องค์ประกอบทางเคมีและฤทธิ์ยับยั้งไวรัสเฮอร์ปีส์ซิมเพล็กซ์ของสมุยและส้มชื่น

นายชัยศักดิ์ จันศรีนิยม

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

CHEMICAL CONSTITUENTS AND ANTI-HERPES SIMPLEX VIRUS ACTIVITY OF *MICROMELUM HIRSUTUM* AND *GLYCOSMIS PARVA*

Mr. Chaisak Chansriniyom

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Pharmacognosy
Department of Pharmacognosy and Pharmaceutical Botany
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2009

Copyright of Chulalongkorn University

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Thesis Title	CHEMICAL CONSTITUENTS AND ANTI-HERPES SIMPLEX VIRUS ACTIVITY OF *MICROMELUM HIRSUTUM* AND *GLYCOSMIS PARVA*
By	Mr. Chaisak Chansriniyom
Field of Study	Pharmacognosy
Thesis Advisor	Associate Professor Nijsiri Ruangrungsi, Ph.D.
Thesis Co-Advisor	Professor Tsutomu Ishikawa, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

Pintip Pongpech ......Dean of the Faculty of Pharmaceutical Sciences
(Associate Professor Pintip Pongpech, Ph.D.)

THESIS COMMITTEE

Kittisak Likhit ......Chairman
(Associate Professor Kittisak Likhitwitayawuid, Ph.D.)

Nijsiri Ruangrungsi ......Thesis Advisor
(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

....Thesis Co-Advisor
(Professor Tsutomu Ishikawa, Ph.D.)

Vimolmas Lipipun ......Examiner
(Associate Professor Vimolmas Lipipun, Ph.D.)

Wandee Gritsanapan ......External Examiner
(Associate Professor Wandee Gritsanapan, Ph.D.)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

ชัยศักดิ์ จันศรีนิยม : องค์ประกอบทางเคมีและฤทธิ์ยับยั้งไวรัสเฮอร์ปีส์ซิมเพล็กซ์ของสมุยและส้มชื่น (CHEMICAL CONSTITUENTS AND ANTI-HERPES SIMPLEX VIRUS ACTIVITY OF *MICROMELUM HIRSUTUM* AND *GLYCOSMIS PARVA*) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. นิจศิริ เรืองรังษี, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : Professor Tsutomu Ishikawa, Ph.D., 279 หน้า.

การศึกษาองค์ประกอบทางเคมีของสมุยและส้มชื่น สามารถแยกสารในกลุ่มคูมารินได้ 3 ชนิด อะคริโดน อัลคาลอยด์ 6 ชนิด ลิโมนอยด์ 3 ชนิด และสารที่เป็นอนุพันธ์ของ *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide 5 ชนิด และ ควิโนโลน อัลคาลอยด์ 1ชนิด การพิสูจน์โครงสร้างของสารทั้งหมดที่แยกได้ อาศัยการวิเคราะห์เชิงสเปคโตสโคปีของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลกับสารที่ทราบโครงสร้างแล้ว พบสารที่แยกได้จากกิ่งของสมุยประกอบด้วยสารในกลุ่มควิโนโลน อัลคาลอยด์ที่พบครั้งแรกในธรรมชาติ 1 ชนิด คือ 1,2-dimethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **[262]** สารกลุ่มคูมารินที่เคยมีรายงานมาแล้ว 2 ชนิด คือ scopoletin **[41]** และ micromelin **[5]** ส่วนสารที่แยกได้จากใบของสมุยประกอบด้วยสารในกลุ่มคูมารินที่เคยมีรายงานมาแล้ว 2 ชนิด คือ micromelin **[5]** และ (–)-(2′*S*, 3′*R*)-3′-senecioyloxymarmesin **[263]** สารที่แยกได้จากกิ่งของส้มชื่นประกอบด้วยสารในกลุ่มอะคริโดน อัลคาลอยด์ชนิดใหม่ 1 ชนิด คือ glycospavarine (1,3,5-trihydroxy-2-methoxy-*N*-methyl-9-acridone) **[266]** สารในกลุ่มอะคริโดน อัลคาลอยที่เคยมีรายงานมาแล้วจำนวน 4 ชนิด คือ *N*-methylatalaphylline **[258]**, glycofolinine **[163]**, citramine **[264]** และ *N*-methylcyclo-atalaphylline-A **[265]** สารในกลุ่มลิโมนอยด์ 3 ชนิด คือ limonin **[267]** และ สารผสมของ limonexic acid **[268]** และ isolimonexic acid **[269]** ส่วนสารที่แยกได้จากใบของส้มชื่นประกอบด้วยสารในกลุ่ม *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives ที่เป็นสารชนิดใหม่จำนวน 3 ชนิด คือ (+)-*S*-deoxydihydroglyparvin **[270]**, (+)-*S*-deoxytetrahydroglyparvin **[271]** และ (+)-tetrahydroglyparvin **[272]** สารในกลุ่ม *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives ที่เคยมีรายงานมาแล้ว 2 ชนิด คือ glyparvin-A **[214]** and (+)-dihydroglyparvin **[213]** และสารในกลุ่มอะคริโดน อัลคาลอยด์ที่เคยมีรายงานมาแล้ว 1 ชนิด คือ arborinine **[87]** สารที่แยกได้ถูกนำไปทดสอบฤทธิ์ทางชีวภาพ คือ ฤทธิ์ยับยั้งไวรัสเฮอร์ปีส์ซิมเพล็กซ์ไทป์ 1 และไทป์ 2 พบว่า glycosparvarine **[266]**, glycofolinine **[163]** และ (+)-tetrahydroglyparvin **[272]** มีฤทธิ์ยับยั้งไวรัสเฮอร์ปีส์ซิมเพล็กซ์ไทป์ 1 และไทป์ 2 ในระดับปานกลางโดยที่มีค่าความเข้มข้นที่ยับยั้งไวรัสเฮอร์ปีส์ซิมเพล็กซ์ 50 เปอร์เซ็นต์ เท่ากับ 151, 348 และ 229 ไมโครโมลาร์ ตามลำดับ ในขณะที่ (+)-*S*-deoxydihydroglyparvin **[270]** มีค่าความเข้มข้นที่ยับยั้งไวรัสเฮอร์ปีส์ซิมเพล็กซ์ 50 เปอร์เซ็นต์ เท่ากับ 29.8 และ 44.6 ไมโครโมลาร์ ต่อไวรัสเฮอร์ปีส์ซิมเพล็กซ์ไทป์ 1 และ ไทป์ 2 ตามลำดับ

ภาควิชา เภสัชเวทและเภสัชพฤกษศาสตร์ ลายมือชื่อนิสิต ชัยศักดิ์ จันศรีนิยม

สาขาวิชา เภสัชเวท ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก

ปีการศึกษา 2552 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4876951233 : MAJOR PHARMACOGNOSY
KEYWORDS : COUMARINS / ACRIDONE ALKALOIDS / SULFUR-CONTAINING PROPANAMIDE DERIVATIVES / LIMONIODS / QUINOLONE ALKALOID / ANTI-HERPES SIMPLEX VIRUS ACTIVITY / *MICROMELUM HIRSUTUM / GLYCOSMIS PARVA*

CHAISAK CHANSRINIYOM : CHEMICAL CONSTITUENTS AND ANTI-HERPES SIMPLEX VIRUS ACTIVITY OF *MICROMELUM HIRSUTUM* AND *GLYCOSMIS PARVA*. THESIS ADVISOR : ASSOCIATE PROFESSOR NIJSIRI RUANGRUNGSI, Ph.D., THESIS CO-ADVISOR : PROFESSOR TSUTOMU ISHIKAWA, Ph.D., 279 pp.

Chemical investigation of *Micromelum hirsutum* Oliv. and *Glycosmis parva* Criab led to the isolation of three coumarins, six acridone alkaloids, three limoniods, five *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives and a 4-quinolone alkaloid. The structure determination of these compounds was accomplished by spectroscopic analyses (UV, IR, MS and NMR properties) and by comparison with previously reported data of known compounds. The branches of *Micromelum hirsutum* Oliv. provided a new natural quinolone alkaloid, namely 1,2-dimethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **[262]**, two known coumarins identified as scopoletin **[41]** and micromelin **[5]** whereas the leaves afforded two known coumarins, micromelin **[5]** and (–)-(2′*S*, 3′*R*)-3′-senecioyloxymarmesin **[263]**. The branches of *Glycosmis parva* Craib yielded a new acridone alkaloid, namely glycosparvarine (1,3,5-trihydroxy-2-methoxy-*N*-methyl-9-acridone) **[266]**, together with four known acridone alkaloids (*N*-methylatalaphylline **[258]**, glycofolinine **[163]**, citramine **[264]** and *N*-methylcyclo-atalaphylline-A **[265]**) and three limonoids (limonin **[267]** and a mixture of limonexic acid **[268]** and isolimonexic acid **[269]**). From the leaves of *Glycosmis parva* Craib, three new *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives, namely (+)-*S*-deoxydihydroglyparvin **[270]**, (+)-*S*-deoxytetrahydroglyparvin **[271]** and (+)-tetrahydroglyparvin **[272]**, two known *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives (glyparvin-A **[214]** and (+)-dihydroglyparvin **[213]**) and a known acridone alkaloid, arborinine, were obtained. The isolated compounds were evaluated for their antiviral activity against HSV-1 and HSV-2. Among them, glycosparvarine **[266]**, glycofolinine **[163]** and (+)-tetrahydroglyparvin **[272]** exhibited moderate activities against both HSV-1 and HSV-2 with EC$_{50}$ of 151 μM, 348 μM and 229 μM, respectively. (+)-*S*-deoxydihydroglyparvin **[270]** exhibited activities with lower EC$_{50}$ of 29.8 and 44.6 μM against HSV-1 and HSV-2, respectively.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Department : Pharmacognosy and Pharmaceutical Botany
Student's Signature Chaisak Chansriniyom
Field of Study : Pharmacognosy
Advisor's Signature
Academic Year : 2009
Co-Advisor's Signature

ACKNOWLEDGEMENTS

The author wishes to express his deepest gratitude to his thesis advisor, Associate Professor Dr. Nijsiri Ruangrungsi, for his guidance, suggestion, and support throughout the course of this study.

His gratitude is particularly extended to Professor Dr. Tsutomu Ishikawa and Associate Professor Dr. Takuya Kumamoto, Department of Medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University, Japan, for providing an opportunity for conduct of experiments, and their help on NMR measurements, discussion on structure elucidation, constructive suggestions and valuable critical comments. Without their help the completion of this work would not have been possible.

His appreciations are also extended to Associate Professor Dr. Vimolmas Lipipun, Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for her help with anti-herpes simplex virus activity tests, kindness and valuable suggestions on antiviral activity test.

His gratitude is sincerely grateful to the dissertation committee for their important and constructive suggestions and crucial review of his dissertation.

A large debt of his gratitude is owed to Dr. Mayuree Kanlayavattanakul, School of Cosmetic Science, Mae Fah Luang University, Thailand, Dr. Daisuke Watanabe, Mr. Yuh-suke Akagi, Mr. Kazuya Suzuki and all members in Department of Medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University, Japan, and his friends at the department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, who kindly offer their assistance, encouragement and helpful comments throughout his research.

For the financial support, he is grateful to the Thailand Research Fund under the Royal Golden Jubilee (RGJ) Ph.D. program 2004 (PHD/0212/2547).

For the provision of laboratory facilities, he is grateful to Department of Medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University, Japan and department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

The author’s final thank goes to his family. They provided him with every opportunity to succeed in life, encourage and support the author.

CONTENTS

Page

CHAPTER

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Page

Page

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Page

Page

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLES

LIST OF TABLES (Continued)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF SCHEMES

LIST OF ABBREVIATIONS

α = Alpha
$[\alpha]^{t}_{D}$ = Specific rotation at t°C and sodium D line (589 nm)
Acetone-d_6 = Deuterated acetone
ATR-IR = Attenuated Total Reflection Infrared Spectroscopy
β = Beta
br = Broad (for NMR spectra)
c = Concentration (Molar)
conc. = Concentration
$^{\circ}$C = Degree Celsius
CC = Column Chromatography
CC_{50} = 50% Cytotoxic Concentration
CD = Circular Dichroism
$CDCl_3$ = Deuterated chloroform
CH_2Cl_2 = Dichloromethane
calcd for = Calculated for
cm = Centimeter
cm^{-1} = Reciprocal centimeter (unit of wave number)
^{13}C-NMR = Carbon-13 Nuclear Magnetic Resonance
δ = Chemical shift
d = Doublet (for NMR spectra)
dd = Doublet of doublets (for NMR spectra)
ddd = Doublet of doublets of doublets (for NMR spectra)
dddd = Doublet of doublets of doublets of doublets (for NMR spectra)
DEPT = Distortionless Enhancement by Polarization Transfer
DMSO-d_6 = Deuterated dimethyl sulfoxide
D_2O = Deuterated water
dt = Doublet of triplets (for NMR spectra)
EC_{50} = 50% Effective Concentration
ED_{50} = 50% Effective Dose
EIMS = Electron Impact Mass Spectrometry
EtOAc = Ethylacetate
EtOH = Ethanol

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

FABMS = Fast Atom Bombardment Mass Spectrometry

γ = Gamma

g = Gram

h = Hour

^{1}H-NMR = Proton Nuclear Magnetic Resonance

^{1}H-^{1}H COSY = Homonuclear (Proton-Proton) Correlation Spectroscopy

HMBC = ^{1}H-detected Heteronuclear Multiple Bond Coherence

HMQC = ^{1}H-detected Heteronuclear Multiple Quantum Coherence

HRESIMS = High Resolution ElectroSpray Ionization Mass Spectrometry

HRFAB = High Resolution Fast Atom Bombardment Mass Spectrometry

HSV-1 = Herpes Simplex Virus Type 1

HSV-2 = Herpes Simplex Virus type 2

HPLC = High Pressure Liquid Chromatography

Hz = Hertz

IR = Infrared spectroscopy

J = Coupling constant

K = Kalvin (unit increment of temperature)

Kg = Kilogram

L = Liter

Lit. = Literature

λ_{max} = Wavelength at maximal absorption

m = Multiplet (for NMR spectra)

ε = Molar absorptivity

$[\Phi]$ = Molar ellipticity

μg = Microgram

μM = Micromolar

$[M]^{+}$ = Molecular ion

$[M+H]^{+}$ = Pseudo molecular ion

$[M+Na]^{+}$ = Pseudo molecular ion

$[M+K]^{+}$ = Pseudo molecular ion

MeOH = Methanol

MeCN = Acetonitrile

mg = Milligram

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

MHz = Megahertz

mm = Millimeter

mp = Melting point

MS = Mass Spectrometry

m/z = Mass to charge ratio

nm = Nanometer

NMR = Nuclear Magnetic Resonance

No. = Number

NOE = Nuclear Overhauser Effect

ν_{max} = Wave number of maximal absorption

ppm = Part-per-million

rel. int. = Relative intensity

s = Singlet (for NMR spectra)

Si-60 = Silica gel

spp. = Species

t = Triplet (for NMR spectra)

td = Triplet of doublets (for NMR spectra)

TLC = Thin Layer Chromatography

TMS = Tetramethylsilane

UV = Ultraviolet Spectroscopy

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER I

INTRODUCTION

The genus *Micromelum* belongs to Aurantioideae sub-family in Rutaceae family. The genus *Micromelum* is a member in the subtribes Micromelinae in the tribe Clauseneae, which is very restricted to tropical East Asia and Malesiana (Kong, *et al*., 1988). The botanical characteristics of plants in the genus of "*Micromelum*, Blume." in "the Flora of Malay Peninsula" (Hooker, 1875) were described as follows:

"Shrubs or small trees. Leaves imparipinnate. Leaflets alternate oblique, membranous, Flowers small white in corymbose cyme large and terminal. Calyx copular 3-to 5- toothed. Petals 5, valvate. Stamens 10 inserted round a disc; filaments linearsubulate, alternate ones shorter. Ovary 5-celled (rarely 2- to 6-celled); ovules 2 in each cell; style 1. Berry pulpy 1- or 2- seeded. Seed oblong. Species 4 to 5, Asia and Polynesia."

There are at least eighteen valid species of *Micromelum* in the Index Kewensis and its supplements according to (Varisara Vaisiriroj, 1980: 1-2), as listed below:

1. *Micromelum caudatum* Merr.
2. *M. ceylanicum* Wight
3. *M. compressum* Merr.
4. *M. coriaceum* Seem.
5. *M. curranii* Elmer
6. *M. diversifolium* Miq.
7. *M. falcatum* Tanaka
   [*M. glabrescens* Benth. (= *M. pubescens* Blume)]
   [*M. glabrescens* Villar (= *M. tephrocarpum* Turcz.)]
8. *M. glabrum* Guillamin
9. *M. globosum* Elmer ex Tanaka
10. *M. hirsutum* Oliver
11. *M. integerrimum* Wight & Arn.
    [*M. minutum* Seem., *M. minutum* Wight & Arn. and *M. minutum* (G. Forst) Wight & Arn. (= *M. pubescens* Blume)]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

12. *M. molle* Turcz.
13. *M. monophyllum* Wight
14. *M. octandrum* Turcz.
15. *M. pubescens* Blume
16. *M. scandens* Rechinger
17. *M. sorsogonense* Elmer ex Tanaka
18. *M. tephrocarpum* Turcz.

[*M. timoriense* Zipp. ex Span. (= *M. pubescens* Blume)]

The species of *Micromelum* in Thailand according to Smitinand (2001) are as follows:

Micromelum glanduliferum B. Hansen	ขี้ผึ้ง Khi phueng (Nakorn Ratchasima); ชมัด Chamat, ชมัดน้อย Chamat noi, สมัดน้อย Samat noi Loei).
M. hirsutum Oliv.	= M. minutum (G. Forst.) Wight & Arn.
M. integerrimum Roxb.	นมวัว Nom wua (Chiang Mai).
M. mimutum (G. Forst.) Wight & Arn.	กะม่วง Kamuang, สมุยช้าง Samui chang, หมุยช้าง Mui chang (Yala), กันโทร้ก Kan-throk (Khmer-Surin); กาจับลัก Ka chap lak, จี้ปุกตัวผู้ Chipuk tua phu, จี้ย้อย Chiyoi, มองคอง Mong khong, หญ้าสาบฮิ้น Ya sap hin (Northern); คอมขน Khom khon, สามโซก Sam sok (Chiang Mai), ฉี้ Chi, ลิ้นชี่ Linchi, สาบแร้งสาบกา Sap raeng sap ka (Chanthaburi); ชะมุย Chamui (Chumphon); ดอกสมัด Dok samat, สะแบก Sa baek (Ubon Ratchathani);

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

M. mimutum (G. Forst.) Wight & Arn.	เพี้ยฟานดง Phia fan dong, สมัดดง Samat dong, สมัดต้น Samat ton, สมัดใหญ่ Samat yai (Loei); มรุยช้าง Marui chang, ลำผีพ่าย Lam phi phai (Trang); มุยขาว Mui khao (Prachuap Khiri Khan); สมุย Samui (Surat Thani); หมอน้อย Mo noi (Uttaradit); หมุยขน Mui khon (Nakhon Si Thammarat); หวด Huat (Lampang); หัสคุณ Hatsa Khun (Saraburi).
M. pubescens Blume	= *M. minutum* (Forst.f.) Wight & Arn.

According to The Flora of Malay Peninsula (Hooker, 1875), the key distinguishing features between *M. hirsutum* Oliv. and *M. pubescens* Blume have been described as follows:

Leaflets ovate lanceolate, glabrescent; cymes pubescent…. 1. *M. pubescens*
Leaflets lanceolate, base very oblique tomentose; cymes very tomentose …. 2. *M. hirsutum*

The botanical features of *M. hirsutum* were described in The Flora of Malay Peninsula (Hooker, 1875), as the following quote.

"*M. hirsutum* Oliv. *Journ. Linn. Soc. V. Supp.* (2), 40; *Hook. fil. F. B. I.* i. 502; *King, l.c.* 219.

shrub or small tree tomentose. Leaves 6 to12 in. long; leaflets 9 to 25, lanceolate to oblong-lanceolate, base very oblique, edges obscurely serrate, tomentose beneath; nerves 5 to 10 pairs, 1.5 to 3.5 in. long, 0.8 to 1.5 in. wide; petioles up to 2 in. long. Cymes very tomentose, lax, 4 to 6 in. across or less. Flowers 25 in. across, white. Calyx deeply 5-lobed. Ovary very villous. Berry orange. *Hab*. Open country, less common than the last, Malaca, Mt. Ophir (Hullett). Perak, Gunong Keledang. Penang (Jack, Porter). Kelantan Kota Bharu (Ridley). *Distrib*. Indo-Malaya. *Native name*: Chemama."

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Although *M. hirsutum* is considered to be synonymous with *M. minutum* according to Smitinand (2001), these two species can be discriminated by the following dichotomous key (Humbert and Gagnepain, 1945).

A. Pétales abondamment velus en dehors.
  a. Pétales à poils hirsute; feuilles veloutées en dessous.…. 1. *M hirsutum*
  b. Pétales à poils couchés, très courts.
    α. Folioles très inégales à la base …. 2. *M. minutum*
    β. Folioles peu ou pas inégals à la base …. 3. *M. integerrimum*
B. Pétales peu ou pas velus en dehors; feuilles glabres ou seulement avec quelques poils sur la còte en dessous. …. 4. *M. falcatum*

The genus *Glycosmis* belongs to the tribe Clauseneae of Aurantioideae sub-family in Rutaceae family. There are forty-three accepted species of plants in the genus *Glycosmis* distributed in India, Malaysia, Thailand, Laos, Viet Nam, Burma, China, Nepal, Tibet, Bangladesh, Sri Lanka, Philippines, Indonesia and Taiwan (Stone, 1985).

According to Ridley and Hutchinson (1922) and Merrill (1986), the plants in the genus *Glycosmis* are evergreen shrubs or undertrees. Leaves alternate, 1-5 foliate. Flowers usually small, axillary panicles. Calyx 4 or 5 partial imbricate. Petals 4 or 5 imbricate. Stamen 8 to 10 free, filaments dilated below. Ovary 2 to 5 celled, the style very short, not jointed, ovule 1 in each cell. Fruits globose, freshy, berry. Seeds 1 to 3 oblong, testa membranous.

The species of *Glycosmis* in Thailand according to Smitinand (2001) are as follows:

Glycosmis chlorosperma (Blume) Spreng	น้ำข้าวเขา Nam khao khao (Nakorn Si Thammarat).
G. cochinchinensis (Lour.) Pierre	เขยโค Khoei kho (General).
G. dinhensis Pierre ex Guillaumin	เขยดิน Khoei din (Nakorn Ratchasima).
G. esquirolii (Lév.) Tanaka	เขยทาน Khoei tai long (General).
G. longipes (Craib) Tanaka	เขยตายลอง Khoei kho(General).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

G. macrophylla (Blume) Miq.	ประยงค์ใบใหญ่ Prayong bai yai (General).
G. ovoidea Pierre	ประยงค์ไข่ Prayong khai (General).
G. parkinsonii Tanaka	เขยแม่ลาน Khoei mae lan (General).
G. parva (Craib)	ประยงค์เกลื่อน Prayong kluean, สัมชื่น Som chuen (General).
G. pentaphylla (Retz.) DC.	กระรอกน้ำ Krarok nam, กระรอกน้ำข้าว Krarok nam khao (Chon Buri); กระโรกน้ำข้าว Krarok nam khao, เขยตาย Khoei tai, ลูกเขยตาย Luk Khoei tai (Central); เขนทะ Khen tha (Northern); ตาระแป Ta-ra-pae (Malay-Yala); น้ำข้าว Nam khao (Central, Peninsular); ประยงค์ใหญ่ Prayong yai (Bangkok); พุทธรักษา Phuttharaksa (Sukhothai); มันหมู Man mu (Prachuap Khiri Khan); สัมชื่น Som chuen (Northeastern, Northern).
G. pierrei Tanaka	เขยเพลีย Khoei plia (Southeastern).
G. puberula Lindl. var. puberula.	ประยงค์ขนบาง Prayong khon bang (Peninsular).
var. subsessilis (Craib) B.C.Stone	ค้างคาวหนู Khangkhao nu (Nakorn Ratchasima).
G. subsessilis Craib	= *G. puberula* Lindl. var. subsessilis (Craib) B.C.Stone
G. tricanthera Guillaumin	เขยตรี Khoei tri (Peninsular).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

G. trifolia (Blume) Spreng.	ขะยงป่า Khayong pa, ประยงค์ป่า Prayong pa, พะยงป่า Phayong pa (Northern).

The botanical characteristics of *Glycosmis parva* Craib were described in Bullentin of Miscellaneous Information of Royal Botanic Garden Kew (1926), as the following quote.

“*Glycosmis parva* Craib [Rutaceae-Aurantieae]; a *G. Montana* Pierre foliis angustioribus, filamentis inferne ampliatis, a *G. dinhense* Pierre ex Guillaumin petalis haud dorso pilosis, foliis haud longe acuminates recedit.

Frutex circa 1.5 m. altus (ex *Kerr*); ramuli graciles, primo subferrugineo-tomentosi vel pubescentes, compressi, mox puberuli, cortice cinereo vel brinneo-cinereo obtecti, lenticellis haud conspicuis. *Folia* alterna, interdum subopposita, lanceolata, apice obtuse, interdum retusa, rarius subacuminata, 3.5-9 cm. longa, 1-2.5 cm. lata, chataceo-coriacea, supra viridia, ad costam breviter subtomentella vel matura fere glabra, subtus pallid viridia, subglabra, costa supra conspicua subtus prominente, nervis lateralibus utrinque 11-14 rectis intra marginem anastomo-santibus supra obscuris vel subconspicuis subtus subprominentibus, aliis paulo minus validis interpositis, nervulis subtus prominulis, margine integra, petiolo 2-6 mm. longo primo puberulo mox fere glabro supra canaliculato suffulta. *Infloerscentia* axillaris, petiole subaequilonga vel eo paululo longior, pedunculo communi ferrugineo-pubescente perbrevi vel sub fructu circa 1 mm. longo suffulata; flores albi (ex *Kerr*), pedicello brevi articulato subglabro bracteolato suffulti. *Sepala* 5, subrotundata, vix 1.5 mm. diametro, dorsoglabra, glandula unica prominente instructa, ciliate. *Petala* 5, oblongo-lanceolata, apice obtusa, basi angustata, 4 mm. longa, 1.5 mm. lata, glabra, glandulosa. *Discus* brevis, crenulatus, glaber. *Filamenta* complanata, apice acuminata, ad 2.5 mm. longa, glabra, antheris 0.75 mm. longis apice glandula parva conspicua globosa ornatis. *Pistillum* glabrum, 1.75 mm. altum, glandulosum, stylo valido vix distincto. *Fructus* subellipsoideus, circa 7 mm. longus.

Krabin, Sakêo, 50 m., evergreen forest, *Kerr* 9766.”

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

In addition, Stone (1985) described the 3-celled ovary as the important recognition feature of *G. parva* which was not mentioned by Craib. Moreover, the reliable key to *G. parva* was available, as quoted below.

“ Group E

Leaves simple or unifoliolate, or some of them 2- or 3- foliolate.
Ovary finely densely puberulent; leaves 1-3-foliolate…. 33. *G. puberula*
Ovary glabrous; style glabrous.
Stamens puberulent; leaves usually lanceolate; ovary 2-locular
…. 28. *G. petelotii*
Stamens glabrous.
Ovary 2- or 3-locular, rarely imperfectly 4-locular; petiolule present (i.e. petiole articulated). except in *G parva.*

Leaves very thick coriaceous …. 6. *G. crassifolia*
Leaves chartaceous to pergamentaceous,
Leaves ovate to elliptic; ovary pustular …. 15. *G. lanceolata*
Leaves narrowly elliptic; ovary smooth …. 24. *G. parva*
Ovary 5- (sometimes 4-) locular.
Petiole not articulated …. 4. *G. cochinchinensis* and 9. *G. dinhensis*
Petiole articulated.
Inflorescence longer than petiole. cymose-paniculate, about half as long as leaf. …. 40. *G. tetracronia*
Inflorescence condensed cymose, as long as or shorter than petiole.
Petiole + petiole 25-40 mm long …. 16. *G. longipes*
Petiole + petiole shorter.
Lamina narrowly lanceolate, about 3 ½ times as long as wide or more …. 31. *G pseudoracemosa*
Lamina proportionally wider, sub-caudately acuminate; leaflets 1-3 …. 20. *G mansiana*
Ovary glabrous (?); style puberulent; flowers tetramerous
…. 23. *G parkinsonii*”

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Recently, *G. longipetala* F. J. Mou & D. X. Zhang was discovered in Guangxi and Yunnan province in southwestern China. The new species is similar to *G. cochinchinensis* (Lour.) Pierre ex Engl. by its simple leaves, but distinguishable in having long-elliptic or oblanceolate leaves, long-ovoid to ellipsoid floral buds, ovaries with many tubercles and glabrous stamens (Mou and Zhang, 2009).

So far, three phytochemical and biological studies on *M. hirsutum* have been published. Six carbazole alkaloids and a lactone derivative of oleic acid with anti-tuberculosis activity from the stem bark (Ma *et al.*, 2005), two coumarins, i.e. micromelin and magnolioside, and three flavonoids from the leaves (Trinh, Tran and Adam, 2004), and essential oils of the branches and leaves, flowers, and fruits (Diep *et al.*, 2007) were reported. For *G. parva*, only one publication on phenethylamides with 4-oxo-2-oxolenyl terpenoid side chain from the lipophilic leaf extracts (Hofer, Vajrodaya and Greger, 1998) was reported.

A number of interesting phytochemical studies on coumarins, acridone alkaloids and sulfur-containing propanamides of these two genus and the promising anti-viral activity against herpes simplex virus type 1 and type 2 of the extracts (see the Results and Discussion section) motivated the author to believe that there were possibilities of finding new compounds and/or new biological active compounds from these two plants. Therefore, the following objectives are put forwards:

1. Isolation and purification of compounds from the branches and leaves of *M. hirsutum* and *G. parva*
2. Determination of the chemical structure of each isolated compound
3. Evaluation of each isolate for its anti-herpes simplex virus activity

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Fruit

Figure 1 *Micromelum hirsutum* Oliver.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Fruit

Flower

Figure 2 *Glycosmis parva* Craib.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

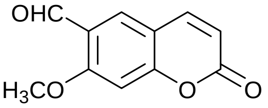
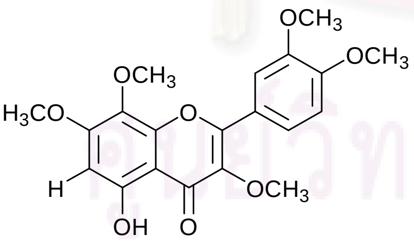
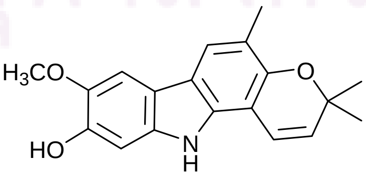
CHAPTER II

HISTORICAL

1. Chemical Constituents of *Micromelum* spp.

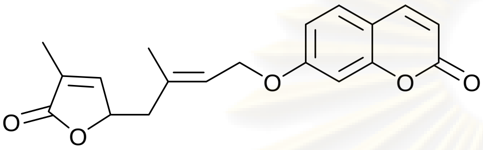
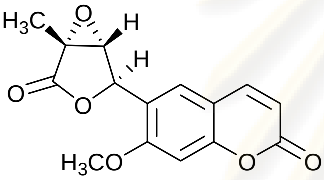
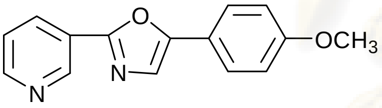
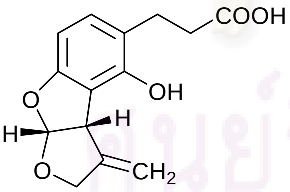
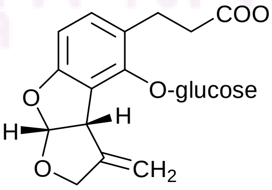
Chemical investigations of the genus *Micromelum* have revealed the widespread occurrence of coumarins. Prenylated coumarins are the unique feature of *Micromelum*. In addition, other classes of substances such as carbazole alkaloids and polyoxygenated flavonoids have also been isolated from this genus. The compounds isolated from *Micromelum*.spp. are shown in **Table 1**.

Table 1 Chemical constituents in *Micromelum* spp.

Plant and compound	Category	Plant part	Reference
Micromelum ceylanicum			
Angelical [**1**]  	Coumarin	Stem	Bowen and Perera, 1982
5-Hydroxy-3,3′,4′,7,8-pentamethoxyflavone [**2**]  	Flavonoid	Leaf and Stem	Bowen and Perera, 1982
Koenigine [**3**]  	Carbazole alkaloid	Leaf	Bowen and Perera, 1982

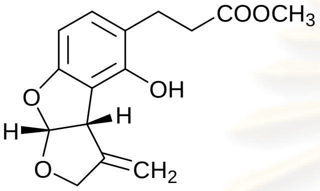
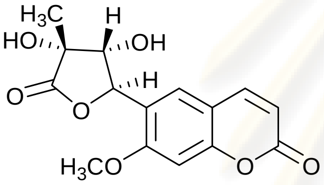
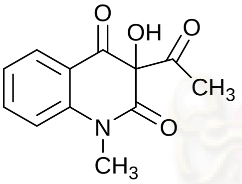
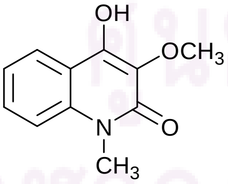
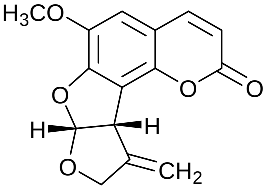
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum ceylanicum			
7-[4′-(4″-Methyl-5″-oxo-2″,5″-dihydro-2″-furyl)-3′-methyl-2′-butenyloxy]coumarin [**4**] 	Coumarin	Leaf	De Silva *et al.*, 1980
Micromelin [**5**] 	Coumarin	Leaf and Stem	Bowen and Perera, 1982
O-Methylhalfordinol [**6**] 	Oxazole alkaloid	Leaf and Stem	Bowen and Perera, 1982
Micromelum falcatum			
3,4-Dihydro-1,2-secomicrominutinin [**7**] 	Dihydrocinnamic acid derivative	Leaf	Kamperdick *et al.*, 1999
3,4-Dihydro-1,2-secomicrominutinin-9-*O*-glucoside [**8**] 	Dihydrocinnamic acid derivative	Leaf	Kamperdick *et al.*, 1999

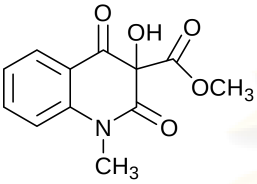
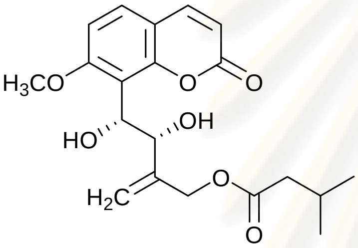
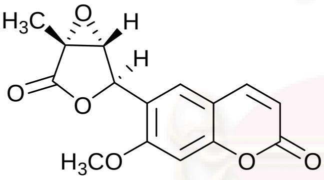
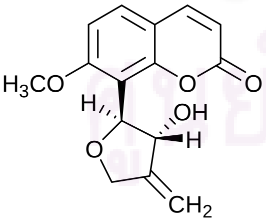
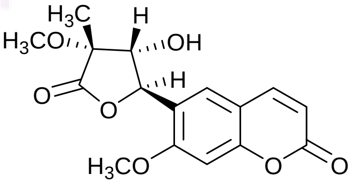
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Micromelum falcatum			
3,4-Dihydro-1,2-secomicrominutinin methylester [**9**] 	Dihydrocinnamic acid derivative	Leaf	Kamperdick *et al.*, 1999
Hydramicromelin A [**10**] 	Coumarin	Stem bark	Luo *et al.*, 2009
3-Hydroxy-1-methyl-3-(2-oxopropyl)-quinoline-2,4(1*H*,3*H*)-dione [**11**] 	Quinolone alkaloid	Stem bark	Luo *et al.*, 2009
4-Hydroxy-3-methoxy-1-methyl-2(1*H*)-quinolinone [**12**] 	Quinolone alkaloid	Stem bark	Luo *et al.*, 2009
6-Methoxymicrominutinin [**13**] 	Coumarin	Leaf	Kamperdick *et al.*, 1999

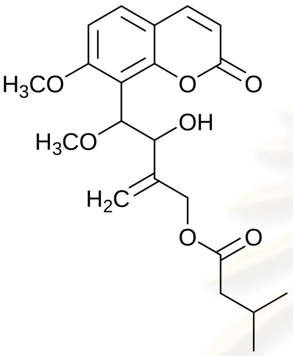
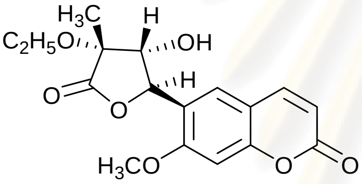
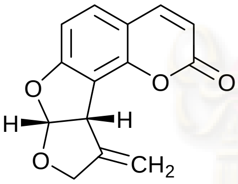
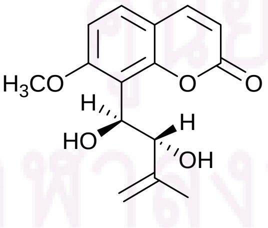
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum falcatum			
Methyl 2-(3-hydroxy-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinolin-3-yl)acetate [**14**] 	Quinolone alkaloid	Stem bark	Luo *et al.*, 2009
Microfalcatin isovalerate [**15**] 	Coumarin	Leaf	Kamperdick *et al.*, 1999
Micromelin [**5**] 	Coumarin	Root	Kong *et al.*, 1988
Micromeloside A [**16**] 	Coumarin	Stem bark	Luo *et al.*, 2009
Micromeloside B [**17**] 	Coumarin	Stem bark	Luo *et al.*, 2009

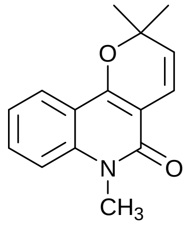
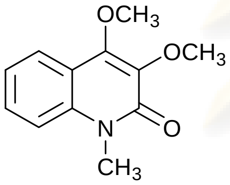
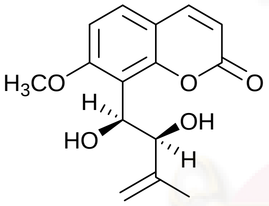
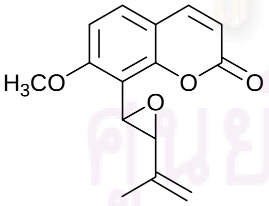
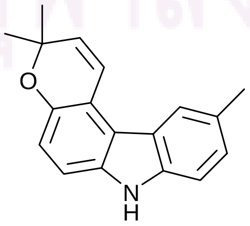
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum falcatum			
Micromeloside C [**18**] 	Coumarin	Stem bark	Luo *et al.*, 2009
Micromeloside D [**19**] 	Coumarin	Stem bark	Luo *et al.*, 2009
Microminutinin [**20**] 	Coumarin	Leaf	Kamperdick *et al.*, 1999
Minumicrolin [**21**] 	Coumarin	Root	Kong *et al.*, 1988 Luo *et al.*, 2009

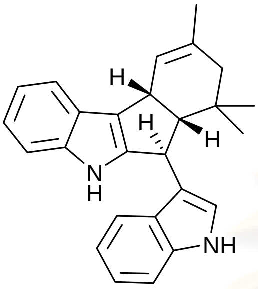
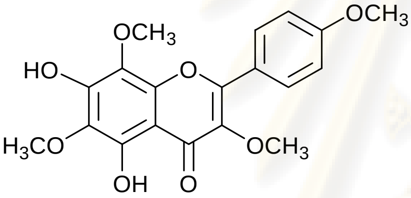
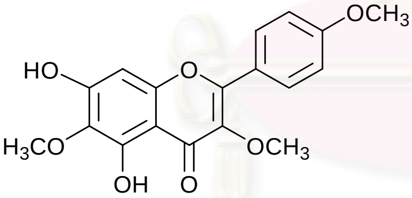
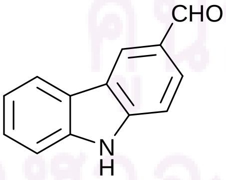
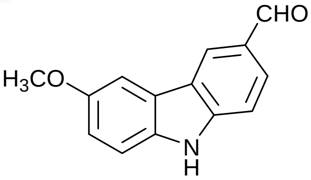
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Micromelum falcatum			
N-Methylflindersine [**22**] 	Quinolone alkaloid	Stem bark	Luo *et al*., 2009
N-Methylswietenidine-B [**23**] 	Quinolone alkaloid	Stem bark	Luo *et al*., 2009
Murrangatin [**24**] 	Coumarin	Stem bark	Luo *et al*., 2009
Phebalosin [**25**] 	Coumarin	Root	Kong *et al.*, 1988
5,6-Pyranoglycozoline [**26**] (= Glycomaurin) 	Carbazole alkaloid	Root	Kong *et al.*, 1988

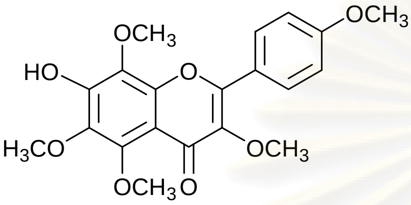
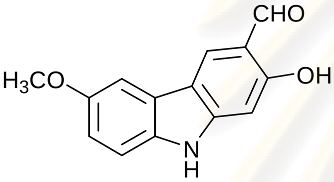
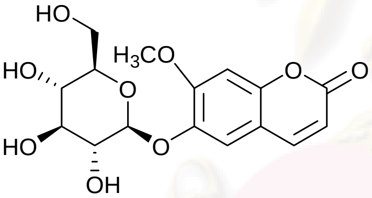
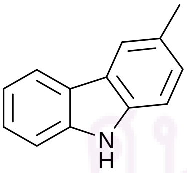
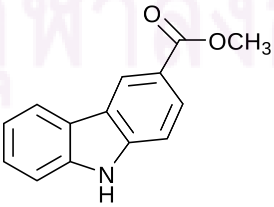
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum falcatum			
Yuehchukene [**27**]  	Dimeric indole alkaloid	Root	Kong *et al.*, 1988
Micromelum hirsutum			
5,7-Dihydroxy-3,6,8,4′-tetramethoxyflavone [**28**]  	Flavonoid	Leaf	Trinh, Tran and Adam, 2004
5,7-Dihydroxy-3,6,4'-trimethoxyflavone [**29**]  	Flavonoid	Leaf	Trinh, Tran and Adam, 2004
3-Formylcarbazole [**30**]  	Carbazole alkaloid	Stem bark	Ma *et al.*, 2005
3-Formyl-6-methoxycarbazole [**31**]  	Carbazole alkaloid	Stem bark	Ma *et al.*, 2005

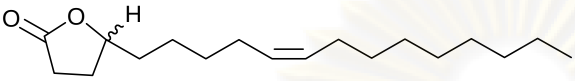
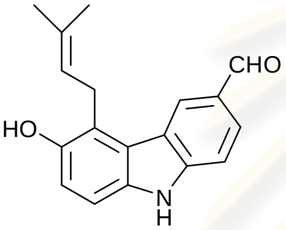
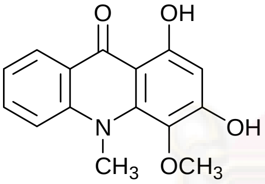
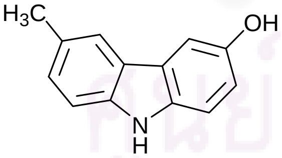
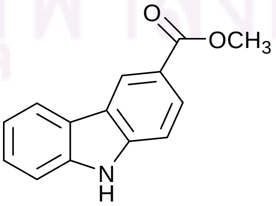
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Micromelum hirsutum			
7-Hydroxy-3,5,6,8,4′-pentamethoxyflavone [**32**] 	Flavonoid	Leaf	Trinh, Tran and Adam, 2004
Lansine [**33**] 	Carbazole alkaloid	Stem bark	Ma *et al.*, 2005
Magnolioside [**34**] 	Coumarin	Leaf	Trinh, Tran and Adam, 2004
3-Methylcarbazole [**35**] 	Carbazole alkaloid	Stem bark	Ma *et al.*, 2005
Methyl carbazole-3-carboxylate [**36**] 	Carbazole alkaloid	Stem bark	Ma *et al.*, 2005

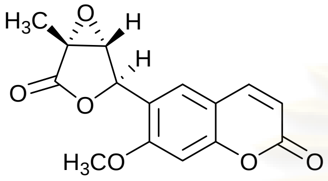
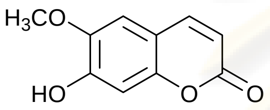
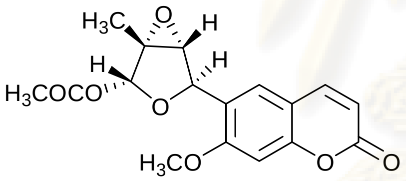
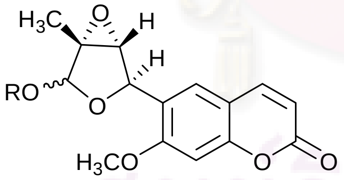
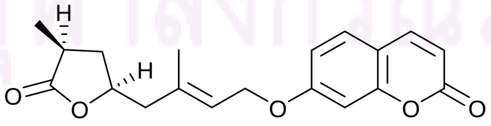
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Micromelum hirsutum			
Micromelide [**37**] 	Lactone derivative of oleic acid	Stem bark	Ma *et al.*, 2005
Micromeline [**38**] 	Carbazole alkaloid	Stem bark	Ma *et al.*, 2005
Micromelum integerrimum			
1,3-Dihydroxy-4-methoxy-10-methylacridone [**39**] 	Acridone alkaloid	Leaf	Yang *et al.*, 2009
Glycozolinol [**40**] 	Carbazole alkaloid	Leaf	Yang *et al.*, 2009
Methyl carbazole-3-carboxylate [**36**] 	Carbazole alkaloid	Leaf	Yang *et al.*, 2009

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum integerrimum			
Micromelin [**5**] 	Coumarin	Stem and leaf	Cassady *et al.*, 1979
Scopoletin [**41**] 	Coumarin	Stem and leaf	Cassady *et al.*, 1979
Micromelum minutum			
Acetyldihydromicromelin A [**42**] 	Coumarin	Aerial part	Das *et al.*, 1984
a mixture of Dihyromicromelin A and B  A; R = α-OH [**43**] B; R = β-OH [**44**]	Coumarin	Aerial part	Das *et al.*, 1984
3″,4″-Dihydrocapnolactone [**45**] 	Coumarin	Leaf	Rahmani *et al.*, 2003

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

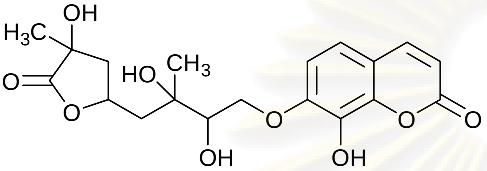
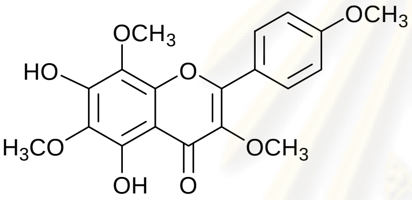
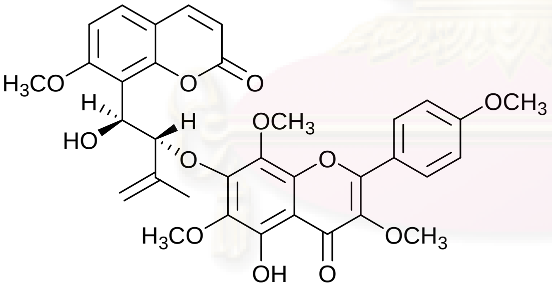
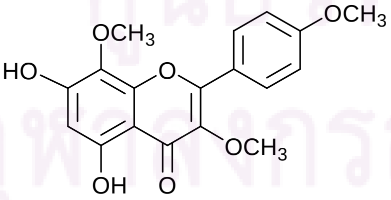
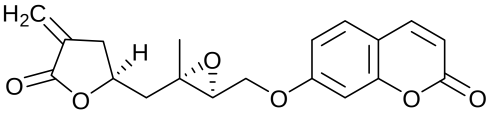
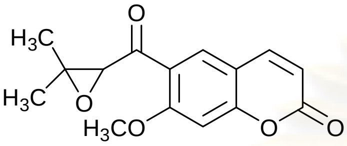
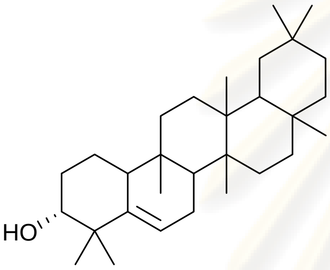
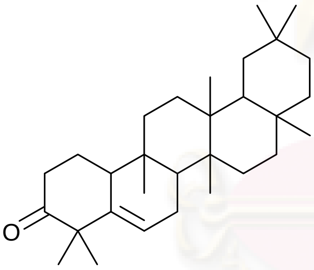
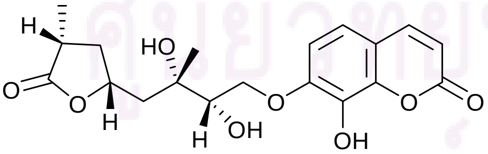
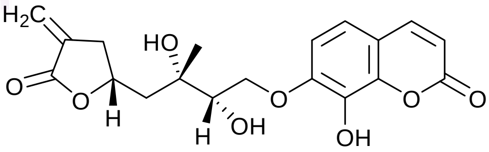
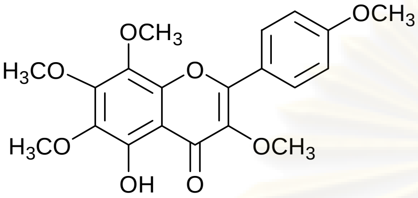
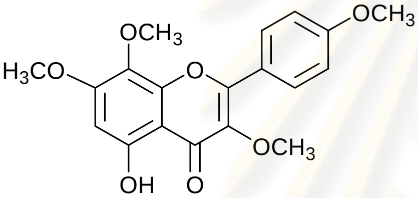
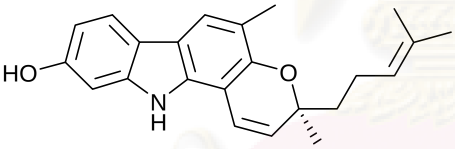
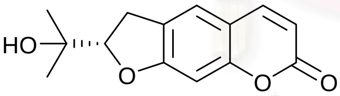
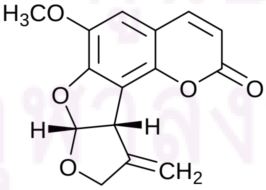
Plant and compound	Category	Plant part	Reference
Micromelum minutum			
8,4″-Dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol [**46**] 	Coumarin	Leaf	Susidarti *et al.*, 2006
5,7-Dihydroxy-3,6,8,4′-tetramethoxyflavone [**28**] 	Flavonoid	Leaf	Sohrab, Hasan and Rashid, 1999
7,12-Ether of 5,7-dihydroxy-3,6,8,4′-tetramethoxyflavone and murrangatin [**47**] 	Dimeric compound of coumarin and flavonoid	Aerial part	Das *et al.*, 1984
5,7-Dihydroxy-3,4′,8-trimethoxyflavone [**48**] 	Flavonoid	Leaf	Sohrab, Hasan and Rashid, 1999
2′,3′-Epoxyisocapnolactone [**49**] 	Coumarin	Leaf	Rahmani *et al.*, 2003

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum minutum			
Hopeyhopin [**50**] 	Coumarin	Leaf	Sohrab, Hasan and Rashid, 1999
5(6)-Gluten-3α-ol [**51**] 	Triterpenoid	Leaf	Susidarti *et al*., 2006
5(6)-Gluten-3-one [**52**] 	Triterpenoid	Leaf	Susidarti *et al*., 2006
8-Hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol [**53**] 	Coumarin	Leaf	Rahmani *et al*., 2003
8-Hydroxyisocapnolactone-2′,3′-diol [**54**] 	Coumarin	Leaf	Rahmani *et al*., 2003

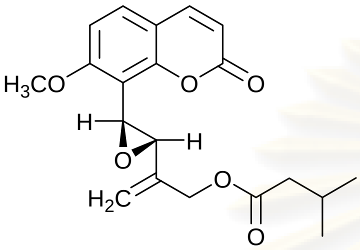
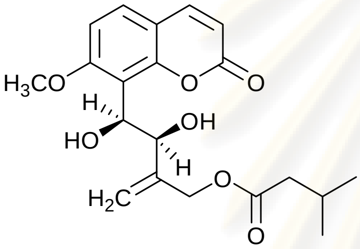
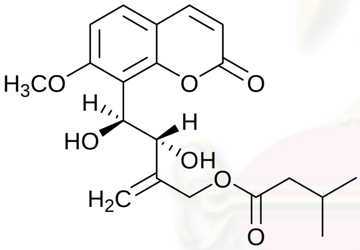
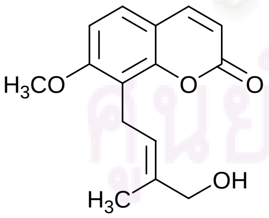
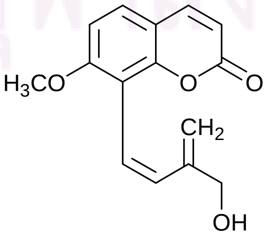
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum minutum			
5-Hydroxy-3,4′,6,7,8-pentamethoxyflavone [**55**] 	Flavonoid	Leaf	Sohrab *et al.*, 2004
5-Hydroxy-3,4′,7,8-tetramethoxyflavone [**56**] 	Flavonoid	Leaf	Sohrab *et al.*, 2004
Mahanine [**57**] 	Carbazole alkaloid	Aerial part	Nakahara *et al.*, 2002
Marmesin [**58**] 	Coumarin	Leaf	Sohrab *et al.*, 2004
6-Methoxymicrominutinin [**13**] 	Coumarin	Leaf and twigs Stem	Rahmani *et al.*, 1994 Ito *et al.*, 2000b

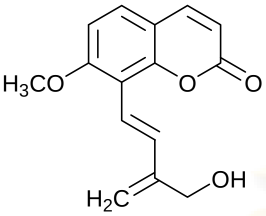
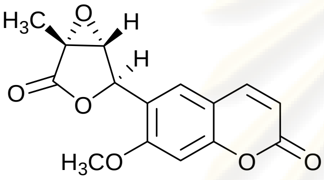
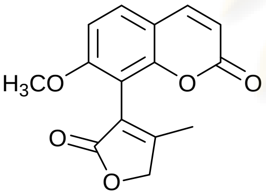
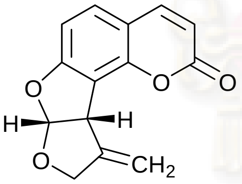
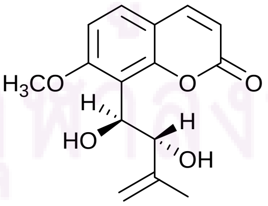
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum minutum			
Micromarin-A [**59**] 	Coumarin	Stem	Ito *et al*., 2000b
Micromarin-B [**60**] 	Coumarin	Stem	Ito *et al*., 2000b
Micromarin-C [**61**] 	Coumarin	Stem	Ito *et al*., 2000b
Micromarin-F [**62**] 	Coumarin	Stem	Ito *et al*., 2000b
Micromarin-G [**63**] 	Coumarin	Stem	Ito *et al*., 2000b

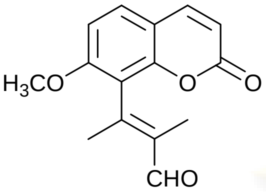
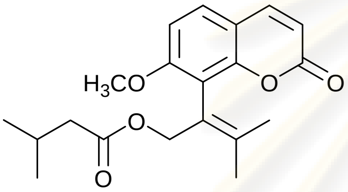
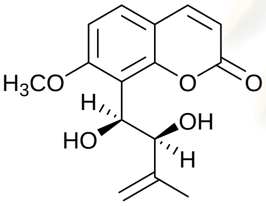
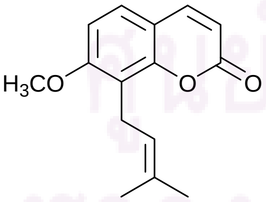
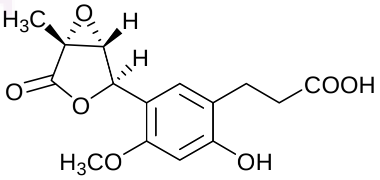
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum minutum			
Micromarin-H [**64**] 	Coumarin	Stem	Ito *et al.*, 2000b
Micromelin [**5**] 	Coumarin	Aerial part Leaf and twigs	Das *et al.*, 1984 Rahmani *et al.*, 1994
Microminutin [**65**] 	Coumarin	Aerial part Leaf and twigs	Das *et al.*, 1984 Rahmani *et al.*, 1994
Microminutinin [**20**] 	Coumarin	Leaf and twigs	Rahmani *et al.*, 1993, 1994
Minumicrolin [**21**]  (Das *et al.*, 1984 reported the *threo* isomer; however, the relative stereochemistry of minumicrolin was proved to be *erythro* isomer based on NOE experiments. (Ito and Furukawa, 1987)	Coumarin	Aerial part	Das *et al.*, 1984

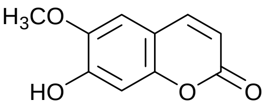
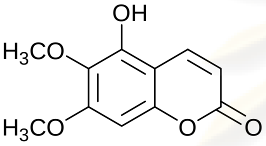
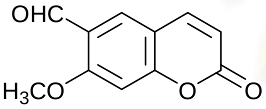
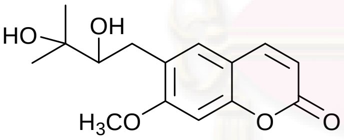
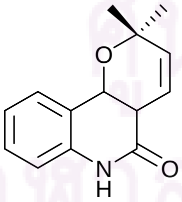
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum minutum			
Murralongin [**66**]  	Coumarin	Aerial part	Das *et al*., 1984
Murralonginol isovalerate [**67**]  	Coumarin	Stem	Ito *et al*., 2000b
Murrangatin [**24**]  (Das *et al*., 1984 reported the *erythro* isomer; however, the relative stereochemistry of murrangatin was proved to be *threo* isomer based on NOE experiments. (Ito and Furukawa, 1987)	Coumarin	Aerial part	Das *et al*., 1984
Osthol [**68**]  	Coumarin	Aerial part	Das *et al*., 1984
1,2-seco-dihydromicromelin [**69**]  	Dihydrocinnamic acid derivative	Leaf	Rahmani *et al*., 1994

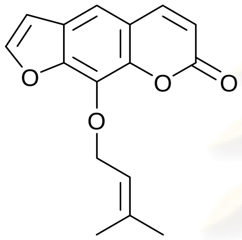
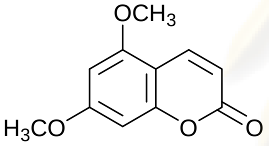
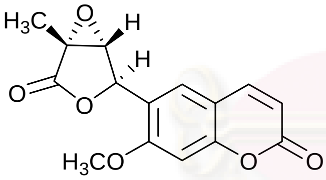
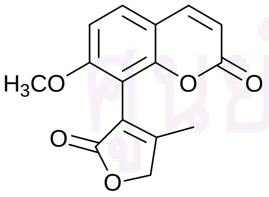
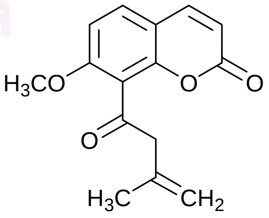
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum minutum			
Scopoletin [**41**] 	Coumarin	Leaf and twigs	Rahmani *et al.*, 1994
Tomentin [**70**] 	Coumarin	Leaf	Sohrab *et al.*, 2004
Micromelum pubescens			
Angelical [**1**] 	Coumarin	Root	Banerji *et al.*, 1988
6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin [**71**] 	Coumarin	Leaf and stem	Joshi *et al.*, 1975
Flindersine [**72**] 	Quinolone alkaloid	Leaf	Tantivatana *et al.*, 1983
Hentriacontane [**73**] $CH_3-(CH_2)_{29}-CH_3$	Long chain hydrocarbon	Leaf and stem	Bhakuni *et al.*, 1971

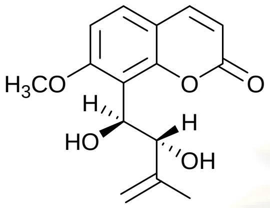
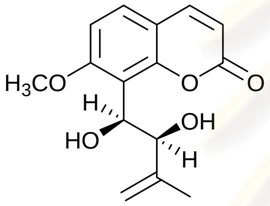
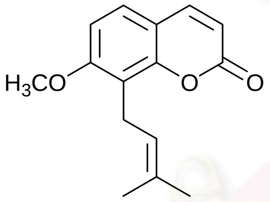
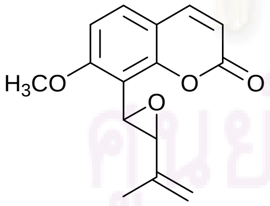
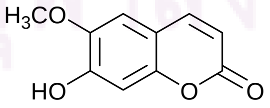
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

Plant and compound	Category	Plant part	Reference
Micromelum pubescens			
1-Hentriacontanol [**74**] $CH_3-(CH_2)_{30}-OH$	Long chain hydrocarbon	Leaf and stem	Bhakuni *et al.*, 1971
Imperatorin [**75**] 	Coumarin	Root	Banerji *et al.*, 1988
Limettin [**76**] 	Coumarin	Root	Banerji *et al.*, 1988
Micromelin [**5**] 	Coumarin	Leaf Leaf and branch	Croft and Toia, 1989 Lamberton, Price and Redcliffe, 1967
Microminutin [**65**] 	Coumarin	Leaf Root	Tantivatana *et al.*, 1983 Banerji, *et al.*, 1988
Micropubescin [**77**] 	Coumarin	Stem bark	Chatterjee, Dutta and Bhattacharyya, 1967

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 (Continued)

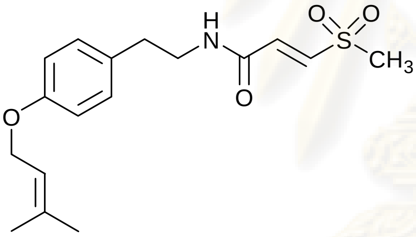
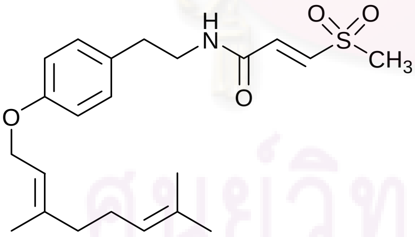
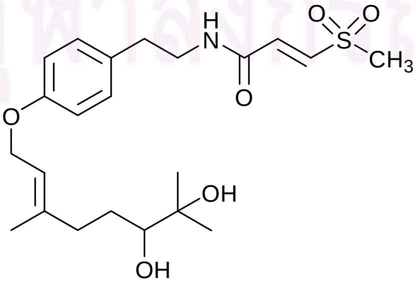
Plant and compound	Category	Plant part	Reference
Micromelum pubescens			
Minumicrolin [**21**] 	Coumarin	Root	Banerji *et al.*, 1988
Murrangatin [**24**] 	Coumarin	Root	Banerji *et al.*, 1988
Osthol [**68**] 	Coumarin	Leaf Leaf and branch	Croft and Toia, 1989 Lamberton, Price and Redcliffe, 1967
Phebalosin [**25**] 	Coumarin	Stem bark Stem bark Root	Tantishaiyakul *et al.*, 1986 Croft and Toia, 1989 Banerji *et al.*, 1988
Scopoletin [**41**] 	Coumarin	Leaf Root	Tantivatana *et al.*, 1983 Banerji *et al.*, 1988

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

2. Chemical Constituents of *Glycosmis* spp.

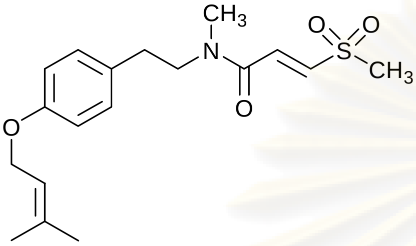
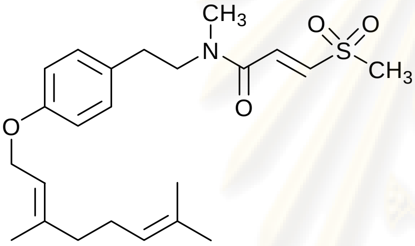
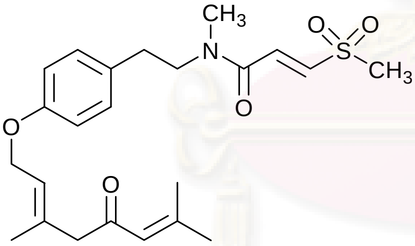
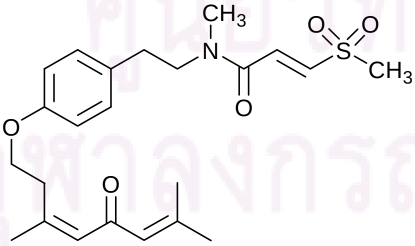
The genus *Glycosmis* is a rich source of various classes of alkaloids and sulfur-containing amides. Quinolone, acridone, and carbazole alkaloids are preferably accumulated in the stem and root bark (Vajrodaya *et al*., 1998), whilst sulfur-containing amides have been mainly detected in leaves. In addition, furoquinoline and quinazoline alkaloids and flavonoids have been usually isolated from leaves (Lukaseder *et al*., 2009). The compounds isolated from *Glycosmis*. spp. are shown in **Table 2**.

Table 2 Chemical constituents in *Glycosmis* spp.

Plant and compound	Category	Plant part	Reference
Glycosmis angustifolia			
Dambullin [**78**] 	Phenylethyl amide	Leaf	Greger *et al.*, 1994
Gerambullin [**79**] 	Phenylethyl amides	Leaf	Greger *et al.*, 1994
Gerambullindiol [**80**] 	Phenylethyl amides	Leaf	Greger *et al.*, 1994

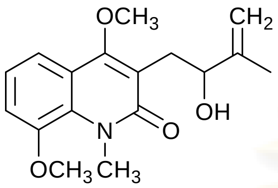
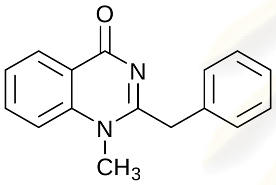
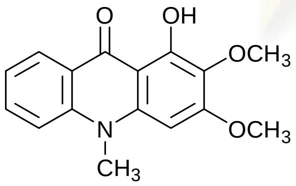
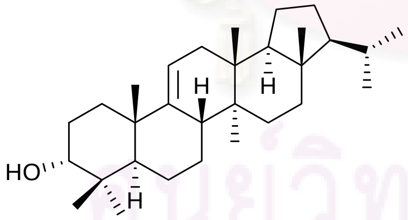
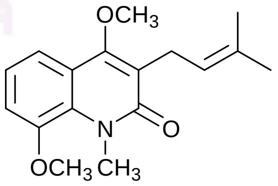
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis angustifolia			
Methyldambullin [**81**] 	Phenylethyl amides	Leaf	Greger *et al.*, 1994
Methylgerambullin [**82**] 	Phenylethyl amides	Leaf	Greger *et al.*, 1994
Methylgerambullone [**83**] 	Phenylethyl amides	Leaf	Greger *et al.*, 1994
Methylisogerambullone [**84**] 	Phenylethyl amides	Leaf	Greger *et al.*, 1994

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis arborea			
Acutifolin [**85**] 	Quinolone alkaloid	Stem	Ito *et al.*, 2004
Arborine [**86**] 	Quinazolone alkaloid	Leaf Leaf	Banerjee *et al.*, 1961 Chakravarti *et al.*, 1961
Arborinine [**87**] 	Acridone alkaloid	Leaf	Banerjee *et al.*, 1961
Arborinol [**88**] 	Triterpenoid	Leaf	Kennard *et al.*, 1965 Pakrashi and Samanta, 1967
4,8-Dimethoxy-1-methyl-3-(3-methylbut-2-enyl)quinolin-2(1*H*)-one [**89**] 	Quinolone alkaloid	Root	Chakravarty *et al.*, 1999

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

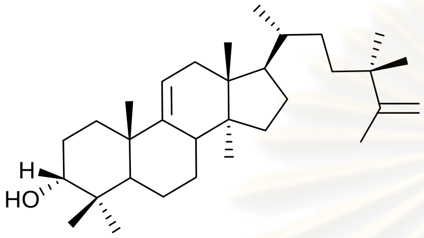
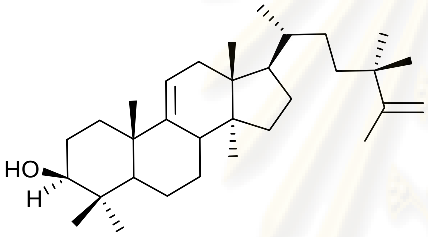
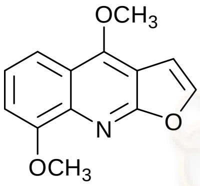
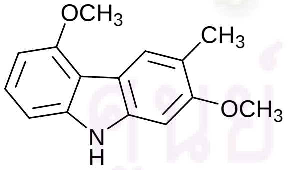
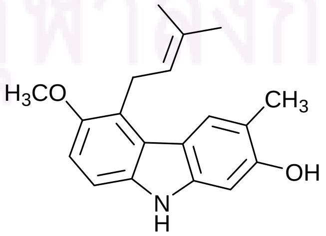
Plant and compound	Category	Plant part	Reference
Glycosmis arborea			
24,24-Dimethyl-5α-lanosta-9(11),25-dien-3α-ol [**90**] 	Triterpenoid	Aerial part	Chakravarty *et al*., 1996
24,24-Dimethyl-5α-lanosta-9(11),25-dien-3β –ol [**91**] 	Triterpenoid	Aerial part	Chakravarty *et al*., 1996
γ-Fagarine [**92**] 	Quinoline alkaloid	Stem	Ito *et al*., 2004
Glybomine-A [**93**] 	Carbazole alkaloid	Stem	Ito *et al*., 2004
Glybomine-B [**94**] 	Carbazole alkaloid	Stem	Ito *et al*., 2004

Table 2 (Continued)

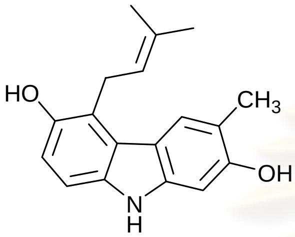
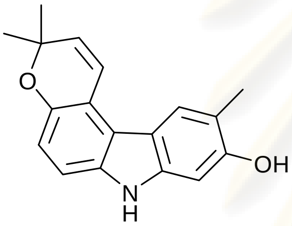
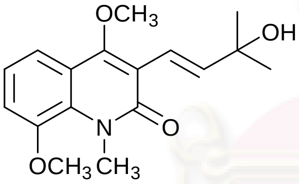
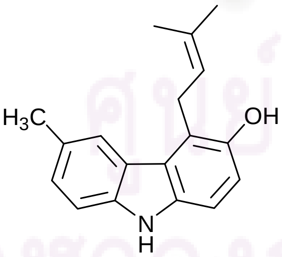
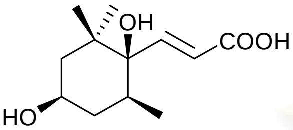
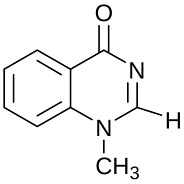
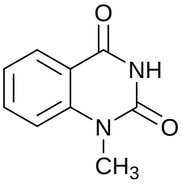
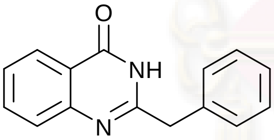
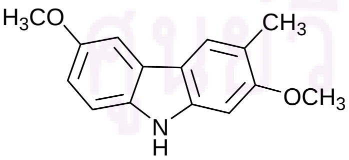
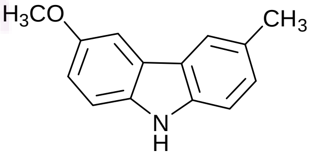
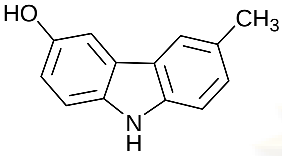
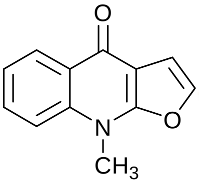
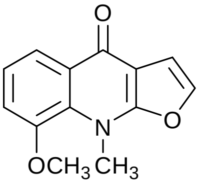
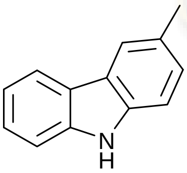
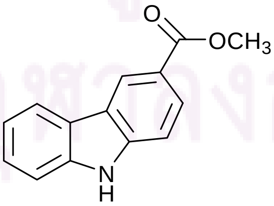
Plant and compound	Category	Plant part	Reference
Glycosmis arborea			
Glybomine-C [**95**]  	Carbazole alkaloid	Stem	Ito *et al*., 2004
Glycoborinine [**96**]  	Carbazole alkaloid	Root	Chakravarty *et al*., 1999
Glycocitlone-C [**97**]  	Quinolone alkaloid	Stem	Ito *et al*., 2004
Glycomaurrol [**98**]  	Carbazole alkaloid	Root	Ito *et al*., 2004

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis arborea			
Glycoric acid [**99**] 	10-normegastigmane skeleton	Aerial part	Chakravarty *et al*., 1996
Glycorine [**100**] 	Quinazolone alkaloid	Leaf	Pakrashi and Bhattacharyya, 1963
Glycosmicine [**101**] 	Quinazolone alkaloid	Leaf	Pakrashi and Bhattacharyya, 1963
Glycosminine [**102**] 	Quinazolone alkaloid	Leaf	Pakrashi and Bhattacharyya, 1963
Glycozolidine [**103**] 	Carbazole alkaloid	Root	Chakravarty *et al*., 1999
Glycozoline [**104**] 	Carbazole alkaloid	Root	Chakravarty *et al*., 1999

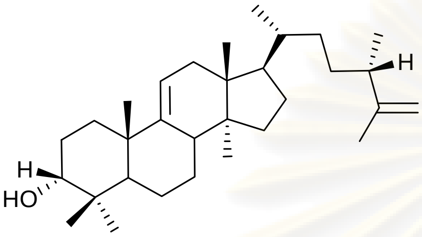
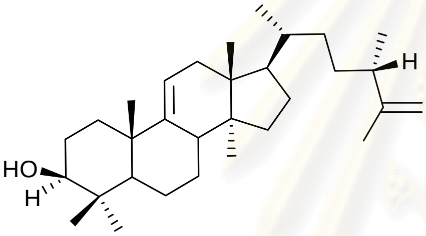
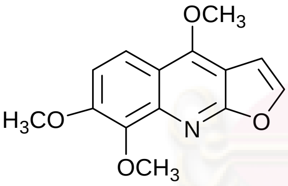
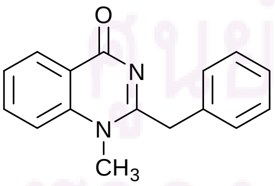
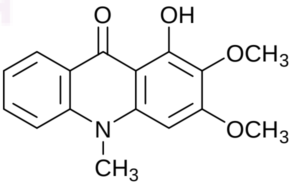
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis arborea			
Glycozolinine [**105**] 	Carbazole alkaloid	Stem	Ito *et al.*, 2004
Isodictamnine [**106**] 	Quinolone alkaloid	Stem	Ito *et al.*, 2004
Iso-*γ*-fagarine [**107**] 	Quinolone alkaloid	Stem	Ito *et al.*, 2004
3-Methylcarbazole [**35**] 	Carbazole alkaloid	Stem	Ito *et al.*, 2004
Methyl carbazole-3-carboxylate [**36**] 	Carbazole alkaloid	Stem	Ito *et al.*, 2004

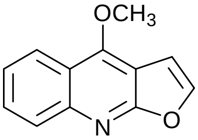
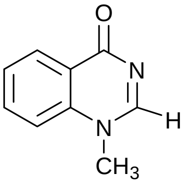
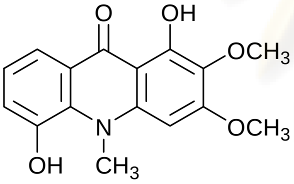
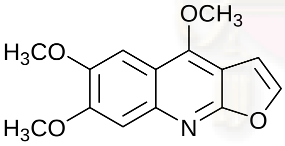
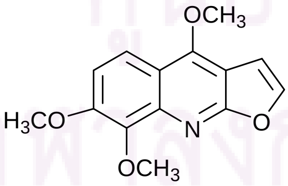
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis arborea			
(24*S*)-24-Methyl-5α-lanosta-9(11),25-dien-3α-ol [**108**] 	Triterpenoid	Aerial part	Chakravarty *et al.*, 1996
(24*S*)-24-methyl-5α-lanosta-9(11),25-dien-3β-ol [**109**] 	Triterpenoids	Aerial part	Chakravarty *et al.*, 1996
Skimmianine [**110**] 	Quinoline alkaloid	Root	Chakravarty *et al.*, 1999
Glycosmis bilocularis			
Arborine [**86**] 	Quinazolone alkaloid	Leaf	Bowen, Perera and Lewis, 1978
Arborinine [**87**] 	Acridone alkaloid	Leaf	Bowen, Perera and Lewis, 1978

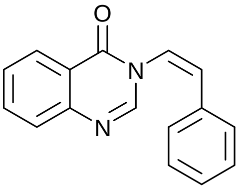
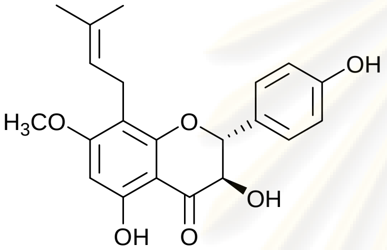
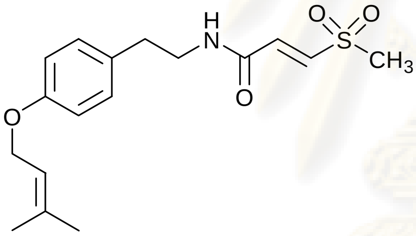
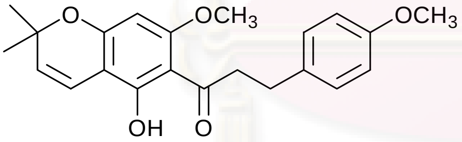
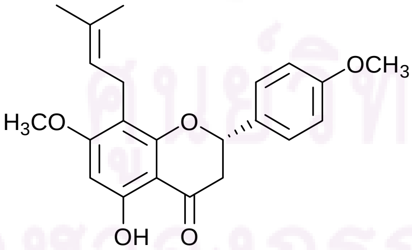
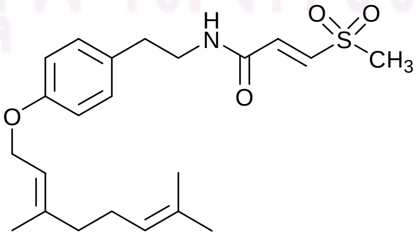
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis bilocularis			
Dictamnine [**111**]  	Quinoline alkaloid	Stem	Bowen, Perera and Lewis, 1980
Glycorine [**100**]  	Quinazolone alkaloid	Leaf	Bowen, Perera and Lewis, 1978
5-Hydroxy-arborinine [**112**]  	Acridone alkaloid	Leaf and stem	Bowen, Perera and Lewis, 1978, 1980
Kokusaginine [**113**]  	Quinoline alkaloid	Leaf and stem	Bowen, Perera and Lewis, 1978, 1980
Skimmianine [**110**]  	Quinoline alkaloid	Laef and stem	Bowen, Perera and Lewis, 1978, 1980

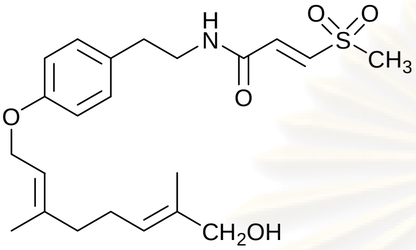
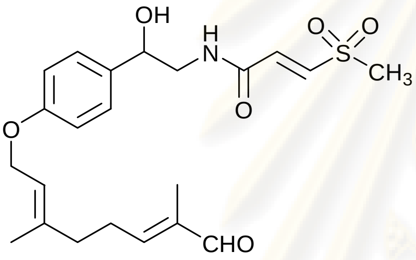
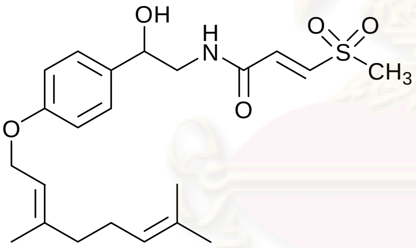
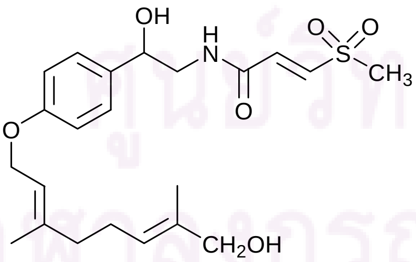
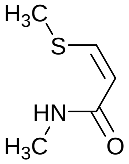
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis chlorosperma			
(*Z*)-Bogorin [**114**]  	Quinazolone alkaloid	Leaf	Seger *et al*., 1998
Chlorospermin [**115**]  	Flavanonol	Leaf	Lukaseder *et al*., 2009
Dambullin [**78**]  	Phenylethylamide derivative	Leaf Leaf	Hofer *et al*., 2000 Rahmani *et al*., 2004
Dihydroglychalcone-A [**116**]  	Chalcone	Leaf	Rahmani *et al*., 2004
Flowerine [**117**]  	Flavanone	Leaf	Lukaseder *et al*., 2009
Gerambullin [**79**]  	Phenylethylamide derivative	Leaf	Rahmani *et al*., 2004

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis chlorosperma			
Gerambullol [**118**] 	Phenylethylamide derivative	Leaf	Hofer *et al.*, 2000
β-Hydroxygerambullal [**119**] 	Phenylethylamide derivative	Leaf	Hofer *et al.*, 2000
β-Hydroxygerambullin [**120**] 	Phenylethylamide derivative	Leaf	Hofer *et al.*, 2000
β-Hydroxygerambullol [**121**] 	Phenylethylamide derivative	Leaf	Hofer *et al.*, 2000
Isopenangin [**122**] 	Amidosulfide	Leaf	Greger *et al.*, 1993a

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

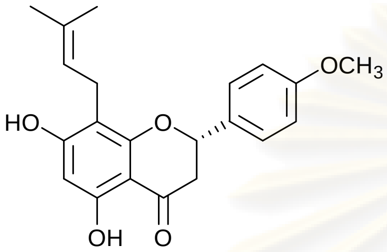
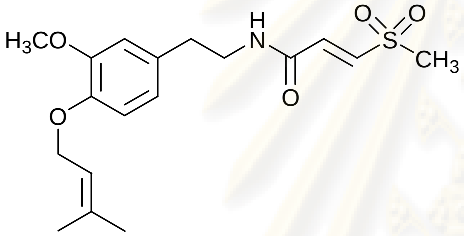
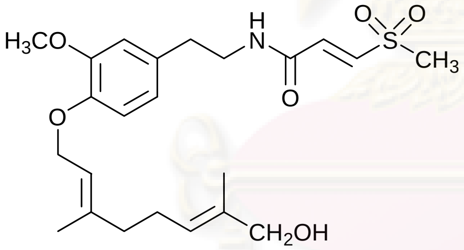
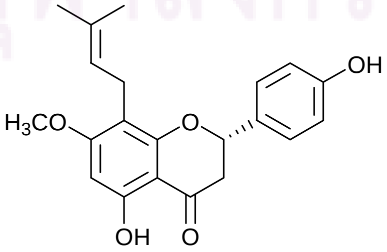
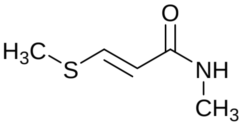
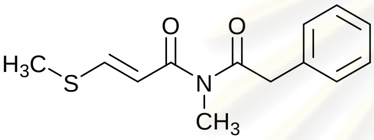
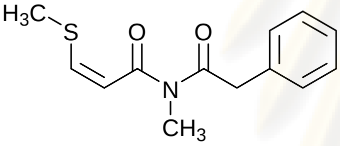
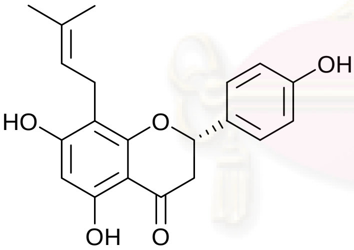
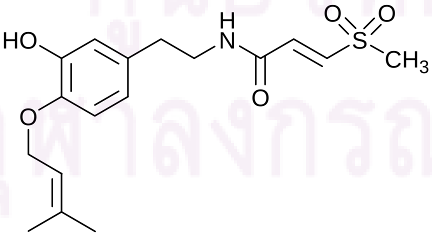
Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis chlorosperma			
4′-*O*-Methyl-8-prenylnaringenin [**123**] 	Flavanone	Leaf	Lukaseder *et al*., 2009
O-Methylsakambullin [**124**] 	Phenylethylamide derivative	Leaf	Hofer *et al*., 2000
O-Methylsakerinol-A [**125**] 	Phenylethylamide derivative	Leaf	Hofer *et al*., 2000
7-*O*-Methylselinone [**126**] 	Flavanone	Leaf	Lukaseder *et al*., 2009
Mundulea-flavanone-B [**127**] 	Flavanone	Leaf	Lukaseder *et al*., 2009

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis chlorosperma			
Penangin [**128**]  	Amidosulfide	Leaf	Greger *et al.*, 1993a
Penimide-A [**129**]  	Sulfur-containing imide	Leaf	Greger *et al.*, 1993a Hinterberger, Hofer and Greger, 1994
Penimide-B [**130**]  	Sulfur-containing imide	Leaf	Greger *et al.*, 1993a Hinterberger, Hofer and Greger, 1994
8-Prenylnaringenin [**131**]  	Flavanone	Leaf	Lukaseder *et al.*, 2009
Sakambullin [**132**]  	Phenylethyl amide derivative	Leaf	Hofer *et al.*, 2000

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

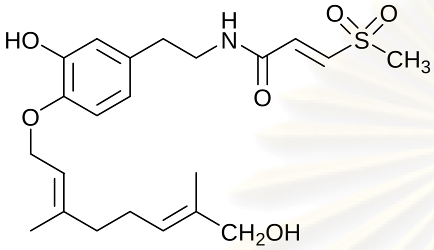
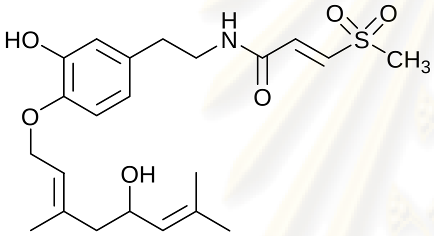
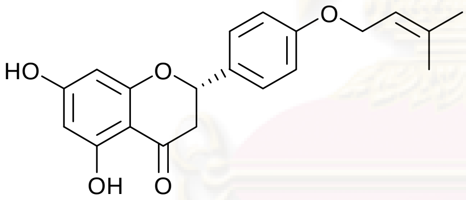
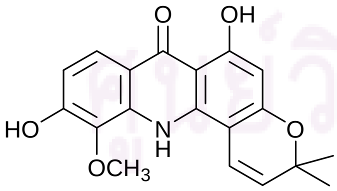
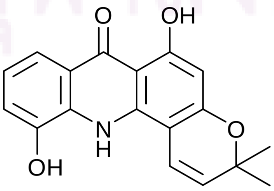
Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis chlorosperma			
Sakerinol-A [**133**]  	Phenylethylamide derivative	Leaf	Hofer *et al*., 2000
Sakerol [**134**]  	Phenylethylamide derivative	Leaf	Hofer *et al*., 2000
Selinone [**135**]  	Flavanone	Leaf	Lukaseder *et al*., 2009
Glycosmis citrifolia			
Acrifoline [**136**]  	Acridone alkaloid	Root and stem bark	Ono, Ito and Furukawa, 1995
Atalaphyllidine [**137**]  	Acridone alkaloid	Root and stem bark	Wu *et al*., 1983

Table 2 (Continued)

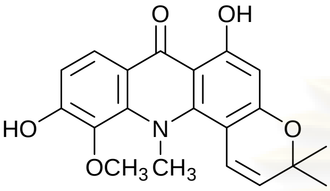
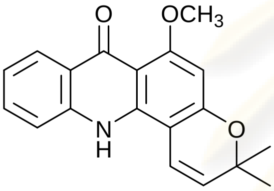
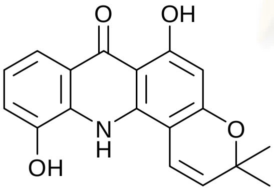
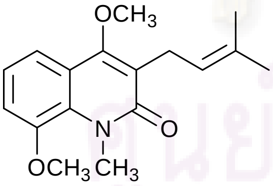
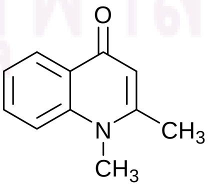
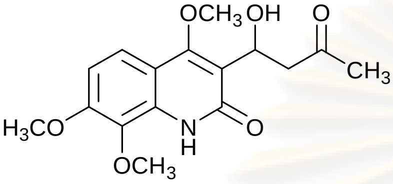
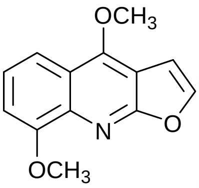
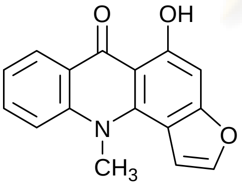
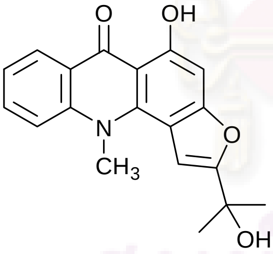
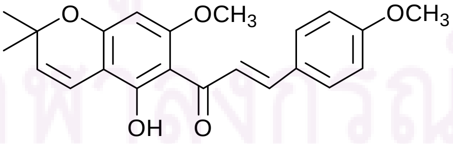
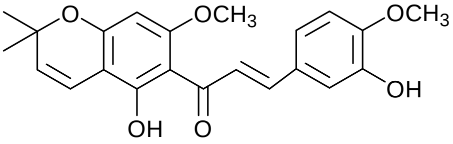
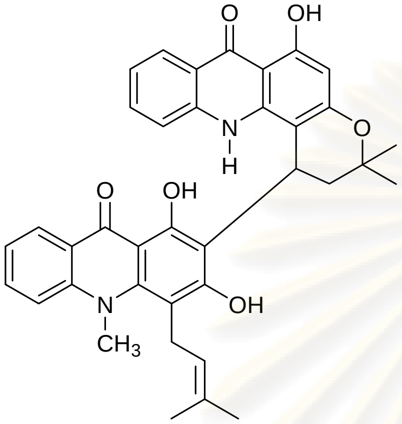
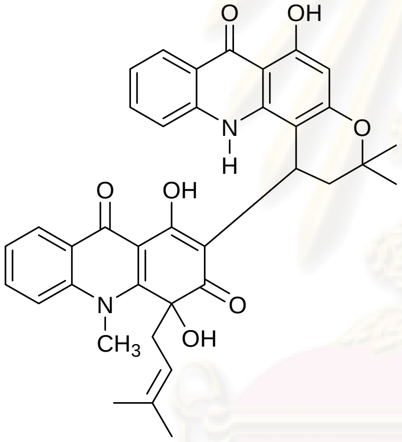
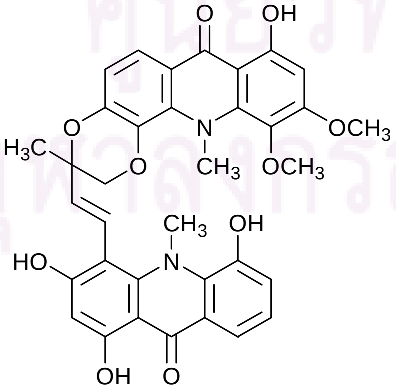
Plant and compound	Category	Plant part	Reference
Glycosmis citrifolia			
Citracridone-I [**138**] 	Acridone alkaloid	Root and stem bark	Wu *et al.*, 1983
Des-*N*-methylacronycine [**139**] 	Acridone alkaloid	Root and stem bark	Wu *et al.*, 1983
Des-*N*-methylnoracronycine [**140**] 	acridone alkaloid	Root and stem bark	Wu *et al.*, 1983
4,8-Dimethoxy-1-methyl-3-(3-methylbut-2-enyl)quinolin-2(1*H*)-one [**89**] 	Quinolone alkaloid	Root and stem bark	Wu *et al.*, 1983
1,2-Dimethylquinolin-4(1*H*)-one [**141**] 	Quinolone alkaloid	Leaf	Wu, Chang and Wu, 1995

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis citrifolia			
Evomeliaefolin [**142**] 	Quinolone alkaloid	Leaf	Wu, Chang and Wu, 1995
γ-Fagarine [**92**] 	Quinoline alkaloid	Leaf	Wu, Chang and Wu, 1995
Furofoline-I [**143**] 	Acridone alkaloid	Root and stem bark	Wu, Furukawa and Hsu 1982
Furofoline-II [**144**] 	Acridone alkaloid	Root and stem bark Root and stem bark	Wu, Furukawa and Hsu, 1982 Wu *et al*., 1983
Glychalcone-A [**145**] 	Chalcone	Leaf	Wu, Chang and Wu, 1995
Glychalcone-B [**146**] 	Chalcone	Leaf	Wu, Chang and Wu, 1995

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis citrifolia			
Glycobismine-A [**147**] 	Dimeric acridone alkaloid	Bark Root and stem bark	Furukawa *et al.*, 1984 Furukawa *et al.*, 1993
Glycobismine-B [**148**] 	Dimeric acridone alkaloid	Root and stem bark	Furukawa *et al.*, 1993
Glycobismine-C [**149**] was considered to be a diastereoisomer of glycobismine-B, even hitherto unknown stereochemistry.			
Glycobismine-D [**150**] 	Dimeric acridone alkaloid	Root and stem bark	Ito *et al.*, 2000a

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

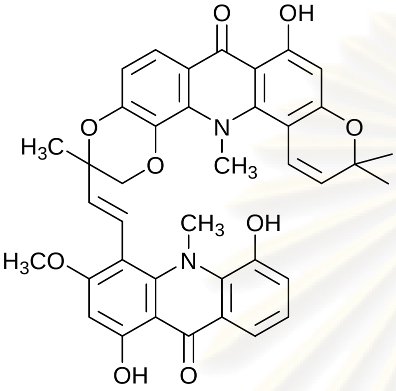
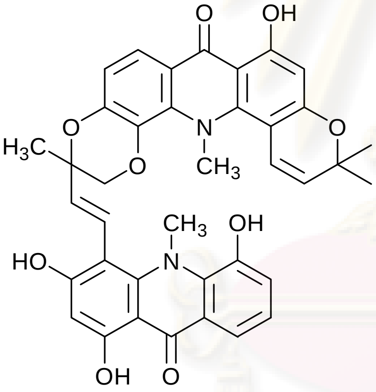
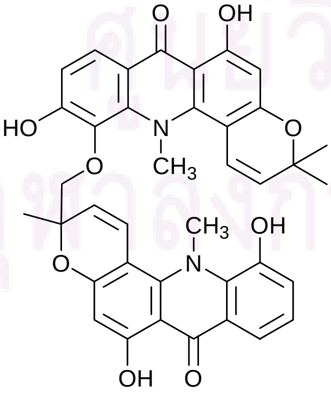
Plant and compound	Category	Plant part	Reference
Glycosmis citrifolia			
Glycobismine-E [**151**] 	Dimeric acridone alkaloid	Root and stem bark	Ito *et al*., 2000a
Glycobismine-F [**152**] 	Dimeric acridone alkaloid	Root	Negi *et al*., 2004
Glycobismine-G [**153**] 	Dimeric acridone alkaloid	Root	Negi *et al*., 2004

Table 2 (Continued)

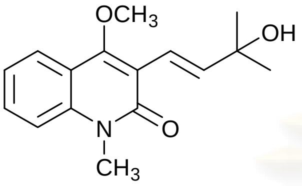
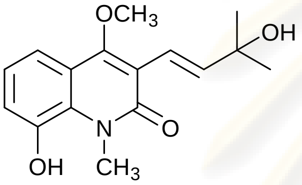
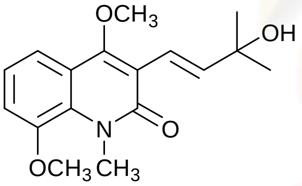
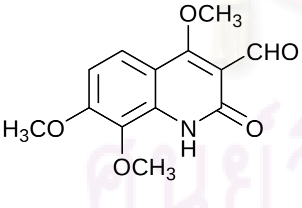
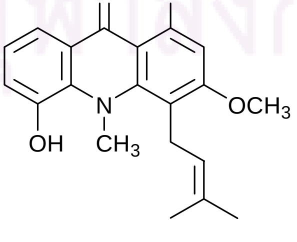
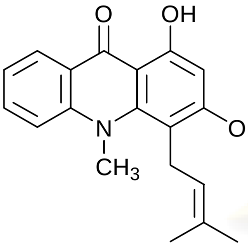
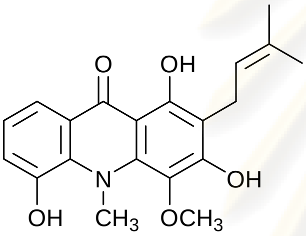
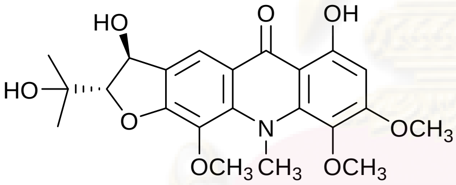
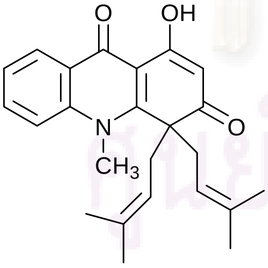
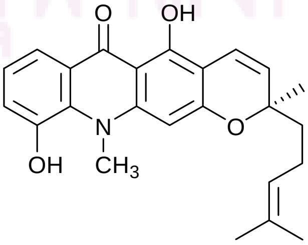
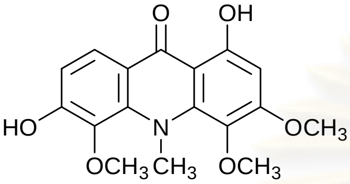
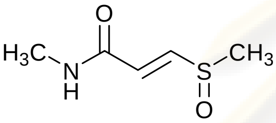
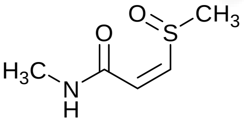
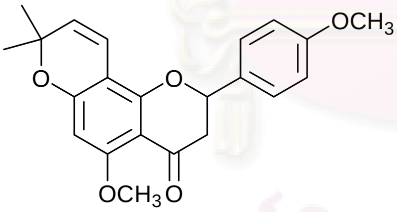
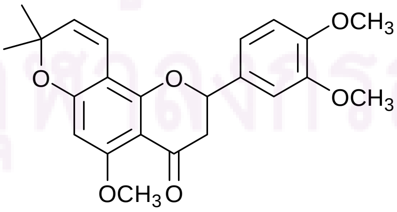
Plant and compound	Category	Plant part	Reference
Glycosmis citrifolia			
Glycocitlone-A [**154**] 	Quinolone alkaloid	Root and stem bark	Ito *et al*., 2000a
Glycocitlone-B [**155**] 	Quinolone alkaloid	Root and stem bark	Ito *et al*., 2000a
Glycocitlone-C [**97**] 	Quinolone alkaloid	Root and stem bark	Ito *et al*., 2000a
Glycocitridine [**156**] 	Quinolone alkaloid	Leaf	Wu, Chang and Wu, 1995
Glycocitrine-I [**157**] 	Acridone alkaloid	Root and stem bark Root and stem bark	Wu, Furukawa and Kuoh, 1982a Wu *et al*., 1983

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis citrifolia			
Glycocitrine-II [**158**] 	Acridone alkaloid	Root and stem bark Root and stem bark	Wu, Furukawa and Kuoh, 1982a Wu *et al*., 1983
Glycocitrine-IV [**159**] 	Acridone alkaloid	Root and stem bark	Ito *et al*., 2000a
Glycocitrine-V [**160**] 	Acridone alkaloid	Root and stem bark	Ito *et al*., 2000a
Glycocitrine-VI [**161**] 	Acridone alkaloid	Root and stem bark	Ito *et al*., 2000a
Glycofoline [**162**] 	Acridone alkaloid	Root and stem bark Root and stem bark	Wu and Furukawa, 1982 Wu *et al*., 1983

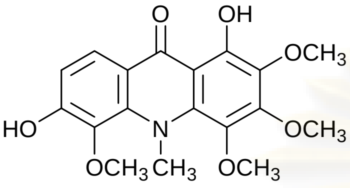
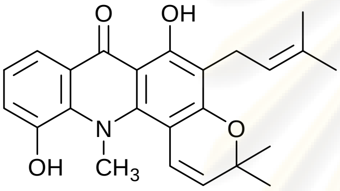
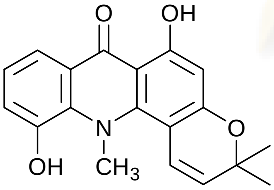
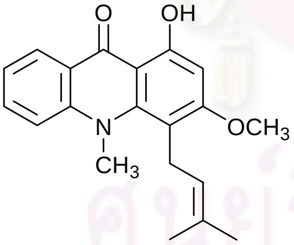
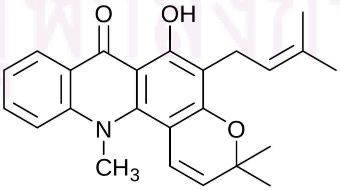
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis citrifolia			
Glycofolinine [**163**] 	Acridone alkaloid	Root and stem bark	Ono, Ito and Furukawa, 1995
Glycothiomin-A [**164**] 	Amidosulfoxide	Leaf	Wu, Chang and Wu, 1995
Glycothiomin-B [**165**] 	Amidosulfoxide	Leaf	Wu, Chang and Wu, 1995
Glyflavanone-A [**166**] 	Flavonoid	Leaf	Wu, Chang and Wu, 1995
Glyflavanone-B [**167**] 	Flavonoid	Leaf	Wu, Chang and Wu, 1995

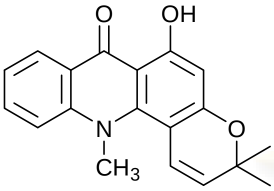
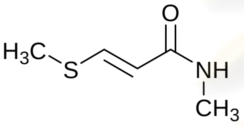
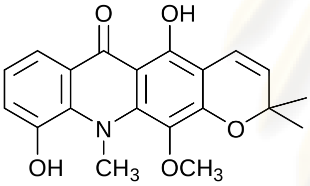
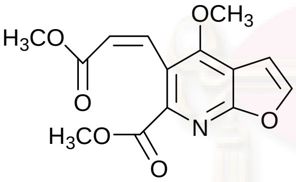
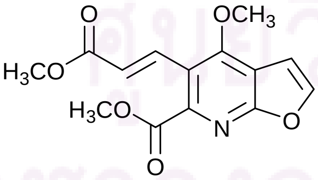
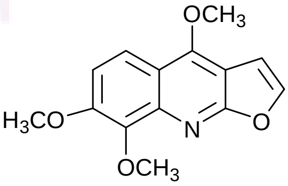
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis citrifolia			
Glyfoline [**168**] 	Acridone alkaloid	Root and stem bark Root and stem bark	Wu, Furukawa and Kuoh, 1982a Wu *et al*., 1983
5-Hydroxy-*N*-methylseverifoline [**169**] (= *N*-methylatalaphyllinine) 	Acridone alkaloid	Root and stem bark	Wu *et al*., 1983
5-Hydroxy-noracronycine [**170**] 	Acridone alkaloid	Root and stem bark	Wu *et al*., 1983
3-*O*-Methylglycocitrine-II [**171**] 	Acridone alkaloid	Root and stem bark Root and stem bark	Wu, Furukawa and Kuoh, 1982a Wu *et al*., 1983
N-Methylseverifoline [**172**] 	Acridone alkaloid	Root and stem bark	Wu *et al*., 1983

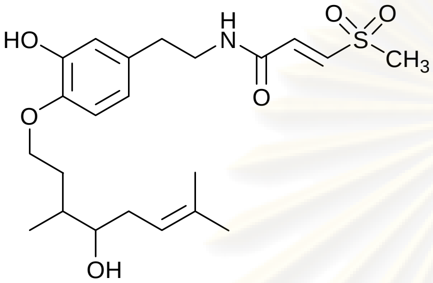
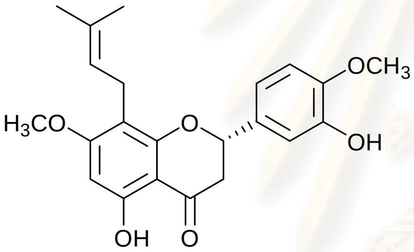
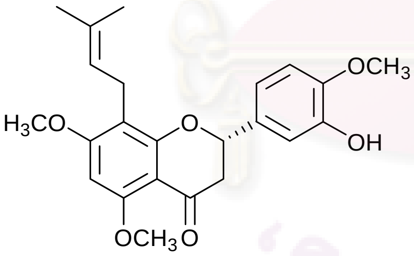
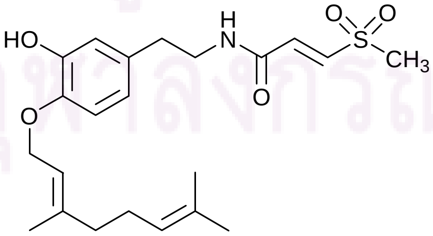
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis citrifolia			
Noracronycine [**173**] 	Acridone alkaloid	Root and stem bark	Wu *et al*., 1983
Penangin [**128**] 	Amidosulfide	Leaf	Wu, Chang and Wu, 1995
Pyranofoline [**174**] 	Acridone alkaloid	Root and stem bark Root and stem bark	Wu, Furukawa and Hsu, 1983 Wu *et al*., 1983
(*Z*)-Rhoifolic acid methyl ester [**175**] 	Furopyridine alkaloid	Leaf	Wu, Chang and Wu, 1995
(*E*)-Rhoifolic acid methyl ester [**176**] 	Furopyridine alkaloid	Leaf	Wu, Chang and Wu, 1995
Skimmianine [**111**] 	Quinoline alkaloid	Leaf	Wu, Chang and Wu, 1995

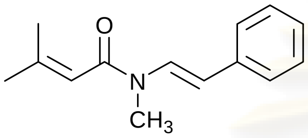
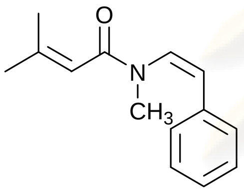
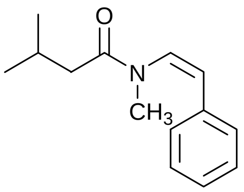
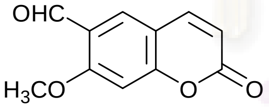
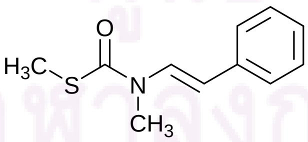
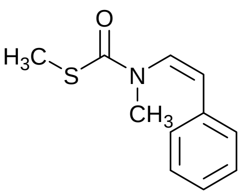
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis craibii			
Dihydroisosakerol [**177**] 	Phenylethylamide derivertive	Leaf	Hofer *et al*., 1995a
Glovanon [**178**] 	Flavanone	Leaf	Lukaseder *et al*., 2009
5-*O*-Methylglovanon [**179**] 	Flavanone	Leaf	Lukaseder *et al*., 2009
Sakerine [**180**] 	Phenylethylamide derivertive	Leaf	Hofer *et al*., 1995a

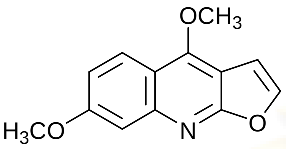
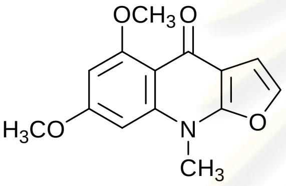
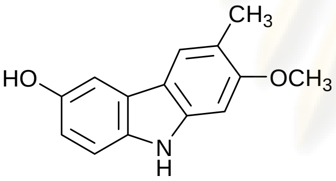
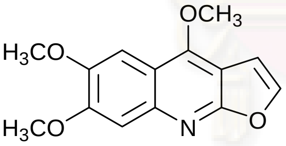
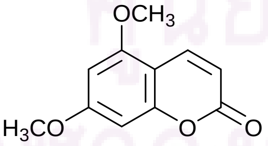
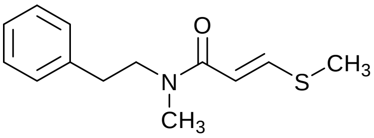
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis crassifolia			
Dehydrothalebanin-A [**181**]  	Phenylethenylamide derivative	Leaf	Greger, *et al*., 1996
Dehydrothalebanin-B [**182**]  	Phenylethenylamide derivative	Leaf	Greger, *et al*., 1996
Thalebanin-B [**183**]  	Phenylethenylamide derivative	Leaf	Greger, *et al*., 1996
Glycosmis cyanocarpa			
Angelical [**1**]  	Coumarin	Leaf	Sarkar, Kundu and Chakraborty, 1978
Dehydroniranin-A [**184**]  	Phenylethenylamide derivative	Leaf	Greger, *et al*., 1996
Dehydroniranin B-[**185**]  	Phenylethenylamide derivative	Leaf	Greger, *et al*., 1996

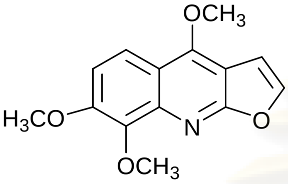
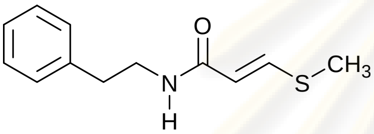
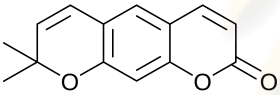
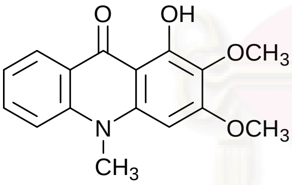
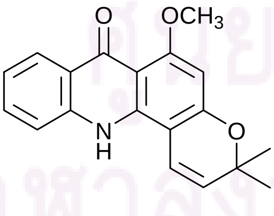
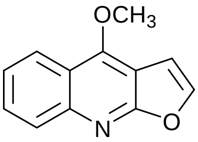
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis cyanocarpa			
Evolitrine [**186**] 	Quinoline alkaloid	Leaf	Sarkar, Kundu and Chakraborty, 1978
Glycarpine [**187**] 	Quinolone alkaloid	Leaf	Sarkar, Kundu and Chakraborty, 1978
Glycozolidol [**188**] 	Carbazole alkaloid	Leaf	Greger *et al*, 1992
Kokusaginine [**113**] 	Quinoline alkaloid	Leaf	Greger *et al*, 1992
Limettin [**76**] 	Coumarin	Leaf	Sarkar, Kundu and Chakraborty, 1978
Methylsinharine [**189**] 	Phenylethylamide	Leaf	Greger *et al*, 1992 Hinterberger, Hofer and Greger, 1994

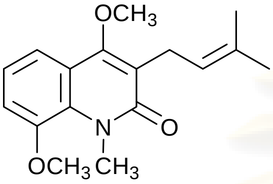
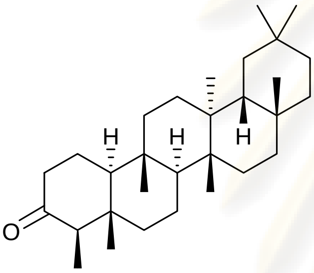
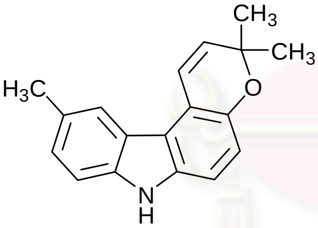
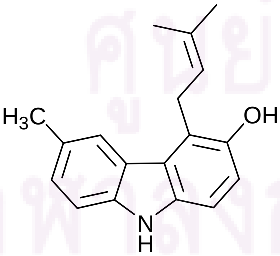
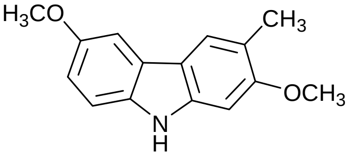
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis cyanocarpa			
Skimmianine [**110**] 	Quinoline alkaloid	Leaf	Greger *et al*, 1992
Sinharine [**190**] 	Phenylethylamide	Leaf	Greger *et al*., 1992 Hinterberger, Hofer and Greger, 1994
Xanthyletin [**191**] 	Coumarin	Leaf	Sarkar, Kundu and Chakraborty, 1978
Glycosmis mauritiana			
Arborinine [**86**] 	Acridone alkaloid	Root	Rastogi, Kapil and Popli, 1980
Des-*N*-methylacronycine [**139**] 	Acridone alkaloid	Stem bark	Kumar, Reisch and Wickramasinghe, 1989
Dictamnine [**111**] 	Quinoline alkaloid	Root	Rastogi, Kapil and Popli, 1980

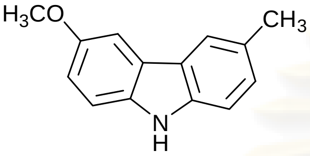
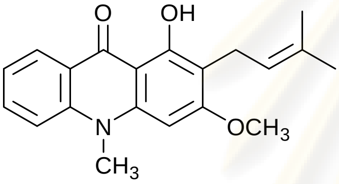
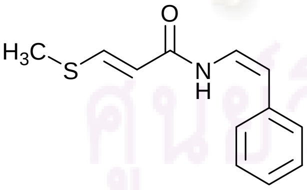
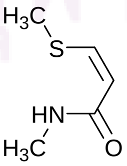
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis mauritiana			
4,8-Dimethoxy-1-methyl-3-(3-methylbut-2-enyl)quinolin-2(1*H*)-one [**89**] 	Quinolone alkaloid	Root	Rastogi, Kapil and Popli, 1980
Friedelin [**192**] 	Triterpenoid	Leaf and stem	Bhakuni *et al*., 1971
Glycomaurin [**26**] (= 5,6-pyranoglycozoline) 	Carbazole alkaloid	Stem bark	Kumar, Reisch and Wickramasinghe, 1989
Glycomaurrol [**98**] 	Carbazole alkaloid	Stem bark	Kumar, Reisch and Wickramasinghe, 1989
Glycozolidine [**103**] 	Carbazole alkaloid	Root	Rastogi, Kapil and Popli, 1980

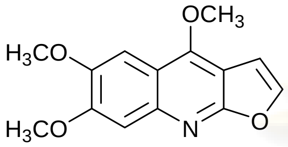
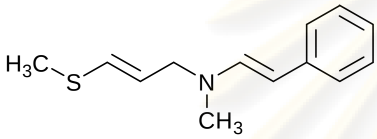
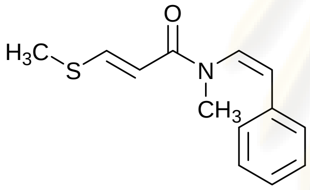
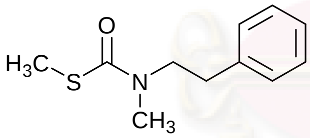
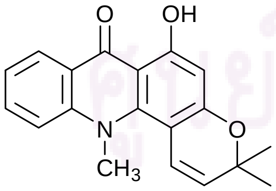
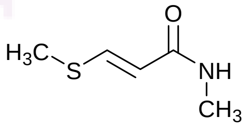
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis mauritiana			
Glycozoline [**104**] 	Carbazole alkaloid	Root	Rastogi, Kapil and Popli, 1980
1-Hydroxy-3-methoxy-10-methyl-2-(3-methylbut-2-enyl)acridan-9(10*H*)-one [**193**] 	Acridone alkaloid	Root	Rastogi, Kapil and Popli, 1980
Hentriacontane [**73**] $CH_3-(CH_2)_{29}-CH_3$	Long chain hydrocarbon	Leaf and stem	Bhakuni *et al.*, 1971
1-Hentriacontanol [**74**] $CH_3-(CH_2)_{30}-OH$	Long chain hydrocarbon	Leaf and stem	Bhakuni *et al.*, 1971
Illukumbin-B [**194**] 	Phenylethyl amide derivative	Leaf	Greger *et al.*, 1993b Hinterberger, Hofer and Greger, 1994
Isopenangin [**122**] 	Amidosulfide	Leaf	Greger *et al.*, 1993b

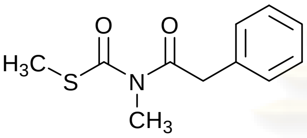
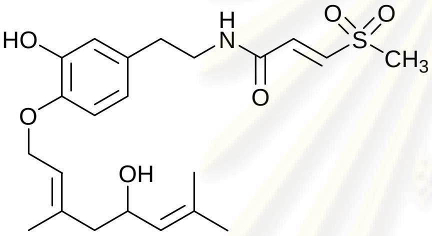
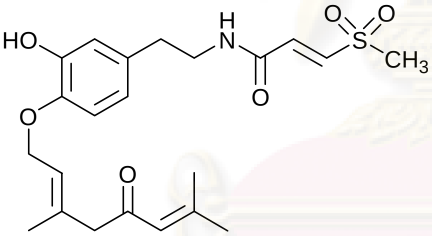
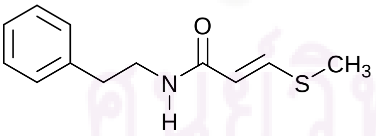
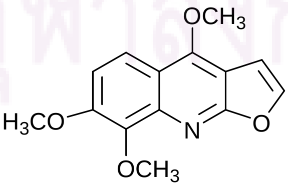
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis mauritiana			
Kokusaginine [**113**] 	Quinoline alkaloid	Leaf	Greger *et al*., 1993b
Methylillukumbin-A [**195**] 	Phenylethylamide derivative	Leaf	Greger *et al*., 1993b Hinterberger, Hofer and Greger, 1994
Methylillukumbin-B [**196**] 	Phenylethylamide derivative	Leaf	Greger *et al*., 1993b Hinterberger, Hofer and Greger, 1994
Niranin [**197**] 	Phenylethylamide derivative	Leaf	Greger, *et al*., 1996
Noracronycine [**173**] 	Acridone alkaloid	Stem bark	Kumar, Reisch and Wickramasinghe, 1989
Penangin [**128**] 	Amidosulfide	Leaf	Greger *et al*., 1993b

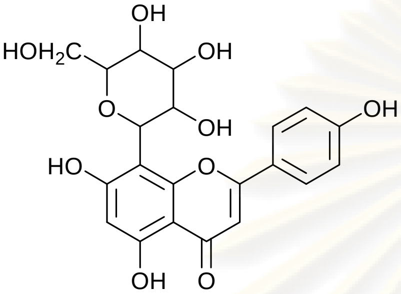
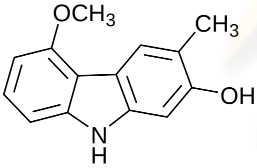
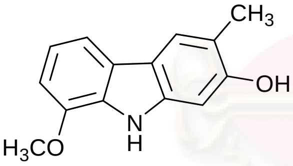
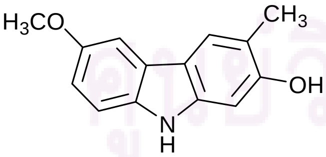
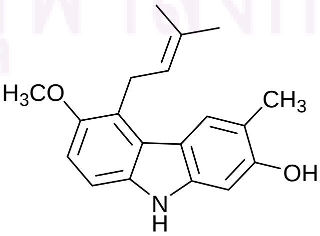
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis mauritiana			
Ritigalin [**198**] 	Sulfur-containg imide	Leaf	Hofer *et al*., 1995b
Sakerol [**134**] 	Phenylethylamide derivative	Leaf	Hofer *et al*., 1995a
Sakerone [**199**] 	Phenylethylamide derivative	Leaf	Hofer *et al*., 1995a
Sinharine [**190**] 	Phenylethylamide derivative	Leaf	Greger *et al*., 1993b
Skimmianine [**110**] 	Quinoline alkaloid	Root	Rastogi, Kapil and Popli, 1980

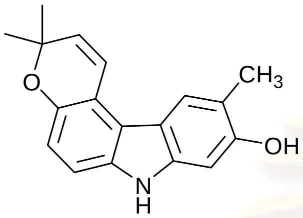
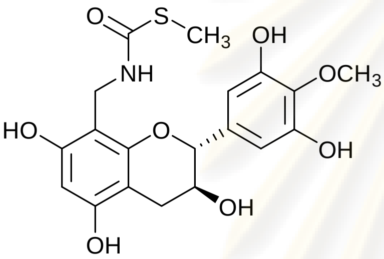
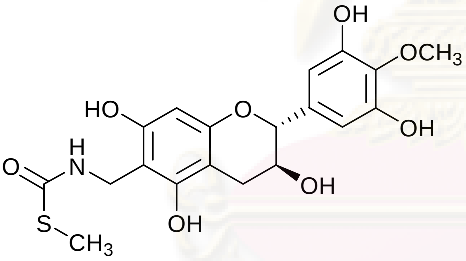
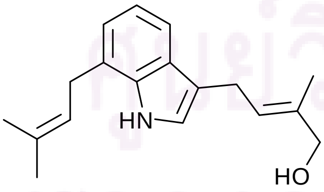
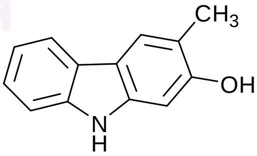
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis mauritiana			
Vitexin [**200**] (8-*C*-*β*-glucopyranosylapigenin) 	Flavonoid	Leaf and stem	Bhakuni *et al*., 1971
Glycosmis montana			
Carbalexin-A [**201**] 	Carbazole alkaloid	Leaf and twig	Wang *et al*., 2005
Carbalexin-B [**202**] 	Carbazole alkaloid	Leaf and twig	Wang *et al*., 2005
Carbalexin-C [**203**] 	Carbazole alkaloid	Leaf and twig	Wang *et al*., 2005
Glybomine-B [**94**] 	Carbazole alkaloid	Leaf and twig	Wang *et al*., 2005

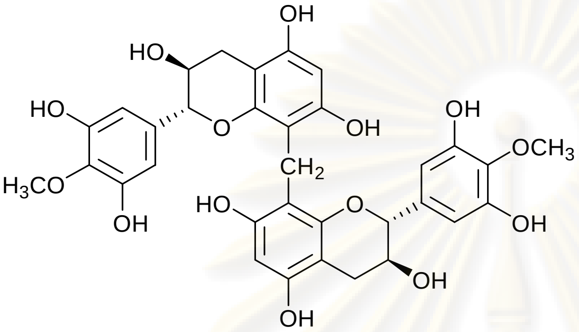
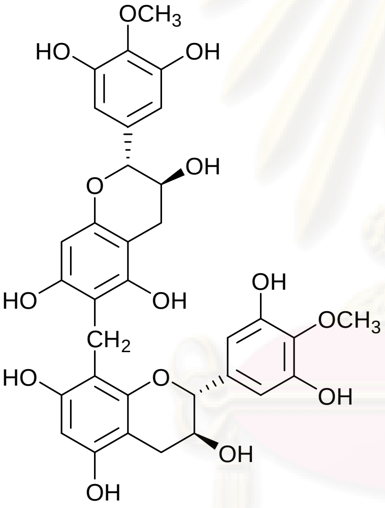
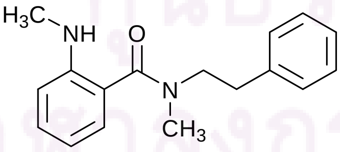
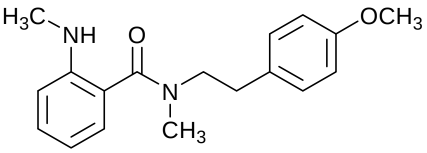
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis montana			
Glycoborinine [**96**] 	Carbazole alkaloid	Leaf and twig	Wang *et al.*, 2005
Glymontanine-A [**204**] 	Sulfur-containing flavanols	Leaf and twigs	Wang *et al.*, 2004
Glymontanine-B [**205**] 	Sulfur-containing flavanols	Leaf and twigs	Wang *et al.*, 2004
(*E*)-2-Methyl-4-(7-(3-methylbut-2-enyl)-1*H*-indol-3-yl)but-2-en-1-ol [**206**] 	Diprenylated indole alkaloid	Leaf and twig	Wang *et al.*, 2005
3-Methyl-9*H*-carbazol-2-ol [**207**] 	Carbazole alkaloid	Leaf and twig	Wang *et al.*, 2005

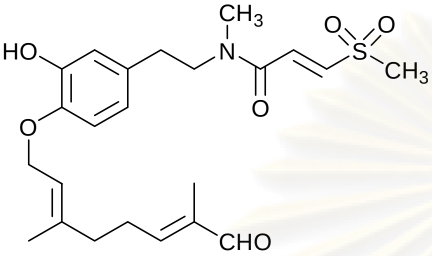
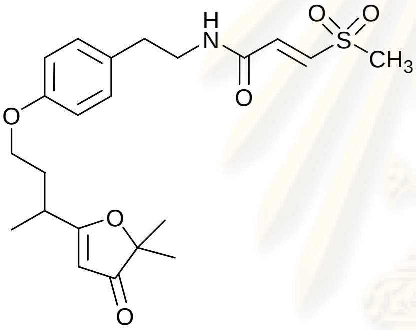
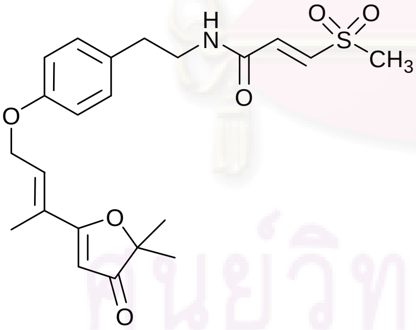
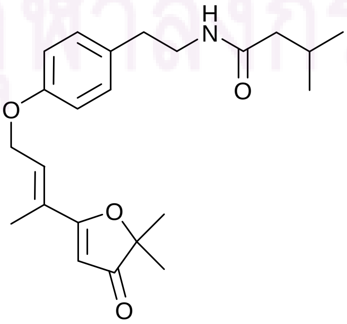
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis montana			
Montahomobisflavan-A [**208**] 	Flavanol dimer	Leaf and twigs	Wang *et al.*, 2004
Montahomobisflavan-B [**209**] 	Flavanol dimer	Leaf and twigs	Wang *et al.*, 2004
Glycosmis ovoidea			
Doisuthine [**210**] 	Phenylethyl amide derivative	Leaf	Hofer *et al.*, 1995a
Methoxydoisuthine [**211**] 	Phenylethyl amide derivative	Leaf	Hofer *et al.*, 1995a

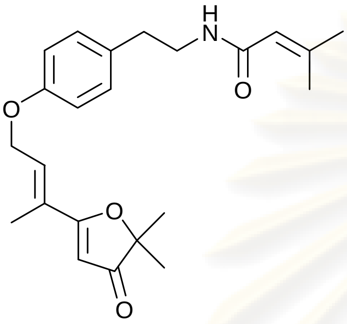
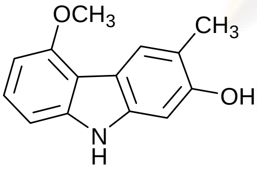
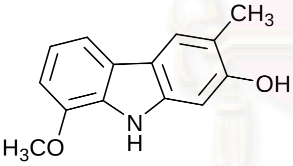
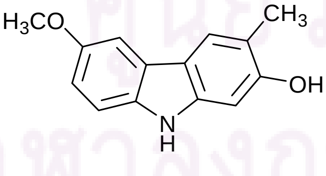
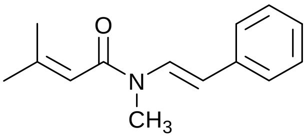
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis ovoidea			
Methylgerambullal [**212**] 	Phenylethylamide derivative	Leaf	Hofer *et al.*, 1995a
Glycosmis parva			
Dihydroglyparvin [**213**] 	Phenylethylamide derivative	Leaf	Hofer, Vajrodaya and Greger, 1998
Glyparvin-A [**214**] 	Phenylethylamide derivative	Leaf	Hofer, Vajrodaya and Greger, 1998
Khaochamide [**215**] 	Phenylethylamide derivative	Leaf	Hofer, Vajrodaya and Greger, 1998

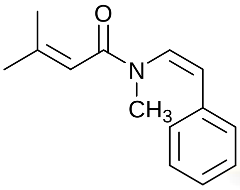
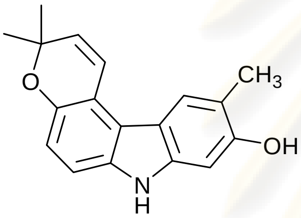
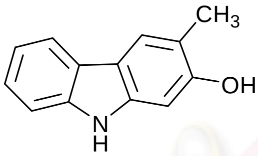
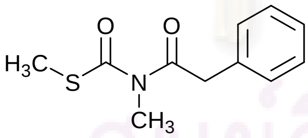
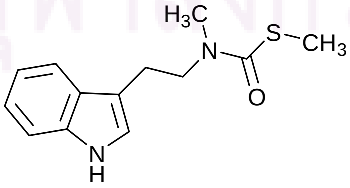
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis parva			
Puhinamide [**216**] 	Phenylethylamide derivative	Leaf	Hofer, Vajrodaya and Greger, 1998
Glycosmis parviflora			
Carbalexin-A [**201**] 	Carbazole alkaloid	Leaf	Pacher *et al.*, 2001
Carbalexin-B [**202**] 	Carbazole alkaloid	Leaf	Pacher *et al.*, 2001
Carbalexin-C [**203**] 	Carbazole alkaloid	Leaf	Pacher *et al.*, 2001
Dehydrothalebanin-A [**181**] 	Phenylethenylamide derivative	Leaf	Pacher *et al.*, 2001

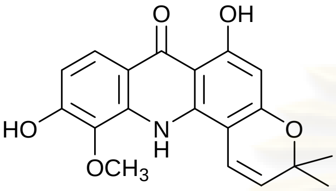
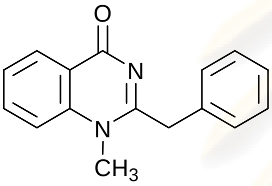
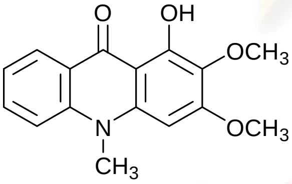
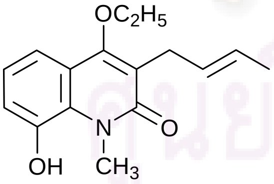
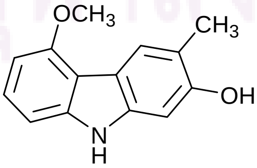
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis parviflora			
Dehydrothalebanin-B [**182**] 	Phenylethenylamide derivative	Leaf	Pacher *et al*., 2001
Glycoborinine [**96**] 	Carbazole alkaloid	Leaf	Pacher *et al*., 2001
3-Methyl-9*H*-carbazol-2-ol [**207**] 	Carbazole alkaloid	Leaf	Pacher *et al*., 2001
Ritigalin [**198**] 	Sulfur-containing Imide	Leaf	Hofer *et al*., 1995b
Glycosmis petelotii			
Glypetelotine [**217**] 	Sulfur-containing indole alkaloid	Leaf	Cuong, Taylor and Sung, 1999

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis pentaphylla			
Acrifoline [**136**] 	Acridone alkaloid	Stem	Ito *et al*., 1999
Arborine [**86**] 	Quinazolone alkaloid	Leaf	Sarkar and Chakraborty, 1979
Arborinine [**87**] 	Acridone alkaloid	Stem	Ito *et al*., 1999
(*E*)-3-(But-2-enyl)-4-ethoxy-8-hydroxy-1-methylquinolin-2(1*H*)-one [**218**] 	Quinolone alkaloid	Root bark	Sinha and Kumar, 1988
Carbalexin-A [**201**] 	Carbazole alkaloid	Leaf	Pacher *et al*., 2001

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

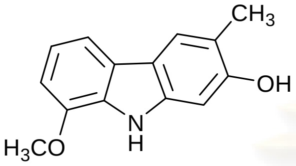
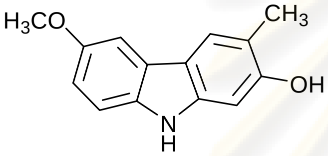
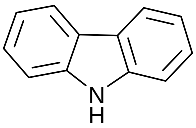
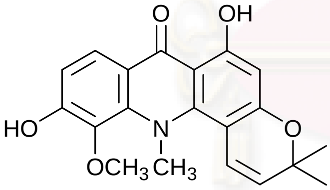
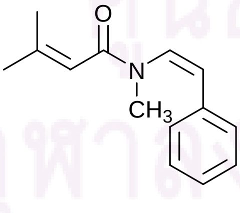
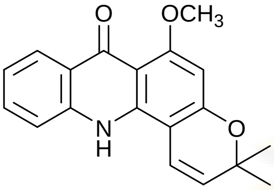
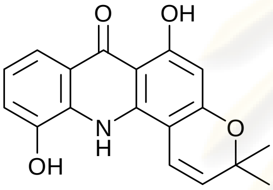
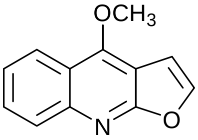
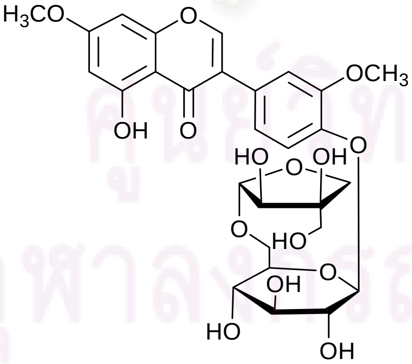
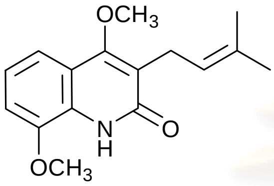
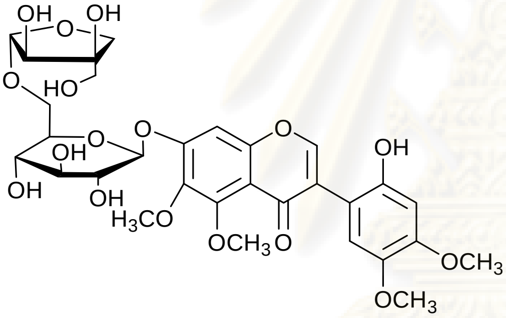
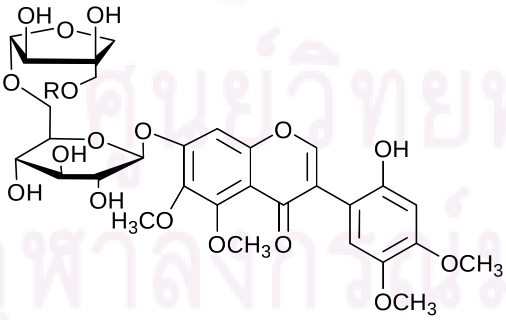
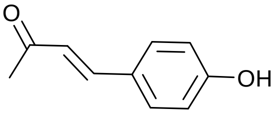
Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis pentaphylla			
Carbalexin-B [**202**] 	Carbazole alkaloid	Leaf	Pacher *et al*., 2001
Carbalexin-C [**203**] 	Carbazole alkaloid	Leaf	Pacher *et al*., 2001
Carbazole [**219**] 	Carbazole alkaloid	Root bark	Chowdhury *et al*., 1987
Citracridone-I [**138**] 	Acridone alkaloid	Stem	Ito *et al*., 1999
Dehydrothalebanin-B [**182**] 	Phenylethenyl amide derivative	Root	Shapiro, Bowman and Lapointe, 2000

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis pentaphylla			
Des-*N*-methylacronycine [**139**] 	Acridone alkaloid	Stem	Ito *et al*., 1999
Des-*N*-methylnoracronycine [**140**] 	Acridone alkaloid	Stem	Ito *et al*., 1999
Dictamnine [**111**] 	Quinoline alkaloid	Root bark	Chakraborty, 1966
4´,5-Dihydroxy-3´,7-dimethoxyisoflavone 4´-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside [**220**] 	Isoflavone diglycosides	Stem	Wang *et al*., 2006

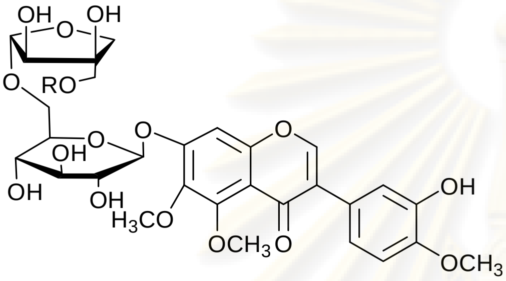
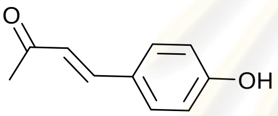
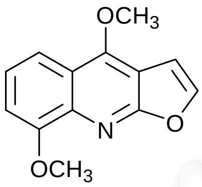
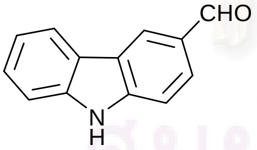
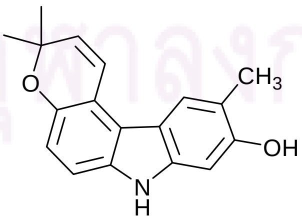
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis pentaphylla			
4,8-Dimethoxy-3-(3-methylbut-2-enyl)quinolin-2(1*H*)-one [**221**] 	Quinolone alkaloid	Leaf	Bhattacharyya and Chowdhury, 1985a
2´,7-Dihydroxy-4´,5´,5,6-tetramethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside [**222**] 	Isoflavone diglycosides	Stem	Wang *et al*., 2006
2´,7-Dihydroxy-4´,5´,5,6-tetramethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*-coumaroyl)-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside [**223**]  R =  (coumaroyl)	Isoflavone diglycosides	Stem	Wang *et al*., 2006

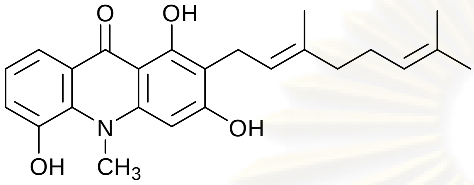
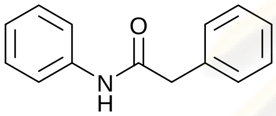
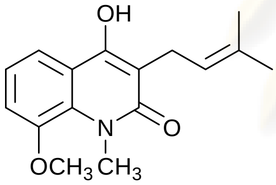
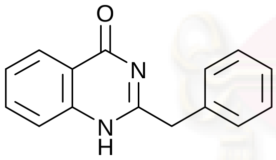
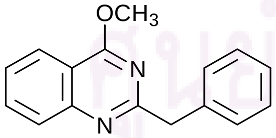
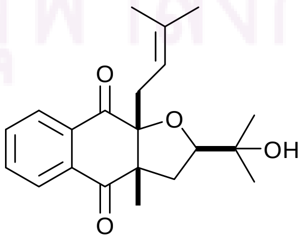
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis pentaphylla			
3´,7-Dihydroxy-4´,5,6-trimethoxyisoflavone 7-*O*-(5-*O*-*trans*-*p*coumaroyl)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside [**224**]  R =  (coumaroyl) 	Isoflavone diglycosides	Stem	Wang *et al.*, 2006
γ-Fagarine [**92**]  	Quinoline alkaloid	Root bark	Chakraborty, 1966
3-Formylcarbazole [**30**]  	Carbazole alkaloid	Root	Jash *et al.*, 1992
Glycoborinine [**96**]  	Carbazole alkaloid	Leaf	Pacher *et al.*, 2001

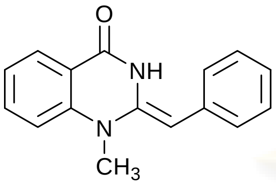
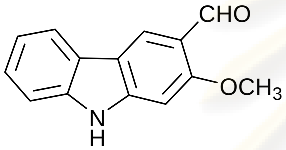
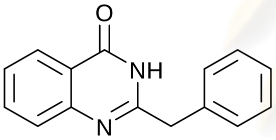
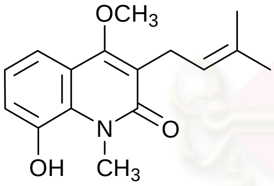
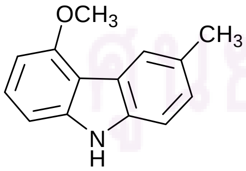
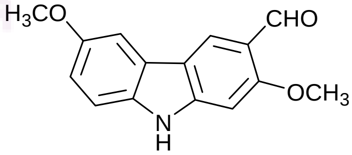
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis pentaphylla			
Glycocotrine-III [**225**] 	Acridone alkaloid	Stem	Ito *et al.*, 1999
Glycomide [**226**] 	Amide	Flower	Sarkar and Chakraborty, 1977
Glycophylone [**227**] 	Quinolone alkaloid	Seed	Bhattacharyya and Chowdhury, 1984
Glycophymine [**228**] 	Quinazolone alkaloid	Flower	Sarkar and Chakraborty, 1977
Glycophymoline [**229**] 	Quinazoline alkaloid	Flower	Sarkar and Chakraborty, 1979
Glycoquinone [**230**] 	Naphthoquinone	Stem	Ito *et al.*, 1999

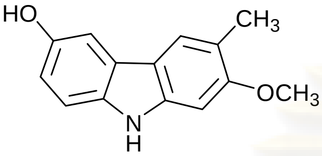
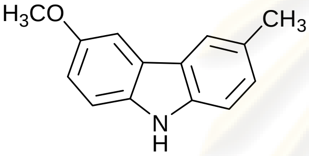
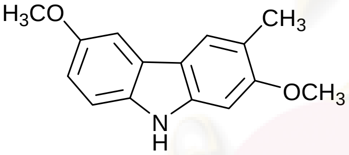
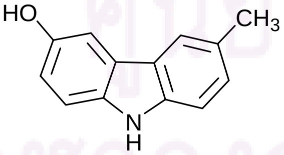
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis pentaphylla			
Glycosine [**231**] 	Quinazolone alkaloid	Leaf	Chatterjee and Majumdar, 1954
Glycosinine [**232**] 	Carbazole alkaloid	Root	Jash *et al*., 1992
Glycosminine [**102**] 	Quinazolone alkaloid	Leaf	Chatterjee and Majumdar, 1954
Glycosolone [**233**] 	Quinolone alkaloid	Root bark	Das *et al*., 1982
Glycozolicine [**234**] 	Carbazole alkaloid	Leaf Root	Muthukrishman *et al*., 1999 Jash *et al*., 1992
Glycozolidal [**235**] 	Carbazole alkaloid	Root	Bhattacharyya and Chowdhury, 1985b

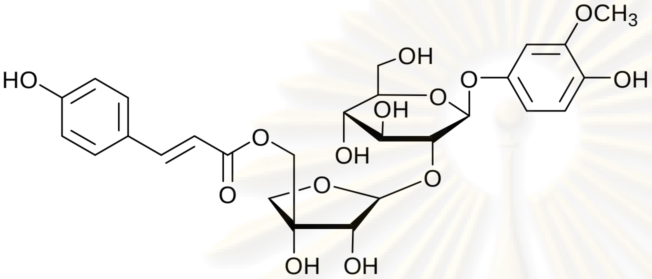
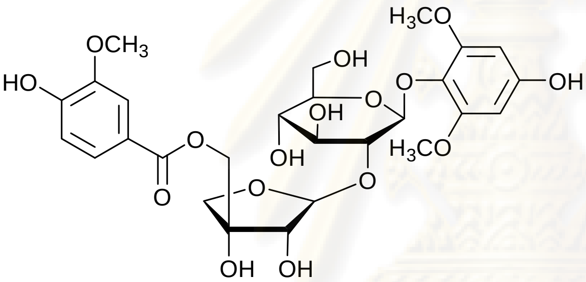
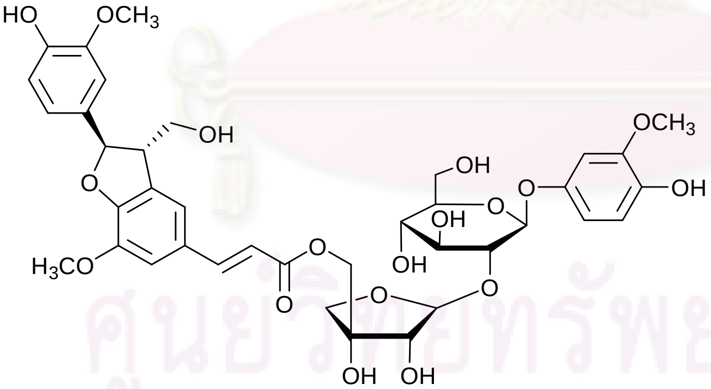
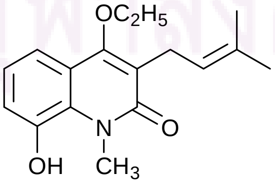
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis pentaphylla			
Glycozolidol [**188**] 	Carbazole alkaloid	Root	Bhattacharyya, Chakrabartty and Chowdhury, 1985
Glycozoline [**104**] 	Carbazole alkaloid	Leaf Root bark	Chakraborty, Roy and Chakraborty, 1989 Chakraborty, 1966, 1969
Glycozolidine [**103**] 	Carbazole alkaloid	Leaf Root bark	Chakraborty, Roy and Chakraborty, 1989 Anwer, Kapil and Popli, 1972
Glycozolinine [**105**] (= Glycozolinol) 	Carbazole alkaloid	Seed Root	Mukherjee, Mukherjee and Ganguly, 1983 Bhattacharyya *et al.*, 1984

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category, plant part and reference**
Glycosmis pentaphylla	
Glypentoside-A [**236**] 	Hydroquinone diglycoside acyl esters Stem Wang *et al.*, 2006
Glypentoside-B [**237**] 	Hydroquinone diglycoside acyl esters Stem Wang *et al.*, 2006
Glypentoside-C [**238**] 	Hydroquinone diglycoside acyl esters Stem Wang *et al.*, 2006
Homo-glycosolone [**239**] 	Quinolone alkaloid Root-bark Kumar, Das and Sinha, 1986

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

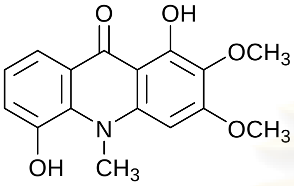
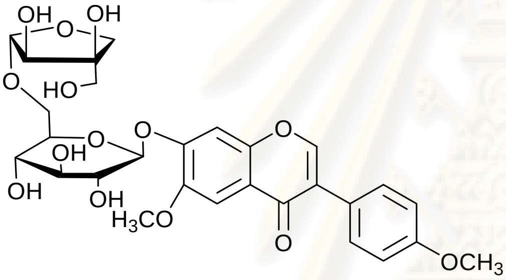
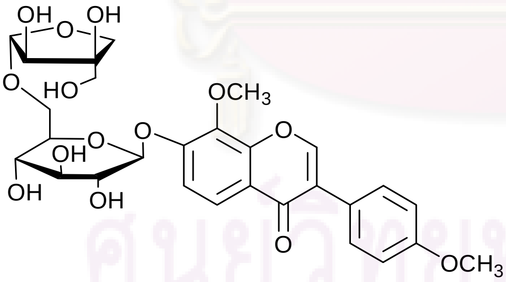
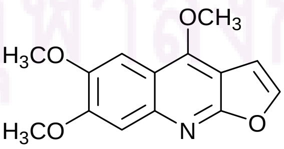
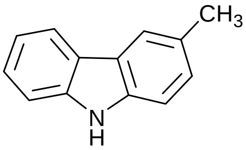
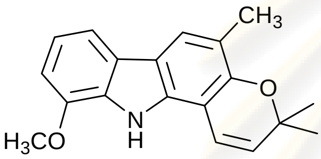
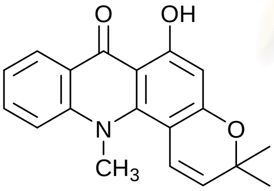
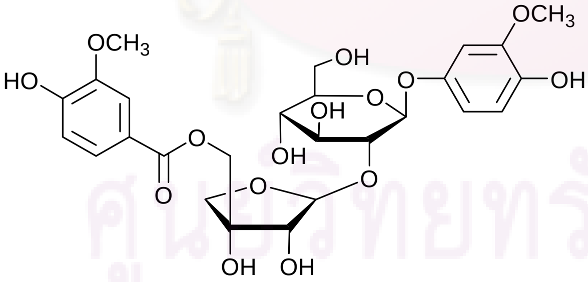
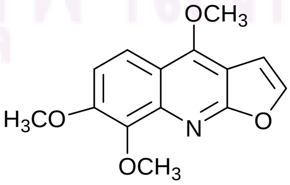
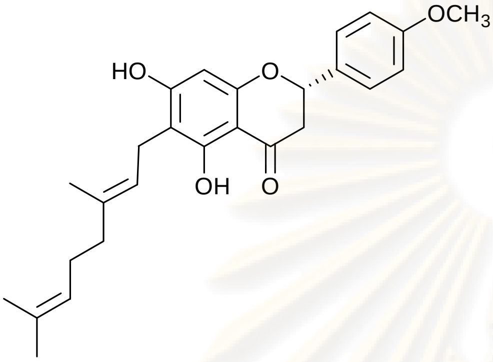
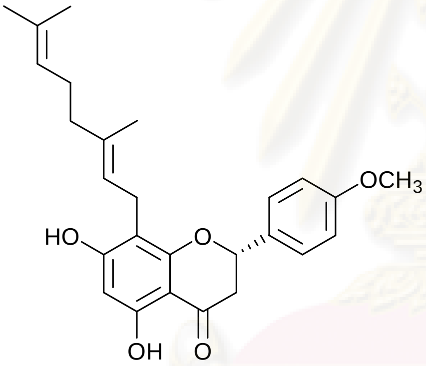
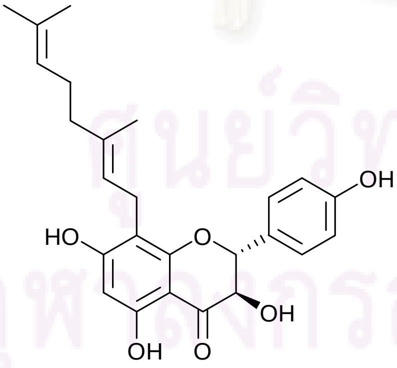
Plant and compound	Category	Plant part	Reference
Glycosmis pentaphylla			
5-Hydroxy-arborinine [**112**] 	Acridone alkaloid	Stem	Ito *et al*., 1999
7-Hydroxy-4´,6-dimethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside [**240**] 	Isoflavone diglycosides	Stem	Wang *et al*., 2006
7-Hydroxy-4´,8-dimethoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside [**241**] 	Isoflavone diglycosides	Stem	Wang *et al*., 2006
Kokusaginine [**113**] 	Quinoline alkaloid	Stem	Ito *et al*., 1999

Table 2 (Continued)

Plant and compound	**Category, plant part and reference**
Glycosmis pentaphylla	
3-Methylcarbazole [**35**] 	Carbazole alkaloid Root bark Chowdhury *et al.*, 1987
Mupamine [**242**] 	Carbazole alkaloid Leaf Chakraborty, Roy and Chakraborty, 1989
Noracronycine [**173**] 	Acridone alkaloid Stem Ito *et al.*, 1999
Seguinoside F [**243**] 	Hydroquinone diglycoside acyl esters Stem Wang *et al.*, 2006
Skimmianine [**110**] 	Quinoline alkaloid Stem Ito *et al.*, 1999 Root bark Chakraborty, 1966

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis pseudoracemosa			
4′-*O*-Methylbonannione [**244**]  	Flavanone	Leaf	Lukaseder *et al*., 2009
Puyanin [**245**]  	Flavanone	Leaf	Lukaseder *et al*., 2009
Puyanol [**246**]  	Flavanonol	Leaf	Lukaseder *et al*., 2009

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

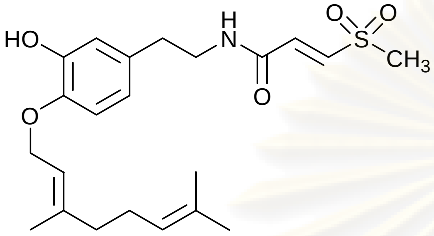
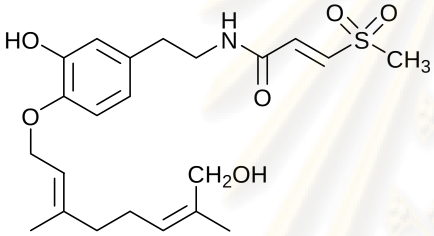
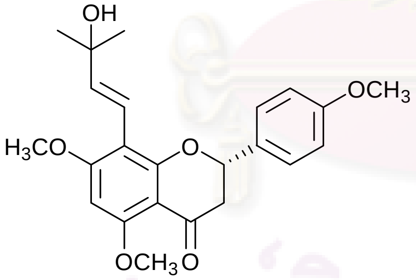
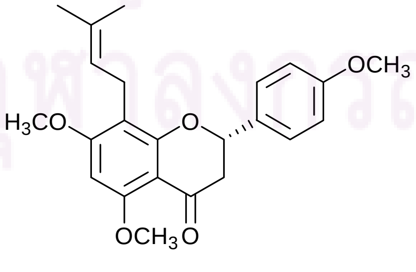
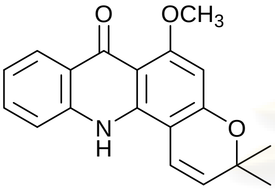
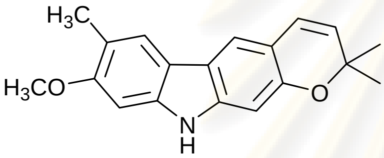
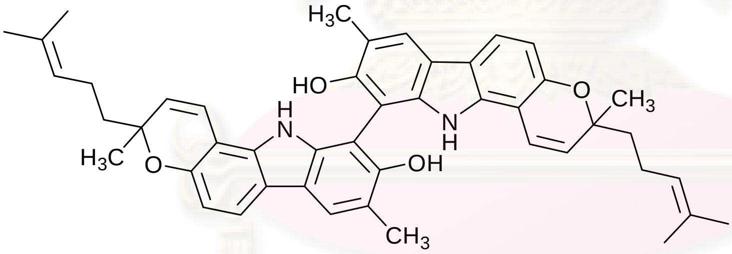
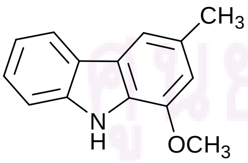
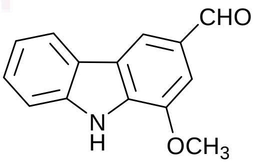
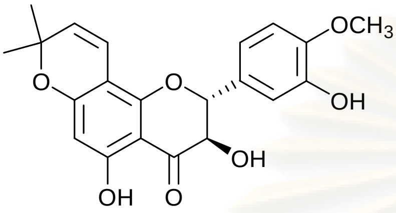
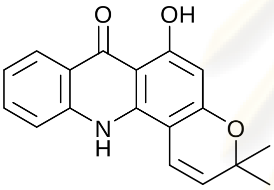
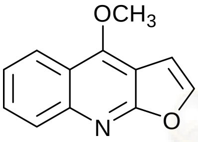
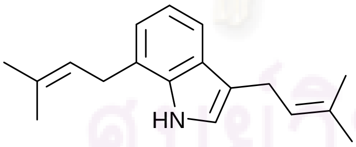
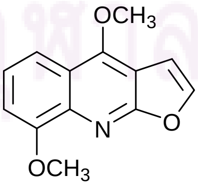
Plant and compound	Category	Plant part	Reference
Glycosmis pseudoracemosa			
Sakerine [**180**] 	Phenylethylamide derivative	Leaf	Hofer *et al*., 2000
Sakerinol-B [**247**] 	Phenylethylamide derivative	Leaf	Hofer *et al*., 2000
Glycosmis puberula			
Glypuberol [**248**] 	Flavanone	Leaf	Lukaseder *et al*., 2009
Mepuberin [**249**] 	Flavanone	Leaf	Lukaseder *et al*., 2009

Table 2 (Continued)

Plant and compound	Category, plant part Reference
Glycosmis rupestris	
Des-*N*-methylacronycine [**139**] 	Acridone alkaloid Bark Rahmani *et al*., 1998
7-Methoxyglycomaurin [**250**] 	Carbazole alkaloid Bark Rahmani *et al*., 1998
Glycosmis stenocarpa	
Bisisomahanine [**251**] 	Dimeric carbazole alkaloid Root Cuong *et al*., 2004
Murrayafoline-A [**252**] 	carbazole alkaloid Root Choi *et al*, 2010 Root Cuong *et al*., 2004
Murrayanine [**253**] 	carbazole alkaloid root Cuong *et al*., 2004

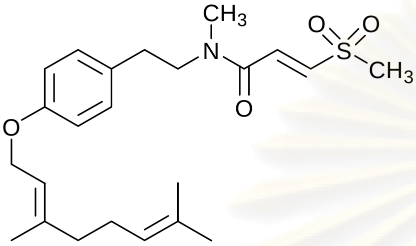
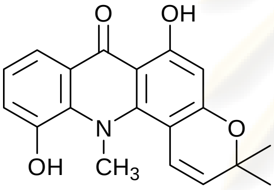
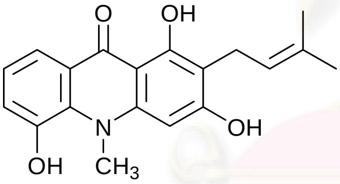
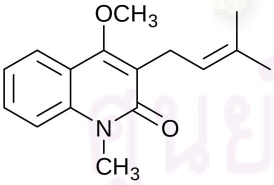
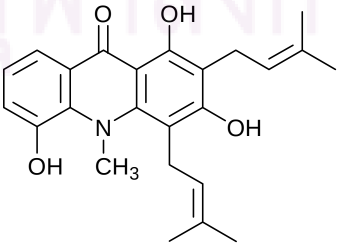
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis sapindoides Glysapinol [**254**] 	Flavanonol	Leaf	Lukaseder *et al*., 2009
Glycosmis trichanthera Des-*N*-methylnoracronycine [**140**] 	Acridone alkaloid	Root bark	Vajrodaya *et al*., 1998
Dictamnine [**111**] 	Quinoline alkaloid	Root bark	Vajrodaya *et al*., 1998
3,7-Diprenyl indole [**255**] 	Indole alkaloid	Root bark	Vajrodaya *et al*., 1998
γ-Fagarine [**92**] 	Quinoline alkaloid	Root bark	Vajrodaya *et al*., 1998

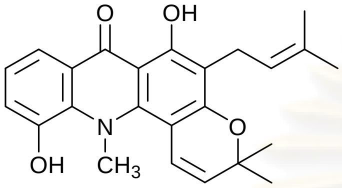
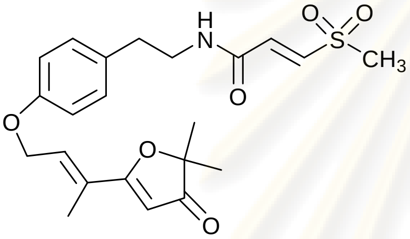
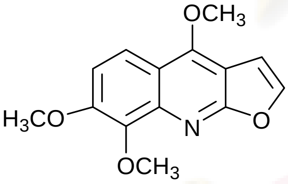
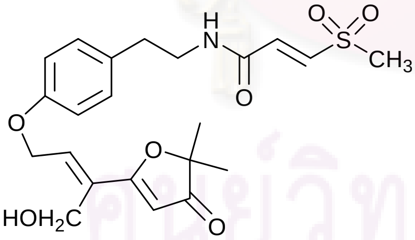
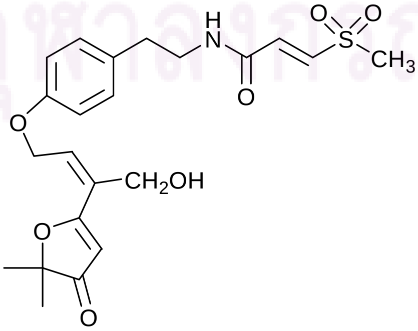
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis trichanthera			
Glyparvin-A [**214**]  	Phenylethylamide derivative	Leaf	Vajrodaya *et al*., 1998
5-Hydroxynoracronycine [**170**]  	Acridone alkaloid	Stem bark	Vajrodaya *et al*., 1998
Junosin [**256**]  	Acridone alkaloid	Stem bark	Vajrodaya *et al*., 1998
N-Methylatanine [**257**]  	Quinolone alkaloid	Root bark	Vajrodaya *et al*., 1998
N-Methylatalaphylline [**258**]  	Acridone alkaloid	Stem bark	Vajrodaya *et al*., 1998

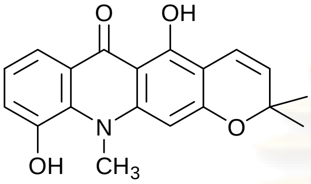
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	**Category**	**Plant part**	**Reference**
Glycosmis trichanthera			
N-methylatalaphyllinine [**169**] (= 5-Hydroxy-*N*-methylseverifoline) 	Acridone alkaloid	Stem bark	Vajrodaya *et al.*, 1998
Methylgerambullin [**82**] 	Phenylethylamide derivative	Leaf	Vajrodaya *et al.*, 1998
Skimmianine [**110**] 	Quinoline alkaloid	Root bark	Vajrodaya *et al.*, 1998
Trichanthins-A [**259**] 	Phenylethylamide derivative	Leaf	Vajrodaya *et al.*, 1998
Trichanthins-B [**260**] 	Phenylethylamide derivative	Leaf	Vajrodaya *et al.*, 1998

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 (Continued)

Plant and compound	Category	Plant part	Reference
Glycosmis trichanthera Yukocitrin [**261**] 	Acridone alkaloid	Stem bark	Vajrodaya *et al.*, 1998

3. Biological Activities of *Micromelum* spp.

3.1 Traditional Uses and Biological Activities of *Micromelum* spp.

Micromelum plants have been used in traditional medicine in many countries with several purposes. In Thailand, many parts of *M. minutum* (Forst. f.) Wight & Arn. have been used. For example, the roots have been used for the treatment of abscess and hemorrhoids (ชยันต์ พิเชียรสุนทร, แม้นมาส ชวลิต และ วิเชียร จีรวงส์, 2542). A decoction of the leaves has been used as a drink for anti-asthmatic property (Panthong, Kanjanapothi and Taylor, 1986). Moreover, the ethanolic extract of the root of *M. minutum* (Forst. f.) Wight & Arn, which has been used for the treatment of tumor, was examined for cytotoxicity against the human cancer cell lines: large cell lung carcinoma (COR-L23), breast adenocarcinoma (MCF-7) and colon adenocarcinoma (LS-174T). The percentage survival of COR-L23, MCF-7 and LS-174T cells treated with extract (concentration 50 μg/mL) were 72.7, 65.5 and 36.3, respectively (Itharat *et al.*, 2004). In Malaysia, *M. minutum* (Forst. f.) Seem. has been used for the treatment of fever and giddiness and as a poultice of the roots for ague (Rahmani *et al.*, 1993). In the islands of Rotuma group (in the Pacific), the bark of *M. minutum* (Forst. f.) Seem. has been used for the treatment of amenorrhea and thoracic pain, while the leaves have been used for toothache, tonic (leaves were incorporated into tonics given to prevent illness, particularly in children) and infection (McClatchey, 1996). Also in the kingdom of Tonga, the bark infusion of *M. minutum* (Forst. f.) Seem. is internally taken for the treatment of stomachache. In addition, the leaf infusion is gargled in the mouth for the treatment of toothache (Whistler, 1991).

3.2 Biological Activities of Compounds Isolated from *Micromelum* spp.

Micromelin [**5**] demonstrated a significant cytotoxicity against *in vivo* P-388 lymphocytic leukemia cells with T/C 149% at 10 mg/Kg [%T/C (median survival of treated animals: median survival of control animals x 100)] (Cassady *et al*., 1979).

Microminutin [**66**] showed activity with an ED$_{50}$ of 3.7 μg/mL against *in vitro* P-388 lymphocytic leukemia cells (Tantivatana *et al*., 1983).

Mahanine [**58**] showed a wide variety of biological activities. Thus it showed cytotoxicity against HL-60 tumor cell line (human promyelocytic leukemia cells) with a MIC$_{100}$ of 4.0 μg/mL (Nakahara *et al*., 2002). Mahanine also induced apoptosis in human leukemia cells (HL-60). At the concentration of 10 μM, mahanine caused a complete inhibition of cell proliferation and the induction of apoptosis in a time dependent manner (Roy *et al*., 2004). Moreover, mahanine inhibited growth and induced apoptosis in prostate cancer cells in a dose and time-dependent manner (Sinha *et al*., 2006). In addition, mahanine exhibited antimutagenicity against heterocyclic amines such as Trp-P-1 with an IC$_{50}$ of 5.2 μM (Nakahara *et al*., 2002). Mananine also displayed antimicrobial activity against *Bacillus cereus* and *Staphylococcus aureus* with MIC$_{100}$ values of 6.25 and 12.5 μg/mL, respectively (Nakahara *et al*., 2002).

Micromolide [**37**] showed potent in vitro anti-tuberculosis activity against *Mycobacterium tuberculosis* strain H37Rν with an MIC value of 1.5 μg/mL. Also, micromeline [**38**], lansine [**33**], 3-formylcarbazole [**30**] and 3-formyl-3-methylcarbazole [**31**] showed anti-TB activity with MIC values of 31.5, 14.3, 42.3 and 15.6 μg/mL, respectively (Ma *et al*., 2005)

4. Biological Activities of *Glycosmis* spp.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.1 Traditional Uses and Biological Activities of *Glycosmis* spp.

G. pentaphylla and *G. cochinchinensis* have been used in Thai traditional medicine: barks, flowers and fruits, and roots for the treatment of abscess scabies and snakebite, respectively (นิจศิริ เรืองรังษี และ ธวัชชัย มังคละคุปต์, 2547, วุฒิ วุฒิธรรมเวช และ ธนศักดิ์ วุฒิธรรมเวช, 2540). *G. arborea* has been used in India against fever and liver complaints (Chakravarty *et al*., 1999). The leaves of *G. arborea* has been used extensively in the

Ayurvedic system of medicine as febrifuge and anthelmintic (Chakravarty *et al*., 1961). *G. citrifolia* is known in Chinese traditional medicine for the treatment of skin itch, scabies, boils and ulcers (Wu *et al*., 1983). The root of *G. pentaphylla* was reported to have pesticidal property (Das *et al*., 1982). In Malaysia, the root of *G. rupestris* has been medicinally used for the treatment of fever and swollen spleen, and as a stimulant to digestion (Rahmani *et al*., 1998).

4.2 Biological Activities of Compounds Isolated from *Glycosmis* spp.

Several biological activities of the compounds isolated from *Glycosmis* spp. have been reported. Most of them are cytotoxicity, antitumor activity, antiviral activity and antimalarial activity of acridone alkaloids. For sulfur-containing propanamides, antifungal activities were reported.

Glycocitrine-I [**157**], des-*N*-methylnoracronycine [**140**], 5-hydroxy-*N*-methylseverifoline [**169**], atalaphyllidine [**137**], *N*-methylatalaphylline [**258**] and glycobismine-A [**147**] exhibited antimalarial activities that were comparable to or greater than that of chloroquine diphosphate. At a concentration of 10 μg/mL *in vitro*, 5-hydroxynoracronycine [**170**] suppressed almost 90% of *Plasmodim yoelii*, which causes malaria in rodents (Fujioka *et al*., 1989).

Atalaphillidine [**137**], citracridone-I [**138**] and 5-hydroxy-*N*-methylseverifoline [**169**] showed antiviral activity against herpes simplex virus type 2 (HSV-2) by plaque reduction assays with ED_{50} values of 0.73, 1.3 and 2.0 μg/mL, respectively (Yamamoto *et al*., 1989)

Sulfur-containing amide compounds, illukumbin-B [**194**], methylillukumbin-B [**196**] and methylillukumbin-A [**195**], exhibited antifungal activity against *Cladosporium cladosporioides*. Methylillukumbin-A [**195**], an all-*trans* orientated isomer, was the most active compound of this series; a clearly visible inhibition zone, even at the lowest concentration at 10 μg/mL was observed (Greger *et al*., 1993b). Later, seven sulfur-containing amide compounds, dehydroniranin-A [**184**], dehydroniranin-B [**185**], methylillukumbin-A [**195]**, methylsinharin [**189]**, niranin [**197**], penangin [**128**] and ritigalin [**198**], were examined for the antifungal activity against phytopathogenic fungus *Cladosporium herbarum* in germtube inhibition test and insecticidal activity against neonate larvae of *Spodoptera littoralis*. The most active antifungal amide was found to be methylillukumbin-A [**195**] with an ED_{50} of 5.5 μg/mL. In parallel tests against neonate larvae of *S. littoralis*, ritigalin [**198**] possessed pronounced contact toxicity with a LC_{50} of 0.05 μmol/dm^2 (Greger *et al*., 1996)

Atalaphyllidine [**137**] and citracridone-I [**138**] almost completely inhibited platelet aggregation induced by arachidonic acid (100 μM), collagen (100 μg/mL) and PAF (2 ng/mL). Atalaphyllidine [**137**], des-*N*-methylnoracronycine [**140**] and 4,8-dimethoxy-1-methyl-3-(3-methylbut-2-enyl)quinolin-2(1*H*)-one [**89**] also showed an inhibitory effect on arachidonic acid and collagen-induced rabbit platelet aggregation (Leu *et al*., 1998).

The EtOAc fraction of *G. pentaphylla* leaf extract inhibited the juvenile hormone III-biosynthesis of the field cricket *Gryllus bimaculatus in vitro*. The bioactive compound responsible for this activity was identified as arborine [**86**] which also showed a larvicidal activity against the mosquito *Culex quinquefasciatus* (Muthukrishnan *et al*., 1999).

Several acridone alkaloids have been reported to possess antiproliferative effect. Atalaphyllidine [**137**] and des-*N*-methylnoracronycine [**140**] inhibited clonal proliferation of HL-60 cell (human promyelocytic leukemia cells) and induced HL-60 cell differentiation (Kawaii *et al*., 1999a). Moreover, atalaphyllidine [**137**], 5-hydroxy-*N*-methylseverifoline [**169**] and des-*N*-methylnoracronycine [**140**] showed antiproliferative effect against several cancer cell lines: human lung carcinoma (A-549), melanin pigment-producing mouse melanoma (B16-melanoma 4A5), T-cell leukemia (CCRF-HSB-2) and human gastric cancer cell and lymph-node metastasized (TGBC11TKB) (Kawaii *et al*., 1999b). Arborinine [**87**] showed potent antiproliferative effect against cervix adenocarcinoma (HeLa) with an IC_{50} value of 1.84 μM which was lower than that of cisplatin (12.43 μM) (Réthy *et al*., 2006).

Glycocitrine-II **[158]** and *O*-methylglycocitrine-II **[171]** which had potent cancer prevention properties inhibited Epstein-Barr virus early antigen activation with IC_{50} values of 280 and 281 mol ratio/32 pmol TPA (12-*O*-tetradecanoylphorbol 13-acetate), respectively (Itoigawa *et al*., 2003).

Glyfoline **[168]** exhibited a significant inhibition of leukemic HL-60 cell growth *in vitro* with an IC_{50} of 1.1 μM (Su, Dziewiszek and Wu, 1991). Mechanistically, glyfoline **[168]** induced nasopharyngeal carcinoma (NPC) cell growth arrest at G2/M cell cycle phase and induced apoptosis by leakage of cytochrome C into the cytosol (Su *et al*., 2005).

CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

Micromelum hirsutum was collected twice from the area near Suratthani Rajabhat University, Muang district, Suratthani province, Thailand in January 2006 (first batch) and August 2007 (second batch).

Glycosmis parva was collected at Sakaerat, Wang Nam Khieo district, Nakorn Ratchasima province, Thailand in December 2007.

Both plants were identified by Assosciate Professor Dr. Nijsiri Ruangrungsi. Voucher specimens (NSR 490121 and NSR 500812) of *Micromelum hirsutum* and a voucher specimen (NSR 510209) of *Glycosmis parva* have been deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

2. General Techniques

2.1 Chromatographic Technique

2.1.1 Analytical Thin-Layer Chromatography (TLC)

Technique : One dimension, ascending
Adsorbent : Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness : 0.25 mm
Solvent system : Various solvent systems depending on materials
Distance : 5.0 cm
Temperature : Laboratory temperature 25-35 °C
Detection : 1) UV light at the wavelengths of 254 and 365 nm
2) phosphomolybdic acid reagent, heating at 110 °C for 2-5 minutes
3) Iodine vapor

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

2.1.2 Preparative Thin-Layer Chromatography (Preparative TLC)

Technique : One dimension, ascending

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

Layer thickness : 0.5 mm

Solvent system : Various solvent systems depending on materials

Distance : 20.0 cm

Temperature : Laboratory temperature 25-35 $^{\circ}$C

Detection : UV light at the wavelengths of 254 and 365 nm

2.1.3 Column Chromatography (CC)

Column : Flat bottom glass column (various diameters)

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

Packing method : Wet packing

Sample loading : 1) Dry packing

The sample was dissolved in a small volume of organic solvent, mixed with a small quantity of adsorbents, triturated, dried and then loaded on top of the column.

: 2) Wet packing

The sample was dissolved in a small volume of the eluent, then loaded on top of the column.

Solvent system : Various solvent systems depending on samples

Detection : Fractions were examined by TLC technique in the same manner as described in section 2.1.1

2.1.4 Flash Column Chromatography (Flash CC)

Column : Flat bottom glass column (various diameters) applied with positive pressure from pump at the top of column

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

Packing method : Wet packing

Sample loading : Dry packing and wet packing as described in section 2.1.3

Solvent system : Various solvent systems depending on samples

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Detection : Fractions were examined by TLC technique in the same manner as described in section 2.1.1

2.1.5 Vacuum Liquid Column Chromatography

Column : sintered glass column (various diameters) applied with negative pressure from aspirator pump

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

Packing method : Dry packing, 4 cm height.

Sample loading : Dry packing as described in section 2.1.3

Solvent system : Various solvent systems depending on samples

Detection : Fractions were examined by TLC technique in the same manner as described in section 2.1.1

2.1.6 Gel Filtration Chromatography

Gel Filter : Sephadex LH-20 (Pharmacia)

Packing method : Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.

Sample loading : The sample was dissolved in a small volume of the eluent and then applied gently on top of the column.

Detection : Fractions were examined by TLC technique in the same manner as described in section 2.1.1

Solvent system : 1) 50% CH_2Cl_2 in MeOH
2) 50% $CHCl_3$ in MeOH

2.1.7 Gel Filtration Chromatography (Recycling Preparative HPLC)

Instrument model : LC-9201 recycling preparative HPLC (Japan Analytical Industry)

Column : JAIGEL-2.5H column (600 × 200 mm^2)

Detector : UV-50

Sample loading : The sample was dissolved in a small volume of the eluent($CHCl_3$) and then injected into injection position and loaded it into the column

Detection : Fractions were examined by TLC technique in the same manner as described in section 2.1.1

Solvent system : $CHCl_3$

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Flow rate : 3.5 mL/min

2.2 Crystallization Technique

The compounds were crystallized from various solvents. Each compound was dissolved in appropriate solvents in test tube and stirred by a small spatula in water bath (about 60°C) until the stretch of crystal appeared and then left standing in ice bath until amorphous powder or crystals were grown.

Another crystallization technique was performed by the following: each compound was dissolved in appropriate solvents until saturated and left standing at room temperature until amorphous powder or crystals were formed.

2.3 Spectroscopy

2.3.1 Ultraviolet (UV) Absorption Spectra

UV spectra were recorded on a JASCO V-530 UV spectrophotometer (Department of Medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University).

2.3.2 Infrared (IR) Absorption Spectra

IR spectra were recorded on a JASCO IR-230E spectrophotometer (Department of Medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University).

2.3.3 Mass Spectra

Molecular ions were measured by Electron Impact Mass Spectrometry (EIMS). Low-resolution and high-resolution measurements were measured on a JEOL GC-Mate spectrometer (Department of Medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University).

Pseudo molecular ions were measured by Fast Atom Bombardment Mass Spectrometry (FABMS) or Electrospray Ionization Mass Spectrometry (ESIMS). Low-resolution and high-resolution measurements were measured on JEOL JMS-AX 500 and JEOL JMS-HX 110 spectrometers, respectively (Chemical Analysis Center, Chiba University). High-resolution ESI measurements were measured on Thermo Scientific Exactive (Chemical Analysis Center, Chiba University).

2.3.4 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H- and ^{13}C-NMR) Spectra

^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were obtained on a Bruker Avance DPX-300 FT-NMR spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded with a JEOL JNM ECP 400 spectrometer (Graduate School of Pharmaceutical Sciences, Chiba University).

^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded with a JEOL JNM ECP 600 spectrometer (Graduate School of Pharmaceutical Sciences, Chiba University).

Solvents for NMR spectra were deuterated chloroform ($CDCl_3$), deuterated acetone (acetone-d_6) or deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts were reported in ppm scale using the chemical shift of the solvent and internal standard (TMS) as the reference signals.

2.4 Physical Property

2.4.1 Melting Points

Melting points were measured on a micromelting point hot-stage apparatus (Yanagimoto) (Department of Medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University).

2.4.2 Optical Rotations

Optical rotations were recorded on a JASCO P-1020 polarimeter (Graduate School of Pharmaceutical Sciences, Chiba University).

2.4.3 Circular Dichroism Spectra

Circular dichroism spectra were obtained from a JASCO J-715 spectropolarimeter (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5 Solvents

Throughout this work, all organic solvents used in the extraction and isolation procedure were of commercial grade and were redistilled prior to use.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

3. Extraction and Isolation

3.1 Extraction and Isolation of Compounds from *Micromelum hirsutum*

3.1.1 Extraction

For the first batch of *Micromelum hirsutum*, the dried branches (260 g) were ground and then extracted successively with dichloromethane (CH_2Cl_2, 4 × 2 L) and methanol (MeOH, 4 × 2 L) to give, after removal of the organic solvent, a dichloromethane extract (2.7 g, 1.04% of dried weight) and a methanol extract (17.3 g, 6.65% of dried weight), respectively, as shown in **Scheme 1**.

The dried leaves of *Micromelum hirsutum* (280 g) were ground and then extracted successively with CH_2Cl_2 (4 × 2 L) and MeOH (4 × 2 L) to give, after removal of the organic solvent, a CH_2Cl_2 extract (21.7 g, 7.75% of dried weight) and a MeOH extract (39.6 g, 14.14% of dried weight), respectively, as shown in **Scheme 2**.

For the second batch of *Micromelum hirsutum*, the dried branches (4.5 Kg) were ground and extracted with hexane (4 × 12 L), CH_2Cl_2 (4 × 12 L) and MeOH (4 × 12 L) to give, on evaporation, 14.50 g of hexane extract (0.32% of dried weight), 13.1 g of CH_2Cl_2 extract (0.29% of dried weight) and 171.0 g of MeOH extract (3.80% of dried weight), respectively, as shown in **Scheme 3**.

The dried young branches (0.7 Kg) were ground and extracted with hexane (4 × 3 L), CH_2Cl_2 (4 × 3 L) and MeOH (4 × 3 L) to give, on evaporation, 5.55 g of hexane extract (0.79% of dried weight), 3.17 g of CH_2Cl_2 extract (0.45% of dried weight) and 56.30 g of MeOH extract (8.04% of dried weight), respectively, as shown in **Scheme 4**.

The dried leaves of *Micromelum hirsutum* (1.0 Kg) were ground and extracted with hexane (4 × 5 L), CH_2Cl_2 (4 × 5 L) and MeOH (4 × 5 L) to give, on evaporation, 36.0 g of hexane extract (3.60% of dried weight), 47.1 g of CH_2Cl_2 extract (4.7% of dried weight) and 170.0 g of MeOH extract (17.0% of dried weight), respectively, as shown in **Scheme 5**.

3.1.2 Separation of CH_2Cl_2 Extract of Branches (First Batch)

The CH_2Cl_2 extract (2.7 g) was dissolved in a small amount of CH_2Cl_2, triturated with silica gel 60 (No. 7734) and dried under room temperature. It was then fractionated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (No. 7334). Elution was completed in a polarity gradient manner with mixtures of hexane, acetone and MeOH (0%, 1%, 2%, 4%, 6%, 8%, 10%, 15%, 20%, 25%, 30% and 100% acetone in hexane, 50% MeOH in acetone, and 100% MeOH). The eluate was collected 100 mL per fraction and examined by TLC. Fractions (42 fractions) with similar chromatographic pattern were combined to yield four major fractions: Fractions MBC1-MBC4, as shown in **Scheme 6**.

3.1.2.1 Isolation of Compound MH1 (Scopoletin)

Fraction MBC4 (1.3 g) was further separated on a silica gel column (2% acetone in CH_2Cl_2). Fractions with similar chromatographic pattern were combined to yield six fractions (MBC41-MBC46). Fraction MBC43 (80 mg) was further fractionated on a silica gel column using 4% EtOAc in CH_2Cl_2 to give three fractions (MBC431-MBC433). Fraction MBC432 (65 mg) was subsequently separated by a silica gel (No.9385) column using 4% EtOAc in CH_2Cl_2 to give three fractions (MBC4321-4323). Fraction MBC4321 (50 mg) was further purified on a silica gel column (50% EtOAc in hexane) to give white solid of compound **MH1** (13 mg). This compound was eventually identified as scopoletin [**41**].

3.1.2.2 Isolation of Compound MH2 (Micromelin)

Fraction MBC42 (103 mg) was fractionated on a silica gel column eluted with 4% EtOAc in CH_2Cl_2 to give three fractions (MBC421-MBC423). Fraction MBC422 was repurified by a silica gel column using 4% EtoAc in CH_2Cl_2 again to give compound **MH2** (31 mg). This compound was later identified as micromelin [**5**].

3.1.3 Separation of CH_2Cl_2 Extract of Branches (Second Batch)

The CH_2Cl_2 extract (13.1 g) was dissolved in a small amount of CH_2Cl_2, triturated with silica gel 60 (No. 7734) and dried under room temperature. It was then subjected to vacuum liquid column chromatography using a sintered glass filter column of silica gel (No. 7334) eluted with mixtures of hexane, acetone and MeOH in a polarity gradient manner (0%, 6%, 8%, 12%, 15%, 20%, 30%, 60% and 100% acetone in hexane, 50% MeOH in acetone, and 100% MeOH). Fifty-seven 300-mL fractions were collected and combined according to their TLC patterns into ten combined fractions (MBC(2)1-MBC(2)10) as shown in **Scheme 7**.

3.1.3.1 Isolation of Compound MH3 (1,2-dimethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid)

Fraction MBC(2)10 (206 mg) was separated by silica gel column chromatography. Elution was performed in a polarity gradient manner with mixtures of CH_2Cl_2 and MeOH to give five fractions (MBC(2)101-MBC(2)105). Fraction MBC(2)102 (43 mg), after drying, was washed with MeOH to give compound MH3 (20 mg) as a white solid. This compound was identified as 1,2-dimethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [**262**], a new natural compound.

3.1.4 Separation of CH_2Cl_2 Extract of Leaves (First Batch)

The CH_2Cl_2 extract (21.6 g) was dissolved in a small amount of CH_2Cl_2, triturated with silica gel 60 (No. 7734) and dried under room temperature. It was then separated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (No. 7334). Elution was completed in a polarity gradient manner with mixtures of hexane, EtOAc and MeOH (0%, 1%, 5%, 10%, 15%, 20% and 25% EtOAc in hexane, 30% MeOH in EtOAc, and 100% MeOH). The eluate was collected 200 mL per fraction and examined by TLC. Thirty-seven fractions were combined to yield seven major fractions (MLC1-MLC7) as shown in **Scheme 8** and **9**.

3.1.4.1 Isolation of Compound MH2 (micromelin)

Fraction MLC7 (10.9 g) was fractionated by vacuum liquid column chromatography using sintered glass filter column of silica gel (No. 7334) eluted in a polarity gradient manner with mixtures of hexane, EtOAc, acetone and MeOH to give seven combined fractions (MLC71-MLC77). Fraction MLC74 (8.3 g) was further purified by silica gel column chromatography (2-100% EtOAc in CH_2Cl_2, gradient). Nine combined fractions (MLC741-MLC749) were obtained. Fraction MLC744 (355 mg) was crystallized from CH_2Cl_2-MeOH mixture to yield compound **MH2** (90 mg) as needles. It was identified as micromelin [**5**].

3.1.4.2 Isolation of Compound MH4 ((–)-(2′*S*, 3′*R*)-3′-Senecioyloxymarmesin)

Fraction MLC747 (1.3 g) was chromatographed on a silica gel column using 30% EtOAc in hexane to give five combined fractions (MLC7471-MLC7474). Fraction MLC7472 (695 mg) was further fractionated on Sephadex LH20 (50% CH_2Cl_2 in MeOH) to give five combined fractions (MLC74721-74725). The Major fraction MLC 74722 (474 mg) was purified on a silica gel column using 30% EtOAc in hexane to yield five combined fractions (MLC747221-747225). Fraction 747223 (377 mg) was crystallized from CH_2Cl_2-hexane mixture to give a white mixture (203 mg). A part (32 mg) of this mixture (203 mg) was purified by preparative TLC (thickness 0.5 mm; 10 x 20 cm^2; 20% EtOAc in toluene) to give compound **MH4** (11 mg). This compound was identified as (–)-(2′*S*, 3′*R*)-3′-Senecioyloxy marmesin [**263**].

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

3.2 Extraction and Isolation of Compounds from *Glycosmis parva*

3.2.1 Extraction

The dried, coarsely powdered branches of *Glycosmis parva* (12.4 Kg) were extracted with MeOH (4 × 30 L) to give, after removal of organic solvent, a syrupy MeOH extract (850.0 g, 6.85% of dried weight). The MeOH extract (850 g) was partitioned with hexane to give a hexane extract (72 g, 0.58% of dried weight). The remaining organic layer was added with water and further partitioned with ethylacetate (EtOAc) to yield an EtOAc extract (321 g, 2.59% of dried weight). The aqueous layer was partitioned with butanol (*n*-BuOH) to give an *n*-BuOH extract (85 g, 0.69% of dried weight). The remaining aqueous layer was dried by lyophilization to yield an aqueous extract (266 g, 2.15% of dried weight), as shown in **Scheme 10**.

The dried, coarsely powdered leaves of *Glycosmis parva* (4.8 Kg) were ground and extracted with MeOH (4 × 10 L) to give, after removal of organic solvent, a syrupy MeOH extract (650 g, 13.54% of dried weight). The MeOH extract (650 g) was partitioned with hexane to give a hexane extract (135 g, 2.81% of dried weight). The remaining organic layer was added with water and further partitioned with EtOAc to yield an EtOAc extract (100 g, 2.08% of dried weight). The aqueous layer was partitioned with *n*-BuOH to give an *n*-BuOH extract (93 g, 1.94% of dried weight). The remaining aqueous layer was dried by lyophilization to yield an aqueous extract (271 g, 5.65% of dried weight), as shown in **Scheme 11**.

3.2.2 Separation of EtOAc Extract of Branches

The EtOAc extract (29.3 g) of branches was divided into four portions (portion-I 5.1 g, portion-II 9.5 g, portion-III 8.2 g and portion-IV 6.4 g). Each portion was subjected to silica gel column chromatography eluted with a gradient solvent system of MeOH and $CHCl_3$ (0%, 1%, 2%, 5%, 10%, 20%, 30%, and 100% MeOH in $CHCl_3$). All fractions from four column chromatographies were combined based on chromatographic pattern to give thirteen combined fractions (GBE1-GBE13) as shown in **Scheme 12 and 13**.

3.2.2.1 Isolation of Compound GP1 (*N*-methylatalaphylline)

Fraction GBE4 (7.0 g) was triturated and washed by Et_2O. After filtration through a kiriyama funnel equipped with a vacuum set, compound **GP1** (850 mg, dried weight) was obtained as a yellow wet cake on a kiriyama filter paper. It was identified as *N*-methylatalaphylline [**258**].

3.2.2.2 Isolation of Compound GP2 (glycofolinine)

The evaporated filtrate (4.0 g) obtained from the filtration of fraction GBE4 was fractionated by silica gel column chromatography using 20-100% acetone in hexane to give ten combined fractions (GBE41-410). Fraction GBE45 (0.4 g) was further purified by silica gel column chromatography eluted with 20% acetone in hexane to furnish compound **GP2** (19 mg) as glycofolinine [**163**].

3.2.2.3 Isolation of Compound GP3 (citramine)

Fraction GBE6 (150 mg) was further purified by silica gel column chromatography using 30-40% acetone in hexane to give compound **GP3** (10.6 mg). This compound was identified as citramine [**264**].

3.2.2.4 Isolation of Compound GP4 (*N*-methylcyclo-atalaphylline-A)

Fraction GBE43 (300 mg) was washed with Et_2O to afford **GP4** (35 mg). This compound was identified as *N*-methylcyclo-atalaphylline-A [**265**].

3.2.2.5 Isolation of Compound GP5 (glycosparvarine)

Fraction GBE9 (590 mg) was triturated and washed with Et_2O. The soluble part (280 mg) was subjected to silica gel column chromatography (2% acetone in $CHCl_3$) to yield compound **GP5** (6.8 mg). This compound was newly identified as glycosparvarine [**266**].

3.2.2.6 Isolation of Compound GP6 (limonin) and Mixture GP7 (a mixture of limonexic acid and isolimonexic acid)

Fraction GBE5 (500 mg) was fractionated by silica gel column chromatogramphy using 20-30% acetone in hexane to give twelve combined fractions (GBE51-512). Fraction GBE59 (110 mg) was further purified by silica gel column chromatography eluted with 20-30% acetone in hexane to give compound **GP6** (6.5 mg) and mixture **GP7** (8 mg) which was obtained from later frations. Compound **GP6** was identified as limonin [**267**], while **GP7** was identified as a mixture of limonexic acid [**268**] and isolimonexic acid [**269**].

3.2.3 Separation of EtOAc Extract of Leaves

The syrupy EtOAc extract (11.6 g) of leaves was triturated with CH_2Cl_2 to give the soluble part (5.4 g) and the remaining (6 g). The soluble part was subjected to silica gel column chromatography eluted with a gradient solvent system of MeOH and $CHCl_3$ (0%, 0.5%, 1%, 2%, 5%, 10%, 20%, 30%, and 100% MeOH in $CHCl_3$). Fractions (twenty-five fractions) with silimar chromatographic pattern were combined to afford twelve fractions (GLE1-GLE12) as shown in **Scheme 14**, **15** and **16**.

3.2.3.1 Isolation of Compound GP8 (arborinine)

Fraction GLE3 (1.0 g) was washed with acetone and crystallized from CH_2Cl_2-acetone mixture to afford compound **GP8** (122.7 mg) as yellow needles. It was identified as arborinine [**87**].

3.2.3.2 Isolation of Compound GP9 ((+)-*S*-deoxydihydroglyparvin) and compound GP13 ((+)-tetrahydroglyparvin)

Fraction GLE6 (1.1 g) was separated to nine combined fractions (GLE61-GLE69) by Sephadex LH-20 column chromatography using 50% $CHCl_3$ in MeOH. Fraction GLE65 (184 mg) was crystallized from CH_2Cl_2-Et_2O mixture to give a solid (60 mg). The solid was subjected to silica gel flash column chromatography (40% Me_2CO/hexane) to give compound **GP9** (28 mg) and compound **GP13** (8 mg) which was obtained from earlier frations. Compound **GP9** was newly identified as (+)-*S*-deoxydihydroglyparvin [**270**], while the compound **GP13** was newly identified as (+)-tetrahydroglyparvin [**272**].

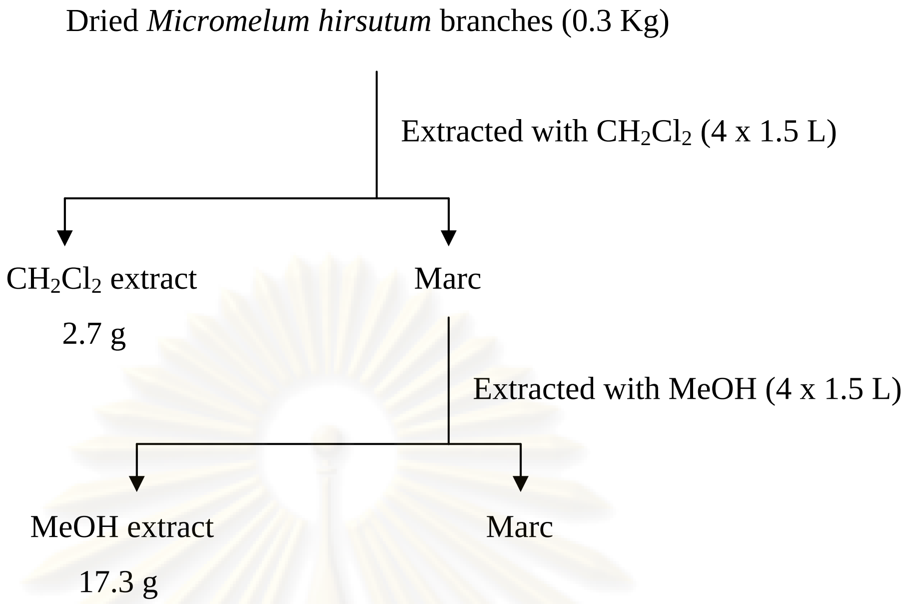
3.2.3.3 Isolation of Compound GP10 ((+)-*S*-deoxytetrahydroglyparvin)

A part (357 mg) of GLE7 (669 mg) was separated into six fractions (GLE71-GLE76) by Sephadex LH-20 column chromatography using 50% $CHCl_3$ in MeOH. GLE73 (255 mg) was further purified by silica gel column chromatography using 2% MeOH in $CHCl_3$ to afford six fractions (GLE731-GLE736). Also, GLE8 (244 mg) was separated into six fractions (GLE81-GLE86) by Sephadex LH-20 column chromatography using 50% $CHCl_3$ in MeOH. Fractions GLE734 (70 mg) and GLE84 (187 mg) were combined as GLE0708 (257 mg). Fraction GLE0708 was chromatographed on silica gel column (3%, 4%, 10%, 20%, 30%, and 100% MeOH/$CHCl_3$) to afford eight combined fractions (GLE07081-07088). Fraction GLE07083 (94 mg) was subjected to recycling preparative HPLC ($CHCl_3$ as a mobile phase) followed by twice silica gel column chromatography (2% MeOH in $CHCl_3$) to afford compound **GP10** (15 mg). Compound **GP10** was newly identified as (+)-*S*-deoxytetrahydroglyparvin [**271**].

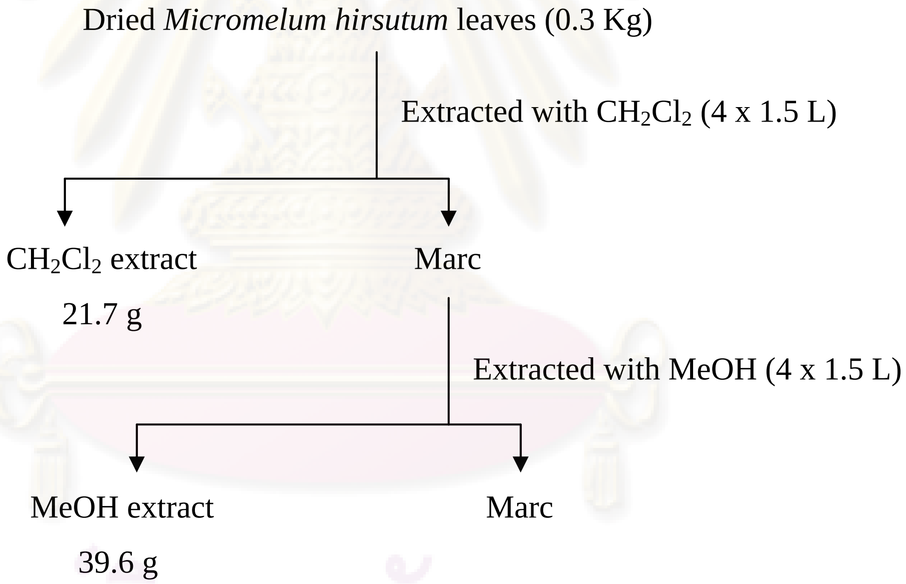
3.2.3.4 Isolation of Compound GP11 (glyparvin-A) and Compound GP12 ((+)-dihydroglyparvin)

GLE5 (550 mg) was separated into five fractions (GLE51-GLE55) by Sephadex LH-20 column chromatography using 50% $CHCl_3$ in MeOH. A part (79 mg) of GLE53 (260 mg) was washed with Et_2O and then recrystallized from CH_2Cl_2-Et_2O mixture to afford compound **GP11** (15 mg) as colorless needles. This compound was identified as glyparvin-A [**214**]. The evaporated mother liquor (44 mg) was further purified by repeated column chromatographies using different solvent systems (4% MeOH/$CHCl_3$, 60-70% EtOAc/hexane, and 45% EtOAc/$CHCl_3$) to furnish compound **GP12** (8 mg) as (+)-dihydroglyparvin [**213**].

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

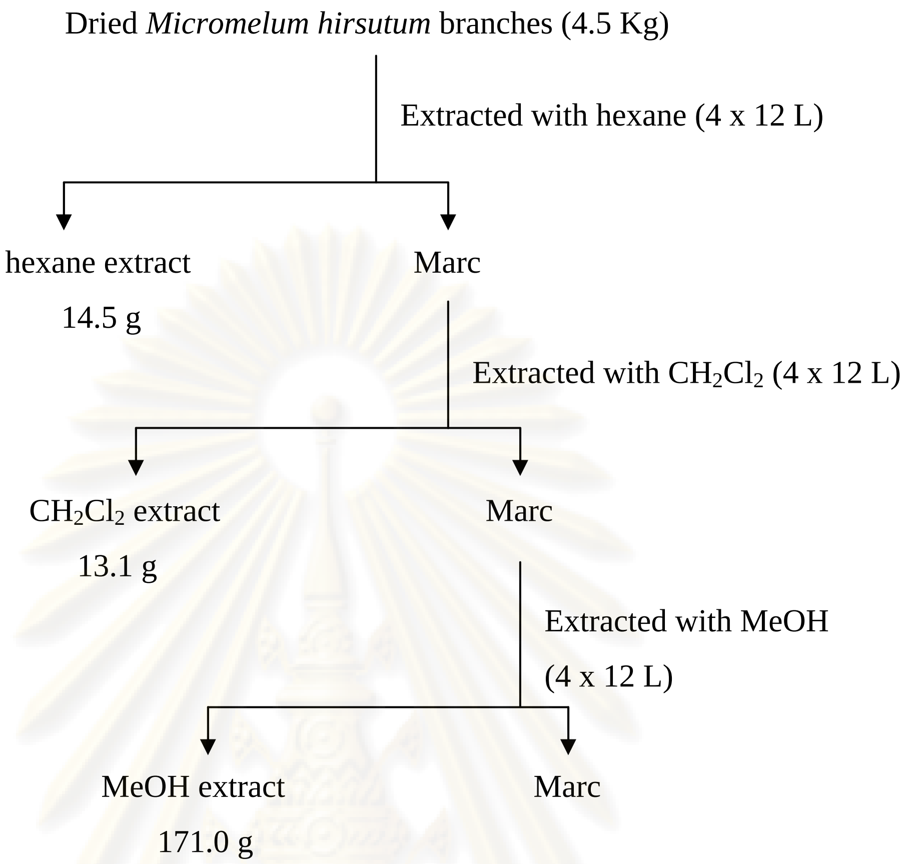


Scheme 1 Extraction of *Micromelum hirsutum* branches (first batch)



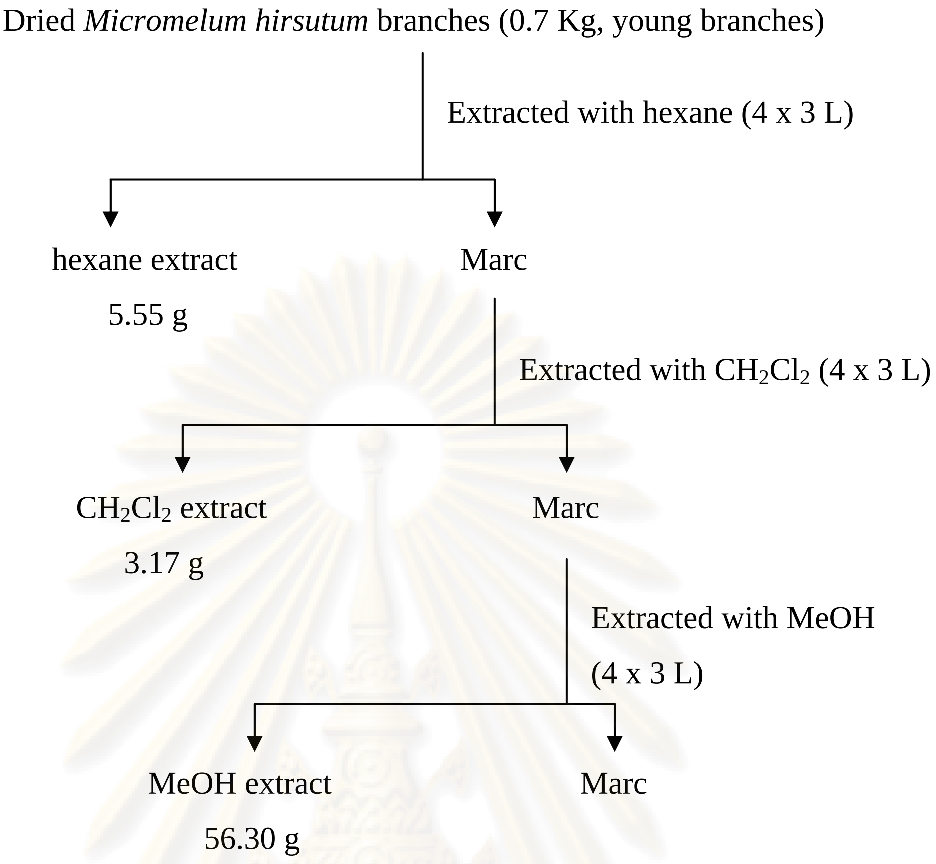
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Scheme 2 Extraction of *Micromelum hirsutum* leaves (first batch)



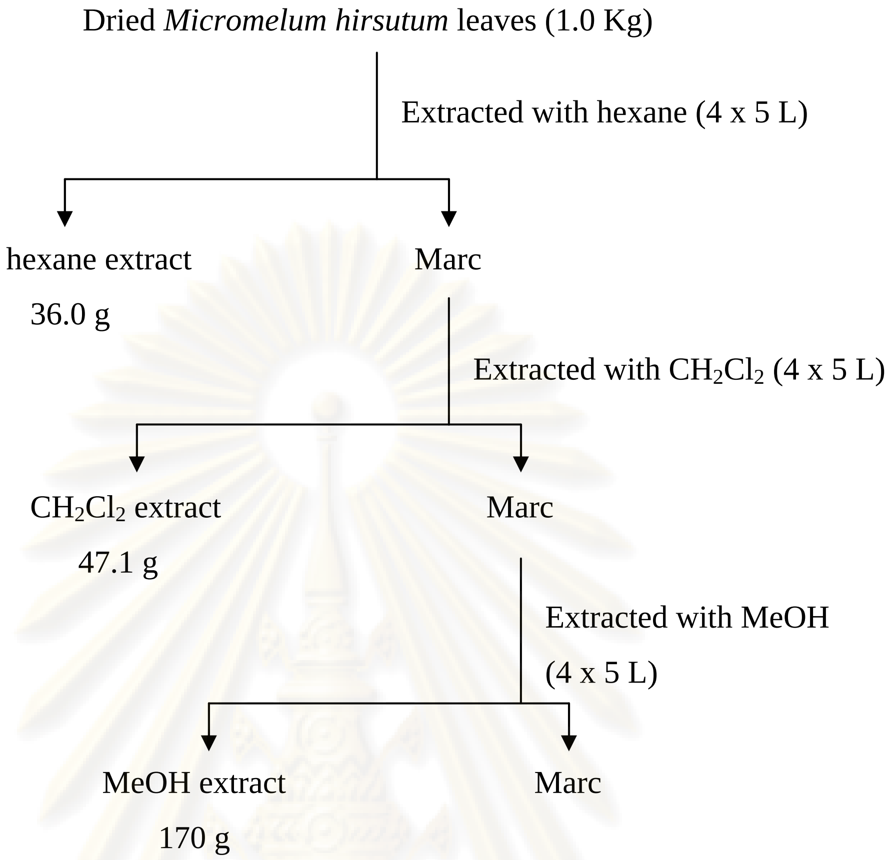
Scheme 3 Extraction of *Micromelum hirsutum* branches (second batch)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



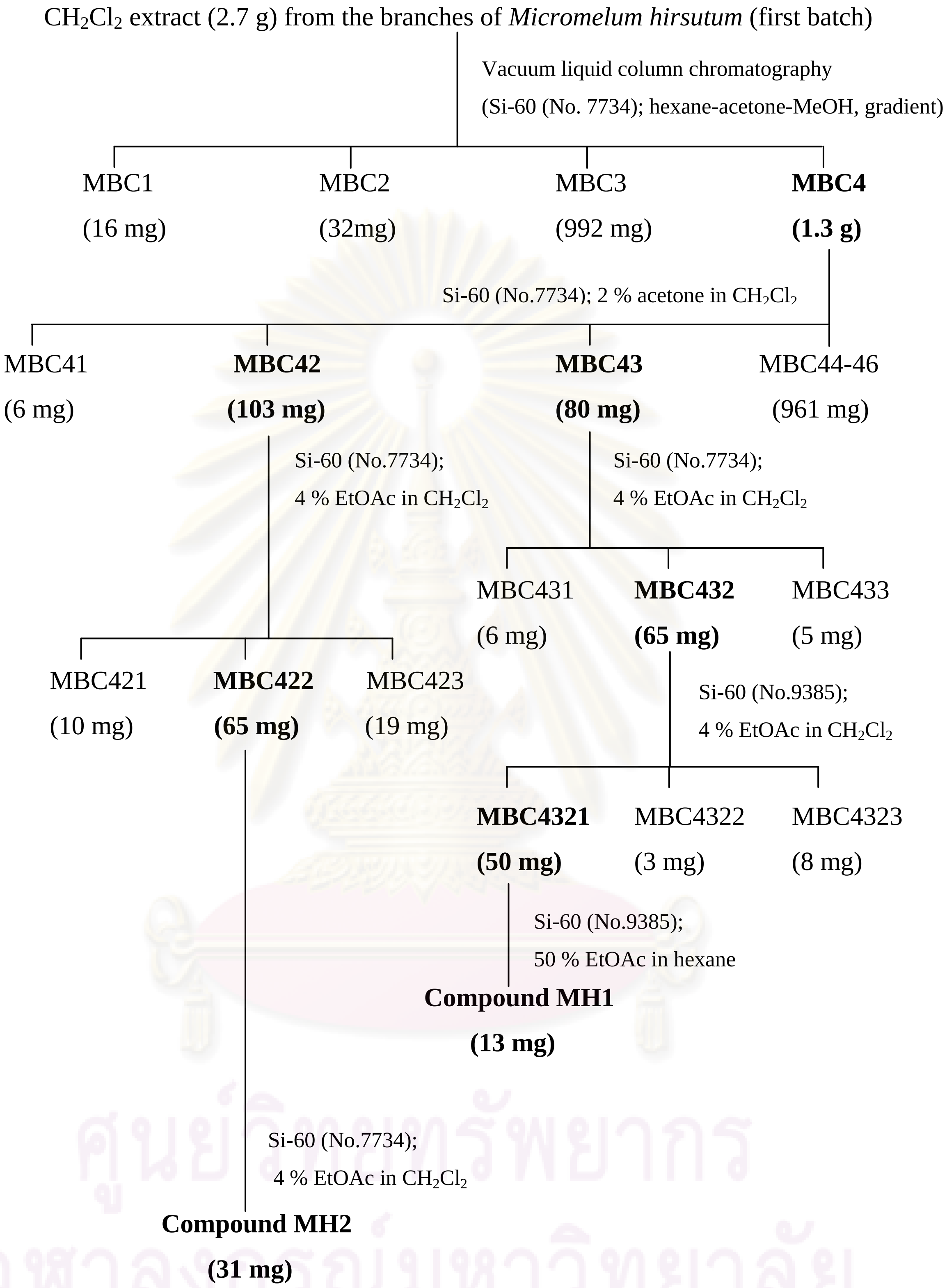
Scheme 4 Extraction of *Micromelum hirsutum* branches (young branches, second batch)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



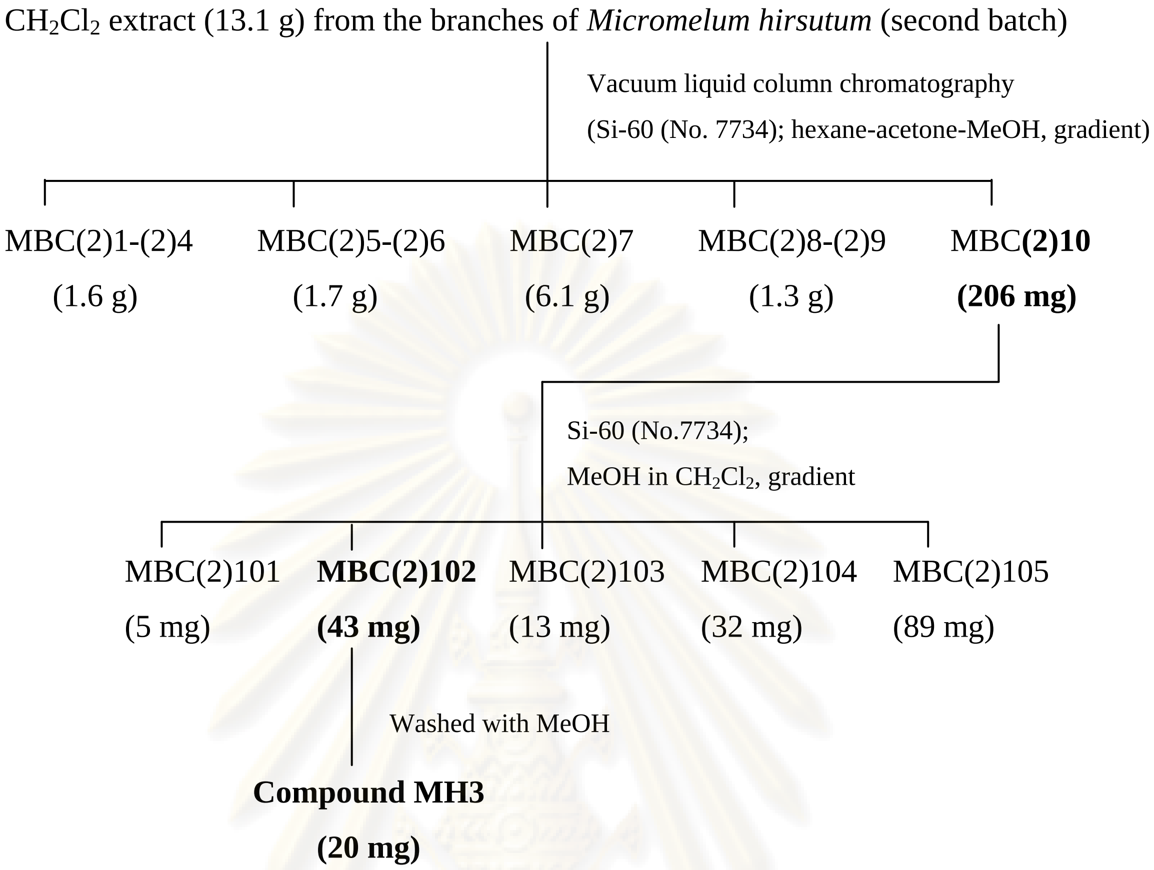
Scheme 5 Extraction of *Micromelum hirsutum* leaves (second batch)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



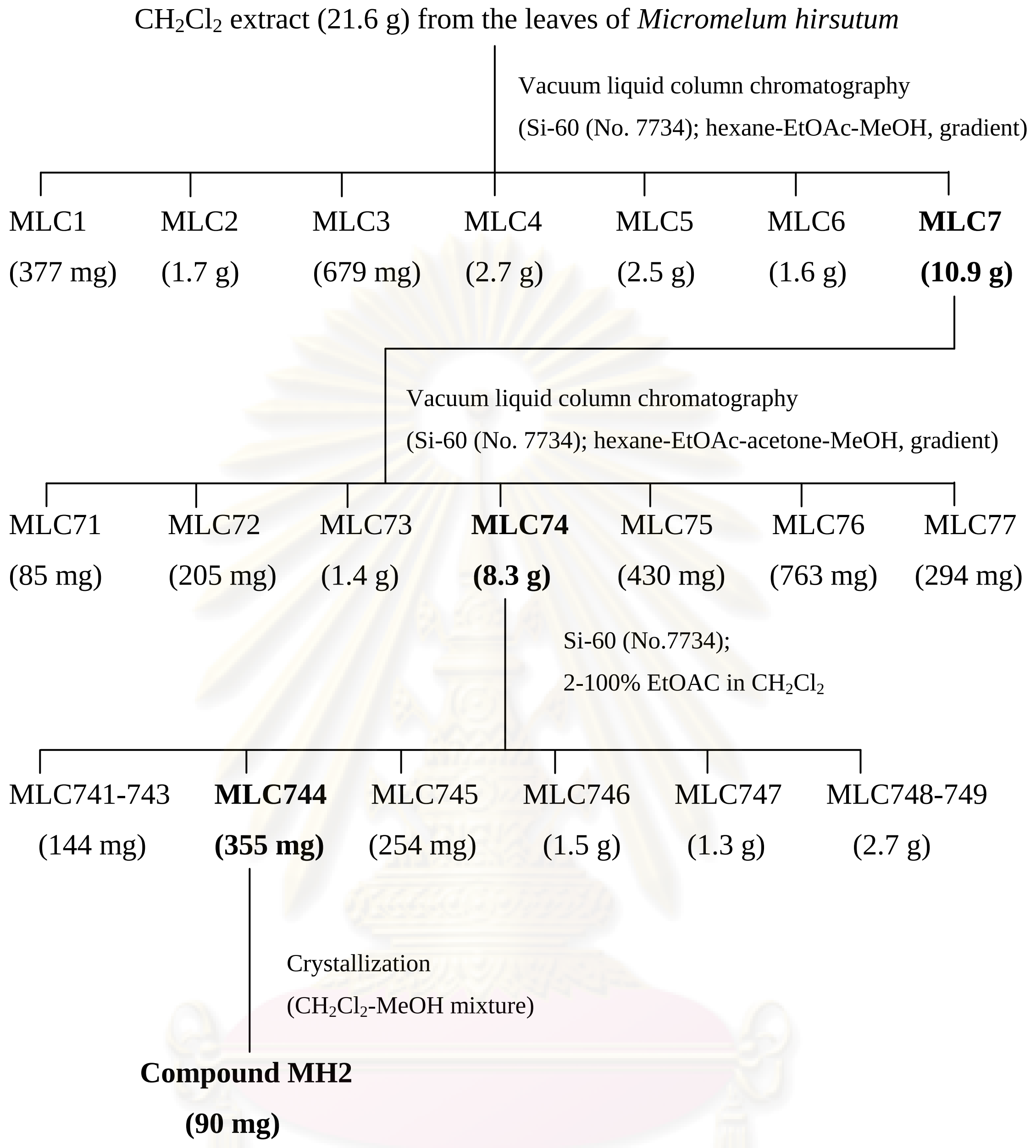
Scheme 6 Separation of CH_2Cl_2 extract (first batch) of the branches of *Micromelum hirsutum*

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



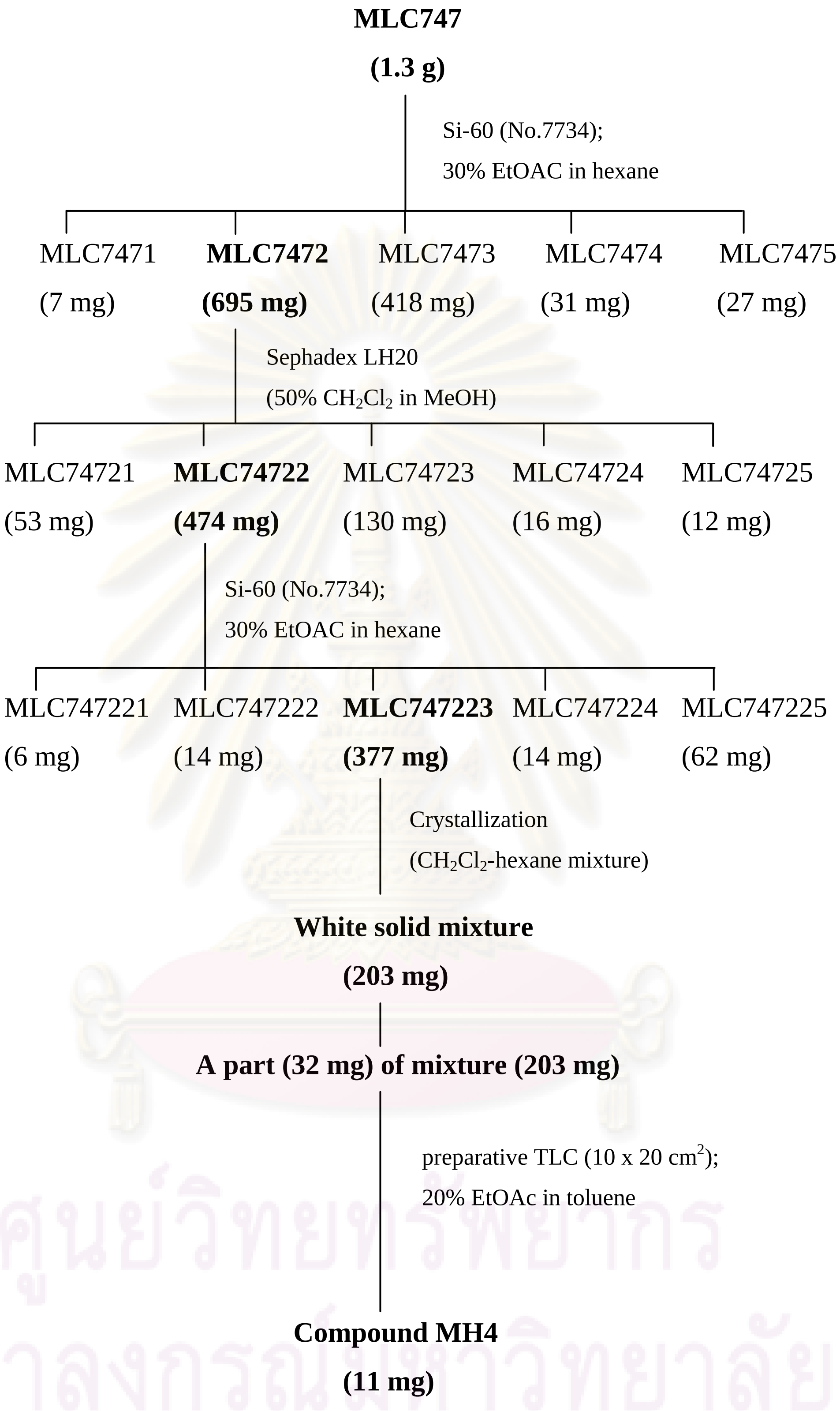
Scheme 7 Separation of CH_2Cl_2 extract (second batch) of the branches of *Micromelum hirsutum*

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



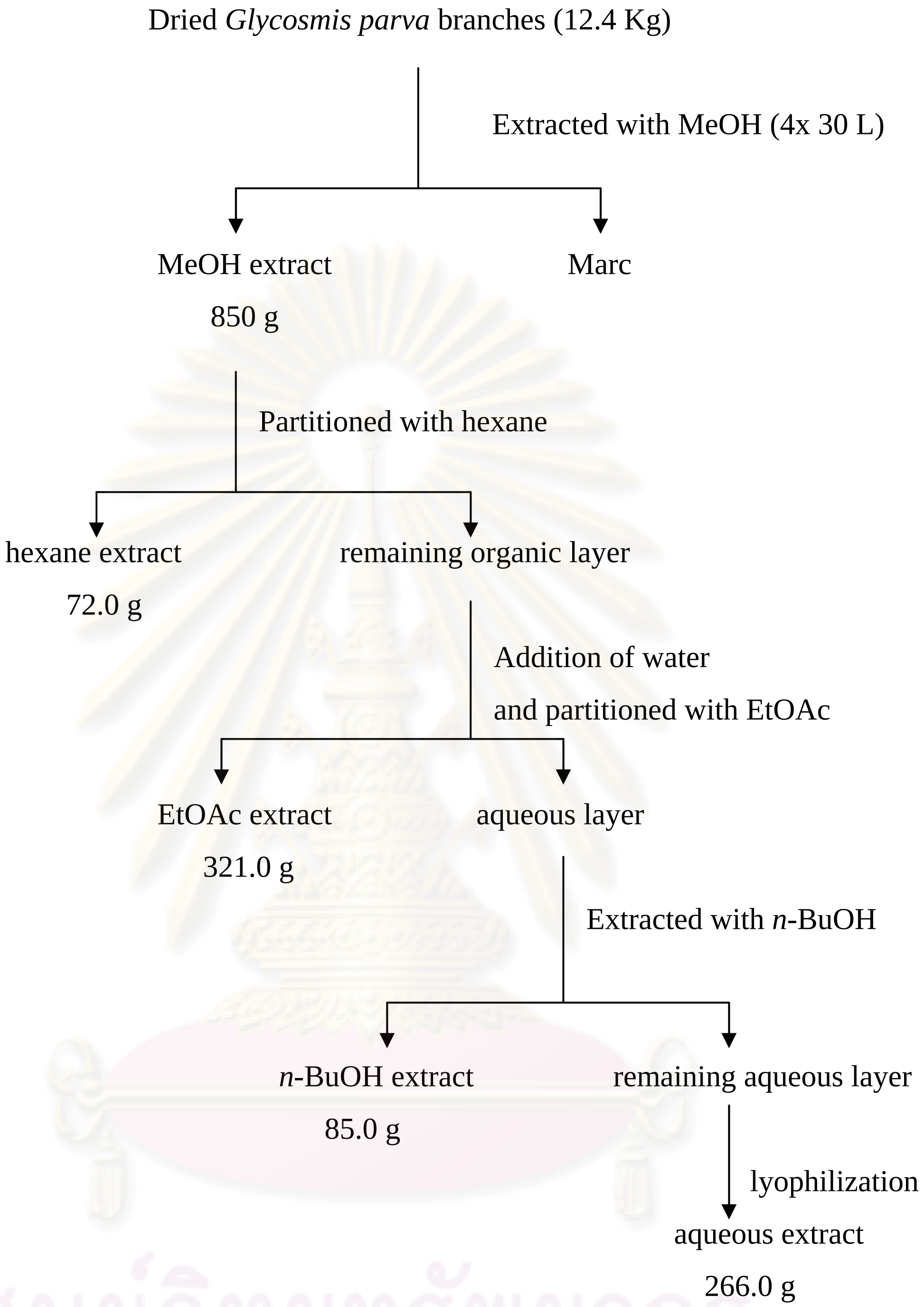
Scheme 8 Separation of CH_2Cl_2 extract of the leaves of *Micromelum hirsutum*

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



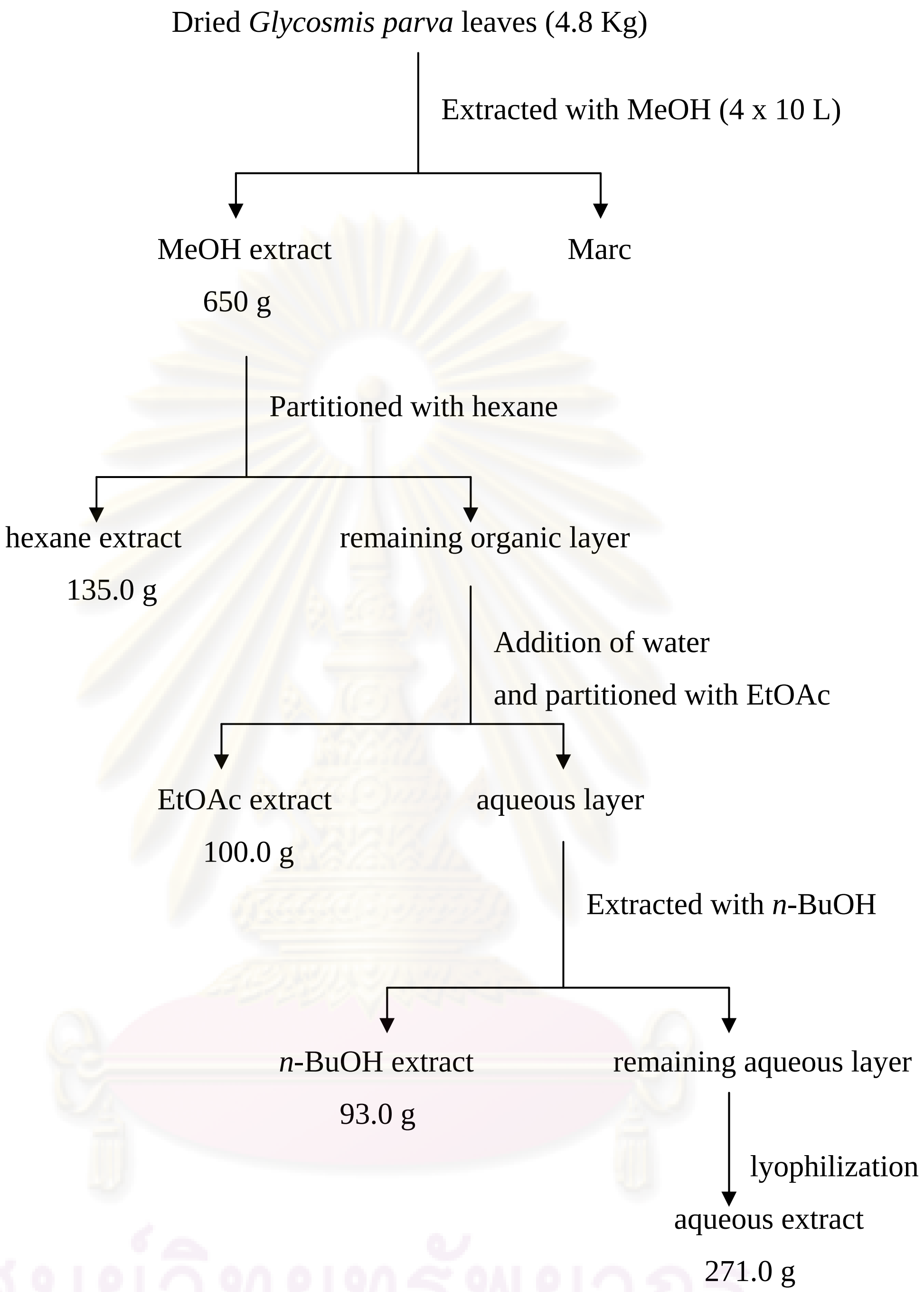
Scheme 9 Separation of fraction MLC 747 from CH_2Cl_2 extract of the leaves of *Micromelum hirsutum*

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



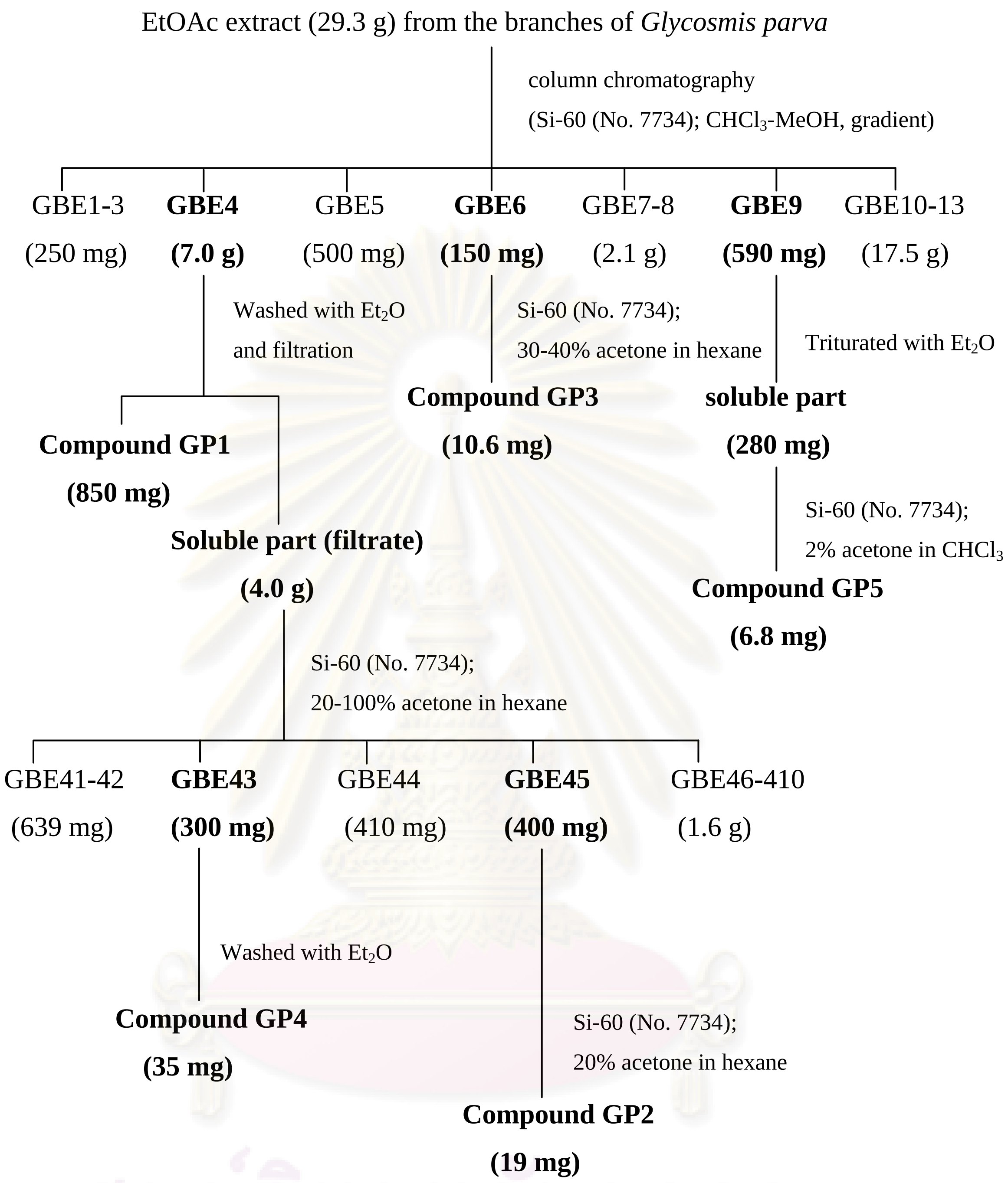
Scheme 10 Extraction of *Glycosmis parva* branches

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



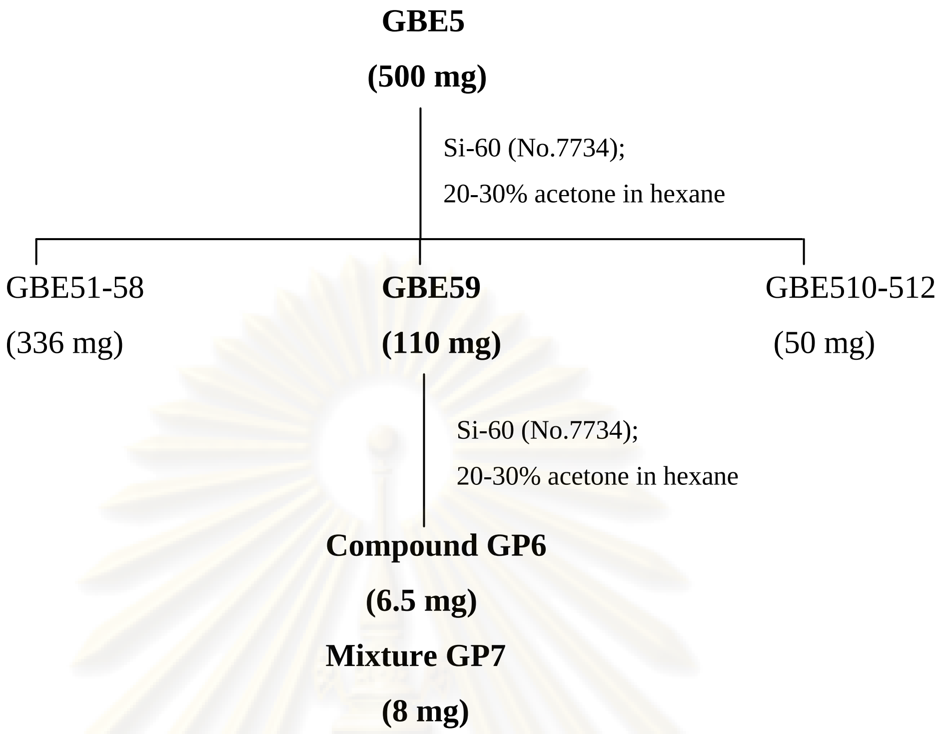
Scheme 11 Extraction of *Glycosmis parva* leaves

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



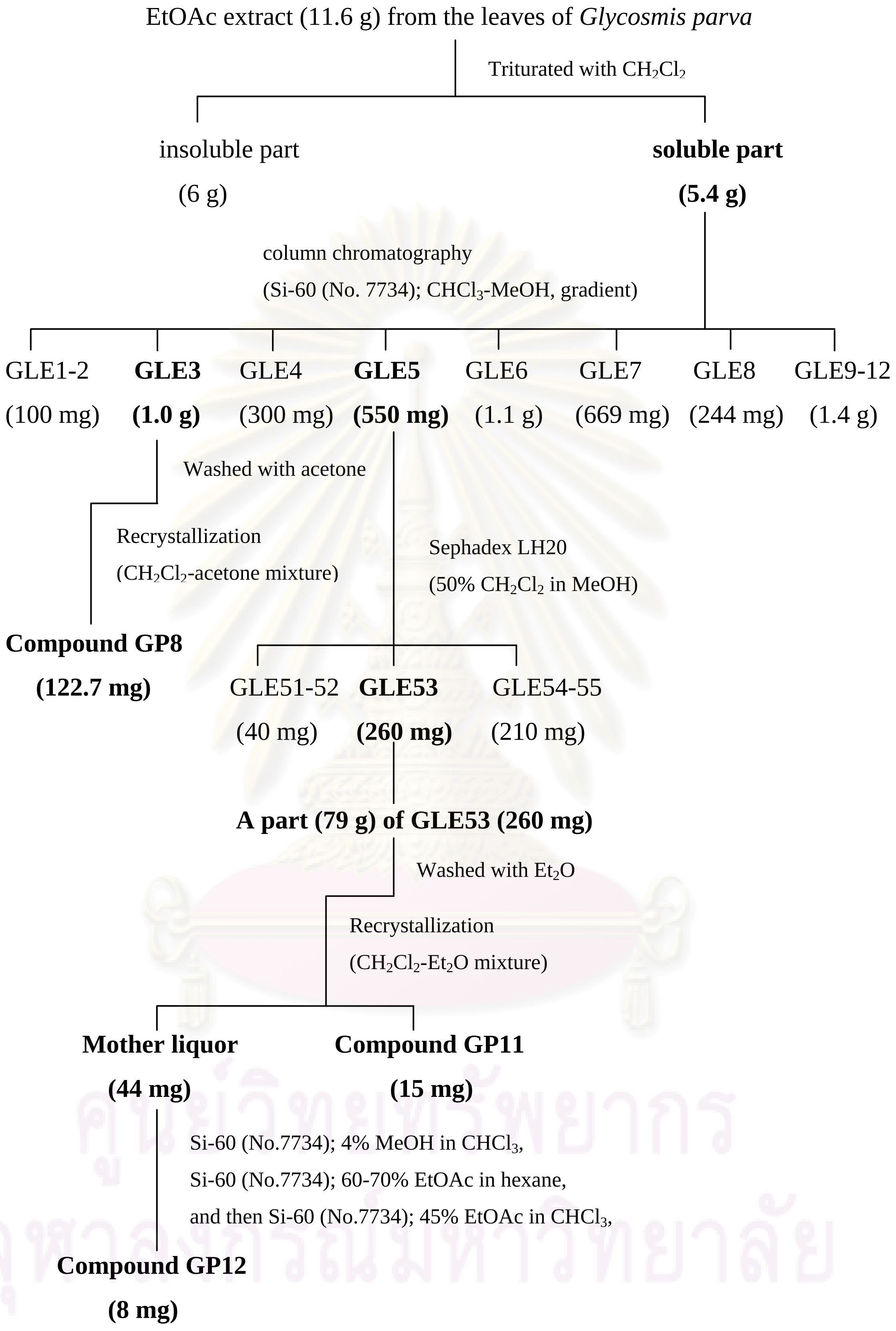
Scheme 12 Separation of EtOAc extract of the branches of *Glycosmis parva*

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



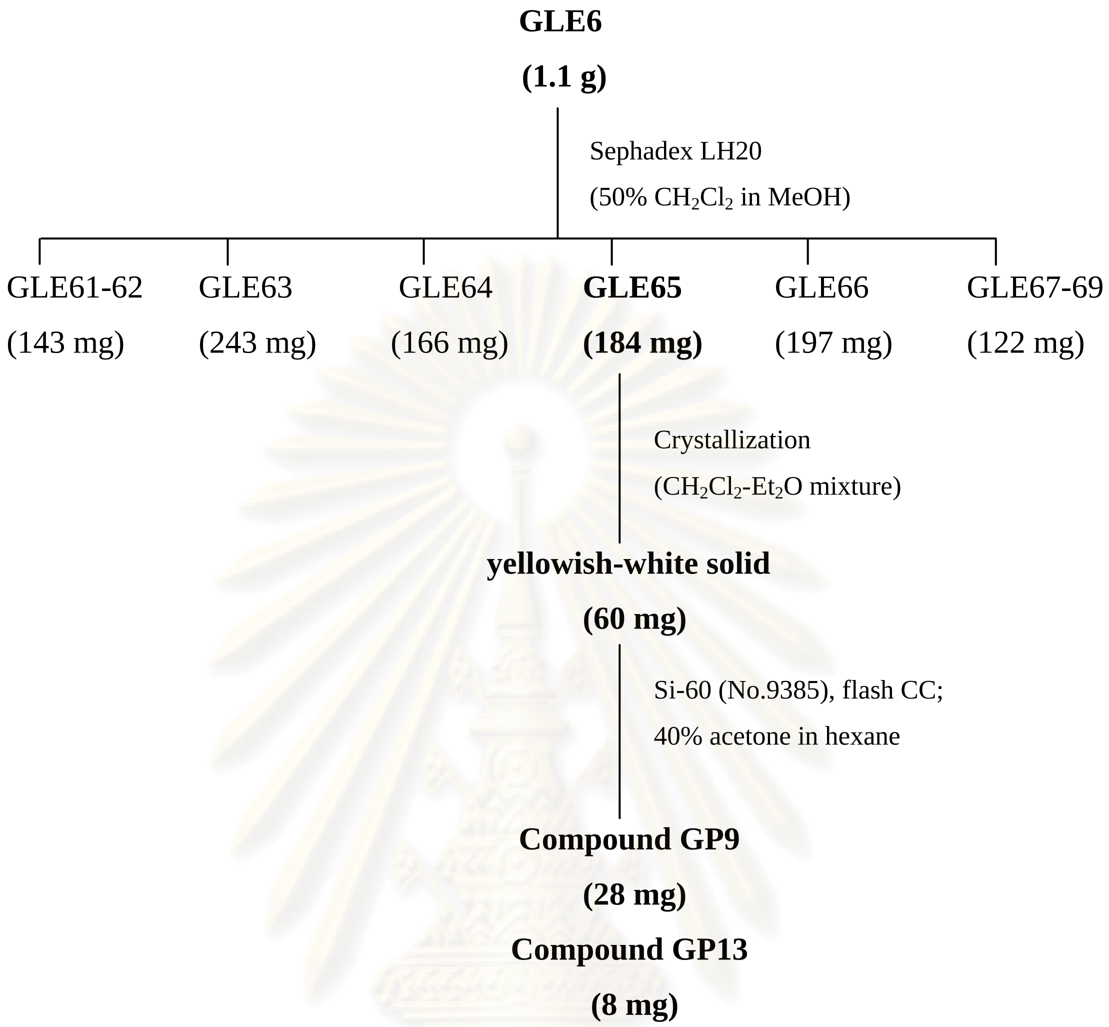
Scheme 13 Separation of fraction GBE5 from EtOAc extract of the branches of *Glycosmis parva*

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



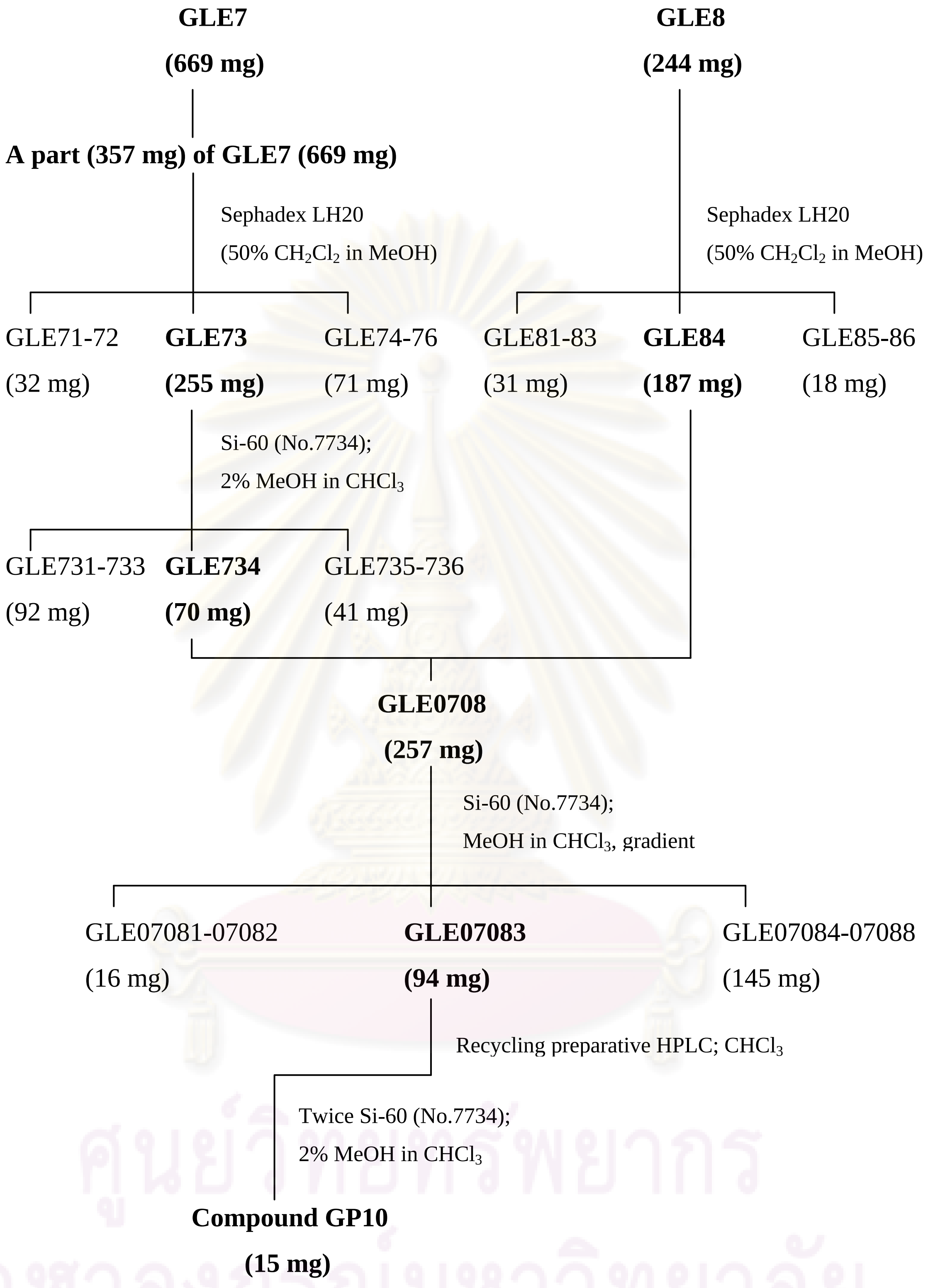
Scheme 14 Separation of EtOAc extract of the leaves of *Glycosmis parva*

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



Scheme 15 Separation of fraction GLE6 from EtOAc extract of the leaves of *Glycosmis parva*

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



Scheme 16 Separation of fraction GLE7 and GLE8 from EtOAc extract of the leaves of *Glycosmis parva*

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4. Physical and Spectral Data of Isolated Compounds

4.1 Compound MH1 (Scopoletin)

Compound **MH1** was obtained as white solid with m.p. 186-190°C, soluble in $CHCl_3$ (13 mg, 4.33×10^{-3}% based on dried weight of the branches).

FABMS : $[M+H]^+$ m/z 193, **Figure 3**

UV : λ_{max} nm (log ε), in $CHCl_3$; 251 (3.77), 259 (3.72), 295 (3.77), 341 (4.07), **Figure 4**

IR : ν_{max} cm^{-1}, ATR; 3332 (board), 1703, 1608, 1436, **Figure 5**

^{1}H-NMR : δ_H ppm, 400 MHz, in $CDCl_3$, **Table 5** and **Figure 6**

^{13}C-NMR : δ_C ppm, 100 MHz, in $CDCl_3$, **Table 5** and **Figure 7**

4.2 Compound MH2 (Micromelin)

Compound **MH2** was obtained as yellowish needles with m.p. 221-224°C, soluble in $CHCl_3$ (31 mg, 1.0×10^{-2}% based on dried weight of the branches, 90 mg, 3.0×10^{-2} % based on dried weight of the leaves).

FABMS : $[M+H]^+$ m/z 289, **Figure 10**

EIMS : m/z (rel. int.); 287 $[M]^+$ (100), 229 (72.4), 214 (22.2), 213 (55.1), 203 (20.5), 186 (21.9), **Figure 11**

$[\alpha]^{23}_D$: –75.9° (c 0.04, $CHCl_3$)

UV : λ_{max} nm (log ε), in $CHCl_3$; 244 (3.82), 251 (3.77), 295 (4.03), 314 (4.15), **Figure 12**

IR : ν_{max} cm^{-1}, ATR; 3065, 2926, 1767, 1732, 1623, 1568, 1473, 1271, 923, 820, **Figure 13**

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$, **Table 6** and **Figure 14**

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$, **Table 6** and **Figure 15**

4.3 Compound MH3 (1,2-dimethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid)

Compound **MH3** was obtained as white solid with m.p. 226-227°C with decomposition, soluble in acetonitrile, DMSO, and mixture of $CHCl_3$ and MeOH (20 mg, 4.4×10^{-4} % based on dried weight of the branches)

FABMS : $[M+H]^+$ m/z 218, **Figure 20**

EIMS : m/z (rel. int.); 217 $[M]^+$ (13.2), 199 (4.3), 173 (24.6), 83 (100), **Figure 21**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

HRESIMS : [M-H]$^-$ *m/z* 216.0666 (calcd for $C_{12}H_{10}NO_3$, 216.0661)

UV : λ_{max} nm (log ε), in CH_3CN; 253 (4.27), 315 (4.13), 329 (4.02), **Figure 22**

IR : ν_{max} cm^{-1}, ATR; 3126, 3065,1696, 1600, 1435, **Figure 23**

^{1}H-NMR : δ_H ppm, 300 MHz, in DMSO-d_6, **Figure 24**

: δ_H ppm, 600 MHz, in DMSO-d_6, **Table 7** and **Figure 25**

^{13}C-NMR : δ_C ppm, 150 MHz, in DMSO-d_6, **Table 7** and **Figure 26**

4.4 Compound MH4 ((–)-(2′*S*, 3′*R*)-3′-Senecioyloxymarmesin)

Compound **MH4** was obtained as white substances with m.p. 149-152°C, soluble in $CHCl_3$ (11 mg, 3.66×10^{-3}% based on dried weight of the leaves)

FABMS : [M+H]$^+$ *m/z* 345, **Figure 29**

HRESIMS : [M+H]$^+$ *m/z* 345.1326 (calcd for $C_{19}H_{21}O_6$, 345.1338)

$[\alpha]^{23}_D$: –314.1° (*c* 0.09, $CHCl_3$)

UV : λ_{max} nm (log ε), in $CHCl_3$; 243 (3.14), 259 (2.91), 298 (3.23), 327 (3.45), **Figure 30**

IR : ν_{max} cm^{-1}, ATR; 3494, 3089, 2984, 2924, 1727, 1651, 1631, 1569, 1485, 1443, 1136, 962, 853, **Figure 31**

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$, **Table 8** and **Figure 32**

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$, **Table 8** and **Figure 33**

4.5 Compound GP1 (*N*-methylatalaphylline)

Compound **GP1** was obtained as yellow solid with m.p. 192-194°C, soluble in $CHCl_3$ and acetone (850 mg, 6.85×10^{-3}% based on dried weight of the branches).

FABMS : [M+H]$^+$ *m/z* 394, **Figure 37**

EIMS : *m/z* (rel. int.); 393 [M]$^+$ (32.4), 350 (14.1), 338 (18.8), 322 (30.2), 294 (14.9), 282 (19.1), 280 (10.1), 268 (10.2), 207 (34.7), 105 (31.9), 97 (28.8), 91 (67.8), 83 (34.7), 77 (39.1), 73 (100), 58 (73.4), **Figure 38**

UV : λ_{max} nm (log ε), in EtOH; 205 (4.23), 273 (4.27), 340 (3.85), 410 (3.48), **Figure 39**

IR : ν_{max} cm^{-1}, ATR; 3489, 3400-2400 (board), 1603, 1566, 1539, 1441, 1290, 1223, 1194, 837,744, **Figure 40**

^{1}H-NMR : δ_H ppm, 600 MHz, in acetone-d_6, **Table 9** and **Figure 41**

^{13}C-NMR : δ_C ppm, 150 MHz, in acetone-d_6, **Table 9** and **Figure 43**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.6 Compound GP2 (glycofolinine)

Compound **GP2** was obtained as orange solid with m.p. 161-163°C, soluble in $CHCl_3$ and acetone (19 mg, 1.5×10^{-4}% based on dried weight of the branches).

FABMS : $[M+H]^+$ *m/z* 332, **Figure 49**

EIMS : *m/z* (rel. int.); 332 $[M+H]^+$ (19.1), 331 M^+ (89.5), 317 (36.9), 316 (100), 301 (86.0), 300 (34.2), 286 (12.9), 165 (13.2), 91 (12.5), **Figure 50**

UV : λ_{max} nm (log ε), in MeOH; 201 (4.28), 220 (3.56), 260 (3.96), 268 (3.95), 332 (3.49), 393 (3.14), **Figure 51**

IR : ν_{max} cm^{-1}, ATR; 3732, 3626, 3383, 1626, 1566, 1495, 1460, 1201, 1140, 671, **Figure 52**

^{1}H-NMR : δ_H ppm, 600 MHz, in acetone-d_6, **Table 10** and **Figure 53**

^{13}C-NMR : δ_C ppm, 150 MHz, in acetone-d_6, **Table 10** and **Figure 54**

4.7 Compound GP3 (citramine)

Compound **GP3** was obtained as yellow amorphous solid, soluble in $CHCl_3$ and acetone (10.6 mg, 8.0×10^{-5}% based on dried weight of the branches).

EIMS : *m/z* (rel. int.); 317 $[M]^+$ (64.3), 302 (49.6), 274 (41.7), 259 (32.7), 105 (16.7), 91 (40.9), 87 (17.3), 77 (22.0), 69 (14.2), 58 (100), **Figure 57**

UV : λ_{max} nm (log ε), in EtOH; 202 (3.45), 221 (3.39), 273 (3.89), 332 (3.30), 381 (3.01), **Figure 58**

IR : ν_{max} cm^{-1}, ATR; 3419, 3126, 2935, 1610, 1541, 1483, 1450, **Figure 59**

^{1}H-NMR : δ_H ppm, 600 MHz, in acetone-d_6, **Table 11** and **Figure 60**

^{13}C-NMR : δ_C ppm, 150 MHz, in acetone-d_6, **Table 11** and **Figure 62**

4.8 Compound GP4 (*N*-methylcyclo-atalaphylline-A)

Compound **GP4** was obtained as orange amorphous solid, soluble in $CHCl_3$ and acetone (35 mg, 2.8×10^{-4}% based on dried weight of the branches).

FABMS : $[M+H]^+$ *m/z* 392, $[M+Na]^+$ *m/z* 414, **Figure 66**

EIMS : *m/z* (rel. int.); 392 $[M+H]^+$ (25.5), 391 $[M]^+$ (92.4), 377 (26.4), 376 (100.0), 349 (15.5), 348 (61.3), 322 (40.6), 318 (26.1), 91 (23.1), **Figure 67**

UV : λ_{max} nm (log ε), in MeOH; 201 (5.12), 229 (4.40), 305 (4.81), 338 (4.28), 420 (3.81), **Figure 68**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

IR : ν_{max} cm^{-1}, ATR; 3627, 3134, 2972, 2918, 1640, 1601, 1564, 1462,1284, 1198, 1140, 752, **Figure 69**

^{1}H-NMR : δ_H ppm, 600 MHz, in acetone-d_6, **Table 12** and **Figure 70**

^{13}C-NMR : δ_C ppm, 150 MHz, in acetone-d_6, **Table 12** and **Figure 71**

4.9 Compound GP5 (glycosparvarine)

Compound **GP5** was obtained as orange amorphous solid, soluble in acetone (6.8 mg, 5.0× 10^{-5}% based on dried weight of the branches).

FABMS : $[M+H]^+$ *m/z* 288, **Figure 77**

EIMS : *m/z* (rel. int.); 288 $[M+H]^+$ (14.7), 287 $[M]^+$ (84.7), 273 (10.3), 272 (62.7), 245 (16.2), 244 (100), 229 (14.1), 130 (24.2), 91(11), 84 (8.1), 77 (12.6), 58 (42.4), **Figure 78**

HREIMS : $[M]^+$ *m/z* 287.0799 (calcd for $C_{15}H_{13}NO_5$, 287.0793)

UV : λ_{max} nm (log ε), in MeOH; 204 (4.36), 232 (4.29), 266 (4.60), 286 (4.49), 321 (sh) (4.11), 409 (3.79), **Figure 79**

IR : ν_{max} cm^{-1}, ATR; 3500-3181 (broad), 1644, 1595, 1557, 1452, 1287, 1223, 1134, 991, 833, **Figure 80**

^{1}H-NMR : δ_H ppm, 600 MHz, in acetone-d_6, **Table 13** and **Figure 81**

^{13}C-NMR : δ_C ppm, 150 MHz, in acetone-d_6, **Table 13** and **Figure 82**

4.10 Compound GP6 (limonin)

Compound **GP6** was obtained as colorless crystals with m.p. 283-285°C with decomposition, soluble in $CHCl_3$ (6.5 mg, 5.0× 10^{-5}% based on dried weight of the branches).

FABMS : $[M+H]^+$ *m/z* 471, **Figure 88**

EIMS : *m/z* (rel. int.); 391 (22.3), 376 (24.5), 347 (35.1), 207(18.1), 95 (26.2), **Figure 89**

$[\alpha]^{24}_D$: −75.0° (*c* 0.12, acetone)

UV : λ_{max} nm (log ε), in MeOH; 207 (3.92), 272 (3.57), **Figure 90**

IR : ν_{max} cm^{-1}, ATR; 2972, 1747, 1718, 1286, 1022, 669, **Figure 91**

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$, **Table 14** and **Figure 92**

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$, **Table 14** and **Figure 94**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.11 Mixture GP7 (a mixture of limonexic acid and isolimonexic acid)

Mixture **GP7** was obtained as white amorphous solid, soluble in MeOH and DMSO (8 mg, 6.5× 10^{-5}% based on dried weight of the branches).

FABMS : $[M+H]^+$ *m/z* 503, $[M+Na]^+$ *m/z* 525, $[M+K]^+$ *m/z* 541, **Figure 103**

EIMS : *m/z* (rel. int.); 391 (4.7), 376 (4.7), 347 (11.2), 207(14.2), 95 (16.4), **Figure 104**

$[\alpha]^{23}_{D}$: –41.8° (*c* 0.09, MeOH)

UV : λ_{max} nm (log ε), in MeOH; 218 (3.55), **Figure 105**

IR : ν_{max} cm^{-1}, ATR; 3640, 3246, 2966, 1745, 1456,1290, 1014, 679, **Figure 106**

^{1}H-NMR : δ_H ppm, 600 MHz, in DMSO-d_6, **Table 15** and **16**, and **Figure 107**

^{13}C-NMR : δ_C ppm, 150 MHz, in DMSO-d_6, **Table 15** and **16**, and **Figure 111**

4.12 Compound GP8 (arborinine)

Compound **GP8** was obtained as yellow needles with m.p. 179-181°C, soluble in CHCl$_3$ (112.7 mg, 2.56× 10^{-3}% based on dried weight of the leaves).

FABMS : $[M+H]^+$ *m/z* 286, **Figure 127**

EIMS : *m/z* (rel. int.); 286 $[M+H]^+$ (13.9), 285 $[M]^+$ (74.2), 271 (16.1), 270 (100.0), 242 (43.2), 199 (29.5), 117 (10.3), 59 (25.1), **Figure 128**

UV : λ_{max} nm (log ε), in MeOH; 205 (3.94), 229 (3.94), 274 (4.36), 326 (3.40), 399 (3.48), **Figure 129**

IR : ν_{max} cm^{-1}, ATR; 3734, 2936, 1635, 1587, 1555, 1455, 1249, 1105, 988, 752, **Figure 130**

^{1}H-NMR : δ_H ppm, 600 MHz, in CDCl$_3$, **Table 17** and **Figure 131**

^{13}C-NMR : δ_C ppm, 150 MHz, in CDCl$_3$, **Table 17** and **Figure 132**

4.13 Compound GP9 ((+)-*S*-deoxydihydroglyparvin)

Compound **GP9** was obtained as colorless amorphous mass, soluble in CHCl$_3$ (28 mg, 5.83× 10^{-4}% based on dried weight of the leaves).

FABMS : $[M+H]^+$ *m/z* 420, $[M+Na]^+$ *m/z* 442, **Figure 135**

HRFABMS : $[M+H]^+$ *m/z*: 420.1831 (calcd for $C_{22}H_{30}NO_5S$, 420.1845)

EIMS : *m/z* (rel. int.); 404 (11.7), 386 (18.4), 286 (13.7), 187 (24.6), 167 (34.2), 139 (53.7), 103 (86.6), 91 (100.0), 77 (90.5), 64 (70.9), **Figure 136**

$[\alpha]^{21}_{D}$: +68.2° (*c* 0.10, CHCl$_3$)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

UV : λ_{max} nm (log ε), in MeOH; 204 (4.31), 225 (4.29), 261 (4.30), **Figure 137**

CD : $[\Phi]_{256}$ +6173; (*c* 1.1×10^{-3}, MeOH), **Figure 138**

IR : ν_{max} cm^{-1}, ATR; 3287, 3051, 2930, 1698, 1648, 1616, 1584, 1548, 1511, 1456, 1379, 1362, 1328, 1240, 1174, 1039, 984, 803, **Figure 139**

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$, **Table 18** and **Figure 140**

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$, **Table 18** and **Figure 141**

4.14 Compound GP10 ((+)-*S*-deoxytetrahydroglyparvin)

Compound **GP10** was obtained as colorless oil, soluble in $CHCl_3$ (15 mg, 3.13×10^{-4}% based on dried weight of the leaves).

FABMS : $[M+H]^+$ *m/z* 422, $[M+Na]^+$ *m/z* 444, $[M+K]^+$ *m/z* 460, **Figure 146**

HRFABMS : $[M+H]^+$ *m/z*: 422.1985 (calcd for $C_{22}H_{32}NO_5S$, 422.2001)

EIMS : *m/z* (rel. int.); *m/z*: 357 (13.8), 286 (94.8), 167 (65.3), 146 (27.1), 140 (22.3), 139 (100), 120 (31.5), 107 (26.9), 69 (24.5), 64 (17.2), **Figure 147**

$[\alpha]^{18}_D$: +24.1° (*c* 0.04, $CHCl_3$)

UV : λ_{max} nm (log ε), in MeOH; 205 (4.15), 225 (4.16), 261 (4.16), **Figure 148**

CD : $[\Phi]_{260}$ +2272; (*c* 8.4×10^{-4}, MeOH), **Figure 149**

IR : ν_{max} cm^{-1}, ATR; 3291, 3075, 2975, 2929, 1695, 1662, 1581, 1511, 1457, 1379, 1362, 1300, 1241, 1176, 1035, 933, 806, **Figure 150**

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$, **Table 19** and **Figure 151**

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$, **Table 19** and **Figure 152**

4.15 Compound GP11 (glyparvin-A)

Compound **GP11** was obtained as colorless needles with m.p. 136-138 °C, soluble in $CHCl_3$ (15 mg, 3.13×10^{-4}% based on dried weight of the leaves).

FABMS : $[M+H]^+$ *m/z* 434, **Figure 158**

EIMS : *m/z* (rel. int.); 435 $[M+2]^+$ (1.1), 433 $[M]^+$ (6.8), 356 (4.5), 286 (14.7), 268 (7.9), 165 (45.2), 139 (23.7), 120 (100), 69 (60.1), **Figure 159**

UV : λ_{max} nm (log ε), in MeOH; 202 (4.91), 225 (4.48), 246 (4.40), 286 (4.44), **Figure 160**

IR : ν_{max} cm^{-1}, ATR; 3295, 1671, 1636, 1551, 1512, 1304, 1131, **Figure 161**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$, **Table 20** and **Figure 162**

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$, **Table 20** and **Figure 163**

4.16 Compound GP12 ((+)-dihydroglyparvin)

Compound **GP12** was obtained as colorless amorphous mass, soluble in $CHCl_3$ (15 mg, 1.67×10^{-4}% based on dried weight of the leaves).

FABMS : $[M+H]^+$ *m/z* 436, **Figure 168**

EIMS : *m/z* (rel. int.); 437 $[M+2]^+$ (0.5), 435 $[M]^+$ (4.2), 356 (33.2), 287 (12.6), 286 (56.8), 167 (62.0), 146 (19.4), 139 (100), 120 (37.0), 69 (34.6), **Figure 169**

$[\alpha]^{19}_{D}$:+20.7° (*c* 0.11, $CHCl_3$)

UV : λ_{max} nm (log ε), in MeOH; 202 (4.91), 224 (4.36), 261 (4.36), **Figure 170**

CD : $[\Phi]_{262}$ +3397; (*c* 8.7×10^{-4}, MeOH), **Figure 171**

IR : ν_{max} cm^{-1}, ATR; 3309, 3067, 2977, 2929, 1696, 1655, 1635, 1556, 1511, 1302, 1130, 967, 826, **Figure 172**

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$, **Table 21** and **Figure 173**

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$, **Table 21** and **Figure 174**

4.17 Compound GP13 ((+)-tetrahydroglyparvin)

Compound **GP13** was obtained as colorless amorphous mass, soluble in $CHCl_3$ (8 mg, 1.67×10^{-4}% based on dried weight of the leaves).

FABMS : $[M+H]^+$ *m/z* 438, $[M+Na]^+$ *m/z* 460, **Figure 179**

HRFABMS : $[M+Na]^+$ *m/z*: 460.1780 (calcd for $C_{22}H_{31}NO_6SNa$, 460.1770)

$[\alpha]^{22}_{D}$:+43.8° (*c* 0.02, $CHCl_3$)

UV : λ_{max} nm (log ε), in $CHCl_3$; 261 (4.27), **Figure 180**

CD : $[\Phi]_{264}$ +1651; (*c* 1.0×10^{-3}, MeOH), **Figure 181**

IR : ν_{max} cm^{-1}, ATR; 3328, 3091, 2922, 1686, 1644, 1573, 1514, 1457, 1294, 1246, 1122, 828, **Figure 182**

^{1}H-NMR : δ_H ppm, 600 MHz, in $CDCl_3$, **Table 22** and **Figure 183**

^{13}C-NMR : δ_C ppm, 150 MHz, in $CDCl_3$, **Table 22** and **Figure 184**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

5. Evaluation of Antiviral Activity against Herpes Simplex Virus Type 1 and Type 2

5.1 Viruses and Cells HSV strains used were HSV-1 (KOS) and HSV-2 (Baylor186). Vero cells (ATCC CCL81) were grown and maintained in Eagle's minimum medium supplemented with 10% fetal bovine serum.

5.2 Plaque Reduction Assay Anti-HSV activity of the compound was determined by plaque reduction assay modified from the reported method (Lipipun *et al*., 2003). Briefly, in post-treatment assay, Vero cells, in 96-well tissue culture plate, were infected with 30 plaque forming units of HSV-1 (KOS) or HSV-2 (Baylor186). After 1 h incubation at room temperature for virus adsorption, the cells were added with overlay media containing various concentrations of the compound. The infected cultures were incubated at 37°C for 2 days. The infected cells were fixed and stained, and then the number of plaques was counted. The 50% effective concentration (EC_{50}) was determined from the curve relating the plaque number to the concentration of the compound. Acyclovir was used as a positive control. In inactivation assay, each of 30 plaque forming units of HSV-1 (KOS) or HSV-2 (Baylor 186) was mixed with various concentrations of compound and incubated for 1 h, then the mixture was added to Vero cells in 96-well tissue culture plate. After 1 h incubation for virus adsorption, the overlay media containing various concentrations of the compound were added. The infected cultures were incubated at 37°C for 2 days. The infected cells were fixed, stained, and the plaques were counted. The 50% effective concentration (EC_{50}) was determined.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER IV

RESULTS AND DISCUSSION

1. Antiviral Activity against Herpes Simplex Virus (HSV) Type 1 and Type 2 of Extracts

Antiviral activities against herpes simplex virus (HSV) type 1 and type 2 of the extracts of *Micromelum hirsutum* and *Glycosmis parva* were examined using plaque reduction assay. The results are shown in **Table 3** and **Table 4**.

Table 3 Antiviral activities of the hexane, CH_2Cl_2 and MeOH extracts prepared from branches, young branches and leaves of *M. hirsutum* against HSV-1 and HSV-2

	inactivation treatment			post-treatment		
	final conc.[a] (µg/mL)	% inhibition[b]		final conc.[a] (µg/mL)	% inhibition[b]	
part/ extract		HSV-1	HSV-2		HSV-1	HSV-2
branches/						
hexane	100	0	0	100	0	0
CH_2Cl_2	50	40	40	100	0	0
MeOH	100	0	0	100	0	0
young branches/						
hexane	50	40	0	50	30	30
	100	70	50	100	70	60
CH_2Cl_2	12.5	40	40	100	30	0
	25	70	70			
MeOH	100	0	0	100	0	0
leaves/						
hexane	100	0	0	100	0	0
CH_2Cl_2	12.5	50	20	50	50	0
	50	70	70	100	70	60
MeOH	100	0	0	100	0	0

a) concentration of extract that was not toxic to the cells used in assay.

b) no inhibition was given by 0.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 4 Antiviral activities of the hexane, EtOAc, *n*-BuOH, and aqueous extracts prepared from branches and leaves of *G. parva* against HSV-1 and HSV-2

part/ extract	inactivation treatment			post-treatment		
	final conc.[a] (μg/mL)	% inhibition[b] HSV-1	% inhibition[b] HSV-2	final conc.[a] (μg/mL)	% inhibition[b] HSV-1	% inhibition[b] HSV-2
branches/						
hexane	50	50	20	50	0	0
EtOAC	25	30	0	25	0	0
n-BuOH	100	0	0	100	0	0
aqueous	100	0	0	100	0	0
leaves/						
hexane	12.5	20	20	25	0	0
EtOAC	12.5	50	30	25	70	60
n-BuOH	100	0	0	100	0	0
aqueous	100	0	0	100	0	0

a) concentration of extract that was not toxic to the cells used in assay.

b) no inhibition was given by 0.

The CH_2Cl_2 extract of the branches of *M. hirsutum* showed anti-herpes simplex virus activity in the inactivation method, whilst the CH_2Cl_2 extract of the leaves showed the anti-herpes simplex virus activities in both inactivation treatment and post-inhibition. In the young branches, the hexane and CH_2Cl_2 extracts showed anti-herpes simplex virus activities in both inactivation treatment and post-inhibition.

For *G. parva*, the hexane and EtOAc extracts of the branches, and the hexane extract of leaves displayed anti-herpes simplex virus activities in the inactivation inhibition, while the EtOAc extract of leaves exhibited anti-herpes simplex virus activities in both inactivation treatment and post-inhibition.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

2. Extracts and Isolated Compounds

The difference of using of solvents in the extraction procedure of *Micromelum hirsutum* between the first and second batch was due to the amount of *Micromelum hirsutum*. The sequential extraction in a polar gradient manner (hexane, CH_2Cl_2 and MeOH) was suitable for large amounts of the second batch of *Micromelum hirsutum* in order to give the good yield of polarity-fractionated extracts. Even though, *Micromelum hirsutum* was collected twice in different month, the chemical constituents of the branches and leaves of these two batch were similar, as evidenced by their TLCs.

Repeated chromatography of the CH_2Cl_2 extracts of *M. hirsutum* using several solvent systems led to the isolation of two coumarins (**MH1** and **MH2**) and a new natural quinolone alkaloid (**MH3**) from the branches and two coumarins (**MH2** and **MH4**) from the leaves. The EtOAc extract of branches of *G. parva* was subjected to separation using several chromatographic techniques to afford a new acridone alkaloid (**GP5**), together with four known acridone alkaloids (**GP1**-**GP4**) and three known limonoids (**GP6** and **GP7**). In addition, the examination of the EtOAc extract of leaves resulted in the isolation of three new *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives (**GP9**, **GP10** and **GP13**), together with two known derivatives (**GP11** and **GP12**) and a known acridone alkaloid (**GP8**).

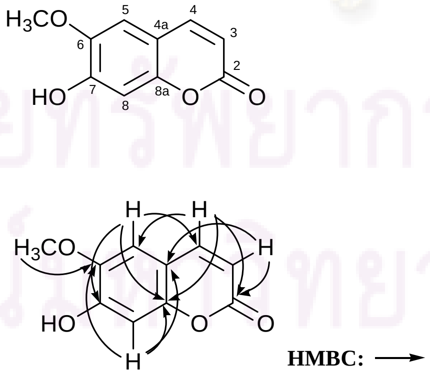
The structures of all of these isolates were determined by interpretation of their UV, IR, NMR and MS data and further confirmed by comparison with literature values. Additionally, their anti-herpes simplex virus activities were also investigated.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

3. Structure Determination of Compounds Isolated from *Micromelum hirsutum*

3.1 Structure Determination of Compound MH1

Compound **MH1** was obtained as white solid with m.p. 186-190°C. The FABMS **(Figure 3)** showed a pseudo molecular ion peak ($[M+H]^+$) at *m/z* 193, consistent with its molecular formula $C_{10}H_8O_4$. The UV spectrum **(Figure 4)** showed absorption maxima at 251, 259, 295 and 341 nm. The IR spectrum **(Figure 5)** displayed strong absorption bands at 3332 (O-H stretching), 1703 (conjugated C=O stretching), 1608 and 1436 (C=C stretching, aromatic ring) cm^{-1}. The ^1H-NMR spectrum of compound **MH1 (Figure 6)** showed a pair of doublet signals at δ_H 6.25 and 7.58 (1H each, *d*, J = 9.6 Hz) assigned to H-3 and H-4 of a coumarin skeleton, respectively. The typical chemical shift of H-4 in compound **MH1** suggested the lack of an oxygen substituent at C-5 (Steck and Mazurek, 1972). The two singlet signals at δ_H 6.83 (H-8, *s*) and 6.90 (H-5, *s*) suggested that the positions of C-6 and C-7 were substituted with electron donating groups. Signals for a methoxy group (δ_H 3.93) and an exchangeable hydroxyl proton (δ_H 6.15) were also found. The ^{13}C-NMR spectrum of compound **MH1 (Figure 7)** showed characteristic signals of *α,β*-unsaturated carbonyl at δ_C 161.4, 113.4 and 143.3 attributed to C-2, C-3 and C-4, respectively, on the coumarin nucleus. The HMBC correlation of δ_H 3.93 with δ_C 144.0 pointed that the methoxy group was substituted at C-6 (δ_C 144.0). The ^1H-NMR and ^{13}C-NMR data of compound **MH1** were completely assigned from HMQC **(Figure 8)** and HMBC **(Figure 9)** correlations in comparison with those of the authentic scopoletin. Thus, compound **MH1** was identified as scopoletin [**41**].



[**41**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 5 NMR spectral data of compound **MH1** and scopoletin

position	compound **MH1** *a)*		scopoletin (authentic) *b)*	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz) *c)*	δ_C (ppm) *c)*
2	-	161.4	-	161.5
3	6.25 (1H, *d*, 9.6)	113.4	6.25 (1H, *d*, 9.5)	113.4
4	7.58 (1H, *d*, 9.6)	143.3	7.58 (1H, *d*, 9.5)	143.3
4a	-	111.5	-	111.5
5	6.90 (1H, *s*)	107.5	6.89 (1H, *s*)	107.5
6	-	144.0	-	144.0
7	-	149.7	-	149.7
8	6.83 (1H, *s*)	103.2	6.82 (1H, *s*)	103.2
8a	-	150.2	-	150.2
OCH_3-6	3.93(3H, *s*)	56.4	3.93 (3H, *s*)	56.4
OH-7	6.15 (1H, *s*)	-	6.18 (1H, *s*)	-

a) ^{1}H-NMR (400 MHz, $CDCl_3$); ^{13}C-NMR (100 MHz, $CDCl_3$).

b) Authentic scopoletin (m.p. 203-205°C) purchased from Aldrich (Lot No. 06010TP) was available in Department of Medicinal Organic Chemistry, Graduate school of Pharmaceutical Sciences, Chiba University.

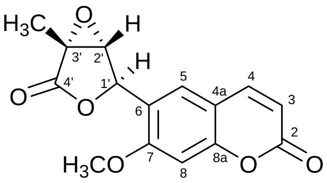
c) ^{1}H-NMR (400 MHz, $CDCl_3$); ^{13}C-NMR (100 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

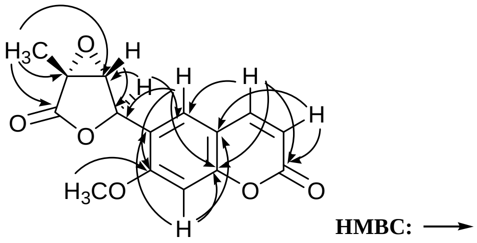
3.2 Structure Determination of Compound MH2

Compound **MH2** was obtained as yellowish needles with m.p. 221-224°C (Lit. Kong, *et al*., 1988; 218-219°C). The FABMS **(Figure 10)** showed $[M+H]^+$ at *m/z* 289, consistent with its molecular formula $C_{15}H_{12}O_6$. The UV spectrum **(Figure 12)** showed absorption maxima at 244, 251, 295 and 314 nm. The IR spectrum **(Figure 13)** displayed absorption bands at 1732, 1623 and 1568 cm^{-1} owing to coumarin framework. In addition, other IR absorption bands were found at 1767, 1271, 923 and 820 cm^{-1}. The ^{1}H-NMR spectrum of compound **MH2 (Figure 14)** showed a typical pair of doublets at δ_H 6.30 and 7.63 (1H each, *d*, *J* = 9.5 Hz) due to the H-3 and H-4 of a coumarin skeleton, respectively. The presence of 6,7-disubstituted coumarin moiety was suggested by two aromatic protons at δ_H 6.85 and 7.35 (each 1H, *s*), referring to H-8 and H-5, respectively. The position of a methoxy group at δ_H 3.92 (3H, *s*) should be placed on C-7 according to the upfield carbon chemical shift of C-8 (δ_C 99.8). The ^{13}C-NMR and DEPT spectra of compound **MH2 (Figures 15** and **16)** showed four relatively downfield signals for sp^3 carbons [two oxygenated methine carbons (δ_c 63.5 and 77.2), one quaternary carbon (δ_c 57.2) and a methoxy group (δ_c 56.5, OCH_3-7)]. The carbon signal at δ_C 172.2 and the IR absorption band at 1767 cm^{-1} suggested the presence of a γ-lactone ring which was established by detection of HMBC correlations [δ_H 1.65 (3H, *s*) with δ_C 172.2 (C-4′), δ_C 57.2 (C-3′) and δ_C 63.5 (C-2′); δ_H 4.01 with δ_C 77.2 (C-1′); δ_H 5.53 with δ_C 63.5 (C-2′)] **(Figures 18** and **19)**. The epoxide ring was pointed on C-2′ (δ_C 63.5) and C-3′(δ_C 57.2) of γ-lactone ring. The characteristic IR absorptions (ν_{max} 1271, 923 and 820 cm^{-1}) supported the presence of epoxide ring. The HMBC correlations of δ_H 6.30 with δ_C 77.2 (C-1′) and of δ_H 5.53 with δ_C 127.5 (C-5) indicated the γ-lactone ring was attached to C-6 (δ_C 120.2). The lack of coupling between two methine protons (δ_H 5.53 (H-1′) and 4.01 (H-2′)) is consistent with *trans* relationship between these protons which result in an approximate 90° dihedral angle. Compound **MH2** was identified as micromelin [**5**]. The stereochemistry of chiral carbons was identical with that of micromelin due to the same minus sign of specific rotation.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



[**5**]



[**5**]

Table 6 NMR spectral data of compound **MH2** and micromelin

position	compound **MH2** [a]		micromelin [c]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
2	-	160.4	-	160.0
3	6.30 (1H, *d*, 9.5)	114.2	6.31 (1H, *d*, 9.6)	114.3
4	7.63 (1H, *d*, 9.5)	142.9	7.66 (1H, *d*, 9.6)	142.8
4a	-	112.4	-	112.5
5	7.35 (1H, *s*)	127.5	7.38 (1H, *s*)	127.5
6	-	120.2	-	120.3
7	-	159.9	-	160.2
8	6.85 (1H, *s*)	99.8	6.88 (1H, *s*)	99.9
8a	-	156.5	-	156.6
1′	5.53(1H, *s*)	77.2 [b]	5.56 (1H, *s*)	77.2
2′	4.01(1H, *s*)	63.5	4.04 (1H, *s*)	63.6
3′	-	57.2	-	57.3
4′	-	172.2	-	172.2
OCH_3-7	3.92 (3H, *s*)	56.5	3.95 (3H, *s*)	56.4
CH_3-3′	1.65 (3H, *s*)	11.3	1.67 (3H, *s*)	11.2

a) ^{1}H-NMR (600 MHz, $CDCl_3$); ^{13}C-NMR (150 MHz, $CDCl_3$).

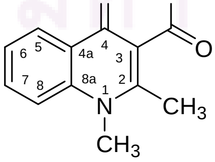
b) The signal was overlapped with solvent peak.

c) The ^{1}H-NMR (90 MHz, $CDCl_3$) and ^{13}C-NMR (22.5 MHz, $CDCl_3$) data were reported by Kong *et al.*, 1988.

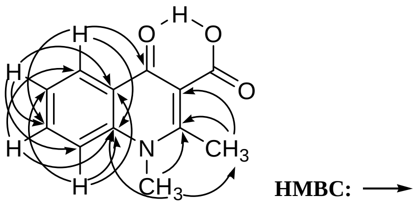
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

3.3 Structure Determination of Compound MH3

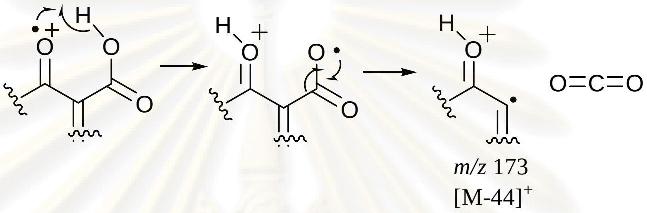
Compound **MH3** was obtained as white solid with m.p. 226-227°C (decomposition). The molecular formula $C_{12}H_{11}NO_3$ was established by the HRESIMS ($[M-H]^-$ at *m/z* 216.0666, calculated for $C_{12}H_{10}NO_3$, 216.0661). The UV spectrum **(Figure 22)** showed absorption maxima at 253, 315 and 329 nm. The IR spectrum **(Figure 23)** displayed absorption bands at 3126 (=C-H stretching), 3065, 1696 (conjugated C=O stretching), 1600 and 1435 (C=C stretching, aromatic ring) cm^{-1}. The ^1H-NMR spectrum of compound **MH3 (Figures 24** and **25)** exhibited the presence of *N*-methyl (δ_H 3.98) and downfield methyl (δ_H 3.11) groups. In the aromatic region, four protons displayed sequentially mutual coupling [δ_H 7.62 (1H, *dd*, *J* = 8.0, 7.1 Hz), 7.95 (1H, *ddd*, *J* = 8.8, 7.1, 1.7 Hz), 8.09 (1H, *d*, *J* = 8.8 Hz) and 8.37 (1H, *dd*, *J* = 8.0, 1.7 Hz)] assignable to H-6, H-7, H-8 and H-5 of a quinolone skeleton, respectively. A downfield signal at δ_H 16.66 (1H, *br s*) was assigned as an intramolecularly hydrogen-bonded acidic proton to carbonyl oxygen. The ^{13}C-NMR spectrum of compound **MH3 (Figure 26)** showed the methyl carbon at δ_C 19.1 and *N*-methyl carbon at δ_C 36.5. The carbon signals at δ_C 177.4 (C-4) and 167.1 (COOH-3) were assigned as a conjugated carbonyl carbon and carboxylic acid carbon, respectively. Even the lack of evidence for O-H stretching of carboxylic acid in the IR spectrum, the intramolecularly hydrogen-bonded signal in ^1H-NMR spectrum and the presence of peak at *m/z* 173 $[M-44]^+$ in EIMS **(Scheme 17)** supported the existence of carboxylic acid carbon. The HMBC correlations [δ_H 8.37 (H-5) with δ_C 177.4 (C-4); δ_H 3.11 (CH_3-2) with δ_C 108.7 (C-3) and 161.2 (C-2); δ_H 3.98 (*N*-CH_3) with δ_C 177.4 (C-4), 140.2 (C-8a) and 19.1 (CH_3-2)] sugguested the 2-methyl-3-carboxylic acid substituents **(Figure 28)**. The lower-field shifted signal of methyl protons (δ_H 3.11, CH_3-2) was due to the anisotropic effect of the carboxylic carbonyl group (COOH-3). Compound **MH3** was reasonably deduced to be 1,2-dimethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [**262**], as a new natural compound.



[**262**]



[**262**]



Scheme 17 Proposed EIMS fragmentation mechanism of compound **MH3**

Table 7 NMR spectral data of compound **MH3**

position	Compound **MH3** [a)]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)
2	-	161.2
3	-	108.7
4	-	177.4
4a	-	123.8
5	8.37 (1H, *dd*, 8.0, 1.7)	125.7
6	7.62 (1H, *dd*, 8.0, 7.1)	125.7
7	7.95 (1H, *ddd*, 8.8, 7.1, 1.7)	134.2
8	8.09 (1H, *d*, 8.8)	117.9
8a	-	140.2
N-CH_3	3.98 (3H, *s*)	36.5
CH_3-2	3.11 (3H, *s*)	19.1
COOH-3	16.66 (1H, *br s*) [b)]	167.1

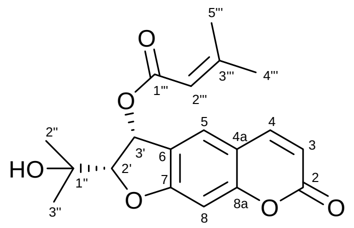
a) ^{1}H-NMR (600 MHz, DMSO-d_6); ^{13}C-NMR (150 MHz, DMSO-d_6).

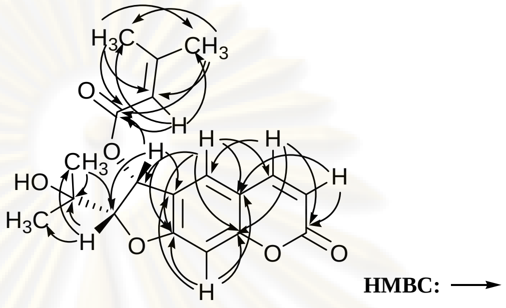
b) Exchangeable proton, this signal was observed in ^{1}H-NMR 300 MHz (DMSO-d_6) spectrum.

3.4 Structure Determination of Compound MH4

Compound **MH4**, white substance with m.p. 149-152°C (Lit. Jiménez, *et al.*, 2000; 148-149°C), showed $[M+H]^+$ at *m/z* 345 in the FABMS **(Figure 29)**, suggesting the molecular formula $C_{19}H_{20}O_6$. The UV spectrum **(Figure 30)** showed absorption maxima at 243, 259, 298 and 327 nm. The IR spectrum **(Figure 31)** exhibited absorption bands of O-H stretching (3494 cm^{-1}), conjugated C=O stretching (1727 cm^{-1}), alkene C=C stretching (1651 cm^{-1}), and aromatic C=C stretching (1631, 1569 and 1436.cm^{-1}). The ^{1}H-NMR spectrum of compound **MH4 (Figure 32)** showed two signals at δ_H 6.23 (*d*, *J* = 9.6 Hz) and 7.60 (*d*, *J* = 9.6 Hz) assigned to H-3 and H-4 of a coumarin skeleton, respectively. Two singlet signals at δ_H 7.58 and 6.86 (each 1H, *s*) were positioned at H-5 and H-8, respectively. The presence of a dihydrofuran ring which is linearly connected with coumarin nucleus at C-6 and C-7 was suggested by the following HMBC correlations: δ_H 7.58 (H-5) with δ_C 163.2 (C-7) and 71.4 (C-3′); δ_H 6.85 (H-8) with δ_C 124.1 (C-6) and 163.2 (C-7); δ_H 6.35 (H-3′) with δ_C 91.1 (C-2′), 124.1 (C-6) and 163.2 (C-7) **(Figure 36)**. A pair of doublet signals at δ_H 4.49 and 6.35 (each 1H, *J* = 6.3 Hz) was assigned to H-2′ and H-3′, respectively. The HMBC correlations [δ_H 1.44 (6H, H-2″ and H-3″) with δ_C 71.3 (C-1″) and 91.1 (C-2′); δ_H 4.49 (H-2′) with δ_C 71.3 (C-1″), 26.7 (C-2″) and 26.5 (C-3″)] and a downfield sp^3 quaternary carbon at C-1″ (δ_C 71.3) suggested that 2-hydroxy-2-methyl-propane was substituted at C-2′. The ^{13}C-NMR spectrum of compound **MH4 (Figure 33)** showed a set of signals [δ_C 165.0 (C-1‴), 114.5 (C-2‴), 160.9 (C-3‴), 27.6 (C-4‴) and 20.6 (C-5‴)] assignable to senecioate (2-methyl-2-butenoate) subunit. The proton signals at δ_H 5.57 (1H, *br s*), 2.21 (3H, *br s*) 1.90 (3H, *br s*) were assigned to H-2‴, H-4‴ and H-5‴ in the senecioate subunit, respectively. The HMBC correlation between δ_H 6.35 (H-3′) and δ_C 165.0 (C-1‴) indicated that senecioate subunit was connected at C-3′ of the dihydrofuran ring. The remaining board signal at δ_H 2.72 (1H) was assigned as hydroxyl proton at C-1″. For the relative stereochemistry, *cis* relationship between the C-2′ and C-3′ substituents was suggested due to the minus sign of specific rotation (Lit. Jiménez, *et al.*, 2000; $[\alpha]^{20}_D$ –236° (*c* 1.3, $CHCl_3$)). Compound **MH4** was identified as (–)-(2′*S*, 3′*R*)-3′-senecioyloxymarmesin [**263**].

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย





[**263**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 8 NMR spectral data of compound **MH4** and (−)-(2′*S*, 3′*R*)-3′-senecioyloxymarmesin

position	Compound **MH4** $^{a)}$		(−)-(2′*S*, 3′*R*)-3′-senecioyloxymarmesin $^{f)}$	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
2	-	161.0 $^{c)}$	-	160.8
3	6.23 (1H, *d*, 9.6)	113.0	6.24 (1H, *d*, 9.5)	113.0
4	7.60 (1H, *d*, 9.6)	143.7	7.61 (1H, *d*, 9.5)	143.6
4a	-	113.5	-	113.4
5	7.58 (1H, *s*)	126.7	7.59 (1H, *s*)	126.6
6	-	124.1	-	124.1
7	-	163.2	-	163.2
8	6.85 (1H, *s*)	99.1	6.86 (1H, *s*)	99.1
8a	-	157.1	-	157.1
2′	4.49 (1H, *d*, 6.3)	91.1	4.51 (1H, *d*, 6.2)	91.0
3′	6.35 (1H, *d*, 6.3)	71.4 $^{d)}$	6.37 (1H, *d*, 6.2)	71.4
1″ /OH-1″	2.72 (1H, *br s*) $^{b)}$	71.3 $^{d)}$	-	71.2
2″	1.44 (3H, *s*)	26.7 $^{e)}$	1.46 (3H, *s*)	26.6
3″	1.44 (3H, *s*)	26.5 $^{e)}$	1.46 (3H, *s*)	26.5
1‴	-	165.0	-	165.0
2‴	5.57 (1H, *br s*)	114.5	5.69 (1H, *br s*)	116.0
3‴	-	160.9 $^{c)}$	-	159.0
4‴	2.21 (3H, *br s*)	27.6	2.18 (3H, *br s*)	27.0
5‴	1.90 (3H, *br s*)	20.6	1.90 (3H, *br s*)	20.0

a) ^{1}H-NMR (600 MHz, $CDCl_3$); ^{13}C-NMR (150 MHz, $CDCl_3$).

b) Exchangeable proton.

c), d) and *e)* Values with same superscript may be interchanged.

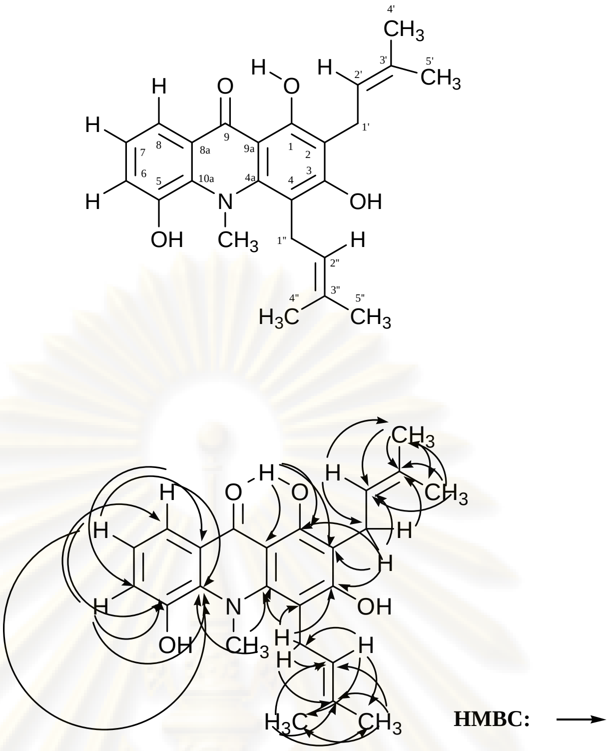
f) The ^{1}H-NMR and ^{13}C-NMR data were reported by Jiménez, *et al.*, 2000.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4. Structure Determination of Compounds Isolated from *Glycosmis parva*

4.1 Structure Determination of Compound GP1

Compound **GP1** was isolated as yellow solid with m.p. 192-194°C (Lit. Govindachari *et al.*, 1970; 192-193°C). The FABMS **(Figure 37)** demonstrated $[M+H]^+$ at *m/z* 394, harmonizing with the molecular formula $C_{24}H_{27}NO_4$. The UV spectrum **(Figure 39)** (λ_{max} 273, 340 and 410 nm) suggested the presence of 1-hydroxy-9-acridone nucleus (Reisch *et al.*, 1971). The IR spectrum **(Figure 40)** showed absorption at 3488 (hydroxyl group), and 3135, 3074, 2960, 2918, 1603, 1566, 1541, 1507, 1438 and 1417 (aromatic and hydrocarbon system) cm^{-1}. The ^1H-NMR spectrum of compound **GP1** **(Figure 41)** showed a lower-field shifted signal at δ_H 14.56 (OH-1, *s*, exchangeable) due to the intramolecularly hydrogen-bonded proton to carbonyl oxygen. Two protons at δ_H 9.26 and 7.91 (each 1H, *s*), which were washed out by D_2O, were assigned to hydroxyl protons (OH-5 and OH-3). In the aromatic region, three protons displayed ABX coupling system [δ_H 7.16 (H-7, *t*, *J* = 7.7 Hz), 7.26 (H-6, *dd*, *J* = 7.7, 1.1 Hz) and 7.77 (H-8, *dd*, *J* = 7.7, 1.1 Hz)]. The doublet of doublet at δ_H 7.77 must belong to H-8 due to the deshielded effect caused by the neighbouring 9-carbonyl group. Two sets of prenyl group were suggested by the signals at δ_H 3.45 (H_2-1′, *d*, *J* = 6.9 Hz), 5.25 (H-2′, *br t*, *J* = 6.1 Hz), 1.80 (H_3-4′, *s*) and 1.66 (H_3-5′, *s*) for one group, and δ_H 3.60 (H_2-1″, *d*, *J* = 5.9 Hz), 5.35 (H-2″, *br t*, *J* = 7.0 Hz), 1.79 (H_3-4″, *s*) and 1.71 (H_3-5″, *s*) for the other. In addition, *N*-methyl signal was found at δ_H 3.67 (3H, *s*). The signal at δ_H 14.56 (OH-1) had HMBC correlations with δ_C 107.9, 160.6 and 110.1, assignable to C-9a, C-1 and C-2, respectively **(Figures 46-48)**. The ^{13}C-NMR spectrum of compound **GP1 (Figure 43)** showed a conjugated ketone signal at δ_C 183.4 (C-9), even though, the C=O stretching of conjugated ketone around 1620 cm^{-1} could not be observed in IR spectrum. Connection of the two prenyl groups at C-2 and C-4 was evidenced by the HMBC correlations [prenyl group attached at C-2: δ_H 3.45 (H-2′) with δ_C 160.6 (C-1), 110.1 (C-2) and 162.0 (C-3); prenyl group attached at C-4: δ_H 3.60 (H-2″) with δ_C 162.0 (C-3), 108.3 (C-4) and 149.8 (C-4a)]. Thus, compound **GP1** was identified as *N*-methylatalaphylline [**258**].



[**258**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 9 NMR spectral data of compound **GP1** and *N*-methylatalaphylline

position	Compound **GP1** [a]		*N*-methylatalaphylline [c]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1/ OH-1	14.56 (1H, *s*) [b]	160.6	14.43 (1H, *s*)	159.1
2	-	110.1	-	106.6
3/ OH-3	7.91 (1H, *s*) [b,*]	162.0	-	161.4
4	-	108.3	-	108.3
4a	-	149.8	-	148.4
5/ OH-5	9.26 (1H, *s*) [b, *]	149.3	9.32 (1H,*br s*)	148.9
6	7.26 (1H, *dd*, 7.7, 1.1)	120.3	7.16 (1H, *m*)	123.9
7	7.16 (1H, *t*, 7.7)	123.6	7.07 (1H, *t*, 8)	119.7
8	7.77 (1H, *dd*, 7.7, 1.1)	117.1	7.78 (1H, *br d*)	115.5
8a	-	125.8	-	124.4
9	-	183.4	-	182.1
9a	-	107.9	-	109.7
10a	-	139.0	-	138.0
N-CH_3	3.67 (3H, *s*)	48.6	3.61 (3H, *s*)	47.8
1′	3.45 (2H, *d*, 6.9)	22.1	3.48 (2H, *m*)	21.5
2′	5.25 (1H, *br t*, 6.1)	123.2	5.28 (1H, *m*)	122.9
3′	-	132.2	-	130.7
4′	1.80 (3H, *s*)	18.0	1.72 (3H, *s*)**	18.0
5′	1.66 (3H, *s*)	25.9	1.77 (3H, *s*)**	25.6
1″	3.60 (2H, *d*, 5.9)	27.1	3.48 (2H,*m*)	26.3
2″	5.37 (1H, *br t*, 7.0)	124.2	5.36 (1H, *m*)	123.0
3″	-	133.4	-	131.2
4″	1.79 (3H, *s*)	18.1	1.82 (3H, *s*)**	18.0
5″	1.71 (3H, *s*)	25.8	1.82 (3H, *s*)**	25.6

a) ^{1}H-NMR (600 MHz, acetone-*d*$_6$); ^{13}C-NMR (150 MHz, acetone-*d*$_6$).

b) Exchangeable proton, they were clearly observed in ^{1}H-NMR 400 MHz **(Figure 42)**.

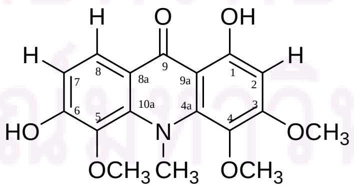
c) The ^{1}H-NMR (100 MHz, $CDCl_3$) data were reported by Wu, Kuoh and Furukawa, 1982b. The ^{13}C-NMR (DMSO-*d*$_6$) data were reported by Banerji *et al.*, 1981.

*, ** Values with same superscript may be interchanged.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

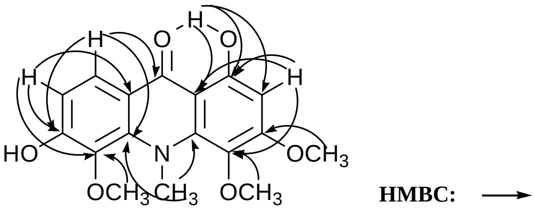
4.2 Structure Determination of Compound GP2

Compound **GP2** was characterized as orange solid with m.p. 161-163°C (Lit. Ono *et al.*, 1995; 161-163°C). The FABMS **(Figure 49)** exhibited $[M+H]^+$ at *m/z* 332, indicating the molecular formula $C_{17}H_{17}NO_6$. The presence of 1-hydroxy-9-acridone skeleton in compound **GP2** was suggested by the UV (λ_{max} 220, 260, 268, 332, 393 nm**, Figure 51**) and IR (3383 (hydroxy group), 1626 (Conjugated carbonyl) cm^{-1}, **Figure 52**) bands (Reisch *et al.*, 1971), together with a strongly hydrogen-bonded proton signal at δ_H 14.20 in the ^{1}H-NMR spectrum **(Figure 53)**. In the aromatic region of ^{1}H-NMR spectrum, the two protons displayed *ortho*-coupling [δ_H 6.94 (H-7, *d*, *J* = 9.2 Hz) and 7.93 (H-8, *d*, *J* = 9.2 Hz)] and a remaining signal as singlet was observed at relatively higher field (δ_H 6.39, H-2). In addition, three methoxy groups and one *N*-methyl group were observed at δ_H 3.78 (6H, *s*), 3.87 (3H, *s*) and 3.97 (3H, *s*). The ^{13}C-NMR spectrum **(Figure 54)** showed a conjugated ketone carbon at δ_C 182.4 (C-9), five oxygenated sp^2 carbons at δ_C 161.2, 160.7, 131.2, 137.2 and 157.0, four quaternary carbons at δ_C 106.2, 118.2, 142.7 and 143.6, and four methyl groups at δ_C 46.7, 56.6, 60.7 and 60.9. The signal at δ_H 14.20 (OH-1) had HMBC correlations with δ_C 106.3, 161.3 and 94.9, assigned to C-9a, C-1 and C-2, respectively **(Figure 56)**. The HMBC correlations [δ_H 7.93 (H-8) and 6.94 (H-7) with δ_C 157.0 (C-6); δ_H 6.94 (H-7) with δ_C 137.3 (C-5); δ_H 3.87 (CH_3-5) with δ_C 137.3 (C-5)] indicated that the hydroxyl group at δ_H 9.03 (1H, *s*) and a methoxy group at δ_H 3.87 were located at C-6 and C-5, respectively. The signals at δ_H 3.78 (6H) assigned as methoxy and *N*-methyl groups were evidenced by the HMBC correlations of δ_H 3.78 (δ_C 60.9) with δ_C 131.2 (C-4) and δ_H 6.39 (H-2) with δ_C 131.2 (C-4) for OCH_3-4 and the HMBC correlations of δ_H 3.78 (δ_C 46.7) with δ_C 142.7 (C-4a) and δ_C 143.6 (C-10a) for *N*-methyl group. The HMBC correlation of δ_H 3.97 with δ_C 160.7 pointed that the remaining methoxy group was substituted at C-3 (δ_C 160.7). Compound **GP2** was identified as the known compound glycofolinine [**163**].



[**163**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



[**163**]

Table 10 NMR spectral data of compound **GP2** and glycofolinine

position	Compound **GP2** [a]		glycofolinine [c]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1/ OH-1	14.20 (1H, *s*)	161.2	14.20 (1H, *s*)	161.6
2	6.39 (1H, *s*)	94.9	6.37 (1H, *s*)	95.3
3	-	160.7	-	161.1
4	-	131.2	-	131.6
4a	-	142.7	-	143.1
5	-	137.2	-	137.6
6 / OH-6	9.03 (1H, *s*) [b]	157.0	-	157.4
7	6.94 (1H, *d*, 9.2)	113.2	6.92 (1H, *d*, 8.8)	117.0
8	7.93 (1H, *d*, 9.2)	123.2	7.91 (1H, *d*, 8.8)	123.7
8a	-	118.2	-	118.6
9	-	182.4	-	182.8
9a	-	106.2	-	113.7
10a	-	143.6	-	144.0
N-CH_3	3.78 (3H, *s*)	46.7	3.77 (3H, *s*)	47.1
OCH_3-3	3.97 (3H, *s*)	56.5	3.97 (3H, *s*)	57.0
OCH_3-4	3.78 (3H, *s*)	60.9	3.77 (3H, *s*)	61.3
OCH_3-5	3.87 (3H, *s*)	60.7	3.86 (3H, *s*)	61.1

a) ^{1}H-NMR (600 MHz, acetone-d_6); ^{13}C-NMR (150 MHz, acetone-d_6).

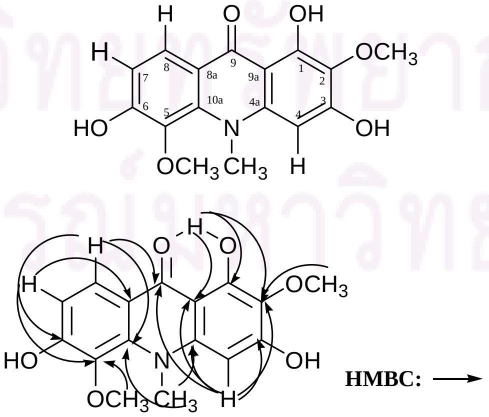
b) Exchangeable proton.

c) The ^{1}H-NMR and ^{13}C-NMR (acetone-d_6) data were reported by Ono *et al.*, 1995.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

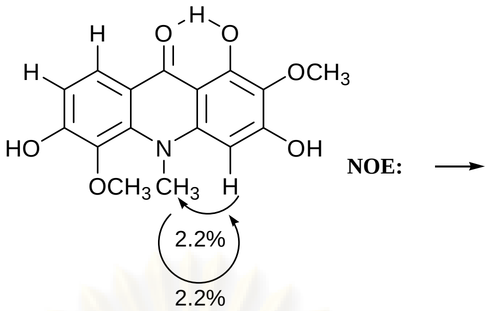
4.3 Structure Determination of Compound GP3

Compound **GP3** was obtained as yellow amorphous solid. It showed molecular ion $[M]^+$ peak at *m/z* 317 in the EIMS **(Figure 57)**, corresponding to the molecular formula $C_{16}H_{15}NO_6$. The UV (λ_{max} 221, 273, 332 and 381 nm, **Figure 58**) and IR spectra (3419, 3126, 1610 and 1541 cm^{-1} **Figure 59**) showed the characteristic absorptions of 1-hydroxy-9-acridone system (Reisch *et al.*, 1971). The ^{1}H-NMR spectrum **(Figures 60** and **61)** exhibited a lower-field shifted signal at δ_H 15.02 (OH-1, *s*), two exchangeable protons at δ_H 8.96 (2H, *br s*, overlapped), two *ortho*-coupled doublets at δ_H 6.95 (H-7, *d*, *J* = 9.1 Hz) and 8.05 (H-8, *d*, *J* = 9.1 Hz), a lone aromatic proton at δ_H 6.48 (H-4, *s*), *N*-methyl group at δ_H 4.04 (3H, *s*) and two methoxy groups at 3.78 (OCH_3-5, *s*) and 3.87 (OCH_3-2, *s*). The ^{13}C-NMR spectrum **(Figure 62)** showed a conjugated ketone carbon at δ_C 180.8 (C-9), five oxygenated sp^2 carbons at δ_C 129.7, 136.0, 156.0, 157.0 and 158.1, four quaternary carbons at δ_C 104.8, 116.7, 139.6 and 143.9, and four methyl group at δ_C 40.1, 60.4 and 61.3. The HMBC correlations between δ_H 15.02 (OH-1, *s*) and δ_C 104.8, 156.0 and 129.7, and between proton signal at δ_H 3.87 and carbon signal at δ_C 129.7 suggested the carbon signals at δ_C 104.8, 156.0 and 129.7 as C-9a, C-1 and C-2, respectively. The HMBC correlations of δ_H 6.48 (1H, *s*) with δ_C 104.8 (C-9a, strong), 129.7 (C-2, strong), 158.1 (C-3, medium), 143.9 (C-4a, weak) and 180.8 (C-9, weak) indicated that the aromatic proton at δ_H 6.48 was positioned at C-4 **(Figure 64)**. The other methoxy proton δ_H 3.78 was located at C-5, as evidenced by the HMBC correlations of protons at δ_H 3.78 and 6.95 (H-7, *d*, *J* = 9.1 Hz) with the carbon at δ_C 136.0 (C-5). The position of proton signal (δ_H 6.48) at C-4 was confirmed by NOE experiments which revealed NOE interaction of H-4 (δ_H 6.48) with the *N*-methyl group (δ_H 4.04) **(Figure 65)**. Thus, compound **GP3** was identified as citramine [**264**].



[**264**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



[**264**]

Table 11 NMR spectral data of compound **GP3** and citramine

position	Compound **GP3** [a)]		citramine [c)]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1/ OH-1	15.02 (1H, *s*) [b)]	156.0	15.03 (1H, *s*)	155.3
2	-	129.7	-	129.0
3 /OH-3	8.96 (1H, *br s*) [b)]	158.1	9.04 (1H, *s*)	157.4
4	6.48 (1H, *s*)	92.4	6.49 (1H, *s*)	91.9
4a	-	143.9	-	142.6
5	-	136.0	-	135.3
6 / OH-6	8.96 (1H, *br s*) [b)]	157.0	8.91 (1H, *s*)	156.1
7	6.95 (1H, *d*, 9.1)	113.1	6.95 (1H, *d*, 8.8)	112.7
8	8.05 (1H, *d*, 9.1)	123.2	8.05 (1H, *d*, 8.8)	121.9
8a	-	116.7	-	115.3
9	-	180.8	-	179.4
9a	-	104.8	-	103.7
10a	-	139.6	-	138.6
N-CH_3	4.04 (3H, *s*)	40.1	4.04 (3H, *s*)	39.5
OCH_3-2	3.87 (3H, *s*)	60.4	3.87 (3H, *s*)	59.8
OCH_3-5	3.78 (3H, *s*)	61.3	3.78 (3H, *s*)	60.7

a) ^{1}H-NMR (600 MHz, acetone-d_6); ^{13}C-NMR (150 MHz, acetone-d_6).

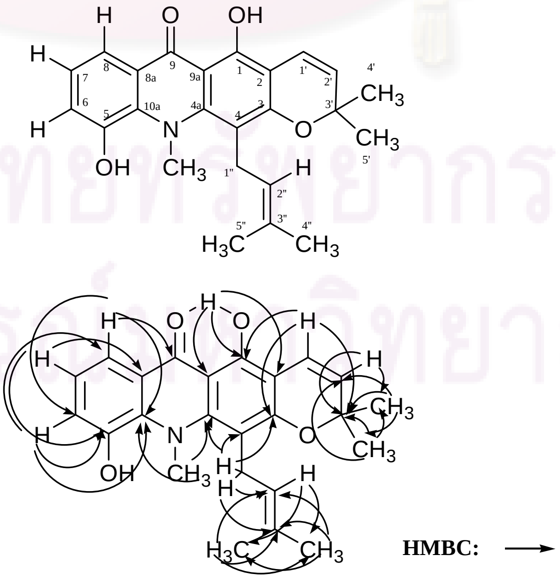
b) Exchangeable proton, they were clearly observed in ^{1}H-NMR 400 MHz **(Figure 61)**.

c) The ^{1}H-NMR (acetone-d_6) and ^{13}C-NMR (DMSO-d_6) data were reported by Ju-ichi *et al.*, 1988.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.4 Structure Determination of Compound GP4

Compound **GP4**, orange amorphous solid, showed $[M+H]^+$ at *m/z* 392 in the FABMS **(Figure 66)**, suggesting the molecular formula $C_{24}H_{25}O_4$. The basic skeleton was characterized to have a 1-hydroxy-9-acridone chromophore by UV (229, 305, 338 and 420 nm, **Figure 68**) and IR (3627 (O-H stretching), 1640 (conjugated carbonyl) cm^{-1}, **Figure 69**) absorptions, together with a strongly hydrogen-bonded proton signal at δ_H 14.67 in the ^{1}H-NMR spectrum **(Figure 70)**. The ^{13}C-NMR and DEPT spectroscopic data **(Figures 71** and **72)** exhibited 24 carbons, attributable to five methyl, one methylene, six methine and twelve quaternary carbons. In the aromatic region of the ^{1}H-NMR spectrum, three mutually coupling ABX signals at δ_H 7.18 (1H, *t*, *J* = 7.7 Hz), 7.27 (1H, *dd*, *J* = 7.7, 1.1 Hz) and 7.75 (1H, *dd*, *J* = 7.7, 1.1 Hz) were attributed to H-7, H-6 and H-8, respectively. The presence of a prenyl group was suggested by the signals at δ_H 3.51 (H_2-1″, *d*, *J* = 6 Hz), 5.35 (H-2″, *m*), 1.70 (H_3-4″, *s*) and 1.79 (H_3-5″, *s*). A set of signals at δ_H 6.72 (H-1′, *d*, *J* = 9.9 Hz), 5.70 (H-2′, *d*, *J* = 9.9 Hz) and 1.47 (6H, *s*) indicated the presence of a 2,2-dimethylpyrano moiety. The HMBC correlations of δ_H 14.67 (OH-1, *s*) with δ_C 107.8, 157.9 and 104.2 suggested the carbon signals at δ_C 107.8, 157.9 and 104.2 as C-9a, C-1 and C-2, respectively. The 2,2-dimethylpyrano moiety was linearly attached to the acridone nucleus at C-2 and C-3 by evidence of HMBC correlations [δ_H 6.72 (H-1′) with δ_C 157.9 (C-1) and 159.7 (C-3); δ_H 5.70 (H-2′) with δ_C 104.2 (C-2)]. The prenyl group at C-4 was suggested by the HMBC correlations of δ_H 3.51 (H-1″) with δ_C 159.7 (C-3), 109.3 (C-4) and 151.2 (C-4a). Thus, compound **GP4** was identified as *N*-methylcyclo-atalaphylline-A [**265**].



[**265**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 12 NMR spectral data of compound **GP4** and *N*-methylcyclo-atalaphylline-A

position	Compound **GP4** [a)]		*N*-methylcyclo-atalaphylline-A [c)]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1/ OH-1	14.67 (1H, *s*) [b)]	157.9	14.63 (1H, *s*)	157.5
2	-	104.2	-	103.4
3	-	159.7	-	158.8
4	-	109.3	-	108.5
4a	-	151.2	-	150.0
5/ OH-5	9.29 (1H, *br s*) [b)]	149.3	9.41 (1H, *br s*)	148.6
6	7.27 (1H, *dd*, 7.7, 1.1)	120.6	7.29 (1H, *br d*, 7.5)	119.7
7	7.18 (1H, *t*, 7.7)	123.9	7.18 (1H, *br d*, 7.5)	123.1
8	7.75 (1H, *dd*, 7.7, 1.1)	117.1	7.76 (1H, *d*, 7.5)	116.1
8a	-	125.7	-	124.9
9	-	183.5	-	182.7
9a	-	107.8	-	106.9
10a	-	138.9	-	138.0
N-CH_3	3.72 (3H, *s*)	48.6	3.71 (3H, *s*)	47.7
1′	6.72 (1H, *d*, 9.9)	116.5	6.73 (1H, *d*, 9.9)	115.6
2′	5.70 (1H, *d*, 9.9)	127.8	5.70 (1H, *d*, 9.9)	126.9
3′	-	78.6	-	77.7
4′	1.47 (3H, *s*)	28.5	1.48 (3H, *s*)	27.6
5′	1.47 (3H, *s*)	28.5	1.48 (3H, *s*)	27.6
1″	3.51 (2H, *d*, 6)	26.6	3.51 (2H, *br d*, 6.3)	25.7
2″	5.35 (1H, *m*)	124.7	5.36 (1H, *m*)	123.9
3″	-	131.7	-	130.8
4″	1.70 (3H, *s*)	25.8	1.70 (3H,*s*)	24.9
5″	1.79 (3H, *s*)	18.2	1.80 (3H,*s*)	17.3

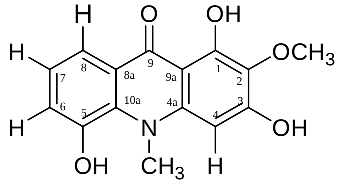
a) ^{1}H-NMR (600 MHz, acetone-d_6); ^{13}C-NMR (150 MHz, acetone-d_6).

b) Exchangeable proton.

c) The ^{1}H-NMR (300 MHz, acetone-d_6) and ^{13}C-NMR (75 MHz, acetone-d_6) data were reported by Chukaew *et al.*, 2008.

4.5 Structure Determination of Compound GP5

Compound **GP5**, orange amorphous solid, showed the molecular ion peak at *m/z* 287.0799 in the HREIMS, providing evidence for a molecular formula of $C_{15}H_{13}NO_5$ (calculated for $C_{15}H_{13}NO_5$, 287.0793). The IR spectrum **(Figure 80)** showed absorption bands at 3500-3181 (broad) and 1644 cm^{-1} due to hydroxy and carbonyl groups, respectively. Characteristic absorptions of acridone alkaloid were observed in the UV spectrum (266, 286, 321 and 409 nm, **Figure 79**). The ^{1}H-NMR spectrum **(Figure 81)** showed the existence of methoxy (δ_H 3.88) and *N*-methyl (δ_H 4.08) groups. In the aromatic region, the three out of four protons displayed sequentially mutual coupling as ABX pattern [δ_H 7.14 (1H, *dd*, *J* = 8.1, 7.7 Hz), 7.29 (1H, *dd*, *J* = 7.7, 1.5 Hz) and 7.91 (1H, *dd*, *J* = 8.1, 1.5 Hz)] and a remaining signal appeared as singlet at relatively higher field (δ_H 6.50). A lower-field shifted signal at δ_H 14.99 was assigned as an intramolecularly hydrogen-bonded proton to carbonyl oxygen and two signals at δ_H 8.95 and 9.31 as exchangeable protons. The presence of the above intramolecularly hydrogen-bonded signal and ABX pattern signals including a deshielded proton (δ_H 7.91) indicated that compound **GP5** was constructed based on a 6,7,8-unsubstituted 1-hydroxy-10-methylacridone skeleton. In the ^{13}C-NMR spectrum **(Figure 82)** twelve signals were observed as aromatic carbons in addition to methoxy (δ_C 60.5), *N*-methyl (δ_C 41.3), and carbonyl (δ_C 181.6) functions. In HMBC experiment, significant correlations from the proton at δ_H 14.99 to the carbons at δ_C 105.8, 156.4, and 129.5, and from the proton at δ_H 3.88 to the carbon at δ_C 129.5 indicated the carbon signals at δ_C 105.8, 156.4, and 129.5 as C-9a, C-1, and C-2, respectively **(Figures 84-86)**. *O*-Quaternary carbons were deduced by HMBC correlations [δ_H 8.95 (3-OH) with δ_C 158.4 (C-3) and 129.5 (C-2); δ_H 9.31 (5-OH) with δ_C 147.8 (C-5) and 134.7 (C-10a)]. The strong HMBC cross-peaks of δ_H 6.50 with δ_C 105.8 (C-9a), 129.5 (C-2) and 158.4 (C-3) assigned the signal at δ_H 6.50 to be H-4. NOE difference experiments revealed NOE correlation of H-4 (δ_H 6.50) with the *N*-methyl group (δ_H 4.08), and that of H-7 (δ_H 7.14) with H-8 (δ_H 7.91) and H-6 (δ_H 7.29) **(Figure 87)**. These data allowed us to deduce the alignment of 1,3,5-trihydroxy-2-methoxy substituents. Thus, compound **GP5** was deduced to be 1,3,5-trihydroxy-2-methoxy-*N*-methyl-9-acridone. It was named to be glycosparvarine [**266**], as a new acridone alkaloid.



glycosparvarine

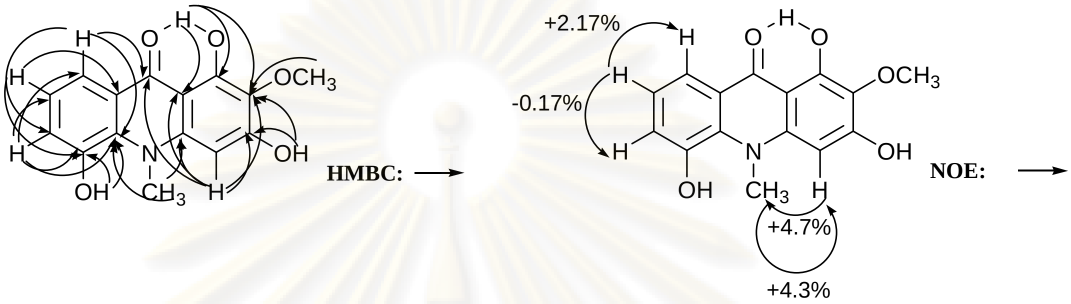


Table 13 NMR spectral data of compound **GP5**

position	Compound **GP5** [a)]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1/ OH-1	14.99 (1H, *s*) [b)]	156.4
2	-	129.5
3 / OH-3	8.95 (1H, *s*) [b)]	158.4
4	6.50 (1H, *s*)	91.8
4a	-	144.3
5/ OH-5	9.31 (1H, *s*) [b)]	147.8
6	7.29 (1H, *dd*, 7.7, 1.5)	120.6
7	7.14 (1H, *dd*, 8.1, 7.7)	122.8
8	7.91 (1H, *dd*, 8.1, 1.5)	117.6
8a	-	124.1
9	-	181.6
9a	-	105.8
10a	-	134.7
N-CH_3	4.08 (3H, *s*)	41.3
OCH_3-2	3.88 (3H, *s*)	60.5

a) ^{1}H-NMR (600 MHz, acetone-d_6);^{13}C-NMR (150 MHz, acetone-d_6).

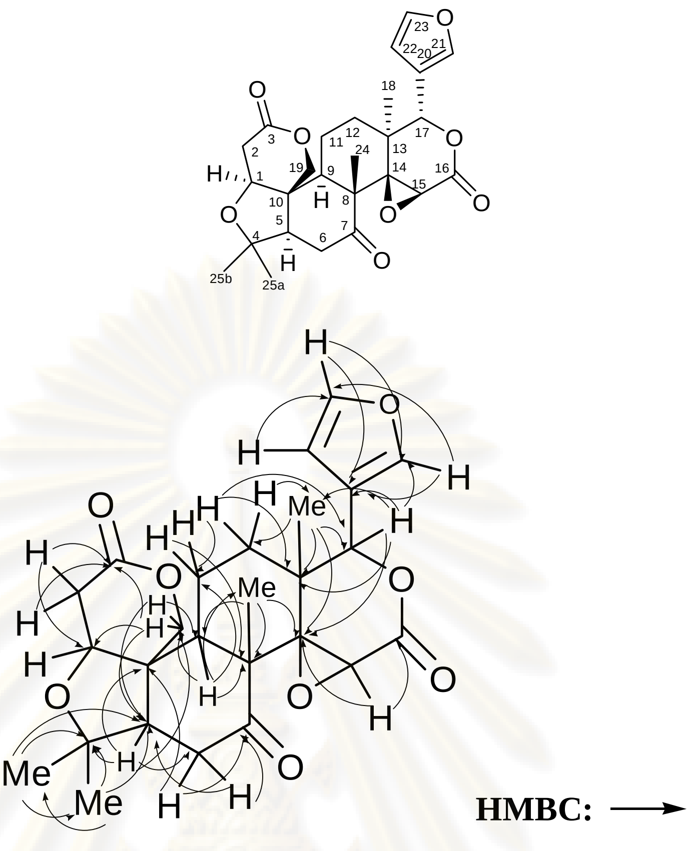
b) Exchangeable proton.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

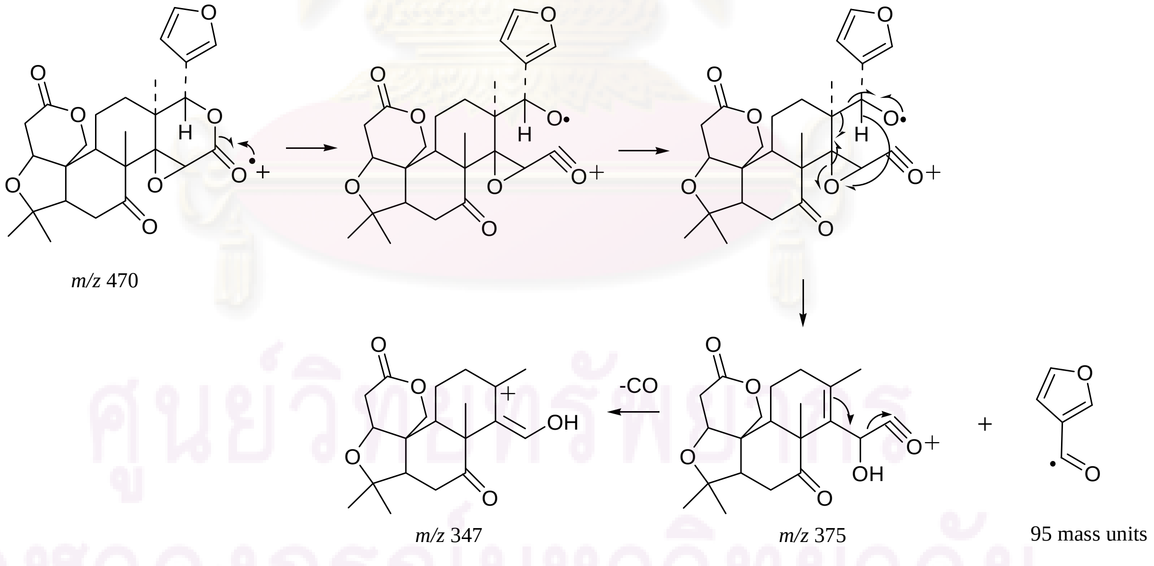
4.6 Structure Determination of Compound GP6

Compound **GP6** was obtained as colorless crystals with m.p. 283-285°C with decomposition (Lit. Breksa III, Dragull and Wong, 2008; 284-294°C with decomposition). It showed $[M+H]^+$ at *m/z* 471 in the FABMS **(Figure 88)**, corresponding to the molecular formula $C_{26}H_{30}O_8$. The UV spectrum **(Figure 90)** exhibited the absorption maxima at 207 and 272 nm. The IR spectrum **(Figure 91)** showed the absorption band at 2972, 1747 (C=O ester group), 1718 (C=O ketone group), 1286, 1022 and 669 cm^{-1}. The signals due to furan ring were shown at δ_H 6.33 (H-22, *dd*, *J* = 1.6, 0.8 Hz), 7.39 (H-23, *t*, *J* = 1.7 Hz) and 7.41 (H-21, *br d*, *J* = 0.8 Hz) in the ^1H NMR spectrum **(Figures 92** and **93)**, and at δ_C 109.6 (C-22), 119.9 (C-20), 141.1 (C-21) and 143.2 (C-23) in the ^{13}C-NMR spectrum. Signals attributed to four methyl protons at δ_H 1.06 (H_3-24), 1.16 (H_3-25b) 1.17 (H_3-18) and 1.29 (H-25a), five methylene protons at δ_H 1.74-1.83 (H-12a), 1.74-1.91 (H_2-11), 1.49-1.52 (H-12b), 2.46 (H-6a), 2.67 (H-2a), 2.85 (H-6b), 2.97 (H-2b), 4.45 (H-19b) and 4.76 (H-19a), and five methine protons at δ_H 2.22 (H-5), 2.54 (H-9), 4.02 (H-15), 4.03 (H-1) and 5.46 (H-17) were found. The ^{13}C-NMR and DEPT135 spectra **(Figures 94-96)** showed twenty-six carbon signals, assignable to four methyl carbons at δ_C 17.6 (C-24), 20.7 (C-18), 21.3 (C-25b), and 30.1 (C-25a), five methylene carbons at δ_C 18.9 (C-11), 35.6 (C-2), 36.4 (C-6), 30.8 (C-12) and 65.3 (C-19), five methine carbons at δ_C 60.5 (C-5), 79.1 (C-1), 48.1 (C-9), 53.8 (C-15) and 77.8 (C-17), eight quaternary carbons at δ_C 37.9 (C-13), 45.9 (C-10), 51.3 (C-8), 65.6 (C-14), 80.3 (C-4), 166.7 (C-16), 169.2 (C-3) and 206.1 (C-7), and four carbons for furan ring. Assignment of signals was made on the basis of 1- and 2-D NMR data acquired for limonin. The fragmentation at *m/z* 347 and 95 suggested the compound **GP6** was a member of limonin-typed limonoids (**Scheme 18**, Manners and Breksa III, 2004). The ^1H and ^{13}C-NMR spectra of limonin and epilimonin, C-17 limonin epimer, showed expected differences in the chemical shift of H-15, H-17 and H-18, and carbon C-17 and C-18 (Breksa III, Dragull and Wong, 2008). In comparison to limonin, the chemical shift of C-17 and C-18 of epilimonin were relatively downfield-shifted at δ_C 88.5 (C-17) and 28.2 (C-18). The melting point of limonin was 284-295°C with decomposition whereas that of epilimonin was 316-318°C. In addition, limonin showed a negative specific rotation whereas that epilimonin showed a positive value. Thus, compound **GP6** was identified as limonin [**267**] by analysis of above spectral data (1- and 2-D NMR experiments, **Figures 97-102**) and confirmed by the reported physicochemical data of limonin (Breksa III, Dragull and Wong, 2008).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



[**267**]



Scheme 18 Proposed EIMS fragmentation mechanism of compound **GP6**

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 14 NMR spectral data of compound **GP6** and limonin

position	Compound **GP6** a)		limonin b)	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	4.03 (1H, *m*)	79.1	4.03 (1H, *m*)	79.2
2a	2.67 (1H, *dd*, 16.8, 1.9)	35.6	2.67 (1H, *dd*, 16.8, 2.0)	35.7
2b	2.97 (1H, *dd*, 16.8, 3.8)	-	2.98 (1H, *dd*, 16.8, 4.0)	-
3	-	169.2	-	169.0
4	-	80.3	-	80.3
5	2.22 (1H, *dd*, 15.9, 3.0)	60.5	2.22 (1H, *dd*, 15.8, 3.4)	60.7
6a	2.46 (1H, *dd*, 14.5, 3.5),	36.4	2.46 (1H, *dd*, 14.4, 3.2)	36.4
6b	2.85 (1H, *dd*, 15.9, 14.5)	-	2.85 (1H, *dd*, 15.8, 14.6)	-
7	-	206.1	-	206.0
8	-	51.3	-	51.4
9	2.54 (1H, *dd*, 12.5, 2.6)	48.1	2.55 (1H, *dd*, 12.2, 3.0)	48.2
10	-	45.9	-	46.0
11	1.74-1.91 (2H, *m*)	18.9	1.72-1.95 (2H, *m*)	19.0
12a	1.74-1.83 (1H, *m*)	30.8	1.46-1.58 (2H, *m*)	30.9
12b	1.49-1.52 (1H, *m*)	-		-
13	-	37.9	-	38.0
14	-	65.6	-	65.7
15	4.02 (1H, *s*)	53.8	4.05 (1H, *s*)	53.9
16	-	166.7	-	166.5
17	5.46 (1H, *s*)	77.8	5.47 (1H, *s*)	77.8
18	1.17 (3H, *s*)	20.7	1.18 (3H, *s*)	20.7
19a	4.76 (1H, *d*, 13.1)	65.3	4.76 (1H, *d*, 13.0)	65.4
19b	4.45 (1H, *d*, 13.1)	-	4.46 (1H, *d*, 13.0)	-
20	-	119.9	-	120.1
21	7.41 (1H, *br d*, 0.8)	141.1	7.40 (1H, *m*)	143.3
22	6.33 (1H, *dd*, 1.6, 0.8)	109.6	6.34 (1H, *m*)	109.7
23	7.39 (1H, *t*, 1.7)	143.2	7.41 (1H, *m*)	141.2
24	1.06 (3H, *s*)	17.6	1.08 (3H, *s*)	17.7
25a	1.29 (3H, *s*)	30.1	1.29 (3H, *s*)	30.2
25b	1.16 (3H, *s*)	21.3	1.18 (3H, *s*)	21.4

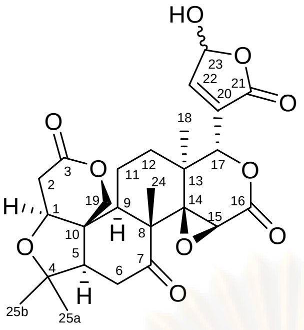
a) ^{1}H NMR ($CDCl_3$, 600 MHz, TMS); ^{13}C NMR ($CDCl_3$, 150 MHz, TMS).

b) The ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS) and ^{13}C NMR ($CDCl_3$, 100 MHz, TMS) data were reported by Breksa III, Dragull and Wong, 2008.

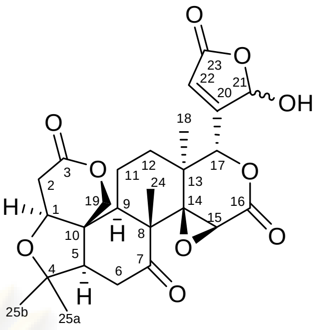
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.7 Structure Determination of Mixture GP7

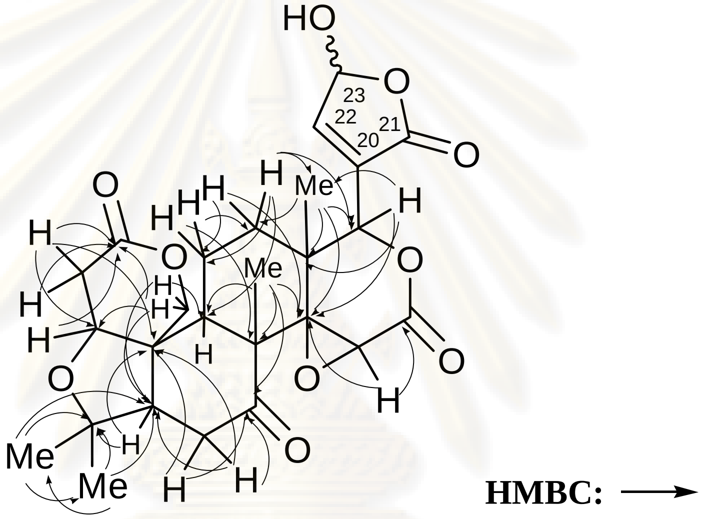
Mixture **GP7**, white amorphous solid, was obtained as a mixture of isomers (compounds **GP7-I** and **GP7-II**). The FABMS **(Figure 103)** demonstrated $[M+H]^+$ ion at *m/z* 503, corresponding to the molecular formula $C_{26}H_{30}O_{10}$. The UV spectrum showed the absorption maxima at 218 nm. The EIMS and IR spectra were quite similar to limonin, a common limonoid compound among plants in the family Rutaceae. The EI mass spectrum **(Figure 104)** showed prominent fragment ions at *m/z* 347 and 95, which also suggested the limonin-type limonoids (**Scheme 18**, Manners and Breksa III, 2004). The IR spectrum **(Figure 106)** showed strong absorption bands at 3640 $cm^{-1}$and 1745 cm^{-1} due to free O-H stretching and C=O stretching, respectively. In the ^{1}H- and ^{13}C-NMR spectra **(Figures 107-116)**, sixty protons and fifty-two carbons were found and could be divided into two sets (compounds **GP7-I** and **GP7-II**) by ^{1}H-^{1}H COSY, HMQC and HMBC correlations **(Figures 117-126)**. The signals due to γ-hydroxybutenolide ring were shown at δ_H 6.29 (1H, *br s*, overlapping), 7.48 (1H, *br s*) and 8.02 (1H, *br s*) in the ^{1}H-NMR spectrum, and at δ_C 98.4, 131.4, 152.2, and 169.0 in the ^{13}C-NMR spectrum for compound **GP7-I**. For the compound **GP7-II**, The signals due to γ-hydroxybutenolide ring were observed at δ_H 6.10 (1H, *s*), 6.29 (1H, *br s*) and 8.02 (1H, *br s*) in the ^{1}H-NMR spectrum, and at δ_C 98.4, 122.1, 163.6, and 169.7 in the ^{13}C-NMR spectrum. In comparison with the ^{1}H- and ^{13}C-NMR data of limonexic acid and isolimonexic acid (Lee *et al*., 1999), the signals of mixture **GP7** consisted of those of limonexic acid [**268**] (compound **GP7-I**) and isolimonexic acid [**269**] (compound **GP7-II**). Thus, mixture **GP7** was identified as a mixture of limonexic acid and isolimonexic acid. Although, limonexic acid and isolimonexic acid had their epimers at C-23 and C-21, respectively, the stereochemistry of mixture **GP7** at C-23 for limonexic and C-21 for isolimonexic acid remained to be undetermined. The mixture **GP7** showed the minus sign of specific rotation ($[\alpha]^{18}_{D}$ −41.8° (MeOH)), which was the same as those of limonexic acid ($[\alpha]_D$ −65° (MeOH)) and isolimonexic acid ($[\alpha]_D$ −140° (MeOH)).



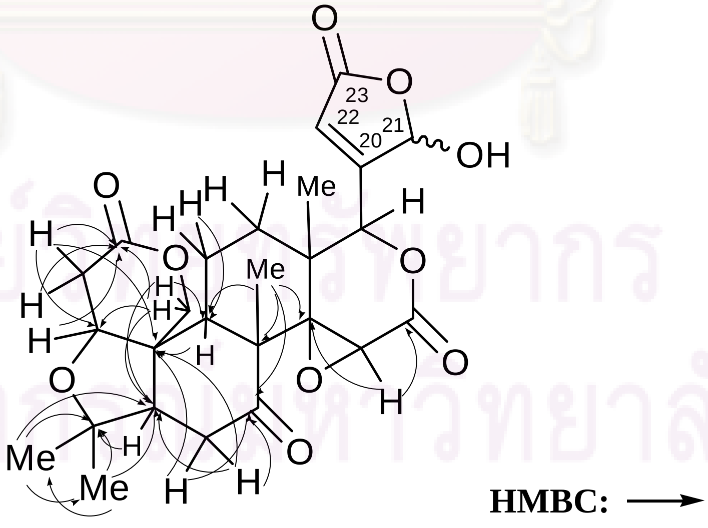
limonexic acid [**268**]



isolimonexic acid [**269**]



limonexic acid [**268**]



isolimonexic acid [**269**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 15 NMR spectral data of compound **GP7-I** and limonexic acid

position	Compound **GP7-I** a)		limonexic acid c)	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	4.12 (1H, *d*, 4.8)	78.4	4.13 (1H, *d*, 3.5)	78.4
2a	2.67 (1H, *dd*, 16.2, 4.8)	35.6	2.65 (1H, *dd*, 3.5, 15.8)	35.5
2b	2.81 (1H, *d*, 16.2)	-	2.78 (1H, *d*, 15.8)	-
3	-	170.1	-	169.8
4	-	79.5	-	79.4
5	2.53 (1H, *dd*, 15.1, 3.1)	57.3	2.52 (1H, *dd*, 3.3, 15.1)	57.3
6α	2.26 (1H, *dd*, 15.1, 3.1)	36.1	2.29 (1H, *dd*, 3.3, 15.1)	36.0
6β	3.08 (1H, *t*, 15.1)	-	3.08 (1H, *t*, 15.1)	-
7	-	207.8	-	207.6
8	-	49.8	-	49.8
9	2.60 (1H, *dd*, 12.4, 3.4)	45.8	2.62 (1H, *dd*, 3.4, 12.4)	45.8
10	-	45.1	-	45.1
11α	1.63-1.80 (1H, *m*)	17.1	1.74 (1H, *m*)	17.0
11β	1.87-1.95 (1H, *m*)	-	1.93 (1H, *m*)	-
12α	1.28-1.31 (1H, *m*)	27.1	1.32 (1H, *m*)	27.1
12β	1.63-1.80 (1H, *m*)	-	1.79 (1H, *m*)	-
13	-	38.6	-	38.4
14	-	67.1	-	66.9
15	4.24 (1H, *s*)	54.2	4.25 (1H, *s*)	54.1
16	-	166.8	-	166.5
17	5.26 (1H, *s*)	75.1	5.27 (1H, *s*)	74.7 75.0 d)
18	1.16 (3H, *s*)	18.6	1.19 (3H, *s*)	18.5
19 a	4.49 (1H, *d*, 13.7)	64.6	4.51 (1H, *d*, 13.1)	64.5
19b	4.90 (1H, *d*, 13.7)	-	4.88 (1H, *d*, 13.1)	-
20	-	131.4	-	130.6
21	-	169.0	-	169.4
22	7.48 (1H, *br s*)	152.2	7.51 (1H, *s*)	152.2 152.9 d)
23	6.29(1H, *br s*, overlapping))	98.4	6.19 (1H, *br s*)	97.4 97.8 d)
28	1.19 (3H, *s*)	29.6	1.21 (3H, *s*)	29.6
29	1.02 (3H, *s*)	21.4	1.05 (3H, *s*)	21.2
30	1.02 (3H, *s*)	17.5	1.05 (3H, *s*)	17.4
OH-23	8.02 (1H, *br s*) b)	-	7.79 (1H, *br s*)	-

a) ^{1}H NMR (DMSO-d_6, 600 MHz); ^{13}C NMR (DMSO-d_6, 150 MHz).

b) Exchangeable proton.

c) The ^{1}H-NMR (DMSO-d_6, 400 MHz at 320 K) and ^{13}C NMR (DMSO-d_6, 100 MHz, at 320 K) data were reported by Lee *et al*., 1999.

d) Pair signals was considered to be 23*R*-limonexic acid. Accordingly, limonexic acid was obtained as a mixture of 23*S* and 23*R* compounds (Lee *et al*., 1999).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 16 NMR spectral data of compound **GP7-II** and isolimonexic acid

position	Compound **GP7-II** a)		isolimonexic acid c)	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	4.11 (1H, *d*, 4.5)	78.5	4.12 (1H,*d*, 3.8)	78.8
2a	2.63 (1H, *dd*, 16.5, 4.5)	35.6	2.67 (1H, *dd*, 16.5, 3.8)	36.1
2b	2.77 (1H, *d*, 16.5)	-	2.82 (1H, *d*, 16.5)	-
3	-	170.1	-	169.5
4	-	79.6	-	80.0
5	2.47 (1H, *dd*, 15.1, 3.1)	58.2	2.48 (1H, *dd*, 2.9, 15.1)	58.7
6*α*	2.29 (1H, *dd*, 15.1, 3.1)	36.1	2.28 (1H, *dd*, 2.9, 15.1)	36.6
6*β*	3.14 (1H, *t*, 15.1)	-	3.16 (1H, *t*, 15.1)	-
7	-	207.9	-	208.3
8	-	50.6	-	51.1
9	2.54 (1H, *dd*, 10.3, 3.2)	46.4	2.54 (1H, *br d*, 11.6)	46.8
10	-	45.2	-	45.7
11*α*	1.63-1.85 (2H, *m*)	17.6	1.69-1.77 (2H, *m*)	18.1
11*β*	-	-	-	-
12*α*	1.63-1.85 (2H, *m*)	28.7	1.69-1.77 (2H, *m*)	29.1
12*β*	-	-	-	-
13	-	37.8	-	38.3
14	-	66.1	-	66.5
15	4.07 (1H, *s*)	53.1	4.06 (1H, *s*)	53.5
16	-	166.1	-	166.6
17	5.19 (1H, *br s*)	77.7	5.18 (1H, *s*)	78.2
18	1.12 (3H, *s*)	19.7	1.10 (3H, *s*)	20.2
19a	4.46 (1H, *d*, 13.1)	64.8	4.47 (1H, *d*, 13.0)	65.3
19b	4.94 (1H, *d*, 13.1)	-	4.95 (1H, *d*, 13.0)	-
20	-	163.6	-	164.2
21	6.10 (1H, *s*)	98.4	6.08 (1H, *s*)	98.6
22	6.29 (1H, *br s*)	122.1	6.29 (1H, *s*)	122.4
23	-	169.7	-	170.6
28	1.20 (3H, *s*)	29.7	1.19 (3H, *s*)	30.1
29	1.04 (3H, *s*)	21.4	1.03 (3H, *s*)	21.9
30	0.99 (3H, *s*)	16.5	0.98 (3H, *s*)	16.9
OH-21	8.02 (1H, *br s*) b)	-	-	-

a) ^{1}H NMR (DMSO-d_6, 600 MHz); ^{13}C NMR (DMSO-d_6, 150 MHz).

b) Exchangeable proton.

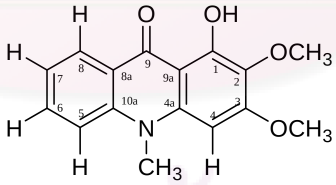
c) The ^{1}H-NMR (DMSO-d_6, 400 MHz at 320 K) and ^{13}C NMR (DMSO-d_6, 100 MHz, at 320 K) data were reported by Lee *et al*., 1999.

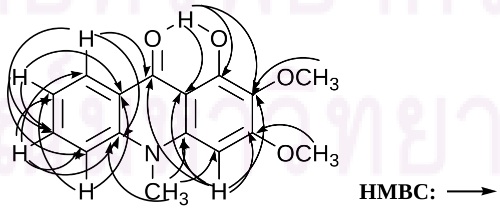
d) The three pair carbon signals observed in the NMR spectrum of limonexic acid were not observed in isolimonexic acid, but isolimonexic acid was considered to be a mixture of 21*R* and 21*S* from the reason related to limonexic acid (Lee *et al*., 1999).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.8 Structure Determination of Compound GP8

Compound **GP8** was isolated as yellow needles with m.p. 179-181°C (Lit. Banerjee *et al.*, 1961; 175°C). It showed $[M+H]^+$ at *m/z* 286 in the FABMS **(Figure 127)**, suggesting the molecular formula $C_{16}H_{15}NO_4$. The UV (229, 274, 326 and 399 nm, **Figure 129**) and IR (3734 and 1635 cm^{-1}, **Figure 130**) absorption bands, in addition to proton signal at δ_H 14.74 in ^{1}H-NMR spectrum **(Figure 131)** indicated the presence of 1-hydroxy-9-acridone nucleus. In the aromatic region of ^{1}H-NMR spectrum, the four among five protons mutaully coupled signals at δ_H 7.23 (1H, *dd*, J = 8.3, 7.3 Hz), 7.44 (1H, *d*, J = 8.3 Hz), 7.67 (1H, *dd*, J = 8.2, 7.3 Hz) and 8.33 (1H, *d*, J = 8.2 Hz) were attributed to H-7, H-5, H-6 and H-8, respectively. One *N*-methyl and two methoxy signals were found at δ_H 3.76 (3H, *s*), 3.90 (3H, *s*) and 3.98 (3H, *s*), respectively. The conjugated ketone signal was detected at δ_C 180.6 (C-9) in the ^{13}C-NMR spectrum **(Figure 132)**. The HMBC correlations of the proton at δ_H 14.74 (OH-1, *s*) with the carbons at δ_C 105.6, 156.0 and 130.0, and the methyl protons at δ_H 3.90 (δ_C 60.7) with the carbon at δ_C 130.0 suggested that the carbon signals at δ_C 105.6, 156.0, 130.0 and 60.8 should be assigned to C-9a, C-1, C-2 and OCH_3-2, respectively **(Figure 132)**. The other methoxy group at δ_H 3.98 (δ_C 55.9) was located at C-3 by the evidence of HMBC correlation between δ_H 3.98 and δ_C 159.2 (C-3). The aromatic proton singlet at δ_H 6.17 (1H, *s*) was assigned to H-4 by HMBC correlations of δ_H 6.17 (H-4) with δ_C 105.6 (C-9a, strong), 130.0 (C-2, strong), 159.2 (C-3, medium), 140.3 (C-4a, weak) and 180.6 (C-9, weak). Thus, compound **GP8** was identified as arborinine [**87**].





[**87**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 17 NMR spectral data of compound **GP8** and arborinine

position	Compound **GP8** [a)]		arborinine [b)]
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_C (ppm)
1/ OH-1	14.74 (1H, *s*)	156.0	155.7
2	-	130.0	129.9
3	-	159.2	159.1
4	6.17 (1H, *s*)	86.6	86.7
4a	-	140.3	140.1
5	7.44 (1H, *d*, 8.3)	114.6	114.5
6	7.67 (1H, *dd*, 8.2, 7.3)	133.9	133.7
7	7.23 (1H, *dd*, 8.3, 7.3)	121.4	121.2
8	8.33 (1H, *d*, 8.2)	126.4	126.0
8a	-	120.5	120.3
9	-	180.6	180.4
9a	-	105.6	105.3
10a	-	141.8	141.6
N-CH_3	3.76 (3H, *s*)	34.0	33.8
OCH_3-2	3.90 (3H, *s*)	60.8	60.6
OCH_3-3	3.98 (3H, *s*)	55.9	55.8

a) ^{1}H NMR ($CDCl_3$, 600 MHz, TMS); ^{13}C NMR ($CDCl_3$, 150 MHz, TMS).

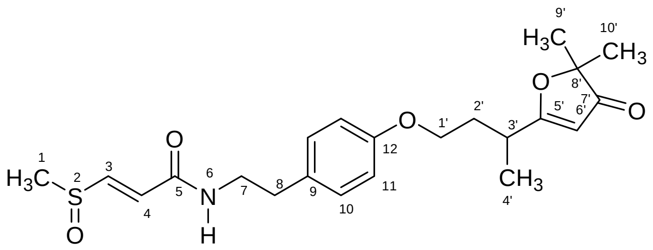
b) The ^{13}C-NMR ($CDCl_3$, TMS) data were reported by Bergenthal *et al*., 1979.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

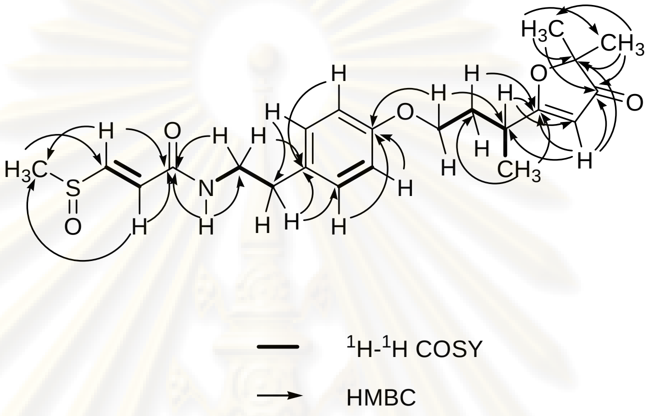
4.9 Structure Determination of Compound GP9

Compound **GP9** was obtained as colorless amorphous mass. The pseudo molecular ion ($[M+H]^+$) peak at *m/z* 420.1831 in the HRFABMS of compound **GP9** suggested the molecular formula $C_{22}H_{29}NO_5S$ (calculated for $C_{22}H_{30}NO_5S$, 420.1845). The optical rotation was positive, $[\alpha]^{21}_D$ +68.2° (*c* 0.10, $CHCl_3$). The UV spectrum **(Figure 137)** showed three absorption maxima at 204, 225 and 261 nm. In the IR spectrum **(Figure 139)** conjugated amide (3287 and 1648 cm^{-1}) and *α,β*-unsaturated ketone (1698 cm^{-1}) functions were observed in addition to a strong absorption due to sulfoxide stretching at 1039 cm^{-1}. The ^1H-NMR spectrum **(Figure 140)** showed three tertiary and one secondary methyl groups, and two ethylene units, one of which was connected to the root of the secondary methyl group, in the aliphatic proton region. In the lower field, the spectrum showed seven protons, six of which were assignable to 1,4-disubstituted benzene [δ_H 6.80 (2H, *d*, *J* = 8.8 Hz) and 7.09 (2H, *d*, *J* = 8.5 Hz)] and *trans-α,β*-unsaturated carbonyl [δ_H 6.69 (1H, *dd*, *J* = 14.5, 1.6 Hz) and 7.52 (1H, *d*, *J* = 14.5 Hz)] systems. The ^{13}C-NMR spectrum **(Figure 141)** supported the above assignments and showed the presence of one additional quaternary carbon (δ_C 88.6). These spectral data were similar to those of a known dihydroglyparvin (Hofer, Vajrodaya and Greger, 1998) which was formally composed of monoterpene, *p*-hydroxyphenethylamine, and *trans-β*-methylsulfonylacrylic acid subunits, except the surrounding of sulfur atom. The presence of 5-(1-methylpropyl)-3(2*H*)-furanone system as a monoterpene unit was suggested by HMBC correlations [δ_H 1.35 (H_3-9′) and 1.36 (H_3-10′) with δ_C 88.6 (C-8′) and 207.6 (C-7′); δ_H 5.35 (H-6′) with δ_C 32.7 (C-3′), 195.0 (C-5′), 207.6 (C-7′), and 88.6 (C-8′); δ_H 2.14 (H-2′a), 2.96 (H-3′), and 1.28 (H_3-4′) with δ_C 195.0 (C-5′); δ_H 2.96 (H-3′) with δ_C 100.3 (C-6′)] **(Figure 145)**. Additional HMBC correlations of δ_H 3.99 (H-1′a) with δ_C 157.6 (C-12), and δ_H 7.52 (H-3), 6.69 (H-4), 6.80 (*N*-H signal overlapped with the aromatic proton signal at δ_H 6.80 (H-11)), and 3.55 (H_2-7) with δ_C 162.6 (C-5) indicated the connection of the monoterpene part with *trans-β*-methylsulfinylacrylic acid one through *p*-hydroxyphenethylamine one. Thus, this could be deduced to be *S*-deoxydihydroglyparvin [**270**], a new *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivative, which was supported by the appearances of characteristic fragment ions at *m/z* 64 and 167 in the EIMS due to methylsulfide (H_3CSOH) and monoterpene ($C_{10}H_{15}O_2$) functions, respectively **(Scheme 19)**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

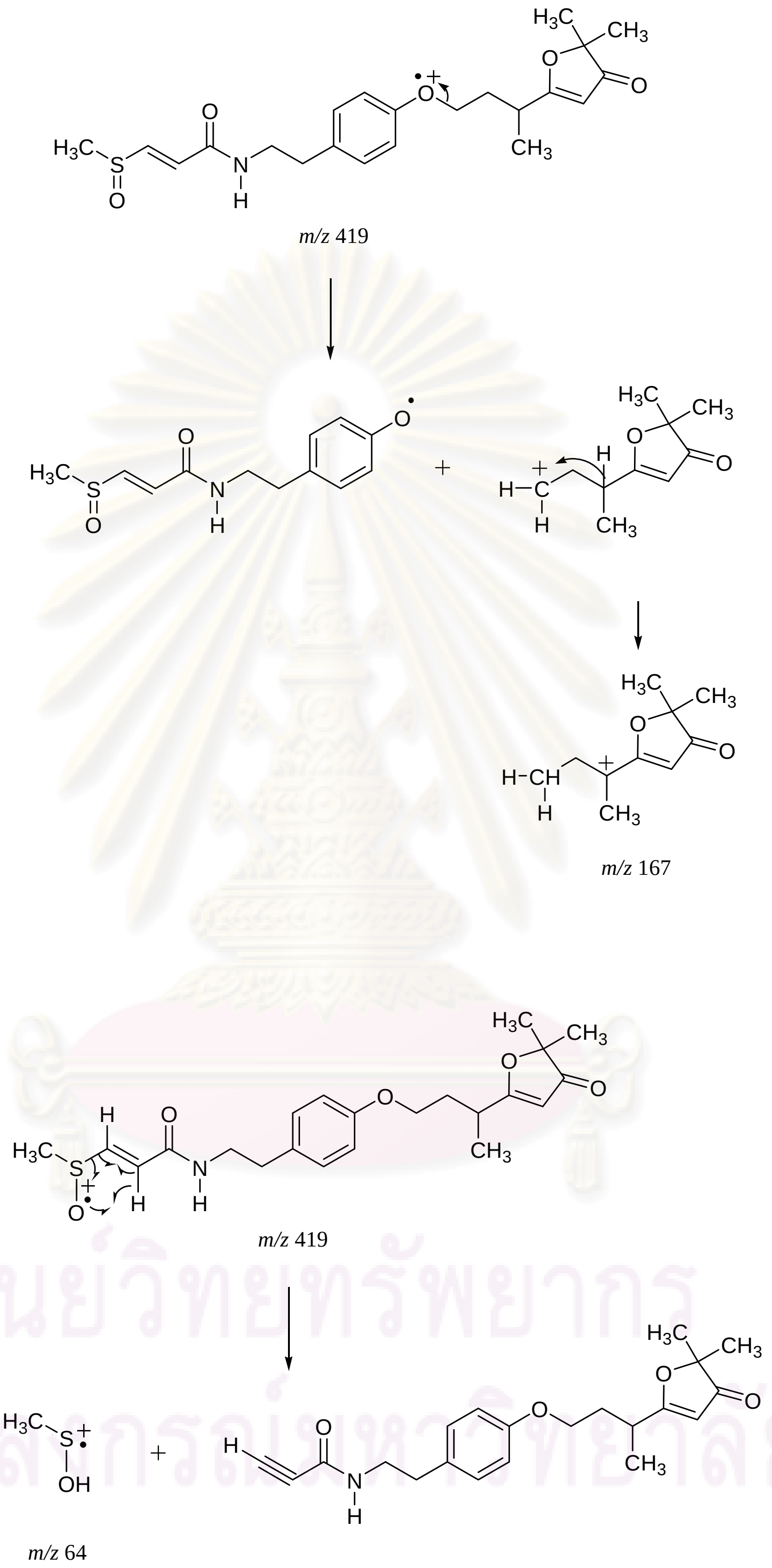


(+)-*S*-deoxydihydroglyparvin



[**270**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Scheme 19 Proposed EIMS fragmentation mechanism of compound **GP9**

Table 18 NMR spectral data of compound **GP9**

position	compound **GP9** [a)]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	2.63 (3H, *s*)	39.9
3	7.52 (1H, *d*, 14.5)	146.3
4	6.69 (1H, *dd*, 14.5, 1.6)	128.6
5	-	162.6
6	6.80 (1H, overlapped signal) [b)]	-
7	3.55 (2H, *dt*, 6.8, 6.5)	41.2
8	2.79 (2H, *t*, 6.9)	34.6
9	-	131.0
10	7.09 (2H, *d*, 8.5)	129.6
11	6.80 (2H, *d*, 8.8)	114.8
12		157.6
1′a	3.99 (1H, *ddd*, 9.5, 6.3, 6.1)	65.3
1′b	3.95 (1H, *ddd*, 9.5, 6.3, 6.1)	
2′a	2.14 (1H, *m*)	33.5
2′b	1.96 (1H, *m*)	-
3′	2.96 (1H, *sextet*, 7.2)	32.7
4′	1.28 (3H, *d*, 7.2)	17.7
5′	-	195.0
6′	5.35 (1H, *s*)	100.3
7′	-	207.6
8′	-	88.6
9′	1.35 (3H, *s*)	23.0
10′	1.36 (3H, *s*)	23.0

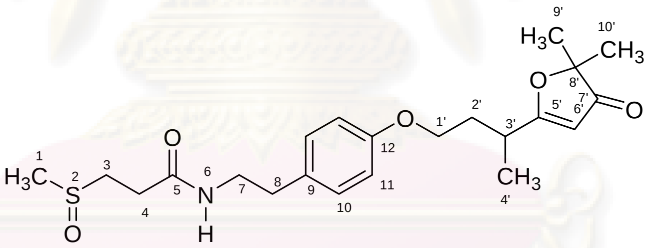
a) ^{1}H-NMR(600 MHz, CDCl$_3$, TMS);^{13}C-NMR (150 MHz, CDCl$_3$, TMS).

b) Exchangeable proton.

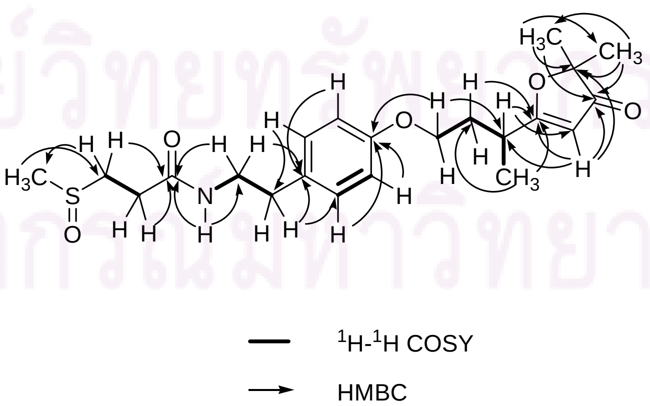
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.10 Structure Determination of Compound GP10

Compound **GP10** was obtained as colorless oil. It possessed the molecular formula $C_{22}H_{29}NO_5S$, as established from the HREIMS, *m/z* 422.1985 $[M+H]^+$ (calcd 422.2001), revealing 2 mass units more than compound **GP9** (*S*-deoxydihydroglyparvin). The optical rotation was positive, $[\alpha]^{18}_{D}$ +24.1° (*c* 0.04, $CHCl_3$). The UV spectrum **(Figure 148)** revealed absorption maxima at λ_{max} 205, 225 and 261 nm. The IR spectrum **(Figure 150)** showed absorption bands at ν_{max} 3291 and 1662 (amide) 1695 (*α,β*-unsaturated ketone) and 1035 (sulfoxide group) cm^{-1}. The ^{1}H-NMR **(Figure 151)** and ^{13}C-NMR **(Figure 152)** spectral data were similar to those of compound **GP9** except for the signals for the acid subunit. Compound **GP10** was reasonably supposed to be *S*-deoxytetrahydroglyparvin [**271**], a new *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivative, carrying a methylsulfinylpropanoic acid subunit instead of *trans-β*-methylsulfinylacrylic acid subunit in compound **GP9** by the following data: δ_H 2.57 (3H, *s*, SMe), 2.64-2.66 (2H, *m*, H_2-4), 2.85 (1H, *ddd*, *J* = 13.2, 6.6, 6.6 Hz, H-3b), and 3.11 (1H, *ddd*, *J* = 13.2, 7.7, 7.7 Hz, H-3a) in the ^{1}H-NMR spectrum; δ_c 38.6 (SMe), 28.8 (C-4), and 49.3 (C-3) in the ^{13}C-NMR spectrum. The ^{1}H-^{1}H COSY and HMBC correlations were shown below **(Figures 154** and **156-157)**.



(+)-*S*-deoxytetrahydroglyparvin



[**271**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 19 NMR spectral data of compound **GP10**

position	compound **GP10** *a)*	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	2.57 (3H, *s*)	38.6
3a	3.11 (1H, *ddd*, 13.2, 7.7, 7.7)	49.3
3b	2.85 (1H, *ddd*, 13.2, 6.6, 6.6)	
4	2.64-2.66 (2H, *m*)	28.8
5	-	169.9
6	6.13 (1H, *br s*) *b)*	-
7	3.46-3.48 (2H, *m*)	41.0
8	2.75 (2H, *t*, 6.9)	34.6
9	-	130.9
10	7.08 (2H, *d*, 8.5)	129.7
11	6.80 (2H, *d*, 8.5)	114.6
12		157.4
1′a	3.98 (1H, *ddd*, 9.4, 6.3, 6.3)	65.2
1′b	3.96 (1H, *ddd*, 9.4, 6.3, 6.3)	
2′a	2.14 (1H, *m*)	33.4
2′b	1.97 (1H, *m*)	-
3′	2.96 (1H, *sextet*, 7.1)	32.5
4′	1.29 (3H, *d*, 7.2)	17.6
5′	-	194.8
6′	5.35 (1H, *s*)	100.1
7′	-	207.5
8′	-	88.5
9′	1.36 (3H, *s*)	22.8
10′	1.36 (3H, *s*)	22.8

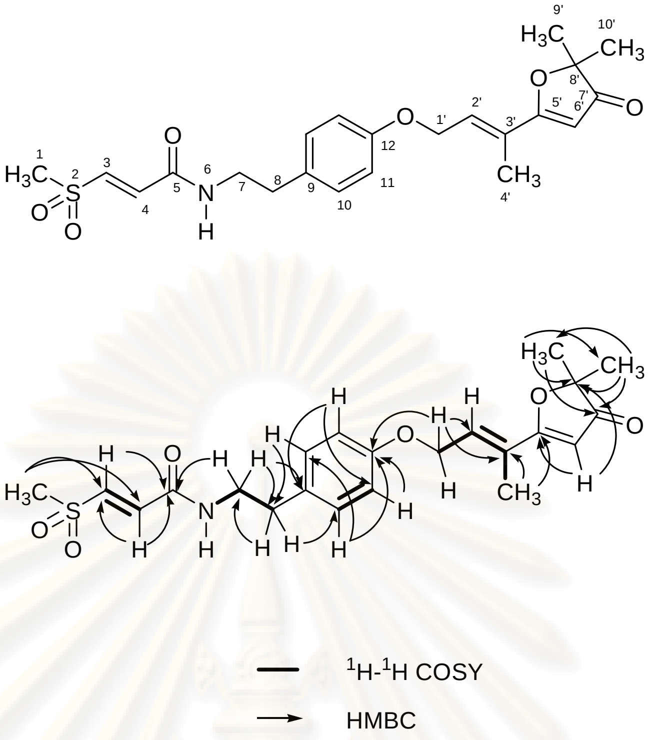
a) ^{1}H-NMR (600 MHz, $CDCl_3$, TMS);^{13}C-NMR (150 MHz, $CDCl_3$, TMS).

b) Exchangeable proton.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.11 Structure Determination of Compound GP11

Compound **GP11** was obtained as colorless needles with m.p. 136-138 °C (Lit. Hofer, Vajrodaya and Greger, 1998; 134-137 °C). The pseudo molecular ion ($[M+H]^+$) peak at *m/z* 434 in the FABMS **(Figure 158)** of the compound **GP11** corresponded to be the molecular formula of $C_{22}H_{27}NO_6S$. The UV spectrum exhibited absorption maxima at λ_{max} 202, 225, 246 and 286 nm **(Figure 160)**. The IR spectrum showed the absorption bands at ν_{max} at 3295 and 1636 (conjugated amide), 1671 (*α,β*-unsaturated ketone), and 1304 and 1131 (sulfonyl group) cm^{-1} **(Figure 161)**. The ^1H-NMR spectrum **(Figure 162)** showed the signals of a AA′BB′ coupling pattern at δ_H 6.88 and 7.13 (each 2H, *d*, *J* = 8.5 Hz, H_2-10 and H_2-11, respectively) attributed to 1,4-disubstituted benzene. Ethylamine and (*Z*)-1-methyl-1-propene fragments were established from the ^1H-^1H COSY cross-peaks between δ_H 2.83 (2H, *t*, *J* = 6.9 Hz)**,** 3.61 (2H, *dt*, *J* = 6.8, 6.6 Hz) and 6.12 (1H, *br t*, *J* = 5.8 Hz), and between δ_H 2.00 (3H, *br s*)**,** 6.79 (1H, *br t*, *J* = 5.8 Hz) and 4.75 (2H, *d*, *J* = 6.0 Hz), respectively **(Figure 165)**. The monoterpene subunit which was formed by the extension of (*Z*)-1-methyl-1-propene fragment with 3(2*H*)-furanone system was evidenced by the HMBC correlations [δ_H 2.00 (H-4′) with δ_C 183.3 (C-5′); δ_H 5.59 (H-6′) with δ_C 183.3 (C-5′) and 207.4 (C-7′); δ_H 1.40 (H-9′ and H-10′) with δ_C 23.1 (C-9′ and C-10′), 88.6 (C-8′) and 207.4 (C-7′)] **(Figure 167)**. The connection of the monoterpene unit with C-12 via ether linkage was suggested by the HMBC correlation between δ_H 4.75 (H-1′) with δ_C 157.2 (C-12) and the chemical shifts of C-10 (δ_C 114.8), C-12 (δ_C 157.2) and C-1′ (δ_C 64.8). The presence of amide group was suggested by the evidence of carbonyl amide signal at δ_C 161.5 (C-4), *N*-H signal at δ_H 6.12 (H-5) and IR absorption. Two remaining coupled olefinic protons at δ_H 6.83 and 7.36 (each 1H, *d*, *J* = 14.6 Hz, H-4 and H-3, respectively) were linked with carbonyl carbon at δ_C 161.5 (C-4) [HMBC correlations of δ_H 6.83 and 7.36 with carbonyl amide signal at δ_C 161.5 (C-4)] and sulfonyl methyl protons at δ_H 2.99 (H_3-1) [HMBC correlations of δ_H 2.99 (H_3-1) with δ_C 138.9 (C-2) and 135.4 (C-3)].These signals revealed that the acid component of amide was (*E*)-3-(methylsulfonyl)propenoic acid. The ethylamine fragment was connected the carbonyl signal at δ_C 161.5 (C-4) and 1,4-disubstituted benzene was suggested by HMBC correlations [δ_H 3.61 (H_2-7) with δ_C 161.5 (C-4); δ_H 2.83 (H_2-8) with δ_C 129.8 (C-10); δ_H 7.13 (H-10) with δ_C 34.3 (C-8)]. Thus, compound **GP11** was identified as a glyparvin-A [**214**].



[**214**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 20 NMR spectral data of compound **GP11** and glyparvin-A

position	compound **GP11** a)		glyparvin-A c)	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	2.99 (3H, *s*)	42.5	2.99 (3H, *s*)	42.5
3	7.36 (1H, *d*, 14.6)	138.9	7.36 (1H, *d*, 14.7)	139.0
4	6.83 (1H, *d*, 14.6)	135.4	6.80 (1H, *d*, 14.7)	135.4
5	-	161.5	-	161.5
6	6.12 (1H, *br t*, 5.8) b)	-	5.92 (1H, *br t*, 6.0)	-
7	3.61 (2H, *dt*, 6.8, 6.6)	41.3	3.61 (2H, *dt*, 6.9, 6.0)	41.3
8	2.83 (2H, *t*, 6.9)	34.3	2.82 (2H, *t*, 6.9)	34.3
9	-	130.7	-	130.7
10	7.13 (2H, *d*, 8.5)	129.8	7.12 (2H, *d*, 8.5)	129.8
11	6.88 (2H, *d*, 8.5)	114.8	6.88 (2H, *d*, 8.5)	114.9
12	-	157.2	-	157.2
1′	4.75 (2H, *d*, 6.0)	64.8	4.75 (2H, *d*, 5.9)	64.8
2′	6.79 (1H, *br t*, 5.8)	132.0	6.78 (1H, *br t*, 5.9)	132.0
3′	-	127.9	-	127.9
4′	2.00 (3H, *br s*)	13.7	2.00 (3H, *br s*)	13.7
5′	-	183.3	-	183.3
6′	5.59 (1H, *s*)	100.0	5.59 (1H, *s*)	100.0
7′	-	207.4	-	207.4
8′	-	88.6	-	88.6
9′	1.40 (3H, *s*)	23.1	1.40 (3H, *s*)	23.1
10′	1.40 (3H, *s*)	23.1	1.40 (3H, *s*)	23.1

a) ^{1}H-NMR (600 MHz, $CDCl_3$, TMS);^{13}C-NMR (150 MHz, $CDCl_3$, TMS).

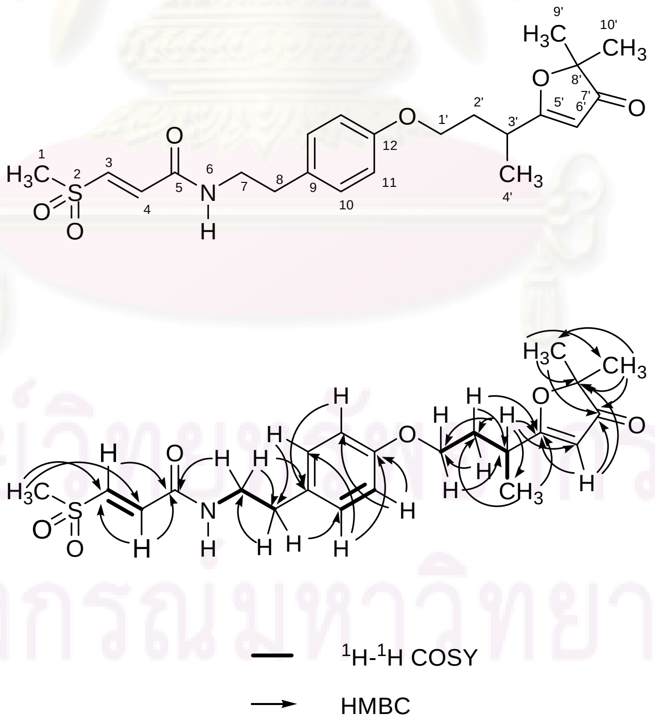
b) Exchangeable proton.

c) The ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) and ^{13}C-NMR (100 MHz, $CDCl_3$, TMS) data were reported by Hofer, Vajrodaya and Greger, 1998.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.12 Structure Determination of Compound GP12

Compound **GP12** was obtained as colorless amorphous mass. It possessed the molecular formula $C_{22}H_{29}NO_6S$, as determined by the FABMS **(Figure 168)**, $[M+H]^+$ at *m*/*z* 436, revealing 2 mass units more than compound **GP11**. Compound **GP12** is optically active with an $[\alpha]^{19}_{D}$ of +20.7° (*c* 0.11, $CHCl_3$). The UV spectrum **(Figure 170)** showed absorption maxima at 202, 224 and 261 nm. The IR spectrum **(Figure 172)** exhibited absorption bands due to conjugated amide (3309 and 1655 cm^{-1}), *α,β*-unsaturated ketone (1696 cm^{-1}) and sulfone (1302 and 1130 cm^{-1}) functions. By comparing the ^1H-NMR and ^{13}C-NMR spectra **(Figures 173** and **174)** with those of compound **GP11**, it was found that the structures of these two compounds were closely related. The only difference was observed around C-2′ and C-3′. The proton signals at δ_H 4.01 (H-1′a), 3.96 (H-1′b), 2.14 (H-2′a), 1.98 (H-2′b) and 2.97 (H-3′) and their ^1H-^1H COSY correlations **(Figure 176)**, in addition to carbon signals at δ_C 33.4 (C-2) and 32.6 (C-3) indicated that the bond between C-2′ and C-3′ was a saturated one comparing with those of compound **GP11**. Thus, compound **GP12** was reasonably deduced to be dihydroglyparvin [**213**].



[**213**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 21 NMR spectral data of compound **GP12** and dihydroglyparvin

position	compound **GP12** [a]		dihydroglyparvin [d]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	2.99 (3H, *s*)	42.5	2.99 (3H, *s*)	42.5
3	7.36 (1H, *d*, 14.7)	138.8	7.37 (1H, *d*, 14.7)	139.0
4	6.88 (1H, *d*, 14.7)	135.6	6.82 (1H, *d*, 14.7)	135.4
5	-	161.6	-	161.5
6	6.41 (1H, *br t*, 5.7) [b]	-	6.08 (1H, *br t*, 6.0)	-
7	3.58 (2H, *q*, 6.6)	41.4	3.60 (2H, *dt*, 6.8, 6.0)	41.4
8	2.80 (2H, *t*, 6.9)	34.3	2.81 (2H, *t*, 6.8)	34.3
9	-	130.5	-	130.4
10	7.08 (2H, *d*, 8.6)	129.7	7.08 (2H, *d*, 8.6)	129.7
11	6.81 (2H, *d*, 8.6)	114.8	6.82 (2H, *d*, 8.6)	114.9
12	-	157.7	-	157.8
1′a	4.01 (1H, *ddd*) [c]	65.3	4.01 (1H, *ddd*) [e]	65.3
1′b	3.96 (1H, *ddd*) [c]		3.98 (1H, *ddd*) [e]	
2′a	2.14 (1H, *m*)	33.4	2.14 (1H, *dddd*) [e]	33.5
2′b	1.98 (1H, *m*)	-	2.00 (1H, *dddd*) [e]	-
3′	2.97 (1H, overlapped signal)	32.6	2.98 (1H, *sextet*)	32.6
4′	1.29 (3H, *d*, 7.2)	17.6	1.30 (3H, *d*, 7.0)	17.6
5′	-	194.9		*
6′	5.30 (1H, *s*)	100.2	5.29 (1H, *s*)	100.3
7′	-	207.6		*
8′	-	88.6		88.5
9′	1.36 (3H, *s*)	22.8	1.37 (3H, *s*)	22.8
10′	(3H, *s*)	22.8	1.37 (3H, *s*)	22.8

a) ^{1}H-NMR (600 MHz, CDCl$_3$, TMS); ^{13}C-NMR (150 MHz, CDCl$_3$, TMS)

b) Exchangeable proton.

c) The ^{1}H-NMR (400 MHz, CDCl$_3$, TMS) and ^{13}C-NMR (100 MHz, CDCl$_3$, TMS) data were reported by Hofer, Vajrodaya and Greger, 1998.

d) proton 1′a at δ_H 4.01 (1H, *ddd*, *J* = 9.5, 6.1, 6.1 Hz), proton 1′b at δ_H 3.96 (1H, *ddd*, *J* = 9.5, 7.1, 5.7 Hz).

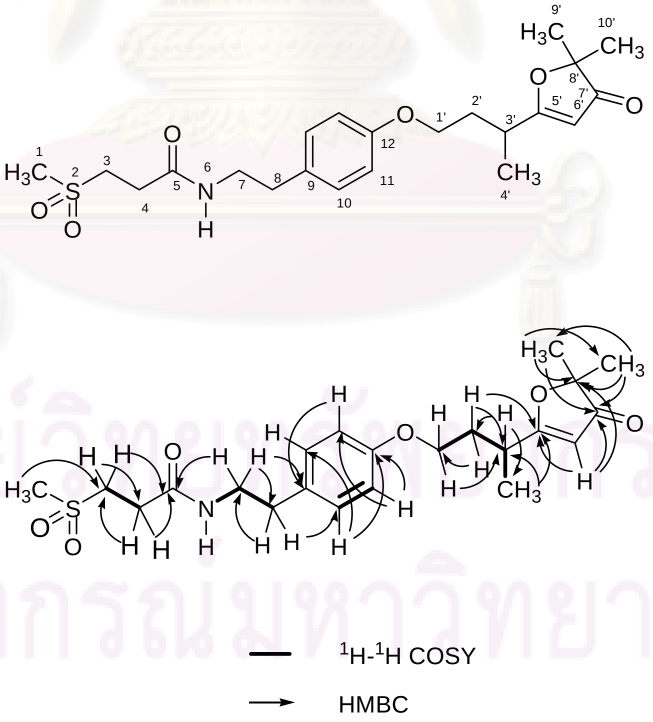
e) Precise assignment of coupling constant; $J_{(1'a,\ 1'b)}$ = 9.6 Hz, $J_{(1'a,\ 2'a)}$ = 5.9 Hz, $J_{(1'a,\ 2'b)}$ = 5.8 Hz, $J_{(1'b,\ 2'a)}$ = 5.6 Hz, $J_{(1'b,\ 2'b)}$ = 7.0 Hz, $J_{(2'a,\ 2'b)}$ = 14.0 Hz, $J_{(2'a,\ 3')}$ = 7.3 Hz, $J_{(2'b,\ 3')}$ = 6.8 Hz, $J_{(3',\ 4')}$ = 7.0 Hz (Hofer, Vajrodaya and Greger, 1998).

* The literature did not report the chemical shift of these signals.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.13 Structure Determination of Compound GP13

Compound **GP13** was obtained as colorless amorphous mass. It was shown to have the molecular formula $C_{22}H_{31}NO_6S$, as deduced from the observed $[M+Na]^+$ at m/z 460.1780 (calculated for $C_{22}H_{31}NO_6SNa$, 460.1770) in the HRFABMS, revealing 2 mass units more than compound **GP12**. The UV spectrum **(Figure 180)** showed absorption maximum at 261 nm. The IR spectrum **(Figure 182)** exhibited the absorption bands due to amide (3328 and 1644 cm^{-1}), *α,β*-unsaturated ketone (1686 cm^{-1}) and sulfone (1294 and 1122 cm^{-1}) functions. Compound **GP13** is optically active with an $[\alpha]^{22}_{D}$ of +43.8° (*c* 0.02, $CHCl_3$). By comparing the ^{1}H-NMR and ^{13}C-NMR spectra **(Figures 183** and **184)** with those of compound **GP12**, it was found that the structures of these two compounds are closely related. However, the signals of proton and carbon around C-3 and C-4 were different. The coupled proton signals at δ_H 3.38 (H_2-3) and 2.67 (H_2-4) and carbon signals at δ_C 50.3 (C-3) and 28.8 (C-4) indicated that a bond between C-3 and C-4 was saturated. Thus, compound **GP13** was reasonably deduced to be tetrahydroglyparvin [**272**], a new *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivative.



[**272**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 22 NMR spectral data of compound **GP13**

position	compound **GP13** [a]	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	2.92 (3H, *s*)	41.5
3	3.38 (2H, *t*, 7.2)	50.3
4	2.67 (2H, *t*, 7.2)	28.8
5	-	168.7
6	5.76 (1H, *br t*, 5.4) [b]	-
7	3.50 (2H, *dt*, 6.9, 6.1)	41.1
8	2.75 (2H, *t*, 6.9)	34.6
9	-	130.7
10	7.08 (2H, *d*, 8.7)	129.7
11	6.81 (2H, *d*, 8.7)	114.7
12	-	157.5
1′a	4.01 (1H, *ddd*, 9.7, 6.0, 6.0)	65.2
1′b	3.95 (1H, *ddd*, 9.7, 6.9, 5.5)	
2′a	2.15 (1H, *m*)	33.4
2′b	1.98 (1H, *m*)	-
3′	2.97 (1H, *sextet*, 7.1)	32.5
4′	1.29 (3H, *d*, 6.9)	17.6
5′	-	194.8
6′	5.34 (1H, *s*)	100.2
7′	-	207.5
8′	-	88.5
9′	1.36 (3H, *s*)	22.9
10′	1.37 (3H, *s*)	22.9

a) ^{1}H-NMR (600 MHz, $CDCl_3$, TMS);^{13}C-NMR (150 MHz, $CDCl_3$, TMS).

b) Exchangeable proton.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

5. Aspect of Stereochemistry of *N*-[(4-Monoterpenyloxy)phenylethyl]-Substituted Sulfur-Containing Propanamide Derivatives

Regarding the stereochemistry of compound **GP9** (*S*-deoxydihydroglyparvin) [**270**], and **GP10** (*S*-deoxytetrahydroglyparvin) [**271**], there are two chiral centers which were a sulfur chiral center and a carbon chiral center at C-3′. For compound **GP12** (dihydroglyparvin) [**213**] and **GP13** (tetrahydroglyparvin) [**272**], there is a carbon chiral center at C-3′. Compound **GP9**, **GP10** and **GP13** were isolated as optically active compounds with the same positive sign of specific rotation; $[\alpha]^{21}_{D}$ +68.2° ($CHCl_3$) in compound **GP9**, $[\alpha]^{18}_{D}$ +24.1° ($CHCl_3$) in compound **GP10** and $[\alpha]^{22}_{D}$ +43.8° ($CHCl_3$) in compound **GP13**. Co-isolation of a known (+)-dihydroglyparvin (compound **GP12**, $[\alpha]^{19}_{D}$ +20.7° ($CHCl_3$)), lacking the sulfur chiral center, suggested that the carbon chiral center of the monoterpene units in these four might be the same absolute stereochemistry, even remaining unknown. (+)-Entadamide C [**273**] had been isolated from *Entada phaseoloides* as a related and simple *β*-methylsufinylcarboxyamide derivative, and the absolute configuration of the sulfur atom had been reported to be an *R* configuration (Ikegami *et al*., 1989). Thus, the positive sign of $[\alpha]_{D}$ in compound **GP9** and compound **GP10** could suggest the same *R*-configuration of the sulfur chiral center which is in (+)-entadamide C. To support this proposal, the circular dichroism (CD) spectra of compound **GP9**, **GP10**, **GP12** and **GP13** were examined and compared with the molar ellipticity of (+)-entadamide C, $[\Phi]_{252}$ +6500 (MeOH), reported by Ikegami *et al*., 1989. Compound **GP9**, **GP10**, **GP12** and **GP13** exhibited the positive cotton effect; $[\Phi]_{256}$ +6173 in compound **GP9 (Figure 138)**, $[\Phi]_{260}$ +2272 in compound **GP10 (Figure 149)**, $[\Phi]_{262}$ +3397 in compound **GP12 (Figure 171)** and $[\Phi]_{264}$ +1651 in compound **GP13 (Figure 181)**. All four compounds showed the positive cotton effect at the wavelength near the absorption maxima in UV spectrum. Even though the compound **GP12** and **GP13** lacked the sulfur chiral atom, they exhibited the positive maxima at the wavelength near those of compound **GP9** and **GP10**. This finding suggested that the positive maxima due to the sulfur chiral center and the carbon chiral center were quite close together. These results supported the proposal that the absolute configuration of the sulfur atom of compound **GP9** and **GP10** could be *R*-configuration and the absolute configuration of carbon chiral center in compound **GP9**, **GP10**, **GP12** and **GP13** might be the same absolute stereochemistry, even hitherto unknown.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

6. Antiviral Activity against Herpes Simplex Virus (HSV) Type 1 and Type 2 of the Isolated Compounds

Isolated compounds were subjected to antiviral activity tests using HSV-1 and HSV-2 and results are shown in **Table 23**.

Table 23 Antiviral activities of isolated compounds against HSV-1 and HSV-2 determined by plaque reduction assay

compound	final conc. (µg/mL)	EC$_{50}$ [a] (µg/mL, ((µM)) inactivation treatment		EC$_{50}$ [a] (µg/mL, ((µM)) post-treatment		CC$_{50}$ [b] (µg/mL, ((µM))
		HSV-1	HSV-2	HSV-1	HSV-2	
MH1	100	0	0	0	0	
MH2	100	0	0	0	0	
MH3	100	0	0	0	0	
MH4	100	0	0	0	0	
GP1	100	0	0	0	0	
GP2	-	50 (151)	50 (151)	50 (151)	50 (151)	150 (453)
GP3	100	0	0	0	0	
GP4	100	0	0	0	0	
GP5	-	100 (348)	>100 (348)	100 (348)	>100 (348)	>100 (348)
GP6	100	0	0	0	0	
GP7	100	0	0	0	0	
GP8	100	0	0	0	0	
GP9	-	12.5 (29.8)	18.7 (44.6)	12.5 (29.8)	18.7 (44.6)	37.5 (89.4)
GP10	100	0	0	0	0	
GP11	1.56	0	0	0	0	3.12 (7.2)
GP12	6.25	0	0	0	0	12.5 (28.7)
GP13	-	100 (229)	>100 (>229)	0	0	>100 (>229)

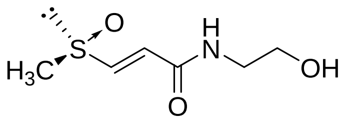
a) EC$_{50}$ (50% effective concentration, µg/mL, (µM)) was determined from three independent assays. Maximum concentration tested were 100 µg/mL. The EC$_{50}$ of acyclovir against HSV-1 was 0.5 µg/mL and 0.63 µg/mL in post-treatment and inactivation treatment, used as positive control.

b) CC$_{50}$ (50% cytotoxic concentration), the concentration that was 50% cytotoxic to the cells used in assay, was determined from three independent assays.

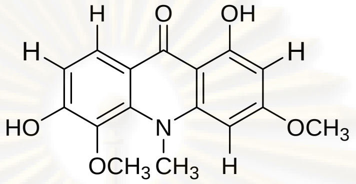
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Anti-HSV activities against HSV-1 and HSV-2 of the isolates were assessed (**Table 23**). Coumarins (compound **MH1**, **MH2** and **MH4**) and a 4-quinolone (compound **MH3**) showed no activity against HSV-1 and HSV-2. Compound **GP2** (glycofolinine) [**163**] exhibited moderate activity with EC_{50} of 151 µM against both HSV-1 and HSV-2 in inactivation treatment and post-treatment, and the 50% cytotoxic concentration (CC_{50}) to the cells used in assay was 453 µM. A new alkaloid, compound **GP5** (glycosparvarine) [**266**] also exhibited activity even with higher EC_{50} (348 µM). In 3-methoxyacridone series, structure-activity relationship (SAR) for anti-herpes simplex virus activity has been suggested the responsibility of the hydroxyl group at either the C-5 or C-6 positions such as citpressine-I [**274**] and citrusinine-I [**275**] (Yamamoto *et al*., 1989). Furthermore, potential anti-herpes simplex virus activity has been observed in the 2,2-dimethylpyranoacridones (Yamamoto *et al*., 1989). These studies showed that oxygen-substituted acridones could act as an additional acridone potential for anti-HSV activity. The limonoid-type compounds (compound **GP6** and mixture **GP7**) showed no activity against HSV-1 and HSV-2.

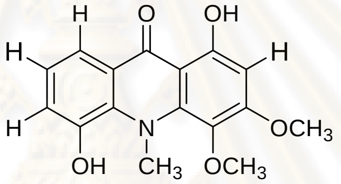
In addition to *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives, compound **GP9** (*S*-deoxydihydroglyparvin) [**270**] exhibited antiviral activities against HSV-1 and HSV-2 with EC_{50} of 29.8 and 44.6 µM in activation treatment and post-treatment, respectively. However, the EC_{50} of compound **GP9** was close to the CC_{50} (89.4 µM). Compound **GP13** (tetrahydroglyparvin) [**272**] showed moderate activity with EC_{50} of 229 µM against both HSV-1 and HSV-2 in inactivation treatment. Compound **GP10** (*S*-Deoxytetrahydroglyparvin) [**271**] showed no activity at concentration tested (100 µg/mL) in inactivation treatment and post-treatment. In contrast, compound **GP11** (glyparvin-A) and compound **GP12** (dihydroglyparvin) showed no activity at the non-toxic concentration. The cytotoxicity against Vero cell of *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives could result from the presence of 2,2-dimethyl-3(2*H*)-furanone system which possesses antitumor activity in natural product antitumor agents such as geiparvarin [**276**] (Smith III *et al*., 1981).



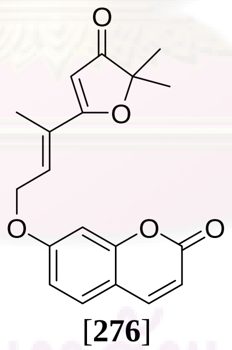
[**273**]



[**274**]



[**275**]



[**276**]

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER V

CONCLUSION

This is the first report on anti-herpes simplex virus activity of extracts of *M. hirsutum* and *G. parva*. There was no mention of anti-herpes simplex virus activity of plants in *Micromelum* spp. and *Glycosmis* spp. that have been used in Thai traditional medicine.

In this investigation, from the branches of *Micromelum hirsutum* Oliv., a new natural product, namely 1,2-dimethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [**262**] was isolated along with two known compounds, scopoletin [**41**] and micromelin [**5**]. For the leaves of *Micromelum hirsutum* Oliv., two known compounds, micromelin [**5**] and (–)-(2′*S*, 3′*R*)-3′-senecioyloxymarmesin [**263**] were isolated.

Chemical examination of the branches of *Glycosmis parva* Craib led to the isolation of a new acridone alkaloid, namely glycosparvarine (1,3,5-trihydroxy-2-methoxy-*N*-methyl-9-acridone) [**266**], together with four known acridone alkaloids and three limonoids. Known compounds were *N*-methylatalaphylline [**258**], glycofolinine [**163**], citramine [**264**], *N*-methylcyclo-atalaphylline-A [**265**], limonin [**267**], and a mixture of limonexic acid [**268**] and isolimonexic acid [**269**]. From the leaves of *Glycosmis parva* Craib, three new *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives, namely (+)-*S*-deoxydihydroglyparvin [**270**], (+)-*S*-deoxytetrahydroglyparvin [**271**] and (+)-tetrahydroglyparvin [**272**] were isolated along with two known derivatives [glyparvin-A [**214**] and (+)-dihydroglyparvin [**213**]] and a known acridone alkaloid, arborinine [**87**].

Isolated compounds were evaluated the anti-herpes simplex virus activity. Glycosparvarine [**266**], glycofolinine [**163**] and (+)-tetrahydroglyparvin [**272**] exhibited moderate activities against both HSV-1 and HSV-2 with EC_{50} of 151 µM, 348 µM and 229 µM, respectively. On the other hand, (+)-*S*-deoxydihydroglyparvin [**270**] exhibited more potent activities with lower EC_{50} of 29.8 and 44.6 µM against HSV-1 and HSV-2, respectively. (+)-*S*-Deoxydihydroglyparvin [**270**] is interesting to investigate the anti-herpes simplex virus mechanism.

REFERENCES

ชยันต์ พิเชียรสุนทร, แม้นมาส ชวลิต และวิเชียร จีรวงส์. 2548. คำอธิบายตำราพระโอสถพระนารายณ์. พิมพ์ครั้งที่ 2. กรุงเทพมหานคร: สำนักพิมพ์อมรินทร์.

นิจศิริ เรืองรังษี และธวัชชัย มังคละคุปต์. 2547. สมุนไพรไทย เล่ม 1. พิมพ์ครั้งที่ 1. กรุงเทพมหานคร: ฐานการพิมพ์.

วุฒิ วุฒิธรรมเวช และธนศักดิ์ วุฒิธรรมเวช. 2540. สารานุกรมสมุนไพร รวมหลักเภสัชกรรมไทย. พิมพ์ครั้งที่ 1. กรุงเทพมหานคร: สำนักพิมพ์โอเดียนสโตร์.

Anwer, M. F., Kapil, R. S. and Popli, S. P. 1972. Identity of glycozolidine. Indian J. Chem. 10: 959.

Banerjee, S. K., Chakravarti, D., Chakravarti, R. N., Fales, H. M. and Klayman, D. L. 1961. Alkaloids of *Glycosmis arborea*-III: Structure of arborinine. Tetrahedron 16: 251-254.

Banerji, J., Das, A. K., Ghoshal, N. and Das, B. 1988. Studies on Rutaceae. Part VIII. Chemical investigation on the constituents of *Atalantia wightii* Tanaka, *Aegle marmelos* Correa ex Koen, *Ruta graveolens* Linn. and *Micromelum pubescens* Blume. Indian J. Chem., Sect. B 27B: 594-596.

Banerji, J., Ghoshal, N., Sarkar, S., Patra, A., Abraham, K. and Shoolery, J. N. 1981. Studies on Rutaceae: Part I–Carbon-13 NMR studies on acridone bases & structure of an alkaloid from *Atalantia wightii*. Indian J. Chem., Sect. B 20B: 835-838.

Bergenthal, D., Mester, I., Rózsa, Z. and Reisch, J. 1979. ^{13}C-NMR-spektren einiger acridon-alkaloide. Phytochemistry 18: 161-163.

Bhakuni, D. S., Gupta, N. C., Satish, S., Sharma, S. C., Shukla, Y. N. and Tandon, J. S. 1971. Medicinal plants. III. Ericaceae and other families. Chemical constituents of *Actinodaphne augustifolia*, *Croton sparsiflorus*, *Duabanga sonneratiodes*, *Glycosmis mauritiana*, *Hedyotis auricularia*, *Lyonia ovalifolia*, *Micromelum pubescens*, *Pyrus pashia* and *Rhododendron niveum*. Phytochemistry 10: 2247-2249.

Bhattacharyya, P., Chakrabartty, P. K. and Chowdhury, B. K. 1985. Glycozolidol, an antibacterial carbazole alkaloid from *Glycosmis pentaphylla*. Phytochemistry 24: 882-883.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Bhattacharyya, P. and Chowdhury, B. K. 1984. Glycophylone: a new quinolone alkaloid from *Glycosmis pentaphylla* (Retz) DC. Chem. Ind. 352.

Bhattacharyya, P. and Chowdhury, B. K. 1985. Glycozolidal, a new carbazole alkaloid from *Glycosmis pentaphylla*. J. Nat. Prod. 48: 465-466.

Bhattacharyya, P. and Chowdhury, B. K. 1985. Glycolone, a quinolone alkaloid from *Glycosmis pentaphylla*. Phytochemistry 24: 634-635.

Bhattacharyya, P., Sarkar, T., Chakraborty, A. and Chowdhury, B. K. 1984. Structure and synthesis of glycozolinol, a new carbazole alkaloid from *Glycosmis pentaphylla* (Retz) DC. Indian J. Chem., Sect. B 23B: 49-51.

Bowen, I. H. and Perera, K. 1982. Alkaloids, coumarins and flavonoids of *Micromelum zeylanicum*. Phytochemistry 21: 433-437.

Bowen, I. H., Perera, K. and Lewis, J. R. 1980. Alkaloids from the stem of *Glycosmis bilocularis* (Rutaceae). Phytochemistry 19: 1566-1568.

Bowen, I. H., Perera, K. P. W. C. and Lewis, J. R. 1978. Alkaloids of the leaves of *Glycosmis bilocularis*. Phytochemistry 17: 2125-2127.

Breksa III A. P., Dragull K. and Wong R. Y. 2008. Isolation and identification of the first C-17 limonin epimer, epilimonin. J. Agric. Food. Chem. 56: 5595-5598.

Cassady, J. M., Ojima, N., Chang, C. and McLaughlin, J. L. 1979. An investigation of the antitumor activity of *Micromelum integerrimum* (Rutaceae). J Nat Prod. 42: 274-278.

Chakraborty, D. P. 1966. Chemical taxonomy. V. Glycozoline, a carbazole derivative from *Glycosmis pentaphylla*. Tetrahedron Lett. 661-664.

Chakraborty, D. P. 1969. Chemical taxonomy. XII. Glycozoline, a carbazole derivative, from *Glycosmis pentaphylla*. Phytochemistry 8: 769-772.

Chakraborty, K., Roy, S. and Chakraborty, D. P. 1989. Mupamine from *Glycosmis pentaphylla*. Phytochemistry 28: 677-678.

Chakravarti, D., Chakravarti, R. N., Cohen, L. A., Dasgupta, B., Datta, S. and Miller, H. K. 1961. Alkaloids of *Glycosmis arborea* [*G. pentaphylla*]. II. Structure of arborine. Tetrahedron 16: 224-250.

Chakravarty, A. K., Das, B., Masuda, K. and Ageta, H. 1996. Tetracyclic triterpenoids from *Glycosmis arborea*. Phytochemistry 42: 1109-1113.

Chakravarty, A. K., Sarkar, T., Masuda, K. and Shiojima, K. 1999. Carbazole alkaloids from roots of *Glycosmis arborea*. Phytochemistry 50: 1263-1266.

Chatterjee, A., Dutta, C. P. and Bhattacharyya, S. 1967. Micromelumin and micropubescin two new coumarins from *Micromelum pubescens*. Sci. Cult. (Calcutta) 33: 371-373.

Chatterjee, A. and Majumdar, S. G. 1954. Alkaloids of *Glycosmis pentaphylla* I. J. Am. Chem. Soc. 76: 2459-2463.

Choi, H., Gwak, J., Cho, M., Ryu, M.-J., Lee, J.-H., Kim, S. K., Kim, Y. H., Lee, G. W., Yun, M.-Y., Cuong, N. M., Shin, J.-G., Song, G.-Y. and Oh, S. 2010. Murrayafoline A attenuates the Wnt/β-catenin pathway by promoting the degradation of intracellular *β*-catenin proteins. Biochem. Biophys. Res. Commun. 391: 915-920.

Chowdhury, B. K., Mustapha, A., Garba, M. and Bhattacharyya, P. 1987. Carbazole and 3-methylcarbazole from *Glycosmis pentaphylla*. Phytochemistry 26: 2138-2139.

Chukaew A., Ponglimanont C., Karalai C. and Tewtrakul S. 2008. Potential anti-allergic acridone alkaloids from the roots of *Alantia monophylla*. Phytochemistry 69: 2616-2620.

Croft, K. D. and Toia, R. F. 1989. Coumarins from *Micromelum minutum*. Planta Med. 55: 401.

Cuong, N. M., Hung, T. Q., Van Sung, T. and Taylor, W. C. 2004. A new dimeric carbazole alkaloid from *Glycosmis stenocarpa* roots. Chem. Pharm. Bull. 52: 1175-1178.

Cuong, N. M., Taylor, W. C. and Sung, T. V. 1999. Glypetelotine, a sulphur-containing indole alkaloid from *Glycosmis petelotii*. Phytochemistry 52: 1711-1714.

Das, B. P., Chowdhury, D. N., Choudhury, B. and Mester, I. 1982. Isolation and structure of glycosolone, a new 2-quinolone alkaloid from *Glycosmis pentaphylla* (Retz) DC. Indian J. Chem., Sect. B 21B: 176-178.

Das, S., Baruah, R. H., Sharma, R. P., Barua, J. N., Kulanthaivel, P. and Herz, W. 1984. 7-Methoxycoumarins from *Micromelum minutum*. Phytochemistry 23: 2317-2321.

De Silva, L. B., De Silva, U. L. L., Mahendran, M. and Jennings, R. C. 1980. Isolation of 7-[4'-(4''-methyl-5''-oxo-2'',5''-dihydro-2''-furyl)-3'-methyl-2'-butenyloxy]coumarin from *Micromelum ceylanicum* Swingle. Indian J. Chem., Sect. B 19B: 820-821.

Diep, P. T. M., Pawlowska, A. M., Cioni, P. L., Nghi, D. H., Huong, L. M., Van Minh, C. and Braca, A. 2007. Chemical and biological studies of the essential oils of *Micromelum hirsutum*. Nat. Prod. Commun. 2: 691-694.

Fujioka, H., Nishiyama, Y., Furukawa, H. and Kumada, N. 1989. In vitro and in vivo activities of atalaphillinine and related acridone alkaloids against rodent malaria. Antimicrob. Agents Chemother. 33: 6-9.

Furukawa, H., Ito, C., Ono, T., Wu, T. S. and Kuoh, C. S. 1993. Spectroscopic elucidation of glycobismines, first naturally occurring binary acridone alkaloids containing a carbon-carbon linkage. J. Chem. Soc., Perkin Trans. 1 471-475.

Furukawa, H., Wu, T. S., Kuoh, C. S., Sato, T., Nagai, Y. and Kagei, K. 1984. Acridone alkaloids. Part XII. The structure of glycobismine-A, the first naturally occurring "binary" acridone alkaloid containing a carbon-carbon linkage. Chem. Pharm. Bull. 32: 1647-1649.

Govindachari T. R., Viswanathan N., Pai B. R., Ramachandran V. N. and Subramaniam P. S. 1970. Alkaloids of *Alantia monophylla* Correa. Tetrahedron 26: 2905-2910.

Greger, H., Hadacek, F., Hofer, O., Wurz, G. and Zechner, G. 1993. Different types of sulfur-containing amides from *Glycosmis cf. chlorosperma*. Phytochemistry 32: 933-936.

Greger, H., Hofer, O., Kaehlig, H. and Wurz, G. 1992. Sulfur containing cinnamides with antifungal activity from *Glycosmis cyanocarpa*. Tetrahedron 48: 1209-1218.

Greger, H., Hofer, O., Zechner, G., Hadacek, F. and Wurz, G. 1994. Sulfones derived from methylthiopropenoic acid amides from *Glycosmis angustifolia*. Phytochemistry 37: 1305-1310.

Greger, H., Zechner, G., Hofer, O., Hadacek, F. and Wurz, G. 1993. Sulfur-containing amides from *Glycosmis* species with different antifungal activity. Phytochemistry 34: 175-179.

Greger, H., Zechner, G., Hofer, O. and Vajrodaya, S. 1996. Bioactive amides from *Glycosmis* species. J. Nat. Prod. 59: 1163-1168.

Hinterberger, S., Hofer, O. and Greger, H. 1994. Synthesis and corrected structures of sulphur-containing amides from *Glycosmis* species: Sinharines, penimides, and. Tetrahedron 50: 6279-6286.

Hofer, O., Greger, H., Lukaseder, B., Vajrodaya, S. and Bacher, M. 2000. Prenylated sulfonyl amides from *Glycosmis* species. Phytochemistry 54: 207-213.

Hofer, O., Vajrodaya, S. and Greger, H. 1998. Phenethylamides with an unusual 4-oxo-2-oxolenyl terpenoid side chain from *Glycosmis* species. Monatsh. Chem. 129: 213-219.

Hofer, O., Zechner, G., Vajrodaya, S., Lutz, G. and Greger, H. 1995. New anthranilic and methylsulfonylpropenoic acid amides from Thai *Glycosmis* species. Liebigs Ann. 1789-1794.

Hofer, O., Zechner, G., Wurz, G., Hadacek, F. and Greger, H. 1995. Ritigalin, a new thiocarbonic acid imide from *Glycosmis* species. Monatsh. Chem. 126: 365-368.

Hooker, J. D. 1875. The Flora of British India Vol. Ranunculaceae to Sapindaceae. 501-502.

Humbert, H. 1945. Supplément à la flore générale de l'Indo-Chine. 616-618.

Ikegami, F., Sekine, T., Duangteraprecha, S., Matsushita, N., Matsuda, N., Ruangrungsi, N. and Murakoshi, I. 1989. Entadamide C, a sulphur-containing amide from *Entada phaseoloides*. Phytochemistry 28: 881-882.

Itharat, A., Houghton, P. J., Eno-Amooquaye, E., Burke, P. J., Sampson, J. H. and Raman, A. 2004. In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. J. Ethnopharmacol. 90: 33-38.

Ito, C. and Furukawa, H. 1987. Constituents of *Murraya exotica* L. Structure elucidation of new coumarins. Chem. Pharm. Bull. 35: 4277-4285.

Ito, C., Itoigawa, M., Sato, A., Hasan, C. M., Rashid, M. A., Tokuda, H., Mukainaka, T., Nishino, H. and Furukawa, H. 2004. Chemical constituents of *Glycosmis arborea*: three new carbazole alkaloids and their biological activity. J. Nat. Prod. 67: 1488-1491.

Ito, C., Kondo, Y., Rao, K. S., Tokuda, H., Nishino, H. and Furukawa, H. 1999. Chemical constituents of *Glycosmis pentaphylla*. Isolation of a novel naphthoquinone and a new acridone alkaloid. Chem. Pharm. Bull. 47: 1579-1581.

Ito, C., Kondo, Y., Wu, T.-S. and Furukawa, H. 2000. Chemical constituents of *Glycosmis citrifolia* (Willd.) Lindl. Structures of four new acridones and three new quinolone alkaloids. Chem. Pharm. Bull. 48: 65-70.

Ito, C., Otsuka, T., Ruangrungsi, N. and Furukawa, H. 2000. Chemical constituents of *Micromelum minutum*. Isolation and structural elucidation of new coumarins. Chem. Pharm. Bull. 48: 334-338.

Itoigawa, M., Ito, C., Wu, T. S., Enjo, F., Tokuda, H., Nishino, H. and Furukawa, H. 2003. Cancer chemopreventive activity of acridone alkaloids on Epstein-Barr virus activation and two-stage mouse skin. Cancer Lett. 193: 133-138.

Jash, S. S., Biswas, G. K., Bhattacharyya, S. K., Bhattacharyya, P., Chakraborty, A. and Chowdhury, B. K. 1992. Carbazole alkaloids from *Glycosmis pentaphylla*. Phytochemistry 31: 2503-2505.

Jiménez, B., Grande, M. C., Anaya, J., Torres, P. and Grande, M. 2000. Coumarins from *Ferulago capillaris* and *F. brachyloba*. Phytochemistry 53: 1025-1031.

Joshi, P. P., Shukla, Y. N., Bhakuni, D. S. and Dhar, M. M. 1975. 6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin, a new coumarin from *Micromelum pubescens*. Indian J. Chem. 13: 772-774.

Ju-ichi M., Kaga H., Muraguchi M., Inoue M., Kajiura I., Omura M. and Furukawa H. 1988. New acridone alkaloid and coumarin from *Citrus* plants. Heterocycles 27: 2197-2200.

Kamperdick, C., Phuong, N. M., Van Sung, T., Schmidt, J. and Adam, G. 1999. Coumarins and dihydrocinnamic acid derivatives from *Micromelum falcatum*. Phytochemistry 52: 1671-1676.

Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., Yano, M., Takemura, Y., Ju-ichi, M., Ito, C. and Furukawa, H. 1999. Acridones as inducers of HL-60 cell differentiation. Leukemia Res. 23: 263-269.

Kawaii S., Tomono Y., Katase E., Ogawa K., Yano M., Takemura Y., Ju-ichi M., Ito C., Furukawa H. 1999. The Antiproliferative Effect of Acridone Alkaloids on Several Cancer Cell Lines. J. Nat. Prod. 62: 587-589.

Kennard, O., di Sanseverino, L. R., Vorbrueggen, H. and Djerassi, C. 1965. Terpenoids. LVI. Complete structure of the triterpene arborinol. Tetrahedron Lett. 3433-3438.

Kong, Y. C., But, P. P. H., Ng, K. H., Li, Q., Cheng, K. F. and Waterman, P. G. 1988. *Micromelum*: a key genus in the chemosystematics of the Clauseneae. Biochem. Syst. Ecol. 16: 485-489.

Kumar, P., Das, B. P. and Sinha, S. K. P. 1986. Homo-glycosolone: a new quinolone alkaloid from *Glycosmis pentaphylla* (Retz) DC. Chem. Ind. 669-670.

Kumar, V., Reisch, J. and Wickramasinghe, A. 1989. Glycomaurin and glycomaurrol, new carbazole alkaloids from *Glycosmis mauritiana* (Rutaceae) bark. Aust. J. Chem. 42: 1375-1379.

Lamberton, J. A., Price, J. R. and Redcliffe, A. H. 1967. Micromelin, a new coumarin from *Micromelum minutum*. Aust. J. Chem. 20: 973-979.

Lee S.-Y., Morita H., Takeya K., Itokawa H., and Fukaya H. 1999. Limonoids from *Citrus nippokoreana*. Nat. Med. 53: 255-258.

Leu, Y.-L., Chan, Y.-Y., Wu, T.-S., Teng, C.-M. and Chen, K.-T. 1998. Antiplatelet aggregation principles from *Glycosmis citrifolia*. Phytother. Res. 12: S77-S79.

Lipipun V., Kurokawa M., Suttisri R., Taweechotipatr P., Pramyothin P., Hattori M. and Shiraki K. 2003. Efficacy of Thai medicine plant extracts against herpes simplex virus type 1 infection in vitro and in vivo. Antiviral Res. 60: 175-180.

Lukaseder, B., Vajrodaya, S., Hehenberger, T., Seger, C., Nagl, M., Lutz-Kutschera, G., Robien, W., Greger, H. and Hofer, O. 2009. Prenylated flavanones and flavanonols as chemical markers in *Glycosmis* species (Rutaceae). Phytochemistry 70: 1030-1037.

Luo, X., Qi, S., Yin, H., Xiao, Z. and Zhang, S. 2009. Micromelosides A-D, four new coumarins from the stem bark of *Micromelum falcatum*. Magn. Reson. Chem. 47: 1110-1114.

Luo, X. M., Qi, S. H., Yin, H., Gao, C. H. and Zhang, S. 2009. Alkaloids from the stem bark of *Micromelum falcatum*. Chem. Pharm. Bull. 57: 600-602.

Ma, C., Case Ryan, J., Wang, Y., Zhang, H.-J., Tan Ghee, T., Van Hung, N., Cuong Nguyen, M., Franzblau Scott, G., Soejarto Djaja, D., Fong Harry, H. and Pauli Guido, F. 2005. Anti-tuberculosis constituents from the stem bark of *Micromelum hirsutum*. Planta Med. 71: 261-267.

Manners G. D. and Breksa III A. P. 2004. Identifying *Citrus* limonoid aglycones by HPLC-EI/MS and HPLC-APCI/MS techniques. Phytochem. Anal. 15: 372-381.

McClatchey, W. 1996. The ethnopharmacopoeia of Rotuma. J. Ethnopharmacol. 50: 147-156.

Merrill, E. D. 1968. A flora of Manila. first Edition. pp. 268-269. Manila: Department of the interior, Bureau of Science.

Mou, F. J. and Zhang, D. X. 2009. *Glycosmis longipetala* F. J. Mou & D. X. Zhang, a new species of Rutaceae from China. J. Syst. Evol. 47: 162-167.

Mukherjee, S., Mukherjee, M. and Ganguly, S. N. 1983. Glycozolinine, a carbazole derivative from *Glycosmis pentaphylla*. Phytochemistry 22: 1064-1065.

Muthukrishnan, J., Seifert, K., Hoffmann, K. H. and Lorenz, M. W. 1998. Inhibition of juvenile hormone biosynthesis in *Gryllus bimaculatus* by *Glycosmis pentaphylla* leaf compounds. Phytochemistry 50: 249-254.

Nakahara, K., Trakoontivakorn, G., Alzoreky, N. S., Ono, H., Onishi-Kameyama, M. and Yoshida, M. 2002. Antimutagenicity of Some Edible Thai Plants, and a Bioactive Carbazole Alkaloid, Mahanine, Isolated from *Micromelum minutum*. J. Agric. Food Chem. 50: 4796-4802.

Negi, N., Jinguji, Y., Ushijima, K., Ikeda, S., Takemura, Y., Ju-ichi, M., Wu, T.-S., Ito, C. and Furukawa, H. 2004. Two new dimeric acridone alkaloids from *Glycosmis citrifolia*. Chem. Pharm. Bull. 52: 362-364.

Ono, T., Ito, C., Furukawa, H., Wu, T.-S., Kuoh, C.-S. and Hsu, K.-S. 1995. Two new acridone alkaloids from *Glycosmis* species. J. Nat. Prod. 58: 1629-1631.

Pacher, T., Bacher, M., Hofer, O. and Greger, H. 2001. Stress induced carbazole phytoalexins in *Glycosmis* species. Phytochemistry 58: 129-135.

Pakrashi, S. C., Bhattacharyya, J., Johnson, L. F. and Budzikiewicz, H. 1963. Studies on indian medicinal plants-VI. Structures of glycosmicine, glycorine and glycosminine, the minor alkaloids from *Glycosmis arborea* (roxb.) DC. Tetrahedron 19: 1011-1026.

Pakrashi, S. C. and Samanta, T. B. 1967. Indian medicinal plants. XVI. Acid induced epimerization and rearrangements of arborinol, the novel triterpene from *Glycosmic arborea*. Tetrahedron Lett. 3679-3684.

Panthong, A., Kanjanapothi, D., and Taylor, W. C. 1986. Ethnobotanical review of medicinal plants from Thai traditional books, Part I: Plants with anti-inflammatory, anti-asthmatic and antihypertensive properties. J. Ethnopharmacol. 18: 213-228.

Rahmani, M., Hin Taufiq-Yap, Y., Ismail, H. B. M., Sukari, A. and Waterman, P. G. 1994. New coumarin and dihydrocinnamic acid derivatives from two malaysian populations of *Micromelum minutum*. Phytochemistry 37: 561-564.

Rahmani, M., Hin, T. Y., Ismail, H. B., Sukari, M. A. and Manas, A. R. 1993. Microminutinin: A Novel Coumarin from *Micromelum minutum*. Planta Med. 59: 93-94.

Rahmani, M., Leng, K. W., Ismail, H. B. M., Hin, T.-Y. Y., Sukari, M. A., Ali, A. M. and Kulip, J. 2004. A new flavonoid and sulphur-containing amides from *Glycosmis chlorosperma*. Nat. Prod. Res. 18: 85-88.

Rahmani, M., Ling, C. Y., Sukari, M. A., Ismail, H. B., Meon, S. and Aimi, N. 1998. 7-Methoxyglycomaurin: a new carbazole alkaloid from *Glycosmis rupestris*. Planta Med. 64: 780.

Rahmani, M., Susidarti, R. A., Ismail, H. B. M., Sukari, M. A., Hin, T. Y. Y., Lian, G. E. C., Ali, A. M., Kulip, J. and Waterman, P. G. 2003. Coumarins from Malaysian *Micromelum minutum*. Phytochemistry 64: 873-877.

Rastogi, K., Kapil, R. S. and Poli, S. P. 1980. New alkaloids from *Glycosmis mauritiana*. Phytochemistry 19: 945-948.

Reisch J., Szendrei K., Minker E. and Novak I. 1971. Spektroskopische methoden zur strukturermittlung von acridon-alkaloiden. Pharmazie 26: 108-216.

Réthy, B., Zupkó, I., Minorics, R., Hohmann, J., Ocsovszki, I. and Falkay, G. 2007. Investigation of cytotoxic activity on human cancer cell lines of arborinine and furanoacridones isolated from Ruta graveolens. Planta Med. 73: 41-48.

Ridley, H. N. 1922. The flora of the Malay Peninsula. Vol. I, first Edition., pp. 348-351. London: L. Reeve & Co., Ltd.

Roy, M. K., Thalang, V. N., Trakoontivakorn, G. and Nakahara, K. 2004. Mechanism of mahanine-induced apoptosis in human leukemia cells (HL-60). Biochem. Pharmacol. 67: 41-51.

Sarkar, M. and Chakraborty, D. P. 1977. Chemical taxonomy (molecular taxonomy). Part 40. Some minor constituents from *Glycosmis pentaphylla*. Phytochemistry 16: 2007-2008.

Sarkar, M. and Chakraborty, D. P. 1979. Chemical taxonomy. Part 45. Glycophymoline, a new minor quinazoline alkaloid from *Glycosmis pentaphylla*. Phytochemistry 18: 694-695.

Sarkar, M., Kundu, S. and Chakraborty, D. P. 1978. Chemical taxonomy. Part 42. Glycarpine, a new alkaloid from *Glycosmis cyanocarpa*. Phytochemistry 17: 2145-2146.

Seger, C., Vajrodaya, S., Greger, H. and Hofer, O. 1998. Structure elucidation and synthesis of a new bioactive quinazolone derivative obtained from *Glycosmis cf. chlorosperma*. Chem. Pharm. Bull. 46: 1926-1928.

Shapiro, J. P., Bowman, K. D. and Lapointe, S. L. 2000. Dehydrothalebanin: A Source of Resistance from *Glycosmis pentaphylla* against the Citrus Root Weevil *Diaprepes abbreviatus*. J. Agric. Food Chem. 48: 4404-4409.

Sinha, S., Pal, B. C., Jagadeesh, S., Banerjee, P. P., Bandyopadhaya, A. and Bhattacharya, S. 2006. Mahanine inhibits growth and induces apoptosis in prostate cancer cells through the deactivation of Akt and activation of caspases. The Prostate 66: 1257-1265.

Sinha, S. K. P. and Kumar, P. 1988. Glycolone: a new 2-quinolone alkaloid from *Glycosmis pentaphylla* (Retz) DC. Indian J. Chem., Sect. B 27B: 460-461.

Smith III, A. B., Levenberg, P. A., Jerris, P. J., Scarborough Jr., R. M. and Wovkulich, P. M. 1981. Synthesis and reactions of simple 3(2*H*)-furanones. J. Am. Chem. Soc. 103: 1501-1513.

Smitinand, T. 2001. Thai Plant Names (Botanical Names – Vernacular Names). revised Edition. Bangkok: The Forest Herbarium, Royal Forest Department.

Sohrab, M. H., Chowdhury, R., Hasan, C. M. and Rashid, M. A. 2004. Chemotaxonomic significance of polyoxygenated flavonoids from the leaves of *Micromelum minutum*. Biochem. Syst. Ecol. 32: 829-831.

Sohrab, M. H., Hasan, C. M. and Rashid, M. A. 1999. 6-Substituted-7-oxygenated coumarins from the leaves of *Micromelum minutum*. Biochem. Syst. Ecol. 27: 535-537.

Su, T. L., Dziewiszek, K. and Wu, T. S. 1991. Synthesis of glyfoline, a constituent of *Glycosmis citrifolia* (Willd.) Lindl. and a potential anticancer agent. Tetrahedron Lett. 32: 1541-1544.

Su, T. L., Huang, H. M., Wang, C. K., Wu, H. C. and Lin, C. T. 2005. Cytochrome c release induces apoptosis of nasopharyngeal carcinoma (NPC) by antitumor glyfoline. Planta Med. 71: 28-32.

Susidarti, R. A., Rahmani, M., Ismail, H. B. M., Sukari, M. A., Hin, T. Y. Y., Lian, G. E. C., Ali, A. M., Kulip, J. and Waterman, P. G. 2006. A new coumarin and triterpenes from Malaysian *Micromelum minutum*. Nat. Prod. Res. 20: 145-151.

Steck, W. and Mazurek, M. 1972. Identification of Natural Coumarins by NMR Spectroscopy. Lloydia 35: 418-439.

Stone, B. C. 1985. A conspectus of the genus *Glycosmis* Correa: studies in Malesian Rutaceae, III. Proc. Acad. Nat. Sci. Phila. 137: 1–27.

Tantishaiyakul, V., Pummangura, S., Chaichantipyuth, C., Ma, W. W. and McLaughlin, J. L. 1986. Phebalosin from the bark of *Micromelum minutum*. J. Nat. Prod. 49: 180-181.

Tantivatana, P., Ruangrungsi, N., Vaisiriroj, V., Lankin, D. C., Bhacca, N. S., Borris, R. P., Cordell, G. A. and Johnson, L. F. 1983. Microminutin, a novel cytotoxic coumarin from *Micromelum minutum* (Rutaceae). J. Org. Chem. 48: 268-270.

Trinh, T. T., Tran, V. S. and Adam, G. 2004. Study on chemical constituents of *Micromelum hirsutum*. Tap Chi Hoa Hoc 42: 177-181.

Varisara Vaisiriroj, "Alkaloid(s) from *Micromelum minutum* leaves," (Master's thesis, Pharmaceutical Botany, Graduate School, Chulalongkorn University, 1980), p. 71.

Vajrodaya, S., Bacher, M., Greger, H. and Hofer, O. 1998. Organ-specific chemical differences in *Glycosmis trichanthera*. Phytochemistry 48: 897-902.

Wang, J., Di, Y., Yang, X., Li, S., Wang, Y. and Hao, X. 2006. Hydroquinone diglycoside acyl esters from the stems of *Glycosmis pentaphylla*. Phytochemistry 67: 486-491.

Wang, J., He, H., Shen, Y. and Hao, X. 2004. Sulfur-containing and dimeric flavanols from *Glycosmis montana*. Tetrahedron Lett. 46: 169-172.

Wang, J., Yang, X., Di, Y., Wang, Y., Shen, Y. and Hao, X. 2006. Isoflavone diglycosides from *Glycosmis pentaphylla*. J. Nat. Prod. 69: 778-782.

Wang, J., Zheng, Y., Efferth, T., Wang, R., Shen, Y. and Hao, X. 2005. Indole and carbazole alkaloids from *Glycosmis montana* with weak anti-HIV and cytotoxic activities. Phytochemistry 66: 697-701.

Whistler, W. A. 1991. Herbal medicine in the Kingdom of Tonga. J. Ethnopharmacol. 31: 339-372.

Wu, T.-S., Chang, F.-C. and Wu, P.-L. 1995. Flavonoids, amidosulfoxides and an alkaloid from the leaves of *Glycosmis citrifolia*. Phytochemistry 39: 1453-1457.

Wu, T. S. and Furukawa, H. 1982. Acridone alkaloids. III. Structure of glycofoline, a new monoterpenoid acridone alkaloid from *Glycosmis citrifolia* (Willd.) Lindl. Heterocycles 19: 825-827.

Wu, T. S., Furukawa, H. and Hsu, K. S. 1982. Acridone alkaloids. V. New furo- and pyranoacridone alkaloids from *Glycosmis citrifolia* (Willd.) Lindl. Heterocycles 19: 1227-1231.

Wu, T. S., Furukawa, H. and Kuoh, C. S. 1982. Acridone alkaloids. IV. Structures of four new acridone alkaloids from *Glycosmis citrifolia* (Willd.) Lindl. Heterocycles 19: 1047-1051.

Wu, T. S., Furukawa, H., Kuoh, C. S. and Hsu, K. S. 1983. Acridone alkaloids. Part 9. Chemical constituents of *Glycosmis citrifolia* (Willd.) Lindl. Structures of novel linear pyranoacridones, furoacridones, and other new acridone alkaloids. J. Chem. Soc., Perkin Trans. 1 1681-1688.

Wu T.-S., Kuoh C.-S. and Furukawa H. 1982. Acridone alkaloids from Severinia buxifolia. Phytochemistry 21: 1771-1773.

Yamamoto, N., Furukawa, H., Ito, Y., Yoshida, S., Maeno, K. and Nishiyama, Y. 1989. Anti-herpesvirus activity of citrusinine-I, a new acridone alkaloid, and related compounds. Antiviral Res. 12: 21-36.

Yang, X.-L., Xie, Z.-H., Jiang, X.-J., Huang, Y.-B. and Liu, J.-K. 2009. A new acridone alkaloid from *Micromelum integerrimum*. Chem. Pharm. Bull. 57: 734-735.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

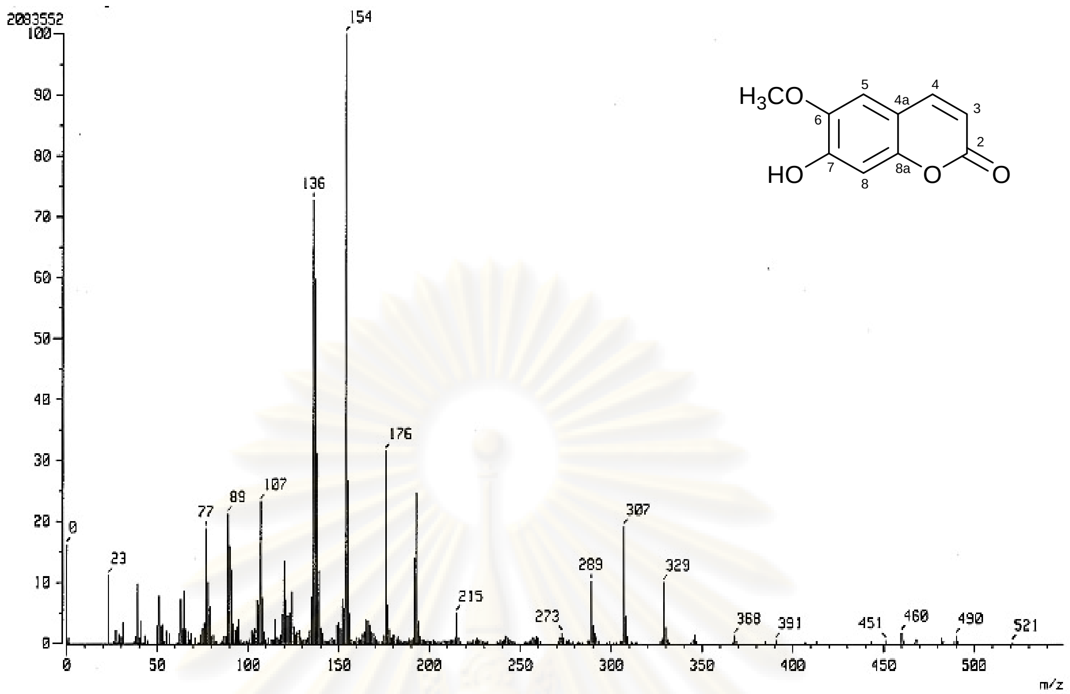


Figure 3 FABMS of compound **MH1**.

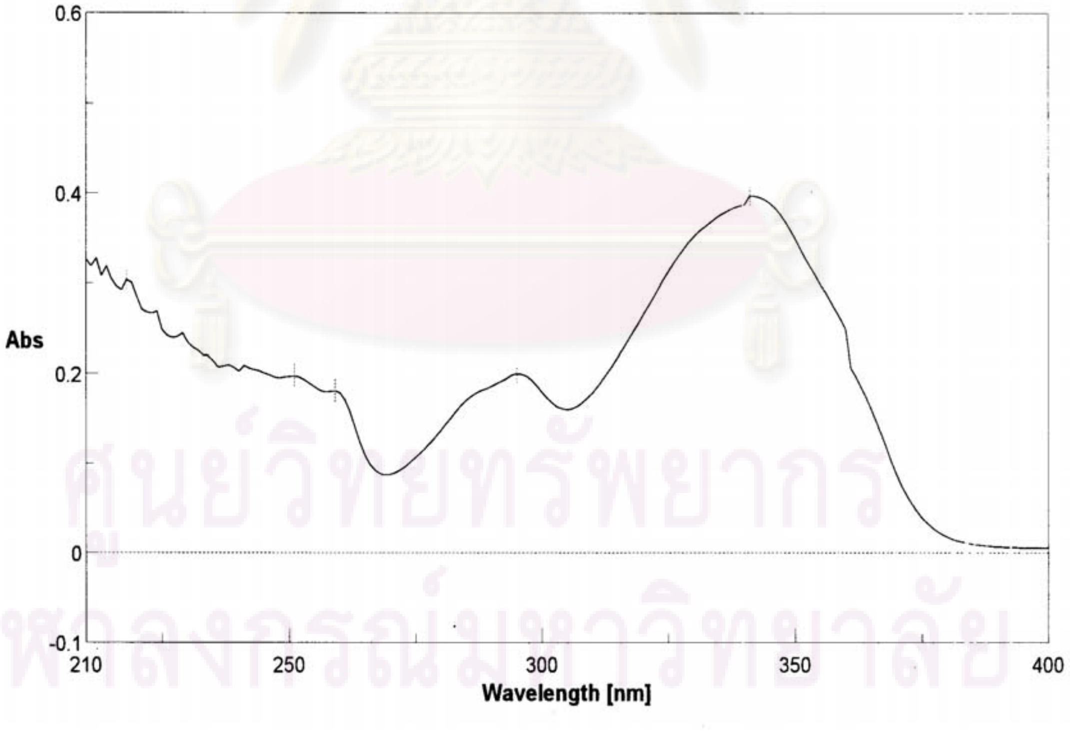


Figure 4 UV spectrum of compound **MH1** ($CHCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

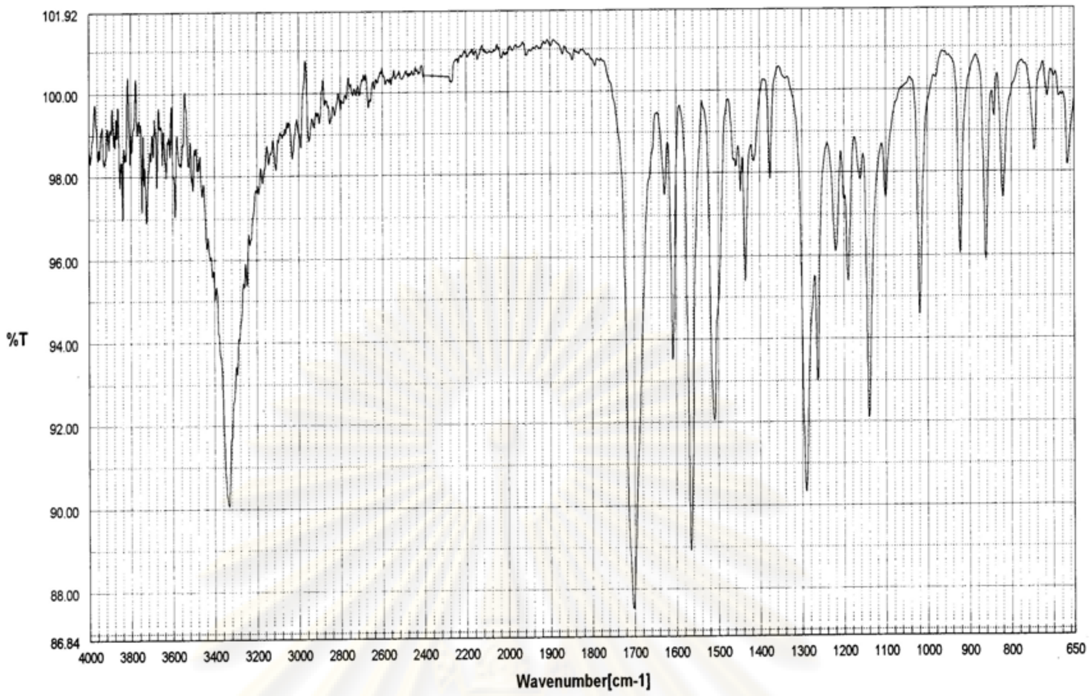


Figure 5 IR spectrum of compound **MH1** (ATR).

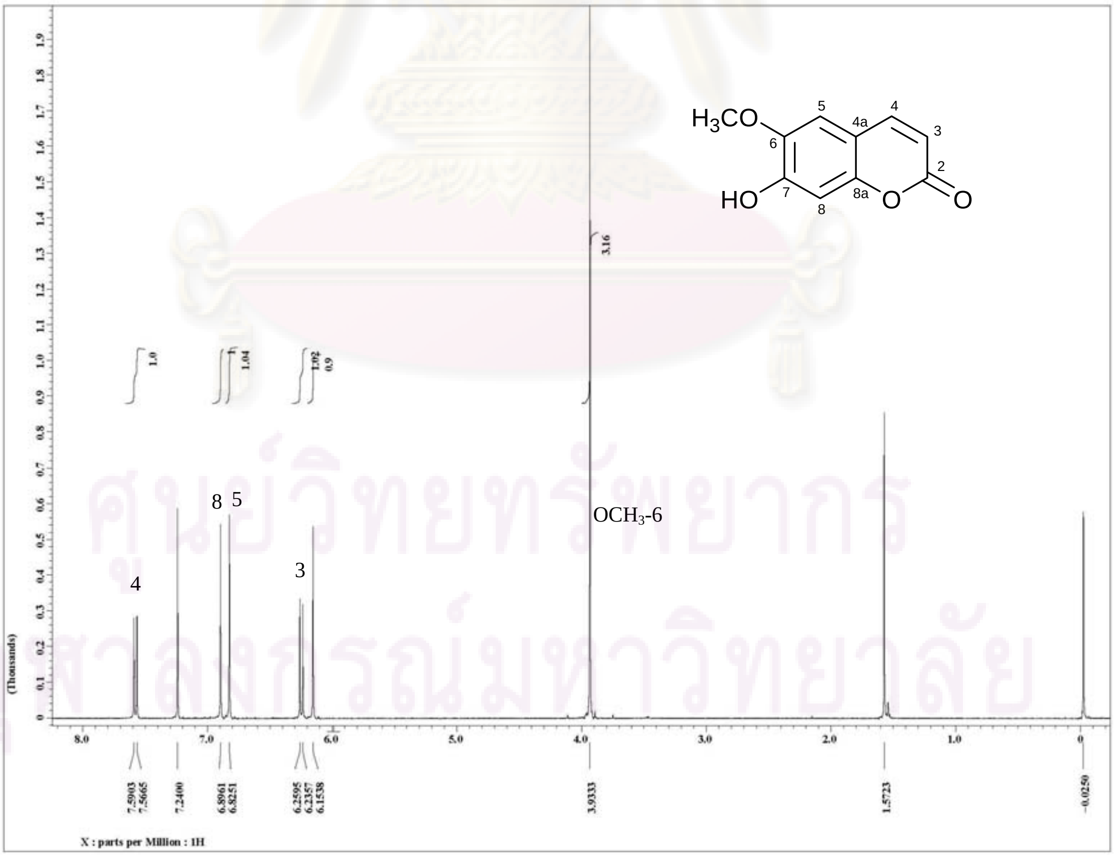


Figure 6 ^{1}H-NMR spectrum of compound **MH1** (400 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

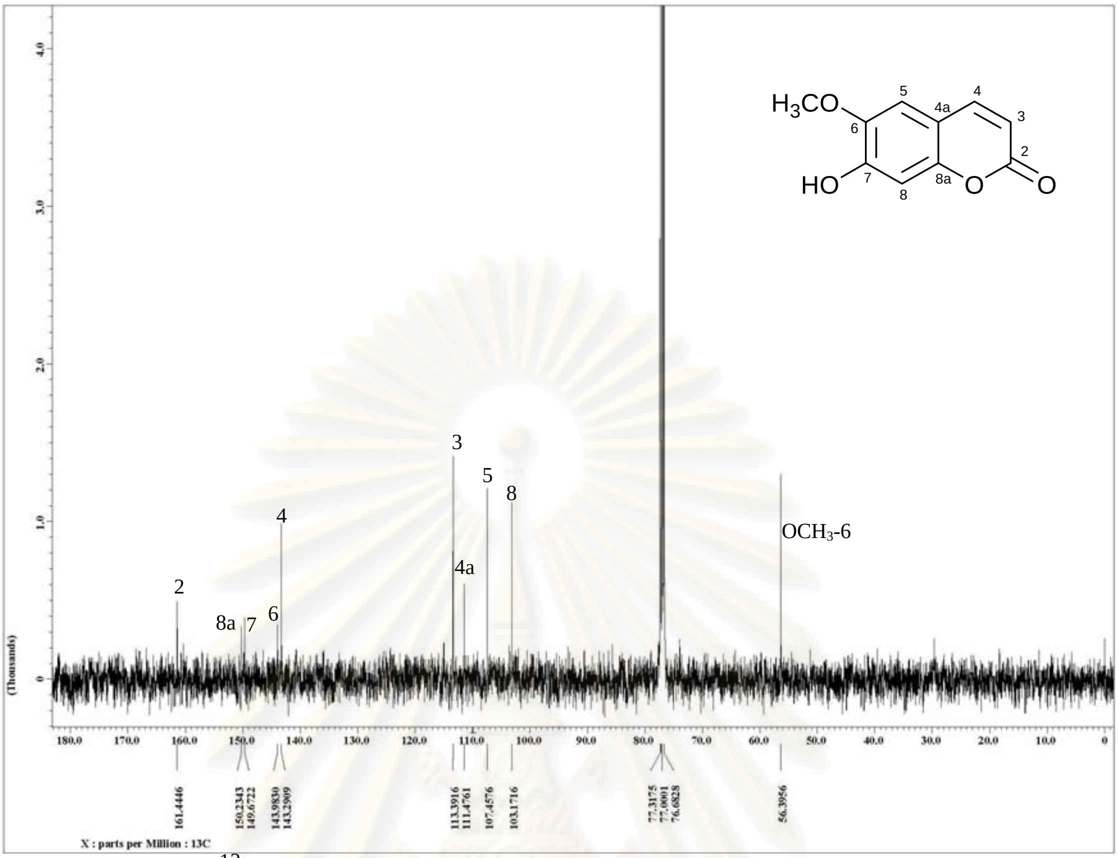


Figure 7 ^{13}C-NMR spectrum of compound **MH1** (100 MHz, $CDCl_3$).

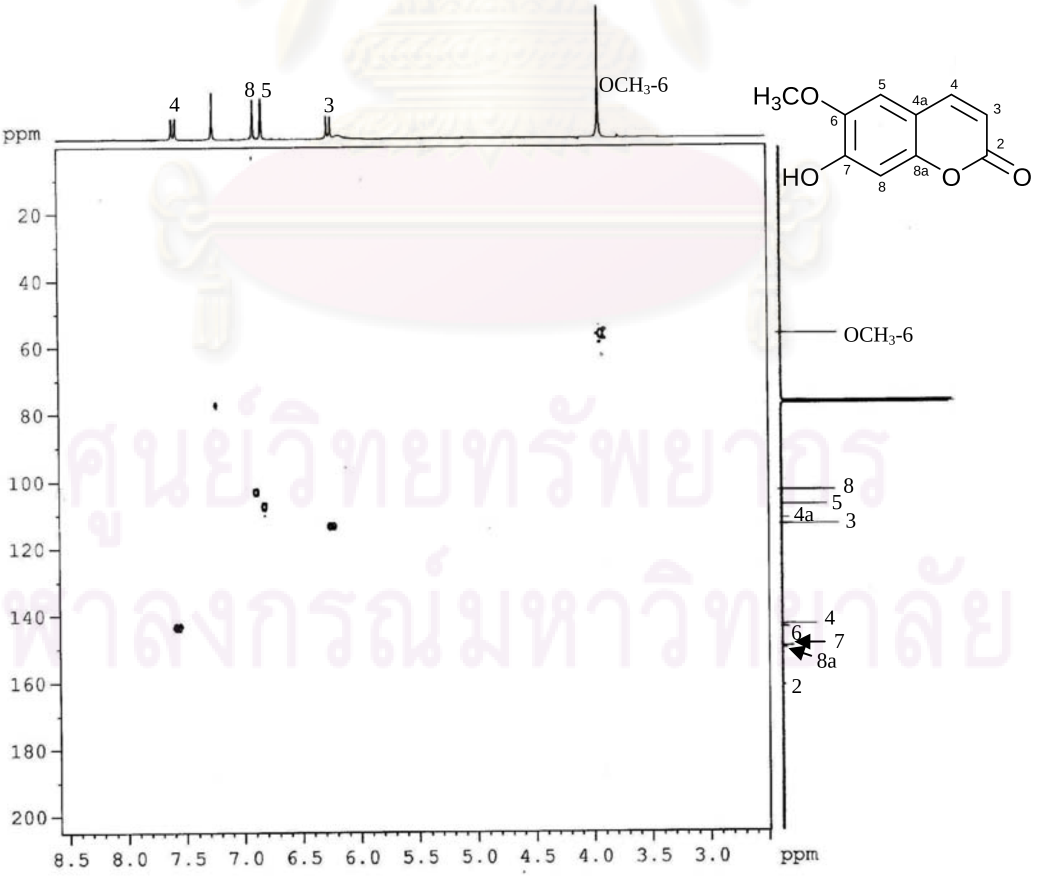


Figure 8 HMQC spectrum of compound **MH1** ($CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

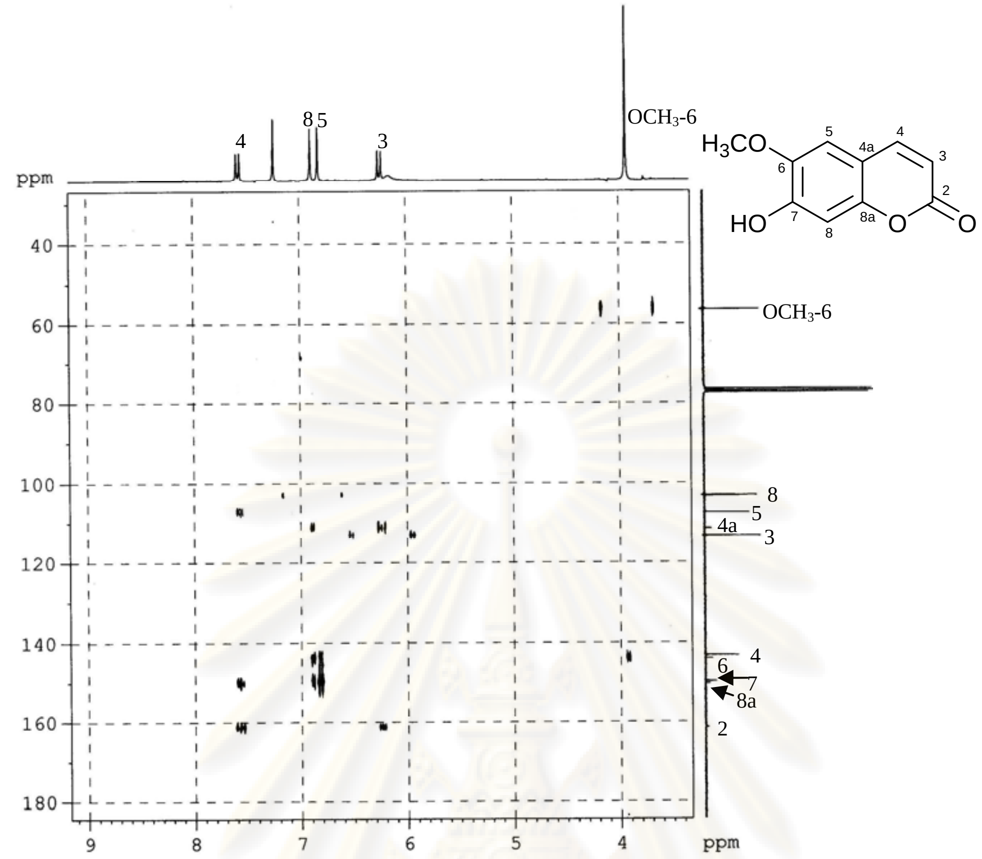


Figure 9 HMBC spectrum of compound **MH1** ($CDCl_3$).

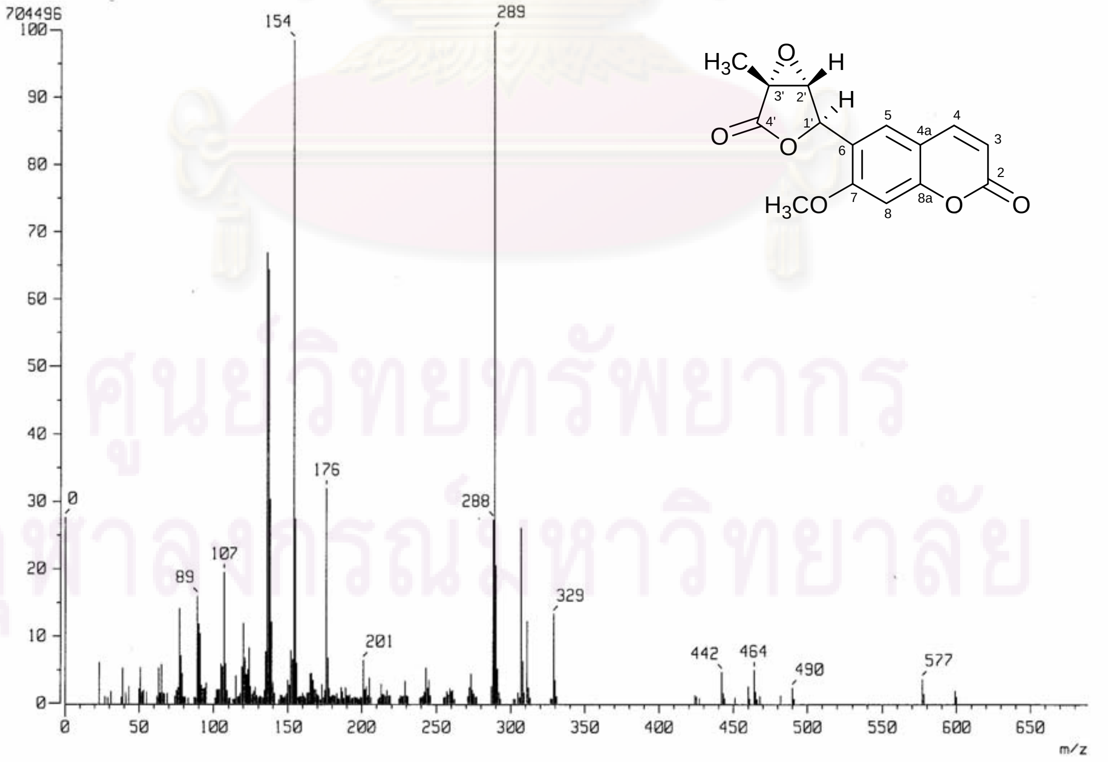


Figure 10 FABMS of compound **MH2**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

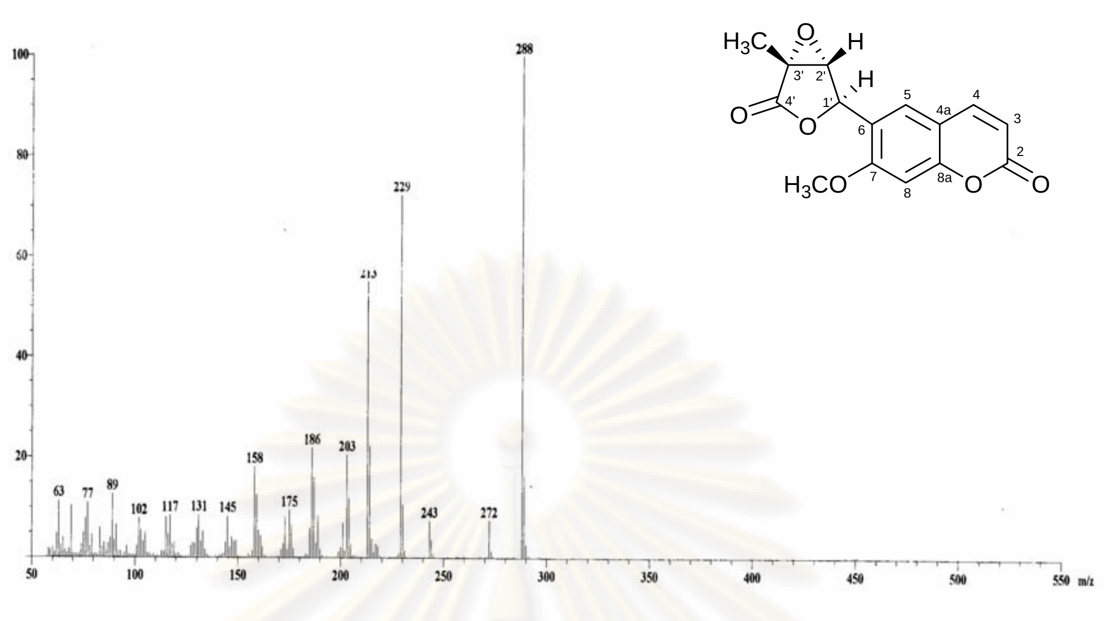


Figure 11 EIMS of compound **MH2**.

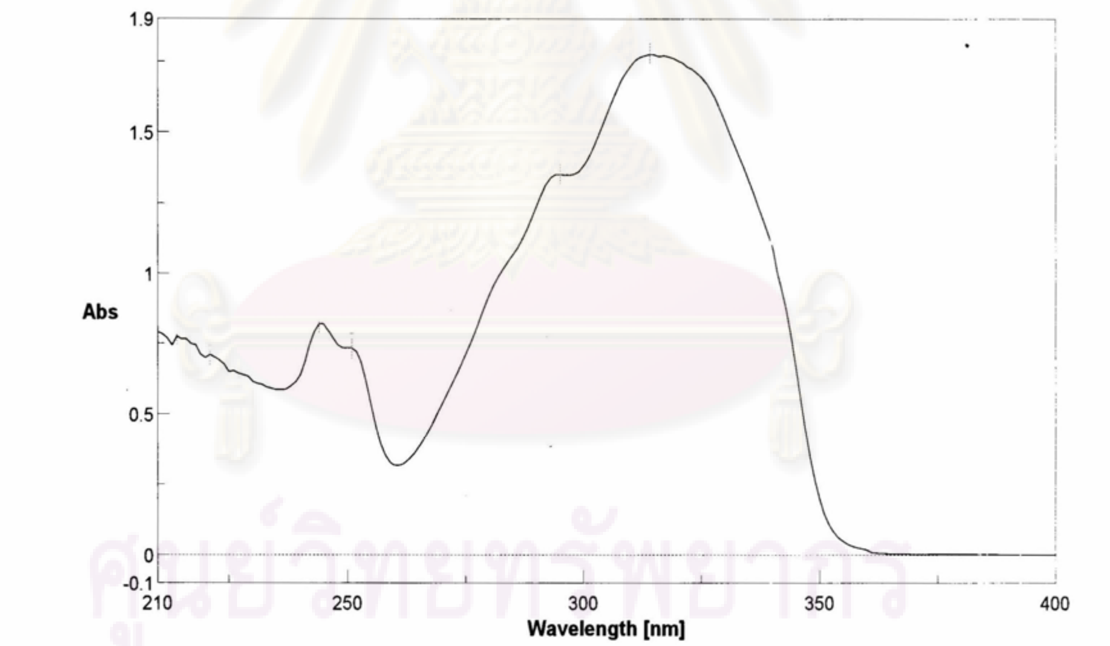


Figure 12 UV spectrum of compound **MH2** ($CHCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

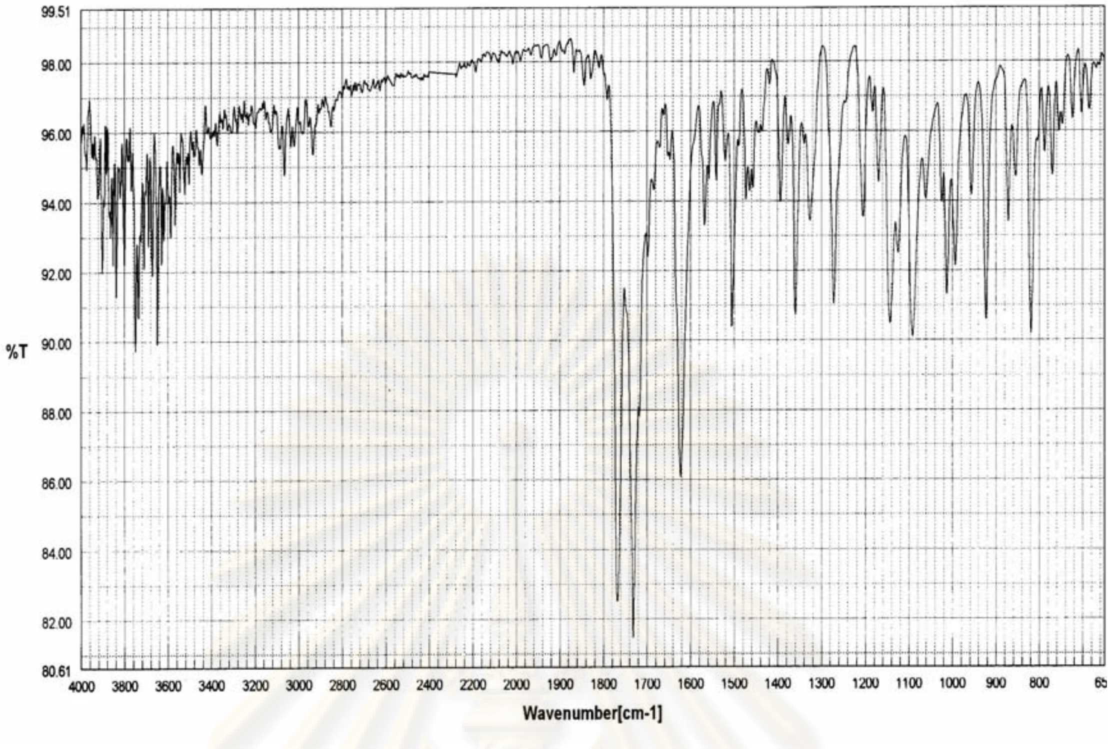


Figure 13 IR spectrum of compound **MH2** (ATR).

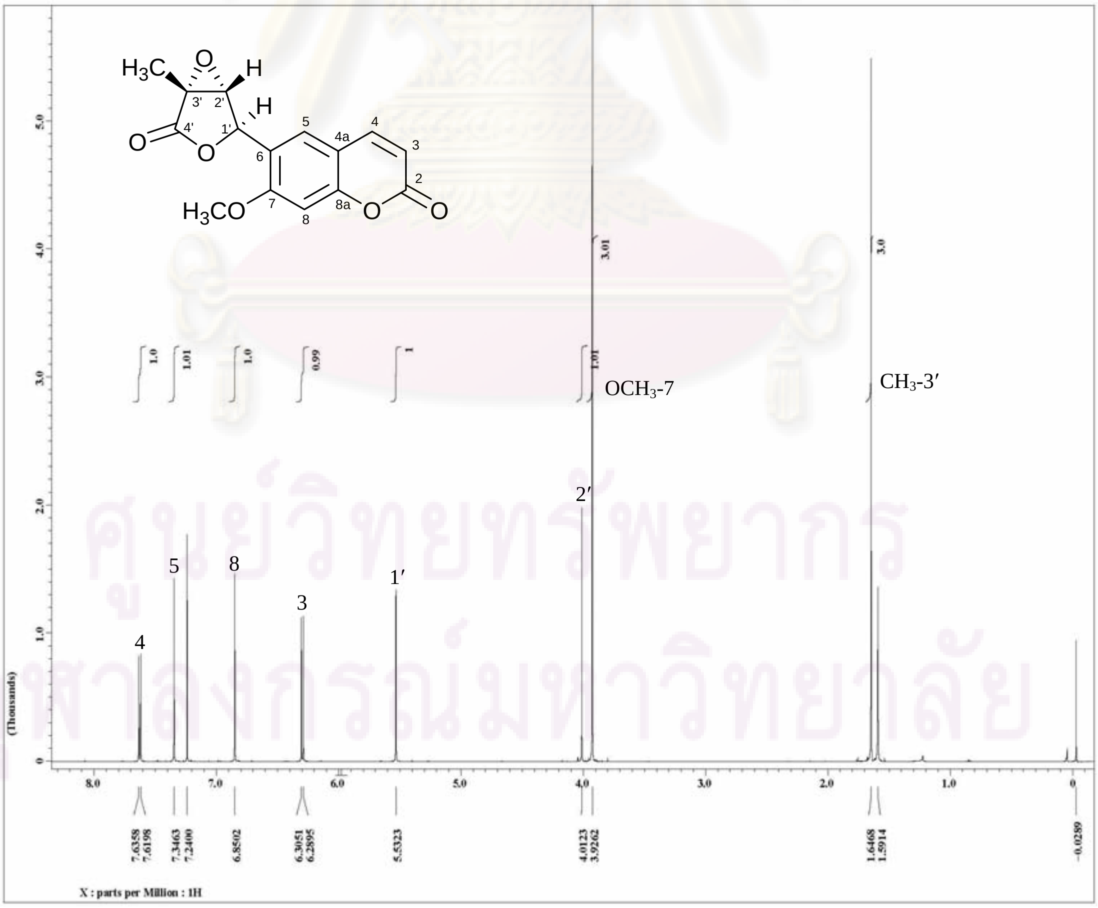


Figure 14 ^{1}H-NMR spectrum of compound **MH2** (600 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

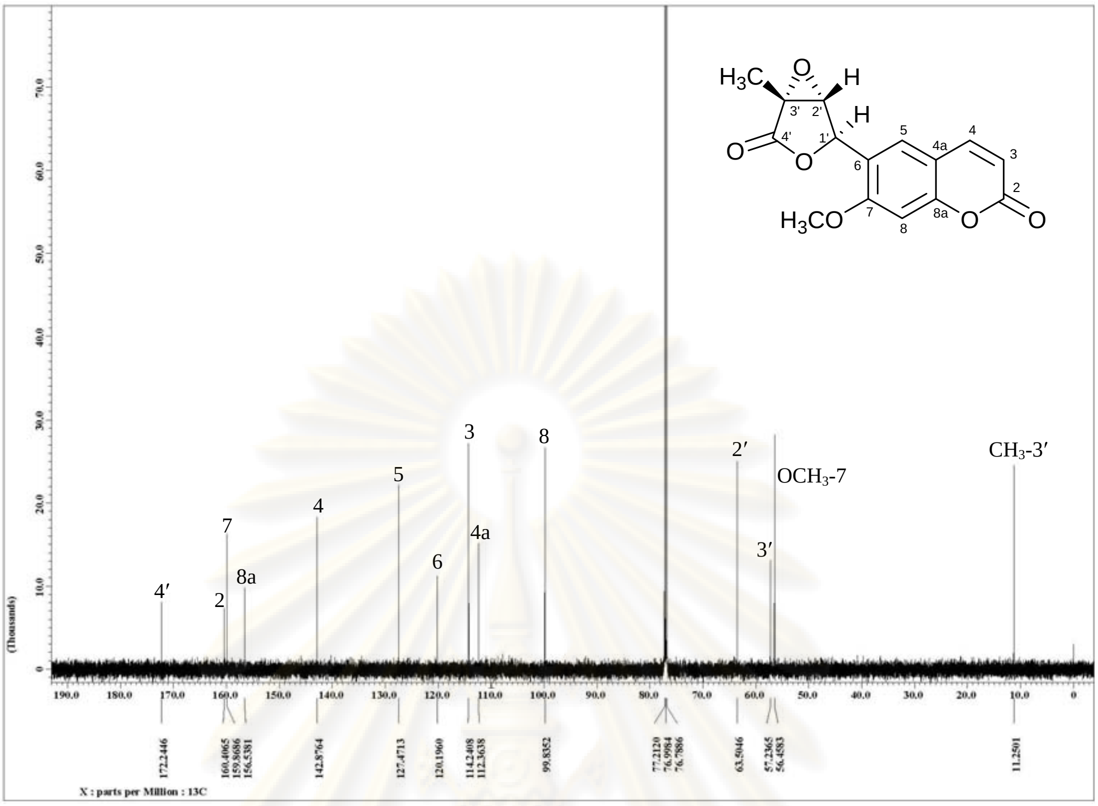


Figure 15 ^{13}C-NMR spectrum of compound **MH2** (150 MHz, $CDCl_3$).

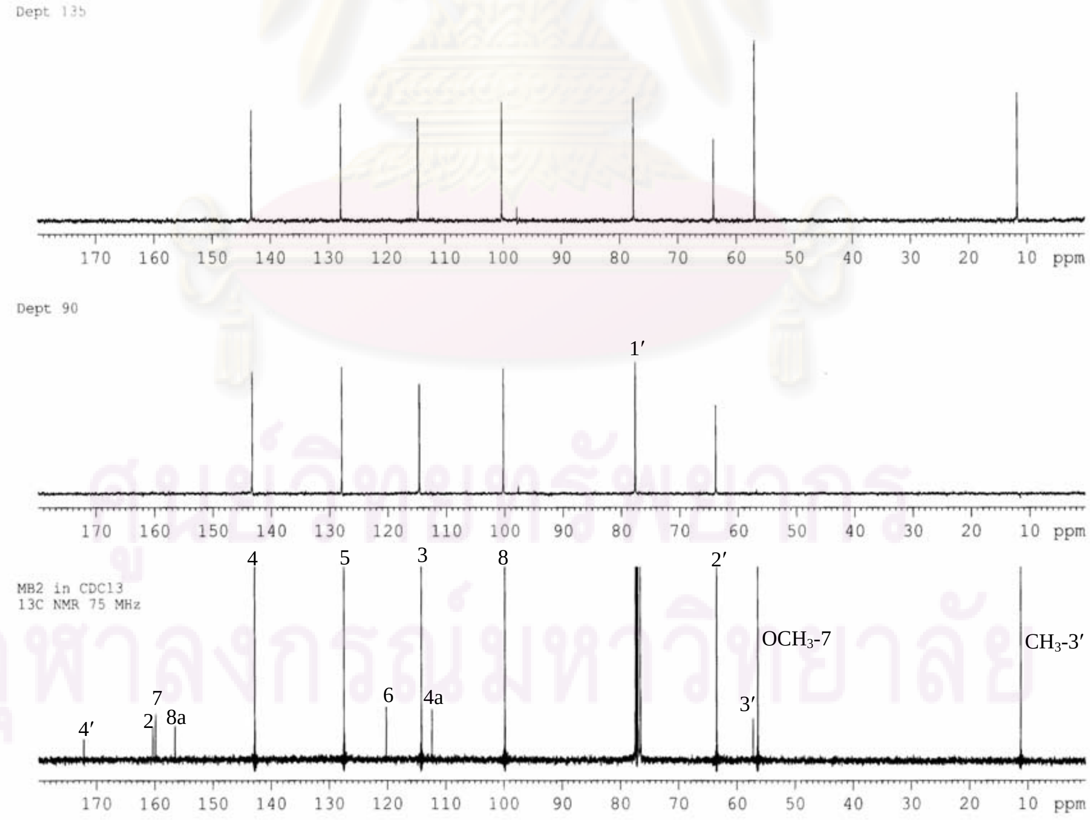


Figure 16 DEPT135 spectrum of compound **MH2** (75 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

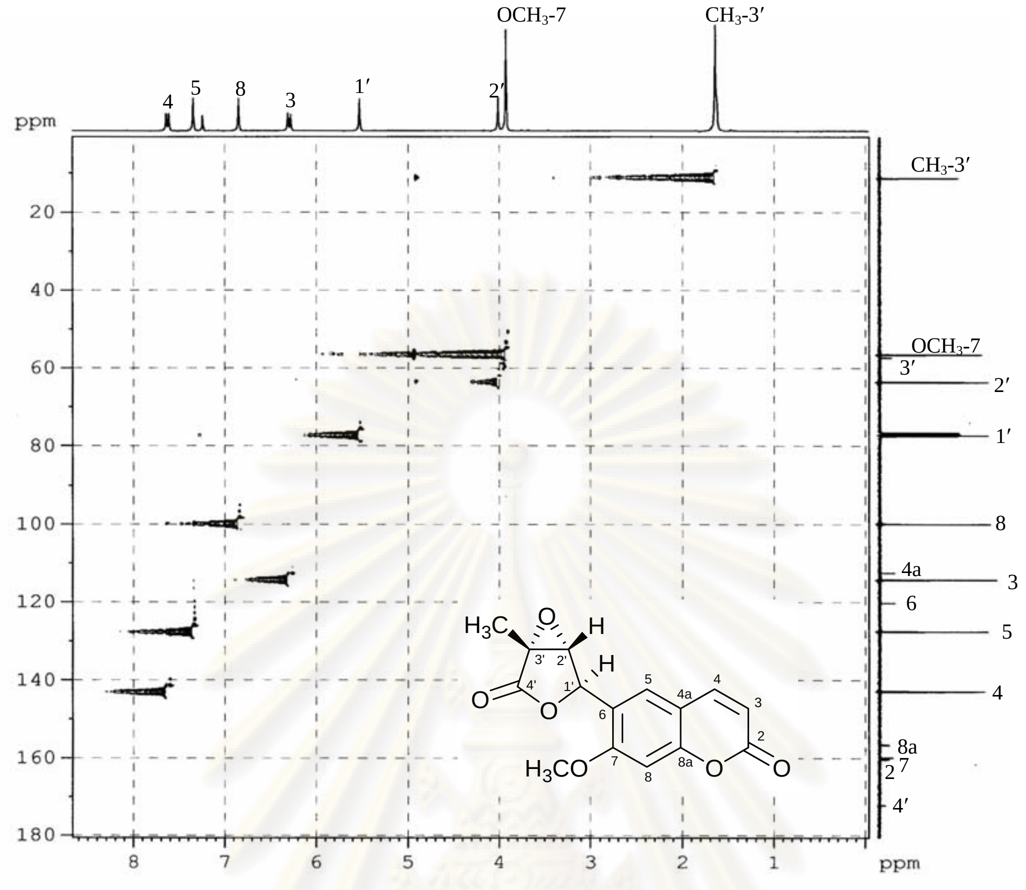


Figure 17 HMQC spectrum of compound **MH2** (CDCl$_3$).

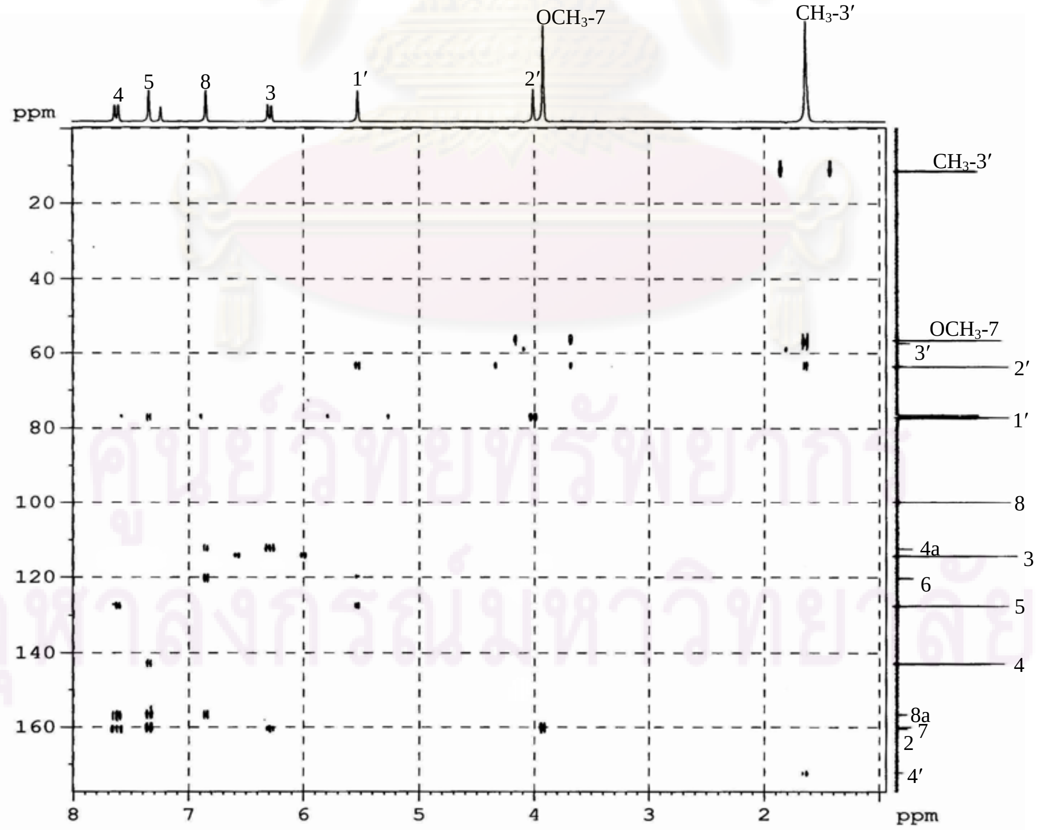


Figure 18 HMBC spectrum of compound **MH2** (CDCl$_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

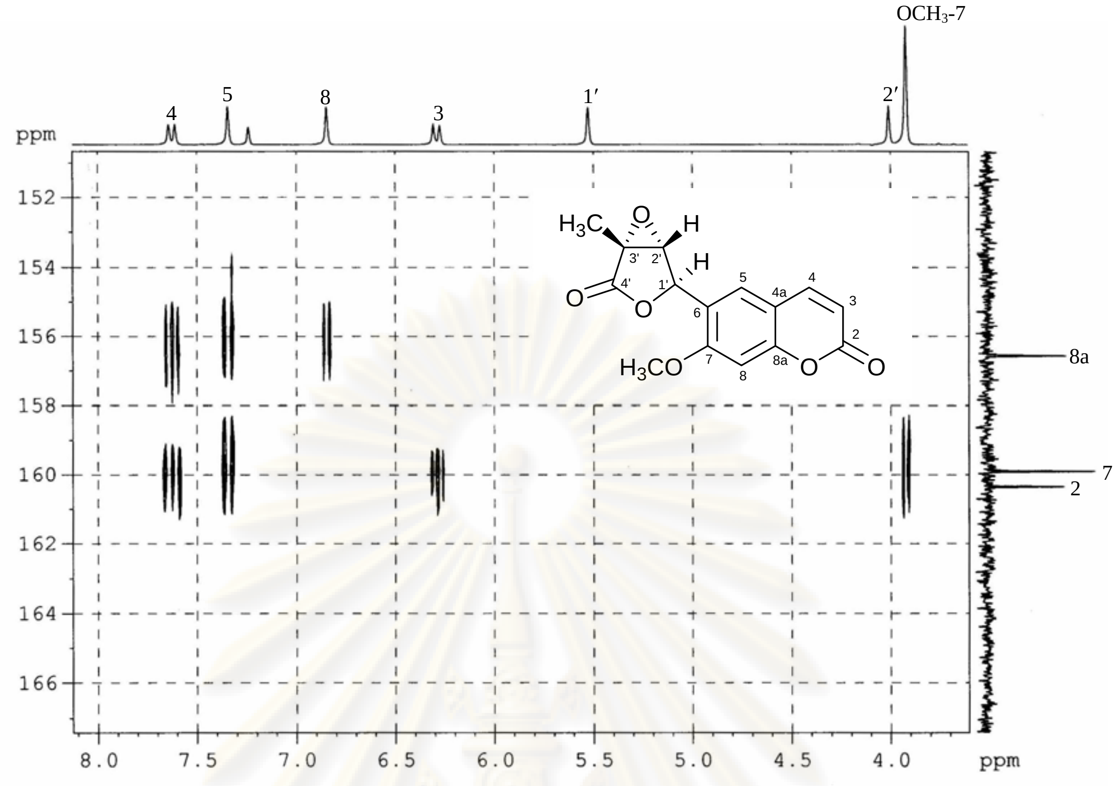


Figure 19 Expanded HMBC spectrum of compound **MH2** ($CDCl_3$).

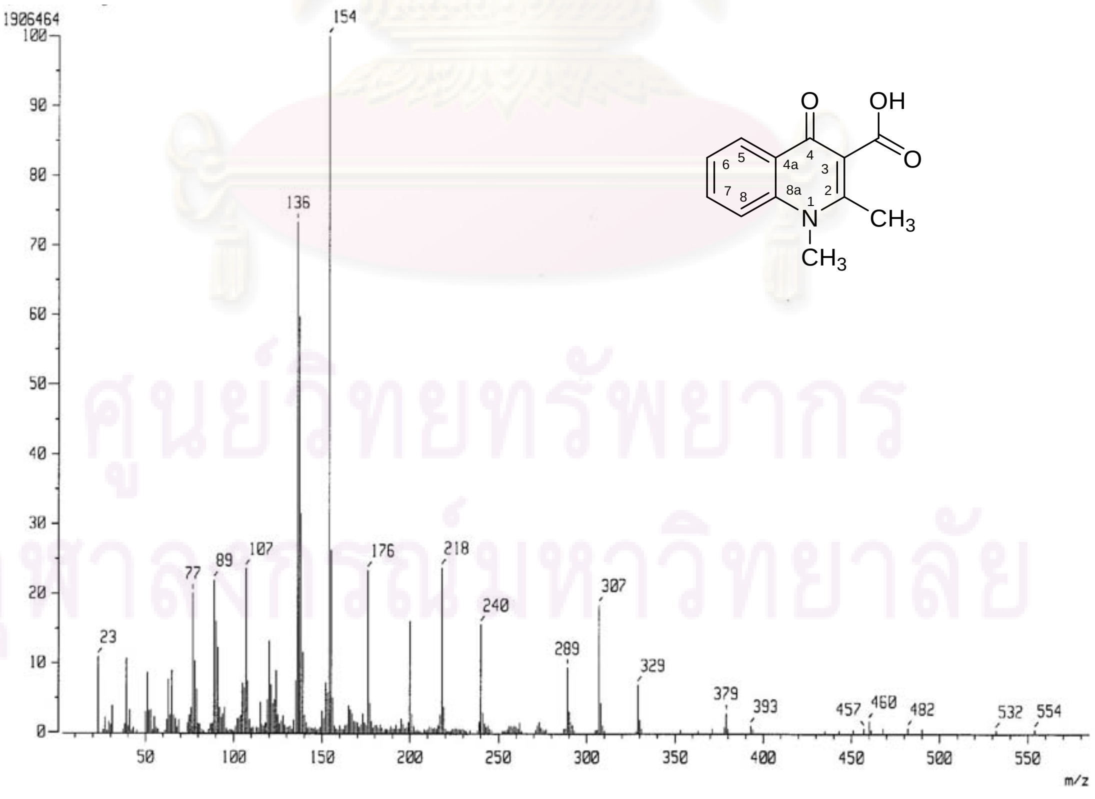


Figure 20 FABMS of compound **MH3**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

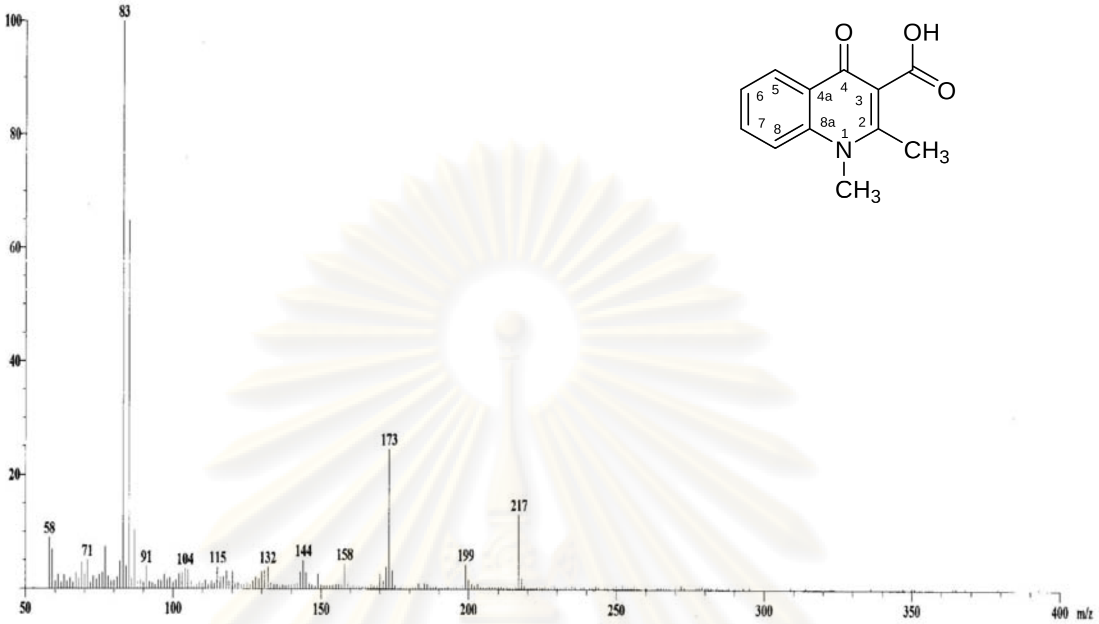


Figure 21 EIMS of compound **MH3**.

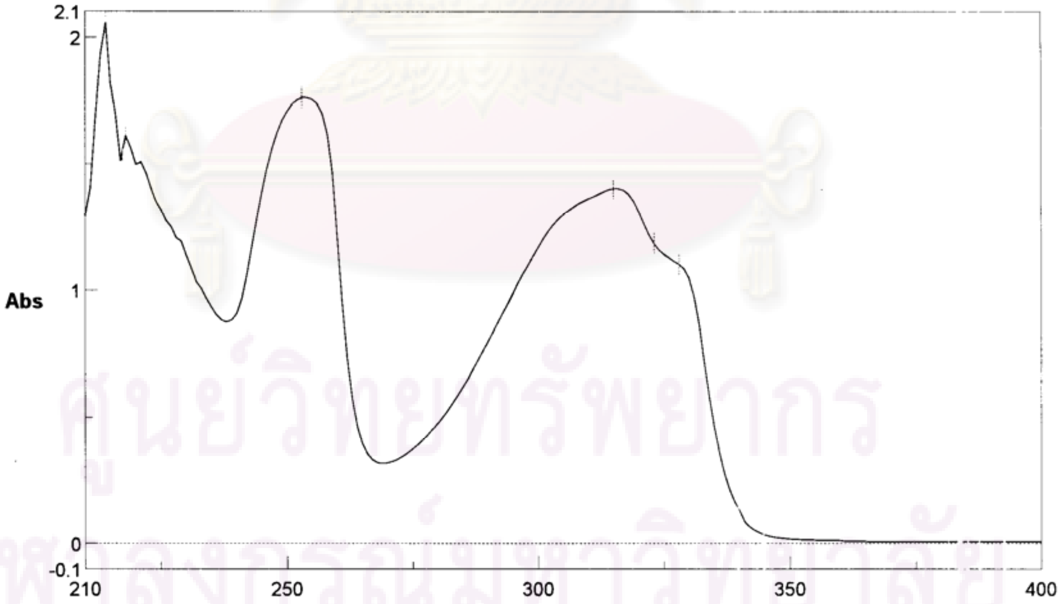


Figure 22 UV spectrum of compound **MH3** (CH_3CN).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

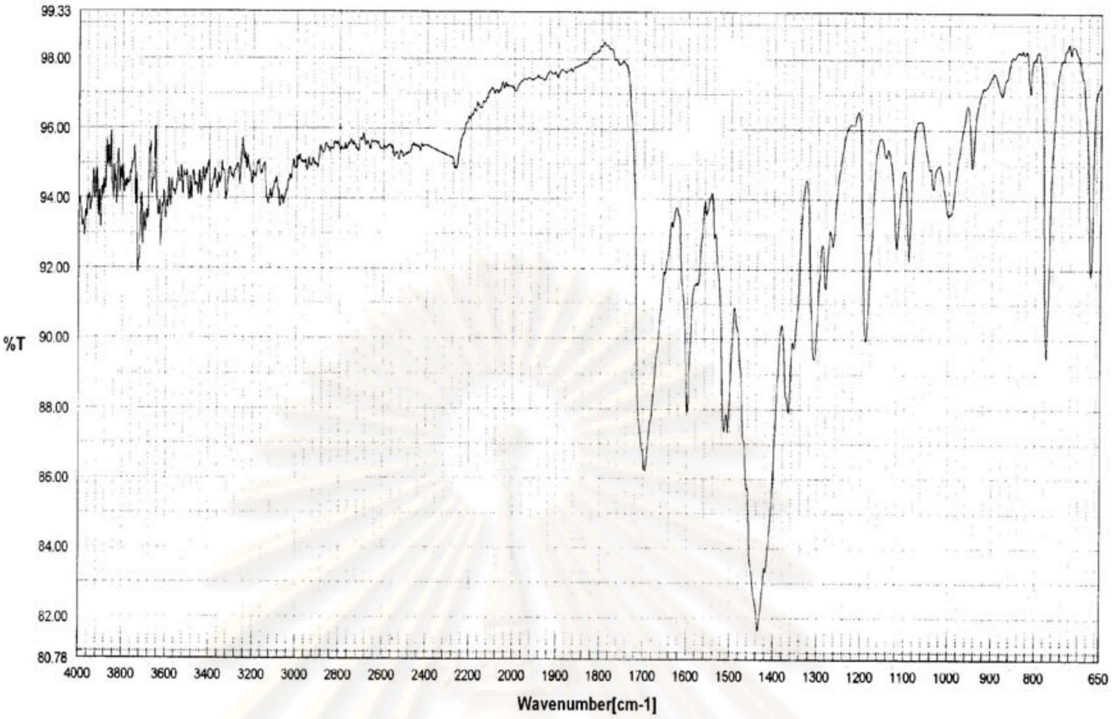


Figure 23 IR spectrum of compound **MH3** (ATR).

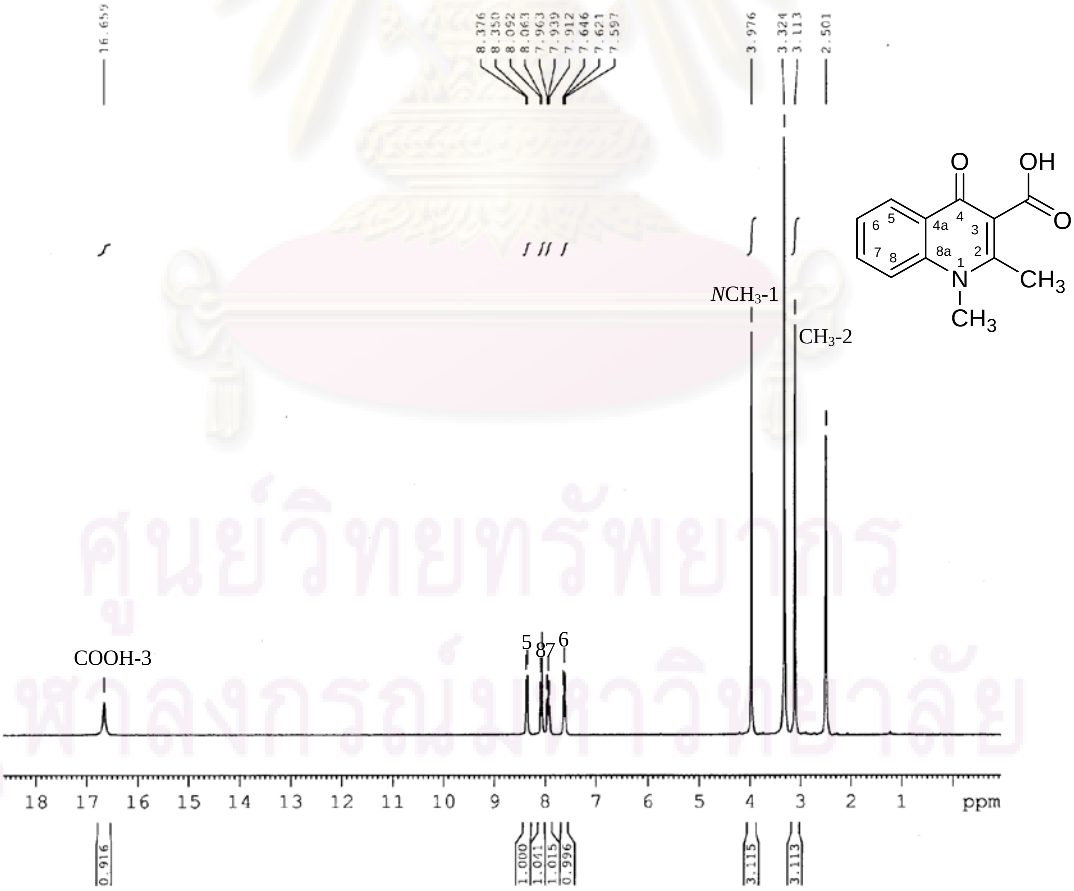


Figure 24 ^{1}H-NMR spectrum of compound **MH3** (300 MHz, DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

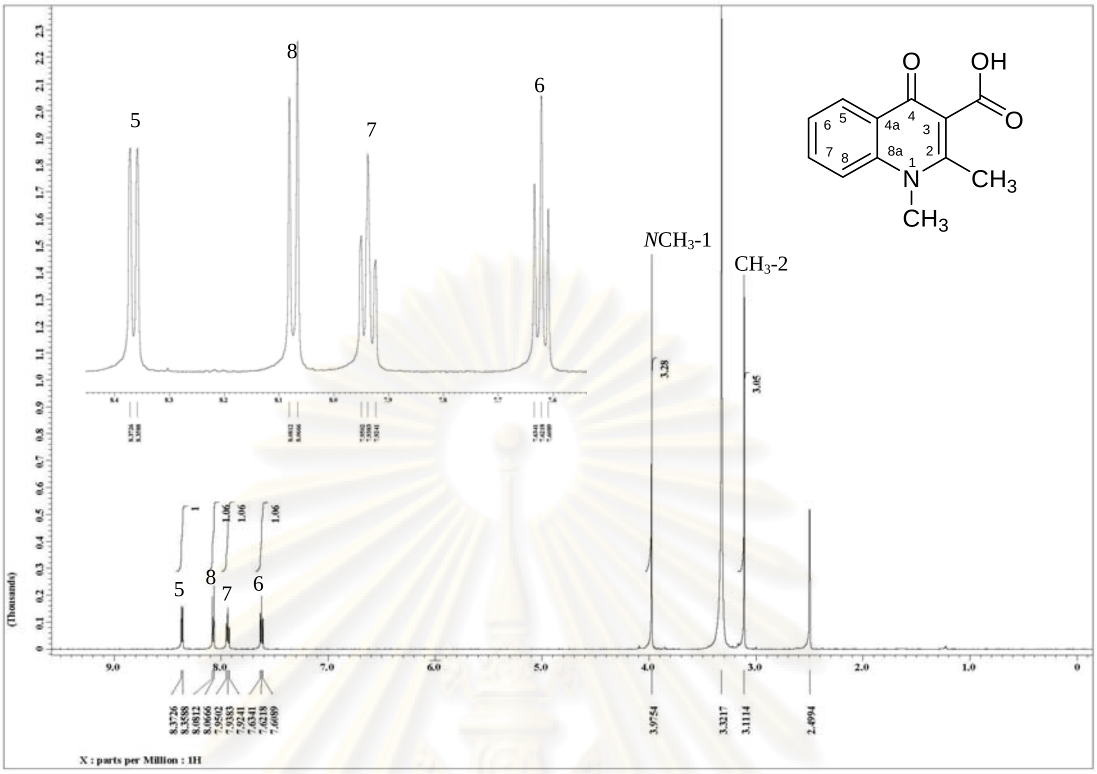


Figure 25 ^{1}H-NMR spectrum of compound **MH3** (600 MHz, DMSO-d_6).

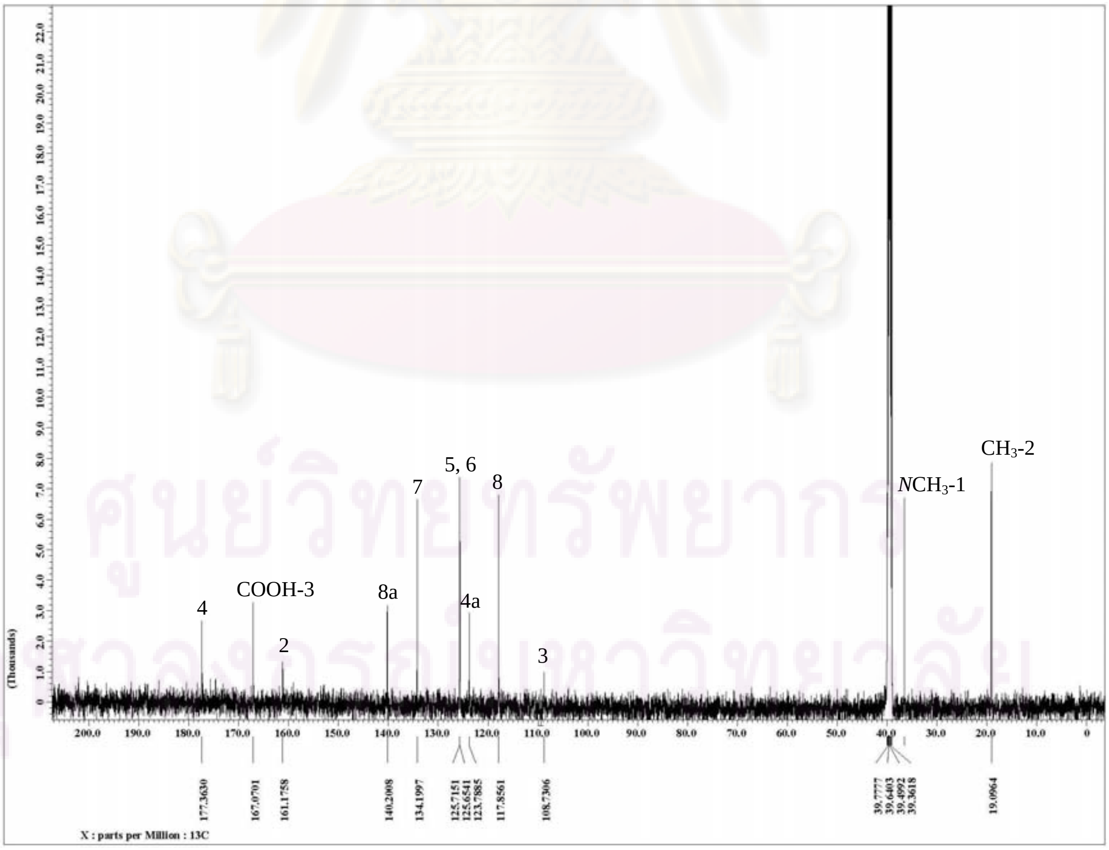


Figure 26 ^{13}C-NMR spectrum of compound **MH3** (150 MHz, DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

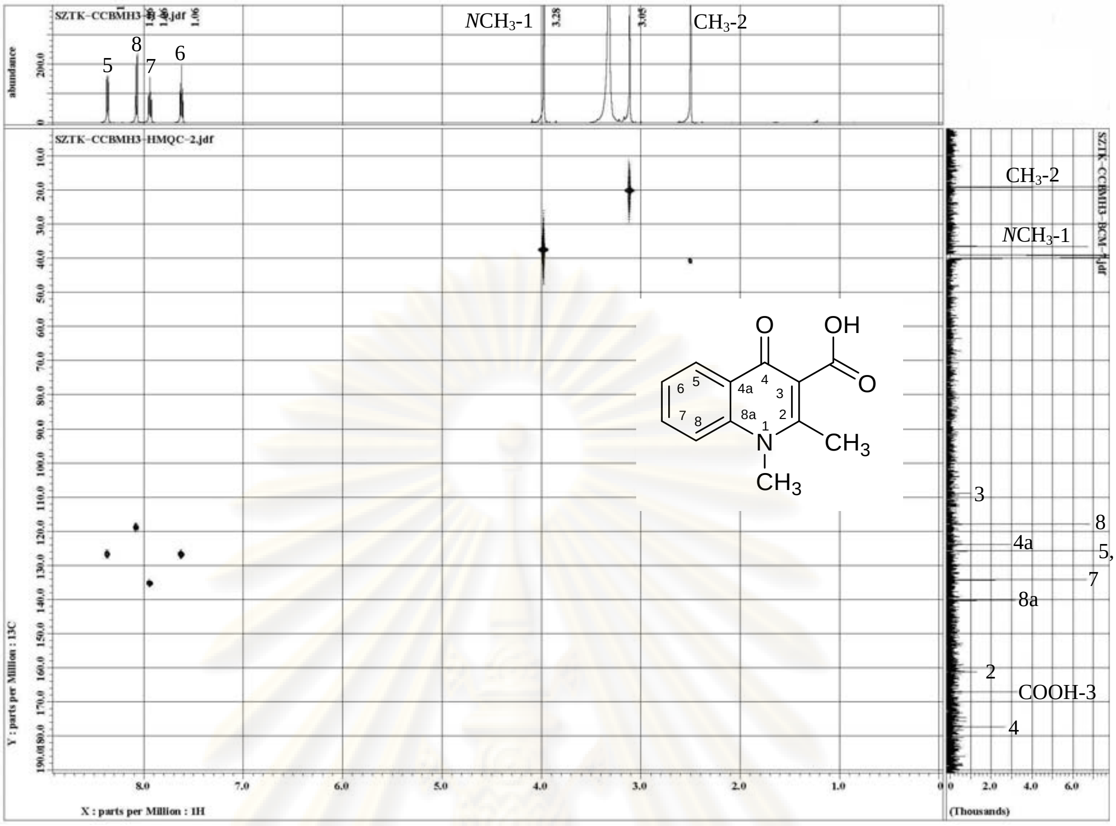


Figure 27 HMQC spectrum of compound **MH3** (DMSO-d_6).

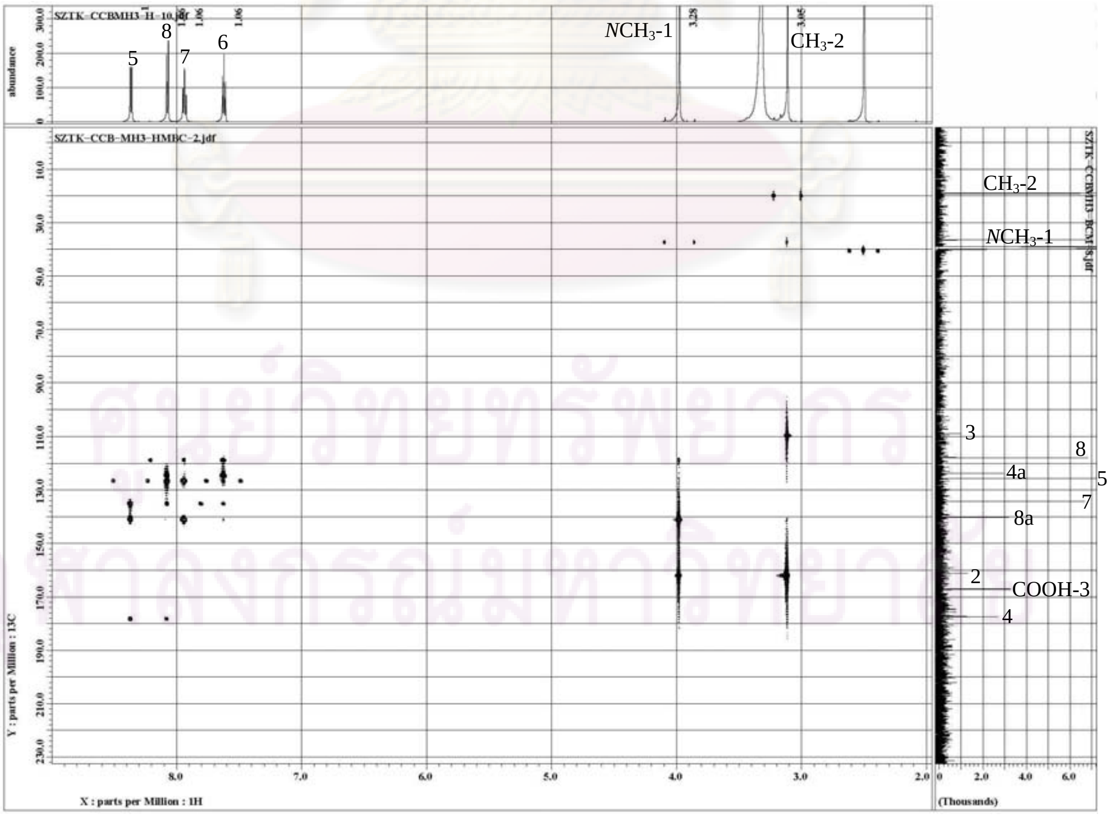


Figure 28 HMBC spectrum of compound **MH3** (DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

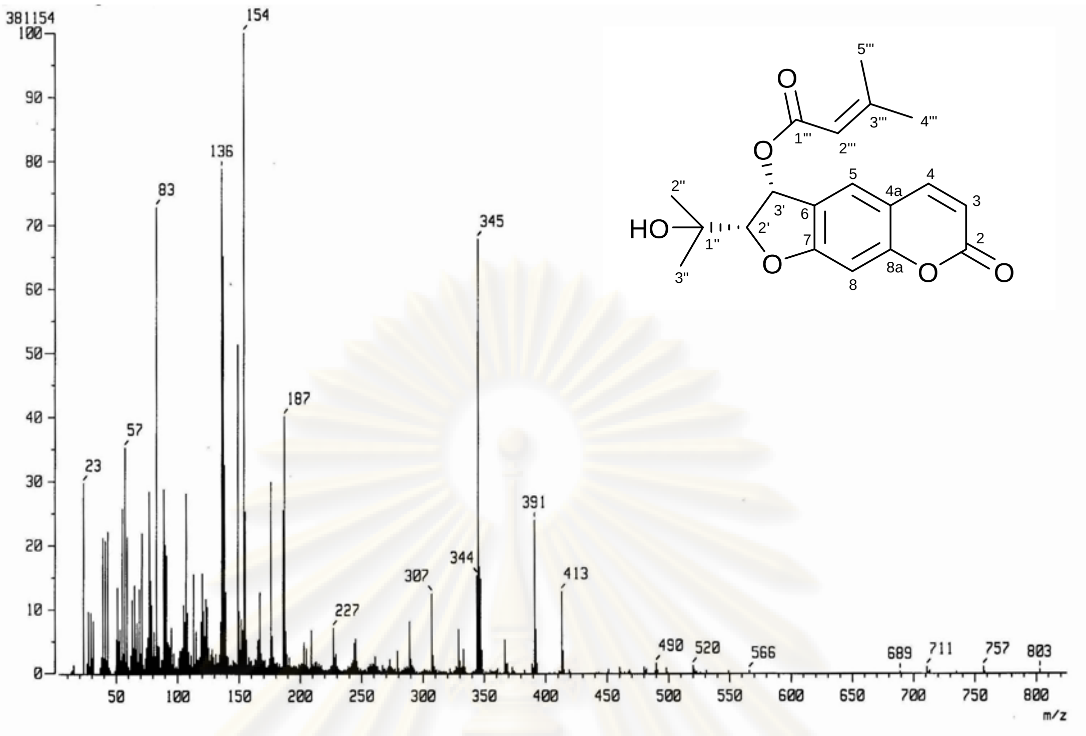


Figure 29 FABMS of compound **MH4**.

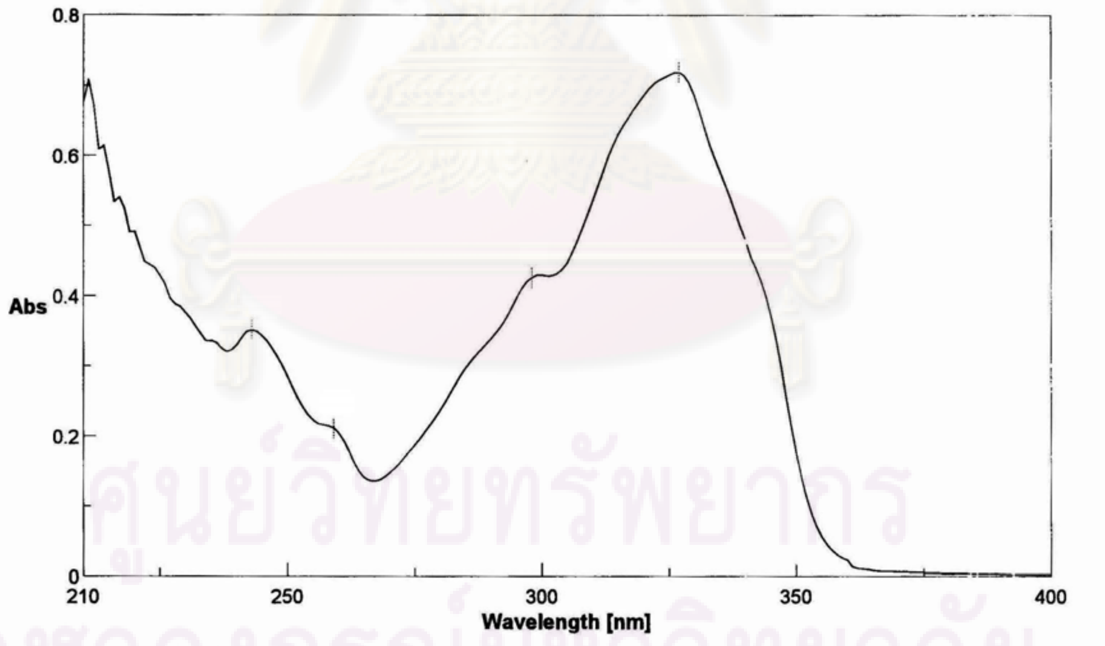


Figure 30 UV spectrum of compound **MH4** ($CHCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

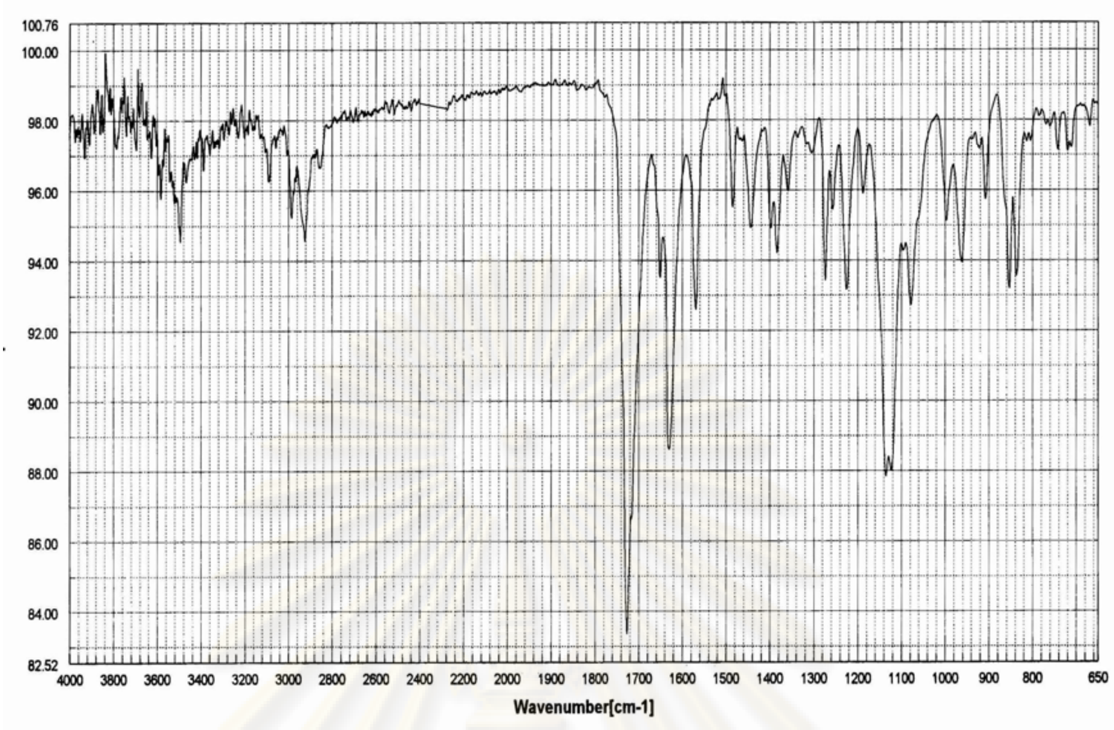


Figure 31 IR spectrum of compound **MH4** (ATR).

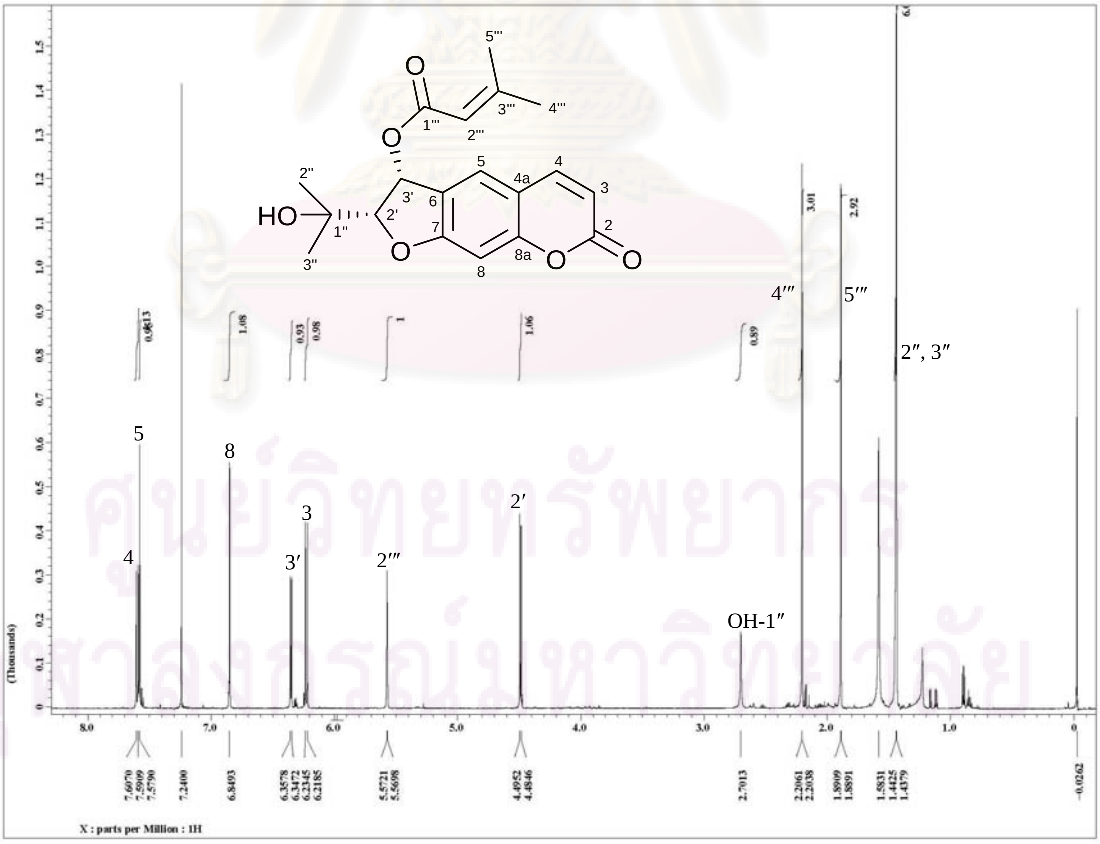


Figure 32 ^{1}H-NMR spectrum of compound **MH4** (600 MHz, $CDCl_3$).

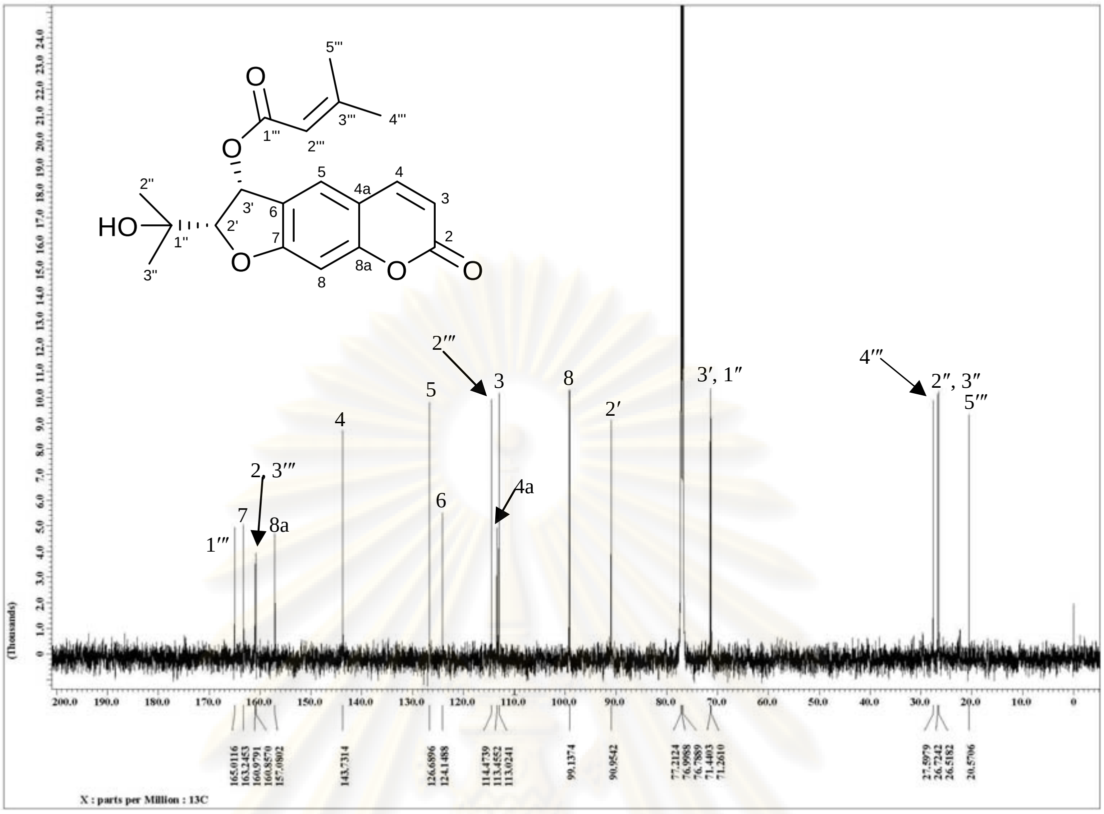


Figure 33 ^{13}C-NMR spectrum of compound **MH4** (150 MHz, $CDCl_3$).

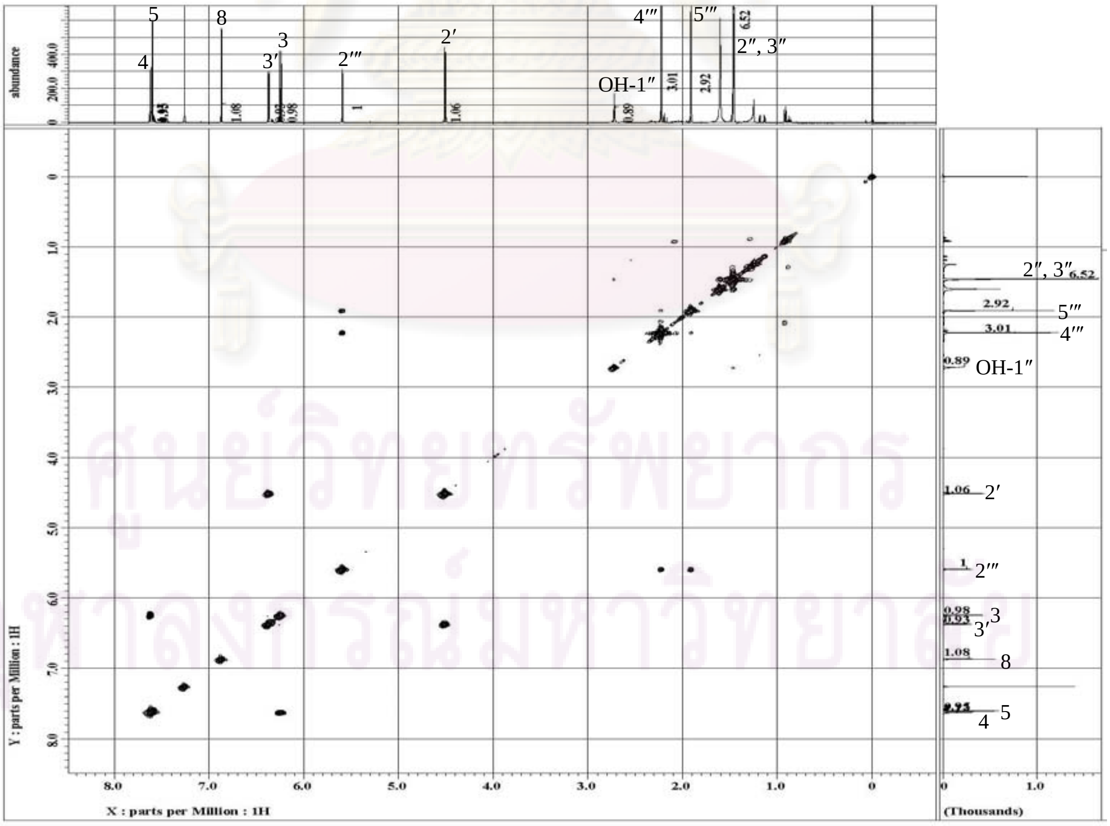


Figure 34 ^{1}H-^{1}H COSY spectrum of compound **MH4** ($CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

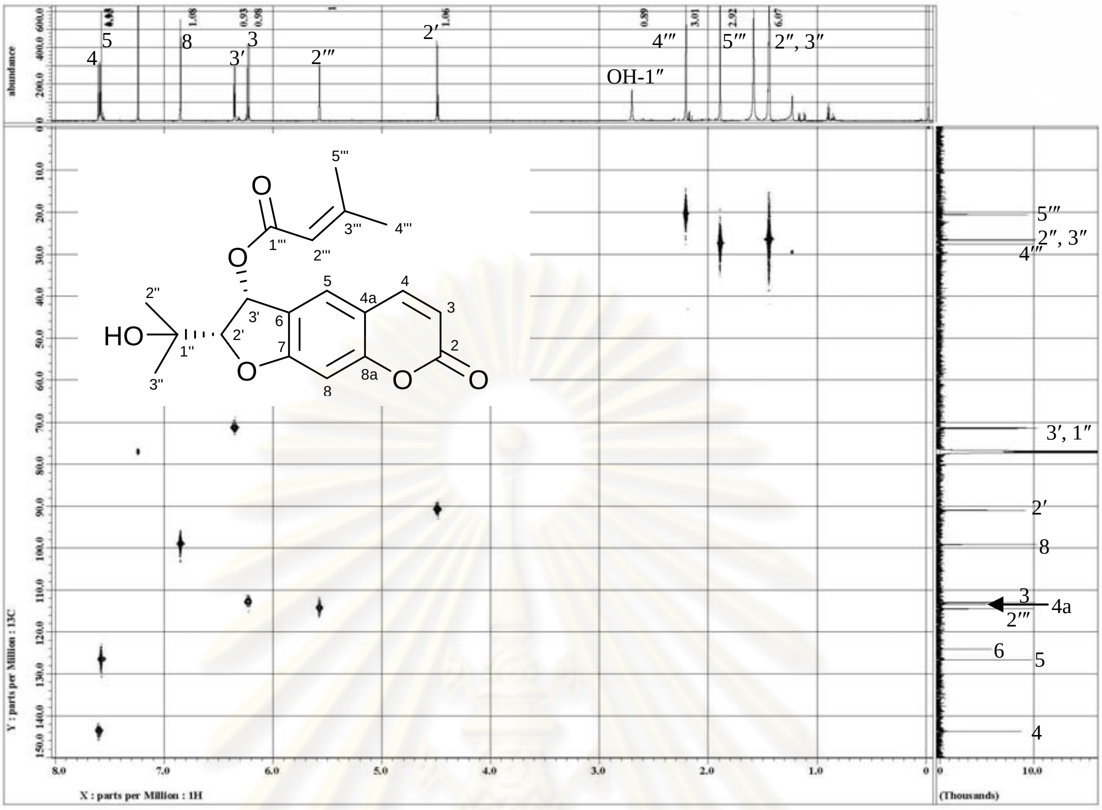


Figure 35 HMQC spectrum of compound **MH4** ($CDCl_3$).

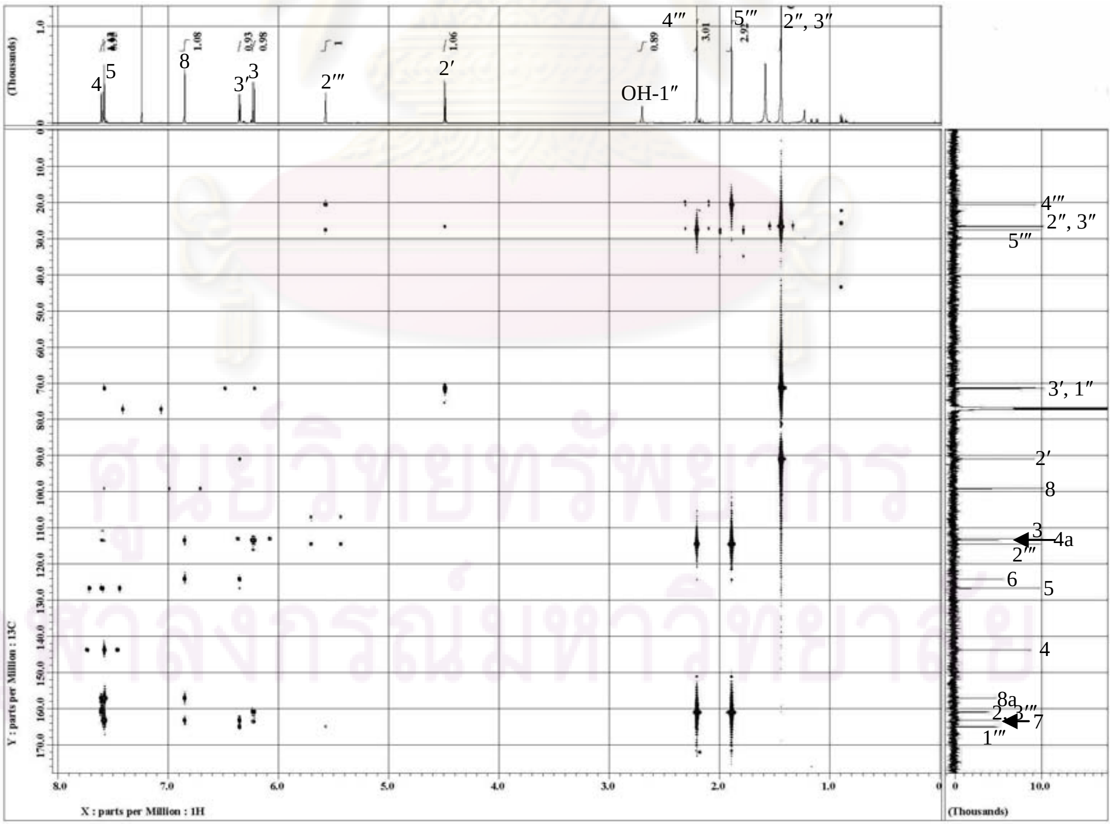


Figure 36 HMBC spectrum of compound **MH4** ($CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

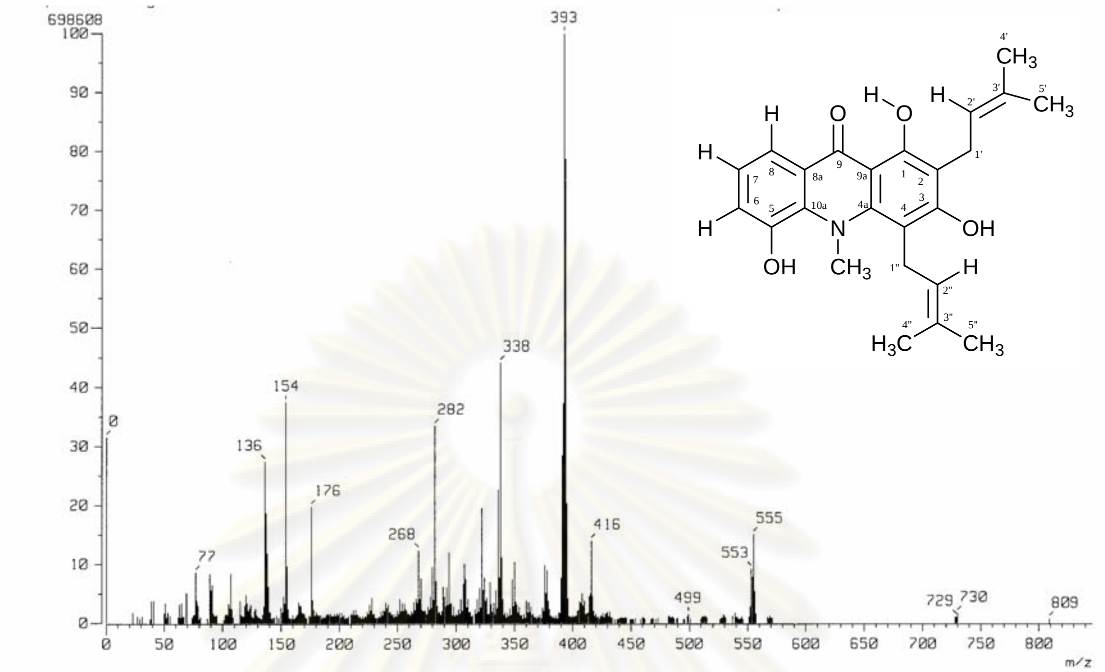


Figure 37 FABMS of compound **GP1**.

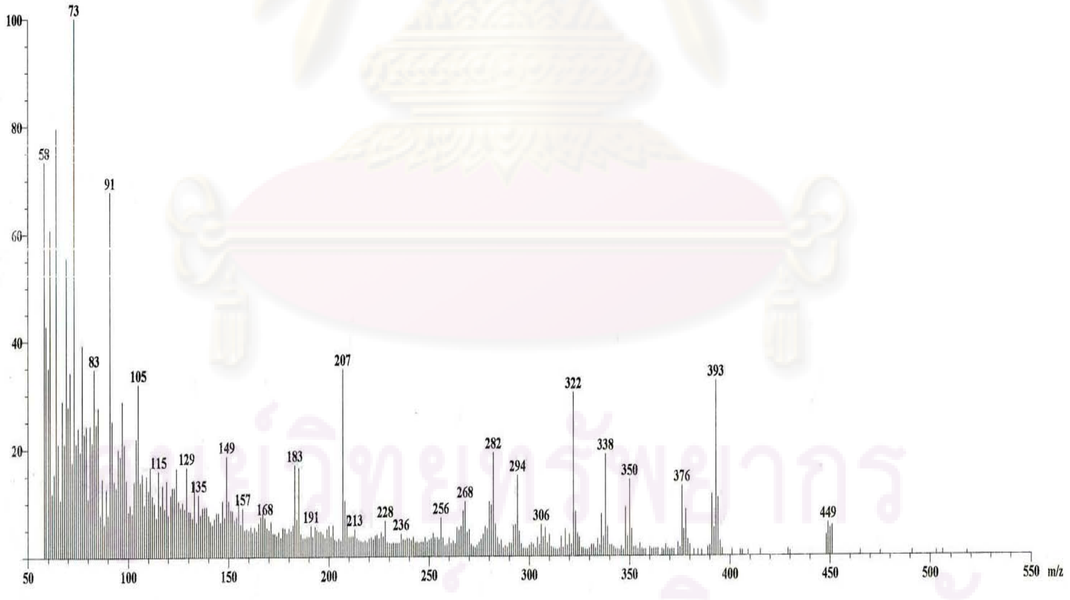


Figure 38 EIMS of compound **GP1**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

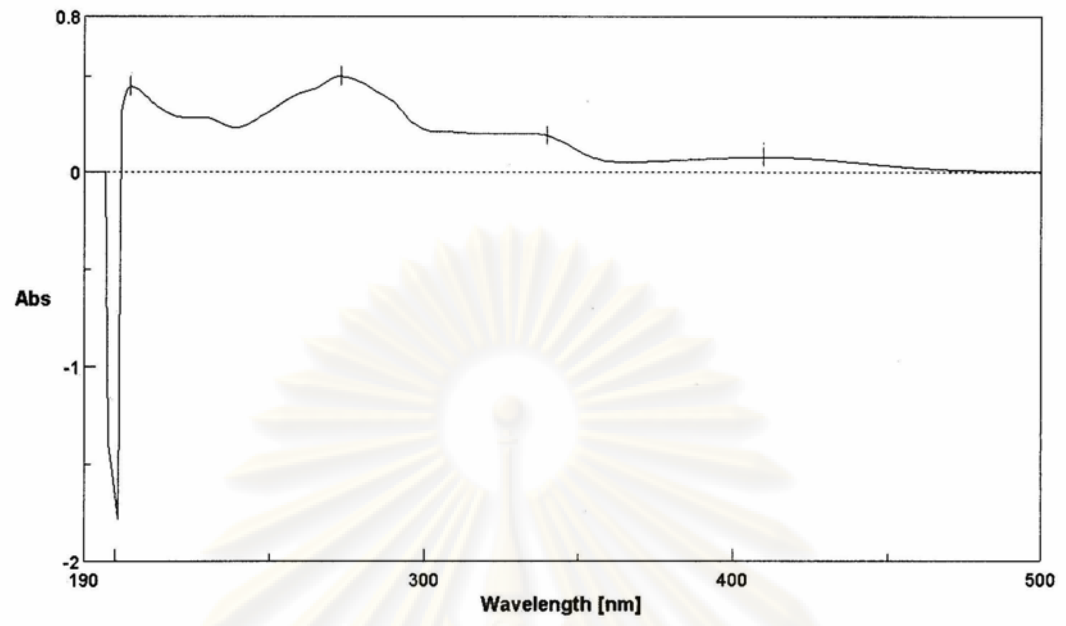


Figure 39 UV spectrum of compound **GP1** (EtOH).

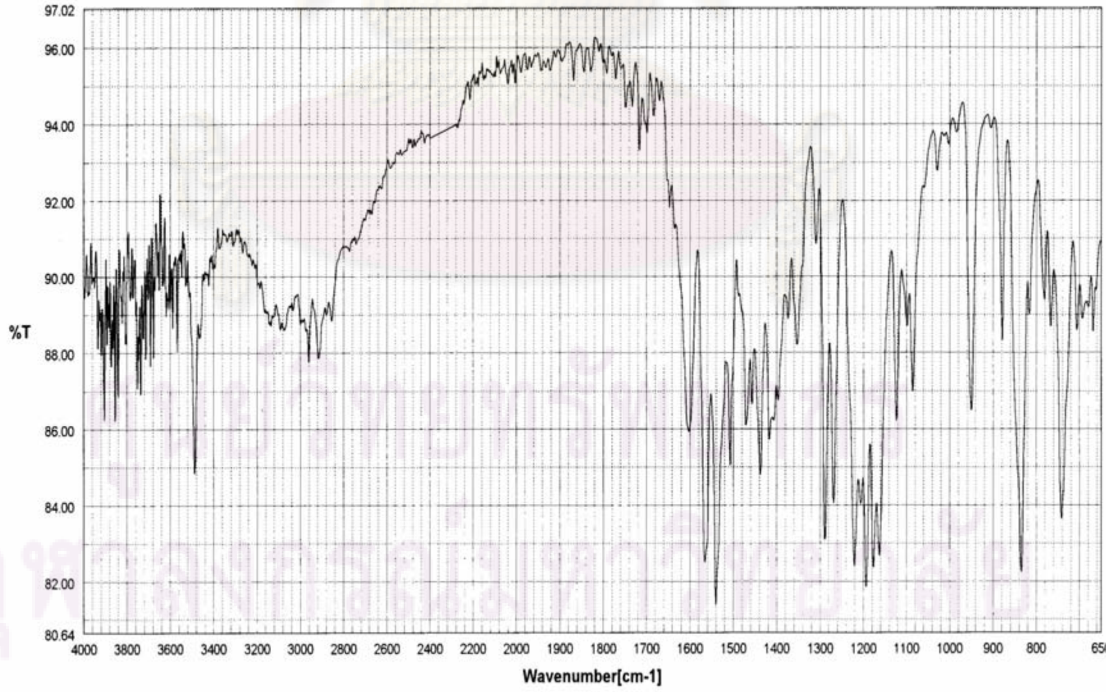


Figure 40 IR spectrum of compound **GP1** (ATR).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

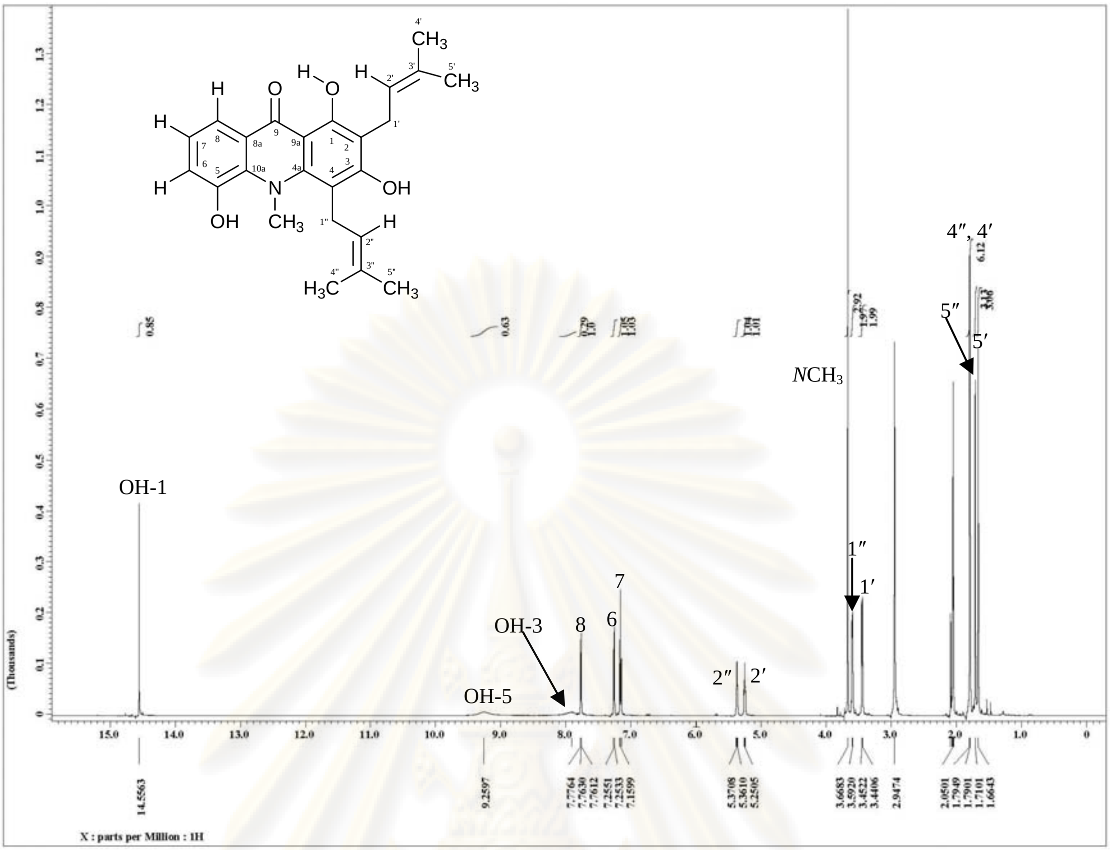
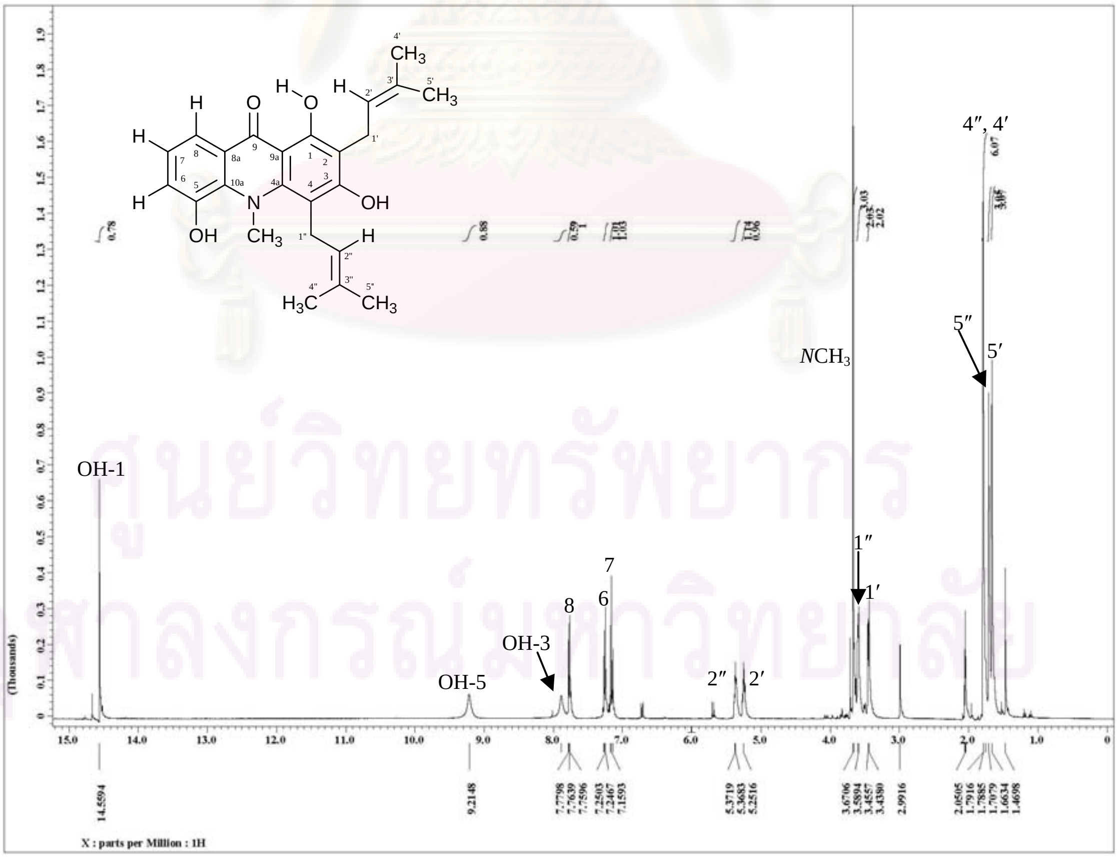


Figure 41 ^{1}H-NMR spectrum of compound **GP1** (600 MHz, acetone-d_6).



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 42 ^{1}H-NMR spectrum of compound **GP1** (400 MHz, acetone-d_6).

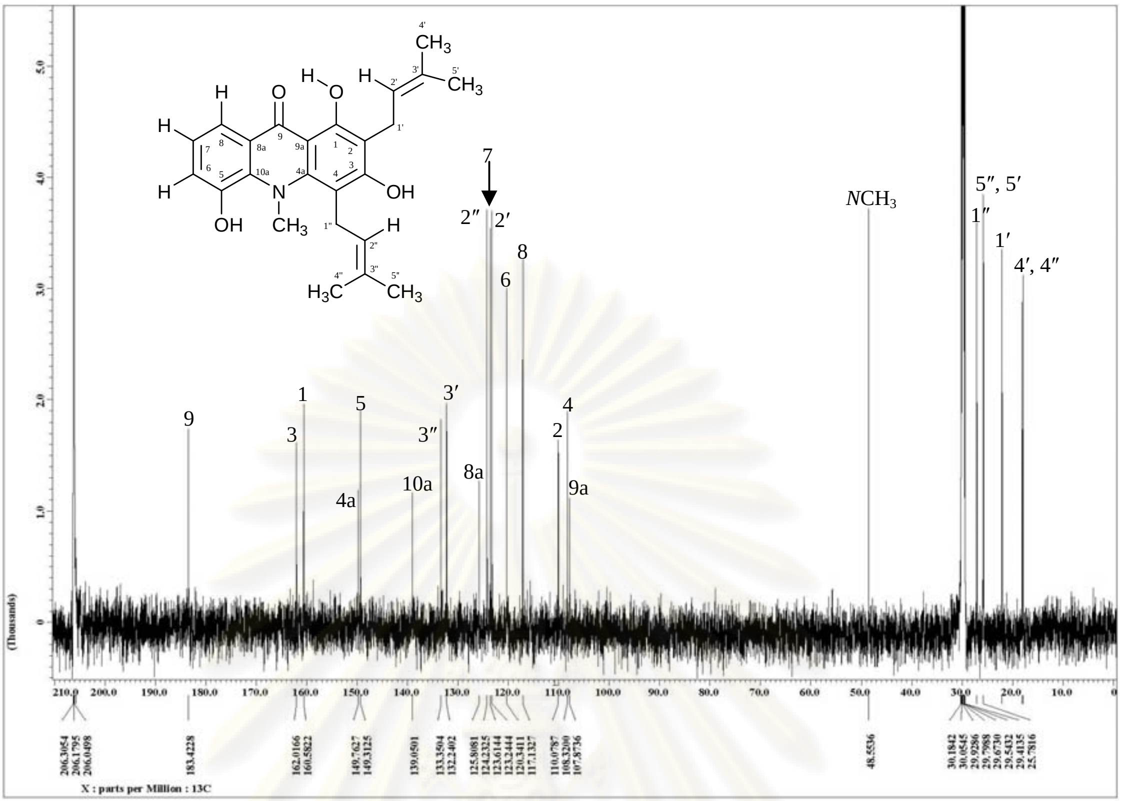


Figure 43 ^{13}C-NMR spectrum of compound **GP1** (150 MHz, acetone-d_6).

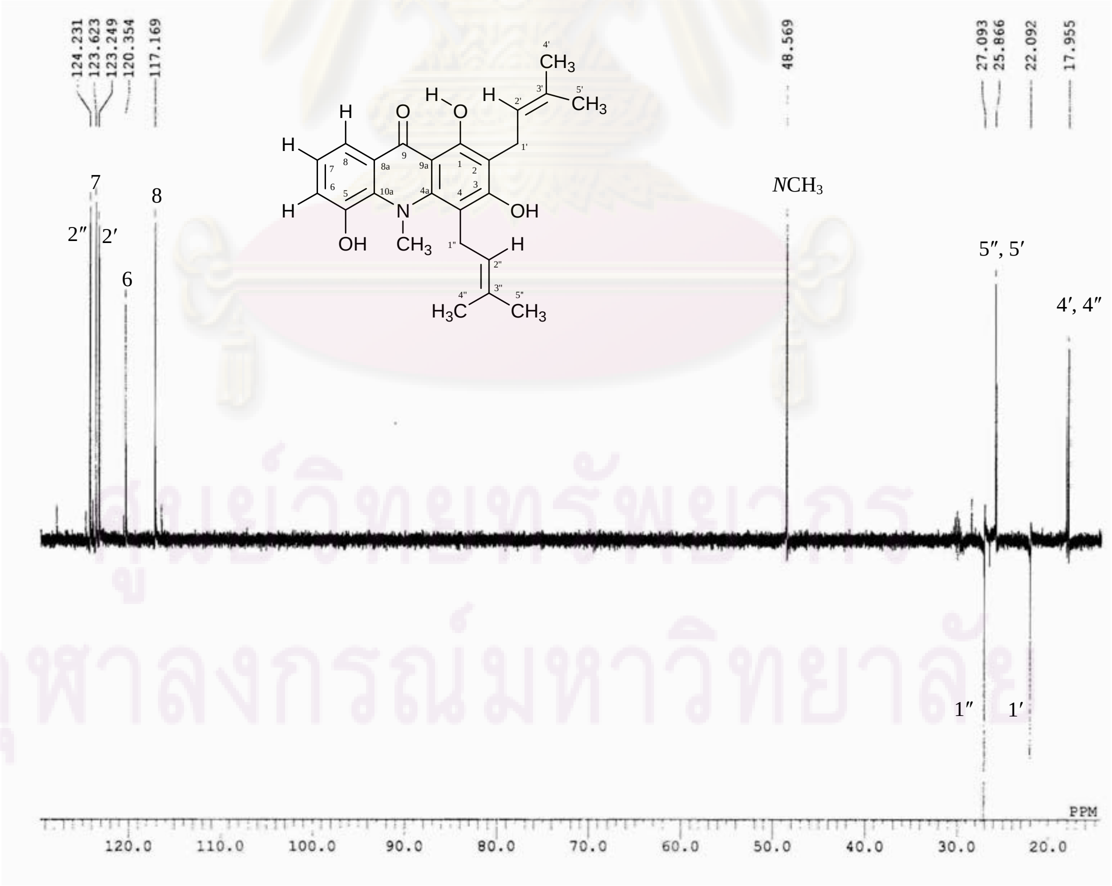


Figure 44 DEPT135 spectrum of compound **GP1** (100 MHz, acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

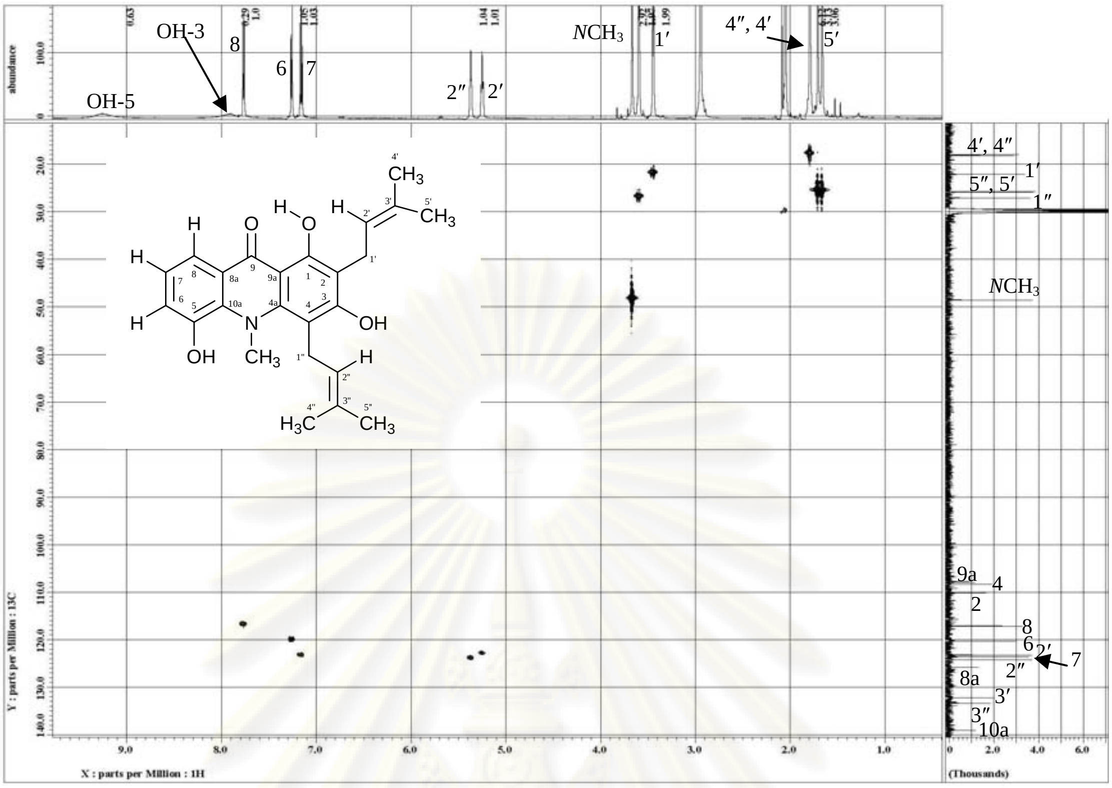


Figure 45 HMQC spectrum of compound **GP1** (acetone-d_6).

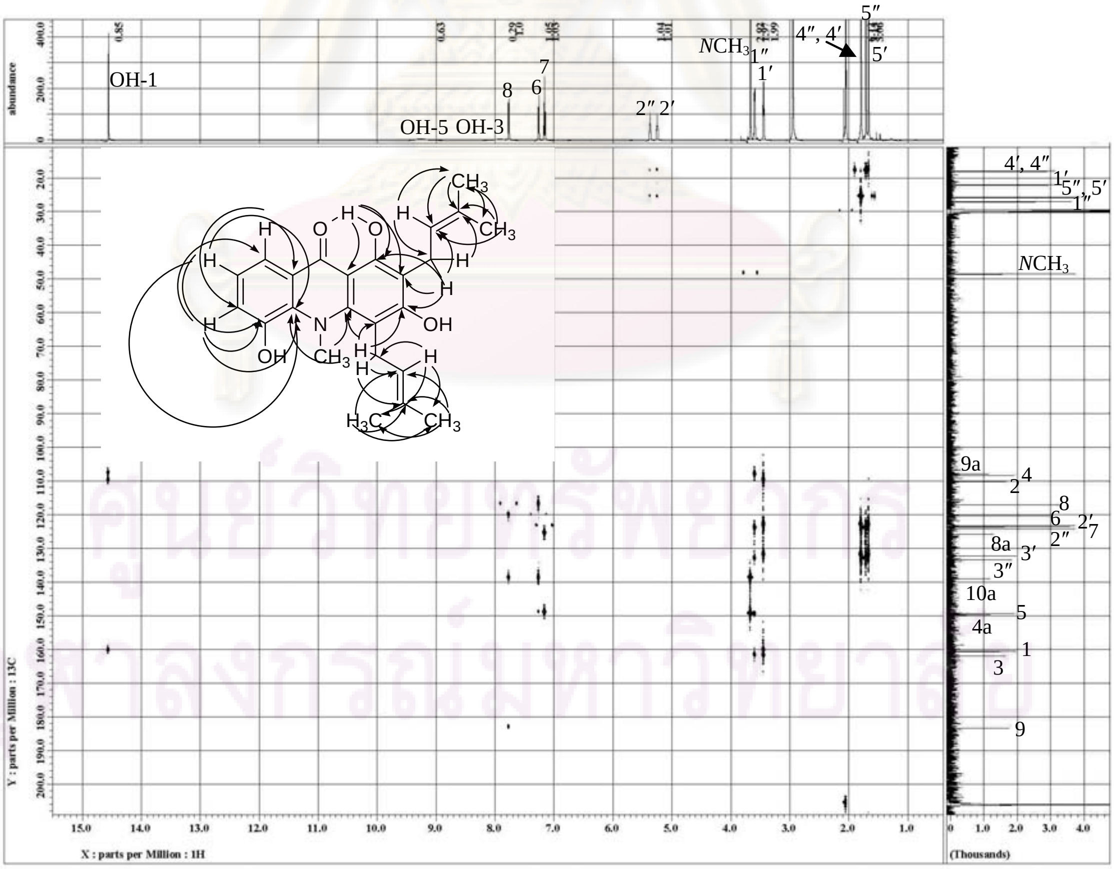


Figure 46 HMBC spectrum of compound **GP1** (acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

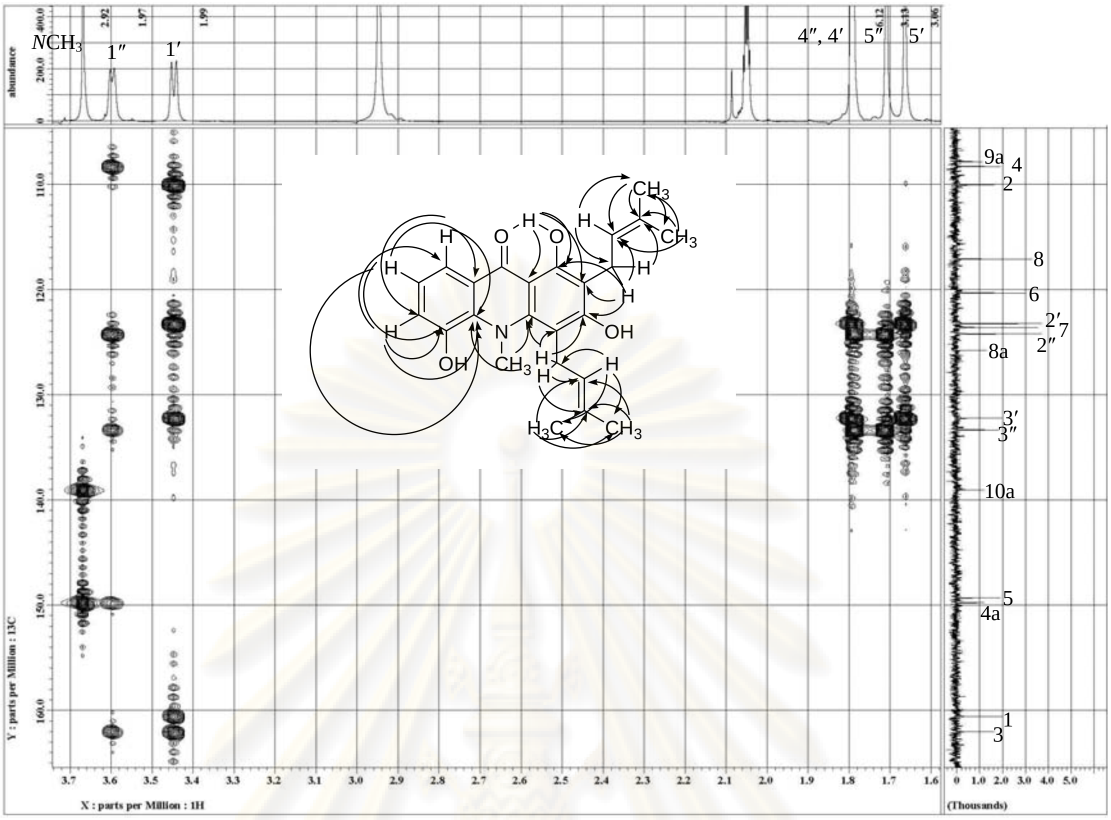


Figure 47 Expanded HMBC spectrum of compound **GP1** (acetone-d_6).

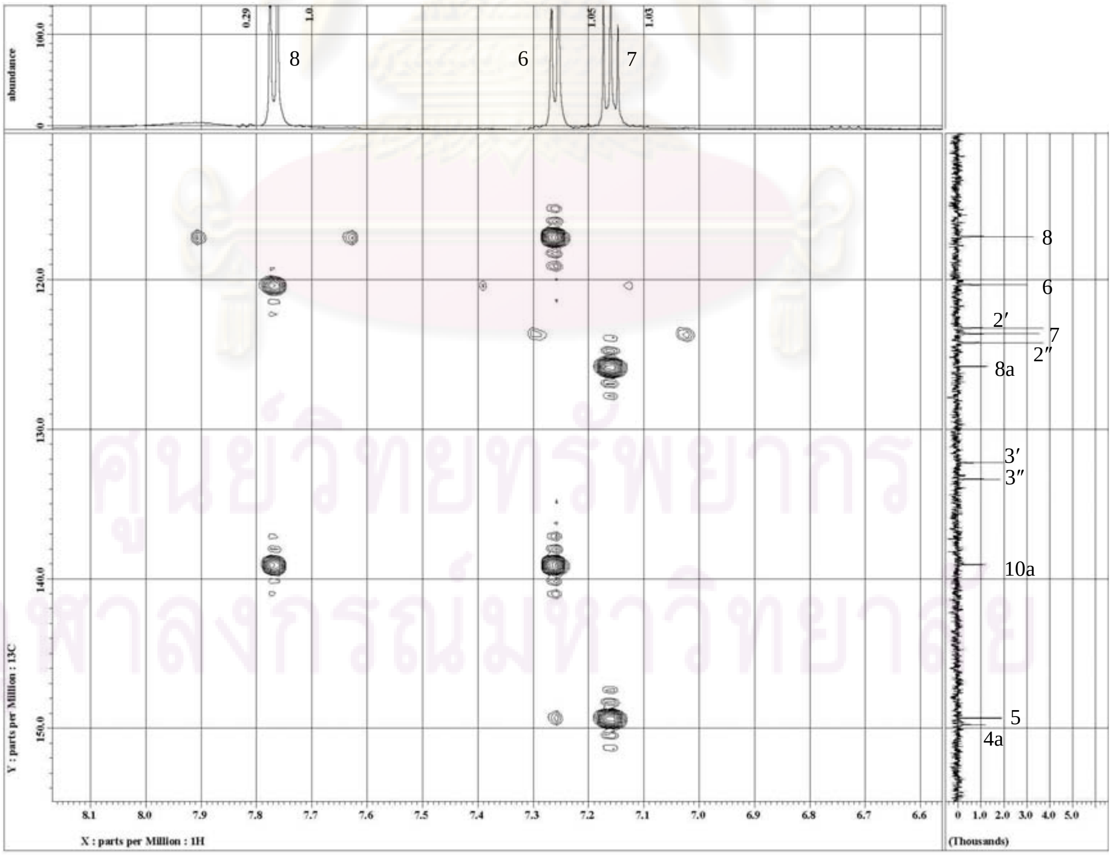


Figure 48 Expanded HMBC spectrum of compound **GP1** (acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

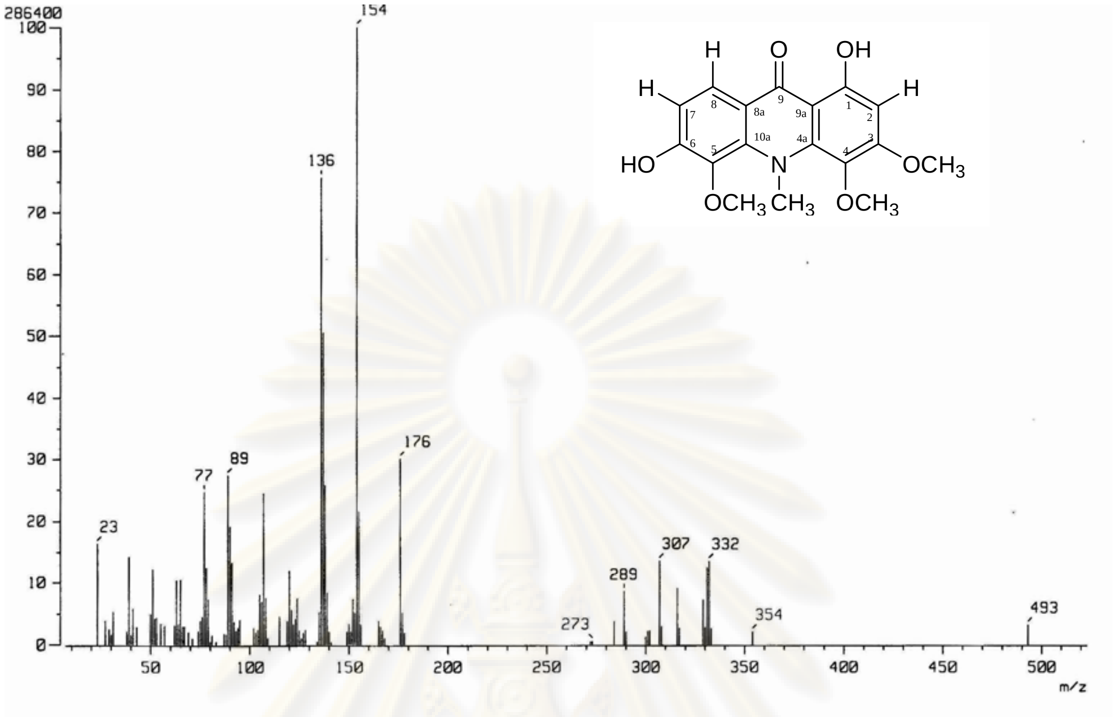


Figure 49 FABMS of compound **GP2**.

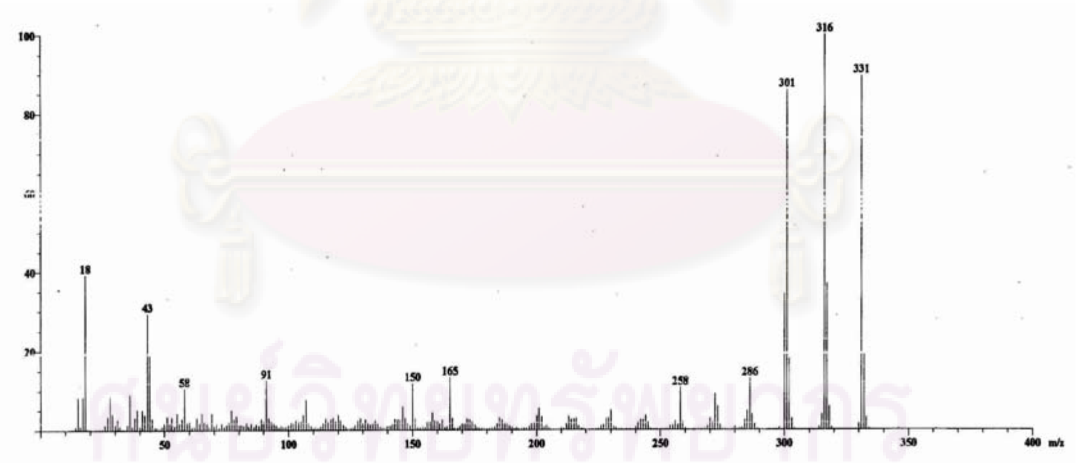


Figure 50 EIMS of compound **GP2**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

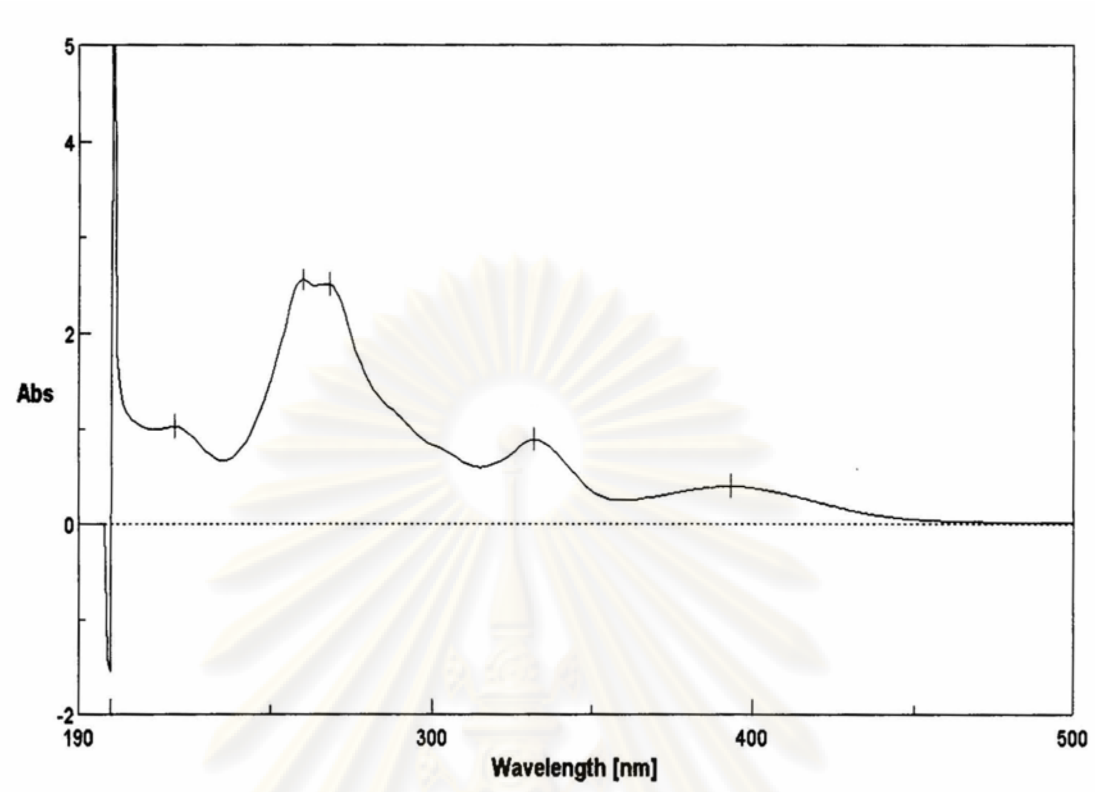


Figure 51 UV spectrum of compound **GP2** (MeOH).

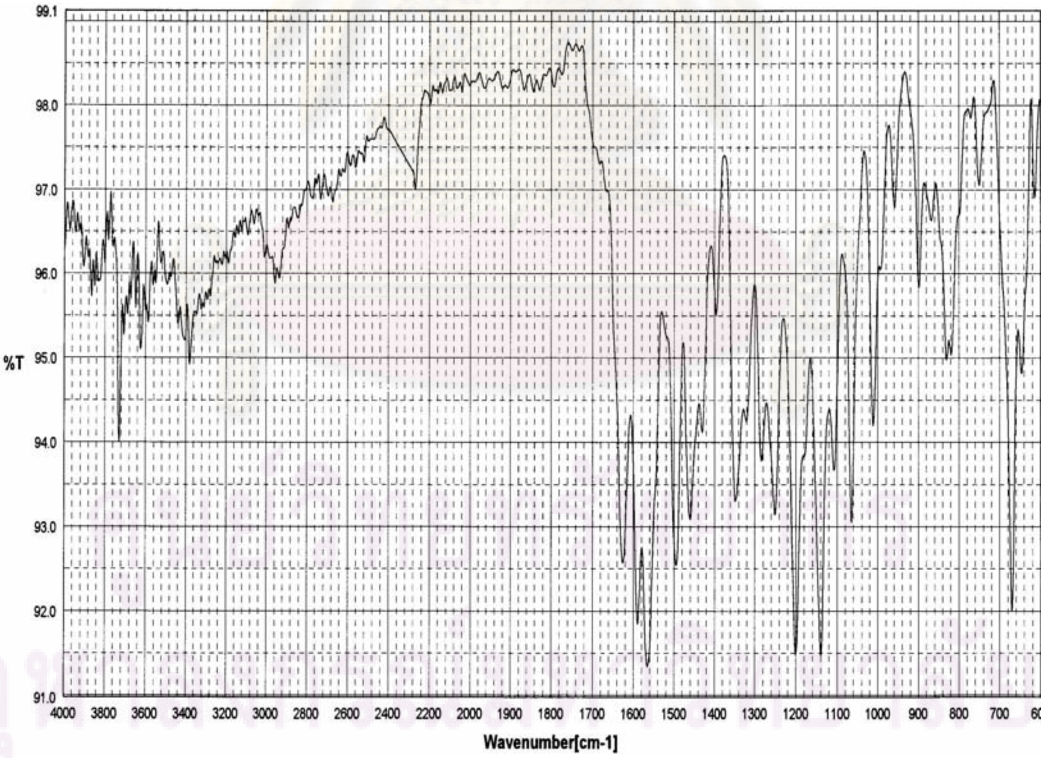


Figure 52 IR spectrum of compound**GP2** (ATR).

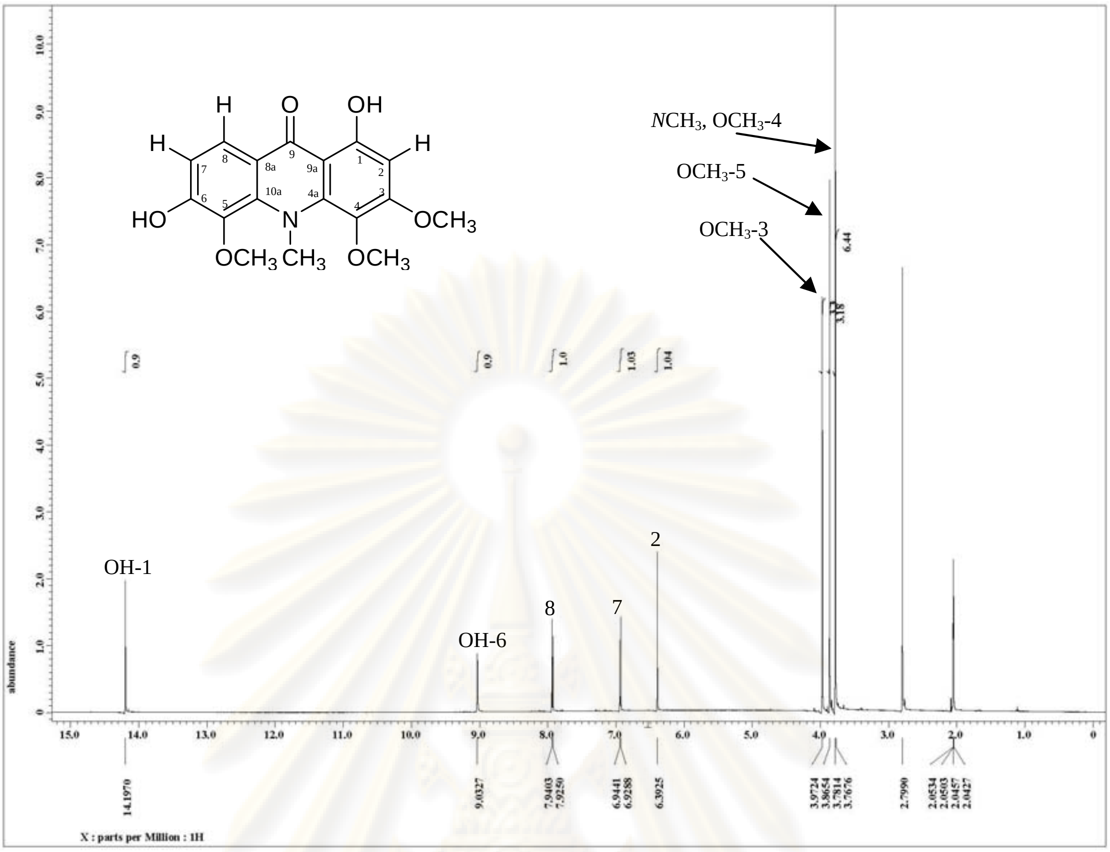


Figure 53 ^{1}H-NMR spectrum of compound **GP2** (600 MHz, acetone-d_6).

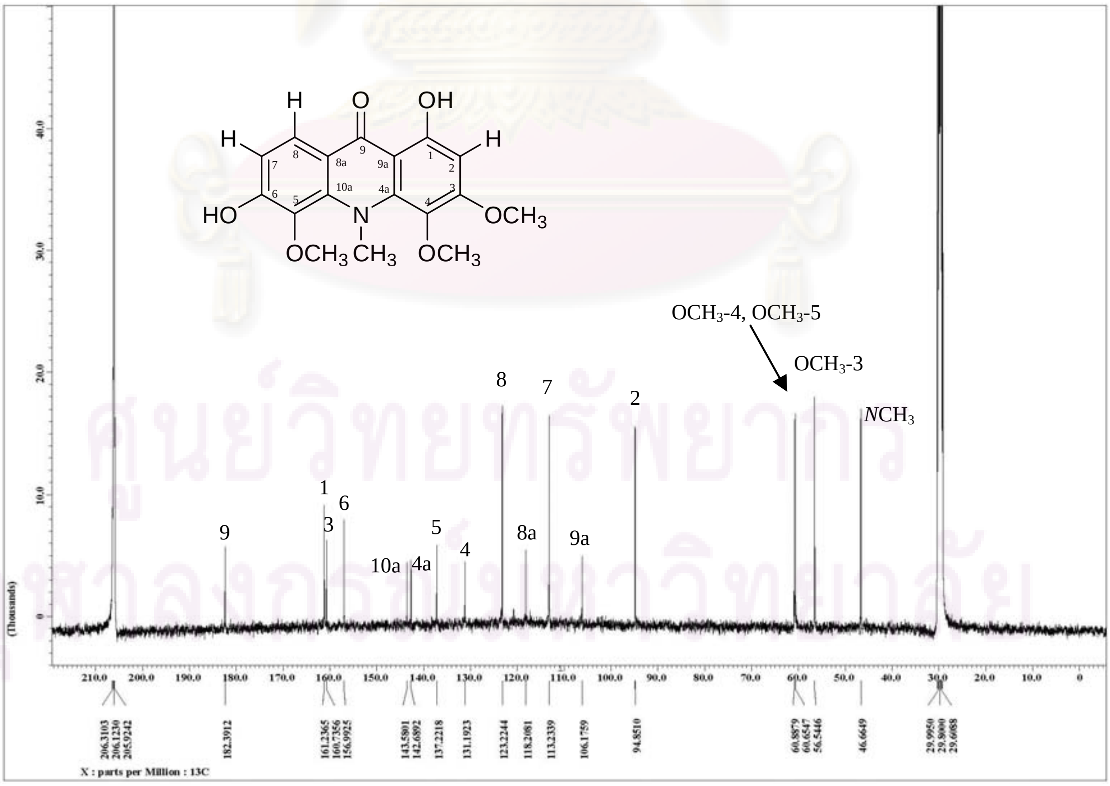


Figure 54 ^{13}C-NMR spectrum of compound **GP2** (150 MHz, acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

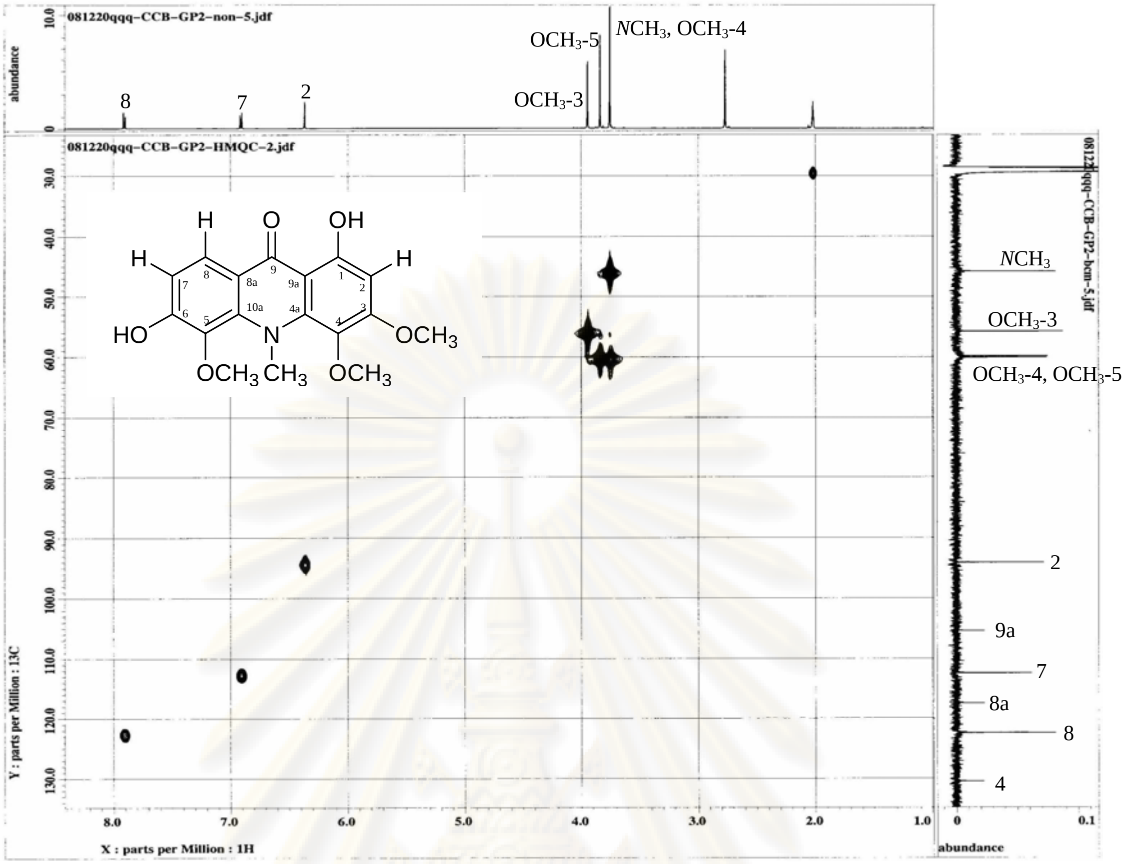


Figure 55 HMQC spectrum of compound **GP2** (acetone-d_6).

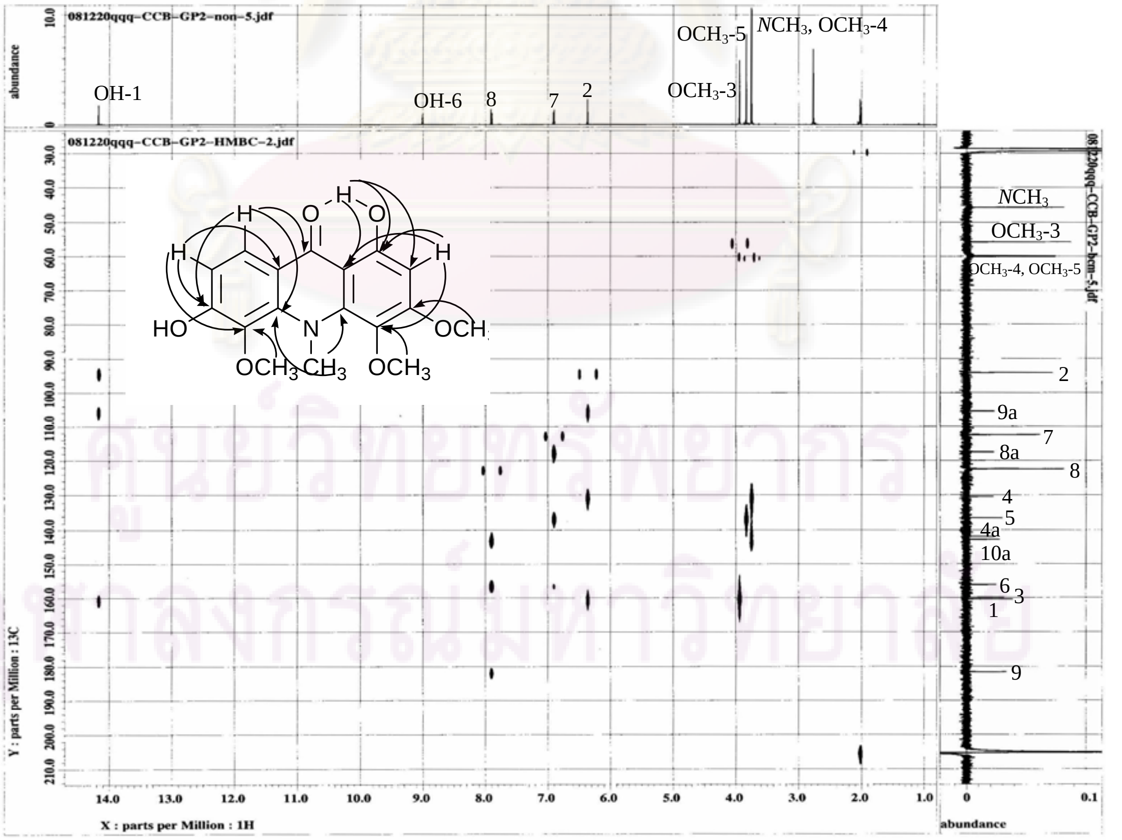


Figure 56 HMBC spectrum of compound **GP2** (acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

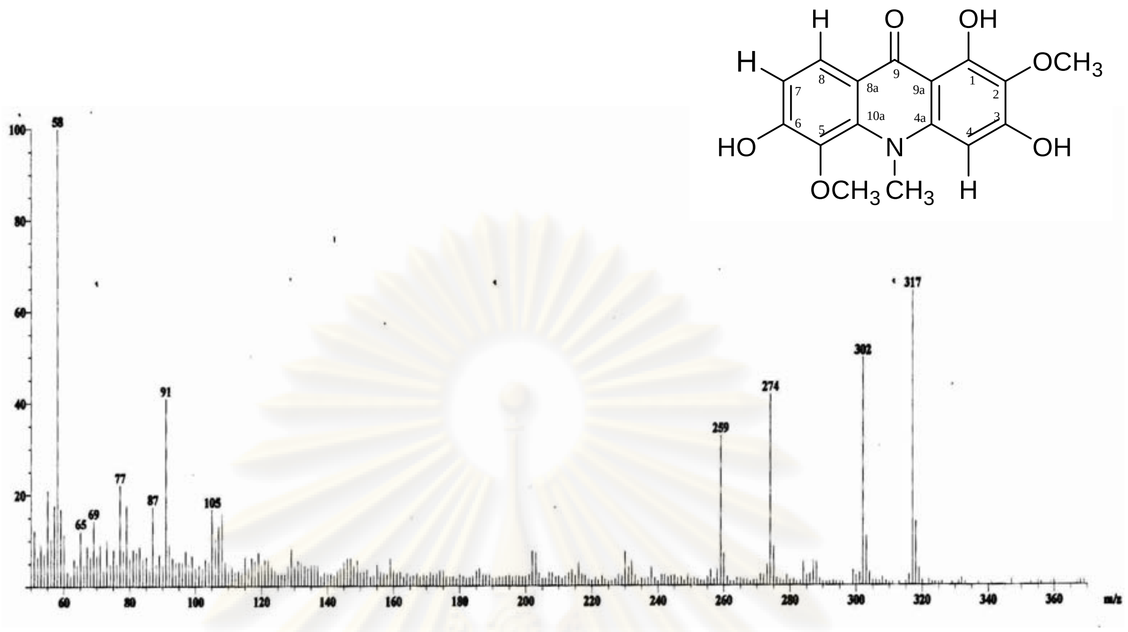


Figure 57 EIMS of compound **GP3**.

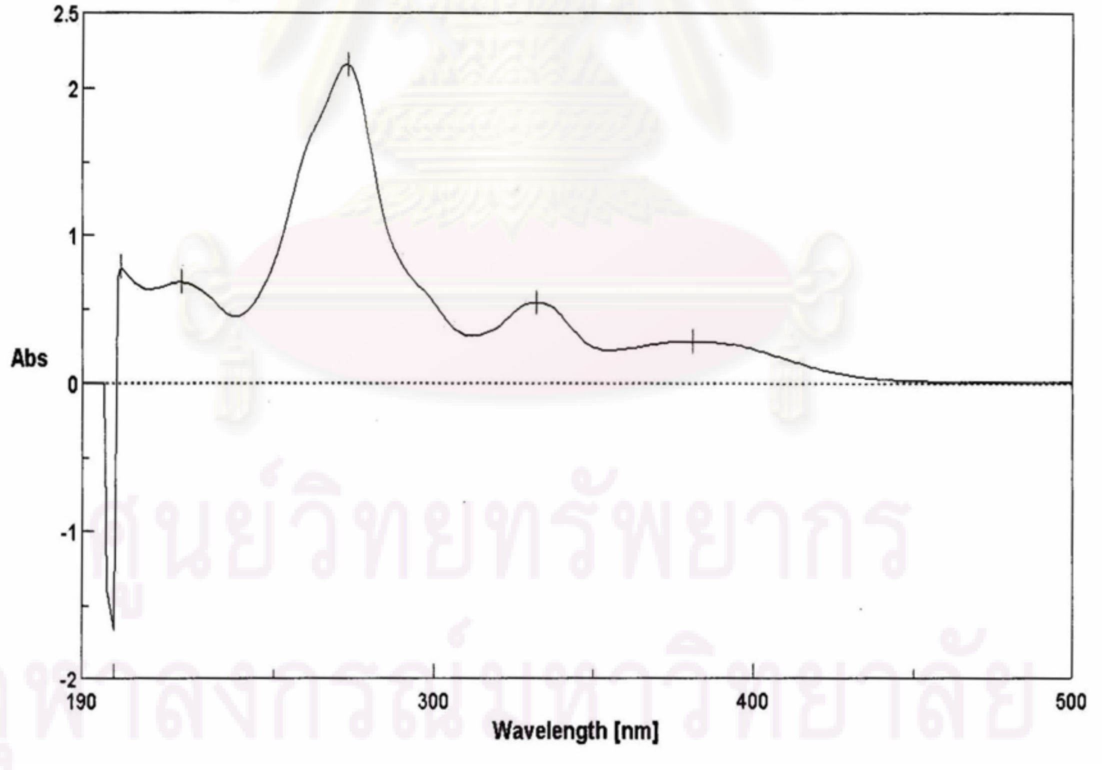


Figure 58 UV spectrum of compound **GP3** (EtOH).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

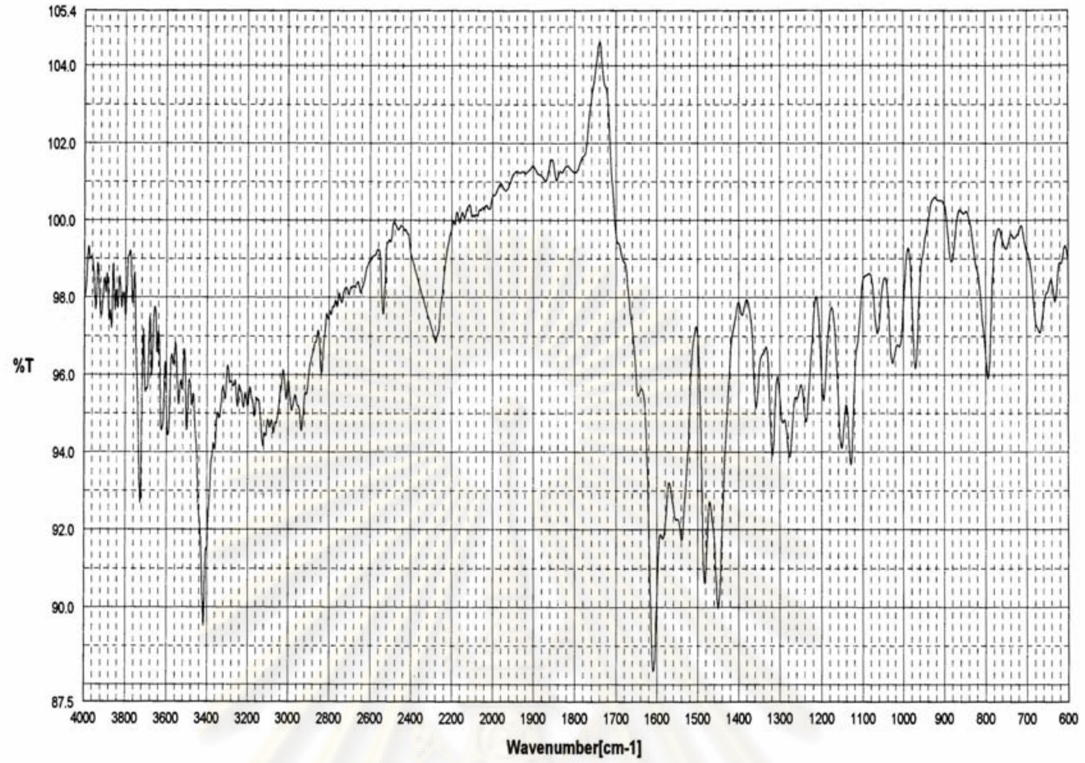


Figure 59 IR spectrum of compound **GP3**.

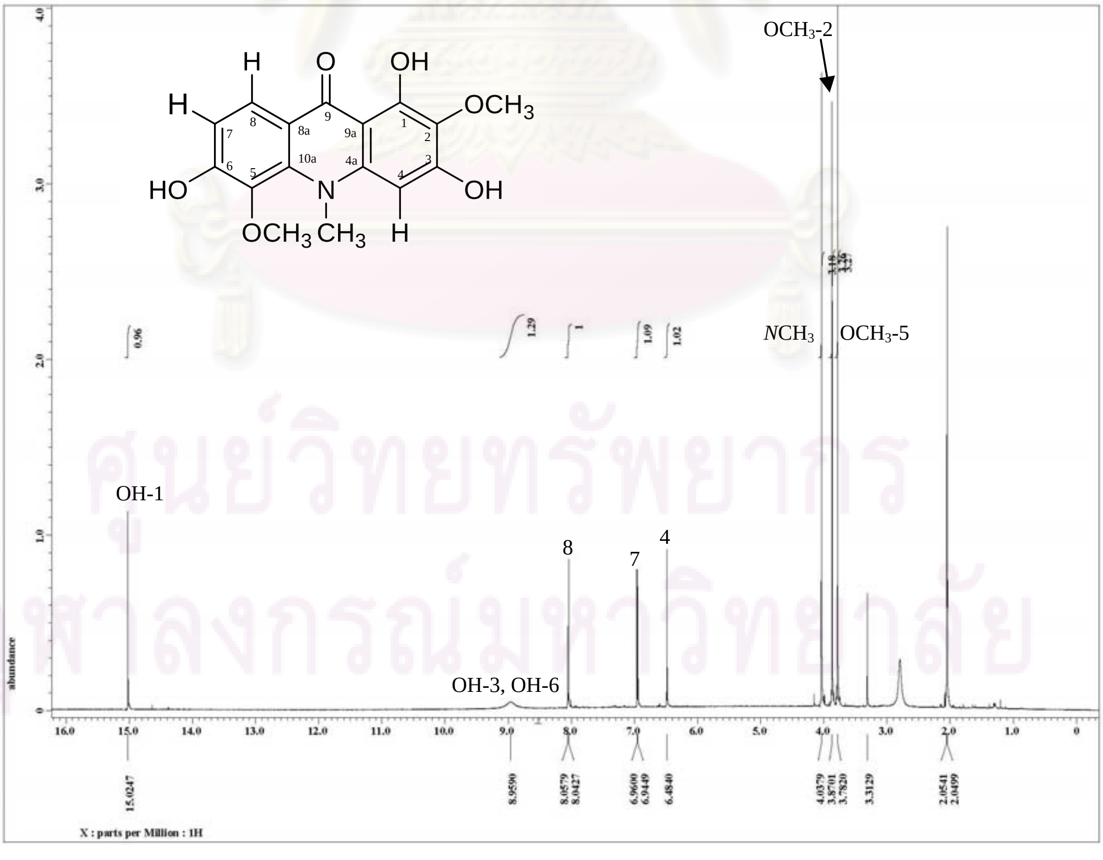


Figure 60 ^{1}H-NMR spectrum of compound **GP3** (600 MHz, acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

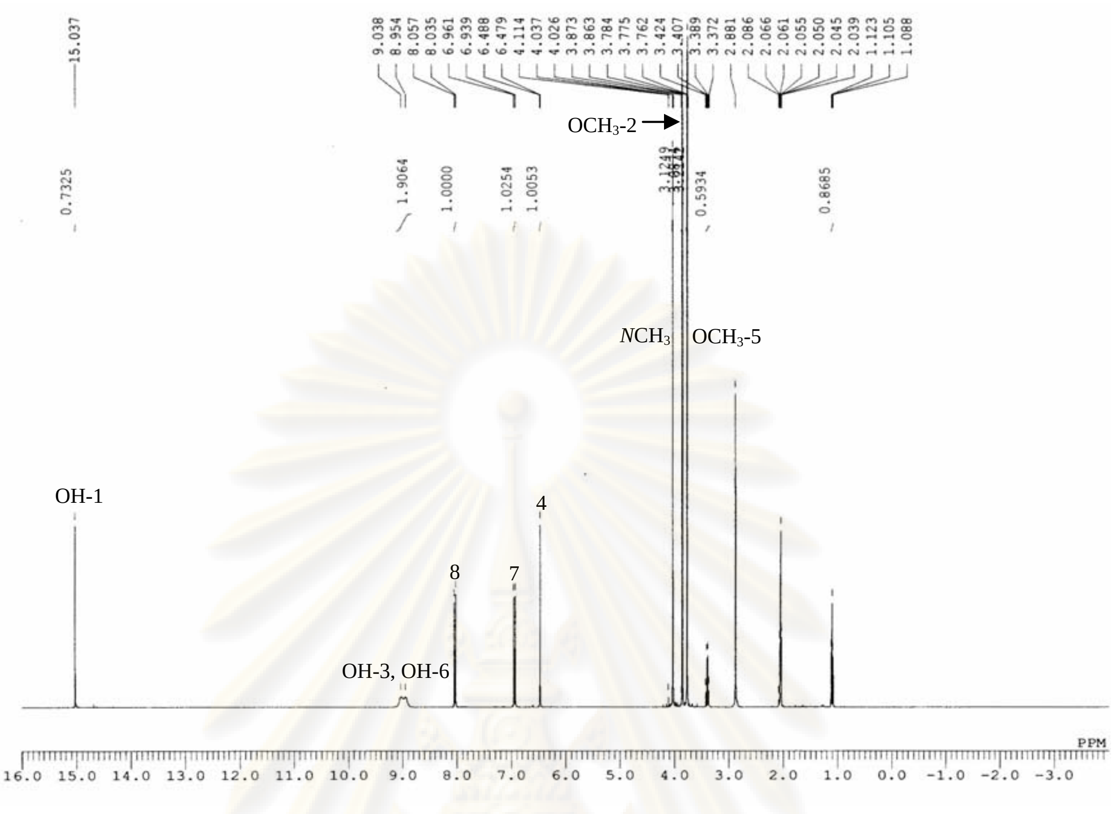


Figure 61 ^{1}H-NMR spectrum of compound **GP3** (400 MHz, acetone-d_6).

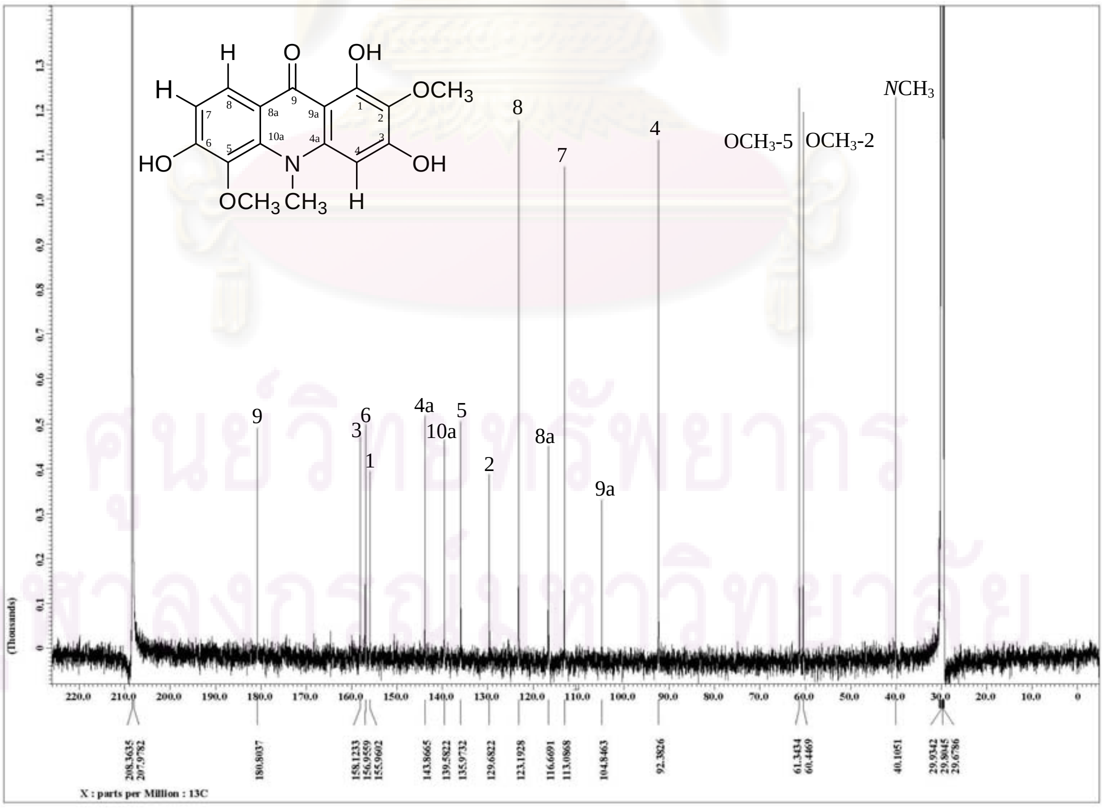


Figure 62 ^{13}C-NMR spectrum of compound **GP3** (150 MHz, acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

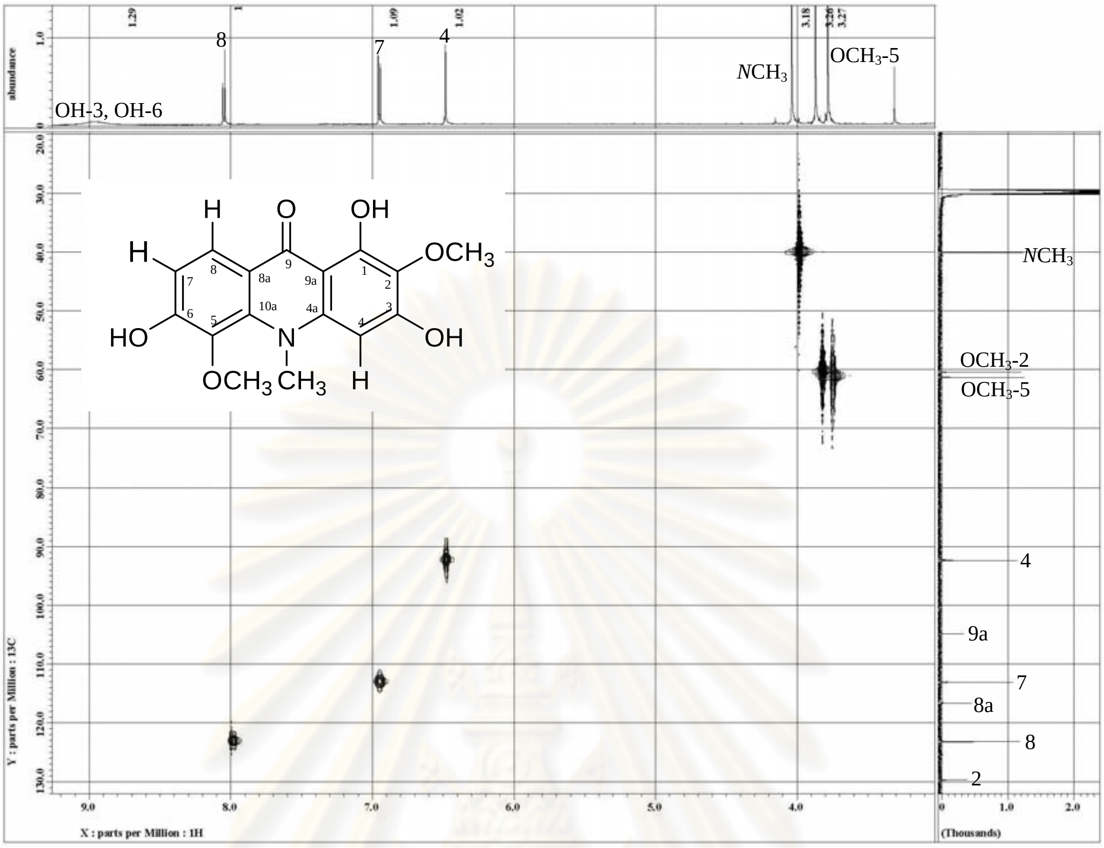


Figure 63 HMQC spectrum of compound **GP3** (acetone-d_6).

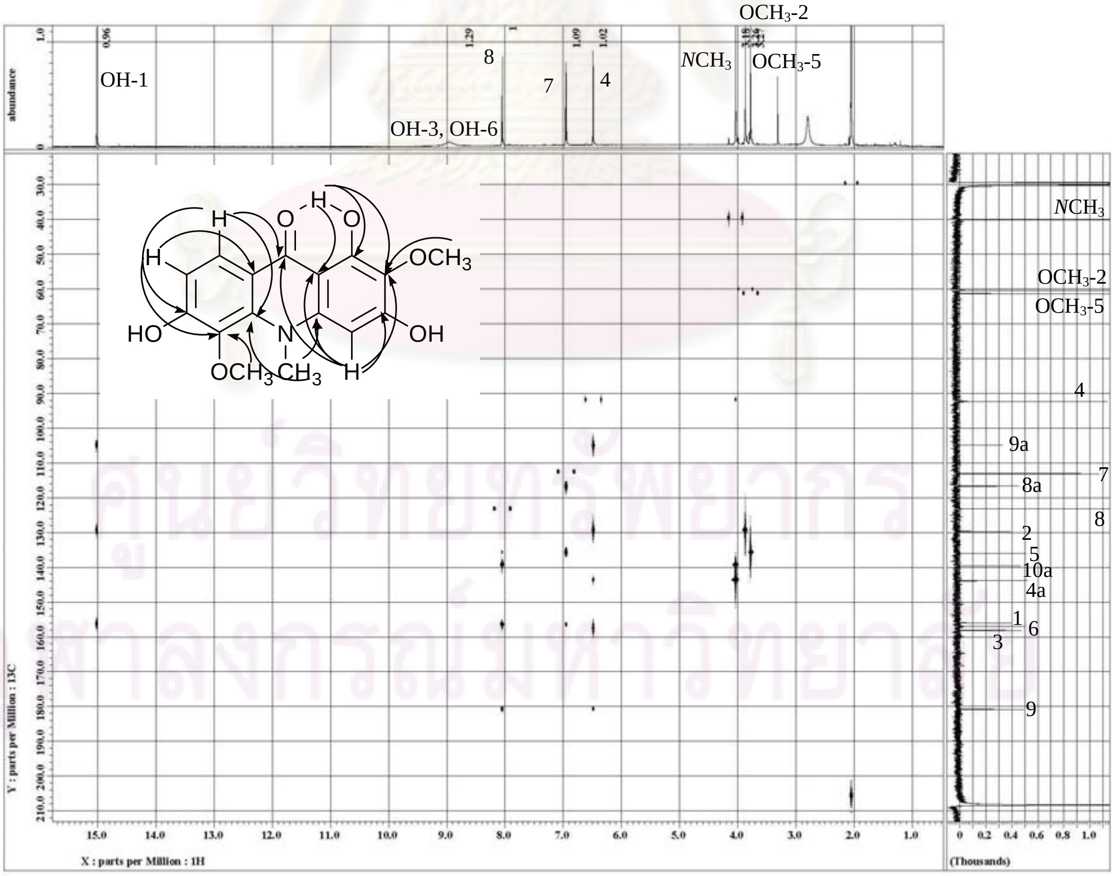


Figure 64 HMBC spectrum of compound **GP3** (acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

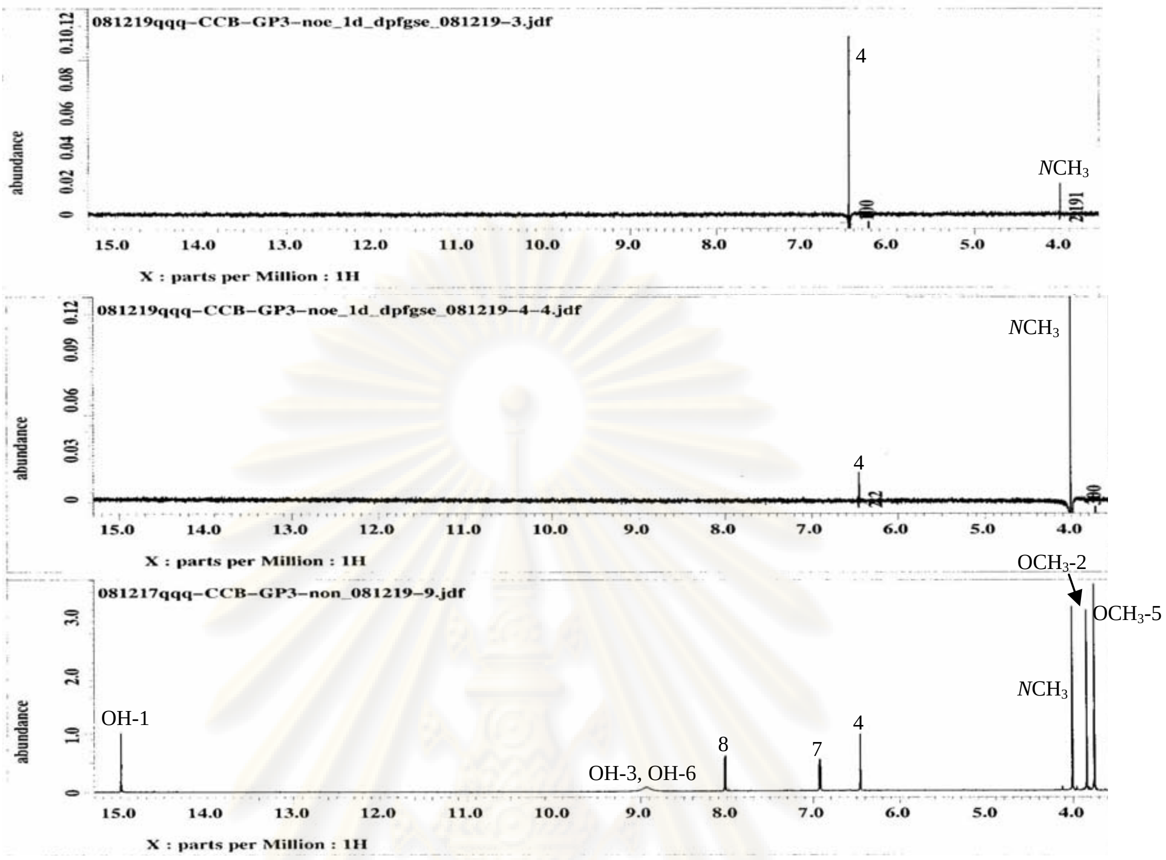


Figure 65 NOE experiments of compound **GP3** (acetone-d_6).

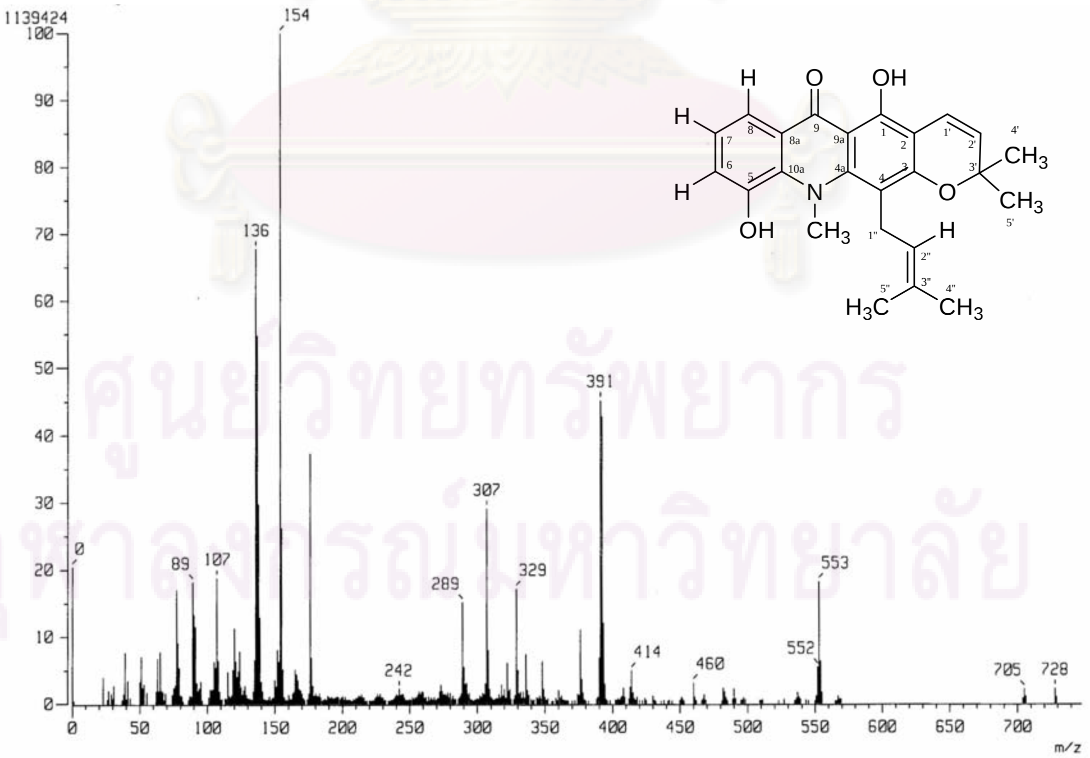


Figure 66 FABMS of compound **GP4**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

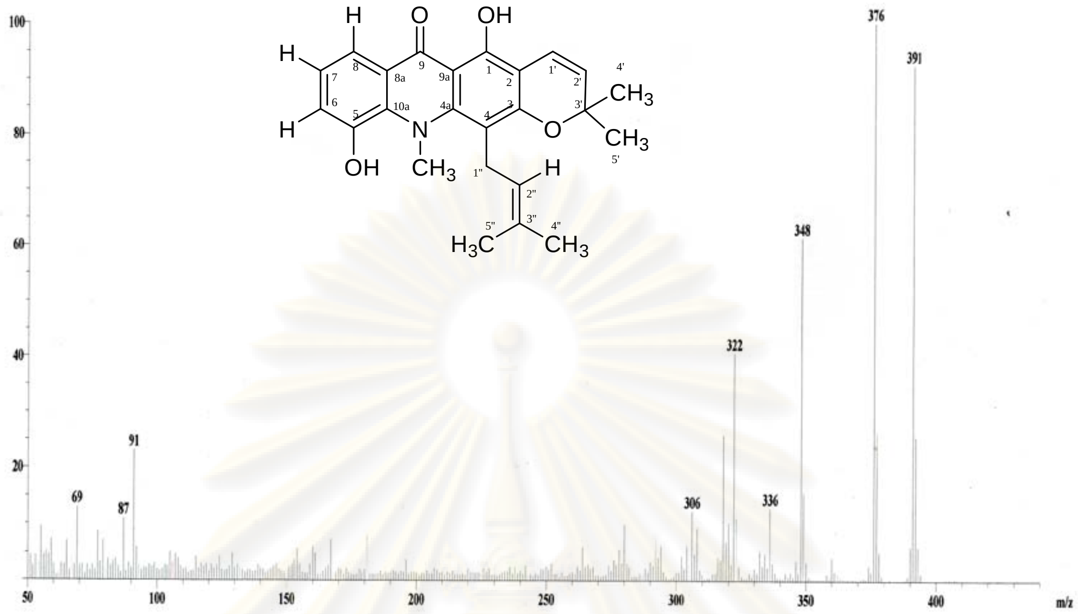


Figure 67 EIMS of compound **GP4**.

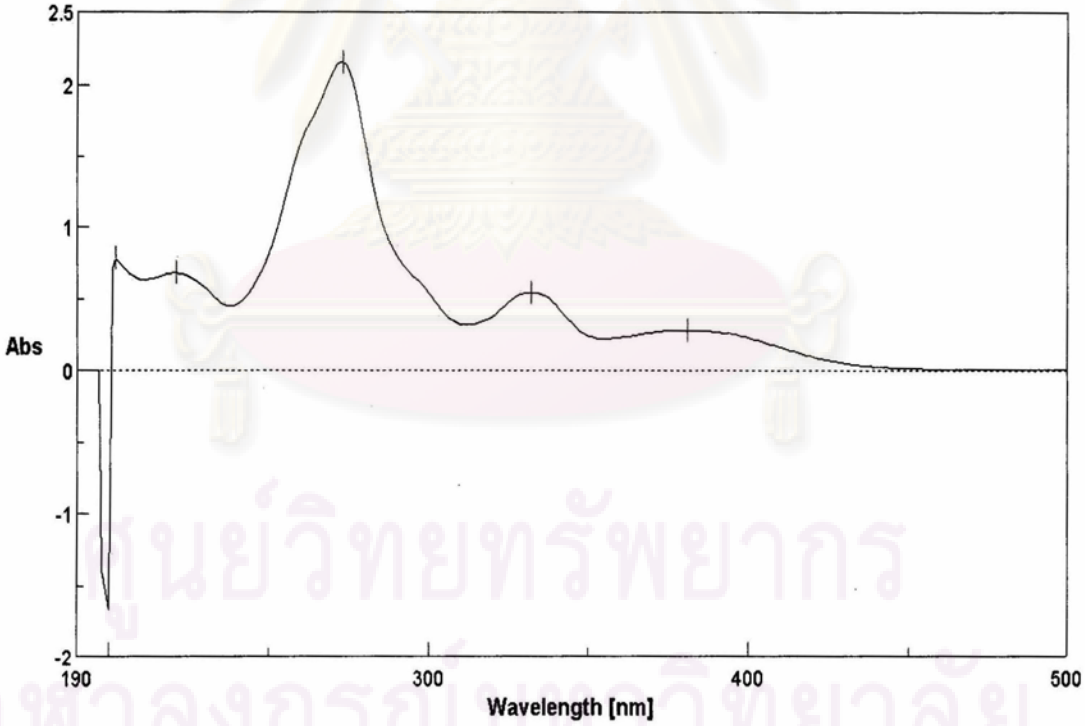


Figure 68 UV spectrum of compound **GP4** (MeOH).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

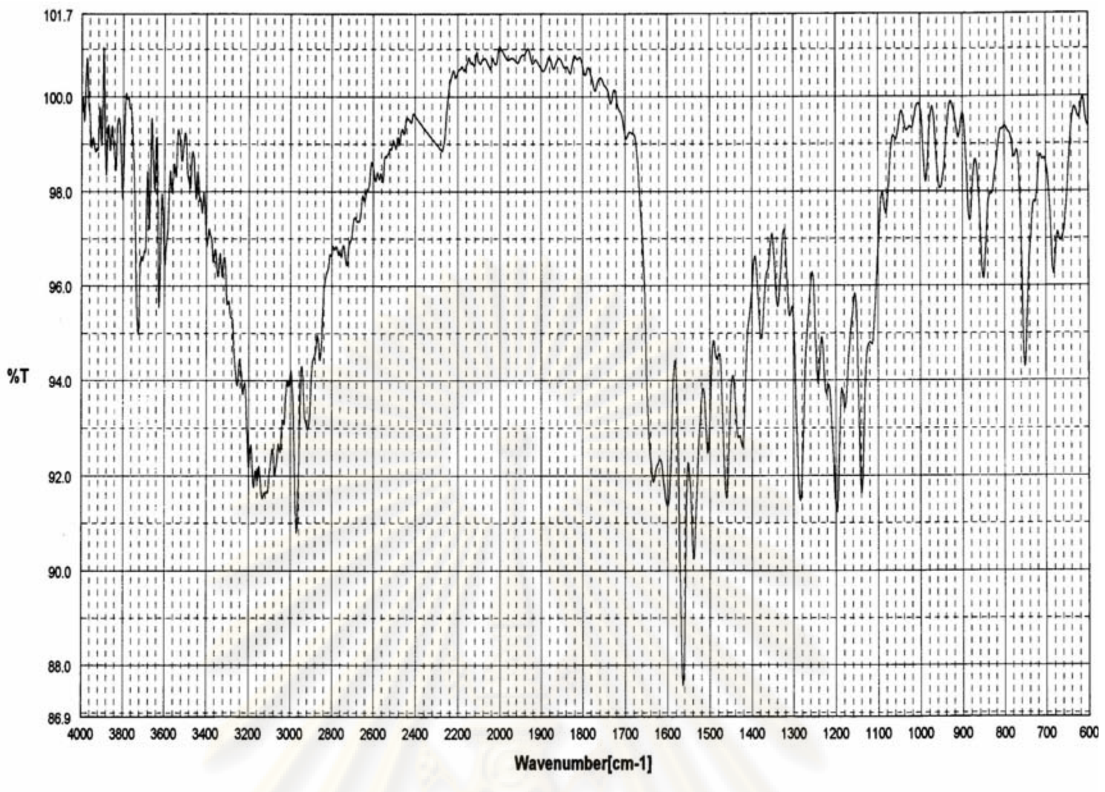


Figure 69 IR spectrum of compound **GP4** (ATR).

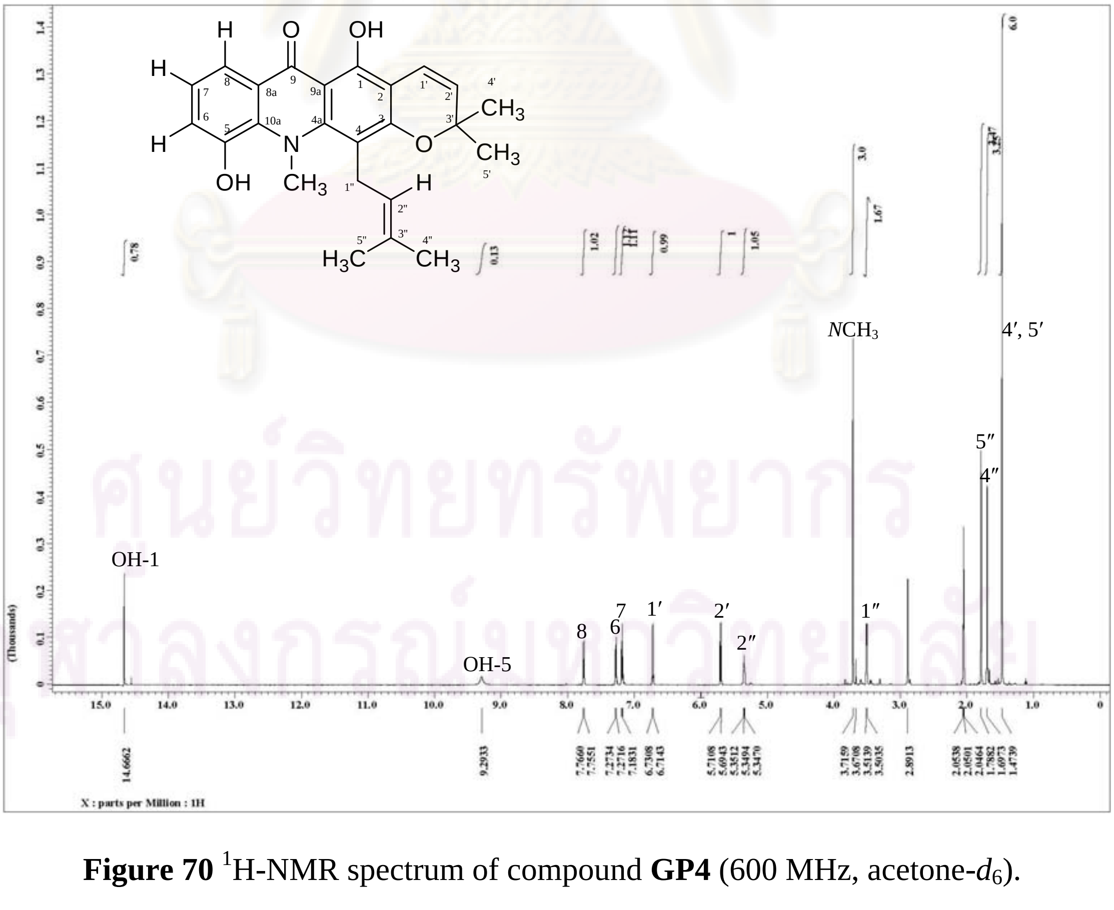


Figure 70 ^{1}H-NMR spectrum of compound **GP4** (600 MHz, acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

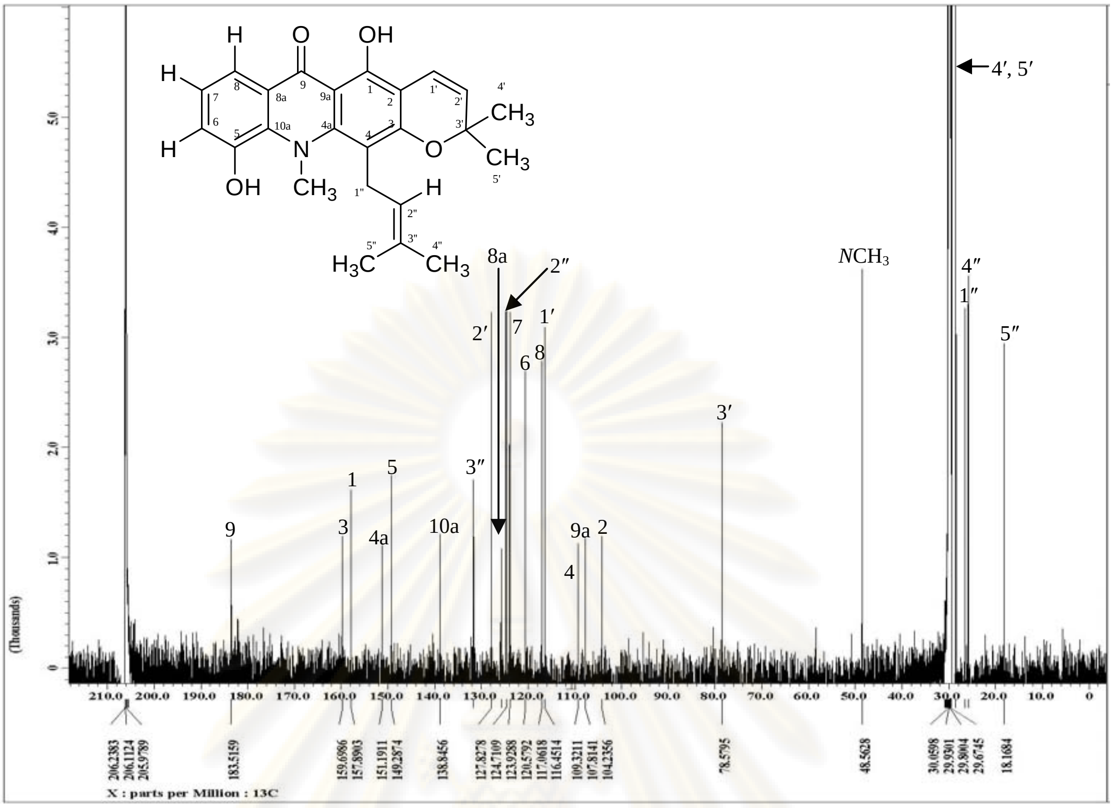


Figure 71 ^{13}C-NMR spectrum of compound **GP4** (150 MHz, acetone-d_6).

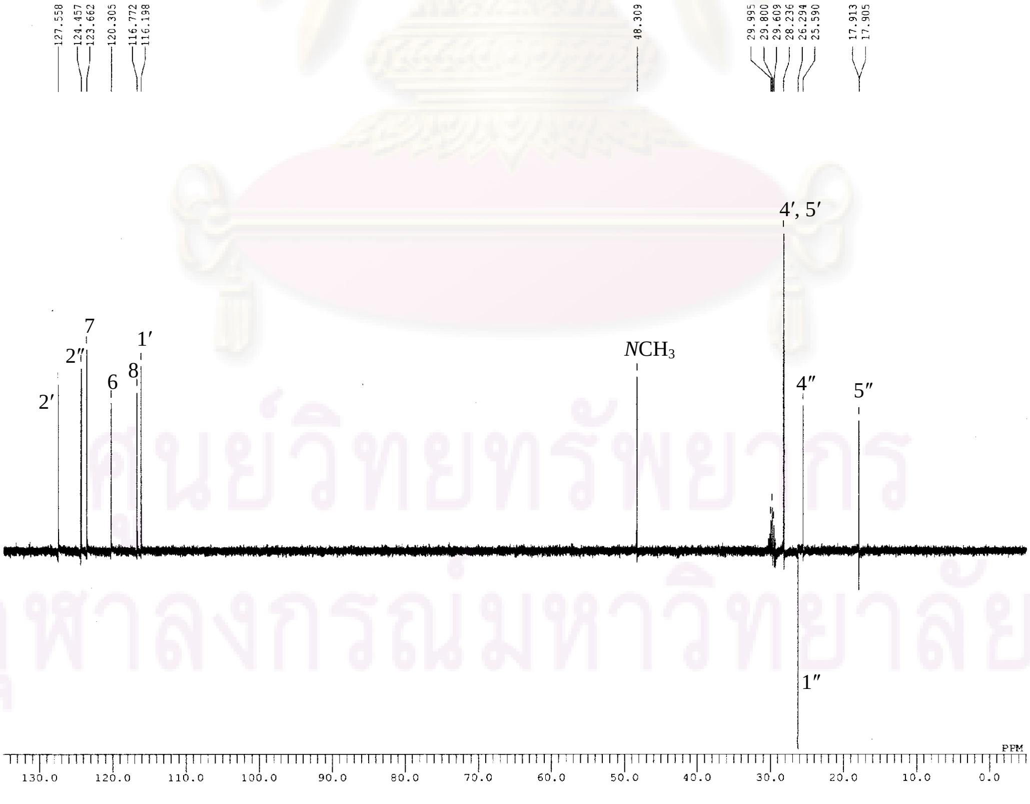


Figure 72 DEPT135 spectrum of compound **GP4** (100 MHz, acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

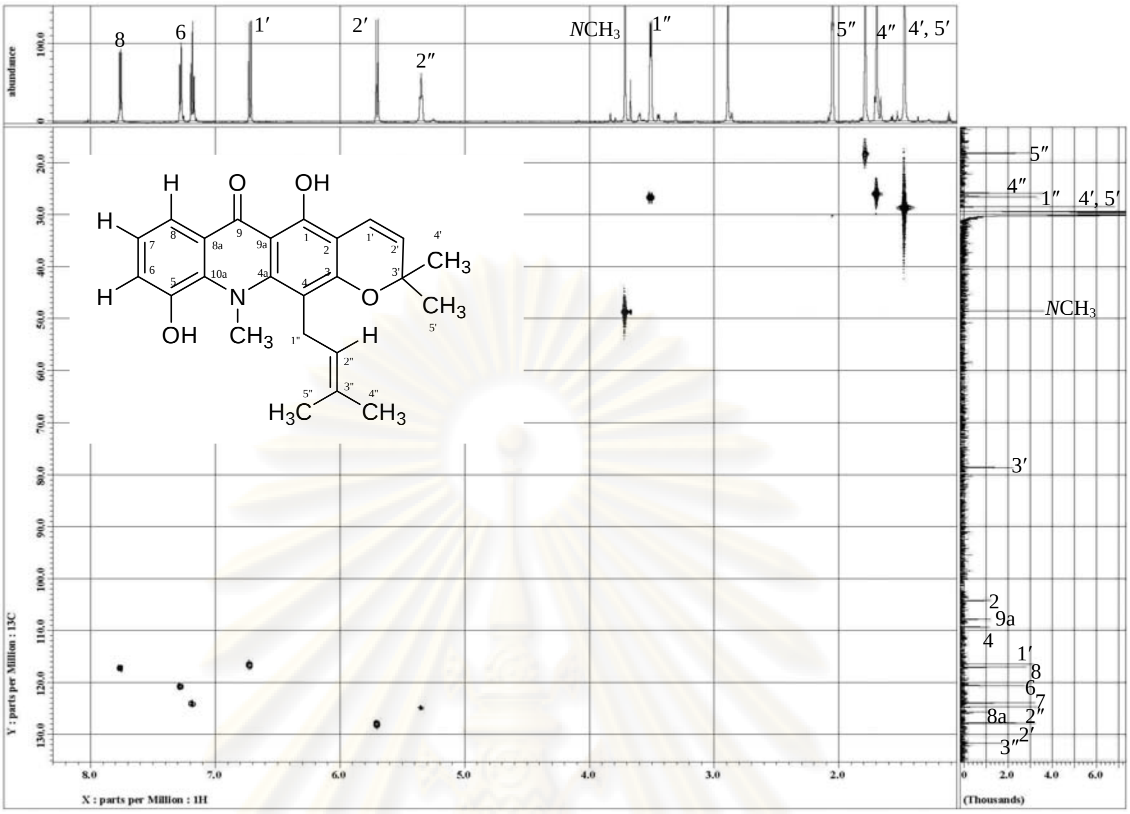


Figure 73 HMQC spectrum of compound **GP4** (acetone-d_6).

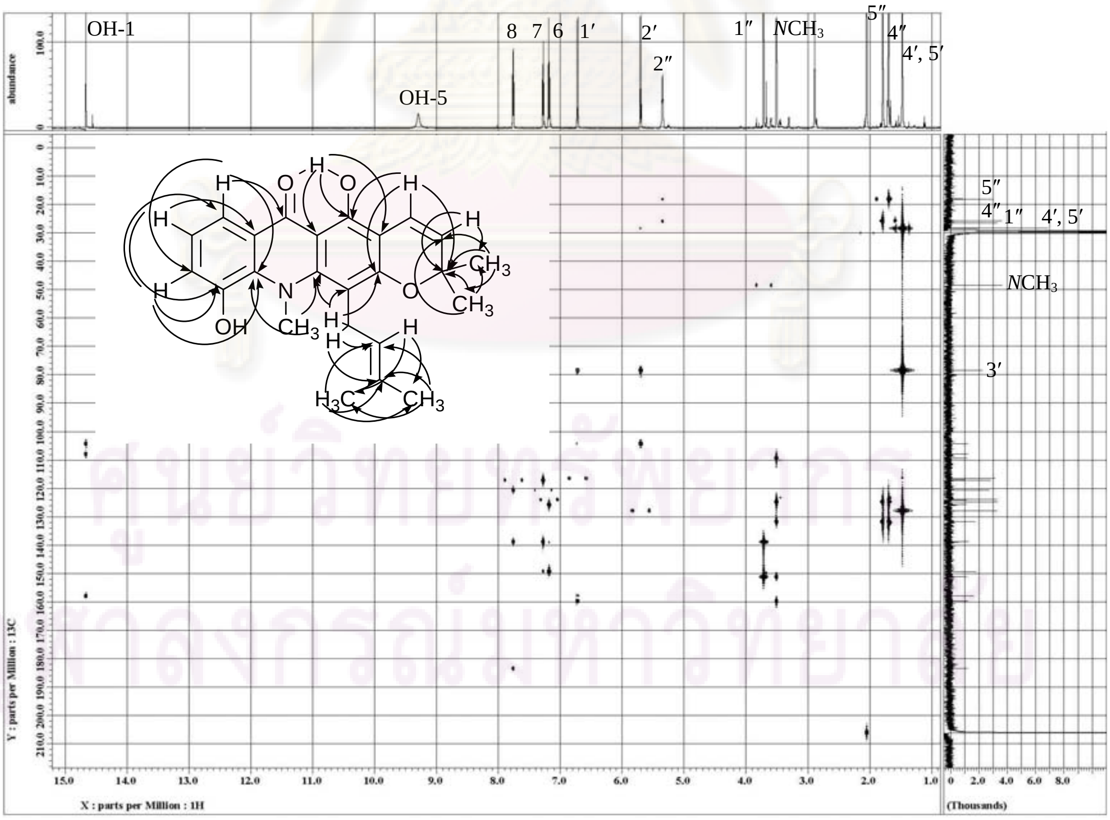


Figure 74 HMBC spectrum of compound **GP4** (acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

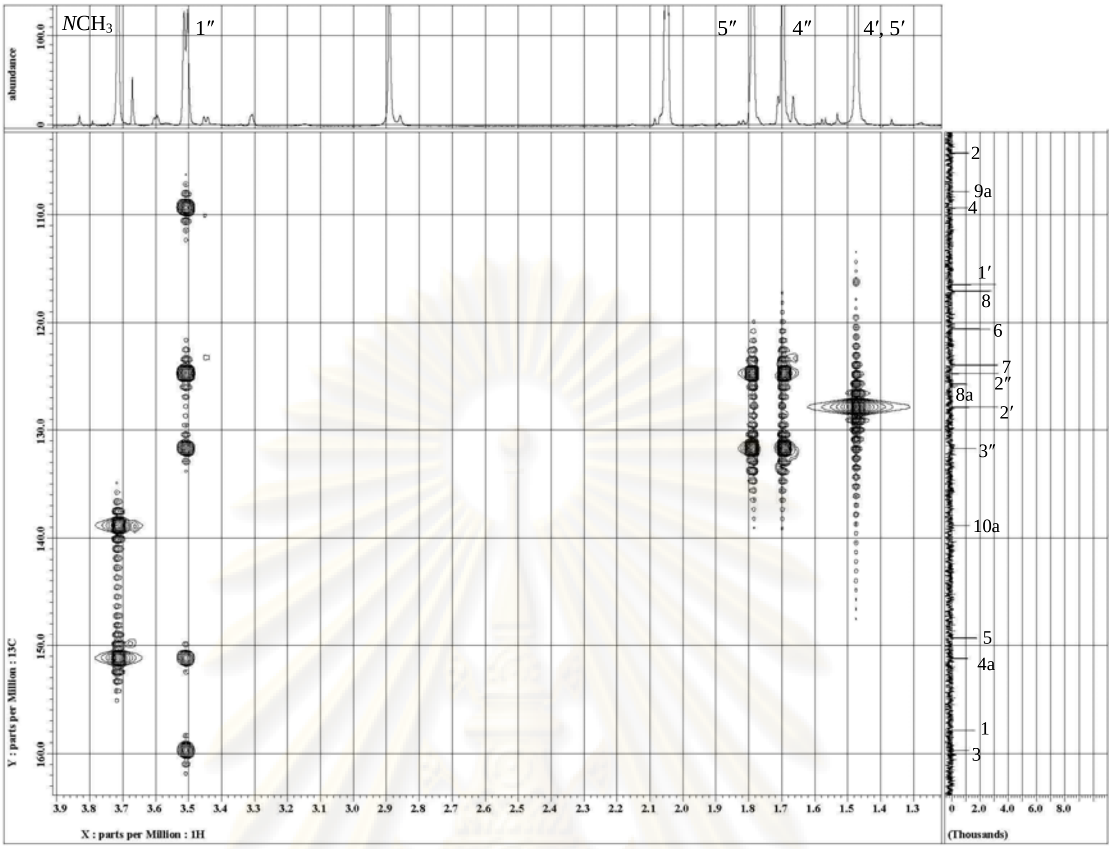


Figure 75 Expanded HMBC spectrum of compound **GP4** (acetone-d_6).

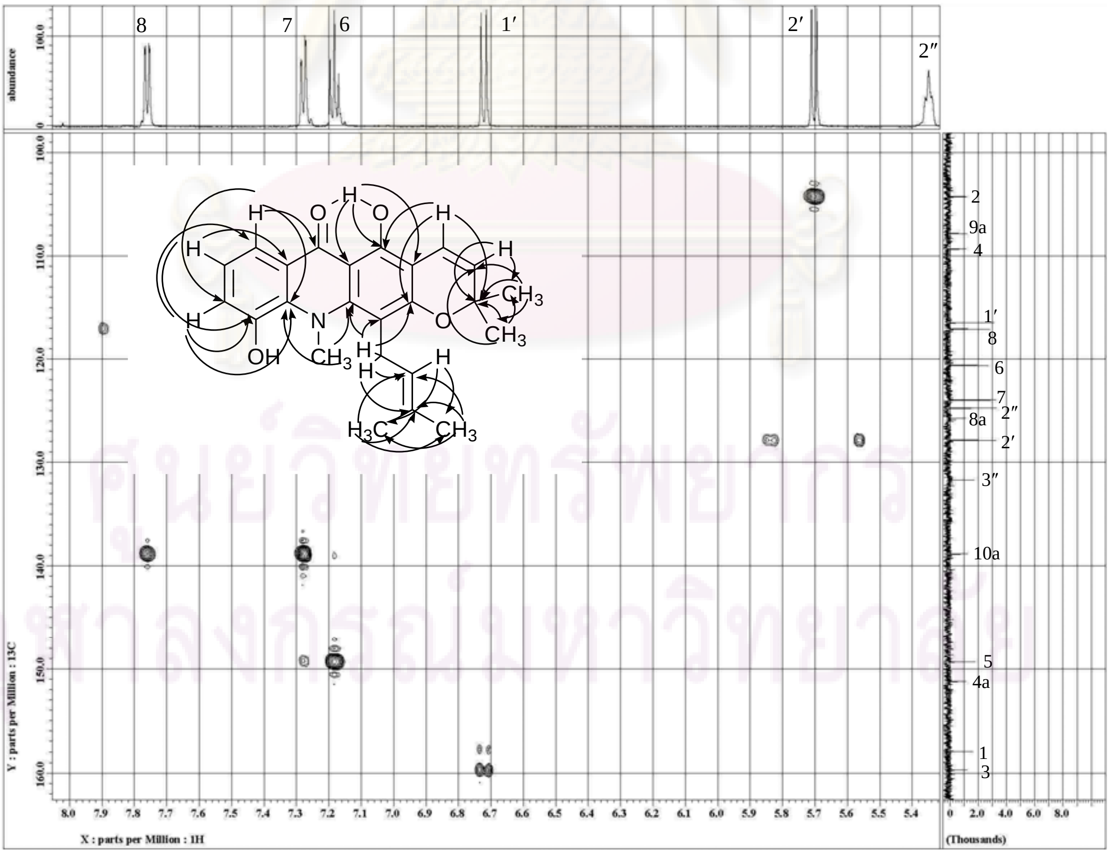


Figure 76 Expanded HMBC spectrum of compound **GP4** (acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

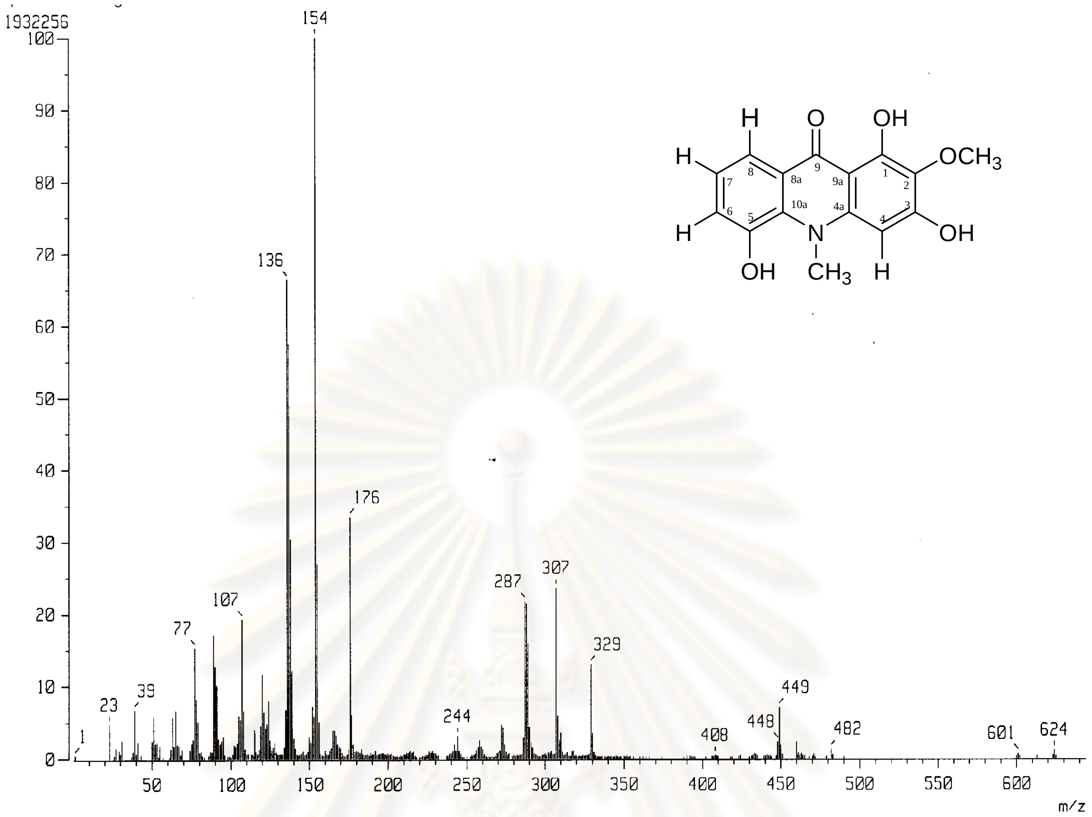


Figure 77 FABMS of compound **GP5**.

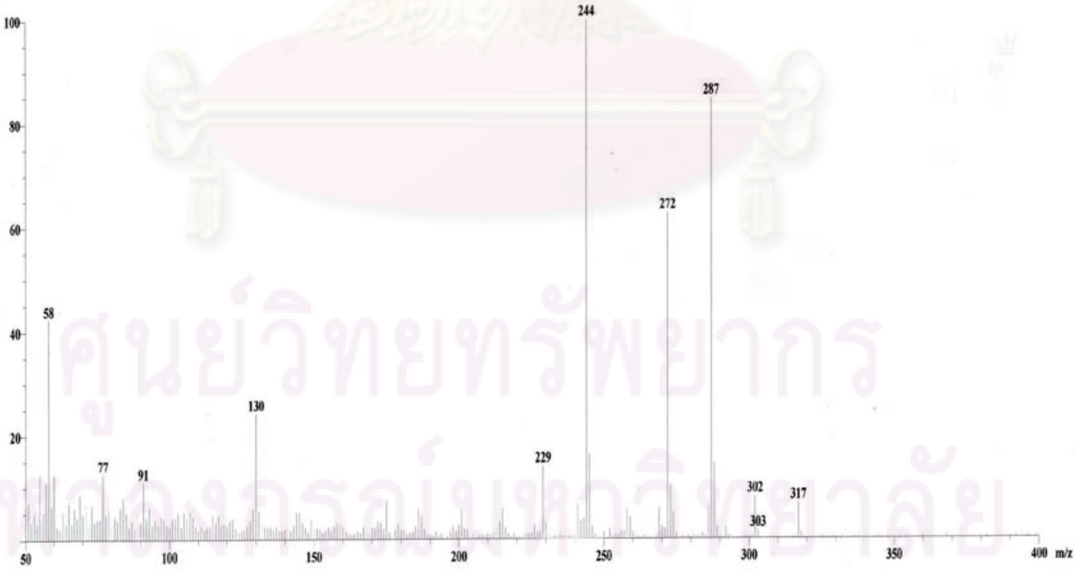


Figure 78 EIMS of compound **GP5**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

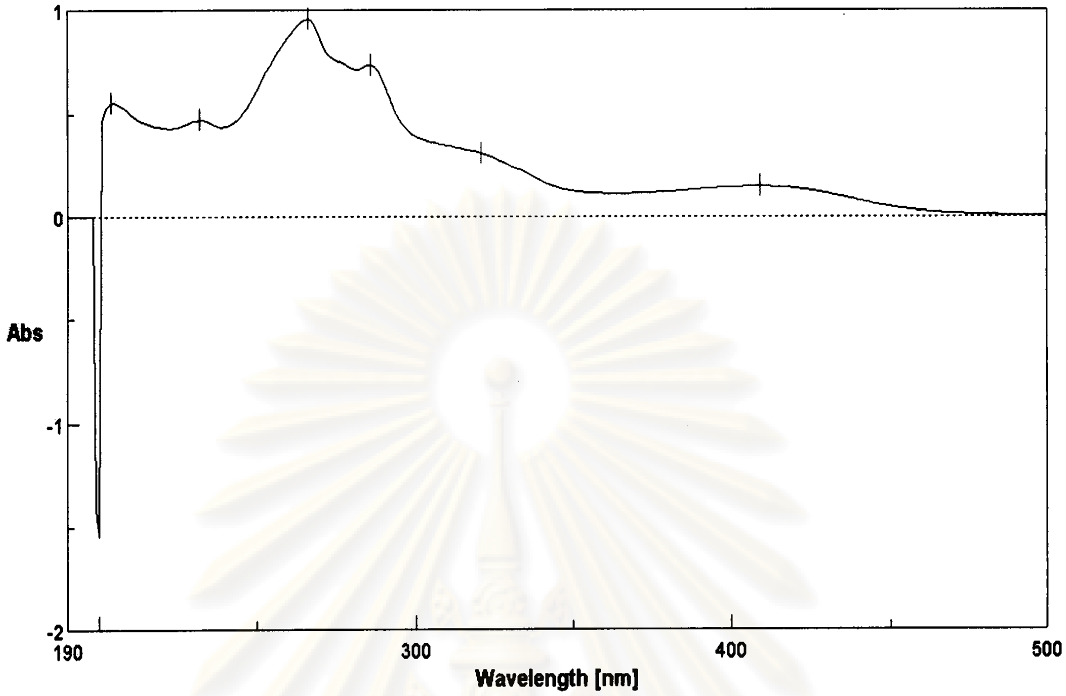


Figure 79 UV spectrum of compound **GP5** (MeOH).

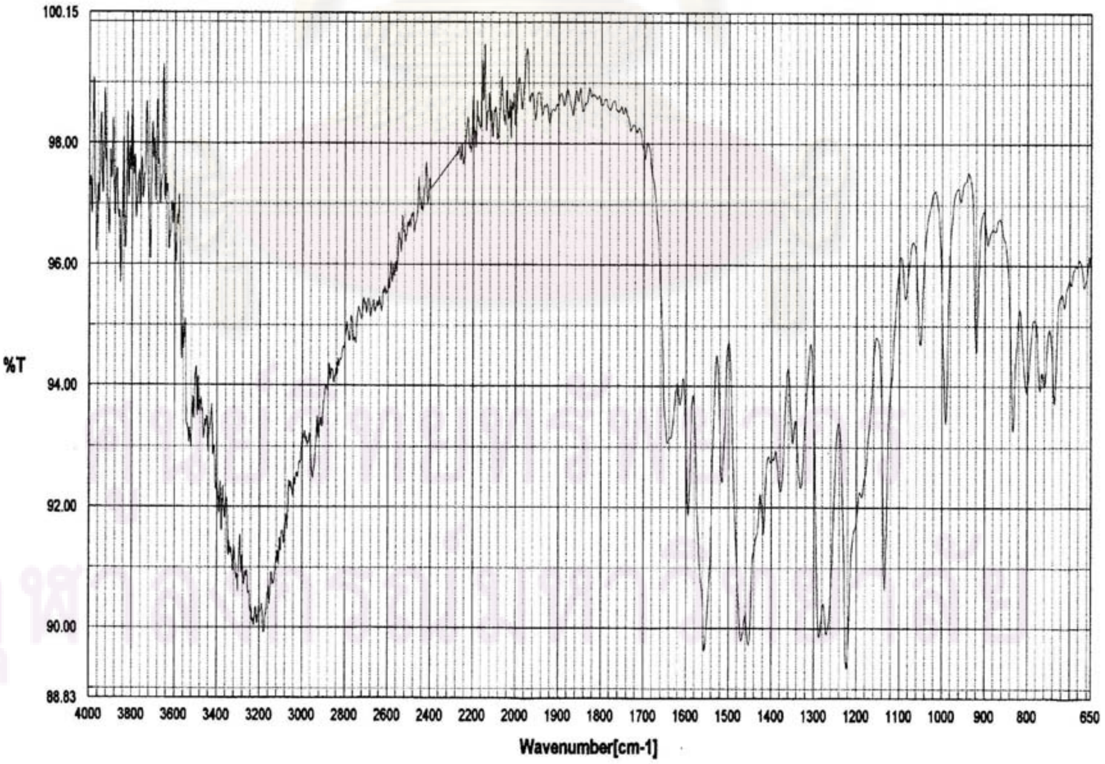


Figure 80 IR spectrum of compound **GP5** (ATR).

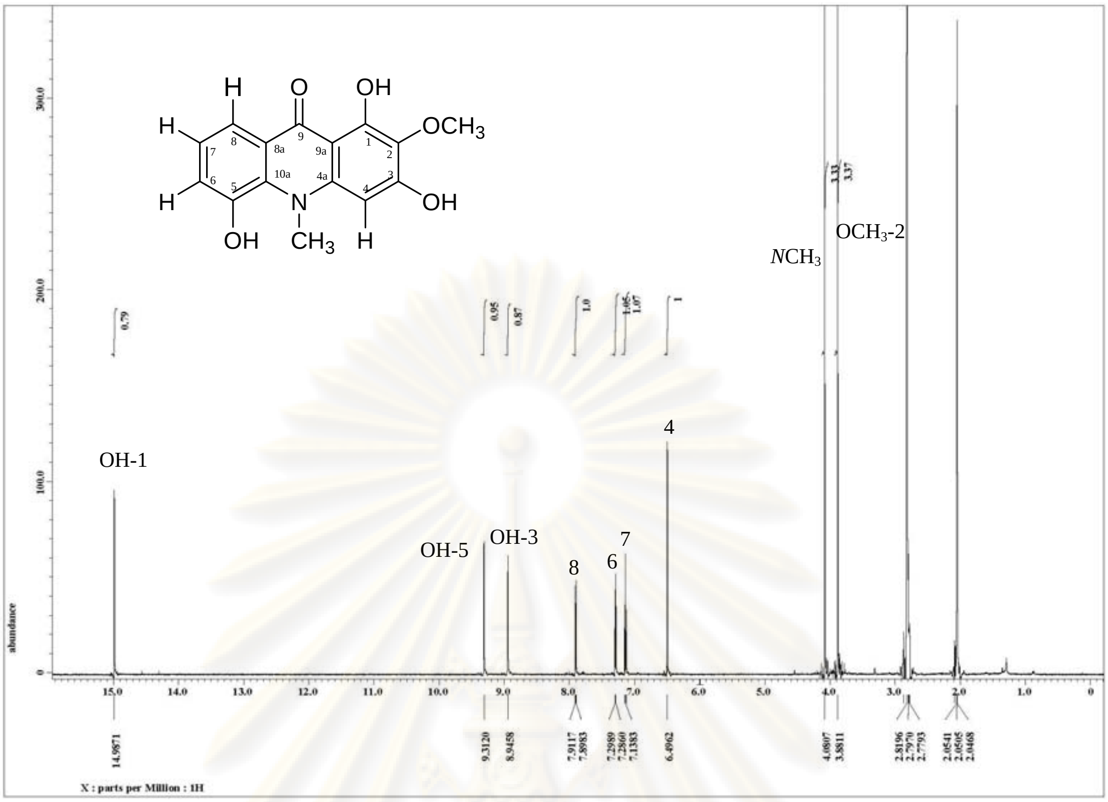


Figure 81 ^{1}H-NMR spectrum of compound **GP5** (600 MHz, acetone-d_6).

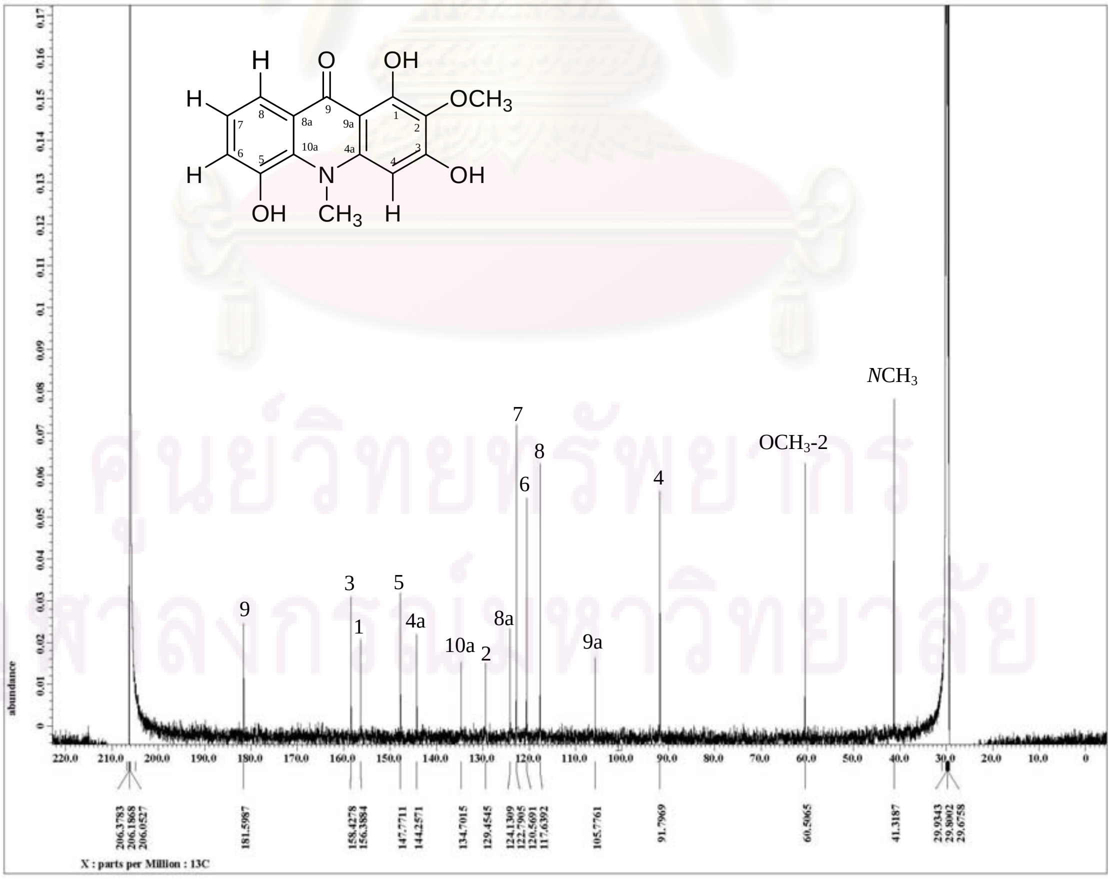


Figure 82 ^{13}C-NMR spectrum of compound **GP5** (150 MHz, acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

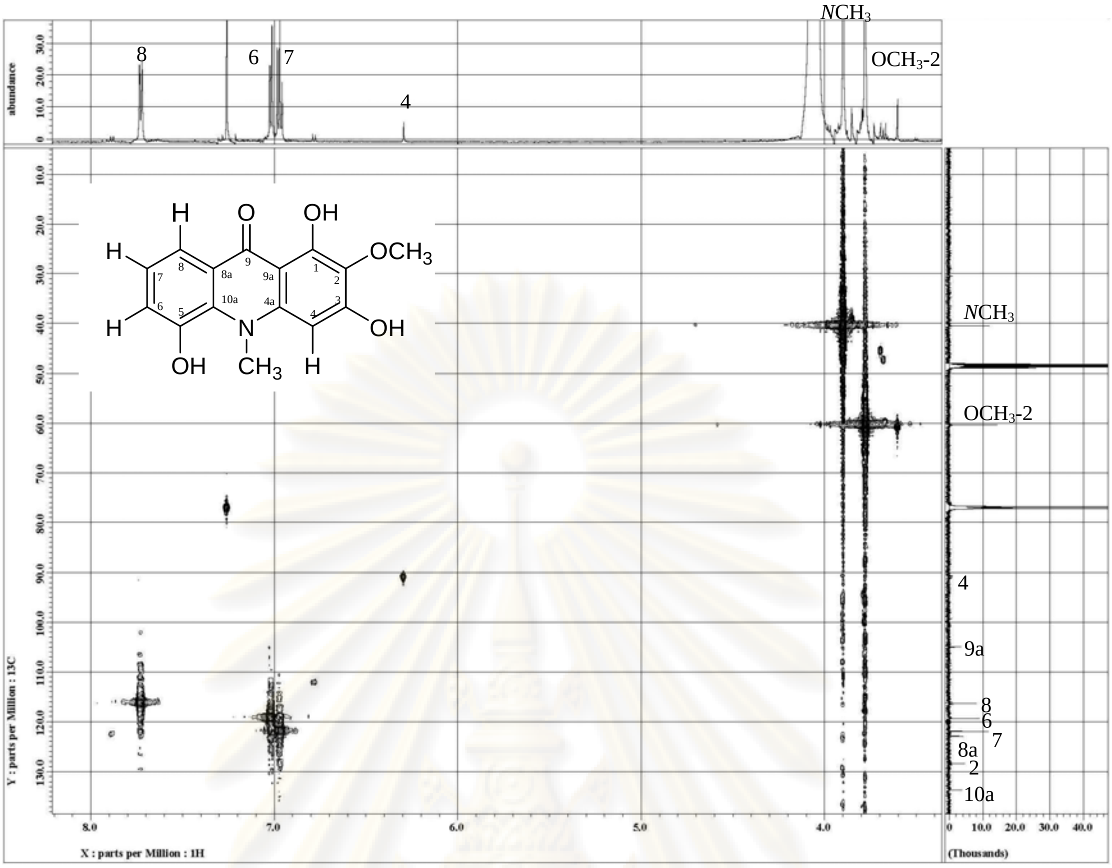


Figure 83 HMQC spectrum of compound **GP5** (CDCl$_3$+CD$_3$OD).

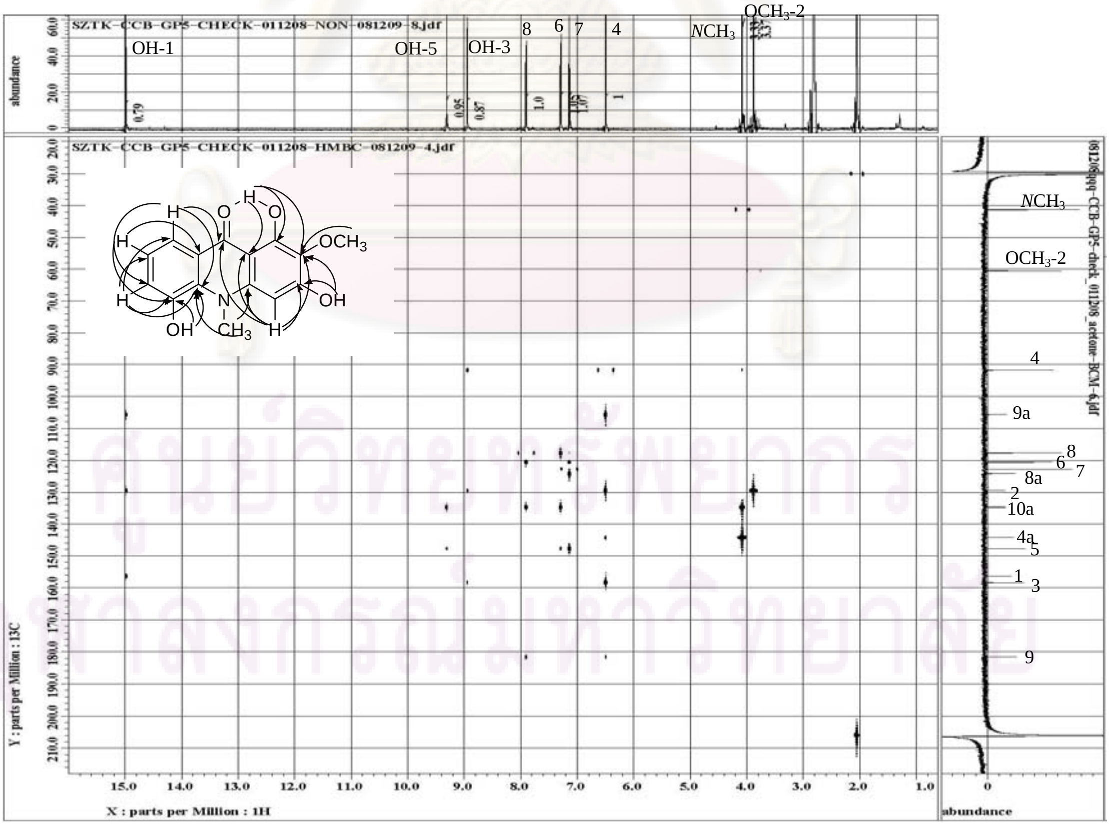


Figure 84 HMBC spectrum of compound **GP5** (acetone-*d*$_6$).

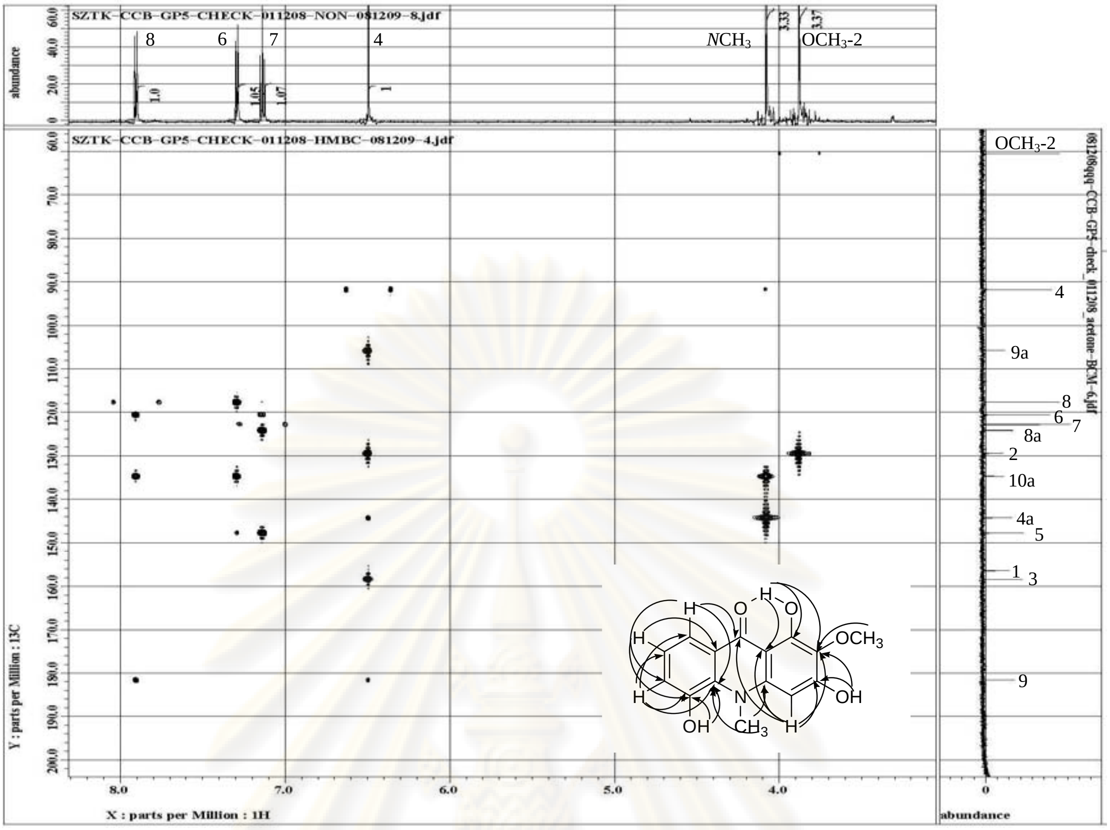


Figure 85 Expanded HMBC spectrum of compound **GP5** (acetone-d_6).

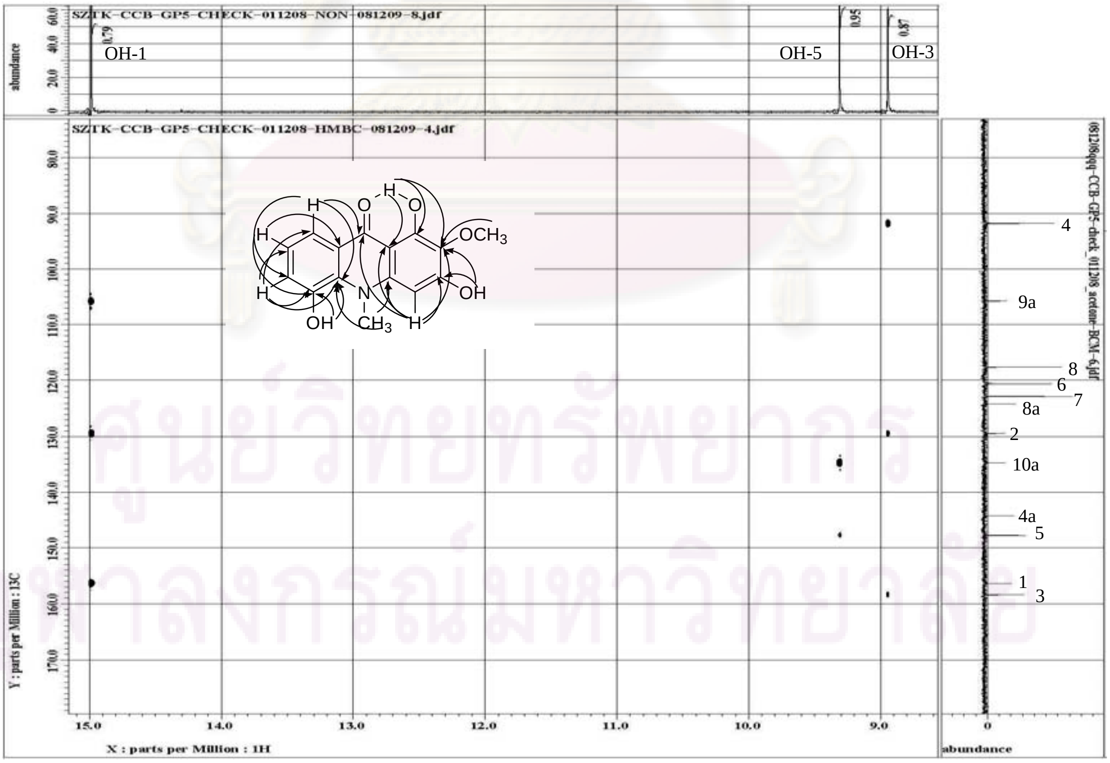


Figure 86 Expanded HMBC spectrum of compound **GP5** (acetone-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

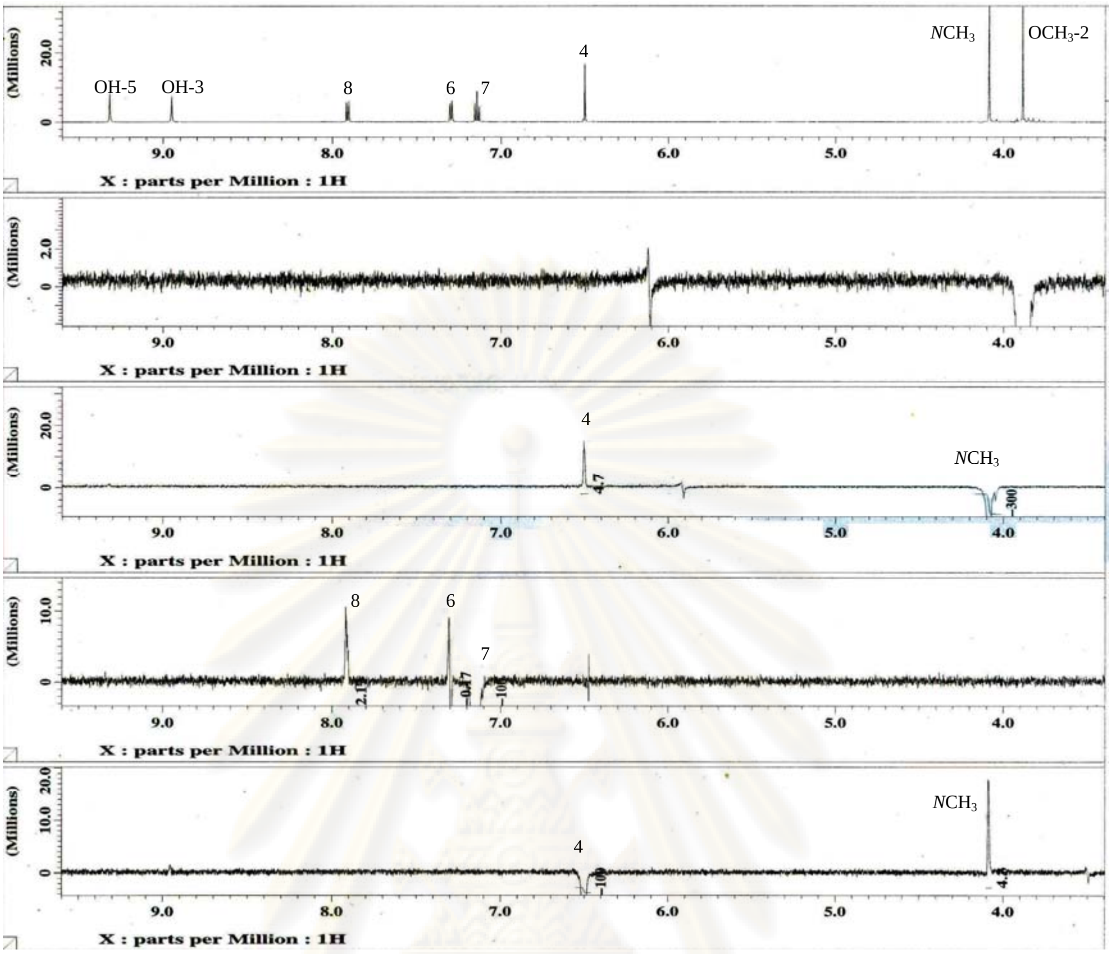


Figure 87 NOE experiments of compound **GP5** (acetone-d_6).

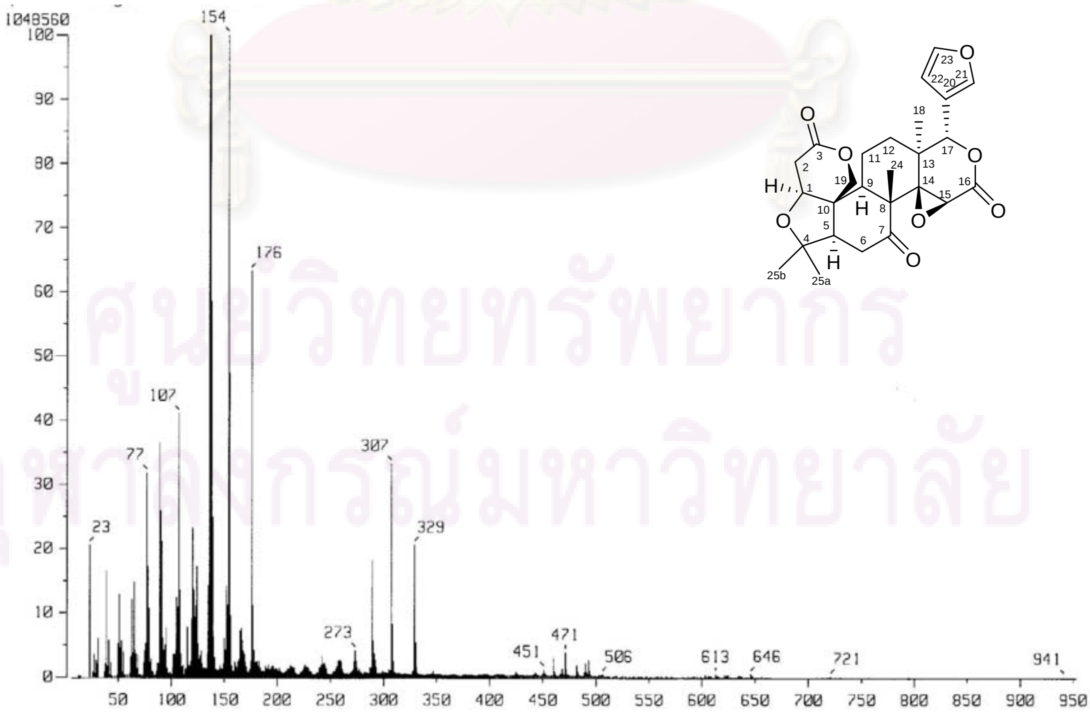


Figure 88 FABMS of compound **GP6**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

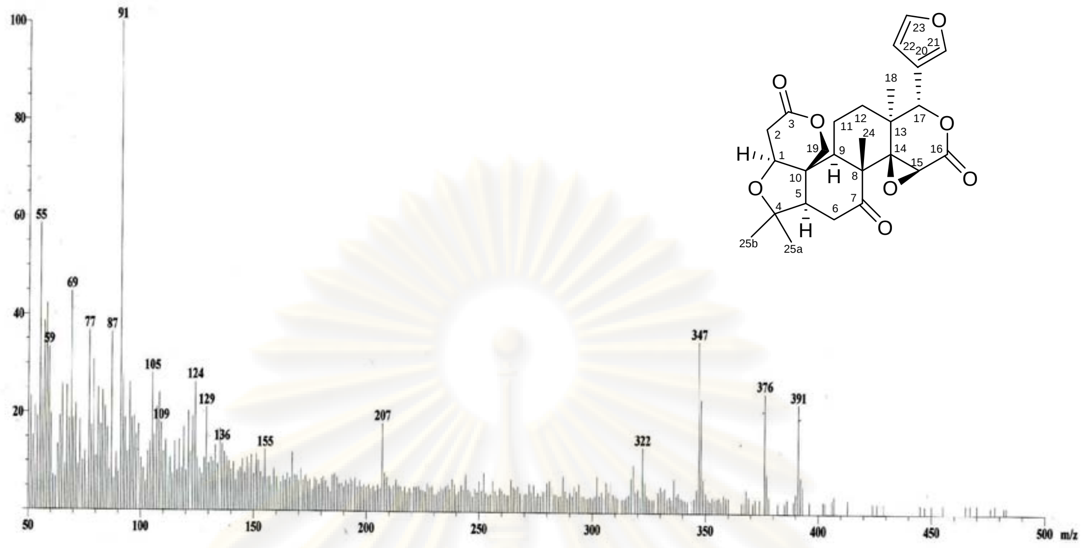


Figure 89 EIMS of compound **GP6**.

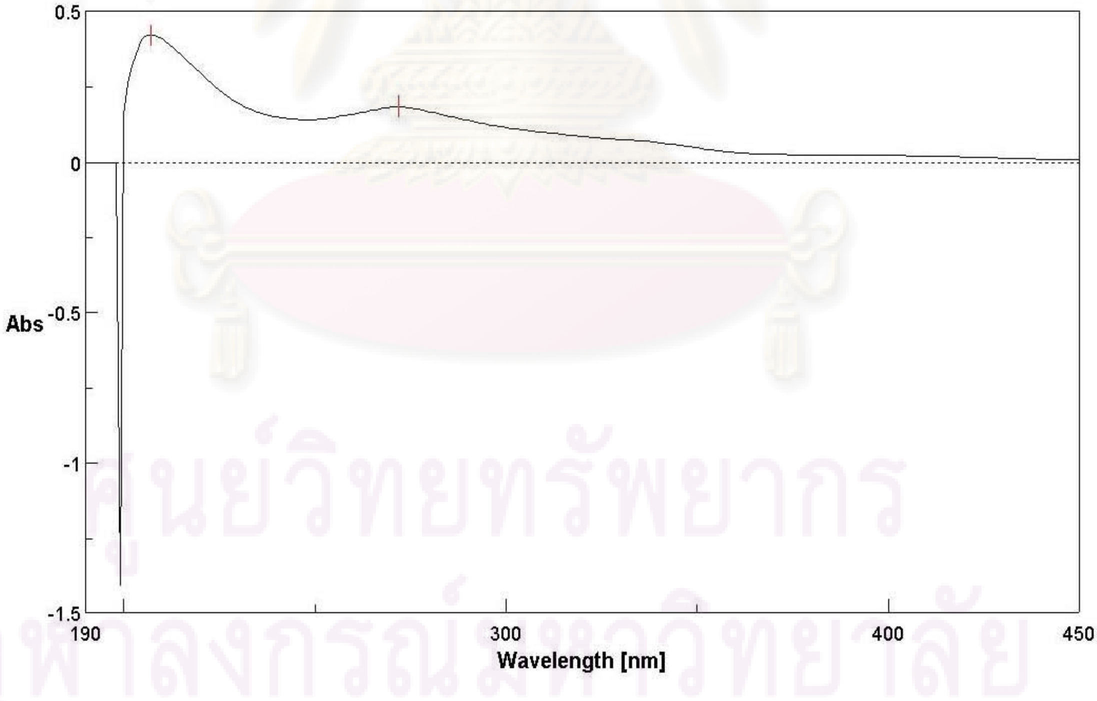


Figure 90 UV spectrum of compound **GP6** (MeOH).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

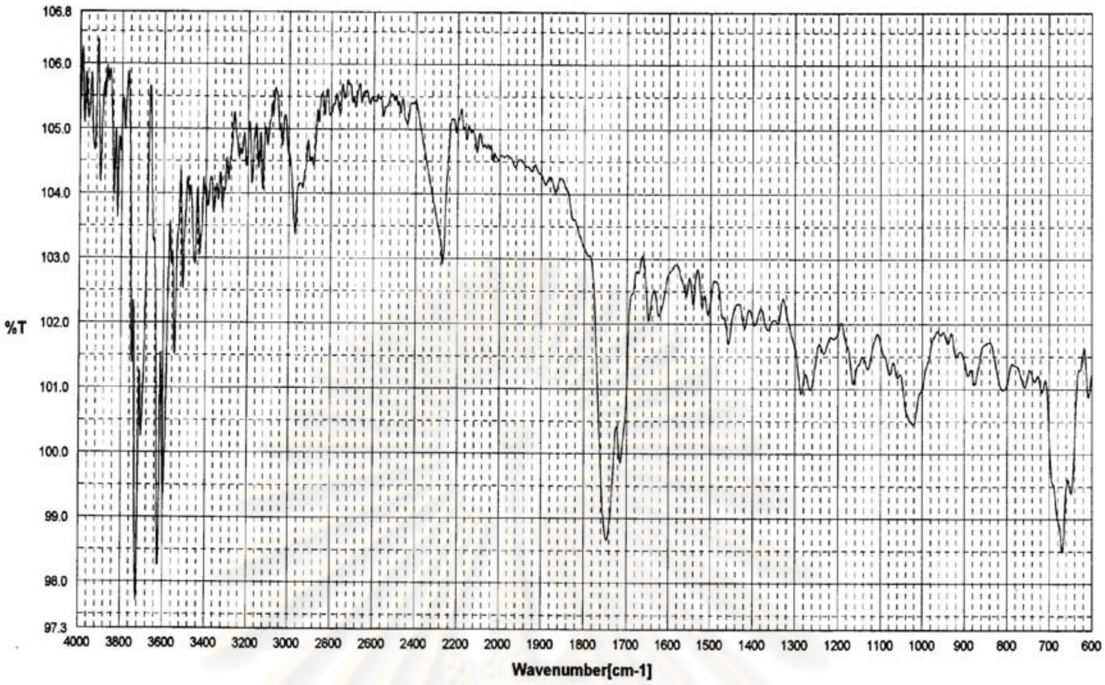


Figure 91 IR spectrum of compound **GP6** (ATR).

(The peak at 2280 cm-^{1} is an artifact produced by the process of subtraction the absorption owing to the CO_2.)

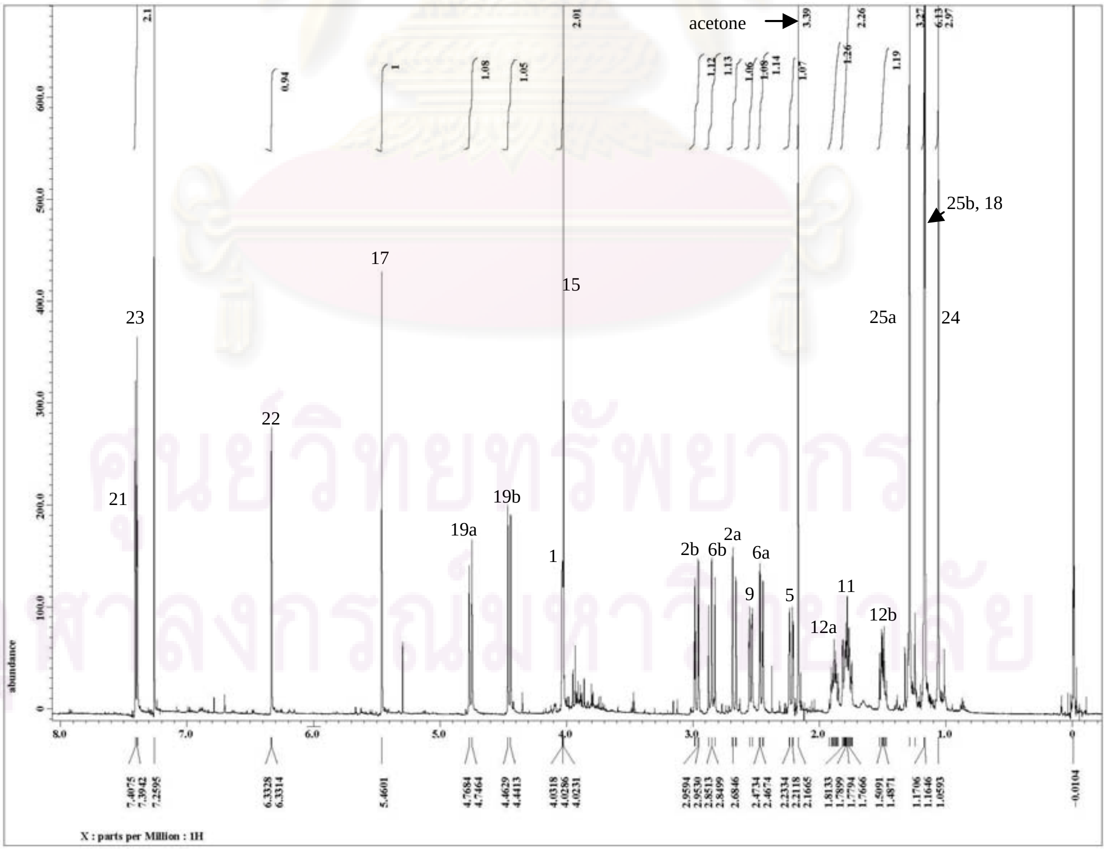


Figure 92 ^{1}H-NMR spectrum of compound **GP6** (600 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

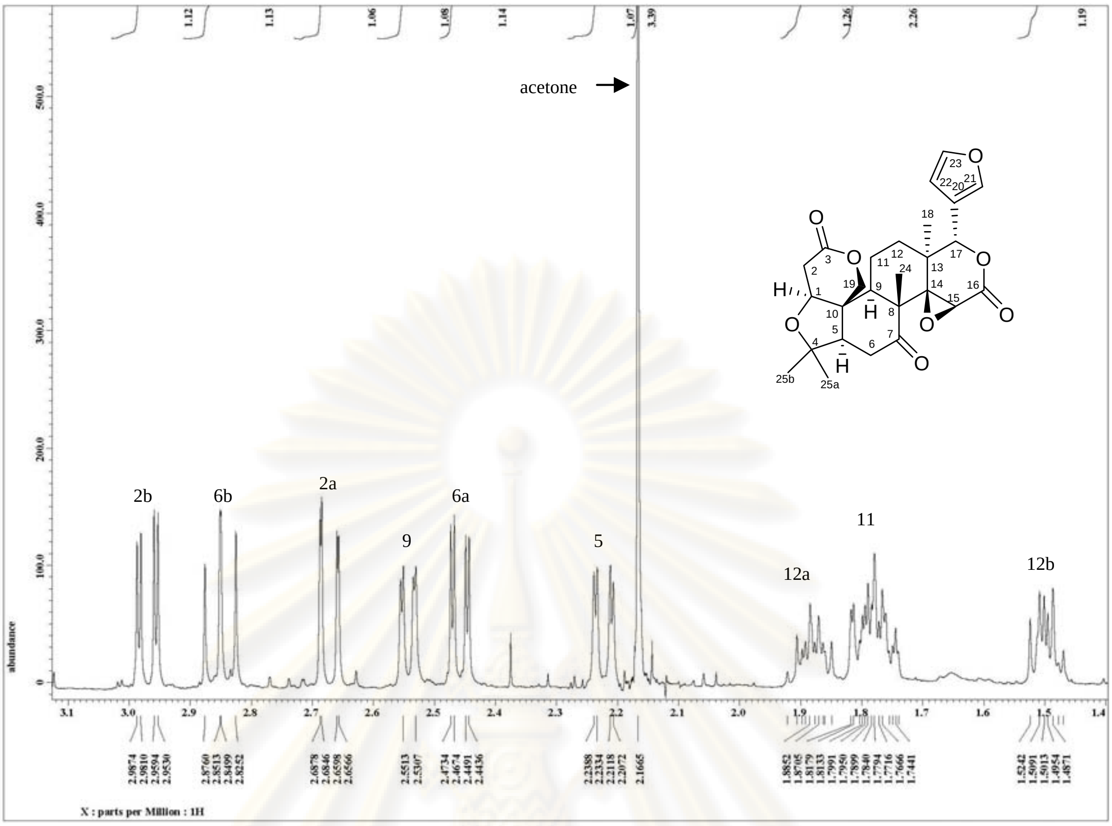


Figure 93 Expanded ^{1}H-NMR spectrum of compound **GP6** (600 MHz, $CDCl_3$).

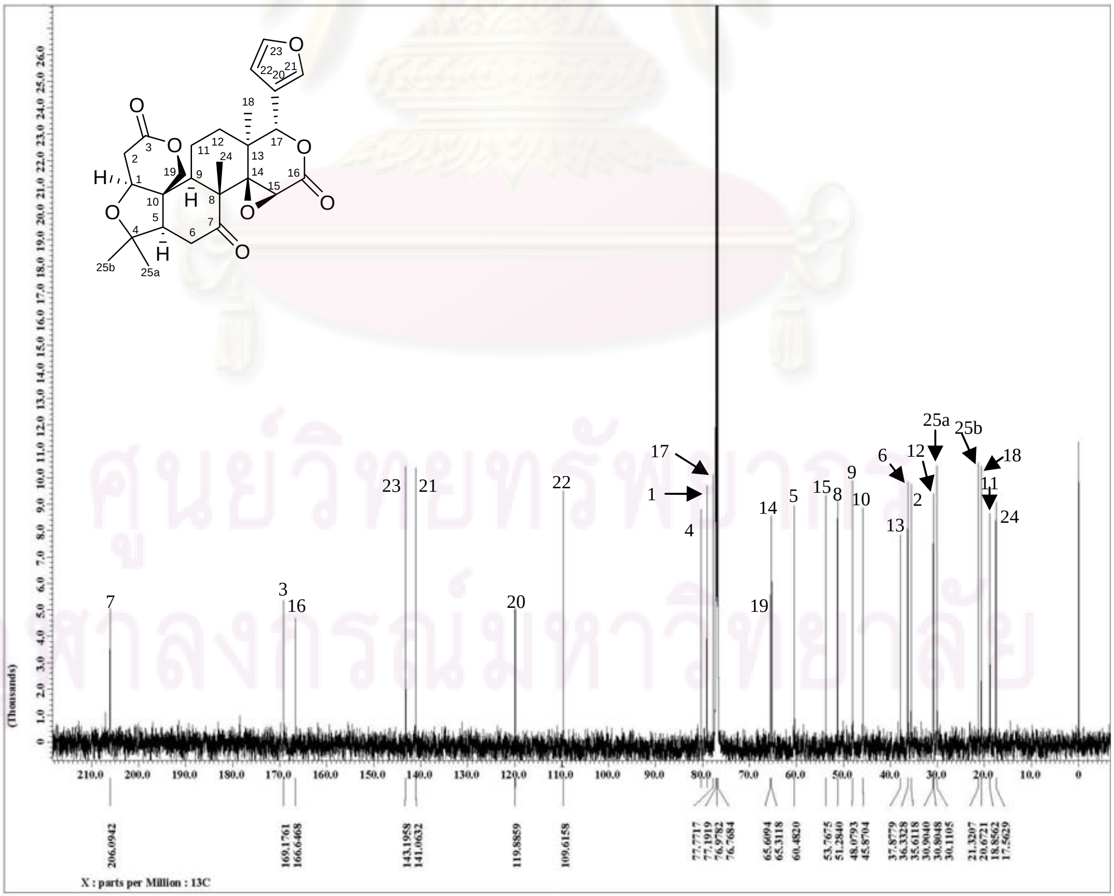


Figure 94 ^{13}C-NMR spectrum of compound **GP6** (150 MHz, $CDCl_3$).

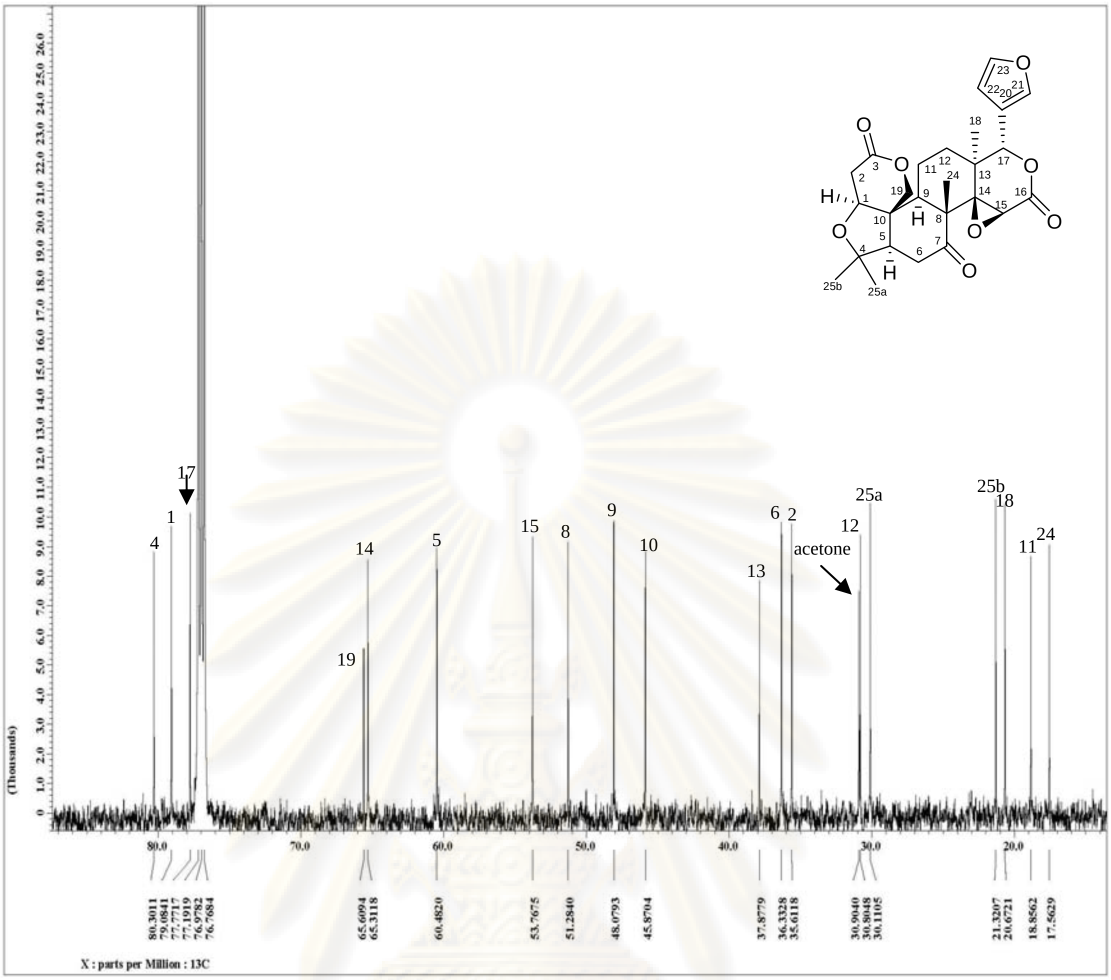


Figure 95 Expanded ^{13}C-NMR spectrum of compound **GP6** (150 MHz, $CDCl_3$).

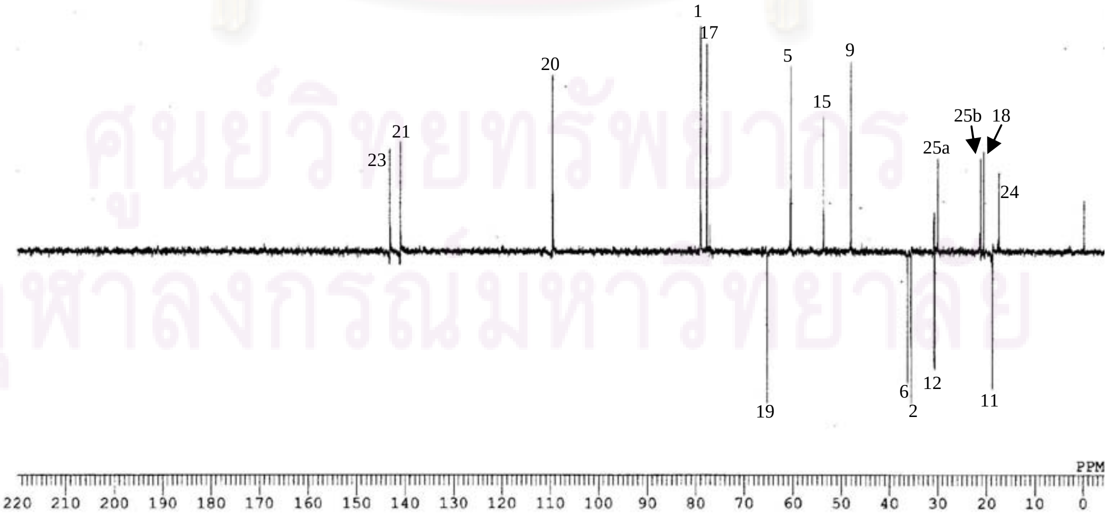


Figure 96 DEPT135 spectrum of compound **GP6** (100 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

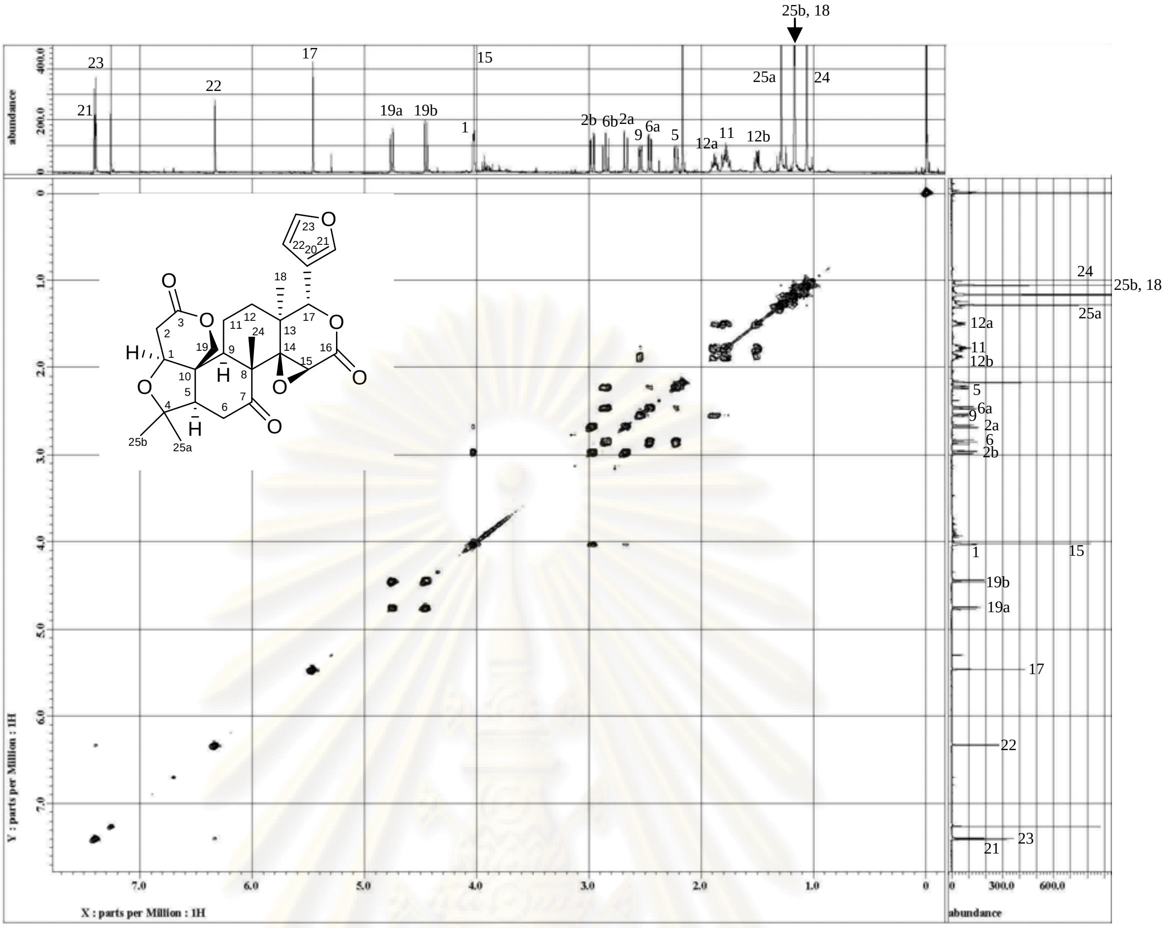


Figure 97 ^{1}H-^{1}H COSY spectrum of compound **GP6** (CDCl$_3$).

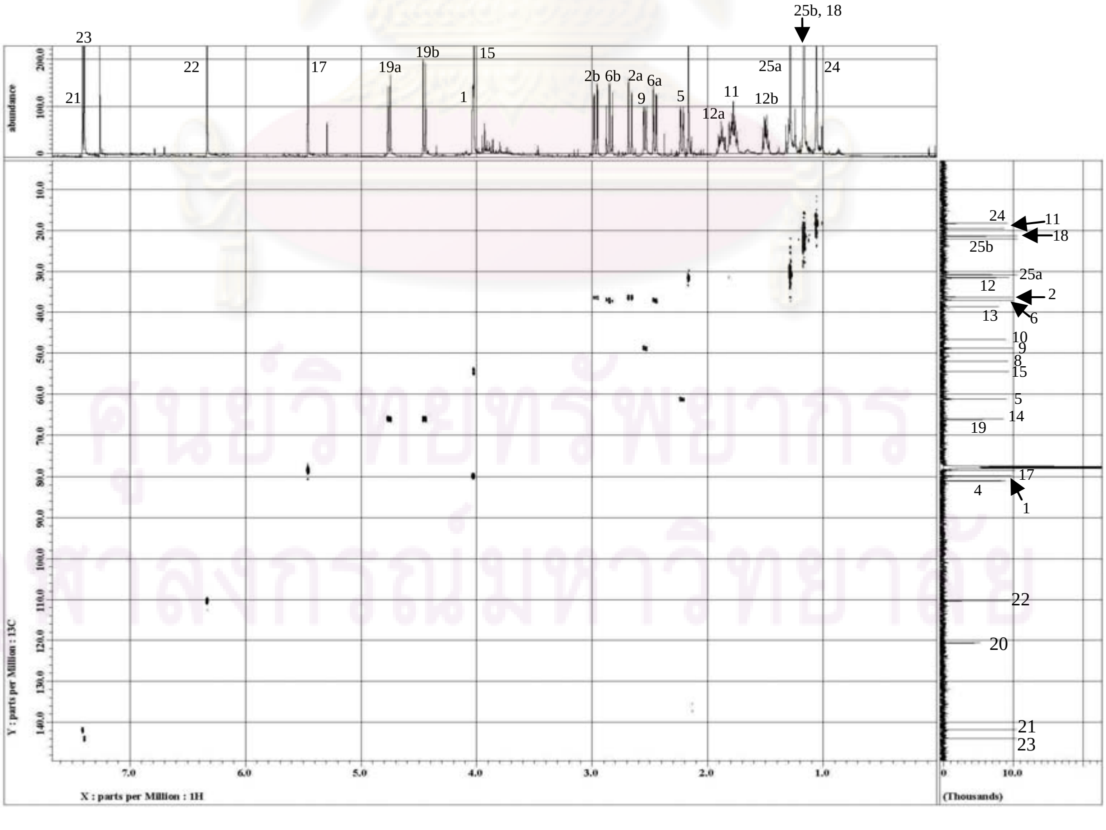


Figure 98 HMQC spectrum of compound **GP6** (CDCl$_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

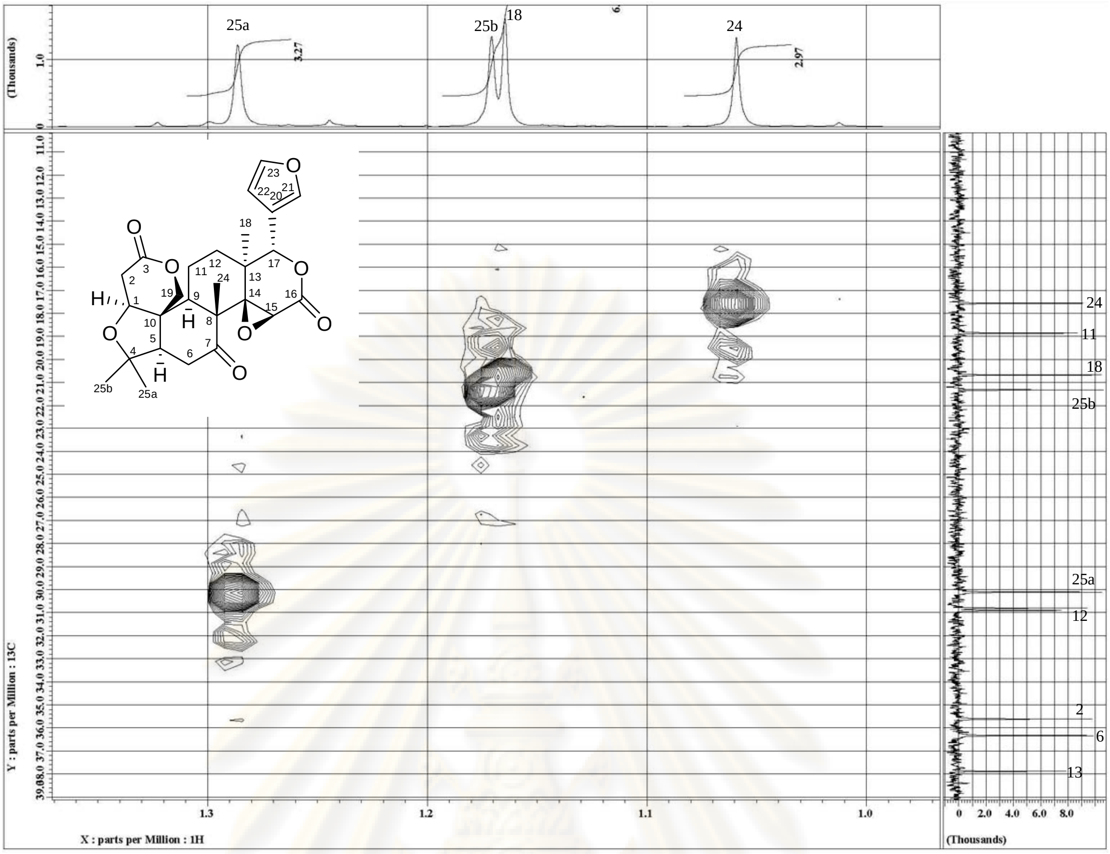


Figure 99 Expanded HMQC spectrum of compound **GP6** ($CDCl_3$).

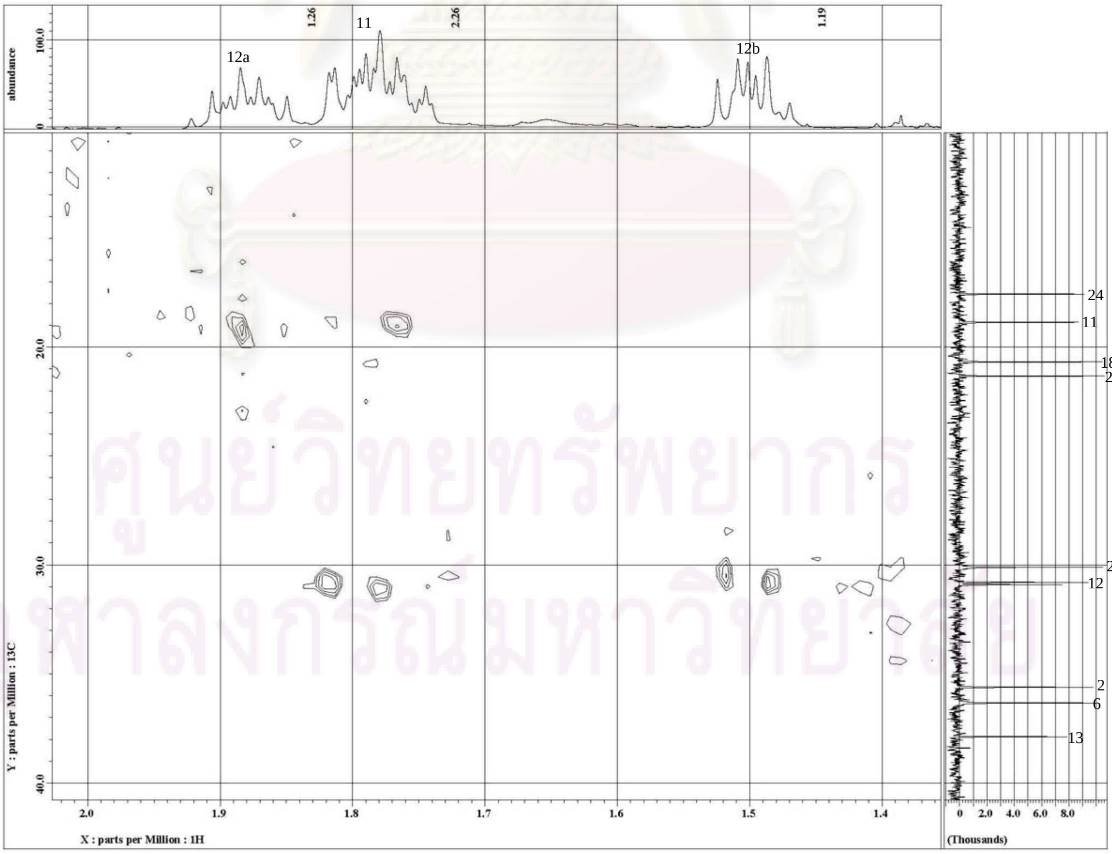


Figure 100 Expanded HMQC spectrum of compound **GP6** ($CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

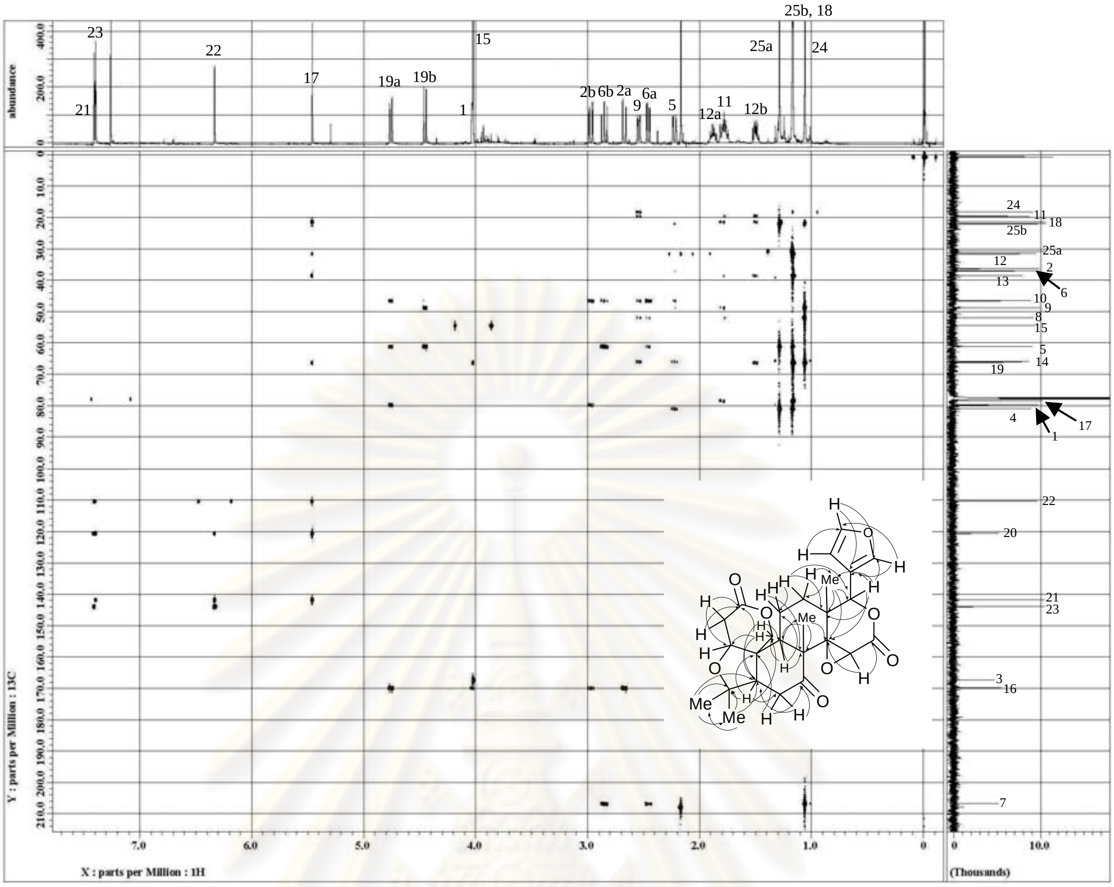


Figure 101 Expanded HMBC spectrum of compound **GP6** $(CDCl_3)$.

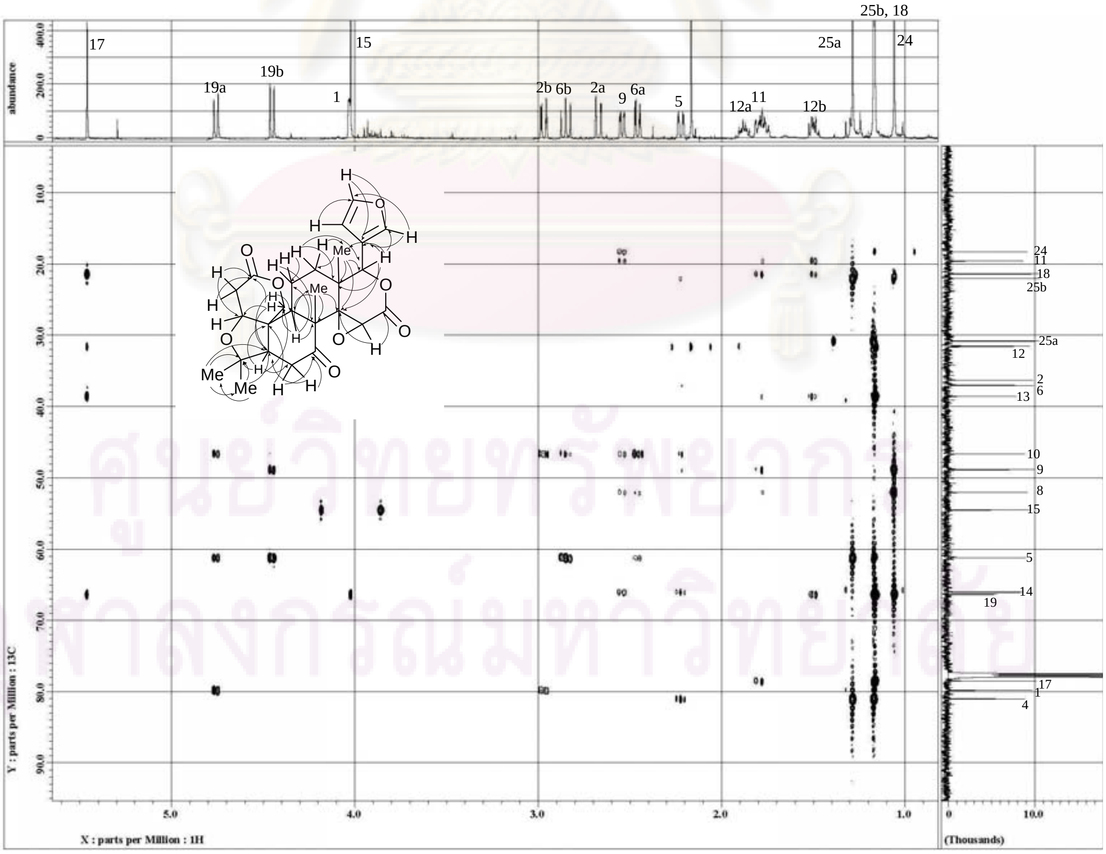


Figure 102 Expanded HMBC spectrum of compound **GP6** $(CDCl_3)$.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

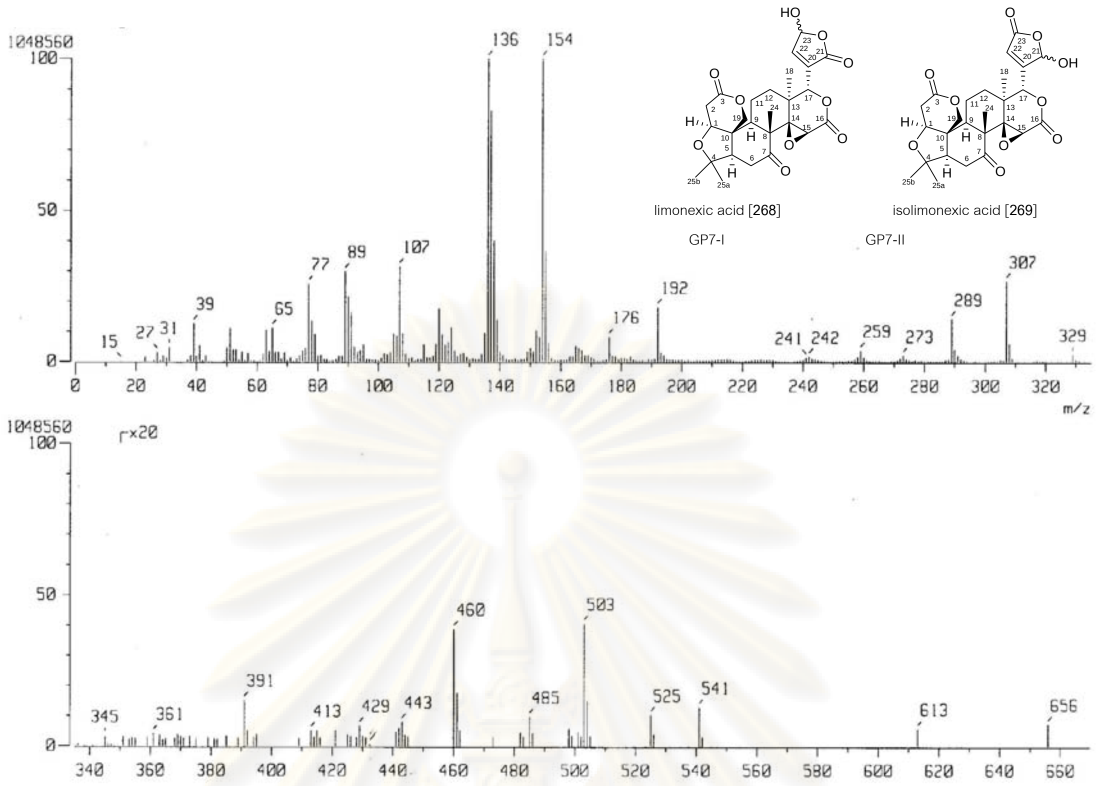


Figure 103 FABMS of mixture **GP7**.

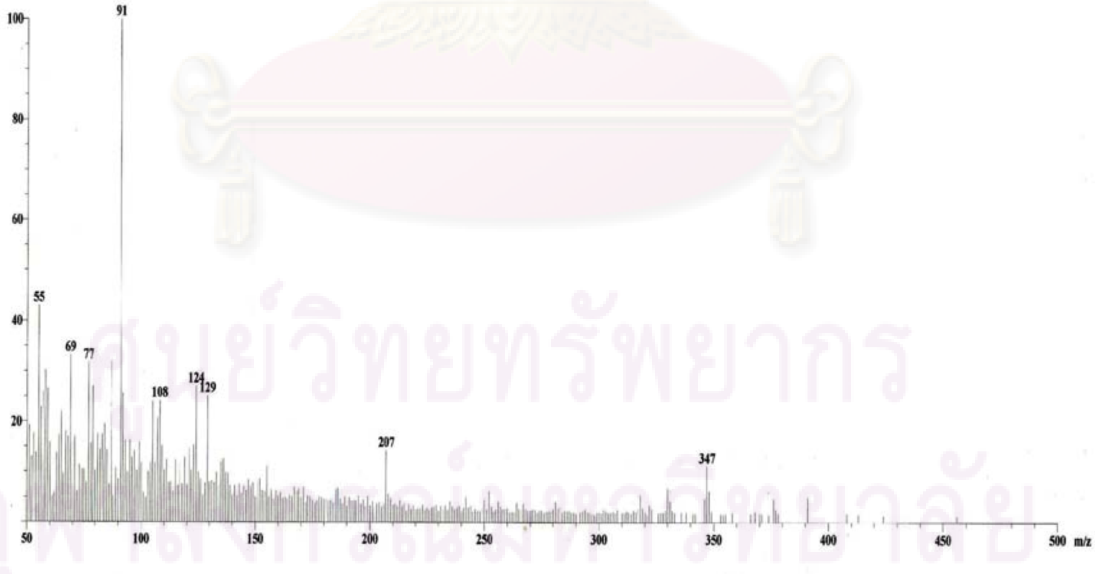


Figure 104 EIMS of mixture **GP7**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

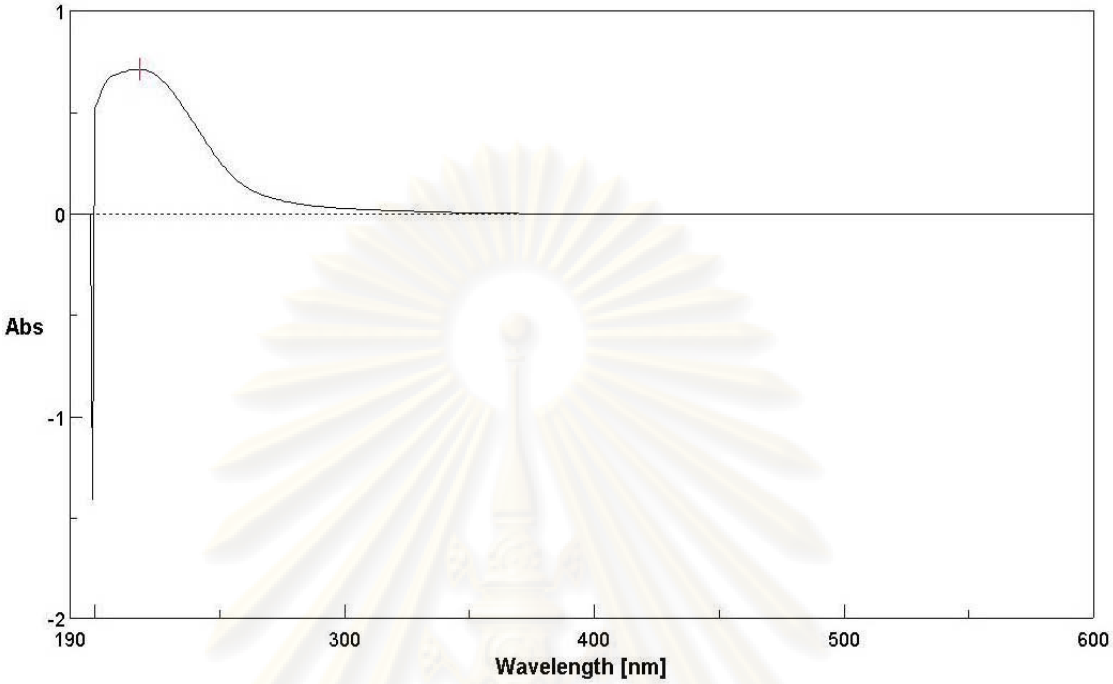


Figure 105 UV spectrum of mixture **GP7** (MeOH).

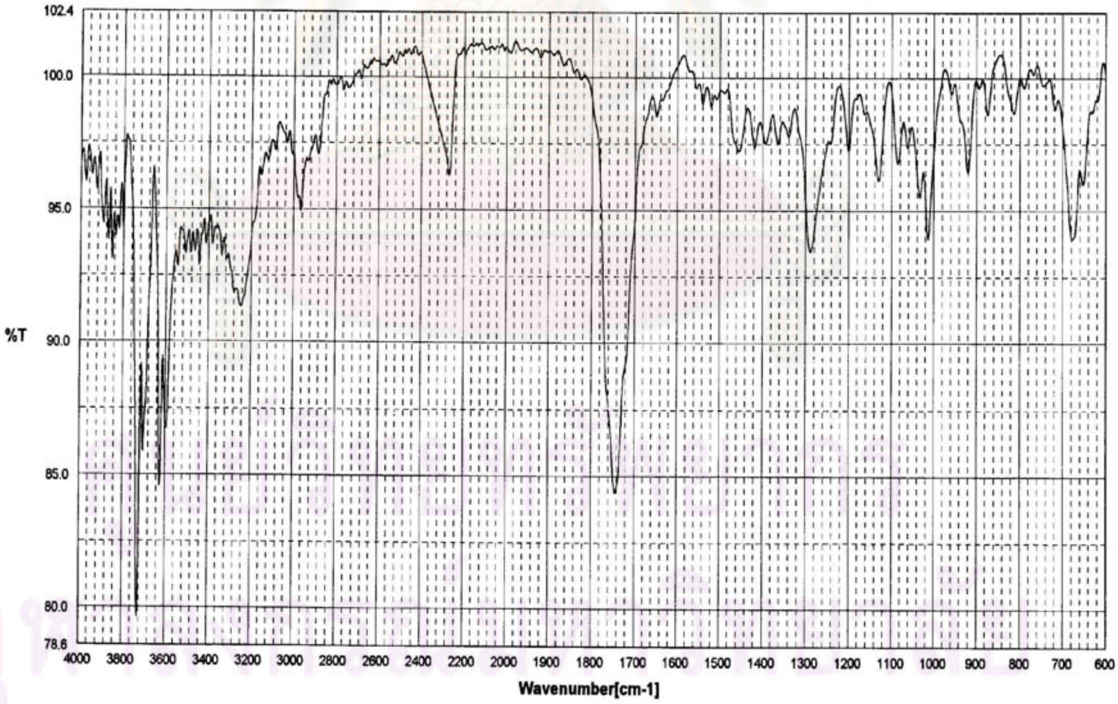


Figure 106 IR spectrum of mixture **GP7** (ATR).

(The peak at 2280 cm-^{1} is an artifact produced by the process of subtraction the absorption owing to the CO_2.)

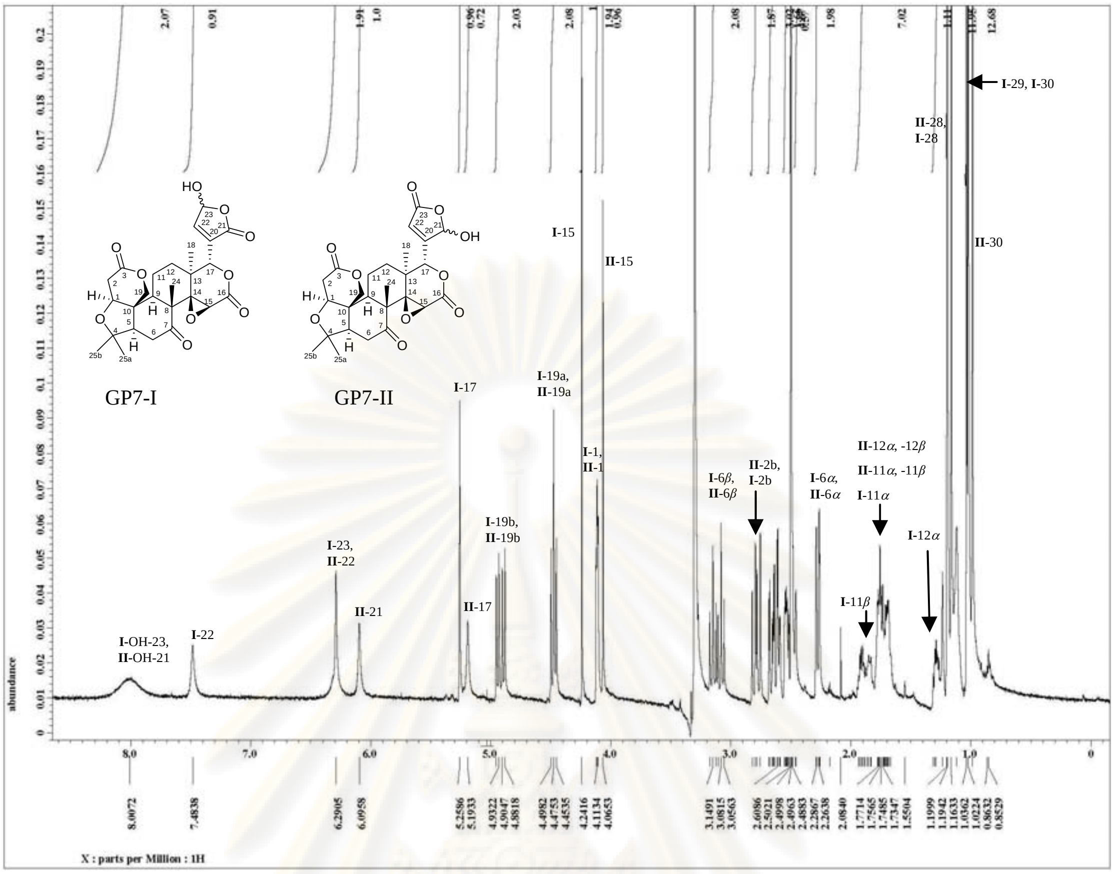


Figure 107 ^{1}H-NMR spectrum of mixture **GP7** (600 MHz, DMSO-d_6).

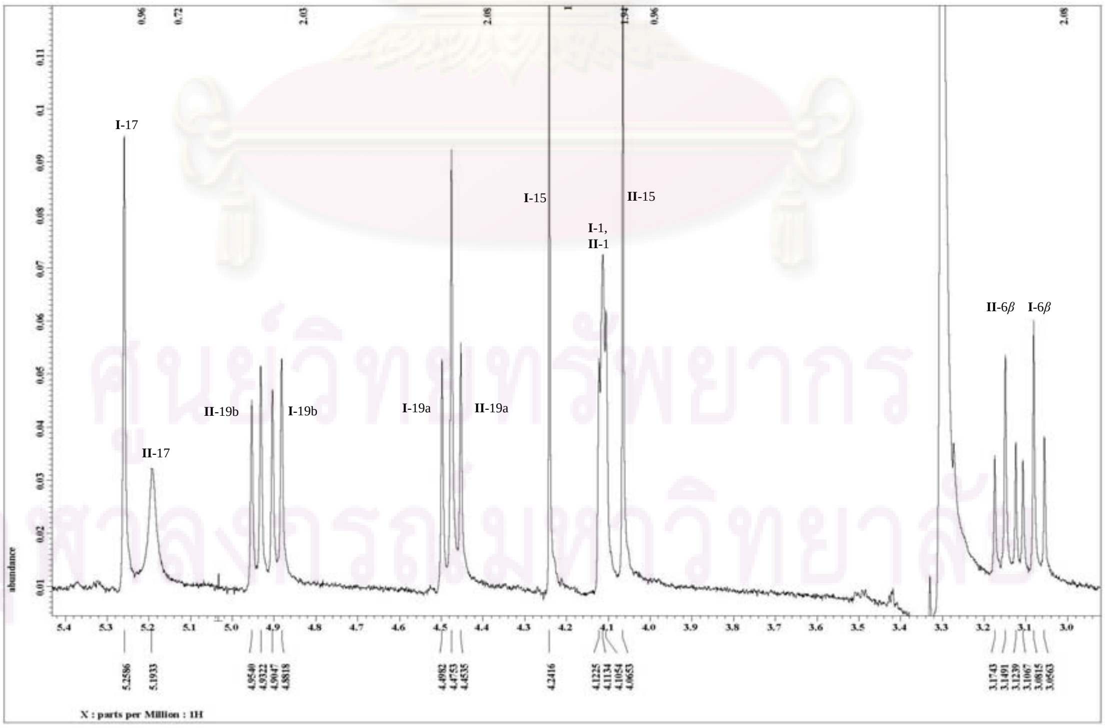


Figure 108 Expanded ^{1}H-NMR spectrum of mixture **GP7** (600 MHz, DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

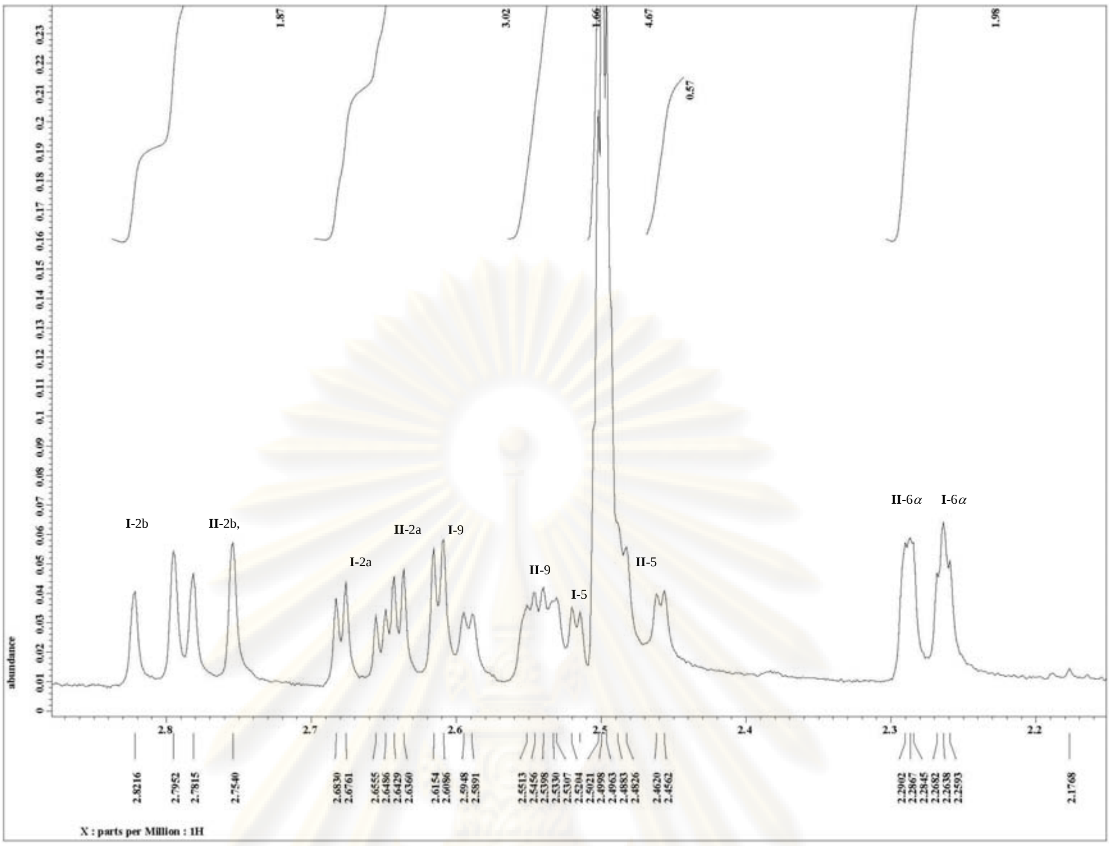


Figure 109 Expanded ^{1}H-NMR spectrum of mixture **GP7** (600 MHz, DMSO-d_6).

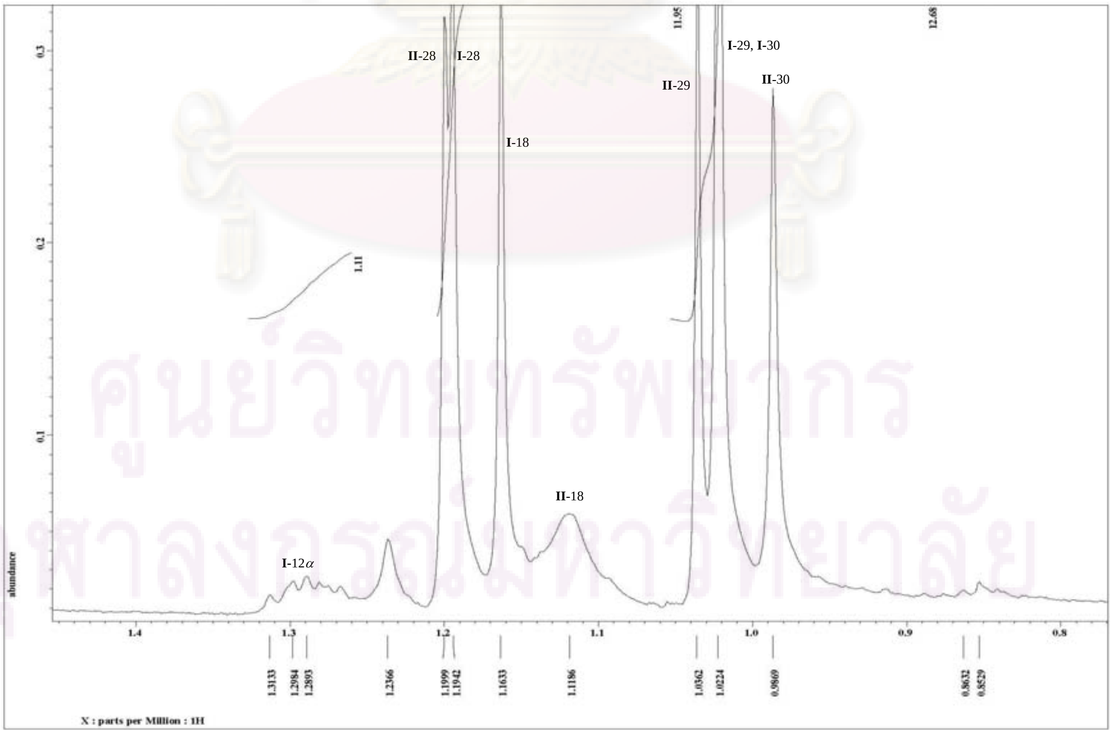


Figure 110 Expanded ^{1}H-NMR spectrum of mixture **GP7** (600 MHz, DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

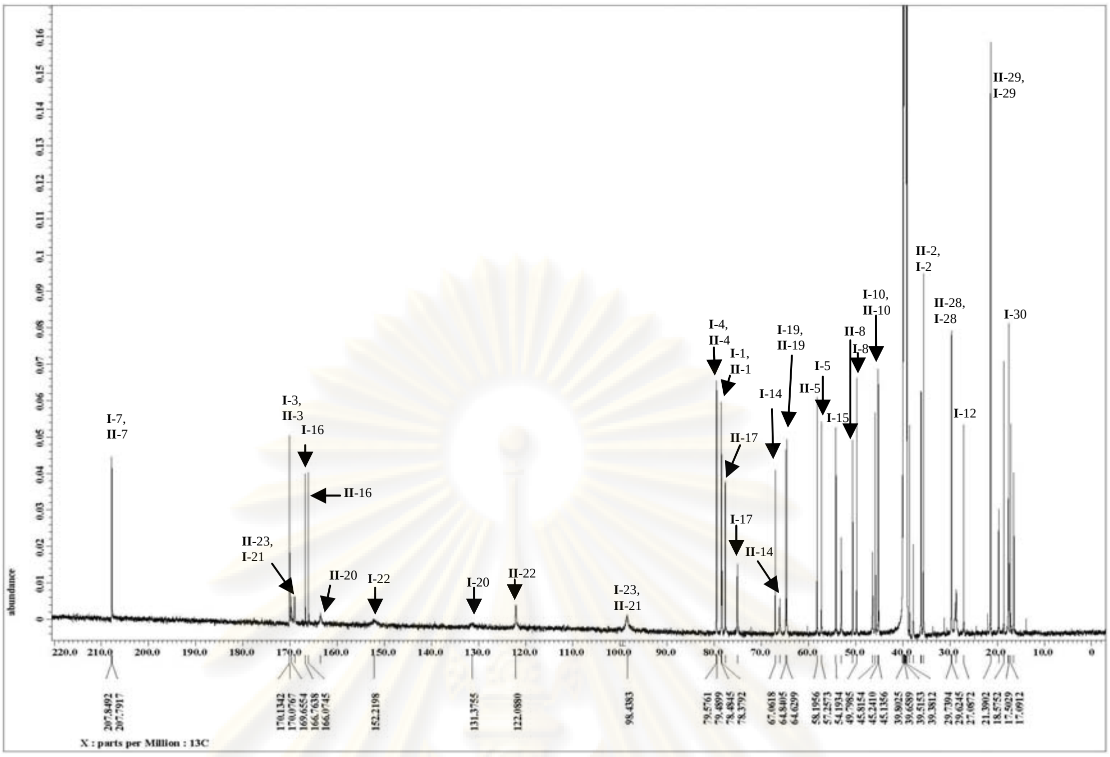


Figure 111 ^{13}C-NMR spectrum of mixture **GP7** (150 MHz, DMSO-d_6).

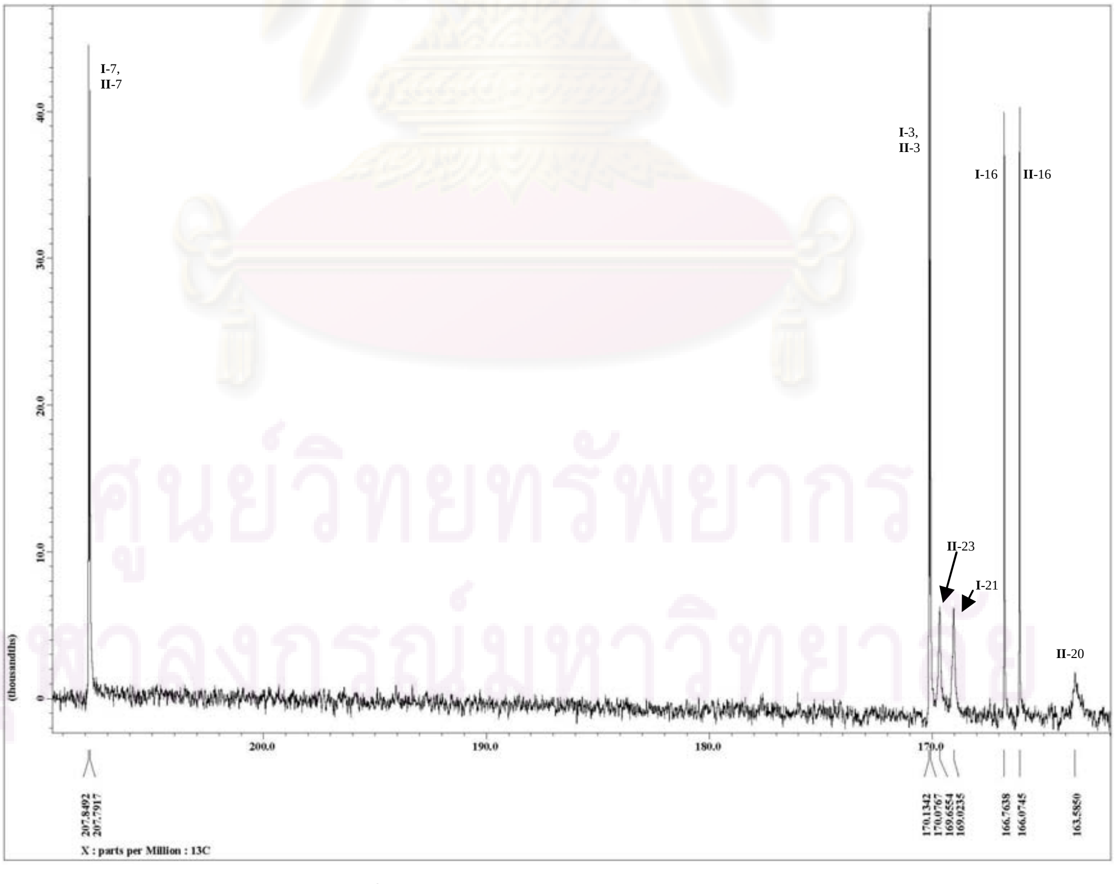


Figure 112 Expanded ^{13}C-NMR spectrum of mixture **GP7** (150 MHz, DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

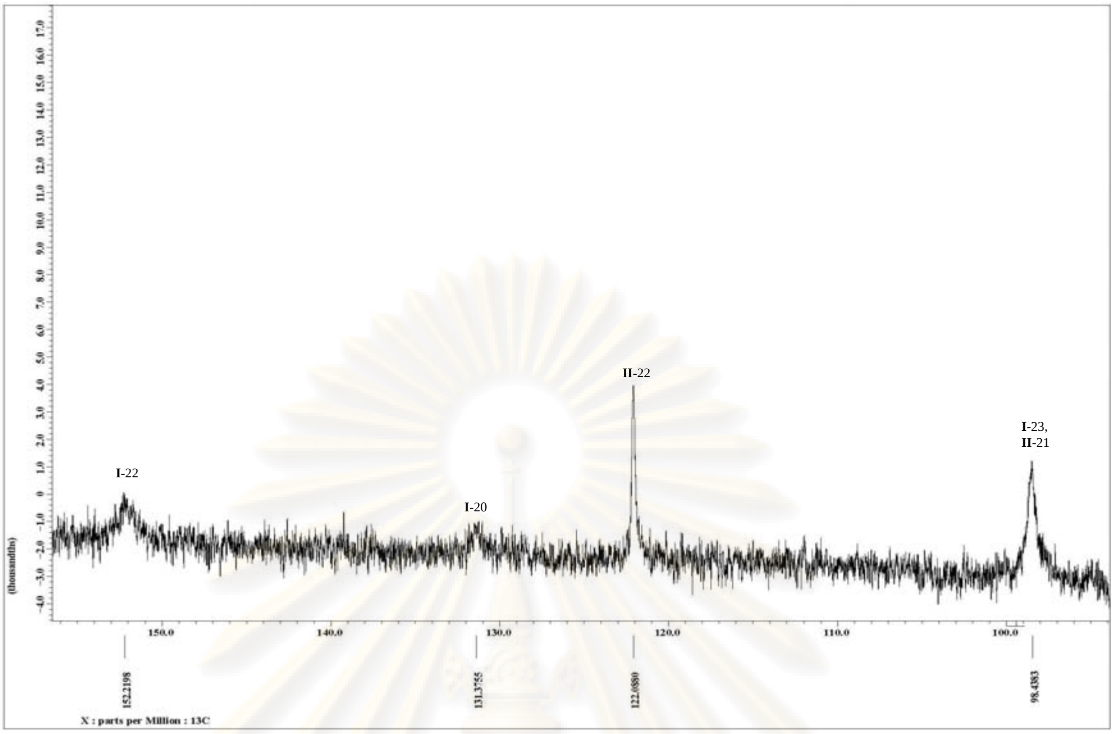


Figure 113 Expanded ^{13}C-NMR spectrum of mixture **GP7** (150 MHz, DMSO-d_6).

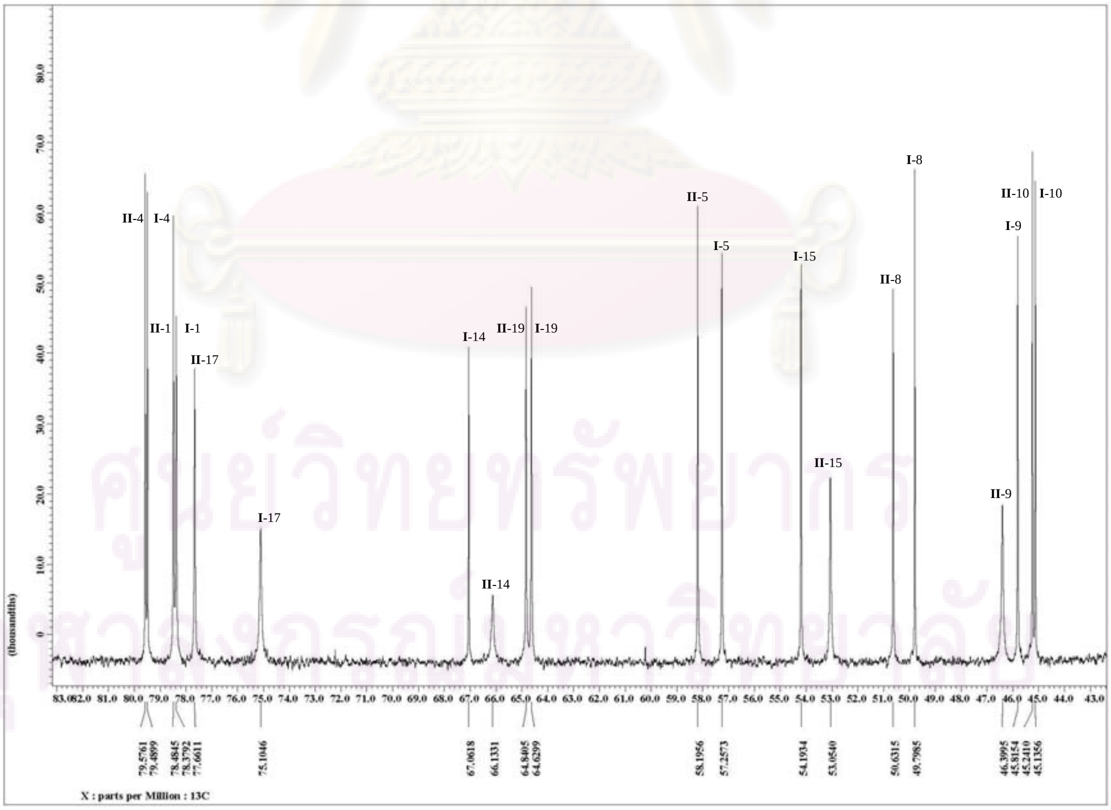


Figure 114 Expanded ^{13}C-NMR spectrum of mixture **GP7** (150 MHz, DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

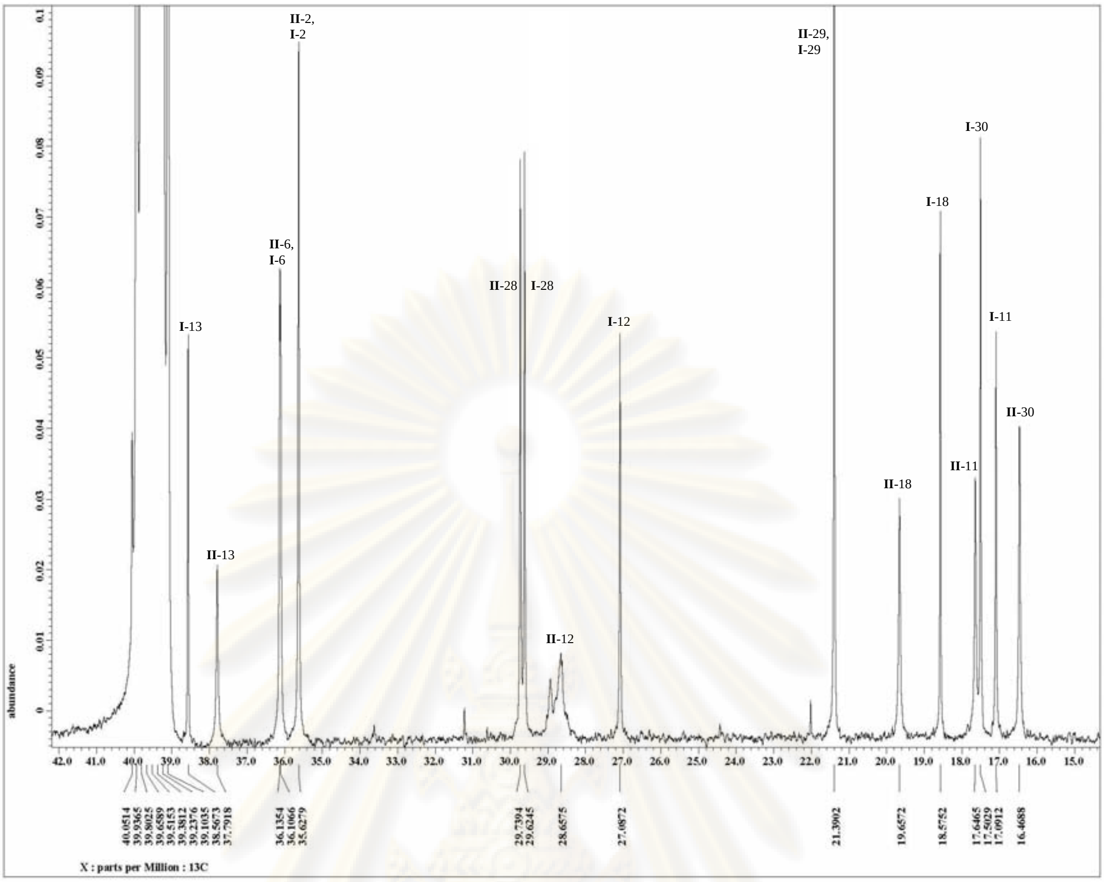


Figure 115 Expanded ^{13}C-NMR spectrum of mixture **GP7** (150 MHz DMSO-d_6).

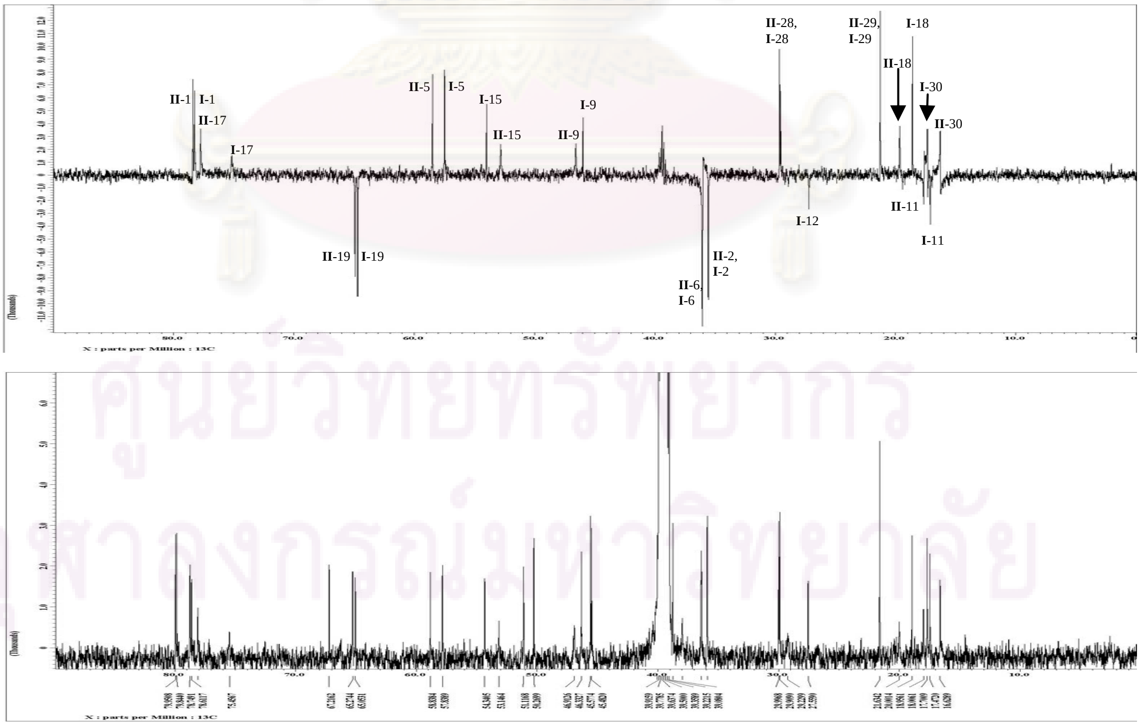


Figure 116 DEPT135 spectrum of mixture **GP7** (150 MHz, DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

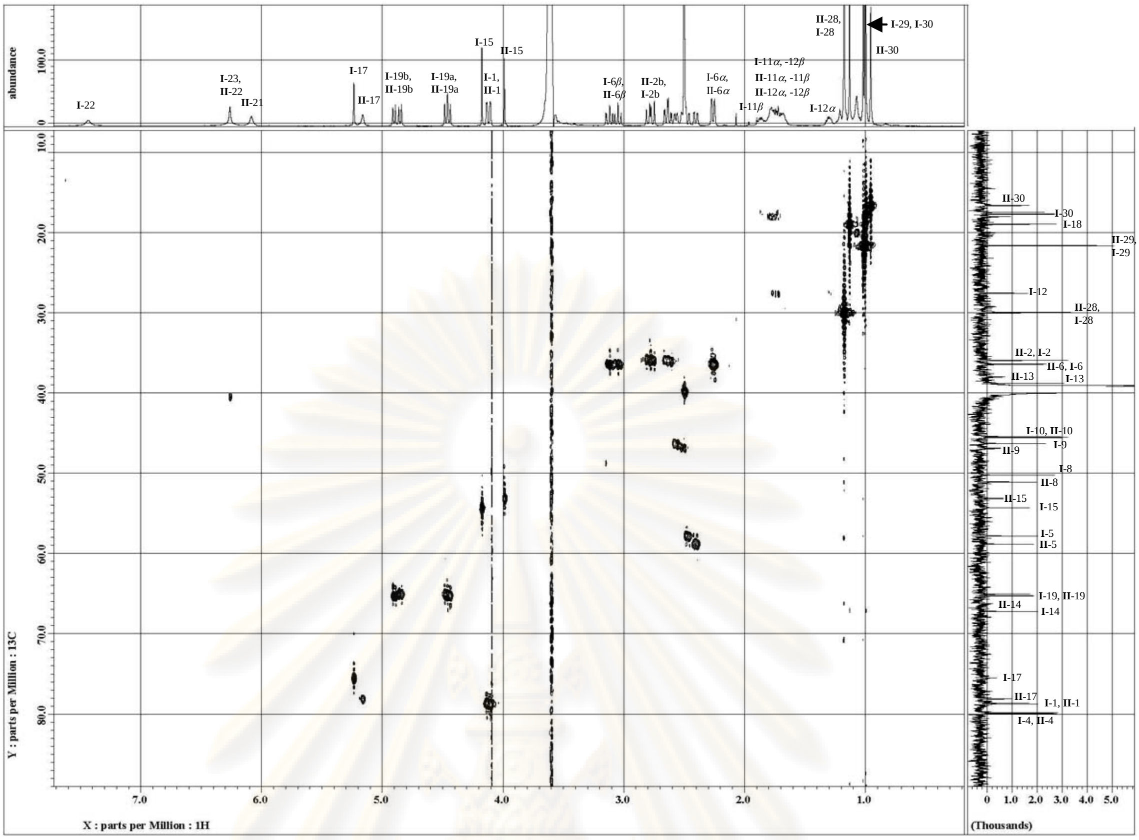
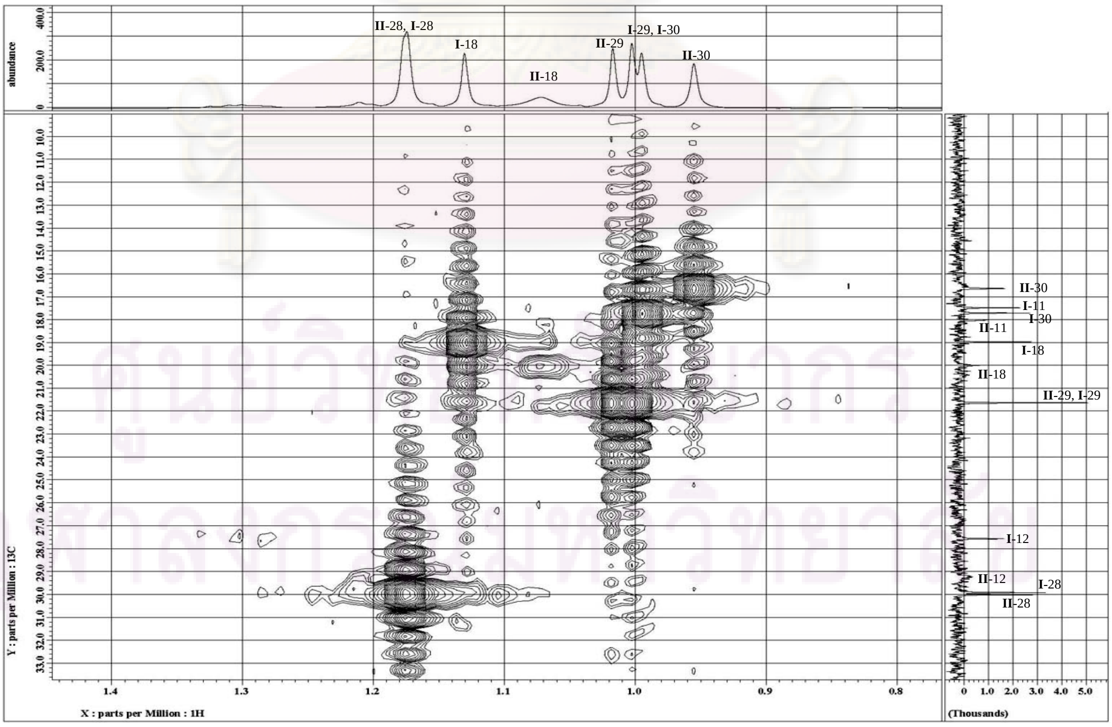


Figure 117 HMQC spectrum of mixture **GP7** (DMSO-d_6).



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 118 Expanded HMQC spectrum of mixture **GP7** (DMSO-d_6).

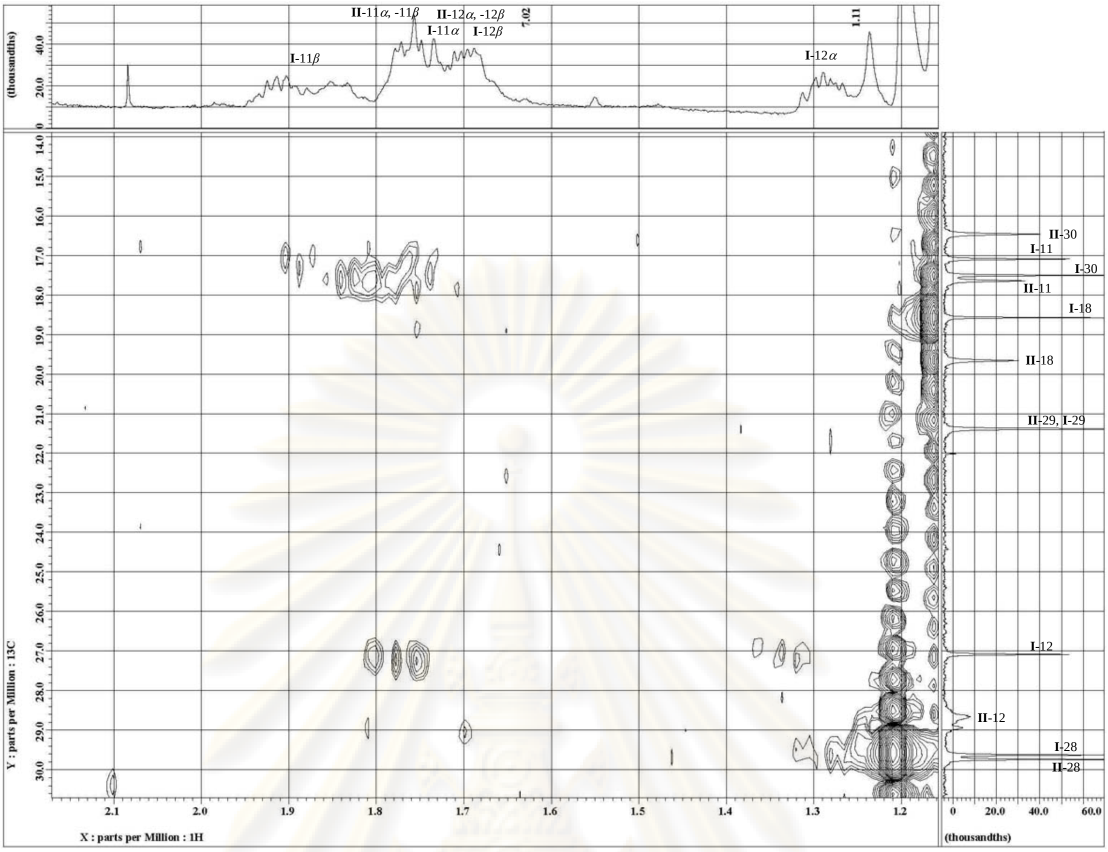


Figure 119 Expanded HMQC spectrum of mixture **GP7** (DMSO-d_6).

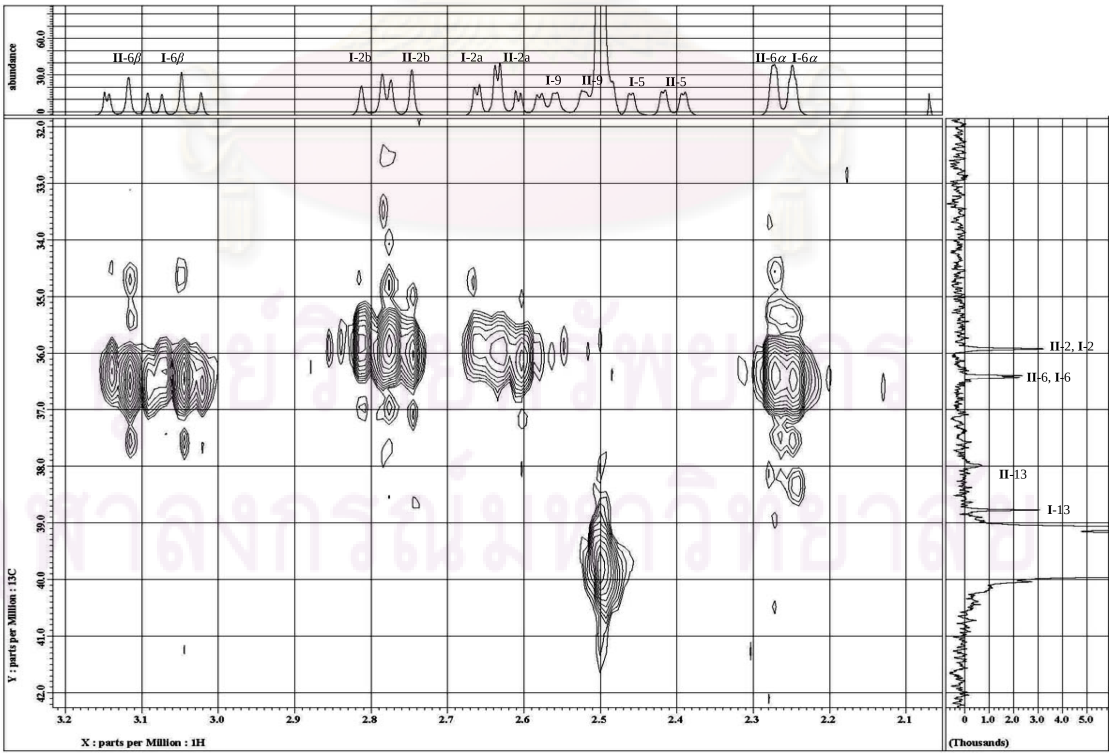


Figure 120 Expanded HMQC spectrum of mixture **GP7** (DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

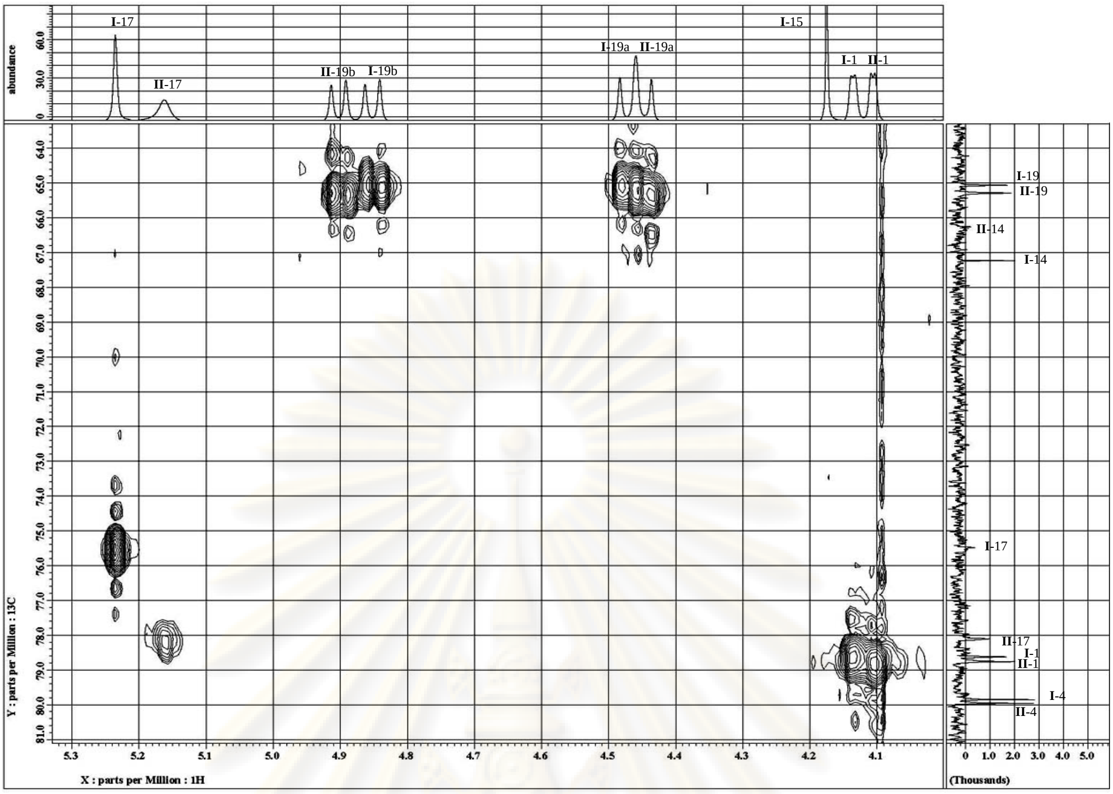


Figure 121 Expanded HMQC spectrum of mixture **GP7** (DMSO-d_6).

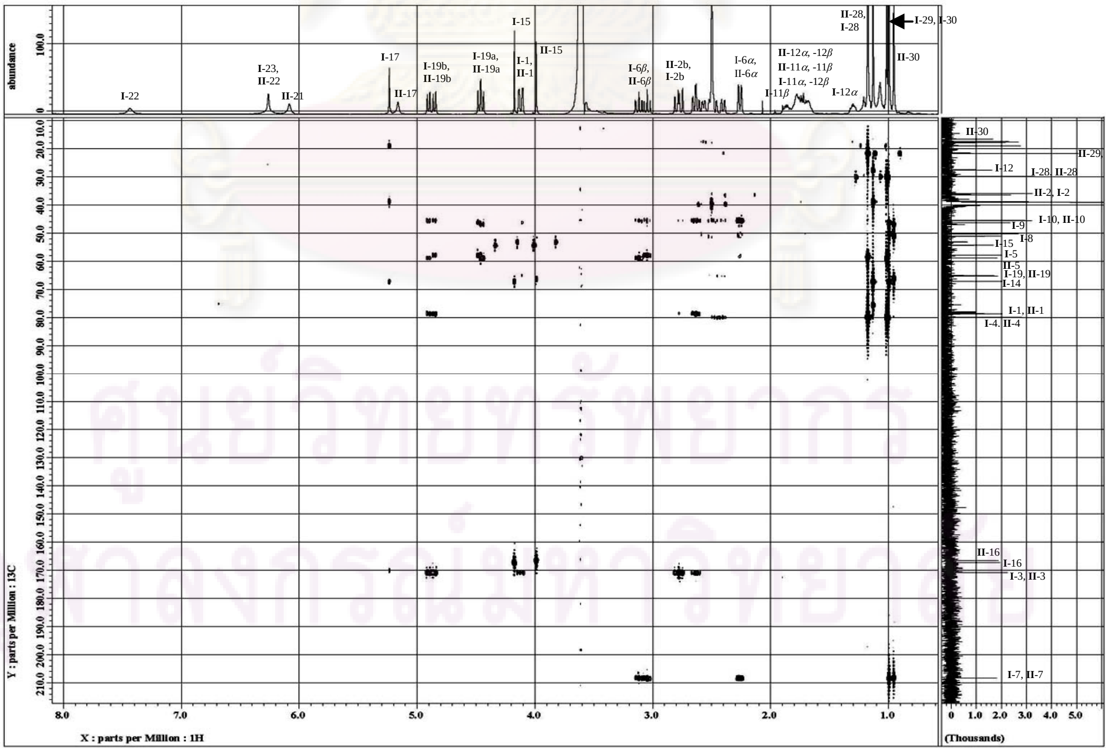


Figure 122 HMBC spectrum of mixture **GP7** (DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

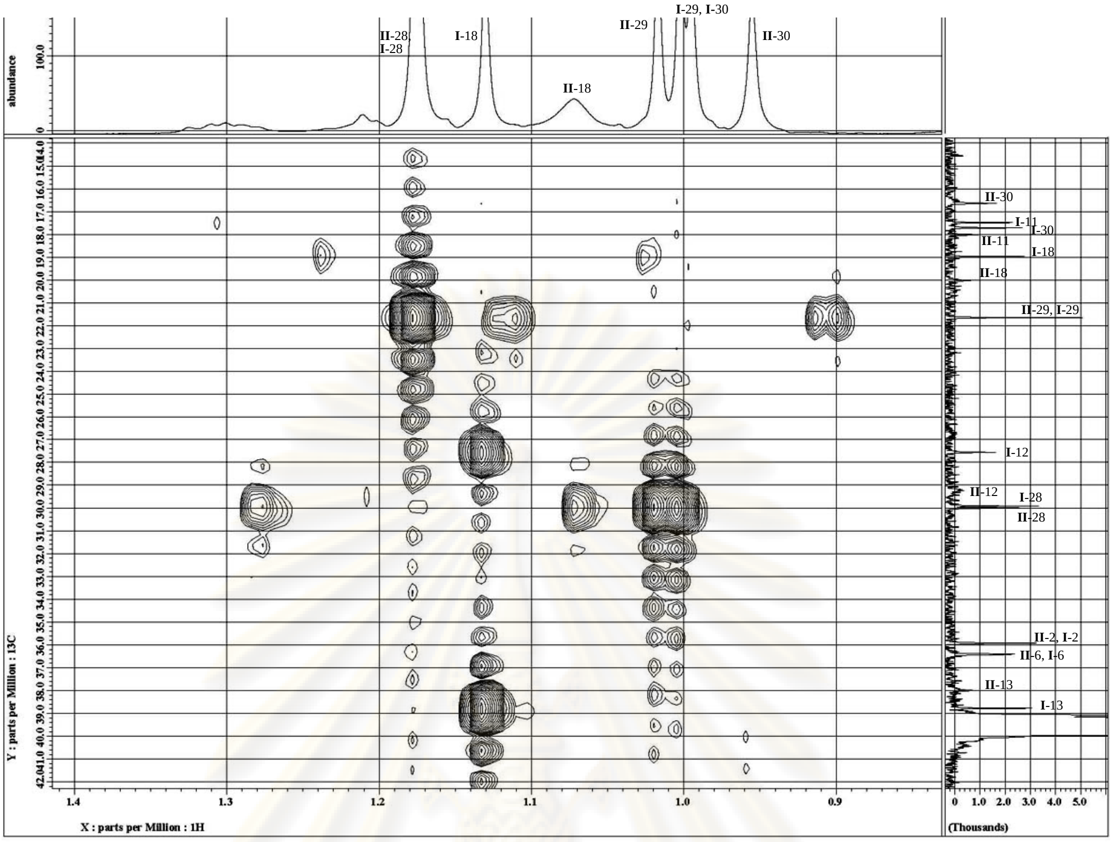


Figure 123 Expanded HMBC spectrum of mixture **GP7** (DMSO-d_6).

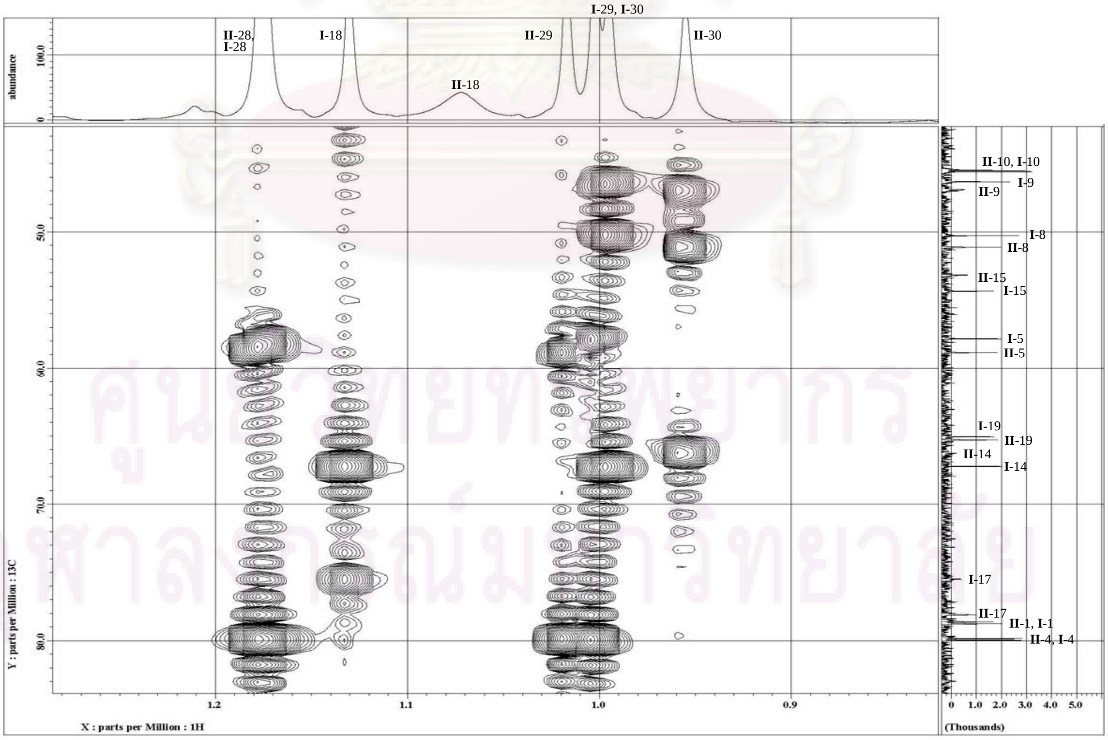


Figure 124 Expanded HMBC spectrum of mixture **GP7** (DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

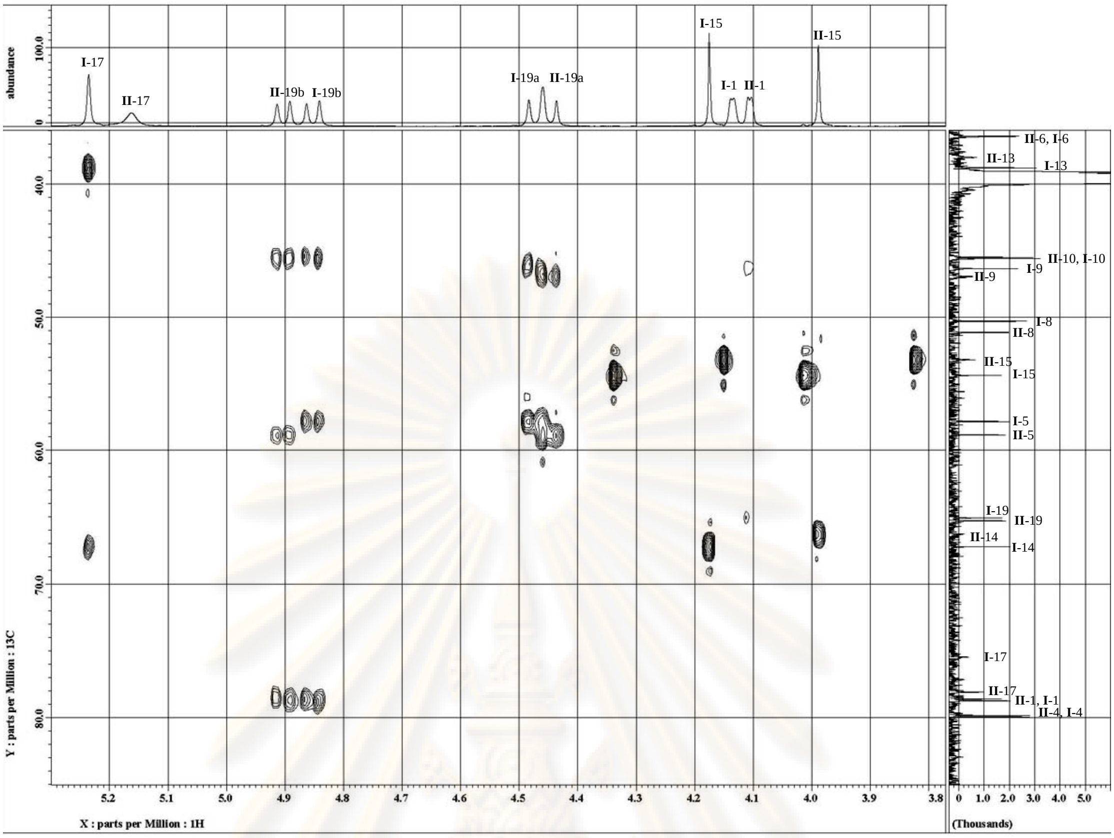


Figure 125 Expanded HMBC spectrum of mixture **GP7** (DMSO-d_6).

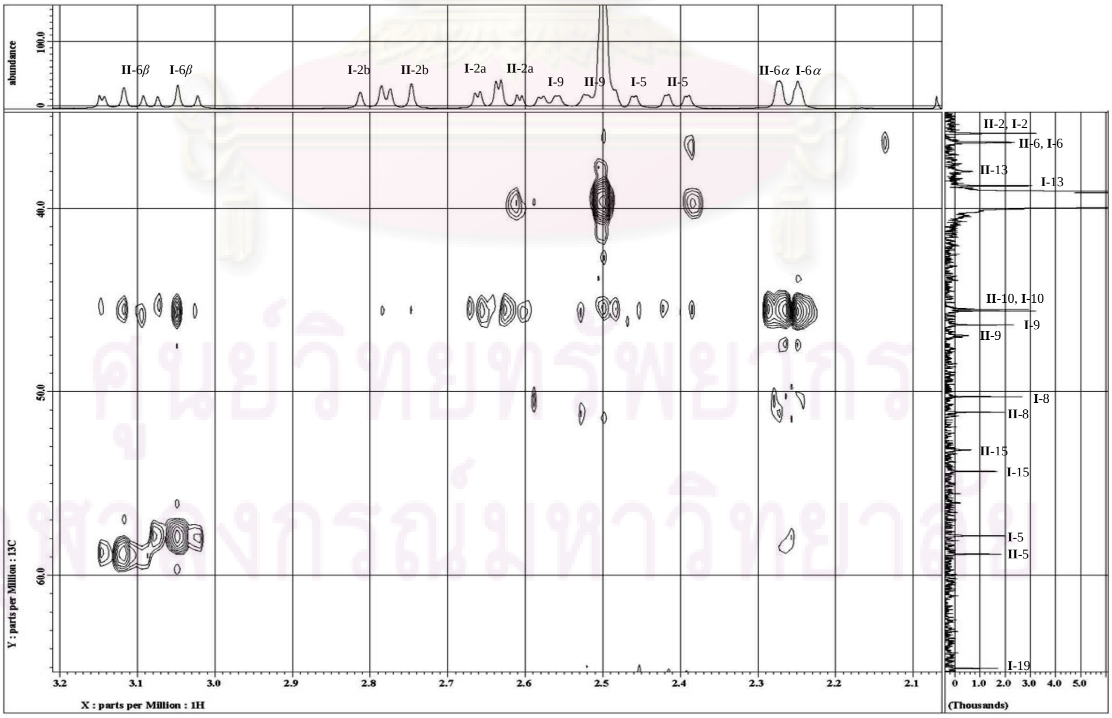


Figure 126 Expanded HMBC spectrum of mixture **GP7** (DMSO-d_6).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

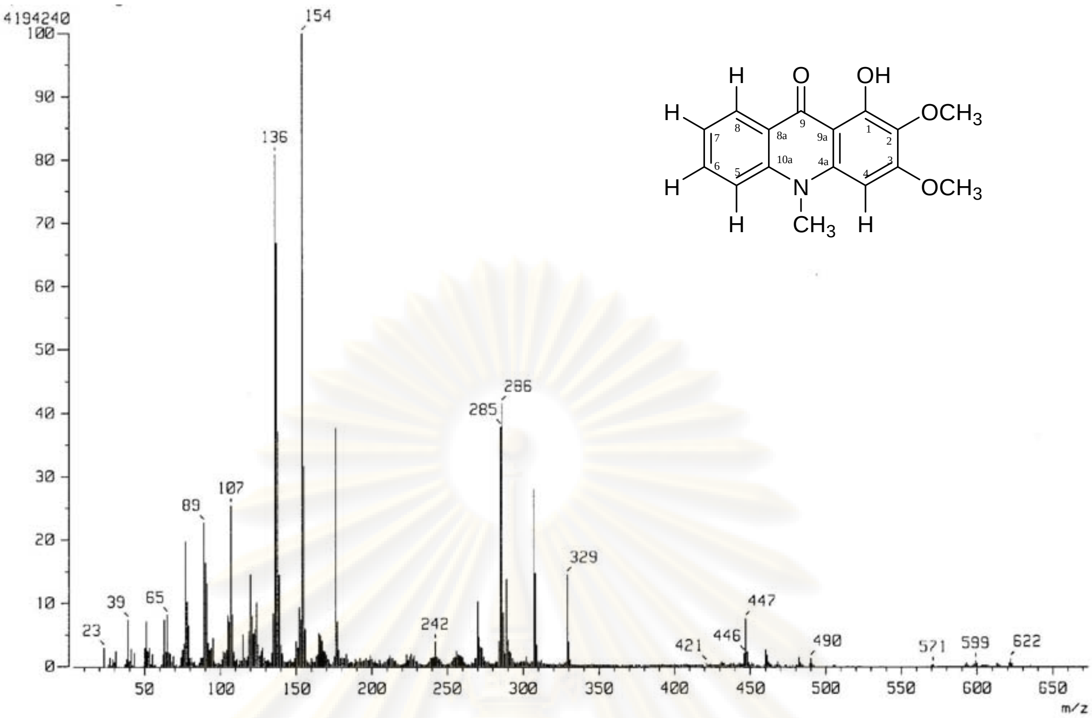


Figure 127 FABMS of compound **GP8**.

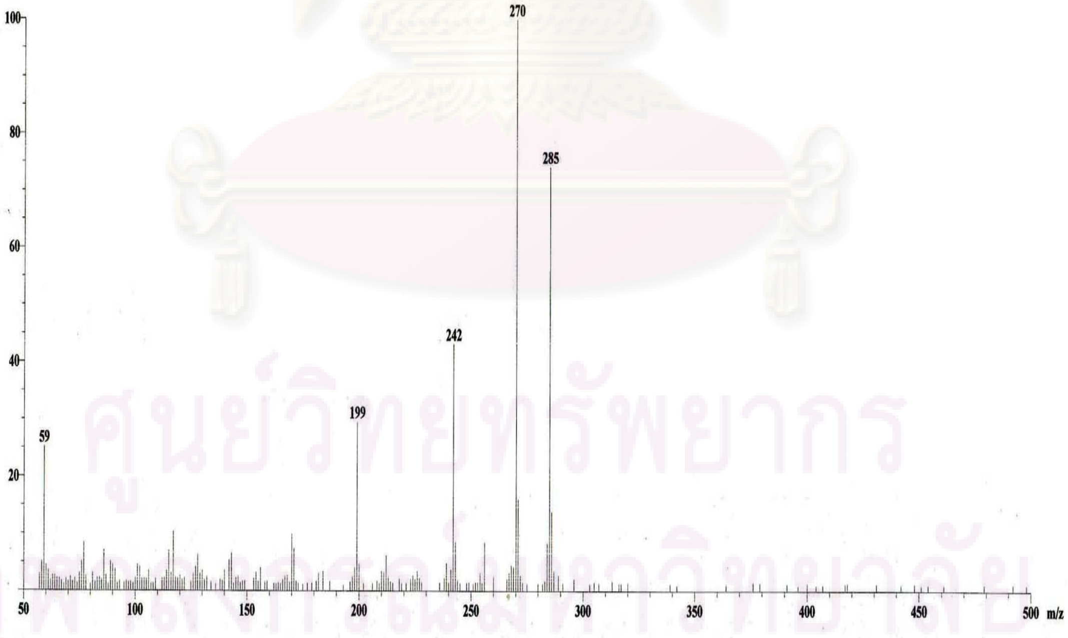


Figure 128 EIMS of compound **GP8**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

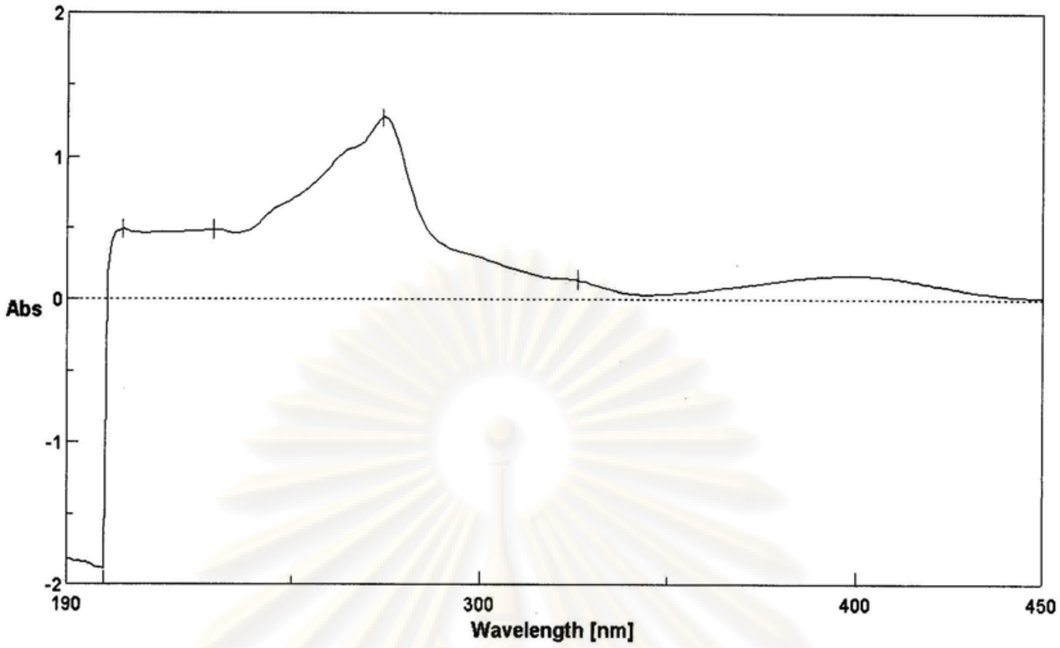


Figure 129 UV spectrum of compound **GP8** (MeOH).

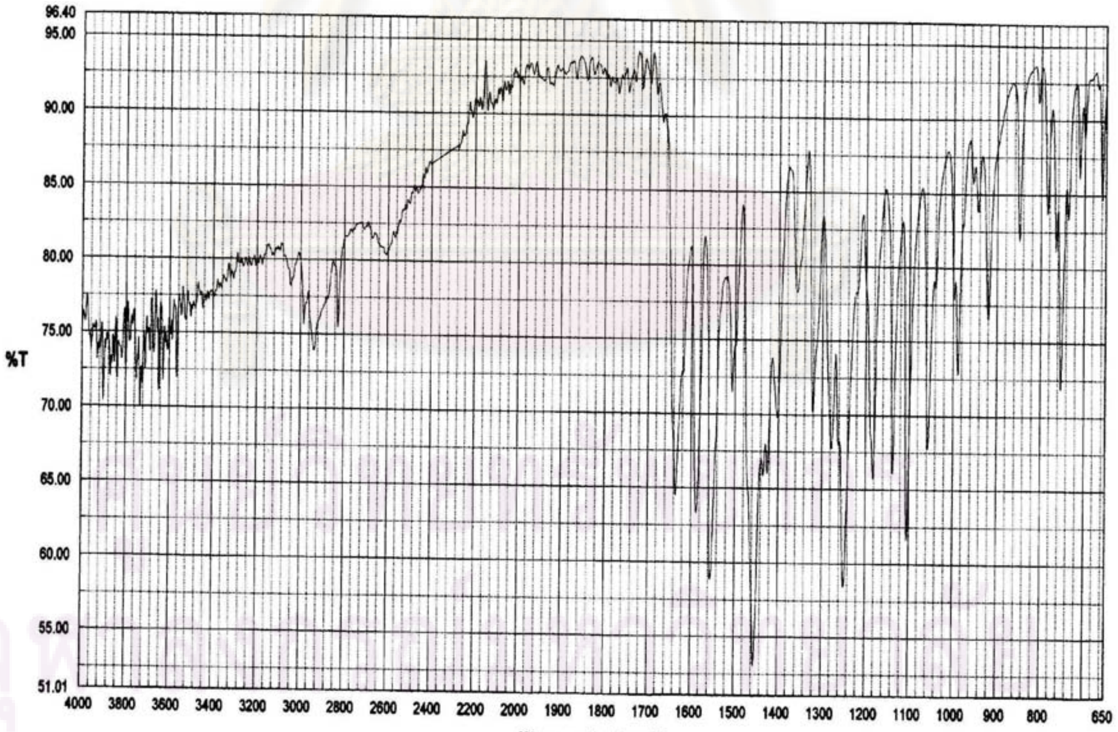


Figure 130 IR spectrum of compound **GP8** (ATR).

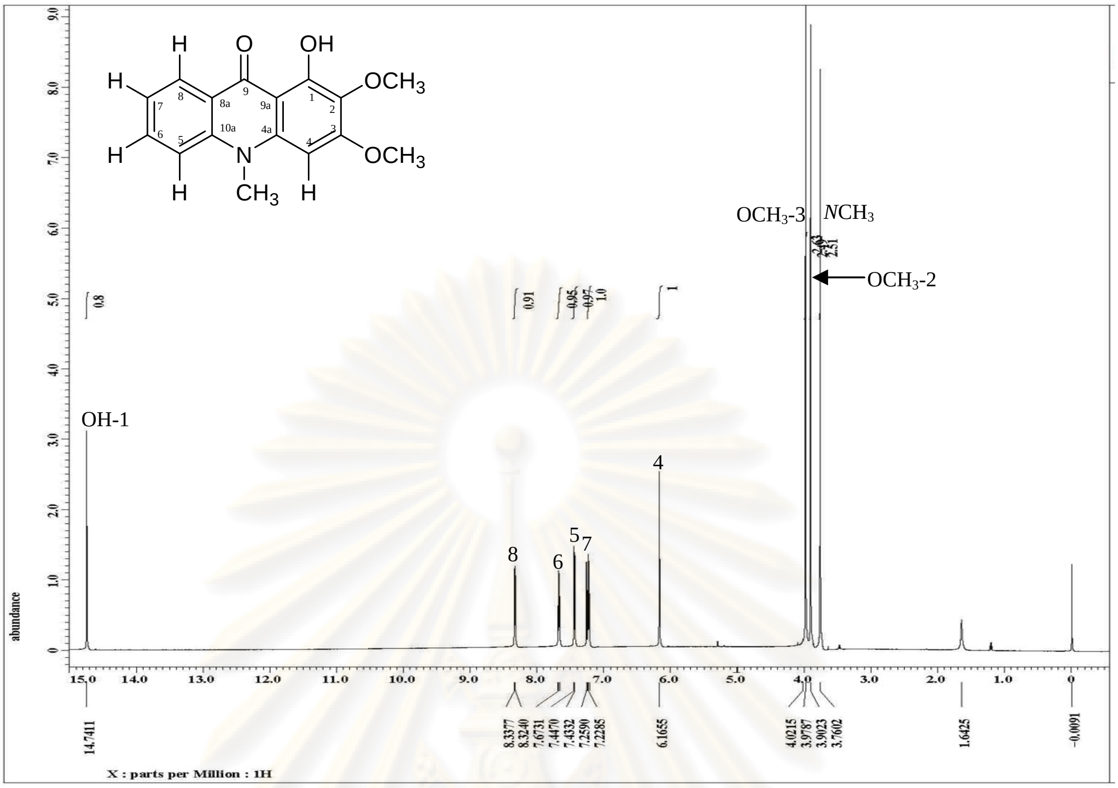


Figure 131 ^{1}H-NMR spectrum of compound **GP8** (600 MHz, $CDCl_3$).

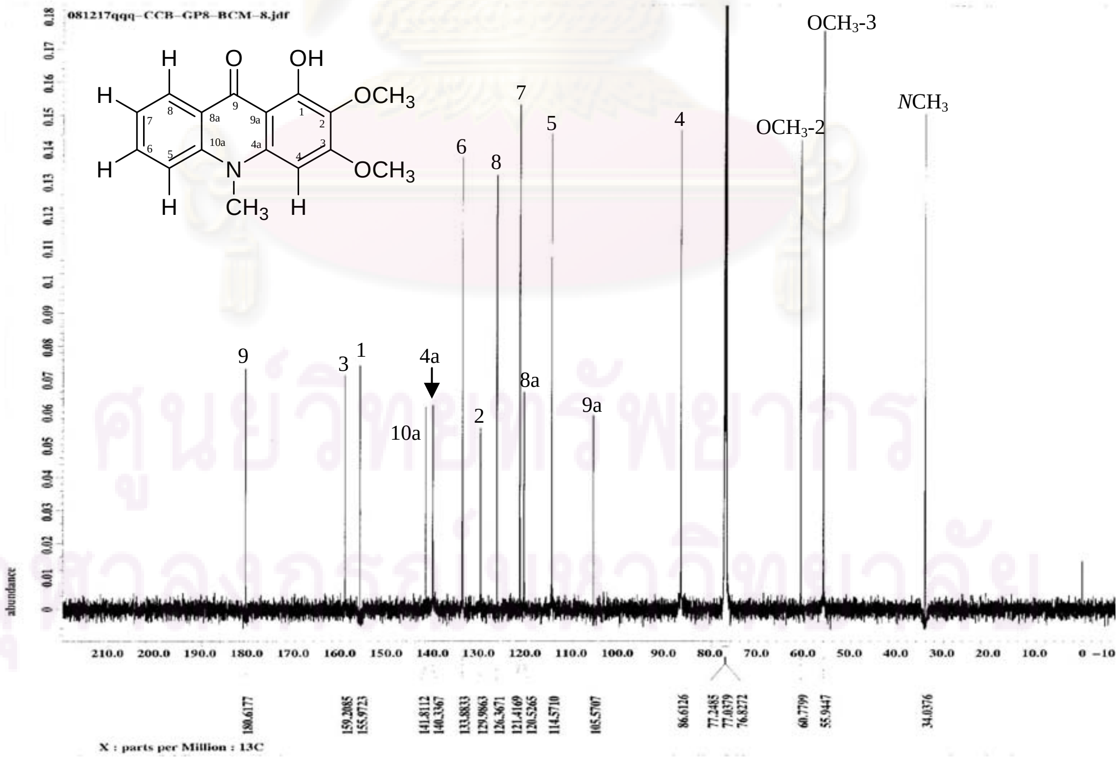


Figure 132 ^{13}C-NMR spectrum of compound **GP8** (150 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

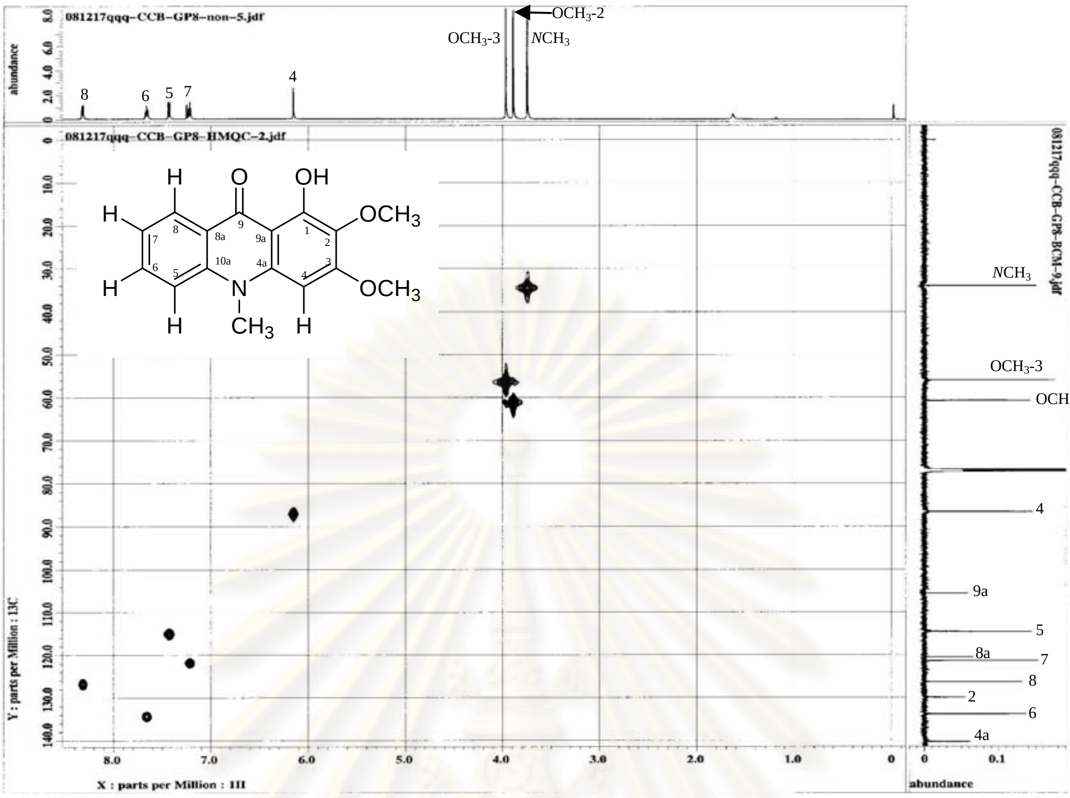


Figure 133 HMQC spectrum of compound **GP8** ($CDCl_3$).

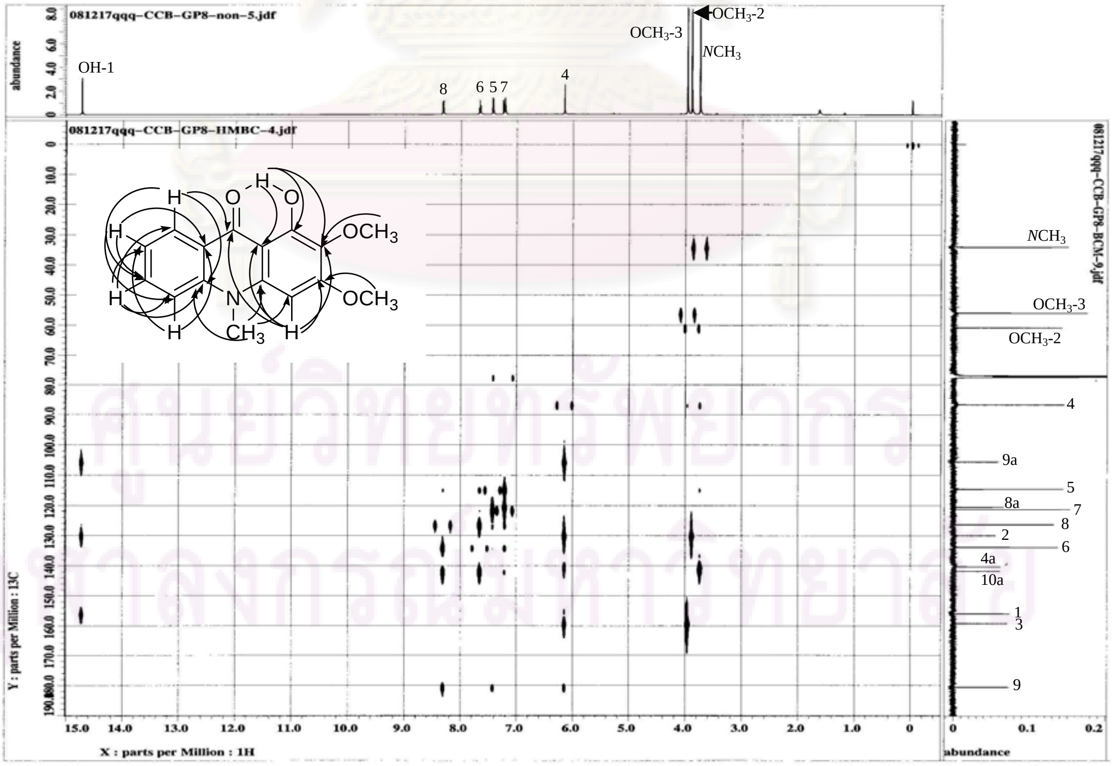


Figure 134 HMBC spectrum of compound **GP8** ($CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

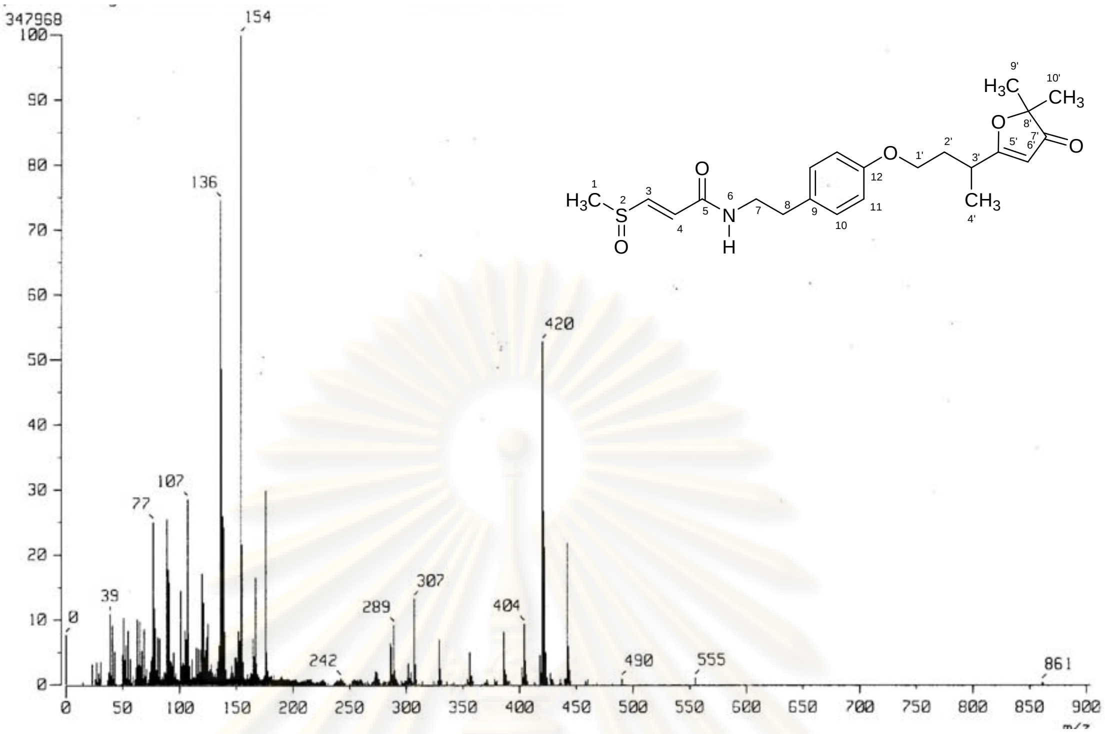


Figure 135 FABMS of compound **GP9**.

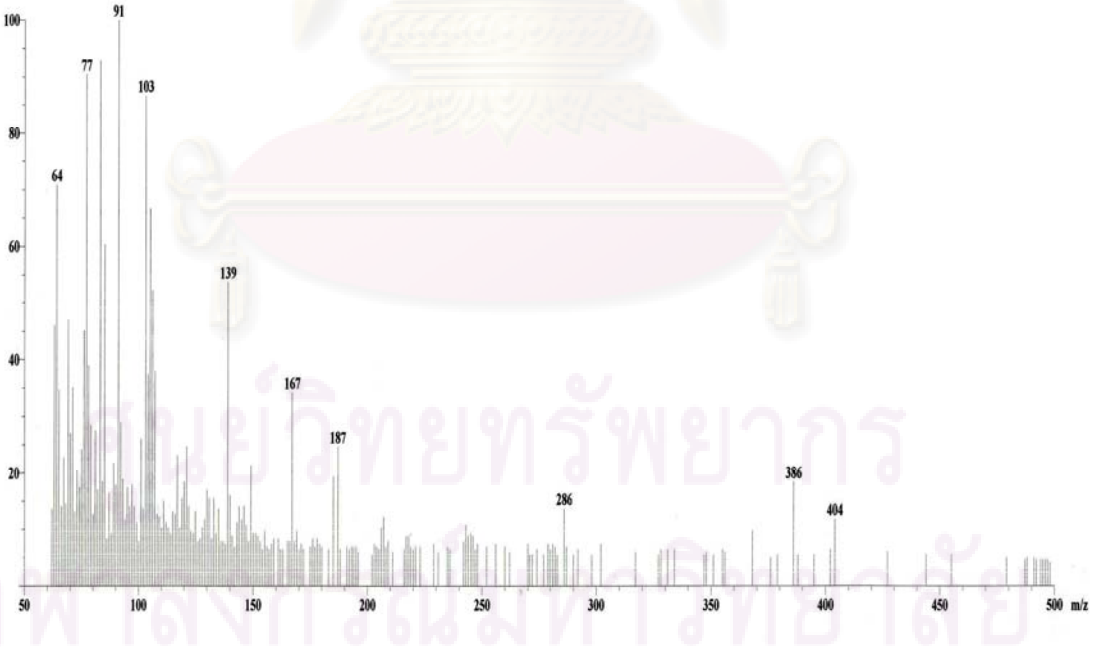


Figure 136 EIMS of compound **GP9**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

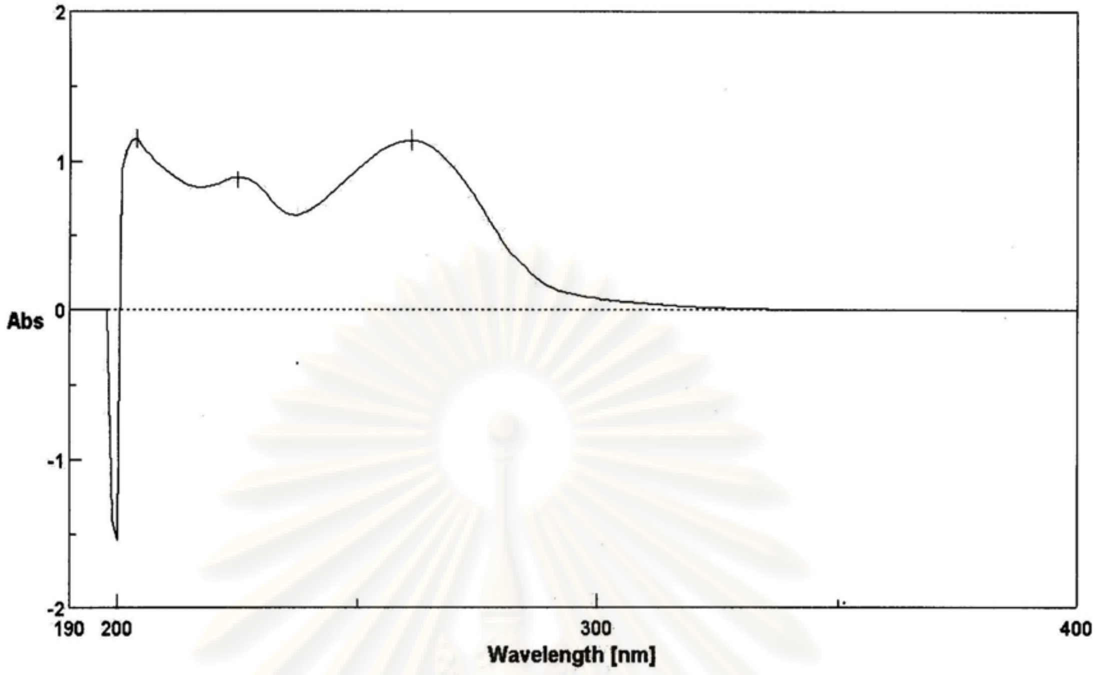


Figure 137 UV spectrum of compound **GP9** (MeOH).

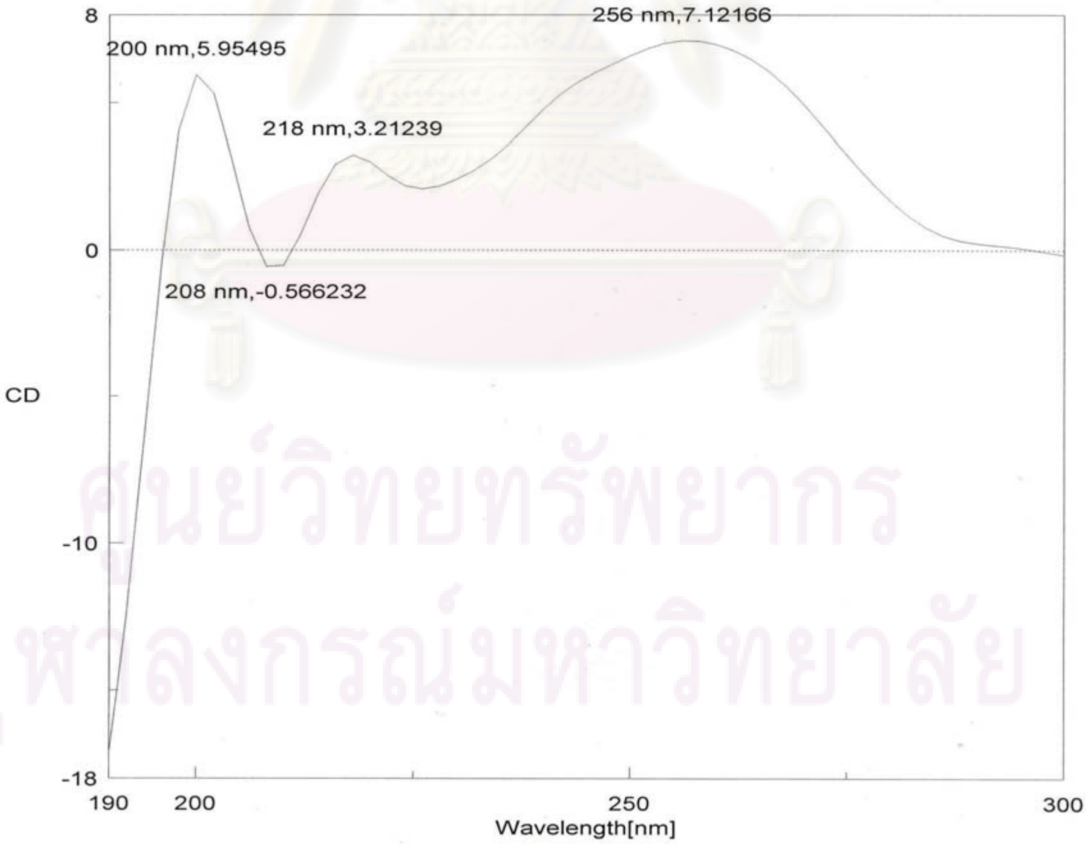


Figure 138 CD spectrum of compound **GP9** (MeOH).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

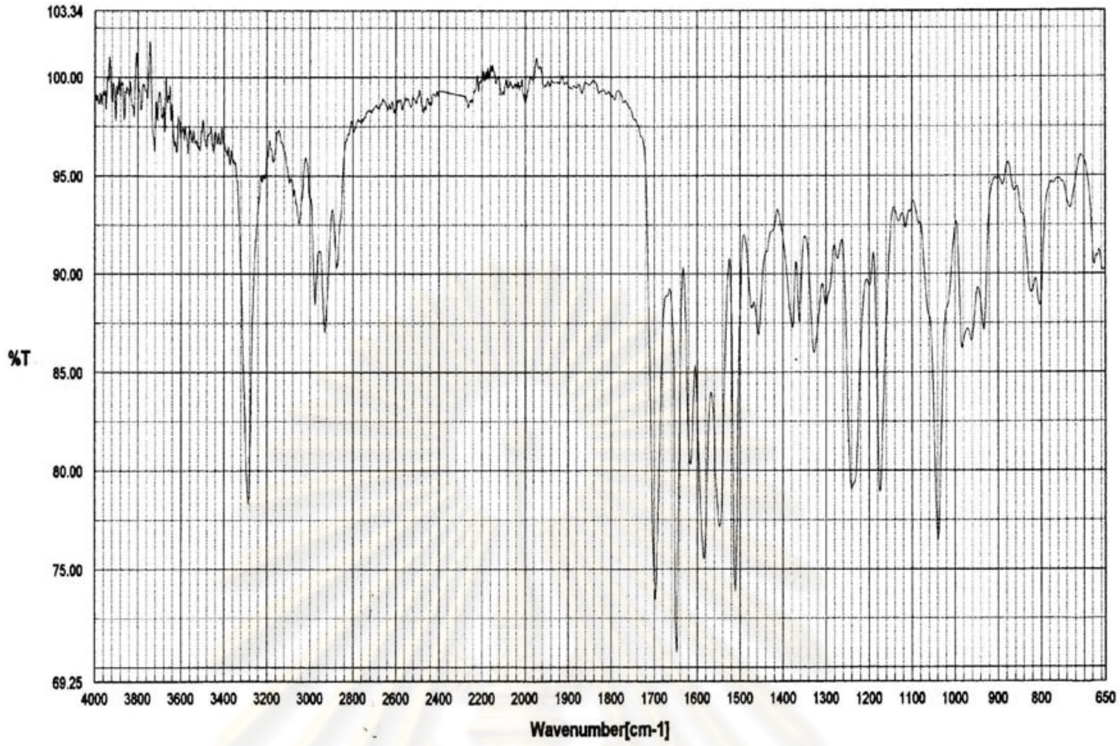


Figure 139 IR spectrum of compound **GP9** (ATR).

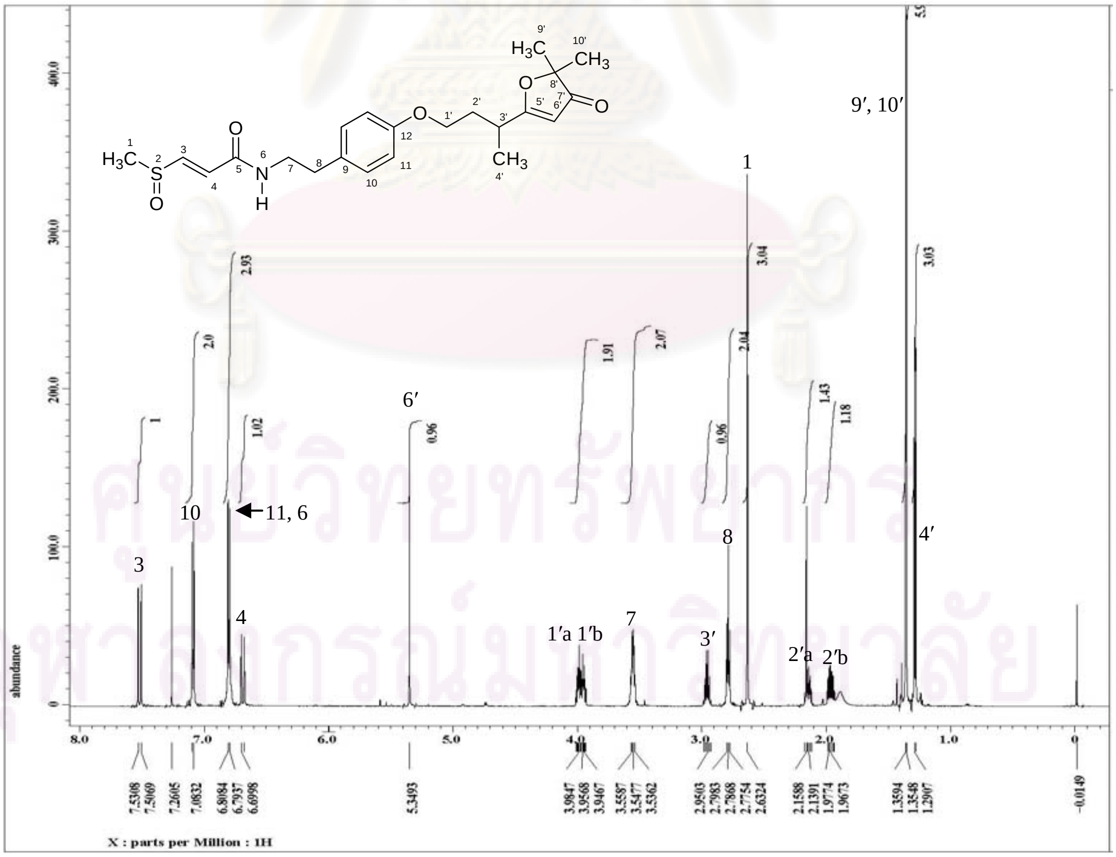


Figure 140 ^{1}H-NMR spectrum of compound **GP9** (600 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

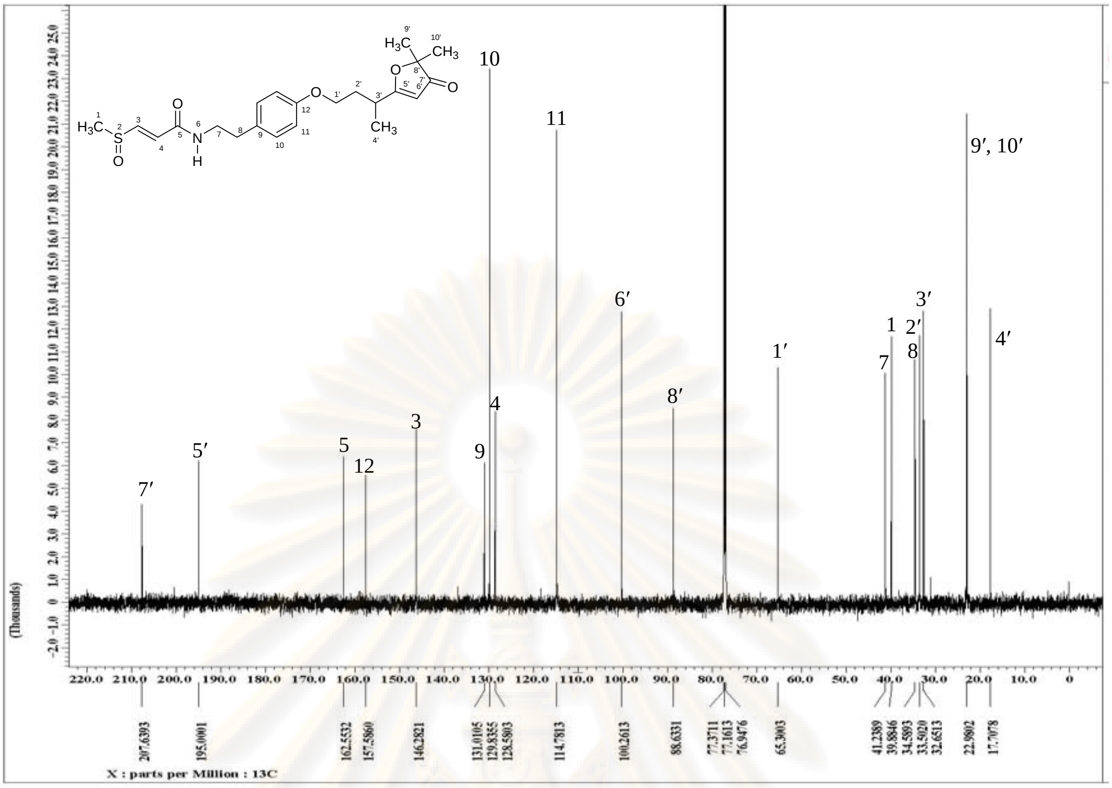


Figure 141 ^{13}C-NMR spectrum of compound **GP9** (150 MHz, $CDCl_3$).

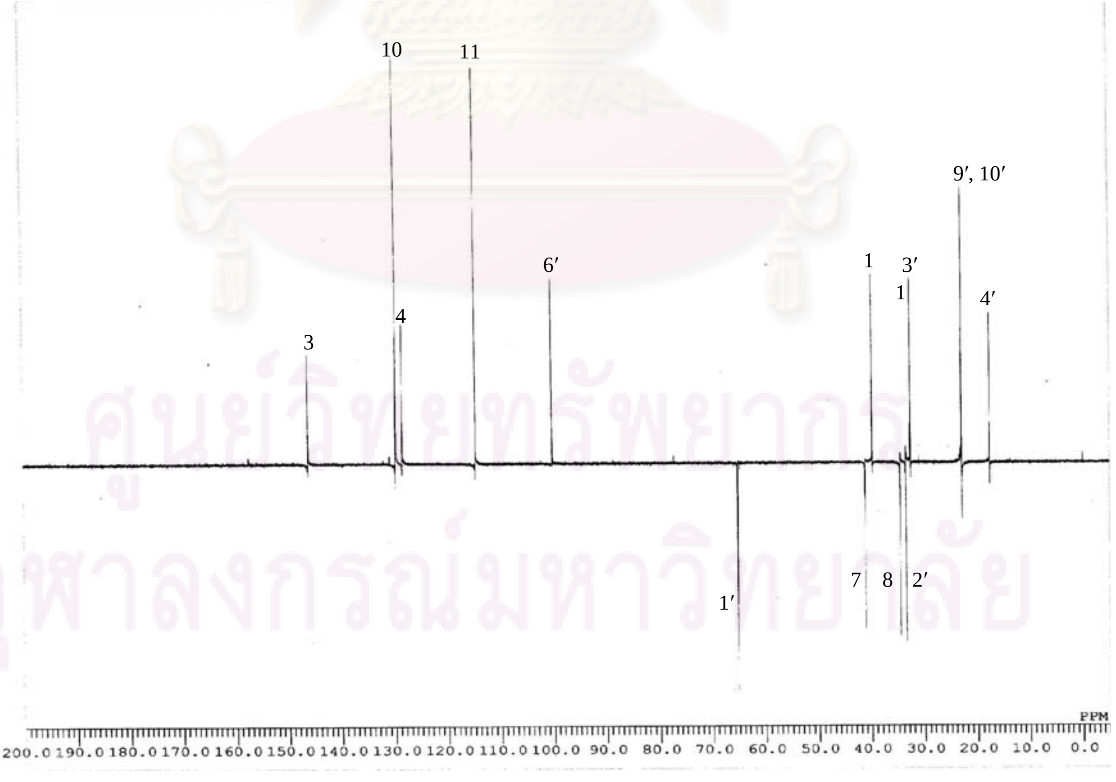


Figure 142 DEPT135 spectrum of compound **GP9** (100 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

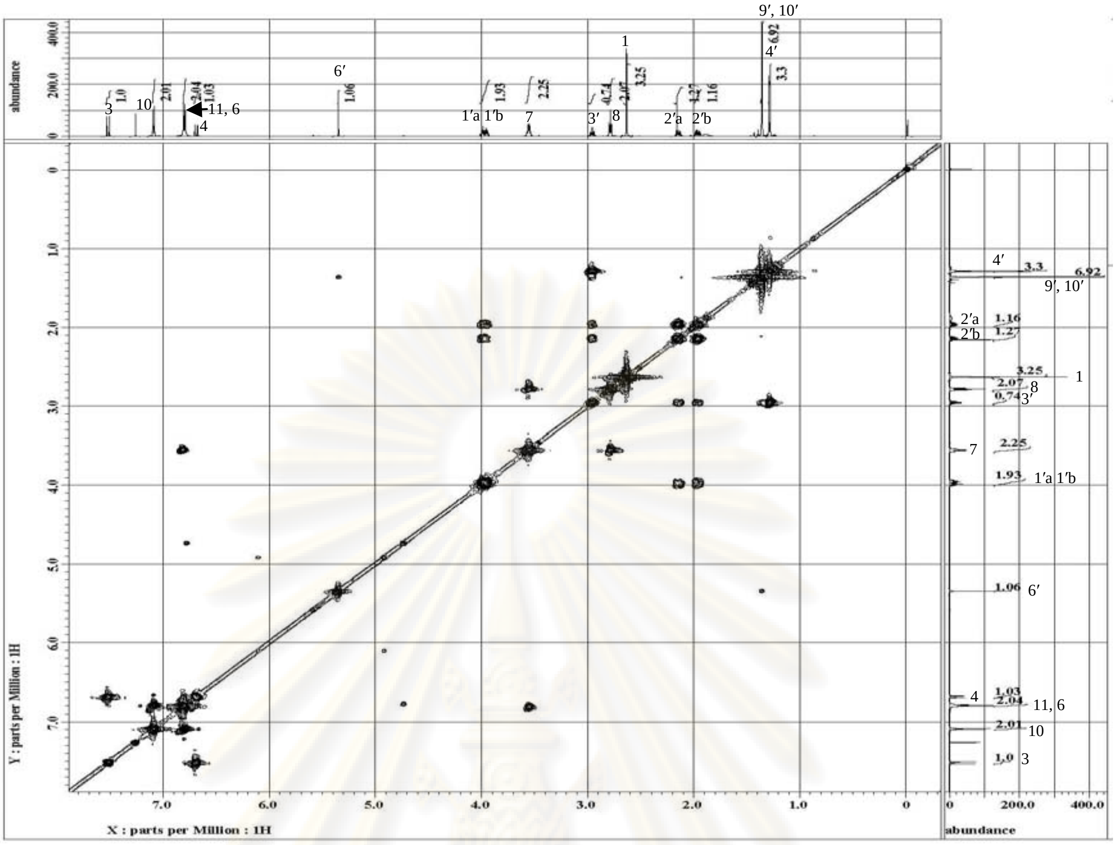


Figure 143 ^{1}H-^{1}H COSY spectrum of compound **GP9** (CDCl$_3$).

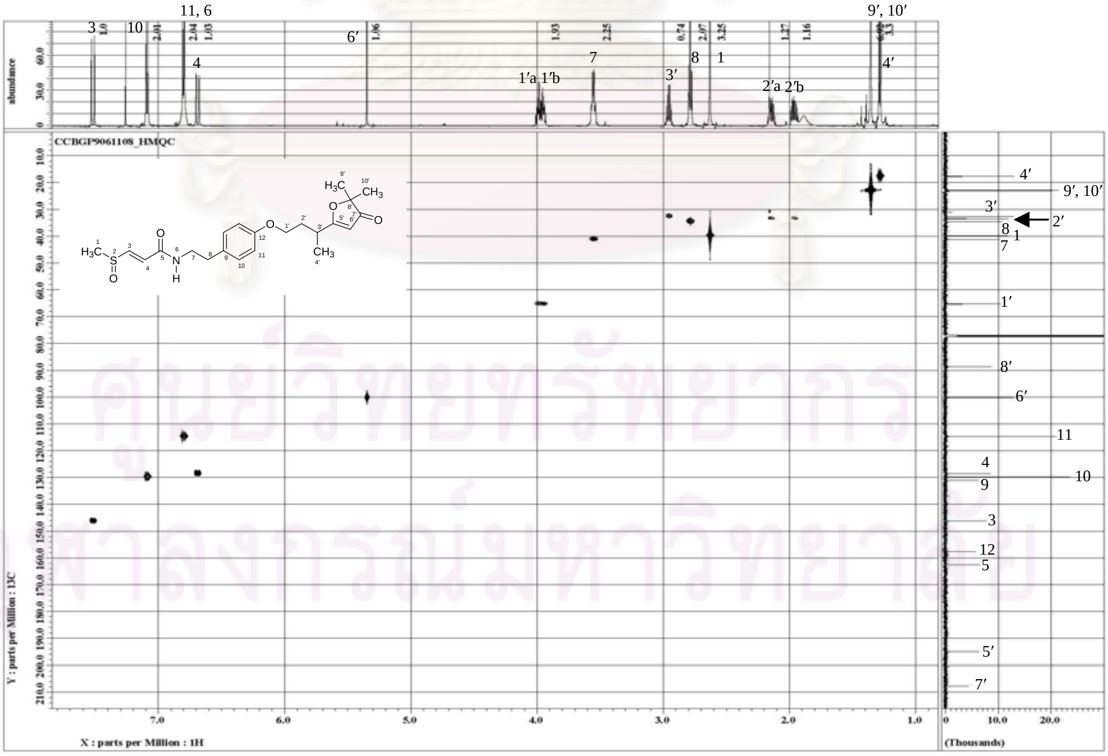


Figure 144 HMQC spectrum of compound **GP9** (CDCl$_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

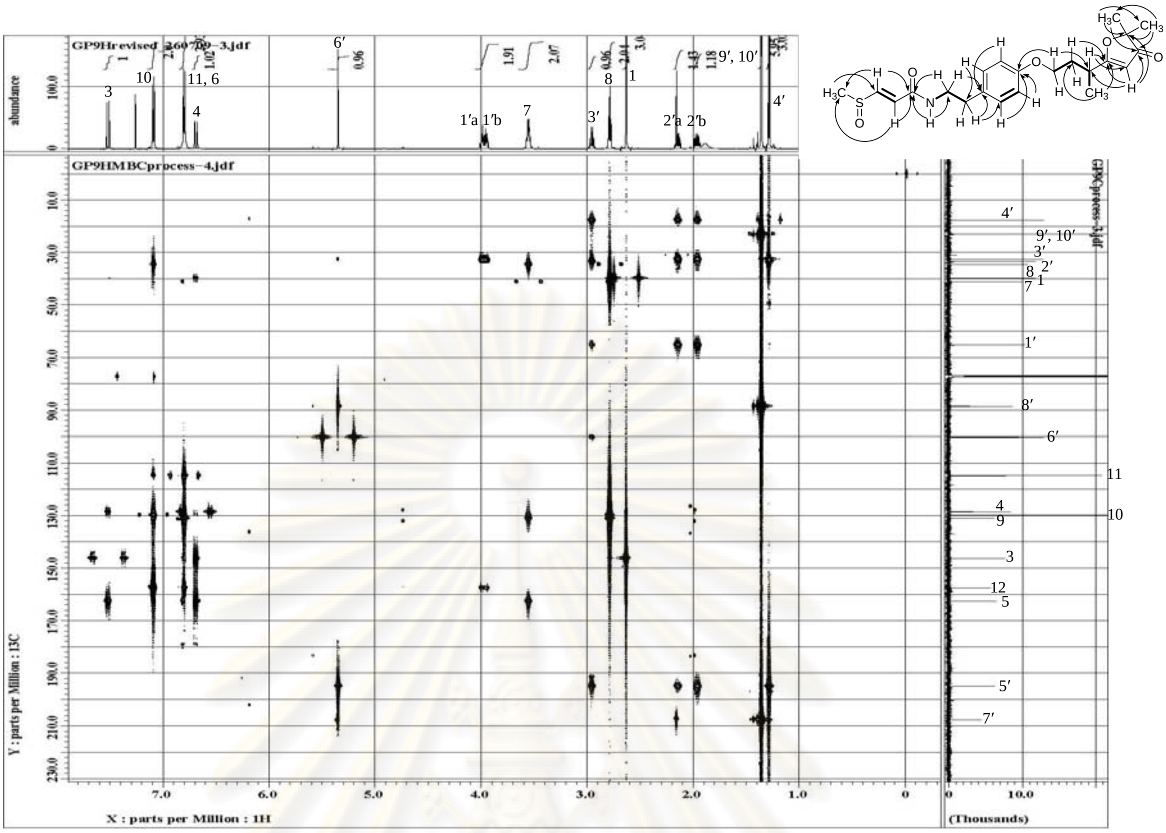


Figure 145 HMBC spectrum of compound **GP9** ($CDCl_3$).

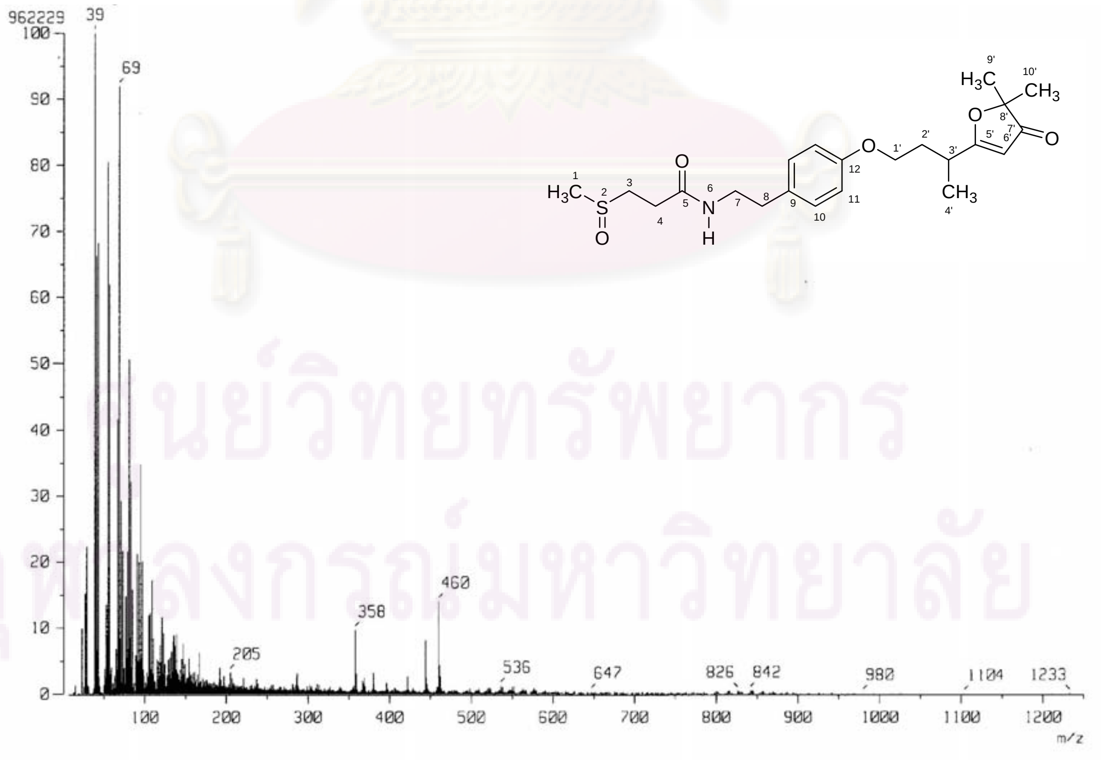


Figure 146 FABMS of compound **GP10**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

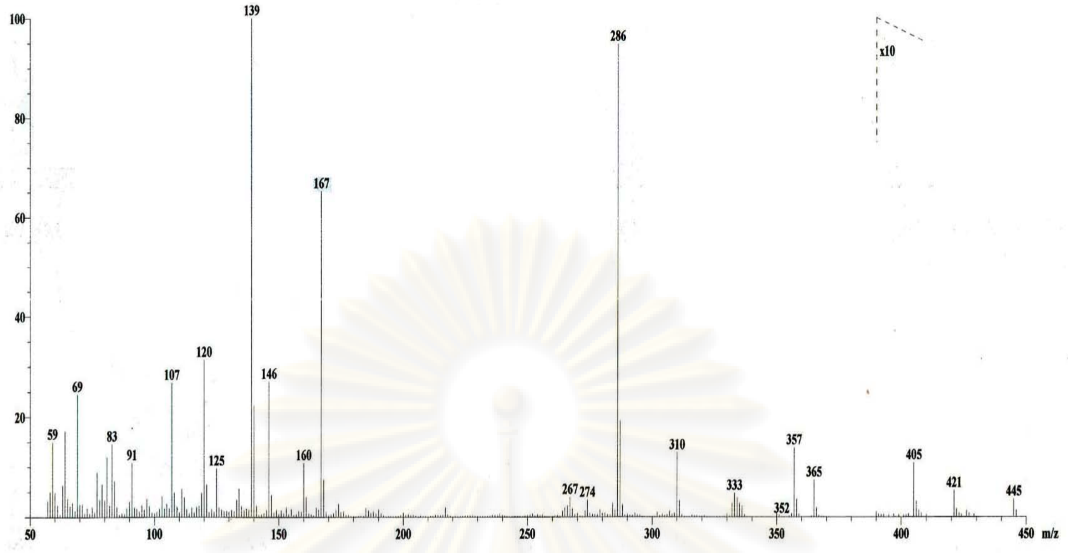


Figure 147 EIMS of compound **GP10**.

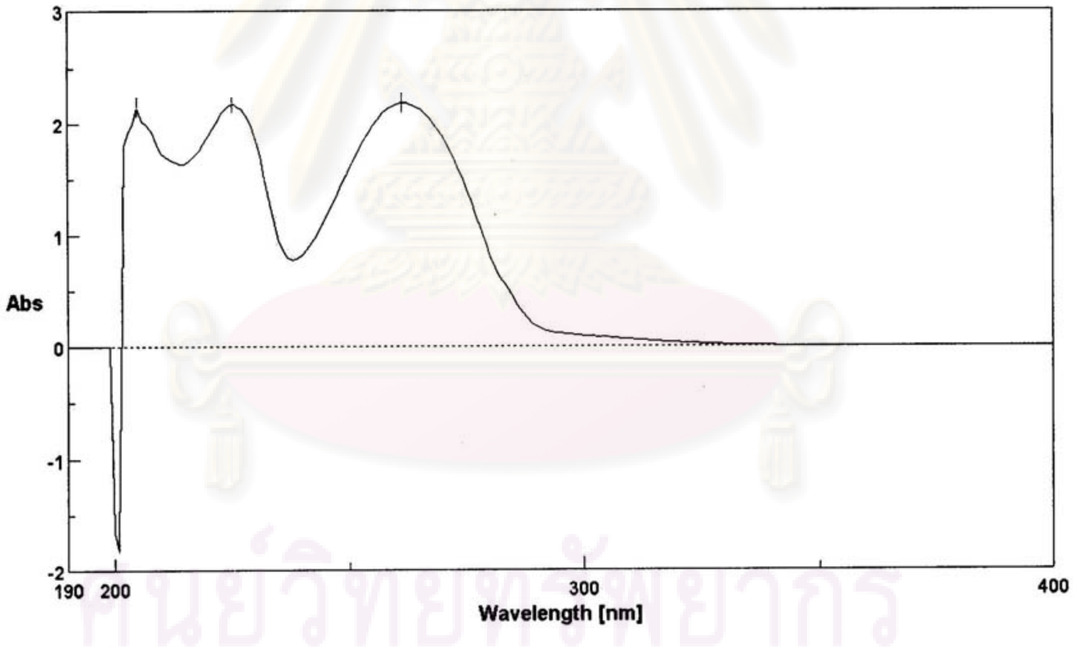


Figure 148 UV spectrum of compound **GP10** (MeOH).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

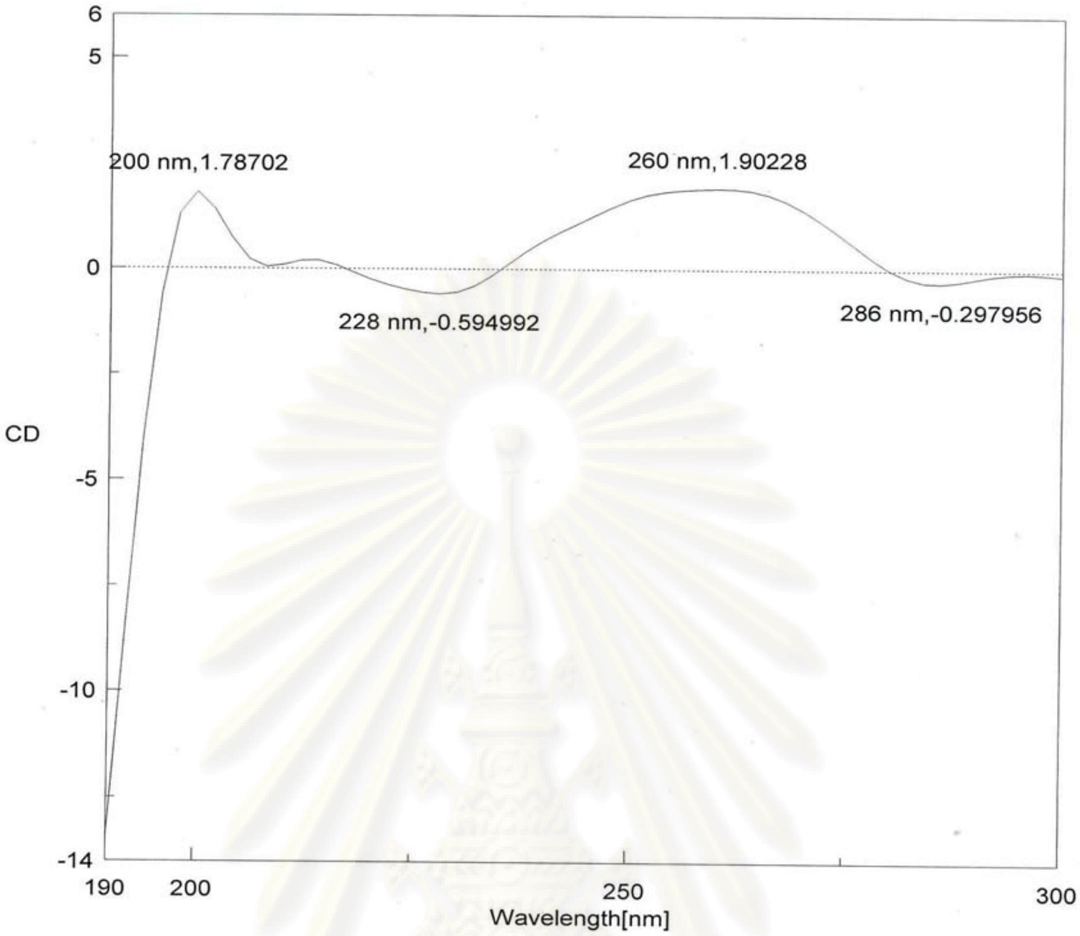


Figure 149 CD spectrum of compound **GP10** (MeOH).

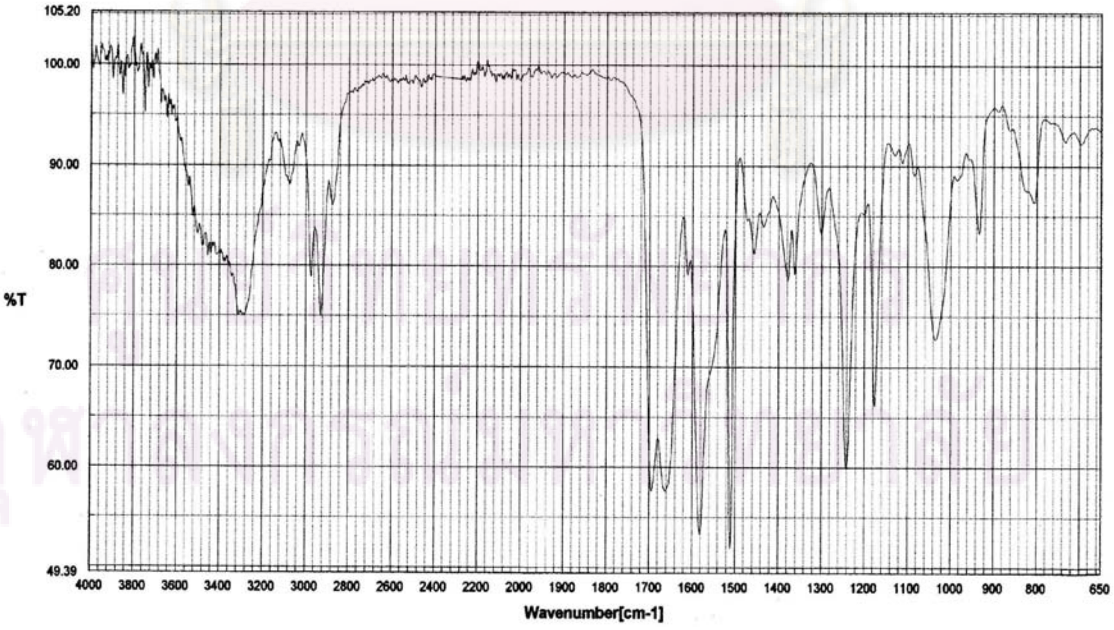


Figure 150 IR spectrum of compound **GP10** (ATR).

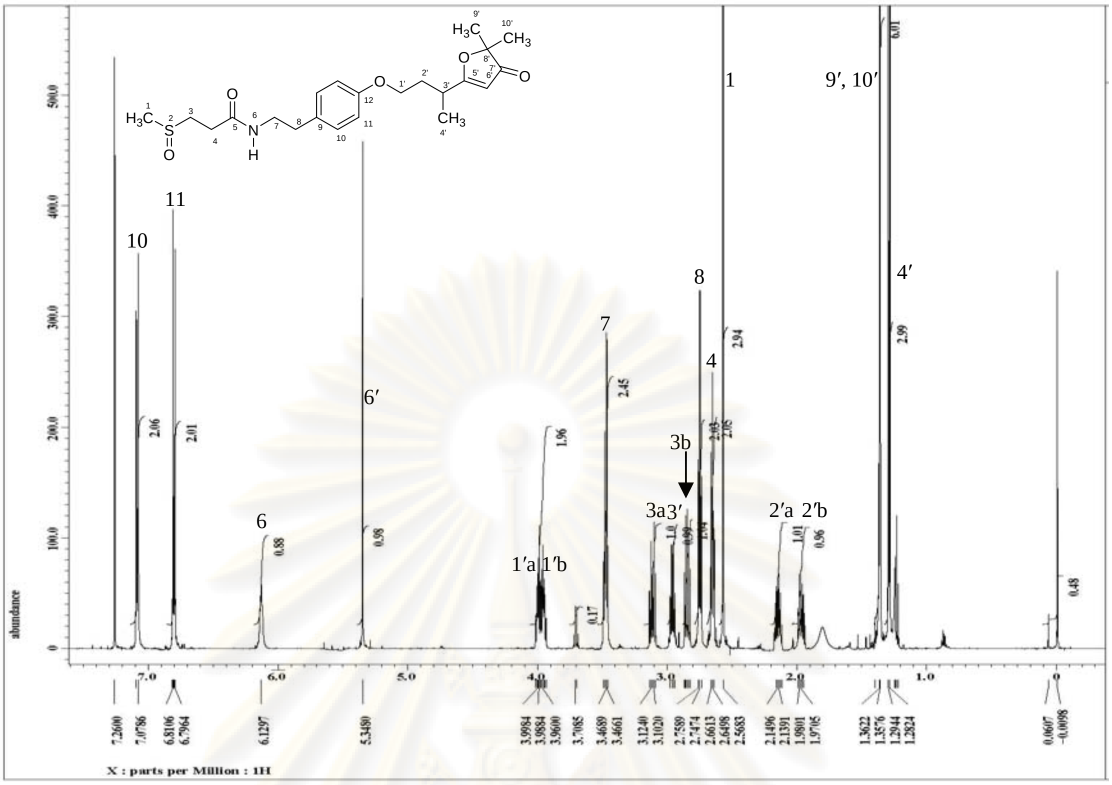


Figure 151 ^{1}H-NMR spectrum of compound **GP10** (600 MHz, $CDCl_3$).

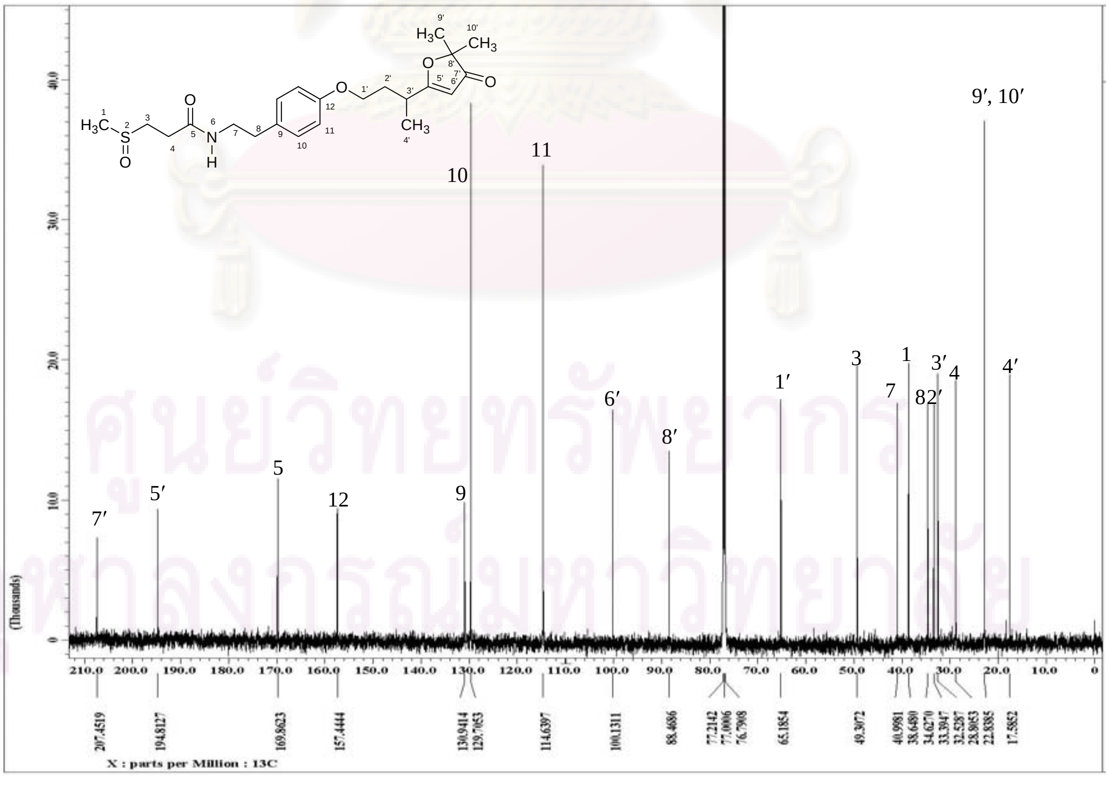


Figure 152 ^{13}C-NMR spectrum of compound **GP10** (150 MHz, $CDCl_3$).

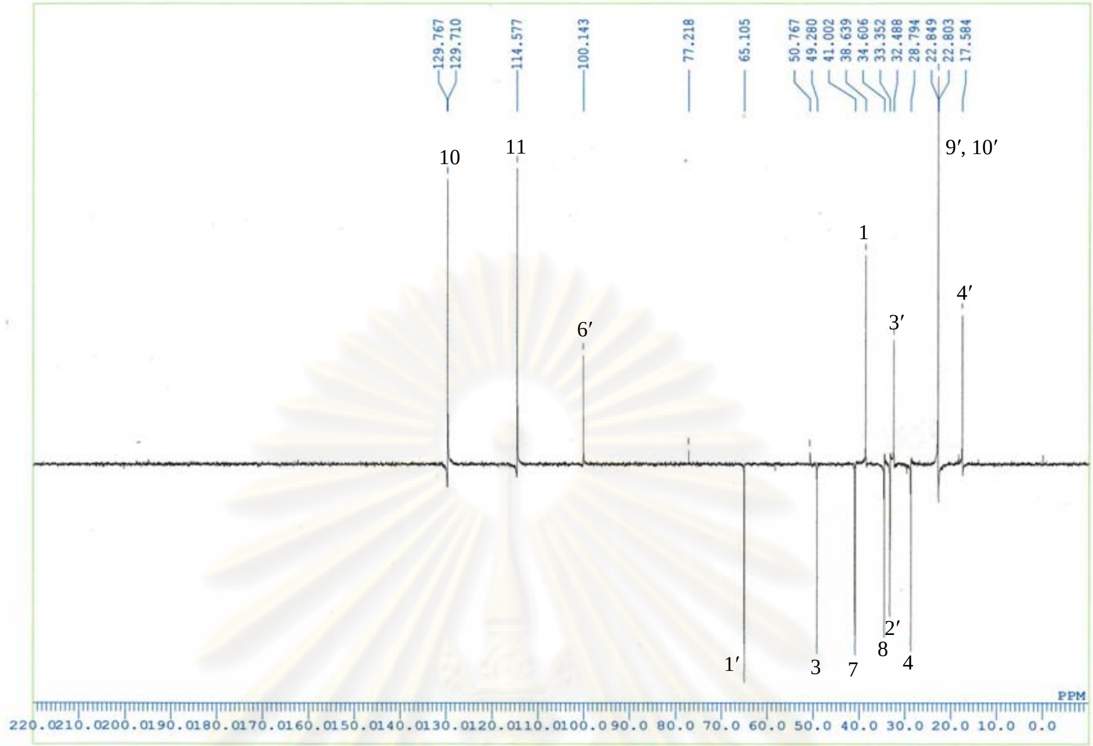


Figure 153 DEPT135 spectrum of compound **GP10** (100 MHz, $CDCl_3$).

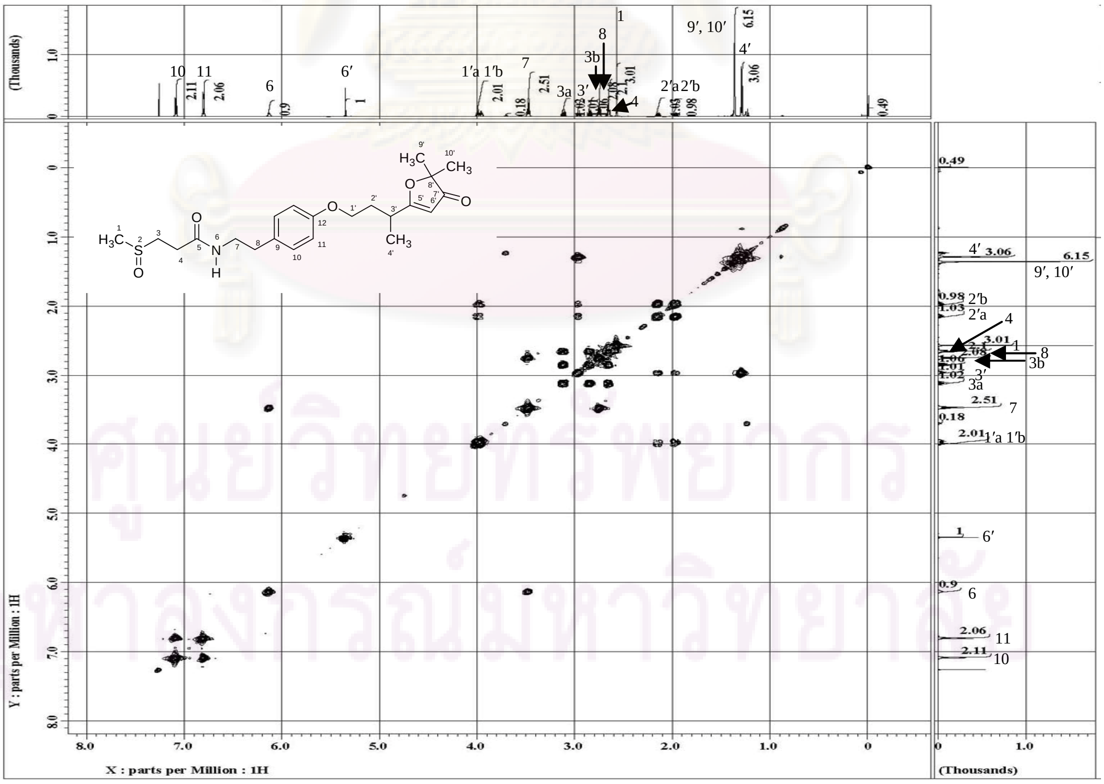


Figure 154 ^{1}H-^{1}H COSY spectrum of compound **GP10** ($CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

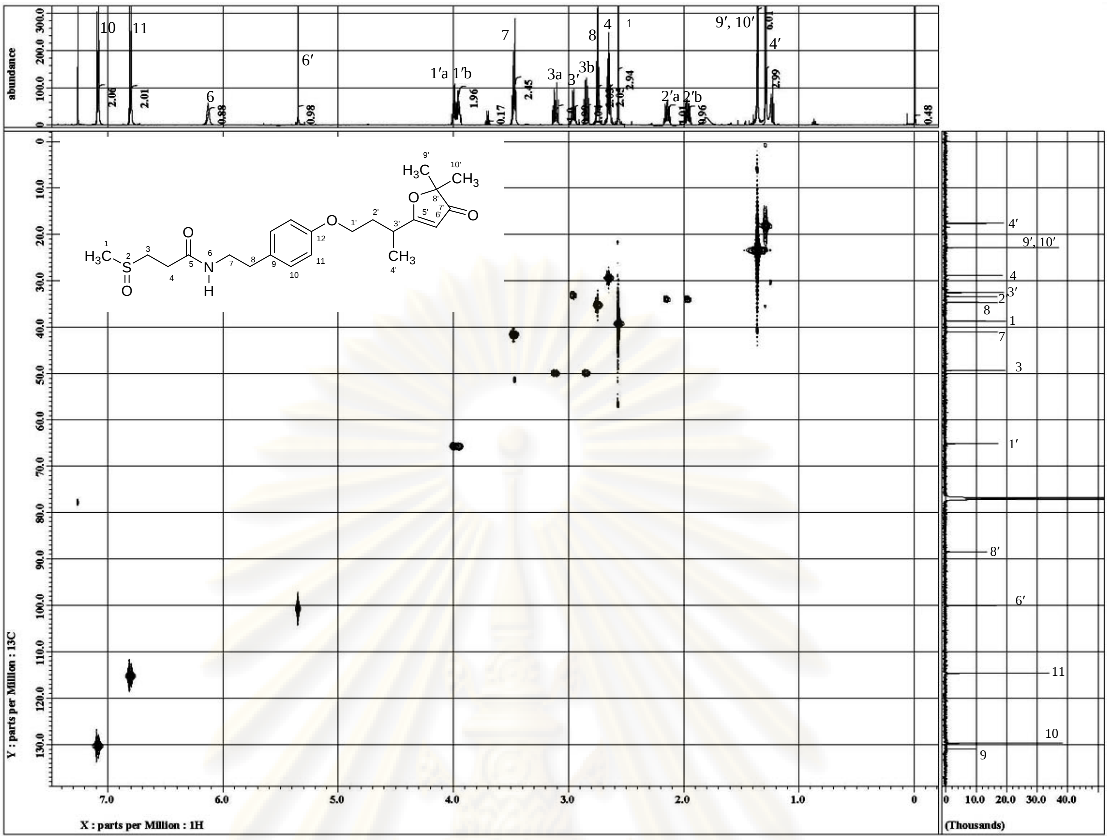


Figure 155 HMQC spectrum of compound **GP10** (CDCl$_3$).

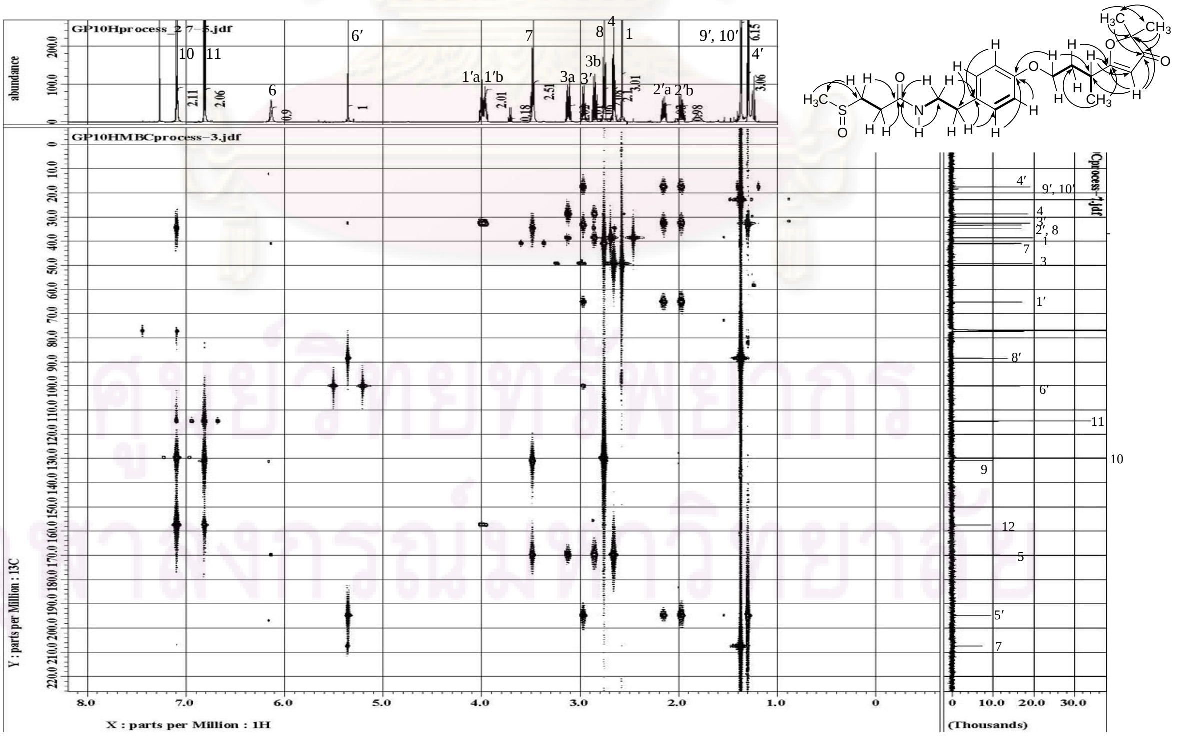


Figure 156 HMBC spectrum of compound **GP10** (CDCl$_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

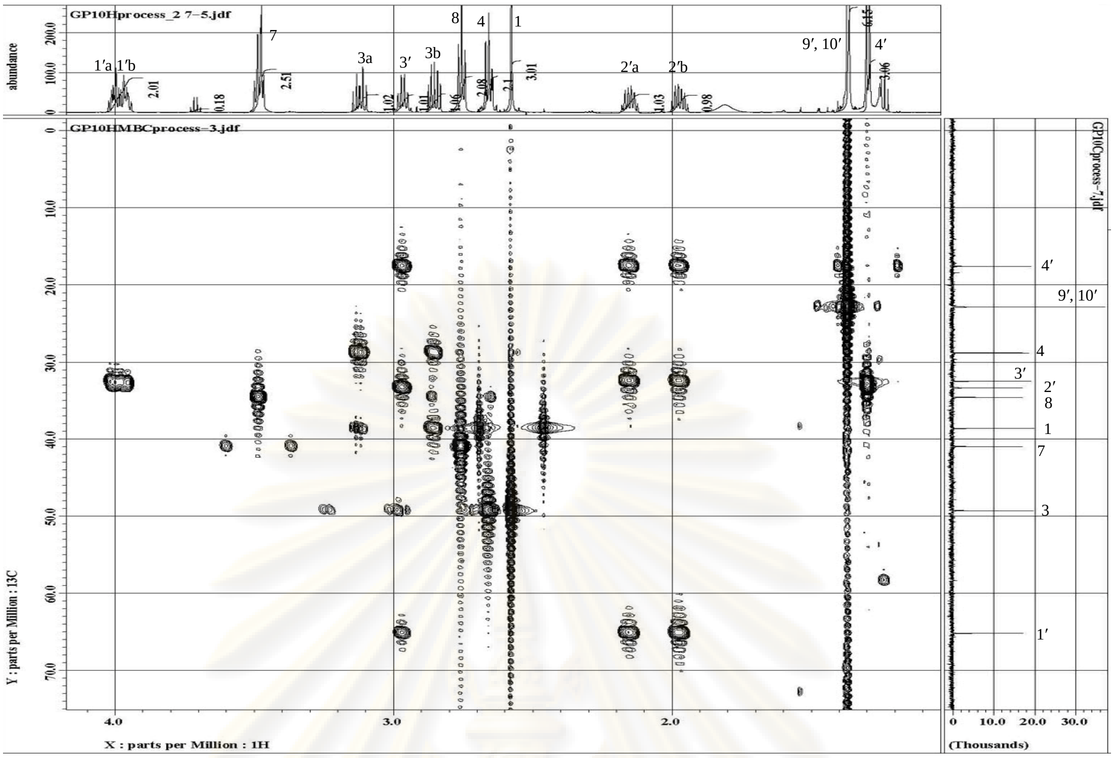


Figure 157 Expanded HMBC spectrum of compound **GP10** ($CDCl_3$).

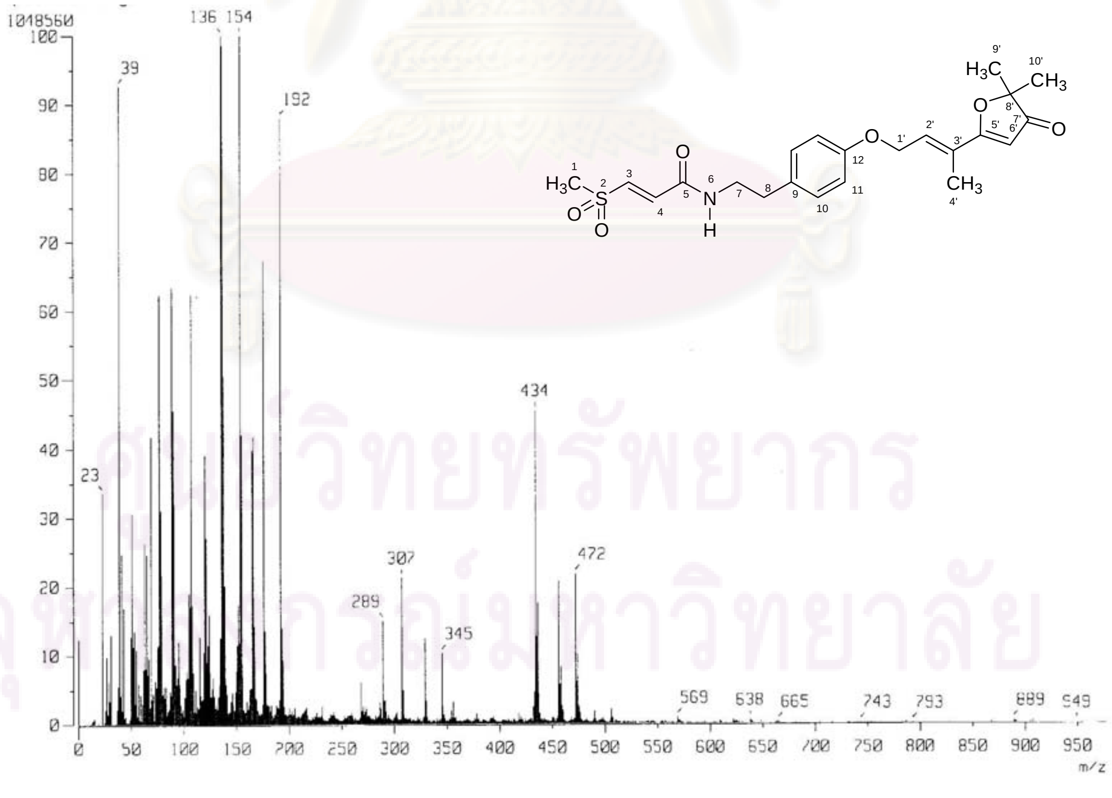


Figure 158 FABMS compound **GP11**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

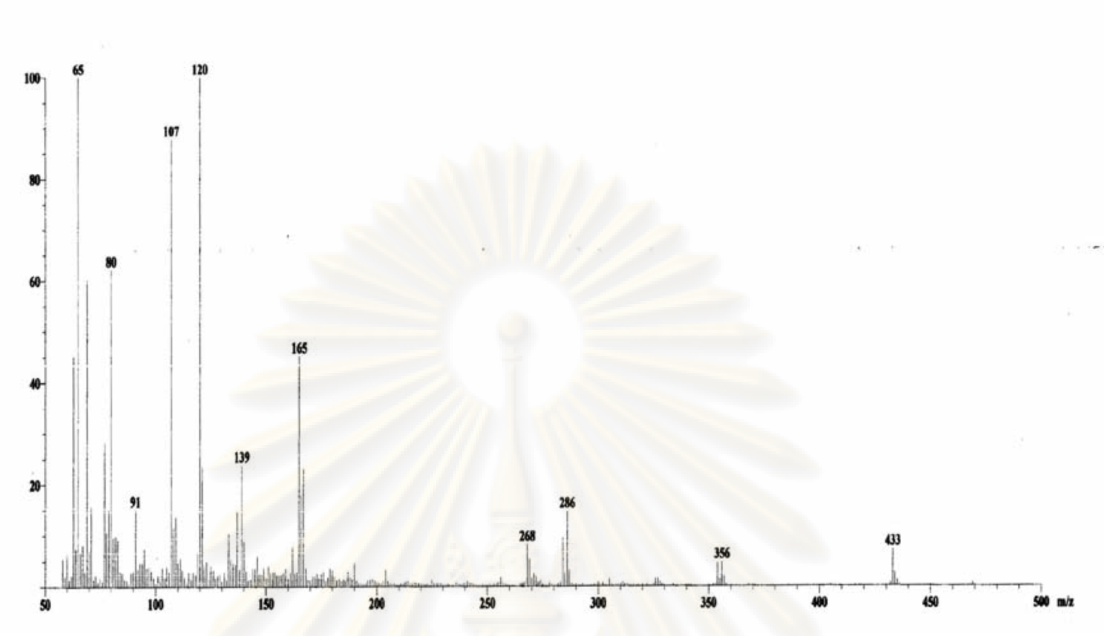


Figure 159 EIMS compound **GP11**.

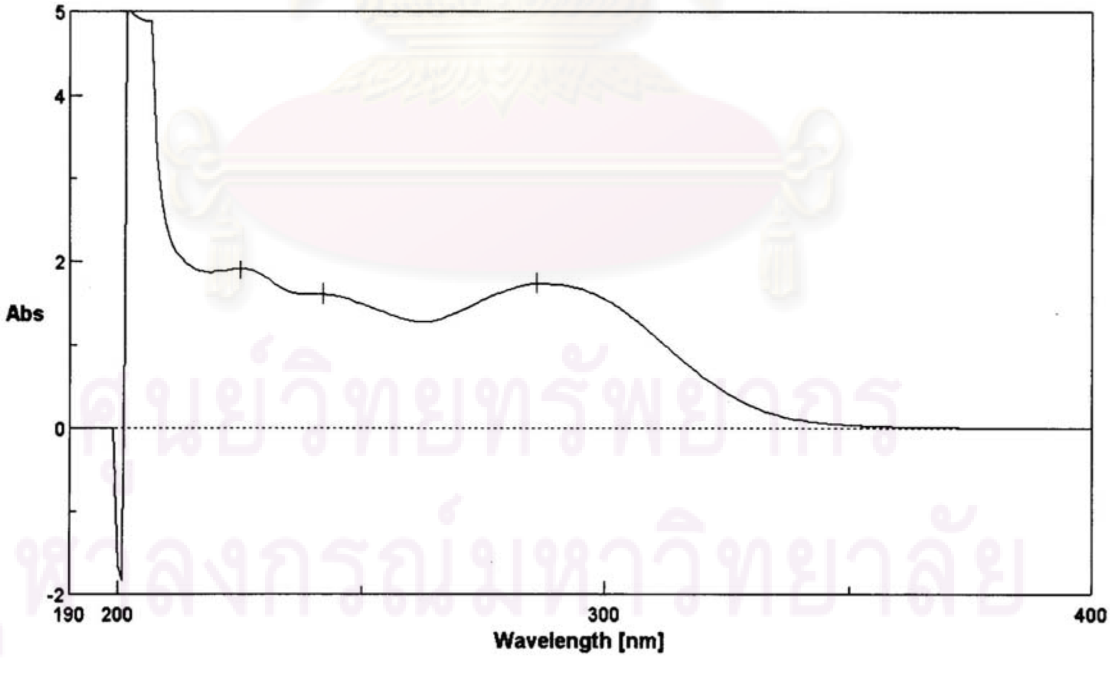


Figure 160 UV spectrum of compound **GP11** (MeOH).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

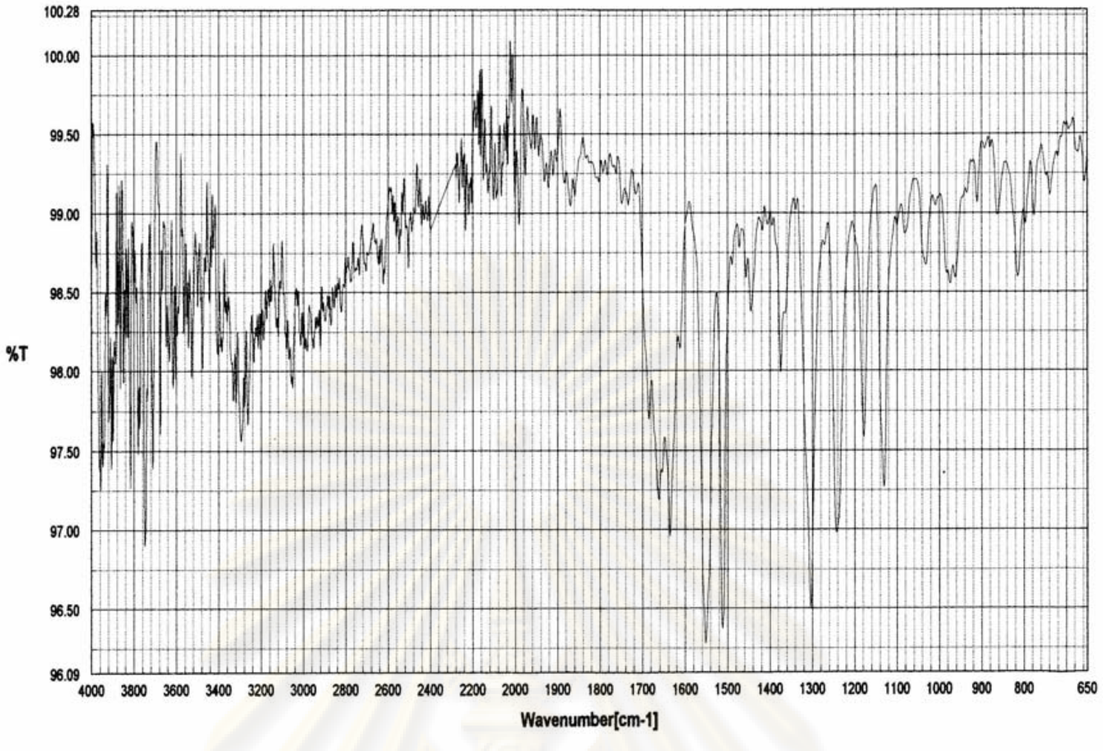


Figure 161 IR spectrum of compound **GP11** (ATR).

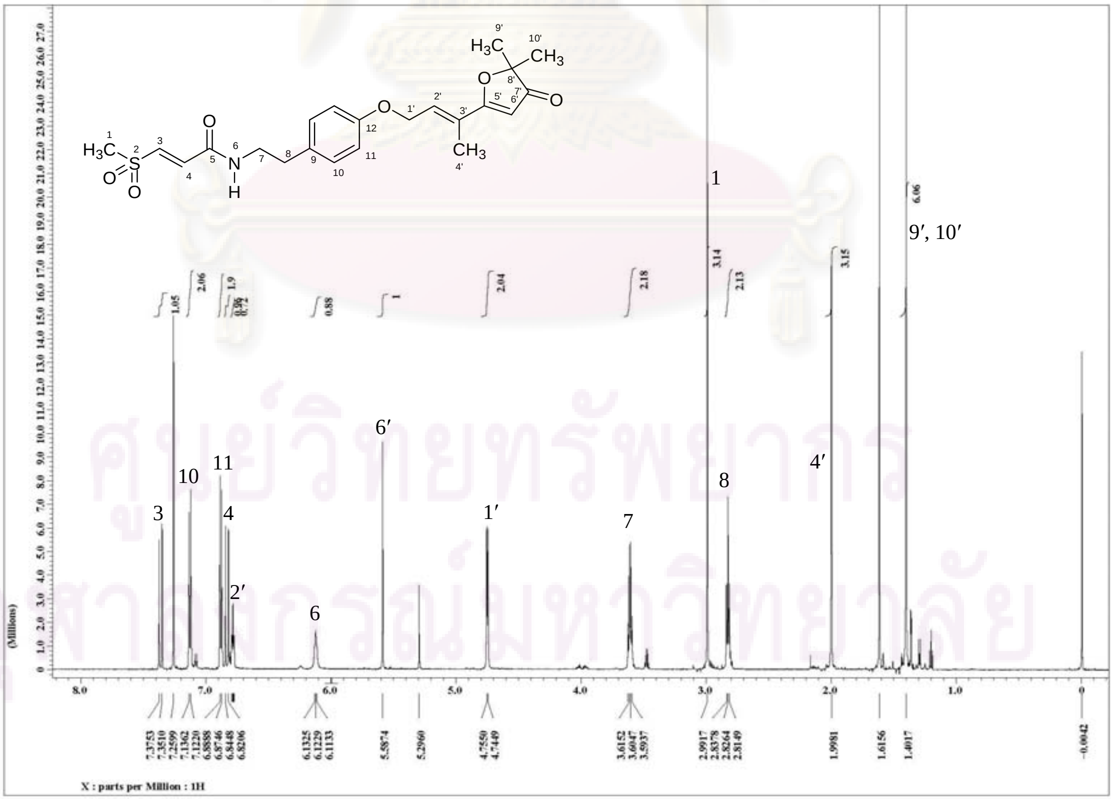


Figure 162 ^{1}H-NMR spectrum of compound **GP11** (600 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

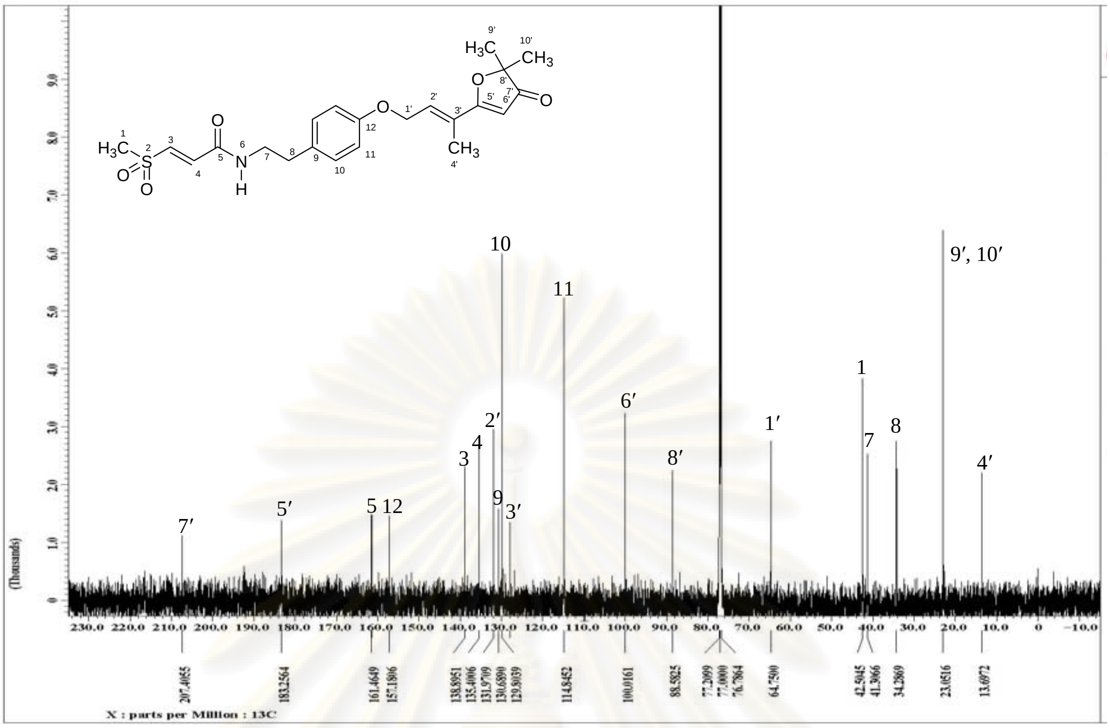


Figure 163 ^{13}C-NMR spectrum of compound **GP11** (150 MHz, $CDCl_3$).

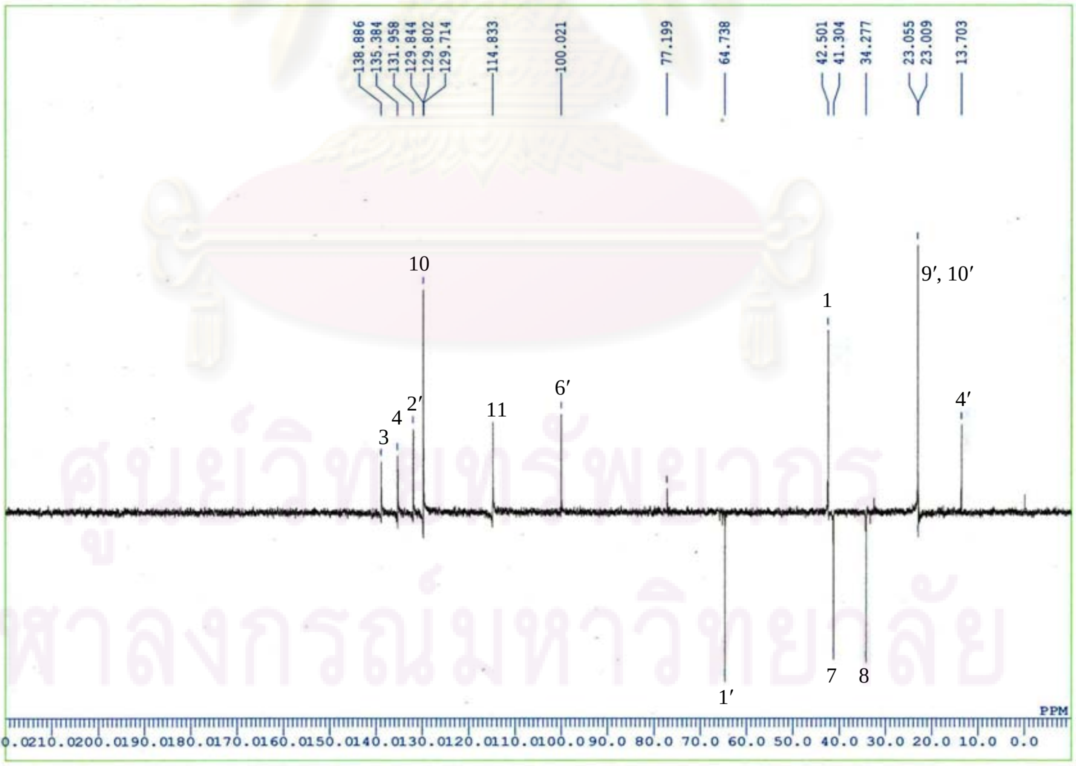


Figure 164 DEPT135 spectrum of compound **GP11** (100 MHz, $CDCl_3$).

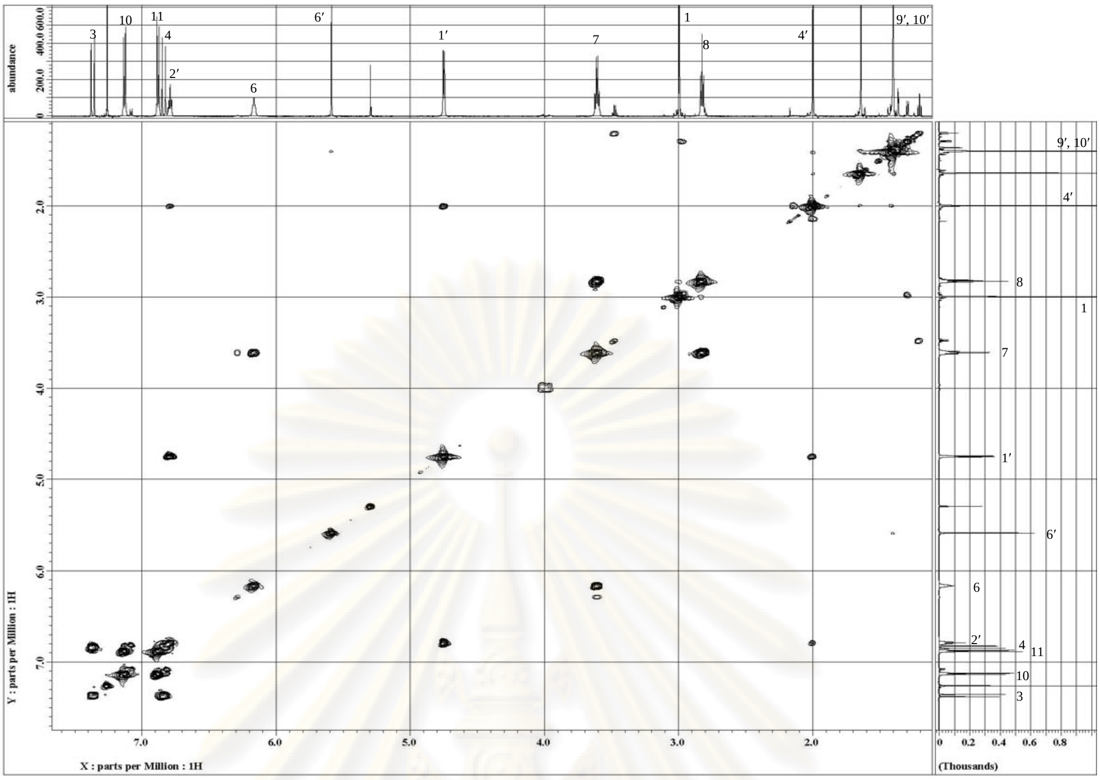


Figure 165 ^{1}H-^{1}H COSY spectrum of compound **GP11** (CDCl$_3$).

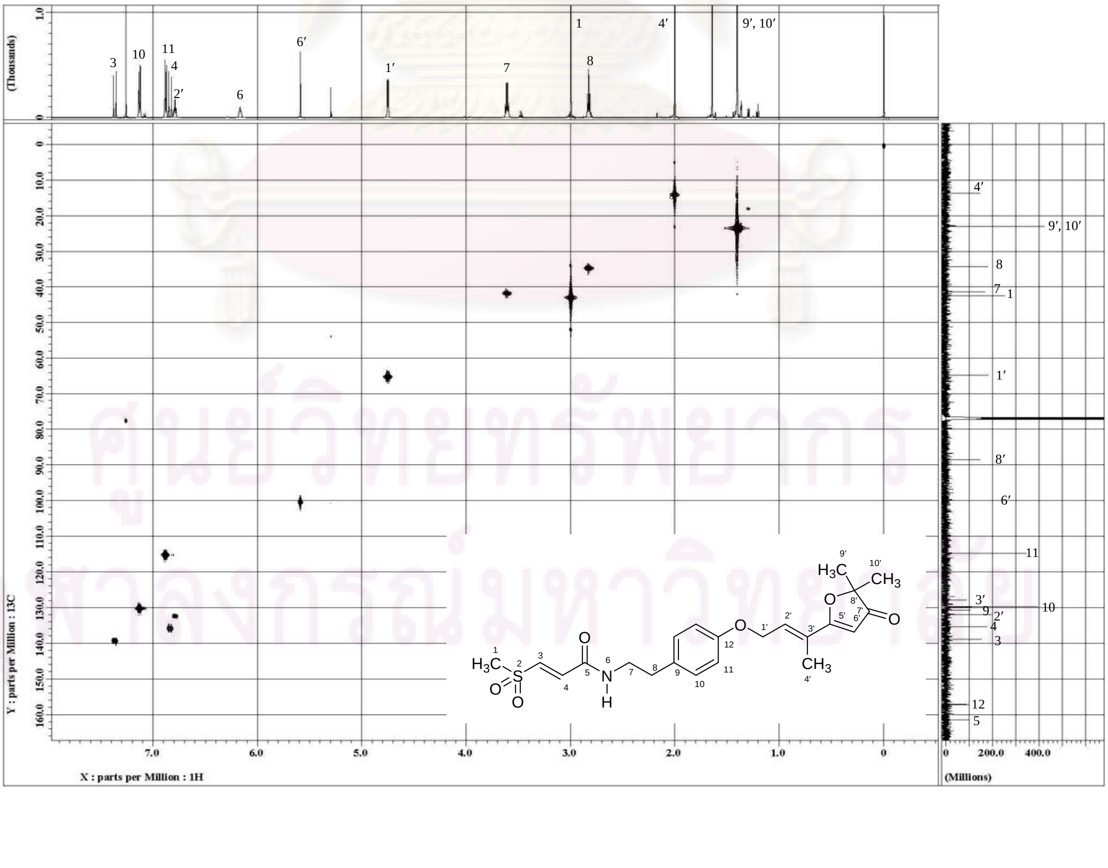


Figure 166 HMQC spectrum of compound **GP11** (CDCl$_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

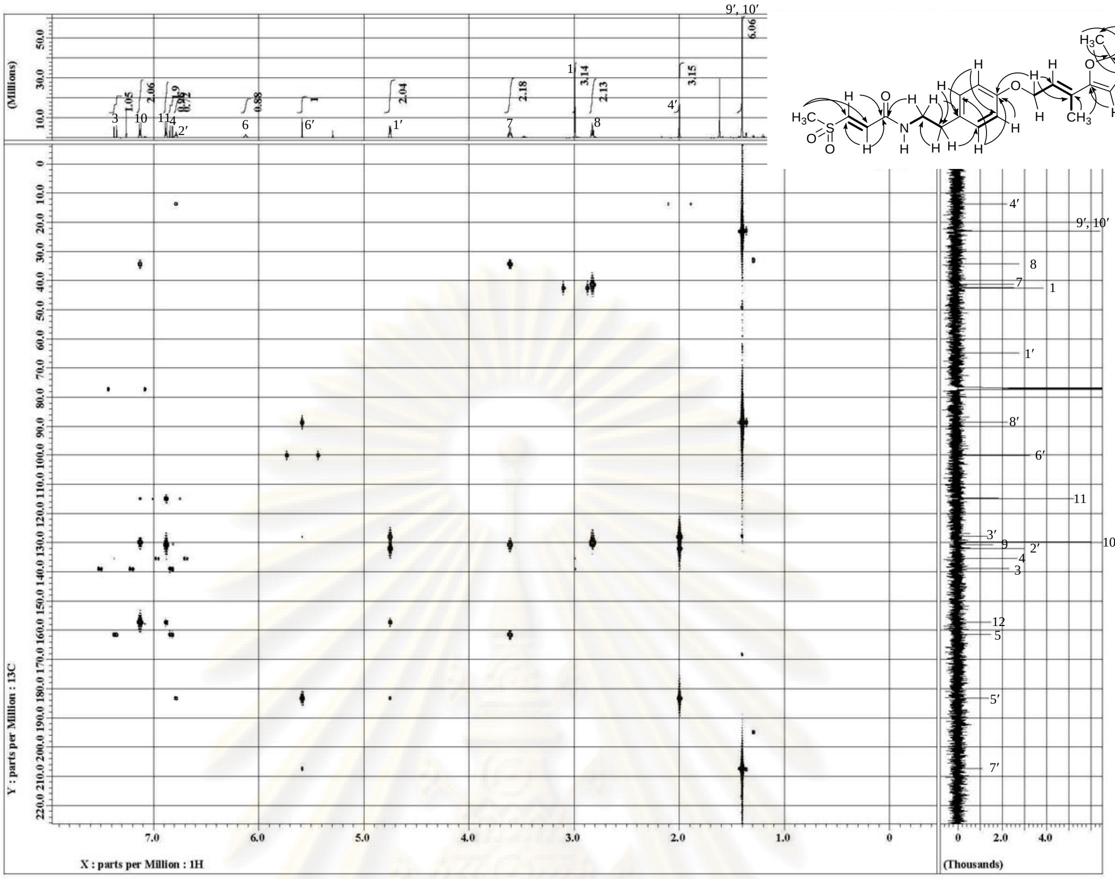


Figure 167 HMBC spectrum of compound **GP11** ($CDCl_3$).

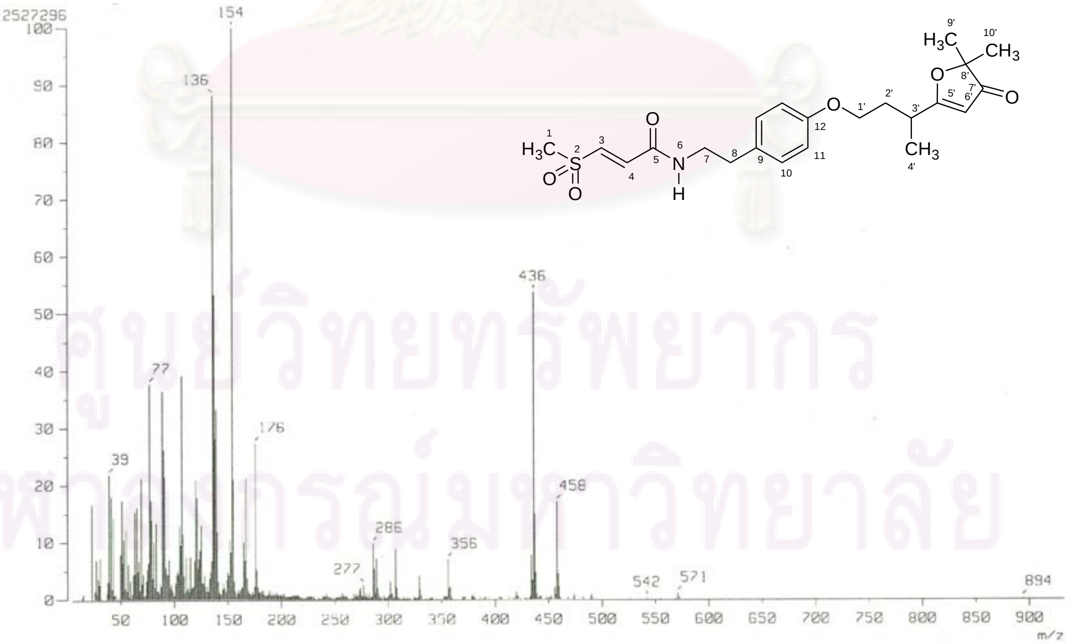


Figure 168 FABMS of compound **GP12**.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

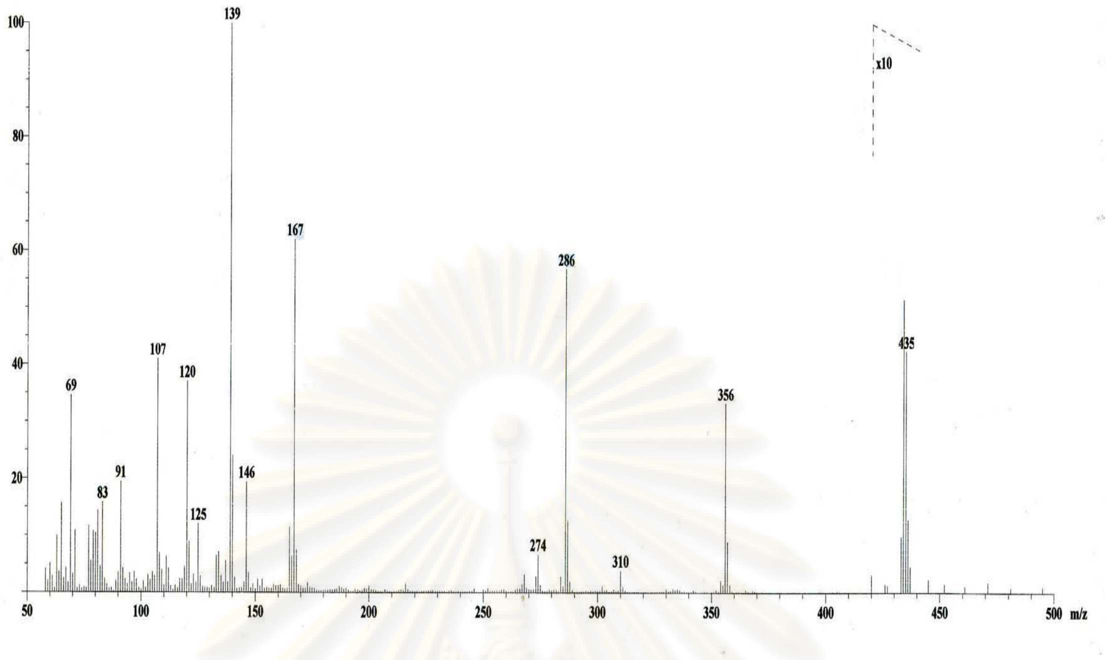


Figure 169 EIMS of compound **GP12**.

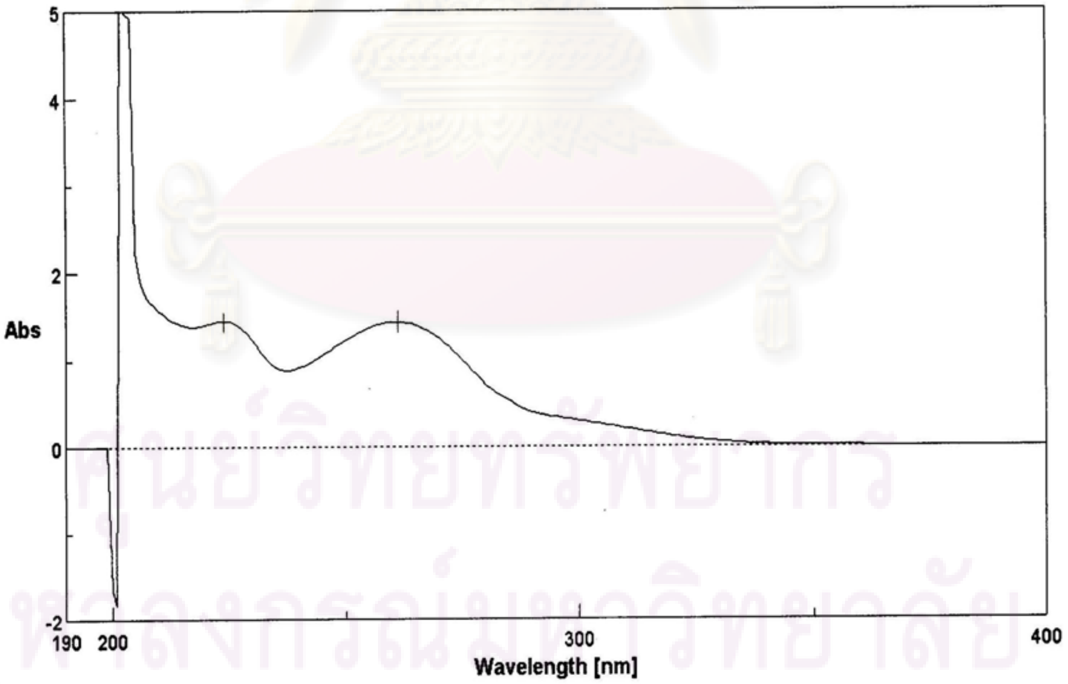


Figure 170 UV spectrum of compound **GP12** (MeOH).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

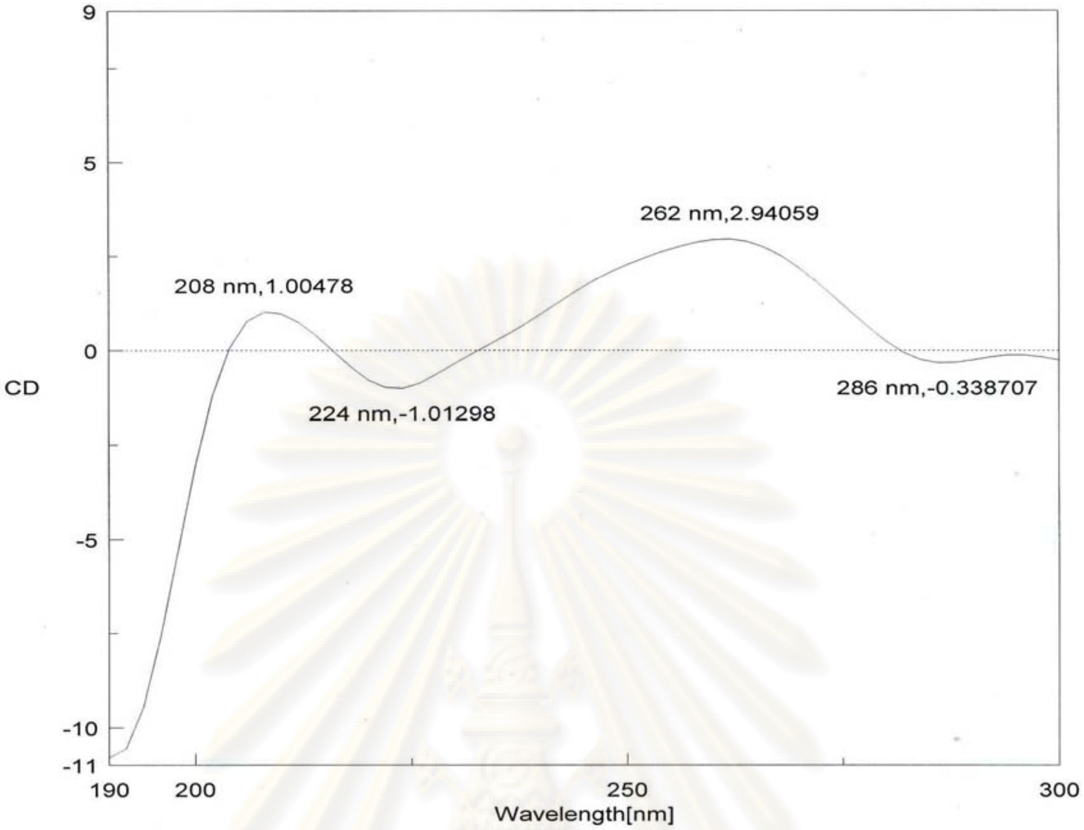


Figure 171 CD spectrum of compound **GP12** (MeOH).

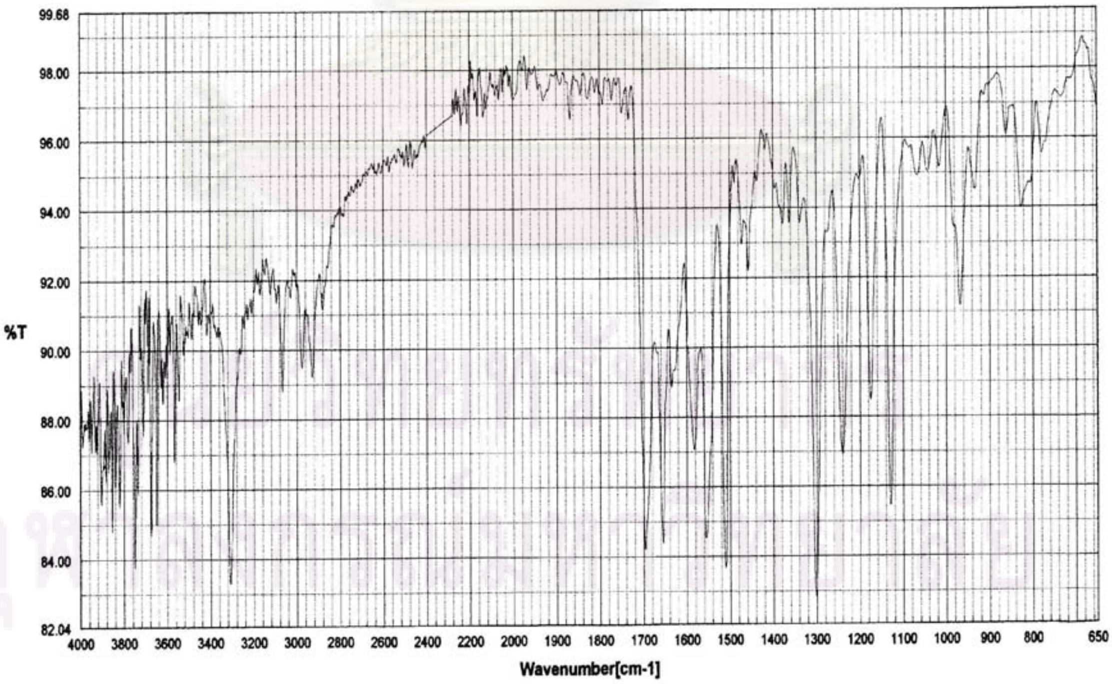


Figure 172 IR spectrum of compound **GP12** (ATR).

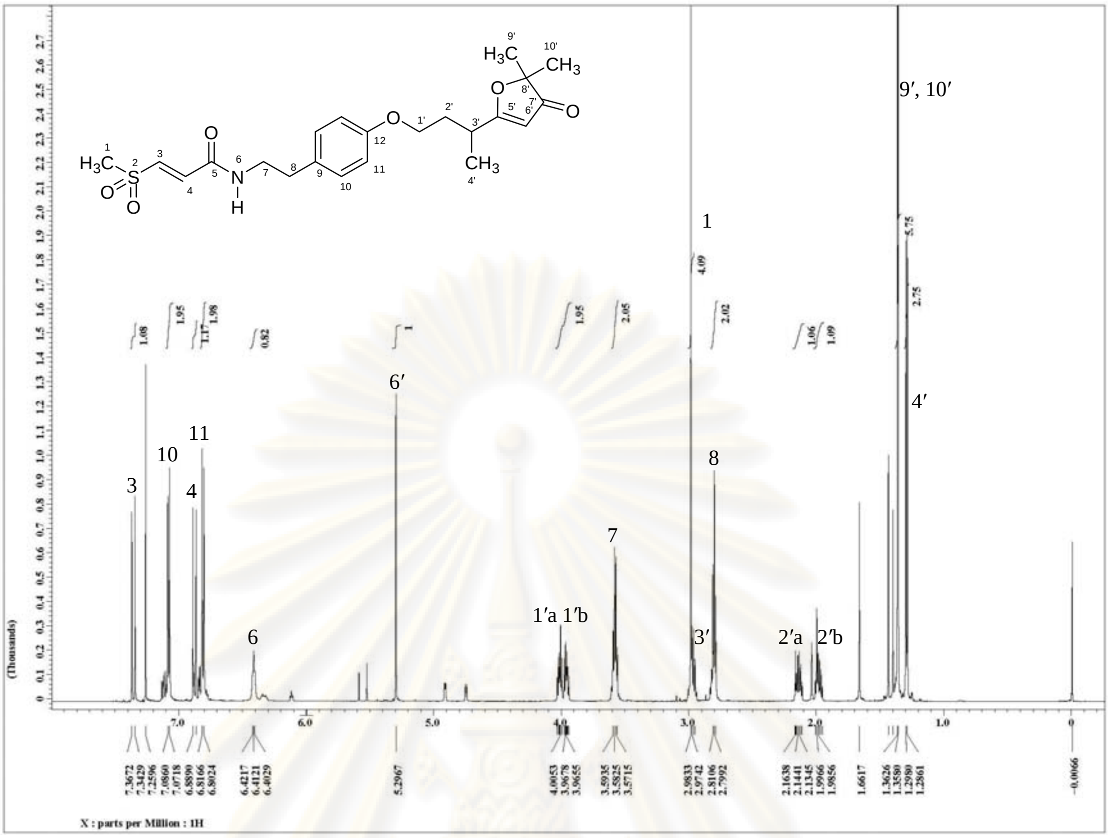


Figure 173 ^{1}H-NMR spectrum of compound **GP12** (600 MHz, CDCl$_3$).

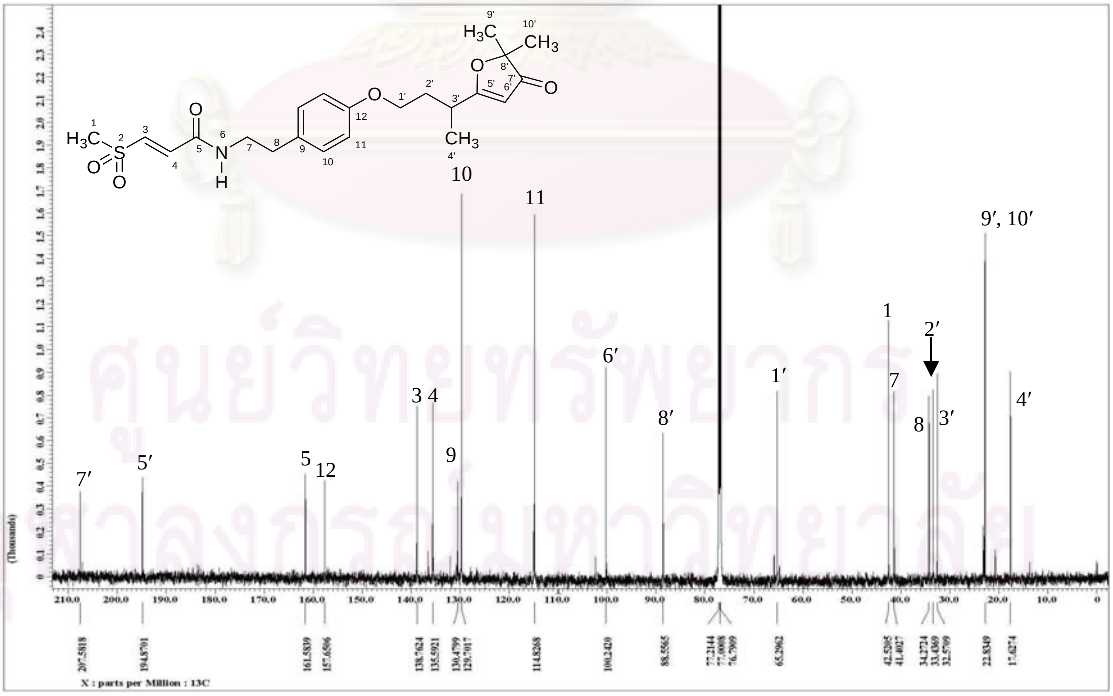


Figure 174 ^{13}C-NMR spectrum of compound **GP12** (150 MHz, CDCl$_3$).

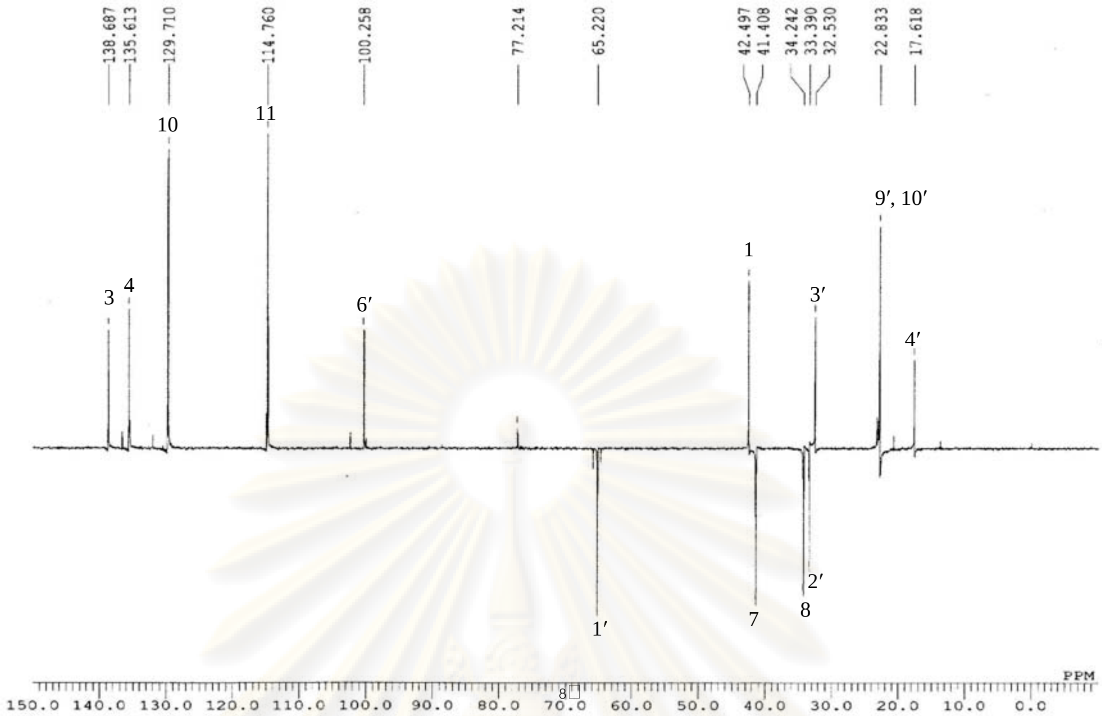


Figure 175 DEPT135 spectrum of compound **GP12** (100 MHz, $CDCl_3$).

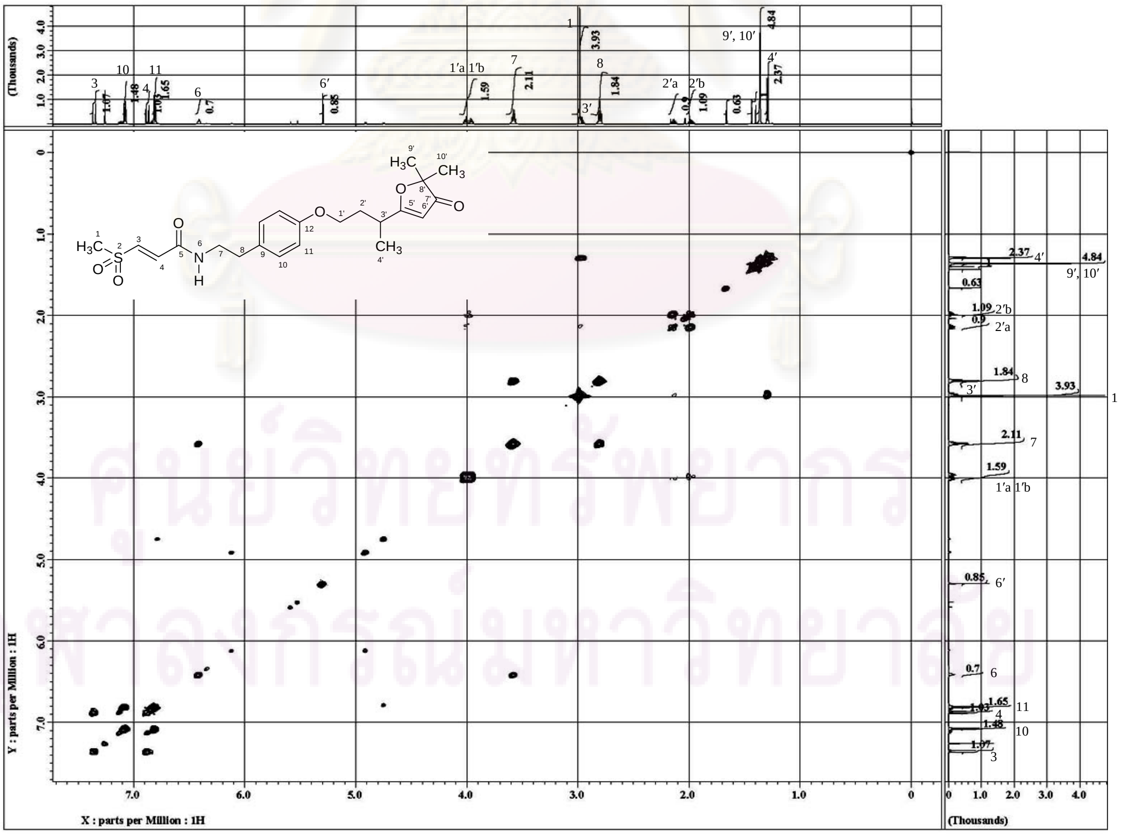


Figure 176 ^1H-^1H COSY spectrum of compound **GP12** ($CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

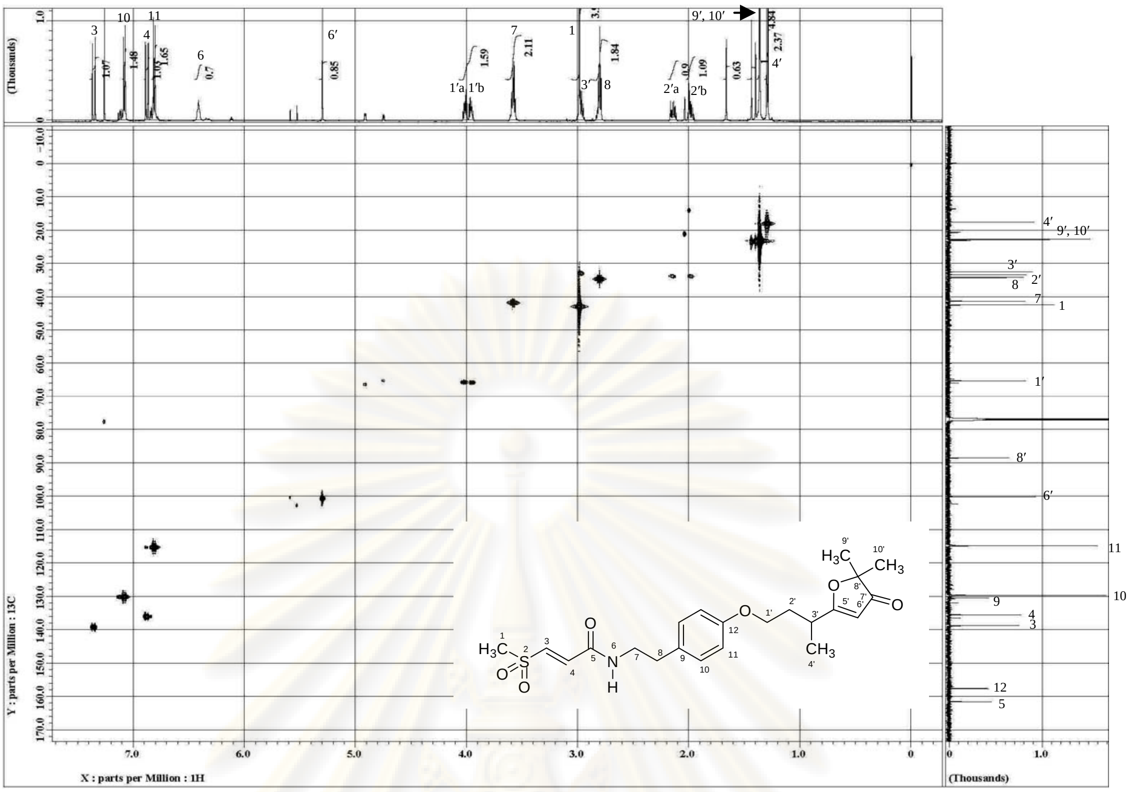


Figure 177 HMQC spectrum of compound **GP12** ($CDCl_3$).

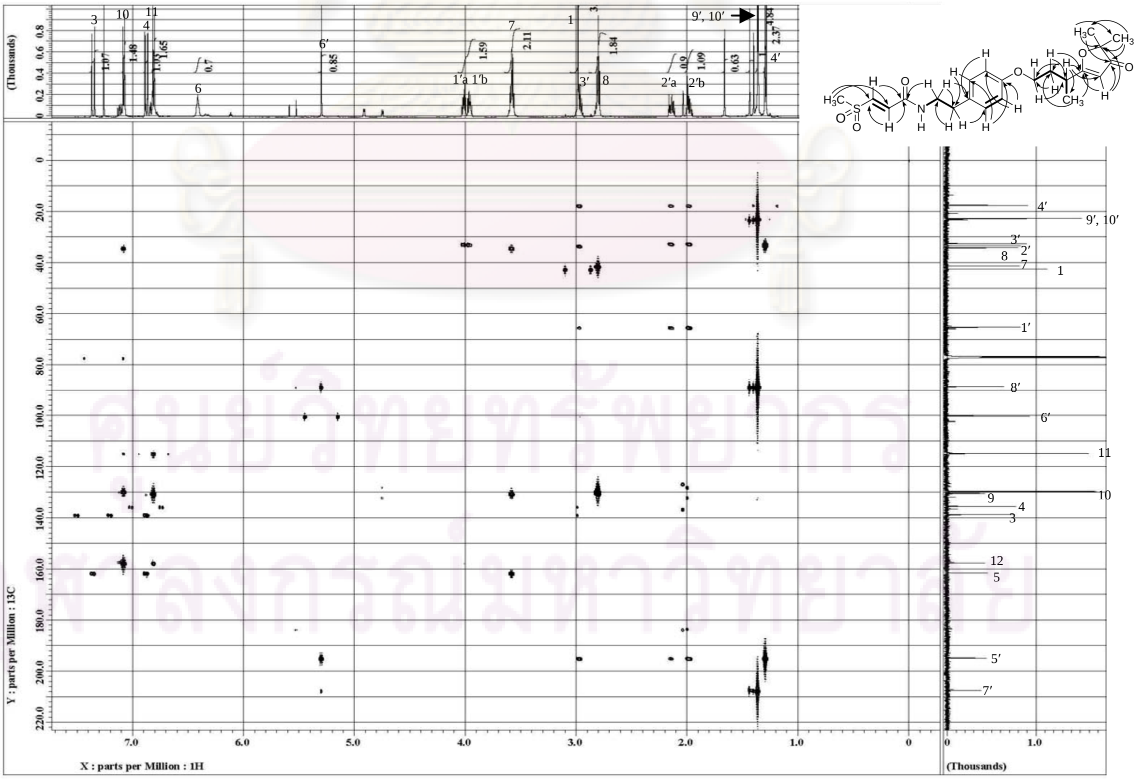


Figure 178 HMBC spectrum of compound **GP12** ($CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

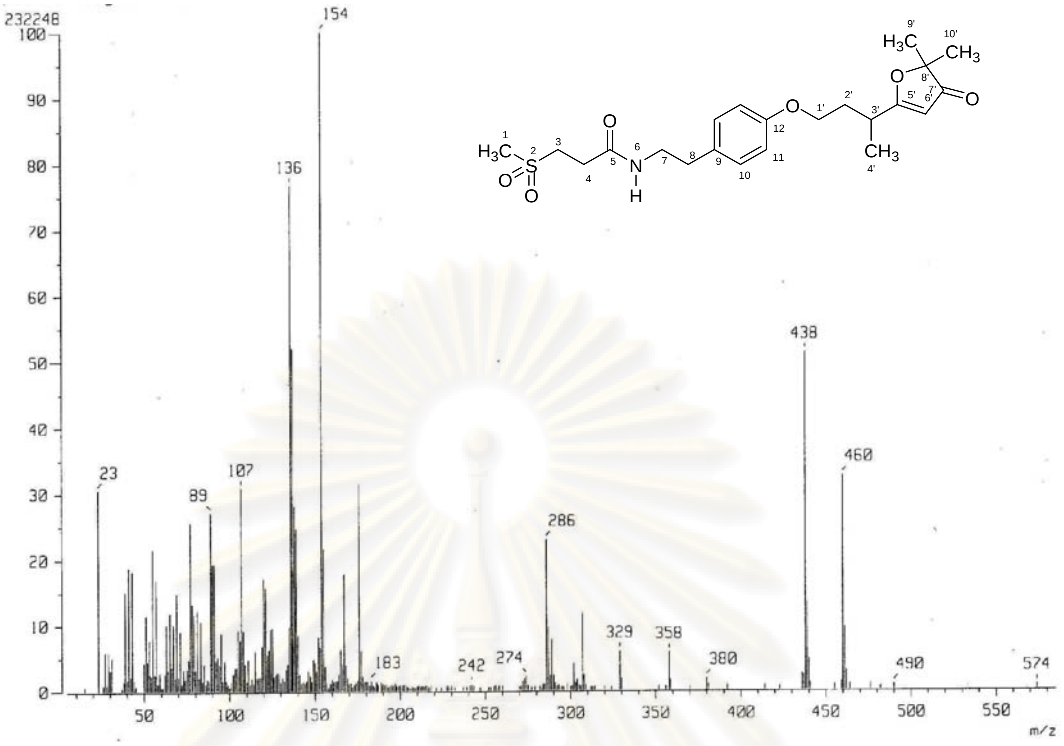


Figure 179 FABMS of compound **GP13**.

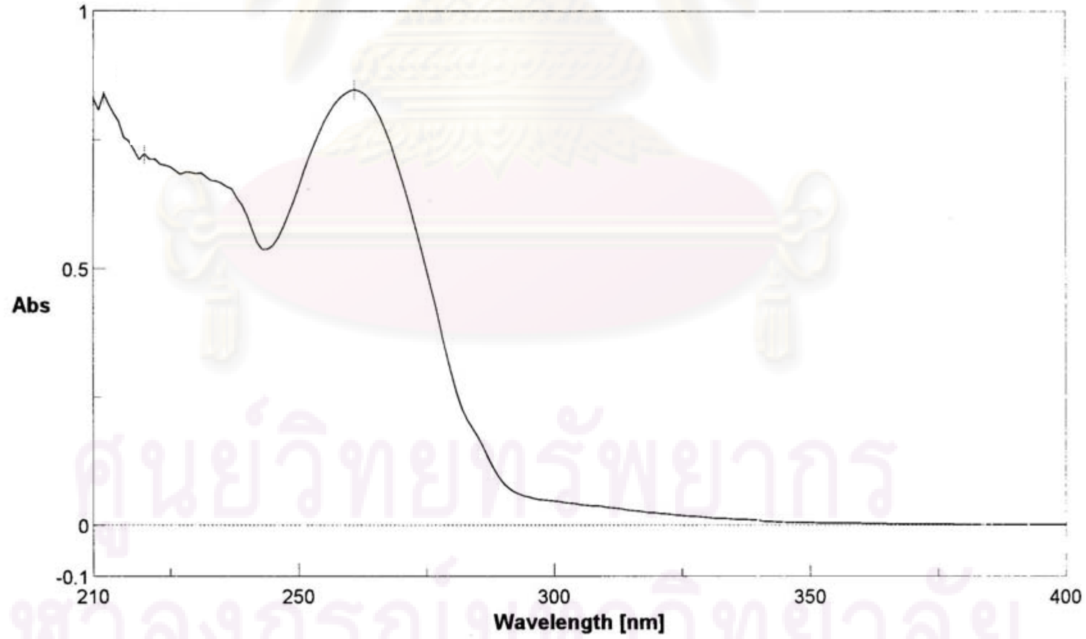


Figure 180 UV spectrum of compound **GP13** ($CHCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

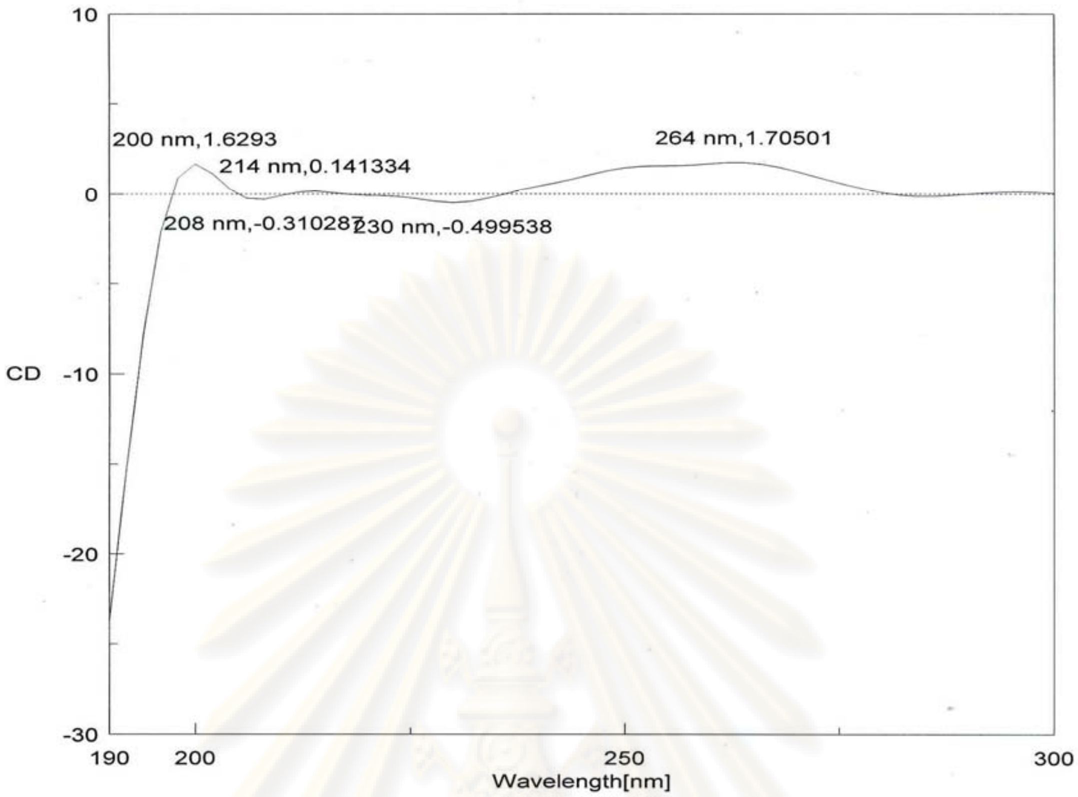


Figure 181 CD spectrum of compound **GP13** (MeOH).

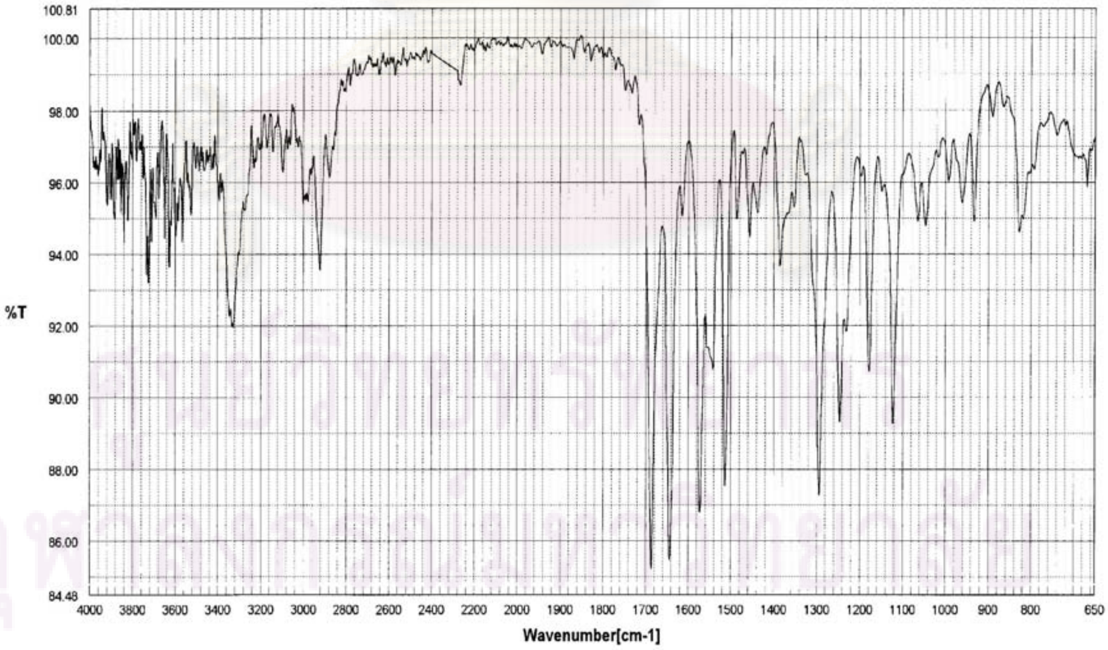


Figure 182 IR spectrum of compound **GP13** (ATR).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

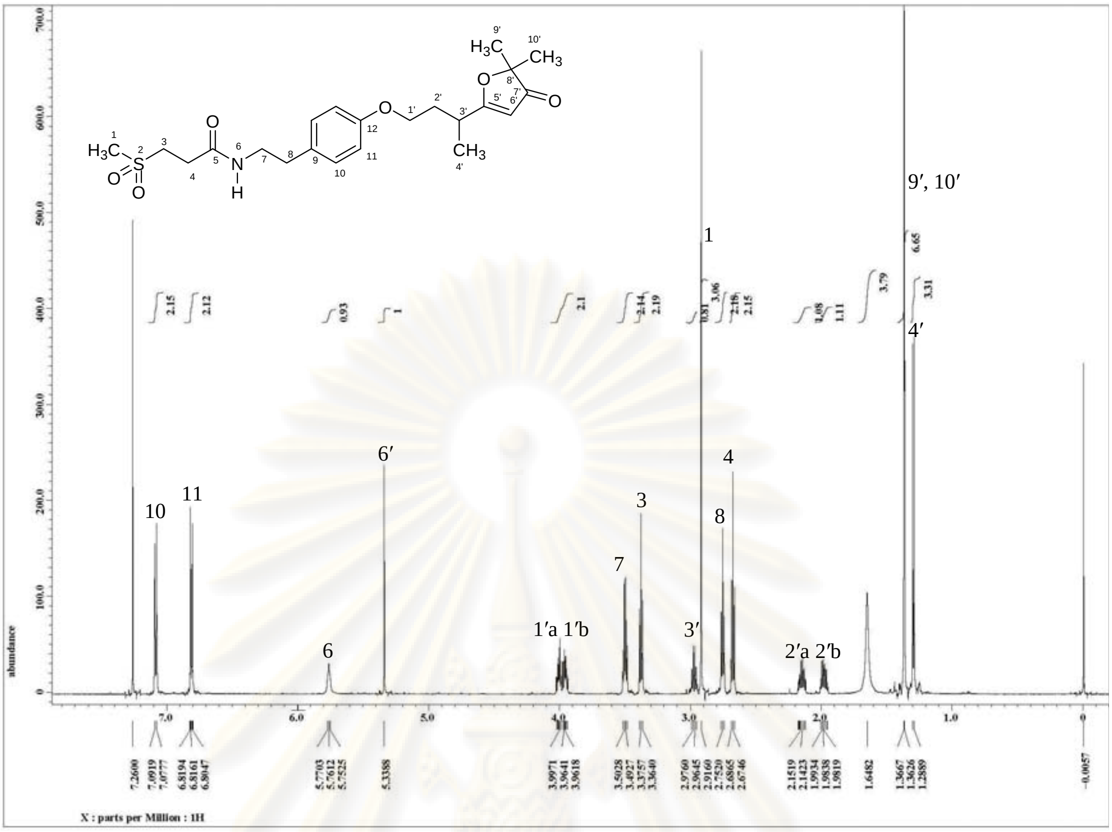


Figure 183 ^{1}H-NMR spectrum of compound **GP13** (600 MHz, $CDCl_3$).

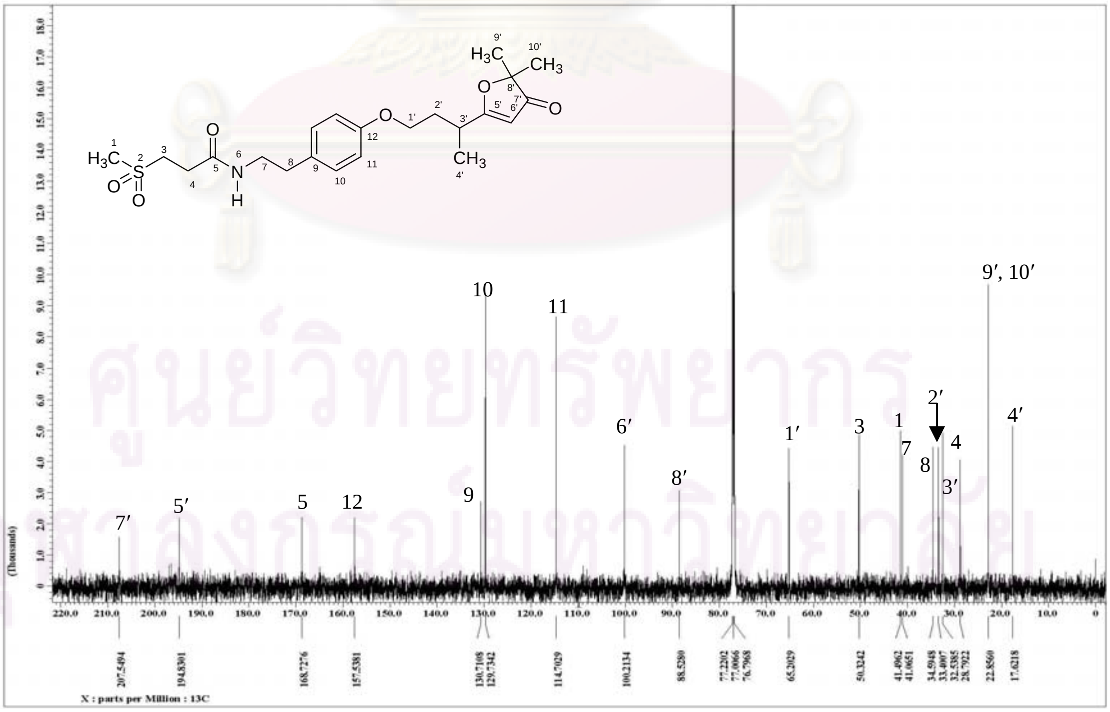


Figure 184 ^{13}C-NMR spectrum of compound **GP13** (150 MHz, $CDCl_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

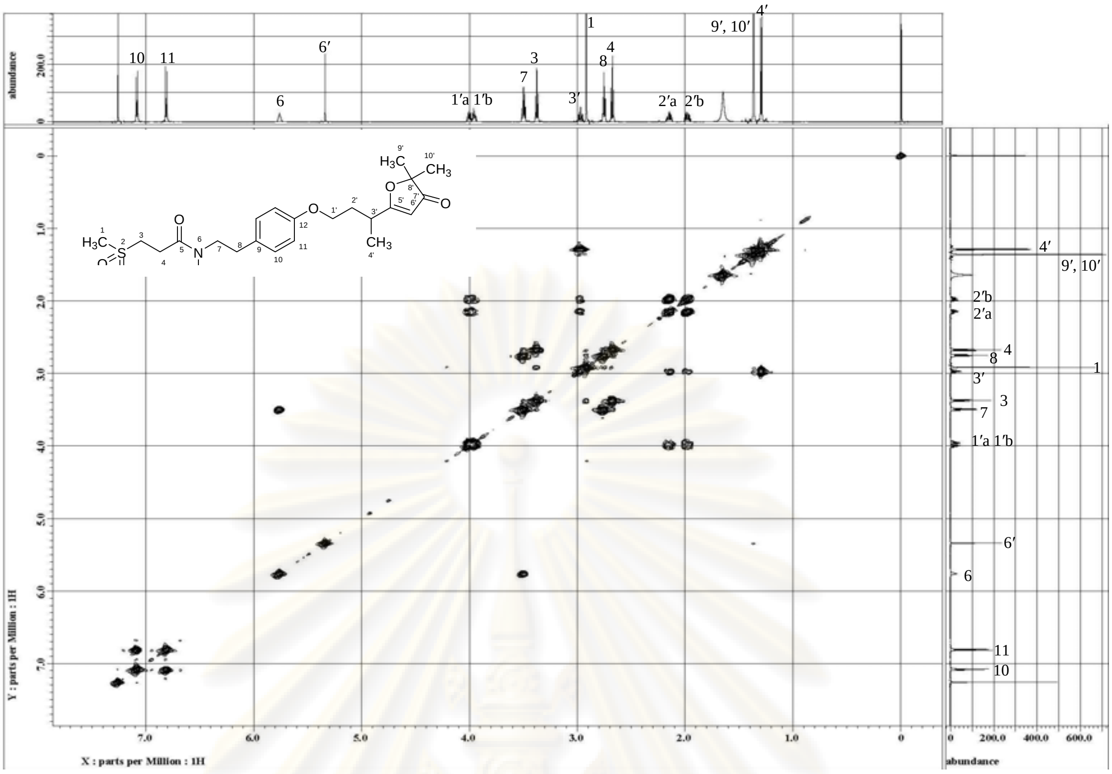


Figure 185 ^{1}H-^{1}H COSY spectrum of compound **GP13** (CDCl$_3$).

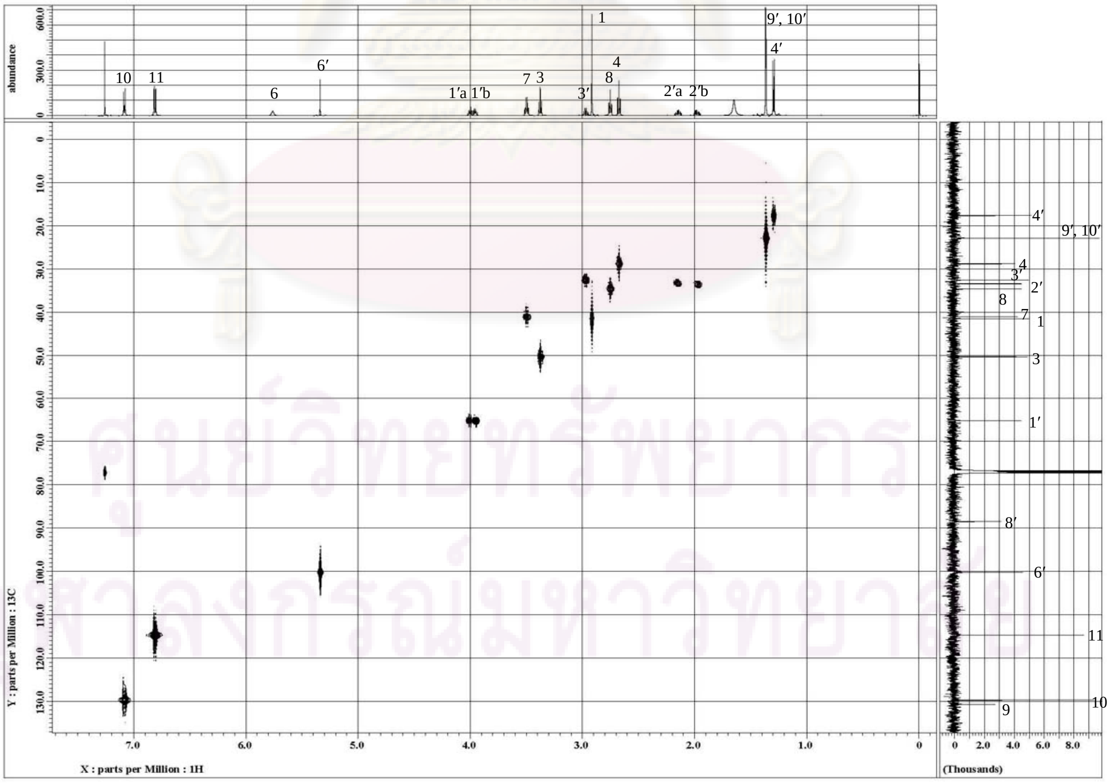


Figure 186 HMQC spectrum of compound **GP13** (CDCl$_3$).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

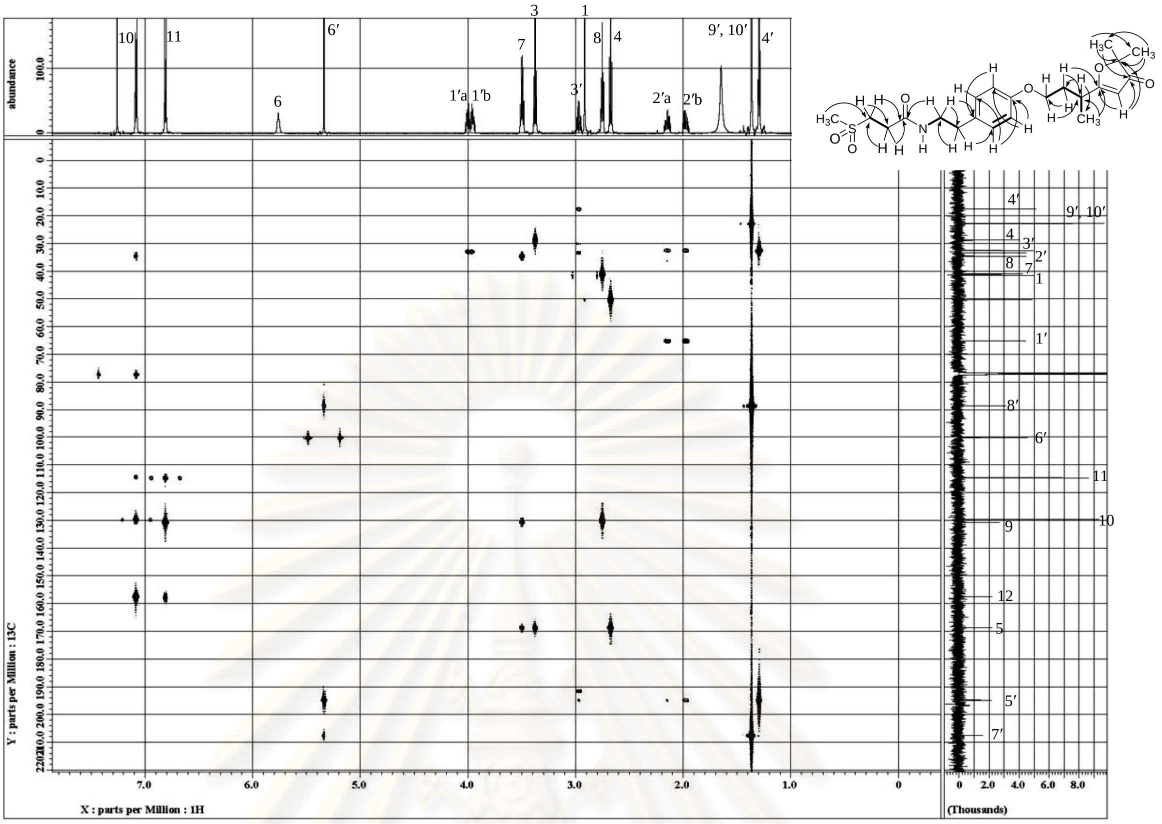


Figure 187 HMBC spectrum of compound **GP13** ($CDCl_3$).

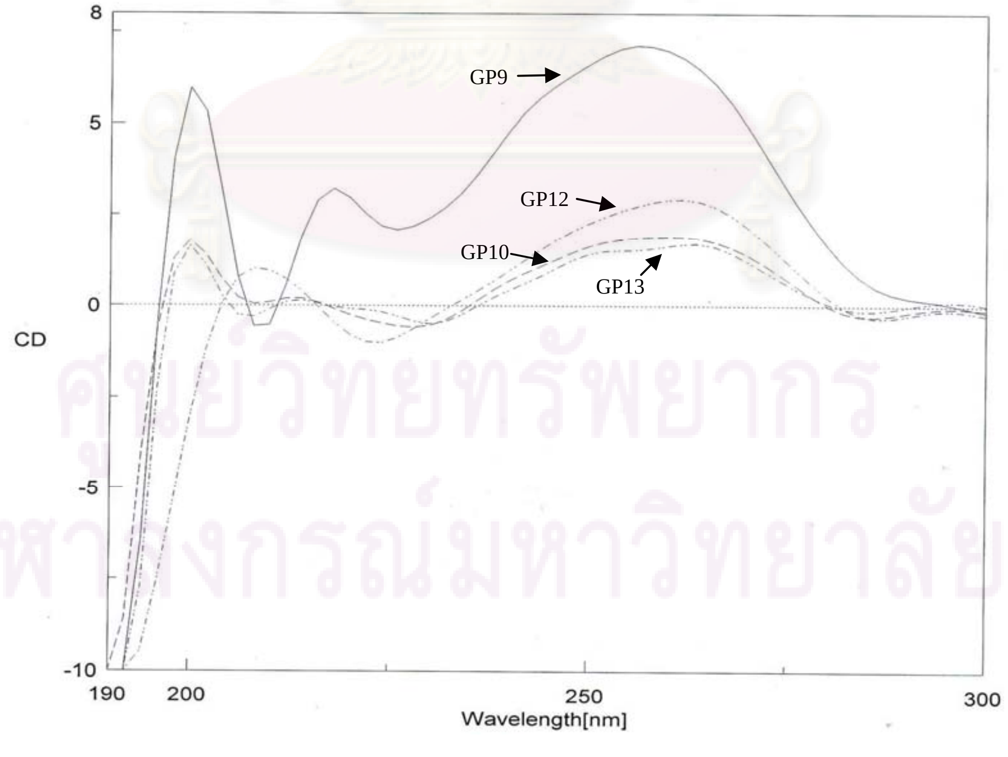


Figure 188 Overlay of CD spectrum of compound **GP9 - GP13** (MeOH).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

VITA

Mr. Chaisak Chansriniyom was born on July 6, 1982 in Bangkok, Thailand. He received his Bachelor's degree of Pharmaceutical Sciences (1st class honor) from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in 2004. He was granted a 2005 Royal Golden Jubilee Ph.D. Scholarship from Thailand Research Fund (TRF).

Publication

1. Chansriniyom, C., Ruangrungsi, N., Lipipun, V., Kumamoto, T., and Ishikawa, T. 2009. Isolation of acridone alkaloids and *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives from *Glycosmis parva* and their anti-herpes simplex virus activity. Chem. Pharm. Bull. 57: 1246-1250.

Proceedings from the meeting

1. Chumseng, S., Itthipanichpong, C., Ruangrungsi, N., Chansriniyom, C., and Limpanasithikul, W. Effects of the Hexane Extract from *Glycosmis parva* on LPS-InducedMacrophage Activation. Proceedings of the 32nd Pharmacological and Therapeutic Society of Thailand Meeting, pp. 184-187. Bangkok, 2010.
2. Rodphukdeekul, S., Sueblinvong, T., Ruangrungsi, N., Chansriniyom, C., and Limpanasithikul, W. Anti-tumor activity of *Micromelum hirsutum* extract. Proceedings of the 32nd Pharmacological and Therapeutic Society of Thailand Meeting, pp. 106-110. Bangkok, 2010.

Oral presentations

1. Chansriniyom, C., Ruangrungsi, N., Lipipun, V., Kumamoto, T., and Ishikawa, T. 2010. Chemical constituents of *Glycosmis parva* and their anti-herpes simplex virus activities. RGJ-Ph.D. Congress XI, April, 1-3, 2010, Pattaya, Chonburi, Thailand.
2. Chansriniyom, C., Ruangrungsi, N., Lipipun, V., Kumamoto, T., and Ishikawa, T. 2009. Chemical constituents of *Glycosmis parva* and their anti-herpes simplex virus activities. JSPS-NRCT Graduated Student Seminar in Natural Medicine Research, December, 14, 2009, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Poster presentation

1. Chansriniyom, C., Ruangrungsi, N., Kumamoto, T., and Ishikawa. 2008. Chemical constituents of *Glycosmis parva*. Joint Seminar between Department of Medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences of Chiba University, Kobe University and Toho University. August, 18-20, 2008, Chiba, Japan.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย