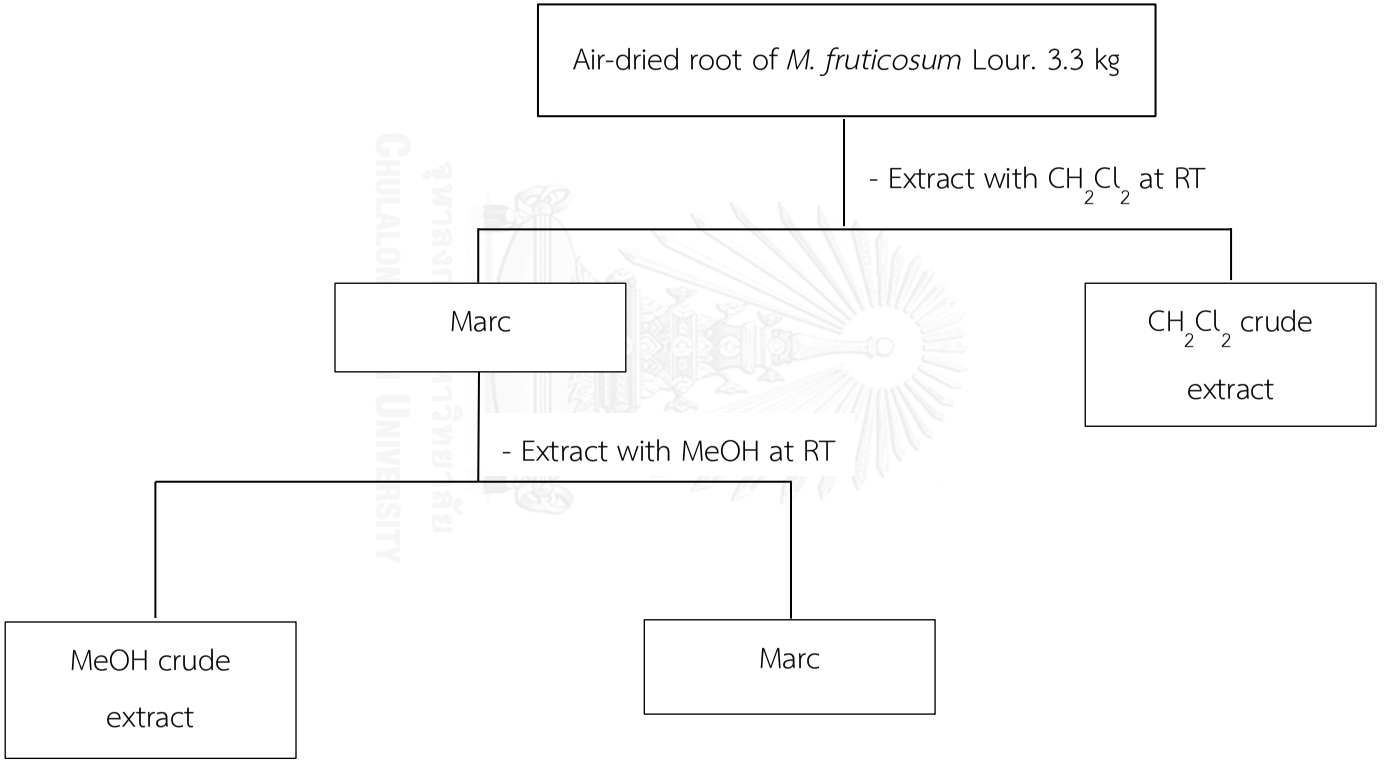
The ^{1}H-NMR spectra (at 400 MHz) and ^{13}C spectra (at 100 MHz) were recorded on a Bruker 400 ADVANCE spectrometer and chemical shifts are reported in part per million (ppm), referenced to solvent residues (δ_H 7.25, δ_C 77.0 ppm for $CDCl_3$, δ_H 2.04, δ_C 29.8, 206.5 ppm for Acetone-d_6 and δ_H 4.78, δ_C 49.0 ppm for CD_3OD). Mass spectra were measured by ESI-MS and high resolution (HR)-ESI-MS. Radical chromatography was performed on chromatotron (model 7924 T, Horrison Research) with silica gel plate of 1 mm thickness. Silica gel (60 Merck cat no. 7730, 7734 and 7749) were used for quick column chromatography, opened column chromatography and centrifugal thin layer chromatography (chromatotron). Chromatorex ODS (100—200 mesh, Fuji Silysia Chemical Ltd.) was used for reversed-phase opened column chromatography.

2.3 Extraction and purification

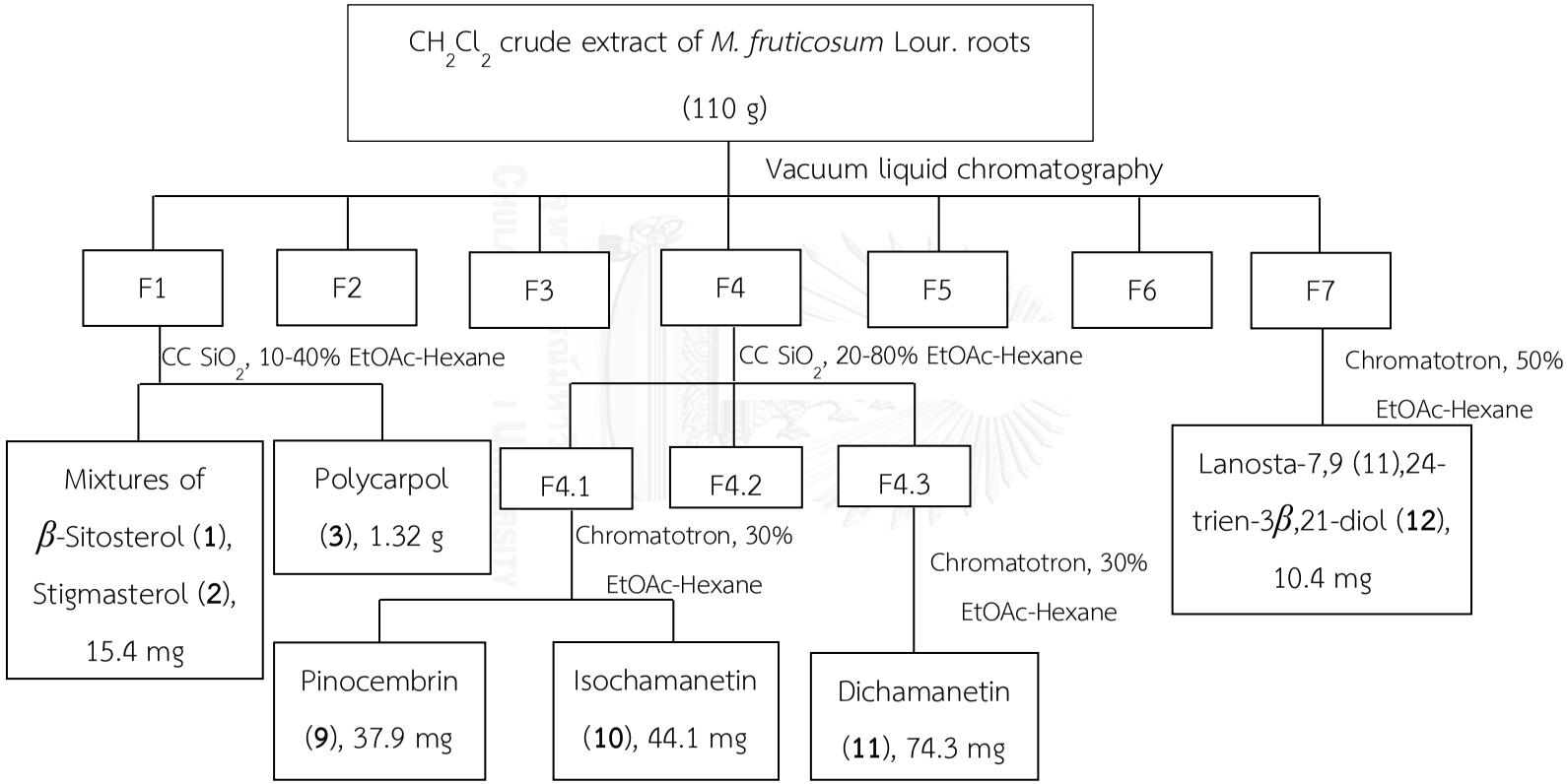
The air-dried roots of *M. fruticosum* Lour. (3.3 kg) were successively extracted with CH_2Cl_2 (3X5L) and MeOH (3X5L) at room temperature. The solvents were evaporated to afford CH_2Cl_2 crude extract (110 g) and afford MeOH crude extrct (24.4 g). The CH_2Cl_2 crude extract was subject to vacuum liquid chromatography (VLC) over silica gel (Merck Art 7730), using successive eluents of hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity to afford seven fractions, F1-F7. Fraction F1 (7.26 g) was fractioned on a silica gel column (using hexane-EtOAc, gradient system, as

eluent) to give two subfractions, mixtures of β-sitosterol (**1**) and stigmasterol (**2**) and pure polycarpol (**3**, 1.32 g). Fraction F2 (52.5 g) was chromatographed on a silica gel column with gradient system of hexane and EtOAc to give three subfractions (F2.1-F2.3) on the basis of TLC. Subfraction F2.1 was further chromatographed on a silica gel column eluted with 30% EtOAc-hexane to afford acetylmelodorinol (**4**, 2.72 g). Subfraction F2.2 was purified by chromatotron eluting with 35% EtOAc-hexane to give chamanetin (**5**, 57.9 mg) and chrysin (**6**, 30.1 mg). Subfraction F2.3 was further separated by chromatotron (using 40% EtOAc-hexane, as eluent) to give melodorinol (**7**, 42.0 mg) and (4*Z*)-6-benzoyloxy-7-hydroxy-2,4-heptadien-4-olide (**8**, 11.3 mg). Fraction F4 was subject to silica gel column using gradient of hexane and EtOAc providing three subfractions (F4.1-F4.3). Subfraction F4.1 was rechromatographed by chromatotron eluting with 30% EtOAc-hexane to yield pinocembrin (**9**, 39.7 mg) and isochamanetin (**10**, 44.1 mg). Subfraction F4.3 was further fractioned using chromatotron (using 30% EtOAc-hexane, as eluent) to afford dichamanetin (**11**, 74.3 mg). F6 was separated by chromatotron (using 50% EtOAc-hexane, as eluent) providing lanosta-7,9(11),24-trien-3β,21-diol (**12**, 10.4 mg). The MeOH crude extract was dissolved in water and loaded onto the Dianion HP-20 column. The crude on the Dianion HP-20 was washed with water to remove any amino acid, salt and sugar out from Dianion HP-20 and organic material was collected by eluted with MeOH. The organic material was chromatographed by silica gel column using gradient system of CH_2Cl_2 and MeOH providing three fractions (M1-M3) on the basis of TLC. The fraction M1 was purified by chromatotron eluted with 50% EtOAc-hexane to afford (4*Z*)-6,7-dihydroxy-2,4-heptadien-4-olide (**13**, 16.3 mg). The fraction M2 was applied to a C18 reversed-phase silica gel column chromatography eluted with 30% water-MeOH yielded catechin (**14**, 15.0 mg). The fraction M3 was separated chromatotron using CH_2Cl_2 and MeOH (9:1) to give ampelopsisionoside (**15**, 7.7 mg).

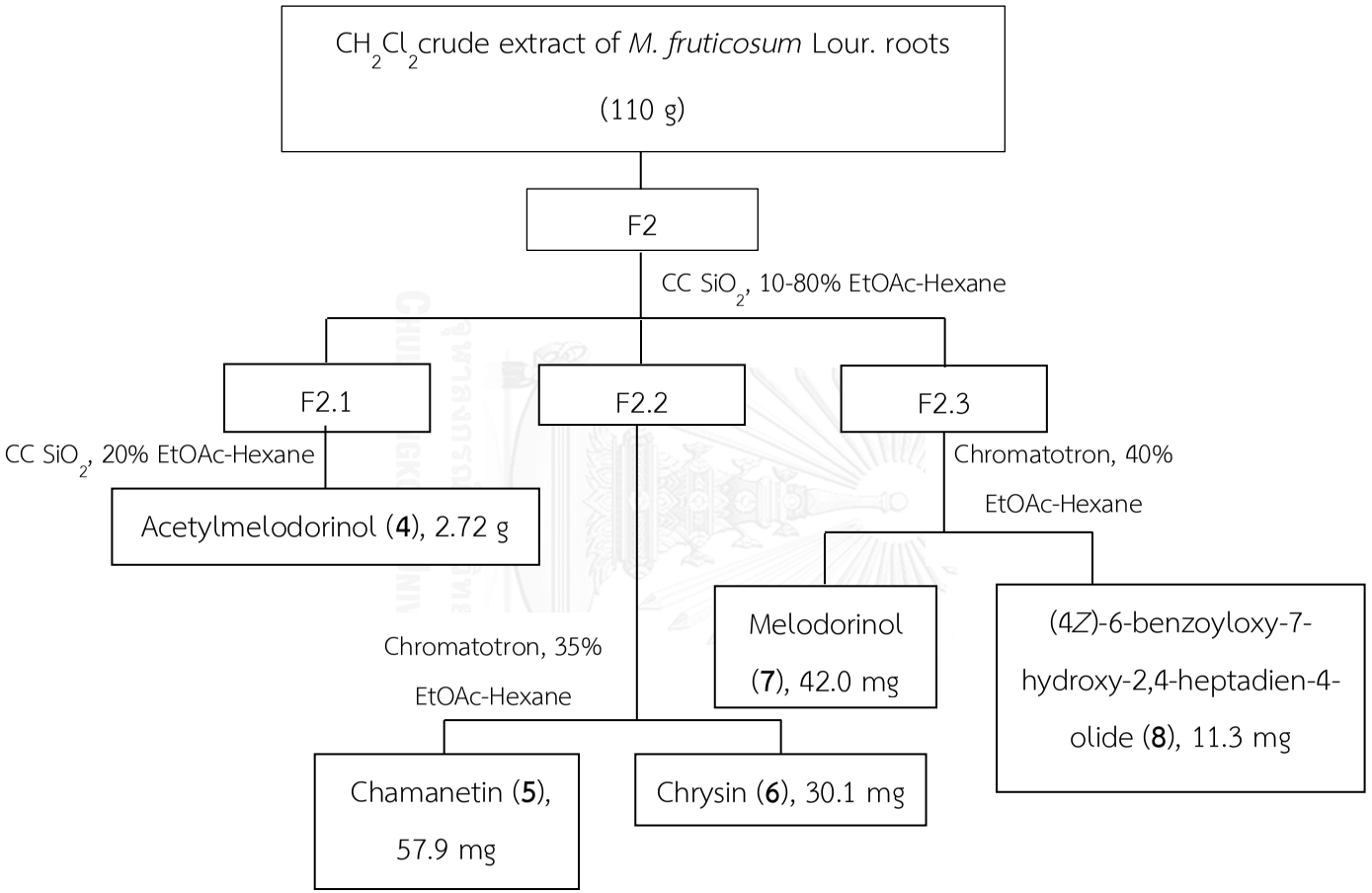
The isolation and purification of all isolated compounds from the CH_2Cl_2 and MeOH extracts of the root of *M. fruticosum* were briefly summarized in scheme 2.1-2.4.



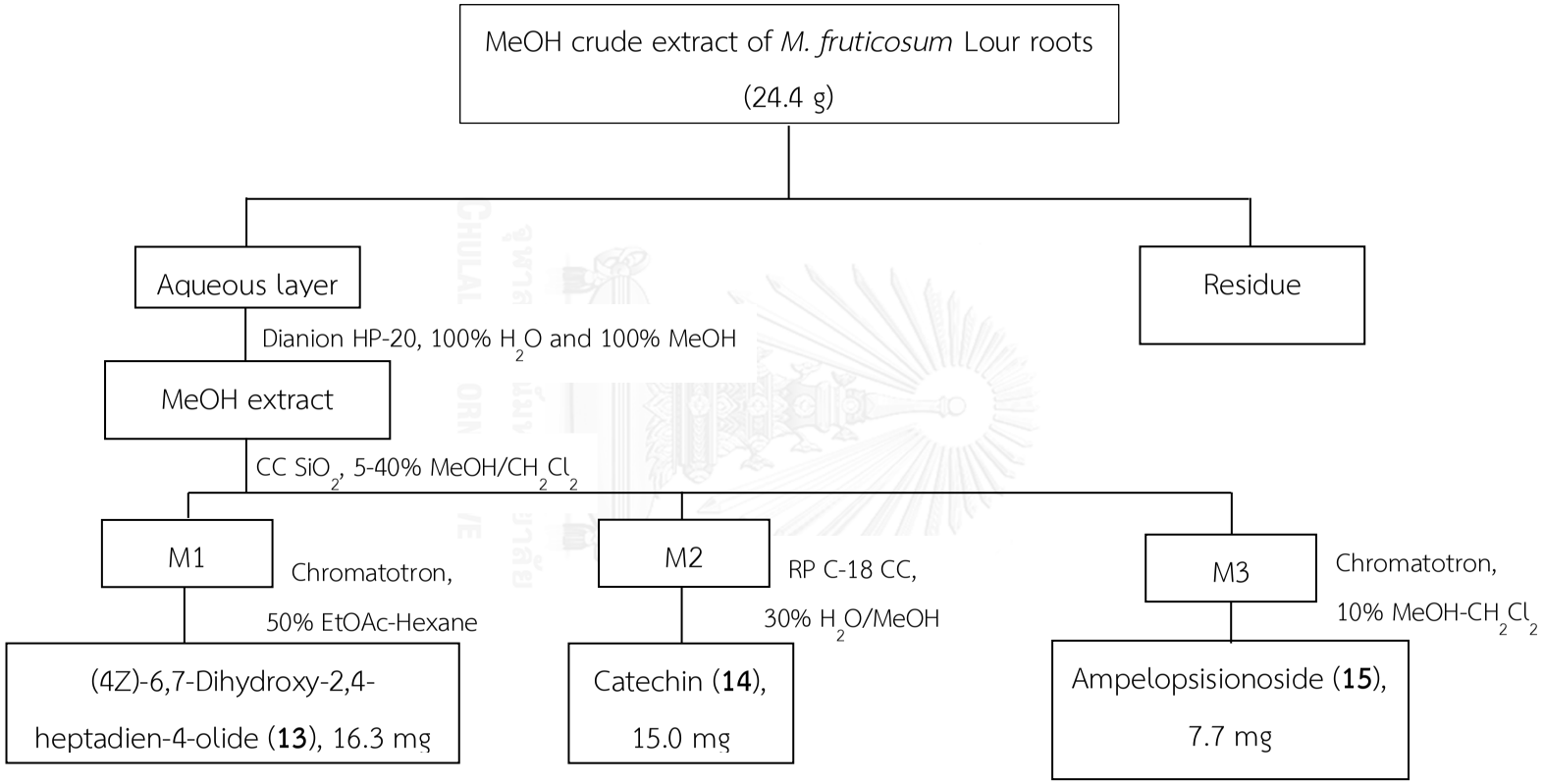
Scheme 2.1 Extraction procedure of *M. fruticosum* Lour. roots



Scheme 2.2 Isolation procedure of F1, F4 and F6 of the CH_2Cl_2 crude extract of *M. fruticosum* Lour. roots



Scheme 2.3 Isolation procedure of F2 of the CH_2Cl_2 crude extracts of *M. fruticosum* Lour. roots



Scheme 2.4 Isolation procedure of MeOH crude extract of *M. fruticosum* Lour. roots

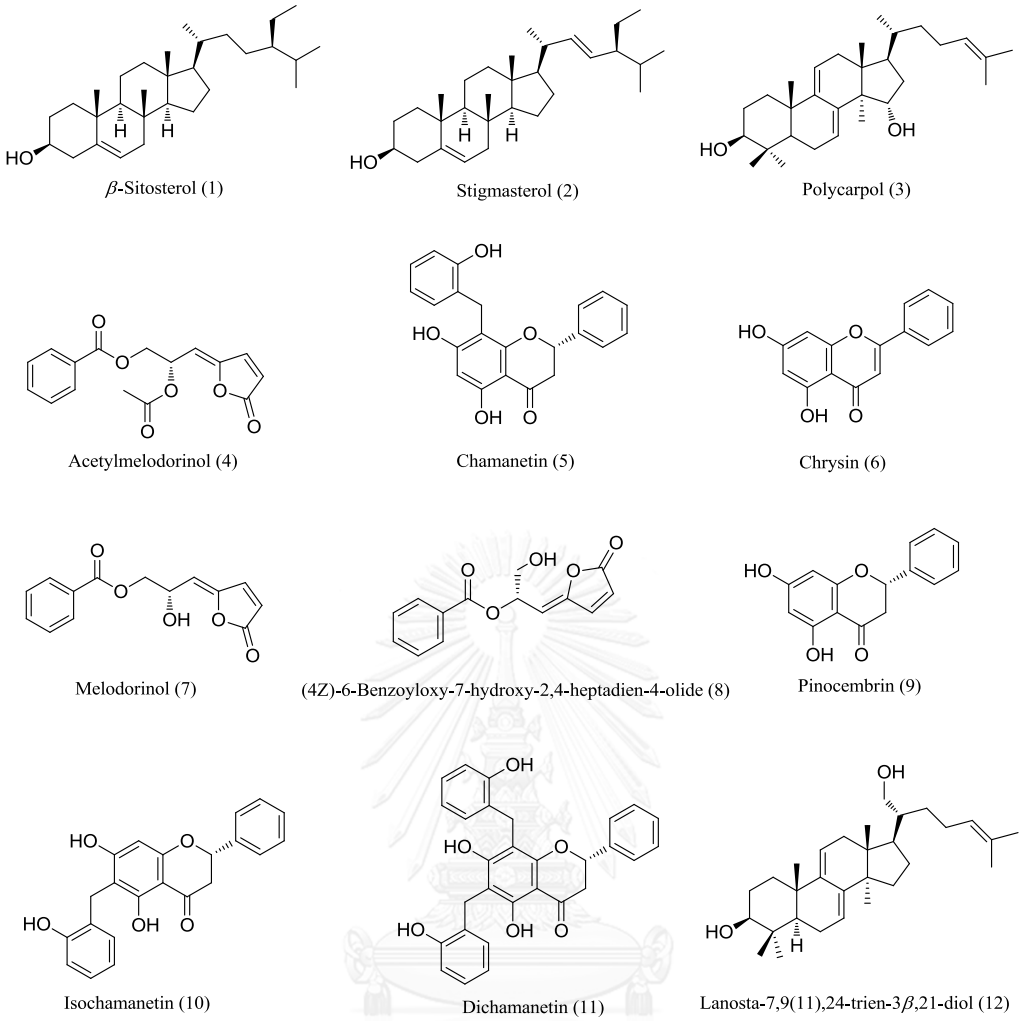


Figure 2.1 Isolated compounds from the CH_2Cl_2 crude extract of *M. fruticosum* roots

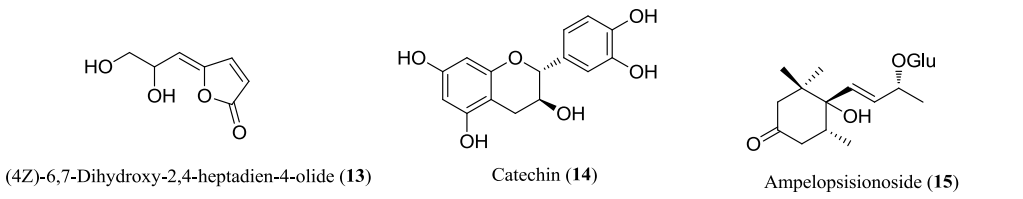


Figure 2.2 Isolated compounds from the MeOH crude extract of *M. fruticosum* Lour. roots

2.4 Synthesis of melodorinol derivatives (7a-7g)

General procedure for synthesis of melodorinol derivatives (7a-7f)

Melodorinol (0.19 mmol) in CH_2Cl_2 (3.84 mL) was added triethylamine (TEA, 1.54 mmol), 4-(dimethylamino)pyridine (DMAP, trace amount) and propionic anhydride (0.58 mmol). The solution was stirred at room temperature for 1 h. The reaction was diluted by CH_2Cl_2, washed with brine and dried over anhydrous Na_2SO_4. After removal solvent, the residue was purified by chromatotron to give Melodorinol derivatives.

Propanoylmelodorinol (7a)

Following the general procedure above, reaction of **7** (50.0 mg, 0.19 mmol), propionic anhydride (74.2 µL, 0.58 mmol), triethylamine (TEA, 155 mg, 1.54 mmol), 4-(dimethylamino)pyridine (DMAP, trace amount) in CH_2Cl_2 (3.84 mL) after 1 h yielded compound **7a** (21.1mg, 34.7%) as a yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.95 (2H, d, *J* = 7.1 Hz, H-10, 14), 7.51 (1H, t, *J* = 7.4 Hz, H-12), 7.38 (2H, t, *J* = 7.7 Hz, H-11,13), 7.30 (1H, d, *J* = 5.5 Hz, H-3), 6.21 (1H, d, *J* = 5.5 Hz, H-2), 6.09 (1H, m, H-6), 5.26 (1H, d, *J* = 8.0 Hz, H-5), 4.48 (2H, m, H-7), 2.31 (2H, q, *J* = 7.5 Hz, H-16), 1.07 (3H, t, *J* = 7.5 Hz, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 173.2 (C-15), 168.5 (C-1), 166.0 (C-8), 150.7 (C-4), 143.3 (C-3), 133.3 (C-12), 129.7 (C-9, 10, 14), 128.5 (C-11, 13), 121.6 (C-2), 109.0 (C-5), 67.2 (C-6), 64.6 (C-7), 27.5 (C-16), 9.0 (C-17); HRMS *m/z* 339.0842 $[M+Na]^+$ (calcd for $C_{17}H_{16}O_6Na$, 339.0845).

Butanoylmelodorinol (7b)

Following the general procedure above, reaction of **7** (50.0 mg, 0.19 mmol), butyric anhydride (94.2 µL, 0.58 mmol), triethylamine (TEA, 155 mg, 1.54 mmol), 4-(dimethylamino)pyridine (DMAP, trace amount) in CH_2Cl_2 (3.84 mL) after 1 h yielded compound **7b** (21.3 mg, 33.6%) as a yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.05 (2H, d, *J* = 7.2 Hz, H-10, 14), 7.60 (1H, t, *J* = 7.4 Hz, H-12), 7.47 (2H, t, *J* = 7.7 Hz, H-11, 13), 7.40 (1H, d, *J* = 5.5 Hz, H-3), 6.30 (1H, d, *J* = 5.5 Hz, H-2), 6.18 (1H, m, H-6), 5.34 (1H, d, *J* = 8.0 Hz, H-5), 4.57 (2H, m, H-7), 2.35 (2H, t, *J* = 7.4 Hz, H-16), 1.67 (2H, m, H-17) 0.95 (3H, t, *J* = 7.4 Hz, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 172.4 (C-15), 168.4 (C-

1), 166.0 (C-8), 150.7 (C-4), 143.3 (C-3), 133.3 (C-12), 129.7 (C-9, 10, 14), 128.5 (C-11, 13), 121.6 (C-2), 109.0 (C-5), 67.1 (C-6), 64.6 (C-7), 36.0 (C-16), 18.2 (C-17), 13.6 (C-18); HRMS *m/z* 353.1000 $[M+Na]^+$ (calcd for $C_{18}H_{18}O_6Na$, 353.1001).

Pentanoylmelodorinol (7c)

Following the general procedure above, reaction of **7** (50.0 mg, 0.19 mmol), pentanoic anhydride (116 µL, 0.58 mmol), triethylamine (TEA, 155 mg, 1.54 mmol), 4-(dimethylamino)pyridine (DMAP, trace amount) in CH_2Cl_2 (3.84 mL) after 1 h yielded compound **7c** (21.9 mg, 33.1%) as a yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.02 (2H, d, *J* = 7.0 Hz, H-10, 14), 7.58 (1H, t, *J* = 7.4 Hz, H-12), 7.45 (2H, t, *J* = 7.7 Hz, H-11, 13), 7.37 (1H, d, *J* = 5.5 Hz, H-3), 6.28 (1H, d, *J* = 5.5 Hz, H-2), 6.15 (1H, m, H-6), 5.32 (1H, d, *J* = 8.0 Hz, H-5), 4.54 (2H, d, *J* = 5.7 Hz, H-7), 2.35 (2H, t, *J* = 7.5 Hz, H-16), 1.59 (2H, m, H-17), 1.30 (2H, m, H-8), 0.86 (3H, t, *J* = 7.3 Hz, H-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 172.6 (C-15), 168.4 (C-1), 166.0 (C-8), 150.7 (C-4), 143.3 (C-3), 133.3 (C-12), 129.7 (C-9, 10, 14), 128.5 (C-11, 13), 121.6 (C-2), 109.0 (C-5), 67.1 (C-6), 64.6 (C-7), 33.9 (C-16), 26.9 (C-17), 22.2 (C-18), 13.6 (C-13.6); HRMS *m/z* 367.1159 $[M+Na]^+$ (calcd for $C_{19}H_{20}O_6Na$, 367.1158).

Hexanoylmelodorinol (7d)

Following the general procedure above, reaction of **7** (50.0 mg, 0.19 mmol), hexanoic anhydride (133 µL, 0.58 mmol), triethylamine (TEA, 155 mg, 1.54 mmol), 4-(dimethylamino)pyridine (DMAP, trace amount) in CH_2Cl_2 (3.84 mL) after 1 h yielded compound **7d** (20.5 mg, 29.8%) as a yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.02 (2H, d, *J* = 7.1 Hz, H-10, 14), 7.57 (1H, t, *J* = 7.4 Hz, H-12), 7.44 (1H, t, *J* = 7.7 Hz, H-11, 13), 7.37 (1H, d, *J* = 5.5 Hz, H-3), 6.28 (1H, d, *J* = 5.5 Hz, H-2), 6.15 (1H, m, H-6), 5.32 (1H, d, *J* = 8.0 Hz, H-5), 4.54 (2H, d, *J* = 5.7 Hz, H-7), 2.33 (2H, t, *J* = 7.5 Hz, H-16), 1.61 (2H, m, H-17), 1.26 (4H, m, H-18, 19), 0.84 (2H, t, *J* = 6.9 Hz, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 172.6 (C-15), 168.4 (C-1), 166.0 (C-8), 150.7 (C-4), 143.3 (C-3), 133.4 (C-12), 129.7 (C-9, 10, 14), 128.5 (C-11, 13), 121.6 (C-2), 109.0 (C-5), 67.1 (C-6), 64.6 (C-7), 34.2 (C-16), 31.2 (C-17), 24.5 (C-18), 22.2 (C-19), 13.8 (C-20); HRMS *m/z* 381.1315 $[M+Na]^+$ (calcd for $C_{20}H_{22}O_6Na$, 381.1314).

Benzoylmelodorinol (7e)

Following the general procedure above, reaction of **7** (50.0 mg, 0.19 mmol), benzoic anhydride (128 mg, 0.58 mmol), triethylamine (TEA, 155 mg, 1.54 mmol), 4-(dimethylamino)pyridine (DMAP, trace amount) in CH_2Cl_2 (3.84 mL) after 1 h yielded compound **7e** (22.8 mg, 32.6%) as a yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.06 (4H, t, *J* = 8.1 Hz, H-,10, 14, 17, 21), 7.59 (2H, m, H-12, 19), 7.46 (4H, m, H-11, 13, 18, 20), 7.42 (1H, d, *J* = 5.5 Hz, H-3), 6.39 (1H, m, H-6), 6.32 (1H, d, *J* = 5.5 Hz, H-2), 5.47 (1H, d, *J* = 8.1 Hz, H-5), 4.73 (2H, m, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 168.4 (C-1), 166.0 (C-8), 165.4 (C-15), 150.9 (C-4), 143.3 (C-3), 133.4 (C-12), 133.3 (C-19), 129.8 (C-17, 21), 129.7 (C-10, 14), 129.6 (C-16), 129.5 (C-9), 128.5 (C-11, 13, 18, 20), 121.7 (C-2), 108.9 (C-5), 68.0 (C-6), 64.6 (C-7) HRMS *m/z* 387.0847 $[M+Na]^+$ (calcd for $C_{21}H_{16}O_6Na$, 387.0845).

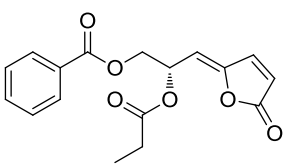
Succinoylmelodorinol (7f)

Following the general procedure above, reaction of **7** (50.0 mg, 0.19 mmol), benzoic anhydride (128 mg, 0.58 mmol), triethylamine (TEA, 155 mg, 1.54 mmol), 4-(dimethylamino)pyridine (DMAP, trace amount) in CH_2Cl_2 (3.84 mL) after 1 h yielded compound **7f** (18.2 mg, 65.8%) as a yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) 7.92 (2H, d, *J* = 7.4 Hz, H-10, 14), 7.48 (1H, t, *J* = 7.4 Hz, H-12), 7.35 (2H, t, *J* = 7.8 Hz, H-11, 13), 7.32 (1H, d, *J* = 5.5 Hz, H-3), 6.19 (d, *J* = 5.5 Hz, H-2), 6.10 – 6.03 (1H, m, H-6), 5.26 (1H, d, *J* = 8.0 Hz, H-5), 4.46 (2H, m, H-7), 2.58 (4H, s, H-16, 17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 177.6 (C-19), 171.1 (C-15), 168.6 (C-1), 166.1 (C-8), 150.8 (C-4), 143.5 (C-3), 133.3 (C-12), 129.7 (C-10, 14), 129.4 (C-9), 128.5 (C-11, 13), 121.6 (C-2), 108.6 (C-5), 67.8 (C-6), 64.6 (C-7), 28.9 (C-16), 28.8 (C-17); HRMS *m/z* 383.0742 $[M+Na]^+$ (calcd for $C_{18}H_{16}O_8Na$, 383.0743).

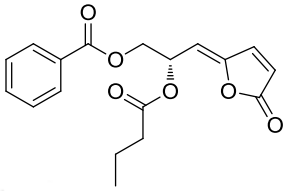
3,6-Dimethoxy-2,5-dihydromelodienone (7g)

To a solution of **7** (50 mg, 0.19 mmol) in 1 M methanolic HCl (3.84 mL) was stirred at room temperature for 24 h. yielded compound **7g** (10.1mg, 15.5%) as a yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.97 (2H, d, *J* = 7.0 Hz, H-10, 14), 7.51 (1H, t, *J* = 7.4 Hz, H-12), 7.38 (2H, t, *J* = 7.7 Hz, H-11, 13), 4.37 (1H, dd, *J* = 11.7, 4.2 Hz, H-7), 4.25 (1H, m, H-7), 4.18 (1H, m, H-3), 3.99 (1H, m, H-6), 3.69 (3H, s, 1-OCH_3), 3.38 (3H, s,

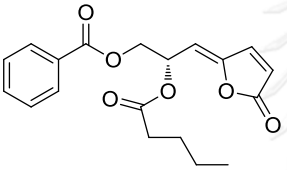
6-OCH_3), 3.36 (3H, s, 3-OCH_3), 2.81 (2H, m, H2), 2.74 (1H, ddd, *J* = 17.4, 8.5, 4.4 Hz, H-5), 2.62 (1H, ddd, *J* = 17.0, 8.1, 4.9 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 204.3 (C-4), 172.3 (C-1), 166.3 (C-8), 113.1 (C-12), 129.7 (C-9, 10, 14), 128.4 (C-11, 13), 76.0 (C-3), 74.8 (C-6), 65.0 (C-7), 58.8 (3-OCH_3), 58.0 (6-OCH_3), 52.1 (1-OCH_3), 46.1 (C-2), 45.7 (C-5); HRMS *m/z* 361.1265 $[M+Na]^+$ (calcd for $C_{17}H_{22}O_7Na$, 361.1263).



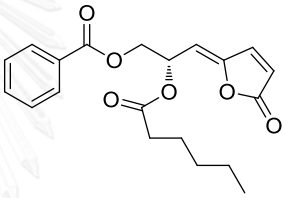
Propanoylmelodorinol (**7a**)



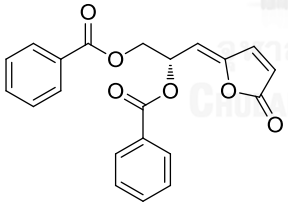
Butanoylmelodorinol (**7b**)



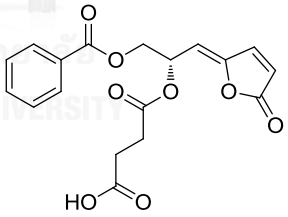
Pentanoylmelodorinol (**7c**)



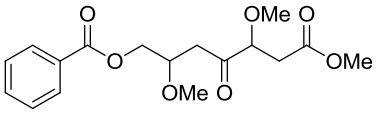
Hexanoylmelodorinol (**7d**)



Benzoylmelodorinol (**7e**)



Succinoylmelodorinol (**7f**)



3,6-Dimethoxy-2,5-dihydromelodienone
(7-Benzoyloxy-1,3,6-trimethoxy-heptan-1,4-dione)
(**7g**)

Figure 2.3 Melodorinol derivatives (**7a**-**7g**)

2.5 Bioassay procedure

All tested compounds were subjected to cytotoxic evaluation against KB (Human epidermoid carcinoma), HeLa (Human cervix adenocarcinoma), MCF-7 (Human breast adenocarcinoma) and HepG-2 (Human hepatocellular carcinoma) cell lines employing the MTT colorimetric assay. Doxorubicin was used as standard antibiotic antitumor agent which exhibits activity against KB, HeLa, MCF-7 and HepG-2 cell lines according to the method of Kongatgip *et al* [17]. This assay was kindly performed by Natural Products Research Section, Research Division, National Cancer Institute, Thailand.

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CHAPTER III

RESULTS AND DISCUSSION

3.1 Properties and structural elucidation of isolated compounds

Isochamanetin (10)

Isochamanetin was obtained as a yellow solid. The chemical formula was established as $C_{22}H_{18}O_5$ from ^{1}H and ^{13}C NMR (Table 3.1). In the ^{1}H NMR data of compound **10**, proton signals at δ_H 2.85 (1H, dd, *J* = 17.1, 2.9 Hz, H-3β), 3.20 (1H, dd, *J* = 17.1, 12.8 Hz, H-3α) and 5.59 (1H, dd, *J* = 12.8, 2.9 Hz, H-2), were used to characterized the C ring of the dihydroflavone moiety. Proton signal at δ_H 3.91 (2H, s, H-11) was methylene proton connected between 2-hydroxylphenyl and dihydroflavone at position 6. Signals of the other ten protons in range δ_H 6.13-7.59 were assigned to aromatic protons of ring A, B and 2-hydroxyphenyl. A singlet signal δ_H 12.56 indicated the presence of an OH group at C-5, which formed an intramolecular hydrogen bond with the oxygen atom at C-4. In the ^{13}C NMR spectrum 22 carbon signals were observed, two methylene carbons at δ_C 22.4 and 43.7, one methine carbon at δ_C 80.0, 18 aromatic carbons in aromatic rings region from 103.3-165.0, and one carbonyl carbon at δ_C 197.2 (Figure3.1). The HMBC correlations of H-11 (δ_H 3.91) to C-1″ (δ_C 127.6), C-2″ (δ_C 155.1) and C-6 (δ_C 108.2) confirmed that these methylene protons connected between 2-hydroxylphenyl and dihydroflavone at position 6 (Figure 3.2). Based on 1D and 2D NMR including to comparison with previous literature data revealed that **10** is isochamanetin [18]. To the best of our knowledge, this compound was isolated for the first time from this plant.

Table 3.1 ^{1}H, ^{13}C NMR HMBC data of **10** in $CDCl_3$ (400 MHz for ^{1}H, 100 MHz for ^{13}C)

Position	δ_H (mult, *J* in Hz)	δ_C	HMBC
2	5.59 (1H, *J* = 12.8, 3.0 Hz)	80.0	C-1′, C-2′, C-6′
3	3.20 (1H, dd, *J* = 17.1, 12.8 Hz, H-3α)	43.7	C-2, C-4, C-1′
	2.85 (1H, dd, *J* = 17.1, 2.9 Hz, H-3β),		
4	-	197.2	-
5	-	103.3	-
6	-	108.2	-
7	-	165.0	-
8	6.13 (1H, s)	95.9	C-6, C-7, C-9, C-10
9	-	162.3	-
10	-	103.3	-
11	3.90 (2H, s)	22.3	C-6, C-1″, C-2″, C-6″
1′	-	140.1	-
2′, 4′	7.58 (2H, d, *J* = 7.2 Hz)	127.3	C-2, C-2′, C-4′, C-6′
3′, 5′	7.46 (2H, t, *J* = 7.2 Hz)	129.5	C-1′, C-3′, C-5′
6′	7.42 (1H, m)	129.4	C-2′, C-4′
1″	-	127.6	-
2″	-	155.2	-
3″	6.86 (1H, d, *J* = 8.0, 0.9 Hz)	115.9	C-2″, C-5″
4″	7.03 (1H, d, *J* = 8.0, 1.4 Hz)	127.9	C-2″, C-6″
5″	6.75 (1H, d, *J* = 7.5, 0.9 Hz)	120.7	C-3″, C-4″
6″	7.15 (1H, d, *J* = 7.5, 1.4 Hz)	130.8	C-11, C-2″, C-4″

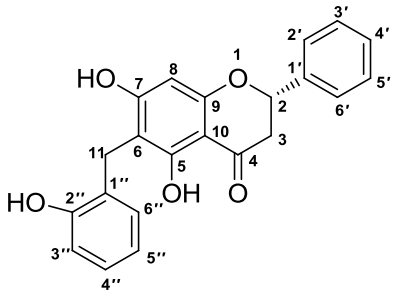


Figure 3.1 Structure of Isochamanetin (**10**)

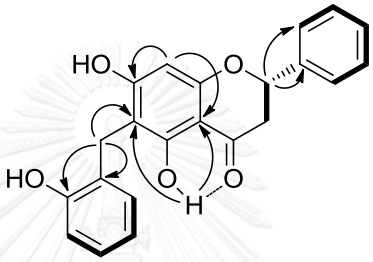


Figure 3.2 Selected HMBC (arrow curve) and COSY (bold lines) correlations of **10**

(4Z)-6,7-dihydroxy-2,4-heptadien-4-olide (13)

(4Z)-6,7-dihydroxy-2,4-heptadien-4-olide was afforded as a pale yellow oil. (4Z)-6,7-dihydroxy-2,4-heptadien-4-olide has molecular formula $C_7H_8O_4$ as established by 1D and 2D NMR (Table 3.3). ^{13}C NMR showed seven signals derived from one methylene carbon, one methine carbon, three olefinic carbons, one quaternary carbon and one carbonyl carbon. Olefinic protons were used to confirm five member ring α,β unsaturated lactone by the correlations from HMBC (Figure 3.3). The H-2, 6.19 (1H, d, *J* =5.5 Hz), revealed the correlations with C-1, C-3 and C-4. Furthermore, H-5, 5.32 (1H, d, *J* = 7.9 Hz), also showed correlations with C-3, C-4 and C-7 (Figure 3.4). Based on 1D and 2D NMR revealed that **13** is (4Z)-6,7-dihydroxy-2,4-heptadien-4-olide. To the best of our knowledge, this compound was isolated for the first time from this plant.

Table 3.2 ^{1}H, ^{13}C NMR HMBC data of **13** in $CDCl_3$ (400 MHz for ^{1}H, 100 MHz for ^{13}C)

Position	δ_H (mult, *J* in Hz)	δ_C	HMBC
1	-	169.0	-
2	6.19 (1H, d, *J* =5.5 Hz)	120.8	C-1, C-3, C-4
3	7.32 (1H, d, *J* = 5.5 Hz)	143.7	C-1, C-2, C-4
4	-	149.8	-
5	5.32 (1H, d, *J* = 7.9 Hz)	113.9	C-3, C-4, C-7
6	4.80 (1H, m)	67.9	C-4
7	3.75 (1H, d, *J* = 11.2, 3.5 Hz)	65.6	C-5
	3.65 (1H, d, *J* = 11.2, 6.8 Hz)		

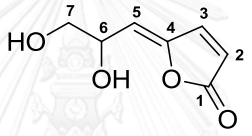


Figure 3.3 Structure of (4Z)-6,7-dihydroxy-2,4-heptadien-4-olide (**13**)

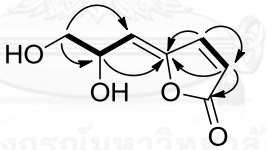


Figure 3.4 Selected HMBC (arrow curve) and COSY (bold lines) correlations of **13**

***β*-sitosterol (1)**: colorless needles, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 5.39 (1H, m, H-6), 1.05 (3H, s, H-19), 0.96 (3H, d, *J* = 6.5 Hz, H-21), 0.89 (3H, t, *J* =7.4 Hz, H-29), 0.87 (3H, d, *J* =6.7 Hz, H-26), 0.85 (3H, d, *J* =6.7 Hz, H-27), 0.72 (3H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$); δ_C 141.2 (C-5), 122.1 (C-6), 72.2 (C-3), 57.2 (C-14), 56.5 (C-17), 50.6 (C-9), 46.2 (C-24), 42.8 (C-4, 13), 40.2 (C-12), 37.7 (C-1), 36.9 (C-10), 36.6 (C-20), 34.4 (C-22), 32.3 (C-2, 8), 32.1 (C-7), 29.6 (C-25), 28.7 (C-16), 26.5 (C-23), 24.7 (C-15), 23.3 (C-28), 21.5 (C-11), 20.2 (C-26), 19.8 (C-19), 19.5 (C-27), 19.5 (C-27), 19.2 (C-21), 12.4 (C-18), 12.3 (C-29). Compound **1** was characterized as *β*-sitosterol by comparison of the physical and spectral data with the literature [19].

Stigmasterol (2): colorless needles, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 5.39 (1H, m, H-6), 5.15 (1H, m, H-23), 5.01 (1H, m, H-22), 3.56 (1H, m, H-3), 1.05 (3H, s, H-19), 0.96 (3H, d, *J* = 6.5 Hz, H-21), 0.89 (3H, t, *J* = 7.4 Hz, H-29), 0.87 (3H, d, *J* = 6.7 Hz, H-26), 0.85 (3H, d, *J* = 6.7 Hz, H-27), 0.72 (3H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$); δ_C 141.2 (C-5), 122.1 (C-6), 72.2 (C-3), 57.2 (C-14), 56.5 (C-17), 50.6 (C-9), 46.2 (C-24), 42.8 (C-4, 13), 40.7 (C-22), 40.2 (C-12), 37.7 (C-1), 36.9 (C-10), 36.6 (C-20), 32.3 (C-2, 8), 32.1 (C-7), 29.6 (C-25), 28.7 (C-16), 24.7 (C-15), 23.3 (C-28), 21.5 (C-11), 21.4 (C-23), 20.2 (C-26), 19.8 (C-19), 19.5 (C-27), 19.2 (C-21), 12.4 (C-18), 12.3 (C-29). Compound **2** was characterized as stigmasterol by comparison of the physical and spectral data with the literature [19].

Polycarpol (3): white needles, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 5.87 (1H, d, *J* = 6.3 Hz, H-7), 5.33 (1H, d, *J* = 6.1 Hz, H-11), 5.11 (1H, t, *J* = 6.3 Hz, H-24), 4.29 (1H, dd, *J* = 9.6, 5.7 Hz, H-15β), 3.27 (1H, dd, *J* = 11.3, 4.4 Hz, H-3), 2.31 (1H, d, *J* = 17.8 Hz, H-12α), 2.21 (1H, dd, *J* = 6.5, 4.1 Hz, H-6β), 2.16 (1H, dd, *J* = 6.5, 4.1 Hz, H-6α), 2.10 (1H, d, *J* = 6.1 Hz, H-12β, 2.05 (1H, m, H-1β), 1.99 (1H, m, H-16β), 1.88 (1H, m, H-23), 1.74 (1H, m, H-16α), 1.70 (3H, m, H-26), 1.67 (1H, m, H-2α), 1.66 (1H, m, H-17), 1.62 (3H, s, H-27), 1.46 (1H, m, H-1α), 1.37 (1H, m, H-20), 1.12 (2H, dd, *J* = 11.8, 3.7 Hz, H-5), 1.06 (2H, m, H-22), 1.03 (3H, s, H-28), 1.00 (3H, s, H-19), 0.96 (3H, s, H-30), 0.91 (3H, d, *J* = 5.0 Hz, H-21), 0.90 (3H, s, H-29), 0.63 (3H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$); δ_C 146.2 (C-9), 140.9 (C-8), 131.1 (C-25), 124.9 (C-24), 121.3 (C-7), 116.1 (C-11), 78.9 (C-3), 74.8 (C-15), 52.0 (C-14), 49.0 (C-5), 48.9 (C-17), 44.4 (C-13), 40.1 (C-16), 38.7 (C-4), 38.5 (C-12), 37.4 (C-10), 36.2 (C-22), 35.8 (C-20), 35.8 (C-1), 28.2 (C-28), 27.8 (C-2), 25.7 (C-26), 24.9 (C-23), 22.9 (C-6), 22.8 (C-19), 18.4 (C-21), 17.6 (C-27), 17.1 (C-30), 15.9 (C-29), 15.8 (C-18). Compound **3** was characterized as polycarpol by comparison of the physical and spectral data with the literature [12].

acetylmelodorinol (4): yellow liquid, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 7.94 (2H, m, H-10, 14), 7.49 (1H, t, *J* = 7.4 Hz, H-12), 7.39 (2H, m, H-11, 13), 7.35 (1H, d, *J* = 5.4 Hz, H-3), 6.20 (1H, d, *J* = 5.4 Hz, H-2), 6.08 (1H, ddd, *J* = 8.1, 6.3, 4.1 Hz, H-6), 5.33 (1H, d, *J* = 8.1 Hz, H-5), 4.50 (1H, dd, *J* = 11.7, 4.1 Hz, H-7β), 4.43 (1H, dd, *J* = 11.7, 6.3 Hz, H-7α), 2.01 (3H, s, H-16); ^{13}C-NMR (100 MHz, $CDCl_3$); δ_C 169.7 (C-15), 168.6 (C-1), 165.9

(C-8), 150.8 (C-4), 143.7 (C-3), 133.3 (C-12), 129.6 (C-10, C-14), 129.5 (C-9), 128.4 (C-11, C-13), 121.4 (C-2), 108.8 (C-5), 67.2 (C-6), 64.6 (C-7), 20.8 (C-16). Compound **4** was characterized as acetylmelodorinol by comparison of spectral data with the literature [14].

Chamanetin (5): yellow solid, ^{1}H-NMR (400 MHz, Acetone-d_6); δ_H 12.00 (1H, s, OH-5), 9.57 (1H, s, OH-7), 7.38 (2H, d, *J* = 7.2 Hz, H-2′, 6′), 7.27 (2H, t, *J* = 7.2 Hz, H-3′, 5′), 7.22 (1H, d, *J* = 7.2 Hz, H-4′), 6.89 (1H, dd, *J* = 7.7, 1.2 Hz, H-6″), 6.84 (1H, td, *J* = 7.8, 1.4 Hz, H-4‴), 6.68 (1H, dd, *J* = 8.0, 1.0 Hz, H-3‴), 6.54 (1H, td, *J* = 7.4, 1.0 Hz, H-5‴), 5.96 (1H, s, H-6), 5.42 (1H, dd, *J* = 12.6, 3.2 Hz, H-2), 3.74 (2H, s, H-11), 2.98 (1H, dd, *J* = 17.1, 12.6 Hz, H-3α), 2.70 (1H, dd, *J* = 17.1, 3.2 Hz, H-3β); ^{13}C-NMR (100 MHz, Acetone-d_6); δ_C 195.8 (C-4). 163.7 (C-7), 161.9 (C-5), 159.9 (C-9), 153.8 (C-2‴), 138.6 (C-1′), 129.2 (C-6‴), 128.0 (C-3′, C-5′), 127.9 (C-4′), 126.4 (C-4‴), 126.2 (C-1‴), 125.7 (C-2′, C-6′), 119.1 (C-5‴), 114.4 (C-3‴), 105.9 (C-8), 102.1 (C-10), 95.6 (C-6), 78.5 (C-2), 42.0 (C-3), 21.6 (C-11). Compound **5** was characterized as chamanetin by comparison of the physical and spectral data with the literature [18].

Chrysin (6): yellow solid; ^{1}H-NMR (400 MHz, Acetone-d_6); δ_H 8.08 (2H, dd, *J* = 7.8, 1.8 Hz, H-2′, 6′), 7.59-7.66 (3H, m, H-3′, 4′, 5′), 6.81 (1H, s, H-3), 6.59 (1H, d, *J* = 2.1 Hz, H-8), 6.30 (1H, d, *J* = 2.1 Hz, H-6); ^{13}C-NMR (100 MHz, Acetone-d_6); δ_C 183.2 (C-4), 165.5 (C-7), 164.7 (C-2), 163.3 (C-5), 159.0 (C-9), 132.7 (C-4′), 132.3 (C-1′), 130.2 (C-3′, 5′), 127.3 (C-2′, 6′), 106.2 (C-3), 105.4 (C-10), 100.0 (C-6), 94.9 (C-8). Compound **6** was characterized as chrysin by comparison of the physical and spectral data with the literature [14].

Melodorum (7): colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 7.93 (2H, d, *J* = 7.4 Hz, H-10, H-14), 7.46 (1H, t, *J* = 7.4 Hz, H-12), 7.32 (2H, m, H-11, H-13), 7.30 (1H, d, *J* = 5.4 Hz, H-3), 6.12 (1H, d, *J* = 5.4 Hz, H-2), 5.34 (1H, d, *J* = 8.3 Hz, H-5), 5.08 (1H, m, H-6), 4.35 (2H, d, *J* = 5.3 Hz, H-7); ^{13}C-NMR (100 MHz, $CDCl_3$); 169.4 (C-1), 166.7 (C-8), 150.0 (C-4), 144.0 (C-3), 133.3 (C-12), 129.7 (C-10, 14), 129.5 (C-9), 128.4 (C-11, 13), 120.8 (C-2), 113.6 (C-5), 67.4 (C-7), 65.4 (C-6). Compound **7** was characterized as melodorinol by comparison of spectral data with the literature [14].

(4*Z*)-6-benzoyloxy-7-heptadien-4-olide (8): yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 7.99 (2H, d, *J* = 7.8 Hz, H-10, 14), 7.51 (1H, t, *J* = 7.4 Hz, H-12), 7.38 (2H, t, *J* = 7.5 Hz, H-11, 13), 7.32 (1H, d, *J* = 5.4 Hz, H-3), 6.20 (1H, d, *J* = 5.4 Hz, H-2), 6.00 (1H, m, H-6), 5.39 (1H, d, *J* = 8.0 Hz, H-5), 3.84 (2H, m, H-7); ^{13}C-NMR (100 MHz, $CDCl_3$); δ_C 168.8 (C-1), 165.9 (C-8), 150.5 (C-4), 143.5 (C-3), 133.4 (C-12), 129.8 (C-10, C-14), 129.6 (C-9), 128.5 (C-11, C-13), 121.3 (C-2), 109.9 (C-5), 71.3 (C-6), 64.2 (C-7). Compound **8** was characterized as (4Z)-6-benzoyloxy-7-heptadien-4-olide by comparison of the physical and spectral data with the literature [14].

Pinocembrin (9): white needles; ^{1}H-NMR (400 MHz, Acetone-d_6); δ_H 12.18 (1H, s, OH-5), 9.67 (s, 1H, OH-7), 7.57 (d, 2H, *J* = 7.2 Hz, H-2′, H6′), 7.46 (t, 2H, *J* = 7.2 Hz, H-3′, H-5′), 7.40 (d, 1H, *J* = 7.2 Hz, H-4′), 6.03 (d, 1H, *J* = 2.2 Hz, H-8), 6.00 (d, 1H, *J* = 2.2 Hz, H-6), 5.56 (dd, 1H, *J* = 12.8, 3.1 Hz, H-2), 3.17 (dd, 1H, *J* = 17.1, 12.8 Hz, H-3α), 2.82 (dd, 1H, *J* = 17.1, 3.1 Hz, H-3β); ^{13}C-NMR (100 MHz, Acetone-d_6); δ_C 196.8 (C-4), 167.4 (C-7), 165.3 (C-5), 164.2 (C-9), 140.1 (C-1′), 129.5 (C-3′, 5′), 129.4 (C-4′), 127.3 (C-2′, 6′), 103.3 (C-10), 97.0 (C-6), 96.0 (C-8), 80.0 (C-2), 43.6 (C-3). Compound **9** was characterized as pinocembrin by comparison of the physical and spectral data with the literature [13].

Dichamanetin (11): Yellow solid; ^{1}H-NMR (400 MHz, Acetone-d_6); δ_H 12.89 (1H, s, OH-5), 7.57 (2H, d, *J* = 7.4 Hz, H-2′, 6′), 7.74-7.36 (4H, m, H-3′, 4′, 5′, 6″, 6‴), 7.23 (1H, m, H-6‴), 7.07 (2H, m, H-4″, 4‴), 6.96 (2H, m, 3″, 3‴), 6.81 (2H, m, H-5″, 5‴), 5.52 (1H, d, *J* = 12.7 Hz, H-2), 4.04 (2H, s, H-12), 4.00 (2H, s, H-11), 3.14 (1H, dd, *J* = 16.1, 12.7 Hz, H-3α), 2.84 (d, *J* = 16.1 Hz, H-3β); ^{13}C-NMR (100 MHz, Acetone-d_6); δ_C 197.7 (C-4), 162.6 (C-7), 160.7 (C-5), 159.6 (C-9), 154.7 (C-2″, 2‴), 140.0 (C-1′), 131.8 (C-6‴), 131.5 (C-6″), 129.7 (C-3′, 5′), 129.5 (C-4′), 128.3 (C-3″, 3‴), 127.8 (C-1‴), 127.7 (C-1″), 127.3 (C-2′, 6′), 121.4 (C-5″), 121.3 (C-5‴), 116.2 (C-3‴), 116.0 (C-3″), 108.9 (C-6), 107.9 (C-8), 103.7 (C-10), 80.1 (C-2), 43.6 (C-3), 23.7 (C-11), 23.1 (C-12) Compound **11** was characterized as dichamanetin by comparison of the physical and spectral data with the literature [18].

Lanosta-7,9(11),24-trien-3β,21-diol (12): white needles; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.51 (2H, m, H-7), 5.34 (2H, d, *J* = 5.7 Hz, H-11), 5.14 (1H, t, *J* =6.4 Hz, H-24),

3.76 (1H, dd, *J* = 11.2, 4.4 Hz, H-21β), 3.67 (1H, dd, *J* = 11.2, 2.6 Hz, H-21α), 3.27 (1H, dd, *J* = 11.3, 4.6 Hz, H-3), 1.71 (3H, s, H-27), 1.64 (3H, s, H-26), 1.03 (3H, s, H-28), 1.00 (3H, s, H-19), 0.92 (3H, s, H-30), 0.91 (3H, s, H-18), 0.61 (3H, s, H-29); ^{13}C-NMR (100 MHz, $CDCl_3$); δ_C 146.2 (C-9), 142.5 (C-8), 131.4 (C-25), 124.8 (C-24), 120.5 (C-7), 115.9 (C-11), 78.9 (C-3), 62.6 (C-21), 50.4 (C-14), 49.1 (C-5), 44.9 (C-17), 43.5 (C-13), 42.7 (C-20), 38.7 (C-4), 37.4 (C-10), 37.2 (C-12), 35.7 (C-1), 31.4 (C-15), 29.8 (C-22), 28.1 (C-28), 27.8 (C-2), 27.5 (C-16), 25.7 (C-30), 25.6 (C-27), 25.0 (C-23), 23.0 (C-6), 22.7 (C-19), 17.7 (C-26), 16.0 (C-29), 15.8 (C-18). Compound **12** was characterized as lanosta-7,9(11),24-trien-3β,21-diol by comparison of the physical and spectral data with the literature [20].

Catechin (14): red solid; ^{1}H NMR (400 MHz, CD_3OD); δ_H 6.87 (1H, d, *J* = 1.5 Hz, 2'), 6.71-6.65 (2H, m, H-5′, 6′), 5.85 (1H, d, *J* = 2.2 Hz, H-6), 5.82 (1H, *J* = 2.2 Hz, H-8), 4.71 (1H, br s, H-2), 4.08 (1H, m, H-3), 2.76 (1H, dd, *J* = 16.8, 4.5 Hz, H-4α), 2.63 (dd, *J* = 16.8, 2.7 Hz, H-4β); ^{13}C NMR (100 MHz, CD_3OD); δ_H 158.0 (C-5), 157.7 (C-7), 157.4 (C-9), 146.0 (C-3′), 145.8 (C-4′), 132.3 (C-1′), 119.5 (C-2′), 116.0 C-5′), 115.4 (C-6), 100.2 (C-10), 96.6 (C-6), 96.0 (C-8), 79.0 (C-2), 67.5 (C-3) 29.3 (C-4).). Compound **14** was characterized as catechin by comparison of the physical and spectral data with the literature [21].

Ampelopsisionoside (15): white powder; ^{1}H NMR (400 MHz, CD_3OD); δ_H 5.93 (1H, dd, *J* =15.8, 6.4 Hz, H-8), 5.75 (1H, d, *J* =15.8 Hz, H-7), 4.46 (1H, q, *J* = 6.4 Hz, H-9), 4.39 (1H, d, *J* = 7.8 Hz, H-1′), 3.87 (1H, dd, J = 11.7, 2.3 Hz, H-6′), 3.66 (1H, dd, *J* = 11.7, 5.4 Hz, H-6″), 3.36 (1H, m, H-3′), 3.31 (1H, m, H-4′), 3.25 (1H, dd, *J* = 5.5, 2.3 Hz, H-5′), 3.20 (1H, m, H-2′), 2.89 (1H, d, *J* = 13.5 Hz, H-2_{ax}), 2.46 (1H, t, *J* = 13.6 Hz, H-4_{ax}) 2.29 (1H, m, H-5), 2.14 (1H, ddd, *J* = 13.6, 4.4, 2.1 Hz, H-4_{eq}), 1.84 (1H, d, *J* = 13.5, 2.1 Hz, H-2_{eq}), 1.34 (3H, d, *J* = 6.4 Hz, H-10), 1.00 (3H, s, H-11), 0.94 (3H, s, H-12), 0.91 (3H, d, *J* = 6.6 Hz, H-13); ^{13}C-NMR (100 MHz, CD_3OD); δ_C 214.9 (C-3), 134.9 (C-8), 134.0 (C-7), 102.6 (C-1′), 78.2 (C-3′), 78.1 (C-6), 78.0 (C-5′), 77.8 (C-9), 75.4 (C-2′), 71.6 (C-4′), 62.8 (C-6′), 52.5 (C-2), 46.2 (C-4), 44.0 (C-1), 37.8 (C-5), 25.4 (C-11), 25.1 (C-12), 21.5 (C-10), 16.5 (C-13). Compound **15** was characterized as ampelopsisionoside by comparison of the physical and spectral data with the literature [22].

The *in vitro* cytotoxicity of 15 isolated compounds and 7 analogues of melodorinol were evaluated against KB (Human epidermoid carcinoma), HeLa (Human cervix adenocarcinoma), MCF-7 (Human breast adenocarcinoma) and HepG-2 (Human hepatocellular carcinoma). Cytotoxic evaluation of isolated compounds was showed in table 3.3.

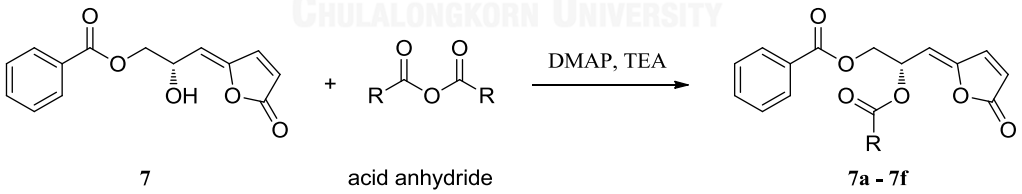
3.2 Bioassay activity of isolated compounds

Table 3.3 *In vitro* cytotoxicity of isolated compounds

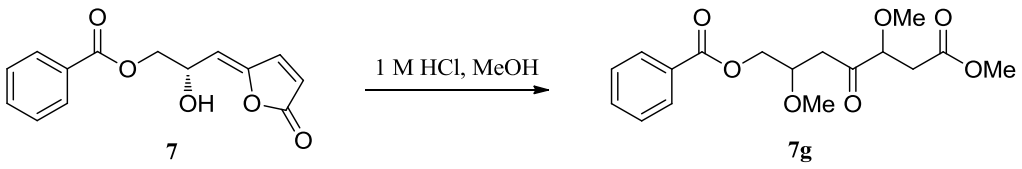
Compound	IC_{50} (µg/mL)			
	KB	HeLaS3	MCF-7	HepG-2
A mixture of β-Sitosterol (**1**) and Stigmasterol (**2**)	NT[a]	NT[a]	NT[a]	NT[a]
Polycarpol (**3**)	28.70	> 100	8.34	6.10
Acetylmelodorinol (**4**)	0.66	0.66	1.26	4.92
Chamanetin (**5**)	0.86	12.70	13.60	7.78
Chrysin (**6**)	6.85	8.61	8.27	7.35
Melodorinol (**7**)	3.71	2.60	1.93	4.21
(4*Z*)-6-Benzoyloxy-7-hydroxy-2,4-heptadien-4-olide (**8**)	5.41	11.10	3.79	9.93
Pinocembrin (**9**)	38.90	71.60	65.20	> 100
Isochamanetin (**10**)	5.67	28.30	7.06	11.80
Dichamanetin (**11**)	5.22	25.70	20.20	15.40
Lanosta-7,9(11),24-trien-3β,21-diol (**12**)	22.20	58.20	23.20	22.70
(4Z)-6,7-Dihydroxy-2,4-heptadien-4-olide (**13**)	65.70	82.80	52.10	49.60
Catechin (**14**)	NT[a]	NT[a]	NT[a]	NT[a]
Ampelopsisionoside (**15**)	> 100	> 100	> 100	> 100
Doxorubicin (standard)	0.13	0.05	0.10	0.31

[a]Not test

The isolated heptenes were **4**, **7**, **8** and **13**. Among them, compound **4** revealed the most cytotoxicity against KB, HeLa and MCF-7 cell lines with IC_{50} values of 0.655, 0.655 and 1.26 µg/mL, respectively (table 3.1). Heptene **13**, which lacked benzoyl moiety at position 7, showed inactive activities against all four cell lines with IC_{50} values of 65.7, 82.8, 52.1 and 49.1 µg/mL, respectively. It is noteworthy that benzoyl moiety seem to play a pivotal role in antiproliferative activity of cell lines. Compound **4** was naturally acetylated at position 6 of compound **7** shown significantly increasing cytotoxicity values of KB and HeLa cell lines. Based on these results, acylation of compound **7** seem to be involved in antiproliferative activity of KB and HeLa cell lines. The isolated flavonoid compounds were **5**, **6**, **9**, **10** and **11**. Compound **5** showed selective cytotoxicity against KB cell with IC_{50} value of 0.86 µg/mL and showed moderate activity cytotoxicity against HeLa, MCF-7 and HepG-2 with IC_{50} value of 12.70, 13.60 and 7.78 µg/mL, respectively. Compound **6** showed moderate cytotoxicity, while compound **9** revealed no activity cytotoxicity against all four cell lines. Compounds **10** and **11** showed weak to no cytotoxicity. Triterpenoid **3** presented low and no effect to KB and HeLa with IC_{50} value of 28.70 and more than 100 µg/mL but showed moderate activities against MCF-7 and HepG-2 with IC_{50} value of 8.34 and 6.10 µg/mL. Triterpenoid **12** revealed weak to no cytotoxicity against all cell lines. Glycoside **15** was inactive.



Scheme 3.1 Nucleophilic acyl substitution reaction of melodorinol (**7**)



Scheme 3.2 Methylation reaction of melodorinol (**7**)

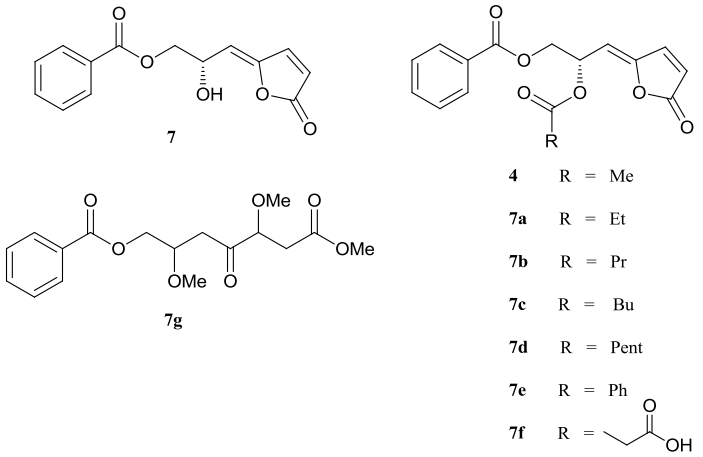


Figure 3.5 Acetylmelodorinol (**4**), melodorinol (**7**) and melodorinol derivatives (**7a**-**7g**)

Table 3.4 *In vitro* cytotoxicity of melodorinol, acetylmelodorinol and melodorinol derivatives

Compound	IC_{50} (μg/mL)			
	KB	HeLaS3	MCF-7	HepG-2
Melodorinol (**7**)	3.71	2.60	1.93	4.21
Acetylmelodorinol (**4**)	0.66	0.66	1.26	4.92
Propanoylmelodorinol (**7a**)	0.92	1.01	0.90	4.26
Butanoylmelodorinol (**7b**)	0.64	0.75	0.78	3.57
Pentanoylmelodorinol (**7c**)	1.07	1.77	2.82	4.64
Hexanoylmelodorinol (**7d**)	2.30	3.03	3.86	8.42
Benzoylmelodorinol (**7e**)	1.46	1.32	1.64	4.98
Succinoylmelodorinol (**7f**)	18.70	33.50	37.10	39.20
3,6-dimethoxy-2,5-dihydro-melodienone (**7g**)	49.20	46.10	31.40	39.20
Doxorubicin (standard)	0.13	0.05	0.10	0.31

According to significant increasing of cytotoxicity against KB and HeLa cells of natural acetylation of acetylmelodorinol (**4**) led to structural modification and cytotoxicity evaluation of melodorinol derivatives (**7a**-**7g**). The melodorinol (**7**) was

selected for further derivatization. The nucleophilic acylation of OH group at position 6 of compound **7** with vary alkyl (**7a**-**7d** and **7f**) and phenyl (**7e**) side chains of acyl group led to the isolation of six acyl melodorinol derivatives (**7a**-**7f**). In addition, compound **7g** was prepared from methylation of compound **7**. Table 3.4 showed *in vitro* cytotoxicity of melodorinol, acetylmelodorinol and melodorinol derivatives against KB, HeLa, MCF-7 and HepG-2. Compounds **7a**-**7b** presented lower IC_{50} of KB, HeLa and MCF-7 cells than compound **7**. It seems that enhancing of hydrophobicity (**7a**-**7b**) led to increasing of antiproliferative activity of KB, HeLa and MCF-7 cells nevertheless more length side chain of acyl group (**7c**-**7d)** did not show significant different in antiproliferative activity. In contrast, acylation of compound **7** with hydrophilic moiety (**7f**) evidently showed low to inactive against four cell lines. While compound **7g**, which was broken lactone ring of heptene skeleton, also revealed inactive against all cell lines. It is noteworthy that lactone ring and hydrophobic moiety seem to be involved in inhibition of proliferative activity rather than length of side chain of hydrophobic acyl moiety. Among melodorinol derivatives, the analogue **7b** exhibited the most active compound against KB, HeLa, MCF-7 and HepG-2 with IC_{50} values of 0.64, 0.75, 0.781 and 3.57 µg/mL, respectively.

.

CHAPTER IV

Conclusion

In conclusion, Compounds **1**-**15** were isolated and purified form CH_2Cl_2 and MeOH crude extracts of *M. fruticosum* Lour. by various chromatographic techniques such as vacuum liquid chromatography, column chromatography as well as centrifugal chromatography. The isolated compounds consisted of four heptenes, acetylmelodorinol (**4**), melodorinol (**7**), (4*Z*)-6-benzoyloxy-7-hydroxy-2,4-heptadien-4-olide (**8**) and (4Z)-6,7-dihydroxy-2,4-heptadien-4-olide (**13**), six flavonoids, chamanetin (**5**), chrysin (**6**), pinocembrin (**9**), isochamanetin (**10**), dichamanetin (**11**), and catechin (**15**), four terpenoids, a mixture of β-sitosterol (**1**) and stigmasterol (**2**), polycarpol (**3**) and lanosta-7,9(11),24-trien-3β,21-diol (**12**), one glycoside, ampelopsisionoside (**15**) figure 4.1. In addition, melodorinol (**7**) was modified to give seven analogues, propanoyl-melodorinol (**7a**), butanoylmelodorinol (**7b**), pentanoylmelodorinol (**7c**), hexanoyl-melodorinol (**7d**), benzoylmelodorinol (**7e**), succinoylmelodorinol (**7f**) and 3,6-dimethoxy-2,5-dihydro-melodienone (**7g**) figure 4.2. The structural elucidations of all isolated and modified compounds were characterized by means of spectroscopic data as well as comparison with previous literature data. Moreover, isolated and modified compounds were tested for cytotoxicity on KB, HeLa, MCF-7 and HepG-2 cell lines.

Chamanetin (**5**) showed good selectivity on cytotoxicity against only KB cell with IC_{50} value of 0.86 µg/mL, while acetylmelodorinol (**4**) showed the highest cytotoxicity against both KB and HeLa with equal IC_{50} values of 0.66 µg/mL. The triterpenoids displayed moderate to inactive cytotoxicity against all cell lines. Based on their cytotoxicity results, heptenes presented the lowest cytotoxicity values against all of four cell lines. The derivative **7b** presented the most active compound against KB, HeLa, MCF-7 and HepG-2 with IC_{50} values of 0.64, 0.75, 0.78 and 3.57 µg/mL, respectively. Preliminary structure activity relationships analysis of functional group on cytotoxicity effects were benzoyl, lactone ring and hydrophobic ester groups in heptene core scaffolds.

In this investigation presented natural products which were extracted from the roots of *Melodorum fruticosum* Lour. Moreover, modification of some natural compounds led to enhancing activity. Therefore, in the future work may involve diverse functional group modifications for increasing biological activity that could be developed into new drugs.

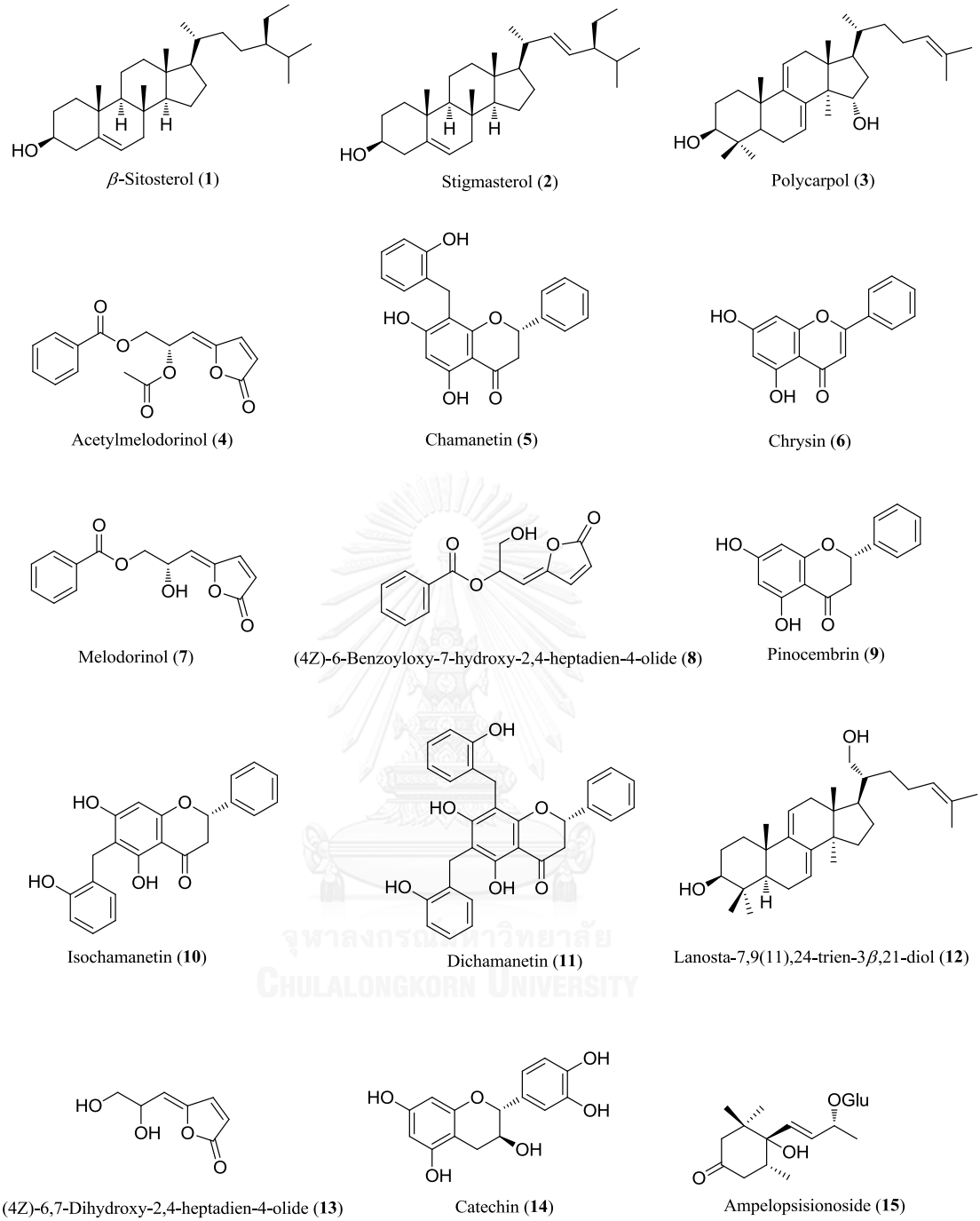
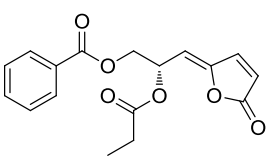
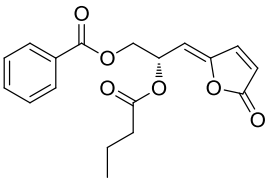


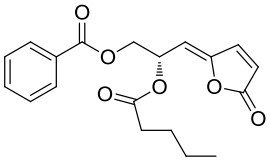
Figure 4.1 All of the isolated compounds (**1**-**15**) from the CH_2Cl_2 and MeOH crude extracts of *M. fruticosum* Lour. roots



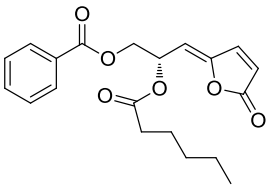
Propionylmelodorinol (**7a**)



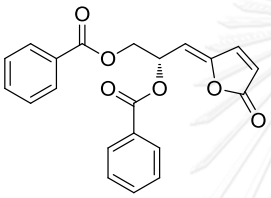
Butyrylmelodorinol (**7b**)



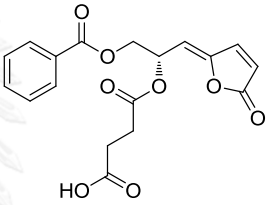
Pentanoylmelodorinol (**7c**)



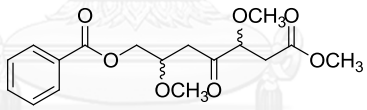
Hexanoylmelodorinol (**7d**)



Benzoylmelodorinol (**7e**)



Succinoylmelodorinol (**7f**)



3,6-Dimethoxy-2,5-dihydromelodienone
(7-Benzoyloxy-1,3,6-trimethoxy-heptan-1,4-dione)
(**7g**)

Figure 4.2 All melodorinol analogues (**7a**-**7g**)

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APPENDIX

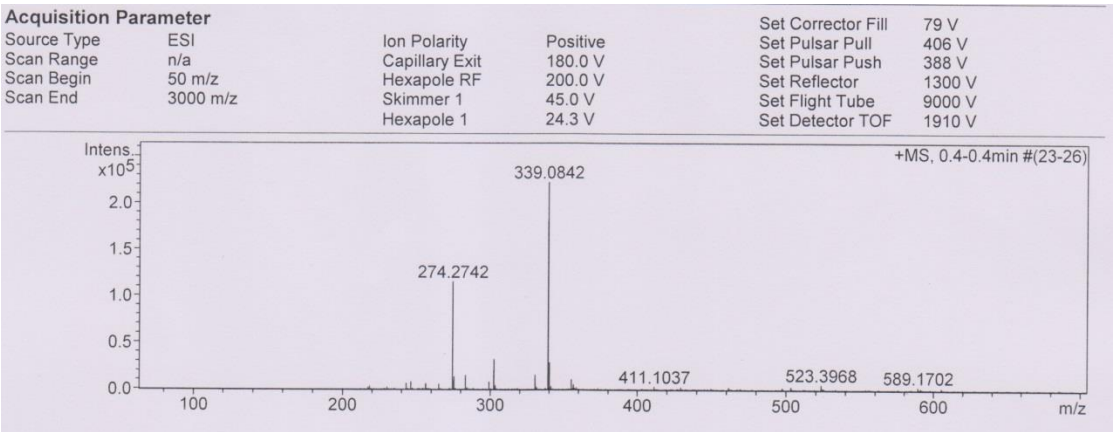

Figure 1 High resolution mass spectrum of Propanoylmelodorinol (**7a**)

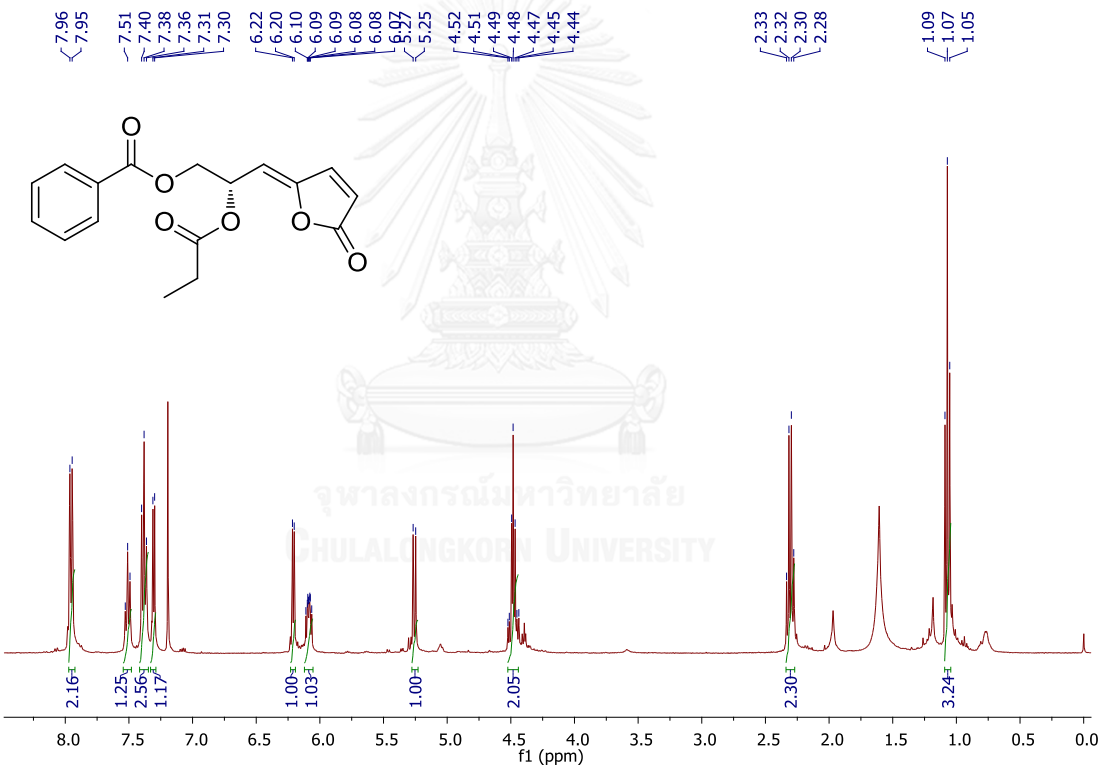

Figure 2 ^{1}H NMR spectrum ($CDCl_3$) of Propanoylmelodorinol (**7a**)

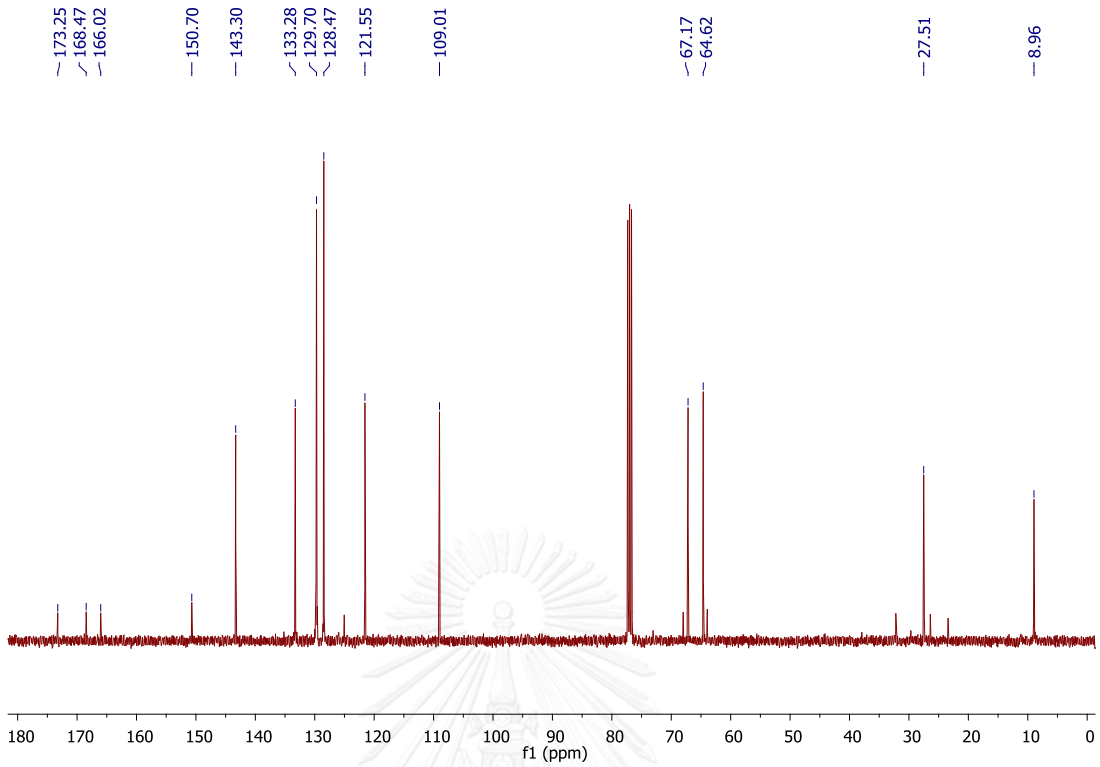

Figure 3 ^{13}C NMR spectrum ($CDCl_3$) of Propanoylmelodorinol (**7a**)

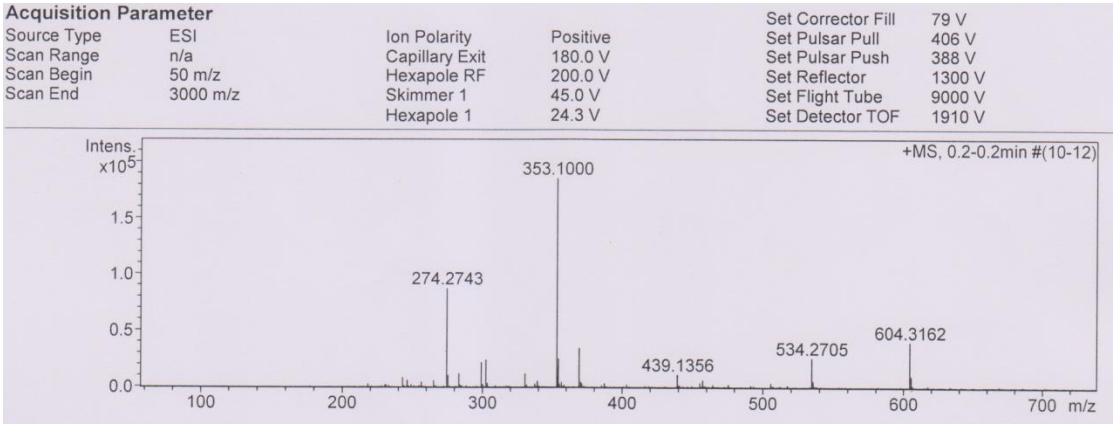

Figure 4 High resolution mass spectrum of Butanoylmelodorinol (**7b**)

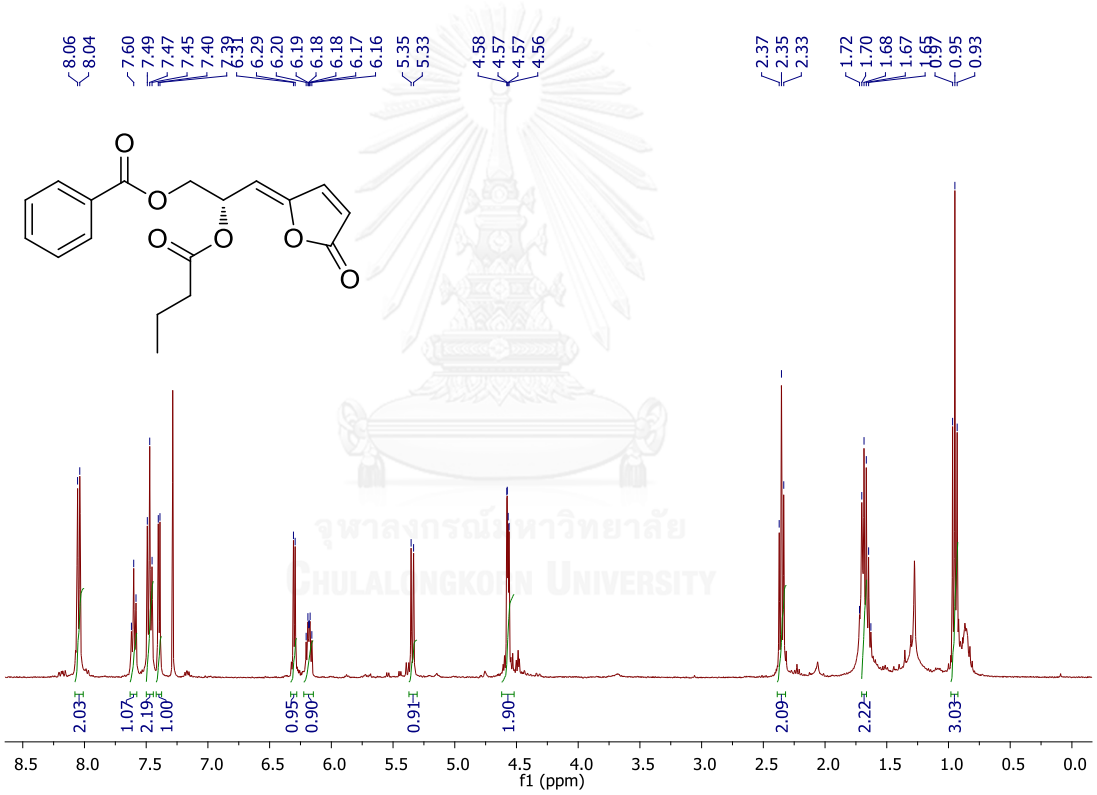

Figure 5 ^{1}H NMR spectrum ($CDCl_3$) of Butanoylmelodorinol (**7b**)

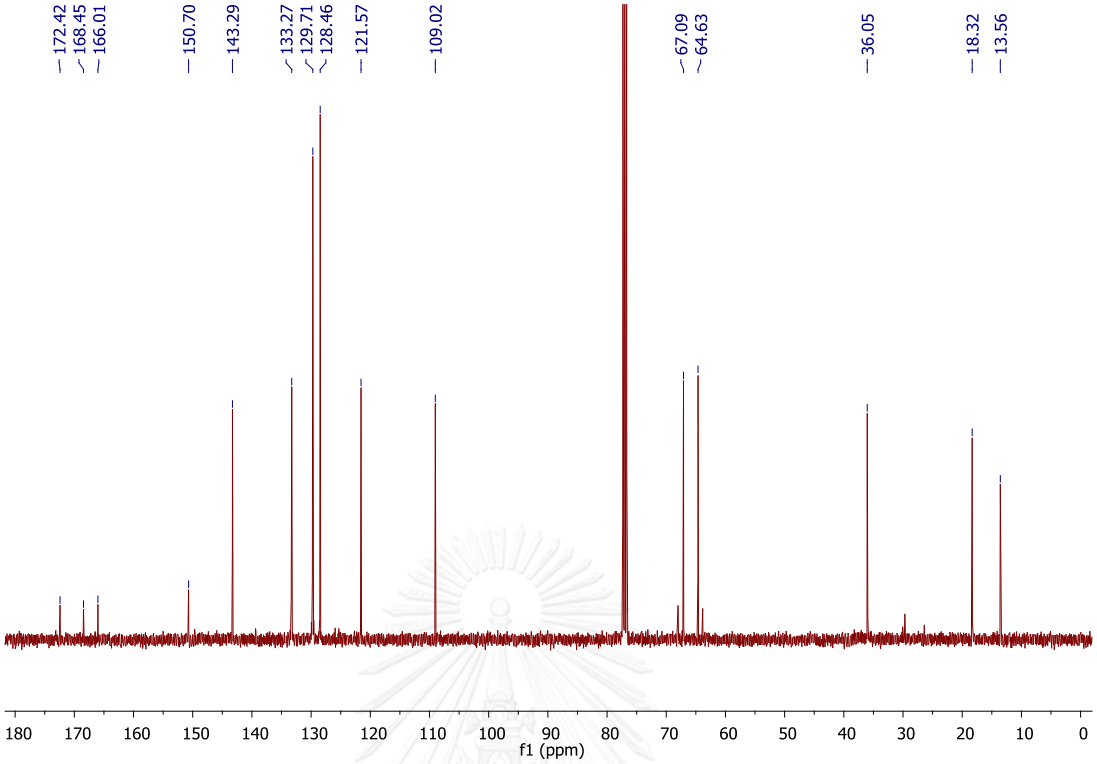

Figure 6 ^{13}C NMR spectrum ($CDCl_3$) of Butanoylmelodorinol (**7b**)

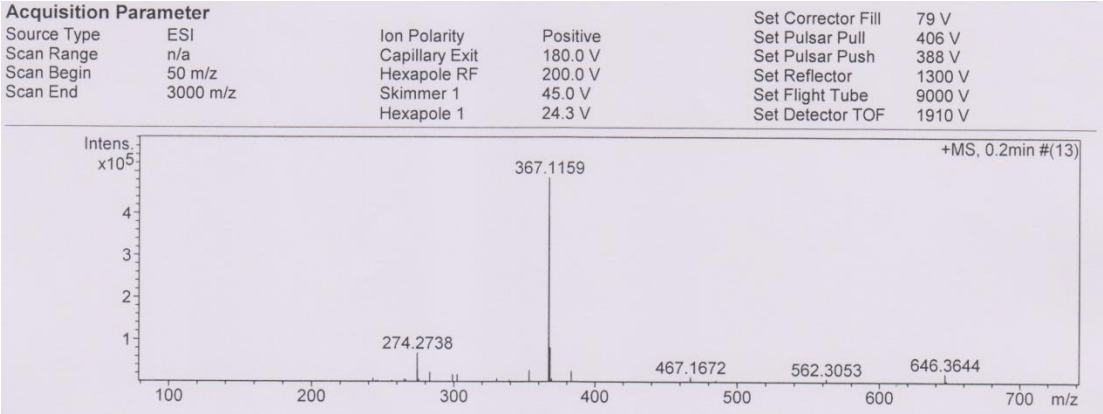

Figure 7 High resolution mass spectrum of Pentanoylmelodorinol (**7c**)

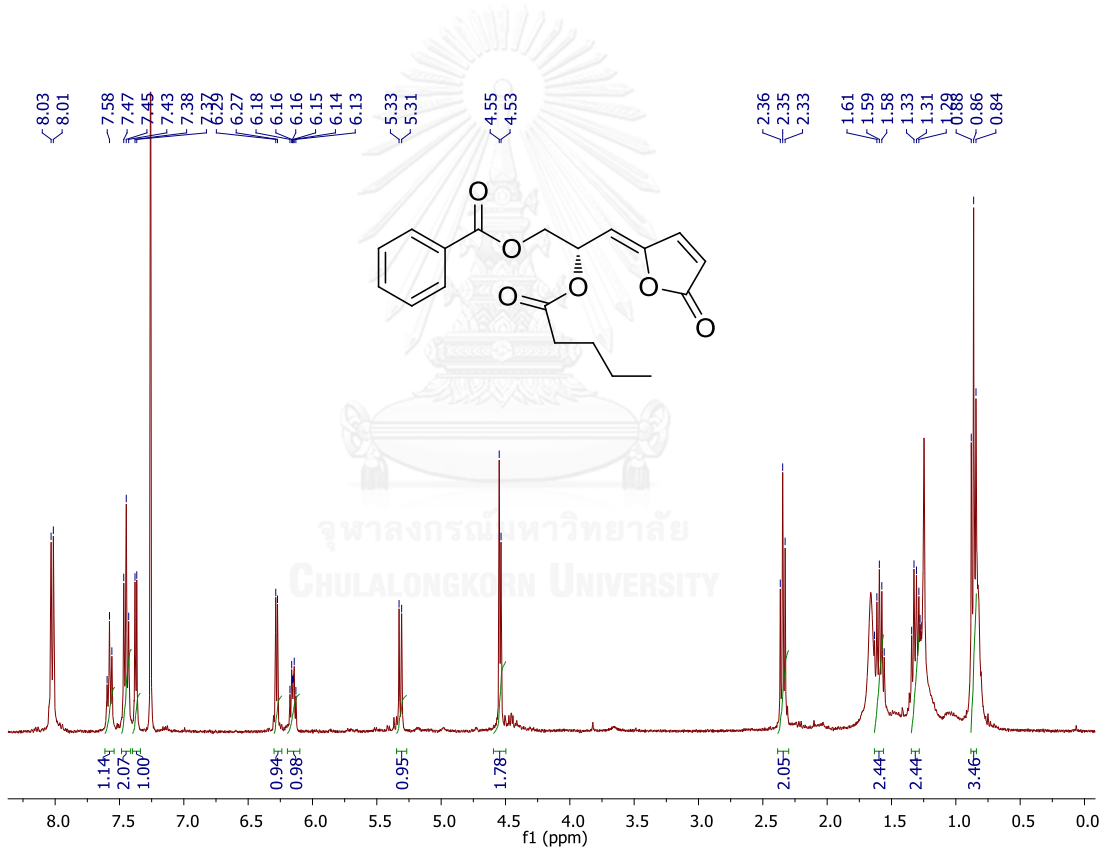

Figure 8 ^{1}H NMR spectrum ($CDCl_3$) of Pentanoylmelodorinol (**7c**)

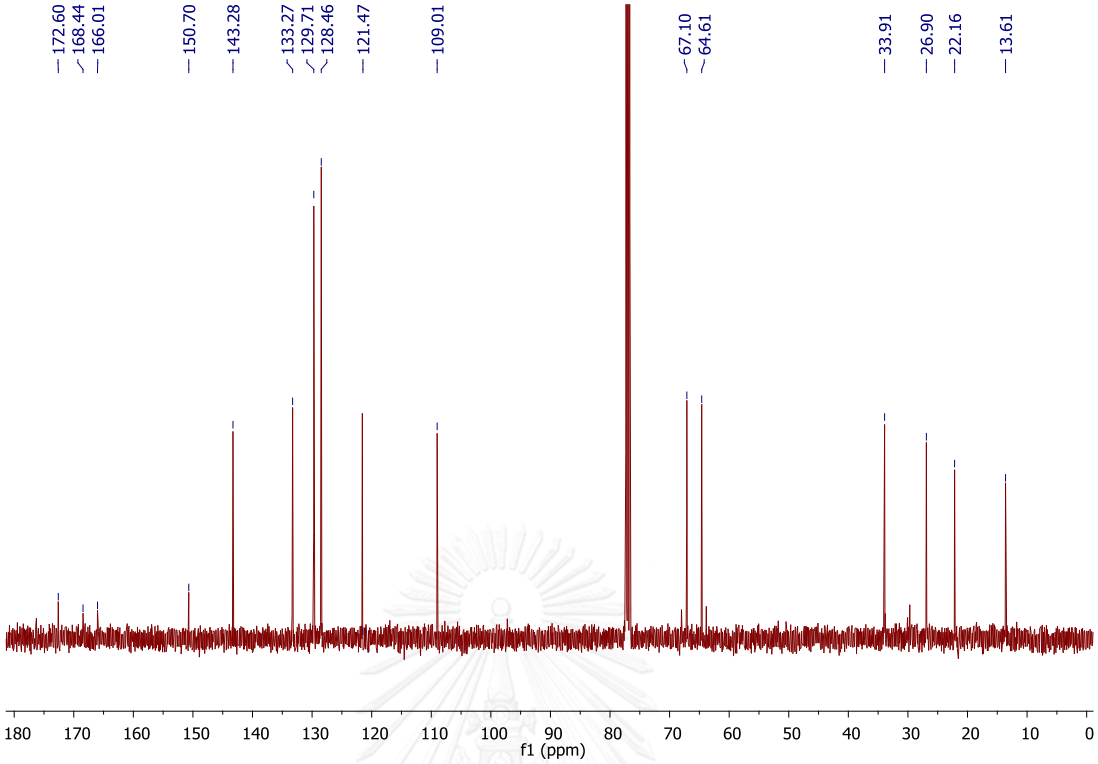

Figure 9 ^{13}C NMR spectrum ($CDCl_3$) of Pentanoylmelodorinol (**7c**)

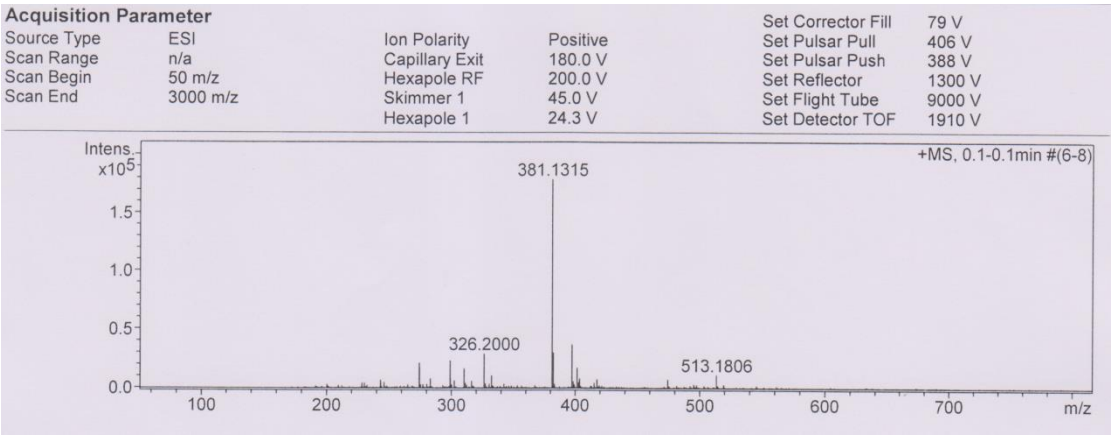

Figure 10 High resolution mass spectrum of Hexanoylmelodorinol (**7d)**

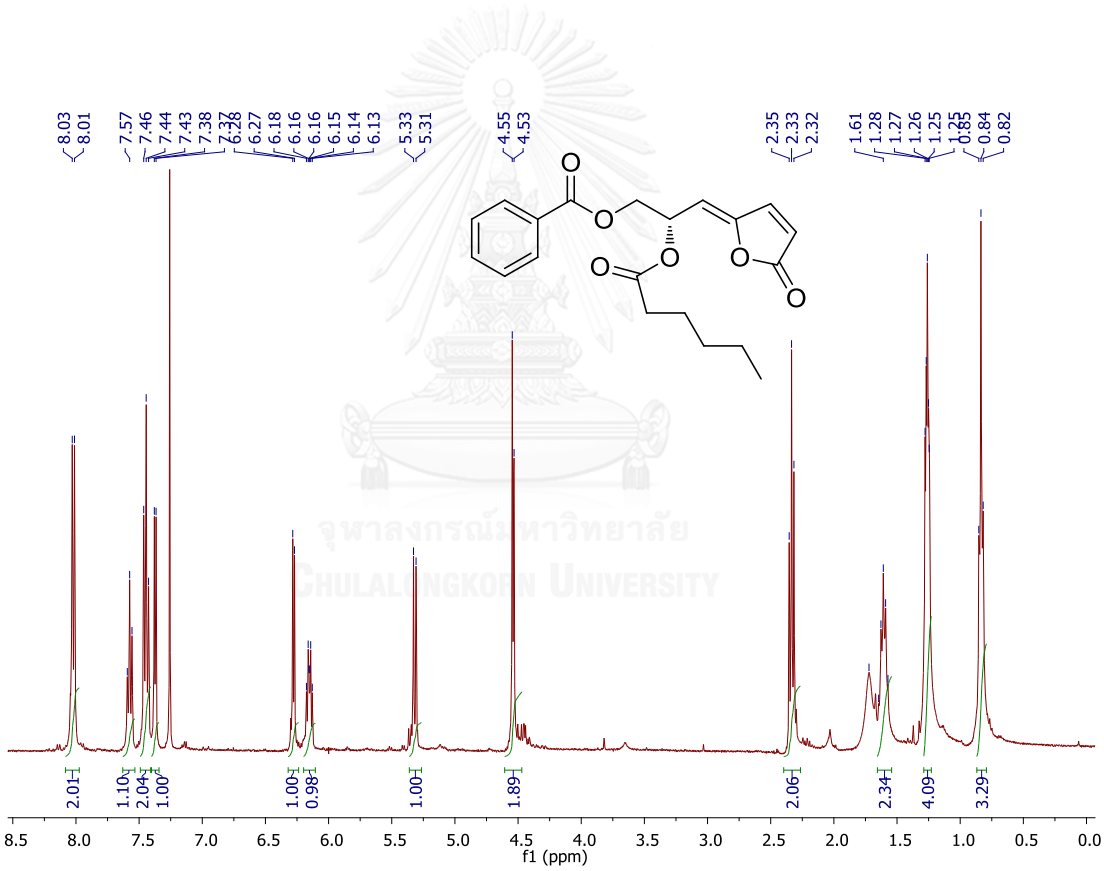

Figure 11 ^{1}H NMR spectrum ($CDCl_3$) of Hexanoylmelodorinol (**7d**)

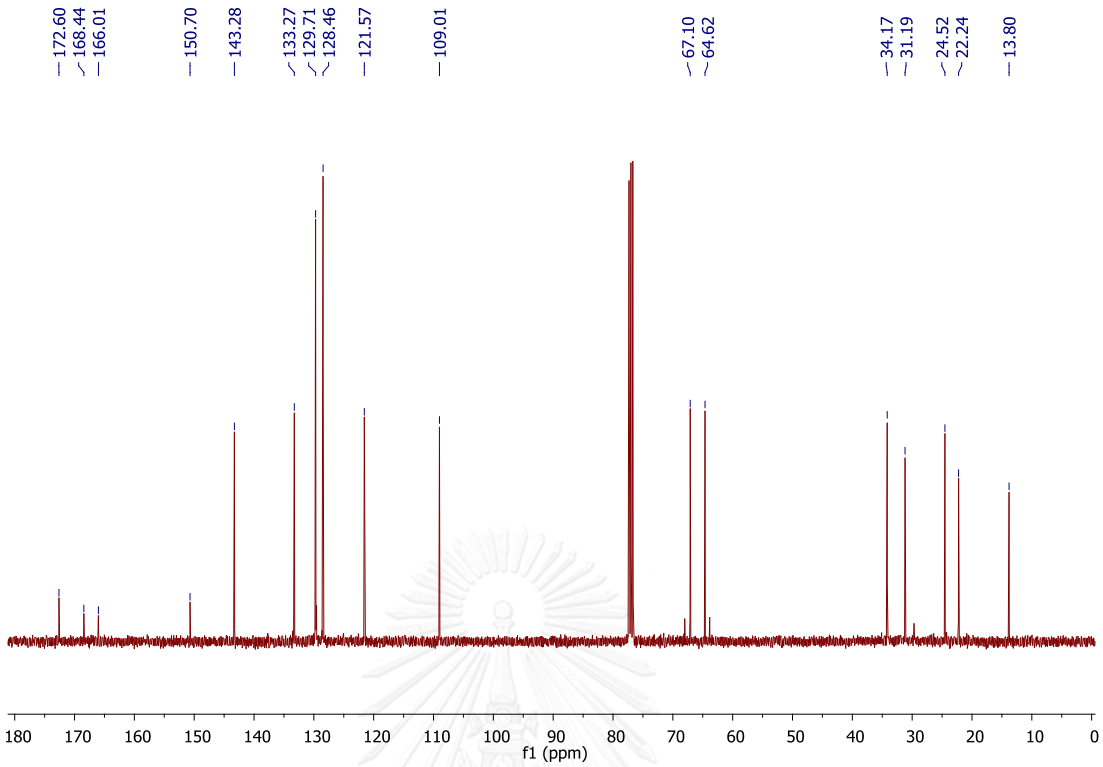

Figure 12 ^{13}C NMR spectrum ($CDCl_3$) of Hexanoylmelodorinol (**7d**)

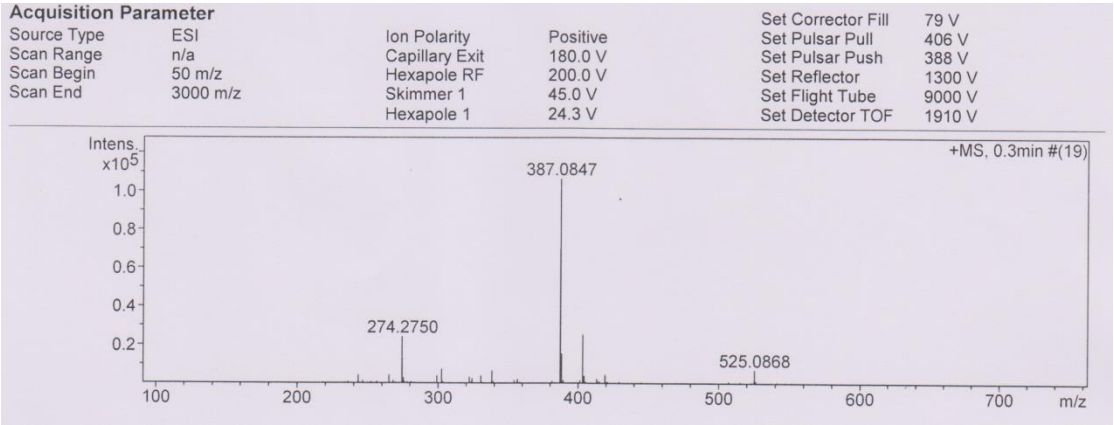

Figure 13 High resolution mass spectrum of Benzoylmelodorinol (**7e**)

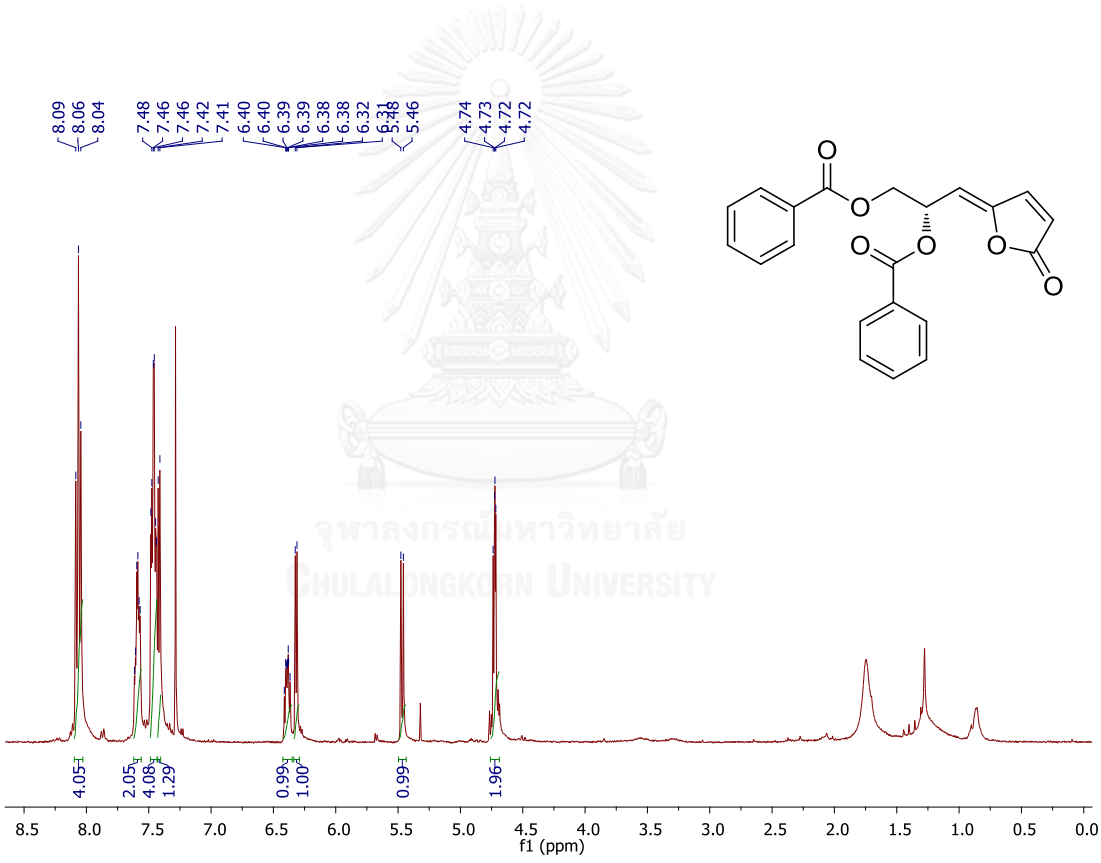

Figure 14 ^{1}H NMR spectrum ($CDCl_3$) of Benzoylmelodorinol (**7e**)

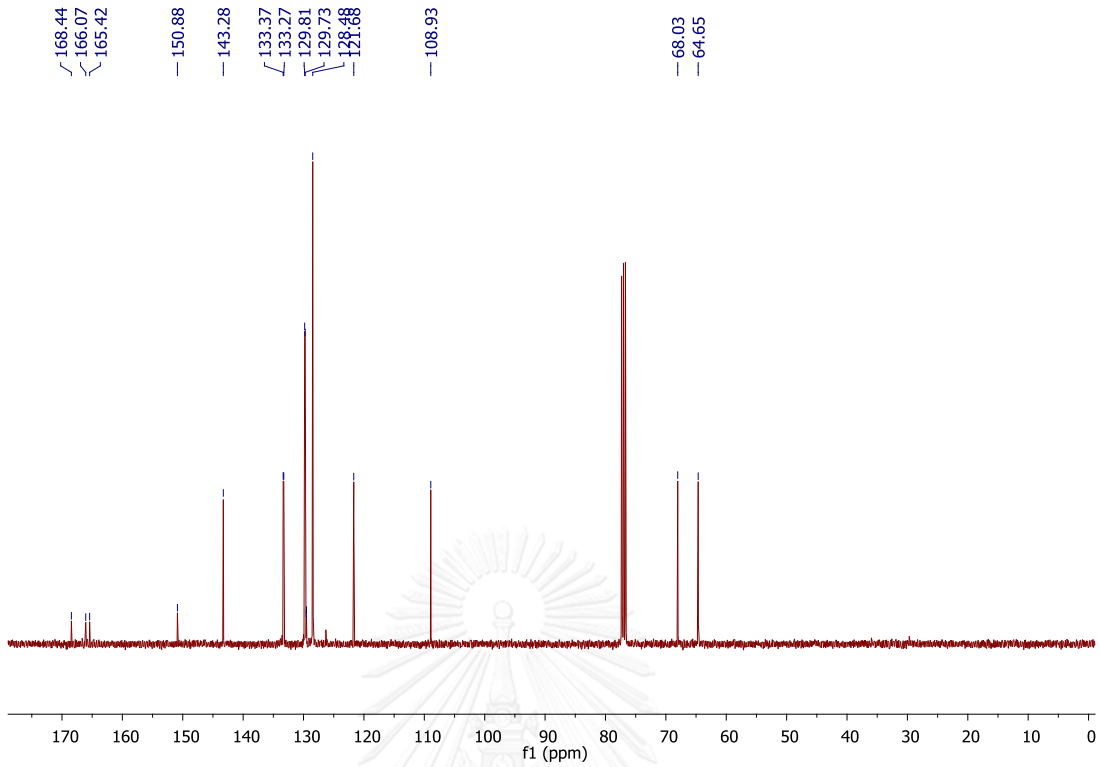

Figure 15 ^{13}C NMR spectrum ($CDCl_3$) of of Benzoylmelodorinol (**7e**)

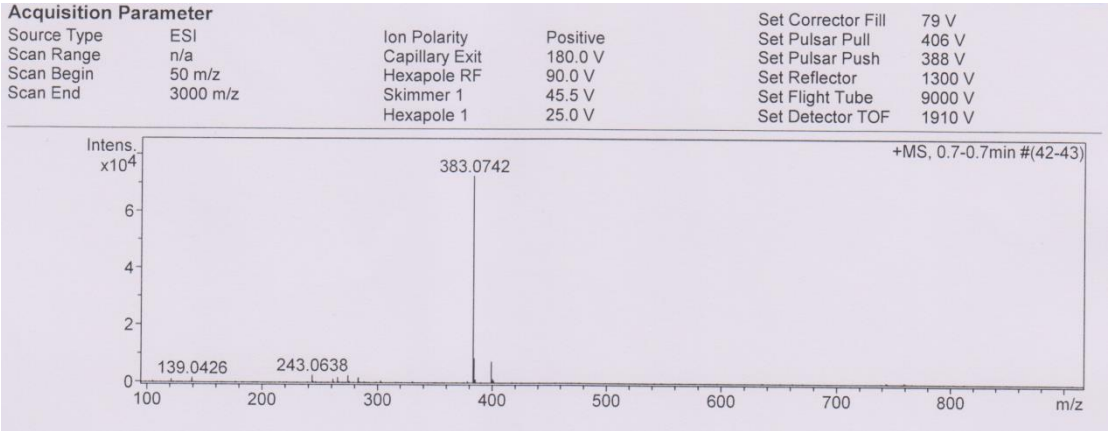

Figure 16 High resolution mass spectrum of Succinoylmelodorinol (**7f**)

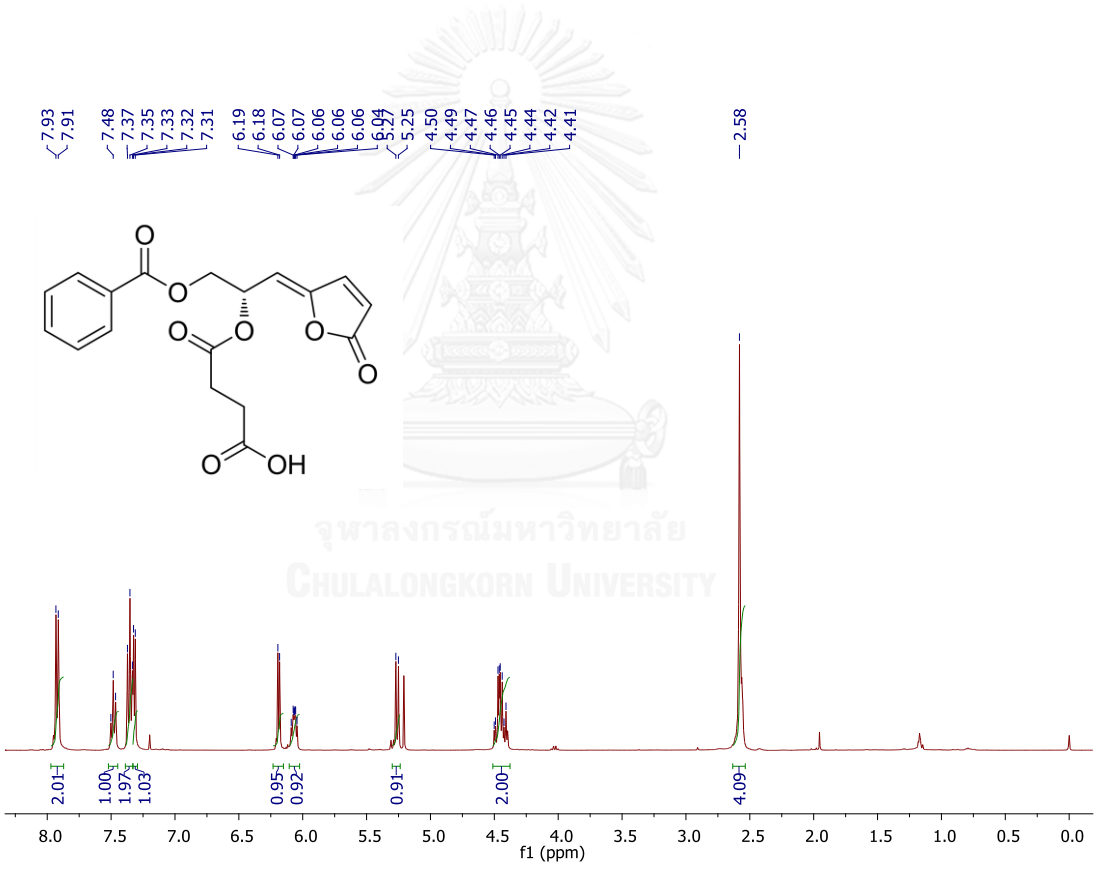

Figure 17 ^{1}H NMR spectrum ($CDCl_3$) of Succinoylmelodorinol (**7f**)

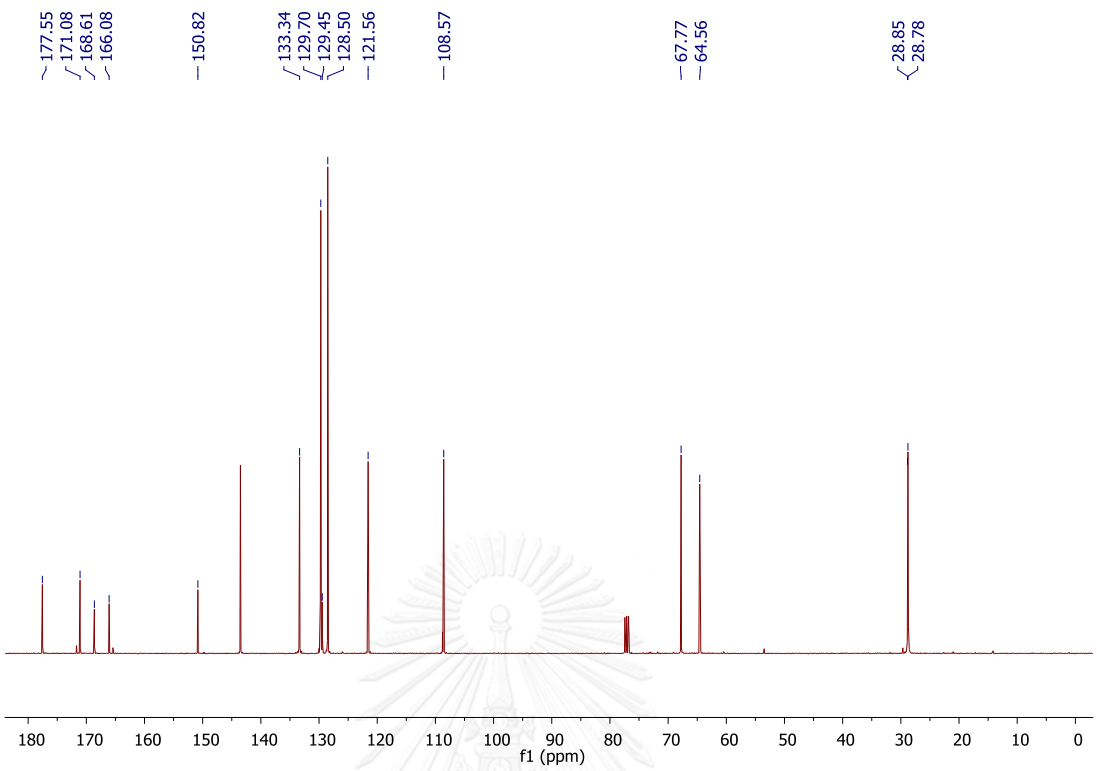

Figure 18 ^{13}C NMR spectrum ($CDCl_3$) of Succinoylmelodorinol (**7f**)

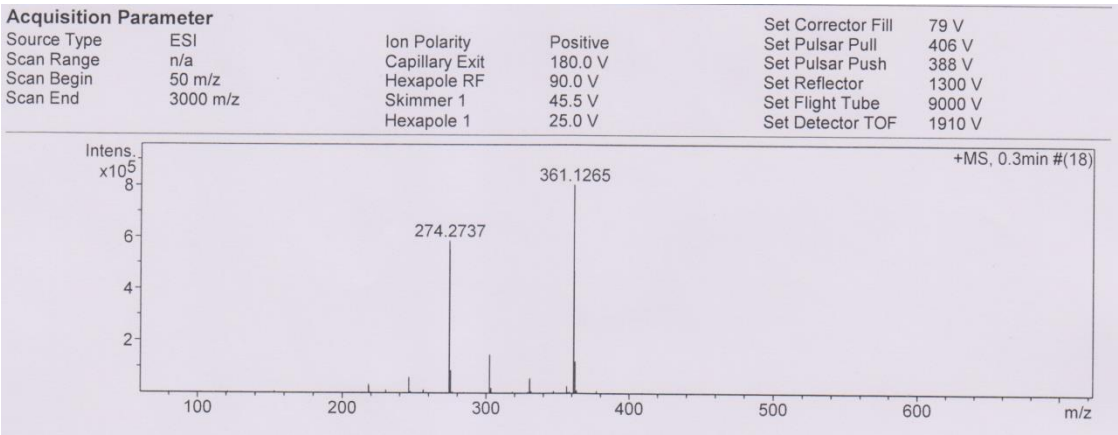

Figure 19 High resolution mass spectrum of 3,6-dimethoxy-2,5-dihydromelodienone (**7g**)

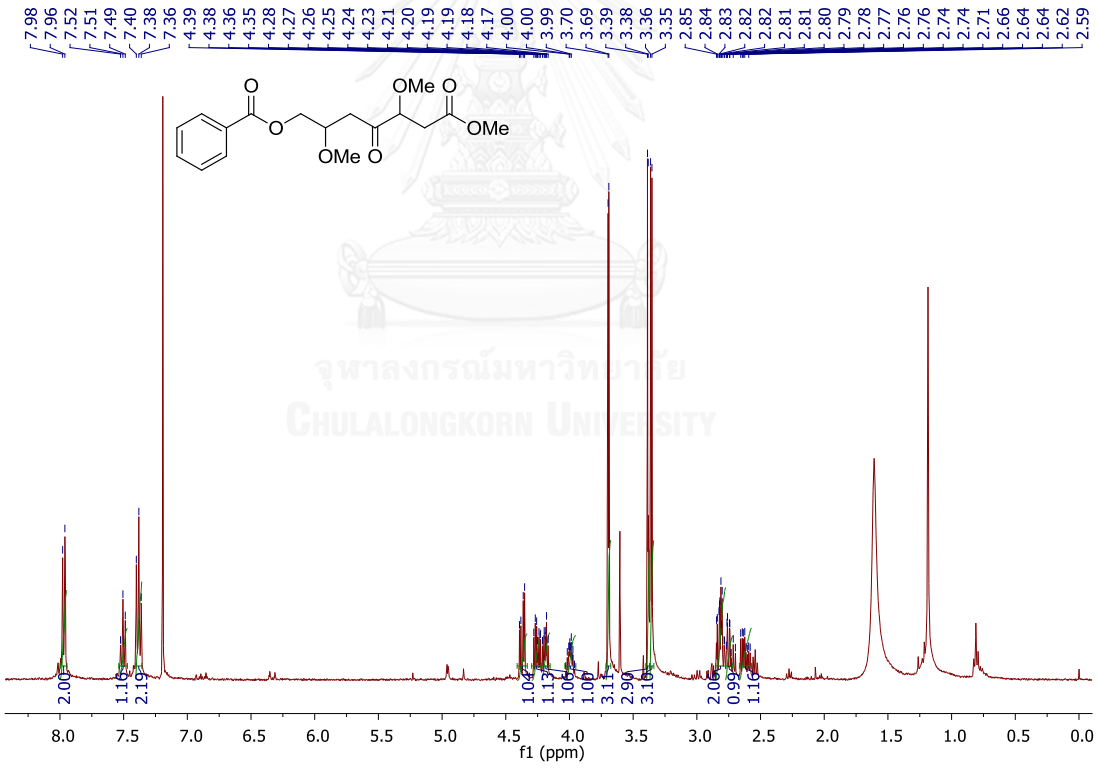

Figure 20 ^{1}H NMR spectrum ($CDCl_3$) of 3,6-dimethoxy-2,5-dihydromelodienone (**7g**)

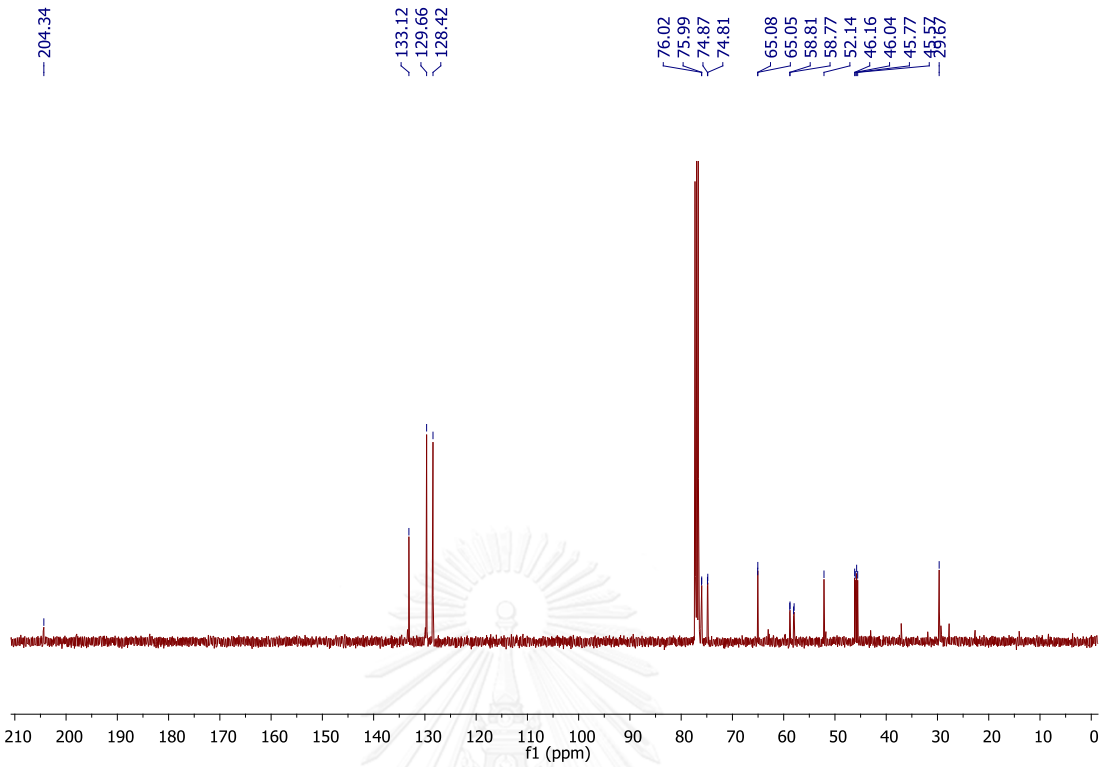

Figure 21 ^{13}C NMR spectrum ($CDCl_3$) of 3,6-dimethoxy-2,5-dihydromelodienone (**7g**)

VITA

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