องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของต้นสิรินธรวัลลีและต้นเปล้าแพะ

นางสาวศิริวรรณ อธิคมกุลชัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเภสัชเคมีและผลิตภัณฑ์ธรรมชาติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547

ISBN : 974-17-5964-9

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *BAUHINIA SIRINDHORNIAE* AND *CROTON HUTCHINSONIANUS*

Miss Sirivan Athikomkulchai

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Pharmaceutical Chemistry and Natural Products
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2004

ISBN : 974-17-5964-9

Thesis Title	CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *BAUHINIA SIRINDHORNIAE* AND *CROTON HUTCHINSONIANUS*
By	Miss Sirivan Athikomkulchai
Field of Study	Pharmaceutical Chemistry and Natural Products
Thesis Advisor	Associate Professor Nijsiri Ruangrungsi, Ph.D.
Thesis Co-Advisor	Professor Somsak Ruchirawat, Ph.D.
Thesis Co-Advisor	Associate Professor Nongluksna Sriubolmas, Ph.D.

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

……Dean of the Faculty of Pharmaceutical Sciences
(Associate Professor Boonyong Tantisira, Ph.D.)

THESIS COMMITTEE

…….. Chairman
(Associate Professor Sumphan Wongseripipatana, Ph.D.)
…….……. Thesis Advisor
(Associate Professor Nijsiri Ruangrungsi, Ph.D.)
…….. Thesis Co-Advisor
(Professor Somsak Ruchirawat, Ph.D.)
…….. Thesis Co-Advisor
(Associate Professor Nongluksna Sriubolmas, Ph.D.)
…….. Member
(Associate Professor Ekarin Saifah, Ph.D.)
…….. Member
(Police Captain Suchada Sukrong, Ph.D.)
…….. Member
(Associate Professor Wandee Gritsanapan, Ph.D.)

ศิริวรรณ อธิคมกุลชัย: องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของต้นสิรินธรวัลลีและต้นเปล้าแพะ. (CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *BAUHINIA SIRINDHORNIAE* AND *CROTON HUTCHINSONIANUS*) อาจารย์ที่ปรึกษา: รศ. ดร. นิจศิริ เรืองรังษี, อาจารย์ที่ปรึกษาร่วม: ศ.ดร. สมศักดิ์ รุจิรวัฒน์, รศ.ดร. นงลักษณ์ ศรีอุบลมาศ, 284 หน้า. ISBN : 974-17-5964-9.

การศึกษาทางพฤกษเคมีของลำต้นและรากสิรินธรวัลลี สามารถแยกสารที่เคยมีรายงานมาแล้วได้ทั้งหมด 17 ชนิด ได้แก่ cyanoglucoside 2 ชนิด (lithospermoside และ menisdaurin), flavan 1 ชนิด ((-)-epicatechin), flavanone 2 ชนิด ((2*S*)-naringenin และ (2*S*)-eriodictyol), flavanonol 1 ชนิด ((+)-taxifolin), flavone 1 ชนิด (luteolin), chalcone 1 ชนิด (isoliquiritigenin), chromone 1 ชนิด (5,7-dihydroxychromone), chromone glucoside 1 ชนิด (5-hydroxychromone 7-β-D-glucoside), lignan glycoside 2 ชนิด ((+)-isolariciresinol 3α-*O*-α-L-rhamnoside และ (+)-lyoniresinol 3α-*O*-α-L-rhamnoside), triterpenoid 2 ชนิด (lupeol และ glutinol), steroid glucoside 1 ชนิด (sitosteryl-3-*O*-β-D-glucoside) และ สารกลุ่ม phenolic 2 ชนิด (3,4,5-trimethoxyphenolic-1-*O*-β-D-glucoside และ protocatechuic acid) สำหรับการศึกษาทางพฤกษเคมีของกิ่งและใบเปล้าแพะสามารถแยกสารได้ 6 ชนิดซึ่งเป็นสารใหม่ 2 ชนิด คือ 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate และ 3′-(4′′-hydroxyphenyl)-propyl benzoate นอกจากนี้ยังพบสารที่มีรายงานมาแล้วอีก 4 ชนิด ได้แก่ farnesyl acetone, poilaneic acid 4-hydroxybenzaldehyde และ dihydroconiferylbenzoate การพิสูจน์สูตรโครงสร้างทางเคมีของสารที่แยกได้นี้ อาศัยการวิเคราะห์สเปคโตรสโคปี ร่วมกับการเปรียบเทียบข้อมูลของสารที่ทราบโครงสร้างแล้ว นอกจากนี้ยังได้นำสารที่แยกได้ไปทดสอบฤทธิ์ทางชีวภาพ ได้แก่ ฤทธิ์ต้านแบคทีเรีย, ฤทธิ์ต้านเชื้อรา, ฤทธิ์ความเป็นพิษต่อเซลล์ และฤทธิ์จับอนุมูลอิสระ พบว่า (+)-isolariciresinol 3α-*O*-α-L-rhamnoside และ (+)-lyoniresinol 3α-*O*-α-L-rhamnoside มีฤทธิ์ในการจับอนุมูลอิสระ (2*S*)-eriodictyol และ isoliquiritigenin มีฤทธิ์ในการยับยั้งเชื้อ *Bacillus subtilis* และ *Staphylococcus aureus* ในขณะที่ (2*S*)-naringenin และ luteolin มีฤทธิ์ในการยับยั้งเชื้อ *B. subtilis* นอกจากนี้ 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl, dihydroconiferyl benzoate และ 3′-(4′′-hydroxyphenyl)-propyl benzoate แสดงฤทธิ์ระดับปานกลางในการยับยั้งเชื้อรา *Candida albicans* ส่วนการตรวจสอบฤทธิ์ความเป็นพิษต่อเซลล์นั้นพบว่า 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl มีฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง NCI-H187 ในระดับต่ำ ในขณะที่ dihydroconiferylbenzoate และ 3′-(4′′-hydroxyphenyl)-propyl benzoate ไม่มีฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง NCI-H187

สาขาวิชา เภสัชเคมีและผลิตภัณฑ์ธรรมชาติ
ปีการศึกษา 2547

ลายมือชื่อนิสิต.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4276962633 : MAJOR: PHARMACEUTICAL CHEMISTRY AND NATURAL PRODUCTS
KEY WORD: *BAUHINIA SIRINDHORNIAE*/ *CROTON HUTCHINSONIANUS*/ FLAVONOID/ CYANOGLUCOSIDE/ PHENYLPROPYL BENZOATE/ FREE RADICAL SCAVENGING ACTIVITY/ ANTIBACTERIAL ACTIVITY/ ANTIFUNGAL ACTIVITY/ CYTOTOXICITY

SIRIVAN ATHIKOMKULCHAI: CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *BAUHINIA SIRINDHORNIAE* AND *CROTON HUTCHINSONIANUS*. THESIS ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph. D. THESIS CO-ADVISOR: PROF. SOMSAK RUCHIRAWAT, ASSOC. PROF. NONGLUKSNA SRIUBOLMAS, Ph. D., pp. 284 ISBN: 974-17-5964-9

Phytochemical study of the stems and roots of *Bauhinia sirindhorniae* K. & SS. Larsen led to the isolation of seventeen known compounds, two cyanoglucosides (lithospermoside and menisdaurin), one flavan ((-)-epicatechin), two flavanones ((2*S*)-naringenin and (2*S*)-eriodictyol), one flavanonol ((+)-taxifolin), one flavone (luteolin), one chalcone (isoliquiritigenin), one chromone (5,7-dihydroxychromone), one chromone glucoside (5-hydroxychromone 7-β-D-glucoside), two lignan glycosides ((+)-isolariciresinol 3α-*O*-α-L-rhamnoside and (+)-lyoniresinol 3α-*O*-α-L-rhamnoside), two triterpenoids (lupeol and glutinol), one steroid glucoside (sitosteryl-3-*O*-β-D-glucoside) and other phenolic compounds (3,4,5-trimethoxyphenolic-1-*O*-β-D-glucoside and protocatechuic acid). Additionally, six compounds were obtained from the phytochemical investigation of the branches and leaves of *Croton hutchinsonianus* Hosseus. These included two new compounds 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate and 3′-(4′′-hydroxyphenyl)-propyl benzoate and other four known compounds, namely farnesyl acetone, poilaneic acid, 4-hydroxybenzaldehyde and dihydroconiferylbenzoate. The structure determination of all isolates were accomplished by spectroscopic methods and compared with the previously reported data of known compounds. The isolated compounds were also subjected to biological evaluations, for antibacterial, antifungal, cytotoxic and free radical scavenging activities. (+)-Isolariciresinol 3α-*O*-α-L-rhamnoside and (+)-lyoniresinol 3α-*O*-α-L-rhamnoside showed free radical scavenging activity. (2*S*)-Eriodictyol and isoliquiritigenin showed activity against *Bacillus subtilis* and *Staphylococcus aureus* whereas (2*S*)-naringenin and luteolin exhibited activity against *Bacillus subtilis*. Furthermore, 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate, dihydroconiferyl benzoate and 3′-(4′′-hydroxyphenyl)-propyl benzoate revealed moderate antifungal activity against *Candida albicans*. In addition, 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate showed weak cytotoxic activity against NCI-H187 cell line while dihydroconiferylbenzoate and 3′-(4′′-hydroxyphenyl)-propyl benzoate were inactive.

Field of study Pharmaceutical Chemistry	Student's signature……..
and Natural Products	Advisor's signature……..
Academic year 2004	Co-Advisor's signature……
	Co-Advisor's signature……

ACKNOWLEDGEMENTS

The author would like to express her sincere and grateful thanks to the following institutions and people who encouraged and supported her research fulfillment:

Associate Professor Dr. Nijsiri Ruangrungsi, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his valuable advices, keen interests and encouragements throughout this study.

Professor Dr. Somsak Ruchirawat, Chulabhorn Research Institute, for his excellent advice and kind assistance.

Associate Professor Dr. Nongluksna Sriubolmas, Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for her kindness, polite suggestions on the determination of antimicrobial activities.

The thesis committee for their constuctive suggestions and criticial review for her thesis.

The Thailand Research Fund, for a 1999 Royal Golden Jubilee Scholarship and the Association of International Education, Japan, for a research grant.

Professor Dr. Kazuei Igarashi, Department of Clinical Biochemistry, Faculty of Pharmaceutical Sciences, Chiba University, for his kindly help and suggestions.

Associate Professor Dr. Fumio Ikegami, Laboratory of Plant Chemistry, Faculty of Pharmaceutical Sciences, Chiba University, for his kind assistance and useful suggestions.

Research Associate Dr. Toshikazu Sekine, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chiba University, for his valuable guidance and advices.

Dr. Megumi Sumino, Laboratory of Plant Chemistry, Faculty of Pharmaceutical Sciences, Chiba University, for her kindly help and encouragement.

Dr. Hunsa Prawat and Miss Vilailak Prachayavarakorn, Laboratory of Natural products, Chulabhorn Research Institute, for their kind assistance and guidance.

Dr. Nopporn Thasana, Dr. Thumnoon Mutarapat, Dr. Poonsakdi Ploypradith and Dr. Duanpen Lertpibulpanya, Laboratory of Medicinal Chemistry, Chulabhorn Research Institute, for their warm suggestions.

Staffs of the Chemical Analytical Center, Chiba University and the staffs of Chulabhorn Research Institute (CRI) for collaboration and excellent support. The Biodiversity Research and Training Program (BRT), Thailand for cytotoxicity and antifungal tests.

All members of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Laboratory of Plant Chemistry, Faculty of Pharmaceutical Sciences, Chiba University, Laboratory of Medicinal Chemistry and Laboratory of Natural Products, Chulabhorn Research Institute for their friendship, assistance and understanding.

Finally, the most special thanks to the author's family and friends for their love and continuous support.

CONTENTS

CONTENTS (continued)

CONTENTS (continued)

Page

CONTENTS (continued)

CONTENTS (continued)

CONTENTS (continued)

Page

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF SCHEMES

LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]_D^{23}$	=	Specific rotation at 23 °C and sodium D line (589 nm)
α	=	Alpha
acetone-d_6	=	Deuterated acetone
β	=	Beta
br	=	Broad
°C	=	Degree Celsius
calcd	=	Calculated
CD	=	Circular Dichroism
$CDCl_3$	=	Deuterated chloroform
CD_3OD	=	Deuterated methanol
$CHCl_3$	=	Chloroform
CH_3CN	=	Acetronitrile
cm	=	Centimeter
cm^{-1}	=	Reciprocal centimeter (unit of wave number)
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
d	=	Doublet (for NMR spectra)
1D	=	One Dimentional
2D	=	Two Dimentional
dd	=	Doublet of doublets (for NMR spectra)
ddd	=	Doublet of doublet of doublet (for NMR spectra)
dddd	=	Doublet of doublet of doublet of doublet (for NMR spectra)
dq	=	Doublet of quartet (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
D_2O	=	Deuterated Water
DPPH	=	1,1-Diphenyl-2-picrylhydrazyl
δ	=	Chemical shift
ED_{50}	=	50% Effective Dose
EIMS	=	Electron Impact Mass Spectrometry
EtOAc	=	Ethyl acetate
EtOH	=	Ethanol

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

FABMS	=	Fast Atom Bombardment Mass Spectrometry
FAB^+MS	=	Fast Atom Bombardment Mass Spectrometry (positive mode)
FAB^-MS	=	Fast Atom Bombardment Mass Spectrometry (negative mode)
Fr.	=	Fraction
g	=	Gram
GGPP	=	Geranylgeranyl pyrophosphate
hr	=	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
H_2O	=	Water
HPLC	=	High Performance Liquid Chromatography
HRFABMS	=	High Resolution Fast Atom Bombardment Mass Spectrometry
Hz	=	Hertz
IC_{50}	=	Median Inhibitory Concentration
IR	=	Infrared Spectrum
J	=	Coupling constant
KBr	=	Potassium bromide
KB	=	Human oral epidermoid carcinoma cell line
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
m	=	Multiplet (for NMR spectra)
μg	=	Microgram
μL	=	Microliter
μM	=	Micromolar
MeOH	=	Methanol
mg	=	Milligram

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

$[M+H]^+$	=	Protonated molecule
$[M-H]^-$	=	Deprotonated molecule
$[M+Na]^+$	=	Sodium adduct ion
$[M+K]^+$	=	Potassium adduct ion
MBC	=	Minimum Bactericidal Concentration
MHz	=	Megahertz
MIC	=	Minimum Inhibition Concentration
min	=	Minute
mL	=	Milliliter
mM	=	Millimolar
m.p.	=	Melting point
MW	=	Molecular weight
m/z	=	Mass to charge ratio
MS	=	Mass Spectrometry
MTT	=	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
mult.	=	Multiplicity
NCI-H187	=	Human small cell lung cancer cell line
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Effect Spectroscopy
o	=	Ortho
p	=	Para
P-388	=	Murine leukemia cell line
ppm	=	Part per million
PTLC	=	Preparative Thin Layer Chromatography
pyridine-d_5	=	Deuterated pyridine
ν_{max}	=	Wave number at maximal absorption
s	=	Singlet (for NMR spectra)
spp.	=	Species
t	=	Triplet (for NMR spectra)

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

t-DCTN	=	Trans-dehydrocrotonin
TEAC	=	Trolox Equivalent Antioxidant Activity
TLC	=	Thin Layer Chromatography
Trolox	=	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
UV	=	Ultraviolet
UV-VIS	=	Ultraviolet and Visible Spectrophotometry
V-79	=	A Chinese hamster lung cell line

CHAPTER I

INTRODUCTION

The genus *Bauhinia* belongs to the family Leguminosae of the subfamily Caesalpinoidae. This genus consists of about 300 species distributed in Africa, Asia, and Latin America.

Plants in the genus *Bauhinia* are trees, shrubs and tendrilled climbers. Leaves are simple, entire, emarginated, bilobed or divided in two free leaflets. The midrib often bristles between the lobes and the base often contains two darker alveoles. Stipules are normally early carducous. Flowers are bisexual (rarely unisexual) with a more or less pronounced receptacle. Calyx is five-merous, cup-shaped, spathaceous or splitting into free segments during anthesis. There are typically five petals. Stamens are 10, 5, 3, 2 or 1 and anthers are released *via* longitudinal slits in all Thai species except *B. bidentata* where they are released *via* a central pore. Ovary is stipitate and is rarely sessile. Pods are dehiscent and are rarely indehiscent (Larsen, Larsen and Vidal, 1984).

According to Smitinand (2001), the species of genus *Bauhinia* found in Thailand are as follows:

B. acuminata L. กาแจ๊ะกูโด Ka-chae-ku-do (Malay-Narathiwat); กาหลง Kalong (Central);โยธิกา Yo thika (Nakhon Si Thammarat); ส้มเสี้ยว Som siao (Central);เสี้ยวน้อย Siao noi (Chaing Mai).

B. aureifolia K. & S.S. Larsen ใบสีทอง Bai si thong, ย่านดาโอ๊ะ Yan da o (Narathiwat).

B. bassacensis Pierre ex Gagnep. เครือเขาหนัง Khruea khao nang (Lampang); ชงโค Chong kho, โยธิกา Yo thi ka (Peninsular); เถากระไดลิง Thao kradai ling (Southeastern).

B. bidentata Jack subp. *bicornuta* (Miq.) ชงโคป่าดอกแดง Chong kho pa dok daeng (Peninsular); เล็บกระรอก Lep krarok (Pattani)

K. & S.S. Larsen		เล็บควายเหล็ก Lep khwai lek (Yala).
B. binata Blanco		แสลงพัน Salaeng phan (Chon Buri).
B. bracteata (Graham ex Benth.) Baker		ปอแก้ว Po-kaeo (Karen-Northern); ปอเจี๋ยน Po chian (Northern); ปอบุ้ง Po bung (Chaing Mai); เสี้ยวเครือ Siao khruea (Nakhon Ratchasima); เสี้ยวดอกขาว Siao dok khao; เสี้ยวเตี้ย Siao tia (Loei); เสี้ยวส้ม Siao som (Uthai Thani, Sakon nakhon); แสลงพัน Saleang phan (Chon buri).
chrysophylla K. & S.S. Larsen	=	*B. aureifolia* K. & S.S. Larsen
B. curtisii Prain		เครือเขาแกบ Khruea khao kaep (Northeastern).
decipiens Craib	=	*B. pottsii* G.Don var. *decipiens* (Craib) K. & S.S. Larsen
detergens Craib	=	*B. bassacensis* Pierre ex Gagnep.
elongata Korth.	=	*B. pottsii* G. Don var. *pottsii*
B. ferruginea Roxb.		ย่านตีนควาย Yan tin khwai (Narathiwat).
flammifera Ridl.	=	*B. integrifolia* Roxb.
B. glauca (Wall. Ex Benth.) subsp. *tenuiflora* (Watt ex C.B. Clarke) K. & S.S. Larsen		ชงโค Chong kho (Penninsular). คางโค Khang kho (Chanthaburi); พาซิว Pha-sio (Karen-Lampang); เสี้ยวเครือ Siao khruea (Chiang Mai, Lampang); เสี้ยวต้น Siao ton (Nan); เสี้ยวป่า Siao pa (Chiang Mai)
B. harmsiana Hosseus		ชงโคขี้ไก่ Chong kho khi kai (Kanchanaburi); เสี้ยว Siao (Phrae); เสี้ยวเคือ Siao khuea (Lamphun).
helferi Craib	=	*B. bracteata* (Graham ex Benth.) Baker
B. hirsuta Weinm.		วุ้งพู Wung-Phu (Karen-Mae Hong Son); เสี้ยวน้อย Siao noi (Northern).

horsfieldii (Miq.) MacBr.	=	*B. scandens* L. var. *horsfieldi*i (Miq.) K. & S.S. Larsen
B. integrifolia Roxb.		กุกูกูด้อ Ku-ku-ku-do, กุกูกูบา Ku-ku-ku-ba (Malay-Pattani); ชงโคย่าน Chong kho yan, ย่านชงโค Yan chong kho (Trang); ชิงโคย่าน Ching kho yan (Peninsular); ดาโอะ Da o (Narathiwat); เถาไฟ Thao fai, โยทะกา Yo thaka (Bangkok); ปอลิง Po ling (Surat thani); เล็บควายใหญ่ Lep khwai yai (Yala, Pattani).
B. involucellata Kurz		แสลงพัน Saleang phan (Kanchanaburi, Saraburi).
kerrii Gagnep.	=	*B. ornata* Kurz var. *kerii* (Gagnep.) K. & S.S. Larsen
B. lakhonensis Gagnep.		ส้มเสี้ยวเถา Som siao thao (Northeastern).
B. malabarica Roxb.		คังโค Khang kho (Suphan Buri); แดงโค Daeng kho (Saraburi); ป้าม Pam (Suai-Surin); ส้มเสี้ยว Som siao (Northern); เสี้ยวส้ม Siao som (Nakhon Ratchasima); เสี้ยวใหญ่ Siao yai (Prachin Buri).
media Craib	=	*B. harmsiana* Hosseus
B. monandra Kurz.		จงโค Chong kho, โยทะกา Yo thaka (Bangkok) One stamened bauhinia.
B. nervosa (Wall. Ex Benth.) Baker		เสี้ยวแก้ว Siao kaeo (General).
B. ornata Kurz var. *kerrii* (Gagnep.) K. & S.S. Larsen		กวาวขน Kwao khon (Chaing Mai); โคคลาน Kho khlan (Prachuap Khiri Khan);ปอมุ้ง Po mung (Chiang Mai); เสี้ยว Siao; ชงโค Chong kho (Phrae); เสี้ยวเครือ Siao khruea (Sukothai); แสลงพันแดง Saleang phan daeng (Loei, Lop Buri).

var. *burmanica* K. & S.S. Larsen	ปอเกี๋ยน Po kian (Northern).
B. penicilliloba Pierre ex Gagnep.	เสี้ยวแดง Siao dang (Loei).
B. pottisii G. Don var. *pottsii*	ชิงโค Ching kho (Ranong, Surat Thani); ชงโคดำ Chong kho dam (Trang).
var. *decipiens* (Craib) K. & S.S. Larsen	ชงโค Chong kho (Trat).
var. *mollissima* (Wall. Ex Prain) K. & S.S. Larsen	ชงโคไฟ Chong kho fai (Penninsular).
var. *subsessilis* (Craib)	ชงโคขาว Chong kho khao (Central); ชงโคป่า Chong kho pa (Chanthaburi); ชั่งโค Chang kho (Trat); ชิงโค Ching kho, ส้มเสี้ยว Som siao (Surat Thani); ชุมโค Chum kho (Chumphon).
var. *velutina* (Wall. Ex Benth.) K. & S.S. Larsen	ชงโค Chong kho (Ranong).
B. pulla Craib.	กาหลง Kalong (Nakhon Sawan); แสลงพัน Saleang phan (Nakhon Ratchasima); แสลงพันเถา Salaeng phan thao (Nakhon Sawan).
B. purpurea L.	กะเฮอ Ka-hoe, สะเปซี Sa-pe-si (Karen-Mae-Hong-Son); ชงโค Chong kho (Central); เสี้ยวดอกแดง Siao dok daeng (Northern); เสี้ยวหวาน Siao wan (Mae Hong Son); Orchid tree, Purple bauhinia.
B. racemosa Lam.	ชงโคขี้ไก่ Chong kho khi kai (Kanchanaburi); ชงโคนา Chong kho na, ชงโคใบเล็ก Chong kho bai lek (Ratchaburi); ชงโคเล็ก Chong kho lek (Saraburi);

		ส้มเสี้ยว Som siao (Lampang); เสี้ยว Siao (Northern); เสี้ยวใหญ่ Siao yai (Prachin Buri).
B. saccocalyx Pierre		คิงโค Khing kho (Nakhon Ratchasima); ชงโค Chong kho (Chanthaburi, Nakhon Ratchasima, Suphan Buri, Uthai Thani); ส้มเสี้ยว Som siao (Nakhon Sawan, Udon Thani); ส้มเสี้ยวโพะ Som siao po, เสี้ยวดอกขาว Siao dok khao; เสี้ยวป่า Siao pa (Nan).
santiwongsei Craib	=	*B. bassacensis* Pierre ex Gagnep.
B. scandens L.		กระไดลิง Kradai ling (Ratchaburi); กระไดวอก
var *horsfieldii* (Miq.)		Kradai wok (Northern); โชกนุ้ย Chok-nui
K. & S.S. Larsen		(Chaobon-Chaiyaphum); มะลืมดำ Ma luem dam (Chaing Mai).
B. sirindhorniae		สามสิบสองประดง Sam sip song pra dong (Nong
K. & S.S. Larsen		Khai); สิรินธรวัลลี Sirinthon wanly (Bangkok).
B. similes Craib.		แสลงพันกระดูก Salaeng phan kraduk (Kanchanaburi).
B. strychnifolia Craib		ขยัน Khayan, เครือขยัน Khruea khayan (Northern); สยาน Sayan (Tak, Lampang); หญ้านางแดง Ya nang daeng (Northeastern).
B. strychnoidea Prain		โชคนุ้ย Chok Nui (Narathiwat).
subsessilis Craib	=	*B. pottsii* G. Don var. *subsessilis* (Craib) de Wit
sulphurea Craib	=	*B. bassacensin* Pierre ex Gagnep.
tenuiflora Watt ex C.B. Clarke	=	*B. glauca* (Wall. Ex Benth.) Benth. Subsp. tenuiflora (Watt ex C.B. Clarke) K. & S.S. Larsen
B. tomentosa L.		ชงโคดอกเหลือง Chong kho dok lueang (Bangkok).

B. variegata L.		เปียงพะโก Piang phako (Sukhothai); โพะเพ่ Pho-phe (Karen-Kanchanaburi); เสี้ยวดอกขาว Siao dok khao (Northern) นางอั้ว Nang ua (Chaing Mai); Mountain ebony tree, St. Thomas tree.
velutina Wall. Ex Benth.	=	*B. pottsii* G.Don var. *velutina* (Wall. Ex Benth.) K. & S.S. Larsen
B. viridescens Desv. var. *viridescens*.		บะหมะคอมี Ba-ma-kho-mi (Karen-Kanchnaburi); ส้มเสี้ยวน้อย Som siao noi (Prachin Buri); ส้มเสี้ยวบาง Som siao bai bang (Prachuap Khiri Khan); เสี้ยวเคี้ยว Siao khiao (Loei); เสี้ยวน้อย Siao noi, เสี้ยวป๊อก Siao pok (Phrae); เสี้ยวฟ่อม Siao fom (Northern).
var. *hirsuta* K. & S.S. Larsen		กาหลงเขา Kalong khao (Kanchanaburi).
B. wallichii J.F. Macbr.		ชงโคภูคา Chong kho phuka (Nan)
B. wintii Craib		คิ้วนาง Khio nang, อรพิม Ora phim (Central).
B. yunnanensis Franch.		เสี้ยวแพะ Siao phae (Lamphun); หญ้าเกล็ดปลามง Ya-klet-pla-mong (Shan-Northern).

Bauhinia sirindhorniae K. & S.S. Larsen is an indigenous plant known in Thai as Sirinthon Wanli or Sam Sip Song Pra Dong and is a trendrilled liana (Figure 1). Young branches are hairy reddish brown and grabrous. Stipules are oblong-elliptic and early caduceus. Leaves are coriaceous and ovate. The apex is slighty bifid to deeply bifid almost to the base. Inflorescences are densely ferrugineous pubescent in which bracts are hairy outside and glabrous inside. Hypanthium is tubular to narrowly funnel-shaped, striate and hairy. Calyx is splited on one side to the base and on the opposite side at the tip only. Petals are densely hairy reddish brown. Stamens are three fertile. The filaments and anthers are glabrous. There are two staminodes with triangular and minute. Ovary is hairy reddish brown. Pods are ferrugineous pubescent. Seeds are 5-7, dark brown, flat and orbicular (Larsen and Larsen, 1997).

The genus *Croton* belongs to the family Euphorbiaceae. They comprise of approximately 700 species which distributed over all warm countries. *Croton* species are reported to possess important medicinal uses and well known as toxic plants.

Most members are trees or shrubs and a few are herbs. Leaves are usually alternate with biglandular at the base. The flowers are small bracts. Male flowers contain five calyx, five petals and a disk of 4-6 glands opposite the sepals. There are many stamens inserted on a hairy receptacle and the anthers are adnate with parallel cells. In female flowers, sepals are usually more ovate than the male and the petals are smaller than the sepals or missing. The disk is annular and consists of 4-6 glands are opposite the sepals. There are three ovaries with solitary ovule in each cells, styles are usually long and slender. Seeds are smooth, albumen copious and broad cotyledon (Shaw 1972).

The species of genus *Croton* which have been recorded in Thailand (Smitinand, 2001), are as follows:

C. acutifolius Esser — จิมิจิยา Chi-mi-chi-ya, เปล้า Plao, เปล้าแพะ Plao phae, มะดอไก่ Mado kai (Northern).

C. argyratus Blume — เปล้า Plao (Prachuap Khiri Khan); เปล้าเงิน Plao ngoen (Nong Khai).

birmanicus Müll.Arg. = *C. tiglium* L.

C. bonplandianus Daillon. — เปล้าทุ่ง Plao thung (General).

C. cascarilloides Raeusch. — เปล้าเงิน Plao ngoen (Songkhla); เปล้าน้ำเงิน Plao nam ngoen (Prachuap Khiri Khan).

C. caudatus Geiseler — กระดอหดใบขน Krado hot bai khon (Chanthaburi); โคคลาน Kho khlan (Nakhon Ratchasima); ปริก Prik (Trang); โคคลาน ใบขน Kho khlan bai khon (General); กูเราะปริยะ Ku-ro-pri-ya (Malay-Narathiwat).

C. columnaris Airy Shaw — เปล้าคำ Plao Kham (Sukhothai).

C. crassifolius Geiseler — ปังคี Pang khi, พังคี Phang khi (Chiang Mai).

cumingii Müll. Arg. = *C. cascarilloides* Raeusch.

C. delpyi Gagnep. เปล้า Plao, เปล้าน้อย Plao noi, นมน้ำเขียว Nom nam khiao (Southeastern).

C. griffithii Hook. f. จิก Chik, เปล้า Plao (Peninsular).

C. hirtus L. Her. เปล้าล้มลุก Plao lom luk (Peninsular).

C. hutchinsonianus Hosseus. เปล้า Plao, เปล้าแพะ Plao phae, เปล้าเลือด Plao lueat, แม่ลาเลือด Mae la lueat, เหมือดฮ้อน Mueat hon (Northern).

C. kerrii Airy Shaw เปล้า Plao (General).

C. kongensis Gagnep. เปล้าเงิน Plao ngoen, เปล้าน้อย Plao noi (Northeastern); เปล้าน้ำเงิน Plao nam ngoen (Eastern); เสปอตุ Se-po-tu (Karen–Chieng Mai).

C. krabas Gagnep. ทรายขาว Sai Khao (Northern); พริกนา Prik na (Central); ฝ้ายน้ำ Fai nam (Eastern).

C. lachnocarpus Benth. ขี้อ้น Khi on (Southwestern).

C. longissimus Airy Shaw เปล้าน้อย Plao noi (Lampang).

C. mekongensis Gagnep**.** เปล้าน้ำเงิน Plao nam ngoen, พริกนา Prik na (Northern).

oblongifolius Roxb. = *C. cascarilloides* Raeusch.

C. poilanei Gagnep. เปล้า Plao, เปล้าใหญ่ Plao yai (Southeastern); เปล้าหลวง Plao luang, เปล้าเลือด Plao lueat (Northern).

pierrei Gagnep. = *C. cascarilloides* Raeusch.

C. robustus Kurz เปล้าเลือด Plao lueat (Lampang).

rottleri Geiseler = *Chrozophora rottleri* (Geiseler) A. Juss ex Spreng.

C. roxburghii N.P. Balakr. ควะวู Khwa-wu (Karen–Kanchanaburi); เซ่งเค่คัง Seng–khe-khang, สะกาวะ Sa-ka-wa, ส่ากูวะ Sa-ku-

wa (Karen-Mae Hong Son); เปาะ Po (Kamphaeng Phet); เปล้าหลวง Plao luang (Nortern); เปล้าใหญ่ Plao yai (Central); ห้าเยิ่ง Ha-yoeng (Shan-Mae Hong Son).

C. santisukii Airy Shaw — เปล้าสันติสุข Plao santisuk (Southwestern).

C. sepalinus Airy Shaw — เปล้าเงิน Plao ngoen (Peninsular).

siamensis Craib = *C. robustus* Kurz

C. stellatopilosus Ohba — เปล้าน้อย Plao noi (Prachin Buri, Prachuap Khiri Khan); เปล้าท่าโพ Plao tha po (Southeastern).

C. thorelii Gagnep. — เปล้าตะวัน Plao tawan (Southeastern).

C. tiglium L. — บะกั้ง Ba kang (Phrae) ; มะข่าง Ma khang, มะคัง Ma khang, มะตอด Matot, หมากทาง Mak thang, หัสคืน, Has sa khuen (Northern); ลูกผลาญศัตรู Luk phlan sattru, สลอด Salot, สลอดต้น Salot ton, หมากหลอด Mak lot (Central); หมากยอดง Mak-yong (Shan-Mae Hong Son); Croton oil plant.

tomentosus Müll.Arg. = *C. crassifolius* Geiseler

C. trachycaulis Airy Shaw — กวะวะ Kwa-wa, กวาโอะวะ Kwa-o-wa (Karen-Kanchanaburi); ขี้อ้น Khi on (Prachuap Khiri Khan).

C. wallichii Müll.Arg. — เปล้า Plao, เปล้านา Plao na (General).

Croton hutchinsonianus Hosseus. has a local name as Plao phae (Figure 2). It is a shrub or small tree reaching 4-5 m, locates commonly in dry mixed deciduous forest or open scrub and grows on lateritic or sandstone soil. Bark is corky and deeply cracked with a deep red sap. It is a coarse plant, with large coriaceous leaves densely minutely steallate-pubescent. The inflorescence is densely whitish steallate-tomentose (Shaw 1972).

During our preliminary evaluation for biological activities, the extract of *Bauhinia sirindhorniae* showed significant scavenging activities towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical activity whereas *Croton hutchinsonianus* showed significant cytotoxicity activity. As for *Bauhinia sirindhorniae*, no phytochemical work has been reported. Therefore, the following objectives are put forwards:

1. To isolate and purify compounds from the stems and the roots of *Bauhinia sirindhorniae*, and from the branches and the leaves of *Croton hutchinsonianus*.
2. To determine the chemical structure of each isolated compound.
3. To evaluate the biological activities of each isolated compound.

Figure 1 *Bauhinia sirindhorniae* K. & S.S. Larsen.

Figure 2 *Croton hutchinsonianus* Hosseus.

CHAPTER II

HISTORICAL

1. Chemical Constituents of *Bauhinia* spp.

A number of compounds has been isolated from the genus *Bauhinia*. They are classified as flavonoids, triterpenoids, steroids, cyanoglucosides, alkaloids, stibenes, lignans, phenylpropanoids and miscellaneous substances (Table 1-3).

Table 1 Distribution of flavonoids in *Bauhinia* spp.

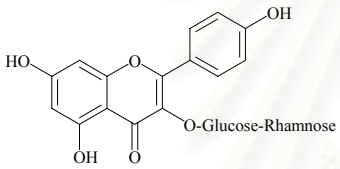
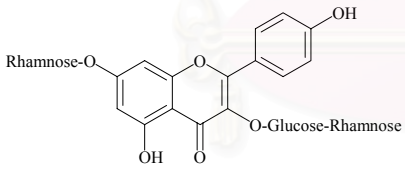
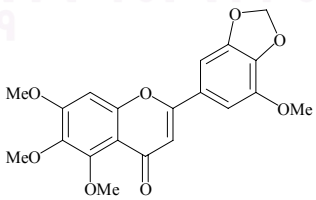
Plant and Chemical compound	Plant part	Reference
Bauhinia candidans		
Kaempferol-3-*O*-β-rutinoside [**1**] 	Leaf	Iribarren and Pomilio, 1983
Kaempferol-3-*O*-β-rutinoside-7-*O*-α-rhamnopyranoside [**2**] 	Leaf	Iribarren and Pomilio, 1983
B. championii		
5,6,7,5′-Tetramethoxy-3′,4′-methylene dioxyflavone [**3**] 	Root	Chen *et al*., 1984

Table 1 (continued)

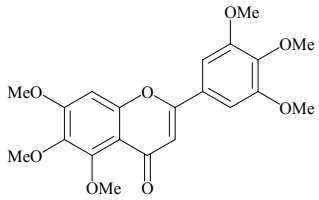
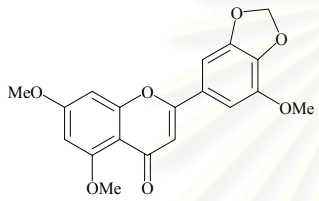
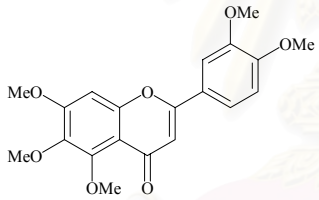
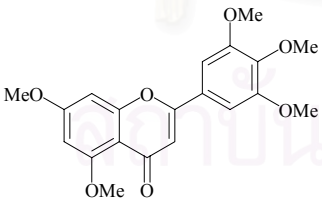
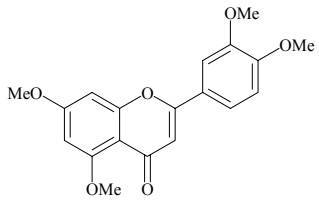
Plant and Chemical compound	Plant part	Reference
5,6,7,3′,4′,5′-Hexamethoxyflavone [**4**] 	Root	Chen *et al*., 1984
5,7,5′-Trimethoxy-3′,4′-methylene dioxyflavone [**5**] 	Root	Chen *et al*., 1984
5,6,7,3′,4′-Pentamethoxyflavone [**6**] 	Root	Chen *et al*., 1984
5,7,3′,4′,5′-Pentamethoxyflavone [**7**] 	Root	Chen *et al*., 1984
5,7,3′,4′-Tetramethoxyflavone [**8**] 	Root	Chen *et al*., 1984

Table 1 (continued)

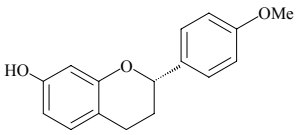
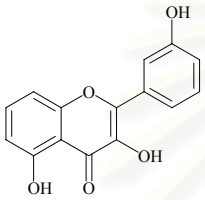
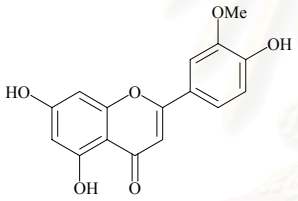
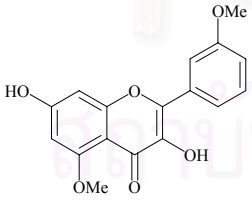
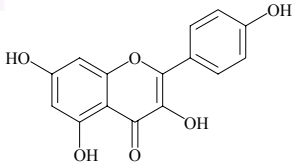
Plant and Chemical compound	Plant part	Reference
B. guianensis		
4′-Hydroxy-7-methoxyflavan [**9**] 	Stem bark	Viana *et al*., 1999
B. manca		
Apigenin [**10**] 	Stem	Achenbach, Stocker and Constenla, 1988
Chrysoeriol [**11**] 	Stem	Achenbach *et al*., 1988
Luteolin-5,3′-dimethyl ether [**12**] 	Stem	Achenbach *et al*., 1988
Kaempferol [**13**] 	Stem	Achenbach *et al*., 1988

Table 1 (continued)

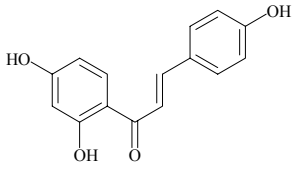
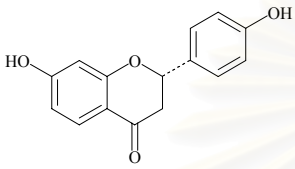
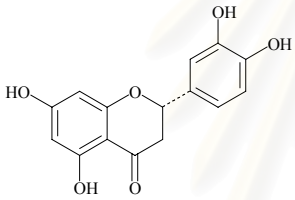
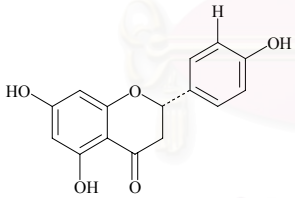
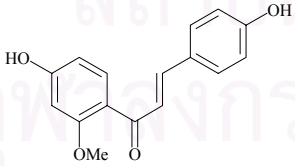
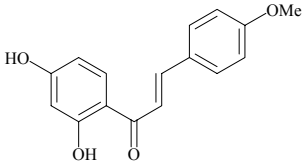
Plant and Chemical compound	Plant part	Reference
Isoliquiritigenin [**14**] 	Stem	Achenbach *et al*., 1988
(2*S*)-Liquiritigenin [**15**] 	Stem	Achenbach *et al*., 1988
(2*S*)-Eriodictyol [**16**] 	Stem	Achenbach *et al*., 1988
(2*S*)-Naringenin [**17**] 	Stem	Achenbach *et al*., 1988
Isoliquiritigenin-2′-methyl ether [**18**] 	Stem	Achenbach *et al*., 1988
Isoliquiritigenin-4-methyl ether [**19**] 	Stem	Achenbach *et al*., 1988

Table 1 (continued)

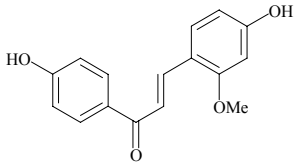
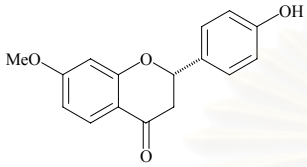
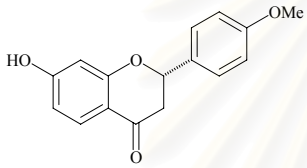
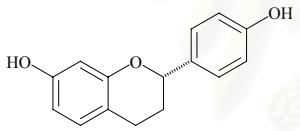
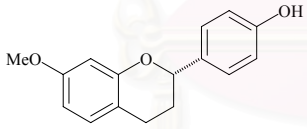
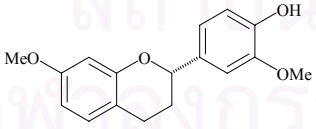
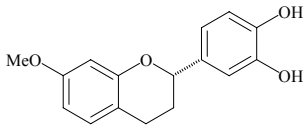
Plant and Chemical compound	Plant part	Reference
Echinatin [**20**] 	Stem	Achenbach *et al*., 1988
(2*S*)-Liquiritigenin-7-methyl ether [**21**] 	Stem	Achenbach *et al*., 1988
(2*S*)-Liquiritigenin-4′-methyl ether [**22**] 	Stem	Achenbach *et al*., 1988
(2*S*)-7,4′-Dihydroxyflavan [**23**] 	Stem	Achenbach *et al*., 1988
(2*S*)-4′-Hydroxy-7-methoxyflavan [**24**] 	Stem	Achenbach *et al*., 1988
(2*S*)-7,3′-Dimethoxy-4′-hydroxy flavan [**25**] 	Stem	Achenbach *et al*., 1988
(2*S*)-3′,4′-Dihydroxy-7-methoxy flavan [**26**] 	Stem	Achenbach *et al*., 1988

Table 1 (continued)

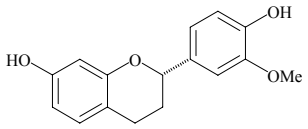
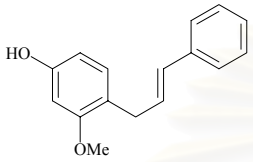
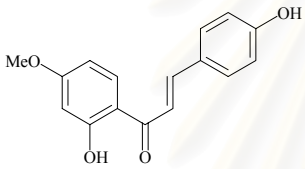
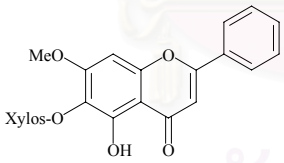
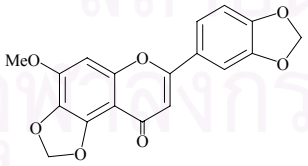
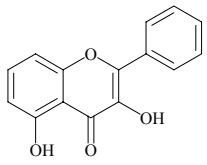
Plant and Chemical compound	Plant part	Reference
(2*S*)-7,4′-Dihydroxy-3′-methoxy flavan [**27**] 	Stem	Achenbach *et al*., 1988
Obtustyrene [**28**] 	Stem	Achenbach *et al*., 1988
2,4′-Dihydroxy-4-methoxy dihydrochalcone [**29**] 	Stem	Achenbach *et al*., 1988
B. purpurea		
5,6-Dihydroxy-7-methoxyflavone-6-*O*-β-D-xylopyranoside [**30**] 	Stem	Yadava and Tripathi, 2000
Bausplendin [**31**] 	Wood	Laux Stefani and Gottlieb, 1985
Chrysin [**32**] 	Bark	Kuo, Chu and Chang, 1998

Table 1 (continued)

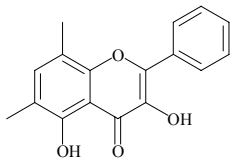
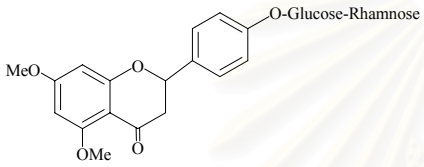
Plant and Chemical compound	Plant part	Reference
6,8-Dimethylchrysin [**33**] 	Bark	Kuo *et al*., 1998
B. variegata		
Naringenin-5,7-dimethylether-4′-rhamnoglucoside [**34**] 	Stem	Gupta, Vidyapati and Chauhan, 1980

Table 2 Distribution of steroids in *Bauhinia* spp.

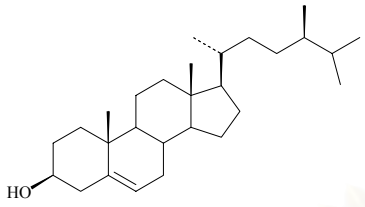
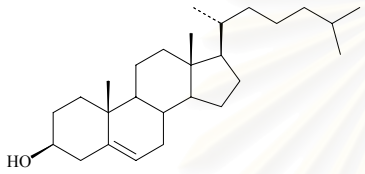
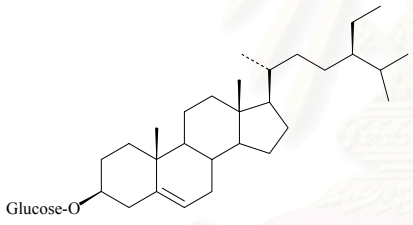
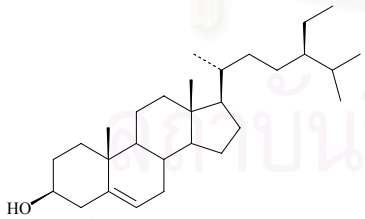
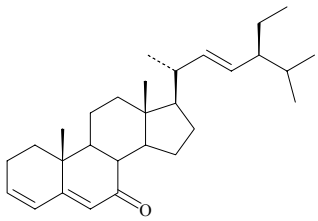
Plant and Chemical compound	Plant part	Reference
B. candidans		
Campesterol [**35**] 	Leaf	Iribarren and Pomilio, 1983
Cholesterol [**36**] 	Leaf	Iribarren and Pomilio, 1983
Daucosterol [**37**] 	Leaf	Iribarren and Pomilio, 1983
β-Sitosterol [**38**] 	Leaf	Iribarren and Pomilio, 1983
Stigmasta-3,5-dien-7-one [**39**] 	Leaf	Iribarren and Pomilio, 1983

Table 2 (continued)

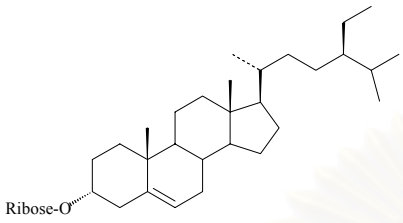
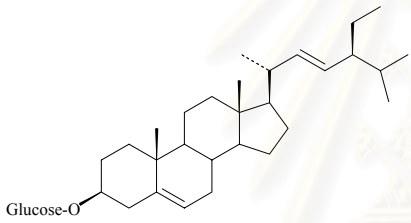
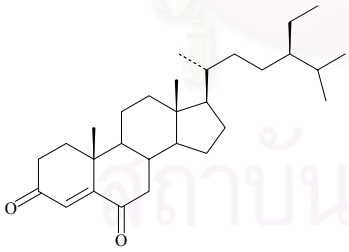
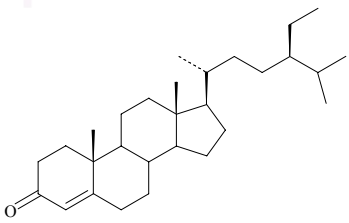
Plant and Chemical compound	Plant part	Reference
B. candidans	Aerial part	Iribarren and Pomilio, 1983
Sitosterol-3-*O*-α-D-riburono furanoside [**40**] 		
B. guianensis	Stem bark	Viana *et al*., 1999
Stigmasta-5,22-dien-3-*O*-β-D-glucopyranoside [**41**] 		
B. manca		
Stigmasta-4-en-3,6-dione [**42**] 	Stem	Achenbach *et al*., 1988
Stigmasta-4-en-3-one [**43**] 	Stem	Achenbach *et al*., 1988

Table 2 (continued)

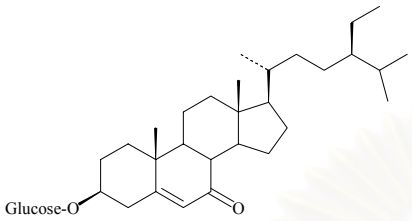
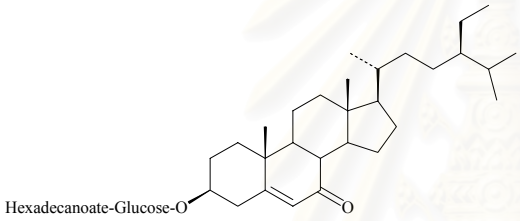
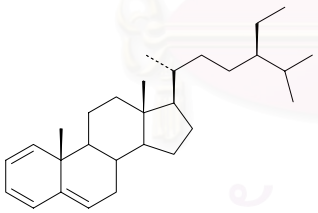
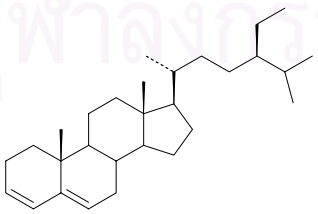
Plant and Chemical compound	Plant part	Reference
B. purpurea		
Stigmasta-5-en-7-one-3-*O*-β-D-glucopyranoside [**44**] 	Bark	Kuo *et al*., 1998
6′-(Stigmasta-5-en-7-one-3-*O*-β-D-glucopyranosidyl) hexadecanoate [**45**] 	Bark	Kuo *et al*., 1998
B. uruguayensis		
Stigmasta-1,3,5-triene [**46**] 	Aerial part	Iribarren and Pomilio, 1989
Stigmasta-3,5-diene [**47**] 	Aerial part	Iribarren and Pomilio, 1989

Table 2 (continued)

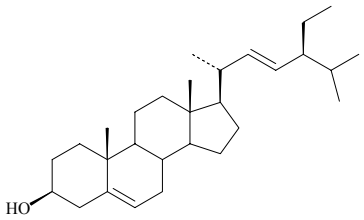
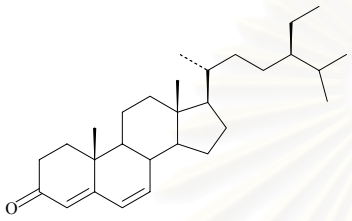
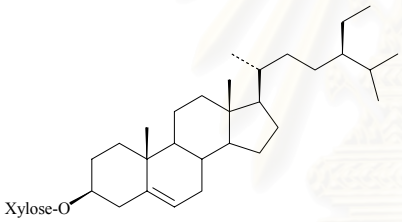
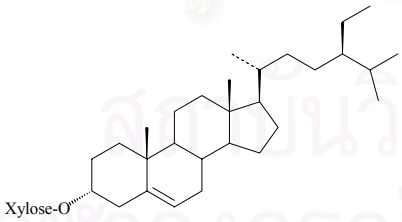
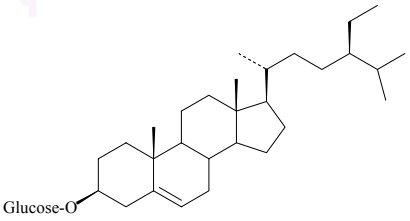
Plant and Chemical compound	Plant part	Reference
Stigmasterol [**48**] 	Aerial part	Iribarren and Pomilio, 1989
Stigmasta-4,6-dien-3-one [**49**] 	Aerial part	Iribarren and Pomilio, 1989
Sitosterol-3-*O*-β-D-xylopyranoside [**50**] 	Aerial part	Iribarren and Pomilio, 1989
Sitosterol-3-*O*-α-D-xylurono furanoside [**51**] 	Aerial part	Iribarren and Pomilio, 1989
Sitosterol-3-*O*-β-D-glucopyranoside [**52**] 	Aerial part	Iribarren and Pomilio, 1989

Table 3 Distribution of miscellaneous compounds in *Bauhinia* spp.

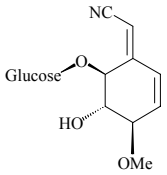
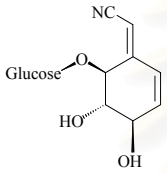
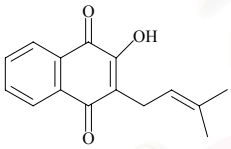
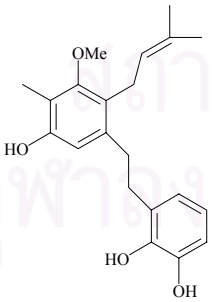
Plant and Chemical compound	Category	Plant part	Reference
B. championii			
Bauhinin [**53**] 	Cyanoglucoside	Root	Chen, Chen and Hsu, 1985
B. fassoglensis			
Lithospermoside [**54**] 	Cyanoglucoside	Root	Fort, Jolad and Nelson, 2001
B. guianensis			
Lapachol [**55**] 	Quinoid	Stem bark	Viana *et al*., 1999
B. malabarica			
Preracemosol A [**56**] 	Stilbene	Root	Kittakoop *et al*., 2000

Table 3 (continued)

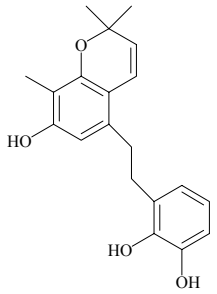
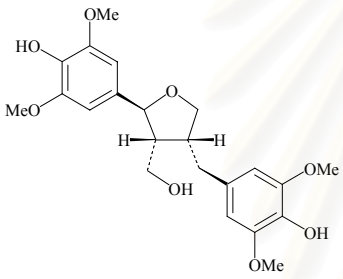
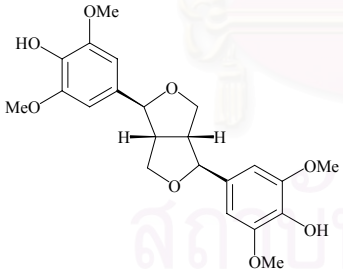
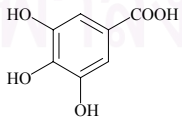
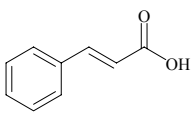
Plant and Chemical compound	Category	Plant part	Reference
Preracemosol B [**57**] 	Stilbene	Root	Kittakoop *et al*., 2000
B. manca			
(7*S*,8*R*,8′*R*)-5-5′-Dimethoxy lariciresinol [**58**] 	Lignan	Stem	Achenbach *et al*., 1988
Syringaresinol [**59**] 	Lignan	Stem	Achenbach *et al*., 1988
Gallic acid [**60**] 	Benzenoid	Stem	Achenbach *et al*., 1988
Cinnamic acid [**61**] 	Phenyl propanoid	Stem	Achenbach *et al*., 1988

Table 3 (continued)

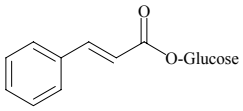
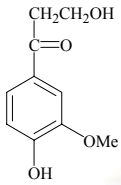
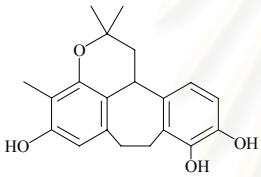
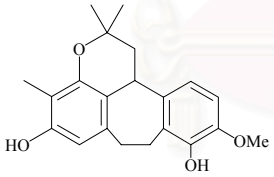
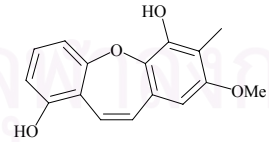
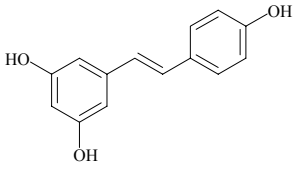
Plant and Chemical compound	Category	Plant part	Reference
Cinnamoyl-β-D-glucoside [**62**] 	Phenyl propanoid	Stem	Achenbach *et al*., 1988
ω-Hydroxypropioguaiacone [**63**] 	Phenyl propanoid	Stem	Achenbach *et al*., 1988
B. racemosa			
Racemosol [**64**] 	Stilbene	Heartwood	Anjaneyulu, Reddy and Reddy, 1986
De-*O*-methylracemosol [**65**] 	Stilbene	Root	Prabhakar *et al*., 1994
Pacharin [**66**] 	Stilbene	Heartwood	Anjaneyulu *et al*., 1984
Resveratrol [**67**] 	Stilbene	Heartwood	Anjaneyulu *et al*., 1984

Table 3 (continued)

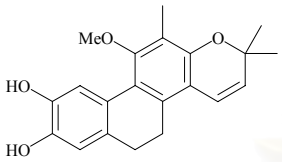
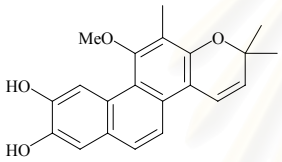
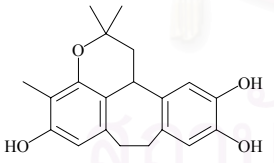
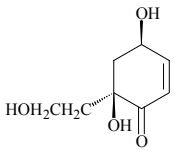
Plant and Chemical compound	Category	Plant part	Reference
B. rufescens			
5,6-Dihydro-11-methoxy-2,2,12-trimethyl-2H-naphthol[1,2-f][1] benzopyran-8,9-diol [**68**] 	Stilbene	Root bark	Millard *et al*., 1991
11-Methoxy-2,2,12-trimethyl-2H-naphthol[1,2-f][1]benzopyran-8,9-diol [**69**] 	Stilbene	Root bark	Millard *et al*., 1991
1,7,8,12b-Tetrahydro-2,2,4-trimethyl-2H-benzo[6,7] cyclohepta[1,2,3-de][1] benzopyran-5,10,11-triol [**70**] 	Stilbene	Root bark	Millard *et al*., 1991
B. tarapotensis			
2,4-Dihydroxy-2-(2-hydroxy ethyl) cyclohexe-5-en-1-one [**71**] 	Cyclohexenone	Leaf	Braca *et al*., 2001

Table 3 (continued)

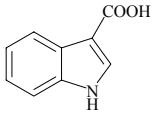
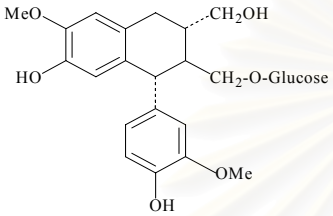
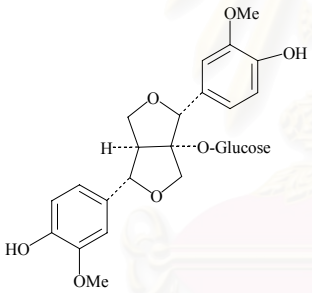
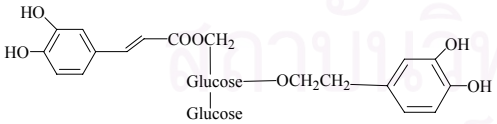
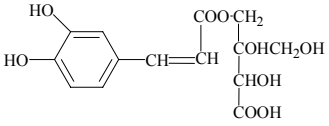
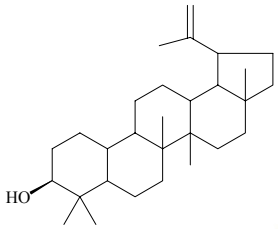
Plant and Chemical compound	Category	Plant part	Reference
Indole-3-carboxylic acid [**72**] 	Alkaloid	Leaf	Braca *et al*., 2001
(-)-Isolariciresinol-3-α-*O*-β-D-glucopyranoside [**73**] 	Lignan	Leaf	Braca *et al*., 2001
(+)-1-Hydroxypinoresinol-1-*O*-β-D-glucopyranoside [**74**] 	Lignan	Leaf	Braca *et al*., 2001
Isoacteoside [**75**] 	Phenyl Propanoid	Leaf	Braca *et al*., 2001
Caffeoyl ester of apionic acid [**76**] 	Phenyl propanoid	Leaf	Braca *et al*., 2001

Table 3 (continued)

Plant and Chemical compound	Category	Plant part	Reference
B. variegata Lupeol [**77**] 	Triterpene	Stem	Gupta *et al*., 1980

2. Chemical Constituents of *Croton* spp.

Chemical investigations of a number of *Croton* species have been shown to be a good source of diterpenes. In addition, other classes of natural compounds such as flavonoids, alkaloids, monoterpenes, triterpenes and miscellaneous substances have been found (Tables 4-7).

Table 4 Distribution of diterpenes in *Croton* spp.

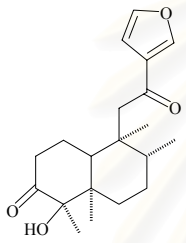
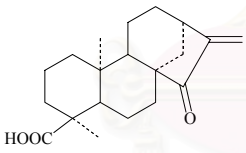
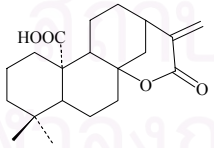
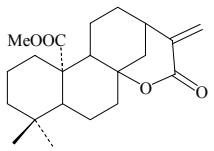
Plant and Chemical compound	Plant part	Reference
Croton argyrophylloides		
3,12-Dioxo-15,16-epoxy-4-hydroxy cleroda-13(16),14-diene [**78**] 	Trunk wood	Monte, Dantas and Braz, 1988
ent-Kaur-16-en-15-oxo-18-oic acid [**79**] 	Trunk wood	Monte *et al*., 1988
Tetracyclic diterpenic acid [**80**] 	Trunk wood	Monte *et al*., 1984
Tetracyclic diterpene ester [**81**] 	Trunk wood	Monte *et al*., 1984

Table 4 (continued)

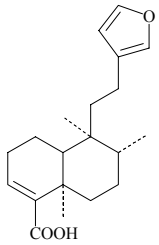
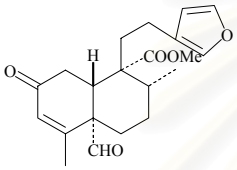
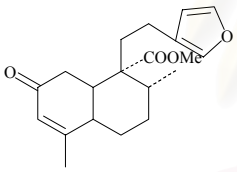
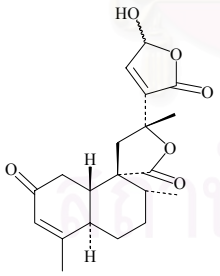
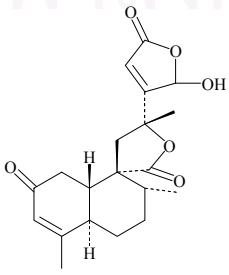
Plant and Chemical compound	Plant part	Reference
C. aromaticus		
(-)-Hardwickiic acid [**82**] 	Root	Bandara, Wimalasiri and Bandara, 1987
C. cajucara		
Cajucarins A [**83**] 	Bark	Itokawa *et al*., 1990
Cajucarins [**84**] 	Bark	Itokawa *et al*., 1990
Cajucarinolide [**85**] 	Bark	Ichihara *et al*., 1991
Isocajucarinolide [**86**] 	Bark	Ichihara *et al*., 1991

Table 4 (continued)

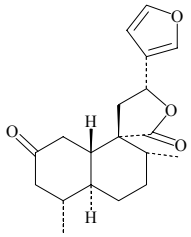
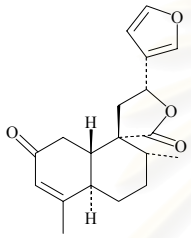
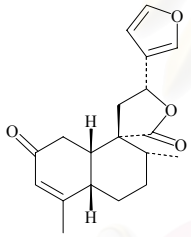
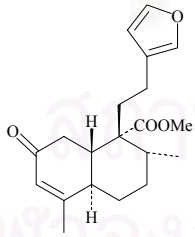
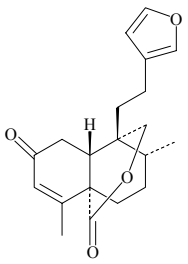
Plant and Chemical compound	Plant part	Reference
trans-Crotonin [**87**] 	Bark	Itokawa *et al*., 1989
Dehydrocrotonin (*trans*-dehydrocrotonin) [**88**] 	Bark	Itokawa *et al*., 1989
cis-Dehydrocrotonin [**89**] 	Bark	Kubo, Asaka and Shibata, 1991
trans-Cajucarin [**90**] 	Bark	Maciel *et al*., 1998
Sacacarin [**91**] 	Bark	Maciel *et al*., 1998

Table 4 (continued)

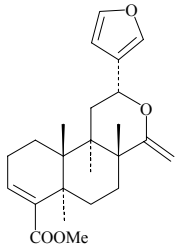
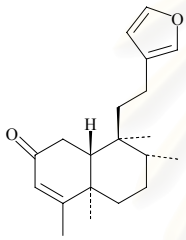
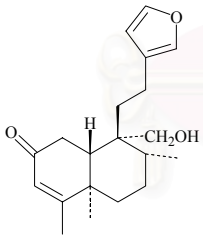
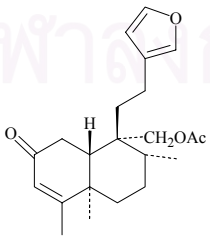
Plant and Chemical compound	Plant part	Reference
C. californicus		
(-)-Methyl barbascoate [**92**] 	Leaf and terminal branch	Wilson, Neubert and Huffman, 1976
C. campestris		
Velamone [**93**] 	Root	Babili *et al*., 1997
Velamolone [**94**] 	Root	Babili *et al*., 1997
Velamone acetate [**95**] 	Root	Babili *et al*., 1997

Table 4 (continued)

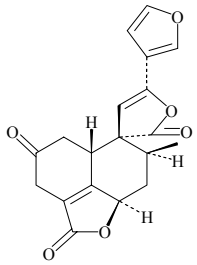
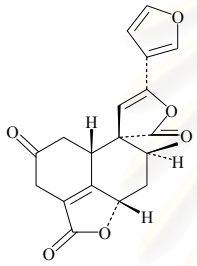
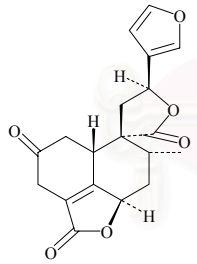
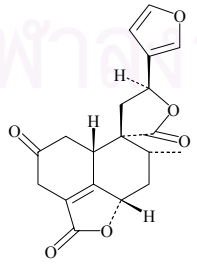
Plant and Chemical compound	Plant part	Reference
C. caudatus		
Crotocaudin [**96**] 	Stem bark	Chatterjee and Banerjee, 1977
Isocrotocaudin [**97**] 	Stem bark	Chatterjee and Banerjee, 1977
Teucvidin [**98**] 	Stem bark	Chatterjee and Banerjee, 1977
Teucin [**99**] 	Stem bark	Chatterjee and Banerjee, 1977

Table 4 (continued)

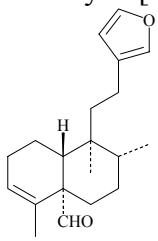
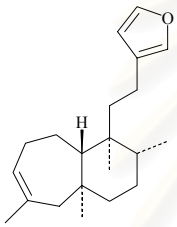
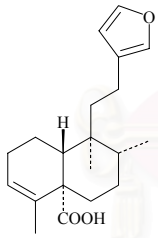
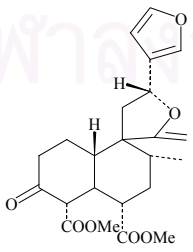
Plant and Chemical compound	Plant part	Reference
C. cortesianus		
Hoffmanniaaldehyde [**100**] 	Aerial part	Seims *et al*., 1992
5,10-Dihydro-5α-hydroxy-10β-printziane [**101**] 	Aerial part	Seims, Dominguez and Jakupovic, 1992
Stigillanoic acid B [**102**] 	Aerial part	Seims *et al*., 1992
C. corylifolius		
Corylifuran [**103**] 	Leaf and twig	Burke, Chan and Pascoe, 1979

Table 4 (continued)

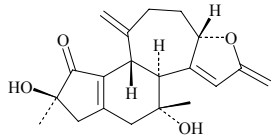
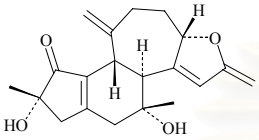
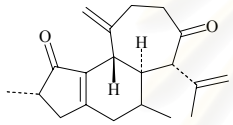
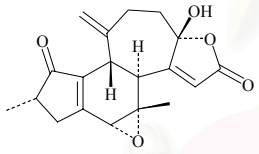
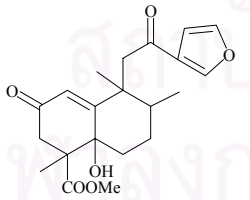
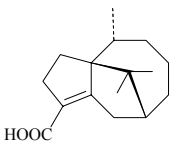
Plant and Chemical compound	Plant part	Reference
Crotofolin A [**104**] 	Leaf and twig	Burke *et al*., 1979
Crotofolin B [**105**] 	Leaf and twig	Burke *et al*., 1979
Crotofolin C [**106**] 	Leaf and twig	Burke *et al*., 1979
Crotofolin E [**107**] 	Leaf and twig	Burke *et al*., 1979
C. crassifolius		
Chettaphanin-I [**108**] 	Root	Boonyaratanakornkit *et al*., 1988
Cyperenoic acid [**109**] 	Root	Boonyaratanakornkit *et al*., 1988

Table 4 (continued)

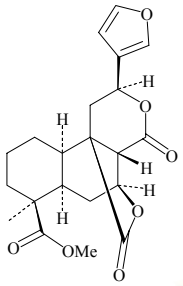
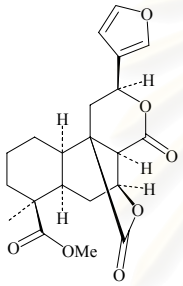
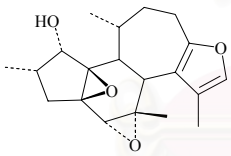
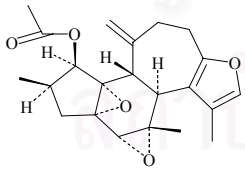
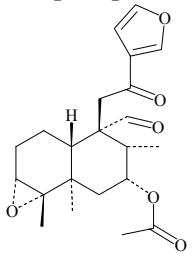
Plant and Chemical compound	Plant part	Reference
C. diasii		
Diasin [**110**] 	Trunk wood	Alvarenga *et al*., 1978
Isodiasin [**111**] 	Trunk wood	Alvarenga *et al*., 1978
C. dichigamus		
Crotoxide A [**112**] 	Leaf	Jogia and Anderson, 1989
Crotoxide B [**113**] 	Leaf	Jogia and Anderson, 1989
C. eluteria		
Cascarillin B [**114**] 	Stem bark	Vigor *et al*., 2001

Table 4 (continued)

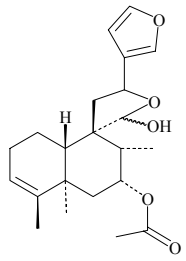
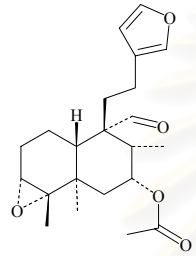
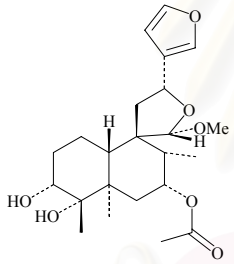
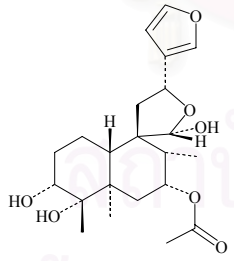
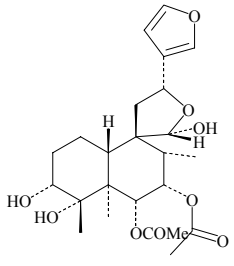
Plant and Chemical compound	Plant part	Reference
Cascarillin C [**115**] 	Stem bark	Vigor *et al*., 2001
Cascarillin D [**116**] 	Stem bark	Vigor *et al*., 2001
Cascarillin E [**117**] 	Stem bark	Vigor *et al*., 2001
Cascarillin F [**118**] 	Stem bark	Vigor *et al*., 2001
Cascarillin G [**119**] 	Stem bark	Vigor *et al*., 2001

Table 4 (continued)

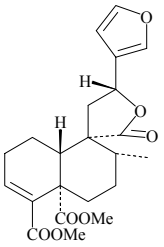
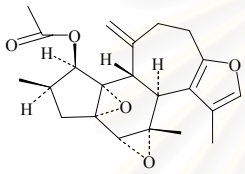
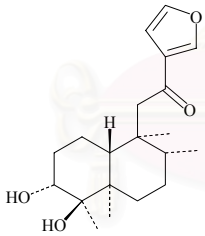
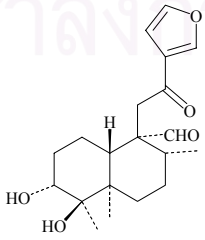
Plant and Chemical compound	Plant part	Reference
C. haumanianus		
Crotocorylifuran [**120**] 	Trunk bark	Tchissambou *et al*., 1990
Crotohaumanoxide [**121**] 	Trunk bark	Tchissambou *et al*., 1990
C. hovarum		
3α,4β-Dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-diene [**122**] 	Stem bark	Krebs and Ramiarantosa, 1996
3α,4β-Dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-diene-9-al [**123**] 	Stem bark	Krebs and Ramiarantosa, 1996

Table 4 (continued)

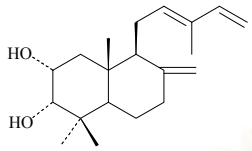
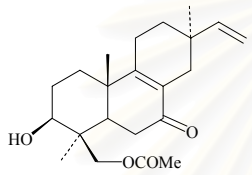
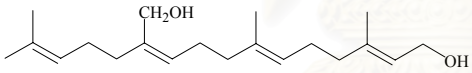
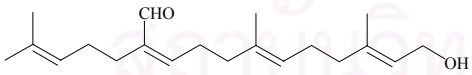
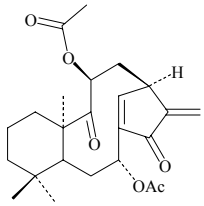
Plant and Chemical compound	Plant part	Reference
C. joufra		
2α,3α-Dihydroxy-labda-8(17),12(13),14(15)-triene [**124**] 	Leaf	Sutthivaiyakit *et al*., 2001
3β-Hydroxy-19-*O*-acetyl-pimara-8(9),15-diene-7-one [**125**] 	Leaf	Sutthivaiyakit *et al*., 2001
C. kerrii		
(*E*,*E*,*Z*)-11-Hydroxymethyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol [**126**] 	Leaf	Sato, Ogiso and Kuwano, 1980
(*E*,*E*,*E*)-11-Formyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol [**127**] 	Leaf	Sato *et al*., 1980
C. kongensis		
ent-8,9-*seco*-7α,11β-Diacetoxykaura-8(14),16-dien-9,15-dione [**128**] 	Leaf	Thongtan *et al*., 2003

Table 4 (continued)

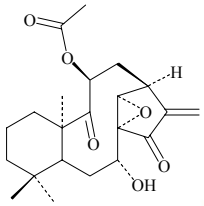
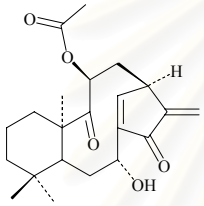
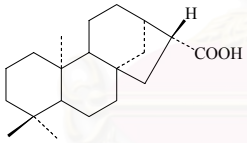
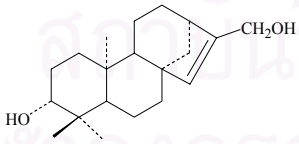
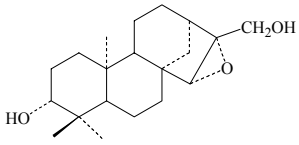
Plant and Chemical compound	Plant part	Reference
ent-8,9-*seco*-8,14-Epoxy-7α-hydroxy-11β-acetoxykaura-16-kauren-9,15-dione [**129**] 	Leaf	Thongtan *et al*., 2003
ent-8,9-*seco*-7α,11β-Diacetoxykaura-8(14),16-dien-9,15-dione [**130**] 	Leaf	Thongtan *et al*., 2003
C. lacciferus		
16α-H-*ent*-Kauran-17-oic acid [**131**] 	Root	Bandara, Wimalasiri and Macleod, 1988
ent-Kaur-15-en-3β,17-diol [**132**] 	Root	Bandara *et al*., 1988
ent-Kaur-15β,16-epoxykauran-17-ol [**133**] 	Root	Bandara *et al*., 1988

Table 4 (continued)

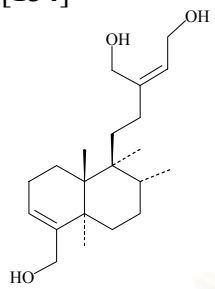
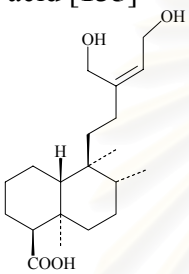
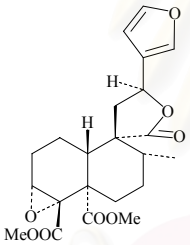
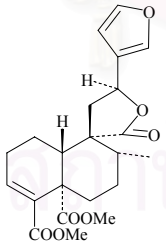
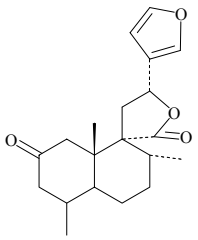
Plant and Chemical compound	Plant part	Reference
C. lechleri		
Bincatriol [**134**] 	Bark	Chen, Cai and Phillipson, 1994
Crolechinic acid [**135**] 	Bark	Chen *et al*., 1994
Korberin A [**136**] 	Bark	Chen *et al*., 1994
Korberin B [**137**] 	Bark	Chen *et al*., 1994
C. lucidus		
Crotonin [**138**] 	Leaf and twig	Chan, Taylor and Willis, 1968

Table 4 (continued)

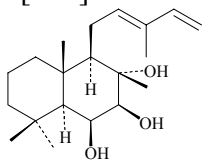
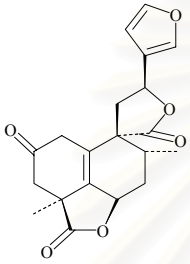
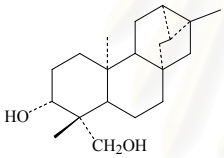
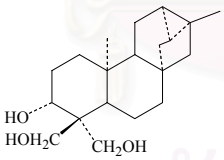
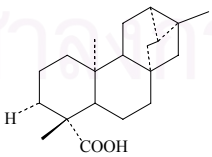
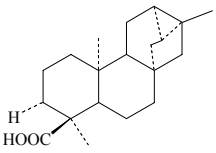
Plant and Chemical compound	Plant part	Reference
C. macrostachys		
Crotomachlin [**139**] 	Seed	Herlem, Huu and Kende, 1993
Neoclerodan-5,10-en-19,6β;20,12-diolide [**140**] 	Root	Kapingu *et al*., 2000
3α,19-Dihydroxytrachylobane [**141**] 	Root	Kapingu *et al*., 2000
3α,18,19-Trihydroxytrachylobane [**142**] 	Root	Kapingu *et al*., 2000
Trachyloban-19-oic acid [**143**] 	Root	Kapingu *et al*., 2000
Trachyloban-18-oic acid [**144**] 	Root	Kapingu *et al*., 2000

Table 4 (continued)

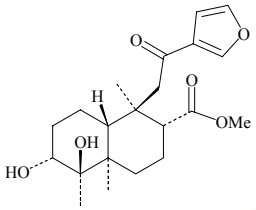
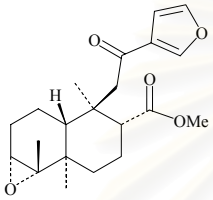
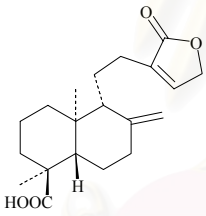
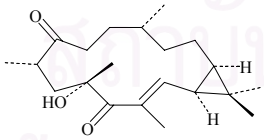
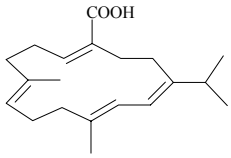
Plant and Chemical compound	Plant part	Reference
C. megalocarpus		
Criromodine [**145**] 	Bark	Mensah *et al*., 1989
Epoxychiromodine [**146**] 	Bark	Mensah *et al*., 1989
C. niveus		
Nivenolide [**147**] 	Leaf	Jas and Hahn, 1978
C. nitens		
Crotonitenone [**148**] 	Leaf and twig	Burke, Chan and Pascoe, 1981
C. oblongifolius		
Crotocembraneic acid [**149**] 	Stem bark	Vilaivan *et al*., 1997

Table 4 (continued)

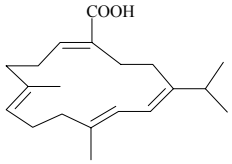
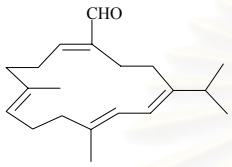
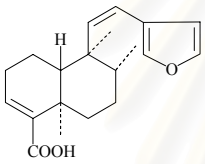
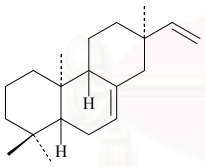
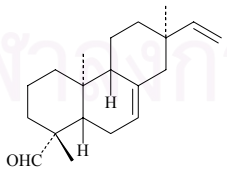
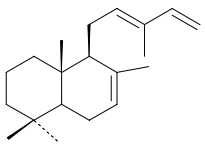
Plant and Chemical compound	Plant part	Reference
Neocrotocembraneic acid [**150**] 	Stem bark	Vilaivan *et al*., 1997
Neocrotocembranal [**151**] 	Stem bark	Roengsumran *et al*., 1999b
11-Dehydro-(-)-hardwickiic acid [**152**] 	Stem bark	Aiyar and Seshadri, 1972
ent-Isopimara-7,15-diene [**153**] 	Stem bark	Aiyar and Seshadri, 1972
ent-Isopimara-7,15-diene-19-aldehyde [**154**] 	Stem bark	Aiyar and Seshadri, 1972
Labda-7,12(*E*),14-triene [**155**] 	Stem bark	Roengsumran *et al*., 1999a

Table 4 (continued)

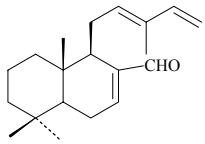
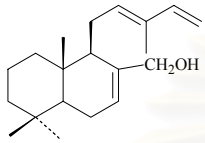
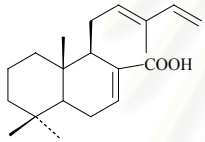
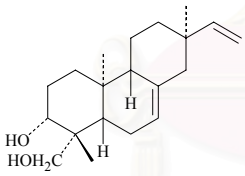
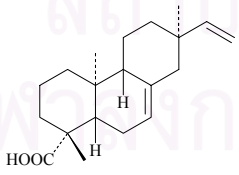
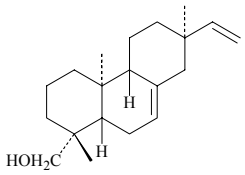
Plant and Chemical compound	Plant part	Reference
Labda-7,12(*E*),14-triene-17-al [**156**] 	Stem bark	Roengsumran *et al*., 1999a
Labda-7,12(*E*),14-triene-17-ol [**157**] 	Stem bark	Roengsumran *et al*., 1999a
Labda-7,12(*E*),14-triene-17-oic acid [**158**] 	Stem bark	Roengsumran *et al*., 1999a
Oblongifoliol [**159**] 	Stem bark	Aiyar and Seshadri, 1970
Oblongifolic acid [**160**] 	Stem bark	Aiyar and Seshadri, 1970
3-Deoxyoblongifoliol [**161**] 	Stem bark	Aiyar and Seshadri, 1972

Table 4 (continued)

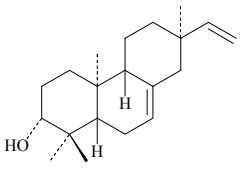
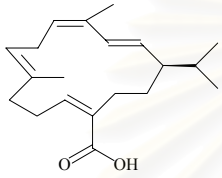
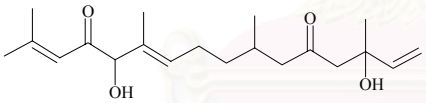
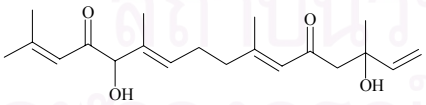
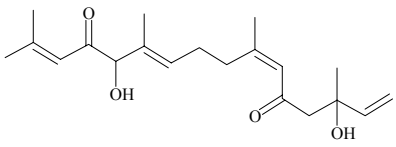
Plant and Chemical compound	Plant part	Reference
19-Deoxyoblongifoliol [**162**] 	Stem bark	Aiyar and Seshadri, 1970
C. poilanei		
Poilaneic acid [**163**] 	Leaf	Sato *et al*., 1981
C. salutaris		
(10*E*)-3,12-Dihydroxy-3,7,11,15-tetramethyl-1,10,14-hexadecatrien-5,13-dione [**164**] 	Twig	Itokawa *et al*., 1991
(6*E*,10*E*)-3,12-Dihydroxy-3,7,11,15-tetramethyl-1,6,10,14-hexadecatrien-5,13-dione [**165**] 	Twig	Itokawa *et al*., 1991
(6*Z*,10*E*)-3,12-Dihydroxy-3,7,11,15-tetramethyl-1,6,10,14-hexadecatrien-5,13-dione [**166**] 	Twig	Itokawa *et al*., 1991

Table 4 (continued)

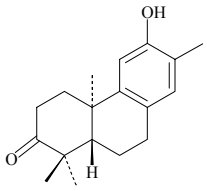
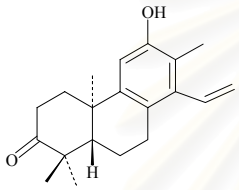
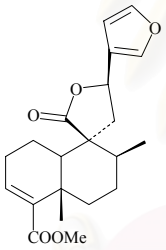
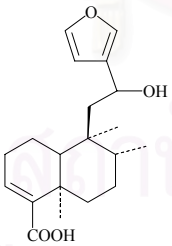
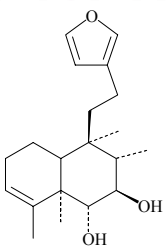
Plant and Chemical compound	Plant part	Reference
12-Hydroxy-13-methylpodocarpa-9, 11,13-trien-3-one [**167**] 	Twig	Itokawa *et al*., 1991
C. sonderianus		
Sonderianol [**168**] 	Heartwood	Craveiro and Silveira, 1982
Sonderianin [**169**] 	Heartwood	Craveiro *et al*., 1981b
12-Hydroxyhardwickiic acid [**170**] 	Root	McChesney, Clarke and Silveira, 1991
6α,7β-Dihydroxyannonene [**171**] 	Root	Silveira and McChesney, 1994

Table 4 (continued)

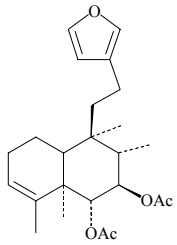
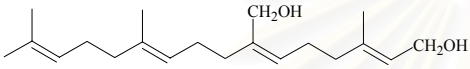
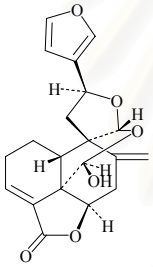
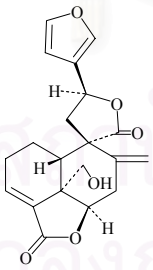
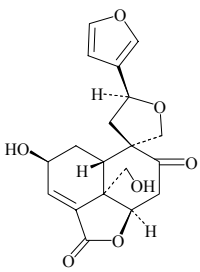
Plant and Chemical compound	Plant part	Reference
6α,7β-Diacetoxyannonene [**172**] 	Root	Silveira and McChesney, 1994
C.sublyratus		
Plaunotol [**173**] 	Stem	Ogiso *et al*., 1978
Plaunol A [**174**] 	Stem	Kitazawa *et al*., 1979
Plaunol B [**175**] 	Stem	Kitazawa *et al*., 1979
Plaunol C [**176**] 	Stem	Kitazawa *et al*., 1980

Table 4 (continued)

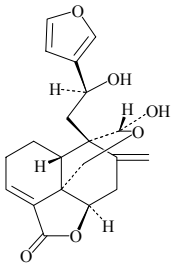
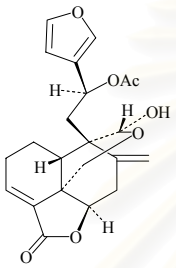
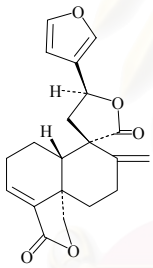
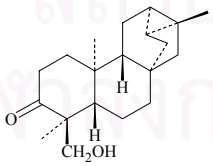
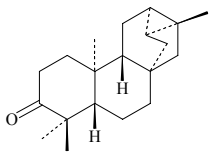
Plant and Chemical compound	Plant part	Reference
Plaunol D [**177**] 	Stem	Kitazawa *et al*., 1980
Plaunol E [**178**] 	Stem	Kitazawa *et al*., 1980
Plaunolide [**179**] 	Stem	Takahashi *et al*., 1983
C. zambesicus		
ent-18-Hydroxy-trachyloban-3-one [**180**] 	Leaf	Block *et al*., 2004
ent-Trachyloban-3-one [**181**] 	Leaf	Block *et al*., 2004

Table 5 Distribution of triterpenes in *Croton* spp.

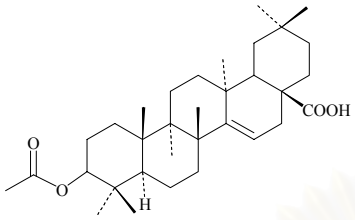
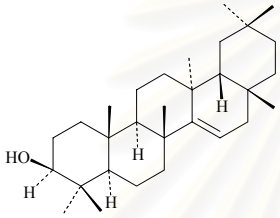
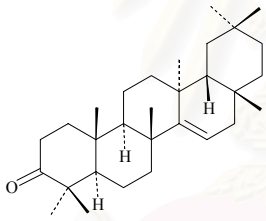
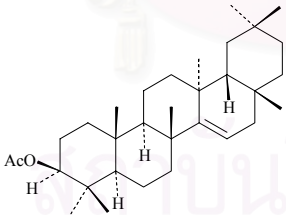
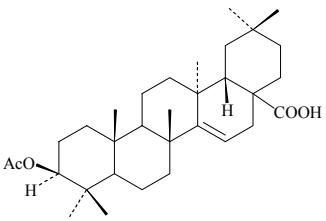
Plant and Chemical compound	Plant part	Reference
Croton cajucara		
Acetyl aleuritolic acid [**182**] 	Bark	Maciel *et al*., 1998
C. caudatus		
Taraxerol [**183**] 	Stem bark	Chatterjee and Banerjee, 1977
Taraxenone [**184**] 	Stem bark	Chatterjee and Banerjee, 1977
Taraxeryl acetate [**185**] 	Stem bark	Chatterjee and Banerjee, 1977
C. lacciferus		
3β-Acetoxy-D-friedolean-14-en-28-oic acid [**186**] 	Root	Bandara *et al*., 1998

Table 6 Distribution of alkaloids in *Croton* spp.

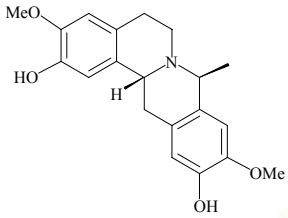
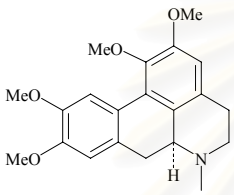
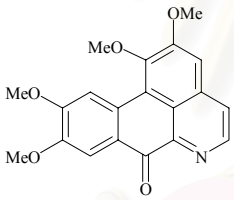
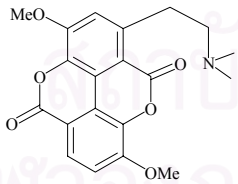
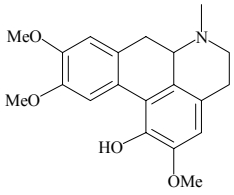
Plant and Chemical compound	Plant part	Reference
Croton hemiargyreus		
Hemiargyrine [**187**] 	Leaf and stem	Amaral and Barnes, 1998
Glaucine [**188**] 	Leaf and stem	Amaral and Barnes, 1998
Oxoglaucine [**189**] 	Leaf and stem	Amaral and Barnes, 1998
C. lechleri		
Taspine [**190**] 	Leaf	Dennis *et al*., 2002
Thaliporphine [**191**] 	Leaf	Dennis *et al*., 2002

Table 6 (continued)

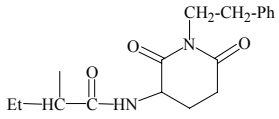
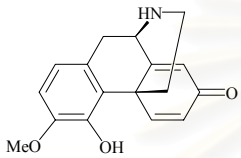
Plant and Chemical compound	Plant part	Reference
C. membranaceus		
Julocrotine [**192**] 	Stem	Aboagye *et al*., 2000
C. salutaris		
N-norsalutaridine [**193**] 	Leaf and twig	Roderick and Orlando, 1981

Table 7 Distribution of miscellaneous compounds in *Croton* spp.

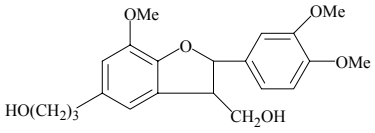
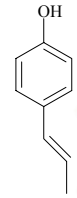
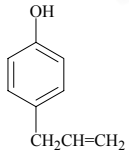
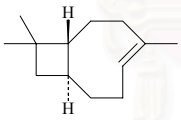
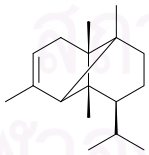
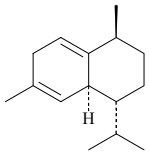
Plant and Chemical compound	Category	Plant part	Reference
C. erythrochilus			
4-*O*-Methyldihydrodehydro diconiferyl alcohol [**194**] 	Lignan	Stem	Pieters , Dirk and Arnold, 1990
C. essequiboensis			
Anethole [**195**] 	Phenyl propanoid	Leaf	Craveiro *et al*., 1981a
Estragole [**196**] 	Phenyl propanoid	Leaf	Craveiro *et al*., 1981a
β-Caryophyllene [**197**] 	Sesquiterpene	Leaf	Craveiro *et al*., 1981a
α-Copaene [**198**] 	Sesquiterpene	Leaf	Craveiro *et al*., 1981a
α-Cubenene [**199**] 	Sesquiterpene	Leaf	Craveiro *et al*., 1981a

Table 7 (continued)

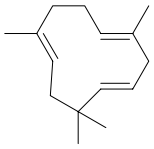
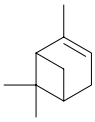
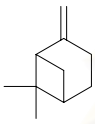
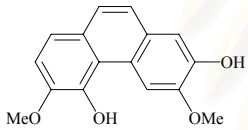
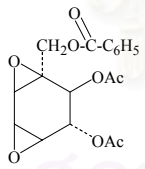
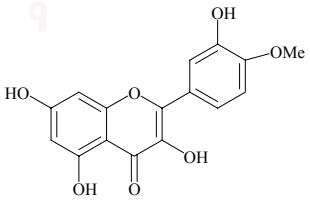
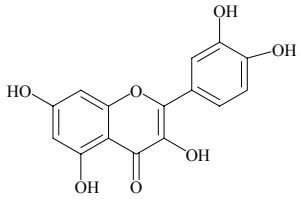
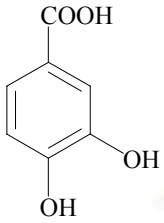
Plant and Chemical compound	Category	Plant part	Reference
α-Humulene [**200**] 	Sesquiterpene	Leaf	Craveiro *et al*., 1981a
α-Pinene [**201**] 	Monoterpene	Leaf	Craveiro *et al*., 1981a
β-Pinene [**202**] 	Monoterpene	Leaf	Craveiro *et al*., 1981a
C. flavens			
Crotoflavol [**203**] 	Phenanthrene	Leaf	Wolfram and Franz, 2001
C. macrostachys			
Crotepoxide [**204**] 	Cyclohexane diepoxide	Fruit	Kupchan, Hemingway and Smith, 1969
C. oblongifolius	Flavonoid	Leaf	Subramanian, Nagarajan and Sulochana, 1971
Isorhamnatin [**205**] 			

Table 7 (continued)

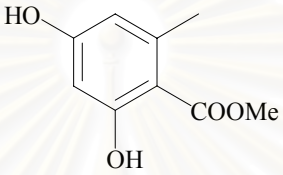
Plant and Chemical compound	Category	Plant part	Reference
Quercetin [**206**] 	Flavonoid	Leaf	Subramanian *et al*., 1971

3. Literature reviews of *Croton hutchinsonianus*

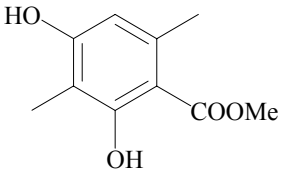
In 1990, Chaoming *et al*. reported the presence of three benzenoid compounds, (protocatechuic acid [**207**], methyl orsellinate [**208**], methyl 2,4-dihydroxy-3,6-dimethylbenzoate [**209**]), two diterpenes (*ent*-kauran-16β,17diole [**210**], and *ent*-kauran-16β,17,19-triol [**211**]) , two steroids (β-sitosterol [**38**], β-sitosterol-D-glucoside [**37**]) and two miscellaneous compounds (triacontanol [**212**], dotriacontanoic acid [**213**]) from the stem bark of *C. hutchinsonianus* (Chaoming *et al*., 1990).



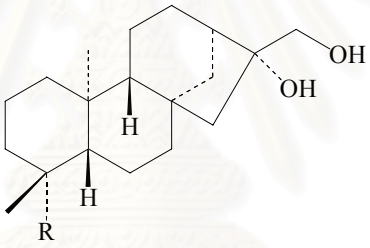
[**207**]



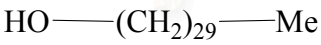
[**208**]



[**209**]



[**210**] R = Me
[**211**] R = CH_2OH

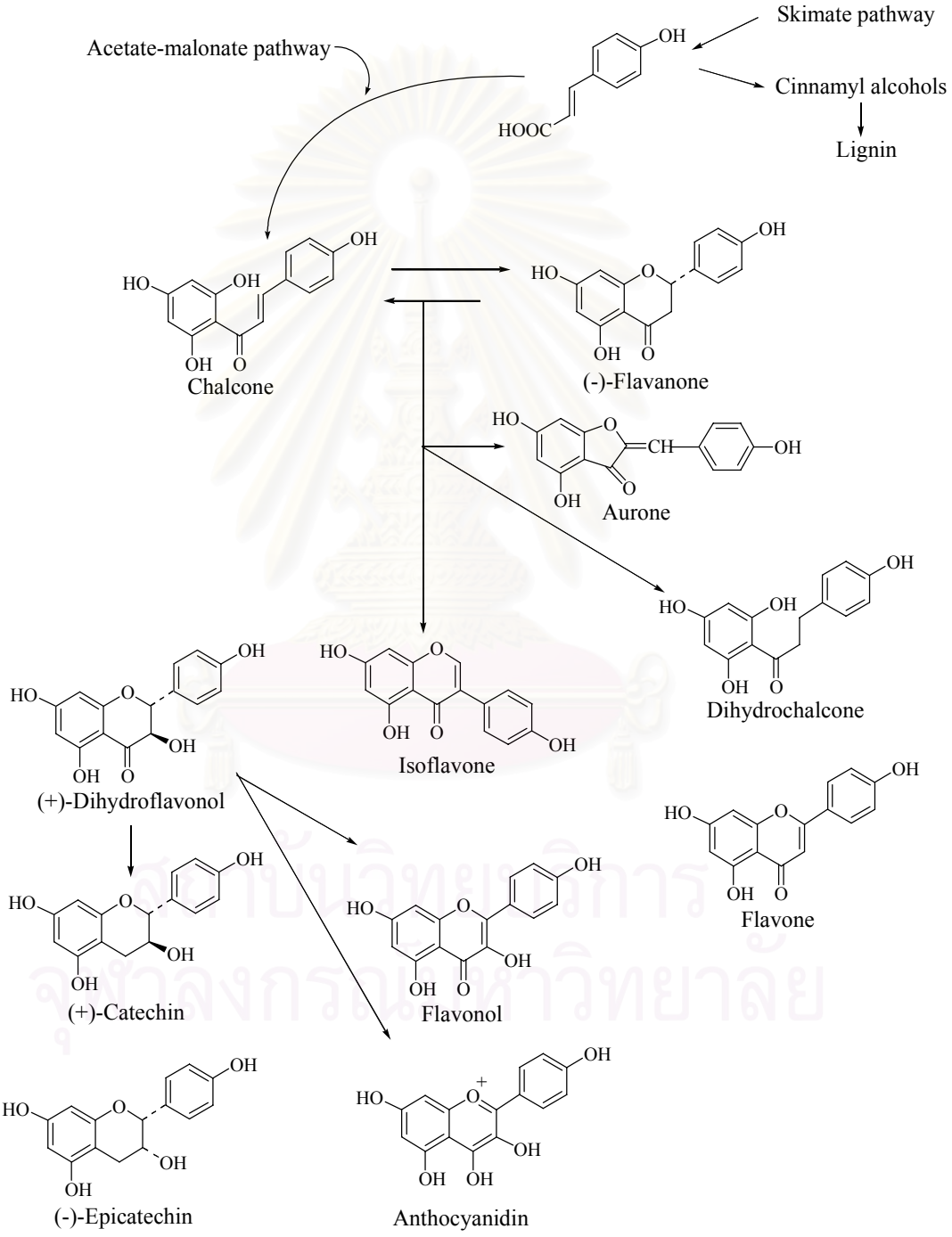


[**212**]

[**213**]

4. Biosynthetic Relationship of Flavonoids in *Bauhinia* spp.

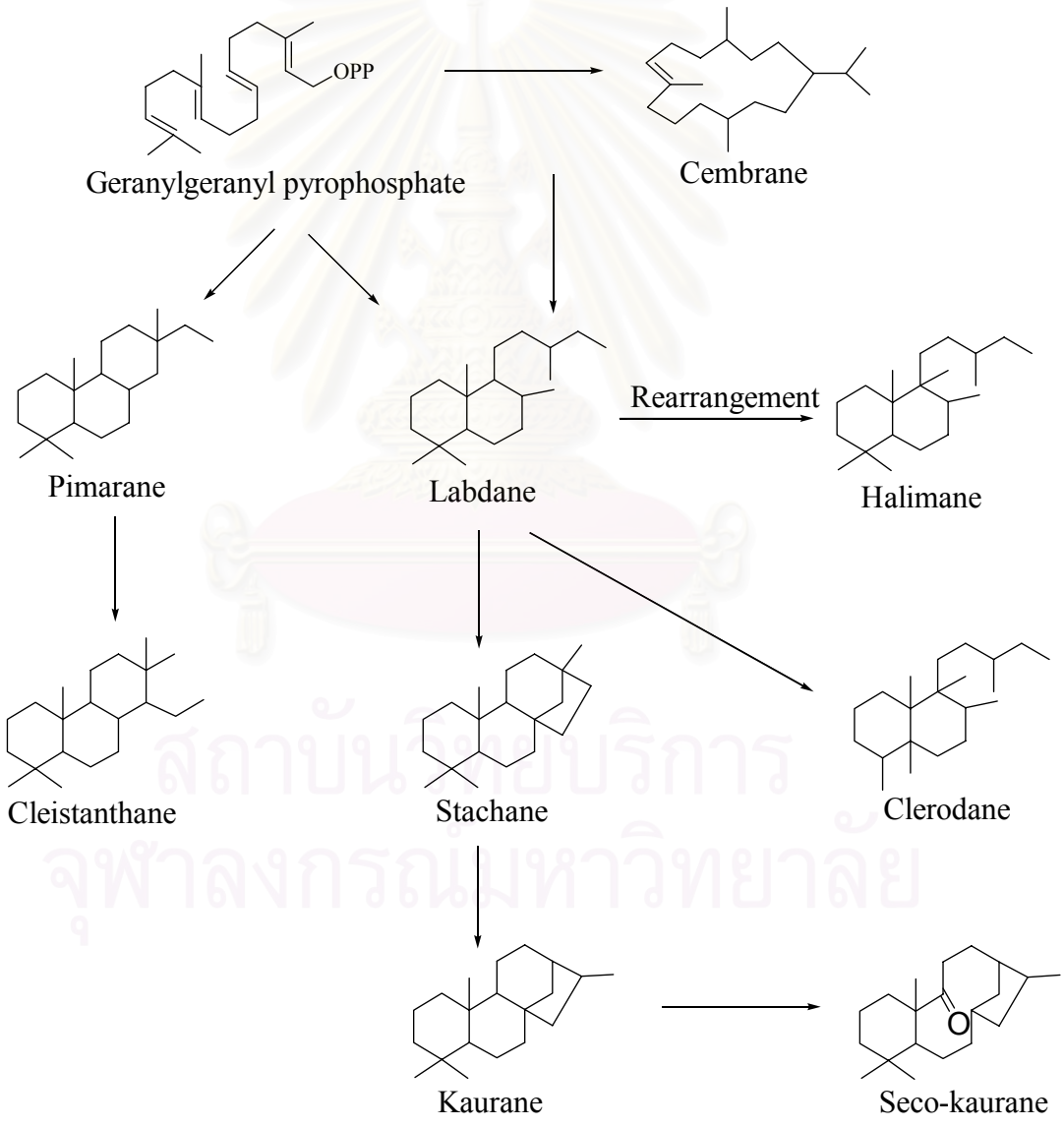
Flavonoids possess fifteen carbons atom in their basic skeletons, which are derived from shikimate and acetate-malonate pathway. The typical flavonoids in *Bauhinia* spp. are chalcone, flavone, flavanone, flavonol, dihydroflavonol and flavan. The relationship of flavonoids is displayed in Scheme 1 (Markham, 1982).



Scheme 1 Currently proposed interrelationships between flavonoid monomer.

5. Biosynthetic Relationship of Diterpenoids in *Croton* spp.

The diterpenes possess twenty carbon atoms in their molecules. They are biogenetically derived from geranylgeranyl pyrophosphate (GGPP). The diterpene skeleton is the fascinating variation encountered in their core structure, these compounds could be classified into several types, such as mono-, bi-, tri-, tetra- and pentacyclic diterpenes. The typical diterpenes in *Croton* spp. are casbane, cembrane, clerodane, cleistanthane, kaurane, labdane, pimarane and halimane. The relationship of diterpenes is displayed in Scheme 2. In addition, the biosynthetic is also proposed (Devon and Scott, 1972).



Scheme 2 Biosynthetic relationship of diterpenes in *Croton* spp.

6. Traditional Uses and Biological Activities of *Bauhinia* spp.

Many plants of the genus *Bauhinia* have been used in traditional medicine in several countries. The decoction of *B. racemosa* leaves has been used in the treatment of headache and malaria, and its bark as an astringent for diarrhea and dysentery in Indian medicine (Anjaneyulu *et al*., 1984). The one handful of grated stem bark of *B. guianensis* is boiled in two liters of water until reduced to 1 liter, then drink half a cup three times per day for stomachache and diarrhea (Mùnoz *et al*., 2000). In Nigeria, the leaves of *B. thonningii* are used to treat diarrhea and fever (Kudi *et al*., 1999). *B. splendens* is a native plant widely distributed in Brazil, being popularly known as "cipo escada", "cipo unha de boi", "escada de jaboti" and "escada demacaco". The leaf and stem bark have been used as traditional remedies in folk medicine for the management of several diseases, *e.g*. infections, inflammatory processes, diabetes and infections of the urinary tracts (Filho *et al*., 1997). In Argentina and southern Brazil, the infusion of *B. candidans* leaves is widely used because of theirs potential hypoglycemic action (Irribarren and Pomilio, 1983).

A famous Thai traditional medicine from *B. sirindhorniae* is known as "Sam Sip Song Pra Dong". The infusion of its stem has been used as anti-inflammatory.

A number of biological investigations of *Bauhinia* species has been reported. The 70% ethanol extract of *B. guianensis* was reported to possess antimalarial activity (Mùnoz *et al*., 2000). The 80% ethanol extract of *B. thonnigii* showed inhibitory effects against parvovirus (Kudi and Myint, 1999), gram-positive bacteria *Staphylococcus aureus*, and gram-negative bacteria *Escherichia coli* (Kudi *et al*., 1999). The 50% ethanol extract of *B. splendens* had a significant analgesic action when assessed against several models of pain. The mechanism underlying its analgesic effect still remains unknown, but seems to be unrelated to interaction with opioid systems (Filho *et al*., 1997).

The antimalarial activities of preracemosol A [**56**], preracemosol B [**57**], racemosol [**64**] and demethylracemosol [**65**] from *B. malabarica* exhibited moderate activities. While only racemosol and demethylracemosol exhibited cytotoxicity against KB and BC cell lines (Kittakoop *et al*., 2000).

As the root bark dichloromethane extract of *B. rufescens* showed antifungal activity in a bioassay with the plant pathogenic fungus *Cladosporium cucumerinum*, a phytochemical investigation was undertaken on material collected in Nigeria. Activity guided fractionation of this extract, using different preparative

chromatographic methods, allowed the isolation of four antifungal tetracyclic compounds: racemosol [**64**], 5,6-dihydro-11-methoxy-2,2,12-trimethyl-2H-naphthol [1,2-f][1]benzopyran-8,9-diol [**68**], 11-methoxy-2,2,12-trimethyl-2H-naphthol[1,2-f][1]benzopyran-8,9-diol [**69**] and 1,7,8,12b-tetrahydro-2,2,4-trimethyl-2H-benzo[6,7]cyclohepta[1,2,3-de][1]benzopyran-5,10,11-triol [**70**] (Millard *et al*., 1991).

The antioxidant activities of *B. tarapotensis* were determined by measuring their free radical scavenging effects using the 1,1-diphenyl-2-picryl hydrazyl free radical (DPPH). Trolox equivalent antioxidant activity (TEAC) methods and the coupled oxidation of β-carotene and linoleic acid. (-) Isolariciresinol-3-α-*O*-β-D-glucopyranoside [**73**], (+)-1-hydroxypinoresinol-1-*O*-β-D-glucopyranoside [**74**] and isoacteoside [**75**] showed good activities in the DPPH and TEAC tests, while 2,4-dihydroxy-2-(2-hydroxy ethyl) cyclohexe-5-en-1-one [**71**] and caffeoyl ester of apionic acid [**76**] were active in the coupled oxidation of β-carotene and linoleic acid bioassay (Braca *et al*., 2001).

7. Traditional Uses and Biological Activities of *Croton* spp.

A great number of species in the genus *Croton* is used in folk medicine for wound infection and also accelerate wound healing. Moreover, they are used to treat rheumatism, cancer (Luzbetak *et al*., 1979), gastric diseases (Craveiro *et al*., 1981a), diarrhea, diabetes (Kubo *et al*., 1991), anthelmintic, purgative, skin rashes, malaria, venereal diseases (Mazzanti *et al*., 1987) and whooping cough (Weckert *et al*., 1992).

Extracts of several species of *Croton* are known to produce anti-inflammatory, antibacterial, antiviral, insecticidal, antifungal, cytotoxicity and other effects. Detailed information on the biological activities of some *Croton* species is exemplified below.

The ethyl acetate extract from bark of *C. cuneatus*, *C. lechleri* and aerial parts of *C. trinititatis* showed antibacterial activity against *Staphylococcus aureus* and antiviral activity against sindbis virus and murine cytomegalovirus (Macrae *et al*., 1988). The ethanol extract from bark of *C. guatamalensis* revealed the antidermatomucosal infections against *Candida albicans* (Caceres *et al*., 1991). A benzene extract of *C. sonderianus* was shown to have antibiotic activity against *Mycobacterium smegmatis* and *Staphylacoccus aureus* (Craveiro and Silveira, 1982). The extract of the cortices of *C. cajucara* showed anti-inflammatory activity against

topical inflammation in the mouse ear induced by teleocidin which was a highly potent irritant and tumor-promoting alkaloid (Ichihara *et al*., 1991). The methanol extract of the bark of *C. cajucara* exhibits strong insect-growth inhibitory activity in the artificial diet feeding bioassay using the lepidopteran pest insect *Pectinophora gossypiela* (pink ballworm) (Kubo *et al*., 1991). The ethanol extract of *C. cajucara* was reported to have a lipid lowering effect in rats fed high fat diet but not in normal rat (Farias *et al*., 1997). The hot petrol extract of the root of *C. lacciferus* and the acetone extract of the root of *C. aromaticus* showed significant insecticidal activity against *Alphis craccivora* (Bandara *et al*., 1988; Bandara *et al*., 1987). The extract of the red sap from *C. palanostigma* was found to be cytotoxic to V-79 cells (Itokawa *et al*., 1991).

An alcoholic extract of the fruits of *C. macrostachys* showed significant inhibitory activity against the Lewis lung carcinoma in mice. Systematic fractionation of the active extract led to characterization of a major active component, crotepoxide [**204**], a cyclohexane diepoxide (Kupchan *et al*., 1969).

An acetone extract of the stem of *C. sublyratus* showed inhibitory activity against reserpine–induced ulcer in mice and Shay-ulcer in rats. Systematic fractionation of the active acetone extract guided by antiulcer activity assay led to the isolation of 18-hydroxy geranylgeraniol or plaunotol [**173**] as the principle constituent with anti-reserpine ulcer activity. This plant known in Thai as “Plau-noi” and has been used as anthelmintic and dermatologic agent (Ogiso *et al*., 1978).

The bioassay-guided fractionation of the crude extract of *C. cajucara* led to the isolation of two clerodane diterpenes, cajucarinolide [**85**] and isocajucarinolide [**86**] which showed inhibitory activities against the topical inflammation in the mouse ear induced by teleocidin (dose = 1 μg/ear) with IC_{50} of 5.6 and 3.0 μg, respectively. In addition, these compounds are potent inhibitors of bee venom phospholipase A_2 *in vitro* (Ichihara *et al*., 1991).

Taspine [**190**] isolated from the chloroform extract of a red viscous sap of the bark of mature trees of *C. lechleri* and its hydrochloride salt were shown to have anti-inflammatory activity in three different standard pharmacological models such as the carrageenan-induced pedal edema method, the cotton pellet-induced granuloma method and the adjuvant polyarthritis model (Perdue *et al*., 1979).

Taspine [**190**] isolated from *C. palanostigma* was found to be cytotoxic to V-79 cells and KB cells with IC_{50} of 0.17 and 0.39 μg/ml, respectively (Itokawa *et al*., 1991).

The kauranoids, *ent*-Kaur-15-en-3β,17-diol [**132**] and *ent*-Kaur-15β,16-epoxykauran-17-ol [**133**] from the hot petrol extracts of the root of *C. lacciferus* showed moderate insecticidal activity against *Alphis craccivora* (Bandara *et al*., 1988)

Trans-dehydrocrotonin (t-DCTN), a 19-nor clerodane diterpene [**88**] was isolated from the bark of *C. cajucara*. This compound exhibited an insect growth inhibitory property with ED_{50} of 30 ppm against the lepidopteran pest insects (*Pectinophora gossypiella* and *Heliothis virescens*) (Kubo *et al*., 1991) and demonstrated a significant hypoglycemic activity in alloxan-induced diabetic rats but not in normal rats. The oral medication with t-DCTN (25 and 50 mg/kg) when administered daily on three consecutive days caused a significant decrease of blood sugar levels when compared to untreated diabetic controls (Farias *et al*., 1997).

8,9-Secokaurane diterpenes, *ent*-8,9-*seco*-7α,11β-Diacetoxykaura-8(14),16-dien-9,15-dione [**128**], *ent*-8,9-*seco*-8,14-Epoxy-7α-hydroxy-11β-acetoxykaura-16-kauren-9,15-dione [**129**] and *ent*-8,9-*seco*-7α,11β-Diacetoxykaura-8(14),16-dien-9,15-dione [**130**] isolated from *C. kongensis* exhibited antimycobacterial activity with minimum inhibitory concentrations (MICs) of 25.0, 6.25 and 6.25 μg/ml, respectively and possessed *in vitro* antimalarial activity against *Plasmodium falciparum* (K_1, multidrug-resistant strain) (Thongtan *et al*., 2003)

Neocrotocembranal [**151**] isolated from the stem of *C. oblongifolius* markedly inhibited platelet aggregation induced by thrombin (0.25 unit/ml). The effect of neocrotocembranal on platelets is probably due to the reactive aldehyde functionality. In addition, neocrotocembranal (6.48 μg/ml) and neocembraneic acid [**150**] (41.47 μg/ml) exhibited cytotoxic activity against P-388 cell culture. It should be mentioned that many cembranoids exhibit cytotoxic activity, especially those highly functionalized cembranoids obtained from marine sources (Roengsumran *et al*., 1999b).

CHAPTER III

EXPERIMENTAL

1. Sources of Plant Materials

The stems and the roots of *Bauhinia sirindhorniae* K & S.S. Larsen were collected from Nongkhai Province, Thailand in January 2001. Authentication of the plant materials was done by comparison with herbarium specimens (BKF No. 124725) at the Botany Section, Technical Division, Department of Agriculture and Cooperatives, Bangkok, Thailand. A voucher specimen has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

The leaves and the branches of *Croton hutchinsonianus* Hosseus were collected from Karnchanaburi Province, Thailand in March 2003. Authentication of the plant materials was done by comparison with herbarium specimens (BKF No. 2225) at the Botany Section, Technical Division, Department of Agriculture and Cooperatives, Bangkok, Thailand. A voucher specimen has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

2. General Techniques

2.1 Analytical Thin Layer Chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	1. Silica gel 60 F_{254} (E. Merck) precoated plate (Aluminium sheet)
		2. ODS, RP-18 F_{254} (E. Merck) precoated plate (Aluminium sheet)
Layer thickness	:	0.25 mm
Distance	:	5 cm
Temperature	:	room temperature (25-35 $^{\circ}$C)
Detection	:	1. Ultraviolet light at 254 and 365 nm
		2. 10% H_2SO_4 in EtOH and heated at 110 $^{\circ}$C for 10 min

2.2 Preparative Thin Layer Chromatography (PTLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	1 mm
Distance	:	15 cm
Temperature	:	room temperature (25-35 $^{\circ}$C)
Detection	:	Ultraviolet light at 254 and 365 nm

2.3 Column chromatography

2.3.1 Vacuum Liquid Column Chromatography

Adsorbent	:	Silica gel 60 (70-230 mesh)
Packing method	:	Dry packing
Sample loading	:	The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on the top of the column.
Detection	:	Fractions were examined by TLC observing under UV light (254 and 356 nm).

2.3.2 Flash Column Chromatography

Adsorbent	:	1. Silica gel 60 (230-400 mesh) 2. Cosmosil 75 C_{18}-OPN (Nacalai tesque)
Packing method	:	Dry packing
Sample loading	:	The sample was dissolved in a small amount of eluent and then applied gently on the top of the column.
Detection	:	Fractions were examined in the same manner as described in section 2.3.1

2.3.3 Gel Filtration Chromatograaphy

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 eluent and applied on top of the column.

2.3.4 High Pressure Liquid Chromatography (HPLC)

Column (Semi-prep.)	:	Inertsil ODS column (20 i.d.×250mm) (gaskurokogyo)
(Analytical)	:	TSK gel ODS120A (4.6 i.d.×150 mm) (TOSOH)
Flow rate	:	1. 5 ml/min for semi-preparative column 2. 1 ml/min for analytical column
Mobile phase	:	1. Isocratic 85% water + 25% methanol 2. Isocratic 70% water + 20% acetonitrile + 10% methanol
Sample preparation	:	The sample was dissolved in a small amount of eluent and filtered through Millipore filter paper before injection.
Injection volume	:	1 ml
Pump	:	LC-9A (Shimadzu)
Detector	:	SPD-6AV UV Detector (Shimadzu)
Recorder	:	C-R6A Chromatopac (Shimadzu)
Temperature	:	Room temperature

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) Absorption Spectra

UV spectra were obtained on Shimadzu UV-2100S UV/vis spectrophotometer (Chulabhorn Research Institute).

2.4.2 Infrared (IR) Absorption spectra

IR spectra were recorded on a JASCO A-302 (Chulabhorn Research Institute) and a JAS FT/IR 230-IR spectrometer (Faculty of Pharmaceutical Sciences, Chiba University).

2.4.3 Mass Spectra

Fast-Atom Bombardment mass spectra (FABMS) and High Resolution Fast Atom Bombardment mass spectra (HRFABMS) were measured on a JEOL JMS-HX-110A spectrometer (The Chemical Analysis Center, Chiba University).

Electron impact mass spectra (EIMS) were measured on a Finnigan INCOS 50 and High Resolution Fast Atom Bombardment mass spectra (HRFABMS) were measured on a MAT 90 (Chulabhorn Research Institute).

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

^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectra were obtained with a Bruker AM 400 (Chulabhorn Research Institute).

^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz) spectra were obtained with a JEOL JNM GSX 500A spectrometer (Faculty of Pharmaceutical Sciences, Chiba University).

2.5 Physical Properties

2.5.1 Optical Rotations

Optical rotation were measured on a JASCO DIP 140 polarimeter (Faculty of Pharmaceutical Sciences, Chiba University) and a Perkin Elmer 341 polarimeter (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5.2 Circular Dichroism (CD) Spectra

Circular Dichroism spectra were measured on a JASCO CD J-720 W spectrometer (Faculty of Pharmaceutical Sciences, Chiba University).

2.5.3 Melting Points

Melting points were obtained on a Yanagimoto Micro Melting Point Apparatus (Faculty of Pharmaceutical Sciences, Chiba University) and Eletrothermal melting point apparatus, Electrothermal 9100 (Chulabhorn Research Institite).

2.6 Solvents

Throughout this work, commercial grade organic solvents were used and redistilled prior to use.

2.7 Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Wako)

6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) (Aldrich)

Quercetin (Aldrich)

2.8 Microtiter Plate Reader

Microtiterplate reader was performed on a Biorad Model 550 (Faculty of Pharmaceutical Sciences, Chiba University).

3. Extraction and Separation

3.1 Extraction and Separation of the Stems of *Bauhinia sirindhorniae*

3.1.1 Extraction

The dried stems of *Bauhinia sirindhorniae* (350 g) were successively extracted with hexane (3×4 L), chloroform (3×4 L), and 95% ethanol (3×4 L). The filtrates were pooled and evaporated under reduced pressure at the temperature not exceeding 40 °C to give the corresponding hexane (620.5 mg), chloroform (515.3 mg) and 95% ethanol extract (62.3 g), respectively.

The 95% ethanol extract (62.3 g) was then partitioned between butanol and water. The butanol layer was dried to yield 22.5 g of a butanol extract while 20.4 g of an aqueous extract was obtained.

3.1.2 Isolation

3.1.2.1 Isolation of Compounds from Chloroform Extract (Sheme 3 and Figure 3)

The chloroform extract (515.3 mg) was dissolved in a small amount of chloroform, triturated with silica gel 60 (70-230 mesh) and dried at room temperature. It was then fractionated by liquid column chromatography. Elution was completed in a polarity gradient manner with mixtures of chloroform-acetone.

The eluates were examined by TLC (silica gel) using mixtures of chloroform-acetone as a developing solvent. Fractions with similar chromatographic pattern were combined to afford five fractions: fractions SC-A (27.6 mg), SC-B (100.2 mg), SC-C (68.9 mg), SC-D (91.8 mg) and SC-E (22.1 mg).

3.1.2.1.1 Isolation of Compound BSC1 (Lupeol)

Fraction SC-B (100.2 mg) was separated by column chromatography (silica gel 60 (230-400 mesh)) using mixtures of hexane-acetone (9:1) as eluent. After combining of collected fractions according to chromatographic pattern (silica gel, hexane-acetone 4:1), three fractions including SC-B1 to SC-B3 were obtained.

Fraction SC-B1 (23.4 mg), after removal of solvents, gave compound BSC1 (5.7 mg, 1.6×10^{-3}% based on dried weight of stems). This compound was identified as lupeol [**77**].

3.1.2.1.2 Isolation of Compound BSC2 (Glutinol)

Fraction SC-B2 (52.3 mg) was subjected to gel filtration chromatography using a Sephadex LH 20 column with mixtures of chloroform-acetone (4:1) as the

eluent. The eluates were collected and combined according to their TLC chromatographic patterns (silica gel, hexane-acetone 4:1).

Fraction SC-B2-2 (35.5 mg), after removal of solvents, gave compound BSC2 (16.3 mg, 4.7×10^{-3}% based on dried weight of stems). This compound was identified as glutinol [**214**].

3.1.2.2 Isolation of Compounds from Butanol Extract (Schemes 4-5 and Figure 3)

The butanol extract (22.5 g) was dissolved in a small amount of methanol, triturated with silica gel 60 (70-230 mesh) and dried at room temperature. It was then fractionated by liquid column chromatography. Elution was completed in a polarity gradient manner with mixtures of chloroform-methanol-water.

The eluates were examined by TLC (silica gel) using mixtures of chloroform-methanol-water as a developing solvent. Fractions with similar chromatographic pattern were combined to afford seven fractions: fractions SB-A (51.2 mg), SB-B (81.4 mg), SB-C (810.6 mg), SB-D (1.2 g), SB-E (393.8 mg), SB-F (2.1 g) and SB-G (16.5 g).

3.1.2.2.1 Isolation of Compound BSB1 (Isoliquiritigenin)

Fraction SB-A (51.2 mg) was fractionated by gel filtration chromatography using a Sephadex LH 20 column with mixtures of chloroform-acetone (1:1) as an eluent. Eluates were collected and combined based on their chromatographic patterns (silica gel, hexane-ethyl acetate 3:2) to give four fractions (SB-A1 to SB-A4).

Fraction SB-A3 (8.2 mg), after removal of solvents, gave compound BSB1 (4.5 mg, 1.3×10^{-3}% based on dried weight of stems). This compound was identified as isoliquiritigenin [**14**].

3.1.2.2.2 Isolation of Compound BSB2 ((+)-Isolariciresinol-3α-*O*-α-L-rhamnoside)

Fraction SB-C (810.6 mg) was separated on a Sephadex LH 20 column (chloroform-methanol 1:1). The eluates were collected and examined by TLC (silica gel, chloroform-methanol-water 8:2:0.1). Fractions with similar chromatographic patterns were combined to yield four fractions (fraction SB-C1 to SB-C4).

Fraction SB-C2 (100.5 mg) was re-separated by column chromatography using silica gel 60 (230-400 mesh) as an adsorbent and eluted with mixtures of chloroform-methanol-water (8:2:0.1). The eluates were collected and combined

according to similarity of chromatographic patterns (chloroform-methanol-water 8:2:0.5) to obtained four fractions (SB-C2-1 to SB-C2-4).

Fraction SB-C2-3 (28.9 mg) was subsequently separated by HPLC using an Inertsil ODS column (20 i.d.×250 mm) with UV 254 nm detection. Elution was performed in an isocratic manner with 70% water + 20% acetonitrile + 10% methanol (flow rate 5 ml/min). Compound BSB2 (2.0 mg, 5.7×10^{-4}% based on dried weight of stems) was obtained at the retention time of 17 minutes. This compound was identified as (+)-isolariciresinol 3α-*O*-α-L- rhamnoside [**215**].

3.1.2.2.3 Isolation of Compound BSB3 (3,4,5-Trimethoxyphenolic-1-*O*-β-D- glucoside)

Fraction SB-C3 (112.8 mg) was re-separated by column chromatography using silica gel 60 (230-400 mesh) as an adsorbent and eluted with mixtures of chloroform-methanol-water (8:2:0.1). The eluates were collected and combined according to the chromatographic patterns (silica gel, chloroform-methanol-water 8:2:0.5) to give four fractions (SB-C3-1 to SB-C3-4).

Fraction SB-C3-3 (10.6 mg) was separated by HPLC using an Inertsil ODS column (20 i.d.×250 mm) eluted with in an isocratic manner with 70% water + 20% acetonitrile + 10% methanol (flow rate 5 ml/min) to give compound BSB3 (3.8 mg, 1.1×10^{-3}% based on dried weight of stems) at retention time 21 minutes. It was identified as 3,4,5-trimethoxyphenolic-1-*O*-β-D- glucoside [**216**].

3.1.2.2.4 Isolation of Compound BSB4 ((-)-Epicatechin)

Fraction SB-C4 (150.3 mg) was subjected to column chromatography using silica gel 60 (230-400 mesh) as an adsorbent and eluted with mixtures of chloroform-methanol (9:1). After combination of collected fractions according to chromatographic pattern (silica gel, chloroform-methanol 4:1), four fractions including fraction SB-C4-1 to SB-C4-4 were obtained.

Recrystallization of fraction SB-C4-2 (22.7 mg) with mixtures of chloroform-methanol yielded a pale yellow needle of compound BRB4 (8.5 mg, 2.4×10^{-3}% based on dried weight of stems). This compound was identified as (-)-epicatechin [**217**].

3.1.2.2.5 Isolation of Compound BSB5 (Protocatechuic acid)

Fraction SB-D (1.2 g) was further purified by gel filtration chromatography using a Sephadex LH 20 column with mixtures of chloroform-methanol (1:1) as the eluent, which resulted in the collection of two fractions SB-D1 and SB-D2.

Fraction SB-D-2 (564.4 mg) was subjected to Cosmosil 75 C_{18}-OPN column chromatography with mixtures of methanol-water (1:4). Eluates were collected and combined based on their chromatographic patterns (silica gel, chloroform-methanol 4:1) to give four fractions (SB-D2-1 to SB-D2-4). Fraction SB-D2-2 (22.6 mg) was recrystallized from methanol to give compound BSB5 (8.0 mg, 2.3×10^{-3}% based on dried weight of stems) as a colorless needle. This compound was identified as protocatechuic acid [**218**].

3.1.2.2.6 Isolation of Compound BSB6 (Lithospermoside)

Fraction SB-E (393.8 mg) was separated by a Cosmosil 75 C_{18}-OPN column chromatography. Elution was performed in a polarity isocratic manner with the mixtures of methanol-water (1: 9). Eluates with similar TLC behavior (silica gel, chloroform-methanol-water 7:3:1) were pooled to give four fractions (SB-E1 to SB-E4).

Fraction SB-E2 (30.2 mg) was re-separated by a Cosmosil 75 C_{18}-OPN column chromatography using and eluted with mixtures of methanol-water (1:9). The eluates were collected and combined according to the chromatographic patterns (silica gel, chloroform-methanol-water 8:2:0.1) to obtain three fractions (SB-E2-1 to SB-E2-3). Fraction SB-E2-1 (12.4 mg), after removal of solvents, gave compound BSB6 (5.5 mg, 1.6×10^{-3}% based on dried weight of stems). This compound was identified as lithospermoside [**54**].

3.2 Extraction and Separation of the Roots of *Bauhinia sirindhorniae*

3.2.1 Extraction

The dried roots of *Bauhinia sirindhorniae* (300 g) were successively extracted with hexane (3×3 L), chloroform (3×3 L), and 95% ethanol (3×3 L). The filtrates were pooled and evaporated under a reduced pressure at the temperature not exceeding 40 °C to give the corresponding hexane (520.6 mg), chloroform (490.5 mg) and 95% ethanol extract (58.2 g), respectively.

The ethanol extract (58.2 g) was then partitioned between butanol and water. The butanol layer was dried to yield 22.3 g of a butanol extract while 19.5 g of an aqueous extract was obtained.

3.2.2 Isolation

3.2.2.1 Isolation of Compounds from Chloroform Extract (Scheme 6 and Figure 4)

The chloroform extract (490.5 mg) was dissolved in a small amount of chloroform, triturated with silica gel 60 (70-230 mesh) and dried at room temperature. It was then fractionated by liquid column chromatography. Elution was completed in a polarity gradient manner with mixtures of chloroform-methanol.

The eluates were examined by TLC using chloroform-methanol as a developing solvent. Fractions with similar chromatographic pattern were combined to afford five fractions: fractions RC-A (62.1 mg), RC-B (121.9 mg), RC-C (157.3 mg), RC-D (32.0 mg) and RC-E (86.0 mg).

3.2.2.1.1 Isolation of Compound BRC1 (5,7-Dihydroxychromone)

Fraction RC-B (121.9 mg) was separated on a Sephadex LH 20 column (chloroform-methanol 1:1). Eluates were collected and combined based on their chromatographic patterns (silica gel, chloroform-ethyl acetate 98:2) to give four fractions (RC-B1 to RC-B4).

Fraction RC-B2 (14.6 mg) was recrystallized from a mixture of chloroform-methanol to give compound BRC1 (7.2 mg, 2.4×10^{-3}% based on dried weight of roots) as a colorless needle. This compound was identified as 5,7 dihydroxychromone [**219**].

3.2.2.1.2 Isolation of Compound BRC2 (Sitosteryl-3-*O*-β-D-glucoside)

Fraction RC-D (32.0 mg) was fractionated by gel filtration chromatography using a Sephadex LH 20 column with mixtures of chloroform-methanol (1:1) as the eluent. Compound BRC2 (10.3 mg, 3.4×10^{-3}% based on dried weight of roots) was finally obtained after the removal of solvent from fraction RC-D2 (19.2 mg). This compound was later identified as sitosteryl-3-*O*-β-D-glucoside [**37**].

3.2.2.3 Isolation of Compounds from Butanol Extract (Schemes 7-8 and Figure 4)

The butanol extract (22.3 g) was dissolved in a small amount of methanol, triturated with silica gel 60 (70-230 mesh) and dried at room temperature. It was then fractionated by liquid column chromatography. Elution was completed in a polarity gradient manner with mixtures of chloroform-methanol-water.

The eluates were examined by TLC using mixtures of chloroform-methanol as a developing solvent. Fractions with similar chromatographic pattern were combined to afford six fractions: fractions RB-A (285.0 mg), RB-B (120.2 mg), RB-C (329.3 mg), RB-D (790.2 mg), RB-E (2.5 g) and RB-F (10.2 g).

3.2.2.3.1 Isolation of Compound BRB1 ((2*S*)-Naringenin)

Fraction RB-A (285.0 mg) was separated on silica gel 60 (230-400 mesh) as an adsorbent. Elution was performed in an isocratic manner with mixtures of chloroform-methanol (98:2). Eluates with similar TLC behavior (silica gel, chloroform-methanol 98:2) were pooled to give four fractions (RB-A1 to RB-A4).

Fraction RB-A2 (52.5 mg) was recrystallized from a mixture of chloroform-methanol to give compound BRB1 (3.7 mg, 1.2×10^{-3}% based on dried weight of roots). It was identified as (2*S*)-naringenin [**17**].

3.2.2.3.2 Isolation of Compound BRB2 (Luteolin)

Fraction RB-B (120.2 mg) was subjected to column chromatography using silica gel 60 (230-400 mesh) as an adsorbent and eluted with mixtures of chloroform-methanol (95:5). After combination of collected fractions according to chromatographic pattern (silica gel, chloroform-methanol 9:1), four fractions including fraction RB-B1 to RB-B4 were obtained.

Fraction RB-B3 (48.0 mg) was further fractionated by gel filtration chromatography using a Sephadex LH 20 column with a mixture of chloroform-methanol (1:1) as the eluent. Fraction RB-B3-2 (24.0 mg) was recrystallization from a mixture of chloroform and methanol to give compound BRB2 (3.0 mg, 1.0×10^{-3}% based on dried weight of roots) as a yellow needle. It was identified as luteolin [**220**].

3.2.2.3.3 Isolation of Compound BRB3 ((2*S*)-Eriodictyol)

Fraction RB-C (329.3 mg) was further purified by repeated column chromatography using silica gel 60 (230-400 mesh) as an adsorbent and eluted with mixtures of chloroform-methanol (9:1), which resulted in the collecting of fractions RB-C1 to RB-C3.

Fraction RB-C1 (43.3 mg) was subjected to gel filtration chromatography using a Sephadex LH 20 column with a mixture of chloroform-methanol (1:1) as the an eluent. Recrystallization of fraction RB-C1-2 (24.6 mg) with a mixture of chloroform and methanol yielded a pale yellow needle of compound BRB3 (7.3 mg, 2.4×10^{-3}% based on dried weight of roots). This compound was identified as (2*S*)-eriodictyol [**16**].

3.2.2.3.4 Isolation of Compound BRB4 ((+)-Taxifolin)

Fraction RB-C3 (97.2 mg) was subjected to column chromatography using silica gel 60 (230-400 mesh) as an adsorbent. Elution with mixtures of chloroform-

methanol (9:1). After combination of collected fractions based on their chromatographic behavior (silica gel, chloroform-methanol 9:1) leading to three fractions: fractions RB-C3-1 to RB-C3-3 were obtained.

Recrystallization of fraction RB-C3-2 (32.1 mg) with mixtures of chloroform and methanol yielded a pale yellow needle of compound BRB4 (8.9 mg, 3.0×10^{-3}% based on dried weight of roots). This compound was identified as (+)-taxifolin [**221**].

3.2.2.3.5 Isolation of Compound BRB5 ((+) Lyoniresinol-3α-*O*-α-L-rhamnoside)

Fraction RB-D (790.2 mg) was fractionated by column chromatography using silica gel 60 (230-400 mesh) as an adsorbent and eluted with mixtures of chloroform-methanol-water (8:2:0.1). The eluates were collected and combined according to the chromatographic patterns (silica gel, chloroform-methanol-water 7:2:1) to obtained four fractions (RB-D1 to RB-D4).

Purification of fraction RB-D1 (166.9 mg) was further performed by gel filtration chromatography using a Sephadex LH 20 column with mixtures of chloroform: methanol (1:1) as an eluent. The eluates were collected and combined based on their TLC chromatographic patterns (silica gel, chloroform-methanol-water 7:3:1) to give two fractions (RB-D1-1 and RB-D1-2). Fraction RB-D1-2 was fractionated by column chromatography (Cosmosil 75C_{18}-OPN, methanol: water 1:4) to give a colorless amorphours mass of compound BRB5 (30.0 mg, 1.0×10^{-2}% based on dried weight of roots). This compound was identified as (+) lyoniresinol-3α-*O*-α-L-rhamnoside [**222**].

3.2.2.3.6 Isolation of Compound BRB6 (5-Hydroxychromone-7-β-D-glucoside)

Fraction RB-D2 (78.5 mg) was further fractionated by gel filtration chromatography using a Sephadex LH 20 column with mixtures of chloroform-methanol (1:1) as an eluent. The eluates were collected and combined based on their TLC chromatographic patterns (silica gel, chloroform-methanol-water 8:2:0.1) to give two fractions (RB-D2-1 and RB-D2-4).

Fraction RB-D2-3 (8.9 mg) was recrystallized from a mixture of chloroform and methanol to give compound BRB6 (1.0 mg, 3.0×10^{-4} % based on dried weight of roots) as a yellow needle. This compound was identified as 5-hydroxychromone-7-β-D-glucoside [**223**].

3.2.2.3.7 Isolation of Compound BRB7 (Menisdaurin)

Fraction RB-D3 (68.2 mg) was fractionated by gel filtration chromatography using a column of a Sephadex LH 20 with mixtures of chloroform: methanol (1:1) as an eluent. The eluates were collected and combined based on their TLC chromatographic patterns (silica gel, chloroform-methanol-water 7:3:1) to give two fractions (RB-D3-1 and RB-D3-2).

Fraction RB-D3-2 (63.7 mg) was purified by HPLC using an Inertsil ODS column (20 i.d.×250 mm) eluted with in an isocratic manner with UV 254 nm detection. Elution was performed in an isocratic manner with 85% water + 25% methanol (flow rate 5 ml/min. A total of compound BRB7 (3.2 mg, 1.1×10^{-3}% based on dried weight of roots) was obtained at the retention time of 25 minutes. This compound was subsequently identified as menisdaurin [**224**].

3.3 Extraction and Separation of the Leaves of *Croton hutchinsonianus*

3.3.1 Extraction

The dried leaves of (2.5 kg) *Croton hutchinsonianus* were successively extracted with hexane (3×20 L), ethyl acetate (3×20 L), and 95% ethanol (3×20 L). The filtrates were pooled and evaporated under reduced pressure at the temperature not exceeding 40 °C to give the corresponding hexane (106.8 g), ethyl acetate (110.7 g) and 95% ethanol extract (112.4 g), respectively.

The 95% ethanol extract (112.4 g) was then partitioned between butanol and water. The butanol layer was dried to yield 75.5 g of a butanol extract whereas 18.4 g of an aqueous extract was obtained.

3.3.2 Isolation

3.3.2.1 Isolation of Compounds from Ethyl Acetate Extract (Scheme 9 and Figure 5)

The ethyl acetate extract (110.7 g) was dissolved in a small amount of chloroform, triturated with silica gel 60 (70-230 mesh) and dried at room temperature. It was then fractionated by vacuum liquid column chromatography. Elution was completed in a polarity gradient manner with mixtures of hexane-ethyl acetate.

The eluates were examined by TLC (silica gel) using hexane-ethyl acetate as a developing solvent. Fractions with a similar chromatographic pattern were combined to afford four fractions: fractions LE-A (4.3 g), LE-B (20.2 g), LE-C (48.6 g) and LE-D (25.8 g).

3.3.2.1.1 Isolation of Compound CBE1 (Farnesyl acetone)

Fraction LE-A (4.3 g) was separated on silica gel 60 (230-400 mesh) as an adsorbent. Elution was performed in an isocratic manner with mixtures of hexane-ethyl acetate (98:2). Eluates with a similar TLC behavior (silica gel, hexane-ethyl acetate 95:5) were pooled to give three fractions (LE-A1 to LE-A3).

Fraction LE-A2 (33.5 mg) was separated by preparative TLC using hexane and ethyl acetate as a developing solvent to give compound CBE1 (19.8 mg, 7.9×10^{-4}% based on dried weight of leaves). It was identified as a farnesyl acetone [**225**].

3.3.2.1.2 Isolation of Compound CBE2 (Poilaneic acid)

Fraction LE-B (20.3 g) was fractionated by gel filtration chromatography using a Sephadex LH 20 column with a mixture of dichloromethane-acetone (1:1) as the eluent. The eluates were collected and combined based on their TLC chromatographic patterns (silica gel, hexane-ethyl acetate 4:1) to give two fractions (LE-B1 and LE-B2).

Fraction LE-B2 (10.1 mg) was re-separated on column chromatography using a Cosmosil 75C_{18}-OPN column and eluted with mixtures of methanol-water (9:1). The eluates were collected and combined according to similarity of chromatographic patterns (silica gel, hexane-ethyl acetate 4:1) to obtained three fractions (LE-B2-1 to LE-B2-3). Fraction LE-B2-2 (98.9 mg), after removal of solvent gave compound CBE2 (48.8 mg, 2.0×10^{-3}% based on dried weight of leaves). This compound was identified as poilaneic acid [**226**].

3.3.2.1.3 Isolation of Compound CBE4 (3-(4-Hydroxy-3,5-dimethoxy phenyl)-propyl benzoate)

Fraction LE-C (48.6 g) was fractionated by gel filtration chromatography using a Sephadex LH 20 column with mixtures of dichloromethane-acetone (1:1) as the eluent. The eluates were collected and combined based on their TLC chromatographic patterns (silica gel, hexane-ethyl acetate 4:1) to give two fractions (LE-C1 and LE-C2).

Fraction LE-C2 (5.8 g) was separated by preparative TLC using hexane-ethyl acetate as a developing solvent to give compound CBE4 (98.5 mg, 3.9×10^{-3}% based on dried weight of leaves). It was identified as 3-(4-hydroxy-3,5-dimethoxyphenyl)-propyl benzoate [227].

3.4 Extraction and Separation of the Branches of *Croton hutchinsonianus*

3.4.1 Extraction

The dried branches of (1.3 kg) *Croton hutchinsonianus* were successively extracted with hexane (3×10 L), ethyl acetate (3×10 L), and 95% ethanol (3×10 L). The filtrates were pooled and evaporated under reduced pressure at the temperature not exceeding 40 °C to give the corresponding hexane (15.0 g), ethyl acetate (20.7 g) and 95% ethanol extract (27.0 g), respectively.

The 95% ethanol extract (27.0 g) was then partitioned between butanol and water. The butanol layer was dried to yield 12.5 g of a butanol extract while 10.4 g of an aqueous extract was obtained.

3.4.2 Isolation

3.4.2.1 Isolation of Compounds from Ethyl Acetate Extract (Schemes 10 and Figure 5)

The ethyl acetate extract (20.7 g) was dissolved in a small amount of chloroform, triturated with silica gel 60 (70-230 mesh) and dried at the room temperature. It was then fractionated by vacuum liquid column chromatography. Elution was completed in a polarity gradient manner with mixtures of hexane-ethyl acetate.

The eluates were examined by TLC (silica gel) using hexane-ethyl acetate as a developing solvent. Fractions with similar chromatographic pattern were combined to afford four fractions: fractions BE-A (1.2 g), BE-B (3.5 g), BE-C (5.7 g) and BE-D (6.9 g).

3.4.2.1.1 Isolation of Compound CBE1 (Farnesyl acetone)

Fraction BE-A (1.2 g) was separated on silica gel 60 (230-400 mesh) as an adsorbent. Elution was performed in a polarity isocratic manner with mixtures of hexane-ethyl acetate (98:2). Eluates with similar TLC behavior (silica gel, hexane-ethyl acetate 95:5) were pooled to give three fractions (BE-A1 to BE-A3).

Fraction BE-A2 (21.4 mg) was separated by preparative TLC using hexane and ethyl acetate as a developing solvent to give compound CBE1 (2.1 mg, 1.8×10^{-4}% based on dried weight of branches). It was identified as farnesyl acetone [**225**].

3.4.2.1.2 Isolation of Compound CBE2 (Poilaneic acid)

Fraction BE-B (3.5 g) was fractionated by gel filtration chromatography using a Sephadex LH 20 column with mixtures of dichloromethane-acetone (1:1) as the

eluent. The eluates were collected and combined based on their TLC chromatographic patterns (silica gel, hexane-ethyl acetate 4:1) to give two fractions (BE-B1 and BE-B2).

Fraction BE-B2 (1.1 g) was re-separated on column chromatography using a Cosmosil 75 C_{18}-OPN column and eluted with the mixtures of methanol-water (9:1). The eluates were collected and combined according to similarity of chromatographic patterns (silica gel, hexane-ethyl acetate 4:1) to obtained three fractions (BE-B2-1 to BE-B2-3). Fraction BE-B2-2 (25.6 mg), after removal of solvents, gave compound CBE2 (5.7 mg, 4.8×10^{-4}% based on dried weight of branches). This compound was identified as poilaneic acid [**163**].

3.4.2.1.3 Isolation of Compound CBE3 (4-Hydroxybenzaldehyde)

Fraction BE-C (5.7 g) was fractionated by gel filtration chromatography using a Sephadex LH 20 column with mixtures of dichloromethane-acetone (1:1) as the eluent. The eluates were collected and combined based on their TLC chromatographic patterns (silica gel, hexane-ethyl acetate 4:1) to give two fractions (BE-C1 and BE-C2).

Fraction BE-C1 (3.4 g) was recrystallized to give compound CBE3 (11.6 mg, 9.7×10^{-4}% based on dried weight of branches) from mixtures of hexane-ethyl acetate as a colorless needle. This compound was identified as 4-hydroxybenzaldehyde [**226**].

3.4.2.1.4 Isolation of Compound CBE4 (3-(4-Hydroxy-3,5-dimethoxy phenyl)-propyl benzoate)

Fraction BE-C2 (1.3 g) was separated on silica gel 60 (230-400 mesh) as an adsorbent. Elution was performed in a polarity isocratic manner with mixtures of hexane-ethyl acetate (9:1). Eluates with similar TLC behavior (silica gel, hexane-ethyl acetate 4:1) were pooled to give three fractions (BE-C2-1 to BE-C2-3).

Fraction BE-C2-2 (27.2 mg) was separated by preparative TLC using the mixtures of hexane-ethyl acetate (3:2) as a developing solvent to give compound CBE4 (35.6 mg, 3.0×10^{-3}% based on dried weight of branches). It was identified as 3-(4-hydroxy-3,5-dimethoxyphenyl)-propyl benzoate [**227**].

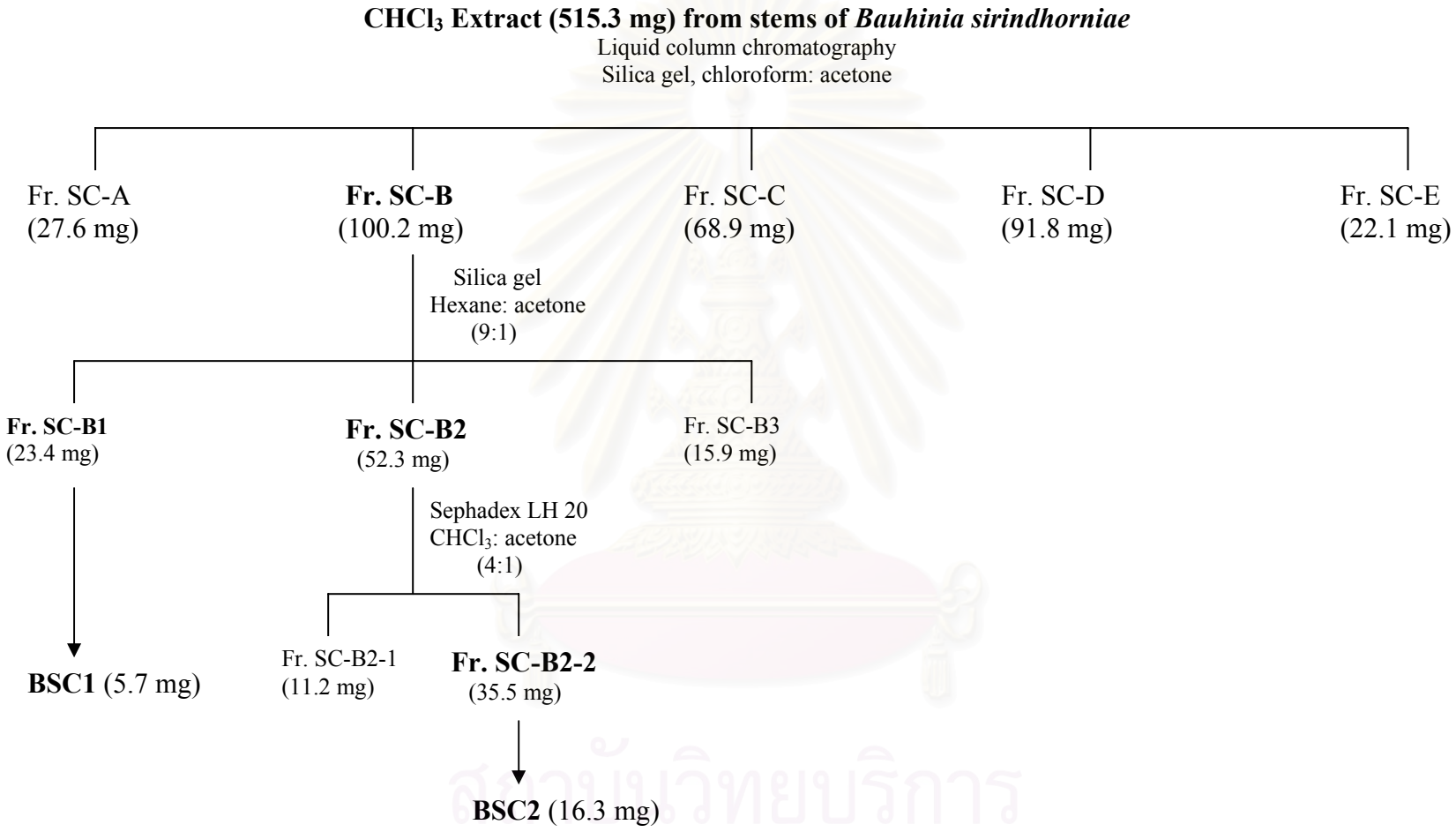
3.4.2.1.5 Isolation of Compound CBE5 (Dihydroconiferyl benzoate)

Fraction BE-C2-1 (27.2 mg) was separated by preparative TLC using mixtures of hexane-ethyl acetate (4:1) as a developing solvent to give compound CBE5 (18.9

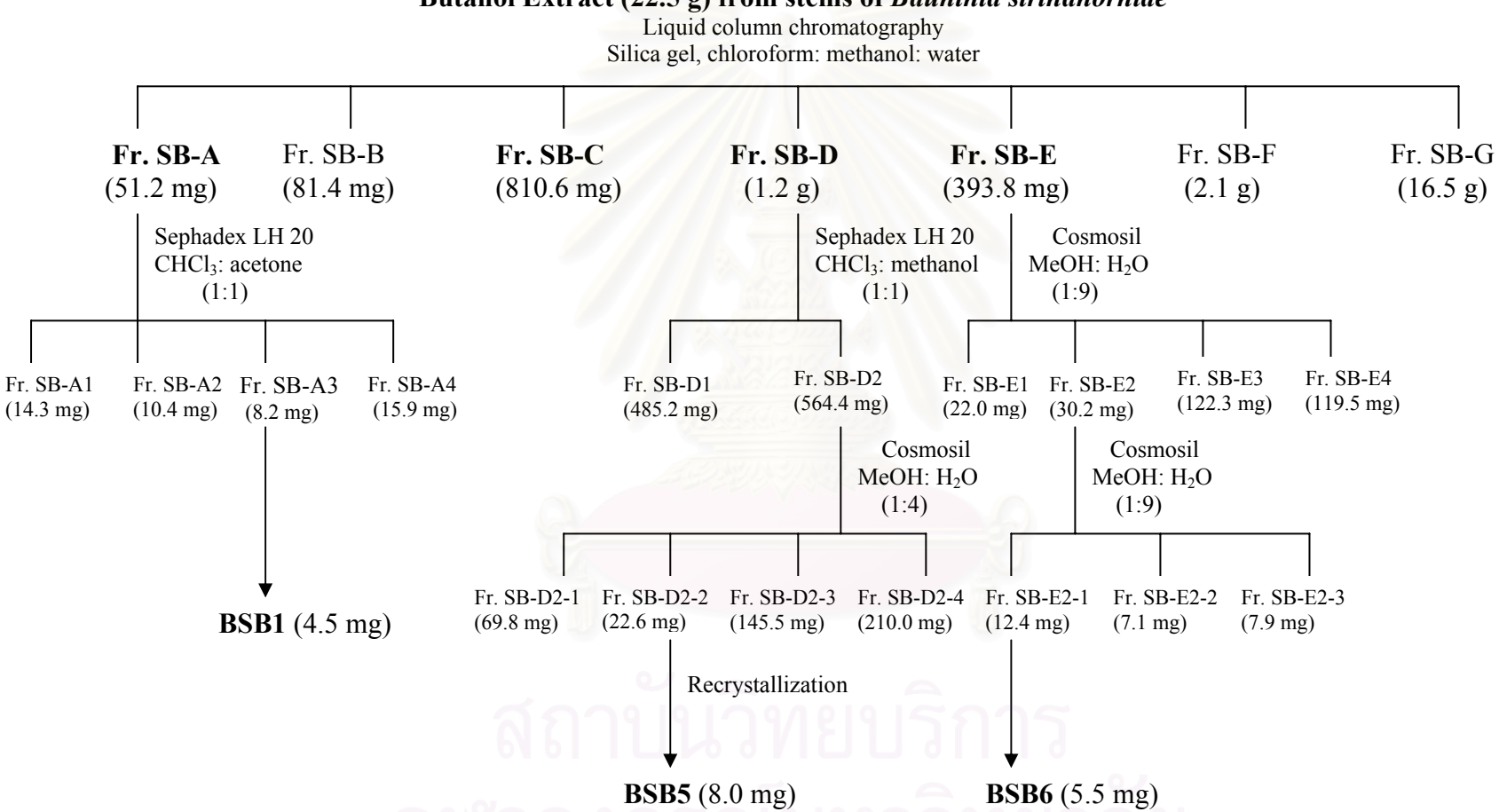
mg, 1.6×10^{-3}% based on dried weight of branches). It was identified as dihydroconiferyl benzoate [**228**].

3.4.2.1.6 Isolation of Compound CBE6 (3-(4-Hydroxyphenyl)-propyl benzoate)

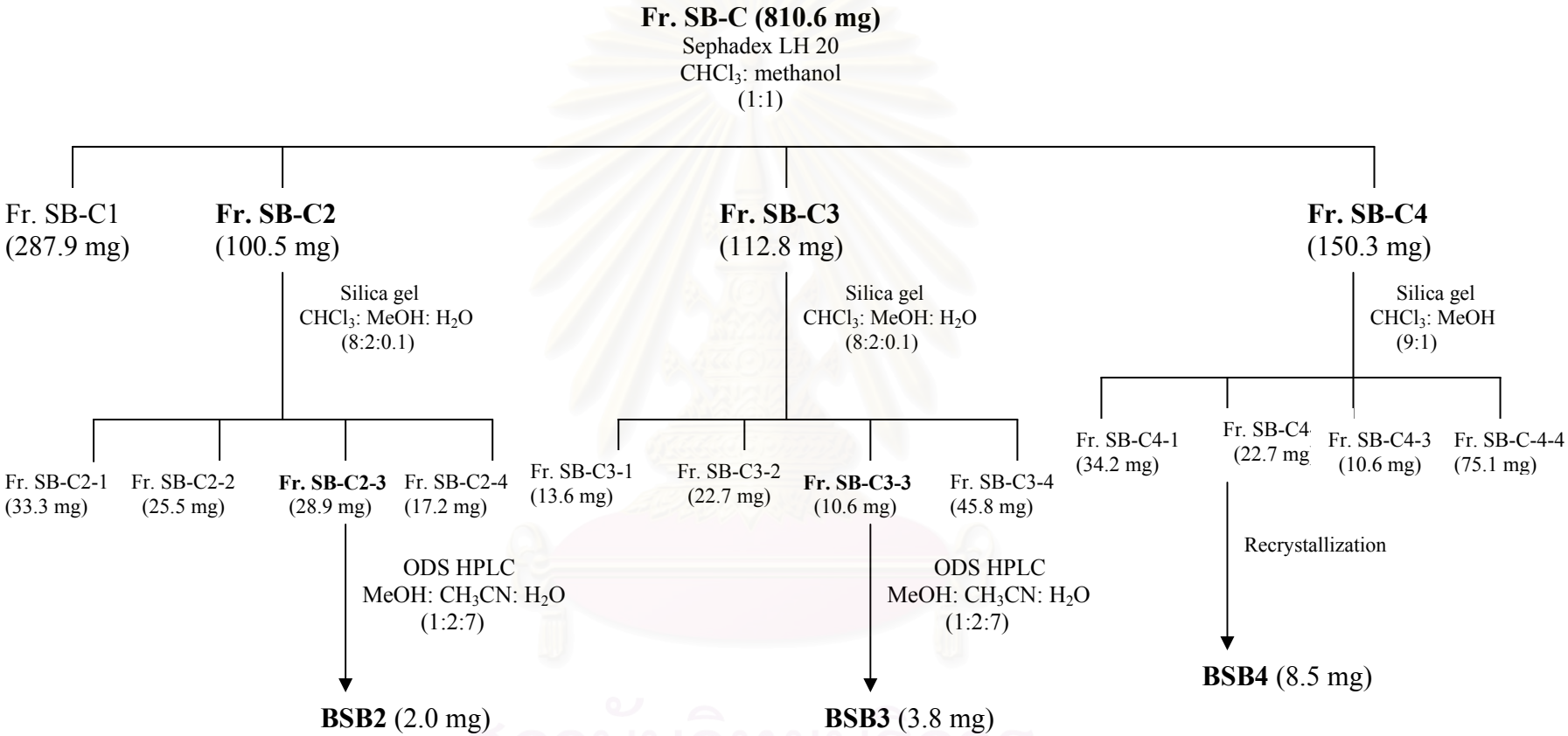
Fraction BE-C2-3 (11.5 mg) was separated by preparative TLC using mixtures of hexane and ethyl acetate (3:2) as a developing solvent to give compound CBE6 (4.9 mg, 4.1×10^{-4}% based on dried weight of branches). It was identified as 3-(4-hydroxyphenyl)-propyl benzoate [**229**].



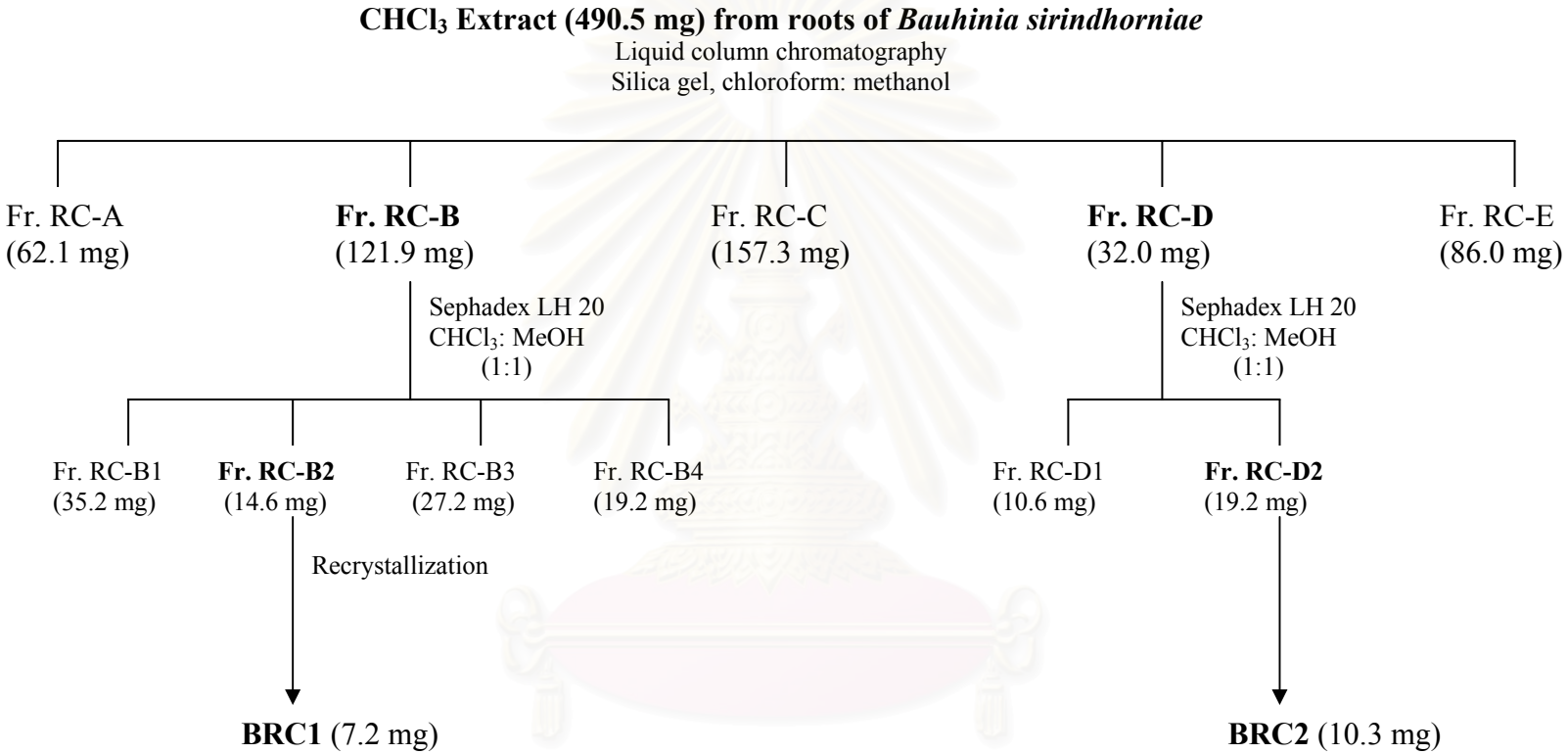
Scheme 3 Separation of the $CHCl_3$ extract of the stems of *Bauhinia sirindhorniae*



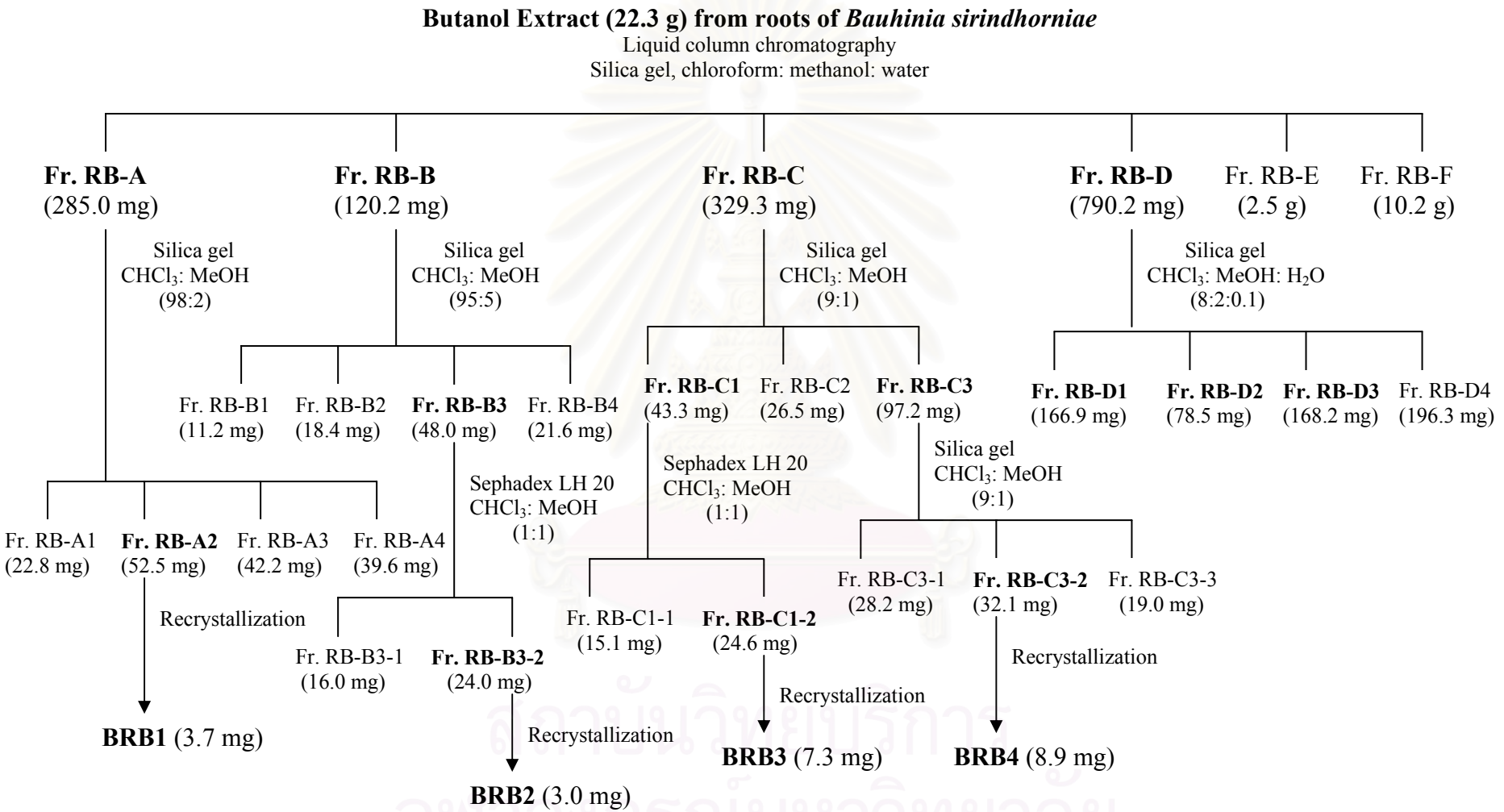
Scheme 4 Separation of the butanol extract of the stems of *Bauhinia sirindhorniae*



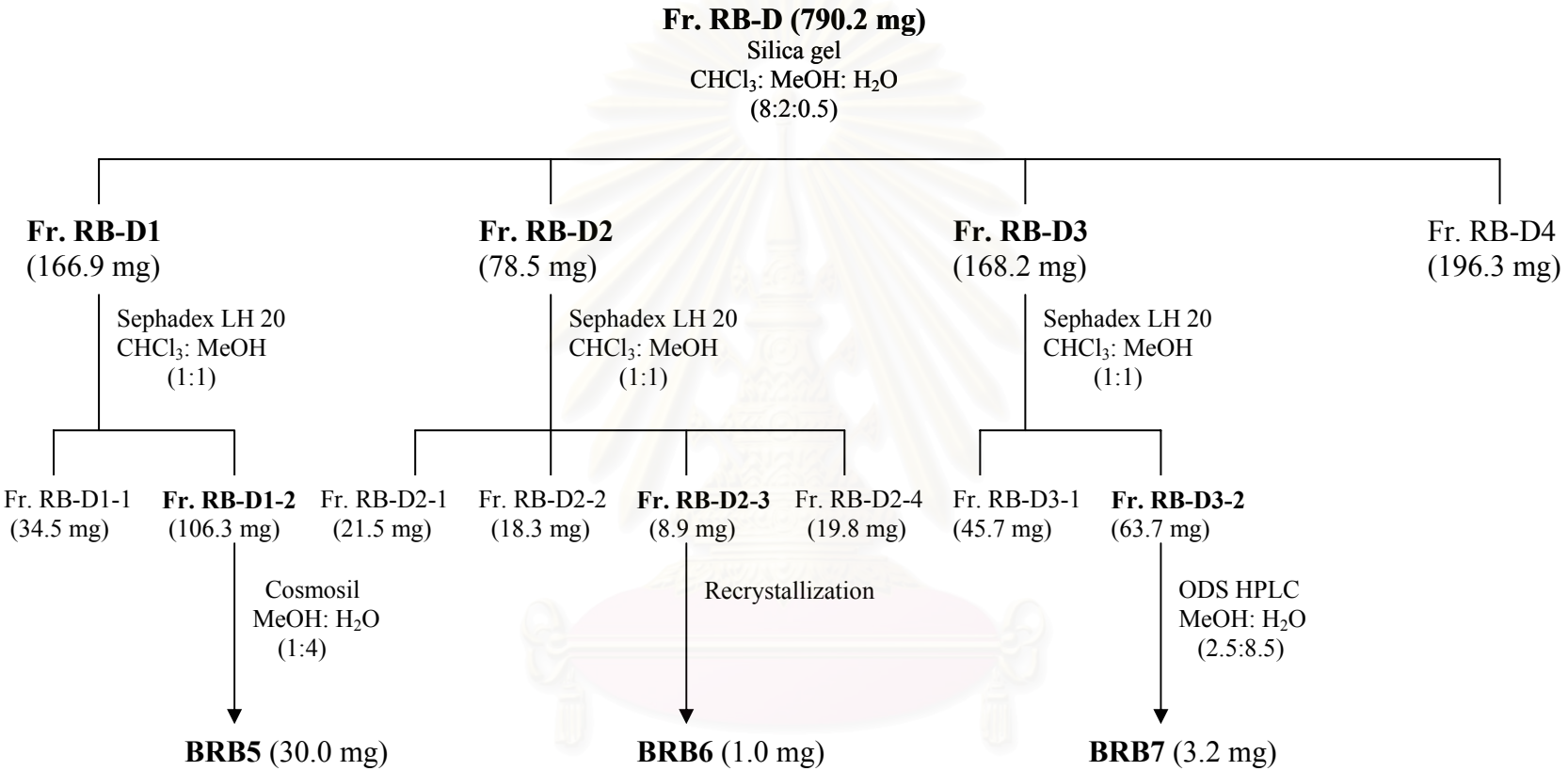
Scheme 5 Separation of fraction SB-C from the butanol extract of the stems of *Bauhinia sirindhorniae*



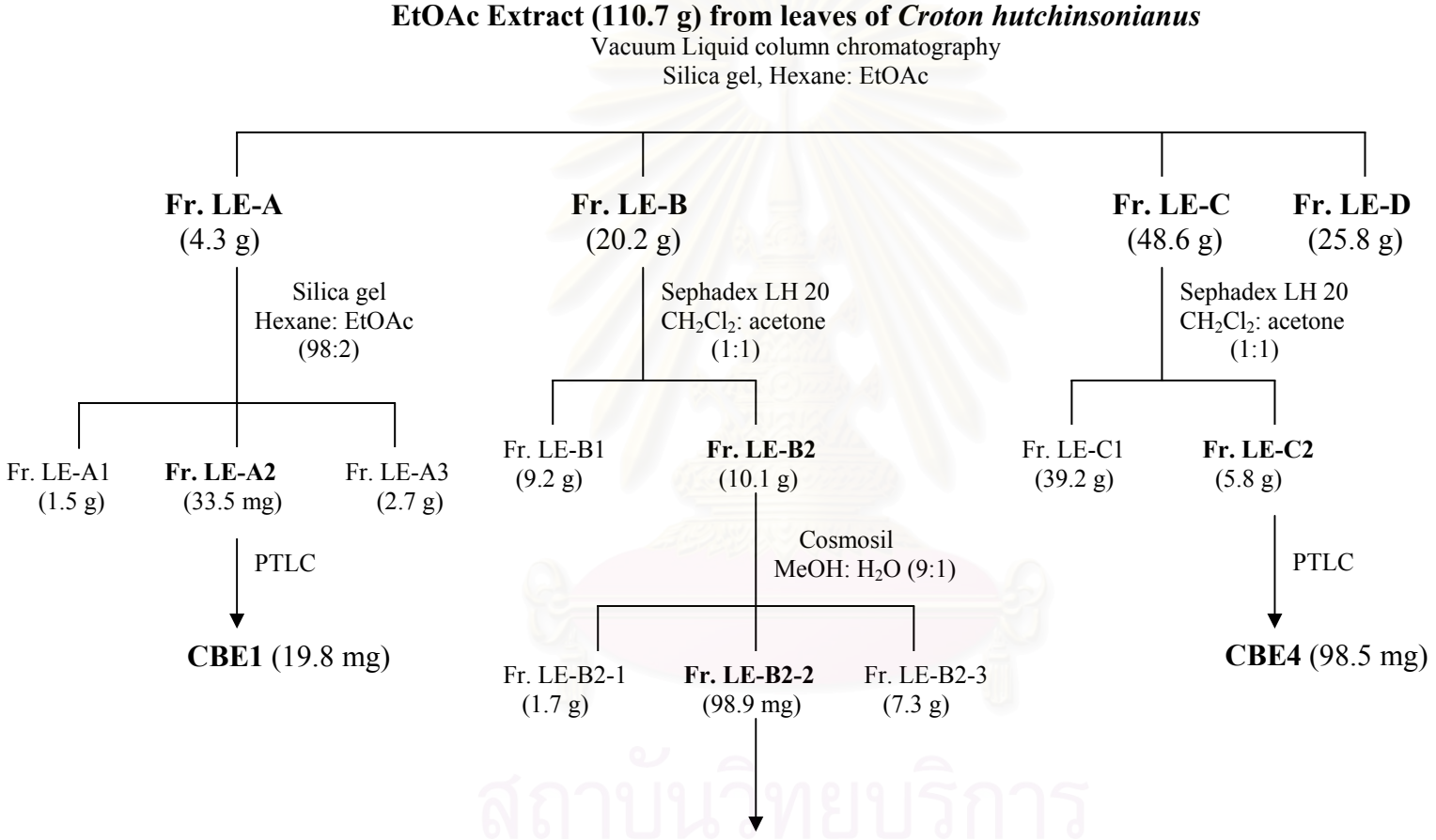
Scheme 6 Separation of the $CHCl_3$ extract of the roots of *Bauhinia sirindhorniae*



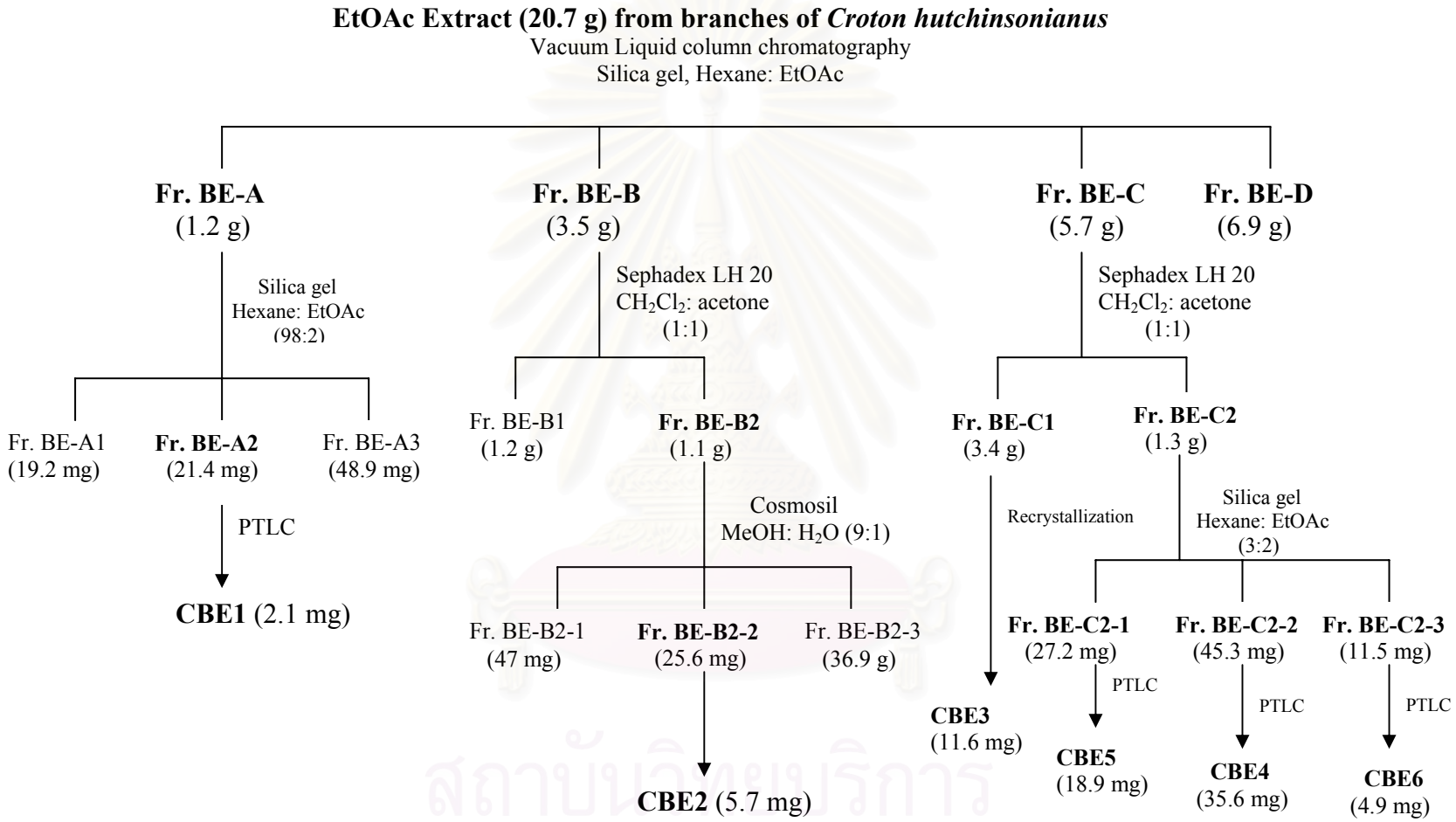
Scheme 7 Separation of the butanol extract of the roots of *Bauhinia sirindhorniae*



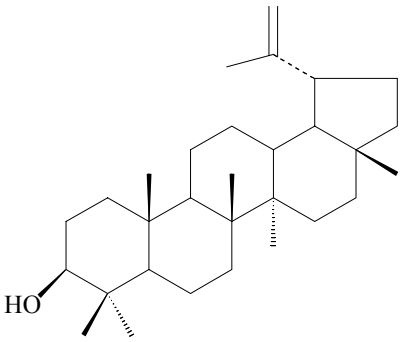
Scheme 8 Separation of fraction RB-D from the butanol extract of the roots of *Bauhinia sirindhorniae*



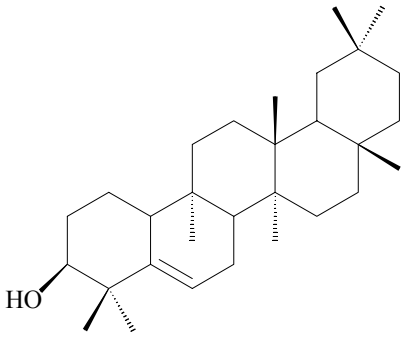
Scheme 9 Separation of the ethyl acetate extract of the leaves of *Croton hutchinsonianus*



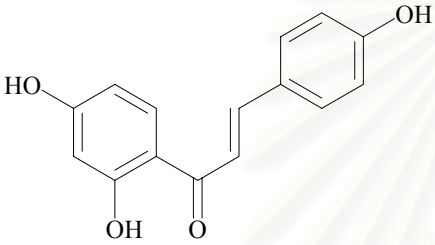
Scheme 10 Separation of the ethyl acetate extract of the branches of *Croton hutchinsonianus*



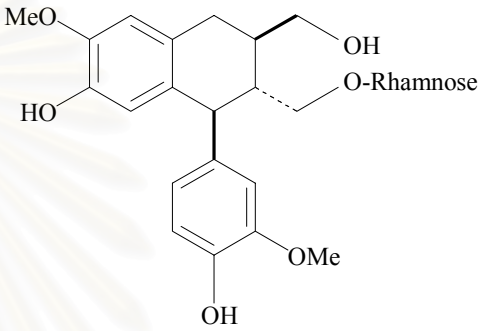
BSC1 [**77**]



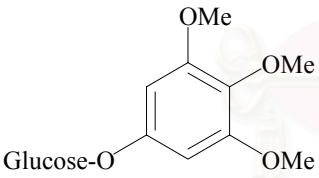
BSC2 [**214**]



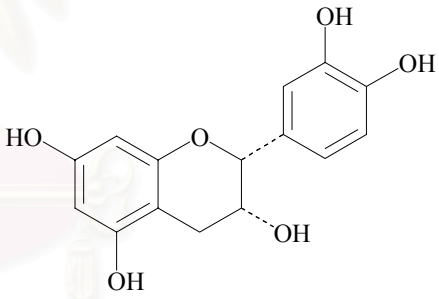
BSB1 [**14**]



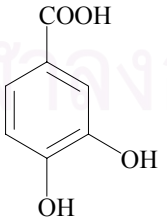
BSB2 [**215**]



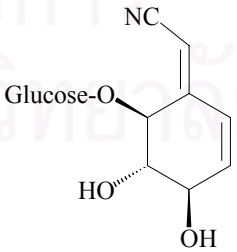
BSB3 [**216**]



BSB4 [**217**]

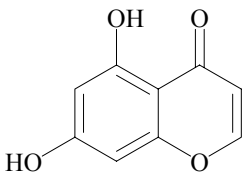


BSB5 [**218**]

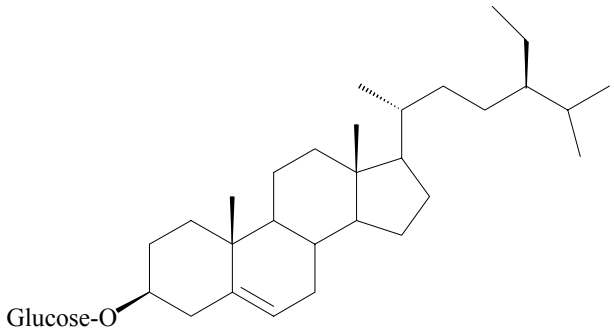


BSB6 [**54**]

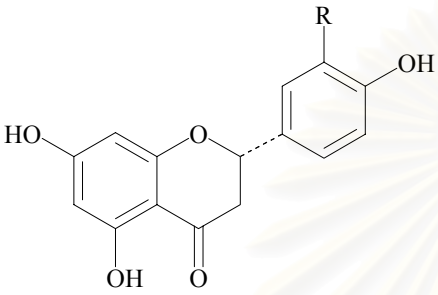
Figure 3 Structures of compounds isolated from the stems of *Bauhinia sirindhorniae*



BRC1 [**219**]

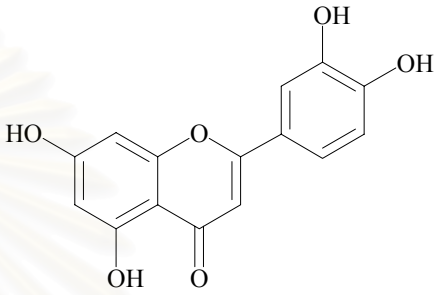


BRC2 [**37**]

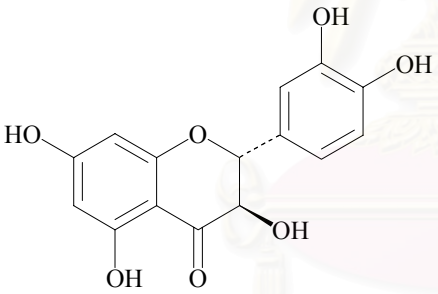


BRB1 [**17**]; R = H

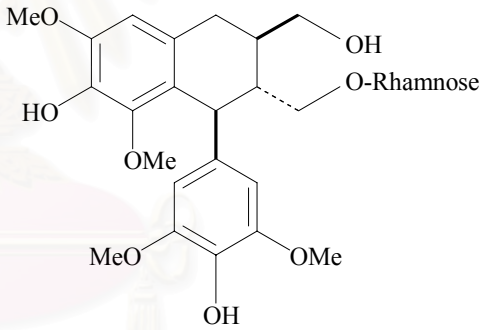
BRB3 [**16**]; R = OH



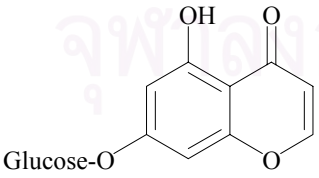
BRB2 [**220**]



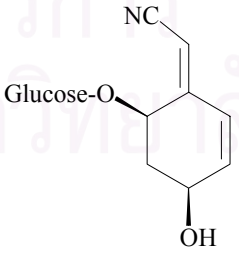
BRB4 [**221**]



BRB5 [**222**]

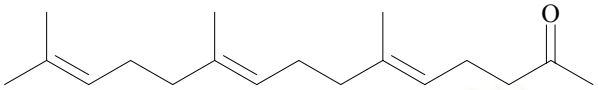


BRB6 [**223**]

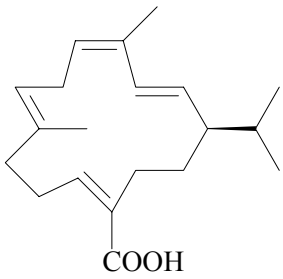


BRB7 [**224**]

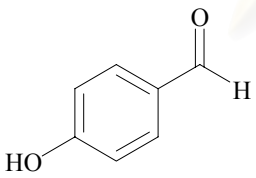
Figure 4 Structures of compounds isolated from the roots of *Bauhinia sirindhorniae*



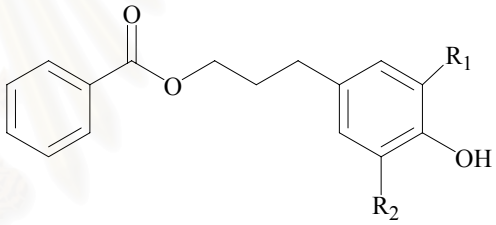
CBE1 [**225**]



CBE2 [**163**]



CBE3 [**226**]



CBE4 [**227**]; R_1 = OMe, R_2 = OMe

CBE5 [**228**]; R_1 = OMe, R_2 = H

CBE6 [**229**]; R_1, R_2 = H

Figure 5 Structures of compounds isolated from the leaves and branches of *Croton hutchinsonianus*

4. Physical and Spectral data of Isolated Compounds

4.1 Compound BSC1 (Lupeol)

Compound BSC1 was obtained as a colorless needle and found to be soluble in chloroform (5.7 mg, 1.6×10^{-3}% base on dried weight of stems).

EIMS : *m/z* (% relative intensity); Figure 11
426 (M^+, 48), 408 (100), 218 (78), 207 (30), 203 (50), 189 (75), 135 (66), 121 (71), 107 (69)

IR : ν_{max} cm^{-1}, KBr disc; Figure 10
3447, 2927, 1650, 1457, 1386

^{1}H NMR : δ ppm, 500 MHz, in chloroform-*d*; Figure 12, Table 8

^{13}C NMR : δ ppm, 125 MHz, in chloroform-*d*; Figure 13, Table 8

4.2 Compound BSC2 (Glutinol)

Compound BSC2 was obtained as a colorless needle and found to be soluble in chloroform (16.3 mg, 4.7×10^{-3}% base on dried weight of stems).

EIMS : *m/z* (% relative intensity); Figure 15
426 (M^+, 20), 408 (100), 274 (98), 259 (76), 173 (63), 161 (58)

IR : ν_{max} cm^{-1}, KBr disc; Figure 14
3461, 2933, 1455, 1385, 1037, 971, 800

^{1}H NMR : δ ppm, 500 MHz, in chloroform-*d*; Figure 16, Table 9

^{13}C NMR : δ ppm, 125 MHz, in chloroform-*d*; Figure 17, Table 9

4.3 Compound BSB1 (Isoliquiritigenin)

Compound BSB1 was obtained as a yellow crystal and found to be soluble in DMSO (4.5 mg, 1.3×10^{-3}% base on dried weight of stems).

FAB$^+$MS : $[M+H]^+$ at *m/z* 257 (positive ion mode); Figure 20

UV : λ_{max} nm (log ε) in methanol; Figure 18
365 (4.30)

IR : ν_{max} cm^{-1}, KBr disc; Figure 19
3301, 1635, 1604, 1564, 1513, 1367, 1294, 1219, 1175, 1128, 1033, 978, 891, 826, 802, 621, 558, 524

Melting point : 182-183 °C

^{1}H NMR : δ ppm, 400 MHz, in DMSO-d_6; Figure 21, Table 10

^{13}C NMR : δ ppm, 100 MHz, in DMSO-d_6; Figure 22, Table 10

4.4 Compound BSB2 ((+)-Isolariciresinol-3α-*O*-α-L-rhamnoside)

Compound BSB2 was obtained as an amorphous powder and found to be soluble in methanol (2.0 mg, 5.7×10^{-4}% base on dried weight of stems).

FAB$^+$MS : $[M+H]^+$ at *m/z* 507 (positive ion mode); Figure 28

UV : λ_{max} nm (log ε) in methanol; Figure 26
221 (4.61), 283 (4.16)

IR : ν_{max} cm^{-1}, KBr disc; Figure 27
3401, 2932, 1602, 1515, 1455, 1380, 1253, 1129, 1049, 879

$[\alpha]_D^{23}$: +20.8° (methanol, *c* 0.25)

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 29, Table 11

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 30, Table 11

4.5 Compound BSB3 (3,4,5-Trimethoxyphenolic-1-*O*-β-D-glucoside)

Compound BSB3 was obtained as a white needle and found to be soluble in methanol (3.8 mg, 1.1×10^{-3}% base on dried weight of stems).

FAB$^+$MS : $[M+H]^+$ at *m/z* 347 (positive ion mode); Figure 33

UV : λ_{max} nm (log ε) in methanol; Figure 31
222 (4.39), 268 (4.01), 288 (3.92)

IR : ν_{max} cm^{-1}, KBr disc; Figure 32
: 3404, 1697, 1614, 1515, 1288, 1072, 763

Melting Point : 199-202 °C

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 34, Table 12

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 35, Table 12

4.6 Compound BSB4 ((-)-Epicatechin)

Compound BSB4 was obtained as a colorless needle and found to be soluble in methanol (8.5 mg, 2.4×10^{-3}% base on dried weight of stems).

FAB$^-$MS : $[M-H]^-$ at *m/z* 289 (negative ion mode); Figure 40

UV : λ_{max} nm (log ε) in methanol; Figure 38
222 (4.96), 280 (4.31)

IR : ν_{max} cm^{-1}, KBr disc; Figure 39
3459, 2932, 1625, 1552, 1442, 1261, 1145, 808, 795

$[\alpha]_D^{23}$: -55° (methanol, *c* 0.25)

CD : $[\theta]_{219}$ –11100.6, $[\theta]_{240}$ +4017.9; $[\theta]_{280}$ –1614.6 (*c* 3.2 × 10^{-4}, methanol) 23 °C

Melting Point : 235-237 °C

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 41, Table 13

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 42, Table 13

4.7 Compound BSB5 (Protocatechuic acid)

Compound BSB5 was obtained as a colorless crystal and found to be soluble in methanol (8.0 mg, 2.3×10^{-3}% base on dried weight of stems).

FAB^{+}MS : $[M+H]^+$ at *m/z* 155 (positive ion mode); Figure 48

UV : λ_{max} nm (log ε) in methanol; Figure 46
222 (4.68), 258 (4.47), 294 (4.23)

IR : ν_{max} cm^{-1}, KBr disc; Figure 47
: 3264, 1673, 1601, 1297, 943, 766, 559

Melting Point : 194-196 °C

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 49, Table 14

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 50, Table 14

4.8 Compound BSB6 (Lithospermoside)

Compound BSB6 was obtained as a fine white needle and found to be soluble in water (5.5 mg, 1.6×10^{-3}% base on dried weight of stems).

FAB^{+}MS : $[M+H]^+$ at *m/z* 330 (positive ion mode); Figure 53

UV : λ_{max} nm (log ε) in water; Figure 51
259 (3.84)

IR : ν_{max} cm^{-1}, KBr disc; Figure 52
3434, 2914, 2224, 1601, 1379, 1256, 1080, 1045, 997, 948, 849, 654

CD : $[\theta]_{263}$ -44030, $[\theta]_{227}$ +35086; (*c* 3.1 × 10^{-4}, water) 23°C

^{1}H NMR : δ ppm, 500 MHz, in water-d_2; Figure 54, Table 15

^{13}C NMR : δ ppm, 125 MHz, in water-d_2; Figure 55, Table 15

4.9 Compound BRC1 (5,7-Dihydroxychromone)

Compound BRC1 was obtained as a colorless needle and found to be soluble in methanol (7.2 mg, 2.4×10^{-3}% base on dried weight of roots).

FAB^{+}MS : $[M+H]^+$ at *m/z* 179 (positive ion mode); Figure 60

UV : λ_{max} nm (log ε) in methanol; Figure 58
224 (4.83), 250 (4.93), 256 (4.95), 295 (4.53)

IR : ν_{max} cm^{-1}, KBr disc; Figure 59
: 3003, 2731, 2628, 1646, 1617, 1500, 1373, 1187, 1032, 845

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 61, Table 16
: δ ppm, 500 MHz, in acetone-d_6; Figure 62

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 63, Table 16

4.10 Compound BRC2 (Sitosteryl-3-*O*-β-D-glucoside)

Compound BRC2 was obtained as a white powder and found to be soluble in chloroform in methanol (10.3 mg, 3.4×10^{-3}% base on dried weight of roots).

FAB$^+$MS : $[M+Na]^+$ at *m/z* 577 (positive ion mode); Figure 67

IR : ν_{max} cm^{-1}, KBr disc; Figure 66
: 3402, 2934, 1463, 1367, 1168, 1073, 1025, 802

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4 + chloroform-*d*; Figure 68, Table 17

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4 + chloroform-*d*; Figure 69, Table 17

4.11 Compound BRB1 ((2*S*)-Naringenin)

Compound BRB1 was obtained as a pale yellow needle and found to be soluble in methanol (3.7 mg, 1.2×10^{-3}% base on dried weight of roots).

FAB$^+$MS : $[M+H]^+$ at *m/z* 273 (positive ion mode); Figure 72

UV : λ_{max} nm (log ε) in methanol; Figure 70
226 (4.75), 288 (4.57), 332 (3.91)

IR : ν_{max} cm^{-1}, KBr disc; Figure 71
: 3268, 1632, 1604, 1463, 1253, 1158, 1084, 832, 728

$[\alpha]_D^{23}$: -13° (MeOH, *c* 0.23)

Melting Point : 249-251 °C

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 73, Table 18

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 74, Table 18

4.12 Compound BRB2 (Luteolin)

Compound BRB2 was obtained as a yellow needle and found to be soluble in DMSO (3.0 mg, 1.0×10^{-3}% base on dried weight of roots).

FAB$^+$MS : $[M+H]^+$ at *m/z* 287 (positive ion mode); Figure 79

UV : λ_{max} nm (log ε) in methanol; Figure 77
221 (4.84), 255 (4.74), 267 (4.71), 350 (4.81)

IR : ν_{max} cm^{-1}, KBr disc; Figure 78
: 3395, 1657, 1608, 1510, 1360, 1259, 1167, 1031, 838, 641

Melting Point : 325-328 °C

^{1}H NMR : δ ppm, 500 MHz, in; Figure 80, Table 19

^{13}C NMR : δ ppm, 125 MHz, in; Figure 81, Table 19

4.13 Compound BRB3 ((2*S*)-Eriodictyol)

Compound BRB3 was obtained as a pale yellow needle and found to be soluble in methanol (7.3 mg, 2.4×10^{-3}% base on dried weight of roots).

FAB$^+$MS : $[M+H]^+$ at *m/z* 289 (positive ion mode); Figure 86

UV : λ_{max} nm (log ε) in methanol; Figure 84
224 (4.98), 288 (4.91), 328 (4.21)

IR : ν_{max} cm^{-1}, KBr disc; Figure 85
: 3366, 1632, 1605, 1452, 1311, 1086, 825, 735

$[\alpha]_D^{23}$: -10° (methanol, *c* 0.39)

Melting Point : 250-153 °C

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 87, Table 20

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 88, Table 20

4.14 Compound BRB4 ((+)-Taxifolin)

Compound BRB4 was obtained as a pale yellow needle and found to be soluble in methanol (8.9 mg, 2.5×10^{-3}% base on dried weight of roots).

FAB$^+$MS : $[M+H]^+$ at *m/z* 305 (positive ion mode); Figure 93

UV : λ_{max} nm (log ε) in methanol; Figure 91
222 (4.99), 290 (4.91), 325 (4.39)

IR : ν_{max} cm^{-1}, KBr disc; Figure 92
: 3412, 1639, 1615, 1476, 1265, 1083, 808, 780

$[\alpha]_D^{23}$: +17° (methanol, *c* 0.32)

CD : $[\theta]_{329}$ +10200.5, $[\theta]_{299}$ –4100.2; (*c* 3.1×10^{-4}, methanol) 23 °C

Melting Point : 238-241 °C

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 93, Table 21

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 94, Table 21

4.15 Compound BRB5 ((+)-Lyoniresinol-3α-*O*-α-L-rhamnoside)

Compound BRB5 was obtained as an amorphous solid and found to be soluble in methanol (30.0 mg, 1.0×10^{-2}% base on dried weight of roots).

FAB$^+$MS : $[M+K]^+$ at *m/z* 605 (positive ion mode); Figure 100

UV : λ_{max} nm (log ε) in methanol; Figure 98
221 (4.85), 278 (3.96)

IR : ν_{max} cm^{-1}, KBr disc; Figure 99
3402, 2937, 1614, 1517, 1461, 1056, 982, 83, 809, 637

$[\alpha]_D^{23}$: +3.3° (methanol, *c* 0.50)

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 101, Table 22

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 102, Table 22

4.16 Compound BRB6 (5-Hydroxychromone-7-β-D-glucoside)

Compound BRB6 was obtained as a white needle and found to be soluble in methanol (1.0 mg, 3.0×10^{-4}% base on dried weight of roots).

FAB$^+$MS : $[M+H]^+$ at *m/z* 341 (positive ion mode); Figure 107

UV : λ_{max} nm (log ε) in methanol; Figure 106
221 (4.06), 252 (4.01), 256 (4.03), 288 (3.42)

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 108, Table 23

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 109, Table 23

4.17 Compound BRB7 (Menisdaurin)

Compound BRB7 was obtained as a white powder and found to be soluble in methanol (3.2 mg, 1.1×10^{-3}% base on dried weight of stems).

FAB$^+$MS : $[M+H]^+$ at *m/z* 314, $[M+Na]^+$ at *m/z* 336, $[M+K]^+$ at *m/z* 352 (positive ion mode); Figure 112

UV : λ_{max} nm (log ε) in methanol; Figure 110
258 (4.82)

IR : ν_{max} cm^{-1}, KBr disc; Figure 111
3404, 2912, 2218, 1520, 1456, 1266, 1044, 843

$[\alpha]_D^{23}$: -195° (methanol, *c* 1.0)

^{1}H NMR : δ ppm, 500 MHz, in methanol-d_4; Figure 113, Table 24

^{13}C NMR : δ ppm, 125 MHz, in methanol-d_4; Figure 114, Table 24

4.18 Compound CBE1 (Farnesyl acetone)

Compounds CBE1 (19.8 mg, 7.9×10^{-4}% base on dried weight of leaves and 2.1 mg, 1.8×10^{-4}% base on dried weight of branches) was obtained as a colorless oil and found to be soluble in chloroform.

EIMS : *m/z* (% relative intensity); Figure 119
262 (M^+, 32), 245 (100), 243 (17), 201 (14), 189 (14), 175 (15), 163 (22), 161 (16), 137 (15), 121 (28), 109 (14), 95 (18)

IR : ν_{max} cm^{-1}, neat; Figure 118
3019, 2974, 2400, 1712, 1221, 762, 730, 457

^{1}H NMR : δ ppm, 400 MHz, in chloroform-*d*; Figure 120, Table 25

^{13}C NMR : δ ppm, 100 MHz, in chloroform-*d*; Figure 121, Table 25

4.19 Compound CBE2 (Poilaneic acid)

Compounds CBE2 (48.8 mg, 2.0×10^{-3}% base on dried weight of leaves and 25.6 mg, 2.0×10^{-3}% base on dried weight of branches) was obtained as a colorless needle and found to be soluble in chloroform.

EIMS : *m/z* (% relative intensity); Figure 127
302 (M^+, 15), 287 (19), 284 (14), 259 (40), 257 (37), 241 (24), 213 (39), 185 (30), 171 (30), 157 (32), 143 (32), 133 (26), 129 (34), 121 (26), 119 (34), 107 (30), 105 (69), 93 (37), 91 (100), 87 (63), 79 (50), 77 (55), 55 (26)

UV : λ_{max} nm (log ε) in methanol; Figure 125
230 (4.62)

IR : ν_{max} cm^{-1}, neat; Figure 126
3445, 2917, 2849, 1699, 1458, 1262, 1033

$[\alpha]_D^{23}$: -140° (chloroform, *c* 0.25)

^{1}H NMR : δ ppm, 500 MHz, in chloroform-*d*; Figure 128, Table 26

^{13}C NMR : δ ppm, 125 MHz, in chloroform-*d*; Figure 129, Table 26

4.20 Compound CBE3 (4-Hydroxybenzaldehyde)

Compound CBE3 was obtained as a colorless needle and found to be soluble in chloroform (11.6 mg, 9.7×10^{-4}% base on dried weight of branches).

EIMS : *m/z* (% relative intensity); Figure 136
122 (10), 121 (100), 105 (16), 93 (15), 77 (15), 74 (11), 66 (5), 65(24), 63 (18), 62 (24), 61 (12)

UV : λ_{max} nm (log ε) in methanol; Figure 134
222 (4.12), 284 (4.24)

IR : ν_{max} cm^{-1}, KBr disc; Figure 135
3164, 1666, 1597, 1454, 1286, 1217, 1160, 835, 705

Melting point : 113-115 °C

^{1}H NMR : δ ppm, 400 MHz, in chloroform-*d*; Figure 138, Table 27

^{13}C NMR : δ ppm, 100 MHz, in chloroform-*d*; Figure 139, Table 27

4.21 Compound CBE4 (3-(4-Hydroxy-3,5-dimethoxyphenyl)-propyl benzoate)

Compounds CBE4 (98.5 mg, 3.9×10^{-3}% base on dried weight of leaves and 35.6 mg, 3.0×10^{-3}% base on dried weight of branches) was obtained as a pale yellow oil and found to be soluble in chloroform.

HREIMS : $[M+H]^+$ at *m/z* 317.1395 calcd for $C_{18}H_{20}O_5$, 317.1389

EIMS : *m/z* (% relative intensity); Figure 144
316 (M^+, 100), 194 (84), 163 (75), 105 (30), 77 (76)

UV : λ_{max} nm (log ε) in methanol; Figure 142
228 (3.85), 272 (2.89)

IR : ν_{max} cm^{-1}, neat; Figure 143
3446, 2921, 1708, 1520, 1300, 1213, 1112, 712

^{1}H NMR : δ ppm, 400 MHz, in chloroform-*d*; Figure 145, Table 28

^{13}C NMR : δ ppm, 100 MHz, in chloroform-*d*; Figure 146, Table 28

4.22 Compound CBE5 (Dihydroconiferyl benzoate)

Compound CBE5 was obtained as a pale yellow oil and found to be soluble in chloroform (18.9 mg, 1.6×10^{-3}% base on dried weight of branches).

HRFABMS : $[M+H]^+$ at *m/z* 287.1289 calcd for $C_{17}H_{18}O_4$, 287.1284

EIMS : *m/z* (% relative intensity); Figure 153
286 (M^+, 100), 164 (100), 133 (34), 105 (23), 77 (36)

UV : λ_{max} nm (log ε) in methanol; Figure 151
229 (4.35), 280 (3.66)

IR : ν_{max} cm^{-1}, neat; Figure 152
3428, 2957, 1718, 1604, 1516, 1273, 1119, 712

^{1}H NMR : δ ppm, 400 MHz, in chloroform-*d*; Figure 154, Table 29

^{13}C NMR : δ ppm, 100 MHz, in chloroform-*d*; Figure 155, Table 29

4.23 Compound CBE6 (3-(4-Hydroxyphenyl)-propyl benzoate)

Compound CBE6 was obtained as a pale yellow oil and found to be soluble in chloroform (4.9 mg, 4.1×10^{-4}% base on dried weight of branches).

HRFABMS : $[M+H]^+$ at *m/z* 257.1179 calcd for $C_{16}H_{16}O_3$, 257.1178

EIMS : *m/z* (% relative intensity); Figure 161

258 (M^+, 3), 134 (38), 133 (100), 105 (50), 103 (17), 77 (36)

UV : λ_{max} nm (log ε) in methanol; Figure 159

228 (4.08), 279 (3.26)

IR : ν_{max} cm^{-1}, neat; Figure 160

3377, 1698, 1633, 1516, 1277, 1118, 712

^{1}H NMR : δ ppm, 400 MHz, in chloroform-*d*; Figure 162, Table 30

^{13}C NMR : δ ppm, 100 MHz, in chloroform-*d*; Figure 163, Table 30

5. Evaluation of Biological Activities

5.1 Antimicrobial Activity

5.1.1 Agar Diffusion Assay

Antimicrobial activity of the crude extracts were screened by agar diffusion method (Jorgensen *et al*, 1999; Ingroff *et al*., 1999).

The bacterial strains used were as follows:

- *Staphylococcus aureus* ATCC 29213
- *Bacillus subtilis* ATCC 6633
- *Pseudomonas aeruginosa* ATCC 27853
- *Escherichia coli* ATCC 25922

The fungal strains used were as follows:

- *Candida albicans* ATCC 10231
- *Trichophyton mentagrophytes* (clinical isolated)

5.1.1.1 Preparation of Sample

The amounts of crude extracts were 10 mg per disk.

5.1.1.2 Preparation of the Inoculum

Each bacterial strain was cultured overnight on trypticase soy agar (TSA) plate at 37 °C. The isolated colonies were inoculated into a 5 ml trypticase soy broth (TSB) and incubated at 37 °C for 2-3 hours. The turbidity of these inocula was adjusted to match that of a 0.5 McFarland standard (approximately 10^8 CFU/ml for bacteria).

Candida albicans ATCC 10231 was grown on Sabouraud dextrose agar (SDA) slant at 30 °C for 24 hours. The inoculum was prepared by suspending the culture in sterile normal saline solution and turbidity of the inoculum was adjusted to match a 0.5 turbidity standard of McFarland.

Spores of *Trichophyton mentagrophytes* grown on SDA slant at 30 °C for five days were washed from the slant culture with sterile 0.05% Tween 80. The turbidity of the spore suspension was adjusted to match 0.5 turbidity standard of McFarland (this produced a fungal suspension containing 1×10^6 to 5×10^6 organisms per ml).

5.1.1.3 Preparation of Test Plates

5.1.1.3.1 Preparation for Testing Bacteria

Mueller Hinton agar (MHA) was melted and allowed to cool at 45-50 °C in a water bath. Then 25 ml of the melted agar medium was dispensed into sterile glass petri dishes, with internal diameters of 9 cm, to yield a uniform depth of 4 mm. The agar was allowed to harden on a flat level surface. The plates were dried for 1 hour at 37 °C.

5.1.1.3.2 Preparation for Testing Fungi

Sabouraud dextrose agar (SDA) was used and prepared as described above.

5.1.1.4 Inoculation of Agar Plates

A sterile cotton swab was dipped in each inoculum and the excess was removed by rotating the swab several times against the inside wall of the tube above the fluid level. The entire surfaces of the MHA plate and the SDA plate for testing bacteria and fungi, respectively, were inoculated by streaking with the swab for three times and each time the plate was rotated 60 degree.

5.1.1.5 Assay Procedure

Within 15 minutes after the plates were inoculated, the sample disks were placed individually then gently pressed down onto the agar surface. This was done in duplicate. After maintaining at room temperature for 1 hour, the bacterial and fungal plates were incubated at 37 °C overnight and 30 °C for 48-72 hours, respectively. The sample disks showing inhibition zone were examined further for their minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC).

5.1.2 Determination of MIC and MBC

Determination of the MIC and MBC of pure compounds against *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633 by broth microdilution test (Jorgensen *et al*., 1999).

5.1.2.1 Preparation of Test Samples

The samples were dissolved in DMSO and diluted with Mueller Hinton broth (MHB) in a two-fold dilution to give the concentrations ranging from 200 μg/ml to 0.39 μg/ml.

5.1.2.2 Preparation of the Inoculum

The inoculum was prepared as described in **5.1.1.2**. The inoculum was further diluted to 1:100 in MHB.

5.1.2.3 Assay Procedure

A 50 μl volume of each concentration of the sample was dispensed to the corresponding well of the sterile multiwell microdilution plate (96-Flat-shaped wells). Another 50 μl volume of diluted inoculum was added into each well. After incubating the tray at 37 °C for 24 hours, the lowest concentration of the sample that showed growth inhibition was considered as the MIC. This determination was done in duplicate. All inhibitory concentrations were re-checked by addition of each solution showing activity into agar plate, and incubated at 37 °C for 24 hours. The lowest concentration of the test compounds which kill these microorganisms were defined as MBC. Penicillin G was used as a positive control.

5.2 Determination of Free Radical Scavenging Activity

5.2.1 TLC Screening Assay (Pezzuto and Kinghorn, 1998)

Free radical scavenging activity of the crude extracts were screened by TLC screening method. The samples were spotted and developed on a TLC plate with suitable developing solvent. After drying, the TLC plate was sprayed with 80 μg/ml solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol. The plate was examined 30 minutes after spraying. Active compounds appear as yellow spots against purple background.

5.2.2 Free Radical Scavenging Activity Assay (Takao *et al*., 1994; Brand-Williams, Cuvelier, and Berset, 1995)

5.2.2.1 Preparation of the Test Sample

Compounds BSB2 [**216**], BSB6 [**54**], BRB5 [**223**] and BRB7 [**225**] from *B. sirindhorniae* were first tested at 40 μg/ml. Compounds exhibiting more than 50% inhibition were further analyzed for their IC_{50} values. Each test sample was prepared as an ethanolic solution with initial concentration of 80 μg/ml. For analysis serial dilution was performed to give seven concentrations (40 μg/ml, 20 μg/ml, 10 μg/ml, 5 μg/ml, 2.5 μg/ml, 1.25 μg/ml and 0.625 μg/ml). Assays were carried out in duplicate. The test sample (100 μl) was added to the reaction mixture (100 μl) to furnish the total volume of 200 μl. The final concentration was calculated by the formula below.

$$N_1V_1 = N_2V_2$$

N_1 = Initial concentration (μM)

V_1 = Initial volume (μl)

N_2 = Final concentration (μM)

V_2 = Final volume (μl)

For example, of test sample (80 μg/ml) was added to the reaction mixture to furnish the total volume of 200 μl.

Thus, final concentration of test sample = 80 μg/ml×100 μl/200 μl

= 40 μg/ml

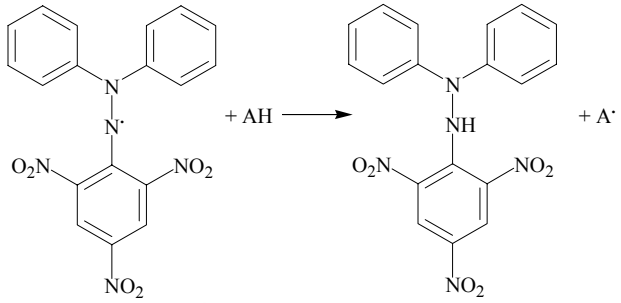
The initial and final concentrations (μg/ml) of test sample

Initial concentration (μg/ml)	80	40	20	10	5	2.50	1.250	0.625
Final concentration (μg/ml)	40	20	10	5	2.5	1.25	0.625	0.312

5.2.2.2 Preparation of the DPPH Solution (200 μM)

1,1-Diphenyl-2-picrylhydrazyl (DPPH) 7.88 mg was dissolved in ethanol 100 ml and the solution (200 μM) was subsequently stirred for 30 minutes.

5.2.2.3 Measurement of Activity



DPPH· (Purple) DPPH-H (Pale yellow)

DPPH = 1,1-diphenyl-2-picrylhydrazyl

AH = antioxidant

The test sample (100 µl) was dissolved in ethanol and mixed with 200 µM DPPH ethanolic solution (100 µl) in a 96-well microtiter plate. The reaction mixture was shaken well and kept in the dark for 20 minutes. The absorbance at 515 nm was measured by microtiter plate reader (Biorad, model 550). The DPPH solution was used as a negative control. Vitamin E derivative Trolox was used as a standard control and quercetin was used as a positive control. The decrease in absorbance per µM of each sample was compared with that of Trolox.

5.3 Cytotoxic Activity

Compounds CBE4 [**227**], CBE5 [**228**] and CBE6 [**229**] were determined for cytotoxicity by employing the colorimetric method against NCI-H187 (human small cell lung cancer cell line) using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method (Skehan *et al*., 1990). The IC_{50} values of the tested compounds were measured in µg/ml. Ellipticine was used as a positive control, exhibiting the activity with the IC_{50} of 0.35 µg/ml.

5.4 Antifungal Activity

Compounds CBE4 [**227**], CBE5 [**228**] and CBE6 [**229**] were evaluated for antifungal activity against *Candida albicans*, employing the colorimetric method (Hawser *et al*., 1998). The IC_{50} values of the tested compounds were measured in µg/ml. Amphotericin B was used as a positive control, exhibiting the activity with the IC_{50} of 0.02 µg/ml.

CHAPTER IV

RESULTS AND DISCUSSION

The pulverized stems and roots of *Bauhinia sirindhorniae* K & S.S. Larsen were successively extracted with hexane, chloroform, and 95% ethanol. The 95% ethanol extracts were investigated by several chromatographic techniques to give seven-teen compounds classified as two cyanoglucosides (BSB6 and BRB7), one flavan (BSB4), two flavanones (BRB1 and BRB3), one flavanonol (BRB4), one flavone (BRB2), one chalcone (BSB1), one chromone (BRC1), one chromone glucoside (BRB6), two lignan glycosides (BSB2 and BRB5), two triterpenoids (BSC1 and BSC2), one steroid glucoside (BRC2) and other phenolic compounds (BSB3 and BSB5). The antimicrobial and free radical scavenging activities of some compounds were evaluated.

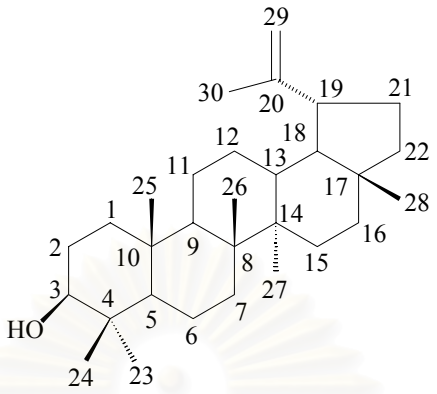
The dried leaves and branches of *Croton hutchinsonianus* Hosseus. were dried, grounded and then sequentially percolated with hexane, ethyl acetate and 95% ethanol, respectively. After successive extraction, the solvents were removed under reduced pressure. The last trace of solvents were eliminated under high vacuum to afford gums which were submitted for cytotoxic assays. Cytotoxic of various extracts are demonstrated result in Table 33.

The hexane and ethyl acetate extract of the leaves and branches showed cytotoxic activity against NCI H-187 cell lines as shown in Table 33. The ethyl acetate extract of the leaves was firstly separated by repeated column chromatography to give one C_{18} terpenoid (CBE1), one diterpene (CBE2) and one phenylpropyl benzoate (CBE4). The chemical investigation of the ethyl acetate extract of the branches has led to the isolation of the same three compounds (CBE1, CBE2 and CBE4), together with one benzaldehyde (CBE3) and two phenylpropyl benzoates (CBE5 and CBE6). No pure compound was isolated from hexane and 95% ethanol extract of the leaves and branches.

The structures of all isolates were determined based on their UV, IR, MS and NMR data, and then discussed by the comparison with the literature values.

1. Structure Determination of Isolated Compounds

1.1 Structure Determination of Compound BSC1

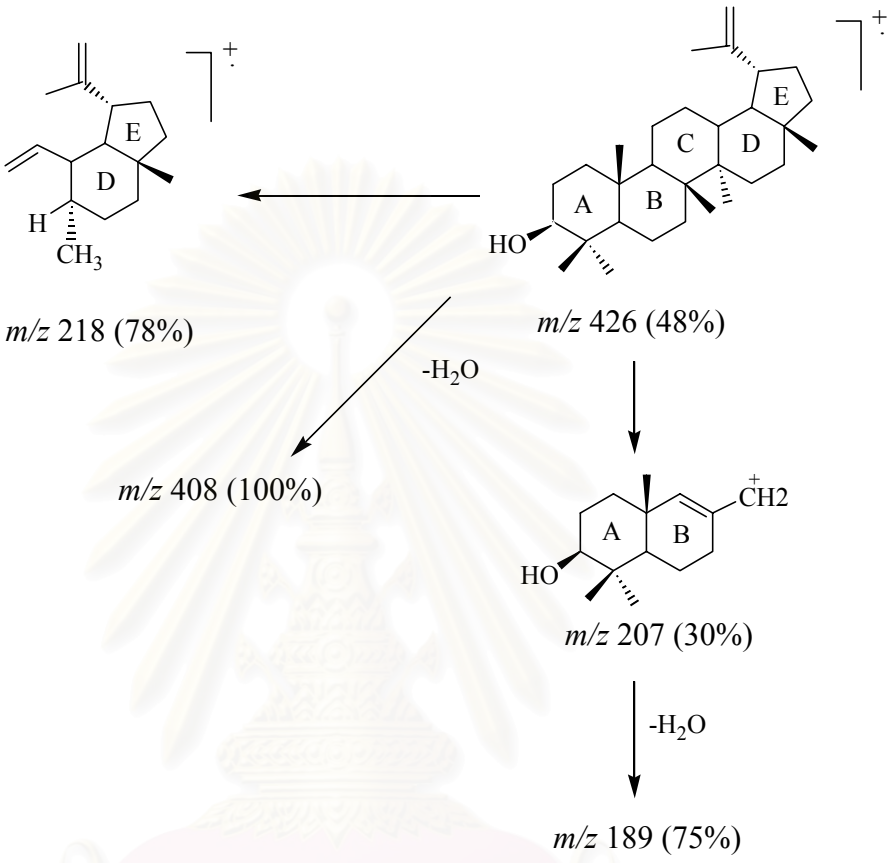


[**77**]

Compound BSC1 was obtained as a colorless needle. It showed a molecular [M^+] ion peak at *m/z* 426 in EIMS (Figure 11), suggesting a molecular formula of $C_{30}H_{50}O$. The fragmentation ions in the mass spectrum of compound BSC1 at *m/z* 426 [M^+] were useful in obtaining the structure of compound BSC1 and were in agreement with those reported in the literature (Hui and Fung, 1969; Hui and Lee, 1971). The ions at *m/z* 408 could reasonably come from [M^+-H_2O]. Other fragmentation pathways are as shown in Scheme 11. The IR spectrum of this compound showed O-H stretching broad band at 3447 cm^{-1} which indicated the presence of hydroxy group (Figure 10).

The ^1H NMR spectrum in $CDCl_3$ (Figure 12 and Table 8) displayed signals for seven methyl groups at δ 0.76, 0.77, 0.85, 0.96, 0.98, 1.04 and 1.66. Signals for several methine and methylene protons appeared at δ 0.90-1.80. In addition, a proton signal at δ 3.19 (*dd*, *J* = 11.2, 4.6 Hz, H-3), a multiplet proton signal at δ 2.35 (H-19) and two broad singlet proton signals at δ 4.54 and δ 4.66 (H-29) were also observed. The ^{13}C NMR spectrum in $CDCl_3$ (Figure 13 and Table 8) showed 30 carbon signals, corresponding to seven methyls, eleven methylenes, six methines and six quaternary carbons.

These ^1H and ^{13}C NMR data were in good agreement with those reported for lupeol [**77**] (Reynolds *et al*., 1986) as shown in Table 8.

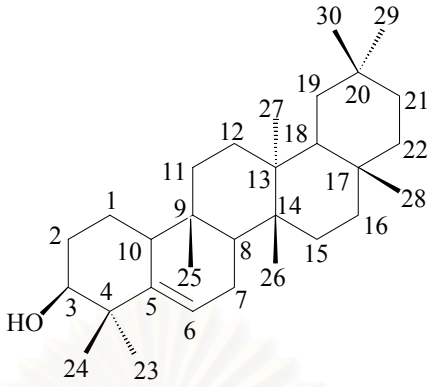


Scheme 11 EIMS Spectra fragmentations of compound BSC1

Table 8 NMR Spectral data of compound BSC1 and lupeol (in $CDCl_3$)

Position	Compound BSC1		Lupeol	
	^{1}H (*mult.*, *J* in Hz)	^{13}C	^{1}H (*mult.*, *J* in Hz)	^{13}C
1a	0.90-1.80	38.7	1.68	38.6
1b	0.90-1.80	-	0.91	-
2a	0.90-1.80	27.4	1.61	27.3
2b	0.90-1.80	-	1.54	-
3	3.19 (*dd*, 11.2, 4.6)	79.0	3.18 (*dd*)	78.9
4	-	38.9	-	38.8
5	0.90-1.80	55.3	0.69	55.2
6a	0.90-1.80	18.3	1.54	18.2
6b	0.90-1.80	-	1.39	-
7	0.90-1.80	34.3	1.41	34.2
8	-	40.8	-	40.7
9	0.90-1.80	50.4	1.28	50.3
10	-	37.2	-	37.1
11a	0.90-1.80	20.9	1.42	20.9
11b	0.90-1.80	-	1.25	-
12a	0.90-1.80	25.2	1.68	25.0
12b	0.90-1.80	-	1.07	
13	0.90-1.80	38.1	1.67	38.0
14	-	42.8	-	42.7
15a	0.90-1.80	27.5	1.71	27.4
15b	0.90-1.80	-	1.01	-
16a	0.90-1.80	35.6	1.49	35.5
16b	0.90-1.80	-	1.38	-
17	-	43.0	-	42.9
18	0.90-1.80	48.3	1.37	48.2
19	2.35 (*m*)	48.0	2.39	47.9
20	-	151.0	-	150.8
21a	0.90-1.80	29.9	1.93	29.8
21b	0.90-1.80	-	1.33	-
22a	0.90-1.80	40.0	1.42	39.9
22b	0.90-1.80	-	1.20	-
23	0.98	28.0	0.98	27.9
24	0.76	15.4	0.77	15.3
25	0.85	16.1	0.84	16.1
26	1.04	16.0	1.04	15.9
27	0.96	14.6	0.97	14.5
28	0.77	18.0	0.79	17.9
29a	4.54 (*br s*)	109.3	4.56	109.3
29b	4.66 (*br s*)	-	4.69	-
30	1.66 (*s*)	19.3	1.69	19.2

1.2 Structure Determination of Compound BSC2



[**214**]

Compound BSC2 was obtained as a colorless needle. The EIMS exhibited [M^+] at *m/z* 426 (Figure 15), corresponding to a molecular formula of $C_{30}H_{50}O$. The IR spectrum of this compound showed O-H stretching band at 3461 cm^{-1} which indicated the presence of hydroxy group (Figure 14).

The ^{1}H NMR spectrum of compound BSC2 in $CDCl_3$ (Figure 16 and Table 9) displayed signals for eight methyls groups at δ 0.88, 0.94, 0.98, 1.02, 1.07, 1.11, 1.14 and 1.20. Signals for several methine and methylene protons appeared at δ 0.88-1.98. In addition, two broad singlet proton signals at δ 3.44 and δ 5.61 were also observed. Other ^{1}H-NMR assignments were illustrated in Table 9.

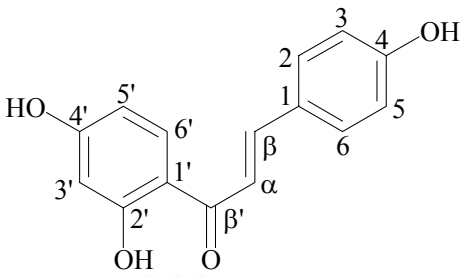
The ^{13}C NMR spectrum of compound BSC2 in $CDCl_3$ (Figure 17 and Table 9) showed 30 carbons signals, corresponding to eight methyls, ten methylenes, five methine and seven quarternary carbons. These ^{13}C NMR data and ^{1}H NMR data which were in good agreement with those reported for glutinol [**214**] (Carvalho and Seita, 1993; Gaind *et al*., 1976) as shown in Table 9.

This compound is a relatively rare triterpenol that is believed to be an intermediate in the biogenetic pathway to friedelin. The triterpenic ketone glutinone (also called alnusenone), isolated from *Alnus glutinosa* (Betulaceae), was the first compound of this class isolated from a natural source (Zhong, Waterman and Jeffreys, 1984). Glutinol (D:B-*friedo*olean-5-en-3β-ol), obtained by reduction of glutinone and which structure was later determined, was isolated for the first time from a natural source from *Euphorbia royleana* (Mahato, Das and Sahu, 1981).

Table 9 NMR Spectral data of compound BSC2 and glutinol (in $CDCl_3$)

Position	Compound BSC2		Glutinol
	^1H (*mult.*, *J* in Hz)	^{13}C	^{13}C
1	0.88-1.98	18.2	18.2
2	0.88-1.98	27.8	27.8
3	3.44 (*br s*)	76.3	76.4
4	0.88-1.98	40.8	40.8
5	0.88-1.98	141.6	141.6
6	5.61 (*br s*)	122.1	122.1
7	0.88-1.98	23.6	23.6
8	0.88-1.98	47.4	47.4
9	0.88-1.98	34.8	34.8
10	0.88-1.98	49.7	49.7
11	0.88-1.98	34.6	34.6
12	0.88-1.98	30.3	30.4
13	0.88-1.98	39.3	39.3
14	0.88-1.98	37.8	37.8
15	0.88-1.98	32.1	32.1
16	0.88-1.98	36.0	36.0
17	0.88-1.98	30.1	30.1
18	0.88-1.98	43.0	43.0
19	0.88-1.98	35.1	35.1
20	0.88-1.98	28.2	28.3
21	0.88-1.98	33.1	33.1
22	0.88-1.98	38.9	39.0
23	0.88-1.20	28.9	29.0
24	0.88-1.20	25.4	25.5
25	0.88-1.20	16.2	16.2
26	0.88-1.20	19.6	19.6
27	0.88-1.20	18.4	18.4
28	0.88-1.20	32.0	32.1
29	0.88-1.20	34.5	34.5
30	0.88-1.20	32.4	32.4

1.3 Structure Determination of Compound BSB1



[**14**]

Compound BSB1 was isolated as a yellow crystal with m.p. 182-183°C. The structure of compound BSB1 was elucidated by spectroscopic methods. Its molecular formula $C_{15}H_{12}O_4$ was established by FAB$^+$MS with the molecular ion $[M+H]^+$ at *m/z* 257 (Figure 20), suggesting ten degrees of unsaturation. The IR spectrum of compound BSB1 exhibited characteristic absorption bands for hydroxyl (3514 cm^{-1}) and carbonyl (1634 cm^{-1}) functionalities (Figure 19). The UV spectrum showed a maximum absorption at 365 nm (Figure 18).

The ^{13}C NMR of compound BSB1 in DMSO-d_6 (Figure 22 and Table 10) exhibited 13 signals. The DEPT spectrum established the existence of nine methine carbons, and six quaternary carbons as shown in Table 10.

The ^1H NMR of compound BSB1 in DMSO-d_6 (Figure 21 and Table 10) showed three protons belonging to 1,2,4-trisubstituted benzene ring system (ABX system) at δ 8.15 (*d*, *J* = 8.8 Hz, H-6′), 6.39 (*dd, J* = 8.8, 2.4 Hz, H-5′), 6.26 (*d, J* = 2.4 Hz, H-3′). Two doublets at δ 7.73 (*J* = 16.0 Hz) and 7.76 (*J* = 16.0 Hz) were observed, suggesting the presence of a double bond between C-α and C-β. In addition, four protons belonging to 1,4 disubstituted benzene ring system (AA′BB′ system) at δ 7.74 (*d, J* = 8.7 Hz, H-2 and H-6) and 6.83 (*d, J* = 8.7 Hz, H-3 and H-5) were noted. Correlations of these protons were observed in the ^1H-^1H COSY spectrum (Figure 23). Connectivity of C-H bond and the connectivity of C-H through two or three bond correlations were shown in the HMQC and HMBC spectrum, respectively (Figures 24-25).

Based on the spectral data of compound BSB1 and comparison of its ^1H and ^{13}C NMR with reported (Saitoh *et al*., 1978, Markham and Ternai, 1976) as shown in

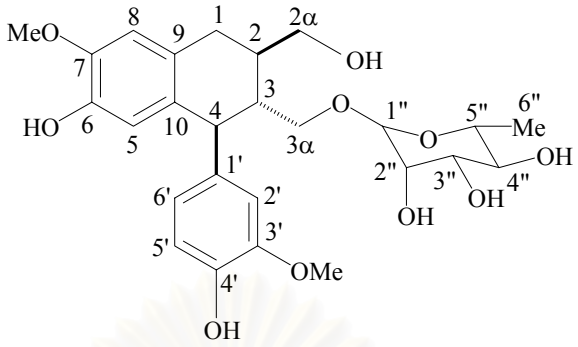
Table 10, structure of compound BSB1 was identified to be isoliquiritigenin [**14**], first found naturally from *Dahlia variabilis* (Smith and Swain, 1953).

Table 10 NMR Spectral data of compound BSB1 and isoliquiritigenin (in DMSO-d_6)

Position	Compound BSB1		Isoliquiritigenin	
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{1}H (*mult.*, *J* in Hz)	^{13}C
1	-	125.8 (C)	-	125.8
2	7.74 (*d*, 8.7)	131.3 (CH)	7.68 (*d*, 8.0)	130.6
3	6.83 (*d*, 8.7)	115.9 (CH)	6.87 (*d*, 8.0)	115.8
4	-	160.3 (C)	-	159.9
5	6.83 (*d*, 8.7)	115.9 (CH)	6.87 (*d*, 8.0)	115.8
6	7.74 (*d*, 8.7)	131.3 (CH)	7.68 (*d*, 8.0)	130.6
β	7.76 (*d*, 16.0)	117.4 (CH)	7.82 (*d*, 16.0)	117.8
α	7.73 (*d*, 16.0)	144.3 (CH)	7.66 (*d*, 16.0)	143.8
β′	-	191.5 (C)	-	191.4
1′	-	112.9 (C)	-	113.2
2′	-	165.1 (C)	-	164.4
3′	6.26 (*d*, 2.4)	102.6 (CH)	6.33 (*d*, 2.5)	102.6
4′	-	165.8 (C)	-	165.4
5′	6.39 (*dd*, 8.8, 2.4)	108.1 (CH)	6.42 (*dd*, 8.0, 2.5)	107.9
6′	8.15 (*d*, 8.8)	132.9 (CH)	8.04 (*d*, 8.0)	132.3
2′-OH	13.61 (*br s*)	-	-	-

*Carbon types were deduced from DEPT experiments.

1.4 Structure Determination of Compound BSB2



[**215**]

Compound BSB2, an amorphous powder, was found to be optically active and was analyzed for $C_{26}H_{34}O_{10}$ from its $[M+H]^+$ at *m/z* 507 in the FAB$^+$MS (Figure 28). Fragments at *m/z* 361 ($[M+H]^+$-146) resulted from cleavage of deoxyhexose unit without the glycosidic oxygen. The IR spectrum of this compound showed the presence of hydroxyl (broad band at 3401 cm^{-1}) and aromatic (1515 cm^{-1}) groups (Figure 27). The UV spectrum revealed the absorption bands at 221 and 283 nm (Figure 26).

The ^{1}H NMR spectrum of compound BSB2 in CD_3OD (Figure 29 and Table 11) showed two peaks at δ 6.10 (1H, *s*) and 6.59 (1H, *s*) due to H-5 and H-8 of the tetrasubstituted aromatic ring, respectively, and peaks at δ 6.51 (*dd*, *J* = 8.0 and 2.0 Hz, H-6′), 6.70 (*d*, *J* = 8.0 Hz, H-5′) and 6.57 (*d*, *J* = 2.0 Hz, H-2′), ascribable to the 3H ABX system of the 3′,4′-disubstituted ring system. The peaks at δ 3.79 and δ 3.72 were attributed to the methoxy groups at C-7 and C-3′. The signal of anomeric proton was found at δ 4.45 (*d*, *J* = 1.4 Hz) and the methyl peak characteristic of rhamnose was observed as a doublet at δ 1.17 (*J* = 6.0 Hz).

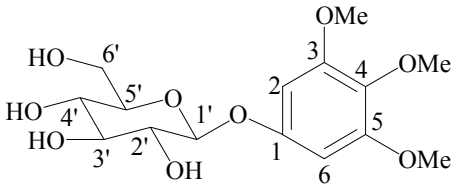
Its ^{13}C NMR data of compound BSB2 in CD_3OD (Figure 30) shown in Table 11 and optical rotation are in good agreement with earlier published data (Kim *et al*., 1994) which supported (+)-isolariciresinol as the aglycone moiety of compound BSB2.

Based on the above spectral data and comparison with reported data (Kim *et al*., 1994), this compound was identified as (+)-isolariciresinol-3α-*O*-α-L-rhamnoside [**215**]. The presence of this compound in this particular species is the second report of this compound obtained as a natural products.

Table 11 NMR Spectral data of compound BSB2 and (+)-isolariciresinol-3α-*O*-α-L- rhamnoside (in CD_3OD)

Position	Compound BSB2		(+)-Isolariciresinol 3-*O*-α-L- rhamnoside	
	^{1}H (*mult.*, *J* in Hz)	^{13}C	^{1}H (*mult.*, *J* in Hz)	^{13}C
Lignan				
1	2.79 (*d*, 8.0)	33.6	2.83 (*d*, 7.8)	33.6
2	1.92 (*m*)	40.1	2.02 (*m*)	40.0
3	1.88 (*m*)	45.5	1.86 (*br t*, 10.2)	45.5
4	3.80 (*d*, 10.2)	48.5	3.85 (*d*, 10.4)	48.3
5	6.10 (*s*)	117.1	6.16 (*s*)	117.1
6	-	146.1	-	146.1
7	-	149.2	-	149.2
8	6.59 (*s*)	112.5	6.66 (*s*)	112.4
9	-	128.9	-	128.9
10	-	138.1	-	138.1
1′	-	134.0	-	133.9
2′	6.57 (*d*, 2.0)	113.5	6.63 (*d*, 1.8)	113.4
3′	-	147.3	-	147.2
4′	-	145.3	-	145.2
5′	6.70 (*d*, 8.0)	116.1	6.75 (1H, 7.9)	116.1
6′	6.51 (*dd*, 8.0, 2.0)	123.2	6.59 (*dd*, 7.9, 1.8)	123.2
2aα	3.60-3.62 (overlapping)	65.4	3.62-3.63 (overlapping)	65.3
2bα	3.62-3.65 (overlapping)	-	3.71 (*dd*, 11.0, 3.7)	-
3aα	3.05 (*m*)	68.0	3.10 (*m*)	67.9
3bα	3.75-3.80 (overlapping)	-	3.80-3.82 (overlapping)	-
7-OMe	3.79 (*s*)	56.4	3.81 (*s*)	56.3
3′-OMe	3.72 (*s*)	56.4	3.77 (*s*)	56.3
Rhamnose				
1″	4.45 (*d*, 1.4)	102.3	4.51 (*d*, 1.5)	102.3
2″	3.82 (*m*)	72.3	3.84 (*dd*, 3.4, 1.6)	72.3
3″	3.62 (*m*)	72.5	3.63 (*dd*, 9.3, 3.3)	72.5
4″	3.32 (*m*)	73.8	3.34 (*t*, 9.0)	73.8
5″	3.49 (*m*)	70.1	3.51 (*dq*, 9.0, 6.0)	70.1
6″	1.17 (*d*, 6.0)	17.9	1.18 (*d*, 6.0)	17.9

1.5 Structure Determination of Compound BSB3



[**216**]

Compound BSB3 was obtained as a white needle. Its showed a molecular ion $[M+H]^+$ at *m/z* 347 in the FAB$^+$MS spectrum (Figure 33), corresponding to the molecular formula of $C_{15}H_{22}O_9$. The IR spectrum showed absorption bands at 3404 (O-H stretching), 1697 (C=O stretching) and 1614 (C=C aromatic) cm^{-1} (Figure 32). The UV spectrum showed the maximal absorptions at 222, 268 and 288 nm (Figure 31).

In addition, ^{1}H and ^{13}C NMR signals in CD_3OD (Figures 34-35 and Table 12) showed peaks assignable for an aromatic ring at δ 6.49 (2H, *s*, H-2, H-6)/ δ 96.1 (C-2, C-6) and for a glucose at δ 3.29-3.48 (4H, overlapping, H-2′, 3′, 4′, 5′)/ δ 75.0 (C-2′), 78.1 (C-3′), 71.7 (C-4′), 78.4 (C-5′), δ 3.65 (1H, *dd*, *J* = 11.9, 2.4 Hz, H-6′a), δ 3.91 (1H, *dd*, *J* = 11.9, 5.2 Hz, H-6′b)/ δ 62.7 (C-6′) and δ 4.80 (1H, *d*, *J* = 7.3 Hz, H-1′)/ δ 103.2 (C-1′). Furthermore, the presence of three methoxy groups were observed at δ 3.69 (3H, *s*, 4-OC$\underline{H}_3$)/ δ 61.2 (4-O$\underline{C}H_3$) and δ 3.80 (6H, *s*, 3- OC$\underline{H}_3$, 5- OC$\underline{H}_3$)/ δ 56.6 (3- O$\underline{C}H_3$, 5- O$\underline{C}H_3$). The assignment of the methoxy groups was accomplished by the analysis of the HMBC correlations.

Regarding the sugar unit, their directly bonded carbons were assigned by the HMQC experiment (Figure 36). The ^{1}H-^{13}C long range correlations in the HMBC spectrum (Figure 37) between anomeric proton H-1′ and C-5′ indicated a pyranose ring with an ether linkage between C-1′ and C-5′. The presence of a diaxial-coupling constants (*J* = 7.3 Hz) indicated that this sugar was a β-D-glucopyranoside. The connection of the sugar and the aromatic ring were determined by HMBC correlations. The sugar unit was attached at C-1 as supported by three-bond coupling of H-1′ with C-1.

From all of the above spectroscopic data in comparison with reported values (Shimura *et al*., 1988; Achenbach, Benirschike and Torrenegra, 1997), compound

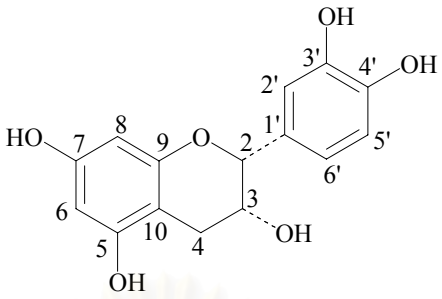
BSB3 was identified as 3,4,5-trimethoxyphenolic-1-*O*-β-D-glucoside [**216**], first isolated from the bark of *Parabenzoin praecox* (Shimura *et al*., 1988).

Table 12 NMR Spectral data of compound BSB3 and 3,4,5-trimethoxyphenolic-1-*O*-β-D-glucoside (in CD_3OD)

Position	Compound BSB3		3,4,5-Trimethoxyphenolic-1-*O*-β-D-glucoside	
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{1}H (*mult.*, *J* in Hz)	^{13}C
Aglycone				
1	-	156.1 (C)	-	156.1
2	6.49 (*s*)	96.1 (CH)	6.48 (*s*)	96.2
3	-	154.8 (C)	-	154.8
4	-	134.5 (C)	-	134.5
5	-	154.8 (C)	-	154.8
6	6.49 (*s*)	96.1 (CH)	6.48 (*s*)	96.1
3, 5-OMe	3.80 (*s*)	56.5 (CH_3)	3.80 (*s*)	56.6
4-OMe	3.69 (*s*)	61.2 (CH_3)	3.69 (*s*)	61.2
Glucose				
1′	4.80 (*d*, 7.3)	103.2 (CH)	4.82 (*d*, 7.6)	103.2
2′	3.29-3.48 (*m*)	75.0 (CH)	3.32-3.47 (*m*)	75.0
3′	3.29-3.48 (*m*)	78.1 (CH)	3.32-3.47 (*m*)	78.1
4′	3.29-3.48 (*m*)	71.7 (CH)	3.32-3.47 (*m*)	71.7
5′	3.29-3.48 (*m*)	78.4 (CH)	3.32-3.47 (*m*)	78.4
6′a	3.65 (*dd*, 11.9, 2.4)	62.7 (CH_2)	3.66 (*dd*, 12.3, 2.6)	62.8
6′b	3.91 (*dd*, 11.9, 5.2)		3.92 (*dd*, 12.3, 5.3)	

*Carbon types were deduced from DEPT experiments.

1.6 Structure Determination of Compound BSB4



[**217**]

Compound BSB4 was obtained as a colorless needle and showed a molecular ion $[M-H]^-$ in the FAB$^-$MS spectrum at *m/z* 289 (Figure 40) corresponding to the molecular formula of $C_{15}H_{14}O_6$. The IR spectrum demonstrated the presence of a hydroxyl (3404 cm^{-1}) but no signal of a carbonyl group was observed (Figure 39). The UV maximal absorptions at 222, 268 and 288 nm (Figure 38) were suggestive of a flavan skeleton (Gómez *et al*., 1985).

The presence of a multiplet signal at δ 4.12 (1H, H-3) and two doublet of doublet signals at δ 2.69 (H-4a) and 2.82 (H-4b) in the ^{1}H-NMR spectrum in CD_3OD (Figure 41 and Table 13) together with the appearance of the oxygen-attached tertiary carbon at δ 79.8 (C-2) and δ 65.1 (C-3) in the ^{13}C NMR spectrum in CD_3OD (Figure 42 and Table 13) indicated that compound BSB4 should be a flavan with oxygenation at C-3. The protons in B-ring (H-2′, H-5′ and H-6′) formed a characteristic ABX pattern at δ 6.94 (*d*, $J_{2',6'}$ = 2.1 Hz , H-2′), 6.72 (*d*, $J_{5',6'}$ = 8.2 Hz, H-5′) and 6.75 (*dd*, $J_{6',5'}$ = 8.2 Hz and $J_{6',2'}$ = 2.1 Hz, H-6′) while the signals of H-6 and H-8 in A-ring appeared as doublets at δ 5.91 (*d, J* = 2.3 Hz) and 5.88 (*d, J* = 2.3 Hz), respectively. The ^{13}C NMR spectrum showed the methylene carbon at δ 29.2 (C-4), two methine carbons at δ 67.4 (C-3), 79.8 (C-2), five aromatic methine at δ 95.9 (C-8), 96.4 (C-6), 115.3 (C-2′), 115.9 (C-5′), 119.4 (C-6′) and seven aromatic quarternary carbons at δ 57.3 (C-7), 100.1 (C-10), 132.2 (C-1′), 145.7 (C-3′), 145.9 (C-4′), 157.3 (C-9), 157.9 (C-5).

The ^{1}H NMR and ^{13}C NMR assignments of the compound BSB4 were performed with the aid of the DEPT method and 2D techniques such as the ^{1}H-^{1}H COSY, HMQC and HMBC experiments (Figures 42-45). All protons and carbons were assigned as shown in Table 13.

The absolute configuration at C-2 and C-3 of compound BSB4 has been proved to be 2*R* and 3*R* by comparing the optical rotation value and CD spectra with those reported in the literature (Harborne, 1982; Korver and Wilkin, 1971).

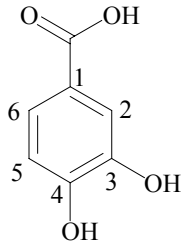
By analysis of the above spectroscopic data and comparison with previously reported data (Agrawal, 1989), compound BSB4 was identified as (-)-epicatechin [**217**], a flavan previously isolated from several plants.

Table 13 NMR Spectral data of compound BSB4 (in CD_3OD) and (-)-epicatechin (in DMSO-d_6)

Position	Compound BSB4		(-)-Epicatechin
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{13}C
A and C ring			
2	4.76 (*br s*)	79.8 (CH)	78.1
3	4.12 (*m*)	67.4 (CH)	65.1
4a	2.69 (*dd*, 16.7, 2.7)	29.2 (CH_2)	28.0
4b	2.82 (*dd*, 16.7, 4.3)	-	-
5	-	157.9 (C)	156.4
6	5.91 (*d*, 2.3)	96.4 (CH)	95.6
7	-	157.3 (C)	156.3
8	5.88 (*d*, 2.3)	95.9 (CH)	94.5
9	-	157.3 (C)	155.7
10	-	100.1 (C)	98.8
B ring			
1′	-	132.2 (C)	130.7
2′	6.94 (*d*, 2.1)	115.3 (CH)	115.0
3′	-	145.7 (C)	144.4
4′	-	145.9 (C)	144.5
5′	6.72 (*d*, 8.2)	115.9 (CH)	115.0
6′	6.75 (*dd*, 8.2, 2.1)	119.4 (CH)	118.1

*Carbon types were deduced from DEPT experiments.

1.7 Structure Determination of Compound BSB5



[**218**]

Compound BSB5 was isolated as a colorless crystal with m.p. 194-196°C. Its molecular formula of $C_7H_6O_4$ was established by FAB$^+$MS spectrum which showed the $[M+H]^+$ peak at *m/z* 155 (Figure 48) suggesting five degrees of unsaturation. The IR spectrum exhibited characteristic absorption bands at 3264 cm^{-1} (O-H stretching), 1673 cm^{-1} (C=O stretching), 1601 cm^{-1} (C=C aromatic stretching) (Figure 47). The UV absorption bands were found at 222, 258 and 294 nm (Figure 46).

The ^{1}H NMR signal of compound BSB5 in CD_3OD (Figure 49 and Table 14) showed three protons belonging to 1,3,4-trisubstituted benzene ring system (ABX system) was observed at δ 7.30 (1H, *br s*, H-2), 7.32 (1H, *d*, *J* = 7.5 Hz, H-6), 6.69 (1H, *d*, *J* = 7.5 Hz, H-5).

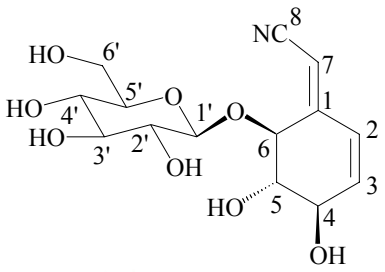
The ^{13}C NMR signal of compound BSB5 in CD_3OD (Figure 50 and Table 14) exhibited seven signals, corresponding to three methine carbons at δ 115.7, 117.7, 123.9 and four quaternary carbons at δ 170.2, 151.5, 146.1, 123.1.

By careful analysis of the obtained spectral data and comparison of the ^{13}C NMR spectral data with the previously reported data (Kaewamatawong, R., 2002) as shown in Table 14, compound BSB5 was determined to be protocatechuic acid [**218**]. This compound has been isolated to be present widely in plants such as *Ochna integerrima* (Kaewamatawong, R., 2002).

Table 14 NMR Spectral data of compound BSB5 (in CD_3OD) and protocatechuic acid (in acetone-d_6)

Position	Compound BSB5		Protocatechuic acid
	^1H (*mult.*, *J* in Hz)	^{13}C	^{13}C
1	-	123.1	122.9
2	7.30 (*br s*)	123.9	123.3
3	-	146.1	145.3
4	-	151.5	150.4
5	6.69 (*d*, 7.5)	115.7	115.4
6	7.32 (*d*, 7.5)	117.7	117.2
C=O	-	170.2	167.4

1.8 Structure Determination of Compound BSB6



[**54**]

Compound BSB6, a fine white needle, was analyzed for $C_{14}H_{19}NO_8$ from its $[M+H]^+$ at *m/z* 330 and the fragment ion at *m/z* 168 $[M+H\text{-glucose}]^+$ in the FAB^+MS (Figure 53). The IR spectrum showed broad adsorption at 3434 cm^{-1} (O-H stretching) together with a very sharp and strong band at 2224 cm^{-1} (C≡N stretching) which are expected for a conjugated nitrile group (Figure 52). The UV spectrum correlated well with those for 1-cyanomethylene-2-cyclohexene at λ_{max} 259 nm (Sosa *et al*., 1977) (Figure 51).

The ^1H NMR spectrum of compound BSB6 in D_2O (Figure 54 and Table 15) displayed three downfield signals corresponding to three olefinic protons at δ 6.23 (H-2) 6.00 (H-3) and 5.50 (H-7), respectively. The signals at δ 6.23 (H-2) and 6.00 (H-3) represent the AB part of an ABX system. The analysis of signals at δ 6.23 (H-2) and 6.00 (H-3) gives the following coupling constants: $J_{2,3}$ = 10.1 Hz and $J_{3,4}$ = 3.0 Hz. The signal at δ 4.75 (H-6) coupled with that at 3.84 (H-5) with $J_{5,6}$ = 8.2 Hz. The signal centered at δ 4.75 (H-6) was then assigned to the allylic proton α to the *O*-glycosyl substituent. The low field value found for the allylic proton at δ 4.75 (H-6) compared to 4.19 (H-4) seems to be in accordance with a stereoisomeric form in which the nitrile group is *syn* with respect to the glycosidic bond. Indeed, in such a configuration the anisotropic effect of the triple bond should deshield H-6.

The ^{13}C NMR spectrum of compound BSB6 in D_2O (Figure 55 and Table 15) displayed a strongly deshielded olefinic carbon at δ 156.1 (C-1) and another strongly deshielded olefinic carbon at δ 97.9 (C-7). The anomeric β-configuration of the glucose moiety is consistent with the chemical shift noted for C-1′ as it appeared at δ 103.3. This value of anomeric carbon resembles more closely to the β-configuration of β-D-glucopyranose (at δ 104.6) than the α configuration of the corresponding

epimer (at δ 100.1) (Sosa *et al*., 1977). The ^{1}H NMR and ^{13}C spectral data of compound BSB6 are in good agreement with earlier published data as shown in Table 15 (Sosa *et al*., 1977 and Wu *et al*., 1979). All protons and carbons were assigned by 2D NMR techniques of the HMQC and HMBC spectra (Figures 56-57).

The CD curve provides spectral information characteristic of this compound. Compound BSB6 showed a positive maximum at 227 nm and a negative maximum at 263 nm, which are consistent with those previously reported data (Wu *et al*., 1979).

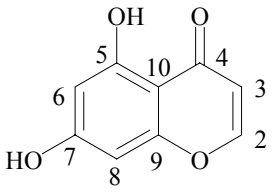
From all of the above spectroscopic data which are in accord with the reported values, compound BSB6 was identified as lithospermoside [**54**]. This compound was first isolated from the roots of *Lithospermum purpureo-caeruleum* (Sosa *et al*., 1977).

Table 15 NMR Spectral data of compound BSB6 and lithospermoside (in D_2O)

Position	Compound BSB6		Lithospermoside
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{13}C
Aglycone			
1	-	156.1 (C)	157.6
2	6.23 (*d*, 10.1)	127.8 (CH)	129.2
3	6.00 (*dd*, 10.1, 3.0)	136.9 (CH)	138.7
4	4.19 (*br s*)	74.7 (CH)	76.2
5	3.84 (*dd*, 8.2, 6.1)	76.6 (CH)	78.5
6	4.75 (*d*, 8.2)	70.7 (CH)	72.3
7	5.50 (*br d*)	97.9 (CH)	99.4
8	-	118.5 (C)	120.1
Glucose			
1′	4.78 (*d,* 7.3)	103.3 (CH)	104.9
2′	3.29-3.41 (*m*)	73.5 (CH)	75.3
3′	3.29-3.41 (*m*)	76.9 (CH)	78.4
4′	3.29-3.41 (*m*)	70.4 (CH)	72.3
5′	3.29-3.41 (*m*)	76.9 (CH)	78.3
6′a	3.75 (*dd,* 12.3, 2.0)	61.6 (CH)	63.5
6′b	3.59 (*dd,* 12.3, 5.2)	-	-

*Carbon types were deduced from DEPT experiments.

1.9 Structure Determination of Compound BRC1



[**219**]

Compound BRC1, was obtained as a colorless crystal, having the molecular formula of $C_9H_6O_4$ which was deduced from FAB$^+$MS spectrum and NMR spectral data. The FAB$^+$MS spectrum exhibited the molecular ion peak $[M+H]^+$ at *m/z* 179 (Figure 60). Its IR spectrum clearly revealed the presence of hydroxyl group (3003 cm^{-1}) and carbonyl group (1646 cm^{-1}) (Figure 59). The UV spectrum showed a maximum absorption at 224, 250, 256, and 295 nm (Figure 58).

The ^{1}H NMR spectrum in CD_3OD of compound BRC1 (Figure 61 and Table 16) exhibited the characteristic signals due to H-2 and H-3 of a chromone skeleton at δ 7.96 and 6.19 (1H each, *d, J* = 6.1 Hz), respectively. The isolated aromatic protons with *meta*-coupling was observed at δ 6.20 (*d, J*=1.8 Hz, H-6) and δ 6.31 (*d, J* = 1.8 Hz, H-8). The ^{1}H NMR spectrum in acetone-d_6 of compound BRC1 (Figure 62) showed a H-bonded phenolic proton at δ 12.74, indicating a 5-hydroxychromone stucture.

The ^{13}C NMR spectrum of compound BRC1 in CD_3OD (Figure 63 and Table 16) displayed the resonance signals for all carbons and the multiplicity of each carbon could assigned by the DEPT spectrum. The carbonyl carbon appeared at δ 182.5 that was found to be similar to those commonly found for chromone (Simon *et al*., 1994). Furthermore, five quarternary carbons at δ 106.5, 159.2, 163.5, 165.2, 182.5 and the presence of four methine carbons at δ 94.7, 99.9, 111.6, 157.6 were also observed.

All protons and carbons were assigned from the HMQC and HMBC experiments (Figures 64-65). The carbonyl carbon of chromone detected at δ 182.5 was correlated with olefinic proton at δ 7.96 (H-2) and 6.19 (H-3) with two-bond and three-bond coupling, respectively in HMBC correlations. This clearly pointed out that the C-2 and C-3 positions in the chromone ring should be unsubstituted.

According to the above results, and comparison of the spectral data of compound BRC1 with those of the previously reported structure (Simon *et al*., 1994),

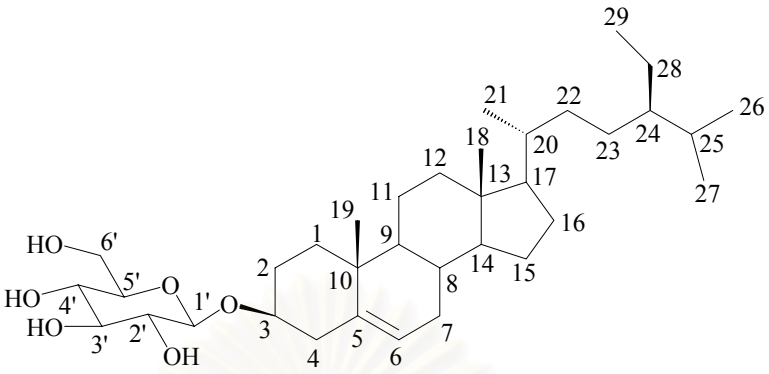
compound BRC1 was verified to be 5,7-dihydroxychromone [**219**]. This compound was obtained previously from *Calluna vulgaris* (Simon *et al*., 1994).

Table 16 NMR spectral data of compound BRC1 and 5,7-dihydroxychromone (in CD_3OD)

Position	Compound BRC1		5,7-Dihydroxychromone	
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{1}H (*mult.*, *J* in Hz)	^{13}C
2	7.96 (*d*, 6.1)	157.6 (CH)	7.94 (*d*, 6.0)	158.0
3	6.19 (*d,* 6.1)	111.6 (CH)	6.16 (*d*, 6.0)	111.7
4	-	182.5 (C)	-	183.4
5	-	163.5 (C)	-	163.5
6	6.20 (*d,* 1.8)	99.9 (CH)	6.17 (*d*, 2.1)	99.9
7	-	165.2 (C)	-	165.2
8	6.31 (*d,* 1.8)	94.7 (CH)	6.30 (*d*, 2.1)	94.7
9	-	159.2 (C)	-	159.2
10	-	106.5 (C)	-	106.5

*Carbon types were deduced from DEPT experiments.

1.10 Structure Determination of Compound BRC2



[**37**]

Compound BRC2 was obtained as a white powder. The $FAB^{+}MS$ spectrum showed molecular fragments ions at *m/z* 577 (Figure 67), corresponding to the molecular formula of $C_{35}H_{60}O_6$. The IR spectrum (Figure 66) exhibited an O-H absorption at ν_{max} 3402 cm^{-1} as well as C-O stretching band at 1073 cm^{-1} indicating the alcohol-containing moiety of this sample. The absorption band at 1642 cm^{-1} suggested the presence of C=C in the structure.

The ^{1}H NMR spectrum in CD_3OD of compound BRC2 (Figure 68 and Table 17) showed the signal in the range of δ 3.15-4.30 corresponding to a sugar moiety. The signal at δ 4.30 was assigned to the anomeric proton (H-1′) with a coupling constant ($J_{axial, axial}$ = 8.0 Hz) which was in agreement with a *trans* diaxial relationship in β-configuration. The spectral data of aglycone part of compound BRC2 were similar to sitosterol. Although the methyl signals of (19-CH_3, 26-CH_3, 27-CH_3 and 29-CH_3) in the range of δ 0.70-0.90 overlapped with each other, their NMR assignments were found almost in accordance with those reported (Kojima *et al*., 1990).

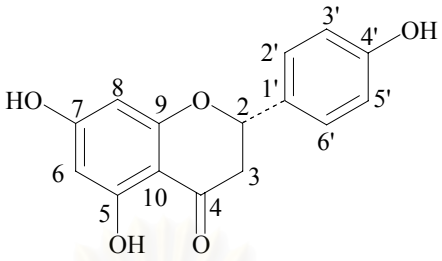
The ^{13}C NMR spectral data of compound BRC2 in CD_3OD (Figure 69 and Table 17) were almost identical with those in the literature (Kojima *et al*., 1990). The comparison of the spectral data are summarized in Table 17.

On the basis of the above data by comparison of the spectral data with those previously reported (Kojima *et al*., 1990), compound BRC2 was identified as sitosteryl-3-*O*-β-D-glucoside [**37**]. This compound is the common sterol in higher plants.

Table 17 NMR Spectral data of compound BRC2 (in $CDCl_3$ + CD_3OD) and sitosteryl-3-*O*-β-D-glucoside (in pyridine-d_5)

Position	Compound BRC2		sitosteryl-3-*O*-β-D-glucoside
	^{1}H (*mult.*, *J* in Hz)	^{13}C	^{13}C
Aglycone			
1	0.90-1.93	37.1	37.6
2	3.15-3.50	29.0	30.3
3	0.90-1.93	79.0	78.3
4a	2.16 (*m*)	38.5	39.4
4b	2.29 (*m*)	-	-
5	-	140.1	141.0
6	5.27 (*br s*)	122.0	122.0
7	0.90-1.93	31.7	32.2
8	0.90-1.93	31.8	32.1
9	0.90-1.93	50.0	50.4
10	-	36.5	37.0
11	0.90-1.93	20.9	21.4
12	0.90-1.93	39.6	40.0
13	-	42.2	42.6
14	0.90-1.93	56.6	57.0
15	0.90-1.93	24.1	24.6
16	0.90-1.93	28.1	28.7
17	0.90-1.93	55.9	56.3
18	0.58 (*s*)	11.6	12.0
19	0.70-0.90	18.8	19.3
20	0.90-1.93	36.0	36.5
21	0.70-0.90	19.1	19.1
22	0.90-1.93	33.8	34.3
23	0.90-1.93	25.9	26.4
24	0.90-1.93	45.7	46.1
25	0.90-1.93	29.4	29.5
26	0.70-0.90	19.1	19.5
27	0.70-0.90	19.6	20.1
28	0.90-1.93	23.0	23.4
29	0.70-0.90	11.7	12.2
Glucose			
1′	4.30 (*d*, 8.0)	100.9	102.6
2′	3.15-3.50	75.5	75.4
3′	3.15-3.50	77.2	78.7
4′	3.15-3.50	70.0	71.7
5′	3.15-3.50	76.2	78.5
6′a	3.73 (*dd*, 11.2, 2.3)	61.7	62.9
6′b	3.60 (*dd*, 11.2, 4.8)		-

1.11 Structure Determination of Compound BRB1



[**17**]

Compound BRB1 was obtained as a yellow needle with m.p. 249-251°C. The FAB^+MS showed its $[M+H]^+$ at *m/z* 273 (Figure 72) suggesting the molecular formula of $C_{15}H_{12}O_5$. The UV spectrum of compound BRB1 (Figure 70) displayed three absorption bands at 226, 288 and 332 nm. The band at 332 nm is refered to Band I and involves the B-ring system. This band appears as a shoulder due to the lack of conjugation between ring A and B. The bands at 288 and 226 nm are typical of Band II which are generally considered to be due to the absorption of the A-ring system (Markham, 1982).

The IR spectrum of compound BRB1 (Figure 71) exhibited the C=O stretching of a conjugated carbonyl group at 1632 cm^{-1} which is slightly shifted to longer wavelength due to the presence of an intramolecular hydrogen bonding between hydroxyl aryl and keto group. The C=C stretching of aromatic ring was observed at 1604 cm^{-1}. The compound was clearly proved to be phenolic by the O-H and C-O stretching bands at 3268 cm^{-1} and 1253 cm^{-1}, respectively.

The ^1H NMR spectrum in CD_3OD of compound BRB1 (Figure 73 and Table 18) was characteristic of as a flavanone. Protons in the B-ring (H-2′, H-6′ and H-3′, H-5′) formed a characteristic AA′BB′ pattern at δ 7.30 (*d*, $J_{2',3'} = J_{6',5'}$ = 8.6 Hz, H-2′ and H-6′) and 6.80 (*d*, $J_{3',2'} = J_{5',6'}$ = 8.6 Hz, H-3′ and H-5′), while the signals of H-6 and H-8 in the A-ring appeared as a doublet at δ 5.85 (*d, J* = 2.0 Hz) and 5.86 (*d, J* = 2.0 Hz), respectively. A doublet of doublet of H-2 indicated the cis-relationship between H-2 and H-3a with $J_{2,3a}$ = 3.0 Hz and trans-relationship between H-2 and H-3b with ($J_{2,3b}$ = 12.9 Hz). The signals at δ 2.68 (*dd*, $J_{3a,3b}$ = 16.2 Hz and $J_{3a,2}$ = 3.0 Hz) and 3.15 (*dd*, $J_{3b,3a}$ = 16.2 Hz and $J_{3b,2}$ = 12.9 Hz) were referred to H-3a and H-3b, respectively.

The ^{13}C NMR spectrum of compound BRB1 in CD_3OD (Figure 74 and Table 18) showed 15 signals for 15 carbon atoms. The types of carbons are classified by the analysis of the DEPT spectrum as shown in Table. The ^{13}C NMR spectral data were in close agreement with the previously published values (Agrawal, 1989) as shown in Table. In order to confirm the chemical shifts of protons and carbons of compound BRB1, the HMQC and HMBC experiments were performed (Figure 75-76).

Compound BRB1 was optically active with the optical rotation of $[\alpha]_D^{23}$ -13° (MeOH, *c* 0.23). The absolute configuration at C-2 of this compound has been proved to be in an *S*-configuration by comparison of the optical rotation value with those reported in the literature (Hsieh, Fang and Cheng, 1998).

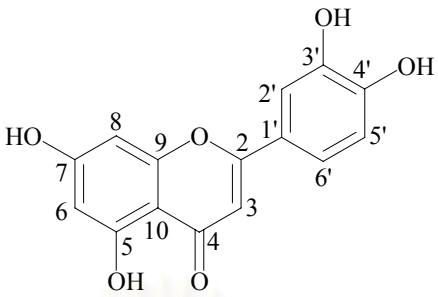
Based on the above data, compound BRB1 was identified as (2*S*)-naringenin [**17**]. This compound was obtained previously from *Artemisia dracunculus* (Balza and Tower, 1984).

Table 18 NMR Spectral data of compound BRB1 (in CD_3OD) and (2*S*)-naringenin (in acetone-d_6)

Position	Compound BRB3		(2*S*)-Naringenin
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{13}C
A and C ring			
2	5.30 (*dd*, 12.9, 3.0)	80.5 (CH)	80.1
3a	2.68 (*dd*, 16.2, 3.0)	44.1 (CH_2)	43.7
3b	3.15 (*dd*, 16.2, 12.9	-	
4	-	197.8 (C)	197.3
5	-	165.5 (C)	165.0
6	5.85 (*d*, 2.0)	97.1 (CH)	97.0
7	-	168.5 (C)	168.0
8	5.86 (*d,* 2.0)	96.2 (CH)	96.1
9	-	164.9 (C)	164.5
10	-	103.3 (C)	103.1
B ring			
1′		131.1 (C)	130.7
2′	7.30 (*d*, 8.6)	129.0 (CH)	128.8
3′	6.80 (*d*, 8.6)	116.3 (CH)	116.2
4′	-	159.1 (C)	158.5
5′	6.80 (*d*, 8.6)	116.3 (CH)	116.2
6′	7.30 (*d*, 8.6)	129.0 (CH)	128.8

*Carbon types were deduced from DEPT experiments.

1.12 Structure Determination of Compound BRB2



[**220**]

Compound BRB2 was obtained as a yellow needle with m.p. 325-328°C. The UV spectrum displayed absorption bands at 221, 255, 267 and 350 nm (Figure 77). The IR spectrum exhibited absorption bands at 3395 (O-H stretching), 1657 (C=O stretching) and 1608 (C=C aromatic ring) cm^{-1} (Figure 78). The $FAB^{+}MS$ showed its $[M+H]^{+}$ at *m/z* 287 (Figure) suggesting the molecular formula of $C_{15}H_{10}O_6$ (Figure 79).

The ^{1}H NMR spectrum in DMSO-d_6 of compound BRB2 (Figure 80 and Table 19) showed a H-bonded phenolic proton at δ 12.97 ppm, indicating a 5-hydroxyflavone structure. The protons in B-ring (H-2′, H-5′ and H-6′) formed a characteristic ABX pattern at δ 7.39 (*d*, $J_{2',6'}$ = 2.0 Hz , H-2′), 6.87 (*d*, $J_{5',6'}$ = 8.5 Hz, H-5′) and 7.41 (*dd*, $J_{6',5'}$ = 8.5 Hz and $J_{6',2'}$ = 2.0 Hz, H-6′) while the signals of H-6 and H-8 in A-ring appeared as a doublet at δ 6.17 (*d, J* = 2.0 Hz) and 6.43 (*d, J* = 2.0 Hz), respectively. An olefinic singlet proton at δ 6.66 was assigned to H-3 by its HMBC correlations with C-10 (103.7) and C-1′ (121.5).

The ^{13}C NMR spectrum of compound BRB2 in DMSO-d_6 (Figure 81 and Table 19) showed 15 signals for 15 carbon atoms. The types of carbons are classified by the analysis of the DEPT spectrum as shown in Table 19.

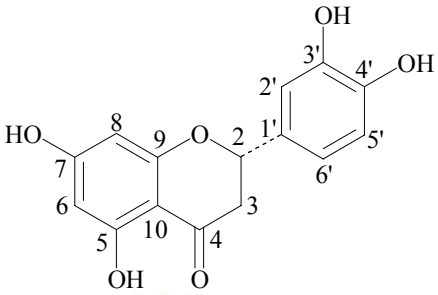
Based on the above spectral evidence, and comparison of the spectral data of compound BRB2 with those previously reported (Agrawal, 1989), together with the information from the HMBC and HMQC experiments (Figures 82-83), compound BRB2 was identified as luteolin [**220**]. This compound occurred in many plants of the family Leguminosae, Resedaceae, Euphorbiaceae, Umbelliferae, Scrophulariaceae, Fabaceae, Asteraceae, Cistaceae, Passifloraceae, Yerbenaceae and Hepaticae (Buckingham, 2001).

Table 19 NMR Spectral data of compound BRB2 and luteolin (in DMSO-d_6)

Position	Compound BRB2		Luteolin
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{13}C
A and C ring			
2	-	163.9 (C)	164.5
3	6.66 (*s*)	102.8 (CH)	103.3
4	-	181.6(C)	182.2
5	-	161.5 (C)	162.1
6	6.17 (*d*, 2.0)	98.8 (CH)	99.2
7	-	164.1 (C)	164.7
8	6.43 (*d*, 2.0)	93.8 (CH)	94.2
9	-	157.3 (C)	157.9
10	-	103.7 (C)	104.2
5-OH	12.97 (*s*)	-	-
B ring			
1′	-	121.5 (C)	122.1
2′	7.39 (*d*, 2.0)	113.3 (CH)	113.8
3′	-	145.7 (C)	146.2
4′	-	149.7 (C)	150.2
5′	6.87 (*d*, 8.5)	116.0 (CH)	116.4
6′	7.41 (*dd*, 8.5, 2.0)	119.0 (CH)	119.3

*Carbon types were deduced from DEPT experiments.

1.13 Structure Determination of Compound BRB3



[**16**]

Compound BRB3 was obtained as a pale yellow needle with m.p. 198-200°C, showed its $[M+H]^+$ at *m/z* 289 in FAB^+MS spectrum (Figure 86) corresponding to the molecular formula of $C_{15}H_{12}O_6$. The IR spectrum showed absorption bands at 3366 cm^{-1} (O-H stretching), and 1632 cm^{-1} (C=O stretching) cm^{-1} (Figure 85). The UV absorptions at 224, 288 and 328 nm (Figure 84) were indicative of a flavanone skeleton (Markham, 1982).

The ^{1}H-NMR spectrum of compound BRB3 in CD_3OD (Figure 87 and Table 20) revealed a doublet of doublet of H-2, indicated the cis-relationship between H-2 and H-3a ($J_{2,3a}$ = 2.8 Hz) and trans-relationship between H-2 and H-3b ($J_{2,3b}$ = 12.6 Hz). The A-ring showed an AB coupling system of the two aromatic protons at H-6 and H-8. The B-ring exhibited signals for an ABX pattern at δ 6.91 (*d*, $J_{2',6'}$ = 2.2 Hz , H-2′), 6.76 (*d*, $J_{5',6'}$ = 8.0 Hz, H-5′) and 6.78 (*dd*, $J_{6',5'}$ = 8.0 Hz and $J_{6',2'}$ = 2.2 Hz, H-6′). The ^{13}C NMR spectrum of compound BRB3 in CD_3OD (Figure 88 and Table 20) showed 15 signals for 15 carbon atoms. The types of carbons are classified by the analysis of the DEPT spectrum as shown in Table. Its ^{13}C NMR data are in good agreement with earlier published data (Agrawal, 1989). The successful assignments of compound BRB3 were accomplished by application of 2D NMR, including the HMQC and HMBC experiments (Figures 89-90).

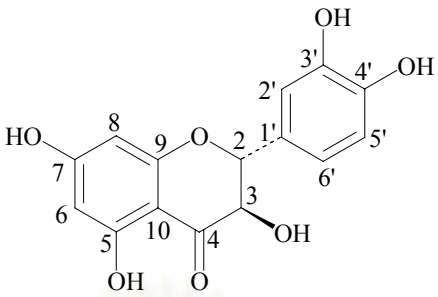
The absolute configuration at C-2 of compound BRB3 has been proved to be *S*-configuration by comparing the optical rotation value with those reported in the literature (Harborne and Mabry, 1982). On the basis of the above spectroscopic data, this compound was identified as (2*S*)-eriodictyol [**16**]. This compound was previously separated from several plants.

Table 20 NMR Spectral data of compound BRB3 (in CD_3OD) and (2*S*)-eriodictyol (in DMSO-d_6)

Position	Compound BRB3		(2*S*)-Eriodictyol
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{13}C
A and C ring			
2	5.25 (*dd*, 12.6, 2.8)	80.5 (CH)	78.3
3a	2.63 (*dd*, 17.0, 2.8)	44.1 (CH_2)	42.2
3b	3.05 (*dd*, 17.0, 12.6)	-	-
4	-	197.7 (C)	196.2
5	-	165.5 (C)	163.4
6	5.87 (*d*, 2.4)	97.0 (CH)	95.7
7	-	168.4 (C)	166.6
8	5.91 (*d*, 2.4)	96.2 (CH)	94.8
9	-	164.8 (C)	162.8
10	-	103.3 (C)	101.7
B ring			
1′	-	131.8 (C)	129.4
2′	6.91 (*d*, 2.2)	114.7 (CH)	114.2
3′	-	146.5 (C)	145.1
4′	-	146.9 (C)	145.6
5′	6.76 (*d*, 8.0)	116.2 (CH)	115.3
6′	6.78 (*dd*, 8.0, 2.2)	119.2 (CH)	117.8

*Carbon types were deduced from DEPT experiments.

1.14 Structure Determination of Compound BRB4



[**221**]

Compound BRB4 was obtained as a pale yellow needle with m.p. 238-241°C. A molecular formula of $C_{15}H_{12}O_7$ was established based on the FAB^+MS which exhibited $[M+H]^+$ at *m/z* 305 (Figure 93). The UV spectrum displayed three absorption bands at 222, 290 and 325 nm (Figure 91). The IR spectrum indicated the presence of hydroxy (3412 cm^{-1}) and carbonyl (1639 cm^{-1}) groups (Figure 92).

The ^1H NMR spectrum in CD_3OD of compound BRB4 (Figure 94 and Table 21) showed the typical AB-coupled protons at δ 4.85 and 4.49 (*J* = 14.0 Hz, 1H each) due to H-2 and H-3 of a dihydroflavonol, respectively. By comparison of ^1H NMR spectral data of compound BRB4 with those of compounds BRB2 and BRB3, similar coupling patterns of protons as an AB pattern at ring A and ABX pattern at ring C could be observed. The ^{13}C NMR spectrum of compound BRB4 in CD_3OD (Figure 95 and Table 21) showed 15 signals for 15 carbon atoms, corresponding to a dihydroflavonol. The ^1H and ^{13}C NMR assignments were performed using the DEPT, HMQC and HMBC experiments (Figures 96-97). Thus, compound BRB4 possessed the 5,7,3′,4′-tetrahydroxy dihydroflavonol skeleton.

The absolute configuration of compound BRB4 is (2*R*, 3*R*). The CD spectra and optical rotation of compound BRB4 are in good agreement with those previously published (Lundgren and Theander, 1988).

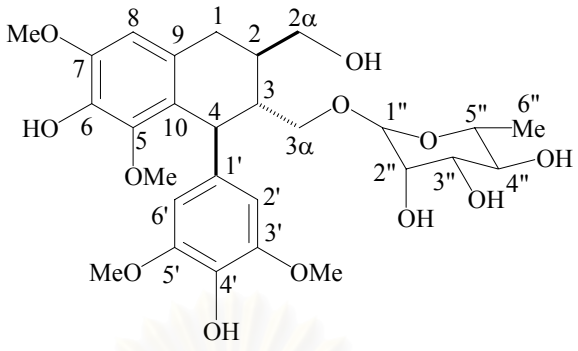
Compound BRB4 was identified as (+)-taxifolin (*trans*-dihydroquercetin) [**221**] based on the above spectral data. The ^1H and ^{13}C NMR spectra were in close agreement with previously published values (Lundgren and Theander, 1988) as shown in Table 21. This compound has been isolated from *Pinus sylvestris* (Lundgren and Theander, 1988).

Table 21 NMR Spectral data of compound BRB4 and (+)-taxifolin (in CD_3OD)

Position	Compound BRB4		(+)-Taxifolin	
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{1}H (*mult.*, *J* in Hz)	^{13}C
A and C ring				
2	4.85 (*d*, 14.0)	85.1 (CH)	4.92 (*d*, 11.3)	84.7
3	4.49 (*d*, 14.0)	73.6 (CH)	4.49 (*d*, 11.3)	73.2
4	-	198.4 (C)	-	197.9
5	-	165.3 (C)	-	164.7
6	5.85 (*d*, 2.5)	97.3 (CH)	5.89 (*d*, 2.2)	97.0
7	-	168.8 (C)	-	168.1
8	5.92 (*d*, 2.5)	96.3 (CH)	5.93 (*d*, 2.2)	95.6
9	-	164.5 (C)	-	164.0
10	-	101.8 (C)	-	101.3
B ring				
1′	-	129.9 (C)	-	129.3
2′	6.91 (*d*, 2.0)	115.9 (CH)	6.97 (*d*, 2.0)	115.6
3′	-	146.3 (C)	-	145.8
4′	-	147.1 (C)	-	146.5
5′	6.78 (*d*, 8.0)	116.0 (CH)	6.81 (*d*, 8.2)	115.6
6′	6.82 (*dd*, 8.0, 2.0)	120.9 (CH)	6.85 (*dd*, 8.2, 2.0)	120.4

*Carbon types were deduced from DEPT experiments.

1.15 Structure Determination of Compound BRB5



[**222**]

Compound BRB5, an amorphous solid, was found to be optically active and was analyzed for $C_{28}H_{38}O_{12}$ from its $[M+K]^+$ at *m/z* 605 in the FAB$^+$MS spectrum (Figure 100). The IR spectrum of compound BRB5 exhibited characteristic absorption bands at 3402 cm^{-1} (O-H stretching) and 1614 cm^{-1} (C=C aromatic) (Figure 99). The UV spectrum showed the absorption bands at 221 and 278 nm (Figure 98).

The ^{1}H NMR spectrum of compound BRB5 in CD_3OD (Figure 101 and Table 22) showed the presence of two singlet peaks at δ 6.50 and 6.25 belonging to the H-8 and H-2′ of the aromatic rings. The peaks at δ 3.15, 3.64 and 3.76 were attributed to the methoxy groups at C-5, C-3′ and C-5′, C-7, respectively, as indicated by HMBC spectra. The signal of anomeric proton was found at δ 4.60 (*d*, *J* = 1.2 Hz) and the methyl peak characteristic of rhamnose was observed as a doublet at δ 1.19 (*J* = 6.1 Hz).

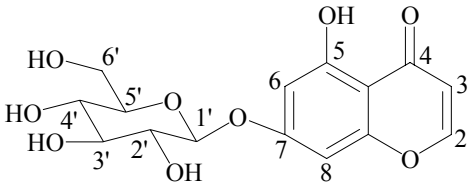
The ^{13}C NMR data in CD_3OD (Figure 102 and Table 22) and DEPT experiment showed 28 carbon signals, corresponding to four methoxyl carbons, one methyl carbon, three methylene carbons, eleven methine carbons and nine quarternary carbons (Figure). In addition, six peaks at δ 18.6, 70.2, 72.5, 73.1, 74.1 and 102.1 were assigned to C-1″ to C-6″ of α-L-rhamnosyl moiety. The location of glycosidic linkage was elucidated by the analysis of 2D NMR spectra, especially the ^{1}H-^{1}H COSY, HMQC and HMBC spectra (Figures 103-105).

By analysis of the above spectroscopic data and comparison of its ^{13}C NMR and optical rotation values with previously reported data (Fuchino *et al*., 1995), compound BRB5 was thus identified as (+)-lyoniresinol-3α-*O*-α-L-rhamnoside [**222**]. This compound has been reported from *Ulmus thomasii* (Hostettler and Seikel, 1969).

Table 22 NMR Spectral data of compound BRB5 (in CD_3OD) and (+)-lyoniresinol-3α-*O*-α-L-rhamnoside (in pyridine-d_5)

Position	Compound BRB5		(+)-Lyoniresinol 3α-*O*-α-L-rhamnoside
	^{1}H (*mult.*, *J* in Hz)	^{13}C	^{13}C
Lignan			
1a	2.50 (*dd*, 15.0, 11.9)	33.7 (CH_2)	33.6
1b	2.65 (*dd*, 15.0, 4.6)	-	-
2	1.55 (*m*)	41.0 (CH)	41.3
3	2.00 (*m*)	46.5 (CH)	46.0
4	4.21 (*d*, 5.8)	43.0 (CH)	42.4
5	-	147.5 (C)	147.9
6	-	138.9 (C)	139.4
7	-	148.7 (C)	148.4
8	6.50 (*s*)	107.8 (CH)	107.5
9	-	130.1 (C)	129.7
10	-	126.0 (C)	126.1
1′	-	139.2 (C)	138.6
2′	6.25 (*s*)	106.7 (CH)	107.1
3′	-	149.1 (C)	149.0
4′	-	134.7 (C)	135.7
5′	-	149.1 (C)	149.0
6′	6.25 (*s*)	106.7 (CH)	107.1
2aα	3.38 (*dd*, 10.8, 7.2)	66.3 (CH_2)	65.6
2bα	3.52-3.54 (overlapping)	-	-
3aα	3.23 (*m*)	69.7 (CH_2)	69.7
3bα	3.50-3.55 (overlapping)	-	-
5-OMe	3.15 (*s*)	60.1 (CH_3)	59.8
7-OMe	3.76 (*s*)	56.6 (CH_3)	56.1
3′, 5′-OMe	3.64 (*s*)	56.8 (CH_3)	56.5
Rhamnose			
1″	4.60 (*d*, 1.2)	102.0 (CH)	102.1
2″	3.79 (*dd*, 3.0, 1.8))	72.4 (CH)	72.5
3″	3.60 (*dd*, 9.5, 3.6)	72.6 (CH)	73.1
4″	3.27 (*t*, 9.4)	73.9 (CH)	74.1
5″	3.45 (*dq*, 9.4, 6.1)	70.1 (CH)	70.2
6″	1.19 (*d*, 6.1)	17.9 (CH_3)	18.6

1.16 Structure Determination of Compound BRB6



[**223**]

Compound BRB6 was obtained as a white powder by crystallization from methanol. The $FAB^{+}MS$ spectrum showed molecular fragments ions at *m/z* 341 (Figure 107), corresponding to $C_{15}H_{16}O_9$. The UV spectrum showed absorption bands at 221, 252, 256 and 288 nm (Figure 106).

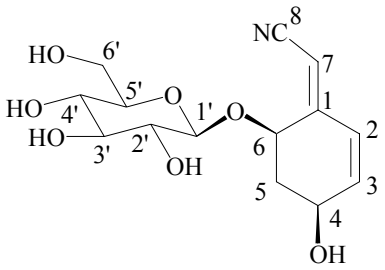
By using the data of ^{1}H and ^{13}C NMR in CD_3OD of compound BRB6 (Figures 108-109 and Table 23), the existence of chromone unit and the glucose unit were established. From ^{13}C NMR spectrum, the sugar moiety showed signals at δ 62.4, 71.2, 74.7, 77.8, 78.4, 101.6 and the chromone unit exhibited signals at δ 96.2, 101.3, 108.4, 112.0, 158.6, 159.5, 163.4, 164.9, 183.6. The mode of glucosidic linkage was determined to be in β-configuration based on the coupling constant of the anomeric proton signal at δ 4.98 (1H, *d, J* = 7.0 Hz).

Based on the comparison of its ^{1}H and ^{13}C NMR data with those reported previously data (Simon *et al*., 1994), compound BRB6 was identified as 5-hydroxychromone-7-β-D-glucoside [**223**]. This compound has been found in *Calluna vulgaris* (Simon *et al*., 1994).

Table 23 NMR spectral data of compound BRB6 and 5-hydroxychromone-7-β-D-glucoside (in CD_3OD)

Position	Compound BRB6		5-Hydroxychromone-7-β-D-glucoside	
	^{1}H (*mult.*, *J* in Hz)	^{13}C	^{1}H (*mult.*, *J* in Hz)	^{13}C
Chromone				
2	7.96 (*d*, 6.1)	158.6	7.94 (*d*, 6.0)	158.0
3	6.19 (*d,* 6.1)	112.0	6.16 (*d*, 6.0)	111.7
4	-	183.6	-	183.4
5	-	163.4	-	163.5
6	6.20 (*d,* 2.1)	101.3	6.17 (*d*, 2.1)	99.9
7	-	164.9	-	165.2
8	6.31 (*d,* 2.1)	96.2	6.30 (*d*, 2.1)	94.7
9	-	159.5	-	159.2
10	-	108.4	-	106.5
Glucose				
1′	4.98 (*d,* 7.0)	101.6	5.00 (*d,* 7.0)	101.6
2′	3.35-3.45 (*m*)	74.7	3.35-3.50 (*m*)	74.7
3′	3.35-3.45 (*m*)	77.8	3.35-3.50 (*m*)	77.9
4′	3.35-3.45 (*m*)	71.2	3.35-3.50 (*m*)	71.2
5′	3.35-3.45 (*m*)	78.4	3.35-3.50 (*m*)	78.4
6′a	3.81 (*dd,* 12.0, 2.0)	62.4	3.87 (*dd,* 12.1, 1.9)	62.4
6′b	3.62 (*dd,* 12.0, 5.5)	-	3.67 (*dd,* 12.1, 5.6)	-

1.17 Structure Determination of Compound BRB7



[**224**]

Compound BRB7, a white powder, showed a typical strong nitrile absorption (C≡N stretching) at 2220 cm^{-1} together with a C=C stretching vibration at 1620 cm^{-1} in the IR spectrum (Figure 111) and also gave an absorption maximum at 258 nm in the UV spectrum (Figure 110), suggesting of the presence of an α, β, γ, δ-unsaturated nitrile group in the molecule (Nakanishi, K. *et al*., 1978). The FAB^+MS afforded the $[M+H]^+$ peak at *m/z* 314 and an intense fragment ion at *m/z* 152 ($[M+H]^+$-162 [glucose unit]), indicating that compound BRB7 is a monoglycoside and carries a glucose as a sugar unit (Figure 112).

The ^1H NMR and ^{13}C NMR of compound BRB7 in CD_3OD (Figures 113-114 and Table 24) differed from those of compound BSB6 at position 5. From ^1H NMR of compound BRB7, the doublet of doublet of doublet signals at δ 1.92 ($J_{5a,5b}$ = 13.7, $J_{5a,6}$ = 7.8 and $J_{5a,4}$ = 6.4 Hz) and 2.16 ($J_{5b,5a}$ = 13.7, $J_{5b,4}$ = 5.0 and $J_{5b,6}$ = 3.1 Hz) were assigned as H-5a and H-5b, respectively. Detailed ^1H NMR and ^{13}C NMR assignments of the compound BRB7 were performed with the aid of the DEPT method and 2D techniques such as the ^1H-^1H COSY, HMQC and HMBC experiments (Figures 115-117) and all protons and carbons were assigned as shown in Table 24. The location of the β–D-glucopyranosyl residue on the aglycone was then determined. The sugar moiety exhibited signals at δ 63.2, 71.8, 74.5, 78.0, 78.2 and 101.6. The observed vicinal coupling constants of J = 7.3 Hz between the trans diaxial oxymethine protons H-1′ and H-2′ suggested that H-1′ were β-anomeric protons. The stereochemistry for this compound was assigned based on the comparison of the optical rotation with reported data (Nakanishi *et al*., 1994).

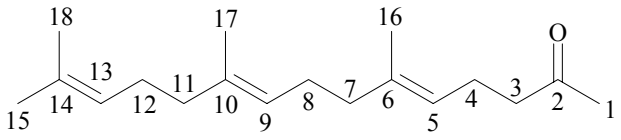
Thus, compound BRB7 was identified as menisdaurin [**224**], a cyanoglucoside previously isolated from *Purshia tridentata* (Nakanishi *et al*., 1994).

Table 24 NMR Spectral data of compound BRB7 and menisdaurin (in CD_3OD)

Position	Compound BRB7		Menisdaurin	
	^1H (*mult.*, *J* in Hz)	$^{13}C^*$	^1H (*mult.*, *J* in Hz)	^{13}C
Aglycone				
1	-	157.1 (C)	-	157.8
2	6.18 (*d*, 10.1)	127.8 (CH)	6.29 (*ddd*, 10.0, 1.5, 0.8)	128.4
3	6.10 (*dd*, 10.1, 3.6)	140.6 (CH)	6.21 (*ddd*, 10.0, 3.5, 0.8)	141.3
4	4.26 (*br s*)	65.4 (CH)	4.36 (*dddd*, 6.3, 5.5, 3.5, 1.5)	66.0
5a	1.92 (*ddd*, 13.7, 7.8, 6.4)	36.1 (CH_2)	2.04 (*ddd*, 13.2, 8.0, 6.3)	36.7
5b	2.16 (*ddd*, 13.7, 5.0, 3.1)	-	2.25 (*ddd*, 13.2, 5.5, 3.5)	-
6	4.82 (*br s*)	72.5 (CH)	4.93 (*ddd*, 8.0, 3.5, 1.3)	73.2
7	5.41 (*br d*)	96.8 (CH)	5.50 (*ddd*, 0.3, 0.8, 1.3)	97.6
8	-	118.0 (C)	-	118.7
Glucose				
1′	4.45 (*d,* 7.3)	101.6 (CH)	4.55 (*ddd*, 8.0, 3.5, 1.3)	102.3
2′	3.29-3.40 (*m*)	74.5 (CH)	3.34 (*dd*, 9.0, 7.5)	75.2
3′	3.29-3.40 (*m*)	78.0 (CH)	3.39 (*dd*, 9.0, 9.0)	78.7
4′	3.29-3.40 (*m*)	71.8 (CH)	3.29 (*dd*, 9.0, 9.0)	72.4
5′	3.29-3.40 (*m*)	78.2 (CH)	3.34 (*ddd*, 9.0, 6.2, 2.2)	78.8
6′a	3.56 (*dd,* 12.3, 6.1)	63.2 (CH)	3.67 (*dd,* 11.8, 6.2)	63.8
6′b	3.79 (*dd,* 12.3, 2.1)	-	3.89 (*dd,* 11.8, 2.2)	-

*Carbon types were deduced from DEPT experiments.

1.18 Structure Determination of Compound CBE1



[**225**]

Compound CBE1 was obtained as a colorless oil. Its molecular formula of $C_{18}H_{30}O$ was deduced from $[M]^+$ ion of the EIMS spectrum at *m/z* 262 (Figure 119) suggesting four degrees of unsaturation. The IR spectrum exhibited characteristic absorption bands at 3019 cm^{-1} (C-H stretching) and 1712 cm^{-1} (C=O stretching) (Figure 118).

The ^1H NMR spectrum in $CDCl_3$ of compound CBE1 (Figure 120 and Table 25) revealed the methyl protons at δ 1.59 (H-17), 1.60 (H-18), 1.62 (H-16), 1.68 (H-15) and 2.14 (H-1), methylene protons at δ 1.96-2.45 and olefinic protons at δ 5.08-5.09.

The ^{13}C NMR spectrum of compound CBE1 in $CDCl_3$ (Figure 121 and Table 25) showed the carbonyl carbon of the ketone at δ 208.8 (C-2) and the olefinic carbons at δ 122.5 (C-5), 124.0 (C-9), 124.4 (C-13), 131.2 (C-14), 134.9 (C-10), and 136.2 (C-6). The carbonyl group could be placed at C-2 according to the HMBC correlations of C-2 with H-1, H-3 (two-bond correlation) and H-4 (three-bond correlation). The olefinic carbons, C-5, C-9 and C-13 showed long-range (^3J) coupling with the methyl protons, H-16, H-17 and H-15, respectively. The types of carbons are classified by the analysis of the DEPT experiment as shown in Table. All protons and carbons were assigned by analysis of the ^1H-^1H COSY, HMQC and HMBC spectra (Figures 122-124).

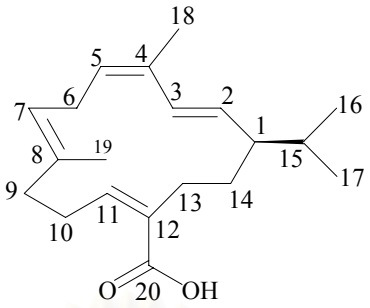
Compound CBE1 was identified as farnesyl acetone [**225**] according to the above spectral data, which as confirmed by comparing them with the previously published data (Ravi *et al*., 1982). This compound was commonly found in plants and also found in the brown alga *Cystophora moniliformis* (Ravi *et al*., 1982).

Table 25 NMR Spectral data of compound CBE1 and farnesyl acetone (in $CDCl_3$)

Position	Compound CBE1		Farnesyl acetone
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{13}C
1	2.14 (*s*)	29.9 (CH_3)	29.8
2	-	208.8 (C)	208.4
3	2.45 (*t*, 7.5)	43.8 (CH_2)	43.7
4	2.26 (*q*, 7.3)	22.5 (CH_2)	22.5
5	5.08 (*m*)	122.5 (CH)	122.5
6	-	136.4 (C)	136.2
7	1.96 (*m*)	39.6 (CH_2)	39.7
8	1.98 (*m*)	26.7 (CH_2)	26.7
9	5.08 (*m*)	124.0 (CH)	124.3
10	-	135.0 (C)	134.9
11	2.07 (*m*)	39.7 (CH_2)	39.7
12	2.06 (*m*)	26.5 (CH_2)	26.5
13	5.09 (*m*)	124.4 (CH)	124.3
14	-	131.2 (C)	131.0
15	1.68 (*s*)	25.7 (CH_3)	25.7
16	1.62 (*s*)	16.0 (CH_3)	16.0
17	1.59 (*s*)	16.0 (CH_3)	16.0
18	1.60 (*s*)	17.7 (CH_3)	17.7

*Carbon types were deduced from DEPT experiments.

1.19 Structure Determination of Compound CBE2



[**163**]

Compound CBE2 was obtained as a colorless oil. Its molecular formula of $C_{20}H_{30}O_2$ was established by EIMS with the $[M]^+$ peak at *m/z* 302, suggesting five degrees of unsaturation (Figure 127). The IR spectrum exhibited characteristic absorption bands at 3445 cm^{-1} (O-H stretching) and 1699 cm^{-1} (C=O stretching) (Figure 126). The UV spectrum exhibited absorption bands at 230 nm (Figure 125).

The ^1H NMR spectrum in $CDCl_3$ of compound CBE2 (Figure 128 and Table 26) showed the presence of two almost overlapped doublets at δ 0.80 (*J* = 6.8 Hz, H-17) and 0.83 (*J* = 6.7 Hz, H-16) together with multiplet at δ 1.48 (H-15) corresponding to non-equivalent methyl protons in an isopropyl group which is probably bonded to an asymmetric carbon. Two singlets of three protons each at δ 1.65 (H-19) and 1.81 (H-18) corresponded to two methyl groups bonded to olefinic carbons. Multiplets between δ 1.35 to 3.08 corresponded to methylene and methine protons. The two mutually coupled trans-olefenic protons at δ 5.21 (*dd*, $J_{2,1}$ = 9.8 and $J_{2,3}$ = 15.6 Hz, H-2) and 6.07 (*d*, *J* = 15.6 Hz, H-3) and three olefenic protons at δ 5.19 (H-7), 5.58 (H-5) and δ 6.05 (H-11) could be detected. The downfield one proton at δ 6.07 (H-11) was assigned to be the β-proton of an α, β-unsaturated carbonyl group (-CH=C-CO).

The ^{13}C NMR spectrum of compound CBE2 in $CDCl_3$ (Figure 129 and Table 26) revealed the presence of 20 carbons consisting of four methyl, five methylene, seven methine and four quarternary carbons from the DEPT experiment. Among the quarternary carbons, one was the carboxyl carbon (C=O). The presence of 20 carbons led to a conclusion that compound CBE2 was a diterpene. Considering the main skeletons of all diterpenes summarized in literatures (Devon and Scott, 1972), only cembrane and abietane-type diterpenes posses an isopropyl group. These two

skeletons were therefore taken into consideration. Further literature reviews showed that several cembrane-type diterpenes were found as constituents in some *Croton* species (Roengsumran *et al*., 1998) and none of the abietane-type diterpenes were reported so far.

The 2D NMR technique, ^{1}H-^{1}H COSY (Figure 130) clearly showed coupling between signals at δ 0.80 (H-17) and 0.83 (H-16) and multiplet of a methine proton at δ 1.48 (H-15). This evidence suggested the presence of an isopropyl group. The methyl signals at δ 1.65 (H-19) and 1.81 (H-18) corresponding to methyl groups bonded to olefinic carbons, gave a correlation to olefinic protons at δ 5.19 (H-7) and 5.58 (H-5), respectively. From the above evidence together with the HMBC and HMQC spectra (Figures 132-133) the presence of the two methyl groups could therefore be placed at C-4 and C-8. In addition to the correlation between signals at δ 6.05 (H-11), which was assigned to the β-proton of an α,β-unsaturated carbonyl moiety, showed cross-peak with multiplet at δ 1.99 (H-13) in HMBC spectral data was observed. The above data led to place double bonds at C-2/ C-3, C-4/ C-5, C-7/ C-8 and C-11/ C-12 in a cembrane skeleton. The trans-double bond was assigned at C-2/ C-3. The double bond located at C-11/ C-12 implying that C-20 of the cembrane skeleton should be a carboxyl group. After the placement of double bond locations, it was therefore possible to assign all correlated protons as shown in Table 26.

The NOESY experiment (Figure 6 and 131) was performed between H-2, H-5/ H-18, H-2/ H-16, H-3/ H-19, H-7/ H-9, H-11/ H-13 and H-14/ H-17 were observed. The NOESY experiment of compound CBE2 was suggested the configuration of all double bonds. The stereochemistry for this compound was established based on the comparing of the optical rotation with the reported data (Sato *et al*., 1991).

The structure of compound CBE2 were proposed to be poilaneic acid [**163**], based on the above spectral evidence and reported data (Sato *et al*., 1991). This compound was previously found in *Croton poilanei* (Sato *et al*., 1991).

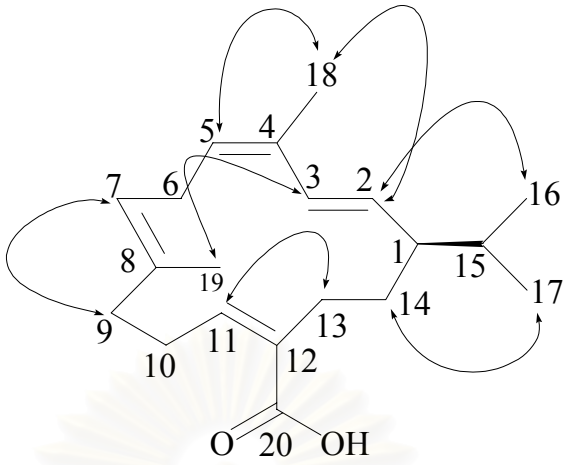


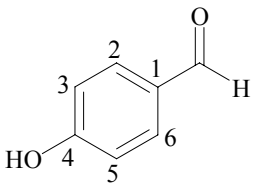
Figure 6 NOESY experiment in compound CBE2

Table 26 NMR Spectral data of compound CBE2 and poilaneic acid (in $CDCl_3$)

Position	Compound CBE2		Poilaneic acid	
	^{1}H (*mult.*, *J* in Hz)	^{13}C*	^{1}H (*mult.*, *J* in Hz)	^{13}C
1	1.73 (*m*)	47.9 (CH)	1.5-2.5 (*m*)	48.0
2	5.21 (*dd*, 9.8, 15.6)	131.3 (CH)	5.21 (*dd*, 9.5, 15.5)	131.3
3	6.07 (*d*, 15.6)	131.0 (CH)	6.05 (*d*, 15.5)	131.0
4	-	135.1 (C)	-	135.2
5	5.58 (*br t*)	125.7 (CH)	5.56 (*dd*, 6.0, 9.5)	125.7
6a	3.08 (*m*)	26.2 (CH_2)	3.05 (*ddd*, 6.0, 9.5, 15.5)	26.3
6b	2.45 (*m*)	-	1.5-2.5 (*m*)	-
7	5.19 (*br d*)	127.9 (CH)	5.21 (*dd*, 6.0, 9.5)	128.0
8	-	131.3 (C)	-	131.3
9a	2.28 (*m*)	38.5 (CH_2)	1.5-2.5 (*m*)	38.6
9b	2.01 (*m*)	-	1.5-2.5 (*m*)	-
10a	2.91 (*m*)	25.8 (CH_2)	1.5-2.5 (*m*)	25.9
10b	2.49 (*m*)	-	1.5-2.5 (*m*)	-
11	6.05 (*dd*, 5.0, 6.7)	147.8 (CH)	6.05 (*dd*, 4.5, 6.5)	147.8
12	-	128.7 (C)	-	128.9
13a	2.52 (*m*)	32.1 (CH_2)	1.5-2.5 (*m*)	32.2
13b	1.99 (*m*)	-	1.5-2.5 (*m*)	-
14a	1.78 (*m*)	29.4 (CH_2)	1.5-2.5 (*m*)	29.5
14b	1.35 (*m*)	-	1.5-2.5 (*m*)	-
15	1.48 (*m*)	32.7 (CH)	1.5-2.5 (*m*)	32.8
16	0.83 (*d*, 6.7)	21.0 (CH_3)	0.83 (*d*, 6.5)	20.9
17	0.80 (*d*, 6.8)	19.3 (CH_3)	0.80 (*d*, 6.5)	19.4
18	1.81 (*br s*)	20.0 (CH_3)	1.82 (*t*, 1.5)	19.9
19	1.65 (*br s*)	14.5 (CH_3)	1.66 (*t*, 1.5)	14.5
20	-	173.1 (C)	-	173.7

*Carbon types were deduced from DEPT experiments.

1.20 Structure Determination of Compound CBE3



[**226**]

Compound CBE3 was a colorless needle with m.p. 195-198°C. Its molecular formula of $C_7H_6O_2$ was established by EIMS which showed the $[M]^+$ peak at *m/z* 122, suggesting five degrees of unsaturation (Figure 136). The IR spectrum exhibited characteristic absorption bands at 3164 cm^{-1} (O-H stretching), 1666 cm^{-1} (C=O stretching), 1597 cm^{-1} (C=C stretching aromatic) (Figure 133). The UV spectrum exhibited absorption bands at 222 and 284 nm (Figure 132).

The ^1H NMR spectrum in $CDCl_3$ of compound CBE3 (Figure 137 and Table 27) showed the protons on the aromatic ring. The protons on the aromatic ring (H-2, H-6 and H-3, H-5) formed a characteristic AA′BB′ pattern at δ 7.82 (*d*, $J_{2,3} = J_{6,5} = 8.7$ Hz, H-2 and H-6) and 6.98 (*d*, $J_{3,2} = J_{5,6} = 8.7$ Hz, H-3 and H-5).

The ^{13}C NMR spectrum of compound CBE3 in $CDCl_3$ (Figure 138 and Table 27) showed signals for seven carbon atoms. The types of carbons are classified by the analysis of the DEPT spectral spectrum as shown in Table 27.

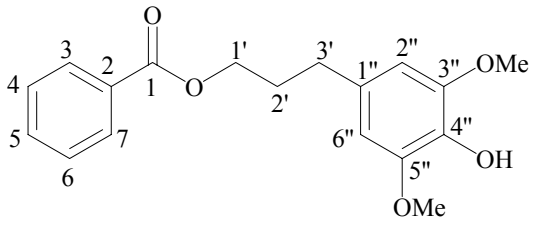
All protons and carbons were assigned by analysis of the ^1H-^1H COSY, HMQC and HMBC spectra (Figures 139-141). Compound CBE3 was identified as the known compound 4-hydroxybenzaldehyde [**226**].

Table 27 NMR Spectral data of compound CBE3 (in $CDCl_3$)

Position	Compound CBE3	
	^{1}H (*mult.*, *J* in Hz)	^{13}C*
1	-	129.2 (C)
2	7.82 (*d*, 8.7)	132.5(CH)
3	6.98 (*d*, 8.7)	115.9 (CH)
4	-	161.6 (C)
5	6.98 (*d*, 8.7)	115.9 (CH)
6	7.82 (*d*, 8.7)	132.5 (CH)
4-OH	9.85 (*s*)	-
C=O	-	191.3

*Carbon types were deduced from DEPT experiments.

1.21 Structure Determination of Compound CBE4



[**227**]

HRFABMS of compound CBE4 suggested a molecular formula of $C_{18}H_{20}O_5$ from its $[M+H]^+$ at *m/z* 317.1395 (calcd for 317.1389) corresponding to nine degrees of unsaturation within the molecule. From the EIMS spectrum, the fragment ion at *m/z* 194 could be formed by the rearrangement of the benzoate group and phenylpropyl moiety (Figure 144 and Sheme 12). The IR spectrum demonstrated the presence of a hydroxyl group at 3446 cm^{-1} (O-H stretching) and a carbonyl group at 1708 cm^{-1} (C=O stretching) (Figure 143). The UV spectral data exhibited absorption bands at 228 and 272 nm (Figure 142).

The ^1H NMR spectrum of compound CBE4 in $CDCl_3$ (Figure 145 and Table 28) showed the presence of two benzene rings which was readily confirmed by analysis of the ^1H-^1H COSY, HMQC and HMBC spectra (Figures 148-150). The ^1H NMR spectral data showed three methylene groups at δ 4.35 (H-1′), 2.09 (H-2′), 2.72 (H-3′) bridged between benzoate and aromatic rings, two methoxyls at δ 3.86 (each 3H, *s*) and one D_2O-exchageable hydroxyl proton at δ 5.40 (*br s*) suggestive of the phenylpropyl benzoate moiety with the substituents of hydroxyl and methoxyl on the other aromatic ring (Figure 146).

In addition, HMBC data for compound CBE4 conclusively demonstrated correlations of methylene protons at H-1′ to C-1 and H-2′ to C-1′′ and H-3′ to C-2′′ and C-1′ respectively. The ^{13}C NMR spectrum of compound CBE4 in $CDCl_3$ (Figure 147 and Table 28) showed the signal of carbonyl ester at δ 166.6. The position of two methoxyl groups (3′′, 5′′-OMe) and one hydroxyl group (4′′-OH) were established employing the HMBC technique as shown in Figure 7. All data are consistent with the structure of compound CBE4 was thus assigned as a new compound, 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate [**227**].

Phenylpropyl benzoates have been previously reported as essential oil in plants such as *Wisteria floribunda* (Ichiro *et al*., 1988). To our knowledge, the work described here is the first report on phenylpropyl benzoates from plants in the genus *Croton*. Moreover, there has been no report on the biological activities of phenylpropyl benzoate.

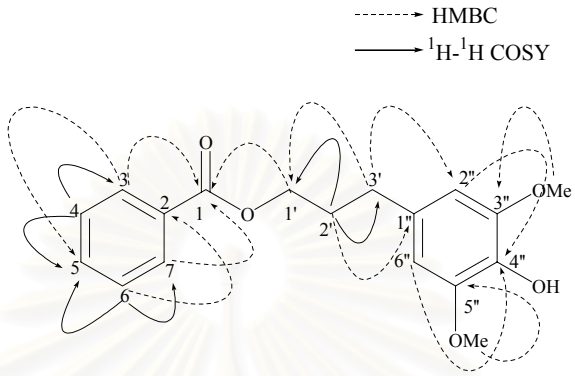
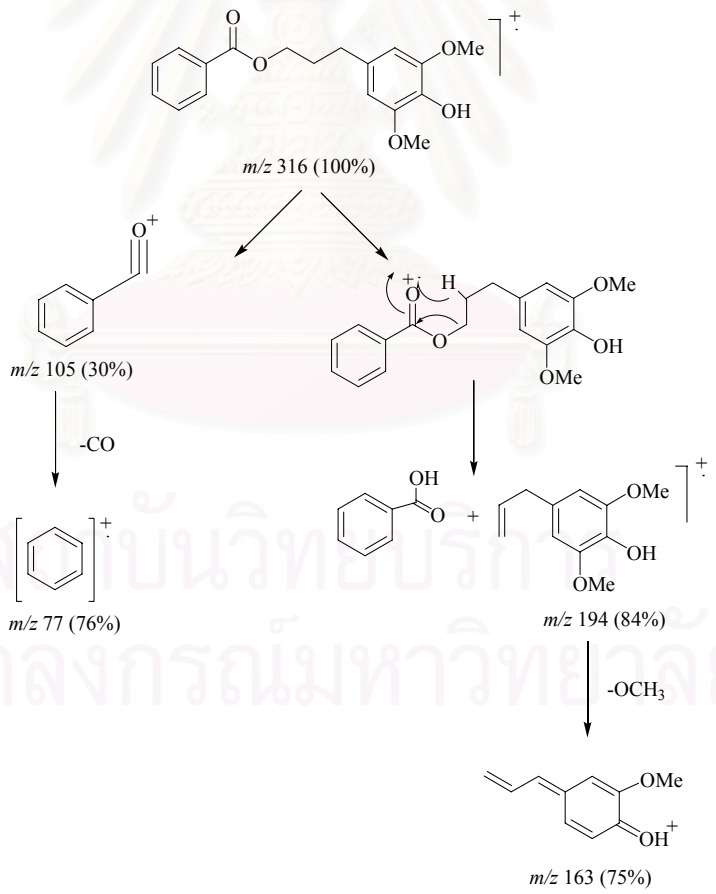


Figure 7 ^{1}H-^{1}H COSY and HMBC correlations of compound CBE4



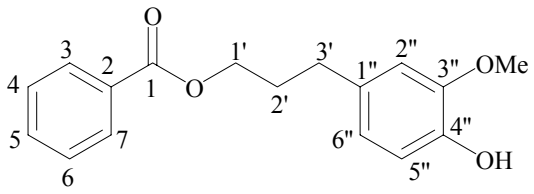
Scheme 12 EIMS Spectra fragmentations of compound CBE4

Table 28 NMR Spectral data of compound CBE4 (in $CDCl_3$)

Position	^{1}H (*mult*., *J* in Hz)	^{13}C*
Benzoate		
1	-	166.6
2	-	130.3
3	8.05 (*dd*, 8.4, 1.3)	129.5
4	7.45 (*tt*, 7.4, 1.7)	128.3
5	7.57 (*tt*, 7.4, 1.3)	132.9
6	7.45 (*tt*, 7.4, 1.7)	128.3
7	8.05 (*dd*, 8.4, 1.3)	129.5
Propyl		
1′	4.35 (*t*, 6.5)	64.3
2′	2.09 (*m*)	30.5
3′	2.72 (*br t*)	32.5
Phenyl		
1″	-	132.3
2″	6.43 (*s*)	104.9
3″	-	146.9
4″	-	132.9
5″	-	146.9
6″	6.43 (*s*)	104.9
3″-OMe	3.86 (*s*)	56.2
4″-OH	5.40 (*s*)	-
5″-OMe	3.86 (*s*)	56.2

*Carbon types were deduced from DEPT experiments.

1.22 Structure Determination of Compound CBE5



[**228**]

Compound CBE5 was given the formula $C_{17}H_{18}O_4$ from its $[M+H]^+$ at *m/z* 287.1289 (calcd for 287.1284) in the HRFABMS. From the EIMS spectrum, the fragment ion at *m/z* 164 could be formed by the rearrangement of the benzoate group and phenylpropyl moiety (Figure 153 and Scheme 13). The IR spectrum exhibited characteristic absorption bands at 3428 cm^{-1} (O-H stretching), 1718 cm^{-1} (C=O stretching) (Figure 152). The UV spectrum revealed the absorption bands at 228 and 272 nm (Figure 151).

The ^1H and ^{13}C NMR spectrum of compound CBE5 in $CDCl_3$ (Figures 154-155 and Table 29) were in good agreement with those of compound CBE4 except for the absence of the signal of one of the methoxyl groups. The ^1H NMR spectral data of CBE5 showed peaks at δ 6.71 (1H, *d*, *J* = 1.3 Hz, H-2′′), δ 6.84 (1H, *d*, *J* = 8.3 Hz, H-5′′) and δ 6.70 (1H, *dd*, *J* = 8.3, 1.3 Hz, H-6′′) ascribable to the three protons in an ABX system of the 3′-phenyl- 3′′, 4′′-disubstituted ring system. On the basis of the above spectroscopic data together with the 2D NMR such as the ^1H-^1H COSY, HMQC and HMBC spectra (Figures156-158 and 8), compound CBE5 was identified as 3′-(4′′-hydroxy-3′′-methoxyphenyl)-propyl benzoate or trivially known as dihydroconiferyl benzoate [**228**]. This compound has already been isolated from the flower of *Gardenia taitensis* DC (Lafontaine, Raharivelomanana and Bianchini, 1991), however no NMR spectral data have been reported.

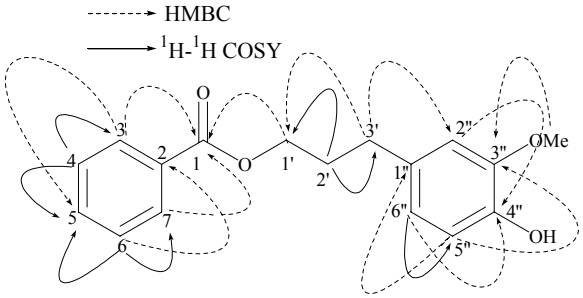
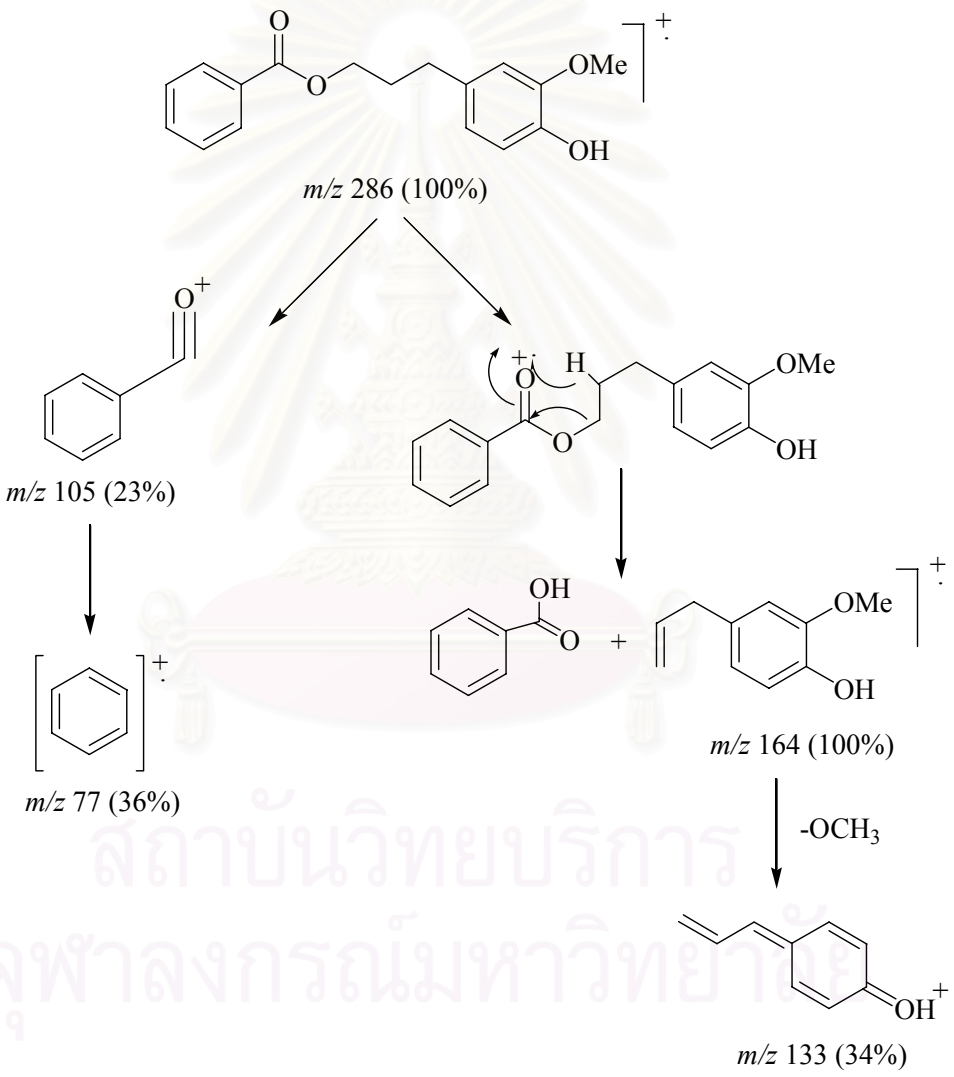


Figure 8 ^{1}H-^{1}H COSY and HMBC correlations of compound CBE5



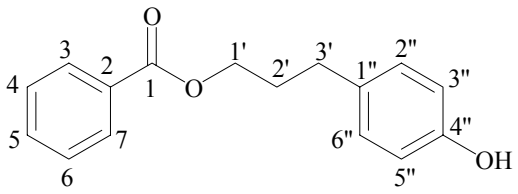
Scheme 13 EIMS Spectra fragmentations of compound CBE5

Table 29 NMR Spectral data of compound CBE5 (in $CDCl_3$)

Position	^{1}H (*mult.*, *J* in Hz)	^{13}C*
Benzoate		
1	-	166.6
2	-	130.3
3	8.04 (*dd*, 8.4, 1.3)	129.5
4	7.44 (*tt*, 7.4, 1.7)	128.3
5	7.56 (*tt*, 7.4, 1.3)	132.9
6	7.44 (*tt*, 7.4, 1.7)	128.3
7	8.04 (*dd*, 8.4, 1.3)	129.5
Propyl		
1′	4.34 (*t*, 6.5)	64.2
2′	2.07 (*m*)	30.5
3′	2.72 (*br t*)	31.9
Phenyl		
1″	-	133.0
2″	6.71 (*d*, 1.3)	120.9
3″	-	146.4
4″	-	143.8
5″	6.84 (*d*, 8.3)	114.3
6″	6.70 (*dd*, 8.3, 1.3)	110.9
3″-OMe	3.84 (*s*)	55.8
4″-OH	5.59 (*br s*)	-

*Carbon types were deduced from DEPT experiments.

1.23 Structure Determination of Compound CBE6



[**229**]

Compound CBE6 had a molecular formula of $C_{16}H_{16}O_3$ from its $[M+H]^+$ at *m/z* 257.1178 (calcd for 257.1179) based on HRFABMS. From the EIMS spectrum, the fragment ion at *m/z* 134 was formed by the rearrangement of the benzoate group and phenylpropyl moiety (Figure 161 and Scheme 14). The UV absorptions exhibited the absorption bands at 228 and 279 nm (Figure 159). The IR spectrum revealed the absorption bands at 3377 cm^{-1} (O-H stretching) and carbonyl group at 1698 cm^{-1} (C=O stretching) (Figure 160).

The ^1H and ^{13}C NMR data of compound CBE6 in $CDCl_3$ (Figure 162-163 and Table 30) was similar to those of compound CBE6 except for the absence of two methoxyl groups at C-5″ and C-3″. The ^1H NMR spectral data of compound CBE6 showed the presence of the *para*-substituted benzene ring at δ 7.08 (2H, *d*, *J* = 8.4 Hz, H-2″ and H-6″) and δ 6.77 (2H, *d*, *J* = 8.4 Hz, H-3″ and H-5″). The ^1H-^1H COSY, HMQC and HMBC experiment on CBE6 (Figures 164-166 and 9) also produced very similar results, indicating that compound CBE4, CBE5 and CBE6 were closely related. Based on the above spectral evidence, compound CBE6 was identified as a new compound and has been named 3′-(4″-hydroxyphenyl)-propyl benzoate [**229**].

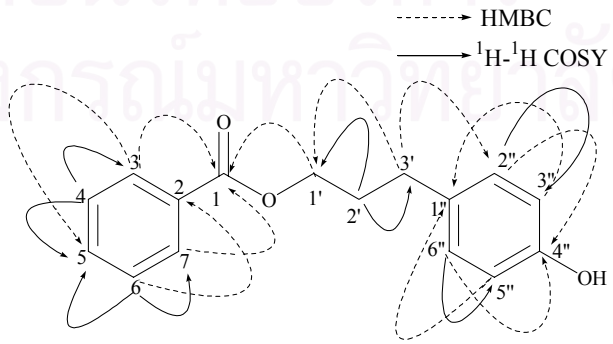
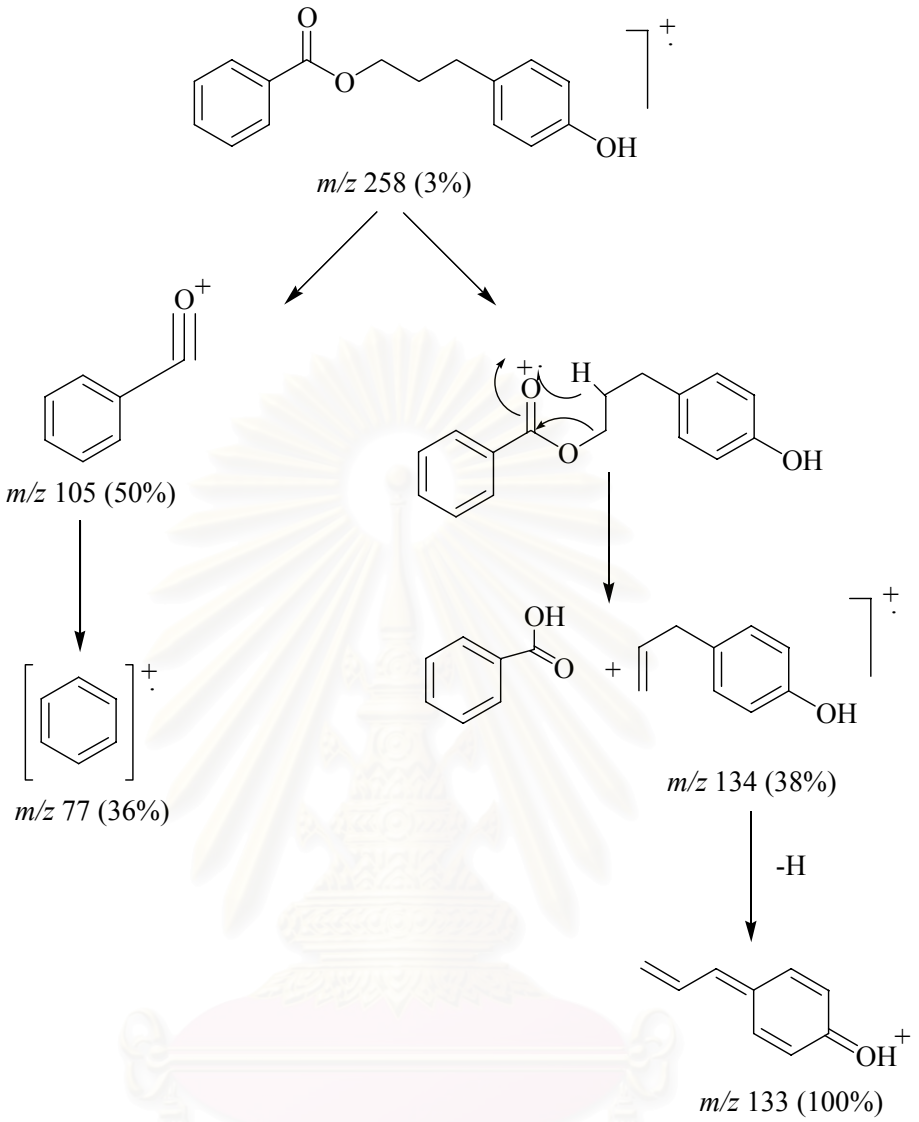


Figure 9 ^1H-^1H COSY and HMBC correlations of compound CBE6



Scheme 14 EIMS Spectra fragmentations of compound CBE6

Table 30 NMR Spectral data of compound CBE6 (in $CDCl_3$)

Position	^{1}H (*mult.*, *J* in Hz)	^{13}C*
Benzoate		
1	-	166.8
2	-	130.3
3	8.04 (*dd*, 8.6, 1.1)	129.5
4	7.45 (*tt*, 7.5, 1.6)	128.4
5	7.57 (*tt*, 7.5, 1.1)	132.9
6	7.45 (*tt*, 7.5, 1.6)	128.4
7	8.04 (*dd*, 8.6, 1.1)	129.5
Propyl		
1′	4.33 (*t*, 6.5)	64.3
2′	2.07 (*m*)	30.5
3′	2.72 (*br t*)	31.3
Phenyl		
1″	-	133.2
2″	7.08 (*d*, 8.4)	129.5
3″	6.77 (*d*, 8.4)	115.3
4″	-	153.9
5″	6.77 (*d*, 8.4)	115.3
6″	7.08 (*d*, 8.4)	129.5
4″-OH	5.59 (*br s*)	-

*Carbon types were deduced from DEPT experiments.

2. Biological Activities of Compounds from *Bauhinia sirindhorniae*

2.1 Antimicrobial Activity

The crude extracts obtained from *Bauhinia sirindhorniae* were examined for this activity against *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231 and *Trichophyton mentagrophytes* (clinical isolated). It was found that some crude extracts possessed antibacterial activities. The 95% ethanol extracts of stems and roots showed activities against *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 29213. The 95% ethanol extracts of the stems showed inhibition zone diameters of 18.55 and 15.55 mm, respectively and the same extracts of the roots showed inhibition zone diameters of 16.05 and 13.60 mm, respectively. Isoliquiritigenin [**14**], (+)-isolariciresinol-3α-*O*-α-L-rhamnoside [**215**], trimethoxyphenolic-1-*O*-β-D-glucoside [**216**], lithospermoside [**54**], (2*S*)-naringenin [**17**], luteolin [**220**], (2*S*)-eriodictyol [**16**], (+)-lyoniresinol-3α-*O*-α-L-rhamnoside [**222**] and menisdaurin [**224**] from the extracts of the roots and stems were subjected to determination of the MIC and MBC. Studies of antibacterial activity of the isolated compounds are shown in the Table 31. It was found that (2*S*)-eriodictyol [**16**] and isoliquiritigenin [**14**] showed the activity against *B. subtilis*. Anti-*S. aureus* activity of both compounds was found to be equal in term of MIC at 200 μg/ml. (2*S*)-Naringenin [**17**] and luteolin [**220**] exhibited activity against *B. subtilis*.

Table 31 Antibacterial activity of some isolated compounds from *Bauhinia sirindhorniae*

Compound	*S. aureus* ATCC 29213		*B. subtilis* ATCC 6633	
	MIC	MBC	MIC	MBC
[**14**]	200	>200	100	100
[**215**]	NA	NA	NA	NA
[**216**]	NA	NA	NA	NA
[**54**]	NA	NA	NA	NA
[**17**]	NA	NA	100	>200
[**220**]	NA	NA	200	200
[**16**]	200	200	50	>200
[**222**]	NA	NA	NA	NA
[**224**]	NA	NA	NA	NA
Penicillin G	0.0625	0.0625	0.031	0.031

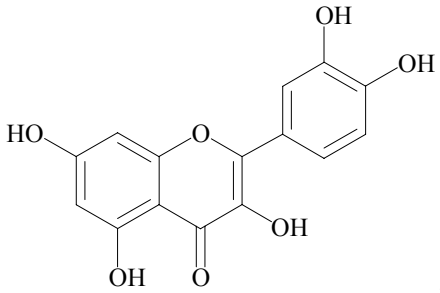
MIC: Minimum Inhibitory Concentration (μg/ml)

MBC: Minimum Bactericidal Concentration (μg/ml)

NA: No Activity

2.2 Free Radical Scavenging Activity

By TLC screening assay, the 95% ethanol extracts from stems and roots of *Bauhinia sirindhorniae* showed free radical scavenging activity. The free radical scavenging activity was evaluated as IC_{50} and Trolox equivalent antioxidant capacity (TEAC) for some isolated compounds from *B. sirindhorniae* that have not been reported before. (+)-Isolariciresinol-3α-*O*-α-L-rhamnoside [**215**], (+)-lyoniresinol-3α-*O*-α-L-rhamnoside [**222**], lithospermoside [**54**] and menisdaurin [**224**] were subjected for this activity**.** The cyanoglucosides, lithospermoside [**54**] and menisdaurin [**224**] showed very weak free radical scavenging activity and thus the TEAC value and IC_{50} values could not be determined. The lignan glycosides, (+)-isolariciresinol-3α-*O*-α-L-rhamnoside [**215**] and (+)-lyoniresinol-3α-*O*-α-L-rhamnoside [**222**] showed moderate activity in comparison with quercetin as a positive control, as shown in Table 32.



Quercetin

Table 32 The DPPH radical scavenging activity of compounds [222] and [215]

Compound	TEAC*	IC_{50} (μM)
[**222**]	0.95	67
[**215**]	0.99	76
Quercetin	1.91	17

*TEAC: Trolox Equivalent Antioxidant Capacity

3. Biological Activities of Compounds from *Croton hutchinsonianus*

3.1 Cytotoxic Activity

Preliminary bioactivity screening revealed that *Croton hutchinsonianus* exhibited cytotoxic activity. The results are summarized in Table 33.

Table 33 The cytotoxic activity against NCI H-187 cell lines of the crude extracts of *Croton hutchinsonianus*

Crude extract	Activity	IC_{50} (μg/ml)
The hexane leaves extract	Moderately active	5.8
The ethyl acetate leaves extract	Moderately active	8.6
The 95% ethanol leaves extract	Inactive	-
The hexane branches extract	Strongly active	1.1
The ethyl acetate branches extract	Weakly active	13.8
The 95% ethanol branches extract	Inactive	-

The compounds investigated for cytotoxic activity were 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate [**227**], dihydroconiferyl benzoate [**228**] and 3′-

(4″-hydroxyphenyl)-propyl benzoate [**229**] , all of which were isolated from *C. hutchinsonianus*.

3′-(4″-Hydroxy-3″,5″-dimethoxyphenyl)-propyl benzoate [**227**] displayed weak cytotoxic activity with the IC_{50} of 11.38 μg/ml while dihydroconiferyl benzoate [**228**] and 3′-(4″-hydroxyphenyl)-propyl benzoate [**229**] were inactive.

3.2 Antifungal Activity

3′-(4″-Hydroxy-3″,5″-dimethoxyphenyl)-propyl benzoate [**227**], dihydro coniferylbenzoate [**228**] and 3′-(4″-hydroxyphenyl)-propyl benzoate [**229**] isolated from *C. hutchinsonianus*, were subjected to biological evaluation for antifungal activity against *Candida albicans*.

3′-(4″-Hydroxy-3″,5″-dimethoxyphenyl)-propyl benzoate [**227**], dihydro coniferyl benzoate [**228**] and 3′-(4″-hydroxyphenyl)-propyl benzoate [**229**] showed moderate antifungal activity with the IC_{50} of 12.43, 7.48 and 5.35 μg/ml, respectively. It should be noted that phenylpropyl benzoate displayed antifungal activity against *C. albicans*.

CHAPTER V

CONCLUSION

The present investigation deals with the isolation of several biogenetically related compounds from the stems and roots of *Bauhinia sirindhorniae* K. & S.S. Larsen. Two cyanoglucosides (lithospermoside [**54**] and menisdaurin [**224**]), one flavan ((-)-epicatechin [**217**]), two flavanones ((2*S*)-naringenin [**17**] and (2*S*)-eriodictyol [**16**]), one flavanonol ((+)-taxifolin [**221**]), one flavone (luteolin [**220**]), one chalcone (isoliquiritigenin [**14**]), one chromone (5,7-dihydroxychromone [**219**]), one chromone glucoside (5-hydroxychromone 7-β-D-glucoside [**223**]), two lignan glycosides ((+)-isolariciresinol-3α-*O*-α-L-rhamnoside [**215**] and (+)-lyoniresinol-3α-*O*-α-L-rhamnoside [**222**]), two triterpenoids (lupeol [**77**] and glutinol [**214**]), one steroid glucoside (sitosteryl-3-*O*-β-D-glucoside [**37**]) and other phenolic compounds (3,4,5-trimethoxyphenolic-1-*O*-β-D-glucoside [**216**] and protocatechuic acid [**218**]) were isolated. Scavenging activity of some isolated compounds from *B. sirindhorniae* towards DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was also described. The lignan glycosides ((+)-isolariciresinol-3α-*O*-α-L-rhamnoside [**215**] and (+)-lyoniresinol-3α-*O*-α-L-rhamnoside [**222**]) showed moderate activity in comparison with quercetin as a positive control. (2*S*)-Eriodictyol [**16**] and isoliquiritigenin [**14**] showed activity against *Bacillus subtilis* and *Staphylococcus aureus* whereas (2*S*)-naringenin [**17**] and luteolin [**220**] exhibited activity against *Bacillus subtilis*. Chemical examination of the branches and leaves of *Croton hutchinsonianus* Hosseus led to the isolation of two new compounds 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate [**227**] and 3′-(4′′-hydroxyphenyl)-propyl benzoate [**229**] and other four known compounds, namely farnesyl acetone [**225**], poilaneic acid [**163**], 4-hydroxybenzaldehyde [**226**] and dihydroconiferylbenzoate [**228**]. The isolated compounds from *C. hutchinsonianus* were subjected for biological activities evaluation, involving antifungal activity and cytotoxicity. 3′-(4′′-Hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate [**227**], dihydroconiferyl benzoate [**228**] and 3′-(4′′-hydroxyphenyl)-propyl benzoate [**229**] revealed moderate antifungal activity against *Candida albicans.* In addition, 3′-(4′′-hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate [**227**] showed weak cytotoxic activity against

NCI-H187 cell line while dihydroconiferylbenzoate [**228**] and 3′-(4′′-hydroxyphenyl)-propyl benzoate [**229**] were inactive.

Table 34 Compounds isolated from chloroform extract of the stems of *Bauhinia sirindhorniae*

Compound	Antibacterial Activity	Free Radical Scavenging Activity
Triterpenes		
Lupeol [**77**]	ND	ND
Glutinol [**214**]	ND	ND

ND: Not Determined

Table 35 Compounds isolated from butanol extract of the stems of *Bauhinia sirindhorniae*

Compound	Antibacterial Activity	Free Radical Scavenging Activity
Chalcone		
Isoliquiritigenin [**14**]	Active	ND
Lignan Glycoside		
(+)-Isolariciresinol-3α-*O*-α-L-rhamnoside [**215**]	Inactive	Active
Flavan		
((-)-Epicatechin [**217**]	ND	ND
Phenolic Compounds		
3,4,5-Trimethoxyphenolic-1-*O*-β-D-glucoside [**216**]	ND	Inactive
Protocatechuic acid [**218**]	ND	ND
Cyanoglucoside		
Lithospermoside [**54**]	Inactive	Inactive

Table 36 Compounds isolated from chloroform extract of the roots of *Bauhinia sirindhorniae*

Compound	Antibacterial Activity	Free Radical Scavenging Activity
Chromone		
5,7-Dihydroxychromone [**219**]	ND	ND
Steroid Glycoside		
Sitosteryl-3-*O*-β-D-glucoside [**37**]	ND	ND

Table 37 Compounds isolated from butanol extract of the roots of *Bauhinia sirindhorniae*

Compound	Antibacterial Activity	Free Radical Scavenging Activity
Flavone		
Luteolin [**220**]	Active	ND
Flavanones		
(2*S*)-Naringenin [**17**]	Active	ND
(2*S*)-Eriodictyol [**16**]	Active	ND
Flavanonol		
(+)-Taxifolin [**221**]	ND	ND
Lignan Glycoside		
(+)-Lyoniresinol-3α-*O*-α-L-rhamnoside [**222**]	Inactive	Active
Chromone Glucoside		
5-Hydroxychromone 7-β-D-glucoside [**223**]	ND	ND
Cyanoglucoside		
Menisdaurin [**224**]	Inactive	Inactive

Table 38 Compounds isolated from ethyl acetate extract of *Croton hutchinsonianus*

Compound	Cytotoxic Against NCI H-187	Antifungal Against *Candida albicans*
C_{18} Terpenoid		
Farnesyl acetone [**225**]	ND	ND
Diterpene		
Poilaneic acid [**163**]	ND	ND
Benzaldehyde		
4-Hydroxybenzaldehyde [**226**]	ND	ND
Phenylpropyl Benzoates		
3′-(4′′-Hydroxy-3′′,5′′-dimethoxyphenyl)-propyl benzoate [**227**]	Weakly Active	Moderately Active
Dihydroconiferyl benzoate [**228**]	Inactive	Moderately Active
3′-(4′′-Hydroxyphenyl)-propyl benzoate [**229**]	Inactive	Moderately Active

REFERENCES

Aboagye, F. A., Sam, G. H., Massiot, G. and Lavaud, C. 2000. Julocrotine, a glutarimide alkaloid from *Croton membranaceus*. Fitoterapia. 71: 461-462.

Achenbach, H., Benirschike, G. and Torrenegra, R. 1997. Alkaloids and other compounds from seeds of *Tabernaemontana cymosa*. Phytochemistry 45: 325-335.

Achenbach, H., Stocker, M. and Constenla, M. A. 1988. Flavonoids and other constituents of *Bauhinia manca*. Phytochemistry 27: 1835-1841.

Agrawal, P. K. 1989. Carbon-13 NMR of flavonoids. Amsterdam, Oxford, New York, Tokyo: Elsevier.

Aiyar, V. N. and Seshadri, T. R. 1970. Chemical Components of *Croton oblongifolius*-III constitution of oblongifolic acid. Tetrahedron 26: 5275-5279.

Aiyar, V. N. and Seshadri, T. R. 1972. Components of *Croton oblongifolius* Part VIII. Curr. Sci. 41: 839-840.

Alvarenga, M. A. D., Gottlieb, H. E., Cottlieb, O. R. Magalhaes, M. T. and Silva, V. O. D. 1978. Diasin, a diterpene from *Croton diasii*. Phytochemistry 17: 1773-1776.

Amaral, A. C. F. and Barnes, R. A. 1998. A tetrahydroprotoberberine alkaloid from *Croton hemiargyreus*. Phytochemistry 47: 1445-1447.

Anjaneyulu, A. S. R., Reddy, A. V. R. and Reddy, D. S. K. 1984. Pacharin : A new dibenzo (2, 3-6, 7) oxepin derivative from *Bauhinia racemosa* Lamk. Tetrahedron 40: 4245-4252.

Anjaneyulu, A. S. R., Reddy, A. V. R. and Reddy, D. S. K. 1986. Racemosol: A novel tetracyclic phenol from *Bauhinia racemosa* Lamk. Tetrahedron 42: 2417-2420.

Babili, F. E., Moulis, C., Bon, M., Respaud, M. J. and Fouraste, I. Three furano-diterpenes from the bark of *Croton campestris*. Phytochemistry 48: 165-169.

Balza F and Tower G. H. N. 1984. Dihydroflavonols of *Artemisia dracunculus*. Phytochemistry 23: 2333-37.

Bandara, B. M. R., Wimalasiri, W. R. and Bandara, K. N. P. 1987. Isolation and insecticidal activity of (-)-hardwickiic acid from *Croton aromaticus*. Planta Med. 53: 575.

Bandara, B. M. R., Wimalasiri, W. R. and Macleod, J. K. 1988. ent-Kauranes and oleananes from *Croton lacciferus*. Phytochemistry 27: 869-871.

Block, S., Baccelli, C., Tinant, B., Meervelt., L.V., Rozenberg, R., Jiwan, J. L. H., Llabres, G., Pauw-Gillet, M. C. D. and Quetin-Leclercq, J. 2004. Diterpenes from the leaves of *Croton Zambesicus*. Phytochemistry 65: 1165-1171.

Boonyarathanakornkit, L., Che, C. T., Fong, H. S. and Farnswoth, N. R. 1988. Constituents of *Croton crassifolius* roots. Planta Med. 54: 61-63.

Braca, A., Tommasi, N. D., Bari, L. D., Pizza, C., Politi, M. and Morelli, I. 2001. Antioxidant principles from *Bauhinia tarapotensis*. J. Nat. Prod. 64: 892-895.

Brand-Williams W., Cuvelier, M. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm.-Wiss. U.-technol. 28: 25-30.

Buckingham, J. 2001. Dictionary of Natural Products [Computer software]. Chapman & Hall/ CRC netbase.

Burke, B. A., Chan, W. R. and Pascoe, K. O. 1979. The structure of crotofolin E, A novel tricyclic diterpene from *Croton corylifolius* Lam. Tetrahedron 32: 1881-1884.

Burke, B. A., Chan, W. R. and Pascoe, K. O. 1981. The structure of crotonitenone, a novel casbane diterpene from *Croton nitens* Sw. (Euphorbiaceae). J. Chem. Soc. Perkin I 25: 2666-2669.

Caceres, A., Jauregui, E., Herrera, D. and Logemann. 1991. Plants used in Guatemala for the treatment of dermatomucosal infections. 1: screening of 38 plants extracts for anticandidal activity. J. Ethnopharmacol. 33: 277-283.

Carvalho, L. and Seita, J. 1993. ^{13}C NMR assignments of D:B-Friedolean-5-en-3β-ol. Nat. Prod. Lett. 2: 57-60.

Chan, W. R., Taylor, D. R. and Willis, C. R. 1968. Terpenoids from Euphorbiaceae. Part I. The structure of crotonin, a norditerpene from Croton lucidus L. J. Chem. Soc. (C) 12: 2781-2785.

Chaoming, L., Shuguang, W., Guoda, T and Handong, S. 1990. Chemical constituents from the stem bark of *Croton hutchinsonianus*. Yunnan Zhiwu Yanjiu. 12: 457-459.

Chatterjee, M. A. and Banerjee, A. 1977. Crotocaudin; a rearranged labdane type norditerpene from *Croton caudatus* Geisel. Tetrahedron 33: 2407-2414.

Chen, C. C., Chen, Y. P. and Hsu, H. Y. 1985. Bauhinin, a new nitrile glucoside from *Bauhinia championii*. J. Nat. Prod. 48: 933-937.

Chen, Z. P., Cai, Y. and Phillipson, J. D. 1994. Studies on the anti-tumour, anti-bacterial and wound-healing properties of dragon's blood. Planta Med. 60: 541-545.

Craveiro, A. A. and Silveira, E. R. 1982. Two cleistanthane type diterpenes from *Croton sonderianus*. Phytochemistry 21: 2571-2574.

Craveiro, A. A., Rodrigues, A. S., Andrade, C. H. S., Matos, F. J. A., Alencar, J. W. and Machado, M. I. L. 1981a. Volatile constituents of Brazilian Euphorbiaceae. Genus *Croton*. J. Nat. Prod. 44: 602-608.

Craveiro, A. A., Silveira, E. R., Filho, R. B. and Mascarenhas, I. P. 1981b. Sonderianin, a furanoid ditepene from *Croton sonderianus*. Phytochemistry 20, 4 : 852-854

Dennis, M., Rudolph, W., Memory, E. L. and Walter, L. 2002. Geographic distribution of three alkaloid chemotypes of *Croton lechleri*. J. Nat. Prod. 65: 814-819.

Devon, T. K. and Scott, A. L. 1972. Handbook of naturally occurring compounds: New York and London: Academic press.

Farias, R. A. F., Rao, V. S. N., Viana, G. S. B., Silveira, E. R., Maciel, M. A. M. and Pinto, A. C. 1997. Hypoglycemic effect of *trans*- dehydrocrotonin, a nor-clerodane diterpene from *Croton cajucara*. Planta Med. 63: 558-560.

Filho, A. W., Breviglieri, E., Filho, V. C. and Santos, A. R. S. 1997. Antinociceptive effect of the Hydroalcoholic extract of *Bauhinia splendens* stems in mice. J. Pharm. Pharmacol. 49: 823-827.

Fort, D. M., Jolad, S. D. and Nelson, S. T. 2001. Lithospermoside from *Bauhinia fassoglensis* (Fabaceae). Biochem. Syst. Ecol. 29: 439-441.

Fuchino, H., Satoh, T. and Tanaka, N. 1995. Chemical evaluation of *Betula* Species in Japan. I. Constituents of *Betula ermanii*. Chem. Pharm. Bull. 43: 1937-1942.

Gaind, K. N., Singla, A. K., Boar, R. G. and Copsey, D. B. 1976. Triterpenoids and sterols of *Kalanchoe spathulata*. Phytochemistry 15: 1999-2000.

Gómez, F., Quijano, L., Calderon, J. S., Rodriquez, C. and Rios, T. 1985. Prenylflavans from *Tephrosia watsoniana*. Phytochemistry 24: 1057-1059.

Gupta, A. K., Vidyapati, T. J. and Chauhan, J. S. 1980. Chemical examination of the stem of *Bauhinia variegata.* Planta Med. 38: 174-176.

Harborne, J. B. and Mabry, T. J. 1982. The Flavonoids: Advances in Research. London, New York: Chapman and Hall.

Hawser, S. P., Norris, H., Jessup, C. J. and Ghannoum, M. A. 1998. Comparison of a 2,3-Bis(2-Methoxy-4-Nitro-5-Sulfophenyl)-5-[(Phenyl-amino) Carbonyl]-2H-Tetrazolium Hydroxide (XTT) Colorimetric Method with the Standardized National Committee for Clinical Laboratory Standards Method of Testing Clinical Yeast Isolates for Susceptibility to Antifungal Agents. J. Clin. Microbiol. 36: 1450-1452.

Herlem, D., Huu, F. K. and Kende, A. S. 1993. Total synthesis of crotomachlin. Tetrahedron Lett. 34: 5587-5590.

Hostettler, F. D. and Seikel, M. K. 1969. Lignans of *Ulmus thomasii* heartwood-II. Tetrahedron 25: 2325-2337.

Hsieh, Y. L., Fang, J. M., and Cheng, Y. S. 1998. Terpenoids and flavonoids from *Pseudotsuga wilsoniana*. Phytochemistry 47: 845-850.

Hui, W. H. and Fung, M. L. 1969. An examination of Euphorbiaceae of Hong Kong. *Glochidion wrightii* Benth. J. Chem. Soc. (C). 13: 1710-12.

Hui, W. H. and Lee, W. K. 1971. An examination of the Euphorbiaceae of Hong Kong. Part VIII. Lup-20(29)-ene-3α,23-diol, A new triterpene from *Glochidion macrophyllum* Benth. J. Chem. Soc. (C). 5: 1004-06.

Ichihara, Y., Takeya, K., Hitotsuyanagi, Y., Morita, H., Okuyama, S., Suganuma, M., Fujiki, H., Motidome, M. and Itokawa, H. 1991. Cajucarinolide and isocajuicarinolide: anti-inflammatory diterpenes from *Croton cajucara*. Planta Med. 58: 549-551.

Ichiro, W., Tetsuya, Y., Kenichi, A., Kunio, K. and Kazuo, H. 1988. Volatile components of *Wisteria* flower. Developments in Food Science. 18: 425-437.

Ingroff, A. E., White, T. and Pfaller, M. A. 1999. Antifungal agents and susceptibility tests. In Baron, E. J. (ed) Manual of Clinical Microbiology. 7th ed.. pp. 1643-1650. Washington, D.C.: ASM Press.

Iribarren, A. M. and Pomilio, A. B. 1983. Components of *Bauhinia candidans.* J. Nat. prod. 46: 752-753.

Iribarren, A. M. and Pomilio, A. B. 1985. Sitosterol 3-*O*-α-D-riburonofuranoside from *Bauhinia candidans.* Phytochemistry 24: 360-361.

Iribarren, A. M. and Pomilio, A. B. 1989. Steroidal glycosides, flavonoids and other components of *Bauhinia uruguayensis*. An. Asoc. Quim. Argent. 77: 461-466.

Itokawa, H., Ichihara, Y., Kojima, H., Watanabe, K. and Takeya, K. 1989. *Nor*-clerodane diterpenes from *Croton cajucara*, and their conformations. Phytochemistry 28: 1667-1669.

Itokawa, H., Ichihara, Y., Shimizu, M., Takeya, K. and Motidome, M. 1990. Cajucarins A and B, new clerodane diterpenes from *Croton cajucara*, and their conformations. Chem. Pharm. Bull. 38: 701-705.

Itokawa, H., Ichihara, Y., Takeya, K., Morita, H. and Motidome, M. 1991. Diterpenes from Croton salutaris. Phytochemistry 30: 4071-4073.

Jas, E. T. and Hahn, L. R. 1978. Nivenolide, a diterpene lactone from *Croton niveus*. Phytochemistry 19: 574-575.

Jogia, M. K. and Anderson, R. J. 1989. Crotofolane diterpenoids from the African shrub *Croton dichogamus* Pax. J. Org. Chem. 54: 1654-1657.

Jorgensen, J.H., Turnidge, J.D. and Washington, J.A. 1999. Antibacterial susceptibility tests: dilution and disk diffusion methods. In Baron, E. J. (ed) Manual of Clinical Microbiology. 7th ed.. pp. 1534-1535. Washington, D.C.: ASM Press.

Kaewamatawong, R. 2002. Free radical scavenging compounds from *Ochna integerrima*. Ph.D.'s Thesis, Pharmaceutical Chemistry and Natural products, Graduate School, Chulalongkorn University.

Kapingu, M. C., Guillaume, D., Mbwambo, Z. H., Moshi, M., Uliso, F. C. and Mahunnah, R. L. A. 2000. Diterpenoids from the roots of *Croton macrostachys*. Phytochemistry 54: 767-770.

Kim, H. J., Woo, E. R. and Park, H. 1994. A novel lignan and flavonoids from *Polygonum aviculare*. J. Nat. Prod. 57: 581-586.

Kitazawa, E., Sato, A., Takahashi, S., Kuwano, H. and Ogiso, A. 1980. Novel diterpenelactones with anti-peptic ulcer activity from *Croton sublyratus*. Chem. Pharm. Bull. 28: 227-234.

Kitazawa, E., Sato, A., Takahashi, S., Sato, A., Kurabayashi, M., Kuwano, H., Hata, T. and Tamura, C. 1979. Plaunol A and B, new anti-ulcer diterpenelactones from *Croton sublyratus*. Tetrahedron Lett. 13: 1117-1120.

Kittakoop, P., Kirtikara, K., Tanticharoen, M. and Thebtaranonth, Y. 2000. Antimalarial preracemosols A and B, possible biogenic precursors of racemosol from *Bauhinia malabarica* Roxb. Phytochemistry 55: 349-352.

Kojima, H., Sato, N., Hatano, A. and Ogura, H. 1990. Sterol glucosides from *Prunella vulgaris*. Phytochemistry 29: 2351-2355.

Kover, O. and Wilkins, C. K. 1971. Circular dichroism spectra of flavanols. Tetrahedron. 27: 5459-5465.

Krebs, H. C. and Ramiarantosa, H. 1996. Clerodane diterpenes and other constituents of *Croton hovarum*. Phytochemistry 41: 561-563.

Kubo, I., Asaka, Y. and Shibata, K. 1991. Insect growth inhibitor *nor*-diterpenes, *cis*-dehydrocrotonin and *trans*-dehydrocrotonin, from *Croton cajucara*, Phytochemistry 30: 2545-2546.

Kudi, A. C. and Myint, S. H. 1999. Antiviral activity of some Nigerian medicinal plant extracts. J. Ethnopharmacol. 68: 289-294.

Kudi, A. C., Umoh, J. U., Eduvie, L. O. and Gefu, J. 1999. Screening of some Nigerian medicinal plants for antibacterial activity. J. Ethnopharmacol. 67: 225-228.

Kuo, Y. H., Chu, P. H. and Chang, C. I. 1998. Chemical studies of the bark of *Bauhinia purpurea*. Chem. Pharm. Bull. 46: 1630-1631.

Kuo, Y. H., Yeh, M. H. and Huang, S. L. 1998. A novel 6-butyl-3-hydroxyflavanone from heartwood of *Bauhinia purpurea*. Phytochemistry 49: 2529-2530.

Kupchan, S. M., Hemingway, R. J., and Smith, R. M. 1969. Tumor inhibitors. XLV. Crotepoxide, a novel cyclohexane diepoxide tumor inhibitor from *Croton macrostachys*. J. Org. Chem. 34: 3898-3902.

Larsen, K., Larsen, S. S. and Vidal, J. E. 1984. "Leguminosae-Caesalpinoideae" in Smitinand, T. and Larsen, K (eds) Flora of Thailand, TISTR Press, Bangkok, p. 40.

Larsen, K. and Larsen, S. S. 1997. *Bauhinia sirindhorniae* sp. nov. (Leguminosae-Caesalpinoideae) a remarkable new species from Thailand. Nord. J. Bot 17: 113-118.

Laux, D. O., Stefani, G. M. and Gottlieb, O. R. 1985. Bausplendin, a dimethylenedioxyflavone from *Bauhinia splendens*. Phytochemistry 24: 1081-1084.

Lundgren, N. L. and Theander, O. 1988. *Cis* and *trans*-dihydroquercetin glucoside from needles of *Pinus sylvestris*. Phytochemistry 27: 829-832.

Luzbetak, D. J., Torrance, S. J., Hoffmann, J. J. and Cole, J. R. 1979. Isolation of (-)-hardwichiic acid and 1-triacontanol from *Croton californicus*. J. Nat. Prod. 42: 315-316.

Lafontaine, A. C., Raharivelomanana, P. and Bianchini, J. P. 1991. Volatile constituents of the flower concrete of *Gardenia taitensis* DC. J. Ess. Oils Res. 4: 335-343.

Maciel, M. A. M., Pinto, A. C. Brabo, S. N. and Silva, M. N. D. 1998. Terpenoids from *Croton cajucara*. Phytochemistry 49: 823-828.

Macrae, W. D., Hudson, J. B. and Towers, G. H. N. 1988. Studies on the pharmacological activity of Amazonian Euphorbiaceae. J. Ethnopharmacol. 22: 143-172.

Mahato, S. B., Das, M. C. and Sahu, N. P. 1981. Triterpenoids of *Scoparia dulcis*. Phytochemistry 20: 171-173.

Markham, K. R. 1982. Techniques of flavonoid Identification. Academic press, New York.

Markham, K. R. and Ternai, B. 1976. ^{13}C NMR of flavonoids-II: flavonoids other the flavone and flavonol aglycones. Tetrahedron 32: 2607-2612.

Mazzanti, G., Bolle, P., Martinoli, D., Piccinelli, D., Grgurina, I., Annati, F. and Mugne, Y. 1987. *Croton macrostachys*, a plant used in traditional medicine: purgative and inflammatory activity. J. Ethnopharmacol. 19: 213-219.

McChesney, J. D., Clark A. M. and Silveira E. R. 1991. Antimicrobial diterpenes of *Croton sonderianus*, hardwickiic and 3,4-secotrachylobanoic acids. J. Nat. Prod. 54, 6:1625-1633.

Mensah, I. A., Waibel, R., Achenbach, H., Muriuki, G., Pearce, C. and Sanders, J. K. M. 1989. A clerodane diterpene and other constituents of *Croton megalocarpus*. Phytochemistry 28: 2759-2761.

Millard, M. P., Recio-Igelesias, M. C., Saadou, M., Stoeckli-Evans, H. and Hostettman, K. 1991. Novel antifungal tetracyclic compounds from *Bauhinia rufescens* Lam. Helv. Chim. Acta. 74: 791-799.

Monte, F. J. Q., Andrade, C. H. S. and Craveiro, A. A. 1984. New tetracyclic diterpenes from *Croton argyrophylloides*. J. Nat. Prod. 47: 55-58.

Monte, F. J. Q., Dantas, E. M. G. and Braz, R. F. 1988. New diterpenoids from *Croton argyrophylloides*. Phytochemistry 27: 3209-3212.

Mùnoz, V., Sauvain, M., Bourdy, G., Callapa, J., Bergeron, S., Rojas, I., Bravo, J. A., Balderrama, L., Ortiz, B., Gimenez, A. and Deharo, E. 2000. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. J. Ethnopharmacol. 69: 127-137.

Nakanishi, T., Nishi, M., Somekawa, M., Murata, H., Mizuno, M., Iinuma, M., Tanaka, T., Murata, J., Lang, F. A., and Inada, A. 1994. Structures of new and known cyanoglucosides from a north American plant, *Purshia tridentata* DC. Chem. Pharm. Bull. 42: 2251-2255.

Ogiso, A., Kitazawa, E., Kurabayashi, M., Sato, A., Takahashi, H., Noguchi, H., Kuwano, H., Kobayashi, S. and Mishima , H. 1978. Isolation and structure of antipeptic ulcer diterpene from Thai medicianal plant. Chem. Pharm. Bull. 26: 3117-3123.

Olsson, E., Berg, J. E. and Wahlberg, I. 1993. Eight new cembranoids from tobacco structural elucidation and conformational studies. Tetrahedron 49: 4075-4992.

Perdue, G. P., Blomster, R. N., Blake, D. A. and Farnsworth, N. R. 1979. South American plants II: Taspine isolation and anti-inflammatory activity. J. Pharm. Sci. 68: 124-125.

Pieters, L. A. C., Dirk, V. B. and Arnold, V. 1990. A dihydrobenzofuran lignan from *Croton erythrochilus*. Phytochemistry 29: 348-349.

Pezzuto, J. M. and Kinghorn, A. D. 1998. Antioxidant flavonoid glycosides from *Daphniphyllum calycinum*. J. Nat. Prod. 61: 706-708.

Prabhakar, P., Gandhidasan, R., Raman, P. V., Krishnasamy, N. R. and Nanduri, S. 1994. De-*O*-methylracemosol : A tetracyclic 2,2-dimethylchroman from the roots of *Bauhinia racemosa*. Phytochemistry 36: 817-818.

Ravi, B. N., Murphy, P. T., Lidgard, R. O., Warren, R. G. and Wells, R. J. 1982. C_{18} Terpenoid metabolites of the brown alga *Cystophora moniliformis*. Aust. J. Chem. 35: 171-182.

Reynolds, W. F., Mclean, S., Poplawski, J., Enriquez, R. G., Escobar, L. I., and Leon, 1986. Total assignment of ^{13}C and ^{1}H spectra of three isomeric triterpenol

derivatives by 2D-NMR: an investigation of the potential utility of ^{1}H chemical shift in structure investigations of complex natural products. Tetrahedron 13: 3419-3428.

Roderick, B. and Orlando, S. 1981. The alkaloids of *Croton salutaris*. Phytochemistry 20: 543-544.

Roengsumran, S., Achayindee, S., Petsom, A., Pudhom, K., Singtothong, P., Surachetapan, C. and Vilaivan, T. 1998. Two new cembranoids from *Croton oblongifolius*. J. Nat. Prod. 61: 652-654.

Roengsumran, S., Petsom, A., Sommit, D. and Vilaivan, T. 1999a. Labdane diterpenoids from *Croton oblongifolius*. Phytochemistry 50: 449-453.

Roengsumran, S., Singtothong, P., Pudhom, K., Ngamrochanavanich, N., Petsom, A. and Chaichantipyuth, C. 1999b. Neocembranal from *Croton oblongifolius*. J. Nat. Prod. 62: 1163-1164.

Saitoh, T., Noguchi, H. and Shibata, S. 1978. A new isoflavone and the corresponding isoflavone of licorice root. Chem. Pharm. Bull. 26: 144-147.

Sato, A., Kurabayashi, M., Ogiso, A. and Kuwano, H. 1981. Poilaneic acid, a cembranoid diterpene from *Croton poilanei*. Phytochemistry 20: 1915-1918.

Sato, A., Ogiso, A. and Kuwano, H. 1980. Acyclic diterpenes from *Croton kerrii*. Phytochemistry 19: 2207-2209.

Seims, K., Dominguez, X. A. and Jakupovic, J. Diterpenes and other constituents from *Croton cortesianus*. Phytochemistry 31: 4363-4365.

Shaw, H. K. A. 1972. The Euphorbiaceae of Siam. Kew Bull. 26: 247.

Shimomura, H., Sashida, Y., Oohara, M., and Tenma, H. 1988. Phenolic glucosides from *Parabenzoin praecox*. Phytochemistry 27: 644-646.

Silveira, E. R. and McChesney, J. D. 1994. 6,7-Oxygenated neo-clerodane furan diterpenes from *Croton sonderianus*. Phytochemistry 36: 1457-1463.

Simon, A., Chulia, A. J., Kaouadji, M. and Delage, C. 1994. Quercetin 3-[triacetylarabinosyl (1→6)] galactoside and chromones from *Calluna vulgaris*. Phytochemistry 36: 1043-1045.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., Mamahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82: 1107-1112.

Smith, B. E. C. and Swain, T. 1953. The isolation of 2′,4,4′-trihydroxychalcone from yellow varieties of *Dahlia variabilis*. J. Chem. Soc. 2185-2187.

Smitinand, T. 2001. Thai plant names (botanical names vernacular names) revised edition. Bangkok: The Forest herbarium, Royal Forest Department. pp. 68-72, 155-157.

Sosa, A., Winternitz, F., Wylde, R. and Pavia, A. A. 1977. Structure of cyanoglucoside of *Lithospermum purpureo-caeruleum*. Phytochemistry 16: 707-709.

Subramanian, S. S., Nagarajan, S. and Sulochana, N. 1971. Flavonoids of some Euphorbiaceous plants. Phytochemistry 10: 2548-2549.

Sutthivaiyakit, S., Nareeboon, P., Ruangrungsi, N., Ruchirawat, S., Pisutjaroenpong, S. and Mahidol, C. 2001. Labdane and pimarane diterpenes from *Croton joufra*. Phytochemistry 56: 811-814.

Takahashi., Kurabayashi, M., Kitazawa, E., Haruyama, H. and Ogiso, A. 1983. Plaunolide, a furanoid diterpene from *Croton sublyratus*. Phytochemistry 22: 302-303.

Takao, T., Kitatani, F., Watanabe, N., Yagi, A. and Sakata, K. 1994. A simple screening method for antioxidants and isolation of several antioxidants proceduced by marine bacteria from fish and shellfish. Biosci. Biotech. Biochem. 58: 1780-1783.

Tchissambou, L., Chaiaroni, A., Riche, C. and Khuong-Huu, F. 1990. Crotocorylifuran and crotohaumanoxide, new diterpenes from *Croton haumanianus* J. Leonard. Tetrahedron 46: 5199-5202.

Thongtan, J., Kittakoop, P., Ruangrungsi, N., Saenboonrueng, J. and Thebtaranonth, Y. 2003. New antimycobacterial and antimalarial 8,9-secokaurane diterpenes from *Croton kongensis*. J. Nat. Prod. 66: 868-870.

Viana, E. P., Santa-Rosa, R. S., Almeida, S. S. M. S. and Santos, L. S. 1999. Constituents of the stem bark of *Bauhinia guianensis*. Fitoterapia 70: 111-112.

Vigor, C., Fabre, N., Fouraste, I. and Moulis, C. 2001. Three clerodane diterpenoids from *Croton eluteria* Bennett. Phytochemistry 57: 1209-1212.

Vilaivan, T., Achayindee, A., Petsom, A., Pudhom, K., Roengsumran, S. and Surachethapan, C. 1997. A new cembranoids from stem barks of *Croton oblongifolius* Roxb. J. Nat. Prod. 60: 270.

Weckert, E. K., Hummer, K., Mensah, I. A. and Achenbach, H. 1992. The absolute configuration of chiromodine. Phytochemistry 31: 2170-2172.

Wilson, S. R., Neubert, L.A. and Huffman, J. C. 1976. The chemistry of the Euphorbiaceae. A new diterpene from *Croton californicus*. J. Am. Chem. Soc. 98: 3669-3674.

Wolfram, E. and Franz, B. 2001. Crotoflavol, a new phenanthrene from *Croton flavens*. Nat. Prod. Lett. 15: 147-150.

Wu, J., Fairchild, E. H., Beal, J. L., Tomimatsu, T. and Doskotch, R. W. 1979. Lithospermoside and dasycarponin, cyanoglucosides from *Thalictrum*. J. Nat. Prod. 42: 500-511.

Yadava, R. N. and Tripathi, P. 2000. A novel flavone glycoside from the stem of *Bauhinia purpurea*. Fitoterapia 71: 88-90.

Zhong, S. M., Waterman, P. G. and Jeffreys, J. A. D. 1984. Naphthoquinone and triterpenes from African *Diospyros* species. Phytochemistry 23: 1067-1072.

APPENDICES

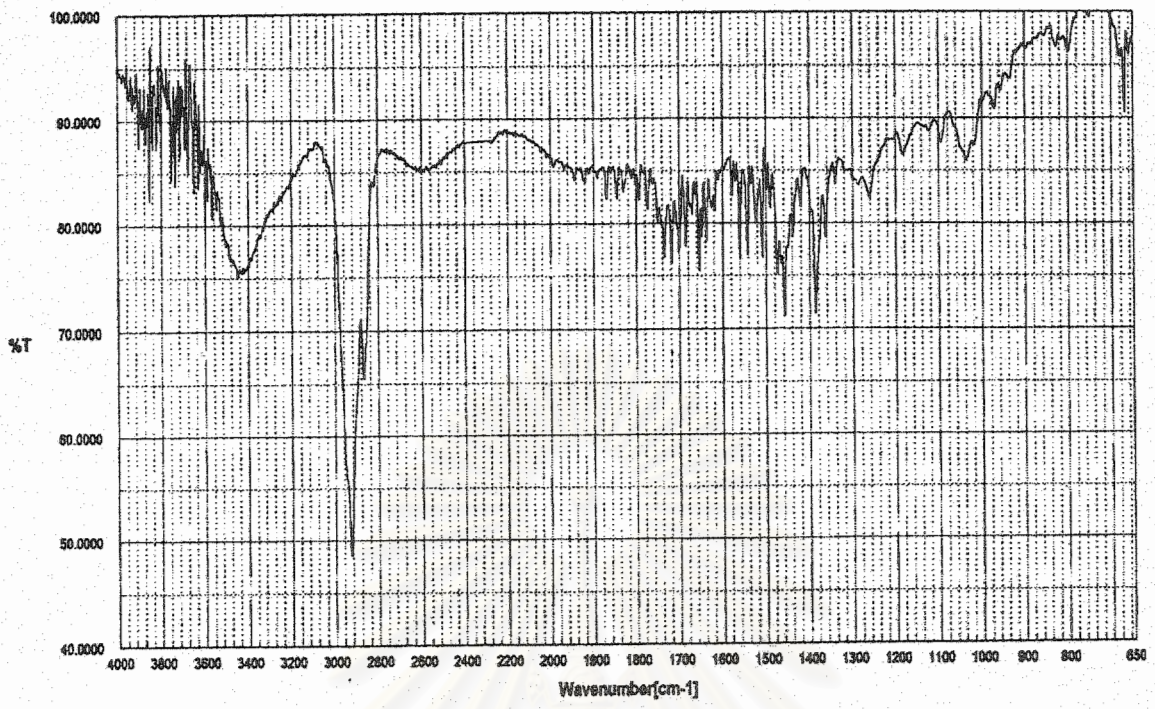


Figure 10 IR Spectrum of compound BRC1 (KBr disc)

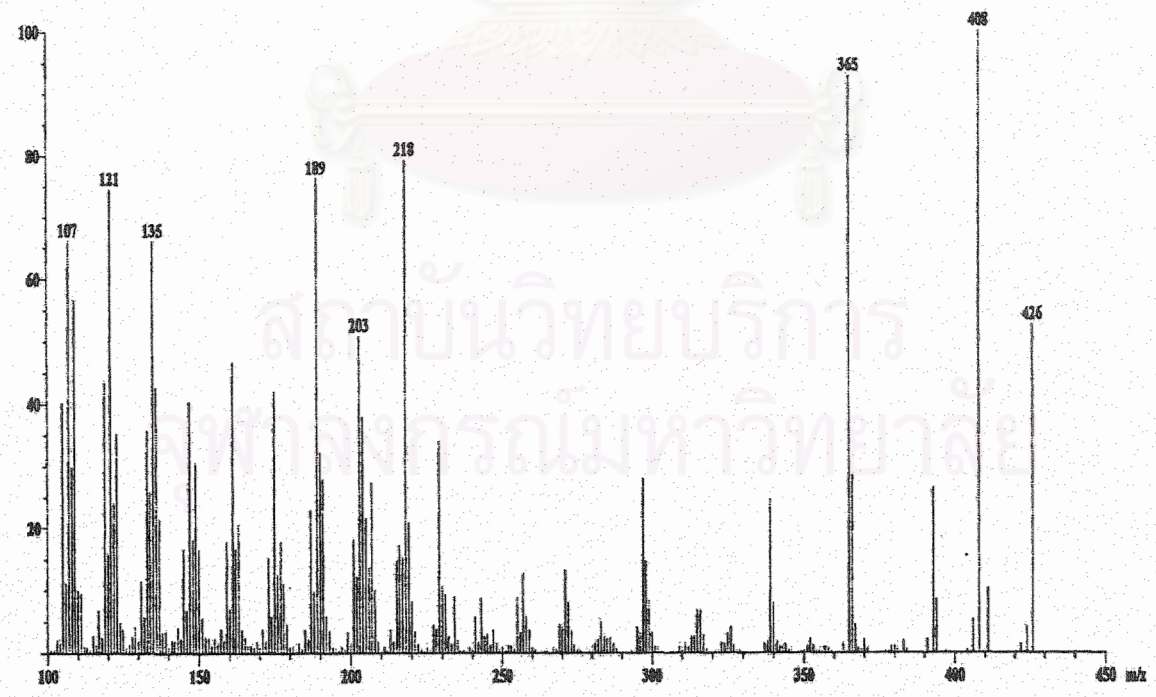


Figure 11 EIMS Mass spectrum of compound BSC1

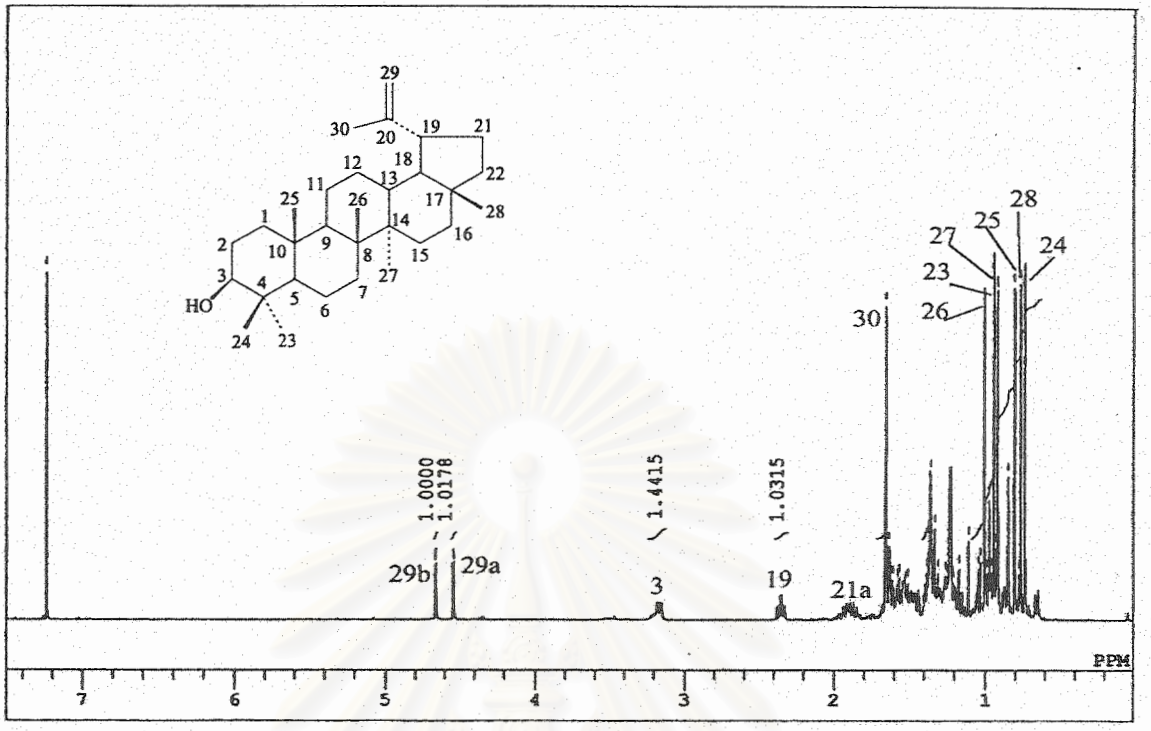


Figure 12 ^{1}H NMR (500 MHz) Spectrum of compound BSC1 (CD_3OD)

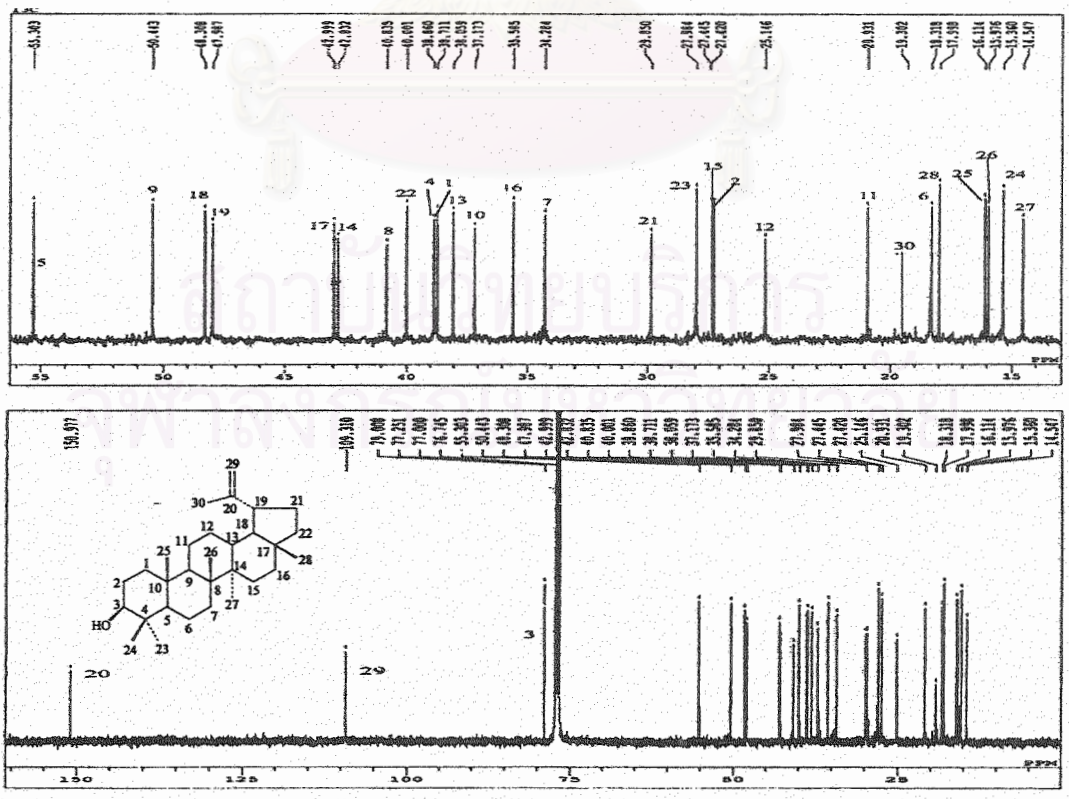


Figure 13 ^{13}C NMR (125 MHz) Spectrum of compound BSC1 (CD_3OD)

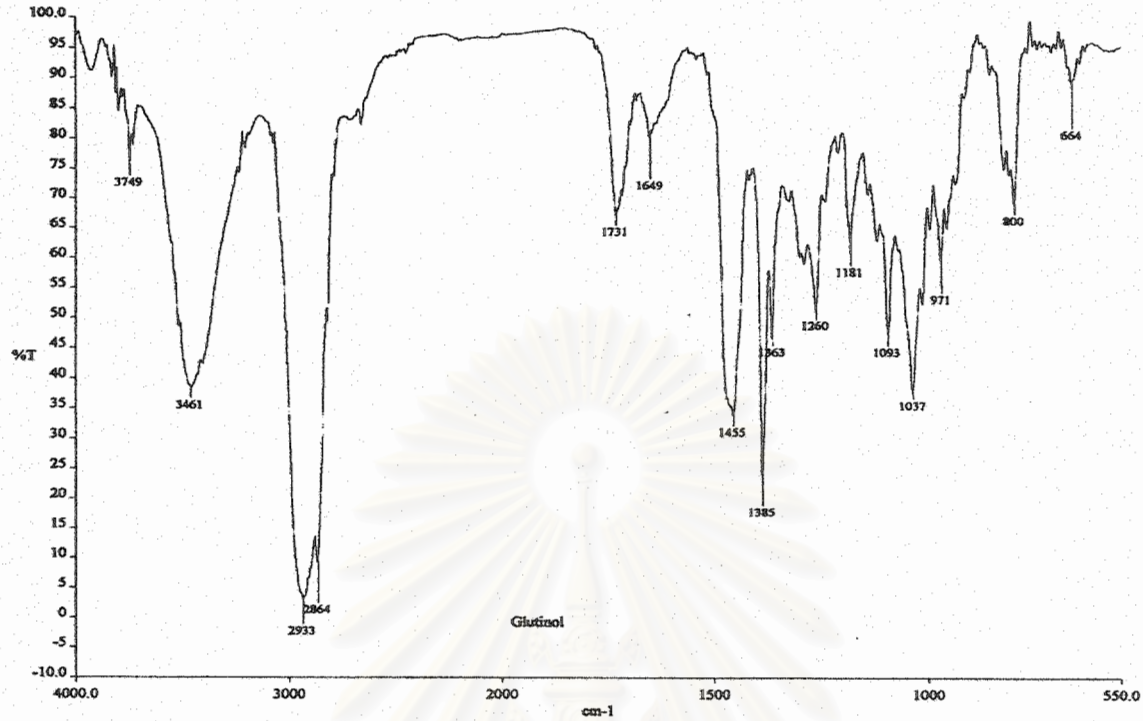


Figure 14 IR Spectrum of compound BSC2 (KBr disc)

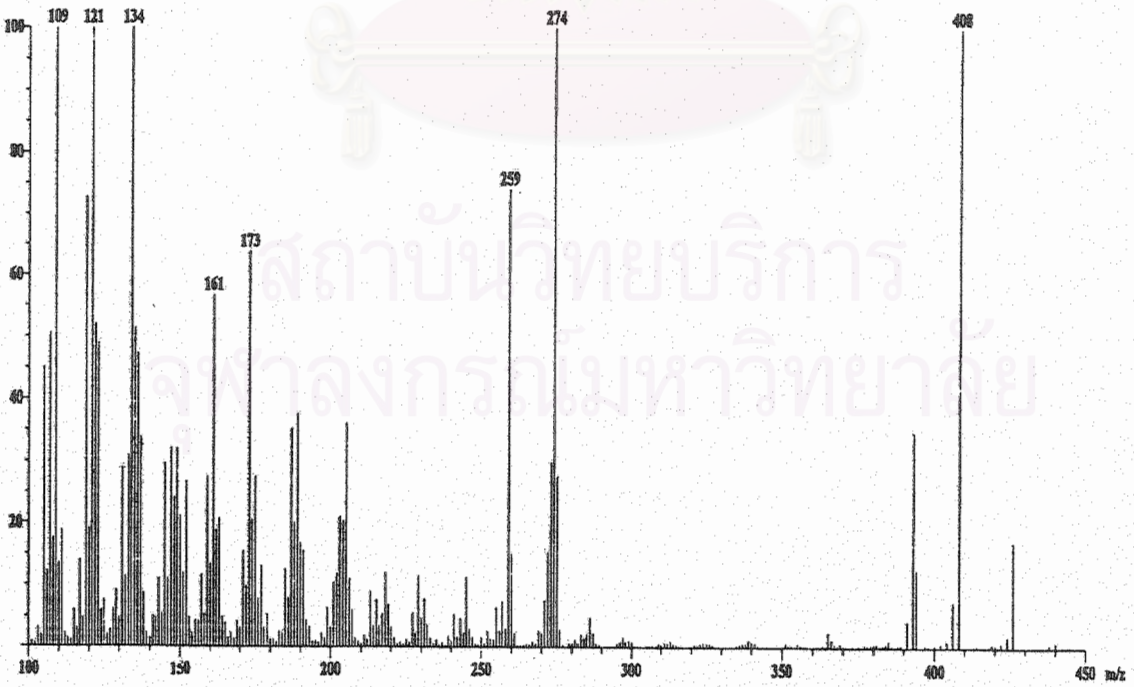


Figure 15 EIMS Mass spectrum of compound BSC2

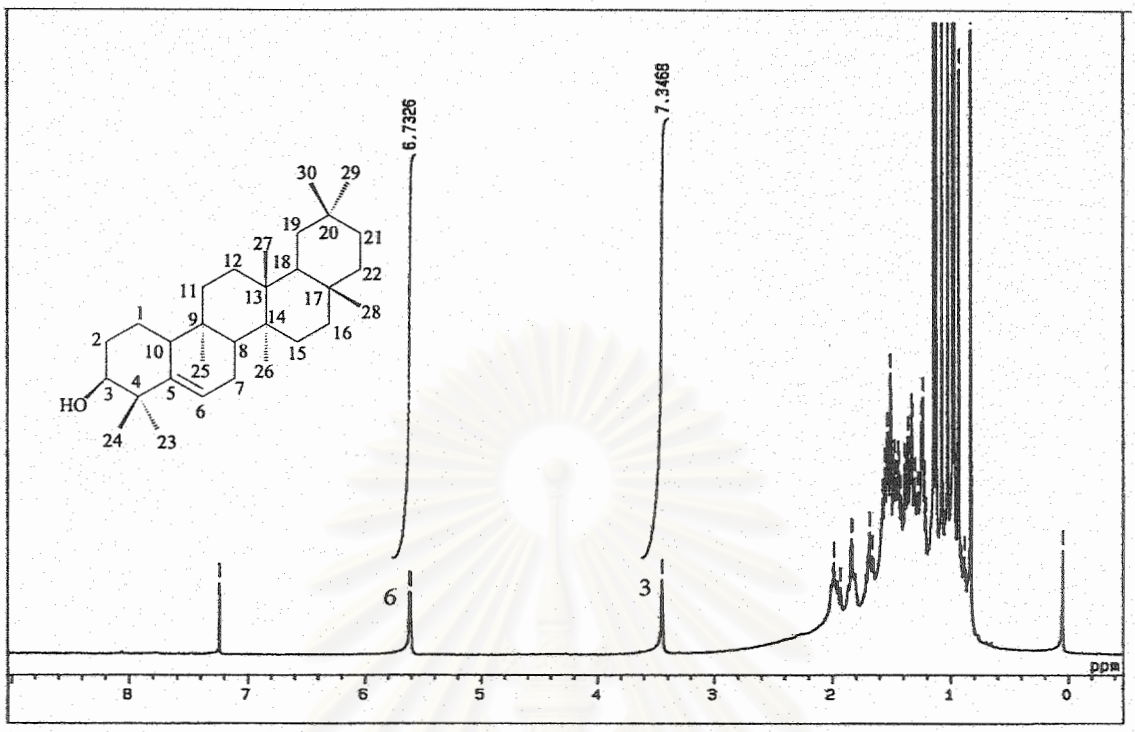


Figure 16 ^{1}H NMR (500 MHz) Spectrum of compound BSC2 (CD_3OD)

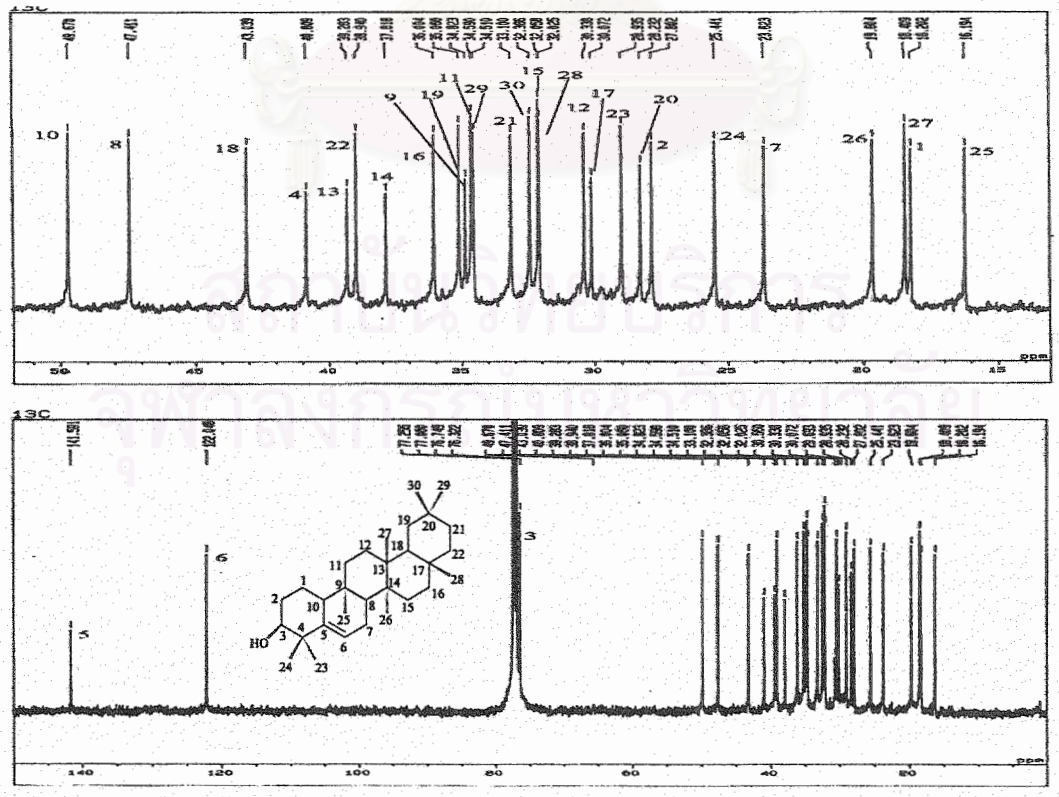


Figure 17 ^{13}C NMR (125 MHz) Spectrum of compound BSC2 (CD_3OD)

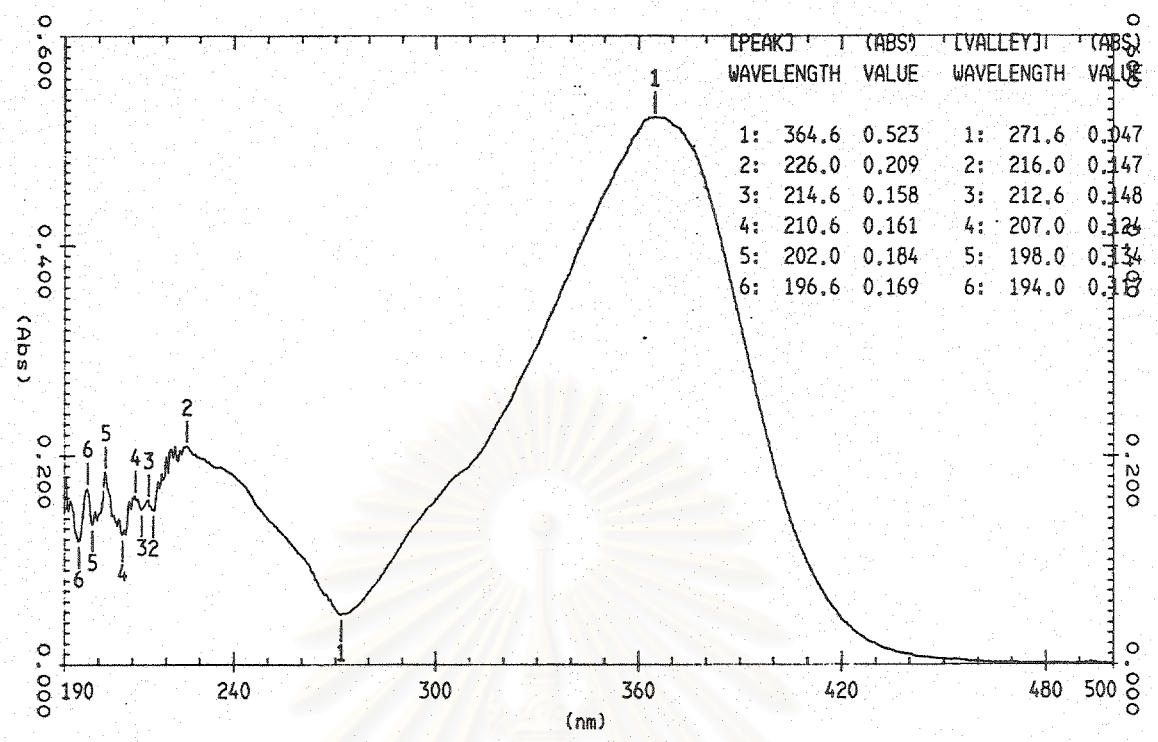


Figure 18 UV Spectrum of compound BSB1 (methanol)

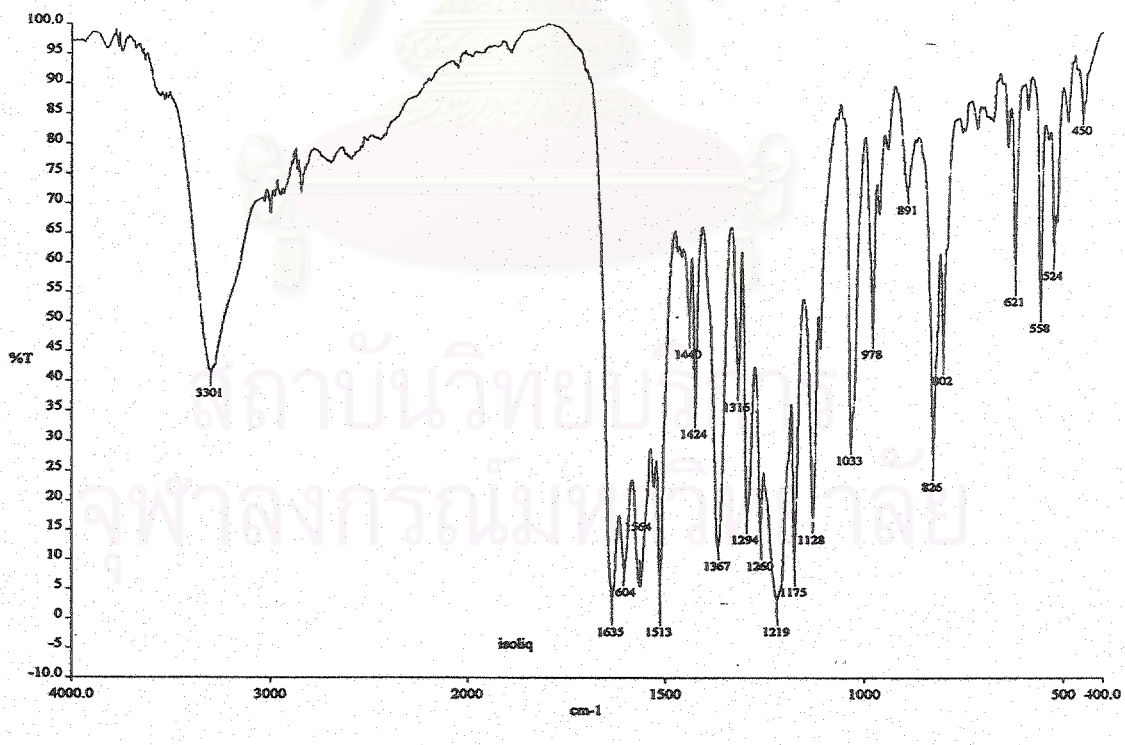


Figure 19 IR Spectrum of compound BSB1 (KBr disc)

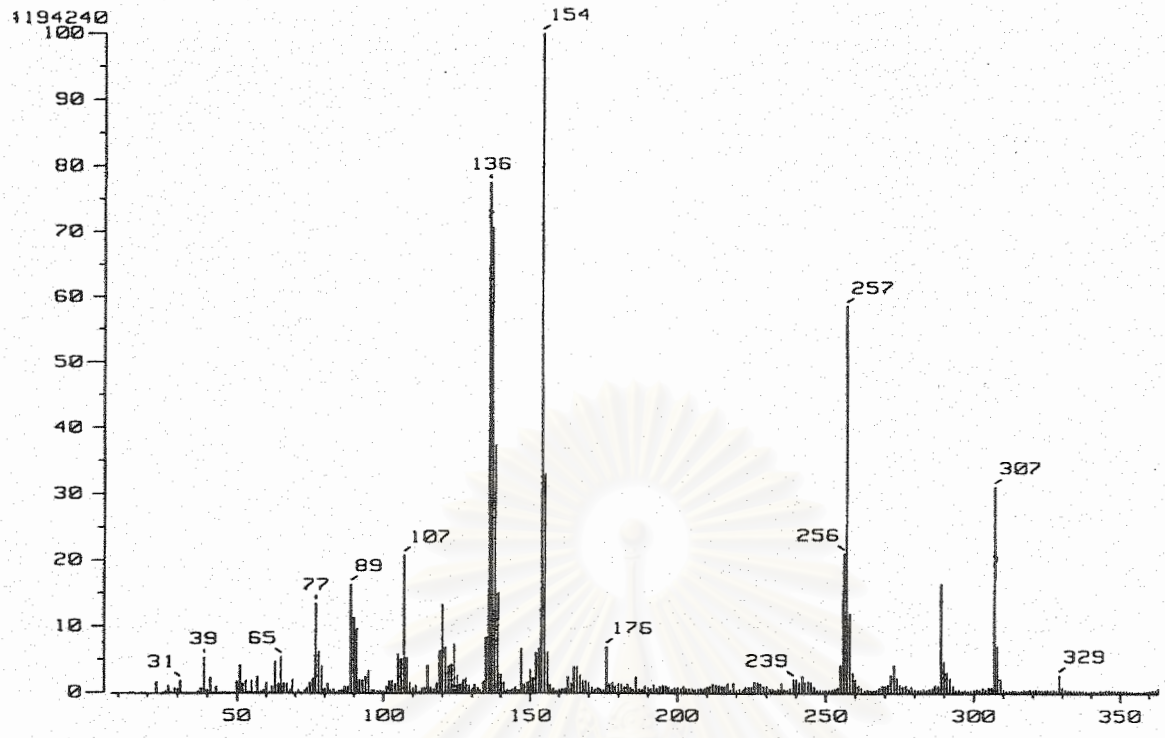


Figure 20 FAB$^+$MS Mass spectrum of compound BSB1

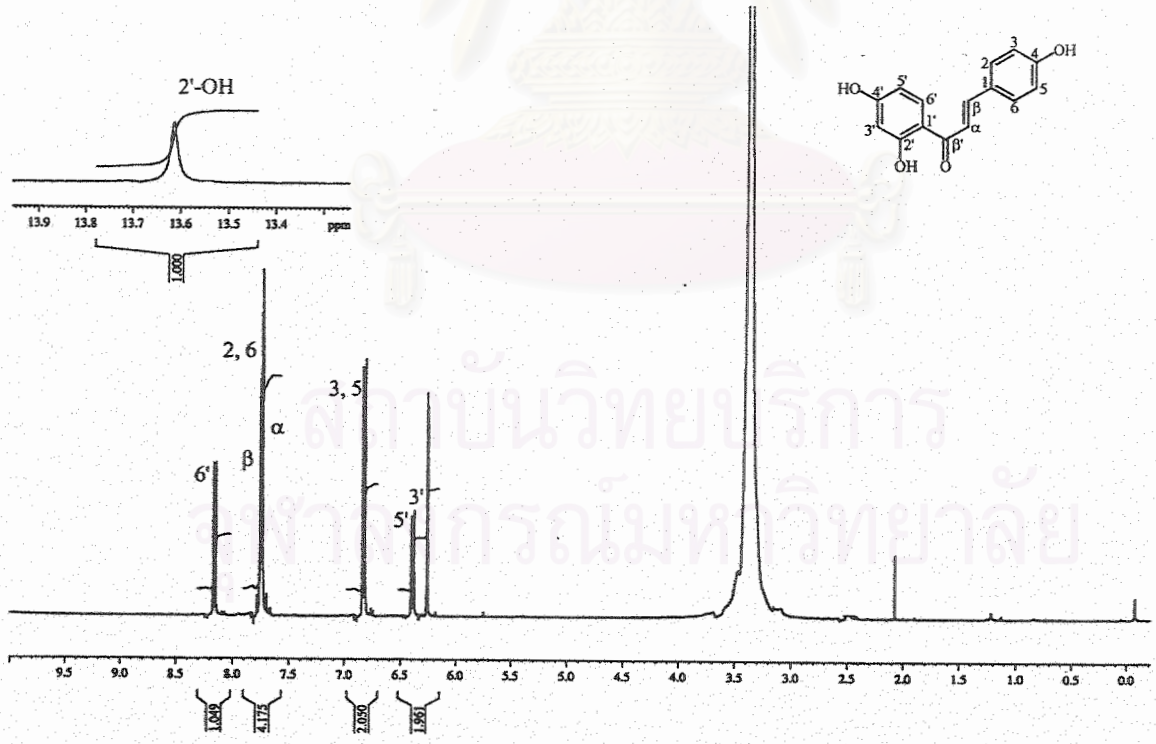


Figure 21 ^{1}H NMR (400 MHz) Spectrum of compound BSB1 (DMSO-d_6)

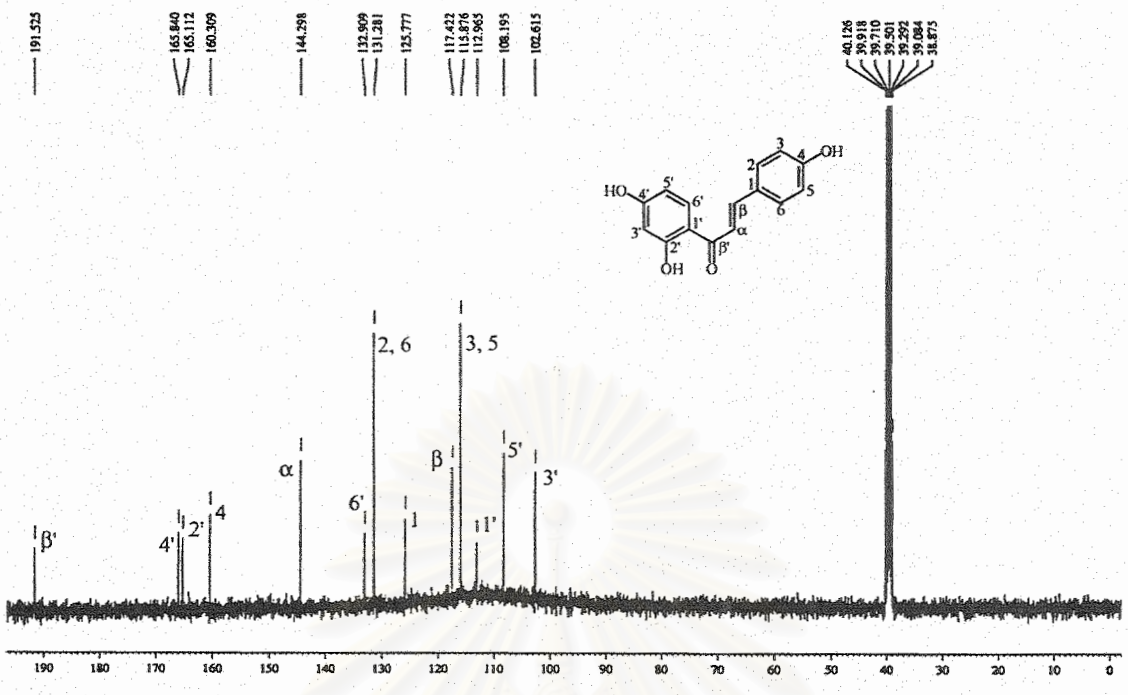


Figure 22 ^{13}C NMR (100 MHz) Spectrum of compound BSB1 (DMSO-d_6)

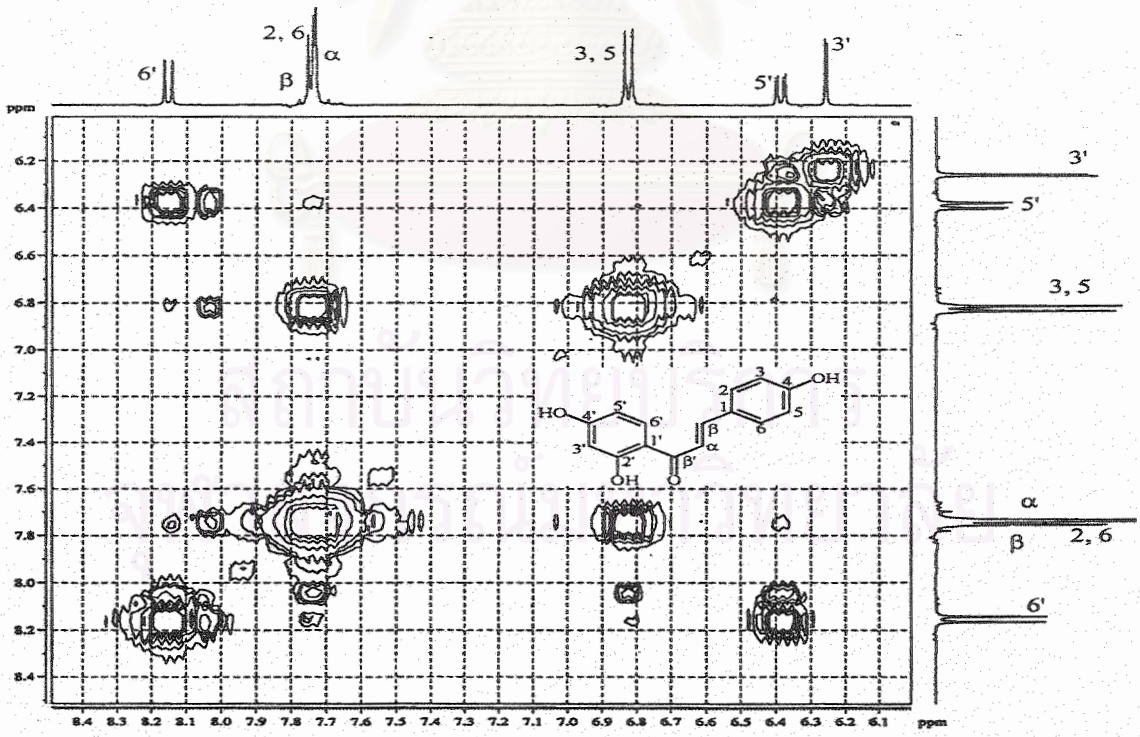


Figure 23 ^{1}H-^{1}H COSY Spectrum of compound BSB1 (DMSO-d_6)

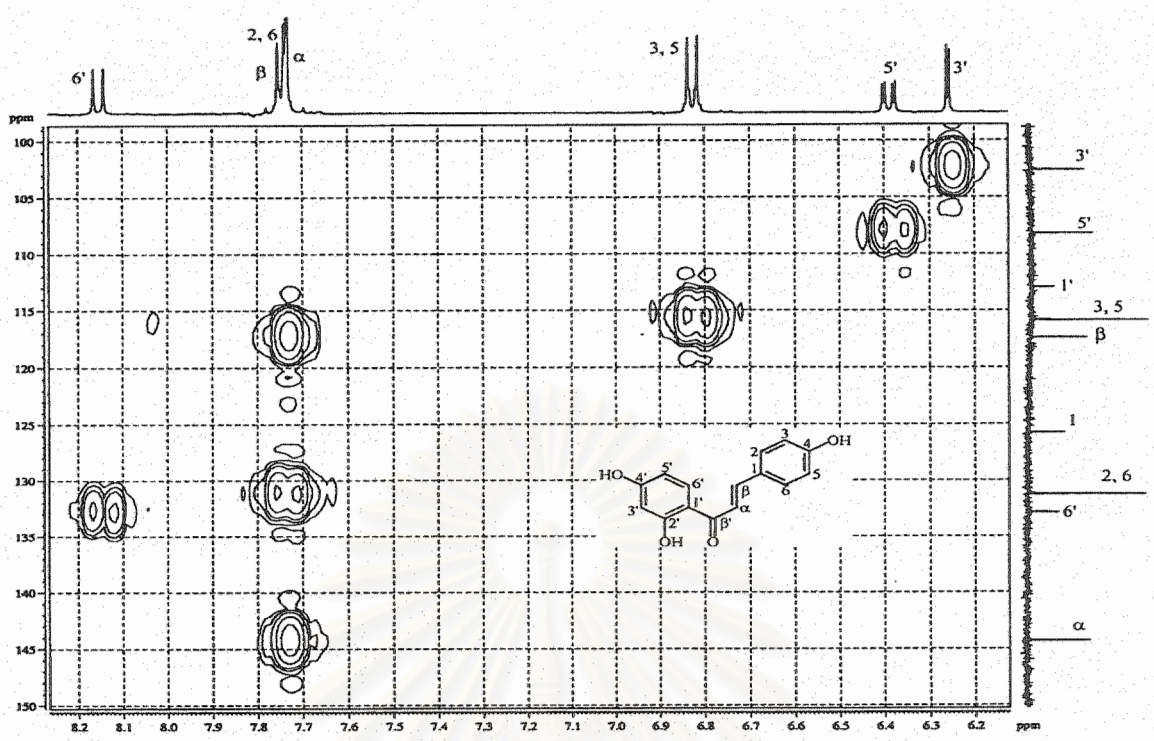


Figure 24 HMQC Spectrum of compound BSB1 (DMSO-d_6)

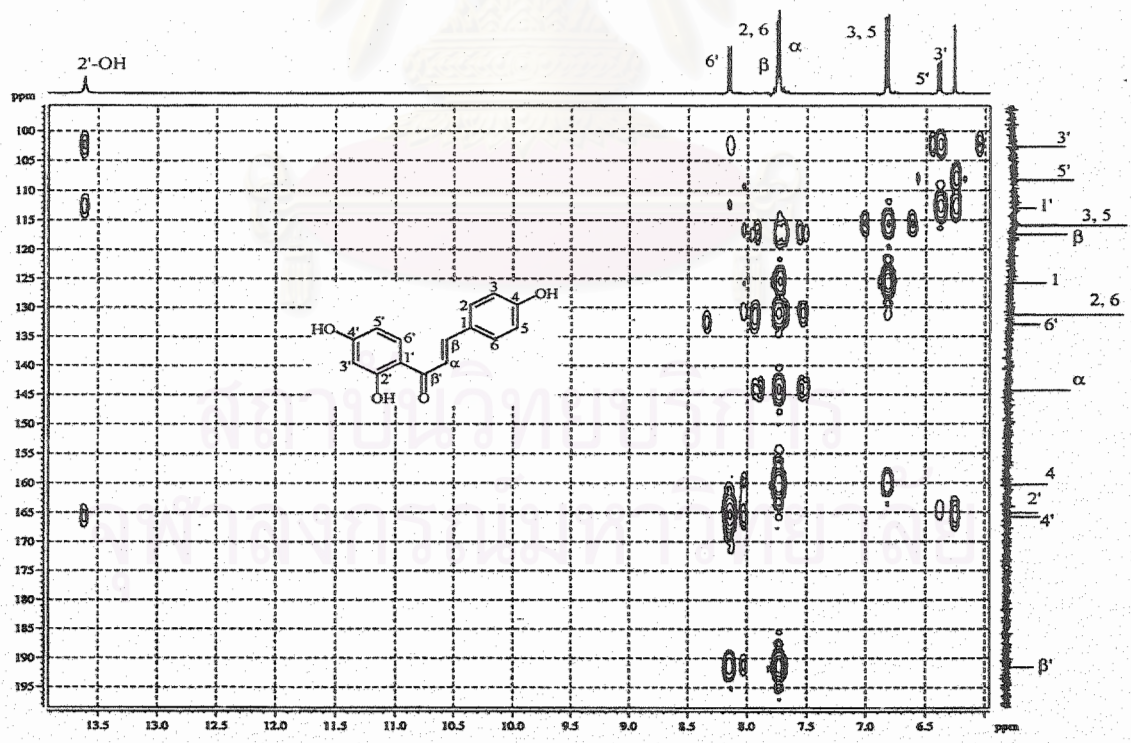


Figure 25 HMBC Spectrum of compound BSB1 (DMSO-d_6)

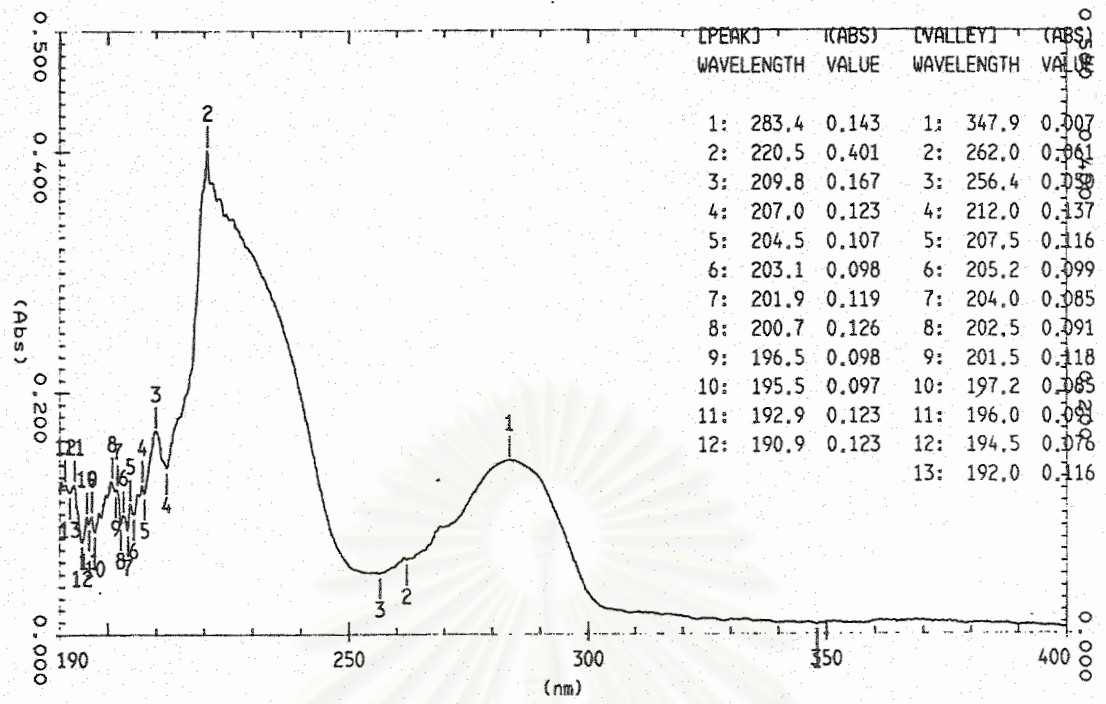


Figure 26 UV Spectrum of compound BSB2 (methanol)

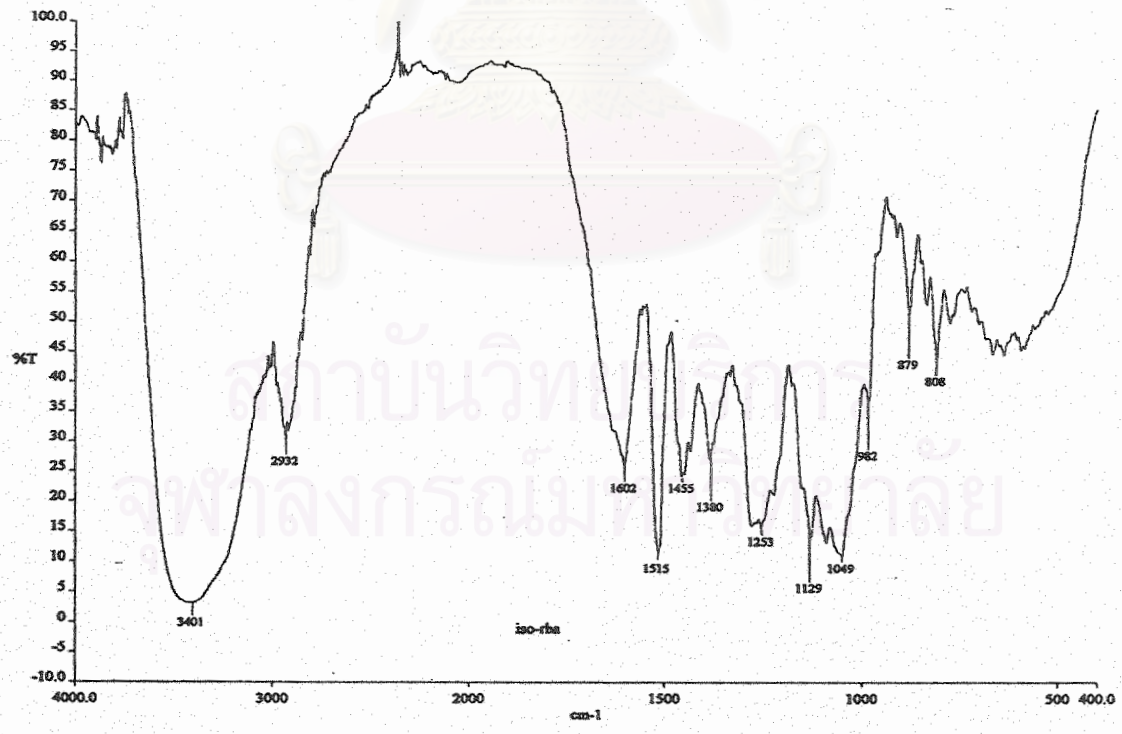


Figure 27 IR Spectrum of compound BSB2 (KBr disc)

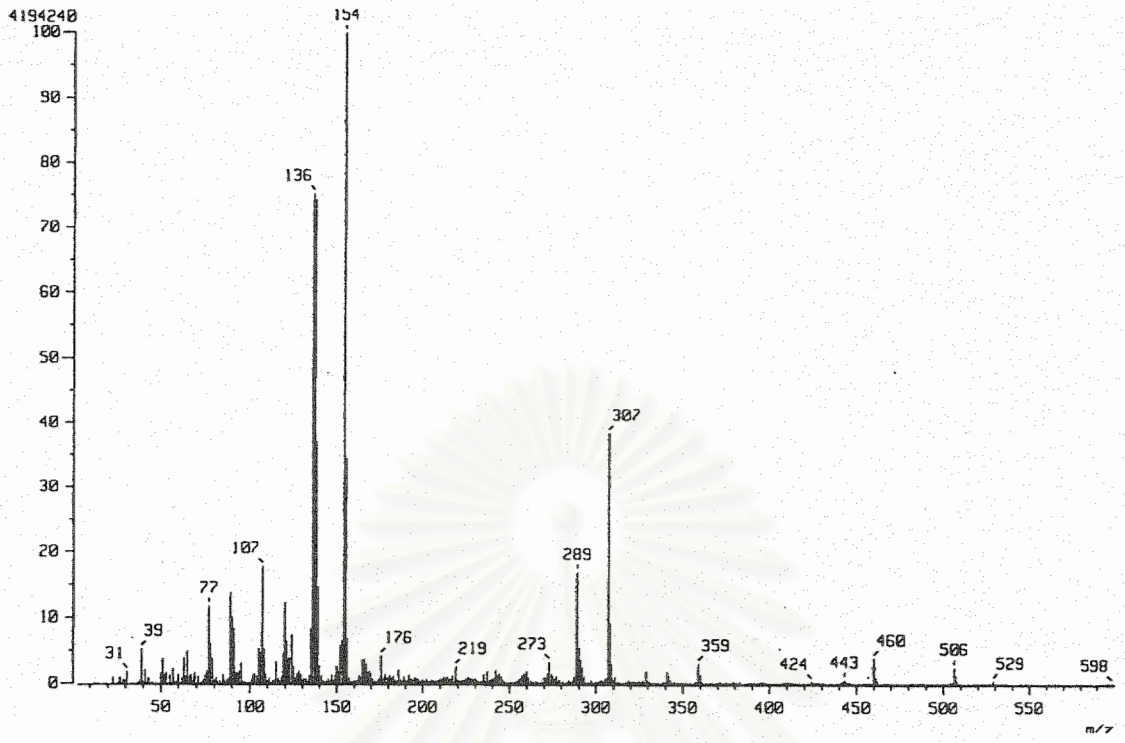


Figure 28 FAB$^+$MS Mass spectrum of compound BSB2

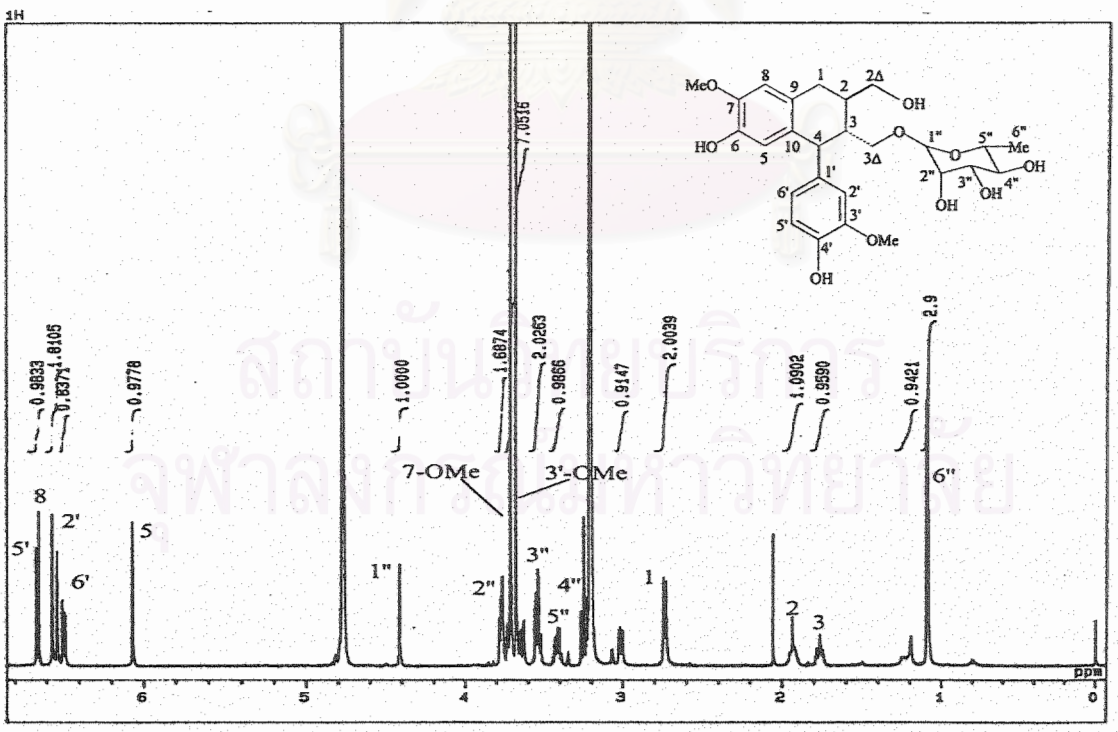


Figure 29 ^{1}H NMR (500 MHz) Spectrum of compound BSB2 (CD_3OD)

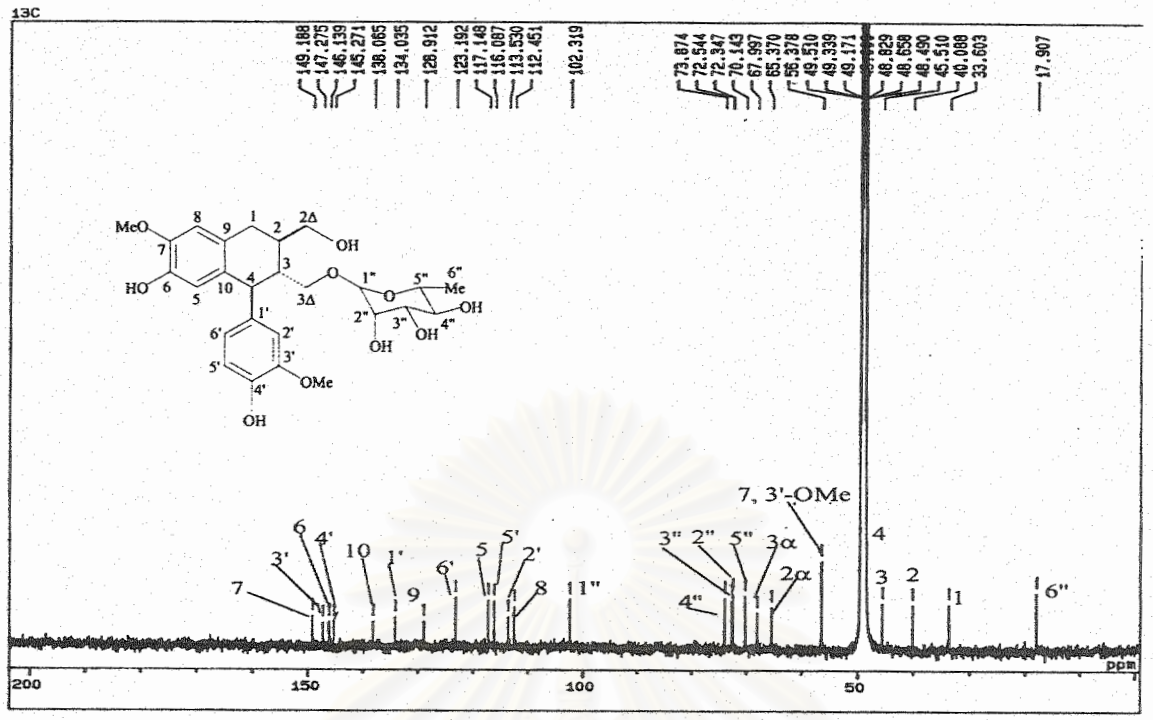


Figure 30 ^{13}C NMR (125 MHz) Spectrum of compound BSB2 (CD_3OD)

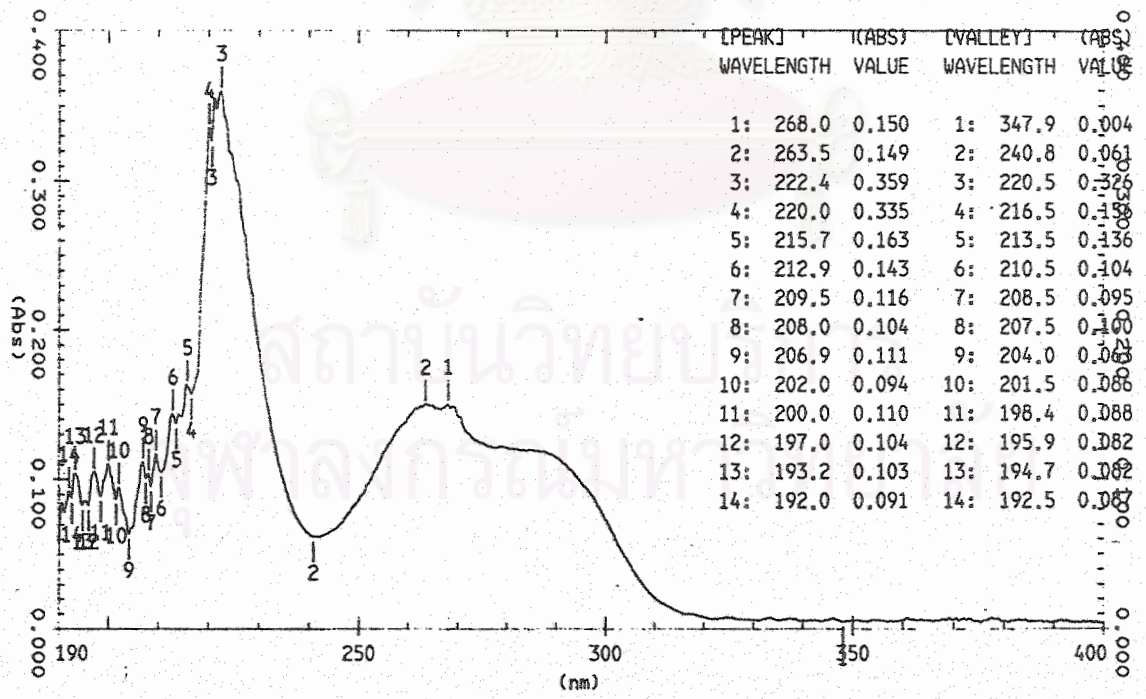


Figure 31 UV Spectrum of compound BSB3 (methanol)

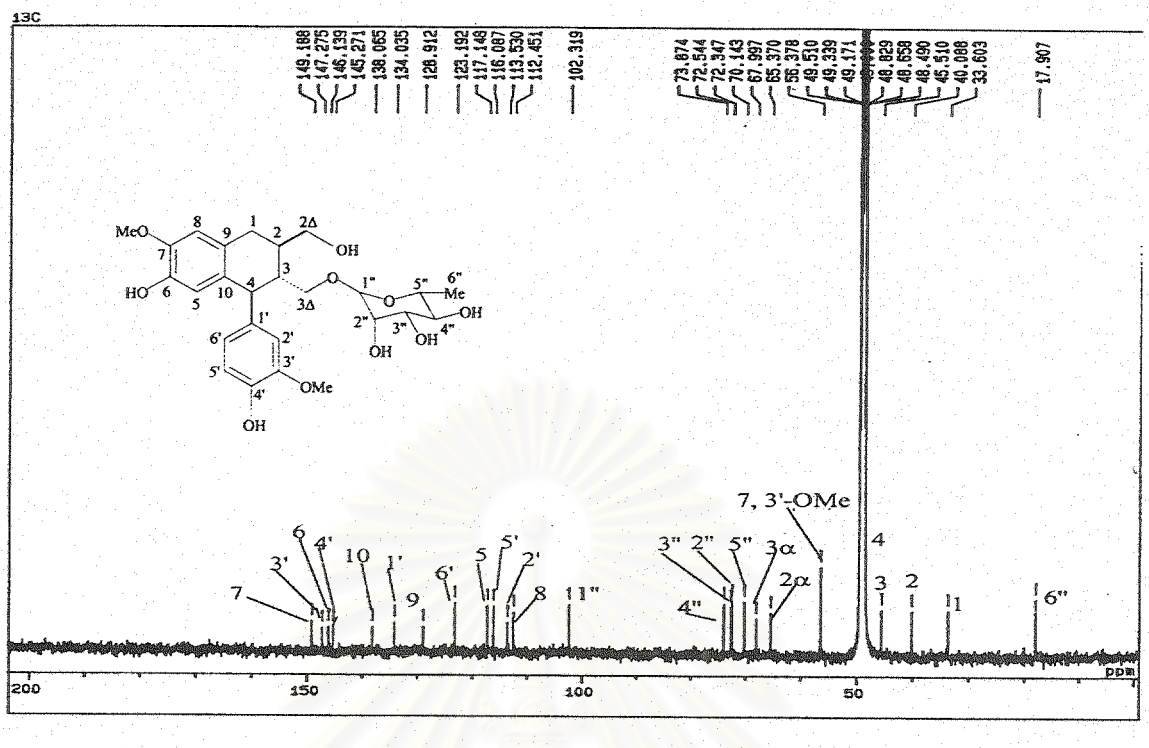


Figure 30 ^{13}C NMR (125 MHz) Spectrum of compound BSB2 (CD_3OD)

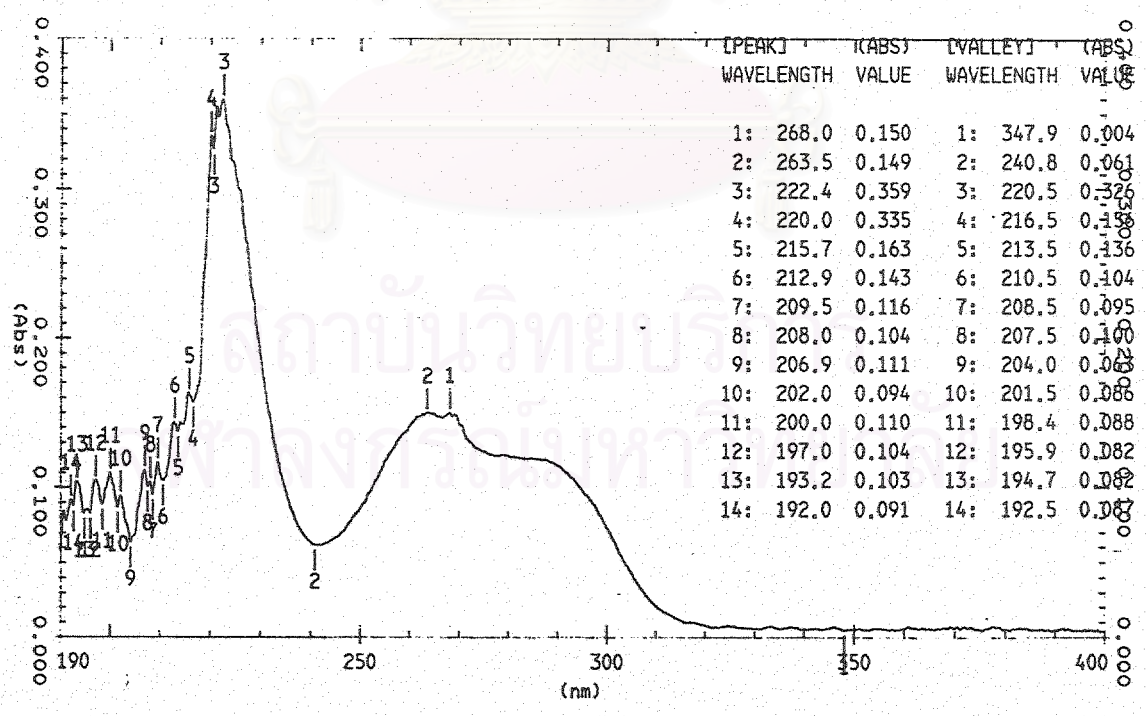


Figure 31 UV Spectrum of compound BSB3 (methanol)

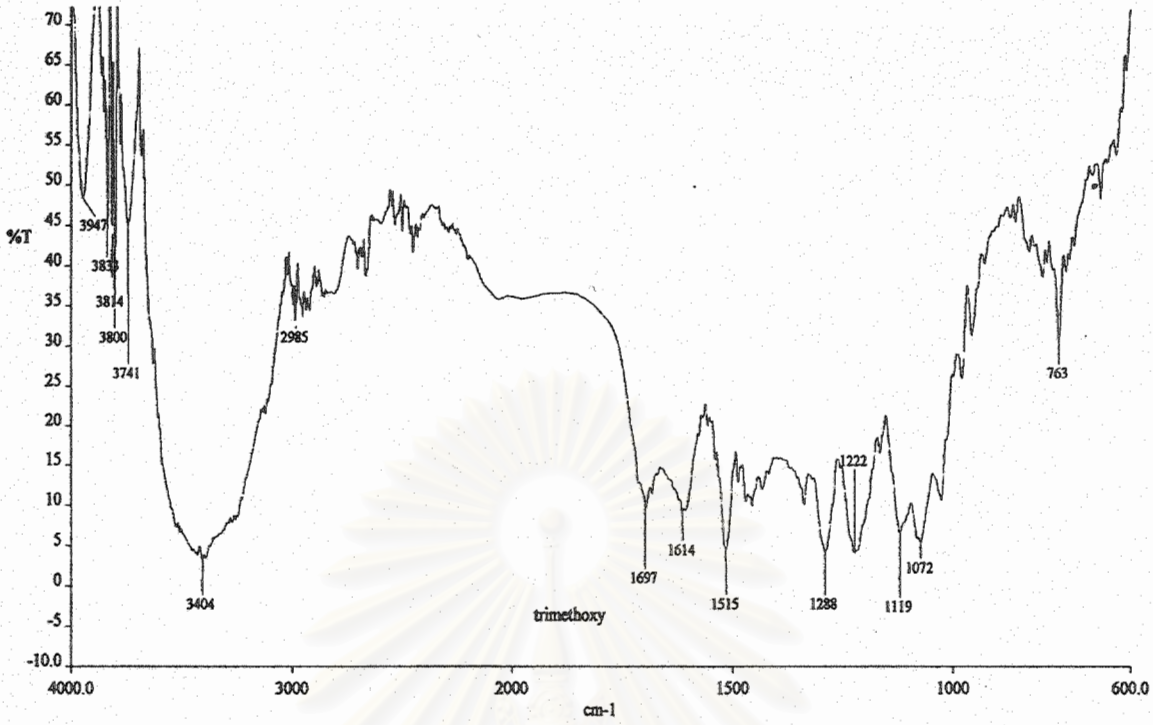


Figure 32 IR Spectrum of compound BSB3 (KBr disc)

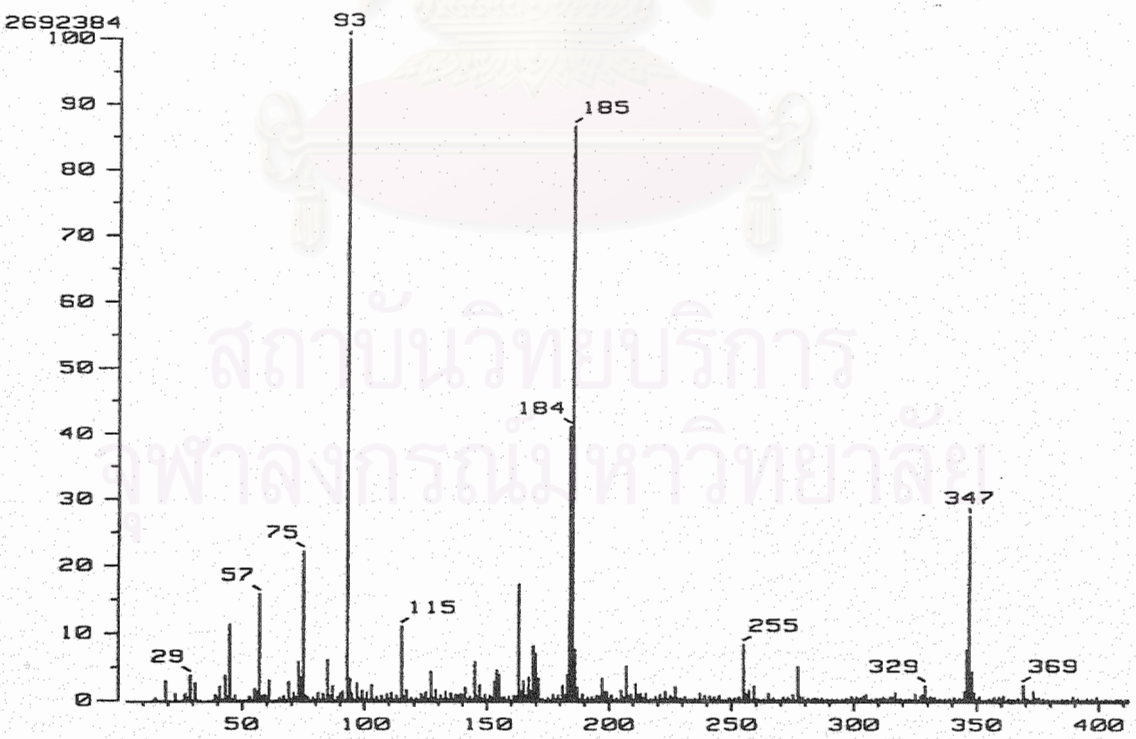


Figure 33 FAB$^+$MS Mass spectrum of compound BSB3

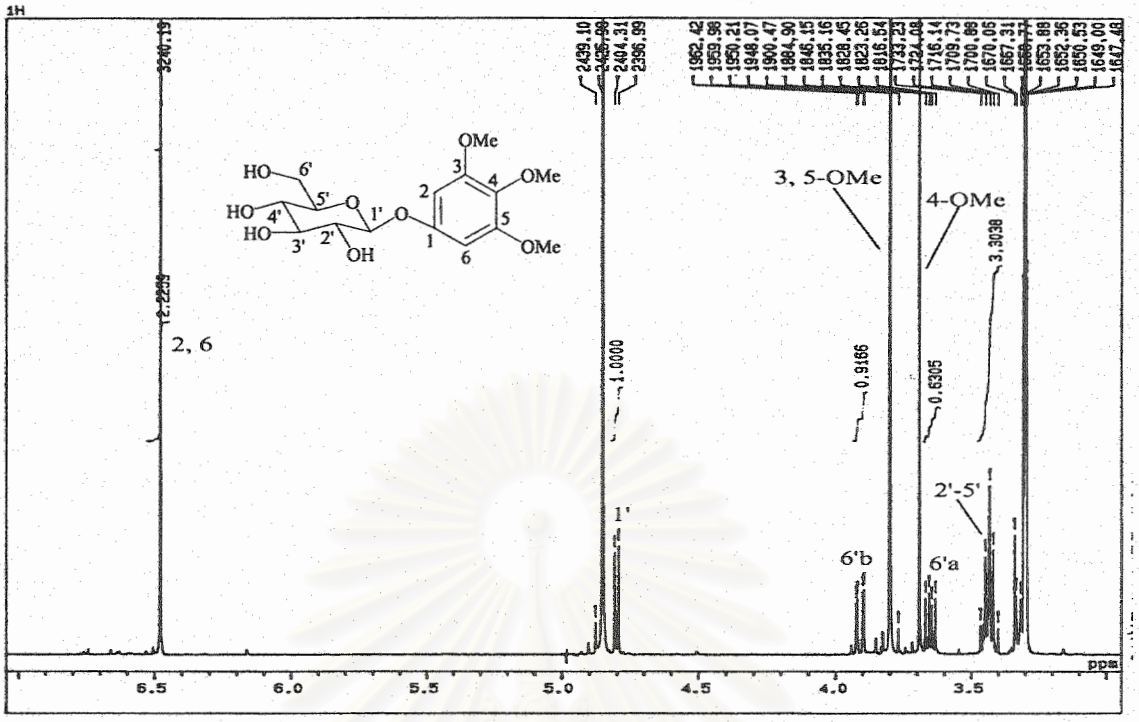


Figure 34 ^{1}H NMR (500 MHz) Spectrum of compound BSB3 (CD_3OD)

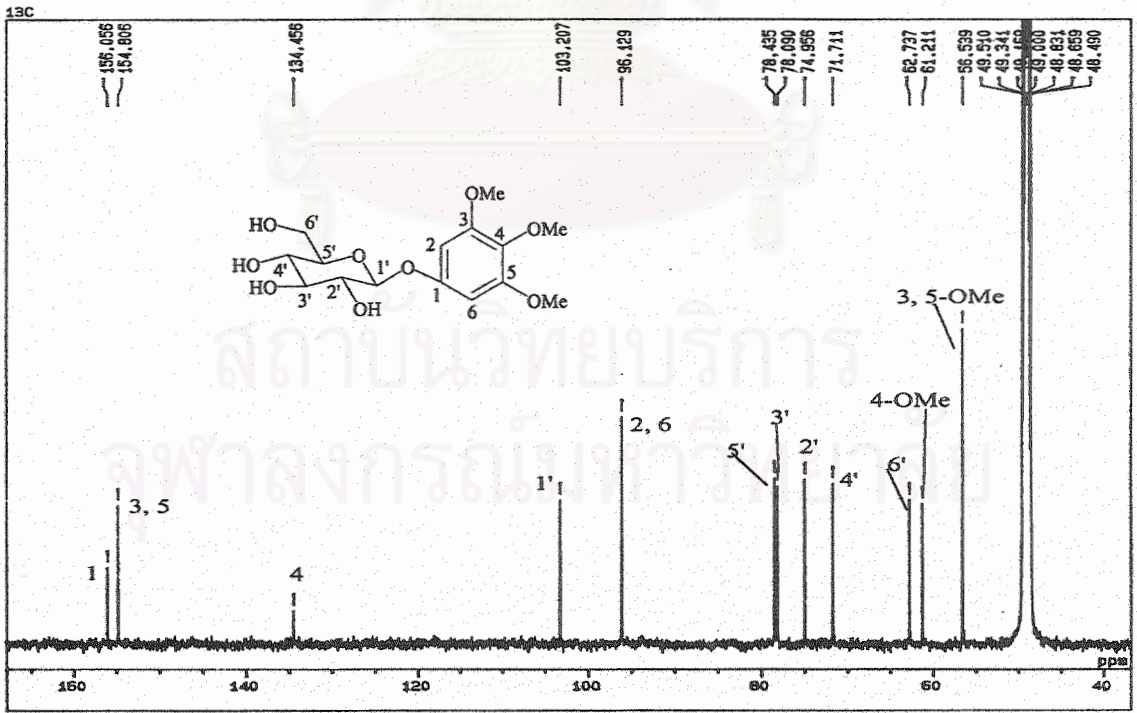


Figure 35 ^{13}C NMR (125 MHz) Spectrum of compound BSB3 (CD_3OD)

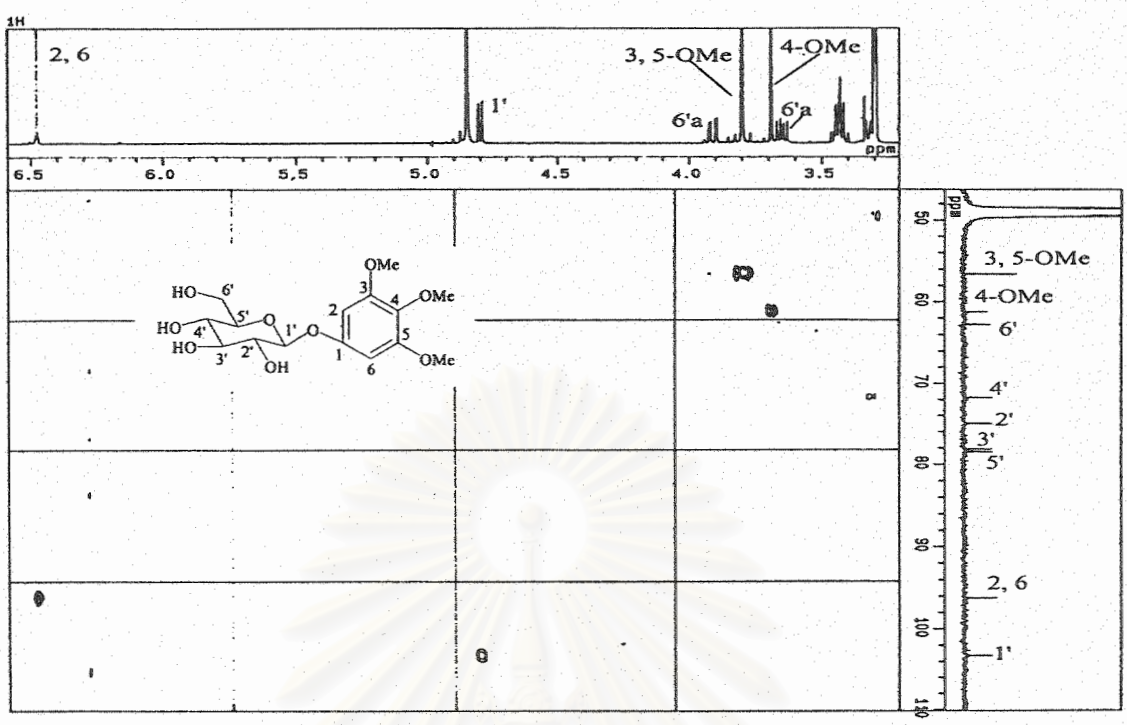


Figure 36 HMQC Spectrum of compound BSB3 (CD_3OD)

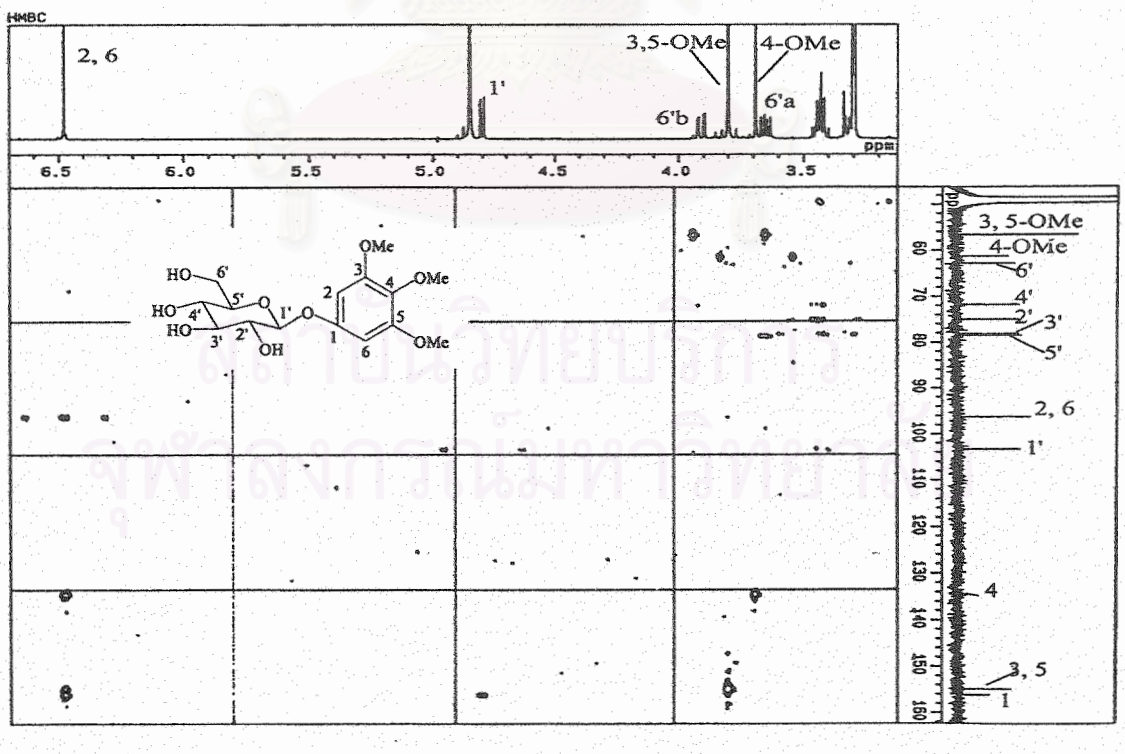


Figure 37 HMBC Spectrum of compound BSB3 (CD_3OD)

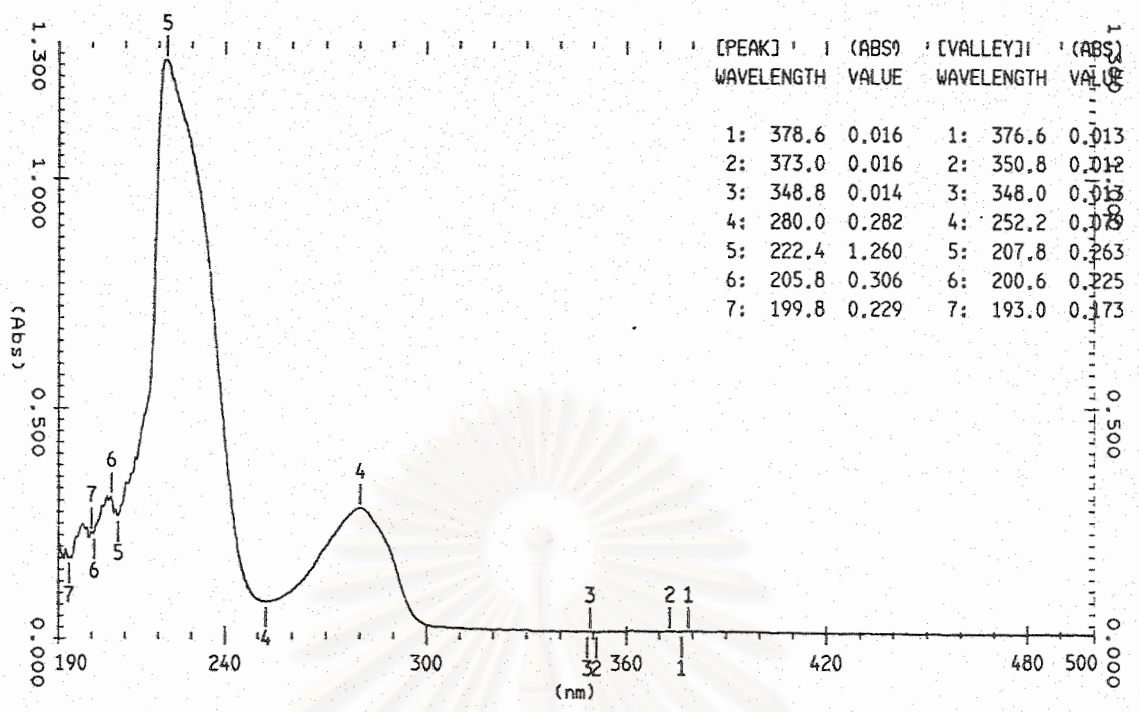


Figure 38 UV Spectrum of compound BSB4 (methanol)

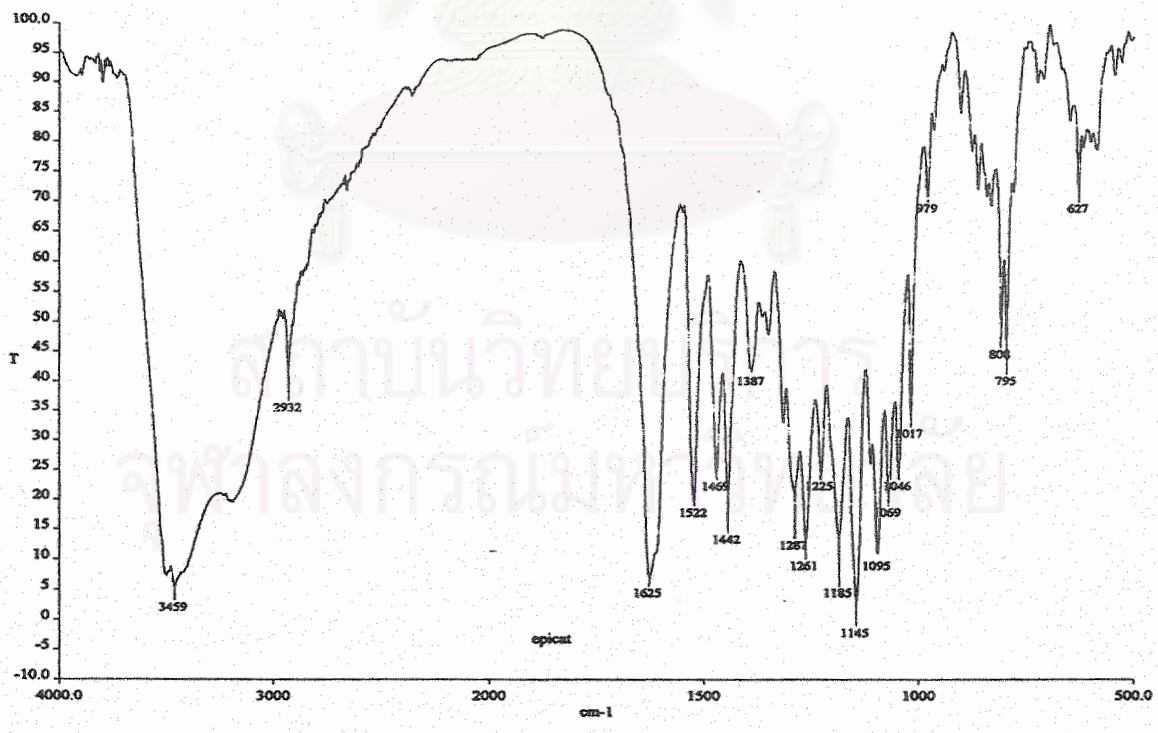


Figure 39 IR Spectrum of compound BSB4 (KBr disc)

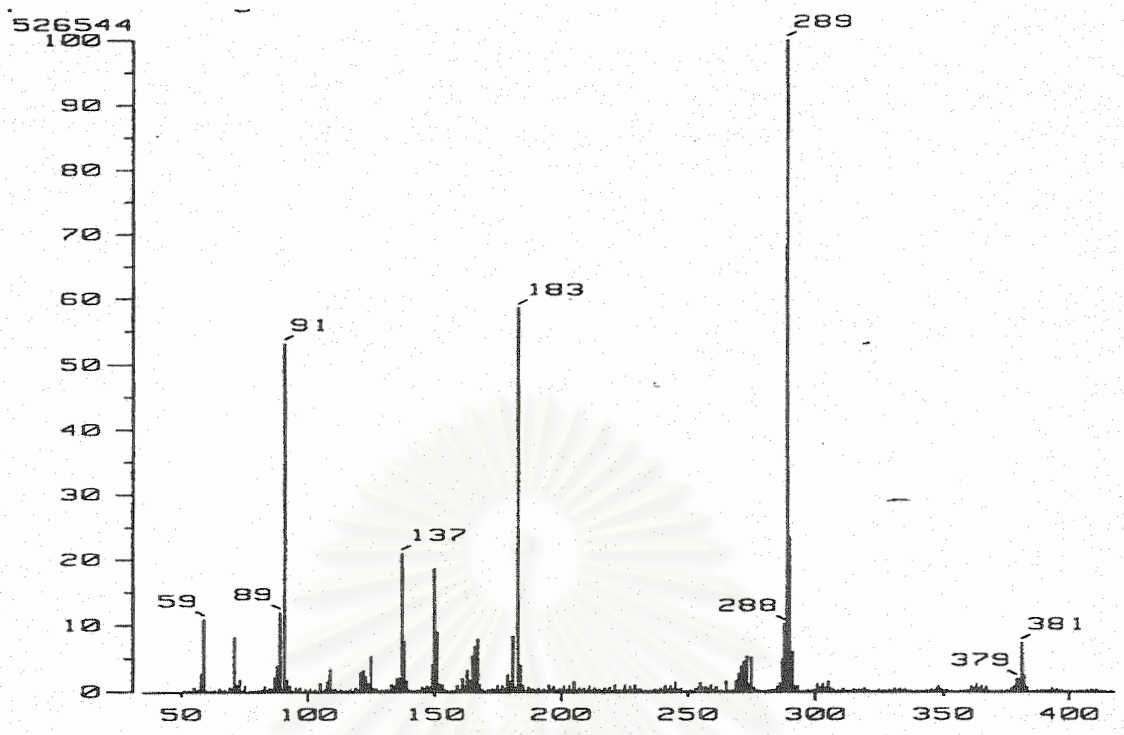


Figure 40 FAB MS Mass spectrum of compound BSB4

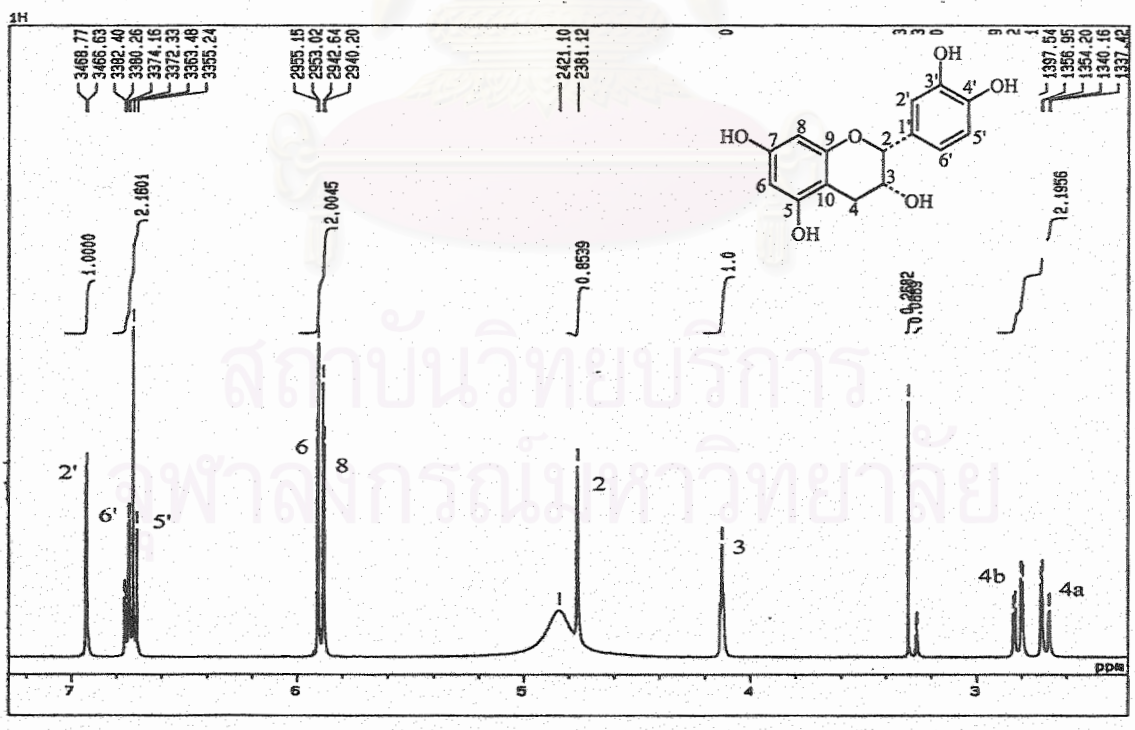


Figure 41 ^{1}H NMR (500 MHz) Spectrum of compound BSB4 (CD_3OD)

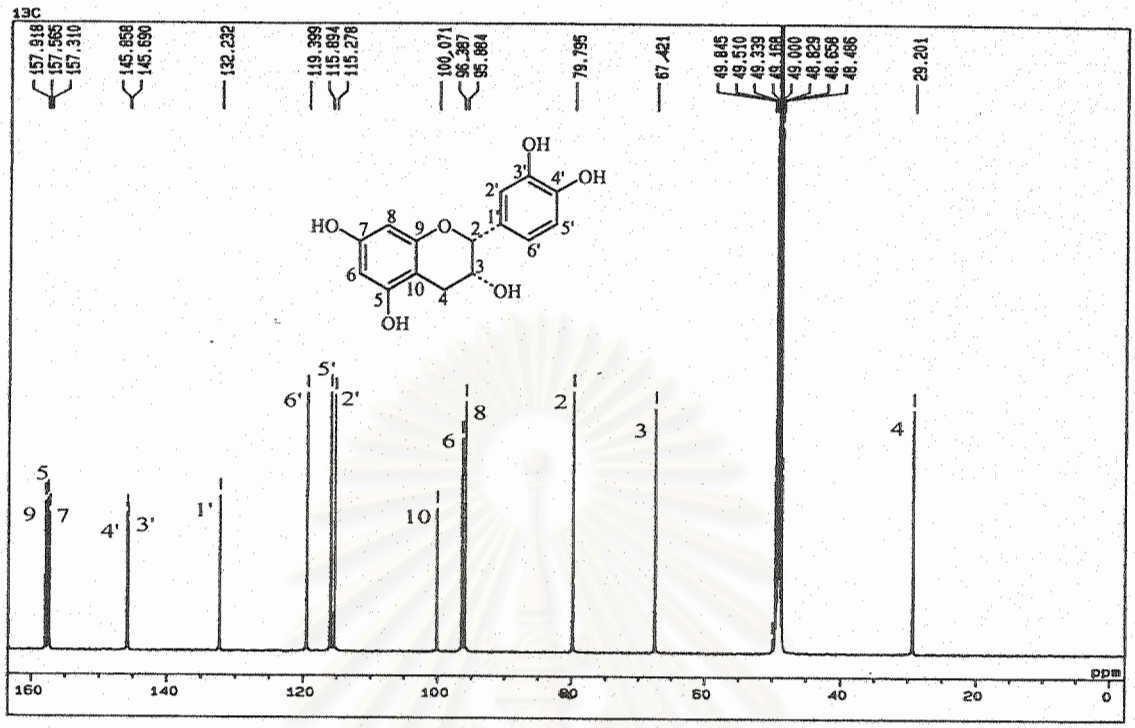


Figure 42 ^{13}C NMR (125 MHz) Spectrum of compound BSB4 (CD_3OD)

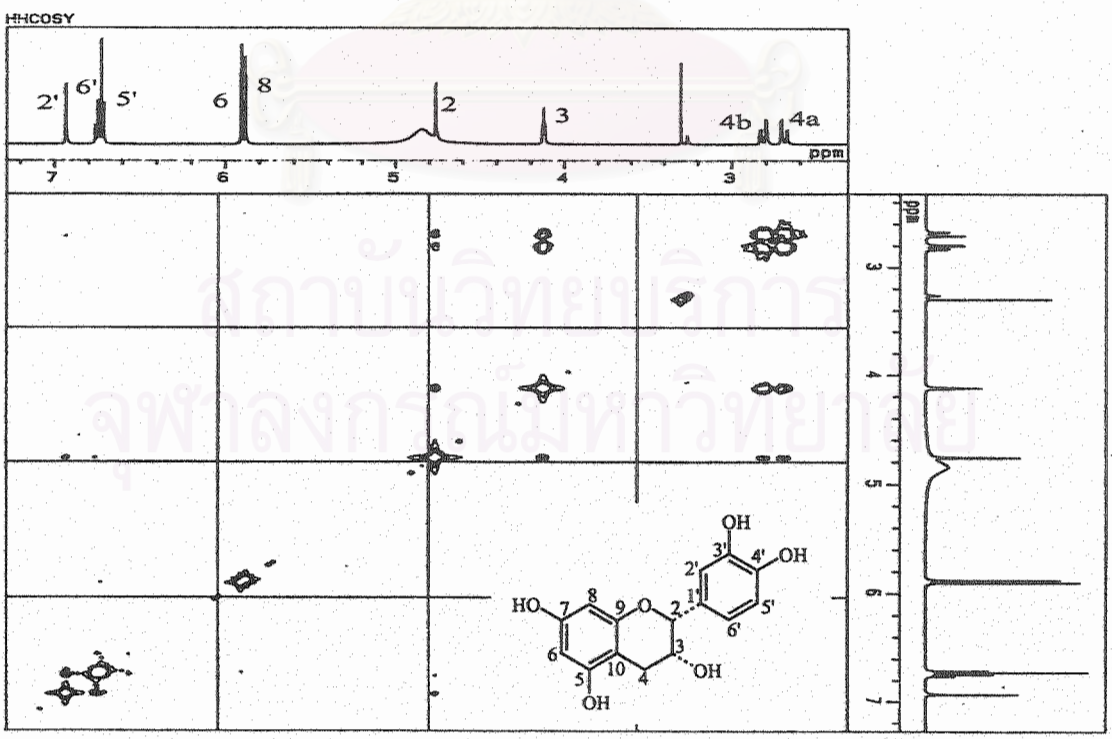


Figure 43 ^{1}H-^{1}H COSY Spectrum of compound BSB4 (CD_3OD)

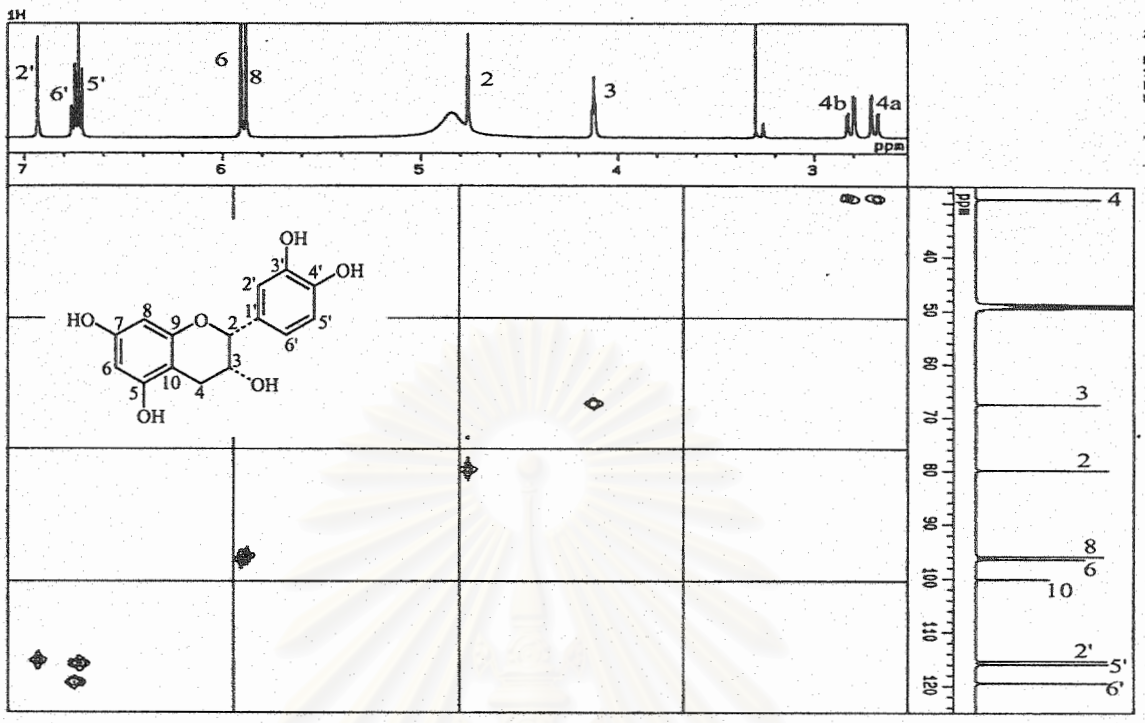


Figure 44 HMQC Spectrum of compound BSB4 (CD_3OD)

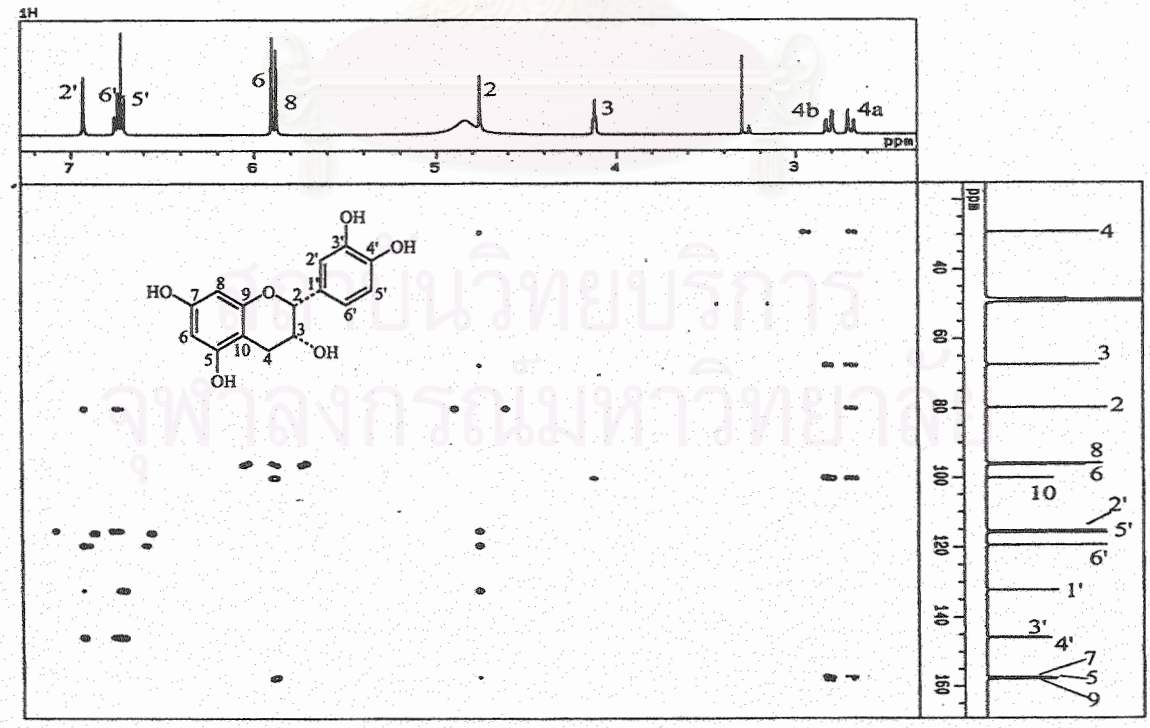


Figure 45 HMBC Spectrum of compound BSB4 (CD_3OD)

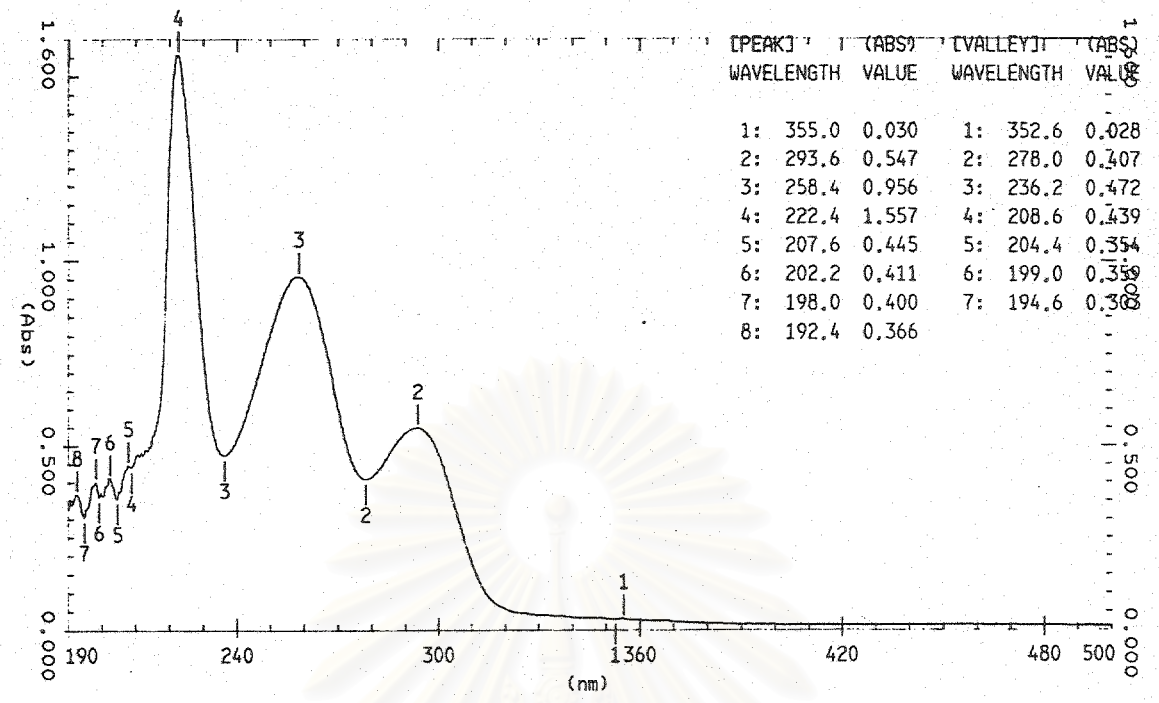


Figure 46 UV Spectrum of compound BSB5 (methanol)

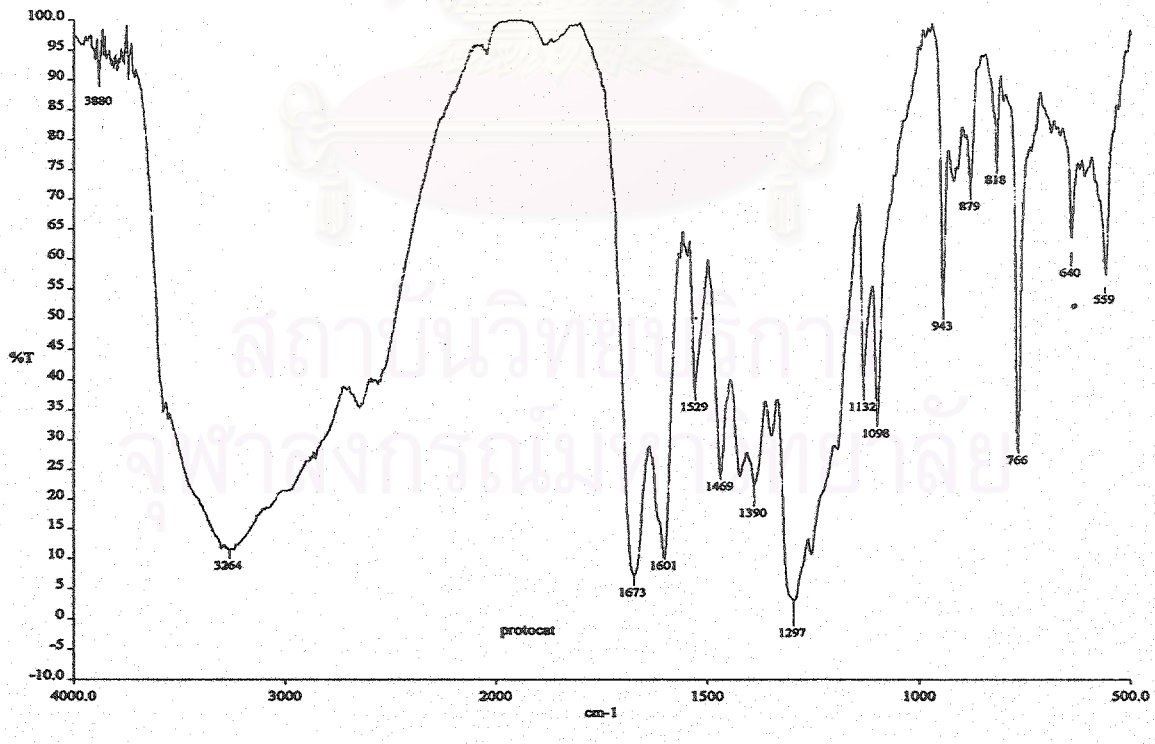


Figure 47 IR Spectrum of compound BSB5 (KBr disc)

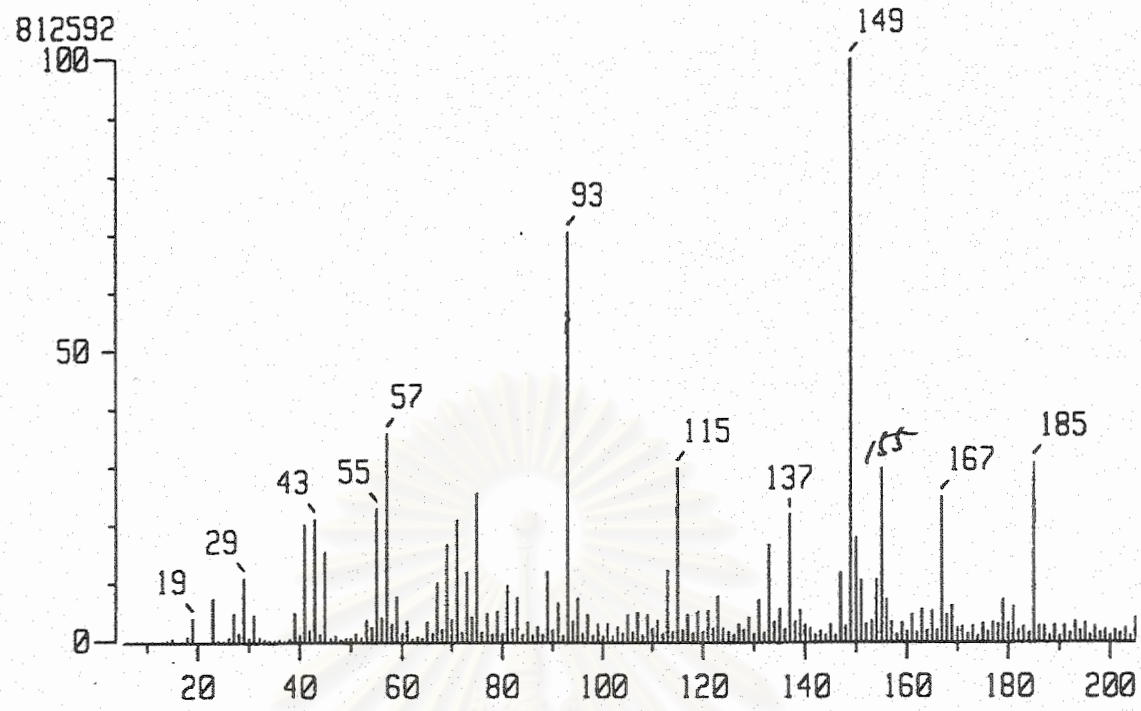


Figure 48 FAB$^+$MS Mass spectrum of compound BSB5

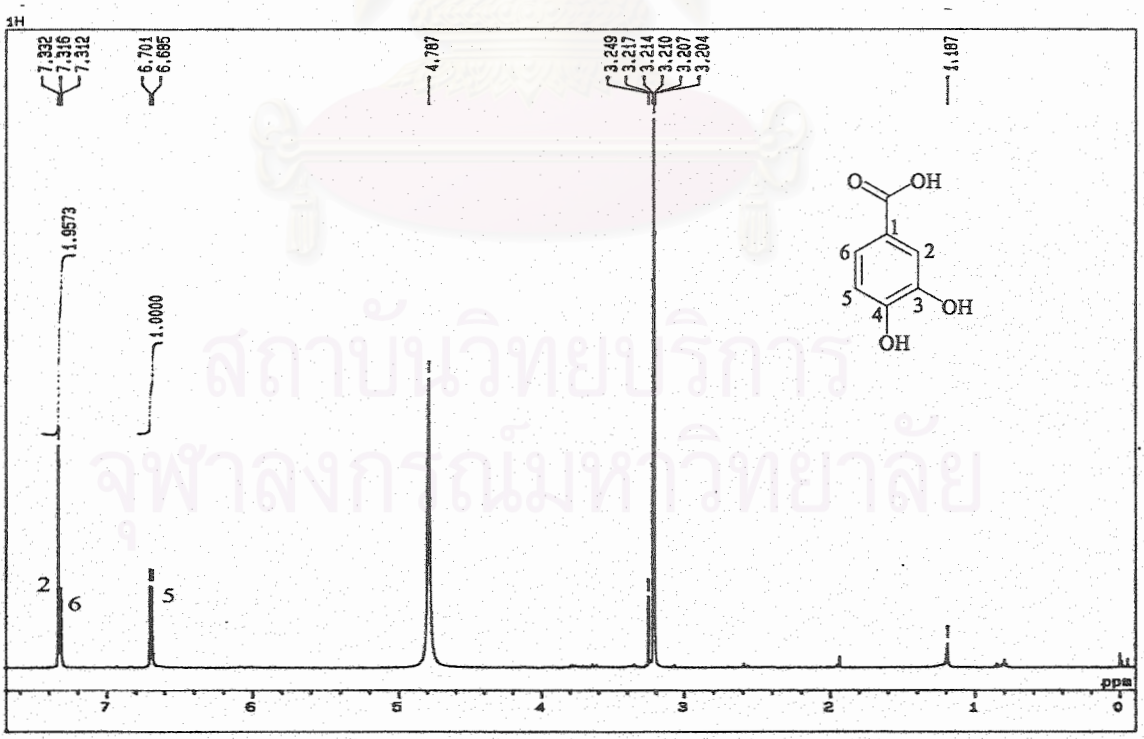


Figure 49 ^{1}H NMR (500 MHz) Spectrum of compound BSB5 (CD_3OD)

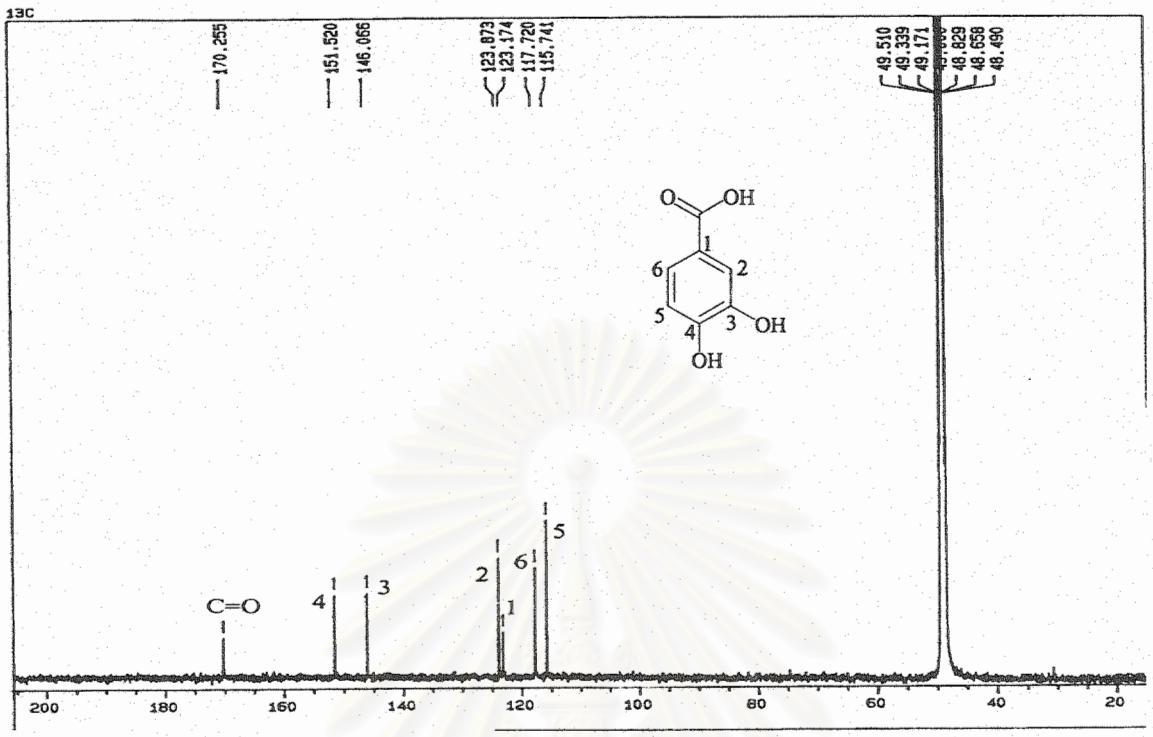


Figure 50 ^{13}C NMR (125 MHz) Spectrum of compound BSB5 (CD_3OD)

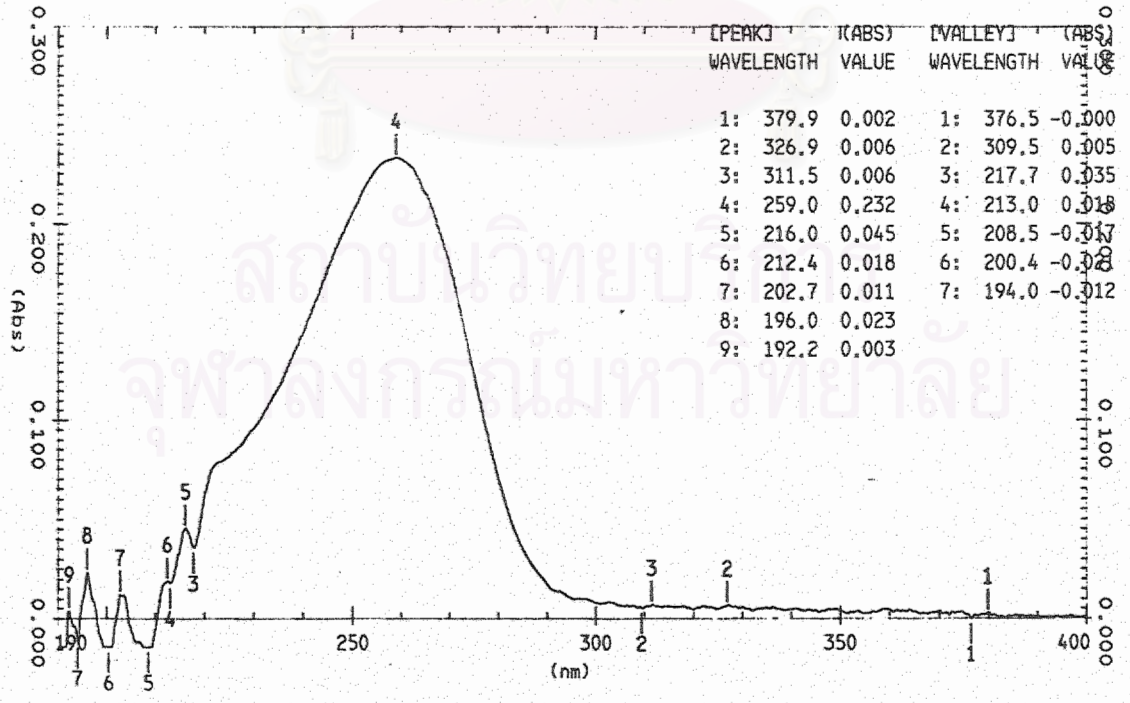


Figure 51 UV Spectrum of compound BSB6 (water)

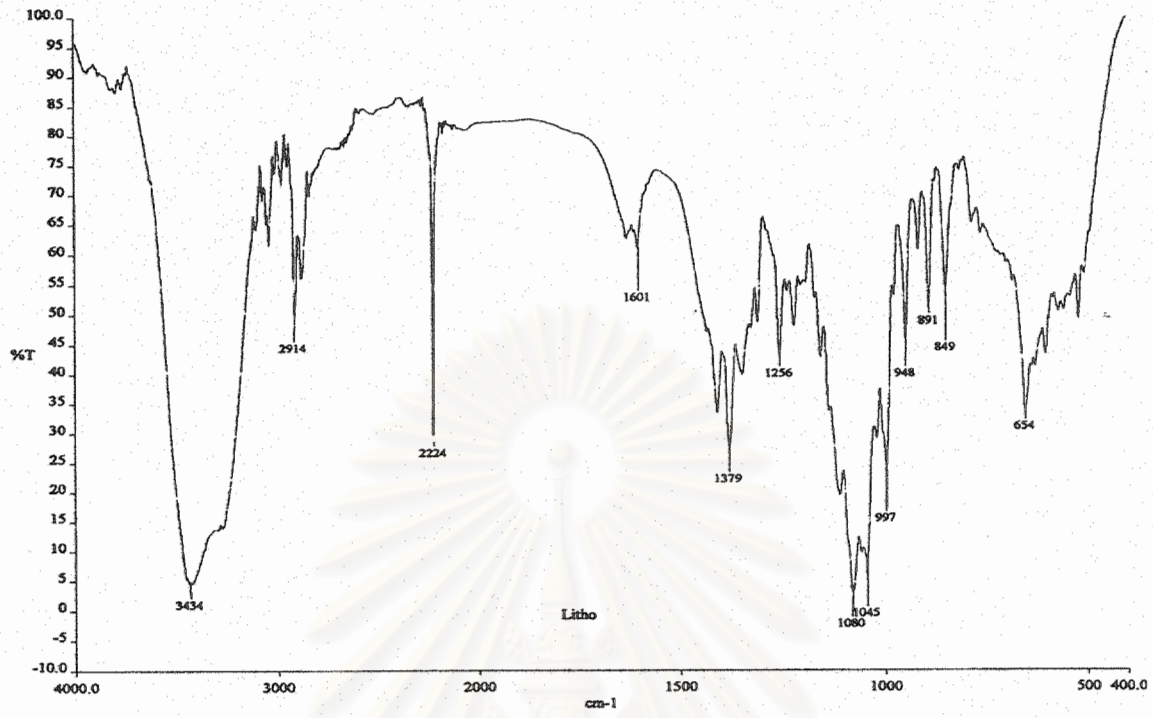


Figure 52 IR Spectrum of compound BSB6 (KBr disc)

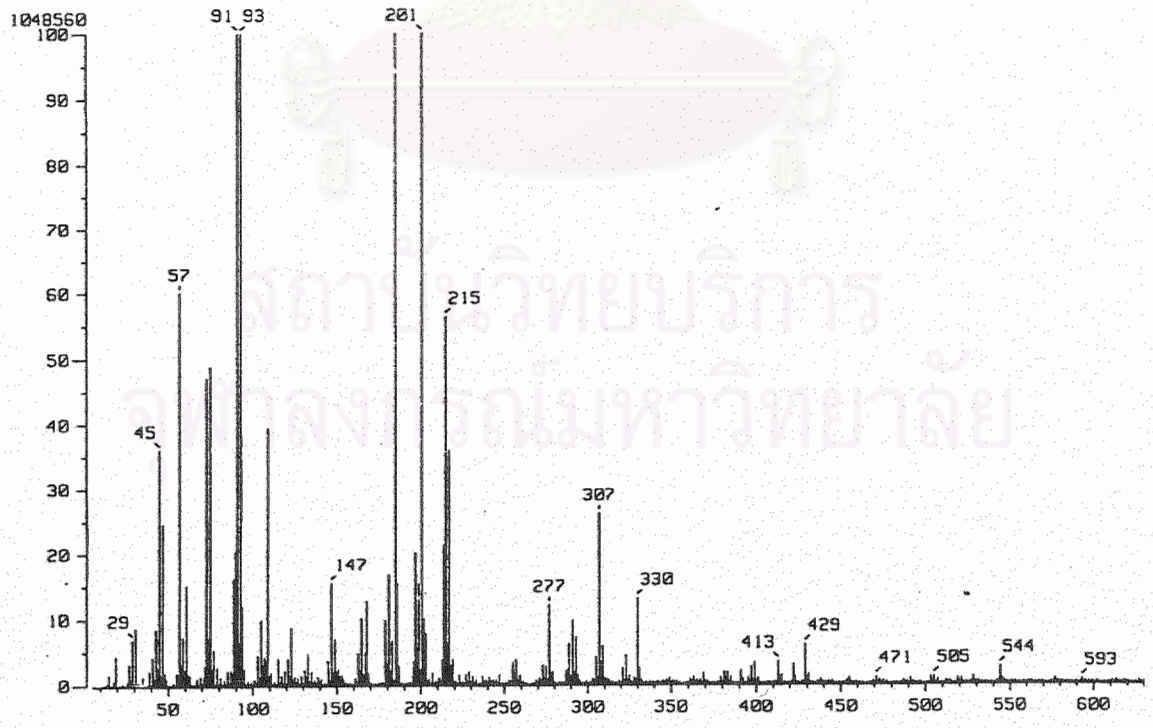


Figure 53 FAB^{+}MS Mass spectrum of compound BSB6

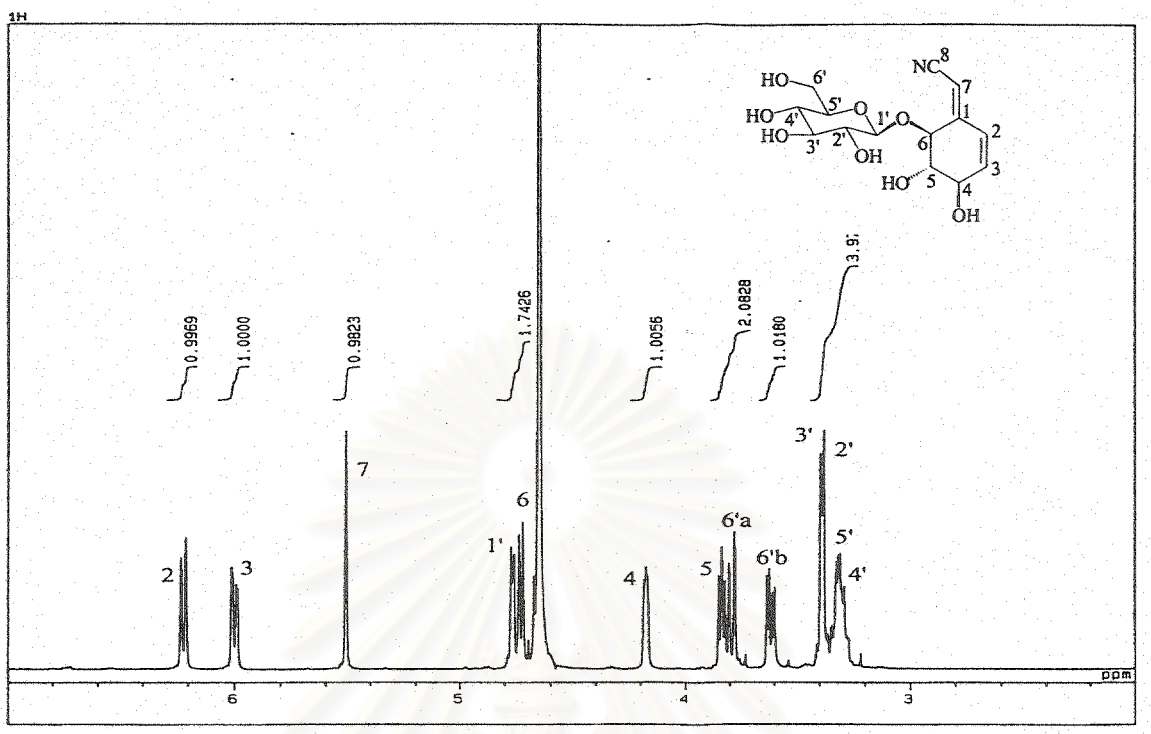


Figure 54 ^{1}H NMR (500 MHz) Spectrum of compound BSB6 (D_2O)

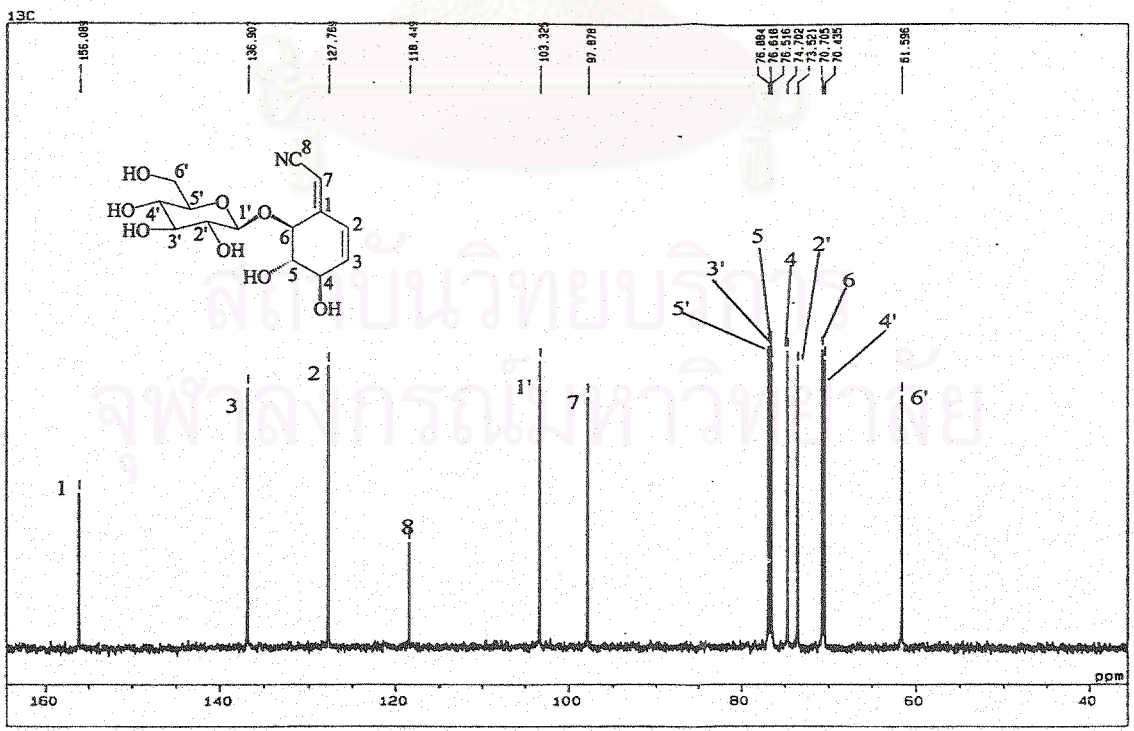


Figure 55 ^{13}C NMR (125 MHz) Spectrum of compound BSB6 (D_2O)

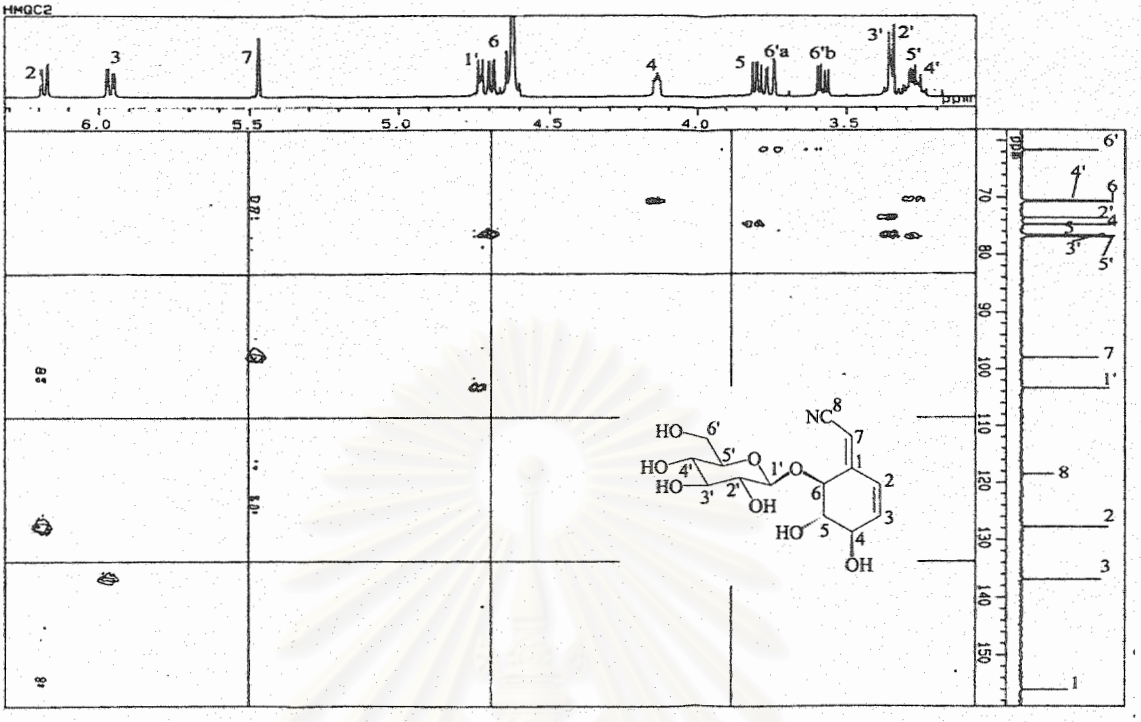


Figure 56 HMQC Spectrum of compound BSB6 (D_2O)

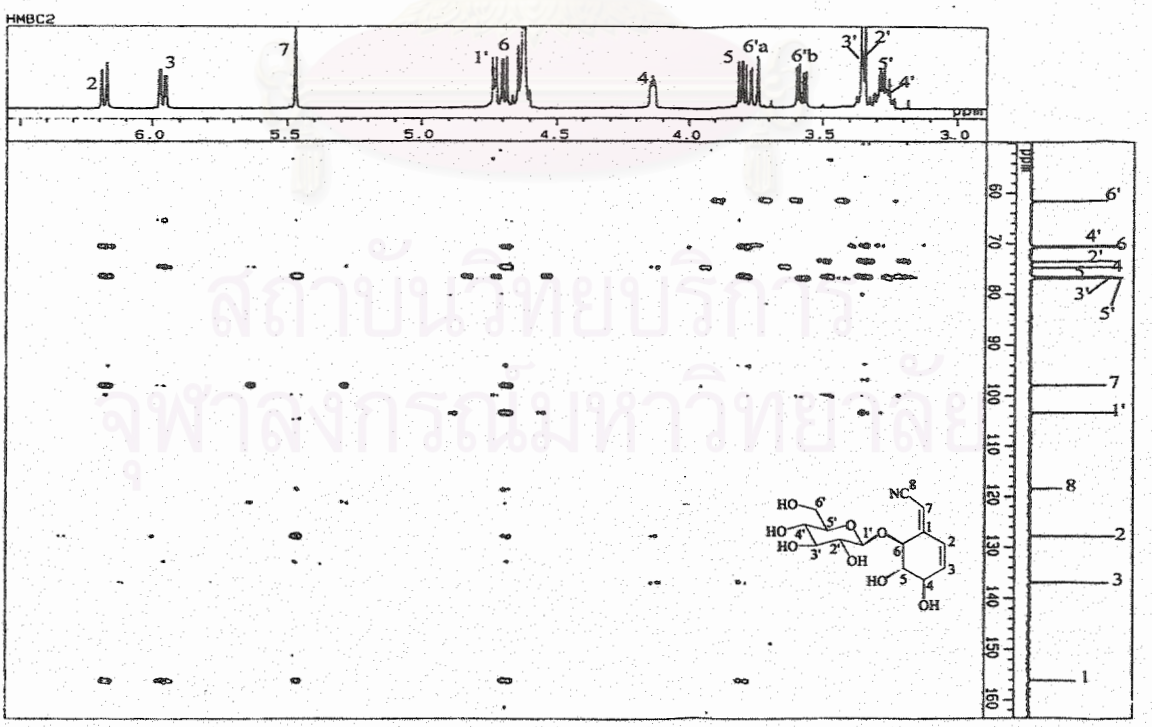


Figure 57 HMBC Spectrum of compound BSB6 (D_2O)

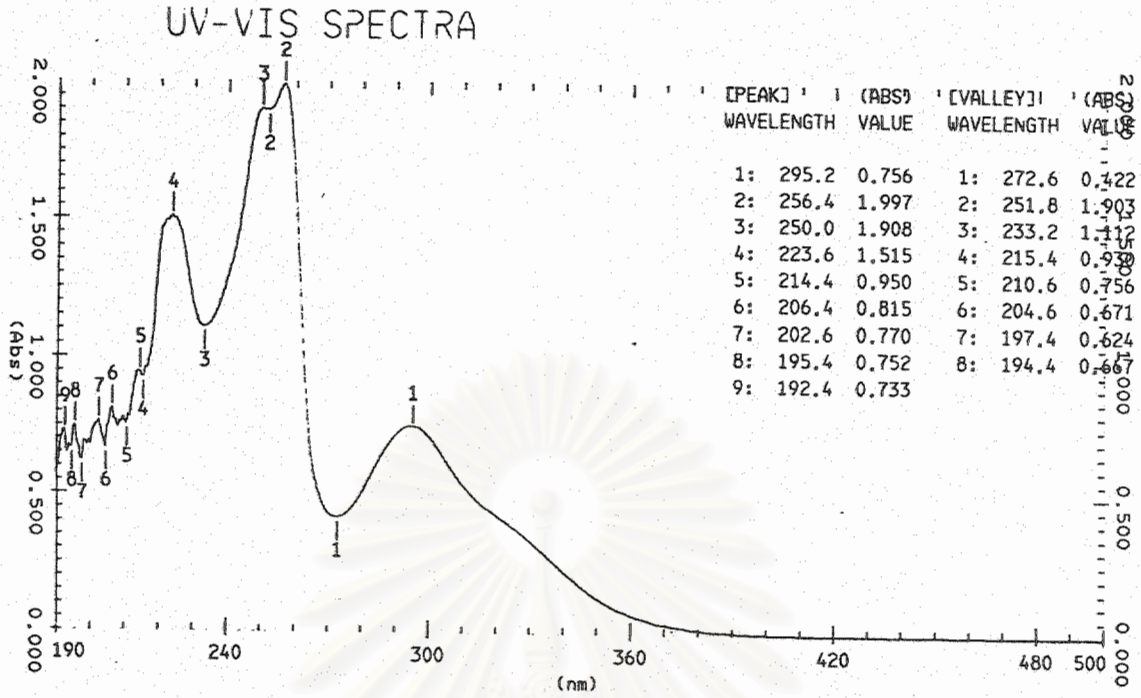


Figure 58 UV Spectrum of compound BRC1 (methanol)

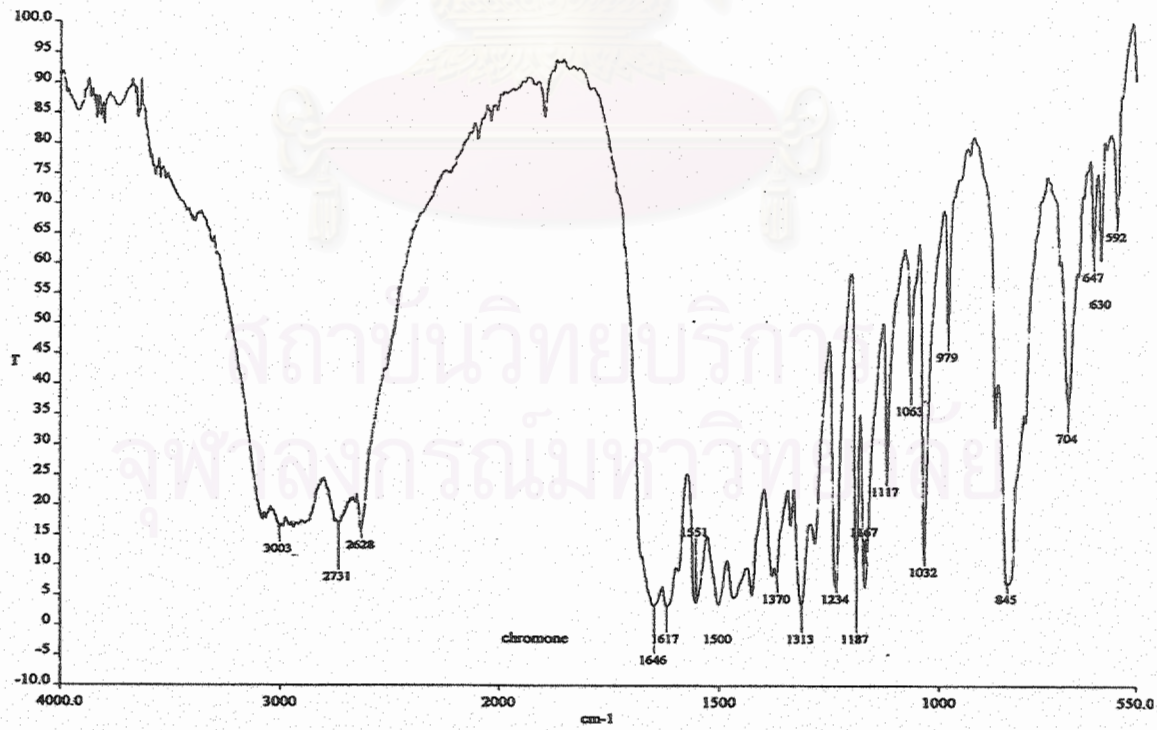


Figure 59 IR Spectrum of compound BRC1 (KBr disc)

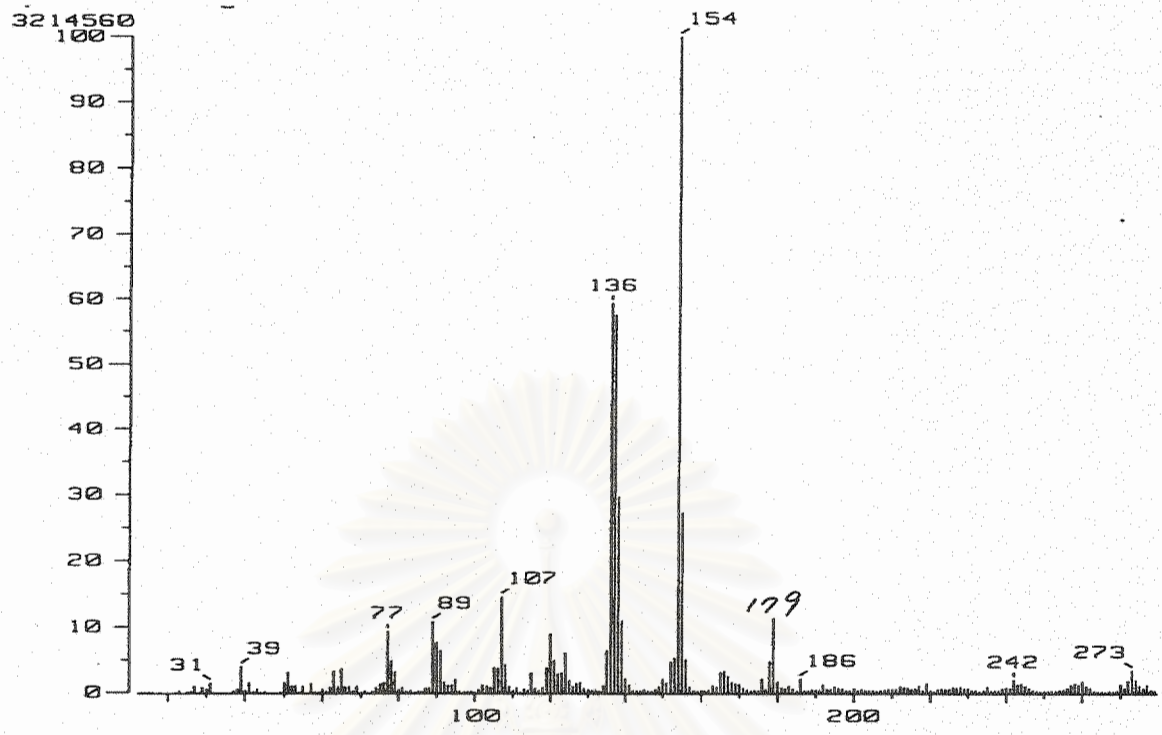


Figure 60 FAB$^+$MS Mass spectrum of compound BRC1

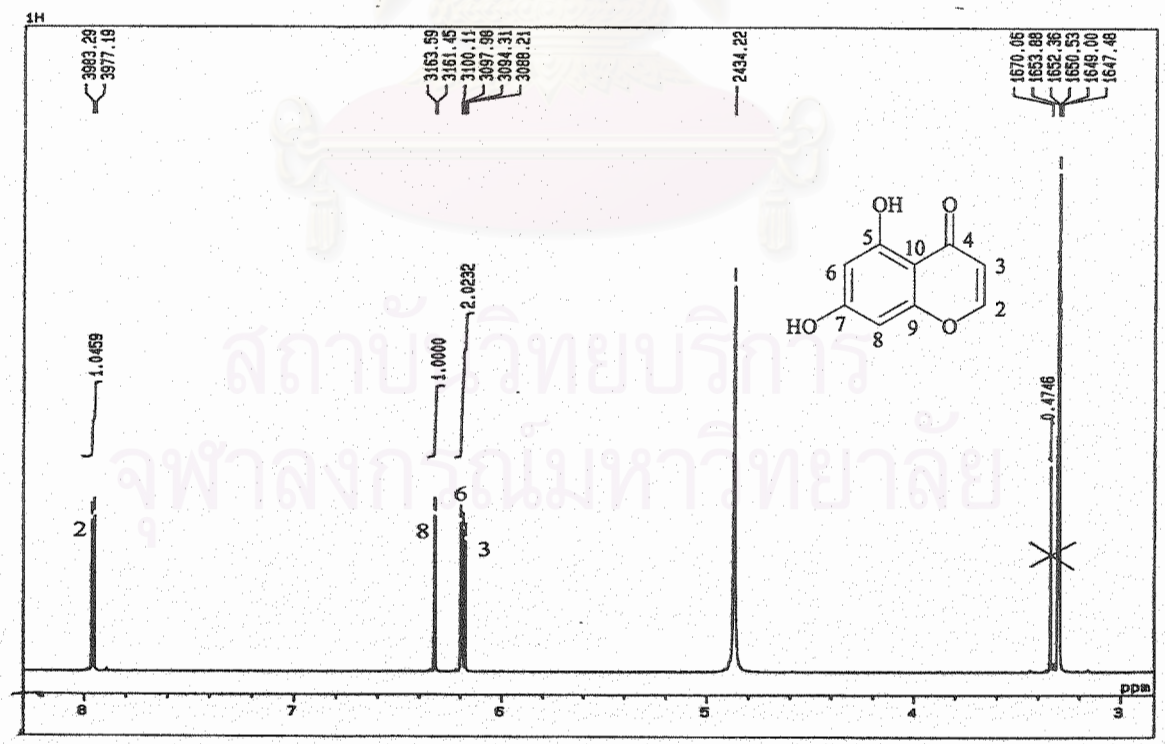


Figure 61 ^{1}H NMR (500 MHz) Spectrum of compound BRC1 (CD_3OD)

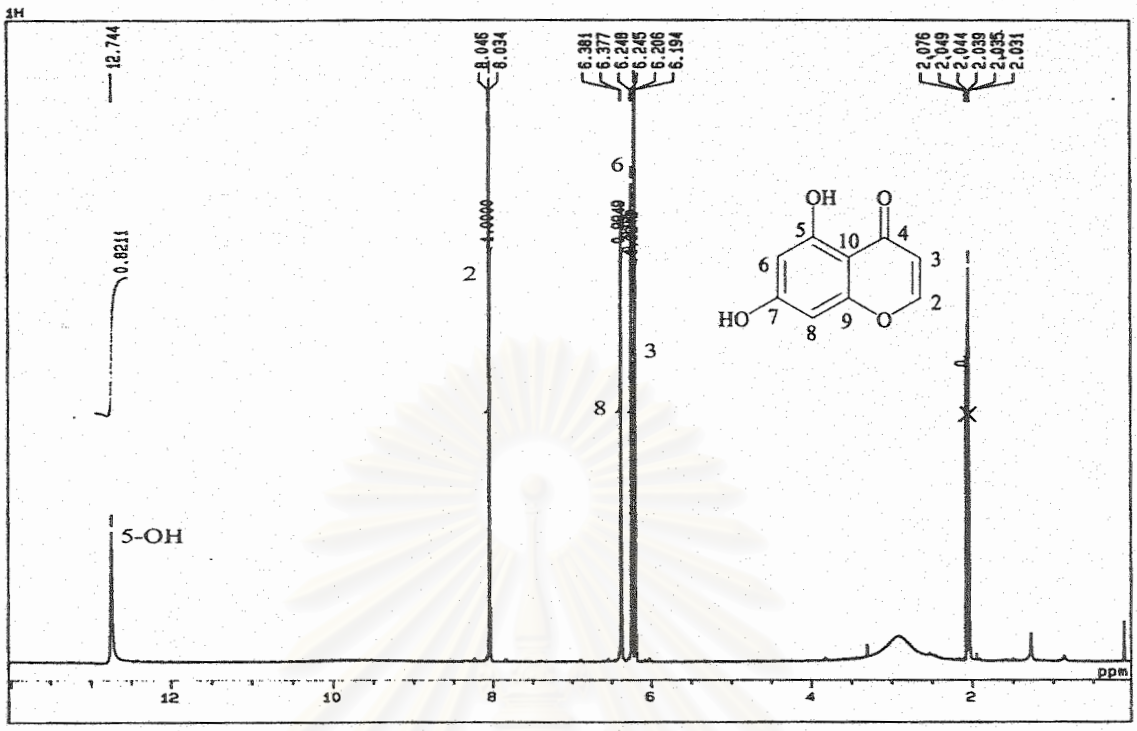


Figure 62 ^{1}H NMR (500 MHz) Spectrum of compound BRC1 (acetone-d_6)

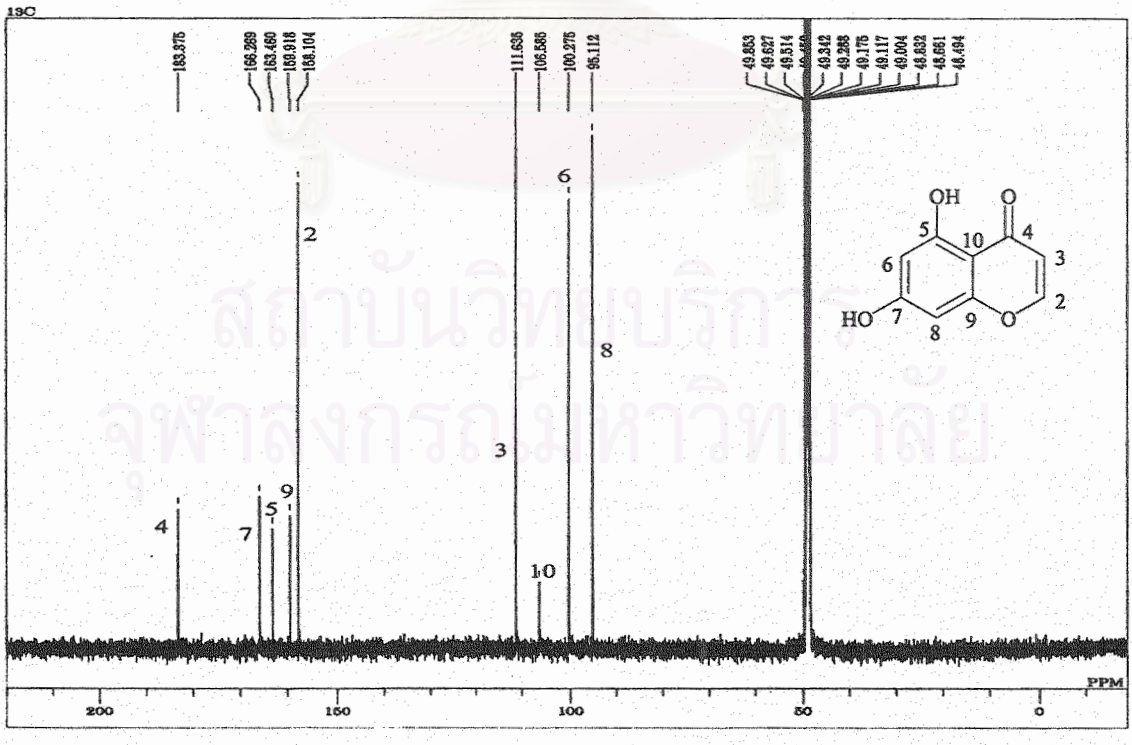


Figure 63 ^{13}C NMR (125 MHz) Spectrum of compound BRC1 (CD_3OD)

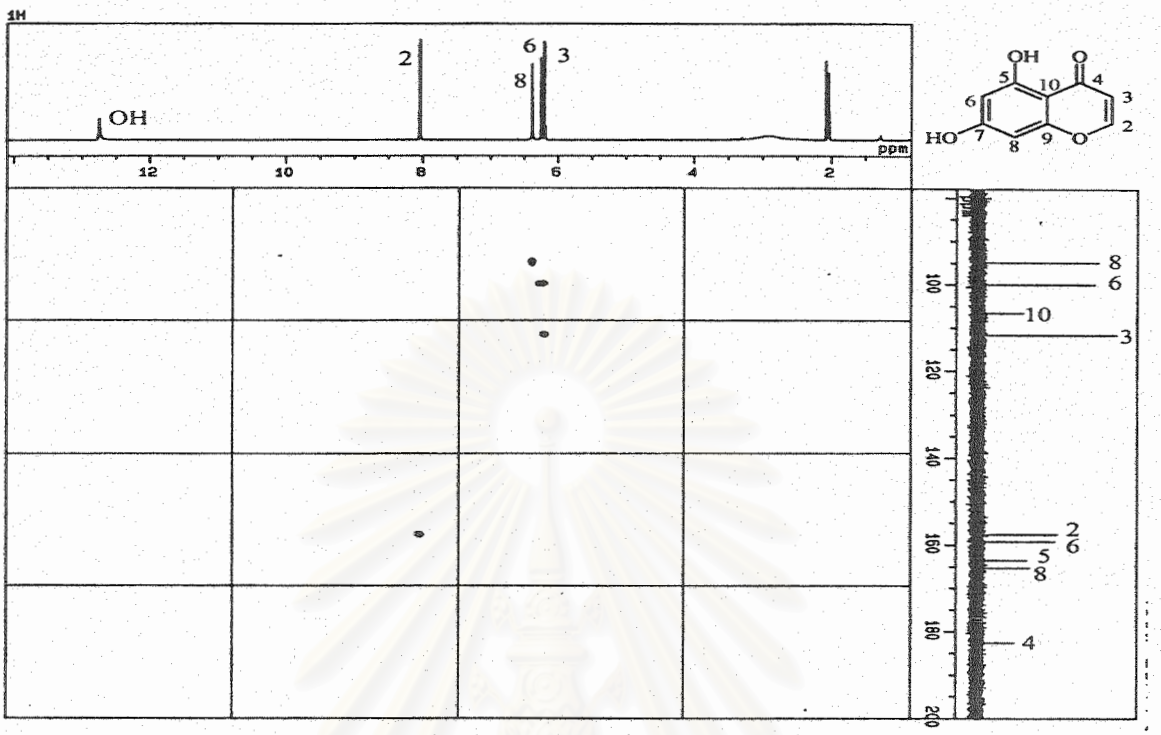


Figure 64 HMQC Spectrum of compound BRC1 (acetone-d_6)

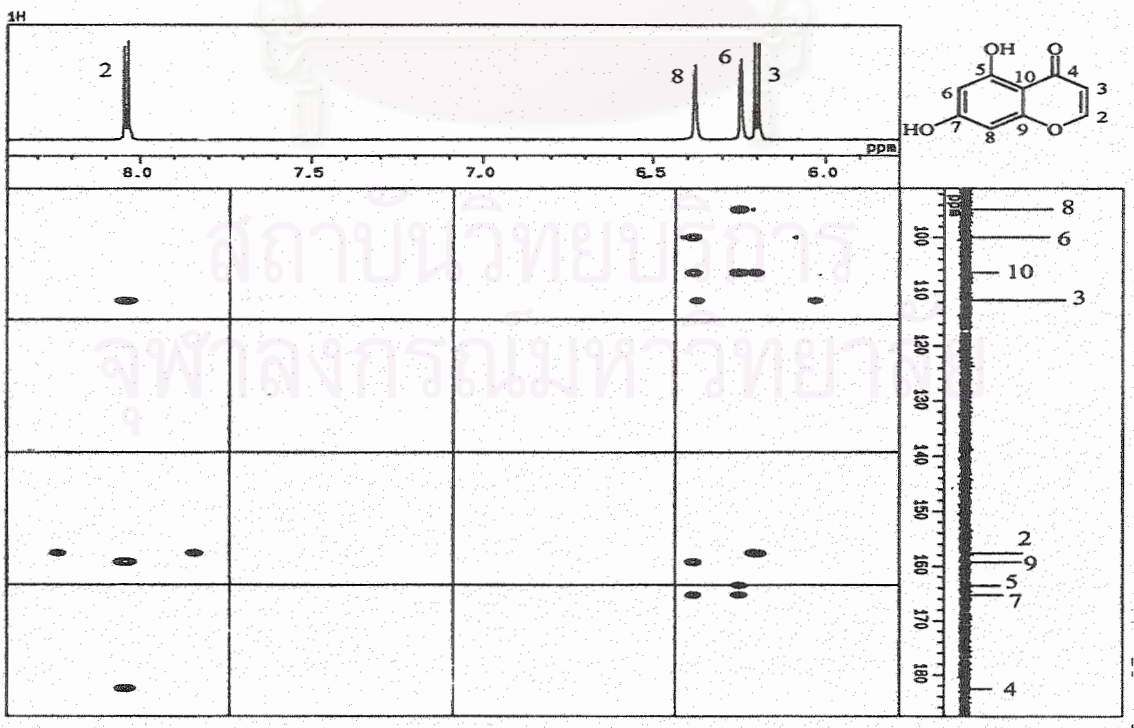


Figure 65 HMBC Spectrum of compound BRC1 (acetone-d_6)

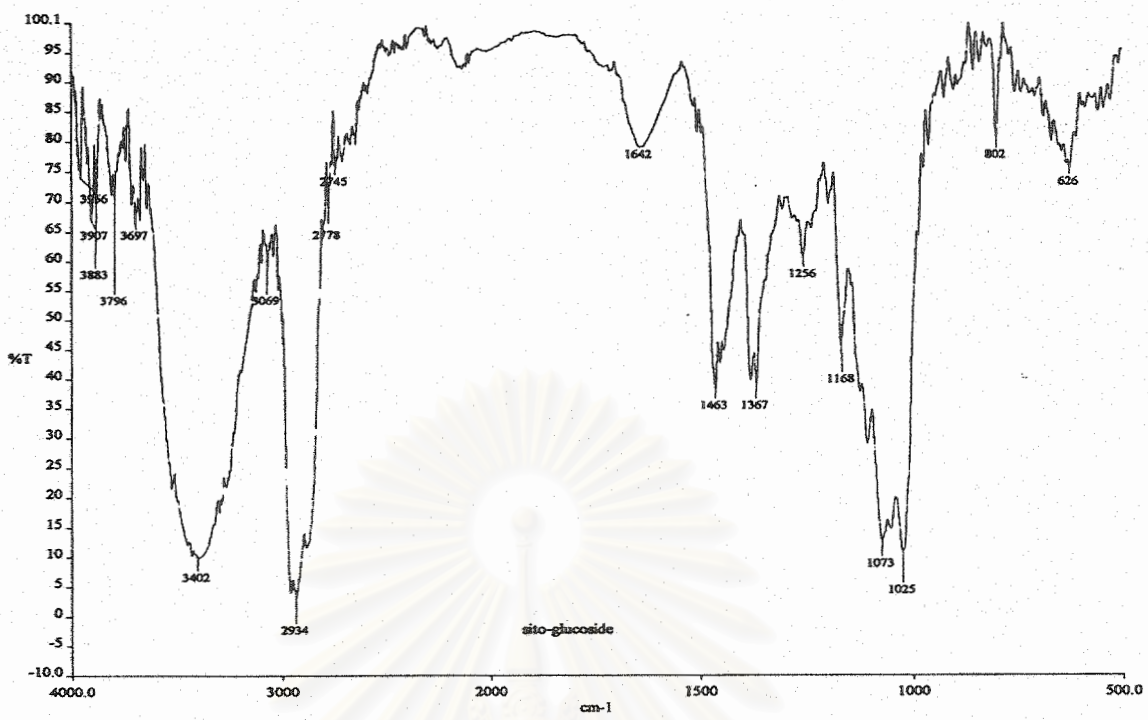


Figure 66 IR Spectrum of compound BRC2 (KBr disc)

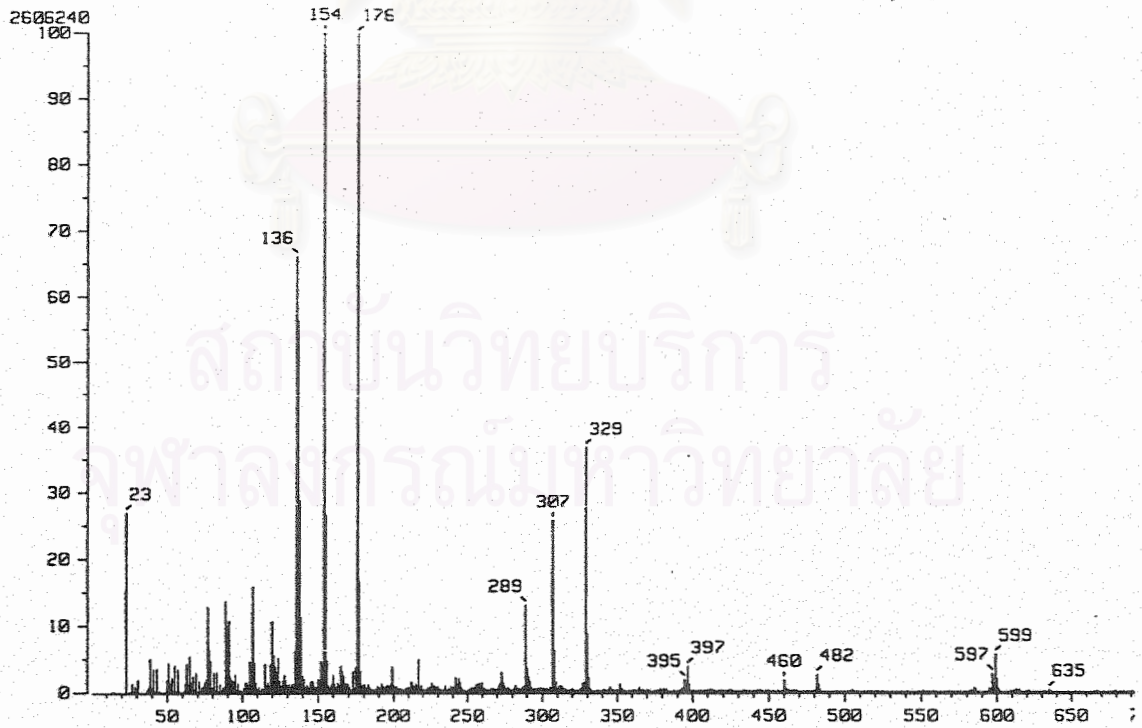


Figure 67 FAB$^+$MS Mass spectrum of compound BRC2

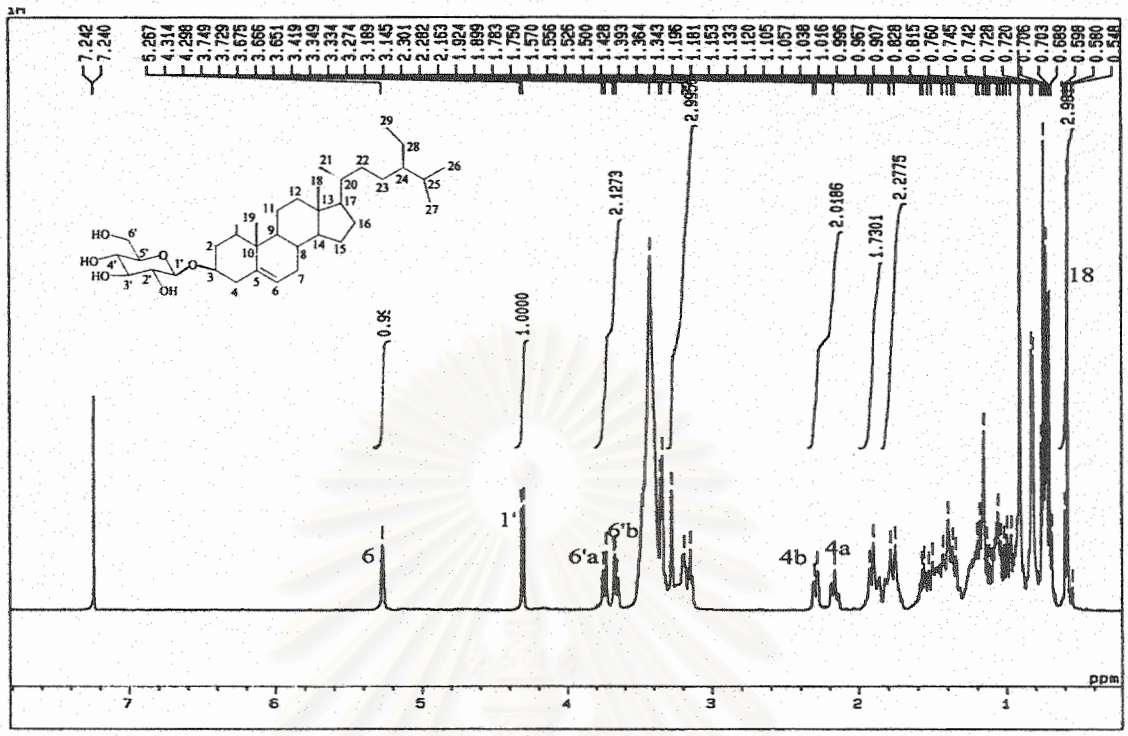


Figure 68 ^{1}H NMR (500 MHz) Spectrum of compound BRC2 ($CDCl_3$ + CD_3OD)

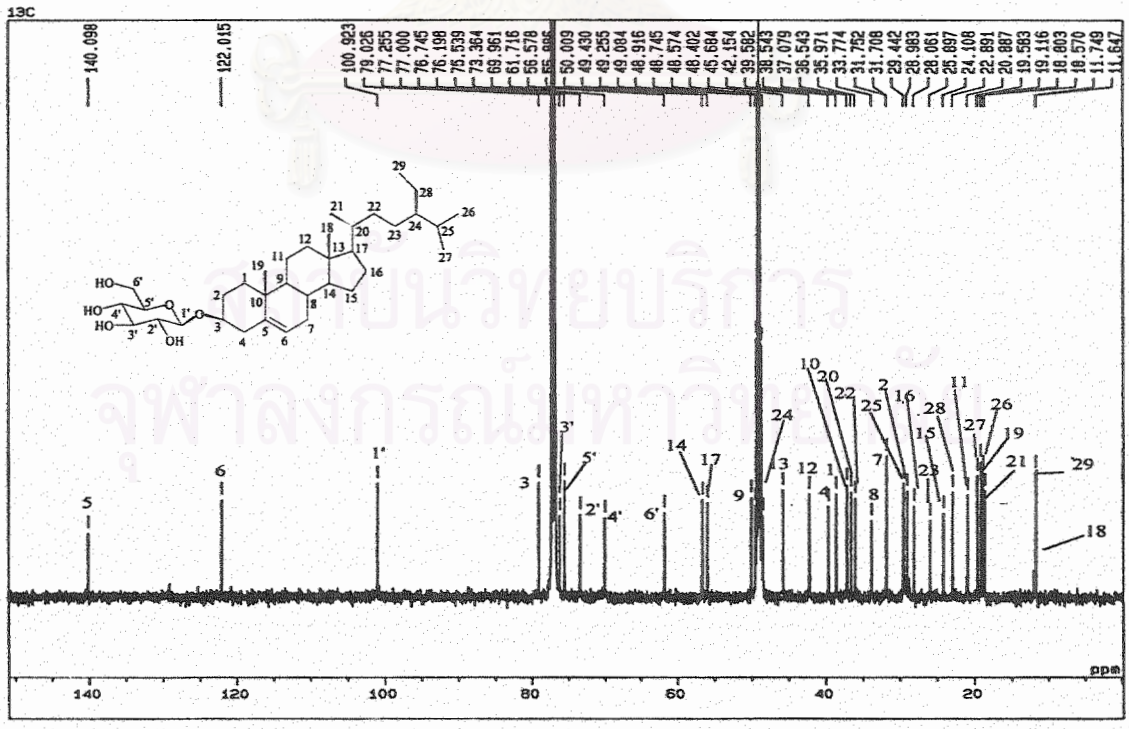


Figure 69 ^{13}C NMR (125 MHz) Spectrum of compound BRC2 ($CDCl_3$ + CD_3OD)

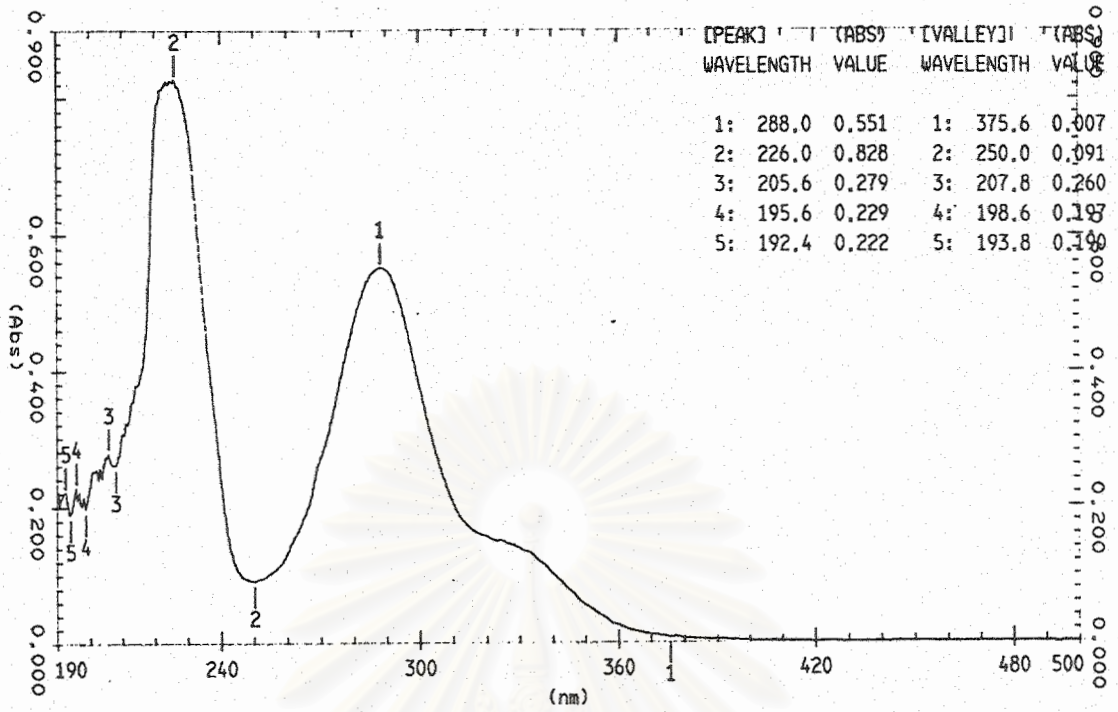


Figure 70 UV Spectrum of compound BRB1 (methanol)

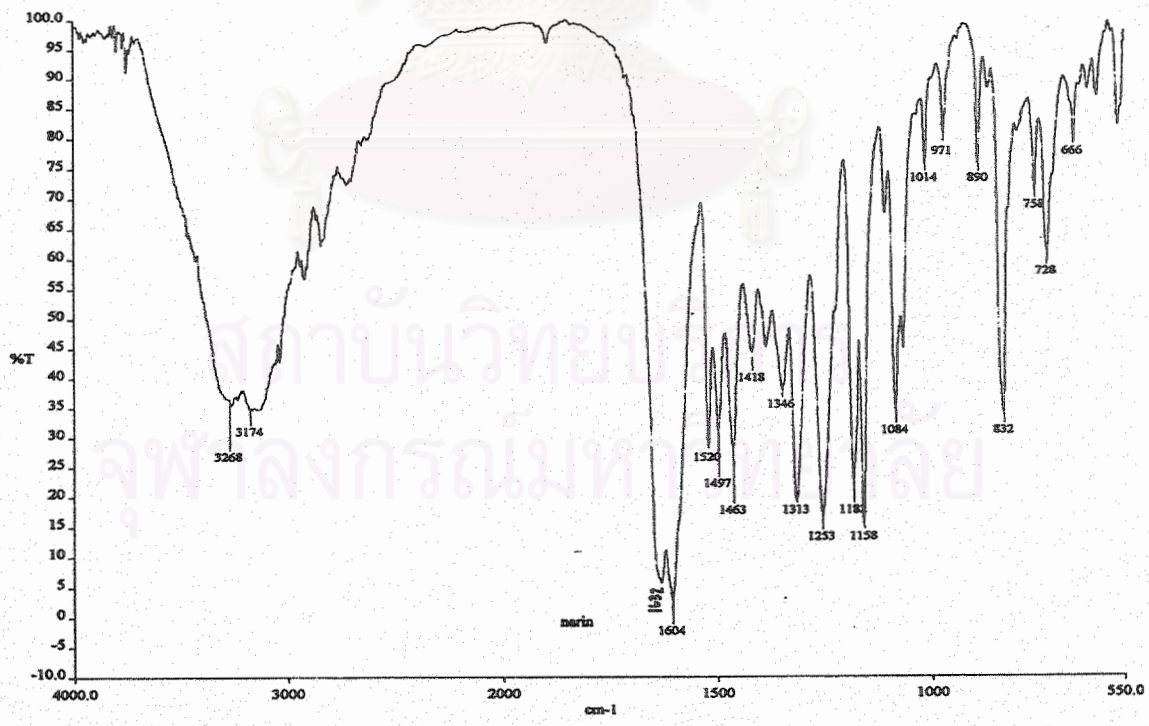


Figure 71 IR Spectrum of compound BRB1 (KBr disc)

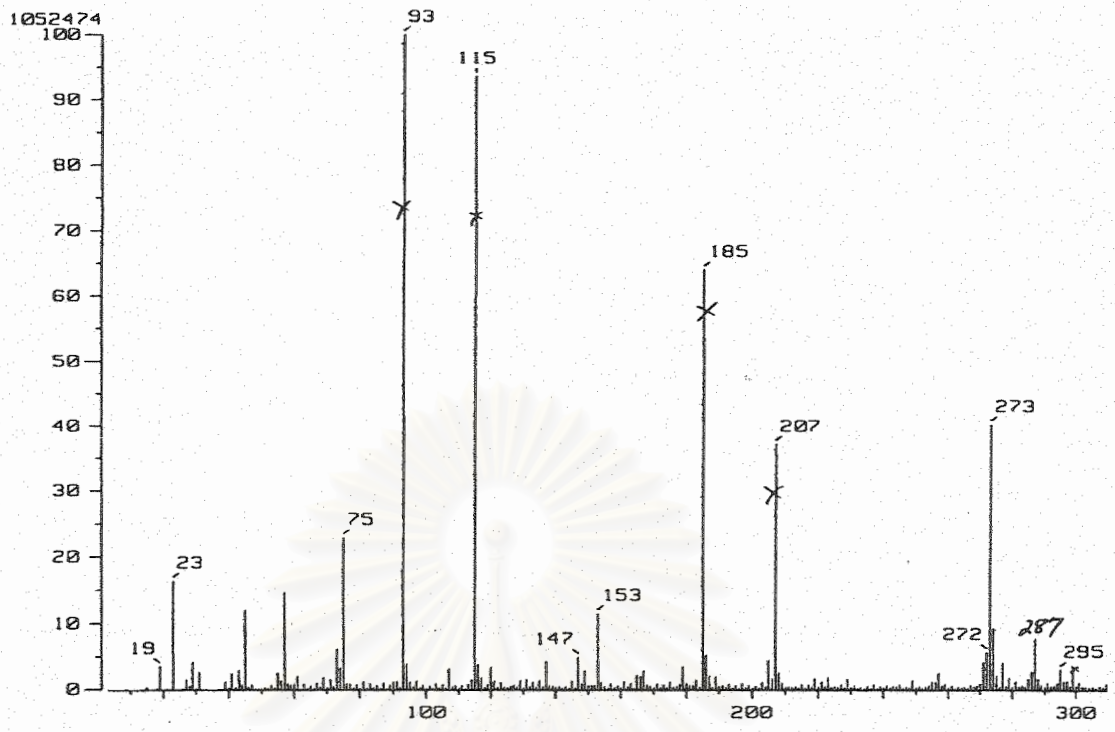


Figure 72 FAB^{+}MS Spectrum of compound BRB1

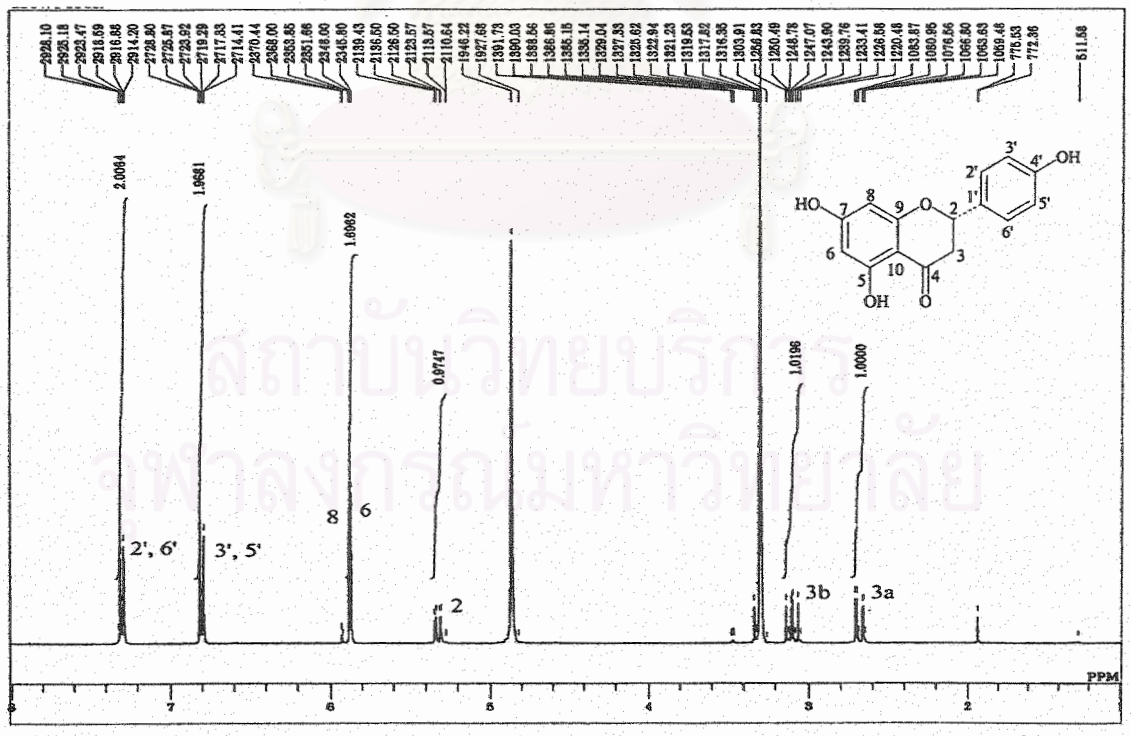


Figure 73 ^{1}H NMR (500 MHz) Spectrum of compound BRB1 (CD_3OD)

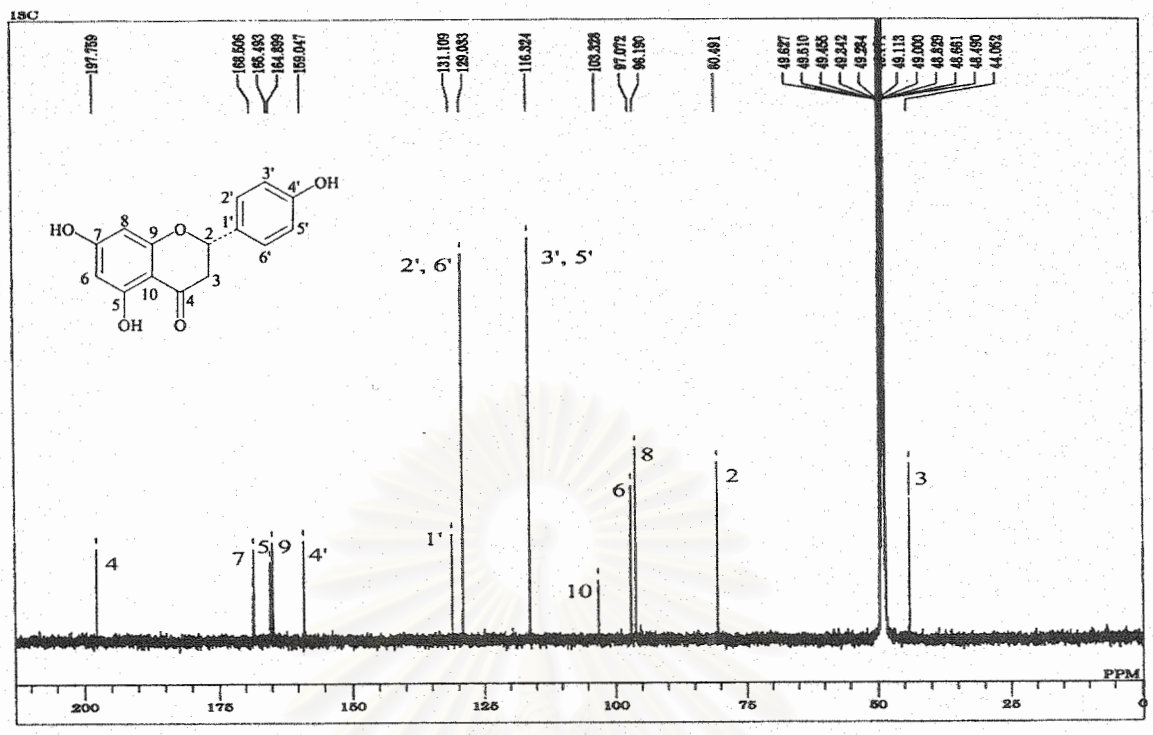


Figure 74 ^{13}C NMR (125 MHz) Spectrum of compound BRB1 (CD_3OD)

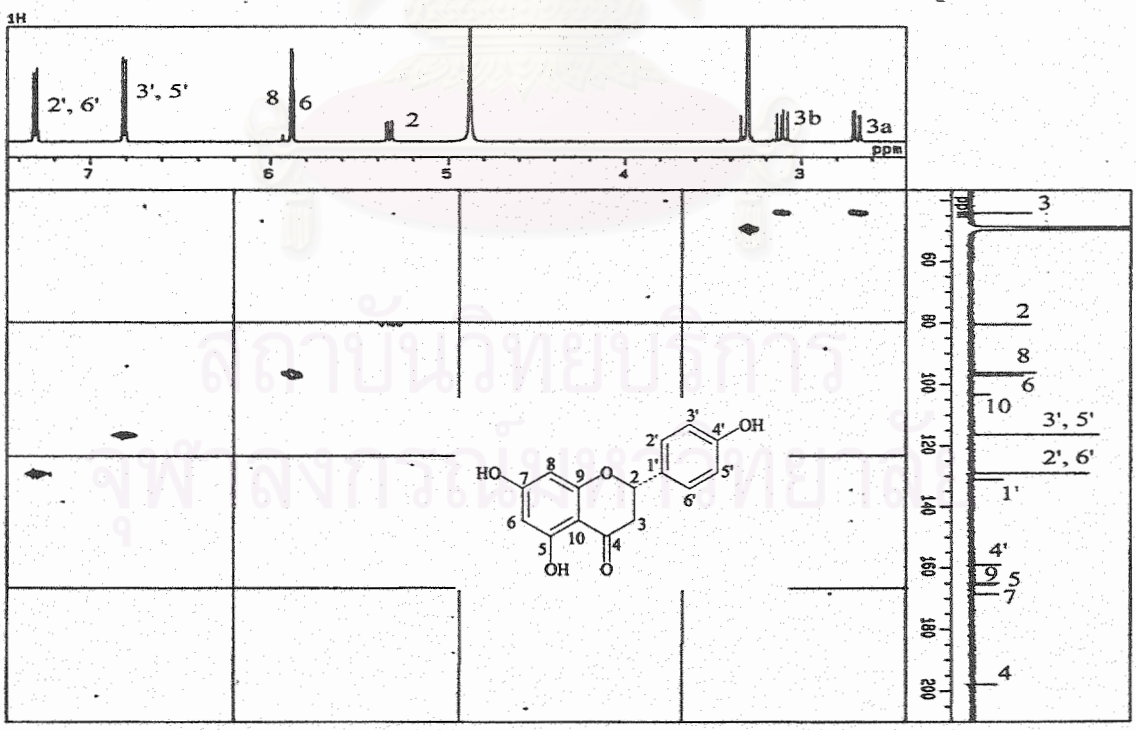


Figure 75 HMQC Spectrum of compound BRB1 (CD_3OD)

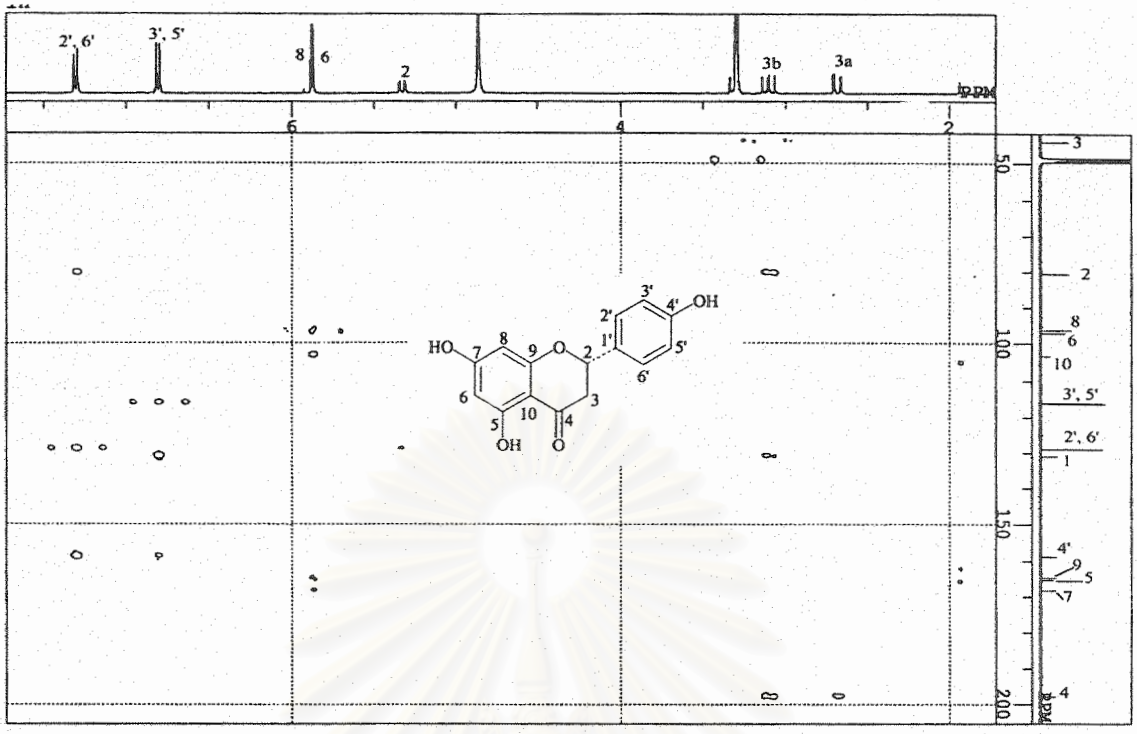


Figure 76 HMBC Spectrum of compound BRB1 (CD_3OD)

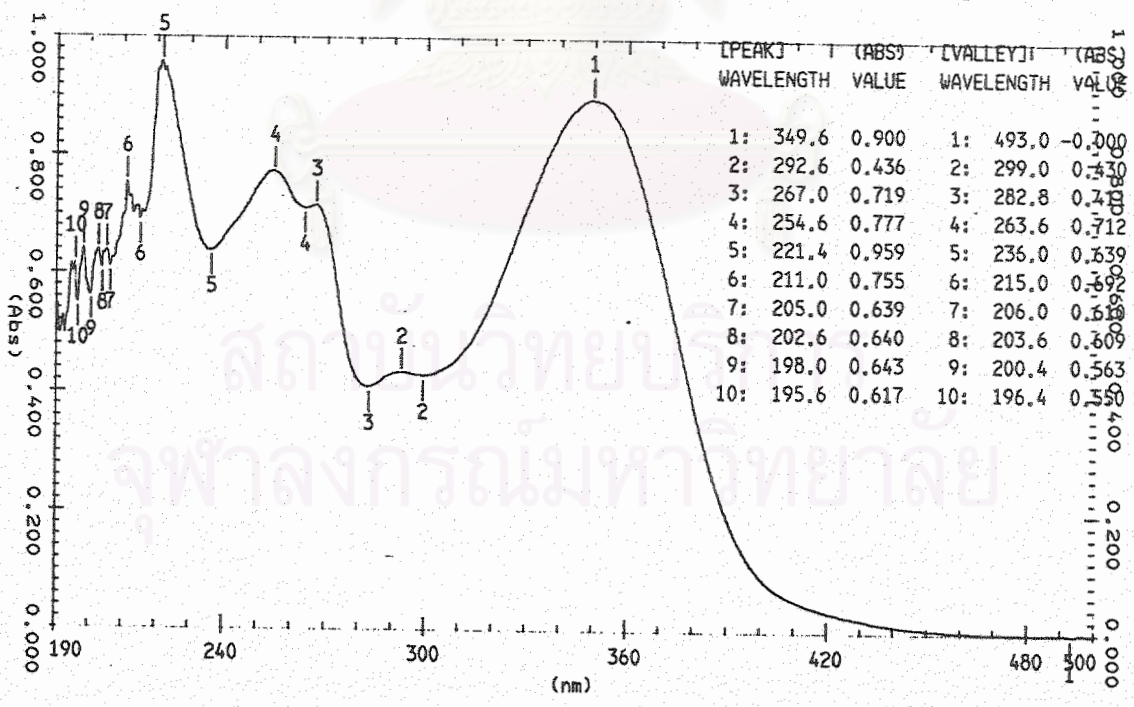


Figure 77 UV Spectrum of compound BRB2 (methanol)

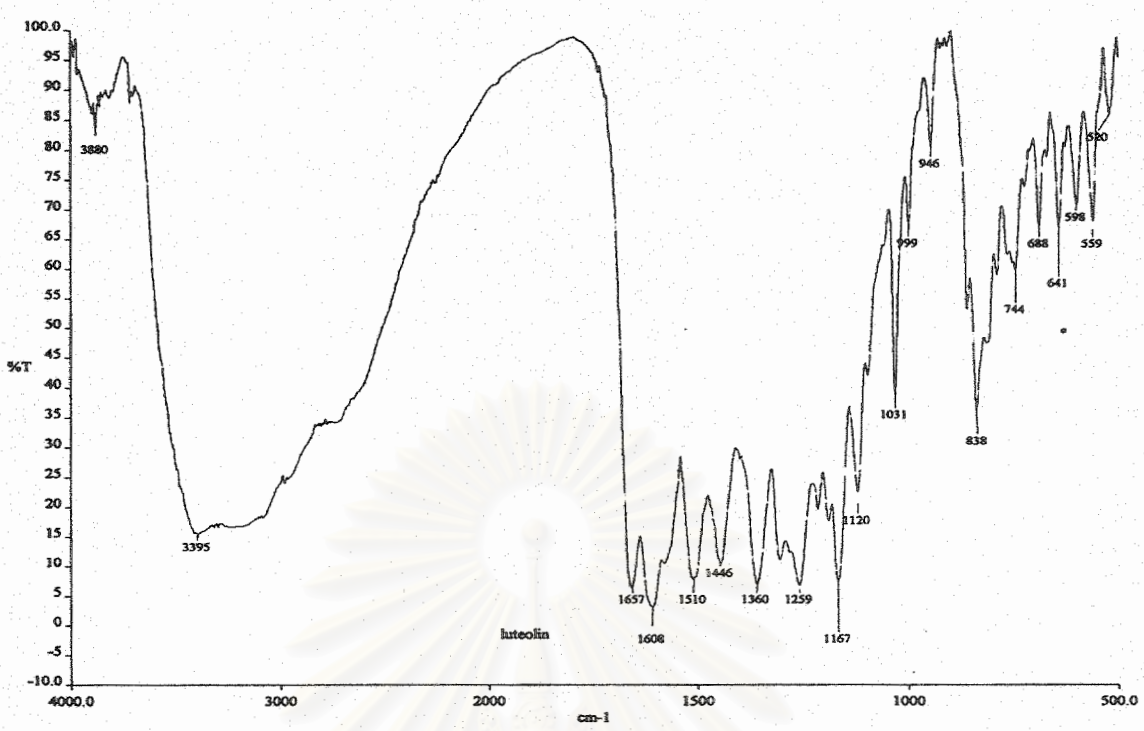


Figure 78 IR Spectrum of compound BRB2 (KBr disc)

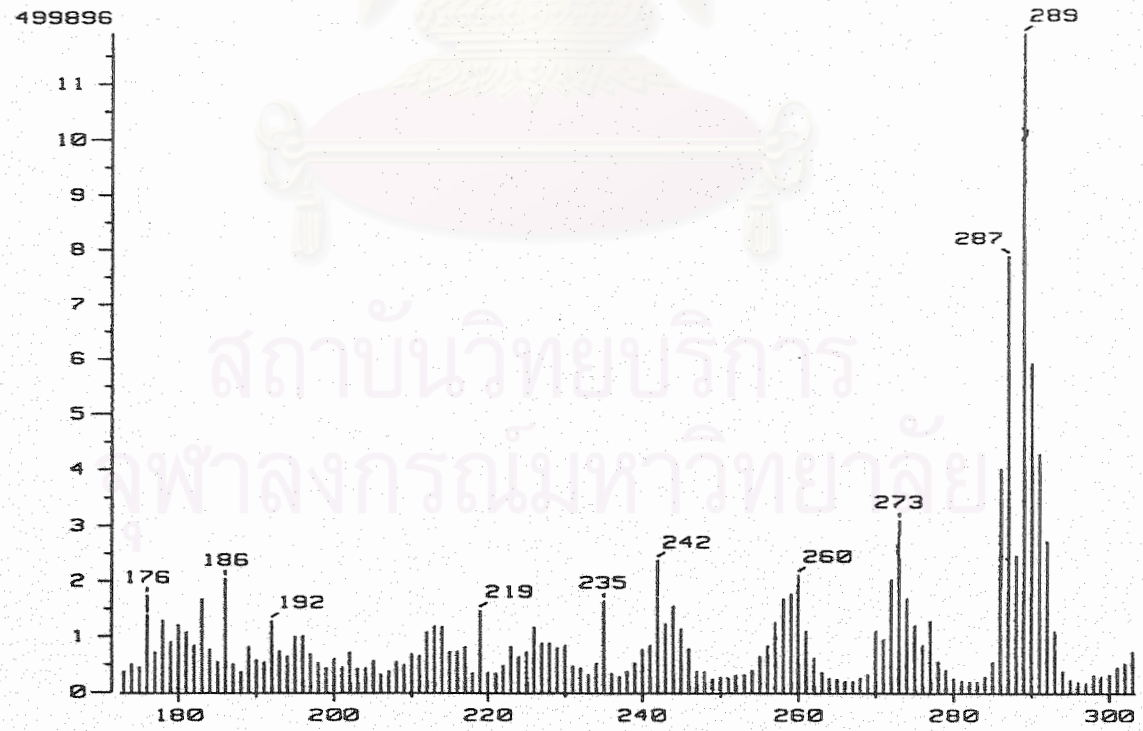


Figure 79 FAB MS Spectrum of compound BRB2

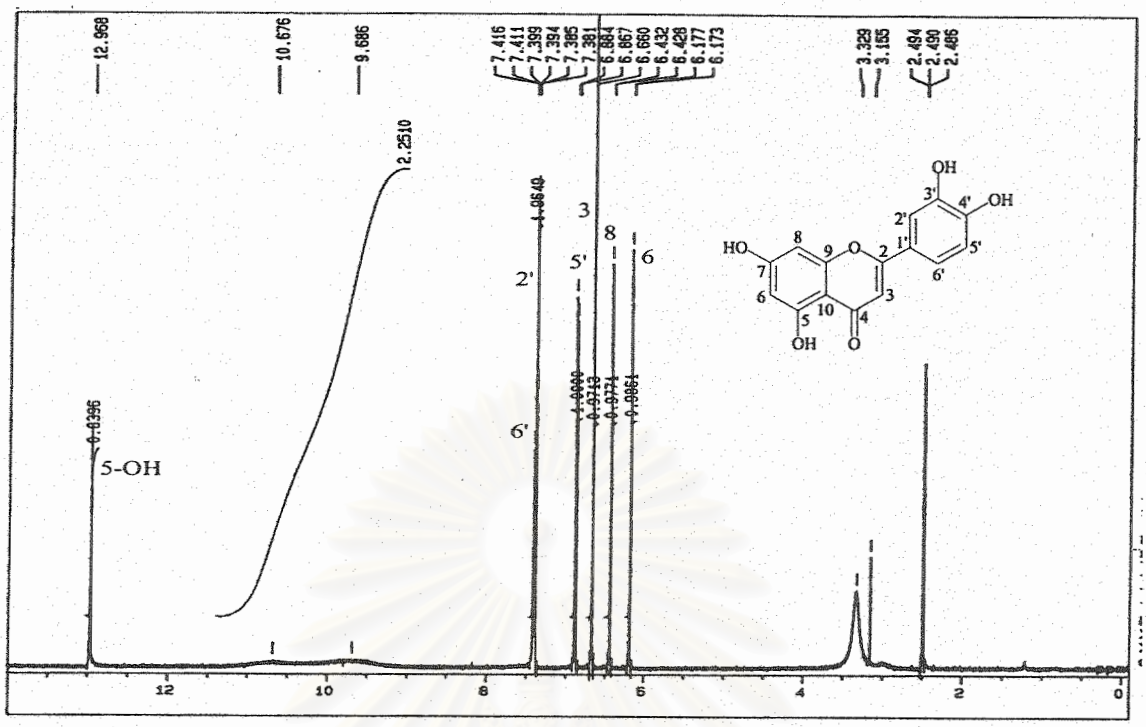


Figure 80 ^{1}H NMR (500 MHz) Spectrum of compound BRB2 (DMSO-d_6)

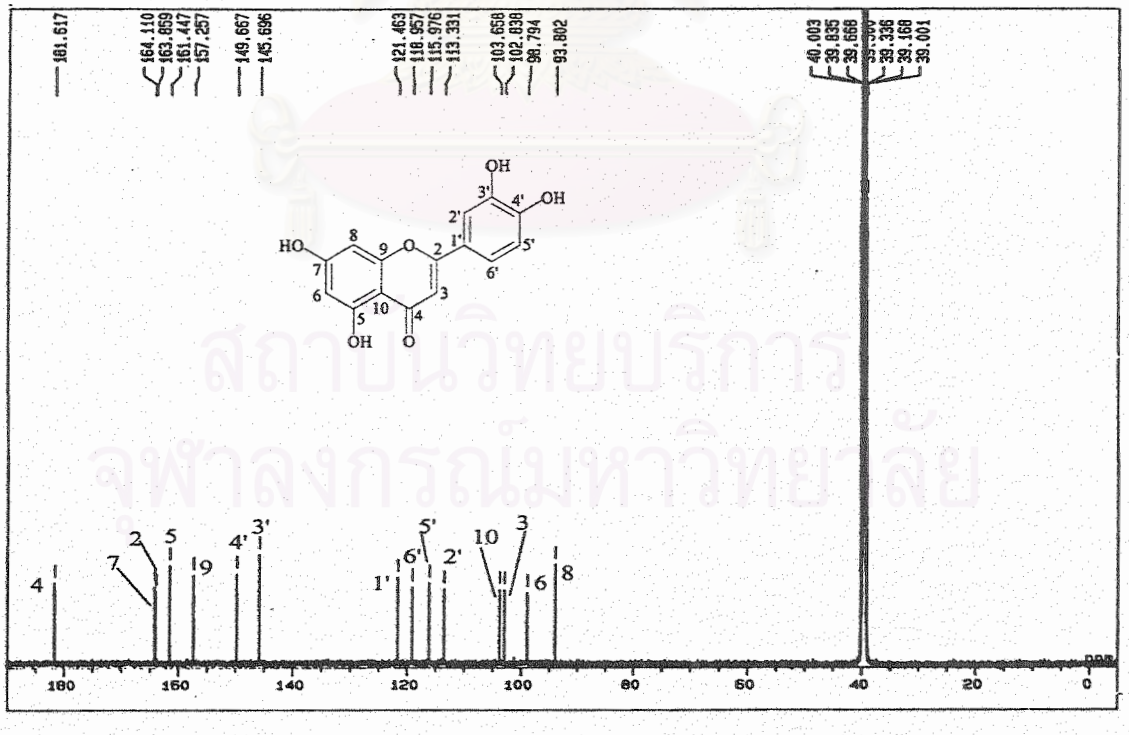


Figure 81 ^{13}C NMR (125 MHz) Spectrum of compound BRB2 (DMSO-d_6)

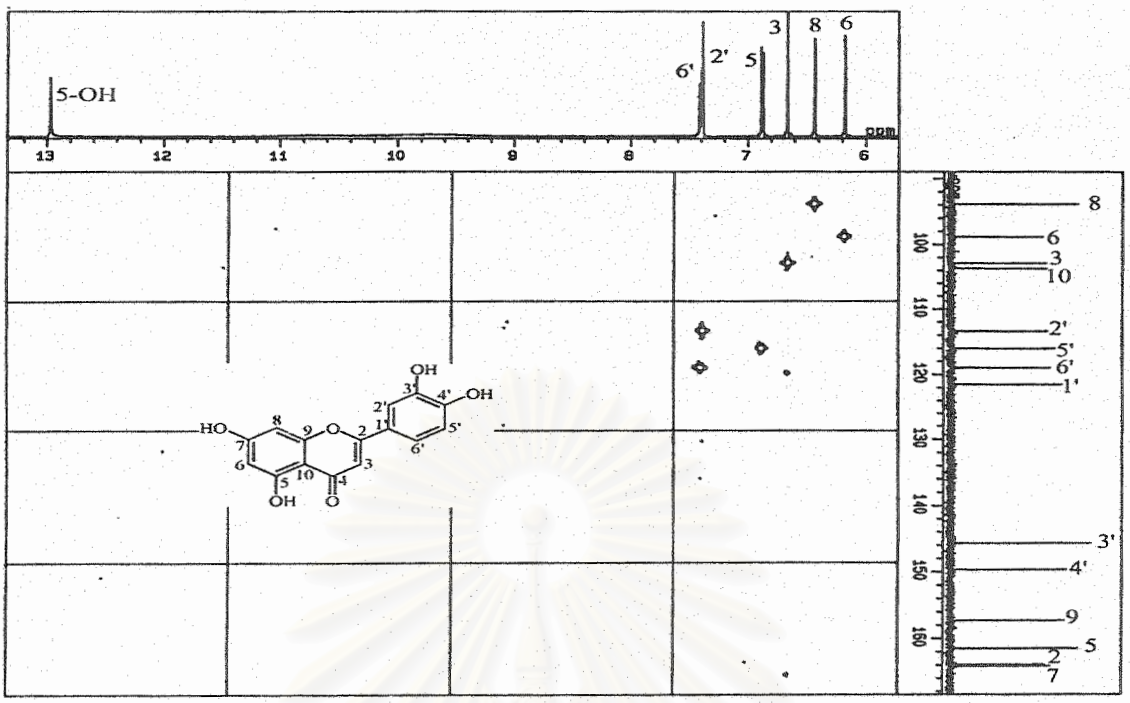


Figure 82 HMQC Spectrum of compound BRB2 (DMSO-d_6)

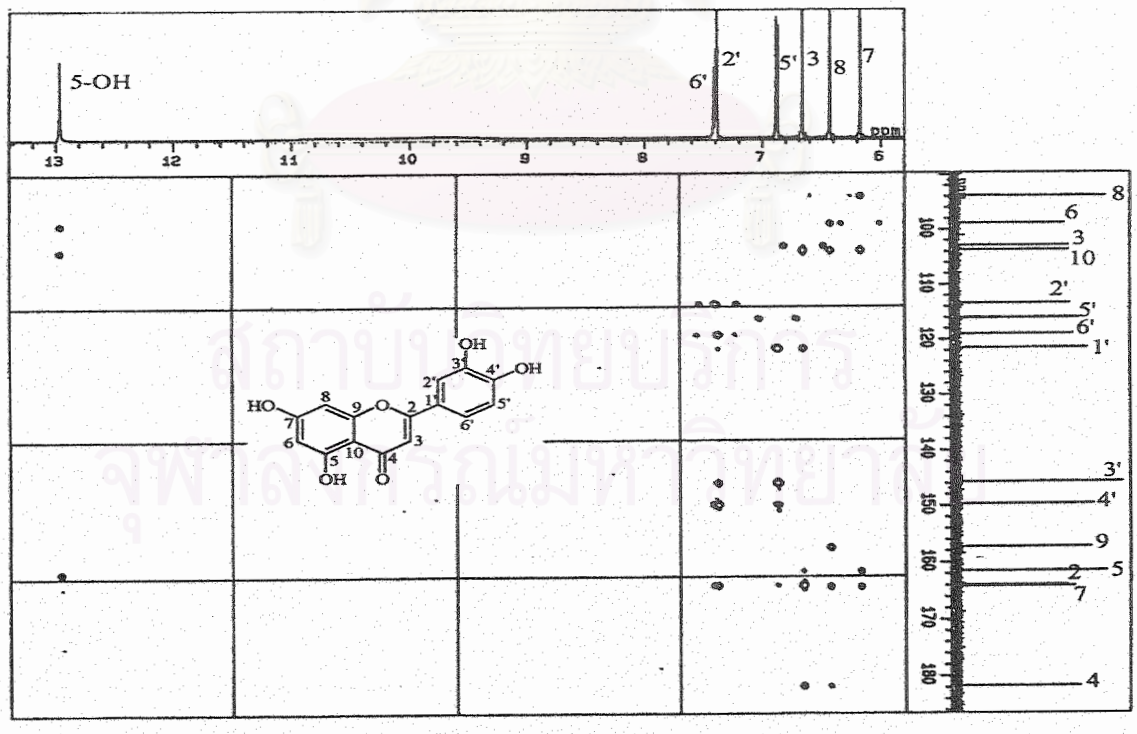


Figure 83 HMBC Spectrum of compound BRB2 (DMSO-d_6)

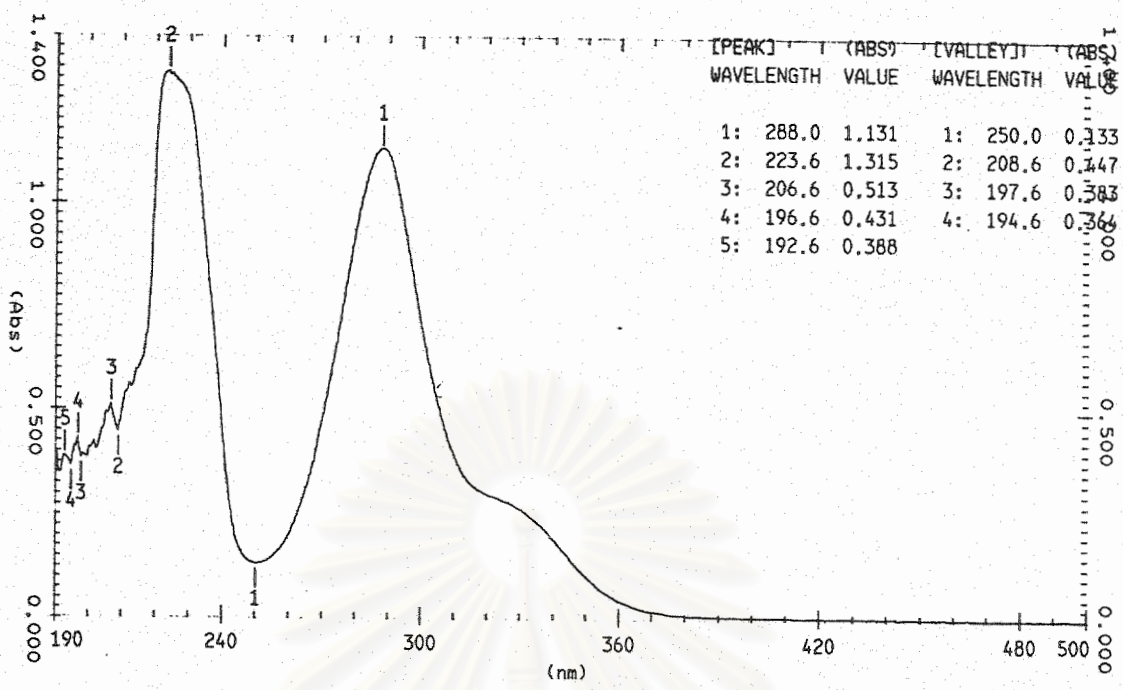


Figure 84 UV Spectrum of compound BRB3 (methanol)

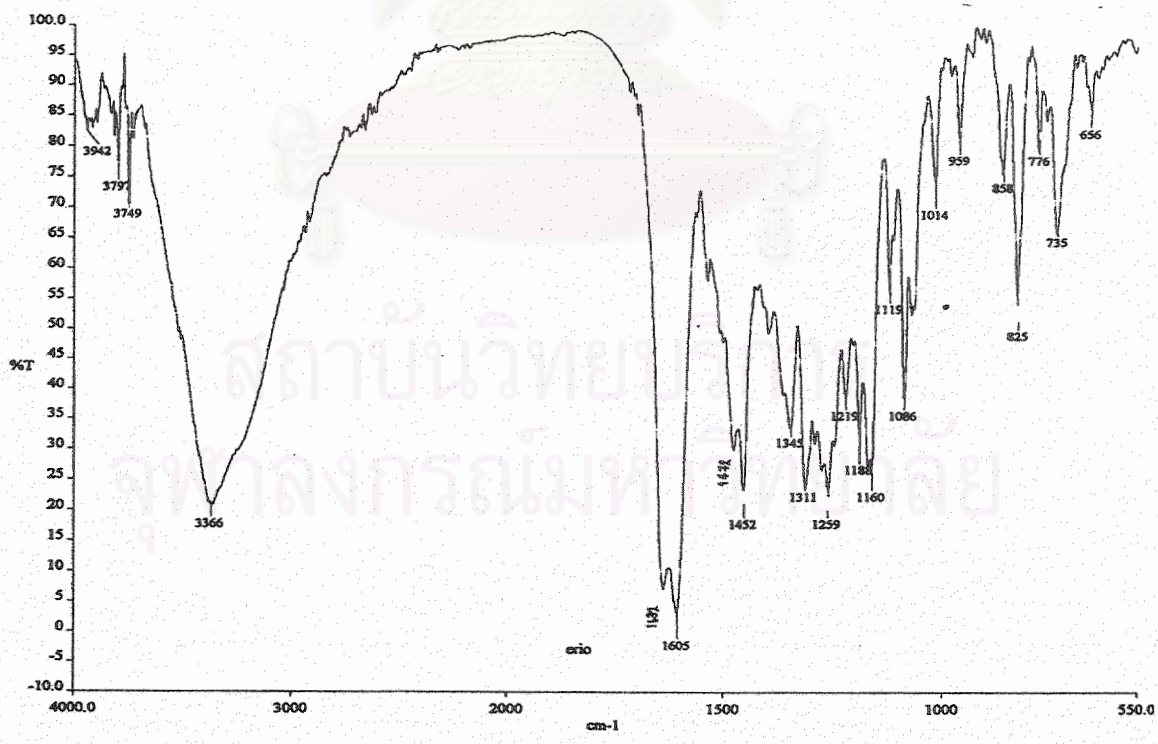


Figure 85 IR Spectrum of compound BRB3 (KBr disc)

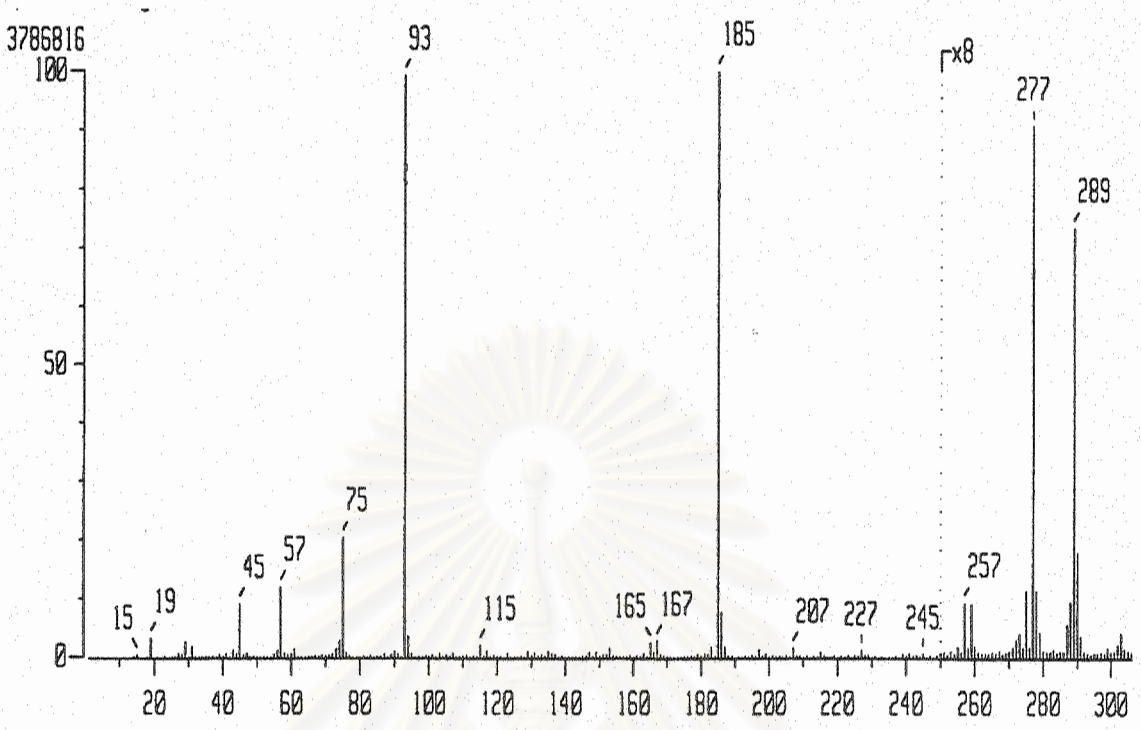


Figure 86 FAB$^+$MS Spectrum of compound BRB3

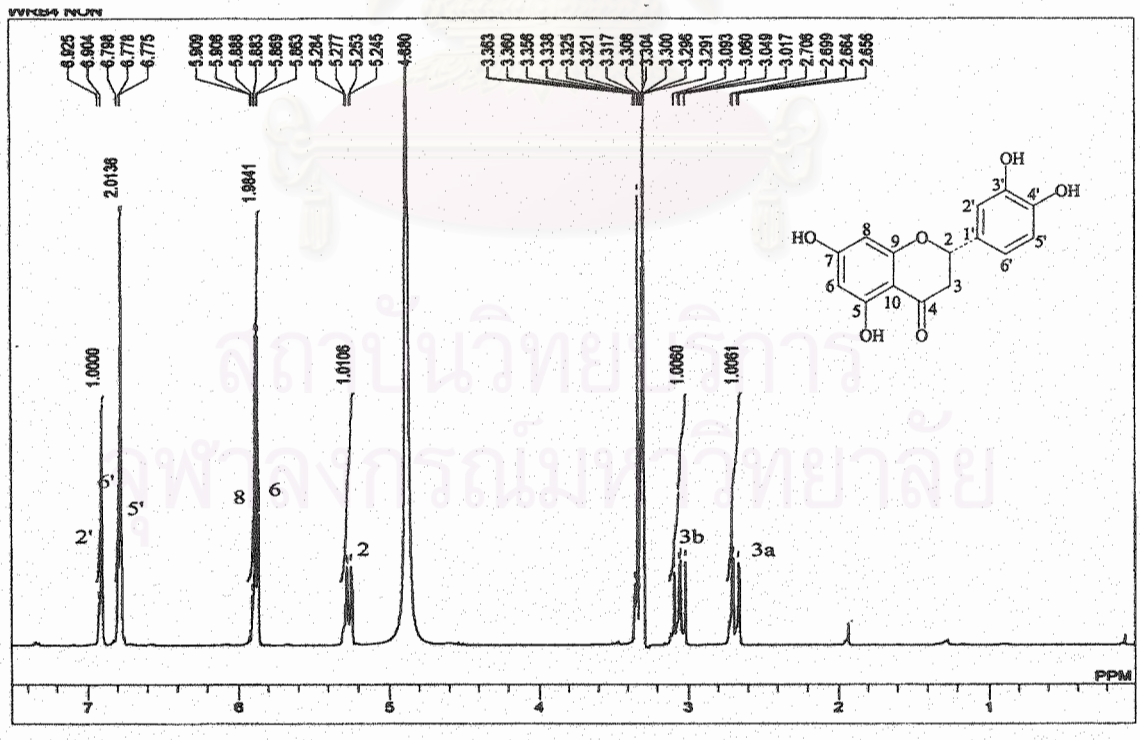


Figure 87 ^{1}H NMR (500 MHz) Spectrum of compound BRB3 (CD_3OD)

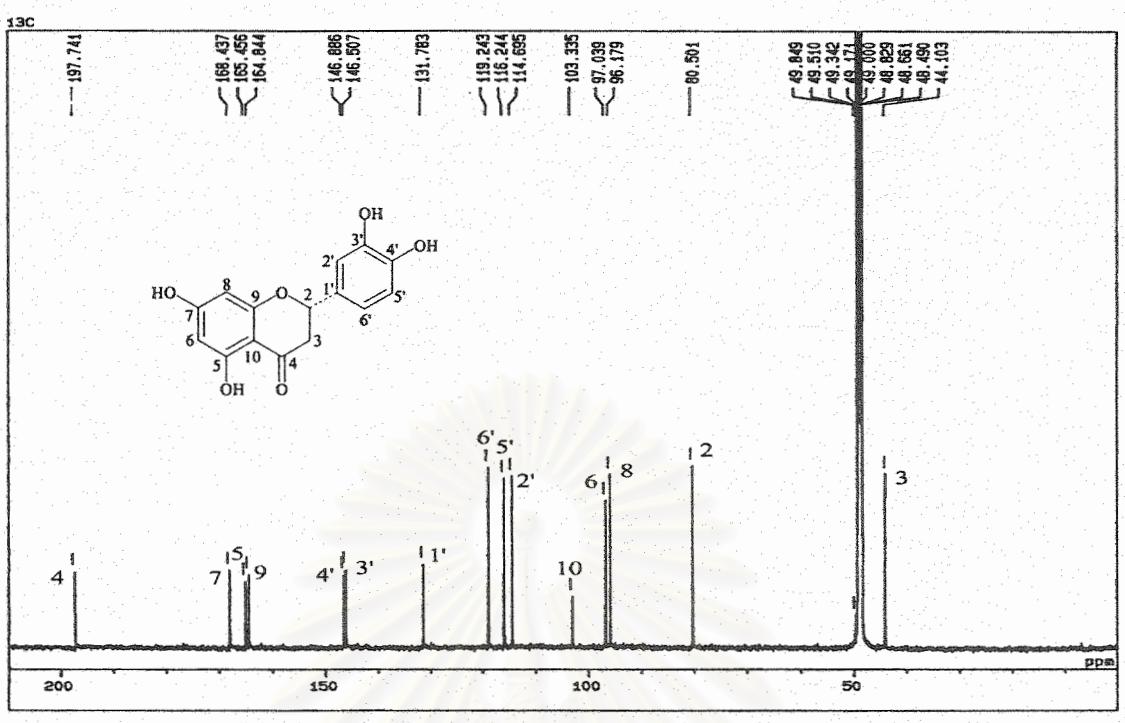


Figure 88 ^{13}C NMR (125 MHz) Spectrum of compound BRB3 (CD_3OD)

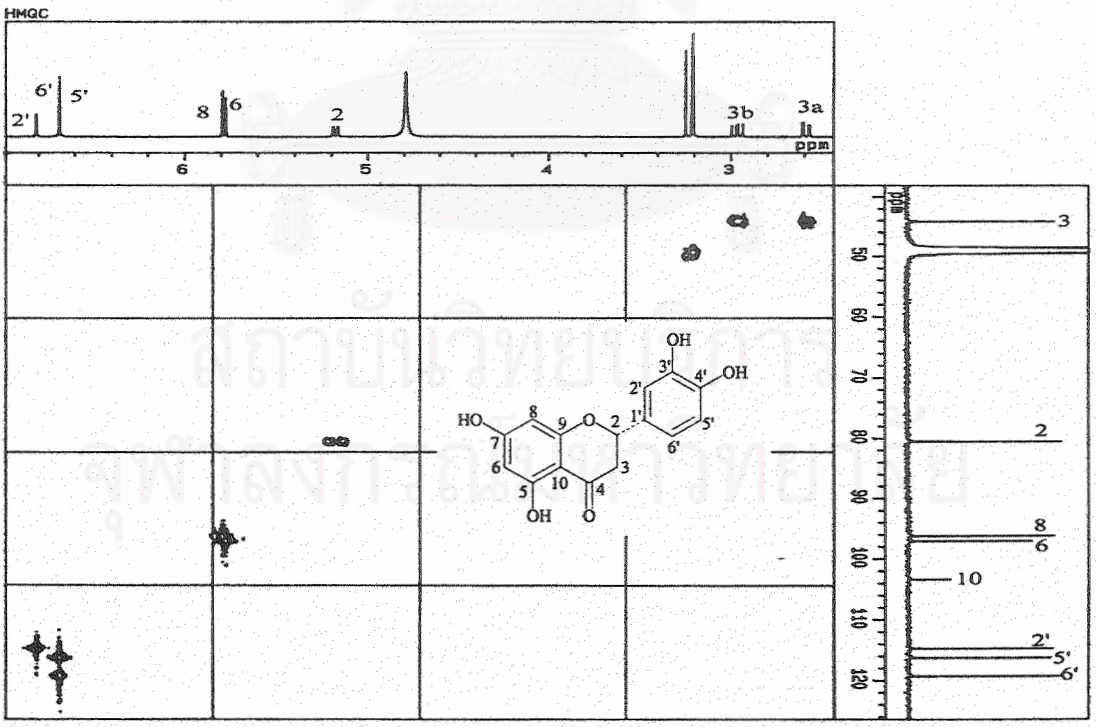


Figure 89 HMQC Spectrum of compound BRB3 (CD_3OD)

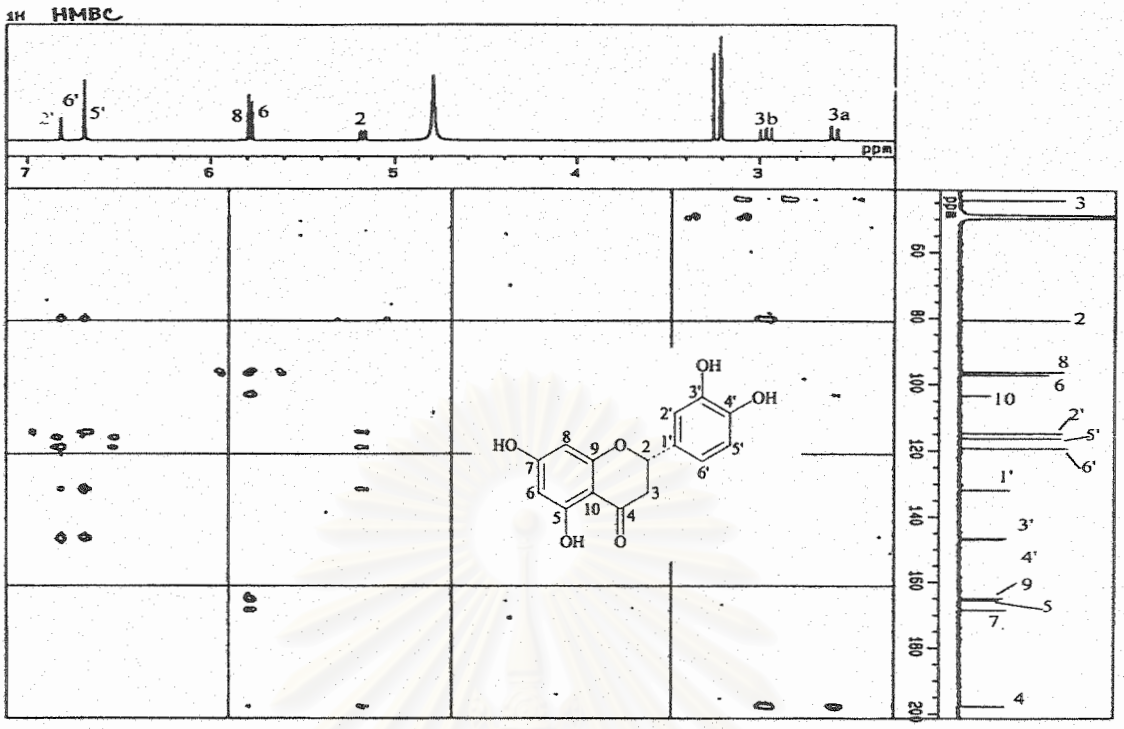


Figure 90 HMBC Spectrum of compound BRB3 (CD_3OD)

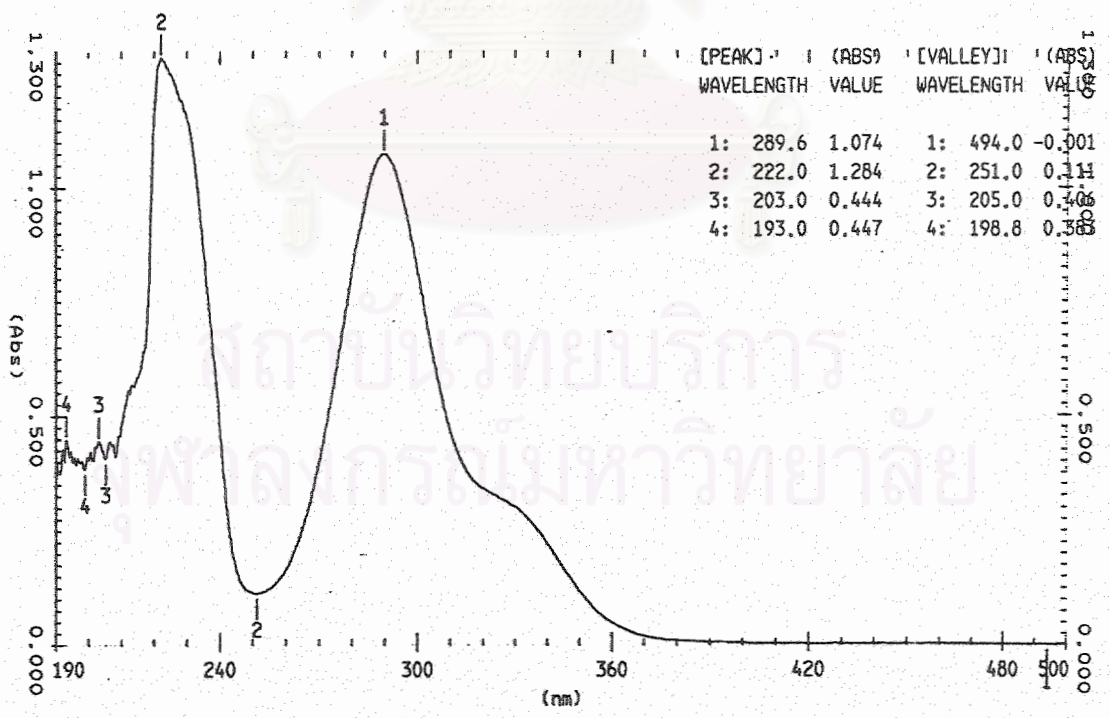


Figure 91 UV Spectrum of compound BRB4 (methanol)

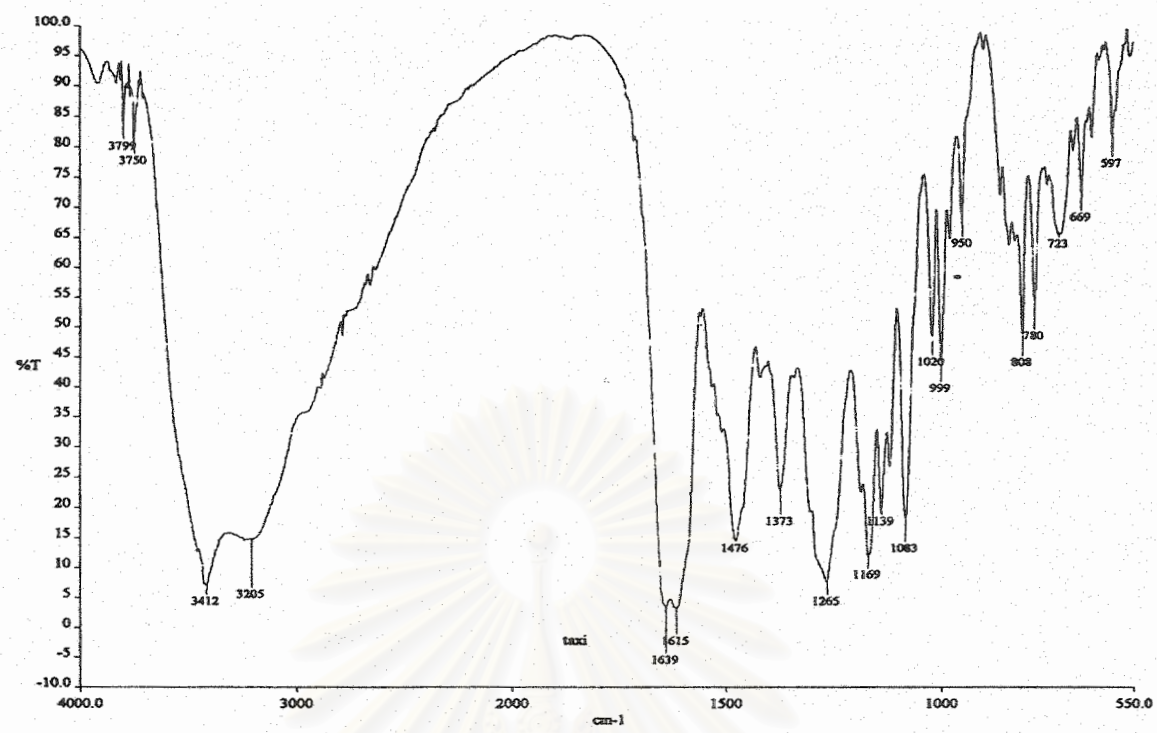


Figure 92 IR Spectrum of compound BRB4 (KBr disc)

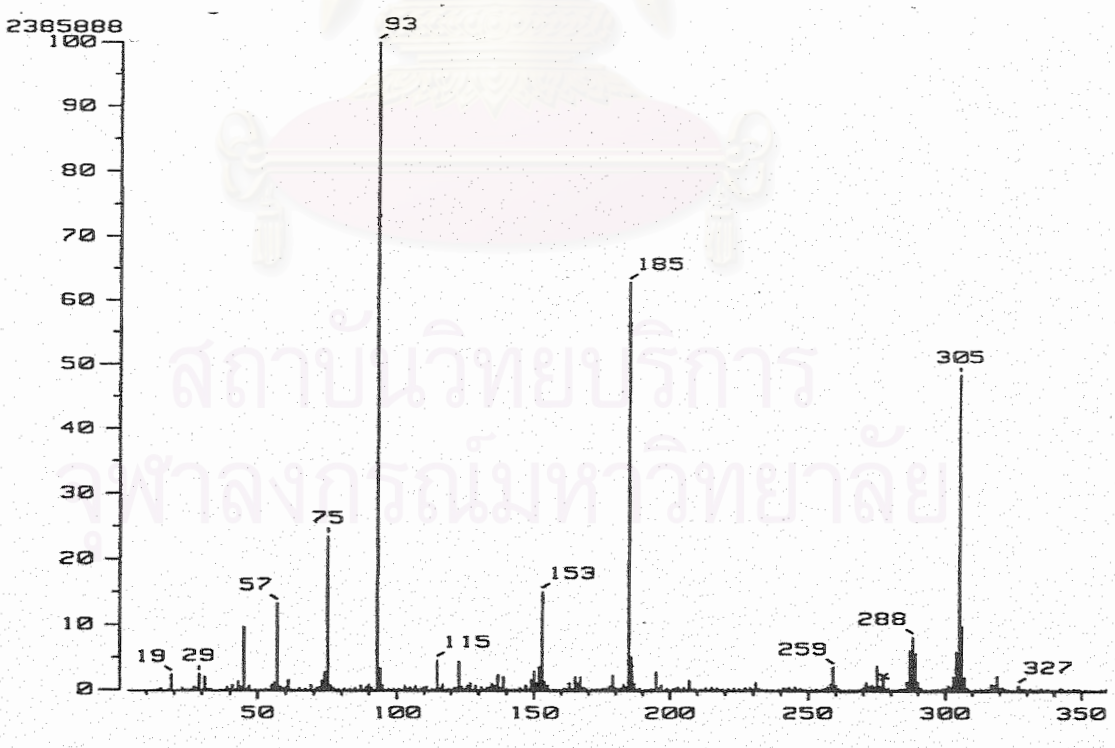


Figure 93 FAB^{+}MS Spectrum of compound BRB4

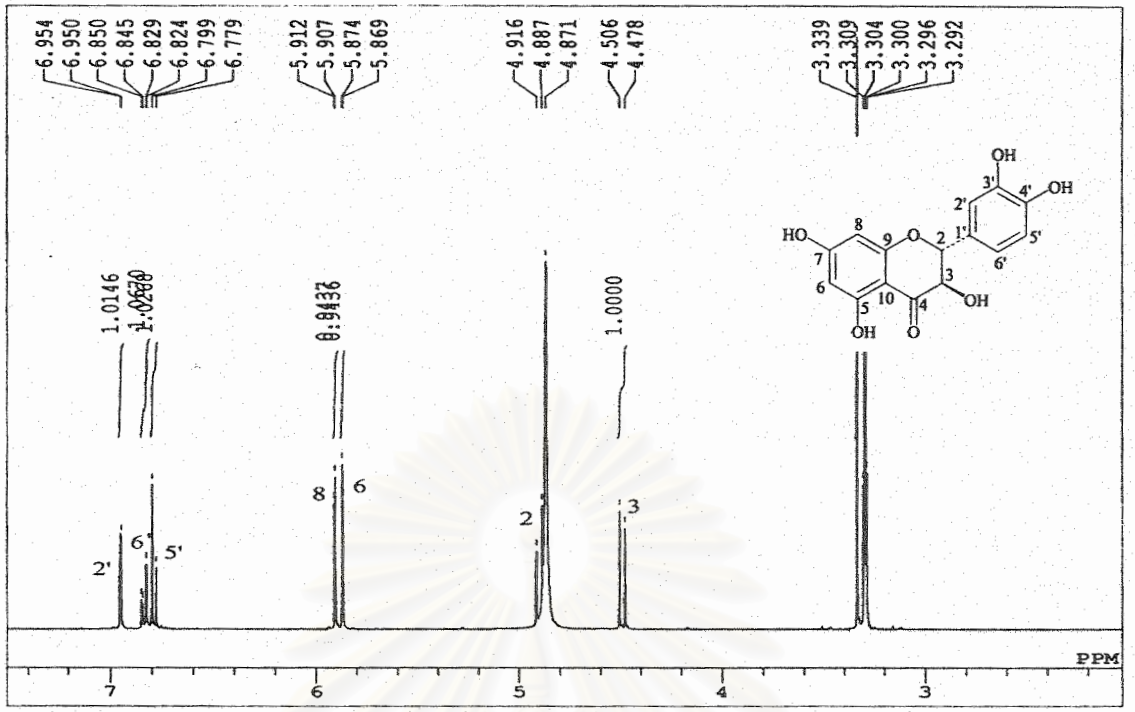


Figure 94 ^{1}H NMR (500 MHz) Spectrum of compound BRB4 (CD_3OD)

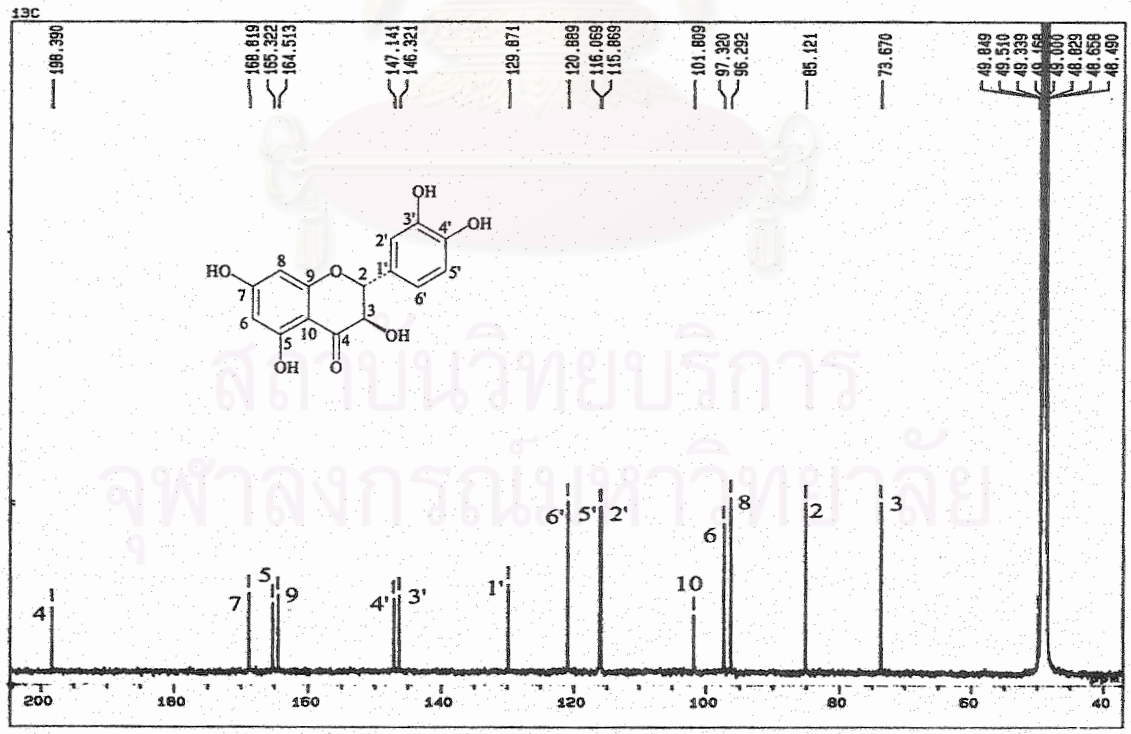


Figure 95 ^{13}C NMR (125 MHz) Spectrum of compound BRB4 (CD_3OD)

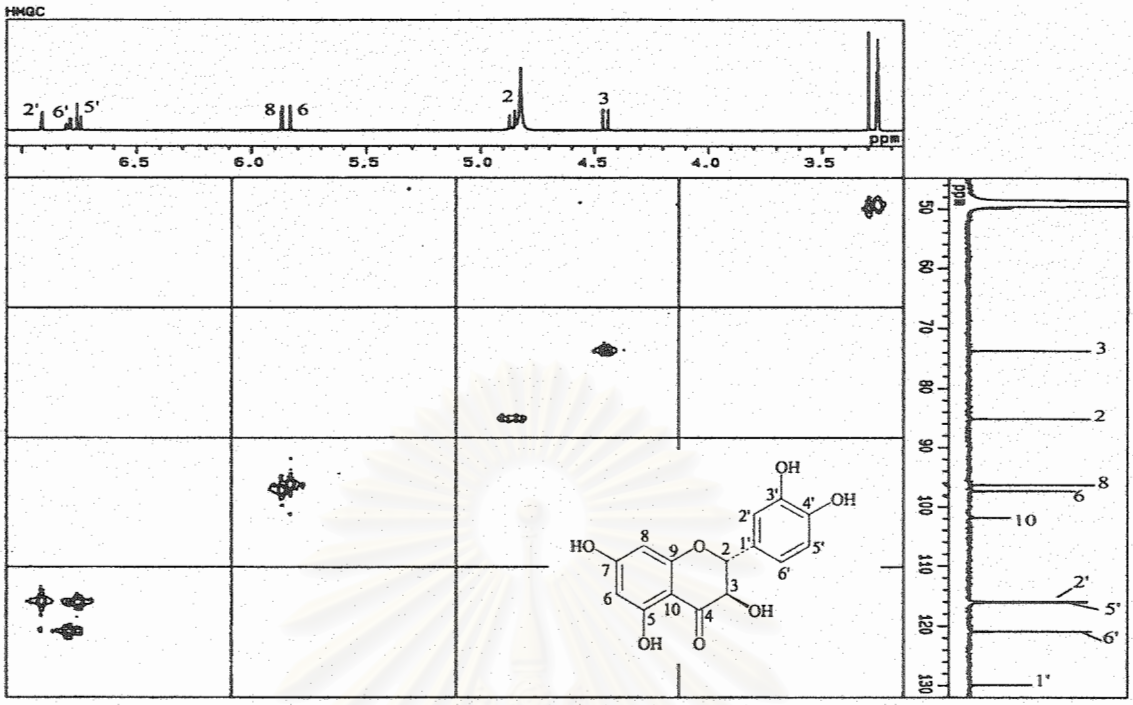


Figure 96 HMQC Spectrum of compound BRB4 (CD_3OD)

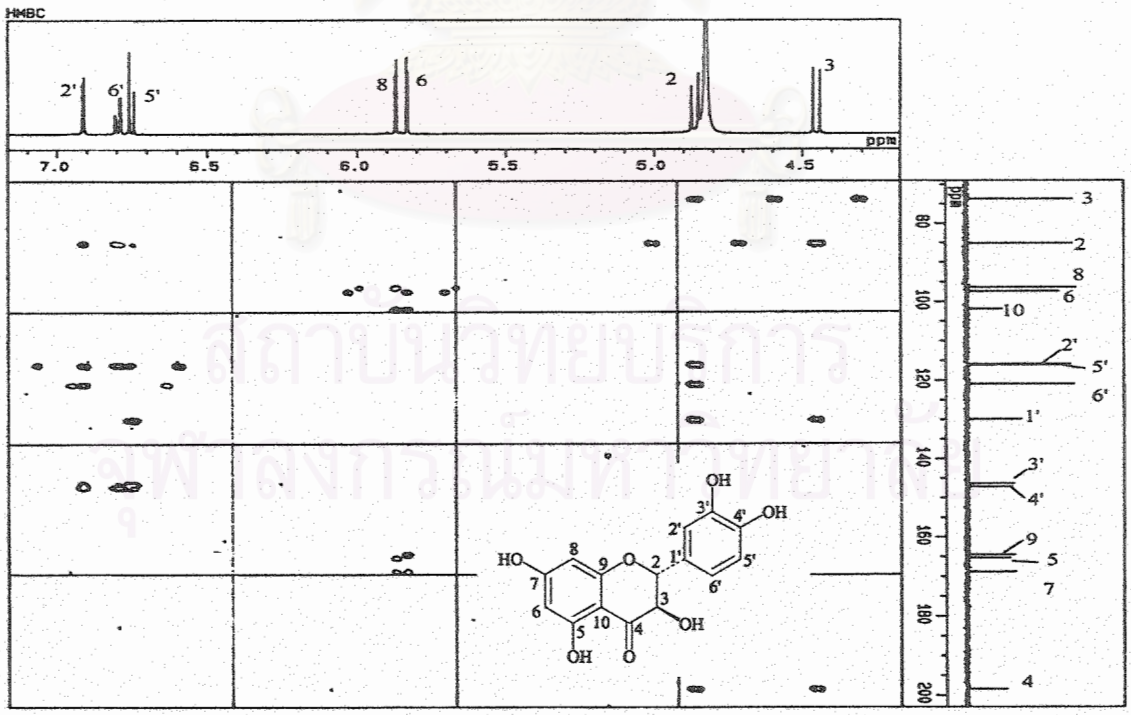


Figure 97 HMBC Spectrum of compound BRB4 (CD_3OD)

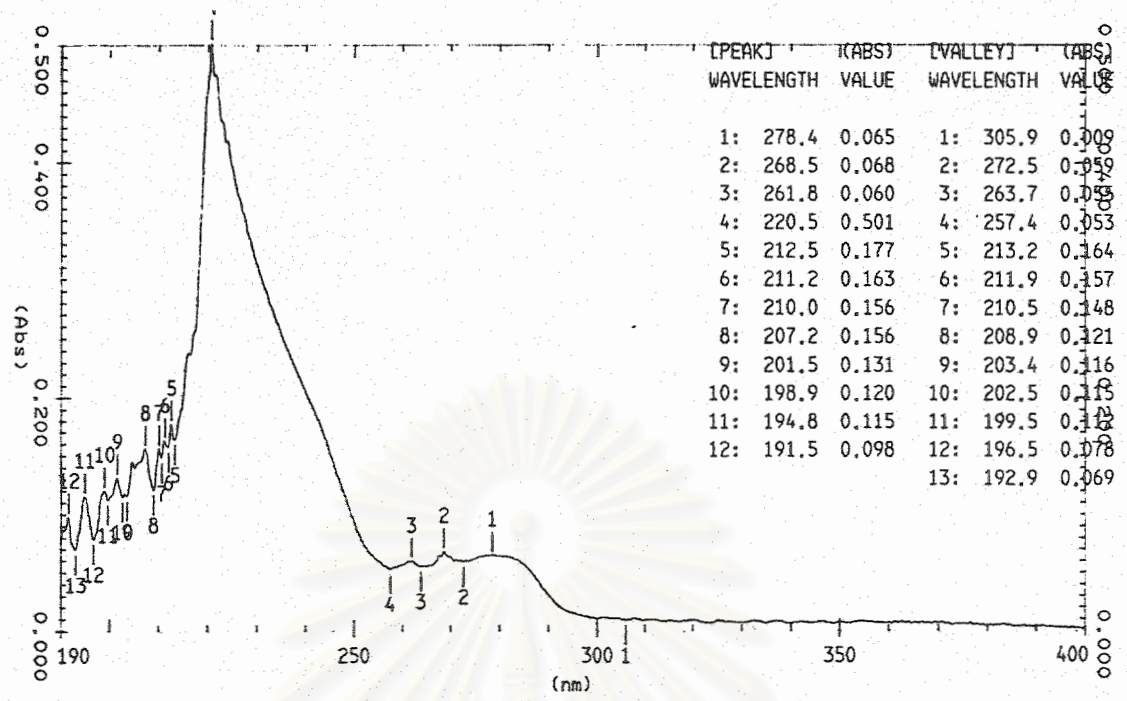


Figure 98 UV Spectrum of compound BRB5 (methanol)

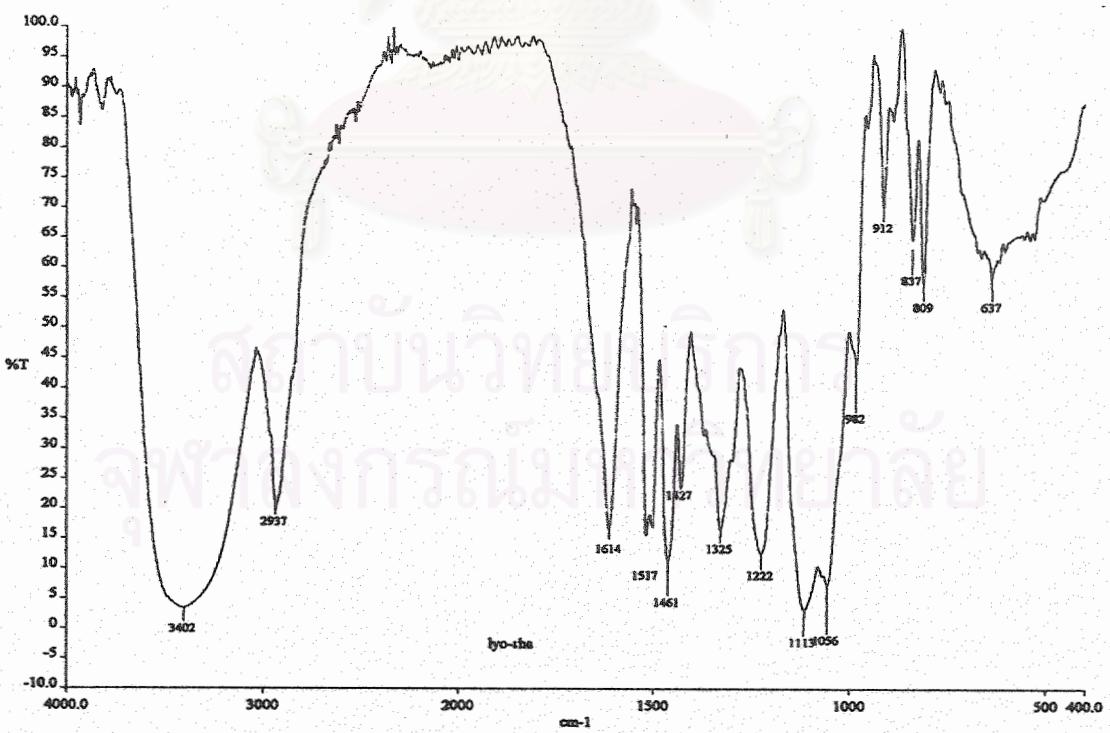


Figure 99 IR Spectrum of compound BRB5 (KBr disc)

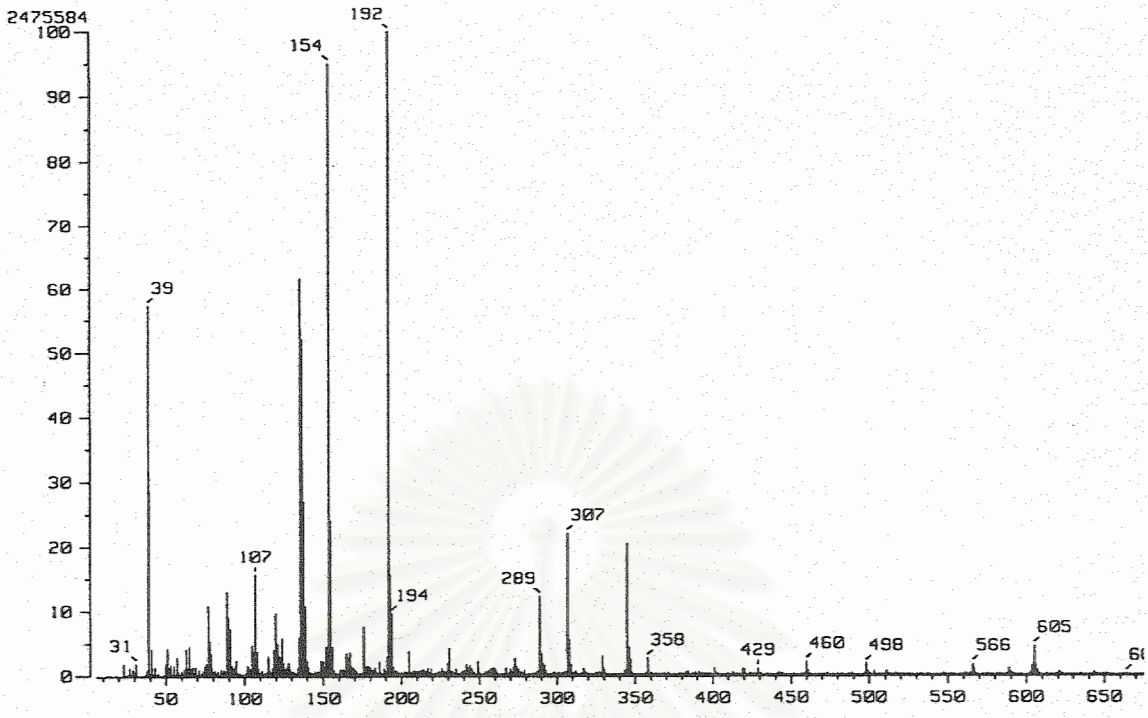


Figure 100 FAB$^+$MS Mass spectrum of compound BRB5

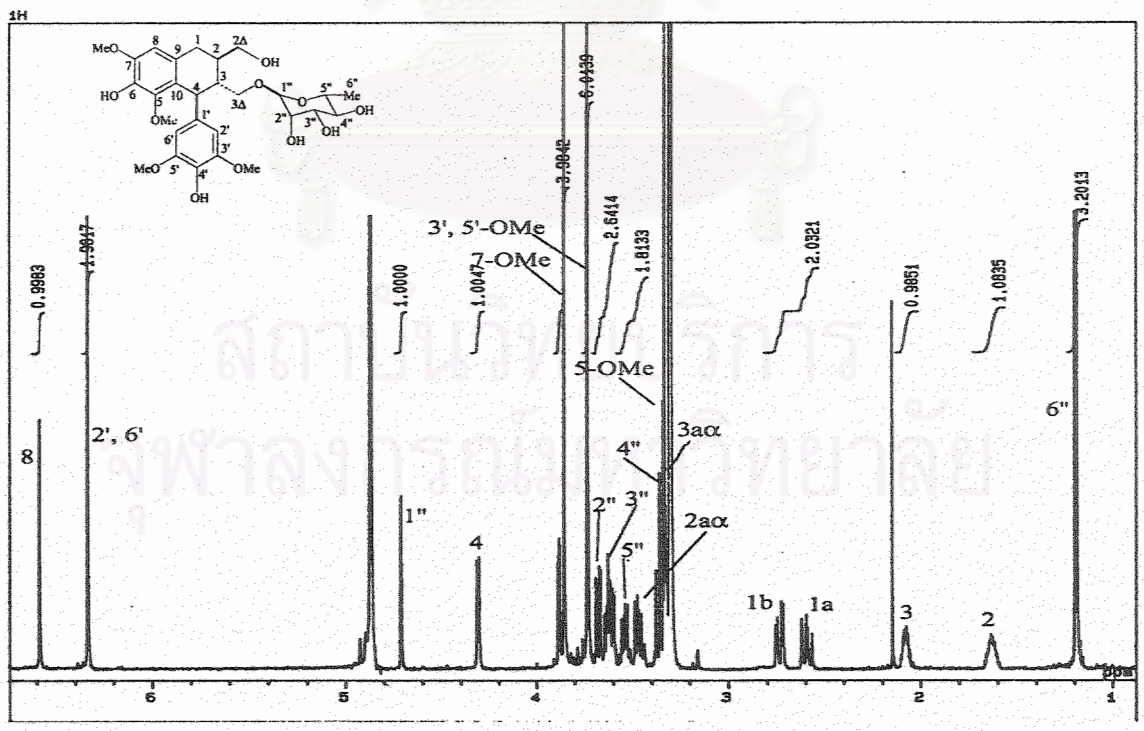


Figure 101 ^{1}H NMR (500 MHz) Spectrum of compound BRB5 (CD_3OD)

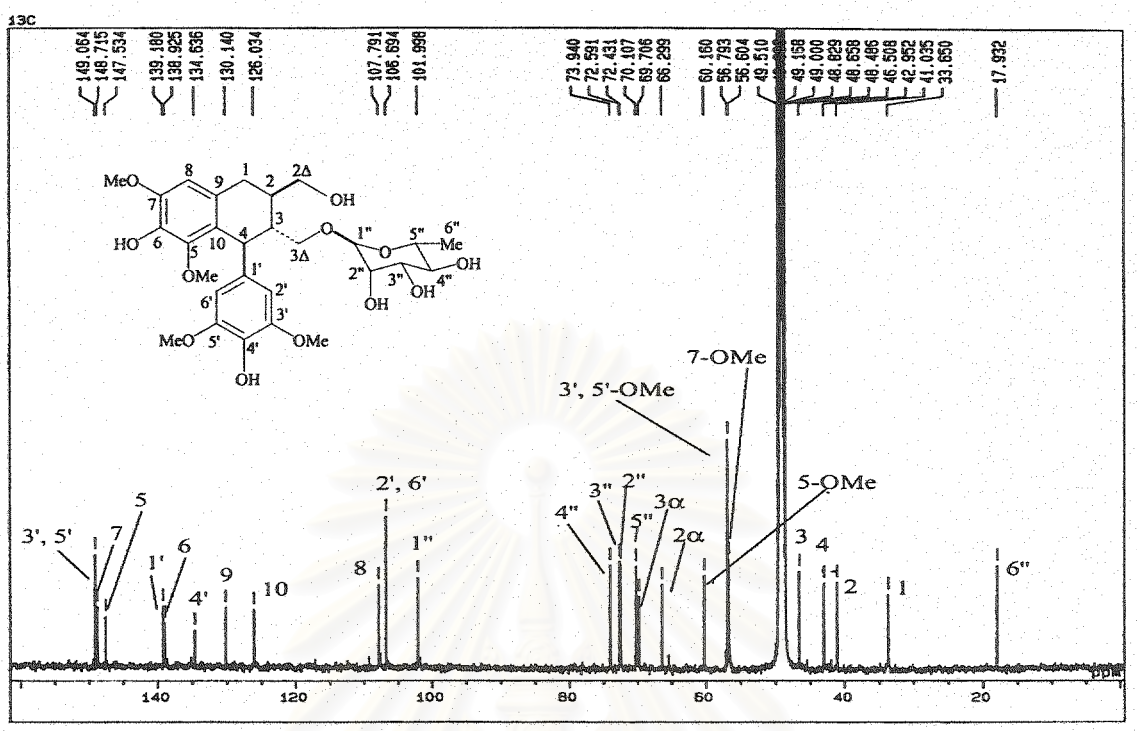


Figure 102 ^{13}C NMR (125 MHz) Spectrum of compound BRB5 (CD_3OD)

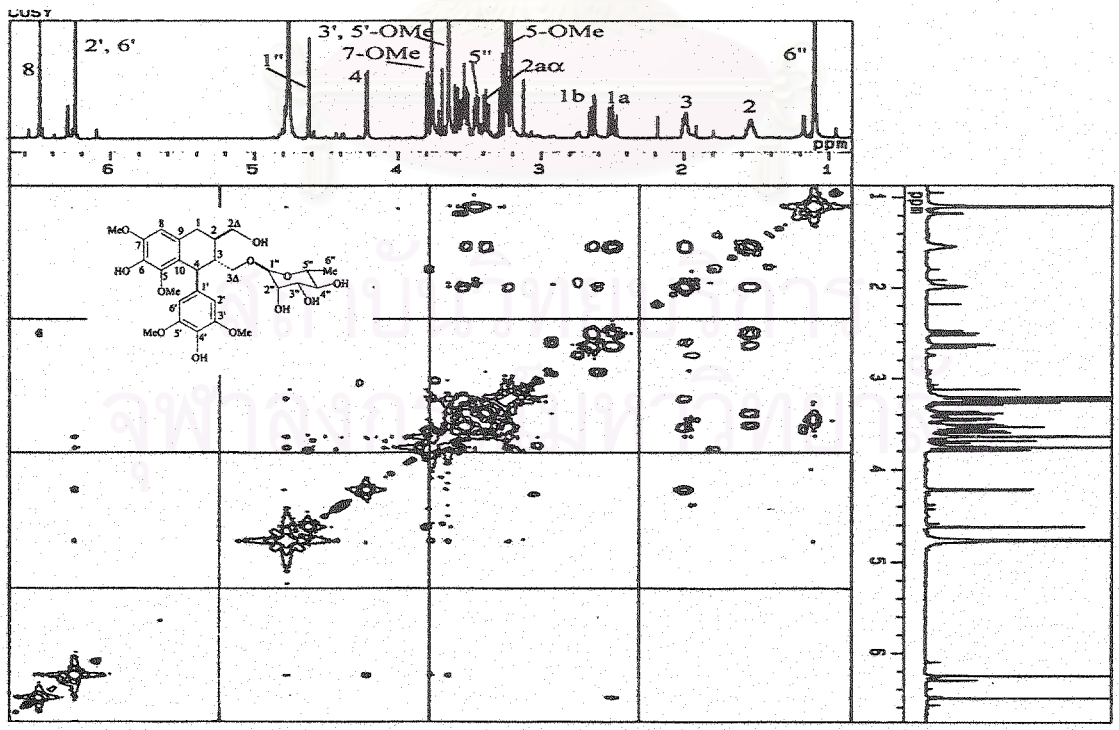


Figure 103 ^{1}H-^{1}H COSY Spectrum of compound BRB5 (CD_3OD)

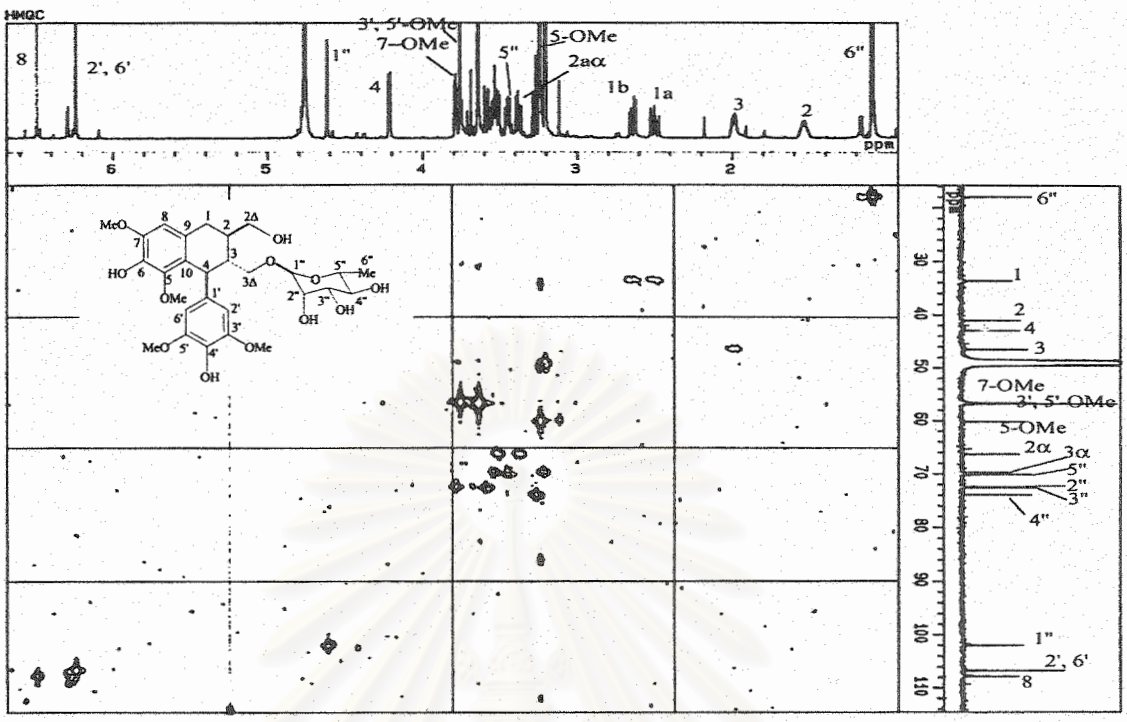


Figure 104 HMQC Spectrum of compound BRB5 (CD_3OD)

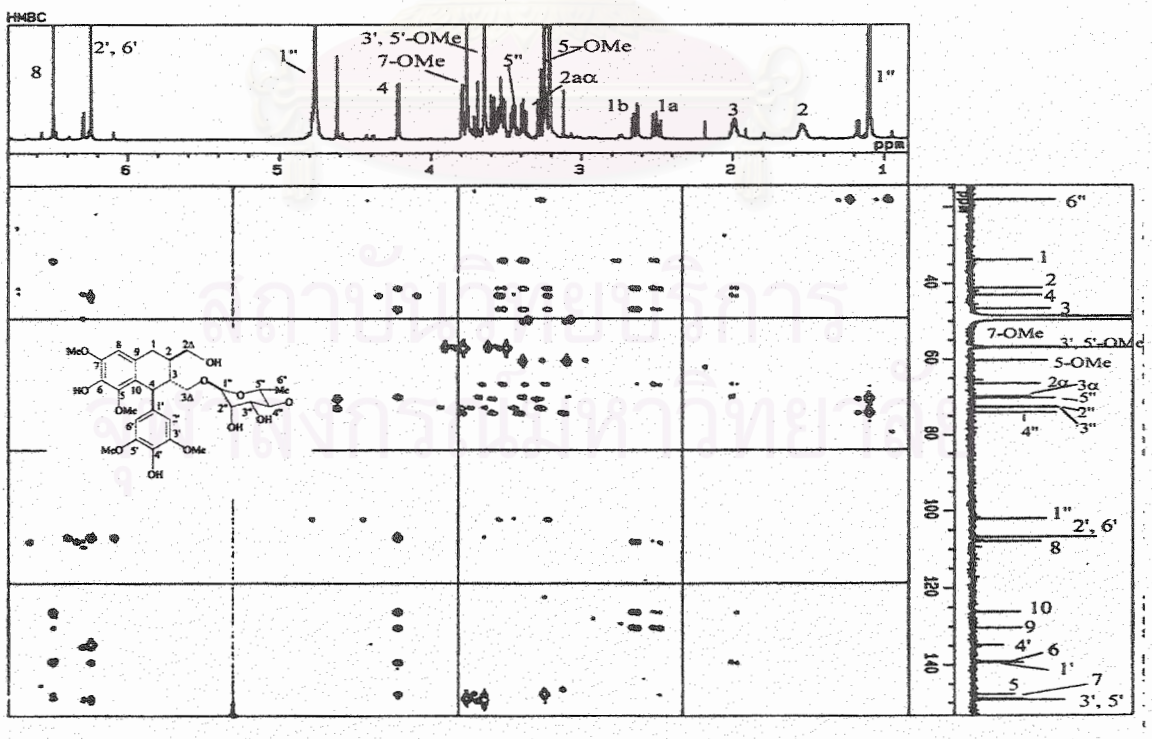


Figure 105 HMBC Spectrum of compound BRB5 (CD_3OD)

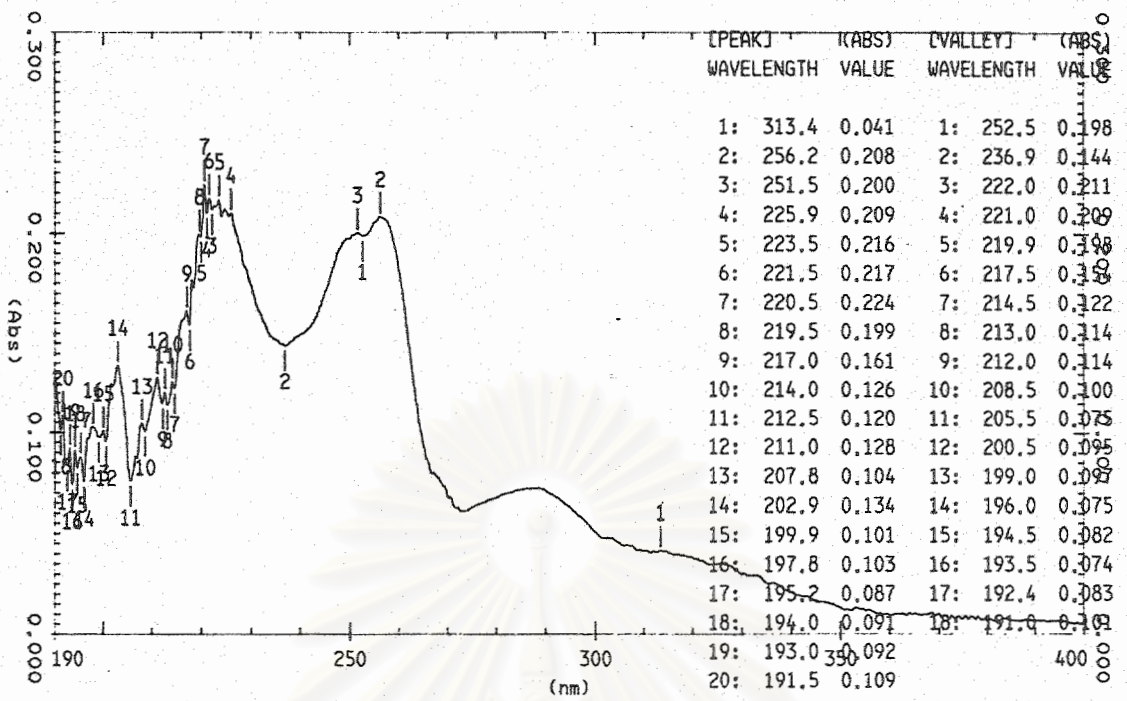


Figure 106 UV Spectrum of compound BRB6 (methanol)

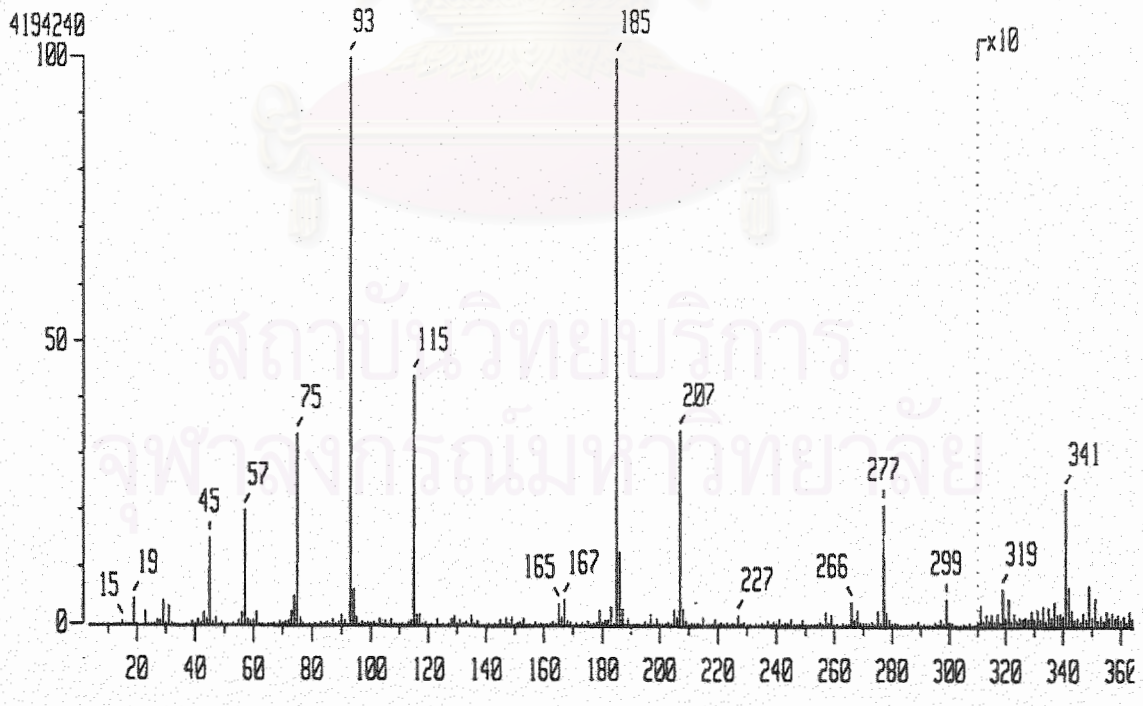


Figure 107 FAB$^+$MS Mass spectrum of compound BRB6

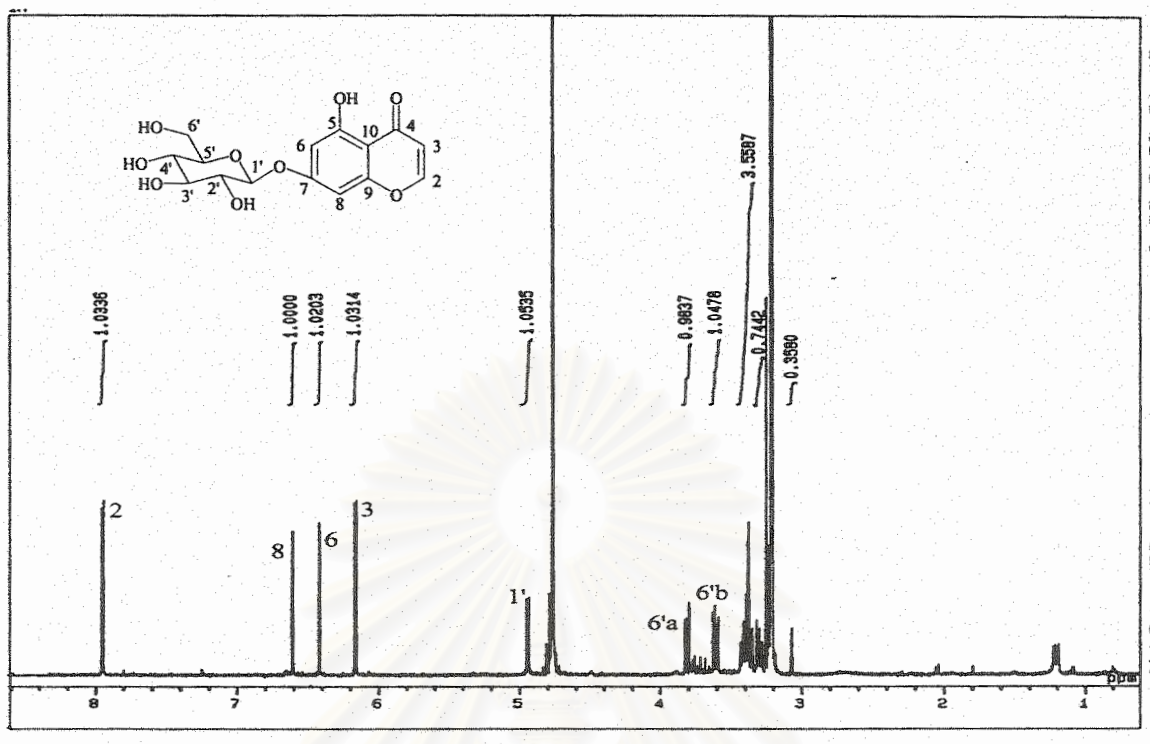


Figure 108 ^{1}H NMR (500 MHz) Spectrum of compound BRB6 (CD_3OD)

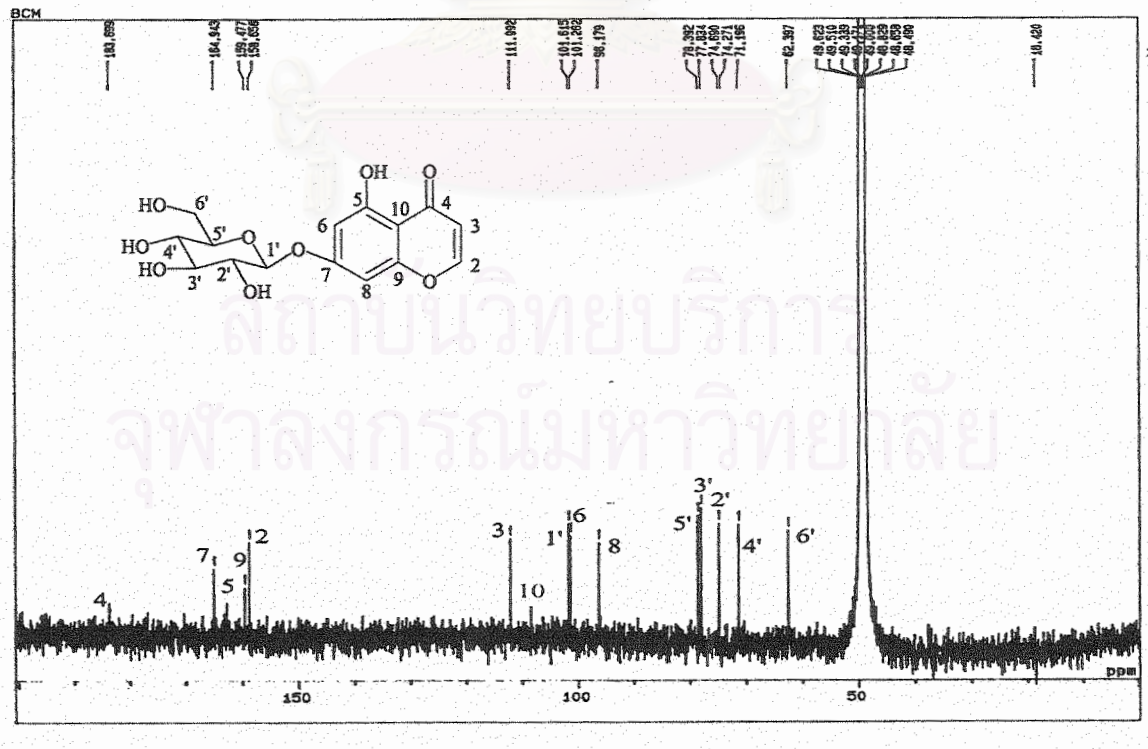


Figure 109 ^{13}C NMR (125 MHz) Spectrum of compound BRB6 (CD_3OD)

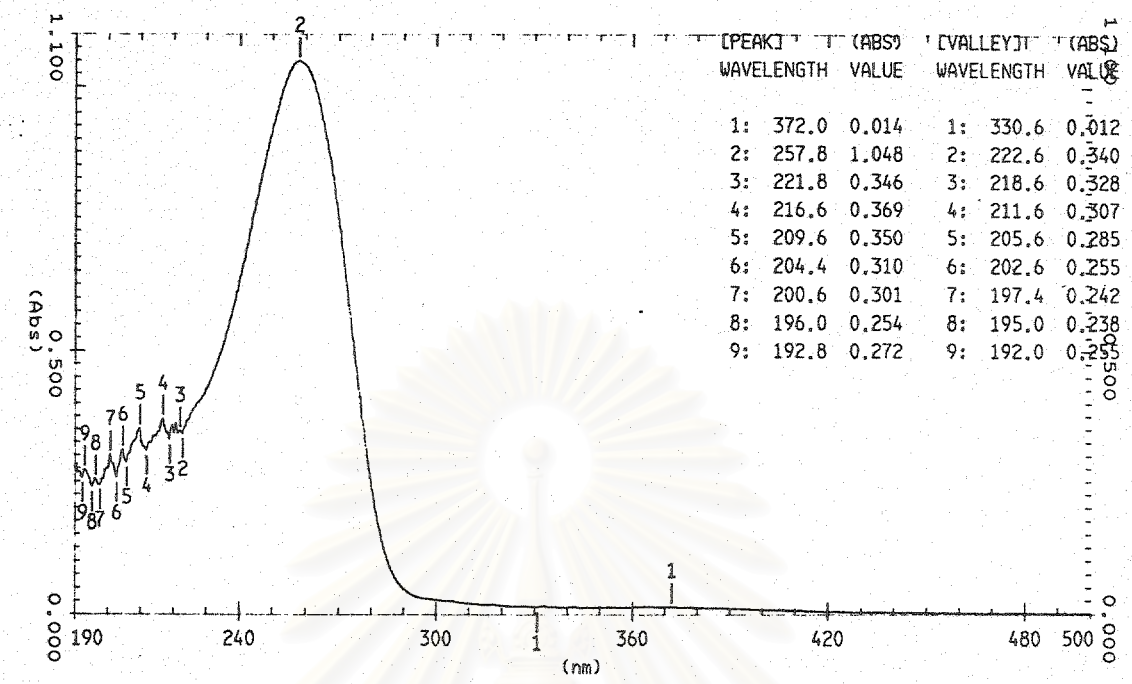


Figure 110 UV Spectrum of compound BRB7 (methanol)

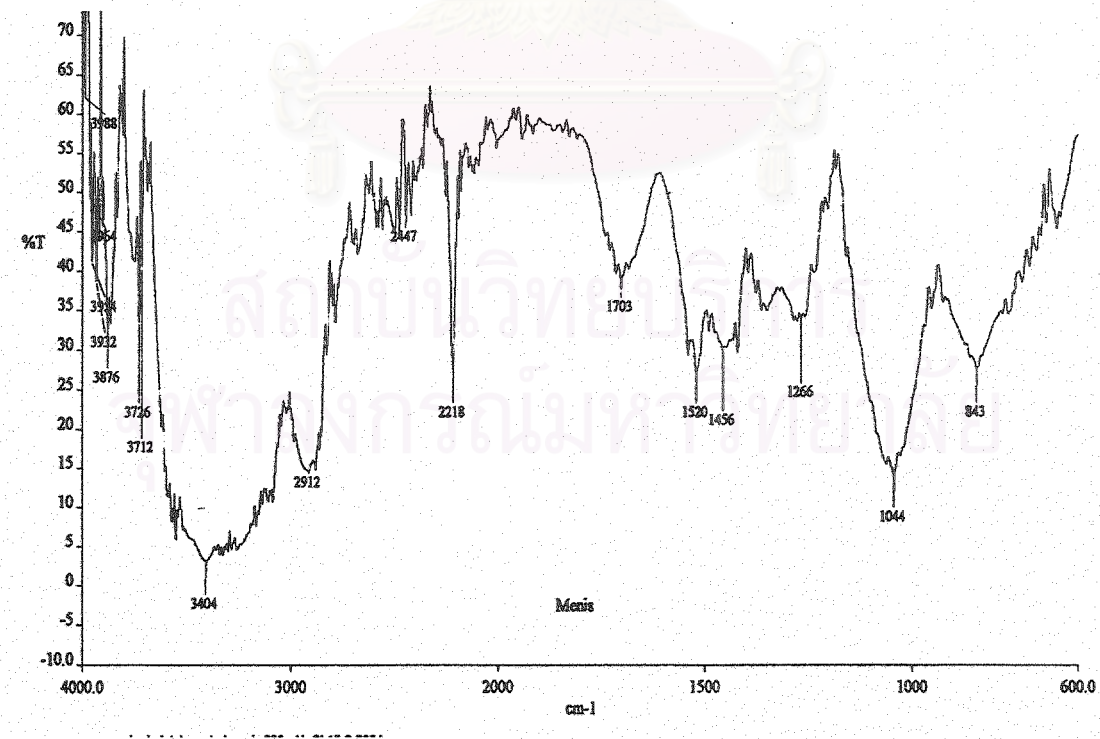


Figure 111 IR Spectrum of compound BRB7 (KBr disc)

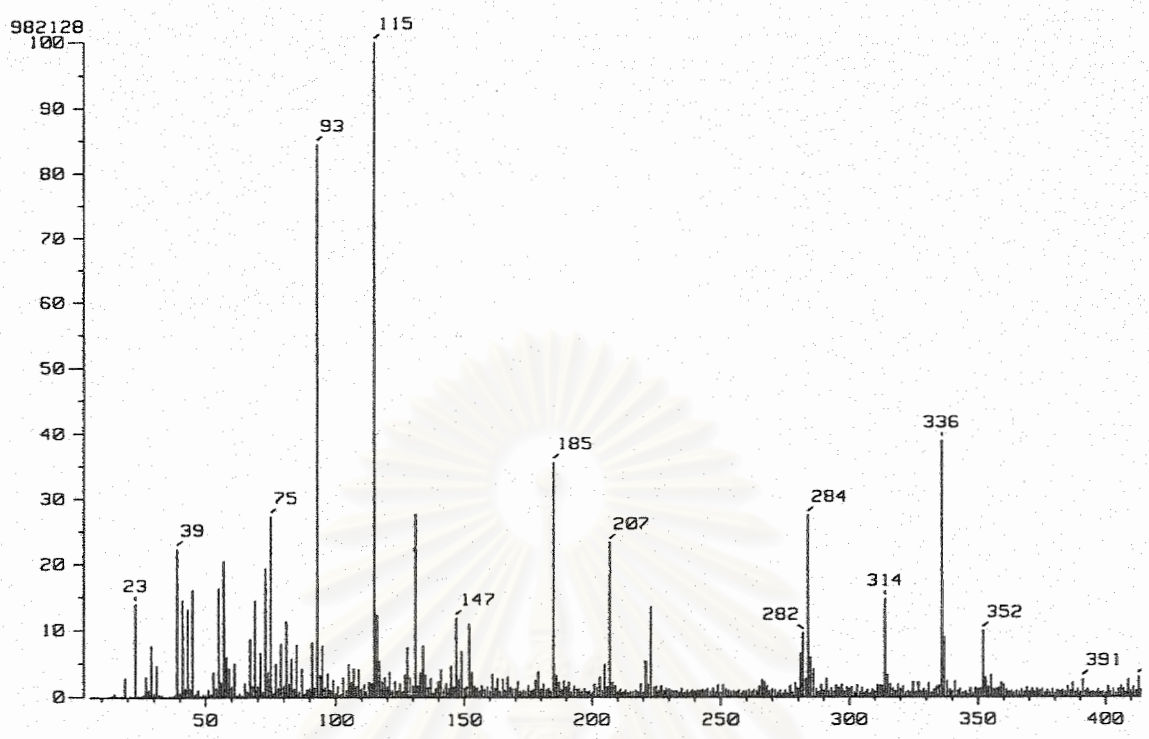


Figure 112 FAB^{+}MS Mass spectrum of compound BRB7

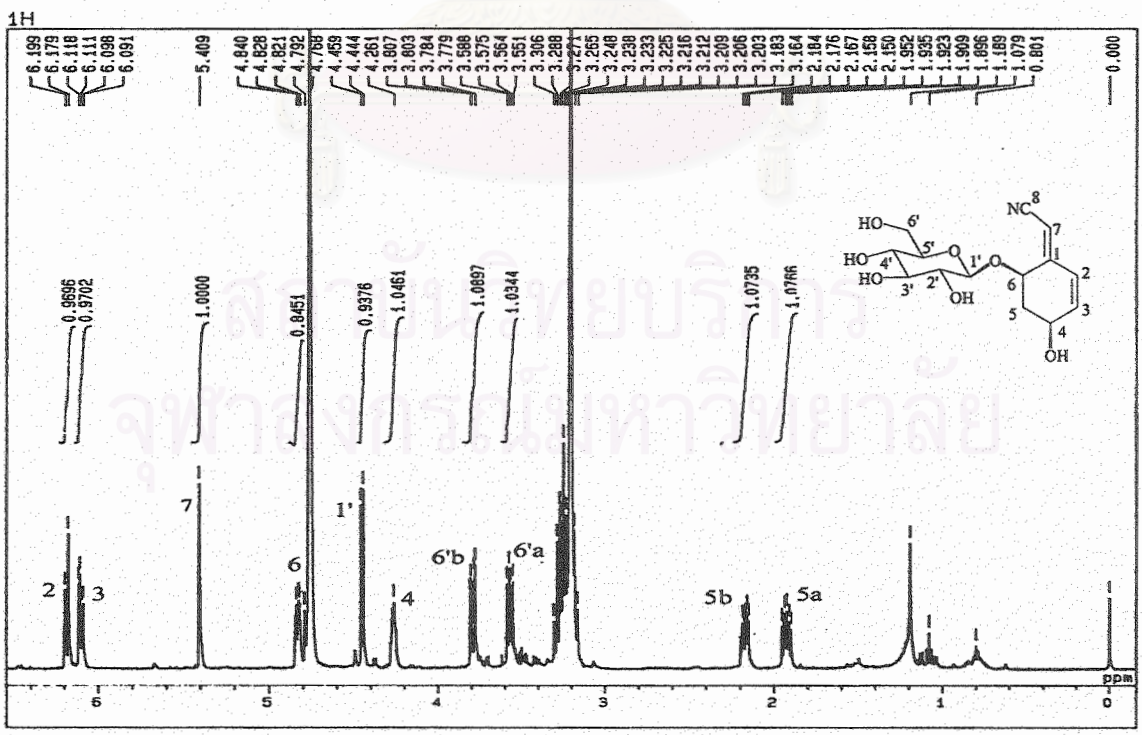


Figure 113 ^{1}H NMR (500 MHz) Spectrum of compound BRB7 (CD_3OD)

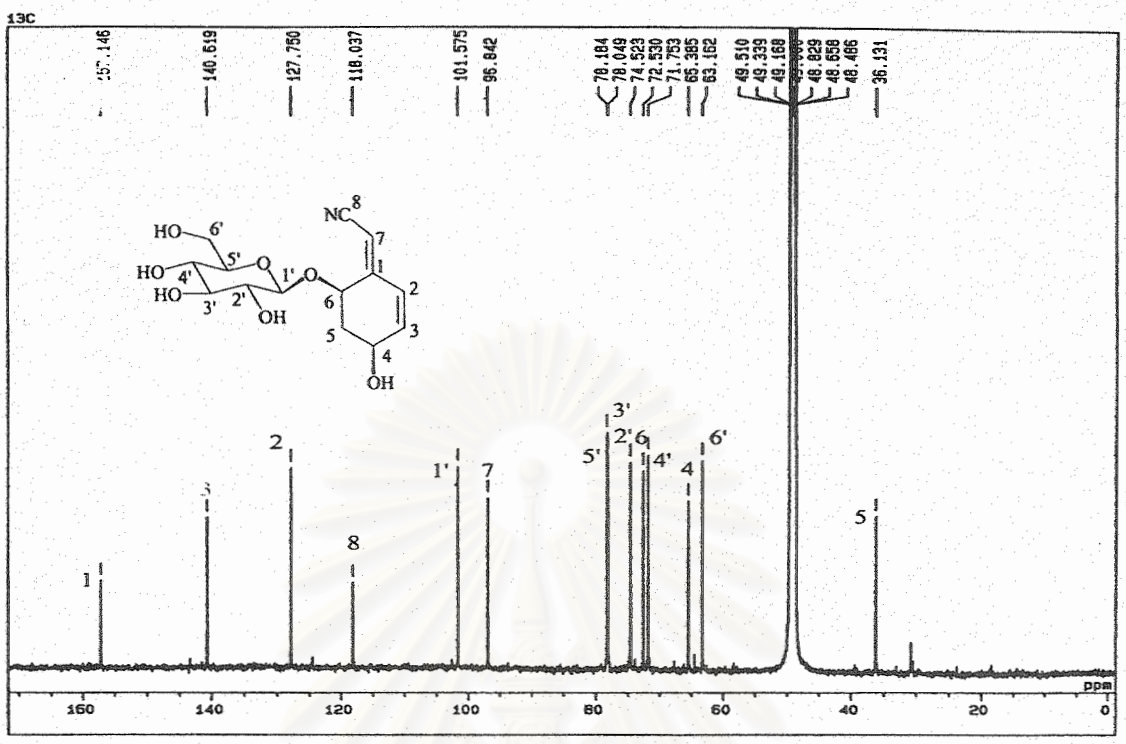


Figure 114 ^{13}C NMR (125 MHz) Spectrum of compound BRB7 (CD_3OD)

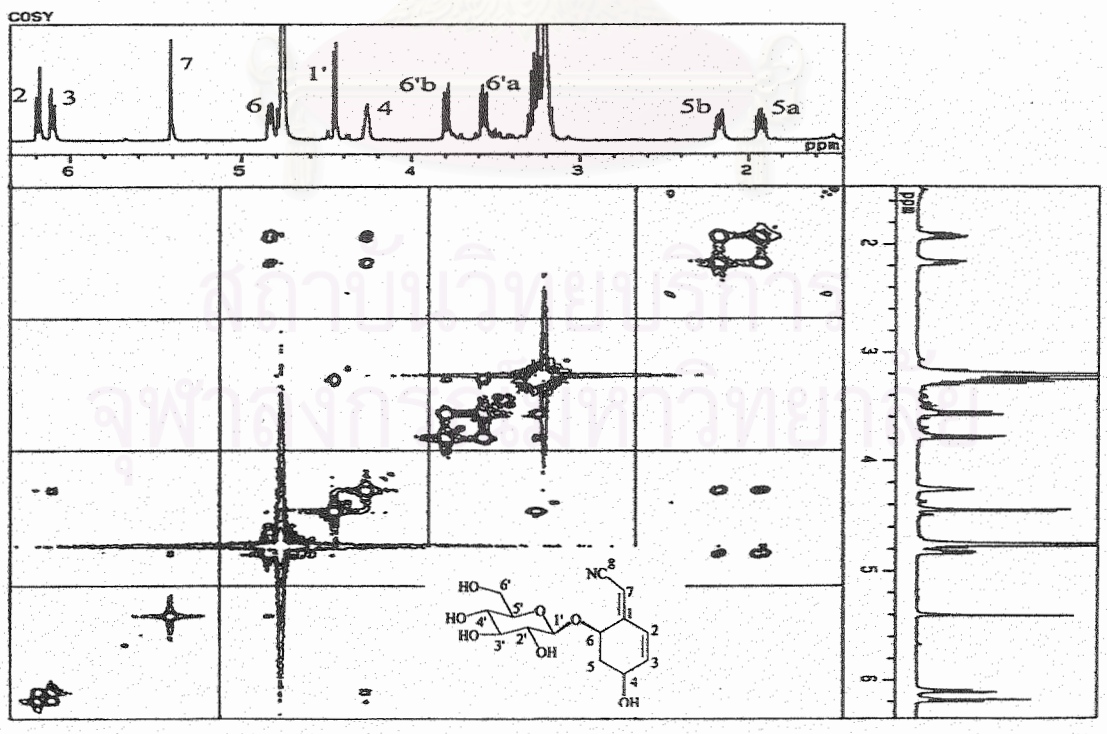


Figure 115 ^{1}H-^{1}H COSY Spectrum of compound BRB7 (CD_3OD)

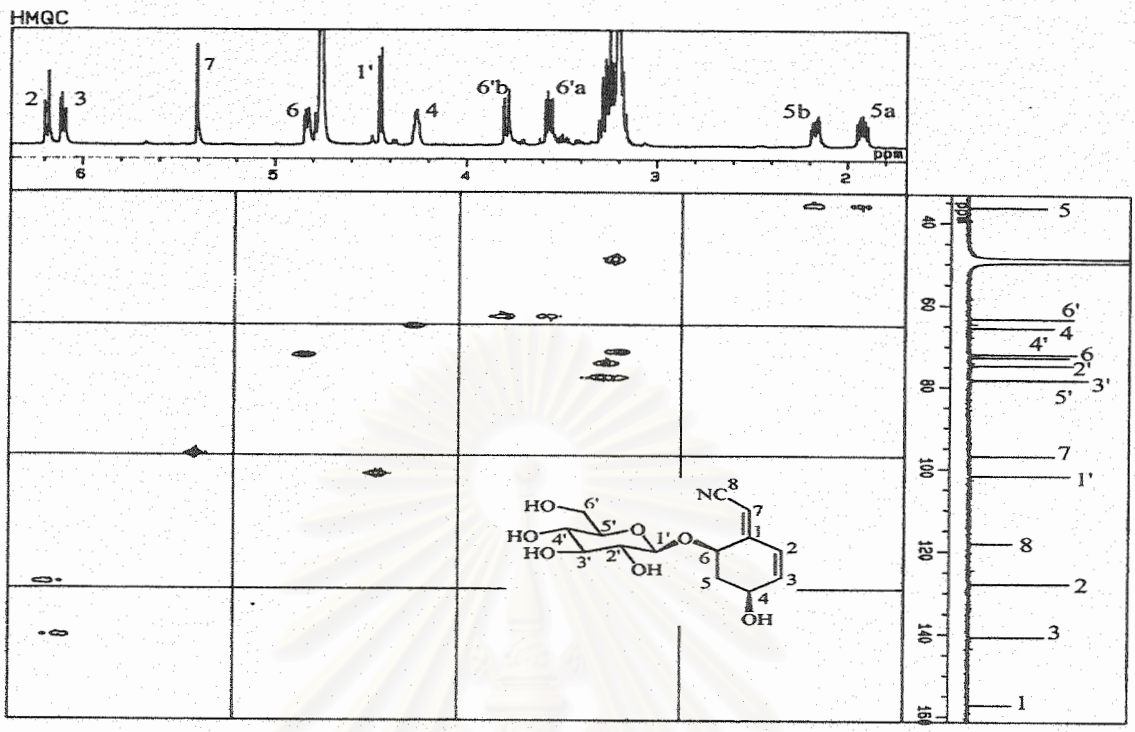


Figure 116 HMQC Spectrum of compound BRB7 (CD_3OD)

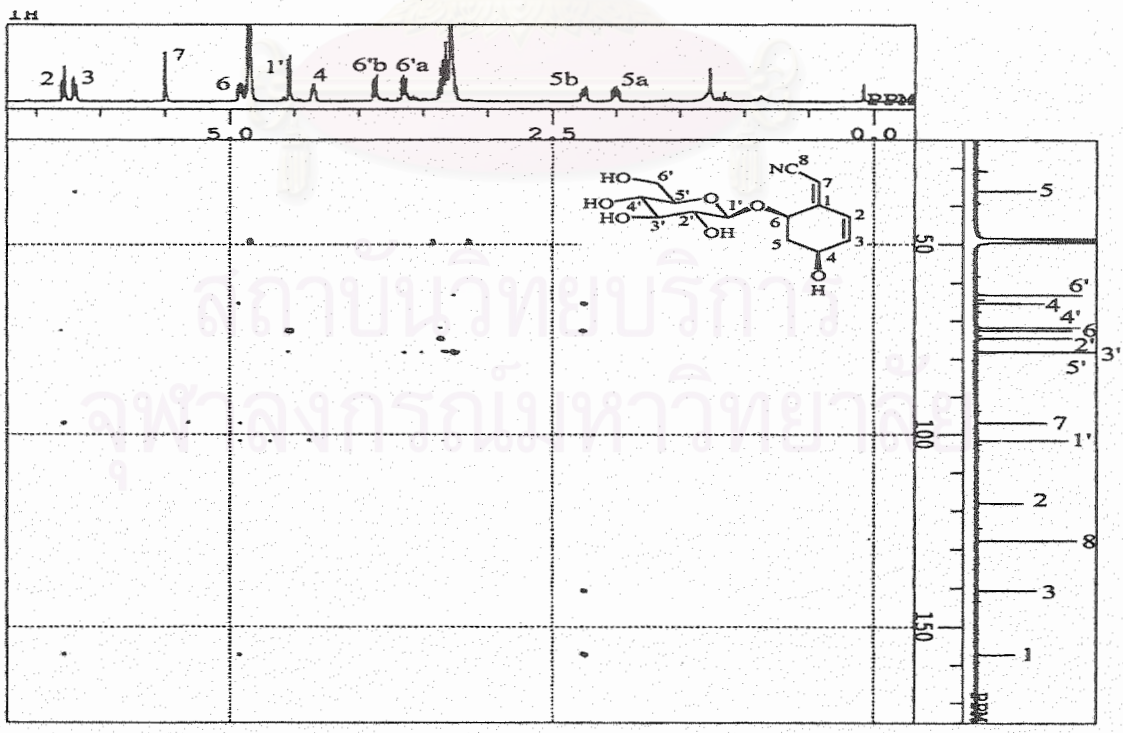


Figure 117 HMBC Spectrum of compound BRB7 (CD_3OD)

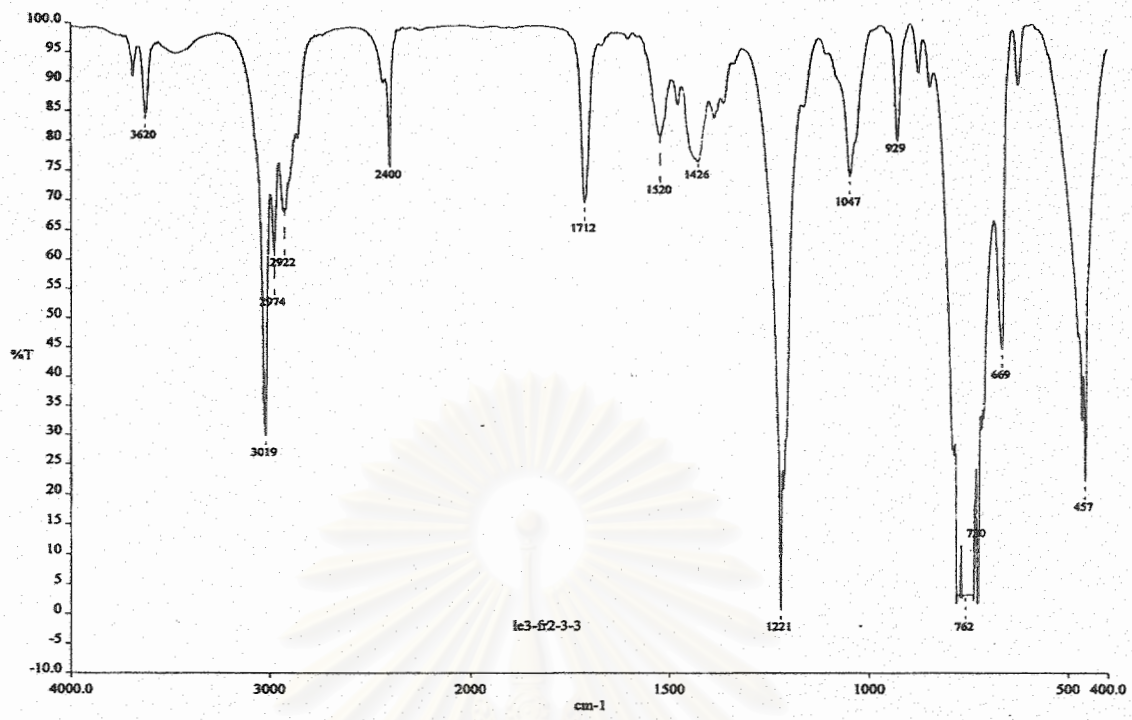


Figure 118 IR Spectrum of compound CBE1 (neat)

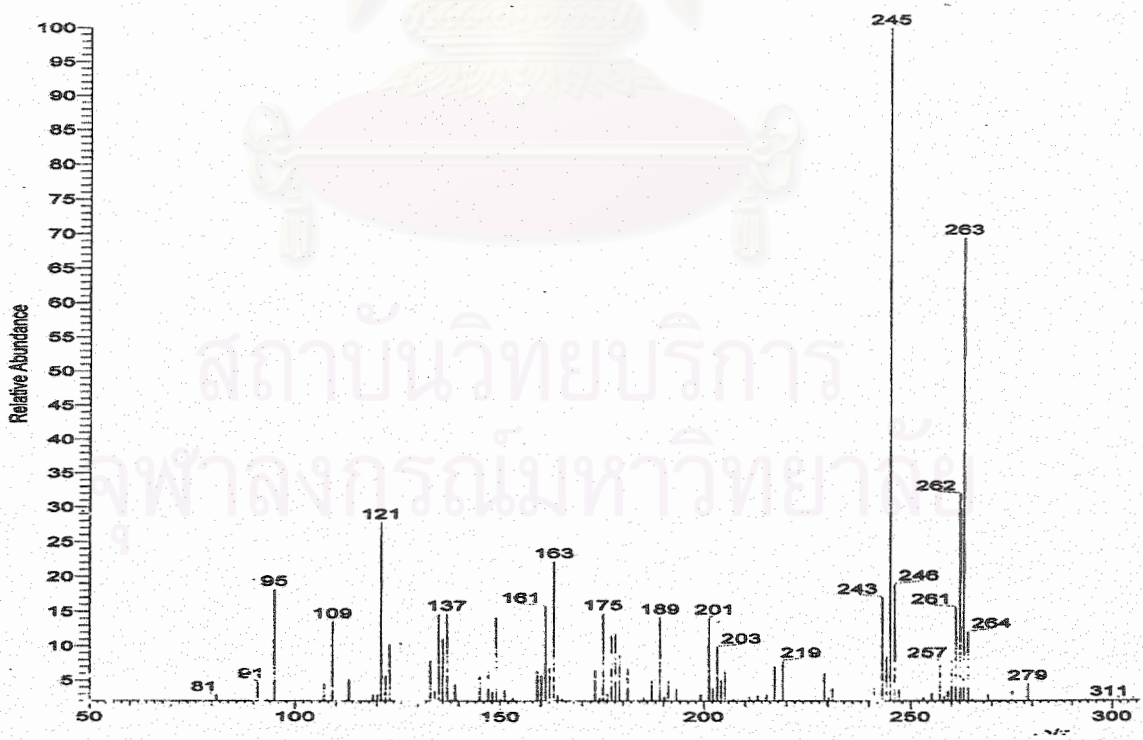


Figure119 EIMS Mass spectrum of compound CBE1

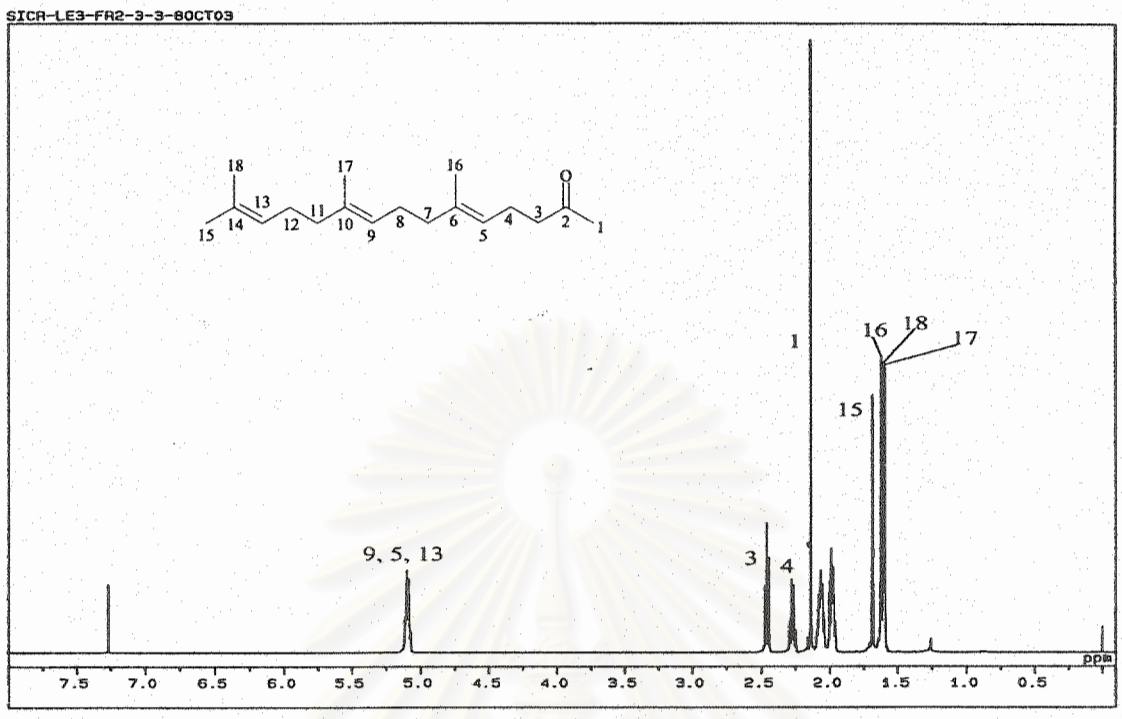


Figure 120 ^{1}H NMR (400 MHz) Spectrum of compound CBE1 ($CDCl_3$)

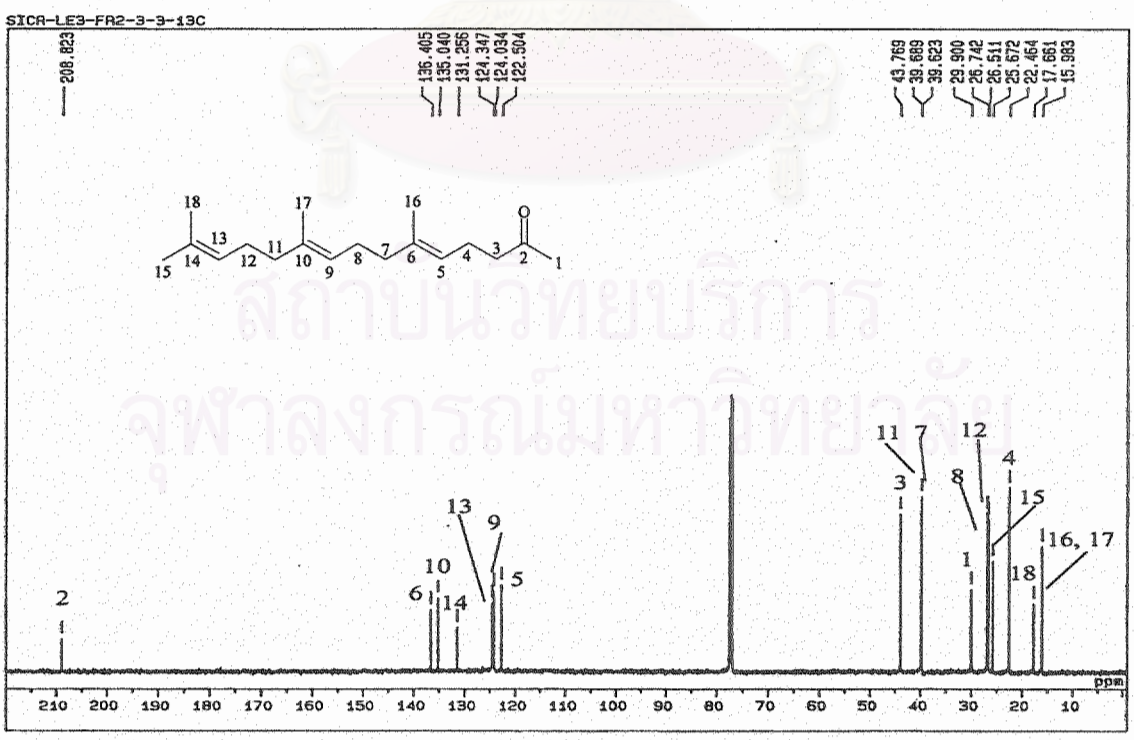


Figure 121 ^{13}C NMR (100 MHz) Spectrum of compound CBE1 ($CDCl_3$)

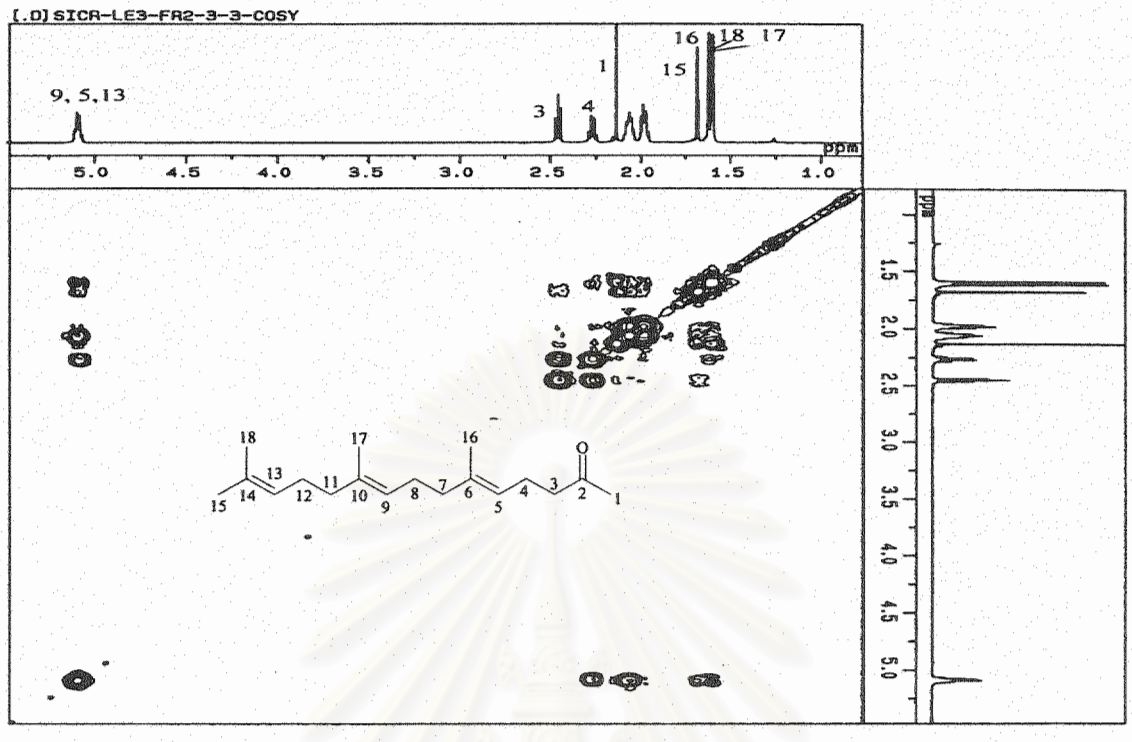


Figure 122 ^{1}H-^{1}H COSY Spectrum of compound CBE1 ($CDCl_3$)

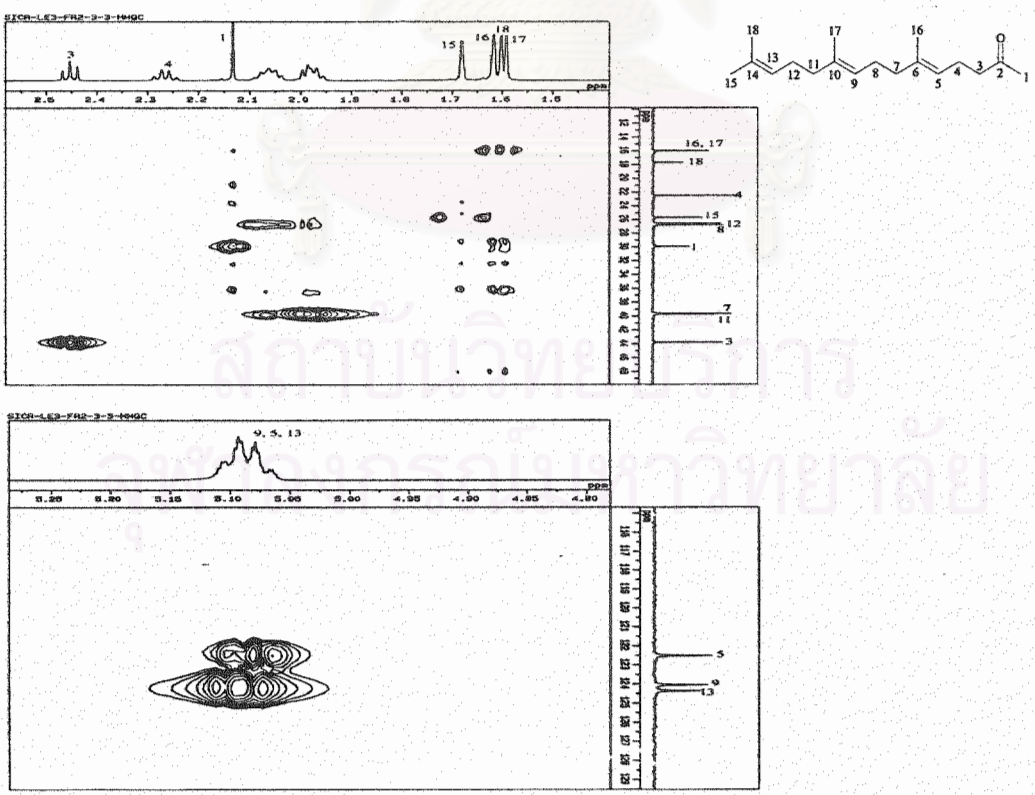


Figure 123 HMQC Spectrum of compound CBE1 ($CDCl_3$)

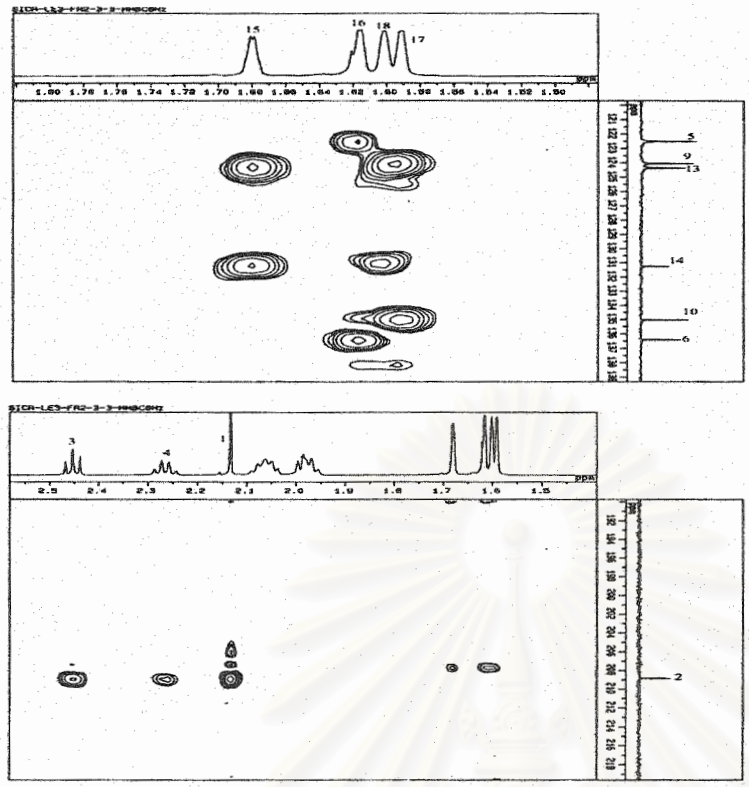


Figure 124 HMBC Spectrum of compound CBE1 ($CDCl_3$)

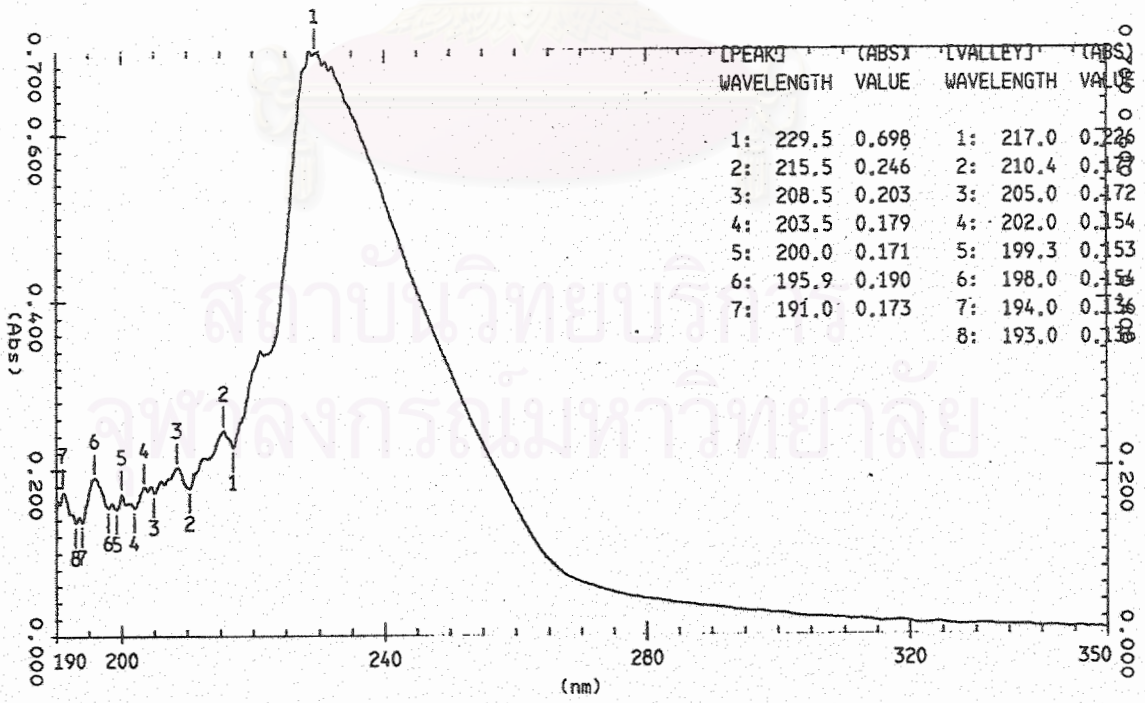


Figure 125 UV Spectrum of compound CBE2 (methanol)

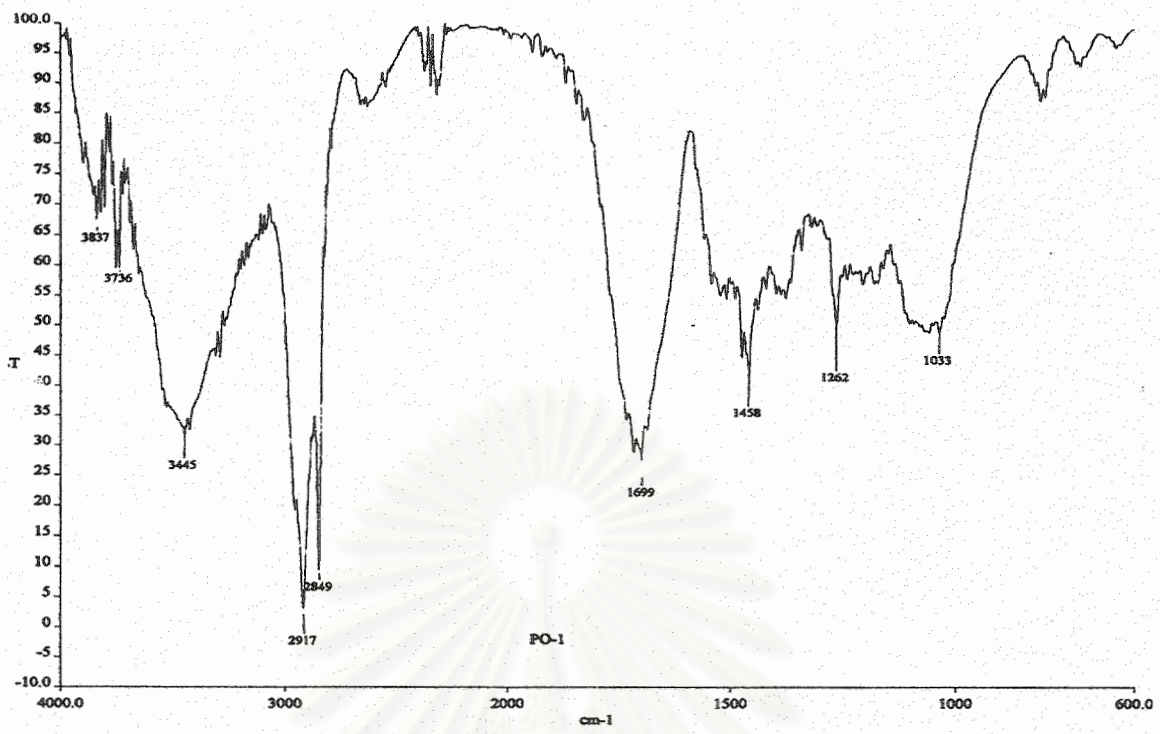


Figure 126 IR Spectrum of compound CBE2 (neat)

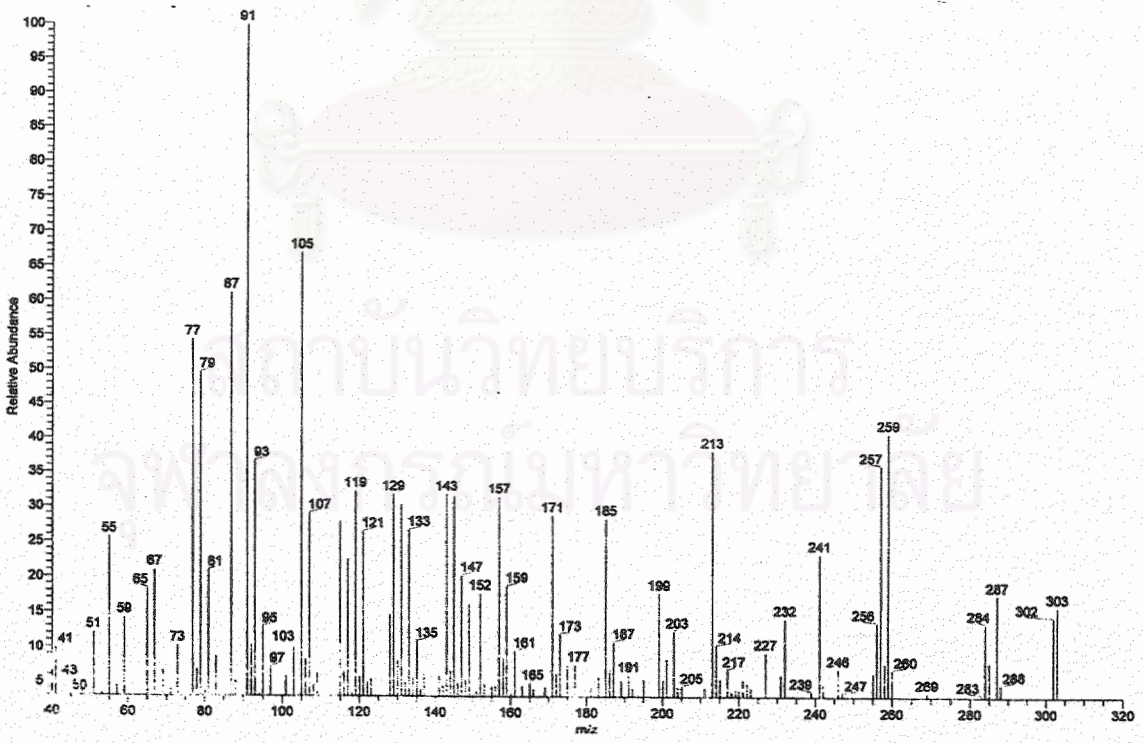


Figure 127 EIMS Mass spectrum of compound CBE2

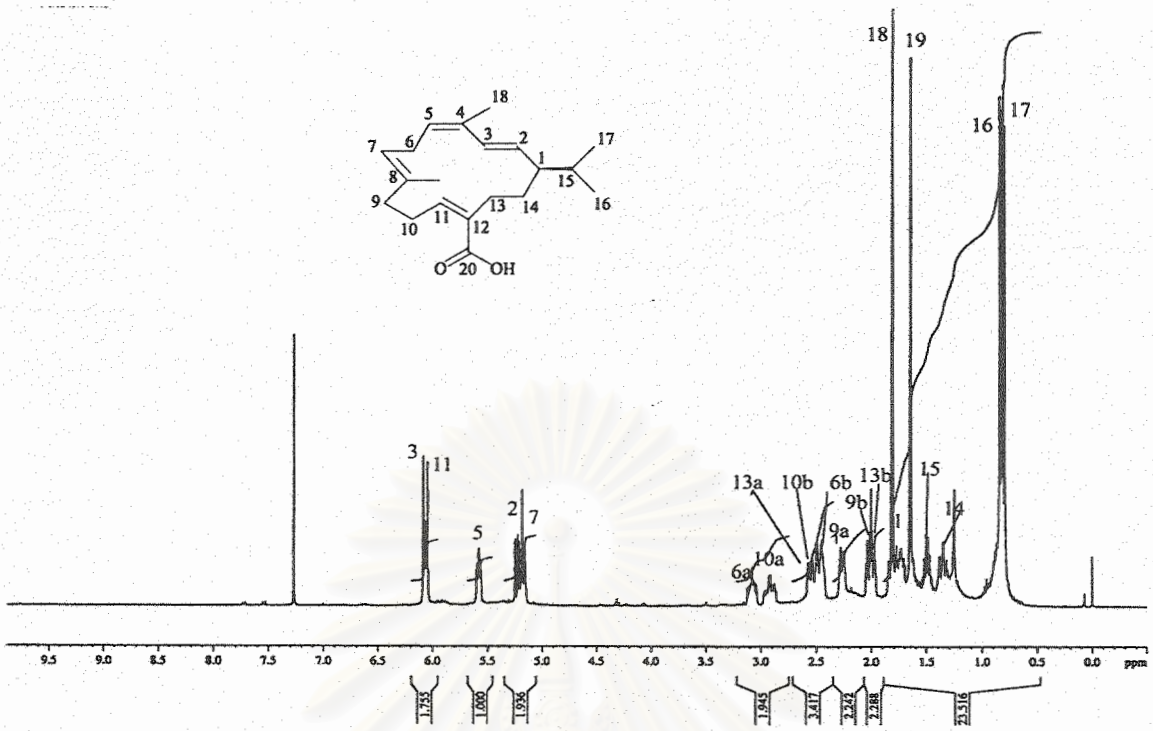


Figure 128 ^{1}H NMR (400 MHz) Spectrum of compound CBE2 ($CDCl_3$)

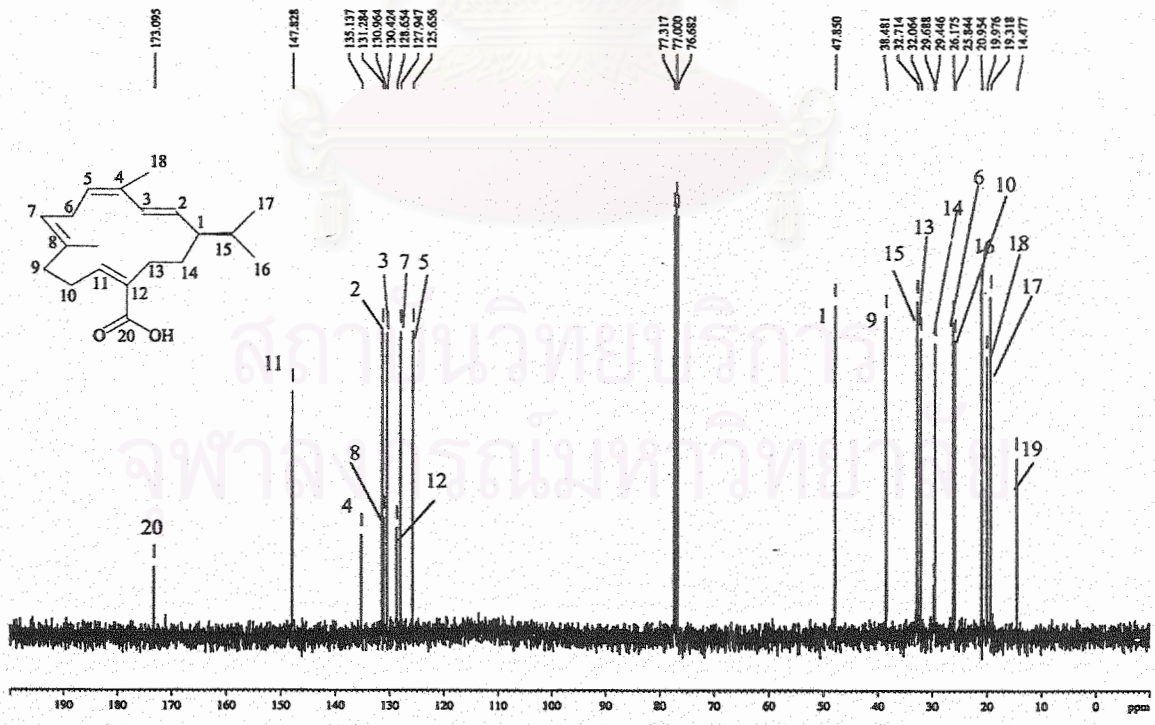


Figure 129 ^{13}C NMR (100 MHz) Spectrum of compound CBE2 ($CDCl_3$)

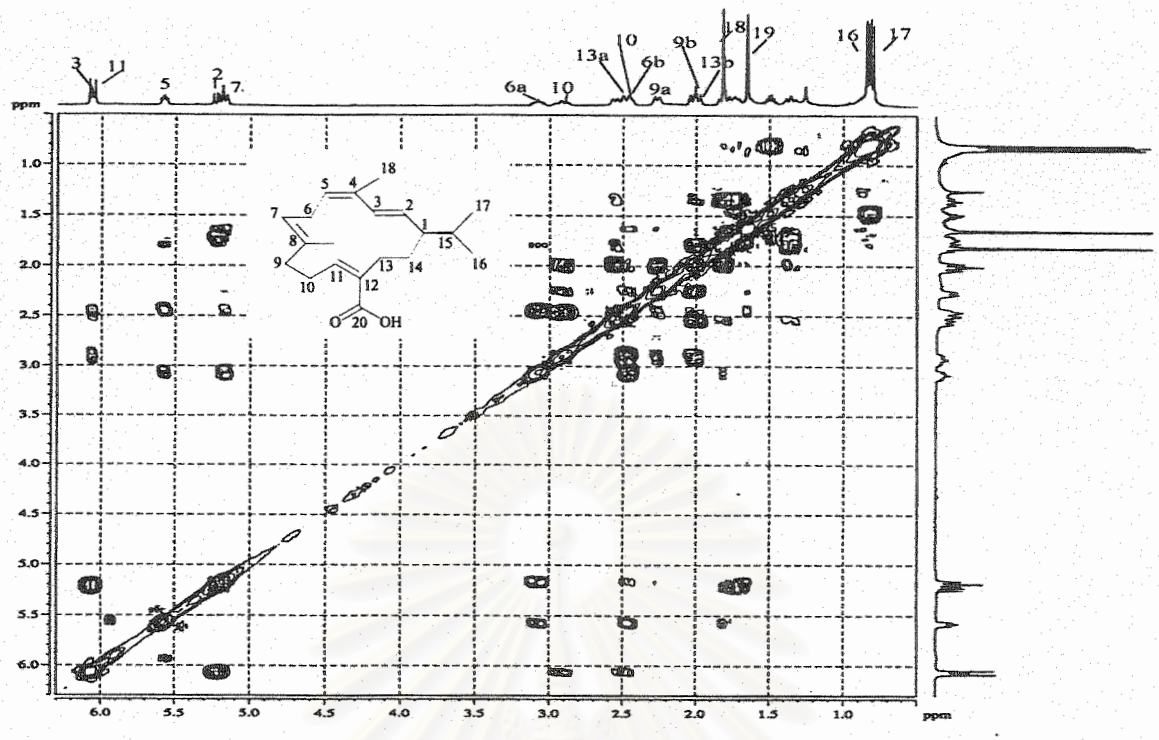


Figure 130 ^{1}H-^{1}H COSY Spectrum of compound CBE2 ($CDCl_3$)

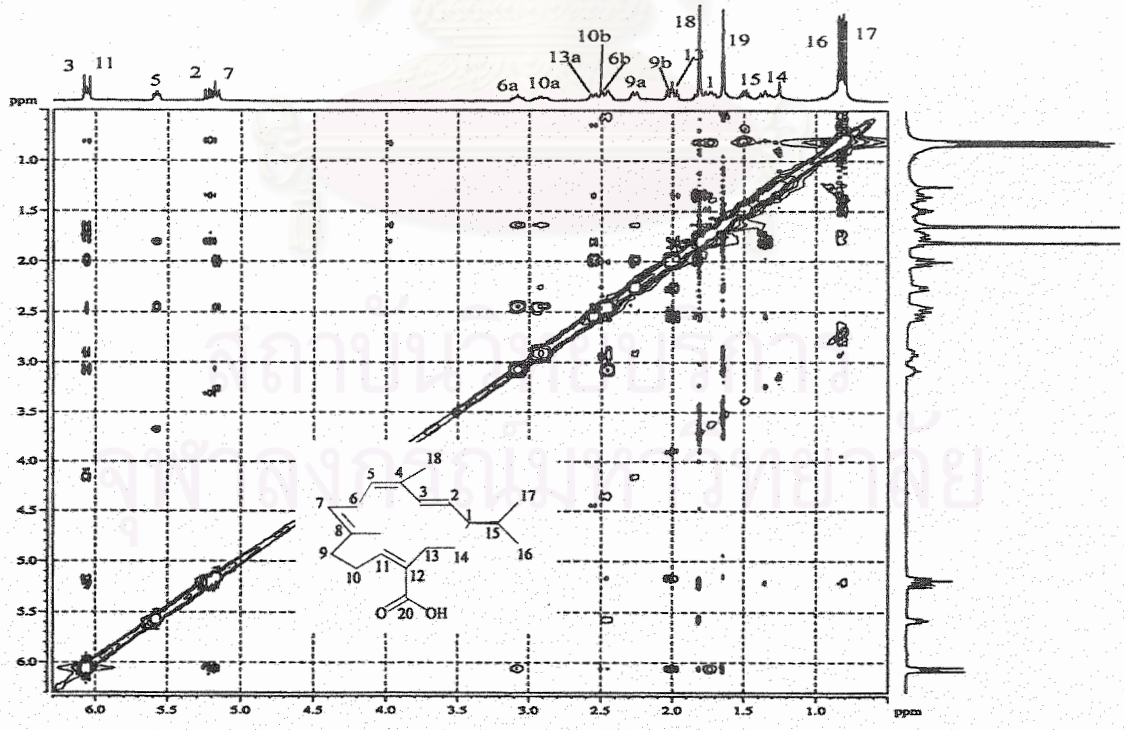


Figure 131 NOESY Spectrum of compound CBE2 ($CDCl_3$)

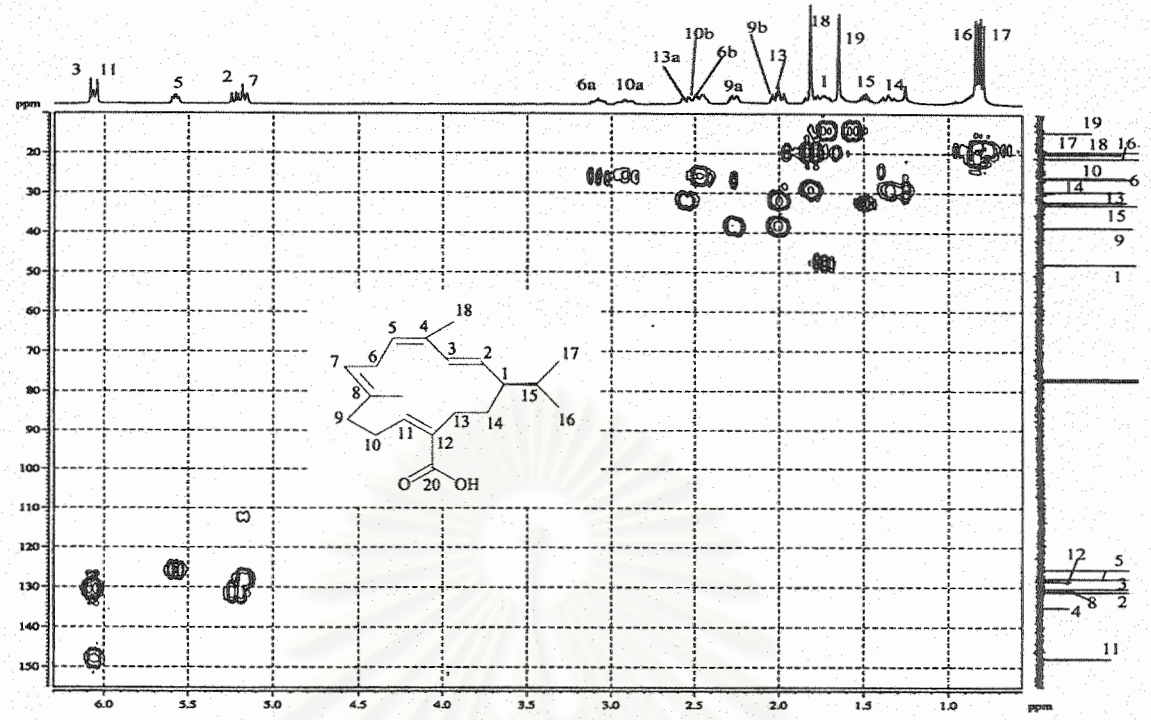


Figure 132 HMQC Spectrum of compound CBE2 ($CDCl_3$)

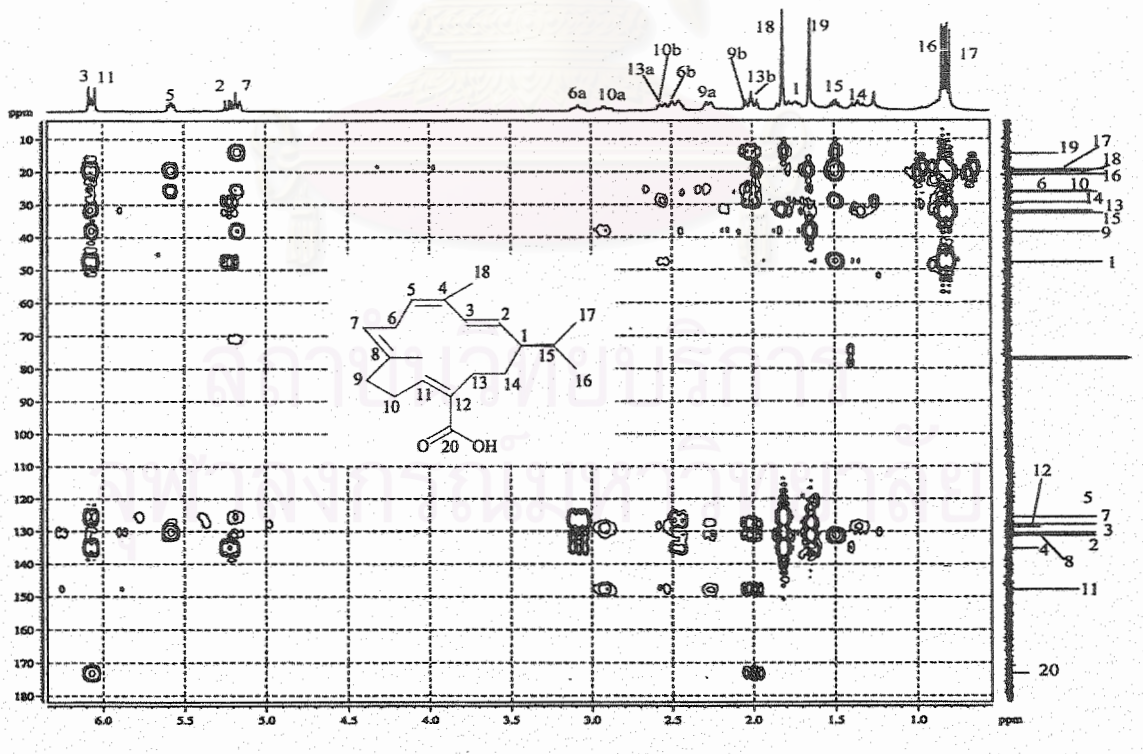


Figure 133 HMBC Spectrum of compound CBE2 ($CDCl_3$)

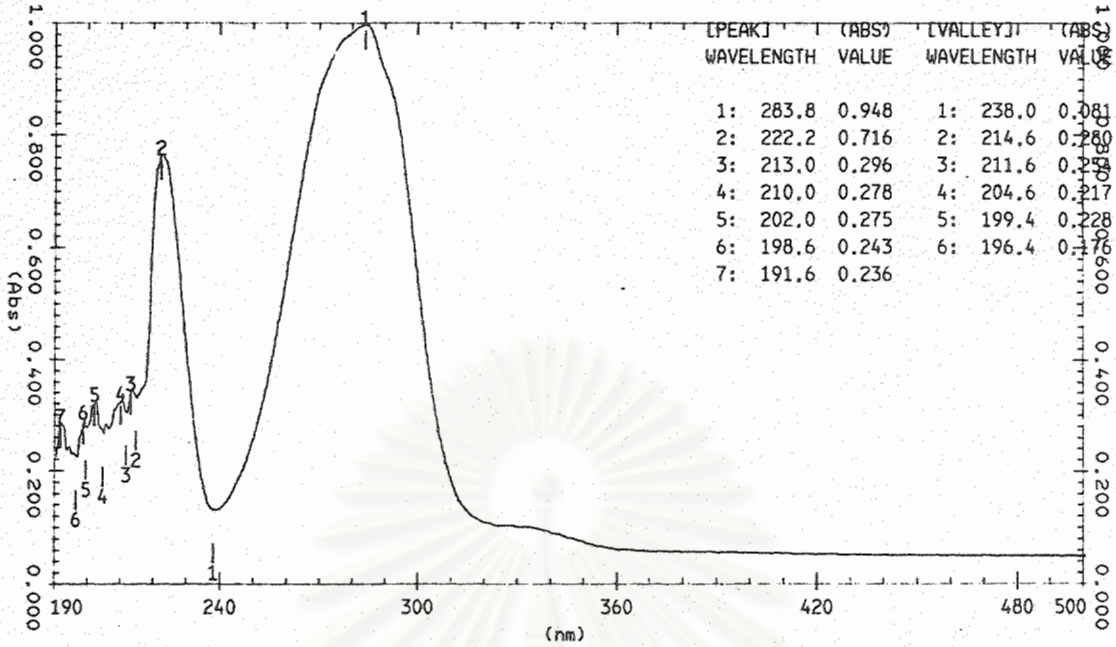


Figure 134 UV Spectrum of compound CBE3 (methanol)

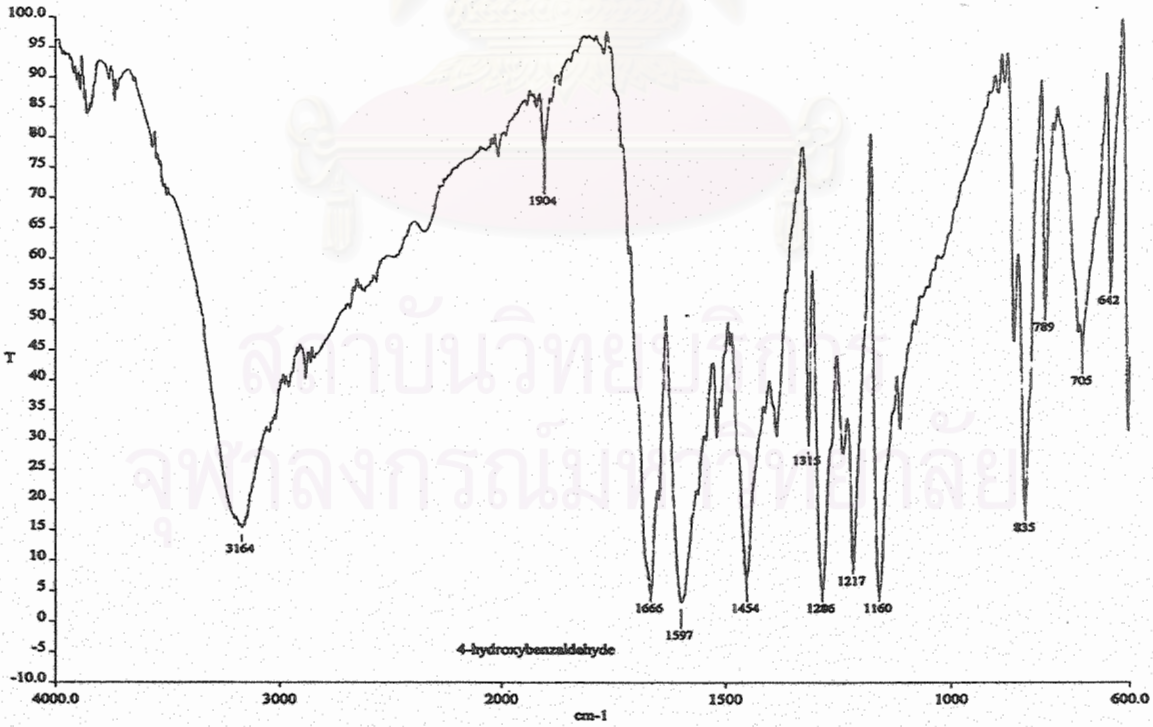


Figure 135 IR Spectrum of compound CBE3 (KBr disc)

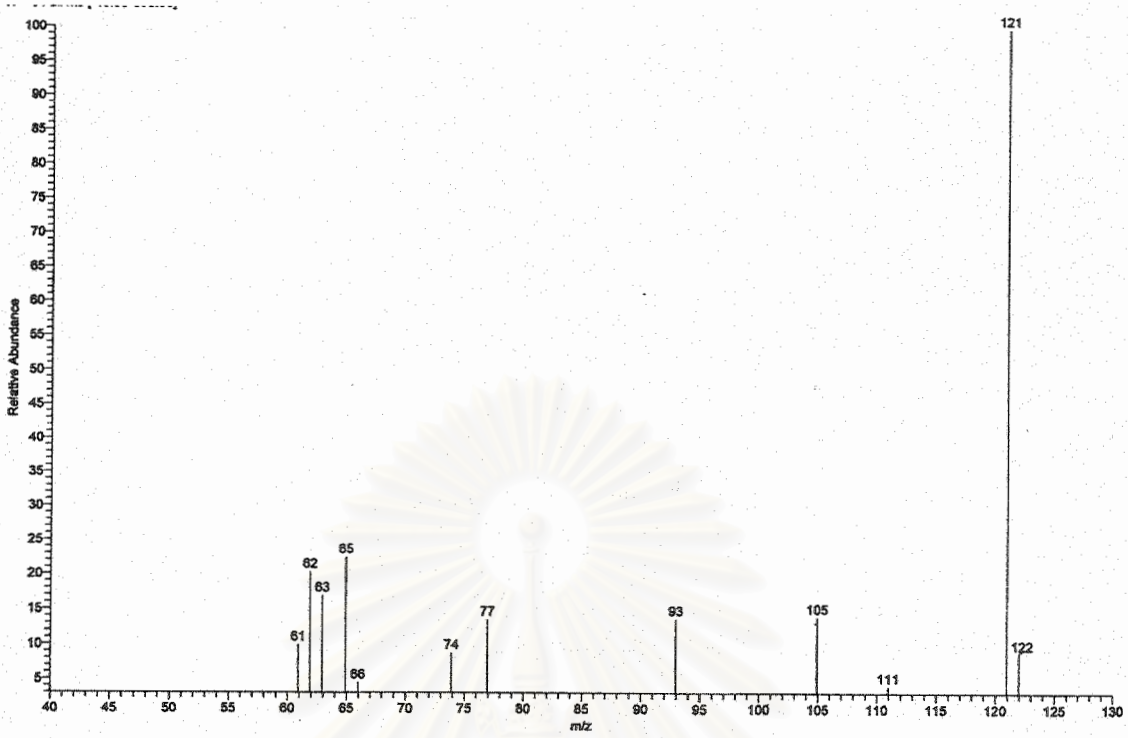


Figure 136 EIMS Mass spectrum of compound CBE3

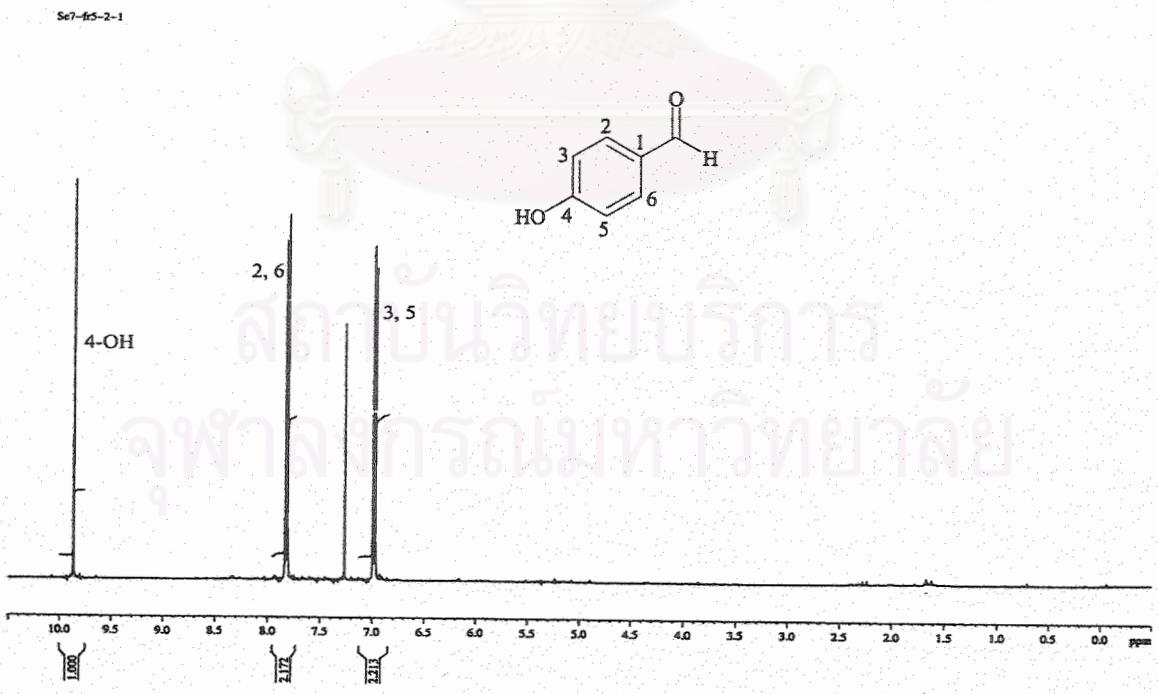


Figure 137 ^{1}H NMR (400 MHz) Spectrum of compound CBE3 ($CDCl_3$)

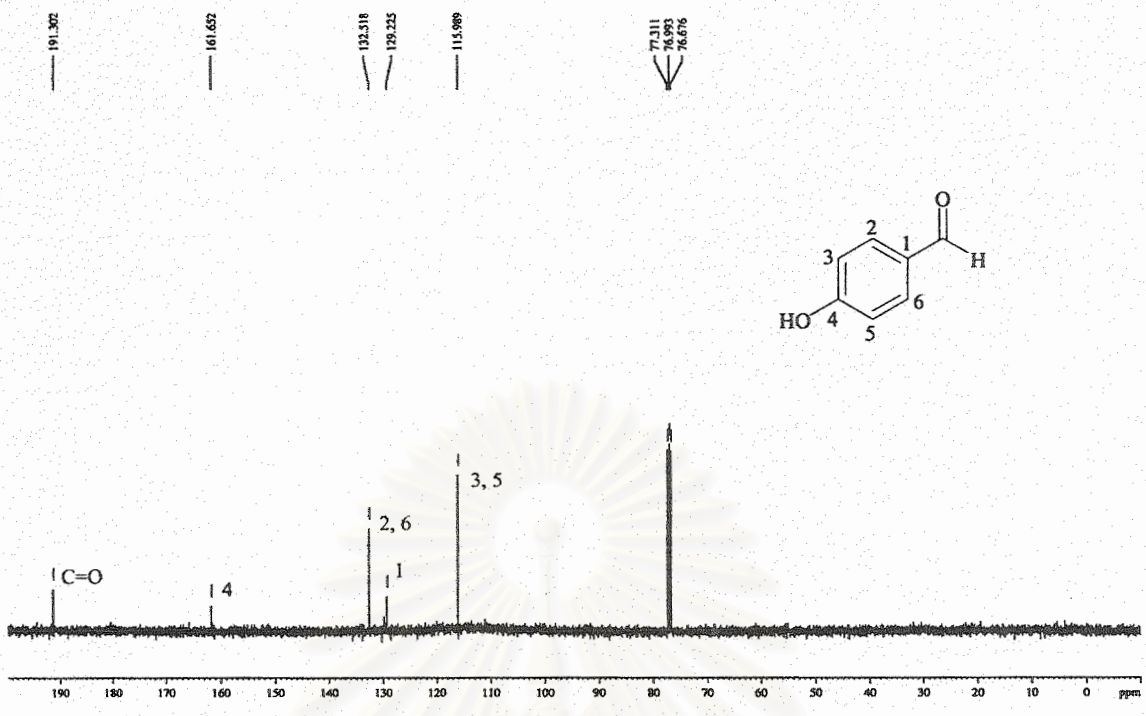


Figure 138 ^{13}C NMR (100 MHz) Spectrum of compound CBE3 ($CDCl_3$)

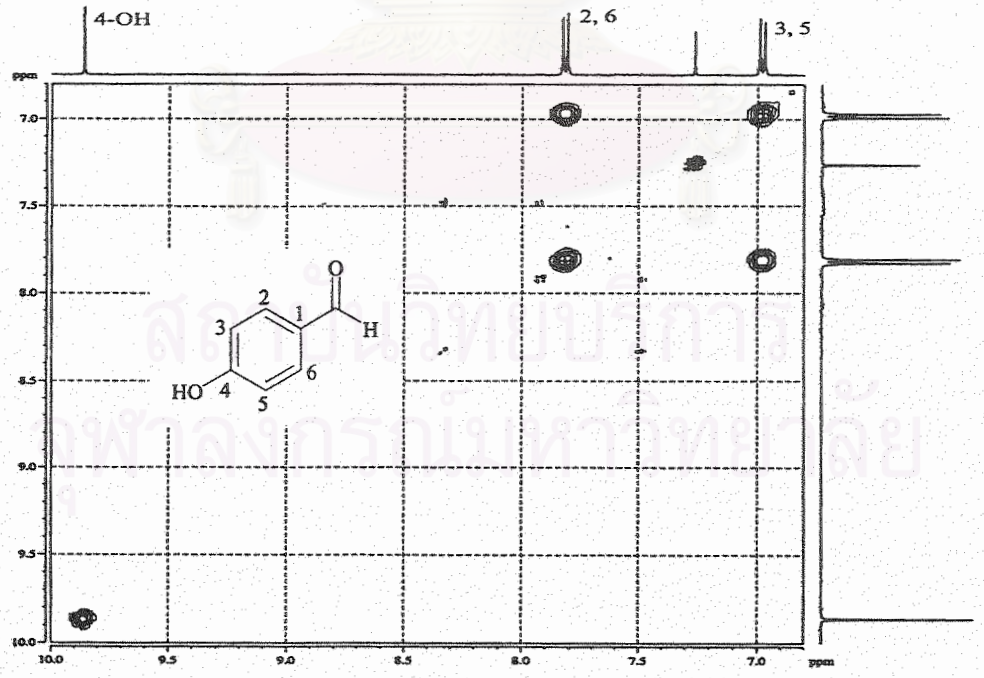


Figure 139 ^{1}H-^{1}H COSY Spectrum of compound CBE3 ($CDCl_3$)

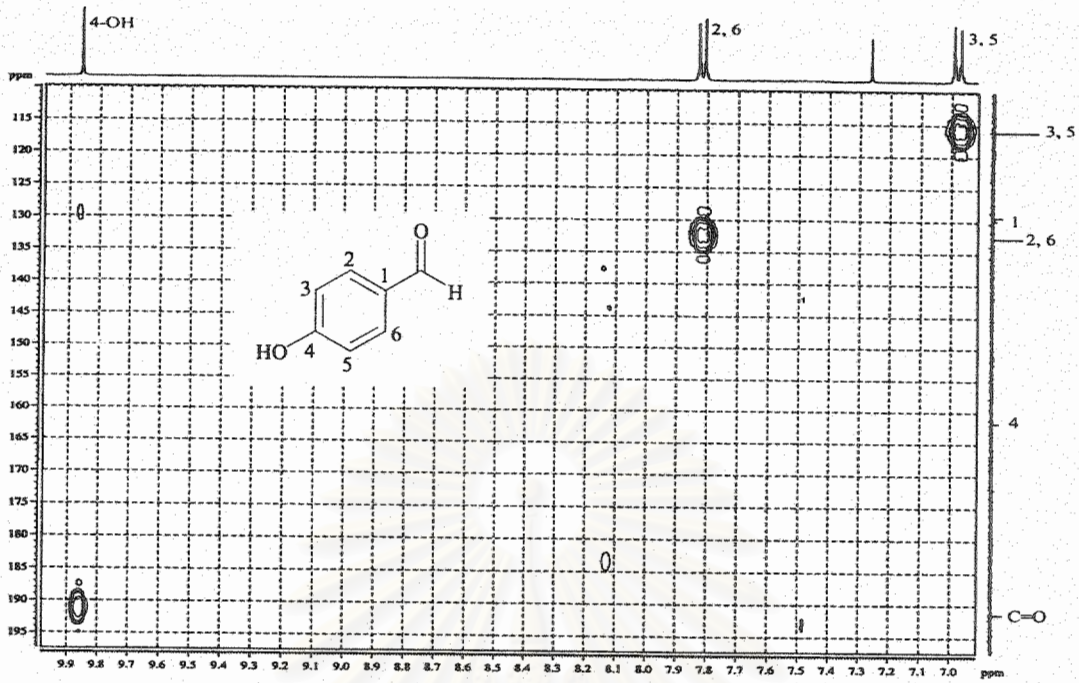


Figure 140 HMQC Spectrum of compound CBE3 ($CDCl_3$)

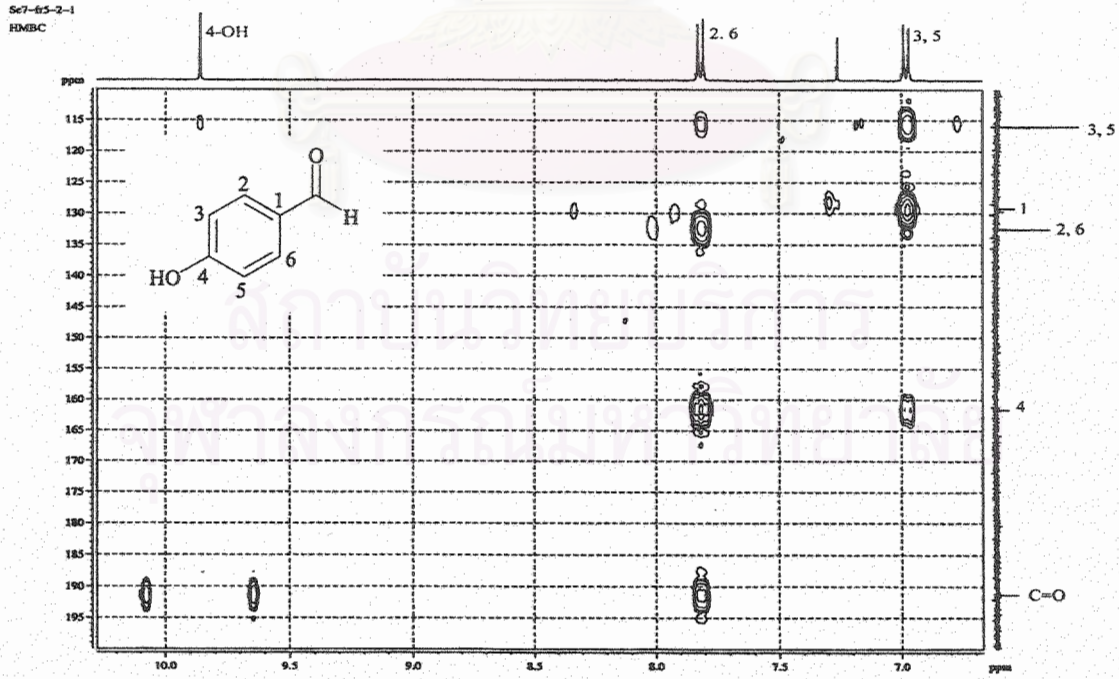


Figure 141 HMBC Spectrum of compound CBE3 ($CDCl_3$)

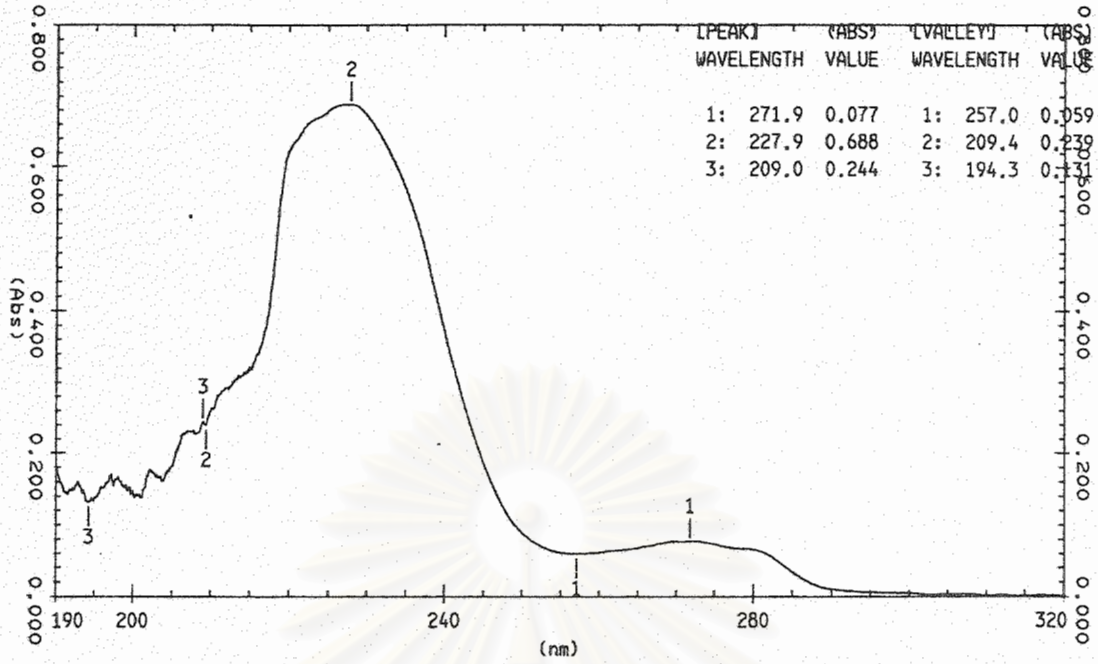


Figure 142 UV Spectrum of compound CBE4 (methanol)

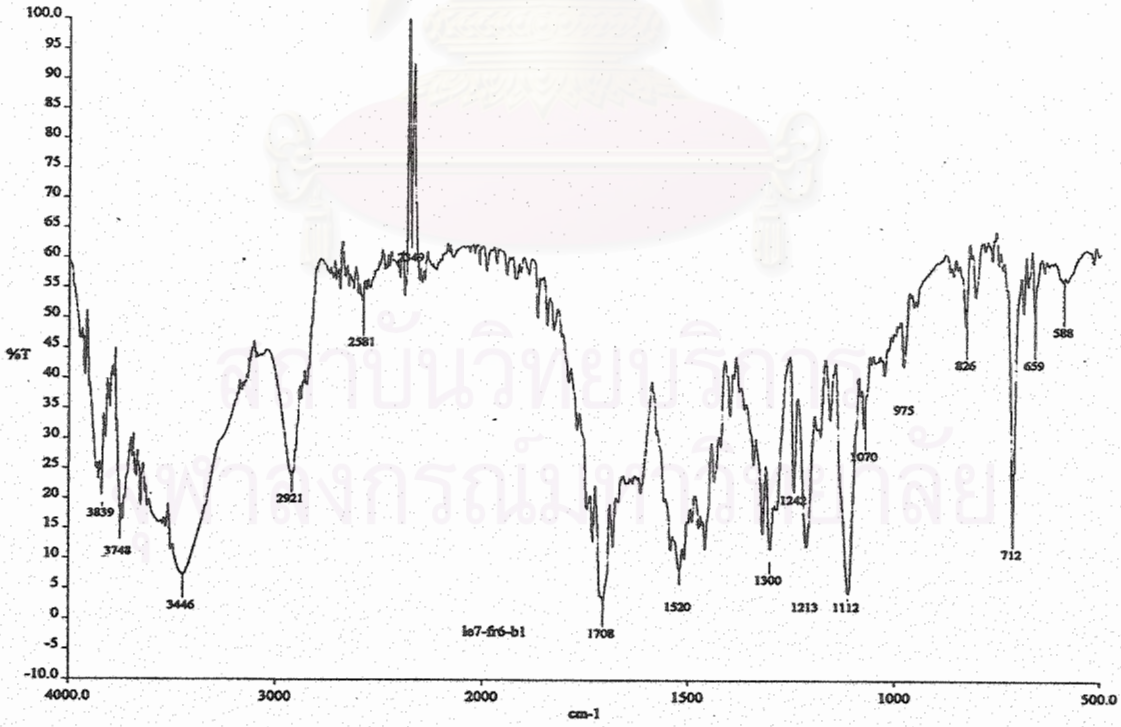


Figure 143 IR Spectrum of compound CBE4 (neat)

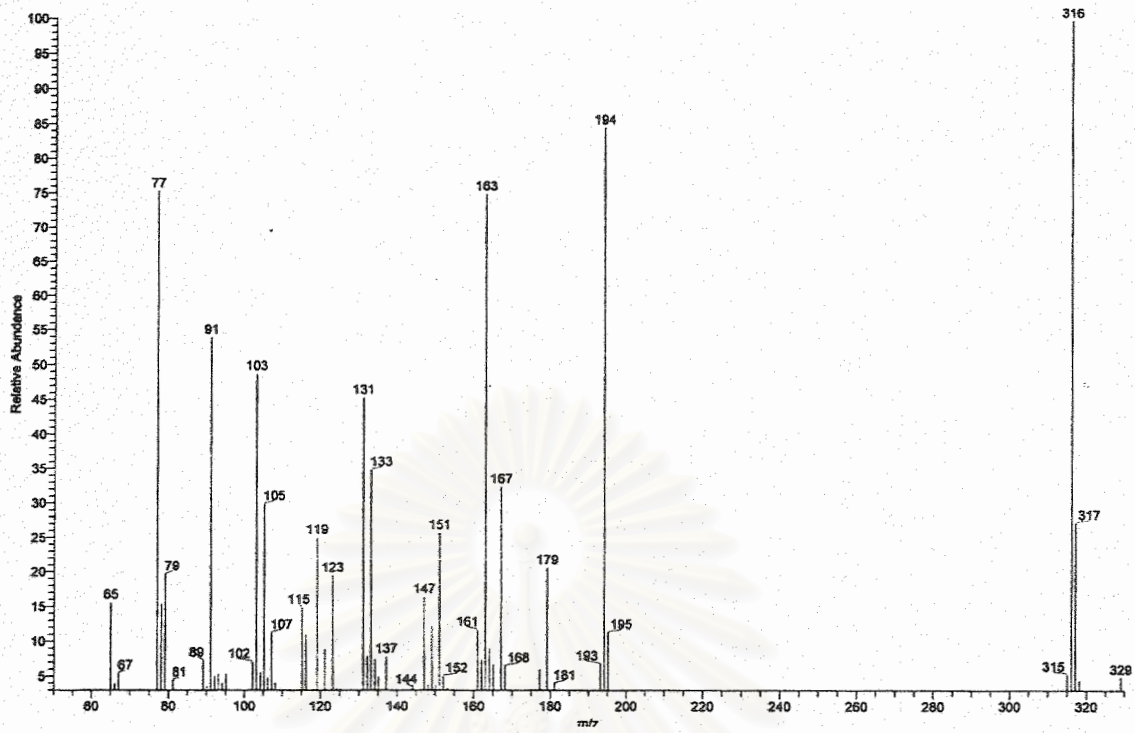


Figure 144 EIMS Mass spectrum of compound CBE4

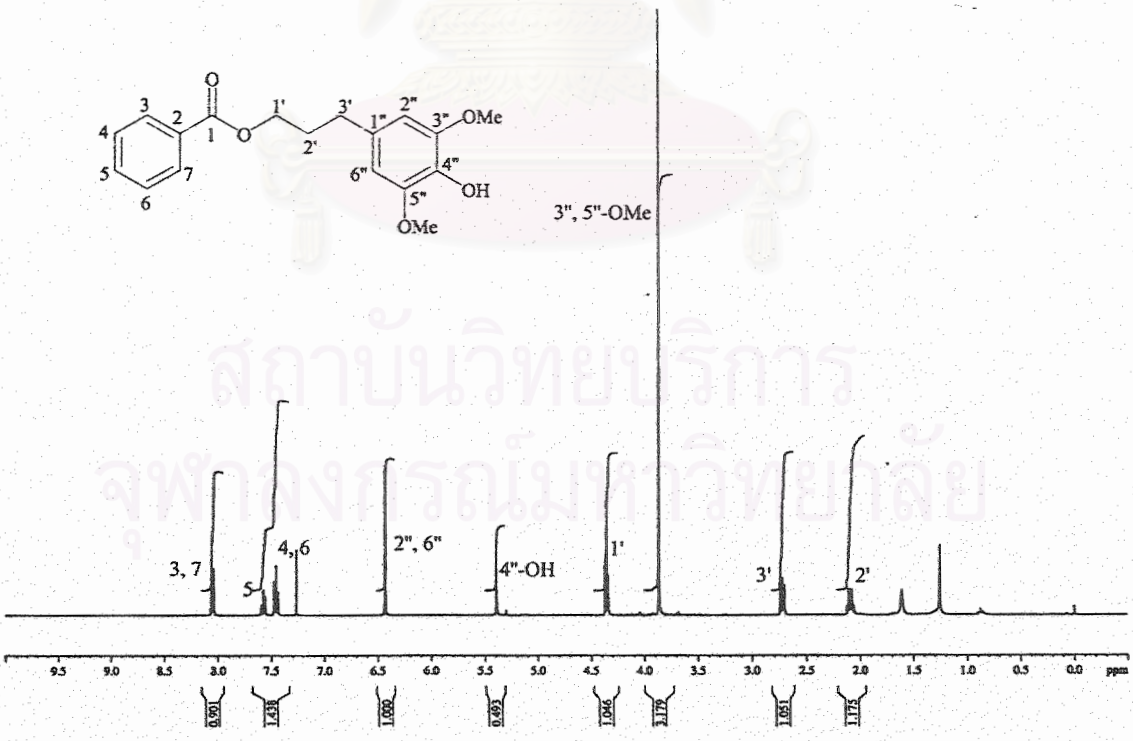


Figure 145 ^{1}H NMR (400 MHz) Spectrum of compound CBE4 ($CDCl_3$)

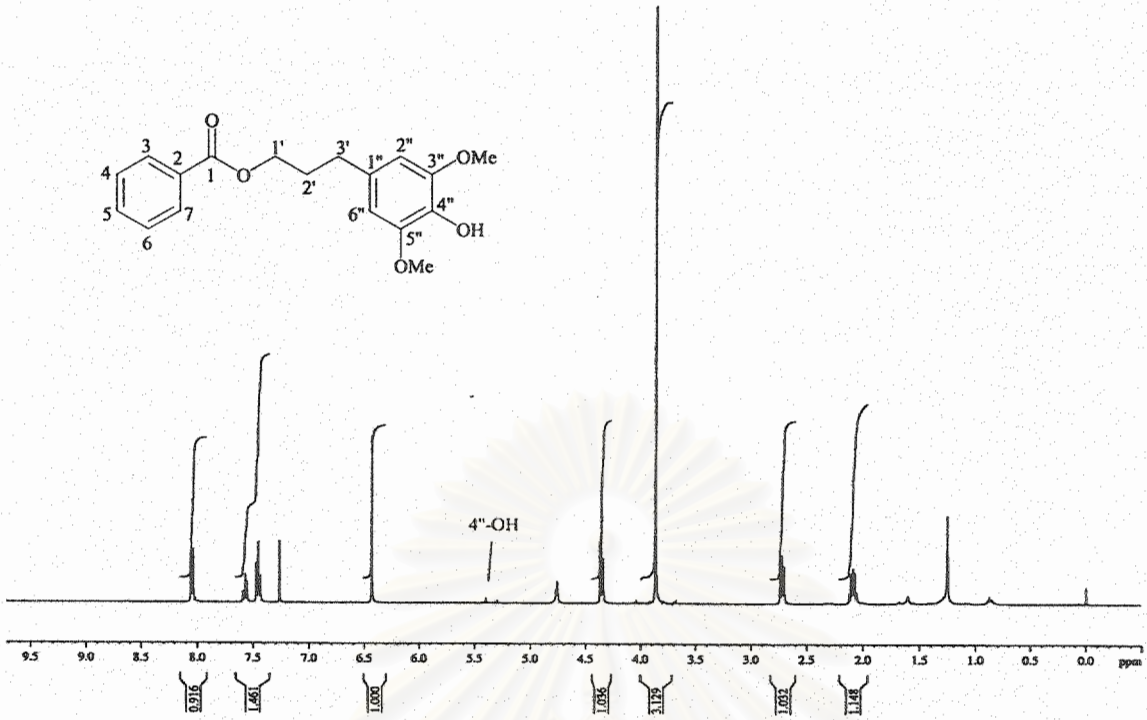


Figure 146 ^{1}H NMR (400 MHz) Spectrum of compound CBE4 ($CDCl_3$+D_2O)

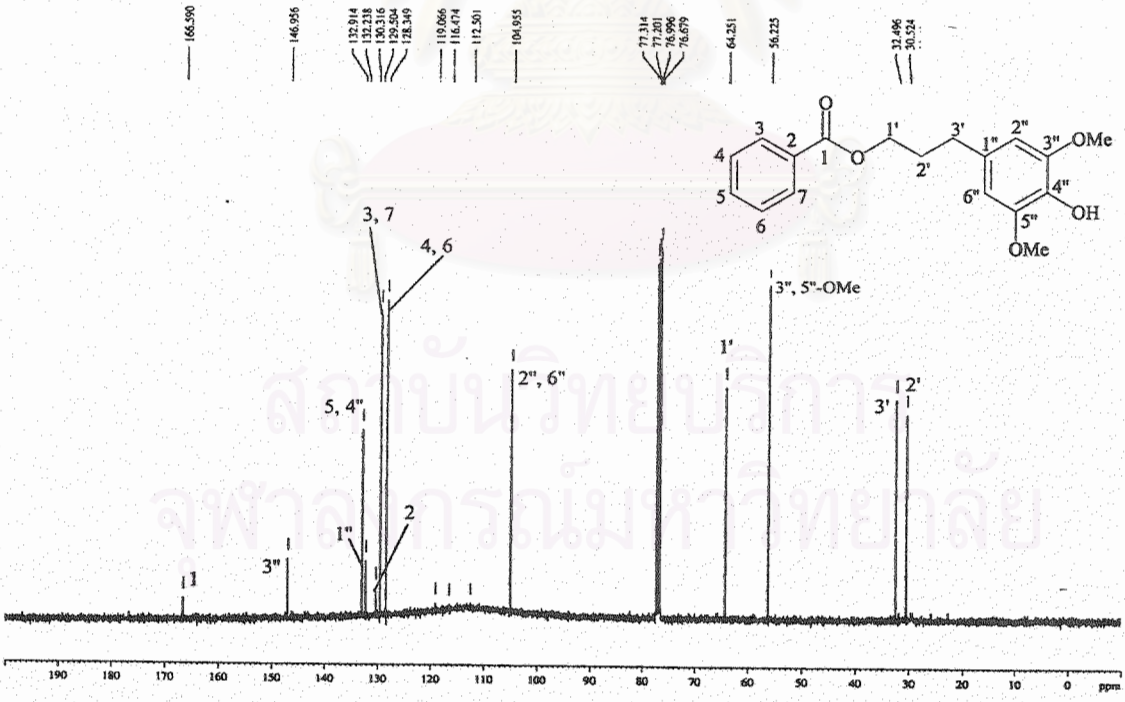


Figure 147 ^{13}C NMR (100 MHz) Spectrum of compound CBE4 ($CDCl_3$)

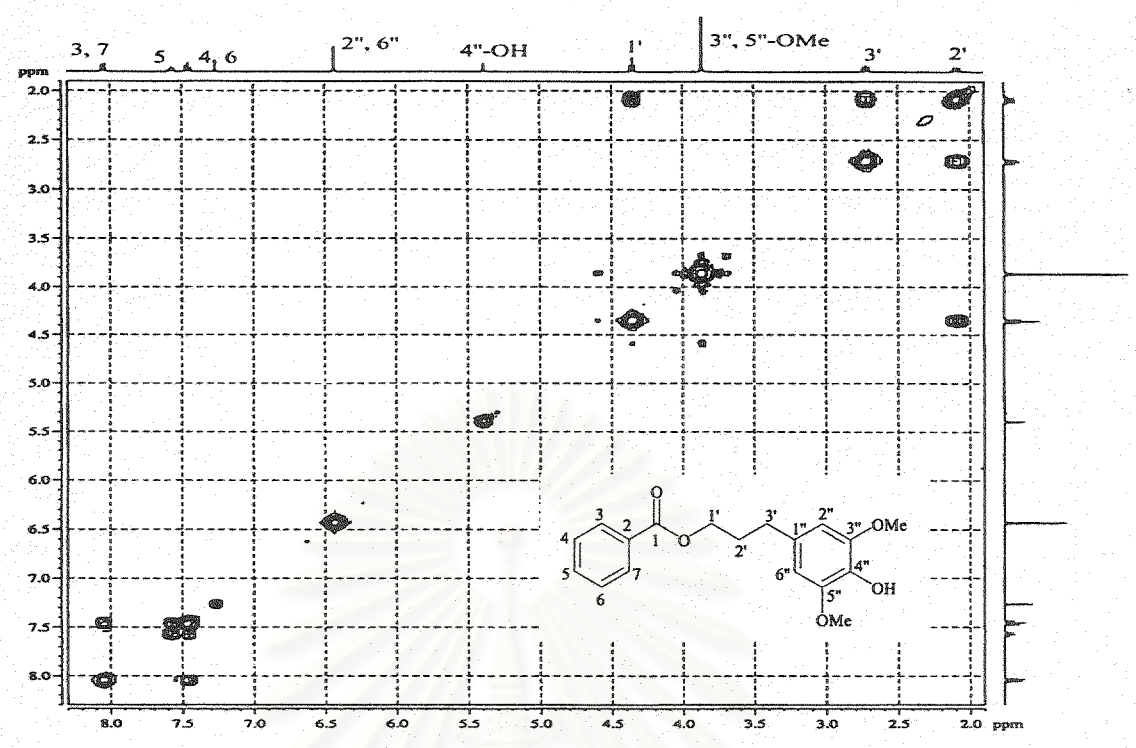


Figure 148 ^{1}H-^{1}H COSY Spectrum of compound CBE4 ($CDCl_3$)

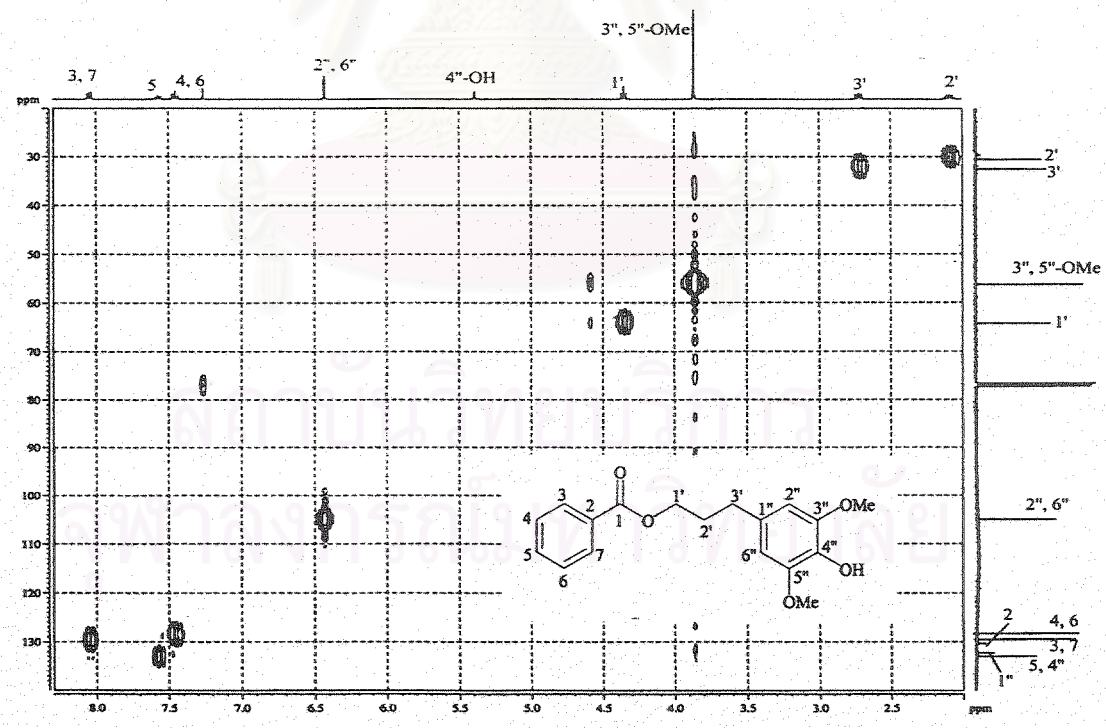


Figure 149 HMQC Spectrum of compound CBE4 ($CDCl_3$)

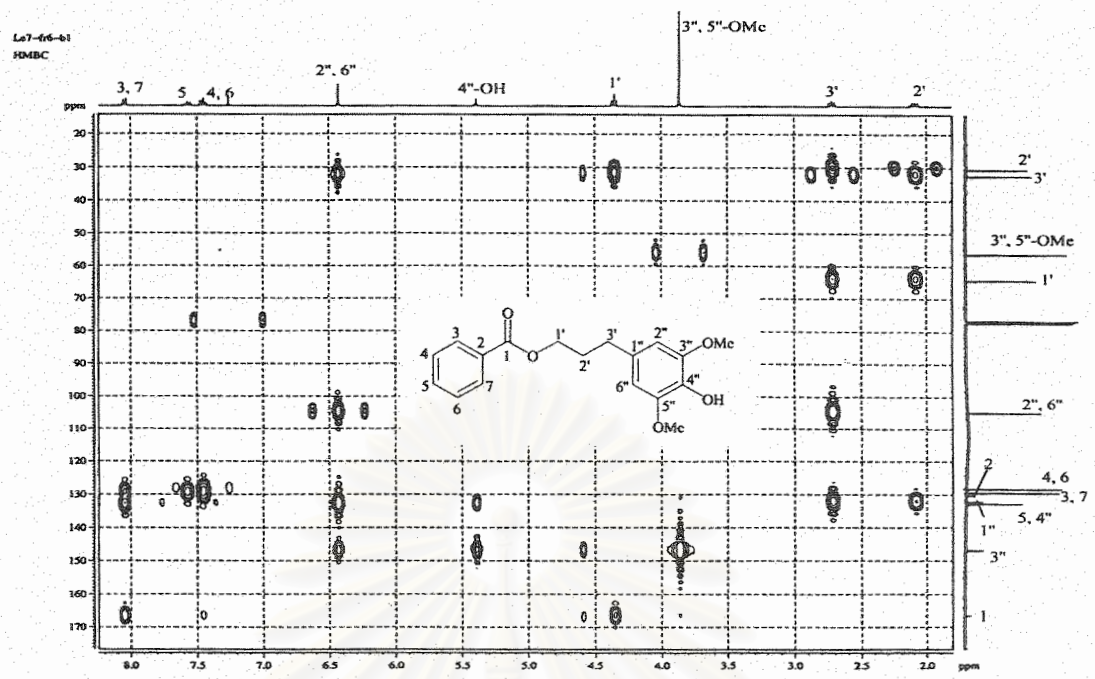


Figure 150 HMBC Spectrum of compound CBE4 ($CDCl_3$)

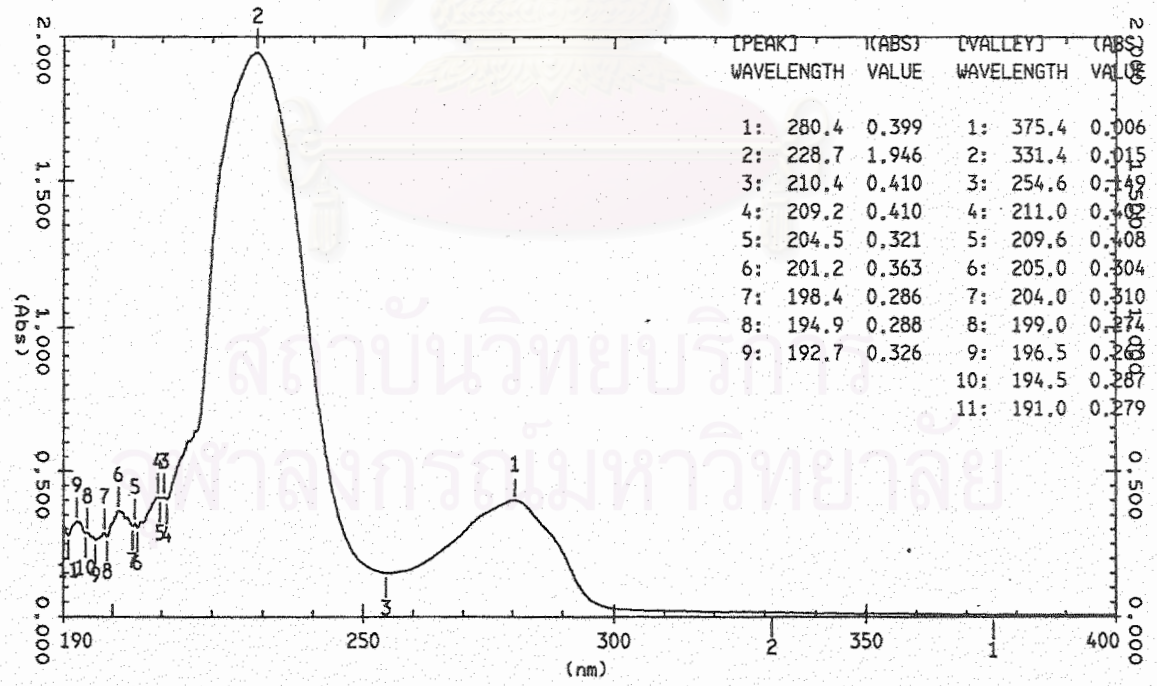


Figure 151 UV Spectrum of compound CBE5 (methanol)

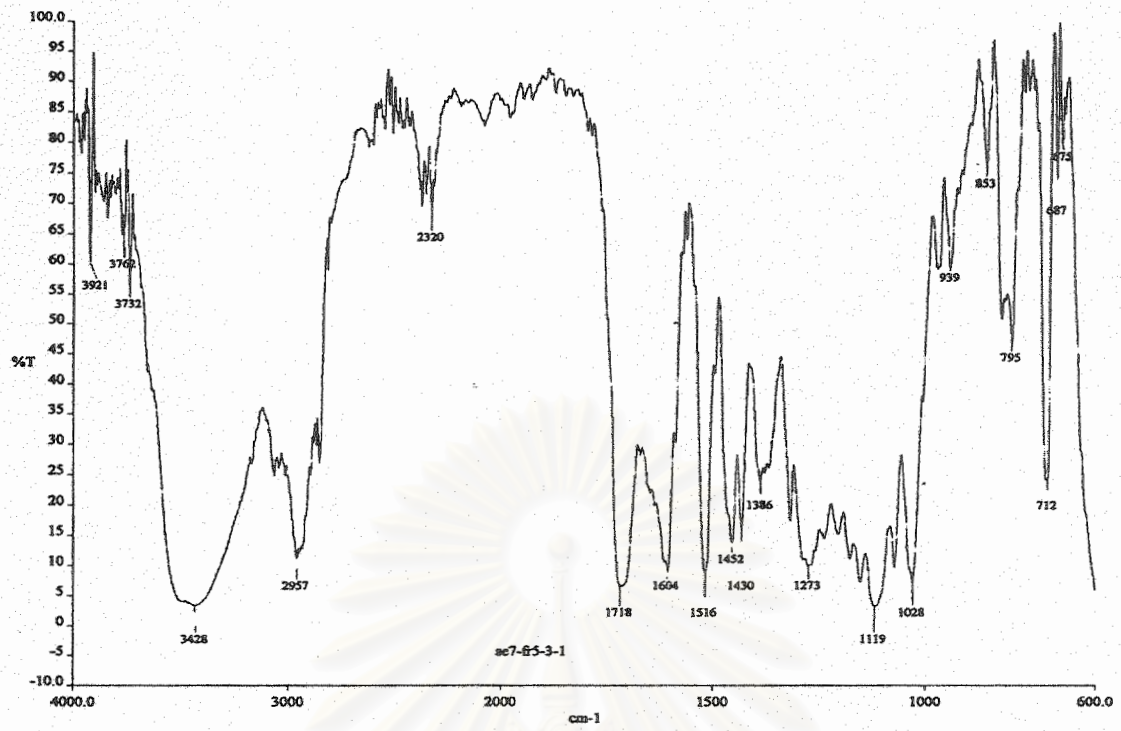


Figure 152 IR Spectrum of compound CBE5 (neat)

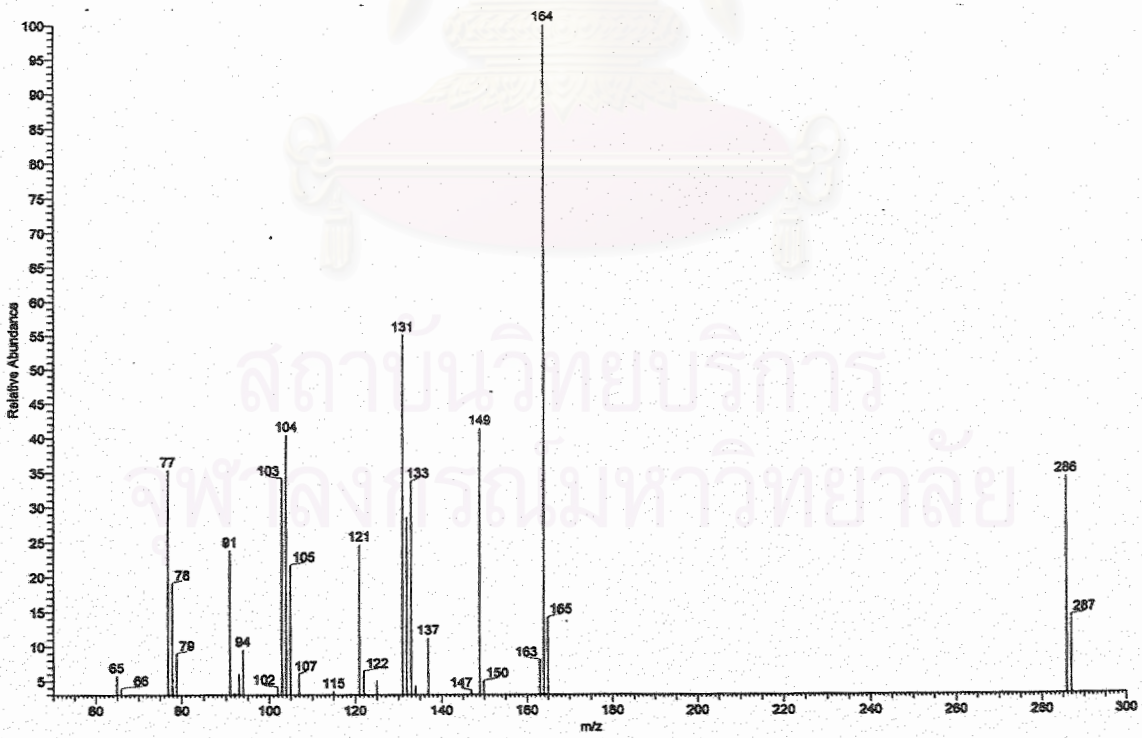


Figure 153 EIMS Mass spectrum of compound CBE5

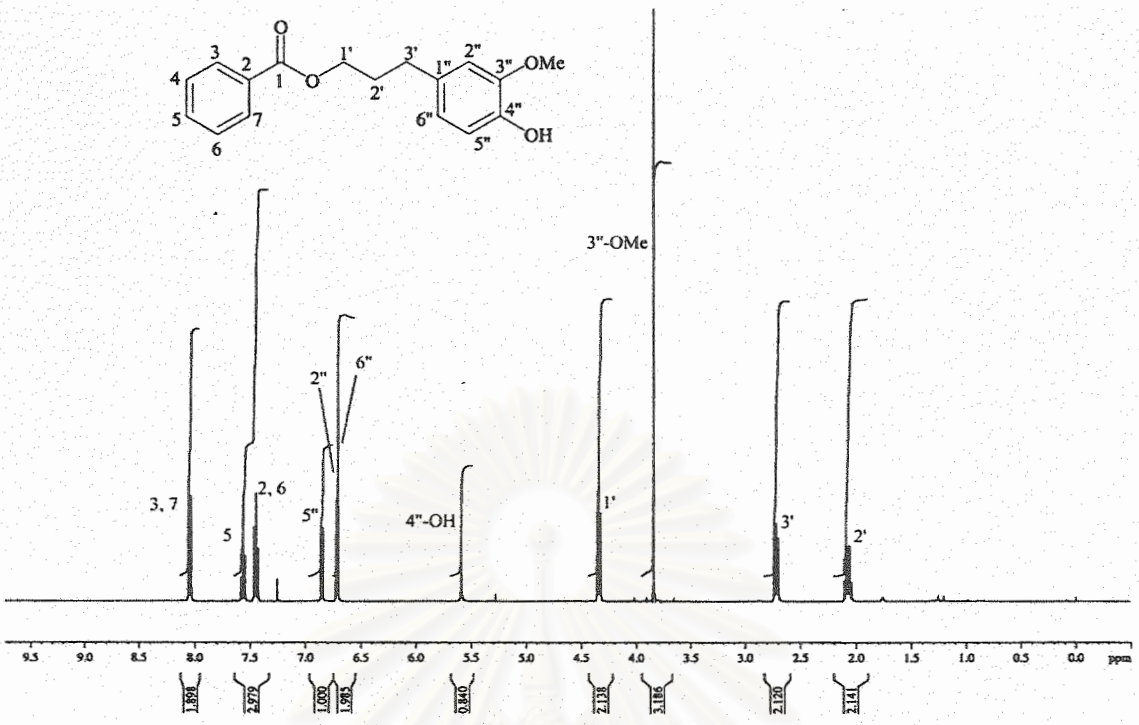


Figure 154 ^{1}H NMR (400 MHz) Spectrum of compound CBE5 ($CDCl_3$)

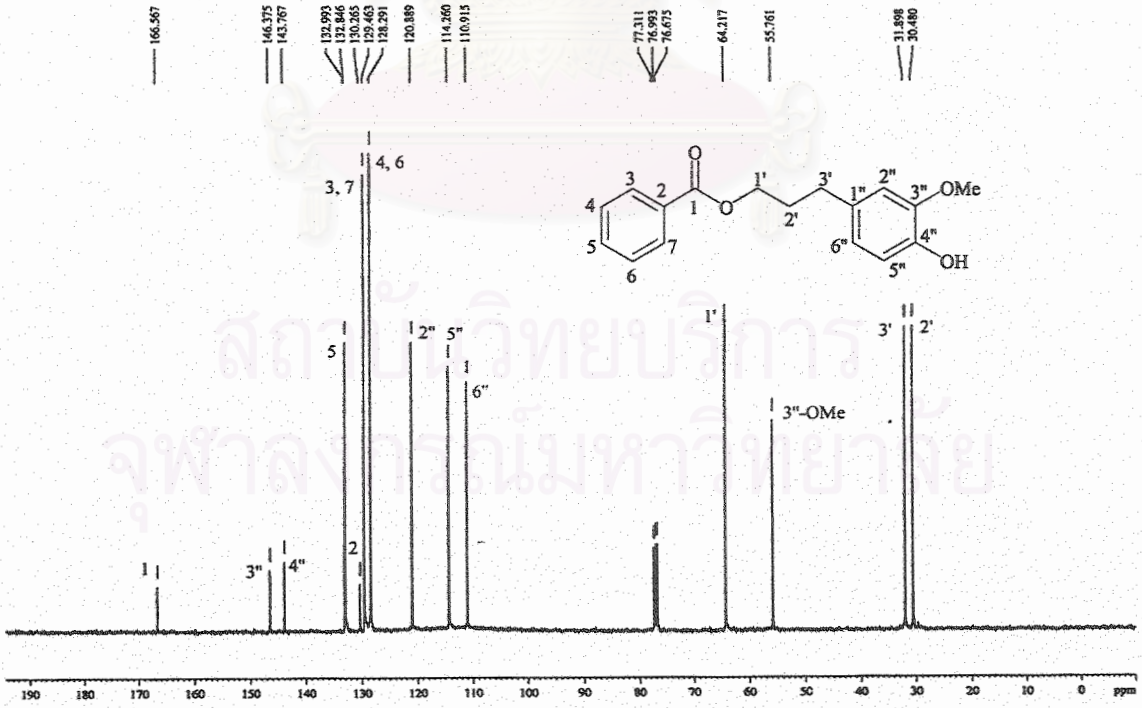


Figure 155 ^{13}C NMR (100 MHz) Spectrum of compound CBE5 ($CDCl_3$)

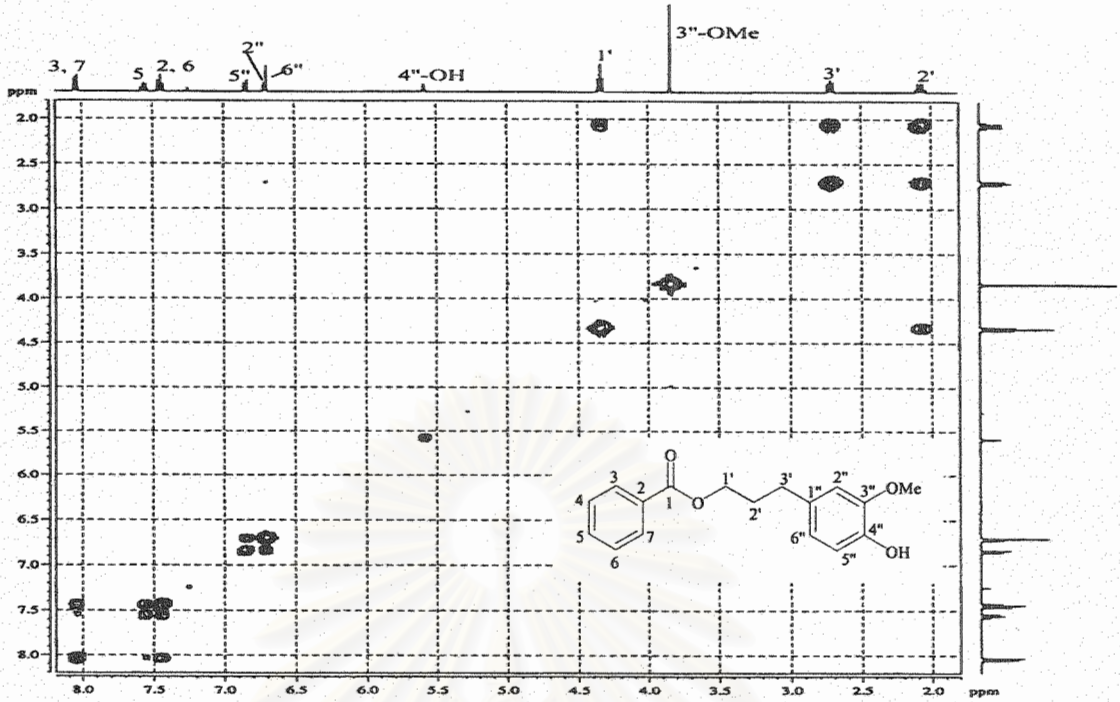


Figure 156 ^{1}H-^{1}H COSY Spectrum of compound CBE5 ($CDCl_3$)

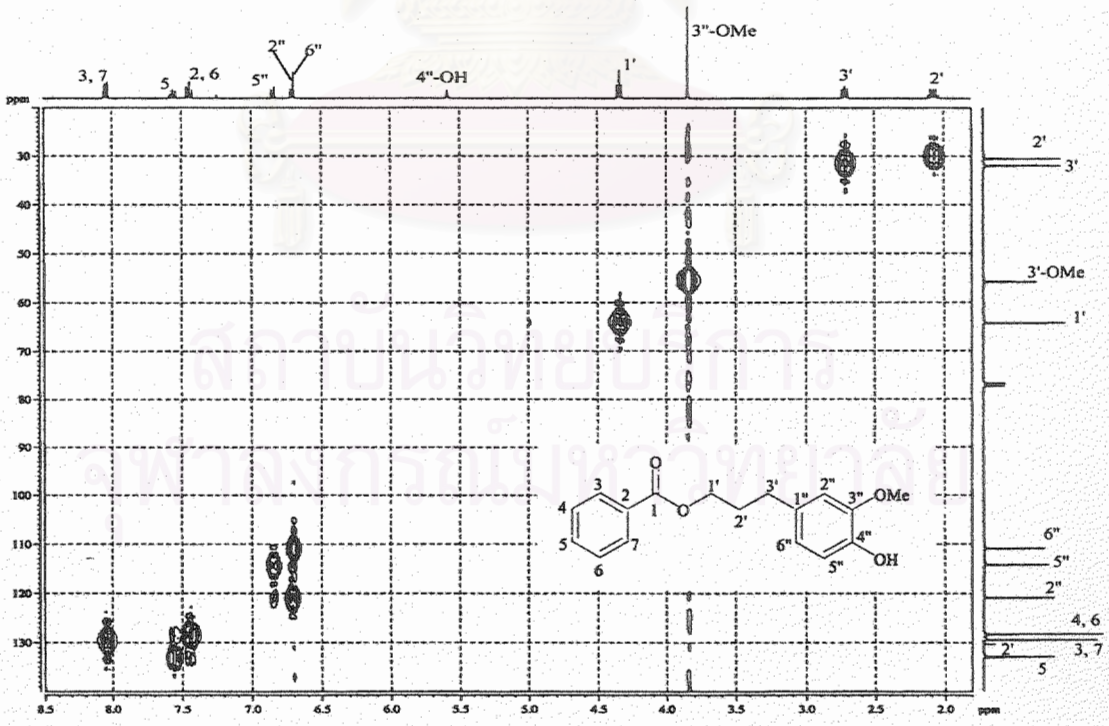


Figure 157 HMQC Spectrum of compound CBE5 ($CDCl_3$)

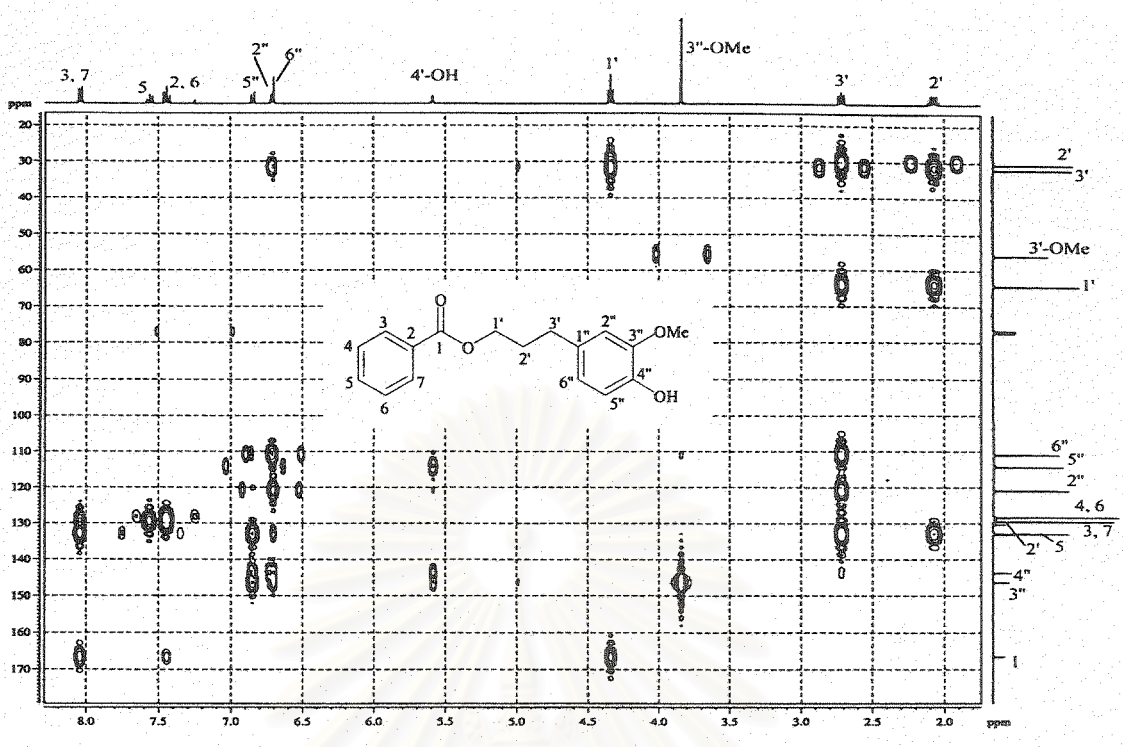


Figure 158 HMBC Spectrum of compound CBE5 ($CDCl_3$)

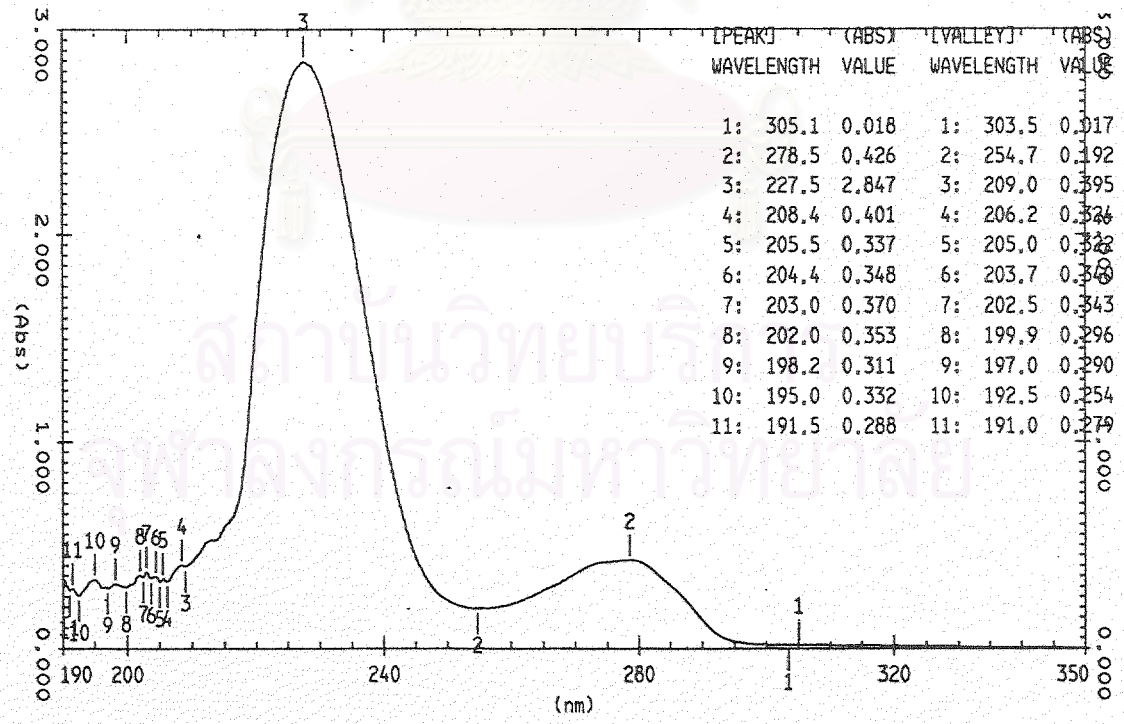


Figure 159 UV Spectrum of compound CBE6 (methanol)

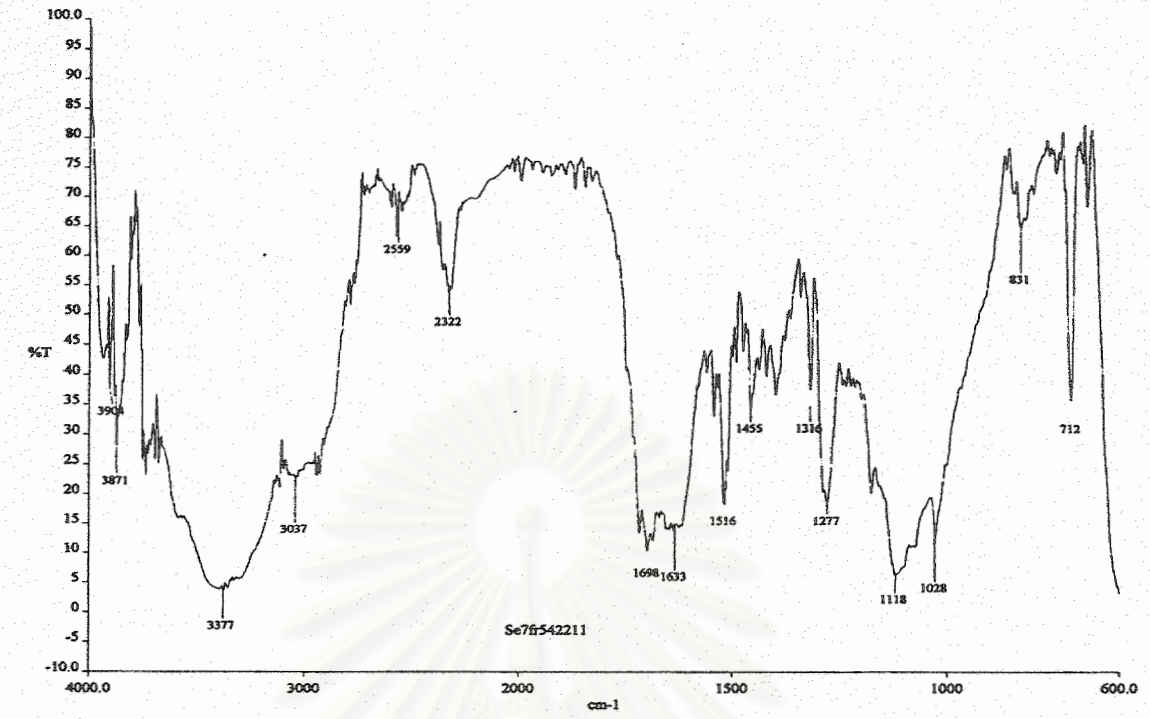


Figure 160 IR Spectrum of compound CBE6 (neat)

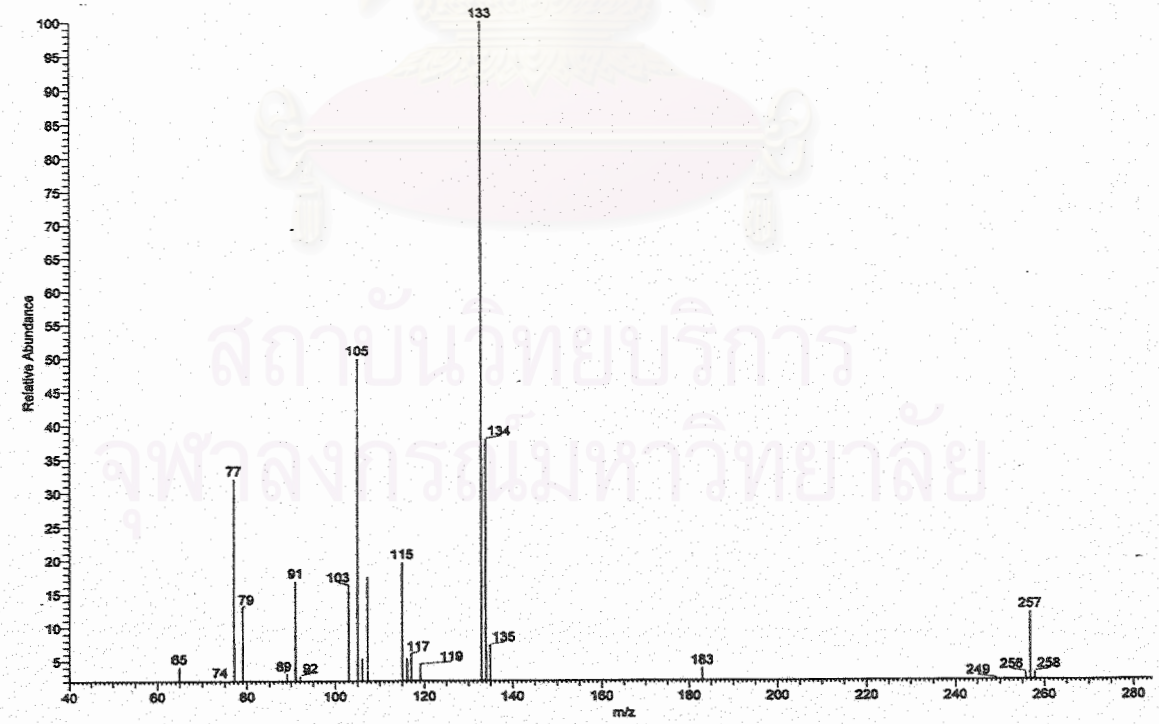


Figure 161 EIMS Mass spectrum of compound CBE6

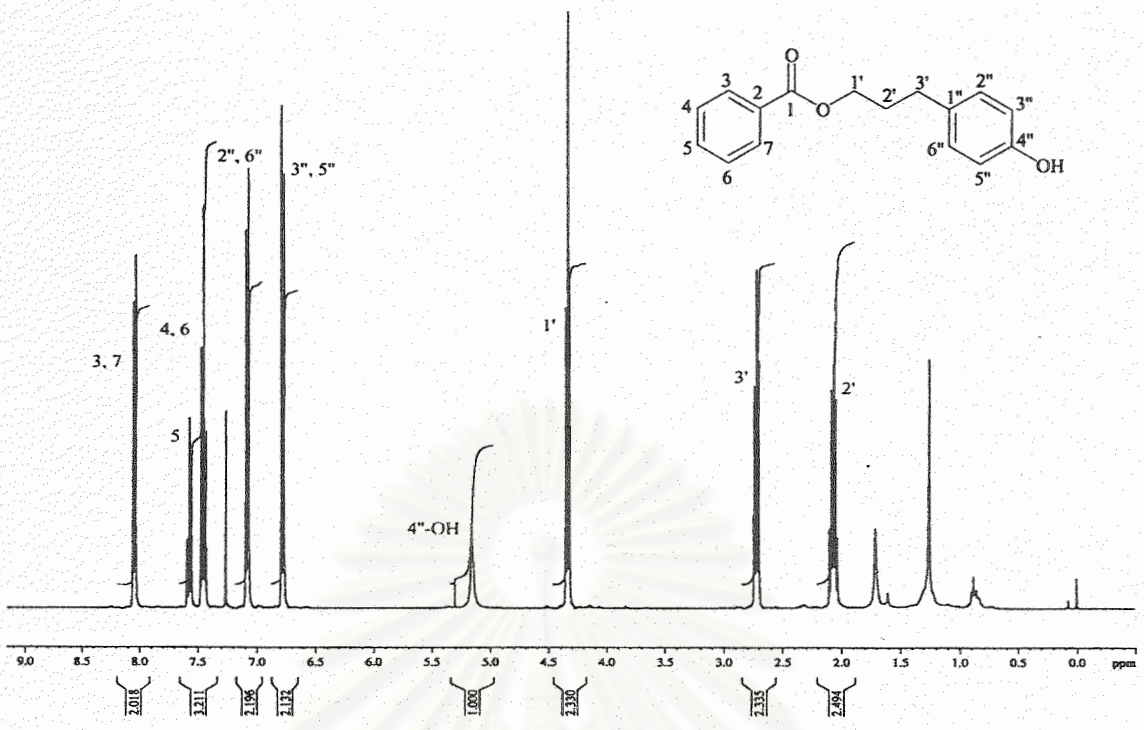


Figure 162 ^{1}H NMR (400 MHz) Spectrum of compound CBE6 ($CDCl_3$)

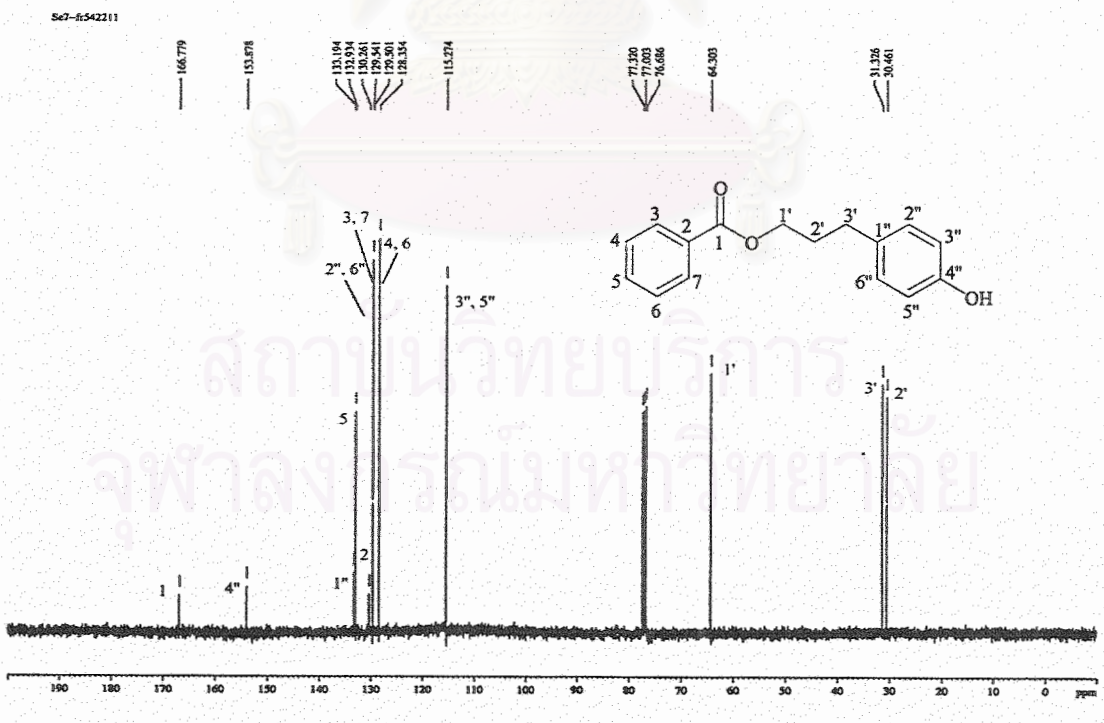


Figure 163 ^{13}C NMR (100 MHz) Spectrum of compound CBE6 ($CDCl_3$)

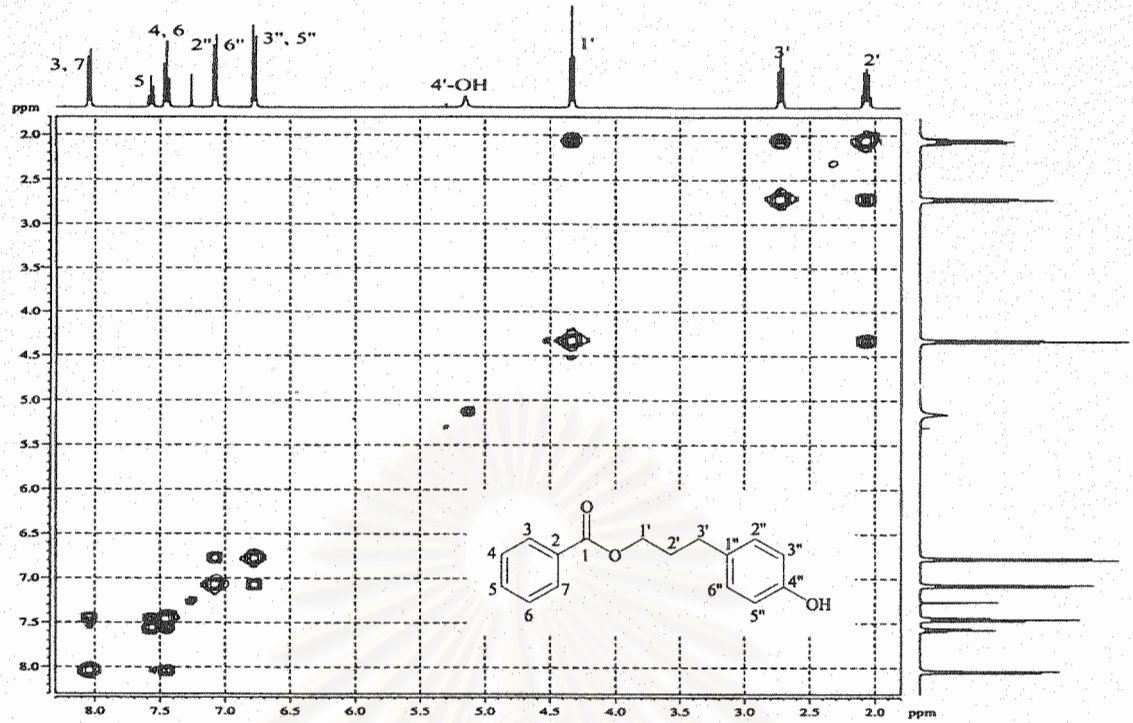


Figure 164 ^{1}H-^{1}H COSY Spectrum of compound CBE6 ($CDCl_3$)

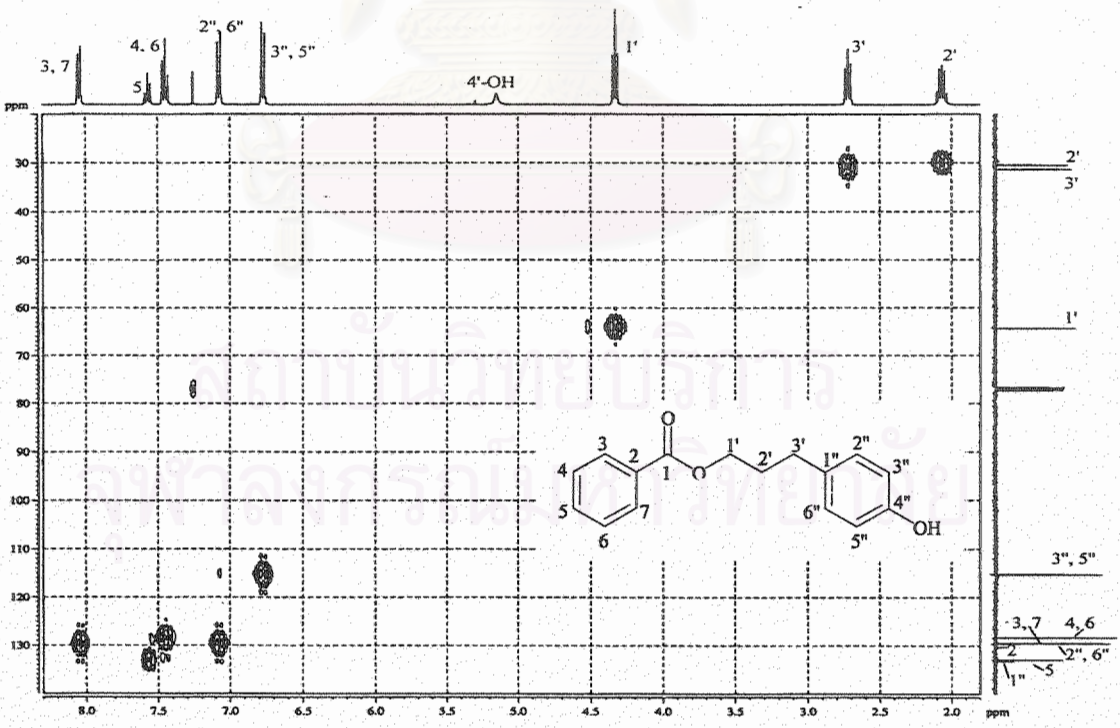


Figure 165 HMQC Spectrum of compound CBE6 ($CDCl_3$)

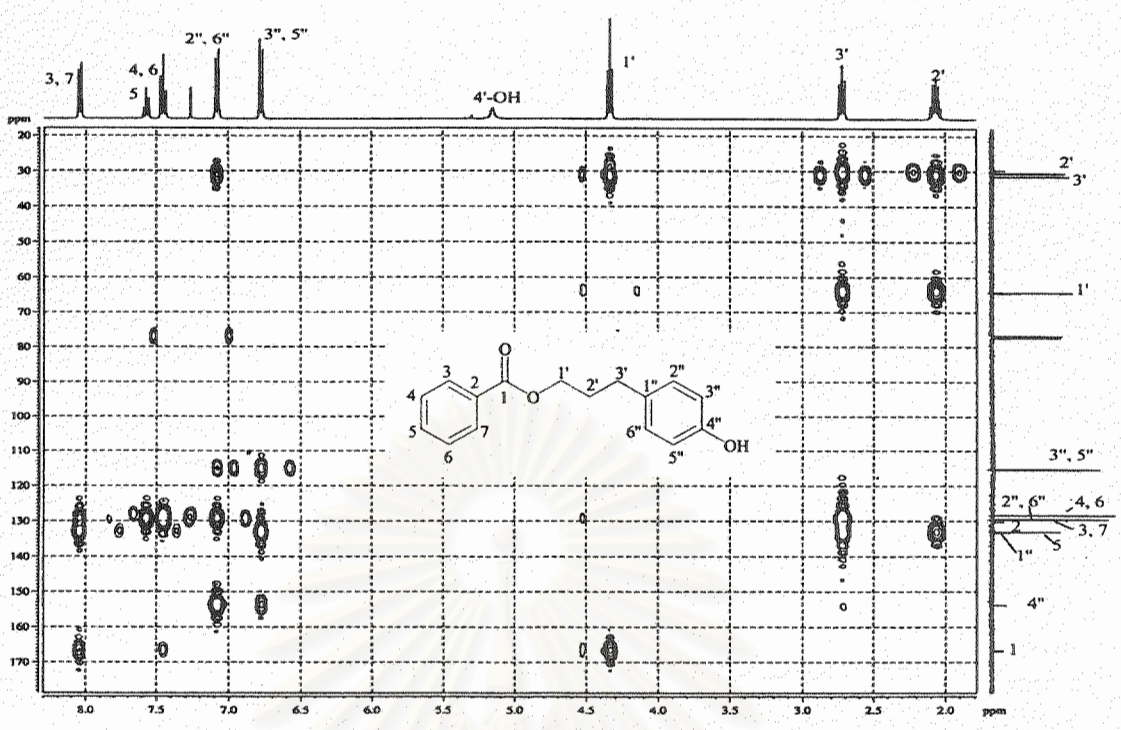


Figure 166 HMBC Spectrum of compound CBE6 ($CDCl_3$)

VITA

Miss Sirivan Athikomkulchai was born on December 2, 1975 in Bangkok, Thailand. She received her Bachelor's degree of Science in Pharmacy (2nd class honor) from the Faculty of Pharmacy, Mahidol University in 1997. She was granted a 1999 Royal Golden Jubilee Ph.D. Scholarship from Thailand Research Fund (TRF).

Publications

1. Athikomkulchai, S., Ruangrungsi, N., Sekine, T., Sumino, M., Igarashi, K., and Ikegami, F. 2003. Chemical Constituents of *Bauhinia sirindhorniae*. Natural Medicines. 57(4): 150-153.
2. Athikomkulchai, S., Prawat, H., Thasana, N., Ruangrungsi, N and Ruchirawat, S. Antifungal Agents from *Croton hutchinsonianus*. Planta Med (Submitted).
3. Athikomkulchai, S., Sriubolmas, N. and Ruangrungsi, N. Antibacterial Activity of *Bauhinia sirindhorniae*. J. Med. Assoc. Thai (Submitted).

Poster Presentations

1. Athikomkulchai, S., and Ruangrungsi, N. "Microscopic Characters of *Aristolochia pothieri* root" p.42, The 18th Annual Research Meeting in Pharmaceutical Sciences, December 7, 2001, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.
2. Athikomkulchai, S., Ruangrungsi, N., Sekine, T., Sumino, M., Igarashi, K., and Ikegami, F. "Chemical Constituents of *Bauhinia sirindhorniae*". p. 26, The Third Indochina Conference on Pharmaceutical Sciences, May 21-23, 2003, Twin Towers Hotel, Bangkok.
3. Athikomkulchai, S., Sriubolmas, N., and Ruangrungsi, N. "Chemical Constituents of *Bauhinia sirindhorniae* and Their Antibacterial Activities". p. 119, 29th Congress on Science and Technology of Thailand, October 20-22, 2003, Golden Jubilee Convention Hall, Khon Kean University, Khon Kean.
4. Athikomkulchai, S., Prawat, H., Ruangrungsi, N. and Ruchirawat, S. "Chemical Constituents of *Croton hutchinsonianus*" p.193, RGJ-Ph.D. Congress V, April 23-25, 2004, Pattaya, Chonburi.